Natural Products from Microorganisms

H. Boyd Woodruff

Summary. Microorganisms are capable of producing natural products with widely divergent chemical structures. Greatest attention in the past has been paid to natural products that have antibiotic properties. Natural products accumulate in fermentation broths during secondary metabolism, a characteristic of the incomplete metabolic control operative in growth-inhibited microorganisms. With this general mechanism of biosynthesis, the natural products synthesized by microorganisms would be expected to have a broad range of pharmacological activities. The directed screening for nonantibiotic natural products has been of limited scope. The expectation that new compounds of interest would be found has been validated. The pharmacologically active natural products provide previously unrecognized structures as tools for fundamental research programs, as well as offering the possibility of direct use in medicine or in industrial processes.

Microbiologists, especially those searching for new biologically active products, have long accepted a statement of Lamarck

> The most important discoveries of the laws, methods and progress of nature have nearly always sprung from the examination of the smallest objects which she contains.
>
> —J. B. LAMARCK
> *Philosophie Zoologique* (1809)

as an article of faith. The well-known diversity of microorganisms with more than 50,000 defined species, and their widespread occurrence in soils, millions per gram, provide practically an unlimited reservoir for study. For this reason, the opportunity exists to make major discoveries of new natural products useful in medicine. For success, however, the opportunity must be clearly recognized, and then special skills must be brought to bear on the problem—those of the microbiologist, the natural-product chemist, the applied scientist.

Economic Significance

The discovery of each new category of medicinal agents has potential economic significance to the national economy because it can yield a more productive population. If successful, it benefits the discoverers and their licensed manufacturers. The antibiotics are obvious examples of economically significant natural products.

A recent listing of pharmaceutical products sold in Japan, ranked in order of market size, demonstrates clearly the economic impact of natural products. The utility extends beyond the conventional antibiotics (Table 1) (*1*). Of the first ten commercial products listed, four with total market value exceeding 300 million U.S. dollars per year are derived directly from microorganisms. These four are sold for purposes other than antibiotic action. Five others are antibacterials, produced as chemical modification of cephalosporin C. Only one substance of the ten was discovered by total synthesis. Expanding the list of economically important products marketed in Japan to the first 50, more than 40 percent are derived from natural products.

Dominance of Antibiotics

In spite of the practical evidence, of which the above example is typical, recognition of the full significance of natural products for all aspects of medicine has been delayed. Consideration of natural products for application in industrial processes or in cultivation of farm crops is almost nonexistent. One reason for the neglect of broader applications may be the overwhelming interest in antibiotic activity on the part of layman and scientist alike.

The fact that interest in the natural-product antibiotics exists should not be surprising. The cures of serious infection, which often occur with miraculous speed—at a price assessed by the druggist directly against the family—cannot help from impressing the layman. Many scientists have used antibiotics as reagents, as targets for research and observation, or as aids in elucidation of biochemical pathways. This interest has led to the development of highly efficient techniques for antibiotic detection and isolation, resulting in the great preponderance of antibiotically active natural products.

The interest, however, has led to the supposition that the reason microorganisms produce so many antibiotics is that the microorganisms are benefited directly in their competitive environment by the antibiotics produced. Thus, one expects the microbes to be the continuing source of new antibiotics helpful to them. In contrast there is no similar rationale apparent for the direct benefit to the microbial cell from other natural products produced, thus there is no expectation that microorganisms should produce a similar variety of nonantibiotic natural products.

The expectation that antibiotics benefit the producing organism seems logical. By definition, antibiotics are substances produced by microorganisms which in very dilute solution kill other microorganisms (*2*). Surely a microbe, exposed as it is to the competitive forces of millions of rivals for its space in nature, releases its own specific venom for its own advantage.

