*Journal of Natural Products*
*Vol. 52, No. 6, pp. 1189-1208, Nov-Dec 1989*



# WHY ARE SECONDARY METABOLITES (NATURAL PRODUCTS) BIOSYNTHESIZED?

DUDLEY H. WILLIAMS,* MARTIN J. STONE, PETER R. HAUCK, and SHIRLEY K. RAHMAN

*University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK*

ABSTRACT.—We adopt the definition of a natural product as a substance that has no known role in the internal economy of the producing organism. The literature abounds with conflicting views for the existence of such natural products. We propose that all such structures serve the producing organisms by improving their survival fitness. We argue that this conclusion is necessitated by the fact that natural products are normally complex structures, whose biosynthesis is programmed by many kilobases of DNA. If it were otherwise, the pressures of Darwinian natural selection would have precluded the expenditure of so much metabolic energy in their construction and the development of such complexity. We further conclude that a natural product improves the producer's survival fitness by acting at specific receptors in competing organisms. Current studies of natural products interacting with receptors support this view, in terms of both the sophistication of the molecule/molecule recognition and the mechanistic details of physiological action. By the application of Occam's razor and general weaknesses of other hypotheses, these other hypotheses are rejected. It is a consequence of our proposal that natural product/receptor interactions of sophistication comparable to enzyme/substrate interactions will be commonplace. Additionally, structures that are candidates to interact with known receptors (e.g., double helical DNA) can on occasion be suggested by inspection of the structures. A range of evidence to support the general conclusions is presented.



Natural products are substances that traditionally lie at the heart of organic chemistry. As such, they are commonly recognized as substances of limited molecular weight (normally $< 3000$ Daltons) and of great structural variety. However, the most pragmatic definition of a natural product is a substance appearing to have no explicit role in the internal economy of the organism that produces it. Substances that apparently have no such role are known as secondary metabolites. Thus, we shall equate natural products with secondary metabolites.

In a relatively recent and authoritative review, Haslam (1) has discussed characteristic features of primary and secondary metabolism. Of particular relevance in the present context is his summary of hypotheses as to why natural products are made. The various hypotheses presented are as follows:

1. "Mutations may well arise (and indeed persist for many generations) which are neither expressly beneficial nor harmful." Such characteristics have been called "ballast" by Krebs (2); he gave as an example alkaloids in plants, the presence of which generally baffles all attempts to account for them satisfactorily.

2. One speculation concerning the profusion and diversity of natural products (now numbered in the tens of thousands) is that they represent "an example of evolution in progress" (1). This is the hypothesis of Muller (3), based on the general idea that secondary metabolism provides "a reservoir of non-functional variety out of which new functional processes can emerge" at some future time by continuing natural selection (4).

3. "The secondary metabolites are waste or detoxification products."

4. "The synthesis of enzymes that are designed to execute the processes of secondary metabolism permits the network of enzymes that is operative in primary metabolism to continue to function until such time as circumstances are propitious for renewed metabolic activity and growth. It is the *processes* of secondary metabolism, rather than the *products* (secondary metabolites), which are important. Such propositions do not preclude the possibility that the secondary metabolites . . . have subsequently acquired a functional role."

5. "At some point in the life of the organism, the secondary metabolites had (have) a functional (metabolic) role."

6. "The secondary metabolites are a measure of the fitness of the organism to survive. The ability to synthesize an array of secondary metabolites which may repel or attract other organisms has *evolved* as one facet of the organism's strategy for survival."

In the following, we argue that hypothesis 6 is by far the most likely to be the one of greatest applicability, and that "repel or attract" must be extended to any result in which the survival of a producer A is enhanced when its product acts on organism B. Such effects which are beneficial to A are potentially enormously diverse, and obviously include the killing of B, if B poses a threat to the survival of A. The reasons for our view are now presented.

DISCUSSION OF THE HYPOTHESES.—We first present arguments which point to the weaknesses of hypotheses 1 to 5 as hypotheses of generality: that is, as proposals that would satisfactorily account for the currently known properties of a wide range of natural products. In relation to hypothesis 1, neutral mutations may indeed persist for many generations in a population, but by definition they are not normally an efficient route for increasing the fitness of an organism to survive. The alkaloids, and indeed natural products in general, are generally biosynthesized by the use of several enzymes. As such, their production is programmed by many kilobases of DNA. To suggest that such a highly ordered and complex system could come about by a series of neutral mutations flies in the face of the accepted basis of Darwinian selection. Additionally, it has gradually come to light that alkaloids not only have the common property of bitterness, which plausibly represents an antifeedant property towards herbivores, but also may have precise physiological effects on other organisms (e.g., strychnine, curare, and morphine).

Hypothesis 2 implies that many of the natural products of today are nonfunctional, but that they have a utility in natural selection because they may in the future be modified to become functional in aiding the producer's survival. Bu'Lock has observed (4) that this hypothesis of Muller's (3) "is either an extremely profound insight into the mechanism of evolution by natural selection at a molecular level, or it is a teleological fantasy; frankly, I am not sure which . . . ." We unambiguously associate ourselves with the proposal that it is a teleological fantasy. This conclusion requires justification. Teleology is defined as the doctrine that developments are due to the purpose or design that is served by them. Note that the definition requires that the developments are caused by the purpose, in the sense that the purpose of design precedes the developments. We argue that this interpretation of teleology makes the Muller argument a concept that is contrary to all the available evidence on natural selection. Indeed Mayr (5) has noted that in such an interpretation, teleology means "goal-directed," and, on the contrary, that "natural selection is strictly an *a posteriori* process which rewards current success but never sets up future goals." We conclude that if any group of natural products is currently non-functional, it is contrary to natural selection that such highly programmed structures could have arisen to serve a future goal. Although neutral mutations of DNA may provide a reservoir out of which new functional processes may emerge, natural selection demands that the current product of the DNA translation be functional if it is complex and produced by a highly ordered and complex pathway. Because natural products are typically produced by pathways involving multi-functional enzymes, or several enzymes, their production does indeed involve "complexity."

Hypothesis 3 does in a sense give a function to natural products—they are the vehicle for clearing unwanted materials from the organism. Yet numerous aspects of this hypothesis seem completely unsatisfactory. Why would enormous diversity and com-

plexity arise if clearance is the function? Indeed, Haslam (1) has cited natural products that accumulate in the producing organism in substantial amounts, the site of storage often being quite separate from the site of synthesis. Like hypothesis 1, hypothesis 3 also appears implausible when it is considered that many kilobases of DNA are used to program the synthesis of a substance destined to be a waste product.

Hypothesis 4 appears to find its origins in an early proposal by Bu'Lock and Powell (6) and later by Woodruff (7). In this proposal, secondary metabolism is regarded as an overflow process, giving rise to shunt metabolites that are produced to reduce abnormal concentrations of normal cellular constituents. The abnormal metabolic flows could arise by an overriding of normal regulatory mechanisms. More recently, Bu'Lock (4) has noted, in relation to this hypothesis, the same objection that he and we have discussed in relation to hypotheses 1 and 3 and others—namely, that "our picture of primary metabolism is that it is too finely adjusted to permit such an explanation, which in any case explains neither the elaboration nor the variety of secondary metabolite structures." He has therefore elaborated (4) a somewhat different proposal in which secondary biosynthesis maintains primary metabolism at a time when the products of primary metabolism cannot be used for cell replication. The significant difference in this proposal appears to be that a rather arbitrary "overriding of normal regulatory mechanisms" (of primary metabolism) is replaced by situations where the products of primary metabolism are stipulated to be not needed. Our objections to all these type 4 hypotheses are that none of them appears to satisfy the diversity of natural product structures, nor are they consistent with the sophisticated functions now being uncovered for many natural products. On the other hand, Bu'Lock has argued (4) that the fact that some organisms demonstrably utilize some secondary metabolites "is *not* an argument against the view that the general function of secondary metabolism has little or nothing to do with the properties of the products." In contrast, we argue below that current work on the mechanism of action of natural products points to the properties of these products being crucial in interfering with life processes.

We interpret hypothesis 5 as implying that natural products exist because they were primary metabolites at some time in the past. Inasmuch as the metabolic role has in this case by definition disappeared, it is a hypothesis that is essentially impossible to test. However, it is not consistent with the available evidence (e.g., the increasing evidence for a nonmetabolic function of the products, cited above); nor is it a hypothesis which leads to testable predictions. Therefore, by applying Occam's razor (hypotheses should not be allowed to multiply unnecessarily), we reject it. The modified statement of hypothesis 5, that at some time secondary metabolites have a metabolic role, is, on the basis of the available evidence, not true.

