Peptide Antibiotics

Do they represent only a few of many yet undiscovered microbial peptides?

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The dominant role played by peptide antibiotics in the early part of the antibiotic era (1928–1968) is often unappreciated by physicians, chemists, and microbiologists who have only comparatively recently become interested in antibiotics. Nearly all of the early antibiotics studied have turned out to be peptides: penicillin; gliotoxin; tyrothricin, tyrocidine, and gramicidin A; and actinomycin. Indeed, many of the techniques used in the study of antibiotics were in part developed in the course of examination of these peptide antibiotics: (i) isolation of antibiotic-producing microorganisms by the crowded-plate method; (ii) the tube dilution and agar-diffusion bioassays; (iii) the controlled experimental infections in mice; (iv) the submerged culture method for producing antibiotics; (v) the counter-current distribution method for analysis of antibiotic mixtures; (vi) filter paper chromatographic separation techniques; and (vii) methods of determination of peptide structure and synthesis.

Despite the continued search during the past 20 years for new antimicrobial agents, only a limited number of peptide antibiotics were found to be of practical value for use in the clinic for chemotherapeutic purposes and in agriculture as animal feed supplements; penicillin G (Fig. 1), cephalosporin C (Fig. 2), bacitracin A (Fig. 3), gramicidin A (Fig. 4), and polymyxin B (Fig. 5) are the most noteworthy among these.

Comparatively few structures of peptide antibiotics have been fully elucidated and even fewer proved by synthesis or other methods such as x-ray crystallography. Nevertheless, there is sufficient information available to form hypotheses concerning their biogenesis and the relation of chemical structure to physiological activity.

Among the common chemical features of peptide antibiotics first stressed by Abraham (*1*) are that, in contrast to proteins, most peptide antibiotics are cyclic in character and contain both D- and L-amino acid residues. A survey of the structures described recently was carried out in an attempt to find additional common features in order to clarify some fundamental questions. What are the mechanisms of biogenesis of peptide antibiotics? What, if any, are the roles of these peptides in the life processes of the producing cells? And what is the origin of the antimicrobial activity?

Newly Recognized Structural Features

In addition to the similarities mentioned by Abraham, other unusual characteristics could be found in many peptide antibiotics. Certain amino acids commonly found in proteins, for example, arginine, histidine, and methionine, apparently occur rarely in peptide antibiotics. Not only is the occurrence of D-amino acids quite general, but D- and L-isomers of the same amino acids occur in the same compound, often as next neighbors. Cyclic peptide antibiotics include both heterodetic (*2*) and homodetic (*3*) structure. Many peptide antibiotics contain proline, *N*-methylated amino acids, and imino acids, which probably preclude helix formation and hence facilitate cyclization. Finally, the formation of small cyclic areas within the macrocyclic structure of the peptide molecule is a common feature of several microbial peptides.

Examples for the last-mentioned feature are penicillin G (Fig. 1) and cephalosporin C (Fig. 2). These peptides are possibly formed as compact structures on a specific surface. Such compactness may facilitate cyclization, and, if the amino acid constituents or their side chains are held in proximity, addition reactions or dehydrogenation can lead to smaller rings. The cyclization of amino acid residues has already been considered as the origin of some of the heterocyclic constituents of peptide antibiotics; the thiazoline moiety of bacitracin is probably formed from an *N*-acylcysteinyl residue, and the thiazoles which occur in bottromycin, micrococcin P, and thiostrepton are probably dehydrogenation products of thiazolines. We have proposed the term "hypercyclization" for the formation of smaller rings within a larger cycle of the whole molecule (*4*).

Some of the more than 40 unusual amino acids and about 30 nonamino acid moieties found in peptide antibiotics are listed in Table 1. The presence of such components differentiates the peptide antibiotics from proteins.