From the beginning of the study of antibiotics, attempts were made to take advantage of this assumed biological warfare in the search for new antibiotics. In 1940 I studied the interaction of the microbes of the soil. Large numbers of living *Escherichia coli*, a species foreign to the soil, were added at weekly intervals to fresh garden soils to encourage enrichment of antibiotic producers, presumably those at advantage by their ability to kill the foreign intruders. In fact, the added *E. coli* did die in the soil, and at an increasing rate as the experiment proceeded. From the enriched soil, bacteria producing the previously known antibiotic substance pyocyanase and a streptomycete producing the new broad-spectrum antibiotic actinomycin were isolated. As was anticipated, plate counts of

The author is Executive Administrator, Merck Sharp & Dohme Research Laboratories Division, MSD (Japan) Co., Ltd., Tokyo, Japan. Reprint requests should be addressed to H.B.W., Merck & Co., Inc., Post Office Box 2000, Rahway, New Jersey 07065.

0036-8075/80/0613-1225$01.25/0

the antibiotic producers of the soil showed that the addition of living *E. coli* had truly enhanced the number of antibiotic producers, from 1.5 million per gram of soil to nearly 6 million. But, unexpectedly, the increase in total microbial population was exactly comparable, 10 million to 40 million recovered by standard plating methods. Fifteen percent of the soil population produced an antibiotic in each situation, regardless of the competitive pressure.

Certain instances in which antibiotic production has occurred in the natural environment and in which the microbial population of the soil has been modified have been reported. Noteworthy is an early observation that mycorrhizal fungi are lacking in the Wareham Heath soils of England, which are rich in gliotoxin-producing fungi. The examples have been evaluated in two critical studies (*3*, *4*) in which those situations are identified where antibiotic production is believed to be truly influential as a protective mechanism.

Many reviewers, however, believe antibiotics do not affect the natural soil environment (*5–8*). Many antibiotics are too labile to exist in the soil. Others are bound in an inactive state by the soil colloids. Of even greater importance, soil conditions are not conducive to antibiotic formation. Antibiotics are produced in quantity only when specialized laboratory procedures are used to emphasize those aspects of metabolism necessary for their formation.

Table 1. Ranking of pharmaceutical products in Japan on the basis of the sales volume between 1 August 1977 and 31 July 1978; NP, natural products.

Rank	Product	Type	Source
1	Cefamezin	Antibiotic	NP; semisynthetic
2	Keflin	Antibiotic	NP; semisynthetic
3	Keflex	Antibiotic	NP; semisynthetic
4	Futraful	Antitumor	Synthetic
5	Krestin	Immune enhancer	NP
6	Neuquinone	Coenzyme	NP; also semisynthetic
7	Larixin	Antibiotic	NP; semisynthetic
8	Syncl	Antibiotic	NP; semisynthetic
9	Dasen	Enzyme	NP
10	Picibanil	Immune enhancer	NP

Table 2. Enzyme inhibitors isolated at the Institute for Microbial Chemistry, January 1972 to May 1979. All compounds listed.