Having pointed out weaknesses of hypotheses 1 to 5, we now turn to strengths of hypothesis 6, as modified to confer any survival advantage on the producing organism due to the structure of the natural product itself. This hypothesis is not only consistent (as are some of the other hypotheses) with use by the producer of much metabolic energy to convert building blocks to highly complex substances, but also with the existence of much genetic information to program their production. Most importantly, hypothesis 6 is the only one that accounts for the fact that many natural products trigger very specific physiological responses and in many cases by binding to receptors with a remarkable complementarity. To regard this latter outcome as an accidental complementarity, as required by all the other hypotheses (unless they conveniently mix in a degree of hypothesis 6), would deny a great strength of this hypothesis. Natural products also in some cases exert their structure-dependent functions in a mechanistically subtle and sophisticated way. Some examples of these phenomena are now presented.

THE MOLECULAR BASIS OF ACTION OF SOME NATURAL PRODUCTS.—Traditionally, organic chemists have classified natural products according to the similarities in their structures (and implicitly the similarities in their biosyntheses). An alternative classification based on the biological functions of these compounds was not possible because these functions were, and in most cases still are, unknown. However, the evolution of a compound by natural selection depends on the function(s) that it performs. A compound that has a beneficial effect for the producing organism will be selected ahead of one whose effect is neutral, which in turn will be favored over a compound which has a detrimental effect.

We now discuss a selection of natural products grouped according to the types of functions that they perform. Each group contains compounds of various structures that are produced by different organisms, but nevertheless perform similar functions for the producing organisms. The functional similarity of these structurally diverse natural products is a clear indication that such functions are advantageous to the producer. Organisms that produce compounds to perform such functions will be rewarded by natural selection.



Perhaps the most thoroughly studied group of natural products with an identified target receptor is that of DNA-binding antibiotics. These have been of special interest due to their potential as anticancer drugs, which results from their ability to inhibit DNA replication and/or transcription. Nature has evolved a diverse set of compounds that attack in a wide variety of ways, including cleavage of the DNA strands, alkylation of the bases, cross-linking of strands, binding into the major or minor groove, and intercalating between consecutive base pairs.

The bleomycins (8) are a group of glycopeptide antibiotics, isolated from *Streptomyces verticillus* (9), which are used clinically against a variety of cancers—squamous cell carcinomas, testicular cancers, cancers of the head and neck, Hodgkin's disease and other lymphomas. They have the structure shown in Figure 1 (10), differing only in the nature of the terminal amine R, and they act by binding to and cleaving DNA. This activity requires the presence of both Fe (II) and molecular oxygen as cofactors, and specific regions of the molecule have been identified as necessary for the DNA-binding and metal-chelating properties (see Figure 1).

The binding of bleomycins to DNA and the nature of the DNA damage are highly specific (11–14). Bleomycins are known to interact most strongly with guanine-containing nucleic acids and those containing alternating purine-pyrimidine sequences. Bases or base propenals, derived from deoxyribonucleotides, are released in the relative order T>C>A>G while d(GpC) and d(GpT) sequences show the highest susceptibility to cleavage, which occurs at the pyrimidine sugar. Both single- and double-stranded breaks are observed in the approximate ratio 9:1.

The details of DNA binding and cleavage have not been fully elucidated. However, kinetic, electron paramagnetic resonance, and visible spectroscopic evidence suggest that the active species in cleavage is formed from a ternary complex between bleomycin, Fe (II), and oxygen. This species is postulated to abstract a hydrogen atom from the deoxyribose C-4; the resulting radical captures molecular oxygen and then decomposes to yield the observed base propenals. Clearly this specificity of hydrogen abstraction requires a highly ordered transition state complex between DNA, bleomycin, Fe (II), and oxygen. Bleomycin-induced DNA cleavage is efficient and selective and results from a sophisticated sequence of chemical reactions. We believe that this degree of sophistication is strong evidence, albeit circumstantial, that bleomycins have evolved specifically to cleave DNA.

Calichemycin γ [**1**] (15,16) is a representative of a class of compounds which also includes esperamycin [**2**] (17,18). These compounds have been found to cleave double-

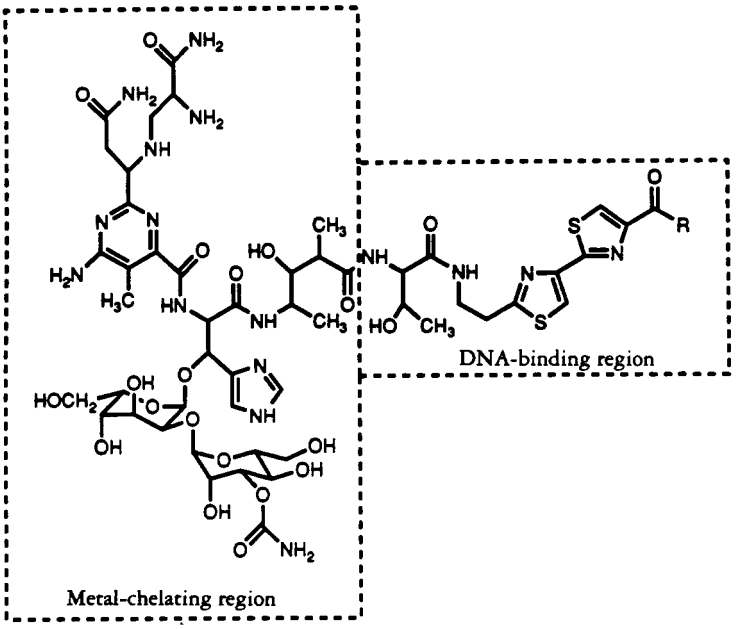
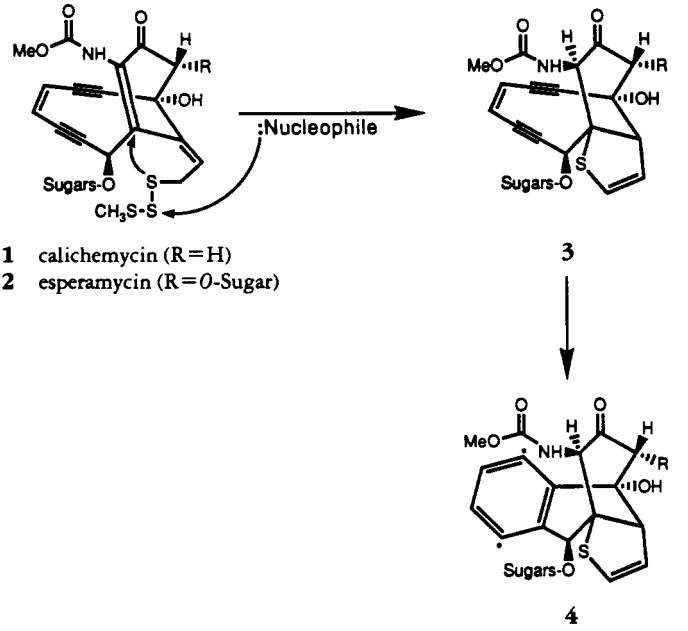



FIGURE 1. Structure of the bleomycins.

stranded DNA (ds-DNA) in the presence of thiol cofactors (17) and hold great promise as anticancer drugs (18). They contain an unusual diyne-ene moiety that is postulated to cyclize in two steps (**2** to **4**), in the presence of reducing agents, to form a 1,4-dehydro-benzene diradical (Scheme 1). This highly reactive species then initiates ds-DNA cleavage by hydrogen abstraction from the deoxyribose sugar. Support for this mechanism of action derives from the observation that when a pseudo-aglycone of **1** is




**1** calichemycin (R=H)
**2** esperamycin (R=*O*-Sugar)

**3**

**4**

SCHEME 1

treated with triphenylphosphine, dihydro-4 is isolated (17), and from experiments (20) in which a calichemycin mimic (containing a cyclic diyne-ene moiety) cleaves ds-DNA in the absence of added catalysts. Recent observations suggest that the cleavage of ds-DNA by calichemycin is sequence specific (19). The reaction of **1** with various DNA samples shows that selective cleavage occurs at the 5′-end of a TCCT sequence and supports the idea that the drug is bound in the minor groove. The authors make the comment that this suggests a "unique fit of the drug and DNA" (19).

These compounds appear to be constructed in such a way that the DNA-cleaving functionality (the benzenoid diradical **4**) remains latent until it is activated by nucleophilic attack (**1** to **3**). It has been suggested (18) that the bridgehead double bond in **1** keeps the ends of the diyne system from approaching near enough to each other to cyclize. However, after reductive cleavage of the methyltrisulfide functionality and cyclization to **3**, the geometrical constraints forbidding cyclization to **4** are removed. We feel that this high degree of sophistication in the mode of action of a natural product argues strongly in favor of the hypothesis that it has evolved to perform a specific task: i.e., ds-DNA cleavage directed against non-self organisms.

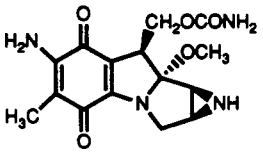
Mitomycin C [**5**] is one of a variety of natural antibiotics containing an aziridine ring that have been isolated from *Streptomyces caespitonis* (26). It is used clinically in the treatment of malignancies of the breast, lung, colon, and stomach. It has been described by Lown (27) as "a model of structural economy" as it contains quinone and urethane moieties as well as the aziridine ring, and all of these groups are involved in the interaction with DNA.

