# THE PFIZER HANDBOOK OF MICROBIAL METABOLITES

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*By*

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# Foreword

THE IMPRESSIVE ADVANCES achieved in fermentation techniques have created new and often highly efficient methods for the synthesis of organic compounds. It seems clear that in addition to antibiotics and steroids, an ever-increasing number of structurally less complicated chemicals will be synthesized most economically by fermentation of abundant starting materials of natural or synthetic origin.

The purpose of this handbook is to list the source and physical, chemical and physiological properties of metabolic products isolated from bacteria, molds, fungi and lichens. In addition to this collection of facts and references, it contains chapters outlining the biogenesis of various structural types elaborated mainly by microorganisms. Although some of our present-day views on biogenetic pathways may have to be revised in the future, these chapters should prove to be exceedingly helpful not only to chemists working on the structures of new substances but also to biochemists investigating the mode of action of physiologically active compounds.

There certainly was an urgent need for such a compilation because the original reports are scattered through a wide variety of scientific journals rarely assembled in one place but distributed in chemical, pharmaceutical and medical libraries. It seems highly appropriate that an attempt to cover the literature in this rapidly expanding field should come from the Research Division of Chas. Pfizer & Co., Inc. The group deserves a great deal of credit for pioneering work in industrial fermentation as well as in isolation and structure elucidation of many antibiotics.

G. BÜCHI
*Cambridge, Massachusetts*

# Acknowledgment

A COMPILATION of this sort was suggested by Dr. Ernest M. Weber in 1956, and the first draft was issued as an intra-company report the following year. Later, publication was suggested by Dr. Gilbert M. Shull and urged by a number of university people interested in microbial metabolites.

Most importantly, publication would not have been possible without the consent and support of Dr. Karl J. Brunings and Dr. I. A. Solomons. Other staff members of the Pfizer Medical Research Laboratories have also been very cooperative. Dr. Frank A. Hochstein has been most helpful throughout the preparation for publication, and I wish to thank him especially as well as Dr. Walter D. Celmer for reading the manuscript at an early stage and for their comments on the chapter on macrolide antibiotics.

In addition, Dr. Francis X. Murphy read the entire galley proof and made many constructive suggestions.

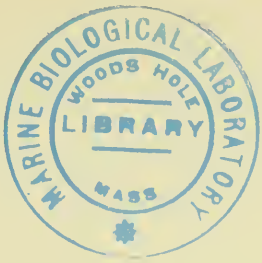
Several other authorities have been kind enough to review their specialties. Professor Hans Brockmann of Göttingen contributed information on the actinomycins; Professor Konrad Bloch of Harvard read the sections dealing with lipides; Dr. T. G. Halsall of Oxford reviewed fungal steroids; Dr. Herchel Smith of Manchester, sections concerned with the biosynthesis of various mold metabolites; Professor F. G. Holliman of Capetown, the section on phenazines; Dr. J. D. Bu'Lock of Manchester, the section on acetylenic substances; and Dr. Edward Borowsky of the Institut Medycyny Moskiej, Gdansk, the sec-

tion on polyene macrolides. Professor George Büchi of Massachusetts Institute of Technology read nearly all of the galley proof and contributed a generous foreword.

We cannot begin to acknowledge all of the assistance received, particularly from the Pfizer library staff and other libraries, from our colleagues on the chemical staff, and from the secretarial staff. Most of the manuscript typing was done by Miss Kathryn Beck, Mrs. Loretta Michaud, Mrs. Terry Lunt, Mrs. Hedy Korst, Mrs. Judith Neff, and Miss Patricia Goepfert. The references were corrected and much indexing was done by Miss Claudette Parent, Miss Grace Olimski, and Miss Patricia French. All of the copy-editing was done by Mrs. Margaret Thompson. Patricia Curtis of Editorial Projects, Inc. was very helpful in coordinating and expediting publication operations.

MAX W. MILLER
*Groton, Connecticut*

# Contents



# THE PFIZER HANDBOOK OF MICROBIAL METABOLITES

# Introduction

THE CULTURE of bacteria and molds, the collection of higher fungi and lichens and the isolation and characterization of their metabolites is a sophisticated sort of research involving several distinct sciences. As a result the reports of such work are scattered through a variety of chemical, biochemical, microbiological, botanical, medical and pharmaceutical journals as well as general scientific journals and those devoted to antibiotics and fermentation technology. The published reviews of the structures of microbial metabolites have been limited in scope. It is difficult for the novice to gain a total impression of the progress that has been made, and difficult even for the specialist in this area to see the forest entire as well as the trees about him.

Having monitored the literature for several years incidental to our own work, we felt that it would be useful to publish a more general list of chemicals produced by microorganisms. More specifically, what has been attempted is a compilation of data on the structural and simpler physical properties of all of the primary microorganism metabolites which have been reported to be produced by the organisms growing either in the wild state or in culture on artificial sugar-based media. Although many structures are incomplete, generally the compounds in this list have been purified, and at least some physical properties observed. In view of the difficulties mentioned above we do not presume to have achieved absolutely complete coverage, and we should be pleased to receive structures or references to appropriate compounds which have been overlooked. Corrections of errors would be appreciated also. The literature available to us has been watched until the beginning of printing operations in December 1960.

Organization is by general similarity of chemical structures, but not in the strictest sense. For example, all carotenes and carotenoids were grouped together rather than grouping a caro-

tene alcohol with, *e.g.*, a steroid alcohol. Many substances are ambiguous and could have been classified in any of several different chapters. A substance which contains a sugar, a benzene ring, a terpenoid fragment and a heterocycle will most likely be found under the appropriate heterocycle classification. Some arbitrary decisions have been necessary, but indexing by name, by empirical formula and by producing microorganism should serve most purposes. Again quite generally, progression is from the simple to the complex; sugarlike compounds being considered simple because they resemble the substrate, glucose.

In order to make the list more coherent a background has been sketched in, emphasizing occurrence and biosynthetic origin. A considerable literature on the biosynthetic origin of microbial metabolites has accumulated. Familiarity with it is valuable in interpreting experimental results in structure determinations. Several old structures have been revised in the light of this new knowledge.

Many of the biosynthetic and other metabolic schemes worked out in microorganisms are quite general in occurrence and have been found to be operative in mammalian metabolism. Because bacteria and fungi grow rapidly and are easy and inexpensive to handle, they are among the most useful tools in the exploration of metabolic routes. Many of the chemicals in this list were isolated incident to such studies.

Some chemicals of metabolic significance and of a suitable degree of complexity can be produced economically in quantity by fermentation methods and have found industrial uses. An example is citric acid, which now finds an annual market of thousands of tons.

The discovery of the effectiveness of the mold product, penicillin, in treating many bacterial infections in man gave tremendous impetus to the isolation and screening of microorganisms and their metabolites for antibiotics. The isolation and study of microbial metabolites, formerly a scholarly pursuit in a few academic laboratories, suddenly was supported by the resources of a great industry. Experience showed that a genus of filamentous soil organism, the actinomycete (streptomycete), was a

particularly prolific source of organisms adaptable to antibiotics production when grown in suitable media.

Research with the actinomycetes resulted in the discovery of agents effective against a broad spectrum of pathogens. The first of these were chloramphenicol, chlortetracycline and oxytetracycline. Since the discovery of oxytetracycline, no antibiotics of broader antibacterial range have been developed.

Prior to the discovery of antibiotics, much work had been done on the structures of lichen substances, and, as mentioned above, a few academic laboratories were interested in mold metabolites. Notable among these was Professor Harold Raistrick's group at the London School of Hygiene and Tropical Medicine. Raistrick, now retired, and his collaborators have published over 100 papers on this topic.

The academic investigators were impelled by no practical motive except perhaps a hope that comparison of the chemical metabolites of various ill-defined groups of fungi would assist in their classification. Some generalizations did become apparent, but on the whole this hope was disappointed. It was found that the same chemical might even be produced by both bacteria and fungi. Some of the old classification schemes based on pigmentation were found to be obsolete.

The structures of the large molecules produced by microorganisms have proved to be more specific and of real value to taxonomy. Since the advent of paper chromatography, the identification of amino acids, sugars and other fragments from cell tissue hydrolysates has been facilitated. From the ensuing proliferation of literature on this subject it is manifest that the compositions of various cell tissues (capsule, wall, protoplast membrane, internal proteins), as well as exotoxins and other high molecular weight exudates, are much more specific. Even strains of species can sometimes be distinguished by the presence or absence of one of these fragments, and these molecules are important in immunology. Work of this sort has become more important since the discovery of evidence that certain antibiotics, *e.g.*, penicillin, interrupt growth and cell division in the bacteria against which they are effective by interfering with

normal cell wall synthesis. Although we were unable to pursue this fascinating topic, an appendix of literature titles on the structure of higher molecular weight products of microorganisms and their cell wall structures has been attached.

In comparing the structures of the hundreds of microorganism metabolites which have been characterized thoroughly it is well to remember that the statistical emphasis may be misleading. It is likely that insoluble compounds, lipophilic materials easily extractible from aqueous cultures, organic acids which can be precipitated as insoluble salts and pigments that are easily observed have probably received a disproportionate degree of attention. The same, of course, could be said for antibiotics, which are conspicuous for their biological activity. The most difficultly discoverable metabolites are the relatively inconspicuous, low molecular weight, hydrophilic, perhaps phosphorylated compounds. Eventually many of the precursors of more elaborate metabolites will be found in this category.

Also, the metabolites of certain microorganisms have received disproportionate study. Examples are *Mycobacterium tuberculosis*, the tuberculosis pathogen, and *Claviceps purpurea*, the ergot fungus. By permission of Dr. Esmond R. Long and the Williams and Wilkins Publishing Company a review of the known metabolites of the former organism has been reproduced as an appendix, although many of the compounds included in this review are also to be found in the body of the text and others in the text which were not in the review. Also an appendix dealing with the confusing subject of microbial carotenoids has been attached by permission of the Chemical Publishing Company and of Professor T. W. Goodwin of the University of Liverpool.

Referencing is not exhaustive. It was kept on the lean side intentionally, and we feel that it is more useful that way. On some topics the literature is vast. It would have been virtually impossible to offer complete referencing of, for example, acetic acid, or even of some of the more complex substances such as the gibberellins or $\beta$-carotene. Much attention has been given to choice of useful references, although no doubt there have

been lapses, and differences of opinion will probably arise. For some of the substances carrying a large literature a review article often is cited. In general an attempt has been made to cite the isolation, final structure determination and synthesis papers insofar as they exist. In the references cited care has been taken to include the complete list of authors as given on the paper. A bibliography of books, general references and reviews is included at the end.

Occasional comments may be found at the bottom of an entry, reflecting the manner in which this material evolved from a card file with a few notes. These comments were allowed to stand without expansion for what they are worth. For the most part the work is uncritical, structures and properties having been transcribed just as given in the literature. Structures and empirical formulas designated as tentative or approximate by the authors have been so designated here.

The indexes were not available prior to printing, and it is hoped that they will point out hitherto unrecognized relationships.

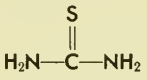
# 1

# Simple Hydrocarbons, Ketones, Aldehydes, Esters, etc.

The simple compounds listed here cannot be treated as a class. The biogenetic origins of many of them should become apparent from the introductions to later chapters. Besides the hydrocarbons shown it might be mentioned that lactarius species sporophores contain *cis*-polyisoprene, a rubber-like substance.

W. D. Stewart, W. L. Wachtel, J. J. Shipman and J. A. Yanko, *Science* 122 1271 (1955).

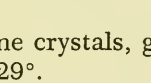
1 **Thiourea,** $CH_4N_2S$, white crystals, m.p. 180–182°.



*Verticillium albo-atrum, Botrytis cinerea*

K. Ovcharov, *Compt. rend. acad. sci.*, U.S.S.R. 16 461 (1937).

2 **Guanidine,** $CH_5N_3$, alkaline crystals, generally isolated as salts, *e.g.* acetate, m.p. 229°.

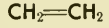


*Boletus edulis, Hydnum aspratum* Berk.

E. Winterstein, C. Reuter and R. Korolev, *J. Chem. Soc.* 104 433 (1913).

Seijiro Inagaki, *J. Pharm. Soc. Japan* 54 824 (1934).

3 **Ethylene,** $C_2H_4$, colorless gas, b.p. −103°.



*Penicillium digitatum, Blastomyces dermatitidis, B. brasiliensis, Histoplasma capsulatum*

Walter J. Nickerson, *Arch. Biochem.* 17 225 (1948).

Erston V. Miller, J. R. Winston and D. F. Fisher, *J. Agr. Research* 60 269 (1940).

Ray E. Young, Harlan K. Pratt and J. B. Biole, *Plant Physiol.* 26 304 (1951).

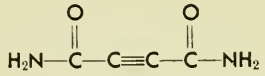
4 **Dimethylsulfone,** $C_2H_6O_2S$, colorless prisms, m.p. 107–109°.

$CH_3SO_2CH_3$

*Cladonia deformis* Hoffm.

Torger Bruun and Nils Andreas Sorensen, *Acta Chem. Scand.* 8 703 (1954).

5 **Cellocidin** (Aquamycin), $C_4H_4O_2N_2$, white crystals, m.p. 216–218° (dec.).



*Streptomyces chibaensis, S. reticuli* var. *aquamyceticus*

The yield was 16.5 g. from 420 liters of culture fluid.

Saburo Suzuki, Goto Nakamura, Kazuhiko Okuma and Yoko Tomiyama, *J. Antibiotics* (Japan) 11A 81 (1958).

Hyozo Taniyama, Shoji Takemura, Kimiko Kageyama and Masanao Funaki, *J. Pharm. Soc. Japan* 79 1510 (1959).

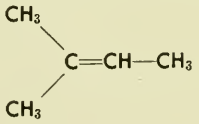
6 **Ethyl Acetate,** $C_4H_8O_2$, colorless liquid, b.p. 77°, $n_D^{20}$ 1.3719.

$CH_3COOC_2H_5$

*Penicillium digitatum*

J. H. Birkinshaw and H. Raistrick, *Trans. Roy. Soc.* (London) B220 331 (1931).

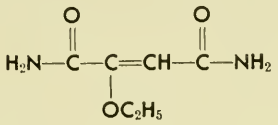
7 **2-Methyl-2-butene,** $C_5H_{10}$, colorless liquid, b.p. 38.4°.



*Puccinia graminis* Pers. var. *tritici* Erikas. and Henn. (uredospores)

F. R. Forsyth, *Can. J. Botany* 33 363 (1955).

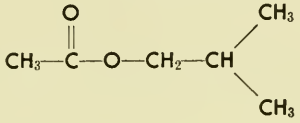
8 **1-Ethoxy-1,2-ethylenedicarboxamide**, $C_6H_{10}O_3N_2$,



*Streptomyces sp.*

Yasuharu Sekizawa, *J. Biochem.* Japan 45 73 (1958).

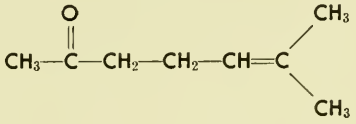
9 **Isobutyl Acetate**, $C_6H_{12}O_2$, colorless liquid, b.p. 61°, $n_D^{15}$ 1.3936.



*Endoconidiophora coerulescens*

J. H. Birkinshaw and E. N. Morgan, *Biochem. J.* 47 55 (1950).

10 **2-Methyl-2-heptene-6-one**, $C_8H_{14}O$, colorless liquid, b.p. 172–174°, 58° (10 mm.), $n_D^{20}$ 1.4445.

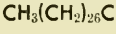


*Endoconidiophora coerulescens* Münch, *E. virescens* Davidson (artificial medium)

Isobutyl acetate and a mixture of methylheptenols were isolated from the same culture.

J. H. Birkinshaw and E. N. Morgan, *Biochem. J.* 47 55 (1950).

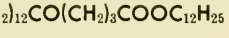
11 **Octacosane**, $C_{28}H_{58}$, colorless crystals, m.p. 61°.



*Amanita phalloides*

Heinrich Wieland and Gustav Coutelle, *Ann.* 548 270 (1941).

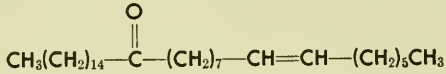
12 **Actinomycin $J_2$** (Waksman's Actinomycin B, Dodecyl Ester of 5-Oxostearic Acid), $C_{30}H_{58}O_3$, colorless crystals, m.p. 81.5°.



*Actinomyces* (*Streptomyces*) *flavus*

Yoshimasa Hirata and Koji Nakanishi, *Bull. Chem. Soc. Japan* 22 121 (1949).

13 ***cis*-Palmitenone**, $C_{31}H_{60}O$, colorless microcrystals, m.p. 40°.

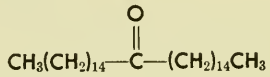


*Corynebacterium diphtheriae*

J. Pudles and E. Lederer, *Biochim. et Biophys. Acta* 11 602 (1953).

*Idem., Bull. soc. chim. biol.* 36 759 (1954).

14 **Palmitone**, $C_{31}H_{62}O$, colorless leaflets, m.p. 82°.



*Corynebacterium diphtheriae*

J. Pudles and E. Lederer, *Bull. soc. chim. biol.* 36 759 (1954).

2

# Alcohols, Glycols and Compounds Related to Sugars

Two of the most important routes of sugar metabolism are the Embden-Meyerhof pathway of anaerobic glycolysis and the oxidative pentose phosphate cycles. Both occur widely in nature, and microorganisms were useful in the discovery of each. Many of the metabolites of this chapter can be pictured as arising from one of these schemes, which are also the main known routes of glucose metabolism in mammals. It should be understood that other paths and fragments of paths of glucose metabolism have been found in various microorganisms.

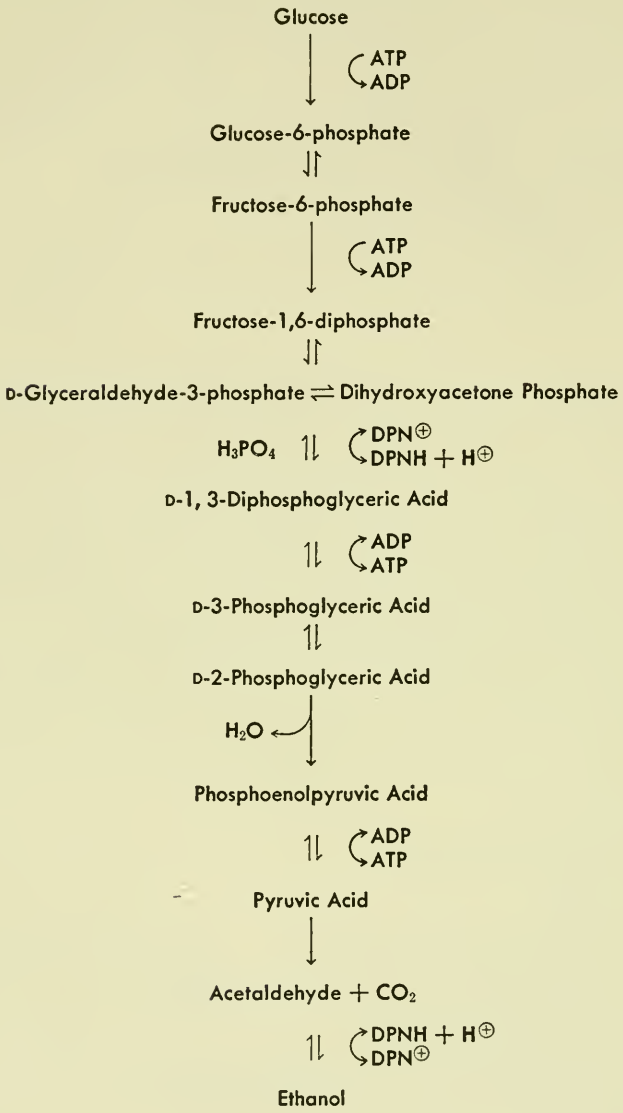
Yeast was instrumental in the elucidation of the Embden-Meyerhof route[1] and the yeast alcohol fermentation is represented as follows, each step catalyzed by a specific enzyme:

Embden-Meyerhof Route of Anaerobic Glycolysis in Yeast

Enzymes

1. Hexokinase
2. Phosphohexoisomerase
3. Phosphohexokinase
4. Aldolase
5. Triosephosphate isomerase
6. Triosephosphate dehydrogenase (Inhibited by iodoacetate)
7. ATP-Phosphoglyceric transphosphorylase
8. Phosphoglyceromutase
9. Enolase (Inhibited by fluoride)
10. ATP-Phosphopyruvic transphosphorylase
11. Carboxylase
12. Alcohol dehydrogenase

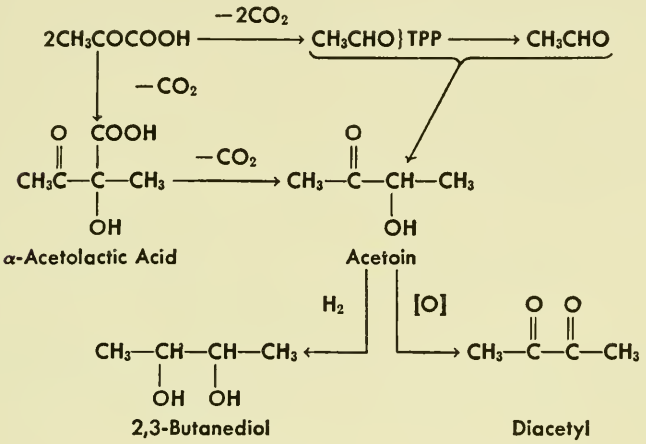
[1] A. J. Kluyver and C. B. Van Niel, "The Microbe's Contribution to Biology," Harvard University Press, Cambridge, Massachusetts, 1956.




Many molds, actinomycetes and bacteria use this system to some degree. Variations occur, and intermediates may feed in from other sources, for example, triose phosphate from the pen-

tose phosphate cycle. Some bacteria are able to produce alcohol by other means.

The pyruvate from anaerobic glycolysis can meet a variety of fates. In some cases it is transformed into acetoin and its oxidation and reduction products, diacetyl and 2,3-butanediol (thiamine pyrophosphate coenzyme). α-Acetolactic acid has been shown to be an intermediate in certain instances:[2]



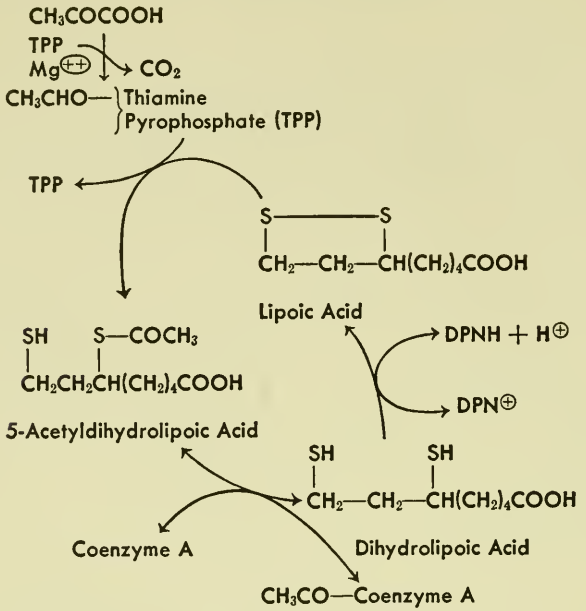

Acetoin has been found in yeast, in other fungi and in bacteria. Large yields of mixtures of these condensation products can be obtained from some bacteria.

Pyruvate is reduced to D-lactic acid in the homofermentative bacteria and lower phycomycetes (and to L-lactic acid in mammalian muscle).

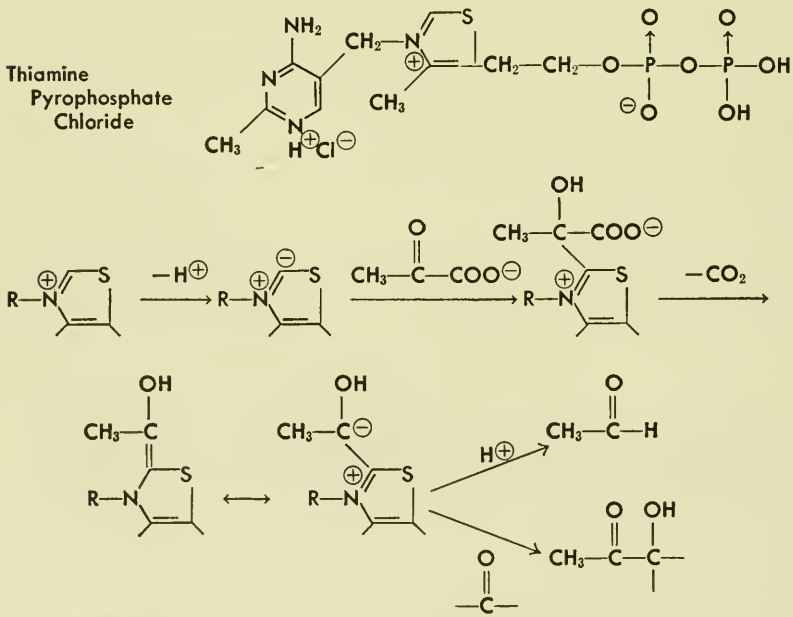
Another reaction of pyruvate is its conversion to acetylcoenzyme A with the participation of lipoic acid; the probable mechanism being:[3]

[2] Elliot Juni, *J. Biol. Chem.* 195 715 (1952); Yutaka Kobayashi and George Kalnitsky, *ibid.* 211 473 (1954).

[3] I. A. Gunsalus, Lois S. Barton and H. Gruber, *J. Am. Chem. Soc.* 78 1763 (1956).




The nature of the actual catalysis of pyruvate decarboxylation and of aldol condensations by thiamine pyrophosphate coenzyme has been elucidated.[4] It is shown below:




[4] Ronald Breslow, *Chem. and Ind.*, 893 (1957).

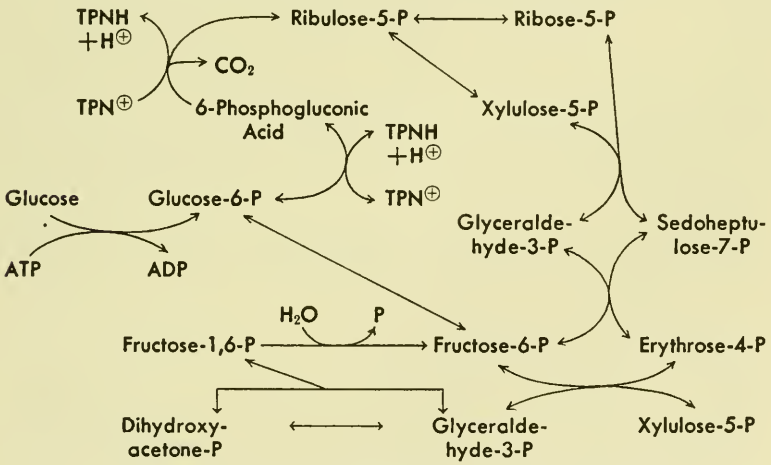
Thus, the production of acetaldehyde (and subsequently alcohol) by yeast, the production of acetoin by certain bacteria, etc.

Although the lipoic acid mechanism was first demonstrated in *Streptococcus faecalis*, all bacteria do not require the cofactor for this transformation.

The role of acetylcoenzyme A in cellular synthesis of fatty acids will be seen later. Butanol is probably formed by reduction of acetoacetylcoenzyme A. It is interesting to note that some microorganisms can synthesize a variety of carbohydrates by using acetate as the sole carbon source, in effect reversing the process (*e.g.*[5]). Pyruvate is also converted to succinate by fixation of $CO_2$.

Various other fates of pyruvate are known. For example, there are bacteria which dismutate 2 moles of pyruvate to 1 mole each of acetic and lactic acids.[6] Also *Bacillus coli* is known to convert pyruvate to a mixture of acetic and formic acids.[7]

The pentose phosphate cycle mentioned earlier probably occurs in many microorganisms. It is outlined below:




Enzyme-catalyzed reactions of the pentose phosphate pathway*

* This diagram together with the summarizing equations is reprinted with permission from Joseph S. Fruton and Sofia Simmonds, "General Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1958, p. 531.

[5] V. I. Lyubimov, *Doklady Akad. Nauk* SSSR **III** No. 4 (1956).

[6] Seymour Karkes, Alice del Campillo, I. C. Gunsalus and Severo Ochoa, *J. Biol. Chem.* 193 721 (1952).

[7] Kenneth V. Thimann, "The Life of Bacteria," Macmillan Co., New York, N. Y. 1955, pp. 441–465.

These reactions in summary are:

6 Hexose phosphate + $6O_2$ → 6 Pentose phosphate + $6CO_2$ + $6H_2O$
4 Pentose phosphate → 2 Hexose phosphate + 2 Tetrose phosphate
2 Pentose phosphate + 2 Tetrose phosphate →
2 Hexose phosphate + 2 Triose phosphate
2 Triose phosphate + $H_2O$ → Hexose phosphate + phosphate

---

Hexose phosphate + $6O_2$ → $6CO_2$ + $5H_2O$ + phosphate

This is, then, a route for the complete degradation of glucose to carbon dioxide and water. The statistical significance and prevalence of this oxidative degradation system among microorganisms remains to be determined.

Ribose can be synthesized by way of the pentose phosphate cycle. In *B. coli* it appears that deoxyribose arises from direct reduction of ribose.[8]

Gluconic acid occurs widely, especially in fungi, and can be formed by enzyme-catalyzed oxidation of the unphosphorylated glucose substrate.[9] In some oxidative bacteria the following scheme occurs:[10]

Glucose → Gluconic Acid → 6-Phosphogluconic Acid →
2-Keto-3-deoxy-6-phosphogluconic Acid → Pyruvic Acid
+
Glyceraldehyde-3-phosphate

The glyceraldehyde phosphate is easily convertible to another mole of pyruvic acid.

Both glucuronic acid[11] and fucose (6-deoxy-L-galactose)[12] seem to be formed from glucose without cleavage of the carbon skeleton.

Glucosamine is probably most commonly formed by glutamine amination of fructose-6-phosphate,[13] although glucosone

[8] Fillmore K. Bagatell, Elmer M. Wright and Henry Z. Sable, *J. Biol. Chem.* 234 1369 (1959).

[9] Vincent W. Cochrane, "Physiology of Fungi," John Wiley and Sons, Inc., New York, N. Y. 1958, pp. 131–135.

[10] Nathan Entner and Michael Doudoroff, *J. Biol. Chem.* 196 853 (1952); Joseph MacGee and Michael Doudoroff, *ibid.* 210 617 (1954).

[11] Frank Eisenberg, Jr. and Samuel Gurin, *J. Biol. Chem.* 195 317 (1952); Frank Eisenberg, Jr., *ibid.* 212 501 (1955).

[12] J. F. Wilkinson, *Nature* 180 995 (1957); Stanton Segal and Yale J. Topper, *Biochim. et Biophys. Acta* 25 419 (1957).

[13] Luis F. Leloir and Carlos E. Cardini, *Biochim. et Biophys. Acta* 12 15 (1953).

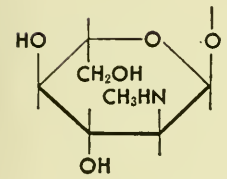
(a logical precursor) has been shown to be formed by some aspergilli.

Mannitol, which is accumulated in quantity by some microorganisms and occurs widely, is known in some cases to be in a reversible equilibrium with fructose, and it probably serves as a reserve food.[14] This reserve function may be true also of other reduced sugars.

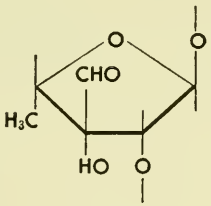
The inositols are not formed by direct hexose cyclization, but their detailed biosynthesis is not known.

Many uncommon sugars have been found as moieties of streptomycete antibiotics. Some of these antibiotics which are predominantly sugar-like in composition are included at the end of this chapter. It might be useful to list the individual sugars here for comparison, including those which occur in streptomycete antibiotics classified in other chapters:

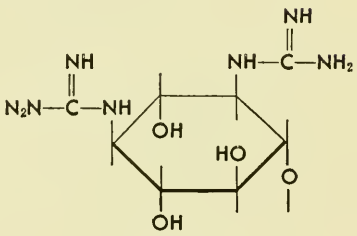
*Sugars from Streptomycete Antibiotics*
(showing points of attachment and stereochemistry where known)



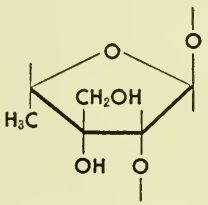

N-Methyl-L-glucosamine
(streptomycins)



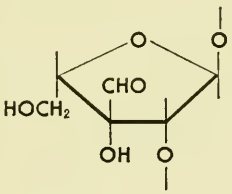

Streptose
(streptomycin)



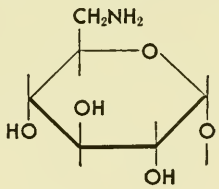

Streptidine
(streptomycin)




Dihydrostreptose
(dihydrostreptomycin)

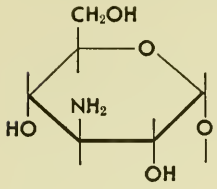



Hydroxystreptose
(hydroxystreptomycin)

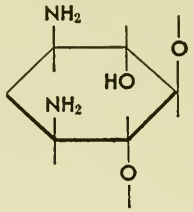



6-Glucosamine
(kanamycin)

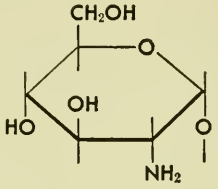
[14] Vincent W. Cochrane, "Physiology of Fungi," John Wiley and Sons, Inc., New York, 1958, p. 122.



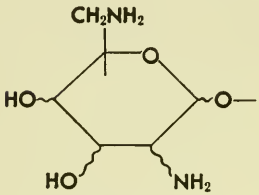

Kanosamine
(kanamycin)



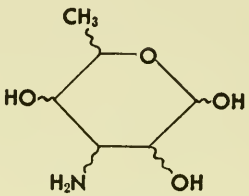

2-Deoxystreptamine
(kanamycin, paromomycin)




D-Glucosamine
(paromomycin,
trehalosamine)

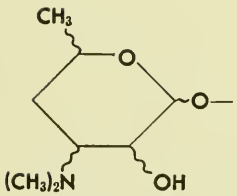



Paromose
(paromomycin)



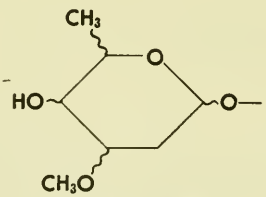

Mycosamine
(nystatin,
amphotericin B,
pimaricin)

(Neosamine C from
neomycin is also a
2,6-diaminohexose.)

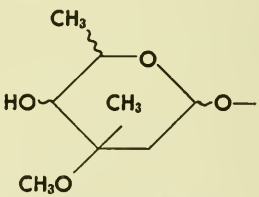



Desosamine
or
Picrocin

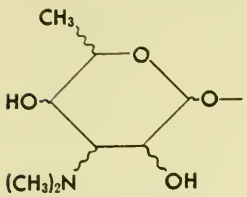
(picromycin, methy-
mycin, neomethymy-
cin, narbomycin, ole-
andomycin, erythro-
mycins A, B, and C.)



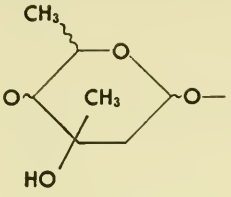

Oleandrose
(oleandomycin)



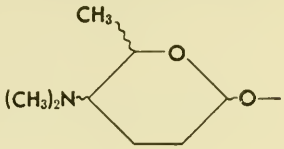

Cladinose
(erythromycins A, B)



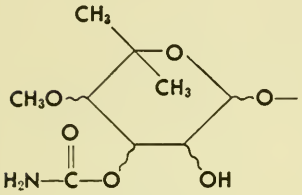
Mycaminose
(carbomycins A, B)



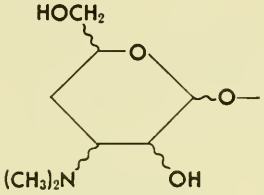
Mycarose
(carbomycins A, B,
spiramycins)



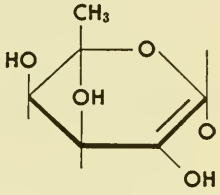
2,3,4,6-Tetradeoxy-4-
dimethylaminohexopy-
ranose (spiramycins)



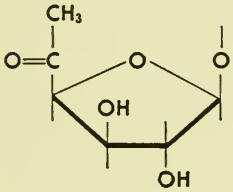
Noviose
(novobiocin)



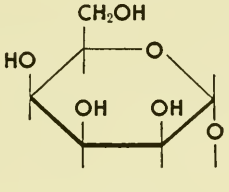
Amosamine
(amicetin)



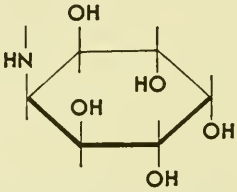
L-2-Ketofucopyranose
(angustmycin A)



5-Keto-6-deoxy-
arabohexose
(hygromycin A)

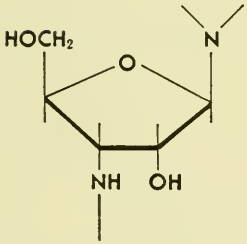


D-Talose
(hygromycin B)

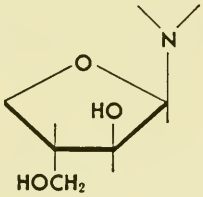


Neoinosamine-2
(hygromycin A)

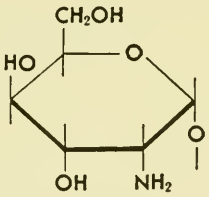
(Two hydroxyl groups in neoinosamine-2 of hygromycin A are connected in a methylenedioxy bridge. Homomycin contains a similar sugar.)



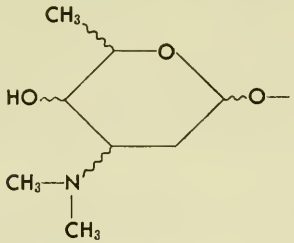
3-Deoxy-3-amino-D-
ribose
(puromycin)



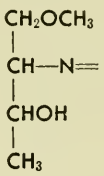
Cordycepose
(cordycepin)



α-D-Gulosamine
(streptothricin,
roseothricin)




Rhodosamine
(rhodomycin)



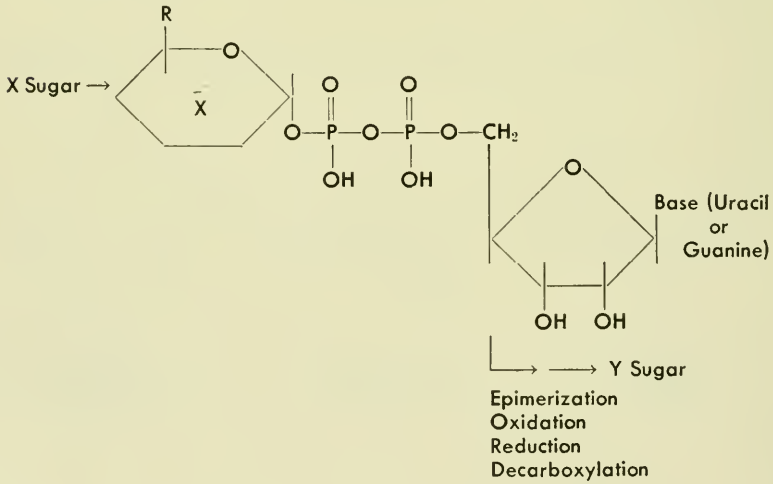

Methyl-2,4-dideoxy-2-aminotetroside
(elaiomycin)

Good reviews of aminosugars have been published.[15,16]

Other unusual sugars have been identified as components of the polysaccharides, mucopolysaccharides, etc., which occur in microbial cell walls and other cell tissues. Information can be obtained on these by way of Appendix A.

No attempt will be made here to discuss thoroughly the polysaccharides. Many references to this subject are listed in Appendix A and in the Bibliography.

As mentioned above many of the large molecules of microorganisms are mucopolysaccharides, etc., which contain sugars other than glucose. Glucose is in fact a relatively rare component of such molecules, but galactose, galacturonic acid, fucose, mannose and other sugars are common. Many hexoses and pentoses can be formed from the parent sugar without chain rupture. The intermediates in these interconversions are known to be sugar nucleotides:[17]




[15] T. Naito, *Jap. J. Pharm. and Chem.* 31 23 (1959).

[16] A. B. Foster and D. Horton, "Advances in Carbohydrate Chem-

Some of these reactions are reversible. Some of the less common aminohexoses are formed also in this way from glucosamine.

Certain fatty alcohols are classified in this chapter because of their functional groups, although biosynthetically they are more compatible with the fatty acids.

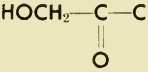
15 **Ethanol**, $C_2H_6O$, colorless liquid, b.p. 78.5°, $n_D^{20}$ 1.3610.

$$CH_3CH_2OH$$

Yeasts, fusaria, mucors, penicillia, aspergilli, etc.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. I pp. 17–196.

16 **Dihydroxyacetone**, $C_3H_6O_3$, colorless microcrystalline powder, m.p. 75–80° (polymorphic).



*Acetobacter suboxydans* (on glycerol)

Aurél Puskás, *Yearbook Inst. Agr. Chem. Technol. Univ. Tech. Sci. Budapest, Hung.* 3 (1952).

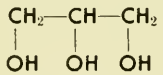
*Idem., ibid.* 8 150 (1954).

A 90% yield of crude and a 70% recovery on recrystallization was reported.

Dihydroxyacetone has been reported also in cultures of *Penicillium brevi-compactum* and *Corynebacterium diphtheriae* (on glucose).

Michizo Asano and Hideo Takahashi, *J. Pharm. Soc. Japan* 68 186 (1948); Paul Godin, *Biochim. et Biophys. Acta* 11 114 (1953).

17 **Glycerol** (Glycerin, 1,2,3-Propanetriol), $C_3H_8O_3$, m.p. 17.8°, b.p. 290° (dec.), $n_D^{20}$ 1.4746.



Yeasts, *Bacillus subtilis, Aspergillus wentii, Clasterosporia, Helminthosporia, penicillia, etc.*

Numerous recent patents. The glycerol situation is well summarized in Underkofler and Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. I; L. A. Underkofler, *Glycerol*, chap. 8, pp. 252–270.

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istry," *Aspects of the chemistry of the amino sugars*, Academic Press, New York, N. Y., 1959 Vol. 14 pp. 224–233.

[17] Saul Roseman, *Federation Proc.* 18 984 (1959). (A review)

18 ***n*-Butanol**, $C_4H_{10}O$, colorless liquid, b.p. 117°, $n_D^{20}$ 1.3993.

$$CH_3CH_2CH_2CH_2OH$$

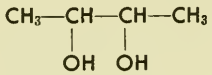
*Clostridium acetobutylicum, Cl. propylbutylicum, Cl. saccharobutylicum*

Yields of about 30% mixed solvents, mainly butanol, but containing also acetone, isopropanol and ethanol are common.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. I; W. N. McCutchan and R. J. Hickey, *The butanol-acetone fermentations,* chap. 11, pp. 347–388.

19 **2,3-Butanediol**, $C_4H_{10}O_2$, colorless liquid, b.p. 180°.

The optical isomer produced depends on the microorganism.



*Aerobacter aerogenes, Serratia marcescens, Bacillus polymyxa, Bacillus subtilis, Pseudomonas hydrophila, Bacillus mesentericus,* yeasts

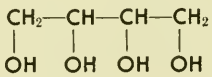
Acetoin, diacetyl and alcohol are often produced at the same time. Approximately 90% yields of butanediol have been reported.

J. A. Wheat, *Ind. Eng. Chem.* 45 2387 (1953).

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. II; G. A. Ledingham and A. C. Neish, *Fermentative production of 2,3-butanediol,* chap. 2, pp. 27–93.

Heikki Suomalainen and Lauri Jännes, *Nature* 157 336 (1946).

20 **Erythritol**, $C_4H_{10}O_4$.



*Armillaria mellea*

J. H. Birkinshaw, C. E. Stickings and P. Tessier, *Biochem. J.* 42 329–332 (1948).

Thirteen % of dry mycelium was the D-threitol isomer, colorless needles, m.p. 88.5°, $[\alpha]_D^{25}$ +4.3° (c 1 in water), −11.1° (in 95% ethanol). Other isomers have been re-

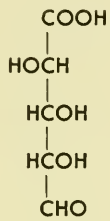
ported, especially *i*-erythritol (*meso*-erythritol). Colorless prisms, m.p. 120° (121.5°) from:

*Roccella montagnei* (yield 2%) and other *Roccella* species, *Penicillium brevi-compactum, P. cyclopium, Aspergillus terreus,* etc.

Albert E. Oxford and Harold Raistrick, *Biochem. J.* 29 1599 (1935).

Yosio Sakurai, *J. Pharm. Soc. Japan* 61 108 (1941).

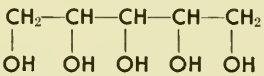
21 **D-Lyxuronic Acid** (isolated as the calcium salt) $C_5H_7O_6Ca/2{\cdot}2H_2O$, $[\alpha]_D^{20}$ −23° $\xrightarrow{30\ \text{minutes}}$ −53° (in water).




*Acetobacter melanogenum*

Minoru Ameyama and Keiji Kondo, *Bull. Agr. Chem. Soc.* (Japan) 22 271 (1958).

22 ***d*-Arabitol**, $C_5H_{12}O_5$, colorless spheroid crystals, m.p. 103°, $[\alpha]_D^{20}$ +7.7° (c 9.26 in saturated borax solution).



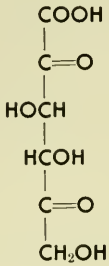

*Lobaria pulmonaria* Hoffm., *Ramalina geniculata* Tayl., *R. sinensis, R. tayloriana, R. scopulorum* (Retz.) Nyl., *Cladonia impexa* Harm., *Fistulina hepatica, Lecanora gangaleoides, Parmelia latissima* Fée, *Umbilicaria pustulata*

Yasuhiko Asahina and Masaichi Yanagita, *Ber.* 67B 799 (1934).

T. W. Breaden, J. Keane and T. J. Nolan, *Sci. Proc. Roy. Dublin Soc.* 23 6 (1942).

Yngve Johannes Solberg, *Acta Chem. Scand.* 9 1234 (1955).

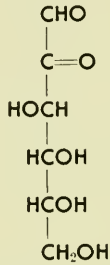
23 **2,5-Diketogluconic Acid**, $C_6H_8O_7$, isolated as Ca salt. No good m.p.



*Acetobacter melanogenum, Pseudomonas, Phytomonas* spp.

H. Katznelson, S. W. Tanenbaum and E. L. Tatum, *J. Biol. Chem.* 204 43 (1953).

24 **Glucosone**, $C_6H_{10}O_6$, levorotatory syrup with reducing properties.



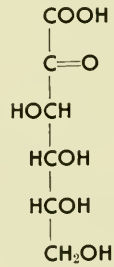
*Aspergillus parasiticus, A. flavus*, some algae

Yields of 13–17% from sucrose have been reported.

Cecil R. Bond, Edwin C. Knight and Thomas K. Walker, *Biochem. J.* 31 1033 (1937).

Ross C. Bean and W. Z. Hassid, *Science* 124 171 (1956).

25 **2-Ketogluconic Acid**, $C_6H_{10}O_7$, colorless crystals, m.p. 152° (Me ester, m.p. 172°).



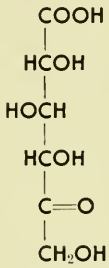
*Acetobacter melanogenum, Pseudomonas, Phytomonas* spp.

The yields of 2-ketogluconic acid are better than 70%. 2,5-Diketogluconic acid can be made the principal product. This diketo acid is unstable, but can be isolated as a salt.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. II, Lewis B. Lockwood, *Ketogenic fermentation processes,* chap. 1, pp. 13–14.

H. Katznelson, S. W. Tanenbaum and E. L. Tatum, *J. Biol. Chem.* 204 43 (1953).

26 **5-Ketogluconic Acid,** $C_6H_{10}O_7$, generally isolated as the Ca salt (no sharp m.p.).




*Acetobacter suboxydans*

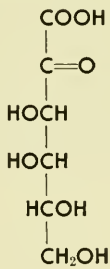
Yields of about 90% have been reported.

Shiro Teramato, Riichiro Yagi and Ichiro Hori, *J. Fermentation Technol.* (Japan) 24 22 (1946).

Joseph J. Stubbs, Lewis B. Lockwood, Edward T. Roe and George E. Ward, U. S. Patent 2,318,641 (1943).

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. II, Lewis B. Lockwood, *Ketogenic fermentation processes,* chap. 1, pp. 10–12.

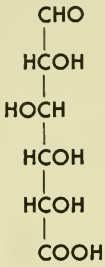
27 **2-Ketogalactonic Acid,** $C_6H_{10}O_7$, colorless crystals, m.p. 170° (K salt, m.p. 139°; Me ester, m.p. 138°).



*Pseudomonas* species (on galactose)

Toshinohu Asai, Ko Aida and Yashuiro Ueno, *J. Agr. Chem. Soc. Japan* 25 625 (1951–1952).

28 D-Glucuronic Acid, $C_6H_{10}O_7$, colorless needles, m.p. 165°, $[\alpha]_D^{24}$ +11.7° → +36.3° (2 hours, c 1 in water).




*Ustulina vulgaricus*

H. Wunchendoroff and C. Killian, *Compt. rend.* 187 572 (1928).

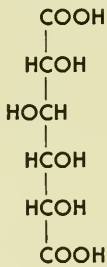
Not isolated—manner of identification not mentioned.

*Penicillium* sp.

Gizin Itto, *J. Agr. Chem. Soc. Japan* 9 552 (1933).

K. Sivarama Sastry and P. S. Sarma, *Nature* 179 44 (1957).

29 Saccharic Acid, $C_6H_{10}O_8$, colorless needles, m.p. 125°, $[\alpha]_D^{19}$ +6.86° → 20.6° (c 1 in water).



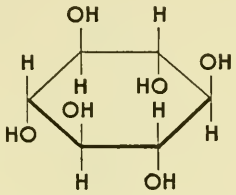

*Aspergillus niger*

T. K. Walker, Vira Subramanian and Frederick Challenger, *J. Chem. Soc.*, 3044 (1927).

About 3.6 g. of the potassium salt were obtained from 120 g. of glucose by interrupting the fermentation before the appearance of much citric or oxalic acids. Also fermentation of 20 g. of calcium gluconate gave 3.7 g. of calcium saccharate.

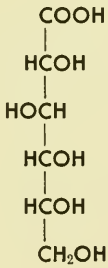
Also reported formed from glucose by two yeasts, *Anthomyces renkaufi* and *Amphierna rubra:*
J. Grüss, *Jahrb. wiss. Botan.* 66 109 (1926).

30 *meso*-**Inositol,** $C_6H_{12}O_6$ (dihydrate), colorless crystals, m.p. 218° (anhydrous) 250–253°.



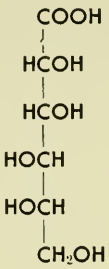

*Pseudomonas fluorescens, Serratia marcescens, Proteus vulgaris, Clostridium butylicum,* yeasts
Yields of 2700–5000 μg. per gram of dry cell weight are obtained in brewers' yeast.
Inositol Literature Briefs Tech. Bull. Y3–101, Corn Products Refining Co., 1953, 44 pp. (A bibliography with titles and abstracts)

31 D-**Gluconic Acid,** $C_6H_{12}O_7$, colorless syrup, cannot be isolated, but readily forms (principally) the δ-lactone, white crystals, m.p. 153°, $[\alpha]_D$ +63.5° → +6.2° (c 1 in water).




Wide variety of mold species, acetobacter species, etc.
Yields 95% with *Aspergillus niger.*
A. J. Mayer, E. J. Umberger and J. J. Stubbs, *Ind. Eng. Chem.* 32 1379 (1940).
Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. I, L. A. Underkofler, *Gluconic acid,* chap. 14, pp. 446–469.

32 **D-Mannonic Acid**, $C_6H_{12}O_7$, forms γ- or δ-lactones, but the free acid cannot be isolated pure.

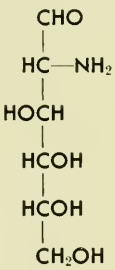



*P. purpurogenum* var. *rubrisclerotium* (on D-mannose) *Acetobacters*

Galactonic acid, etc., can be produced similarly from the corresponding sugar.

A. Angeletti and C. F. Cerruti, *Ann. chim. applicata* **20** 424 (1930).

33 **D-Glucosamine** (Chitosamine) $C_6H_{13}O_5N$, white needles, m.p. 110° (dec.), $[\alpha]_D^{20}$ +47.5° (c 1 in water).

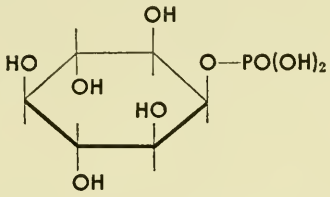



Many bacteria, fungi and lichens. Present in bound form in mold mycelium. Produced by the action of certain streptomyces species on chitin.

Joseph J. Noval and Walter J. Nickerson, *Bacteriol. Proc.*, 125 (1956).

Leslie Ralph Berger and Donald M. Reynolds, *Biochim. et Biophys. Acta* **29** 522 (1958).

34 **Mesoinositol Monophosphate,** $C_6H_{13}O_9P \cdot 3H_2O$, colorless tablets, m.p. 201° (dec.).

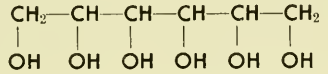



*Mycobacterium tuberculosis* var. *hominis*

Michael A. Macheboeuf, Georgette Lévy and Marguerite Faure, *Compt. rend.* **204** 1843 (1937). (Occurred as a fatty acid ester)

James Cason and R. J. Anderson, *J. Biol. Chem.* **126** 527 (1938). (As a constituent of a polysaccharide)

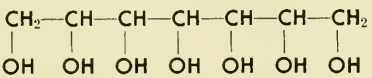
35 **D-Mannitol,** $C_6H_{14}O_6$, colorless prisms, m.p. 163° (166°) $[\alpha]_D^{25}$ −0.49°. (C 1 in water. Addition of borax → strong dextrorotation.)




Aspergilli, penicillia, other fungi, many lichens, algae and bacteria

For example: Mitizo Asano, Chunoshin Ukita and Tomoyoshi Komai, *Japanese Patent* 180,442 (1949) describe extraction of mannitol and ergosterol from *Penicillium* mycelium. See W. Karrer's compilation (listed in the general reference bibliography) for other references.

36 **D-Volemitol,** $C_7H_{16}O_7$, silky needles, m.p. 153.5° $[\alpha]_D^{20}$ +17.08° (1.001 g. + 0.7 g. of Borax in 15 ml. of $H_2O$).

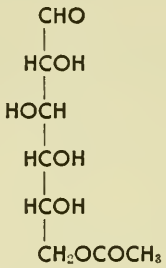



*Dermatocarpon miniatum* (L.) Mann.

Yasuhiko Asahina and Motoyasu Kagitani, *Ber.* **67B** 804 (1934).

Bengt Lindberg, Alfons Misiorny and Carl Axel Wachtmeister, *Acta Chem. Scand.* **7** 591 (1953). (A survey of the occurrence of low molecular weight carbohydrate constituents in lichens)

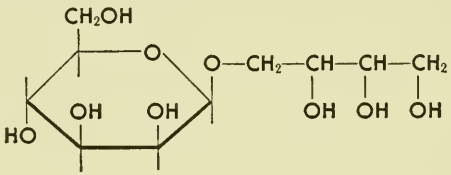
37 **6-O-Acetylglucose,** $C_8H_{14}O_7$, minute colorless prisms, m.p. 133°, $[\alpha]_D^{20}$ +48° (c 4.0 in water at equilibrium).




*Bacillus megaterium*

R. B. Duff, D. M. Webley and V. C. Farmer, *Nature* **179** 103 (1957).

38 **D-Mannopyranosyl-1-*meso*-erythritol,** $C_{10}H_{20}O_9$, colorless crystals, m.p. 160°, $[\alpha]_D$ −36.7°.

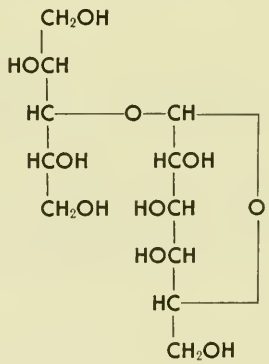



*Ustilago* sp.

Besides this water-soluble compound the fungus produces 15 g. per liter of an oil, consisting of a mixture of fatty acid esters of D-mannopyranosyl-1-*meso*-erythritol.

B. Boothroyd, J. A. Thorn and R. H. Haskins, *Can. J. Biochem. and Physiol.* 34 10 (1956).

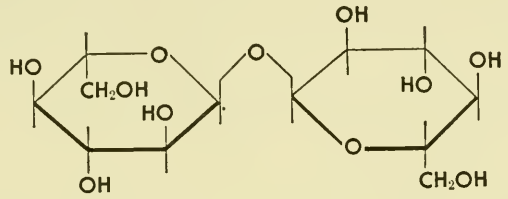
39 **Umbilicin (3-β-D-Galactopyranosido-D-arabitol),** $C_{11}H_{22}O_{10}$, colorless crystals, m.p. 138°, $[\alpha]_D^{20}$ −81° (c 2 in water).



*Umbilicaria pustulata*

Bengt Lindberg, Carl A. Wachtmeister and Börje Wickberg, *Acta Chem. Scand.* 6 1052 (1952).

40 **Trehalosamine**, $C_{12}H_{22}O_{10}N$ (Hydrochloride) white microcrystalline powder, $[\alpha]_D^{25}$ +176° (c 2.0 in water).

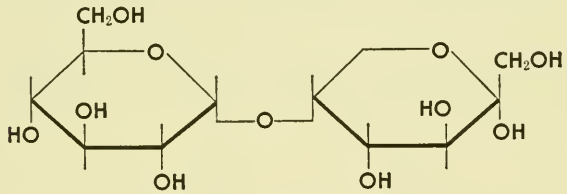



A streptomycete

A yield of about 5 g. per liter was obtained.

Frederico Arcamone and Franco Bizioli, *Gazz. chim. ital.* 87 896 (1957).

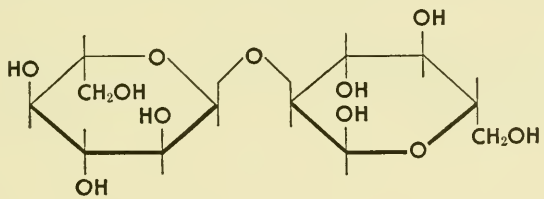
41 **Leucrose** (5-0-α-D-Glucopyranosyl-D-fructopyranose), $C_{12}H_{22}O_{11}$, colorless hygroscopic bars, m.p. 161–163° (anhydrous), 156–158° (monohydrate), $[\alpha]_D^{25}$ −8.2° → −7.6° (<1 hour, c 4 in water).




*Leuconostoc mesenteroides*

Frank H. Stodola, E. S. Sharpe and H. J. Koepsell, *J. Am. Chem. Soc.* 78 2514 (1956).

42 **Kojibiose** (2-0-α-D-Glucopyranosyl-D-glucose), $C_{12}H_{22}O_{11}$, m.p. (Octaacetate) 166°, $[\alpha]_D$ +150° (c 2.1 in chloroform). Free sugar: $[\alpha]_D$ +136° (equil., c 0.5 in water).

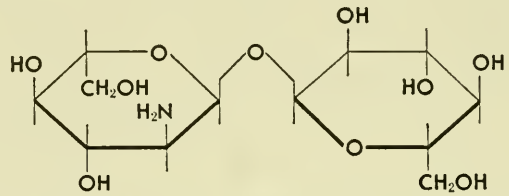


*Aspergillus niger*

Stanley Peat, W. J. Whelan and Kathleen A. Hinson, *Chem. and Ind.*, 385 (1955).

A. Sato and K. Aso, *Nature* **180** 984 (1957).

43 **Trehalose** (Mycose, α-D-Glucosido-α-D-glucoside), $C_{12}H_{22}O_{11}$, colorless, hygroscopic crystals, m.p. ~210° (dec.) (anhydrous), 97° (hydrate), $[\alpha]_D^{20}$ (hydrate) +178° (in water).




*Amanita muscaria*, other mushrooms and molds, mycobacteria, yeasts and algae. First isolated from rye ergot (*Claviceps purpurea* (Fr.) Tul.).

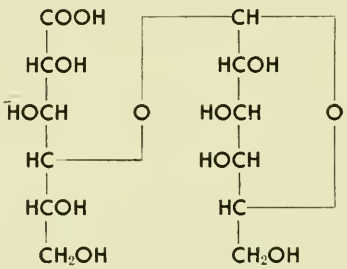
Trehalose is present in young mushrooms, but as the plants develop it is replaced by mannitol. It also occurs in seaweeds and higher plants.

E. Bourquelot, *Compt. rend.* **108** 568 (1889).

H. Bredereck, *Ber.* **63B** 959 (1930). (Structure)

Bengt Lindberg, *Acta Chem. Scand.* **9** 917 (1955).

44 **Lactobionic Acid**, $C_{12}H_{22}O_{12}$, Calcium Salt: granular white powder, $[\alpha]_D^{25}$ +25.1° (c 5.2 in water).




*Pseudomonas* species, other oxidative bacteria (on lactose)

A 77% yield has been reported. Maltobionic acid was prepared similarly from maltose.

Frank H. Stodola and Lewis B. Lockwood, *J. Biol. Chem.* **171** 213 (1947).

Lewis B. Lockwood and Frank H. Stodola, U. S. Patent 2,496,297 (1950).

45 **Hygromycin B**, $C_{15}H_{28}O_{10}N_2$, amorphous powder, m.p. ~180°.

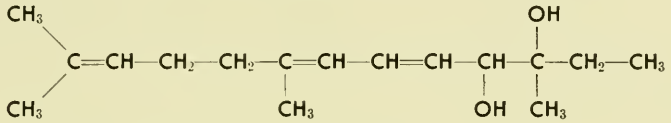
D-Talose has been shown to be one moiety of this antibiotic.

*Streptomyces hygroscopicus*

Robert L. Mann and W. W. Bromer, *J. Am. Chem. Soc.* **80** 2715 (1958).

Paul F. Wiley and Max V. Sigal, Jr., *ibid.* **80** 1010 (1958).

46 **Grifolin**, $C_{16}H_{28}O_2$, fine colorless needles, m.p. 40°.



*Grifola confluens* (=*Polyporus confluens*)

Other components of the extract were mannitol, sterols, a hemin-like substance, a compound $C_8H_{14}O$ (m.p. 145°) and a compound $C_{15}H_{24}O_2$ (m.p. 151°).

Y. Hirata and K. Nakanishi, *J. Biol. Chem.* **184** 135 (1950).

47 **Cetyl Alcohol**, $C_{16}H_{34}O$, colorless crystals, m.p. 50°, $n_D^{79}$ 1.4283.

$$CH_3(CH_2)_{14}CH_2OH$$

*Amanita phalloides*

Heinrich Wieland and Gustav Coutelle, *Ann.* **548** 270 (1941).

48 **Clavicepsin**, $C_{18}H_{34}O_{16}$, colorless crystals, m.p. (anhydr.) 198°, $[\alpha]_D^{20}$ +142°.

A glucoside hydrolyzing to 1 mole of mannitol and 2 moles of glucose. The detailed structure was not determined.

*Claviceps purpurea*

F. Marino-Zuco and U. Pasquero, *Gazz. chim. ital.* **41** 368 (1912).

49 **Stearyl Alcohol**, $C_{18}H_{38}O$, colorless leaflets, m.p. 59°.

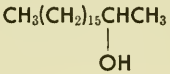
$$CH_3(CH_2)_{16}CH_2OH$$

*Penicillium notatum*

A yield of 0.13 g. was obtained from 300 g. of dry mycelium.

A. Angeletti, G. Tappi and G. Biglino, *Ann. chim.* (Rome) 42 502 (1952).

50 *d*-2-**Octadecanol,** $C_{18}H_{38}O$, colorless needles, m.p. 56°, $[\alpha]_D$ +5.7° (in chloroform).



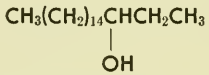
*Mycobacterium tuberculosis* var. *hominis, M. avium, M. phlei*

Mary C. Panghorn and R. J. Anderson, *J. Am. Chem. Soc.* 58 10 (1936).

R. E. Reeves and R. J. Anderson, *ibid.* 59 858 (1937).

R. J. Anderson, J. A. Crowder, M. S. Newman and F. H. Stodola, *J. Biol. Chem.* 113 637 (1936).

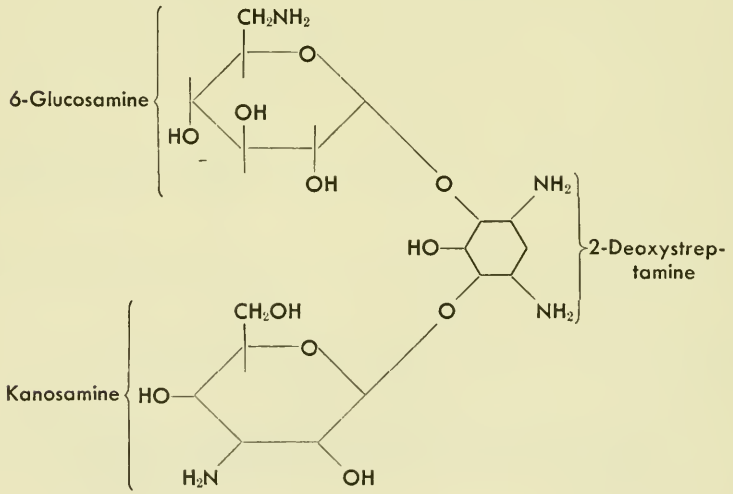
51 *d*-3-**Octadecanol,** $C_{18}H_{38}O$, colorless crystals, m.p. 56°.



*Corynebacterium diphtheriae*

A. A. Kanchukh, *Ukraïn. Biokhim. Zhur.* 26 186 (1954).

52 **Kanamycin,** $C_{18}H_{36}O_{11}N_4$, Sulfate: white prisms which decompose over a wide range above 250°, $[\alpha]_D^{24}$ +146° (c 1 in 0.1 N sulfuric acid).




*Streptomyces kanamyceticus*

Tomio Takeuchi, Tokuro Hikiji, Kazuo Nitta, Seiro Yamazaki, Sadao Abe, Hisaro Takayama and Hamao Umezawa, *J. Antibiotics* (Japan) **10A** 107 (1957).

Hamao Umezawa, Mashiro Ueda, Kenji Maeda, Koki

Yagishita, Shinichi Kondo, Yoshiro Okami, Ryozo Utahara, Yasuke Osato, Kazuo Nitta and Tomio Takeuchi, *ibid.* **10A** 181 (1957).

Kenji Maeda, Masahiro Ueda, Koki Yagishita, Shohei Kawaji, Shinichi Kondo, Masao Murase, Tomio Takeuchi, Yoshiro Okami and Hamao Umezawa, *ibid.* **10A** 228 (1957).

M. J. Cron, D. L. Johnson, F. M. Palermiti, Y. Perron, H. D. Taylor, D. F. Whitehead and I. R. Hooper, *J. Am. Chem. Soc.* **80** 752 (1958).

M. J. Cron, O. B. Fardig, D. L. Johnson, D. F. Whitehead, I. R. Hooper and R. U. Lemieux, *ibid.* **80** 4115 (1958).

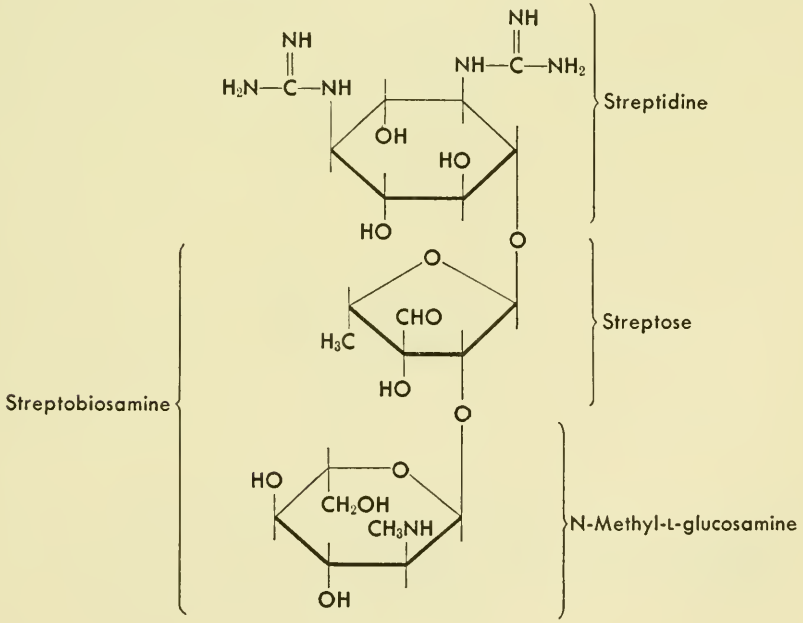
53 **Kanamycin B,** colorless crystals, m.p. dec. from 170°, $[\alpha]_D^{24}$ +135° (c 0.63 in water).

Acid hydrolysis yields 2-deoxystreptamine and kanosamine, but no 6-glucosamine as from kanamycin. An unidentified ninhydrin-positive compound was obtained instead. Positive Schiff, Molisch, Elson-Morgan tests.

*Streptomyces kanamyceticus*

H. Schmitz, O. B. Fardig, F. A. O'Herron, M. A. Rousche and I. R. Hooper, *J. Am. Chem. Soc.* **80** 2911 (1958).

54 **Streptomycin,** $C_{21}H_{39}O_{12}N_7$, m.p. (Reineckate) 164° dec. (Helianthate) 220–226° dec., $[\alpha]_D$ (Hydrochloride) −84° (c 0.5 in water), $[\alpha]_D^{26.6}$ (Trihydrochloride) −86.1° (c 1.0 in water), $[\alpha]_D^{25}$ (Sulfate) −79° (c 1.0 in water). Salts are deliquescent.



*Streptomyces griseus* (Krainsky) Waksman et Henrici
*S. bikiniensis, S. mashuensis*

Albert Schatz, Elizabeth Bugie and Selman A. Waksman, *Proc. Soc. Exptl. Biol. Med.* 55 66 (1944). (Isolation)

Selman A. Waksman, "Streptomycin, Its Nature and Applications," Williams and Wilkins Co., Baltimore, Md., 1949. (A review)

Herbert E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *J. Biol. Chem.* 160 337 (1945).

Frederick A. Kuehl, Jr., Robert L. Peck, Charles E. Hoffhine, Jr., Robert P. Graber and Karl Folkers, *J. Am. Chem. Soc.* 68 1460 (1946).

Frederick A. Kuehl, Jr., Edwin H. Flynn, Norman G. Brink and Karl Folkers, *ibid.* 68 2096, 2679 (1946). (Structure)

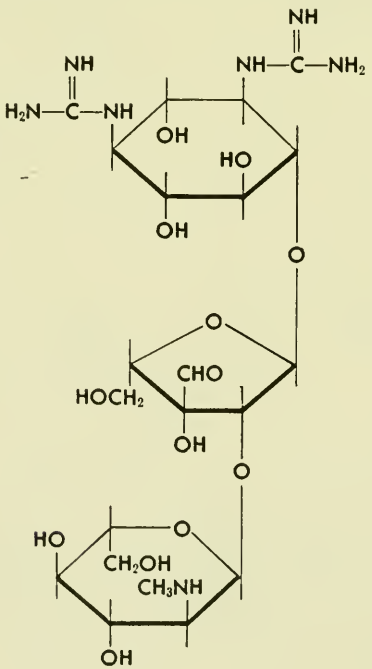
I. R. Hooper, L. H. Klemm, W. J. Polglase and M. L. Wolfrom, *ibid.* 69 1052 (1947).

H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *Science* 103 540 (1946).

E. P. Abraham and H. W. Florey, "Antibiotics," Oxford University Press, London, 1949 Vol. II chap. 41, pp. 1297–1309.

E. P. Abraham, *ibid.* chap. 42, pp. 1310–1326.

55 **Hydroxystreptomycin** (Reticulin) $C_{21}H_{39}O_{13}N_7$, Helianthate: red-brown crystals, m.p.: darkening at 200° (dec.), Trihydrochloride: $[\alpha]_D^{28}$ −91° (c 1.0 in water).



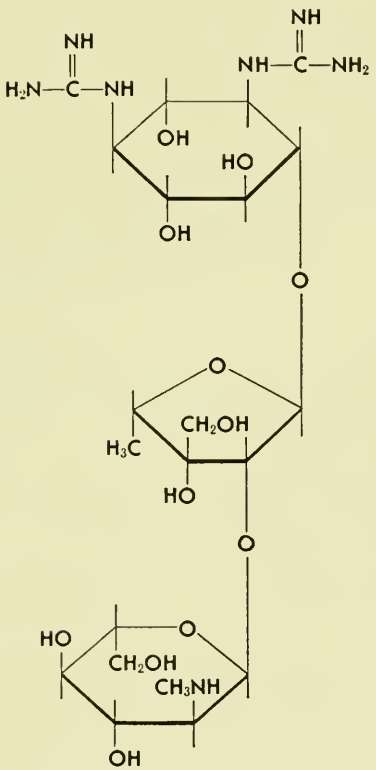
*Streptomyces griseocarneus*

Seigo Hosaya, Momoe Soeda, Nobuhiko Komatsu and Yoko Sonoda, *Japan. J. Exptl. Med.* 20 327 (1949).

Robert G. Benedict, Frank H. Stodola, Odette L. Shotwell, Anne Marie Borud and Lloyd A. Lindenfelser, *Science* 112 77 (1950).

Frank H. Stodola, Odette L. Shotwell, Anne Marie Borud, Robert G. Benedict and Arthur C. Riley, Jr., *J. Am. Chem. Soc.* 73 2290 (1951). (Structure)

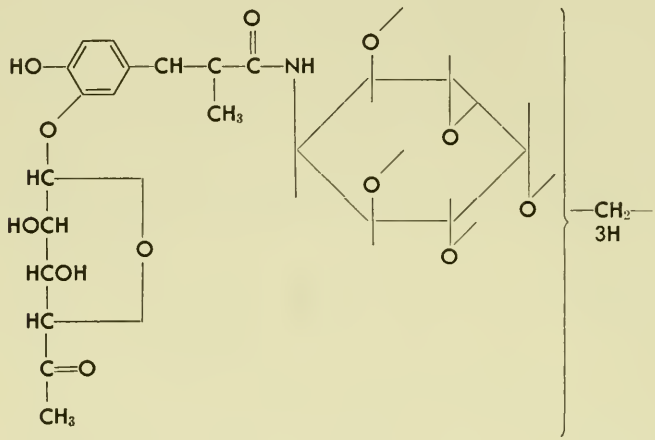
56 **Dihydrostreptomycin,** $C_{21}H_{41}O_{12}N_7$, non-deliquescent white powder $[\alpha]_D^{25}$ −94.5°. Hydrochloride and sulfate were used.




*Streptomyces humidus*

Sueo Tatsuoka, Tsunaharu Kusaka, Akira Miyake, Michitaka Inoue, Hiromu Hitomi, Yutaka Shiraishi, Hidesuke Iwasaki and Masahiko Imanishi, *Pharm. Bull.* 5 343 (1957). (Primary fermentation product)

57 **Hygromycin A**, $C_{23}H_{29}O_{12}N$, amorphous (some crystalline derivatives have been prepared). $[\alpha]_D^{25}$ −126° (c 1 in water). Partial structure:



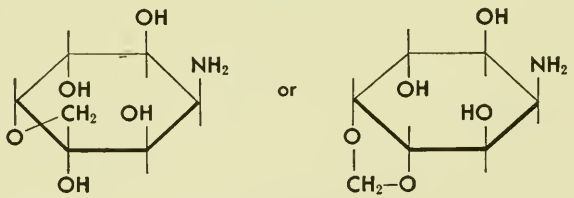

*Streptomyces hygroscopicus* (Jensen) Waksman and Henrici

R. L. Mann, R. M. Gale and F. R. Van Abeele, *Antibiotics and Chemotherapy* 3 1279 (1953). (Isolation)

Robert L. Mann and D. O. Wolf, *J. Am. Chem. Soc.* 79 120 (1957). (Structure)

58 **Homomycin**, white powder, m.p. 105–109° (dec. >160°).

Homomycin has been shown to be the same as hygromycin except that the homomycin amino sugar moiety is:

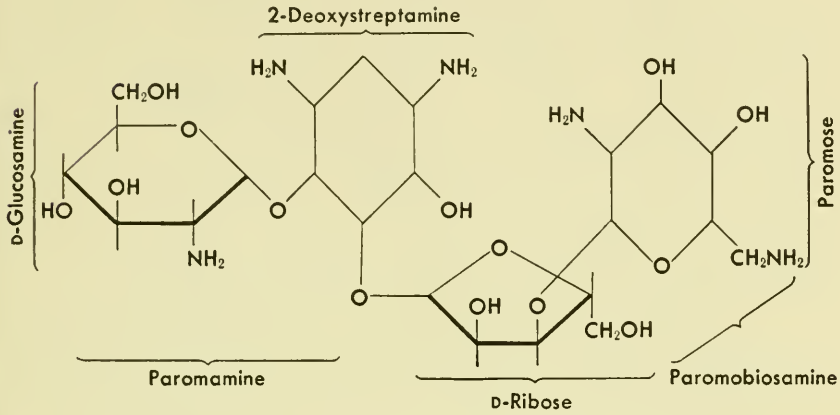



*Streptomyces noboritoensis* n. sp.

Yusuke Sumiki, Gotaku Nakamura, Makoto Kawasaki, Satoru Yamashita, Kentaro Anzai, Kiyoshi Isono, Yoshiko Serizawa, Yoko Tomiyama and Saburo Suzuki, *J. Antibiotics* (Japan) 8A 170 (1955). (Isolation)

Mitsuo Namiki, Kiyoshi Isono, Kentaro Anzai and Saburo Suzuki, *ibid.* 10A 36 (1957). (Structure)

59 **Paromomycin**, $C_{23}H_{45}O_{14}N_5$, white amorphous solid, $[\alpha]_D^{25}$ +64° (c 1.0 in water), Hydrochloride $[\alpha]_D^{25}$ +56.5° (c 1.0 in water).




*Streptomyces rimosus* forma *paromomycinus*

Paromomycin seems to be identical with catenulin.

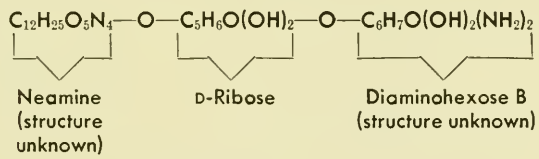
Theodore H. Haskell, James C. French and Quentin R. Bartz, *J. Am. Chem. Soc.* 81 3480 (1959).

*Ibid.* Belgian Patent 547,976.

60 **Neomycins.** (Fradiomycins, Streptothricins, Neomins, Mycifradin, Nivemycins, Myacins)

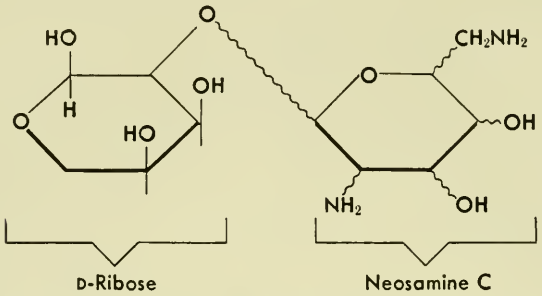
Neomycin A is identical with neamine, a moiety of neomycins B and C. Neomycins B and C are identical except for the diaminohexose components.

Neomycin B (Streptothricin B II), $C_{23}H_{46}O_{12}N_6$, amorphous hygroscopic white powder, no definite m.p., $[\alpha]_D^{25}$ +83° (in 0.2 N $H_2SO_4$).




Neomycin C (Streptothricin B I), $C_{23}H_{46}O_{12}N_6$, amorphous, hygroscopic white powder, no definite m.p., $[\alpha]_D^{25}$ +121° (in 0.2 N $H_2SO_4$).

Also contains neamine. The disaccharide portion (neobiosamine C) has been characterized, however, as:




*Streptomyces fradiae*, other *Streptomyces* spp.

Selman A. Waksman and Hubert A. Lechevalier, *Science* 109 305 (1949). (Isolation)

Byron E. Leach, William H. DeVries, Harrison A. Nelson, William G. Jackson and John S. Evans, *J. Am. Chem. Soc.* 73 2797 (1951). (Isolation)

Jared H. Ford, Malcolm E. Bergy, A. A. Brooks, Edward R. Garrett, Joseph Alberti, John R. Dyer and H. E. Carter, *ibid.* 77 5311 (1955).

Kenneth L. Rinehart, Jr., Peter W. K. Woo, Alexander D. Argoudelis and Astrea M. Giesbrecht, *ibid.* 79 4567 (1957).

Kenneth L. Rinehart, Jr., Peter W. K. Woo and Alexander D. Argoudelis, *ibid.* 79 4568 (1957).

*Idem., ibid.* 80 6461 (1958).

Kenneth L. Rinehart, Jr., and Peter W. K. Woo, *ibid.* 80 6463 (1958). (Structure)

61 **Catenulin** (Sulfate) $[\alpha]_D^{25}$ +51.9° (c 1 in water).

A substance resembling paromomycin. Acid hydrolysis yields neamine.*

*Streptomyces catenulensis*

J. W. Davisson, I. A. Solomons and T. M. Lees, *Antibiotics and Chemotherapy* 2 460 (1952).

62 **Dextromycin**, Helianthate: m.p. 227°, Hydrochloride: $[\alpha]_D^{25}$ +61° (c 1 acetone).

Similar to neomycin B.*

*Streptomyces* sp. resembling *S. fradiae*

Koichi Ogata and Koichi Nakazawa, *J. Antibiotics* (Japan) 3 440 (1950).

* Probably identical with paromomycin. (Private communication from Drs. W. Celmer and C. Shaffner)

Toyonari Araki, Akira Miyake, Yoshitamo Aramaki, Hiroshi Kojima, Hajime Yokotani, Koichi Ogata and Koichi Nakazawa, *Ann. Repts. Takeda Research Lab.* 13 1 (1954).

* Identical with neomycin B. See addendum.

63 **Framycetin** (Actilin, Soframycin, Antibiotic E.F. 185), Hydrochloride: white powder, $[\alpha]_D$ +57° (c 1.0 in water), m.p. (picrate) 189° (dec.).

Framycetin resembles neomycin and streptomycin in some respects, but is distinct. Hydrolysis yields neamine, a pentose, and a diaminohexose. Framycetin forms peptide derivatives such as a reineckate and a picrate. The molecular weight is about 1400–1500. No guanidine tests were observed, and all the nitrogen is present as primary amine groups.

*Streptomyces* sp. resembling *S. lavendulae*

Louis Jacques Decaris, *Ann. pharm. franç.* 11 44 (1953).

Maurice Marie Janot, Henry Pénau, Digna van Stolk, Guy Hagemann and Lucien Pénasse, *Bull. Soc. chim. France*, 1458 (1954).

A. Lutz and M. A. Witz, *Compt. rend. soc. biol.* 149 1467 (1955).

A. Saito and C. P. Schaffner, *Congr. intern. biochim.*, Résumés communs., 3$^{e}$ *Congr.*, Brussels, 1955, p. 98.

64 **Hydroxymycin**, probable empirical formula $C_{25}H_{47}O_{15}N_5$, white powder, $[\alpha]_D^{20}$ 63° ± 2° (c. 1.0 in water) (Sulfate) white powder, $[\alpha]_D^{20}$ +51° (c 1.0 in water).

A basic antibiotic similar to streptomycin and neomycin. Contains 6.2% total nitrogen and 6.0% amino nitrogen. It is water soluble and insoluble in most organic solvents with a molecular weight of about 610. Hydrolysis yields a fragment called pseudoneamine and others which show pentose and 2-aminohexose reactions.

An antifungal substance was produced in the same culture.

*Streptomyces paucisporogenes*

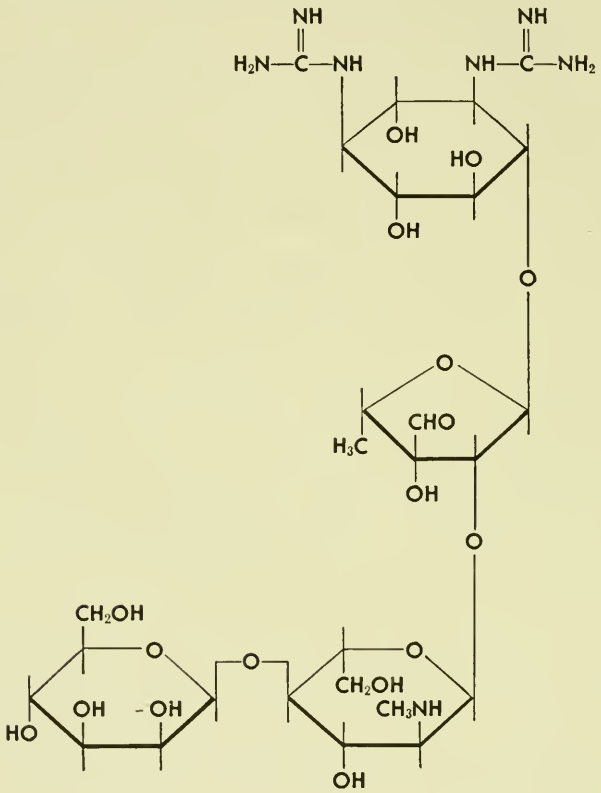
M. M. Janot, H. Pénau, G. Hagemann, H. Velu, J. Teillon and G. Bouet, *Ann. pharm. franç.* 12 440 (1954).

G. Hagemann, G. Nominé and L. Pénasse, *Ann. pharm. franç.* 16 585 (1958).

H. Pénau, G. Hagemann and H. Velu, *Bull. soc. chim. biol.* 41 761 (1959).

J. Bartos, *Ann. pharm. franç.* 16 596 (1958).

65 **Mannosidostreptomycin** (Streptomycin B), $C_{27}H_{49}O_{17}N_7$, colorless crystals, m.p. (Anhydrous Reineckate) 178° dec. (Trihydrochloride) 190–200° dec., $[\alpha]_D^{25}$ (Trihydrochloride) −47° (c 1.35 in water).

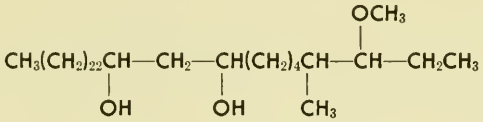



Occurs together with streptomycin in some cultures. *Streptomyces griseus*

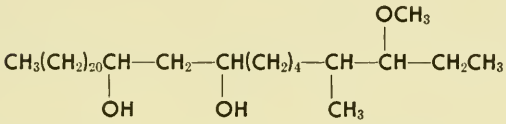
Josef Fried and Homer E. Stavely, *J. Am. Chem. Soc.* 74 5461 (1952). (Structure)

66 **Phthiocerol**, $C_{36}H_{74}O_3$, colorless plates, m.p. 71.5–73°, $[\alpha]_D$ −4.50° (c 11.48 in chloroform).

It is claimed (in the most recent reference below) that phthiocerol, as ordinarily isolated, is a mixture of the following two substances:



and



*Mycobacterium tuberculosis* (human, bovine and avian)

In the wax of the mycobacteria phthiocerol is present mainly as the dimycoceranate.

J. A. Hall, J. W. Lewis and N. Polgar, *J. Chem. Soc.*, 3971 (1955).

Hans Noll, *J. Biol. Chem.* 224 149 (1957).

H. Demarteau-Ginsburg, E. Lederer, R. Ryhage, S. Ställberg-Stenhagen and E. Stenhagen, *Nature* 183 1117 (1959).

3

# Aliphatic Acids and Glycolipides

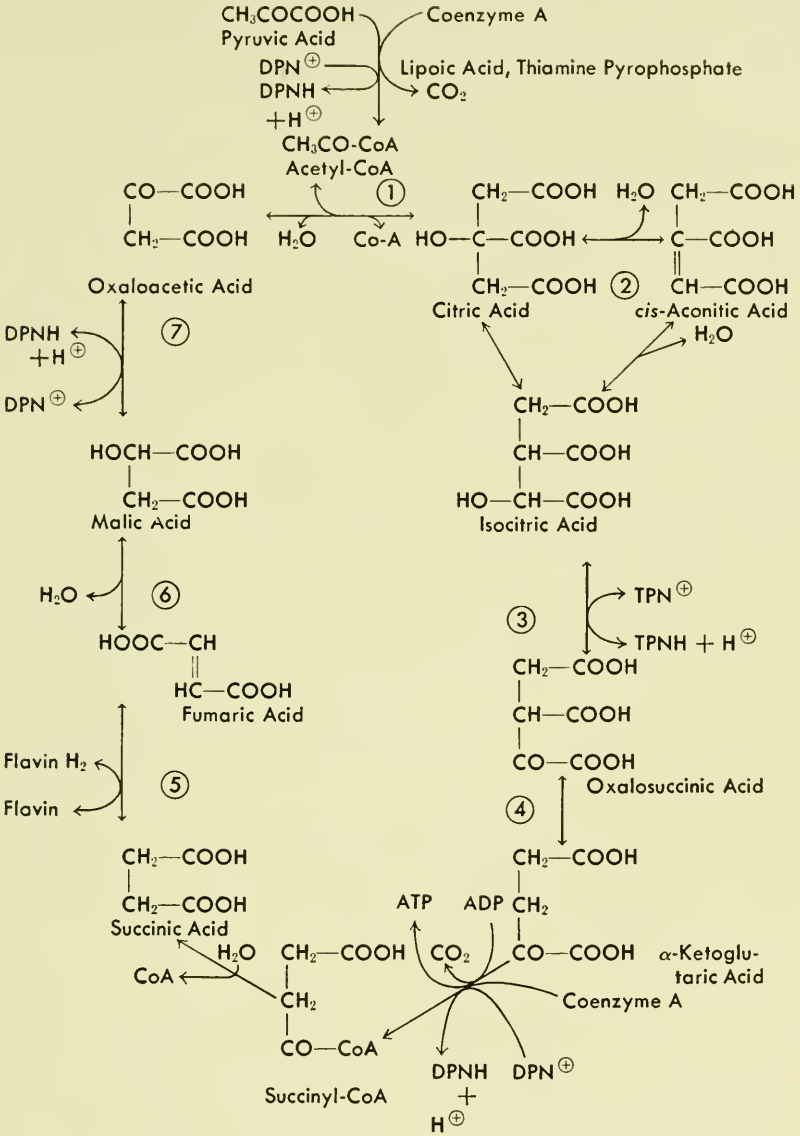
The metabolic origins of some of the acids in this section can be deduced from the foregoing chapter. Among these are pyruvic, glyceric, acetic, formic, propionic and lactic acids.

Many of the other simpler acids are recognizable as members of the citric acid cycle and ancillary routes. The citric acid cycle (tricarboxylic acid cycle or Krebs cycle) is outlined below:

- *The Citric Acid Cycle*

Enzymes:

1. Condensing enzyme
2. Aconitase
3. Isocitric dehydrogenase
4. Oxalosuccinic decarboxylase
5. Succinic dehydrogenase
6. Fumarase
7. Malic dehydrogenase

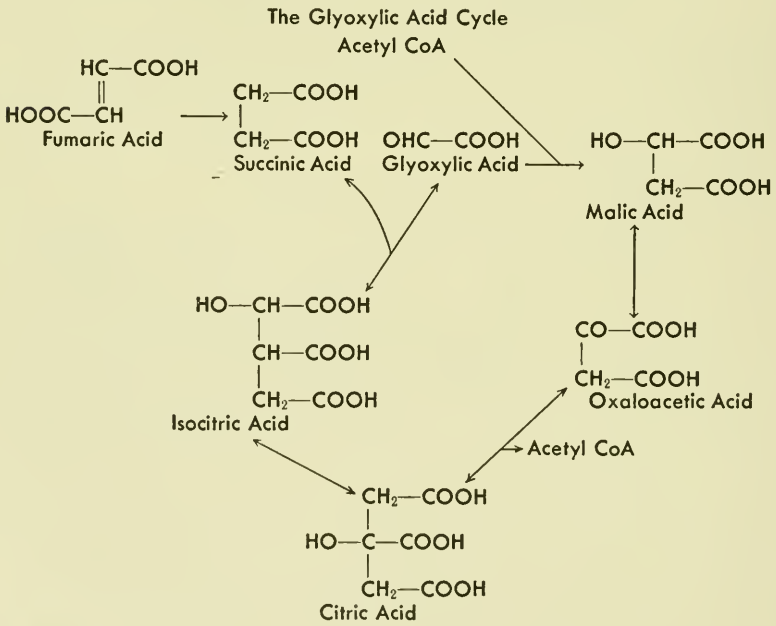

CH3COCOOH
Pyruvic Acid
Coenzyme A
DPN⊕
DPNH
Lipoic Acid, Thiamine Pyrophosphate
CO2
+H⊕
CH3CO-CoA
Acetyl-CoA
CO—COOH
CH2—COOH
1
H2O
Co-A
CH2—COOH
HO—C—COOH
CH2—COOH
H2O
CH2—COOH
C—COOH
CH—COOH
2
Citric Acid
cis-Aconitic Acid
H2O
Oxaloacetic Acid
DPNH
+H⊕
DPN⊕
7
CH2—COOH
CH—COOH
HO—CH—COOH
Isocitric Acid
HOCH—COOH
CH2—COOH
Malic Acid
H2O
6
3
TPN⊕
TPNH + H⊕
HOOC—CH
HC—COOH
Fumaric Acid
CH2—COOH
CH—COOH
CO—COOH
Oxalosuccinic Acid
Flavin H2
Flavin
5
4
CH2—COOH
CH2—COOH
Succinic Acid
CH2—COOH
CH2
CO—CoA
Succinyl-CoA
ATP
ADP
CH2
CO—COOH
α-Ketoglu-
taric Acid
H2O
CoA
CO2
Coenzyme A
DPNH
+
H⊕
DPN⊕

The net effect of the cycle is to oxidize pyruvic acid to carbon dioxide and water:

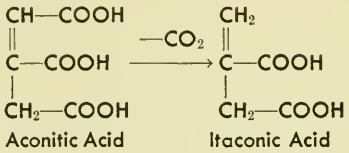
$$CH_3COCOOH + 5O \rightarrow 3CO_2 + 2H_2O$$

Enzymes of the citric acid cycle occur widely among microorganisms, and it is likely that the cycle and variants of it are equally ubiquitous. Its primary physiological function in microorganisms (if a primary function can be singled out) is less clear, two possibilities being: (a) an energy source and (b) a source of amino acid skeletons. Interruption of the cycle or imbalances under certain conditions lead to accumulation of certain acids. Thus high yields of citric, isocitric, $\alpha$-ketoglutaric, fumaric and malic acids can be obtained in controlled fungal fermentations.

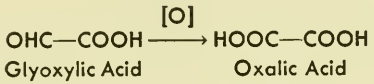
It was mentioned in the preceding chapter that certain microorganisms are capable of growing on a medium containing acetate as the sole carbon source, synthesizing all their carbohydrate requirements from it. In some of these microorganisms, at least, this ability may be due to possession of a pair of enzymes (malate synthetase and isocitritase) which permit operation of a cycle ancillary to the citric acid cycle or replacement of the steps from isocitric acid to malic acid and commonly called the glyoxylic acid cycle:



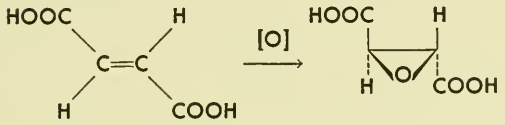
The origin of certain other acids can be deduced; for example, itaconic acid by decarboxlration of aconitic, oxalic acid by oxidation of glyoxylic and epoxysuccinic by oxidation of fumaric.




Aconitic Acid — Itaconic Acid

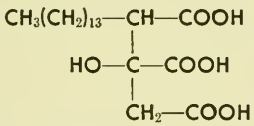



Glyoxylic Acid — Oxalic Acid

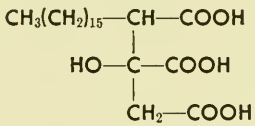



Fumaric Acid — *trans*-Epoxysuccinic Acid

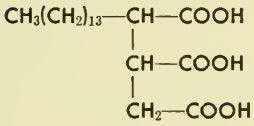
Certain higher fungi and some molds produce acids such as caperatic, agaricic, rangiformic, mineoluteic, roccellic, and spiculisporic, which appear to be essentially aldol condensation products of various keto acids of the citric acid cycle with long chain fatty acids.



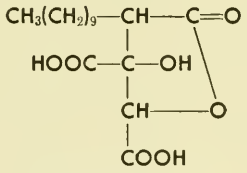

Caperatic Acid
(one carboxyl group a methyl ester)



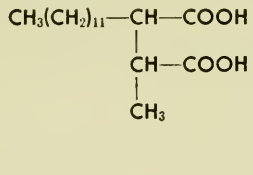

Agaricic Acid



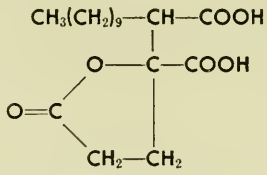

Rangiformic Acid
(one carboxyl group a methyl ester)




Miniolutеic Acid



Roccellic Acid



Spiculisporic Acid

Lipide production by microorganisms varies widely, some yeasts and molds producing up to 50% of their dry weight. Yeasts were used for commercial submerged culture production of fat during World War II in Germany.

It has been estimated that 80–90% of all fatty acids in plants and higher animals occur as esters—triglycerides and phospholipides. In microorganisms a high percentage of the lipides seem to be bound in some way, perhaps as lipoproteins, liposaccharides, sterol esters, etc., and a preliminary acid hydrolysis is required before complete extraction.

The fatty acid contents of the fats produced by a few molds and yeasts have been studied in detail, and several of these are reproduced in the following table.

TABLE I

*Component Fatty Acids of Fats Produced by Microorganisms*

| | *Aspergillus nidulans*[1] | *Penicillium soppii.*[2] | *Penicillium lilacinum*[3] | *Penicillium spinulosum*[4] | Yeast Strain No. 72[5] | *Rhodotorula* sp.[6] | *Torulopsis* sp.[7] |
|---|---|---|---|---|---|---|---|
| Free acidity (% oleic) | 0 8 | 0.6 | 0.2 | 5.8 | 33 | 18 | 51.2 |
| Component Acids | | | | | | | |
| Myristic | 0.7 | 0.3 | 0.1 | — | 0.1 | 1.1 | 0.3 |
| Palmitic | 20.9 | 22.0 | 32.3 | 18.0 | 25.6 | 29.8 | 7.9 |
| Stearic | 15.9 | 7.6 | 9.4 | 11.9 | 5.9 | 8.8 | 3.8 |
| Arachidic, Behenic, Lignoceric | 1.4 | 0.9 | 1.4 | 1.4 | 5.1 | 1.4 | 0.2 |
| Hexadecenoic | 1.2 | 3.3 | 3.4 | 3.8 | 1.3 | 1.8 | 7.6 |
| Oleic | 40.3 | 45.2 | 38.6 | 43.3 | 54.5 | 40.1 | 21.5 |
| Linoleic | 17.0 | 20.0 | 13.4 | 21.1 | 5.7 | 11.2 | 49.7 |
| Linolenic | 0.2 | 0.3 | — | 0.3 | 0.7 | 4.8 | 4.4 |
| Unsaturated $C_{20}$ | 2.4 | 0.4 | 1.4 | 0.2 | 1.1 | 1.0 | — |

Generally microorganism lipides have a higher free fatty acid content than those of animals. Bacterial fats seem to have received less quantitative study. *cis*-Vaccenic and lactobacillic acids have been shown to be major constituents of the lipides of lactobacilli,[8] streptococci[9] and *Agrobacterium tumefaciens*.[10] An analysis of the fatty acids of two strains of *Mycobacterium tuberculosis* has been published:[11]

TABLE II

*Higher Fatty Acid Content (%) in the Phosphatides and Fats of Mycobacterium tuberculosis $H_{37}$ Rv and BCG*

| | Phosphatide | | Fat | |
|---|---|---|---|---|
| | $H_{37}$Rv | BCG | $H_{37}$Rv | BCG |
| Mycolic Acid | 20.0 | 20.4 | — | — |
| I. Unknown Acid | 3.0 | 3.0 | 0.7 | 3.1 |
| II. " " | 13.8 | 8.6 | 1.1 | 2.1 |
| III. " " | — | — | 2.7 | 1.5 |
| III. Phthioic Acid | 5.7 | 12.3 | 20.0 | 5.5 |
| III. Unknown Acid | 3.7 | 14.0 | — | — |
| IV. " " | — | — | 8 0 | — |
| Arachidonic Acid | — | — | — | 2.3 |
| Stearic Acid | 13.0 | 13.0 | 24.5 | 22.1 |
| Oleic and Palmitic Acids | 28.0 | 19.2 | 34.0 | 48.2 |
| Linoleic Acid | 12.8 | 10.4 | 10.0 | 15.2 |

The waxes and fats in which the acid-fast mycobacteria and corynebacteria abound have been investigated extensively, and a variety of oxidized, methylated and branched chain fatty acids and alcohols isolated and characterized. In the oxidized and

[1] J. Singh, T. K. Walker and M. L. Meara, *Biochem. J.* 61 85 (1955).

[2] J. Singh, Sudha E. Philip and T. K. Walker, *J. Sci. Food and Agr.* 8 697 (1957).

[3] J. Singh, Sudha Shah and T. K. Walker, *Biochem. J.* 62 222 (1956).

[4] I. Shimi, Ph.D. Thesis, Univ. of Manchester, 1955.

[5] T. P. Hilditch and R. K. Shrivastava, *Biochim. et Biophys. Acta* 2 80 (1948).

[6] John Holmberg, *Svensk Kem. Tidskr.* 60 14 (1948).

[7] R. Reichert, *Helv. Chim. Acta* 28 484 (1945).

[8] Klaus Hofmann and Sylvan M. Sax, *J. Biol. Chem.* 205 55 (1953).

[9] Klaus Hofmann and Fred Tausig, *ibid.* 213 415 (1955).

[10] *Idem., ibid.* 213 425 (1955).

[11] Josef Pokorný, *Naturwissenschaften* 10 241 (1958).

methylated acids the oxygen and methyl groups usually appear in positions consistent with the acetate theory of fatty acid biogenesis. These bacteria seem to be able also (in effect) to couple two long chain fatty acids to form ketones and branched chain acids.

Bacterial lipopolysaccharides are irritating pyrogens, relatively toxic to higher animals. The polysaccharide component is the carrier of serological effects, while the lipide moiety has an affinity for the surface of erythrocytes and produces the toxic and pyrogenic effect.[12] The high molecular weight wax called cord factor from mycobacteria is quite toxic (quantitatively comparable to diphtheria toxin) and is believed by some to be the principal factor responsible for the virulence of tuberculosis pathogens. Some of the simpler liposaccharides are shown in this section. References to those of higher molecular weight are included in an appendix.

Phosphatides are widely distributed in nature, though generally in small quantities. They are difficult to handle intact, and few have been well characterized. The metabolism, theories of function and biosynthesis of phospholipides have been reviewed.[13]

For many years chemists speculated on the reason for the predominance of compounds with an even number of carbon atoms among natural fatty acids. The mystery was intensified by such animal feeding experiments as those of Knoop and Dakin,[14] which showed that in mammalian metabolism stepwise degradation of fatty acids and similar substances occurred two carbon atoms at a time.

Microorganisms have been instrumental in the discovery of the significance of acetate in the catabolism and in the biosynthesis of fatty acids. The enzymatic methods, particularly those of anaerobic microorganisms, may differ in detail from those of higher animals. This work has been well reviewed.[15]

Great advances were made in the discovery of coenzyme A,[16]

[12] O. Westphal, O. Lüderitz, E. Eichenberger and E. Neter, *Deut. Z. Verdauungs-u. Stoffwechselkrankh.* 15 170 (1955).

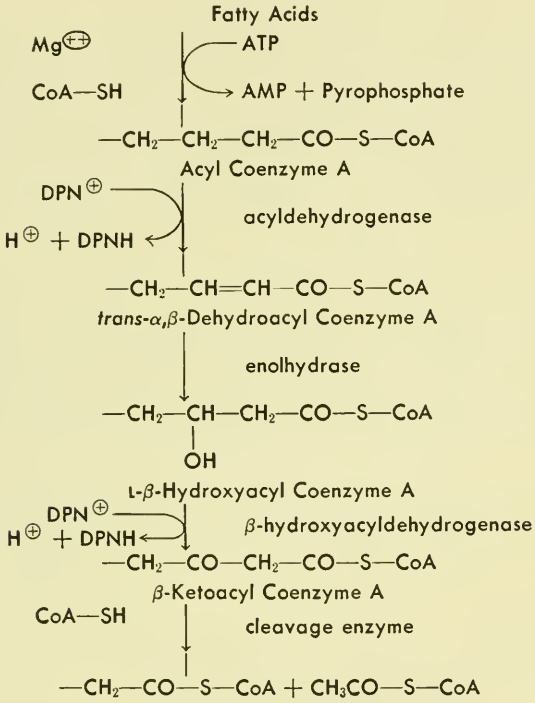
[13] E. P. Kennedy, *Ann. Rev. Biochem.* 26 130 (1957).

[14] H. D. Dakin, "Oxidations and Reductions in the Animal Body," Longmans, Green and Co., London, 1922.

[15] H. A. Barker, "Bacterial Fermentations," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 30.

[16] Fritz A. Lipmann, "Les Prix Nobel," Stockholm, 1954.

the isolation of acetyl coenzyme A (from yeast), the demonstration that the acetyl group was attached to its sulfur atom in a thioester linkage and that acetyl coenzyme A was an active acetylating agent.[17] The enzymic steps in what must be a very general scheme of fatty acid catabolism now can be written as follows:[18]

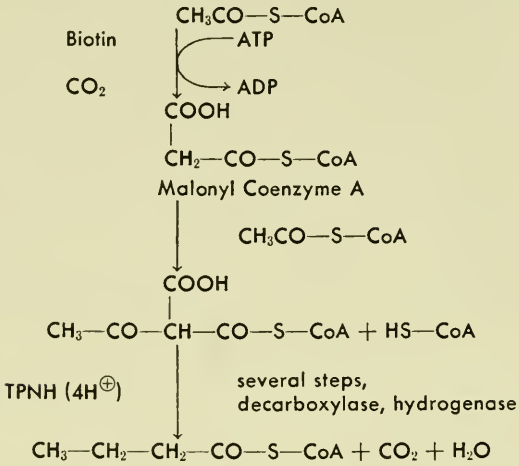



At first this process was thought to be reversible or cyclic. It has since been shown that a separate set of enzymes controls fatty acid biosynthesis. The required enzymes and cofactors for the synthetic process have been isolated, and in outline the

[17] Feodor Lynen, Ernestine Reichert and Luistraud Rueff, *Ann.* 574 1 (1951).

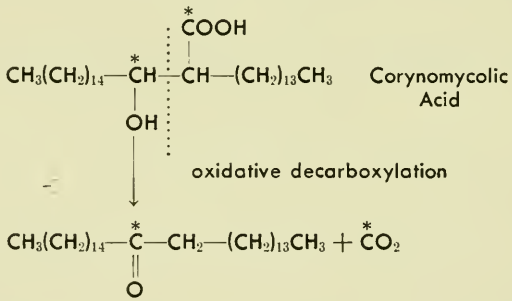
[18] Feodor Lynen, *Ann. Rev. Biochem.* 24 653 (1955).

process is at present believed to be represented by the scheme:[19]




The butyryl coenzyme A can then react with another molecule of malonyl coenzyme A and the process repeats. There is a statistical distribution peak at 14–18 carbon atom length chains.

Certain bacteria can couple chains of considerable length as, for example, in corynomycolic acid produced by corynebacteria:



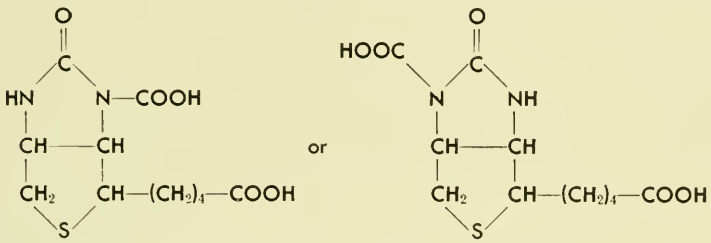

This compound is formed by the coupling of two palmitic acid molecules as shown by a labeling experiment.[20] $C^{14}$-1-Labeled

[19] Salih J. Wakil, Edward B. Titchener and David M. Gibson, *Biochim. et Biophys. Acta* 29 225 (1958); Salih J. Wakil, *J. Am. Chem. Soc.* 80 6465 (1958); David M. Gibson, Edward B. Titchener and Salih J. Wakil, *Biochim. et Biophys. Acta* 30 376 (1958).

[20] Mireille Gastambide-Odier, E. Lederer, *Nature* 184 1563 (1959).

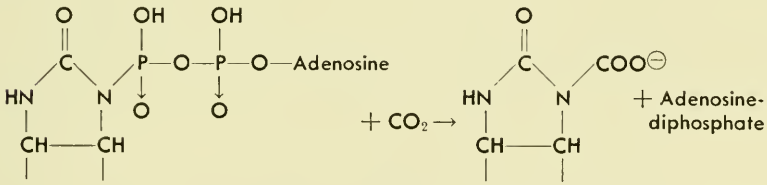
palmitic acid was incorporated into mycolic acid, and the product degraded to show that the carboxyl group and the oxidized C-atom $\beta$-to it in the corynomycolic acid were labeled. A similar biosynthetic path was suggested for the higher molecular weight mycolic acids produced by mycobacteria. Thus, condensation of 2 moles of $n$-$C_{26}$ and 2 moles of $n$-$C_{18}$ acids would yield the $C_{88}$ mycolic acids of cord factor. A $C_{26}$ acid is known to be produced by mycobacteria, and a $C_{52}$ acid, corynine, by corynebacteria.

The biotin requirement for enzymatic carboxylations is becoming generally recognized. It was in connection with his studies in lipide metabolism that Lynen isolated and synthesized a reaction product of biotin and carbon dioxide in which $CO_2$ had reacted at one of the nitrogen atoms to give an allophanic acid type of intermediate, the side-chain carboxyl group perhaps




being bound to the protein apoenzyme by an amide bond.

An intermediate may be adenosine diphosphoryl biotin (from ATP):

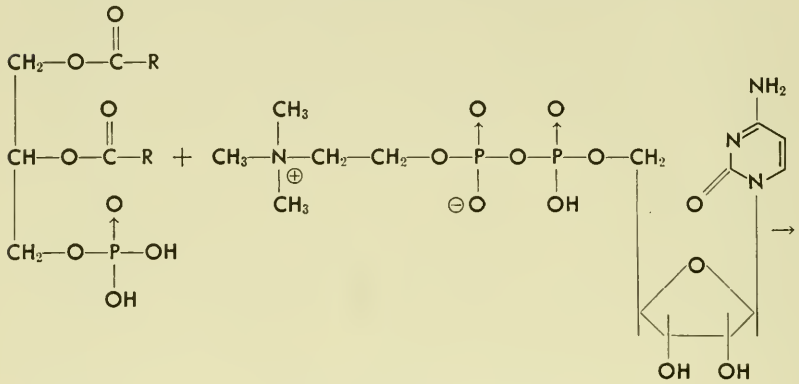



Other suggestions concerning the detailed function of this carboxylase cofactor were made.[21]

The lecithins are formed by initial ATP phosphorylation of one glycerol hydroxyl group followed by esterification of the re-

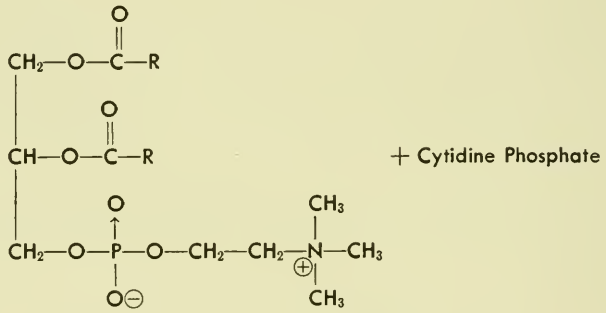
[21] F. Lynen, J. Knappe, E. Lorch, G. Jütting and E. Ringelmann, *Angew. Chem.* 71 481 (1959).

maining two hydroxyls by fatty acids as their coenzyme A esters. The phosphate group is then displaced by a choline phosphate group contributed by a coenzyme, cytidine diphosphocholine:



Diglyceride Phosphate

Cytidine-5′-diphosphatecholine



+ Cytidine Phosphate

The mechanism for cephalin formation is probably similar.

67 **Formic Acid,** $CH_2O_2$, colorless liquid, b.p. 100.5°, $n_D^{20}$ 1.3714.

HCOOH

*Pseudomonas formicans* n. sp., etc.
See the reference below for earlier work.
Irving P. Crawford, *J. Bacteriol.* 68 734 (1954).

68 **Oxalic Acid,** $C_2H_2O_4$ (Dihydrate), colorless tablets, m.p. 101°.

HOOC—COOH

*Aspergillus niger, Penicillium oxalicum, Citromyces* spp., many other fungus species and most lichens.

It occurs as the calcium salt in most lichens and higher fungi, but occasionally also as the free acid.

Jackson W. Foster, "Chemical Activities of Fungi," Academic Press Inc., New York, N. Y., 1949, chap. 10, pp. 326–350.

G. Walter, "Organic Acid Production by some Wood-Rotting Basidiomycetes," Univ. Microfilms Pub. 10,417, 1955, 99 pp.

69 **Acetic Acid,** $C_2H_4O_2$, colorless liquid, b.p. 118°, $n_D^{20}$ 1.3718.

$CH_3COOH$

*Saccharomyces cerevisiae*, other yeasts. Present in small quantities in many microorganisms.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954, Vol. I, Ruse H. Vaughn, *Acetic acid-vinegar*, chap. 17, pp. 498–535.

70 **Pyruvic Acid,** $C_3H_4O_3$, colorless liquid, b.p. 165° (dec.), $n_D^{20}$ 1.4138.

$CH_3COCOOH$

*Pseudomonas saccharophila*, etc.

Approximately 2 moles of pyruvic acid were produced per mole of glucose.

Nathan Entner and Michael Doudoroff, *J. Biol. Chem.* **196** 853 (1952).

71 **Malonic Acid,** $C_3H_4O_4$, colorless plates, m.p. 135°.

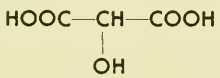
$HOOC—CH_2—COOH$

*Penicillium funiculosum, P. islandicum* Sopp, other fungi

D-Mannitol was isolated from the same culture.

Takeo Yamamoto, *J. Pharm. Soc. Japan* **75** 761 (1955).

72 **Tartronic Acid,** $C_3H_4O_5$, colorless crystals, m.p. 163° (dec.).



*Acetobacter acetosum, Gluconoacetobacter liquefaciens*

The first organism also produced 2-keto-D-gluconic acid and 5-keto-D-gluconic acid. The second organism also produced acetaldehyde, formic acid, acetic acid, 5-keto-gluconic acid, glycolic acids, other reducing acids, rubiginol, rubiginic acid and 3,5-dihydroxy-1,4-pyrone.

D. Kulka, A. N. Hall and T. K. Walker, *Nature* **167** 905 (1951).

Ko Aida, Toshio Kojima and Toshinobu Asai, *J. Gen. and Appl. Microbiol.* 1 18 (1955).

73 **β-Nitropropionic Acid**, $C_3H_5O_4N$, colorless crystals, m.p. 65°.

$$O_2N—CH_2CH_2COOH$$

*Aspergillus flavus, A. oryzae*

Milton T. Bush, Oscar Touster and Jean Early Brockman, *J. Biol. Chem.* 188 685 (1951).

Seiji Nakamura and Chuji Shimoda, *J. Agr. Chem. Soc. Japan* 28 909 (1954).

H. Raistrick and A. Stössl, *Biochem. J.* 68 647 (1958). See addendum for reference on biosynthesis.

74 **Propionic Acid**, $C_3H_6O_2$, colorless liquid with sharp odor, b.p. 140.5°.

$$CH_3CH_2COOH$$

*Amanita muscaria L., Propionibacteria, Clostridium propionicum*

Julius Zellner, *Monatsh.* 26 727 (1905).

Kenneth V. Thimann, "The Life of Bacteria," The Macmillan Company, New York, 1955, pp. 429–440.

75 **L(+)-Lactic Acid** (*d*-Lactic Acid, Sarcolactic Acid), $C_3H_6O_3$, colorless crystals, m.p. 52.8°, $[\alpha]_D^{15}$ +3.33° (c 5.022 in water), hygroscopic, polymerizes.

$$\begin{array}{l} CH_3CHCOOH \\ \quad\quad | \\ \quad\quad OH \end{array}$$

*Lactobacilli, Rhizopus* species, etc.

Yields of 90% or better have been reported.

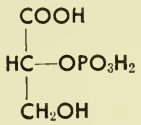
Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. I, Ruse H. Vaughn, *Acetic acid-vinegar*, chap. 17, pp. 498–535; H. H. Shopmeyer, *Lactic acid*, chap. 12, pp. 391–419.

76 **L(−)-Glyceric Acid**, $C_3H_6O_4$, unstable, usually isolated as a salt. Ca salt (dihydrate), m.p. 138°, $[\alpha]_D^{20}$ +13.3° (c 4.5 in water).

$$\begin{array}{l} COOH \\ \quad | \\ HCOH \\ \quad | \\ CH_2OH \end{array}$$

We have observed (by paper chromatographic comparison with an authentic sample on several solvent systems) the production of this acid by a wide variety of fungi. It is always accompanied by gluconic acid.

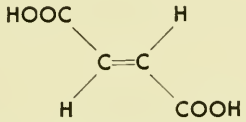
77 **2-Phosphoglyceric Acid**, $C_3H_7O_7P$.




Yeast

O. Meyerhof and W. Kiessling, *Biochem. Z.* 276 239 (1935).

78 **Fumaric Acid**, $C_4H_4O_4$, colorless crystals, m.p. 290° (subl.) (dec.).

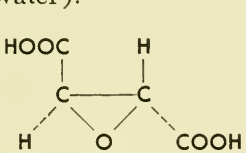



*Rhizopus* species, also *Mucor*, *Cunninghamella* and *Circinella* species, *Aspergillus* and *Penicillium* species, *Boletus* spp., *Fusaria*, etc.

Yields are about 59%.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. I, Ruse H. Vaughn, *Acetic acid-vinegar*, chap. 17, pp. 498–535; Jackson W. Foster, *Fumaric acid*, chap. 15, pp. 470–487.

79 ***l-trans*-Ethylene Oxide α,β-Dicarboxylic Acid** (Epoxysuccinic Acid), $C_4H_4O_5$, colorless crystals, m.p. 185° (dec.) $[\alpha]_D^{24}$ −117° (c 1 in water).

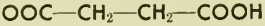



*Aspergillus fumigatus, Monilia formosa, Penicillium viniferum*

Yields greater than 20 g. per liter have been obtained.

Andrew J. Moyer, U. S. Patent 2,674,561 (1950).

80 **Succinic Acid**, $C_4H_6O_4$, colorless prisms, m.p. 185–187°.



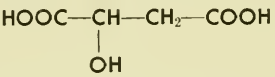
*Mucor stolonifer, Aspergillus terreus, Ustilina vulgaris, Penicillium aurantio-virens, Fusarium oxysporum,* lichens, etc.

Occurrence is wide, but yields are generally rather low.

Ve. S. Butkevich and M. V. Fedorov, *Biochem. Z.* 219 103 (1930).

Jackson W. Foster, "Chemical Activities of Fungi," Academic Press Inc., New York, N. Y., 1949, p. 373.

81 *l*-**Malic Acid**, $C_4H_6O_5$, colorless crystals, m.p. 99°, $[\alpha]_D^{30}$ −1.43° (c 21.65 in water).



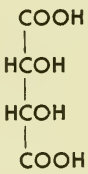
White aspergilli, clasterosporium spp., many other fungi.

Yields are high in some cases.

Reinhold Schreyer, *Biochem. Z.* 240 295 (1931).

John L. Yuill, *Chem. Ind.* 55 155 (1936).

82 L(+)-**Tartaric Acid**, $C_4H_6O_6$, colorless powder or crystals, m.p. 168–170° (dec.), $[\alpha]_D^{20}$ +11.98° (c 20 in water).



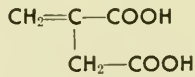
*Gibberella saubinetii, Acetobacter suboxydans*

Citric and acetic acids were produced also.

Lyle E. Hessler and Ross A. Gortner, *J. Biol. Chem.* 119 193 (1937).

Jonas Kamlet, U. S. Patent 2,314,831 (1943).

83 **Itaconic Acid**, $C_5H_6O_4$, colorless crystals, m.p. 162–164°.



*Aspergillus terreus, Ustilago zeae, Helicobasidium monpa,* other fungi

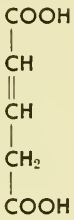
Jasper H. Kane, Alexander C. Finlay and Philip F. Amann, U. S. Patent 2,385,283 (1945).

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. I, Lewis B. Lockwood, *Itaconic acid,* chap. 16, pp. 488–498.

Yields are high in the case of *A. terreus*. *Ustilago zeae* is reported to produce 15 g. per liter as well as some dianthrone and glycolipides.

R. H. Haskins, J. A. Thorn and B. Boothroyd, *Can. J. Microbiol.* I 749 (1955).

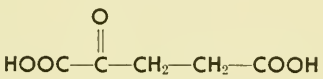
84 ***trans*-Glutaconic Acid,** $C_5H_6O_4$, colorless needles, m.p. 138°.



*Aspergillus niger* (on *l*-xylose)

Shinichiro Baba and Kinichiro Sakaguchi, *Bull. Agr. Chem. Soc.* (Japan) 18 93 (1942).

85 **α-Ketoglutaric Acid,** $C_5H_6O_5$, colorless crystals, m.p. 115–116°.

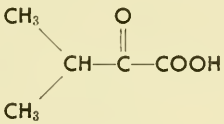


*Pseudomonas fluorescens*

Harold J. Koepsell, Frank H. Stodola and Eugene S. Sharpe, U. S. Patent 2,724,680 (1955).

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. II, Lewis B. Lockwood, *Ketogenic fermentation processes,* chap. 1, pp. 18–19.

86 **Dimethylpyruvic Acid,** $C_5H_8O_3$, leaflets, m.p. ~24°, b.p. 76–78°.



*Aspergillus* spp., *Piricularia oryzae* (biotin-deficient medium)

K. Ramachandran and V. Radha, *Current Sci.* (India) 24 50 (1955).

Hirohiko Katsuki, *J. Am. Chem. Soc.* 77 4686 (1955).

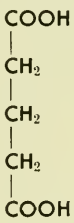
87 **Other Keto-Acids:**

Many of the transitory α-keto-acids present in cultures of microorganisms can be isolated by means of interceptors such as 2,4-dinitrophenylhydrazine. One recent paper reported the following acids identified principally in lactic and propionic bacteria cultures:

| | |
|---|---|
| Glyoxylic Acid | *p*-Hydroxyphenylpyruvic Acid |
| Pyruvic Acid | Hydroxypyruvic Acid |
| α-Ketoisovaleric Acid | Oxalacetic Acid |
| α-Ketoisocaproic Acid | α-Ketoglutaric Acid |
| α-Ketocaproic Acid | |

Matti Kreula and Artturi I. Virtanen, *Acta. Chem. Scand.* 11 1431 (1957).

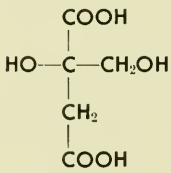
88 **Glutaric Acid,** $C_5H_8O_4$, colorless needles, m.p. 97°.



*Aspergillus niger* (on *l*-xylose)

Shinichirō Baba and Kinichiro Sakaguchi, *Bull. Agr. Chem. Soc.* (Japan) 18 93 (1942).

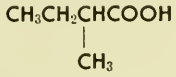
89 **Itatartaric Acid,** $C_5H_8O_6$, occurs as a gummy equilibrium mixture of lactone and free acid. Characterized as the methyl ester derivative.



*Aspergillus terreus* mutant

Frank H. Stodola, M. Friedkin, Andrew J. Moyer and Robert D. Coghill, *J. Biol. Chem.* 161 739 (1945).

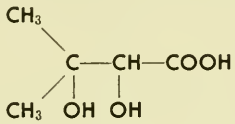
90 α-**Methylbutyric Acid,** $C_5H_{10}O_2$, colorless crystals, m.p. 176°, $[\alpha]_D^{21}$ +17.6°.



*Penicillium notatum*

Donald J. Cram and Max Tishler, *J. Am. Chem. Soc.* **70** 4238 (1948).

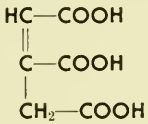
91 α,β-**Dihydroxyisovaleric Acid,** $C_5H_{10}O_4$, colorless syrup, $[\alpha]_D^{23}$ −12.4° (c 2 in dilute HCl, pH 1) and +10° (c 2 in water, pH 5.5–6.5). Forms crystalline quinine salt.



A valine precursor isolated from a *Neurospora crassa* mutant

John R. Sjölander, Karl Folkers, Edward A. Adelberg and E. L. Tatum, *J. Am. Chem. Soc.* **76** 1085 (1954).

92 *cis*-**Aconitic Acid,** $C_6H_6O_6$, colorless crystals, m.p. 125°.

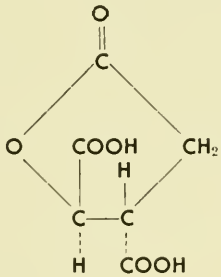


*Aspergillus niger*

This acid presumably is present to some extent in all organisms with the citric acid cycle.

Kinichiro Sakaguchi and Shinichiro Baba, *Bull. Agr. Chem. Soc.* (Japan) **18** 95 (1942). (Not isolated)

93 *allo*-**Isocitric Acid (Lactone),** $C_6H_6O_6$, m.p. 140–141° $[\alpha]_D^{190}$ +42.3° (c 4.83 in water).

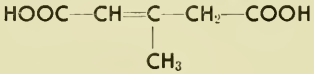


*Penicillium purpurogenum* Stoll *var. rubrisclerotium* Thom.

Yields greater than 20% of the glucose substrate supplied have been reported. Probably the isomer normal to the mammalian citric acid cycle also occurs in some microorganisms, but it has not been reported to accumulate.

Teruhiko Beppu, Shigeo Abe and Kinichiro Sakaguchi, *Bull. Agr. Chem. Soc.* (Japan) 21 263 (1957).

94 *trans*-β-Methylglutaconic Acid, $C_6H_8O_4$, colorless crystals, m.p. 131–134°.



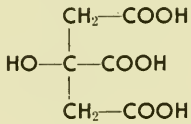
*Ustilago sphaerogena*

This substance is a component of ferrichrome A pigment,* in which its monohydroxamate is complexed with iron.

Thomas Emery and J. B. Neilands. (In press)

* See addendum.

95 Citric Acid, $C_6H_8O_7$ (occurs as monohydrate), colorless crystals or white powder, m.p. (monohydrate) ~100°, (anhydrous) 153°.

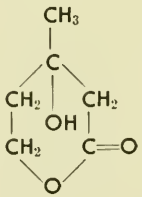


Wide variety of fungi, *e.g.*, *Aspergillus niger*.

Yields are high.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, N. Y., 1954 Vol. I; Marvin J. Johnson, *The citric acid fermentation*, chap. 13, pp. 420–445.

96 Mevalonic Acid Lactone (Hiochic Acid, β-Hydroxy-β-methyl-δ-valerolactone), $C_6H_{10}O_3$, colorless, hygroscopic crystals, m.p. 27°, b.p. 90° (0.3 mm.). (Synthetic racemate.)



Yeasts (Isolated from Distillers' Dried Solubles).

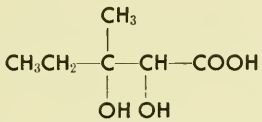
Donald E. Wolf, Carl H. Hoffman, Paul E. Aldrich, Helen R. Skeggs, Lemuel D. Wright and Karl Folkers, *J. Am. Chem. Soc.* 78 4499 (1956).

Helen R. Skeggs, Lemuel D. Wright, Emlen L. Cresson, Gloria D. E. MacRae, Carl H. Hoffman, Donald E. Wolf and Karl Folkers, *J. Bact.* 72 519 (1956).

Carl H. Hoffman, Arthur F. Wagner, Andrew N. Wilson, Edward Walton, Clifford H. Shunk, Donald E. Wolf, Frederick W. Holly and Karl Folkers, *J. Am. Chem. Soc.* 79 2316 (1957).

Clifford H. Shunk, Bruce O. Linn, Jesse W. Huff, James L. Gilfillan, Helen R. Skeggs and Karl Folkers, *ibid.* 79 3294 (1957).

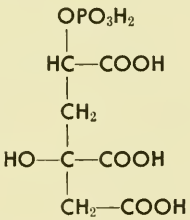
97 **α,β-Dihydroxy-β-methylvaleric Acid,** $C_6H_{12}O_4$, colorless syrup, $[\alpha]_D^{23}$ +3° (c 2.3 in water containing 1 equiv. of $Ca(OH)_2$) and −16.7° (c 2.3 in dilute HCl, pH 1). Forms crystalline quinine salt.



A precursor of isoleucine isolated from a *Neurospora crassa* mutant.

John R. Sjölander, Karl Folkers, Edward A. Adelberg and E. L. Tatum, *J. Am. Chem. Soc.* 76 1085 (1954).

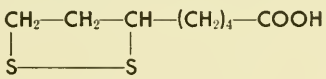
98 **2-Phospho-4-hydroxy-4-carboxyadipic Acid,** $C_7H_{11}O_{11}P$.



*Escherichia coli*

W. W. Umbreit, *J. Bacteriol.* 66 74 (1953).

99 **Lipoic Acid** (6,8-Thioctic Acid), $C_8H_{14}O_2S_2$, pale yellow crystals, m.p. 47°, $[\alpha]_D^{23}$ +10.4°.



Yeast, *E. coli* mutant

Lester J. Reed, Quentin F. Soper, Geo. H. F. Schnakenberg, Stanley F. Kern, Harold Boaz and I. C. Gunsalus, *J. Am. Chem. Soc.* 74 2383 (1952); Lester J. Reed, I. C. Gunsalus, G. H. F. Schnakenberg, Quentin F. Soper, Harold E. Boaz, Stanley F. Kern and Thomas V. Parke, *ibid.* 75 1267 (1953). (Isolation)

Edward Walton, Arthur F. Wagner, Louis H. Peterson, Frederick W. Holly and Karl Folkers, *ibid.* 76 4748 (1954); Edward Walton, Arthur F. Wagner, Frank W. Bachelor, Louis H. Peterson, Frederick W. Holly and Karl Folkers, *ibid.* 77 5144 (1955). (Synthesis)

100 **2-Decene-1,10-dioic Acid,** $C_{10}H_{16}O_4$, colorless crystals, m.p. 172°.

$$HOOC—CH{=}CH—(CH_2)_6—COOH$$

*Penicillium notatum*

Donald J. Cram and Max Tishler, *J. Am. Chem. Soc.* **70** 4238 (1948). (Isolation)

101 **10-Undecynoic Acid,** $C_{11}H_{18}O_2$, colorless crystals, m.p. 39°.

$$HC{\equiv}C—(CH_2)_8—COOH$$

*Rhodotorula glutinis* var. *lusitanica*

Undecenoic acid was isolated from the same culture.

Nagueira Prista, *Anais. fac. farm. Porto* 14 19 (1954).

102 **10-Undecenoic Acid** (10-Undecylenic Acid), $C_{11}H_{20}O_2$, colorless crystals, m.p. 24°, $n_D^{24}$ 1.4464.

$$CH_2{=}CH(CH_2)_8COOH$$

*Rhodotorula glutinis* var. *lusitanica*

Nogueira Prista, *Anais. fac. farm. Porto* 14 19 (1954).

103 **Myristic Acid,** $C_{14}H_{28}O_2$, colorless soft leaflets, m.p. 54°.

$$CH_3(CH_2)_{12}COOH$$

Widely distributed, especially as its triglyceride.

104 **D-β-Hydroxymyristic Acid,** $C_{14}H_{28}O_3$, colorless crystals, m.p. 73°, $[\alpha]_D^{25}$ −16° (c 2.0 in chloroform).

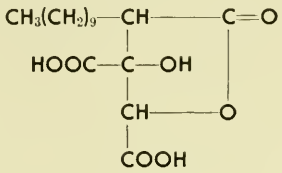
$$CH_3(CH_2)_{10}\underset{\displaystyle OH}{\underset{|}{C}}HCH_2COOH$$

*Escherichia coli*

Obtained together with lauric, myristic and palmitic acids from an acid hydrolysate of the phospholipide fraction.

Miyoshi Ikawa, J. B. Koepfli, S. G. Mudd and Carl Niemann, *J. Am. Chem. Soc.* 75 1035 (1953).

105 **Mineoluteic Acid,** $C_{16}H_{26}O_7$, colorless needles, m.p. 171°, $[\alpha]_{5461}^{16}$ +108.1° (c 1.07 in acetone)




*Penicillium minioluteum* Dierckx

Spiculisporic acid is produced in the same culture.

John H. Birkinshaw and Harold Raistrick, *Biochem. J.* 28 828 (1934).

106 **Palmitoleic Acid** (Physetolic Acid, 9-Hexadecenoic Acid), $C_{16}H_{30}O_2$, colorless crystals, m.p. 30–33°.

$$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$$

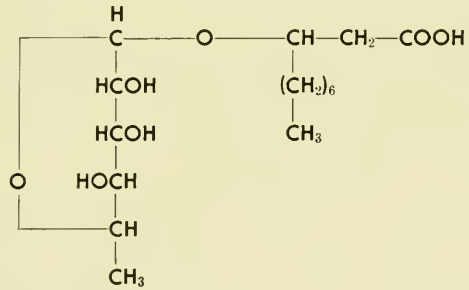
Yeast, *Corynebacterium diphtheriae, Streptococcus* spp., *Penicillium lilacinum* occurs widely.

E. Chargaff, Z. *physiol. Chem.* 218 223 (1933).

Klaus Hofmann and Fred Tausig, *J. Biol. Chem.* 213 415 (1955).

J. Singh, Sudha Shah and T. K. Walker, *Biochem. J.* 62 222 (1956).

107 **Pyolipic Acid,** $C_{16}H_{30}O_7$, colorless, viscous oil.




*Pseudomonas pyocyanea*

The yield was 1-2 g. per liter.

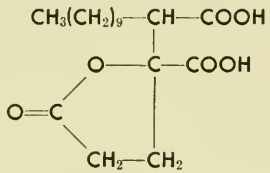
Sune Bergström, Hugo Theorell and Hans Davide, *Arch. Biochem.* 10 165 (1946).

108 **Palmitic Acid,** $C_{16}H_{32}O_2$, soft white crystals, m.p. 62.5°.

$$CH_3(CH_2)_{14}COOH$$

Widely distributed, especially as esters.

109 **Spiculisporic Acid,** $C_{17}H_{28}O_6$, colorless crystals, m.p. 145°, $[\alpha]_{5461}$ −14.76° (in alcohol).

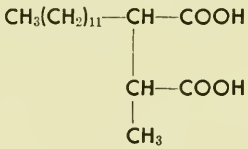


*Penicillium spiculisporum* Lehman, *P. crateriforme* Gilman and Abbott and *P. minioluteum* Dierckx

P. W. Clutterbuck, H. Raistrick and M. L. Pintoul, *Trans. Roy. Soc.* (London) B220 301 (1931). (Isolation and structure)

Albert E. Oxford and Harold Raistrick, *Biochem. J.* 28 1321 (1934). (Isolations)

110 **Roccellic Acid,** $C_{17}H_{32}O_4$, colorless crystals, m.p. 131°, $[\alpha]_D^{26}$ +16.80°.



*Roccella tinctoria* (L.), *R. montagnei* Bel., etc., also *Lecanora* species

Yields 1–4%. Erythrin and *i*-erythritol also were present.

G. Kennedy, J. Breen, J. Keane and T. J. Nolan, *Sci. Proc. Roy. Dublin Soc.* 21 557 (1937).

111 ***cis*-Vaccenic Acid,** $C_{18}H_{34}O_2$, soft white platelets, m.p. 43°.

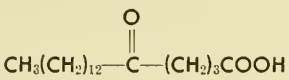
$$CH_3(CH_2)_5CH{=}CH(CH_2)_9COOH$$

*Lactobacillus arabinosus, L. casei, Agrobacterium tumefaciens, Streptococcus* spp.

Klaus Hofmann, Robert A. Lucas and Sylvan M. Sax, *J. Biol. Chem.* 195 473 (1952).

Klaus Hofmann and Fred Tausig, *ibid.* 213 425 (1955).

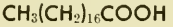
112 **Lactarinic Acid** (5-Ketostearic Acid), $C_{18}H_{34}O_3$, colorless plates, m.p. 87°.



*Lactarius rufus* Scopol.

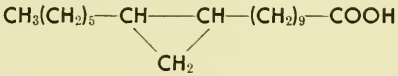
A. K. Schneider and M. A. Spielman, *J. Biol. Chem.* 142 345 (1942).

113 **Stearic Acid**, $C_{18}H_{36}O_2$, colorless leaflets m.p. 69°.



Widely distributed.

114 **Lactobacillic Acid** (Phytomonic Acid), $C_{19}H_{36}O_2$, colorless crystals, m.p. 33.6–35°.

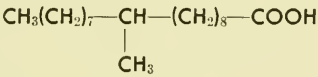


*Lactobacillus arabinosus, L. casei, Agrobacterium (Phytomonas) tumefaciens*

Klaus Hofmann, Otto Jucker, William R. Miller, Alfred C. Young, Jr. and Fred Tausig, *J. Am. Chem. Soc.* 76 1799 (1954).

Klaus Hofmann, Gino J. Marco and George A. Jeffrey, *ibid.* 80 5717 (1958). (Structure)

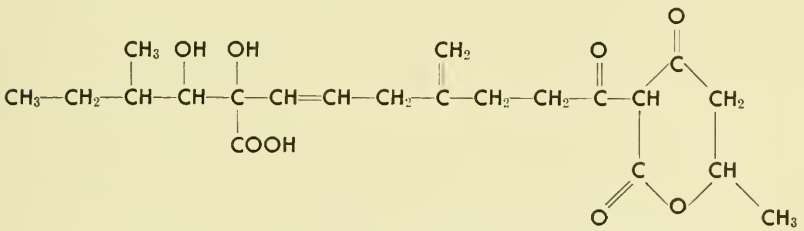
115 **Tuberculostearic Acid** (*l*-10-Methyloctadecanoic Acid), $C_{19}H_{38}O_2$, colorless oil, m.p. 12.8–13.4°, $n_D^{25}$ 1.4514, $[\alpha]_D^{26}$ −0.045°.



*Mycobacterium tuberculosis* var. *hominis*

Franklin S. Prout, James Cason and A. W. Ingersoll, *J. Am. Chem. Soc.* 70 298 (1948). (Synthesis)

116 **Alternaric Acid**, $C_{21}H_{30}O_8$, colorless crystals, m.p. 138°.

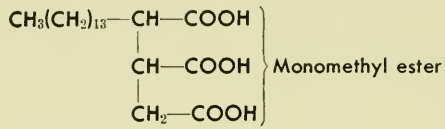


*Alternaria solani* Ell. and Mart., Jones and Grout

John Frederick Grove, *J. Chem. Soc.*, 4059 (1952). (Isolation)

J. R. Bartels-Keith and John Frederick Grove, *Proc. Chem. Soc.*, 398 (1959). (Structure)

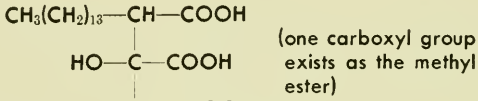
117 **Rangiformic Acid**, $C_{21}H_{38}O_6$, colorless needles, m.p. 106°, $[\alpha]_D^{24}$ +16.2°.




*Cladonia rangiformis* Hoffm., *C. mitis* Sandst.

Masaru Aoki, *J. Pharm. Soc. Japan* 66A 52 (1946).

118 **Caperatic Acid**, $C_{21}H_{38}O_7$, colorless leaflets, m.p. 132°, $[\alpha]_D^{10}$ −3.85°.



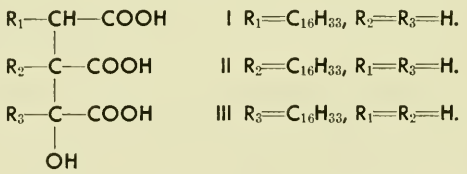

*Parmelia caperata* (L.), *Nephromopsis stracheyi, f. ectocarpisma* Hue.

Protocetraric acid also was present.

Michizo Asano, Yukio Kameda and Osamu Tamemasa, *J. Pharm. Soc. Japan* 64 203 (1944).

119 **Ungulinic Acid**, $C_{22}H_{38}O_6$, colorless microcrystalline needles, m.p. 78–80°.

Tentative structure of hydrate (ordinarily a γ-lactone):

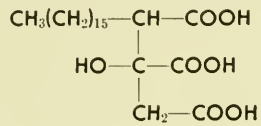



*Polyporus betulinus*

J. H. Birkinshaw, E. N. Morgan and W. P. K. Findlay, *Biochem. J.* 50 509 (1952).

Sidonie Marcus, *ibid.* 50 516 (1952).

120 **Agaricic Acid** (Agaricin, Laricic Acid, Agaric Acid) $C_{22}H_{40}O_7$, colorless microcrystalline powder, m.p. 142° (dec.), $[\alpha]_D^{19}$ −9° (in dilute NaOH solution).



*Polyporus officinalis* (=*Fomes officinalis, Fomes laricis*)

A yield of 18% of the weight of the fruiting body has been reported.

H. Thomas and J. Vogelsang, *Ann.* 357 145 (1907).

121 **Ventosic Acid,** $C_{22}H_{44}O_6$, white amorphous powder, m.p. 183°. A tetrahydroxybehenic acid.

*Haematomma ventosum,* other lichens

Thamnolic acid was isolated from the same source.

Yngve Johannes Solberg, *Acta Chem. Scand.* 11 1477 (1957).

122 **Tetracosanoic Acid** (Lignoceric Acid), $C_{24}H_{48}O_2$, colorless plates, m.p. 87.5°.

$CH_3(CH_2)_{22}COOH$

*Mycobacterium tuberculosis, Phycomyces blakesleeanus, Penicillium chrysogenum*

Robert L. Peck and R. J. Anderson, *J. Biol. Chem.* 140 89 (1941).

Karl Bernhard and Hans Albrecht, *Helv. Chim. Acta* 31 977 (1948).

Yoshiro Abe, *Proc. Fac. Eng. Keiogijuku Univ.* 2 15 (1949). (*Chem. Abstr.* 47 4949i)

123 **Pentacosanoic Acid,** $C_{25}H_{50}O_2$, colorless crystals, m.p. 84°.

$CH_3(CH_2)_{23}COOH$

*Mycobacterium tuberculosis* var. *hominis*

A. Aebi, J. Asselineau and E. Lederer, *Bull. soc. chim. biol.* 35 661 (1953).

124 **Hexacosanoic Acid** (Phthioic Acid, Cerotic Acid, Cerinic Acid), $C_{26}H_{52}O_2$, colorless crystals, m.p. 88°.

$CH_3(CH_2)_{24}COOH$

*Mycobacterium tuberculosis, Phycomyces blakesleeanus*

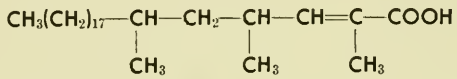
Obtained together with palmitic, tuberculostearic and mycoceranic acids.

R. J. Anderson, *J. Biol. Chem.* 83 505–519 (1929).

Karl Bernhard and Hans Albrecht, *Helv. Chim. Acta* 31 977 (1948).

Jean Asselineau, *Compt. rend.* 237 1804 (1953).

125 **Mycolipenic Acid** ((+)-2,4L,6L-Trimethyltetracos-2-enoic Acid), $C_{27}H_{52}O_2$, low melting solid $[\alpha]_D^{20}$ +7.9° (c 25.2 in ether), $n_D^{36}$ 1.4600.



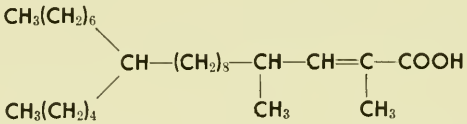
*Mycobacterium tuberculosis* var. *hominis*

J. D. Chanley and N. Polgar, *J. Chem. Soc.*, 1003 (1954). (Isolation)

D. J. Millin and N. Polgar, *Proc. Chem. Soc.*, 122 (1957). (Synthesis)

126 **$C_{27}$-Phthienoic Acid** (*trans*-2,4-Dimethyl-13-*n*-amyl-2-eicosenoic Acid), $C_{27}H_{52}O_2$, soft white crystals, m.p. 26° and 39° (polymorphic), $[\alpha]_D^{25}$ +17.8° ±0.2°, $n_D^{25}$ 1.4666.

Tentative structure:



The author emphasizes the difference of this compound from mycolipenic acid.

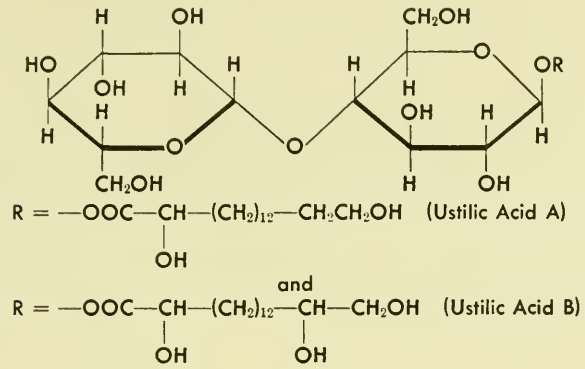
*Mycobacterium tuberculosis* var. *hominis*

James Cason, Hans-Ruedi Urscheler and C. Freeman Allen, *J. Org. Chem.* 22 1284 (1957). (Structure) and earlier papers in this series.

127 **Ustilagic Acids.**

The corn smut fungus produces a group of related glycolipides. As originally isolated, the properties of the partially purified mixture were given as: $C_{37}H_{62-66}O_{17}$, colorless, needle-like crystals, m.p. 144–147°, $[\alpha]_D^{23}$ +7° (c 1.0

in pyridine). Two of the component structures have been characterized as shown:




*Ustilago zeae*, other *Ustilaginales* spp.

Yields of 12–33% of the glucose supplied were reported.

R. H. Haskins and J. A. Thorn, *Can. J. Botany* 29 585 (1951).

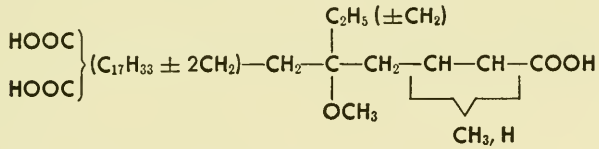
R. U. Lemieux, J. A. Thorn, Carol Brice and R. H. Haskins, *Can. J. Chem.* 29 409 (1951). (Isolation)

R. U. Lemieux, *ibid.* 29 415 (1951).

R. U. Lemieux, J. A. Thorn and H. F. Bauer, *ibid.* 31 1054 (1953).

128 **Bongkrekic Acid**, $C_{29}H_{40}O_7$, unstable, resinous, $[\alpha]_D^{22}$ +165° (c 2.0 in $NaHCO_3$).

The stabler hydrogenated compound, $C_{29}H_{54}O_7$, was given the following partial structure.

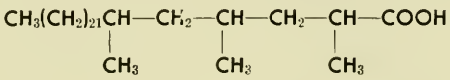



*Pseudomonas cocovenenans* (on a special copra-containing medium)

Bongkrekic acid is a toxin and has antibiotic properties.

D. H. Nugteren and W. Berends, *Rec. trav. chim.* 76 13 (1957).

129 **Mycoceranic Acid** (Mycocerosic Acid), $C_{31}H_{62}O_2$, white solid, m.p. 30°, $[\alpha]_D^{21}$ −6.9° (c 16.8 in ether).



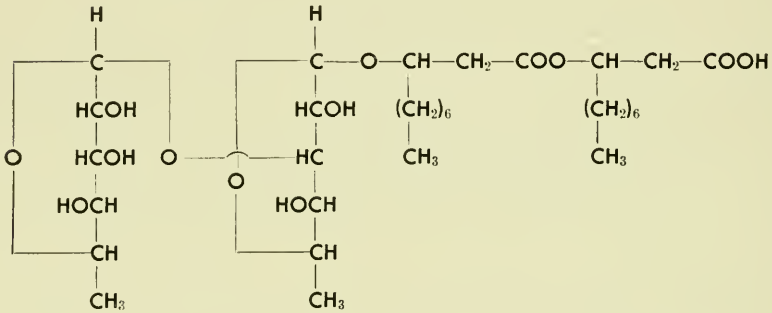
*Mycobacterium tuberculosis*

Occurs esterified with phthiocerol.

J. D. Chanley and N. Polgar, *J. Chem. Soc.*, 1003, 1011 (1954).

130 **Glycolipide from** *Pseudomonas aeruginosa*, $C_{32}H_{60}O_{14}$ (Monohydrate), colorless rectangular platelets, m.p. 86°, $[\alpha]_D$ −84° (c 3.0 in chloroform).

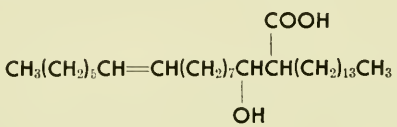
Probable structure:



*Pseudomonas aeruginosa* (three different strains)

F. G. Jarvis and M. J. Johnson, *J. Am. Chem. Soc.* 71 4124 (1949). (Isolation)

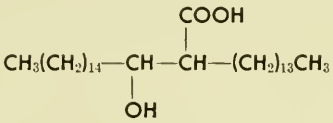
131 **Corynomycolenic Acid**, $C_{32}H_{62}O_3$, colorless oil, $n_D^{19}$ 1.4758. Methyl ester: $[\alpha]_{5461}^{20}$ +9.0 ±0.3°.



*Corynebacterium diphtheriae*

J. Pudles and E. Lederer, *Biochim. et Biophys. Acta* 11 163 (1953).

132 **Corynomycolic Acid**, $C_{32}H_{64}O_3$, colorless crystals, m.p. 70°, $[\alpha]_D$ 7.5°.



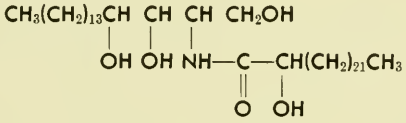
*Corynebacterium diphtheriae, C. ovis*

E. Lederer, J. Pudles, S. Barbezat and J. J. Trillat, *Bull. soc. chim. France* 93 (1952).

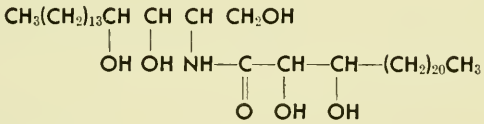
Anne Diara and Julia Pudles, *Bull. soc. chim. biol.* 41 481 (1959).

133 **Fungal Cerebrins**

A. $C_{42}H_{85}O_5N$



B. $C_{42}H_{85}O_6N$



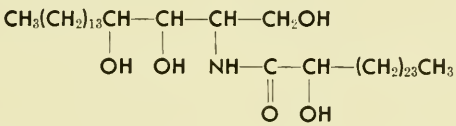
*Penicillium* spp., yeasts

Takeshi Oda, *J. Pharm. Soc. Japan* 72 136 (1952). (Isolation); *idem., ibid.* 72 142 (1952). (Structure)

A. H. Cook, "The Chemistry and Biology of Yeasts," A. A. Eddy, *Aspects of the chemical composition of yeast*, Academic Press, Inc., New York, N. Y., 1958, p. 203.

134 **Yeast Cerebrin**, $C_{44}H_{89}O_5N$, colorless crystals, m.p. 87–89°, $[\alpha]_D$ +31°.

Tentative structure:



Yeasts

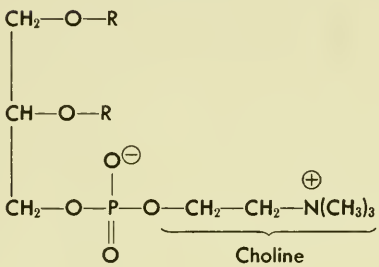
Fritz Reindel, A. Weichmann, S. Picard, Karl Luber and Paul Turula, *Ann.* 544 116 (1940).

A. H. Cook, "The Chemistry and Biology of Yeasts," A. A. Eddy, *Aspects of the chemical composition of yeast*, Academic Press, Inc., New York, N. Y., 1958, p. 203.

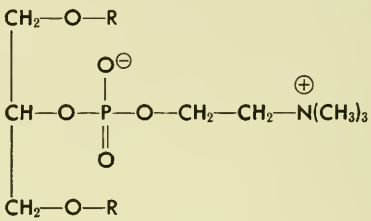
135 **Lecithins and Cephalins**

The lecithins and cephalins are widely occurring phospholipides. They are generally oily or partially crystalline materials with mixed fatty acids. Lecithin and Cephalin Structures (R = various fatty acids).

α-Lecithin




β-Lecithin




The cephalins are similar except that the choline residue is replaced by ethanolamine.

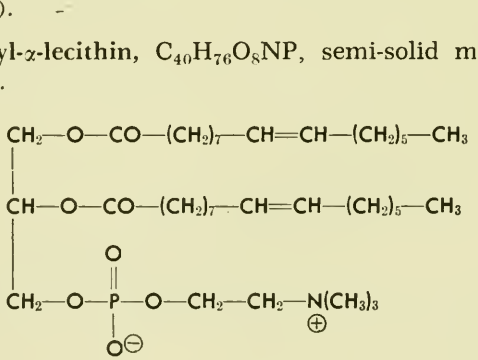
Yeast, *Aspergillus sydowi*, etc.

F. M. Strong and W. H. Peterson, *J. Am. Chem. Soc.* 56 952 (1934).

D. W. Woolley, F. M. Strong, W. H. Peterson and E. A. Prill, *ibid.* 57 2589 (1935).

L. F. Salisbury and R. J. Anderson, *J. Biol. Chem.* 112 541 (1936).

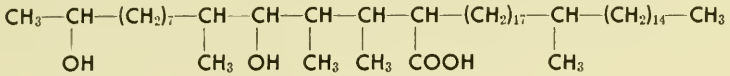
136 **Dipalmitoleyl-α-lecithin,** $C_{40}H_{76}O_8NP$, semi-solid material, $[\alpha]_D$ +6.6°.




Yeast

Donald J. Hanahan and Michael E. Jayko, *J. Am. Chem. Soc.* 74 5070 (1952). (Isolation)

137 **Corynine** (Corynodic Acid), $C_{52}H_{104}O_4$, colorless crystals, m.p. 70°.

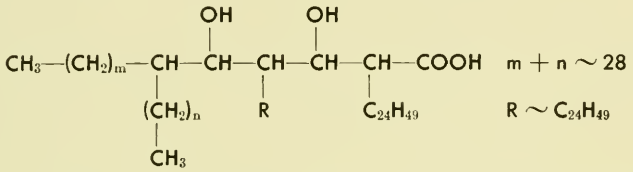


*Corynebacterium diphtheriae*

Obtained from the saponification of the phospholipide fraction.

Hideo Takahashi, *J. Pharm. Soc. Japan* 68 292 (1948).

138 **A Mycolic Acid,** $C_{84}H_{174}O_4$ ($\pm 5CH_2$), colorless microcrystals, m.p. 56–58°, $[\alpha]_D$ +2° (c 2.446 in chloroform).

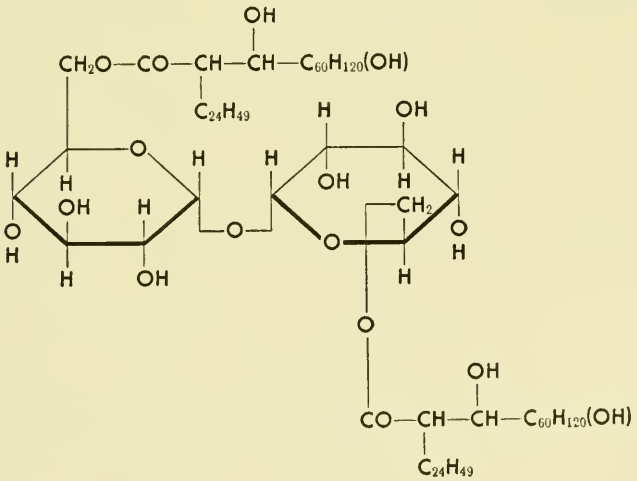


*Mycobacterium tuberculosis* human Canetti strain

This acid was isolated by chromatography from a saponification of the chloroform soluble wax.

Jean Asselineau, *Bull. soc. chim. France* 135 (1960).

139 **Cord Factor,** $C_{186}H_{366}O_{17}$ $\pm 10$ $CH_2$, nearly colorless wax, m.p. 43–45°, $[\alpha]_J$ +40 ±5° (c 1.37 in chloroform).



*Mycobacterium tuberculosis* (six different virulent human and bovine strains as well as the BCG strain).

Hydrolysis yields 1 mole of trehalose and 2 moles of mycolic acid.

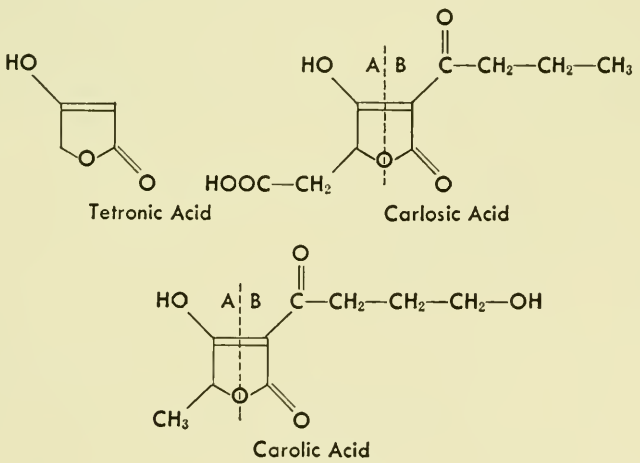
H. Noll, H. Bloch, J. Asselineau and E. Lederer, *Biochim. et Biophys. Acta* **20** 299 (1956).

4

# Tetronic Acids and Other Lactones and Lactams

This chapter includes derivatives of tetronic acid as well as some related lactones. Ascorbic acid is included in this section because it is structurally similar to the tetronic acids, although it might equally well have been placed with the sugar acids.

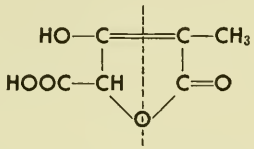
The tetronic acids appear to be condensation products of two simple molecules. Ehrensvärd and his collaborators have obtained experimental confirmation of this in two cases.[1] By labeled acetate studies on carlosic and carolic acids, they have shown the B portions of the molecules as indicated below to be



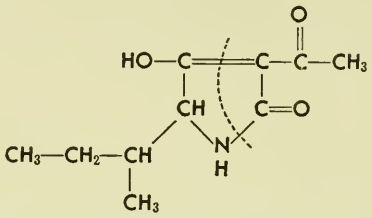

[1] Gösta Ehrensvärd, "Chemical Society Symposia," Special Publication No. 12, The Chemical Society, London, 1958, p. 14.

composed of three acetate units, while the A part is probably derived from another source related to carbohydrate biosynthesis. It would seem as if in the case of carlosic acid the A portion were derived from oxaloacetic acid, and in carolic acid from lactic or pyruvic acids.

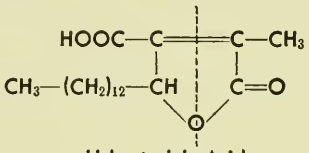
Inspecting other structures it appears (formally at least) that in zymonic acid, isolated by Stodola from many yeasts, the A portion could be from tartronate.



Zymonic Acid

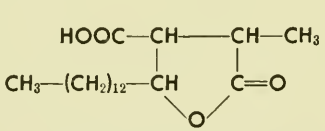


Tenuazonic Acid

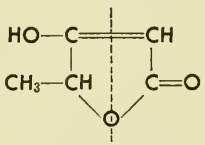


Lichesterinic Acid

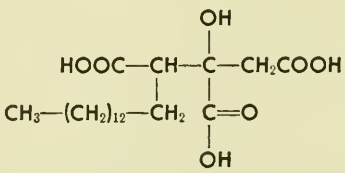
There are other possibilities in this case, however. Tenuazonic acid, a lactam similar to the tetronic acids, must surely be derived from isoleucine and acetoacetate.* Lichesterinic acid apparently is the result of a condensation between pyruvate and 3-oxypalmitate. Nephromopsic acid, which sometimes is found with lichesterinic acid, may be a reduction product.



Nephromopsic Acid



γ-Methyltetronic Acid



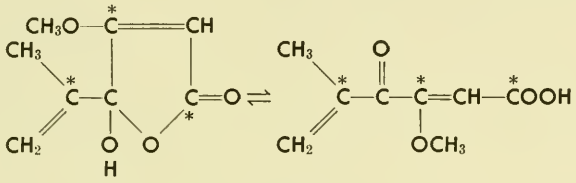
Caperatic Acid

It is interesting to note the co-occurrence of nephromopsic acid and caperatic acid, the former being (apparently) a condensa-

* See addendum.

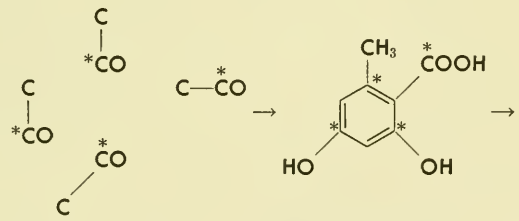
tion product of a $C_{15}$ fatty acid and pyruvate while the latter seems to be the condensation product of a $C_{16}$ fatty acid with oxaloacetate. Many other such apparent biosynthetic origins can be detected.

The biosynthesis of penicillic acid has been studied.[2] At first glance this would appear to be derived from acetate and dimethylpyruvate, β-methylglutaconate or a similar unit. It was found that 2-$C^{14}$-mevalonic acid lactone was not incorporated into the penicillic acid molecule when added to the growth medium of *Penicillium cyclopium* Westling. However, $CH_3C^{14}OOH$ was incorporated with equal labeling at the sites shown:

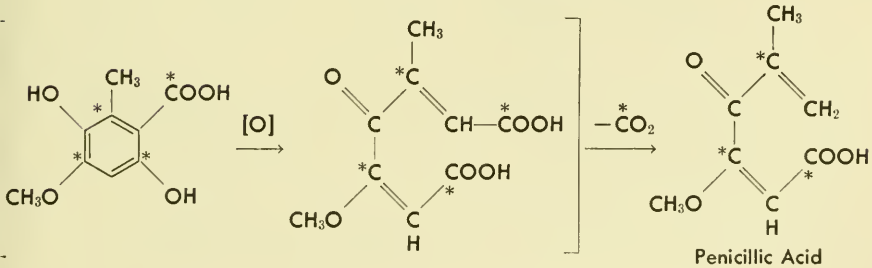


Penicillic Acid

With a relationship to the terpene biosynthetic route ruled out and a similarity to the valine biogenetic pathway also unlikely, the authors suggested a precursor of the orsellinic acid type, perhaps the 4-methyl ether:†



Orsellinic Acid



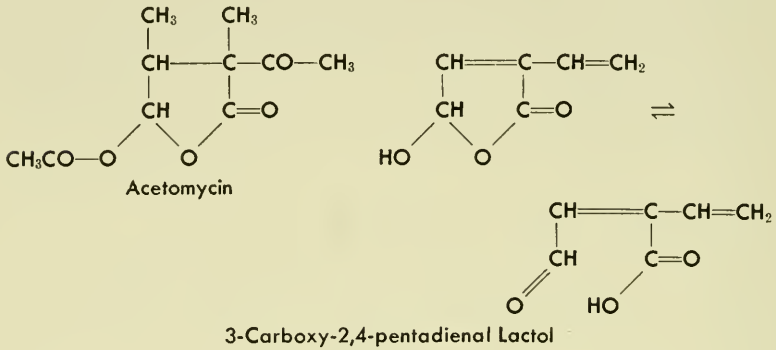
Penicillic Acid

[2] A. J. Birch, G. E. Blance and Herchel Smith, *J. Chem. Soc.*, 4582 (1958).

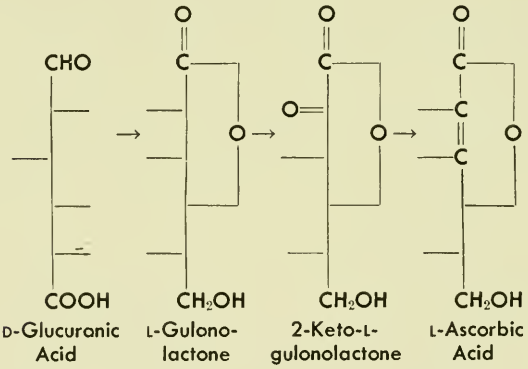
† See addendum.

A somewhat similar aromatic ring cleavage has been proposed[3] in the biosynthesis of patulin.

It is likely that the biosynthetic origins of the two recently reported streptomycete antibiotics, acetomycin and 3-carboxy-2,4-pentadienal lactol (PA-147) are mutually related.



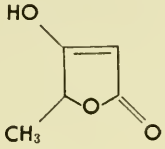

The biosynthesis of ascorbic acid in *Aspergillus niger* is known to involve the following stages:[4]




The glucuronic acid probably quite generally can arise from glucose by a hexose interconversion of the type discussed earlier in the section on sugars. In muscle tissue it may also come from myoinositol.

[3] J. D. Bu'Lock and A. J. Ryan, *Proc. Chem. Soc.*, 222 (1958).
[4] K. Sivarama Sastry and P. S. Sarma, *Nature* 179 44 (1957).

140 **γ-Methyltetronic Acid**, $C_5H_6O_3$, colorless crystals, m.p. 115°, $[\alpha]_{5461}$ −21° (c 0.526 in water).



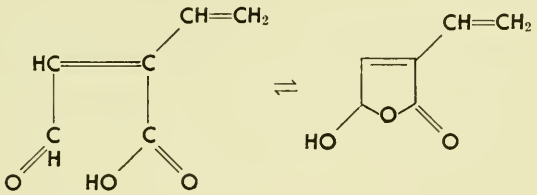
*Penicillium charlesii* G. Smith, *P. fellutanum*

The yield of this and the following tetronic acids from *P. charlesii* totaled 14% of the glucose consumed.

Percival Walter Clutterbuck, Harold Raistrick and Fritz Reutter, *Biochem. J.* 29 1300 (1935).

V. C. Vora, *J. Sci. Ind. Research* (India) 13B 504 (1954).

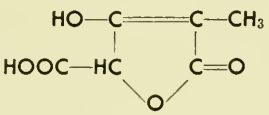
141 **3-Carboxy-2,4-pentadienal Lactol** (PA-147), $C_6H_6O_3$, viscous oil which polymerizes on standing at room temperature, $[\alpha]_D$ 0 ±2° (c 2 in $CHCl_3$).



*Streptomyces* sp.

Hans Els, B. A. Sobin and W. D. Celmer, *J. Am. Chem. Soc.* 80 878 (1958).

142 **Zymonic Acid**, $C_6H_6O_5$, isolated as the stable methyl ester, b.p. 118–123° (1 mm.), $n_D^{28}$ 1.4640.

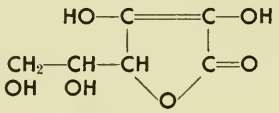


*Trichosporon capitatum, Hansenula subpelliculosa, Kloeckera brevis, Sporobolomyces salmonicolor, Cryptococcus laurentii, Debaryomyces hansenii, Nematospora coryli, Torula mellis*

Frank H. Stodola, Odette L. Shotwell and Lewis B. Lockwood, *J. Am. Chem. Soc.* 74 5415 (1952).

Frank H. Stodola, "Chemical Transformations of Microorganisms," Squibb Lectures on Chemistry of Microbial Products, John Wiley and Sons, New York, N. Y., 1958, pp. 97–102.

143 **Ascorbic Acid** (Vitamin C), $C_6H_8O_6$, colorless crystals, m.p. 190–192°, $[\alpha]_D^{23}$ +48° (c 1 in methanol).



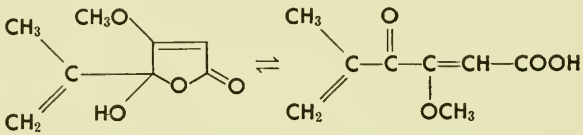
*Serratia marcescens* (on xylose), *Aspergillus niger* (Up to 140 mg. per liter yields have been reported from *A. niger.*)

M. Geiger-Huber and H. Galli, *Helv. Chim. Acta* 28 248 (1945).

Adelheid Galli, *Ber. schweiz. botan. Ges.* 56 113 (1946).

J. M. Van Lanen and F. W. Tanner, Jr., *Vitamins and Hormones* 6 163 (1948).

144 **Penicillic Acid**, $C_8H_{10}O_4$, colorless crystals, m.p. 87° (anhydrous), 64° (hydrate).



*Penicillium cyclopium* Westling, *P. puberulum* Bainier, *P. thomii, P. baarnense, Aspergillus ochraceus*

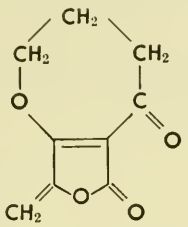
John H. Birkinshaw, Albert E. Oxford and Harold Raistrick, *Biochem. J.* 30 394 (1936). (Structure)

O. F. Black and C. L. Alsberg, *U. S. Dept. Agr., Bur. Plant Ind. Bull.* No. 199 (1910); Carl L. Alsberg and Otis F. Black, *Bur. Plant Ind. Bull.* No. 270 (1913). (Isolation)

R. A. Raphael, *J. Chem. Soc.*, 805 (1947). (Synthesis of dihydropenicillic acid)

E. O. Karow, H. B. Woodruff and J. W. Foster, *Arch. Biochem.* 5 279 (1944). (Isolations)

145 **Dehydrocarolic Acid,** $C_9H_8O_4$, colorless fine platelets, polymerizes above 80°.

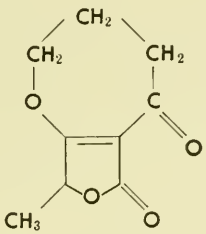


*Penicillium cinerascens* Biourge

Carlosic acid, spinulosin and gliotoxin also were produced.

A. Bracken and H. Raistrick, *Biochem. J.* 41 569 (1947).

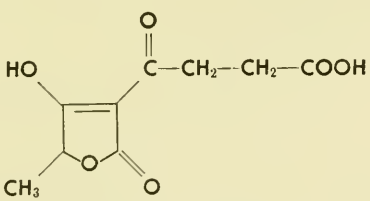
146 **Carolic Acid,** $C_9H_{10}O_4$, colorless needles, m.p. 132° $[\alpha]_{5461}$ +84° (c 0.50 in water).



*P. charlesii* G. Smith

Percival W. Clutterbuck, Walter N. Haworth, Harold Raistrick, Geo. Smith and Maurice Stacey, *Biochem. J.* 28 94 (1934).

147 **Carolinic Acid,** $C_9H_{10}O_6$, colorless prisms, m.p. 123° (dec.), $[\alpha]_{5461}$ +60° (c 0.34 in water).

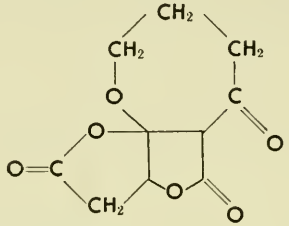


*Penicillium charlesii* G. Smith

L. J. Haynes, J. R. Plimmer, and (in part) A. H. Stanners, *J. Chem. Soc.*, 4661 (1956). (Synthesis)

Percival W. Clutterbuck, Walter N. Haworth, Harold Raistrick, Geo. Smith and Maurice Stacey, *Biochem. J.* 28 94 (1934).

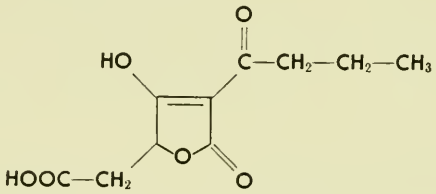
148 **Carlic Acid**, $C_{10}H_{10}O_6$, colorless needles, m.p. 176° (dec.) $[\alpha]_{5461}$ −160° (c 0.28 in water).



*P. charlesii* G. Smith

Percival W. Clutterbuck, Walter N. Haworth, Harold Raistrick, Geo. Smith and Maurice Stacey, *Biochem. J.* 28 94 (1934). (Isolation)

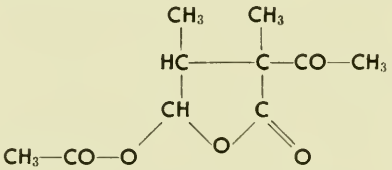
149 **Carlosic Acid**, $C_{10}H_{12}O_6$, colorless needles, m.p. 181°, $[\alpha]_{5461}$ −160° (c 0.21 in water).



*P. charlesii* G. Smith

Percival W. Clutterbuck, Walter N. Haworth, Harold Raistrick, Geo. Smith and Maurice Stacey, *Biochem. J.* 28 94 (1934). (Isolation)

150 **Acetomycin**, $C_{10}H_{14}O_5$, colorless rods, m.p. 115° (subl. 70°), $[\alpha]_D$ −167° (in ethanol).

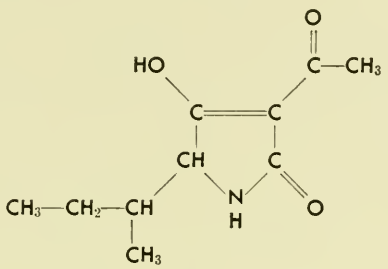


*Streptomyces ramulosus* n. sp.

The yield was about 1 g. per liter.

L. Ettlinger, E. Gäumann, R. Hütter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog and H. Zähner, *Helv. Chim. Acta* 41 216 (1958). (Isolation)

151 **Tenuazonic Acid**, $C_{10}H_{15}O_3N$, straw-colored gum, b.p. 117° (0.035 mm.), $[\alpha]_{5461}^{20}$ −136 ±5° (c 0.2 in chloroform).



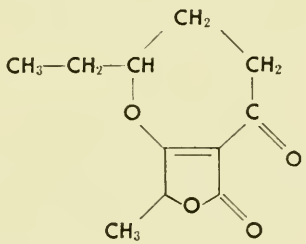
*Alternaria tenuis* Auct.

Tenuazonic acid is one of several compounds isolated from culture filtrates of this fungus. The other substances (structures still unknown) were: Altenuic acids I, II and III, altenusin, dehydroaltenusin and altertenuol. Alternariol and its methyl ether were isolated from the mycelium.

T. Rosett, R. H. Sankhala, C. E. Stickings, M. E. U. Taylor and R. Thomas, *Biochem. J.* 67 390 (1957). (Isolation)

C. E. Stickings, *ibid.* 72 332 (1959). (Structure)

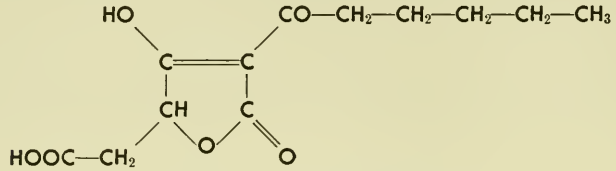
152 **Terrestric Acid**, $C_{11}H_{14}O_4$, colorless crystals, m.p. 89°, $[\alpha]_{5461}^{20}$ +61.1° (c 0.53 in water).



*Penicillium terrestre* Jensen

John Howard Birkinshaw and Harold Raistrick, *Biochem. J.* 30 2194 (1936).

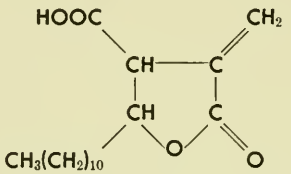
153 **Viridicatic Acid** (Ethylcarlosic Acid), $C_{12}H_{16}O_6$, colorless platelets, m.p. 174.5°, $[\alpha]_{5461}^{20}$ −105° (c 1.0 in ethanol).




*Penicillium viridicatum* Westling

J. H. Birkinshaw and M. S. Samant, *Biochem. J.* 74 369 (1960).

154 **Nephrosterinic Acid**, $C_{17}H_{28}O_4$, colorless leaflets, m.p. 96°, $[\alpha]_D^{10}$ +10.81°.

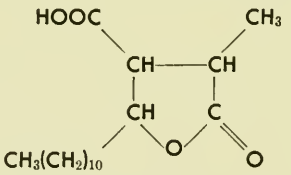



*Nephromopsis endocrocea* Asahina (=*Cetraria endocrocea* (Asahina) Sato)

Nephrosteranic acid, endocrocin and caperin were also present.

Yasuhiko Asahina, Masaiti Yanagita and Y. Sakurai, *Ber.* **70B** 227 (1937).

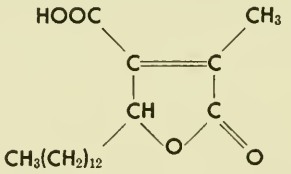
155 **Nephrosteranic Acid**, $C_{17}H_{30}O_4$, colorless plates, m.p. 95°.




*Nephromopsis endocrocea* Asahina

Yasuhiko Asahina, Masaiti Yanagita and Y. Sakurai, *Ber.* **70B** 227 (1937).

156 ***l*-Lichesterinic Acid**, $C_{19}H_{32}O_4$, colorless needles, m.p. 124°, $[\alpha]_D^{25}$ −32.66°.

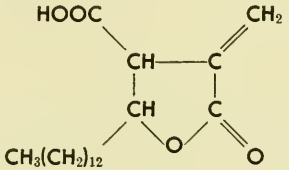


*Cetraria islandica* f. *tenuifolia*, *Nephromopsis stracheyi* f. *ectocarpisma* Hue.

Yasuhiko Asahina and Masaiti Yasue, *Ber.* **70B** 1053 (1937).

Yukio Kameda, *J. Pharm. Soc. Japan* **61** 266 (1941). (German abstract)

157 *d*-**Protolichesterinic Acid**, $C_{19}H_{32}O_4$, colorless leaflets, m.p. 107.5°, $[\alpha]_D^{19.5}$ +12.1°.




*Cetraria islandica* Ach., *Parmelia sinodensis* Asahina, *Cladonia papillaria* (Ehrh.) Hoffm.

Yasuhiko Asahina, *J. Japan. Botan.* **18** 489 (1942).

The *l*-isomer, m.p. 107.5°, $[\alpha]_D^{19.5}$ −12.7°, has been isolated from *Cetraria crispa* Nyl. (=*C. tenuifolia* Howe).

Y. Asahina and M. Asano, *J. Pharm. Soc. Japan* No. 539, 1 (1927).

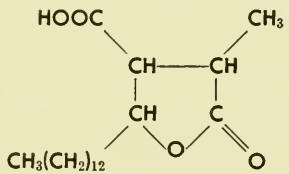
Eugene E. van Tamelen and Shirley Rosenberg Bach, *J. Am. Chem. Soc.* **80** 3079 (1958). (Synthesis)

158 *l-allo*-**Protolichesterinic Acid**, $C_{19}H_{32}O_4$, colorless plates, m.p. 107°, $[\alpha]_D^{18}$ −102°.

*Cetraria islandica* Ach. var. *orientalis* Asahina

Yasuhiko Asahina and Masaiti Yasue, *Ber.* **70B** 1053 (1937).

159 **Nephromopsic Acid**, $C_{19}H_{34}O_4$, colorless leaflets, m.p. 137°.




*Nephromopsis stracheyi* f. *ectocarpisma* Hue.

Occurs with usnic acid, *l*-lichesterinic acid, *l*-protolichesterinic acid and caperatic acid.

Michizo Asano and Tiaki Azumi, *Ber.* **68B** 995 (1935).

5

# Carotenes and Carotenoids

Carotene pigments are widely distributed throughout nature, and many microorganism pigments are carotenoid. Their isolation and characterization are often complicated by the co-occurrence of closely related compounds, and in some cases by poor stability. Many identifications have been made on the basis of ultraviolet absorption spectra alone.

For these reasons, and because of duplications in nomenclature, the literature dealing with microorganism carotenoids is confused. The situation has been reviewed by T. W. Goodwin,[1] and to augment the entries in this section some pertinent tables and references from this book have been incorporated as an appendix.

Carotenoids occur in both photosynthetic and non-photosynthetic microorganisms, and their functions are not established clearly. In fungi they may stimulate photokinetic responses such as phototropic bending. In sarcina and staphylococcus species there may be some protection of the cell from ultraviolet light. In photosynthetic genera it has been suggested that carotenoids may serve as blue-light energy absorbers, as oxygen carriers and in the prevention of chlorophyll-catalyzed photooxidations.

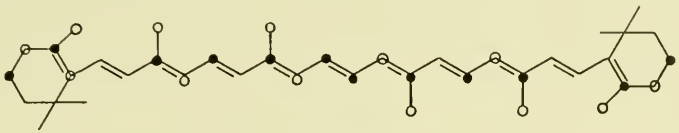
The work that has been done on carotene biogenesis in microorganisms has been well summarized.[2, 3] It has been found[4, 5]

[1] T. W. Goodwin, "Comparative Biochemistry of Carotenoids," Chemical Publishing Co., Inc., New York, N. Y., 1954.

[2] G. E. W. Wolstenholme and Maeve O'Connor, "CIBA Foundation Symposium on the Biosynthesis of Terpenes and Sterols," E. C. Grob, *The biosynthesis of carotenoids by microorganisms,* Little, Brown and Co., Boston, Mass., 1959, pp. 267–278.

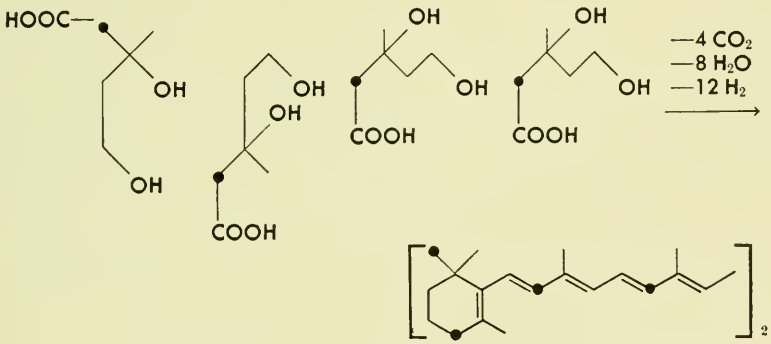
[3] T. W. Goodwin, *ibid.,* pp. 279–294.

that *Mucor hiemalis* uses acetate for the production of β-carotene. The product derived from $C^{14}$-labeled acetate has been partially degraded, and the following partial distribution pattern demonstrated:



β-carotene
○ Carbon atom from the methyl group of acetate
● Carbon atom from the carboxyl group of acetate

Mevalonic acid is an effective carotene precursor in at least certain microorganisms.[6, 7] In this connection it is noteworthy that in *Phycomyces blakesleeanus* and in *Mucor hiemalis* the production of sterols and carotenoids always runs proportionally.[8] The scheme shown below has been proposed for the mode of condensation.[2]

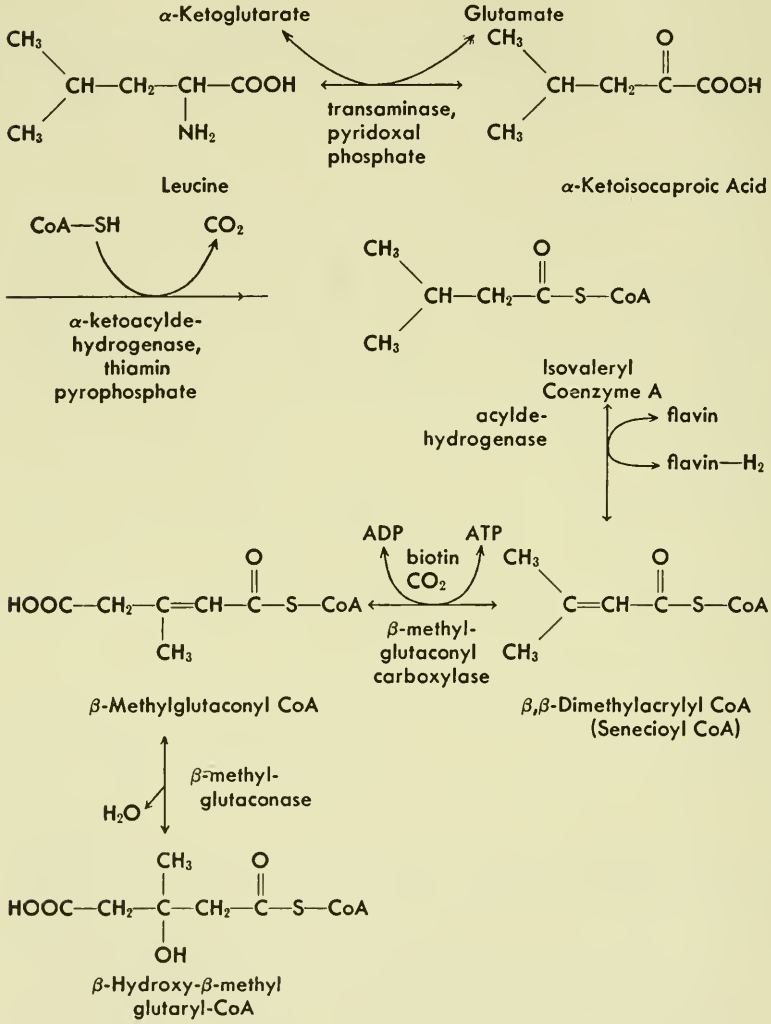



Leucine has been known for many years to have ketogenic and carotenogenic properties to a greater extent than other amino acids. The discovery of mevalonic acid facilitated an

[4] E. C. Grob and R. Bütler, *Helv. Chim. Acta* 39 1975 (1956).
[5] E. C. Grob, *Chimia* 10 73 (1956).
[6] G. D. Braithwaite and T. W. Goodwin, *Biochem. J.* 66 31p (1957).
[7] E. C. Grob, *Chimia* 11 338 (1957).
[8] E. C. Grob, M. Bein and W. H. Schopfer, *Bull. soc. chim. biol.* 33 1236 (1951).