Producing culture	Enzyme inhibited*	*Journal of Antibiotics* (reference)
	Bacterium	
Pseudomonas sp.	Dopamine-β-hydroxylase	**25**, 497 (1972)
	Actinomycete (filamentous bacteria)	
Streptomyces fulvoviridis var. *acarbodicus*	Alkaline phosphatase	**31**, 244 (1978)
Streptomyces sp.	Aminopeptidase A	**31**, 636 (1978)
S. olivoreticuli	Aminopeptidase B	**29**, 97 (1976)
S. filipiensis	Catechol-*O*-methyltransferase	**26**, 112 (1973)
S. roseolus	Catechol-*O*-methyltransferase	**28**, 619 (1975)
S. mobaraensis	Cyclic AMP-diesterase	**28**, 558 (1975)
S. neyagawaensis var. *orobolere*	Dopa decarboxylase	**28**, 947 (1975)
S. griseoruber	Elastase	**26**, 787 (1973)
S. lavendulae	Esterase	**31**, 639 (1978)
S. alboverticillatus	β-Galactosidase	**28**, 555 (1975)
S. nigellus	β-Galactosidase	**32**, 91 (1979)
S. xanthophaeus	β-Galactosidase	**28**, 1006 (1975)
S. griseosporeus	Glyoxalase I	**28**, 737 (1975)
S. fulvoviridis	β-Lactamase	**30**, 770 (1977)
Streptomyces sp.	*N*-Methyltransferase	**27**, 726 (1974)
Streptomyces sp.	Monoamine oxidase	**26**, 162 (1973)
S. testaceus	Pepsin	**26**, 615 (1973)
S. michiganensis	Protease	**25**, 263 (1972)
S. mauvecolor	Protease	**25**, 267 (1972)
S. violaceus	Protease	**25**, 267 (1972)
S. yokosukaensis	Protease	**25**, 263 (1972)
S. verticillus var. *quintum*	Sialidase	**27**, 963 (1974)
S. tanashiensis	Thermolysin	**26**, 621 (1973)
S. luteogriseus	Tryptophan hydroxylase	**30**, 675 (1977)
Streptoverticillium parvisporogenes	Pepsin	**26**, 539 (1973)
	Basidiomycete (mushroom)	
Inonotus sp.	Catechol-*O*-methyltransferase	**29**, 882 (1976)
Gloeophyllum striatum	Dopamine-β-hydroxylase	**25**, 239 (1972)
Coriolus maximus	Monoamine oxidase	**26**, 162 (1973)
Lentinus sp.	Monoamine oxidase	**26**, 162 (1973)
Pogonomyces sp.	Monoamine oxidase	**26**, 162 (1973)
	Fungi imperfecti (no sexual stage)	
Corynespora cassicola	Catechol-*O*-methyltransferase	**26**, 618 (1973)
Aspergillus niger	Dopa decarboxylase	**28**, 947 (1975)
Piricularia sp.	Histidine decarboxylase	**27**, 587 (1974)

*For inhibitors discovered at the Institute for Microbial Chemistry prior to 1972 see Umezawa (*22*).

A Broader Concept

The understanding that microorganisms in the soil are not necessarily dependent for their survival directly on the antagonistic activity of the products that they produce leads to a suggestion that there is little reason to expect a priori that microorganisms will preferentially synthesize only substances having antibiotic activity. As living cells, they should be expected to synthesize a potpourri of natural substances, many of which, because they originate from biochemical pathways, can influence the biological activities of higher forms of life. In practical terms, microbial products can be expected to influence various human disease syndromes, for example, by acting as neuroleptics, cardiotonics, hypotensives, and antilipemics. Similarly, they may be expected to stimulate growth, improve efficiency, raise mental acuity, or influence behavior. That limited attention has been paid to the search for such nonantibiotic microbial products implies that recognition of the probability is still limited. Acceptance by the full range of laboratory scientists necessary to operate effective screening programs has not been achieved.

Possibly what is required for the full acceptance is logical support for the idea that microbes should produce natural medicinal products, a concept that proves as compelling as the assumed reason for antibiotic formation and that provides the driving force for devising the screening techniques necessary for the detection, isolation, structure determination, and evaluation of natural products. From the accounts of steps involved in successful antibiotic screening programs, it is clear that such endeavors cannot be undertaken without adequate funding. They are expensive and require a well-organized and dedicated staff (*9*).

Secondary Metabolism

Experience has shown that biologically active natural products, sufficient in amount to facilitate detection and isolation, are produced in high frequency by microorganisms. More than 3000 different antibiotics have been isolated from cultures of a single group of microorganisms, the actinomycetes (*10*). These easily cultivated filamentous bacteria are a relatively homogeneous group, consisting of about 600 defined species. If evolutionary development favors conservation of energy from available nutrients, there must be an explanation why so much cellular energy in this group of simple organisms is devoted to anabolic activities that occur during the late stages of a fermentation.

An explanation for the formation of natural products by microorganisms in quantity sufficient for easy detection can be found in a peculiarity of their cultivation in the laboratory. At first, when exposed to rich nutrient media with appropriate physical conditions they multiply rapidly, reaching large numbers. Then follows abrupt cessation of growth, occurring while abundant supplies of most nutrients remain, with environmental conditions continuing to be favorable for metabolism. Bu'Lock (*11, 12*) has named the two phases the trophophase and idiophase, describing in detail the characteristics of each, as well as the factors that cause change of phase.