Mitomycin C acts by alkylating DNA, and about 10% of these covalent interactions result in the cross-linking of double-helical DNA. However, the alkylation reaction is preceded by a series of reactions that convert the antibiotic to an activated form (28,29). It is proposed that enzymic reduction of the quinone inside the victim cell gives rise to loss of MeOH, activating both the carbamate and aziridine moieties to alkylation by the guanine bases of DNA. It is also thought that the production of hydroxy radicals from the intermediate semiquinone leads to single strand breakages which enhance the carcinostatic properties of the antibiotic (30,31). The chemical stability of mitomycin C under acidic conditions is unusually high for an aziridine but serves to increase the antibiotic's half-life and hence its efficiency. This property is of interest to medical researchers because the pH of cancer cells is generally lower than that of normal cells. In addition to the modification of DNA that mitomycin C causes, low concentrations of mitomycin cross-linked DNA have been observed to inhibit DNA polymerase even when excess DNA template is present (32). This effect may be important in preventing the repair of damaged DNA.

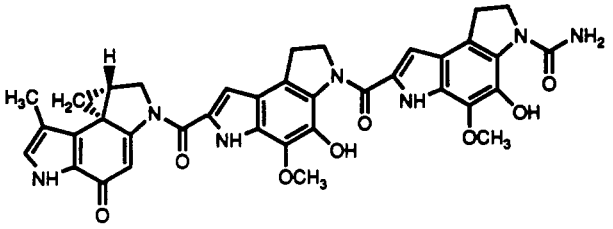
The tetracyclic skeleton, as well as the aziridine, quinone, and carbamate moieties, is conserved throughout all naturally occurring mitomycins, and it is these features which are necessary for anti-DNA activity. This conservation of functional structure supports the hypothesis that mitomycins have evolved specifically for their useful function of alkylating and cross-linking DNA.

It is noteworthy that neither mitomycin C [**5**] nor calichemycin [**1**] reacts directly with DNA. Each compound undergoes a chemical transformation that activates it towards DNA attack. This latent potency may be a subtle way of producing a compound that is not toxic while inside the producer, but which becomes toxic after penetrating the victim cell. As such, it represents an extra degree of sophistication among functional natural products.

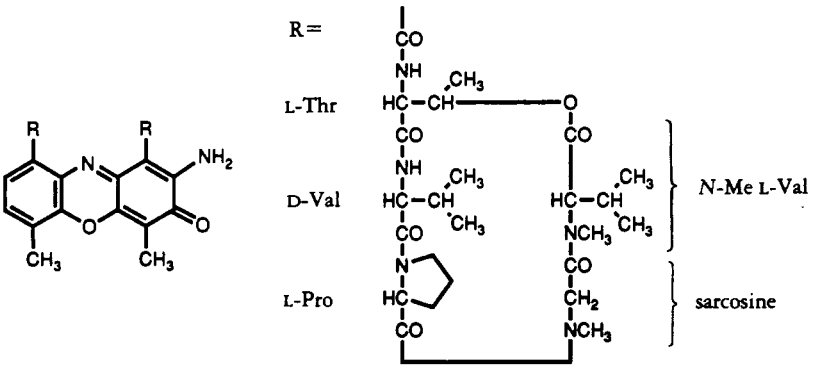
CC-1065 [**6**] is a potent antitumor antibiotic produced by *Streptomyces zelensis* (21–23). It has a right-handed twist along its length with hydrophobic and hydrophilic groups along the concave and convex edges of the molecule, respectively (24). It has



**5** mitomycin C



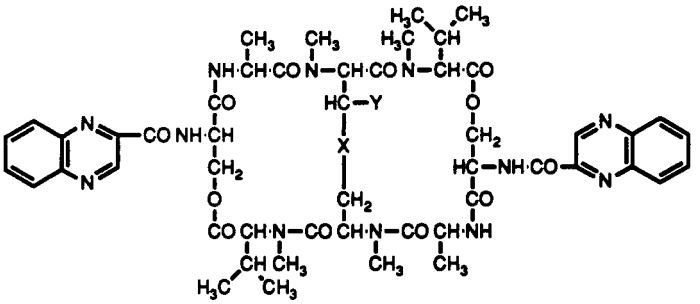
**6** CC-1065




**7** actinomycin D

been shown that DNA is the cellular target of CC-1065, and the structure of the resulting adduct has been elucidated (25). CC-1065 bonds covalently to N-3 of adenine in the minor groove of double-helical DNA. In addition, it is highly selective for AT-rich sites in DNA, binding with greatest affinities over the 5-base regions 5′PuNTTA and 5′AAAAA (Pu = purine; N = any base). Studies on analogues of CC-1065 in conjunction with molecular models of the antibiotic have shown that alternative stereoisomers of CC-1065 do not bind to DNA. This implies that CC-1065 has been selected over other possible stereoisomers specifically for its ability to bind to DNA.

Actinomycin D [7] is an anticancer antibiotic which consists of a substituted phenoxazone chromophore bearing two identical pentapeptide lactones [L-Thr-D-Val-L-Pro-Sar-L-MeVal]. It is active against Gram-positive bacteria and inhibits RNA synthesis, and it is used clinically against various forms of cancer. The observed activity of actinomycin D results from its interaction with double helical DNA: the chromophore between G-C and C-G base pairs, while the two pentapeptide loops occupy the minor groove (34–36). This structure is stabilized by specific hydrogen bonding from the L-threonine amide hydrogen and carbonyl oxygen atoms in the drug to N-2 and N-3 of a guanine base in the DNA. Furthermore, specific intramolecular hydrogen bonds in the antibiotic force the molecule into a conformation "strikingly adapted to intercalation into a right handed DNA helix" (35). Such a conformation allows the formation of several favorable hydrophobic interactions (directly observable by nOe's in $^1$H-nmr spectra) which can stabilize the DNA/antibiotic complex.



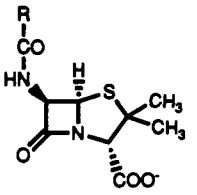
**8** echinomycin (X=S, Y=SMe)
**9** triostin A (X=$S_2$, Y=H)



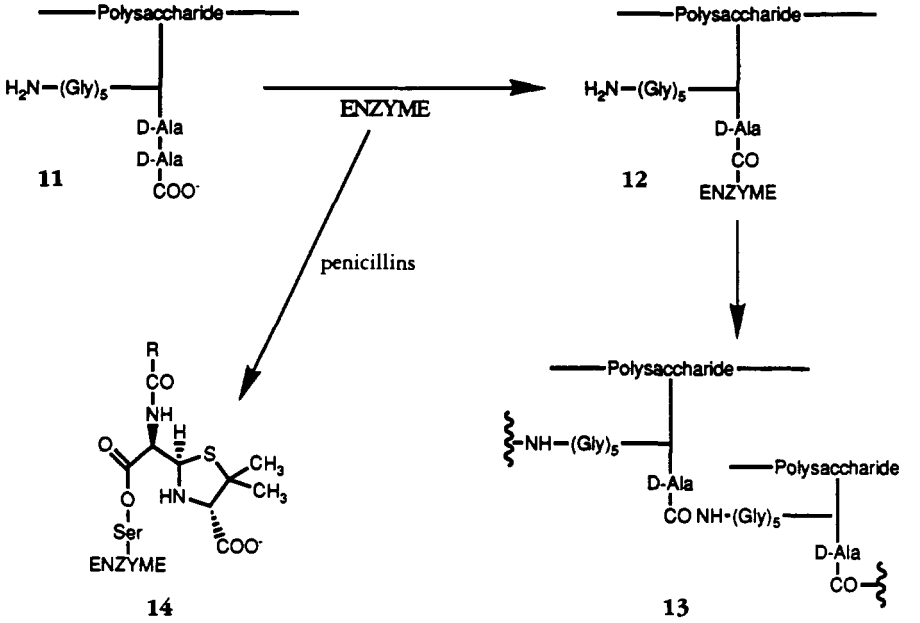
The property of intercalation, the binding of a chromophore to DNA by being "sandwiched" between two base pairs, has received much attention in recent years as a starting point for the understanding and design of anticancer drugs. Particularly interesting examples are the quinoxaline antitumor antibiotics which comprise not one but two quinoxaline chromophores joined by a cross-linked cyclic octadepsipeptide (37). The two best-studied examples of these are echinomycin [**8**] and triostin A [**9**]. These antibiotics are believed to function by bis-intercalation into a DNA double helix. The antibiotics form a staple-like shape in which the aromatic chromophores are parallel and positioned 10–11 Å apart, the distance required to span two DNA base pairs (38); this binding stabilizes the double helix. Foot-printing studies have utilized the fact that base pairs bound to quinoxaline antibiotics have reduced susceptibility to DNA cleavage. Such studies (39) have revealed a strong preference of these compounds for G-C base pairs, which is supported by crystal structures of echinomycin and triostin A complexes with the self-complementary hexanucleotide d(CGTACG) (40). According to these crystal structures, the drug-DNA complex is stabilized not only by chromophore-base pair stacking and hydrogen bonding between a guanine base and the peptide backbone, but more significantly by approximately twenty van der Waals interactions! Such a close fit points strongly to the idea that echinomycin and triostin A have evolved specifically because of their selective binding to CpG dinucleotide sequences in DNA double helices.