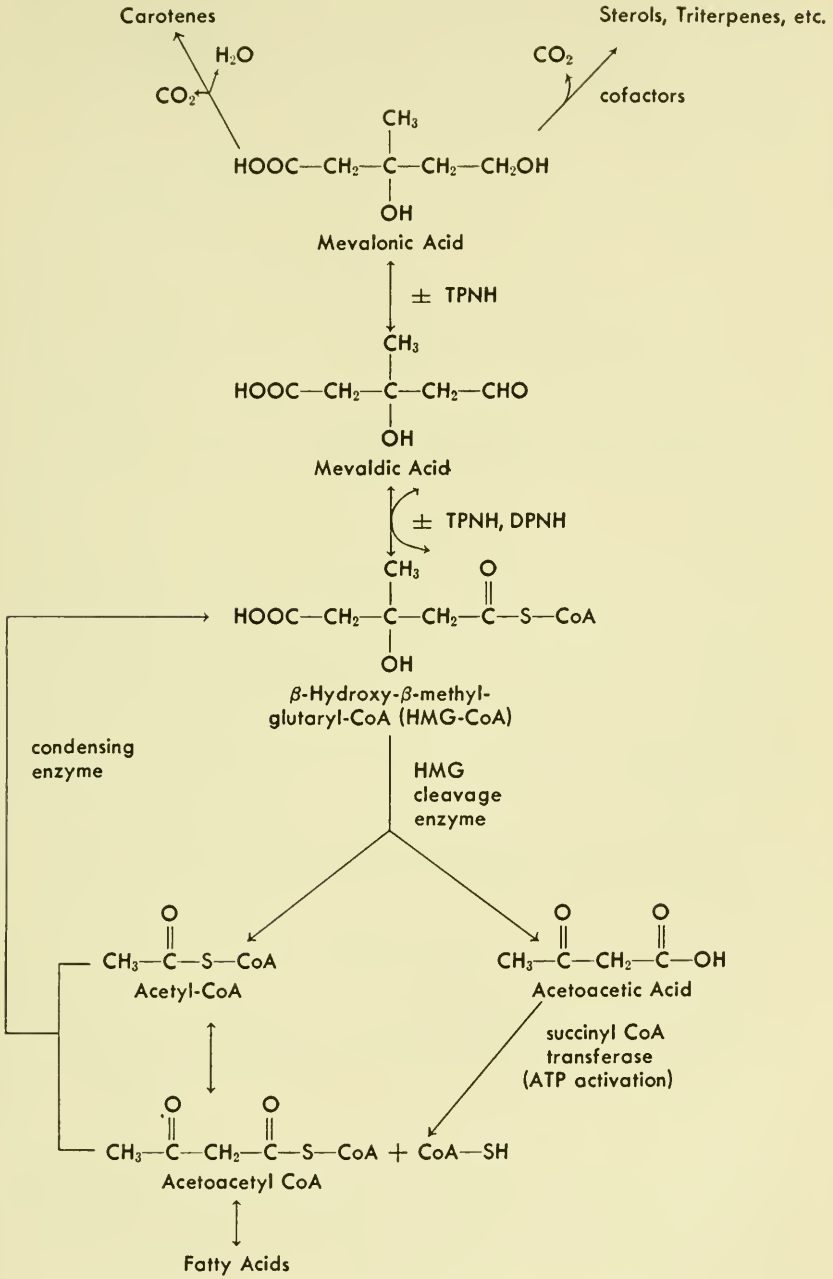
explanation of this effect, and this interesting work has been reviewed.[9, 10]

Some of the relationships thought to exist are:



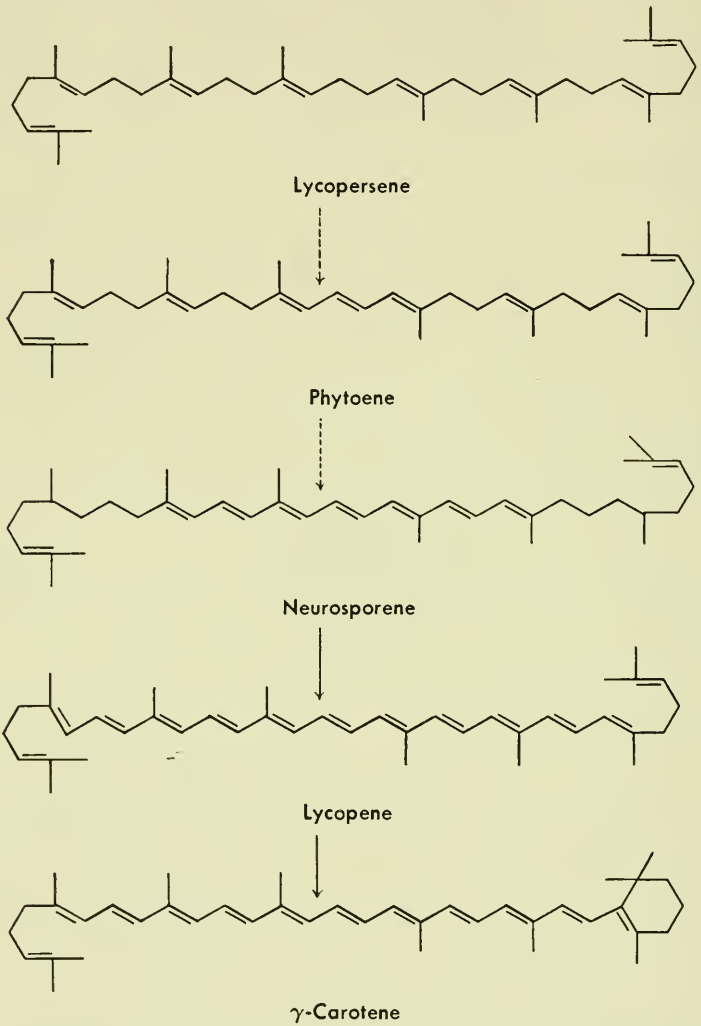

[9] G. E. W. Wolstenholme and Maeve O'Connor, "CIBA Foundation Symposium on the Biosynthesis of Terpenes and Sterols," M. J. Coon, F. P. Kupiecki, E. E. Dekker, M. J. Schlesinger and Alice del Campillo, *The enzymic synthesis of branched-chain acids,* Little, Brown and Co., Boston, Mass., 1959, pp. 62–74.

[10] *Idem., ibid.,* Harry Rudney, *The biosynthesis of β-hydroxy-β-methylglutaryl coenzyme A and its conversion to mevalonic acid,* pp. 75–94.




The precursors of the carotenes are colorless, more reduced compounds. These substances then are dehydrogenated in a stepwise fashion, a process which requires light and oxygen.

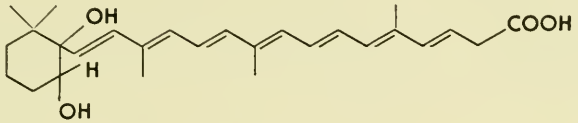
Oxygen-containing carotenoids appear at an early stage in the biosynthetic scheme. Based on the order of appearance in cultures of *Neurospora crassa*, Grob has proposed* the following partial pathway of carotenoid formation:




Lycopersene has not been isolated from a natural source, but this colorless polyene has been synthesized and seems to be a logical early member of this sequence.

* See addendum.

160 **Azafrin** (Escobedin), $C_{27}H_{38}O_4$, orange crystals, m.p. 213°, $[\alpha]_{6438}^{20}$ −75° (c 0.28 in alcohol), U.V. 428, 458 mμ in chloroform.

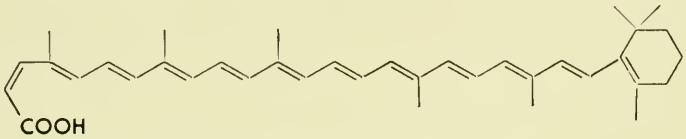



*Mycobacterium phlei*

Mary A. Ingraham and Harry Steenbock, *Biochem. J.* 29 2553 (1935).

Richard Kuhn, Alfred Winterstein and Hubert Roth, *Ber.* 64A 333 (1931).

161 **Torularhodin** (May = Lusomycin), $C_{37}H_{48}O_2$, red needles, m.p. 202° (vac.) (dec.), U.V. 554, 515, (483) mμ in chloroform.




*Rhodotorula rubra, R. sanniei*

The yield from *R. sanniei* was 2900 γ per gram of dry cells. Also obtained were torulene (143 γ per gram) and β-carotene (10 γ per gram) and traces of γ-carotene and lycopene.

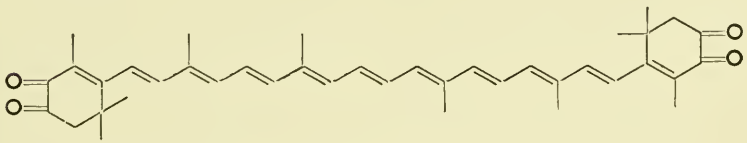
Edgar Lederer, *Bull. soc. chim. biol.* 20 611 (1938).

Claude Fromageot and Joué Léon Tchang, *Arch. Mikrobiol.* 9 424 (1938).

L. Nogueira Prista, *Congr. Luso-Espan. farm.* 2 274 (1952). (Chem. Abstr. 48 13807a)

R. Entschel and P. Karrer, *Helv. Chim. Acta* 42 466 (1959).

162 **Astacin** (3,4,3′,4′-Tetraoxo-β-carotene), $C_{40}H_{48}O_4$, violet, metalloid needles, m.p. 240–243°, U.V. 500 mμ in carbon disulfide.



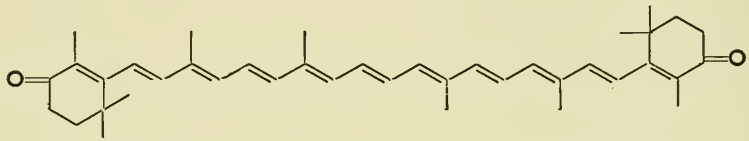

*Mycobacterium laticola*

H. F. Haas and L. D. Bushnell, *J. Bacteriol.* 48 219 (1944). (Isolation)

R. Kuhn, E. Lederer and A. Deutsch, *Hoppe-Seylers Z.* 220 229 (1933).

R. Kuhn and E. Lederer, *Ber.* 66 448 (1933).

163 **Canthaxanthin** (4,4′-Dioxo-β-carotene) $C_{40}H_{52}O_2$, red crystals, m.p. 218°, U.V. 480 mμ in benzene.



*Cantharellus cinnabarinus, Corynebacterium michiganense*

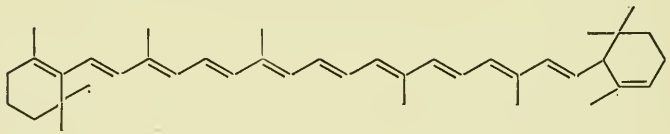
Francis Haxo, *Botan. Gaz.* 112 228 (1950). (Isolation)

S. Saperstein and M. P. Starr, *Biochem. J.* 57 273 (1954).

F. J. Petracek and L. Zechmeister, *Arch. Biochem. and Biophys.* 61 137 (1956). (Structure)

C. K. Warren and B. C. L. Weedon, *J. Chem. Soc.*, 3986 (1958). (Synthesis)

164 **α-Carotene**, $C_{40}H_{56}$, deep purple prisms, m.p. 187.5° (vac.), $[\alpha]_D^{18}$ +385° (c 0.08 in benzene), U.V. 446, 473 mμ in light petroleum ether.



*Dacromyces stillatus, Neurospora crassa* (mutants), *Mycobacterium phlei, Phycomyces blakesleeanus, Rhodotorula rubra, Gymnosporangium juniperi-virginianae, Puccinia coronifera, Aleuria aurantia, Cantharellus cibarius, Coleosporium senecionis, Penicillium sclerotiorum*

Edgar Lederer, *Bull. soc. chim. biol.* 20 611 (1938).

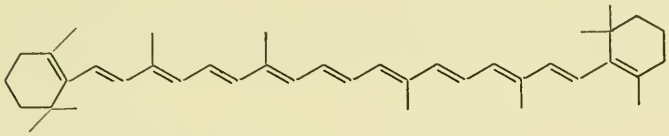
Harry Willstaedt, *Svensk. Kem. Tidskr.* 49 318 (1937).

B. L. Smits and W. J. Peterson, *Science* 96 210 (1942).

J. Bonner, A. Sandoval, W. Tang and L. Zechmeister, *Arch. Biochem.* 10 113 (1946).

T. W. Goodwin, *Biochem. J.* 53 538 (1953).

165 $\beta$-Carotene, $C_{40}H_{56}$ dark violet prisms from benzene-methanol, red leaflets from petroleum ether, m.p. 183° (vac.), U.V. 425, 450, 476 m$\mu$ in light petroleum ether.



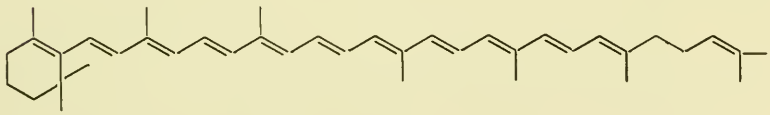
*Phycomyces blakesleeanus, Neurospora crassa, Rhodotorula rubra, R. sanniei, R. glutinis, Sporobolomyces roseus, S. salmonicolor, Cantharellus cibarius, C. cinnabarinus, Allomyces javanicus, Coleosporium senecionis, Mitrula paludosa, Penicillium sclerotiorum, Fremella mesenterica, Puccinia coronifera, Pilobolus bleinii, Gymnosporangium juniperi-virginianae, Dacromyces stillatus, Aleuria aurantia, Cryptococcus laurentii, C. luteolus, Monilia sitophila, Corynebacterium michiganense* (mutants), *Mycobacterium phlei, Sarcina aurantiaca*

For references see:

T. W. Goodwin, *Ann. Rev. Biochem.* 24 497 (1955).

*Idem.*, "Carotenoids," Chemical Publishing Co., Inc., New York, N. Y. 1954, p. 108 etc.

166 $\gamma$-Carotene, $C_{40}H_{56}$, fine deep red crystals with a blue luster from benzene-methanol, m.p. 177.5°, U.V. 493, 462, 437 m$\mu$ in petroleum ether.



*Allomyces arbuscula, A. javanicus, A. macrocygna, A. moniliformis, Puccinia coronifera, Phycomyces blakesleeanus, Neurospora crassa, Cantharellus cibarius, Coleosporum senecionis, Dacromyces stillatus, Gymnosporangium juniperi-virginianae, Cryptococcus laurentii, C. luteolus, Mycobacterium phlei, Chlorobium* spp. *Penicillium sclerotiorum*

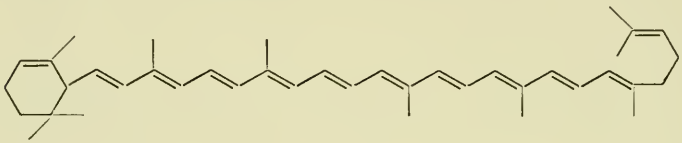
For references see:

T. W. Goodwin, *Ann. Rev. Biochem.* 24 497 (1955).

*Idem.*, "Carotenoids," Chemical Publishing Co., Inc., New York, N. Y. 1954, p. 108 etc.

J. Bonner, A. Sandoval, W. Tang and L. Zechmeister, *Arch. Biochem.* 10 113 (1946).

167 δ-Carotene, $C_{40}H_{56}$, fine orange to red needles, m.p. 140.5°, U.V. 488, 456, 430, 280 mμ in isooctane.
Proposed structure:



*Cantharellus cibarius, Neurospora crassa* (mutants), *Staphylococcus aureus*

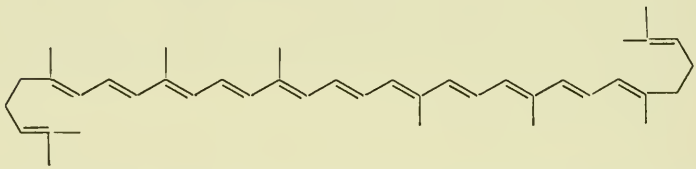
Harry Willstaedt, *Svensk Kem. Tidskr.* 49 318 (1937).

Ben Sobin and Grant L. Stahly, *J. Bacteriol.* 44 265 (1942).

J. W. Porter and M. M. Murphy, *Arch. Biochem. and Biophys.* 32 21 (1951). (Isolation)

Francis Haxo, *Biol. Bull.* 103 268 (1952).

168 Lycopene (Solanorubin, Rhodopurpurene) $C_{40}H_{56}$, brownish red to carmine crystals, m.p. 174°, U.V. 446, 474, 506 mμ in petroleum ether.



*Phycomyces blakesleeanus,* certain *Cantharellus* spp., *Neurospora crassa, Micrococcus tetragenus* (pink type), *Anthurus aserioformis, Allomyces javanicus, Rhodotorula glutinis, R. rubra, R. sanniei, Corynebacterium michiganense, C. diphtheriae, Mycobacterium phlei, Staphylococcus aureus, Coleosporium senecionis, Sarcina aurantiaca*

Harry Willstaedt, *Svensk. Kem. Tidskr.* 49 318 (1937).

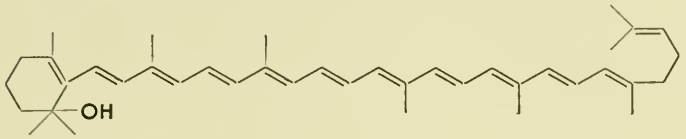
Francis Haxo, *Arch. Biochem.* 20 400 (1949).

P. Karrer, C. H. Eugster and E. Tobler, *Helv. Chim. Acta* 33 1349 (1950). (Synthesis)

T. W. Goodwin, *Ann. Rev. Biochem.* 24 497 (1955).

Synnove Liaaen Jensen, Germaine Cohen-Bazire, T. O. M. Nakayama and R. Y. Stanier, *Biochim. et Biophys. Acta* 29 477 (1958).

169 **Rhodopin,** $C_{40}H_{56}O$, violet-red needles, m.p. 168° (171°), U.V. 440, 470, 501 m$\mu$ in light petroleum.



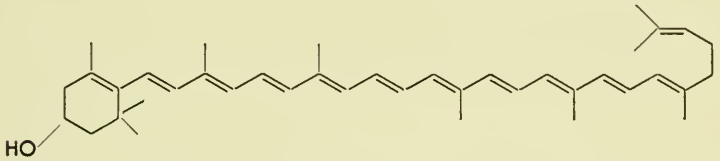

*Polystigma rubrum*

Edgar Lederer, *Bull. soc. chim. biol.* 20 611 (1938).

Synnöve Liaaen Jensen, *Acta Chem. Scand.* 13 842 (1959). (Structure)

Paul Karrer and Ulrich Solmssen, *Helv. Chim. Acta* 18 25, 1306 (1935); 21 454 (1938).

170 **Rubixanthin** (3-Hydroxy-$\gamma$-carotene), $C_{40}H_{56}O$, coppery red needles, m.p. 160°, U.V. 432, 462, 494 m$\mu$ in hexane.




*Staphylococcus aureus, Coleosporium senecionis, Micrococcus tetragenus*

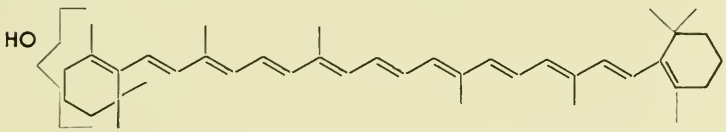
E. Lederer, *Bull. soc. chim. biol.* 20 611 (1938).

Ben Sobin and Grant L. Stahly, *J. Bacteriol.* 44 265 (1942).

H. A. Reimann and C. M. Eklund, *J. Bact.* 42 605 (1941).

Richard Kuhn and Christoph Grundmann, *Ber.* 67 339 (1934).

171 **Cryptoxanthin** (Cryptoxanthol, 3- or 4-Oxy-$\beta$-carotene, $C_{40}H_{56}O$) deep red prisms, m.p. 169° (vac.), optically inactive, U.V. 425s, 450, 480 m$\mu$ in hexane.




*Mycobacterium phlei, Dacromyces stillatus, Vibrio adaptatus, Pseudomonas xanthochrus, P. aestumarina, Rocella montagnei*

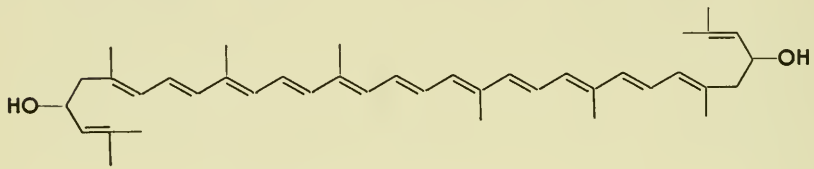
Richard Kuhn and Christoph Grundmann, *Ber.* 66 174 (1933).

Mary A. Ingraham and Harry Steenbock, *Biochem. J.* 29 2553 (1935).

F. P. Zscheile, J. W. White, B. W. Beadle and J. R. Roach, *Plant Physiol.* 17 331 (1942).

T. R. Seshadry and S. S. Subramanian, *Proc. Indian Acad. Sci.* 30A (1949).

172 **Lycophyll** (3,3′-Dihydroxylycopene), $C_{40}H_{56}O_2$, purple crystals, m.p. 179°, U.V. 444, 473, 504 mμ in petroleum ether.

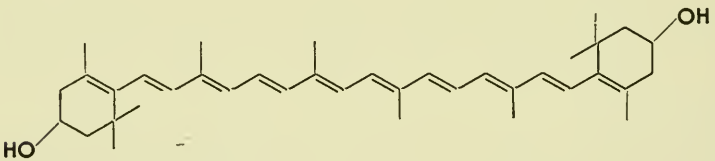



*Rhodospirillum rubrum, Chromatium* spp.

M. S. Barber, L. M. Jackson and B. C. L. Weedon, *Proc. Chem. Soc.*, 96 (1959). (Structure)

L. Zechmeister and L. V. Cholnoky, *Ber.* 69B 422 (1936).

173 **Zeaxanthin** (Zeaxanthol), $C_{40}H_{56}O_2$, yellow crystals, m.p. 207° (215°), optically inactive, U.V. 451, 476 mμ in petroleum ether.




*Mycobacterium phlei, Dacromyces stillatus, Staphylococcus aureus, Pseudomonas xanthochrus, P. aestumarina, Vibrio adaptatus*

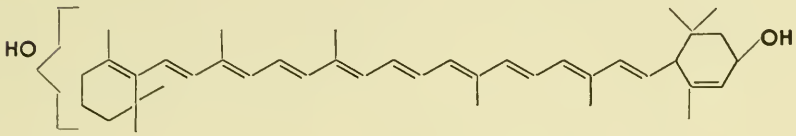
Erwin Chargaff and Joseph Dieryck, *Naturwissenschaften* 20 872 (1932).

Mary A. Ingraham and Harry Steenbock, *Biochem. J.* 29 2553 (1935).

Walter Steuer, *Zentr. Bakteriol. Parasitenk.* 167 210 (1956).

T. W. Goodwin, *Biochem. J.* 53 538 (1953).

174 **Lutein** (Xanthophyll, Luteol), $C_{40}H_{56}O_2$, yellow prisms, m.p. 190°, $[\alpha]_{Cd}^{18}$ +165° (c 0.7 in benzene), U.V. 420, 446.5, 476 m$\mu$ in petroleum ether.




*Mycobacterium phlei, Staphylococcus aureus, Sarcina lutea, Micrococcus lysodeikticus*

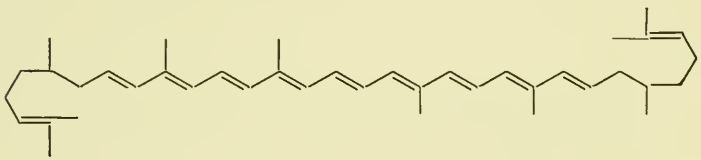
Erwin Chargaff, *Compt. rend.* 197 946 (1933).

Mary A. Ingraham and Harry Steenbock, *Biochem. J.* 29 2553 (1935).

Tatsuo Ohta, *J. Pharm. Soc. Japan* 71 1319 (1951). (Isolation)

A. R. Gilby and A. V. Few, *Nature* 182 55 (1958).

175 **Neurosporene** (6,7,6′,7′-Tetrahydrolycopene), $C_{40}H_{60}$, yellow-orange or yellow-brown crystals, m.p. 124°, U.V. 414, 438.5, 469 m$\mu$ in petroleum ether.



*Neurospora crassa, Rhodotorula rubra,* etc.

Neurosporene and hydroxylated neurosporenes are probable intermediates in the biogenesis of other carotenoids occurring in microorganisms.

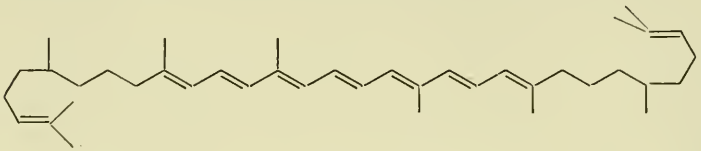
J. Bonner, A. Sandoval, W. Tang and L. Zechmeister, *Arch. Biochem.* 10 113 (1946).

Francis Haxo, *ibid.* 20 400 (1949).

L. Zechmeister and B. Kenneth Koe, *J. Am. Chem. Soc.* 76 2923 (1954).

Synnöve Liaaen Jensen, Germaine Cohen-Bazire, T. O. M. Nakayama and R. Y. Stanier, *Biochim. et Biophys. Acta* 29 477 (1958).

176 $\eta$-Carotene, $C_{40}H_{64}$, probably has not been entirely purified, U.V. 376 (380), 396 (404), 418 (424), 450.



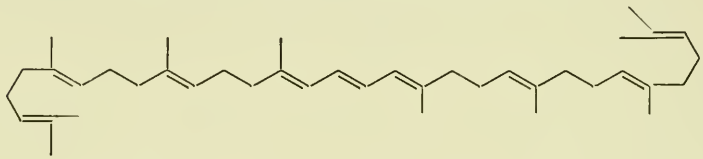
*Phycomyces blakesleeanus, Neurospora crassa* (mutants), *Dacromyces stillatus*

H. A. Nash and F. P. Zscheile, *Arch. Biochem.* 7 305 (1945).

T. W. Goodwin, "Carotenoids," Chemical Publishing Co., Inc., New York, N. Y. 1954, p. 108, etc.

G. MacKinney, C. O. Chichester and Patricia S. Wong, *Arch. Biochem. and Biophys.* 53 480 (1954).

177 **Phytoene** (7,8,11,12,12′,11′,8′,7′-Octahydrolycopene), $C_{40}H_{64}$, colorless, viscous oil with a strong fluorescence in ultraviolet light, U.V. 275s, 283, 295s in isooctane.



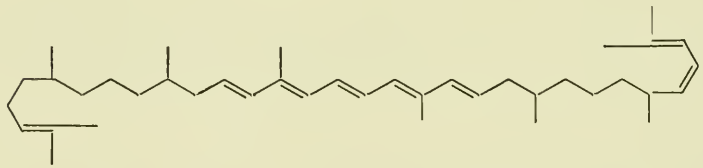
*Mycobacterium phlei, Rhodopseudomonas spheroides* (mutant), *Rhodospirillum rubrum*

J. W. Porter and F. P. Zscheile, *Arch. Biochem. and Biophys.* 10 537 (1946).

W. J. Rabourn and F. W. Quackenbush, *Arch. Biochem. and Biophys.* 61 111 (1956). (Structure)

T. W. Goodwin, and Malini Jamikorn, *Biochem. J.* 62 269 (1956).

178 **Phytofluene** (5,6,7,8,9,10,10′,9′,8′,7′,6′,5′-Dodecahydrolycopene), $C_{40}H_{68}$, colorless, viscous oil with a strong fluorescence in ultraviolet light, U.V. 332, 347, 367 m$\mu$ in petroleum ether.



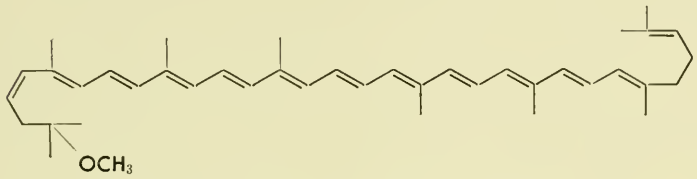
*Neurospora crassa, N. sitophila, Mycobacterium phlei, Phycomyces blakesleeanus,* etc.

Phytofluene probably occurs widely among microorganisms. It is a probable precursor of many of the carotene pigments.

L. Zechmeister and F. Haxo, *Arch. Biochem.* 11 539 (1946). (Isolation from neurospora)

L. Zechmeister, *Experientia* 10 1 (1954). (Structure)

179 **P-481,$C_{41}H_{58}O$,** U.V. 455, 482, 514 m$\mu$ in petroleum ether.
Tentative structure:

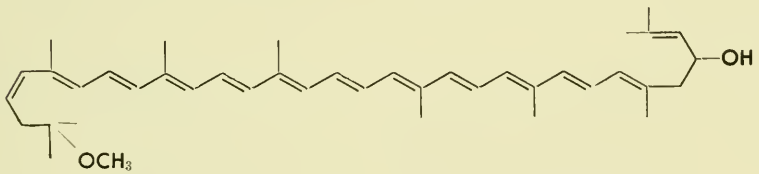



*Rhodospirillum rubrum, Chromatium* spp.

M. S. Barber, L. M. Jackson and B. C. L. Weedon, *Proc. Chem. Soc.*, 96 (1959). (Structure)

Synnöve Liaaen Jensen, *Acta Chem. Scand.* 12 1698 (1958).

180 **Hydroxy-P-481** (May = Rhodovibrin), $C_{41}H_{58}O_2$, U.V. 455, 482, 515 m$\mu$ in petroleum ether.
Tentative structure:

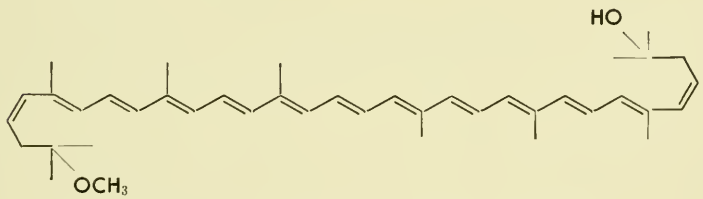



*Rhodospirillum rubrum, Chromatium* spp.

M. S. Barber, L. M. Jackson and B. C. L. Weedon, *Proc. Chem. Soc.*, 96 (1959).

Synnöve Liaaen Jensen, *Acta Chem. Scand.* 12 1698 (1958).

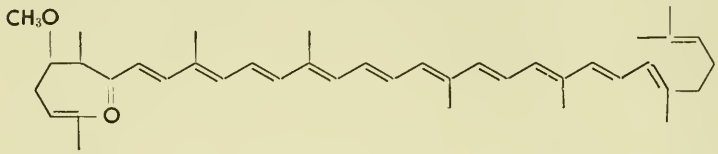
181 **Hydroxyspirilloxanthin** (May = Bacteriopurpurin, Bacterioerythrin) $C_{41}H_{58}O_2$, U.V. 489, 523 m$\mu$ in petroleum ether.
Tentative structure:




*Rhodospirillum rubrum, Chromatium* spp.

M. S. Barber, L. M. Jackson and B. C. L. Weedon, *Proc. Chem. Soc.*, 96 (1959).

182 **Pigment R** (Spheroidenone), $C_{41}H_{58}O_2$, red crystals, m.p. 155.5–158°, U.V. 460 (455), 482 (487), 513 (516.5) mμ in light petroleum.

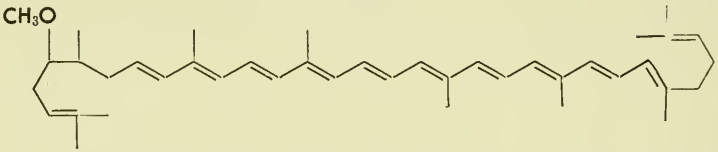



*Rhodopseudomonas spheroides*, other purple bacteria

C. B. Van Niel, *Antonie Van Leeuwenhoek J. Microbiol. Serol. Jubilee Vol. Albert J. Kluyver* 12 156 (1947). (Isolation)

T. W. Goodwin, D. G. Land and M. E. Sissins, *Biochem. J.* 64 486 (1956). (Structure)

183 **Pigment Y,** $C_{41}H_{60}O$, yellow unstable crystals, m.p. 116–135° (dec.). Stable in solution. U.V. 426.5, 452 (454), 484 (486) mμ in petroleum ether.




*Rhodopseudomonas spheroides*, other purple bacteria

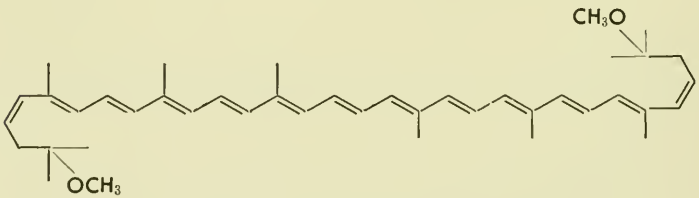
A hydroxylated pigment Y was produced in the same fermentation, but could not be crystallized.

C. B. Van Niel, *Antonie Van Leeuwenhoek J. Microbiol. Serol. Jubilee Vol. Albert J. Kluyver* 12 156 (1947). (Isolation)

T. W. Goodwin, D. G. Land and M. E. Sissins, *Biochem. J.* 64 486 (1956). (Structure)

Synnöve Liaaen Jensen, *Acta Chem. Scand.* 12 1698 (1958).

184 **Spirilloxanthin** (Rhodoviolascin), $C_{42}H_{60}O_2$, violet spindle-form crystals, m.p. 218°, U.V. 464, 491, 524 mμ in petroleum ether.



*Rhodospirillum rubrum*, other purple bacteria, *Neurospora crassa* (mutants), *Chromatium* spp.

P. Karrer and U. Solmssen, *Helv. Chim. Acta* 18 1306 (1935).

C. B. Van Niel and James H. C. Smith, *Arch. Mikrobiol.* 6 219 (1935). (Isolation)

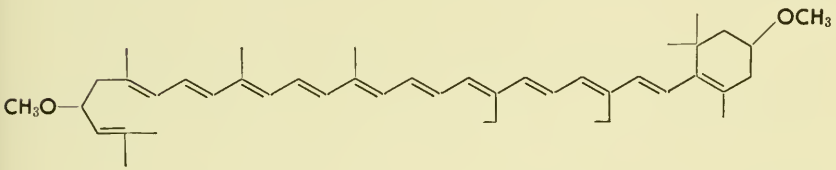
A. Polgar, C. B. Van Niel and L. Zechmeister, *Arch. Biochem.* 5 243 (1944).

Synnöve Liaaen Jensen, Germaine Cohen-Bazire, T. O. M. Nakayama and R. Y. Stanier, *Biochim. et Biophys. Acta* 29 477 (1958). (Synthesis)

M. S. Barber, L. M. Jackson and B. C. L. Weedon, *Proc. Chem. Soc.*, 96 (1959).

185 **Torulene**, $C_{42}H_{60}O_2$, dark red crystals, m.p. 185°, U.V. 460, 486, 519 m$\mu$ in petroleum ether.

Tentative structure:




*Rhodotorula rubra*

Occurs together with $\beta$-carotene, torularhodin and an unstable, uncharacterized carotene.

Edgar Lederer, *Bull. soc. chim. biol.* 20 611 (1938).

J. Bonner, A. Sandoval, W. Tang and L. Zechmeister, *Arch. Biochem.* 10 113 (1946).

186 **Sarcinaxanthin**, yellow crystals, m.p. 149°, U.V. 415, 440, 469 m$\mu$ in petroleum ether.

About 3.4 mg. of this mono-hydroxy xanthophyll were obtained from 385 g. of dried *Sarcina lutea* cells. It is also produced by *Flavobacterium marinotypicum* and by *Staphylococcus citreus*.

A closely related hydrocarbon, sarcinene, occurs in all these species as well as in *Flavobacterium sulfureum*.

Yoshiharu Takeda and Tatuo Ota, *Z. physiol. Chem.* 268 1 (1941). (Isolation)

Doris P. Courington and T. W. Goodwin, *J. Bacteriol.* 70 568 (1955).

Tatsuo Ohta, Toshio Miyazaki and Teruo Minomiya, *Chem. Pharm. Bull.* 7 254 (1959).

187 **Neurosporaxanthin,** dark grayish purple leaflets, m.p. 192° (vac.), U.V. 472 $m\mu$ in hexane (486 $m\mu$ in benzene).

An uncharacterized carotenoid which gives yellow solutions and a red color adsorbed on sucrose.

*Neurospora crassa*

Marko Zalokar, *Arch. Biochem. and Biophys.* **70** 568 (1957). (Isolation)

188 **Leprotene** (Leprotin), coppery red needles, m.p. 197°, U.V. 429, 452, 479 $m\mu$ in petroleum ether.

The principal carotene of *Mycobacterium phlei* and other mycobacteria. It contains no ionone rings and does not function as a provitamin A.

Yoshiharu Takeda and Tatsuo Ohta, *J. Biochem.* Japan **36** 535 (1944). (Isolation)

Tatsuo Ohta, *J. Pharm. Soc. Japan* **71** 462 (1951).

189 **Mycoxanthin,** U.V. 385, 406, 430 $m\mu$ in petroleum ether.

A new yellow carotenoid with a relatively short chromophore.

*Mycobacterium phlei, M. marianum, M. battaglini*

Aldo Gaudiano, *Atti. accad. nazl. Lincei, Rend., Classe sci. fis., mat. e nat.* **21** 308 (1956). (*Chem. Abstr.* **51** 8876 f) (Isolation)

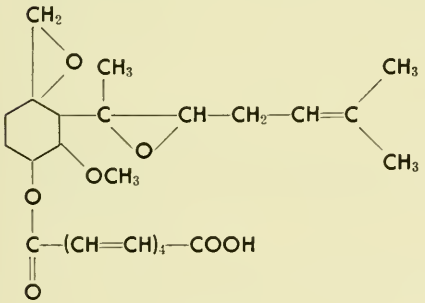
# 6

# Polyenes and Polyynes, Excluding Polyene Macrolides

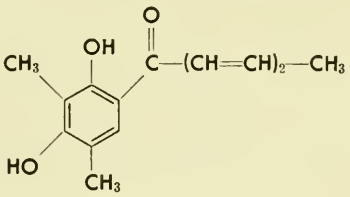
The polyenes of this section somewhat resemble crocetin, bixin and the carotenes in their long systems of conjugated double bonds with the resultant color and other physical properties, but they lack the isoprenoid structure.

The acetylenic compounds often occur in low yields and in complex mixtures. While generally colorless, they are conspicuous by their strong and characteristic ultraviolet absorption spectra. Many of them are unstable.

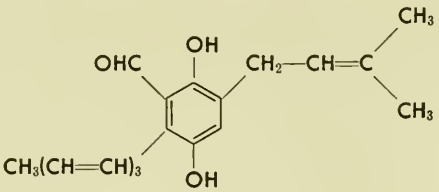
From the examples reported to date it seems that basidiomycetes are the principal producers of such metabolites among microorganisms, although such substances occur widely in higher plants. That lower fungi are capable of forming polyenes is demonstrated, however, by the side-chains of metabolites classified elsewhere, for example fumagillin, sorbicillin and auroglaucin:




Fumagillin

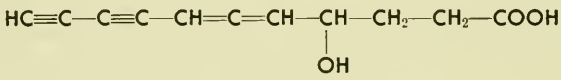



Sorbicillin

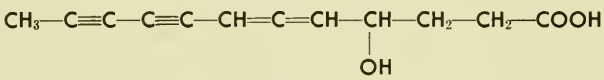



Auroglaucin

It is likely that both polyenes and polyynes are acetate-derived. It has been demonstrated[1] that nemotinic acid with 11 carbon atoms is formed from 6 moles of an acetic acid derivative, with head to tail linkage and elimination of the terminal methyl group.



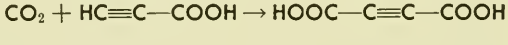

Nemotinic Acid




Odyssic Acid

Odyssic acid was presumed to be formed similarly, but with terminal methyl group retention.

In the examples available the acetylenic acids with an odd number of carbon atoms terminate in an acetylenic bond. This seems to indicate elimination of the terminal methyl group by oxidation and decarboxylation. It is interesting to note that the reverse process has been demonstrated in the conversion of propynoic acid to acetylenedicarboxylic acid by a soil isolate.[2]

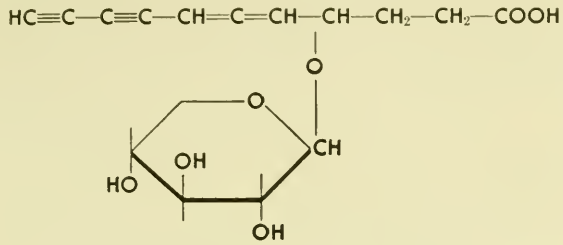



The xyloside of nemotinic acid also has been isolated.[3] When isolated from a culture grown on glucose with 1-$C^{14}$-labeled acetic acid added to the medium, labeling is found in the polyacetylenes but not in the xylose moiety. When isolated from

[1] J. D. Bu'Lock and H. Gregory, *Biochem. J.* 72 322 (1959).

[2] Akira Hanaoka, Tokuya Harada and Takeo Takizawa, *J. Agr. Chem. Soc. Japan* 26 151 (1952).

[3] J. D. Bu'Lock and H. Gregory, *Experientia* 15 420 (1959).



a culture grown on ethanol with 1-$C^{14}$-labeled acetic acid added to the medium, labeling was found in the xylose as well as in the acetylenic acid. It was assumed that in the latter case, where the molecule was synthesized entirely from $C_2$ units, the xylose was produced by way of intermediates closely related to glucose. Glucose itself acted as the xylose precursor, then, in the first experiment. A closer analysis of the labeling pattern of the xylose moiety led to the suggestion that the pentose was formed from glucose by way of glucuronic acid followed by decarboxylation.

Many of the acetylenic acids have antibiotic properties.

A review of polyacetylenes was published recently.[4]

About a dozen more compounds of this type are listed in the addendum.

190 **Agrocybin,** $C_8H_5O_2N$, unstable compound white crystals, darkening in air, m.p. 130–140° (dec. explosively), U.V. 216, 224, 269, 286, 304, 325 m$\mu$ in 95% ethanol.

$$HOCH_2—C{\equiv}C—C{\equiv}C—C{\equiv}C—CONH_2$$

*Agrocybe dura*

Marjorie Anchel, *J. Am. Chem. Soc.* 74 1588 (1952).

J. D. Bu'Lock, E. R. H. Jones, G. H. Mansfield, J. W. Thompson and M. C. Whiting, *Chem. and Ind.*, 990 (1954). (Structure)

P. J. Ashworth, E. R. H. Jones, G. H. Mansfield, K. Schlögl, J. M. Thompson, M. C. Whiting, *J. Chem. Soc.*, 950 (1958). (Synthesis)

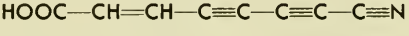
191 **Diatretyne 1,** $C_8H_5O_3N$, unstable crystals, m.p. 198° (dec. explosively), U.V. 223, 260, 275, 290, 309 m$\mu$ in 95% ethanol.

$$HOOC—CH{=}CH—C{\equiv}C—C{\equiv}C—CONH_2$$

and

[4] E. R. H. Jones, *Proc. Chem. Soc.*, 199–211 (1960).

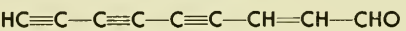
192 **Diatretyne 2** (Nudic Acid B), $C_8H_3O_2N$, short colorless needles, m.p. 179° (dec.), U.V. 228, 238, 268, 283, 299, 322 mμ in 95% ethanol.



*Clitocybe diatreta*

Marjorie Anchel, J. *Am. Chem. Soc.* 74 1588 (1952).
*Idem., ibid.* 75 4621 (1953).
*Idem. Science* 121 607 (1955). (Structure)
P. J. Ashworth, E. R. H. Jones, G. H. Mansfield, K. Schlögl, J. M. Thompson and M. C. Whiting, *J. Chem. Soc.*, 950 (1958). (Synthesis)

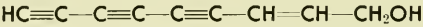
193 *trans*-**Non-2-ene-4,6,8-triyn-1-al,** $C_9H_4O$, colorless needles, which rapidly decompose in light at room temperature. U.V. 210.5 (220), 228, 240, 257, 271, 287, 306, 327 mμ in ethanol.



*Coprinus quadrifidus*

Six related compounds occurred in the same culture.
E. R. H. Jones and J. S. Stephenson, *J. Chem. Soc.*, 2197 (1959).

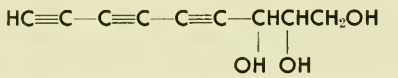
194 *trans*-**Non-2-ene-4,6,8-triyn-1-ol,** $C_9H_6O$, colorless crystals, decomposing at ordinary conditions, U.V. 233, 243, 255, 283, 300, 320 mμ in hexane.



*Coprinus quadrifidus*

E. R. H. Jones and J. S. Stephenson, *J. Chem. Soc.*, 2197 (1959).

195 **(2d,3d)-Nona-4,6,8-triyn-1,2,3-triol,** $C_9H_8O_3$, colorless crystals (dec.) ~40°, $[\alpha]_D$ +6° (c 0.82 in ethanol), U.V. 208, 254, 269.5, 286.5, 305 mμ in ethanol.



*Coprinus quadrifidus*

E. R. H. Jones and J. S. Stephenson, *J. Chem. Soc.*, 2197 (1959).

196 **Biformin**, highly unstable crystals.

Probably a straight-chain, nine carbon atom glycol, containing two acetylenic and two ethylenic bonds in conjugation.

*Polyporus biformis*

A similar substance, biforminic acid, occurred in the same culture.

William J. Robbins, Frederick Kavanagh and Annette Hervey, *Proc. Nat. Acad. Sci.* 33 176 (1947).

Marjorie Anchel and Marvin P. Cohen, *J. Biol. Chem.* 208 319 (1954).

197 *trans*-**Dec-2-ene-4,6,8-triyn-1-al**, $C_{10}H_6O$, pale yellow needles, m.p. 108°, U.V. (225) (234.5), 245.5, 258 (272), 288, 306, 326, 350, m$\mu$ in hexane.

$$CH_3—C{\equiv}C—C{\equiv}C—C{\equiv}C—CH{=}CH—CHO$$

*Pleurotus ulmarius*

J. N. Gardner, E. R. H. Jones, P. R. Leeming and J. S. Stephenson, *J. Chem. Soc.*, 691 (1960).

198 **Diatretyne-3** (*trans*-10-Hydroxydec-2-ene-4,6,8-triynoic Acid), $C_{10}H_6O_3$, nearly colorless rods from ethyl acetate, rapidly becoming coated with blue-green polymer, U.V. 253, 280, 297, 316, 339 m$\mu$.

$$HOCH_2—C{\equiv}C—C{\equiv}C—C{\equiv}C—CH{=}CH—COOH$$

*Clitocybe diatreta*

The author noted the similarity to the antibiotic principle of the royal jelly of bees:

$$HOCH_2CH_2CH_2CH_2CH_2CH_2CH_2—\overset{trans}{CH{=}CH}—COOH$$

Helen Flon and Marjorie Anchel, *Arch. Biochem. and Biophys.* 78 111 (1958).

Marjorie Anchel, *Arch. Biochem. and Biophys.* 85 569 (1959).

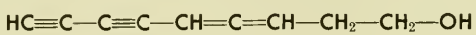
199 **Deca-*trans*-2,*trans*-8-diene-4,6-diyne-1,10-dioic Acid**, $C_{10}H_6O_4$, amorphous powder, m.p. (dec.) ~200°, U.V. 216 (258), 267, 296, 315, 338 m$\mu$ in ethanol.

$$HOOC—CH{=}CH—C{\equiv}C—C{\equiv}C—CH{=}CH—COOH$$

*Polyporus anthracophilus*

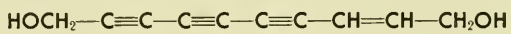
J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

200 **Marasin** [(-)-Nona-3,4-diene-6,8-diyne-1-ol], $C_{10}H_8O$, unstable oily substance, polymerized spontaneously, $[\alpha]_D^{25}$ about −325° (c 0.2).



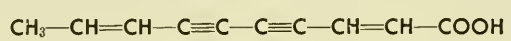
*Marasmius ramealis*
Gerd Benz, *Arkiv for Kemi* 14 305 (1959).

201 ***trans*-Dec-2-ene-4,6,8-triyn-1,10-diol**, $C_{10}H_8O_2$, colorless needles, (dec.) 138°, U.V. 205, 212, 231, 243.5, 259, 279, 290.5, 309.5, 330.5 mμ in ethanol.



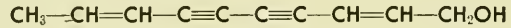
*Coprinus quadrifidus*
E. R. H. Jones and J. S. Stephenson, *J. Chem. Soc.*, 2197 (1959).

202 ***trans,trans*-Matricaria Acid**, $C_{10}H_8O_2$, colorless plates, m.p. 175° (dec.), U.V. 245, 256, 310, 329 mμ in ethanol.



*Polyporus anthracophilus*
J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

203 ***trans,trans*-Matricarianol**, $C_{10}H_{10}O$, colorless needles, m.p. 105.5°, U.V. 217.5, 231.5, 237, 247, 261, 276, 293, 312 mμ in ethanol.



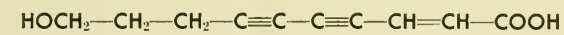
*Polyporus anthracophilus*
J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

204 **Deca-*cis*-2-*trans*-8-diene-4,6-diyn-1-ol**, $C_{10}H_{10}O$, m.p. <20°, U.V. 213.5, 230, 237.5, 246.5, 261.5, 276.5, 293.5, 312.5 mμ.



*Polyporus guttalatus*
J. N. Gardner, E. R. H. Jones, P. R. Leeming and J. S. Stephenson, *J. Chem. Soc.*, 691 (1960).

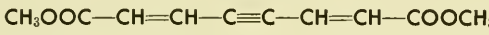
205 **10-Hydroxydec-*trans*-2-ene-4,6-diynoic Acid,** $C_{10}H_{10}O_3$, colorless plates, m.p. 154.5°, U.V. 215, 222 (243) (225), 270, 285, 303 mμ in ethanol.



*Polyporus anthracophilus*

J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

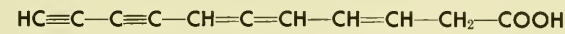
206 **Dimethyl Octa-*trans*-2,*trans*-6-dien-4-yne-1,8-dioate,** $C_{10}H_{10}O_4$, colorless plates, m.p. 117–119.5°, U.V. (205), 214 (240) (278), 292, 307 mμ in ethanol.



*Polyporus anthracophilus*

J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

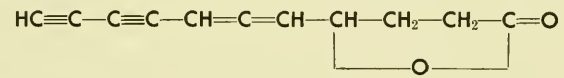
207 **Undec-3,5,6-triene-8,10-diynoic Acid,** $C_{11}H_8O_2$.



*Drosophila semivestita*

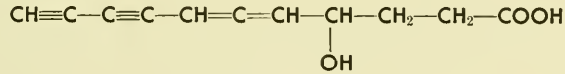
Marjorie Anchel, *Science* 126 1229 (1957).

208 **Nemotin,** $C_{11}H_8O_2$, unstable except in solutions, $[\alpha]_D^{17}$ +380° (c 0.3 in ether), U.V. 207, 236, 248, 262, 276 mμ in water.



and

209 **Nemotinic Acid,** $C_{11}H_{10}O_3$, unstable except in solutions, $[\alpha]_D^{17}$ +320° (c 0.2 in ether), U.V. 208, 237, 249, 263, 277 mμ in water.



*Poria corticola, P. tenuis* and another unidentified basidiomycete

Yields of mixed acetylenes from one of the fungi were:

TABLE I

| Compound | Concentration in the medium (mg. per liter) | Per cent of total |
|---|---|---|
| Nemotinic Acid..... | 110 | 67.5 |
| Nemotin..... | 14 | 8.5 |
| Odyssic Acid..... | 34 | 21 |
| Odyssin..... | 5 | 3 |

J. D. Bu'Lock, E. R. H. Jones and P. R. Leeming, *J. Chem. Soc.*, 4270 (1955). (Structure)

210 **Methyl *trans*-10-Hydroxydec-2-ene-4,6,8-triyn-1-oate,** $C_{11}H_8O_3$, needles (dec. ~115°), U.V. 245, 256.5, 283, 301, 320.5, 343.5 mμ in carbon tetrachloride.

$$HOCH_2—C\equiv C—C\equiv C—C\equiv C—CH=CH—COOCH_3$$

*Pleurotus ulmarius, Merulius lachrymans*

J. N. Gardner, E. R. H. Jones, P. R. Leeming and J. S. Stephenson, *J. Chem. Soc.*, 691 (1960).

211 ***trans,trans*-Matricaria Ester,** $C_{11}H_{10}O_2$, colorless needles, m.p. 62°, U.V. (234), 246, 258 (296), 314, 333 mμ in ethanol.

$$CH_3—CH=CH—C\equiv C—C\equiv C—CH=CH—COOCH_3$$

*Polyporus anthracophilus*

J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

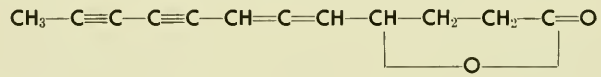
212 **Methyl 10-Hydroxydec-*trans*-2-ene-4,6-diynoate,** $C_{11}H_{12}O_3$, nearly colorless oil, U.V. 215, 223 (243), 258, 273, 287, 305 mμ in ethanol.

$$HOCH_2—CH_2—CH_2—C\equiv C—C\equiv C—CH=CH—COOCH_3$$

*Polyporus anthracophilus, Merulius lachrymans*

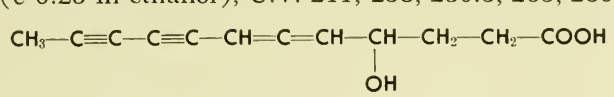
J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

213 **Odyssin,** $C_{12}H_{10}O_2$, unstable except in solutions, $[\alpha]_D^{20}$ +360° (c 0.2 in ethanol), U.V. 210, 237.5, 250, 264, 280 mμ.



and

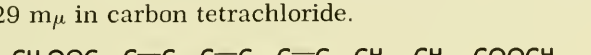
214 **Odyssic Acid,** $C_{12}H_{12}O_3$, unstable except in solutions, $[\alpha]_D^{20}$ +300° (c 0.25 in ethanol), U.V. 211, 238, 250.5, 265, 280.5 mμ.



*Poria corticola, P. tenuis*

J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

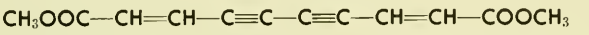
215 **Dimethyl Deca-2,4,6-triyne-1,10-dioate,** $C_{12}H_{10}O_4$, colorless needles, m.p. 45°, U.V. 209, 217, 226, 257, 272, 288, 307, 329 mμ in carbon tetrachloride.



*Merulius lachrymans*

J. N. Gardner, E. R. H. Jones, P. R. Leeming and J. S. Stephenson, *J. Chem. Soc.*, 691 (1960).

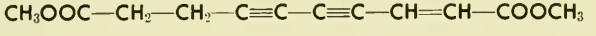
216 **Dimethyl Deca-*trans*-2,*trans*-8-diene-4,6-diyne-1,10-dioate,** $C_{12}H_{10}O_4$, colorless plates, m.p. 104.5–107.5°, U.V. 216, 269, 298, 317, 339 mμ in ethanol.



*Polyporus anthracophilus*

J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

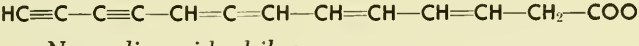
217 **Dimethyl Dec-*trans*-2-ene-4,6-diyne-1,10-dioate,** $C_{12}H_{12}O_4$, colorless crystals, m.p. 56.5–58°, U.V. 214.5, 223 (243) (255), 270, 285, 303 mμ in ethanol.



*Polyporus anthracophilus*

J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

218 **Mycomycin,** $C_{13}H_{10}O_2$, colorless needles, m.p. 75° (dec. explosively), $[\alpha]_D^{25}$ −130° (c 0.4 in ethanol), U.V. 256, 267, 281 mμ in diethyl ether.

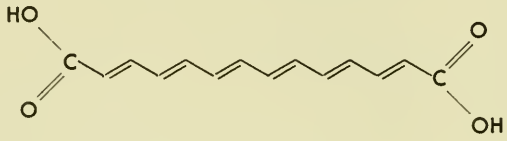


*Nocardia acidophilus*

Walter D. Celmer and I. A. Solomons, *J. Am. Chem. Soc.* 74 1870, 3838 (1952). (Structure)

Edwin A. Johnson and Kenneth L. Burdon, *J. Bacteriol.* 54 281 (1947).

219 **Corticrocin,** $C_{14}H_{14}O_4$, orange-red, amorphous powder or yellow needles and prisms, m.p. subl. 270°, m. 317° (sealed tube), U.V. 374, 393, 416 mμ in ethanol.




*Corticeum croceum* Bres. (= *Corticium sulfureum* (Fr.) Fr.)

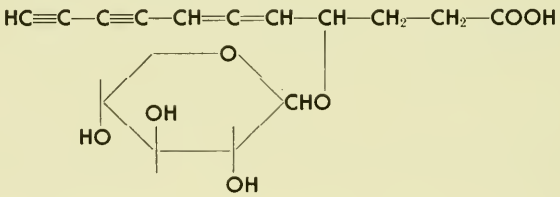
Yields of about 4% of the mycorrhizal weight have been reported.

Holger Erdtman, *Acta Chem. Scand.* 2 209 (1948). (Isolation and Structure)

B. L. Shaw and M. C. Whiting, *J. Chem. Soc.*, 3217 (1954). (Synthesis)

B. C. L. Weedon, *ibid.* 4168 (1954). (Synthesis)

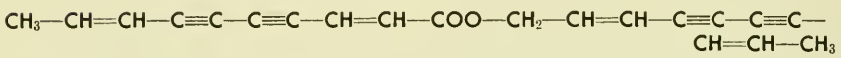
220 **Nemotinic Acid Xyloside,** $C_{18}H_{18}O_7$, $[\alpha]_D^{25}$ +237° (c 0.1 ethanol).




Basidiomycete B-841

J. D. Bu'Lock and H. Gregory, *Experientia* 15 420 (1959).

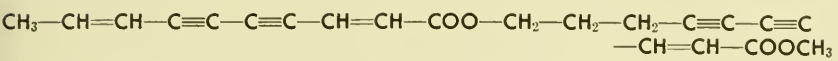
221 **Deca-*trans*-2-*trans*-8-diene-4,6-diynyl Deca-*trans*-2,*trans*-8-diene-4,6-diynoate,** $C_{20}H_{16}O_2$, colorless crystals, m.p. 124–126°, U.V. 213 (233), 238.5, 246, 259, 277.5, 295, 314, 335 mμ in ethanol.




*Polyporus anthracophilus*

J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

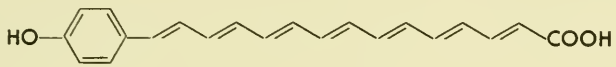
222 **Methyl 10-(Deca-*trans*-2,*trans*-8-diene-4,6-diyn-1-oyloxy)-dec-*trans*-2-ene-4,6-diynoate,** $C_{21}H_{18}O_4$, colorless plates, m.p. 91–93°, U.V. 223, 246.5, 259, 287, 305, 334 $m\mu$ in ethanol.



*Polyporus anthracophilus*

J. D. Bu'Lock, E. R. H. Jones and W. B. Turner, *J. Chem. Soc.*, 1607 (1957).

223 **Cortisalin,** $C_{21}H_{20}O_3$, violet-red needles, m.p. dec. >290°, U.V. (318), 345 (420), 443 (462) $m\mu$ in pyridine.



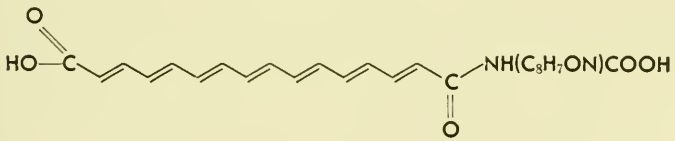
*Corticium salicinum* Fries

A yield of 2.6 g. of crude material was obtained from 222 g. of fungal fruiting body.

Jarl Gripenberg, *Acta Chem. Scand.* 6 580 (1952).

D. Marshall and M. C. Whiting, *J. Chem. Soc.*, 537 (1957). (Synthesis)

224 **Limocrocin,** $C_{26}H_{26}O_6N_2$, a yellow actinomycete pigment. Dark red crystals from AcOH m.p. 316° (dec.). Dimethyl ester of perhydo-deriv., fine, colorless needles, m.p. 146–147°. Partial structure:



A demethylcrocetin derivative with the $C_8H_7ON$ probably a heterobicyclic residue. Eq. wt. 225 (232).

*Streptomyces limosus* (Glycine-glycerol substrate)

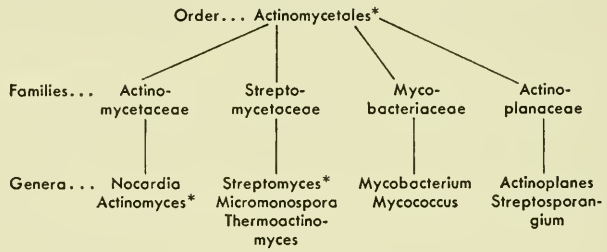
Hans Brockmann and Hans-Ulrich May, *Chem. Ber.* 88 419 (1955).

Hans Brockmann and Gerhard Grothe, *Chem. Ber.* 86 1110 (1953).

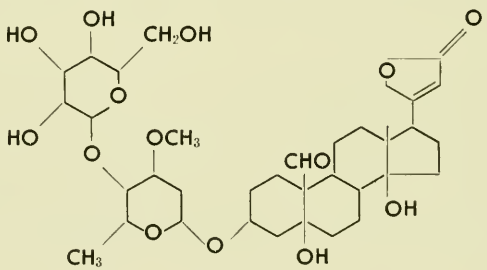
7

# Macrocyclic Lactones (Macrolides)

The macrolide (macrocyclic lactone) antibiotics are an interesting new class of compounds elaborated by members of the order actinomycetales and particularly by the genus streptomyces. The lactone moieties of these molecules resemble the partially oxidized and alkylated aliphatic acids characteristic of the related mycobacterium genus. A partial listing according to Bergey's Manual of the members of the order actinomycetales is shown below to clarify these relationships.



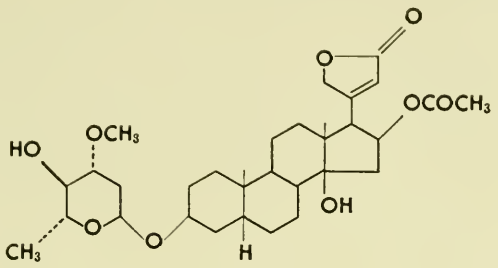

A resemblance to the steroid glycosides, for example strophanthin and oleandrin shown below, also has been noted.[1]




Strophanthin

* In the vernacular usage streptomycete-streptomyces and actinomycete may indicate either order or genus, perhaps more commonly the order.

[1] R. B. Woodward, *Festschr. Arthur Stoll.*, 524 (1957).




Oleandrin

In this regard it is striking that the sugar L-oleandrose occurs in both oleandrin and in the macrolide oleandomycin.

The macrolide antibiotics are most effective against gram-positive bacteria. In the introduction to the section on steroids and terpenoids, it was mentioned that no true steroids have ever been detected conclusively in bacteria. It was noted also that certain investigators exploring the utilization of mevalonic acid by gram-positive bacteria (especially lactobacilli) found that partially oxidized aliphatic substances with more than 15 carbon atoms were produced.[2] While these products were not thoroughly characterized, the properties as described were reminiscent of the lactone portions of the macrolides. It also has been mentioned elsewhere that the general chemical structure and metabolism of the actinomycetales seem to be more closely related to that of the bacteria than to that of the fungi, which they resemble superficially. From these premises, it is tempting to speculate that the macrolide antibiotics may interfere in some way with a primitive kind of hormonal or steroid metabolism in gram-positive bacteria. In this connection it should be noted, however, that the sugar portions of most of the known macrolide antibiotics are essential to their antibacterial activity. Tylosin and lankamycin may be exceptions.

Several of the many macrocyclic lactones which have been isolated from streptomycete cultures have been well characterized structurally. Complete structures have been reported for picromycin, methymycin, neomethymycin, erythromycin, erythromycin B, erythromycin C, carbomycin (Magnamycin), carbomycin B, oleandomycin and pimaricin. A considerable amount of information has been reported concerning the structures of narbomycin, the foromacidins (spiramycins) and the pentaenes lagosin and filipin.*

The few cases available for comparison fall into a general pattern. This involves the lactone of a long chain aliphatic

[2] E. Kodicek, Abstracts of the Gordon Conference on Vitamins and Metabolism, 1958.

* See addendum.

acid, quite evidently acetate-derived, in conjugation with one or more sugar-like moieties. These sugars are uncommon ones, and one of them is usually an amino-sugar, desosamine being particularly prevalent so far. Several of the incompletely characterized macrolides, especially those of the polyene type, have been reported to contain no nitrogen, however. Among these are lagosin, fungichromin, A-246, miamycin and filipin. One macrolide, celesticetin,* contains sulfur. Lankamycin also contains no nitrogen.

Of all the macrolides the biosynthesis of erythromycin has been investigated most thoroughly. One of the questions to be answered was whether the erythronolide moiety is derived from acetate or from propionate. A labeling and degradation study with $C^{14}$-containing precursors has shown that propionate or its biological equivalent is the true precursor.[3] Propionate-C-1 was incorporated only into the "methylene" carbon atoms, while propionate C-2 was incorporated largely into the tertiary carbon atoms and not at all into the carbon-bound methyl groups. Additional evidence against the acetate hypothesis was the fact that $C^{14}$-labeled formate or $C^{14}$-methyl methionine did not label the terminal three carbon atom subunit of erythronolide.

A previous study[4] had shown that $C^{14}$-1-labeled propionate caused labeling of erythronolide, but not of the sugars desosamine and cladinose. The reverse was true when the labeled precursor was $C^{14}$-methyl methionine.[5]

Other evidence which has been published suggests or is consistent with derivation of erythronolide from propionate.[6]

A notice has been published that a labeling study on the biogenesis of erythromycin is in progress with the use of propionic acid-1-$C^{14}$-$H^3$.[7]

It remains to be seen whether or not some of the less highly

[3] John W. Corcoran, Toshi Kaneda and John C. Butte, *J. Biol. Chem.* 235 pc29 (1960).

[4] Z. Vanek, J. Majer, A. Babický, J. Liebster, K. Vereš and L. Doležilová, Abstr. IVth Intern. Congr. Biochem., Vienna, 1958; *cf. Angew. Chem.* 71 40 (1959).

[5] Z. Vanek, J. Majer, J. Liebster, K. Vereš and L. Doležilová, Symposium on Antibiotics, Prague, 1959.

[6] V. Musilek and V. Ševcik, *Naturwissenschaften* 45 86 215 (1958); *idem.*, Symposium on Antibiotics, Prague, 1959.

[7] H. Grisebach, H. Achenbach and U. C. Grisebach, *Naturwissenschaften* 47 206 (1960).

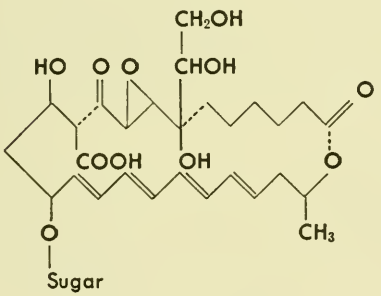
* See entry 923 for non-macrolide structure.

branched lactones are derived in whole or in part from acetate.

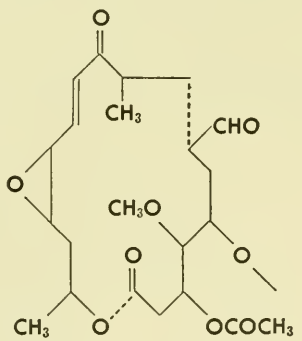
It is obvious that in each case many modifications of the macrolide moiety have occurred from the simplest intermediate ring which could be envisaged. These include complete or partial reduction of carbonyl groups, dehydration of the corresponding secondary alcohols, epoxidation or reduction of carbon-carbon double bonds, oxidation of tertiary carbon atoms, cleavage of epoxides to glycols, etc. Yet, despite the confusing detail, the fundamental pattern of oxidation and reduction remains apparent, just as it does in many of the metabolites of the mycobacteria and corynebacteria.

It will be interesting to see how much of the information concerning the biogenesis of the macrolides can be transposed to metabolites of the mycobacteria and corynebacteria and vice versa.

In the cases of picromycin, methymycin, erythromycin, narbomycin and oleandomycin it is possible to follow the course of alternate oxidation throughout the lactone rings with remarkable regularity, the hypothetical intermediate being, apparently, a single continuous chain, unbranched except for the methyl groups. In the cases of carbomycin and pimaricin, anomalies occur. These could be explained by a junction of shorter chains, perhaps as shown below, in a manner similar to the formation of corynomycolic acid by the coupling of 2 moles of palmitic acid:

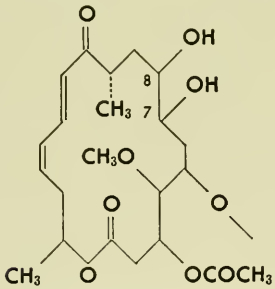



Pimaricin

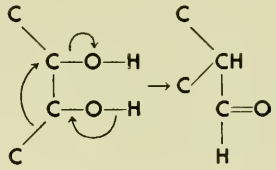



Lactone Portion of Carbomycin

Another suggestion has been made in the case of carbomycin,[1] namely that a protocarbomycin may occur which later rearranges by a glycol-aldehyde shift:




Proposed Precursor of the Lactone Portion of Carbomycin




Proposed Glycol-aldehyde Rearrangement at 7,8-Positions

Such a precursor is the more plausible because it would have an 18-membered carbon atom chain and a C-19 carbon skeleton, the same as that of the known tuberculostearic acid, even including the stereochemistry of the branching methyl group.

Streptomyces species produce many antifungal antibiotics which have in common chains of conjugated olefinic bonds. By means of the ultraviolet absorption spectra it is possible to classify them according to the length of the conjugated chain. Generally these substances are rather intractable with low solubilities and indefinite melting points.

A structure has been proposed for pimaricin, a tetraene. Whether or not this structure proves to be entirely correct, there is evidence from several sources that at least certain of these substances are macrocyclic lactones.

So many of these compounds have been reported lately that any listing is likely to be incomplete. The following table must include most of them, however, grouped by number of conjugated olefinic bonds.

TABLE I

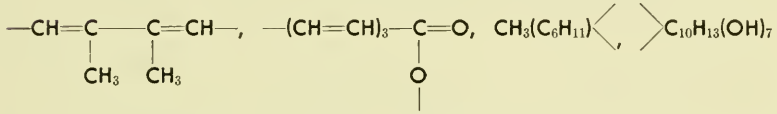
| Tetraene | Hexaene | Pentaene | Heptaene |
|---|---|---|---|
| Nystatin (Fungicidin) | Fradicin | Eurocidin | Amphotericin B |
| Rimocidin | Flavacid | Fungichromatin | Candidin |
| Pimaricin | Mediocidin | Fungichromin | Candicidin |
| Amphotericin A | Endomycin B (Helixin B) | Filipin | Candimycin |
| Protocidin | | PA-153 | Ayfactin |
| Chromin | | Pentamycin | Ascosin |
| Antimycoin | | | Trichomycin |
| Sistomycosin | | | PA-150 |
| Endomycin A (Helixin A) | | | Antibiotic 1968 |
| Etruscomycin | | | |
| PA-166 | | | |
| Tennecetin | | | |
| Flavofungin | | | |

Various other substances, *e.g.* the etamycin, valinomycin and actinomycin types of antibiotics, could be classed as macrolides since they all contain large rings in which lactone groups participate.

*a.* POLYENE MACROLIDES

225 **Flavofungin,** $C_{30}H_{48}O_9$ Dihydrate.

A polyene macrolide containing 7 acetylatable hydroxyl groups, 5 hydrogenatable carbon-carbon double bonds of which at least 4 are conjugated, contains no alicyclic ring, has at least 2 and probably 3 C—$CH_3$. Ozonolysis indicates a $CH_3(C_6H_{11})$ group. The most important structural elements are:

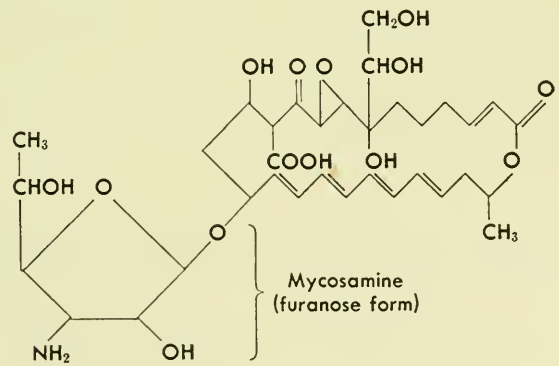



Shown to be distinct from pimaricin, nystatin, amphotericin-B, fungichromin, lagosin, filipin and fumagillin.

A streptomycete

R. Bognar, *Angew. Chem.* 72 139 (1960).

226 **Pimaricin** (Myprozine), $C_{34}H_{49}O_{14}N$, colorless crystals, m.p. 200° (dec.), U.V. 279, 290, 303, 318 m$\mu$ in methanol. Proposed structure:




*Streptomyces natalensis* n. sp.

A. P. Struyk, I. Hoette, G. Drost, J. M. Waisvisz, T. Van Eek and J. C. Hoogerheide, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 878.

James B. Patrick, Richard P. Williams and John S. Webb, *J. Am. Chem. Soc.* 80 6689 (1958). (Structure)

227 **PA-166,** $C_{35}H_{53}O_{14}N$ (proposed), colorless powder, m.p. gradual dec. up to 260°, $[\alpha]_D^{25}$ +275° (c 0.2 in pyridine).

An amphoteric tetraene. U.V. maxima: 291, 304, 319 in aqueous methanol. Positive ninhydrin, 2,4-DNPH and Fehling's tests. Three C-methyl groups.

*Streptomyces* n. sp.

B. K. Koe, F. W. Tanner, Jr., K. V. Rao, B. A. Sobin and W. D. Celmer, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 897.

228 **Etruscomycin,** $C_{36}H_{57}O_{14}N$, white crystals, $[\alpha]_D^{20}$ +296° (c 1 in pyridine).

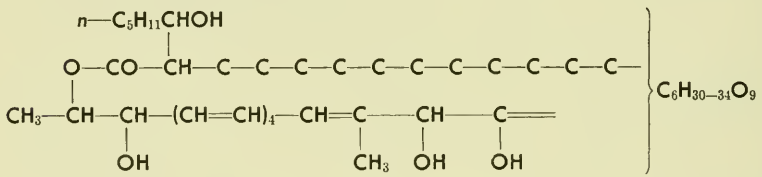
A tetraene antibiotic. I.R. peaks at: 2.91, 3.38, 5.83, 6.30, 9.44, 9.55, 11.85$\mu$. U.V. peaks at: 290, 300, 316 m$\mu$.

*Streptomyces lucensis* n. sp.

F. Arcamone, C. Bertazzoli, G. Canevazzi, A. DiMarco, M. Ghione and A. Grein, *Giorn. Microbiol.* 4 119 (1957).

229 **Lagosin** (Antibiotic A-246), $C_{41}H_{66\text{-}70}O_{14}$, m.p. ~235° (dec.), $[\alpha]_D^{20}$ −160° (c 0.2 in methanol).

An antifungal pentaene macrolide antibiotic with the following partial structure:*




*Streptomyces* sp.

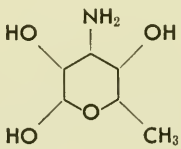
M. L. Dhar, V. Thaller and M. C. Whiting, *Proc. Chem. Soc.*, 148 (1958).

M. L. Dhar, V. Thaller, M. C. Whiting, Ragnar Ryhage, Stina Stälberg-Stenhagen and Einar Stenhagen, *ibid.*, 154 (1959). (Structure)

S. Ball, Christine J. Bessell and Aileen Mortimer, *J. Gen. Microbiol.* 17 96 (1957). (Isolation)

230 **Nystatin** (Fungicidin, Mycostatin) $C_{46}H_{77}O_{19}N$ (tentative), yellow powder, m.p. dec. above 160°, but no definite m.p., $[\alpha]_D^{25}$ +10° (in glacial acetic acid).

An amphoteric tetraene. U.V. maxima at: 280, 291, 304, 318 m$\mu$. Contains a mycosamine moiety:




* See addendum.

*Streptomyces noursei*

Elizabeth L. Hazen and Rachel Brown, *Proc. Soc. Expl. Biol. Med.* 76 93 (1951).

James D. Dutcher, Gerald Boyack and Sidney Fox, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, p. 191.

David R. Walter, James D. Dutcher and O. Wintersteiner, *J. Am. Chem. Soc.* 79 5076 (1957). (Structure)

231 **Rimocidin** (Sulfate heptahydrate), large fragile plates, m.p. ~151° (dec.), $[\alpha]_D^{25}$ (sulfate) +75° (c 1 in methanol).

An amphoteric tetraene. U.V. maxima at: 279, 291, 304, 318 mμ. Analysis (hydrated sulfate): C 57.65, H 7.82, N 1.81, S 2.03.

*Streptomyces rimosus*

J. W. Davisson, F. W. Tanner, Jr., A. C. Finlay and I. A. Solomons, *Antibiotics and Chemotherapy* 1 289 (1951).

232 **Protocidin,** m.p. dec. from 120°.

A polyene antifungal agent. U.V. maxima 277, 290, 303 and 318 mμ. Reduces $KMnO_4$. Green Fehling. Negative biuret, Sakaguchi, Molisch, ninhydrin, anthrone, $FeCl_3$.

*Streptomyces* sp.

The yield was about 100 mg. per liter.

Jean Marie Sakimoto, *J. Antibiotics* (Japan) **10A** 128 (1957).

233 **Amphotericin-A,** m.p. gradual dec. above 153°, $[\alpha]_D^{23.5}$ +32° (in acid dimethylformamide).

An amphoteric tetraene. U.V. maxima: 291, 305, 320 mμ. Analysis: C 60.32, H 8.39, N 1.72.

*Streptomyces* sp.

J. Vandeputte, J. L. Wachtel and E. T. Stiller, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 587.

234 **Sistomycosin,** buff or light yellow microcrystals, m.p. ~230° (browning from 130°).

A neutral tetraene. U.V. maxima: 218, 292.5, 306, 320.5 mμ in aqueous solution. Positive Benedict and Molisch tests.

*Streptomyces viridosporus* n. sp.

J. Ehrlich, M. Knudsen and Q. Bartz, Canadian Patent 514,894 (1955).

235 **Endomycin A** (Helixin A), yellow-brown powder.

An acidic tetraene. U.V. maxima at 292, 301, 319 mμ.

*Streptomyces hygroscopicus* (*S. endus*)

A yield of 11.7 g. of mixed endomycins from about 15 liters of broth has been reported.

L. C. Vining and W. A. Taber, *Can. J. Chem.* 35 1461 (1957).

David Gottlieb, P. K. Bhattacharyya, H. E. Carter and H. W. Anderson, *Phytopathology* 41 393 (1951). (Isolation)

Curt Leben, G. J. Stessel and G. W. Keitt, *Mycologia* 44 159 (1952).

R. R. Smeby, Curt Leben, G. W. Keitt and F. M. Strong, *Phytopathology* 42 506 (1952).

236 **Tennecetin,** yellow amorphous powder.

A tetraene antibiotic. U.V. absorption peaks at 288, 300–302, and 315–318 mμ.

*Streptomyces chattanoogensis*

James Burns and D. Frank Holtman, *Antibiotics and Chemotherapy* 9 398 (1959).

237 **Antimycoin,** organic acid, U.V. maxima: 291, 304–305, 318 mμ in ethanol. Similar to fungicidin. (A tetraene)

*Streptomyces aureus* Waksman and Curtis

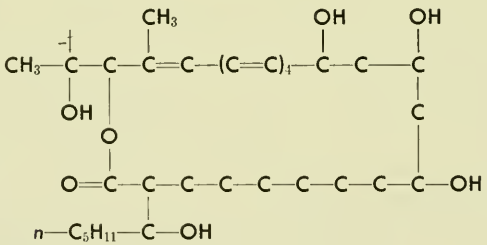
Carl P. Schaffner, Irwin D. Steinman, Robert S. Safferman and Hubert Lechevalier, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, pp. 5869–5873.

Frederick Raubitscheck, Robert F. Acker and Selman A. Waksman *Antibiotics and Chemotherapy* 2 179 (1952).

238 **Filipin,** $C_{32}H_{54}O_{10}$, fine yellow needles, m.p. 195–205° (dec.) (s. 147°), $[\alpha]_D^{22}$ −148.3° (c 0.89 in methanol).

A neutral pentaene. U.V. maxima at 322, 338, 355 mμ. Contains 7–8 acetylatable non-vicinal hydroxyl groups and 3–4 C—$CH_3$ groups.

Possible partial structure:*



*Streptomyces filipinensis* n. sp.

Geo. B. Whitfield, Thomas D. Brock, Alfred Ammann, David Gottlieb and Herbert E. Carter, *J. Am. Chem. Soc.* 77 4799 (1955). (Isolation)

Alfred Ammann, David Gottlieb, Thomas D. Brock, Her-

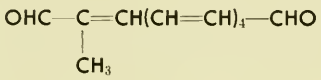
* See addendum.

bert E. Carter and George B. Whitfield, *Phytopathology* 45 559 (1955).

Belig Berkoz and Carl Djerassi, *Proc. Chem. Soc.*, 316 (1959). (Structure)

239 **Fungichromin,** $C_{35}H_{60}O_{13}$, pale yellow crystals, m.p. 205–210°.

A pentaene. U.V. maxima: 322.5, 338.5, 356.5 m$\mu$. The following moiety has been obtained by alkaline hydrolysis followed by periodate oxidation:



*Streptomyces cellulosae*

A similar substance, fungichromatin, occurred in the same culture.

Alfred A. Tytell, Frank J. McCarthy, W. P. Fisher, William A. Balhofer and Jesse Charney, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, p. 716.

Arthur C. Cope and Herbert E. Johnson, *J. Am. Chem. Soc.* 80 1504 (1958).

240 **PA-153,** $C_{37}H_{61}O_{14}N$ (proposed), colorless powder, m.p. gradual dec. up to 260° (triethylamine salt dec. 126–129°), $[\alpha]_D^{25}$ +398° (c 0.2 in pyridine).

An amphoteric pentaene. U.V. maxima: 303, 317, 332, 349 in aqueous methanol. Positive ninhydrin, 2,4-DNPH and Fehlings tests. Three C-methyl groups.

*Streptomyces* n. sp.

B. K. Koe, F. W. Tanner, Jr., K. V. Rao, B. A. Sobin and W. D. Celmer, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 897.

241 **Pentamycin,** pale yellow needles, m.p. 237° (dec.).

An antifungal pentaene antibiotic resembling filipin in some properties. U.V. maxima at: 322, 338, 356 m$\mu$. Contains only C, H, O.

About 60 g. of fairly pure material were obtained from 100 liters of culture (mycelium).

*Streptomyces penticus*

Sumio Umezawa and Yoshiaki Tanaka, *J. Antibiotics* (Japan) 11A 26 (1958).

242 **Eurocidin.**

A pentaene. U.V. maxima: 318, 333, 351 m$\mu$.

*Streptomyces alboreticuli* n. sp.

Yashiro Okami, Ryazo Utahara, Shashiro Nakamura and Hamao Umezawa, *J. Antibiotics* (Japan) 7A 98 (1954).

Ryozo Utahara, Yashiro Okami, Shashiro Nakamura and Hamao Umezawa, *ibid.* 7A 120 (1954).

243 **Fradicin,** $C_{30}H_{34}O_4N_4$, pale greenish yellow crystals, m.p. darkens without melting 180–300°, $[\alpha]_D^{25}$ +65° (c 1 dioxane).

Weakly basic hexaene. U.V. maxima: 290–295. Two methoxyls.

*Streptomyces fradiae*

E. Augustus Swart, Antonio H. Romano and Selman A. Waksman, *Proc. Soc. Exptl. Biol. Med.* 73 376 (1950).

Richard J. Hickey and Phil Harter Hidy, *Science* 113 361 (1951).

244 **Flavacid,** pale yellow microcrystalline powder, m.p. 102–105° (dec.).

A weakly acidic hexaene. U.V. maxima: 340, 360, 380 mμ. A tetraene with peaks at 293, 306 and 324 is also present.

A streptomycete resembling *S. flavus*

Isao Takahashi, *J. Antibiotics* (Japan) 6A 117 (1953).

L. C. Vining and W. A. Taber, *Can. J. Chem.* 35 1461 (1957).

245 **Mediocidin,** yellow amorphous powder.

A hexaene. U.V. maxima: 340, 357, 378 mμ. A tetraene, probably identical with that in the flavacid complex, is also present. U.V. maxima: 293, 306, 324.

*Streptomyces mediocidicus,* n. sp.

Ryazo Utahara, Yoshiro Okami, Shashiro Nakamura and Hamao Umezawa, *J. Antibiotics* (Japan) 7A 120 (1954).

L. C. Vining and W. A. Taber, *Can. J. Chem.* 35 1461 (1957).

246 **Endomycin B** (Helixin B), yellow-brown powder.

An acidic hexaene. For U.V. spectrum see first reference below.

*Streptomyces hygroscopicus* (*S. endus*)

L. C. Vining and W. A. Taber, *Can. J. Chem.* 35 1461 (1957).

David Gottlieb, P. K. Bhattacharyya, H. E. Carter and H. W. Anderson, *Phytopathology* 41 393 (1951). (Isolation)

Curt Leben, G. J. Stessel and G. W. Keitt, *Mycologia* 44 159 (1952).

R. R. Smeby, Curt Leben, G. W. Keitt and F. M. Strong, *Phytopathology* 42 506 (1952).

247 **Helixins.**

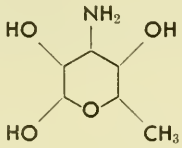
A complex of three or four compounds. Helixin B is identical with endomycin B.

*Streptomyces* sp.

Curt Leben, G. J. Stessel and G. W. Keitt, *Mycologia* 44 159 (1952).

248 **Amphotericin-B,** $C_{46}H_{73}O_{20}N$ (tentative) deep yellow prisms or needles from dimethylformamide, m.p: gradual dec. above 170°, $[\alpha]_D$ +333° (in acid dimethylformamide).

An amphoteric heptaene, U.V. maxima at: 364, 383, 408 m$\mu$. Contains a mycosamine moiety:




*Streptomyces nodosus*

J. Vandeputte, J. L. Wachtel and E. T. Stiller, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 587. (Isolation)

David R. Walters, James D. Dutcher and O. Wintersteiner, *J. Am. Chem. Soc.* 79 5076 (1957). (Structure)

249 **Zaomycin,** m.p. 242–246° (dec.).

An amphoteric antibiotic said to resemble amphotericin. Positive ninhydrin, Millon, biuret, $FeCl_3$ tests. Negative Fehling and Liebermann reactions.

*Streptomycin zaomyceticus*

Yorio Hinuma, *J. Antibiotics* (Japan) 7A 134 (1954).

250 **PA-150,** $C_{54}H_{82}O_{18}N_2$ (proposed), yellow powder, m.p. gradual dec. up to 260°, $[\alpha]_D^{25}$ +294° (c 0.2 in pyridine).

An amphoteric heptaene. U.V. maxima: 340, 358, 377, 397 m$\mu$ in aqueous methanol. Positive 2,4-DNPH and Fehlings tests. Four C-methyl groups.

*Streptomyces* n. sp.

B. K. Koe, F. W. Tanner, Jr., K. V. Rao, B. A. Sobin and W. D. Celmer, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 897.

251 **Trichomycin,** yellow powder, m.p. 155° (dec.).

A heptaene. U.V. maxima: 286, 346, 364, 384, 405 m$\mu$. May be a mixture of two heptaenes.

*Streptomyces hachijoensis* n. sp.

Seigo Hosoya, Nobuhiko Komatsu, Momoe Soeda and Yoko Sonoda, *Japan J. Exptl. Med.* 22 505 (1952).

Seigo Hosoya, Nobuhiko Komatsu, Momoe Soeda, Tatsuro Yuwaguchi and Yoko Sonoda, *J. Antibiotics* (Japan) 5 564 (1952).

252 **Candidin,** yellow powder.

Acidic heptaene. U.V. maxima: (Na salt) 234, 282, 345, 360, 383, 405 mμ in aqueous solution. The free acid lacks the 345 peak. Contains nitrogen and gives positive ketone tests.

*Streptomyces viridoflavus*

Willard A. Taber, Leo C. Vining and Selman A. Waksman, *Antibiotics and Chemotherapy* 4 455 (1954).

Leo C. Vining, Willard A. Taber and Francis J. Gregory, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, p. 980.

**Candicidins.**

Heptaenes. U.V. maxima:

253 **Candicidin A:** 360, 380, 403 mμ.

254 **Candicidin B:** 362, 381, 404 mμ.

255 **Candicidin C:** 358, 379, 402 mμ.

*Streptomyces griseus,* other *Streptomyces* spp.

Hubert A. Lechevalier, R. F. Acker, C. T. Corke, C. M. Haenseler and S. A. Waksman, *Mycologia* 45 155 (1953).

256 **Ascosin,** yellow-orange powder.

A weakly acidic heptaene. U.V. maxima: 234, 288, 340, 357, 376, 398 mμ in methanol.

*Streptomyces canescus*

Richard J. Hickey, Cyril J. Corum, Phil H. Hidy, I. Ray Cohen, Urs F. B. Nager and Eleonore Kropp, *Antibiotics and Chemotherapy* 2 472 (1952).

Isadore R. Cohen, U. S. Patent 2,723,216, (1955).

### *b.* OTHER MACROLIDES

257 **Nitrosporin,** $C_{20}H_{26}O_6N_2$, colorless crystals, m.p. 130–140° (dec.). Crystals brown on exposure to air.

A basic substance, apparently a macrolide.

*Streptomyces nitrosporeus*

Hamao Umezawa and Tomio Takeuchi, *J. Antibiotics* (Japan) 5 270 (1952).

258 **Celesticetin I,** amphoteric, crystalline and dextrorotatory, $C_{24}H_{36-40}O_9N_2S$ (suggested empirical formula), Oxalate and Salicylate water soluble. Oxalate m.p. 149–154°; Salicylate m.p. 139° (tabular monoclinic crystals).

Erythromycin-like. (See entry 923, however)

Positive tests—$FeCl_3$, Molisch, Ekkert

White ppt.—$Br_2$ water, Millon's Reagent, $HgCl_2$

Negative tests—$AgNO_3$, PbAc, Benedict, ninhydrin, iodoform, nitroprusside (becomes + after standing several days in 6 N hydrochloric acid)

No immediate reaction with $Br_2$—$CCl_4$.

*Streptomyces caelestis*

Herman Hoeksema, Glen F. Crum, William H. DeVries, *Antibiotics Annual* 2 837–841 (1954–1955). (Isolation and purification)

259 **Amaromycin,** $C_{27}H_{39}O_7N$ (proposed), colorless prisms, m.p. 164.5°, $[\alpha]_D^{25}$ +6.19° (c 1 in ethanol).

Basic substance, analysis: C 63.66, H 8.73, N 3.0. Negative $FeCl_3$, biuret, ninhydrin, Sakaguchi, Schiff. Positive Tollens, Fehlings. Precipitated by Reinecke's salt. Probably a macrolide.

*Streptomyces flavochromogenes*

Toju Hata, Yashimoto Sano, Hideo Tatsuta, Ryazo Sugawara, Akihiro Matsumae and Kokichi Kanamori, *J. Antibiotics* (Japan) 8A 9 (1955).

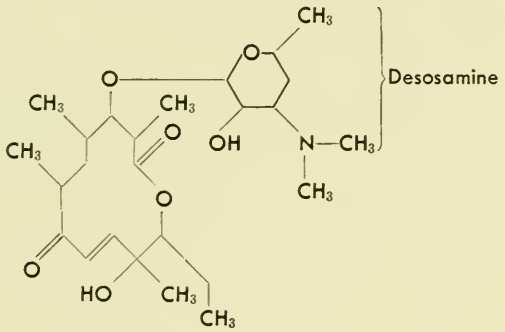
260 **PA-133 A,** $C_{25}H_{43}O_6N$, colorless amorphous solid, $[\alpha]_D^{25}$ +39.6° (c 0.5 in methanol).

A macrolide antibiotic.

*Streptomyces* sp.

K. Murai, B. A. Sobin, W. D. Celmer and F. W. Tanner, *Antibiotics and Chemotherapy* 9 485 (1959).

261 **Methymycin,** $C_{25}H_{43}O_7N$, colorless prisms, needles, m.p. 195–197° (203°), $[\alpha]_D^{25}$ +61° (in methanol).

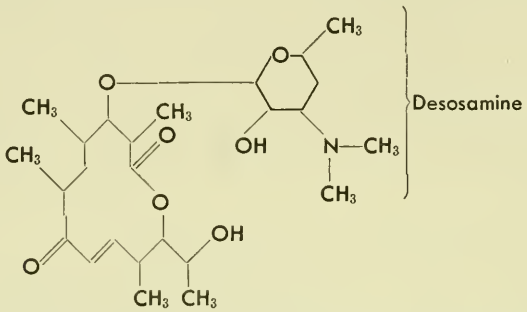


A streptomycete

Carl Djerassi and John A. Zderic, *J. Am. Chem. Soc.* 78 2907 (1956). (Structure)

Milton N. Donin, Joseph Pagano, James D. Dutcher and Clara M. McKee, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, p. 179. (Isolation)

262 **Neomethymycin,** $C_{25}H_{43}O_7N$, colorless crystals, m.p. 156°, $[\alpha]_D^{25}$ +93° (in chloroform).

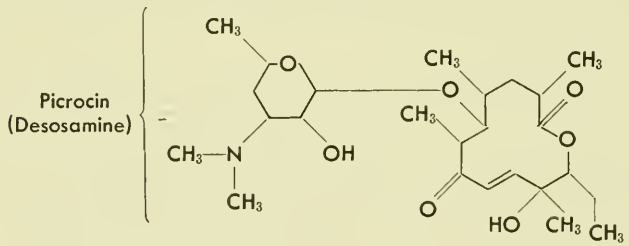



Same streptomycete which produces Methymycin

Carl Djerassi and O. Halpern, *J. Am. Chem. Soc.* 79 2022 (1957). (Structure)

J. Vandeputte, unpublished. (Isolation)

263 **Picromycin,** $C_{25}H_{43}O_7N$, colorless crystals, m.p. 169.5°, $[\alpha]_D^{20}$ −33.5° (c 2.07 in chloroform).




*Streptomyces felleus* n. sp.

Hans Brockmann and Rudolf Oster, *Chem. Ber.* 90 605 (1957). (Partial structure)

R. Anliker and K. Gubler, *Helv. Chim. Acta* 40 119 (1957). (Structure)

Hans Brockmann and Willfried Henkel, *Chem. Ber.* 84 284 (1951). (Isolation)

*Ibid., Naturwissenschaften* 37 138 (1950). (Isolation)

264 **PA-133-B**, $C_{25}H_{45}O_{10}N$, colorless crystals, m.p. 99.8–101°, $[\alpha]_D^{25}$ +22.5° (c 0.5 in methanol).

A macrolide antibiotic.

*Streptomyces* sp.

K. Murai, B. A. Sobin, W. D. Celmer and F. W. Tanner, *Antibiotics and Chemotherapy* 9 485 (1959).

265 **Griseomycin** (Lomycin) (Hydrochloride) $C_{25}H_{46}O_8NCl$, white powder, m.p. 76–80° (dec.), $[\alpha]_D^{25}$ +32° (c 1 in chloroform).

Precipitated by Reinecke salt, bromine water, picric acid. Thought to be a macrolide.

*Streptomyces griseolus*

P. J. Van Dijck, H. P. Van de Voorde and P. DeSomer, *Antibiotics and Chemotherapy* 3 1243 (1953).

*Ibid.* Belgian Patent 522,647 (1954).

266 **Proactinomycin A**, $C_{27}H_{47}O_8N$ (proposed), colorless crystals, m.p. 168°.

267 **Proactinomycin B**, $C_{28}H_{49}O_8N$ (proposed), colorless crystals, m.p. 83–87°.

268 **Proactinomycin C**, $C_{24}H_{41}O_6N$ (proposed), amorphous.

Basic substances, precipitated by Reineckes salt, picric or flavianic acids, etc. Probably macrolides.

*Nocardia gardneri*

A. D. Gardner and E. Chain, *Brit. J. Exptl. Path.* 23 123 (1942).

R. Q. Marston, *ibid.* 30 398 (1949). (Isolation)

## Antimycins (Antipiriculins)*

269 **Antimycin $A_1$**, $C_{28}H_{40}O_9N_2$, colorless crystals, m.p. 149–150°, $[\alpha]_D^{26}$ +76 (c 1 in chloroform).

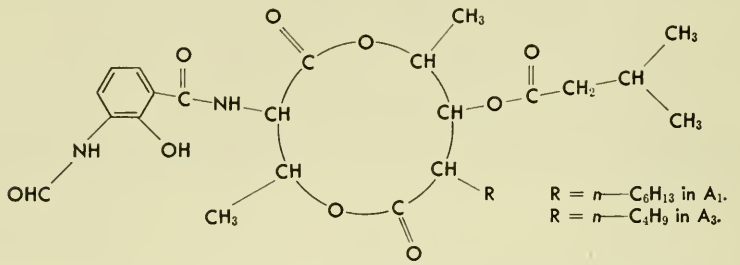
270 **Antimycin $A_{2a}$**, $C_{26}H_{36}O_9N_2$, colorless crystals, m.p. 143–149°.

271 **Antimycin $A_{2b}$** (may be isomeric with $A_{2a}$), colorless crystals, m.p. 168°.

272 **Antimycin $A_3$** (Blastmycin), $C_{26}H_{36}O_9N_2$, colorless crystals, m.p. 170.5–171.5°, $[\alpha]_D^{26}$ +64.3° (c 1 in chloroform).

* The antimycins might also be classified as depsipeptides (peptolides).

273 Antimycin $A_4$, oily.




At least seven streptomyces species produce antimycins, including *S. kitazawaensis* Harada et Tanaka nov. sp. and *S. blastmyceticus*. The former organism also produces carzinocidin. Blastmycin is identical with antimycin $A_3$. Virosin is probably a mixture of antimycin components. Certain antimycin-producing cultures also contain actinomycin B.

Wen-chik Liu and F. M. Strong, *J. Am. Chem. Soc.* 81 4387 (1959).

Wen-chik Liu, E. E. Van Tamelen and F. M. Strong, *ibid.* 82 1652 (1960). (Degradations, etc.)

F. M. Strong, J. P. Dickie, M. E. Loomans, E. E. Van Tamelen and R. S. Dewey, *ibid.* 82 1513 (1960). (Structure)

Bryant R. Dunshee, Curt Leben, G. W. Keitt and F. M. Strong, *ibid.* 71 2436 (1949). (Isolation)

Yoshio Sakagami, Setsuo Takeuchi, Hiroshi Yonehara, Heiichi Sakai and Matso Takashima, *J. Antibiotics* (Japan) 9A 1 (1956).

Kiyoshi Nakayama, Fukusaburo Okamoto and Yujiro Harada, *ibid.* 9A 63 (1956).

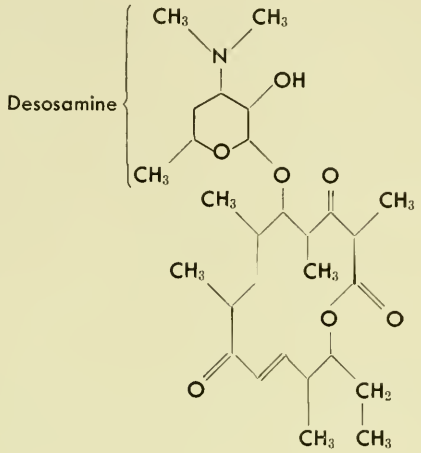
Yujiro Harada, Keizo Uzu and Masaru Asai, *ibid.* 11A 32 (1958).

Hiroshi Yonehara and Setsuo Takeuchi, *ibid.* 11A 122 (1958). (Proposed structure)

Kiyoshi Watanabe, Tsutomo Tanaka, Keiko Fukuhara, Norisama Miyairi, Hiroshi Yonehara and Hamao Umezawa, *ibid.* 10A 39 (1957).

F. M. Strong, "Topics in Microbial Chemistry" (Squibb Lectures on the Chemistry of Microbial Products), John Wiley and Sons, Inc., New York, 1956, pp. 1–44. (A review to that date)

274 **Narbomycin**, $C_{28}H_{49}O_7N$, colorless crystals, m.p. 113.5–115°, $[\alpha]_D$ +68.5° (c 1.35 in chloroform).




*Streptomyces narboensis* n. sp.

R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, P. Reusser, and H. Zähner, *Helv. Chim. Acta* 38 935 (1955).

R. Anliker, D. D. Dvornik, K. Gubler, H. Heusser and V. Prelog, *ibid.* 39 1785 (1956).

V. Prelog, A. M. Gold, G. Talbot and A. Zamojski. (To be published)

275 **Leucomycin**, $C_{33-38}H_{54-66}O_{11-13}N$, colorless crystals, m.p. 124–125.5°, $[\alpha]_D^{20}$ −67.1° (c 1 in ethanol).

Leucomycin appears to be a macrolide antibiotic.*

*Streptomyces kitasatoensis* n. sp.

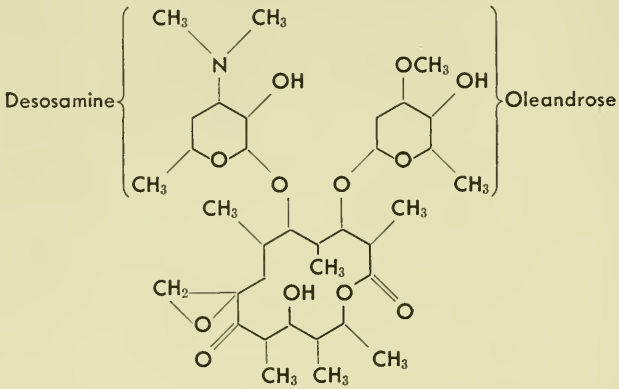
Toju Hata, Yoshimoto Sano, Natsuo Ohki, Yasuhiku Yokoyama, Akihiro Matsumae and Shinya Ito, *J. Antibiotics* (Japan) 6A 87 (1953).

Yoshimoto Sano, Tadashi Hoshi and Toju Hata, *ibid.* 7A 88 (1954).

Yoshimoto Sano, *ibid.* 7A 93 (1954).

* See addendum.

276 **Oleandomycin (PA-105)**, $C_{35}H_{61}O_{12}N$, colorless prisms, m.p. 110° (dec.), $[\alpha]_D^{25}$ −65° (c 1 in methanol).




*Streptomyces antibioticus*

B. A. Sobin, A. R. English and W. D. Celmer, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, p. 827.

W. D. Celmer, H. Els and K. Murai, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 476.

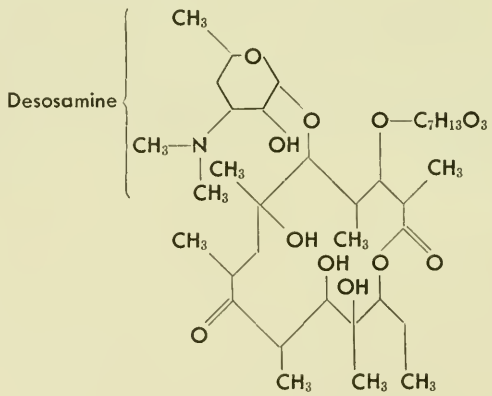
Hans Els, Walter D. Celmer and Kotaro Murai, *J. Am. Chem. Soc.* 80 3777 (1958).

W. D. Celmer, "Antibiotics Annual 1958–1959," Medical Encyclopedia, Inc., New York, p. 277. (Biochemical correlations)

F. A. Hochstein, H. Els, W. D. Celmer, B. L. Shapiro and R. B. Woodward, *J. Am. Chem. Soc.* 82 3225 (1960). (Structure)

277 **Erythromycin C**, $C_{36}H_{65}O_{13}N$, white needles, m.p. 121–125°.

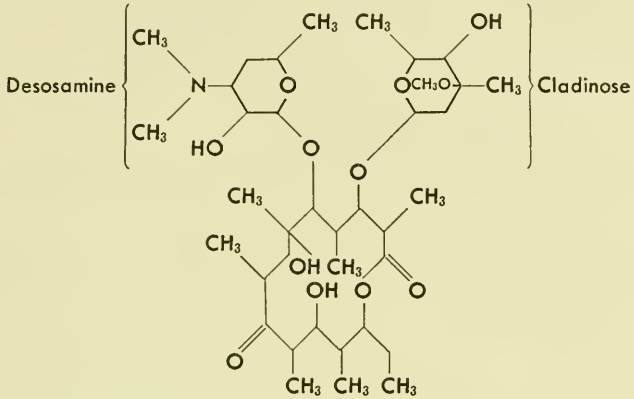
Erythromycin C differs from erythromycin only in the neutral sugar moiety, so that the following partial structure can be written:



*Streptomyces erythreus*

Paul F. Wiley, Richard Gale, C. W. Pettinga and Koert Gerzon, *J. Am. Chem. Soc.* 79 6074 (1957). (Structure and isolation)

278 **Erythromycin B,** $C_{37}H_{67}O_{12}N$, colorless crystals, m.p. 198°, $[\alpha]_D^{25}$ −78° (c 2 in ethanol).

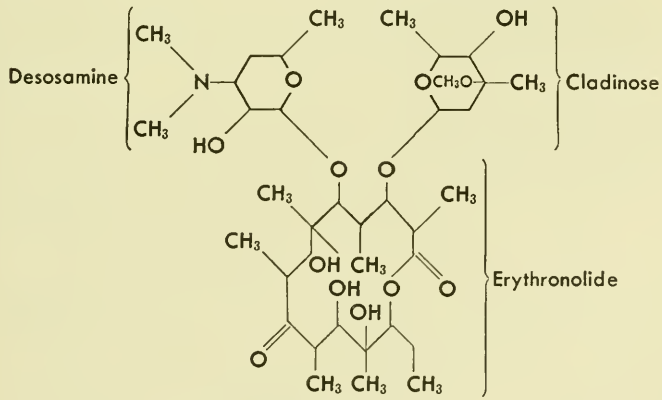



*Streptomyces erythreus*

Paul F. Wiley, Max V. Sigal, Jr., Allidene Weaver, Rosemarie Monahan and Koert Gerzon, *J. Am. Chem. Soc.* 79 6070 (1957). (Structure)

C. W. Pettinga, W. M. Stark and F. R. Van Abeele, *ibid.* 76 569 (1954). (Isolation)

279 **Erythromycin** (Ilotycin, Erythrocin), $C_{37}H_{67}O_{13}N$, white needles, m.p. 136–140°, $[\alpha]_D^{25}$ −78° (c 1.99 in alcohol).




*Streptomyces erythreus*

R. K. Clark, Jr. *Antibiotics and Chemotherapy* 3 663 (1953). (Isolation)

Paul F. Wiley, Koert Gerzon, Edwin H. Flynn, Max V. Sigal, Jr., Allidene Weaver, U. Carol Quarck, Robert R. Chau-

vette and Rosemarie Monahan, *J. Am. Chem. Soc.* 79 6062 (1957). (Structure)

280 **PA-108,** $C_{38}H_{63}O_{14}N$, colorless solid, m.p. 121–123°, $[\alpha]_D^{25}$ −36.8° (c 1 in chloroform).

A macrolide antibiotic.

*Streptomyces* sp.

K. Murai, B. A. Sobin, W. D. Celmer and F. W. Tanner, *Antibiotics and Chemotherapy,* 9 485 (1959).

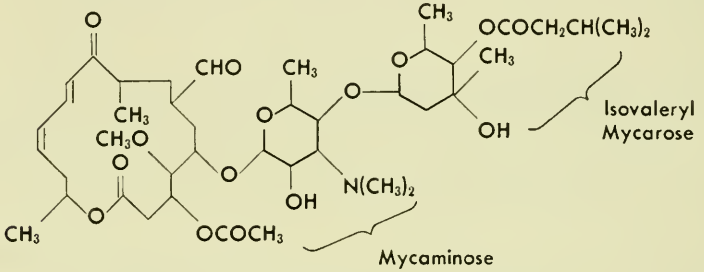
281 **PA-148,** $C_{38}H_{65}O_{15}N$, colorless amorphous solid, m.p. 115–118°, $[\alpha]_D^{25}$ −69.3° (c 0.5 in methanol).

A macrolide antibiotic.

*Streptomyces* sp.

K. Murai, B. A. Sobin, W. D. Celmer and F. W. Tanner, *Antibiotics and Chemotherapy,* 9 485 (1959).

282 **Carbomycin B,** $C_{42}H_{67}O_{15}N$, colorless plates, m.p. 141–144° (dec.), Hydrochloride 164–166° (dec.), $[\alpha]_D^{25}$ −35° (c 2.0 in chloroform).

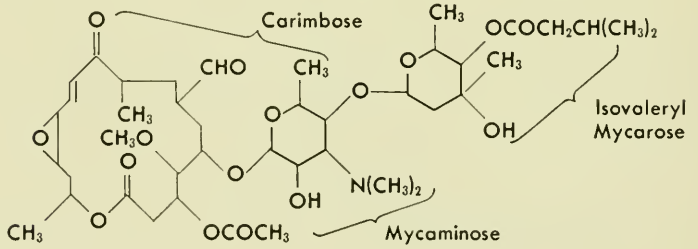



*Streptomyces halstedii*

F. A. Hochstein and Kotaro Murai, *J. Am. Chem. Soc.* 76 5080 (1954). (Isolation)

R. B. Woodward, *Angew. Chem.* 69 50 (1957). (Structure)

283 **Carbomycin** (Magnamycin), $C_{42}H_{67}O_{16}N$, colorless laths, m.p. 212–214° (dec.), $[\alpha]_D^{25}$ −58.6° (c 1 in chloroform).



*Streptomyces halstedii, S. alboreticuli*

R. B. Woodward, *Angew. Chem.* 69 50 (1957). (Structure)

Richard L. Wagner, F. A. Hochstein, Kotaro Murai, N. Messina and Peter B. Regna, *J. Am. Chem. Soc.* 75 4684 (1953). (Isolation)

284 **Tertiomycin A,** $C_{42}H_{49}O_{16}N$, white needles, m.p. 215–217° (s. 208°) (dec.), $[\alpha]_D^{17}$ −49° (c 1 in chloroform) $[\alpha]_D^{16}$ −47° (c 1.0 in ethanol).

A macrolide antibiotic. Carbomycin produced also by *S. alboreticuli.*

*Streptomyces eurocidicus, S. alboreticuli*

Teisuke Osato, Masahiro Ueda, Setsuko Fukuyama, Koki Yagishita, Yoshiro Okami and Hamao Umezawa, *J. Antibiotics* (Japan) 8A 105 (1955).

285 **Tertiomycin B,** $C_{43}H_{71}O_{17}N$ (proposed), white needles, m.p. 97°, $[\alpha]_D^{22}$ −56° (c 1 in ethanol).

A macrolide antibiotic.

*Streptomyces eurocidicus*

The same organism produces eurocidin, tertiomycin A and azomycin.

Teisuke Osato, Koki Yagishita and Hamao Umezawa, *J. Antibiotics* (Japan) 8A 161 (1955).

Akira Miyoke, Hidesuke Iwasaki and Torao Tawewaka, *J. Antibiotics* (Japan) 12A 59 (1959).

286 **Foromacidin A** (Spiramycin I): $C_{45}H_{78}O_{15}N_2$, colorless powder, m.p. 134–138°, $[\alpha]_D$ −81° (c 0.34 in methanol).

287 **Foromacidin B** (Spiramycin II): $C_{47}H_{80}O_{16}N_2$, colorless powder, m.p. 130–132°, $[\alpha]_D$ −83° (c 0.82 in ethanol).

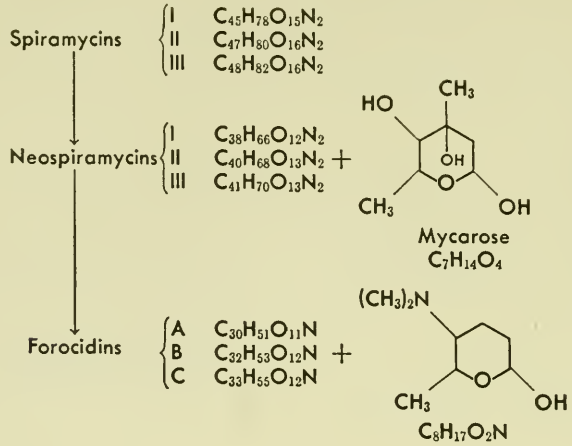
288 **Foromacidin C** (Spiramycin III): $C_{48}H_{82}O_{16}N_2$, colorless powder, m.p. 124–128°, $[\alpha]_D$ −79° (c 1.19 in ethanol).

289 **Foromacidin D:** Equiv. Wt. 452, colorless powder, m.p. 135–140°, $[\alpha]_D$ −75° (c 0.81 in ethanol).

Two streptomycetes

R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, A. Wettstein and H. Zähner, *Helv. Chim. Acta* 39 304 (1956).

The foromacidins (or spiramycins) are apparently macrolide antibiotics. On degradation they yield three sugars typical of this class.




Raymond Paul and Serge Tchelitcheff, *Bull. soc. chim. France* 442, 734 (1957).

*Idem., ibid.*, 150 (1960).

290 **Tylosin,** $C_{45}H_{79}O_{17}N$, colorless crystals, m.p. 128–132°, $[\alpha]_D^{25}$ −46° (c 2 in methanol).

A macrolide antibiotic, containing the sugars mycarose and mycaminose. Also has an $\alpha$, $\beta$, $\gamma$, $\delta$-unsaturated carbonyl system.

*Streptomyces fradiae*

R. L. Hamill, M. E. Haney, Martha C. Stamper and Paul Wiley, Abstr. Atlantic City Meeting, Am. Chem. Soc., September, 1959. (To be published)

J. M. McGuire, W. S. Boniece, W. A. Daily, C. E. Higgens, M. M. Hoehn, W. M. Stark, W. B. Sutton, J. Westhead and R. N. Wolfe. (To be published)

291 **Angolamycin,** $C_{49\text{-}50}H_{87\text{-}91}O_{18}N$, colorless crystals, m.p. 165–168°, $[\alpha]_D^{21}$ −64° (c 1.3 in chloroform).

A macrolide antibiotic apparently similar to carbomycin, but with characteristic sugars.

*Streptomyces eurythermus*

R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, L. Neipp, V. Prelog, P. Reusser and H. Zähner, *Helv. Chim. Acta* 38 1202 (1955).

292 **Miamycin,** colorless crystals, m.p. 221° (dec.), $[\alpha]_D^{25}$ −18° (c 1.0 in 0.02 N hydrochloric acid).

A macrolide antibiotic. Analysis: C 61.4, 61.5, H 8.7, 8.6. Mol. wt. ~609.

*Streptomyces ambofaciens*

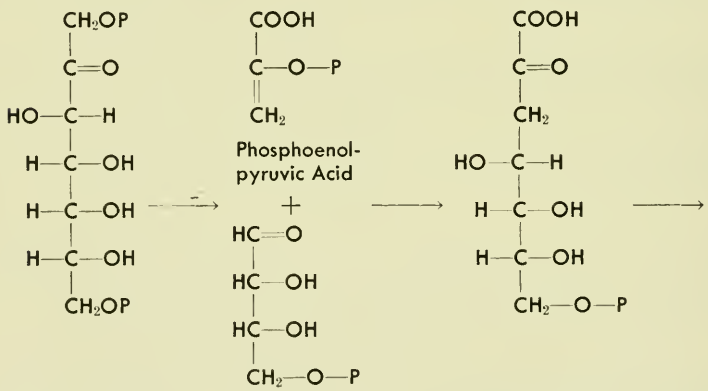
H. Schmitz, M. Misiek, B. Heinemann, J. Lein and I. R. Hooper, *Antibiotics and Chemotherapy* 7 37 (1957).

# 8

# Alicyclic Compounds Other Than Terpenoids and Steroids

This section contains non-terpenoid, non-steroid alicyclics of diverse biosynthetic origin. Many of these, especially the streptomycete products, were antibiotic isolates.

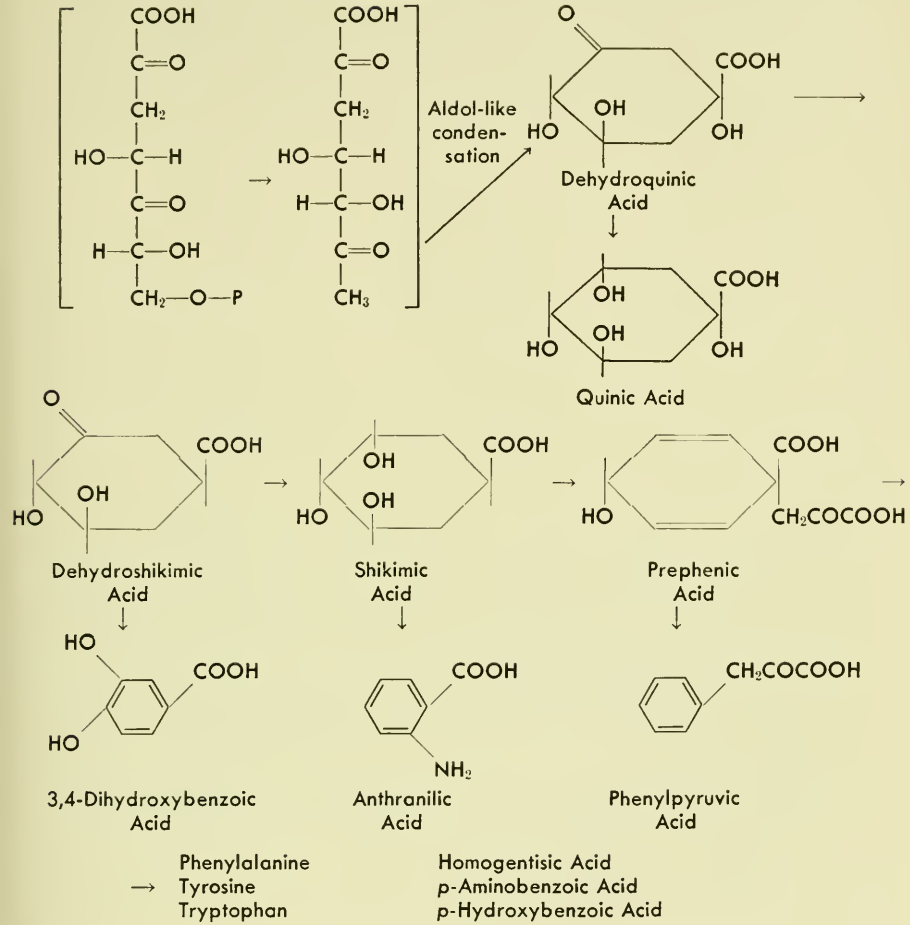
Included here are some of the intermediates in the biosynthetic route from carbohydrates to aromatic amino acids and to certain other aromatic compounds. Part of this sequence, worked out largely by Tatum, Davis, Sprinson and collaborators,[1, 2, 3] is reproduced below in brief outline only since it has been widely reviewed and publicized. (P indicates phosphorylation):




[1] Bernard D. Davis, *Intermediates in amino acid biosynthesis, Advances in Enzymology* 16 247–312 (1955). (A review)

[2] Alton Meister, "Biochemistry of the Amino Acids," Academic Press, Inc., New York, 1957, pp. 346–349.

[3] P. R. Srinivasan, Masayuki Katagiri and David B. Sprinson, *J. Biol. Chem.* 234 713 (1959); P. R. Srinivasan and David B. Sprinson, *ibid.* 234 716 (1959).

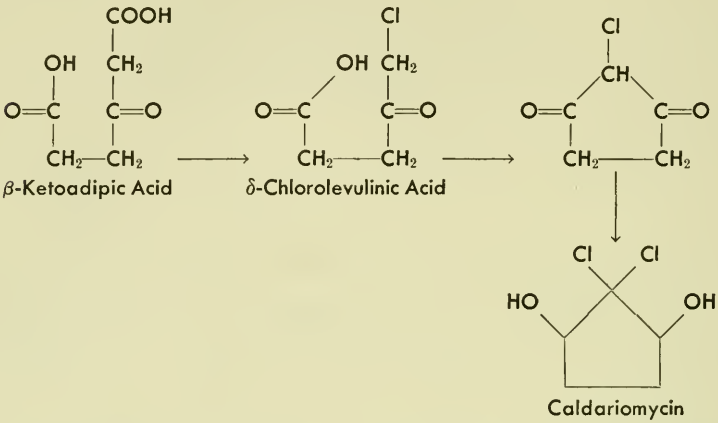



Microorganisms were the principal tools in this work, especially the mold *Neurospora crassa* and the bacteria *Escherichia coli* and *Aerobacter aerogenes* mutated so that the biosynthesis of aromatic amino acids was blocked at various points. These mutants accumulated intermediates in the sequence prior to the blocks, and these substances could then be isolated. Also when such mutants (auxotrophs) were supplied with the critical substance whose biosynthesis was blocked, the microorganisms were capable of completing the sequence to the aromatic acids.

This route from carbohydrates to certain types of aromatic substances has been established as quite general in metabolism.

Biosynthesis of the chlorinated cyclopentane, caldariomycin,

has been studied.[4] β-Ketoadipic acid and δ-chlorolevulinic acid were found to be intermediates. The sequence shown here, then, probably represents at least part of the biogenetic scheme for this metabolite.

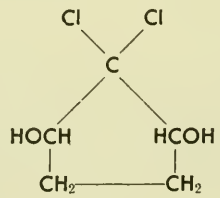



Palitantin appears to be an interesting example of an unaromatized acetate derivative. Its origin is revealed by the 14-carbon atoms, the uneven-numbered side-chains and the pattern of oxidation and unsaturation.

The cycloheximides also seem to be acetate derivatives, although apparently no study of their biosynthesis has been published.

Without having made a detailed analysis of the experimental work it would seem that the proposed structures for the glauconic acids are unique if not improbable.

293 **Caldariomycin**, $C_5H_8O_2Cl_2$, colorless needles, m.p. 121°, $[\alpha]_{5461}^{20}$ +59.2° (c 0.338 in water).

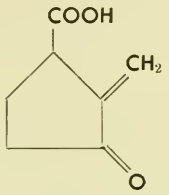



*Caldariomyces fumago*

[4] Paul D. Shaw, Jonathon R. Beckwith and Lowell P. Hager, *J. Biol. Chem.* 234 2560 (1959).

Percival W. Clutterbuck, Sudhir L. Mukhopadhyay, Albert E. Oxford and Harold Raistrick, *Biochem. J.* 34 664 (1940).

294 **Sarkomycin,** $C_7H_8O_3$, oil (dihydro-derivative), m.p. 99° with sublimation, $[\alpha]_D^{25}$ +66.7° (in water).




*Streptomyces erythrochromogenes*

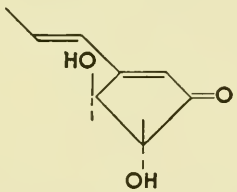
A yield of about 5 g. from 2 liters of broth has been reported.

Hamao Umezawa, Tadashi Yamamato, Tomio Takeushi, Teisuke Osato, Yashiro Okami, Seizaburo Yamaoka, Tomoharu Okuda, Kazuo Nitta, Koki Yagishita, Ryazo Utahara and Sumio Umezawa, *Antibiotics and Chemotherapy* 4 514 (1954). (Isolation)

I. R. Hooper, L. C. Cheney, M. J. Cron, O. B. Fardig, D. A. Johnson, D. L. Johnson, F. M. Palermiti, H. Schmitz and W. B. Wheatley, *ibid.* 5 585 (1955). (Structure)

M. M. Shemyakin, L. A. Shchukina, E. I. Vinogradova, M. N. Kolosov, R. G. Vdovina, M. G. Karapetyan, V. Ya. Rodionov, G. A. Ravdel, Yu. B. Shvetsov, E. M. Bamdas, E. S. Chaman, K. M. Ermolaev and E. P. Semkin, *Zhur. Obschchei Khim.* 27 742 (1957). (Synthesis of dihydrosarkomycin)

295 **Terrein,** $C_8H_{10}O_3$, m.p. 127°, $[\alpha]_{5461}^{20}$ +185° (c 1 in water).

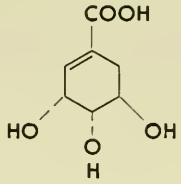



*Aspergillus terreus* Thom, *Penicillium raistrickii*

Harold Raistrick and Geo. Smith, *Biochem. J.* 29 606 (1935). (Isolation)

D. H. R. Barton and E. Miller, *J. Chem. Soc.*, 1028 (1955). (Structure)

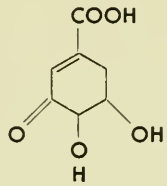
296 **5-Dehydroshikimic Acid,** $C_7H_8O_5$, colorless prisms, m.p. 150–152°, $[\alpha]_D^{28}$ −57° (in ethanol).




*Escherichia coli* mutants

Ivan I. Salamon and Bernard D. Davis, *J. Am. Chem. Soc.* 75 5567 (1953).

297 **Shikimic Acid,** $C_7H_{10}O_5$, colorless crystals, m.p. 184°, $[\alpha]_D^{20}$ −246° (in water).

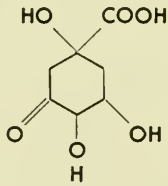



*Escherichia coli*

Yields of about 0.5 g. per liter have been reported.

P. R. Srinivasan, Harold T. Shigeura, Milton Sprescher, David B. Sprinson and Bernard D. Davis, *J. Biol. Chem.* 220 477 (1956).

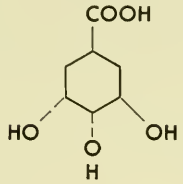
298 **5-Dehydroquinic Acid,** $C_7H_{10}O_6$, colorless crystals, m.p. 140–142°.




*Escherichia coli*

Ulrich Weiss, Bernard D. Davis and Elizabeth S. Mingioli, *J. Am. Chem. Soc.* 75 5572 (1953).

299 **Dihydroshikimic Acid,** $C_7H_{12}O_5$, colorless prisms, m.p. 135°, $[\alpha]_D^{25}$ −63° (c 10 in water).

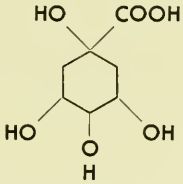



*Lactobacillus pastorianus* var. *quinicus*

A 96% yield was reported.

J. G. Carr, A. Pollard, G. C. Whiting and A. H. Williams, *Biochem. J.* 66 283 (1957).

300 **Cordycepic Acid,** $C_7H_{12}O_6$, colorless needles, m.p. 168°, $[\alpha]_D^{26}$ +6.8° (in water).

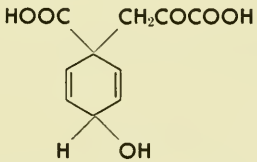



*Cordyceps sinensis* (Berkeley) Saccardo

The yield was 7% of the weight of the dried and defatted mycelium.

R. Chatterjee, K. S. Srinivasan and P. C. Maiti, *J. Am. Pharm. Assoc.* 46 114 (1957).

301 **Prephenic Acid,** $C_{10}H_{10}O_6$, unstable in aqueous solution, isolated as the barium salt.




Mutants of *Escherichia coli* and *Neurospora crassa*

Ulrich Weiss, Charles Gilvarg, Elizabeth S. Mingioli and Bernard D. Davis, *Science* 119 774 (1954).

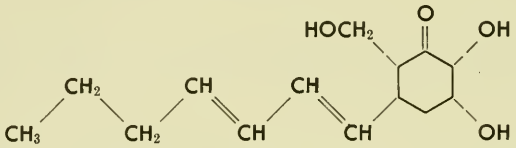
302 **Frequentin,** $C_{14}H_{20}O_4$, colorless needles, m.p. 128°, $[\alpha]_D^{26}$ +68° (0.5 in chloroform).

Probably similar to palitantin in structure.

*Penicillium frequentans* Westling, *P. cyclopium*

P. J. Curtis, H. G. Hemming and W. K. Smith, *Nature* **167** 557 (1951).

303 **Palitantin,** $C_{14}H_{22}O_4$, colorless needles, m.p. 163°, $[\alpha]_{5461}^{23}$ +4.4° (c 0.8 in chloroform).




*Penicillium palitans* Westling, *P. frequentans, P. cyclopium*

John Howard Birkinshaw and Harold Raistrick, *Biochem. J.* **30** 801 (1936).

P. J. Curtis, H. G. Hemming and W. K. Smith, *Nature* **167** 557 (1951).

A. Bracken, Anna Pocker and H. Raistrick, *Biochem. J.* **57** 587 (1954).

K. Bawden, B. Lythgoe and D. J. S. Marsden, *J. Chem. Soc.,* 1162 (1959). (Structure)

304 **B-73,** $C_{15}H_{16}O_2N_2$, colorless plates, m.p. 275°, $[\alpha]_D^{25}$ +3.43° (c 0.4 in dimethylformamide).

Negative ferric chloride test, non-fluorescent under U.V. light, soluble in aqueous sodium hydroxide.

*Streptomyces albulus*

Non-antibiotic compound isolated from a broth containing cycloheximide, 4-acetoxycycloheximide, C-73, and fungicidin.

K. Rao, Abstracts, 134th Meeting of the American Chemical Society, Chicago, September 1958.

305 **C-73,** $C_{15}H_{17}O_4N$, pale yellow needles, m.p. 199°, $[\alpha]_D^{25}$ +5.06° (c 0.4 in dimethylformamide).

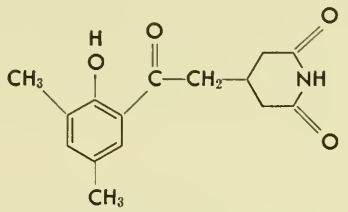
Green ferric chloride test, bright yellow fluorescence in U.V. light, soluble in sodium hydroxide solution.

*Streptomyces albulus*

This antibiotically inert compound was isolated from a culture containing cycloheximide and stereoisomers, 4-acetoxycycloheximide, fungicidin, E-73 and B-73.

K. Rao, Abstracts, 134th Meeting of the American Chemical Society, Chicago, September 1958.

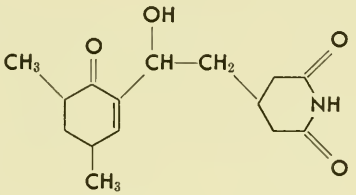
306 **Actiphenol,** $C_{15}H_{17}O_4N$, colorless crystals, m.p. 199°.




An actidione-producing streptomycete (ETH 7796).

R. J. Highet and V. Prelog, *Helv. Chim. Acta* 42 1523 (1959).

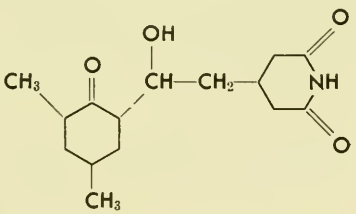
307 **Inactone,** $C_{15}H_{21}O_4N$, colorless needles, m.p. 116°, $[\alpha]_D^{26}$ −55° (c 2 in water).




*Streptomyces griseus*

Raymond Paul and Serge Tchelitcheff, *Bull. soc. chim. France* 1316 (1955).

308 **Cycloheximide** (Actidione, Naramycin A), $C_{15}H_{23}O_4N$, colorless crystals, m.p. 119.5–121°, $[\alpha]_D^{29}$ −3.4° (c 9.47 in ethanol).




*Streptomyces griseus, S. noursei*

Byron E. Leach, Jared H. Ford and Alma J. Whiffen, *J. Am. Chem. Soc.* 69 474 (1947).

Jared H. Ford and Byron E. Leach, *ibid.* 70 1223 (1948).

Edmund C. Kornfeld, Reuben G. Jones and Thomas V. Parke, *ibid.* 71 150 (1949). (Structure)

Tomoharu Okuda, *Chem. Pharm. Bull.* (Japan) 7 659 (1959). (Stereochemistry)

309 **Cycloheximide Diasterioisomer,** $C_{15}H_{23}O_4N$, colorless rectangular plates, m.p. 100–105°, $[\alpha]_D^{25}$ +12°.

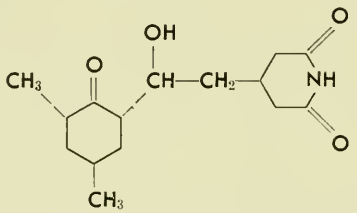
The crystal form differed from that of cycloheximide, and a mixture with authentic cycloheximide melted at 85–95°.

*Streptomyces albulus*

Cycloheximide, 4-acetoxycycloheximide, two antibiotically inert compounds B-73 and C-73 and fungicidin were isolated from the same culture.

K. Rao. Abstracts, 134th Meeting of the American Chemical Society, Chicago, September 1958.

310 **Naramycin B,** $C_{15}H_{23}O_4N$, colorless plates, m.p. 109°, $[\alpha]_D^{12.5}$ +50.2° (c 2.0 in methanol).



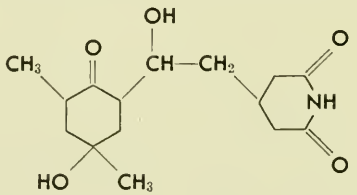

*Streptomyces* sp.

A stereoisomer of cycloheximide.

Tomoharu Okuda, Makato Suzuki, Yoshiyuki Egawa and Kokichi Ashino, *Chem. Pharm. Bull.* (Japan) 6 328 (1958). (Isolation)

Tomoharu Okuda, *ibid.* 7 659 (1959). (Stereochemistry)

311 **Streptovitacin A,** $C_{15}H_{23}O_5N$, colorless crystals, m.p. 156–159°.

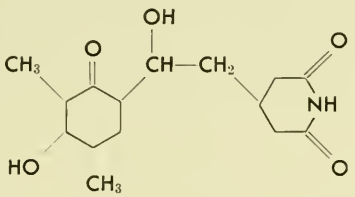



*Streptomyces griseus*

T. E. Eble, M. E. Bergy, C. M. Large, R. R. Herr and W. G. Jackson, "Antibiotics Annual 1958–1959," Medical Encyclopedia, Inc., New York, p. 555. (Isolation)

Ross R. Herr, *J. Am. Chem. Soc.* **81** 2595 (1959). (Structure)

312 **Streptovitacin B,** $C_{15}H_{23}O_5N$, colorless crystals, m.p. 124–128°.

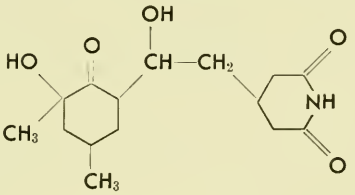



*Streptomyces griseus*

T. E. Eble, M. E. Bergy, C. M. Large, R. R. Herr and W. G. Jackson, "Antibiotics Annual 1958–1959," Medical Encyclopedia, Inc., New York, p. 555. (Isolation)

Ross R. Herr, *J. Am. Chem. Soc.* **81** 2595 (1959). (Structure)

313 **Streptovitacin $C_2$,** $C_{15}H_{23}O_5N$, colorless crystals, m.p. 91–96°.




*Streptomyces griseus*

Ross R. Herr, *J. Am. Chem. Soc.* **81** 2595 (1959). (Structure)

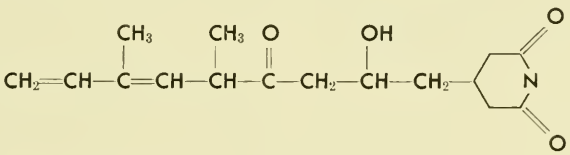
314 **Streptovitacin D,** $C_{15}H_{23}O_5N$, colorless crystals, m.p. 67–69°.

A ring-hydroxylated cycloheximide of unknown structure.

*Streptomyces griseus*

Ross R. Herr, *J. Am. Chem. Soc.* **81** 2595 (1959).

315 **Streptimidone,** $C_{16}H_{23}O_4N$, colorless crystals, m.p. 72°.

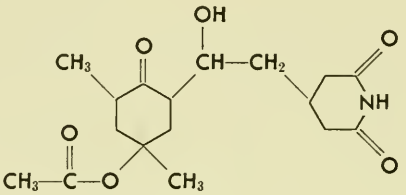



A streptomycete

Roger P. Frohardt, Henry W. Dion, Zbigniew L. Jukabowski, Albert Ryder, James C. French and Quentin R. Bartz, *J. Am. Chem. Soc.* 81 5500 (1959).

E. E. Van Tamelen and V. Haarstad, *J. Am. Chem. Soc.* 82 2974 (1960). (Revised structure)

316 **3-[2-(3,5-Dimethyl-5-acetoxy-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide (4-Acetoxycycloheximide, E-73),** $C_{17}H_{25}O_6N$, colorless crystals, m.p. 140°, $[\alpha]_D^{25}$ −8.8° (c 1.0 in methanol.)



*Streptomyces albulus*

Two diastereoisomers of cycloheximide were isolated from the same broth. Fungicidin and two unknown compounds also were isolated.

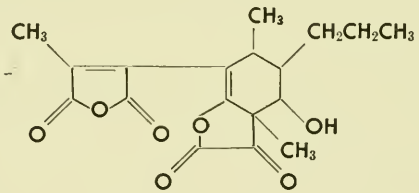
Koppaka V. Rao and Walter P. Cullen, *J. Am. Chem. Soc.* 82 1127 (1960). (Isolation)

Koppaka V. Rao, *ibid.* 82 1129 (1960). (Structure)

317 **Glauconic Acids.**

Glauconic Acid I, $C_{18}H_{20}O_7$, colorless crystals, m.p. 202°, optically inactive.

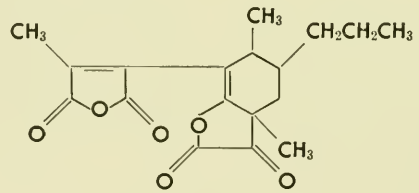
Proposed structure:



and

Glauconic Acid II, $C_{18}H_{20}O_6$, colorless crystals, m.p. 186°, optically inactive.

Proposed structure:



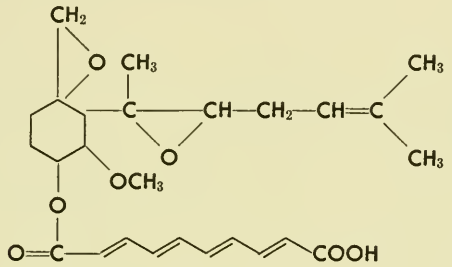
*Penicillium glaucum, P. purpurogenum*

Nadine Wijkman, *Ann.* 485 61 (1931). (Isolation)

Kurt Kraft, *ibid.* 530 20 (1937). (Structure)

Matao Takashima, Akira Kitajima and Kenichi Otsuka, *Nippon Nôgei-kagaku Kaishi* 29 25 (1955). (Isolation from *P. purpurogenum*) (*Chem. Abstr.* 52 20379d)

318 **Fumagillin** (Amebacilin, Fumidil) $C_{26}H_{34}O_7$, colorless or pale yellow crystals, m.p. 189–194° (dec.), $[\alpha]_D^{25}$ −26.6° (c 0.25 in methanol).




*Aspergillus fumigatus* Fres.

J. Landquist, *J. Chem. Soc.*, 4237 (1956).

J. McNally and D. Tarbell, *J. Am. Chem. Soc.* 80 3676 (1958).

D. Chapman and D. Tarbell, *ibid.* 80 3679 (1958).

A. Cross and D. Tarbell, *ibid.* 80 3682 (1958).

R. Carman, D. D. Chapman, N. J. McCorkindale, D. S. Tarbell, F. H. L. Varino, R. L. West and D. L. Wilson, *J. Am. Chem. Soc.* 81 3151 (1959).

D. S. Tarbell, R. M. Carman, D. D. Chapman, K. R. Huffman and N. J. McCorkindale, *J. Am. Chem. Soc.* 82 1005 (1960). (Structure)

T. E. Eble and F. R. Hanson, *Antibiotics and Chemotherapy* 1 54 (1951). (Isolation)

9

# Terpenoids and Steroids

Ergosterol is the principal fungal sterol. It was named for its occurrence in ergot, and it has been isolated from a wide variety of other fungi as well as from lichens. It has been reported to be the only sterol in certain molds,[1] but it is often accompanied by related compounds. It has been identified also in algae. Some yeasts produce several per cent of their dry cell weight in ergosterol. Yeasts which produce large quantities of fat do not necessarily produce a higher proportion of ergosterol.

There have been few reports of the isolation or detection of sterols in bacteria, and there is doubt as to whether bacteria produce sterols. A critical historical review of this question has been published.[2] Mevalonic acid is an acetate-replacing factor in lactobacilli, and a labeling study[3] with paper chromatography and spectral work on the labeled non-saponifiable lipides showed the presence of non-steroid, hydroxylated and unsaturated compounds with more than 15 carbon atoms. It may be that simpler substances of this sort replace sterols in bacteria. An artificial requirement for vitamin $D_2$ can be induced in some bacteria. The resulting inhibition of growth can be reversed by vitamins $D_2$, $D_3$ or suprasterol, but not by 7-dehydroergosterol nor by cholesterol.[3]

Yeasts and higher fungi produce squalene and $C_{27}$ to $C_{31}$ compounds, some of which have been shown to be precursors of cholesterol in mammalian metabolism. Some higher fungi and many lichens produce triterpenes or close derivatives.

Since the availability of isotopes, which permit the tracing of small quantities of material, much of the biosynthetic route to

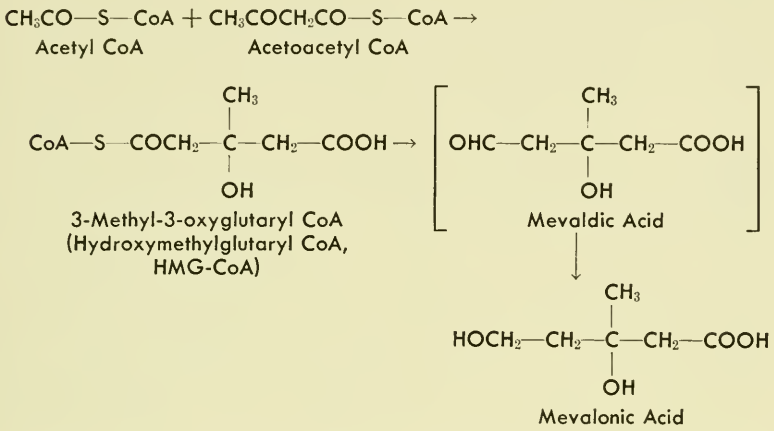
[1] Joseph V. Fiore, *Arch. Biochem.* 16 161 (1948).

[2] Audrey Fiertel and Harold P. Klein, *J. Bacteriol.* 78 738 (1959).

[3] E. Kodicek, Abstracts of the Gordon Conference on Vitamins and Metabolism, 1958.

the principal mammalian sterol, cholesterol, has been worked out. Good reviews of this work are available.[4] Many of the proved intermediates in this route have been isolated from fungi, and evidently the biogenesis of ergosterol and the triterpenes is quite similar to that of cholesterol up to the later stages.[5]

The conversion of acetate to mevalonate follows the course:[6, 6a]




In the light of the newer knowledge concerning the role of malonyl CoA in fatty acid biosynthesis there may eventually be some minor modifications in this scheme. It should be mentioned that mevalonic acid has been shown to be an irreversible intermediate in the biosynthesis of terpenoids.[7, 8, 9]

Isopentenyl pyrophosphate, a further intermediate in the bio-

[4] Louis F. Fieser and Mary Fieser, "Steroids," Reinhold Publishing Corp., New York, 1959, pp. 403–420.

[5] Pierre Crabbé, *Record of Chemical Progress* 20 189 (1959).

[6] J. W. Cornforth, R. H. Cornforth, A. Pelter, M. G. Horning and G. Popják, *Tetrahedron* 5 311 (1959).

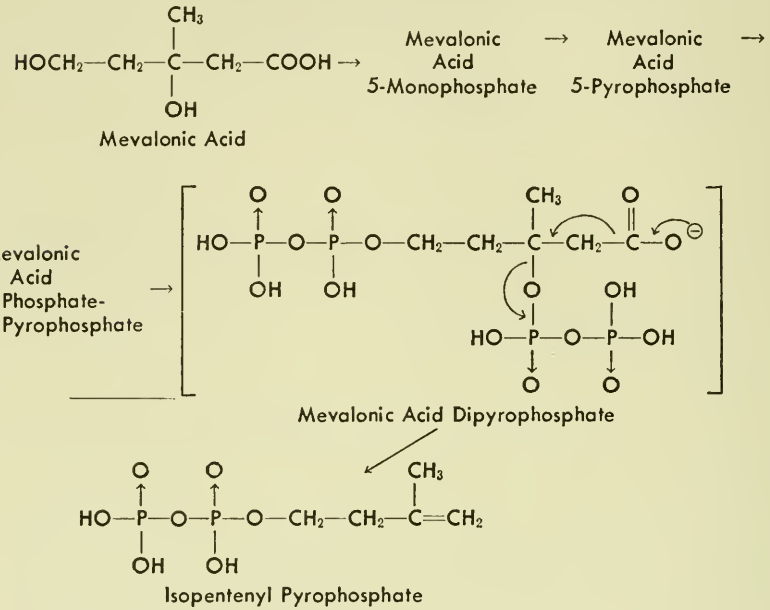
[6a] G. E. W. Wolstenholme and Maeve O'Conner (Eds.), "CIBA Foundation Symposium on the Biosynthesis of Terpenes and Sterols," Harry Rudney, *The biosynthesis of β-hydroxy-β-methylglutaryl coezyme A and its conversion to mevalonic acid,* Little, Brown and Co., Boston, 1959, pp. 75–94.

[7] A. J. Birch, R. J. English, R. A. Massy-Westropp and Herchel Smith, *Proc. Chem. Soc.*, 233 (1957).

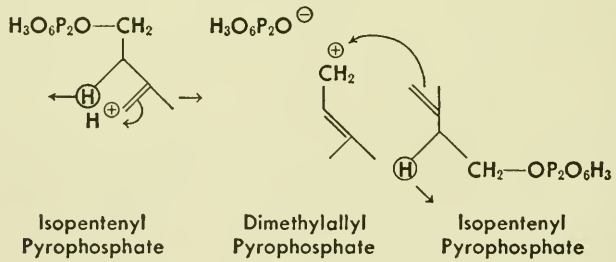
[8] *Idem., J. Chem. Soc.,* 369 (1958).

[9] J. Fishman, E. R. H. Jones, G. Lowe and M. C. Whiting, *Proc. Chem. Soc.,* 127 (1959).

synthetic process, apparently arises from phosphorylated mevalonic acid by a concerted decarboxylation with elimination of the C-3-hydroxyl group, since it has been shown that no protonation of the carbon chain occurs during decarboxylation.[10]



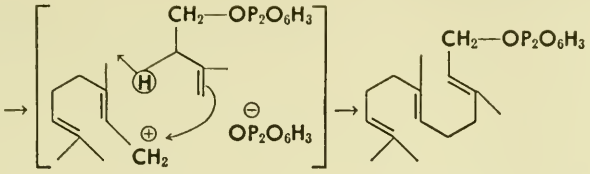

Since both $\gamma,\gamma$-dimethylallyl pyrophosphate[11] and farnesyl pyrophosphate[12] have been isolated, it is possible to envisage a continuation:




[10] A. de Waard, A. H. Phillips and Konrad Bloch, *J. Am. Chem. Soc.* 81 2913 (1959).

[11] B. W. Agranoff, H. Eggerer, U. Henning and F. Lynen, *J. Am. Chem. Soc.* 81 1254 (1959).

[12] F. Lynen, H. Eggerer, U. Henning and Ingrid Kessel, *Angew. Chem.* 70 738 (1958).

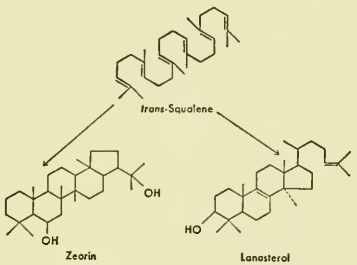



Farnesyl Pyrophosphate

Two moles of farnesyl pyrophosphate then unite head-to-head in what, deuterium experiments indicate,[13, 14] is probably a reductive process to form squalene.* All *trans*-squalene is formed, and this is the only isomer which can cyclize to triterpenes and steroids.[15]

The significance of the stereoisomer has been considered, and a generalized scheme devised for the various modes of cyclization of squalene, supported by the current theories of conformational analysis and ionic cyclization.[16, 17, 18]

Squalene can cyclize with no skeletal rearrangement to form compounds such as the lichen substance, zeorin. It also can rearrange to the lanostane skeleton found so frequently among the steroids of the higher fungi. Lanosterol itself, a known intermediate in the biosynthetic route to cholesterol, has been found in yeast, as has squalene.




[13] H. Rilling, T. T. Tchen and Konrad Bloch, *Proc. Nat. Acad. Sci.* 44 163 (1958).

[14] H. C. Rilling and Konrad Bloch, *J. Biol. Chem.* 234 1424 (1959).

* See addendum for a recent modification of this scheme.

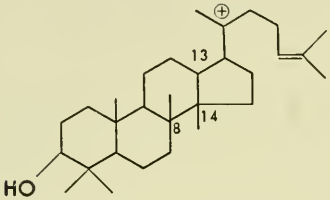
[15] Robert G. Langdon and Konrad Bloch, *ibid.* 200 135 (1953).

[16] L. Ruzicka, A. Eschenmoser and H. Heusser, *Experientia* 9 362 (1953).

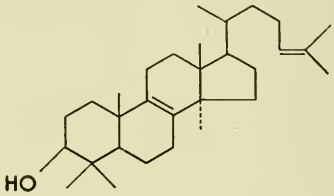
[17] A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, *Helv. Chim. Acta* 38 1890 (1955).

[18] Alexander Todd, "Perspectives in Organic Chemistry," L. Ruzicka, *Bedeutung der theoretischen organischen Chemie für die Chemie der Terpenverbindungen,* Interscience Publishers, Inc., New York, 1956, pp. 265–315; L. Ruzicka, *Proc. Chem. Soc.,* 341–360 (1959); Faraday Lecture, *History of the isoprene rule.*

It is likely that the cyclization of squalene to form such structures occurs by a concerted mechanism which proceeds from ring to ring until complete and that this all occurs on one enzyme surface. Thus, isolation of intermediates between squalene and an initial cyclization product such as the one shown is improbable. The cyclization is oxygen-initiated, explaining the frequent occurrence of the 3-hydroxyl groups in natural steroids.



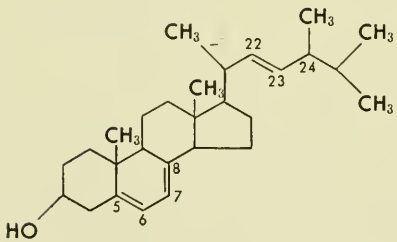

Proposed initial cyclization product of the Squalene → Lanosterol route



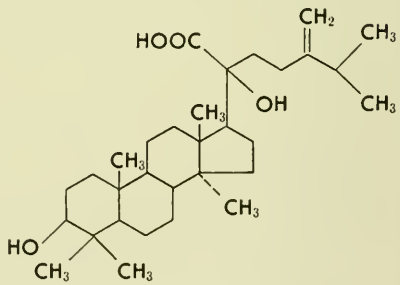

Lanosterol

Transformation of the proposed tetracyclic precursor to lanosterol involves two 1,2-methyl group migrations (14 → 13 and 8 → 14) as shown by tracer experiments.[6, 19]

Eburicoic acid has the lanostane skeleton, but with a methylene group attached to carbon atom 24 of the side-chain. Similarly, ergosterol has a methyl group in this position. Labeling




Ergosterol



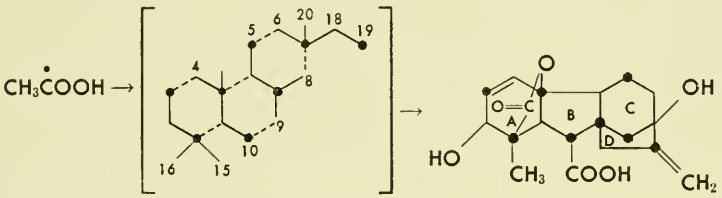

Eburicoic Acid

[19] R. K. Maudgal, T. T. Tchen and Konrad Bloch, *J. Am. Chem. Soc.* **80** 2589 (1958).

experiments[20, 21, 22, 23] have shown that this "extra" carbon atom is not derived from acetate, but is furnished by formate and, more efficiently, by methionine.

Progressing along the biosynthetic route from squalene to ergosterol (and cholesterol), it is obvious that lanosterol must lose the two methyl groups at C-4 and one at C-14. These are probably removed oxidatively, and eventually some of the intermediates may be isolated. Zymosterol has been considered as an intermediate in the biosynthesis of cholesterol; but while it occurs together with ergosterol in yeasts, it has been found[20] that squalene, but not zymosterol, is converted to ergosterol by yeast homogenates.

The biogenesis of the interesting diterpenoids gibberellic acid, rosenonolactone and trichothecin has been studied. In the case of gibberellic acid[24] studies with $CH_3C^{14}OOH$ and with C-2-la-




beled mevalonate gave the labeling pattern shown. A precursor was inferred, and the following deductions made: (a) The methyl carbon atom attached to ring A is derived specifically from position 2 of mevalonic acid lactone. (b) The carboxyl carbon atom is derived specifically from position 9 of the precursor. (c) The phyllocladene ring system of gibberellic acid is formed by migration of C-6 to C-18.

Rosenonolactone, rosololactone and trichothecin are all produced by the fungus *Trichothecium roseum*.

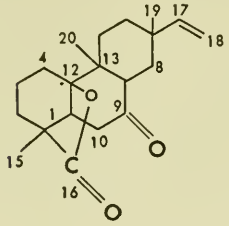
[20] George J. Alexander, Allen M. Gold and Erwin Schwenk, *ibid.* 79 2967 (1957).

[21] William G. Dauben and John H. Richards, *ibid.* 78 5329 (1956).

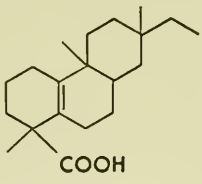
[22] William G. Dauben, Yoshio Ban and John H. Richards, *ibid.* 79 968 (1957).

[23] William G. Dauben, Gerhard J. Fonken and George A. Boswell, *ibid.* 79 1000 (1957).

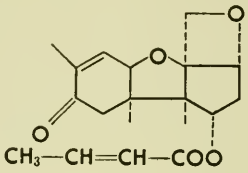
[24] A. J. Birch, R. W. Rickards and Herchel Smith, *Proc. Chem. Soc.*, 192 (1958).




Rosenonolactone



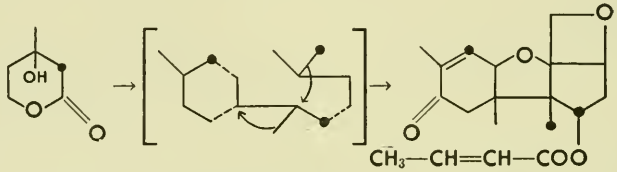

Rosololactone




Trichothecin

The carbon skeleton of rosenonolactone[9] is apparently derived from the same kind of precursor as gibberellic acid, but in a simpler way. All that is required is the migration of a methyl group from C-12 to C-13 in the same manner as in the biosynthesis of steroids.

The carbon skeleton of trichothecin[10] can be derived from a sesquiterpenoid intermediate by way of two 1,2-methyl group shifts:




2-$C^{14}$-Mevalonate

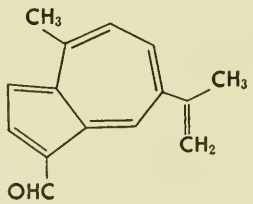
Proposed sesquiterpenoid intermediate

Trichothecin

When labeled acetate was used in this study, 95% of the activity appeared in the crotonic acid moiety. These results, considered together, are another confirmation of the irreversibility of the acetate-mevalonate process.

A symposium has been published thoroughly reviewing current research on the biosynthesis of terpenes and sterols.[25]

319 **Lactaroviolin,** $C_{15}H_{14}O$, red-violet crystals, m.p. 53°.




[25] G. E. W. Wolstenholme and Maeve O'Conner (Eds.), "CIBA Foundation Symposium on the Biosynthesis of Terpenes and Sterols," Little, Brown and Co., Boston, 1959.

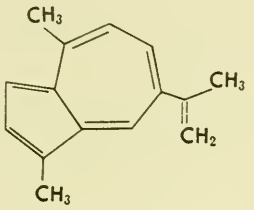
*Lactarius deliciosus* L.

Harry Willstaedt and B. Zetterberg, *Svensk Kem. Tidskr.* 58 306 (1946).

Pl. A. Plattner, E. Heilbronner, R. W. Schmid, R. Sandrin and A. Fürst, *Chem. and Ind.*, 1202 (1954). (Structure)

E. Heilbronner and R. W. Schmid, *Helv. Chim. Acta* 37 2018 (1954).

320 Lactarazulene, $C_{15}H_{16}$, blue liquid, b.p. 155–160° (2.5–3 mm.).




*Lactarius deliciosus* L.

321 Occurs together with lactaroviolin (q.v.) and a green crystalline compound, **Verdazulene**, $C_{15}H_{16}$, m.p. 90°.

Frantisek Sorm, Vera Benesova and Vlastimil Herout, *Chem. Listy* 47 1856 (1953). (Structure)

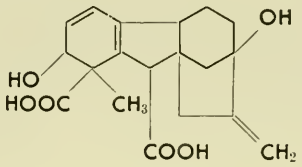
## Gibberellins and Gibberellenic Acid

Although gibberellic acid is the gibberellin produced in highest yield by *Gibberella fujikuroi*, three minor gibberellins are produced also, and the crude mixture is commonly isolated. The minor gibberellins are called $A_1$, $A_2$ and $A_4$, gibberellic acid being $A_3$. (It also has been called gibberellin X.) Their structures are similar to those of gibberellic acid.

Gibberellin $A_1$ has been found in plants as well as in fungi. All of the four gibberellins show plant hormone activity. A fifth, inactive, compound called gibberellenic acid has been isolated recently. It may be an artifact.

A structure for gibberellic acid was advanced in 1956 by an English group. Structure work has continued in Japan, where the gibberellins were originally isolated, and recently structures for all the gibberellins have been published which differ somewhat from the one first advanced in England. Even more recently a third set of structures, complete with stereochemistry, has been proposed by the English school. It is these structures which are shown here.

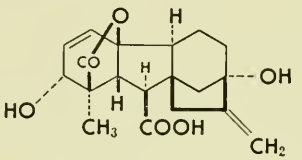
322 **Gibberellenic Acid**, $C_{19}H_{22}O_6$, colorless crystals, m.p. 185° (dec.). Strong U.V. absorption at 253 m$\mu$ ($\epsilon$ = 19,200).




*Fusarium moniliforme*

Koert Gerzon, Harold L. Bird, Jr. and Don O. Woolf, Jr., *Experientia* 13 487 (1957).

323 **Gibberellic Acid** (Gibberellin $A_3$, Gibberellin X), $C_{19}H_{22}O_6$, colorless crystals, m.p. 235° (dec.), $[\alpha]_D^{20}$ +92°. Proposed complete stereochemical structure:




P. J. Curtis and B. E. Cross, *Chem. and Ind.*, 1066 (1954). (Isolation)

B. E. Cross, John Frederick Grove, J. MacMillan and T. P. C. Mulholland, *Chem. and Ind.*, 954 (1956). (Structure)

Brian E. Cross, *J. Chem. Soc.*, 4670 (1954).

Nobutaka Takahashi, Yasuo Seta, Hiroshi Kitamura and Yusuke Sumiki, *Bull. Agr. Chem. Soc.* (Japan) 23 405 (1959).

Hiroshi Kitamura, Yasuo Seta, Nobutaka Takahashi, Akira Kawarada and Yusuke Sumiki, *ibid.* 23 408 (1959).

Yasuo Seta, Nobutaka Takahashi, Akira Kawarada, Hiroshi Kitamura and Yusuke Sumiki, *ibid.* 23 412 (1959).

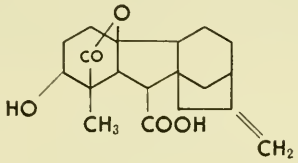
Nobutaka Takahashi, Yasuo Seta, Hiroshi Kitamura, Akira Kawarada and Yusuke Sumiki, *ibid.* 23 493 (1959).

Yasuo Seta, Nobutaka Takahashi, Hiroshi Kitamura, Makoto Takai, Sahuro Tamura and Yusuke Sumiki, *ibid.* 23 499 (1959).

Nobutaka Takahashi, Yasuo Seta, Hiroshi Kitamura and Yusuke Sumiki, *ibid.* 23 509 (1959).

B. E. Cross, J. F. Grove, P. McCloskey and T. P. C. Mulholland, *Chem. and Ind.*, 1345 (1959); B. E. Cross, John Frederick Grove, J. MacMillan, J. S. Moffatt, T. P. C. Mulholland and J. C. Seaton, *Proc. Chem. Soc.*, 302 (1959).

324 **Gibberellin $A_4$**, $C_{19}H_{24}O_5$, colorless crystals, m.p. 222° (dec.), $[\alpha]_D^{20}$ −20.8° (c 0.34 in methanol).

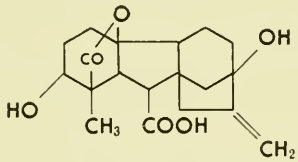



*Gibberella fujikuroi* (Saw.) Wollenweber

Nobutaka Takahashi, Yasuo Seta, Hiroshi Kitamura and Yusuke Sumiki, *Bull. Agr. Chem. Soc.* (Japan) 21 396 (1957). (Isolation of Gibberellin $A_4$)

See other references under Gibberellin $A_1$ for structure work.

325 **Gibberellin $A_1$**, $C_{19}H_{24}O_6$, colorless crystals, m.p. 255–258° (dec.), $[\alpha]_D^{25}$ +36°.




*Gibberella fujikuroi* (Saw.) Wollenweber

Nobutaka Takahashi, Hiroshi Kitamura, Akira Kawarada, Yasuo Seta, Makato Takai, Suburo Tamura and Yusuke Sumiki, *Bull. Agr. Chem. Soc.* (Japan) 19 267 (1955). (Isolation of gibberellins and their properties)

Nobutaka Takahashi, Yasuo Seta, Hiroshi Kitamura and Yusuke Sumiki, *ibid.* 23 405 (1959).

Hiroshi Kitamura, Yasuo Seta, Nobutaka Takahashi, Akira Kawarada and Yusuke Sumiki, *ibid.* 23 408 (1959). (Structures of the gibberellins)

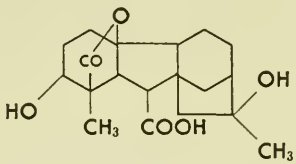
Nobutaka Takahashi, Yasuo Seta, Hiroshi Kitamura, Akira Kawarada and Yusuke Sumiki, *ibid.* 23 493 (1959). (Structures of the gibberellins).

Yasuo Seta, Nobutaka Takahashi, Hiroshi Kitamura, Makato Tokai, Saburo Tamura and Yusuke Sumiki, *ibid.* 23 499 (1959). (Structures of the gibberellins)

Nobutaka Takahashi, Yasuo Seta, Hiroshi Kitamura and Yusuke Sumiki, *ibid.* 23 509 (1959). (Structures of the gibberellins)

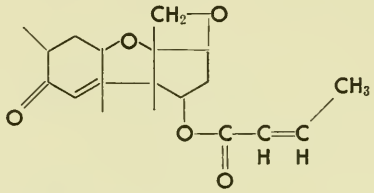
B. E. Cross, John Frederick Grove, J. MacMillan, J. S. Moffatt, T. P. C. Mulholland and J. C. Seaton, *Proc. Chem. Soc.*, 302 (1959). (Above structure)

326 **Gibberellin $A_2$**, $C_{19}H_{26}O_6$, colorless crystals, m.p. 235–237° (dec.), $[\alpha]_D$ +11.7°.




*Gibberella fujikuroi* (Saw.) Wollenweber
See references under Gibberellin $A_1$.

327 **Trichothecin**, $C_{19}H_{24}O_5$, colorless needles, m.p. 118°, $[\alpha]_D^{18}$ +44° (c 1.0 in chloroform).



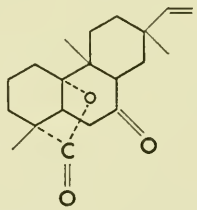

*Trichothecium roseum* (Link)

G. G. Freeman and R. I. Morrison, *Nature* 162 30 (1948).

G. G. Freeman, J. E. Gill and W. S. Waring, *J. Chem. Soc.*, 1105 (1959). (Structure)

J. Fishman, E. R. H. Jones, G. Lowe and M. C. Whiting, *Proc. Chem. Soc.*, 127 (1959). (Structure)

328 **Rosenonolactone**, $C_{20}H_{28}O_3$, white prisms, m.p. 208°, $[\alpha]_D^{18}$ −116° (c 2.0 in chloroform).



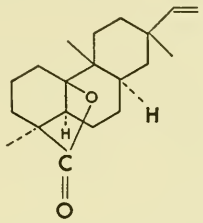

*Trichothecium roseum* (Link)

About 6 g. of dry mycelium were obtained from a liter of culture solution, and from this about 0.2 g. of rosenonolactone was extracted.

Alexander Robertson, W. R. Smithies and Eric Tittensor, *J. Chem. Soc.*, 879 (1949). (Isolation)

Adelaide Harris, Alexander Robertson and W. B. Whalley, *ibid.*, 1799 (1958). (Structure)

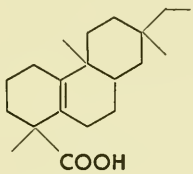
329 **9-Deoxorosenonolactone,** $C_{20}H_{30}O_2$, colorless crystals, m.p. 115°, $[\alpha]_D$ +57° (in chloroform).




*Trichothecium roseum* (Link)

W. B. Whalley, B. Green, D. Arigoni, J. J. Britt and Carl Djerassi, *J. Am. Chem. Soc.* 81 5520 (1959).

330 **Rosololactone,** $C_{20}H_{30}O_3$, white crystals, m.p. 186°, $[\alpha]_D^{18}$ +6.3° (c 2.3 in chloroform).



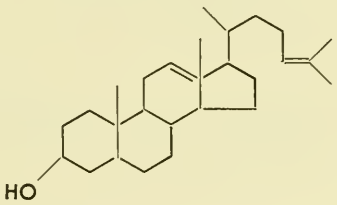

*Trichothecium roseum* (Link)

Rosololactone is a minor product of this fermentation. It occurs in the mycelium together with rosenonolactone and mannitol.

Alexander Robertson, W. R. Smithies and Eric Tittensor, *J. Chem. Soc.*, 879 (1949). (Isolation)

Adelaide Harris, Alexander Robertson and W. B. Whalley, *ibid.*, 1807 (1958). (Structure)

331 **Zymosterol,** $C_{27}H_{44}O$, colorless crystals, m.p. 110°, $[\alpha]_D$ +49°.




Zymosterol is second to ergosterol in abundance in yeast fat.

Ida Smedley-MacLean, *Biochem. J.* 22 22 (1928). (Isolation)

332 **Hyposterol,** tentatively $C_{27}H_{42}O$ or $C_{27}H_{44}O$, colorless unstable crystals, m.p. 100–102°, $[\alpha]_D^{20}$ +12.5° (in chloroform).

Structure unknown. May be a $C_{28}$ sterol.

Yeasts

Heinrich Wieland and G. A. C. Gaugh, *Ann.* 482 36 (1930).

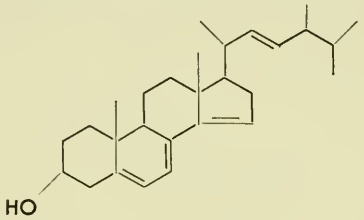
333 **Anasterol,** $C_{27}H_{44}O$, colorless crystals, m.p. 157–159°, $[\alpha]_D^{25}$ −8.1° (in chloroform).

Structure unknown. May be a $C_{28}$ sterol.

Yeasts

Heinrich Wieland and G. A. C. Gaugh, *Ann.* 482 36 (1930).

334 **14-Dehydroergosterol,** $C_{28}H_{42}O$, colorless needles, m.p. 198–201°, $[\alpha]_D$ −396° (in carbon tetrachloride).



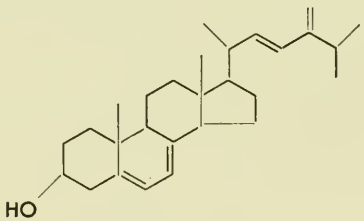

*Aspergillus niger*

Ergosterol was isolated from the same culture.

D. H. R. Barton and T. Bruun, *J. Chem. Soc.*, 2728 (1951).

335 **24(28)-Dehydroergosterol (5,7,22,24(28)-Ergostatetraen-3-β-ol),** $C_{28}H_{42}O$, colorless crystals (Monohydrate), m.p. 118–120°, $[\alpha]_D^{25}$ −78° (1% in chloroform).

Probable structure:

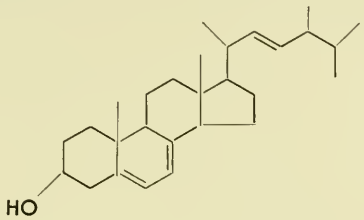



Yeasts

Under appropriate growth conditions, yields of this sterol equal those of ergosterol.

O. N. Breivek, J. L. Owades and R. F. Light, *J. Org. Chem.* 19 1734 (1954).

336 **Ergosterol,** $C_{28}H_{44}O$, colorless crystals, m.p. 165°, $[\alpha]_D^{25}$ −130° (in chloroform).




Ergosterol is the most abundant sterol of yeasts and molds. It occurs widely and was isolated first from ergot (*Claviceps purpurea*). It also occurs in lichens and has been detected in *Euglena* spp. There is much literature, one recent example being:

Akira Saito, *J. Fermentation Technol.* (Japan) 31 140 (1953).

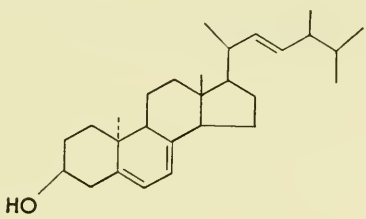
Yields as high as 2–2.7% of dry cell weight have been reported, by using *Saccharomyces carlsbergensis*. Ergosterol is reported to be the only sterol occurring in several species of Fusaria. It occurs as the palmitate in *Penicillium* spp. and in *Aspergillus fumigatus*.

Albert E. Oxford and Harold Raistrick, *Biochem. J.* 27 1176 (1933).

P. Wieland and V. Prelog, *Helv. Chim. Acta* 30 1028 (1947).

Joseph V. Fiore, *Arch. Biochem.* 16 161 (1948).

337 **Pyrocalciferol,** $C_{28}H_{44}O$, colorless needles, m.p. 93–95°, $[\alpha]_D^{20}$ +502° (in alcohol).



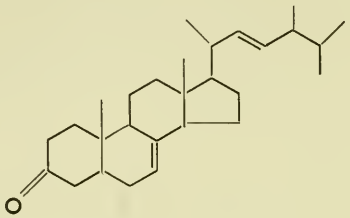

*Penicillium notatum*

A yield of 12 mg. was obtained from 450 g. of dry mycelium.

A. Angeletti, G. Tappi and G. Biglino, *Ann. Chim.* (Rome) 42 502 (1952).

J. Castells, E. R. H. Jones, G. D. Meakins and R. W. J. Williams, *J. Chem. Soc.*, 1159 (1959). (Structure)

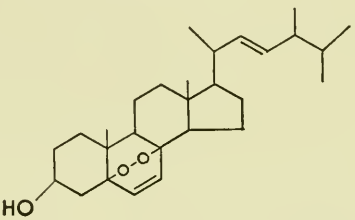
338 **Ergosta-7,22-dien-3-one,** $C_{28}H_{44}O$, m.p. 184–187°, $[\alpha]_D$ +6° (in chloroform).




*Fomes fomentarius*

H. R. Arthur, T. G. Halsall and R. D. Smith, *J. Chem. Soc.*, 2603 (1958).

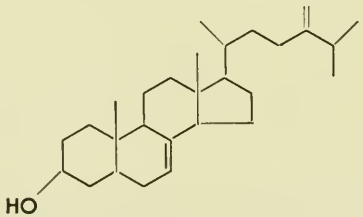
339 **Ergosterol Peroxide,** $C_{28}H_{44}O_3$, colorless crystals, m.p. 178°, $[\alpha]_D$ −36°.




*Aspergillus fumigatus* (mycelium)

P. Wieland and V. Prelog, *Helv. Chim. Acta* 30 1028 (1947).

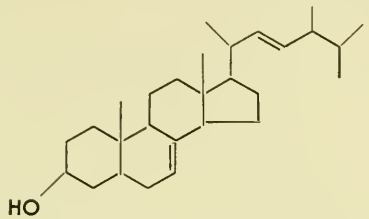
340 **Episterol** ($\Delta^{7,24(28)}$-Ergostadien-3$\beta$-ol), $C_{28}H_{46}O$, colorless crystals, m.p. 150°, $[\alpha]_D$ −5° (in chloroform).




Yeasts

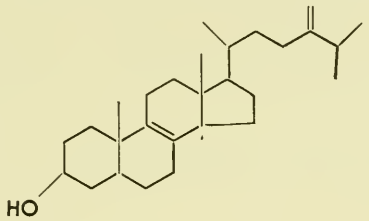
Heinrich Wieland, Fridolf Rath and Horst Hesse, *Ann.* 548 34 (1941).

341 **5,6-Dihydroergosterol** ($\Delta^{7,22}$-Ergostadien-3$\beta$-ol), $C_{28}H_{46}O$, colorless crystals, m.p. 176°, $[\alpha]_D^{20\pm5}$ −19° (in chloroform).



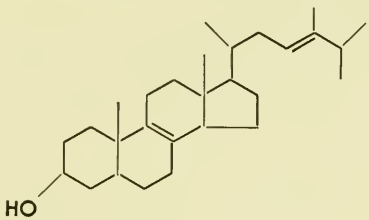

Yeasts, *Claviceps purpurea*
Heinrich Wieland and Willi Benend, *Ann.* 554 1 (1943).
D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1354 (1948).

342 **Fecosterol** ($\Delta^{8,24(28)?}$-Ergostadien-3$\beta$-ol), $C_{28}H_{46}O$, colorless crystals, m.p. 161–163°, $[\alpha]_D^{25}$ +42° (in chloroform).




Yeasts
Heinrich Wieland, Fridolf Rath and Horst Hesse, *Ann.* 548 34 (1941).
D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 214 (1949).

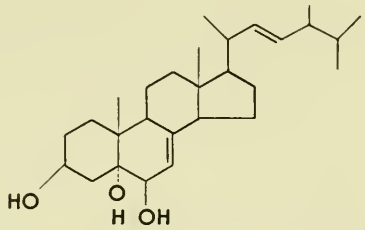
343 **Ascosterol** ($\Delta^{8,23(?)}$-Ergostadien-3$\beta$-ol), $C_{28}H_{46}O$, colorless crystals, m.p. 146°, $[\alpha]_D^{23}$ +45° (in chloroform).




Yeasts

Heinrich Wieland, Fridolf Rath and Horst Hesse, *Ann.* 548 34 (1951).

344 **Cerevisterol** ($\Delta^{7,22}$-Ergostadiene-3$\beta$,5$\alpha$,6$\beta$-triol), $C_{28}H_{46}O_3$, colorless crystals, m.p. 256–259°, $[\alpha]_D$ −83° (c 0.89 in pyridine).

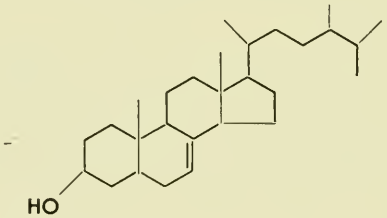



Yeasts, *Claviceps purpurea* (ergot), *Amanita phalloides* About 10 g. were obtained from 4500 kg. of dried yeast. Some 20 g. were obtained from 17 kg. of dry *Amanita phalloides.*

Heinrich Wieland and Gustav Coutelle, *Ann.* 548 275 (1941). (Isolation)

G. H. Alt and D. H. R. Barton, *J. Chem. Soc.,* 1356 (1954). (Structure)

345 **Fungisterol** ($\Delta^7$-Ergosten-3$\beta$-ol), $C_{28}H_{48}O$, colorless crystals, m.p. 149°, $[\alpha]_D^{23}$ −0.2° (in chloroform).

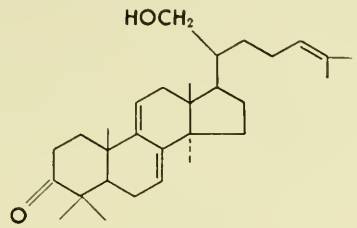



Fungisterol accompanies ergosterol in ergot, occurs in *Amanita phalloides, Penicillium chrysogenum, Rhizopus saponicus, Calocera viscosa, Polyporus confluens* Fr., P. *sulfureus* (Bull.) Fr., *Hydnum imbricatum* L., *Geaster fimbriatus* Fr.

Heinrich Wieland and Gustav Coutelle, *Ann.* 548 270 (1941). (Structure)

Akira Saito, *J. Fermentation Technol.* (Japan) 29 310 (1951).

346 **21-Hydroxylanosta-7,9(11)-24-triene-3-one,** $C_{30}H_{46}O_2$, colorless needles, m.p. 119–121°, $[\alpha]_D$ +56° (c 0.97 in chloroform).

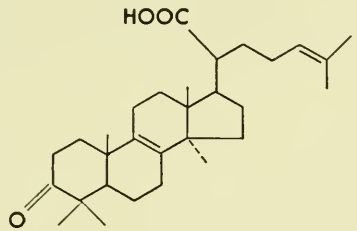



*Polyporus pinicola* Fr.

The derivative reduced and acetylated in the 3-position was isolated from the same specimen as were fungisterol and ergosta-7,22-diene-3-one.

T. G. Halsall and G. C. Sayer, *J. Chem. Soc.*, 2031 (1959).

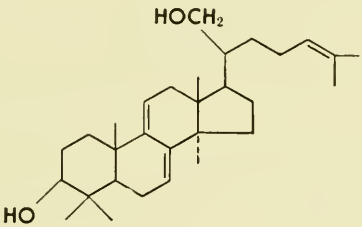
347 **Pinicolic Acid A,** $C_{30}H_{46}O_3$, colorless needles, m.p. 197–202°, $[\alpha]_D$ +68° (c 0.83 in chloroform).




*Polyporus pinicola* Fr.

Joyce M. Guider, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.*, 4471 (1954).

348 **Lanosta-7,9(11)-24-triene-3β,21-diol,** $C_{30}H_{48}O_2$, colorless needles, m.p. 194–197°, $[\alpha]_D$ +72° (c 1.06 in chloroform).

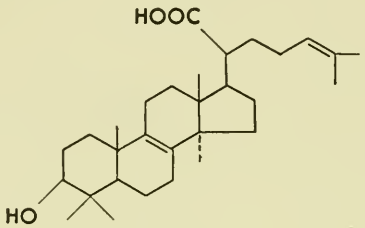


*Polyporus pinicola* Fr.

The corresponding 3-ketone was isolated from the same specimen as well as a mixture of fungisterol and ergosta-7,22-diene-3-one.

T. G. Halsall and G. C. Sayer, *J. Chem. Soc.*, 2031 (1959).

349 **3β-Hydroxylanosta-8,24-diene-21-oic Acid** (Trametenolic Acid B), $C_{30}H_{48}O_3$, colorless needles, m.p. 253–258°, $[\alpha]_D$ +43° (c 0.94 in pyridine).



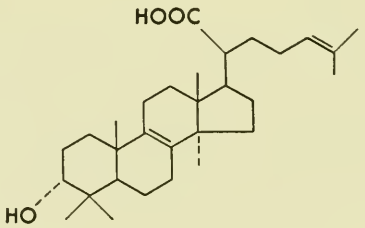

*Trametes odorata* (Wulf.) Fr.

Three other acids were isolated as their methyl esters from the same specimen: Ester A; m.p. 159–165°, $[\alpha]_D$ +49°. Ester B, m.p. 152°, $[\alpha]_D$ +66° and Ester C, m.p. 197–199°.

T. G. Halsall, R. Hodges and G. C. Sayer, *J. Chem. Soc.*, 2036 (1959).

T. G. Halsall and E. R. H. Jones, *Fortschr. Chem. org. Naturst.* 12 95 (1955). (A review)

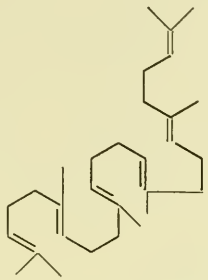
350 **3α-Oxylanosta-8,24-diene-21-oic Acid,** $C_{30}H_{48}O_3$, isolated as the methyl ester-acetate.




*Polyporus pinicola* Fr.

J. J. Beereboom, H. Fazakerley and T. G. Halsall, *J. Chem. Soc.*, 3437 (1957).

351 **Squalene**, $C_{30}H_{50}$, pale yellow oil with blue fluorescence, b.p. 203° (0.15 mm.), $n_D^{20}$ 1.4965. Often isolated as the hydrogen chloride addition product.



Yeasts, *Claviceps purpurea* (ergot), *Amanita phalloides*

Squalene may constitute as much as 16% of brewers' yeast lipide.

A. H. Cook, "The Chemistry and Biology of Yeasts," A. A. Eddy, *Aspects of the chemical composition of yeast,* Academic Press, New York, 1958, pp. 207–208.

K. Täufel, H. Thaler and H. Schreyegg, *Fettchem. Umschau* 43 26 (1936).

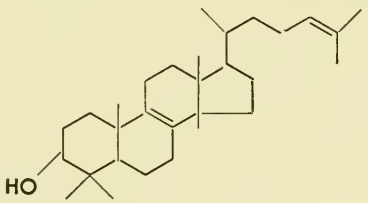
About 3 g. were obtained from 17 kg. of *Amanita phalloides.*

Heinrich Wieland and Gustav Coutelle, *Ann.* 548 275 (1941).

Nearly 25% of the unsaponifiable fraction of the fat of *Torula utilis* were found to be squalene.

R. Reichert, *Helv. Chim. Acta* 28 484 (1945).

352 **Lanosterol** (Kryptosterol, $\Delta^{8,24}$-Lanostadien-3-ol), $C_{30}H_{50}O$, colorless crystals, m.p. 138°, $[\alpha]_D$ +62° (in chloroform).




Yeasts

Heinrich Wieland, Heinrich Pasedach and Albert Ballauf, *Ann.* 529 68 (1937).

L. Ruzicka, R. Denss and O. Jeger, *Helv. Chim. Acta* 29 204 (1946).

W. Voser, M. V. Mijovic, H. Heusser, O. Jeger and L. Ruzicka, *ibid.* 35 2414 (1952).

353 **Physarosterol,** $C_{30}H_{52}O_3$, colorless crystals, m.p. 137–139°, $[\alpha]_D^{28}$ −55.3° (c 0.5 in chloroform).

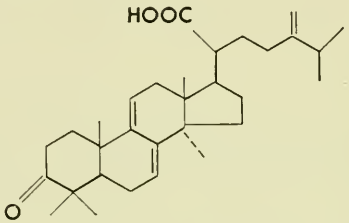
Probably a $C_{30}$, unsaturated, trihydroxy sterol with one hydroxyl group in the 3β-position.