As long as microorganisms are growing in the trophophase, there is demand for metabolites as basic cell constituents. The amino acids, Krebs-cycle intermediates, and purines are fixed into the cellular structures. The microbial cells undergo a change, however, at the beginning of the idiophase when cell replication ceases, as it does in commercial fermentation vessels, as a result of nutrient deficiency, accumulation of a toxic substance, deficiency of oxygen, or some other limiting factor. In spite of repression of enzymes and the feedback inhibition of anabolic pathways, metabolic intermediates continue to accumulate. These intermediates can serve as the initiators for secondary metabolism, the process by which many of the pharmacologically active natural products are synthesized.

There is little justification for assuming that these secondary metabolites should be antibiotic only. Moreover, considering their source, namely, metabolites common to both animals and microorganisms, and the fact that many antibiotics are harmful for the producing cell, as well as for pathogens, it should be expected that secondary products which

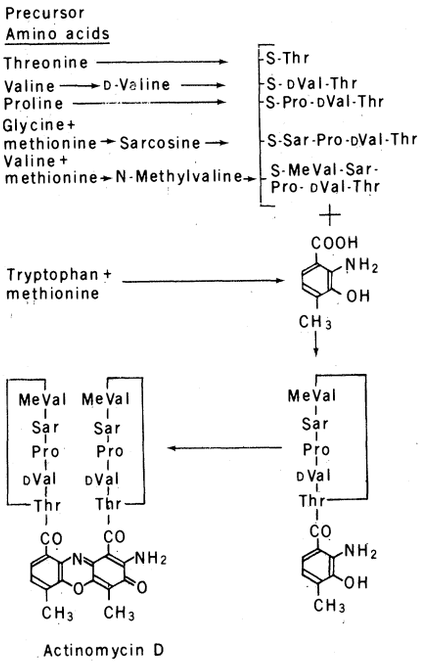


Fig. 1. Scheme of natural-product formation from amino acids.

accumulate during the idiophase will show a wide range of biological activities in addition to the inhibition of growth alone.

Whether the products themselves benefit the producing organisms by acting as protectants, by shifts in microbial status such as in spore formation, by serving as storage products, or by other favorable functions, the secondary metabolic process by which they are formed does appear to provide an advantage to the growth-inhibited microorganisms. Alternating feast and famine is normal in soils, as green manures are plowed under or organic composts are added to cultivated soils, or as leaves fall and plants are killed by the frosts of seasonal changes. The soil population responds accordingly, with shifts from autochthonous to zymogenic populations, repeatedly exposing the soil microbes to the stress of shifting from idiophase to trophophase and back again. Herein may lie the primary evolutionary advantage of secondary metabolism, and the reason why microbes should be considered as producers of a variety of biologically interesting compounds.

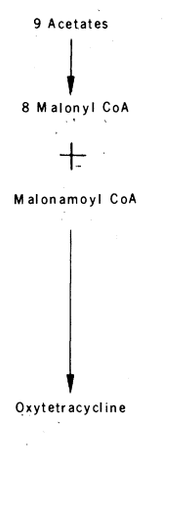
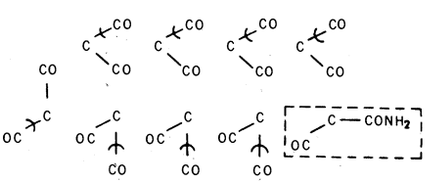


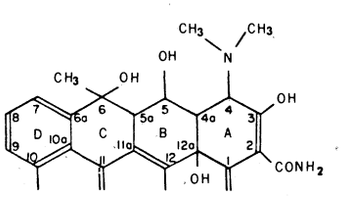
Fig. 2. Scheme of natural-product formation from acetate.