Another group of natural product antibiotics is that which inhibits bacterial cell wall synthesis in Gram-positive bacteria. If an organism is competing with a Gram-positive bacterium, then it would be to that organism's advantage to kill the bacterium. One conceivable way to do this is by preventing the synthesis of the bacterial cell wall, thus causing lysis of the bacterium, and several important groups of antibiotics function by doing just this. For example, the target of both the penicillins and the vancomycin group of antibiotics is the final cross-linking step in Gram-positive bacterial cell wall biosynthesis. However, their mechanisms of action are quite different.

The penicillins [**10**] contain a β-lactam ring to which a variable group R is attached via a peptide bond (see Scheme 2) (41). They function by inhibiting the transpeptidase enzyme that catalyzes cross-linking of peptidoglycan strands in Gram-positive bacterial cell walls. The cell wall of Gram-positive bacteria consists of a linear polysaccharide bearing tetrapeptide sidechains that are cross-linked to adjacent polysaccharide strands, e.g., in the case of *Staphylococcus aureus* via a pentaglycine bridge **13**. The final steps in the biosynthesis of this peptidoglycan involve displacement of a terminal D-alanine residue from the pentapeptide intermediate **11** and cross-linking of different strands. The latter process is catalyzed by a transpeptidase enzyme which forms an acyl-enzyme intermediate **12** by displacing the terminal residue. Subsequent reaction of this inter-



**10** penicillins




SCHEME 2

mediate with an *N*-terminal Gly residue on an adjacent polysaccharide strand results in the cross-linked peptidoglycan **13**. However, penicillin inhibits the transpeptidase enzyme by forming a covalent bond to a serine residue sidechain hydroxyl group at the enzyme active site **14** (42); consequently, cross-linking is prevented. As an extension of the original Strominger hypothesis [that pencillin is a -D-Ala-D-Ala mimic], it has been postulated that the effectiveness of transpeptidase inhibition by pencillins is due to the fact that penicillin is an analogue of the transition state in the enzymic cleavage of acyl-D-Ala-D-Ala (43).

A major problem in the clinical use of penicillins has been the fact that bacteria can develop resistance to these antibiotics. This resistance involves the secretion of a penicillase enzyme, which cleaves the β-lactam bond in penicillin, rendering the antibiotic ineffective (44). In an effort to overcome resistance, many synthetic or semisynthetic penicillins have been developed (45). Recently, the vancomycin group antibiotics have become important as an alternative approach.

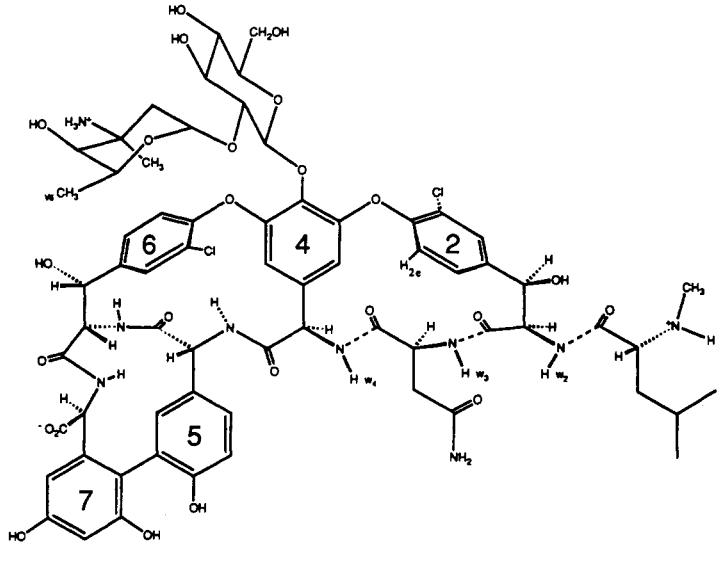
Vancomycin [**15**] is the most closely studied of a class of antibiotics which inhibit bacterial cell-wall biosynthesis by binding to the *C*-terminal peptide sequence -L-amino acid-D-Ala-D-Ala in the cell wall precursor **11** (46). Receptor/antibiotic binding studies have been carried out using the cell wall receptor analogues $Ac_2$-L-Lys-D-Ala-D-Ala or Ac-D-Ala-D-Ala. As the details of the interaction have been elucidated, it has become increasingly apparent that there is a remarkable complementarity between vancomycin and the cell wall peptide (47,48). The site of peptide binding in vancomy-

cin is a cleft which runs the length of the molecule along its peptide backbone. Lying at the bottom of this groove is a series of hydrogen-bonding sites that firmly anchor residues 1 and 2 of the cell-wall analogue to vancomycin (see **16**). There are also three distinct "pockets" in the antibiotic that determine the selectivity and strength with which it binds its target peptides.

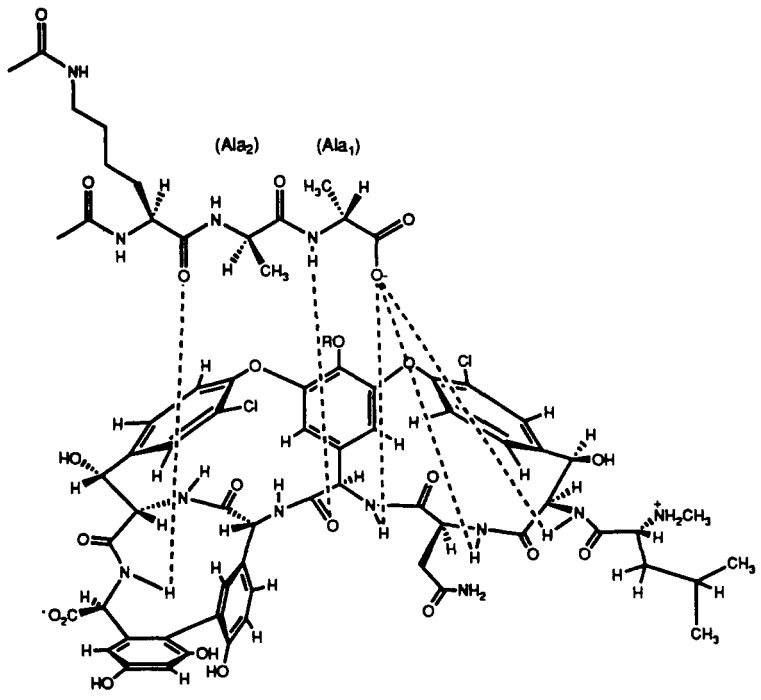
The first pocket binds the carboxylate anion of the target peptide; it consists of three amide NH's ($w_2$, $w_3$, and $w_4$) surrounded by hydrophobic groups. Instead of pointing in opposite directions, as would be expected for a normal β-strand, these three protons can easily point in roughly the same direction, enabling them to hydrogen-bond simultaneously to the peptide carboxylate. It has been proposed (49) that this unusual arrangement has been achieved by the selection of *R*, *R*, *S*, and *R* as the configurations of amino acids 1 to 4 (counting from the amino end) in vancomycin. Furthermore, the hydrophobic groups (rings 2 and 4, and the Leu and Asn side chains) that line the cavity serve to create a region of low local dielectric constant which strengthens the hydrogen bonds within it.

Vancomycin [**15**] is highly selective in its binding of peptides. It achieves this in part by having a pocket that will snugly fit the D-Ala methyl group of a *C*-terminal peptide ($Ala_1$ in **16**). This pocket is defined by proton 2e, ring 4, the chlorine on ring 6, and the $V_6$ Me group on the remote sugar. These atoms leave little space for any group larger than methyl, and it has been shown that the energy of binding for $Ac_2$-L-Lys-D-Ala-D-Leu compared to $Ac_2$-L-Lys-D-Ala-D-Ala is 3 kcal/mol lower (50), illustrating a remarkable selectivity. The binding site for the side chain of the second amino acid in the cell-wall analogue is less restrictive (and consequently less selective) but operates on the same principles (51,52). The remarkable complementarity between the cell wall analogue *N*-Ac-D-Ala-D-Ala and vancomycin is illustrated in Figure 2.

The vancomycin-group antibiotics and the penicillins are among the best understood of all clinically used antibiotics. Each group of compounds targets a specific reactant in the final step of peptidoglycan cell wall biosynthesis and gives rise to lysis of the target cell. We believe these are clear indications that the organisms that produce these antibiotics do so because of the selectional advantage over competing organisms which they thereby achieve.