*Physarum polycephalum*

This organism also produces a yellow pigment.

C. F. Emanuel, *Nature* 182 1234 (1958).

354 **Polyporenic Acid C,** $C_{31}H_{46}O_4$, colorless crystals, m.p. 273–276°, $[\alpha]_D$ +8° (in pyridine).




*Polyporus betulinus*, *P. benzoinus*, *P. pinicola*

A. Bowers, T. G. Halsall and G. C. Sayer, *J. Chem. Soc.*, 3070 (1954).

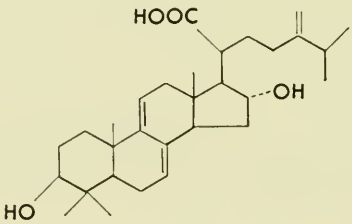
355 **Agaricolic Acid,** $C_{31}H_{48}O_3$ (probably), colorless crystals, m.p. 226°, $[\alpha]_D^{20}$ +34.4° (in pyridine).

Probably a monohydroxy triterpene acid. It occurs together with ergosterol and eburicoic acid, agaricic acid and other metabolites.

*Polyporus officinalis*

J. Valentin and S. Knülter, *Pharm. Zentralhalle* 96 478 (1957).

356 **Dehydrotumulosic Acid,** $C_{31}H_{48}O_4$.

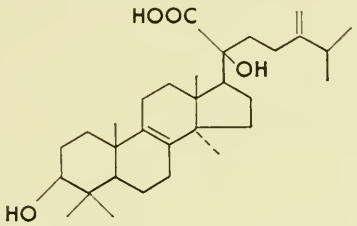


*Polyporus tumulosus* Cooke, *P. australiensis* Wakefield, *P. betulinus*, *Poria cocos*

This acid has never been separated completely from its mixture with tumulosic aicd, but the structure has been deduced from physical measurements.

L. A. Cort, R. M. Gascoigne, J. S. E. Holker, B. J. Ralph, Alexander Robertson and J. J. H. Simes, *J. Chem. Soc.*, 3713 (1954).

357 **Eburicoic Acid,** $C_{31}H_{50}O_3$, colorless crystals, m.p. 292–293°, $[\alpha]_D^{17}$ +50° (c 1.2 in chloroform).

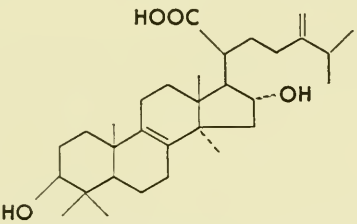



*Polyporus officinalis* Fr., *P. anthracophilus*, Cooke, *P. eucalyptorum* Fr., *P. sulfureus* (Bull.) Fr., *P. hispidus* (Bull.) Fr., *Poria cocos* (Schw.) Wolf, *Lentinus dactyloides* Cleland.

The yield is 50% of the dry mycelial weight in some cases. The 3$\beta$-acetate also occurs naturally in at least some of these basidiomycetes.

J. S. E. Holker, A. D. G. Powell, Alexander Robertson, J. J. H. Simes, R. S. Wright and (in part) R. M. Gascoigne, *J. Chem. Soc.*, 2422 (1953). (Structure)

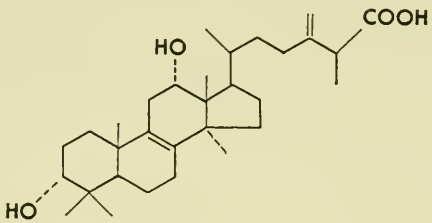
358 **Tumulosic Acid,** $C_{31}H_{50}O_4$, colorless fine needles, m.p. 306° (dec.), $[\alpha]_D$ +8.1° (c 3.30 in pyridine).




*Polyporus tumulosus* Cooke, *P. australiensis* Wakefield, *P. betulinus* Fr., *Poria cocos* Wolf, *Poria cocos* (Schw.) Wolf (syn. *Pachyma hoelen* Rumph.)

L. A. Cort, R. M. Gascoigne, J. S. E. Holker, B. J. Ralph, Alexander Robertson and J. J. H. Simes, *J. Chem. Soc.*, 3713 (1954).

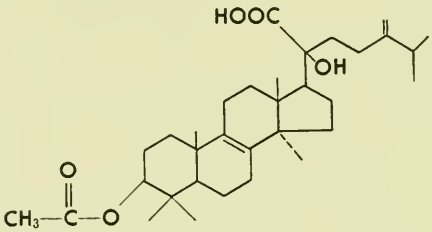
359 **Polyporenic Acid A** (Ungulinic Acid), $C_{31}H_{50}O_4$, colorless needles, m.p. 199–200° (dec.), $[\alpha]_D^{20}$ +64° (c 1.28 in pyridine).




*Polyporus betulinus* Fr.

T. G. Halsall and R. Hodge, *J. Chem. Soc.*, 2385 (1954). (Structure)

360 **O-Acetyleburicoic Acid,** $C_{33}H_{52}O_4$, colorless needles, m.p. 256–259°, $[\alpha]_D^{25}$ +33.4° (in pyridine).

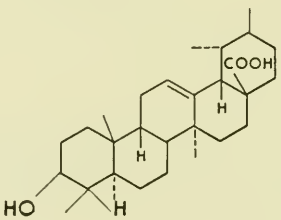



*Polyporus anthracophilus*

R. M. Gascoigne, J. S. E. Holker, B. J. Ralph and Alexander Robertson, *J. Chem. Soc.*, 2346 (1951).

F. N. Lakey and P. H. A. Strasser, *ibid.*, 873 (1951). (Structure)

361 **Ursolic Acid** (probable structure), $C_{30}H_{48}O_3$, colorless crystals, m.p. 291–292°, $[\alpha]_D^{27}$ +72° (in 1:1 chloroform-methanol).



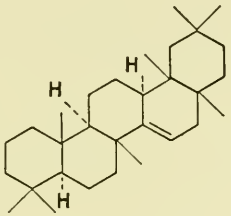
*Cladonia sylvatica* L. Harm., *Cl. impexa* Harm.

This acid also occurs in animals and plants. Since pentacyclic triterpenes are not characteristic of molds, they may be produced by the algal partner of the symbiont lichen.

T. W. Breaden, J. Keane and T. J. Nolan, *Sci. Proc. Roy. Dublin Soc.* 23 197 (1944).

A. Zürcher, O. Jerger and L. Ruzicka, *Helv. Chim. Acta* 37 2145 (1954).

362 **Taraxerene,** $C_{30}H_{50}$, colorless crystals, m.p. 237°, $[\alpha]_D$ +1° (c 0.81 in chloroform).

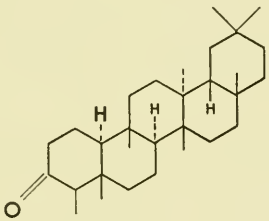



*Cladonia deformis* Hoffm.

About 15 mg. of pure hydrocarbon were obtained from 2.9 kg. of dry lichen.

Torger Bruun, *Acta Chem. Scand.* 8 71 (1954).

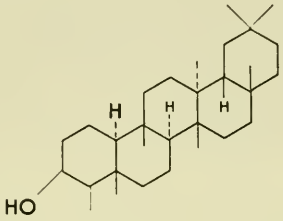
363 **Friedelin,** $C_{30}H_{50}O$, colorless crystals, m.p. 267–269° (dec.) (vac.), $[\alpha]_D$ −21° (c 2.34 in chloroform).




*Cetraria nivalis* (L.) Ach., *C. islandica* (L.) Ach., *C. cucullata* (Bell.) Ach., *C. crispa* (Ach.) Nyl., *C. delisei* (Bory) Th. Fr. (syn. *hiascens* Fr.), *Cladonia alpestris* (L.) Rabh., *Alectoria ochroleuca* (Ehrh.) Nyl. and *Stereocaulon paschale* (L.) Fr.

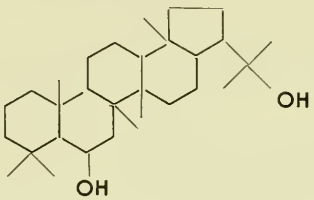
Torger Bruun, *Acta Chem. Scand.* 8 71 (1954).

364 **epi-Friedelinol**, $C_{30}H_{52}O$, colorless crystals, m.p. 280° (vac.), $[\alpha]_D$ +23° (c 0.52 in chloroform).




*Cetraria nivalis* (L.) Ach.
Torger Bruun, *Acta Chem. Scand.* 8 71 (1954).

365 **Zeorin**, $C_{30}H_{52}O_2$, colorless crystals, m.p. 223–227°, $[\alpha]_D$ +54° (c 0.50 in chloroform).




*Lecanora sordida, L. thiodes, L. epanora, L. sulfurea, Physcia caesia, Ph. endococcina, Anaptychia speciosa, A. hypoleuca, Parmelia leucotyliza, Dimelaena oreina, Haematomma coccineum, H. leiphaemum, H. prophyrium, Placodium saxicolum, Peltigera malacea, P. horizontalis, P. propagulifera, Nephroma arcticum, N. antarcticum, N. laevigatum, N. parile, Cladonia deformis, Coccifera pleurota, C. bellidiflora, Urceolaria cretacea, Lepraria latebrarum*

"Elsevier's Encyclopedia of Organic Chemistry," 14 Suppl., Elsevier Publishing Co., London, 1952, p. 1197S. (Occurrence)

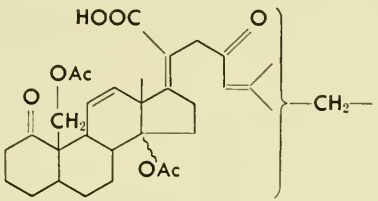
D. H. R. Barton and T. Bruun, *J. Chem. Soc.*, 1683 (1952).

D. H. R. Barton, P. de Mayo and J. C. Orr, *ibid.*, 2239 (1958).

366 **Leucotylin**, $C_{30}H_{52}O_3$, colorless prisms, m.p. 333°, $[\alpha]_D^{24}$ +49.43°. A triterpenoid compound accompanying zeorin.
*Parmelia leucotyliza* Nyl.
Yasuhiko Asahina and Hirosi Akagi, *Ber.* 71B 980 (1938).

367 **Helvolic Acid** (Fumigacin, May = Mycocidin), $C_{32}H_{42}O_8$, colorless fine needles, m.p. 211° (dec.), $[\alpha]_D^{25}$ −124° (in chloroform).

Tentative partial structure:




*Aspergillus fumigatus* mut. *helvola* Yuill

Donald J. Cram and Norman L. Allinger, *J. Am. Chem. Soc.* 78 5275 (1956). (Structure)

E. Chain, H. W. Florey, M. A. Jennings and T. I. Williams, *Brit. J. Exp. Pathol.* 24 108 (1943). (Isolation)

CIBA Lectures in Microbial Chemistry, E. P. Abraham, "Biochemistry of Some Peptide and Steroid Antibiotics," *The cephalosporins*, John Wiley and Sons, New York, 1957, pp. 30–63. (A review)

## Cephalosporins P

These non-peptide compounds accompany cephalosporins N and C in *Cephalosporium salmosynnematum* fermentations.

TABLE I

| | Compound | Crystal form | Melting point | $[\alpha]_D$ | Molecular formula |
|---|---|---|---|---|---|
| 368 | **Cephalosporin $P_1$** | colorless needles | 147° | +28° (c 2.7 in chloroform) | $C_{32}H_{38}O_8$ |
| 369 | **Cephalosporin $P_2$** | | 151° | | |
| 370 | **Cephalosporin $P_3$** | white, amorphous solid | | | |
| 371 | **Cephalosporin $P_4$** | Fawn-colored crystals | 220–230° | | |

Cephalosporin $P_1$ resembles helvolic acid (from *Aspergillus fumigatus*). A complete (steroid) structure has been determined by T. G. Halsall and associates, but has not been published yet.

H. S. Burton and E. P. Abraham, *Biochem. J.* 50 168 (1951). (Isolation)

H. S. Burton, E. P. Abraham and H. M. E. Cardwell, *ibid.* 62 171 (1956).

CIBA Lectures in Microbial Biochemistry, E. P. Abraham, "Biochemistry of Some Peptide and Steroid Antibiotics," *The cephalosporins,* John Wiley and Sons, New York, 1957, pp. 30–63. (A review)

368 Cephalosporin $P_1$, $C_{32}H_{48}O_8$, colorless crystals, m.p. 147°, $[\alpha]_D^{20}$ +28° (2.7 in chloroform).

*Cephalosporium* spp.

A number of similar substances, called cephalosporins $P_2$, $P_3$, $P_4$ and $P_5$ were isolated from the same fermentation, but were not obtained in high enough yields to permit much structure work.

H. S. Burton and E. P. Abraham, *Biochem. J.* 50 168 (1951). (Isolation)

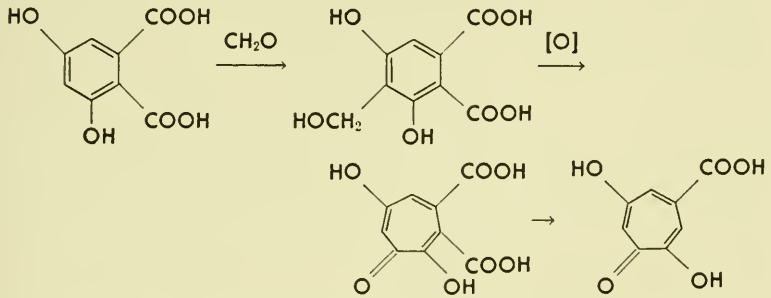
H. S. Burton, E. P. Abraham and H. M. E. Cardwell, *Biochem. J.* 62 171 (1956).

CIBA Lectures in Microbial Chemistry, E. P. Abraham, "Biochemistry of Some Peptide and Steroid Antibiotics," *The cephalosporins,* John Wiley and Sons, New York, 1957, pp. 30–63. (A review)

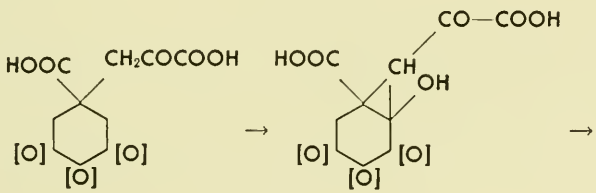
10

# Tropolone Acids

The detailed biosynthetic origin of the tropolone acids remains obscure. Various suggestions have been made. One of these[1, 2] proposed enlargement of the aromatic ring of 3,5-dihydroxyphthalic acid, a known mold metabolite:



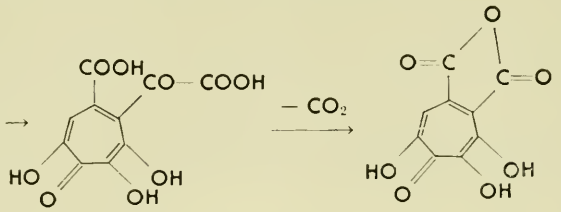

Another[3] proposed enlargement of an alicyclic ring in a $C_6$—$C_3$ type of intermediate from the shikimic acid route:



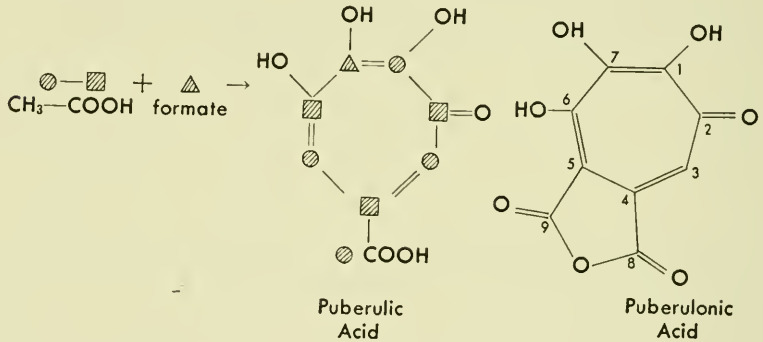

[1] T. R. Seshadri, *J. Sci. Ind. Research* (India) 14B 248 (1955).

[2] R. Robinson, "The Structural Relations of Natural Products," Oxford Univ. Press, London, 1955.

[3] A. J. Birch, *Fortschr. Chem. org. Naturstaffe* 14 186 (1957)




Labeling studies[4, 5, 6] show that acetate and formate are the primary precursors rather than glucose. Tanenbaum, Bassett and Kaplan found that stipitatic acid isolated from a *Penicillium stipitatum* culture grown on 1-$C^{14}$-glucose had an activity about five times as great as phenylalanine or tyrosine (shikimic acid route) isolated from the same culture. Richards and Ferretti grew *Penicillium aurantio-virens* on media containing (a) 1-$C^{14}$-acetate, (b) 2-$C^{14}$-acetate and (c) 1-$C^{14}$-glucose. Puberulic acid and puberulonic acid were isolated, separated and degraded. Their results, in agreement with Bentley's where the same precursors were used, indicate the incorporation of formate and acetate as follows:




That is, $C_1$, $C_3$, $C_5$ and $C_8$ of the tropolones (as numbered in the puberulonic acid structure shown) are derived from the methyl carbon atom of acetate, while $C_2$, $C_4$ and $C_6$ are from the acetate carboxyl group carbon atom. The $C_7$ carbon atom of the tropolones was shown by Bentley[5] to be derived from formate.

The origin of the $C_9$ carbon atoms present in puberulonic and

[4] John H. Richards and Louis D. Ferretti, *Biochem. and Biophys. Res. Comms.* 2 107 (1960).

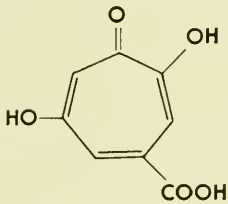
[5] Ronald Bentley, *Biochim. et Biophys. Acta* 29 666 (1958).

[6] S. W. Tanenbaum, E. W. Bassett and M. Kaplan, *Arch. Biochem. and Biophys.* 81 169 (1959).

stipitatonic acids remains undetermined. It, too, may arise from formate. A study has been made[7] of the enzymatic decarboxylation of stipitatonic and puberulonic acids. A biochemical relationship was concluded by way of this enzyme, and the suggestion made that the intermediate tropolone precursors must be at least $C_9$ compounds, and that direct closure of an acyclic to a seven-membered ring structure must occur.

The results of Richards and Ferretti seem to leave it an open question as to whether the tropolone ring is formed by direct cyclization of a long-chain acyclic compound or by expansion of a six-membered ring, and the exact nature of the intermediate precursors of this interesting series of mold metabolites remains a mystery.

372 **Stipitatic Acid,** $C_8H_6O_5$, pale yellow plates, m.p. 282° (dec.).

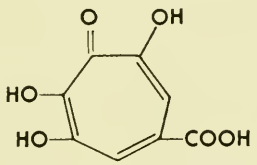



*Penicillium stipitatum* Thom

J. R. Bartels-Keith, A. W. Johnson and W. I. Taylor, *J. Chem. Soc.*, 2352 (1951). (Synthesis)

Peter L. Pauson, *Chem. Revs.* 55 9 (1955). (A review of tropolones)

373 **Puberulic Acid,** $C_8H_6O_6$, nearly colorless plates, m.p. 318°.

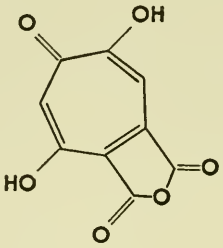



*Penicillium puberulum* Bainier, *P. aurantio-virens* Biourge, *P. cyclopium-viridicatum* and *P. johannioli* Zaleski

R. E. Corbett, C. H. Hassall, A. W. Johnson and A. R. Todd, *J. Chem. Soc.*, 1 (1950).

[7] Ronald Bentley and Clara P. Thiessen, *Nature* 184 552 (1959).

374 **Stipitatonic Acid,** $C_9H_4O_6$, yellow crystals, m.p. 237° (dec.).



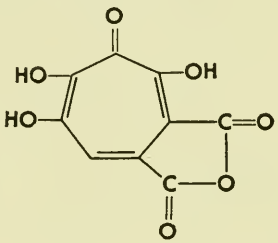
*Penicillium stipitatum* Thom

W. Segal, *Chem. and Ind.*, 1040 (1957). (Isolation)

Kozo Doi and Yoshio Kitahara, *Bull. Chem. Soc. Japan* 31 788 (1958). (Structure)

W. Segal, *Chem. and Ind.*, 1726 (1958). (Corrected structure)

375 **Puberulonic Acid,** $C_9H_4O_7$, fine yellow needles, m.p. 298° (dec.).



*Penicillium johannioli* Zaleski, *P. cyclopium-viridicatum, P. puberulum* Bainier and *P. aurantio-virens* Biourge

See preceding reference.

Gunhild Aulin-Erdtman, *Chem. and Ind.*, 29 (1951).

*Idem., Acta Chem. Scand.* 5 301 (1951). (Structure)

376 **Compound T,** $C_{10}H_8O_4$ or $C_{10}H_{10}O_4$, colorless crystals, m.p. 150°.

This compound shows the typical tropolone spectrum, and it is apparently a new tropolone acid.

*Penicillium stipitatum*

S. W. Tanenbaum, E. W. Bassett and M. Kaplan, *Arch. Biochem. and Biophys.* 81 169 (1959).

11

# Phenolic Substances

## *a.* PHENOLS AND PHENOL ETHERS (GENERAL)

Phenolic substances are commonly encountered as microorganism metabolites. Besides the compounds listed in this chapter phenolic moieties are present in other structures such as the xanthones, alternariol, blastmycin, hygromycin, fulvic acid, citromycetin, atrovenetin, the tetracyclines, mycobactin, anthraquinones and naphthoquinones. Benzoquinones are undoubtedly oxidation products of phenolic precursors.

Practically all of the phenolic materials in this section are mold metabolites. Perhaps that is because more isolation work has been done with fungi than with bacteria. It is evident that similar compounds are produced by bacteria, since 6-methylsalicylic acid, a typical penicillium metabolite, also occurs as a moiety of mycobactin from *Mycobacterium phlei.* Also, 2,3-dihydroxybenzoic acid occurs as a moiety of a metabolite from *Bacillus subtilis,* and 2,6-dihydroxybenzoic acid as a part of pyoluteorin from *Pseudomonas aeruginosa.* It is interesting that these bacterial phenolic acids are conjugates of nitrogen-containing substances.

The phenolic acid production of certain cultures has been studied in depth. *Penicillium brevi-compactum,* for example, has been found to produce the following:

3,5-Dihydroxyphthalic Acid $C_8H_6O_6$

1-Carboxy-2,5-dioxyphenyl Acetyl Carbinol $C_{10}H_{10}O_6$

2,4-Dioxy-6-pyruvylbenzoic Acid $C_{10}H_8O_6$

Mycophenolic Acid $C_{17}H_{20}O_6$

Another investigation[1] in fact found a total of 11 different phenolic substances in a culture of this organism. In addition to the above were found a compound $C_{10}H_{10}O_7$, two derivatives of mycophenolic acid, two "intermediates between $C_{10}H_{10}O_7$ and $C_8H_6O_6$" and two reduction products of $C_{10}H_{10}O_5$.

The mold *Penicillium griseofulvum* produces:

6-Methylsalicylic Acid $C_8H_8O_3$
Orsellinic Acid $C_8H_8O_4$
Griseofulvin $C_{17}H_{17}O_6Cl$
Dechlorogriseofulvin $C_{17}H_{18}O_6$
Bromogriseofulvin $C_{17}H_{17}O_6Br$
Gentisic Acid $C_7H_6O_4$
Fulvic Acid $C_{14}H_{12}O_8$
Mycelianamide $C_{22}H_{28}O_5N_2$

Another study[2] found three more unidentified phenolic substances in this culture.

A *Penicillium patulum* culture has been found[3] to produce:

Patulin $C_7H_6O_4$
Gentisaldehyde $C_7H_6O_3$
Gentisic Acid $C_7H_6O_4$
Gentisyl Alcohol $C_7H_8O_3$
6-Methylsalicylic Acid $C_8H_8O_4$
6-Formylsalicylic Acid $C_8H_6O_4$
3-Hydroxyphthalic Acid $C_8H_6O_5$
Pyrogallol $C_6H_6O_3$
p-Hydroxybenzoic Acid $C_7H_6O_3$
Anthranilic Acid $C_7H_7O_2N$

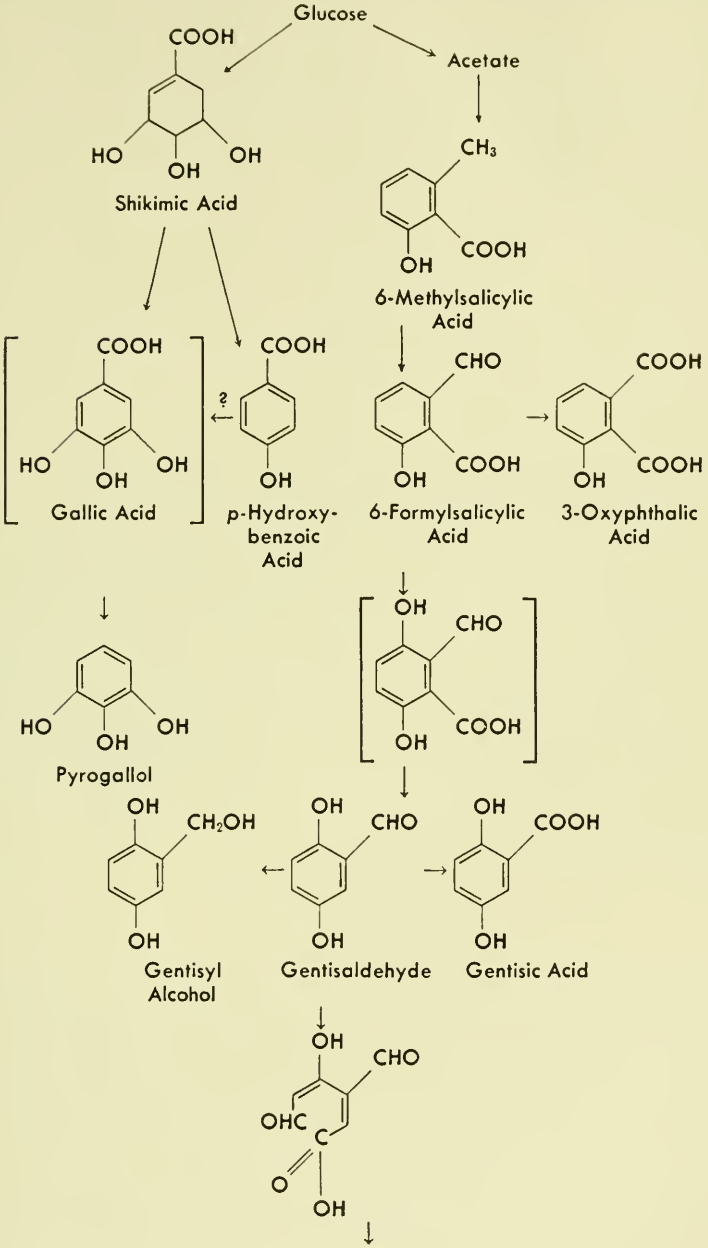
Also an "aliphatic precursor of patulin" and a depside-like compound were detected but not entirely characterized.

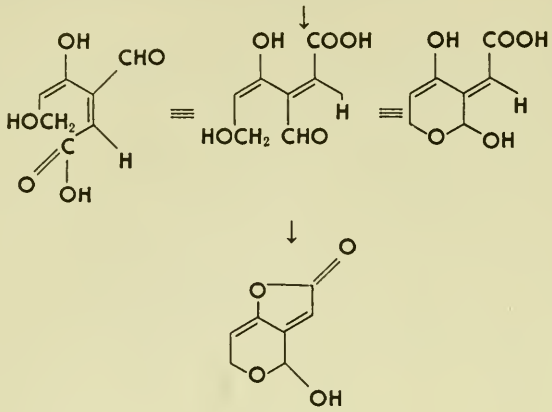
Many such families of metabolites can be assembled by reference to the microorganism index. Studies such as those above facilitate the development of biosynthetic routes. For example, Bassett and Tanenbaum suggest the following interrelationships among the *Penicillium patulum* phenolic metabolites:

[1] Paul Godin, *Antonie van Leeuwenhoek J. Microbiol. Serol.* 21 215 (1955).

[2] Paul Simonart and Renaat de Lathouwer, *Zentr. Bakteriol., Parasitenk.*, Abt. II 110 339 (1957).

[3] E. Bassett and S. Tanenbaum, *Experientia* 14 38 (1958).


Glucose
Acetate
Shikimic Acid
6-Methylsalicylic Acid
Gallic Acid
p-Hydroxy-benzoic Acid
6-Formylsalicylic Acid
3-Oxyphthalic Acid
Pyrogallol
Gentisyl Alcohol
Gentisaldehyde
Gentisic Acid




Patulin

Thus, the acetate and shikimic acid routes to aromatic compounds seem to be operating in a single microorganism.

It was a kind of statistical consideration of the structures of natural products which led to the revival of the acetate hypothesis of biogenesis as applied to substances other than fatty acids.

Phenolic compounds were particularly instrumental since the frequent occurrence of *meta*-hydroxyl groups (resorcinol and phloroglucinol types) was easy to recognize and challenging to explain. The case first was stated clearly by Collie many years ago.[4] Lately Birch and others have developed a firm experimental basis for the theory by isotopic labeling and chemical degradation studies.

Some phenolic compounds which have been shown in this way to be acetate-derived are:

- 6-Methylsalicylic Acid[5]
- Griseofulvin[6]
- Mycophenolic Acid[7]
- Emodin[8]

[4] John Norman Collie, *Proc. Chem. Soc.* 23 230 (1907); *idem., J. Chem. Soc.* 91 1806 (1907).

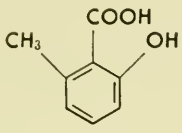
[5] A. J. Birch, R. A. Massy-Westropp and C. J. Moye, *Australian J. Chem.* 8 539 (1955).

[6] A. J. Birch, R. A. Massy-Westropp, R. W. Rickards and Herchel Smith, *J. Chem. Soc.*, 360 (1958).

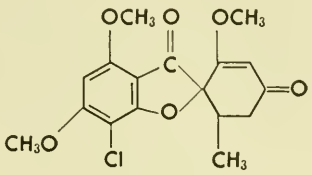
[7] A. J. Birch, R. J. English, R. A. Massy-Westropp, M. Slaytor and Herchel Smith, *ibid.*, 365 (1958); A. J. Birch, R. J. English, R. A. Massy-Westropp and Herchel Smith, *ibid.*, 369 (1958).

[8] Sten Gatenbeck, *Acta Chem. Scand.* 12 1211 (1958).

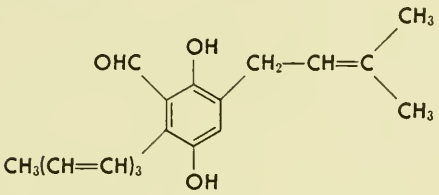
Auroglaucin[9]
Helminthosporin[10]



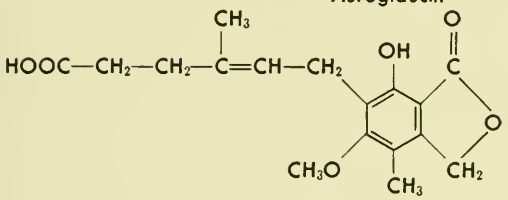

6-Methylsalicylic Acid



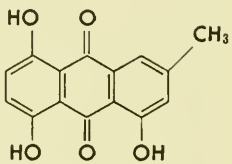

Griseofulvin



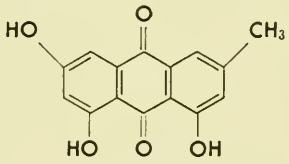

Auroglaucin




Mycophenolic Acid




Helminthosporin




Emodin

Interesting details have been discovered. For example,[7] the methyl group attached to the aromatic ring in mycophenolic acid is furnished by methionine, probably at a relatively early stage in the synthetic sequence. The methoxyl methyl group also is furnished by methionine. The aromatic nucleus is acetate-derived, while mevalonic acid was shown to be a specific

[9] A. J. Birch, J. Schofield and Herchel Smith, *Chem. and Ind.*, 1321 (1958).

[10] A. J. Birch, A. J. Ryan and Herchel Smith, *J. Chem. Soc.*, 4773 (1958).

and irreversible intermediate for the terpenoid side-chain. There was no incorporation of mevalonic acid into the aromatic nucleus. Mevalonic acid also was incorporated exclusively into the isopentene side-chain of auroglaucin.

Both bacteria and fungi are able to hydroxylate aromatic rings, and the acetate pattern of alternate oxidation often is confused by further oxidations of this sort.

Other details remain to be determined. The predominance of metabolites indicating derivation from an even number of acetate units has led to speculation concerning a four-carbon intermediate such as acetoacetate. Even larger intermediates have been proposed, such as orsellinic acids as precursors of anthraquinones.[11] So far this possibility has not been ruled out in each case[8] by rigorous experimental evidence although there is an intuitive tendency to favor the simplest and most flexible unit and to apply the accumulated body of knowledge about intermediary metabolism. The co-occurrence in a natural source of the anthraquinone and related phenanthrenequinone mentioned in the introduction to the section on quinones is presumptive evidence against orsellinic acid intermediates, since the two quinone molecules appear to be formed merely by a different mode of folding or arrangement on an enzyme surface of the same intermediate polyketomethylene chain. On the other hand the isolation of such orsellinic acids from isolated fungus members of lichens incapable of completing the anthraquinone synthesis is interesting.

The structural relationships (some obvious, others more obscure) among the mold products fulvic acid, citromycetin, fusarubin, purpurogenone, etc.[12, 13] argue in favor of a flexible intermediate in the sense of a single polyketomethylene chain that could be folded and modified in various ways to give related metabolites. Comparison of the structures of the lactone moieties of the macrolide antibiotics with those of the tetracyclines (both classes of compounds produced by streptomycetes) also seems to point to intermediates of this type. While this is a good working hypothesis, such intermediates have not been isolated and in fact could not long exist in the free state. Perhaps eventually a better knowledge of enzymes will let us know

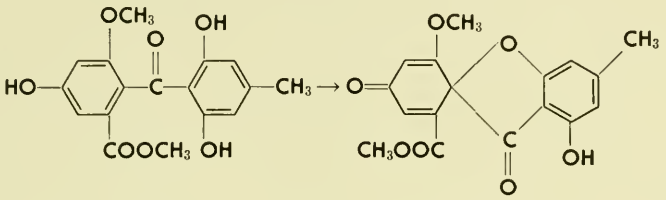
[11] K. Aghoramurthy and T. R. Seshadri, *J. Sci. Ind. Research* (India) 13A 114 (1954).

[12] F. M. Dean, R. A. Eade, R. A. Moubasher and A. Robertson, *Nature* 179 366 (1957).

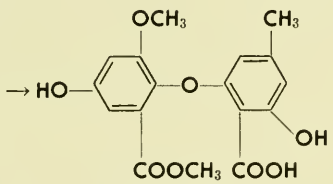
[13] W. B. Whalley, *Chem. and Ind.*, 131 (1958).

in more detail how such acetate-derived mold metabolites are formed, and why the chain lengths seldom exceed 14 to 18 carbon atoms.

The recent discovery and characterization of asterric acid, a mold metabolite in which two phenolic units are joined by an ether linkage, have inspired the suggestion that the final phases of its biogenetic scheme may involve a geodin-like intermediate and sulochrin as follows:




Sulochrin Dechlorogeodin

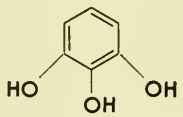



Asterric Acid

The authors believe that the known occurrence of sulochrin and geodin as mold metabolites supports this argument.[14]

The transformation of sulochrin to dechlorogeodin, incidentally, is an example of intramolecular phenol coupling, a phenomenon discussed at greater length under Part *b* of this section.

377 **Pyrogallol,** $C_6H_6O_3$, colorless crystals which turn brown in air, m.p. 133°.

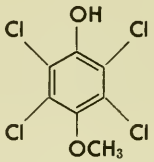



*Penicillium patulum*

E. W. Bassett and S. W. Tanenbaum, *Biochim. et Biophys. Acta* 28 247 (1958).

[14] R. F. Curtis, C. H. Hassall and D. W. Jones, *Chem. and Ind.*, 1283 (1959).

378 *p*-Methoxytetrachlorophenol (Drosophilin A), $C_7H_4O_2Cl_4$, yellow crystals, m.p. 118°.

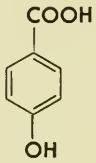



*Drosophila subatrata* (Batsch ex Fr.) Quel.

The yield was 100 mg. from 31 liters of culture solution.

Frederick Kavanagh, Annette Hervey and William J. Robbins, *Proc. Natl. Acad. Sci. U. S.* 38 555 (1952).

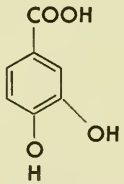
379 *p*-Hydroxybenzoic Acid, $C_7H_6O_3$, colorless crystals, m.p. 213°.




*Penicillium patulum*

E. W. Bassett and S. W. Tanenbaum, *Biochim. et Biophys. Acta* 28 247 (1958).

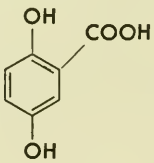
380 Protocatechuic Acid, $C_7H_6O_4$, white or tan crystalline powder which darkens in air, m.p. ~200° (dec.). Monohydrate from water.




*Phycomyces blakesleeanus* (sugar substrate)

H. B. Schröter, *Angew. Chem.* 68 158 (1956).

381 Gentisic Acid, $C_7H_6O_4$, colorless crystals, m.p. 199°.



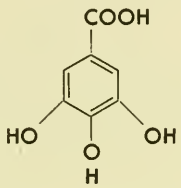
*Penicillium griseofulvum* Dierckx, *P. jenseni, P. divergens*

Harold Raistrick and Paul Simonart, *Biochem. J.* 27 628 (1933).

J. Barta and R. Mecir, *Experientia* 4 277 (1948).

A. Brack, *Helv. Chim. Acta* 30 1 (1947). (Isolation)

382 **Gallic Acid,** $C_7H_6O_5$, colorless or pale tan crystals (Monohydrate from water), m.p. 225–250° (dec.).

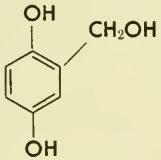



*Phycomyces blakesleeanus* (sugar substrate)

Protocatechuic acid and another unidentified phenol also were shown to be present by paper chromatography.

H. B. Schröter, *Angew. Chem.* 68 158 (1956).

383 **Gentisyl Alcohol,** $C_7H_8O_3$, colorless crystals, m.p. 100°.



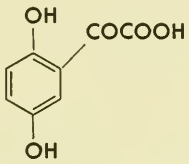

*Penicillium patulum* Bainier, *P. divergens* Bainier and Sartory

A. Brack, *Helv. Chim. Acta* 30 1 (1947). (Isolation)

B. G. Engel and W. Brzeski, *ibid.* 30 1472 (1947).

J. Barta and R. Mecir, *Experientia* 4 277 (1948).

384 **2,5-Dihydroxyphenylglyoxylic Acid,** $C_8H_6O_5$, yellow needles, m.p. 141°.



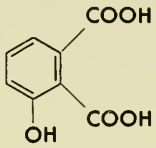

*Polyporus tumulosus* Cooke (artificial medium)

Oxalic acid, homoprotocatechuic acid and 2,4,5-trihydroxyphenylglyoxylic acid are produced in the same culture.

Otto Neubauer and L. Flatow, *Hoppe-Seyler's Zeitschrift für physiol. Chem.* 52 375 (1907).

G. F. J. Moir and B. F. Ralph, *Chem. and Ind.*, 1143 (1954).

385 **3-Hydroxyphthalic Acid,** $C_8H_6O_5$, colorless crystals m.p.: anhydride formation near 150°, melting 166°. Sublimes.



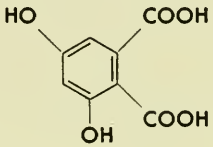

*Penicillium islandicum, P. patulum*

A yield of only 1–2 mg. per liter was obtained.

Sten Gatenbeck, *Acta Chem. Scand.* 11 555 (1957).

E. W. Bassett and S. W. Tanenbaum, *Experientia* 14 38 (1958).

386 **3,5-Dihydroxyphthalic Acid,** $C_8H_6O_6$, colorless prisms, m.p. 188° (resolidifying at 206°).

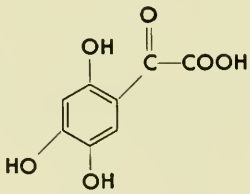



*Penicillium brevi-compactum* Dierckx

Albert E. Oxford and Harold Raistrick, *Biochem. J.* 26 1902 (1932). (Isolation)

John Howard Birkinshaw and Arthur Bracken, *J. Chem. Soc.*, 368 (1942). (Synthesis)

387 **2,4,5-Trihydroxyphenylglyoxylic Acid,** $C_8H_6O_6$, bright red prisms, m.p. 193°.

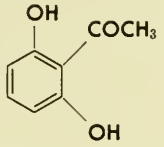



*Polyporus tumulosus* (artificial medium)

Homoprotocatechuic acid and oxalic acid were present in the same culture.

B. J. Ralph and Alexander Robertson, *J. Chem. Soc.*, 3380 (1950).

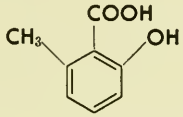
388 2,6-Dihydroxyacetophenone, $C_8H_8O_3$, yellow needles, m.p. 154–158°.




*Daldinia concentrica*

D. C. Allport and J. D. Bu'Lock, *J. Chem. Soc.*, 654 (1960).

389 6-Methylsalicylic Acid (2,6-Cresotic Acid, 3-Hydroxy-5-toluic Acid, 6-Hydroxy-2-methylbenzoic Acid), $C_8H_8O_3$, colorless needles, m.p. 170°.

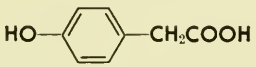



*Penicillium griseofulvum* Dierckx, *P. flexuosum*, *P. patulum* Bainier, *P. urticae*

Winston Kennay Anslow and Harold Raistrick, *Biochem. J.* 25 39 (1931).

E. W. Bassett and S. W. Tanenbaum, *Experientia* 14 38 (1958).

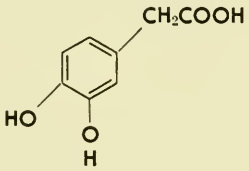
390 *p*-Hydroxyphenylacetic Acid, $C_8H_8O_3$, colorless crystals, m.p. 148° (subl.).




*Hypochnus sasakii* Shirai (*Corticium sasakii*, *Pellicularia sasakii*)

Ysu Shik Chen, *Bull. Agr. Chem. Soc.* (Japan) 22 136 (1958).

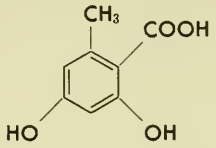
391 Homoprotocatechuic Acid, $C_8H_8O_4$, colorless plates, m.p. 128.5°.



*Polyporus tumulosus* (artificial medium)

B. J. Ralph and Alexander Robertson, *J. Chem. Soc.*, 3380 (1950).

392 **Orsellinic Acid,** $C_8H_8O_4$, colorless crystals, m.p. (Monohydrate) 176°.




*Penicillium griseofulvum, Chaetomium cochlioides*

L. Reio, *J. Chromatography* 1 338 (1958).

Klaus Mosbach, *Zeitschr. Naturforsch.* 14b 69 (1959).

393 **Compound D,** $C_9H_8O_5$, cream-colored prisms, m.p. 259° (dec.).

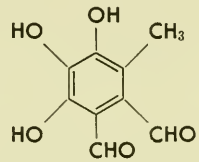
A meta diphenol with a carboxyl group para to a hydroxyl and an aldehyde group ortho to a hydroxyl.

*Paecilomyces victoriae* V. Szilvinyi

Ustic acid, dehydroustic acid and 4,6-dihydroxy-3-methoxyphthalic acid were isolated from the same culture.

V. C. Vora, *J. Sci. Ind. Research* (India) 13B 842 (1954).

394 **Flavipin,** $C_9H_8O_5$, pale yellow light-sensitive rods, m.p. 233° (dec.).

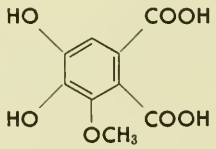



*Aspergillus flavipes* (Bainier and Sartory) Thom and Church, *A. terreus* Thom

P. Rudman, "Metabolic Products of *A. flavipes*, *A. terreus* and Certain Other Molds," Doctoral Thesis, Univ. of London, London, 1955.

H. Raistrick and P. Rudman, *Biochem. J.* 63 395 (1956).

395 **4,6-Dihydroxy-3-methoxyphthalic Acid,** $C_9H_8O_7$, colorless prisms, m.p. 193°.

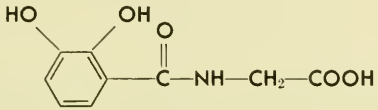


*Paecilomyces victoriae* V. Szilvinyi

Ustic acid, dehydroustic acid and another incompletely characterized phenolic acid were isolated from the same culture.

V. C. Vora, *J. Sci. Ind. Research* (India) 13B 842 (1954).

396 **2,3-Dihydroxybenzoylglycine,** $C_9H_9O_5N$, colorless needles, m.p. 210°.

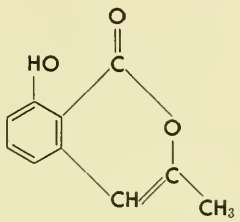



*Bacillus subtilis* (iron-deficient medium)

Coproporphyrin and succinic acid were also produced.

Takeru Ito and J. B. Neilands, *J. Am. Chem. Soc.* 80 4645 (1958).

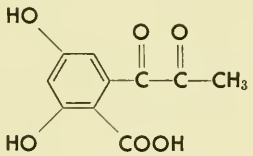
397 **8-Hydroxy-3-methylisocoumarin,** $C_{10}H_8O_3$, colorless needles, m.p. 99°.




*Marasmius ramealis*

Gerd Benz, *Arkiv för Kemi* 14 511 (1959).

398 **2,4-Dioxy-6-pyruvylbenzoic Acid,** $C_{10}H_8O_6$, fine colorless crystals, m.p. 125–135°.

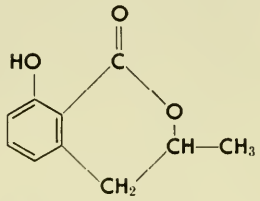



*Penicillium brevi-compactum* (syn. *P. stoloniferum* Thom)

Percival W. Clutterbuck, Albert E. Oxford, Harold Raistrick and Geo. Smith, *Biochem. J.* 26 1441 (1932). (Isolation)

Albert E. Oxford and Harold Raistrick, *ibid.* 27 634 (1933).

399 **Mellein** (Ochracin), $C_{10}H_{10}O_3$, colorless prisms, m.p. 58°, $[\alpha]_D$ −124.86° ($[\alpha]_D^{12}$ −108.15° in chloroform).



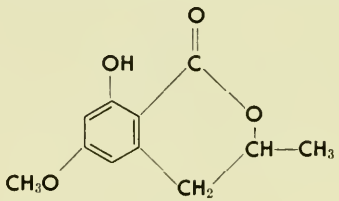

*Aspergillus melleus* Yugawa, *A. ochraceus*

Eijiro Nishikawa, *J. Agr. Chem. Soc. Japan* 9 772 (1933). (Isolation) (*Chem. Abstr.* 28 2751)

Teijiro Yabuta and Yusuke Sumiki, *ibid.* 9 1264 (1933). (Isolation) (*Chem. Abstr.* 28 2350)

John Blair and G. T. Newbold, *J. Chem. Soc.*, 2871 (1955). (Structure)

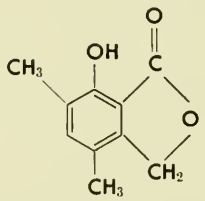
It is interesting to note that a similar compound:




has been isolated from carrots which had developed a bitter taste during cold storage.

Ernest Sondheimer, *J. Am. Chem. Soc.* 79 5036 (1957). (Isolation)

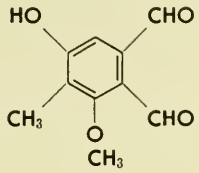
400 **3,5-Dimethyl-6-oxyphthalide**, $C_{10}H_{10}O_3$, colorless needles, m.p. 156–158°.




*Penicillium gladioli*

H. Raistrick and D. J. Ross, *Biochem. J.* 50 635 (1952).

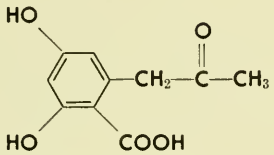
401 **Quadrilineatin,** $C_{10}H_{10}O_4$, colorless needles, m.p. 172° (dec.).




*Aspergillus quadrilineatus* Thom and Raper

J. H. Birkinshaw, P. Chaplen and R. Lahoz-Oliver, *Biochem. J.* 67 155 (1957).

402 **1-Carboxy-2,5-dioxybenzyl Methyl Ketone,** $C_{10}H_{10}O_5$, large diamond-shaped crystals, m.p. 152–156° (dec.), remelting at 220–230°.

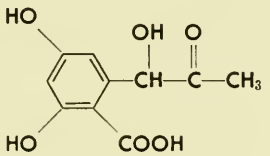



*Penicillium brevi-compactum* (syn. *P. stoloniferum* Thom)

Percival W. Clutterbuck, Albert E. Oxford, Harold Raistrick and Geo. Smith, *Biochem. J.* 26 1441 (1932).

Albert E. Oxford and Harold Raistrick, *ibid.* 27 634 (1933).

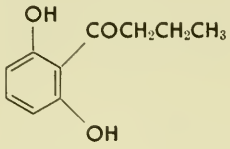
403 **1-Carboxy-2,5-dioxyphenyl Acetyl Carbinol,** $C_{10}H_{10}O_6$, colorless rhombs, m.p. 202–204° (dec.).




*Penicillium brevi-compactum* (syn. *P. stoloniferum* Thom)

Percival W. Clutterbuck, Albert E. Oxford, Harold Raistrick and Geo. Smith, *Biochem. J.* 26 1441 (1932).

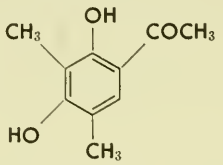
404 2,6-Dihydroxybutyrophenone, $C_{10}H_{12}O_3$, yellow needles, m.p. 116.5–118°.




*Daldinia concentrica*

D. C. Allport and J. D. Bu'Lock, *J. Chem. Soc.*, 654 (1960).

405 Clavatol, $C_{10}H_{12}O_3$, colorless plates, m.p. 183°.



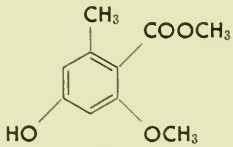

*Aspergillus clavatus*

Occurs as a minor product with patulin in this culture.

F. Bergel, A. C. Morrison, A. R. Moss and H. Rinderknecht, *J. Chem. Soc.*, 415 (1944). (Isolation)

C. H. Hassall and A. R. Todd, *ibid.*, 611 (1947). (Structure)

406 Sparassol, $C_{10}H_{12}O_4$, colorless microcrystals, m.p. 67°.




*Sparassis ramosa, Evernia prunastri*

John Stenhouse, *Ann.* 68 55 (1848).

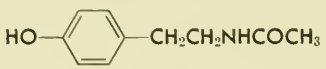
Emil Fischer and Kurt Hoesch, *ibid.* 391 347 (1912). (Structure)

Richard Falck, *Ber.* 56B 2555 (1923).

E. Wedekind and K. Fleischer, *ibid.* 56B 2556 (1923). (Structure)

Ernst Späth and Karl Jeschki, *ibid.* 57A 471 (1924).

407 N-Acetyltyramine, $C_{10}H_{13}O_2N$, colorless crystals, m.p. 135° (s. 128°).

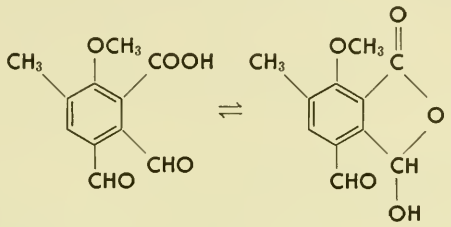



*Streptomyces griseus* (Krainski) Waksman et Henrici, *Mycobacterium tuberculosis*

J. Comin and W. Keller-Schierlein, *Helv. Chim. Acta* 42 1730 (1959).

Yutaka Shirai, *Kekkaku* (Tuberculosis) 30 628 (1955). (*Chem. Abstr.* 50 5839g)

408 **Gladiolic Acid,** $C_{11}H_{10}O_5$, colorless needles, m.p. 158–160°.



*Penicillium gladioli* McCull. and Thom

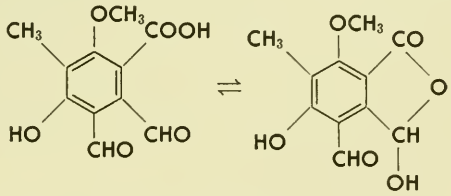
Yield 300 mg. per liter.

Besides dihydrogladiolic acid and 3,5-dimethyl-6-oxyphthalide, a third "contaminant," $C_{11}H_{10}O_4$ (a lactone), was present in the culture.

John Frederick Grove, *Biochem. J.* 50 648 (1952). (Structure)

P. W. Brian, P. J. Curtis and H. G. Hemming, *J. Gen. Microbiol.* 2 341 (1948). (Isolation)

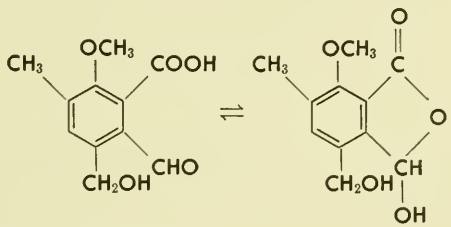
409 **Cyclopaldic Acid,** $C_{11}H_{10}O_6$, colorless needles, m.p. 224° (subl.).



*Penicillium cyclopium* Westling

J. H. Birkinshaw, H. Raistrick, D. J. Ross and C. E. Stickings, *Biochem. J.* 50 610 (1952).

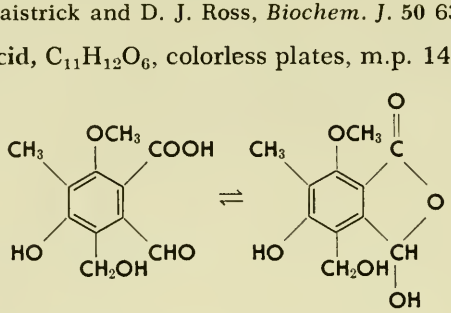
410 **Dihydrogladiolic Acid,** $C_{11}H_{12}O_5$, colorless crystals, m.p. 135° (dec.).



*Penicillium gladioli*

H. Raistrick and D. J. Ross, *Biochem. J.* 50 635 (1952).

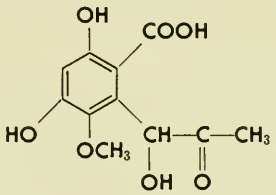
411 **Cyclopolic Acid,** $C_{11}H_{12}O_6$, colorless plates, m.p. 147° (dec.).



*Penicillium cyclopium*

J. H. Birkinshaw, H. Raistrick, D. J. Ross and C. E. Stickings, *Biochem. J.* 50 610 (1952).

412 **Ustic Acid,** $C_{11}H_{12}O_7$, colorless crystals, m.p. 169° (dec.).



*Aspergillus ustus, Paecilomyces victoriae, Ustilago zeae*

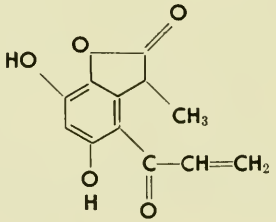
H. Raistrick and C. E. Stickings, *Biochem. J.* 48 53 (1951). Yield about 0.5 g. per liter.

V. C. Vora, *J. Sci. Ind. Research* (India) 13B 842 (1954).

Occurred together with dehydroustic acid, 4,5-dihydroxy-3-methoxyphthalic acid and a fourth compound, $C_9H_8O_5$, m.p. 259°; an *m*-dihydroxyphenol with a carbonyl group and a carboxyl group.

413 **Radicinin,*** $C_{12}H_{10}O_5$, optically active crystals.

Proposed Structure:



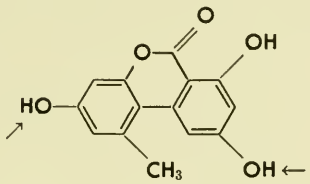
*Stemphylium radicinum*

D. D. Clarke and F. F. Nord, *Arch. Biochem. and Biophys.* 59 269–284 (1955).

* See also entry 871.

414 **Alternariol,** $C_{14}H_{10}O_5$, colorless needles, m.p. 350° (dec.)
and

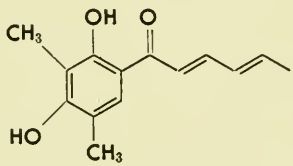
415 **Alternariol Methyl Ether,** $C_{15}H_{12}O_5$, colorless needles, m.p. 267° (dec.).




The methyl ether is at one of the positions indicated.
*Alternaria tenuis*
The yield was about ½ g. per liter.
H. Raistrick, C. E. Stickings and R. Thomas, *Biochem. J.* 55 421 (1953).

416 **Altertenuol,** $C_{14}H_{10}O_6$, buff-colored rods, m.p. 284° (dec. and subl.).
Forms a triacetate and a trimethyl derivative. Probably related to alternariol.
*Alternaria tenuis*
T. Rosett, R. H. Sankhala, C. E. Stickings, M. E. U. Taylor and R. Thomas, *Biochem. J.* 67 390 (1957).

417 **Sorbicillin,** $C_{14}H_{16}O_3$, orange plates, m.p. 113° (remelting at 129°).




*Penicillium notatum* Westling
Donald J. Cram and Max Tishler, *J. Am. Chem. Soc.* 70 4238 (1948). (Isolation from Clinical Sodium Penicillin)
Donald J. Cram, *ibid.* 70 4240 (1948). (Structure)
Besides sorbicillin several other compounds were isolated from careful investigation of a sample of early clinical sodium penicillin. In view of the source it is hard to say which of these may be considered true metabolites. The other compounds were:
Tiglic Acid, $C_5H_8O_2$, m.p. 63°
*d*-α-Methylbutyric Acid, $C_5H_{10}O_2$ b.p. 175°, $[\alpha]_D^{20}$ +15.2°
Furoic Acid, m.p. 129°

β-Indoleacetic Acid, m.p. 167°
Phenylacetic Acid, m.p. 76°
2-Decenedioic Acid, $C_{10}H_{16}O_4$, m.p. 172°
Pigment I (β-Penetrin), m.p. 207°.

β-Penetrin is identical with an alkaline hydrolysis product of penetrinic acid, a metabolite of *P. notatum* reported earlier

Pigment II, $C_{10}H_{11}O_6N$, orange prisms, m.p. 105°, N.E. indicates a dicarboxylic acid. Optically inactive. Negative $FeCl_3$ test. Decolorizes permanganate. Decolorized by sodium hydrosulfite and apparently reduced to a hydroquinone, m.p. 129°.

Frank H. Stodola, Jacques L. Wachtel, Andrew J. Moyer and Robert D. Coghill, *J. Biol. Chem.* 159 67 (1945).

418 **Dehydroaltenusin,** $C_{15}H_{12}O_6$, yellow needles, m.p. 189° (dec.).
An acidic compound probably related to altenusin.
*Alternaria tenuis*
T. Rosett, R. H. Sankhala, C. E. Stickings, M. E. U. Taylor and R. Thomas, *Biochem. J.* 67 390 (1957).

419 **Altenusin,** $C_{15}H_{14}O_6$, colorless prisms, m.p. 202° (dec.).
An acidic compound which forms a tetramethyl derivative. Probably related to alternariol.
*Alternaria tenuis*
T. Rosett, R. H. Sankhala, C. E. Stickings, M. E. U. Taylor and R. Thomas, *Biochem. J.* 67 390 (1957).

420 **Altenuic Acid I,** $C_{15}H_{14}O_8$, colorless needles, m.p. 183°, second m.p. 224–230° (dec.).
A dibasic acid probably related to alternariol.
*Alternaria tenuis*
T. Rosett, R. H. Sankhala, C. E. Stickings, M. E. U. Taylor and R. Thomas, *Biochem. J.* 67 390 (1957).

421 **Altenuic Acid II,** $C_{15}H_{14}O_8$, colorless plates, m.p. 245° (dec.).
A dibasic acid probably related to alternariol.
*Alternaria tenuis*
T. Rosett, R. H. Sankhala, C. E. Stickings, M. E. U. Taylor and R. Thomas, *Biochem. J.* 67 390 (1957).

422 **Altenuic Acid III,** $C_{15}H_{14}O_8$, colorless prisms, m.p. 198–202°, second m.p. 225° (dec.).
A dibasic acid probably related to alternariol.
*Alternaria tenuis*
T. Rosett, R. H. Sankhala, C. E. Stickings, M. E. U. Taylor and R. Thomas, *Biochem. J.* 67 390 (1957).

423 **Penitrinic Acid,** $C_{15}H_{17}O_5N$, pale yellow bars, m.p. 217–223° (dec.), $[\alpha]_D^{23}$ −549° (in dimethylformamide).

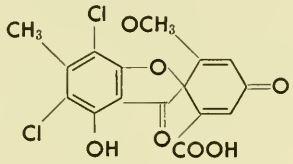
Similar in structure to sorbicillin. The two pigments occur together.

*Penicillium notatum* Westling

Frank H. Stodola, Jacques L. Wachtel, Andrew J. Moyer and Robert D. Coghill, *J. Biol. Chem.* 159 67 (1945).

Kei Arima, Kazuo Kamagata and Hideo Nakamura, *J. Agr. Chem. Soc. Japan* 27 389 (1953). (Structure work)

424 ***d,l*-Erdin,** $C_{16}H_{10}O_7Cl_2$, yellow crystals, m.p. 210–212°.



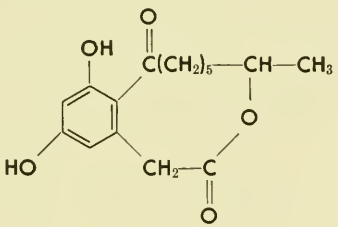

*Aspergillus terreus* Thom

Erdin occurs naturally as the racemate although the closely related geodin, which is present in the same culture, is the *d*-isomer.

Harold Raistrick and George Smith, *Biochem. J.* 30 1315 (1936). (Isolation)

D. H. R. Barton and A. I. Scott, *J. Chem. Soc.*, 1767 (1958). (Structure)

425 **Curvularin,** $C_{16}H_{20}O_5$, colorless crystals, m.p. 206°, $[\alpha]_D^{18}$ −36.3° (c 3.8 in ethanol).




*Curvularia* sp.

The yield was 0.40 to 0.48 g. per liter of culture broth. A second compound $C_{16}H_{18}O_5$, m.p. 224.5°, $[\alpha]_D^{18}$ −83°, (also phenolic) was isolated from the same culture.

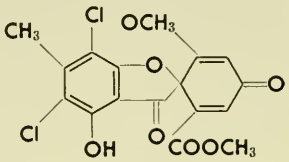
C. Calam (Imperial Chemical Industries), unpublished. (Isolation)

O. C. Musgrave, *J. Chem. Soc.*, 4301 (1956). (Isolation)

*Idem., ibid.*, 1104 (1957).

A. J. Birch, O. C. Musgrave, R. W. Rickards and Herchel Smith, *ibid.*, 3146 (1959). (Structure)

426 ***d*-Geodin**, $C_{17}H_{12}O_7Cl_2$, yellow crystals, m.p. 228–231°, $[\alpha]_D$ +140° (c 0.80 in chloroform).

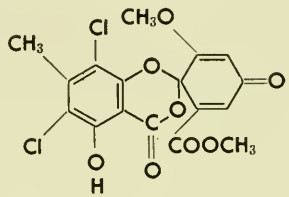



*Aspergillus terreus* Thom

Harold Raistrick and George Smith, *Biochem. J.* 30 1315 (1936). (Isolation)

D. H. R. Barton and A. I. Scott, *J. Chem. Soc.*, 1767 (1958). (Structure)

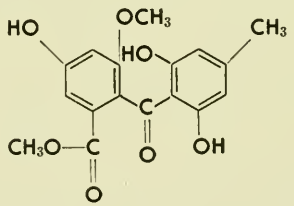
427 **Geodoxin**, $C_{17}H_{12}O_8Cl_2$, yellow needles, m.p. 216° (dec.).




*Aspergillus terreus* Thom

C. H. Hassall and T. C. McMorris, *J. Chem. Soc.*, 2831 (1959).

428 **Sulochrin**, $C_{17}H_{16}O_7$, colorless crystals, m.p. 262°.




*Oospora sulfurea-ochracea*

Hidejiro Nichikawa, *Bull. Agr. Chem. Soc.* (Japan) 12 47 (1936).

*Idem., J. Agr. Chem. Soc. Japan* 13 1 (1937).

*Idem., Bull. Agr. Chem. Soc.* (Japan) 16 97 (1940).

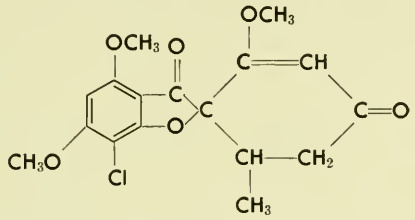
429 **Geodin-like Antibiotic,** yellow crystals, m.p. 229° (subl. 175° at 3 mm.), $[\alpha]_D^{20}$ +175° (in chloroform).

The chlorine-containing part of the molecule is the same as that of geodin as shown by hydrolysis fragments. Other chemical and physical properties are similar to those of geodin.

*Aspergillus flavipes*

Paul Delmotte, Julia Delmotte-Plaquée and René Bastin, *J. Pharm. Belg.* 11 200 (1956).

430 **Griseofulvin** (Fulvicin, Grisovin) $C_{17}H_{17}O_6Cl$, colorless crystals, m.p. 220°, $[\alpha]_D^{21}$ +337° (c 1.0 in acetone).




*Penicillium griseofulvum* Dierckx, *P. patulum, P. albidum* Sopp., *P. raciborskii* Zal., *P. melinii* Thom, *P. urticae* Bain., *P. raistrickii, P. janczewski* Zal. (*P. nigricans* Thom and Bainier), *Carpenteles brefeldianum* Dodge (Shear)

Albert Edward Oxford, Harold Raistrick and Paul Simonart, *Biochem. J.* 33 240 (1939). (Isolation)

J. C. McGowan, *Trans. Brit. Mycol. Soc.* 29 188 (1946).

P. J. Curtis and J. F. Grove, *Nature* 160 574 (1947).

P. W. Brian, P. J. Curtis and H. G. Heming, *Brit. Mycol. Soc. Trans.* 32 30 (1949).

John Frederick Grove, Doreen Ismay, J. MacMillan, T. P. C. Mulholland, M. A. Thorold Rogers, *Chem. and Ind.,* 219 (1951). (Structure)

*Idem., J. Chem. Soc.,* 3958 (1952).

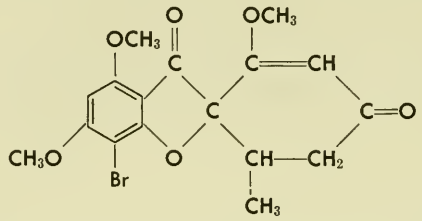
John Frederick Grove, J. MacMillan, T. P. C. Mulholland and M. A. Thorold Rogers, *ibid.,* 3949, 3977 (1952). (Structure)

John Frederick Grove, J. MacMillan, T. P. C. Mulholland and (Mrs.) J. Zealley, *ibid.,* 3967 (1952).

T. P. C. Mulholland, *ibid.,* 3987, 3994 (1952).

A. J. Birch, R. A. Massy-Westropp, R. W. Rickards and Herchel Smith, *Proc. Chem. Soc.,* 98 (1957). (Biosynthesis)

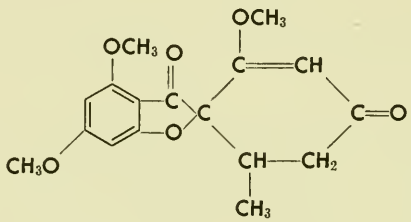
431 **Bromogriseofulvin,** $C_{17}H_{17}O_6Br$, colorless crystals, m.p. 204°.



On the proper medium bromogriseofulvin generally can be produced by the same molds which produce griseofulvin.

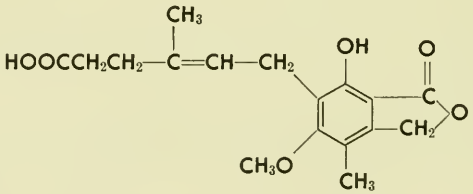
J. MacMillan, *J. Chem. Soc.*, 2585 (1954). (Isolation)

432 **Dechlorogriseofulvin,** $C_{17}H_{18}O_6$, colorless needles, m.p. 179–181°, $[\alpha]_D^{19}$ +390° (c 1 in acetone).



*Penicillium griseofulvum* Dierckx, *P. janczewski* Zal.
J. MacMillan, *Chem. and Ind.*, 719 (1951).
*Idem., J. Chem. Soc.*, 1697 (1953).
D. H. R. Barton and T. Bruun, *J. Chem. Soc.*, 603 (1953).

433 **Mycophenolic Acid,** $C_{17}H_{20}O_6$, colorless needles, m.p. 141°.

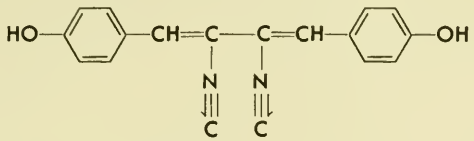


*Penicillium brevi-compactum* Dierckx
C. L. Alsberg and O. F. Black, *Bull. U. S. Bur. Pl. Ind.*, No. 270 (1913). (Isolation)
Percival Walter Clutterbuck, Albert Edward Oxford, Harold Raistrick and George Smith, *Biochem. J.* 26 1441 (1932).

J. H. Birkinshaw, A. Bracken, E. N. Morgan and H. Raistrick, *ibid.* 43 216 (1948).

J. H. Birkinshaw, H. Raistrick and D. J. Ross, *Biochem. J.* 50 630 (1952). (Structure)

434 **Xanthocillin-X**, $C_{18}H_{12}O_2N_2$, yellow crystals, m.p. ~200° (dec.).




*Penicillium notatum* Westling

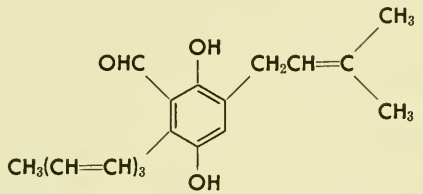
Xanthocillin constitutes about 70% of a mixture containing a second constituent, xanthocillin-Y.

W. Rothe, *Deutsche Med. Wochenschr.* 79 1080 (1954). (Isolation)

I. Hagedorn and H. Tönjes, *Pharmazie* 11 409 (1956). (Structure)

Ilse Hagedorn, Ulrich Eholzer and Arthur Luttringhaus, *Chem. Ber.* 93 1584 (1960). (Experimental work)

435 **Auroglaucin**, $C_{19}H_{22}O_3$, orange-red crystals, m.p. 153°.

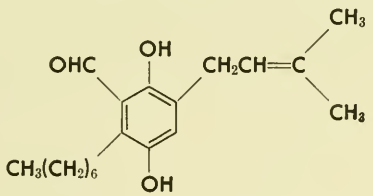



*Aspergillus glaucus, A. mangini,* other aspergilli

H. Raistrick, Robert Robinson and A. R. Todd, *J. Chem. Soc.*, 80 (1937).

Adolfo Quilico, Cesare Cardani and Luigi Panizzi, *Atti accad. nazl. Lincei Rend., Classe sci. fis., Mat. e nat. sci.* 14 358 (1953). (Structure)

436 **Flavoglaucin**, $C_{19}H_{28}O_3$, pale yellow crystals, m.p. 103°.

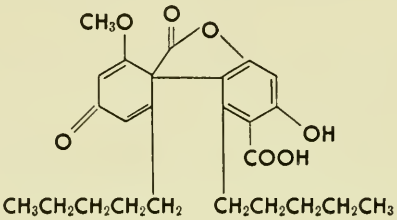


*Aspergillus glaucus*, other aspergilli

H. Raistrick, Robert Robinson and A. R. Todd, *J. Chem. Soc.*, 80 (1937).

Adolfo Quilico, C. Cardani and G. Stagno d'Alcontres, *Gazz. chim. ital.* 83 754 (1953). (Structure)

437 **Picrolichenic Acid**, $C_{25}H_{30}O_7$, colorless crystals, m.p. 178° (dec.). Proposed structure:

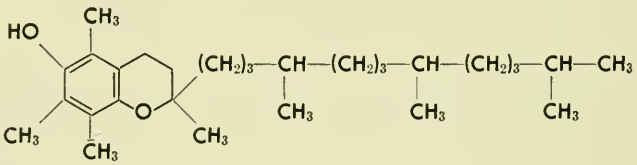



*Pertusaria amara* (Ach.) Nyl., *Variola amara* (Ach.) The yield was 5–10% of the dry weight of the lichen.

H. Erdtman and C. A. Wachtmeister, *Chem. and Ind.*, 1042 (1957).

Carl Axel Wachtmeister, *Acta Chem. Scand.* 12 147 (1958). (Structure)

438 **α-Tocopherol** (Vitamin E), $C_{29}H_{50}O_2$, viscous oil, b.p. 200–220° (0.1 mm.), $n_D^{25}$ 1.5045, U.V. max. 294 mμ.

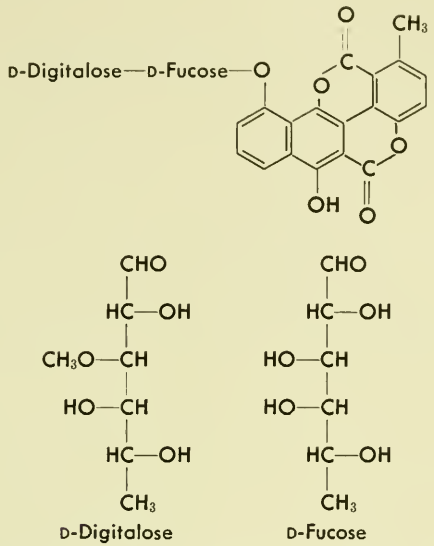



Identified in about a dozen varieties of chlorophyll-containing bacteria by paper chromatographic comparisons. (Not isolated.)

J. Green, S. A. Price and L. Gare, *Nature* 184 1339 (1959).

439 **Chartreusin** (Antibiotic X-465A), $C_{32}H_{32}O_{14}$, greenish yellow crystals, m.p. 184–186°, $[\alpha]_D^{25}$ +132° ±6° (c 0.2 in pyridine).

Proposed Structure:




*Streptomyces chartreusis* and probably other *Streptomyces* spp.

Byron E. Leach, Kenneth M. Calhoun, LeRoy E. Johnson, Charlotte M. Teeters and William G. Jackson, *J. Am. Chem. Soc.* 75 4011 (1953). (Isolation)

K. M. Calhoun and L. E. Johnson, *Antibiotics and Chemotherapy* 6 294 (1956).

Julius Berger, L. H. Sternbach, R. G. Pollock, E. R. LaSala, S. Kaiser and M. W. Goldberg, *J. Am. Chem. Soc.* 80 1636 (1958).

L. H. Sternbach, S. Kaiser and M. W. Goldberg, *ibid.* 80 1639 (1958).

E. Simonitsch, W. Eisenhuth, O. A. Stamm and H. Schmid, *Helv. Chim. Acta* 43 58 (1960). (Structure)

440 **Chartreusin-like Antibiotic,** $C_{32}H_{34}O_{14}$, m.p. 186°.

A weakly acidic glucoside.

*Streptomyces* sp.

F. Arcamone, F. Bizioli and T. Scotti, *Antibiotics and Chemotherapy* 6 283 (1956).

## *b.* DEPSIDES AND DEPSIDONES

Lichens are symbiotic partnerships of fungi and algae. While this slow-growing combination is visible without the aid of lenses, the extractable metabolites so resemble those of microorganisms that they are included in this listing for comparison.

Lichens and the fruiting bodies of the higher fungi were long used in folk medicine in the damp northern lands where they are prominent members of the flora. It was only natural, then, that the tool of organic chemistry was applied at an early date in these locations to elucidate the structures of their metabolites. Thus, historically, a large body of knowledge on such structures existed long before systematic work was begun on the fungi and streptomycetes, which have been so much more rewarding to modern medicine.

Depsides, *e.g.* microphyllic acid and olivetoric acid, frequently contain aliphatic side-chains attached to their phenolic rings. The fact that these invariably consisted of an uneven number of carbon atoms was soon recognized and used as a rule in structure determinations. It was considered a curious phenomenon until it became apparent that such molecules are particularly obvious examples of derivation from acetate.

Certain lichen metabolites, for example some of the anthraquinone pigments, have been found also in fungi. Moreover, some of the fungal partners have been isolated from lichens and grown alone in pure culture. In a few such cases the same metabolites have been isolated which are produced by the partnership itself. Examples are the anthraquinones physcion (parietin) and rhodocladonic acid, the dibenzofurans usnic and didymic acids, as well as pulvic anhydride (stictaurin) and the nidulins.[1, 2, 3]

In contrast there is evidence that depsides and depsidones cannot be produced by the isolated fungus partner, but are the unique products of a collaborative effort.[4] In the work just cited it was found that the fungal components of various cla-

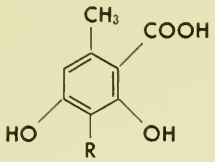
[1] E. Thomas, *Beitr. z. Kryptogamenflora der Schweiz* 9 1 (1939).

[2] Hempstead Castle and Flora Kubsch, *Arch. Biochem.* 23 158 (1949).

[3] F. M. Dean, A. D. T. Erni and Alexander Robertson, *J. Chem. Soc.*, 3545 (1956).

[4] Dieter Hess, Z. *Naturforsch.* 14b 345 (1959).

donia, parmelia and placodium species, grown alone in pure culture, produced no depsides nor depsidones. Orsellinic and

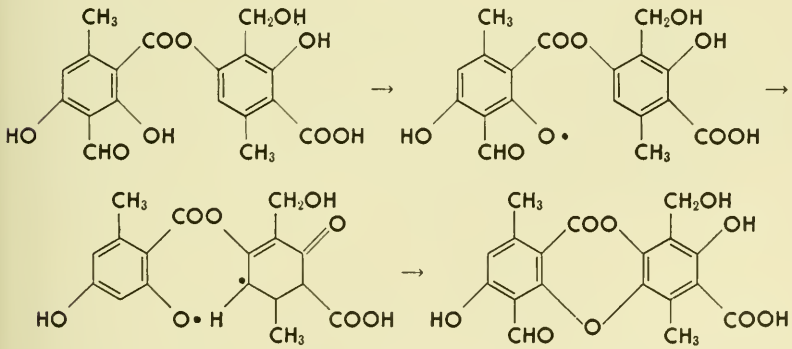



R=H=Orsellinic Acid

R=CHO=Haematommic Acid

haematommic acids, simpler moieties which could not be shown to be present as such in the parent lichens, were isolated. This could indicate that these phenols are precursors, and that the algae are necessary to effect coupling as well as final, characteristic modifications. It is interesting that orsellinic acid (q.v.) has been isolated recently from other fungus cultures. Phenolic acids of this sort are obviously acetate-derived.

Depsidones probably are formed by phenol coupling of the depsides. Phenol coupling (phenol dehydrogenation) is undoubtedly a widespread phenomenon among natural products. It involves the removal of one electron from the phenol with formation of a phenol-free radical. Such radicals are relatively stable due to the resonance possibilities. In complex natural products such phenol radicals can form new bonds by intramolecular attack. Thus the formation of a depsidone (in this case protocetraric acid) from a depside might be represented as follows:




Another example of intramolecular carbon-oxygen coupling was noted earlier in this chapter in the formation of the geodin, griseofulvin type of skeleton.

Carbon-carbon bonds can be formed similarly (by coupling

of the ortho and para resonance isomers of the phenol-free radical). Biphenyl, binaphthyl and *bis*-anthraquinone skeletons might be formed in this way.

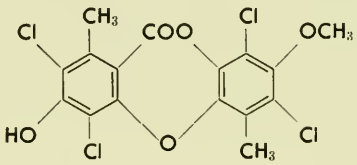
A combination of the two types of bond formation (*i.e.* first an intermolecular carbon-carbon coupling followed by an intramolecular oxygen-carbon coupling) probably occurs in the biosynthesis of compounds such as the dibenzofurans, etc.

More thorough considerations of phenol coupling as a biosynthetic process have been published.[5, 6]

*In vitro* couplings of phenolic compounds have been accomplished in the laboratory, by using simple electron acceptors such as molecular oxygen or ferric chloride, and natural products have been prepared in this way. Yields under such conditions are generally low, and the orienting influence of the enzyme surface seems to be required for real efficiency.

Referencing of this section is lean because of the very thorough existing work.[7] In general the final structure determination or synthesis is mentioned.

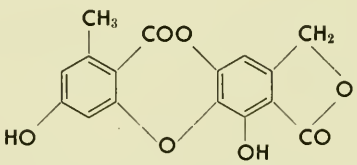
441 **Diploicin,** $C_{16}H_{10}O_5Cl_4$, colorless crystals, m.p. 232°.




*Buellia canescens* (Dicks.) DeNot.

Thomas J. Nolan, Joseph Algara, Eugene P. McCann, Wm. A. Manahan and Niall Nolan, *Sci. Proc. Roy. Dublin Soc.* 24 319 (1948).

442 **Variolaric Acid** (Ochrolechaic Acid, Parellic Acid), $C_{16}H_{10}O_7$, colorless crystals, m.p. 296°.




[5] D. H. R. Barton and T. Cohen, *Festschrift Arthur Stoll,* 117 (1957).

[6] Holger Erdtman and Carl Axel Wachmeister, *ibid.,* 144 (1957).

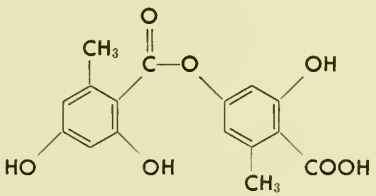
[7] Yasuhiko Asahina and Shoji Shibata, "Chemistry of Lichen Substances," Japan Society for the Promotion of Science, Tokyo, 1954. (In English)

*Lecanora parella* Ach.

The yield was about 1%. Mannitol also was present.

D. Murphy, J. Keane and T. J. Nolan, *Sci. Proc. Roy. Dublin Soc.* 23 71 (1943).

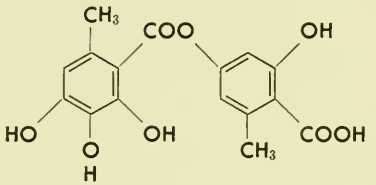
443 **Lecanoric Acid** (Glabratic Acid), $C_{16}H_{14}O_7$, colorless needles, m.p. 175°.




*Parmelia tinctorum* Despr., *P. borreri* Turm., *P. scortea* Ach. and *P. latissima* Fee.

Emil Fischer and Hermann O. L. Fischer, *Ber.* 46 1138 (1913). (Synthesis)

444 **Diploschistesic Acid,** $C_{16}H_{14}O_8$, colorless leaflets, m.p. 174°.

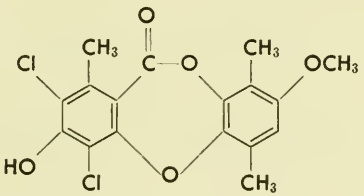



*Diploschistes scruposus* (*L.*) and *D. bryophilus* (Ehrh.)

Lecanoric acid was isolated from the same source.

Yasuhiko Asahina and Masaichi Yasue, *Ber.* **69B** 2327 (1936). (Synthesis)

445 **Vicanicin,** $C_{17}H_{14}O_5Cl_2$, colorless needles, m.p. 248–250°.

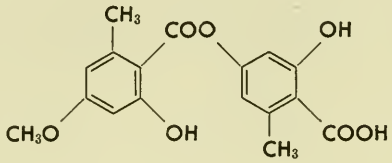



*Teloschistes flavicans*

A yield of about 1% of the dry lichen weight was obtained.

S. Neelakantan, T. R. Seshadri and S. S. Subramanian, *Tetrahedron Letters* No. 9, pp. 1–4 (1959).

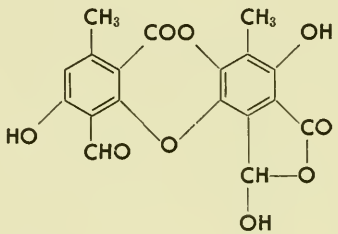
446 **Evernic Acid,** $C_{17}H_{16}O_7$, colorless prisms, m.p. 169°.




*Evernia prunastri* L., *Ramalina pollinaria* Wests., *Usnea jesoensis* Asahina

Fukuziro Fuzikawa and Kumao Ishiguro, *J. Pharm. Soc. Japan* 56 837 (in German, 149) (1936). (Synthesis)

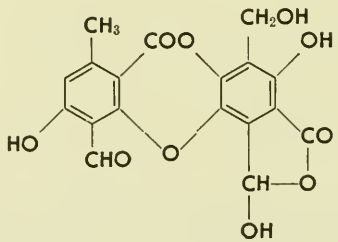
447 **Norstictic Acid,** $C_{18}H_{12}O_9$, nearly colorless needles, m.p. 283° (dec.).




*Lobaria pulmonaria* Hoffm., *Parmelia acetabulum* Duby., *Usnea japonica*, Wain., etc.

Yasuhiko Asahina and Masaichi Yanagita, *Ber.* **67B** 799 (1934).

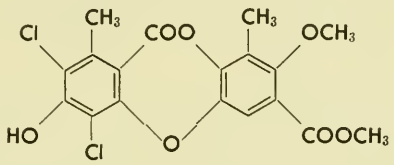
448 **Salazinic Acid** (Saxatilic Acid), $C_{18}H_{12}O_{10}$, colorless needles, m.p. 260° (dec. from 240°).



*Parmelia cetrata* Ach., *P. conspersa* Ach., *P. marmariza* Nyl., *P. saxatilis* Ach., *P. abyssinica* Kremp.

Yasuhiko Asahina and Juntaro Asano, *Ber.* **66B** 689, 893, 1215 (1933).

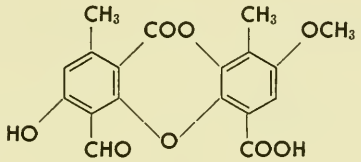
449 **Gangaleoidin**, $C_{18}H_{14}O_7Cl_2$, colorless needles, m.p. 213°.




*Lecanora gangaleoides* Nyl.

V. E. Davidson, J. Keane and T. J. Nolan, *Sci. Proc. Roy. Dublin Soc.* 23 143 (1943). (Structure)

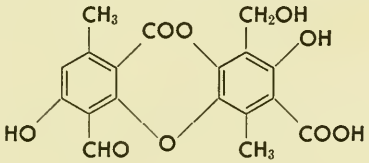
450 **Psoromic Acid** (Sulcatic Acid, Parellic Acid), $C_{18}H_{14}O_8$, colorless needles, m.p. 265°.




*Psoroma crassum* Körber, *Alectoria zopfii* Asahina, etc.

Syozi Shibata, *J. Pharm. Soc. Japan* 59 323 (in German, 111) (1939). (Synthesis)

451 **Protocetraric Acid** (Capraric Acid, Ramalinic Acid), $C_{18}H_{14}O_9$, colorless fine needles, m.p. 250° (dec. from 220°).

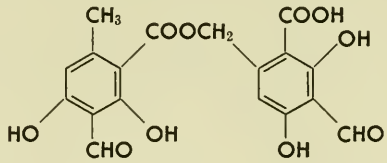



*Parmelia caperata, Ramalina farinacea,* etc.

Yasuhiko Asahina and Yaichiru Tanase, *Ber.* **66B** 700 (1933).

Yasuhiko Asahina and Juntaro Asano, *ibid.* 66B 893, 1215 (1933).

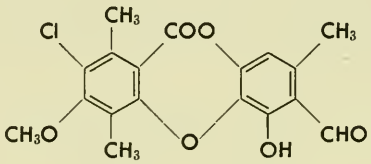
452 **Barbatolic Acid**, $C_{18}H_{14}O_{10}$, colorless crystals, m.p. 206° (dec.) (s. 190°).




*Usnea barbata, Alectoria implexa* (Hoffm.) Nyl. f. *fuscidula* Arn.

Eero E. Suominen, *Suomen Kemistileht;* 12B 26 (1939).

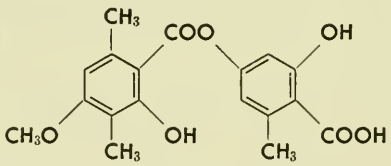
453 **Pannarin**, $C_{18}H_{15}O_6Cl$, colorless prisms, m.p. 216°.




*Pannaria lanuginosa* Korb., *P. fulvescens* Nyl., *P. lurida* Nyl.

Itiro Yosioka, *J. Pharm. Soc. Japan* 61 332 (1941).

454 **Obtusatic Acid** (Ramalic Acid), $C_{18}H_{18}O_7$, colorless needles, m.p. 208° (dec.).

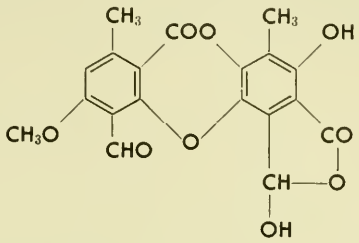



*Ramalina pollineria* Ach. other *Ramalina* species

Evernic acid, usnic acid and sometimes sekikaic acid were isolated from the same sources.

Fukuziro Fuzikawa, *J. Pharm. Soc. Japan* 56 237 (in German, 25) (1936). (Synthesis)

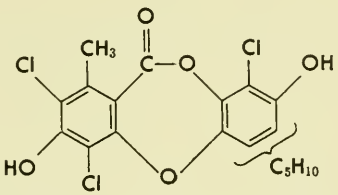
455 **Stictic Acid** (Stictaic Acid, Pseudopsoromic Acid, Scopularic Acid), $C_{19}H_{14}O_9$, colorless microcrystals, m.p. 268° (dec.).




*Lobaria pulmonaria* Hoffm., *L. oregana* Müll. Arg., *Stereocaulon nabewariense* Zahlb., etc.

Yasuhiko Asahina and Masaiti Yanagita, *Ber.* **67B** 1965 (1934).

456 **Nornidulin** (Ustin), $C_{19}H_{15}O_5Cl_3$, hexagonal plates or prisms, m.p. 185°.



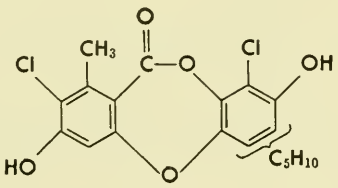

*Aspergillus nidulans*, NRRL No. 2006

A little succinic acid was isolated from the same culture.

F. M. Dean, John C. Roberts and Alexander Robertson, *J. Chem. Soc.*, 1432 (1954).

457 **Dechloronornidulin** (Ustin II), $C_{19}H_{16}O_5Cl_2$, needles, m.p. 212–214°.

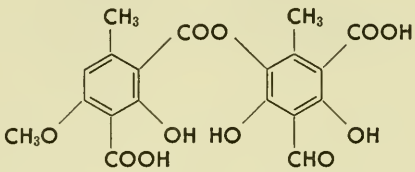
Partial structure:



*Aspergillus nidulans* NRRL No. 2006

F. M. Dean, A. D. T. Erni and Alexander Robertson, *J. Chem. Soc.*, 3545 (1956).

458 **Thamnolic Acid,** $C_{19}H_{16}O_{11}$, pale yellow crystals, m.p. 223° (dec.).

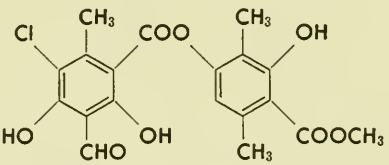



*Thamnolia vermicularis* (Sw.) Schaer., *Cladonia polydactyla* Flk., *Cl. digitata*, other *Cladonia*, *Parmeliopsis* and *Pertusaria* spp.

Yasuhiko Asahina and Michio Hiraiwa, *Ber.* **69B** 330 (1936).

*Idem., ibid.* 72 1402 (1939).

459 **Chloroatranorin,** $C_{19}H_{17}O_8Cl$, colorless crystals, m.p. 208°.

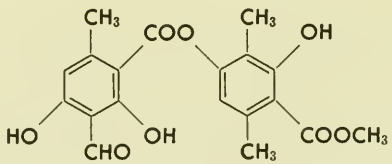



*Parmelia furfuracea* Ach., *P. physodes* Ach., *Evernia prunastri*, etc., wide occurrence

Georg Koller and Karl Pöpl, *Monatsh.* 64 106 (1934).

*Idem., ibid.* 64 126 (1934).

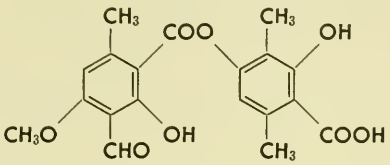
460 **Atranorin** (Atranoric Acid, Usnarin, Parmelin), $C_{19}H_{18}O_8$, colorless prisms, m.p. 196°.




Atranorin occurs in about 90 different lichens. *d*-Usnic acid also often is present.

Alexander St. Pfau, *Helv. Chim. Acta* 9 650 (1926).

461 **Baeomycesic Acid,** $C_{19}H_{18}O_8$, colorless crystals, m.p. 233°.

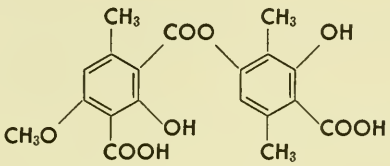



*Baeomyces roseus* Pers., *B. fungoides* Ach., *Thamnolia subvermicularis* Asahina

Squamatic acid also was present in some cases.

Georg Koller and Walter Maass, *Monatsh.* 66 57 (1935).

462 **Squamatic Acid** (Sphaerophoric Acid), $C_{19}H_{18}O_9$, colorless crystals, m.p. 228° (dec.).

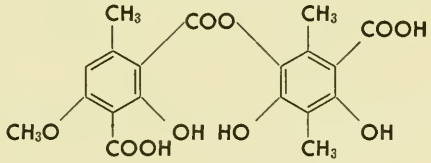



*Cladonia bellidiflora* var. *coccocephala* Ach., *Cl. squamosa* Hoffm., *Cl. uncialis* (L.) Web., *Thamnolia subvermicularis* Asahina

A little *l*-usnic acid was present also.

Yasuhiko Asahina and Yoshio Sakurai, *Ber.* 70B 64 (1937). (Synthesis)

463 **Hypothamnolic Acid,** $C_{19}H_{18}O_{10}$, colorless needles, m.p. 217.5°.

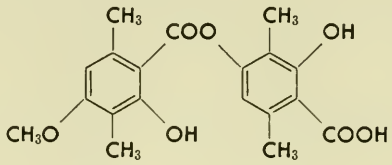



*Cladonia pseudostellata* Asahina

The yield was about 0.1%. Usnic acid was present also.

Yasuhiko Asahina, Masaru Aoki and Fukuziro Fuzikawa, *Ber.* 74B 824 (1941).

464 **Barbatic Acid** (Rhizoic Acid, Coccellic Acid), $C_{19}H_{20}O_7$, colorless prisms, m.p. 187° (dec.).

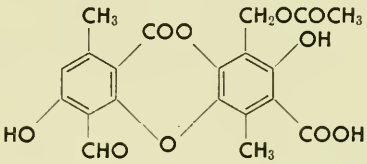



*Cladonia floerkeana* Sommerf., *Cl. bacillaris* Nyl., *Cl. macilenta* (Hoff.) Flk., *Cl. coccifera* (L.), *Cl. amaurocraea* (Fl.) Schaer., *Rhizocarpon geographicum* (L.), *Usnea longissima* Ach.

Usnic acid also was present.

Fukuziro Fuzikawa, *J. Pharm. Soc. Japan* 56 237 (in German, 25) (1936). (Synthesis)

465 **Physodalic Acid** (Monoacetylprotocetraric Acid), $C_{20}H_{16}O_{10}$, colorless plates, m.p. 260° (dec. from 230°).

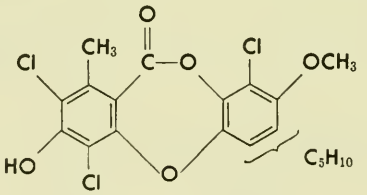



*Parmelia physodes* Ach., *P. hypotrypella* Asahina

Wilhelm Zopf, *Ann.* 295 287 (1897).

*Idem., ibid.* 300 350 (1898).

466 **Nidulin,** $C_{20}H_{17}O_5Cl_3$, colorless crystals, m.p. 180°.

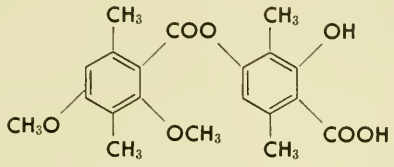



*Aspergillus nidulans* NRRL, No. 2006

The yield was about 6 g. from 126 g. of dry mycelium; a little mannitol also was found.

F. M. Dean, John C. Roberts and Alexander Robertson, *J. Chem. Soc.*, 1432 (1954).

467 **Diffractaic Acid** (Dirhizonic Acid), $C_{20}H_{22}O_7$, colorless needles, m.p. 189°.

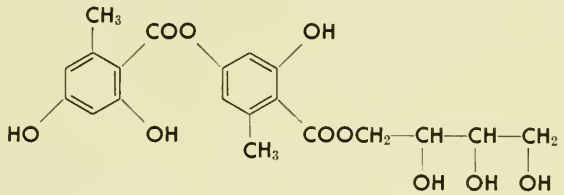


*Usnea diffracta* Wain., *Usnea longissima* Ach., *Alectoria ochroleuca* Mass.

The yield was 3.6%.

Yasuhiko Asahina and Fukuziro Fuzikawa, *Ber.* 65B 583 (1932). (Synthesis)

468 **Erythrin**, $C_{20}H_{22}O_{11}$, colorless needles, m.p. 148°, $[\alpha]_D^{29}$ +10.63°.

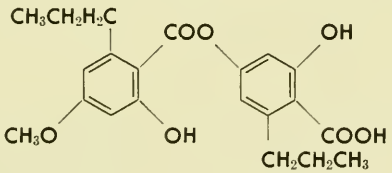


*Roccella montagnei* Bel. and *R. fuciformis* DC

This is an erythritol ester of lecanoric acid. The yield was about 5% of the weight of the lichen. Free erythritol and rocellic acid were isolated from the same source.

Yosio Sakurai, *J. Pharm. Soc. Japan* 61 108 (in German, 45) (1941).

469 **Divaricatic Acid**, $C_{21}H_{24}O_7$, colorless needles, m.p. 137°.

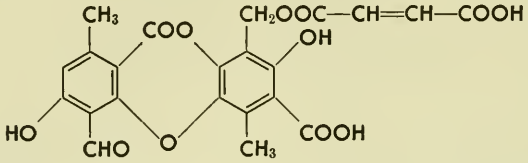


*Evernia divaricata* L., *E. mesomorpha* f. *esorediosa* Müll., Arg.

The yield from *E. mesomorpha* was recorded as 2.5% of the lichen weight. Usnic acid was isolated from the same source.

Yasuhiko Asahina and Michio Hiraiwa, *Ber.* **70B** 1826 (1937). (Synthesis)

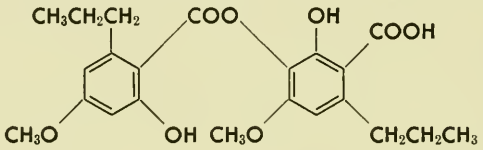
470 **Fumarprotocetraric Acid,** $C_{22}H_{16}O_{12}$, colorless needles, m.p. 250–260° (dec. from 230°).




*Cetraria islandica* Ach., *Cladonia rangiferina* (L.) Web., *Cl. sylvatica* (L.) Hoffm.

Yasuhiko Asahina and Yaichiro Tanase, *Ber.* **67B** 766 (1934).

471 **Sekikaic Acid,** $C_{22}H_{26}O_8$, colorless needles, m.p. 147° (dec.).

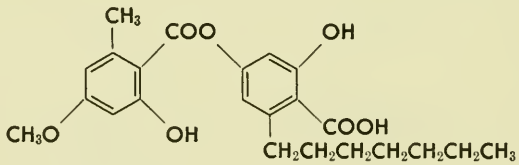



*Ramalina geniculata* Hook et Tayl., *R. calicaris* Rohl, and *R. intermediella* Wain.

The yield was about 1%. A little *d*-usnic acid also was present as well as ramalinolic acid.

Yasuhiko Asahina and Masaichi Yasue, *Ber.* **68B** 132 (1935). (Synthesis)

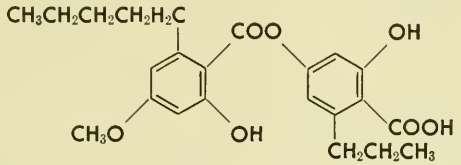
472 **Sphaerophorin,** $C_{23}H_{28}O_7$, colorless crystals, m.p. 137°.




*Sphaerophorus fragilis* Pers., *S. coralloides* Pers., *S. melanocarpus*

Akira Hasimoto, *J. Pharm. Soc. Japan* 58 776 (in German, 221) (1938). (Synthesis)

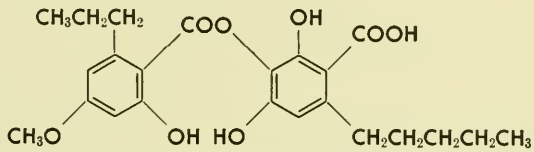
473 **Imbricaric Acid**, $C_{23}H_{28}O_7$, colorless needles, m.p. 125°.




*Parmelia perlata* Ach., *Cladonia impexa* Harm., *Cl. evansi* f. Abb., *Cl. pseudoevansi* Asahina

Yasuhiko Asahina and Itiro Yoshioka, *Ber.* **70B** 1823 (1937). (Synthesis)

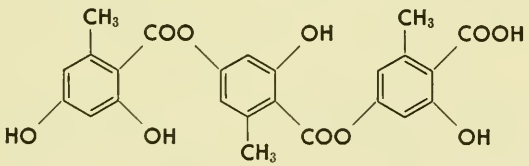
474 **Ramalinolic Acid**, $C_{23}H_{28}O_7$, colorless crystals, m.p. 163°.




*Ramalina intermediella* Wain., *R. calicaris* Rohl, *R. geniculata* Hook et Tayl. and *R. usneoides* Mont.

Yasuhiko Asahina and Tunaharu Kusaka, *Ber.* **69B** 1896 (1936). (Synthesis)

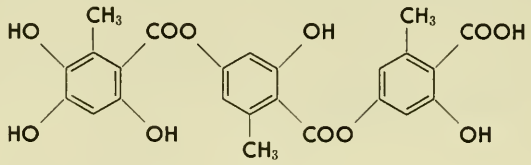
475 **Gyrophoric Acid**, $C_{24}H_{20}O_{10}$, colorless needles, m.p. 220°.




*Gyrophora esculenta* Miyoshi, *G. proboscidea* L., *Umbilicaria pustulata* L. Hoffm. *Ochrolechia pallescens*, *Lobaria pulmonaria* var. *meridionalis* (Wain.) Zahlbr.

Yasuhiko Asahina and Itiro Yasioka, *Ber.* **70B** 200 (1937). (Synthesis)

476 **Hiascic Acid,** $C_{24}H_{20}O_{11}$, colorless crystals, m.p. 190.5° (dec.).

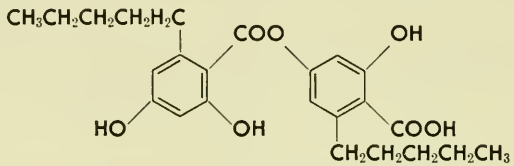



*Cetraria hiascens* Th. Fr.

Gyrophoric acid also was present.

Yasuhiko Asahina and Tunaharu Kusaka, *Bull. Chem. Soc. Japan* 17 152 (in German) (1942).

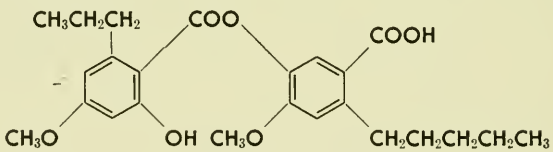
477 **Anziaic Acid,** $C_{24}H_{30}O_7$, colorless, fine needles, m.p. 124° (dec.).




*Anzia opuntiella* Müll. Arg., *A. gracilis, A. leucobatoides* f. *hypomelaena* and *Cetraria sanguinea*

Yasuhiko Asahina and Michio Hiraiwa, *Ber.* **70B** 1826 (1937). (Synthesis)

478 **Homosekikaic Acid** (Nemoxynic Acid), $C_{24}H_{30}O_8$, colorless prisms, m.p. 137.5°.

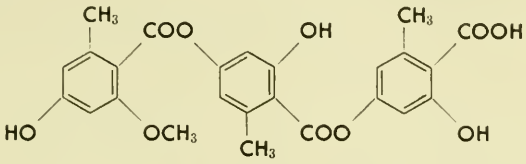



*Cladonia pityrea* Flk. f. *phyllophora* Mudd, *Cladonia nemoxyna* (Ach.) Nyl.

The yield was about 0.1%. A little fumarprotocetraric acid also was present.

Yasuhiko Asahina and Tsunakaru Kusaka, *Ber.* **70B** 1815 (1937). (Synthesis)

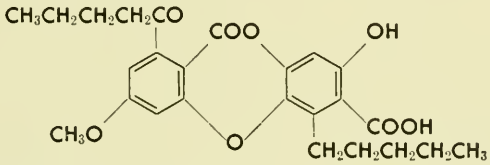
479 **Umbilicaric Acid**, $C_{25}H_{22}O_{10}$, colorless crystals, m.p. 203° (dec.). Synthetic sample m.p. 189°.




*Gyrophora polyphylla* (L.), *G. deusta* (L.) and *G. vellea* (L.)

Yasuhiko Asahina and Itiro Yosioka, *Ber.* **70B** 200 (1937). (Synthesis)

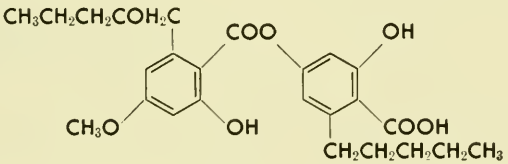
480 **Lobaric Acid** (Stereocaulic Acid, Usnetic Acid), $C_{25}H_{28}O_8$, colorless needles, m.p. 192°.




*Stereocaulon paschale* Ach., *S. exutum* Nyl., etc. (wide occurrence)

Yasuhiko Asahina and Masaiti Yasue, *Ber.* **69B** 643 (1936).

481 **Glomelliferic Acid**, $C_{25}H_{30}O_8$, colorless prisms, m.p. 143°.



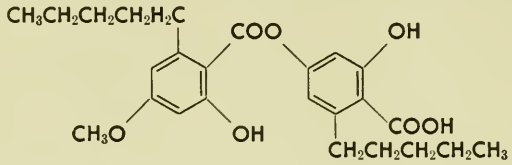

*Parmelia glomellifera* Nyl.

W. Zopf, *Ann.* **297** 303 (1897), **313** 341 (1900).

Yasuhiko Asahina and Hisasi Nogami, *Ber.* **70B** 1498 (1937).

K. Minami, *J. Pharm. Soc. Japan* **64** 315 (1944).

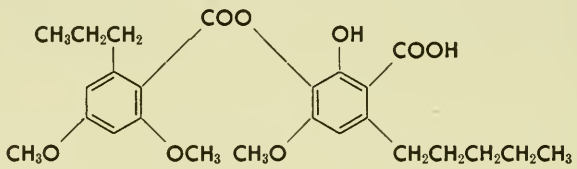
482 **Perlatolic Acid,** $C_{25}H_{32}O_7$, colorless needles, m.p. 108°.




*Parmelia perlata* Ach., *Cladonia impexa* Harm., *Cl. evansi* f. Abb., *Cl. pseudoevansi* Asahina

Yasuhiko Asahina and Itiro Yoshioka, *Ber.* **70B** 1823 (1937). (Synthesis)

483 **Boninic Acid,** $C_{25}H_{32}O_8$, colorless plates, m.p. 134.5°.

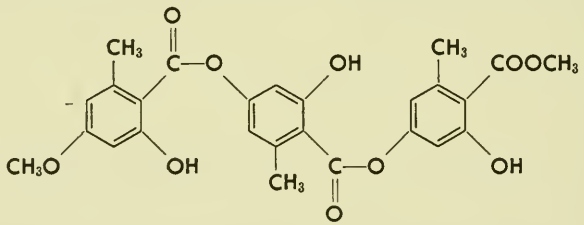



*Ramalina boninensis* Asahina

The yield was about 0.5%, and a little *d*-usnic acid was present.

Yasuhiko Asahina and Tsunaharu Kusaka, *Ber.* **70B** 1815 (1937). (Synthesis)

484 **Tenuiorin,** $C_{26}H_{24}O_{10}$, colorless crystals, m.p. 238° (dec.) s. 180°.

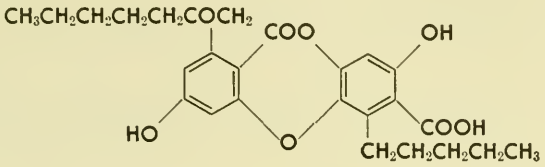



*Lobaria pulmonaria* Hoffm. f. *tenuior* Hue.

Mannitol also was present.

Yasuhiko Asahina and Masaiti Yanagita, *Ber.* **66B** 1910 (1933).

485 **Physodic Acid** (Farinacic Acid), $C_{26}H_{30}O_8$, colorless prisms, m.p. 205° (dec.).



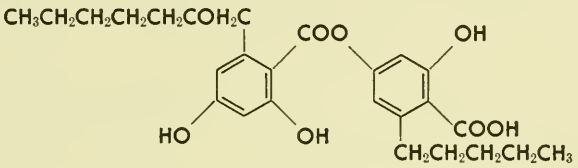

*Parmelia physodes* Ach., *P. furfuracea* Ach.

A yield of 5% was reported.

Yasuhiko Asahina and Hirasi Nogami, *Ber.* **67B** 805 (1934).

*Idem., ibid.* **68B** 77, 1500 (1935).

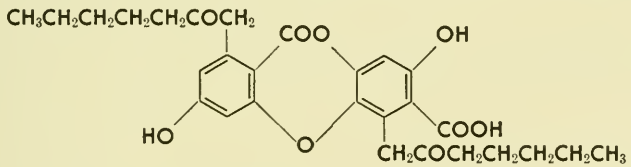
486 **Olivetoric Acid,** $C_{26}H_{32}O_8$, colorless crystals, m.p. 151°.




*Parmelia olivetorum* Nyl., *Cornicularia pseudosatoana* Asahina and *C. divergens* Ach.

Yasuhiko Asahina and Fukuziro Fuzikawa, *Ber.* **67B** 163 (1934).

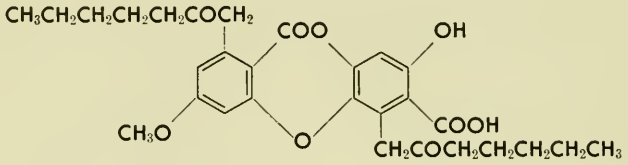
487 **Alectoronic Acid,** $C_{28}H_{32}O_9$, colorless prisms, m.p. 193°.




*Alectoria japonica* Tuck., *A. sarmentosa* Ach., *Cetraria pseudocomplicata* Asahina, *Nephromopsis cilialis* Hue.

Yasuhiko Asahina, Yoshinari Kanaoka and Fukuziro Fuzikawa, *Ber.* **66B** 649 (1933).

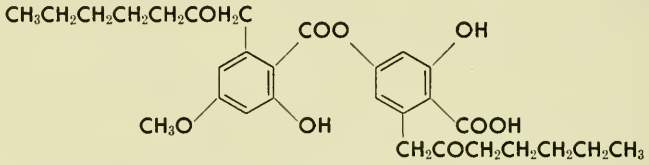
488 α-Collatolic Acid (Lecanorolic Acid, Lecanoral), $C_{29}H_{34}O_9$ colorless needles, m.p. 124°.




*Cetraria collata* Müll. Arg., *Lecanora atra* (Hudson) Ach., *L. grumosa* (Pers.) Röhl.

Yasuhiko Asahina, Yoshinari Kanaoka and Fukuziro Fuzikawa, *Ber.* 66B 649 (1933).

489 Microphyllic Acid, $C_{29}H_{36}O_9$, colorless needles, m.p. 116°.




*Cetraria japonica* Zahlbr.

Some chloroatranorin was isolated from the same extract. The yield of microphyllic acid was about 4% of the lichen weight.

Yasuhiko Asahina and Fukuziro Fuzikawa, *Ber.* 68B 2022 (1935).

12

# Quinones and Related Compounds

Quinones occur widely in nature, and this topic has been reviewed.[1, 2, 3] Even allowing for their conspicuousness due to color, solubility characteristics and (often) quantity, it seems that they are broadly distributed among plants, and fungi are no exceptions.

Anthraquinones, in particular, have been isolated frequently from fungus cultures. Some 80 anthraquinones and related substances of known structure were listed by W. Karrer[3] as having been reported from plant sources in general. About half of this number have been isolated and characterized from fungi and lichens. Since no anthraquinones have been reported from algae, it may be that those present in lichens are formed primarily by the fungus component. There is some evidence, however, that in lichens both partners are required for the biosynthesis of depsides and depsidones.[4]

In fungi anthraquinones occur mainly in the mycelium, often as mixtures of closely related materials. It is likely, for this reason, that some of the quinones reported in the early literature were impure.

The frequent identification of anthraquinone pigments in molds has caused some speculation on their function. Arguments in favor of a biological function have been summarized

[1] S. Shibata, *Kagaku (Science)* 26 391–396 (1956).

[2] R. H. Thomson, "Naturally Occurring Quinones," Academic Press, New York, 1957, 302 pp.

[3] W. Karrer, "Konstitution und Vorkommen der Organischen Pflanzenstoffe," Birkhauser Verlag, Basel, 1958.

[4] Dieter Hess, *Zeitschr. Naturforsch.* 14b 345 (1959).

as follows:[5] (1) Some pigment complexes are produced in large quantities, up to 30 percent of the total dry weight of the mycelium. (2) In many cases, the maximal pigment content is reached while usable carbohydrate is still present. If harvesting is delayed, pigment disappears as autolysis sets in. (3) The same pigment often is present in different genera or families, suggesting solution of a metabolic problem in the same way. (4) Reduction products such as anthranols, anthrones and quinhydrones sometimes are present together with the parent quinone, perhaps indicating a hydrogen or electron transport function. (5) Several mold pigments are antibiotic toward other fungi and bacteria.

On the other hand, it has been pointed out[6] that, in fungi, induced mutations leading to full blocking of the production of acetate-derived aromatic compounds such as anthraquinones do not seem to affect the vitality of the organism. The antifunctionalists believe that anthraquinones and perhaps some other mold metabolites are merely waste or storage products due to an overflow of acetate metabolism. If some of these products happen to inhibit competitors, they facilitate species survival.

A similar concept of the significance of such mold metabolites has been mentioned by Dalgliesh.[7] He proposed that enzyme systems unable to deal with substrate because it is in large excess, or for some other reason, might convert it to anthraquinones and other substances, which are eliminated, then, in a kind of "detoxication" disposal mechanism.

An enzyme chemist, F. F. Nord, has suggested[8] that many of the metabolites produced in yields exceeding functional requirements, or for which there is no function, accumulate because some of the enzyme systems involved in the oxidative sequences become saturated with respect to their substrates. They are thus, in his opinion, probably products of anaerobic

[5] G. Smith, Congr. intern. botan. Paris, Rapps. et communs., 8 Sec. 83–89 (1954).

[6] Gösta Ehrensvärd, "Developments in Aromatic Chemistry," Special Publication No. 12, English Chemical Society, London, 1958, p. 29.

[7] C. E. Dalgliesh, *ibid.*, p. 14.

[8] F. F. Nord and D. D. Clarke, *Arch. Biochem. and Biophys.* 59 285 (1955).

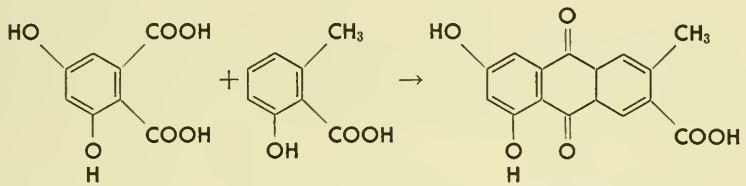
metabolism, and arise in a manner analogous to the accumulation of citric acid, which is induced under the same conditions.[9]

There is no convincing experimental evidence that anthraquinones are important in electron transport.

It has been suggested[10] that anthraquinones are acetate-derived, and there is some experimental confirmation.[11, 12, *]

This proof was obtained by growing the mold in the presence of $C^{14}$-labeled acetate, isolating the metabolite, which incorporated the label to some degree, then degrading the molecule by ingenious chemical methods to determine the sites of labeling.

Although an acetate origin is indicated, the detailed natures of the intermediates in the biosynthetic mechanism are still unknown. Intermediates such as orsellinic acid,[13] dihydroxyphthalic acid,[14] and 6-methylsalicylic acid[15] (all known mold metabolites) have been proposed, *e.g.*:




3,5-Dihydroxyphthalic Acid | 6-Methylsalicylic Acid | Endocrocin

Birch prefers to think in terms of an intermediate formally resembling a polyketomethylene chain, which can be modified in various ways on an enzyme surface to yield related metabolites. This concept is supported by the occasional discovery of related metabolites in the same culture or plant. For example, the co-occurring anthraquinone and phenanthrenequinone

[9] H. A. Krebs, *Biochem. J.* 31 2095 (1937).

[10] A. J. Birch and F. W. Donovan, *Austral. J. Chem.* 6 360 (1953).

[11] Sten Gatenbeck, *Acta Chem. Scand.* 12 1211 (1958).

[12] A. J. Birch, A. J. Ryan and Herchel Smith, *J. Chem. Soc.*, 4473 (1958).

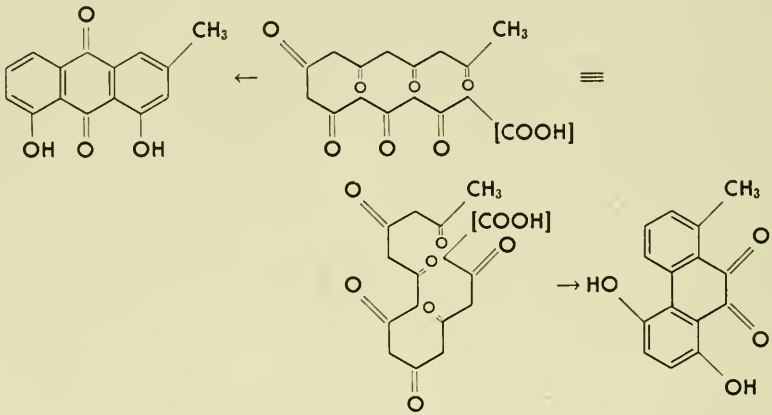
* Also see addendum for later work.

[13] K. Aghoramurthy and T. R. Seshadri, *J. Sci. Ind. Res.* (India) 13A 114 (1959).

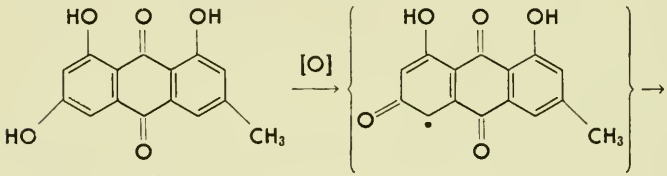
[14] E. L. Tatum, *Ann. Rev. Biochem.* 13 667 (1944).

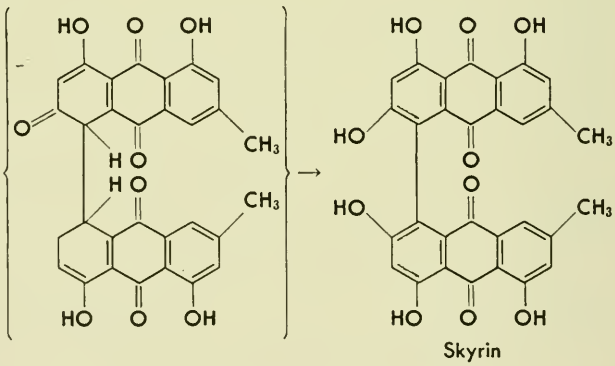
[15] Harold Raistrick, *Acta Chem. Fenn.* 10A 237 (1950).

shown below could be envisaged as derivatives of a common precursor chain, which is laid down upon the enzyme surface in different patterns before cyclization.[16]




It is likely that the dianthraquinones are formed by oxidative phenolic radical coupling, *e.g.*:*



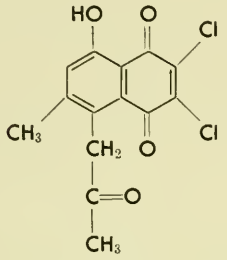




[16] A. J. Birch, private communication.

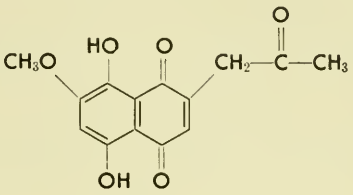
* See addendum for evidence to the contrary.

Other metabolites, such as actinorhodin and the perylenequinones may be formed similarly.

Structures such as mollisin and javanicin seem to indicate an acetate derivation for naphthoquinones.

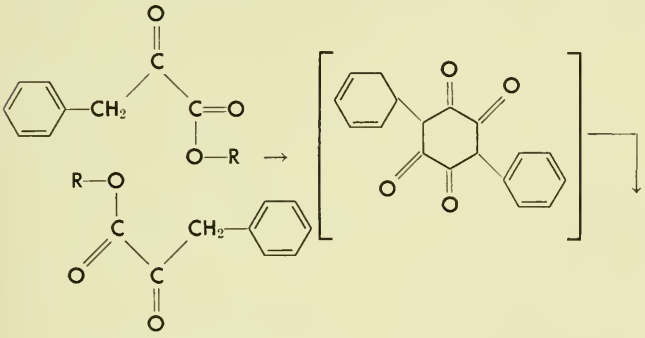



Mollisin

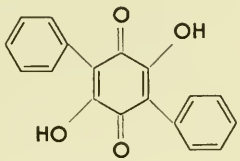



Javanicin

The suggestion has been made[17] that the terphenylquinones might be formed by autocondensation of a phenylpyruvic acid type of molecule in the following sense:




Phenylpyruvate

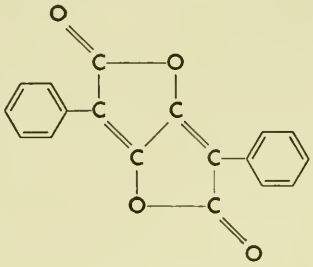



Polyporic Acid

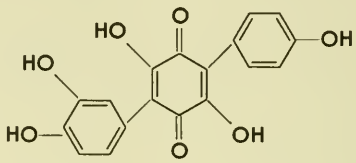
Similarly *p*-hydroxyphenylpyruvate would form atromentin. Polyporic acid might be transformed by oxidation to pulvinic

[17] G. Read and L. Vining, *Chem. and Ind.*, 1546 (1959).

acid, and by further hydroxylations to leucomelone or other terphenylquinones.



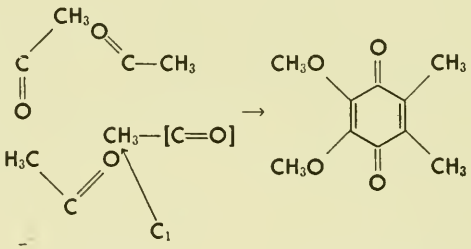
Pulvinic Acid



Leucomelone

If this suggestion can be confirmed experimentally, it will relate this type of benzoquinone metabolite to the shikimic acid route of biogenesis.

The biosynthesis of a benzoquinone, aurantiogliocladin, has been studied, by using $C^{14}$-labeled formate and acetate.[18] The results demonstrated formation from 4 moles of acetate with decarboxylation, C-methylation, post-oxidation in the aromatic



Acetate

Aurantiogliocladin

ring and O-methylation of the phenolic hydroxyl groups.

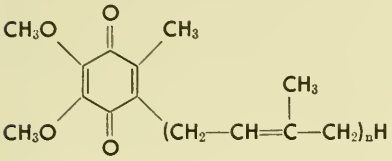
6-Methylsalicylic acid appears to be an intermediate.[19]

Aurantiogliocladin, isolated from a gliocladium specimen, resembles the coenzymes Q. These substances occur in the cell mitochondria of a wide variety of organisms. They are benzoquinones substituted similarly to aurantiogliocladin, but with

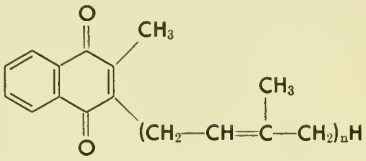
[18] A. J. Birch, R. I. Fryer and Herchel Smith, *Proc. Chem. Soc.*, 343 (1958).

[19] Private communication from Herchel Smith.

additional polyisoprenoid side-chains. There is a marked re-




Coenzyme $Q_n$




Vitamin $K_n$

semblance to the previously discovered vitamins K. The following substances have been isolated, purified, and the structures determined:

TABLE I

| Origin | Numbers of side-chain isoprene units (n) | Number of carbon atoms | Melting point (°C) | Designation | References |
|---|---|---|---|---|---|
| *Saccharomyces cerevisiae* | 6 | 39 | 16° | Coenzyme $Q_6$ | 20 |
| *Torula utilis* | 7 | 44 | 30.5° | Coenzyme $Q_7$ | 20, 21 |
| *Azotobacter vinelandii* | 8 | 49 | 37° | Coenzyme $Q_8$ | 20, 21 |
| *Torula utilis* | 9 | 54 | 45.2° | Coenzyme $Q_9$ | 20, 21 |
| Beef heart | 10 | 59 | 48° | Coenzyme $Q_{10}$ | 21, 22 |

A survey was made, by using methods sometimes short of isolation and purification (paper chromatographic comparisons, spectra, etc.) of the occurrence of coenzyme Q and of vitamin K in a wide variety of biological types.[23] Many bacteria contain coenzyme Q. The mycobacteria and streptomycetes seem to contain vitamin K instead. *Escherichia coli* and chromatium species contain both. Obligate anaerobes such as the clostridia

[20] R. L. Lester, F. L. Crane and Y. Hatefi, *J. Am. Chem. Soc.* 80 4751 (1958).

[21] R. L. Lester and F. L. Crane, *Biochim. et Biophys. Acta* 32 492 (1959).

[22] F. L. Crane, Y. Hatefi, R. L. Lester and Carl Widmer, *Biochim. et Biophys. Acta* 25 220 (1957); *idem., ibid.* 32 73 (1959).

[23] R. L. Lester and F. L. Crane, *J. Biol. Chem.* 234 2169 (1959).

contain neither, and facultative anaerobes such as *Saccharomyces cerevisiae* and *E. coli* contain neither when grown anaerobically. A chart of microbial occurrence was published:

TABLE II

| Organism | Coenzyme Q | Vitamin K |
|---|---|---|
| *Saccharomyces cerevisiae* (anaerobic) | — | — |
| *Saccharomyces cerevisiae* (aerobic) | $Q_6$ | |
| *Saccharomyces cavalieri* | $Q_6$ | |
| *Saccharomyces fragilis* | $Q_6$ | |
| *Neurospora crassa* | $Q_{10}$ | |
| *Mucor corymbifer* | $Q_9$ | |
| *Streptomyces griseus* | | + |
| *Mycobacterium smegmatis* | | + |
| *Mycobacterium tuberculosis* | | + |
| *Bacillus mesentericus* | | + |
| *Escherichia coli* | $Q_8$ | |
| *Chromatium* spp. | $Q_7$ | |
| *Rhodospirillum rubrum* | $Q_9$ | |
| *Pseudomonas fluorescens* | $Q_9$ | |
| *Hydrogenomonas* sp. | $Q_8$ | |

Basidiomycetes contain neither coenzyme Q nor vitamin K, but produce another quinone which seems to have the same function in this family. It has been extracted and purified to some extent and called basidioquinone.

A comparison of all the animal, plant and microorganism sources indicated that, in general, lower organisms contain lower homologues of coenzyme Q.

Evidence has been obtained for the coenzyme function of the Q (and K) quinones: (1) Extraction from mitochondria destroys enzymatic activity, which is restored by restoration of the coenzymes. (2) Inhibitors of electron transport, such as the antibiotic, antimycin A, affect the oxidation state of the quinones in a predictable manner. (3) The rate of oxidation or reduction in mitochondria is what might be anticipated for participation in electron transport. The pattern of occurrence in aerobic and anaerobic microorganisms also is suggestive.

The general structure of the electron transport system in cell mitochondria in the light of the new discoveries has been reviewed.[24]

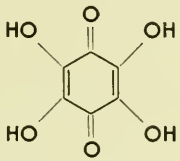
Apparently coenzyme Q is formed by a combination of the

[24] D. E. Green and R. L. Lester, *Federation Proc.* 18 987–1000 (1959).

simple acetate and terpenoid biosynthetic routes. Mevalonic acid was incorporated into the molecule by rats (especially vitamin A-deficient rats) and by rat liver, while 2,3-dimethoxy-5-methyl-1,4-benzoquinone and D,L-tocopherol were not.[25] This contrasts with evidence that 2-methyl-1,4-naphthoquinone is used as a precursor of vitamin K by rats.[26] Evidently, no experimental work has been published on biosynthesis in microorganisms.

*a.* BENZOQUINONES

490 **Tetrahydroxybenzoquinone,** $C_6H_4O_6$, bluish black plates, no melting point.



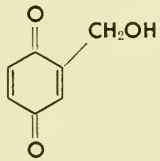

*Pseudomonas beijerinckii* Hof grown on salted beans. The substrate is *meso*-inositol, which probably is a normal constituent of beans.

T. Hof, *Rec. Trav. Botan. Néerland.* 32 92 (1935). (Isolation)

A. J. Kluyver, T. Hof and A. G. J. Boezaardt, *Enzymologia* 7 257 (1939). (Structure)

Paul W. Preisler and Louis Berger, *J. Am. Chem. Soc.* 64 67 (1942).

491 **Gentisylquinone,** $C_7H_6O_3$, yellow needles, m.p. 76°.



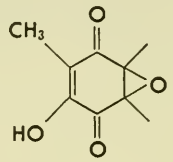

*Penicillium patulum* Bainier probably produces a little of this quinone under certain conditions, although it may be an artifact, since larger quantities of the corresponding hydroquinone are produced. It has been isolated as a deep violet colored complex, m.p. 86–89°, with the hydroquinone.

[25] U. Gloor and O. Wiss, *Arch. Biochem. and Biophys.* 83 216 (1959).

[26] C. Martius and H. O. Esser, *Biochem. Z.* 331 1 (1958).

B. G. Engel and W. Brzeski, *Helv. Chim. Acta* 30 1472 (1947).

492 Terreic Acid, $C_7H_6O_4$, pale yellow, large, glistening plates, m.p. 127–127.5°, $[\alpha]_D^{22}$ −28.6° (c 1 in 50% methanol-benzene). Sublimes.



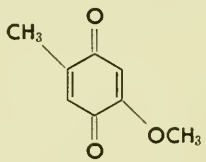

*Aspergillus terreus* grown in a glucose and corn-steep liquor-cottonseed meal medium.

H. M. Florey, E. Chain, N. G. Heatley, M. A. Jennings, A. G. Sanders, E. P. Abraham and M. E. Florey, "Antibiotics," Oxford University Press, London, 1949 Vol. I p. 388.

Murray A. Kaplan, Irving R. Harper and Bernard Heinemann, *Antibiotics and Chemotherapy* 4 746 (1954). Yield 138 g. from 200 liters.

J. Sheehan, W. Lawson and R. Gaul, *J. Am. Chem. Soc.* 80 5536–5538 (1958). (Structure)

493 4-Methoxytoluquinone (Coprinin), $C_8H_8O_3$, yellow spangles, m.p. 175°.

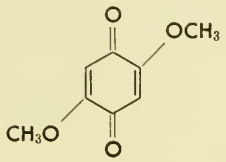



*Coprinus similis* B. and Br., *Lentinus degener* Kalchbr. grown on a Czapek-Dox medium, containing glucose and corn-steep solids.

Marjorie Anchel, Annette Hervey, Frederick Kavanagh, Jerome Polatnick and William J. Robbins, *Proc. Nat. Acad. Sci. U. S.* 34 498 (1948). (Isolation)

R. B. Woodward, Franz Sondheimer, David Taub, Karl Heusler and W. M. McLamore, *J. Am. Chem. Soc.* 74 4234 (1952). (Synthesis)

494 2,5-Dimethoxybenzoquinone, $C_8H_8O_4$, yellow prisms, m.p. 250° (dec.).



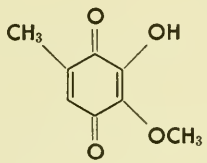
*Polyporus fumosus* (Pers.) Fries grown on an artificial medium including glucose and corn-steep liquor.

Yield: 0.1 g. from 2 liters of culture broth.

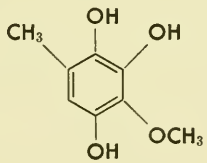
J. D. Bu'Lock, *J. Chem. Soc.*, 575 (1955). (Isolation)

E. Knoevenagel and C. Bückel, *Ber.* 34 3993 (1901). (Synthesis)

495 **Fumigatin**, $C_8H_8O_4$, maroon needles, m.p. 116°.



496 **Fumigatin Hydroquinone** is produced as well, the ratio of the two compounds varying with the age of the culture.

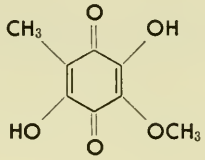


*Aspergillus fumigatus* Fres. grown on a Raulin-Thom medium.

Winston Kennay Anslow and Harold Raistrick, *Biochem. J.* 32 687 (1938). (Isolation)

W. K. Anslow, J. N. Ashley and H. Raistrick, *J. Chem. Soc.*, 439 (1938). (Synthesis)

497 **Spinulosin,** $C_8H_8O_5$, purple-black plates, m.p. 203°.




First isolated from three strains of *Penicillium spinulosum* Thom grown on a modified Czapek-Dox-glucose medium. Later isolated from two out of seven strains of *Aspergillus fumigatus* examined. *Spinulosin* as well as an orange pigment, m.p. 184–185°, with antibiotic properties resembling those of fumigatin, also has been isolated from an unidentified *Penicillium* (perhaps *Penicillium spinulosum*). *Penicillium cinerascens* Biourge is another producer.

J. H. Birkinshaw and H. Raistrick, *Trans. Roy. Soc.* (London) **B220** 245 (1931).

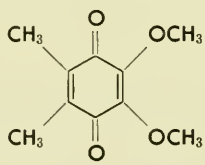
Winston K. Anslow and Harold Raistrick, *Biochem. J.* 32 687, 2288 (1938). (Isolation)

A. Bracken and H. Raistrick, *ibid.* 41 569 (1947).

Keichiro Hoshishima, *Tohuku J. Exptl. Med.* 52 273 (1950).

Winston K. Anslow and Harold Raistrick, *Biochem. J.* 32 803 (1938). (Synthesis)

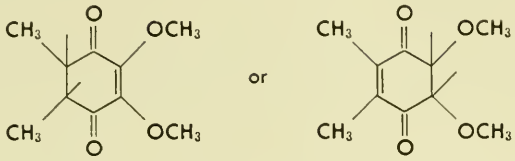
498 **Aurantiogliocladin,** $C_{10}H_{12}O_4$, orange plates, m.p. 62.5°.




The corresponding quinhydrone, a dark red compound called rubrogliocladin, occurs together with aurantiogliocladin.

A *Gliocladium* specimen, probably *G. roseum* Bainier produces these substances as well as:

499 **Gliorosein**, $C_{10}H_{14}O_4$, colorless crystals, m.p. 48°.

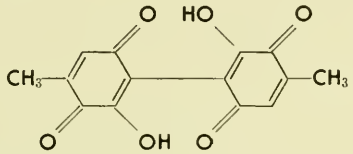



P. W. Brian, P. J. Curtis, S. R. Howland, E. G. Jeffreys and H. Raudnitz, *Experientia* 7 266 (1951). (Isolation)

E. B. Vischer, *J. Chem. Soc.*, 815 (1953). (Structure)

Wilson Baker, J. F. W. McOmie and D. Miles, *ibid.*, 820 (1953). (Synthesis)

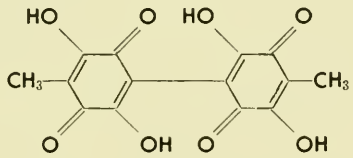
500 **Phoenicin**, $C_{14}H_{10}O_6$, yellow-brown tablets, m.p. 231°.




*Penicillium phoeniceum* van Beyma, *P. rubrum* O. Stoll.

Theodore Posternak, Hans W. Ruelius and Jacques Tcherniak, *Helv. Chim. Acta* **26** 2031 (1943). (Synthesis)

501 **Oosporein** (Chaetomidin), $C_{14}H_{10}O_8$, bronze plates, m.p. 260–275°.



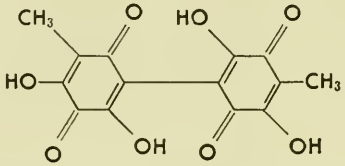

*Oospora colorans* van Beyma, *Chaetomium aureum* Chivers, *Verticillium psalliotae*, *Acremonium* sp.

F. Kögl and G. C. Van Wessem, *Rec. trav. chim.* **63** 5 (1944). (Isolation)

F. M. Dean, A. M. Osman and Alexander Robertson, *J. Chem. Soc.*, 11 (1955).

G. Lloyd, Alexander Robertson, G. B. Sankey and W. B. Whalley, *ibid.*, 2163 (1955).

502 **Isoöösporein,*** $C_{14}H_{10}O_8$, purple crystals, no m.p., subl. 220–250°, dec. 250°.

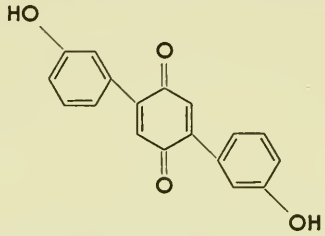


Unclassified citric acid-forming fungus.

Maximal yield 2.5 g. per liter.

Nobuyo Shigematsu, *J. Inst. Polytech.*, Osaka City Univ. Ser. C 5 100 (1956).

503 **Volucrisporin,** $C_{18}H_{12}O_4$, red plates, m.p. >300°.

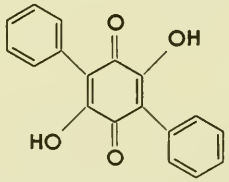


*Volucrispora aurantiaca*

Occasionally small quantities of the leuco derivative (hydroquinone) occur with the pigment.

P. V. Divekar, G. Read and L. C. Vining, *Chem. and Ind.*, 731 (1959).

504 **Polyporic Acid,** $C_{18}H_{12}O_4$, bronze leaflets, m.p. 305–307° (dec.).



*Polyporus nidulans* Fries, *P. rutilans* (Pers.) Fries, *Peniophora filamentosa* (B. and C.) Burt, *Sticta coronata* Muell., *S. colensoi* Bab.

Fritz Kögl, *Ann.* 465 243 (1928).

J. Murray, *J. Chem. Soc.*, 1345 (1952).

* See addendum.

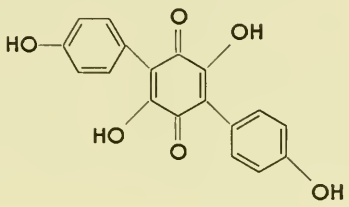
The air-dried fruiting body of *P. rutilans* contains 23%. It is not produced by the fungal mycelium in artificial culture.

Robert L. Frank, George R. Clark and James N. Coker, *J. Am. Chem. Soc.* 72 1824 (1950).

Polyporic acid is probably identical with the lichen pigment, orygmaeic acid, first described by Zopf.

Wilhelm Zopf, *Ann.* 317 124 (1901).

505 **Atromentin,** $C_{18}H_{12}O_6$, bronze leaflets, no m.p.

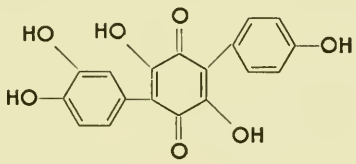



*Paxillus atromentosus* (Batsch.) Fr.

This basidiomycete often grows on old tree trunks and produces the pigment first in a leuco-form, which air-oxidizes to the colored form on the outer portions of the fruiting body and during isolation. The yield was about 2% of the weight of the air-dried fruiting body.

Fritz Kögl, *Ann.* 465 243 (1928).

506 **Leucomelone,** $C_{18}H_{12}O_7$, brown leaflets, m.p. 320° (dec.).



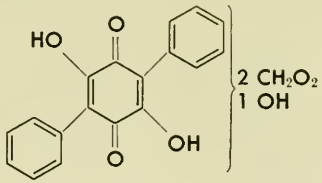

*Polyporus leucomelas* Pers. ex Fr.

Yield 3 g. per kilogram of fruiting body.

Masuo Akagi, *J. Pharm. Soc. Japan* 62 129 (1942). Synthesis)

507 **Thelephoric Acid,** $C_{20}H_{12}O_9$, lustrous, nearly black prisms, no m.p.

Partial structure:

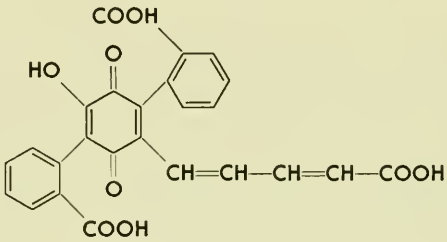



*Thelephora palmata*, other *Thelephora* spp., *Lobaria retigera* Trev., *L. pulmonaria* (L.) Hoffm., *Hydnum* spp., *Cantharellus multiplex* Underw., *Polystictus versicolor* (L.) Fr.

Fritz Kögl, Hanni Erxleben and Ludwig Jänecke, *Ann.* 482 105 (1930).

K. Aghoramurthy, K. G. Sarma and T. R. Seshadri, *Tetrahedron Letters* No. 8 20 (1959). (Revised structure)

508 **Muscarufin**, $C_{25}H_{16}O_9 \cdot H_2O$, orange-red needles, m.p. 275.5°.

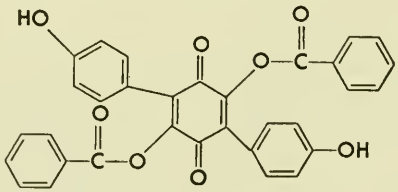



*Amanita muscaria* (Linn.) Fries

This pigment causes the red color of the caps of this common poisonous toadstool (fly agaric), yet 500 kg. of the fungus yielded only 850 mg. of pure material.

Fritz Kögl and Hanni Erxleben *Ann.* 479 11 (1930).

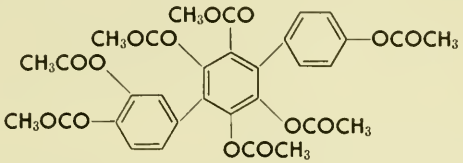
509 **Auriantiacin** (Atromentin-3,6-dibenzoate), $C_{32}H_{20}O_8$, dark red needles, m.p. 285–295°.




*Hydnum aurantiacum* Batsch.

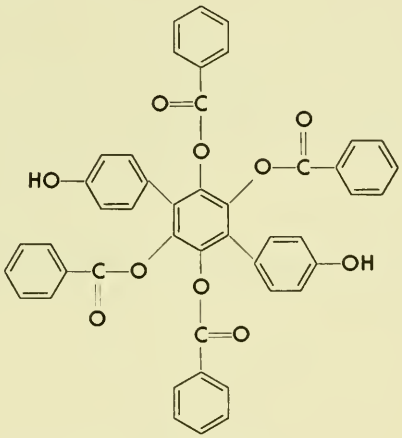
Jarl Gripenberg, *Acta Chem. Scand.* 10 1111 (1956).

510 **Protoleucomelone,** $C_{32}H_{28}O_{14}$, colorless crystals, m.p. 203–205°. Probable structure:




*Polyporus leucomelas* Pers. ex Fr.
Yield 3–4 g. per kilogram of mushrooms.
Masuo Akagi, *J. Pharm. Soc. Japan* 62 129 (1942).

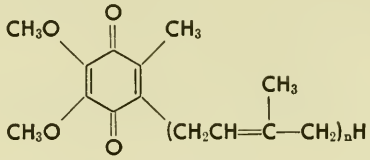
511 **Metabolite of *Hydnum aurantiarum*,** $C_{46}H_{30}O_{10}$, colorless needles. m.p. 305–307°.




*Hydnum aurantiacum* Batsch.
Aurantiacin and thelephoric acid are produced by the same organism.
Jarl Gripenberg, *Acta Chem. Scand.* 12 1411 (1958).

**Coenzymes Q** (Mitoquinone, Ubiquinone, $Q_{275}$, SA).
These compounds occur widely in the cell mitochondria of microorganisms and higher animals, where they play a part in the electron transport system. Variations in side-chain length occur as in the case of vitamin K. Compounds in which n = 6, 7, 8 and 9 have been isolated from microbial sources. The quinone moiety resembles aurantiogliocladin.

General structure:



512 **Coenzyme $Q_6$**, $C_{39}H_{58}O_4$, m.p. 16°.
*Saccharomyces cerevisiae*

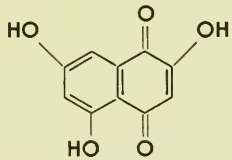
513 **Coenzyme $Q_7$**, $C_{44}H_{66}O_4$, orange crystals, m.p. 30.5°.
*Torula utilis*

514 **Coenzyme $Q_8$**, $C_{49}H_{74}O_4$, orange crystals, m.p. 37°.
*Azotobacter vinelandii*

515 **Coenzyme $Q_9$**, $C_{54}H_{82}O_4$, orange crystals, m.p. 45.2°.
*Torula utilis*
R. L. Lester, F. L. Crane and Y. Hatefi, *J. Am. Chem. Soc.* 80 4751 (1958). (Isolation)
F. W. Heaton, J. S. Lowe and R. A. Morton, *J. Chem. Soc.*, 4094 (1956).

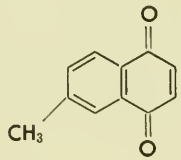
### *b.* NAPHTHOQUINONES

516 **Flaviolin**, $C_{10}H_6O_5$, garnet red rhombs containing solvent of crystallization, m.p.: dec. near 250°.



*Aspergillus citricus* (Wehmer) Mosseray
J. E. Davies, F. E. King and John C. Roberts, *Chem. and Ind.*, 1110 (1954). (Structure)

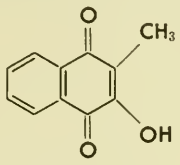
517 **6-Methyl-1,4-naphthoquinone**, $C_{11}H_8O_2$, golden yellow needles, m.p. 90–91°.



*Marasmius gramineum* Lib.

Gerd Bendz, *Acta Chem. Scand.* 2 192 (1948).
*Idem., ibid.* 5 489 (1951).

518 **Phthiocol,** $C_{11}H_8O_3$, yellow prisms, m.p. 173–174°.




*Mycobacterium tuberculosis* var. *hominis, Corynebacterium diphtheriae*

R. J. Anderson and M. S. Newman, *J. Biol. Chem.* 103 197 (1933).

Rudolph J. Anderson, R. L. Peck and M. M. Creighton, *ibid.* 136 211 (1940).

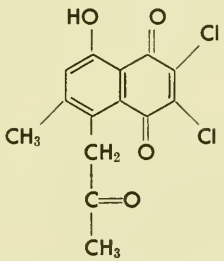
Michizo Asano and Hideo Takahashi, *J. Pharm. Soc. Japan* 65 17 (1945).

M. Terni, *Boll. soc. ital. biol. sper.* 25 60 (1949).

There is evidence that phthiocol is an artifact, and that the precursor is a compound related to vitamin $K_2$, but of higher molecular weight.

J. Francis, J. Madinaveitia, H. M. Macturk and G. A. Snow, *Nature* 163 365 (1949).

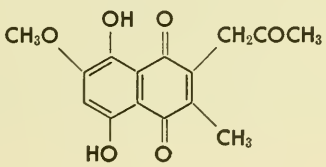
519 **Mollisin,** $C_{14}H_{10}O_4Cl_2$, orange-yellow needles, m.p. 202° (dec.).




*Mollisia caesia*, Sacc. sensu Sydow, *M. gallens* Karst.

G. J. M. van der Kerk and J. C. Overum, *Rec. trav. chim.* 76 425 (1957).

520 **Javanicin,** $C_{15}H_{14}O_6$, red laths, m.p. 208°.

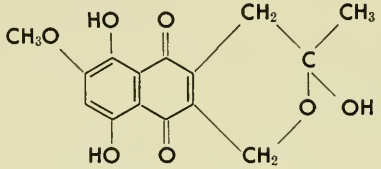


*Fusarium javanicum* Koorders

Yield about 20 mg. per liter (purified pigment). Occurs together with fusarubin.

H. R. V. Arnstein and A. H. Cook, *J. Chem. Soc.*, 1021 (1947). (Isolation)

521 **Fusarubin** (Oxyjavanicin), $C_{15}H_{14}O_7$, red prisms, m.p. 218° (preheated block).




*Fusarium solani* (Mart.) App. and Wr.

Yield about 50 mg. per liter (mixed with javanicin).

H. R. V. Arnstein and A. H. Cook, *J. Chem. Soc.*, 1021 (1947).

Hans W. Ruelius and Adeline Gauhe, *Ann.* **569** 38 (1950).

After ether extraction of the acidified broth, a water-soluble derivative of fusarubin remains behind. This has been identified as a sulfate ester occurring at one of the hydroquinone hydroxyl groups and was called fusarubinogen. Fusarubinogen actually is present in the broth in a reduced form, which is probably a derivative of β-hydronaphthazarin.

Hans W. Ruelius and Adeline Gauhe, *Ann.* **570** 121 (1951).

522 **Bostrycoidin,** $C_{18}H_{14}O_7$ (proposed), red or brown lath clusters, m.p. 243°.

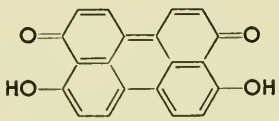
A substituted naphthoquinone similar to javanicin.

*Fusarium bostrycoides* Wr. and Rkg.

Mary Alice Hamilton, Marjorie S. Knorr and Florian A. Cajori, *Antibiotics and Chemotherapy* 3 853 (1953).

F. A. Cajori, Theodore T. Otani and Mary Alice Hamilton, *J. Biol. Chem.* **208** 107 (1954). (Isolation)

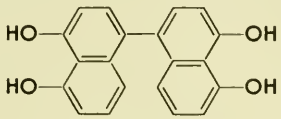
523 **4,9-Dihydroxyperylene-3,10-quinone,** $C_{20}H_{10}O_4$, dark red needles, dec. near 350°.




*Daldinia concentrica* (Bolt) Ces. and de Not.

J. M. Anderson and J. Murray, *Chem. and Ind.*, 376 (1956). (Isolation)

524 It has since been reported that this perylenequinone is probably an artifact of **4,5,4′5′-tetrahydroxy-1,1′-dinaphthyl.**




This polyphenol was obtained from the same organism. It was found to oxidize in part to a dark, melanin-like polymer, and in part to the perylenequinone. The structure was proved by synthesis.

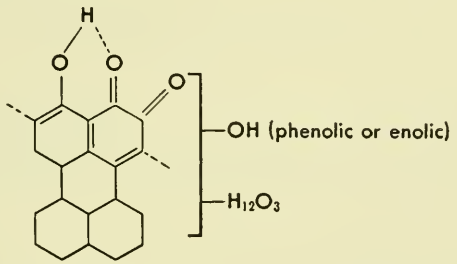
J. D. Bu'Lock and D. C. Allport, *Proc. Chem. Soc.*, 264 (1957).

D. C. Allport and J. D. Bu'Lock, *J. Chem. Soc.*, 654 (1960).

525 **Mycochrysone,** $C_{20}H_{14}O_7$, orange-red crystals, m.p.: slow dec. above 180°.

No N, $—OCH_3$, $C—CH_3$ nor halogen. Three active hydrogens.

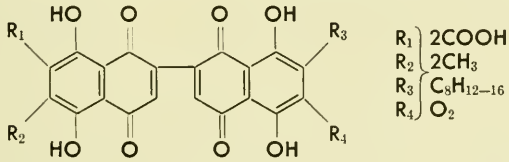
Partial structure:




An inoperculate discomycetous fungus.

G. Read, P. Shu, L. C. Vining and R. H. Haskins, *Can. J. Chem.* 37 731 (1959).

526 **Actinorhodin,** $C_{32}H_{26\text{-}30}O_{14}$, bright red needles, dec. 270°.

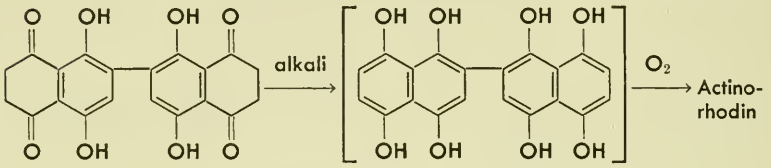



*Streptomyces coelicolor* (Müller) Waksman and Henrici

The yield was about 15% of the mycelial weight.

Hans Brockmann and Ernst Hieronymus, *Chem. Ber.* 88 1379 (1955).

This compound has been shown to be an artifact, and by careful isolation under acidic conditions the precursor, protoactinorhodin, with the nucleus below, can be isolated.

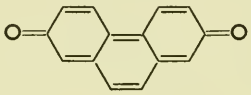



527 **Protoactinorhodin** was isolated as pale red prisms, dec. near 330°, probably $C_{32}H_{32}O_{14}$.

Hans Brockmann and Volkmar Loeschcke, *Chem. Ber.* 88 778 (1955).

528 **Xylindein,** $C_{34}H_{26}O_{11}$, deep brown high-melting, pleochroic leaflets.

The structure is obscure, but an extended quinone system of the type



was postulated.

*Chlorosplenium aeruginosum* (Oeder ex Fries) De Not

Fritz Kögl and G. von Taeuffenbach, *Ann.* 445 170 (1925).

Fritz Kögl and Hanni Erxleben, *ibid.* 484 65 (1930).

529 **Rhodomycetin,** gradual darkening at 300°.

Dark red powder, red in acid solution and blue in alkaline. U.V. 235, 540, 580 mμ.

Reddish violet in $H_2SO_4$, positive $FeCl_3$, $H_2O_2$ and $Na_2S_2O_4 \cdot 2H_2O$ reduction.

Resembles actinorhodin.

*Streptomyces griseus*

Gerald Shockman and Selman A. Waksman, *Antibiotics and Chemotherapy* 1 68 (1951).

530 **Naphthoquinone from *Mycobacterium phlei,*** yellow oil, U.V. 243, 249, 261, 270, 328 mμ in isooctane.

Appears to have about 30 carbon atoms and is probably a vitamin $K_1$. Mol. wt. about 620.

*Mycobacterium phlei*

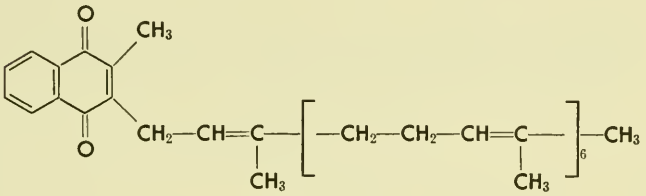
Ten mg. were obtained from 450 g. of wet cells.

A. F. Brodie, B. R. Davis and L. G. Fieser, *J. Am. Chem. Soc.* 80 6454 (1958).

**Vitamins $K_2$:**

Vitamin $K_2$ was first isolated from putrefied fish meal in 1939 by Doisy and collaborators. Tishler and Sampson later found that it was produced by pure cultures of *Bacillus brevis*. Isler and collaborators corrected the structure originally proposed to A. below. They also isolated a lower isoprenolog, B., from putrefied fish meal. Both structures were proved by synthesis. The later group also determined the structure of (and synthesized) a higher isoprenolog C. isolated earlier in England.

531 **A.**, $C_{46}H_{64}O_2$, light yellow plates, m.p. 54°.

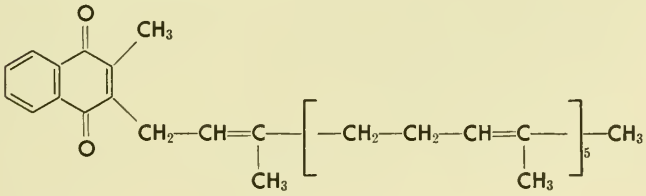


*Bacillus brevis*

R. W. McKee, S. B. Binkley, Sidney A. Thayer, D. W. Maccorquodale and Edward A. Doisy, *J. Biol. Chem.* 131 327 (1939).

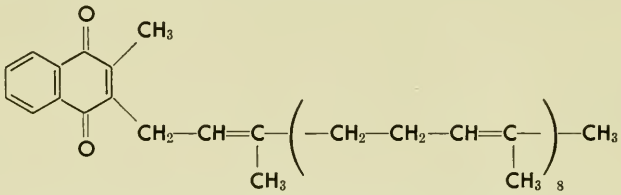
M. Tishler and W. Sampson, *Proc. Soc. Exp. Biol.* 68 136 (1948).

532 **B.**, $C_{41}H_{56}O_2$, light yellow plates, m.p. 50°.



O. Isler, R. Rüegg, L. Chopard-dit-Jean, A. Winterstein and O. Wiss, *Helv. Chim. Acta* 41 786 (1958).

533 C. $C_{56}H_{80}O_2$, yellow crystals, m.p. 58–59°.



*Mycobacterium tuberculosis* (Brevannes)

This substance constituted about 0.5% of the dry cell weight.

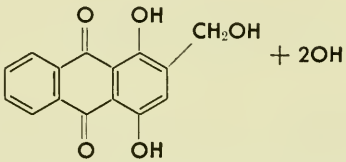
J. Francis, J. Madinaveitia, H. M. Macturk and G. A. Snow, *Nature* 163 365 (1949). (Isolation)

H. Noll, R. Rüegg, U. Gloor, G. Ryser and O. Isler, *Helv. Chim. Acta* 43 433 (1960). (Structure and synthesis)

## C. ANTHRAQUINONES

534 **Anthraquinone pigment from *Gibberella fujikuroi*,** probably $C_{14}H_{10}O_7$, red crystals, m.p. 325° (sealed tube).

Partial and tentative structure:



The structure may resemble that of cynodontin.

*Gibberella fujikuroi* (Saw.) Wollenweber

Yukihiko Nakamura, Tokuji Shimomura and Joji Ono, *J. Agr. Chem. Soc. Japan* 31 669 (1957). (Isolation)

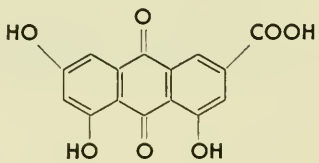
535 **Clavorubin,** $C_{14}H_{12}O_9$, red crystals.

Has one C—$CH_3$ group. U.V. absorption resembles a 1,5,8-trihydroxyanthraquinone. The leuco-acetate (like that of chrysergonic acid) has a diphenyl-like absorption.

*Claviceps purpurea*

B. Franck and T. Reschke, *Angew. Chem.* 71 407 (1959).

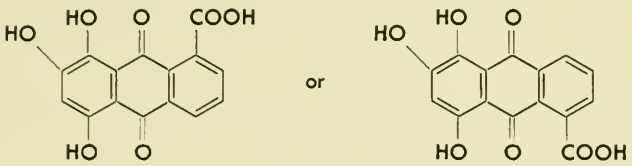
536 **Emodic Acid,** $C_{15}H_8O_7$, orange needles, m.p. 363–365°.



*Penicillium cyclopium* Westling

Winston K. Anslow, John Breen and Harold Raistrick, *Biochem. J.* 34 159 (1940).

537 **Boletol**, $C_{15}H_8O_7$, red needles, m.p. 275–280° (dec.).

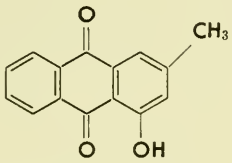



*Boletus luridus* Schaeff. ex Fries, *B. badius* Fr., *B. chrysenteron* Bull., *B. satanas* Lenz, *B. subtomentosus* Linn.

The higher yielding species gave about 1 g. of pure material from 20 kg. of fruiting body.

Fritz Kögl and W. B. Deijs, *Ann.* 515 10, 23 (1935). (Synthesis)

538 **Pachybasin**, $C_{15}H_{10}O_3$, yellow needles, m.p. 78°.

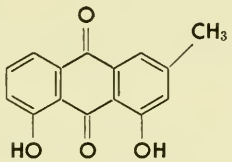



*Pachybasium candidum* (Sacc.) Peyronel

Pachybasin, like most of the other anthraquinone pigments, occurs as one constituent of a mixture of pigments. Chrysophanol was identified as one of the other constituents of this mixture.

Shoji Shibata and Michio Takido, *Pharm. Bull.* (Tokyo) 3 156 (1955).

539 **Chrysophanol** (Chrysophanic Acid), $C_{15}H_{10}O_4$, dark yellow leaflets, m.p. 196°.

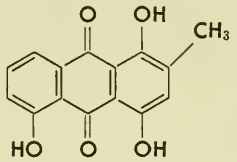



*Penicillium islandicum* Sopp, *Pachybasium candidum* (Sacc.) Peyronel, *Chaetomium affine* Corda

The 9-anthrone corresponding to chrysophanol has been isolated from higher plants.

B. H. Howard and H. Raistrick, *Biochem. J.* 46 49 (1950).
Shoji Shibata, *Kagaku* (*Science*) 26 391 (1956).

540 **Islandicin,** $C_{15}H_{10}O_5$, dark red plates, m.p. 218°.




*Penicillium islandicum* Sopp.

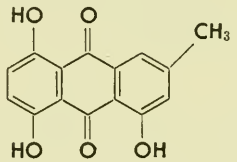
This mold produces a complex mixture of pigments constituting up to 20% of the mycelial weight.

B. H. Howard and H. Raistrick, *Biochem. J.* 44 227 (1949).

Islandicin seems to be identical with funiculosin, a trihydroxyanthraquinone pigment of the same melting point and empirical formula isolated from *Penicillium funiculosum* Thom, a species closely related to *P. islandicum.*

Hisanao Igarasi, *J. Agr. Chem. Soc. Japan* 15 225 (1939).

541 **Helminthosporin,** $C_{15}H_{10}O_5$, dark maroon needles, m.p. 227°.

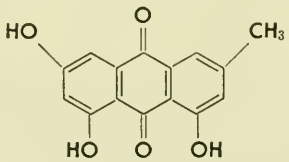



*Helminthosporium gramineum* Rabenhorst, *H. cynodontis* Marignoni, *H. catenarium, H. triticivulgaris* Nisikadɔ

About 30% of the dry mycelium of *H. gramineum* consisted of anthraquinone pigments, mainly helminthosporin and catenarin.

Harold Raistrick, Robert Robinson and Alexander R. Todd, *J. Chem. Soc.,* 488 (1933).

542 **Emodin** (Frangula-Emodin), $C_{15}H_{10}O_5$, orange needles, m.p. 255°.



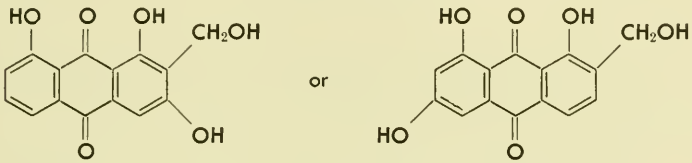
*Cortinarius sanguineus* (Wulf.) Fries, *Chaetomium affine* Corda.

A yield of about 3% of the dry mycelial weight has been mentioned.

Fritz Kögl and J. J. Postowcky, *Ann.* 444 1 (1925).

R. A. Jacobson and Roger Adams, *J. Am. Chem. Soc.* 46 1312 (1934). (Synthesis)

543 **Versicolorin**, $C_{15}H_{10}O_6$, yellow-orange needles, m.p. 282°.

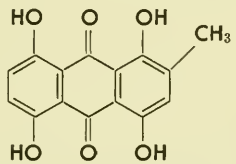



*Aspergillus versicolor* (Vuillemin) Tiraboschi

The same organism produces an uncharacterized xanthone pigment.

Yuishi Hatsuda and Shimpei Kuyama, *J. Agr. Chem. Soc. Japan* 29 11 (1955).

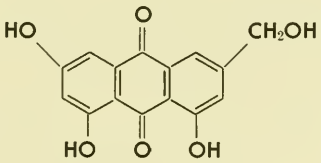
544 **Cynodontin**, $C_{15}H_{10}O_6$, bronze plates, m.p. 260°.




*Helminthosporium cynodontis* Marignoni, *H. euclaenae* Zimmermann, *H. avenae* Ito and Kurib, *H. victoriae*

Winston Kennay Anslow and Harold Raistrick, *Biochem. J.* 34 1546 (1940). (Synthesis)

545 **ω-Hydroxyemodin** (Citreorosein), $C_{15}H_{10}O_6$, orange needles, m.p. 288°.

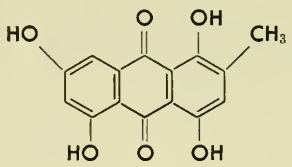



*Penicillium cyclopium* Westling, *P. citreo-roseum* Dierckx.

Winston K. Anslow, John Breen and Harold Raistrick, *Biochem. J.* 34 159 (1940).

Theodore Posternak, *Compt. rend. soc. phys. his. nat. Genève* 56 28 (1939).

546 **Catenarin,** $C_{15}H_{10}O_6$, red plates, m.p. 246°.

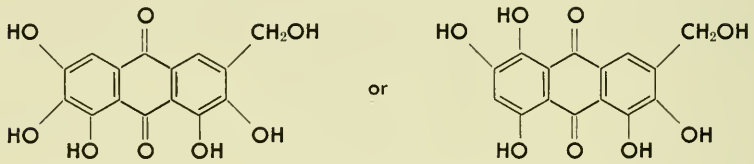



*Helminthosporium catenarium* Drechsler, *H. gramineum* Rabenhorst, *H. velutinum* Link, *H. triticivulgaris* Nisikado, *Penicillium islandicum* Sopp, *Aspergillus amstelodami* (Mangin) Thom and Church

More than 15% of the mycelial weight of *H. catenarium* was catenarin.

Winston Kennay Anslow and Harold Raistrick, *Biochem. J.* 35 1006 (1941). (Synthesis)

547 **Asperthecin,** $C_{15}H_{10}O_8$, chestnut brown needles, no m.p.



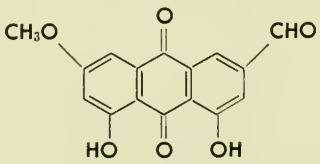

*Aspergillus quadrilineatus* Thom and Raper and other species of the *Aspergillus nidulans* group

S. Neelakantan, Anna Pocker and H. Raistrick, *Biochem. J.* 66 234 (1957).

A closely related pigment has been observed, which may have been a tautomeric or reduced form of asperthecin. It could not be isolated because of its ready conversion to asperthecin.

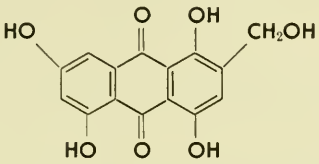
B. H. Howard and H. Raistrick, *Biochem. J.* 59 475 (1955).

548 **Fallacinal,** $C_{16}H_{10}O_6$, orange-yellow needles, m.p. 251°.



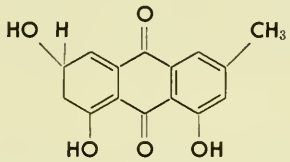
*Xanthoria fallax* (Hepp.) Arn.
Takao Murakami, *Pharm. Bull.* (Tokyo) 4 298 (1956).

549 **Tritisporin** ($\omega$-Hydroxycatenarin), $C_{15}H_{10}O_7$, brown needles, m.p. 260–262°.




*Helminthosporium triticivulgaris* Nisikado
S. Neelakantan, Anna Pocker and H. Raistrick, *Biochem. J.* 64 464 (1956).

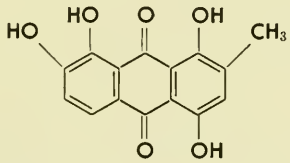
550 **Flavoskyrin**, $C_{15}H_{12}O_5$, yellow crystals, m.p. 208° (dec.), $[\alpha]_D$ −295° (in dioxane).




*Penicillium islandicum* Sopp.
Shoji Shibata, Takao Murakami and Michio Takito, *Pharm. Bull.* (Tokyo) 4 303 (1956). (Structure)

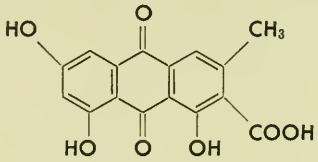
551 **Compound A** (1,4,7,8-Tetrahydroxy-2-methylanthraquinone), $C_{15}H_{12}O_7$.

An optically inactive compound (no melting point given). Treatment with conc. $H_2SO_4$ yields an anthraquinone, $C_{15}H_{10}O_5$, red crystals, m.p. 255°, with the following structure:




*Penicillium islandicum*
Sten Gatenbeck, *Acta Chem. Scand.* 12 1985 (1958).
*Idem., ibid.* 13 705 (1959).

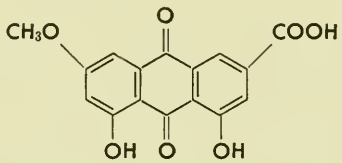
552 **Endocrocin,** $C_{16}H_{10}O_7$, copper-red leaflets, m.p. 318° (dec.).




*Nephromopsis endocrocea* Asahina
Yasuhiko Asahina and Fukuziro Fuzikawa, *Ber.* **68B** 1558 (1935).
*Aspergillus amstelodami* (Mangin) Thom and Church.
Shoji Shibata and Shinsaku Natori, *Pharm. Bull.* (Tokyo) **1** 160 (1953).

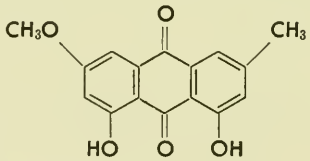
553 **Clavoxanthin,** $C_{16}H_{10}O_7$, yellow needles, m.p. 340° (dec.).
Apparently similar to endocrocin.
*Claviceps purpurea*
B. Franck and T. Reschke, *Angew. Chem.* **71** 407 (1959).

554 **Parietinic Acid,** $C_{16}H_{10}O_7$, yellow needles, m.p. ~300° (sublimes).




*Xanthoria parietina* (L.) Th. Fr.
Walter Escherich, *Biochem. Z.* **330** 73 (1958).

555 **Physcion** (Parietin), $C_{16}H_{12}O_5$, orange-yellow leaflets, m.p. 207°.




*Aspergillus glaucus* spp., *A. chevalieri, A. ruber* (Mangin) Raper and Thom, *Penicillium herquei* Bainier and Sartory, *Xanthoria parietina* (L.) Beltram, *X. fallax, Teloschistes flavicans* (Sw.) Norm., *T. exilis* Wainio, *Placodium* spp., *Caloplaca elegans* (Link)

F. Rochleder and W. Heldt, *Ann.* 48 1 (1843).

Harold Raistrick, *Enzymologia* 4 76 (1937).

H. Raistrick, Robert Robinson and A. R. Todd, *J. Chem. Soc.*, 80 (1937).

Julius Nicholson Ashley, Harold Raistrick and Taliesin Richards, *Biochem. J.* 33 1291 (1939).

T. R. Seshadri and S. Sankara Subramanian, *Proc. Indian Acad. Sci.* 30A 67 (1949).

Walter B. Mors, *Bol. Inst. Quim. Agric.* No. 23 7 (1951).

S. Neelakantan and T. R. Seshadri, *J. Sci. Ind. Research* (India) 11B 126 (1952).

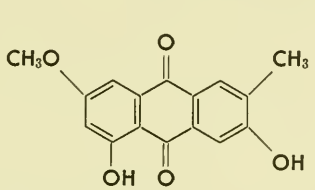
Shoji Shibata and Shinsaku Natori, *Pharm. Bull.* (Tokyo) 1 160 (1953).

Mitizo Asano and Yosio Arata, *J. Pharm. Soc. Japan* 60 521 (1940).

J. A. Galarraga, K. G. Mill and H. Raistrick, *Biochem. J.* 61 456 (1955).

Jiro Kitamura, Uzuhiko Kurimoto and Matatsugu Zokoyama, *J. Pharm. Soc. Japan* 76 972 (1956).

556 **Macrosporin**, $C_{16}H_{12}O_5$, orange-yellow rhombic crystals, m.p. 300° (dec.).

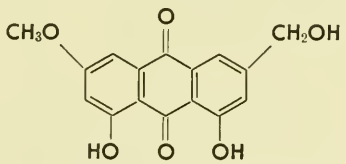



*Macrosporium porri* Elliott

R. Suemitsu, Y. Matsui and M. Hiura, *Bull. Agr. Chem. Soc.* (Japan) 21 1–4, 337 (1957). (Isolation)

R. Suemitsu, M. Nakajima and M. Hiura, *ibid.* 23 547 (1959).

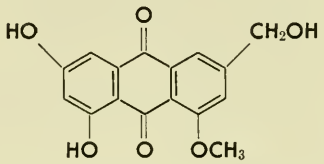
557 **Teloschistin** (Fallacinol), $C_{16}H_{12}O_6$, orange plates, m.p. 245–247°.




*Teloschistes flavicans* (Sw.) Norm., *Xanthoria fallax* (Hepp.) Arn.

T. R. Seshadri and S. Sankara Subramanian, *Proc. Indian Acad. Sci.* 30A 67 (1949).

558 **Roseopurpurin** (Carviolin), $C_{16}H_{12}O_6$, yellow needles, m.p. 286°.

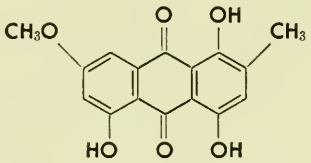



*Penicillium roseopurpureum* Dierckx

559 A second pigment, **carviolacin**, $C_{20}H_{16}O_7$, light brown needles, m.p. 243° (dec.), was isolated from this mold. It is apparently closely related in structure.

Theodore Posternak, *Helv. Chim. Acta* 23 1046 (1940).
H. G. Hind, *Biochem. J.* 34 67, 577 (1940).

560 **Erythroglaucin** (Catenarin 6-Methyl Ether), $C_{16}H_{12}O_6$, deep red plates or needles, m.p. 205°.




*Aspergillus glaucus* (ten spp.)

The former rubroglaucin was shown to be a mixture of physcion and erythroglaucin.

Julius Nicholson Ashley, Harold Raistrick and Taliesin Richards, *Biochem. J.* 33 1291 (1939).

561 **Neophromin**, $C_{16}H_{12}O_6$, ocher colored needles, m.p. 198° (dec.). A quinone-like pigment.

*Neophromium lusitanicum*

O. Hesse, *J. prakt. Chem.* 57 409 (1898).

562 **Dermocybin**, $C_{16}H_{12}O_7$, red needles, m.p. 228°.

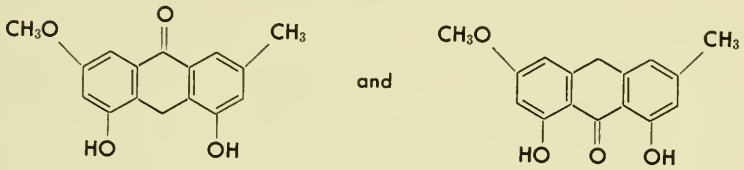
This is an incompletely characterized anthraquinone pigment. It has five nuclear hydroxyl groups, one of them methylated. It is produced along with emodin by

*Cortinarius sanguineus* (Wulf.) Fries and constitutes 0.2–0.4% of the mycelial weight.

*Cortinarius cinnabarinus* Fries produces a pigment which is the same or similar.

Fritz Kögl and J. J. Postowsky, *Ann.* 444 1 (1925).

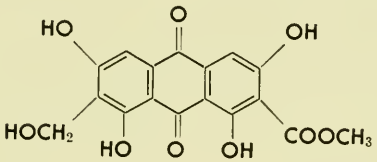
563, 564 **Physcion Anthranols,** $C_{16}H_{14}O_4$, m.p.'s 260° and 181°.




*Aspergillus glaucus* (five types)

Julius Nicholson Ashley, Harold Raistrick and Taliesin Richards, *Biochem. J.* 33 1291 (1939).

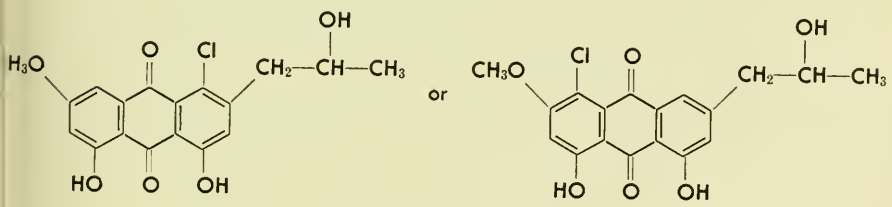
565 **Rhodocladonic Acid,** $C_{17}H_{12}O_9$, red needles, m.p. >360°.




Thirteen *Cladonia* species

Shoji Shibata, Michio Takido and Osamu Tanaka, *J. Am. Chem. Soc.* 72 2789 (1950).

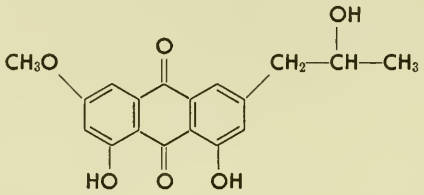
566 **Nalgiolaxin,** $C_{18}H_{15}O_6Cl$, yellow plates or needles, m.p. 248°, $[\alpha]_{5790}^{22}$ +40.3° (in chloroform).




*Penicillium nalgiovensis* Laxa

H. Raistrick and J. Ziffer, *Biochem. J.* 49 563 (1951).

567 **Nalgiovensin,** $C_{18}H_{15}O_6$, orange needles or plates, m.p. 199–200°, $[\alpha]_{5790}^{20}$ +39.7° (in chloroform).




*Penicillium nalgiovensis* Laxa

H. Raistrick and J. Ziffer, *Biochem. J.* 49 563 (1951). (Isolation)

A. J. Birch and R. A. Massy-Westropp, *J. Chem. Soc.*, 2215 (1957). (Structure)

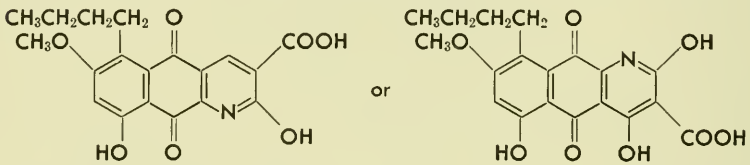
568 **Thermophillin,** $C_{18}H_{18}O_9$, golden plates, m.p. subl. 245° (dec. 260° sealed tube).

Quinonoid properties.

*Lenzites thermophila*

H. S. Burton, *Nature* 166 570 (1950).

569 **Phomazarin,*** $C_{19}H_{17}O_8N$, orange needles, m.p., 197° (dec.).



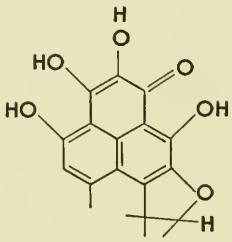

*Phoma terrestris* Hansen

F. Kögl and J. Sparenburg, *Rec. trav. chim.* 59 1180 (1940).

F. Kögl and F. S. Quackenbush, *ibid.* 63 251 (1944).

F. Kögl, G. C. van Wessem and O. I. Elsbach, *ibid.* 64 23 (1945). (Synthesis)

570 **Atrovenetin,** $C_{19}H_{18}O_6$, brownish yellow prisms, m.p. 295° (dec.), $[\alpha]_{5461}^{21}$ +154° (c 0.486 in dioxan).




*Penicillium atrovenetum* G. Smith

* See addendum.

K. G. Neill and H. Raistrick, *Chem. and Ind.*, 551 (1956). (Isolation)

*Idem., Biochem. J.* 65 166 (1957). (Isolation)

D. H. R. Barton, P. de Mayo, G. A. Morrison and H. Raistrick, *Tetrahedron* 6 48 (1959). (Structure)

571 **Norherqueinone,** $C_{19}H_{18}O_7$, dark red needles, m.p. 279° (dec.), $[\alpha]_D^{23}$ +1080° ±60° (c 0.048 in pyridine).

Structure: Unmethylated herqueinone

See herqueinone for organism, structure and references.

572 **Herquein,** $C_{19}H_{20}O_8$ (proposed), yellow-brown crystals, m.p. 129° (dec.).

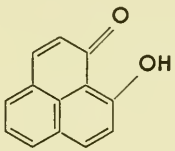
Water-soluble. Fluoresces in alkali.

*Penicillium herquei*

H. Stowar Burton, *Brit. J. Exptl. Path.* 30 151 (1949).

573 **Herqueinone,** $C_{20}H_{20}O_7$, red needles, m.p. 226° (dec.) (sublimes), $[\alpha]_D^{22}$ +440° ±40° (c 0.063 in ethanol).

Partial structure:




*Penicillium herquei* Bainier and Sartory

A crude pigment yield of 17% of the weight of the dry mycelium was obtained. The major constituents were norherqueinone and its methyl ether, herqueinone. Minor constituents were physcion and *meso*-erythritol.

The plant pigment, haemocorin, also contains the perinaphthenone nucleus.

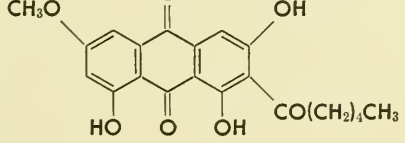
Frank H. Stodola, Kenneth B. Raper and Dorothy I. Fennell, *Nature* 167 773 (1951). (Isolation)

J. A. Galarraga, K. G. Neill and H. Raistrick, *Biochem. J.* 61 456 (1955).

D. H. R. Barton, P. de Mayo, G. A. Morrison, W. H. Schaeppi and H. Raistrick, *Chem. and Ind.*, 552 (1956). (Structure)

Robert E. Harman, James Cason, Frank H. Stodola and A. Lester Adkins, *J. Org. Chem.* 20 1260 (1955).

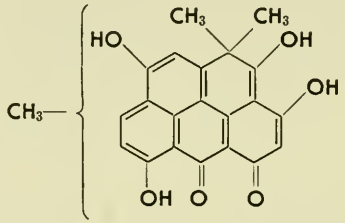
574 **Solorinic Acid,** $C_{21}H_{20}O_7$, red-brown plates, m.p. 203.5°.



*Solorina crocea* (L.) Ach.

G. Koller and H. Russ, *Monatsh.* 70 54 (1937).

575 **Resistomycin,** $C_{22}H_{16}O_6$, yellow needles, m.p. 315° (dec.) (sublimes from 215°).




*Streptomyces resistomycificus*

Hans Brockmann and Günter Schmidt-Kastner, *Chem. Ber.* 87 1460 (1954). (Isolation)

H. Brockmann, E. Meyer and K. Schrempp, Dissertations, University of Göttingen, 1954, 1958. (Partial structure by courtesy of Prof. Brockmann)

576 **Granatacin,** $C_{22}H_{20}O_{10}$, pomegranate-red crystals, m.p. 204–206° (dec.).

A tricyclic tetrahydroxyquinonedicarboxylic acid with antibiotic properties.

*Streptomyces olivaceus* (Waksman) Waksman and Henrici

R. Corbaz, L. Ettlinger, E. Gäumann, J. Kalvoda, W. Keller-Schierlein, F. Kradolfer, B. K. Manukian, L. Neipp, V. Prelog, P. Reusser and H. Zähner, *Helv. Chim. Acta* 40 1262 (1957).

577 **Luteomycin** (Antibiotic 289), $C_{26}H_{33}O_{12}N$ (proposed), (Hydrochloride) orange-yellow crystals, m.p. 199° (dec.).

Color changes to purple in alkali. Positive quinone-$Na_2CO_3$, $FeCl_3$. Negative ninhydrin, biuret, Molisch, Fehling, Sakaguchi. Can be precipitated as reineckate, helianthate or picrate.

*Streptomyces flaveolus, S. tanashiensis* related to *S. antibioticus*

Toju Hata, Tomojiro Higuchi, Yoshimoto Sano and Katuko Sawashi, *Kitasato Arch. Exptl. Med.* 22 229 (1949).

Hamao Umezawa, Tomio Takeuchi, Kazuo Nitta, Kenji

Maeda, Tadashi Yamamoto and Seizaburo Yamaoka, *J. Antibiotics* (Japan) 6A 45 (1953).

Teisuke Osato, Koki Yagishita, Ryozo Utahara, Masahiro Ueda, Kenji Maeda and Hamao Umezawa, *ibid.* 6A 52 (1953).

Berislav Govorčin, *Tehnički Pregled* 8 43 (1956).

578 **Luteoleersin,** $C_{26}H_{38}O_7$, yellow crystals, m.p. 135°, $[\alpha]_{5461}^{18}$ 214° (c 0.456 in ethanol).

