BIOLOGY OF ANTIBIOTICS

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Preface

This book is based on Hans Zähner's Biologie der Antibiotica, published in 1965.

There is a vast literature on antibiotics, covering chemical, pharmacological, and clinical aspects. We have made no attempt to cover this literature comprehensively. Our effort is directed toward discussing antibiotics as biological agents. They are substances produced by living cells, yet they are able to inhibit the growth of living cells — in many cases even the cells that produce them. We have taken this apparent biological paradox as our point of departure and have tried to look in this light at the production of antibiotics and at their mode of action. In a sense antibiotics are comparable to mutations. They are useful as tools in the study of metabolism by blocking specific reactions. At the same time their mode of origin and their effects on the organisms that produce them are interesting problems in their own right. We have tried to incorporate both aspects into our considerations.

This little book, designed for biology students and medical students, provides them with a framework into which to fit more specialized and detailed information on antibiotics. We have tried to present an overall picture of the actions of antibiotics on sensitive organisms and of the problems that arise during their use (for example, drug resistance) and at the same time to sketch out the biological background against which to consider the actions of antibiotics, such as the sources of antibiotics in nature and their biosyntheses in relation to the metabolism of the organisms that produce them.

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Chapter 1

Introduction

What are antibiotics? Why are they formed in nature? These two questions are discussed in this introductory chapter.

Antibiotics can be defined as substances produced by living organisms, which in low concentrations are able to inhibit growth of other organisms. This is a formal definition, and by and large it is valid. As with most definitions though, there are exceptions. For example, enzymes that are excreted by microorganisms and that by catalyzing the breakdown of proteins or carbohydrates inhibit the growth of other microbes are not considered to be antibiotics.

Over the years, as more and more antibiotics have been discovered, it has been found that most of them, especially those of medical importance, fall into the general category of *secondary metabolites*. Perhaps the simplest way to acquire an impression of these substances is to observe their formation. Among microorganisms their taxonomic distribution is restricted, and among bacteria we find them to be produced largely by certain spore-forming groups (Bacilli and Actinomycetes). During its normal life cycle such an organism will grow in an appropriate culture medium until it has produced the maximum number of cells it is capable of forming under the particular conditions. The limitation may be set by the supply of oxygen, by the amount of the carbon and energy source supplied, or by other nutritional or environmental factors. Once the culture has stopped growing, it enters the stationary phase, followed eventually by death or alternatively by spore formation. More is said about the growth of bacteria in Chapter 3. Usually at this stage — after the cells have stopped dividing — secondary metabolites begin to be produced. Their production continues for a certain length of time, which may be either longer or shorter than the active growth period of the culture, and it then ceases. Secondary metabolites are often produced in large

Table 1-1 Classes of Organic Compounds in Which Secondary Metabolites Are Found

Amino sugars	Lactones	Pyrones
Anthocyanins	Macrolides	Pyrroles
Anthraquinones	Naphthalenes	Pyrrolines
Aziridines	Naphthoquinones	Pyrrolizines
Benzoquinones	Nucleosides	Quinolines
Coumarins	Oligopeptides	Quinolinols
Diazines	Phenazines	Quinones
Epoxides	Phenoxazinones	Salicylates
Ergoline alkaloids	Phthaldehydes	Terpenoids
Flavonoids	Piperazines	Tetracyclines
Glutaramides	Polyacetylenes	Tetronic acids
Glycosides	Polyenes	Triazines
Hydroxylamines	Pyrazines	Tropolones
Indole derivatives	Pyridines	

amounts, of the same order of magnitude as the dry weight of the culture, and for the most part they are excreted into the culture medium.

What is the chemical nature of secondary metabolites? They are a diverse group of compounds, most of them of relatively low molecular weight. An awareness of this diversity may be gained from an inspection of Table 1-1, which lists some of the classes of compounds found among secondary metabolites, including classes that contain antibiotics. It should be noted that, in spite of the large variety of compounds, classes to which primary metabolites belong (amino acids, sugars, and intermediates of carbohydrate metabolism, fatty acids, and others) are largely absent. A characteristic feature of secondary metabolism is that any given organism usually produces an array of related compounds belonging to the same class. Another feature of this type of metabolism is that a large number of substances arises from relatively few intermediates of primary metabolism. These aspects of secondary metabolism are discussed in Chapter 4.