Biosynthesis of Peptide Antibiotics

The main problems encountered in determining the mechanisms involved in the biosynthesis of the peptide antibiotics include (i) the mechanisms by which the amino acids are combined to form antibiotics; (ii) the formation and incorporation into the peptide chain of D-amino acid residues; and (iii) the formation of the macrocyclic systems. These problems have been widely studied, particularly with respect to the bacitracins, the gramicidins, the tyrocidines (including gramicidin S), the polymyxins and circulins, the nisins, the malformins, and mycobacillin. Although it is unlikely that the same enzyme systems are involved in the microorganisms producing these antibiotics, it seems reasonable to conclude that similar mechanisms are involved. Under these circumstances we feel justified in presenting a unified picture rather than treating the biosynthesis of each antibiotic as a separate entity.

Peptide Antibiotic Biosynthesis in Relation to Protein Synthesis

Many of the peptide antibiotics synthesized by bacteria are produced when the cultures have passed through the logarithmic growth phase and have begun to sporulate. In these fermenta-

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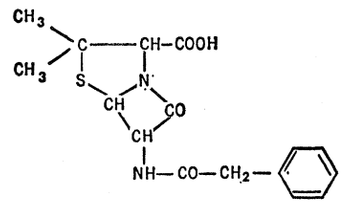

Fig. 1. Penicillin G.

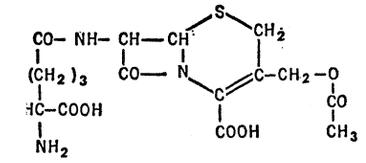

Fig. 2. Cephalosporin C.

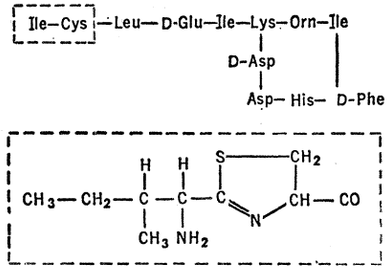

Fig. 3. Bacitracin A. The isoleucyl-cysteine sequence is present in the form of thiazoline.

tions, the antibiotic production phase is correlated with formation of sporangia and spores, and no antibiotic activity is found in nonsporulating cultures. All of the antibiotic activity produced by these bacteria (bacitracins, tyrocidins, polymyxins) is associated with the spores and cells, and none apparently is found "free" in the medium.

Although some preliminary data had suggested that, in the case of bacitracin, the antibiotic might be a component of the spore coat, more recent investigations have not confirmed this hypothesis for either bacitracin (*5*), mycobacillin (*6*), or polymyxin (*7*). We have no firm evidence that these or the other peptide antibiotics have a function in the metabolism of the antibiotic-producing microorganism.

The possible relation of antibiotic production to synthesis of cell protein has been studied by adding to antibiotic-forming cultures a variety of substances which inhibit cell protein synthesis, and then determining their effect on antibiotic production. In most cases antibiotic production was not affected when cell protein synthesis was inhibited; gramicidin S continued to accumulate in *Bacillus brevis* fermentations when growth and protein synthesis had been almost entirely inhibited by the addition of 5-fluorouracil, 5-bromouridine, puromycin, mitomycin C, or chloramphenicol to the antibiotic-producing cultures. The incorporation of constituent amino acids into actinomycins by *Streptomyces antibioticus* was increased from two- to threefold when incorporation of these amino acids into cell protein was essentially prevented by added chloramphenicol (*8*). Incorporation of threonine into polymyxin B was not affected by chloramphenicol or actinomycin D under conditions affecting incorporation of this amino acid into cell protein (*9*); and chloramphenicol, puromycin, and tetracycline did not affect bacitracin (*10*) or tyrocidine (*11*) synthesis in cultures producing these antibiotics.

Antimetabolites of the nucleic acid bases such as 6-azauracil, 5-bromouracil, 5-fluorouracil, 8-azaguanine, and 6-azathymine have been added to cultures producing polymyxin (*12*) and mycobacillin (*13*), and were found, at concentrations inhibiting the growth of the bacteria, to have no effect on antibiotic production. In one study addition of 8-azaguanine at higher than the minimum inhibitory concentrations for growth resulted in inhibition of polymyxin biosynthesis, but no 8-azaguanine was found in the nucleic acid of the bacteria. It was evident that polymyxin production is independent of the ribonucleic acids participating in protein synthesis.