Believed to be a substituted quinone, containing two active hydrogens. It was accompanied by a reduction product:

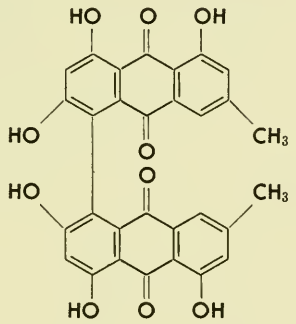
579 **Alboleersin,** $C_{26}H_{40}O_7$, colorless crystals, m.p. 215°, $[\alpha]_{5461}^{18}$ 274° (c 0.430 in ethanol).

Contains three active hydrogens.

*Helminthosporium leersii* Atkinson

Julius N. Ashley and Harold Raistrick, *Biochem. J.* 32 449 (1938).

580 **Skyrin** (Endothianin), $C_{30}H_{18}O_{10}$, dark orange rods, m.p. >380°.




*Penicillium islandicum* Sopp, *P. wortmanni* Klocker, *P. tardum* Thom, *P. rugulosum* Thom, *Endothia parasitica* (Murr.) Anderson and Anderson, *E. fluens* Shear and Stevens

All of these fungi produce a mixture of skyrin with rugulosin.

F. Kögl and F. S. Quackenbush, *Rec. trav. chim.* 63 251 (1944).

Shoji Shibata, Osamu Tanaka, Goro Chihara and Horoshi Mitsuhashi, *Pharm. Bull.* (Tokyo) **1** 302 (1953).

Shoji Shibata, Takao Murakami, Osamu Tanaka, Goro Chihara, Isao Kitagawa, Masashi Sumimoto and Chikara Kaneko, *ibid.* **3** 160 (1955). (Structure)

Shoji Shibata, Takao Murakami, Osamu Tanaka, Goro Chihara and Masashi Sumimoto, *ibid.* **3** 274 (1955).

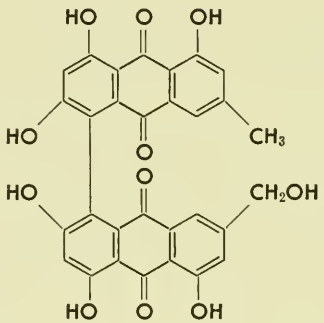
J. Breen, J. C. Dacre, H. Raistrick and G. Smith, *Biochem. J.* **60** 618 (1955).

Shoji Shibata, Michio Takido and Terumi Nakajima, *Pharm. Bull.* (Tokyo) **3** 286 (1955).

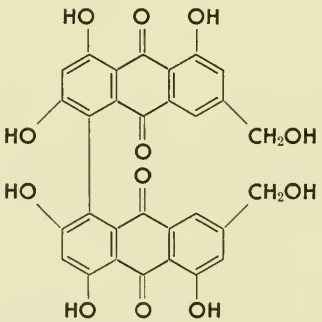
Yuzuru Yamamoto, Takeo Yamamoto, Skoichi Kanatomo and Kiyoshi Tanimichi, *J. Pharm. Soc. Japan* **76** 192 (1956).

Yazuru Yamamoto, Akira Hamaguchi, Isao Yamamoto and Sumie Imai, *ibid.* **76** 1428 (1956).

581 **Pigment B:** $C_{30}H_{18}O_{11}$.



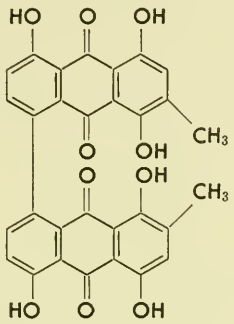

582 **Pigment C:** $C_{30}H_{18}O_{12}$.




These are oxidized skyrins.

*Penicillium islandicum* N.R.R.L. 1175
Shoji Shibata, Michio Takido and Terumi Nakajima, *Pharm. Bull.* (Tokyo) 3 286 (1955).

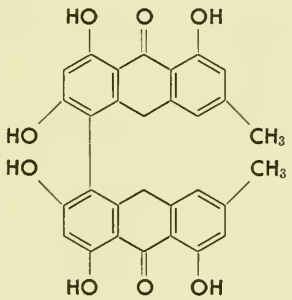
583 **Iridoskyrin,** $C_{30}H_{18}O_{18}$, irridescent red rods or plates, m.p. 358°.




*Penicillium islandicum* Sopp.
B. H. Howard and H. Raistrick, *Biochem. J.* 57 212 (1954).

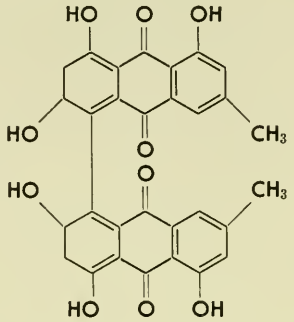
584 **Aurofusarin,** $C_{30}H_{20}O_{12}$, m.p. >360°.
This incompletely characterized pigment produced by *Fusarium culmorum* W. G. Smith may be a dianthraquinone.
Julius N. Ashley, Betty C. Hobbs and Harold Raistrick, *Biochem. J.* 31 385 (1937).

585 **Penicilliopsin,** $C_{30}H_{22}O_8$, orange crystals, m.p. 330° (dec.).




*Penicilliopsis clavariaeformis* Solms-Laubach
H. Brockmann and H. Eggers, *Angew. Chem.* 67 706 (1955).

586 **Rugulosin** (Radicalisin), $C_{30}H_{22}O_{10}$, yellow prisms, m.p. 293° (dec.), $[\alpha]_{5461}^{18}$ +605° (dioxane).




*Penicillium rugulosum* Thom, *P. tardum* Thom, *P. wortmanni* Klöcker, *Endothia parasitica* (Murr.) Anderson and Anderson, *E. fluens* Shear and Stevens.

About 20% of the dry weight of *P. rugulosum* mycelium is rugulosin.

J. Breen, J. C. Dacre, H. Raistrick and G. S. Smith, *Biochem. J.* 60 618 (1955).

Shoji Shibata, Osamu Tanaka, Goro Chihara and Horoshi Mitsuhashi, *Pharm. Bull.* (Tokyo) 1 302 (1953).

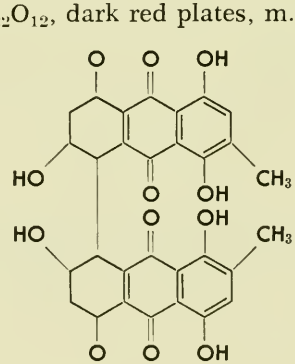
Shoji Shibata, Takao Murakami, Osamu Tanaka, Goro Chihara, Isao Kitagawa, Masashi Sumimoto and Chikara Kaneko, *ibid.* 3 160 (1955). (Structure)

Shoji Shibata, Takao Murakami, Osamu Tanaka, Goro Chihara and Masashi Sumimoto, *ibid.* 3 274 (1955).

Yazuru Yamamoto, Akira Hamaguchi, Isao Yamamoto and Sumie Imai, *J. Pharm. Soc. Japan* 76 1428 (1956).

Shoji Shibata and Isao Kitagawa, *Pharm. Bull.* (Tokyo) 4 309 (1956). (Structure)

587 **Rubroskyrin**, $C_{30}H_{22}O_{12}$, dark red plates, m.p. 289° (dec.).

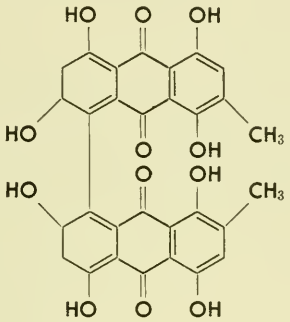


*Penicillium islandicum* Sopp.

This pigment is produced in a mixture including islandicin, iridoskyrin, erythroskyrin, catenarin, luteoskyrin and skyrin. The weight of the pigment mixture is about 10% of the weight of the dry mycelium.

Shoji Shibata and Isao Kitagawa, *Pharm. Bull.* (Tokyo) 4 309 (1956).

588 **Luteoskyrin,** $C_{30}H_{22}O_{12}$, yellow needles, m.p. 273° (dec.), $[\alpha]_D^{25}$ −880° (in acetone).





*Penicillium islandicum* Sopp.

Shoji Shibata and Isao Kitagawa, *Pharm. Bull.* (Tokyo) 4 309 (1956). (Structure)

589 **Cercosporin,** $C_{30}H_{28}O_{10}$, red crystals, m.p. 241°, $[\alpha]_{7000}^{20}$ +470° (c 0.5 in chloroform).

This pigment contains two methoxyl groups, two quinoid carbonyls, two phenolic hydroxyls and two alcoholic hydroxyls. The yield was 79 mg. per gram of dry mycelium.

Shimpei Kuyama and Teiichi Tamura, *J. Am. Chem. Soc.* 79 5725, 5726 (1957).

590, 591 592 **Chaetochrysin** and **Chaetoflavin,** $C_{31}H_{26}O_{11}$, yellow crystals, no melting point, and **Chaetoalbin,** $C_{30}H_{28-30}O_{11}$, white crystals, no melting point.

These uncharacterized compounds were isolated from mycelial extracts along with chrysophanol. They seem to be modified dianthraquinones. They yield some chrysophanol on alkaline oxidation, contain one methoxyl group and have high optical rotations.

*Chaetomium affine* Corda

Vincent Arkley, F. M. Dean, Peter Jones, Alexander Robertson and John Tetaz, *Croat. Chem. Acta* 29 141 (1957).

593 **Rifomycin B,** $C_{39}H_{51}O_{14}N$, m.p. 160–164° (dec.).
A dibasic acid (pKs 2.8, 6.7). Probably a quinone (U.V. peaks at 400–460, also at 223, 234).
*Streptomyces mediterranean*
P. Sensi, A. Greco and R. Ballotta, 7th Annual Symposium on Antibiotics, Washington, 1959.

594 **Vinacetin,** yellow platelets, m.p. 157°.
Apparently quinoid. Positive $FeCl_3$, violet color in alkali, positive Molisch, Liebermann, Fehling. Negative ninhydrin, Millon, Sakaguchi.
*Streptomyces* sp.
Kyuzo Omachi, *J. Antibiotics* (Japan) **6A** 73 (1953).

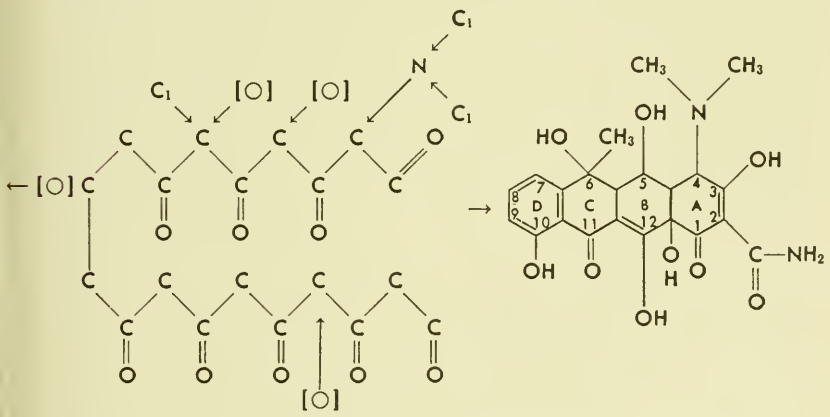
595 **Rhodophyscin,** red leaflets, m.p. 260° (dec.).
A quinone-like substance.
*Physica endococcina*
Wilhelm Zopf, *Ann.* **340** 276 (1905).

13

# Tetracycline, Analogues and Related Substances

The tetracycline antibiotics display features indicative of an acetate origin. The oxygenation pattern is generally consistent as are the points of occurrence of methyl groups and halogen atoms. There is also at least a superficial resemblance to proved acetate derivatives such as the anthraquinones. So far the experimental evidence published concerning the biosynthetic origin of the tetracyclines has been limited, and some interesting obscurities remain.

The general concept of an acetate-derived precursor in the sense of a polyketomethylene chain is, in the case of oxytetracycline as follows:



A 6-demethyltetracycline has been isolated from a fermentation broth, and tetracycline itself is a 5-deoxyoxytetracycline as well as a 7-dechlorochlortetracycline; so sometimes some of the steps in the biosynthetic scheme are omitted.

The production by *Streptomyces rimosus* of oxytetracycline-X[1], a modification of Terramycin in which there is an acetyl group instead of a carboxamide group at position 2, supports the acetate theory since terramycin-X is more directly in the line of descent from a polyketomethylene chain (ten head to tail condensed acetate units) than is Terramycin itself.

The dehydro derivatives which have been isolated[2] also may be considered as precursors of the other tetracyclines since the additional double bond may be the (as yet unreduced) result of an aldol type of condensative ring closure with elimination of a water molecule.

More experimental work has been reported on the biosynthetic origin of oxytetracycline than on that of related substances. Addition of $C^{14}H_3$-methionine and 2-$C^{14}$-acetic acid to oxytetracycline-producing fermentations yields radioactive oxytetracycline (Terramycin). Quantitative degradation and counting studies show that methionine furnishes the $C_6$-methyl and the N-methyl groups. The radioactivity of the degradation fragments from the molecule which had incorporated 2-$C^{14}$-acetic acid indicated that most of the molecule is in quantitative agreement with the theoretical requirements for acetate derivation.[3, 4]

Results are entirely consistent with formation of the ring skeleton at least from $C_5$ to $C_{12}$ by head to tail linkage of acetate units. Glutamic acid has been considered as a possible precursor of part of the A-ring (carboxamide side-chain, carbon atoms 2, 3, 4, 4a and the 4-amino nitrogen) and 2-$C^{14}$-labeled glutamic acid yielded a labeled oxytetracycline.[5] Later evidence[6] indicates that acetate also is capable of furnishing these carbon atoms although the level of activity in the A-ring seems to be somewhat lower than the theoretical, particularly in Terramycin isolated from older fermentations. The degradation fragments

[1] F. A. Hochstein, M. Schach von Wittenau, Fred W. Tanner, Jr. and K. Murai, *J. Am. Chem. Soc.* 82 (1960). (In press)

[2] J. R. D. McCormick, Philip A. Miller, John A. Growich, Newell O. Sjölander and Albert P. Doerschuk, *ibid.* 80 5572 (1958).

[3] A. J. Birch, J. F. Snell and P. L. Thompson, *ibid.* 82 2402 (1960).

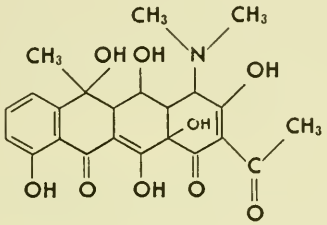
[4] A. J. Birch and P. L. Thompson, *ibid.* 82 (1960). (In press)

[5] J. F. Snell, R. L. Wagner, Jr. and F. A. Hochstein, *Internat. Conf. on Peaceful Uses of Atomic Energy*, 431 (1955); J. F. Snell, Symposium on Uses of Isotopes, Uniontown, Pa., 1957.

[6] A. J. Birch and P. L. Thompson, *J. Am. Chem. Soc.* 82 (1960). (In press)

from this portion of the molecule are not satisfactory for the clarification of the origin of the A-ring. It remains to be seen whether or not a less direct mechanism of acetate incorporation prevails in this area.

The isolation and identification of oxytetracycline-X (2-acetyl-2-decarboxamidooxytetracycline), a lower potency antibiotic, from cultures of *Streptomyces rimosus*, the Terramycin producer, seem to support in a general way the idea of the acetate derivation of ring A. It is tempting to speculate that oxytetra-




cycline-X may be a precursor of oxytetracycline, but this has not been proved.

With the acetate theory as a guide, it is possible to extrapolate some predictions from the tetracyclines isolated and characterized to date. It would seem probable that other mutations of the producing organisms might be obtained in which one minor biosynthetic step is blocked. Thus, retention of an oxygen atom at position 8 might be expected. Similarly, other tetracyclines lacking the $C_6$-methyl and/or hydroxyl groups, the $C_{12a}$-hydroxyl group and perhaps the N-methyl groups may be found. It is also possible that glycosides may be isolated as in the pyrromycinones.

The pyrromycinones are produced by streptomyces species, and they bear some resemblance to the tetracyclines. The four linear rings appearing in various states of oxidation and the similarity in the number of carbon atoms make it seem that their biogenetic origin may be similar to that of the tetracyclines. Apparently no experimental work has been published on this point. There is probably a close relationship among the pyrromycinones, rhodomycinones and quinocyclines. All of these pigments are found occasionally as glycosides, but no tetracycline glycosides have been reported yet.

The rhodomycins are a complex of red pigments produced by *Streptomyces purpurascens*. The original complex was sepa-

rated into four components; rhodomycin A, isorhodomycin A, rhodomycin B and isorhodomycin B. The first three were isolated in the crystalline state. These substances contained nitrogen, and, on mild acid hydrolysis, yielded an amino sugar, rhodosamine, $C_8H_{17}O_3N$, plus the aglycones (rhodomycinones, isorhodomycinones).

The same organism has yielded a number of other pigments which do not contain nitrogen. These also have been designated rhodomycinones. Three of these, $\beta$, $\epsilon$ and *iso*-$\epsilon$ have been obtained crystalline. It has been reported (no experimental details) that a $\gamma$-rhodomycinone and six other rhodomycinones have been isolated "in substance" and that three others have been demonstrated by paper chromatography. The rhodomycinones seem to resemble the pyrromycinones, quinocyclines, cinerubins and rutilantinone.

596 **Rhodomycin A** (Hydrochloride), $C_{20}H_{29}O_7N \cdot HCl$, fine, dark red needles, m.p. 193° (dec.) (preheated block).

Hans Brockmann and Ilse Borchers, *Chem. Ber.* 86 261 (1953).

597 **Isorhodomycin A** (Hydrochloride), $C_{20}H_{29}O_8N \cdot HCl$ (proposed), m.p. 220°, $[\alpha]_{606\text{-}760}^{18}$ +268 ±30° (c 0.1 in methanol).

Hans Brockmann and Peter Patt, *Chem. Ber.* 88 1455 (1955).

598 **Rhodomycin B** (Hydrochloride), $C_{19}H_{27}O_7N \cdot HCl$, red prisms, m.p. 180°, $[\alpha]_{606\text{-}760}^{18}$ +174 ±10° (c 0.05 in methanol).

An isorhodomycin B also was present.

Hans Brockmann and Peter Patt, *Chem. Ber.* 88 1455 (1955).

599 **$\beta$-Rhodomycinone**, $C_{20}H_{14}O_5$ (proposed), dark red needles, m.p. 225°.

Hans Brockmann and Burchard Franck, *Chem. Ber.* 88 1792 (1955). (Isolation)

Hans Brockmann and P. Boldt, *Naturwissenschaften* 44 616 (1957). (Revised empirical formula)

600 **$\epsilon$-Rhodomycinone**, $C_{21}H_{22}O_8$, thick red prisms, m.p. 185° (dec. at 208°)

and

601 **$\epsilon$-Isorhodomycinone**, $C_{20}H_{20}O_9$, dark red leaflets, m.p. 245° (dec.).

Hans Brockmann and Burchard Franck, *Chem. Ber.* 88 1792 (1955). (Isolation)

Other references:

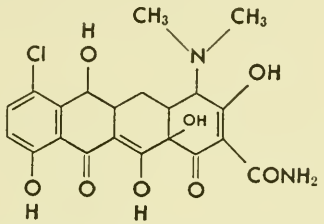
Hans Brockmann and Klaus Bauer, *Naturwissenschaften* 37 492 (1950).

Hans Brockmann, Klaus Bauer and Ilse Borchers, *Chem. Ber.* 84 700 (1951).

Hans Brockmann and Enno Spohler, *Naturwissenschaften* 42 154 (1955). (Characterization of rhodosamine)

Hans Brockmann, German Patent 913,813 (1954).

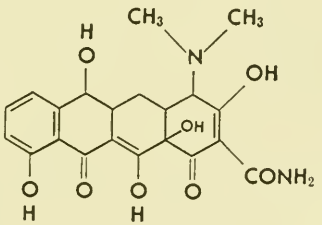
602 **7-Chloro-6-demethyltetracycline,** $C_{21}H_{21}O_8N_2Cl$ (isolated as the sesquihydrate), yellow crystals, m.p. 174–178° (dec.), $[\alpha]_D^{25}$ −258° (0.5% in 0.1 N sulfuric acid).




*Streptomyces aureofaciens* Duggar (mutant)

J. R. D. McCormick, Newell O. Sjölander, Ursula Hirsh, Elmer R. Jensen and Albert P. Doerschuk, *J. Am. Chem. Soc.* 79 4561 (1957).

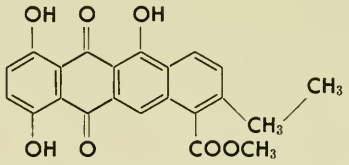
603 **6-Demethyltetracycline,** $C_{21}H_{22}O_8N_2Cl$ (isolated as the hydrochloride hemihydrate), yellow crystals, m.p. 203–209° (dec.), $[\alpha]_D$ −259° (c 0.5 in 0.1 N sulfuric acid).




*Streptomyces aureofaciens* Duggar (mutant)

J. R. D. McCormick, Newell O. Sjölander, Ursula Hirsh, Elmer R. Jensen and Albert P. Doerschuk, *J. Am. Chem. Soc.* 79 4561 (1957).

604 **$\eta$-Pyrromycinone**, $C_{22}H_{16}O_7$, red needles, m.p. 236° (sublimes).

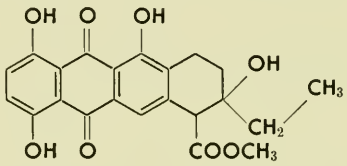



*Streptomyces* spp.

Hans Brockmann and Werner Lenk, *Chem. Ber.* 92 1880 (1959). (Structure)

Hans Brockmann and Hans Brockmann, Jr., *Naturwissenschaften* 47 135 (1960). (Revised structure)

605 **$\zeta$-Pyrromycinone**, $C_{22}H_{20}O_8$, orange-red needles, m.p. 216° (sublimes), $[\alpha]_{Cd}^{20}$ +74 ±6° (in chloroform).




*Streptomyces* spp.

Brockmann and collaborators have isolated about a dozen pigments of this type from various unclassified streptomycetes.

Hans Brockmann and Burchard Franck, *Chem. Ber.* 88 1792 (1955).

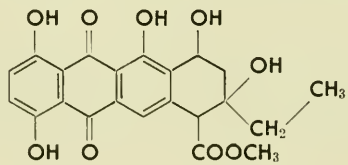
H. Brockmann, Luis Costa Plà and W. Lenk, *Angew. Chem.* 69 477 (1957).

H. Brockmann and P. Boldt, *Naturwissenschaften* 44 616 (1957).

Hans Brockmann and Werner Lenk, *Chem. Ber.* 92 1880 (1959). (Structure)

*Idem., Naturwissenschaften* 47 135 (1960). (Revised structure)

606 **$\epsilon$-Pyrromycinone** (Rutilantinone), $C_{22}H_{20}O_9$, orange-red needles, m.p. 213°, $[\alpha]_{Cd}^{20}$ +143 ±7° (c 1.0 in chloroform).




*Streptomyces* spp.

ε-Pyrromycinone occurs as such and also as the chromophore of the antibiotics pyrromycin and the cinerubins. It is identical with rutilantinone.

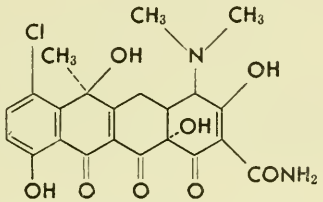
Hans Brockmann and Werner Lenk, *Chem. Ber.* 92 1880 (1959). (Structure)

*Idem., Naturwissenschaften* 47 135 (1960). (Revised structure)

H. Brockmann, H. Brockmann, Jr., J. J. Gordon, W. Keller-Schierlein, W. Lenk, W. D. Ollis, V. Prelog and I. O. Sutherland, *Tetrahedron Letters* No. 8, p. 25 (1960).

W. D. Ollis, I. O. Sutherland and J. J. Gordon, *Tetrahedron Letters* No. 16, p. 17 (1959).

607 **7-Chloro-5a(11a)-dehydrotetracycline,** $C_{22}H_{21}O_8N_2Cl$, $[\alpha]_D^{25}$ 15.5° (c 0.65 in 0.03 N hydrochloric acid).

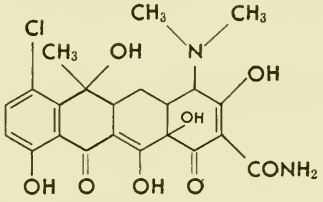



*Streptomyces aureofaciens* Duggar mutant

The analogous compounds in which the chlorine atom is replaced by H and Br are also claimed.

J. R. D. McCormick, Philip A. Miller, John A. Growich, Newell O. Sjölander and Albert P. Doerschuk, *J. Am. Chem. Soc.* 80 5572 (1958).

608 **Chlortetracycline** (Aureomycin, Biomycin), $C_{22}H_{23}O_8N_2Cl$, fine yellow crystals, m.p. 168°, $[\alpha]_D^{23}$ −274.9° (in methanol).




*Streptomyces aureofaciens*

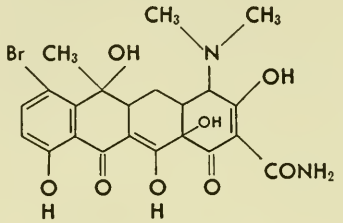
R. W. Broschard, A. C. Dornbush, S. Gordon, B. L. Hutchings, A. R. Kohler, G. Krupka, S. Kuchner, D. V. Lefemine and C. Pidacks, *Science* 109 199 (1949). (Isolation)

Benjamin M. Duggar, U. S. Patent 2,482,055 (1949).

C. R. Stephens, L. H. Conover, F. A. Hochstein, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *J. Am. Chem. Soc.* 74 4976 (1952).

C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, *ibid.* 74 4981 (1952).

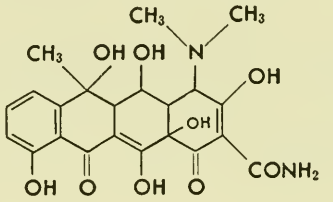
609 **Bromotetracycline,** $C_{22}H_{23}O_8N_2Br$, m.p. 170–172°, $[\alpha]_D^{20}$ −196° (in 0.1 N hydrochloric acid).




*Streptomyces aureofaciens*

P. Sensi, G. A. DeFerrari, G. G. Gallo and G. Rolland, *Il Farmaco Ed. sci.* (Pavia) 10 337 (1955).

610 **Oxytetracycline** (Terramycin), $C_{22}H_{24}O_9N_2$, light-yellow crystals, m.p. (anhydride) ~185° (dec.), $[\alpha]_D^{25}$ (dihydrate) −196.6° (c 1.0 in 0.1 N hydrochloric acid).




*Streptomyces rimosus*

A. C. Finlay, G. L. Hobby, S. Y. P'An, P. P. Regna, J. B. Routien, D. B. Seeley, G. M. Shull, B. A. Sobin, I. A. Solomons, J. W. Vinson and J. H. Kane, *Science* 111 85 (1950). (Isolation)

Ben A. Sobin, Alexander C. Finlay and Jasper H. Kane, U. S. Patent 2,516,080 (1950).

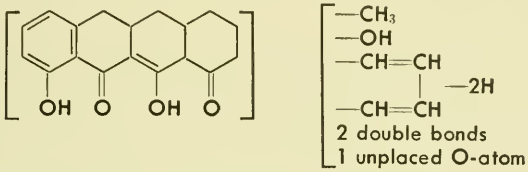
Peter P. Regna, I. A. Solomons, Kotaro Murai, Albert E. Timreck, Karl J. Brunings and W. A. Lazier, *J. Am. Chem. Soc.* 73 4211 (1951).

C. R. Stephens, L. H. Conover, F. A. Hochstein, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.* 74 4976 (1952).

F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.* 75 5455 (1953). (Structure)

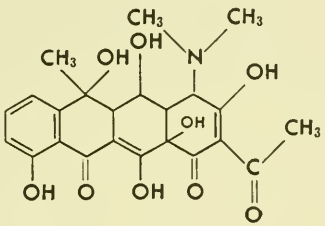
611 **Antibiotic X-340,** $C_{23}H_{20}O_6$, yellow needles, m.p. 330° (dec.). An antibiotic isolated from the mycelium of an uniden-

tified streptomycete. The molecular weight was about 390. Contained 3 —OH groups (one acidic) and one C—$CH_3$ group. Monomethyl derivative with diazomethane. Mono- and tri-acetates were formed, depending on method. The infrared absorption pattern was similar to that of Terramycin. The following partial structure was proposed:




V. C. Vora, K. Shete and M. M. Dhar, *J. Sci. Ind. Research* (India) 16C 182 (1957). (Isolation)

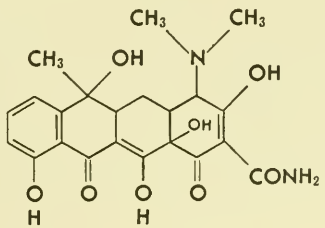
612 **2-Acetyl-2-decarboxamidooxytetracycline** (Terramycin-X) (Hydrochloride), $C_{23}H_{25}O_9N \cdot HCl$, yellow crystals, m.p. 201–203°, $[\alpha]_D^{25}$ −46.6° (c 0.9 in 0.1 N hydrochloric acid).




*Streptomyces rimosus*

F. A. Hochstein, M. Schach von Wittenau, F. W. Tanner, Jr. and K. Murai, *J. Am. Chem. Soc.* 82 (1960). (In press)

613 **Tetracycline** (Achromycin, Tetracyn, Polycycline, Panmycin), $C_{22}H_{24}O_8N_2$, yellow crystals, m.p. 170–175° (dec.), $[\alpha]_D^{25}$ −239° (c 1.0 in methanol).




*Streptomyces* sp.

Tetracycline was first prepared by catalytic dechlorination of chlortetracycline but was later isolated as a primary fermentation product.

P. Paul Minieri, Melvin C. Firman, A. G. Mistretta, Anthony Abbey, Clark E. Bricker, Neil E. Rigler and Herman Sokol, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, p. 81. (Isolation)

614 **Quinocyclines** (PA-121)

A complex of tetracyclic amphoteric antibiotic yellow pigments, which in some respects resemble nitrogen-containing hydroxyanthraquinones.

Six active components have been separated and analyses and color reactions were determined.

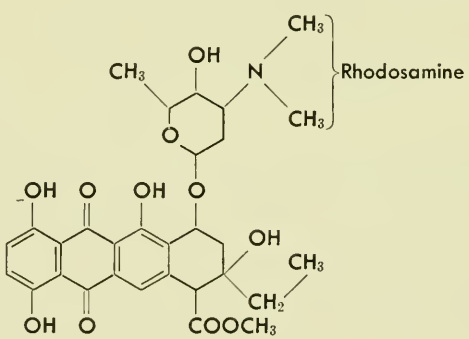
Two components have an aglycone with the probable empirical formula $C_{25}H_{20}O_6N_2$.

*Streptomyces* sp.

W. D. Celmer, K. Murai, K. V. Rao, F. W. Tanner, Jr. and W. S. Marsh, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 484. (Isolation)

Charles R. Stephens, unpublished. (Empirical formula)

615 **$\eta$-Pyrromycin,** $C_{30}H_{35}O_{11}N$ (Hydrochloride), red crystals, m.p. 162° (dec.), $[\alpha]_{Cd}^{20}$ +132 ±27° (c 0.4 in methanol).




A streptomycete

The relationship to $\epsilon$-pyrromycinone and to the cinerubins should be noted.

Hans Brockmann and Werner Lenk, *Chem. Ber.* 92 1904 (1959). (Structure)

Hans Brockmann and Hans Brockmann, Jr., *Naturwissenschaften* 47 135 (1960).

616 **Aklavin**, $C_{30}H_{37}O_{11}N$ (Hydrochloride) orange crystals, m.p. 197°.

Amphoteric. Contains an amino sugar, $C_8H_{17}O_4N$, isomeric with mycaminose or amosamine linked glycosidically to the secondary hydroxyl group.

*Streptomyces* sp.

F. Strelitz, H. Flon, U. Weiss and I. N. Asheshor, *J. Bacteriol.* 72 90 (1956).

617 **Cinerubins**

Cinerubins A and B are isomeric red bases, with the empirical formula $C_{44}H_{59}O_{18}N \pm CH_2$. The chromophoric aglycone has been shown to be identical with $\epsilon$-pyrromycinone. Both cinerubins also contain three sugars, two of these being the same in both compounds, but the third one being characteristic. The structures of these sugars have not been reported yet.

*Streptomyces antibioticus* (Waksman and Woodruff) Waksman et Henrici, *S. galiloeus* Ettlinger *et al.*, *S. niveoruber* Ettlinger *et al.*

Leopold Ettlinger, Ernest Gäumann, Ralf Hütter, Walter Keller-Schierlein, Friederich Kradolfer, Lucien Neipp, Vlado Prelog, Pierre Reusser and Hans Zähner, *Chem. Ber.* 92 1867 (1959).

14

# Aromatic Compounds Not Classified Elsewhere

This chapter includes a heterogeneous group of aromatic compounds which arise from different biosynthetic routes. Cinnamic acid and its derivatives undoubtedly are formed by way of the shikimic acid pathway.[1, 2] The occurrence of anisaldehyde and anisic acid derivatives in the same fermentation with methyl *p*-methoxycinnamate suggests that the former may be degradation products of the latter.

Chloramphenicol, too, has a $C_6$-$C_3$ skeleton which seems to relate it to the shikimic acid pathway. It has been shown that *p*-nitrophenylserinol does not act as a precursor, and, when it is added to fermentations, it is acetylated but not dichloroacetylated. $C^{14}$-Labeled *p*-nitrophenylserinol is not incorporated into the chloramphenicol molecule nor is $C^{14}$-labeled dichloroacetic acid. Thus, what appears to be a logical step in the biosynthesis—the dichloroacetylation of *p*-nitrophenylserinol—does not occur.[3]

The tricarboxylic acid produced by *Chaetomium indicum* is evidently formed by the condensation of $\alpha$-ketoglutaric acid with phenylpyruvic acid.

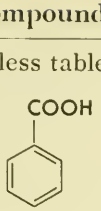
The lichen acids of this chapter show a provocative symmetry, and the incorporation of amino acids into two of them is interesting. The diphenylbutadiene structure has been found also in xanthocillin. Apparently there has been no experimental study of their biogenesis.

[1] Friedrich Weygand and Heinz Wendt, Z. *Naturforsch.* 14b 421 (1959).

[2] T. A. Geissman and T. Swain, *Chem. and Ind.*, 984 (1957).

[3] David Gottlieb, P. W. Robbins and H. E. Carter, *J. Bacteriol.* 72 153 (1956).

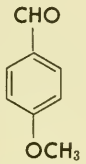
618 **Benzoic Acid,** $C_7H_6O_2$, colorless tablets, m.p. 122.5°.




Yeast

Richard Kuhn and Klaus Schwarz, *Ber.* 74 1617 (1941).

619 **Anisaldehyde,** $C_8H_8O_2$, oily liquid, b.p. 248°, $n_D^{13}$ 1.5764.

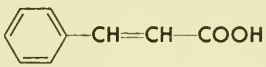



*Trametes suavolens* (Linn.) Fr., *Lentinus lepideus, Daedalea juniperina*

J. H. Birkinshaw, A. Bracken and W. P. K. Findlay, *Biochem. J.* 38 131 (1944).

J. H. Birkinshaw and P. Chaplen, *ibid.* 60 255 (1955).

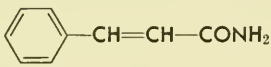
620 ***trans*-Cinnamic Acid,** $C_9H_8O_2$, colorless crystals, m.p. 133°.




*Ceratostomella fimbriata* (on sweet potato culture)

Takashi Kubota and Keizo Naya, *Chem. and Ind.*, 1427 (1954).

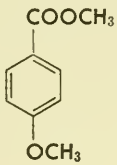
621 ***trans*-Cinnamic Acid Amide,** $C_9H_9ON$, colorless crystals, m.p. 147–149°.




*Streptomyces* sp.

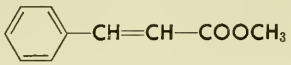
Yasuharu Sekizawa, *J. Biochem.* Japan 45 9 (1958). (Isolation)

622 **Methyl Anisate,** $C_9H_{10}O_3$, colorless crystals, m.p. 48°, b.p. 256°.



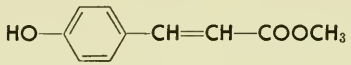
*Trametes suavolens* (Linn.) Fr., *Lentinus lepideus*
J. H. Birkinshaw, A. Bracken and W. P. K. Findlay, *Biochem. J.* 38 131 (1944).

623 **Methyl *trans*-Cinnamate,** $C_{10}H_{10}O_2$, clear, pale yellow oil, b.p. 94–110° (2–3 mm.) or white crystals, m.p. 35–37°, $n_D^{21}$ 1.5766



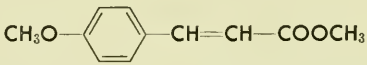
*Lentinus lepideus* Fr. (artificial medium)
John Howard Birkinshaw and Walter Philip Kennedy Findlay, *Biochem. J.* 34 82 (1940).

624 **Methyl *p*-Coumarate,** $C_{10}H_{10}O_3$, colorless crystals, m.p. 137–139°.



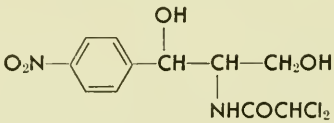
*Lentinus lepideus*
H. Shimazono and F. F. Nord, *Arch. Biochem. and Biophys.* 78 263 (1958).

625 **Methyl *p*-Methoxycinnamate,** $C_{11}H_{12}O_3$, colorless crystals, m.p. 88°.



*Lentinus lepideus* Fr. (artificial medium)
John Howard Birkinshaw and Walter Philip Kennedy Findlay, *Biochem. J.* 34 82 (1940).

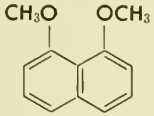
626 **Chloramphenicol** (Chloromycetin, Levomycetin), $C_{11}H_{12}O_5N_2Cl_2$, colorless crystals, m.p. 149.7°, $[\alpha]_D^{25}$ −25.5° (in ethyl acetate).



*Streptomyces venezuelae*
John Ehrlich, Quentin R. Bartz, Robert M. Smith, Dwight A. Joslyn and Paul R. Burkholder, *Science* 106 417 (1947). (Isolation)
John Controulis, Mildred C. Rebstock and Harry M. Crooks, Jr., *J. Am. Chem. Soc.* 71 2463 (1949). (Synthesis)

Loren M. Long and H. D. Troutman, *ibid.* 71 2469 (1949).

627 **1,8-Dimethoxynaphthalene,** $C_{12}H_{12}O_2$, colorless crystals, m.p. 158–161°.

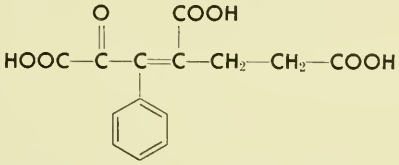


*Daldinia concentrica*

8-Methoxyl-1-naphthol also was identified (by paper chromatography).

D. C. Allport and J. D. Bu'Lock, *J. Chem. Soc.*, 654 (1960).

628 **4-Carboxy-2-oxo-3-phenylhept-3-enedioic Acid,** $C_{14}H_{12}O_7$, colorless prisms, m.p. 170° (dec.).



*Chaetomium indicum* Corda

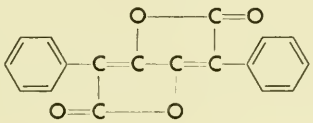
The yield was 250–500 mg. per liter. In addition to the acid above, two uncharacterized compounds were isolated in smaller quantities: Metabolite A, $C_{26}H_{37}O_6N$, pale yellow needles, m.p. 159°, $[\alpha]_D^{20}$ +11.4° (c 1.022 in chloroform). Yield 1.5–2.0 g. from 100 l. of broth. Soluble in aqueous $NaHCO_3$. Wine red $FeCl_3$ test. Formed an insoluble green-blue copper derivative.

Metabolite B, colorless prisms, m.p. 146°, $[\alpha]_D^{20}$ +120° (c 1.01 in chloroform).

Analysis: C 68.1, H 8.2, N 2.7, C-methyl 12%. Same color tests as A above.

D. H. Johnson, Alexander Robertson and W. B. Whalley, *J. Chem. Soc.*, 2429 (1953).

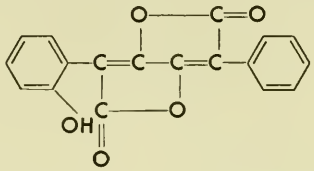
629 **Pulvic Anhydride,** $C_{18}H_{10}O_4$, yellow needles, m.p. 222–224°.



*Sticta aurata* Ach.

O. Hesse, *J. prakt. Chem.* 170 334 (1900).

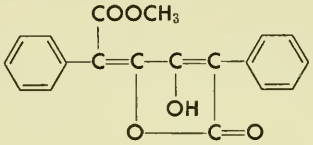
630 **Calycin,** $C_{18}H_{10}O_5$, orange-red crystals, m.p. 244°.



*Lepraria candelaris* Schaer., *Sticta aurata* Ach. and *Sticta crocata* Ach.

Mitizo Asano and Yukio Kameda, *Ber.* 68 1568 (1935).

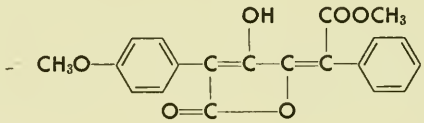
631 **Vulpinic Acid,** $C_{19}H_{14}O_5$, yellow crystals, m.p. 148°.



*Evernia vulpina* L., *Cyphelium chrysocephalum* Ach., *Calicium chlorinum* Körper, *Cetraria juniperina* Fr. var. *tubulosa* Schaer and *Cetraria pinastri* (Scop.)

P. Karrer, K. A. Gehrckens and W. Heuss, *Helv. Chim. Acta* 9 446 (1926). (Structure)

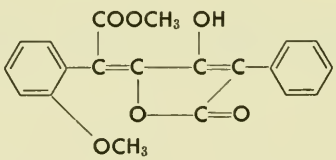
632 **Pinastric Acid** (Chrysocetraric Acid), $C_{20}H_{16}O_6$, orange needles, m.p. 200–203°.



*Lepraria flava* (Schreber.) f. *quercina, Cetraria pinastri* (Scop.), *Cetraria tubulosa* (Schreb.), *Cetraria juniperina* L. (Ach.)

Mitizo Asano and Yukio Kameda, *Ber.* 68 1565 (1935). (Structure)

633 **Leprapic Acid** (Leprapinic Acid, Methyl 2-Methoxypulvinate), $C_{20}H_{16}O_6$, golden plates, m.p. 159°.



*Lepraria citrina*

O. P. Mittal and T. R. Seshadri, *J. Chem. Soc.*, 3053 (1955). (Isolation)

*Idem., ibid.*, 1734 (1956). (Synthesis)

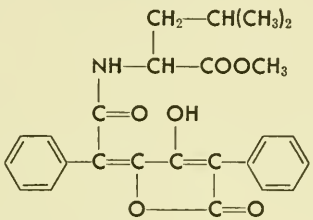
634 **Mycolutein,** $C_{22}H_{24}O_6N$ (proposed), bright yellow tablets, m.p. 157°, $[\alpha]_D^{25}$ +54° (c 1 in chloroform).

Contains an aromatic nucleus. Alkali-unstable. Negative $FeCl_3$. Decolorizes bromine with HBr evolution.

*Streptomyces* sp.

Henry Schmitz and Robert Woodside, *Antibiotics and Chemotherapy* 5 652 (1955).

635 **Epanorin,** $C_{25}H_{25}O_6N$, yellow needles, m.p. 135°, $[\alpha]_D^{26}$ −1.86 ±0.2° (c 6.48 in chloroform).

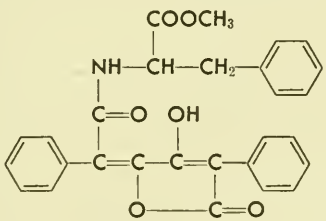



*Lecanora epanora* Ach.

Zeorin was found in the same extract.

Robert L. Frank, S. Mark Cohen and James N. Coker, *J. Am. Chem. Soc.* 72 4454 (1950). (Structure and synthesis)

636 **Rhizocarpic Acid,** $C_{28}H_{23}O_6N$, yellow needles, m.p. 177°, $[\alpha]_D^{20}$ +110.4° ±2.1° (c 1.22 in chloroform).




*Rhizocarpon geographicum* L., *R. viridiatrum* Flk., *Calicium hyperellum* Ach.

Robert L. Frank, S. Mark Cohen and James N. Coker, *J. Am. Chem. Soc.* 72 4454 (1950). (Synthesis)

15

# Amines

Much remains to be learned concerning the earlier stages of nitrogen metabolism in microorganisms. Practically, the ability of certain soil bacteria (in combination with legumes) to fix gaseous nitrogen has been exploited for many years. Research in this area has been reviewed.[1] Ammonia, methane, hydrogen and water probably were present in the atmosphere of the primitive earth, and it has been shown[2] that amino acids can be formed by electric discharges through such mixtures.

While we are primarily concerned in this compilation with metabolites isolated from microorganisms growing in the wild state or cultivated on an essentially glucose medium, the more complex amines are generally only remotely derived from sugar, often by way of the amino acids. A large literature exists on the ability of bacteria to decarboxylate amino acids to amines, these experiments generally involving addition of the amino acid to the medium. It has been shown,[3] however, that many bacteria which produce amines on a casein hydrolysate medium do not do so on a synthetic medium with ammonium salts the only nitrogen source. Studies with *Escherichia coli*[4] indicate that aspartic acid and alanine and perhaps glutamic acid serve as important nitrogen entry vehicles. These acids can supply the total nitrogen requirement if no ammonium ion is available,

[1] William D. McElroy and Bentley Glass, "Inorganic Nitrogen Metabolism," Johns Hopkins Press, Baltimore, 1956.

[2] Stanley L. Miller, *Science* 117 528 (1953); *idem.*, *J. Am. Chem. Soc.* 77 2351 (1955).

[3] H. Proom and A. J. Woiwod, *J. Gen. Microbiol.* 5 930 (1951).

[4] "Studies of Biosynthesis in *E. coli*," Carnegie Institute of Washington Publication 607, Washington, 1955.

and, even when it is, much of the cellular nitrogen is derived from them by transamination.

Within the frame of our present endeavor there seems to have been little systematic, comparative study of the amine metabolites of microorganisms. This has been true particularly of the fungi, which generally have been considered to have a poorer nitrogen metabolism than the bacteria. Apparently this situation is being remedied, at least for higher fungi. Recently the amine content of 105 species, representing 18 families of higher fungi, was investigated.[5] It was found that ammonia was distributed universally, and that the ammonia content increased with the age of the fruiting body. Methylamine occurred in 22 species, dimethylamine in 10, trimethylamine in 8, isoamylamine in 19 and β-phenylethylamine in 4. Earlier work was reviewed also, a distinction being made between the amines present in fresh fruiting bodies and those present after autolysis.

Also an exceptionally thorough analysis was made recently of the basic constituents of the fruiting body of a single basidiomycete, *Polyporus sulfureus*.[6] These included amines, basic amino acids, nucleotides and betaines. Many of the simple amines produced by *Claviceps purpurea* have been identified during the extensive studies of ergot, and these are listed in the introduction to the section on ergot alkaloids in the chapter on Heterocycles.

Muscarine, a compound which might have been classified under several different chapter headings, is apparently a derivative of a 1-amino-3,6-desoxyhexose and is probably more directly connected with sugar metabolism than many of the amines listed here.

Amino sugars and other complex amines are listed elsewhere under more appropriate classifications.

It has been shown that putrescine furnishes the 4-carbon atom moiety of spermine and spermidine in *Neurospora crassa*,[7] and that methionine supplies the 3-carbon chain of spermidine in the same organism.[8] It is known that ATP and $Mg^{++}$ are re-

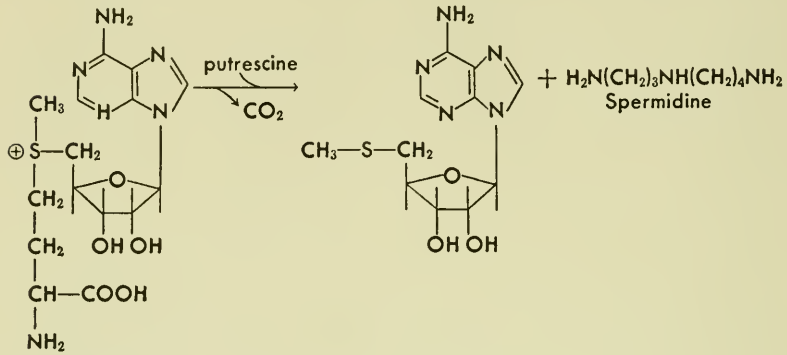
[5] Elard Stein von Kamienski, *Planta* 50 331 (1958).

[6] P. H. List, *Planta Med.* 6 424 (1958).

[7] H. Tabor, S. M. Rosenthal and C. W. Tabor, *Federation Proc.* 15 367 (1956).

[8] Ronald C. Greene, *J. Am. Chem. Soc.* 79 3929 (1957).

quired. A mechanism such as the one shown here (abbreviated)




may be operative.

637 **Ammonia,** $NH_3$, colorless gas.

$NH_3$

Widely distributed in the fruiting bodies of the higher fungi and lichens. The content increases with age.

Elard Stein von Kamienski, *Planta* 50 331 (1958).

638 **Methylamine,** $CH_5N$, colorless gas.

$CH_3NH_2$

*Russula* (11 spp.), *Lactarius deliciosus, L. vellereus, L. helvus, Boletus edulis, B. appendiculatus, Scleroderma vulgare, Anthurus muellerianus, Mutinus caninus, Trachypus versipellis, Dermocybe (Cortinarius) cinnamomea, Lepiota clypeolaria, Pholiota mutabilis, Sticta fuliginosa, S. sylvatica, Polyporus sulfureus*

Elard Stein von Kamienski, *Planta* 50 331 (1958).

P. H. List, *Planta Med.* 6 424 (1958).

639 **Ethylamine,** $C_2H_7N$, volatile liquid, b.p. 16.6°.

$CH_3CH_2NH_2$

*Claviceps purpurea, Polyporus sulfureus*

Maximilian Steiner and Elard Stein von Kamienski, *Naturwissenschaften* 42 345 (1955).

P. H. List, *Planta Med.* 6 424 (1958).

640 **Dimethylamine,** $C_2H_7N$, colorless gas, b.p. 7°.

$CH_3—NH—CH_3$

*Phallus impudicus, Clathrus ruber, Russula aurata*
Gustav Klein and Max Steiner, *Jahrb. wiss. Bot.* 68 602 (1928).

*R. sardonia, R. turci, R. lepida, R. cyanoxantha, R. grisea, R. olivacea, R. vesca, R. alutacea, Sticta sylvatica, Polyporus sulfureus*
Elard Stein von Kamienski, *Planta* 50 333 (1958).
P. H. List, *Planta Med.* 6 424 (1958).

641 **Ethanolamine,** $C_2H_7ON$, colorless oil, b.p. 171°, $n_D^{20}$ 1.4539.

$HOCH_2CH_2NH_2$

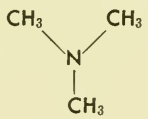
*Neurospora crassa* (and probably in) *Boletus edulis, B. versipellis, Xerocomus badius, Lepiota clypeoloris, Pholiota mutabilis, Tricholoma nudum, Russula maculata, R. turci, Lactarius vellercus, Amanita muscaria, Polyporus sulfureus*
George L. Ellman and Herschel K. Mitchell, *J. Am. Chem. Soc.* 76 4028 (1954).
Elard Stein von Kamienski, *Planta* 50 331 (1958).
P. H. List, *Planta Med.* 6 424 (1958).

642 **Aminoacetone,** $C_3H_7ON$, colorless crystals, m.p. 130.5°.

$CH_3COCH_2NH_2$

*Staphylococcus aureus*
W. H. Elliot, *Nature* 183 1051 (1959).

643 **Trimethylamine,** $C_3H_9N$, colorless gas (fishy odor), b.p. 3°.




*Boletus edulis, Ustilago maydis, Phallus impudicus, Claviceps purpurea, Tilletia laevis, T. tritici, Clathrus ruber, Russula* spp. *Sticta* spp.
J. Zellner, *Monatsh.* 31 617 (1910).
William Fielding Hanna, Hubert Bradford Vickery and George W. Pucher, *J. Biol. Chem.* 97 351 (1932). (Isolation)
Maximilian Steiner and Elard Stein von Kamienski, *Naturwissenschaften* 42 345 (1955).

644 ***n*-Propylamine**, $C_3H_9N$, liquid, b.p. 50°.

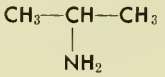
$$CH_3CH_2CH_2NH_2$$

*Claviceps purpurea*, *Polyporus sulfureus*

Maximilian Steiner and Elard Stein von Kamienski, *Naturwissenschaften* 42 345 (1955).

P. H. List, *Planta Med.* 6 424 (1958).

645 **Isopropylamine**, $C_3H_9N$, liquid, b.p. 33°.



*Claviceps purpurea*

Maximilian Steiner and Elard Stein von Kamienski, *Naturwissenschaften* 42 345 (1955).

646 **Methylaminoethanol**, $C_3H_9ON$, slightly viscous liquid, b.p. 159°.

$$HOCH_2CH_2NHCH_3$$

*Neurospora crassa* mutant

N. H. Horowitz, *J. Biol. Chem.* 162 413 (1946).

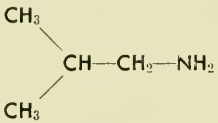
647 ***n*-Hexylamine**, $C_6H_{15}N$, liquid, b.p. 129°.

$$CH_3(CH_2)_5NH_2$$

*Claviceps purpurea*

Maximilian Steiner and Elard Stein von Kamienski, *Naturwissenschaften* 42 345 (1955).

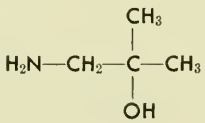
648 **Isobutylamine**, $C_4H_{11}N$, liquid, b.p. 68°.



*Claviceps purpurea*

Maximilian Steiner and Elard Stein von Kamienski, *Naturwissenschaften* 42 345 (1955).

649 **1-Amino-2-methyl-2-propanol**, $C_4H_{11}ON$, liquid, b.p. 151°.



*Neurospora crassa*

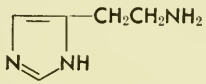
George L. Ellman and Herschel K. Mitchell, *J. Am. Chem. Soc.* 76 4028 (1954).

650 **Putrescine,** $C_4H_{12}N_2$, crystals, m.p. 27°.

$$H_2NCH_2CH_2CH_2CH_2NH_2$$

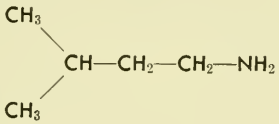
*Boletus edulis, B. luteus, B. elegans, Amanita muscaria*
C. Reuter, Z. *physiol. Chem.* 78 167, 223 (1912).
Albert Küng, *ibid.* 91 241 (1914).
Werner Keil and Hans Bartmann, *Biochem. Z.* 280 58 (1935).

651 **Histamine,** $C_5H_9N_3$, deliquescent needles, m.p. 83° (Hydrochloride) m.p. 244–246° (Picrate) m.p. 160°.



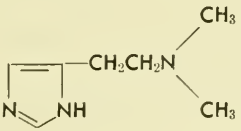

*Claviceps purpurea, Coprinus comatis* Gray
Paul Heinz List, *Arch. Pharm.* 291 502 (1958).

652 **Isoamylamine,** $C_5H_{13}N$, liquid, b.p. 95–97°.




*Boletus edulis, B. sanguineus, B. queletii, B. luridus, B. regius, B. appendiculatus, Phallus impudicus, Claviceps purpurea, Amanita phalloides, Marasmium peronatus, Russula foetens, R. turei, R. maulata, Trachypus scaber, Xeroeomus sanguineus, X. subtomentosus, Mutinus caninus, Lycoperdon piriforme, L. gemmatum, Phlegmacium mellioleus, Nematoloma fasciculare, Polyporus sulfureus*
Maximilian Steiner and Elard Stein von Kamienski, *Naturwissenschaften* 40 483 (1953).
Elard Stein von Kamienski, *Planta* 50 334 (1958).
P. H. List, *Planta Med.* 6 424 (1958).

653 **Dimethylhistamine,** $C_7H_{13}N_3$ (Dihydrochloride) m.p. 245–250° (dec.).



*Coprinus comatis* Gray

Paul Heinz List, *Arch. Pharm.* 291 502 (1958).

654 **Acetylcholine,** $C_7H_{17}O_3N$, colorless, hygroscopic powder.

*Streptobacterium plantarum*

Acetylcholine is produced also by the ergot fungus, *Claviceps purpurea,* and probably by many other microorganisms.

Yield: about 160 γ per milliliter from the first organism above.

Adolf Wacker, Adolf Roth, Heinz Sucker and Otto Dann, *Ann.* 601 202 (1957).

655 **Spermidine,** $C_7H_{19}N_3$, unstable oil, b.p. 128° (14 mm.).



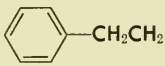
Yeast, *Neurospora crassa*

Occurs as the phosphate.

H. Tabor, S. M. Rosenthal and C. W. Tabor, *Federation Proc.* 15 367 (1956).

Ronald C. Greene, *J. Am. Chem. Soc.* 79 3929 (1957).

656 **β-Phenylethylamine,** $C_8H_{11}N$, liquid, b.p. 196–198°.



*Boletus edulis, B. luteus, Claviceps purpurea, Polyporus sulfureus, Marasmius peronatus, Phlegmacium melliolus, Nematoloma fasciculare, Pholiota mutabilis*

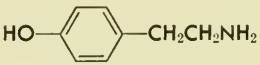
C. Reuter, Z. *physiol. Chem.* 78 167 (1912).

Werner Keil and Hans Bartmann, *Biochem. Z.* 280 58 (1935).

Elard Stein von Kamienski, *Planta* 50 335 (1958).

P. H. List, *Planta Med.* 6 424 (1958).

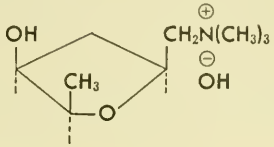
657 **Tyramine,** $C_8H_{11}ON$, colorless crystals, m.p. 164°. (Picrate), m.p. 206°.



*Coprinus comatis* Gray, *Claviceps purpurea*

Paul Heinz List, *Arch. Pharm.* 291 502 (1958).

658 **Muscarine,** $C_9H_{19}O_2N$, white crystals, Hydrochloride $[\alpha]_D^{20}$ +1.57° (in water).



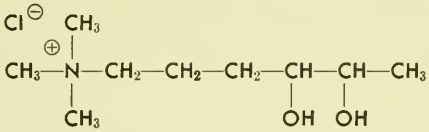
*Amanita muscaria*

F. Kögl, C. A. Salemink, H. Schouten and F. Jellinck, *Rec. trav. chim.* 76 109 (1957). (Structure)

E. Hardegger and F. Lohse, *Helv. Chim. Acta* 40 2383 (1957). (Synthesis and configuration)

P. J. Fraser, *Brit. J. Pharmacol.* 12 47 (1957). (Pharmacology)

659 **Muscaridine,** $C_9H_{22}O_2NCl$, isolated as the chloroaurate, $C_9H_{22}AuCl_4O_2N$, m.p. 129–131° (dec.), $[\alpha]_D^{19}$ +20.5° ±0.5° (c 8.3 in water).

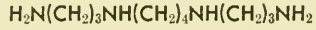


*Amanita muscaria* L.

F. Kögl, C. A. Salemink and P. L. Schuller, *Rec. trav. chim.* 79 485 (1960). (Isolation)

C. A. Salemink and P. L. Schuller, *ibid.* 79 278 (1960). (Synthesis)

660 **Spermine,** $C_{10}H_{26}N_4$, deliquescent, $CO_2$-absorbing crystals. Phosphate: m.p. 230–234° (dec.).



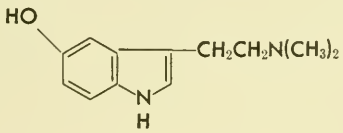
Yeast, *Neurospora crassa*

Occurs phosphorylated.

H. Tabor, S. M. Rosenthal and C. W. Tabor, *Federation Proc.* 15 367 (1956).

Ronald C. Greene, *J. Am. Chem. Soc.* 79 3929 (1957).

661 **Bufotenin,** $C_{12}H_{16}ON_2$, colorless crystals, m.p. 146°.

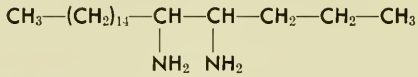


*Amanita mappa* and certain related species

Bufotenin occurs also in the skin secretions of toads.

Theodor Wieland and Werner Motzel, *Ann.* 581 10 (1953).

662 Necrosamine, $C_{20}H_{44}N_2$ (Hydrochloride) crystals, m.p. ~275° (dec.).



*Escherichia coli*

This amine was a component of the phospholipide fraction.

Miyoshi Ikawa, J. B. Koepfli, S. G. Mudd and Carl Niemann, *J. Am. Chem. Soc.* 75 3439 (1953).

16

# Amino Acids and Related Compounds

A general review of the intermediary metabolism of amino acids would be disproportionate to the scope of this book. It is only possible to sketch in here some relationships and biosynthetic sequences which may serve as reminders or as guides for the novice.

As in acetate metabolism microorganisms have been used to explore the network of metabolic relationships among the amino acids. Many of these have proved quite general, yet it is only necessary to consider the unusual amino acids which have been isolated from microbial sources to realize the differences from human metabolism.

In this section principally free amino acids are considered. Polypeptides are listed and discussed in the succeeding section. Amino acid isolation and assay formerly were tedious and generally confined to analysis of hydrolysates of total proteins. Paper chromatography and reliable microbiological assays have made possible the separation and assay of the low concentrations of amino acids evolved into fermentation broths.

The older work on fungi has been reviewed.[1] A semiquantitative survey of the free amino acids of a taxonomic range of fungi gave the results shown in Table I[2] on page 300. In general there were found no outstanding differences in the quantities or types of amino acids produced by the different fungi, nor in the types produced by fungi as compared with those of higher plants. The absence of tryptophan in all species examined is noteworthy. Four unidentified compounds were found in various fungi. These were suggested tentatively as

[1] Jackson W. Foster, "Chemical Activities of Fungi," Academic Press, New York, 1949.

[2] R. Close, *Nature* 185 609 (1960).

TABLE I

*Free Amino Acids Present in the Hyphal Extract of Certain Fungi*

| | *Pythium ultimum* (on Dox) | *Phyto-phthora cactorum* | *Phyco-myces nitens* + | *Tham-nidium elegans* | *Chroma-crea spinulosa* | *Fusarium culmorum* | *Fusarium javanicum* | *Stereum purpureum* |
|---|---|---|---|---|---|---|---|---|
| Aspartic acid | ++ | ++ | +++ | +++ | ++ | +++ | +++ | +++ |
| Glutamic acid | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| α-Alanine | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| β-Alanine | − | ++ | + | + | − | − | + | − |
| γ-Aminobutyric acid | ++ | +++ | ++ | ++ | +++ | ++ | + | − |
| Serine | + | ++ | ++ | + | ++ | +++ | ++ | ++ |
| Glycine | ++ | ++ | ++ | + | ++ | ++ | ++ | + |
| Threonine | ++ | ++ | ++ | − | + | + | ++ | + |
| Proline | − | +++ | − | ++ | − | − | − | − |
| Tyrosine | − | + | + | − | + | − | + | − |
| Arginine | +++ | +++ | +++ | + | ++ | ++ | ++ | ++ |
| Histidine | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ |
| Lysine | ++ | ++ | − | − | ++ | − | ++ | ++ |
| Phenylalanine | − | − | + | + | + | − | − | − |
| Leucine and/or *iso*-leucine | ++ | ++ | ++ | + | ++ | ++ | ++ | + |
| Methionine and/or valine | ++ | ++ | ++ | + | +++ | ++ | ++ | + |
| Ornithine | − | + | − | − | − | + | − | + |
| Cysteic acid | ++ | + | − | + | ++ | ++ | + | − |
| Asparagine | ++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ |
| Glutamine | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Initial pH | 3.7 | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 |
| Final pH | 5.5 | 4.7 | 6.7 | 3.9 | 5.1 | 7.0 | 6.9 | 6.5 |
| Incubation time (days) | 10 | 29 | 16 | 26 | 14 | 14 | 24 | 19 |

+++, Strong ninhydrin color; ++, moderate color; +, weak color; −, not detected.

α-aminoadipic acid, 3,4-dihydroxyphenylalanine, ethanolamine and taurine.

The amino acids of some algae have been reported,[3] and also those of *Fusarium lycopersici*.[4] A quantitative study was made of the amino acid composition of *Ustilago maydis* fermentation broth.[5] Of the 3.5 mg. per milliliter of $NH_4^+$ nitrogen added, 2.9 mg. per milliliter remained extracellular. This extracellular nitrogen contained 1.17 mg. per milliliter of organic nitrogen and 1.74 mg. of residual $NH_4^+$ nitrogen.

TABLE II

*Amino Acid Composition of Ustilago maydis Fermentation Broth*

| Amino acid* | Unhydrolyzed broth | | Hydrolyzed broth | |
|---|---|---|---|---|
| | μgm /ml. | μgm.N/ml. | μgm./ml.† | μgm.N/ml.† |
| Lysine..... | 387 | 64.2 | 413 | 79.2 |
| Arginine..... | 997 | 320.5 | 1136 | 365.3 |
| Histidine..... | 155 | 42.0 | 182 | 49.3 |
| Aspartic acid... | 200 | 21.0 | 506 | 53.2 |
| Glutamic acid... | 894 | 85.1 | 945 | 90.0 |
| Glycine..... | 200 | 38.1 | 295 | 55.0 |
| Alanine‡..... | | | 406 | 63.8 |
| Valine..... | 290 | 25.7 | 279 | 33.4 |
| Leucine..... | 387 | 41.3 | 368 | 39.3 |
| Isoleucine..... | 276 | 29.5 | 212 | 22.7 |
| Serine..... | 276 | 30.1 | 307 | 40.9 |
| Threonine‡..... | | | 237 | 27.9 |
| Proline..... | 263 | 32.0 | 289 | 35.2 |
| Phenylalanine... | 267 | 22.6 | 389 | 33.0 |
| Tyrosine..... | 139 | 10.7 | 383 | 29.6 |
| Tryptophan..... | 40 | 5.6 | | |
| Methionine..... | 65 | 6.0 | | |
| | 4.79 mgm. | 0.774 mgm. | 6.35 mgm. | 1.02 mgm. |

* The amino acids in the hydrolyzed broth and the basic amino acids in the unhydrolyzed broth were separated chromatographically and assayed colorimetrically. The other amino acids in the unhydrolyzed broth were assayed microbially.

† Values expressed as μgm. per milliliter in terms of original broth.

‡ Valid microbial assays were not obtained.

[3] L. Fowden, *ibid.* 167 1030 (1951); Borje Wickberg, *Acta Chem. Scand.* 11 506 (1957).

[4] V. Flück and K. H. Richle, *Phytopath. Z.* 24 455 (1955).

[5] Eugene L. Dulaney, E. Bilinski and W. B. McConnell, *Can. J. Biochem. and Physiol.* 34 1195 (1956).

In this case the broth was hydrolyzed and compared with the original to eliminate interference by small peptides in the microbial assays. Tryptophan and methionine were destroyed by the hydrolysis and chromatography procedure and are absent from the second part of the table. It was found that 53% of the extracellular organic nitrogen was represented by free amino acids. Some strains of *Ustilago maydis* produce 200–300 μg. of lysine per milliliter.[6]

A study of the extracellular nitrogen of several molds[7] gave the results in the accompanying table.

TABLE III

*Amount of Nitrogen Assimilated Which Appeared in the Medium After Seven Days Growth*

| Fungus | Nitrogen source | Extracellular nitrogen (as % initially added nitrogen) |
|---|---|---|
| | $NH_4^+$ | 7.5 |
| *Aspergillus niger* | $NO_3^-$ | 3.5 |
| | $NH_4^+$ | 20.0 |
| *Penicillium chrysogenum* | $NO_3^-$ | 7.5 |
| | $NH_4^+$ | 27.0 |
| *Trichoderma viride* | $NO_3^-$ | 36.0 |
| *Botrytis alii* | $NH_4^+$ | 23.5 |

The extracellular nitrogen was related to the nitrogen supplied in two cases:

TABLE IV

*Formation of Extracellular Nitrogen in Relation to Initially Added Nitrogen Which Disappeared*

| Fungus | Nitrogen Source | Amount of nitrogen supplied (mg./flask) | | |
|---|---|---|---|---|
| | | 6.6 | 13.2 | 6.6 + 6.6 |
| | | Extracellular N as % N assimilated | | |
| | $NH_4^-$ | 25.35 ± 5.58 | 25.80 ± 2.86 | |
| *Scopulariopsis brevicaulis* | $NO_3^-$ | 20.03 ± 4.47 | 16.62 ± 2.08 | 16.50 ± 1.98 |
| *Penicillium griseofulvum* | $NO_3^-$ | 13.00 ± 3.35 | 12.50 ± 0.70 | 12.34 ± 2.73 |

[6] M. Richards and R. H. Haskins, *Can. J. Microbiol.* 3 543 (1957).
[7] A. G. Morton and D. Broadbent, *J. Gen. Microbiol.* 12 248 (1955).

In this earlier study most of the extracellular nitrogen appeared to be peptide in nature, yielding some 14 amino acids on hydrolysis. In the one case tested one of the fungi was unable to use the extracellular nitrogen formed, but assimilated the constituent amino acids when these were liberated by acid hydrolysis.

A quantitative report has been made on the free amino acids present in an alcohol extract of *Mucor mucedo*.[8] They were as follows:

TABLE V

*Amino Acids Present in 75% Alcohol Extracts of Mucor mucedo (as % Total Nitrogen)*

| Amino ocid | Alcohol extract | Hydrolysate of insoluble residue |
|---|---|---|
| Alanine | 1.8 | 6.7 |
| β-Alanine | ... | ... |
| Arginine | 4.8 | 12.2 |
| Asparagine | 9.5 | ... |
| Aspartic acid | 22.8 | 16.4 |
| γ-Aminobutyric acid | 6.8 | ... |
| Citrulline | ... | ... |
| Cystine | ... | 2.2 |
| Glutamine | 15.2 | ... |
| Glutamic acid | 11.6 | 35.2 |
| Glycine | 1.9 | 3.8 |
| Histidine | ... | 1.6 |
| *iso*-Leucine | 1.9 | 0.7 |
| Leucine | 2.4 | 5.2 |
| Lysine | 3.7 | 3.8 |
| Methionine | 0.6 | 1.1 |
| Proline | 1.4 | 4.2 |
| Phenylalonine | 0.9 | 1.0 |
| Serine | 2.4 | 3.3 |
| Threonine | 1.1 | 2.2 |
| Tyrosine | 1.2 | 2.1 |
| Valine | 2.9 | 4.4 |

These values were compared with those of other plants over a taxonomic range.

A report of the free amino acids produced by *Penicillium roquefortii* indicated the following to be most prominent:[9]

[8] K. Mansford and R. Raper, *Nature* 174 314 (1954).

[9] J. Koloušek and S. Michalik, *Sbornik Československ. Akad. Zeměděl Ved.* 27A 281 (1954). (*Chem. Abstr.* 50 4295c)

| | |
|---|---|
| Aspartic Acid | Valine |
| Glutamic Acid | Methionine |
| Serine | Leucine |
| Threonine | Isoleucine |
| α-Alanine | |

The free and combined amino acids of the uredospores of ten wheat rust strains have been determined quantitatively.[10]

The intracellular amino acids of microorganisms have been studied. Gale demonstrated the presence of such a pool in *Streptococcus faecalis*.[11] Gale and Taylor extended the investigation to a variety of bacteria and yeasts with particular attention to lysine and glutamic acid.[12] Fuerst studied several fungi.[13] The free intracellular amino acids of certain strains of *Neurospora crassa* have been explored.[14] The relative quantities of amino acids present varied widely among the various mutants. In all some 35 ninhydrin-positive substances were encountered among the 28 different strains studied. The free amino acids of *Staphylococcus aureus* have been determined, and the ability of bacteria to concentrate amino acids strikingly demonstrated by comparison of the concentrations of internal and external acids.[15]

TABLE VI

*Free Amino Acids in Exponentially Growing Staphylococcus aureus Cells Growing in Synthetic Medium*

| Amino Acid | Quantity (μmole/g.) in internal pool | Ratio of internal to external concentration |
|---|---|---|
| Glutamic acid | 39.6 | 25.4 |
| Aspartic acid | 38 | 22.6 |
| Proline | 16.8 | 23.2 |
| Isoleucine | 8.3 | 13.3 |
| Leucine | 2.6 | 4.5 |
| Methionine | 6.7 | 8.3 |

[10] M. E. McKillican, *Can. J. Chem.* 38 244 (1960).

[11] E. F. Gale, *J. Gen. Microbiol.* 1 53 (1947).

[12] E. F. Gale and E. S. Taylor, *ibid.* 1 77 (1947); E. S. Taylor, *ibid.* 1 86 (1947).

[13] R. Fuerst and J. Awapara, *Texas Repts. Biol. and Med.* 10 424 (1952).

[14] Robert Fuerst and Robert P. Wagner, *Arch. Biochem. and Biophys.* 70 311 (1957).

[15] R. Hancock, *Biochim. et Biophys. Acta* 28 402 (1958).

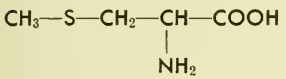
TABLE VI—Continued

*Free Amino Acids in Exponentially Growing Staphylococcus aureus Cells Growing in Synthetic Medium*

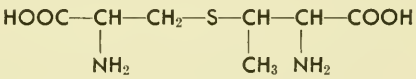
| Amino Acid | Quantity (μmole/g.) in internal pool | Ratio of internal to external concentration |
|---|---|---|
| Alanine | 8.1 | 5.4 |
| Cystine and cysteine | 5.5 | |
| Serine | 3.4 | 5.4 |
| Glycine | 2.8 | 2.3 |
| Tyrosine | 2.4 | 3.1 |
| Lysine | 2.2 | 4.6 |
| Arginine | 2.2 | 3.0 |
| Histidine | 1.7 | 2.2 |
| Phenylalanine | 1.3 | 1.8 |
| Threonine | 1.0 | 1.6 |
| Tryptophan | 0.3 | |

All the amino acids found in the internal protein of the cell were present in the internal pool of free amino acids.

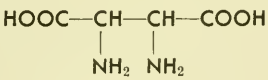
A new amino acid, S-methyl-L-cysteine, has been isolated from *Neurospora crassa*.[16] An isomer of β-methyllanthionine has been isolated from yeast.[17] Urocanic acid has been detected in *Micrococcus lysodeikticus*.[18]



S-Methylcysteine



β-Methyllanthionine



α,β-Diaminosuccinic Acid

New, partially characterized α-amino acids have been isolated from boletus and lactarius species.[19, 20, 21] α,β-Diaminosuccinic acid has been isolated from production filtrates of the antibiotic

[16] James B. Ragland and James L. Liverman, *Arch. Biochem. and Biophys.* 65 574 (1956).

[17] Phyllis F. Downey and Simon Black, *J. Biol. Chem.* 228 171 (1957).

[18] Jana Gregoire and Jean Gregoire, *Compt. rend.* 245 2553 (1957).

[19] A. I. Virtanen and O. Ayräpää, *Suomen Kem.* 31B 190 (1958).

[20] Atsushi Komamine and Artturi I. Virtanen, *Acta Chem. Scand.* 13 2141 (1959).

[21] J. Casimir and Artturi I. Virtanen, *ibid.* 13 2139 (1959).

oxytetracycline (*Streptomyces rimosus*).[22] The structures of certain other unusual amino acids are listed in this section.

Production of glutamic acid by streptomycetes on synthetic medium containing glycine has been investigated.[23] Yields of extracellular glutamic acid were 0.25–1.75 g. per liter. It was the only amino acid or nitrogenous material present after four and seven days, but after ten days some alanine, phenylalanine, aspartic acid and glycine appeared. Strains examined were: *Streptomyces annulatus, S. aureofaciens, S. fradiae, S. olivaceus* and *S. rimosus.*

The high proportions and amounts of L-glutamic acid synthesized by microorganisms have led to the development of an economical process for its commercial production. Certain micrococcus and bacillus species produce more than a 20% yield (molar basis) from the glucose supplied.[24] A similar yield of valine has been reported.[25]

L-Lysine is also produced commercially by a direct process (micrococcus, bacillus)[26] and by a two-stage process (*Escherichia coli, Aerobacter aerogenes*),[27] 2,6-diaminopimelic acid being the intermediate in the latter case.

Tryptophan production by *E. coli* and by *Salmonella typhi* has been reported as small unless indole is added.[28] The indole apparently competitively inhibited tryptophanase. Many microorganisms are able to synthesize tryptophan from indole and serine.

A survey of 20 genera, 72 species and 334 strains of aerobic bacteria for amino acid accumulation revealed no marked taxonomic difference except that facultative aerobes such as escherichia, aerobacter and bacillus species were superior to obligatory aerobes such as pseudomonas. Production and accumulation were more dependent on strain and conditions.[29]

The biosynthesis and metabolic interrelationships of amino acids can be considered here only in briefest summary because of the breadth and complexity of the subject. More thorough re-

[22] F. A. Hochstein, *J. Org. Chem.* 24 679 (1959).

[23] D. Perlman and E. O'Brien, *J. Bact.* 75 611 (1958).

[24] Toshinobu Asai, Ko Aida and Kunio Oishi, *Bull. Agr. Chem. Soc.* (Japan) 21 134 (1957).

[25] Zenjirô Sugisaki, *Nippon Nôgei-kagaku Kaishi* 34 153 (1960).

[26] Shukuo Kinoshita, Kiyoshi Nakayama and Sohei Kitada, *J. Gen. Appl. Microbiol.* 4 128 (1958).

[27] Lester E. Casida, Jr., U. S. Patent 2,711,396 (1956).

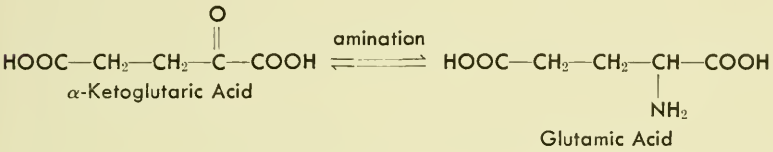
[28] P. Fildes, *J. Gen. Microbiol.* 15 636 (1956).

[29] Hiroshi Iizuka and Kazuo Komagata, *Nippon Nôgei-kagashu Kaishi* 34 27 (1960).

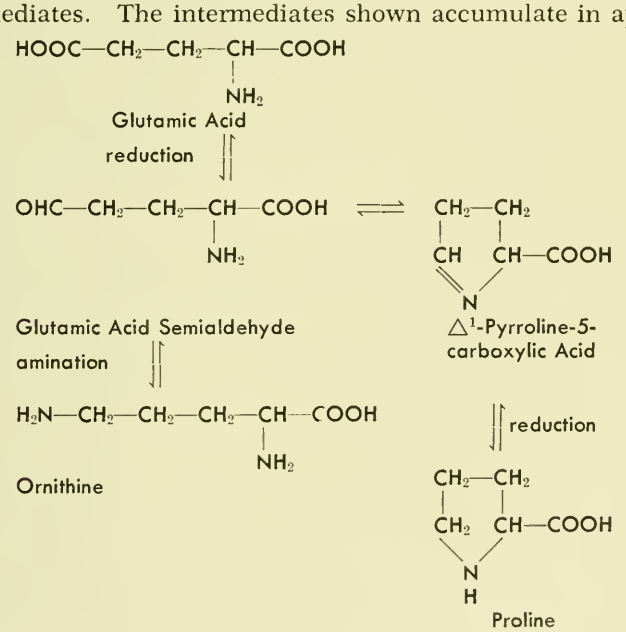
views are available[30, 31, 32] and references to some of the vast literature on this subject can be found there.

The occurrence studies cited demonstrate the importance of glutamic acid. It is a constituent of folic acid and related substances, and of glutathione, and various antibiotics. It occurs in the cell wall of bacteria and, as a polypeptide, is the sole capsular substance of certain bacilli. Its wide distribution reflects its cross-roads position in nitrogen metabolism.

Synthesis of glutamic acid by most aerobic microorganisms involves amination of α-ketoglutaric acid (a reversible reaction), thus tying it in with the citric acid cycle. It is a precursor of ornithine, proline and in some cases lysine.




In *E. coli,* at least, the route to ornithine involves N-acetylated intermediates. The intermediates shown accumulate in appro-



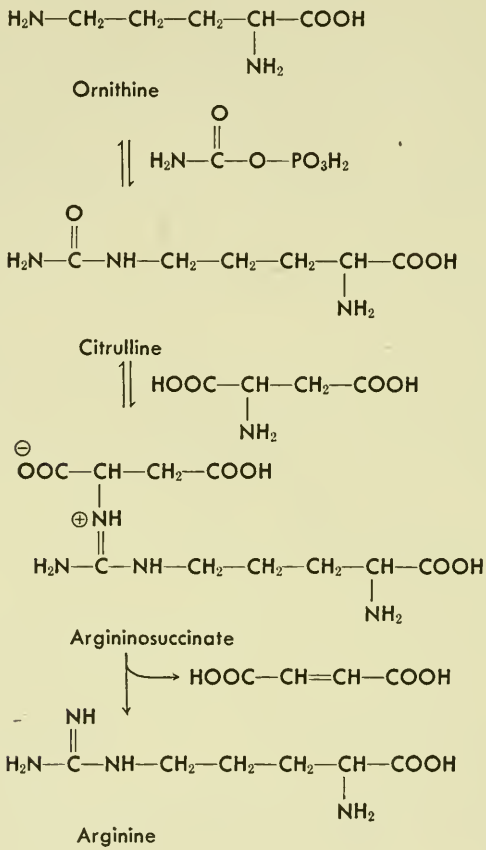

[30] Bernard D. Davis, *Advances in Enzymol.* 16 247–312 (1955).

[31] Alton Meister, "Biochemistry of the Amino Acids," Academic Press, New York, 1957, pp. 256–394.

[32] Joseph S. Fruton and Sofia Simmonds, "General Biochemistry," John Wiley and Sons, Inc., New York, 1958, pp. 771–896.

priate auxotrophs and can be isolated. This scheme has been found in a variety of molds, yeasts and bacteria.

Ornithine reacts with carbamyl phosphate to form citrulline, an intermediate in the biosynthesis of arginine:

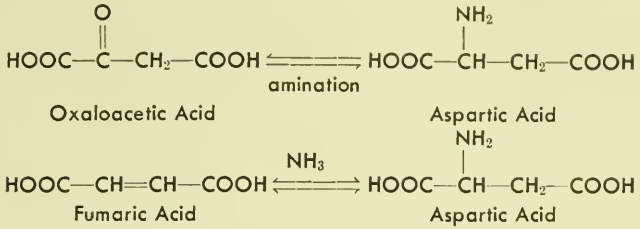



Arginine can complete the "urea cycle" by losing urea to form ornithine. Enzymes for all these steps have been found in various microorganisms.

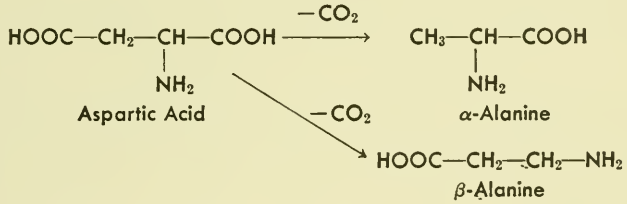
Glutamic acid acts as an ammonia carrier by formation of its half amide, glutamine, and in this way contributes nitrogen to the biosynthesis of purines and amino sugars.

Aspartic acid also occupies a central position in nitrogen metabolism. In microorganisms it can be synthesized either by amination of oxaloacetic acid or by the addition of ammonia

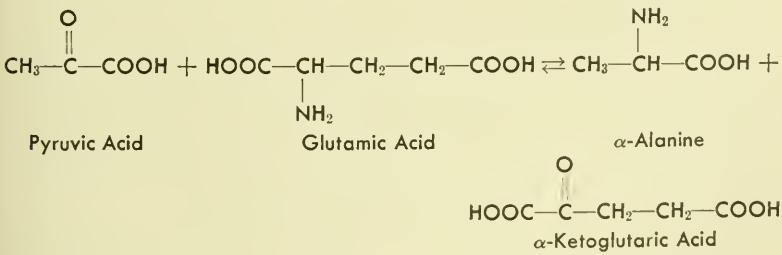
to fumaric acid, the former process probably being more prevalent.




Either equation ties aspartic acid in with the citric acid cycle. Like glutamic acid, aspartic acid acts as an ammonia carrier through its half amide, asparagine. One role of aspartic acid was seen above in the biosynthesis of arginine. Aspartic acid has been proved a precursor of pyrimidines in certain microorganisms. It is also a precursor of threonine and of both $\alpha$- and $\beta$-alanines. Separate enzymes control the selective decarboxylations to form the alanines.

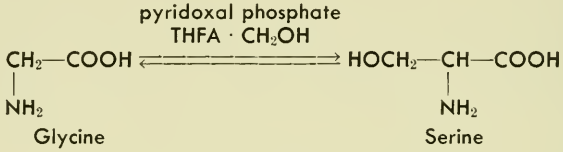


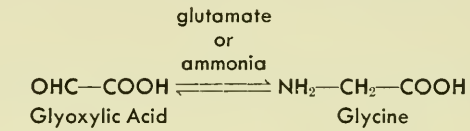

$\alpha$-Alanine (either isomer) can be synthesized, too, from pyruvic acid by a wide variety of biological systems. Some microorganisms effect this amination directly from ammonia, but the transamination from glutamate is probably more prevalent. Alanine, therefore, is also closely connected with carbohydrate and fat metabolism, and it is used as an energy source by many microbes. Through pyruvate it also may be considered a precursor of glycine, serine, cysteine and of valine, leucine and



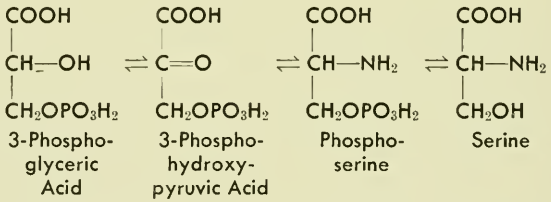
isoleucine. D-Alanine occurs in bacterial cell walls and spores and frequently in antibiotics. Some bacteria even require an exogenous source of D-alanine, particularly on a medium devoid of pyridoxine, since pyridoxal phosphate is a coenzyme for the racemase. $\beta$-Alanine is a component of coenzyme A. A related substance, $\beta$-nitropropionic acid has been isolated from an aspergillus species.

Glycine and serine are reversibly interconvertible in most organisms, tetrahydrofolic acid transferring the hydroxymethyl group. Glycine also is formed by amination of glyoxylate in some microorganisms.




In *E. coli* serine is probably to be regarded as the precursor of glycine. The origin of serine is still obscure. There is a possibility that it may arise from phosphoglyceric acid from the glycolysis scheme:

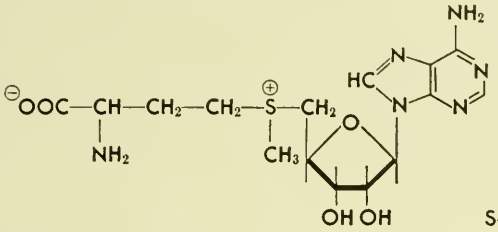



Glycine is a precursor of the porphyrins, purines, glutathione and sarcosine.

Serine contributes the carbon skeleton of cysteine in most organisms. Most microorganisms can use sulfate but not methionine as a sulfur source, while mammals require methionine for this purpose but cannot use sulfate. The conversion route of methionine to cysteine has been worked out for higher animals, but is not entirely understood in microorganisms.

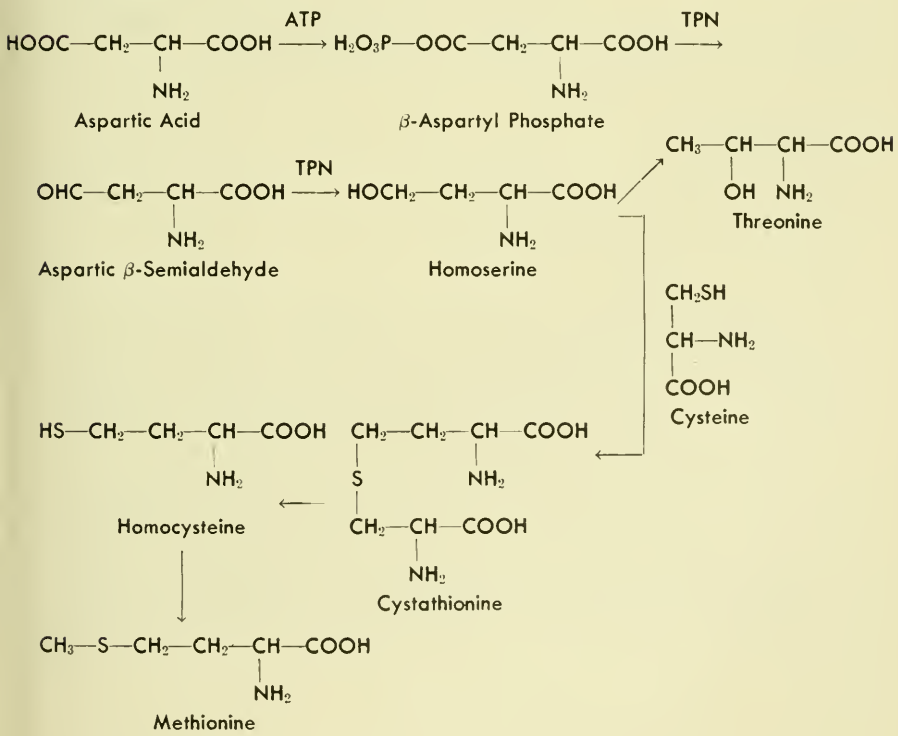
Thiosulfate is used by some molds, and cysteine-S-sulfonate has been found to be an intermediate. Hydrogen sulfide has been reported as a precursor in yeast. Threonine has been isolated as an intermediate to cysteine in a neurospora auxotroph.

Cysteine is a component of glutathione and of penicillin. Methionine is important in transmethylation reactions. The entire topic of one-carbon metabolism cannot be reviewed here. The transfer of methyl groups from methionine to oxygen and nitrogen atoms, and probably to carbon atoms in biosynthetic sequences requires ATP, and the active complex has been identified as S-adenosylmethionine.



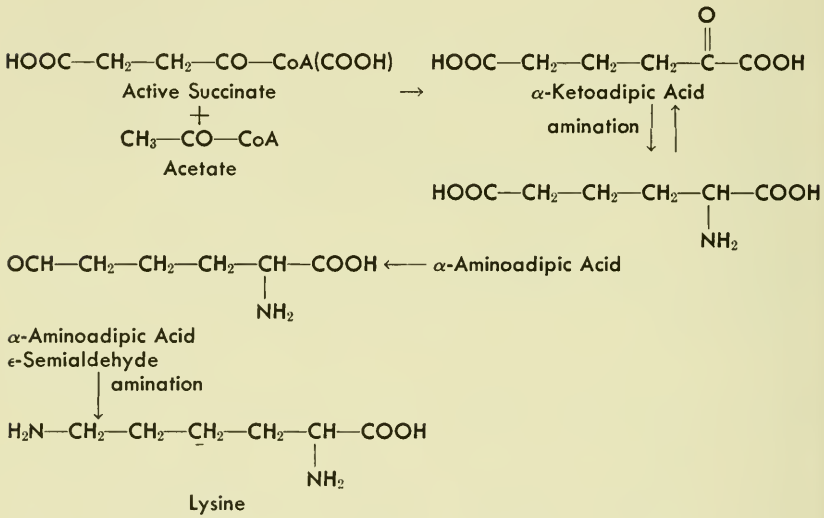
S-Adenosylmethionine

Several labile methyl group compounds (choline, betaine, serine) probably can contribute the methyl group of methionine by way of the proper coenzymes ($B_{12}$, THFA). Some neurospora mutants have been found which seem to synthesize methionine from cysteine and, ultimately, from aspartic acid. The following scheme has been suggested:



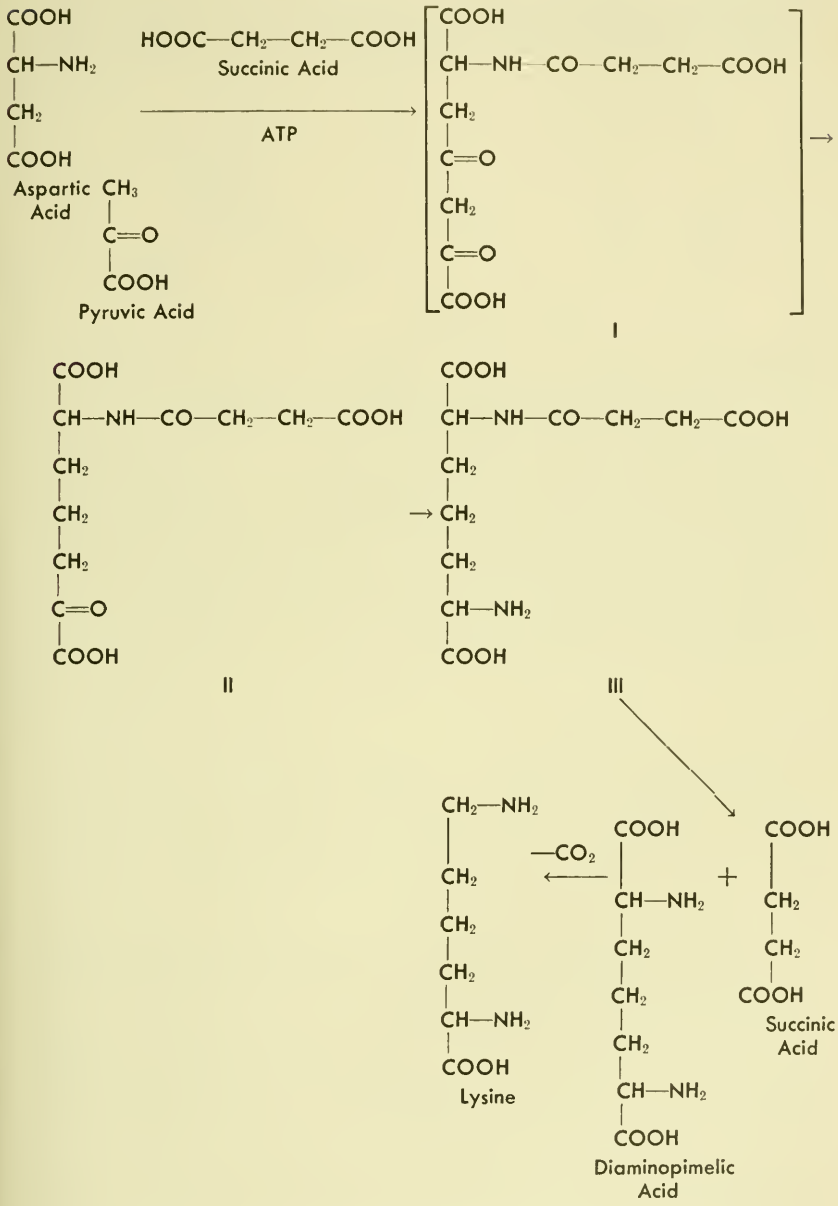
Homoserine also is a precursor of threonine in neurospora mutants, with ATP and pyridoxal phosphate required. Threonine is synthesized by most microorganisms although it is an essential in mammalian diets.

The fact that lysine-requiring neurospora mutants use α-aminoadipic acid makes probable a biosynthetic scheme in which the terminal carboxyl group is reduced and aminated as in the biosynthesis of ornithine from glutamic acid. Some molds even are able to use α-ketoadipic acid, which strengthens the argument. Labeling studies indicate formation of the α-ketoadipic acid by condensation of acetate with either α-ketoglutarate or with the "active succinate" from the citric acid cycle, the acetate carboxyl furnishing the carboxyl group of lysine. Proposed lysine biosynthesis in molds:



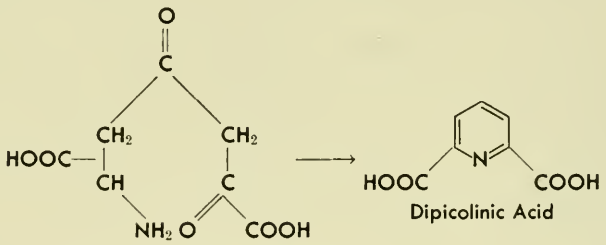

α-Aminoadipic acid is produced by *Penicillium chrysogenum* as a component of a tripeptide isolated from the mycelium. It also occurs as a moiety of the antibiotic synnematin-B (cephalosporin N) produced by the mold *Cephalosporium salmosynnematum,* and it has been isolated from *Aspergillus oryzae.*

α,ε-Diaminopimelic acid is a precursor of lysine in *E. coli* and in many other bacteria. L,L-Diaminopimelic acid is formed in *E. coli* by condensation of pyruvic acid with aspartic acid. Later a specific racemase converts it to the *meso*-form. A complete mechanism for lysine biosynthesis in bacteria has been proposed:




Intermediate III has been isolated and identified, and there is some evidence for the existence of II. Rather similar interme-

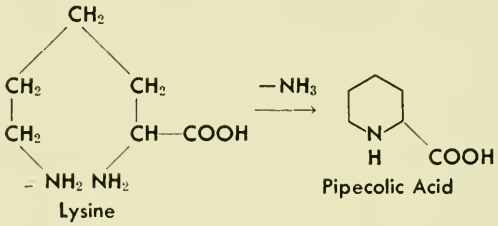
diates have been suggested as precursors of 2,6-dipicolinic acid, which is formed in some bacterial spores. Free diaminopimelic




acid has been isolated from vegetative cells of such spore-formers. It has never been found in yeasts and molds.

α,ε-Diaminopimelic acid replaces lysine in the repeating pentapeptide unit of the bacterial cell wall in *Corynebacterium diphtheriae*, *E. coli* and certain other bacteria (especially gram negatives). Some *E. coli* strains accumulate considerable quantities of diaminopimelic acid, and this faculty has been exploited in a two-step commercial production process.

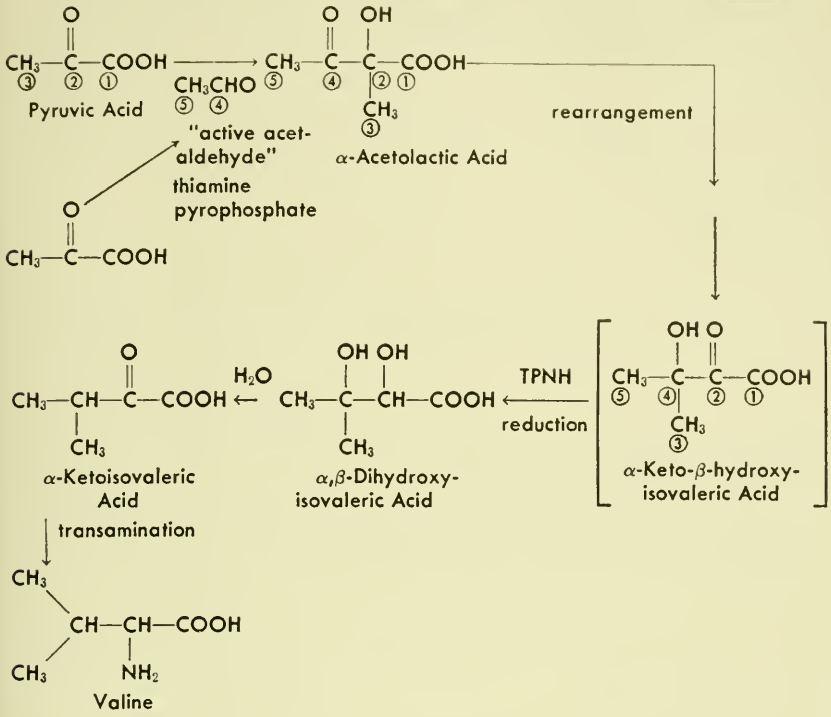
Many microorganisms metabolize lysine to pipecolic acid, a component of several antibiotics.




The amino acids discussed to date are closely integrated with carbohydrate and fat metabolism. Those remaining to be considered are more remotely derived.

Valine, isoleucine and leucine are essential to the mammalian diet and are required also by many microorganisms. This seems to indicate enzymatic difficulties in the biosynthesis of these branched-chain amino acids.

Much evidence has accumulated concerning the biosynthesis of valine and isoleucine, and the following pathway is indicated (for valine):

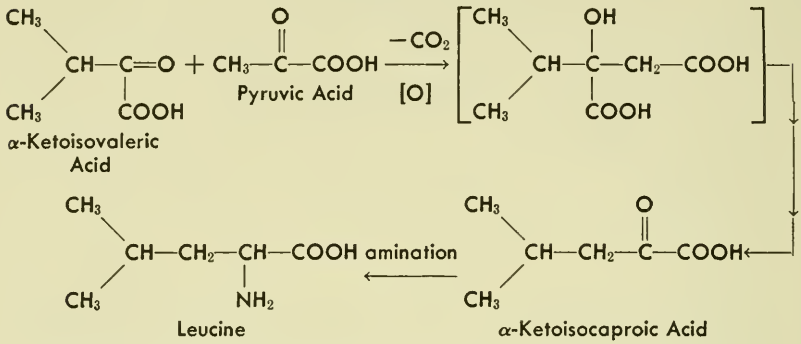



The intermediates, α-acetolactic acid and α,β-dihydroxyisovaleric acid, have been isolated from a variety of microorganisms and are well characterized. α-Keto-β-hydroxyisovaleric acid has not been reported yet, although when it is mixed with enzyme preparations from molds and yeasts together with TPNH, it is reduced to α,β-dihydroxyisovaleric acid. α-Ketoisovaleric acid is aminated by numerous microorganisms.

The scheme for isoleucine is believed to be analogous, but with α-ketobutyric acid replacing pyruvic acid as the initial substance. This four-carbon acid is, in turn, derived from homoserine or threonine, and ultimately from aspartic acid. Some of the steps of the valine and isoleucine syntheses are known to share common enzymes.

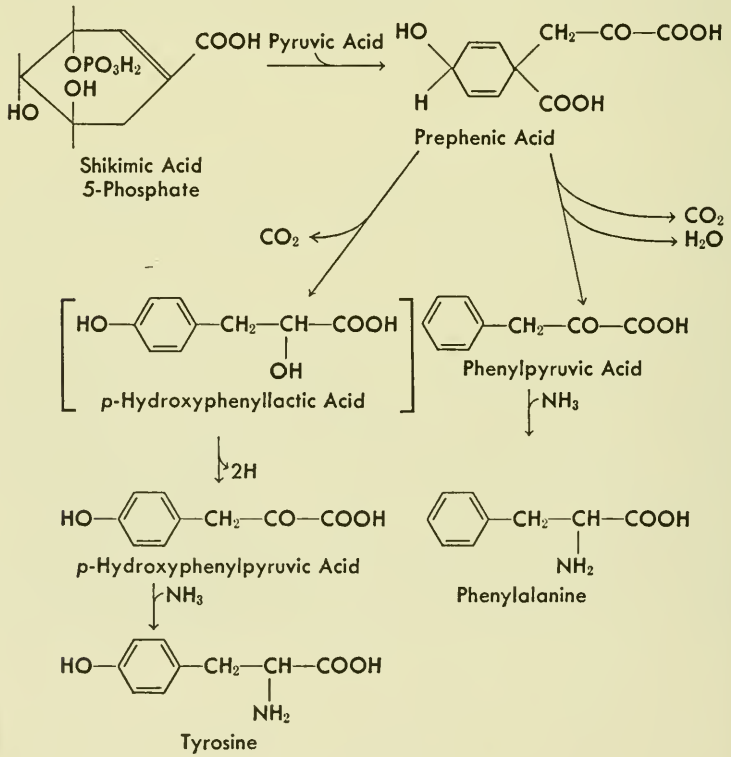
Leucine biosynthesis is apparently the same as that of valine up to the final amination step. Leucine, however, requires 3 moles of pyruvate for its 6-carbon atom chain rather than the

2 required by valine. The remaining steps of the proposed leucine biosynthesis in microorganisms are:

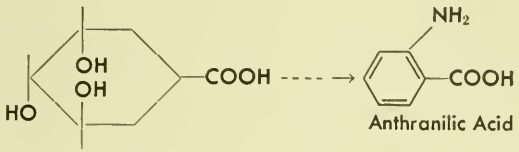



This partial scheme is based on labeled media experiments in yeasts, molds and bacteria.

The biogenetic scheme of the aromatic amino acids phenylalanine and tyrosine was briefly outlined in the introduction to the section on simpler alicyclic compounds. The final stages of this route are shown here, beginning with shikimic acid:

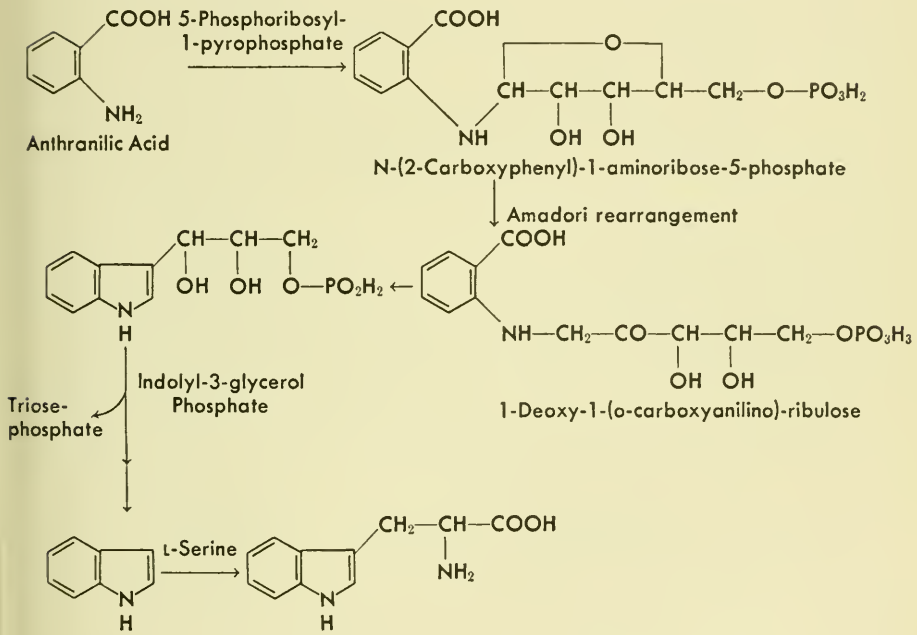


The benzene ring of tryptophan also arises from the shikimic acid route. The intermediates are unknown between shikimic acid and the first aromatic member of the sequence, anthranilic acid:




Shikimic Acid

The remainder of the sequence in its present state of development is as follows:

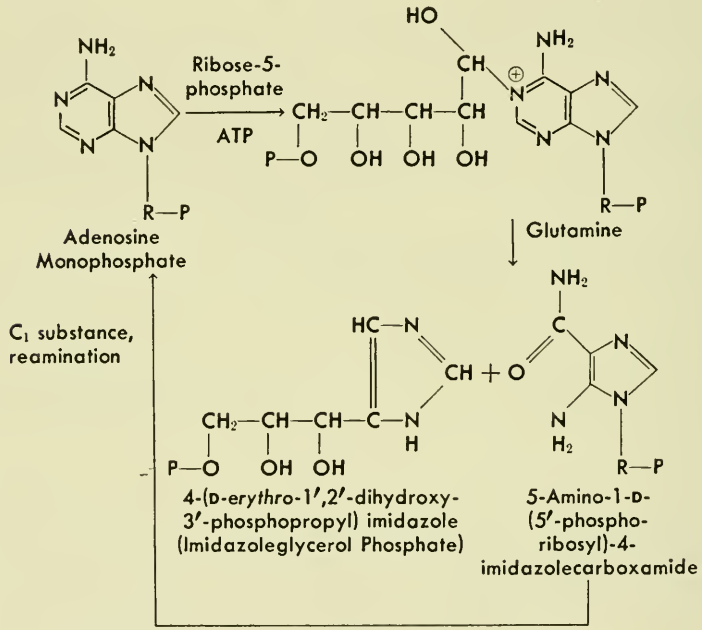



There appears to be some question as to whether the Amadori rearrangement product is a bona fide member of this sequence. It has been isolated from *Aerobacter aerogenes* and characterized as derivatives, and it substitutes for anthranilic acid in bacterial mutants requiring the latter.

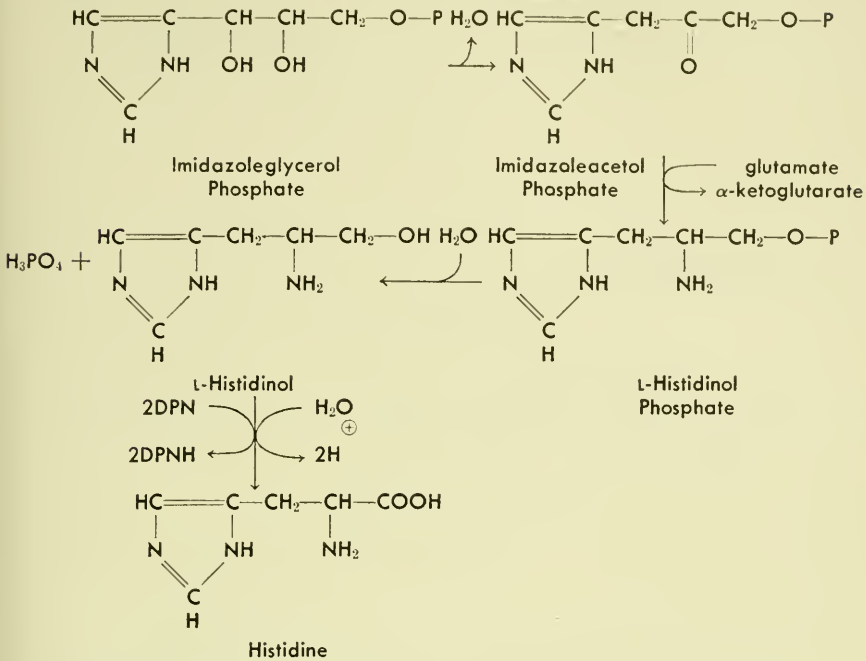
The anthranilic acid carboxyl group is known to be lost as carbon dioxide during the formation of the pyrrole ring, and the first two carbon atoms of ribose are known to form the 2 and 3 positions of the indole ring. Glucose also can furnish these two carbon atoms. In this connection it should be men-

tioned that N-fructosylanthranilic acid has been isolated from a yeast. Probably indole never exists in the free state to any appreciable extent during the tryptophan synthesis, but is enzyme-bound.

Ribose contributes also to the biosynthesis of histidine. Here purines are catalytic, furnishing a carbon atom and a nitrogen atom from the pyrimidine ring to form positions 2 and 3 of the histidine ring. The purine is then regenerated by reaction with a $C_1$ substance. Adenine is the most efficient purine for this purpose. The following scheme has been worked out, largely on the basis of auxotroph work: (P = phosphate, R = ribose).




Some chemicals which inhibit purine synthesis also cause accumulation of such intermediates. To continue with the biosynthesis of histidine:




It is interesting that the final stages of this synthesis differ from those in the tryptophan sequence when some of the intermediates are so closely related. Perhaps in some species a lesser difference will be found.

Histidine is converted to ergothioneine in microorganisms by methylation to form hercynine, followed by direct introduction of the thiol group.

663 **Glycine,** $C_2H_5O_2N$, colorless crystals, m.p. ~280–290° (dec.) (rapid heating).

$H_2N—CH_2—COOH$

Widely distributed.

664 **Sarcosine,** $C_3H_7O_2N$, colorless crystals, m.p. 212° (dec.).

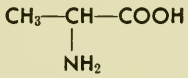
$CH_3NHCH_2COOH$

*Cladonia sylvatica*

Also a component of the actinomycin antibiotics.

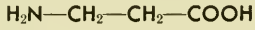
P. Linko, M. Alfthan, J. K. Miettinen and Artturi I. Virtanen, *Acta Chem. Scand.* 7 1310 (1953).

665 **L-Alanine,** $C_3H_7O_2N$, colorless crystals, m.p. 297° (dec.), $[\alpha]_D^{26}$ +8.5° (9.3% solution of the hydrochloride in water).



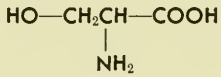
Widely distributed.

666 **β-Alanine,** $C_3H_7O_2N$, colorless crystals, m.p. 207° (dec.) (preheated bath).



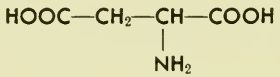
Widely distributed.

667 **L-Serine,** $C_3H_7O_3N$, colorless crystals, m.p. 228° (dec.) (sublimes 150° at $10^{-4}$ mm. Hg), $[\alpha]_D^{25}$ +14.45° (0.5 g. per 5.6 g. of 1 N hydrochloric acid).



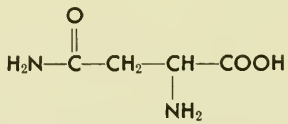
Widely distributed.

668 **L-Aspartic Acid,** $C_4H_7O_4N$, colorless crystals, m.p. 270° (sealed capillary, preheated bath) (dec.), $[\alpha]_D^{24}$ +24.6° (c 2 in 6 N hydrochloric acid).



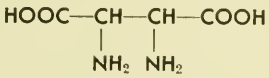
Widely distributed.

669 **L-Asparagine,** $C_4H_8O_3N_2$, colorless crystals (Monohydrate), m.p. 234°, (dec.) (preheated bath), $[\alpha]_D^{20}$ −5.5° (c 1.3 in water).



Widely distributed.

670 *d*-**Diaminosuccinic Acid,** $C_4H_8O_4N_2$, colorless crystals, m.p. (dec.), 240–290°, $[\alpha]_D^{25}$ +28° (c 2.0 in 5% sodium hydroxide solution).

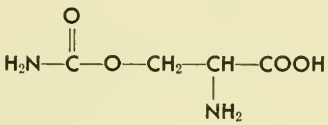


*Streptomyces rimosus*

This amino acid sometimes crystallizes from oxytetracycline broth concentrates. The yield is about 250–500 mg. per liter.

F. A. Hochstein, *J. Org. Chem.* 24 679 (1959).

671 **O-Carbamyl-D-serine,** $C_4H_8O_4N_2$, colorless needles, m.p. 226–234° (dec.), $[\alpha]_D$ −19.6° (c 2 in N hydrochloric acid).

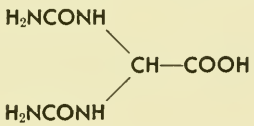


*Streptomyces polychromogenes*

D-Serine or derivatives is also present in polymyxin, echinomycin, cycloserine and amicetin.

G. Hagemann, L. Pénasse and J. Teillon, *Biochim. et Biophys. Acta* 17 240 (1955).

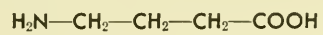
672 **Allantoic Acid,** $C_4H_8O_4N_4$, colorless needles, m.p. 165° (dec.).



*Coprinus miraceus, Collybia dryophila*

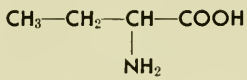
R. Fosse and A. Brunel, *Compt. rend.* 197 288 (1933).

673 γ-**Aminobutyric Acid,** $C_4H_9O_2N$, colorless crystals, m.p. 202° (dec.) rapid heating.



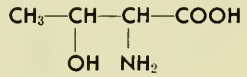
Widely distributed.

674 L-(+)-α-Aminobutyric Acid, $C_4H_9O_2N$, colorless crystals, m.p. 270–280° (dec.), $[\alpha]_D^{20}$ +8.0° (c 1.0 in water).



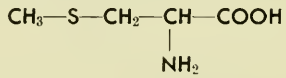
*Escherichia coli, Corynebacterium diphtheriae*
A. Polson, *Nature* 161 351 (1948).
Elizabeth Work, *Biochim. et Biophys. Acta* 3 400 (1949).

675 L-Threonine, $C_4H_9O_3N$, colorless crystals, m.p. 255–257° (dec.), $[\alpha]_D^{26}$ −28.3° (c 1.1 in water).



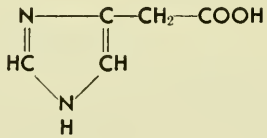
Widely distributed.

676 S-Methyl-L-cysteine, $C_4H_9O_2NS$, colorless crystals, m.p. ~164° (dec.), $[\alpha]_D^{25}$ +125° (c 2.5 in water).



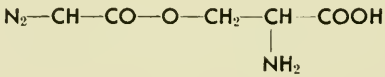
*Neurospora crassa*
James B. Ragland and James L. Livermore, *Arch. Biochem. and Biophys.* 65 574 (1956). (Isolation from neurospora)
Clayton J. Morris and John P. Thompson, *J. Am. Chem. Soc.* 78 1605 (1956). (Physical properties)

677 4-Imidazolyacetic Acid, $C_5H_6O_2N_2$, colorless needles (Hydrate), m.p. 222° (dec.).



*Polyporus sulfureus*
P. H. List, *Planta Med.* 6 424 (1958).

678 Azaserine (Diazoacetyl-L-serine), $C_5H_7O_4N_3$, light yellow-green crystals, dec. 146–162°, $[\alpha]_D^{27.5}$ −0.5° (c 8.46 in water at pH 5.18).



An unclassified streptomycete

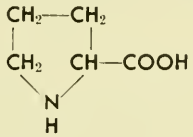
James A. Moore, John R. Dice, Ernest D. Nicolaides, Roger D. Westland and Eugene L. Wittle, *J. Am. Chem. Soc.* 76 2884 (1954). (Synthesis)

C. Chester Stock, H. Christine Reilly, Sonja M. Buckley, Donald A. Clarke and C. P. Rhoads, *Nature* 173 71 (1954).

John Ehrlich, Lucia E. Anderson, George L. Coffey, Arthur B. Hillegas, Mildred P. Knudsen, Harold J. Koepsell, Dorothy L. Kohberger and Julian E. Oyaas, *ibid.* 173 72 (1954).

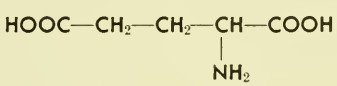
Quentin R. Bartz, Carole C. Elder, Roger P. Frohardt, Salvatore A. Fusari, Theodore H. Haskell, Doris W. Johannessen and Albert Ryder, *ibid.* 173 72 (1954). (Isolation)

679 **L-Proline,** $C_5H_9O_2N$, colorless crystals, m.p. 220–222° (dec.) (rapid heating), $[\alpha]_D^{25}$ −80° (c 1.0 in water).



Widely distributed.

680 **L-Glutamic Acid,** $C_5H_9O_4N$, colorless crystals, m.p. 247° (dec.), $[\alpha]_D^{22.4}$ +31.4° (c 1 in 6 N hydrochloric acid).

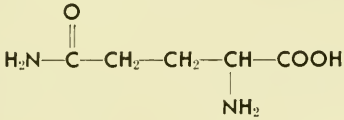


*Micrococcus varians*

A 17% molar yield (from glucose) was reported.

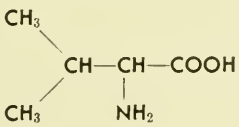
Toshinobu Asai, Ko Aida, Kunio Oishi, *Bull. Agr. Chem. Soc.* (Japan) 21 134 (1957).

681 **L-Glutamine,** $C_5H_{10}O_3N_2$, colorless crystals, m.p. 185° (dec.), $[\alpha]_D^{25}$ +5.9° (c 4.0 in water).



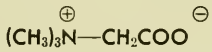
Widely distributed.

682 **L-Valine,** $C_5H_{11}O_2N$, colorless crystals, m.p. 315° (dec.) (closed capillary). Sublimes, $[\alpha]_D^{26}$ +14° (c 0.9 in water).



Widely distributed.

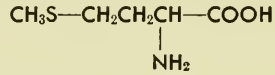
683 **Betaine**, $C_5H_{11}O_2N$, white prisms or leaflets, m.p. 293° (dec.).



*Aspergillus oryzae, Patella vulgata, Claviceps purpurea* (Fries) Tul. and other fungi

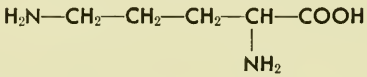
Jacqueline Etiènne-Petitfils, *Bull. soc. chim. biol.* 38 1315 (1956).

684 **L-Methionine**, $C_5H_{11}O_2NS$, colorless crystals, m.p. ~280° (dec.) (sealed capillary), $[\alpha]_D^{25}$ −8° (c 1.0 in water).



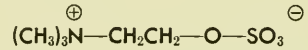
Widely distributed.

685 **L-Ornithine**, $C_5H_{12}O_2N_2$, colorless crystals, m.p. 140° (subl. 120°), $[\alpha]_D^{25}$ +12° (c 6.5 in water).



Widely distributed.

686 **Choline Sulfate**, $C_5H_{13}O_4NS$



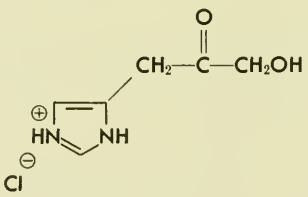
*Aspergillus sydowi, Penicillium chrysogenum,* lichens, yeasts

Choline yields of 6000–7000 μg. per gram of dry cell weight are available in certain Distillers' Dried Solubles.

D. W. Woolley and W. H. Peterson, *J. Biol. Chem.* 122 213 (1937).

J. deFlines, *J. Am. Chem. Soc.* 77 1676 (1955).

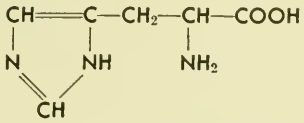
687 **Imidazoleacetol (Hydrochloride)**, $C_6H_8O_2N_2 \cdot HCl$, white needles, m.p. 171–174° (dec.).



*Neuorspora crassa* and *E. coli* mutants

Bruce N. Ames, Herschel K. Mitchell and Mary B. Mitchell, *J. Am. Chem. Soc.* 75 1015 (1953).

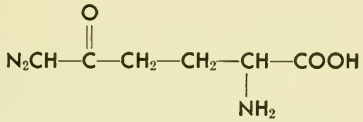
688 **L-Histidine,** $C_6H_9O_2N_3$, colorless crystals, m.p. 287° (dec.), $[\alpha]_D^{20}$ −39.7° (c 1.13 in water).



*Claviceps purpurea* (Fries) Tul.

H. Heath and Jennifer Wildy, *Biochem. J.* 64 612 (1956).

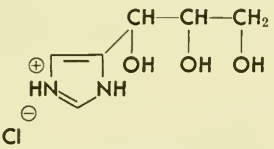
689 **6-Diazo-5-oxo-L-norleucine** (DON), $C_6H_9O_3N_3$, pale greenish yellow crystals, m.p. 145–155° (dec.), $[\alpha]_D^{26}$ +21° (c 5.4 in water).



An unclassified streptomycete

Henry W. Dion, Salvatore A. Fusari, Zbigniew L. Jakubowski, John G. Zora and Quentin R. Bartz, *J. Am. Chem. Soc.* 78 3075 (1956). (Isolation and characterization)

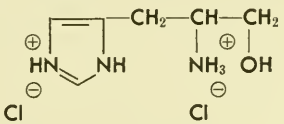
690 **Imidazoleglycerol** (Hydrochloride), $C_6H_{10}O_3N_2 \cdot HCl$, colorless crystals, m.p. 103° (dec.), $[\alpha]_D^{25.6}$ +13.3° (c 7.5 in water).



*Neurospora crassa* mutant

Bruce N. Ames and Herschel K. Mitchell, *J. Biol. Chem.* 212 687 (1955).

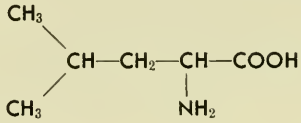
691 **L-Histidinol** (Hydrochloride), $C_6H_{11}ON_3 \cdot 2HCl$, colorless crystals, m.p. 194° (dec.).



*E. coli* mutant

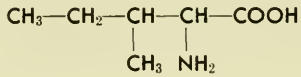
Henry J. Vogel, Bernard D. Davis and Elizabeth S. Mingioli, *J. Am. Chem. Soc.* 73 1897 (1951).

692 **L-Leucine,** $C_6H_{13}O_2N$, colorless crystals, m.p. ~295° (dec.) (sealed tube) (subl. from 140°), $[\alpha]_D^{25}$ −11° (c 2.0 in water).



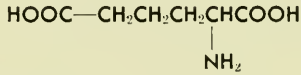
Widely distributed.

693 **L-Isoleucine,** $C_6H_{13}O_2N$, colorless crystals, m.p. 284° (dec.) (subl. from 160°), $[\alpha]_D^{20}$ +11° (c 3.0 in water).



Widely distributed.

694 **L-α-Aminoadipic Acid,** $C_6H_{11}O_4N$, white crystals, m.p. 206° (dec.).

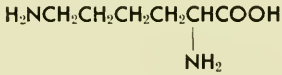


*Aspergillus oryzae*

Also a component of several antibiotics.

Emmanuel Windsor, *J. Biol. Chem.* 192 595 (1951).

695 **L-Lysine,** $C_6H_{14}O_2N_2$, white needles, m.p. 224°.

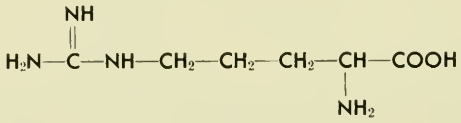


*Ustilāgo maydis* PRL 1092

The yield was 200–300 mg. per liter of free lysine in the broth as determined by a bioassay (not isolated).

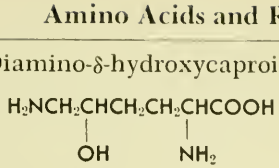
M. Richards and R. H. Haskins, *Can. J. Microbiol.* 3 543 (1957).

696 **L-Arginine,** $C_6H_{14}O_2N_4$, colorless crystals (Dihydrate), m.p. 245° (dec.) (browning above 200°), $[\alpha]_D^{20}$ +13° (c 3.5 in water).



Widely distributed.

697 **δ-Oxy-L-lysine** (α,ε-Diamino-δ-hydroxycaproic acid), $C_6H_{14}O_3N_2$.

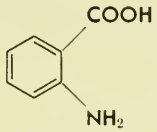


*Mycobacterium phlei*

Occurs bound in a phosphatide (yellow powder, m.p. 180–190°), molecular weight about 16,000. It is the sole amino acid, and constitutes about 1% of the phosphatide.

M. Barbier and E. Lederer, *Biochim. et Biophys. Acta* 8 590 (1952).

698 **Anthranilic Acid,** $C_7H_7O_2N$, leaflets, m.p. 144°.



*Corynebacterium diphtheriae*

Detected by paper chromatography.

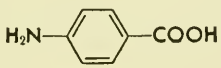
A. J. Woiwood and F. V. Linggood, *Intern. Congr. Biochem., Abstrs. of Communs.* 1st Congr., Cambridge, England, 320 (1949).

Anthranilic acid has been isolated also from a pseudomonas culture:

Rokuro Takeda and I. Nakanishi, *J. Fermentation Technol.* 37 No. 2 (1959).

It also accumulates in certain bacterial auxotrophs.

699 ***p*-Aminobenzoic Acid,** $C_7H_7O_2N$, yellowish red crystals, m.p. 186°.

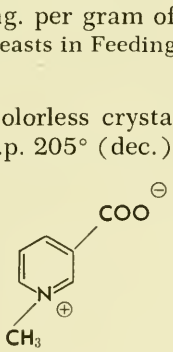


*Hansenula anomala, Mycotorula lipolytica*

Yields about 1 mg. per gram of dry cells.

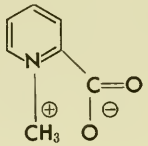
W. H. Peterson, "Yeasts in Feeding" Symposium, Milwaukee, 1948.

700 **Trigonelline,** $C_7H_7O_2N$, colorless crystals, m.p. (anhyd.) 218° (dec.) (Picrate) m.p. 205° (dec.).



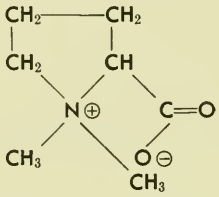
*Polyporus sulfureus*
P. H. List, *Planta Med.* 6 424 (1958).

701 **Homarine,** $C_7H_7O_2N$ (Hydrochloride), m.p. 170–175° (dec.) (Picrate) m.p. 155–160°.



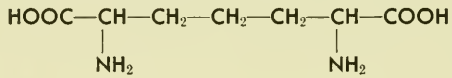
*Polyporus sulfureus*
P. H. List, *Planta Med.* 6 424 (1958).

702 **Stachydrine,** $C_7H_{13}O_2N$, white monohydrated crystals, m.p. (anhydr.) 235° (dec.).



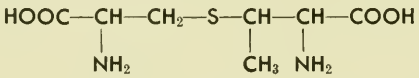
*Aspergillus oryzae,* other fungi (in small yields)
R. Takata, *J. Soc. Chem. Ind. Japan* 32 155B (1929).

703 **2,6-Diaminopimelic Acid** (Both L,L- and *meso* forms occur naturally), $C_7H_{14}O_4N_2$, colorless needles, m.p. >305°.



*Corynebacterium diphtheriae, Mycobacterium tuberculosis, Bacillus anthracis, E. coli* mutants
Elizabeth Work, *Biochem. J.* 49 17 (1951).
H. Smith, R. E. Strange and H. T. Zwartouw, *Nature* 178 865 (1956).
Lester E. Casida, Jr., U. S. Patent 2,771,396 (1956).

704 **β-Methyllanthionine,** $C_7H_{14}O_4N_2S$, $[\alpha]_D^{20}$ +37.6° (c 0.5 in 1 N hydrochloric acid).

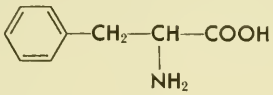


Yeast
This isomer is not the same as the one isolated from

antibiotic hydrolysates. Desulfurization with Raney nickel yields L-alanine and D-α-amino-*n*-butyric acid.

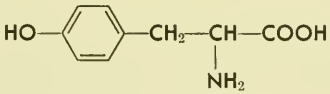
Phyllis F. Downey and Simon Black, *J. Biol. Chem.* 228 171 (1957).

705 **L-Phenylalanine,** $C_9H_{11}O_2N$, colorless crystals, m.p. 283° (dec.) (rapid heating), $[\alpha]_D^{20}$ −35° (c 2 in water).



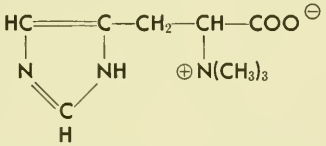
Widely distributed.

706 **L-Tyrosine,** $C_9H_{11}O_3N$, colorless crystals, m.p. 342–344° (sealed capillary, preheated bath) (dec.), $[\alpha]_D^{22}$ −10.6° (c 4 in 1 N hydrochloric acid).



Widely distributed.

707 **Hercynine** (Histidine Betaine), $C_9H_{15}O_2N_3$, white crystals, no sharp m.p., forms mono- and dipicrates.



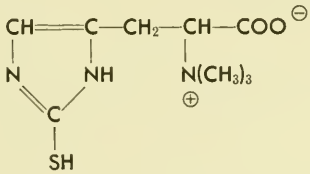
*Amanita muscaria, Agaricus campestris, Boletus edulis* Bull., *Polyporus sulfureus*

Fr. Kutscher, *Zentr. Physiol.* 24 775 (1910).

R. Engeland and F. Kutscher, *ibid.* 26 569 (1912). (Synthesis)

Albert Küng, *Z. physiol. Chem.* 91 241 (1914).

708 **Ergothioneine,** $C_9H_{15}O_2N_3S$, colorless crystals, m.p. 290° (dec.), $[\alpha]_D$ +116.5°.



*Claviceps purpurea* (Fries), Tul. *Coprinus comatus, Mycobacterium tuberculosis*

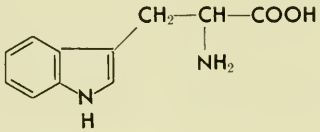
C. Tanret, *J. pharm. chim.* **30** 145 (1909).

H. Heath and Jennifer Wildy, *Biochem. J.* **64** 612 (1956). (Biosynthesis)

Paul Heinz List, *Arch. Pharm.* **290** 517 (1957).

Dorothy S. Genghof, *Bact. Proc.*, 190 (1960).

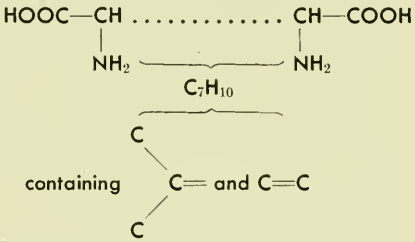
709 L-Tryptophan, $C_{11}H_{12}O_2N_2$, colorless crystals, m.p. 289° (dec.) (rapid heating), $[\alpha]_D^{23}$ −31.5 (c 1.0 in water).




Widely distributed.

710 **Amino Acid from *Lactarius helvus*,** $C_{11}H_{18}O_4N_2$, colorless crystals, yellowing near 200° and darkening to 300°. Molecular weight 251 by isothermal distillation. Adds 2 $H_2$ and 2 $Br_2$.

Partial structure:

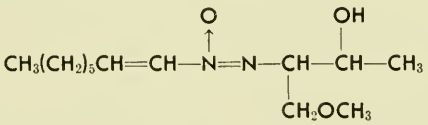



*Lactarius helvus*

Ateushi Komamine and Artturi Virtanen, *Acta Chem. Scand.* **13** 2141 (1959).

J. Casimir and A. I. Virtanen, *ibid.* **13** 2139 (1959). (Isolation)

711 Elaiomycin, $C_{13}H_{26}O_3N_2$, pale yellow oil, $[\alpha]_D^{26}$ +38.4° (c 2.8 in absolute ethanol).




*Streptomyces hepaticus*

Theodore H. Haskell, Albert Ryder and Quentin R. Bartz, *Antibiotics and Chemotherapy* 4 141 (1954). (Isolation)

John Ehrlich, Lucia E. Anderson, George L. Coffey, William H. Feldman, Myron W. Fisher, Arther B. Hillegas, Alfred G. Karlson, Mildred P. Knudsen, Jean K. Weston, Anne S. Youmans and Guy P. Youmans, *ibid.* 4 338 (1954).

C. L. Stevens, B. T. Gillis, J. C. French and T. H. Haskell, *J. Am. Chem. Soc.* 78 3229 (1956). (Structure)

17

# Polypeptides and Related Compounds

Polypeptides are often intractable, difficultly crystallizable substances. The newer techniques of chromatography, end-group analysis and electrophoresis have facilitated their investigation.

Most of the polypeptides and related compounds listed in this section are antibiotic isolates. Antibiosis may be a primary or only a secondary function of these materials. Polypeptides, of course, have hormonal and other functions in higher animals. Among microorganisms streptomycetes and bacteria have been the richest sources so far, perhaps in part because they have been examined more extensively for antibiotic activity than other microorganisms.

Special types of polypeptides have been isolated from bacterial cell walls by fragmentation with lysozyme or bacteriophage. They also tend to accumulate when bacteria are inhibited by certain antibiotics. Determination of their structures is beginning to elucidate the nature of the bacterial cell wall as well as the mode of action of the antibiotics involved.

Some attention has been given to intracellular peptides, principally in connection with their role in protein synthesis. The fundamental process of polypeptide and protein biosynthesis is just beginning to yield some of its secrets. Before discussing it, some earlier work on simpler polypeptide biosynthesis will be reviewed.

Glutathione is a widely distributed tripeptide which has a rapid metabolic turnover in yeast and also in mammalian tissues. Partly for this reason it has been suggested as an intermediate in protein biosynthesis, but because of its reversible oxidation-reduction properties, a respiratory role also has been proposed. In fact, it has not been proved satisfactorily that polypeptides serve as direct precursors for protein synthesis in

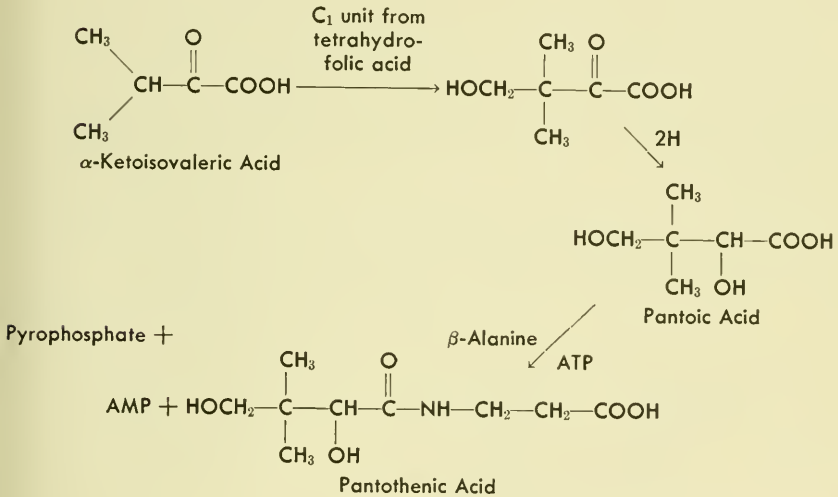
microorganisms, although strepogenins (glutamic acid containing oligopeptides from the enzymic digests of certain proteins) stimulate the growth of some bacteria. There is evidence for the occurrence of independent uptake mechanisms for glycine and glycine peptides in *Lactobacillus casei*.[1]

Glutathione formation takes place in two separate reactions, each involving ATP:[2]

(1) L-Glutamic Acid + L-Cysteine + ATP→
L-γ-Glutamylcysteine + ADP + $H_3PO_4$.

(2) L-γ-Glutamylcysteine + Glycine + ATP→
L-Glutathione + ADP + $H_3PO_4$.

The biosynthesis of pantothenic acid probably proceeds as follows, the last step also being coupled with ATP cleavage, but with different products:[3, 4]



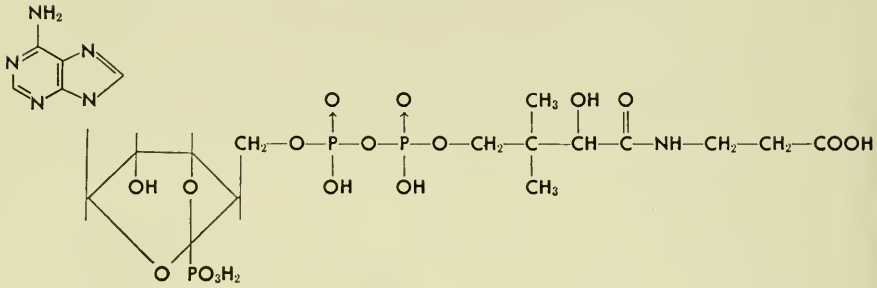

[1] Franklin R. Leach and Esmond E. Snell, *Biochim. et Biophys. Acta* 34 292 (1959).

[2] John E. Snoke and Konrad Bloch, *J. Biol. Chem.* 199 407 (1952); John E. Snoke, *ibid.* 213 813 (1955); John E. Snoke and Konrad Bloch, *ibid.* 213 825 (1955); Stanley Mandeles and Konrad Bloch, *ibid.* 214 639 (1955).

[3] Werner K. Maas and Henry J. Vogel, *J. Bacteriol.* 65 388 (1953); M. Purko, W. O. Nelson and W. A. Wood, *J. Biol. Chem.* 207 51 (1954); E. Nelson McIntosh, M. Purko and W. A. Wood, *ibid.*, 228 499 (1957).

[4] Werner K. Maas, *J. Biol. Chem.* 198 23 (1952); Akira Matsuyama, *Bull. Agr. Chem. Soc.* (Japan) 21 47 (1957) and earlier papers in this series; Herbert S. Ginoza and Robert A. Altenbern, *Arch. Biochem. and Biophys.* 56 537 (1955).

It appears that an adenylic acid-pantoate complex is the intermediate which couples with the enzyme.



Pantothenylcysteine is a precursor of pantetheine in *Lactobacillus helveticus*.[5]

The red actinomycins were the first antibiotics isolated crystalline from actinomycetes.[6] In the ensuing 20 years about a dozen named species of streptomycetes have been found to produce actinomycins, and probably many other isolates have gone unreported.

TABLE I

*Chronological List of Microorganisms Reported to Produce Actinomycins**

| Year reported | Name given to microorganism | Actinomycin complex† |
|---|---|---|
| 1941 | *Streptomyces antibioticus* | A |
| 1946 | Non-chromogenic species | A |
| 1947 | *S. flavus* | A (J) |
| 1948 | *S. parvus* | A |
| | *S. flavovirens* | — |
| 1949 | *Streptomyces* sp. | B |
| | *S. chrysomallus* | C |
| 1951 | *S. flaveolus* | A (J) |
| | *Micromonospora globosa* | — |
| 1952 | *Streptomyces* sp. | X |

[5] Gene M. Brown, *J. Biol. Chem.* 226 651 (1957).

[6] S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exp. Biol.* 45 609 (1940).

TABLE 1—Continued

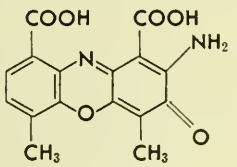
| Year reported | Name given to microorganism | Actinomycin complex† |
|---|---|---|
| 1954 | *S. flavus* | X (B) |
| | *S. flavus* | X |
| | *S. antibioticus* | X |
| | *S. flavus-parvus* | X (B) |
| | *S. parvullus* | D |
| | *S. cellulosae* | — |
| | *S. michiganensis* | X |
| | *S. antibioticus* | M |
| 1956 | *Streptomyces* sp. | E |
| | *Streptomyces* sp. | F |
| 1958 | *S. fradiae* | Z, X |

* By courtesy of Dr. H. Boyd Woodruff, Merck, Sharpe and Dohme, and the New York Academy of Science.

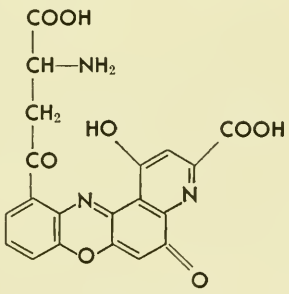
† See the discussian of nomencloture preceding the actinomycin entries.

Often these polypeptide pigments occur in closely related complexes, the individual members varying only by slight changes in the side-chains.

The chromophore, actinocinin, resembles that of the ommochromes, a group of insect pigments studied by Butenandt,[7] and it is similar to the pigment cinnabarin from a higher fungus.

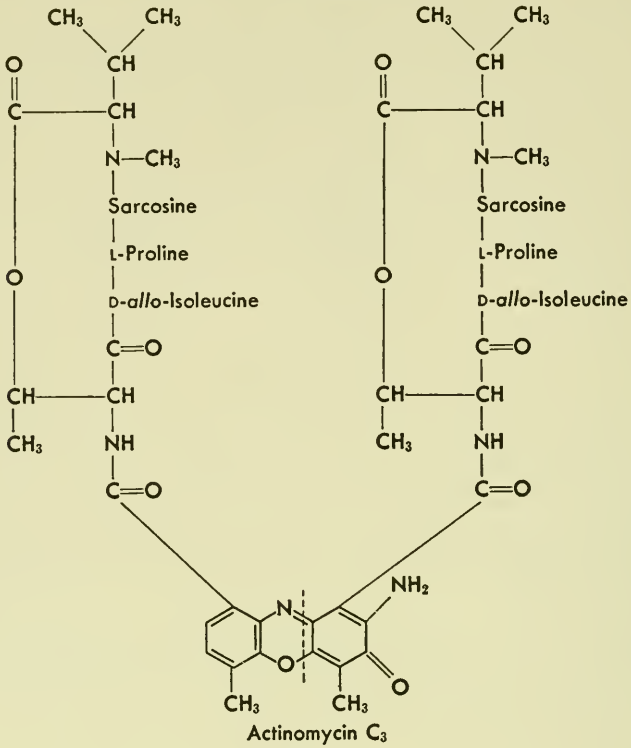


Actinocinin



Xanthommatin

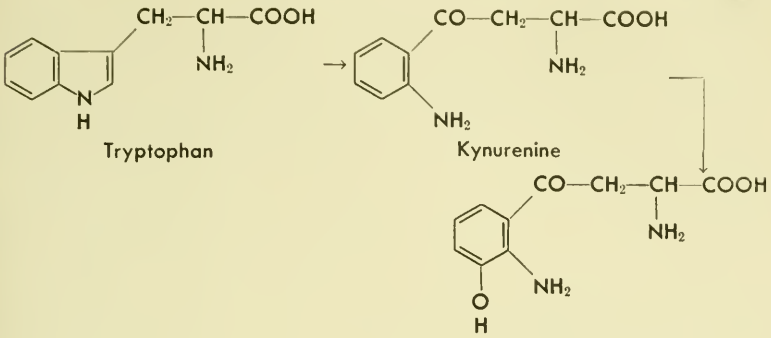
[7] Adolph Butenandt, Ulrich Schiedt, Ernst Biekert and Pierre Kornmann, *Ann.* 586 217 (1954); Adolph Butenandt, Ulrich Schiedt and Ernst Biekert, *Ann.* 586 229, 588 106 (1954); Adolph Butenandt, Ulrich Schiedt, Ernst Biekert and R. Jan. T. Cromartie, *Ann.* 590 75 (1954). (Structure)



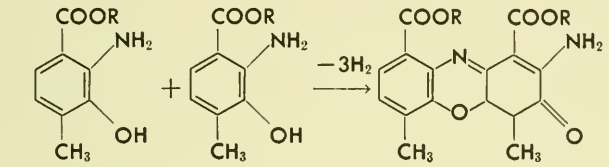

Actinomycin $C_3$

The dual nature of the actinomycin molecules makes it rather obvious that they must be formed by condensation of two similar halves. Butenandt showed that xanthommatin was derived from tryptophan by feeding experiments with the labeled amino acid. Similarly Katz has shown[8] that actinocinin is derived from tryptophan. Thus the entire actinomycin molecule is composed of amino acid derivatives. In the case of xanthommatin the intermediate is kynurenine, a known degradation product of tryptophan. Kynurenine may also be an intermediate in actinocinin biosynthesis, although the assumed intermediate, 3-oxy-4-methyl-anthranilic acid might equally well arise through a variation in the biosynthetic route to tryptophan.

[8] Edward Katz, N. Y. Acad. Sci. Conference on Actinomycins, March 31 to April 1, 1960. (Unpublished)




It is likely that the peptide side-chain is attached before condensation to form the chromophore.




3-Oxy-4-methyl-anthranilic Acid (R = Polypeptide Moiety)

Actinomycins

It is interesting that methyltryptophans (α,5,6-methyls) are inhibitory to actinomycin production. Methionine is probably the donor of the methyl groups in N-methylvaline and sarcosine.[8]

In two streptomycete species D-valine inhibits actinomycin synthesis while L-valine stimulates it, although D-valine is the isomer present in the side-chains.[8] This behavior is reminiscent of the results of similar earlier experiments with penicillin and with valinomycin.

Schmidt-Kastner found that addition of a large quantity of sarcosine to the medium caused replacement of part or all of the side-chain proline by sarcosine. Analogously, addition of isoleucine caused replacement of N-methylvaline by N-methylisoleucine.[9] Since then many new actinomycins have been prepared by addition of various amino acids to the medium. Even

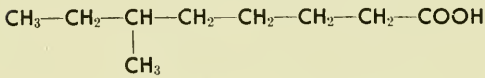
[9] G. Schmidt-Kastner, *Naturwissenschaften* 43 131 (1956).

pipecolic acid can be incorporated.[10] It should be noted that certain amino acid analogues can be incorporated into enzymes and other proteins without impairing their normal functions.[11]

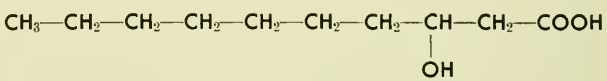
Professor Hans Brockmann and his collaborators at Göttingen, who were primarily responsible for determining the structure of the first well-characterized actinomycin, actinomycin $C_3$,[12] have succeeded in synthesizing this substance[13] and it should be possible now to prepare an even wider variety of actinomycins.

Valinomycin, shown opposite, can be hydrolyzed to its constituents: L-valine, D-valine, D-α-hydroxyisovaleric acid and L-lactic acid. It has been found[14] that L-valine-1-$C^{14}$ in the medium was incorporated to an equal extent into the D-valyl and L-valyl portions of the molecule, to a lesser extent into the D-α-hydroxyisovaleric acid, and not at all into the lactic acid. D-Valine-1-$C^{14}$ was incorporated only to a slight extent. Similar results have been obtained in studies on the origin of the D-valine moieties in penicillin and in actinomycin.

The co-occurrence of valine with the biosynthetically related α-hydroxyisovaleric acid in several polypeptides is noteworthy. Also conjugated with certain polypeptides are 6-methyloctanoic



6-Methyloctanoic Acid



3-Oxydecanoic Acid

acid and 3-oxydecanoic acid. The latter substance has been found conjugated with bacterial carbohydrates too.

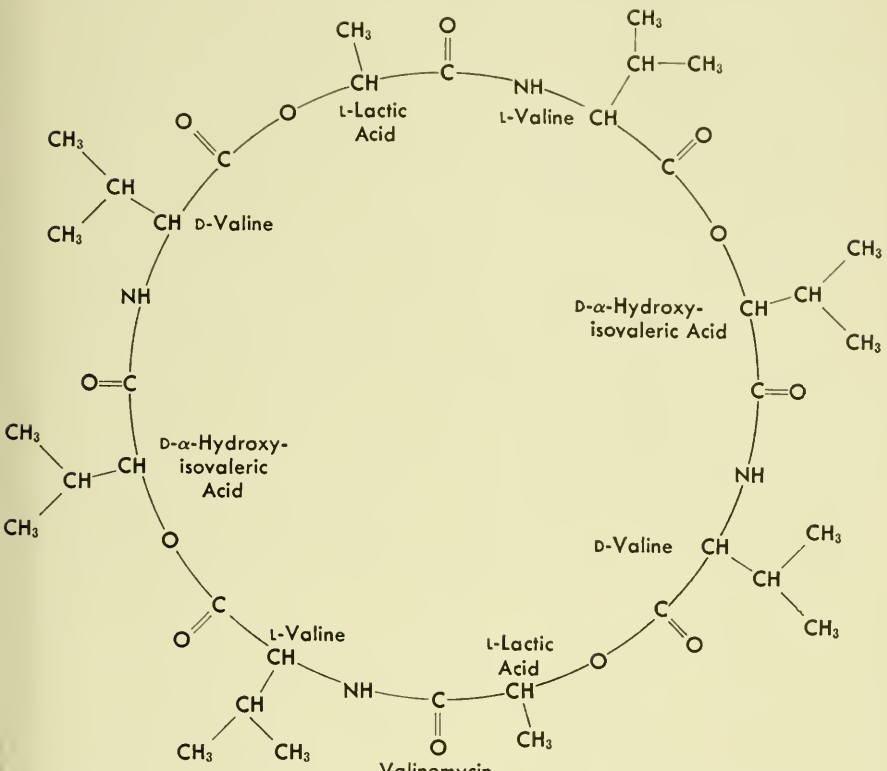
[10] Edward Katz and William Goss, *Nature* 182 1668 (1958); S. A. Waksman, E. Katz, W. A. Goss, L. H. Pugh, M. Solvtorowsky, and N. A. Auerbach, *Science* 129 1290 (1959); E. Katz and W. A. Goss, *Biochem. J.* 73 458 (1959); A. W. Johnson and A. B. Mauger, *ibid.* 73 535 (1959); William A. Goss and Edward Katz, *Antibiotics and Chemotherapy* 10 221 (1960).

[11] *E.g.*, Akira Yoshida and Mekoto Yamasaki, *Biochim. et Biophys. Acta* 34 158 (1959).

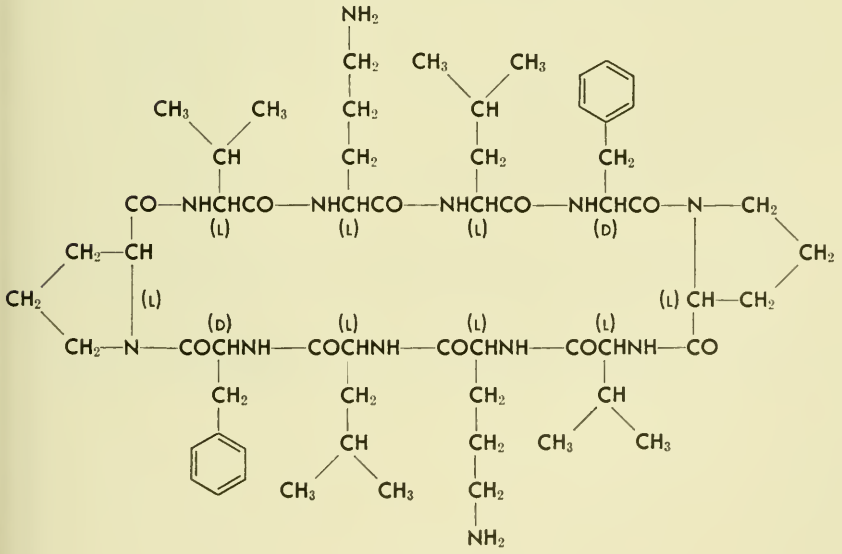
[12] H. Brockmann, G. Bohnsack, B. Franck, H. Gröne, H. Muxfeldt and C. Süling, *Angew. Chem.* 68 70 (1956); H. Brockmann, N. Grubhofer, H. Kalbe and W. Kass, *Chem. Ber.* 84 260 (1951); H. Brockmann, *Angew. Chem.* 66 1 (1954); H. Brockmann and B. Franck, *ibid.* 68 70 (1956) and other papers in this series.

[13] H. Brockmann, W. Sunderkötter, K. W. Ohly and P. Boldt, *Naturwissenschaften* 47 230 (1960); H. Brockmann and H. Lackner, *ibid.* 47 320 (1960).

[14] J. C. MacDonald, *Can. J. Microbiol.* 6 27 (1960).




Valinomycin



Gramicidin S

The biosynthesis of gramicidin S has been studied.[15] The conclusions were: (a) The five amino acids of the cyclic decapeptide pass through a number of intermediates before or during incorporation. (b) Final formation of gramicidin S is a simple reaction not requiring free amino acids which occurs readily in cell-free suspensions. (c) Three peptides were isolated containing fragments of the amino acid sequences of the antibiotic. These may or may not have been intermediates.

It is possible to extract intracellular peptides with suitable solvents. This has been done with mammalian pituitary tissue,[16, 17] with plant seeds[18] and with yeast[19] and bacteria.[20, 21] In all cases cited care was taken to obviate contamination by fragments of proteolysis. There is some indication that yields are higher from rapidly growing bacteria than from resting cells.

The intracellular peptides of the torula yeast studied were found to be predominantly acidic with glutamic acid the principal amino acid. About 40 peptides were purified in adequate quantity to permit hydrolysis and identification of constituent amino acids. These are tabulated below (x indicates an unidentified ninhydrin-positive substance):

TABLE II

*Some Intracellular Peptides of Torula Yeast*

| Peptide No. | Amino acid content | Peptide No. | Amino acid content |
|---|---|---|---|
| 1 | Glu, x-6, Gly, Ala, Asp, Arg, Val | 21 | Glu (Gly, x-3, Ala) |
| | | 22 | Glu, Gly, Cys (Glutathione) |

[15] J. M. Barry and Elizabeth Ishihara, *Nature* 181 1274 (1958).

[16] T. Winnick, R. E. Winnick, R. Acher and C. Fromageot, *Biochim. et Biophys. Acta* 18 488 (1955).

[17] L. K. Ramachandran and T. Winnick, *ibid.* 23 533 (1957).

[18] H. Borriss and G. Schneider, *Naturwissenschaften* 42 103 (1955).

[19] F. Turba and H. Esser, *Biochem.* Z. 327 93 (1956).

[20] G. E. Connell and R. W. Watson, *Biochim. et Biophys. Acta* 24 226 (1957).

[21] R. B. Roberts, P. H. Abelson, D. C. Cowie, E. T. Bolton and R. J. Britten, "Studies of Biosynthesis in *E. coli*," Carnegie Institute, Washington, D. C., 1955.

TABLE II–Continued

| Peptide No. | Amino acid content | Peptide No. | Amino acid content |
|---|---|---|---|
| 2 | Glu, Gly, Ala, Asp, Ser, Val, x-7, Arg | 23 | Glu, Gly, Ala, His, Arg or. Cys, x-6, Asp, Lys, Val |
| 3 | Glu, Gly, Asp, Ala, Thr? x-6, Arg? | 24 | Glu, Gly, Ala, x-7, x-11, Asp, Ser, Leu, Val, Arg, Lys |
| 4 | Glu, Gly, Ala, Thr? Asp, Arg, His, Val, x-5 | 25 | Gly, Glu, x-6, Ser, Ala, His, Val, Leu, Asp |
| 5 | Glu, Gly, Ala, His, Asp, x-4 | 26 | Glu, Gly, x-4, Ala, His, Lys, Leu |
| 6 | Glu, Gly, Ala, x-5, Asp, Arg, Val, His | 27 | Gly, Ala, Glu, x-4, Val, Arg |
| 7 | Glu, Gly, His, Ala, x-5, Asp, Arg, Val | 28 | Glu, Gly, x-11, Ser, Ala, Arg, Thr, X-7, Asp, Val, Lys |
| 8 | Glu, Gly, Ala, Asp, x-4, His, Arg | 29 | Glu, Gly, Ser, Ala, x-11, Asp, Thr, Val, Lys, Arg, Leu |
| 9 | Asp, Gly, Glu, Ala, x-5, Val, Arg | 30 | Glu, Gly, Ser, Ala, x-8, Asp, Thr, x-11, Val, Leu, Arg |
| 10 | Ala, Gly, Glu, x-5, x-6, Val | 31 | Gly, Glu, x-4, Ser, Ala, Asp, Leu |
| 11 | x-4, x-7, x-5, Gly, Glu, Ala, Asp | 32 | Gly, x-5, Glu, Ala, Asp, Arg |
| 12 | Asp (Gly, Glu, Ala, x-5, x-6) | 33 | Glu, Gly, x-6, Ala, α-But, Leu |
| 13 | Glu, Gly, Ala, Asp, x-5 | 34 | x-3, Ala, Gly, Glu, x-7, Ser, Asp, Val, Arg, Leu |
| 14 | Glu, Gly, Ala, x-5, x-8, Ser, Asp, Val, Arg? | 35 | Gly, Glu, Ala, x-7, Arg, Asp, Val, Leu |
| 15 | Ala, Gly, Glu, x-4, x-6, Asp | 36 | Arg? x-3, Gly, Glu, Ser., Ala, x-8, x-12, Asp, Thr, Val, Leu |
| 16 | Glu, Gly, His, Ala, Cys, x-4 | 37 | Gly, Glu, Ser, Ala, Asp, x-5, Arg, x-9, Thr? Val |
| 17 | Glu, Gly, Cys, x-10, Ala, Ser, x-6, Asp, Arg, Val, Leu | 38 | Gly, Ser, Gly, Ala, Asp, Val, x-6, Thr, Arg, Lys, His, x-12 |
| 18 | Glu, Gly, x-9, Ser, Ala, Asp, Thr, Cys? Arg, x-5 | 39 | Gly, Glu, Ala, x-6, Leu, Val, Thr, Asp, x-11, His, Lys |
| 19 | Gly, Glu, x-6, Ala, Ser, Asp, Val, Leu, His | 40 | Gly, Glu, x-5, Ala, α-But, Val |
| 20 | Glu, Gly, Ala, Asp, x-7, Ser, Tyr, Val, Leu, His | | |

In a similar study with the use of *E. coli* ten intracellular peptides were purified in sufficient amounts to allow amino acid determination.[22] In this case the N-terminal amino acids were

[22] D. Grünberger, Jiřina Černá and F. Šorm, *Experientia* 16 54 (1960).

distinguished by formation of their dinitrophenyl derivatives. The results are shown in the following table:

TABLE III

*Some Intracellular Peptides of Escherichia coli*

| Peptide No. | Terminal amino acid | (Other amino acids) |
|---|---|---|
| 1 | Glu | (Cys, Gly, Lys) |
| 2 | Glu | (Ala, Cys, Gly, Lys) |
| 3 | Asp | (Cys, Gly, Lys) |
| 4 | Lys | (Ala, Arg, Asp, Cys, Gly, Glu, Ser) |
| 5 | Asp | (Arg, Gly, Glu, $\gamma$-$NH_2$But, Lys, Val) |
| 6 | Ser | (Asp, Gly, Lys) |
| 7 | Ala | (Asp, Lys) |
| 8 | Glu | (Ala, Asp, Cys, Gly, Lys, Leu, Val) |
| 9 | Glu | (Ala, Asp, Lys, Cys, Gly) |
| 10 | Glu | (Cys, Gly) |

It has been reported that gram-negative bacteria contain much less intracellular free ninhydrin-positive substances than do gram-positive ones.[21]

A basic polypeptide has been extracted from dried cells of the human strain of *Mycobacterium tuberculosis* $H_{37}R_v$, purified, crystallized and quantitatively analyzed for amino acid constituents.[23] The pure peptide showed activity in the tuberculin test at least equal to that of standard old tuberculin. The amino acid content was as follows, subscripts indicating number of moles:

$$Arg_{10}\ His_1\ Lys_2\ Phe_1\ Tyr_1\ Leu_3\ Ileu_2\ Val_5$$
$$Ala_9\ Gly_6\ Glu_8\ Pro_5\ Ser_2\ Thr_3\ Asp_6\ Try_1$$

The molecular weight was calculated to be 7180.

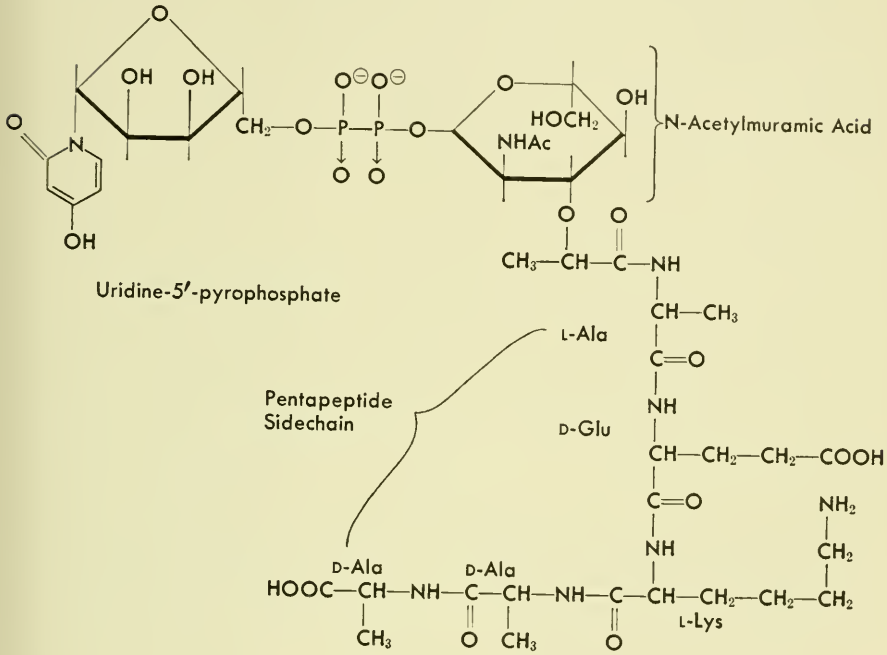
Certain polypeptides accumulate in *E. coli* cells grown in the presence of chloramphenicol (a protein synthesis inhibitor). Two of these have been isolated and purified.[24]

[23] Yuichi Yamamura, Seisi Morizawa, Atsushi Tanaka, and Kenji Shojima, *Proc. Jap. Acad. Sci.* 35 295 (1959); Seisi Morizawa, Atsushi Tanaka, Kenji Shojima and Yuichi Yamamura, *Biochim. et Biophys. Acta* 38 252 (1960).

[24] F. Šorm and Jiřina Černá, *Collection Czechoslov. Chem. Commun.* 25 565 (1960).

Synthesis of the cell wall mucopeptides of staphylococci is unaffected by chloramphenicol, but inhibited (at least indirectly) by penicillin, bacitracin, cycloserine, novobiocin and gentian violet. None of these inhibits protein synthesis.

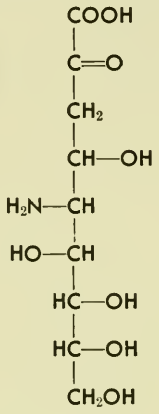
Penicillin-inhibited *Staphylococcus aureus* accumulates three closely related uridine nucleotides.[25] One of these has been assigned the structure:[26, 27, 28]




This fragment may be the repeating unit of an activated cell wall precursor, since the ratio of muramic acid:alanine:glutamic acid:lysine is 1:3:1:1, the same ratio found in lysozyme digests of whole bacteria. In *E. coli* and *Corynebacterium diphtheriae* the lysine in the peptide chain is replaced by its biosynthetic precursor, *meso*-diaminopimelic acid.

[25] J. T. Park and N. J. Johnson, *J. Biol. Chem.* 179 585 (1949).
[26] J. T. Park and J. L. Strominger, *Science* 125 99 (1957).
[27] J. L. Strominger, *J. Biol. Chem.* 234 1520 (1959).
[28] *Idem., Federation Proc.* 18 334 (1959); Eiji Ito and Jack L. Strominger, *J. Biol. Chem.* 235 PC5 (1960).

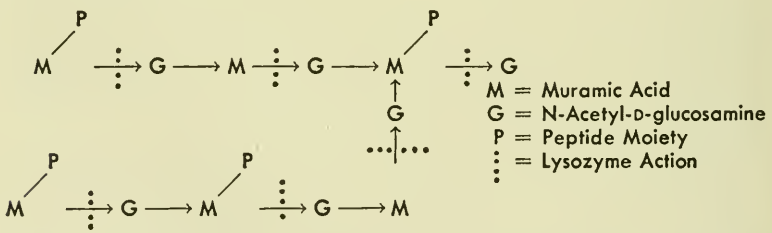
There is increasing evidence that the antibiotics mentioned, lysozyme and bacteriophages, all bring about a similar result, the accumulation or liberation of a fundamental cell wall unit such as the one shown. Lysozyme and bacteriophages are able to liberate the unit from pre-formed walls, while the antibiotics merely block wall synthesis. Also, the unit obtained by lysozyme or phage action seems to contain glucosamine as well as muramic acid, and sometimes diaminopimelic acid (a lysine precursor) rather than lysine. There is evidence that N-acetyl-D-glucosamine is a direct precursor of muramic acid.



Neuraminic Acid

Several neuraminopeptides have been isolated from an *E. coli* mutant culture, and one of these has been purified.[29] It is composed of N-acetylneuraminic acid, glucosamine, alanine, lysine and glutamic acid.[30]

A model of cell wall structure in gram-positive bacteria has been postulated:[31]




[29] P. J. O'Brien and F. Zilliken, *Biochim. et Biophys. Acta* 31 543 (1959).

[30] E. Kean, Dissertation. (In press)

[31] Friedrich Zilliken, *Federation Proc.* 18 966–973 (1959).

The spine is composed of alternating muramic acid and N-acetylglucosamine units with branching to adjacent chains from muramic acid, the latter bearing the peptide chain. Penicillin (and perhaps the other antibiotics mentioned) prevents incorporation of M-P units, and cycloserine prevents incorporation of the terminal two D-alanine units into the side-chain. There is evidence that the dipeptide D-alanyl-D-alanine is preformed before attachment to the peptide chain.

A review of the chemistry of bacterial cell walls has been published.[31]

The newer general theory of polypeptide and protein synthesis can be sketched in only briefly here.[32] It is thought that the DNA of the cell nucleus lays out the pattern for replication of the ribosomal RNA, and this pattern is characteristic of each genus, species and type of organism. The ribosomal RNA in turn serves as the template for protein construction. Smaller, more soluble molecules, which seem to be RNA fragments ending in the nucleotide adenylic acid, attach themselves at this end to individual amino acids. This attachment requires an enzyme specific for each of the 20 or more amino acids plus ATP. There is also a different transfer RNA molecule for each amino acid. These activated amino acids can be isolated and purified. In this form the amino acid is able to fit into the proper place on the RNA template, probably due to the unique geometry of a short sequence of nucleotides in the chain. Once attached to RNA, condensation of the amino acids to form polypeptides or proteins is facilitated by the favorable arrangement and proximity of the reacting groups. This scheme is believed to be quite general in metabolism.

A more specific discussion by E. F. Gale of current knowledge about the incorporation of amino acids into bacterial proteins and polypeptides has been published.[33] It is obvious that considerable differences must exist between mechanisms of polypeptide synthesis in microbial and mammalian metabolism in view of the D-amino acids and other abnormal amino acids which occur in microbial polypeptides. It is apparently these differences

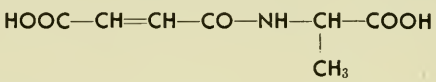
[32] Robert B. Loftfield, *Prog. Biophys., Biophys. Chem.* No. 8 348 (1957); F. H. C. Crick, *Symposia of the Society for Exp. Biol.* No. 12 138 (1958); Mahlon B. Hoagland, *Scientific American* 201 55 (1959); Alton Meister, *Rev. Mod. Phys.* 31 210–220 (1959); Leo Szilard, *Proc. Nat. Acad. Sci. U. S.* 46 277 (1960).

[33] "CIBA Lectures in Microbial Chemistry," E. F. Gale, *Synthesis and organization in the bacterial cell,* John Wiley and Sons, New York, 1959, 106 pp.

which are exploited by some of the more successful antibiotics.

Certain compounds listed elsewhere might have been classed as polypeptides. Examples are: penicillins, gliotoxin, certain ergot alkaloids, various diketopiperazines, netropsin, amicetin, Vitamin $B_c$ conjugate and other folic acids.

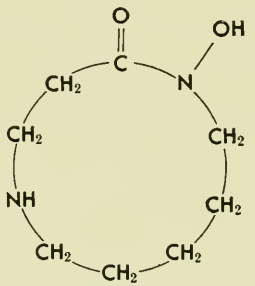
712 **DL-Fumarylyl Alanine** (Fumaromono-D,L-alanide), $C_7H_9O_5N$, colorless needles, m.p. 229° (dec.).



*Penicillium resticulosum*

John Howard Birkinshaw, Harold Raistrick and George Smith, *Biochem. J.* 36 829 (1942).

713 **Nocardamin,** $C_8H_{16}O_2N_2$, white needles, m.p. 184°, no optical activity.

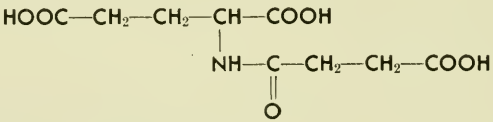


*Actinomyces buchanan*

A. Stoll, J. Renz and A. Brack, *Helv. Chim. Acta* 34 862 (1951).

R. F. C. Brown and G. Büchi, unpublished. (Revised structure)

714 **N-Succinyl-L-glutamic Acid,** $C_9H_{13}O_7N$ (Monohydrate), colorless hygroscopic crystals, m.p. 62–64°, $[\alpha]_D^{20}$ −11° (c 1.07 in water).

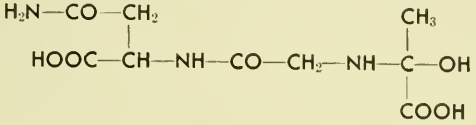


*Bacillus megatherium*

This substance appears during the sporulating phase before the appearance of dipicolinic acid.

Jean Paul Aubert, Jacqueline Millet, Elisabeth Pineau and Gerard Milhaud, *Compt. rend.* 249 1956 (1959).

715 **Lycomarasmine,** $C_9H_{15}O_7N_3$, white powder, m.p. 227–229 (dec.). Tentative structure:



*Fusarium lycopersici* Sacc.

This is the toxin of fusarium wilt. A second compound, $C_9H_{12}O_7N_2$, white powder, m.p. 273–276° (dec.), has been isolated from the mother liquors. It is produced in up to three times the yield of lycomarasmine, but is biologically inactive. It is also produced (with evolution of ammonia) by boiling lycomarasmine with water.

The yield of lycomarasmine in the initial isolation was 80–110 mg. per liter.

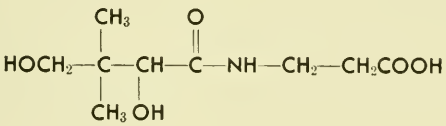
There is still some dissatisfaction with this structure.

Pl. A. Plattner and N. Clauson-Kaas, *Helv. Chim. Acta* 28 188 (1945). (Isolation)

D. W. Woolley, *J. Biol. Chem.* 176 1291 (1948). (Structure)

M. Brenner, R. Tamm and P. Quitt, *Helv. Chim. Acta* 41 763 (1958). (Criticism of structure)

716 ***d*-Pantothenic Acid,** $C_9H_{17}O_5N$, viscous oil, $[\alpha]_D^{25}$ +37.5° (in water).



Penicillin liquors yield 600–800 $\mu$g. per gram of dry cell weight.

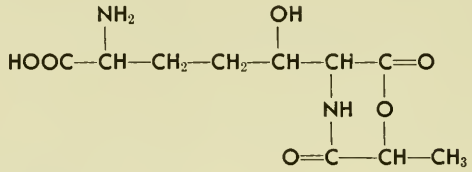
Yeasts contain 150–300 $\mu$g. per gram of dry cell weight.

D. W. Woolley, *J. Am. Chem. Soc.* 62 2251 (1940). (Synthesis)

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. II, J. M. Van Lanen, *Production of vitamins other than riboflavin,* chap. 6, pp. 191–216.

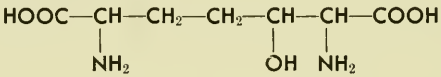
717 **Toxin of tobacco wild-fire disease**, $C_{10}H_{16}O_6N_2$.

Probable structure:



*Pseudomonas tabaci*

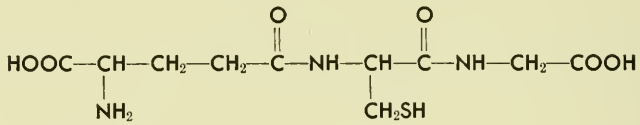
The toxin can by hydrolyzed to lactic acid and the amino acid, tabtoxinin, $C_7H_{14}O_5N_2$, ($\alpha,\epsilon$-diamino-$\beta$-hydroxypimelic acid):



D. W. Woolley, G. Schaffner and Armin C. Braun, *J. Biol. Chem.* 198 807 (1952). (Isolation)

*Idem., ibid.* 215 485 (1955). (Structure)

718 **Glutathione** (Glutamylcysteinylglycine) $C_{10}H_{17}O_6N_3S$, colorless crystals, m.p. 190–192° (dec.). Unstable. $[\alpha]_{Hg.}^{28.5}$ −9.4° in water, −85° in 10% hydrochloric acid.

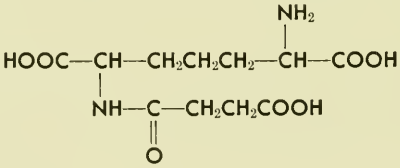


Yeasts

F. G. Hopkins, *Biochem. J.* 15 286 (1921). (Isolation)

Charles Robert Harington and Thomas Hobson Mead, *ibid.* 29 1602 (1935). (Synthesis)

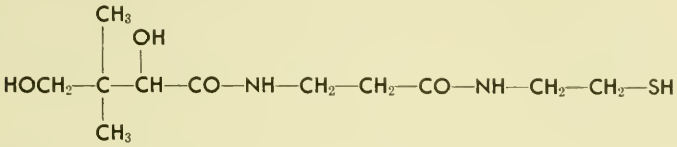
719 **N-Succinyl-L-diaminopimelic Acid**, $C_{11}H_{18}O_7N_2$.



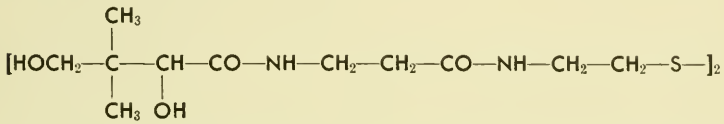
Charles Gilvarg, *Biochim. et Biophys. Acta* 24 216 (1957).

**Lactobacillus bulgaricus Factor** (Pantetheine and Pantethine), $C_{11}H_{22}O_4N_2S$ and $C_{22}H_{42}O_8N_4S_2$.

720 **Pantetheine:** Colorless, hygroscopic, amorphous powder, $[\alpha]_D^{20}$ +12.9° (in water).



721 **Pantethine:** viscous oil.



Yeasts, *Ashbya gossypi,* many other microorganisms

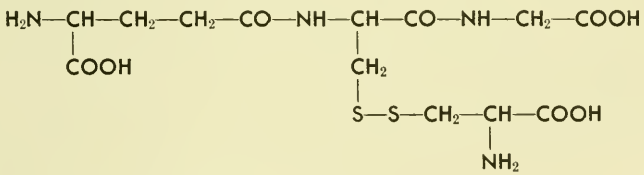
William L. Williams, E. Hoff-Jörgensen and Esmond E. Snell, *J. Biol. Chem.* 177 933 (1949).

Esmond E. Snell, Gene M. Brown, Vincent J. Peters, Jean A. Craig, E. L. Wittle, J. A. Moore, V. M. McGlohon and O. D. Bird, *J. Am. Chem. Soc.* 72 5349 (1950).

Vincent J. Peters, Gene M. Brown, William L. Williams and Esmond E. Snell, *ibid.* 75 1688 (1953).

Gene M. Brown and Esmond E. Snell, *ibid.* 75 1691 (1953).

722 **Glutathione-Cysteine Disulfide,** $C_{13}H_{22}O_8N_4S_2$.

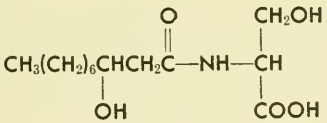


*Saccharomyces cerevisiae*

Glutathione itself occurs in yeasts. The disulfide above was not isolated.

Arthur H. Livermore and Edward C. Muecke, *Nature* 173 265 (1954).

723 **Serratamic Acid,** $C_{13}H_{25}O_5N$, colorless crystals, m.p. 138° (dec.), $[\alpha]_D^{20}$ −10.2° (c 5.0 in ethanol).



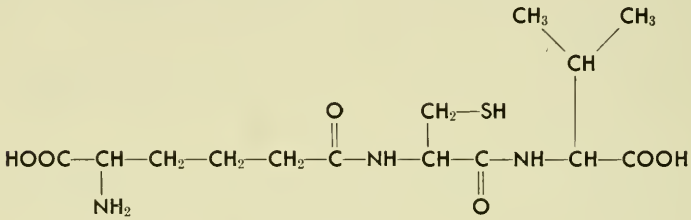
*Serratia* species

Yields as high as 8 g. per liter have been reported. Hydrolysis gives L-serine and (−)-3-oxydecanoic acid. The latter acid also has been found in conjugation with rhamnose and with other amino acids (see Viscosin).

N. J. Cartwright, *Biochem. J.* **60** 238 (1955).

*Idem., ibid.* **67** 663 (1957). (Structure)

724 **δ-(α-Aminoadipyl) cysteinylvaline,** $C_{14}H_{25}O_6N_3S$.



*Penicillium chrysogenum*

This tripeptide was isolated from the mycelium of the penicillin-producing mold. It may be a penicillin precursor since cyclization in the proper way would yield synnematin-B (cephalosporin-N) which differs from penicillin only in its side-chain. Synnematin never has been isolated from *P. chrysogenum*, however.

H. R. V. Arnstein, D. Morris and E. J. Toms, *Biochim. et Biophys. Acta* **35** 561 (1959).

725 **Alazopeptin,** $C_{15}H_{21}O_6N_7$, no definite m.p., $[\alpha]_D^{25}$ +9.5° (c 4.7 in water).

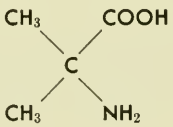
A peptide containing 1 mole of α-alanine and 2 moles of 6-diazo-5-oxoaminohexanoic acid (DON) or an isomer.

*Streptomyces griseoplanus*

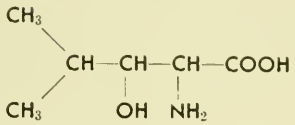
S. E. DeVoe, N. E. Rigler, A. J. Shay, J. H. Martin, T. C. Boyd, E. J. Backus, J. H. Mowat and N. Bohonos, "Antibiotics Annual 1956–1957," Medical Encyclopedia, Inc., New York, p. 730.

726 **Antibiotic I.C.I. 13,959.**

Acid hydrolysis yielded:
α-Aminoisobutyric Acid



β-Hydroxyleucine



as well as L-leucine, β-alanine and γ-methylproline. The β-hydroxyleucine, which had not been reported previously as a natural product, has either the D- or L-*threo* but not the *erythro* configuration.

A *Paecilomyces* strain

G. W. Kenner and R. C. Sheppard, *Nature* 181 48 (1958).

727 **Viomycin** (Vinactin A, Vinactane, Celiomycin, Viocin), $C_{17\text{-}18}H_{31\text{-}35}O_8N_9$, Sulfate: m.p. (anhydrous) 252° (dec.) (hydrated) 280° (dec.), $[\alpha]_D^{25}$ −32° (c 1 in water). Rotation varies with pH.

A strongly basic polypeptide. The following components have been identified: α,β-diaminopropionic acid, β-lysine, L-serine and a guanidino compound. Salts are neutral.

*Streptomyces floridae*, *S. puniceus*, *S. vinaceus*

A. C. Finlay, G. L. Hobby, F. Hochstein, T. M. Lees, T. F. Lenert, J. A. Means, S. Y. P'An, P. P. Regna, J. B. Routien, B. A. Sobin, K. B. Tate and J. H. Kane, *Am. Rev. Tuberc.* 63 1 (1951).

Quentin R. Bartz, John Ehrlich, James D. Mold, Mildred A. Penner and Robert M. Smith, *ibid.* 63 4 (1951).

Theodore H. Haskell, Salvatore A. Fusari, Roger P. Frohardt and Quentin R. Bartz, *J. Am. Chem. Soc.* 74 599 (1952).

R. L. Mayer, P. C. Eisman and E. A. Konopka, *Experientia* 10 335 (1954).

728 **Phthiomycin**, white powder.

A basic polypeptide resembling viomycin.

*Streptomyces luteochromogenes* n. sp.

Kenji Maeda, Yoshiro Okami, Ryozo Utahara, Hiroko Kosaka and Hamao Umezawa, *J. Antibiotics* (Japan) 6A 183 (1953).

Yasushi Miyamoto and Kenji Maeda, *ibid.* 7A 17 (1954).

729 **Streptolin A**, $C_{17}H_{31}O_8N_5$ or $C_{24}H_{45}O_{11}N_7$, m.p. 206° (dec.), sulfate $[\alpha]_D^{25}$ −20°.

Streptolins A and B are similar. They resemble streptothricin, viomycin, geomycin and roseothricin in their acid hydrolysates, which contain L-β-lysine, α-D-gulosamine, streptolidine, ammonia and carbon dioxide.

*Streptomyces* spp.

R. W. Rivett and W. H. Peterson, *J. Am. Chem. Soc.* 69 3006 (1947). (Isolation)

Edward E. Smissman, Robert W. Sharpe, B. F. Aycock, Eugene E. van Tamelen and W. H. Peterson, *ibid.* 75 2029 (1953).

Eugene E. van Tamelen and Edward E. Smissman, *ibid.* 75 2031 (1953).

Eugene E. van Tamelen, John R. Dyer, Herbert E. Carter, Jack V. Pierce and Edward E. Daniels, *ibid.* 78 4817 (1956).

730 **Noformicin*** (Sulfate), $C_{17}H_{34}O_5N_{10}(SO_4)_2$, m.p. (Hydrochloride) 265° (dec.).

Hydrolysis yields glutamic acid, ammonia and two other ninhydrin-positive compounds which are not ordinary amino acids.

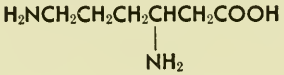
*Nocardia formica*

Dale A. Harris and H. Boyd Woodruff, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, p. 609.

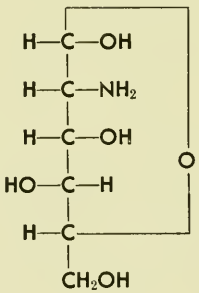
731 **Streptothricin,** $C_{20}H_{36}O_9N_8$, platelets (Reineckate), m.p. 192–194° (Hydrochloride) $[\alpha]_D^{25}$ −51.3°.

A basic polypeptide. Hydrolysis yields:

L-β-Lysine:



D-Gulosamine:



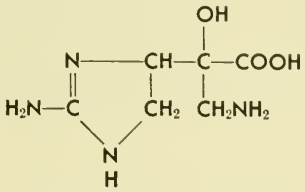
Streptolidine:

$C_6H_{12}N_4O_3$

Several structures have been proposed for this moiety. See C. Sweeley, Ph.D. Dissertation, Univ. of Illinois, 1955.

* See entry 915 for structure.

It may be identical with the amino acid known as roseonine or geamine.



Carbon dioxide and ammonia also have been identified in hydrolysates.

