THE PFIZER HANDBOOK OF MICROBIAL METABOLITES

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.

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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.

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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 β-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.

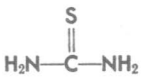
1

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).

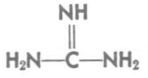
1 **Thiourea,** CH_4N_2S, white crystals, m.p. 180–182°.



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°.