Experiments with cell-free systems have shown that synthesis of gramicidin S occurs independently of protein synthesis in preparations from *Bacillus brevis* and related organisms. Several studies showed that the solution after removal of cell walls and structural units by centrifugation synthesized gramicidin S from the five-constituent amino acids [when supplemented with an energy source, for example, phosphoenolpyruvate + pyruvate kinase (*14*)]. These results ruled out any major role for ribosomes or messenger RNA in the gramicidin S synthesis. The isolation of a messenger RNA which contradicted these findings (*15*) has recently been revised (*16*), and now all investigators confirm synthesis of gramicidin S by a soluble enzyme system.

In similar experiments with the edeine-forming organism (*17*), cellular fractions (obtained by ammonium sulfate precipitation of the sonicates) formed the antibiotic when supplemented with pyridoxal phosphate, spermidine, diaminopropionic acid, glycine, and tyrosine (all constituents of the antibiotic), and the edeine syn-

Table 1. Some unusual components of peptide antibiotics.*

Amino acids and related compounds
D-α-Aminoadipic acid
L-α-Amino-β-phenylbutyric acid
α,β-Diaminobutyric acid
Dehydrobutyrine
δ-Amino-valeric acid
2-Aminohexenoic acid
6-Diazo-5-oxo-aminohexanoic acid
L-α,β-Diaminopropionic acid
N,N'-Dimethyl-L-cysteine
N-Hydroxy-L-ornithine
Allo-D-hydroxyproline
cis-3-Hydroxy-L-proline
trans-3-Hydroxy-L-proline
Allo-D-isoleucine
N-Methyl-L-isoleucine
L-Lanthionine
β-Methyl-L-lanthionine
L-β-Lysine
N-Methyl-L-alanine
β-Alanine
L-β-(2-Thiazolyl)-β-alanine
Dehydroalanine
L-Methyl-4-aminopyrrole-2-carboxylic acid
L-β-Methyl aspartic acid
N-Methyl-L-leucine
β-*N*-Methyl-L-leucine
L-β-Hydroxyleucine
N-Methyl-L-phenylalanine
N-Methyl-L-*p*-dimethylaminophenylalanine
3-Nitro-4-hydroxy-L-proline
N-Methyl-L-valine
L-Norvaline
L-γ-Formylmethylnorvaline
D-Penicillamine (β-mercapto-D-valine)
D-α-Pipecolic acid
4-Ketopipecolic acid
Sarcosine
L-α-Phenylsarcosine
L-Phenylglycine
α-(2-Iminohexahydro-4-pyrimidyl)-glycine
Allo-D-threonine
β-Methyl-L-tryptophan
Amines
1-Amino-5-hydroxyaminopentane
Cadaverine
Ethanolamine
Spermidine
Sugars
D-Glucosamine
Glucose
Xylose
Acids (fatty acids, hydroxy acids, heterocyclic carboxylic acids)
6-Diazo-5-oxo-hexanoic acid
Formic acid
α-*n*-Hexyl-β,γ-dihydroxylpentanoic acid
3-Hydroxydecanoic acid
D-α-Hydroxyisovaleric acid
3-Hydroxypicolinic acid
β-Hydroxytridecanoic acid
Lactic acid
6-Methylheptanoic acid
(+)-6-Methyloctanoic acid
β-Methyltetradecanoic acid
(+)-12-Methyltetradecanoic acid
Propionic acid
2-Propionylthiazole-4-carboxylic acid
Quinoxaline-2-carboxylic acid
Succinic acid

* For the antibiotics in which the unusual components occur, compare reference *52*.

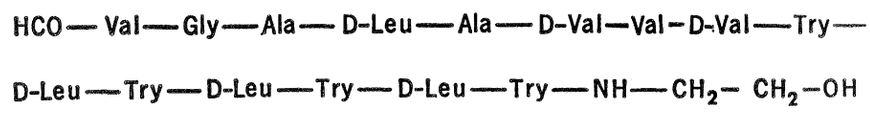

Fig. 4. Gramicidin A.

thesis with these enzyme preparations was not inhibited by either chloramphenicol or chlortetraycycline.