*Streptomyces lavendulae* and other streptomyces species

Selman A. Waksman and H. Boyd Woodruff, *Proc. Soc. Exp. Biol. Med.* 49 207 (1942). (Isolation)

Herbert E. Carter, Walter R. Hearn, Edwin M. Lansford, Jr., A. C. Page, Jr., Norman P. Salzman, David Shapiro and W. R. Taylor, *J. Am. Chem. Soc.* 74 3704 (1952). (Structure)

H. E. Carter, R. K. Clark, Jr., Paul Kohn, John W. Rathrock, W. R. Taylor, C. A. West, George B. Whitfield and William G. Jackson, *ibid.* 76 566 (1954).

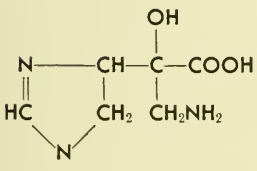
Koji Nakamishi, Tashito Ito and Yoshimasa Hirata, *ibid.* 76 2845 (1954).

Eugene E. van Tamelen, John R. Dyer, Herbert E. Carter, Jack V. Pierce and Edward E. Daniels, *ibid.* 78 4817 (1956).

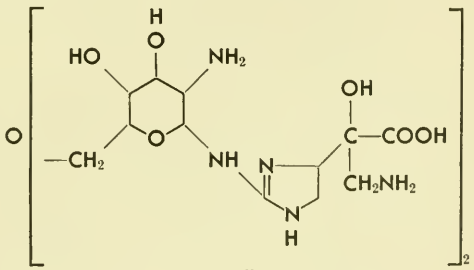
R. Cölln, Ph.D. Dissertation, Göttingen, 1957.

**Roseothricins.**

732 A polypeptide antibiotic complex of the streptothricin type. Acid hydrolysis of **Roseothricin A** yields $\beta$-lysine and roseonine (geamine) I in a 1:1 ratio, an isomer of



I



II

glucosamine, and a substance resistant to further hydrolysis which was assigned structure II.

*Streptomyces roseochromogenes*

Seigo Hosoya, Momoe Soeda, Nobuhiko Komatsu and Susumu Imamura, *J. Antibiotics* (Japan) 4 79 (1951).

Y. Saburi, *ibid.* 6B 402 (1953).

Tashio Goto, Yosimasa Hirata, Seigo Hosoya and Nabukiko Komatsu, *Bull. Chem. Soc. Japan* 30 304, 729 (1957). (Structure)

733 **Pleocidin,** a hygroscopic white powder.

A polypeptide resembling streptothricin.

*S. lavendulae* or related sp.

Jesse Charney, Wm. S. Roberts and W. P. Fisher, *Antibiotics and Chemotherapy* 2 307 (1952).

734, 735 **Mycothricins (A and B).**

Basic polypeptides related to streptothricin. Acid hydrolysis yielded β-lysine, (present in streptothricin, pleocidin, geomycin and viomycin), roseonine (geamine) present in streptothricin, geomycin and pleocidin, and serine (present in viomycin).

*Streptomyces lavendulae* type

G. Rangaswami, C. P. Schaffner and S. A. Waksman, *Antibiotics and Chemotherapy* 6 675 (1956).

736 **Grasseriomycin,** pale yellow hydrochloride, m.p. (Reineckate) 187–190° (dec.). Molecular weight 610.

A polypeptide resembling streptothricin. Negative biuret, Millon, $FeCl_3$. Positive ninhydrin, Molisch, Fehling.

*Streptomyces lavendulae, S. griseolavendus*

Kasububo Ueda, Youichiro Okimoto, Heiichi Sakai and Kei Arima, *J. Antibiotics* (Japan) 8A 91 (1955).

Yusuke Sumiki, Kinichiro Sakaguchi and Takenori Asai, Japanese Patent 6296 (1957).

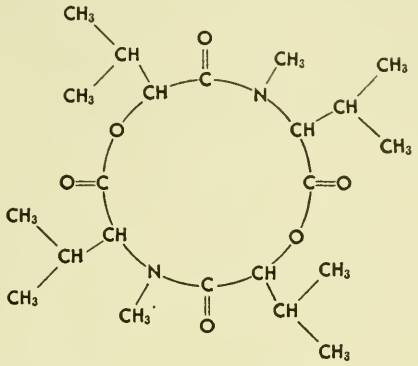
737 **Actinorubin** ($C_6H_{14}O_3N_2$ or $C_9H_{22}O_4N_5$) (Helianthate), reddish orange clusters, m.p. 206–214° (dec.).

A basic polypeptide related to streptothricin. Positive biuret, reduces $KMnO_4$, Fehlings solution. Negative Molisch, Sakaguchi.

*Streptomyces* spp. resembling *S. erythreus, S. fradii, S. albosporeus*

Renate Junowicz-Kocholaty and Walter Kocholaty, *J. Biol. Chem.* 168 757 (1947).

738 **Enniatin-B,** $C_{33}H_{38}O_6N_3$, colorless needles, m.p. 174°, $[\alpha]_D^{21}$ −106.3° (c 0.695 in chloroform).



*Fusaria* species

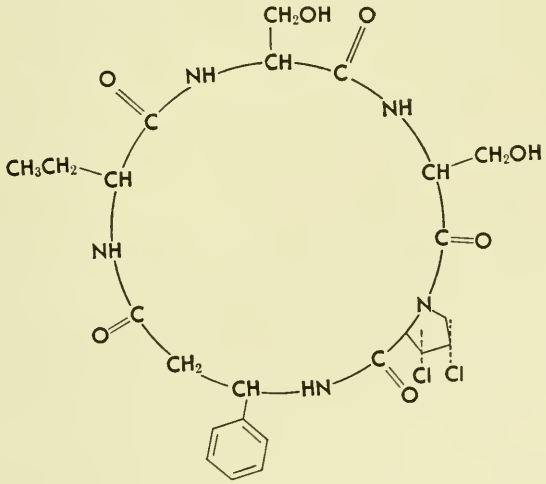
Yield about 0.5 g. per liter.

Pl. A. Plattner and U. Nager, *Experientia* 3 325 (1947).

Pl. A. Plattner, U. Nager and A. Boller, *Helv. Chim. Acta* 31 594 (1948).

Pl. A. Plattner and U. Nager, *ibid.* 31 665 (1948).

739 **Islanditoxin,** $C_{24}H_{31}O_7N_5Cl_2$, colorless, amorphous solid, m.p. 258°.



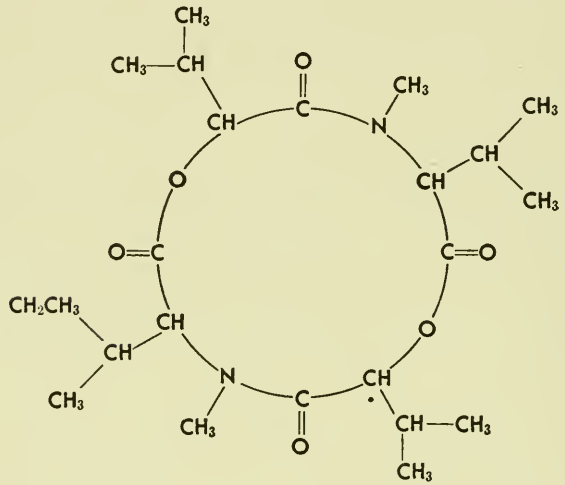
*Penicillium islandicum* Sopp.

Shingo Marumo and Yusuke Sumiki, *J. Agr. Chem. Soc. Japan* 29 305 (1955). (Isolation)

Shingo Marumo, *Bull. Agr. Chem. Soc.* (Japan) 19 258 (1955).

*Idem., ibid.* 23 428 (1959). (Structure)

740 **Enniatin-A** (Lateritiin-I), $C_{24}H_{42}O_6N_2$, colorless needles, m.p. 122°, $[\alpha]_D^{18}$ −91.9° (c 0.926 in chloroform).




*Fusarium orthoceras* var. *enniatinum, F. scirpi* Lamb. et Fautr., *F. lateritium*

The yield was about 1 g. per liter.

E. Gaümann, Stephi Roth, L. Ettlinger, Pl. A. Plattner and U. Nager, *Experientia* 3 202 (1947). (Isolation)

Pl. A. Plattner and U. Nager, *ibid.* 3 325 (1947).

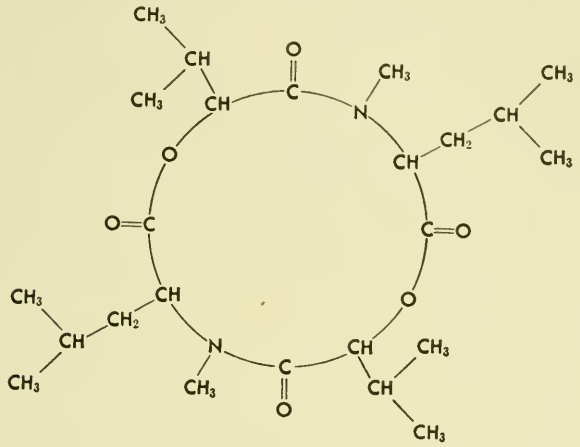
Pl. A. Plattner, U. Nager and A. Boller, *Helv. Chim. Acta* 31 594 (1948).

Pl. A. Plattner and U. Nager, *ibid.* 31 2192, 2203 (1948).

A. H. Cook, S. F. Fox and T. H. Farmer, *J. Chem. Soc.*, 1022 (1949).

741 **Enniatin-C**, $C_{24}H_{42}O_6N_2$, m.p. 123°, $[\alpha]_D^{22}$ −83° (c 1.162 in chloroform).

Proposed structure:

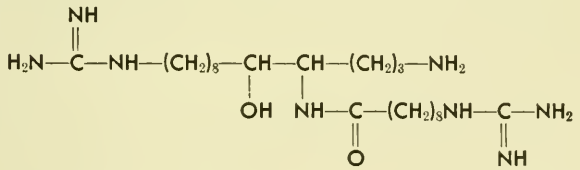


*Fusaria* species

The yield was about 0.6 g. per liter.

Pl. A. Plattner and U. Nager, *Helv. Chim. Acta* 31 2203 (1948).

742 **Eulicin**, $C_{24}H_{52}O_2N_8$, m.p. (Helianthate) 139°.



*Streptomyces* sp. resembling *S. parvus*

An actinomycin and a basic substance also were produced.

Jesse Charney, Roy A. Machlowitz, Frank J. McCarthy, Gertrude A. Rutkowski, Alfred A. Tytell and W. P. Fisher, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 228. (Isolation)

Robert E. Harman, Edward A. Ham, William A. Bolhofer and Norman G. Brink, *J. Am. Chem. Soc.* 80 5173 (1958). (Structure)

PA 114 Antibiotics.

743 **PA 114A,*** $C_{25}H_{31}O_6N_3$ or $C_{35}H_{42}O_9N_4$ (proposed), colorless needles, m.p. 200° (dec.), $[\alpha]_D^{25}$ −207° (c 0.5 in methanol).

A neutral substance, green $FeCl_3$ test. Negative ninhydrin, carbohydrate tests.

744 **PA 114B,†** $C_{52}H_{63}O_{12}N_9$ (proposed), colorless crystals, m.p. 265° (dec.), $[\alpha]_D^{25}$ −59.7° (c 0.5 in methanol).

A weak acid, red $FeCl_3$ test. Negative ninhydrin, carbohydrate tests, 2,4-DNPH.

*Streptomyces olivaceus*

Walter D. Celmer and Ben A. Sobin, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 437.

745 **PA 114B-3,** colorless needles, m.p. 207°, $[\alpha]_D^{20}$ −37.2° (in methanol).

A polypeptide antibiotic similar to PA-114B. Analysis: C 62.77, H 6.52, N 12.61.

A *Streptomyces olivaceus* strain

D. C. Hobbs and W. D. Celmer, *Federation Proc.* 18 246 (1959).

746 **Streptogramin,** approximate formula $C_{26}H_{33}O_7N_3$, m.p. 155°, $[\alpha]_D$ −134°.

Neutral compound.

*Streptomyces graminofaciens*

Jesse Charney, W. P. Fisher, Charles Curran, Roy A. Machlowitz and Alfred A. Tytell, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, p. 171.

Lateritiin Group

Several colorless compounds similar to the enniatins were isolated from fusaria species in England. One of these, lateritiin I, has been shown identical with enniatin A. The others are:

| | Name | Suggested formula | Melting point | $[\alpha]_D^{20}$ |
|---|---|---|---|---|
| 747 | Lateritiin II | $C_{26}H_{46}O_7N_2$ | 125° | −92° |
| 748 | Avenacein | $C_{25}H_{44}O_7N_2$ | 139° | −101° |
| 749 | Fructigenin | $C_{26}H_{44-46}O_7N_2$ | 129° | −103° |
| 750 | Sambucinin | $C_{24}H_{42}O_7N_2$ | 86° | −83° |

All these compounds yield D(−)-α-hydroxyisovaleric

* May be identical with staphylomycin $M_1$, E-129A (ostreogrycin A).

† See addendum for structure.

acid, $C_5H_{10}O_3$, m.p. 65°, $[\alpha]_D^{13}$ −21° (c 1.25 in chloroform), and N-methyl-L-valine on acid hydrolysis.

The enniatins also uniformly contain D(−)-α-hydroxyisovaleric acid, but each contains a characteristic N-methylamino acid. (*cf.* valinomycin, amidomycin).

A. H. Cook, S. F. Cox, T. H. Farmer and M. S. Lacey, *Nature* **160** 31 (1947).

A. H. Cook, S. F. Cox and T. H. Farmer, *ibid.* **162** 61 (1948). *Idem., J. Chem. Soc.,* 1022 (1949).

751 **Chlorine-containing Peptide,** $C_{25}H_{36}O_8N_5Cl_2$, white needles, m.p. 251° (dec.), $[\alpha]_D^{16}$ −92.9° (in methanol).

Positive biuret and Pauly reactions, negative Sakaguchi, Neubauer-Rhode, ninhydrin, Millon reactions.

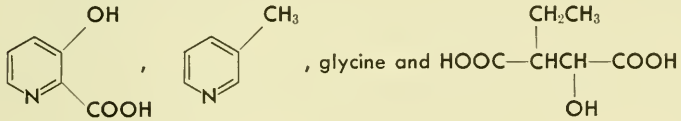
Acid hydrolysis yielded serine (2 to 3 moles), α-aminobutyric acid (1 mole), β-phenyl-β-aminopropionic acid (1 mole) and an unidentified substance yielding a positive Ehrlich reaction.

*Penicillium islandicum* Sopp.

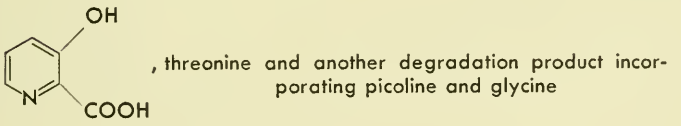
Yoshita Kobayashi, Kenji Uraguchi, Takashi Tatsuno, Fuminori Sakai, Michio Tsukioka, Yutaka Sakai, Osamu Yonemitsu, Taiko Sato, Masashi Miyake, Mamoru Saito, Makoto Enomoto, Toshio Shikata and Toshitaka Ishiko, *Proc. Japan Acad.* **34** 736 (1958).

752 **Pyridomycin,** $C_{26-27}H_{32}O_8N_4$, colorless needles, m.p. 218–222°.

Apparently rather closely related to etamycin. Alkaline fusion yields:




Acid hydrolysis yields:




*Streptomyces pyridomyceticus*

Kenji Maeda, *J. Antibiotics* (Japan) **10A** 94 (1957) and earlier papers in the series.

753 **Levomycin,** $C_{27}H_{38}O_{10}N_6$ (proposed), colorless crystals, m.p. 222–224°, $[\alpha]_D^{25}$ −323° (c 1 in chloroform).

A polypeptide containing an aromatic group.

*Streptomyces* sp.

Herbert E. Carter, Carl P. Schaffner and David Gottlieb, *Arch. Biochem. and Biophys.* 53 282 (1954).

754 **Staphylomycin $M_1$**, $C_{28}H_{36}O_8N_3$ (probable), m.p. 165–167° (dec.), $[\alpha]_D$ −190° ±2° (c 0.5 in ethanol).

A neutral compound. Carbonyl group present. Glycine and proline liberated on acid hydrolysis. Related to PA 114A.*

755 **Staphylomycin S**, $C_{38\text{-}39}H_{47\text{-}48}O_9N_6$ (proposed, but see structure below), white crystals, m.p. 240–242° $[\alpha]_D$ −28.0° (c 1.0 in ethanol).

A weak acid. Threonine, norvaline, α-aminobutryic acid, phenylalanine and proline were produced on acid hydrolysis. Related to PA 114B (or identical).

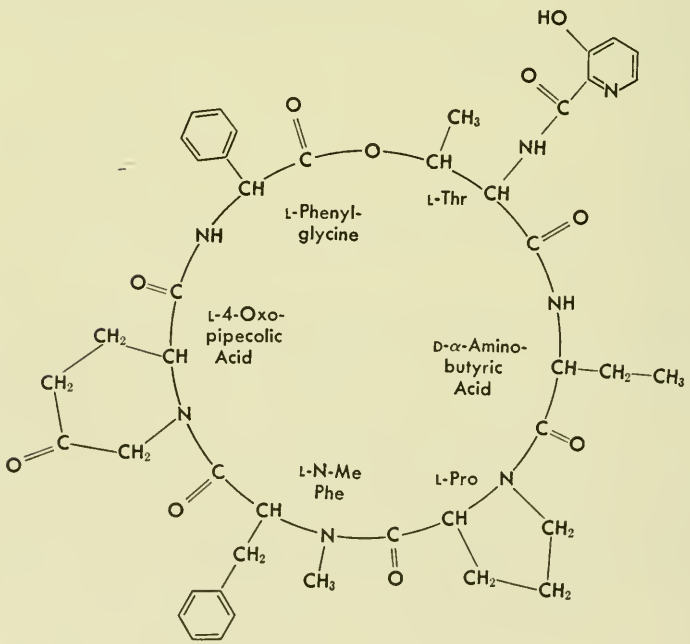
Staphylomycin $M_2$. This third factor has not been obtained pure.

There appears to be a relationship between the staphylomycin complex and streptogramin, etamycin, etc.

*Streptomyces* sp. resembling *S. virginiae*

H. Vanderhaeghe, P. Van Dijck, G. Parmentier and P. De Somer, *Antibiotics and Chemotherapy* 7 606 (1957).

The probable structure of one of the staphylomycins recently was reported to be:

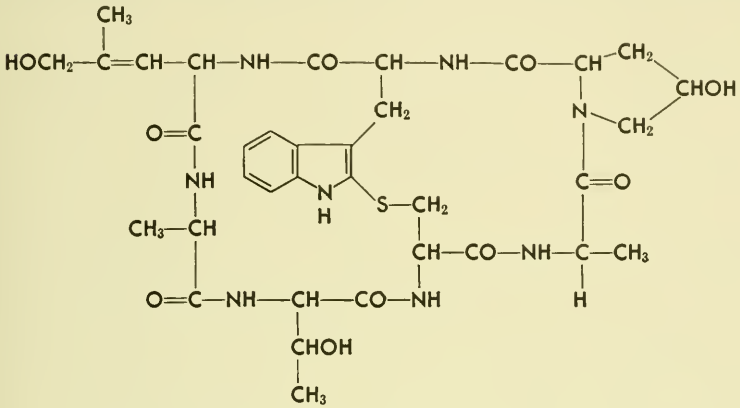



* Identical with PA 114A.

H. Vanderhaege, Abstr. Biochem. Symposium, XVIIth Internat. Congress Pure and Appl. Chem., Munich 1959.

H. Vanderhaege and G. Parmentier, *Bull. Soc. Chim. belges* 68 716 (1959).

756 **Phalloidin,** $C_{35}H_{46}O_{10}N_8S + 6H_2O$.



*Amanita phalloides*

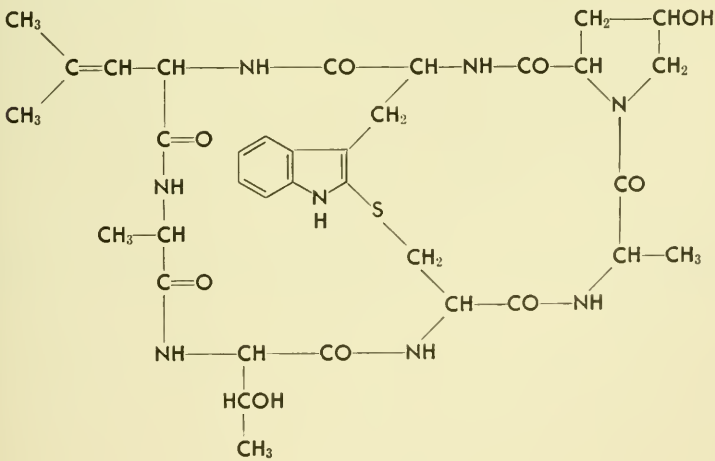
From 100 g. of fresh fungus were obtained 10 mg. of phalloidin, 8 mg. of $\alpha$-amanitin, 5 mg. of $\beta$-amanitin and about 0.5 mg. of $\gamma$-amanitin. The amanitins have not been characterized thoroughly, but seem to be related to phalloidin.

Theodor Wieland, *Angew. Chem.* 69 44 (1957).

Theodor Wieland and Werner Schön, *Ann.* 593 157 (1955).

Theodor Wieland and Christoph Dudensing, *ibid.* 600 156 (1956).

757 **Phalloin,** $C_{35}H_{46}O_9N_8S$, colorless needles, m.p. 250–280° (dec.). Probable structure:

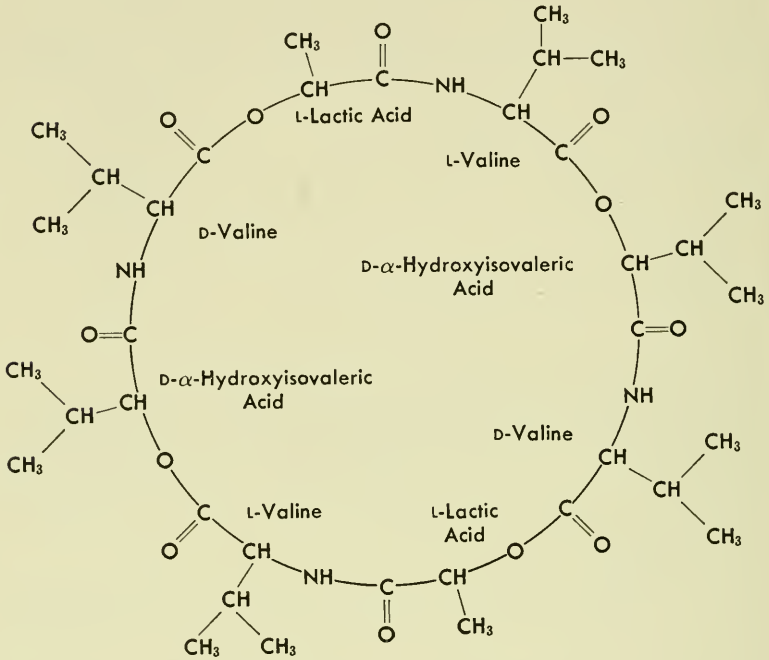


*Amanita phalloides*

Theodor Wieland and Karl Mannes, *Angew. Chem.* 69 389 (1957).

*Idem., Ann.* 617 152 (1958).

758 **Valinomycin**, $C_{36}H_{60}O_{12}N_4$, colorless platelets, m.p. 190°, $[\alpha]_D^{20}$ +31° (c 1.6 in benzene).



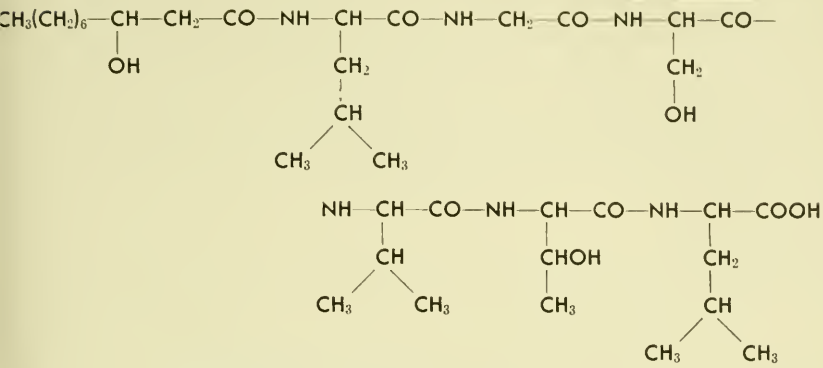

*Streptomyces fulvissimus*

The yield was about 100 mg. per liter. Acid hydrolysis gives 2 moles of L(+)-valine, 2 moles of D(−)-valine, 2 moles of L(−)-lactic acid and 2 moles of D(−)-α-hydroxyisovaleric acid. (*Cf.* the enniatin and lateritiin groups, and amidomycin.)

H. Brockmann and G. Schmidt-Kastner, *Chem. Ber.* 88 57 (1955).

Hans Brockmann and Hermann Geeren, *Ann.* 603 213 (1957).

759 **Viscosin**, $C_{36}H_{66}O_{10}N_6$, amorphous white powder, m.p. 269° (dec.), $[\alpha]_D^{20}$ −162°.



*Pseudomonas viscosa*

Mitsuyuki Kochi, Vincent Groupé, Leonora H. Pugh and David Weiss, *Bact. Proc.*, 29 (1951).

Takashi Ohno, Shigeru Tajima and Katsuyuki Toki, *J. Agr. Chem. Soc. Japan* 27 665 (1953).

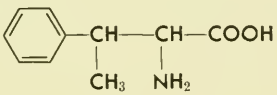
Doki and Ohno (unpublished). Total structure determination. Reported by S. Otani in a lecture on polypeptide antibiotics in 1957.

Takashi Ohno, Shigeru Tajima and Katsuyuki Toki, *J. Agr. Chem. Soc. Japan* 27 665 (1953).

760 **Bottromycin** (B-Mycin), $C_{38}H_{57-61}O_{7-8}N_7S$, white amorphous material, $[\alpha]_D^{25}$ −14.2° (c 0.5 in 96% ethanol).

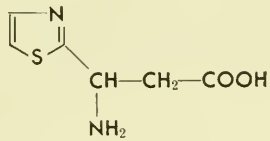
Bottromycin is a weakly basic polypeptide. Acid hydrolysis yields six ninhydrin-positive compounds. Two of these are glycine and valine. Two others are new amino acids:

α-Amino-β-phenylbutyric Acid



and

β-(2-Thiazole)-β-alanine



*Streptomyces bottropensis*

J. M. Waisvisz, M. G. van der Hoeven, J. van Peppen and W. C. M. Zwennis, *J. Am. Chem. Soc.* 79 4520 (1957). (Isolation)

J. M. Waisvisz, M. G. van der Hoeven, J. F. Hölscher and B. te Nijenhuis, *ibid.* 79 4522 (1957).

J. M. Waisvisz, M. G. van der Hoeven and B. te Nijenhuis, *ibid.* 79 4524 (1957). (Structure)

**Micrococcins.**

761 **Micrococcin,** white needles, m.p. 222–228° (dec.), $[\alpha]_D^{21}$ 116° ±1° (c 5.0 in 90% ethanol), molecular weight >2000.

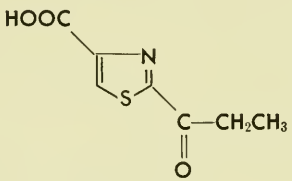
A *Micrococcus* sp.

T. L. Su, *Brit. J. Exptl. Path.* 29 473 (1948).

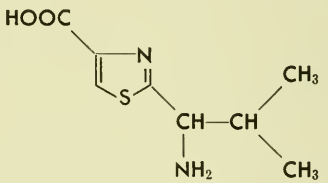
N. G. Heatley and Hazel M. Doery, *Biochem. J.* 50 247 (1951).

762 **Micrococcin-P,** white crystals, yellowing in light, m.p. 252° (dec. from 232°), $[\alpha]_D^{21}$ +63.7° (c 1.19 in 90% ethanol), molecular weight ~2200.

Two fragments have been identified as:




2-Propionylthiazole-4-carboxylic Acid




2-(1-Amino-2-methylpropyl) thiazole-4-carboxylic Acid

Acid-catalyzed esterification gave a dimethyl ester, $C_{24}H_{23}O_5N_5S_4$ and a base $C_{16}H_{19}O_3N_3S_3$. Also threonine, ammonia and propionic acid were isolated.

This antibiotic seems to be similar to or identical with the earlier one, but is distinguished by the letter P until identity is proved.

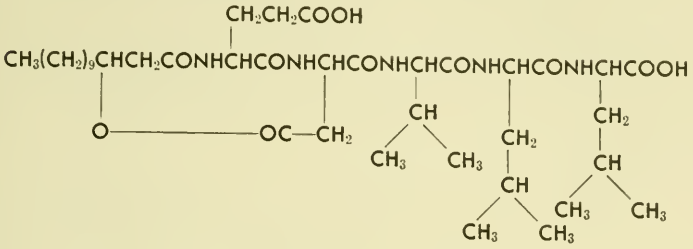
*Bacillus pumilis*

A. T. Fuller, *Nature* 175 722 (1955). (Isolation)

E. P. Abraham, N. G. Heatley, P. Brookes, A. T. Fuller and James Walker, *ibid.* 178 44 (1956).

P. Brookes, A. T. Fuller and James Walker, *J. Chem. Soc.*, 689 (1957).

763 **Esperin,** $C_{39}H_{67}O_{11}N_5$, colorless crystals, m.p. 238° (dec.), $[\alpha]_D^{15}$ −24° (c 0.66 in methanol).



*Bacillus mesentericus*

Hiroshi Ogawa and Teiichiro Ito, *J. Agr. Chem. Soc. Japan* 24 191 (1950). (Isolation)

*Idem., ibid.* 26 432 (1952).

*Idem., Bull. Agr. Chem. Soc.* (Japan) 23 536 (1959). (Structure)

764 **Actinochrysin,** $C_{40}H_{57}O_{11}N_7$, a brick red pigment.

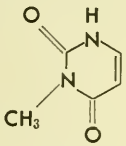
Similar to but distinct from actinomycins. A weak base with two acid groups. Molecular weight 811. Soluble in acetone.

*Streptomyces chrysomallus*

Hans Brockmann and Arnold Bohne, German Patent 912,-010 (1954). (*Chem. Abstr.* 52 12334g)

765 **Grisein,** $C_{40}H_{61}O_{20}N_{10}SFe$, red, amorphous powder.

Isolated from acid hydrolysate:



3-Methyluracil

+ Glutamic Acid
+ An unidentified amino acid

The iron is $Fe^{III}$ and can be removed and readded to the complex.

*Streptomyces griseus*

The Russian antibiotic, albomycin, produced by *Streptomyces subtropicus* seems to be similar to or identical with grisein.

Donald M. Reynolds, Albert Schatz and Selman A. Waksman, *Proc. Soc. Exp. Biol.* 64 50 (1947). (Isolation)

Donald M. Reynolds and Selman A. Waksman, *J. Bact.* 55 739 (1948).

Frederick A. Kuehl, Jr., Mary Neale Bishop, Louis Chaiet and Karl Folkers, *J. Am. Chem. Soc.* 73 1770 (1951).

766 **Albomycin** (Sulfate), red amorphous powder, molecular weight >1300.

Partial Constitution:

Albomycin is a basic, cyclic polypeptide containing iron (~4% by weight). Iron can be removed with acetone (color loss) and restored with $FeCl_3$. Hydrolysis yields: ornithine, serine, glutamic acid, alanine, glycine, proline and one unidentified amino acid.

*Streptomyces subtropicus* n. sp.

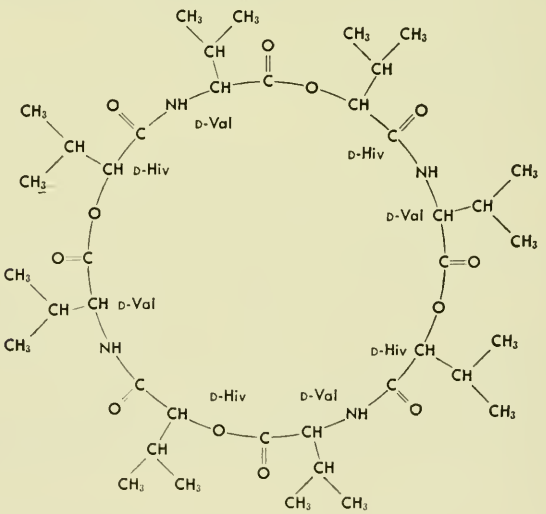
Albomycin may be identical with grisein, produced by *Streptomyces griseus*.

M. G. Brazhnikova, N. N. Lomakina and L. I. Murayeva, *Doklady Akad. Nauk S.S.S.R.* 99 827 (1954).

G. F. Gause, *Brit. Med. J.* 2 1177 (1955).

Yu. O. Sazykin, *Mikrobiologiya* 24 75 (1955).

767 **Amidomycin**, $C_{40}H_{68}O_{12}N_4$, colorless needles, m.p. 192°, $[\alpha]_D^{26}$ +19.2° (c 1.2 in ethanol).




*Streptomyces species* (PRL 1642)

Amidomycin contains 4 moles each of D(−) valine and D(−) α-hydroxyisovaleric acid. (*Cf.* Valinomycin, lateritiins, enniatins.)

L. C. Vining and W. A. Taber, *Can. J. Chem.* 35 1109 (1957).

768 **Toxin of *Helminthosporium victoriae*.**

This toxin consists of two loosely connected moieties. The first is a tricyclic secondary amine called victoxinine, and the second a pentapeptide. The intact toxin shows a negative ninhydrin test, and a molecular weight of 800 was assumed.

Victoxinine, $C_{17}H_{29}ON$ (Hydrochloride), colorless needles, m.p. 172°, $[\alpha]_D^{25}$ −78° (c 3.2 in 95% alcohol). Negative U.V.

Pentapeptide:

On acid hydrolysis yielded:

Aspartic acid, glutamic acid, glycine, valine and one of the leucines.

*Helminthosporium victoriae*

Ross B. Pringle and Armin C. Braun, *Nature* 181 1205 (1958).

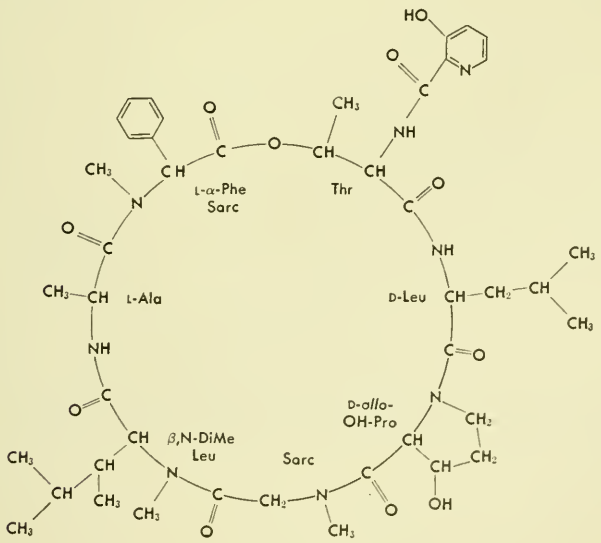
769 **Telomycin**, cream colored amorphous solid.

A polypeptide antibiotic, containing glycine, alanine, threonine and aspartic acid. Molecular weight about 1000. Contains no sulfur. Negative Fehling, ninhydrin, biuret. Similar to etamycin.

*Streptomyces* sp.

M. Misiek, O. B. Fardig, A. Gourevitch, D. L. Johnson, I. R. Hooper and J. Lein, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 852.

770 **Etamycin** (Viridogrisein), $C_{44}H_{62}O_{10}N_8$, white crystals, hydrochloride m.p. 163–170° (dec.), $[\alpha]_D^{25}$ conflicting reports.



*Streptomyces* sp. resembling *S. lavendulae*

*Cf.* Pyridomycin staphylomycin, osteogrycin, PA-114, mikamycin, streptogramin, telomycin, echinomycin.

This class of polypeptides appears to be related biogenically to the actinomycins.

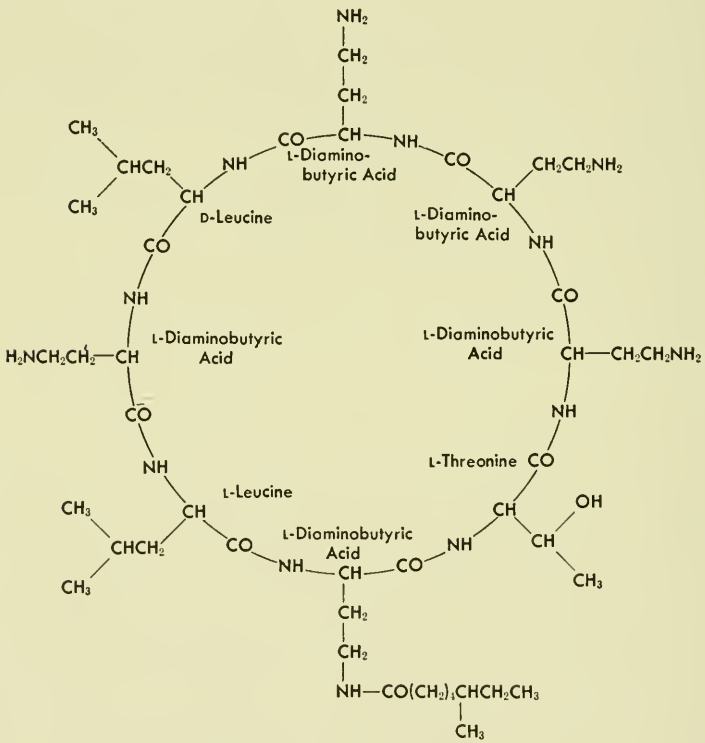
B. Heinemann, A. Gourevitch, J. Lein, D. L. Johnson, M. A. Kaplan, D. Vanas and I. R. Hooper, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, p. 728.

Quentin R. Bartz, Jean Standiford, James D. Mold, Doris W. Johannessen, Albert Ryder, Andrew Maretzki and Theodore H. Haskell, *ibid.*, p. 777.

Theodore H. Haskell, Andrew Maretzki and Quentin R. Bartz, *ibid.*, p. 784.

John C. Sheehan, Hans Georg Zachau and William B. Lawson, *J. Am. Chem. Soc.* 79 3933 (1957). (Structure)

771 **Colistin,** $C_{45}H_{85}O_{10}N_{13}$.




Yasuo Koyama, Akio Kurosasa, Atsushi Tsuchiya and Kinsuke Takakuta, *J. Antibiotics* (Japan) 3 457 (1950).

Taiichi Ito, Sadao Miyamura, Seihachiro Niwayama, Masanobu Oishi, Nobuhiro Igarashi, Hiromichi Hoshino and Shozo Muto, *ibid.* 7B 147 (1954).

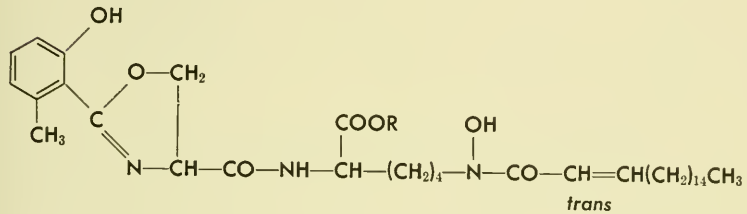
Yasuo Kayama, Japanese Patent 1546 (1952).

Takeshi Oda, Mitsuhiro Kinoshita, Osamu Yamanaka and Fumio Ueda, *J. Pharm. Soc. Japan* 74 1234 (1954).

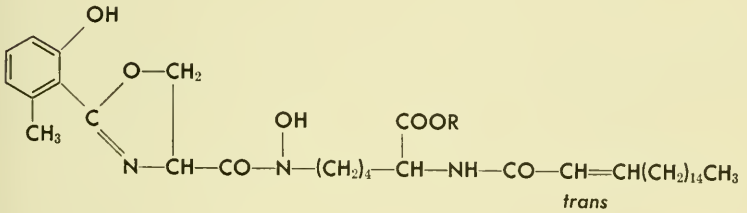
Takeshi Oda and Fumio Ueda, *ibid.* 74 1246 (1954).

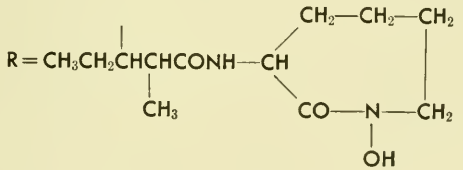
772 **Mycobactin,** $C_{47}H_{75}O_{10}N_5$, microcrystalline white powder with pale green fluorescence, m.p. 165–166.5°, $[\alpha]_D^{25}$ −19° (c 4.9 in chloroform).

Mycobactin is a weak acid believed to have one of the following structures:




or




*Mycobacterium phlei*

The yield was about 67 g. from 41 kg. of moist cells.

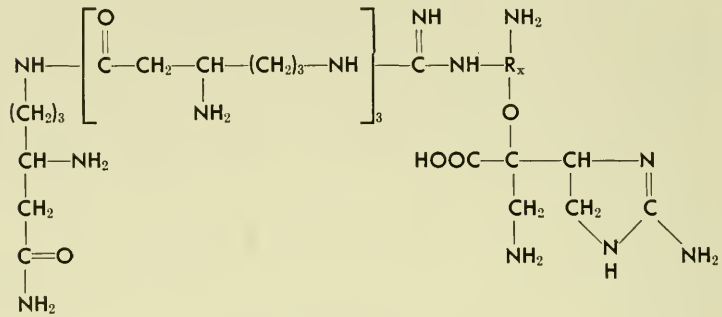
G. A. Snow, *J. Chem. Soc.*, 4080 (1954) and earlier papers in the series.

773 **Geomycin** $(C_6H_{12}O_2N_2)_{8\text{-}10}$, Helianthate red platelets, m.p. 205–215° (dec.), Hydrochloride $[\alpha]_D^{20}$ +16°.

A basic polypeptide. Acid hydrolysis yields: geamine, β-lysine, and an amino sugar, plus small amounts of

aspartic acid, glutamic acid, serine, threonine, glycine and alanine.

The structural evidence has been well summarized and a partial structure postulated by R. Cölln, Ph.D. Dissertation, Göttingen, 1957. The partial structure is:



*Streptomyces xanthophaeus*, n.sp.

Hans Brockmann and Burchard Franck, *Naturwissenschaften* 41 451 (1954).

Hans Brockmann and Hans Musso, *Chem. Ber.* 87 1779 (1954).

*Idem., ibid.* 88 648 (1955).

774 **Lavendulin** (Helianthate), $C_{49}H_{63}O_{18}N_{13}S$ (proposed), orange crystals, m.p. 212–220° (dec.).

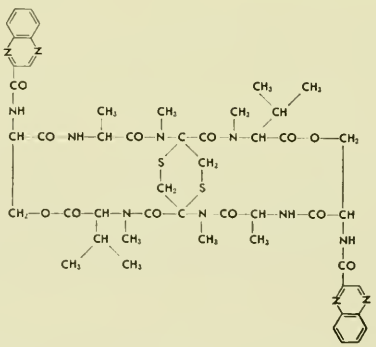
A basic polypeptide. Positive $FeCl_3$, Fehling, biuret, $KMnO_4$. Negative Molisch, Sakaguchi.

*Streptomyces* sp. similar to *S. lavendulae*

Albert Kelner and Harry E. Morton, *J. Bact.* 53 695 (1947). (Isolation)

Harry E. Morton, *Proc. Soc. Exp. Biol. Med.* 64 327 (1947).

775 **Echinomycin** (X-948),* $C_{50}H_{60}O_{12}N_{12}S_2$, granular, nearly colorless hygroscopic powder, m.p. 217°, $[\alpha]_D$ −310° (c 0.86 in chloroform).



* Antibiotic X-1008 (unclassified) resembles echinomycin.

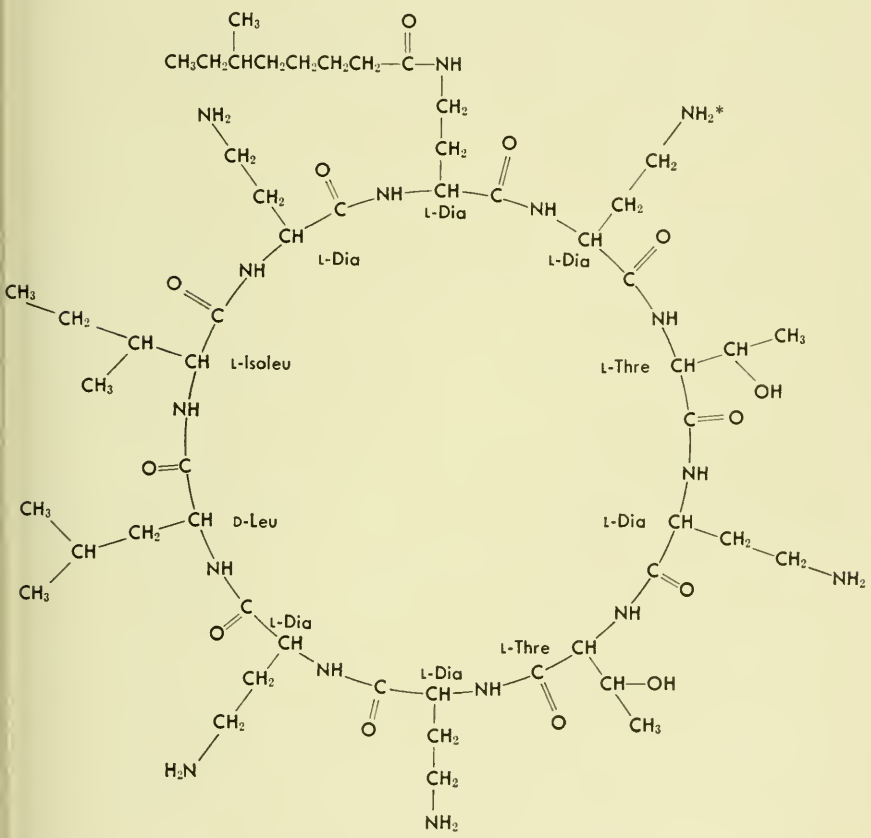
*Streptomyces echinatus* n. sp.

R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog, P. Reusser and H. Zähner, *Helv. Chim. Acta* 40 199 (1957). (Isolation)

W. Keller-Schierlein, M. Lj. Mikhailovich and V. Prelog, *ibid.* 42 305 (1959). (Structure)

**Circulins**, $C_{53}H_{100}O_{13}N_{16}$ (Sulfate), amorphous solid, m.p. 226–228° (dec.), $[\alpha]_D^{25}$ −61.6°.

776 **Circulin A:**




*Bacillus circulans*

Hydrolysis yields 6 moles of L-α, γ-diaminobutyric acid, 2 moles of L-threonine, 1 mole of D-leucine, 1 mole of L-isoleucine and 1 mole of (+)6-methyloctanoic acid.

77 **Circulin B** has essentially the same structure, but the 6-methyloctanoic acid moiety is attached at the starred amino

group. There may be other similar compounds in the complex also.

F. J. Murray, P. A. Tetrault, O. W. Kaufman, H. Koffler, D. H. Peterson and D. R. Colingsworth, *J. Bact.* 57 305 (1949).

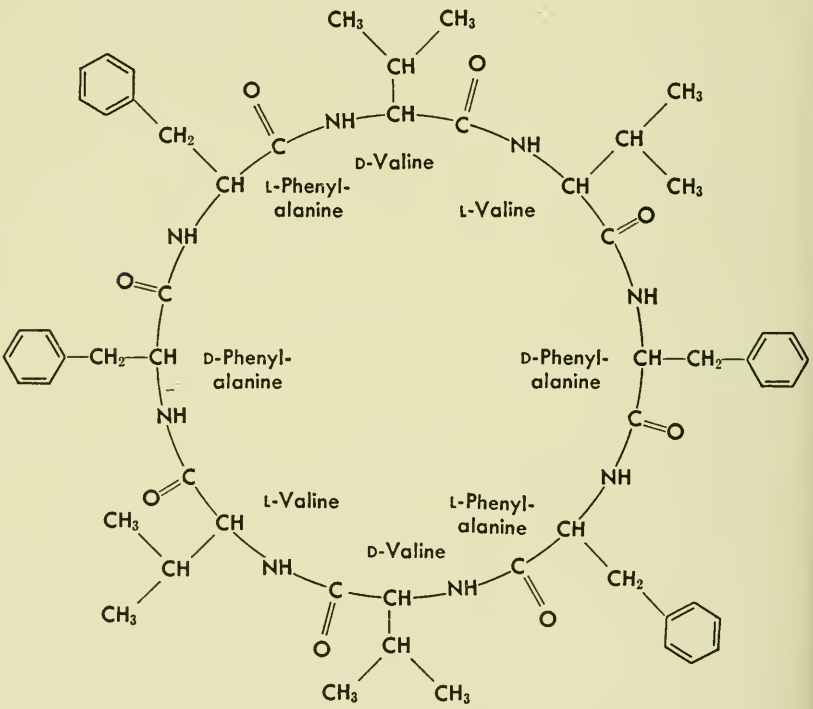
D. H. Peterson and L. M. Reineke, *J. Biol. Chem.* 181 95 (1949).

Tashio Kobayashi, J. E. Grady, J. L. Parsons, Henry Koffler and P. A. Tetrault, *Abstr. 133rd Meeting Am. Chem. Soc.*, 25C (1958).

H. Koffler and T. Kobayashi, *Abstr. 4th Intern. Congr. Biochem.*, 9 (1958).

Henry Koffler, *Science* 130 1419 (1959).

778 **Fungisporin,** $C_{56}H_{72}O_8N_8$, colorless crystals, m.p. 355–360° (dec.) (subl. from 250°), molecular weight 980. Proposed structure:




*Penicillium* and *Aspergillus* spp.

This polypeptide was obtained by destructive distillation of spores, when it separated by sublimation.

U. Sumiki and K. Miyao, *J. Agr. Chem. Soc. Japan* 26 27 (1952).

*Idem., Bull. Agr. Chem. Soc.* (Japan) 19 86 (1955).

Kohei Miyao, *ibid.* 24 23 (1960).

779 **Polypeptin** (formerly called circulin, but not identical with the polypeptide now known as circulin), $C_{56}H_{96}O_{13}N_{12}$, colorless crystals, m.p. 176°, $[\alpha]_D^{20}$ (Sulfate) −93.3° (c 3.0 in 70% isopropyl alcohol).

A basic polypeptide, containing: three α,γ-diaminobutyric acids, one L-threonine, one D-valine, one L-isoleucine, two L-leucines and one D-phenylalanine.

*Bacillus krzemieniewski*, a *B. circulans* mucoid variant

Stacey F. Howell, *J. Biol. Chem.* 186 863 (1950).

Werner Hausmann and Lyman C. Craig, *ibid.* 198 405 (1952).

**Polymyxins:**

780 **Polymyxin A** (Aerosporin)

781 **Polymyxin $B_1$**

782 **Polymyxin $B_2$**

783 **Polymyxin C**

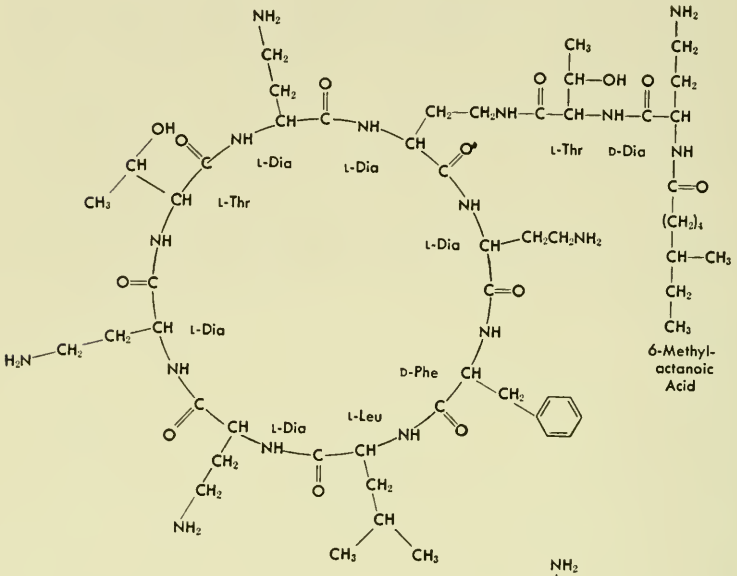
784 **Polymyxin D**

785 **Polymyxin E**

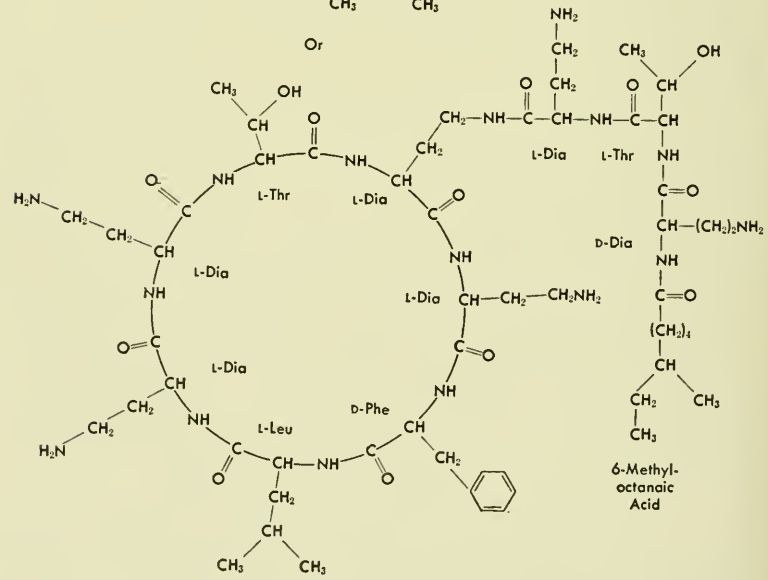
A complex of related polypeptides produced by *Bacillus polymyxa*. Initially five components, A, B, C, D and E were separated. Then B was resolved into two components $B_1$ and $B_2$, differing only in the fatty acid moiety. All polymyxins contain L-α, γ-diaminobutyric acid and L-threonine. All but $B_2$ apparently contain *d*-6-methyloctanoic acid, and it contains a C-8 acid instead. Polymyxin A has been reported to contain D-leucine but no phenylalanine. It is also known as aerosporin because it is produced by *Bacillus aerosporus*. Polymyxin C contains phenylalanine but no leucine. Polymyxin D contains leucine but no phenylalanine, and it also has been reported to contain D-serine. Polymyxin E has the same qualitative composition as A, but is distinct.

Two alternative structures have been suggested for polymyxin $B_1$ as the result of degradative studies. These structures are shown here, the amino acids being abbreviated in the following manner:

Dia = α,γ-Diaminobutyric Acid
Thr = Threonine
Phe = Phenylalanine
Leu = Leucine




Or



Commercial polymyxin is essentially polymyxin B sulfate, a white powder, m.p. 228–232° (dec.), $[\alpha]_D^{25}$ −45° (c 0.1). The empirical formula of the free base is $C_{56}H_{96\text{-}98}O_{13}N_{16}$.

G. C. Ainsworth, A. M. Brown and G. Brownlee, *Nature* **160** 263 (1947). (Isolation)

George Brownlee, *Ann. N. Y. Acad. Sci.* **51** 875 (1949). (Polymyxin A)

P. H. Bell, J. F. Bone, J. P. English, C. E. Fellows, K. S. Howard, M. M. Rogers, R. G. Shepherd and R. Winterbottom, *ibid.* **51** 897 (1949). (Degradations, identification of amino acids)

Tudor S. G. Jones, *ibid.* **51** 909 (1949). (Separations, degradations, identification of amino acids)

J. R. Catch, Tudor S. G. Jones and S. Wilkinson, *ibid.* **51** 917 (1949).

P. P. Regna, I. A. Solomons, B. K. Forscher and A. E. Timreck, *J. Clin. Invest.* **28** 1022 (1949). (Purification of B)

Werner Hausmann and Lyman C. Craig, *J. Am. Chem. Soc.* **76** 4892 (1954). (Resolution of B into two parts)

Werner Hausmann, *ibid.* **78** 3663 (1956). (Proposal of detailed cyclic structures)

Gerard Biserte and Michel Dautrevaux, *Bull. soc. chim. biol.* **39** 795 (1957). (Structure)

**Gramicidins.**

A mixture of polypeptides produced by *Bacillus brevis* and originally called tyrothricin was separated into two groups, the tyrocidines (about 80%) and the gramicidins (about 20%). Each of these groups has been fractionated further into pure polypeptides.

The original gramicidin consisted of a mixture of three closely related neutral polypeptides. It was assigned an average empirical formula of $C_{148}H_{210}O_{26}N_{30}$, colorless platelets, m.p. 228–231°, $[\alpha]_D^{20}$ +3°.

| | Fraction A | Fraction B | Fraction C |
|---|---|---|---|
| D-Leucine | + | + | + |
| L-Trypotophan | + | + | + |
| L-Alanine | + | + | + |
| DL-Valine | + | + | + |
| Glycine | + | + | + |
| Phenylalanine | | + | − |
| Tyrosine | | | + |

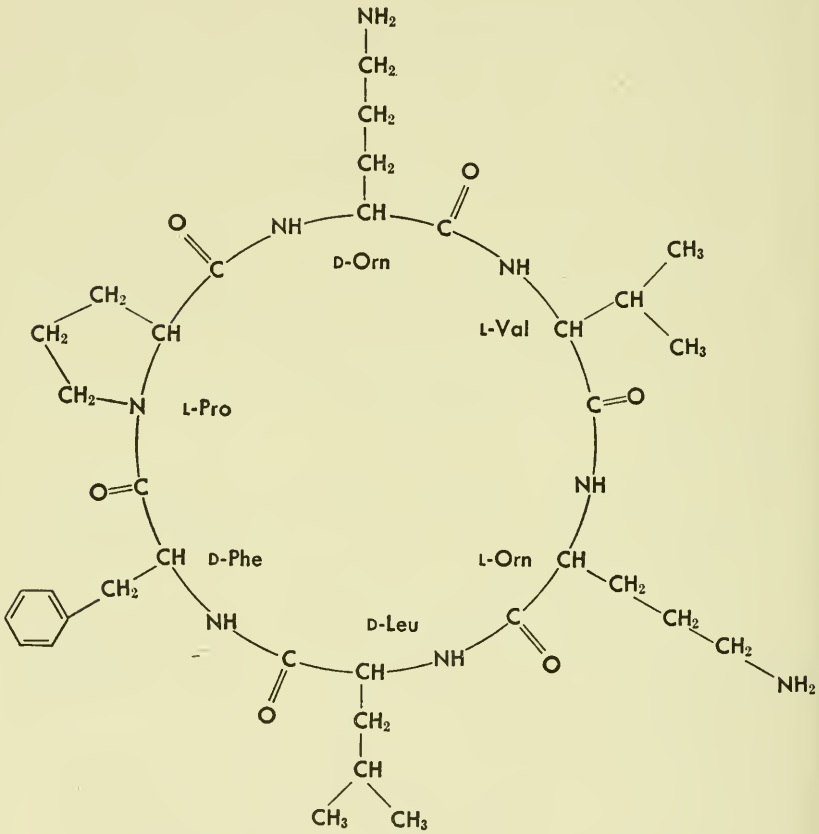
Rollin D. Hotchkiss and René J. Dubos, *J. Biol. Chem.* 132 791 (1940).

*Idem., ibid.* 141 155 (1941). (Isolation)

Max Tishler, J. L. Stokes, N. R. Trenner and John B. Conn, *ibid.* 141 197 (1941).

Rollin D. Hotchkiss, *Advances in Enzymol.* 4 153 (1944).

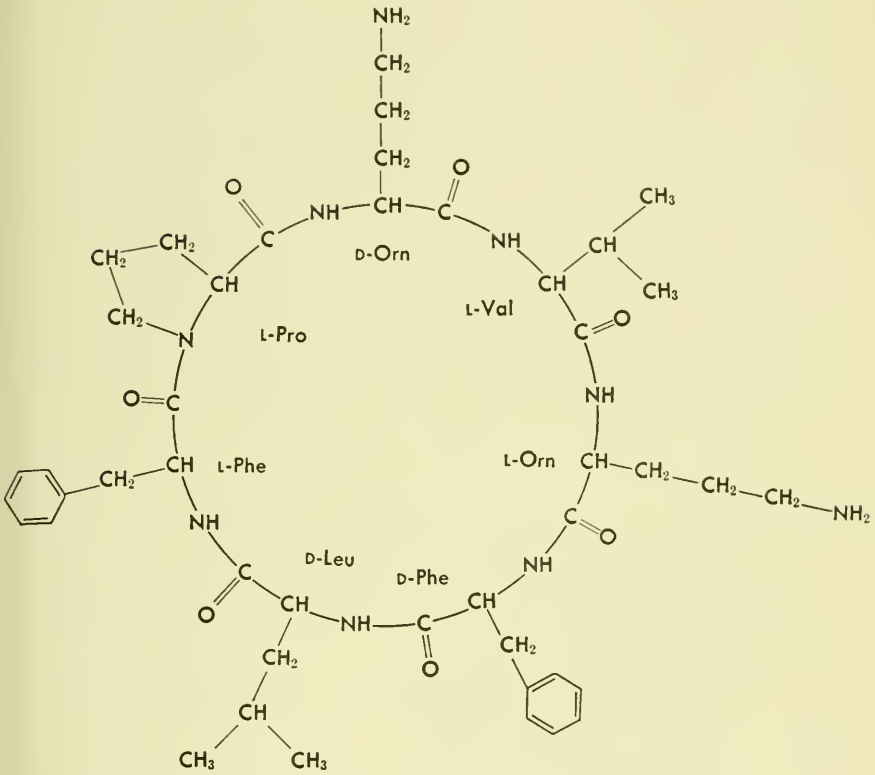
786 **Gramicidin $J_2$**, $C_{35}H_{56}O_6N_8$.




*Bacillus brevis*

Shokei Otani, H. Nagano and Y. Saito, *Osaka Shiritsu Daigaku Igaku Zasshi* 7 640–650 (1958). (*Chem. Abstr.* 12403g)

787 **Gramicidin $J_1$, $C_{44}H_{65}O_7N_9$.**

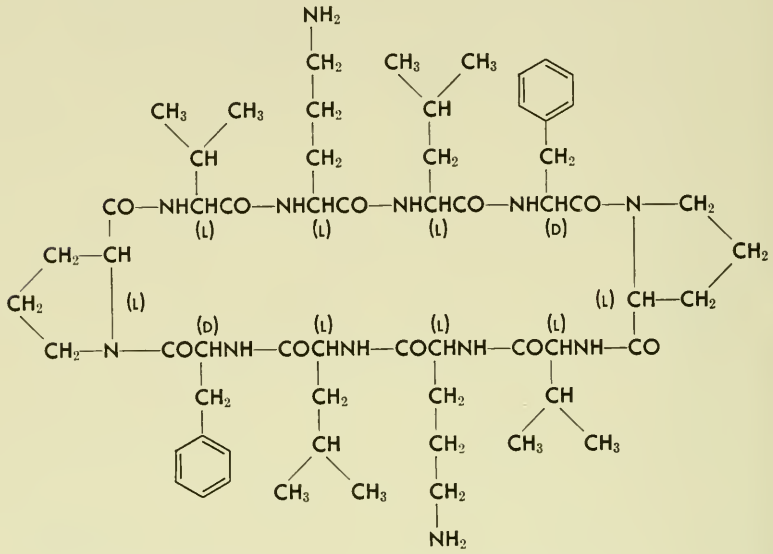



*Bacillus brevis*

Shokei Otani and Yoshitaka Saito, *Proc. Japan. Acad.* 30 991 (1954).

*Idem., Congr. intern. biochim., Résumés Communs.,* 3e Congr., Brussels, 88 (1955).

788 **Gramicidin S** (Gramicidin C), $C_{60}H_{92}O_{10}N_{12}$, colorless needles, m.p. 277° (dec.), $[\alpha]_D^{24}$ −289° ±10° (c 0.43 in 70% ethanol).



*Bacillus brevis* var. Gause-Brazhnikova

G. F. Gause and M. G. Brazhnikova, *Am. Rev. Soviet Med.* 2 134 (1944).

R. L. M. Synge, *Biochem. J.* 39 363 (1945). (Characteristics)

F. Sanger, *ibid.* 40 261 (1946).

R. Consden, A. H. Gordon, A. J. P. Martin and R. L. M. Synge, *ibid.* 40 xciii (1946).

*Idem.*, *ibid.* 41 596 (1947).

Alan R. Battersby and Lyman C. Craig, *J. Am. Chem. Soc.* 73 1887 (1951).

R. Schwyzer and P. Sieber, *Helv. Chim. Acta* 40 624 (1957). (Synthesis)

789 **Gramicidin D** (Gramicidin Dubos), colorless crystals, m.p. 229° (dec.).

A crystalline component of tyrothricin. A cyclic polypeptide composed of 4 moles of D-Leucine, 4 moles of L-tryptophan, 2 moles of D-Valine, 2 moles of L-Valine, 2 moles of L-alanine, 1 mole of glycine and 1 mole of ethanolamine.

*Bacillus brevis*

René J. Dubos and Rollin D. Hotchkiss, *J. Exptl. Med.* 73 629 (1941). (Isolation)

A. H. Gordon, A. T. P. Martin and R. L. M. Synge, *Biochem. J.* 37 86 (1943).

Rollin D. Hotchkiss, *Advances in Enzymol.* 4 153 (1944).
R. L. M. Synge, *Biochem. J.* 39 355 (1945).
T. S. Work, *The relation of optical form to biological activity in the amino acid series,* Biochem. Soc. Symposia 1 61 (1948).

790 **Racemomycin B,** $C_{60}H_{128}O_{32}N_{20}$, white, hygroscopic powder, m.p. 150°, $[\alpha]_D^{19}$ −34° (c 0.5 in water).

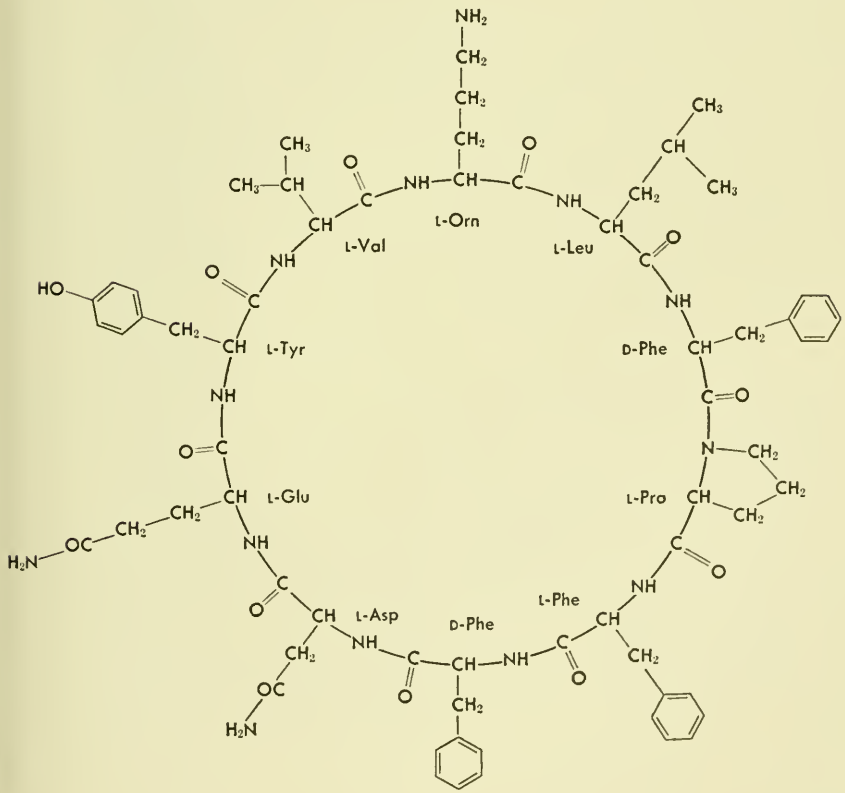
A basic antibiotic resembling streptothricin. Acid hydrolysis gives a reducing sugar and carbon dioxide, β-lysine and roseonine in the ratio 2:3:2. Racemomycin B occurs in a complex with two (apparently similar) substances, racemomycins A and C.

*Streptomyces racemochromogenus* n. sp.

Hyozo Taniyama and Shoji Takemura, *J. Pharm. Soc. Japan* 77 1210 (1957).

*Idem., ibid.* 78 742 (1958).

791 **Tyrocidine A,** $C_{66}H_{86}O_{13}N_{13}$, colorless needles or rods, m.p. 240–242° (dec.), $[\alpha]_D^{25}$ −111°. A component of the tyrothricin complex.



*Bacillus brevis*

Rollin D. Hotchkiss and René J. Dubos, *J. Biol. Chem.* 132 791 (1940). (Isolation)

R. L. M. Synge and A. Tiselius, *Acta Chem. Scand.* 1 749 (1947).

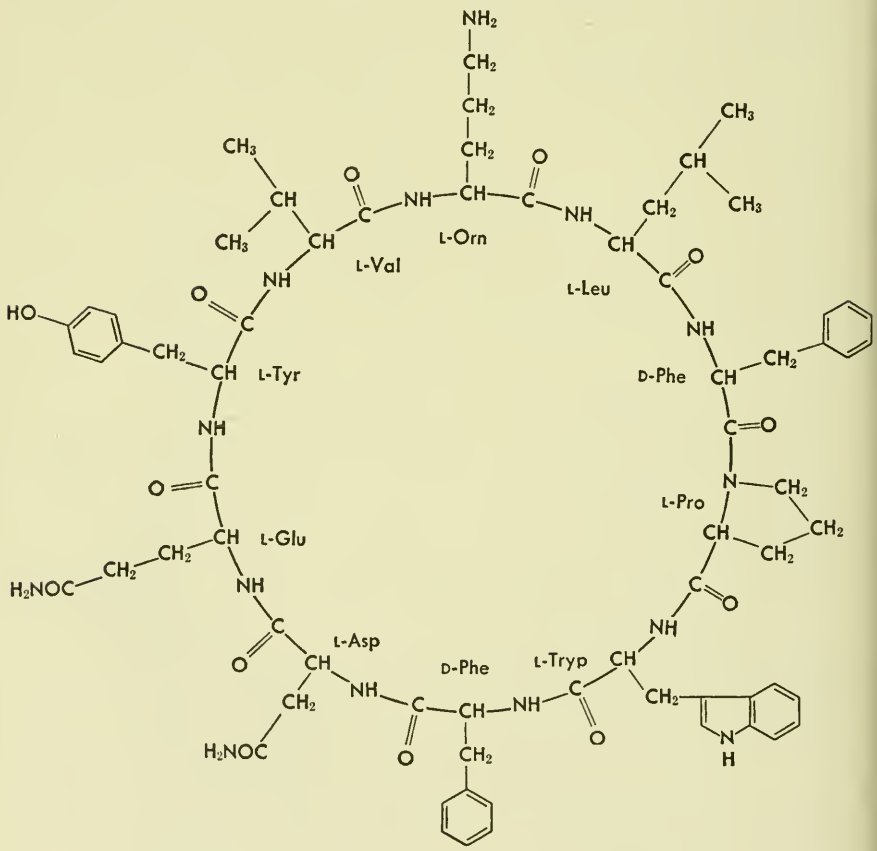
R. L. M. Synge, *Quart. Rev.* 3 245 (1949). (Review of work to that date)

Alan R. Battersby and Lyman C. Craig, *J. Am. Chem. Soc.* 74 4019, 4023 (1952). (Separation)

Alejandro Paladini and Lyman C. Craig, *ibid.* 76 688 (1954). (Structure)

792 **Tyrocidine B,** $C_{68}H_{88}O_{13}N_{14}$.

A component of the tyrothricin complex.



*Bacillus brevis*

T. P. King and L. C. Craig, *J. Am. Chem. Soc.* 77 6627 (1955). (Final structure)

**Actinomycins.**

The nomenclature of the actinomycins is confused because they occur in difficulty separable complex mixtures, several different research groups have investigated them, and, even when pure, one substance cannot be compared with another by techniques as simple as a mixed melting point. This problem has been discussed by Brockmann in a review of the actinomycins.

L. Zechmeister (editor), "Fortschritte der Chemie organischer Naturstoffe" XVIII, Hans Brockmann, *The actinomycins,* Springer Verlag, Vienna, 1960.

At first actinomycins A, B and C were isolated, but later these were found to be mixtures. As such complexes were resolved by paper chromatography, Arabic numeral subscripts were attached to the capital Roman letter in order of appearance on the developed chromatogram, the origin on the paper being zero (*e.g.*, $C_1$, $C_2$, $C_3$). When some of the separated actinomycins were resolved even further, a further subdivision in nomenclature was required; so a lower case Roman letter was attached to give, *e.g.*, $C_{2a}$ which appeared between $C_2$ and $C_3$. When the $X_0$ complex at the origin was resolved, a slightly different system was used, Greek letters being attached to the Arabic numeral subscript, *e.g.*, $X_{0\gamma}$ was less polar than $X_{0\beta}$.

Few series are complete because often names have been eliminated due to duplication, further resolution, etc. Thus, a complex designated I was resolved into $I_1$ and $I_2$, but these later were shown to be the same as $C_1$ and $C_2$ and the I names eliminated.

Still this method of nomenclature does have a rationale, although it may not be readily apparent, and it is used in Germany and in Switzerland.

Other groups continue to refer to various complexes as A, B or D types. These consist essentially of various ratios of actinomycin $X_2$ and its reduction product, actinomycin $C_1$, actinomycin D being nearly pure $C_1$.

The E and F series arose when it was discovered that addition of certain amino acids to the medium in large amounts caused displacement of certain other amino acids in the peptide side-chains, thus creating new "biosynthetic" actinomycins.

Beyond historical interest there seems to be little point in attempting to standardize the nomenclature of actinomycin mixtures. Waksman has proposed that a Roman numeral be assigned to each pure actinomycin, and Johnson's group has taken up this practice, actinomycins II and III being distinct from those characterized elsewhere, while IV is identical with $C_1$ or D, etc. Brockmann views this as one more contribution to the confusion of the literature and, claiming the right of discoverer of many of the actinomycins, has made the suggestion that no nomenclature system will relieve the confusion unless it makes apparent the amino acid sequences of the side-chains.

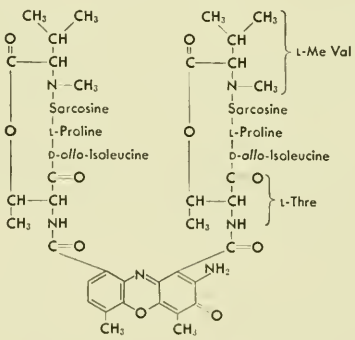
Although this does not solve the problem of trivial nomenclature, Brockmann uses a shorthand method of demonstrating the structures of the actinomycins in which a symbol ——< represents the actinocinin moiety, the branches at the right symbolizing the amino and quinonoid carbonyl groups. The abbreviated amino acid names are then attached in proper sequence. In most of the asymmetric actinomycins the chains in which the differing amino acids occur have not yet been specified, and this is indicated by an ⊐-symbol, indicating possible reversal of position.

The structure of actinomycin $C_3$ (which has been synthesized) is given somewhat more fully to show structural details. The custom of arrangement by empirical formula is ignored here to permit grouping by related structures.

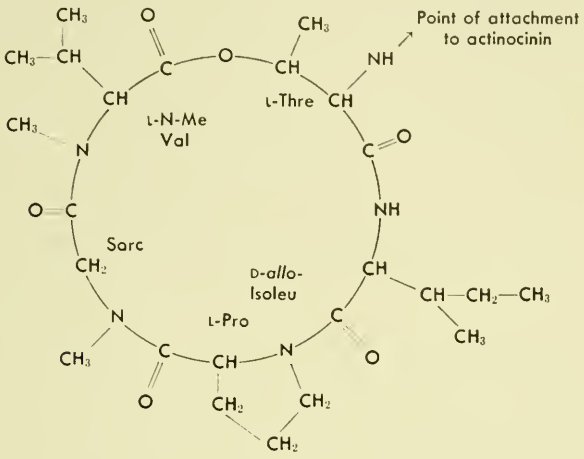
The mitomycins (unclassified) may be actinomycins.

There is an apparent striking biogenetic similarity among the etamycin, staphylomycin, etc. group of polypeptides on the one hand and the actinomycins on the other.

793 **Actinomycin $C_3$** (VII) $C_{64}H_{90}O_{16}N_{12}$, red crystals, m.p. 232–235° (dec.), $[\alpha]_D^{19}$ −321° ±10°.



Below is shown one of the peptide side-chains of actinomycin $C_3$ to permit comparison with etamycin, etc.



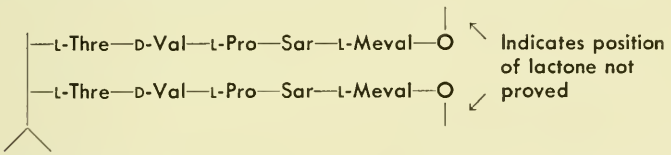

*Streptomyces antibioticus, S. chrysomallus*

H. Brockmann, G. Bohnsack, B. Franck, H. Gröne, H. Muxfeldt and C. Süling, *Angew. Chem.* 68 70 (1956) and preceding papers. (Structure)

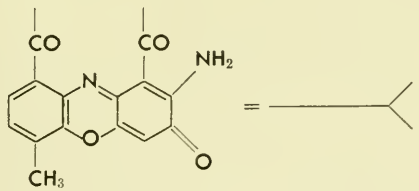
H. Brockmann, W. Sunderkötter, K. W. Ohly and P. Boldt, *Naturwissenschaften* 47 230 (1960).

H. Brockmann and L. Lackner, *ibid.*, 47 230 (1960).

794 **Actinomycin $C_1$** (D,IV,$X_1$,$B_1$,$I_1$) $C_{61}H_{90}O_{16}N_{12}$ red prisms, m.p. 241° (235.5–236.5) (dec.) $[\alpha]_D^{20}$ −349° ±10° (337°).




Where



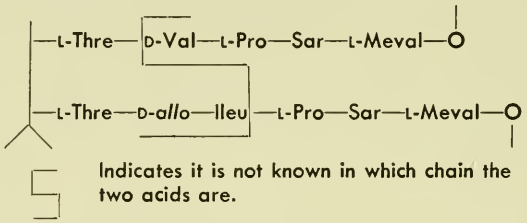
*Streptomyces chrysomallus, S. antibioticus; S. parvullus*

A. W. Johnson and A. B. Mauger, *Biochem. J.* 73 535 (1959).

Hans Brockmann and Hans-Sieghard Petras, *Naturwissenschaften* 46 400 (1959).

Hans Brockmann, P. Boldt and Hans-Sieghard Petras, *ibid.* 47 62 (1960).

795 **Actinomycin $C_2$** (VI) $C_{62}H_{92}O_{16}N_{12}$ red crystals, m.p. 237° (dec.), $[\alpha]_D^{19}$ −325° ±10°.




*Streptomyces chrysomallus*

A. W. Johnson and A. B. Mauger, *Biochem. J.* 73 535 (1959).

Hans Brockmann and Hans-Sieghard Petras, *Naturwissenschaften* 46 400 (1959).

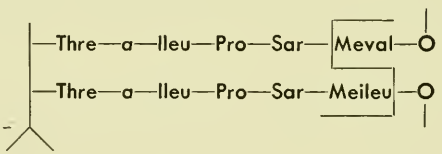
Hans Brockmann, P. Boldt and Hans-Sieghard Petras, *ibid.* 47 62 (1960).

$C_{2a}$ $C_{62}H_{92}O_{26}N_{12}$ an isomer of $C_2$ found by paper chromatography.

*Streptomyces chrysomallus*

Hans Brockmann and B. Franck, *ibid.* 47 15 (1960).

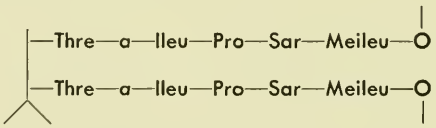
796 **Actinomycin $E_1$**, $C_{64}H_{96}O_{16}N_{12}$.




*Streptomyces* sp.

Günther Schmidt-Kastner, *Naturwissenschaften* 43 131 (1956).

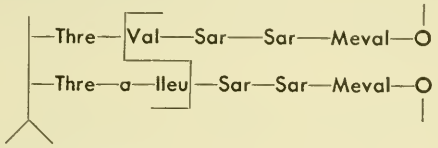
797 **Actinomycin $E_2$**, $C_{65}H_{98}O_{16}N_{12}$.




*Streptomyces* sp.

Günther Schmidt-Kastner, *Naturwissenschaften* 43 131 (1956).

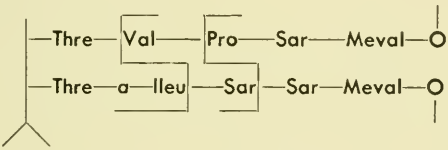
798 **Actinomycin $F_1$**, $C_{58}H_{88}O_{16}N_{12}$.




*Streptomyces* sp.

Günther Schmidt-Kastner, *Naturwissenschaften* 43 131 (1956).

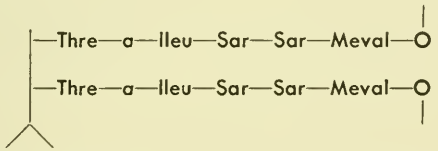
799 **Actinomycin $F_2$**, $C_{60}H_{90}O_{16}N_{12}$.




*Streptomyces* sp.

Günther Schmidt-Kastner, *Naturwissenschaften* 43 131 (1956).

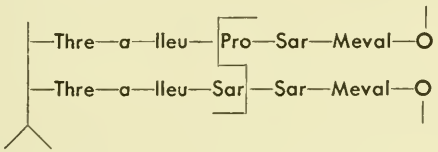
800 **Actinomycin $F_3$**, $C_{59}H_{90}O_{16}N_{12}$.




*Streptomyces* sp.

Günther Schmidt-Kastner, *Naturwissenschaften* 43 131 (1956).

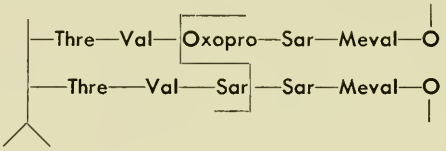
801 **Actinomycin $F_4$**, $C_{61}H_{92}O_{16}N_{12}$.




*Streptomyces* sp.

Günther Schmidt-Kastner, *Naturwissenschaften* 43 131 (1956).

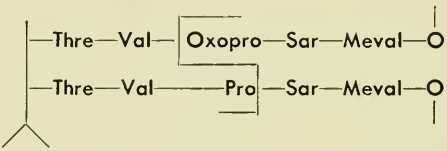
802 **Actinomycin $X_{1a}$**, $C_{59}H_{87}O_{17}N_{12}$.




*Streptomyces chrysomallus, S. fradiae*

Hans Brockmann and H. Gröne, *Chem Ber.* 87 1036 (1954).

803 **Actinomycin $X_2$** (V, $B_2$) $C_{61}H_{89}O_{17}N_{12}$ red plates, m.p. 244–246°, $[\alpha]_D^{19}$ −341° ±10°.




*Streptomyces chrysomallus, S. fradiae*

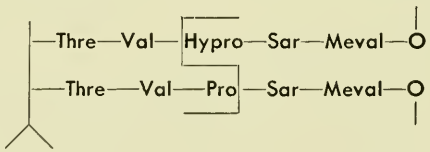
Hans Brockmann and Hans Gröne, *Chem. Ber.* 87 1036 (1954).

804 **Actinomycin $X_3$**, needles.

An actinomycin $X_3$, containing threonine, sarcosine, proline, valine, isoleucine and N-methylvaline, also has been isolated.

H. Brockmann and H. Gröne, *Chem. Ber.* 87 1036 (1954).
Werner Frommer, *Arch. Mikrobiol.* 34 1 (1959).

805 **Actinomycin $X_{0\beta}$** (I) $C_{61}H_{90}O_{17}N_{12}$ yellow needles, m.p. 245–247, $[\alpha]_D^{20}$ −260° ±10° (c 0.22 acetone).

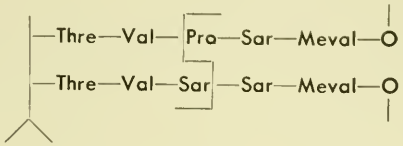



*Streptomyces chrysomallus, S. fradiae*

Hans Brockmann, Gottfried Pampus and Jost H. Manegold, *Chem. Ber.* 92 1294 (1959).

Hans Brockmann and H. Gröne, *Chem Ber.* 87 1036 (1954).

806 **Actinomycin $X_{0\gamma}$**, $C_{59}H_{88}O_{16}N_{12}$.

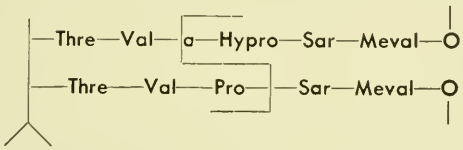



*Streptomyces chrysomallus, S. fradiae*

Hans Brockmann and Gottfried Pampus, *Angew. Chem.* 67 519 (1955).

H. H. Martin and Gottfried Pampus, *Arch. Mikrobiol.* 25 90 (1956).

807 **Actinomycin $X_{0\delta}$**, $C_{64}H_{90}O_{17}N_{12}$.




*Streptomyces chrysomallus, S. fradiae*

Same references as Actinomycin $X_{0\gamma}$.

**Actinomycins Z.**

All contain the same five amino acids on hydrolysis: threonine, sarcosine, N-methylalanine, valine and N-methylvaline.

808 **Actinomycin $Z_0$**, amorphous orange-red powder, m.p. 250° (dec.).

*Streptomyces fradiae*

R. Bossi, R. Hütter, W. Keller-Schierlein, L. Neipp and H. Zähner, *Helv. Chim. Acta* 41 1645 (1958).

809 **Actinomycin $Z_1$**, orange-red crystals, m.p. 256–260 (dec.), $[\alpha]_D$ −362° (c 0.185 in $CHCl_3$).

*Streptomyces fradiae*

R. Bossi, R. Hütter, W. Keller-Schierlein, L. Neipp and H. Zähner, *Helv. Chim. Acta* 41 1645 (1958).

**Actinomycins $Z_2$,$Z_3$,$Z_4$**, an inseparable mixture, m.p. 256–260° (dec.), $[\alpha]_D$ −246 (c 0.257 in $CHCl_3$).

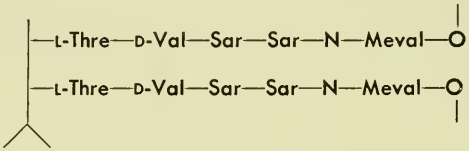
R. Bossi, R. Hütter, W. Keller-Schierlein, L. Neipp and H. Zähner, *Helv. Chim. Acta* 41 1645 (1958).

810 **Actinomycin $Z_5$**, short red staffs, m.p. 261–267 (dec.), $[\alpha]_D$ −284° (c 0.244 in $CHCl_3$).

*Streptomyces fradiae*

R. Bossi, R. Hütter, W. Keller-Schierlein, L. Neipp and H. Zähner, *Helv. Chim. Acta* 41 1645 (1958).

811 **Actinomycin II**, $C_{57}H_{86}O_{16}N_{12}$ red plates, m.p. 215° $[\alpha]_D^{17}$ −157° (c 0.24 in $CHCl_3$).

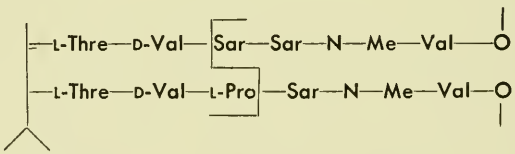



*Streptomyces chrysomallus*

A. W. Johnson and A. Mauger, *Biochem. J.* 73 535 (1959).

William A. Goss and Edward Katz, *Antibiotics and Chemotherapy* 10 221 (1960).

812 **Actinomycin III**, $C_{59}H_{86}O_{16}N_{12}$ red prisms, m.p. 237°, $[\alpha]_D^{19}$ −205° (c 0.22 in $CHCl_3$).

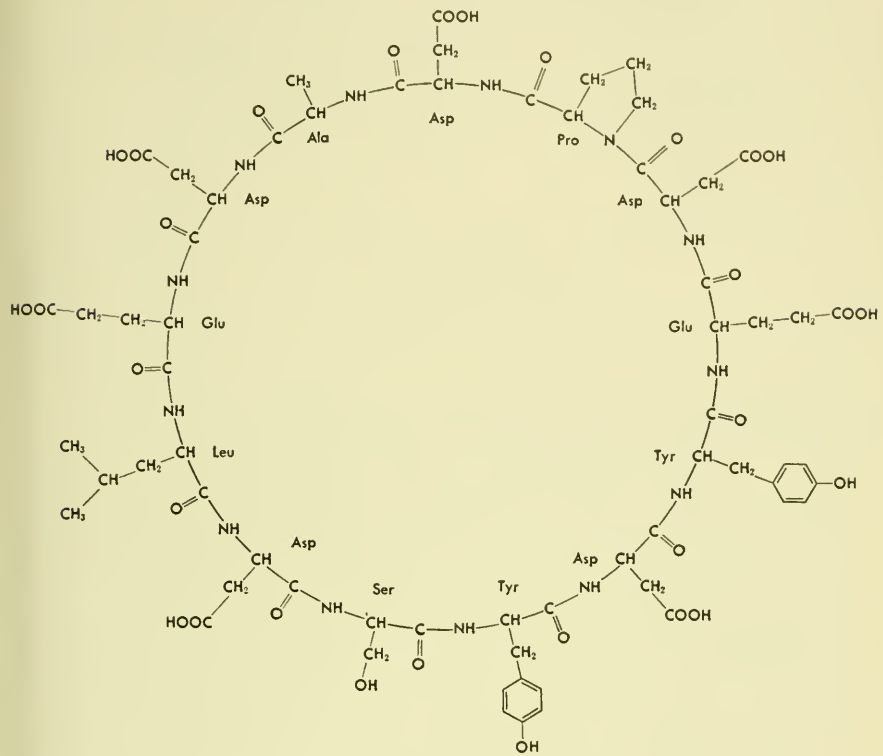



*Streptomyces chrysomallus*

A. W. Johnson and A. Mauger, *Biochem. J.* 73 535 (1959).

William A. Goss and Edward Katz, *Antibiotics and Chemotherapy* 10 221 (1960).

813 **Mycobacillin**, $C_{65}H_{85}O_{30}N_{13}$, colorless needles.



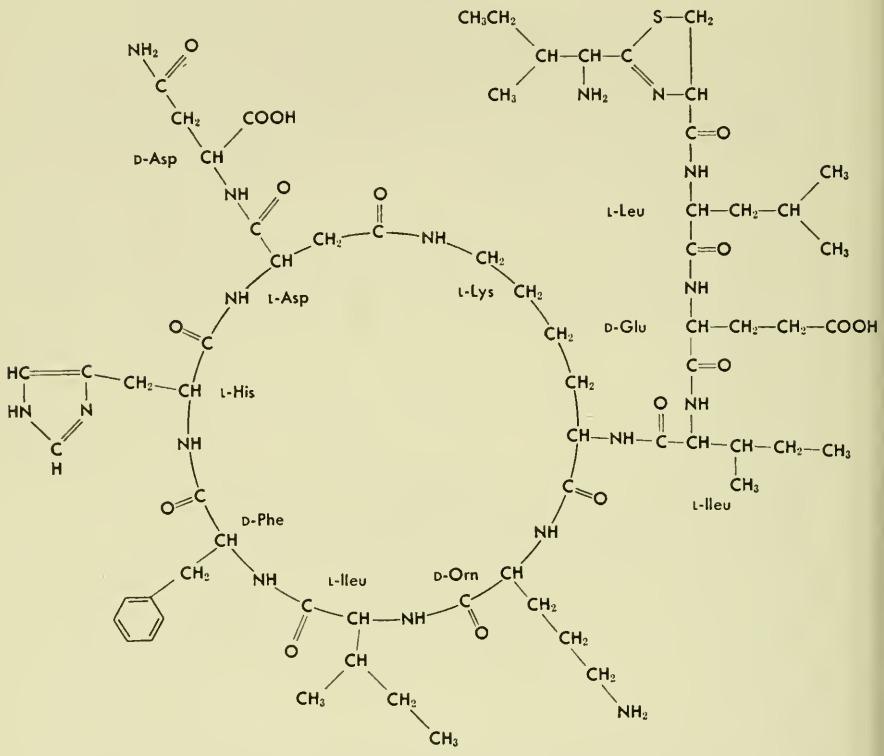

*Bacillus subtilis*

Hydrolysis yields five aspartic acids, two glutamic acids, two tyrosines, one proline, one serine, one leucine and one alanine. (Unspecified configurations)

S. K. Majumdar and S. K. Bose, *Nature* 181 134 (1958). (Isolation)

*Idem., Biochem. J.* 74 596 (1960). (Structure)

814 Bacitracin A, $C_{66}H_{103}O_{16}N_{17}S$, white, hygroscopic, amorphous powder, $[\alpha]_D^{23}$ +5° (±2.5°).




*Bacillus subtilis, B. licheniformis*

The bacitracins are a difficultly separable polypeptide complex. Bacitracins A, B, C, D, E, $F_1$, $F_2$, $F_3$ and G have been differentiated. The F series may be artifacts. The structure of bacitracin A has received the most attention. In certain of the other bacitracins isoleucine is replaced by valine. The complex from *B. licheniformis* was originally called ayfivin.

I. M. Lockhart, E. P. Abraham and G. G. F. Newton, *Biochem. J.* 61 534 (1955).

J. R. Weisiger, W. Hausmann and L. C. Craig, *J. Am. Chem. Soc.* 77 731, 3123 (1955).

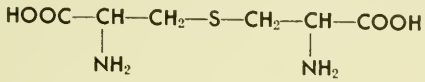
Dorothy Wrinch, *Nature* 179 536 (1957).

E. P. Abraham, "CIBA Lectures in Microbial Biochemistry,"

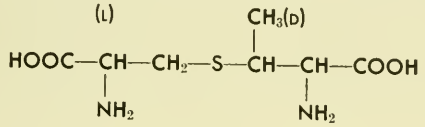
*The bacitracins*, John Wiley and Sons, New York, 1957, pp. 1–30. (A review which also covers the earlier work)

815 **Subtilin**, amorphous white powder, $[\alpha]_D^{23}$ −29° to −35°.

Subtilin is a basic polypeptide, molecular weight 3188, which yields 11 common amino acids, lanthionine:



and a new S-amino acid, probably β-methyllanthionine:



The common amino acids identified are: glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, lysine, asparagine and glutamic acid.

*Bacillus subtilis*

Eugene F. Jansen and Doris J. Hirschmann, *Arch. Biochem.* 4 297 (1944).

A. J. Salle and Gregory J. Jann, *Proc. Soc. Exp. Biol.* 60 60 (1945).

W. Steenken, Jr. and E. Wolinsky, *J. Bact.* 57 453 (1949).

J. C. Lewis and N. S. Snell, *J. Am. Chem. Soc.* 73 4812 (1951).

Gordon Alderton, *ibid.* 75 2391 (1953).

**Nisins**, nearly white needles.

Consist of four active cyclic polypeptides. All contain lanthionine and β-methyllanthionine. These amino acids also occur in the antibiotics, subtilin, cinnamycin and duramycin.

816, 817, 818 **Nisins A, B** and **C** contain leucine and/or isoleucine, valine, alanine, glycine, proline, aspartic acid, histidine, lysine and methionine.

819 **Nisin D** contains glutamic acid, but no valine or methionine.

Nisin A has a molecular weight of ~7000 and also contains serine.

*Streptococcus lactis, S. cremoris*

N. J. Berridge, G. G. F. Newton and E. P. Abraham, *Biochem. J.* 52 529 (1952).

G. G. F. Newton and E. P. Abraham, *Nature* 171 606 (1953).

G. Cheeseman and N. Berridge, *Biochem. J.* 71 185 (1959).

820 **Duramycin,** colorless amorphous solid, no definite m.p., Hydrochloride: $[\alpha]_D^{25}$ −6.4° (c 3.9 in water).

Duramycin is a polypeptide, containing at least one free amino group and several free carboxyl groups. Acid hydrolysis yielded: lanthionine, β-methyllanthionine, aspartic acid, glutamic acid, glycine, valine, proline, phenylalanine and possibly ornithine and hydroxyproline. Duramycin is related to, but distinct from, cinnamycin.

*Streptomyces cinnamoneus* f. *azacoluta*

Odette L. Shotwell, Frank H. Stodola, William R. Michael, Lloyd A. Lindenfelser, Robert G. Dworschack and Thomas G. Pridham, *J. Am. Chem. Soc.* 80 3912 (1958).

821 **Cinnamycin.**

A polypeptide containing (probably): glutamic acid, aspartic acid, proline, phenylalanine, valine, arginine, lanthionine and β-methyllanthionine.

*Streptomyces cinnamoneus*

Robert G. Benedict, William Dvonch, Odette L. Shotwell, Thomas G. Pridham and Lloyd A. Lindenfelser, *Antibiotics and Chemotherapy* 2 591 (1952).

Robert G. Benedict, *Bot. Rev.* 19 229 (1953).

822 **Matamycin,** colorless crystals, m.p. 173° (dec.), $[\alpha]_D^{20}$ +36.6° (c 0.11 in methanol).

An essentially neutral antibiotic of low solubility. Analysis: C 43.95, H 4.06, N 14.45, S 13.57. Halogen-free. Positive Fehlings, Tollens, permanganate, DNPH, and (after hydrolysis) ninhydrin tests. Negative ferric chloride and Sakaguchi tests. A hydrolysate contained: cysteic acid, glycine, serine, alanine, arginine and two other amino acids.

*Streptomyces matensis* n. sp.

P. Sensi, R. Ballotta and G. G. Gallo, *Antibiotics and Chemotherapy* 9 76 (1959).

An inactive compound, "Compound I," evidently of analogous structure was isolated from the same culture:

823 **Compound I,** colorless crystals, m.p. 189° (dec.), $[\alpha]_D^{20}$ +151.6° (c 0.1 in dioxane).

Analysis: C 45.84, H 3.90, N 14.99, S 14.64. It may be a dehydration product of matamycin.

824 **Comirin,** nearly colorless powder, m.p. 230–235° (dec.).

A polypeptide containing the following amino acids: serine, aspartic acid, glycine, α,γ-diaminobutyric acid, lysine, leucine, isoleucine, tyrosine and arginine. An ether-soluble moiety also was present. Negative ninhydrin, positive biuret. No free amino acid groups.

*Pseudomonas antimycetica*

W. G. C. Forsyth, *Biochem. J.* 59 500 (1955).

825 **Colimycin.**

A crystalline polypeptide, containing mainly D-leucine and L-threonine.

*Bacillus colistinus*

P. V. Forni and E. Guidetti, *Minerva med.* II 823 (1956).

826 **Brevin.**

Brevin is a polypeptide containing: aspartic acid, glycine, tyrosine, serine, an unidentified basic substance (and also a fatty acid component?).

*Bacillus brevis*

Ella M. Barnes and G. G. F. Newton, *Antibiotics and Chemotherapy* 3 866 (1953).

827 **Brevolin,** Hydrochloride yellowish white amorphous, $[\alpha]_D^{26}$ −18.9°.

Brevolin is a polypeptide, probably related to brevin.

*Bacillus brevis*

Junichi Kawamata and Yutaka Motomura, *J. Antibiotics* (Japan) 7A 25 (1954).

**Antibiotics from Yeast.**

Two amorphous compounds have been isolated from bakers' yeast. They have antibacterial and antifungal effects, and seem to be cyclic polypeptides. Acid hydrolysis
828 of one of these ($Y_1$) gave leucine, valine, alanine,
829 glutamic acid and glycine. Acid hydrolysis of $Y_2$ gave the same amino acids plus γ-aminobutyric acid.

Werner Motzel and Elton S. Cook, *Nature* 182 455 (1958).

830 **Alvein.**

A basic polypeptide containing arginine.

*Bacillus alvei*

K. Gilliver, A. M. Holmes and E. P. Abraham, *Brit. J. Exptl. Path.* 30 209 (1949).

831 **Thiostrepton,** colorless crystals, m.p. 246–256° (dec.), $[\alpha]_D^{23}$ −98.5° (c 1 in glacial acetic acid).

A weakly basic polypeptide. Probable amino acid content: leucine (or isoleucine), valine, alanine, threonine, proline, lysine, glycine, aspartic acid, glutamic acid, cystine and tryptophan.

*Streptomyces* sp.

John Vandeputte and James D. Dutcher, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 560.

832 **Antibiotic 899,** reddish yellow amorphous powder, m.p. 115–120°.

A neutral compound with spectra similar to those of streptogramin.

*Streptomyces* sp. resembling *S. virginiae*

P. De Somer and P. Van Dijck, *Antibiotics and Chemotherapy* 5 632 (1955).

833 **Amphomycin,** colorless crystals, $[\alpha]_D^{25}$ +7.5° ±5 (c 1 in water at pH 6).

An acidic (amphoteric) polypeptide, minimal molecular weight about 1500.

*Streptomyces canus*

Bernard Heinemann, Murray A. Kaplan, Robert D. Muir and Irving R. Hooper, *Antibiotics and Chemotherapy* 3 1239 (1953).

834 **Aspartocin.**

An acidic polypeptide similar to amphomycin. C 53.2, H 7.6, N 13.2, S 0.42, no halogen. Hydrolyzes to 4 moles of L-aspartic acid, 2 moles of glycine, 1 mole of L-proline, 1 mole of L-valine, α,β-diaminobutyric acid, α-[L],β-methylaspartic acid, D-α-pipecolic acid and an unsaturated fatty acid.

*Streptomyces griseus* var. *spiralis, S. violaceus*

Yields of 1 to 10 g. per liter were obtained.

A. J. Shay, J. Adam, J. H. Martin, W. K. Hausmann, P. Shu and N. Bohonos, 7th Annual Symposium on Antibiotics, Washington, D. C., 1959.

J. H. Martin and W. K. Hausmann. *J. Am. Chem. Soc.* 82 2079 (1960).

835 **Zaomycin,** m.p. 242–246° (dec.).

A polypeptide resembling amphomycin.

*Streptomyces zaomyceticus* n. sp.

Yorio Hinuma, *J. Antibiotics* (Japan) 7A 134 (1954).

836 **Bacillomycin** (Fungocin, Bacillomycin R, Bacillomycin A), colorless microcrystals.

An acidic polypeptide, molecular weight ~1000. Analysis: C 52.69, H 7.20, N 12.29. Contains glutamic acid, aspartic acid, serine, threonine and tyrosine. Similar to or identical with eumycin.

*Bacillus subtilis*

Maurice Landy, Sanford B. Rosenman and George H. Warren, *J. Bact.* 54 24 (1947).

Howard Tint and Wilhelm Reiss, *J. Biol. Chem.* 190 133 (1951).

Robert A. Turner, *Arch. Biochem.* 60 364 (1956).

837 **Bacillomycin B,** amorphous yellow material.

A polypeptide containing glutamic acid, aspartic acid, proline, tyrosine and leucine.

*Bacillus subtilis*

Isao Shibasaki and Gyozo Terui, *J. Fermentation Technol.* (Japan) 31 339 (1953).

838 **Bacillomycin C.**

A polypeptide containing glutamic acid, aspartic acid, tyrosine, leucine and valine.

*Bacillus subtilis*

Isao Shibasaki and Gyozo Terui, *J. Fermentation Technol.* (Japan) 32 115 (1954).

839 **Fungistatin.**

An amphoteric polypeptide, containing aspartic acid, lysine, serine, threonine, proline, alanine, isoleucine, valine, tryptophan, tyrosine, other unidentified amino acids. Molecular weight about 2400.

*Bacillus subtilis*

Gladys L. Hobby, Peter P. Regna, Nancy Dougherty and William E. Steig, *J. Clin. Invest.* 28 927 (1949).

P. P. Regna, R. A. Carboni and W. E. Steig, *Am. Chem. Soc. Meeting-in-Miniature,* Brooklyn (1950).

Robert L. Peck and John E. Lyons, *Ann. Rev. Biochem.* 20 367 (1951).

840 **Bryamycin,** m.p. 223–235° (dec.), $[\alpha]_D^{27}$ −68.5° (c 1 in chloroform).

A polypeptide containing alanine, glycine, isoleucine, threonine, cystine and unidentified compounds.

*Streptomyces hawaiiensis* n. sp.

M. J. Cron, D. F. Whitehead, I. R. Harper, B. Heinemann and J. Lein, *Antibiotics and Chemotherapy* 6 63 (1956).

841 **Coliformin.**

A polypetide, molecular weight 4000 ± 400, containing glutamic acid, aspartic acid, lysine, valine, leucine, serine, alanine and glycine. Positive Molisch. Contains traces of phosphorus and sulfur.

An *E. coli-Aerobacter aerogenes* type of bacterium

Stig K. L. Freyschuss, Stig O. Pehrson and Borje Steinberg, *Antibiotics and Chemotherapy* 5 218 (1955).

842 **Mycosubtilin,** white crystals, m.p. 256°.

A polypeptide, C 55.31, H 7.61, N 15.15.

*Bacillus subtilis*

Robert P. Walton and H. Boyd Woodruff, *J. Clin. Invest.* 28 924 (1949).

843 **Grizein** (Helianthate) homogeneous brown powder, m.p. 194–196° (dec.) (hydrochloride) white, hygroscopic powder.

A basic polypeptide complex. Positive biuret, ninhydrin, glucosamine reactions. Negative maltol, histidine, Sakaguchi tests.

*Streptomyces griseus*-like strains

N. A. Krasilnikov, A. N. Belozerskii, Ya. I. Rautenshtein, A. I. Korenyako, N. I. Nikitina, A. I. Sokolova and S. O. Uryson, *Mikrobiologiya* 26 418 (1957).

**Licheniformins,** amorphous white powders, no m.p.

844 **Licheniformin A,** hydrochloride: $[\alpha]_D^{20}$ −37.4° (c 1 in chloroform).

845 **Licheniformin B,** hydrochloride: $[\alpha]_D^{20}$ −37.7° (c 1 in chloroform).

846 **Licheniformin C,** hydrochloride: $[\alpha]_D^{20}$ −36.8° (c 1 in chloroform).

A rather high molecular weight polypeptide complex. Negative glucosamine and Molisch. Positive Sakaguchi, biuret.

Licheniformins A and B contain: aspartic acid, glycine, serine, lysine, arginine, valine, proline and phenylalanine.

*Bacillus licheniformis*

R. K. Callow, R. E. Glover, P. D'Arcy Hart and G. M. Hills, *Brit. J. Exptl. Path.* 28 418 (1947).

R. K. Callow and T. S. Work, *Biochem. J.* 51 558 (1952).

847 **Carcinomycin**, dark green, amorphous.

A polypeptide antibiotic. Sulfur-free.

*Streptomyces carcinomycicus*

Shogo Hosotani and Momoe Soeda, Japanese Patent 6893 (1959). (*Chem. Abstr.* 54 831g)

848 **Carcinocidin**, $[\alpha]_D^{25}$ −20° (c 1 in water).

A polypeptide antibiotic, containing cystine, lysine, glycine and glutamic acid. Molecular weight >6000.

*Streptomyces kitazawaensis*

This organism also produces antimycin A.

F. Okamoto, Shigeo Kubo, Takahashi Nara and Shiro Tanaka, Jap. Patent Appl. 6894 (1959). (*Chem. Abstr.* 54 832c)

849 **Melanomycin** (Sodium Salt), brown, amorphous powder.

A polypeptide antibiotic yielding on hydrolysis: phenylalanine, leucine, valine, proline, alanine, glutamic acid and histidine.

*Streptomyces melanogenes*

Fujiki Hata, Ryozo Sugawara, Akihiro Matsumae and Takamoto Sano, Japanese Patent 5899 (1959). (*Chem. Abstr.* 54 833b)

850 **Notatin** (Penicillin B, Penatin), buff colored powder, water soluble, $[\alpha]_D^{22}$ −4.8° (c 0.012 in water).

A flavoprotein enzyme (glucose-oxidase), molecular weight about 152,000.

*Penicillium notatum*, other *Penicillium* spp.

R. Cecil and A. G. Ogston, *Biochem. J.* 42 229 (1948).

18.

# Heterocycles

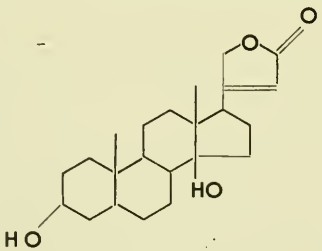
## *a.* FURANS AND RELATED SUBSTANCES

Apparently there has been no investigation of the biosynthetic origin of the furans listed here, but it is known that furans can be formed in several different ways.

The relationship of furans to sugars is recognized in the designation of the five-membered ring hemi-acetal form of sugars as the furanose form. Dilute acid converts glucose to 5-hydroxymethylfurfural. The latter compound may be a precursor of Sumiki's acid, although the transformations are probably enzymatic. The four carbon atom sugar erythrose also is a likely furan precursor as pointed out by Wenkert.[1]

The furans with carbon chains at the 2-position are obviously terpenoid. Since they were isolated from a sweet potato medium, their direct derivation from glucose cannot be assumed. The simpler substances may arise from oxidation of the more complex.

It is interesting to note that the lactone side-chain of digitoxigenin is derived from acetate rather than from mevalonic acid.[2] Such lactones as well as the related tetronic acids, would seem



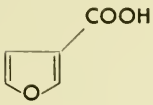

Digitoxigenin

to be potential furan precursors.

[1] Ernest Wenkert, *Experientia* 15 165 (1959).

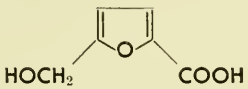
[2] E. Leete, *Seventh Medicinal Chemistry Symposium of the American Chemical Society*, Kingston, Rhode Island, 1960.

851 **Furan-3-carboxylic Acid**, $C_5H_4O_3$, colorless crystals, m.p. 121°.



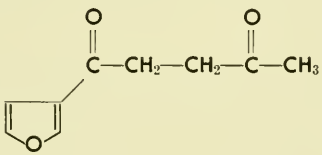

*Ceratostomella fimbriata* (sweet potato substrate)
Takashi Kubota and Keizo Naya, *Chem. and Ind.*, 1427 (1954).

852 **5-Hydroxymethylfuran-2-carboxylic Acid** (Sumiki's Acid), $C_6H_6O_4$, colorless crystals, m.p. 164° (dec.).



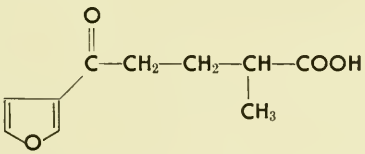

*Aspergillus glaucus, A. clavatus, A. niger, A. oryzae, A. wentii, Gibberella fujikuroi*
Yusuke Sumiki, *J. Agr. Chem. Soc. Japan* 7 819 (1931).
Akira Kawarada, Nobutaka Takahashi, Hiroshi Kitamura, Yasuo Seta, Makoto Takai and Saburo Tamura, *Bull. Agr. Chem. Soc.* (Japan) 19 84 (1955).

853 **Ipomeanine**, $C_9H_{10}O_3$, oil, $b._{0.001}$ 74–79°, $n_D^{15}$ 1.4975, $[\alpha]_D$ +3.9°.



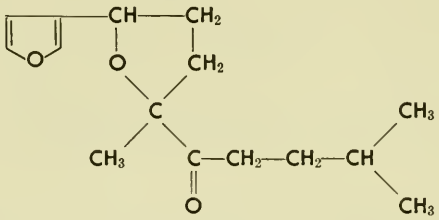

*Ceratostomella fimbriata* (sweet potato substrate)
Takashi Kubota and Nobutaka Ichikawa, *Chem. and Ind.*, 902 (1954).

854 **Batatic Acid**, $C_{10}H_{12}O_4$, colorless crystals, m.p. 88.5°, $[\alpha]_D^{10}$ +17.5° (in ethanol).




*Ceratostomella fimbriata* (sweet potato substrate)
Takashi Kubota and Keizo Naya, *Chem. and Ind.*, 1427 (1954).

855 Ipomeamarone, $C_{15}H_{22}O_3$, colorless oil, $b._{0.001}$ 103°, $n_D^{15}$ 1.4827, $[\alpha]_D^{25}$ +28°.



*Ceratostomella fimbriata* (sweet potato substrate)

T. Kubota and T. Matsuura, *Chem. and Ind.*, 521 (1956). (Synthesis)

There is a marked resemblance between ipomeamarone and dendrolasin, an oil $C_{15}H_{22}O$, isolated from ants. It is an enantiomer of ngaione, isolated from *Myoporum* spp. (higher plant).

A. Quilico, F. Piozzi and M. Pavan, *Tetrahedron* 1 177 (1957). (Structure)

A. J. Birch, R. A. Massy-Westropp and S. E. Wright, *Chem. and Ind.*, 902 (1954).

Ipomeamarone is thought to be formed by the host (sweet potato) tissue to resist invasion by *Ceratostomella fimbriata.**

## *b.* DIBENZOFURANS AND RELATED SUBSTANCES

Dibenzofurans constitute a class of natural products found only in lichens. Usnic acid is the most widely distributed dibenzofuran. Its structure, which was controversial for some time, now has been proved by synthesis.[1]

The dibenzofurans are formed from 2 moles of the acetate-derived resorcinolic substances typical of lichens. Results of chemical experiments, including the method of synthesis of usnic acid, make it quite probable that phenol coupling of the sort mentioned in connection with depsides and depsidones also is involved here.[2, 3] Thus,

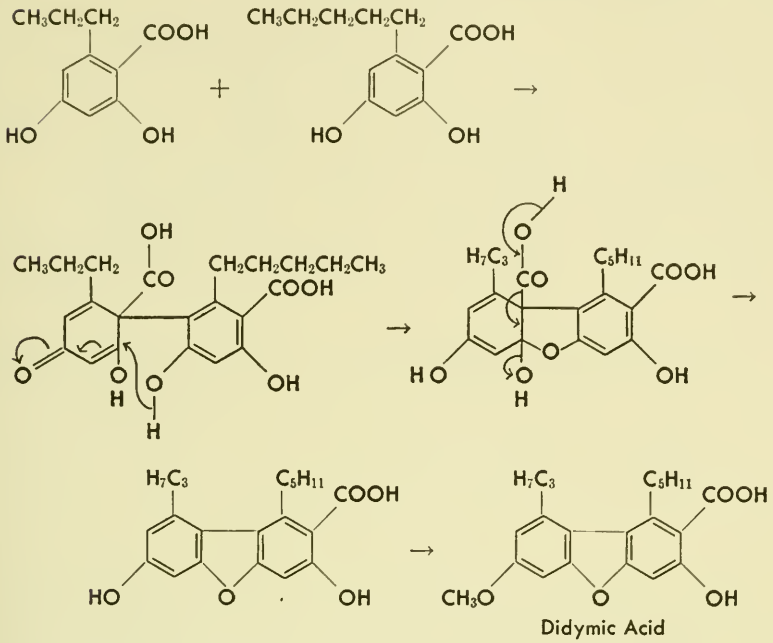
* T. Akazawa, *Arch. Biochem. and Biophys.* 90 82 (1960).

[1] D. H. R. Barton, A. M. Deflorin, O. E. Edwards and J. B. Hendrickson, *Chem. and Ind.*, 1670 (1955).

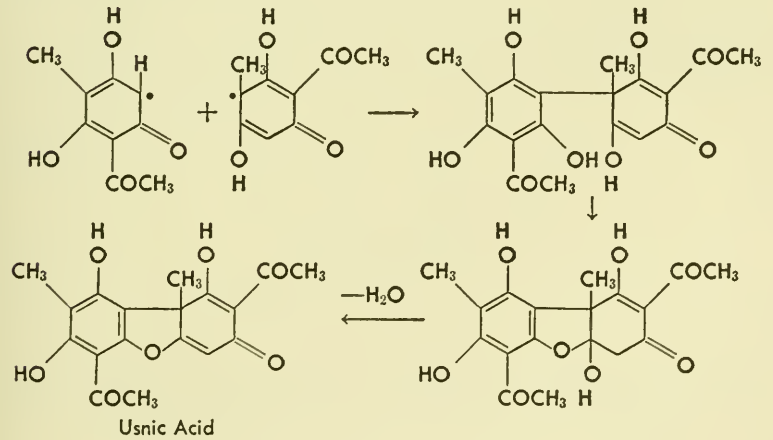
[2] D. H. R. Barton and T. Cohen, *Festschr. Arthur Stoll*, 117 (1957).

[3] Holger Erdtman and Carl Axel Wachtmeister, *ibid.*, 144 (1957).

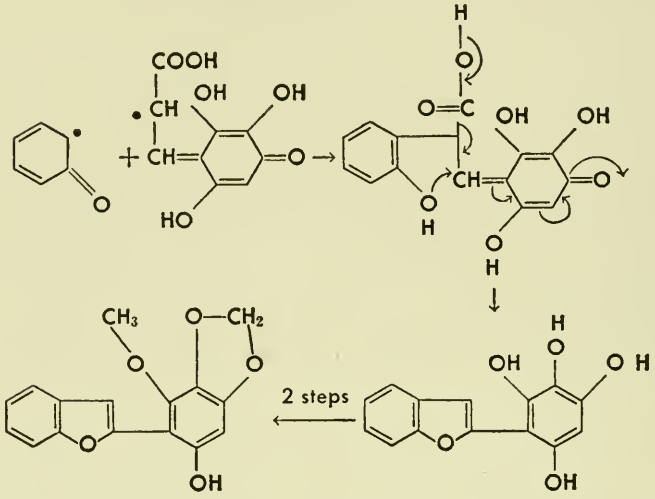
dydymic acid would be formed by coupling of two similar orsellinic acids:




And in the case of usnic acid:

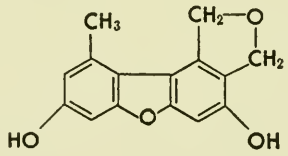


Formation of the monobenzofuran shown also may involve phenol coupling, if not precisely as indicated at least in the same general fashion:




Apparently, many lichens contain an enzyme system which can promote phenolic coupling of this type. Neither the dibenzofurans nor the depsides and depsidones are produced by molds alone (although some of their resorcinolic precursors are), and the algal partners must be required in the coupling process.

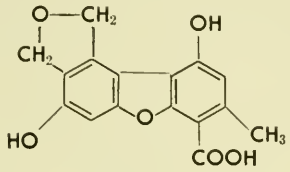
856 **Strepsilin**, $C_{15}H_{10}O_5$, colorless crystals, m.p. 324°.




*Cladonia strepsilis* Wain.

Shoji Shibata, *J. Pharm. Soc. Japan* 64 20 (1944). (Structure)

857 **Porphyrilic Acid,** $C_{16}H_{10}O_7$, colorless needles, m.p. 280–283° (dec.).

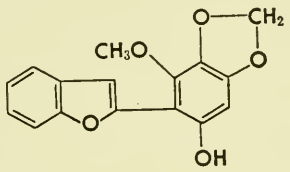



*Haematomma coccineum* (Dicks.), *H. porphyrium* (Pers.)

Porphyrilic acid occurs together with *l*-usnic acid and atranorin.

Carl Axel Wachtmeister, *Acta Chem. Scand.* **10** 1404 (1956). (Structure)

858 **2-(6-Hydroxy-2-methoxy-3,4-methylenedioxyphenyl)-benzofuran,** $C_{16}H_{12}O_5$, colorless crystals, m.p. 118°.

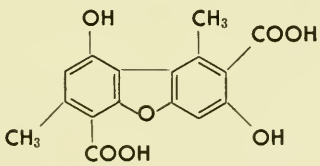



Yeast

A yield of 0.5–2.0 mg. per pound of bakers' yeast was reported.

M. A. P. Meisinger, Frederick A. Kuehl, Jr., E. L. Rickes, Norman G. Brink, Karl Folkers, Martin Forbes, Friederich Zilliken and Paul Gyorgy, *J. Am. Chem. Soc.* **81** 4979 (1959). (Structure)

859 **Pannaric Acid,** $C_{16}H_{12}O_7$, colorless needles, m.p. 243–245°.

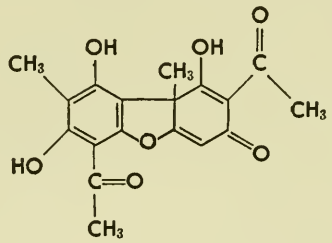



*Crocynea membranacea* (Dicks.) Zahlbr. = *Pannaria lanuginosa* Ach.

O. Hesse, *J. prakt. Chem.* **70** 1 (1904). (Isolation)

Äkermark H. Erdtman and C. A. Wachtmeister, *Acta Chem. Scand.* 13 1855 (1959). (Structure)

860 *d*- and *l*-Usnic Acid, $C_{18}H_{16}O_7$, yellow crystals, m.p. 203°, $[\alpha]_D^{17}$ (*d*-form) +492°, (*l*-form) −495°. M.p. *d,l*-form 195°.




*Usnea, Alectoria, Ramalina, Evernia, Cetraria, Parmelia, Cladonia, Lecanora* and *Haematomma* species (most yellow lichens). Long known.

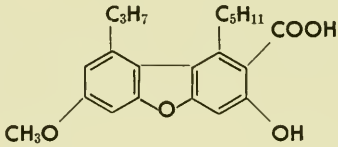
Both *d*- and *l*-forms occur in lichens. Relatively high yields are available from some species.

Clemens Schöpf and Friedrich Ross, *Naturwissenschaften* 26 772 (1938).

*Idem., Ann.* 546 1 (1941). (Structure)

D. H. R. Barton, A. M. Deflorin, O. E. Edwards and J. B. Hendrickson, *Chem. and Ind.*, 1670 (1955). (Synthesis)

861 Didymic Acid (Incrassatic Acid), $C_{22}H_{26}O_5$, colorless crystals, m.p. 172°.




*Cladonia* species (occurs together with squamatic and barbatic acids)

Yasuhiko Asahina and Masaru Aoki, *J. Pharm. Soc. Japan* 64 41 (1944).

## C. PYRANS AND RELATED SUBSTANCES

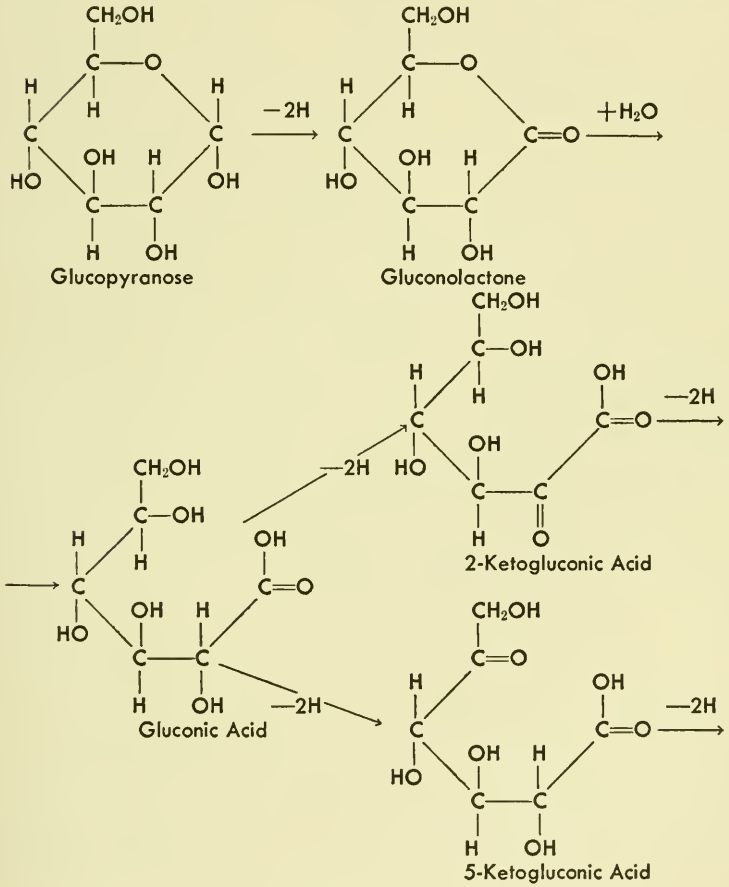
### The γ-Pyrones and Patulin

The biosynthesis of patulin was discussed in the introduction to the chapter on phenolic substances.

Kojic acid has long attracted interest because it is produced in such high yields by certain *Aspergillus* species. Within the past few years isokojic acid and several other related γ-pyrones have been isolated from *Gluconoacetobacter* cultures.

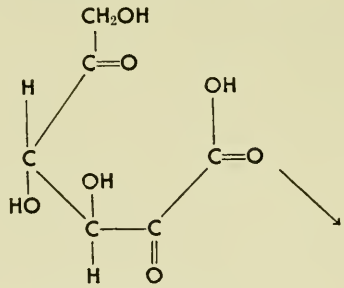
The fungi are able to use pentose and triose substrates as well as glucose, although labeling studies have shown conversion of glucose to kojic acid without cleavage of the 6-carbon chain.[1]

*Gluconoacetobacter liquefaciens* seems to be more selective in its substrate and uses only glucose, gluconate and 2-ketogluconate. The variety of γ-pyrones produced is useful in deducing the kind of intermediate involved. The foregoing considerations plus the isolation of 2,5-diketogluconic acid from cultures of this bacterium have led to formulation of the following biosynthetic route to the pyrones produced by *Gluconoacetobacter liquefaciens*:[2]

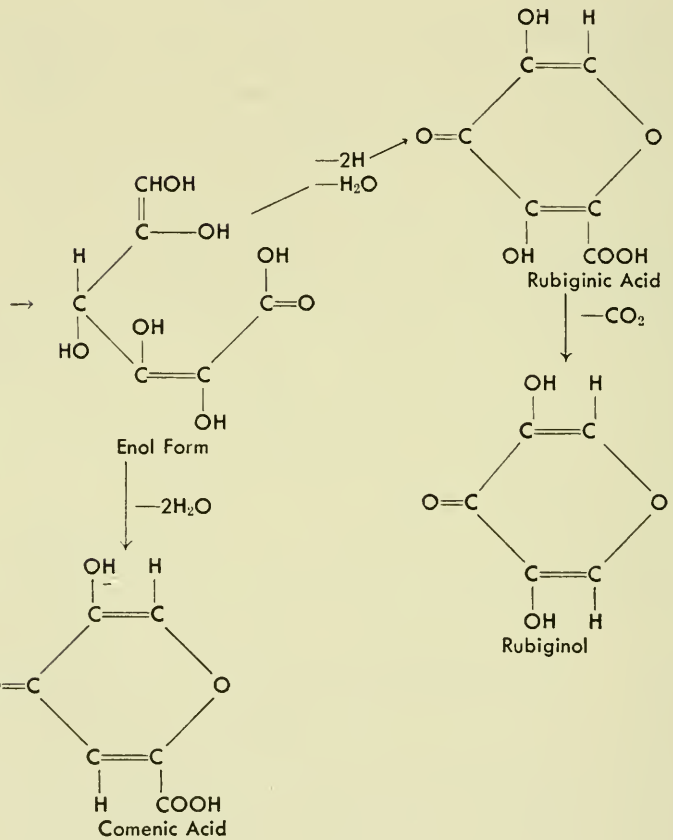



[1] H. R. V. Arnstein and R. Bentley, *Biochem. J.* 62 403 (1956).

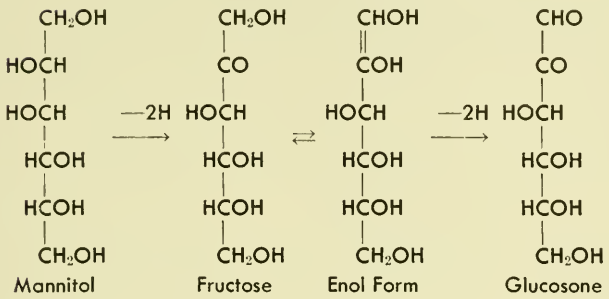
[2] Ko Aida, Mitsuko Fujii and Toshinobu Asai, *Bull. Agr. Chem. Soc.* (Japan) 21 30 (1957).

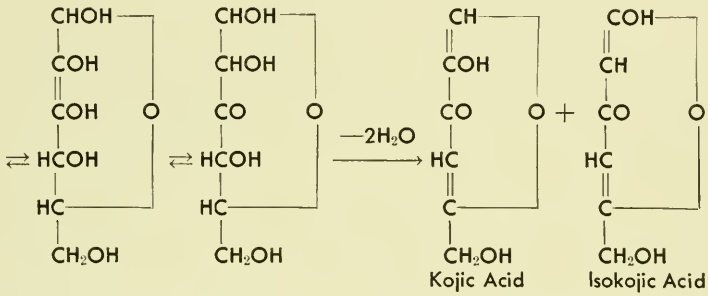



2, 5-Diketogluconic Acid



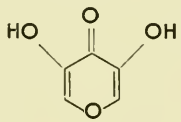
Another bacterial species, *Gluconoacetobacter roseum*, studied by the Japanese, produces kojic and isokojic acids and only from a fructose, sucrose or mannitol substrate. The two products are always found together. The proposed route by which these two pyrones are formed from fructose by *Gluconoacetobacter roseum* is shown below:[3]




Kojic acid is potentially an inexpensive chemical because of high yields from aspergilli.

862 **Rubiginol**, $C_5H_4O_4$, colorless plates, m.p. 203.5°.



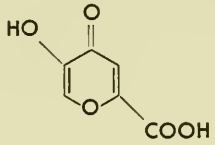
*Gluconoacetobacter liquefaciens*

A yield of 1.2 g. of rubiginol from 140 g. of glucose substrate was reported.

Ko Aida, *J. Gen. and Appl. Microbiol.* (Japan) 1 30 (1955).

[3] Ko Aida, Mitsuko Fujii and Toshinobu Asai, *Proc. Japan Acad.* 32 595 (1956).

863 **Comenic Acid**, $C_6H_4O_5$, colorless plates, m.p. 276° (dec.).

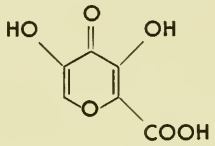



*Gluconoacetobacter liquefaciens*

A yield of 1.1 g. from 140 g. of glucose has been reported.

Ko Aida, *Bull. Agr. Chem. Soc.* (Japan) 19 97 (1955).

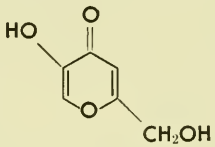
864 **Rubiginic Acid**, $C_6H_4O_6$, colorless needles, m.p. 230° (dec.).




*Gluconoacetobacter liquefaciens*

Ko Aida, *Bull. Agr. Chem. Soc.* (Japan) 19 97 (1955).

865 **Kojic Acid**, $C_6H_6O_4$, colorless prisms, m.p. 152°.



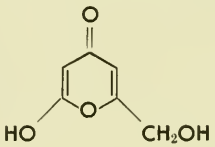

*Aspergillus flavus, A. oryzae, A. tamarii, A. glaucus, Gluconoacetobacter roseum* (fructose substrate)

High yields (45 g. or more per 100 g. of glucose substrate) are produced by some aspergillus strains.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. II, Lewis B. Lockwood, *Ketogenic fermentation processes*, chap. 1, pp. 19–20. (A review)

Andrew Bielik, *Advances in Carbohydrate Chem.* 11 145 (1956). (A review)

866 **Isokojic Acid**, $C_6H_6O_4$, colorless plates, m.p. 183°.

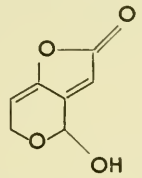


*Gluconoacetobacter roseum* (fructose substrate)

Isokojic acid was produced together with kojic acid and an unidentified substance.

Ko Aida, Mitsuko Fujii and Toshinobu Asai, *Proc. Japan. Acad.* 32 600 (1956).

867 **Patulin** (Clavacin, Clavatin, Claviformin, Penicidin, Expansine, Mycoin), $C_7H_6O_4$, colorless crystals, m.p. 111°.



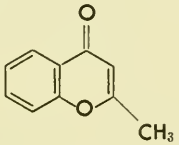
*Penicillium patulum* Bainier (*P. urticae*), *P. griseofulvum, P. claviforme, P. expansum, P. melinii, P. equinum, P. novae-zeelandiae, P. leucopus, Aspergillus clavatus, A. terreus, A. giganteus, Gymnoascus* spp.

H. W. Florey, E. Chain, N. G. Heatley, M. A. Jennings, A. G. Sanders, E. P. Abraham and M. E. Florey, "Antibiotics," Oxford University Press, London, 1949, pp. 223–272. (Reviews earlier work)

R. B. Woodward and Gurbakhsh Singh, *J. Am. Chem. Soc.* 72 1428 (1950). (Synthesis)

868 **5-Hydroxy-2-methylchromone,** $C_{10}H_8O_3$, yellow needles, m.p. 72–76°.

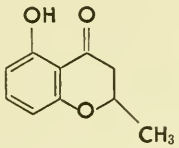
Proposed structure:



*Daldinia concentrica*

D. C. Allport and J. D. Bu'Lock, *J. Chem. Soc.*, 654 (1960).

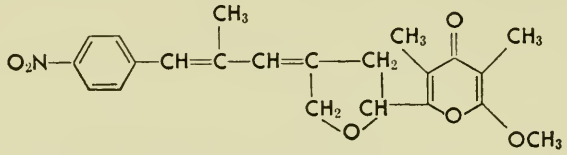
869 **5-Hydroxy-2-methylchromanone,** $C_{10}H_{10}O_3$, pale yellow needles, m.p. 30–33°.



*Daldinia concentrica*

D. C. Allport and J. D. Bu'Lock, *J. Chem. Soc.*, 654 (1960).

870 Aureothin, $C_{22}H_{23}O_6N$, yellow crystals, m.p. 158°.



*Streptomyces thioluteus*

Aureothin occurs as a by-product in the aureothricin fermentation.

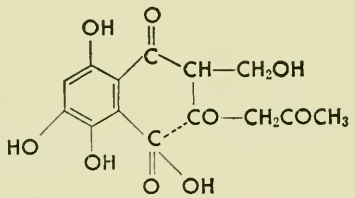
Kenji Maeda, *J. Antibiotics* (Japan) 6A 137 (1953). (Isolation)

Y. Hirata, H. Nakata and K. Yamada, *J. Chem. Soc. Japan* 79 1390 (1958) and preceding papers. (Structure)

## Quinonoid Compounds.

This section includes a group of colored compounds, many of which have chromophores resembling those of quinones. These unusual substances presented some interesting structural problems. In many cases there was a long time interval between isolation and complete structure determination.

The relationship between fulvic acid and citromycetin is obvious. The relationship of both of these compounds to fusarubin has been pointed out recently.[1] This is less obvious, but a precursor such as (I) was envisaged for all three compounds, the formation of fusarubin involving ring closure at the dotted line.



I

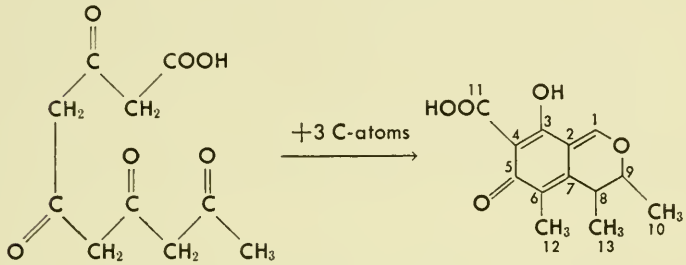
*Penicillium griseofulvum*, which is one of the producers

[1] F. M. Dean, R. A. Eade, R. A. Moubasher and A. Robertson, *Nature* 179 366 (1957).

of fulvic acid, also produces a variety of other metabolites, including griseofulvin and mycelianamide. There seems to be no close relationship between these compounds and the three mentioned above, however.

The biosyntheses of sclerotiorin,[2] citromycetin[2] and citrinin[2, 3] have been investigated by using $C^{14}$-labeled acetate, formate and methionine.

The two studies of citrinin (III) were in agreement, the results of both indicating origin from a 10-carbon atom polyketomethylene chain in the sense of (II).

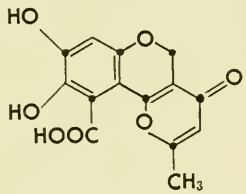



II III

The carbon atoms 11, 12 and 13 in (III) were contributed by methionine or formate.

Sclerotiorin also is acetate derived with contribution of three carbon atoms by formate.

Citromycetin (V) is derived entirely from seven acetic acid units, $CH_3—C^{14}OOH$ ($CH_3—\dot{C}OOH$) and yields the labeling pattern shown below.



V

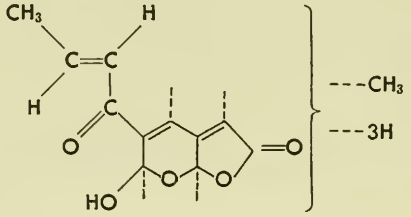
It would appear that purpurogenone should also be derived from seven acetate units.

[2] A. J. Birch, P. Fitton, E. Pride, A. J. Ryan, Herchel Smith and W. B. Whalley, *J. Chem. Soc.*, 4576 (1958).

[3] Erwin Schwenk, George J. Alexander, Allen M. Gold and Dean F. Stevens, *J. Biol. Chem.* 233 1211 (1958).

871 **Radicinin,*** $C_{12}H_{12}O_5$, yellow crystals, m.p. 220° (dec.), $[\alpha]_D^{27}$ −217.4° (c 2.37 in pyridine).

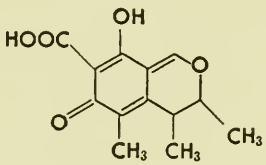
Proposed partial structure:




*Stemphylium radicinum* Sterad (formerly *Alternaria radicina*)

D. D. Clarke and F. F. Nord, *Arch. Biochem. and Biophys.* 59 269, 285 (1955).

872 **Citrinin,** $C_{13}H_{14}O_5$, long yellow prisms, m.p. 179° (dec.), $[\alpha]_D^{20}$ −34.5° (c 0.60 in alcohol).



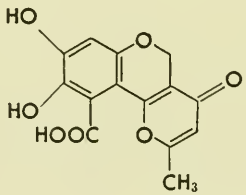

*Penicillium citrinum, P. expansum, P. implicatum, P. chrzaszszi, P. citreo-sulfuratum, P. lividum, P. phaeojanthinellum, Aspergillus terreus, A. candidus, A. niveus*

A. C. Hetherington and H. Raistrick, *Trans. Roy. Soc.* (London) B220 269 (1931). (Isolation)

D. H. Johnson, Alexander Robertson and W. B. Whalley, *J. Chem. Soc.*, 2971 (1950).

H. H. Warren, Gregg Dougherty and Everett S. Wallis, *J. Am. Chem. Soc.* 71 3422 (1949). (Synthesis)

873 **Citromycetin** (Frequentic Acid), $C_{14}H_{10}O_7$, lemon yellow hydrated needles, m.p. 283–285° (dec.).




* See entry 413.

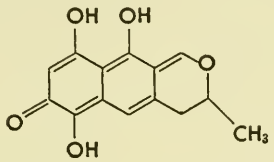
*Penicillium frequentans, P. roseo-purpurogenum, P. glabrum, P. pfefferianum, Citromyces* strains, *Corynebacterium diphtheriae*

A. C. Hetherington and H. Raistrick, *Trans. Roy. Soc.* (London) B220 209 (1931). (Isolation)

Alexander Robertson, W. B. Whalley and J. Yates, *J. Chem. Soc.*, 2013 (1951). (Structure)

Michizo Asano and Hideo Takahashi, *J. Pharm. Soc. Japan* 65 81 (1945). (Isolation from the corynebacterium)

874 **Purpurogenone,** $C_{14}H_{12}O_5$, crimson prisms, m.p. 310° (dec.).



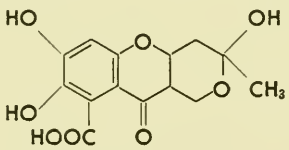

*Penicillium purpurogenum* Stoll

Yield 8–14 g. of crude pigment from about 250 g. of dry mycelium, which was obtained from about 70 liters of culture solution.

Ergosteryl palmitate, m.p. 104–106°, and mannitol, m.p. 166°, also were isolated from this fermentation.

John C. Roberts and C. W. H. Warren, *J. Chem. Soc.*, 2992 (1955).

875 **Fulvic Acid,** $C_{14}H_{12}O_8$, yellow crystals, m.p. 246° (dec.).



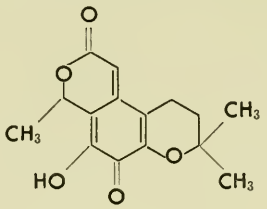

*Penicillium flexuosum, P. brefeldianum, P. griseofulvum*

876 *P. griseofulvum* produced a nitrogen-containing compound, m.p. 165°, in the same broth. 877 *P. brefeldianum* produced a neutral nitrogen-containing compound, m.p. 132–135°, in the same culture.

Albert Edw. Oxford, Harold Raistrick and Paul Simonart, *Biochem. J.* 29 1102 (1935). (Isolation)

F. M. Dean, R. A. Eade, R. A. Moubasher and A. Robertson, *Nature* 179 366 (1957). (Structure)

878 **Fuscin,** $C_{15}H_{16}O_5$, orange plates, m.p. 230°.



*Oidiodendron fuscum* Robak

879 A colorless **dihydrofuscin,** m.p. 206°, was also produced.

S. E. Michael, *Biochem. J.* 43 528 (1948). (Isolation)

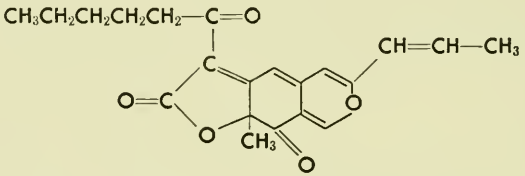
D. H. R. Barton and J. B. Hendrickson, *J. Chem. Soc.,* 1028 (1956). (Synthesis)

**Azaphilones.**

This group of mold pigments, so named because most of them react avidly with ammonia, includes monascorubrin, sclerotiorin, rotiorin, rubropunctatin and monascin.

A. Powell, A. Robertson and W. Whalley, "Chemical Society Symposia," Special Publication No. 5, The Chemical Society, London, 1956, p. 27. (Survey of the chemistry of the azaphilones to that date)

880 **Rubropunctatin,** $C_{21}H_{22}O_5$, orange needles, m.p. 156.5° (dec.), $[\alpha]_D$ −3481° (c 1.07 in chloroform).

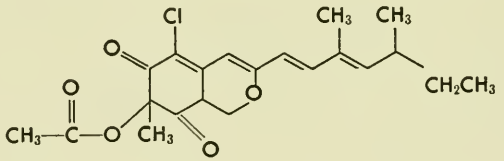


*Monascus rubropunctatus* Satô

E. J. Haws, J. S. E. Holker, A. Kelly, A. D. G. Powell and Alexander Robertson, *J. Chem. Soc.,* 3598 (1959). (Structure)

A. Powell, Dissertation, Liverpool, 1954. (Isolation)

881 **Sclerotiorin,** $C_{21}H_{22}O_5Cl$, yellow crystals, m.p. 206° $[\alpha]_D$ +500°.



*Penicillium sclerotiorum* van Beyma, *P. multicolor* G.M.P., *P. implicatum* Biourge

Timothy P. MacCurtin and Joseph Reilly, *Biochem. J.* 34 1419 (1940). (Isolation)

H. C. Fielding, Alexander Robertson, R. B. Traners and W. B. Whalley, *J. Chem. Soc.*, 1814 (1958).

F. M. Dean, J. Staunton and W. B. Whalley, *ibid.*, 3004 (1959). (Structure)

882 **Monascin,** $C_{21}H_{26}O_5$, yellow crystals, m.p. 142°, $[\alpha]_D$ +544°.

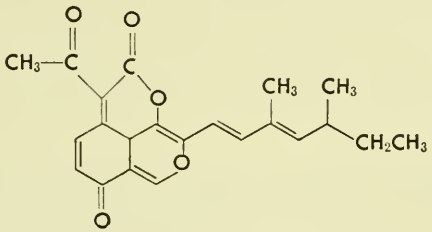
*Monascus rubriginosus* Satô, *M. purpureus* Wentii, *M. rubropunctatus* Satô

Hidijiro Nishikawa, *J. Agr. Chem. Soc. Japan* 8 1007 (1932).

H. Solomon and P. Karrer, *Helv. Chim. Acta* 15 18 (1932).

883 **Rotiorin,** $C_{23}H_{24}O_5$, red needles, m.p. 246° (dec.) (sublimes), $[\alpha]_D^{22}$ +5080° (c 0.002 in chloroform).

Tentative structure:

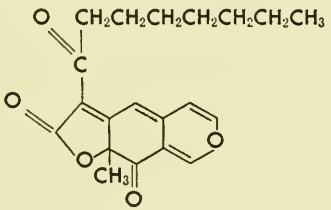



*Penicillium sclerotiorum* van Beyma

Eight kilograms of dry mycelium yielded 300–350 g. of sclerotiorin and 100–150 g. of rotiorin.

G. B. Jackman, Alexander Robertson, R. B. Traners and W. B. Whalley, *J. Chem. Soc.*, 1825 (1958). (Structure)

884 **Monascorubrin,** $C_{23}H_{26}O_5$, orange crystals, m.p. 134–136°, $[\alpha]_{700}^{16}$ −1500° (c 0.1 in ethanol).



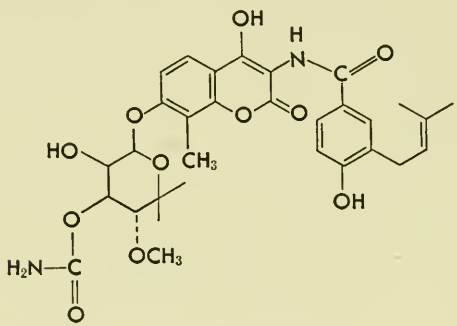

*Monascus purpureus* Wentii

H. Nishikawa, *J. Agr. Chem. Soc. Japan* 5 1007 (1932). (Isolation)

K. Nakanishi, M. Ohashi, S. Kumasaki and S. Yamamura, *J. Am. Chem. Soc.* 81 6339, 6340 (1959). (Structure)

B. C. Fielding, E. J. Haws, J. S. E. Holker, A. D. G. Powell, A. Robertson, D. N. Stanway and W. B. Whalley, *Tetrahedron Letters* No. 5 24 (1960). (Proposed revised structure shown)

885 **Novobiocin** (Streptonivicin, Cathomycin, Albamycin, Spheromycin, Vulcamycin, Crystallinic Acid, Antibiotic PA-93, Cardelmycin), $C_{31}H_{36}O_{11}N_2$, pale yellow crystals, m.p. 152–156° (dec.) and 174–178° (dec.) (polymorphic), $[\alpha]_D^{24}$ −63° (c 1 in ethanol).




*Streptomyces spheroides, S. niveus, S. griseus*

Herman Hoeksema, James L. Johnson and Jack W. Hinman, *J. Am. Chem. Soc.* 77 6710 (1955).

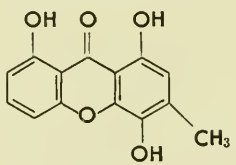
Jack W. Hinman, Herman Hoeksema, E. Louis Caron and W. G. Jackson, *ibid.* 78 1072 (1956).

Clifford H. Shunk, Charles H. Stammer, Edward A. Kaczka, Edward Walton, Claude F. Spencer, Andrew N. Wilson, John W. Richter, Frederick W. Holly and Karl Folkers, *ibid.* 78 1770 (1956). (Structure)

Herman Hoeksema, E. Louis Caron and Jack W. Hinman, *ibid.* 78 2019 (1956). (Structure)

*d.* XANTHONES

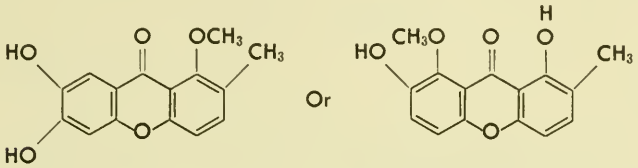
886 **Ravenelin**, $C_{14}H_{10}O_5$, yellowish crystals, m.p. 267°.



*Helminthosporium ravenelii*

F. F. Nord and Robert P. Mull, *Advances in Enzymol.* 5 194 (1945). (Synthesis)

887 **Rubrofusarin,** $C_{15}H_{12}O_5$, orange-red needles, m.p. 210°. Alternative structures:




*Fusarium culmorum* (W.G.Sm.) Sacc., *Fusarium graminearum* Schwabe (*Gibberella saubinettii*)

888 Another pigment, **aurofusarin,*** $C_{30}H_{20}O_{12}$, m.p. >360°
889 and a colorless compound, **culmorin,** $C_{15}H_{26}O_2$, m.p. 175°, $[\alpha]_D^{20}$ −14.45° were isolated from the same cultures.

Julius Nicholson Ashley, Betty Constance Hobbs and Harold Raistrick, *Biochem. J.* 31 385 (1937).

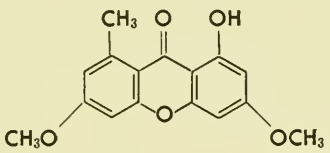
Robert P. Mull and F. F. Nord, *Arch. Biochem.* 4 419 (1944). (Structure)

890 **Asperxanthone,** $C_{16}H_{14}O_5$, yellow needles, m.p. 203°. A 1-hydroxydimethoxymethylxanthone which yields nor-rubrofusarin on demethylation.

*Aspergillus niger* (mycelium)

N. A. Lund, Alexander Robertson and W. B. Whalley, *J. Chem. Soc.*, 2434 (1953).

891 **Lichexanthone,** $C_{16}H_{14}O_5$, yellowish crystals, m.p. 187°.



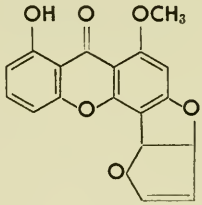

*Parmelia formosana* Zahlbr.

The yield was about ½ g. from 25 g. of lichen.

Yasuhiko Asahina and Hirashi Nogami, *Bull. Chem. Soc. Japan* 17 202 (1942).

* See entry 584.

892 Sterigmatocystin, $C_{18}H_{12}O_6$, pale yellow needles, m.p. 246° (dec.), $[\alpha]_D^{20.5}$ −387° (c 0.424 in chloroform).
Probable structure:




*Aspergillus versicolor* (Vuillemin) Tiraboschi
J. E. Davies, D. Kirkaldy and John C. Roberts, *J. Chem. Soc.*, 2169 (1960). (Structure)
Abou-Zeid, Dissertation, London, 1953. (Isolation)
J. E. Davies, John C. Roberts and S. C. Wallwork, *Chem. and Ind.*, 178 (1956). (Isolation)
J. H. Birkinshaw and I. M. M. Hammady, *Biochem. J.* 65 162 (1957). (Isolation)
Yuichi Hatsuda and Shimpei Kuyama, *J. Agr. Chem. Soc. Japan* 28 989 (1954). (*Chem. Abstr.* 50 15,522) (Isolation)

## *e*. COMPOUNDS RELATED TO THIOPHENE, IMIDAZOLE, THIAZOLE AND ISOXAZOLE.

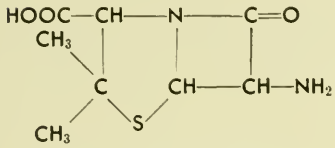
Some of the commercially important compounds in this section are the antibiotics cycloserine and the penicillins and the vitamins, thiamine and biotin.

Penicillin was discovered by Fleming in 1929, and commercial fermentation techniques were developed during the second World War. Penicillins with several different side-chains were found to be produced by various penicillia and aspergilli, and hundreds of unnatural penicillins were prepared by the addition of side-chain precursors to fermentations.

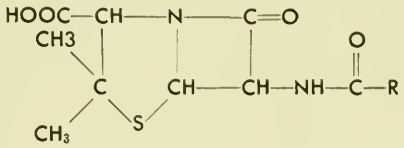
It was not until 1959, however, that the nucleus common to all penicillins, 6-aminopenicillanic acid, was isolated from fermentations.[1] This discovery has made possible the preparation of a new series of penicillins through

[1] Koichi Kato, *J. Antibiotics* (Japan) 6A 130, 184 (1953); F. R. Batchelor, F. P. Doyle, J. H. C. Nayler and G. N. Rolinson, *Nature* 183 257 (1959).

attachment of side-chains by the methods of organic chemistry.




6-Aminopenicillanic Acid




Penicillins

Since 6-aminopenicillanic acid can be isolated from penicillin fermentations in good yields, it is probably an intermediate. Also, the fact that side-chain precursors are so readily incorporated into the molecule indicates attachment of the side-chain to be the final step in penicillin biosynthesis. This is also known to be the rate-limiting step, and, even in commercial fermentations, side-chain precursors are added routinely.

The precursors of the 6-aminopenicillanic acid nucleus have been shown to be (stereospecifically) L-cysteine[2] and L-valine,[3] although additions of these amino acids to fermentations do not cause dramatic improvements in yields or in rates of synthesis. Degradation studies have shown that L-cysteine occurs in the same configuration after incorporation into the penicillin molecule, while valine has been converted to the D-form. Aside from the change in configuration of valine, both amino acids are incorporated intact.

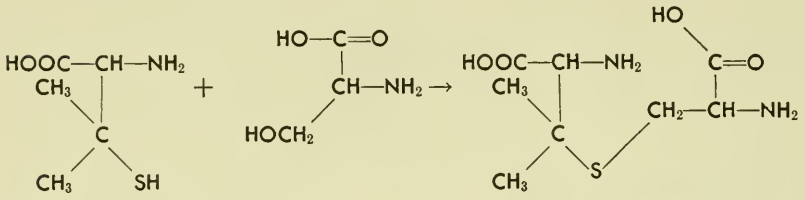
Other substances have been considered as penicillin precursors and intermediates. Among them are penicillamine,[4] β-hydroxyvaline,[3] serine,[2] glycine,[2] homocys-

[2] H. R. V. Arnstein and P. T. Grant, *Biochem. J.* 57 353, 360 (1954); H. R. V. Arnstein and J. C. Crawhall, *ibid.* 67 180 (1957); Carl M. Stevens, Edward Inamine and Chester W. Delong, *J. Biol. Chem.* 219 405 (1956); H. R. V. Arnstein and H. Margreiter, *Biochem. J.* 68 339 (1958); F. H. Grau and W. J. Halliday, *ibid.* 69 205 (1957).

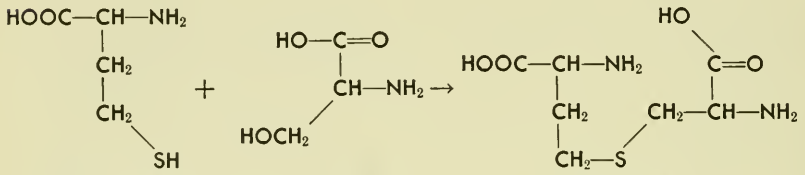
[3] H. R. V. Arnstein and Margaret E. Clubb, *ibid.* 65 618 (1957); Carl M. Stevens and Chester W. Delong, *J. Biol. Chem.* 230 991 (1958).

[4] Carl M. Stevens, Pran Vohra, Edward Inamine and Oliver A. Roholt, Jr., *ibid.* 205 1001 (1953).

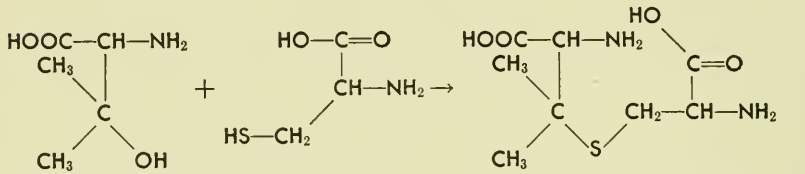
teine,[5] methionine,[4] glutathione[4] and acetate. Some of these rejected intermediates are shown:




Penicillamine Serine β, β-Dimethyllanthionine



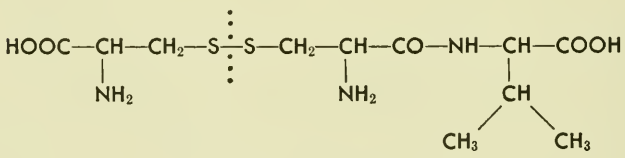

Homocysteine Serine Cystathionine




β-Hydroxyvaline Cysteine β, β-Dimethyllanthionine

Lanthionine and β-methyllanthionine occur in several other polypeptide antibiotics (subtilin, duramycin, cinnamycin, nisins). Certain of these compounds are incorporated to some extent, but only indirectly.

Some evidence is being accumulated concerning the actual peptide intermediate. The dipeptide L-cystinyl-L-(COOH—$C^{14}$) valine is a better penicillin precursor than L-(COOH—$C^{14}$) valine alone, while the reverse is true for protein synthesis.[6] (L-Cystine can be reduced to L-cysteine by the mold.)

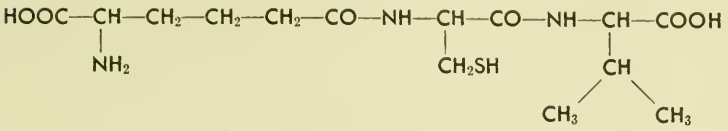



L-Cystinyl-L-(COOH—$C^{14}$) valine

[5] Carl M. Stevens, Pran Vohra, Joseph E. Moore and Chester W. DeLong, *ibid.* 210 713 (1954).

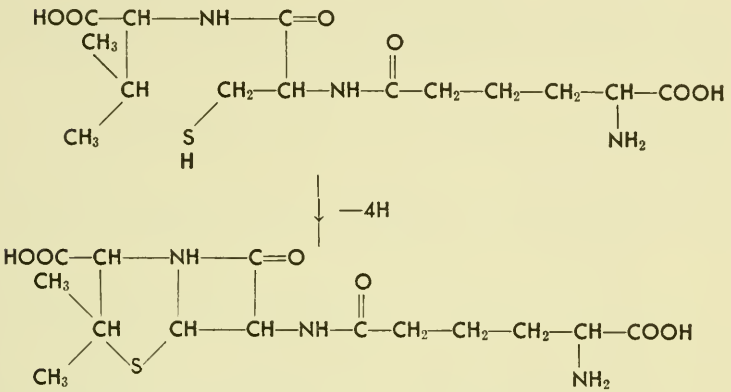
[6] H. R. V. Arnstein and D. Morris, *Biochem. J.* 71 8p (1959).

The same research group (Arnstein and collaborators) has isolated a tripeptide from the mycelium of the commercial penicillin producer, *Penicillium chrysogenum.*[7] It is δ-(α-aminoadipyl) cysteinylvaline:



δ-(α-Aminoadipyl) cysteinylvaline

Consistent with the above evidence, this is a cysteinylvaline. It is not difficult to envisage cyclization to form synnematin B:



Interesting features of this discovery are, first, that a side-chain is attached before cyclization to form 6-aminopenicillanic acid and, second, that the side-chain is α-aminoadipic acid, the side-chain of synnematin B (cephalosporin N) which is not produced by *Penicillium chrysogenum.* Perhaps side-chain exchange occurs after cyclization. The configurations of the amino acids in the acyclic mycelial peptide have not been reported yet.

The structure of cephalosporin C, a substance related to synnematin B, is known, but has not yet been published.

Two reviews of the biosynthesis of penicillin are cited.[8, 9]

[7] H. R. V. Arnstein, D. Morris and E. Toms, *Biochim. et Biophys. Acta* 35 561 (1959).

[8] A. L. Demain, *Advances in Appl. Microbiol.* 1 23 (1959).

[9] D. Hockenhull, *Prog. in Ind. Microbiol.* 1 1 (1959).

Cycloserine (oxamycin) appears to be a cyclized D-serine amide or hydroxamide. As mentioned elsewhere it is known to inhibit the incorporation of D-alanyl-D-alanine into the cell walls of certain bacteria.

Thiamine is an enzyme prosthetic group of fundamental importance, probably occurring in all living things. Many microorganisms are capable of *de novo* synthesis, although the vitamin is required in mammalian diets. Some microorganisms incapable of total synthesis can couple certain pyrimidine and thiazole precursors, others require only one of the heterocycles preformed, and certain yeasts have a requirement for thiamine itself.

Beyond this little is known about the biosynthesis of thiamine. Other naturally occurring thiazoles (*e.g.* those in certain antibiotics) are known to be derivatives of cysteine. Nakayama has proposed the general scheme:[10]

Cysteine → Thiazolidine-4-carboxylic Acid → 4-Methylthiazole → 4-Methyl-5-(2-hydroxyethyl)-thiazole

on the basis of work with mutants. Some work has been done on the biosynthesis of other pyrimidines, but apparently little on the thiamine constituent.

*Bacillus subtilis* incorporates formate $C^{14}$ extensively into the pyrimidine, but not the thiazole moiety of thiamine.[11] In this bacterium the pyrimidine moiety of thiamine restores growth and formate incorporation into purines and thymine in amethopterin treated cultures. The thiazole part restores thiamine synthesis, but does not show the additional effects.

It appears now that all enzymes in which thiamine is the active site have the function of decarboxylating α-ketoacids and of cleaving α-diketones or α-hydroxyketones. These functions were illustrated in an earlier section.

Thiamine, unphosphorylated and detached from its apoenzyme, is capable of carrying out some of its coenzyme functions *in vitro* under favorable conditions.[12, 13, 14]

[10] Hideo Nakayama, *Vitamins* (Japan) 11 20, 169 (1956).

[11] Martin J. Pine and Robert Guthrie, *J. Bacteriol.* 78 545 (1959).

[12] Shunzi Mizuhara and Philip Handler, *J. Am. Chem. Soc.* 76 571 (1954).

[13] Emeteria Yatco-Manzo, Frances Roddy, Ralph G. Yount and David E. Metzler, *J. Biol. Chem.* 234 733 (1959).

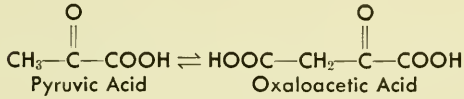
[14] Ralph G. Yount and David E. Metzler, *ibid.* 234 738 (1959).

By selective synthetic substitutions with blocking groups at various positions in the two heterocycles, the active site of the molecule has been located as the 2-position of the thiazole ring.[15, 16] It is here that pyruvic acid, for example, is decarboxylated to form (still in combination with thiamine pyrophosphate) "active acetaldehyde" and α-ketoglutaric acid to form "active succinate." The active acetaldehyde intermediate was shown in Section 2. It is claimed that this intermediate has been isolated from *Escherichia coli*.[16a]

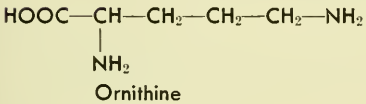
A thorough review of thiamine is available.[17]

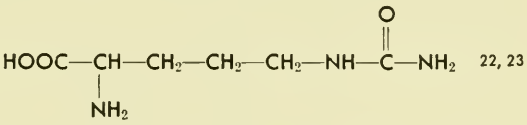
For more than 20 years biotin has been recognized as a dietary requirement in higher animals and yeasts. It was formerly called vitamin H, and animal deficiencies could be induced by feeding raw egg-white. This contains a protein, avidin, which complexes tightly enough with biotin to cause avitaminosis.

The biochemical function and mode of action of biotin long remained obscure. It is now known to be a cocarboxylase or coenzyme component for the transfer of carbon dioxide. Some of the reactions which it catalyzes are:




18, 19, 20, 21




22, 23

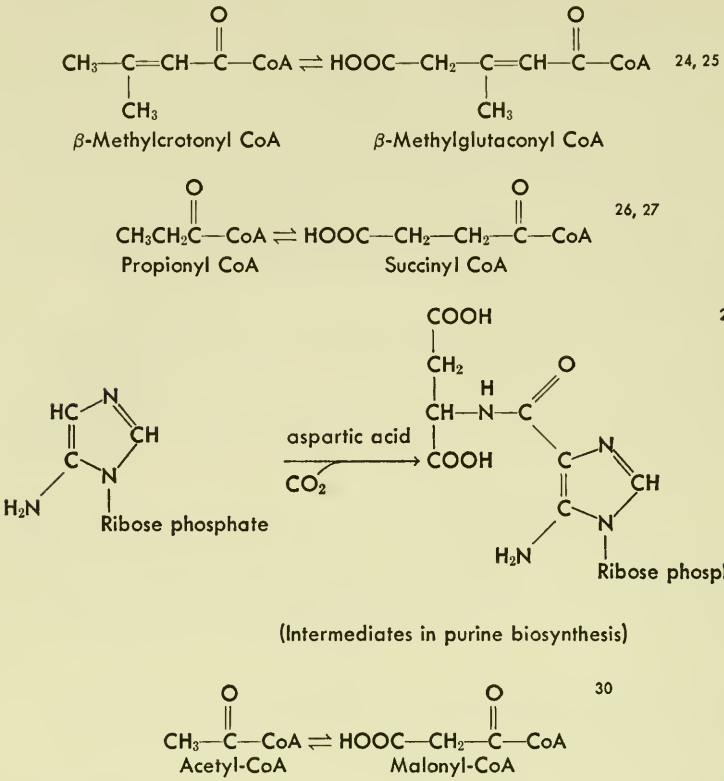
[15] Ronald Breslow, *J. Am. Chem. Soc.* 79 1762 (1957); 80 3719 (1958).

[16] Ronald Breslow and Edward McNelis, *ibid.* 81 3080 (1959).

[16a] Gerald L. Carlson and Gene M. Brown, *J. Biol. Chem.* 235 PC3 (1960).

[17] Paul D. Boyer, Henry Lardy and Karl Myrbäck (Eds.), "The Enzymes" Academic Press, New York, 1960 Vol. II, David E. Metzler, *Thiamine coenzymes*, pp. 295–337.

[18] Henry A. Lardy, Richard L. Potter and C. A. Elvehjem, *J. Biol. Chem.* 169 541 (1947).




[19] William Shive and Lorene Lane Rogers, *ibid.* 169 453 (1947).

[20] Herman C. Lichstein and W. W. Umbreit, *ibid.* 170 329 (1947).

[21] Henry A. Lardy, Richard L. Potter and R. H. Burris, *ibid.* 179 721 (1949).

[22] Patricia R. MacLeod, Santiago Grisolia, Philip P. Cohen and Henry A. Lardy, *ibid.* 180 1003 (1949).

[23] Gladys Feldott and Henry A. Lardy, *ibid.* 192 447 (1951).

[24] Bimal K. Bachhawat, Wm. G. Robinson and Minor J. Coon, *J. Am. Chem. Soc.* 76 3098 (1954); *idem., J. Biol. Chem.* 219 539 (1956).

[25] F. Lynen, J. Knappe, E. Lorch, G. Jütting and E. Ringelmann, *Angew. Chem.* 71 481 (1959).

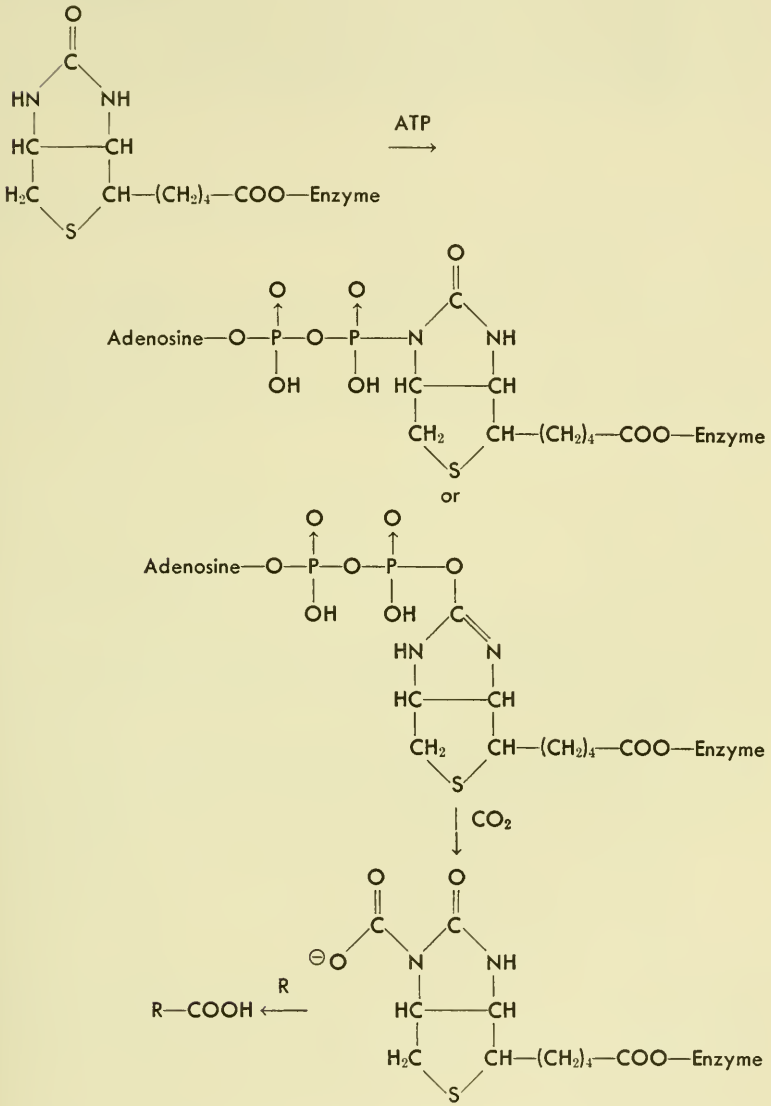
[26] Henry A. Lardy and Robert Peanasky, *Physiol. Rev.* 33 560 (1953).

[27] Henry A. Lardy and Julius Adler, *J. Biol. Chem.* 219 933 (1956).

[28] Patricia R. MacLeod and Henry A. Lardy, *ibid.* 179 733 (1949).

[29] Albert G. Moat, Charles N. Wilkins, Jr. and Herman Friedman, *ibid.* 223 985 (1956); Albert G. Moat and Floyd Nasuti, *Federation Proc.* 19 313 (1960).

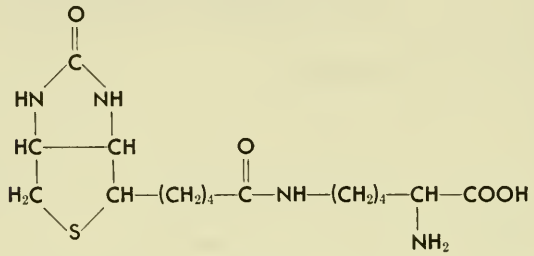
The mode of action of biotin is known now in enough detail to suggest the scheme outlined below. It is still uncertain which nitrogen atom of the biotin molecule participates.[25]



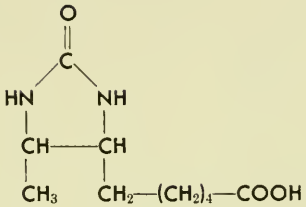

[30] Salih J. Wakil, Edward B. Titchener and David M. Gibson, *Biochim. et Biophys. Acta* 29 225 (1958); Salih J. Wakil, *J. Am. Chem. Soc.* 80 6465 (1958).

It is probable that biotin is attached to the enzyme in an amide linkage, perhaps at the $\epsilon$-amino group of a lysine unit. Evidence indicates that a variety of apoenzymes can use biotin as the prosthetic group in reversible carbon dioxide transfer just as a variety of apoenzymes can use riboflavin in reversible hydrogen transfer.

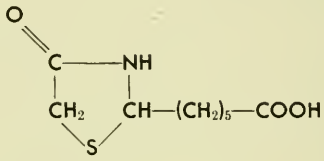
Biocytin is a biotin-lysine conjugate isolated from controlled autolysates of yeast cells.[31, 32]



Biocytin



Dethiobiotin



Actithiazic Acid

It is better utilized by some microorganisms than is biotin itself.

Actithiazic acid is a biotin antimetabolite.

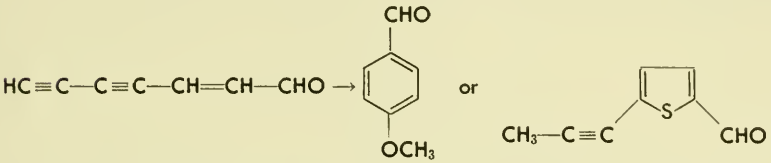
The biosynthetic origin of biotin remains obscure. Pimelic acid is an effective precursor in biotin-producing organisms. Dethiobiotin is produced by a *Penicillium chrysogenum* mutant, and it may be an intermediate in the biosynthetic scheme at least in this and probably in other microorganisms.[33]

[31] Lemuel D. Wright, Emlen L. Cresson, Helen R. Skeggs, Thomas R. Wood, Robert L. Peck, Donald E. Wolf and Karl Folkers, *ibid.* 74 1996 (1952).

[32] Donald E. Wolf, John Valiant, Robert L. Peck and Karl Folkers, *ibid.* 74 2002 (1952).

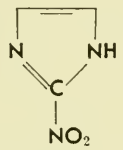
[33] E. L. Tatum, *J. Biol. Chem.* 160 455 (1945).

Junipal appears to be related to the acetylenic substances typical of basidiomycetes which were listed in an earlier section. In some way sulfur seems to have been added, in effect across two acetylenic bonds to form a thiophene ring. It has been suggested[34] that junipal and anisaldehyde, occurring in the same culture and with the same number of carbon atoms, may be derivatives of a common acetylenic aldehyde precursor, perhaps $C_7H_4O$:




Azomycin seems to incorporate a modified guanidine group.

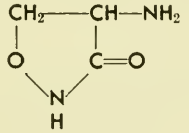
893 **Azomycin** (2-Nitroimidazole), $C_3H_3O_2N_3$, white needles, m.p. 283° (dec.).




*Nocardia* sp. resembling *N. mesenterica*

Shoshiro Nakamura and Hamao Umezawa, *J. Antibiotics* (Japan) 8A 66 (1955) and other papers in this series.

894 **Oxamycin** (Cycloserine, Orientomycin D-4-Amino-3-isoxazolidone, PA-94), $C_3H_6O_2N_2$, colorless crystals, m.p. 156° (dec.), $[\alpha]_{5461}$ 25° +137° ±2° (c 5 in 2 N sodium hydroxide).




*Streptomyces garyphalus, S. orchidaceus, S. lavendulae, S. nagasakiensie* nov. sp., *S.* K-300, etc.

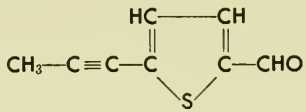
[34] J. H. Birkinshaw and P. Chaplen, *Biochem. J.* 60 255 (1955).

Dale A. Harris, Myrle Ruger, Mary Ann Reagan, Frank J. Wolf, Robert L. Peck, Hyman Wallick and H. Boyd Woodruff, *Antibiotics and Chemotherapy* 5 183 (1955).

Roger L. Harned, Phil Harter Hidy and Eleanore Kropp LaBaw, *ibid.* 5 204 (1955).

Charles H. Stammer, Andrew N. Wilson, Claude F. Spencer, Frank W. Bachelor, Frederick W. Holly and Karl Folkers, *J. Am. Chem. Soc.* 79 3236 (1957). (Synthesis)

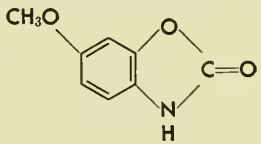
895 **Junipal,** $C_8H_6OS$, thick, colorless needles, m.p. 80°.



*Daedalea juniperina* Murr.

J. H. Birkinshaw and P. Chaplen, *Biochem. J.* 60 255 (1955).

896 **6-Methoxybenzoxazolidone,** $C_8H_7O_3N$, red crystals, m.p. 154°.

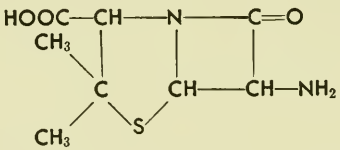


*Ustilago maydis* (spores)

The same compound has been isolated from young corn plants.

P. H. List, *Arch. Pharm.* 292 452 (1959).

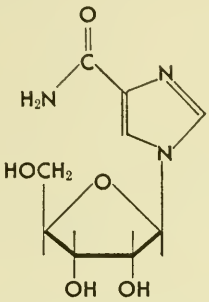
897 **6-Aminopenicillanic Acid,** $C_8H_{12}O_3N_2S$, colorless crystals, m.p. 208° (dec.).



*Penicillium chrysogenum*

F. R. Batchelor, F. P. Doyle, J. H. C. Nayler and G. N. Robinson, *Nature* 183 257 (1959).

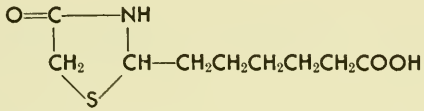
898 5-Amino-4-imidazolecarboxamide Riboside, $C_9H_{14}O_5N_4$, colorless crystals, m.p. 213° (dec., previous browning).




*Escherichia coli* (sulfonamide–inhibited)

G. Robert Greenberg and Edra L. Spilman, *J. Biol. Chem.* 219 411 (1956).

899 Actithiazic Acid (Acidomycin, Mycobacidin PA-95), $C_9H_{15}O_3NS$, colorless needles, m.p. 140°, $[\alpha]_D^{25}$ −60° (c 1 in absolute alcohol).




*Streptomyces virginiae, S. cinnamonensis, S. lavendulae*

Yields of about 0.3 g. per liter have been reported.

Walton E. Grundy, Alma L. Whitman, Elbina G. Rdzok, Edward J. Rdzok, Marjorie E. Hanes and John C. Sylvester, *Antibiotics and Chemotherapy* 2 399 (1952).

J. R. Schenck and A. F. DeRose, *Arch. Biochem. and Biophys.* 40 263 (1952).

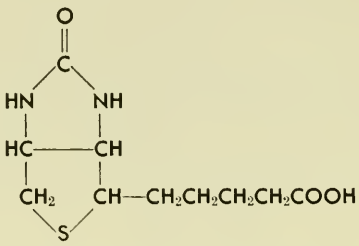
R. K. Clark, Jr. and J. R. Schenck, *ibid.* 40 270 (1952).

W. M. McLamore, Walter D. Celmer, Virgil V. Bogert, Frank C. Pennington and I. A. Solomons, *J. Am. Chem. Soc.* 74 2946 (1952).

B. A. Sobin, *ibid.* 74 2947 (1952).

W. M. McLamore, Walter D. Celmer, Virgil V. Bogert, Frank C. Pennington, B. A. Sobin and I. A. Solomons, *ibid.* 75 105 (1953). (Synthesis)

900 **Biotin,** $C_{10}H_{16}O_3N_2S$, colorless needles, m.p. 230–232° (dec.), $[\alpha]_D^{22}$ +92° (c 0.3 in 0.1 N sodium hydroxide).

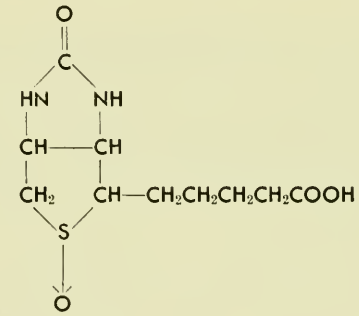


*Torula utilis*, other yeasts (occurs also in molds and bacteria)

Yields of 0.5–3.6 μg. per gram of dry cell weight are obtained from *Torula utilis*.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 **Vol. II**, J. M. Van Lanen, *Production of vitamins other than riboflavin*, chap. 6, pp. 191–216. (A review)

901 **Biotin-l-sulfoxide,** $C_{10}H_{16}O_4N_2S$, colorless crystals, m.p. 238–243°, $[\alpha]_D^{20}$ −40° (in 0.1 N sodium hydroxide).

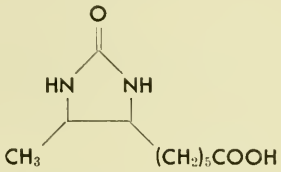


*Aspergillus niger*

Lemuel D. Wright and Emlen L. Cresson, *J. Am. Chem. Soc.* **76** 4156 (1954).

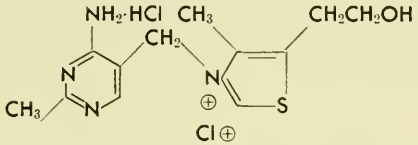
Lemuel D. Wright, Emlen L. Cresson, John Valiant, Donald E. Wolf and Karl Folkers, *ibid.* **76** 4160, 4163 (1954). (Isolation and characterization)

902 **Dethiobiotin** (Desthiobiotin, 5-Methyl-2-oxo-4-imidazolidinecaproic Acid), $C_{10}H_{18}O_3N_2$, colorless needles, m.p. 156–158°, $[\alpha]_D^{21}$ +10.7° (c 2.0 in water).




*Penicillium chrysogenum*
E. L. Tatum, *J. Biol. Chem.* 160 455 (1945).

903 **Thiamin** (Vitamin $B_1$, Aneurin) (Chloride Hydrochloride), $C_{12}H_{18}ON_4Cl_2S$, colorless needles, m.p. ~250° (dec.).

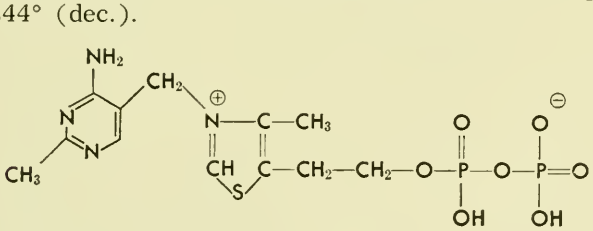



Most yeasts, molds and bacteria

Yields of 120–200 μg. per gram of dry primary-grown yeast cells can be obtained. Much higher yields (600–1200 μg. per gram) can be obtained if all that is required is coupling of supplied precursors.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. II, J. M. Van Lanen *Production of vitamins other than riboflavin,* chap. 6, pp. 191–216. (A review)

904 **Cocarboxylase** (Cozymase II, Vitamin $B_1$-diphosphate, Thiamin diphosphate, Aneurindiphosphate) (Hydrochloride), $C_{12}H_{18}O_7N_4SP_2 \cdot HCl$, nearly colorless needles, m.p. 242–244° (dec.).

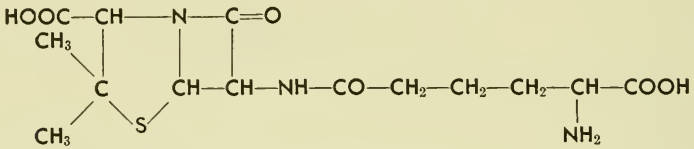


Yeast

K. Lohmann and Ph. Schuster, *Biochem.* Z. 294 188 (1937). (Isolation)

Kurt G. Stern and Jesse W. Hofer, *Science* 85 483 (1937). (Synthesis)

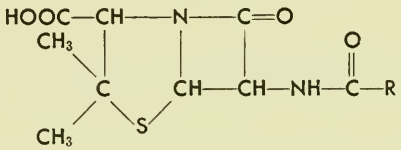
905 **Synnematin-B** (Cephalosporin N, Salmotin), $C_{14}H_{21}O_6N_3S$, barium salt, $[\alpha]_D^{20}$ +187° (c 0.6 in water).



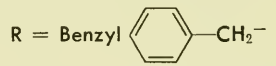
*Cephalosporium salmosynnematum*

E. P. Abraham, "CIBA Lectures in Microbiol. Biochemistry," *Biochemistry of some peptide and steroid antibiotics,* John Wiley and Sons, New York, 1957. (A review)

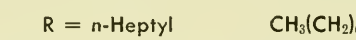
Natural Penicillins. General formula:



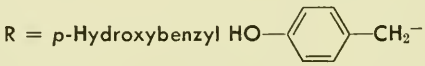
906 **Penicillin G,** $C_{16}H_{18}O_4N_2S$, colorless prisms, m.p. (Na salt) 215° (dec.), $[\alpha]_D^{25}$ +305° (c 0.821 in water).



907 **Penicillin K,** $C_{16}H_{26}O_4N_2S$, colorless prisms (Na salt), $[\alpha]_D^{25}$ +258° (c 0.43 in water).



908 **Penicillin X,** $C_{16}H_{18}O_5N_2S$, colorless crystals, m.p. (Na salt) 228–235° (dec.), $[\alpha]_D$ +267° (c 0.525 in water).



909 **Gigantic Acid** (Dihydro F), $C_{14}H_{22}O_4N_2S$ (Na salt), colorless crystals, m.p. 188° (dec.), $[\alpha]_D^{23}$ +319° (c 1 in water).

910 **Penicillin F** (Flavicidin, Flavicin) $C_{14}H_{20}O_4N_2S$, m.p. (Na salt) 204° (dec.), $[\alpha]_D^{20-25}$ +276–316° (c 0.821 in water).

R = *n*-Pentenyl $CH_3CH_2—CH=CH—CH_2—$

The $\Delta^3$-pentenyl variant is also known.

Penicillium species, especially *P. chrysogenum* and *P. notatum* Westling, and aspergillus species, especially *A. flavus* from which Penicillin F was obtained.

H. Clarke, J. Johnson and R. Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, 1949. (A review)

911 **Cephalosporin C,** proposed molecular formula $C_{16}H_{21}O_8N_3S$, Na salt: $[\alpha]_D^{20}$ +103°.