**15** vancomycin




**16** vancomycin/$Ac_2$-L-Lys-D-Ala-D-Ala complex

A further group of natural products of known function consists of those that disrupt normal ion gradients. One such compound is an ionophore known as lasalocid A [**17**] (53). Ionophores function as antibiotics by binding to metal ions and transporting them across cell membranes. Lasalocid A itself has a complex array of stereochemistry along its backbone as well as a series of oxygens in appropriate positions to coordinate a metal ion. Still *et al.* (54) found that inversion of the stereochemistry of either or both of two different (starred) centers on the lasalocid A backbone produced a drop in the association constant for the modified compound and $Ba^{++}$ of more than two orders of magnitude relative to the natural compound. Furthermore, a computational study was performed that examined lasalocid A and every singly epimerized lasalocid derivative. This study predicted a structure for the natural compound that was nearly identical to an X-ray-derived structure of the Ba-lasalocid complex (53); however, no other epimer was judged by this protocol to be capable of complexing ions as well as lasalocid A. These results suggest that the natural stereochemistry of lasalocid A is essential for the ionophore to complex ions most efficiently. Because this stereochemistry is the result of a long series of enzyme-catalyzed reactions, it is unlikely that this genetically expensive natural product is only coincidentally made with just the right stereochemistry to perform this role, which we presume ultimately aids the producing organism in its competition with other species.

Another natural product that affects ion concentrations in cells is 1α,25-dihydroxycholecalciferol glycoside, which has been isolated from the leaves of *Solanum malacoxylon* Senoltner (55). This species was identified as the cause of "Enteque Seco", a wasting disease of grazing cattle in Argentina typified by abnormally high concentrations of calcium and potassium in the blood (56). 1α,25-Dihydroxycholecalciferol [**18**], the product of vitamin $D_3$ metabolism, is the hormone that controls the translocation of calcium through the intestine; it is essential in the correct dosage for normal growth. However, high levels of this hormone can in turn cause the increased levels of calcium observed

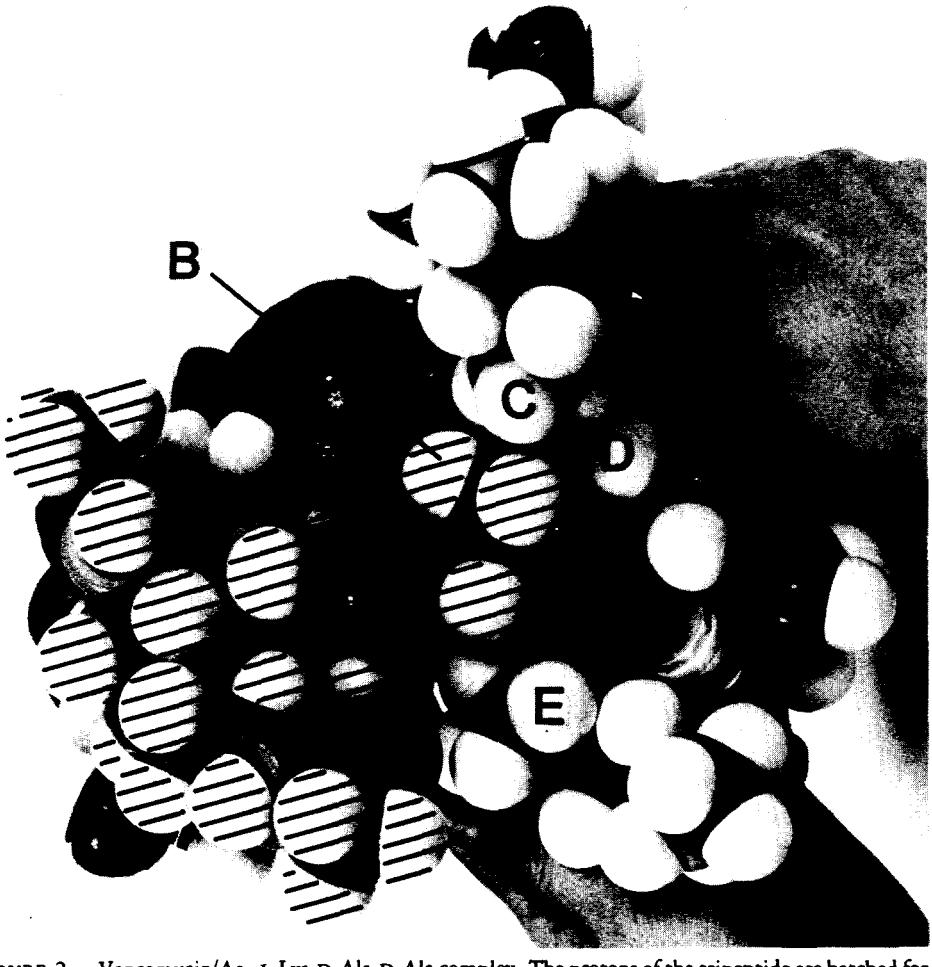
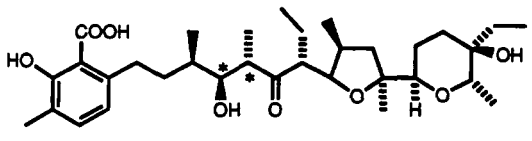


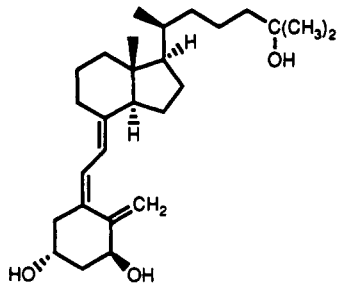

FIGURE 2. Vancomycin/$Ac_2$-L-Lys-D-Ala-D-Ala complex. The protons of the tripeptide are hatched for clarity. A, ring 6 Cl; B, C-terminal Ala methyl group; C, V6 methyl group; D, 2e proton; E, methyl group of Leu.

and the resulting drastic physiological effects in the cattle. We propose that *S. malacoxylon* has evolved the ability to produce 1α,25-dihydroxycholecalciferol as a defense mechanism against grazing animals. The genetic information required to produce 1α,25-dihydroxycholecalciferol could of course be "hijacked" from species to species, rather than having to evolve independently in each case.

Our penultimate example of physiologically active natural products is the phytoecdysones. The process of molting or "ecdysis" is essential for insect growth and is known to be controlled by the steroidal hormones known as ecdysones, the most important being α-ecdysone [**19**] and β-ecdysone [**20**] (57,58). Insects metabolize phytosterols available from plant sources to produce their essential hormonal steroids, vast quan-



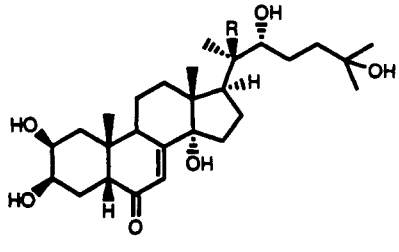

**17** lasalocid A



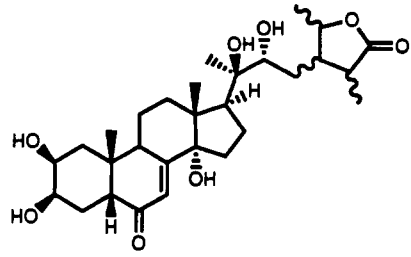
**18** 1α,25-dihydroxycholecalciferol

tities of which have been found in plants (59,60). More significantly, a large number of analogues of α- and β-ecdysone, classified as the phytoecdysones (e.g., cyasterone [**21**]) have also been isolated from plants, in particular ferns and gymnosperms (61). These have enormous hormonal activity compared to the parent compounds and, when administered to insects, cause major abnormalities in growth leading to sterility and death in a few cases (62,63). Some insects detoxify orally ingested ecdysone, but the structural variations in phytoecdysones offer some protection against rapid deactivation, thus rendering these compounds effective pesticides (64). We suggest that these complex steroids have evolved with minor structural modifications when compared to the requisite insect hormones to provide a defense mechanism for the producing organism. Indeed, ferns and gymnosperms are comparatively free of insect predation.