In contrast to primary metabolites, secondary metabolites are not essential for growth of the organism. Primary metabolites are either building blocks for macromolecules, intermediates in reactions generating energy-rich compounds (ATP), or parts of coenzymes. Secondary metabolites have no such vital roles in metabolism. Yet it seems likely that secondary metabolism does have a useful function in the

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life of the organism, as shown in some cases (especially of sporulating bacteria), by inferior viability of mutant strains unable to carry out some steps in secondary metabolism. Presumably this type of metabolism protects the cell against environmental or internally generated hazards that arise during the resting phase. One usually thinks of microorganisms as living in a world in which all they do is grow and divide, but in nature as well as in artificial cultures, sooner or later the suspending medium becomes growth-limiting. It then becomes necessary for the organism to survive under such conditions until the environment changes and will again support its growth.

Secondary metabolism occurs also in fungi, higher plants, and to some extent in animals. Among the latter two, because of the intermingling between growing and differentiating cells, it is not so simple to draw a distinction, in terms of timing, between primary and secondary metabolism, as it is in microorganisms.

As we mentioned, most antibiotics are secondary metabolites, and what we have said about secondary metabolism applies to most of them. There are, however, a few antibiotics that are primary metabolites, being formed during the growth of the organism. The polypeptide antibiotic nisin is an example.

With the background of secondary metabolism presented here we can now ask the second question: Why do organisms produce antibiotics? They are growth-inhibitory substances, and moreover in many instances they inhibit the growth of the organisms that produce them. Before embarking on a further discussion, we admit that we do not know what their functions, if any, are, and what follows is therefore largely speculative.

Some of the possible roles that have been assigned to antibiotics are "useless" ones, such as waste products of cell metabolism or the breakdown products of macromolecules; or "useful" ones for the metabolism of the producing cell, such as food-storage, or useful ones for the life of the cell in its natural environment, such as inhibiting the growth of neighboring organisms. They have also been considered as biochemical vestiges that once had a useful function but have lost it. Without going into a detailed discussion of these possibilities we can say that probably none of these is correct, at least not as a primary function. We must remember that the purpose of secondary

metabolism is to protect the cells against adverse conditions arising during the resting phase. Any function that may be assigned to antibiotics must be sought in this context.

A possible function of secondary metabolism is to prevent during the resting phase the accumulation of primary metabolites that may be harmful to the cell. Secondary metabolites, as we have mentioned, are formed from intermediates of primary metabolism. In that sense antibiotics and other secondary metabolites would be primarily the end products of "detoxification" enzymes, and their biological activity would be accidental, the important biological feature being the process of secondary metabolism per se.

It does not seem likely, however, that among all these biologically active substances, many of which can inhibit growth of the cells that produce them, some do not fulfill some sort of useful function. For example, it may be detrimental for a cell in a poor environment to try to synthesize macromolecules. Many antibiotics are inhibitors of macromolecular synthesis, and they may therefore be useful in preventing abortive attempts of cells to synthesize macromolecules.

As an extension of the idea that antibiotics participate in the control of macromolecular synthesis, we may consider the restricted distribution of these substances in nature. Among microorganisms, antibiotics are mainly found in species that have the ability to sporulate. It is possible, therefore, that the production of some antibiotics is related to the ability of an organism to sporulate. In bacilli, it has been demonstrated that the production of antibiotics occurs during the early stages of spore formation. It is likely that these antibiotics function as regulators of macromolecular synthesis. As sporulation may be considered as a process of differentiation, antibiotics active in this process are thus agents of differentiation.