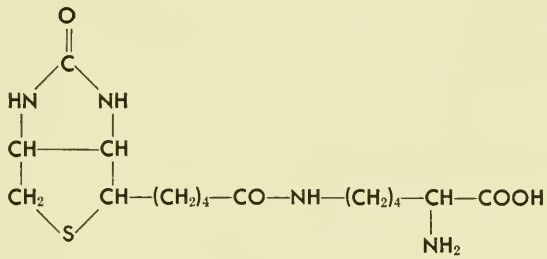
Structural features:

Acid hydrolysis yields 1 D-α-aminoadipic acid, 1 $CO_2$ and 2 $NH_3$. No penicillamine is produced in contrast to cephalosporin N.

*Cephalosporium salmosynnematum*

G. G. F. Newton and E. P. Abraham, *Biochem. J.* 62 651 (1956).

912 **Biocytin,** $C_{16}H_{28}O_4N_4S$, colorless crystals, m.p. 228–230° (dec.) (245–252°).




Yeast

Lemuel D. Wright, Emlen J. Cresson, Helen R. Skeggs, Thomas R. Wood, Robert L. Peck, Donald E. Wolf and Karl Folkers, *J. Am. Chem. Soc.* 72 1048 (1950). (Isolation)

Robert L. Peck, Donald E. Wolf and Karl Folkers, *ibid.* 74 1999 (1952). (Structure)

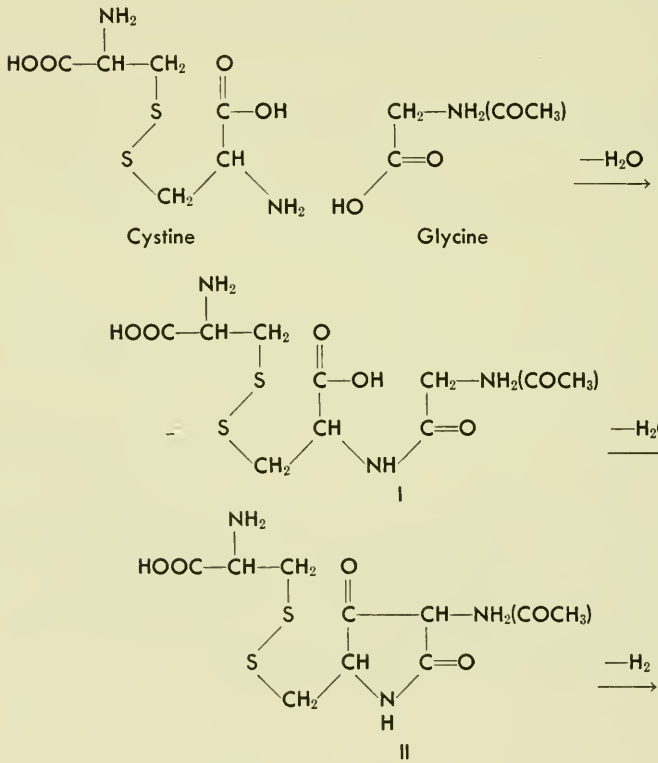
Donald E. Wolf, John Valiant, Robert L. Peck and Karl Folkers, *ibid.* 74 1002 (1952). (Synthesis)

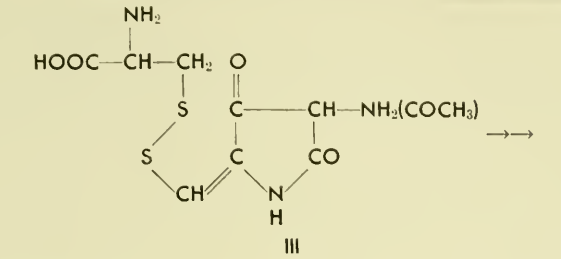
## *f.* PYRROLES, PORPHYRINS AND RELATED COMPOUNDS

Pyrroles occur rather frequently as microorganism metabolites. They are constituents of porphyrins, of vitamin $B_{12}$, of certain bacterial pigments, and of some compounds which have been considered as antibiotics.

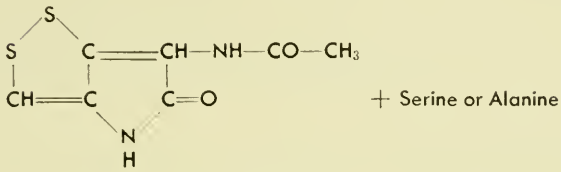
More has been published concerning the biosynthesis of the complex substances because of their more general import in biological systems, but it is tempting to speculate on the origins of the simpler compounds even though little evidence is yet available.

Holomycin is the simplest of three similar substances produced by streptomycetes, although the structures of aureothricin and thiolutin were determined earlier. The skeletons of glycine and cysteine are perceptible within the holomycin molecule, and, superficially, it seems that a biosynthetic route related to the following might take place:





III



Holomycin

A glycylcystine intermediate I is reminiscent of the peptide intermediate now implicated in the biosynthesis of penicillin.[1] It is known that there has been some academic interest in the origin of these compounds, however, and since no publications have been forthcoming, perhaps the problem is more complicated.

Pyoluteorin, with a carbonyl group at the two position of the pyrrole moiety, suggests an origin in the glutamate → proline pathway, perhaps from $\delta^1$-pyrroline-5-carboxylic acid, although the chlorination of the ring may indicate a less obvious derivation. The pyrrolidine moiety of the plant alkaloid, nicotine, has been shown to be biosynthesized from glutamate.[2]

The origins of prodigiosin and netropsin are not obvious. Some work has been done on prodigiosin.[3, 4] Glycine-2-$C^{14}$ was incorporated into prodigiosin, but 5-aminolevulinic acid-5-$C^{14}$ was not.[4] This apparently distinguishes the method of biosynthesis from that of the porphyrins. Moreover, $C^{14}$-labeled L-proline was found to be several times more efficient as a prodigiosin precursor in *Serratia marcescens* than glycine, while the reverse is

[1] H. R. V. Arnstein, D. Morris and E. Toms, *Biochim. et Biophys. Acta* 35 561 (1959).

[2] Thomas Griffith, Kenneth P. Hellman and Richard U. Byerrum, *J. Biol. Chem.* 235 800 (1960).

[3] R. Hubbard and C. Rimington, *Biochem. J.* 46 220 (1950).

[4] Gerald S. Marks and Lawrence Bogorad, *Proc. Nat. Acad. Sci.* 46 25 (1960).

true in heme synthesis (in rats).[5] The biosynthesis at least seems to be related to the metabolism of 5-carbon units such as proline, ornithine and glutamic acid. It was further proposed[4] that the methoxyl group in one pyrrole ring indicated derivation from hydroxyproline, and that the colorless $C_{10}$ pyrrolic substance, which is thought to be a prodigiosin precursor,[6] was also probably derived from two C-5 units and that the *n*-amyl side-chain also might be a rudimentary C-5 amino acid chain. In this connection, the isolation of a $C_{25}$ "prodigiosin-like pigment"[7] from a streptomycete should be mentioned. While all of the proposals made are not entirely compatible with the revised structure published since,[8] the basic tenets seem to be sound.

Orange and blue variants of prodigiosin occur. The latter, which are less soluble, may be metal chelates.

Some work also has been done on the biosynthesis of the pyrrolic pigments of *Bacillus bruntzii,* and glycylglycine was found to be a better precursor than glycine and a number of other peptides.[9]

It is safe to say that natural pyrroles are formed by a variety of methods. Demonstration of the participation of erythrose in the shikimic acid biosynthetic route has inspired the admonition that erythrose and its 4-C-atom derivatives should not be ignored as possible precursors of furans and pyrroles.[10]

Because of their importance in photosynthesis, in hemoglobin, in cytochromes and peroxidases and in the chromophore of vitamin $B_{12}$, there has been much investigation of the general mode of biosynthesis of porphyrins. It is likely that a similar method is operative in all cases.

Porphyrins are present in yeasts, molds and bacteria.

[5] David Shemin and D. Rittenberg, *J. Biol. Chem.* 166 621 (1946).

[6] Ursula V. Santer and Henry J. Vogel, *Biochim. et Biophys. Acta* 19 578 (1956).

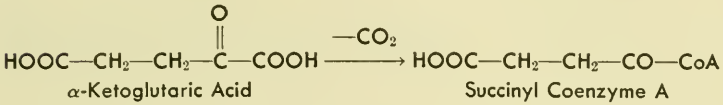
[7] F. Arcamone, A. DiMarco, M. Ghione and T. Scotti, *Giorn. microbiol.* 4 77 (1957).

[8] Harry H. Wasserman, James A. McKeon, Lewis Smith and Peter Forgione, *J. Am. Chem. Soc.* 82 506 (1960).

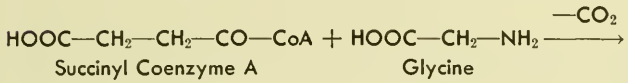
[9] J. G. Marchal and S. Baldo, *Trav. lab. microbiol. fac. pharm.* (Nancy) No. 18 187 (1956).

[10] Ernest Wenkert, *Experientia* 15 166 (1959).

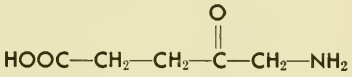
The photosynthetic bacteria, grown aerobically in light, are a rich source, and so are corynebacteria. Part of the biosynthetic pathway to the porphyrins has been explored in photosynthetic bacteria, and it is thought to be of general significance:[11, 12]



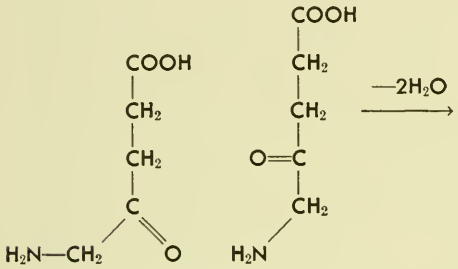

α-Ketoglutaric Acid — Succinyl Coenzyme A



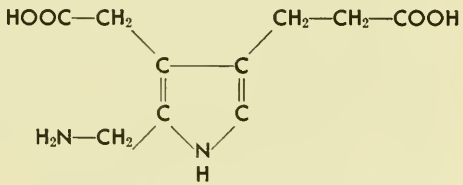

Succinyl Coenzyme A — Glycine




δ-Aminolevulinic Acid




δ-Aminolevulinic Acid



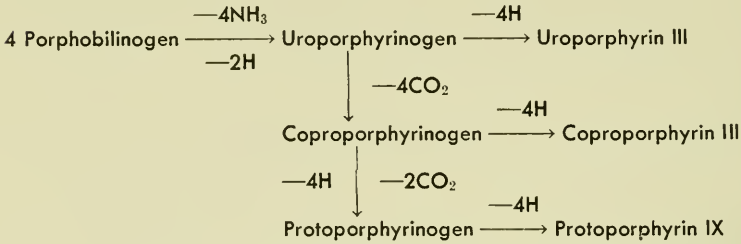

Porphobilinogen

Pyridoxal phosphate is required as a co-factor (glycine activator) in the glycine-succinyl-COA condensation.[12] Porphobilinogen then condenses to form coproporphyrin and protoporphyrin. In certain photosynthetic bacteria,

[11] June Lascelles, *Biochem. J.* 62 78 (1956); *idem., Abstracts of the Gordon Conference on Metabolism,* 1957.

[12] Goro Kikuchi, Abhaya Kumar, Phyllis Talmadge and David Shemin, *J. Biol. Chem.* 233 1214 (1958).

such as *Rhodopseudomonas spheroides*, the following sequence has been shown:




The reduced precursors may be the biologically active species, and the porphyrins by-products stabilized by oxidation.[11]

Higher animals (as well as microorganisms) are capable of porphyrin synthesis, and, in fact, the above work with photosynthetic bacteria was based on earlier labeling experiments in animals,[13] and porphobilinogen was first isolated from the urine of humans with acute porphyria.[14]

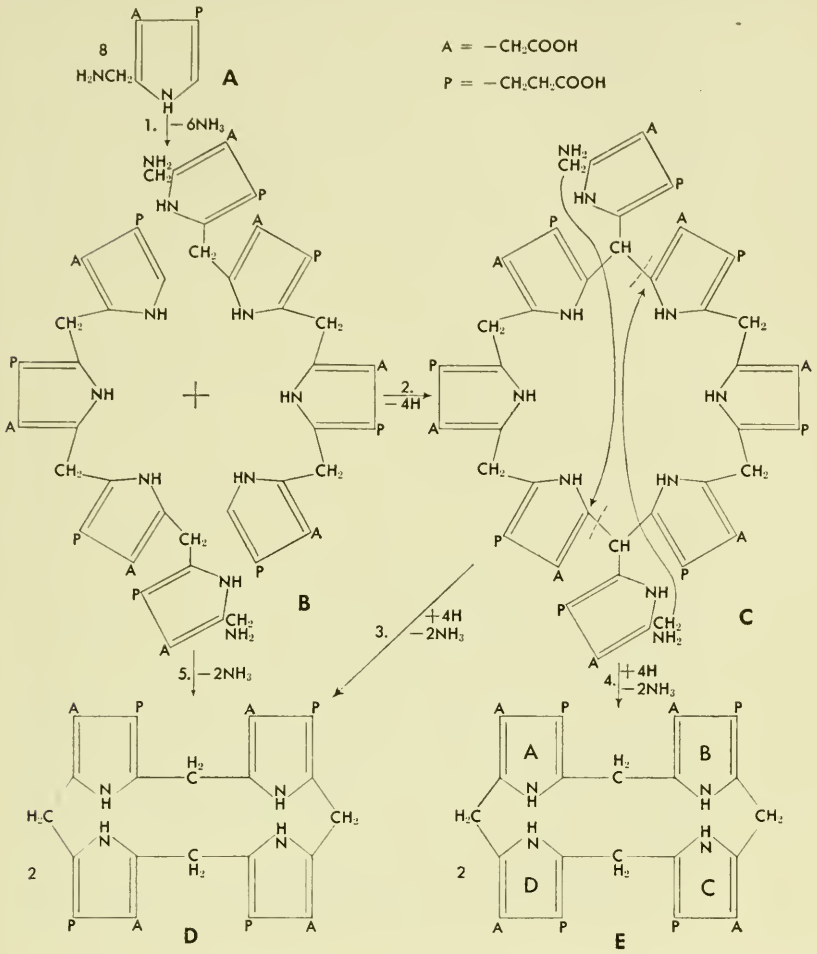
Widely occurring enzymes convert porphobilinogen to uroporphyrins, but it is difficult to isolate and identify the intermediates. Apparently they are quite transitory. Some interesting speculations have been published concerning their nature.[15, 16] The Wittenberg hypothesis, based on the known transformations of porphobilinogen by chemicals and enzymes, the extensive labeling studies that have been published, and on the construction of models, is outlined in the following series of equations:

[13] David Shemin and D. Rittenberg, *J. Biol. Chem.* 166 621, 627 (1946); Norman S. Radin, D. Rittenberg and David Shemin, *ibid.* 184 745 (1950); Jonathan Wittenberg and David Shemin, *ibid.* 185 103 (1950); David Shemin and Jonathan Wittenberg, *ibid.* 192 315 (1951); Helen M. Muir and A. Neuberger, *Biochem. J.* 47 97 (1950); David Shemin, Charlotte S. Russell and Tessa Abramsky, *J. Biol. Chem.* 215 613 (1954); K. D. Gibson, W. G. Lauer and A. Neuberger, *Biochem. J.* 70 71 (1958); K. D. Gibson, A. Neuberger and J. J. Scott, *ibid.* 61 618 (1955); J. E. Falk, E. I. B. Dresel, A. Benson and B. C. Knight, *ibid.* 63 87 (1956); E. I. B. Dresel and J. E. Falk, *ibid.* 63 388 (1956).

[14] R. G. Westall, *Nature* 170 614 (1952); G. H. Cookson and C. Rimington, *Biochem. J.* 57 476 (1954).

[15] David Shemin, *Harvey Lectures* 50 258 (1956).

[16] Jonathan B. Wittenberg, *Nature* 184 876 (1959).




Bogorad found[17] that the enzyme porphobilinogen deaminase converts porphobilinogen (**A**) to uroporphyrinogen (**D**). Because a second enzyme, uroporphyrinogen isomerase, has as its only substrate (not **D**) a product of the action of porphobilinogen deaminase on (**A**), there must have been one or more colorless intermediates. The intermediates must be convertible, spontaneously or under the continuing influence of porphobilinogen deaminase,

[17] Lawrence Bogorad, *J. Biol. Chem.* 233 501, 510, 516 (1958).

to (D) (reaction 5). The linear tetrapyrrole (B) shown is the intermediate proposed by Wittenberg.

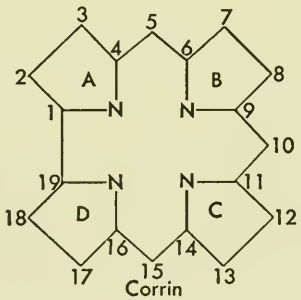
The enzyme, uroporphyrinogen isomerase, acting on porphobilinogen, yields uroporphyrinogen III (E) as its first detectable product. Wittenberg proposed that the function of this enzyme is to condense 2 molecules of (B) (reaction 2), creating the cyclic octapyrrole (C). Model studies indicate that such an intermediate could fold and undergo rearrangement, spontaneously or under continued enzyme influence, to yield 2 molecules of uroporphyrinogen III (E) (reaction 4).

The over-all result of this reaction sequence would be the interchange of the pyrrole moieties destined to form rings D of the porphyrins between two tetrapyrroles, with consequent reversal of the positions of the D rings relative to the other pyrrole rings of the tetrapyrroles.

This hypothesis seems to be in accord with all other known evidence concerning porphyrin biosynthesis, and it would account for their peculiar asymmetry. Many references to related work are cited by Wittenberg. It is notable that appropriate dipyrromethanes were not converted to porphyrinogens or porphyrins by porphobilinogen deaminase.[18]

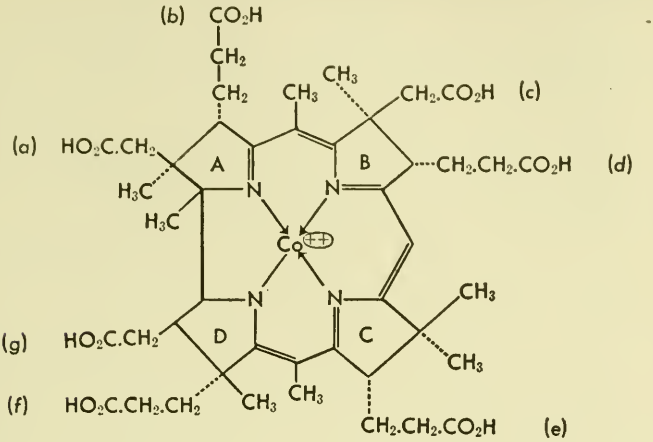
Vitamin $B_{12}$ is the only vitamin produced exclusively by microorganisms, although not all microbes are capable of elaborating it. Most seem to form little more than enough for their own slight requirements, the best organisms for primary production by fermentation being: *Streptomyces olivaceus, S. griseus, Propionibacterium shermanii* and *Bacillus megatherium.*

The nucleus of vitamin $B_{12}$ differs somewhat from that of porphyrins and is called the corrin ring:[55]

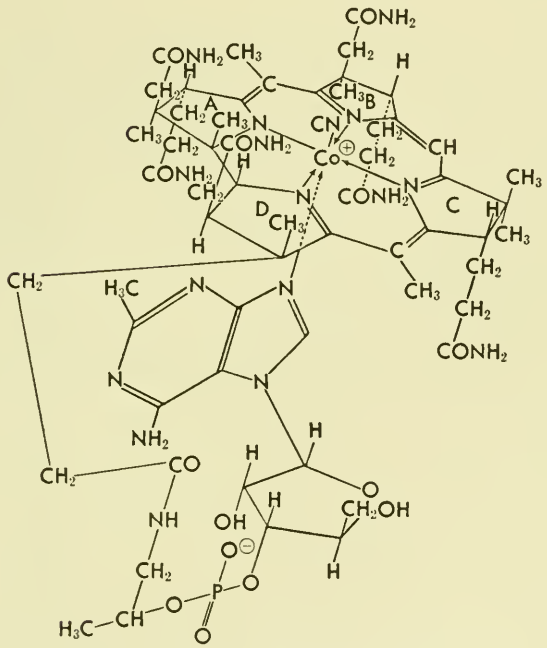


Corrin

[18] D. S. Hoare and H. Heath, *Biochim. et Biophys. Acta* 39 167 (1960).




Cobyrinic Acid




α-(5,6-Dimethylbenzimidazolyl) cobamide Cyanide

The nucleotide-free carboxylic acid form is called cobyrinic acid, the carboxyl groups (amides, etc.) being let-

tered as shown. When the aminopropanol group is incorporated by amide linkage at the f-position, the name is modified to cobinic acid (all carboxyl groups as amides = cobinamide), and when ribose is incorporated, the name is modified to cobamic acid (cobamide). The name of the heterocycle is then inserted at the beginning with the suffix -yl. Thus, vitamin $B_{12}$ is correctly named: α-(5,6-dimethylbenzimidazolyl) cobamide cyanide.

The two principal moieties are called the planar group and the nucleotide, and these are essentially perpendicular in relative steric arrangement.

A number of analogues of vitamin $B_{12}$ have been isolated from natural sources. These sources include $B_{12}$ fermentations, the rumen or the gut of various animals and sewage sludge. The naturally occurring analogues are listed below by trivial name, together with the characteristic heterocycle of the nucleotide.

TABLE I

*Naturally Occurring Vitamin $B_{12}$ Analogues*

| Name | Nucleotide base | Reference |
|---|---|---|
| Vitamin $B_{12}$ | 5,6-Dimethylbenzimidazole | |
| Pseudo (ψ)-Vitamin $B_{12}$ | Adenine | 19, 20, 21, 22, 23 |
| Factor A | 2-Methyladenine | 20, 21, 22, 23 |
| Factor B (Etiocobalamine) | No nucleotide | 21, 24, 25, 26 |
| Factor C (Guanosine Diphosphate Factor B) | Guanine | 21, 24, 25, 27, 37 |
| Factor D* | Unknown | 21, 22 |
| Factor E* | Unknown | 22 |
| Factor F | 2-Methylmercaptoadenine(?) | 21, 22, 25 |
| Factor G | Hypoxanthine | 22 |
| Factor H | 2-Methylhypoxanthine | 22 |
| Factor I ($B_{12}$ factor$_{III}$) | 5-Hydroxybenzimidazole | 22, 28, 29 |
| Factors J, K, L, M | Unknown | 30 |
| ———— | Unknown purine base (?) | 31 |
| ———— (May be factor F) | 2-Methylmercaptoadenine | 32 |
| ———— (May be factor C) | Guanine | 27 |
| Factor "A" Ribose Phosphate | No base | 33 |
| Factor $V_{1a}$ (Cobyrinic Acid a, b, c, d, e, g-hexaamide) | No base | 34, 35 |
| Factor $V_{1b}$ (Cobyrinic Acid Pentamide) | No base | 34, 35 |
| ———— | 5-Methylbenzimidazole | 36 |
| ———— | Benzimidazole | 36† |

* Not crystalline. † About 17 other cobamides were detected in this study.

Besides the natural analogues, many vitamin $B_{12}$ variants have been prepared by addition of analogues of the

[19] H. W. Dion, D. G. Calkins and J. J. Pfiffner, *J. Am. Chem. Soc.* 74 1108 (1952).

[20] J. E. Ford, E. S. Holdsworth, S. K. Kon and J. W. G. Porter, *Nature* 171 148 (1953).

[21] H. G. Heinrich (Editor), "Vitamin $B_{12}$ and Intrinsic Factor, First European Symposium, Hamburg, 1956." M. E. Coates and S. K. Kon, Ferdinand Enke, Stuttgart, 1956, p. 72.

[22] F. B. Brown, J. C. Cain, Dorothy E. Gant, T. F. J. Parker and E. Lester Smith, *Biochem. J.* 59 82 (1955).

[23] H. W. Dion, D. G. Calkins, and J. J. Pfiffner, *J. Am. Chem. Soc.* 76 948 (1954).

[24] S. K. Kon, Biochem. Symposium No. 13, p. 17 (1955).

[25] H. G. Heinrich (Editor), "Vitamin $B_{12}$ and Intrinsic Factor, First European, Hamburg, 1956." J. W. S. Porter, Ferdinand Enke, Stuttgart, 1956, p. 43.

[26] J. B. Armitage, J. R. Cannon, A. W. Johnson, T. F. J. Parker, E. Lester Smith, W. H. Stafford and A. R. Todd, *J. Chem. Soc.*, 3849 (1953).

[27] R. Barchielli, G. Boretti, P. Julita, A. Migliacci and A. Minghetti, *Biochim. et Biophys. Acta* 25 452 (1957).

[28] Wilhelm Friedrich and Konrad Bernhauer, *Chem. Ber.* 89 2030 (1956).

[29] Wilhelm Friedrich and Konrad Bernhauer, *Angew. Chem.* 65 627 (1953).

[30] Clifford H. Shunk, Franklin M. Robinson, James F. McPherson, Marjorie M. Gasser and Karl Folkers, *J. Am. Chem. Soc.* 78 3228 (1956).

[31] G. E. W. Wolstenholm and Maeve O'Connor (Eds.), CIBA Foundation Symposium on "The Chemistry and Biology of Purines," E. Lester Smith, *The chemistry of new purines in the* $B_{12}$ *series of vitamins,* Little, Brown & Co., Boston, 1957, pp. 160–168.

[32] Wilhelm Friedrich and Konrad Bernhauer, *Chem. Ber.* 90 1966 (1957).

[33] Hanswerner Dellweg and Konrad Bernhauer, *Arch. Biochem. and Biophys.* 69 74 (1957).

[34] Konrad Bernhauer, Hanswerner Dellweg, Wilhelm Friedrich, Gisela Gross and F. Wagner, Z. *Naturforsch.* 156 336 (1960).

[34a] K. Bernhauer, H. W. Dellweg, W. Friedrich, G. Gross, F. Wagner, and P. Zeller, *Helv. Chim. Acta* 43 693 (1960).

[35] Konrad Bernhauer, Elisabeth Becher, Gisela Gross and Georg Wilharm, *Biochem.* Z. 332 562 (1960).

[35a] K. Bernhauer, F. Wagner and P. Zeller, *Helv. Chim. Acta* 43 696 (1960).

[36] Wilhelm Friedrich and Konrad Bernhauer, *Chem. Ber.* 91 2061 (1958).

[37] G. Boretti, A. DiMarco, T. Fuoco, M. P. Marnati, A. Migliacci and C. Spalla, *Biochim. et Biophys. Acta* 37 379 (1960).

nucleotide base to fermentations. A review[38] lists about 50 such compounds, some of which have vitamin activity.

There seems to be a fundamental similarity in the biosynthetic routes to vitamin $B_{12}$ and the porphyrins. $C^{14}$-Labeled glycine or δ-aminolevulinic acid are heavily incorporated.[39] Threonine furnishes the aminopropanol moiety as demonstrated by incorporation of the amino acid labeled with $N^{15}$.[40] There seems to be no information yet on the biosynthetic origin of the dimethylbenzimidazole moiety.

Red cobamide-containing polypeptides have been isolated from microorganisms, and some of these can replace cobamide in deficient microorganisms, and in the oral treatment of pernicious anemia.[41, 42]

Cobamides have been implicated in several metabolic processes.[43] In *Escherichia coli* mutants they seem to assist in the formation and transfer of methionine methyl groups[44] (folic acid is also required). They are thought to be involved in the reduction of disulfides to thiols.[45] In

[38] D. Perlman, *Advances in Appl. Microbiol.* 1 87–112 (1952). (A review)

[39] David Shemin, John W. Corcoran, Charles Rosenblum and Ian M. Miller, *Science* 124 272 (1956); John W. Corcoran and David Shemin, *Biochim. et Biophys. Acta* 25 661 (1957).

[40] Alvin I. Krasna, Charles Rosenblum and David B. Sprinson, *J. Biol. Chem.* 225 745 (1957).

[41] H. G. Wijmenga, J. Lens and A. Middlebeek, *Chem. Weekblad* 45 342 (1949); H. G. Wijmenga and B. Hurenkamp, *ibid.* 47 217 (1951); H. G. Wijmenga and W. L. C. Veer, *ibid.* 48 33 (1952); H. G. Wijmenga, K. W. Thompson, K. G. Stern and D. J. O'Connell, *Biochim. et Biophys. Acta* 13 144 (1954); H. G. Wijmenga, J. Lens and S. J. Geerts, *Acta Haematol.* 11 372 (1954).

[42] K. Hausmann, *Lancet* 257 962 (1949); K. Hausmann and K. Mulli, *Acta Haemotol.* 1 345 (1952); *idem., Lancet* 262 185 (1952); K. Hausmann, *Klin. Wochschr.* 31 1017 (1953); K. Hausmann, L. Ludwig and K. Mulli, *Acta Haemotol.* 10 282 (1953); K. Mulli and O. J. Schmid, Z. *Vitamin-, Hormon-u. Fermentforsch.* 8 225 (1956); J. G. Heathcote and F. S. Mooney, *Lancet* 274 982 (1958).

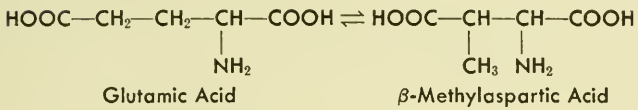
[43] R. D. Williams (Ed.), "The Biochemistry of Vitamin $B_{12}$," June Lascelles and M. J. Cross, *The function of vitamin* $B_{12}$ *in microorganisms,* Biochemical Society Symposia No. 13, Cambridge University Press, London, 1955, pp. 109–123.

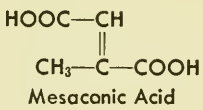
[44] C. W. Helleiner and D. D. Woods, *Biochem. J.* 63 26p (1956).

[45] J. W. Dubnoff and E. Bartroy, *Arch. Biochem. and Biophys.* 62 86 (1956); Chiun T. Ling and Bacon F. Chow, *J. Biol. Chem.* 206 797 (1954).

*Lactobacillus leichmannii* they are required for the reduction of formate to the methyl group of thymine by a pathway not involving methionine nor a hydroxymethyl intermediate.[46] In the same organism they have been reported necessary for the synthesis of deoxyribose.[47]

The isolation of actual coenzyme forms of cobamides has permitted more precise determination of some functions which are known to be direct. Barker and collaborators found that cell-free extracts of the anaerobe *Clostridium tetanomorphum* metabolized glutamate in a way different from the citric acid cycle, catalyzing the equilibrium:




An orange form of pseudovitamin $B_{12}$ was isolated and found to be required for the first step.[48] (It is noteworthy that β-methylaspartic acid occurs in the polypeptide antibiotic, aspartocin.) The entire nature of this coenzyme is still unknown, but the nucleotide base is known to be adenine. Also a second mole of adenine nucleoside is present, bound in such a way as to affect radically the corphyrin spectrum, and cleavable by photolysis. The nucleoside apparently is attached to cobalt, replacing the cyano group. It contains an unusual sugar.[49]

In this isomerization there are two possible migrating

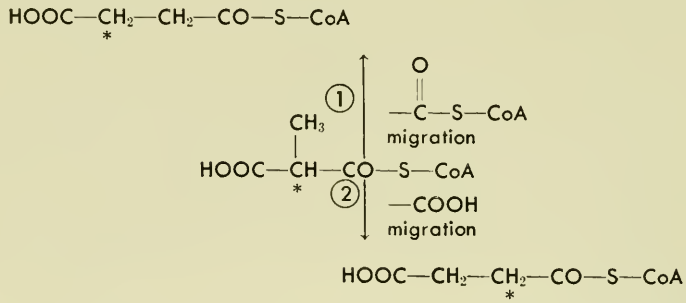
[46] James L. Dinning, Barbara K. Allen, Ruth Young and Paul L. Day, *J. Biol. Chem.* 233 674 (1958).

[47] Mancourt Downing and B. S. Schweigert, *J. Biol. Chem.* 220 521 (1956); W. T. Wong and B. S. Schweigert, *Proc. Soc. Exptl. Biol. Med.* 94 455 (1957).

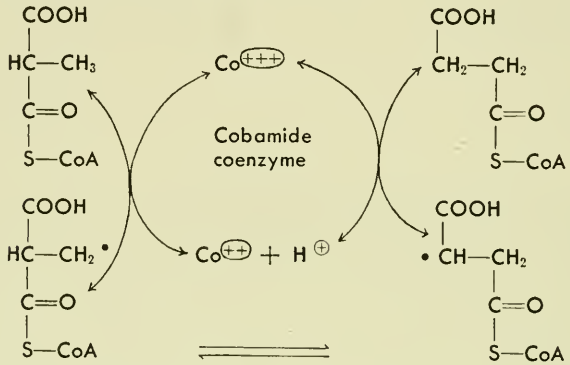
[48] H. A. Barker, H. Weissbach and R. D. Smyth, *Proc. Nat. Acad. Sci. U. S.* 44 1093 (1958).

[49] H. A. Barker, R. D. Smyth, H. Weissbach, J. I. Toohey, J. N. Ladd and B. E. Volcani, *J. Biol. Chem.* 235 480 (1960); H. Weissbach, J. N. Ladd, B. E. Volcani, R. D. Smyth and H. A. Barker, *ibid.* 235 1462 (1960); J. N. Ladd, H. P. C. Hogenkamp and H. A. Barker, *Biochem. and Biophys. Res. Comms.* 2 143 (1960).

groups as shown below. A labeling experiment has shown that




① is the actual process.[50] A free radical mechanism was proposed in which the $Co^{+++}$ of the cobamide coenzyme initiates the one-electron transfer:




This is analogous to a rearrangement reported earlier by Urry and Kharasch.[51]

The same organism (*Clostridium tetanomorphum*) was found capable of producing coenzymes containing the benzimidazole and dimethylbenzimidazole forms of vitamin $B_{12}$.[52] The dimethylbenzimidazole coenzyme has been found[53] to promote the equilibrium rearrangement previously known to exist:[54]

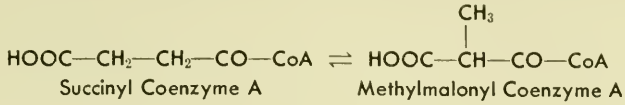
[50] H. Eggerer, P. Overath and F. Lynen, *J. Am. Chem. Soc.* 82 2643 (1960).

[51] W. H. Urry and M. S. Kharasch, *ibid.* 66 1438 (1944).

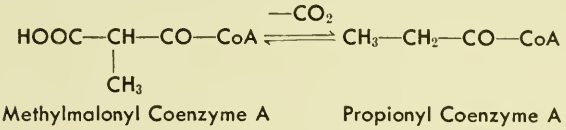
[52] H. Weissbach, J. Toohey and H. A. Barker, *Proc. Nat. Acad. Sci.* 45 521 (1959).

[53] E. R. Stadtman, P. Overath, H. Eggerer and F. Lynen, *Biochem. and Biophys. Res. Comms.* 2 1 (1960); Joseph R. Stern and Daniel L. Friedman, *ibid.* 2 82 (1960); Shantov Gurnani, S. P. Mistry and B. Connor Johnston, *Biochim. et Biophys. Acta* 38 187 (1960).

[54] Robert W. Swick and Harland G. Wood, *Proc. Nat. Acad. Sci. U. S.* 46 28 (1960).




The final step in the conversion of succinate to propionate is the biotin-dependent decarboxylation:[53]




The total process can be written:

(1) Acetyl CoA + Succinate ⇌ Succinyl CoA + Acetate
(2) Succinyl CoA $\xrightleftharpoons{B_{12}\ \text{coenzyme}}$ Methylmalonyl CoA
(3) Methylmalonyl CoA + Biotinenzyme ⇌ $CO_2$ Biotinenzyme + Propionyl CoA
(4) Propionyl CoA + Acetate ⇌ Acetyl CoA + Propionate

Perhaps it is significant that propionibacteria are relatively rich sources of vitamin $B_{12}$ and of biotin. This scheme also shows how propionic acid can be oxidized by entry into the carboxylic acid cycle.

The precise mechanism by which these interesting rearrangements are promoted by the $B_{12}$ coenzymes remains to be determined. It has been pointed out[53] that, in effect, what is accomplished is a transpropionation.

A monograph on vitamin $B_{12}$ has been published.[55]

The cytochromes are heme proteins important in electron transport. The most studied is cytochrome *c*. The commonest source is muscle, but yeast cytochrome *c* has been crystallized.[56] Classification is made by spectrum, and the proteins are species specific.

The prosthetic group of cytochrome *c* is protoporphyrin IX bound firmly to the apoenzyme by covalent bonds between the thiol groups of cysteine and the vinyl groups of the porphyrin.[57] Four of the iron coordination bonds are

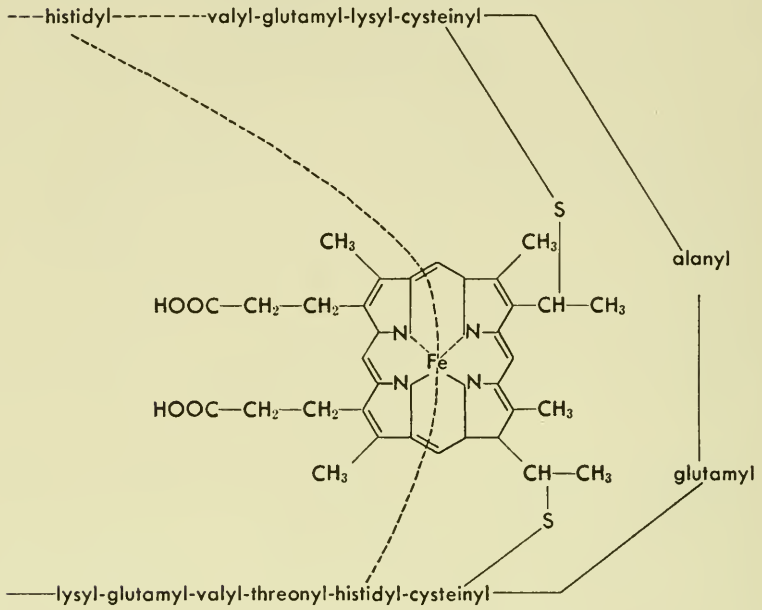
[55] E. Lester Smith, "Vitamin $B_{12}$," John Wiley & Sons, Inc., New York, 1960.

[56] Bunji Hagihara, Takekazu Horio, Kazuo Okunuki, Jinpei Yamashita and Mitsuhiro Nozaki, *Nature* 178 629 (1956).

[57] K. Zeile and H. Meyer, *Hoppe-Seylers Z. physiol. Chem.* 262 178 (1939); H. Theorell, *Enzymologia* 6 88 (1939); Karl-Gustav Paul, *Acta Chem. Scand.* 5 389 (1951).

to porphyrin nitrogen, the other two to histidyl residues in the protein.

Proteolytic enzyme degradation of cytochrome *c* has yielded the polypeptide fragment in the vicinity of the porphyrin, and the amino acid sequence has been determined. It is thought to be:[58]




Cytochrome c Fragment (Hemopeptide)

Bovine cytochrome *c* has a particle weight of about 13,000 and contains about 20 lysine and 3 or 4 histidine residues. A helical model of the Pauling type thus probably showns the entire active region of the enzyme since this cytochrome contains only one prosthetic group.

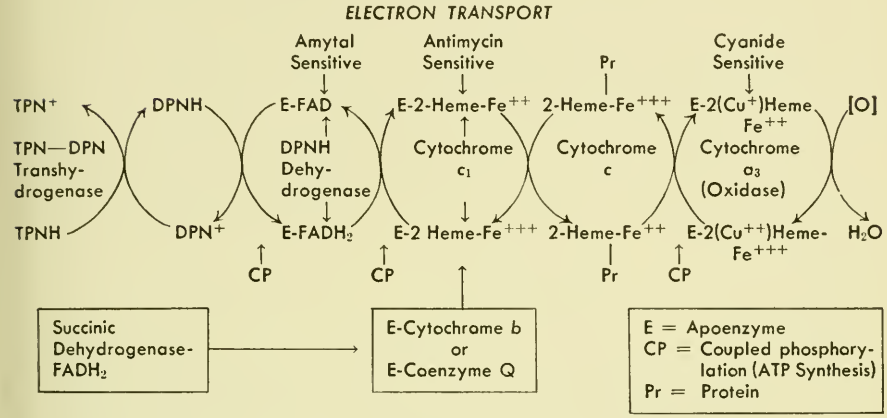
Cytochromes ($c_4$ and $c_5$) isolated from *Azotobacter vinelandii* have a particle weight of about 12,000 and contain 0.46% iron, so that superficially they resemble mammalian cytochrome c.[59] In a comparative study of mammalian and bacterial (*Pseudomonas aeruginosa*) cyto-

[58] Hans Tuppy and G. Bodo, *Monatshefte Chem.* 85 1024, 1182 (1954); Hans Tuppy and Sven Paleus, *Acta Chem. Scand.* 9 353, 365 (1955).

[59] A. Tissieres, *Biochem. J.* 64 582 (1956).

chrome c rather minor spectral differences were noted, but there were gross differences in the amino acid composition of the protein.[60] The prosthetic group of cytochrome $a_2$ from *Aerobacter aerogenes* has been purified but not crystallized.[61] Strict anaerobes such as clostridia seem to lack cytochromes, and some lactobacilli seem to use flavins instead.

Reviews of the role of cytochromes in electron transport have been published.[62, 63, 64, 65] This process is shown in outline in the accompanying diagram.




The role of lipides and quinones in electron transport has been discussed.[66] The mechanism of coupled phosphorylation is not understood in detail, but can be represented as follows:

[60] Martin D. Kamen and Yoshiro Takeda, *Biochim. et Biophys. Acta* 21 518 (1956).

[61] J. Barrett, *Biochem. J.* 64 626 (1956).

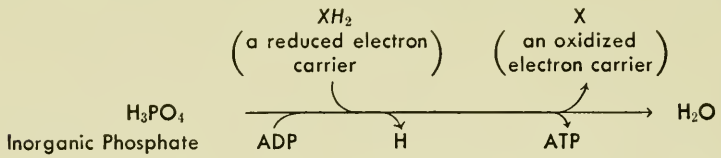
[62] Albert L. Lehninger, *The Harvey Lectures,* 49 176–215 (1955); *idem., Scientific American* 202 102–118 (1960).

[63] Britton Chance and G. R. Williams, *Advances in Enzymol.* 17 65–130 (1956).

[64] Joseph S. Fruton and Sofia Simmons, "General Biochemistry," John Wiley and Sons, New York, 1958, pp. 284–386.

[65] David E. Green and Johan Jarnefelt, *Perspectives in Biol. and Med.* 2 163–184 (1959).

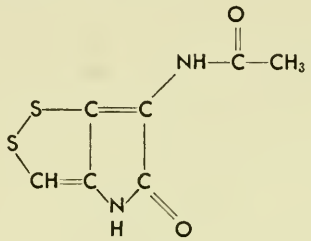
[66] D. E. Green and R. L. Lester, *Federation Proc.* 18 987–1000 (1959).




Some electron transport poisons are shown. Many other poisons also act by interfering somehow with the function of the electron transport enzymes.

A lucid, if rather popularized, exposition has been published of the energy relationships in cell respiration, as well as the gross cell structure involved.[67]

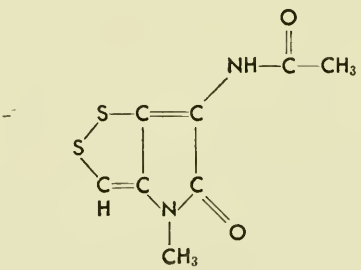
913 **Holomycin** (Des-N-methylthiolutin), $C_7H_6O_2N_2S_2$, orange-yellow leaflets, m.p. 264–271° (dec.).



*Streptomyces griseus* (Krainsky) Waksman et Henrici

L. Ettlinger, E. Gäumann, R. Hütter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog and H. Zähner, *Helv. Chim. Acta* 42 563 (1959).

914 **Thiolutin** (Acetopyrrothine, Farcinicin), $C_8H_8O_2N_2S_2$, yellow crystals, m.p. 260–270° (dec.).



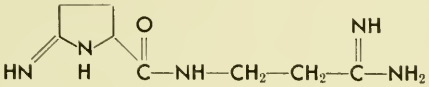
[67] Albert L. Lehninger, *Scientific American* 202 102–117 (1960).

*Streptomyces albus*

Walter D. Celmer, Fred W. Tanner, Jr., M. Harfenist, T. M. Lees and I. A. Solomons, *J. Am. Chem. Soc.* 74 6304 (1952).

Walter D. Celmer and I. A. Solomons, *ibid.* 77 2861 (1955). (Structure)

915 **Noformicin,** $C_8H_{15}ON_5$, Dihydrochloride m.p. 265° (dec.), $[\alpha]_D^{25}$ +7.0° (c 1.0 in water).

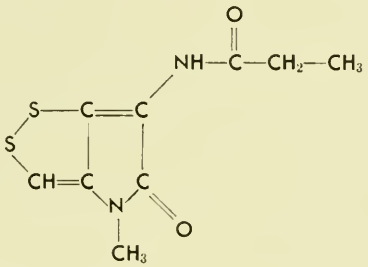


*Nocardia formica*

Reed A. Gray, *Phytopathology* 45 281 (1955).

Robert L. Peck, Henry M. Shafer and Frank J. Wolf, U. S. Patent 2,804,463 (1957).

916 **Aureothricin** (Propiopyrrothine), $C_9H_{10}O_2N_2S_2$, yellow crystals, m.p. 256° (dec.).

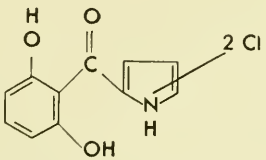


*Streptomyces celluloflavus* n. sp.

Haruo Nishimura, Toshiaki Kimura and Masa Kuroya, *J. Antibiotics* (Japan) 6A 57 (1953).

Walter D. Celmer and I. A. Solomons, *J. Am. Chem. Soc.* 77 2861 (1955). (Structure)

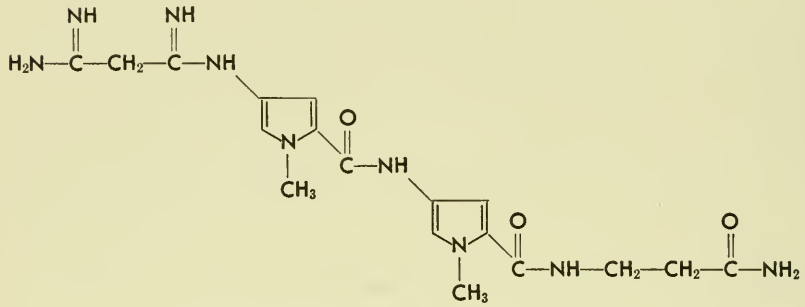
917 **Pyoluteorin,** $C_{11}H_7O_3NCl_2$, m.p. 174° (dec.).
Partial structure:



*Pseudomonas aeruginosa*

Rokuro Takeda, *J. Am. Chem. Soc.* 80 4749 (1958). (Structure)

918 **Netropsin** (Congocidine, Sinanomycin, T1384), $C_{18}H_{26}O_3N_{10}$, the hydrochloride crystallizes as colorless, hygroscopic prisms, m.p. 168–172° (dec.).



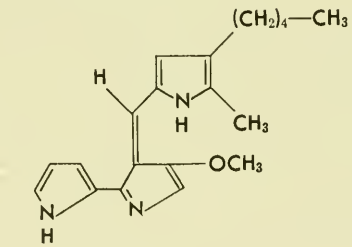
*Streptomyces netropsis, S. chromogenes* n.sp., *S. amboFaciens* n. sp.

A. C. Finlay, F. A. Hochstein, B. A. Sobin and F. X. Murphy, *J. Am. Chem. Soc.* 73 341 (1951).

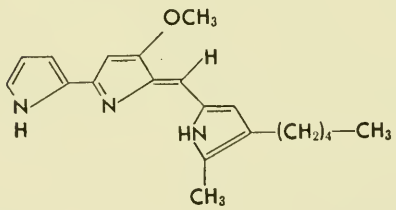
E. E. van Tamelen and A. D. G. Powell, *Chem. and Ind.*, 365 (1957). (Structure)

919 **Prodigiosin**, $C_{20}H_{25}ON_3$, red crystals with a green reflex, m.p. 151.5–152.9° (dec.).

Alternative structures:*



or



* See addendum.

*Serratia marcescens* (*Bacillus prodigiosum*), *S. marinorubrum*

Fritz Wrede and Alexander Rothhaas, *Z. physiol. Chem.* 226 95 (1934).

Other metabolites which have been isolated from cultures of *Serratia marcescens* are:

920 A "prodigiosin precursor," $C_{10}H_{10}O_2N_2$, colorless needles, m.p. > 250° (dec.).

921 A colorless, crystalline compound, not an antibiotic, $C_{34}H_{62}O_{10}N_3$, m.p. 153°.

922 An amide, $C_{24}H_{33}O_2N_7$.

Palmitic acid.

Three other red, one orange and one blue pigments.

A polypeptide, marcescin.

A polysaccharide.

Fritz Wrede and Alexander Rothhaas, *Z. physiol. Chem.* 226 95 (1934).

Ursula V. Santer and Henry J. Vogel, *Biochim. et Biophys. Acta* 19 578 (1956).

O. M. Efimenko, G. A. Kuznetsova and P. A. Yakimov, *Biokhimiya* 21 416 (1956).

A. J. Castro, J. F. Deck, M. T. Hugo, L. R. Williams and M. R. Zingg, *J. Org. Chem.* 23 1232 (1958).

A. J. Castro, A. H. Corwin, F. J. Waxham and A. L. Beilby, *ibid.* 24 455 (1959).

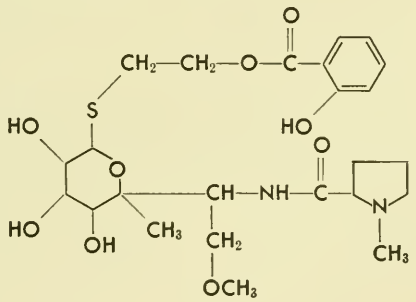
Doris P. Courington and T. W. Goodwin, *J. Bacteriol.* 70 568 (1955).

Harry H. Wasserman, James E. McKeon, Lewis Smith and Peter Forgione, *J. Am. Chem. Soc.* 82 506 (1960). (Structure shown above)

A. Treibs and R. Galler, *Angew. Chem.* 70 57 (1958).

923 **Celesticetin,** $C_{24}H_{36}O_9N_2S$, hygroscopic glass, m.p. (Oxalate): 147–152°, $[\alpha]_D^{24}$ +126.6° (c 0.5 in chloroform), $[\alpha]_D^{24}$ (Oxalate) 106.6° (c 0.5 in water).

Proposed Structure:



*Streptomyces celestis* n. sp., resembling *S. glaucus*

C. DeBoer, A. Dietz, J. R. Wilkins, C. N. Lewis and G. M. Savage, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, p. 831.

Herman Hoeksema, Glen F. Crum and William H. DeVries, *ibid.* p. 837.

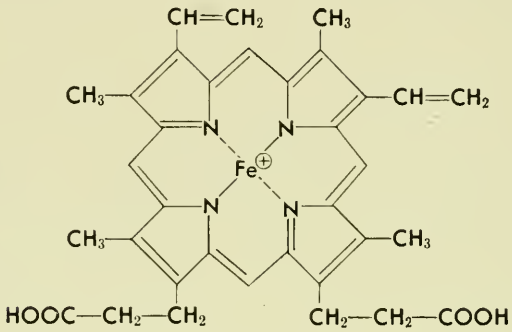
Clarence DeBoer, Alma Dietz and Herman Hoeksema, U. S. Patent 2,928,844 (1960). (Structure)

924 **Prodigiosin-like Pigment,** $C_{25}H_{35}ON_3$, orange crystals, partial melting 147–149°, resolidification, melting 203°.

Streptomycete related to *S. ruber* (Krainsky, Waksman and Henrici) and *S. roseodiastaticus*, Waksman and Lechevalier

F. Arcamone, A. DiMarco, M. Ghione and T. Scotti, *Giorn. microbiol.* 4 77 (1957).

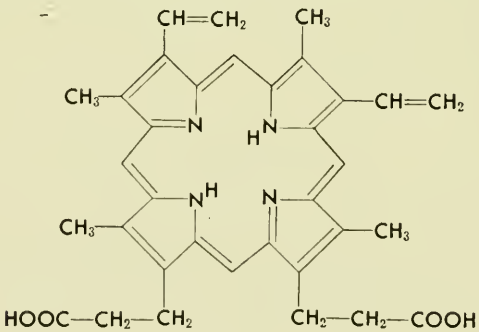
925 **Hematin,** $C_{34}H_{32}O_4N_4Fe^{\oplus}OH^{\ominus}$.




*Saccharomyces anamensis*

H. Fischer and F. Schwerdtel, Z. *physiol. Chem.* 175 248 (1928).

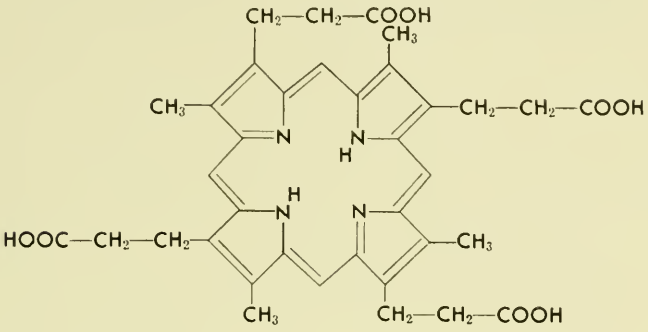
926 **Protoporphyrin,** $C_{34}H_{34}O_4N_4$, deep red crystals, m.p. >300°.



Yeasts, *Rhodopseudomonas spheroides*, other photosynthetic bacteria

Hans Fischer and Hermann Fink, Z. *physiol. Chem.* 140 57 (1924).

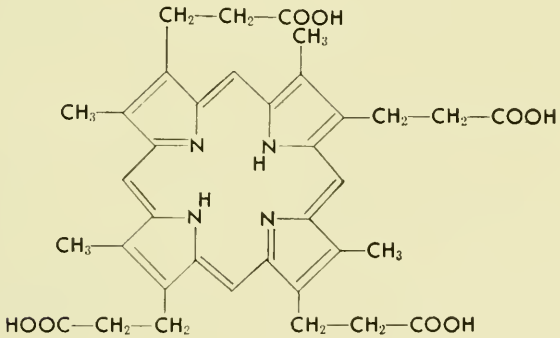
927 **Coproporphyrin I**, $C_{36}H_{38}O_8N_4$.




*Saccharomyces cerevisiae*, *S. anamensis*, other yeasts, *Aspergillus oryzae*, photosynthetic bacteria

Hans Fischer and Hermann Fink, Z. *physiol. Chem.* 150 243 (1925).

928 **Coproporphyrin III**, $C_{36}H_{38}O_8N_4$, dark red crystals.

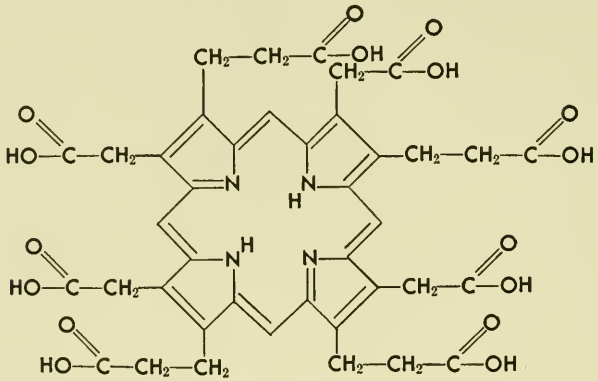



*Mycobacterium tuberculosis* var. *hominis*, *Rhodopseudomonas spheroides*, *Corynebacterium diphtheriae*

M. O'L. Crowe and A. Walker, *Brit. J. Exptl. Path.* 32 1 (1951).

C. M. Todd, *Biochem. J.* 45 386 (1949).

929 **Uroporphyrin III**, $C_{40}H_{38}O_{16}N_4$.

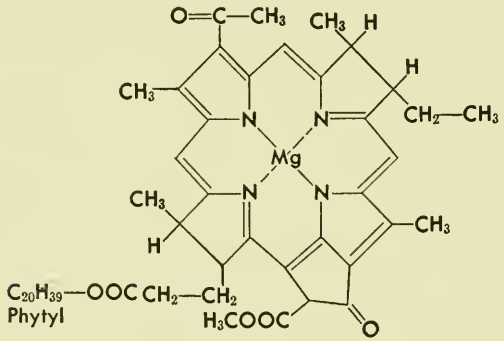


*Rhodopseudomonas spheroides*

June Lascelles, *Abstracts Gordon Research Conference, Vitamins and Metabolism* (1958). (Detection)

H. Fischer and H.-J. Hofmann, *Z. physiol. Chem.* 246 15 (1937); H. Fischer and A. Müller, *ibid.* 246 31 (1937). (Structure)

930 **Bacteriochlorophyll a**, $C_{55}H_{74}O_6N_4Mg$, amorphous, slow decomposition above 94°.



*Rhodospirillum rubrum, R. fulvum, Rhodopseudomonas spheroides, Thiocystis violacea,* other *Rhodovibrio* spp. and sulfur and chlorobacteria

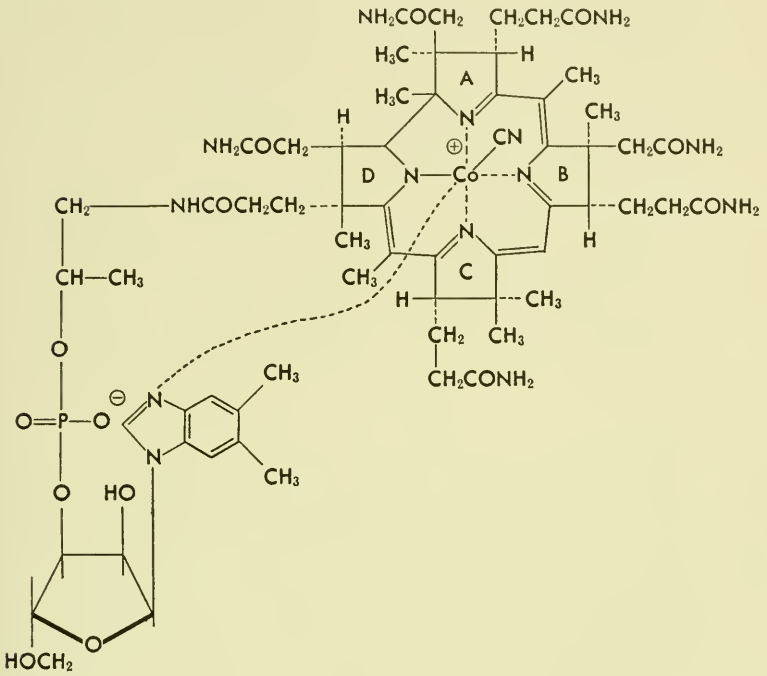
Hans Fischer and Robert Lambrecht, *Z. physiol. Chem.* 249 1 (1937).

Hans Fischer, Robert Lambrecht and Hellmuth Mittenzwei, *ibid.* 253 1 (1938).

John W. Weigl, *J. Am. Chem. Soc.* 75 999 (1953).

A. Seybold and G. Hirsch, *Naturwissenschaften* 41 258 (1954.)

931 **Vitamin $B_{12}$** (Cyanocobalamin, α-(5,6-Dimethylbenzimidazolyl) cobamide cyanide), $C_{63}H_{88}O_{14}N_{14}PCo$, dark red crystals which blacken near 212° and do not melt below 320°, $[\alpha]_{6563}^{23}$ −59 ±9° (dilute aqueous solution).




Vitamin $B_{12}$ activity has been detected in fermentation broths from many microorganisms, *e.g. Streptomyces griseus, S. antibioticus, S. roseochromogenes, Mycobacterium smegmatis, Lactobacillus arabinosus,* propionibacteria. Crystalline material has been isolated from some of these. For primary fermentations, *Streptomyces olivaceus* is probably the best producer (3.3 mg. per liter).

Dorothy Crowfoot Hodgkin, Jennifer Kamper, Maureen MacKay and Jenny Pickworth, *Nature* 178 64 (1956). (Structure)

W. H. Sebrell, Jr. and Robert S. Harris, "The Vitamins," Robert S. Harris, Donald E. Wolf, Karl E. Folkers, H. M. Wuest, Thomas H. Jukes and William L. Williams, *Vitamin $B_{12}$*, Academic Press Inc., New York, 1954 Vol. I Chap. 3, pp. 396–524. (A review)

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. II, J. M. VanLanen, *Production of vitamins other than riboflavin,* chap. 6, pp. 207–8.

932 **Factor B** is vitamin $B_{12}$ from which the nucleotide moiety has been removed. It has been isolated from fermentations, from rumen contents, from sewage, and it can be prepared chemically from vitamin $B_{12}$.

E. Lester Smith, "Vitamin $B_{12}$," John Wiley and Sons, Inc., New York, 1960, 196 pp. (A monograph)

This monograph also explains the new nomenclature system for $B_{12}$ and related compounds.

Other intermediates in the biosynthesis of vitamin $B_{12}$ by *Propionibacterium shermanii* have been detected:

Konrad Bernhauer, Elisabeth Becher, Gisela Gross and Georg Wilharm, *Biochem. Z.* 332 562 (1960).

K. Bernhauer, Hw. Dellweg, W. Friedrich, G. Gross, F. Wagner and P. Zeller, *Helv. Chim. Acta* 43 693 (1960).

K. Bernhauer, F. Wagner and P. Zeller, *ibid.* 43 696 (1960).

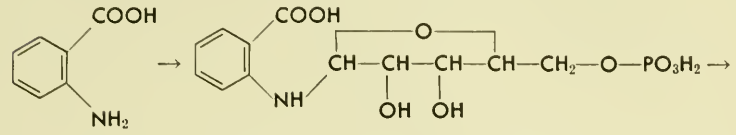
## *g.* INDOLES

The indole nucleus occurs in microorganisms in such forms as tryptophan, one of the less abundant amino acids, in bacterial pigments such as violacein and indigo and in amines from higher fungi such as serotonin and psilocybin, which have strong physiological effects in higher animals. The indole nucleus is incorporated also into bizarre fungal metabolites such as echinulin and gliotoxin, into the mushroom poisons, such as phalloidin, and into the ergot alkaloids listed in the following section.

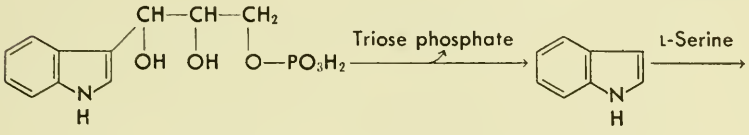
One route to indole and to tryptophan was outlined in the section on amino acids. This is the pathway discovered by Yanofsky and confirmed and elaborated in his and other laboratories.[1] Anthranilic acid from the shikimic acid route combines with ribose phosphate, cyclization occurs to form the pyrrole ring, a triose phosphate is elimi-

[1] C. Yanofsky, *Biochim. et Biophys. Acta* 16 594 (1955); *idem., J. Biol. Chem.* 223 171 (1956); F. Gibson, M. Jones and H. Taltscher, *Biochem. J.* 64 132 (1956); P. A. Trudinger, *ibid.* 62 480 (1956); F. Lingens and H. Hellmann, *Angew. Chem.* 69 97 (1957); L. W. Parks and H. C. Douglas, *Biochim. et Biophys. Acta* 23 207 (1957); J. Gots and S. Ross, *ibid.*, 24 429 (1957); C. Yanofsky and M. Rachmeier, *ibid.* 28 640 (1958).

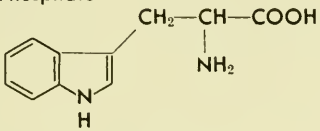
nated and the indole so formed combines with L-serine to form L-tryptophan:




Anthranilic Acid N-(2-Carboxyphenyl)-1-aminoribose-5-phosphate

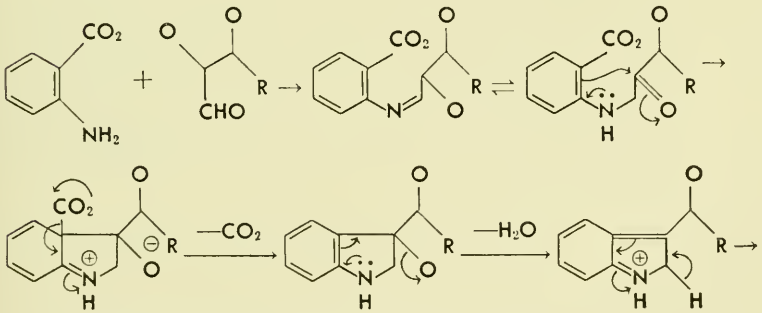



Indolyl-3-glycerol Phosphate

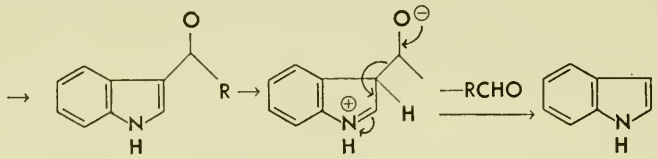



L-Tryptophan

N-Fructosylanthranilic acid has been isolated from a yeast, and it may be another intermediate in indole synthesis. In this case a tetrose would be eliminated. If pentoses and hexoses can both be used in reactions with anthranilic acid, perhaps tetroses can be as well. This possibility is emphasized by Wenkert[2] in a discussion of alkaloid biosynthesis. A reaction of this sort might explain the frequent occurrence in nature of indole derivatives with two carbon atom side-chains in the 3-position. In other words the indole biosynthesis could be generalized:



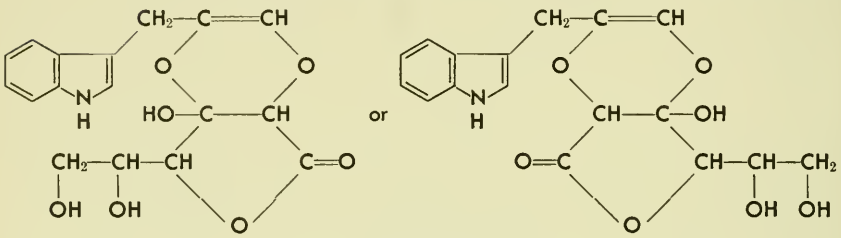

[2] Ernest Wenkert, *Experientia* 15 165 (1959).



in which R—CH(OH)—CH(OH)—CHO is any of several sugars.

It may be that other derivatives of anthranilic acid can participate in this route, too. For example 5-hydroxyanthranilic acid would give rise to the 5-oxyindole derivatives found in nature. It is notable that this acid is a growth promoter for an *Escherichia coli* mutant.[3]

Ascorbigen, a bound form of ascorbic acid isolated from plants of the cabbage family, has one of the structures:[4]



Ascorbigen
(alternate structures)

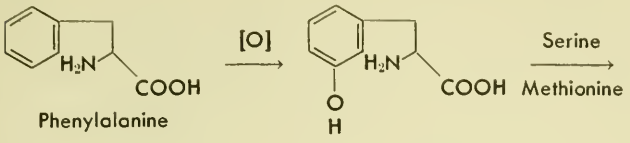
The presumptive precursor is 3-indolylacetol, analogous to an intermediate in histidine biosynthesis, and it is interesting to speculate as to whether this is an offshoot of the biosynthetic route to tryptophan or whether it is formed by way of tryptophan.

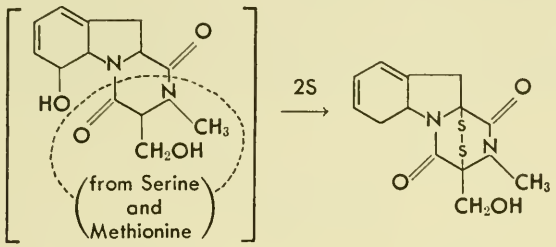
The mold product, echinulin, has an unusual structure, apparently involving the indole synthesis, terpenoid and amino acid precursors. Gliotoxin, on the other hand, is almost entirely derived from amino acids, and it could have been classified as a polypeptide. $C^{14}$-Labeling studies have demonstrated the following biosynthetic pathway for gliotoxin:[5]

[3] H. Niemer and A. Oberdorfer, Z. *physiol. Chem.* 308 51 (1957).

[4] Z. Prochazka, V. Sanda and F. Sorm, *Coll. Czech. Chem. Comm.* 22 654 (1957).

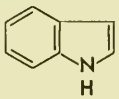
[5] J. A. Winstead and R. J. Suhadolnik, *J. Am. Chem. Soc.* 82 1644 (1960); R. J. Suhadolnik, A. Fischer and J. Wilson, *Federation Proc.* 19 8 (1960).



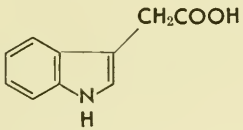

Methionine was the most efficient source of the N-methyl group, the $\beta$-carbon of serine being about one third as effective. Both of the amino acid skeletons were incorporated intact when furnished, and *m*-tyrosine could also be used as a precursor.

933 **Indole**, $C_8H_7N$, colorless leaflets, m.p. 52°.



*Escherichia coli* mutants, yeasts, *Treponema* spp.
P. A. Trudinger, *Biochem. J.* 62 480 (1956).
Charles Yanofsky, *J. Biol. Chem.* 223 171 (1956).
F. Gibson, Marjorie J. Jones and H. Teltscher, *Biochem. J.* 64 132 (1957).
L. W. Parks and H. C. Douglas, *Biochim. et Biophys. Acta* 23 207 (1957).
Michel Moureau and W. Aladame, *Ann. inst. Pasteur* 88 231 (1955).

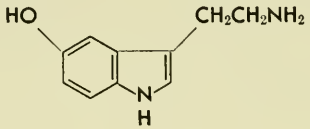
934 **Indole-3-acetic Acid** (Rhizopin), $C_{10}H_9O_2N$, colorless plates, m.p. 164°.



*Rhizopus suinus, R. nigricans, Aspergillus niger, Peni-*

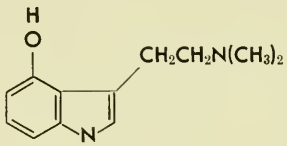
*cillium notatum, Absidia ramosa, Boletus edulis,* Yeasts
Niels Nielsen, *Biochem. Z.* 237 244 (1931); 249 196 (1932).
Fritz Kögl and D. G. F. R. Kostermans with A. J. Haagen-Smit and H. Erxleben, *Z. physiol. Chem.* 228 113 (1934).
Kenneth V. Thimann, *J. Biol. Chem.* 109 279 (1935).
Donald J. Cram and Max Tishler, *J. Am. Chem. Soc.* **70** 4238 (1948). (Isolation)
Ryuichi Honda, Japanese Patent 603 (1950).

935 **Serotonin** (5-Hydroxytryptamine), $C_{10}H_{12}ON_2$ (Hydrochloride), colorless crystals, m.p. 167°.



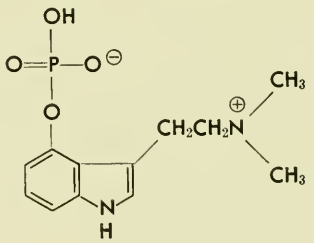

*Panaeolus campanulatus*
Demonstrated by paper chromatography only.
Varro E. Taylor, Jr., *Science* 128 718 (1958).

936 **Psilocin,** $C_{12}H_{16}ON_2$, colorless crystals, m.p. 173–176° (dec.).




*Psilocybe* species
Psilocin is a minor constituent of the mushrooms which contain psilocybin.
A. Hofmann and F. Troxler, *Experientia* 15 101 (1959).

937 **Psilocybin,** $C_{12}H_{17}O_4N_2P$, colorless crystals, m.p. 185–195° (dec.).

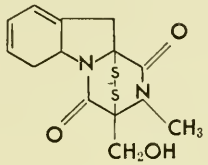


*Psilocybe mexicana* Heim, *P. caerulescens* Murr. var. *Mazecatorum* Heim, *P. aztecorum* Heim, *P. sempervirens* Heim et Cailleux, *P. zapotecorum* Heim, *Stropharia cubensis* Earle

A. Hofmann, R. Heim, A. Brack and H. Kobel, *Experientia* 14 107 (1958).

A. Hofman, A. Frey, H. Ott, Th. Petrzilka and F. Troxler, *ibid.* 14 397 (1958). (Synthesis)

938 **Gliotoxin** (Aspergillin), $C_{13}H_{14}O_3N_2S_2$, m.p. 195° (dec.), $[\alpha]_D^{25}$ −290° ±10° (c 0.078 in ethanol).



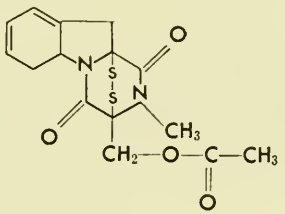

*Trichoderma viride, Aspergillus fumigatus, Penicillium terlikowski* Zaleski, *P. cinerascens, P. jenseni, Gliocladium fimbriatum*

The yield of gliotoxin and its acetate from *P. terlikowski* Zaleski was reported as about 100 mg. per liter.

John R. Johnson, William F. Bruce and James D. Dutcher, *J. Am. Chem. Soc.* 65 2005 (1943) and other papers in this series.

Malcolm R. Bell, John R. Johnson, Bernard S. Wildi and R. B. Woodward, *J. Am. Chem. Soc.* 80 1001 (1958). (Structure)

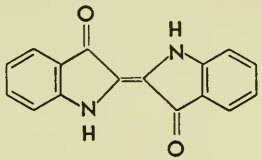
939 **Gliotoxin Acetate,** $C_{15}H_{16}O_5N_2S_2$, pale yellow rhombic crystals, m.p. 159°, $[\alpha]_D^{19}$ −197° (c 0.600 in chloroform).




*Penicillium terlikowski* Zaleski

John R. Johnson, Aklaq R. Kidwai and John S. Warner, *J. Am. Chem. Soc.* 75 2110 (1953).

940 **Indigo,** $C_{16}H_{10}O_2N_2$, blue powder with a coppery luster, sublimes.



*Schizophyllum commune* mutant

Ammonium ion was the only nitrogen source.

Philip G. Miles, Henning Lund and John R. Raper, *Arch. Biochem. and Biophys.* 62 1 (1956).

941 **Chetomin,** $C_{16}H_{17}O_4N_3S_2$ (proposed), amorphous white powder, m.p. 218–220° (dec.), $[\alpha]_D^{22}$ +360° (c 1 in chloroform).

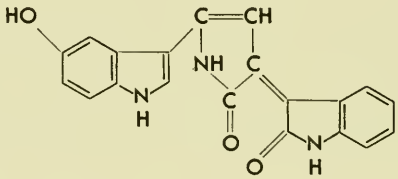
A neutral compound. Positive indole, Hopkins-Cole, negative biuret, Millon.

*Chaetomium cochlioides*

Walton B. Geiger, Jean E. Conn and Selman A. Waksman, *J. Bacteriol.* 48 531 (1944). (Isolation)

Walton B. Geiger, *Arch. Biochem.* 21 125 (1949).

942 **Violacein,** $C_{20}H_{13}O_3N_3$, violet-black microcrystals, m.p. >350° (dec.).



*Chromobacterium violaceum*

F. M. Strong, *Science* 100 287 (1944).

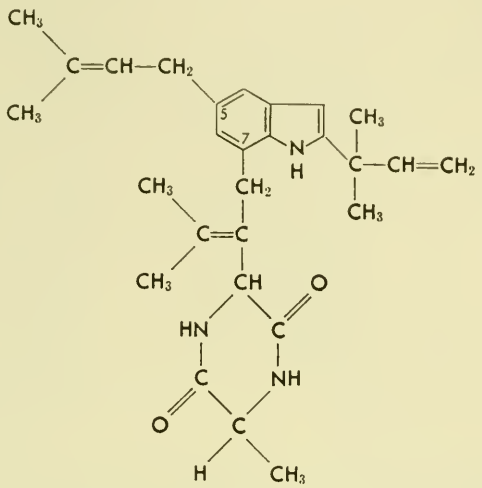
R. T. S. Beer, *Angew. Chem.* 69 676 (1957).

J. A. Ballantine, C. B. Barrett, R. J. S. Beer, B. G. Boggiano, K. Clarke, Stephen Eardley, B. E. Jennings and Alexander Robertson, *J. Chem. Soc.*, 2222 (1957) and preceding papers in this series.

J. A. Ballantine, R. T. S. Beer, D. J. Crutchley, G. M. Dodd and D. R. Palmer, *J. Chem. Soc.*, 2292 (1960). (Synthesis)

R. D. Demoss and N. R. Evans, *J. Bacteriol.* 79 729 (1960). (Biosynthesis)

943 **Echinulin**, $C_{28}H_{37}O_2N_3$, white needles, m.p. 242°.
Probable structure:



*Aspergillus glaucus* types, *A. echinulatus, A. chevalieri*
About 200 g. of pure material were obtained from 5 kg. of dry mycelium. Auroglaucin and flavoglaucin were isolated from the same source.

A. Quilico and L. Panizzi, *Ber.* **76B** 348 (1943). (Isolation)

Adolfo Quilico, Cesare Cardini and Franco Piozzi, *Gazz. Chim. ital.* 86 211 (1956). (Structure)

Ziro Kitamura, Uzukiko Kurimoto and Matatsugu Yokoyama, *J. Pharm. Soc. Japan* 76 972 (1956).

C. Cardani, G. Casnati, F. Piozzi and A. Quilico, *Tetrahedron Letters* No. 16 1 (1959). (Structure)

## *h.* ERGOT ALKALOIDS

The constituents of the sclerotia of the fungus *Claviceps purpurea* (Fries) Tul., a cereal parasite, have been extensively studied. Some of the alkaloids are used in medicine for their oxytocic properties and to relieve migraine.

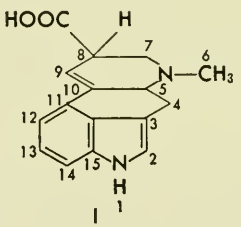
Ergocristine, ergokryptine and ergocornine (and their isomers) constitute a closely related complex formerly thought to be homogeneous and called ergotoxine. Besides the alkaloids which are shown in the succeeding

pages, many other chemicals have been identified. Among them are:

| | | |
|---|---|---|
| Ergothioneine | Leucine | Clavicepsin |
| Histidine | Ammonia | Ergosterol |
| Tyrosine | Methylamine | Oils |
| Betaine | Trimethylamine | Lactic Acid |
| Choline | Ethylamine | Succinic Acid |
| Acetylcholine | *n*-Propylamine | Oxalic Acid |
| Cadaverine | *iso*-Propylamine | Citric Acid |
| Putrescine | *iso*-Butylamine | Formic Acid |
| Agmatine | *iso*-Amylamine | Ethanol |
| Histamine | *n*-Hexylamine | Furfural |
| Tyramine | β-Phenylethylamine | Acetaldehyde |
| Valine | Mannitol | Acetone |
| | | Ergoflavine and other pigments |

Careful work has shown that many of the alkaloids produced in the natural state can be produced in artificial culture as well.[1, 2, 3] Total alkaloid yields of 1000–1500 mg. per liter of culture fluid have been obtained exclusive of mycelial alkaloids.[1]

The conventional ergot alkaloids contain the lysergic acid moiety I or isolysergic acid, the stereoisomer at position 8.




I

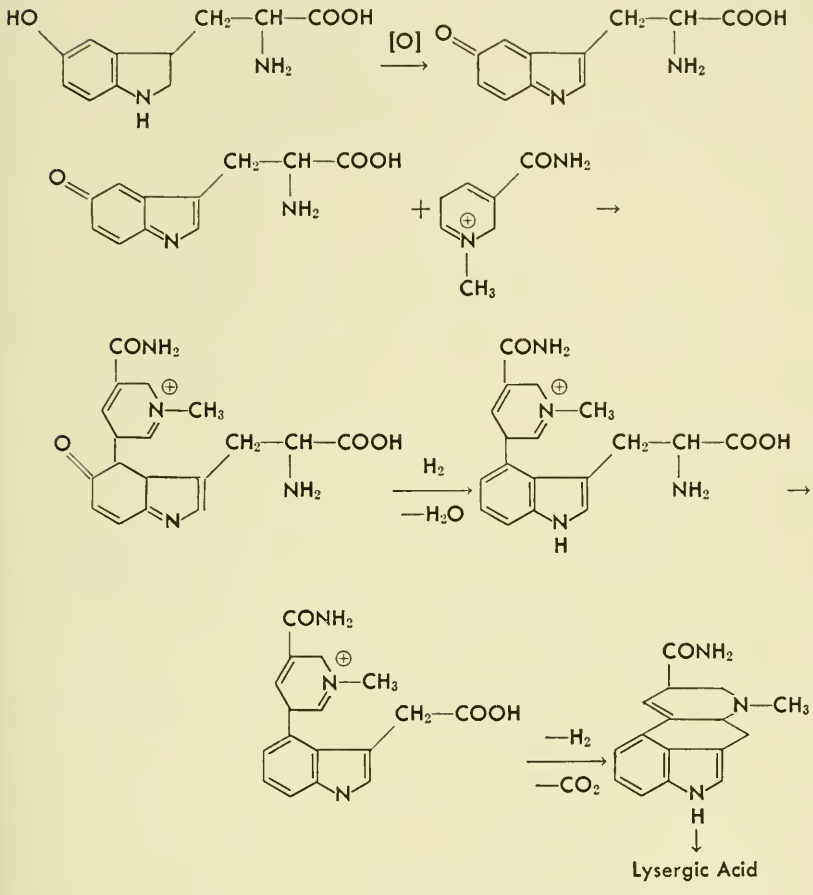
[1] A. Hofmann, R. Brunner, H. Kobel and A. Brack, *Helv. Chim. Acta* 40 1358 (1957).

[2] W. A. Taber and L. C. Vining, *Can. J. Microbiol.* 3 55 (1957).

[3] Ervin Gláz, *Acta Pharm. Hung.* 25 11 (1955).

A number of different hypotheses have been advanced concerning the biosynthetic origin of the ergot alkaloids. These are outlined below:

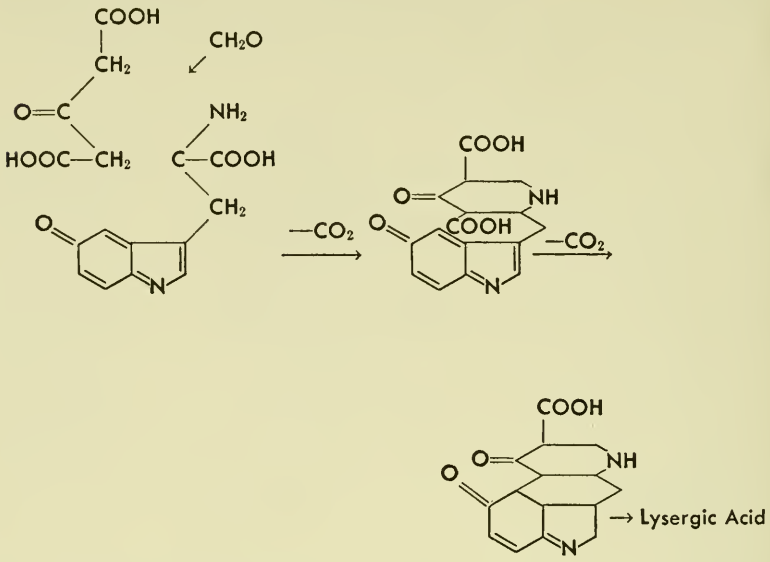
(1) van Tamelen (1953):[4]



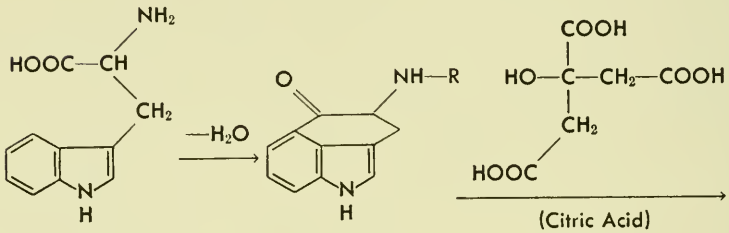

(2) Harley-Mason (1954):[5]

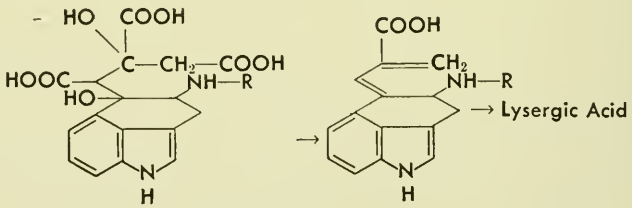
[4] Eugene van Tamelen, *Experientia* 9 457 (1953).

[5] J. Harley-Mason, *Chem. and Ind.*, 251 (1954).



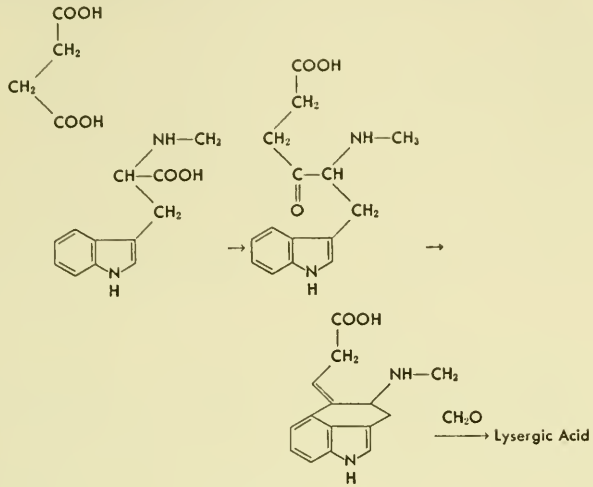

(3) Wendler (1954):[6]



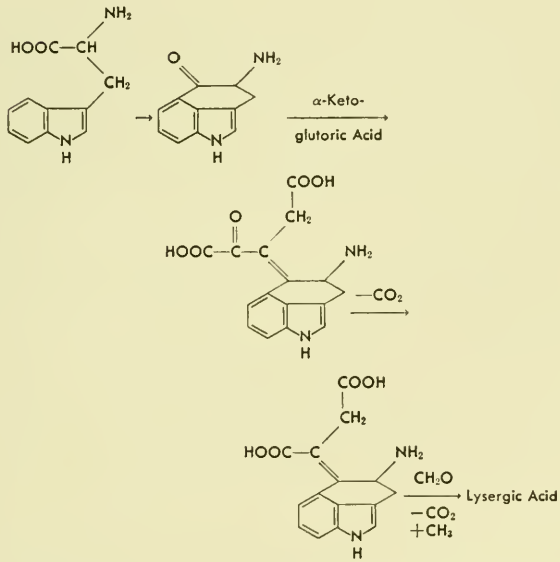




[6] N. L. Wendler, *Experientia* **10** 338 (1954).

(4) Robinson (1955):[7]



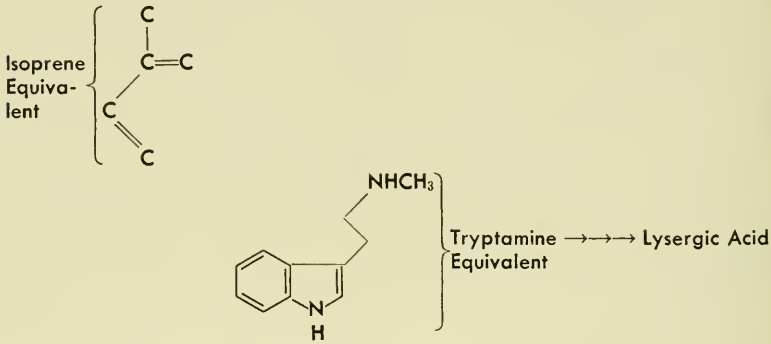

(5) Feldstein (1956):[8]




[7] Sir Robert Robinson, "The Structural Relations of Natural Products," Oxford University Press, Oxford, 1955.

[8] A. Feldstein, *Experientia* 12 475 (1956).

(6) Birch (1958),[9] Mothes, *et al.* (1958):[10]




Each of these hypotheses has had its votaries, but now experimental work is beginning to accumulate. There have been conflicting results, partly because some experimenters have injected labeled precursors into infected rye plants, while others added them to cultures grown on artificial medium.

The 5-hydroxytryptophan proposals have been criticized[7] because no 5-hydroxyindole analogues of lysergic acid have been found in nature, and because (obviously the devices of organic chemists) they suffer from some rather improbable biological intermediates. Brady has found that in artificial culture tryptophan was an efficient precursor for the clavine alkaloids, while 5-hydroxytryptophan was not.[11]

By using parasitic cultures one group reported good incorporation of $\beta$-$C^{14}$-tryptophan,[10] while another reported[12] only weak labeling of the alkaloids isolated from the sclerotia.

By use of a cell homogenate technique, it was found that alanine and phenylalanine were incorporated into ergotamine and the ergotoxine complex, but not into ergonovine, which suggests that these amino acids are precursors of the peptide structure of the water-insoluble

[9] G. E. Wolstenholme and Cecilia M. O'Connor, CIBA Foundation Symposium on "Amino Acids and Peptides with Antimetabolic Activity," A. J. Birch and Herchel Smith, *Oxidative formation of biologically active compounds from peptides*, Little, Brown and Co., Boston, 1958, pp. 254–256.

[10] K. Mothes, F. Weygand, D. Gröger and H. Grisebach, *Z. Naturforsch.* 13b 41 (1958).

[11] Lynn Robert Brady, *Dissertation Abstr.* 20 2526 (1960).

[12] R. J. Suhadolnik, L. M. Henderson, J. B. Hanson and Y. H. Loo, *J. Am. Chem. Soc.* 80 3153 (1958).

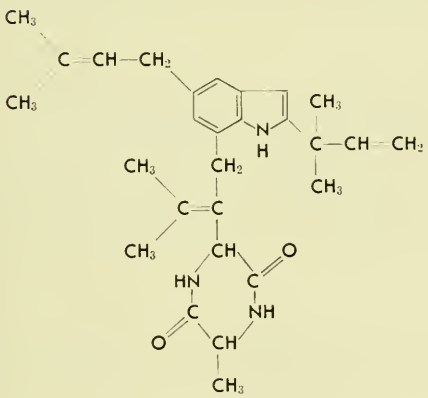
ergot alkaloids.[13] $C_{14}$-Labeled indole and serine, alone or together, were not incorporated.

Another artificial culture study in which the *Claviceps purpurea* culture was grown saprophytically on a simple galactose, ammonium succinate, mineral salts, biotin medium to which D,L-$\beta$-$C^{14}$-tryptophan was added, found that the tryptophan was an efficient precursor.[14] Labeling was about the same throughout the range of alkaloids isolated, thus suggesting a common biogenesis. Supplementation with L-tryptophan increased the yield and caused the formation of elymoclavine and agroclavine, which were not formed otherwise.

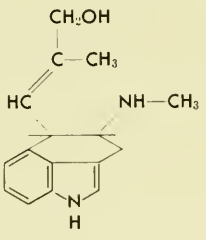
Another (non-tracer) experiment in artificial culture showed no increase in total alkaloid production on supplementation with either tryptophan, hydroxytryptophan, indole, 5-hydroxyindole or serotonin.[15]

The consensus of the labeling experiments seems to be, however, that tryptophan is a rather direct precursor of the lysergic acid skeleton.

Apparently there is no good evidence yet concerning the origin of the remainder of the skeleton. The isoprenoid precursor hypothesis is under investigation.[9, 16] This proposal is buttressed by the structure of the mold metabolite, echinulin, which has an indole nucleus bearing isoprenoid attachments.




Echinulin




Chanoclavine

[13] Aro Garo Paul, *Dissertation Abstr.* 17 2143 (1957).

[14] W. A. Taber and L. C. Vining, *Chem. and Ind.* 1218 (1959).

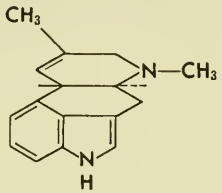
[15] Ross M. Baxter, S. I. Kandel and A. Okany, *Nature* 185 241 (1960).

[16] A. J. Birch, B. J. McLoughlin and Herchel Smith, *Tetrahedron Letters* No. 7 1 (1960).

It is also supported by the structure of chanoclavine, which seems to be not too remotely derived from such an intermediate.

A thorough review of the chemistry of the ergot alkaloids has been published.[17]

944 **Agroclavine**, $C_{16}H_{18}N_2$, colorless crystals, m.p. 210–212° (dec.), $[\alpha]_D^{20}$ −183° (c 1 in pyridine).

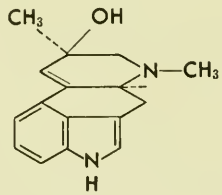



*Claviceps purpurea* (Fries) Tul.

A. Hofmann, R. Brunner, H. Kobel and A. Brack, *Helv. Chim. Acta* 40 1358 (1957).

945 **Setoclavine**, $C_{16}H_{18}ON_2$, colorless crystals, m.p. 229–234° (dec.), $[\alpha]_D^{20}$ +174° (c 1 in pyridine).

946 **Isosetoclavine** (Triseclavine), $C_{16}H_{18}ON_2$ (stereoisomer of setoclavine), colorless crystals, m.p. 234–237° (dec.), $[\alpha]_D^{20}$ +107° (c 1 in pyridine).

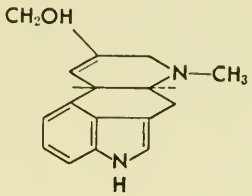



*Clavicepts purpurea* (Fries) Tul.

A. Hofmann, R. Brunner, H. Kobel and A. Brack, *Helv. Chim. Acta* 40 1358 (1957).

[17] Arthur Stoll, *Fortschr. Chem. org. Naturstoffe* 9 114–170 (1952).

947 **Elymoclavine,** $C_{16}H_{18}ON_2$, colorless crystals, m.p. 245–247° (dec.), $[\alpha]_D^{20}$ −152° (c 1 in pyridine).

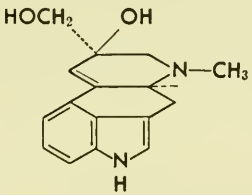



*Claviceps purpurea* (Fries) Tul.

A. Hofmann, R. Brunner, H. Kobel and A. Brack, *Helv. Chim. Acta* **40** 1358 (1957).

948 **Penniclavine,** $C_{16}H_{18}O_2N_2$, colorless crystals, m.p. 222–225° (dec.), $[\alpha]_D^{20}$ +153° (c 1 in pyridine).

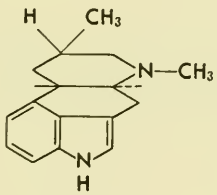
949 **Isopenniclavine,** $C_{16}H_{18}O_2N_2$ (stereoisomer of penniclavine), colorless crystals, m.p. 163–165° (dec.), $[\alpha]_D^{20}$ +146° (c 1 in pyridine).




*Claviceps purpurea* (Fries) Tul.

A. Hofmann, R. Brunner, H. Kobel and A. Brack, *Helv. Chim. Acta* **40** 1358 (1957).

950 **Dihydroagroclavine** (Festuclavine), $C_{16}H_{20}N_2$, colorless crystals, m.p. 242° (dec.), $[\alpha]_D^{20}$ −69° (c 0.5 in chloroform).



*Claviceps purpurea* (Fries) Tul.

Matazo Abe, *Ann. Rept. Takeda Res. Lab.* **10** 73, 83, 90, 110, 126, 129, 145, 152, 167, 171, 179, 190, 205, 210 (1951).

Matazo Abe, Togo Yamano, Yoshiharu Kôzu and Mitsugu Kusumoto, *J. Agr. Chem. Soc. Japan* **24** 416, 471 (1951); **25** 458 (1952); **27** 18, 613, 617 (1953).

Matazo Abe *ibid.* **28** 44, 501 (1954).

Matazo Abe, Togo Yamano, Yochiharu Kozu and Mitsugi Kusumoto, *ibid.* **29** 364 (1955).

Matazo Abe, Saburo Yamatodani, Togo Yamano and Mitsugi Kusumoto, *Bull. Agr. Chem. Soc.* (Japan) **19** 92 (1955).

Saburo Yamatodani and Matazo Abe, *ibid.* **19** 94 (1955).

951 **Pyroclavine,** $C_{16}H_{20}N_2$, colorless crystals, m.p. 204° (dec.), $[\alpha]_D^{20}$ −90° (c 0.2 in pyridine).

and

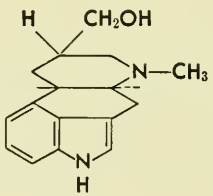
952 **Costaclavine,** $C_{16}H_{20}N_2$, colorless crystals, m.p. 182° (dec.), $[\alpha]_D^{20}$ +44° (c 0.2 in pyridine).

These are thought to be isomers of dihydroagroclavine.

*Claviceps purpurea* (Fries) Tul.

Matazo Abe, Saburo Yamatodani, Togo Yamano and Mitsugi Kusumoto, *Bull. Agr. Chem. Soc.* (Japan) **20** 59 (1956).

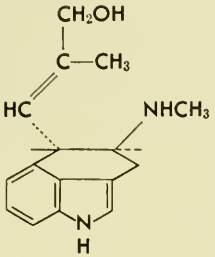
953 **Dihydroelymoclavine,** $C_{16}H_{20}ON_2$, colorless crystals, m.p. 210° (dec.), $[\alpha]_D^{28}$ −167° (c 0.16 in chloroform).




*Claviceps purpurea* (Fries) Tul.

See references under dihydroagroclavine.

954 **Chanoclavine** (Secaclavine), $C_{16}H_{20}ON_2$, colorless crystals, m.p. 220–222° (dec.), $[\alpha]_D^{20}$ −240° (c 1 in pyridine).



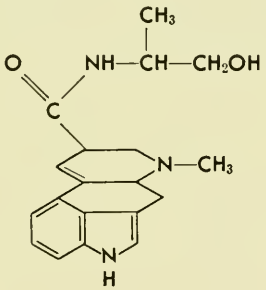

*Claviceps purpurea* (Fries) Tul.

A. Hofmann, R. Brunner, H. Kobel and A. Brack, *Helv. Chim. Acta* 40 1358 (1957).

Matazo Abe, Togo Yamano, Saburo Yamatodani, Yoshiharu Kozu, Mitsugi Kusumoto, Hajime Komatsu and Saburo Yamada, *Bull. Agr. Chem. Soc.* (Japan) 23 246 (1959).

955 **Ergobasine** (Ergometrine, Ergonovine, Ergotocine, Ergostetrine, Ergotrate, Ergoclinine), $C_{19}H_{23}O_2N_3$, colorless crystals, m.p. 162°, $[\alpha]_D^{20}$ +90° (c 1 in water).

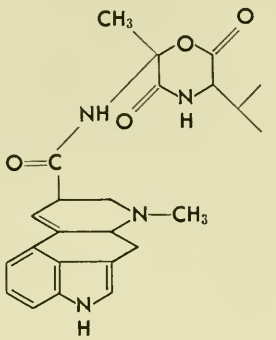
956 **Ergobasinine**, $C_{19}H_{23}O_2N_3$ (stereoisomer of ergobasine), colorless crystals, m.p. 196°, $[\alpha]_D^{20}$ +414° (c 1 in chloroform).




*Claviceps purpurea* (Fries) Tul.

Walter A. Jacobs and Lyman C. Craig, *Science* 82 16 (1935). (Structure)

957 **Ergosecalinine**, $C_{24}H_{28}O_4N_4$, colorless crystals, m.p. 217° (dec.), $[\alpha]_D^{18}$ +298° (c 0.2 in chloroform).

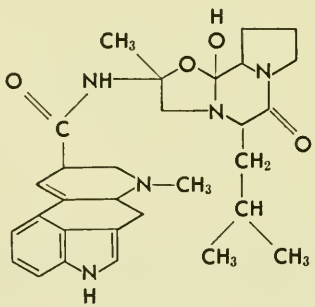



*Claviceps purpurea*

Matazo Abe, Togo Yamano, Saburo Yamatodani, Yoshiharu Kozu, Mitsugi Kusumoto, Hojime Komatsu and Saburo Yamada, *Bull. Agr. Chem. Soc.* (Japan) 23 246 (1959).

958 **Ergosine**, $C_{30}H_{37}O_5N_5$, colorless crystals, m.p. 228° (dec.), $[\alpha]_D^{20}$ −179° (c 1 in chloroform).

959 **Ergosinine**, $C_{30}H_{37}O_5N_5$ (stereoisomer of ergosine), colorless crystals, m.p. 228° (dec.), $[\alpha]_D^{20}$ +420° (c 1 in chloroform).

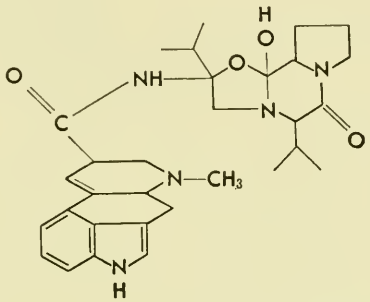



*Claviceps purpurea* (Fries) Tul.

A. Stoll, A. Hofmann and Th. Petzilka, *Helv. Chim. Acta* 34 1544 (1951). (Structure)

960 **Ergocornine,** $C_{31}H_{39}O_5N_5$, colorless crystals, m.p. 182–184° (dec.), $[\alpha]_D^{20}$ −188° (c 1 in chloroform).

961 **Ergocorninine,** $C_{31}H_{39}O_5N_5$ (stereoisomer of ergocornine), colorless crystals, m.p. 228° (dec.), $[\alpha]_D^{20}$ +409° (c 1 in chloroform).

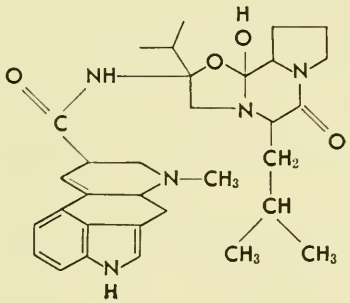



*Claviceps purpurea* (Fries) Tul.

A. Stoll, A. Hofmann and Th. Petzilka, *Helv. Chim. Acta* 34 1544 (1951). (Structure)

962 **Ergokryptine,** $C_{32}H_{41}O_5N_5$, colorless crystals, m.p. 212–214° (dec.), $[\alpha]_D^{20}$ −187° (c 1 in chloroform).

963 **Ergokryptinine,** $C_{32}H_{41}O_5N_5$ (stereoisomer of ergokryptine) colorless crystals, m.p. 240–242° (dec.), $[\alpha]_D^{20}$ +408° (c 1 in chloroform).

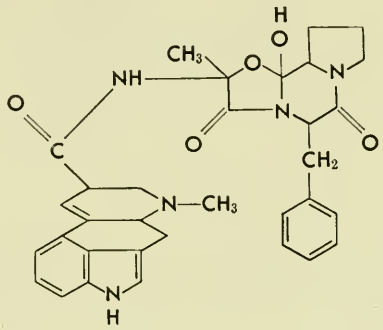


*Claviceps purpurea* (Fries) Tul.

A. Stoll, A. Hofmann and Th. Petzilka, *Helv. Chim. Acta* 34 1544 (1951). (Structure)

964 **Ergotamine,** $C_{33}H_{35}O_5N_5$, colorless prisms, m.p. 212–214° (dec.), $[\alpha]_D^{20}$ −160° (c 1 in chloroform).

965 **Ergotaminine,** $C_{33}H_{35}O_5N_5$ (stereoisomer of ergotamine), colorless plates, m.p. 241–243° (dec.), $[\alpha]_D^{20}$ +369° (c 0.5 in chloroform).



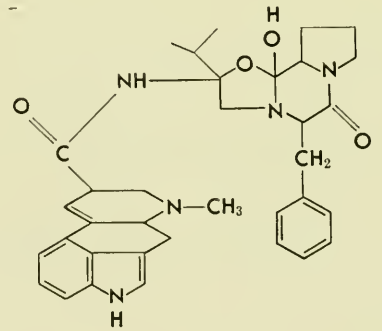

*Claviceps purpurea* (Fries) Tul.

Walter A. Jacobs and Lyman C. Craig, *J. Org. Chem.* 1 245 (1936).

Arthur Stoll, *Helv. Chim. Acta* 28 1283 (1945).

966 **Ergocristine,** $C_{35}H_{39}O_5N_5$, colorless crystals, m.p. 165–170° (dec.), $[\alpha]_D^{20}$ −183° (c 1 in chloroform).

967 **Ergocristinine,** $C_{35}H_{39}O_5N_5$ (stereoisomer of ergocristine), m.p. 226° (dec.), $[\alpha]_D^{20}$ +336° (c 1 in chloroform).



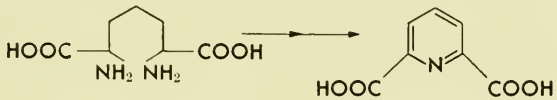
*Claviceps purpurea* (Fries) Tul.

A. Stoll, A. Hofmann and Th. Petzilka, *Helv. Chim. Acta* 34 1544 (1951). (Structure)

## *i.* PYRIDINES

Few pyridines are listed, but two of these, nicotinic acid and pyridoxine, are vitamins. Fusaric acid is a wilt toxin, and 2,6-dipicolinic acid appears in conspicuous quantities in bacterial spores.

Dipicolinic acid[1, 2, 3, 4] probably is formed by cyclization of $\alpha,\epsilon$-diaminopimelic acid, a lysine precursor and cell wall constituent of some bacteria:




Diaminopimelic Acid 2,6-Dipicolinic Acid

The metabolic significance, if any, is unknown. In *Bacillus sphaericus* diaminopimelic acid is present in spores and not in vegetative cells, but in many bacteria it is present in both.

Fusaric and dehydrofusaric acids are by-products of the gibberellin fermentation and are produced by fusarium types. These include plant pathogens, and fusaric acid solutions sprayed on healthy plants of the usual host cause wilting typical of infection. Apparently no study has been made of the mode of biogenesis.

Nicotinic acid in its coenzyme forms occurs in all living cells where it is essential in hydrogen and electron transport. It is used by a variety of apoenzymes as the prosthetic group for various dehydrogenase reactions. It is much less tightly bound to the protein than, for example, flavine adenine dinucleotide, perhaps to facilitate movement of the available supply among the apoenzymes in need of it.

Some of the many microbial reactions known to require diphosphopyridine nucleotide (DPN) or triphosphopyridine nucleotide (TPN) are:

[1] Joan F. Powell, *Biochem. J.* 54 210 (1953).

[2] J. J. Perry and J. W. Foster, *J. Bacteriol.* 72 295 (1956).

[3] William K. Harrell and Emil Mantini, *Can. J. Microbiol.* 3 735 (1957).

[4] Joan F. Powell and R. E. Strange, *Biochem. J.* 65 700 (1957).

$CH_3CH_2OH \rightleftharpoons CH_3CHO$ (in yeast)

R—CHO $\rightleftharpoons$ R—COOH (in yeast)

Glutathione—SH $\rightleftharpoons$ Glutathione—S—S—Glutathione (in yeast)

Isocitrate $\rightleftharpoons$ Oxalosuccinate (bacteria, yeast)

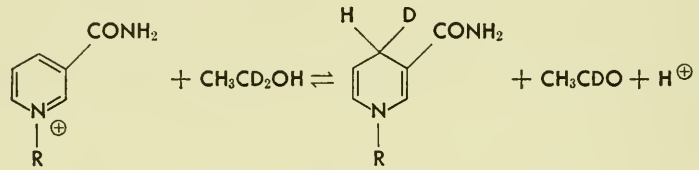
D-Glucopyranose-6-phosphate $\rightleftharpoons$ 6-Phospho-D-gluconolactone (yeast)

L-Glutamate $\rightleftharpoons$ $\alpha$-Ketoglutarate + $NH_4^{\oplus}$ (bacteria)

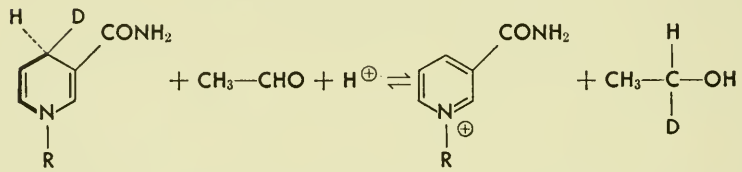
D-Glyceraldehyde-3-phosphate + Phosphate $\rightleftharpoons$ D-1,3-Diphosphoglyceric Acid (yeast)

Some of these reactions occur quite generally. Occasionally DPN and TPN are interchangeable, although one or the other is used more efficiently.

Direct transfer of hydrogen between the substrate and the 4-position of the nicotinamide moiety of DPN (in the presence of yeast alcohol dehydrogenase) has been demonstrated, and the stereochemistry of this reaction studied in exquisite detail by means of deuterated substrate:[5, 6]




(R = the rest of the DPN molecule)



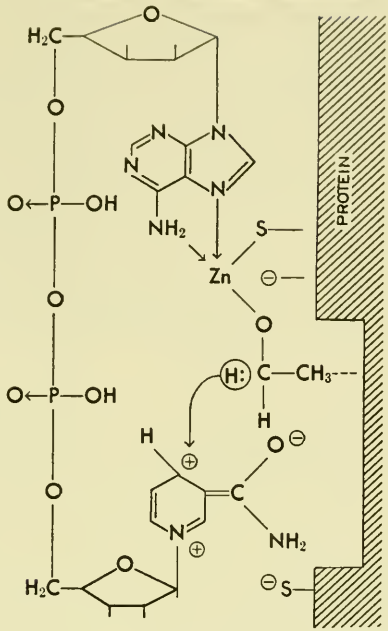

In the second equation the deuterium atom is removed exclusively, leaving deuterium-free DPN. This indicates a marked steric effect, since the deuterium atom projects from one side of the molecule. Moreover, a single stereoisomer of deuterated ethanol is produced.

Speculations have been made concerning the precise nature of the coenzyme-apoenzyme-substrate-metal ion complex. One model[7] is shown below:

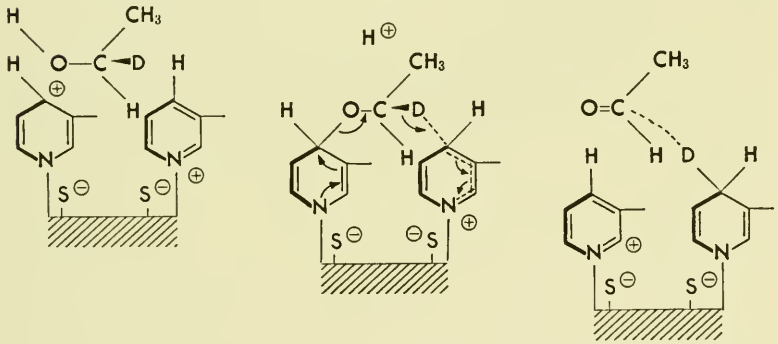
[5] Harvey F. Fisher, Eric E. Conn, Birgit Vennesland and F. H. Westheimer, *J. Biol. Chem.* 202 687 (1953).

[6] H. Richard Levy, Frank A. Loewus and Birgit Vennesland, *J. Am. Chem. Soc.* 79 2949 (1957).

[7] Kurt Wallenfels and Horst Sund, *Biochem. Z.* 329 59 (1957).




The fact that alcohol and lactic acid dehydrogenases all have been found to contain 2 or 4 DPN molecules has also inspired the hypothesis that hydrogen transfer might require a pair of adjoining prosthetic groups in a scheme such as:

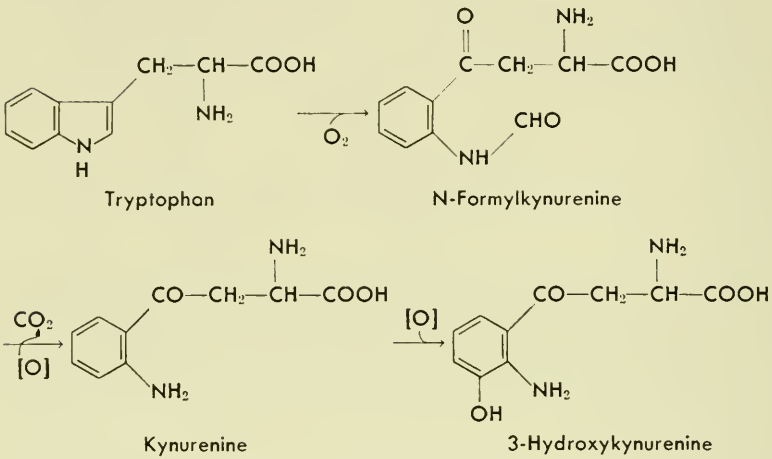


in which a deuterated substrate is shown for clarity.[8] A more detailed discussion has been published of the stereo-

[8] Jan van Eys, Anthony San Pietro and Nathan O. Kaplan, *Science* 127 1443 (1958).

chemistry of microbiological reactions with emphasis on those promoted by dehydrogenases.[9]

The biosynthesis of nicotinic acid has been studied in several different biological systems. In neurospora (and in mammals) tryptophan is the source with 3-oxyanthranilic acid a proved intermediate.[10, 11, 12, 13, 14, 15] The remaining stages of this route are obscure, although α-aminomethyl-α,β-*trans*-γ,δ-*cis*-muconic acid may be an intermediate.[16] It has been shown to be a precursor of nicotinic acid for the bacterium *Xanthomonas pruni*. If it proves to be generally significant, then the following scheme can be written:




Tryptophan — N-Formylkynurenine

Kynurenine — 3-Hydroxykynurenine

[9] G. E. W. Wolstenholme and Cecilia M. O'Connor (Eds.), CIBA Foundation Study Group No. 2, "Steric Course of Microbiological Reactions," Little, Brown and Company, Boston, 1959, 115 pp.

[10] W. A. Krehl, L. J. Teply, P. S. Sarma and C. A. Elvehjem, *Science* **101** 489 (1945).

[11] Fred Rosen, Jesse W. Huff and William A. Perlzweig, *J. Biol. Chem.* **163** 343 (1946).

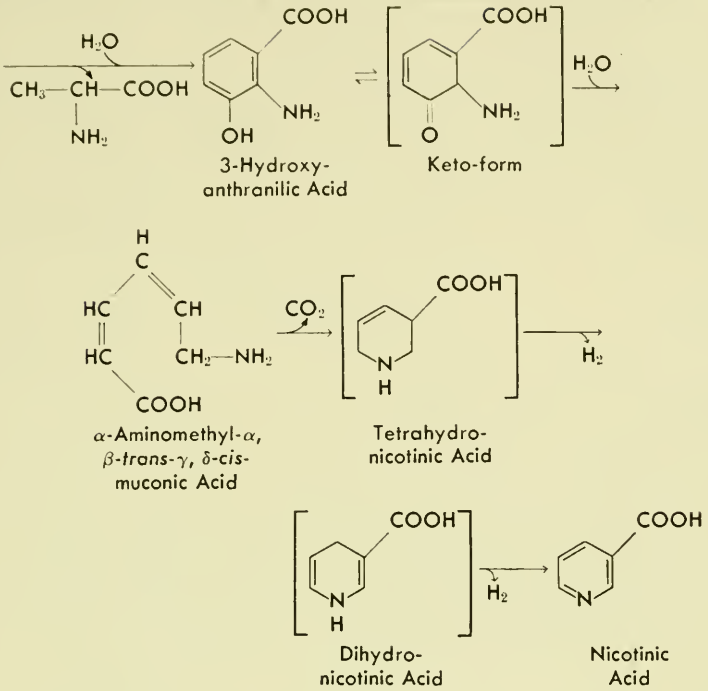
[12] G. S. Beadle, H. K. Mitchell and J. F. Nye, *Proc. Nat. Acad. Sci.* **33** 155 (1947).

[13] Francis A. Haskins and Herschel K. Mitchell, *ibid.* **35** 500 (1949).

[14] Irving L. Miller and Edward A. Adelberg, *J. Biol. Chem.* **205** 691 (1953).

[15] William B. Jakoby and David M. Bonner, *ibid.* **205** 699, 709 (1953).

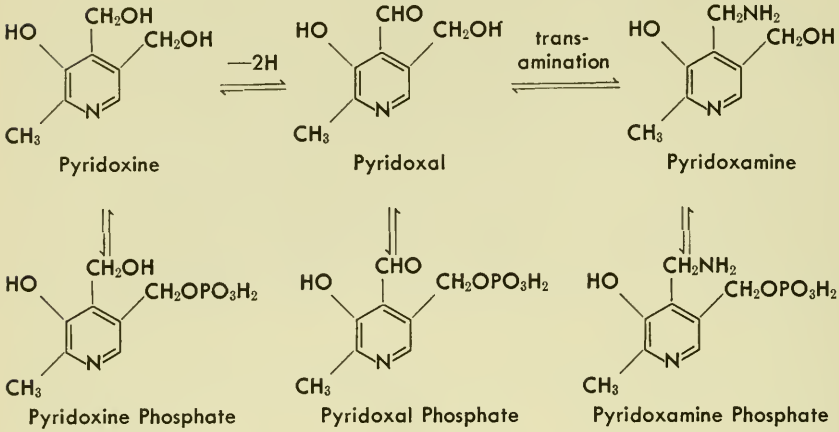
[16] J. O. Harris and F. Binns, *Nature* **179** 475 (1957).



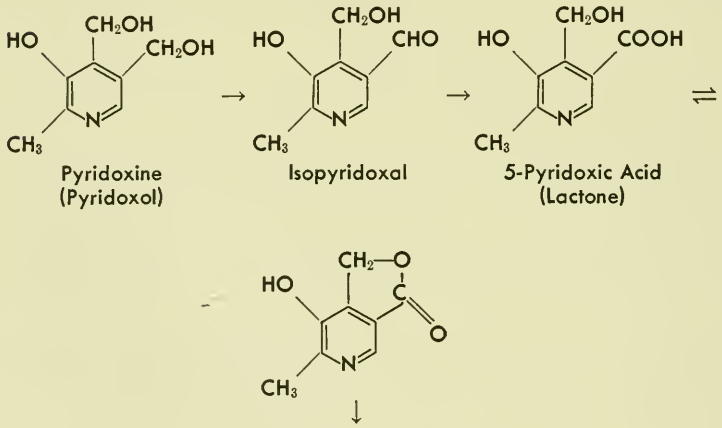

A different method of biosynthesis exists in *Escherichia coli* and *Bacillus subtilis* since tryptophan is not used. Investigation of this route has not progressed so far, but glycerol is capable of supplying all carbon atoms, as are glyceric acid and dihydroxyacetone (but not pyruvate). Succinate, malate, fumarate and oxaloacetate also were used. Ribose and adenine were required, which suggests direct synthesis of the coenzyme.[17]

[17] Manuel V. Ortega and Gene M. Brown, *J. Am. Chem. Soc.* 81 4437 (1959).

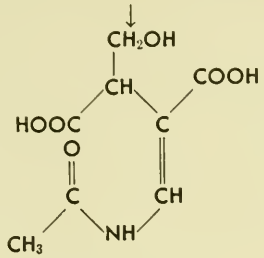
The various forms of pyridoxine are:




Virtually nothing is known concerning the biogenesis of pyridoxine. Since catabolism often furnishes clues useful in the study of biosynthesis, it should be noted that oxidative bacteria degrade pyridoxine as follows:[18]




[18] Victor W. Rodwell, Benjamin E. Volcani, Miyoshi Ikawa and Esmond E. Snell, *J. Biol. Chem.* 233 1548 (1958); Miyoshi Ikawa, Victor W. Rodwell and Esmond E. Snell, *ibid.* 233 1555 (1958).




α-Hydroxymethyl-α'-(N-acetylaminomethylene)-succinic Acid

Acid hydrolysis converts the acyclic product to paraconic acid.

Functions of the vitamin are better understood. The names pyridoxine or vitamin $B_6$ commonly are used in a general sense to refer to the group. Pyridoxal 5-phosphate is the actual prosthetic group in most enzymic reactions. It is a component of transaminases, amino acid decarboxylases, tryptophan synthetase, amino acid racemases, threonine synthetase (homoserine isomerase), δ-aminolevulinate synthetase, phosphorylase and various other enzymes which manipulate amino acids. More thorough discussions of functions of this important vitamin can be found in reviews.[19, 20]

Some pyridoxal-catalyzed reactions can be carried out in aqueous solution without the apoenzymes if heat and the proper metal ions ($Al^{+++}$, $Fe^{++}$, $Cu^{++}$) are supplied. Mechanisms which have been proposed for three such reactions are outlined in the following equations:[21, 22, 23, 24]

[19] Esmond E. Snell, *Vitamins and Hormones* 16 77 (1958).

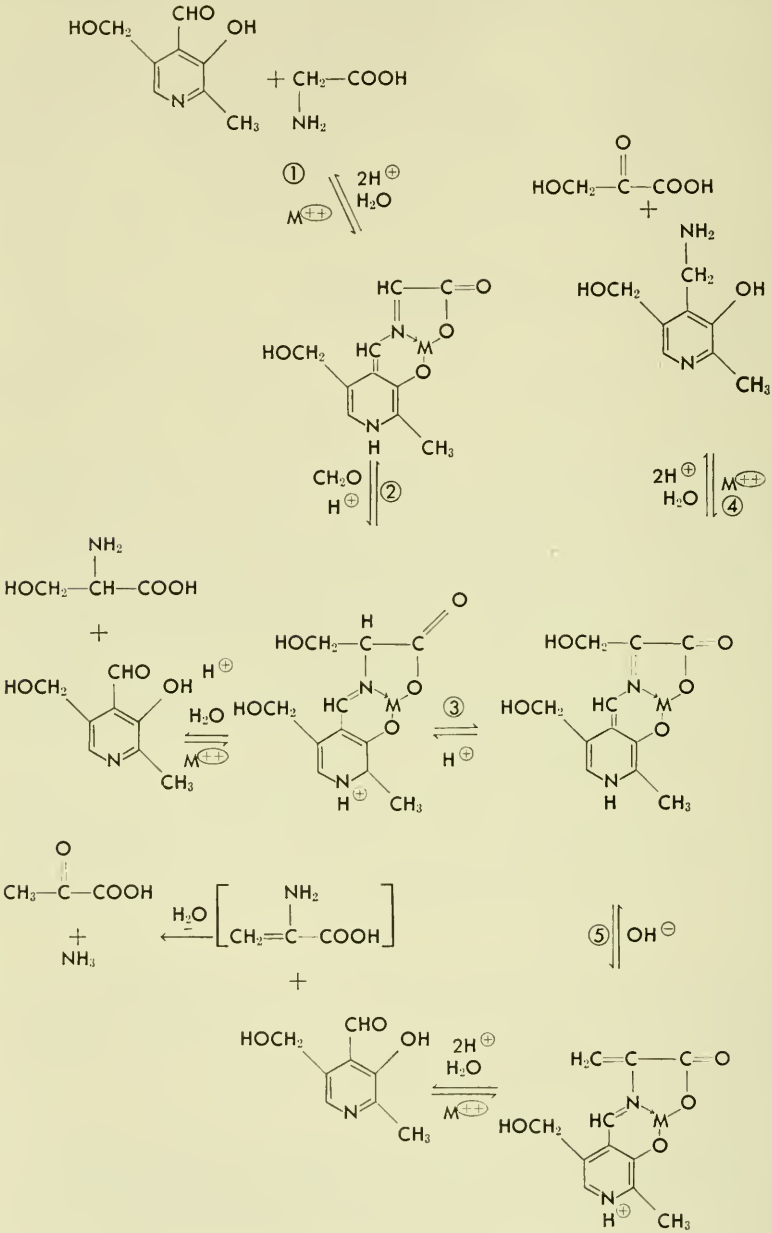
[20] Paul D. Boyer, Henry Lardy and Karl Myrbäck, (Eds.) "The Enzymes," Alexander E. Braunstein, *Pyridoxal phosphate,* Academic Press, New York, 1960, pp. 113–184.

[21] David E. Metzler, Miyoshi Ikawa and Esmond E. Snell, *J. Am. Chem. Soc.* 76 648 (1954).

[22] J. B. Longenecker and Esmond E. Snell, *ibid.* 79 142 (1957).

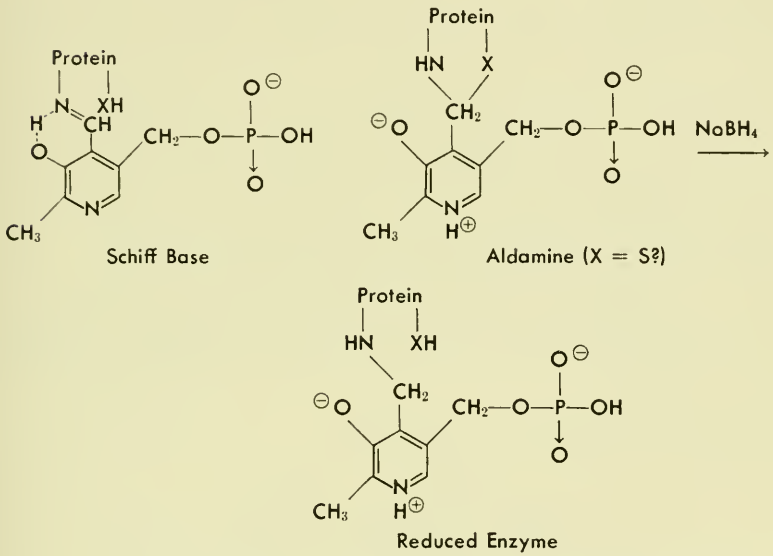
[23] W. Terry Jenkins and Irwin W. Sizer, *ibid.* 79 2655 (1957).

[24] D. S. Hoare and Esmond E. Snell, Proc. Internat. Sympos. Enz. Chem., Tokyo and Kyoto, Pergamon Press, London, 1957, p. 142.



M = Metal: ①, ② = Aldol formation and cleavage
③, ④ = Transamination
③, ⑤ = α, β-Elimination

Attachment to the apoenzyme *in vivo* was assumed to be at the pyridine nitrogen atom. Spectral data from such model systems, however, when applied to purified enzymes, indicate that pyridoxal phosphate is bound to the apoenzyme as a Schiff base in glutamate-aspartate aminopherase[23] and in homoserine deaminase-cystathionase.[25] In crystalline muscle phosphorylase pyridoxal is bound to the apoenzyme, probably at a lysine ε-amino group, as an aldamine, involving an additional side-chain of the protein (perhaps —SH).[26, 27]




Glutamate-aspartate aminopherase contains 2 moles of bound pyridoxal phosphate and muscle phosphorylase 4. It is rather surprising to find the vitamin in an enzyme, such as the latter, unrelated to its ordinary function. Doubt has been cast on its function as a prosthetic group in phosphorylase by several experiments, one of them the reduction shown, which should have inactivated the pyridoxal, but which did not inactivate the enzyme.[28] It

[25] Yoshihiko Matsuo and David M. Greenberg, *J. Biol. Chem.* 230 545, 561 (1958); *idem., ibid.* 234 507, 516 (1959).

[26] Alan B. Kent, Edwin G. Krebs and Edmond H. Fischer, *J. Biol. Chem.* 232 549 (1958).

[27] Barbara Illingworth, Hendrik S. Jansz, David H. Brown and Carl F. Cori, *Proc. Nat. Acad. Sci.* 44 1180 (1958).

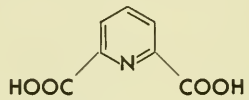
[28] Edmond H. Fischer, Alan B. Kent, Eloise R. Snyder and Edwin G. Krebs, *J. Am. Chem. Soc.* 80 2906 (1958).

may be that it serves a structural or other function here.

D-Cycloserine has been reported to inhibit aspartate aminopherase, indole synthetase and D-alanine-D-glutamate aminopherase in some bacteria.[29, 30] Aspartic analogues, such as diaminosuccinic acid and hydroxyaspartic acid also are effective inhibitors of the first enzyme above.[31]

It has been suggested that pyridoxine may be implicated in the active transport of amino acids across cell walls.[32]

968 **2, 6-Dipicolinic Acid,** $C_7H_5O_4N$, colorless needles, m.p. 236° (dec.).




*Bacillus megatherium, B. cereus* var. *terminalis, B. sphaericus* types

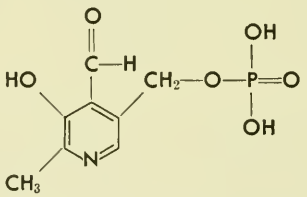
Occurs as the calcuim salt in spores.

Joan F. Powell, *Biochem. J.* 54 210 (1953).

William K. Harrell and Emil Mantini, *Can. J. Microbiol.* 3 735 (1957).

Joan F. Powell and R. E. Strange, *Biochem. J.* 65 700 (1957).

969 **Pyridoxal-5′-phosphate** $C_8H_{10}O_6NP$




Yeasts, molds, bacteria (widely distributed)

[29] Takakazu Aoki, *Kekkaku* 32 544, 605 (1957). (*Chem. Abstr.* 52 7427g).

[30] N. K. Kochetkov, R. M. Khomutov, M. J. Karpeiskii, E. I. Budovskii and E. S. Severin, *Doklady Akad. Nauk S.S.S.R.* 126 1132 (1959).

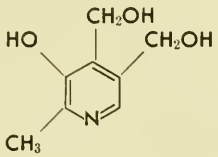
[31] Mario Garcia-Hernandez and Ernest Kun, *Biochim. et Biophys. Acta* 24 78 (1957).

[32] Halvor N. Christensen, Thomas R. Riggs and Barbara R. Coyne, *J. Biol. Chem.* 209 413 (1954); Halvor N. Christensen and Thomas R. Riggs, *ibid.* 220 265 (1956).

I. C. Gunsalus, W. D. Bellamy and W. W. Umbreit, *J. Biol. Chem.* 155 685 (1944).

Dorothea Heyl, Eileen Luz, Stanton A. Harris and Karl Folkers, *J. Am. Chem. Soc.* 73 3430 (1951). (Synthesis)

970 **Pyridoxine** (Vitamin $B_6$), $C_8H_{11}O_3N$, colorless needles from acetone, m.p. 160° (sublimes).



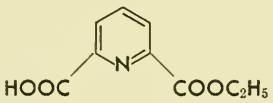

Yeasts, molds.

Yields of 82–114 μg. per gram (dry basis) have been reported from penicillin broth filtrates.

Yields of 23–100 μg. per gram of dry cells have been reported from brewers' yeast.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. II, J. M. VanLanen, *Production of vitamins other than riboflavin*, Chap. 6, pp. 191–216. (A review)

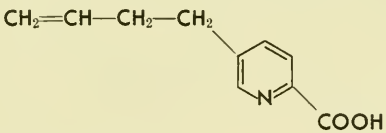
971 **Ethyl Hydrogen 2, 6-Dipicolinate,** $C_9H_9O_4N$, colorless crystals, m.p. 121.5°.




*Bacillus cereus* var. *mycoides* (spores)

J. J. Perry and J. W. Foster, *J. Bacteriol.* 72 295 (1956).

972 **Dehydrofusaric Acid,** $C_{10}H_{11}O_2N$, colorless crystals, m.p. 118°.



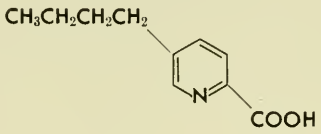

*Gibberella fujikuroi* Saw.

Ernst Gäumann, *Phytopathology* 47 342 (1957).

C. A. Stoll and J. Renz, *Phytopathol. Z.* 27 380 (1957).

John Frederick Grove, P. W. Jeffs and T. P. C. Mulholland, *J. Chem. Soc.*, 1236 (1958).

973 **Fusaric Acid**, $C_{10}H_{13}O_2N$, colorless crystals, m.p. 100°.



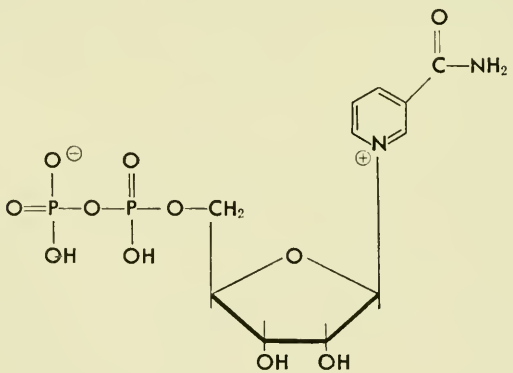

*Gibberella fujikuroi* (Saw.) Wr., *Fusarium heterosporum* Nees, *F. bulbigenum* Cke. *et* Mass. var. *lycopersici* (Bruchi) Wr. *et* Rg., *F. vasinfectum* Atk., *F. orthoceras* App. *et* Wr., *Nectria cinnabarina* (Tode) Fr.

Yields of about 0.5 g. per liter have been reported.

Teijiro Yabuta, Katsuji Kambe and Takeshi Hayashi, *J. Agr. Chem. Soc. Japan* 10 1059 (1934).

John Frederick Grove, P. W. Jeffs and T. P. C. Mulholland, *J. Chem. Soc.*, 1236 (1958).

974 **Coenzyme III** (Nicotinamide Ribose 5′-Diphosphate), $C_{11}H_{16}O_{11}N_2P_2$.

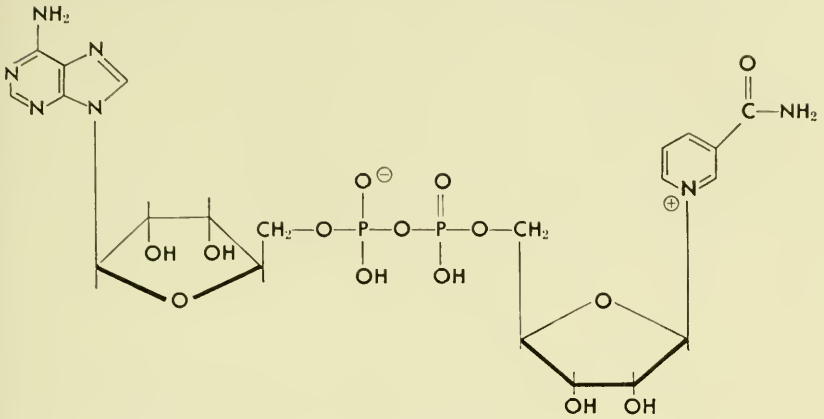



Yeast

Nicotinic acid nucleotides also have been isolated from yeast.

Thomas P. Singer and Edna B. Kearney, *Biochim. et Biophys. Acta* 11 290 (1953).

975 **Diphosphopyridinenucleotide** (DPN), $C_{21}H_{27}O_{14}N_7P_2$.

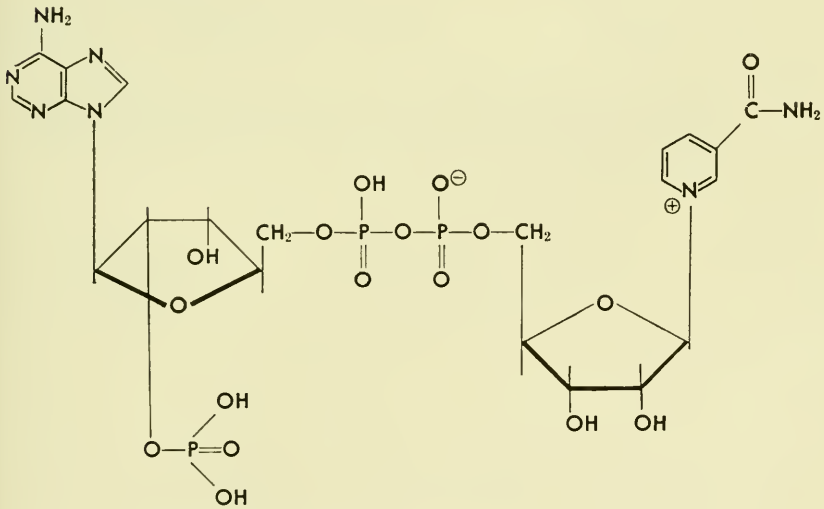


Yeasts, molds (widely distributed)

H. von Euler, P. Karrer and B. Brecker, *Helv. Chim. Acta* 19 1060 (1936). (Structure)

G. A. LePage, *J. Biol. Chem.* 168 623 (1947).

976 **Triphosphopyridinenucleotide** (TPN, Codehydrase II), $C_{21}H_{28}O_{17}N_7P_3$.



Yeasts, molds, etc.

Otto Warburg, Walter Christian and Alfred Griese, *Biochem. Z.* 279 143 (1935); 282 157 (1935). (Isolation)

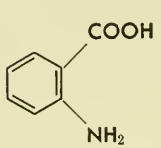
H. von Euler and F. Schlenk, Z. *physiol. Chem.* 246 64 (1937). (Structure)

Arthur Kornberg and W. E. Pricer, Jr., *J. Biol. Chem.* 186 557 (1950).

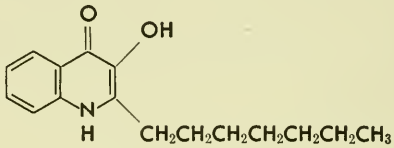
## *j.* QUINOLINES

Quinolines are produced by bacteria and molds, but apparently none has been reported from streptomycetes or lichens. A complex of seven related 4-oxyquinolines is elaborated by the oxidative bacterium *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*). These are commonly called "pyo" compounds.

Evidently no investigations have been made on the mode of biosynthesis of microbial quinolines. The isolation of anthranilic acid and of 2-*n*-heptyl-3-oxy-4-quinolone from "pyo" fermentation broths is suggestive, however.[1] It seems probable that the "pyo" compounds could

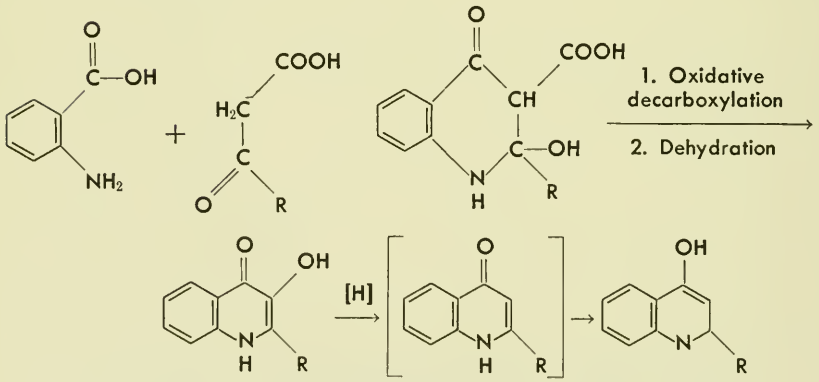



Anthranilic Acid




2-*n*-Heptyl-3-oxy-4-quinolone

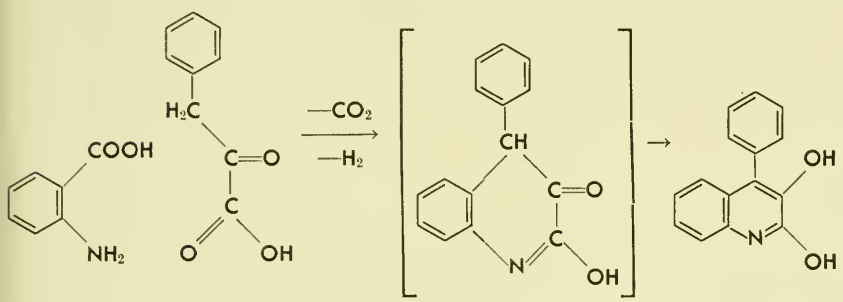
be formed essentially by condensation of anthranilic acid or a biosynthetic precursor with a fatty acid or a fatty acid precursor:




[1] Rokuro Takeda, *J. Fermentation Technol.* 37 59 (1959).

Oxidative decarboxylation would then yield an intermediate of the type isolated, and a one-stage reduction the 4-oxyquinolines. The N-oxides might be formed later by post-oxidation. Quinolines are known to be quite susceptible to N-oxidation by peroxides or oxygen.

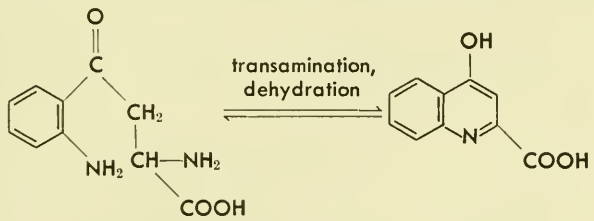
The structure of the mold product, viridicatin, has been verified by synthesis, while that of cyclopenin is still uncertain. It would appear that these substances are also derivatives of anthranilic acid. In this case, condensation probably occurs with an earlier member of the shikimic acid pathway, perhaps prephenic acid or phenylpyruvic acid:




Anthranilic Acid — Phenylpyruvic Acid — Viridicatin

Such condensations have been suggested to explain the origin of certain oxyquinoline plant alkaloids.[2]

There is, of course, a possibility for 4-oxyquinoline formation by way of tryptophan and kynurenine:

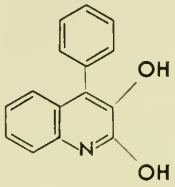



Kynurenine

This seems to be an unnecessarily indirect route, but all of the schemes shown here await experimental test.

[2] Ernest Wenkert, *Experientia* 15 165 (1959).

977 **Viridicatin,** $C_{15}H_{11}O_2N$, colorless needles, m.p. 268°.

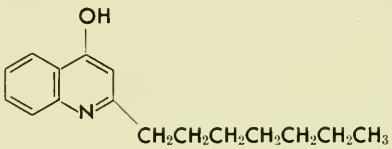



*Penicillium viridicatum* Westling, *P. cyclopium* Westling

See under cyclopenin.

A. Bracken, Anna Pocker and H. Raistrick, *Biochem. J.* 57 587 (1954). (Synthesis)

978 **2-*n*-Heptyl-4-oxyquinoline,** $C_{16}H_{21}ON$, colorless crystals, m.p. 146°.

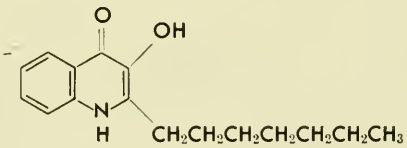



*Pseudomonas aeruginosa*

These quinoline derivatives are called "pyo" compounds.

Ibert C. Wells, *J. Biol. Chem.* 196 331 (1952). (Synthesis)

979 **2-*n*-Heptyl-3-oxy-4-quinolone,** $C_{16}H_{21}O_2N$.

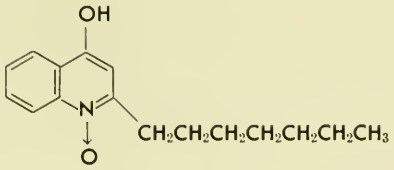



*Pseudomonas aeruginosa* strain T-359

The other "pyo" compounds were isolated as well as anthranilic acid, pyoluteorin, pyocyanine, phenazine-1-carboxylic acid and oxychlororaphine.

Rokuro Takeda, *J. Fermentation Technol.* 37 59 (1959).

980 **2-*n*-Heptyl-4-oxyquinoline N-oxide,** $C_{16}H_{21}O_2N$, colorless leaflets, m.p. 158–160°.

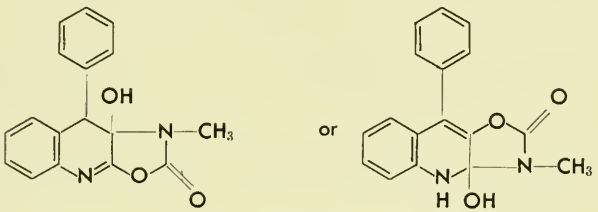



*Pseudomonas aeruginosa*

J. W. Cornforth and A. T. James, *Biochem. J.* 63 124 (1956). (Synthesis)

981 **Cyclopenin,** $C_{17}H_{14}O_3N_2$, colorless tablets, m.p. 207°, $[\alpha]_D^{20}$ −306° (c 1.0 in ethanol).

Proposed structures:

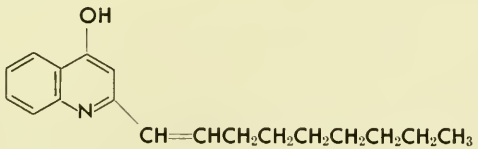



*Penicillium cyclopium* Westling

Usually viridicatin is produced by the same organism. Palitantin and frequentin are also produced by *P. cyclopium*

A. Bracken, Anna Pocker and H. Raistrick, *Biochem. J.* 57 587 (1954).

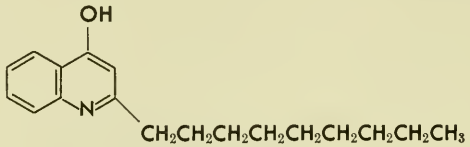
982 **2-(*n*-$\Delta^1$-Nonenyl)-4-oxyquinoline,** $C_{18}H_{23}ON$, colorless crystals, m.p. 153°.




*Pseudomonas aeruginosa*

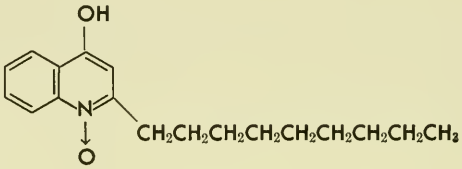
Ibert C. Wells, *J. Biol. Chem.* 196 331 (1952). (Synthesis)

983 2-*n*-Nonyl-4-oxyquinoline, $C_{18}H_{25}ON$, colorless crystals, m.p. 139°.



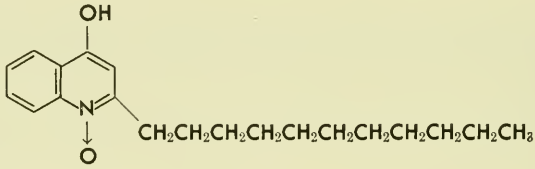

*Pseudomonas aeruginosa*
Ibert C. Wells, *J. Biol. Chem.* 196 331 (1952). (Synthesis)

984 2-*n*-Nonyl-4-oxyquinoline N-Oxide, $C_{18}H_{25}O_2N$, colorless leaflets, m.p. 148°.




*Pseudomonas aeruginosa*
J. W. Cornforth and A. T. James, *Biochem. J.* 63 124 (1956). (Synthesis)

985 2-*n*-Undecyl-4-oxyquinoline N-Oxide, $C_{20}H_{29}O_2N$, colorless leaflets, m.p. 148.5°.




*Pseudomonas aeruginosa*
J. W. Cornforth and A. T. James, *Biochem. J.* 63 124 (1956). (Synthesis)

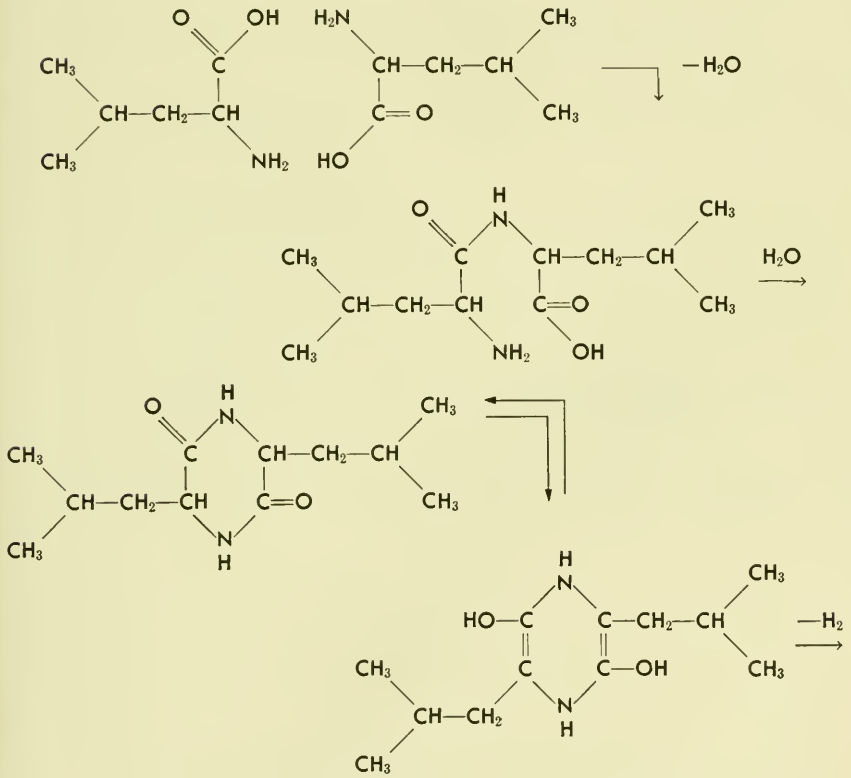
## *k.* PYRAZINES, DIKETOPIPERAZINES

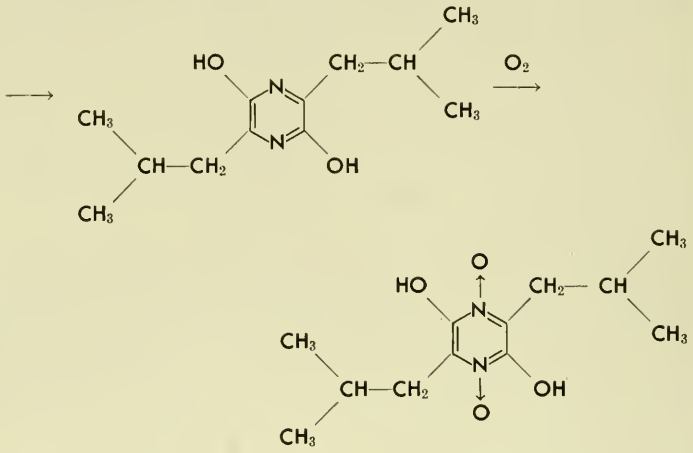
Diketopiperazines are produced by molds, yeasts and lichens, but none has been reported from bacteria. Besides those listed in this section, others are classified elsewhere, for example, echinulin and gliotoxin under indoles.

Flavacol and pulcherriminic acid seem to be derived

from leucine, the echinulin moiety from leucine and alanine, aspergillic acid from leucine and isoleucine, the mycelianamide moiety from tyrosine and alanine, picrorocellin from phenylalanine, and gliotoxin from phenylalanine and serine. It might be mentioned that we have isolated from a *Rhizopus nigricans* culture a diketopiperazine which is a derivative of isoleucine and valine (unpublished).

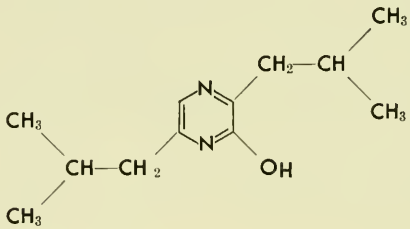
Dehydration, dehydrogenation, oxidation and N- or O-methylation sometimes occur to obscure the origin to some degree. Aromatization to a pyrazine has taken place in flavacol and pulcherriminic acid, aspergillic acid, a dihydropyrazine, representing an intermediate oxidation state. Formation of pulcherriminic acid might be represented as follows:





The addition of sulfur across the diketopiperazine ring in gliotoxin is interesting.

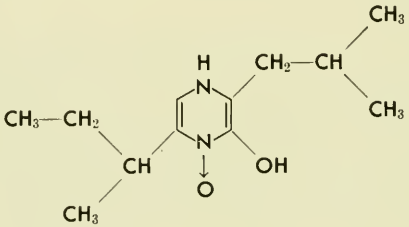
986 **Flavacol**, $C_{12}H_{20}ON_2$, colorless needles, m.p. 147–149°.



*Aspergillus flavus*

George Dunn, G. T. Newbold and F. S. Spring, *J. Chem. Soc.* 2586 (1949). (Synthesis)

987 **Aspergillic Acid**, $C_{12}H_{20}O_2N_2$, pale yellow needles, m.p. 97–99°, $[\alpha]_D^{25}$ +13.4° (c 1 in ethanol).



*Aspergillus flavus*

James D. Dutcher, *J. Biol. Chem.* 232 785 (1958).

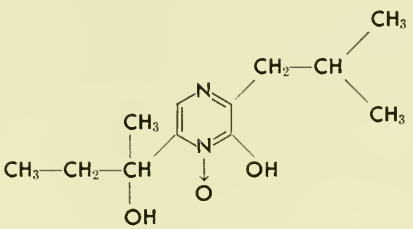
988 **Granegillin,** $C_{12}H_{20}O_2N_2$, pale yellow needles, m.p. 99–100°, optically inactive, the crystals have a characteristic odor (as does Aspergillic Acid).

The only important difference in properties between granegillin and aspergillic acid is the lack of optical activity in the former, and the two compounds may be identical.

A mold resembling *Aspergillus flavus*

A. Csillag, *Acta Microbiol.* (Hungary) 1 321 (1954); Abstr. in *Bull. Hyg.* 30 159 (1955).

989 **Hydroxyaspergillic Acid,** $C_{12}H_{20}O_3N_2$, nearly colorless needles, m.p. 148–150°, $[\alpha]_D^{25}$ +36° (c 1 in ethanol).



*Aspergillus flavus*

James D. Dutcher, *J. Biol. Chem.* 232 785 (1958).

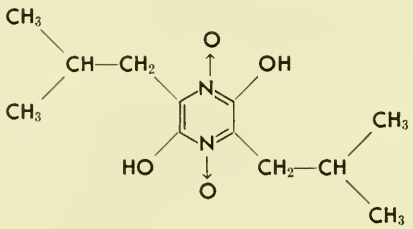
990 **Neohydroxyaspergillic Acid,** $C_{12}H_{20}O_3N_2$, colorless crystals, m.p. 164–166°, $[\alpha]_D^{25}$ −58° (c 1.01 in ethanol).

*Aspergillus sclerotiorum*

A yield of about 300 mg. per liter was obtained.

Ulrich Weiss, Frieda Strelitz, Helen Flon and Igor N. Asheshov, *Arch. Biochem. and Biophys.* 74 150 (1958).

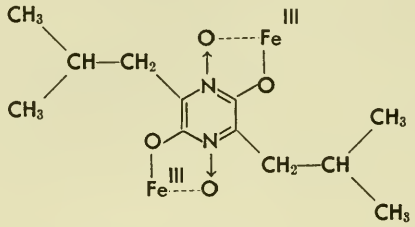
991 **Pulcherriminic Acid,** $C_{12}H_{20}O_4N_2$, m.p. 173°.



*Candida pulcherrima* (Lindner) Windisch

This compound was isolated as a red, iron-complexed

pigment called pulcherrimin, which probably has the structure:

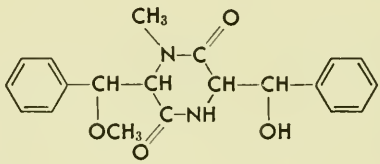


The yield was 30 mg. of pulcherrimin per gram of dry cells.

A. J. Kluyver, J. P. van der Walt and A. J. van Triet, *Proc. Nat. Acad. Sci. U. S.* 39 583 (1953).

A. H. Cook and C. A. Slater, *J. Chem. Soc.*, 4130, 4133, (1956). (Structure)

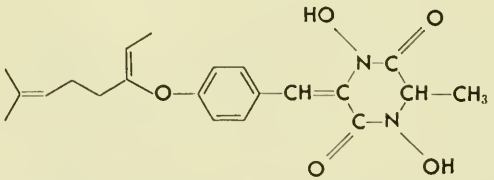
992 **Picrorocellin,** $C_{20}H_{22}O_4N_2$, colorless prisms, m.p. 192–194°, $[\alpha]_D$ +12.5°.



*Roccella fuciformis* Ach.

Martin Onslow Forster and William Bristow Saville, *J. Chem. Soc.*, 816 (1922).

993 **Mycelianamide,** $C_{22}H_{28}O_5N_2$, colorless leaflets, m.p. 170–172° (dec.), $[\alpha]_{5461}^{19}$ −217° (c 0.869 in chloroform).



*Penicillium griseofulvum*

A. J. Birch, R. A. Massy-Westropp and R. W. Rickards, *J. Chem. Soc.*, 3717 (1956).

A. J. Birch, *Proc. Chem. Soc.*, 233 (1957).

## *l.* PHENAZINES AND PHENOXAZONES

The phenazine bacterial pigments have been known for many years. Pyocyanine was probably isolated in the early 1860's, and oxychlororaphine was synthesized in 1930. New pigments of this type continue to be reported, usually from pseudomonas species, but also from streptomycetes. Pyocyanine is responsible for the blue-green color of pus, since *Pseudomonas aeruginosa* is a skin parasite, and certain other blue or green stains on natural materials have been identified with phenazine pigments.

The phenazine bacterial pigments have been reviewed,[1] and this introduction will be confined to a few remarks on biosynthesis. Actually, there is as yet little to be said on this subject. Several studies have been made concerning medium requirements and improvements for optimum pigment production in both growing[2] and stationary cultures.[3] In growing cultures a yield of 231 mg. of pyocyanine per liter was obtained on a medium containing glycerol, D,L-alanine, L-leucine and magnesium, calcium, phosphate, sulfate and ammonium ions.

In resting cultures glutamic acid and $\gamma$-aminobutyric acid were found to be the best substrates, yielding about 250 mg. of pyocyanine per liter. Pigment production was slow and inhibited by respiratory poisons (cyanide, azide) but not by fluoride.

These results are not very helpful in speculations on the biosynthetic intermediates.

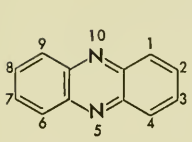
Viewed in aggregate there is a noticeable recurrence of either hydroxyl or carboxyl groups at the 1-position, the 9-position or the 6-position of the phenazine nucleus.

[1] G. A. Swan and D. G. I. Felton, "Phenazines," Interscience Publishers, Inc., New York, 1957, pp. 174–191.

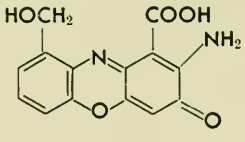
[2] M. O. Burton, J. J. R. Campbell and B. A. Eagles, *Can. J. Res.* **26C** 15 (1948); M. O. Burton, B. A. Eagles and J. J. R. Campbell, *ibid.* **25C** 121 (1947); G. Young, *J. Bacteriol.* 54 109 (1947); Esther Hellinger, *J. Gen. Microbiol.* 5 633 (1951).

[3] N. Grossowicz, Peyuta Hayat and Y. S. Halpern, *J. Gen. Microbiol.* **16** 576 (1957).

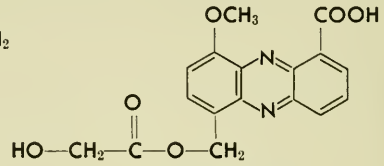
This is reminiscent of the phenoxazones such as cinna-




Phenazine




Cinnabarin

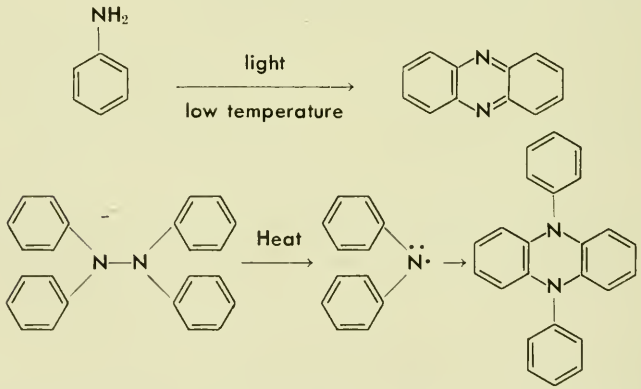



Griseolutein A

barin and actinocinin. The analogy perhaps can be developed farther, since a streptomycete pigment has been found with an amino group in the 2-position.

The resemblance is sufficient to suggest anthranilic acid or related substances as intermediates in the biosynthesis of phenazines. Oxidative decarboxylations of aromatic acids to phenols are not unknown among obligate aerobes of the type that produce phenazines. Also 3-oxyanthranilic acid might account for some of the phenolic hydroxyl groups.

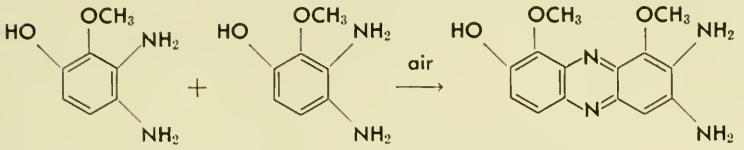
As for the coupling reaction, perhaps something akin to phenolic-free radical coupling takes place. Photoirradiation of aniline at low temperatures has been reported to produce phenazine.[4] Also tetraphenylhydrazine heated to 90° apparently dissociates to a free radical which rearranges to (among other things) a dihydrophenazine.[5]




[4] A. N. Terenin, *Acta Physicochim. S.S.S.R.* 13 1 (1940); *Chem. Abstr.* 35 1701 (1941).

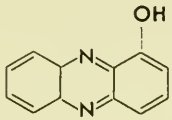
[5] G. W. Wheland, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, 1949, pp. 727–728.

Atmospheric oxidation is enough to cause phenazine formation from 3,4-diaminoguaiacol.[6] This is a favorable case for free radical stabilization.




This argument of course is speculative.

994 **1-Phenazinol** (1-Hydroxyphenazine, Hemipyocyanine), $C_{12}H_8ON_2$, orange crystals, m.p. 157° (sublimes).



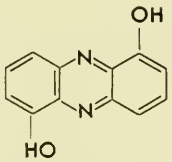

*Pseudomonas aeruginosa*

Fritz Wrede and E. Strack, *Z. physiol. Chem.* 177 177 (1928).

G. M. Badger, R. S. Pearce and R. Pettit, *J. Chem. Soc.* 3204 (1951).

Walter S. Moos and John W. Rowen, *Arch. Biochem. and Biophys.* 43 88 (1953).

995 **1,6-Dihydroxyphenazine,** $C_{12}H_8O_2N_2$, golden yellow prisms, m.p. 274°.

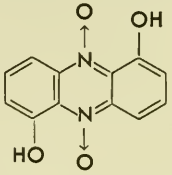



*Streptomyces thioluteus*

Hideshi Akabori and Michikazu Nakamura, *J. Antibiotics* (Japan) 12A 17 (1959).

[6] Fr. Fichter and Julius Schwab, *Ber.* 39 3339 (1906).

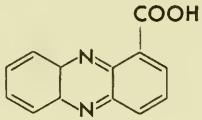
996 **Iodinin** (1,6-Phenazinediol-5,10-dioxide), $C_{12}H_8O_4N_2$, purple crystals with a coppery glint, m.p. 236° (dec.).




*Chromobacterium iodinum*

G. R. Clemo and A. F. Daglish, *J. Chem. Soc.*, 1481 (1950).

997 **Phenazine-1-carboxylic Acid,** $C_{13}H_8O_2N_2$, greenish yellow needles, m.p. 242°.




*Pseudomonas aureofaciens* Kluyver, *Streptomyces misakiensis, Calonectria*

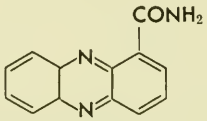
Yields of about 1 g. per liter have been mentioned. The streptomycete produced another phenazine, $C_{17}H_{16}N_2O_2$, called tubermycin A. A pigment closely related to phenazine-1-carboxylic acid was also isolated by Kluyver from the pseudomonas organism.

A. J. Kluyver, *J. Bacteriol.* 72 406 (1956).

Wm. C. Haynes, Frank H. Stodola, Joan M. Locke, Thomas G. Pridham, Howard F. Conway, Virgil E. Sohns and Richard W. Jackson, *ibid.* 72 412 (1956).

Kiyoshi Isono, Kentaro Anzai and Saburo Suzuki, *J. Antibiotics* (Japan) 11A 264 (1959).

998 **Oxychlororaphine,** $C_{13}H_9ON_3$, yellow needles, m.p. 237° (sublimes in the absence of $O_2$, giving yellow crystals, m.p. 241°).

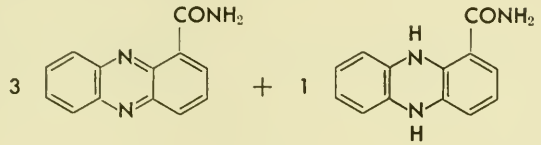



*Pseudomonas chlororaphis*

Fritz Kögl and J. J. Postowsky, *Ann.* 480 280 (1930). (Synthesis)

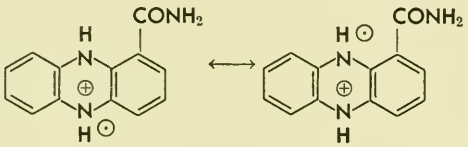
999 **Chlororaphine**, green crystals, m.p. (in the absence of $O_2$) 225° (dec.) (in the presence of $O_2$ sublimes at 210°, giving a yellow sublimate).

Chlororaphine in the crystalline state is a molecular compound of phenazine-1-carboxamide and its dihydro derivative in the ratio of 3:1.




Charles Dufraisse, André Etienne and Edmond Toromanoff, *Compt. rend.* 235 920 (1952).

But in solution in the pH range 1.75–10.85 (particularly at lower pH) chlororaphine exists largely in the semiquinone form:

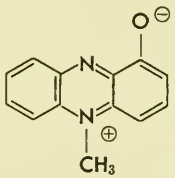



Carlo Cattaneo, Guido Sartori and M. Morellini, *Gazz. chim. ital.* 77 381 (1947).

*Pseudomonas chlororaphis*

Fritz Kögl and J. J. Postowsky, *Ann.* 480 280 (1930).

1000 **Pyocyanine**, $C_{13}H_{12}N_2O$, dark blue needles, m.p. 133°, decomposes to 1-phenazinol on standing in light and air.

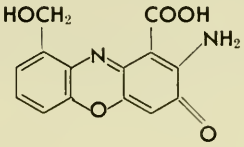



*Pseudomonas aeruginosa* (*Bacillus pyocyaneus*), *Cyanococcus chromospirans*

Heinz Hilleman, *Ber.* **71B** 46 (1938). (Structure)

G. Farber, *Sbornik Ceskoslov. Akad. Zemedelske* 23 355 (1951); *Chem. Abstr.* 45 9605 (1951).

1001 **Cinnabarin** (Polystictin), $C_{14}H_{10}O_5N_2$, red needles, m.p.: dec. >320°.




*Coriolus sanguineus* Fr. [= *Polyporus cinnabarinus* Fr. = *P. sanguineus* Fr. = *P. coccineus* Fr. = *P. puniceus* Kalch. = *Polystictus cinnabarinus* (Jacq.) = *P. sanguineus* L. = *P. semisanguineus* Lloyd = *Trametes cinnabarina* (Jacq.) Fr.]

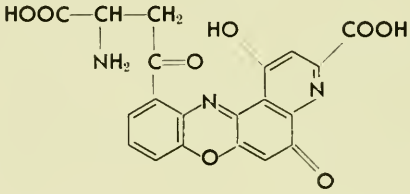
About 100 mg. of red pigment were obtained from 55 g. of mycelium.

Jarl Gripenberg, *Acta Chem. Scand.* 5 590 (1951).

G. W. K. Cavill, B. J. Ralph, J. R. Tetaz and R. W. Werner, *J. Chem. Soc.*, 525 (1953).

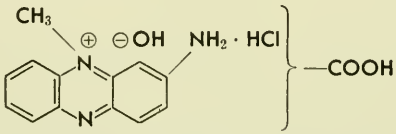
Jarl Gripenberg, *Acta Chem. Scand.* 12 603 (1958). (Structure)

The same phenoxazone chromophore which occurs in cinnabarin and the actinomycins has been found in certain insect pigments called ommatins, *e.g.* xanthommatin:




Adolf Butenandt, Ulrich Schiedt, Ernst Bickert and R. Jan. T. Cromartie, *Ann.* 590 75 (1954).

1002 **Pigment A**, $C_{14}H_{11}O_2N_3 \cdot 2H_2O$, red crystals, dec. without melting. Tentative structure:




Yield 12–20 mg. per liter

and

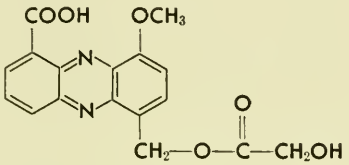
1003 **Pigment B,** $C_{15}H_{15}O_6N_3S$ (may also be hydrated), red crystals, dec. without melting.

An acidic pigment similar to A in structure, but with an additional methyl group and a sulfo group. Yield 30–40 mg. per liter.

Both produced by a red strain of *Pseudomonas aeruginosa.*

F. G. Holliman, *Chem. and Ind.*, 1668 (1957).

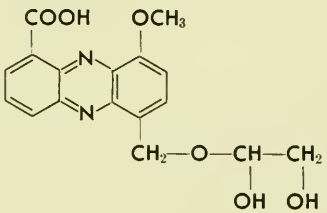
1004 **Griseolutein A,** $C_{17}H_{14}O_6N_2$, reddish yellow needles, m.p. 193° (dec.).




*Streptomyces griseoluteus*

Shoshiro Nakamura, *Chem. and Pharm. Bull.* (Japan) 6 547 (1958).

1005 **Griseolutein B,** $C_{17}H_{16}O_6N_2$, pale yellow crystals, darkening from 150°, dec. above 220°. Griseolutein B is a phenazine with the following proposed structure:




*Streptomyces griseoluteus* n. sp.

Hamao Umezawa, Seiki Hayano, Kenji Maeda, Yasuo Ogata and Yoshiro Okami, *J. Antibiotics* (Japan) 4 34 (1951).

Teisuke Osato, Kenji Maeda and Hamao Umezawa, *ibid.* 7A 15 (1954).

Shoshiro Nakamura, Kenji Maeda, Teisuke Osato and Hamao Umezawa, *ibid.* 10A 265 (1957).

Shoshiro Nakamura, *Chem. and Pharm. Bull.* (Japan) 6 547 (1958).

## *m.* PYRIMIDINES

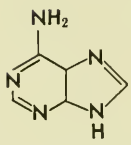
Pyrimidines are fundamental components of living cells. They have long been recognized as constituents of nucleic acids, and more recently other functions have been discovered.

Microorganisms are rather rich in nucleoproteins. Yeast, which has been a common experimental source, contains about 4 percent of its dry weight in nucleic acids, and bacteria up to 15 percent. Bacteriophages are largely nucleoprotein, and certain plant viruses entirely. By contrast, thymus gland, one of the richer animal tissue sources, contains only about 3 percent.

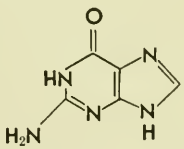
The protein moieties often are relatively low in molecular weight, some of them qualifying as large peptides, and they generally seem to be rich in basic amino acids. The total nucleoprotein molecular weights, however, are very high—often running to many millions. The complexity of the nucleic acid moiety varies with the complexity of the species. Since the DNA carries the genetic information, it might be expected to be more complex and higher in molecular weight for the human species than, for example, in a simple plant virus.

Two types of nucleic acids have been distinguished, both widely distributed. Ribose nucleic acid (RNA) and deoxyribose nucleic acid (DNA) have been mentioned earlier in connection with their roles in protein synthesis and genetics.

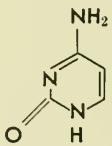
Neither of these substances has been obtained entirely pure, but newer techniques such as electrophoresis and paper chromatography have permitted refinements. The important purine and pyrimidine components of RNA are adenine, guanine, cytosine and uracil. In DNA thymine takes the place of uracil, and 5-methylcytosine is a minor



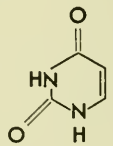

Adenine (6-amino-purine)




Guanine (2-amino-6-oxypurine)

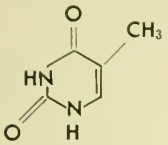



Cytosine (2-oxy-6-amino-pyrimidine)

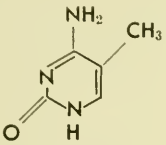



Uracil (2,6-dioxy-pyrimidine)

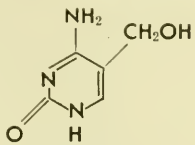
component in some species.




Thymine
(2,6-dioxy-5-methylpyrimidine)



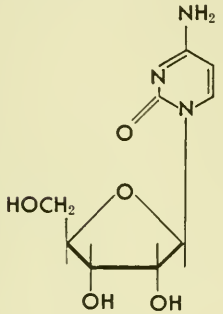

5-Methyl cytosine
(2-oxy-5-methyl-6-aminopyrimidine)



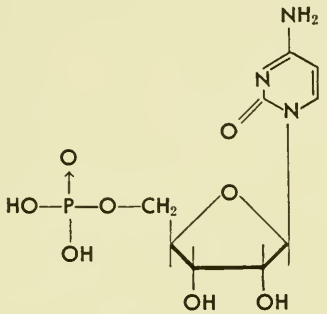

5-Hydroxymethyl cytosine
(2-oxy-5-hydroxymethyl-6-aminopyrimidine)

In some *Escherichia coli* bacteriophages the 5-methylcytosine is replaced by 5-hydroxymethylcytosine. A substance believed to be 5-ribosyluracil has been isolated in considerable quantities from yeast RNA.

The united pyrimidine and ribose moieties are called nucleosides, and the phosphorylated nucleosides are called nucleotides.




Cytidine
(a nucleoside)




Cytidylic Acid
(a nucleotide)

The nucleic acids are, then, polymeric nucleotides, a free phosphoric acid function being esterified by a free pentose alcohol group.

In neither RNA nor DNA are the four main heterocyclic components present in equimolar quantities, and, moreover, there is of course species variation. For example, yeast DNA contains more adenine and thymine than guanosine and cytosine, while the reverse is true for some bacteria. The molar sum of the purines generally equals that of the pyrimidines, and, more specifically, the number of moles of adenine present equals the number of moles of thymine, and the cytosine (and methylcytosine) equals the guanine.

There is good evidence now that most DNA is composed

of a helical coil of paired strands, the strands and coils being associated by hydrogen bonding, *e.g.*, between the amino group of adenine and the carbonyl group of thymine.[1] This structure is supported by roentgen ray diffraction data, by acid-base titration studies and by light-scattering measurements on solutions. There are some recent indications, however, that single-stranded DNA does exist in some cases.

Tobacco mosaic virus, a crystalline substance which has been investigated extensively, consists of a single strand of RNA coiled within a protein sheath. The degree of organization (non-covalent bonding) in the nucleic acid moieties of nucleoproteins has been studied.[2] In some instances the nucleic acids seem to be less organized in the intact protein than in the free state.

Pyrimidine nucleotides also serve as coenzymes in a number of biological reactions. Thus uridine nucleotide is important in the enzymic manipulation of sugars. In recent years, uridine-5′-diphosphate sugar esters have been isolated from a variety of animal, plant and microbial sources.

Confining our attention to microorganisms, uridine diphosphate glucose, UDP-galactose, UDP-acetylglucosamine as well as uridine triphosphate (UTP) and uridine-diphosphate (UDP) have been isolated from yeast.[3, 4, 5] The same substances have been isolated from *Penicillium chrysogenum* mycelium.[6] Other free nucleotides identified from the mold were: diphosphophyridine nucleotide (DPN), cytidine-5′-monophosphate (CMP), adenosine-5′-monophosphate (AMP), triphosphopyridine nucleotide (TPN), guanosine-5′-monophosphate (GMP), inosine-5′-monophosphate (IMP), uridine-5′-monophosphate (UMP),

[1] J. D. Watson and F. H. C. Crick, *Nature* 171 737, 964 (1953).

[2] F. Bonhoeffer and H. K. Schachman, *Biochem. and Biophys. Res. Comms.* 2 366 (1960).

[3] R. Caputto, Luis F. Leloir, C. E. Cardini and A. C. Paladini, *J. Biol. Chem.* 184 333 (1950); E. Cabib, Luis F. Leloir and C. E. Cardini, *ibid.* 203 1055 (1953).

[4] S. H. Lipton, S. A. Morell, Alexander Frieden and Robert M. Bock, *J. Am. Chem. Soc.* 75 5449 (1953).

[5] Hanns Schmitz, *Biochem. Z.* 325 555 (1954).

[6] A. Ballio, C. Casinovi and G. Serlupi-Crescenzi, *Biochim. et Biophys. Acta* 20 414 (1956).

adenosine-5′-diphosphate (ADP), guanosine-5′-diphosphate mannose (GDPM), adenosine-5′-triphosphate (ATP) and guanosine-5′-triphosphate (GTP).

The UTP is an intermediate in the formation of the diphosphate:[7, 8]

$$\text{UTP} + \text{Sugar-1-phosphate} \rightleftharpoons \text{UDP-Sugar} + \text{Pyrophosphate}$$

Once in the form of UDP esters, sugars are susceptible to a variety of enzymic transformations, some of which were mentioned in the section on polypeptides. For example, 4-epimerization may be caused:[3, 9]

$$\text{UDP-Glucose} \rightleftharpoons \text{UDP-Galactose}$$
$$\text{and}$$
$$\text{UDP-D-Xylose} \rightleftharpoons \text{UDP-L-Arabinose}$$

Since there is a DPN requirement in these reactions, it is likely that the 4-hydroxyl group of the sugar is oxidized to a ketone, then reduced stereospecifically. Isotope work supports this hypothesis.[10, 11, 12] UDPG can be oxidized also to UDP-glucuronate:[13, 14]

$$\text{UDP-Glucose} \xrightarrow[H_2O]{2\text{DPN}} \xrightarrow[2\text{DPNH}]{3H^{\oplus}} \text{UDP-Glucuronic Acid}$$

A yeast enzyme catalyzes the reaction:[15, 16]

$$\text{UDP-Glucose} + \text{Glucose-6-phosphate} \longrightarrow \text{Trehalose Phosphate} + \text{UDP}$$

Similarly, di- and polysaccharides seem to be formed

[7] Paul E. Trucco, *Arch. Biochem. and Biophys.* 34 482 (1951).

[8] Agnete Munch-Petersen, Herman M. Kalckar, Enrico Cutolo and Evelyn E. B. Smith, *Nature* 172 1036 (1953).

[9] Luis F. Leloir, *Arch. Biochem. and Biophys.* 33 186 (1951).

[10] Arthur Kowalsky and Daniel E. Koshland, *Biochim. et Biophys. Acta* 22 575 (1956).

[11] Laurens Anderson, Aurora M. Landel and Donald F. Diedrich, *ibid.* 22 573 (1956).

[12] Herman M. Kalckar and Elizabeth S. Maxwell, *ibid.* 22 589 (1956).

[13] V. Ginsburg, E. F. Neufeld and W. Z. Hassid, *Proc. Nat. Acad. Sci. U. S.* 42 333 (1956); V. Ginsburg, *J. Biol. Chem.* 232 55 (1958).

[14] Evelyn E. B. Smith, Agnete Munch-Petersen and George T. Mills, *Nature* 172 1038 (1953).

[15] E. Cabib and Luis F. Leloir, *J. Biol. Chem.* 231 259 (1958).

[16] Luis F. Leloir and E. Cabib, *J. Am. Chem. Soc.* 75 5445 (1953).

in this way. Involvement in chitin (*Neurospora crassa*)[17] and cellulose (*Acetobacter xylinum*)[18] biosynthesis has been shown with labeled UDP-acetylglucosamine and UDP-glucose, respectively, and work with tritium-labeled substrates and cell-free extracts of group A streptococci has shown involvement in hyaluronate biosynthesis.[19] Other evidence indicates involvement in glucuronide[20, 21] and glycogen[22, 23] formation in animals, and glucoside[24, 25] formation in plants. UMP,[26] UDP, UTP[27, 28] and UDP-glucose[29, 30] have been synthesized chemically.

Several cytidine nucleotides have been isolated from natural sources.[31, 32, 33] CDP-Choline and CDP-ethanolamine have been isolated from animals,[33] plants and yeasts[34]

[17] Luis Glaser and David H. Brown, *Biochim. et Biophys. Acta* 23 449 (1957); *idem., J. Biol. Chem.* 228 729 (1957).

[18] Luis Glaser, *Biochim. et Biophys. Acta* 25 436 (1957); *idem., J. Biol. Chem.* 232 627 (1958).

[19] Alvin Markovitz, J. A. Cifonelli and Albert Dorfman, *Biochim. et Biophys. Acta* 28 453 (1958).

[20] Evelyn E. B. Smith and George T. Mills, *Biochim. et Biophys. Acta* 13 386 (1954).

[21] G. J. Dutton and I. D. E. Storey, *Biochem. J.* 57 275 (1954); 59 279 (1955).

[22] Luis F. Leloir and C. E. Cardini, *J. Am. Chem. Soc.* 79 6340 (1957); L. F. Leloir, J. M. Olavarria, Sara H. Goldemberg and H. Carminatti, *Arch. Biochem. and Biophys.* 81 508 (1959).

[23] P. W. Robbins, R. R. Traut and F. Lipmann, *Proc. Nat. Acad. Sci. U. S.* 45 6 (1959).

[24] G. Jacobelli, M. J. Tabone and D. Tabone, *Bull. soc. chim. biol.* 40 955 (1958).

[25] C. E. Cardini and L. F. Leloir, *Nature* 182 1446 (1958).

[26] Alexander R. Todd, "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, Editors) Academic Press, New York, 1957 3 p. 811.

[27] R. B. Hurlbert, *ibid.*, p. 785.

[28] G. W. Kenner, A. R. Todd and F. J. Weymouth, *J. Chem. Soc.*, 3675 (1952); N. Annand, V. M. Clark, R. H. Hall and A. R. Todd, *ibid.*, 3665 (1952).

[29] G. W. Kenner, A. R. Todd and R. F. Webb, *ibid.*, 2843 (1954).

[30] Robert Warner Chambers, J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.* 79 4240 (1957); J. G. Moffatt and H. G. Khorana, *ibid.* 80 3756 (1958).

[31] Rolf Bergquist and Adam Deutsch, *Acta Chem. Scand.* 7 1307 (1953).

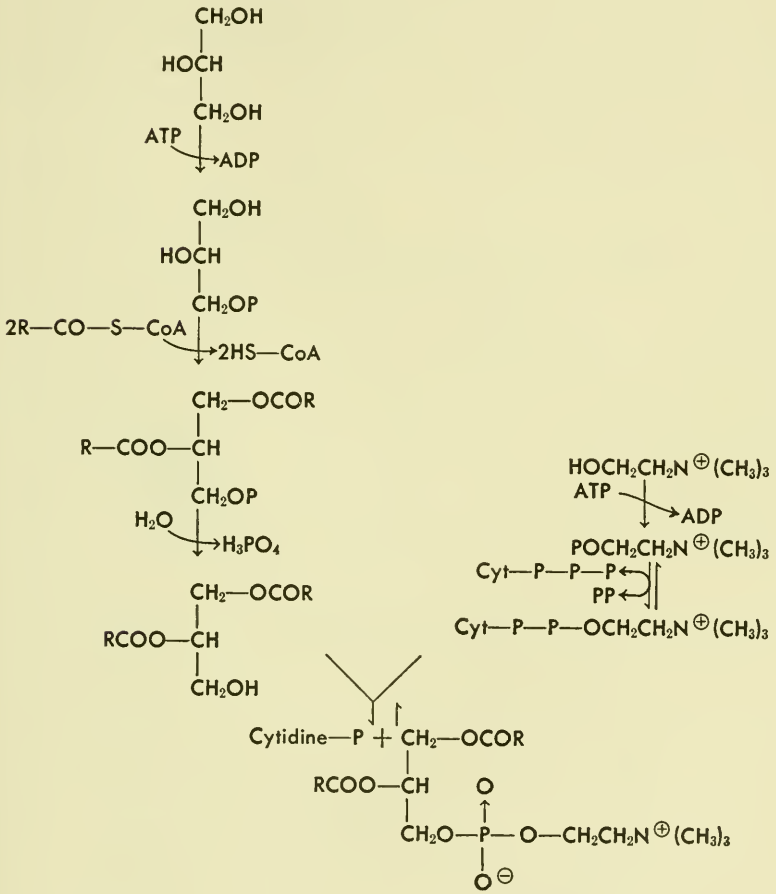
[32] Hanns Schmitz, Robert B. Hurlbert and Van R. Potter, *J. Biol. Chem.* 209 41 (1954).

[33] Eugene P. Kennedy and Samuel B. Weiss, *J. Am. Chem. Soc.* 77 250 (1955); *idem., J. Biol. Chem.* 222 193 (1956).

[34] Irving Lieberman, L. Berger and W. Theodore Gimenez, *Science* 124 81 (1956).

and seem to be nearly ubiquitous, although so far they have not been reported from other microorganisms. CDP-Glycerol and CDP-ribitol have been isolated only from lactobacilli,[35] but probably such substances will be found elsewhere.