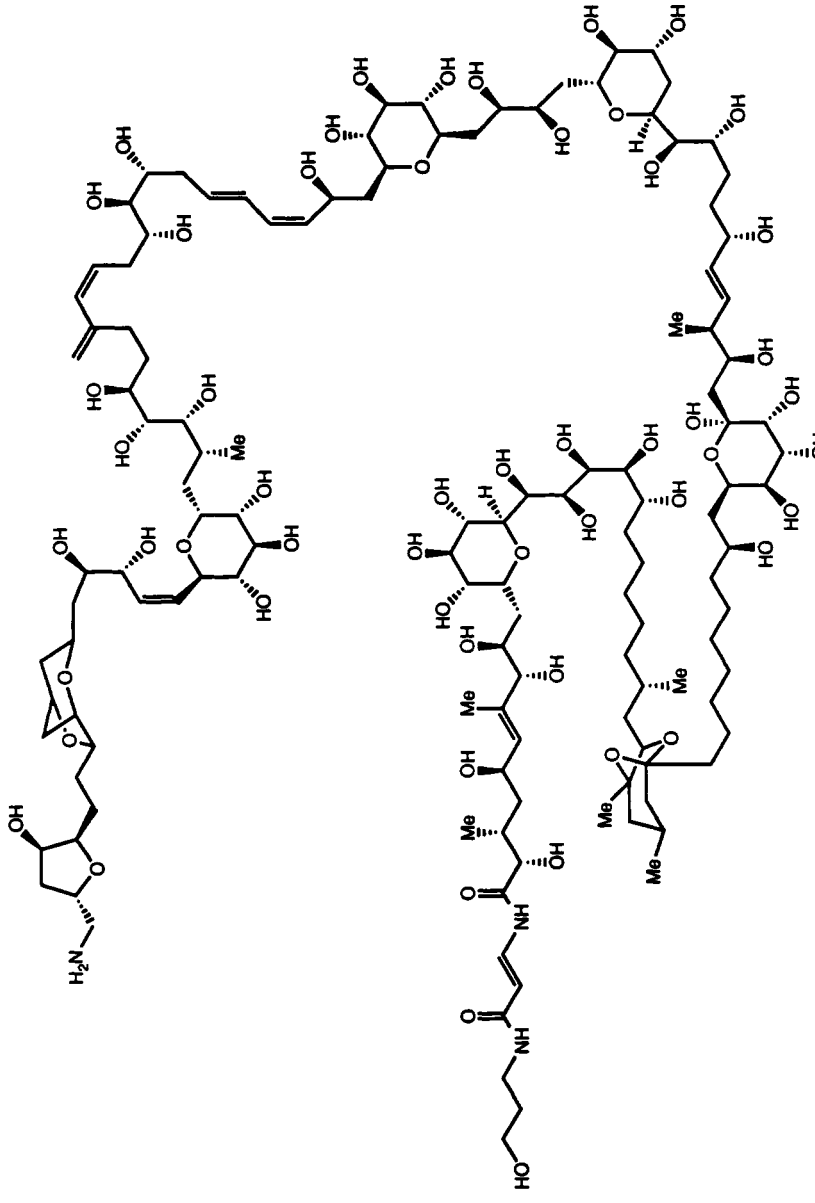
No discussion of physiologically active secondary metabolites would be complete without mention of the natural product toxins. Traditionally, these are considered separately from the antibiotics, because the toxicity is normally observed against complex eukaryotic organisms. These toxins clearly aid the survival of the producing organism (or of an organism that lives symbiotically with the producer) by virtue of their function: potential predators are deterred from eating the producer. Enormous numbers of such toxins are now known, and we refer only to a particularly famous one, palytoxin [**22**], produced by the marine organism *Palythoa tuberculosa* and related species (65). This material contains 64 asymmetric atoms, displays a remarkable toxicity (at concentrations as low as 0.025 μg/kg), and must be produced with a significant cost in metabolic energy. The question arises as to how the final structure was selected. The probability that such a complex molecule was produced by a series of neutral mutations and selected only at the level of the final structure is negligible. Such a hypothesis can be excluded with reasonable certainty. On the other hand, natural selection would be able to bring together several simpler precursors, which might have served earlier useful functions, to build up a newer and more complex functional entity such as palytoxin. Products of great complexity may well be constructed by the fusion of several simpler gene products which evolved earlier in time. Although such speculations are



**19** α-ecdysone (R=H)
**20** β-ecdysone (R=OH)



**21** cyasterone



**22** palytoxin

plausible, the exact routes by which natural products have evolved to their present, often highly complex, structures via the process of natural selection are unknown. This fact need not deter us in our view; it is equally unclear what may have been the functions and structures of the simpler precursors of today's proteins.

DISCUSSION AND CONSEQUENCES OF THE STRUCTURE-FUNCTION HYPOTHESIS.—We have discussed examples of several natural products where the structure-function hypothesis (i.e., that the function of a natural product is a direct consequence of its structure) is relatively easy to accept. Yet we propose that this structure-function hypothesis is equally applicable to plant alkaloids and terpenes, and that in most of these cases it is simply that the target organisms and receptors (either past or present) have not yet been identified (and admittedly in some cases may never be identified). Yet functional roles for many of these substances are already indicated. For example, Gilbert (66) has noted that alkaloids of the olivacine class are inhibitors of DNA synthesis by intercalation (67), and that co-secretion of $H_2O$-insoluble rotenones and $H_2O$-soluble saponins from *Derris* species may allow the dispersion in $H_2O$ and animal penetration of the former toxin (68). Additionally, he notes a variety of physiological functions of terpenes from Brazilian plants, including the ability to kill nematode larvae (69), which parasitize both plants and animals. Haslam (1) provides numerous examples of other striking physiological functions of natural products, which are of course legion in the modern literature. Among all these, we do not of course preclude physiological activities due to accidental fits to receptors with which the natural product did not interact during its evolutionary selection. However, a key feature of our proposal is that, even in these cases, the natural product exists because during the course of the evolution of its complex structure and complex biosynthesis, the current product—and any ancestral precursors—must have provided the producer with a selectional advantage by binding to a receptor in another organism. Thus, complexity of structure and biosynthetic pathway come about only by prolonged evolutionary pressure, but this consideration does not preclude the possibility of later selection due to entirely new interactions.

We emphasize that natural products are common in precisely those organisms (e.g., plants and microorganisms) that lack an immune system. Conversely, natural products are sparse in organisms which possess an immune system. Indeed, Gilbert (66) has earlier noted that in plants, fungi, and invertebrate animals, which lack the sophisticated immune system found in vertebrates, chemical defenses play an important role. Accordingly, we view many natural products as an evolutionary outcome to aid an organism's survival in the absence of an immune system. Although vertebrates in general lack the rich secondary metabolite chemistry of plants and microbes, there are a few notable exceptions. For example, frogs of Central and South America produce a wide variety of highly toxic alkaloids (70,71), and the South African clawed toad *Xenopus laevis* has recently been shown to produce peptide antibiotics, which appear to act by cleaving cell membranes (72,73).

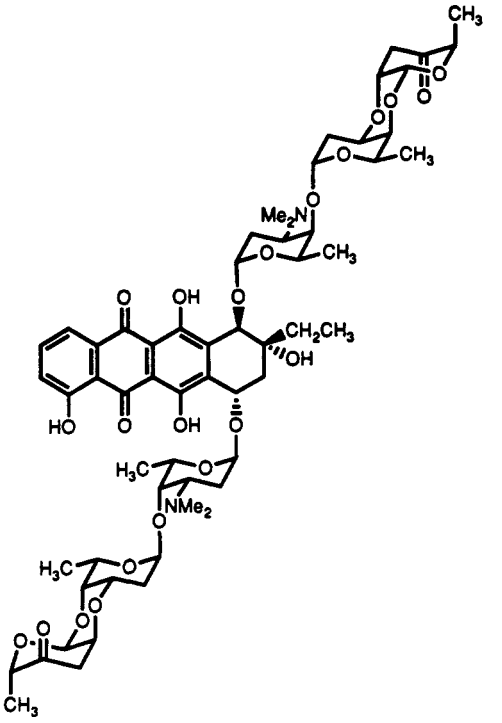
We have alluded earlier to the enormous structural variety of natural products. Possible reasons for this merit consideration. Structures and mechanisms that evolved early in the evolution of living organisms are frequently highly conserved; among structures, one can cite the histones, and among mechanisms, the genetic code. It appears likely that these structures and mechanisms were well defined and honed in distant and common ancestors. Conversely, great diversity of the genotype, and hence of the phenotype, is a product of later evolutionary history. Thus, enormous molecular and phenotypical variation can come about when organisms evolve in niches that are environmentally diverse. If these niches are isolated, then new species may evolve, and at

even later times when organisms become more mobile and/or the densities of organisms on the earth's surface increase, the now distinct species come into competition. According to the structure/function hypothesis, it would be at this time that natural products would evolve. Gottlieb (74) has reviewed the evidence that antibiotics may be produced by members of the soil microflora in their natural environment and function there in antagonistic capacities. The Darwinistic competition necessary to make this a plausible hypothesis appears likely to exist in the soil. Gottlieb cites the estimation of Brian (75) that one gram of surface soil (other than in extreme conditions) contains the following populations: bacteria ($10^6$–$10^8$), actinomycetes ($10^6$–$10^7$), protozoa ($10^5$–$10^6$), fungi ($5 \times 10^4$–$10^6$), and algae ($10^4$–$5 \times 10^4$). Moreover, antagonisms have been demonstrated to exist between members of the soil microflora (76). Despite this evidence, Gottlieb (74) concluded that the then available data (1976) "still do not allow us to accept the thesis that antibiotics are naturally produced in soil and function there in antagonistic capacities." In the light of the data now available on antagonist/receptor complementarity, we propose that the antibiotics described in this article have evolved to act in antagonistic capacities against neighboring organisms.