Types of Natural Products

There are the numerous factors that can determine the type of natural product produced by a microorganism and the chemical substances ultimately found in a fermentation broth. The range of structural types truly is enormous. Among the antibiotics, the most thoroughly investigated of the natural products, 58 structural categories are used in Umezawa's index (*10*) to subdivide the products of the actinomycetes, with hundreds of additional structures listed as single examples. The target of the antibiotic action, such as inhibition of bacterial cell wall or bacterial protein synthesis, disruption of nucleic acid polymers or of bacterial membrane integrity, and action as enzyme antagonists (reversible or irreversible) is important because it is the basis for the specificity of antibiotic action, thereby providing a therapeutic index adequate for human use. The target, however, appears to show little correlation with chemical structure. For example, medicinal products that act by blocking synthesis of the cell wall of pathogenic bacteria include (i) cycloserine, among the simplest and smallest of the antibiotics (102 daltons); (ii) microbial lysozyme, a macromolecular enzyme; (iii) amino acid–derived products in which the β-lactam structure is essential (the penicillins); and (iv) cyclic peptides (bacitracin).

A significant attribute of the natural products of microorganisms is their





structural relationship to the common end products of metabolism—the carbohydrates, lipids, amino acids, purines, and pyrimidines. This relationship sometimes may be complex or somewhat obscure, but in the case of the commercially important products the metabolic origin is usually evident. For example, Figs. 1 and 2, respectively, show the relation of the antitumor agent actinomycin D to precursor amino acids and that of oxytetracycline to malonamoyl-CoA and malonyl-CoA (*13, 14*).

Target for Applied Research

If biologically active natural products are by-products of the normal metabolism of growth-inhibited cells, these cells should be subject to metabolic control and also to genetic manipulation as a means of enhancing productivity to economically significant levels. Both aspects have been investigated although largely on an empirical basis, as part of the developmental research programs of commercial organizations that manufacture and sell antibiotics and other anti-infective agents. The results have been successful, as evidenced by the production of antibiotics in megaton quantities by fermentation to meet worldwide needs. Even the economically competitive field of farm animal production has yielded to natural products, with complex coccidiostats of microbial origin gradually displacing synthetic compounds of relatively simple structure. For example, the steps for improvement in yield of a natural-product coccidiostat isolated from a streptomycete to achieve a yield compatible with economic needs have been described (*15*).

Target for Basic Research

The principal factor determining the type of natural product accumulated is the microorganism itself. The enzymatic complement of the cell is critical in determining the final result and the combination necessary to produce any single substance occurs infrequently among microorganisms. The type of antibiotic produced has been considered sufficiently definitive that proposals have been made to utilize it as a major determinant in microbial species definition. Yet, further searching frequently shows the same natural product can be accumulated by truly different organisms: for example, the production of the β-lactam antibiotic penicillin N by the true fungi and by filamentous bacteria, or the antitubercular antibiotic cycloserine by filamentous and by motile, rod-shaped bacteria. In contrast, separate isolates of a single microbial species, not readily distinguishable from one another by other criteria, can produce many natural products of diverse structure. Thirty-four named antibiotics included in the 1967 edition of Umezawa's index (*10*) are produced by strains of *Streptomyces griseus*, a species originally famous as the source of streptomycin.

Descriptions of fundamental aspects of control mechanisms, with examples of the manipulation of gene expression and outlining the episomatic coding of certain enzymes essential for natural-product synthesis are beginning to appear and rapid progress should be forthcoming. At present the interest in basic aspects of natural-product synthesis is worldwide (*11, 12, 16–20*).

As previously stated, natural-product formation is characteristic of the idiophase, that period in a microbial culture in which growth is inhibited but cells remain metabolically active. The usual failure of natural products to accumulate in the preceding trophophase of batch culture may be due in part during that period of active cellular growth to the repression of the enzymes necessary for natural-product synthesis. For example, workers in the centers referenced (*11, 12, 16–20*) of research have called attention to production of the amidino transferase of streptomycin synthesis, the phenylalanine racemase and gramicidin S synthetase of gramicidin S synthesis, the phenoxazinone synthase of actinomycin biosynthesis, and the guanosine triphosphate (GTP) 8-formylhydrolase of the pyrrolopyrimidine nucleoside antibiotics only at the end of the trophophase.