CDP-Choline and CDP-ethanolamine are coenzymes essential to the biosynthesis of lecithin and phosphotidylethanolamine.[33] The stages in the biosynthesis of lecithin may be outlined:



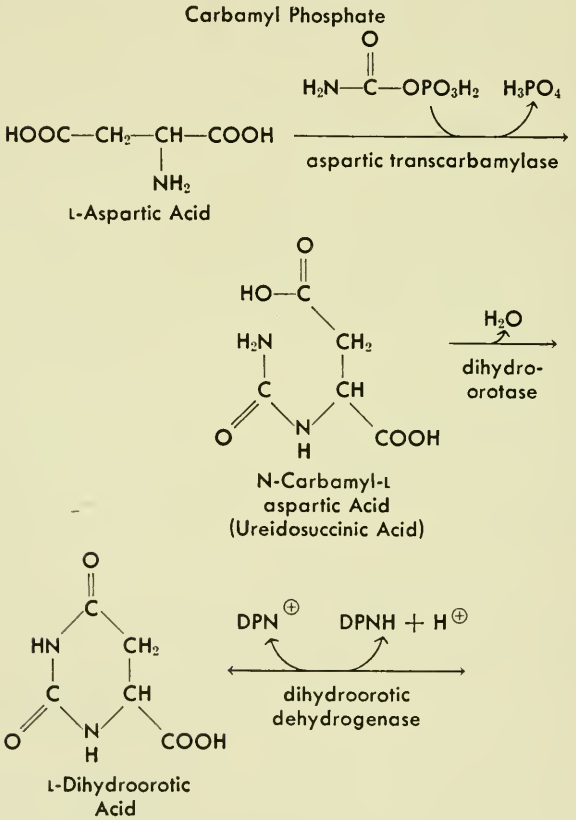

The cytidine monophosphate can then be rephosphoryl-

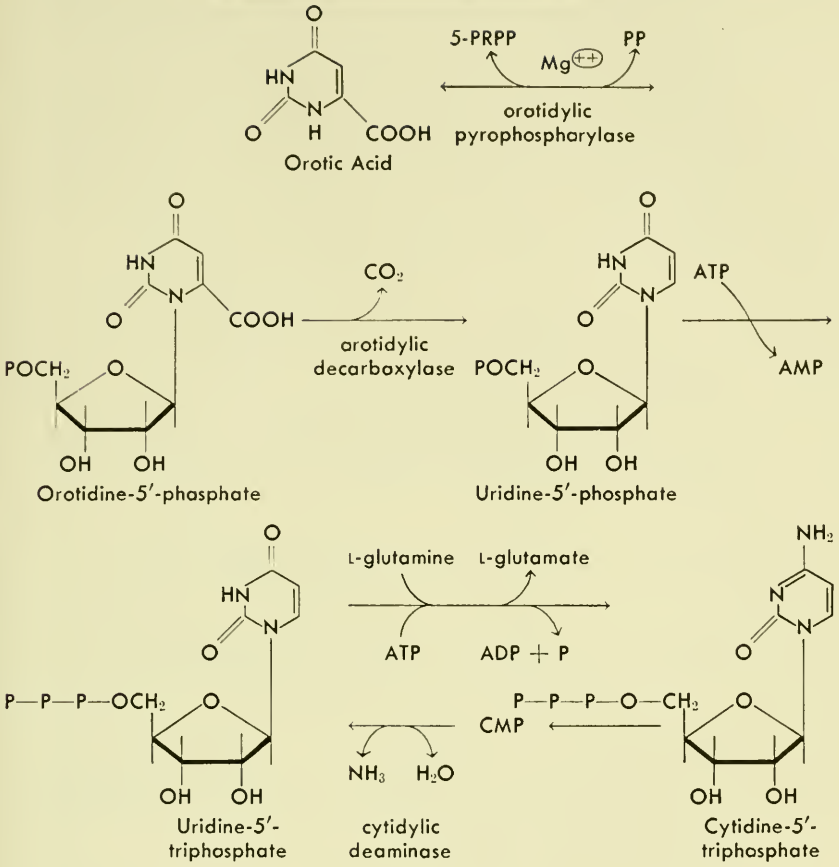
[35] J. Baddiley and A. P. Mathias, *J. Chem. Soc.*, 2723 (1954); J. Baddiley, J. G. Buchanan, B. Cares, A. P. Mathias and A. R. Sanderson, *Biochem. J.* 64 599 (1956).

ated to the triphosphate by ATP, making the process a catalytic one.

The function of the CDP-ribitol and CDP-glycerol in *Lactobacillus arabinosus* seems to be to donate these two reduced sugar phosphates in the formation of polymers. These ribitol-glycerol-phosphate polymers are components of the cell walls of bacteria. Several references are given in Appendix A to structural studies on these substances.

Biosynthesis of the pyrimidines seems to take a similar course in microorganisms and in higher animals. So many workers have contributed to our knowledge of this scheme that referencing cannot be included, but in outline what is now believed to be the important pathway is shown below:

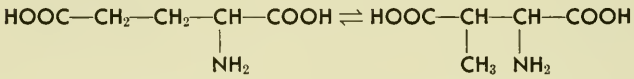





The biosynthesis of the deoxyribonucleotides may proceed similarly as far as uridine-5′-phosphate. Direct transfer into the deoxyribose series (*i.e.* removal of the 2′-hydroxyl from the ribose moiety) can then occur, or hydrolysis to the pyrimidine base and subsequent reaction with 2-deoxyribose-1-phosphate can take place.

There has been much interest in the origin of the 5-methyl group in thymine (5-methyluracil). The occurrence of 5-hydroxymethylcytosine in some species suggested donation (in that series) by a tetrahydrofolic acid derivative. Isotope experiments indicate that the α-C-

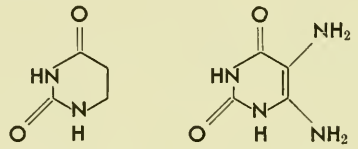
atom of glycine, the $\beta$-C-atom of serine and the C-atom of formate can all serve as donors at least indirectly.[36, 37] There is a vitamin $B_{12}$ requirement for the conversion of formate to the thymine methyl group in *Lactobacillus leichmannii*, and the pathway does not involve methionine or a hydroxymethyl group.[38] It has been suggested that since vitamin $B_{12}$ coenzymes are required to promote the equilibrium




Glutamic Acid $\beta$-Methylaspartic Acid

$\beta$-methylaspartic acid may replace aspartic acid as an intermediate in thymine biosynthesis.[39]

An alternate pathway of pyrimidine biosynthesis involving dihydrouracil, a member of the catabolic route, has been suggested.[40]




4,5-Dihydrouracil 4,5-Diaminouracil

The entire subject of the enzymic synthesis of pyrimidines has been reviewed.[41]

4,5-Diaminouracil has been detected as a metabolite of *Eremothecium ashbyii* and suggested as an intermediate in riboflavin biosynthesis.[42]

[36] David Elwyn and David B. Sprinson, *J. Biol. Chem.* 207 467 (1954); idem., *J. Am. Chem. Soc.* 72 3317 (1950).

[37] J. R. Totter, Elliott Volkin and C. E. Carter, *J. Am. Chem. Soc.* 73 1521 (1951); J. R. Totter and Audrey N. Best, *Arch. Biochem. and Biophys.* 54 318 (1955).

[38] James S. Dinning, Barbara K. Allen, Ruth Young and Paul L. Day, *J. Biol. Chem.* 233 674 (1958).

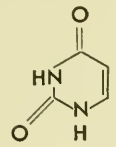
[39] H. D. Isenberg, E. Seifter and J. I. Berkman, *Biochim. et Biophys. Acta* 39 187 (1960).

[40] Lewis C. Mokrasch and Santiago Grisolia, *Biochim. et Biophys. Acta* 27 227 (1958).

[41] Peter Reichard, *Advances in Enzymology* 21 263–294 (1959).

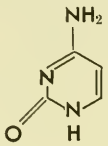
[42] T. W. Goodwin and D. H. Treble, *Biochem. J.* 67 10p (1957).

1006 **Uracil,** $C_4H_4O_2N_2$, colorless needles, m.p. ~335° (dec.).



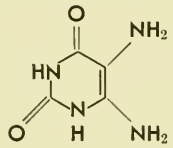
*Agaricus nebularis,* yeasts
Nils Löfgren, Björn Lüning and Harry Hedström, *Acta Chem. Scand.* 8 670 (1954).

1007 **Cytosine,** $C_4H_5ON_3$, large colorless crystals, m.p. ~320° (dec.).



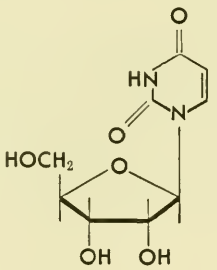
*Agaricus nebularis*
Nils Löfgren, Björn Lüning and Harry Hedström, *Acta Chem. Scand.* 8 670 (1954).

1008 **4,5-Diaminouracil,** $C_4H_6O_2N_4$, has been shown to be a metabolite of *Eremothecium ashbyii* by trapping with diacetyl.



T. W. Goodwin and D. H. Treble, *Biochem. J.* 67 10p (1957).

1009 **Uridine,** $C_9H_{12}O_6N_2$, colorless crystals, m.p. 165°, $[\alpha]_D^{20}$ +6.4° (10°) (in water).

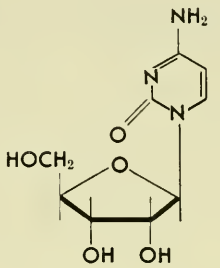


Yeast

Hellmut Bredereck, Annelise Martini and Friedrich Richter, *Ann.* 74 694 (1941).

Hubert S. Loring and James McT. Ploeser, *J. Biol. Chem.* 178 439 (1949).

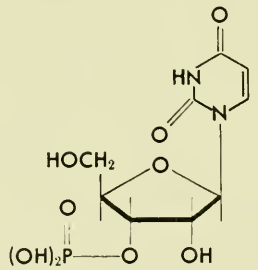
1010 **Cytidine,** $C_9H_{13}O_5N_3$, colorless needles, m.p. 225–230° (dec.), $[\alpha]_D^{20}$ +29.6° (in water).




Yeast

Hellmut Bredereck, Annelise Martini and Friedrich Richter, *Ann.* 74 694 (1941).

1011 **Uridine-3′-phosphate** (Uridylic Acid), $C_9H_{13}O_9N_2P$, colorless prisms, m.p. 200° (dec.), $[\alpha]_D$ +9.5 to 14.5° (in water).




Yeast

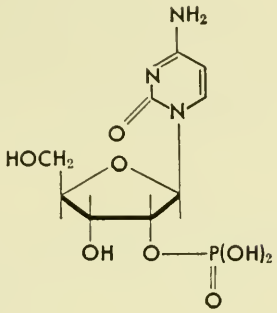
The 5′-di- and triphosphates also have been isolated from microorganisms.

Hellmut Bredereck and Gerd Richter, *Ber.* 71B 718 (1938).

W. E. Cohn and C. E. Carter, *J. Am. Chem. Soc.* 72 2606 (1950).

A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 2476 (1949).

1012 **Cytidine-2′-phosphate** (Cytidylic Acid) $C_9H_{14}O_8N_3P$, colorless crystals, m.p. 238–240° (dec.), $[\alpha]_D$ +20.7° (c 1.0 in water).

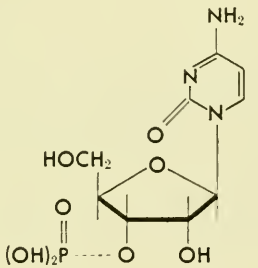



Yeast

Hubert S. Loring, Nydia G. Luthy, Henry W. Bortner and Luis W. Levy, *J. Am. Chem. Soc.* 72 2811 (1950).

Hubert S. Loring and Nydia G. Luthy, *ibid.* 73 4215 (1951).

1013 **Cytidine-3′-phosphate** (Cytidylic Acid), $C_9H_{14}O_8N_3P$, colorless tablets, m.p. 230–234° (dec.), $[\alpha]_D$ +49°. (c 0.5 in water).



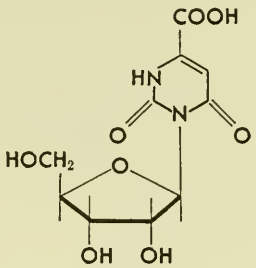

Yeast

The 5′-di- and triphosphates also have been isolated from microorganisms.

Hubert S. Loring, Nydia G. Luthy, Henry W. Bortner and Luis W. Levy, *J. Am. Chem. Soc.* 72 2811 (1950).

Hubert S. Loring and Nydia G. Luthy, *ibid.* 73 4215 (1951).

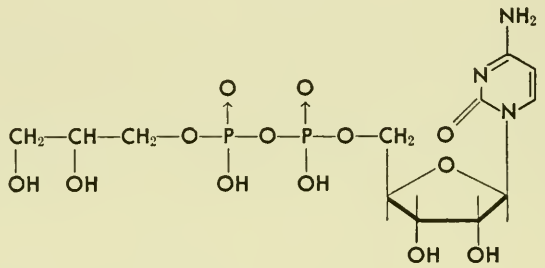
1014 **Orotidine** (Orotic Acid Riboside), $C_{10}H_{12}O_8N_2$, cyclohexylamine salt, m.p. 183°.




*Neurospora crassa* mutant

A. Michael Michelson, William Drell and Herschel K. Mitchell, *Proc. Nat. Acad. Sci. U. S.* 37 396 (1951).

1015 **Cytidine Diphosphate Glycerol**, $C_{12}H_{21}O_{12}N_3P_2$.

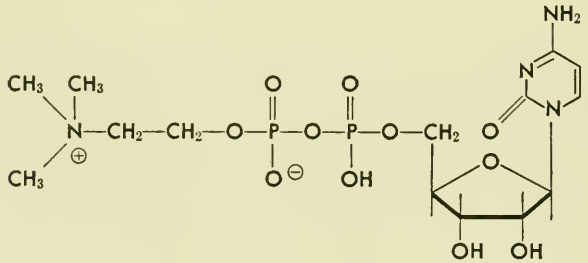



*Lactobacillus arabinosus*

J. Baddiley and R. P. Mathias, *J. Chem. Soc.*, 2723 (1954).

J. Baddiley, J. G. Buchanan, B. Cares, A. P. Mathias and A. R. Sanderson, *Biochem. J.* 64 599 (1956).

1016 **Cytidine-5′-diphosphatecholine** (CDP-Choline), $C_{13}H_{24}O_{11}N_4P_2$, amorphous white, hygroscopic powder.



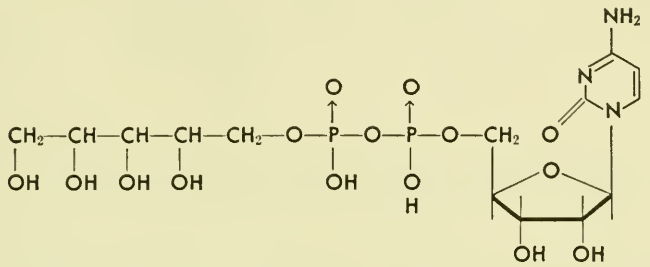
Yeast

This compound is a biogenetic precursor of the lecithins and cephalins.

Irving Lieberman, L. Berger and W. Theodore Gimenez, *Science* 124 81 (1956).

Eugene P. Kennedy and Samuel B. Weiss, *J. Biol. Chem.* 222 193 (1956).

1017 **Cytidine Diphosphate Ribitol,** $C_{14}H_{25}O_{15}N_3P_2$.

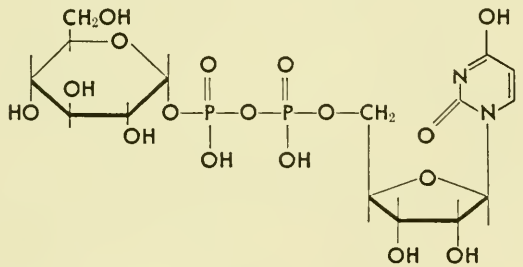


*Lactobacillus arabinosus*

J. Baddiley and A. P. Mathias, *J. Chem. Soc.*, 2723 (1954).

J. Baddiley, J. G. Buchanan, B. Cares, A. P. Mathias and A. R. Sanderson, *Biochem. J.* 64 599 (1956).

1018 **Uridinediphosphateglucose (UDPG),** $C_{15}H_{24}O_{17}N_2P_2$.



Yeast, molds

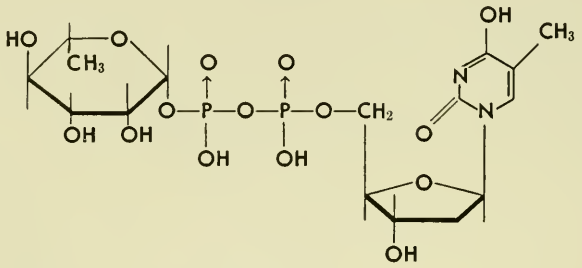
A uridinediphosphateacetylglucosamine also has been isolated from yeast.

R. Caputto, Luis F. Leloir, C. E. Cardini and A. C. Paladini, *J. Biol. Chem.* 184 333 (1950).

E. Cabib, Luis F. Leloir and C. E. Cardini, *ibid.* 203 1055 (1953).

J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.* 80 3756 (1958). (Synthesis)

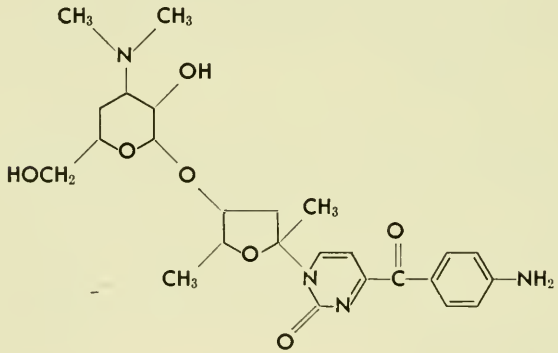
1019 **Thymidine Diphosphate Rhamnose,** $C_{16}H_{26}O_{14}N_2P_2$.



*Lactobacillus acidophilus*

Reiji Okazaki, *Biochem. and Biophys. Res. Comms.* 1 34 (1959).

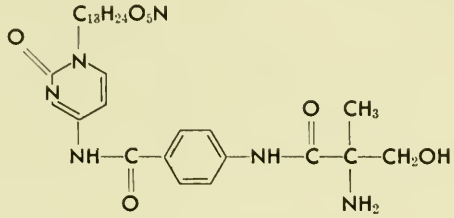
1020 **Plicacetin** (Amicetin B), $C_{25}H_{35}O_7N_5$, colorless needles, m.p. 182–184° from $H_2O$—$CH_3OH$, $[\alpha]_D^{26}$ +181° (c 2.7 in methanol).



*Streptomyces plicatus*

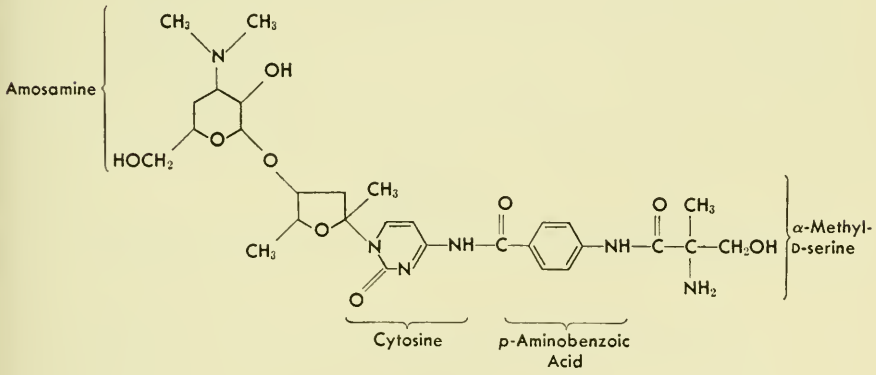
Theodore H. Haskell, Albert Ryder, Roger P. Frohardt, Salvatore A. Fusari, Zbigniew L. Jakubowski and Quentin R. Bartz, *J. Am. Chem. Soc.* 80 743 (1958).

1021 **Bamicetin**, $C_{28}H_{40}O_9N_6$, white microcrystals, m.p. 240° (dec.), $[\alpha]_D^{26}$ +123° (c 0.5 in 0.1 N hydrochloric acid).
Partial Structure:




*Streptomyces plicatus*
Theodore H. Haskell, Albert Ryder, Roger P. Frohardt, Salvatore A. Fusari, Zbigniew L. Jakubowski and Quentin R. Bartz, *J. Am. Chem. Soc.* 80 743 (1958).

1022 **Amicetin** (Sacromycin, Allomycin), $C_{29}H_{42}O_9N_6$, colorless needles, m.p. 165–169°, $[\alpha]_D^{24}$ +116.5° (c 0.5 in 0.1 N hydrochloric acid).



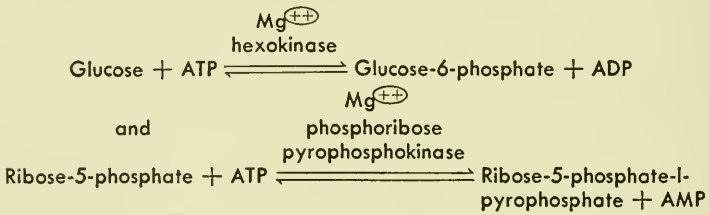

*Streptomyces vinaceus-drappus, S. fasciculatus, S. sindenensis, S. plicatus*
Edwin H. Flynn, J. W. Hinnan, E. L. Caron and D. O. Woolf, Jr., *J. Am. Chem. Soc.* 75 5867 (1953).

Calvin L. Stevens, Robert J. Gasser, Tapan K. Mukherjee and Theodore H. Haskell, *ibid.* 78 6212 (1956).

## *n.* PURINES

The nature of nucleic acids and the participation of purines in their structure were discussed in the preceding section. The process of oxidative phosphorylation also was mentioned although it is not yet entirely understood. In this process inorganic phosphate ions disappear during biological oxidation of substrates and become bound in adenosine triphosphate (ATP), the universal storage molecule for chemical energy within cells. Many examples of ATP as an energy donor were seen in earlier sections.

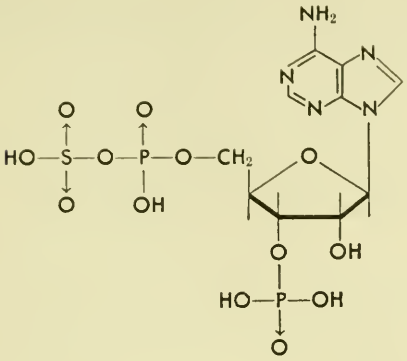
Adenosine polyphosphates have other functions, most of them concerned with the activation and transfer of various chemical moieties with formation of new chemical bonds. ATP, for example, can donate phosphate or pyrophosphate groups to form new phosphate esters. Two such known reactions are:




Adenosine-3′-phospho-5′-phosphosulfate has been established as activated sulfate,[1, 2] and it has been used in the formation of sulfate esters of a number of phenols and

[1] Robert S. Bandurski, Lloyd G. Wilson and Craig L. Squires, *J. Am. Chem. Soc.* 78 6408 (1956).

[2] P. W. Robbins and Fritz Lipmann, *ibid.* 78 2652, 6409 (1956).

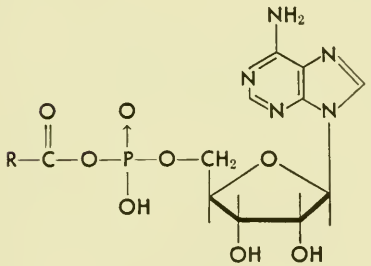


Adenosine-3′-phospho-5′-phosphosulfate

alcohols in the presence of sulfokinases. The generality of the sulfate transfer mechanism has been demonstrated in yeast, neurospora and liver.

The recognition of S-adenosylmethionine as the active complex in methyl group transfer from methionine (and perhaps in its biosynthesis) was noted in the section on amino acids.

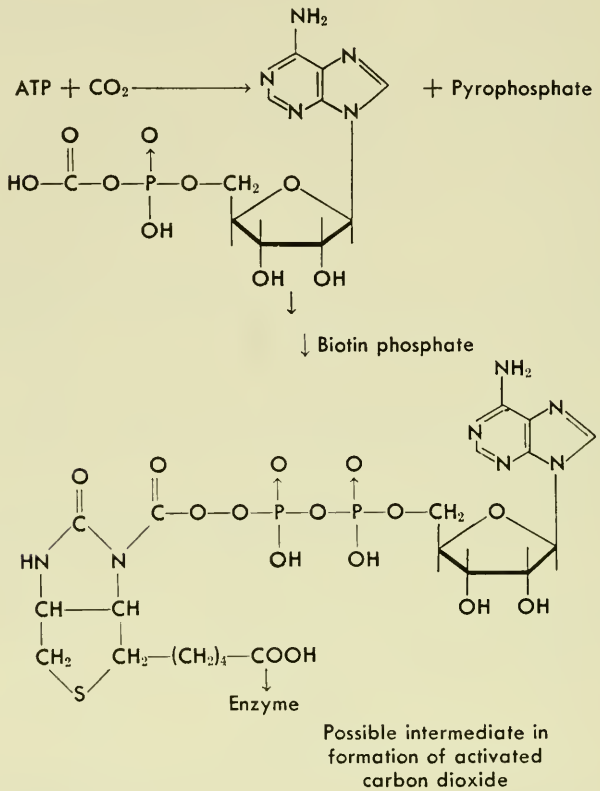
In the section on aliphatic acids an ATP requirement was noted in the formation of acyl coenzyme A. A number of acyl adenylates have been prepared or isolated from natural sources.[3, 4, 5] These can be converted enzymically into acyl coenzyme As. The general structure of these activated acids is:



Acid Anhydrides of Adenosine-5′-phosphate

[3] Paul Berg, *ibid.* 77 3163 (1955).
[4] Preston T. Talbert and F. M. Huennekens, *ibid.* 78 4671 (1956).
[5] C. H. Lee Peng, *Biochim. et Biophys. Acta* 22 42 (1956).

In the same section the mediation of ATP in the formation of active carbon dioxide was seen:




Possible intermediate in formation of activated carbon dioxide

Synthetic adenosyl-5′-phosphoryl carbonate has been prepared.[6]

The role of adenine nucleotide as the terminal or activating nucleotide of transfer RNA in protein synthesis was mentioned in the amino acid section.

[6] B. K. Bachhawat, J. F. Woessner and M. J. Coon, *Federation Proc.* 15 214 (1956).

Finally, the occurrence of the adenine nucleotide moiety in various other coenzymes (coenzyme A, flavine-adenine dinucleotide, DPN, etc.) should not be forgotten. The functions of these coenzymes are considered elsewhere.

Adenine polyphosphates, then, are so ubiquitous and so metabolically important that they nearly all have been encountered prior to this point in our discussions of microbial metabolism.

Guanosine polyphosphates, too, are widespread, and they seem to be able to duplicate some of the less specific functions of those of adenine. One reaction in which a guanine polyphosphate is known to participate is:[7]

$$\alpha\text{-Ketoglutaric Acid} + DPN^{\oplus} + CoA\text{-}SH \rightarrow Succinyl\text{-}S\text{-}CoA + DPNH + H^{\oplus} + CO_2$$

$$Succinyl\text{-}S\text{-}CoA + Guanosine\ Diphosphate + H_3PO_4 \rightleftharpoons Succinic\ Acid + CoA\text{-}SH + GTP$$

The enzyme catalyzing this reaction has been isolated only from tissues of higher animals, and there is evidence that in *Escherichia coli* at least the adenine nucleotide seems to be involved.[8]

Guanosine and inosine nucleotides also participate in the formation of phosphoenolpyruvate from oxaloacetate:[9]

$$Oxaloacetic\ Acid + GTP \rightleftharpoons Phosphoenolpyruvic\ Acid + GDP + CO_2$$

but again this has been shown only in animal tissues.

The general function of GTP as an energy source in the amination of inosinic acid during adenine biosynthesis will be seen later.

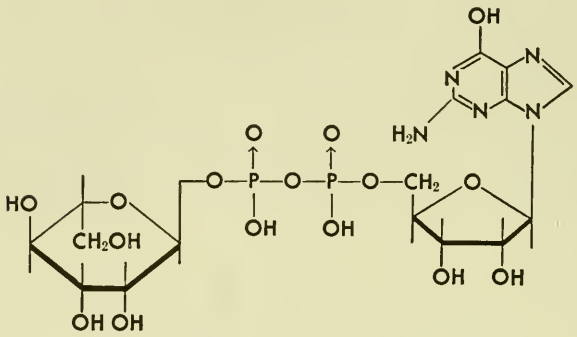
Guanosine diphosphate mannose has been isolated

[7] D. R. Sanadi, David M. Gibson, Padmasini Ayengar and Miriam Jacob, *J. Biol. Chem.* 218 505 (1956).

[8] Roberts A. Smith, Irma F. Frank and I. C. Gunsalus, *Federation Proc.* 16 251 (1957).

[9] M. F. Utter and K. Kurahashi, *J. Biol. Chem.* 207 821 (1954).

from yeast[10] and a penicillium mold[11] as well as from higher animals, and it probably occurs in plants. Guano-



Guanosine Diphosphate Mannose

sine diphosphate fucose has been isolated from *Aerobacter aerogenes*,[12] and this organism has an enzyme which converts GDP-mannose to GDP-fucose. This conversion requires TPNH and must involve several steps to accomplish the requisite epimerizations and reduction of the terminal carbon atom. The functions of these guanosine derivatives are unknown, but yeast elaborates a mannan, and fucose is a proven constituent of bacterial polysaccharides (as well as blood group specific polysaccharides in higher animals). This may then be a form in which sugars are modified and transported for incorporation into polysaccharides.

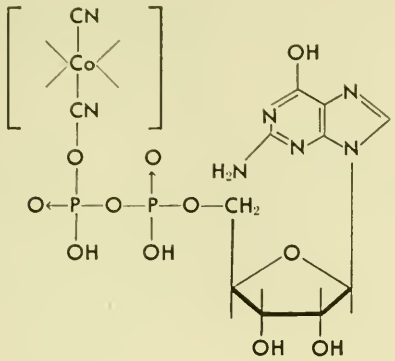
A substance of the vitamin $B_{12}$ group isolated from *Nocardia rugosa* has been identified as guanosine diphosphate factor B, *i.e.* a guanosine-5′-pyrophosphoric ester of factor B in which ribose is linked to N-9 of guanine (partial structure shown).[13]

[10] E. Cabib and Luis F. Leloir, *ibid.* 206 779 (1954).

[11] A. Ballio, C. Casinovi and G. Serlupi-Crescenzi, *Biochim. et Biophys. Acta* 20 414 (1956).

[12] V. Ginsburg and H. N. Kirkman, *J. Am. Chem. Soc.* 80 3481, 4426 (1958).

[13] R. Barchielli, G. Boretti, A. DiMarco, P. Julita, A. Migliacci, A. Minghetti and C. Spalla, *Biochem. J.* 74 382 (1960).



Guanosine Diphosphate Factor B
(Factor B = Vitamin $B_{12}$ minus the dimethylbenzimidazole nucleotide moiety)

This substance has been suggested as an intermediate near the end of the vitamin $B_{12}$ synthesis just prior to introduction of the dimethylbenzimidazole nucleotide.

There is evidence that labeled guanine is an isotopic precursor of riboflavin in *Eremothecium ashbyii.* Adenine also is a precursor of this vitamin. In each case the $C_8$ atom is lost. In the case of adenine, at least, the pyrimidine ring is incorporated intact into riboflavin[14] although pyrimidines such as uracil and thymine are ineffective precursors.[15]

Inosine is an intermediate in the biosynthesis of adenine and guanine, but beyond the phosphoenol pyruvate formation and some of the less specific reactions of the purine nucleotides (phosphate transfer, etc.) few functions have been discovered.

The purine nucleotides have been reviewed.[16, 17, 18, 19, 20, 21]

[14] Walter S. McNutt, Jr., *J. Biol. Chem.* 219 365 (1956).

[15] John A. MacLaren, *J. Bacteriol.* 63 233 (1952).

[16] Paul D. Boyer, Henry Lardy and Karl Myrbäck, "The Enzymes" Vol. II, Robert M. Bock, *Adenine nucleotides and properties of pyrophosphate compounds,* Academic Press, New York, 1960, pp. 3–27.

[17] *Ibid.,* Merton F. Utter, *Guanosine and inosine nucleotides,* pp. 75–87.

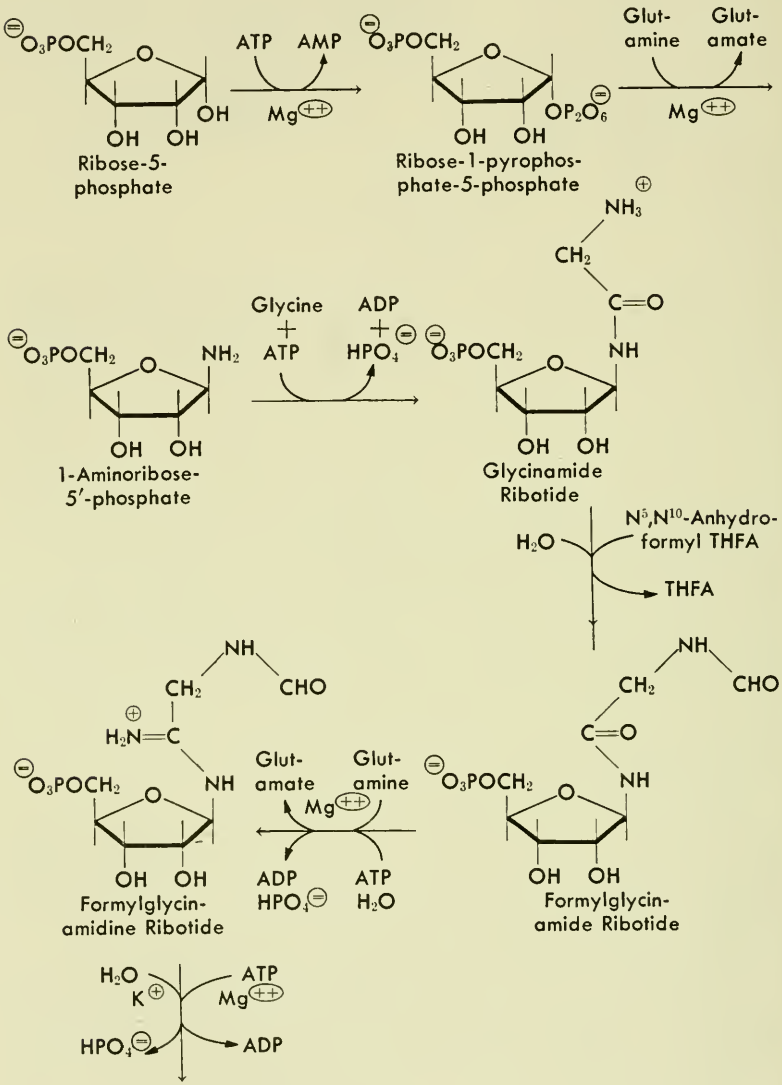
[18] Jack L. Strominger, *Physiol. Rev.* 40 55–111 (1960).

[19] J. Baddiley and J. G. Buchanan, *Quart. Rev.* 12 152–172 (1958).

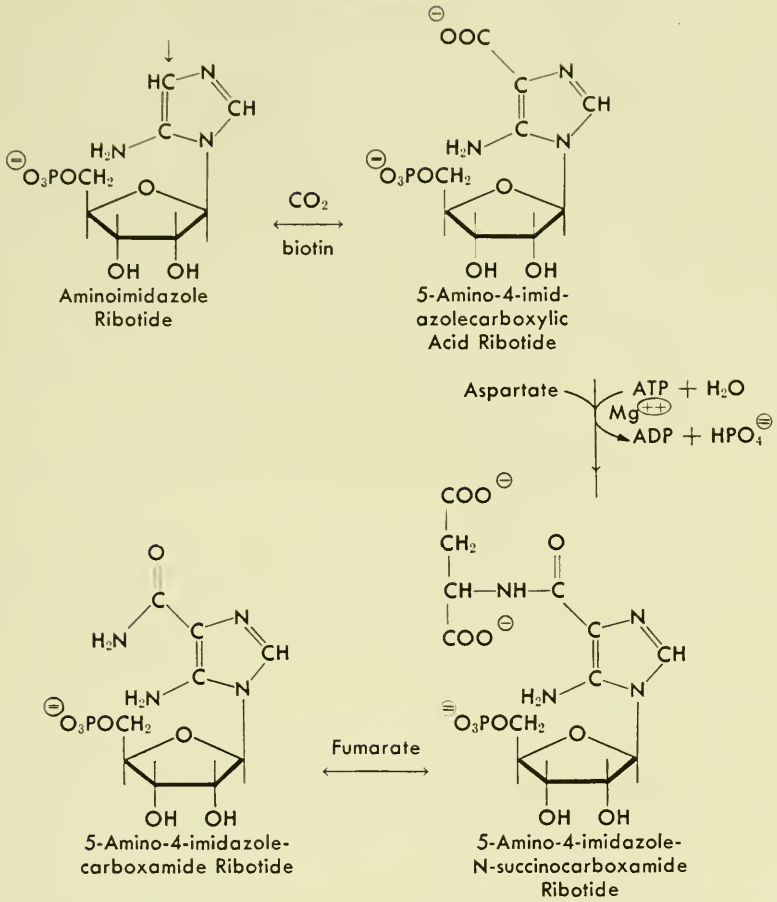
[20] Standish C. Hartman and John M. Buchanan, *Advances in Enzymology* 21 199–261 (1959). (Copyright 1959 by Interscience Publishers, Inc., New York)

[21] G. E. W. Wolstenholme and Cecilia M. O'Connor (Eds.), "CIBA Foundation Symposium on the Chemistry and Biology of Purines," J. M. Buchanan, J. G. Flaks, L. C. Hartman, B. Levenberg, L. N. Lukens and L. Warren, *The enzymatic synthesis of inosinic acid de novo,* Little, Brown and Co., Boston, 1957, pp. 233–255.

The general scheme of purine biosynthesis is understood now. It is outlined in the following equations:[22]




[22] Reproduced from reference 20.




Sulfanilamide and other sulfa drugs inhibit the growth of many bacteria by interfering with the incorporation of *p*-aminobenzoic acid into the folic acid coenzymes (*p*-aminosalicylic acid, etc., may do the same in mycobacteria), and *E. coli* cultures so inhibited accumulate isolable quantities of 5-amino-4-imidazolecarboxamide ribotide.[23]

[23] Joseph S. Gots and Edith G. Gollub, *Proc. Nat. Acad. Sci. U. S.* 43 826 (1957).

Azaserine, a glutamine antagonist, inhibits purine synthesis in some bacteria, and causes accumulation of formylglycinamide ribotide in *E. coli*.[24] Another antibiotic, 6-diazo-5-oxo-L-norleucine, also inhibits purine biosynthesis at this stage. Purine-requiring mutants of *E. coli* and *A. aerogenes* accumulate the following compounds or derivatives: aminoimidazole,[25] 5-aminoimidazolecarboxamide,[26] 5-amino-4-imidazole-N-succinocarboxamide[23] and xanthine.[27] Yeast grown on a biotin-deficient medium gives off aminoimidazole riboside and hypoxanthine.[28]

Cell-free extracts of *Neurospora crassa* are able to promote all the reactions shown in the biosynthetic scheme above. All these facts as well as other evidence indicate that this is the principal biosynthetic route to purines in bacteria and fungi, and probably is quite general.

Inosinic acid is an intermediate in the biosynthetic route to the other purines as shown in the formula sequence on page 533.

Extracts of *Aerobacter aerogenes* convert inosinic acid to xanthylic acid, and there is other evidence that the final stages of purine biosynthesis follow this route in many bacteria and fungi as well as in animal cells.

Other references can be found in some of the reviews of this subject.[20, 21]

There are indications that methylated purines may be minor constituents of yeast and bacterial nucleic acids. Traces of 6-methylaminopurine, 6-hydroxy-2-methylaminopurine and 1-methylguanine were detected in yeast RNA.[29] Small amounts of 6-methylaminopurine, 6,6-di-

[24] A. J. Tomisek, H. J. Kelley and H. E. Skipper, Abstr., 128th Meeting, Am. Chem. Soc., 5C, Minneapolis, Sept., 1955.

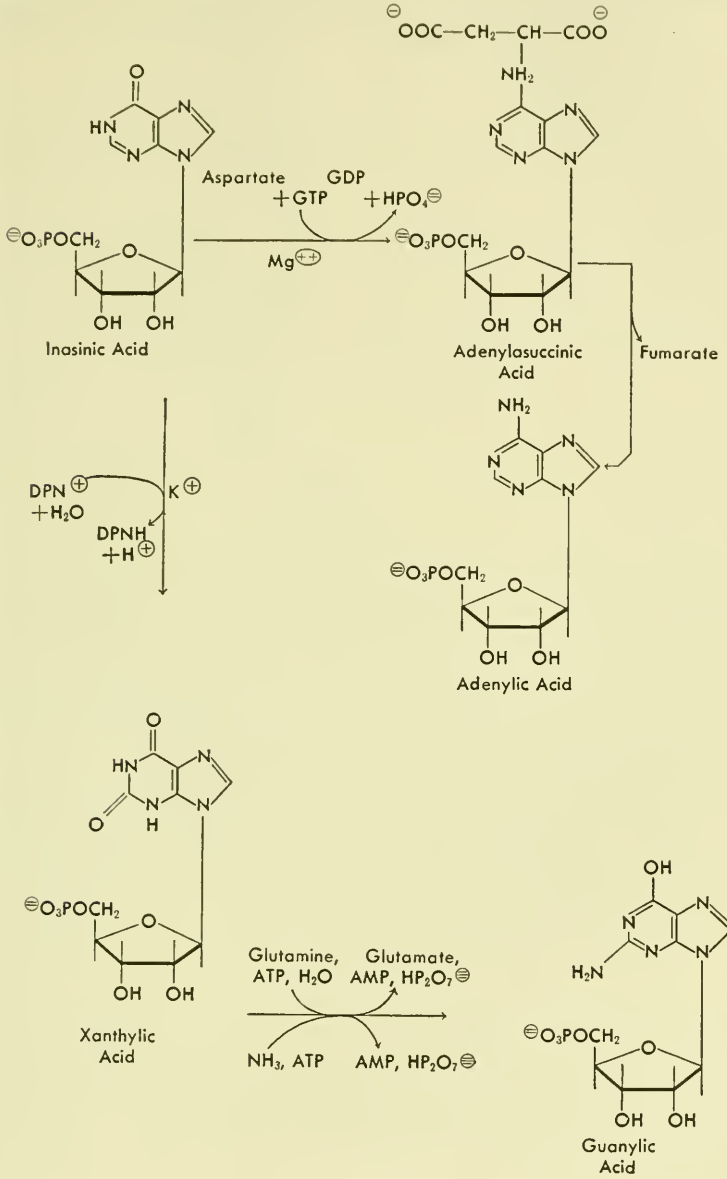
[25] Samuel H. Love and Joseph S. Gots, *J. Biol. Chem.* 212 647 (1955).

[26] Joseph S. Gots, *ibid.* 228 57 (1957).

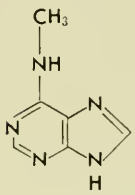
[27] Boris Magasanik, H. S. Moyed and Lois B. Gehring, *ibid.* 226 339 (1957).

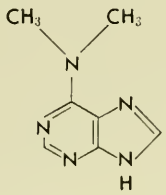
[28] D. P. Lones, C. Rainbow and J. D. Woodward, *J. Gen. Microbiol.* 19 146 (1958).

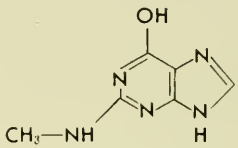
[29] Max Adler, Bernard Weissmann and Alexander B. Gutman, *J. Biol. Chem.* 230 717 (1958).

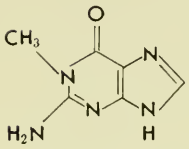

Aspartate
GDP
+GTP
+HPO4
Mg++
Inasinic Acid
Adenylasuccinic Acid
Fumarate
DPN
+H2O
DPNH
+H
K
Adenylic Acid
Xanthylic Acid
Glutamine,
ATP, H2O
Glutamate,
AMP, HP2O7
NH3, ATP
AMP, HP2O7
Guanylic Acid

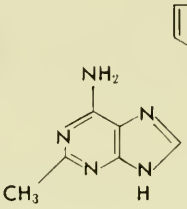
methylaminopurine and 2-methyladenine have been found in bacterial RNA.[30]

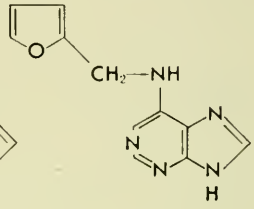

6-Methylamino-purine


6-Dimethylamino-purine


2-Methylamino-6-hydroxy purine


1-Methylguanine


2-Methyladenine


Kinetin

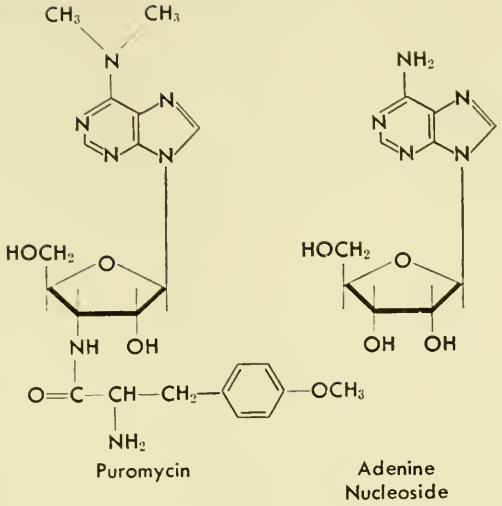
Kinetin is a substance isolated from yeast which stimulates cell division in plant tissues. Work on kinetin and related compounds has been reviewed.[31]

Several antibiotics contain the purine nucleus. Some of these have excited interest as purine analogues for tumor inhibition, but they are all toxic. Puromycin is an inhibitor of protein synthesis.[32] The interference has been shown to occur at the last stage—that is the exchange of the activated amino acid between transfer–RNA and the growing protein chain.

[30] J. W. Littlefield and D. B. Dunn, *Biochem. J.* 68 8P (1958); *idem., Nature* 181 254 (1958).

[31] E. R. Squibb Lectures on Chemistry of Microbial Products, "Topics in Microbial Chemistry," John Wiley and Sons, New York, 1958, F. M. Strong, *Kinetin and kinins*, pp. 98–158.

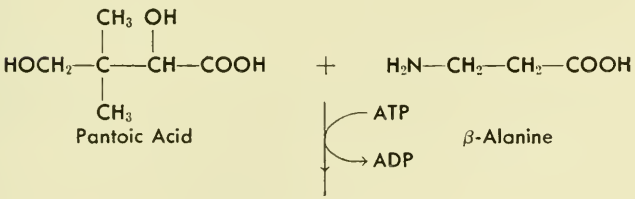
[32] Michael Yarmolinsky and Gabriel de la Haba, *Chem. and Eng. News* April 25, 1960.

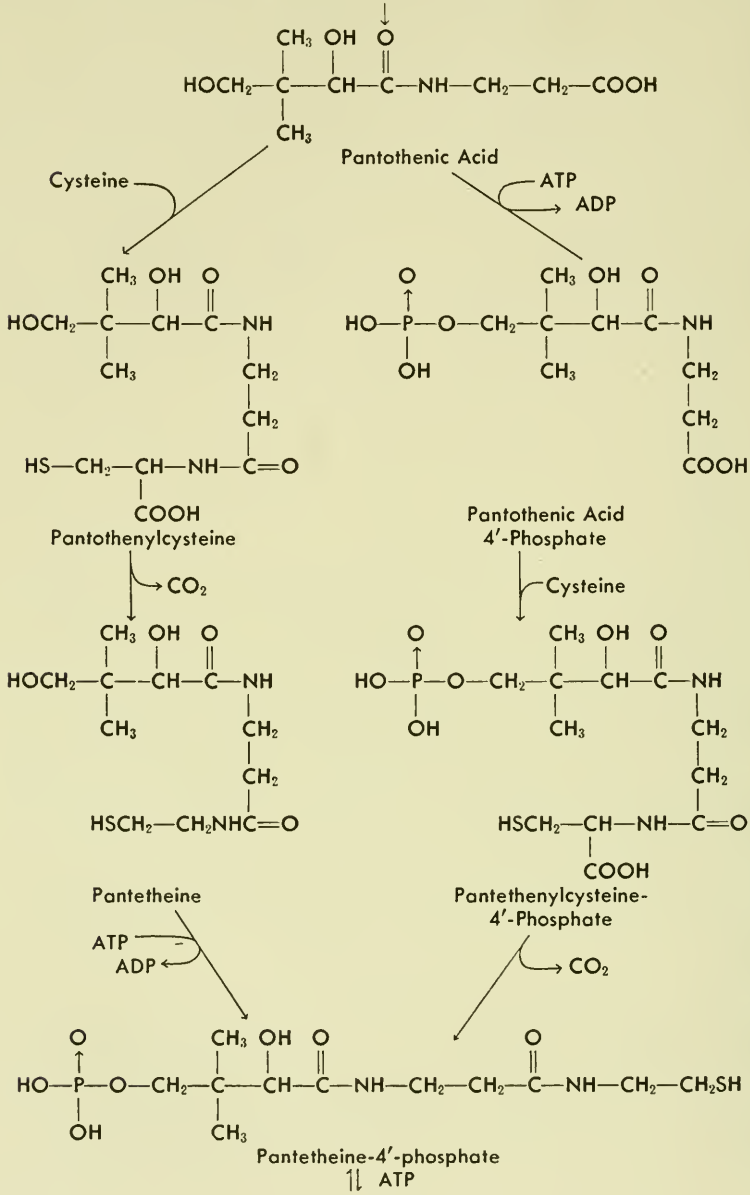


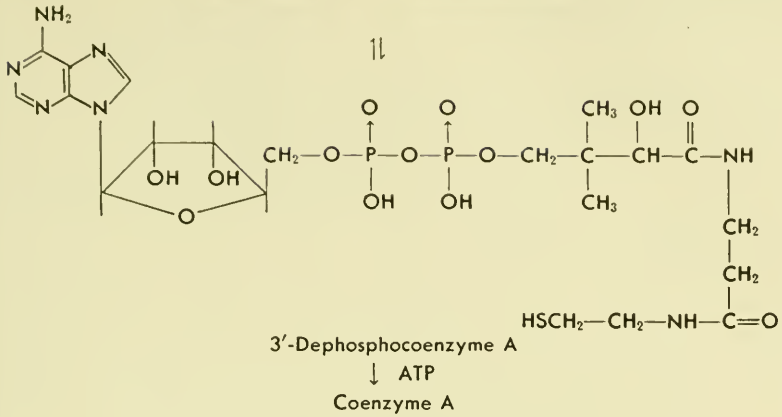

Substitution of other amino acids for the *p*-methoxyphenylalanine moiety gives analogues which still inhibit protein synthesis, although the free nucleoside moiety is a less effective inhibitor. The similarity in structure suggests competition with adenine nucleoside.

Functions of coenzyme A have been discussed throughout the appropriate sections. The biosyntheses of the various moieties of the molecule also have been considered with the possible exception of β-aminoethanethiol, which is derived from cysteine.

The biosynthetic union of these moieties, originally studied in animal tissues, follows the probable course:




Cysteine
Pantothenic Acid
ATP
ADP
Pantothenylcysteine
Pantothenic Acid 4′-Phosphate
Cysteine
Pantetheine
ATP
ADP
Pantethenylcysteine-4′-Phosphate
Pantetheine-4′-phosphate
ATP




Most of these intermediates have been identified in microorganisms, *e.g. Streptobacterium plantarum.*[33] Pantothenic acid is required by some microorganisms, but probably not by man, perhaps because of the excess produced by *E. coli* and other intestinal microbes.

A number of higher fungi and molds have been examined thoroughly for nucleotide content. Some of the organisms which have been studied are: *Penicillium chrysogenum,*[34] *Aspergillus oryzae,*[35] *Polyporus squamosus,*[36] *Amanita muscaria,*[36] *Lycoperdon pratense,*[36] *Hypholoma capnoides,*[36] *Armillaria mellea,*[36] *Pholiota squarrosa,*[36] *Lactarius vellereus,*[36] *Lactarius turpis,*[36] *Torulopsis utilis,*[37] *Micrococcus lysodeikticus,*[38] *Coprinus comatis,*[39] and *Polyporus sulfureus.*[40]

[33] Theodor Wieland, Walter Maul and Ernst Friedrich Möller, *Biochem. Z.* 327 85 (1955).

[34] A. Ballio, C. Casinovi and G. Serlupi-Crescenzi, *Biochim. et Biophys. Acta* 20 414 (1956); Alessandro Ballio and Giovanni Serlupi-Crescenzi, *Nature* 179 154 (1957).

[35] Kazuo Okunuki, Kozo Iwasa, Fumio Imamoto and Tadoyoshi Higashiyama, *J. Biochem.* (Tokyo) 45 795 (1958).

[36] Rolf Bergkvist, *Acta Chem. Scand.* 12 1549, 1554 (1958).

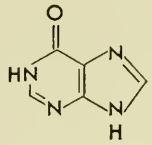
[37] D. Gilbert and E. Yemm, *Nature* 182 1745 (1958).

[38] J. V. Scaletti, *Dissertation Abstr.* 17 1191 (1957).

[39] Paul Heinz List, *Arch. Pharm.* 291 502 (1958).

[40] *Idem., Planta Med.* 6 424 (1958).

1023 **Hypoxanthine**, $C_5H_4ON_4$.



*Amanita muscaria, Boletus edulis, Agaricus nebularis, Polyporus sulfureus*

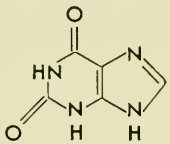
E. Buschmann, *Pharm. Post* 45 453 (1912). (*Chem. Abstr.* 6 2485)

E. Winterstein, C. Reuter and R. Korolev, *J. Chem. Soc.* 104 I 433 (1913).

Nils Löfgren, Björn Lüning and Harry Hedström, *Acta Chem. Scand.* 8 670 (1954).

Paul Heinz List, *Planta Med.* 6 424 (1958).

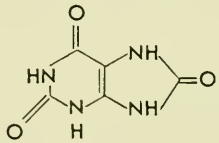
1024 **Xanthine**, $C_5H_4O_2N_4$, colorless crystals, m.p. 220° (dec.).



*Amanita muscaria*

E. Buschmann, *Pharm. Post* 45 453 (1912). (*Chem. Abstr.* 6 2485)

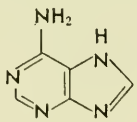
1025 **Uric Acid**, $C_5H_4O_3N_4$, colorless crystals, m.p. >400° (dec.).



*Aspergillus oryzae*

Miazuko Sumi, *Biochem. Z.* 195 161 (1928).

1026 **Adenine**, $C_5H_5N_5$ (Trihydrate), colorless needles, m.p. 360–365° (dec.) (subl. from 220°) (Picrate), dec. 280°.



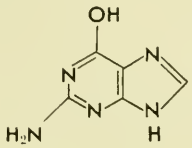
*Coprinus comatis* Gray, *Boletus edulis, Polyporus sulfureus*

Paul Heinz List, *Arch. Pharm.* 291 502 (1958).

E. Winterstein and C. Reuter, *Centr. Bakt. Parasitenk. II Abt.* 34 566 (1912). (*Chem. Abstr.* 6 3279)

Paul Heinz List, *Planta Med.* 6 424 (1958).

1027 **Guanine**, $C_5H_5ON_5$, (Picrate) dec. from 190°.

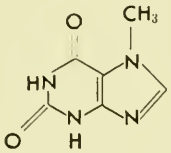



*Coprinus comatis* Gray, *Boletus edulis*

Paul Heinz List, *Arch. Pharm.* 291 502 (1958).

E. Winterstein, C. Reuter and R. Korolev, *J. Chem. Soc.* 104 I 433 (1913).

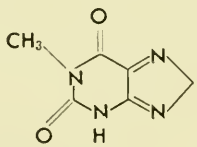
1028 **Heteroxanthine**, $C_6H_6O_2N_4$, colorless crystals, m.p. ~380° (dec.).




Yeast

P. W. Wiardi and B. C. P. Jansen, *Rec. trav. chim.* 53 205 (1934).

1029 **Toxoflavin**, $C_6H_6O_2N_4$, yellow crystals, m.p. 171°.

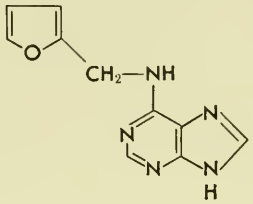



*Pseudomonas cocovenenans*

A. G. van Veen and W. K. Mertens, *Proc. Acad. Sci. Amsterdam* 36 666 (1933). (Isolation) (*Chem. Abstr.* 27 5771)

A. G. van Veen and J. K. Baars, *Rec. trav. chim.* 57 248 (1938). (Structure)

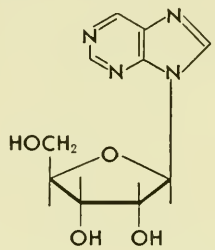
1030 **Kinetin** (6-Furfurylaminopurine), $C_{10}H_9ON_5$, colorless prisms, m.p. 265° (sealed tube to prevent sublimation).



Yeast extracts

E. R. Squibb Lectures on Chemistry of Microbial Products, "Topics in Microbial Chemistry," John Wiley and Sons, New York, 1958, F. M. Strong, *Kinetin and kinins,* pp. 98–157.

1031 **Nebularine** (9-(β-D-Ribofuranosyl) purine), $C_{10}H_{12}O_4N_4$, colorless prisms, m.p. 181°, $[\alpha]_D^{25}$ −48.6° (c 1 in water).



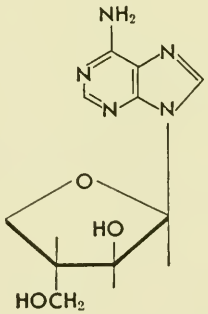
*Agaricus* (*Clitocybe*) *nebularis* Batsch.

Lars Ehrenburg, Harry Hedström, Nils Löfgren and Bertil Takman, *Svensk Kem. Tidskr.* 58 269 (1946).

Nils Löfgren, Björn Lüning and Harry Hedström, *Acta Chem. Scand.* 8 670 (1954).

David I. Magrath and George Bosworth Brown, *J. Am. Chem. Soc.* 79 3252 (1957). (Synthesis)

1032 **Cordycepin,** $C_{10}H_{13}O_3N_5$, colorless needles, m.p. 225°, $[\alpha]_D^{20}$ −47° (in water).

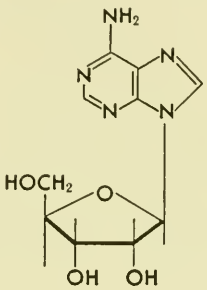


*Cordyceps militaris* (Linn.) Link

K. G. Cunningham, S. A. Hutchinson, William Manson and F. S. Spring, *J. Chem. Soc.*, 2299 (1951).

H. R. Bentley, K. G. Cunningham and F. S. Spring, *ibid.*, 2301 (1951). (Structure)

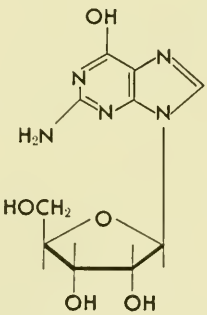
1033 **Adenosine,** $C_{10}H_{13}O_4N_5$, needles, m.p. 229°, $[\alpha]_D^{20}$ −60 to −63° (in water).




*Agaricus nebularis*

Nils Löfgren, Björn Lüning and Harry Hedström, *Acta Chem. Scand.* 8 670 (1954).

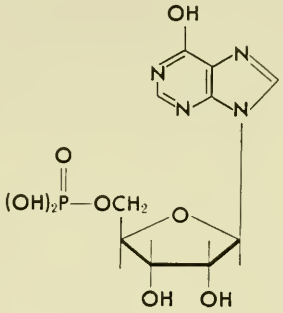
1034 **Guanosine,** $C_{10}H_{13}O_5N_5$, colorless crystals, m.p. 237° (dec.), $[\alpha]_D^{20}$ −60° (in 0.1 N sodium hydroxide).




Yeast

Hellmut Bredereck, Annelise Martini and Friedrich Richter, *Ber.* 74B 694 (1941).

1035 **Inosine-5′-phosphate** (Inosinic Acid), $C_{10}H_{13}O_8N_4P$, a syrup.



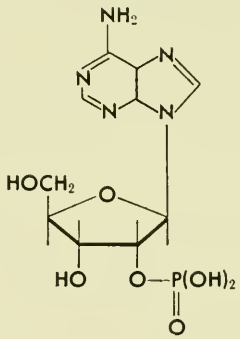
Yeast, *Penicillium chrysogenum*

The 5′-diphosphate also has been isolated.

E. Cabib, Luis F. Leloir and C. E. Cardini, *J. Biol. Chem.* 203 1055 (1953).

A. Ballio, C. Casinovi and G. Serlupi-Crescenzi, *Biochim. et Biophys. Acta* 20 414 (1956).

1036 **Adenosine-2′-phosphate** (Adenylic Acid a), $C_{10}H_{14}O_7N_5P$, colorless crystals, m.p. 187° (dec.).



Yeast

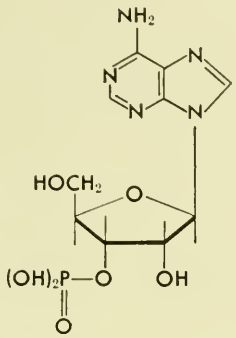
D. M. Brown, G. D. Fasman, D. I. Magrath, A. R. Todd, W. Cochran and M. M. Woolfson, *Nature* 172 1184 (1953).

C. E. Carter, *J. Am. Chem. Soc.* 72 1466 (1950).

Joseph X. Khym, David G. Doherty, Elliot Volkin and Waldo E. Cohn, *ibid.* 75 1262 (1953).

D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 44 (1952).

1037 **Adenosine-3′-phosphate** (3-Adenylic Acid, Yeast Adenylic Acid), $C_{10}H_{14}O_7N_5P$, colorless crystals, m.p. 191–195° (dec.), $[\alpha]_D^{20}$ −66° (c 2 in 5% sodium hydroxide).

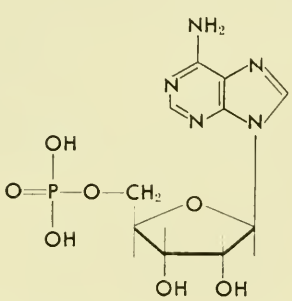


Yeast, *Penicillium chrysogenum*

H. Steudel and E. Peiser, Z. *physiol. Chem.* 127 262 (1923).

D. A. Kita and W. H. Peterson, *J. Biol. Chem.* 203 861 (1953).

1038 **Adenosine-5′-phosphate** (Muscle Adenylic Acid), $C_{10}H_{14}O_7N_5P$, colorless crystals, m.p. 178°, $[\alpha]_D^{25}$ −50° (in formamide).



Yeasts, *Lactobacillus arabinosus, Penicillium chrysogenum*

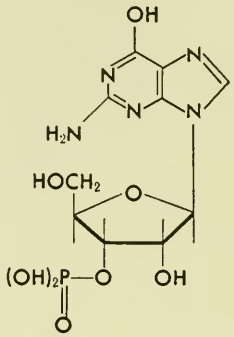
The 5′-diphosphate (ADP) also has been isolated from microorganisms.

E. Cabib, Luis F. Leloir and C. E. Cardini, *J. Biol. Chem.* 203 1055 (1953).

J. Baddiley and A. C. Mathias, *J. Chem. Soc.*, 2723 (1954).

A. Ballio, C. Casinovi and G. Serlupi-Crescenzi, *Biochim. et Biophys. Acta* 20 414 (1956).

1039 **Guanosine-3′-phosphate** (Guanylic Acid), $C_{10}H_{14}O_8N_5P$, colorless crystals, $[\alpha]_D$ −7.5° to −13.5° (in water).

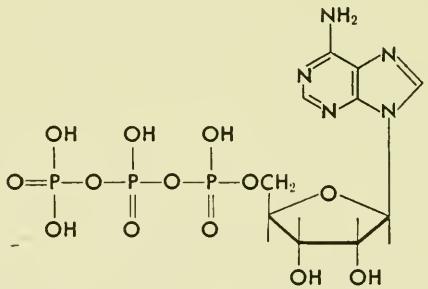


Yeast

The 5′-di- and triphosphates also have been isolated from microorganisms.

Walter Jones and M. E. Perkins, *J. Biol. Chem.* 62 557 (1925).

1040 **Adenosine-5′-triphosphate** (ATP), $C_{10}H_{16}O_{13}N_5P_3$.



Yeasts, molds, bacteria, etc. (widely distributed)

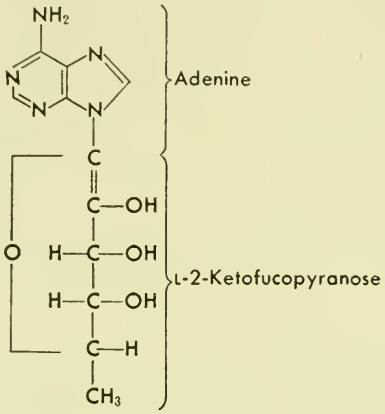
Th. Wagner-Jauregg, *Z. physiol. Chem.* 238 129 (1936). (Isolation)

G. A. LePage and W. W. Umbreit, *J. Biol. Chem.* 148 255 (1943).

D. A. Kita and W. H. Peterson, *ibid.* 203 861 (1953).

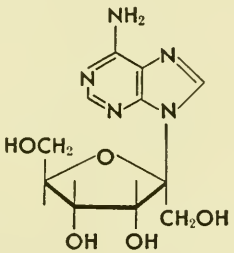
A. Endō, *Ann. Report Takamine Lab.* 11 45 (1959).

1041 **Angustmycin A**, $C_{11}H_{13}O_4N_5$, colorless needles, m.p. (anhydr.) 169.5° (dec.), $[\alpha]_D^{18}$ +48.3°.
Probable structure:




*Streptomyces hygroscopicus*
Hsü Yüntsen and Hiroshi Yonehara, *Bull. Agr. Chem. Soc.* (Japan) 21 261 (1957).
Hsü Yüntsen, Kazuhiko Ohkuma, Yoshio Ishii and Hiroshi Yonehara, *J. Antibiotics* (Japan) 9A 195 (1956). (Isolation and characterization)
Hsü Yüntsen, *ibid.* 11A 79 (1958). (Structure)

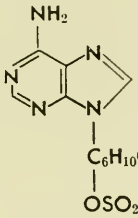
1042 **Angustmycin C** (Psicofuranine), $C_{11}H_{15}O_5N_5$, colorless crystals, m.p. 202–204°, $[\alpha]_D^{19}$ −71.1° (c 1.8 in pyridine).




*Streptomyces hygroscopicus* var. *angustmyceticus*
Hsü Yüntsen, *J. Antibiotics* (Japan) 11A 244 (1958). (Structure)

1043 **Nucleocidin,** $C_{11}H_{16}O_8N_6S$, colorless crystals, no definite m.p., $[\alpha]_D^{24.5}$ −33.3° (c 1.05 in 1:1 ethanol, 0.1 N hydrochloric acid).

Partial structure:



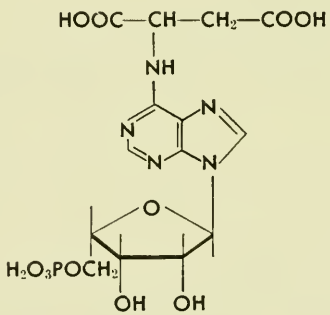

$C_6H_{10}O_5$ is an unusual reducing sugar.

*Streptomyces calvus*

S. O. Thomas, V. L. Singleton, J. A. Lowery, R. W. Sharpe, L. M. Pruess, J. N. Porter, J. H. Mowat and N. Bohonos, "Antibiotics Annual 1956–1957," Medical Encyclopedia Inc., New York, p. 716. (Isolation)

C. W. Waller, J. B. Patrick, W. Fulmor and W. E. Meyer, *J. Am. Chem. Soc.* 79 1011 (1957). (Structure)

1044 **Adenylosuccinic Acid,** $C_{14}H_{18}O_{11}N_5P$, no properties listed.

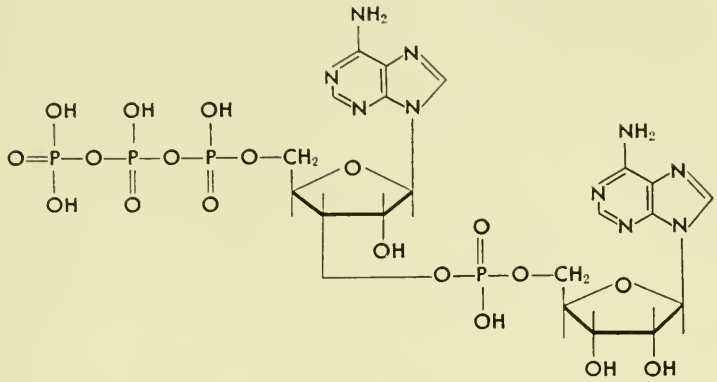



*Penicillium chrysogenum* (mycelium)

About 16 known derivatives of adenine, guanine, cytidine, uracil, etc., also were detected in this study.

Alessandro Ballio and Giovanni Serlupi-Crescenzi, *Nature* 179 154 (1957).

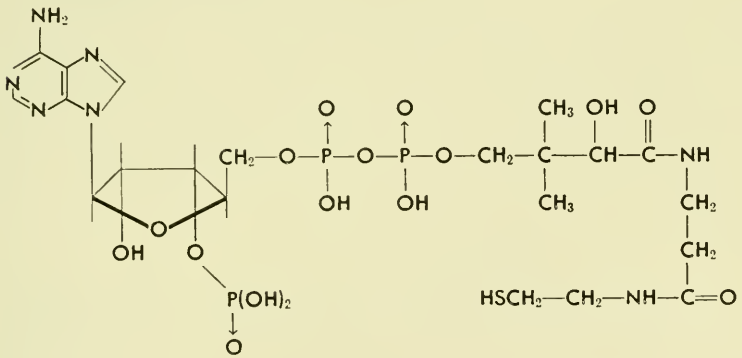
1045 **Diadenosinetetraphosphate**, $C_{20}H_{28}O_{19}N_{10}P$, $[\alpha]_{5446}^{20}$ −39.2° (in N sulfuric acid).



Yeast

W. Kiessling and O. Meyerhof, *Naturwissenschaften* 26 13 (1938).

1046 **Coenzyme A**, $C_{21}H_{36}O_{16}N_7SP_3$, white amorphous powder.

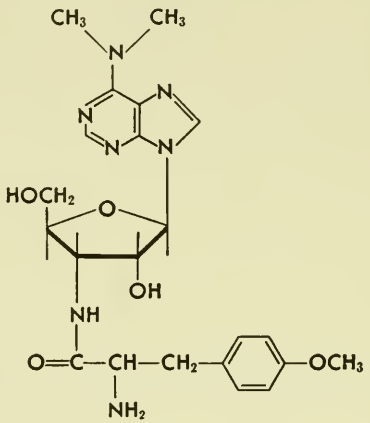


Occurs widely in microorganisms and higher animals. Yeast and certain streptomycetes were early sources.

F. M. Strong, "Squibb Lectures on the Chemistry of Microbial Products," *Coenzyme A and related compounds*, John Wiley and Sons, Inc., New York, 1956, pp. 44–98. (This review lists 117 earlier references.)

J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.* 81 1265 (1959). (Synthesis)

1047 **Puromycin,** $C_{22}H_{29}O_5N_7$, white crystals, m.p. 175.5–177° (uncorr.), $[\alpha]_D^{25}$ −11° (c 1 in ethanol).




*Streptomyces albo-niger*

J. W. Porter, R. I. Hewitt, C. W. Hesseltine, G. Krupka, J. A. Lowery, W. S. Wallace, N. Bohonos and J. H. Williams, *Antibiotics and Chemotherapy* 2 409 (1952).

Coy W. Waller, Peter W. Fryth, Brian L. Hutchings and James H. Williams, *J. Am. Chem. Soc.* 75 2025 (1953). (Structure)

B. R. Baker, Robert E. Schaub, Joseph P. Joseph and James H. Williams, *ibid.* 77 12 (1955). (Synthesis)

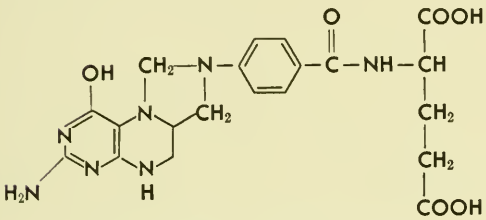
## *O.* PTERIDINES AND FLAVINES

Pteridines (pterins), originally discovered in insects, occur widely, and several have been isolated from microbial sources. The most important of these from the metabolic standpoint is folic acid. This substance, or group of related substances, is a vitamin for most mammals and plants and for some microorganisms unable to produce it. Pure folic acid first was isolated from liver and from yeast. The triglutamyl form was isolated from a corynebacterium, and the heptaglutamyl derivative, first isolated from yeast, since has been found in a variety of microorganisms. The reason for the polypeptide chains is not clear. These forms are as effective as folic acid in

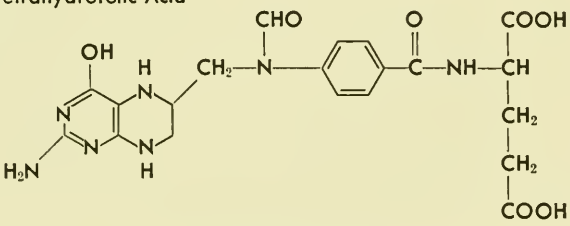
higher animals, but are not so active as folic acid for the bacteria ordinarily used in bioassays.

The functions of folic acid as a B-vitamin have been investigated extensively and are now largely understood. Some of these have been encountered earlier in our discussions, but the role of folic acid derivatives in one-carbon metabolism has not been considered as such.

In its coenzyme form folic acid is attached to a protein apoenzyme, probably at the glutamic acid moiety, and the pteridine ring is reduced. One of these pteroproteins has been crystallized.[1] The "active formate" form of the coenzyme has been shown to be $N^{10}$-formyltetrahydrofolic acid,[2, 3, 4] and the "active formaldehyde" form probably is $N^5$,$N^{10}$-methylenetetrahydrofolic acid.[5, 6, 7, 8]



"Active Formaldehyde" $N^5$, $N^{10}$-Methylenetetrahydrofolic Acid



"Active Formate" $N^{10}$-Formyltetrahydrofolic Acid

[1] Jesse C. Rabinowitz and W. E. Pricer, Jr., *Federation Proc.* 17 293 (1958).

[2] H. M. Rauen and Lothar Jaenicke, Z. *physiol. Chem.* 293 46 (1953).

[3] Lothar Jaenicke, *Biochim. et Biophys. Acta* 17 588 (1955).

[4] H. M. Rauen, *Biochem. Z.* 328 562 (1957).

[5] R. L. Blakley, *Biochem. J.* 58 448 (1954).

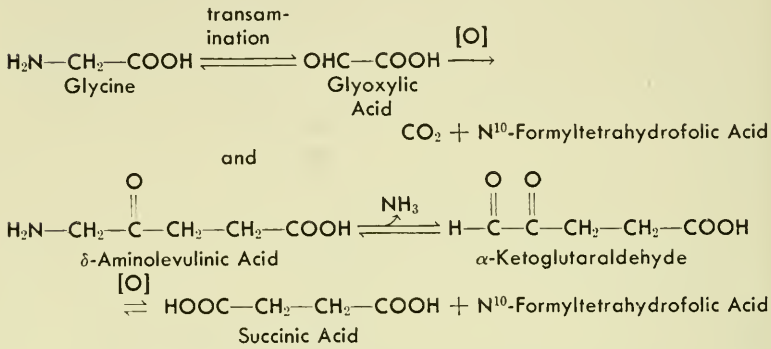
[6] Roy L. Kisliuk, *J. Biol. Chem.* 227 805 (1957).

[7] M. J. Osborn and F. M. Huennekens, *Biochim. et Biophys. Acta* 26 646 (1957).

[8] F. M. Huennekens and M. J. Osborn, *Advances in Enzymology* 21 370 (1959).

The two forms are interconvertible and this oxidation-reduction equilibrium probably is mediated by an enzyme with triphosphopyridine nucleotide (TPN) as the prosthetic group.

Formate added as a substrate is, then, activated in this way. The $N^{10}$-formyl group also can be furnished by glycine, either by way of glyoxylic acid[9, 10] or by way of δ-aminolevulinic acid.[11, 12, 13] The equations are:




Once formed "active formate" is the formylating agent in certain metabolic reactions. The important formylations by this agent which have been discovered to date are the two formylations already noted in the biosynthetic route to the purines. Thus glycineamide ribotide is formylated to furnish C-8 of the purine nucleus and, later, 5-amino-4-imidazolecarboxamide ribotide is formylated to furnish C-2 of the purine nucleus.

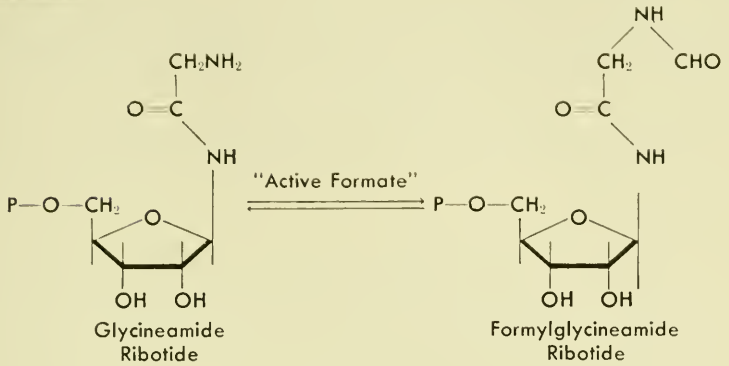
[9] Henry I. Nakada and Sidney Weinhouse, *Arch. Biochem. and Biophys.* 42 257 (1953).

[10] Sidney Weinhouse in W. D. McElroy and H. B. Glass (Editors), "Amino Acid Metabolism," Johns Hopkins Press, Baltimore, 1955, pp. 637–57.

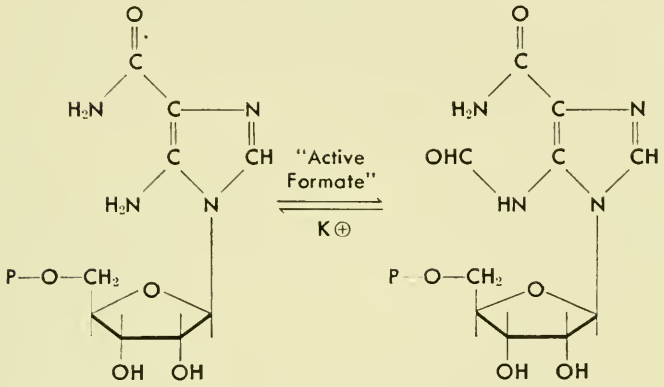
[11] David Shemin, *ibid.*, p. 727.

[12] David Shemin, Tessa Abramsky and Charlotte S. Russell, *J. Am. Chem. Soc.* **76** 1204 (1954).

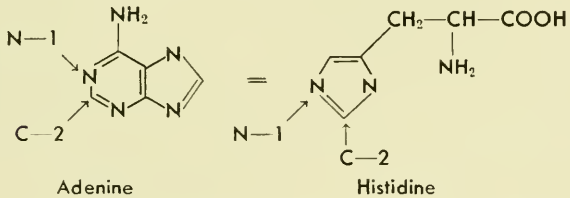
[13] Irving Weliky and David Shemin, *Federation Proc.* **16** 268 (1957).




and

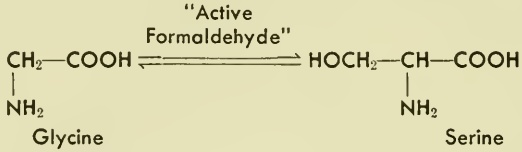



As was seen in the biosynthesis of histidine the N-1 and C-2 atoms of the purine nucleus are donated to this amino acid during its formation so that



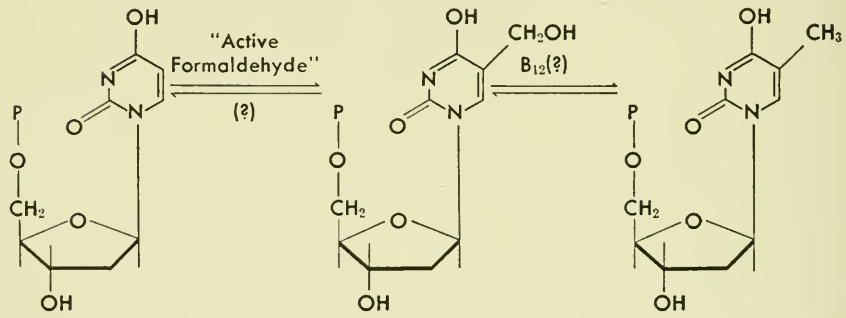

indirectly, at least, these atoms too are furnished by the coenzyme.

The "active formaldehyde" form of the coenzyme is intermediate in the interconversion of glycine and serine:




The large literature on this subject has been reviewed.[8]

The "active formaldehyde" form may also be considered to be a methyl group donor, although much remains to be learned about the mechanisms of these donations. In the biosynthesis of thymine from uracil, serine, formaldehyde or formate are more effective precursors of the introduced methyl group than is methionine, and this precursor effect is inhibited by folic acid antagonists.[8] Actually, the acceptor is probably not uracil, but deoxyuridine or deoxyuridylic acid:



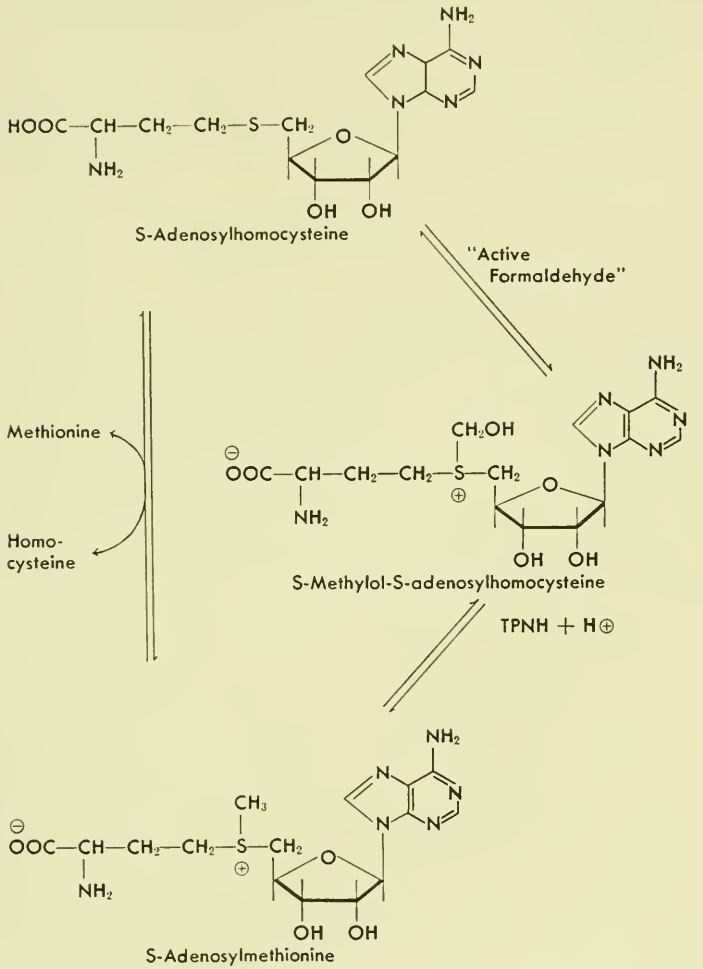

The occurrence of 5-hydroxymethylcytosine in some species has been cited as suggestive of formation of a hydroxymethyl intermediate in this way, at least in the cytosine series.[14, 15] On the other hand it has been reported that in *Lactobacillus leichmannii* there is a vitamin $B_{12}$ requirement for the conversion of formic acid to the thymine methyl group, and that the route does not involve either methionine or a hydroxymethyl group.[16]

[14] Seymour S. Cohen and Lawrence L. Weed, *J. Biol. Chem.* 209 789 (1954).

[15] Maurice Green and Seymour S. Cohen, *ibid.* 225 387 (1957).

[16] James S. Dinning, Barbara K. Allen, Ruth Young and Paul L. Day, *ibid.* 233 674 (1958).

The synthesis of the labile methyl group of methionine has been shown to involve a one-carbon unit at the formaldehyde oxidation level, and the "active formaldehyde" form of the coenzyme has been implicated.[17, 18] Here, again, not everything is known. The following route has been suggested:[8, 19]




[17] David Elwyn, Arthur Weissbach and David B. Sprinson, *J. Am. Chem. Soc.* 73 5509 (1951).

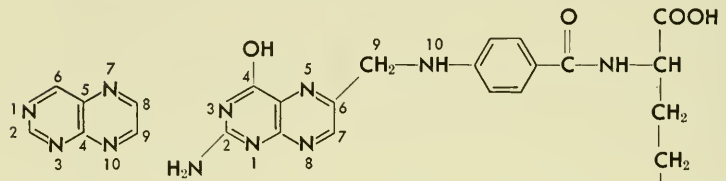
[18] David B. Sprinson in W. D. McElroy and H. B. Glass (Editors), "Amino Acid Metabolism," Johns Hopkins Press, Baltimore, 1955, p. 608.

[19] Audrey Stevens and W. Takami, *Federation Proc.* 17 316 (1958).

In an *Escherichia coli* mutant requiring either methionine or vitamin $B_{12}$ for growth methionine synthesis from homocysteine and serine was stimulated by addition of vitamin $B_{12}$.[20, 21] This suggests that again vitamin $B_{12}$ may be involved in methyl group synthesis.

There is some evidence (from higher animals) that there is a folic acid requirement for the introduction into aminoethanol of some, if not all, of the methyl groups of choline.[22, 23]

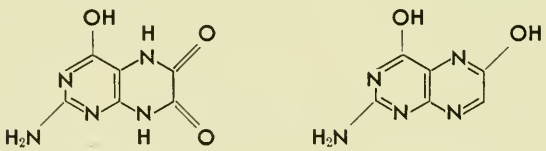
Little is known about the biosynthesis of pteridines in microorganisms. There are suggestions that both pteridines and flavines are related to the purines in this respect.



Pteridine

Pteroyl-L-glutamic Acid
(Folic Acid)

Labeled molecule studies with butterflies indicate that carbon atoms 4 and 5 of the pteridine ring in leucopterin and xanthopterin are derived from glycine (4 from the glycine carboxyl group and 5 from the α-carbon atom).[24]



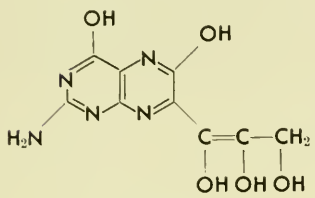
Leucopterin Xanthopterin

The C-6 position seems to be furnished from carbon dioxide and the C-2 position from formate, reminiscent of the purines. Carbon atoms 8 and 9 of the pteridine nucleus

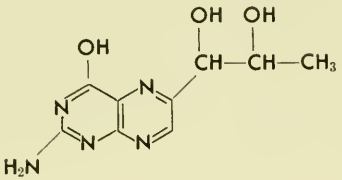
[20] C. W. Helliner and D. D. Woods, *Biochem. J.* 63 26 p (1956).
[21] R. L. Kisliuk and D. D. Woods, *J. Gen. Microbiol.* 18 xv (1957).
[22] Jacob A. Stekol, Sidney Weiss and Ethyl I. Anderson, *J. Am. Chem. Soc.* 77 5192 (1955).
[23] R. Venkataraman and D. M. Greenberg, *ibid.* 80 2025 (1958).
[24] F. Weygand and M. Waldschmidt, *Angew. Chem.* 67 328 (1955).

(in leucopterin from butterflies) are furnished quite directly by glucose. Over 50 percent of the activity of D-glucose-1-$C^{14}$ was found in these two positions, and acetate was excluded as a direct precursor of this part of the molecule.[25]

A sugar origin for this part of the pteridine ring is suggested, too, by the natural occurrence of such substances as erythropterin and biopterin, although, in these cases,

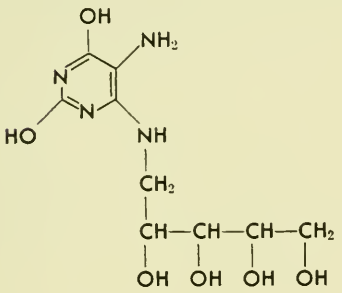



Erythropterin

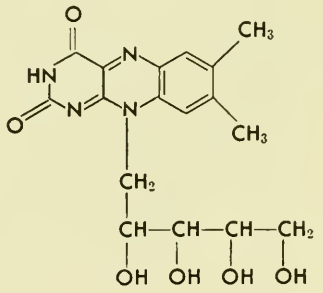



Biopterin

pentoses would be expected. Both erythropterin and biopterin, incidentally, occur as glycosides. If a precursor such as this were assumed, it would relate these substances closely with the riboflavin structure. There is experimental support for the assumption of the pyrimidine shown as a riboflavin precursor.[26]




Assumed pteridine precursor




Riboflavin

Many pteridine derivatives related to the pteridine

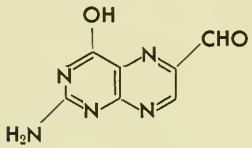
[25] F. Weygand, H.-J. Schliep, H. Simon and G. Dahms, *ibid.* 71 522 (1959).

[26] Toyokazu Kishi, Mitsuko Asai, Toru Masuda and Satoru Kuwada, *Chem. and Pharm. Bull.* (Japan) 7 515 (1959).

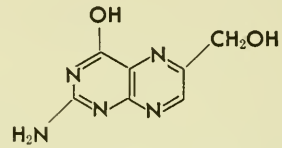
moiety of folic acid have been isolated from non-microbial species. This subject has been reviewed.[8, 27]

Labeled xanthopterin was converted to 5-formyl-5,6–7,8-tetrahydropteroic acid by *Enterococcus stei, Streptococcus fecalis, E. coli* and *Pichia membranaefaciens*.[27a] Folic acid was not formed even when *p*-aminobenzoic acid was added to the medium. Cell extracts of these microorganisms produced folic acid principally.

The assembly of the three moieties of folic acid into the complete molecule has been studied. *Lactobacillus arabinosus* contains enzymes able to couple 2-amino-4-hydroxypteridine-6-carboxaldehyde or the corresponding alcohol with *p*-aminobenzoic acid.[28]




2-Amino-4-hydroxypteridine-6-carboxaldehyde




2-Amino-4-hydroxy-6-hydroxymethylpteridine

These pteridines are even more effective precursors in their reduced forms. Many other pteridines tested were not used. ATP (and $Mg^{++}$) was required. Its role is unknown, although phosphorylation of the alcohol of the pteridine hydroxymethyl group might be necessary to activate it for coupling.

*p*-Aminobenzoic acid was more effective than *p*-aminobenzoylglutamic acid in this coupling reaction in *E. coli*,[29] although *Mycobacterium avium* was able to use the peptide.[30] Apparently adenylo-*p*-aminobenzoic acid was an intermediate in the latter organism (ATP and CoA were required).

The origin of *p*-aminobenzoic acid was considered in an earlier section. It has been known for some time that the anti-infective sulfonamide drugs function by interfering

[27] J. J. Pfiffner and O. D. Bird, *Ann. Rev. Biochem.* 25 416–419 (1956).

[27a] F. Korte and Gotthard Synnatschke, *Ann.* 628 153 (1959).

[28] T. Shiota, *Arch. Biochem. and Biophys.* 80 155 (1959).

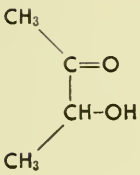
[29] Gene M. Brown, *Federation Proc.* 18 19 (1959).

[30] H. Katunuma, Abstr. 32nd Congr. Japanese Biochem. Assoc., Kyoto, July 1957.

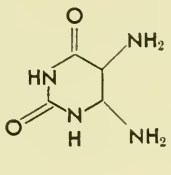
with the incorporation of *p*-aminobenzoic acid into folic acid. Enzyme studies (*E. coli* extracts) now seem to have narrowed this to inhibition of the coupling of the pteridine moiety with *p*-aminobenzoic acid,[31] although in the *Mycobacterium avium* study inhibition of peptide formation by prevention of adenylo-*p*-aminobenzoic acid formation was suggested.

Investigation of the biosynthesis of riboflavin is facilitated by the existence of the two microorganisms, *Eremothecium ashbyii,* a yeast, and *Ashbya gossypii,* a mold, which are prodigious producers of this vitamin, evolving large quantities into the culture medium.

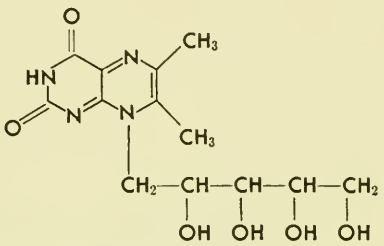
Besides riboflavin several other substances have been isolated from riboflavin fermentations. The structures of these metabolites suggest that they may be biosynthetic precursors of the vitamin.



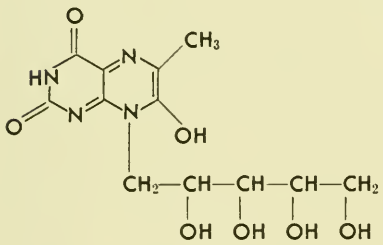
Acetoin



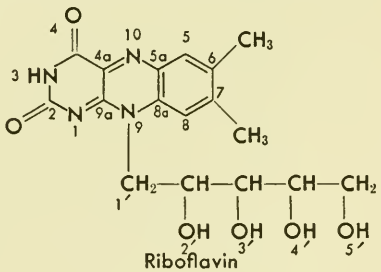
4,5-Diaminouracil



6,7-Dimethyl-8-(D-1′-ribityl)-lumazine
G-Compound (green fluorescence)



6-Methyl-7-oxy-8-(D-1′-ribityl)-lumazine
V-Compound (violet fluorescence)



Riboflavin

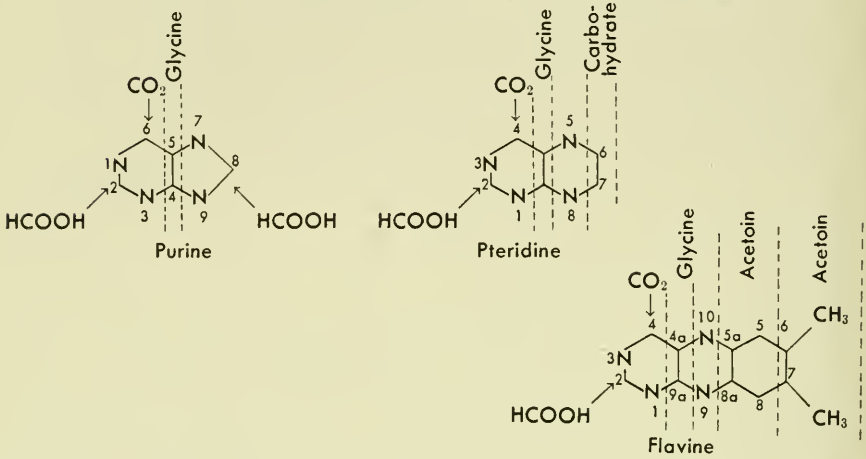
They are shown in the accompanying formulas.

Addition of purines to cultures of growing riboflavin producers increases the yield of riboflavin.[32] $C^{14}$-8-Labeled adenine contributes no radioactivity to the riboflavin mole-

[31] Gene M. Brown, *Physiol. Revs.* 40 359 (1960).
[32] John A. MacLaren, *J. Bacteriol.* 63 233 (1952).

cule,[33] but C-4 of the purine nucleus is equivalent to C-4a in riboflavin, and C-5 of purine to C-9a of riboflavin.[34] The C-4 of riboflavin is furnished by carbon dioxide (*cf.* C-6 in purines), and C-2 from formate (*cf.* C-2 in purines). These relationships are shown in generalized diagram.

*Sources of the Carbon Atoms in Purines, Pteridines and Flavines*




The pyrimidine rings in all these systems seem to have a common origin, and perhaps purines are precursors of the other two classes of heterocycles.

Guanine-5-$C^{14}$ was converted to labeled riboflavin and to labeled G-compound by *Eremothecium ashbyii*, *Ashbya gossypii*, *Candida flareri*, *C. guilliermondii* and *C. parapsilopsis*.[35] Pyrimidines and pteridines were not used directly, and, when labeled G-compound was added to growing cultures, it was not converted to riboflavin by *E. ashbyii* nor was labeled 4,5-diaminouracil. V-Compound was shown to be formed rather easily from G-compound by air oxidation of a stored alkaline solution. While G-compound was not used by growing whole cells, cell-free extracts of *E. ashbyii*, *Ashbya gossypii*, *Mycobacterium smegmatis* and *M. avium* were able to incorporate it into the riboflavin molecule.[36, 37]

[33] Walter S. McNutt, *J. Biol. Chem.* 210 511 (1954).

[34] G. W. E. Plaut, *ibid.* 208 513 (1954).

[35] Friedhelm Korte, Hans Ulrich Aldag, Gerhard Ludwig, Wilfried Paulus and Klaus Störiko, *Ann.* 619 70 (1958).

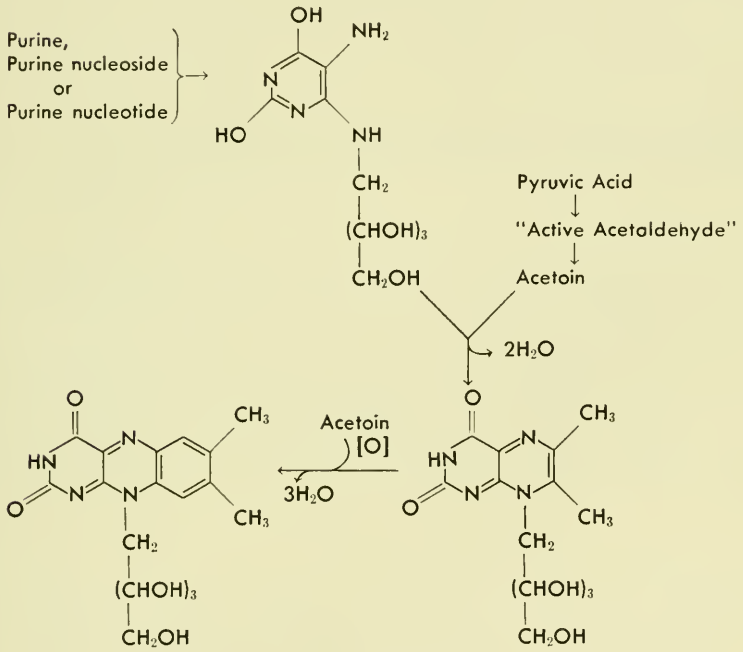
[36] Friedhelm Korte and Hans Ulrich Aldag, *Ann.* 628 144 (1959).

[37] G. F. Maley and G. W. E. Plaut, *J. Am. Chem. Soc.* 81 2025 (1959).

Adenine was found to be a more efficient precursor for riboflavin than G-compound in $C^{14}$-labeling studies,[38] and guanine and xanthine have been found more efficient than adenine.[39]

Acetate[40] and shikimic acid[41] have been shown to be improbable direct precursors of the A ring of riboflavin. Acetoin has been isolated from riboflavin fermentations[42] and is a normal metabolite of these organisms and of other yeasts. On the basis of chemical studies this substance (or near derivatives) was proposed as a precursor of the A ring of riboflavin.[38, 43] It has been confirmed that acetoin is an efficient biological precursor of the vitamin[41] although intermediates cannot be ruled out entirely.

At present, then, the following biosynthetic scheme seems indicated:




[38] R. Cresswell and H. Wood, *Proc. Chem. Soc.*, 386 (1959).

[39] E. G. Brown, T. W. Goodwin and S. Pendleton, *Biochem. J.* 68 40 (1955).

[40] G. W. E. Plaut, *J. Biol. Chem.* 211 111 (1954).

[41] T. W. Goodwin and D. H. Treble, *Biochem. J.* 70 14 p (1958).

[42] Toru Masuda, *Pharm. Bull.* (Japan) 5 136 (1957).

[43] A. J. Birch and C. J. Moye, *J. Chem. Soc.*, 412 (1957); 2622 (1958).

The occurrence of V-compound could be explained as due to a side-reaction in which pyruvate rather than acetoin reacted with the pyrimidine, or it may merely be an oxidation product of G-compound. The close relationship between pyruvate, active acetaldehyde and acetoin, which is mediated by thiamine, has been discussed in an earlier section.

The origin of the ribityl group remains obscure. It is yet to be shown whether this moiety is derived from the ribose of the purine nucleosides or whether it is formed in some other way. Some work has been done on this facet of the biosynthesis.[33, 44, 45, 46]

Riboflavin is phosphorylated by ATP to give riboflavin-5′-phosphate, a coenzyme form. This, in turn, can react again with ATP in the presence of the appropriate enzyme to form flavine-adenine dinucleotide, the other co-

$$\text{Riboflavin} + \text{ATP} \xrightarrow{Mg^{++}} \text{Riboflavin-5′-phosphate} + \text{ADP}$$

$$\text{Riboflavin-5′-phosphate} + \text{ATP} \rightleftharpoons \text{Flavine-adeninedinucleotide} + \text{Pyrophosphate}$$

enzyme form. Flavine-adenine dinucleotide (FAD) is produced commercially in Japan from *E. ashbyii* mycelium.

The principal point of attachment of flavinemononucleotide (FMN) to the apoenzyme seems to be the phosphate group. There may be involvement of the 3-imino group also. FAD is the most prevalent coenzyme form, although FMN occurs in rather large proportions in some microor-

[44] G. W. E. Plaut and Patricia L. Broberg, *J. Biol. Chem.* 219 131 (1956).

[45] Edna B. Kearney and Sasha Englard, *ibid.* 193 821 (1951).

[46] Anthony W. Schrecker and Arthur Kornberg, *ibid.* 182 795 (1950).

ganisms. Obligate anaerobes contain relatively large quantities of flavoproteins. Surveys have been made of the flavine content of microorganisms not used in commercial production.[47, 48] There is variation in the tightness of binding of the coenzyme, and the modes of attachment are not entirely understood.

One of the functions of the flavine enzymes has been mentioned already, namely, the dehydrogenation of reduced DPN in the respiratory chain. Sites of DPNH formation were seen earlier, particularly in the glycolysis route and the citric acid cycle. The enzyme succinic dehydrogenase is a flavoprotein, and the $FADH_2$ formed in this reaction also is fed into the respiratory chain. Besides the direct net synthesis of 2 moles of ATP during glycolysis and of 1 mole of ATP in the citric acid cycle, the remaining energy released during glucose catabolism is transferred in the form of hydrogen or electrons to enzymes with TPN, DPN or FAD as prosthetic groups.

These reduced enzymes are, in turn, oxidized by the metal ion-porphyrin enzymes, which are oxidized by gaseous oxygen. When two hydrogen atoms are passed along the entire respiratory chain, water is formed as well as 3 more molecules of ATP.

The exact number of particles in the chain is not entirely clear, and there are variations with different organisms. In lactobacilli, for example, flavines seem to replace heme proteins in electron transport.[48a] Also obscure is the exact manner in which ATP is formed during respiration and the precise way in which hydrogen is transferred from one coenzyme to the next. There has been interesting speculation in this area of biophysics.

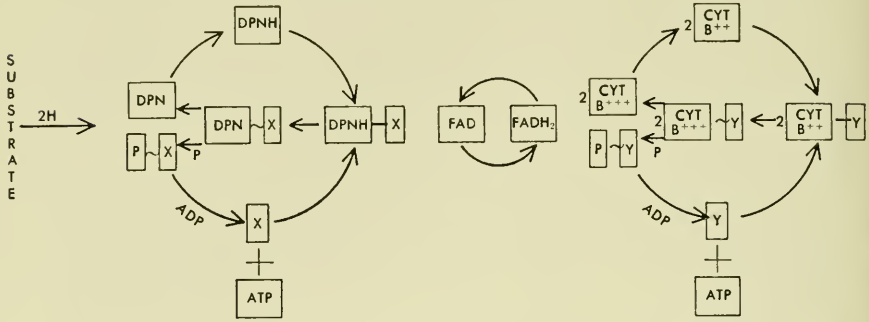
The respiratory chain can be shown in a simplified form as in the accompanying diagram.[49]

[47] J. L. Peel, *Biochem. J.* 69 403 (1958).

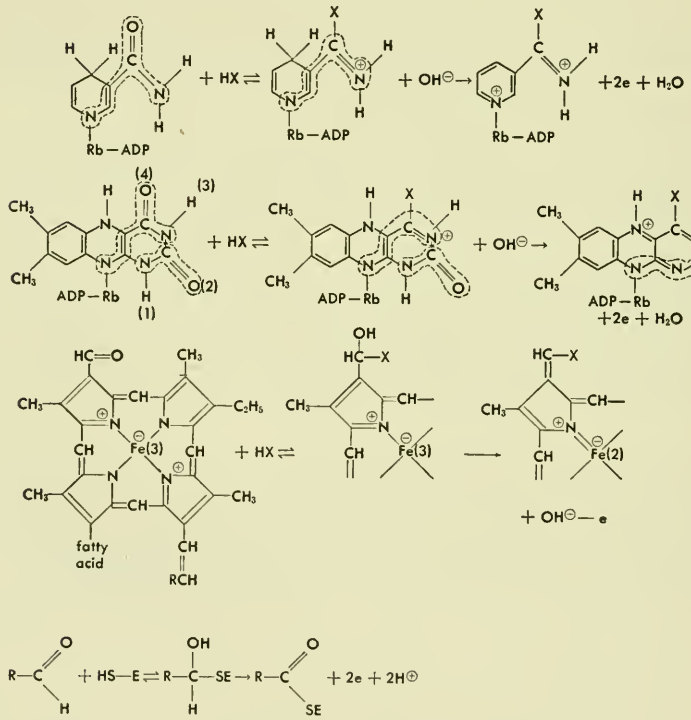
[48] Chester DeLuca, Morton M. Weber and Nathan O. Kaplan, *J. Biol. Chem.* 223 559 (1956).

[48a] Cornelius F. Strittmatter, *Federation Proc.* 17 318 (1958).

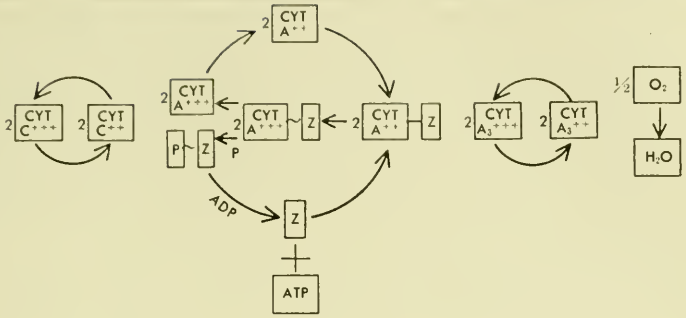
[49] Albert L. Lehninger, *Scientific American* 202 102 (1960).



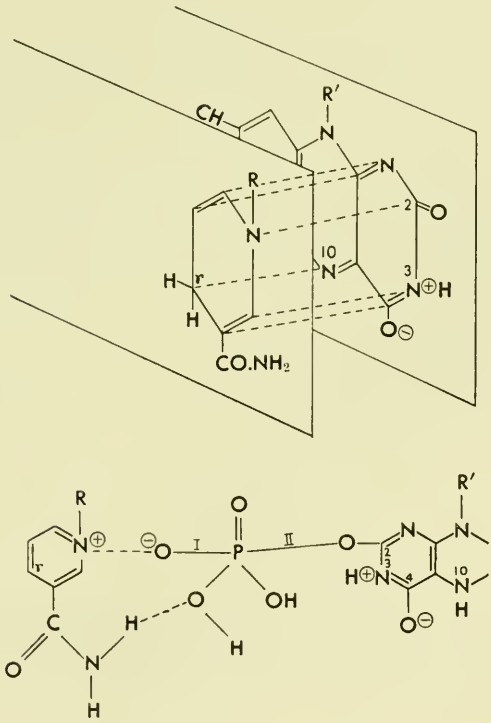

The natures of the entities X, Y and Z are mysterious. If they are assumed to possess nucleophilic groups such as R—$S^-$, R—$COO^-$ or $H_2PO_4^-$, then one scheme has been advanced to show how the requisite energy-rich bonds could be formed in DPNH, FADH and Ferricytochrome $a_3$.[50] The coupling methods and resonance systems involved are shown in the diagram:




[50] Paul E. Glahn and Sigurd O. Nielsen, *Nature* 183 1578 (1959).




Another hypothesis assumes close approach of DPNH and riboflavin in parallel planes with interposition of inorganic phosphate, held perhaps by hydrogen bonding, *e.g.* to the amide moiety of nicotinamide.[51] These geometrical and chemical relationships can be represented as follows:




[51] Barbro Grabe, *Biochim. et Biophys. Acta* 30 560 (1958).

When an electron is transferred from the N-atom of the reduced pyridine ring to an unoccupied $\pi$-orbital of the isoalloxazine ring of FAD, the N-atom assumes a positive charge, which is neutralized by attraction of a proximate, ionized phosphate hydroxyl oxygen. The increased electron density on the O-atom at position 2 in the riboflavin nucleus might cause formation of a bond to phosphorus as shown in the activated complex above, the reaction being:

$$DPNH + FAD + H_2O_3PO^{\ominus} + H^{\oplus} \rightleftharpoons DPN^{\oplus} + H_2O_3P\text{—}FADH + OH^{\ominus}$$

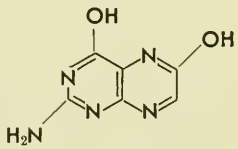
When this substance is oxidized by the subsequent carrier (probably a cytochrome), two electrons, perhaps dislocalized $\pi$-electrons, are withdrawn from the FAD-complex thus permitting dissociation of a proton and activation of the phosphoryl group. In the presence of ADP, then, ATP could be formed according to the equation:

$$FADH\text{—}PO_3H_2 + ADP + 2Fe^{(+++)} \rightleftharpoons FAD + H^{\oplus} + ATP + 2Fe^{(++)}$$

Other flavoprotein dehydrogenase substrates are: aldehydes, $\alpha$-amino acids, $\alpha$-hydroxy acids, purines, fatty acid-coenzyme A esters and certain amines. Flavine enzymes also participate in bacterial hydrogenase systems, in nitrate reduction and assimilation by fungi and higher plants and in photosynthesis and bioluminescence. There is currently much study of flavoprotein reactions, which can often be followed by spectrophotometry and EPR techniques.

Reviews of the flavine coenzymes and their biosynthesis are available.[52, 31]

1048 **Xanthopterin,** $C_6H_5O_2N_5$, yellow amorphous substance, isolated as barium or sodium salts.




[52] Paul D. Boyer, Henry Lardy and Karl Myrbäck (Eds.), "The Enzymes" Vol. II, 2nd ed., Helmut Beinert, *Flavin coenzymes*, Academic Press, New York, 1960, pp. 340–416.

*Mycobacterium tuberculosis*

Also occurs as a butterfly wing pigment.

Marguerite O'L. Crowe and Amy Walker, *Brit. J. Exptl. Pathol.* 35 18 (1954). (Isolation from this organism)

Robert Purrmann, *Ann.* 546 98 (1940), 548 284 (1941). (Synthesis)

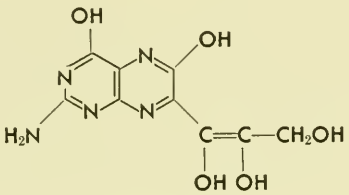
1049 **Pterin-like Substance.**

By paper chromatographic comparisons this purple fluorescent substance was shown to be similar to or identical with 2-amino-4,7-dihydroxypteridine-6-acetic acid ($C_8H_7O_4N_5$).

*Aspergilli*

Yasuyuki Kaneko, *J. Agr. Chem. Soc. Japan* 31 122 (1957).

1050 **Erythropterin,** $C_9H_9O_5N_5$, deep red crystals from 0.01 N hydrochloric acid.

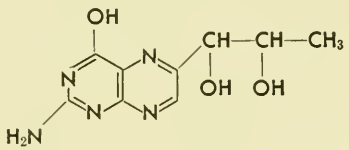



*Mycobacterium tuberculosis* var. *hominis, M. lacticola*

M. O'L. Crowe and A. Walker, *Science* 110 166 (1949).

Rudolf Tschesche and Frederic Vester, *Chem. Ber.* 86 454 (1953).

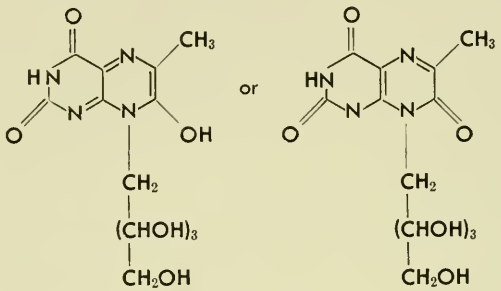
1051 **Biopterin,** $C_9H_{11}O_3N_5$, pale yellow crystals, m.p. 250–280° (dec.), $[\alpha]_D^{25}$ −50° (in 0.1 N hydrochloric acid).




Yeast, *Ochromonas malhamensis*

E. L. Patterson, H. P. Broquist, Alberta M. Albrecht, M. H. von Saltza and E. L. R. Stokstad, *J. Am. Chem. Soc.* 77 3167 (1955).

1052 **V-Compound** (8-Ribityl-6-methyl-7-oxylumazine, Compound A), $C_{12}H_{16}O_7N_4$, colorless crystals, m.p. 263° (dec.), $[\alpha]_D^{20}$ +4.5° (c 3.3 in water) +11.45° (in 0.1 N sodium hydroxide solution).



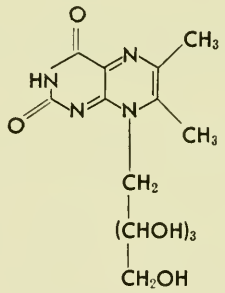

*Eremothecium ashbyii*

Toru Masuda, Toyokazu Kishi and Mitsuko Asai, *Chem. and Pharm. Bull* (Japan) 6 291 (1958). (Structure)

Toru Masuda, Toyokazu Kishi, Mitsuko Asai and Satoru Kuwada, *ibid.* 7 361, 366 (1959). (Synthesis)

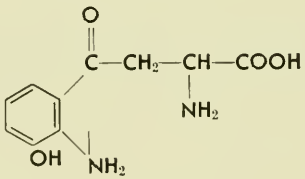
Walter S. McNutt, *J. Am. Chem. Soc.* 82 217 (1960).

1053 **G-Compound** (8-Ribityl-6,7-dimethyllumazine), $C_{13}H_{18}O_6N_4$, light yellow needles, m.p. 273° (dec.), $[\alpha]_D^{20}$ −164°.




*Eremothecium ashbyii*

1054 ***l*-3-Oxykynurenine**, $C_{10}H_{12}O_4N_2$



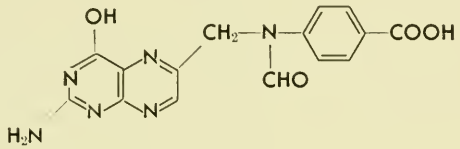
was isolated from the same culture. This metabolite resembles 3-oxyanthranilic acid, known to be a biosynthetic precursor of nicotinic acid.

Toru Masuda, *Pharm. Bull.* (Japan) 4 71 (1956). (Isolation)

*Idem., ibid.* 5 28 (1957). (Structure)

Toru Masuda, Toyokazu Kishi, Mitsuko Asai and Satoru Kuwada, *Chem. and Pharm. Bull.* (Japan) 7 361 (1959). (Synthesis)

1055 **Rhizopterin** ($N^{10}$-Formylpteroic Acid) (*Streptococcus lactis* R Factor) (SLR Factor), $C_{15}H_{12}O_4N_6$, light yellow crystals, m.p. >300°.

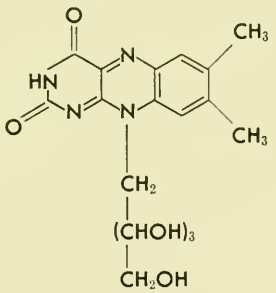



*Rhizopus nigricans*

Edward L. Rickes, Louis Chaiet and John C. Keresztesy, *J. Am. Chem. Soc.* 69 2749 (1947).

Donald E. Wolf, R. Christian Anderson, Edward A. Kaczka, Stanton A. Harris, Glen E. Arth, Philip L. Southwick, Ralph Mozingo and Karl Folkers, *ibid.* 69 2753 (1947). (Synthesis)

1056 **Riboflavin** (Vitamin $B_2$), $C_{17}H_{20}O_6N_4$, yellow-orange microcrystalline powder, m.p. ~280° (rapid heating), $[\alpha]_D^{25}$ −112° to −122° (50 mg. in 2 ml. of 0.1 N alcoholic sodium hydroxide diluted to 10 ml. with water).

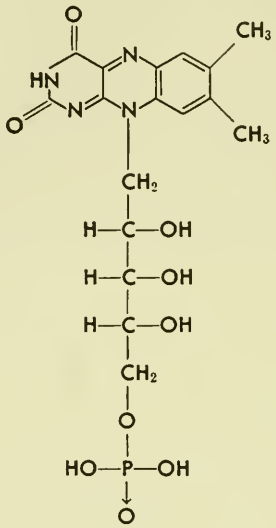





Ascomycetes such as *Eremothecium ashbyii* and *Ashbya gossypi* produce high yields.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. II, Richard J. Hickey, *Production of riboflavin by fermentation*, Chap. 5, pp. 157–190. (A review)

1057 **Riboflavin-5′-phosphate**, $C_{17}H_{21}O_9N_4P$, yellow microcrystals.

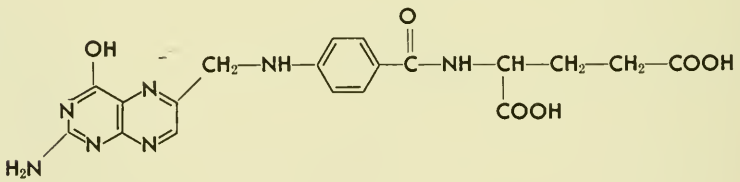


Yeast

Otto Warburg and Walter Christian, *Biochem. Z.* 254 438 (1932); 258 496 (1933); 263 228 (1933). (Isolation)

H. S. Forrest and A. R. Todd, *J. Chem. Soc.*, 3295 (1950). (Synthesis)

1058 **Folic Acid** (Pteroylglutamic Acid Folacin, Vitamin $B_c$), $C_{19}H_{19}O_6N_7$, pale yellow-orange needles, which char above 250°.

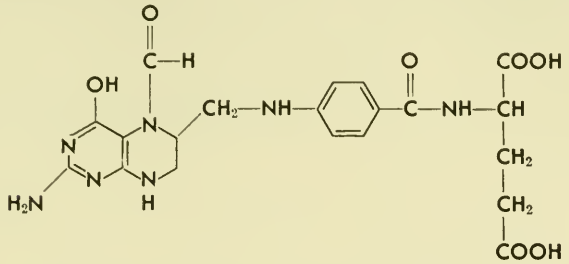


Yeasts and certain higher fungi

Yields of 19–80 $\mu$g. per gram of dry cell weight are obtained from brewers' yeast.

Leland A. Underkofler and Richard J. Hickey, "Industrial Fermentations," Chemical Publishing Co., Inc., New York, 1954 Vol. III, J. M. Van Lanen, *Production of vitamins other than riboflavin,* Chap. 6, pp. 191–216. (A review)

1059 **Citrovorum Factor** (Folinic Acid-SF, Leucovorin, $N^5$-Formyltetrahydrofolic Acid) $C_{20}H_{23}O_7N_7$ (Trihydrate): Buff crystals, m.p. 248–250° (dec.), $[\alpha]_D^{25}$ +16.76 (c 3.52 on anhydrous basis in 5% sodium bicarbonate solution).



Yeasts (probably widely distributed)

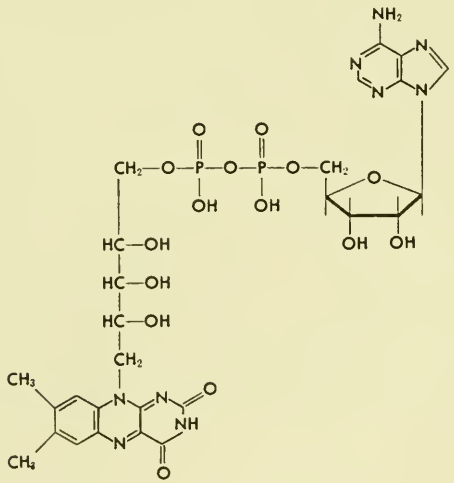
The corresponding compound with the formyl group transferred to the amine group of the *p*-aminobenzoic acid moiety ($N_{10}$) is also known.

C. H. Hill and M. L. Scott, *J. Biol. Chem.* 196 195 (1952). (Isolation from brewers' yeast)

A. G. M. Sjöström and L. E. Ericson, *Acta Chem. Scand.* 7 870 (1953). (Isolation from eight lichens)

Donna B. Cosulick, Barbara Roth, James M. Smith, Jr., Martin E. Hultquist and Robert P. Parker, *J. Am. Chem. Soc.* 74 3252 (1952). (Structure)

1060 **Flavine-Adenine-Dinucleotide,** $C_{27}H_{33}O_{15}N_9P_2$, amorphous white powder.



Yeasts, molds, bacteria (widely distributed)

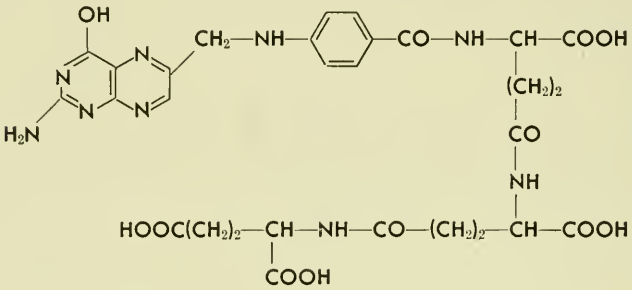
Otto Warburg and Walter Christian, *Biochem. Z.* **298** 150 (1938). (Isolation)

S. M. H. Christie, G. W. Kenner and A. R. Todd, *Nature* **170** 924 (1952).

*Idem., J. Chem. Soc.*, 46 (1954). (Synthesis)

J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.* **80** 3756 (1958). (Synthesis)

1061 **Fermentation "Lactobacillus casei" Factor** (Teropterin, Pteroyl-γ-glutamyl-γ-glutamylglutamic Acid), $C_{29}H_{33}O_{12}N_9$.




*Corynebacterium* sp.

Brian L. Hutchings, E. L. R. Stokstad, Nestor Bohonos, Nathan Sloane and Y. Subbarow, *Ann. N. Y. Acad. Sci.* **48** 265 (1946). (Isolation)

J. H. Boothe, J. H. Mowat, B. L. Hutchings, R. B. Angier, C. W. Waller, E. L. R. Stokstad, J. Semb, A. L. Gazzola and Y. Subbarow, *J. Am. Chem. Soc.* **70** 1099 (1948).

J. H. Boothe, J. Semb, C. W. Waller, R. B. Angier, J. H. Mowat, B. L. Hutchings, E. L. R. Stokstad and Y. Subbarow, *ibid.* **71** 2304 (1949). (Synthesis)

1062 **Vitamin $B_c$ Conjugate** (Pteroylhexaglutamylglutamic Acid), $C_{49}H_{61}O_{24}N_{13}$.

The structure is like that of the preceding formula, but with four more glutamic acid units in the polypeptide side-chain.

Bacteria, yeasts, molds (widely distributed among microorganisms)

P. R. Burkholder, Ilda McVeigh and Katherine Wilson, *Arch. Biochem.* **7** 287 (1945).

J. J. Pfiffner, D. G. Calkins, E. S. Bloom and B. L. O'Dell, *J. Am. Chem. Soc* **68** 1392 (1946). (Structure)

1063 **Pteridine pigment.**

A pigment which fluoresces under U.V. light is produced by *Microsporum* species (some of which cause ringworm). This pigment has been isolated and purified to some extent. The infrared spectrum indicates that it is a pteridine, probably trisubstituted, and possibly 2-$NH_2$ (or —OH), 4—OH and 6—$CH_2OH$ substituted.

*Microsporum gypseum, M. canis*

Frederick T. Wolf, Ernest A. Jones and Helene A. Nathan, *Nature* 182 475 (1958).

## 19.

# Unclassified Metabolites

1064 **Aburamycin** (M5-18903), yellow crystals, m.p. 163–165° (169–171°), $[\alpha]_D^{20}$ +24.56° (c 1 in methanol) $[\alpha]_D^{25}$ −29° (c 0.5 in methanol).

Absorbs 2 moles of $H_2$. Acetylates (m.p. acetate = 205–207°). A weakly acidic antibiotic, apparent molecular weight 1295. Aburamycin and M5-18903 appear to be optical antipodes of the same compound.

*Streptomyces* spp.

Haruo Nichimura, Toshiaki Kimura, Katsuya Tawara, Kunio Sasaki, Kiyoshi Nakajima, Noboru Shimaoka, Saburo Okamoto, Masafumi Shimohira and Jun Isono, *J. Antibiotics* (Japan) 10A 205 (1957).

Richard M. Gale, Marvin M. Hoehn and Mack H. McCormick, "Antibiotics Annual 1958–1959," Medical Encyclopedia, Inc., New York, p. 489.

1065 **Actinobolin,** $C_{13}H_{20\text{-}22}O_6N_2$, amorphous hygroscopic white powder, $[\alpha]_D^{26}$ (Sulfate) +54.5° (c 1 in water).

An amphoteric antibiotic. Forms an acetate: m.p., partial m. at 130°, resolidified 145°, dec. 263–266°, $[\alpha]_D^{26}$ +58° (c 1 in water). Positive ninhydrin, ferric chloride, $KMnO_4$, Fehlings, iodoform tests. Absorbs no hydrogen.

*Streptomyces* sp.

Theodore H. Haskell and Quentin R. Bartz, "Antibiotics Annual 1958–1959," Medical Encyclopedia, Inc., New York, p. 505.

1066 **Actinoleukin** $(C_9H_{12}O_3N_2)_n$, colorless crystals, m.p. 191° (dec.).

Analysis: C 55.53, H 6.05, N 14.05
55.68, 5.98, 14.01

Negative biuret, ninhydrin, Tollens, Fehling. Positive $FeCl_3$.

*Streptomyces aureus*

Masahiro Ueda, Yukio Tanigawa, Yoshiro Okami and Hamao Umezawa, *J. Antibiotics* (Japan) 7A 125 (1954).

1067 **Akitamycin**, $[\alpha]_D^{25}$ +158° (c 0.5 in dimethylformamide), U.V. 291, 303.5, 319 mμ. Tetraene, C 57.26, H 7.68, N 1.64.

*Streptomyces akitaensis*

*J. Antibiotics* (Japan) 12B 293, 295, 297 (1959).

1068 **Albidin**, $C_5H_4O_2$ (proposed), red needles, not melting below 380°.

Unstable. Most stable below pH 3.

*Penicillium albidum*

P. J. Curtis and J. F. Grove, *Nature* 160 574 (1947).

P. J. Curtis, H. G. Hemming and C. H. Unwin, *Brit. Mycol. Soc. Trans.* 34 332 (1951).

1069 **Albofungin**, bright yellow powder, dec. 190°, U.V. 240, 255, 305, 375 mμ. Contains C, H, N, O.

*Streptomyces albus* var. *fungus*

A. S. Chochlov, *Czech. Symposium on Antibiotics* (Prague), 154 (1959).

1070 **Albomycetin**, $C_{32}H_{54}O_9N$ (proposed), colorless crystals, m.p. 166°.

A basic substance precipitated by ammonium reineckate. Positive Fehlings, Tollens, cherry colored Elson-Morgan. Negative $FeCl_3$, Sakaguchi, Molisch, Millon. May be a macrolide.

*Streptomyces albus*

Bunji Takahashi, *J. Antibiotics* (Japan) 7A 149 (1954).

1071 **Alboverticillin** (Hydrochloride), colorless, amorphous, $[\alpha]_D^{20}$ −33.5° (c 1.0 in water).

Negative U.V., Tollens, Molisch, Benedict, maltol, Elson-Morgan, biuret, Millon, Sakaguchi, anthrone and $FeCl_3$. Positive ninhydrin, Fehling.

*Streptomyces* sp.

Kenji Maeda, Sinichi Kondo, Kofumi Ohi, Hiroko Kondo, E. Lin Wang, Yasusuke Osato and Hamao Umezawa, *J. Antibiotics* (Japan) 11A 30 (1958).

1072 **Aliomycin**, yellowish brown powder. Contains C, H, N, O, S. Pentaene. U.V. 321, 330, 351 mμ.

Positive Fehling (on heating, weakly positive Molisch, red purple in concentrated $H_2SO_4$).

*Streptomyces acidomyceticus*

Seizi Igarasi, Koichi Ogata and Akira Miyake, *J. Antibiotics* (Japan) **9B** 101 (1956).

1073 **Allomycin,*** $C_{29}H_{44}O_9$, crystalline, m.p. 237–239° (dec.) $[\alpha]_D^{17}$ $-118.8 \pm 0.5°$ (c 0.98 in 0.1 N hydrochloric acid).

*Streptomyces sindenensis*

Koichi Nakazawa, Shigehiro Fujii, Michitaka Inoue, Hiroshi Hitomi, Ohira Miyake and Jyuzo Kaneko, *J. Antibiotics* (Japan) **7B** 168 (1954).

Sueo Tatsuoka, Koichi Nakazawa, Michitaka Inoue and Shigehiro Fujii, *J. Pharm. Soc. Japan* **75** 1206 (1955).

1074 **Alternarine,** colorless needles, m.p. 230°.

*Alternaria solani*

Herman Darpoux, Albert Faivré-Amiot and Louis Roux, *Compt. rend.* **230** 993 (1950).

1075 **Althiomycin,** $C_{15}H_{14}N_4S_2O_6$, colorless crystals, m.p. 172–174° (dec.) (browning from 120–160°), $[\alpha]_D^{20}$ +20.3 (c 1.33 in methyl cellosolve).

Unstable at pH <5.0 or >7.0.

A streptomycete

Hiroshi Yamaguchi, Yuya Nakayama, Keiichi Takeda, Kosaku Tawara, Kenji Maeda, Tomio Takeuchi and Hamao Umezawa, *J. Antibiotics* (Japan) **10A** 195 (1957).

1076 **Anisomycin** (PA-106, PA-107), $C_{14}H_{19}O_4N$, white needles, m.p. 140°, $[\alpha]_D^{25}$ $-45° \pm 3°$ (c 1.0 in chloroform).

*Streptomyces griseolus,* other *Streptomyces* spp.

Ben A. Sobin and Fred W. Tanner, Jr., *J. Am. Chem. Soc.* **76** 4053 (1954).

Fred W. Tanner, Jr., B. A. Sobin and J. F. Gardocki, "Antibiotics Annual 1954–1955" Medical Encyclopedia, Inc., New York, p. 809.

1077 **Antibiotic A 246,†** $C_{41}H_{66\text{-}70}O_{14}$, crystalline, m.p. 235° (dec.), $[\alpha]_D^{20}$ −160° (c 0.2 in methanol).

Reacts with $HIO_4$.

*Streptomyces* sp.

M. L. Dhar, V. Thaller and M. C. Whiting, *Proc. Chem. Soc.*, 148 (1958).

1078 **Antibiotic B-456,** m.p. 176° (dec.), $[\alpha]_D^{16}$ −22.9°.

C 57.52, H 6.67, N 11.12

Positive biuret, Millon. Negative Molisch, Benedict, Fehling.

* See amicetin.

† Identical with lagosin, entry 229.

Valine, leucine, proline, aspartic, glutamic, D-tyrosine and ornithine produced after hydrolysis.

*Bacillus subtilis*

Yuzuru Tanaka, *J. Antibiotics* (Japan) **9B** 1 (1956).

1079 **Antibiotic C-159,**

U.V. 260–280, 345 mμ in aqueous solution.

C 58.7, H 7.4, N 9.9, O 24.0

Inhibits growth of organisms containing glycine, alanine, threonine, aspartic acid.

*Streptomyces canus*

Bristol Laboratories, British Patent 814,794 (1959).

1080 **Antibiotic D-13,** dense crystals, m.p. 243°.

C 56.91, H 6.97, O 22.61, N 13.51.

*Streptomyces vinaceus-drappus*

Upjohn Co., British Patent 708,686 (1954).

1081 **Antibiotic E-212,** colorless needles, m.p. 233–234°.

U.V. 235, 273 mμ in 0.1 N hydrochloric acid. C 49.14, H 4.34, N 23.77, O 22.55

Negative ninhydrin, biuret, Fehling, $FeCl_3$, Molisch, Millon and Ehrlich.

*Streptomyces* sp. like *S. albus*

Ko Kikuchi, *J. Antibiotics* (Japan) 8A 145 (1955).

1082 **Antibiotic LA-7017,** greenish yellow powder, m.p. 154–157° (dec.), $[\alpha]_D^{25}$ −155° (c 0.4 in ethanol).

Contains only C, H, O (C 56.99, H 7.18). Contains two acidic groups, Equiv. Wt. = 1180. Decolorized $KMnO_4$. Negative Fehling's test.

*Streptomyces* sp. 7017

P. Sensi, A. M. Greco and H. Pagani, *Antibiotics and Chemotherapy* 8 241 (1958).

1083 **Antibiotic M-4209,** $C_{40-42}H_{67-71}O_{16}N$, white crystals m.p. 210–214° (dec.), $[\alpha]_D^{25}$ −54 ± 2° (c 1 in methanol), U.V. 240, 330 mμ.

Methoxyl, acetyl and iso-valeryl groups present.

*Streptomyces hygroscopicus*

James D. Dutcher, John Vandeputte, Sidney Fox and L. J. Heuser, *Antibiotics and Chemotherapy* 3 910 (1953).

1084 **Antibiotic WC 3628,** $C_{42}H_{73}O_{16}N$, white crystals, m.p. 220–222° (Kofler), $[\alpha]_D^{22}$ −57 ± 3° (c 0.5 in ethanol).

*Streptomyces* sp. WC 3628
McCormick, Canadian Patent 513,324 (1955).

1085 **Antibiotic T,** trichothecin-like, crystalline prisms, m.p. 126°, $[\alpha]_D^{20}$ +135° (c 1 in chloroform).
A basidiomycete.
E. T. Glaz, Eszter Scheiber, J. Gyimesi, I. Horwath, Katalin Steczek, A. Szentirmai and G. Bohus, *Nature* 184 908 (1959).

1086 **Antibiotic X-206,** $C_{46}H_{80}O_{13}$, colorless crystals, m.p. 126–128°, $[\alpha]_D^{29}$ +15.0° (c 2.0 in methanol).
*Streptomyces* sp.
Julius Berger, A. I. Rachlin, W. E. Scott, L. H. Sternbach and M. W. Goldberg, *J. Am. Chem. Soc.* 72 5295 (1951).

1087 **Antibiotic X-464,** $C_{25}H_{40}O_7$, white crystals, m.p. 172–174° (dec.), $[\alpha]_D^{27}$ +65.9° (c 2.0 in methanol).
*Streptomyces* sp.
Julius Berger, A. I. Rachlin, W. E. Scott, L. H. Sternbach and M. W. Goldberg, *J. Am. Chem. Soc.* 73 5295 (1951).

1088 **Antibiotic X-537A,** $C_{34}H_{52}O_8$, colorless crystals, m.p. 100–109°, $[\alpha]_D^{26}$ −7.2° (c 1.0 in alcohol), U.V. 317, 249 mμ in isopropyl alcohol.
Positive $FeCl_3$ test.
*Streptomyces* sp.
Julius Berger, A. I. Rachlin, W. E. Scott, L. H. Sternbach and M. W. Goldberg, *J. Am. Chem. Soc.* 73 5295 (1951).

1089 **Antibiotic X-1008,** $C_{29}H_{38}O_7N_6S$, cube-like crystals, m.p. 209–216° (dec.), $[\alpha]_D^{27}$ −282° (c 1 in chloroform).
Resembles echinomycin
*Streptomyces* sp.
J. Berger, E. R. LaSala, W. E. Scott, B. R. Meltsner, L. H. Sternbach, S. Kaiser, S. Teitel, E. Mack and M. W. Goldberg, *Experientia* 13 434 (1957).

1090 **Antibiotic from *B. cepae*,** colorless crystals, m.p. 185° (dec.) C 40.8, H 5.3.
*Bacillus cepae*
Isolated from rotting onion.
M. Fiuczek, *Med. Doswiadczalna i Mikrobiol.* 2 175 (1950). (*Biol. Abstr.* 26 3975).

1091 **Antibiotic from *B. pumilis*,** $C_8H_9N_2O_2S$, white crystals, m.p. 252°.
Negative ninhydrin.

*Bacillus pumilis*

A. T. Fuller, *Nature* 175 722 (1955).

1092 **Antibiotic from *Monosporium bonorden*,** $C_{17}H_{16}O_7$, colorless crystals, m.p. 193.5°, $[\alpha]_D^{20}$ +203° (in chloroform).

Two phenolic hydroxyl groups, one active hydrogen on an aromatic ring, one double link in a side-chain and a free carboxyl group present.

Molecular structure may be closely related to the structure proposed for mycophenolic acid.

*Monosporium bonorden*

P. Delmotte and J. Delmotte-Plaquee, *Nature* 171 344 (1953).

1093 **Antibiotic from *Penicillium spinulosum*,** fine white needles, m.p. 183–185°.

*Penicillium spinulosum*

Shegejii Kondo and Bunji Takahashi, *J. Penicillin* (Japan), 1 147 (1947).

1094 **Antibiotic from *S. abikoensis*,** yellow powder. Heptaene. U.V. 242, 358, 400 mμ in ethanol. Actinoleukin in mycelium.

*Streptomyces abikoensis*

Masahiro Ueda and Hamao Umezawa, *J. Antibiotics* (Japan) 9A 86 (1956).

1095 **Antibiotic from *S. fungicidicus*.** U.V. 290, 303, 317 mμ. Similar to fungicidin or rimocidin.

Positive Fehling, Molisch, Negative Millon, Sakaguchi, Schiff, Tollens, $FeCl_3$. Blue with $FeCl_3$-K ferricyanate; decolorizes $KMnO_4$.

*Streptomyces fungicidicus*

Hamao Umesawa, Yoshio Okami and Ryozo Utahara, Japanese Patent 5744 (1956).

1096 **Antibiotic from *S. griseus*.** Heptaene. U.V. 359–362, 378–382, 401–405 mμ.

*Streptomyces griseus*

Richard A. Pledger and Hubert Lechevalier, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 249.

1097 **Antibiotic 26/1,** yellow crystalline. Heptaene. U.V. 359, 380, 404 mμ in ethanol.

Alcohol solution turns violet with $H_2SO_4$; decolorizes $KMnO_4$. Negative biuret and ninhydrin.

*Actinomyces globisporus*

V. A. Tsyganov, P. N. Golyakov, A. M. Bezborodov, V. P. Namestnikova, G. V. Khopko, S. N. Solov'ev, M. A. Malyshkina and L. O. Bol'shakova, *Antibiotiki* 4 21 (1959).

1098 **Antibiotic 446,** white crystalline powder, m.p. 81–87°, $[\alpha]_D^{22}$ −82° (c 0.5 in ethanol). U.V. 230–231, 280 mμ.

C 60.47, H 7.99, N 2.02

Negative Fehling.

*Nocardia mesenterica*

Masahiro Ueda and Hamao Umezawa, *J. Antibiotics* (Japan) 8A 164 (1955).

1099 **Antibiotic 720-A,*** $C_{28}H_{40}O_9N_2$, white needles, m.p. 139.5–140°, $[\alpha]_D^{18}$ +73.5° (c 1.0 in acetone) U.V. 227, 346 mμ.

Positive $FeCl_3$; negative Molisch, ninhydrin, biuret, Ehrlich and 2:4 DNPH.

*Streptomyces* n. sp.

Yoshio Sakagami, Setsuo Takeuchi, Hiroshi Yonehara, Heüchi Sakai and Matao Takashima, *J. Antibiotics* (Japan) 9A 1 (1956).

1100 **Antibiotic 587/13,** Hydrochloride

C 39.5, H 6.97, N 15.7, Cl 16.75

*Streptomyces lavendulae*

D. M. Trakhtenberg, V. M. Baikina, E. I. Rodionovskaya, I. M. Prosnyakova, O. A. Kalinovskii, Yu V. Zakharova and A. A. Khokhlov, *Antibiotiki* (U.S.S.R.) 4 9 (1959).

1101 **Antibiotic 1037,** crystalline needles, m.p. 283–289°, $[\alpha]_D^{35}$ −51°.

C 49.33–49.47, H 4.56–4.90, N 23.75–24.14, no halogen or sulfur

*Streptomyces* sp.

Hiroshi Yamamoto, Shigehiro Fujii, Koichi Nakazawa, Akira Miyake, Hiromu Hitomi and Masahiko Imanishi, *Ann. Repts. Takeda Research Lab.* 16 26 (1957).

1102 **Antibiotic 6270,†** $C_{29}H_{37}N_6SO_{6-7}$, crystalline.

*Streptomyces flavochromogenes*

M. G. Brazhnikova, Czech. Symposium on Antibiotics (Prague), 140 (1959).

1103 **Antibiotic 6706,‡** $C_{26-27}H_{32}O_8N_4$, colorless needles, m.p. 214–216°, U.V. 304 mμ.

Gives negative $FeCl_3$, Fehling, Tollens, ninhydrin and Millon tests.

* See entry 269 (antimycin $A_1$).

† Cf. entry 1089.

‡ See pyridomycin, entry 752.

*Streptomyces* sp.

Masahiko Kuraya, Bunji Takahashi, Yorio Hinuma, Takaaki Yashima, Kenzo Watanabe, Masa Kuroya and Susumu Hamada, *J. Antibiotics* (Japan) 7A 58 (1954).

1104 **Antifungal Substance**, colorless needles, m.p. 283–289°, $[\alpha]_D^{35}$ −51°.

A water-soluble compound similar to toyokamycin and monilin. Analysis: C 49.33–49.47, H 4.56–4.90, N 23.75–24.14.

*Streptomyces* sp.

Hiroshi Yamamoto, Shigehiro Fujii, Koichi Nakazawa, Akira Miyake, Hiromu Hitomi and Masahiko Imanishi, *Takeda Kenkyusho Nempo* 16 26 (1957).

1105 **Antifungal substance** produced by *Streptomyces* strain No. 1037.

Crystalline needles, m.p. 283–289°, $[\alpha]_D^{35}$ −51°. C 49.33–49.47 H 4.56–4.90 N 23.75–24.14, no halogen or sulfur.

It seems to belong to the same group of substances as toyokamycin and monilin.

Hiroshi Yamamoto, Shigehiro Fujii, Koichi Nakazawa, Akira Miyake, Hiromu Hitomi and Masahiko Imanishi, *Ann. Rept. Takeda Research Lab.* 16 26 (1957).

1106 **Argomycin**, $C_{25}H_{43}O_7N$, m.p. 164°, $[\alpha]_D^{25}$ +8.2° (in ethanol).

May be a macrolide.

*Streptomyces griseolus*

Toji Hata, Yoshimoto Sano, Hideo Tatsuta, Ryozo Sugawara, Akihiro Matsumae and Kokichi Kanamori, *J. Antibiotics* (Japan) 8A 9 (1955).

1107 **Aspelein**, $C_{29}H_{20}O_{10}$, dark red plates, no m.p.

This pigment contained two hydroxyl groups (diacetate, yellow crystals, m.p. 276–285°) and an alkoxyl group. Spectra described.

*Aspergillus elegans*

P. E. Gregoire, *Bull. soc. chim. biol.* 33 1681 (1951).

**Aterrimins**—complex containing aterrimins A and B, exhibiting characteristics of a lactone; contain C, H and O and have no definite m.p.

1108 **Aterrimin A**, $[\alpha]_D^{20}$ +245° in ethanol. U.V. 277, 287, 310–325 mμ in absolute alcohol C. 65.5 H 7.8 O 26.7 (by difference).

1109 **Aterrimin B,** $[\alpha]_D^{20}$ +342° in ethanol. U.V. same as A. C 69.7 H 8.05 O 22.25 (by difference).

*Bacillus subtilis* var. *aterrimus*

Gordon Alderton and Neva S. Snell, U. S. Patent 2,850,427 (1958).

1110 **Aureolic Acid,** Mg salt: $(C_{56\text{-}60}H_{96\text{-}104}O_{29\text{-}31})_2Mg$, yellow crystals, $[\alpha]_D$ +68° (c 1 in methanol).

A weak acid, green $FeCl_3$ test, negative Fehlings, anthrone.

*Streptomyces* sp.

Walton E. Grundy, Alma W. Goldstein, Charles J. Rickher, Marjorie E. Hanes, Halleck B. Warren, Jr. and John C. Sylvester, *Antibiotics and Chemotherapy* 3 1215 (1953).

1111 **Azalomycin B,** $C_{14}H_{24}O_5$, white needles, 185–187° (dec.) $[\alpha]_D^{25}$ −48° (c 1.0 in methanol). U.V. 252.5 mμ.

*Streptomyces hygroscopicus*

Manoru Arai, *J. Antibiotics* (Japan) 13A 51 (1960).

1112 **Azalomycin F,** $C_{30}H_{50}O_{10}N_2$, white needles, m.p. 125–127° (dec.) $[\alpha]_D^{22}$ +46° (c 1.0 in methanol).

U.V. resembles that of musarin and hygrostatin. I.R. differs.

Positive ninhydrin, negative $FeCl_3$, Molisch, anthrone and Millon.

*Streptomyces hygroscopicus*

H. D. Tresner and E. J. Backus, *Appl. Microbiol.* 4 243 (1956).

S. A. Waksman and A. T. Henrici, Bergey's "Manual of Determinative Bacteriology," 1957, pp. 796–797.

Mamoru Arai, *J. Antibiotics* (Japan) 13A 51 (1960).

1113 **Baccatine A,** $C_{26}H_{48}O_6N_2$ (proposed). Colorless crystals, m.p. 135°. Mol. Wt. ~480.

May be a depsipeptide (peptolide).

*Gibberella baccata.*

Jean Guérillot-Vinet, A. Guérillot-Vinet, Lucien Guyot, Jacques Montégut and Louis Roux, *Compt. rend.* 230 1424 (1950).

M. M. Shemyakin, *Angew. Chem.* 72 342 (1960).

1114 **Bacilipin A,** sheaves of needles, m.p. 76–78°.

C 42.6, H 6.3, N 2.5, Ba 24.6.

Negative Molisch, 2,4-DNPH, $AgNO_3$.

Positive $Br_2$.

1115 **Bacilipin B,** crystals, m.p. 105°.
C 52.45, H 6.75, N 2.09, Ba 21.6
Gave same tests as Bacilipin A.
Both A and B gave positive ninhydrin after hydrolysis.
*Bacillus subtilis*
G. G. F. Newton, *Brit. J. Exptl. Biol.* 30 306 (1949).

1116 **Bacilysin,** white powder containing C, H, O and N.
Gives a positive ninhydrin; negative biuret and Molisch tests.
Produced by the soil bacillus NTCC 7197.
E. P. Abraham and H. W. Florey, "Antibiotics," Vol. I *Antibiotics from bacteria in the genus bacillus,* Oxford University Press, London, 1949 Chap. 10, pp. 457–458.

1117 **Biformyne 1** (Biformin), $C_9H_{20}O_2$, white crystalline solid, m.p. 40–43°, U.V. 276, 278, 291 m$\mu$ in alkali.
*Polyporus biformis*
Marjorie Anchel and Marvin P. Cohen, *J. Biol. Chem.* 208 319 (1954).

1118 **Blasticidin A,** $C_{46\text{-}52}$, $H_{8\text{-}12}$ $N_{4\text{-}7}$, light yellow powder, m.p. 197–201°. U.V. 216 m$\mu$. Soluble in $H_2O$.

1119 **Blasticidin B,** colorless liquid, b.p. 36° (0.001 mm.). Insoluble in $H_2O$.

1120 **Blasticidin C,** red-brown powder. Insoluble in $H_2O$.
*Streptomyces griseochromogenes*
Kazuo Fukunaga, Tomomasa Misato, Itaru Iskii and Masaru Asakawa, *Bull. Agr. Chem. Soc.* (Japan) 19 181 (1955).

1121 **Blasticidin-S,** $C_{14}H_{20}O_5N_6$, white needles, m.p. 235° (dec.), $[\alpha]_D^{11}$ +108.4° (c 1.0 in water).
A basic antibiotic (forms a picrate).
Negative $FeCl_3$, Fehling, Tollens, Millon, Ehrlich, Sakaguchi, Molisch, biuret, ninhydrin, aldehyde and ammoniacal $AgNO_3$ tests. Blasticidin-S is a member of a complex with at least three other components, blasticidins A, B and C.
*Streptomyces griseochromogenes*
Setsuo Takeuchi, Kosei Hirayama, Kazaburo Ueda, Heiichi Sakai and Hiroshi Yonehara, *J. Antibiotics* (Japan) 11A 1 (1958).

1122 **Borrelidin,** $C_{28}H_{43}O_6N$, m.p. 145°, $[\alpha]_D^{27}$ −28° (in ethanol). An acidic compound.
*Streptomyces rochei*
J. Berger, L. M. Jampolsky and M. W. Goldberg, *Arch. Biochem.* 22 476 (1949).

1123 **Caerulomycin,** $C_{12}H_{11}O_2N_3$, colorless needles, m.p. 175°. Red $FeCl_3$ test. Contains one methoxyl group.
*Streptomyces caeruleus*
A. Funk and P. V. Divekar, *Can. J. Microbiol.* 5 317 (1959).

1124 **Camphomycin,** white needles, m.p. ~149°. Positive Nessler and Tollens.
*Streptomyces rutgersensis* var. *castelarense*
Augusto P. Cercos, *Rev. argentian agron.* 20 53 (1953).

1125 **Candidulin,** $C_{11}H_{15}O_3N$, white needles, m.p. 88°, $[\alpha]_D^{24}$ +15° ± 2° (c 1 in chloroform).
A neutral, non-aromatic substance. Negative ninhydrin, 2,4-DNPH, $FeCl_3$.
*Aspergillus candidus*
P. G. Stansly and N. H. Ananenko, *Arch. Biochem.* 23 256 (1949).

1126 **Canescin,** $C_{15}H_{14}O_7$, white needles, m.p. 201–202° (dec.). Purple color with $FeCl_3$ in ethanol.
*Penicillium canescens*
Yield 30–110 mg. per liter.
P. W. Brian, H. G. Hemming, J. S. Moffatt and C. H. Unwin, *Trans. Brit. Mycol. Soc.* 36 243 (1953).

1127 **Cardinophyllin** (Carzinophilin), potassium salt: colorless needles, m.p. 220° (dec.).
Contains C, H, O, N. Positive xanthoprotein, negative ninhydrin, diphenylamine. Negative resorcinol, Millon, Liebermann.
*Streptomyces sahachiroi*
Toju Hata, Fumiwaka Koga, Yoshimoto Sano, Kokichi Kanamori, Akihiro Matsumae, Ryozo Sugawara, Tadashi Hoshi and Tatsuo Shima, *J. Antibiotics* (Japan) 7A 107 (1954).
Fujiki Hata and Takamoto Sano, Japanese Patent 7590 (1956).

1128 **Carzinophilin A,** colorless needles, m.p. 217–222° (dec.), $[\alpha]_D^{28}$ +57.8° (in chloroform).
Positive ninhydrin, 2,4-DNPH, bromine uptake, an-

throne, Baeyer, xanthoproteic. Unstable in aqueous solution.

*Streptomyces sahachiroi* n. sp.

Hideo Kamada, Shigetoshi Wakaki, Yasuo Fujimoto, Keitaro Tomioka, Satoshi Ueyama, Hakudai Marumo and Keizo Uzu, *J. Antibiotics* (Japan) 8A 187 (1955).

1129 **Cerevioccidin**, $C_{22}H_{39}O_4N_5$, colorless needles, m.p. 249° (dec.). Negative biuret, ninhydrin, Fehling, Sakaguchi, Tollens, glucosamine. Positive Janovsky.

*Streptomyces* sp. resembling *S. cacaoi*

Satoru Yamashita, Teruzo Sawazaki, Makoto Kawasaki, Goto Nakamura, Kentaro Anzai, Kiyoshi Isono, Yoshiko Serizawa, Yoshiko Sekiyama and Saburo Suzuki, *J. Antibiotics* (Japan) 8A 42 (1955).

**Chlamydosporin**, complex of two closely related antibiotics produced by the fungus *Fusarium* MLF 1230 found in insects and their larvae.

1130 **Chlamydosporin A**, light brown amorphous substance insoluble in water.

1131 **Chlamydosporin B**, colorless, crystalline, soluble in water.

Both contain 4.3% N but no sulfur.

Albert Faivré-Amiot, Hermon Darpoux and Louis Roux, *Compt. rend.* 235 912, 982 (1952).

1132 **Chromomycin $A_3$** (main component of complex), $C_{22\text{-}23}H_{32\text{-}34}O_{11}$, yellow powder, m.p. 183° (dec.), $[\alpha]_D^{20}$ −26° (c 1 in ethanol.

May be related to the actinomycins.

*Streptomyces griseus* No. 7

Yoshitomo Aramaki, Junmei Watanabe, Ichiro Ishikawa, Akira Miyake, Homu Ito, Koichi Nakazawa, Koichi Ogata, Motoo Shibata, Masaji Igarashi and Kazuo Tanabe, *Ann. Repts. Takeda Research Lab.* 14 60 (1955).

Tatsuoka *et al.*, *Gann.* 49 Suppl. 23 (1958).

S. Wakaki *et al.*, *Antibiotics and Chemotherapy* 8 228 (1958).

Motoo Shibata, Kazuo Tanabe, Yoshio Hamada, Koiti Nakazawa, Akira Miyake, Hiroshi Hitoma, Masuo Miyamoto and Komei Mizuno, *J. Antibiotics* (Japan) 13B 1 (1960).

1133 **Chrysergonic Acid**, $C_{32}H_{30\text{-}32}O_{14}$, fine yellow needles, m.p. 268–270° from chloroform (250–257° from acetic acid), $[\alpha]_D^{20}$ −3° → +34° (in pyridine).

*Claviceps purpurea*

A. Stoll, J. Renz and A. Brack, *Helv. Chim. Acta* 35 2022 (1952).

1134 **Chrysomycin,** $C_{22}H_{20}O_7$ (proposed), greenish yellow crystals, m.p. 255–260° (dec.), $[\alpha]_D^{22}$ +16° (c 1 in acetic acid).

Neutral, photosensitive compound. Takes up $4H_2$ with loss of color.

*Streptomyces* sp.

Frieda Strelitz, Helen Flon and Igor V. Asheshov, *J. Bacteriol.* 69 280 (1955).

1135 **Clitocybin,** colorless crystals, m.p. 77°.

*Clitocybe candida*

A. Charles Hollande, *Compt. rend.* 221 361 (1945); 228 1758 (1949).

1136 **Coelicolorin,** purplish red powder, 142–146°.

*Streptomyces coelicolor*

Yuichi Hatsuta, *J. Antibiotics* (Japan) 2 276 (1949).

1137 **Collinomycin,** orange prisms, m.p. 280°.

*Streptomyces collinus* (mycelium)

Hans Brockmann and Karl-Heinz Renneberg, *Naturwissenschaften* 40 166 (1953).

1138 **Compound $C_{11}H_{20}O_9N_2$.**

A basic red pigment, yellow in alkaline, red in acid solutions. Positive Bayer, diazo tests.

*Inocybe patoullardii* Bres.

Helmut Müller, Dissertation, Würzburg, 1959.

1139 **Cosynthetic Factor-1** $C_{14\text{-}15}H_{17}O_7N_3$, crystalline. An acidic compound, Mol. Wt. 340–360.

Thought to be a cofactor in the biosynthesis of tetracyclines.

*Streptomyces aureofaciens* strain W-5, *S. albo-niger, S. griseus, S. albas, S. platensis, S. hygroscopicus, S. rimosus*

Jerry Robert Daniel McCormick, Nancy Hazlett Arnold, Ursula Hirsch, Philip Andrew Miller and Newell Oscar Sjölander, Union of South Africa Patent Application 59–2174 (1959).

1140 **Croceomycin,** $C_{22}H_{18}O_6$, m.p. 325° (subl. 240° at 1–2 mm.), $[\alpha]_D^{18}$ −32 ±4°.

Forms a triacetate. Diazomethane adds two methyl groups.

*Streptomyces arabicus*

Motoo Shibata, Koichi Nakazawa, Akira Miyake, Michitaka Inoue and Akira Akabori, *Takeda Kenkyusho Nempo* 16 32 (1957). (*Chem. Abstr.* 52 10279e)

1141 **Cyanomycin,** $C_{15}H_{12}N_2O_2$ (proposed), dark blue needles, m.p. 128° (dec.).

A basic antibiotic pigment with pH-indicating properties, apparently distinct from other known pigments. Aureothricin occurs in the same culture.

*Streptomyces* strain No. 4738

Masanao Funaki, Fumiyasu Tsuchiya, Kiyoharu Maeda and Takeshi Kamiya, *J. Antibiotics* (Japan) 11A 143 (1958).

1142 **Datemycin,** $C_{58}H_{102}O_6N_4$, colorless powder, m.p. 197° (dec.), $[\alpha]_D^{15}$ −43.7° (c 1 in water).

U.V. maximum at 247 mμ. Negative ninhydrin $\xrightarrow[100°,\ 10\ hrs.]{6N\ HCl}$ positive ninhydrin. Negative Hopkins-Cole, xanthoprotein, Sakaguchi, Millon, Elson-Morgan, Molisch, Fehling, silver mirror tests.

"M-14" strains

Masahiko Kuroya and Yasuo Koyama, Japanese Patent 6648 (1959).

1143 **Desertomycin,** $C_{33}H_{60\text{-}62}O_{14}N$, snow white crystals, m.p. 189°.

Positive ninhydrin, C-methyl. Acetylates, hydrogenates, decolorizes bromine or permanganate.

A crystalline antifungal agent, flavofungin, has been isolated from the same culture.

*Streptomyces flavofungini*

J. Úri and I. Béhési, *Nature* 181 908 (1958).

J. Úri, R. Bognár and B. Varga, *ibid.* 182 401 (1958).

1144 **Diaporthin,** $C_{13}H_{14}O_3$, white crystals, m.p. 91.5–92.5° $[\alpha]_D^{25}$ +58° (c 1 in chloroform).

*Endothia parasitica*

A. Neelameghan, *Hindustan Antibiotics* 2 13 (1959).

1145 **Diplococcin,** antibacterial substance elaborated by certain milk streptococci. In the same category as the sulfur-free polypeptides, gramicidin and tyrocidine. Unlike these polypeptides diplococcin contains arginine residues, shows no tendency to crystallize and is obviously of greater molecular complexity.

C 50.5, H 7.3, N 13.1, no sulfur.

*Streptomyces lactis*
A. E. Oxford, *Biochem. J.* 38 178 (1944).
*Idem., ibid.* 39 xiii (1945).

1146 **Distamycin A,** pure white powder, basic, forms salts.
Positive biuret test.
CIBA, Australian Patent 28,469 (1957).

1147 **D-Substance,** white needles, m.p. 124–125°.
Highly toxic.
*Streptomyces flavus* O-2
Isao Takahashi, *J. Antibiotics* (Japan) 6A 117 (1953).

1148 **Elaiophylin** $(C_6H_{10}O_2)_n$, no N, S, X, white crystals, m.p. 178–183° (dec.) $[\alpha]_D^{20}$ −49° (in chloroform).
*Streptomyces melanosporus* (sine *melanosporofaciens*)
F. M. Arcamone, C. Bertazzoli, M. Ghione and T. Scotti, *Giorn. microbiol.* 7 207 (1959).

1149 **Endosubtilysin,** yellow powder, soluble in alcohol and chloroform. Forms a water-soluble sodium salt. Appears to be an organic acid.
*Bacillus subtilis*
Louis de Saint-Rat and Henry R. Olivier, *Compt. rend.* 222 297 (1946).

1150 **Enteromycin,** m.p. 167–168°, U.V. 282 mμ.
C 38.2, H 4.62, N 4.3.
*Streptococcus albireticuli*
Teisuke Osato, Masahiro Ueda, Setsuko Fukuyama, Koki Yagishita, Yoshiro Okami and Hamao Umezawa, *J. Antibiotics* (Japan) 8A 105 (1955).

1151 **Ergochrysin,** $C_{28}H_{28}O_{12}$, yellow-golden leaflets, m.p. 266° from chloroform (242–244° from alcohol-pyridine).
*Claviceps purpurea*
C. Jacoby, *Arch. exp. Pathol. Pharmakol.* 39 85 (1897).
Werner Bergmann, *Ber.* 65 1486, 1489 (1932).

1152 **Ergoflavin,** $C_{30}H_{26}O_{14}$, yellow needles, m.p. 350° (dec.) from methanol or dioxane, $[\alpha]_D^{21}$ +37.5° (c 1.236 in acetone).
Structural features determined:
4 phenolic hydroxyls
2 alcoholic hydroxyls
2 carbonyls
2 γ-lactones
*Claviceps purpurea*

The yield is 1–2% of the weight of the dry sclerotia.
G. Eglinton, F. E. King, G. Lloyd, J. W. Loder, J. R. Marshall, Alexander Robertson and W. B. Whalley, *J. Chem. Soc.*, 1833 (1958).

The relationship of ergoflavin to the other yellow pigments, secalonic acid, ergochrysin, chrysergonic acid, sclererythrin, scleroxanthin, sclerocristallin and ergoxanthin (some of them identical) is discussed in the above paper as well as in an earlier paper by:
Albert Freeborn, *Pharm. J.* 88 568 (1912).
A. Stoll, J. Renz and A. Brack, *Helv. Chim. Acta.* 35 2022 (1952).

1153 **Estin,** $C_{16}H_{14}O_6Cl_2$, m.p. 223°.
Contains two methoxyl groups. A second and similar compound, Nordin, is produced with it. A 143 mg. sample of the mixture was obtained from 1480 ml. of culture solution.
*Penicillium paxilli* var. *echinulatum*
Eitaro Komatsu, Japanese Patent 4799 (1953).

1154 **Eumycetin,** fine white needles, m.p. 148–150°.
Positive $FeCl_3$, negative biuret, ninhydrin, Molisch, Fehling.
*Streptomyces* sp. similar to *S. purpurochromogenes*
Edwin A. Johnson and Kenneth L. Burdon, *J. Bacteriol.* 51 591 (1946).

1155 **Eumycin,** amorphous precipitate, heat-stable in acid, unstable in alkaline solutions above pH 8.0.
*Bacillus subtilis*
Edwin A. Johnson and Kenneth L. Burdon, *J. Bacteriol.* 51 591 (1946).

1156 **Exfoliatin,** $C_{27}H_{40}O_{16}Cl$, colorless needles, m.p. 172°.
Positive $FeCl_3$, Molisch, Negative Fehling.
*Streptomyces exfoliatus*
Hamao Umezawa, Kiyoshi Oikawa and Motoko Suzuki, *J. Antibiotics* (Japan) 5 466 (1952).

1157 **Fairodin,** crystalline, m.p. 237–239° (dec.) $[\alpha]_D^{25}$ −102° (c 1 in water).
C 59.6, H 6.7, N 14.3.
*Bacillus brevis*

S. Oya, Japanese Patent Application SHO 32–3997 (1957).

1158 **Fermicidin,** $C_{14}H_{21}O_4N$, colorless needles, m.p. 96°, $[\alpha]_D^{18}$ +52.3° ±1.5° (c 0.65 in water).

*Streptomyces* sp. similar to *S. griseolus*

Seizi Igarasi and Shozo Wada, *J. Antibiotics* (Japan) **7B** 221 (1954).

1159 **Fermizin,** $C_{14}H_{21}O_4N$, needles, m.p. 96–98°.

An antifungal agent.

*Streptomyces griseus*

About 10 g. were obtained from 100 l. of fermentation broth. Apparently identical with fermicidin.

Koichi Ogata, Masaji Igarashi and Shozo Wada, Japanese Patent Application 6150 (1958).

1160 **Fervenulin,** $C_7H_7O_2N_5$, yellow crystals, m.p. 178–179° (dec.). Mol. Wt. 193. Acid-stable, base-labile. U.V. peaks at 275, 239 mμ.

*Streptomyces fervens*

T. E. Eble, E. C. Olson, C. M. Large and J. W. Shell, 7th Annual Symposium on Antibiotics, Washington, D. C., 1959.

1161 **Flavensomycin,** pale yellow crystals, m.p. 152°.

A water soluble compound containing nitrogen but not sulfur or halogen. Some carbohydrates tests were positive. U.V. maximum at 251 mμ.

*Streptomyces tanaschiensis* type

R. Craveri and G. Giolitti, *Nature* 179 1307 (1957).

1162 **Flavucidin,** $C_{34}H_{55}NO_9$, colorless needles, m.p. 144–145°, $[\alpha]_D^{20}$ 94°, U.V. 275 mμ.

Positive Molisch. Negative ninhidrin.

*Streptomyces* sp. No. 14420

Motoo Shibata, Koichi Nakazawa, Akira Miyake, Michitaka Inoue, Jiro Terumichi and Hiroshi Kawashima, *Ann. Rept. Takeda Research Lab.* 17 16 (1958).

1163 **Folimycin,** m.p. 163–164° (dec.) agricultural antifungal antibiotic.

*Streptomyces nayagawaensis* n. sp.

Hiroichi Yamamoto, Koiti Nakazawa, Satoshi Horii and Akira Miyake, *J. Agr. Chem. Soc. Japan* 34 268 (1960).

1164 **Fomecin A,** $C_8H_8O_5$, m.p.: dec. >160°.

Weakly acidic, thermostable, alkali labile.

*Fomes* (*Polyporus*) *juniperinus*

Marjorie Anchel, Annette Hervey and William J. Robbins, *Proc. Nat. Acad. Sci. U. S.* 38 655 (1952).

1165 **Fuscomycin**, m.p. 180° (dec.).
*Streptomyces fuscus*
Fujiki Hata and Keigen Sano, Japanese Patent 5046 (1953).

1166 **Glutinosin**, $C_{48}H_{60}O_{16}$ (proposed), colorless plates, gradual dec. to 300°, $[\alpha]_D^{20}$ ~+54° (c 0.2 in benzene).
*Metarrhizium glutinosum*
P. W. Brian and J. C. McGowan, *Nature* 157 334 (1946).

1167 **Grisamine**, $C_{28}H_{38}O_{10}N_6$ or $C_{20}H_{30}O_7N_4$ (proposed), colorless needles, m.p. 167–170°.
Negative Fehling, $FeCl_3$, Sakaguchi, ninhydrin, biuret.
*Streptomyces* sp. similar to *S. griseoflavus*
Teruzo Sawazaki, Goto Nakamura, Makato Kawasaki, Satoru Yamashita, Kiyoshi Isono, Kentaro Anzai, Yoshiko Serizawa, Yoshiko Sekiyama and Saburo Suzuki, *J. Antibiotics* (Japan) 8A 39 (1955).

1168 **Griseoflavin**, colorless needles, m.p. 210–215° (dec.).
Not precipitated by peptide reagents. Negative carbohydrate and amino sugar tests, $FeCl_3$.
*Streptomyces griseoflavus*
Yoshio Waga, *J. Antibiotics* (Japan) 6A 66 (1953).

1169 **Griseoviridin**, $C_{22}H_{29}O_7N_3S$ (proposed), colorless crystals, m.p. (polymorphic) 158–166°, 194–200°, 230°, 240° (dec.), $[\alpha]_D^{27}$ −237° (c 0.5 in methanol).
Neutral compound. Negative $FeCl_3$, Sakaguchi, positive Bayer. Gives cystine on acid hydrolysis. Further structural features are suggested in the last reference below.
*Streptomyces griseus, S. griseoviridus* n. sp.
Quentin R. Bartz, Jean Standiford, James D. Mold, Doris W. Johannessen, Albert Ryder, Andrew Maretzki and Theodore H. Haskell, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, p. 777.
John Ehrlich, George L. Coffey, Myron W. Fisher, Margaret M. Galbraith, Mildred Penner Knudsen, Raymond W. Sarber, A. S. Schlingman, Robert M. Smith and Jean K. Weston, *ibid.*, p. 790 (1954–1955).
Lucia E. Anderson, John Ehrlich, Sung Huang Sun and Paul R. Burkholder, *Antibiotics and Chemotherapy* 6 100 (1956).

D. E. Ames, R. E. Bowman, J. F. Cavalla and D. D. Evans, *J. Chem. Soc.*, 4260 (1955).

D. E. Ames and R. E. Bowman, *ibid.*, 4264 (1955).

1170 **Helenine.**

An unstable, little characterized antiviral agent. A ribonucleoprotein.

*Penicillium funiculosum*

Richard E. Shope, *J. Exp. Med.* 97 601, 639 (1953).

U. J. Lewis, Edward L. Rickes, Laurella McClelland and Norman G. Brick, *J. Am. Chem. Soc.* 81 4115 (1959).

1171 **Heliomycin,** needles, chars >300°, complex U.V., Mol. Wt. 235. Positive $FeCl_3$ and Millon.

May be a polypeptide.

*Actinomyces flavochromogenes* var. *heliomycini*

M. G. Brazhnikova, T. A. Uspenskaya, L. B. Sokolova, T. P. Preobrazhenskaya, G. F. Gauze, R. S. Ukholina, V. A. Shorin, O. K. Rossolimo and T. P. Vertogradova, *Antibiotiki* 3 29 (1958).

1172 **Hirsutic Acid C,** $C_{15}H_{20}O_4$ (proposed), colorless crystals, m.p. 179.5°, $[\alpha]_D^{20}$ +11.9° (in absolute ethanol).

A group of acidic materials. Hirsutic acid C has been best characterized. It is a monobasic acid, only slightly soluble in $H_2O$, soluble in most organic solvents. Negative 2,4-DNPH, $FeCl_3$, Fehling. White precipitate with Br water.

*Stereum hirsutum*

N. G. Heatley, M. A. Jennings and H. W. Florey, *Brit. J. Exp. Path.* 28 35 (1947).

1173 **Hygroscopin A,** $C_{13}H_{24}O_3N_2$, oil, b.p. 64° (0.003 mm.), $n_D^{13}$ 1.4830, $[\alpha]_D^{14}$ 84.7° (in methanol).

1174 **Hygroscopin B,** $C_{15}H_{28}O_3N_2$, oil, b.p. 70° (0.008 mm.), $n_D^{14}$ 1.4935, $[\alpha]_D^{14}$ −38.8° (in ethanol).

*Streptomyces hygroscopicus*

Koichi Nakazawa, Kinzo Oki, Isao Tadokoro, Mikio Honjo, Hiroshi Hitomi and Jisaburo Ueyanagi, *J. Agr. Chem. Soc. Japan* 28 296 (1954).

Sueo Tatsuoka, Akira Miyake, Mikio Honjo, Hiroshi Hitomi, Jisaburo Ueyanagi, Masuo Miyamoto, Koiti Nakazawa and Kinzo Oki, *J. Antibiotics* (Japan) 7B 329 (1954).

1175 **Hygrostatin,** light yellow powder, m.p. 129–131° (dec.), $[\alpha]_D^{20}$ +43° (c 1.21 in methanol).

Contains nitrogen, but no sulfur or halogen. U.V. at 240 mμ.

*Streptomyces hygrostaticus*

Kenzo Furushiro, Kiyotake Shimizu, Heiichi Sakai, Masayuki Minoyata and Toshio Fujisawa, Iyaku, *Shigen Kankyusho Nempo* 24–39 (1958). (*Chem. Abstr.* 54 10048b)

1176 **Illudin M,** $C_{21}H_{32}O_7$, prismatic rods in ethanol, m.p. 216° (cor.). $[\alpha]_D^{20}$ −126° in ethanol. Mol. Wt. 386. U.V. 247, 330 mμ in 95% ethanol.

Contains two acidic groups and an α,β-unsaturated carbonyl group.

Yield 0.08 g. per liter.

1177 **Illudin S,** $C_{15}H_{22}O_4$, crystalline, m.p. 124–125°. Mol. Wt. 264. U.V. 235, 328 mμ in 95% ethanol.

Yield 0.33 g. per liter.

*Clitocybe illudens*

Marjorie Anchel, Annette Hervey and William J. Robbins, *Proc. Nat. Acad. Sci. U. S.* 36 300 (1950); 38 927 (1952).

A third, antibiotically inactive substance, $C_{10}H_{16}O_4$ or $C_{15}H_{24}O_6$, crystals, m.p. 72–74°, $[\alpha]_D^{20}$ −107° (in absolute ethanol) occurred in the same culture.

1178 **Imoticidin,** m.p. 245° (darkening from 210°).

An antibiotic isolate, C 64.71, H 9.50, N 0.0, $H_2O$ 7.63. Mol. Wt. 532–553.

*Streptomyces albus*

Tadao Inouye, Yasuhiro Okamoto and Yosikazu Nishikado, *Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ.* 11 95 (1959). (In English)

1179 **Indigoidine,** deep blue pigment, no melting point.

Low solubility in most solvents. Soluble in dilute hydrochloric acid. Analysis of partially purified compound: C 47.74, H 3.82, N 17.95. Formed a red crystalline acetate, m.p. >300° (dec.), but more soluble: C 49.63, H 3.98, N 16.05, acetyl 16.9. A red benzoate was also prepared.

*Corynebacterium insidiosum* (McCulloch) Jensen, *Pseudomonas indigofera, Erwinia chrysanthemi, Arthrobacter* sp.

B. Elazari-Volcani, *Arch. Mikrobiol.* 10 343 (1939).
D. A. Kuhn and M. P. Starr, *Bacteriol. Proc.* 58 (1956).
Mortimer P. Starr, *Arch. Mikrobiol.* 30 325 (1958).

1180 **Isorhodomycin A,*** $C_{20}H_{29}O_8N$ or $C_{21}H_{31}O_8N$, hydrochloride: deep red prisms, m.p. 220°, $[\alpha]_{6060\text{-}7600}^{18}$ +268° ±30° (c 1 in methanol).

Occurs with rhodomycin A.

Either compound on mild hydrolysis yields a water-soluble, N-containing moiety and a water-insoluble chromophore.

*Streptomyces purpurascens*

Hans Brockmann and Peter Patt, *Chem. Ber.* 88 1455 (1955).

1181 **Itaconitin,** yellow needles, m.p. 169°.

Negative Beilstein, fuchsin, xanthogen, Legal, Ehrlich, Liebermann, $FeCl_3$ tests. Decolorized bromine and $KMnO_4$. Formed an acetate, semicarbazone and 2,4-DNPH. Hydrogenated to hexahydroitaconitin.

*Aspergillus itaconicus*

Kono Kinoshita and Shoichi Nakajima, *Hoshi Yakka Daigaku Kiyo* 7 17 (1958).

1182 **Laterosporin**

Appeared to be a peptide. Isolated as a hydrochloride. Soluble in water. Tendency to precipitate out of solution in NaCl solution or in 0.2 M phosphate buffer.

*Bacillus laterosporus*

Ella M. Barnes, "Antibiotics," Vol. II *Antibiotics from bacteria in the genus bacillus,* Oxford University Press, London, 1949, Chap. 10 appendix, pp. 1540–1541.

1183 **Latumcidin** (Sulfate), $C_{11}H_{13}O_2N \cdot H_2SO_4$, white needles, m.p. 140°, $[\alpha]_D^{21}$ +148.9° (c 0.1 in 0.1 N sodium hydroxide).

A basic, unstable, antifungal agent. Positive diazo, Baeyer, bromine. Negative $FeCl_3$, Fehling, Tollens, Ehrlich, Sakaguchi, ninhydrin, biuret, Molisch.

Somewhat resembles eulicin, and abikoviromycin.

*Streptomyces reticuli* var. *latumcidus*

Yoshio Sakagami, Ichiro Yamaguchi, Hiroshi Yonehara, Zoichiro Okimoto, Sadazi Yamanouchi, Kazuo Takiguchi and Heiichi Sakai, *J. Antibiotics* (Japan) 11A 6 (1958).

* Identical with entry 597.

1184 **Lenamycin**, $C_4H_4O_3N_2$ or $C_4H_4O_2N_2$ (proposed), colorless crystals, m.p. 202–207° (dec.) optically inactive.

Apparently an $\alpha,\beta$-unsaturated amide. Negative ninhydrin, biuret, anthrone, $FeCl_3$, Sakaguchi, Elson-Morgan, nitro and oxime tests.

A streptomycete

The yield was 72 mg. from 5 l. of broth. Occurs together with *trans*-cinnamic acid amide and ethoxyethene-1,2-dicarboamide.

Yasuharu Sekizawa, *J. Biochem.* (Japan) 45 159 (1958).

1185 **Lenzitin**, colorless needles, m.p. 166°.

Contains C, H, O only. Positive $FeCl_3$, $KMnO_4$.

*Lenzites sepiaria* (Wulf)

M. Litvinov and E. Moiseeva, *Priroda* 1 60 (1951).

1186 **Litmocidin**, m.p. 144–146° (dec.).

An acid-base indicator. Decolorized by bisulfite or zinc dust, color restored by air oxidation.

*Proactinomyces cyaneus* var. *antibioticus*

G. F. Gause, *J. Bacteriol.* 51 649 (1946).

M. G. Brazhnikova, *ibid.* 51 655 (1946). (Isolation)

1187 **Longisporin**, $C_{36}H_{58}O_{10}$, crystals, m.p. 99–101°, $[\alpha]_D$ +2.62°.

Alkaline hydrolysis yields a hydroxy acid $C_{10}H_{16}O(OH)(COOH)$. It was suggested that the antibiotic is a cyclic ester of three such acid units.

*Actinomyces longispori*

G. P. Menshikov and M. M. Rubinshtein, *Zhur. Obshchei Khim.* 26 2035 (1956).

1188 **Lustericin**, $C_{40}H_{64}O_{13}$, white crystals, m.p. 130° $[\alpha]_D^{20}$ 0°, mol. wt. 130.

*Streptomyces* sp.

Motoo Shibata, Koichi Nakazawa, Michitaka Inoue, Jiro Terumichi and Akira Miyake, *Ann. Rept. Takeda Research Lab.* 17 19 (1958).

1189 **Lycopersin**, $C_{20}H_{15}O_8$, bright red needles, darkens from 250°, dec. 305°.

*Fusarium lycopersici, F. vasinfectum*

G. Kreitman and F. F. Nord, *Arch. Biochem.* 21 457 (1949).

Gerald Kreitman, Oldrich K. Sebek and F. F. Nord, *ibid.* 28 77 (1950).

1190 **Malucidin,** complex yeast protein, soluble in water, not coagulable, not dialyzable. Contains organic phosphorus to which its activity can be related.

The protein is combined with RNA, while the latter by itself has very little, if any, antibacterial property.

Brewers' and bakers' yeasts

I. A. Parfentjev, *Federation Proc.* 16 428 (1957).

1191 **Marasmic Acid,** $C_{16}H_{20}O_4$ (proposed) colorless needles, m.p. 174° (sealed tube), $[\alpha]_D^{25}$ 176° (c 1.4 in acetone).

A monobasic acid with reducing properties. Negative $FeCl_3$, $Br_2$ in $CCl_4$. Forms a 2,4-dinitrophenylhydrazone.

*Marasmius conigenus*

Frederick Kavanagh, Annette Hervey and William J. Robbins, *Proc. Nat. Acad. Sci. U. S.* 35 343 (1949).

1192 **Marcomycin,** $C_{15}H_{30}O_9N_2$, white crystals, m.p. 160–180° (dec.).

*Streptomyces hygroscopicus*

German Patent 1,027,846 (1958).

1193 **Megacidin,** $C_{24}H_{38}O_{10}$ (proposed), colorless crystals, m.p. 162–164°, $[\alpha]_D$ −51° (c 0.958 in ethanol).

A neutral compound with an easily saponifiable ester or lactone group.

Also isolated from the same fermentation were: **L-leu-**
1194 **cyl-L-proline anhydride,** m.p. 158–165°, $[\alpha]_D$ −128° (c
1195 0.968 in ethanol) and **L-leucyl-L-leucine anhydride.**

*Streptomyces* sp.

L. Ettlinger, E. Gäumann, R. Hütter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog, P. Reusser and H. Zähner, *Monatsh. Chem.* 88 989 (1957).

1196 **Melanosporin,** $C_{56\text{-}63}H_{105\text{-}117}O_{20\text{-}22}N_3$, yellowish white amorphous solid, m.p. 132–134°, $[\alpha]_D^{20}$ +30° (c. 1.57 in methanol).

Strong acid hydrolysis yields three ninhydrin-positive compounds. Negative $FeCl_3$. Positive ninhydrin.

*Streptomyces melanosporus* (*sine melanosporofaciens*) n. sp.

F. M. Arcamone, C. Bertazzoli, M. Ghione and T. Scotti, *Giorn. microbiol.* 7 207 (1959).

1197 **Mesenterin,** colorless needles, m.p. 122–126°.

A basic compound, analysis: C 65.82, H 7.10, N 8.66. Positive Molisch, negative ninhydrin, biuret, Fehling, $FeCl_3$.

Occurs with azomycin and antibiotic 446.

*Nocardia mesenterica*

Masahiro Ueda and Hamao Umezawa, *J. Antibiotics* (Japan) 8A 164 (1955).

1198 Metabolite, $C_{24}H_{50}O_2$, colorless crystals, m.p. 82°.

Negative Liebermann-Burchard, $KMnO_4$, tetranitromethane tests.

*Amanita phalloides*

Heinrich Wieland and Gustav Coutelle, *Ann.* 548 270 (1941).

1199 Metabolite of *Coprinus comatis*, $C_{12}H_{16}ON_2S$, m.p. 157°.

A basic compound, containing a phenolic hydroxyl group. Positive Millon, Pauly diazo tests. Raney nickel desulfurization gave a compound, m.p. 250° (dec.).

*Coprinus comatis* Gray

Paul Heinz List, *Arch. Pharm.* 291 502 (1958).

1200 Metabolite from *Curvularia lunata*, $C_{14}H_{18}O_5$, colorless needles, m.p. 195°.

Insoluble in aqueous sodium carbonate, soluble in aqueous sodium hydroxide. Brown color with alcoholic ferric chloride.

*Curvularia lunata*

Also isolated from the same culture were mannitol and a trace of crystalline material, m.p. 176–178° (dec.).

T. Krishna Murty and S. Sankara Subramanian, *Indian J. Pharm.* 20 72 (1958).

1201 Metamycin, white crystals, m.p. 173° (dec.) $[\alpha]_D^{589}$ +36.6 (c 0.11 in methanol) U.V. 237, 305–307 $m\mu$ in 0.1 N sodium hydroxide.

C 43.95, H 4.06, N 14.45, S 13.57.

Positive Fehling, Tollens, $Br_2$, decolorization of permanganate, 2,4-DNPA tests. Negative $FeCl_3$ and Sakaguchi tests.

*Streptomyces matensis*

P. Sensi, R. Ballotta and G. G. Gallo, *Antibiotics and Chemotherapy* 9 76 (1959).

1202 Microcin A, neutral, reddish violet in color, separated at pH 7.0.

1203 Microcin B, acidic, yellowish red, slightly soluble in water separated at pH 2.0.

Both give negative Molisch and $FeCl_3$; vary from micromonosporin in activity, have much resistance to U.V.

*Micromonospora* sp.

Tomotsune Taira and Shigehiro Fujii, *J. Antibiotics* (Japan) 5 187 (1952).

1204 **Mikamycin A,** $C_{31}H_{39}O_9N_3$, yellowish white crystals, m.p. 147–152° (dec.), $[\alpha]_D^{28}$ −152° (c 0.5 in methanol).

Apparently identical with the principal active component of the streptogramin and antibiotic No. 899 complexes.

A neutral antibiotic. Negative ninhydrin, biuret, glucosamine, maltol and Millon. Green-black $FeCl_3$. Brown precipitate with the Tollens reagent. Positive Benedict. Forms a 2,4-DNPH.

*Streptomyces mitakaensis*

Mamoru Arai, Keiko Karasawa, Shoshiro Nakamura, Hiroshi Yonehara and Hamao Umezawa, *J. Antibiotics* (Japan) 11A 14 (1958).

Mamoru Arai, Koichi Okabe, Hiroshi Yonehara and Hamao Umezawa, *ibid.* 11A 21 (1958).

Koichi Okabe, *ibid.* 12A 86 (1959).

1205 **Mikamycin B,** $C_{45}H_{58}O_{11}N_8$ (proposed), white platelets, m.p. 160°, dec. 262°, $[\alpha]_D^{15}$ −61.3° (c 1.0 in methanol).

Similar to PA-114 B in physical and chemical properties but differs from staphylomycin S. It is thought to be different from both.

Gives a positive $FeCl_3$. Negative Ehrlich, biuret, Fehling, Tollens, nearly negative ninhydrin.

*Streptomyces mitakaensis*

Kiyoshi Watanabe, Hiroshi Yonehara, Nobuo Tanaka and Hamao Umezawa, *J. Antibiotics* (Japan) 12A 112 (1959).

Koyoshi Watanabe, *ibid.* 13A 57 (1960).

**Mitomycins.**

1206 1207 1208 1209 1210 1211 1212 1213 A complex from which several compounds were isolated: colorless fractions **W-1** (m.p. 148°), **W-2** (m.p. 138°) and **W-3** (m.p. 187°). Pigmented fractions **A** (red crystals) m.p. 167°, **B** (violet crystals), **C** (bluish violet crystals), **Y** (yellow crystals) m.p. 180–240° (dec.) and **R** (red-brown amorphous powder). Pigmented fractions are antibiotic.

1214 **Mitomycin C,** $C_{54}H_{61}O_{19}N_{13}$ (tentative), deep bluish violet crystals, m.p.: no melting or dec. noted below 360°.