This evidence, together with the difficulty of adaptation of the generally accepted mechanism of protein synthesis (universal triplet code, messenger RNA, and assembly on ribosomes), leads only to the conclusion that biosynthesis of peptide antibiotics must proceed by a different pathway.

In the absence of an elaborate system for coding, only comparatively small molecules can be formed. The possibility of polymerization can be excluded since without exception a fairly well-defined sequence of the building components is at hand. One should expect, therefore, that the molecular weights of peptide antibiotics should be in a limited range, and an inspection of known examples suggests that the molecular weight of 3000 might be the upper limit. Only two exceptions to this "rule" were found—nisin (*18*) and saramycetin (*19*)—and their reported molecular weights were open to question (*20*), and, indeed, revised figures (*21*) have confirmed our opinion concerning the molecular weights of microbial peptides.

The incorporation of amino acids at certain positions in the actinomycin and tyrocidine molecules seems to be less specific than incorporation in proteins. This may be the reason for the occurrence of families of peptide antibiotics. A summary of the biologically acceptable replacements, that is, replacements yielding biologically active products, is presented in Table 2.

In all of these replacements, a monoamino-monocarboxylic acid is replaced by a monoamino-monocarboxylic acid or an imino acid is replaced by an imino acid (including *N*-methylamino acids). Chemical synthesis of actinomycins (*22, 23*) as well as analogs of vernamycin B (*24*) and pristinamycin I (*25*) also shows that some variations are possible, but yet the requirements for antibiotic activity are rather narrow.

Origin of D-Amino Acid Residues

Among the D-amino acids found on hydrolysis of peptide antibiotics are D-alanine, D-aspartic acid, D-α-aminobutyric acid, D-α-γ-diaminobutyric acid, D-glutamic acid, D-alloisoleucine, D-cysteine, D-leucine, D-ornithine, D-phenylalanine, D-proline, D-serine, D-allothreonine, D-tryptophan, and D-valine.

From experiments on the biosynthesis of bacitracin, actinomycins, valinomycin, tyrocidins, penicillin, and gramicidins, it is evident that the D-amino acid residues in these peptide antibiotics do not arise from D-amino acids, since addition of labeled D-amino acids to antibiotic-producing cells did not result in incorporation of any of the D-amino acid added into the antibiotic. Amino acid racemases have been implicated in the cell-free formation of gramicidin S (*14*), and probably will be found in the systems producing other peptides containing D- and L-isomers. In one study, no activation of the D-amino acids was noted, although the enzyme containing extracts of the actinomycin-producing streptomycete did activate the L-amino acids (*26*). Actually, inhibition of antibiotic synthesis by the D-isomer of an amino acid which is found in the molecule as a D-residue often has been noted (*27*).

During the examination of the configuration of the amino acids in stendomycin (Fig. 6) (*28*), the observation was made that the two threonines and two isoleucines found in this antibiotic have D-configuration and all four belong to the allo series. This suggests that epimerization on the α carbon of these amino acids, possibly inversion in several steps, led to the conversion of an L-amino acid to a D-acid. Since the configuration at the β-carbon atom was not involved, not an antipod (D-isoleucine or D-threonine) was formed but a diastereoisomer (D-alloisoleucine and D-allothreonine).

A survey of amino acid compositions of microbial peptides (Table 3) showed that, while there are many compounds which contain L-isoleucine or D-alloisoleucine, the two other isomers D-isoleucine and L-alloisoleucine do not occur in the peptides described so far. The configuration at the β-carbon atom serves as an internal marker for the detection of epimerization at the α-carbon atom. This "rule of α-epimerization" (*29*) could also be extended to threonine. Here, however, one exception could be found—L-allothreonine in telomycin (*30*). Although in the case of threonine, change of configuration at the β-center is more conceivable than with isoleucine, it is still more likely that the configuration of the threonines in telomycin (only tentatively assigned) needs to be revised.