These speculations lead us to define a central function of some antibiotics in the life of the organism that produces them. Admittedly there is no direct evidence for such a role of antibiotics, but then little is known (in contrast to primary metabolism) about the physiology of sporulation, although it has been studied extensively. A word of caution against generalizing about physiological roles of antibiotics: The production of antibiotics during secondary metabolism is extremely variable, the proportion among secondary metabolites pro-

duced being very much dependent on environmental conditions. This argues against all antibiotics being involved in vital processes, and emphasizes that in regard to biological functions we may be dealing with a very heterogeneous group.

In addition to a role in the metabolism of the organism that produces them, there is the possibility that in some cases the excretion of antibiotics has a selective advantage for the producer because of inhibition of growth of surrounding organisms. This is an incidental role that presumably has evolved secondarily and represents an "extra bonus" of the process of antibiotics production.

In summary, most antibiotics are produced during the resting phase of microbial growth, as a result of a type of metabolism, called secondary metabolism, which is different from that occurring during growth and division and is concerned with maintaining the cell during this phase of its life. This fact, together with the restricted taxonomic distribution of antibiotics, mainly among sporulating organisms, has invited speculation as to a possible role of some antibiotics in protecting the organism during the resting phase and, in some cases, in setting up or maintaining a differentiated state prerequisite to spore formation. There is not direct evidence for such a role of antibiotics, and such functions are suggested mainly on the basis of their being biologically active compounds. The alternative would be that the biological activity of these compounds is fortuitous and of no significance in secondary metabolism. The great variability observed in the production of secondary metabolites calls for a cautious attitude in assigning functions to antibiotics in resting-phase metabolism.

In the pages to follow we first discuss the taxonomic distribution of antibiotic-producing organisms (Chapter 2), then some general methods used in the study of antibiotics, together with the principles underlying these methods (Chapter 3). After this we deal with the biosynthesis (Chapter 4) and the mode of action (Chapter 5) of antibiotics, together with resistance developed to their actions (Chapter 6). Finally (Chapter 7) we deal with possible future studies on antibiotics.

We would like to emphasize again that the theme of this book is "biology of antibiotics," that is, not only a consideration of their uses by man but also a general treatment of their role in nature. However,

a large part of this book is concerned with aspects relevant to medicine and other human endeavors. In addition we have purposely chosen as illustrative examples those antibiotics that are clinically useful.

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Chapter 2

The Taxonomic Distribution of Antibiotic-Producing Organisms

We shall make a few comments on limitations imposed on the detection of antibiotics before discussing the distribution of antibiotic-producers among living organisms.

1. With the commonly used procedures it is not possible to test all types of microorganisms for the production of antibiotics. For example, obligatory parasites are excluded, not because they lack biosynthetic capabilities, but because we have no way of determining antibiotics they may produce.

2. Each group of investigators engaged in the search for new antibiotics usually concentrates its efforts on a limited number of organisms. This has tended to produce a biased selection of organisms, in spite of the availability of generally applicable testing methods.

3. The conditions of testing impose limitations on the detection of antibiotics because of the limited number of indicator strains that can be used, the time of exposure of these strains to the potential antibiotic-producing strains, and the chosen culture conditions in general. Negative evidence for antibiotics-production in this case, therefore, is of much less value than is positive evidence.

4. In the literature on the distribution of antibiotics one often encounters data that were collected for other purposes and in which descriptions of antibiotic activity are fragmentary. For constructing a complete survey of antibiotic-producers it is desirable to have data from as wide a source as possible, but to be reliable, they have to contain a thorough description of an antibiotic, including chemical characterization.

Taking all these limitations into account, it still remains a fact that the taxonomic distribution of antibiotic-producing organisms is

Table 2-1 Distribution of Antibiotic-Producing Organisms

Producing organism	Number	Percent of total number of described producers
Pseudomonadales	11	1.2
Eubacteriales	70	7.7
	(53 are *Bacilli*)	—
Actinomycetales	529	58.2
Fungi	165	18.1
Algae and lichens	8	0.9
Higher plants	110	12.1
Animals	16	1.8
	909	100

(compiled from Korzybski *et al.* 1967)

very much restricted. This is shown in Table 2-1, which lists the distribution of organisms producing well-characterized antibiotics. (The data were compiled in 1967.) If we take all published descriptions of antibiotics into account, the total number of antibiotics would be greater, but it would hardly change the distribution.