Positive $FeCl_3$, Fehling, biuret, Ehrlich, decolorization of permanganate. Negative Benedict, Tollens, ninhydrin, Milton, Raymond. Mol. Wt. ~1120.

The mitomycins may be related to the actinomycins.

*Streptomyces caespitosus*

S. Wakaki, H. Marumo, K. Tomioka, G. Shimizu, E. Kato, H. Kamada, S. Kudo and Y. Fugimoto, *Antibiotics and Chemotherapy* 8 228 (1958).

Toju Hata, Yoshimoto Sano, Ryozo Sugawara, Akihiro Matsumae, Kokichi Kamamori, Tatsuo Shima and Tadashi Hoshi, *J. Antibiotics* (Japan) 9A 141 (1956).

1215 **Moldin**, gives positive Molisch and $FeCl_3$ but negative biuret, ninhydrin, Tollens, Fehling and Sakaguchi tests.

*Streptomyces sp.* res. *S. phalochromogenus*

Kenji Maeda, Yoshiro Okami, Osamu Taya and Hamao Umezawa, *J. Antibiotics* (Japan) 5 465 (1952).

1216 **Monilin**, $C_{15}H_{20}O_3N_6$, colorless needles, m.p. 235–238° (dec.). An antifungal compound. Positive ninhydrin.

*Streptomyces sakaiensis*

Shigehiro Fujii, Hiromu Hitomi, Masahiko Imanishi and Koichi Kakazawa, *Ann. Rept. Takeda Research Lab.* 14 8 (1955).

1217 **Musarin** $(C_{35}H_{60}O_{14}N_2)_n$ (proposed), Mol. Wt. ~5000, yellow powder, m.p. 170° (dec.), $[\alpha]_D^{20}$ +35.1° ±1.6° (c 1.21 in methanol).

An acidic substance.

*Streptomyces* sp.

H. R. V. Arnstein, A. H. Cook and Margaret S. Lacey, *J. Gen. Microbiol.* 2 111 (1948).

1218 **Mutomycin**, $C_7H_{11\text{-}12}O_2$, white crystalline powder, m.p. 141.5–142°.

*Actinomyces atroolivaceus* var. *mutomycini*

G. F. Gauze, T. S. Maksimova, O. L. Popova, M. G. Brazhnikova, T. A. Uspenskaya and O. K. Rossolimo, *Antibiotiki* U.S.S.R. 4 20 (273 in English) (1959).

1219 **Mycelin**, m.p. 263° (dec.).

Water insoluble, contains no nitrogen or sulfur. Negative Molisch. Flavomycin is produced by the same organism. Mycelin has antifungal properties.

*Streptomyces roseoflavus*

Kazuyoshi Aiso, Tadashi Arai, Kazuhiro Washida and Tei Tanaami, *J. Antibiotics* (Japan) 5 217 (1952).

1220 **Mycelin-IMO,** yellow crystalline, m.p. 214–222° (dec.), $[\alpha]_D^{21}$ +70 ±2 (c 1 in 1,4-dioxane) U.V. 243, 294, 335, 355, 373 mμ. Mol. Wt. 335, C 71.29, H 5.96, N 11.31.

*Streptomyces diastatochromogenes*

Koichi Ogata, Masaji Igarashi, Akira Miyake and Hiroichi Yamamoto, Japanese Patent 5898 (1957).

1221 **Mycorhodin,** bright red needles, m.p. 200–202° (dec.) U.V. 420, 471, 250 mμ in ethanol. C 58.7 H 5.2 N 2.1.

Mol. Wt. 698, 635.

Acid-base indicator.

*Streptomyces* sp.

M. Misiek, A. Gourevitch, B. Heinemann, M. J. Cron, D. F. Whitehead, H. Schmitz, I. R. Hooper and J. Lein, *Antibiotics and Chemotherapy* 9 280 (1959).

1222 **Mycospocidin** $(C_{20}H_{32}O_9N_2)_n$, colorless crystals, dec. 233°, $[\alpha]_D^{26}$ +56° (c 1 in pyridine).

Negative ninhydrin, biuret, Tollens, Fehling, ferric chloride tests. Positive diazo reaction.

Acid hydrolysis yielded two ninhydrin-positive substances, one perhaps being glycine.

*Streptomyces bobiliae*

Shoshiro Nakamura, Mamoru Arai, Keiko Karasawa and Hiroshi Yonehara, *J. Antibiotics* (Japan) **10A** 248 (1957).

1223 **Mycothricin,** colorless crystals, complex consists of strong organic bases.

Negative ninhhydrin, biuret, Fehling, Tollens, Molisch, Millon, maltol and Sakaguchi.

*Streptomyces lavendulae*

G. Rangaswami, *Hindustan Antibiotics Bull.* 2 46 (1959).

1224 **Mycoticin,** $C_{18}H_{30}O_5$ (proposed), yellow crystals.

Contains a hydroxyl group, has reducing properties, fluoresces under U.V.

*Streptomyces ruber*

Ruth C. Burke, Jacob H. Swartz, S. S. Chapman and Wei-Yuan Huang, *J. Invest. Dermatol.* 23 163 (1954).

1225 **Nigericin,** $C_{39}H_{69}O_{11}$, colorless needles, m.p. 246–254°.

A monobasic acid.

*Streptomyces* sp. resembling *S. violaceaniger*

Roger L. Harned, Phil Harter Hidy, Cyril J. Corum and Kenneth L. Jones, *Antibiotics and Chemotherapy* 1 594 (1951).

1226 **Nocardianin,** $C_{65-67}H_{96-104}O_{15}N_{18}$, red prisms, m.p. 228–235° (dec.), $[\alpha]_D^{25}$ −223° (c 0.3 in methanol).

Negative biuret, ninhydrin.

*Nocardia* sp.

I. R. Bick, Gregory J. Jann and Donald J. Cram, *Antibiotics and Chemotherapy* 2 255 (1952).

1227 **Nocardorubin,** crimson powder, darkens from 180° (dec.).

*Nocardia narasinoensis*

*J. Antibiotics* (Japan) 8B 253 (1955).

1228 **Nonactin,** $C_{30}H_{48}O_9$, colorless crystals, m.p. 147°, optically inactive.

Slight U.V. at 264 mμ (log ε = 1.5 in ethanol). Inert to chemicals and microbes.

*Streptomyces* spp. which produce cycloheximide.

R. Corbaz, L. Ettlinger, E. Gaümann, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog and H. Zähner, *Helv. Chim. Acta* 38 1445 (1955).

1229 **Nordin,** $C_{18}H_{16}O_8Cl_2$, m.p. 134–136°.

Occurs with estin (q.v.).

*Penicillium paxilli* var. *echinulatum*

Eitaro Komatsu, Japanese Patent 4799 (1953).

1230 **Nudic Acid A,** $C_{14}H_{20}O_3$ (proposed), colorless crystals, m.p. 123.5°.

No reducing properties. Takes up bromine.

*Tricholoma nudum*

H. W. Florey, E. Chain, N. G. Heatley, M. A. Jennings, A. G. Sanders, E. P. Abraham and M. E. Florey, "Antibiotics," Oxford University Press, London, 1949, p. 358.

1231 **Nybomycin,** $C_{16}H_{14}O_4N_2$, colorless crystals, which darken at 330° without melting.

Negative ninhydrin, biuret, $FeCl_3$; sugar tests, Ehrlich, $KM_nO_4$, $Br_2$.

*Streptomyces* sp.

Frieda Strelitz, Helen Flon and Igor N. Asheshov, *Prac. Nat. Acad. Sci. U. S.* 41 620 (1955).

T. E. Eble, G. A. Boyack, C. M. Large and W. H. De Vries, *Antibiotics and Chemotherapy* 8 627 (1958).

1232 **Oligomycin A,** $C_{24}H_{40}O_6$, colorless crystals, m.p. 140° (dec.), 150° (dec.) (polymorphic), $[\alpha]_D^{23}$ −54.5° (c 4.40 in dioxane).

Mol. Wt. = 424. Absorbs 2 moles $H_2$. Four active H. Five C—$CH_3$ groups. Forms a diacetate.

1233 **Oligomycin B,** $C_{22}H_{36}O_6$, colorless crystals, m.p. 160°, 169° (polymorphic), $[\alpha]_D^{23.5}$ −49.5° (c 1.03 in methanol).

Mol. Wt. = 396. Four active H. Five C—$CH_3$ groups. Forms a diacetate.

1234 **Oligomycin C,** $C_{28}H_{46}O_6$, colorless crystals, m.p. 198–200°, $[\alpha]_D^{23}$ −80.7° (c 3.70 in dioxane).

Contains six C—$CH_3$ groups.

*Streptomyces* sp. (may be *S. diastatochromogenes*)

Robert M. Smith, William H. Peterson and Elizabeth McCoy, *Antibiotics and Chemotherapy* 4 962 (1954).

Satoru Masamune, J. M. Sehgal, E. E. van Tamelen, F. M. Strong and W. H. Peterson, *J. Am. Chem. Soc.* 80 6092 (1958).

1235 **Ophiobalin,** $C_{28}H_{32}O_4$, white prisms, m.p. 181–182°.

*Ophiobalus miyabeanus*

A. Neelameghan, *Hindustan Antibiotics* 2 13 (1959).

1236 **Oregonensin,** $C_{20}H_{32}O_8$ (proposed), colorless needles, m.p. 82°.

A neutral substance. Positive 2,4-DNPH.

*Ganoderma oregonense*

H. W. Florey, E. Chain, N. G. Heatley, M. A. Jennings, A. G. Sanders, E. P. Abraham and M. E. Florey, "Antibiotics," Oxford University Press, London, 1949, p. 362.

1237 **Oryzacidin** (Oryzasizine), $C_8H_{13}O_5N$, colorless, hygroscopic needles, m.p. 162° (dec.), $[\alpha]_D$ −138°.

β-Nitropropionic acid also occurs free in the culture broth.

*Aspergillus oryzae*

Chujiro Shimoda, *J. Agr. Chem. Soc. Japan* 25 254 (1951).

Seiji Nakamura and Chuji Shimoda, *ibid.* 28 909 (1954).

1238 **PA-128,** $C_{37\text{-}46}H_{61\text{-}75}O_{13\text{-}16}N$, light yellow rectangular plates, m.p. 143° $[\alpha]_D^{25}$ −2.0° (c 1 in methanol).

Negative $FeCl_3$, no colors in aqueous base nor concentrated $H_2SO_4$. Positive 2,4-DNPH, decolorizes $Br_2$ water and permanganate. Takes up >6 mM of hydrogen per gram of antibiotic.

Unclassified *Streptomycete*

Koppaka V. Rao and John E. Lynch, *Antibiotics and Chemotherapy* 8 437 (1958).

1239 **PA-132,** $C_{16}H_{18\text{-}20}O_5$, free acid is a colorless amorphous powder, $[\alpha]_D^{25}$ −161° (c 1.0 in methanol). Handled as the benzylamine salt: white crystals, m.p. 128–131°, $[\alpha]_D^{25}$ −130° (c 1.0 in methanol).

A lactonic acid containing two C-methyl groups. Decolorizes bromine or permanganate. Negative $FeCl_3$, Fehling, 2,4-DNPH, Tollens, $AgNO_3$ and NaOI.

*Streptomyces* sp.

B. Kenneth Koe, Ben A. Sobin and Walter D. Celmer, "Antibiotics Annual 1956–1957," Medical Encyclopedia, Inc., New York, p. 672.

1240 **Phagolessin A 58,** light yellow hygroscopic powder.

Negative $FeCl_3$, biuret, Millon and ninhydrin test.

*Streptomyces* sp.

Igor N. Asheshov, Freda Strelitz and Elizabeth A. Hall, *Antibiotics and Chemotherapy* 2 366 (1952).

1241 **Phalamycin,** $C_{36}H_{41}O_{14}N_9S$ (proposed), colorless crystals, no sharp m.p.

Positive $FeCl_3$, $Br_2$ absorption. Has primary or secondary alcohol groups.

*Streptomyces noursei* variant

Rachel Brown, *N. Y. State Dept. Health, Ann. Rept. Div. Labs and Research* 18 (1956). (*Chem. Abstr.* 51 16672e)

1242 **Phalofacin** gives positive $FeCl_3$ but negative biuret, Millon, ninhydrin, Molisch, Tollens and Sakaguchi tests.

*Streptomyces* sp. res. *S. aureus*

Kenji Maeda, Yoshiro Okami, Osamu Taya and Hamao Umezawa, *J. Antibiotics* (Japan) 5 465 (1952).

1243 **Phleomycin,** $C_{53}H_{93}O_{32}N_{17}$, white to pale green amorphous powder, isolated as a blue monocopper complex. U.V. 244, 295–300 $m\mu$.

Gives positive ninhydrin and diazo tests. Negative Fehling, Tollens, Sakaguchi and Molisch.

*Streptomyces verticillis*

Tomohisa Takita, Kenji Maeda and Hamao Umezawa, *J. Antibiotics* (Japan) 12A 111 (1959).

Tomohisa Takita, *ibid.* 12 285 (1959).

1244 **Phytonivein,** $C_{29}H_{46}O_2$, colorless needles, m.p. 138°.

*Fusarium bulbigenum*

The watermelon wilt toxin.

Isamu Hirose and Seiyo Aoe, *Ann. Phytopathol. Soc. Japan* 19 162 (1955). (*Chem. Abstr.* 50 14058g)

Isamu Hirose and S. Nishimura, *Nippon Nogêi-kagaku Kaishi* 30 528 (1956).

1245 **Piricularin**, $C_{17}H_{14}N_2O_3$ or $C_{18}H_{14}N_2O_3$, colorless crystals, m.p. 73.5°, $[\alpha]_D^{28}$ −19°.

Absorbs 4 moles of hydrogen over platinum catalyst, contains two phenolic or enolic hydroxyls, no methoxyl. Reacts with 3 moles of 2,4-dinitrophenylhydrazine. Has 1 N-methyl, no NH or $NH_2$. λ max. in $H_2O$ = 240 mμ. $E_{1cm.}^{1\%}$ 2824. A toxin of rice blast disease.

*Piricularia oryzae*

Kinjiro Tamari and Jun Kaji, *Nippon Nogêi-kagaku Kaishi* 31 387 (1957).

1246 **Pleomycin**, $C_{14}H_{12}O_8$, rectangular plates from ethanol, m.p. 235°, U.V. 270, 330, 340 mμ in 0.13 M phosphate buffer.

*Streptomyces pleofaciens*

Roy A. Machlowitz, Jesse Charney, Alfred A. Tytell and W. P. Fisher, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, p. 806.

1247 **Pleuromutilin** (Drosophilin B), $C_{22}H_{34}O_5$, colorless crystals, m.p. 170°, $[\alpha]_D^{24}$ +20° (c 3.0 in absolute ethanol).

Forms a diacetate, non-phenolic, probably has a lactone ring, forms a hydrazone.

*Pleurotus mutilus*

Marjorie Anchel, *J. Biol. Chem.* 199 133 (1952).

1248 **Pleurotin**, $C_{20}H_{22}O_5$, yellow-amber needles, m.p. 220–215° (dec.), $[\alpha]_D^{23}$ −20° (c 0.59 in chloroform).

A neutral, photosensitive compound. Negative $FeCl_3$, oxidized KI.

*Pleurotus griseus*

William J. Robbins, Frederick Kavanaugh and Annette Hervey, *Proc. Nat. Acad. Sci. U. S.* 33 171 (1947).

1249 **Pluramycin A**, orange needle crystals, dec. from 177°, U.V. 208, 245 (265–270) mμ in ethanol.

C 66.63, H 6.30, N 3.66

Negative $FeCl_3$, Fehling, Tollens and 2,4-DNPH.

1250 **Pluramycin B**, reddish brown powder, possible neutral substance. The pluramycins may be related to the actinomycins.

Tomio Takeuchi, Kazuo Nitta and Hamao Umezawa, *J. Antibiotics* (Japan) 9A 22 (1956).

Kenji Maeda, Tomio Takeuchi, Kazuo Nitta, Koki Yagishita, Ryozo Utahara, Teisuke Osato, Masahiro Ueda, Shinichi Kondo, Yoshiro Okami and Hamao Umezawa, *ibid.* 9A 75 (1956).

1251 **Poin,** crystals, m.p. 142–143°.

C 59.70, H 7.77, O 32.53

*Fusarium sporotrichiella* var. *poae*

O. K. Élpidina, *Antibiotiki* U.S.S.R. 4 46 (273 in English) (1959).

1252 **Primycin,** $C_{19}H_{37}O_7N$, white microcrystals, m.p. 166–168° (dec.).

No reducing properties. Can be acetylated. Strong Sakaguchi test.

An unclassified actinomycete

T. Vályi-Nagy, J. Úri and I. Szilágy, *Nature* 174 1105 (1954).

1253 **Psalliotin,** crystalline, water soluble, inactivated by bright light.

*Psalliota xanthoderma*

Nancy Atkinson, *Nature* 174 598 (1954).

*Idem.,* Australian Patent 20,272,156 (1957).

1254 **Pulvilloric Acid,** buff colored needles, turning bright yellow in air.

An acidic, antifungal antibiotic, containing only C, H, O. Blue $FeCl_3$, negative Tollens. Yield 600 mg. per liter.

*Penicillium pulvillorum* Turfitt

P. W. Brian, P. J. Curtis, H. G. Hemming and G. L. F. Norris, *Brit. Mycol. Soc. Trans.* 40 369 (1957).

1255 **Pumilin,** lemon-yellow crystals, m.p. >360°.

Negative $FeCl_3$, copper-red in 5 N hydrochloric acid.

*Bacillus pumilis*

0.7 g. was obtained from 500 gal. of broth.

D. S. Bhate, *Nature* 175 816 (1955).

1256 **Racemomycin A**

1257 **Racemomycin B,*** $C_{60}H_{128}O_{32}N_{20}$, white powder, m.p. (Hydrochloride) 175° (dec.), $[\alpha]_D^{19}$ −45° (c 0.5 in water).

Positive Molisch, Elson-Morgan and biuret. Negative Sakaguchi, maltol, $FeCl_3$, 2,4 DNPH and Fehling. Yields $\beta$-lysine and roseonine on hydrolysis.

1258 **Racemomycin C,** isolated in a small amount as a salt (m.p. 210°).

* See entry 790.

*Streptomyces racemochromogenes* n. sp.

Hyozo Taniyama and Shoji Takemura, *J. Pharm. Soc. Japan* 77 1210, 1217 (1957); 78 742 (1958).

1259 **Ractinomycin A,** $C_{33}H_{30}O_{14}N_3$, orange needles, m.p. browns ~157°, blackens at 205°.

Negative ninhydrin, biuret, Sakaguchi, Millon. Positive Tollens, Molisch, $FeCl_3$. Decolorizes $KMnO_4$. Decolorized by $H_2O_2$. Alkali-unstable. Turns purple above pH 6.5. Contains no amino acids.

1260 **Ractinomycin B,** reddish orange needles, m.p. 172–175° (dec.).

Negative $FeCl_3$.

The ractinomycins are said to resemble the actinomycins in some respects.

*Streptomyces* sp. similar to *S. phaeochromogenes*

Ryozo Utahara, Hideo Oyagi, Koki Yagishita, Yoshiro Okami and Hamao Umezawa, *J. Antibiotics* (Japan) 8A 132 (1955).

Ryozo Utahara, *ibid.* 10A 115 (1957).

S. Wakiki *et al., Antibiotics and Chemotherapy* 8 228 (1958).

1261 **Raisnomycin,** dark yellow basic material, insoluble in water. The hydrochloride and disulfate are slightly soluble. The impure material does not have an end absorption in U.V.

*Streptomyces kentuckensis*

Fred S. Barr and Paul E. Carman, *Antibiotics and Chemotherapy* 6 286 (1956).

1262 **Rammacin,** $C_{26}H_{43}O_8$, crystalline, m.p. 235°, Mol. Wt. 499.

Negative $Br_2$; positive benzenoid.

*Streptomyces* sp.

K. Ahmad and M. F. Islam, *Nature* 176 646 (1955).

1263 **Ramycin** (Mol. Wt. 478, contains only carbon, hydrogen and oxygen), colorless plates, m.p. 158° (dec.), optically inactive.

Structural features:

A non-phenolic hydroxy acid with one or more carbon-carbon double bonds.

*Mucor ramannianus*

P. J. van Dijck and P. deSomer, *J. Gen. Microbiol.* 18 377 (1958).

1264 **Raromycin,** m.p. 211–213° C 57.97, H 8.46, N 0.44, O 33.13 by difference.

*Streptomyces* sp.

Nabuo Tanaka, Hisaji Yamazaki, Koichi Okabe and Hamao Umezawa, *J. Antibiotics* (Japan) **10A** 189 (1957).

1265 **Roseomycin,** crystalline helianthate, m.p. 211–216° (dec.) and reineckate m.p. 114° (dec.).

Positive Molisch, Tollens, indole, glucosamine and Fehling.

Negative maltol, biuret, ninhydrin and Sakaguchi.

*Streptomyces roseochromogenes*

Nakao Ishida, *J. Antibiotics* (Japan) 3 845 (1950).

1266 **Rhizobacidin,** crystalline, m.p. 215–220° (dec.). Contains C, H, O and N but not S. Positive biuret, xanthoproteic, ninhydrin and Sakaguchi. Negative Ehrlich, Molisch and $FeCl_3$.

*Bacillus subtilis*

Carlos Casas-Campillo, *Ciencia* (Mexico) 11 21 (1951).

1267 **Rhodocidin,** red powder, U.V. shows a broad peak at 500–530 $m\mu$. Soluble in water and organic solvents.

*Streptomyces phoenix*

Jesse Charney, Roy A. Machlowitz, W. S. Roberts and W. P. Fisher, *Antibiotics and Chemotherapy* 3 788 (1953).

**Ristocetins** (Spontins, Ristins).

Two closely related amphoteric antibiotics containing amino and phenolic groups and sugars. Each contains four reducing sugars: glucose, mannose, rhamnose and D-arabinose.

Negative biuret, Sakaguchi, maltol. Positive phosphomolybdic acid test for phenols, ninhydrin (after acid hydrolysis), anthrone. Mol. Wt. 2500–5000. Contain C, H, O, N, S.

1268 **Ristocetin A** (Sulfate): $[\alpha]_D^{25}$ −120–133° (in water).

1269 **Ristocetin B** (Sulfate): $[\alpha]_D^{25}$ −144–149° (in water).

*Nocardia lurida*

Julian E. Philip, Jay R. Schenck and Martha P. Hargie, "Antibiotics Annual 1956–1957," Medical Encyclopedia, Inc., New York, p. 699.

1270 **Rotaventin,** white crystals, m.p. 170–175° (dec.).

*Streptomyces reticuli*

Nobukiko Komatsu and Momoe Soeda, *Japan. J. Exp. Med.* 21 279 (1951).

1271 **Rubromycin,** thin square rods, m.p. 215° (dec.) U.V. 518–520, 546, 584 mμ.

C 60.30, H 4.26, O 33.91

Contains no N (differing from rhodomycin). Differs from rhodomycetin in that the latter is found in the culture solution; the present compound is in the mycelium.

*Streptomyces collinus* n. sp.

Hans Brockman and Karl Heinz Renneberg, *Naturwissenschaften* 40 59 (1953).

1272 **Ruticin,** orange needle-like crystals, U.V. 227, 262, 364 mμ.

*Streptomyces* res. *S. rutgersensis*

W. P. Fisher, Jesse Charney, Ray A. Machlowitz, James E. Blair and Alfred A. Tytell, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, p. 174.

1273 **Sarcidin,** m.p. 274–275° (dec.).

C 41.89, H 5.02, N 21.82 and a qualitative sulfur test.

Tamio Takeuchi, Kazuo Nitta and Hamao Umezawa, *J. Antibiotics* (Japan) 6A 31 (1953).

1274 **Secalonic Acid,** $C_{31}H_{30\text{-}32}O_{14}$, lemon-yellow needles, m.p. 244–250° from chloroform, $[\alpha]_D^{20}$ −81° (acetone), −66° (chloroform), −198° → −59° (pyridine).

*Claviceps purpurea*

F. Kraft, *Arch. Pharm.* 244 336 (1906).

A. Stoll, J. Renz and A. Brack, *Helv. Chim. Acta* 35 2022 (1952).

1275 **Seligocidin,** crystalline powder, U.V. 304 mμ in ethanol.

Positive Sakaguchi and ninhydrin; negative biuret.

*Streptomyces* res. *S. roseochromogenes*

Shoshiro Nakamura, Kenji Maeda, Yoshiro Okami and Hamao Umezawa, *J. Antibiotics* (Japan) 7A 57 (1954).

1276 **Sirenin,** $C_{21}H_{36}O_7N$.

Mol. Wt.: found 386, calculated 414. Contains a lactone ring, a carbonyl group and a —C=C— or —C=N— bond. The absence of hydroxyl and carboxyl groups and of aromatic rings was ascertained.

*Allomyces* species

Sirenin is a sex hormone of this water-mold.

Leonard Machlis, *Nature* 181 1790 (1958).

1277 **Sporidesmin** (probably) $C_{19}H_{21}O_6N_3S_2Cl \cdot CCl_4$, colorless crystals (carbon tetrachloride solvate) sintering from 109° → resin → 125° semi-solid → meniscus at 130–134°, $[\alpha]_D^{20}$ −19° (c 2.2 in methanol).

Other formulae without chlorine are not excluded, since the solvent-free compound has not been isolated. The compound is a toxin in animals.

*Sporidesmium bakeri* Syd.

R. L. M. Synge and E. P. White, *Chem. and Ind.*, 1546 (1959).

1278 **Streptocardin**, Crystalline, U.V. 365 (242) (252) mμ in phosphate buffer (pH 6) forms water-soluble alkali salts.

*Streptomyces sp., Nocardia sp.*

W. P. Fisher, Roy A. Machlowitz, Alfred A. Tytell and Jesse Charney, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, p. 177.

1279 **Streptolydigin**, $C_{32}H_{46}O_9N_2$ (or $C_{35}H_{50}O_{10}N_2$), m.p. 144–150° (dec.), $[\alpha]_D^{25}$ −93° (c 1.6 in chloroform).

An enolic acid. Positive $FeCl_3$, iodoform. Negative biuret, ninhydrin, Fehling, Molisch. Reacts with $Br_2$ in $CCl_4$.

*Streptomyces lydicus*

T. E. Eble, C. M. Large, W. H. DeVries, G. F. Crum and J. W. Shell, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 893.

**Streptovaricin** (Dalacin). A complex consisting of at least five active closely related components. These were separated by countercurrent distribution into Streptovaricins:

1280 **A**, $C_{34}H_{47\text{-}49}O_{13}N$, yellow crystals, m.p. 182–184°, $[\alpha]_D^{24}$ +454° ($CHCl_3$).

1281 **B**, $C_{34}H_{47\text{-}49}O_{13}N$, yellow crystals, m.p. 195–200°, $[\alpha]_D^{24}$ +168° ($CHCl_3$).

1282 **C**, $C_{34}H_{47\text{-}49}O_{13}N$, yellow crystals, m.p. 168–171°, $[\alpha]_D^{24}$ +317° ($CHCl_3$).

1283 **D**, yellow crystals, m.p. 115–118°, $[\alpha]_D^{24}$ +102° ($CHCl_3$).

1284 **E**, yellow crystals, m.p. 102–105°, $[\alpha]_D^{24}$ +6.13° ($CHCl_3$).

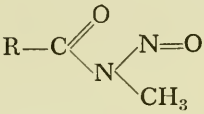
*Streptomyces spectabilis*

Paul Siminoff, Robert M. Smith, Walter T. Sokolski and G. M. Savage, *Am. Rev. Tuberc. Pulmonary Diseases* 75 576 (1957).

George B. Whitfield, Edward C. Olson, Ross R. Herr, John A. Fox, Malcolm E. Bergy and Gerald A. Boyack, *ibid.* 75 584 (1957).

Upjohn Co., British Patent 811,757 (1959).

1285 **Streptozotacin,** $C_{14}H_{17}O_{12}N_5$, m.p. 115–125° (dec.).

Probably still a mixture. Base-unstable neutral substance. Seems to contain the partial structure  Alkaline treatment liberates diazomethane.

*Streptomyces achromogenes*

R. R. Herr, T. E. Eble, M. E. Bergy and H. K. Jahnke, 7th Annual Symposium on Antibiotics, Washington, D. C., 1959.

1286 **Substance 1404,** yellow crystalline, Hexaene. M.p. 210–220° (dec.), $[\alpha]_D^{21}$ +67.5 ±2.0° (c 1 in dioxane).

Contains N 10.47, no sulfur, no halogen.

*Streptomyces diastatochromogenes* (Mycelium)

Masaji Igarashi, Koichi Ogata and Akira Miyake, *J. Antibiotics* (Japan) 8B 113 (1955).

1287 **Sulfactin,** $C_{38}H_{55}O_7N_{11}S_4$ or $C_{27}H_{40}O_5N_8S_3$ (proposed), hygroscopic white needles, m.p. 245–275° (dec.).

Positive Fehling. Reduces $KMnO_4$. Negative biuret, $FeCl_3$, Molisch, Sakaguchi.

*Streptomyces roseus*

Renate Junowicz-Kocholaty, Walter Kocholaty and Albert Kelner, *J. Biol. Chem.* 168 765 (1947).

1288 **Sulfocidin,** yellow-brown crystals, m.p. 166–178°, $[\alpha]_D^{25}$ −58.5° (c 0.51 in chloroform).

Neutral antibiotic, analysis C 64.88, H 8.38, N 4.25, S 1.80. Negative nitroprusside and azide iodine, ninhydrin, $FeCl_3$, Sakaguchi, maltol, biuret, Fehling, 2,4-DNPH. Decolorizes permanganate.

*Streptomyces* sp.

Morris Zief, Robert Woodside and George E. Ham, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 886.

1289 **Taitomycin**, yellow-brown powder, U.V. at 330, 420 mμ.
C 53.57, H 4.87, N 9.50 ash 2.8.
Positive Fehling and ninhydrin (acid hydrolysate).
*Streptomyces afghanensis*
Mitsuo Shimo, Tatsuji Shiga, Takashi Tomosugi and Ikuzo Kamoi, *J. Antibiotics* (Japan) 12A 1 (1959).
Takashi Tomosugi, Ikuzo Kamoi, Tatsuji Shiga and Mitsuo Shimo, *ibid.* 12A 7 (1959).

1290 **Tardin**, $C_{11}H_{15}O_3$ (proposed), pale yellow oil, $[\alpha]_D^{20}$ −11.4° (in alcohol).
Positive $FeCl_3$. Negative 2,4-DNPH. Hydrolyzes to an acidic and a neutral fraction.
*Penicillium tardum*
N. Borodin, F. J. Philpot and H. W. Florey, *Brit. J. Exp. Path.* 28 31 (1947).

1291 **Terrecin**, light yellow prisms, m.p. 219°.
Analysis: C 51.89, H 3.51, N 3.8, Cl 19.1. Alkali soluble. Positive $FeCl_3$.
*Aspergillus terreus*
Kazuo Iwata and Itiro Yosioka, *J. Antibiotics* (Japan) 3 192 (1950).

1292 **Thiactin**, acid and alkali metal salts (previously identified as bryamycin). M.p. 220–234°, $[\alpha]_D^{27}$ −68.5 −69.5° (c 1 in chloroform).
*Streptomyces hawaiiensis*
Bernard Heinemann, Irving R. Hooper and Martin J. Cron, British Patent 790,521 (1958).

1293 **Thioaurin** (Orosomycin, Antibiotic HA-9), $C_7H_6O_2N_2S_2$ or $C_{14}H_{12}O_4N_4S_4$ (proposed), yellow crystals, m.p. 178–180°, optically inactive.
Strong U.V. at 232, 370 mμ. Negative $FeCl_3$.
*Streptomyces* sp. resembling *S. lipmanii*
William A. Bolhofer, Roy A. Machlowitz and Jesse Charney, *Antibiotics and Chemotherapy* 3 382 (1953).
William Eisenman, P. Paul Minieri, Anthony Abbey, John Charlebois, Mary Moncrieff-Yates and Neil E. Rigler, *ibid.* 3 385 (1953).

1294 **Thiomycin**, golden yellow needles, m.p. 176–178° (dec.).
Resembles thioaurin somewhat. May be identical. Analysis: C 49.61, H 5.50, N 8.88, S 16.26. Negative $FeCl_3$, ninhydrin, Fehling.

*Streptomyces* sp. resembling *S. phaeochromogenes* var. *chloromyceticus*

Yorio Hinuma, Susumu Hamada, Takaaki Yashima and Kyoko Ishikara, *J. Antibiotics* (Japan) 8A 118 (1955).

1295 **Totomycin,** $C_{21}H_{29}O_{11}N$, amorphous.

*Streptomyces crystallinus*

Jacques Loewe Research Foundation, Inc., British Patent 758,276 (1956).

1296 **Toyocamycin,** $C_{12}H_{14}O_4N_5$, colorless needles, prisms (monohydrate), m.p. 243°.

Analysis: Negative $FeCl_3$, Fehling, Molisch, Millon, Sakaguchi, Ehrlich. Mol. Wt. 286, 266.

*Streptomyces toyocaensis*

Ko Kikuchi, *J. Antibiotics* (Japan) 8A 145 (1955).

Haruo Nishimura, Ken Katagiri, Kozaburo Sato, Mikao Mayama and Noburo Shimaoka, *ibid.* 9A 60 (1956).

1297 **Tubercidin,** $C_{11}H_{14}O_4N_4$, crystals, m.p. 247° (dec.).

Forms a picrate, reineckate, helianthate, and pentachlorophenolate. A basic substance stable to acid and alkali.

A streptomycete

Kentaro Anzai, Goto Nakamura and Saburo Suzuki, *J. Antibiotics* (Japan) 10A 201 (1957).

1298 **Unclassified Compound,** $C_{17}H_{12}O_2N_2$, m.p. 220° (dec.).

Contains two enolic groups. U.V. bands at 243 and 374 $m\mu$. Photosensitive.

*Penicillium puberulum* (mycelium)

A. H. Campbell, M. E. Foss, E. L. Hirst and J. K. N. Jones, *Nature* 155 141 (1945).

1299 **Unnamed antibiotic,** $C_{11}H_{17}O_3N$, hygroscopic light yellow crystals, m.p. 195° (dec.).

U.V. absorption at 365, 410 $m\mu$.

*Proteus immunitatis anticarcinomatosa* n. sp. (on a special blood plasma-bouillon medium)

Atsuo Ushiyama and Takaaki Miyasaka, Japanese Patent Application 3998 (1957).

1300 **Vancomycin** (Hydrochloride), amphoteric white solid, Mol. Wt. 3200–3500 ±200 (titr.).

*Streptomyces orientalis* n. sp.

M. H. McCormick, W. M. Stark, G. E. Pittenger, R. C. Pittenger and J. M. McGuire, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 606.

H. Nishimura, *Ann. Rept. Shionogi Res. Lab.* I 479 (1957).

1301 Variotin, $C_{18}H_{27}O_4N$, colorless oil, $[\alpha]_D^{25}$ −5.68° (c 1.0 in methanol).

A neutral oil with an ester-like odor. C 67.35, H 8.58, N 4.16, contains no halogen, sulfur or phosphorus. Positive diazo, nitroalkyl and hydroxamic acid reactions; negative ferric chloride, Millon, Ehrlich, Sakaguchi, Molisch, biuret, xanthoprotein and ninhydrin tests.

*Paecilomyces variotis* Bainier var. *antibioticus*

Hiroshi Yonehara, Setsuo Takeuchi, Hakao Umezawa and Yusuke Sumiki, *J. Antibiotics* (Japan) 12A 109, 195 (1959).

1302 Vengicide, $C_{24}H_{29}O_9N_{10}$, white, amorphous, m.p. 241.5–243°, $[\alpha]_D^{20}$ −51.6° (in 0.1 N hydrochloric acid solution).

Mol. Wt. ~600. U.V. λmax. 233.5 and 273.5 mμ in 0.05 N hydrochloric acid. C 47.05, H 4.85, O 24.85, N 23.85.

*Streptomyces vendargensis*

Oxytetracycline is produced also in this fermentation.

N. V. Koninklijke Nederlandsche Gist—en Spiritus—fabriek, British Patent 764,198 (1956). (*Chem. Abstr.* 51 10009a)

A. P. Struyck, Canadian Patent 514,164 (1955).

1303 Vertimycin C, crystalline, m.p. 152–155°. C 62.4, H 6.84, O 21.9, N 8.0.

*Streptomyces verticillatus*

Canadian Patent 575,235 (1959).

1304 Violacetin, fine yellow needles, m.p. (hydrochloride) >210°.

Basic compound. Positive ninhydrin, diazo tests. Precipitated from aqueous solution by picric acid, phosphotungstic acid, forms reineckate. Analysis: C 38.26, H 6.74, N 24.71, Cl 9.33. Negative biuret, Fehling, ninhydrin, glucosamine, maltol, Sakaguchi, Millon, xanthoprotein.

*Streptomyces* sp. resembling *S. purpurochromogenes*

Kazuyoshi Aiso, Tadashi Arai, Ichiro Shidara, Hiroo Kurihara and Yoshiro Morita, *J. Antibiotics* (Japan) 8A 33 (1955).

1305 Violarin, $C_{22-24}H_{32-34}O_{8-9}$, dark violet color or amorphous red powder, dec. 130°, somewhat similar to litmocidin, rubidin and rhodomycetin.

*Streptomyces violaceus*

N. A. Krasilnikov, G. K. Skryabin and O. I. Artamonova, *Antibiotiki* (U.S.S.R.) 3 (1958).

*Idem., J. Antibiotics* (Japan) 13A 1 (1960).

D. M. Trakhtenberg, L. V. Čerenkova and A. S. Chochlov, Symposium on Antibiotics, Prague (1959).

Viridins, $C_{19}H_{16}O_6$ (isomers).

1306 α-Viridin, fine colorless needles, m.p. 208–217° (dec.), $[\alpha]_D^{20}$ −213.4° (in chloroform).

1307 β-Viridin, Fine colorless needles, m.p. 140° (dec.), $[\alpha]_D^{20}$ −50.7° (in chloroform).

Both compounds show: negative Schiff, $FeCl_3$, iodoform. Red-violet color with phloroglucinol-hydrochloric acid. Positive ketone derivative tests, Fehling, Tollens.

*Trichoderma viride*

P. W. Brian and J. C. McGowan, *Nature* 156 144 (1945).

P. W. Brian, P. J. Curtis, H. G. Hemming and J. C. McGowan, *Ann. Appl. Biol.* 33 190 (1946).

E. B. Vischer, S. R. Howland and H. Raudnitz, *Nature* 165 528 (1950).

1308 Virtosin, $C_{27}H_{40}O_9N_2$, colorless needles, m.p. 142.5–143°, $[\alpha]_D^{18}$ +80° ± 0.5° (c 1 in acetone).

Positive Fehling and Sakaguchi reactions; negative ninhydrin and maltol tests.

*Streptomyces olivochromogenes*

Akira Miyake, Shozo Wada, Motoo Shibata, Koichi Nakasawa, Jujo Kaneko and Yasuharu Mamiya (to Takeda Pharmaceutical Industries Ltd.), Japanese Patent Appl. 6149 (1957).

1309 Wortmannin, colorless needles, m.p. 240° (yellowing in sunlight).

A neutral antifungal antibiotic, containing only C, H, O. Yields were about 100 mg. per liter.

*Penicillium wortmanni* Klocker

P. W. Brian, P. J. Curtis, H. G. Hemming and G. L. F. Norris, *Brit. Mycol. Soc. Trans.* 40 365 (1957).

1310 Xanthicin, $C_{13}H_{15}O_5N$, yellowish silky crystals, m.p. 211–213° (dec.), $[\alpha]_D^{15}$ +319° (c 0.25 in acetone).

U.V. maxima at 270 mμ ($CH_3OH$), 260 mμ, 325 mμ (0.1 MKOH). Positive aldehyde, indole, $FeCl_3$ tests. Negative amino, nitro, Fehling's, phosphomolybdic acid tests. Alkaline $KMnO_4$ oxidation gives succinic acid.

*Streptomyces xanthochromogenes*

Yasuji Sekizawa and Keiko Miwa, *Nippon Nôgei-kagaku Kaishi* 30 471 (1956).

1311 **Xanthomycin-like Antibiotic,** $C_{29}H_{42}O_7N_9S_4Cr$ (Reineckate), yellow-orange glass, U.V. 264.5, 335 mμ in water, pH 2.

Positive Benedict, bromine, silver nitrate, potassium iodide, sodium hydrosulfite and periodic acid.

*Streptomyces* sp.

James D. Mold and Quentin R. Bartz, *J. Am. Chem. Soc.* 72 1847 (1950).

1312 **Xanthomycins** (Protomycins), $C_{23}H_{29\text{-}31}O_7N_3$, free base: deep orange-red amorphous solid. Dihydrochloride: bright orange-yellow plates, $[\alpha]_D^{25}$ +115° (c 0.4 in water). Reineckate: long, orange needles, m.p. 165–170° (dec.).

Contains components A and B. Acid hydrolysis yields ethanolamine, methylamine and ammonia. Red-purple color with alkali. Positive $Br_2$ uptake, Benedict, silver nitrate, sodium hydrosulfite, ketone derivatives. Negative ninhydrin, Molisch, Sakaguchi, $FeCl_3$.

*Streptomyces* sp.

C. B. Thorne and W. H. Peterson, *J. Biol. Chem.* 176 413 (1948).

K. V. Rao and W. H. Peterson, *J. Am. Chem. Soc.* 76 1335 (1954).

1313 **Xanthothricin,** yellow needles, m.p. 165° (s. 161–162°).

Analysis: C 43.64, H 3.82, N 35.21, O 17.34.

*Streptomyces* sp. similar to *S. albus*

Roy A. Machlowitz, W. P. Fisher, Betsey S. McKay, Alfred A. Tytell and Jesse Charney, *Antibiotics and Chemotherapy* 4 259 (1954).

## BIBLIOGRAPHY, REVIEWS AND GENERAL REFERENCES

A book closely related to this one in intent and format is Walter Karrer's "Konstitution und Vorkommen der organischen Pflanzenstoffe (exclusive Alkaloide)." This lists over 2600 compounds with simple physical properties and thorough referencing. The emphasis is on metabolites of higher plants, although many fungal products are listed.

Another related book is "Type Reactions in Fermentation Chemistry," by Lowell L. Wallen, Frank H. Stodola and Richard W. Jackson. Here the emphasis is on non-sugar substrates, and classification is by type of reaction (oxidation, reduction, etc.) accomplished. Many microbial transformations of steroids are included, for example. Structural formulas, names of microorganisms and references are listed.

The revised edition of W. W. Umbreit's "Metabolic Maps" should be mentioned. This is essentially a list of equations, outlining various metabolic pathways, with no discussion and little referencing, but including catabolic routes and those in higher organisms.

"Naturally Occurring Quinones," by R. Thomson, is similar in method to our handbook, but is confined to the single class of compounds with more thorough discussion of each entry. "The Comparative Biochemistry of the Carotenoids" by T. W. Goodwin is somewhat similar in its restriction to a single class of chemicals. Both books are broader in scope as far as producing organism is concerned, and are not limited to microorganism products.

"The Chemistry of Microorganisms," by Arthur Bracken, is descriptive in style, showing some of the degradations and syntheses leading to establishment of chemical structures and offering essays on related topics. There is, perhaps, some emphasis on substances isolated and characterized by the Raistrick group.

We have not designated antibiotics as such nor have we attempted to separate the commercial from the non-commercial or to give the trade names or the biological properties. Data on biological properties are difficult to evaluate and, on the newer antibiotics, may conflict. Trade names tend to change due, for example, to improvements in dosage forms.

Many antibiotic spectra as well as physical properties and references are given in the "Handbook of Toxicology, Vol. II, Antibiotics" edited by W. S. Spector.

The "Physicians' Desk Reference" is an annual publication listing antibiotics and other medicines by brand name, by manufacturer and by type of medicine. There is also a therapeutic indications index, listing medicines available for the treatment of a given condition, and an index listing professional information (composition, dosage, etc.) on each product.

The "Antibiotics Annual" series also is a useful reference work on antibiotics.

Various other monographs, reviews and general references are in the list below.


1 "Konstitution und Vorkommen der organischen Pflanzenstoffe (exclusive Alkaloide)," Walter Karrer, Birkhäuser Verlag, Basel, 1958, 1207 pp. An index similar in intent to this book, but with its scope the entire plant kingdom. Thoroughly referenced.

2 "Type Reactions in Fermentation Chemistry," L. Wallen, F. Stodola and R. Jackson, Agricultural Research Service, United States Department of Agriculture (ARS–71–13), Peoria, 1959. A compilation of the types of chemical conversions by microorganisms which have been reported in the literature with emphasis on non-sugar substrates.

3 "Metabolic Maps," W. W. Umbreit, Burgess Publishing Co., Minneapolis, 1960.

4 "The Chemistry of Microorganisms," Arthur Bracken, Pitman and Sons, London, 1955, 343 pp.

5 "Antibiotics and Mold Metabolites," a symposium at the March 26, 1956 meeting of the English Chemical Society. Reprinted as Special Publication No. 5.

6 "Chemical Compounds Formed from Sugars by Molds," B. Gould, Scientific Report Series No. 7 of the Sugar Research Foundation, New York, 1947.

7 "The Microbes Contribution to Biology," Albert J. Kluyver and C. van Niel, Harvard University Press, Cambridge, 1956, 182 pp.

8 "Industrial Fermentations," Leland A. Underkofler and Richard J. Hickey, Chemical Publishing Co., Inc., New York, 1954, Vol. I, 565 pp., Vol. II, 578 pp.

9 *Industrial and Engineering Chemistry Annual Unit Process Review of Fermentation,* Samuel C. Beesch and G. M. Shull, *Ind. and Eng. Chem.* 48 1585 (1956). These reviews list, among other things, new antibiotics and new microbiological transformations of steroids.

10 *Industrial and Engineering Chemistry Annual Unit Processes Review of Fermentation,* Samuel C. Beesch and G. M. Shull, *Ind. and Eng. Chem.* 49 1491 (1957).

11 *Industrial and Engineering Chemistry Annual Unit Process Review of Fermentation*, Samuel C. Beesch and Fred W. Tanner, Jr., *Ind. and Eng. Chem.* 50 1341–1354 (1958).

12 *Biochemistry of microorganisms*, C. B. van Niel, *Ann. Rev. Biochem.* 12 551–586 (1943). A review with 371 references.

13 "Handbook of Toxicology, Vol. II, Antibiotics," W. S. Spector (Ed.), W. B. Saunders and Co., Philadelphia, 1957. This is a compilation of data on physical and biological properties of 340 antibiotics or substances which have been tested as antibiotics. Most of these are microorganism metabolites. Thoroughly referenced. This compilation was prepared under the direction of the Committee on the Handbook of Biological Data, Division of Biology and Agriculture, the National Academy of Sciences, The National Research Council.

14 *Chemistry and biochemistry of antibiotics*, E. B. Chain, *Ann. Rev. Biochem.* 27 167–212 (1958). A review with 297 references.

15 *Structural chemistry of actinomycetes antibiotics*, Eugene van Tamelen, *Fortschr. Chem. org. Naturstoffe* 16 90–138 (1958). A review with 113 references.

16 *Biochemistry of antibiotics*, S. B. Binkley, *Ann. Rev. Biochem.* 24 597–626 (1955). A literature survey complete to October 1954 with 284 references.

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APPENDIX A.

# The Chemical Composition of the Tissues and Large Molecules of Bacteria and Fungi

The composition of the cell wall, the capsule and the protoplast membrane in bacteria and of the mycelial wall in molds is generally more specific to the organism than that of the lower molecular weight metabolites. For that reason these substances are more interesting in taxonomy and immunochemistry. The toxins, pyrogens and lipoproteins are also interesting from these standpoints.

The advent of paper chromatography has so facilitated the identification of amino acids, sugars and other fragments of the hydrolysis of the higher molecular weight components of microorganisms that the literature on this topic has blossomed during recent years.

Some of the results have been unexpected. For example, the actinomycetes, which resemble the molds superficially, have been found closer chemically to the bacteria.

This appendix is a list of references on the subject. While the paper titles may not always so indicate, they are all concerned in some way with the composition or structure of the tissues and macromolecules of bacteria and fungi.

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APPENDIX B.

# Bacterial and Fungal Carotenes

The subject of bacterial and fungal carotenoids is confusing because of the large number of closely related structures and, in some cases, duplications in nomenclature. The following tables were prepared by an authority, Professor T. W. Goodwin of the University of Liverpool. They appeared in his excellent book "The Comparative Biochemistry of the Carotenoids" and are reproduced here with his permission and with the consent of the Chemical Publishing Co., 212 Fifth Ave., New York City.

TABLE I

*The Qualitative Distribution of Caratenoids in Fungi*

| Pigment | α-carotene | β-carotene | γ-carotene | δ-carotene | ζ-carotene | lycopene | neurosporene | phytofluene | phytoene | torulene | rubixanthin | rhodoviolascin | lycoxanthin (or rhodoplin) | rhodopurpurene | torularhodin | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Aleuria aurantia* | + | + | | | | | | | | | | | | | | 1 |
| *Allomyces arbuscula* | | | + | | | | | | | | | | | | | 2 |
| *Allomyces javanicus* | | + | + | | | | | | | | | | | | | 2 |
| *Allomyces macrogyna* | | | + | | | | | | | | | | | | | 2 |
| *Allomyces moniliformis* | | | + | | | | | | | | | | | | | 2 |
| *Cantharellus cibarius* | + | + | + | + | | + | | | | | | | | | | 3 |
| *Cantharellus cinnobarinus** | | + | | | | | | + | | | | | | | | 12 |
| *Cantharellus infunadibiliformis* | | | | | | + | ? | | | | | | | | | 3 |
| *Cantharellus lutescens* | | | | | | + | | | | | | | | | | 3 |
| *Coleosporium senecianis* | + | | + | | | | | | | + | + | | | | | 1 |
| *Dacromyces stillatus* | | + | + | | | | | + | + | + | | | | | | 13 |
| *Gymnosporangium juniperivirginionae* | + | + | + | | | | | | | | | | | | | 14 |
| *Lycogola epidendron* | | | | | | | | | | + | | + | | | | 1 |
| *Neurospora crassa* | + | + | + | | | + | + | + | | | | + | + | + | | 4 |
| *Phycomyces blakesleeanus* | + | + | + | | + | + | + | + | + | | | | | | | 5,6,7,8,9 |
| *Pilabolus kleinii* | | + | | | | | | | | | | | | | | 15 |
| *Palystigma rubrum* | | | | | | | | | | | | | + | | | 1 |
| *Puccinia coranifera* | + | + | + | | | | | | | | | | | | + | 1 |
| *Rhodotorula rubra* | + | + | | | ? | | ? | + | | + | | | | | + | 1,10,11 |
| *Rhodotorula sonniei* | | + | | | | + | | | | + | | | | | + | 10 |
| *Sporobolomyces roseus* | | + | | | | | | | | + | | | | | | 1 |
| *Sporobolomyces salmanicolor* | | + | | | | | | | | + | | | | | | 1 |
| *Tremella mesenterica* | | + | | | | | | | | | | | | | | 1 |

* Also canthaxanthin.

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TABLE II

*Characteristic Fungal Carotenoids**

| Pigment | Melting point | Absorption spectra maxima (mμ) | | |
|---|---|---|---|---|
| | | Carbon disulphide | Light petroleum | Chloroform |
| Torulene[1, 2] | 185° | 563–5, 520–5, 488–91 | | 539,501,469 |
| Torularhodin[2] | 201–203° (decomp.) | 582,541,502 | 537,501,467 | 554,515,483 |
| Neurosporene[3] (See also Tetrahydrolycopene) | 124° | 502.5, 470.5, 439.5 | 470,441.5 | |
| Acid carotenoid[3] from *Neurospora crassa* | — | 512–514 | 516, 482 | |
| Pigment III } from *Cartinarius* | — | — | 520, 470 | 462, 405 |
| Pigment VI } *cinnabarinus*[4] | — | 494 | — | 455 |
| Canthaxanthin | 218° | 500 | — | 462 |

* Pigments first reported in other organisms but also present in fungi are not recorded here.

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TABLE III

*Fungi in Which Early Workers[1,2] Have Reported the Presence of Carotenoids, but Which Have Not Recently Been Investigated*

| | |
|---|---|
| *Ascobolus* spp. (not *A. furfuraceus*[3]) | *Peziza (Lachnea) scutellata* |
| *Calocerca cornea* | *Phragmidium violaceum* |
| *Calocerca palmata* | *Pilobolus crystallimus* |
| *Calocerca viscosa* | *Pilobolus kleinii* |
| *Chytridium* spp. | *Pilobolus oedipus* |
| *Coleosporium pulsatilla* | *Polystigma ochraceum (fulvum)* |
| *Ditiola radicata* | *Puccinia coronata* |
| *Leotia lubrica* | *Saccharomyces* (spp.) |
| *Lycogala flavofuscum* | *Sphaerostilbe coccaphila* |
| *Melampsora aecidioides* | *Spathularia flavida* |
| *Melampsora salicis capreae* | *Stemonitis* spp. |
| *Nectria cinnabarina* | *Triphragmium ulmariae* |
| *Peziza aurantia* | *Uredo (Coleosporium) euphrasie* |
| *Peziza (Lachnum) bicolor* | *Uromyces alchemille* |

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[2] F. G. Kohl, Untersuchungen über das Carotin und seine physiologische Bedeutung in der Pflanze, Bornträger, Leipzig, 1902.

[3] T. W. Goodwin, *Biochem. J.* 50 550 (1952).

TABLE IV

*Fungi from Which Carotenoids Have Been Shown to Be Absent*

| | |
|---|---|
| *Agaricus (Telamoria) armillatus*[1] | *Nephoroma lusitanica*[1] |
| *Agaricus laceatus*[1] | *Oidium violaceum*[1] |
| *Alternaria solani*[2,*] | *Paxillus atratomentosus*[1] |
| *Amanita muscaria*[1] | *Penicilliopsis clavariaeformis*[1] |
| *Amanita pantherina*[1] | *Peziza aeruginosa*[1] |
| *Arthonia* spp.[1] | *Peziza echinospora*[1] |
| *Ascobolus furfuraceus*[3] | *Peziza sanguinea*[1] |
| *Bachospora dryma*[1] | *Phragmidium violaceum*[1] |
| *Bacidia muscorum*[1] | *Pichia* spp.[4] |
| *Biatora fungidula*[1] | *Polyporus grammocephalus*[5] |
| *Bilimbia melaena*[1] | *Polyporus luzonenis*[5] |
| *Boletus luridus*[1] | *Polyporus rubidus*[5] |
| *Boletus scaber*[1] | *Polyporus zonalis*[5] |
| *Buellia* spp.[1] | *Polystictus hirsutus*[5] |
| *Cladonia coccifera*[1] | *Polystictus sanguineus*[5] |
| *Clavaria ternica*[1] | *Polystictus versicolor*[5] |
| *Claviceps* spp.[1] | *Polystictus xanthopus*[5] |
| *Cortinarius bulliardi*[1] | *Pullularia* spp.[4] |
| *Cortinarius violaceus*[1] | *Rhizoctonia solani*[2,*] |
| *Daedalea flavida*[5] | *Rhizopogon rubescens*[1] |
| *Fusarium lycopersici*[2,*] | *Russula alutacea*[1] |
| *Fusarium moniforme*[2,*] | *Russula aurata*[1] |
| *Fusarium oxysporium*[2,*] | *Russula emetica*[1] |
| *Ganoderma (Fomes) lucidus*[5] | *Russula integra*[1] |
| *Gomphidius glutinosus*[1] | *Saccobolus violaceus*[1] |
| *Gomphidius viscidus*[1] | *Sarcogyme pruinosa*[1] |
| *Helminthosporium sativum*[2,*] | *Taphrina deformans*[2] |
| *Helvella esculenta* | *Thalloidima candidum*[1] |
| *Hydnum ferrugineum*[1] | *Thelephorus* spp.[1] |
| *Hydnum repandum*[1] | *Thielavia terricola*[2,*] |
| *Hygrophorus coccineus*[1] | *Torulopsis lipofera*[4] |
| *Hygrophorus conicus*[1] | *Torulopsis luteola*[4] |
| *Hygrophorus punicens*[1] | *Torulopsis pulcherrima*[4,6] |
| *Lactarius deliciosus*[1] | *Trametes persooni*[5] |
| *Lecidea* spp.[1] | *Trametes versatilis*[5] |
| *Lenzites subferruginea*[5] | *Zygosaccharomyces* spp.[4] |

* Only vitamin A-active carotenoids are absent from these species. Inactive carotenoids may possibly be present.

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TABLE V

*Properties of Bacterial Carotenoids*

| Name | Melting point | Absorption maxima in mμ | | |
|---|---|---|---|---|
| | | Light petroleum | Carbon disulphide | Chloroform |
| Sarcinene[1,*] | — | 415,440,469 | | |
| Sarcinaxanthin[2,†] | 149–150° | 415,440,469 | 464,494 | 423,451,480 |
| Xanthophyll[3,†] (Lutein) from *Sarcina lutea* | | — — — | 466,499 | 451,480 |
| Flavorhodene[4,5,‡] (Rhodoviolacein) | 111–113° | 442,470 | 472,503 | 453,482 |
| Rhodopurpurene[4,5,§] | 162° | 472,502 | 479,511,550 | 458,487,527 |
| Rhodopin[4,5] | 171° | 440,470,501 | 478,508,547 | 453,486,521 |
| Rhodovibrin[4,5] | 168° | | 517,556 | |
| Rhodoviolascin[4,5] (= Spirilloxanthin) | 218° | | 496,530,573.5 | 476,507,544 |
| α-Bacteriopurpurin[6,‖] | — | 460,495,528 (in methanol) | 498,532,571 | |
| β-Bacteriopurpurin[7,#] | — | 452,482,502 (in methanol) | | |
| Leprotene[8] | 198–200° | 425,452,484 | 477,499,517 | 428,460,495 |
| Xanthophyll from *Flavobact. esteroaromaticum, F. suaveolens and F. faecale*[3] | — | | 453,482,513 | 460,513 |
| Carotene from *F. sulphureum*[3,*] | — | | 437,466,487 | 451,481 |
| Xanthophyll from *Erwinia loythri*[2] | — | | 478,513 | 458,485 |
| Xanthophyll from *E. ananas*[2] | — | | 474,508 | 460,493 |
| Chrysophlein[9,10] | — | 452 | 487 | — |

* The probable identity of these with neurosporene cannot be ignored.

† These may be identical.

‡ May be identicol with ε-carotene.

§ May be identicol with lycopene.

‖ α-Bacteriopurpurin is probably one of Karrer's rhodocorotenoids.

# β-Bacteriopurpurin is probably identical with rhodoviolascin.

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APPENDIX C.

# The Chemical Constituents of Mycobacteria

A great many metabolites of mycobacteria have been characterized, many of them incidental to the study of tuberculosis. The following referenced list was prepared by Dr. Esmond R. Long and appeared in his recent book, *The Chemistry and Chemotherapy of Tuberculosis.* It is reproduced here by permission of the author and of the Williams and Wilkins Publishing Co. of Baltimore. While many of the compounds in this list appeared earlier in the Handbook, it may be useful to see them in aggregate as well.

CONSTITUENTS OF MYCOBACTERIA

*(The numbers refer to articles in the reference list that follows)*

| Substance identified | Source of material | | | | | | |
|---|---|---|---|---|---|---|---|
| | Culture medium [1] | Bacillary bodies | | | | | |
| | | *Human* | *Bovine* | *Avian* | *Leprosy* | *Timothy* | *Others* |
| ALCOHOLS | | | | | | | |
| *d*-Eicosanol-2 and *d*-octadecanol-2 | | | | 9, 170 | 87 | 162 | |
| Glycerol | | 6, 7, 15, 17, 61, 225 | 42, 44 | | 14 | 163 | |
| Glycol (phytoglycol) | | 202 | | | 62 | | |
| Leprosol | | | | | 62 | | |
| Phthiocerol | | 1, 61, 65, 66, 89, 170, 203, 225 | 44, 65 | | | | |
| CARBOHYDRATES | | | | | | | |
| Arobinose | 73, 94, 147, 171 | 1, 15, 52, 74, 85, 94, 103, 148, 172 | 55 | 9, 74 | 8 | | |
| 2-Desoxyribase | | | | 54 | | 118 | |
| Galactose | 94 | 1, 15, 52, 85, 94, 172 | 39 | 9 | 8 | | |
| Glucosamine | | 100, 172 | | 9 | | | |
| Glucose | | 6, 26, 42 | | 17 | | | |
| Glycogen | | 133 | | 53 | | | |
| Inositol | 168 | 6, 11, 13, 17, 18, 52, 69, 68, 74, 204, 261 | 17, 44 | 9, 17, 18, 74 | 8, 219 | 161 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Mannose | 73, 94, 147 | 1, 11, 13, 15, 16, 17, 18, 52, 68, 74, 94, 103, 148, 172, 225 | 17, 39, 44, 55 | 9, 17, 18, 74 | 8, 219 | 162 | |
| Polysaccharides | 73, 94, 128, 146, 147, 157, 171, 180, 187, 190, 201, 224 | 74, 86, 94, 100, 102, 103, 116, 133, 148, 192, 200 | 39, 55 | 17, 74, 122, 171 | 219 | | |
| Polysaccharide I | 181, 216 | | | | | | |
| Polysaccharide II | 126, 189 | | | | | | |
| Ribose | | 85 | | 54 | | 58 | |
| Trehalose | | 12, 63, 76, 159 | 159 | 170 | 14 | 162 | |
| Uronic acid | | | 39 | | | | |
| ENZYMES | | 34, 71, 92, 155, 175, 176 | 30, 34, 84, 175 | 110, 131, 132, 175, 179, 227, 228, 229 | | 71, 77, 78, 175, 176, 233 | 34, 71, 77, 84, 101, 107, 141, 176, 223, 238, |
| Amidase, Protease | | | | | | 178 | |
| Asparaginase | | 129 | 129 | 125 | | | 129 |
| Catalase | | 60, 83, 154 | 66, 83, 154 | 83 | | 83 | 83 |
| Decarboxylase | | | | | | 99, 206 | |
| Esterase | | 59 | 59 | 105, 160, 230 | | 59, 152, 160 | 59 |
| Lipase | | | | 106 | | 152 | 49 |
| Penicillinase | | 108 | | | | | |
| Peptidase | | 177 | 36, 177 | | | 177 | 177 |
| Transaminase | | | | 111 | | 121 | |
| Urease | | 193 | 193 | 193 | | 193, 233 | 193, 233 |
| Glycerophosphoric acid | | 6, 13, 68, 191 | 17, 144 | 17 | 219 | 162, 191 | |

APPENDIX C—*Continued*

| Substance identified | Source of material | | | | | | |
|---|---|---|---|---|---|---|---|
| | Culture medium | Bacillary bodies | | | | | |
| | | *Human* | *Bovine* | *Avian* | *Leprosy* | *Timothy* | *Others* |
| LIPIDES | | 21, 26, 35, 50, 56, 61, 63, 145, 166, 169, 197, 215 | 21, 35, 51, 144 | 17, 171, 229 | 219 | 162 | 214 |
| Acetone soluble wax | | 7, 12, 35, 76 | 17, 35, 42 | 17 | 14, 219 | 163 | |
| Crotonaldehyde | 3 | | | | | | |
| Fatty acids | | 4, 45, 50 | | | | | |
| Anisic | | 1, 12, 63, 76 | | | | | |
| Caproic | | | | | 14 | | |
| Cerotic | | 7 | 42 | | | | |
| Citric | 138 | | | | | | |
| Leprosinic | | | | | 10, 82, 87 | | |
| Linoleic and Linolenic | | 7 | | | | | |
| Malic and succinic | 209 | | | | | | |
| Mycolic | | 1, 2, 22, 24, 25, 26, 50, 56, 61, 82, 139, 159, 164, 204, 225, | 44, 67, 82, 90, 159 | 8, 9, 82 | | 29, 54, 82, 164 | 29 |
| Myoceranic | | 167 | | | | | |
| Mycocerosic | | 89 | | | | | |
| Myristic | | | | | 10, 14 | | |
| Octadecanoic | | 48 | | | | | |
| Oleic | | 6, 15, 89, 164 | 17 | 17 | | | |
| Oxalic | 208 | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Palmitic..... | | 6, 7, 15, 48, 89, 164, 225 | 17, 42, 51 | 17 | 10, 14, 219 | 162, 163 | |
| Pentacosanoic... | | | | 8 | | | |
| Phthalic..... | | 1 | | | | | |
| Phthioic (hexacosanoic)..... | | 1, 6, 7, 46, 47, 47, 61, 63, 76, 89, 139, 164, 199, 225 | 44 | | | | |
| Stearic..... | | 6, 15, 48, 89, 139, 164, 225 | 42 | 17 | 10, 14, 219 | 163 | |
| Tetracosanoic... | | | 44 | 8, 9 | 10, 14, 87 | 164 | |
| Tuberculenone and hydroxy-acid $C_{60}$..... | | 22 | | | | | |
| Tuberculostearic. | | 7, 15, 48, 61, 63, 68, 76, 89, 164, 198, 220, 225 | 42 | | 219 | 163 | |
| Glycolipide (lipopolysaccharide)..... | | 23, 24, 56, 158 | 23, 158 | | | | |
| Mycobactin..... | | | | | | 195 | 174 |
| Phosphatides..... | | 6, 11, 13, 16, 17, 18, 61, 63, 68, 164, 221 | 17 | 17, 18 | 219 | 29, 162 | 153 |
| Pyridine..... | | | 232 | | | | |
| Sterols..... | | 19 | | | | | |
| Waxes | | | | | | | |
| A and B..... | | 169 | | | | | |
| C and D..... | | 35, 158, 169 | 35, 158 | | | | |
| Purified..... | | 61, 35, 172 | 35, 44 | 170 | | | |
| Soft wax..... | | 6 | | | | | |
| Leprosin..... | | | | | 10 | | |
| Unsaponifiable.. | | 6, 202 | | | | 162 | |

APPENDIX C—*Continued*

| Substance identified | Source of material | | | | | | |
|---|---|---|---|---|---|---|---|
| | Culture medium | Bacillary bodies | | | | | |
| | | *Human* | *Bovine* | *Avian* | *Leprosy* | *Timothy* | *Others* |
| NUCLEIC ACIDS | | 44, 114, 140 | 114 | | | 58 | |
| Adenine | | 41, 114, 127, 142, 194 | 127, 194 | 222 | | 58 | 127 |
| Cytosine | | 41, 114, 127, 194 | 127, 194 | 222 | | 58 | 127 |
| Desoxyribonucleic acid | 190, 210 | 75, 102, 114, 117, 134, 165, 194 | 194 | 51, 54, 222, 229 | | 75, 117, 118, 134, 165 | 165 |
| Guanine | | 127, 142, 194 | 127, 194 | 222 | | 58 | 127 |
| 5-Methyl-cytosine | | 115, 194 | 194 | 222 | | | |
| Ribonucleic acid | | 75, 117, 127 | 127 | 54, 229 | | 75, 117 | 127 |
| Thymidine | | | | | | 118 | |
| Thymine | | 41, 114, 194 | 194 | 222 | | 58 | |
| Uracil | | 114, 127 | 127 | | | 58 | 127 |
| PIGMENTS | 98, 150 | 64, 149 | | | | | |
| Azafrin | | | | | | 109 | |
| Carotene | | 213 | | | | 51, 93, 109 | 218 |
| Coproporphyrin | | 64, 69 | 69 | | | 69, 70, 217 | 69, 70, 217 |
| Cytochrome | | 20 | 20 | 20, 227 | | 20, 70, 217 | 70, 217 |
| Kryptoxanthin | | | | | | 109 | |
| Leprotin | | | | | 97 | | 161 |
| Lutein | | | | | | 51, 109 | 218 |
| Phthiocol | | 12, 61, 63, 76 | | | | | |
| Zeaxanthin | | | | | | 109 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| PROTEINS..... | 31, 33, 38, 39, 40, 95, 104, 112, 113, 119, 120, 128, 135, 146, 180, 181, 182, 184, 185, 186, 187, 188, 196, 201, 211, 214, 215 | 28, 94, 96, 102, 103, 116, 151, 183, 212, 213 | 151 | 151, 171, 217 | | 151 | 195 |
| AMINO ACIDS..... | 112, 181, 188 | 1, 24, 27, 79, 91, 123, 124, 215 | 43, 91 | 91 | | 29, 91, 107 | 91, 107, 215 |
| α-ε-Diaminopimelic acid..... | 188, 226 | 88, 226 | 88, 226 | 88, 226 | | | 88 |
| Ornithine..... | | 88 | 88 | | | 88 | 88 |
| VITAMINS | | | | | | | |
| $B_1$ (aneurine)..... | 143 | | | | | | |
| (thiamine)..... | 168 | | | | | | |
| $B_2$ (riboflavine).... | 32, 37, 168 | 173, 205 | 173 | 205, 231 | | 206 | |
| $B_6$ (pyridoxine).... | 168 | | | | | | |
| $B_{10}$ and $B_{11}$..... | 156 | | | | | | |
| $B_{12}$..... | | 130 | | | | 130 | 144 |
| Biotin..... | 32, 136, 168 | | | | | | |
| Folic acid..... | 168 | | | | | | |
| K (phthiocol)..... | | 5, 195 | | | | 195 | |
| Niacin (nicotinic acid)..... | 32, 168, 209 | 80 | 80 | | | | |
| (PP factor)..... | 72 | | | | | | |
| Pantothenic acid... | 32, 168 | | | | | | |
| p-Aminobenzoic acid..... | 32, 81, 137, 168, 209 | | | | | | |

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## ADDENDUM

In order to cover pertinent literature appearing as late as December, 1960 this addendum is attached. Also included is a little material from earlier dates which was overlooked. Arrangement is by chapter title, and new compounds eligible for inclusion often are given appropriate entry numbers, but with a letter added to the number so that it is evident in the indexes that such entries are located in the addendum. Due to time restrictions these entries may be abbreviated, but references are listed. The addendum is not indexed.

## 2. Alcohols, Glycols and Compounds Related to Sugars

17a **Acetyl Methyl Carbinol** (Acetoin)

This substance, mentioned as a co-product of butanediol, is produced by many microorganisms. It is given off by several streptomycetes, including *Streptomyces erythreus*, an erythromycin producer. It is present in such large quantities in some erythromycin fermentations that it interferes with production of the antibiotic.[1]

A survey has been made of 44 species and strains of acetobacter for ability to convert lactate to acetoin.[1a] *A. rancens* and *A. pasteurianus* were good producers, the former yielding one isomer, the latter the other.

Acetoin metabolism of bacteria in general has been studied.[1b]

Biosynthesis of acetoins has been reviewed.[1c]

47a **Galactosyl Lactose**

This trisaccharide was produced by *Penicillium chrysogenum* Thom on a lactose medium and assigned the structure O-$\beta$-D-galactopyranosyl-(1 → 6)-O-$\beta$-D-galactopyranosyl-(1 → 4)-D-glucopyranose.[2]

Several papers have appeared on the mode of action of

[1] V. Musilek, V. Sevcik, M. Musilkova, J. Rokos and P. Prochazka, *Experientia* 14 323 (1958).

[1a] J. de Ley, *J. Gen. Microbiol.* 21 352–365 (1959).

[1b] Yasuhiro Maeda, *Okayama Igakkai Zasshi* 71 8017 (1959). (*Chem. Abstr.* 55 694i)

[1c] H. Oberman, *Postepy Biochemii* 6 181–195 (1960).

[2] A Ballio and S. Russi, *Tetrahedron* 9 125 (1960).

streptomycin. Its effect on *Escherichia coli* has been studied.[3] The cell permeability barrier was altered, reminiscent of detergents and of polymyxin. Preformed cells were undamaged, but defects were caused in cell membranes formed in its presence by non-resistant cells. When $C^{14}$-labeled streptomycin was used, initial uptake occurred only outside the cell wall and secondary uptake depended on secondary damage to the membrane. The growing membrane was the primary site of action of the antibiotic.

The effect of streptomycin on the excretion of nucleotides by *E. coli* has been investigated.[4] Streptomycin enhanced excretion of 5′-nucleotides and prevented excretion of 2′- or 3′-nucleotides. It was not clear whether streptomycin blocked RNA synthesis *de novo* or whether degradation of RNA to 5′-nucleotides was enhanced.

The same group has published on chloramphenicol-sensitive and chloramphenicol-insensitive phases of the lethal action of streptomycin.[5] It appeared that the lethal effect of streptomycin on *E. coli* was exerted in two phases (1) a preparatory phase, which is markedly less lethal and can be blocked by chloramphenicol (a protein synthesis inhibitor), followed by (2) a more direct lethal phase which is insensitive to chloramphenicol. The induction process might have been due to formation of a permease without which streptomycin could not accumulate in the cell in lethal concentration.

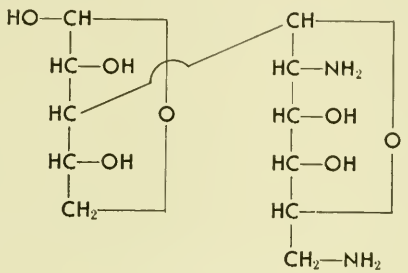
It has been found that, while penicillin inhibits growth of *Staphylococcus aureus* (strain Duncan), it does not cause rapid lysis as, *e.g.*, in the case of *E. coli*. Penicillin and streptomycin added (each at minimally bactericidal concentrations) to exponentially growing cultures caused rapid lysis. Only antibiotically active forms of streptomycin were effective. Under anaerobic conditions lysis was not rapid. (Streptomycin is not ordinarily effective under such conditions.[6])

[3] Nitya Anand and Bernard D. Davis, *Nature* 185 22, 23 (1959).
[4] Carmen L. Rosano, Richard A. Peabody and Charles Hurwitz, *Biochim. et Biophys. Acta* 37 380 (1960).
[5] Charles Hurwitz and Carmen L. Rosano, *ibid.* 41 162 (1960).
[6] R. Hancock, *Nature* 186 658 (1960).

It has been reported that streptomycin inhibits dehydrogenases by influencing the apoenzyme.[7] The conclusion was made that further search for enzymatic reactions susceptible to streptomycin should be aimed at the study of its influence on intracellular synthetic processes, mainly the synthesis of nucleic acids and proteins.

The mode of action of streptomycin in connection with its binding by *Mycobacterium avium* has been studied.[8]

The stereochemistry of neobiosamine B is as shown.[9]




Neobiosamine B

Dextromycin is neomycin B and contains a small amount of neomycin C.[10] Framycetin also is identical with neomycin B.[11]

59a **Aminocidin** (Crestomycin, Antibiotic 1600, Pharmiglucin, F. I. 5853) $C_{23}H_{45}O_{14}N_5$ (Sulfate) $[\alpha]_D^{23} + 51°$ in water. Produced by *Streptomyces crestomyceticus*, n. sp.[12] This antibiotic seems to be similar to or identical with paromomycin.

[7] K. Michalska, Symposium on Antibiotics, Prague, 1959.

[8] Tatsuji Kinoshita, *Nagoya J. Med. Sci.* 21 323 (1958).

[9] Kenneth L. Rinehart, Alexander D. Argoudelis, Townley P. Culbertson, W. Scott Chilton and Klaus Streigler, *J. Am. Chem. Soc.* 82 2970 (1960).

[10] Sueo Tatsuoka, Akira Miyake and Hayao Nawa, *J. Antibiotics* (Japan) 11A 193 (1958).

[11] Kenneth L. Rinehart, Jr., Alexander D. Argoudelis, William A. Goss, Arthur Sohler and Carl P. Schaffner, *J. Am. Chem. Soc.* 82 3938 (1960).

[12] F. Arcamone, C. Bertazzoli, M. Ghione and T. Scotti, *Giorn. Microbiol.* 7 251 (1959).

D-Araboascorbic acid is produced by *Penicillium decumbens*, *P. chrysogenum* mutant *fulvescens*, *P. notatum*, *P. meleagrinum* and *P. cyaneofulvum* growing on sucrose, glucose or D-gluconate.[13]

## 3. Aliphatic Acids and Glycolipides

The name mycoside has been suggested to designate a type-specific glycolipide of mycobacterial origin. To clarify nomenclature it was proposed that $C_A$ from photochromogenic strains be called mycoside A, $G_B$ from bovine strains, mycoside B, and $J_{AV}$ from avian strains mycoside C. Some properties are listed:[14]

Mycoside A:

Nearly colorless solid, m.p. 105°, $[\alpha]_D^{20} - 37°$ (in chloroform). Anal: C 72.2, H 11.3, $-OCH_3$ 8.6, N 0.0, P 0.0. U.V. maxima at 222, 274, 278 m$\mu$ (in hexane). Contains 2-O-methylfucose, 2-O-methylrhamnose and 2,4-di-O-methylrhamnose. The lipide part is a mycocerosate of an aromatic alcohol.

Mycoside B:

Colorless wax, m.p. 25°, $[\alpha]_D^{20} - 22°$ (in chloroform). Anal: C 76.6, H 12.0, $-OCH_3$ 4.3, N 0.0, P 0.0. U.V. maxima at 222, 274, 281 m$\mu$. Contains only one sugar, 2-O-methylrhamnose. The lipide moiety is a diester of 2 molecules of a branched-chain acid fraction of mean molecular weight corresponding to $C_{22}H_{44}O_2$ with a phenolic alcohol. It may also sometimes contain mycocerosic acid.

Mycoside C:

A peptide-glycolipide mixture. One component separated on silica gel had the following properties:
m.p. 200°, $[\alpha]_D^{20} - 31°$ (in chloroform). Anal:
Calculated for $C_{73}H_{133}O_{24}N_5$: C 59.8, H 9.1, N 4.8, $-OCH_3$ 6.3
Found: C 60.1, H 8.7, N 5.1, $-OCH_3$ 6.0.
It contains three deoxyhexoses, one being 6-deoxytalose and one 3,4-dimethoxyrhamnose. The peptide moiety is

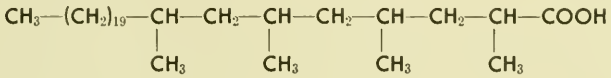
[13] T. Takahashi, M. Mitsumoto and H. Kayamori, *Nature* 188 411 (1960).

[14] Donald W. Smith, H. M. Randall, A. P. MacLennan and E. Lederer, *ibid.* 186 887 (1960).

a pentapeptide containing 1 mole of D-phenylalanine, 2 moles of *allo*-threonine and 2 moles of D-alanine. The lipide moiety was not entirely pure, but may be a hydroxy acid of about $C_{24}H_{48}O_3$. Two O-acetyl groups are present in mycoside C.

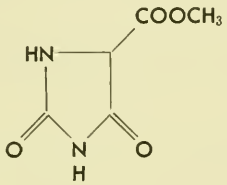
The lipoids of mycobacteria, their chemical structures and biological effects have been reviewed.[15]

132a **Mycocerosic Acid,** $C_{32}H_{64}O_2$, isolated by Anderson and collaborators,[16] has been shown to be 2,4,6,8-tetramethyloctacosanoic acid:[17]



Indications were obtained for the presence in mycobacteria of normal chain acids with 22, 24, 26 and 28 carbon atoms; 2-, 4-, 6-trimethyl-substituted acids with 25, 27 and 29 carbon atoms; and 2-, 4-, 6-, 8-tetramethyl-substituted acids with 30, 32 and 34 carbon atoms.

Succinic, fumaric and acetic acids as well as D,L-5-carboxymethylhydantoin, shown below, have been iso-



lated as extracellular acids from *Mycobacterium ranae* and from *M. tuberculosis* $H37R_v$.[18]

[15] E. Lederer, *Angew. Chem.* 72 372 (1960).

[16] L. G. Ginger and R. J. Anderson, *J. Biol. Chem.* 157 203 (1945) and preceding papers.

[17] Cecile Asselineau, Jean Asselineau, Ragnar Ryhage, Stina Ställberg-Stenhagen and Einar Stenhagen, *Acta Chem. Scand.* 13 822 (1959).

[18] Andree V. Fowler, Merrill N. Camien and Max S. Dunn, *J. Biol. Chem.* 235 1386 (1960).

Lipides of *Corynebacterium ovis* have been studied,[19] as have the component fatty acids of *Sporidesmium bakeri* Syd. lipides.[20]

The oil of wheat stem rust uredospores was found to contain a substantial quantity of an acid not previously reported from natural sources, *cis*-9,10-epoxyoctadecanoic acid, $C_{18}H_{34}O_3$, colorless leaflets, m.p. 58.5–59.5°, *cis*-epoxide peak in the infra-red at 845 cm.$^{-1}$.[21]

The chemistry of naturally occurring 1,2-epoxides, including many microbial products, has been reviewed.[21a]

Another new fatty acid, $C_{17}H_{32}O_2$, containing a cyclopropane ring has been reported (in a preliminary communication) as occurring in *Escherichia coli* lipides.[22]

Bongkrekic acid, at a concentration of $10^{-6}$ molar, is a potent inhibitor of oxidative phosphorylation as carried out by mitochondrial enzymes in heart muscle tissue.[23]

The direct participation of protein-bound biotin in fatty acid biosynthesis has been confirmed.[24]

Both 9- and 10-hydroxystearic acids can replace oleic acid as growth factors for anaerobically grown yeast, which requires unsaturated acid, and these substances may be precursors of oleic acid in yeast.[25]

The role of vitamins in lipide metabolism has been reviewed.[26]

Hydroxypyruvic acid has been isolated as the 2,4-dinitrophenylhydrazone from *Aspergillus niger*. It may arise from 3-phosphoglyceric acid.[27]

[19] A. Diara and J. Pudles, *Bull. soc. chim. biol.* 41 481 (1959).

[20] L. Hartman, J. C. Hawke, Isobel M. Morice and T. B. Shorland, *Biochem. J.* 75 274 (1960).

[21] A. Tulloch, B. Craig and G. Ledingham, *Can. J. Microbiol.* 5 485 (1959).

[21a] A. D. Cross, *Quart. Revs.* 14 317–336 (1960).

[22] Simone Dauchy and Jean Asselineau, *Compt. rend.* 250 2635 (1960).

[23] W. Welling, J. A. Cohen and W. Berends, *Biochem. Pharmacol.* 3 122 (1960).

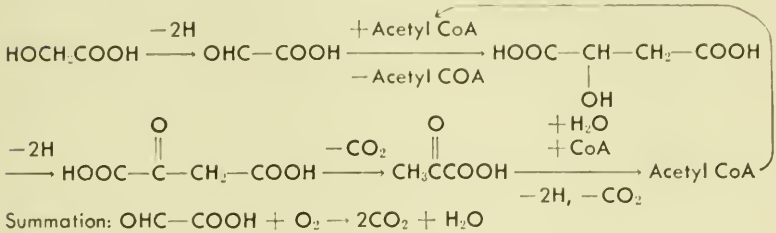
[24] S. J. Wakil and D. M. Gibson, *Biochim. et Biophys. Acta.* 41 122 (1960).

[25] W. J. Lennarz and Konrad Bloch, *J. Biol. Chem.* 235 PC 26 (1960).

[26] Bacon F. Chow, *Am. J. Clinical Nutrition* 8 321 (1960).

[27] Francis J. Behal, *Arch. Biochem. and Biophys.* 88 110 (1960).

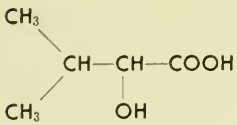
The oxidative degradation of glycolic acid in *E. coli* takes the following course:[28]



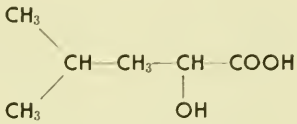

Summation: $OHC—COOH + O_2 \rightarrow 2CO_2 + H_2O$

A study has been made of the synthesis of cell materials from acetate by *Aspergillus niger*,[28a] and by *Escherichia coli*.[28b] Interrelationships of the tricarboxylic acid and glyoxylic acid cycles were discussed.

Lactobacilli produce $\alpha$-hydroxy acids other than lactic. Two of these have been identified as $\alpha$-hydroxy-D-isovaleric and D-isocaproic acids:[29]




D-Isovaleric Acid




D-Isocaproic Acid

These are growth promoters for certain strains of lactobacilli.

*Penicillium atrovenetum*, a $\beta$-nitropropionic acid producer,[30] was grown on $C^{14}$-labeled $\beta$-alanine, on $NaHC^{14}O_3$ and on 4-$C^{14}$-D,L-aspartic acid.[31] Since 96 percent of the label was in the 1-position, apparently aspartic acid was incorporated as a unit.

[28] H. L. Kornberg and J. R. Sadler, *Nature* 185 153 (1960).

[28a] J. F. Collin and H. L. Kornberg, *Biochem. J.* 77 430 (1960).

[28b] H. L. Kornberg, P. J. R. Phizackerly and J. R. Sadler, *ibid.* 77 438 (1960).

[29] Merrill N. Camien, Andree V. Fowler and Max S. Dunn, *Arch. Biochem. and Biophys.* 83 408 (1959).

[30] H. Raistrick and A. Stössl, *Biochem. J.* 68 647 (1958).

[31] A. J. Birch, B. J. McLoughlin, Herchel Smith and J. Winter, *Chem. and Ind.*, 840 (1960).

A review of naturally occurring nitro compounds has been published.[32]

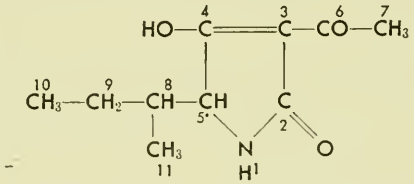
The fatty acids of *B. alcaligenes faecalis*, *S. pullorum*, *B. fluorescens*, *S. typhi-murium* and *B. natta* have been analyzed.[33] Palmitic and unsaturated $C_{18}$-acids were the main components. Unsaturated $C_{16}$-acids were present to some extent, the unsaturated $C_{18}$- and $C_{16}$-acids being largely oleic and palmitoleic. A saturated $C_{15}$-acid was abundant in the fat of *B. natta*.

Two acids, 13-methyltetradecanoic, m.p. 52.5–53°, and 15-methylhexadecanoic, m.p. 61.0–61.5°, were the main components of the fatty acid fraction of *B. subtilis*.[34]

A new monounsaturated, monohydroxy acid, diphtherocorynic, $C_{53}H_{104}O_3$, has been reported produced by *Corynebacterium diphtheriae*.[35] Its relationship to related compounds has been discussed.[36]

## 4. Tetronic Acids and Other Lactones and Lactams

Tenuazonic acid (3-acetyl-5-*sec*-butyltetramic acid) has been biosynthesized incorporating 3.9 percent of the tracer from a medium containing $CH_3C^{14}OONa$.[37] Of the total incorporated radioactivity 96 percent was present in the C-2 and C-6 atoms. The remaining 4 percent was shared by C-4 and C-10, and this was explained on the basis of the manner of biosynthesis of isoleucine.




[32] M. Pailer, *Fortschr. Chem. org. Naturstoffe* 18 55–78 (1960).

[33] Kunihiko Saito, *J. Biochem.* (Tokyo) 47 699 (1960).

[34] *Idem., ibid.* 47 710 (1960).

[35] E. M. Gubarev and L. M. Pustovlova, *Ukrain. Biokhim. Zhur.* 30 569 (1958).

[36] Raoul Toubiana and Jean Asselineau, *Compt. rend.* 251 884 (1960).

[37] C. E. Stickings and R. J. Townsend, *Proc. Biochem. Soc.*, 36P (1960).

It might be pointed out that, formally, some of the vulpinic acids are tetronic acids although we have not classified them as such.

The chemistry of the tetronic acids has been reviewed.[38]

## 5. Carotenes and Carotenoids

Another paper has been published on the incorporation of $C^{14}$-labeled compounds into carotenes by *Neurospora crassa*.[39] Mevalonic acid salts were the best of eight precursors used, but less than 1 percent of the 2-$C^{14}$ activity was incorporated into the carotene fraction. Phytoene, $\gamma$-carotene and its isomers ($\beta$- and $\zeta$-), phytofluene, neurosporene, spirilloxanthin and its isomers and lycopene were isolated. The presence of much phytoene, whose presence in the theoretical biosynthetic sequence has been questioned, was taken as an argument against formation of the carotenes by stepwise interconversions involving either hydrogenation or dehydrogenation and as an indication, rather, of independent synthesis.

The major carotenoids of some ascomycetes and basidiomycetes have been identified.[40] $\beta$-Carotene was predominant in *Epichloë typhina* and *Helotium citrinum*. Cryptoxanthin was second in importance in *Calocera viscosa*. Neurosporene was the major carotenoid in dull yellow *Cantharellus infundibiliformis* with traces of lycopene present. The reverse was true in *Cantharellus lutescens*. No carotenoids, but instead pigments with quinone-like reactions, were detected in the grey *Cantharellus cinereus* and orange-red *Guepinius helvelloides*.

A red pigmented yeast isolated from root nodules of *Lupinus luteus* produced torulene, $\beta$-carotene, $\gamma$-carotene and torularhodin.[41] Diphenylamine inhibited production of $\gamma$-carotene and torularhodin.

*Rhodotorula mucilaginosa* contained, in decreasing or-

[38] L. J. Haynes and J. R. Plimmer, *Quart. Revs.* 14 292 (1960).

[39] Leo F. Krzeminski and F. W. Quackenbush, *Arch. Biochem. and Biophys.* 88 287 (1960).

[40] Gilbert Turian, *Arch. Mikrobiol.* 36 139 (1960).

[41] Gy. Schneider, B. Matkovics and J. Zsolt, *Acta. Univ. Szegediensis, Acta. Phys. et Chem.* 5 55 (1959).

der of quantity, torularhodin, torulene, $\gamma$-carotene and $\beta$-carotene, but no phytoene or phytofluene.[42] Ultraviolet irradiation gave stable strains varying greatly from the parent both in quality and quantity of carotenoid content. One of many inhibitors tested, 2-hydroxybiphenyl, inhibited carotenogenesis without affecting culture growth. Doubt was expressed that the different carotenoids are biosynthetically mutually related.

Oil of wheat rust (*Puccinia graminis* var. *tritici*) uredospores contained $\beta$- and $\gamma$-carotenes with minor amounts of phytoene, lycopene, a *cis*-$\beta$-carotene and a *cis*-carotene.[43]

Mycoxanthin is the principal carotenoid of *Mycobacterium battaglini*.[44] Leprotene, a leprotene derivative, $\beta$-carotene, $\alpha$-carotene and an $\alpha$-carotene monoepoxide probably were present.

A carotenoid pigment in *Spirobacillus cienkowskii* Metchnikoff, a pathogen of cladocera, resembled rhodoviolascin or $\alpha$-bacteriopurpurin.[45] Astacene and astaxanthin also were thought to be present.

*Staphylococcus citreus* contains the orange carotenoid, sarcinaxanthin, and the yellow sarcinene.[46] Reference was made to two other uncharacterized carotenoids which have been isolated from natural sources, neoxanthin and corynexanthin.[47]

A new carotenoid has been isolated, which probably has the structure shown below.[48]

175a **Bacterioruberin a,** $C_{40}H_{56}O_2$, mauve-violet needles, m.p. 182° (vac.), U.V. 369, 385, 461, 494, 528 m$\mu$ in petroleum ether.

[42] Jean Villoutreix, *Biochim. et Biophys. Acta.* 40 434, 442 (1960).

[43] F. Hougen, B. Craig and G. Ledingham, *Can. J. Microbiol.* 4 521 (1958).

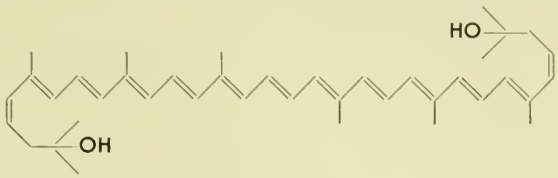
[44] Aldo Gaudiano, *Rend. ist. super. sanita* 22 769 (1959). (*Chem. Abstr.* 54 13253a)

[45] J. Green, *Nature* 183 56 (1959).

[46] Tatsuo Ohta, Toshio Miyazaki and Teruo Ninomiya, *Chem. & Pharm. Bull.* (Tokyo) 7 254 (1959).

[47] W. Hodgkiss, J. Liston, T. W. Goodwin and Malini Jamikorn, *J. Gen. Microbiol.* 11 438 (1954).

[48] Synnöve Liaaen Jensen, *Acta Chem. Scand.* 14 950 (1960).




*Halobacterium* sp.

A mutant of *Staphylococcus aureus* unable to produce bright pigments incorporated the label of 2-$c^{14}$-mevalonic acid into phytoene, which it accumulated.[48a]

The biosynthesis and function of the carotenoid pigments have been reviewed.[49] Also a review of *cis, trans*-isomeric carotenoid pigments has been published.[50]

## 6. Polyenes and Polyynes, Excluding Polyene Macrolides

In a review of polyacetylenes[51] a number of substances not included in our list were mentioned without references or physical properties. These are reproduced here:

193a **Octa-2,6-dien-4-yn-1,8-dioic Acid,** $C_8H_6O_4$.

$$HOOC—CH=CH—C\equiv C—CH=CH—COOH$$

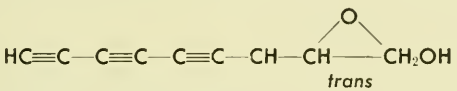
*Polyporus anthracophilus*

194a **Non-2-en-4,6,8-triynoic Acid,** $C_9H_4O_2$.

$$HC\equiv C—C\equiv C—C\equiv C—C\equiv C—CH=CH—COOH$$

*Psilocybe sarcocephala*

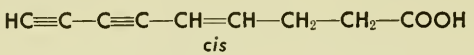
195a **Non-2-*trans*-oxido-4,6,8-triynol** (Biformin?), $C_9H_6O_2$.




*Coprinus quadrifidis* (*Polyporus biformis*?)

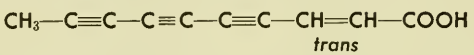
[48a] Ginzaburo Suzue, *Biochim. et Biophys. Acta* 45 616 (1960).
[49] T. W. Goodwin, *Advances in Enzymol.* 21 295–361 (1959).
[50] L. Zechmeister, *Fortschr. Chem. org. Naturstoffe* 18 (1960).
[51] E. R. H. Jones, *Proc. Chem. Soc.*, 199–211 (1960).

195b **Non-4-*cis*-en-6,8-diynoic Acid,** $C_9H_8O_2$.



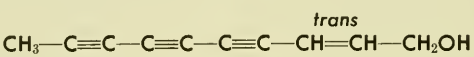
*Drosophila subatrata*

198a **Dec-2-*trans*-en-4,6,8-triynoic Acid,** $C_{10}H_6O_2$.



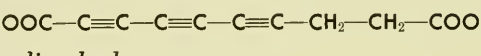
*Pleurotus ulmarius, Tricholoma paneolum*

201a **Dec-2-*trans*-en-4,6,8-triynol,** $C_{10}H_8O$.



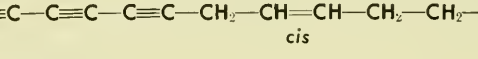
*Pleurotus ulmarius*

200a **Deca-4,6,8-triyn-1,10-dioic Acid,** $C_{10}H_6O_4$.



*Merulius lachrymans*

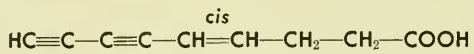
219a **Tetradec-5-*cis*-en-8,10,12-triyn-1,14-dioic Acid,** $C_{14}H_{12}O_4$.



*Poria sinuosa*

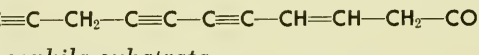
Four other polyacetylenes have been reported, complete with physical properties:[52]

195c **Drosophilin E** (*cis*-Non-4-en-6,8-diynoic Acid), $C_9H_8O_2$, light-sensitive prisms, m.p. 35°, U.V. 279.5, 264, 250, 238, 227, 210 m$\mu$.



*Drosophila subatrata*

209a **Drosophilin C** (*cis*-Undec-3-en-5,7,10-triynoic Acid), $C_{11}H_8O_2$, colorless needles, slowly yellowing in light at 20°, m.p. 97.5–99°, U.V. 280.5, 264.5, 250.5, 238, 226.5, 210.5 m$\mu$.



*Drosophila subatrata*

[52] E. R. H. Jones, P. R. Leeming and W. A. Remers, *J. Chem. Soc.*, 2257 (1960).

209b **Drosophilin D** (*cis*-Undeca-3,9,10-trien-5,7-diynoic Acid), $C_{11}H_8O_2$, colorless plates, m.p. 21–28°, U.V. 303.5, 290.5, 274.5, 259, 217 mμ.

$$H_2C{=}C{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH_2{-}COOH$$

*Drosophila subatrata*

219b **Compound 3040** (Dimethyl *trans*-Undeca-2-en-4,6-diyn-1,11-dioate), $C_{13}H_{14}O_4$, colorless crystals, m.p. 15–16°, U.V. 304, 286, 270, 255, 222.5, 215 mμ.

$$CH_3OOC{-}CH_2{-}CH_2{-}CH_2{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}COOCH_3$$

*Drosophila subatrata*

An Italian review on the chemical aspects of the basidiomycete antibiotics has been published.[53]

## 7. Macrocyclic Lactones (Macrolides)

A new tetraene antibiotic has been reported.[54]

233a **Unamycin A,** white needles, m.p. (dec.) 148–150°, $[\alpha]_D^{25}$ −92° (c 1.0 in 80% methanol-water), U.V. 290, 304, 319 mμ in methanol.

An acidic tetraene. Negative $FeCl_3$, Million, Fehling, Tollens tests. Positive Molisch, $KMnO_4$ and $Br_2$ tests. Pink Schiff test.

A second substance resembling toyocamycin was isolated:

1288a **Unamycin B,** white needles, m.p. 236–238° (dec.), $[\alpha]_D^{15}$ −43° (c 1.0 in acid methanol), N. E. 310.

C 46.4, H 4.46, N 22.25. Gives essentially the same color tests as unamycin A.

The unamycins were produced by *Streptomyces fungicidicus.*

A heptaene which may be new has been reported.[55]

256a **Grubilin** green-yellow, amorphous.

A non-toxic heptaene produced by *Streptomyces* BA-27,

[53] Marcella Magliola, *Annali di Chimica* 50 455–490 (1960).

[54] Masayuke Matsuoka and Hamao Umezawa, *J. Antibiotics* (Japan) 13A 114 (1960).

[55] J. Uri, I. Szilagyi and I. Békési, Symposium on Antibiotics, Prague, 1959.

and differing from amphotericin B, ascosin, aureofacin, AYF, candicidin, candidin, candimycin, PA 150 and trichomycin.

Antimycoin has been separated into A and B components.[56] Mevalonic acid stimulated production of these substances by *Streptomyces aureus*. Of nine other polyene producers tested, *Streptomyces viridoflavus* production of candidin and *Streptomyces* strain 3832 production of a pentaene (antibiotic S-8) of the eurocidin type were stimulated by mevalonic acid addition.

The mechanism of nystatin action on *Candida albicans* has been studied.[57] Respiration was accelerated and glucose uptake diminished, apparently by alteration of cell permeability.

A dissertation has been published (not yet received) entitled *Beitrag zur Kenntnis des Candicidins D*, G. Demuth, Math.-Naturw. Fakultät der Univ. Göttingen, 1959.

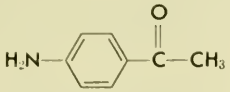
Some generalizations can be made now concerning the structures of polyene macrolides.* Tetraenes and heptaenes generally seem to contain nitrogen, while pentaenes and hexaenes do not. Moldicidin and PA-153 are exceptions since they are nitrogen-containing pentaenes. All tetraenes except PA-166 contain mycosamine. PA-166 contains an amino sugar (not a deoxy type) other than mycosamine. Pentaenes are neutral, containing neither amino sugars nor free carboxyl groups.

Heptaenes have been found so far to contain four different nitrogen-containing moieties. Two of these are the amino sugars previously mentioned. The other two are the aromatic amines, *p*-aminoacetophenone and *p*-aminophenylacetone, which are released by alkaline hydrolysis.

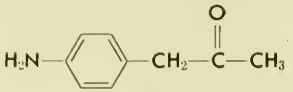
[56] Robert Samuel Safferman, *Dissertation Abstr.* 20 4264 (1960).

[57] J. W. Harman and J. G. Masterson, *Irish J. Med. Sci.* 378 249 (1957).

* Most of the information below on the polyene macrolides was taken from a seminar given by Dr. Edward Borowsky, Visiting Professor at the Institute for Microbiology at Rutgers University from Gdansk, Poland, in August 1960 and will be published.



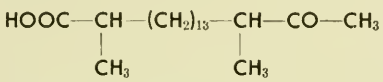
*p*-Aminoacetophenone



*p*-Aminophenylacetone

Amphotericin B and candidin are examples of heptaenes containing mycosamine. Candicidin, trichomycin and PA-150 contain *p*-aminoacetophenone.

Hydrocandidin has yielded an oxidation fragment identified as:



Some studies on the biosynthesis of this heptaene show no incorporation of labeled mevalonic acid, propionic acid or methionine. It seems to be derived from acetate.

The pentaene, moldicidin A, $C_{43}H_{22}O_{19}N$ was omitted.

Moldicidin B is identical with pentamycin.[57a] Candicidin is identical with ascosin. The main component of the PA-150 complex is identical with one component of the candidin complex. Several substances listed in the unclassified section are actually known to be polyene macrolides. These include: 1072-aliomycin (pentaene), 1067-akitamycin (tetraene), 1095-antibiotic from *Streptomyces fungicidicus* (tetraene), 1096-antibiotic from *S. griseus* (heptaene), 1097-antibiotic 26/1 (heptaene), 1294-substance 1404 (hexaene).

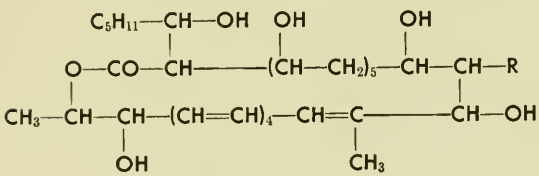
A new heptaene, perimycin (aminomycin), probably $C_{47}H_{75}O_{14}N_2$ and incorporating a *p*-aminophenyl group, has been reported.[58] Another heptaene, antibiotic 2814H, is produced together with a pentaene, antibiotic 2814P, netropsin and aureothin by *Streptomyces* IA 2814 resembling *S. netropsis*.[59] Analytical and optical data were reported on each.

[57a] Hiroshi Ogawa, Teiichiro Ito, Shigeharu Inoue and Motohiro Nishio, *J. Antibiotics* (Japan) 13A 353 (1960).

[58] Edward Borowsky *et al.*, Abstracts 1960 Conference on Antimicrobial Agents, Washington, D. C., October 26–28, 1960.

[59] Heinz Thrum and I-dschang Dcho, *Naturwissenschaften* 20 474 (1960).

The complete structures of the tetraenes, lagosin and filipin have been reported to be:[60]



R=OH=lagosin, $C_{35}H_{58}O_{12}$
R=H=filipin, $C_{35}H_{58}O_{11}$

Humidin $(C_{12}H_{20}O_4)_n$, colorless plates, m.p. 145–146° (dec.), $[\alpha]_D^{34}$ −6° (c 1.0 in ethanol), mol. wt. 550 ± 50, 823 ± 10, is an antifungal antibiotic isolated from the mycelium of *Streptomyces humidus*, which also produces dihydrostreptomycin.[61] It was not clear from the abstract whether or not this substance was of the polyene macrolide type.

Some aspects of the mode of action of polyene antifungal antibiotics have been reviewed.[62]

A nitrogen-containing antifungal polyene antibiotic, capacidin, produced by a streptomycete has been isolated.[63, 64] The substance is levorotatory, has reducing properties, is a primary or secondary alcohol and shows ultraviolet absorption peaks at 318, 332, 350 mμ.

A general review of the polyene antifungal antibiotics has been published.[65]

Two new antibiotics have been reported, one of them, at least, apparently a macrolide.[66]

[60] M. L. Dhar, V. Thaller and M. C. Whiting, *Proc. Chem. Soc.*, 310 (1960).

[61] Koichi Nakazawa, Motoo Shibata, Hiroichi Yamamoto, Toshihiko Kanzaki, Eiji Higashide, Akira Miyake and Satoshi Horii, *Nippon Nôgei Kagaku Kaishi* 32 713 (1958). (*Chem. Abstr.* 54 22843g)

[62] E. Drouhet, L. Hirth and G. Lebeurier, *Annals N. Y. Acad. Sci.* 89 134–155 (1960).

[63] Rachel Brown and Elizabeth Hazen, N. Y. State Dept. Health, Ann. Rept. Div. Labs. and Research 50–52 (1959). (*Chem Abstr.* 54 22824h)

[64] *Idem., Antibiotics and Chemotherapy* 10 702 (1960).

[65] L. C. Vining, *Hindu Antibiotics Bulletin* 3 37–55 (1960).

[66] E. Gäumann, R. Hütter, W. Keller-Schierlein, L. Neipp, V. Prelog and H. Zähner, *Helv. Chim. Acta* 43 601 (1960).

265a **Lankamycin**, $C_{36}H_{62}O_{14}$, colorless crystals, m.p. 147–150° and at 181–182°, $[\alpha]_D^{20}$ −94° (c 1.23 in ethanol). U.V. 289 mμ.

Typical erythromycin color tests were obtained. It is notable that this macrolide contains no amino sugar.

A second, unclassified antibiotic was isolated from the same culture (*Streptomyces violaceoniger* (Waksman et Curtis) (Waksman et Henrici).

1164a **Lankacidin**, $C_{46}H_{66}O_{16}N_2$, pale yellow microcrystalline powder, m.p. 165–168°, $[\alpha]_D^{20}$ −161° (c 0.967 in ethanol), U.V. 227 mμ (log 2.95).

Contained no N − $CH_3$ or −$OCH_3$ groups.

It is interesting that spiramycin contains three sugars.[67]

A paper on the mode of action of erythromycin[68] reports that, when the antibiotic was added to growing cells of *E. coli*, synthesis of protein (but not RNA or DNA) was inhibited, as was adaptive formation of β-galactosidase. Lactose was the substrate. Oxygen uptake of resting cells was inhibited in some organisms but not in others, but in no case did cytochrome oxidase appear to be affected.

The wild strain of *Streptomyces kitasatoensis* Hata produces leucomycin, a complex of six macrolide antibiotics, while a mutant produces only two of these, although total macrolide production was the same in each case.[69, 70, 71] Probable empirical formulas of the members of the complex are shown below:

| leucomycin | formula | melting point |
|---|---|---|
| $A_1$ | $C_{46}H_{81}O_{17}N$ | |
| $A_2$ | $C_{68}H_{111}O_{22}N$ | |
| $B_1$ | $C_{35}H_{59}O_{13}N$ | 214.5–216.5° |
| $B_2$ | $C_{35}H_{65}O_{16}N$ | 214–216° |
| $B_3$ | $C_{34}H_{53}O_{13}N$ | 216–217° |
| $B_4$ | $C_{35}H_{59}O_{16}N$ | 221–223.8° |

[67] Raymond Paul and Serge Tchelitcheff, *Bull. Soc. chim. France,* 150 (1960).

[68] Hiroshi Nakagawa, *Osaka Daigaku Igaku Zasshi* 11 3451 (1959). (*Chem. Abstr.* 54 11154a)

[69] J. Abe, Y. Suzuki, T. Watanabe and K. Satake, *Nippon Kagaku Zasshi* 31 969 (1960).

[70] T. Watanabe *et al., Bull. Chem. Soc. Japan* 33 1100 (1960).

[71] Tetsuo Watanabe, Hisao Nishida, Jinnosuke Abe and Kazuo Satake, *ibid.* 33 1104 (1960).

Methymycin has been found to be biosynthesized principally from propionate, although one mole of acetate may be incorporated.[72]

## 8. Alicyclic Compounds Other Than Terpenoids and Steroids

An investigation of the biosynthesis of palitantin shows that it is acetate-derived, and that neither shikimic acid nor mevalonic acid are involved.[73]

Several compounds have been isolated which may be related to cycloheximide:

302a **Niromycin B,** $C_{14}H_{21}O_4N$ (suggested), white, hygroscopic crystals, m.p. 47–67°.

A neutral substance produced by *Streptomyces albus.*[74]

302b **Niromycin A,** white hygroscopic, amorphous powder, m.p. 98–105°.

Positive 2,4-dinitrophenylhydrazine and Tollens tests. Negative ninhydrin, $FeCl_3$, Fehlings, Benedicts, Molisch, biuret, $KMnO_4$ tests.

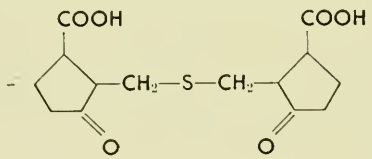
The effect of cycloheximide on the metabolism and growth of *Saccharomyces pastorianus* has been studied.[75]

Some substances related to sarkomycin and produced by the same organism were overlooked.[76] These were:

301a **Sarkomycin $E_2$,** $C_{10}H_{14}O_4$, m.p. 179°.

301c **Sarkomycin $E_1$,** $C_{14}H_{18}O_7$, m.p. 169°.

301d **Sarkomycin $S_2$,** $C_{14}H_{18}O_6S$, m.p. 183°, $[\alpha]_D$ +136°.




[72] A. J. Birch, E. Pride, R. W. Rickards, P. J. Thomson, J. D. Dutcher, D. Perlman and C. Djerassi, *Chem. and Ind.,* 1245 (1960).

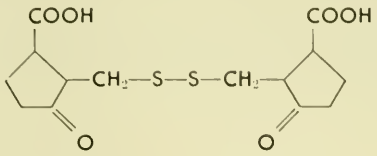
[73] P. Chaplen and R. Thomas, *Biochem. J.* 77 91 (1960).

[74] Teisuke Osato, Yutaka Morikubo and Hamao Umezawa, *J. Antibiotics* (Japan) 13A 110 (1960).

[75] Bradner Wood Coursen, *Dissertation Abstr.* 21 (1960).

[76] Sueo Tatsuoka *et al., J. Antibiotics* (Japan) 9B 104 (1956).

301e **Sarkomycin $S_1$**, $C_{14}H_{18}O_6S_2$, m.p. 161°, $[\alpha]_D^{22}$ +145°.




301f **Sarkomycin $S_3$**, m.p. 148°.

C 50.39, H 5.31, S 15.75.

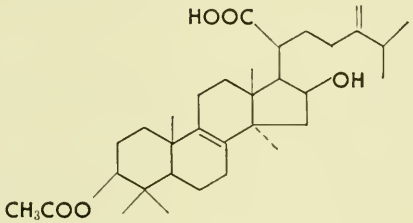
## 9. Terpenoids and Steroids

A new trichothecin-like antibiotic has been isolated from a basidiomycete.[77]

The oil of wheat stem rust uredospores contains $\Delta^7$-ergostenol (fungisterol).[43]

Another steroidal metabolite of *Poria cocos* has been isolated and characterized. It is:[78]

354a **Pachymic Acid** (3$\beta$-0-Acetylpolyporenic Acid B), $C_{33}H_{52}O_5$, colorless crystals, m.p. 296–299°, $[\alpha]_D^{22.5}$ 17.7° (c 0.566 in pyridine).




Cholesterol biosynthesis is inhibited by farnesoic acid and its analogues.[79]

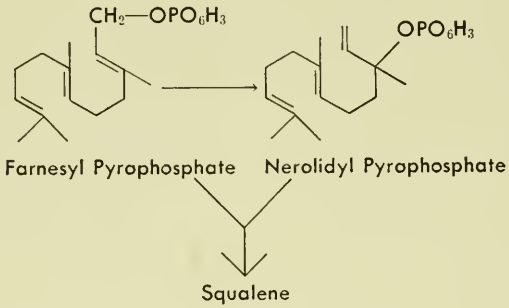
The conversion of mevalonate to a mixture of farnesol and nerolidol (probably as their pyrophosphates) by a

[77] Ervin Gláz, Eszter Scheiber, J. Gyimesi, I. Horvath, Katalin Steczek, A. Szentirmai and G. Bohus, *Nature* 184 Suppl. No. 12, 908 (1959).

[78] Shoji Shibata, Shinsaku Natori, Ko Fujita, Isao Kitagawa and Kazue Watanabe, *Chem. & Pharm. Bull.* (Tokyo) 6 608 (1958).

[79] G. Popják, Rita H. Cornforth and K. Clifford, *Lancet*, 1270 (1960).

liver enzyme preparation has suggested that 1 mole of each is involved in the biosynthesis of squalene.[80] The condensation of these two substances would then be analogous to that of isopentenylpyrophosphate with 3,3-dimethylallyl pyrophosphate (or geranyl pyrophosphate).




The significant points of the chemical mechanism of squalene biosynthesis were summarized as follows: (a) The process is not a concerted reaction, but proceeds in steps with well-defined stable intermediates. (b) During isomerization of isopentenylpyrophosphate there is an uptake of one proton in the terminal methylene group, and this proton appears finally in one of the terminal methyl groups at each end of squalene, which means the entry into each molecule of squalene of two protons not contained originally in mevalonic acid. (c) There are no reductive steps involved in the synthesis of geranyl or farnesyl pyrophosphates. (d) Farnesyl pyrophosphate and the nerolidyl derivative are the two sesquiterpenoids condensing to the symmetrical dihydroterpene, squalene, a stable intermediate being dihydrosqualene. (e) During stabilization of the condensation product of the farnesyl and nerolidyl derivatives, elimination of two protons, originally attached to C-5 of mevalonate occurs. (f) The final step is a reduction, introducing into squalene two further hydrogen atoms not contained originally in mevalonic acid.

[80] J. W. Cornforth and G. W. Popják, *Tetrahedron Letters* No. 19 29 (1959).

## 10. Tropolone Acids

More data have been published on the structure of heliomycin (entry 1173). It is acidic (pK 5.8), forms a diacetate and may contain a benzotropolone ring system. Empirical formulas $C_{19}H_{14\text{-}16}O_5$ or $C_{23}H_{18\text{-}20}O_6$ have been suggested.[81]

## 11. Phenolic Substances

*p*-Hydroxybenzoic acid, found earlier in *Penicillium patulum* has been isolated also from *Penicillium griseofulvum*.[82] Isolated from the same culture were:

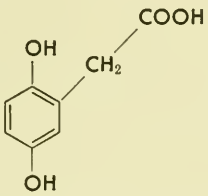
379a ***m*-Hydroxybenzoic Acid, $C_7H_6O_3$, m.p. 201°**

and

379b **Salicylic Acid** (*o*-Hydroxybenzoic Acid), m.p. 159°.

The same mold produces homogentisic acid, a metabolite also found in some of the higher fungi.[83]

391a **Homogentisic Acid, $C_8H_8O_4$, m.p. 152–154°.**




*p*-Hydroxyphenylpyruvic acid and tyrosine were identified in the culture, and occasionally 1,4-hydroquinone was present.

The production of gallic acid by *Phycomyces blakesleeanus* (sporangiophores) has been confirmed.[84] It was suggested that this substance may be the primary photosensitive pigment involved in the strong negative phototropic response to ultraviolet light which such organs show.

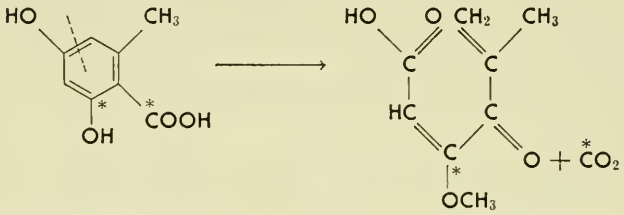
[81] Z. V. Pushkareva, N. M. Voronina, S. I. Omel'chenko, L. B. Radina and Yu. N. Sheinker, *J. Gen. Chem.* (USSR) 29 3469 (English translation) (1960).

[82] P. Simonart, A. Wiaux and H. Verachtert, *Bull. soc. chim. biol.* 41 537, 541 (1959).

[83] Paul Simonart, Anselme Wiaux and Hubert Verachtert, *Zentrl. Bakteriol. Parasitenk. Abt. II* 113 209 (1960).

[84] David S. Dennison, *Nature* 184 2036 (1960).

$C^{14}$-Labeled orsellinic acid has been prepared by using *Chaetomium cochliodes* as the producer. Orsellinic acid was known to be a metabolite of *Penicillium barnense*, which also produces penicillic acid. When *Penicillium barnense* was grown in the presence of the labeled orsellinic acid, it could be shown that orsellinic acid was a precursor of penicillic acid in this organism.[85] The sites of labeling and actual modes of cleavage are shown.




Orsellinic Acid Penicillic Acid

It appears that orsellinic acid is an intermediate in the biogenesis of the xanthone ravenelin.[86] It has been suggested as an intermediate in the biosynthesis of several other types of compounds, *e.g.*, lichen substances, fungal anthraquinones and alternariol.

An uncharacterized substance has been isolated from *Curvularia lunata*.[87]

417a **Substance from *Curvularia lunata*,** $C_{14}H_{18}O_5$, colorless solid, m.p. 195°.

Apparently phenolic. Mannitol was isolated from the same culture.

Curvularin, also produced by *Curvalaria lunata*, is produced by *Penicillium steckii* as well.[88]

A new depsidone has been isolated from an Australian lichen and characterized as norlobaridone:[89]

[85] Klaus Mosbach, *Acta. Chem. Scand.* 14 457 (1960).

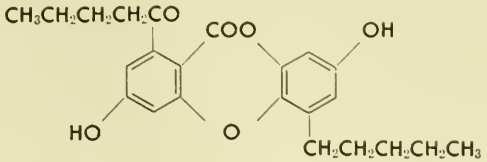
[86] Private communication from Herchel Smith.

[87] T. Krishna Murty and S. Sankara Subramanian, *Indian J. Pharmacy* 20 72 (1958).

[88] D. Fennell, K. B. Raper and F. H. Stodola, *Chem. and Ind.*, 1382 (1959).

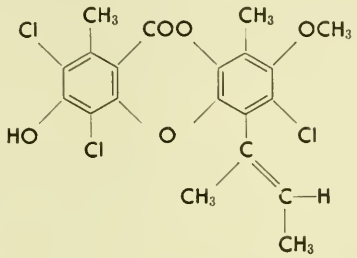
[89] G. P. Briner, G. E. Gream and N. V. Riggs, *Australian J. Chem.* 13 275 (1960).

471a **Norlobaridone**, $C_{23}H_{26}O_6$, colorless crystals, m.p. 188–190°.




A yield of 2.2% was obtained from *Parmelia conspersa.*

The structure of nidulin (and thus of nornidulin) has been completed.[89a] It is:




The chemistry of the uncommon 1-methylpropenyl substituent is greatly modified by the neighboring chlorine atom.

## 12. Quinones and Related Compounds

### *a.* BENZOQUINONES

The growth of a mycobacterium was stimulated by coenzyme $Q_{10}$ which suggests a possible role in energy metabolism.[90]

### *b.* NAPHTHOQUINONES

A variety of bacteria (*Bacillus cereus, B. subtilis, Proteus vulgaris, Sarcina flava, Staphylococcus aureus, Mycobacterium phlei, Pseudomonas* spp., *Azotobacter vinelandii, Nocardia* sp.) were examined for vitamin K content.[91] Three types were identified. Vitamin $K_2$ was

[89a] F. M. Dean, D. S. Deorha, A. D. T. Erni, D. W. Hughes and John C. Roberts, *J. Chem. Soc.*, 4829 (1960).

[90] James O. Norman and Robert P. Williams, *Biochem. and Biophys. Res. Comms.* 2 372 (1960).

[91] Bodil Kruse Jacobsen and Hendrik Dam, *Biochim. et Biophys. Acta* 40 211 (1960).

isolated from *Bacillus cereus* and vitamin $K_1$ or a related substance from *Mycobacterium phlei.*

A lipide cofactor, perhaps a K vitamin or a tocopherol, has been implicated in the conversion of L-gulonolactone into L-ascorbic acid.[92]

### C. ANTHRAQUINONES

In 1955 three substances were isolated from a yellow sterile mold and were called flavomycelin, rhodomycelin and purpurmycelin.[93] Rhodomycelin is identical with islandicin and flavomycelin with luteoskyrin. Acetone solutions of luteoskyrin turn purple on exposure to light, and purpurmycelin was found to be identical with this irradiation product.[94]

The biosynthesis of the pigments of *Penicillium islandicum* has been studied.[95] The acetate origin of islandicin, skyrin, rubroskyrin (luteoskyrin) and iridoskyrin was established. The results of labeling experiments led to the conclusion that, despite the close structural relationship, these pigments are not interconvertible *in vivo*, but seem to be derived from a common pre-aromatic stage. Also mutations fail to block formation of any single pigment. Biogenesis, it was suggested, must not take place by stepwise formations of defined intermediates such as benzene derivatives, but should be dependent throughout on participation of activated acetate.

An acidic substance related to herqueinone has been isolated.[96]

A review of quinones as metabolic products of microorganisms has been published.[97]

There have been two recent publications on the struc-

[92] I. B. Chatterjee, N. C. Kar, N. C. Ghosh and B. C. Guha, *Arch. Biochem. and Biophys.* 86 154 (1960).

[93] H. Nishikawa, *Tohoku J. Agr. Res.* 5 285 (1955).

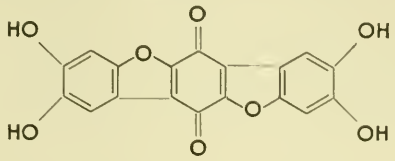
[94] S. Shibata, I. Kitagawa and N. Nishikawa, *Pharm. Bull.* (Tokyo) 5 383 (1957).

[95] Sten Gatenbeck, *Acta Chem. Scand.* 14 102, 230, 296 (1960).

[96] K. S. Gopalkrishnan and N. Narasimhachari, "Antibiotics," Council of Scientific and Industrial Research, New Delhi, 1958, pp. 176–179.

[97] J. H. Birkinshaw, *Planta Med.* 7 367 (1959).

ture of thelephoric acid (entry 493).[98, 99] The second publication cited reported the synthesis of thelephoric acid and seems to establish the structure definitely as:

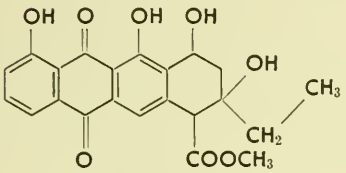



Oösporein (chaetomidin) (entry 487) is reported to be identical with isooösporein (entry 488).[100]

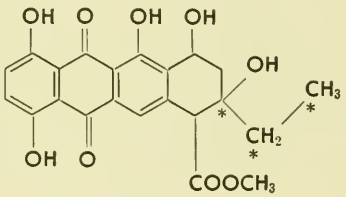
A reinvestigation of quinones produced by *Phoma terrestris* Hansen identified cynodontin and a small amount of another anthraquinone, but found no phomazarin (entry 556).[101]

## 13. Tetracycline, Analogues and Related Substances

The aglycone, aklavinone, of the antibiotic aklavin has been found to differ from rutilantinone (ε-pyrromycinone) only by lacking one hydroxyl group.[102]

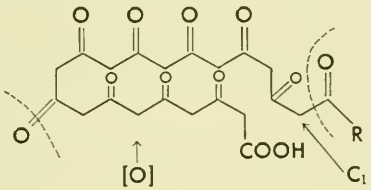



Aklavinone $C_{22}H_{20}O_8$




Rutilantinone $C_{22}H_{20}O_9$

A biogenesis was postulated in the following sense:




[98] K. Aghoramurthy, K. G. Sarma and T. R. Seshadri, *Tetrahedron Letters* No. 16 4 (1960).

[99] J. Gripenberg, *Tetrahedron* 10 135 (1960).

[100] J. Smith and R. H. Thomson, *ibid.* 10 148 (1960).

[101] D. E. Wright and K. Schofield, *Nature* 188 233 (1960).

[102] J. J. Gordon, L. M. Jackman, W. D. Ollis and I. O. Sutherland, *Tetrahedron Letters* No. 8 28 (1960).

A more recent publication indicates that nine acetate units are incorporated into the rutilanlinone molecule, but that the three starred atoms are from propionate.[103] Methionine would not, then, be involved in the side-chain synthesis.

Two investigations have been made on the chlorination mechanism of *Streptomyces aureofaciens* in the production of aureomycin.[104, 105] The authors of the first reference concluded that incorporation of the chlorine atom does not take place on the finished tetracycline molecule, but at an earlier stage of biosynthesis. Wang's results lead to the same conclusion.

The influence of specific enzyme poisons on the production of oxytetracycline has been studied.[106] Iron-containing oxidases and flavine oxidases participated in the biosynthesis of oxytetracycline. Phenoloxidase inhibitors, on the other hand, stimulated production.

There is little agreement on the mode of action of the tetracycline antibiotics, and it may be that they act in a variety of ways. Inhibition of RNA and DNA synthesis and inhibition of enzymic conversion of uracil to thymine,[107] binding by chelation of metal ions required by coenzymes[108] and blocking of unspecified biosynthetic pathways[109] have been mentioned.

A discussion of the mechanisms of action of antibiotics in general has been published.[110]

[103] W. D. Ollis, I. O. Sutherland, R. C. Codner, J. J. Gordon and G. A. Miller, *Proc. Chem. Soc.*, 347 (1960).

[104] J. Kollar and M. Jarai, Symposium on Antibiotics, Prague, 1959.

[105] E. Lin Wang, *J. Antibiotics* (Japan) 12A 31, 41, 50 (1959).

[106] V. Ševčík, V. Musílek and I. Komersová, Symposium on Antibiotics, Prague, 1959.

[107] T. Balakrishna Rao, D. V. Temhane, D. V. Rege and A. Sreenivasan, "Antibiotics," Council of Scientific and Industrial Research, New Delhi, 1958, p. 212.

[108] E. U. Weinberg, *Bacteriol. Revs.* 21 46 (1957).

[109] J. F. Snell, Florence Z. Thanassi and Dorothy Ann Sypowicz, *Antibiotics and Chemotherapy* 8 57 (1958).

[110] S. G. Bradley and L. A. Jones, *Annals N. Y. Acad. Sci.* 89 123 (1960).

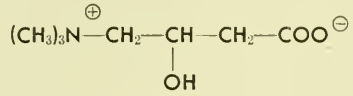
## 14. Aromatic Compounds Not Classified Elsewhere

The cooccurrence of anisaldehyde and junipal in *Daedalea juniperina* cultures has inspired the suggestion that both substances are derived from a common acetylenic precursor.[111, 112] An earlier report that *Polyporus benzoinus* produces considerable quantities of anisaldehyde was not mentioned in our entry on that substance.[113]

## 15. Amines

Although the ordinary source of the amine, carnitine, is mammalian muscle, a publication was overlooked in which it was isolated from the mold *Neurospora crassa* grown on a chemically defined medium.[114]

653a **L-Carnitine**, $C_7H_{15}O_3N$, extremely hygroscopic crystals, m.p. 196–198°, $[\alpha]_D^{20}$ −23.5° (c 0.5 in water).



This amine would not replace choline in choline-less neurospora mutants. It was not found in *E. coli*. The role of carnitine in lipide metabolism has been reviewed.[115, 116]

An amine related to muscarine has been isolated and characterized by synthesis.[117, 118] It is:

658a **(+)-Muscaridine**, $C_9H_{22}O_2NCl$ (Chloroaurate), $C_9H_{22}AuCl_4O_2N$, m.p. 129–131°, $[\alpha]_D^{19}$ +20.5° ±0.5° (c 8.3 in water).

[111] J. H. Birkinshaw and P. Chaplen, *Biochem. J.* 60 255 (1955).

[112] K. E. Schulte and N. Jantos, *Arch. Pharm.* 292 536 (1959).

[113] J. H. Birkinshaw, E. N. Morgan and W. P. K. Findlay, *Biochem. J.* 50 509 (1952).

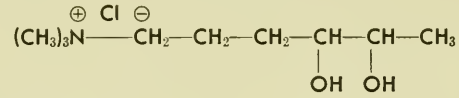
[114] G. Fraenkel, *Biol. Bull.* 104 359 (1953).

[115] G. Fraenkel and S. Freedman, *Vitamins and Hormones* 15 74–115 (1957).

[116] E. P. Adams, P. E. Ballance and A. E. Bender, *Nature* 185 612 (1960).

[117] F. Kögl, C. A. Salemink and P. L. Schuller, *Rec. trav. chim.* 79 278 (1960).

[118] C. A. Salemink and P. L. Schuller, *ibid.* 79 485 (1960).



*Amanita muscaria*

A survey of 32 fungi and nine bacteria indicated that the production of choline sulfate is limited to the higher fungi.[119] All bacteria were negative as were phycomycetes. Of the ascomycetes, sphærioles produced it, but endomycetales did not. Basidiomycetes and all fungi imperfecti examined (except *Torula utilis*) were producers.

List has continued his investigations of the basic constituents of higher fungi. From *Polyporus sulfureus* were isolated the following non-volatile substances: adenine, hypoxanthine, arginine, histidine, lysine, choline, histidine betaine, phenylethylamine, imidazolyl acetate, homarine, trigonelline, γ-butyrobetaine and an uncharacterized hydrochloride, $C_9H_{16}N_2 \cdot 2HCl$.[120]

The mushroom *Coprinus atramentarius* was studied.[121] A prior report that it produced tetraethylthiuram disulfide could not be confirmed. Found, however, were isoamylamine, phenylethylamine, adenine, hypoxanthine, urocanic acid, imidazolyacetic acid, imidazolylpropionic acid, imidazolylethanol, histidine, arginine, choline, lysine, guanidine, ergothioneine, hercynine, glycine, betaine, tyramine, putrescine, cadaverine, δ-aminovaleric acid, α-guanidinobutyric acid, two unidentified bases, glycine, threonine, glutamic acid, aspartic acid, alanine, proline, leucine, valine, isoleucine, citrulline, tyrosine and ornithine.

A dissertation has been published on basic constituents and amino acids of the basidiomycete, *Inocybe patouillardii* Bres.[122]

Found were methylamine, dimethylamine, ethylamine, *n*-propylamine, isoamylamine, β-phenylethylamine, choline, cadaverine, putrescine, hypoxanthine, alanine, pro-

[119] T. Harada and B. Spender, *J. Gen. Microbiol.* 22 520 (1960).

[120] P. List and H. Menssen, *Arch. Pharm.* 292 260–271 (1959).

[121] P. H. List and H. Reith, *Arzneimittel-Forsch.* 10 34–40 (1960).

[122] H. Müller, Dissertation, Naturw. Fakultät Univ. Würzburg, 1959.

line, tyrosine, valine, leucine, cysteine, aspartic acid, glutamic acid, histidine, imidazole-4-acetic acid, arginine, ornithine and the incompletely characterized basic red pigment of the organism, $C_{11}H_{20}O_9N_2$. This was yellow in alkali, red in acid solutions and gave positive Bayer and Pauly diazo tests.

A study of the biogenesis of spermidine (entry 642) in microorganisms has shown that the $C_4$ moiety is derived from putrescine (or ornithine) while the $C_3$ chain has its origin in methionine.[123]

Biochemical pathways in legume root nodule nitrogen fixation have been reviewed.[124]

## 16. Amino Acids and Related Compounds

The lysine, methionine and tryptophan contents of a number of yeasts have been surveyed.[125]

In a study of the interrelationships between folic acid and cobalamin in the synthesis of methionine by extracts of *E. coli*, it was concluded that serine is not on the route of biosynthesis of the methyl group of methionine.[126]

Discussing the two modes of lysine synthesis by lower fungi, Vogel has pointed out that organisms of older evolutionary origin follow the bacterial route.[127] These include eubacteria, pseudomonads and actinomycetes. Ascomycetous and basidiomycetous fungi use the fungal pathway via α-aminoadipic acid.

## 17. Polypeptides and Related Compounds

The ostreogrycin (E-129) complex was isolated in 1958[128] and reported similar to streptogramin, staphylomycin (A-899), PA-114 and mikamycin.

E-129A probably is identical with staphylomycin M and

[123] H. Tabor, S. M. Rosenthal and C. W. Tabor, *J. Biol. Chem.* 233 907 (1958).

[124] F. J. Bergersen, *Bacteriol. Revs.* 24 246 (1960).

[125] G. E. N. Nelson, R. F. Anderson, R. A. Rhodes, Margaret C. Shekleton and H. H. Hall, *Appl. Microbiol.* 8 179 (1960).

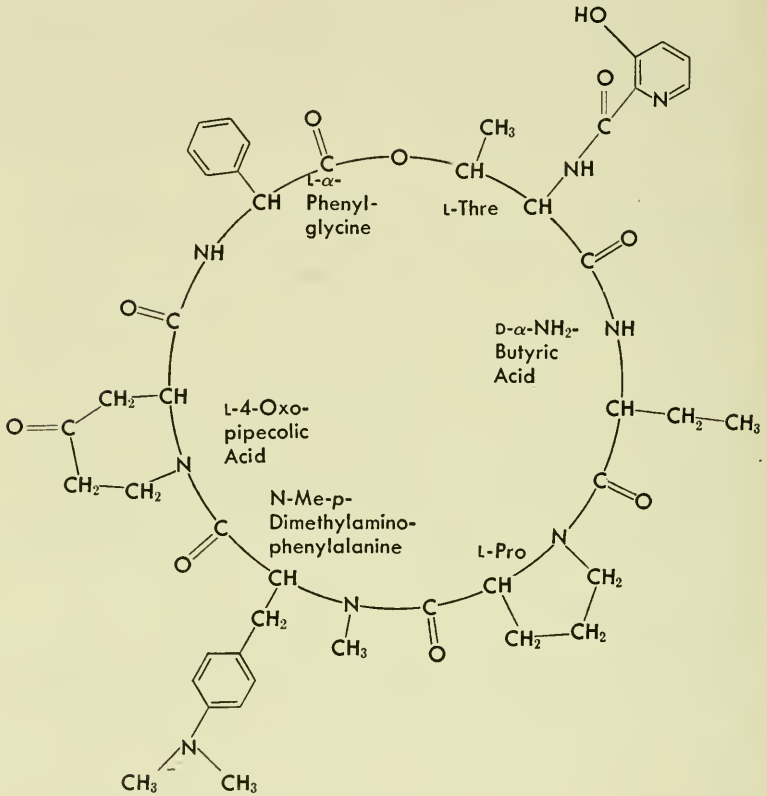
[126] R. L. Kisliuk and D. D. Woods, *Biochem. J.* 75 467 (1960).

[127] H. J. Vogel, *Biochim. et Biophys. Acta* 41 172 (1960).

[128] S. Ball, B. Boothroyd, K. A. Lees, A. H. Raper and E. Lester Smith, *Biochem, J.* 68 24p (1958).

PA-114A. E-129B may be identical with PA-114B, but different from staphylomycin S:[129]

770a **Ostreogrycin B,** (E-129B) $C_{45}H_{54}O_9N_8$, colorless prisms from methanol with solvation, colorless needles from toluene, m.p. 266–268°, $[\alpha]_D^{20}$ −66.8° (c 0.5 in methanol).




*Streptomyces ostreogriseus*

This structure differs from staphylomycin only by substitution of *p*-dimethylamino-N-methylphenylalanine for N-methylphenylalanine.

A similar structure has been proposed for mikamycin B, the only difference being a hydroxyl group in the β-posi-

[129] F. W. Eastwood, B. K. Snell and Alexander Todd, *J. Chem. Soc.*, 2286 (1960).

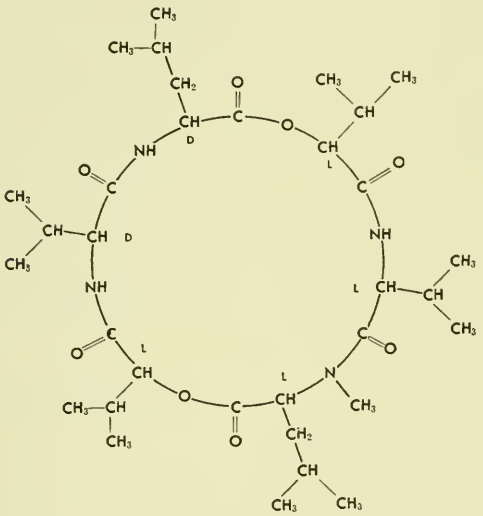
tion of the pyridine moiety adjacent to the carbonyl group in the mikamycin.[129a]

An antiviral polypeptide, cephalomycin, has been reported.[130] It contained leucine, alanine, valine, arginine, glutamic acid, aspartic acid, glycine, threonine, tyrosine, phenylalanine and three unidentified ninhydrin-positive substances.

Some peptide sequences of colimycin have been determined.[131] It resembles polymyxin B, and the sequence L-Dia—L-Dia—D-Leu—L-Leu—L-Dia—L-Dia—L-Dia—L-Dia—L-Thr has been established (L-Dia = $\alpha$, $\gamma$-L-diaminobutyric acid).

An antibiotic named colisan has been isolated from a bacillus.[132, 133]

Sporidesmolide I, a metabolic product of *Sporidesmium bakeri* Syd., colorless needles, m.p. 261–263°, $[\alpha]_D^{17}$ −217° in chloroform (c 1.5) has the empirical formula $C_{33}H_{58}O_8N_4$ and the structure:[133a]



[129a] Kiyoshe Watanabe, Hiroshi Yonehara, Hamao Umezawa and Yusuke Sumiki, *J. Antibiotics* (Japan) 13A 293 (1960).

[130] Akihiro Matsumae, *J. Antibiotics* (Japan) 13A 143 (1960).

[131] Michel Dautrevaux and Gerard Biserte, *Compt. rend. soc. biol.* 153 1346 (1959).

[132] R. Reitler and J. Boxer, *Nature* 158 26 (1946).

[133] R. Reitler and A. Berner, to be published.

[133a] D. W. Russell, *Biochim. et Biophys. Acta* 45 411 (1960).

making it a new member of the depsipeptide or peptolide class. This is the first report of L-α-hydroxyisovaleric acid as a natural product.

A wilt toxin, culmomarasmin, which was 200 times as active as fusaric acid or lycomarasmine, has been isolated from *Fusarium culmorum*.[134] It is a crystalline polypeptide, m.p. 215–218° (dec.), stable below 0°. It is ninhydrin-negative and has the analysis: C 45.31, H 7.08, O 27.56, N 10.36, S 4.76, Cl 4.19, C—$CH_3$ 3.17, —$OCH_3$ 1.08. It also contains iron (1.39% inorganic residue). The amino acids are cystine, leucine, serine, aspartic acid, glutamic acid, alanine, valine, *allo*-isoleucine, proline, glycine, threonine and ammonia.

Two dissertations on wilt toxins have been published.[135, 136]

The antibacterial activities of acyclic decapeptide analogues of gramicidin S have been measured.[137] The mode of action of the acyclic compounds differs from that of the cyclic ones. While gramicidin S causes immediate bacteriostasis, the acyclic analogues are effective only after several cell divisions. The most active analogue was 1/10 as active as gramicidin S against *E. coli* and 1/40 as active against *Staphylococcus aureus*.

The mushroom toxin, phalloidin, has been reported to act by inhibition of oxidative phosphorylation.[138] A more recent study claims that it acts, rather, by interference with protein synthesis.[139]

The neuromuscular blocking properties of various polypeptide antibiotics have been investigated.[139a]

A yellow pigment has been isolated from *E. coli*.[140]

[134] J. Kiss, *Chimia* 14 174 (1960).

[135] Hans Gempeler, *Über welkaktive Inhaltsstoffe von Endopathia parasitica (Murr.) und von Fusarium martii,* Dissertation, Eidgenössische Technische Hochschule, 1959.

[136] Fritz Kugler, *Über welkaktive Inhaltsstoffe von Endopathia parasitica (Murr.) und von Fusarium solani (Mart.) v. Martii,* Dissertation, Eidgenössische Technische Hochschule, 1959.

[137] B. F. Erlanger and L. Goode, *Science* 131 669 (1960).

[138] Benno Hess, *Biochem. Z.* 328 325 (1956).

[139] A. von der Decken, H. Low and T. Hultin, *ibid.* 332 503 (1960).

[139a] R. H. Adamson, F. N. Marshall and J. P. Long, *Proc. Soc. Exptl. Biol. and Med.* 105 494 (1960).

[140] K. Ishii and M. Sevag, *Arch. Biochem. and Biophys.* 77 41 (1958).

Acid hydrolysis yielded *p*-aminobenzoic acid, glutamic acid, alanine, leucine and perhaps another uncharacterized substance (not a pteridine) with an U.V. maximum at 360 $m\mu$.

A total synthesis of gramicidin $J_2$ has been achieved.[141] The biosynthesis of this substance has been investigated.[142] The antibiotic was concentrated in the RNA-rich protoplast precipitate.

The fact that bacitracin A (especially old samples) stimulates growth of *Phycomyces blakesleanus* may be due to conversion of the thiazoline ring of bacitracin A to a thiazole ring (bacitracin F).[143]

Papers have appeared on metabolism and actinomycin production by streptomycetes[144] and on the citric acid cycle and actinomycin formation.[145]

The cytostatic activity of actinomycins is reversed by high concentrations of purines and pyrimidines.[146] The interpretation of this effect was that actinomycin may react with DNA to form dye-polymer complexes.

Mitomycin C causes bacteria to break down their DNA rapidly, acid-soluble products being formed.[146a]

An actinomycin complex, aurantin, colorless crystals, m.p. 255–257°, $[\alpha]_D^{18} - 308°$, has been isolated in Russia.[147] The complex contains threonine, sarcosine, proline, valine, N-methylvaline and isoleucine. It was separated into four biologically active components: $A_1$ m.p. 205°, $A_2$ m.p. 225°, $A_3$ m.p. 226° and $A_4$ m.p. 152°.

Methionine furnishes the methyl groups attached to the aromatic chromophore of the actinomycins as shown by labeling with $C^{14}$.[147a]

[141] Y. Noda, *J. Chem. Soc. Japan* 80 411 (1959).

[142] S. Otani, I. Murakami and S. Chin, Abstr. 118th Meeting, Japanese Antibiotics Association.

[143] Sibor Ebringer, *Naturwissenschaften* 47 210 (1960).

[144] Paul Präve, *Arch. Mikrobiol.* 32 278 (1959).

[145] *Idem., ibid.* 32 286 (1959).

[146] W. Kersten, H. Kersten and H. M. Rauen, *Nature* 187 60 (1960).

[146a] E. Reich, A. J. Shatkin and E. L. Tatum, *Biochim. et Biophys. Acta* 45 608 (1960).

[147] A. B. Cilaev, T. I. Orlova, B. C. Kuznetsova and I. B. Mironova, *Antibiotiki* 3 18 (1960).

[147a] A. J. Birch, D. W. Cameron, P. W. Holloway and R. W. Rickards, *Tetrahedron Letters* No. 25 26 (1960).

A general review of actinomycin structure and synthesis has appeared.[147b]

A colorless, amorphous polypeptide antibiotic, edein, has been isolated from a strain of *Bacillus brevis*.[148] It contained arginine, glycine, glutamic acid, aspartic acid, tyrosine and two unidentified ninhydrin-positive spots.

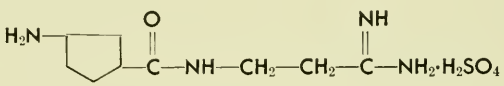
Two heat stable polypeptides, phytoactin and phytostreptin, have been isolated from an unclassified streptomycete.[149] Both contain valine, α-alanine, proline, leucine or isoleucine, arginine, glycine and serine.

Two peptide antibiotics not mentioned before are coliformin[150] and roseocitrins A and B.[151] Coliformin has the analysis: C 47.6, H 8.22, Cl 3.31, S 0.23, P 0.47 and O 33.15 and contains alanine, glycine, serine, glutamic acid, aspartic acid, lysine, valine and leucine. The roseocitrins appear to resemble streptothricin.

In a review of this class the name depsipeptide has been suggested for substances such as amidomycin and valinomycin, which are composed of α-hydroxy acids and amino acids.[152] Synthetic methods have been devised for both regular and irregular sequences of the two types of acids in these antibiotics.

The biosynthesis of α,γ-diaminobutyric acid in *Bacillus circulans* has been studied.[153]

The structure of amidinomycin, $C_9H_{18}ON_4 \cdot H_2SO_4$, has been shown to be:[153a]




[147b] Hans Brockmann, *Angew. Chem.* 72 939–948 (1960).

[148] Z. Kurylo-Borowska, Symposium on Antibiotics, Prague, 1959.

[149] Jack Ziffer, S. J. Ishihara, T. J. Cairney and A. W. Chow, *Phytopathology* 47 539 (1957).

[150] Stig K. L. Freyschuss, Stig O. Pehrson and Borje Steinberg, *Antibiotics and Chemotherapy* 5 218 (1955).

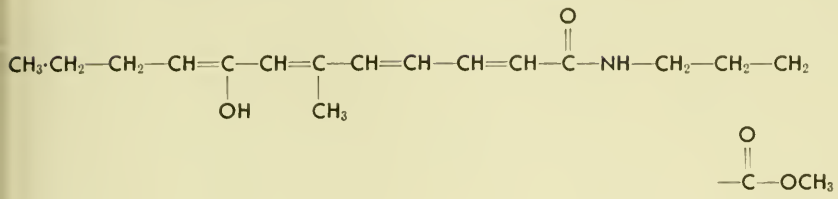
[151] Hisaya Kato, *J. Antibiotics* (Japan) 6A 143 (1953).

[152] M. M. Shemyakin, *Angew. Chem.* 72 342–345 (1960).

[153] Yelahanka Krishnamurthy Murthy, Dissertation, Purdue Univ., 1958.

[153a] Shoshiro Nakamura, Keiko Karasawa, Nobuo Tanaka, Hiroshi Yonehara and Hamao Umezawa, *J. Antibiotics* (Japan) 13A 362 (1960).

The structure of the antifungal antibiotic, variotin, $C_{18}H_{27}O_4N$, is:[153b]



Thus, while it is a tetraene, it is not of the macrolide class.

**Siderochromes.**

A number of microorganisms have been found to produce iron-containing pigments which absorb in the ultraviolet at 420–440 m$\mu$ and have other properties in common. It has been suggested that these be called *siderochromes*.[154]

Some of these substances are antibiotic and are called *sideromycins*. Others are growth factors and may be designated *sideramines*. The antibiotic sideromycins seem to function by inhibiting the growth factor sideramines.

It remains to be seen how broadly the significance of these substances will extend. Some 50 strains of streptomycetes produce sideromycin-like antibiotics.[154] Of 32 common microbial species examined 10 produced coprogen-like substances.[155] The sideramines seem to perform a coenzyme-like function in many microorganisms.

Grisein A and albomycin have broad antibiotic activity. In gram-positive microorganisms, but not in gram-negative ones, their effects are inhibited by sideramines. Ferrimycin is 10 to 50 times as effective as penicillin against gram-positive microorganisms in animal studies.

The following table shows some of the siderochromes which have been best characterized:

[153b] Setsuo Takeuchi, Hiroshi Yonehara, Hamao Umezawa and Yusuke Sumiki, *ibid.* 13A 289 (1960).

[154] H. Bickel, E. Gäumann, W. Keller-Schierlein, V. Prelog, E. Vischer, A. Wettstein and H. Zähner, *Experientia* 16 129–133 (1960).

[155] C. W. Hesseltine, A. R. Whitehill, C. Pidacks, M. Ten Hagen, N. Bohonos, B. L. Hutchings and J. H. Williams, *Mycologia* 45 7 (1953).

| Sideromycins | Producing microorganism | Analyses (%) | | | | | Mol. wt. | pK | Absorption λ max. $E_1^{1\%}$ cm. | Hydrolysis products | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | H | N | Cl | Fe | | | | | |
| Ferrimycin A (may consist of 2 components) | *Streptomyces griseoflavus* (Krainsky) Waksman et Henrici, *S. galilaeus*, *S. lavendulae* | 48.65 | 7.09 | 12.95 | 6.10 | 4.56 | 1106 | 4.18<br>7.88 | 228, 282<br>319, 28<br>425, 22 | Ammonia, Succinic Acid 1-Amino-5-hydroxylaminopentane, δ-aminovaleric Acid, Cadaverine, Cryst. compound (λ max. 227, 323 mμ), Proline and 1 unidentified ninhydrin-positive substance. | 156<br>157<br>158 |
| Grisein A | *Streptomyces griseus* Waksman et Henrici | 43.95 | 5.65 | 12.97 | | 5.14 | 1034 | | 265, 108<br>420, 29 | Methyluracil, Glutamic Acid, Hydroxylamine | 159<br>160 |
| Albomycin (A complex. The main component has been resolved into two parts.) | *Actinomyces subtropicus* Kudrina et Kochetkova | | | | | 4.16 | 1270–1346 | | | Methyluracil, Serine, Ornithine, Hydroxylamine | 161–166 |

| Sideramines | Producing microorganism | Analyses (%) | | | | | Mol. wt. | pK | Absorption λ max. $E_1^{1\%}$ cm. | Hydrolysis products | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | H | N | Cl | Fe | | | | | |
| Ferrichrome | *Ustilago sphaerogena* Burill ex Ellis et Everh. | 44.02 | 5.90 | 16.55 | | 7.35 | 725 | | 425, 39 | Ammonia, Glycine, Ornithine, Hydroxylamine, (3 moles), Acetic Acid (3 moles) | 167 |
| Ferrichrome A | *Ustilago sphaerogena* Burill ex Ellis et Everh. | 44.75 | 5.80 | 11.18 | | 5.3 | 1100 | | 440, 34 | Glycine, Ornithine, Hydroxylamine (3 moles), *trans*-β-Methylglutaconic Acid (3 moles) | 167 |
| Ferrioxamine B | *Streptomyces griseoflavus, S. pilosus; S. viridochromogenes, S. olivaceus, S. aureofaciens, S. galilaeus, S. lavendulae, S. polychromogenes, S. griseus* | 48.04 | 7.41 | 11.21 | 5.25 | 7.67 | 704 | 9.74 | 430, 39 | Ammonia, Succinic Acid, 1-Amino-5-hydroxylaminopentane, δ-Aminovaleric Acid, Cadaverine, Acetic Acid | 168 |
| Coprogen | *Penicillium sp.* | 50.96 | 6.83 | 10.26 | | 6.61 | | | 440, 36 | | 169 |
| Terregens factor | *Arthrobacter terregens* | | | | | | | | | | 170 |

Other less well characterized siderochromes were discussed in reference 156.

In the ferrichromes the iron is bound by coordination with hydroxamic acid derivatives of the aliphatic acid moieties.

[156] H. Bickel, E. Gäumann, W. Keller-Schierlein, V. Prelog, E. Vischer, A. Wettstein and H. Zähner, *Experientia* 16 128 (1960).

[157] H. Bickel, B. Fechtig, G. E. Hall, W. Keller-Schierlein, V. Prelog and E. Fischer, *Helv. Chim. Acta* 43 901 (1960).

[158] H. Bickel, *et al.*, to be published.

[159] D. M. Reynolds, A. Schatz and S. A. Waksman, *Proc. Soc. Exp. Biol. Med.* (New York), 64 50 (1947); D. M. Reynolds and S. A. Waksman, *J. Bacteriol.* 55 739 (1948).

[160] F. A. Kuehl, M. N. Bishop, L. Chaiet and K. Folkers, *J. Am. Chem. Soc.* 73 1770 (1951).

[161] M. G. Brazhnikova, N. N. Lomakina and L. I. Murayeva, *Doklady Akad. Nauk. S.S.S.R.* 99 827 (1954).

[162] E. O. Stapley and R. E. Ormond, *Science* 125 587 (1957).

[163] G. F. Gause, *Brit. Med. J.* 2 1177 (1955); G. F. Gause and M. G. Brazhnikova, *Novosti Med.* (Moscow) 23 3 (1951).

[164] Yu. O. Sazykin, *Mikrobiologiya* 24 75 (1955).

[165] E. S. Kudrina and G. V. Kochetkova, *Antibiotiki* (Moscow) 3 63 (1958).

[166] O. Mikes and F. Sorm, Symposium on Antibiotics, Prague, 1959.

[167] J. B. Neilands, *J. Am. Chem. Soc.* 74 4846 (1952); *idem., J. Biol. Chem.* 205 643, 647 (1953); *idem., Bacteriol. Revs.* 21 101 (1957); J. A. Garibaldi and J. B. Neilands, *J. Am. Chem. Soc.* 77 2429 (1955); Thomas Emery and J. B. Neilands, to be published; T. Emery and J. B. Neilands, *Nature* 184 1632 (1959).

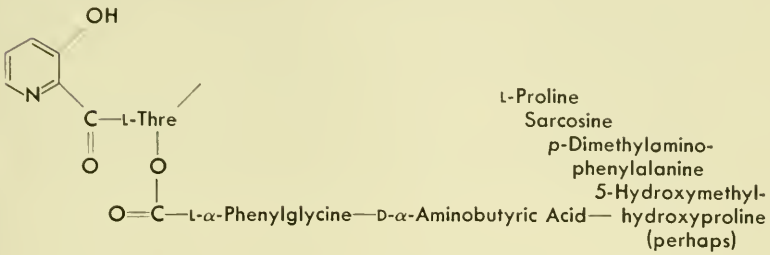
[168] G. E. Hall, unpublished.

[169] C. W. Hesseltine, C. Pidacks, A. R. Whitehall, N. Bohonos, B. L. Hutchings and J. H. Williams, *J. Am. Chem. Soc.* 74 1362 (1952); C. W. Hesseltine, A. R. Whitehall, C. Pidacks, M. T. Hagen, N. Bohonos, B. L. Hutchings and J. H. Williams, *Mycologia* 45 7 (1953); C. Pidacks, A. R. Whitehall, L. Pruess, C. W. Hesseltine, B. L. Hutchings, N. Bohonos and J. H. Williams, *J. Am. Chem. Soc.* 75 6064 (1953).

[170] A. G. Lochhead, M. O. Burton and R. H. Thexton, *Nature* 170 282 (1952); A. G. Lochhead and M. O. Burton, *Can. J. Botany* 31 7 (1953); M. O. Burton, F. J. Sowden and A. G. Lochhead, *Can. J. Biochem. and Physiol.* 32 400 (1954).

Baccatine A (entry 1114) has been shown to be a mixture of enniatins A and B.[171]

A partial structure has been advanced for PA-114-B-1 (entry 729).[172] It is $C_{48}H_{61}O_{12}N_9$:




PA-114-B-3, a minor component of this synergistic complex, contains all the same components except sarcosine. It seems to contain another N-methyl amino acid instead. Other synergistic complexes of this sort are streptogramin, staphylomycin, ostreogrycin and mikamycin. These were classified as follows:

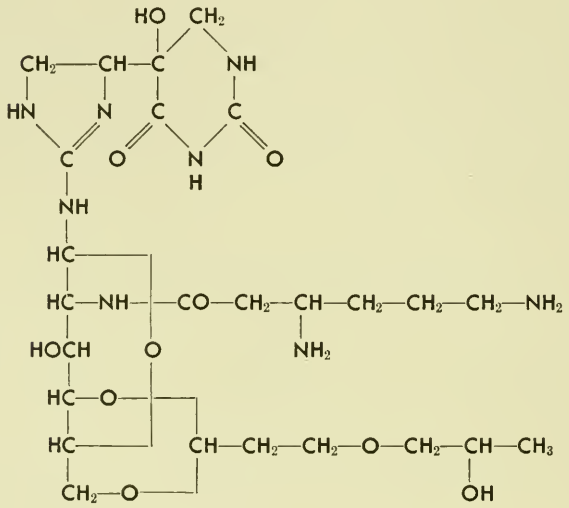
| Type | Specific compound | Synonyms |
|---|---|---|
| A | $A_1$ | PA-114-A-1<br>Ostreogrycin (E-129) Factor A<br>Mikamycin A<br>Streptogramin main component<br>Staphylomycin $M_1$ |
| | $A_2$ | Staphylomycin $M_4$ |
| B | $B_1$ | PA-114-B-1<br>Ostreogrycin (E-129) Factor B<br>Mikamycin B |
| | $B_2$ | Staphylomycin S |
| | $B_3$ | PA-114-B-3 |
| | | Streptogramin, minor component |

[171] G. E. Hall, *Chem. and Ind.*, 1270 (1960).
[172] D. C. Hobbs and W. D. Celmer, *Nature* 187 598 (1960).

More data have been published on the purification and physical properties of mycobacillin (entry 795).[173]

Some degradation studies of thiostreptone (entry 809) have been reported.[174] L-Threonine, L-isoleucine, L-alanine and D-cysteine were identified, and several thiazole-containing fragments were isolated. A minimal molecular weight of 1500 is required.

A structure has been proposed for a new antibiotic, racemomycin O.[175] It is produced by *Streptomyces racemochromogenus,* has the empirical formula $C_{25}H_{44}O_{10}N_8$ and is thought to be:



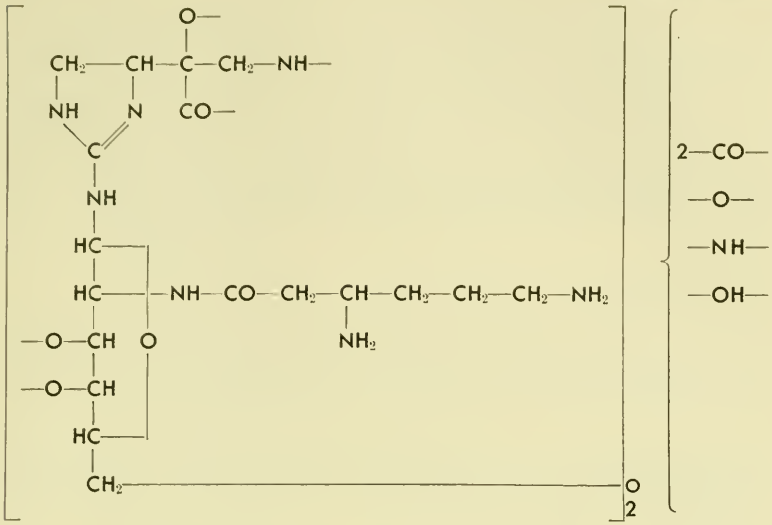
A partial structure has been advanced for roseothricin A (entry 717).[176]

[173] S. K. Majumdar and S. K. Bose, *Arch. Biochem. and Biophys.* 90 154 (1960).

[174] Miklos Bodanszky, John Timothy Sheehan, Josef Fried, Nina J. Williams and Carolyn A. Birkheimer, *J. Am. Chem. Soc.* 82 4747 (1960).

[175] S. Takemura, *Chem. & Pharm. Bull.* (Tokyo) 8 578 (1960).

[176] T. Goto, Y. Hirata, S. Hosoya and N. Komatsu, *Bull. Chem. Soc. Japan* 30 729 (1957).



A new polypeptide antibiotic, glumamycin, has been reported.[177] It consists of colorless powder, m.p. 230° (dec.), mol. wt. ~1800 and is composed of 4-isotridecenoic acid, $C_8H_{17}CH{=}CHC_2H_4{-}COOH$, L-aspartic acid, glycine, L-valine, L-proline, D-pipecolic acid and $\alpha,\beta$-diaminobutyric acid.

A number of compounds listed in the unclassified section are known to be or thought to be polypeptides. These include alboverticillin, antibiotic B-456, bacilipins, bacillomycins, bacilysin, datemycin, diplococcin, distamycin A, laterosporin, melanosporin, mikamycins, mitomycins, monilin, mycospocidin, phleomycin, pluramycins, racemomycins, ractinomycins, roseomycin, taitomycin, violacetin and undoubtedly others.

## 18. Heterocycles

### C. PYRANS AND RELATED SUBSTANCES

8-Hydroxy-3,4-dimethylisocoumarin has been isolated from cultures of a wild *Oospora* specimen.[178]

[177] Michitaka Inoue, Hiroshi Hitomi, Komei Mizuno, Masahiko Fujino, Akira Miyake, Koiti Nakazawa, Motoo Shibata and Toshihiko Kanzaki, *ibid.* 33 1014 (1960).

[178] I. Yamamoto and Y. Yamamoto, *Bull. Agr. Chem. Soc.* (Japan) 24 628 (1960).

A survey has shown that α-tocopherol is the only form of vitamin E found in bacteria.[179] It was found in about a dozen chlorophyll-containing organisms, although not in all such bacteria which were studied. Its production is not limited to any particular type of chlorophyll-containing bacterium. Tocopherol production seemed to parallel chlorophyll production, and it was suggested that the same phytol precursor might be used for both.

### *d.* XANTHONES

A labeled precursor investigation of ravenelin by Birch and associates has shown that orsellinic acid is an intermediate in the biosynthesis of xanthones.[180]

### *e.* COMPOUNDS RELATED TO THIOPHENE, IMIDAZOLE, THIAZOLE AND ISOXAZOLE

A comparison of the effects of D-cycloserine and of D-alanine on the incorporation of D,L-alanine-1-$C^{14}$ into bacterial proteins showed that D-cycloserine acts as a D-alanine antagonist.[181, 182]

A paper has been published on the lability of 2-acetylthiazolium salts and in support of the proposed mode of action of thiamine.[183]

A paper on the enzymatic formation of thiamine and phosphate esters of the pyrimidine moiety seems to be the first of a series on the biosynthesis of thiamine.[184] A dissertation on the biosynthesis of the thiazole moiety has been published.[185]

[179] J. Green, S. Price and L. Gare, *Nature* 184 1339 (1959).

[180] Private communication from Herchel Smith.

[181] P. Barbieri, A. diMarco, L. Fuoco and A. Rusconi, *Biochem. Pharmacol.* 3 101 (1960).

[182] P. Barbieri, A. diMarco, L. Fuoco, P. Julita, A. Migliacci and A. Rusconi, *ibid.* 3 264 (1960).

[183] Ronald Breslow and Edward McNelis, *J. Am. Chem. Soc.* 82 2394 (1960).

[184] Gerald W. Camiener and Gene M. Brown, *J. Biol. Chem.* 235 2411 (1960).

[185] J. Vogel, Dissertation, University of Bonn, 1960.

## *f.* PYRROLES, PORPHYRINS AND RELATED COMPOUNDS

A dissertation has been published on a prodigiosin-like pigment.[186]

A partial synthesis of vitamin $B_{12}$ has been reported.[187, 188]

Guanosine diphosphate factor B and B diphosphate ester have been indentified as intermediates in the biosynthesis of vitamin $B_{12}$.[189]

A dissertation has been published on the biosynthesis of members of the vitamin $B_{12}$ group.[190, 191]

A report has been made on the preparation and properties of purified intrinsic factor. The purified material is a better $B_{12}$ binder than the crude, and it is not a mucoprotein as previously believed.[192]

A publication on the biosynthesis of uroporphyrin III from porphobilinogen reported that uroporphyrinogen I is not an intermediate in the biosynthesis of uroporphyrinogen III.[193]

A pink pigment identified as coproporphyrin III was isolated from *Mycobacterium tuberculosis avium*[194] as it had been earlier from *Mycobacterium karlinski.*[195]

At least two kinds of chlorophylls have been shown to be present in green bacteria.[196]

[186] Roswitha Zimmer-Galler, Dissertation, Technische Hochschule, München, 1960.

[187] K. Bernhauer, F. Wagner, Hw. Dellweg and P. Zeller, *Helv. Chim. Acta* 43 700 (1960).

[188] W. Friedrich, G. Gross, K. Bernhauer and P. Zeller, *ibid.* 43 704 (1960).

[189] G. Boretti, A. diMarco, L. Fuoco, M. Marnati, A. Migliacci and C. Spalla, *Biochim. et Biophys. Acta* 37 379 (1960).

[190] Fred Sanders, *Dissertation Abstr.* 18 2189 (1959).

[191] F. Sanders and Gerald R. Seaman, *J. Bacteriol.* 79 619 (1960).

[192] Leon Ellenbogen and William L. Williams, *Biochem. and Biophys. Res. Comms.* 2 340 (1960).

[193] Lawrence Bogorad and Gerald S. Marks, *Biochim. et Biophys. Acta* 41 358 (1960).

[194] D. S. P. Patterson, *Biochem. J.* 76 189 (1960).

[195] C. M. Todd, *ibid.* 45 386 (1949).

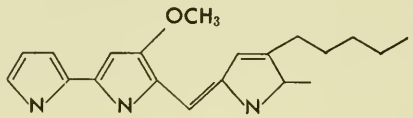
[196] R. Y. Stanier and J. H. C. Smith, *Biochim. et Biophys. Acta* 41 478 (1960).

A b-type cytochrome has been isolated from the fungus *Sclerotiana libertiana* and identified.[197]

Protoporphyrin IX has been isolated from bacterial catalase and characterized.[198]

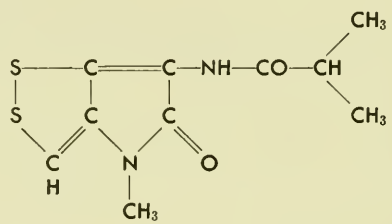
Addition of δ-aminolevulinic acid to cultures of propionibacteria caused large increases in the production of porphyrins, but no rise in vitamin $B_{12}$ production, indicating divergent biosynthetic routes.[199]

The structure of the antifungal pigment prodigiosin has been proved by synthesis.[200] It is




and is thus the second natural product containing a 2,2′-dipyrrole skeleton, vitamin $B_{12}$ being the other.

A streptomycete has been found which produces isobutyropyrrothine, orange-red antibiotic crystals, m.p. 228°:[200a]




Aureothricin, thiolutin, a colorless base, and a heptaene, hamycin, also were produced.

[197] Tateo Yamanaka, Takehazu Horio and Kazuo Okunuki, *Biochim. et Biophys. Acta* 40 349 (1960).

[198] Steve Miller, Davis Hawkins and Robert P. Williams, *J. Biol. Chem.* 235 3280 (1960).

[199] G. V. Pronyakova, *Biokhimiya* (English translation) 25 223 (1960).

[200] Henry Rapoport and Kenneth G. Holden, *J. Am. Chem. Soc.* 82 5510 (1960).

[200a] D. S. Bhata, R. K. Hulyakar and S. K. Menon, *Experientia* 16 504 (1960).

*g.* INDOLES

The structure previously proposed for echinulin has been confirmed, the only reservation being possible exchange of the groups in the 5 and the 7 positions of the indole nucleus.[201]

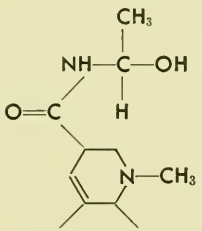
926a **Lysergic Acid Amide** (Ergine), $C_{16}H_{17}ON_3$ (Monomethanolate), m.p. 130–135° (efferv.), resolidifies 140°, m.p. 190° with previous dec.

926b **Isolysergic Acid Amide** (Isoergine), $C_{16}H_{17}ON_3$.

937a **Lysergic Acid Methylcarbinolamide,** $C_{18}H_{21}O_2N_3$, colorless prisms, m.p. 135° (dec.), $[\alpha]_D^{20}$ +29° ±2° (~1.0 in dimethylformamide).

937b **Isolysergic Acid Methylcarbinolamide,** $C_{18}H_{21}O_2N_3$, not crystalline.

A yield of about 2 g. per liter of the above alkaloids was produced by *Claviceps paspali* Stevens T. Hall growing in submerged culture.[202] A partial structure is shown for the carbinolamide isomer corresponding to lysergic acid:




Another new ergot alkaloid, molliclavine, has been reported:[203, 204]

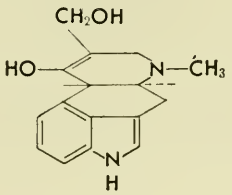
[201] Franco Piozzi, Giuseppe Casnati, Adolfo Quilico and Cesare Cardani, *Gazz. chim. ital.* 90 451, 476 (1960).

[202] F. Arcamone, C. Bonino, E. B. Chain, A. Ferretti, P. Pennella, A. Tonola and Lidia Vero, *Nature* 187 238 (1960).

[203] M. Abe, S. Yamatodani, T. Yamano and M. Kusumoto, *J. Agr. Chem. Soc. Japan* 34 249 (1960).

[204] M. Abe and S. Yamatodani, *Bull. Agr. Chem. Soc.* (Japan) 19 161 (1955).

930a **Molliclavine**, $C_{16}H_{18}O_2N_2$, colorless crystals, m.p. 253° (dec.), $[\alpha]_D^{17}$ +30° (c 1.0 in pyridine).




*Claviceps purpurea*

An antibiotic of novel structure incorporating an indole nucleus is:

391b **PA-155A**, $C_{14}H_{15}O_2N_3$, colorless crystals, m.p. 209°, $[\alpha]_D^{25}$ −214° (c 2.0 in methanol), U.V. 218, 273, 281, 288 mμ.

No reaction with dinitrophenylhydrazine. Negative ninhydrin, $FeCl_3$ tests. Blue Ehrlich's test. Decolorizes $Br_2$, $KMnO_4$. *Streptomyces albus*[73, 73a]

### *i.* PYRIDINES

The plant toxin, fusaric acid, was produced when *Fusarium oxysporum* var. *lini* was grown on artificial medium or on non-resistant flax tissues, but not when the fungus was grown on resistant strain tissues.[205]

A dissertation has been published on dipicolinic acid formation and other chemical aspects of bacterial sporulation.[206]

The mononucleotide of nicotinic acid has been isolated from a fusarium specimen[207] and from a yeast.[208]

### *k.* PYRAZINES, DIKETOPIPERAZINES

Several diketopiperazines have been isolated from the fungus *Rosellinia necatrix* Berlese.[209] They are L-prolyl-

[73] Koppaka V. Rao, *Antibiotics and Chemotherapy* 10 312 (1960).

[73a] Manfred von Schach, private communication.

[205] E. J. Trione, *Phytopathology* 50 480 (1960).

[206] Herbert M. Nakata, *Dissertation Abstr.* 20 3020 (1960).

[207] A. Ballio and S. Russi, *Arch. Biochem. and Biophys.* 85 567 (1959).

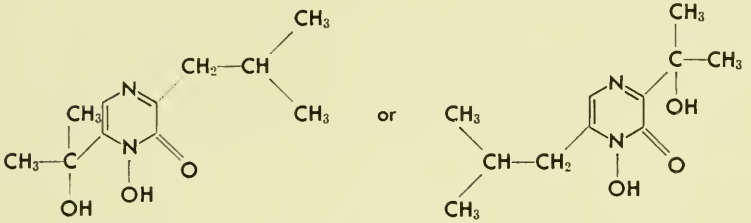
[208] R. W. Wheat, *ibid.* 85 567 (1957).

[209] Yu-Shih Chen, *Bull. Agr. Chem. Soc.* (Japan) 24 372 (1960).

L-leucine anhydride, L-prolyl-L-valine anhydride and an apparently new diketopiperazine, L-prolyl-L-phenylalanine anhydride (compound E) $C_{14}H_{16}O_2N_2$, m.p. 127–128°, $[\alpha]_D^{20}$ −99.8° (c 1.0 in ethanol). A crystalline wax, m.p. 52°, was isolated from the same culture and assumed to be *n*-pentacosane, $C_{25}H_{52}$. Also an uncharacterized substance, white needles, m.p. 206–208°, called rosellinic acid was isolated.

L-Prolyl-L-valine anhydride had been isolated previously from a streptomycete culture.[210] L-Prolyl-L-leucine anhydride had been isolated both from a streptomycete and from *Aspergillus fumigatus*.[211]

Muta-aspergillic acid, $C_{11}H_{18}O_3N_2$, pale yellow needles, m.p. 173° (dec.) (subl.) with alternative structures:

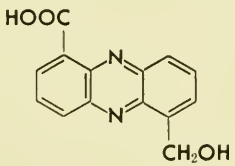



has been reported.[211a]

## *l*. PHENAZINES AND PHENOXAZONES

Three new natural phenazines have been reported.[212]

984b **1-Hydroxymethyl-6-carboxyphenazine**, $C_{15}H_{12}O_3N_2$, light yellow crystals, m.p. 197–201°.



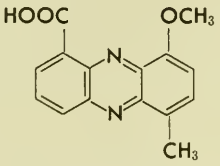

[210] Y. Koaze, *ibid.* 22 98 (1958).

[211] J. L. Johnson, W. G. Jackson and T. E. Elbe, *J. Am. Chem. Soc.* 73 2947 (1951).

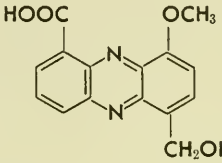
[211a] Seiji Nakamura, *Bull. Agr. Chem. Soc.* (Japan) 24 629 (1960).

[212] Koki Yagishita, *J. Antibiotics* (Japan) 13A 83 (1960).

985a 1-Methoxy-4-methyl-9-carboxyphenazine, $C_{16}H_{14}O_3N_2$, yellow needles, m.p. 124–126°.



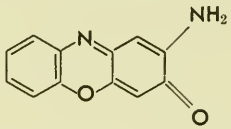

984a 1-Methoxy-4-hydroxymethyl-9-carboxyphenazine (Griseolutic Acid) $C_{15}H_{12}O_3N$.



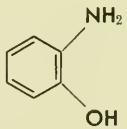

All of these compounds were isolated from a culture of *Streptomyces griseoluteus*.

An unclassified streptomycete produced two substances which were named questiomycins A and B.[213] These have been identified as:

977a 6-Aminophenoxazone (Questiomycin A) $C_{12}H_8O_2N_2$, red crystals, m.p. 241–244° (dec.) subl. from 150°.




377a 2-Aminophenol (Questiomycin B), colorless crystals, m.p. 170–175° (subl. 120°).




The suggestion was made that the aminophenol might be the precursor of the aminophenoxazone.

A purple and a yellow pigment isolated from *Brevibac-*

[213] Kentaro Anzai, Kiyoshi Isono, Kazuhiko Okuma and Saburo Suzuki, *ibid.* 13A 125 (1960).

*terium crystalloiodinum* Sasaki, Yoshida et Sasaki have been identified as iodinin and 1,6-dihydroxyphenazine, respectively.[214]

## *m.* PYRIMIDINES

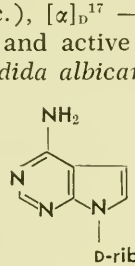
Two dissertations have been published on the biosynthesis of pyrimidines, one with rat liver enzymes,[215] the other with *Neurospora crassa*.[216]

Thymidine diphosphate mannose (as well as the previously reported thymidine diphosphate rhamnose) has been isolated from cultures of *Streptomyces griseus*.[217]

It is possible that this substance is an intermediate in the biosynthesis of streptomycin B (*α-D-mannopyranosylstreptomycin*) which is produced by this organism along with streptomycin.

Tritium labeling experiments indicated that in the case cited, at least, the epimerization of N-acetylglucosamine to N-acetylmannosamine, probably by way of uridine diphosphate N-acetylglucosamine, does not involve oxidation to a ketosugar, followed by stereospecific reduction.[218]

The structure of tubercidin, $C_{11}H_{14}O_4N_4$, m.p. 247° (dec.), $[\alpha]_D^{17}$ −62°, produced by *Streptomyces tubercidicus* and active against *Mycobacterium tuberculosis* and *Candida albicans*, has been reported to be:[218a]




4-amino-7-[D-ribofuranosyl]-pyrrolo-[2,3-d]-pyrimidine

Toyocamycin has a similar structure.[218b]

[214] Tosi Irie, Etsuro Kurosawa and Iwao Nagaoka, *Bull. Chem. Soc. Japan* 33 1057 (1960).

[215] Richard L. Stambaugh, *Dissertation Abstr.* 20 64 (1959).

[216] Kamala P. Chakraborty, *ibid.* 20 3044 (1960).

[217] J. Baddiley and N. L. Blumson, *Biochim et Biophys. Acta* 39 376 (1960).

[218] Luis Glaser, *ibid.* 41 534 (1960).

[218a] Saburo Suzuki and Shingo Marumo, *J. Antibiotics* (Japan) 13A 360 (1960).

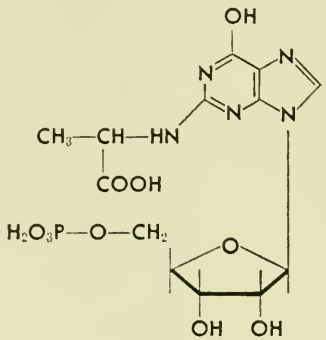
[218b] Kazuhiko Ohkuma, *ibid.* 13A 361 (1960).

*n.* PURINES

Guanosine diphosphate glucose and guanosine diphosphate fructose are produced by *Eremothecium ashbyii.*[219] A dissertation reports the isolation of a new guanine derivative from a riboflavin producer.[220]

A new purine riboside has been isolated from fusarium species.[221] It has been assigned the provisional structure:

2-(1-Carboxyethylamino)-6-hydroxy-9-D-ribofuranosylpurine




Nebularine (9-β-D-ribofuranosylpurine), produced by *Agaricus nebularis*, has been isolated from a streptomycete.[221a]

The nucleotides of *Aspergillus oryzae* have been characterized.[222]

The mechanism of action of the antibiotic, psicofuranine, against *Staphylococcus aureus* has been studied.[223] A possible effect may be interference with the biosynthesis of guanylic acid from xanthylic acid.

[219] H. G. Pontis, A. L. James and J. Baddiley, *Biochem. J.* 75 428 (1960).

[220] Usama A. S. Al-Khalidi, *Dissertation Abstr.* 21 (1960).

[221] Alessandro Ballio, Carlo Delfini and Serena Russi, *Nature* 186 968 (1960).

[221a] Kiyoshi Osono and Saburo Suzuki, *J. Antibiotics* (Japan) 13A 270 (1960).

[222] Kazuo Okunuki, Kozo Iwasa, Fumio Imamoto and Tadayoshi Higashiyama, *J. Biochem.* (Tokyo) 45 795 (1958).

[223] Ladislav J. Hanka, *J. Bacteriol.* 80 30 (1960).

The antibiotic, mitomycin C, blocks DNA synthesis completely in *Escherichia coli*, but does not interfere with RNA synthesis or protein synthesis.[224] Phage-infected bacteria continued DNA synthesis, but no infective particles were produced when high concentrations of mitomycin were present.

A new incompletely characterized electron transport component has been isolated from *Mycobacterium phlei*.[225]

The mode of inhibition of electron transport by antimycin A has been studied.[226]

Evidence has been published for participation of a *vic*-dithiol in oxidative phosphorylation.[227]

A review of ion transport and respiration has been published.[228]

ATP can replace light in bacterial photosynthesis. This discovery was made with the use of the obligate phototroph chromatium. An acetate medium is adequate, and carbon dioxide is not required.[229]

The biosynthesis of nucleic acids has been reviewed.[230]

The biosynthesis and interconversions of purines and their derivatives have been reviewed.[231]

## *O.* PTERIDINES AND FLAVINES

The prosthetic group of a chromoprotein from mycobacteria may be a pteridine.[232]

In the fly, *Drosophila melanogaster*, labeling studies indicate that glucose carbon atoms are specifically in-

[224] M. Sakiguchi and Y. Takagi, *Biochim. et Biophys. Acta* 41 434 (1960).

[225] W. B. Sutton, *Federation Proc.* 19 31 (1960).

[226] A. L. Tappel, *Biochem. Pharmacol.* 3 289 (1960).

[227] Arvan Fluharty and D. R. Sanadi, *Proc. Nat. Acad. Sci.* U.S.A. 46 608 (1960).

[228] R. N. Robertson, *Biol. Revs.* 35 231–265 (1960).

[229] M. Losada, A. V. Trebst, S. Ogata and Daniel I. Arnon, *Nature* 186 753 (1960).

[230] Arthur Kornberg, *Reviews of Modern Physics* 31 200–209 (1959).

[231] Albert G. Moat and Herman Friedman, *Bacteriol. Revs.* 24 309 (1960).

[232] F. B. Cousins, *Biochim. et Biophys. Acta* 40 532 (1960).

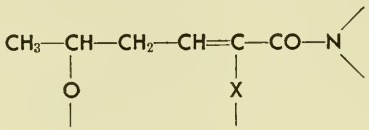
corporated into pteridines, but not into purines produced by the organism.[233]

The structure of "active formaldehyde" ($N^5$,$N^{10}$-methylenetetrahydrofolic acid) has been proved by synthesis.[234]

## 19. Unclassified Metabolites

Streptolydigin probably contains 4 carbon-carbon double bonds conjugated with a $\beta$-diketone system.[235] It also contains at least four hydroxyl groups, at least four C-methyl groups and at least one amide group. Methylamine was a base hydrolysis product of tetradecahydrostreptolydigin.

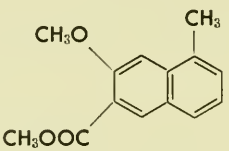
Griseoviridin, empirical formula $C_{22}H_{29\pm2}O_7N_3S$, probably consists of three moieties.[236] A 6 carbon atom fragment has been identified as:



and the sulfur atom may be attached at the X-position.

The probable structure of a methanolysis product of carzinophilin has been published.[237] It is:

Methyl 1-methyl-7-methoxynaphthalene-6-carboxylate



Mikamycin should be classified as a polypeptide of the etamycin type. L-Proline and glycine have been characterized in a hydrolysate. A monoacetate, a di-2,4-dinitrophenylhydrazone derivative, and a decahydro de-

[233] O. Brenner-Holzbach and F. Leuthardt, *Helv. Chim. Acta* 42 2254 (1959).

[234] M. J. Osborn, P. T. Talbot and F. M. Huennekens, *J. Am. Chem. Soc.* 82 4921 (1960).

[235] Jerome Allen Gourse, Dissertation, Univ. of Illinois, 1959.

[236] P. de Mayo and A. Stoessl, *Can. J. Chem.* 38 950 (1960).

[237] Masao Tanaka, Teruo Kishi and Yoshiki Maruta, *J. Antibiotics* (Japan) 12B 361 (1959).

rivative have been prepared. The melting point of the yellow crystals is given as 178° (dec.).[238]

Russian antibiotic 6613 may be identical with etamycin.[239]

Monamycin, $C_{22}H_{36\text{-}38}O_5N$, needles, m.p. 126°. Monohydrochloride: m.p. 187°, $[\alpha]_D^{18}$ −62 ±5° (c 0.9 in ethanol), containing 1 N—$CH_3$, 3 C—$CH_3$ groups, no U.V., I.R. suggestive of amide links, has been isolated from *Streptomyces jamaicensis* n. sp.[240]

Teruchiomycin, $C_{28}H_{43}O_{10}N$, needles, m.p. 202–204° (dec.), a new antibiotic from *Streptomyces antibioticus* has been reported.[241]

A new acidic antibiotic, C-159, U.V. max. 345, 260, 280 mμ, C 58.7, H 7.4, D 24.0, N 9.9% has been patented.[242]

The blue intracellular pigment of *Pseudomonas lemonnieri* has been isolated, purified and characterized.[243]

A preliminary investigation has been made of the pigments of *Trichophyton rubrum*.[244]

Rubidin, a quinoid dark red powder with acid base indicator properties, U.V. 320, 415, 490 mμ in butanol, C 51.9, H 5.56, O 42.54, positive $FeCl_3$ and zinc dust tests, is a substance isolated from an unclassified streptomycete.[245]

A new antibacterial antibiotic has been reported.[246] It had the following properties: yellow needles, m.p. 134°, mol. wt. 397, U.V. maxima at 328.5, 314.5, 298.7 mμ. Positive Millon, Liebermann, Schiff, $FeCl_3$ and $NH_3$-$AgNO_3$ tests. Probably $C_{22}H_{22}O_7$ with hydroxyl, methyl and 2 ketone groups present.

[238] Koichi Okabe, *ibid.* 12A 86 (1959).

[239] M. Brazhnikova *et al.*, *Antibiotics* (USSR) 4 414 (English translation) (1959).

[240] C. H. Hassall and K. E. Magnus, *Nature*, Suppl. 184 1223 (1959).

[241] H. Umezawa *et al.*, Japanese Patent 850 (1958).

[242] British Patent 814,794 (1959).

[243] Werner Blau, Gladys Cosens and Mortimer P. Starr, *Bacteriol. Proc.*, 153 (1960).

[244] Malati Bacchwal and G. C. Walker, *Can. J. Microbiol.* 6 383 (1960).

[245] A. K. Banerjee, G. P. Sen and P. Nandi, "Antibiotics Annual 1955–1956," Medical Encyclopedia, Inc., New York, p. 640.

[246] Thadée Staron and Albert Faivre–Amiot, *Compt. rend.* 250 1580 (1960).

Bold-faced numbers indicate primary microbial metabolite entries, while Arabic numbers signify incidental mention under such entries. Italic numbers are page numbers, and generally indicate occurrence in a chapter or section introduction. The appendixes and addendum are not indexed.

## EMPIRICAL FORMULA INDEX

This index lists the known empirical formulas of microbial metabolites as an aid to future characterizations. Boldfaced numbers are entry numbers, while italic numbers are page numbers reflecting occurrence in a chapter or section introduction. The appendixes and addendum are not indexed.

Boldfaced numbers are entry numbers of metabolites produced by a microorganism. Italic numbers are page numbers and indicate mention in a chapter or section introduction. The appendixes and addendum are not indexed.