Thus, we propose that great natural product diversity would co-evolve with great species variety. In this sense, chlorophyll is quite clearly seen as a product of primary metabolism that is common to a wide variety of organisms, and it is indeed normally so regarded. If this line of reasoning is correct, then it follows that primary metabolism evolved before secondary metabolism. The variety of natural products then becomes a consequence of their relatively late evolution and is associated with competition among an enormous variety of species. In line with this evolutionary argument, Campbell (77) has chosen to define primary and secondary metabolites according to the extent of their distribution in nature.

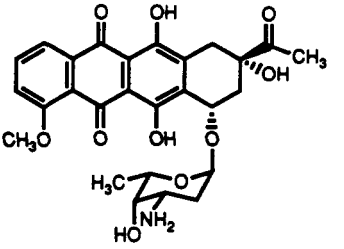
Reasons have been put forward as to why at least some molecules from microbes may have regulatory functions in higher vertebrates. It is suggested (78) that because vertebrate cells such as glands, neurones, and immune cells appeared relatively recently in evolution, some of the molecules through which these cells communicate (e.g., hormones and neuropeptides) may have first appeared in similar or identical form in unicellular or simple multicellular organisms. There is evidence to support this view in many cases (78,79). For example, male and female steroidal sex hormones are produced by the aquatic fungus *Achlya bisexualis* (80). Because numerous receptors of vertebrates and their corresponding ligands may have evolved from the corresponding receptors and ligands in microbes, it follows that the screening of microbial products may be a fruitful approach toward the discovery of new therapeutic agents for clinical use (79). The use of cyclosporin (a product of *Trichoderma polysporum*) (81) as an immunoregulatory agent (82,83), which is important in post-surgery management of organ transplants, supports this view, as does the discovery of the macrolide FK-506 (84), which is more active than cyclosporin in several of its immunoregulatory effects.

A scientific hypothesis should not only be consistent with the available evidence but ideally should be able to make successful predictions. One consequence of our proposal is that where the geometry of natural product receptors is known, a survey of natural products should indicate those which might bind to such a receptor. Unfortunately, extremely few receptor sites are currently known, not only because of the difficulty of locating such receptors but also of isolating them and determining their structures. However, there is a notable exception where the known receptor to produce a physiological response is double helical DNA (85). This receptor has a twofold axis of symmetry, and it is therefore likely that some natural products which recognize DNA will possess such twofold symmetry or at least approach it closely. Therefore, candidate structures can be sought on the basis of twofold symmetry, especially if this occurs in



**23** ditrisarubicin B

the presence of a chromophore which is a potential intercalator. Using this approach, we have identified ditrisarubicin B [**23**] (86) as a structure which should bind to DNA, not only by intercalation but also by binding of the symmetrically placed trisaccharide units into the minor groove of DNA. This mode of action is supported by the X-ray structures of two daunomycin [**24**]/hexanucleotide complexes (87,88), which involve intercalation of the aromatic chromophore while the amino sugar occupies the minor groove. Note that it is the *trans*-1,4-dihydroxy functionalization of the cyclohexene ring of **23** which has evolved (and not *cis*) in order to accommodate the requirement of a twofold axis. Subsequent to these conclusions based on ligand-receptor interactions, Hawley *et al.* (89) have hypothesized that sugars attached to such structures may bind into the minor groove of DNA, especially if these compounds possess twofold symmetry. An almost perfect twofold axis of symmetry is of course also found in actinomycin D [**7**].



**24** daunomycin

CONCLUSIONS.—We are surprised at the extent to which the view that natural products exert specific physiological effects by "accidentally" binding to receptors still holds sway in many quarters. Beliefs along these lines are cited at the beginning of this article, including the summary of Bu'Lock (4) that "it is the process of secondary biosynthesis which is seen as advantageous, and not, in the general case, its products, and in my opinion this is still the general type of explanation". Additionally, there is the view of Zähner *et al.* (90), who state that we should ". . . rid ourselves of the simplistic idea that antibiotics are formed as defense mechanisms, and recognize instead that antibiotics are nothing more than secondary metabolites which possess, more or less incidentally, an antibiotic effect . . . ." In the light of the evidence presented in this paper, we take the precisely contrary stance that, where sophisticated receptor/antibiotic complementarity exists, these antibiotics have evolved under the pressures of natural selection specifically to bind to these receptors. Moreover, because alternative hypotheses are without a comparable body of supporting evidence, we suggest that Occam's razor should be applied. We therefore propose that all natural products have evolved under the pressure of natural selection to bind to specific receptors. If our hypothesis is correct, then we should not be surprised to find many more natural product/receptor interactions which display the same sophistication as substrate/enzyme interactions.



## LITERATURE CITED

1. E. Haslam, *Nat. Prod. Rep.*, **3**, 217 (1986).
2. H.A. Krebs, *Bull. Johns Hopkins Hosp.*, **95**, 19 (1954).
3. E. Muller, in: "Secondary Metabolism and Co-evolution." Ed. by M. Luckner, K. Mothes, and L. Nover, Deutsche Akademie der Naturforscher Leopoldina, Halle/Salle, 1947, p. 123.
4. J.D. Bu'Lock, in: "The Biosynthesis of Mycotoxins." Ed. by P.S. Steyn, Academic Press, 1980, p. 14.
5. E. Mayr, "Towards a New Philosophy of Biology," The Belknap Press of Harvard University Press, Cambridge, MA, 1988, p. 43.
6. J.D. Bu'Lock and H.M. Powell, *Experientia*, **16**, 22 (1964).
7. H.B. Woodruff, *Symp. Soc. Gen. Microbiol.*, **16**, 22 (1966).
8. L.F. Povirk, in: "Molecular Aspects of Anti-Cancer Drug Action." Ed. by S. Neidle and M.J. Waring, Macmillan, London, 1983, Chapter 6.
9. K. Maeda, H. Kosaka, K. Yagishita, and H. Umezawa, *J. Antibiot., Ser. A*, **9**, 82 (1956).
10. T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Umezawa, H. Naganawa, and H. Umezawa, *J. Antibiot.*, **31**, 801 (1978).
11. H. Asakura, H. Umezawa, and M. Hori, *J. Antibiot.*, **31**, 1316 (1978).
12. H. Kasai, H. Naganawa, T. Takita, and H. Umezawa, *J. Antibiot.*, **31**, 156 (1978).
13. A.D. D'Andrea and W.A. Haseltine, *Proc. Natl. Acad. Sci. USA*, **75**, 3608 (1978).
14. M. Takeshita, A.P. Grollman, E. Ohtsubo, and H. Ohtsubo, *Proc. Natl. Acad. Sci. USA*, **75**, 5983 (1978).
15. M.D. Lee, T.S. Dunne, M.M. Siegel, C.C. Chang, G.O. Morton, and D.B. Borders, *J. Am. Chem. Soc.*, **109**, 3464 (1987).
16. M.D. Lee, T.S. Dunne, C.C. Chang, G.A. Ellestad, M.M. Siegel, G.O. Morton, W.J. McGahren, and D.B. Borders, *J. Am. Chem. Soc.*, **109**, 3466 (1987).
17. J. Golik, J. Clardy, G. Dubay, G. Groenwald, H. Kawaguchi, M. Konishi, B. Krishnan, H. Ohkuma, K. Saitoh, and T.W. Doyle, *J. Am. Chem. Soc.*, **109**, 3461 (1987).
18. J. Golik, G. Dubay, G. Groenwald, H. Kawaguchi, M. Konishi, B. Krishnan, H. Ohkuma, K. Saitoh, and T.W. Doyle, *J. Am. Chem. Soc.*, **109**, 3462 (1987).
19. N. Zein, A.M. Sinha, W.J. McGahren, and G.A. Ellestad, *Science*, **240**, 1198 (1988).
20. K.C. Nicolau, Y. Ogawa, G. Zuccarello, and H. Kataoka, *J. Am. Chem. Soc.*, **110**, 7247 (1988).
21. J.J. Hanka, A. Dietz, S.A. Gerpheide, S.L. Kuentzel, and D.G. Martin, *J. Antibiot.*, **31**, 1211 (1978).
22. D.G. Martin, L.H. Hanka, and G.L. Neil, in: "Proceedings." Sixty-ninth Annual Meeting of the American Association for Cancer Research, Waverly Press, Baltimore, 1978, Vol. 19, p. 99.
23. L.H. Hurley and D.R. Nedham-van Devanter, *Acc. Chem. Res.*, **19**, 230 (1986).