Figure 2-1 shows a phylogenetic scheme for the fungi. Chemotherapeutically useful antibiotics have been found only among members of the order *Aspergillales*. Figure 2-1 shows some of the important antibiotics and the species that produce them. A few other groups of fungi also produce antibiotics, though not clinically useful ones. For example, polyacetylenes are produced by certain *Basidiomycetes;* certain *fungi imperfecti,* especially *Fusarium* species, produce enniatins, enniatin-like compounds, and quinone antibiotics. Compared to *Aspergillales* the number of other antibiotic-producers is small.

Bacterial taxonomy is at a much less advanced stage than taxonomy of the fungi, and it is futile to attempt the construction of a phylogenetic scheme comparable to that in Fig. 2-1. A scheme of this kind prepared by Hütter for a single group, the *Actinomycetales,* is shown in Fig 2-2. Again producers of medically and otherwise important antibiotics are indicated, and here they are found mainly among the genera *Streptomyces* and *Nocardia.* These two genera are closely related and belong to the same family. Thus among actinomycetes, as among fungi, only one group of closely related genera produces clinically important antibiotics. What is striking among the antibiotics

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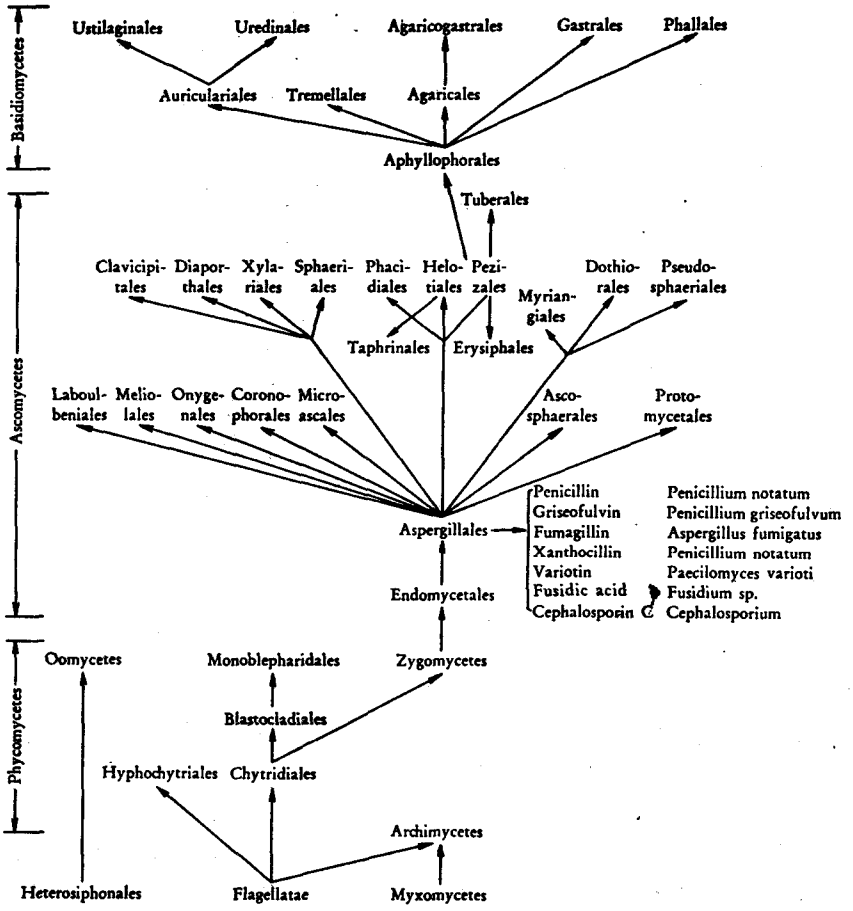

Fig. 2-1. Phylogeny of fungi, according to Gäumann (1964). Chemotherapeutically useful antibiotics, produced by *Aspergillales*, are indicated.

produced by actinomycetes is the great variety of substances, both in regard to chemical structure and mode of action. Actinomycetes have contributed a number of new classes to the catalogue of natural products, such as the macrolides, sideromycins, macrotetrolides, anthracyclines, and others.