Necessary for the formation of large amounts of natural products are initiators. These may be natural metabolites, such as malonamide or acetate, which are incorporated directly into the molecule and determine whether oxytetracycline or the minor antibiotic product 2-acetyl-2-decarboxamide-oxytetracycline is formed. In other instances, initiators serve as inducers for enzymes essential to the synthesis of the natural product; for example, the induction of dimethylallyl tryptophan synthetase, the first enzyme of the pathway to the ergoline alkaloids, by either tryptophan, a natural precursor of the alkaloid, or thiotryptophan, a nonincorporated analog.

The relative significances of other regulatory factors include (i) the feedback regulation of a natural product by a normal metabolite acting on a common precursor (such as the inhibition of penicillin production by L-lysine), (ii) the feedback regulation of phosphatases es-

Table 3. Examples of pharmacologically active natural products discovered by directed searching. Antimicrobial and antitumor products are not included.

Activity*	Product	Producing culture	Reference
Anticoagulant	Phialocin	*Phialocephala repens*	(*23*)
Antidepressant	1,3-Diphenethylurea	*Streptomyces* sp.	(*24*)
Anthelmintic	Avermectin	*Streptomyces avermitilus*	(*25*)
Antilipidemic	Ascofuranone	*Ascochyta viciae*	(*26*)
Antipernicious anemia	Vitamin B_{12}	*Streptomyces griseus*	(*27*)
Coronary vasodilator	Naematolin	*Naematoloma fasciculare*	(*28*)
Detoxicant	Detoxin	*Streptomyces caespitosus*	(*29*)
DNA-transformation inhibitor	Antraformin	*Streptomyces* sp.	(*30*)
Esterogenic	Zearalenone	*Gibberella zeae*	(*31*)
Food pigment	Monascin	*Monascus* sp.	(*32*)
Herbicide	Herbicidin	*Streptomyces saganonensis*	(*33*)
Hypotensive	Fusaric acid	*Fusarium* sp.	(*34*)
Immune enhancer	*N*-acetylmuramyl-tripeptide	*Bacillus cereus*	(*35*)
Insecticide	Piericidin	*Streptomyces mobaraensis*	(*36*)
Miticide	Tetranactin	*Streptomyces aureus*	(*37*)
Plant hormone	Gibberellic acid	*Gibberella fujikuroi*	(*38*)
Salivation inducer	Slaframine	*Rhizoctonia leguminicola*	(*39*)
Serotonin antagonist	HO_{2135}	*Streptomyces griseus*	(*40*)

*Single examples only are given. Many products isolated on the basis of antibiotic action also demonstrate pharmacological action. For more complete discussion, see reviews by Matthews and Wade (*41*) and by Perlman (*42, 43*).

sential in natural-product biosynthesis by inorganic phosphates (such as inhibition of streptomycin formation by phosphates), and (iii) the catabolite repression of natural-product synthesis by a rapidly utilized carbon source (such as repression of phenoxazinone synthase essential for actinomycin synthesis by glucose, or the repression of novobiocin formation by citrate). Other regulatory phenomena, such as the inhibitory effect of phosphate on the biosynthesis of chlortetracycline, in which no phosphatases are thought to be involved, have been interpreted as a regulation induced by adenosine triphosphate (ATP). Inorganic phosphate also has a direct repressive effect on enzymes characteristic of the biosynthesis of naturally occurring alkaloids. Dimethylallyltryptophan synthetase and chanoclavine-I cyclase are repressed by phosphate. Tryptophan derepresses these enzymes, permitting initiation of alkaloid synthesis in the presence of phosphate.

Many natural products and their precursors inhibit their own biosynthesis. The relief of these regulatory effects by mutation or by chemical additives is a primary method used to elucidate the basic phenomena of natural-product biosynthesis, as well as an approach to improved yield in the applied microbiology laboratory (*17*).

Most of the basic studies on factors controlling natural-product synthesis have been performed with antibiotics. However, there are enough examples of factors influencing synthesis of other natural products (such as alkaloids, ubiquinone-10, cyanocobalamin, aflatoxins, and gibberellic acid) to justify the assumption that there will be uniformity in the controlling mechanisms.