24. C.G. Chidester, W.C. Krueger, S.A. Mizsak, D.J. Duchamp, and D.G. Martin, *J. Am. Chem. Soc.*, **103**, 7629 (1981).
25. L.H. Hurley, V.L. Reynolds, D.H. Swenson, G.L. Petzold, and T.A. Scahill, *Science*, **226**, 843 (1984).
26. T. Hata, Y. Sano, R. Sugawara, A. Matsumae, K. Kanamori, T. Shima, and T. Hoshi, *J. Antibiot., Ser. A*, **9**, 141 (1956).
27. J.W. Lown, in: "Molecular Aspects of Anti-Cancer Drug Action." Ed. by S. Neidle and M.J. Waring, Macmillan, London, 1983, pp. 286–287.
28. V.N. Iyer and W. Szybalski, *Proc. Natl. Acad. Sci. USA*, **50**, 355 (1963).
29. H.W. Moore and R. Czerniak, *Med. Res. Rev.*, **1**, 249 (1981).
30. J.W. Lown, A. Begleiter, D. Johnston, and A.R. Morgan, *Can. J. Biochem.*, **54**, 110 (1976).
31. J.W. Lown, S.-K. Sim, and H.-H. Chen, *Can. J. Biochem*, **56**, 1042 (1978).
32. M. Tanaka and S. Yoshida, *Biochem. Pharmacol.*, **30**, 299 (1981).
33. W.A. Remers, "The Chemistry of Antitumor Antibiotics," Wiley, New York, 1979, Vol. 1, Chapter 1.
34. H.M. Sobell and S.C. Jain, *J. Mol. Biol.*, **68**, 21 (1972).
35. D.G. Reid, S.A. Salisbury, and D.H. Williams, *Biochemistry*, **22**, 1377 (1983).
36. E.V. Scott, G. Zon, L.G. Marzelli, and W.D. Wilson, *Biochemistry*, **27**, 7940 (1988).
37. L.P.G. Wakelin, *Med. Res. Rev.*, **6**, 275 (1986).
38. M.J. Waring and K.R. Fox, in: "Molecular Aspects of Anti-Cancer Drug Action." Ed. by S. Neidle and M.J. Waring, Macmillan, London, 1983, Chapter 5.
39. C.M.L. Low, H.R. Drew, and M.J. Waring, *Nucleic Acids Res.*, **12**, 4865 (1984).
40. G. Ughetto, A.H.-J. Wang, G.J. Quigley, G.A. van der Marel, J.H. van Boom, and A. Rich, *Nucleic Acids Res.*, 2305 (1985).
41. N.R. Trenner, *Anal. Chem.*, **22**, 405 (1950).
42. R.R. Yocum, D.J. Waxman, J.R. Rasmussen, and J.L. Strominger, *Proc. Natl. Acad. Sci. USA*, **76**, 2730 (1971).
43. B. Lee, *J. Mol. Biol.*, **61**, 463 (1971).
44. M.R. Pollock, *Proc. R. Soc. London, Ser. B*, **179**, 385 (1971).
45. P.G. Sammes, Ed., "Topics in Antibiotic Chemistry," Vol. 4, Wiley, New York, 1980.
46. H.R. Perkins, *Biochem. J.*, **111**, 195 (1969).
47. D.H. Williams and D.W.J. Butcher, *J. Am. Chem. Soc.*, **103**, 3697 (1981).
48. D.H. Williams, M.P. Williamson, D.W.J. Butcher, and S.J. Hammond, *J. Am. Chem. Soc.*, **105**, 1332 (1983).
49. D.H. Williams and J.P. Waltho, *Biochem. Pharmacol.*, **37**, 133 (1988).
50. M. Nieto and H.R. Perkins, *Biochem. J.*, **123**, 789 (1971).
51. M.P. Williamson, D.H. Williams, and S.J. Hammond, *Tetrahedron*, **40**, 569 (1984).
52. M.P. Williamson and D.H. Williams, *Eur. J. Biochem.*, **138**, 345 (1984).
53. C.C. Chang and I.C. Paul, *Science*, **196**, 1441 (1977).
54. W.C. Still, P. Hauck, and D. Kempf, *Tetrahedron Lett.*, **28**, 2817 (1987).
55. M.R. Haussler, R.H. Wasserman, T.A. McCain, M. Peterlik, K.M. Bursac, and M.R. Hughes, *Life Sci.*, **18**, 1049.
56. N.A. Worker and B.J. Carrillo, *Nature*, **215**, 72 (1967).
57. A. Butenandt and P. Karlson, *Z. Naturforsch.*, **96**, 389 (1954).
58. P. Karlson, *Vitam. Horm. (NY)*, **14**, 227 (1956).
59. K. Nakanishi, *BioScience*, **18**, 791 (1968).
60. T. Takemoto, S. Ogawa, N. Nishimoto, S. Arihari, and K. Blue, *Yakugaku Zasshi*, **87**, 1414 (1967).
61. K. Nakanishi, T. Goto, S. Ho., S. Natori, and S. Noze, "Natural Products Chemistry," Academic Press, New York, 1974, Vol. 1, p. 525.
62. M. Kobayashi, T. Takemoto, S. Ogawa and N. Nishimoto, *J. Insect Physiol.*, **13**, 1395 (1967).
63. M. Kobayashi, K. Nakanishi, and M. Koreeda, *Steroids*, **9**, 529 (1967).
64. W.E. Robbins, J.N. Kaplanis, M.J. Thompson, T.J. Shortino, C.F. Cohen, and S.C. Joyner, *Science*, **161**, 1158 (1968).
65. J.K. Cha, W.J. Christ, J.M. Finan, H. Fujioka, Y. Kishi, L.L. Klein, S.S. Ko, J. Leder, W.W. McWhorter Jr., K.-P. Pfaff, M. Yonaga, D. Uemura, and Y. Hirata, *J. Am. Chem. Soc.*, **104**, 7369 (1982).
66. B. Gilbert, *Pontif. Acad. Sci. Scr. Varia*, **10**, II(9), 225 (1977).
67. B. Festy, J. Poisson, and C. Paoletti, *FEBS Lett.*, **17**, 321 (1971).
68. M.C. do Nascimento, R.L. de Vasconcellos Dias, and W.B. Mors, *Phytochemistry*, **15**, 1553 (1976).

69. E.G. Goulart, M.J. Jourdan, B.G. Brazil, B. Gilbert, J.N. Callegari Lopes, S.J. Sarti, W. Vichnewski, and A.W. Thames, *Rev. Bras. Farm.*, **56**, 123 (1975).
70. C.W. Myers and J.W. Daly, *Sci. Am.*, February, 1983, p. 120.
71. J.S. Bainbridge, *Smithsonian*, January, 1989, p. 70.
72. M.G. Giovannini, L. Poulter, B.W. Gibson, and D.H. Williams, *Biochem. J.*, **243**, 113 (1987).
73. M. Zasloff, *Proc. Natl. Acad. Sci. USA*, **84**, 5449 (1987).
74. D. Gottlieb, *J. Antibiot.*, **29**, 987 (1976).
75. P.W. Brian, *Symp. Soc. Exp. Biol.*, No. 3, Growth, 358 (1979).
76. S.A. Waksman, "Microbial Antagonisms and Antibiotic Substances," The Commonwealth Fund, New York, 1945.
77. I.M. Campbell, *Adv. Microbiol. Physiol.*, **25**, 2 (1984).
78. J. Roth, D. LeRoith, E.S. Collier, A. Watkinson, and M.A. Lesniak, *Ann. N.Y. Acad. Sci.*, **463**, 1 (1986).
79. L.J. Nisbet and N. Porter, *Symp. Soc. Gen. Microbiol.*, **44**, 1 (1989).
80. T.C. McMorris, *Philos. Trans. R. Soc. London, B*, **248**, 459 (1978).
81. M. Dreyfuss, E. Harris, H. Hofmann, H. Kobel, W. Pache, and H. Tscherter, *Eur. J. Appl. Microbiol.*, **3**, 125 (1976).
82. J.F. Borel, C. Feurer, H.U. Gubler, and H. Stahelin, *Agents Actions*, **6**, 468 (1976).
83. D. Bunjes, C. Hardt, M. Rollinghof, and H. Wagner, *Eur. J. Immunol.*, **11**, 657 (1981).
84. T. Kino, H. Hatanaka, M. Hashimoto, M. Nishiyama, T. Goto, M. Okuhara, M. Kohsaka, H. Aoki, and H. Imanaka, *J. Antibiot.*, **40**, 1249 (1987).
85. J. Fisher and P.A. Aristoff, *Progr. Drug.. Res.*, **32**, 411 (1988).
86. S. Kunimoto, Y. Takahoshi, T. Uchida, T. Takeuchi, and H. Umezawa, *J. Antibiot.*, **41**, 655 (1988).
87. A.H.-J. Wang, G. Ughetto, G.J. Quigley, and A. Rich, *Biochemistry*, **26**, 1152 (1987).
88. M.H. Moore, W.N. Hunter, B. Langlois d'Estaintot, and O. Kennard, *J. Mol. Biol.*, **206**, 693 (1989).
89. R.C. Hawley, L.L. Kiesling, and S.L. Schreiber, *Proc. Natl. Acad. Sci. USA*, **86**, 1105 (1989).
90. H. Zähner, H. Drantz, and W. Weber, in: "Bioactive Microbial Products." Ed. by J.D. Bu'Lock, L. Nisbet, and D.J. Winstanley, Academic Press, New York, 1982, Vol. 1, p. 51.

*Received 2 August 1989*