Figure 2-3 shows a classification scheme of the *Eubacteriales* according to Bergey. Antibiotics are produced only by the *Bacillaceae*. Five important antibiotics are indicated, all of which are polypeptides.

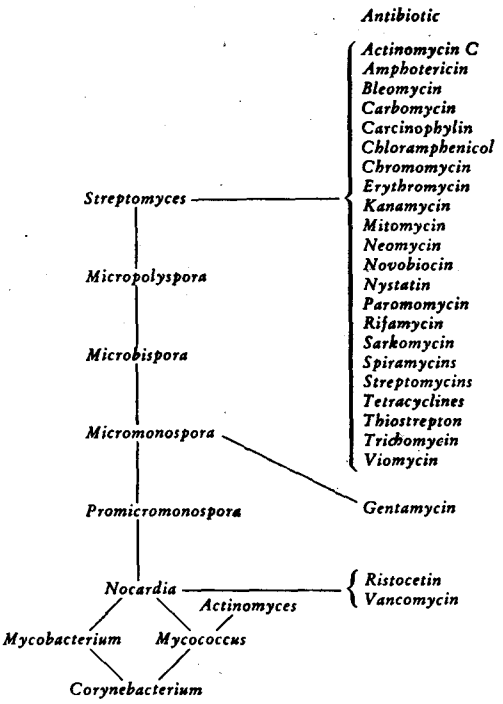

Fig. 2-2. Phylogeny of Actinomycetales and related groups, according to Hütter (1966). (Modified from Hütter, R.; Systematik der Streptomyceten, Karger-Verlag, Basel, 1966.)

Some of these resemble each other structurally, so that the biosynthetic capacity of this group for antibiotic production is probably not as great as may be suggested by their number. These antibiotics are, however, very different from each other in their mode of action. It may be mentioned that polymyxin and colistin are very active against gram-negative bacteria and only weakly active against gram-positive bacteria, which is an unusual situation among antibiotics. Gramicidin S, bacitracin, and tyrothricin, like most other antibiotics, are more active against gram-positive bacteria.

It can be seen from Figs. 2-1, 2-2, and 2-3 that the production of a given antibiotic is usually confined to a single group of closely related organisms. This "rule of specificity" holds for all therapeutically important antibiotics. If we consider other well-characterized antibiotics, the rule still holds for several hundred more. Exceptions to this rule

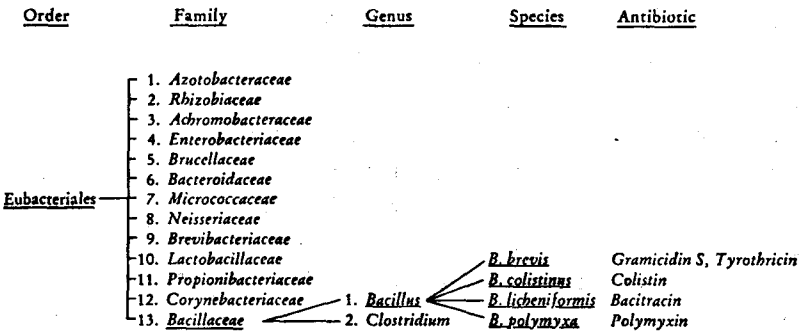

Fig. 2-3. Antibiotic-producers among *Eubacteriales*.