Nonantibiotic Natural Products

Directed search for nonantibiotically active natural products has yielded promising results such as those of Umezawa, whose quest for inhibitors of enzymes has now been extended to the more specialized objective of inhibitors of enzymes located on cell surfaces (Table 2). Specificity in activity is possible, and one need merely select the desired target. For example, elastatinal is a highly effective inhibitor of pancreatic elastase, but has low activity against human granulocyte elastase. A search for a natural-product inhibitor with the latter enzyme as target quickly led to discovery of its specific natural-product inhibitor, elasnin (*21*).

Table 3 is presented as an example of success achieved from limited screening aimed directly at nonantibiotic, physiologically active microbial metabolites; it is not an all-inclusive review, but merely provides evidence that, when a serious attempt is made, with the use of the same creativity of approaches as applied in the successful antibiotic screens, the results are equally promising.

Necessity for Broadened Research

An essential complement to the recognition that fermentation broths contain natural products of great variety in chemical and biological properties is the necessity to perform research to define those properties of products that are useful in the pursuit of food and health. One such property, antibiosis, is of value, and the methods to be used for detection of such activity and proof of its utility against infectious disease are apparent. The essential properties and their methods of evaluation are usually less clear when the objectives are more complex: for example, organic disease of man or the improvement of feed efficiency in farm animals. The understanding that natural products of unusual chemical structure exist in fermentation broths, therefore, implies not only that microbiologists should be aware of the possibilities for significant discoveries and should design efficient screening procedures for their detection, but that biochemists, biologists, and clinicians must also participate in the endeavor by identifying critical biochemical pathways, by developing simplified in vivo evaluation systems, and by demonstrating correlations with human and animal responses that may lead to appropriate screening methods.

The above discussion indicates that paying greater attention to natural products for their applied values in human well-being and animal or plant health is justifiable. Of at least equal significance is the expansion in fundamental knowledge concerning properties of molecules that results when the structures of new natural products are elucidated. From them, medicinal chemists obtain ideas for use in their synthetic endeavors, biophysicists learn more about the topology of active sites of the target proteins on which they act, toxicologists understand more about specificity of action of molecules given in vivo and physicians face new challenges in devising appropriate clinical trials. From such attention the antibiotic age of biochemistry, which has been of such value to basic science, may evolve to the natural-product age.

References and Notes

1. *Japan Medical Gazette* **16** (No. 1), 8 (20 January 1979).
2. S. A. Waksman, *Am. Sci.* **41**, 8 (1953).
3. P. W. Brian, *Symp. Soc. Gen. Microbiol.* **7**, 168 (1957).
4. G. W. Bruehl, R. L. Millar, B. Cunfer, *Can. J. Plant Sci.* **49**, 235 (1969).
5. K. F. Baker and W. C. Snyder, Eds., *Ecology of Soil-Borne Plant Pathogens—Prelude to Biological Control* (Univ. of California Press, Berkeley, 1965).
6. D. Gottlieb, *J. Antibiot.* **29**, 987 (1976).
7. S. A. Waksman, *Biol. Rev.* **23**, 452 (1948).
8. H. B. Woodruff, *Symp. Soc. Gen. Microbiol.* **16**, 22 (1966).
9. _____, S. Hernandez, E. O. Stapley, *Hind. Antibiot. Bull.* **31**, 71 (1979).
10. H. Umezawa, *Index of Antibiotics from Actinomycetes* (University Park Press, Baltimore), vol. 1 (1967), vol. II (1978), vol. II, part 2, in press.
11. J. D. Bu'Lock, *The Biosynthesis of Natural Products: An Introduction to Secondary Metabolism* (McGraw-Hill, New York, 1965).
12. _____, *Essays in Biosynthesis and Microbial Development* (Wiley, New York, 1967).
13. J. Meienhofer and E. Atherton, *Adv. Appl. Microbiol.* **16**, 203 (1973).
14. J. R. D. McCormick, in *Antibiotics*, vol. 2, *Biosynthesis*, D. Gottlieb and P. D. Shaw, Eds. (Springer-Verlag, New York, 1967), p. 113.
15. W. M. Stark, in *Fermentation Advances*, D. Perlman, Ed. (Academic Press, New York, 1969), p. 517.
16. A. L. Demain, *J. Appl. Chem. Biotechnol.* **22**, 345 (1972).
17. _____, *Stadler Genet. Symp.* **8**, 41 (1976).
18. _____, *Adv. Appl. Microbiol.* **16**, 177 (1973).
19. J.-F. Martin, in *Antibiotics and Other Secondary Metabolites*, R. Hütter, T. Leisinger, J. Nüesch, W. Wehrli, Eds. (Academic Press, New York, 1978), p. 19.
20. H. Umezawa, *J. Antibiot.* **30** (Suppl.), S-138 (1977).
21. H. Ohno, T. Saheki, J. Awaya, A. Nakagawa, S. Omura, *J. Antibiot.* **31**, 1116 (1978).
22. H. Umezawa, *Enzyme Inhibitors of Microbial Origin* (University Park Press, Baltimore, 1972).
23. T. Suzuki, N. Meguriya, M. Yokohori, S. Kondo, Japanese patent 71 02,115, 19 January 1971.
24. Y. Iwai, A. Hirano, J. Awaya, S. Matsuo, S. Omura, *J. Antibiot.* **31**, 375 (1978).
25. R. W. Burg, B. M. Miller, E. E. Baker, J. Birnbaum, S. A. Currie, R. Hartman, Y-L. Kong, R. L. Monaghan, G. Olson, I. Putter, J. B. Tunac, H. Wallick, E. O. Stapley, R. Oiwa, S. Omura, *Antimicrob. Agents Chemother.* **15**, 361 (1979).
26. M. Sawada, T. Hosokawa, T. Okutomi, K. Ando, *J. Antibiot.* **26**, 681 (1973).
27. E. L. Rickes, N. G. Brink, F. R. Koniuszy, T. R. Wood, F. K. Folkes, *Science* **108**, 634 (1948).
28. Y. Ito, H. Kurita, M. Sato, Japanese patent 70 16,795, 10 June 1970.
29. H. Yonehara, H. Sato, S. Aizawa, T. Hidaka, A. Shimizu, N. Otake, *J. Antibiot.* **21**, 369 (1968).
30. T. Tanaka, *ibid.* **28**, 567 (1975).
31. C. M. Christensen, G. H. Nelson, C. J. Miroaha, *Appl. Microbiol.* **13**, 653 (1965).
32. B. C. Fielding *et al.*, *J. Chem. Soc.* (1961), p. 4579.
33. M. Arai, T. Haneishi, N. Kitahara, R. Enokita, K. Kawakubo, Y. Kondo, *J. Antibiot.* **29**, 863 (1976).
34. H. Hidaka, T. Nagatsu, K. Takeya, T. Takeuchi, H. Suda, K. Kojiri, M. Matsuzaki, H. Umezawa, *ibid.* **22**, 228 (1969).
35. F. Ellouz, A. Adam, R. Ciorbaru, E. Lederer, *Biochem. Biophys. Res. Commun.* **59**, 1317 (1974).
36. S. Tamura, N. Takahashi, S. Miyamoto, R. Mori, S. Suzuki, J. Nagatsu, *Agric. Biol. Chem.* **27**, 576 (1963).
37. K. Ando, H. Oishi, S. Hirano, T. Okutomi, K. Suzuki, H. Okazaki, M. Sawada, T. Sagawa, *J. Antibiot.* **24**, 347 (1971).
38. P. J. Curtis and B. E. Cross, *Chem. Ind.* (1954), p. 1066.
39. M. H. Crump, E. B. Smalley, J. N. Henning, R. E. Nichols, *J. Am. Vet. Med. Assoc.* **143**, 996 (1963).
40. T. Arai and T. Hayama, *Jpn. J. Med. Progr.* **49**, 813 (1962).
41. H. W. Matthews and B. F. Wade, *Adv. Appl. Microbiol.* **21**, 269 (1977).
42. D. Perlman, *Wis. Pharm. Ext. Bull.* **19**, 22 (1976).
43. _____ and G. P. Peruzzotti, *Adv. Appl. Microbiol.* **12**, 277 (1970).