Table 2-2 Exceptions to the "Rule of Specificity"

Antibiotic	Producing organism
Cephalosporin N	*Cephalosporium* (Fungi Imperfecti
	Streptomyces
Citrinin	Leaves of *Crotalaria crispata*
	Penicillium
	Streptomyces
Fusidic acid (Ramycin)	*Fusidium* (Fungi Imperfecti, belongs probably to the ascomycetes)
	Cephalosporium
	Mucor ramannianus (a phycomycete)
Beta-nitropropionic acid (Bovinocidin)	*Streptomyces*
	Aspergillus
	Constituent of a glycoside in higher plants
Various phenazines	*Streptomyces*
	Pseudomonas iodinum
9-(B-D-riboturanosyl)-purine (Nebularin)	*Agaricus nebularis* (a basidiomycete)

are listed in Table 2-2. The "rule of specificity" applies not only to single substances but also to whole classes of substances and to building blocks of antibiotics, such as sugar moieties. Thus in regard to classes of compounds, macrolide and polyene antibiotics have been demonstrated only in cultures of actinomycetes. There they occur with high frequency, polyenes being found among 75 percent of freshly isolated strains, macrolides among 1 to 3 percent. Another group, the polyacetylene antibiotics, have so far been found only among basidiomycetes. In regard to specific moieties, Table 2-3 lists some of the

sugar moieties occurring in macrolide antibiotics. It can be seen that with the exception of oleandrose, which occurs in glycosides of higher plants, all these sugars are confined to the genus *Streptomyces*. The sugars described in Table 2-3 represent well-studied examples. Other less well-studied cases illustrating the same principle could be cited, such as the sugar moieties of polyenes, anthracyclines, and chromomycins. The whole macrolide molecule is quite complex, and one can understand that the synthesis of such a complicated structure may be confined to only one group of organisms. The sugar moieties, on the other hand, are much simpler structures, and for this reason one may expect them to be distributed more widely. Yet the genetic capabilities for their production have been retained within only a very limited group of organisms. This is reminiscent of a group of dideoxy sugars that are confined in their occurrence mainly to certain enteric bacteria (Salmonella), where they form part of the surface O antigen.

In conclusion, in examining the distribution of antibiotic-producing species, we find it to be very restricted, mainly to certain microorganisms that are able to sporulate, and moreover, we find a great deal of specificity in regard to the kind of antibiotics produced by a given group of organisms. We have already indicated in Chapter 1 a possible explanation for the restricted evolution of antibiotics, namely that antibiotic production is part of the secondary metabolism characteristic of these sporulating organisms. The rule of specificity reflects further specializations among these groups. One final point can be made in regard to the restricted distribution: Considering that antibiotics are not absolutely essential for life and may be produced in copious amounts, organisms that evolved the capacity to produce them must be living in a relatively rich environment, with good supplies of nutrients. Conditions of plenty are not found too often, though they seem to be present in the soil in which antibiotic-producers are found.

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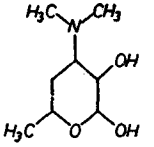
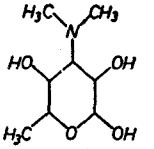
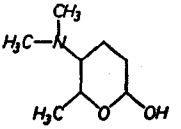
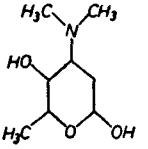
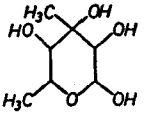
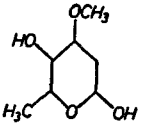
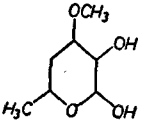
Table 2—3 Some Sugar Moieties of Macrolide Antibiotics

Sugar	Chemical structure	Component of	Formed by
Desosamine (picrosin)		Methymycin Picromycin Griseomycin Narbomycin Erythromycins Oleandomycin	Various species of *Streptomyces*
Mycaminose		Spiramycins Carbomycins Leucomycins Niddamycin Tylosin Acumycin Relomycin	Various species of *Streptomyces*
Torosamine		Spiramycins	*S. aureofaciens*
Angolosamine		Angolamycin	Various species of *Streptomyces*
Mycarose		Erythromycin Carbomycin Spiramycins Angolamycin Tylosin Leucomycin	Various species of *Streptomyces*
Oleandrose		Oleandomycin Cardiac glycosides	*S. antibioticus* Higher plants
Lankavose		Lankamycin Chalcomycin	Various species of *Streptomyces*

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