The absence of D-isoleucine, L-alloisoleucine, D-threonine, and L-allothreonine from microbial peptides should be considered as clear indication for the origin of D-amino acids in these compounds. They all stem from L-amino acids.

Table 2. Amino acid substitutions in polypeptide antibiotics.

Antibiotic family	Amino acid in the major component	Replacement
Actinomycins	D-Valine	D-Alloisoleucine
	L-Proline	4-Hydroxy-L-proline
		4-Keto-L-proline
		Sarcosine
		Pipecolic acid
		Azetidine-2-carboxylic acid
Bacitracins	L-Valine	L-Isoleucine
Bottromycins	L-Proline	3-Methyl-L-proline
Gramicidin A	L-Leucine	L-Isoleucine
Ilamycins	*N*-Methyl-L-leucine	*N*-Methyl-L-γ-formylnorvaline
Polymyxins	D-Phenylalanine	D-Leucine
	L-Isoleucine	L-Leucine
Quinoxaline antibiotics	*N*-Methyl-L-valine	*N*-Methyl-L-isoleucine
Sporidesmolides	D-Valine	D-Alloisoleucine
Tyrocidine	L-Phenylalanine	L-Tryptophan
	D-Phenylalanine	D-Tryptophan
Vernamycin B	D-Alanine	D-Butyrine

Macrocyclic Structures

A macrocyclic structure is a common feature of polypeptide antibiotics. Gramicidin S, a cyclic decapeptide composed of two identical pentapeptide units (L-Val-L-Orn-L-Leu-D-Phe-L-Pro)

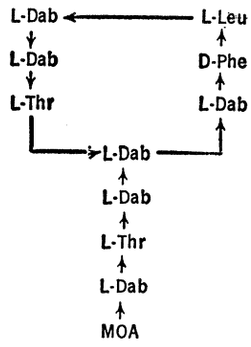

Fig. 5. Polymyxin B.

Table 3. Microbial peptides containing L-isoleucine and D-alloisoleucine.

L-Isoleucine	D-Alloisoleucine
Angolide	Actinomycin C
Bacitracins	Angolide
Destruxin B	Sporidesmolides
Gramicidin A	Peptidolipin N.A.
Polymyxins	Stendomycin
Polypeptin	
Subtilin	
Thiostrepton	

linked head to tail might be thought to be formed by enzymic linkage of two preformed chains (*31*). Schwyzer and Sieber (*32*) showed cyclodimerization of this type to occur in vitro. The structures of some of the peptide antibiotics are such that it seems that peptide synthesis is terminated by the cyclization of a chain via the COOH-terminal residue. In some cases there is an unusual grouping, for example, a fatty acid, on the NH_2-terminal end. As the mechanism of biosynthesis of these peptides differs from that of protein synthesis, the question arises whether the linking of their amino acids in specific sequence involves a multienzyme complex or a series of separate enzymes like those responsible for the biosynthesis of glutathione. There is a wide variety of complicated problems involved, including the pathways of incorporation of D-amino acid residues into the peptide chain, and many critical experiments are yet to be designed.

Importance of Spatial Architecture in Peptide Antibiotics

Although there is only limited evidence available on the mechanisms of action of the peptide antibiotics in inhibiting cellular processes, there is a growing realization that there is a positive relationship between antibiotic topology and the site of action. As more success is achieved in determining the site of action, we can logically expect the preparation of new semisynthetic autibiotics which will have improved properties as compared with the naturally occurring products.

One of the problems receiving the greatest attention has been the study of the mechanism of action of the penicillins. There is considerable evidence supporting the idea that the antibiotic activity is connected with the presence of *N*-acetylmuramic acid in the mucopeptide of bacterial and rickettsial cell walls. The antibiotic seems to act in sensitive strains by interfering with the function of an enzyme which normally handles some derivative of *N*-acetylmuramic acid as an intermediate during mucopeptide synthesis. To see whether *N*-acetylmuramic acid and penicillin showed any similarity in this respect, Collins and Richmond (*33*) constructed wire models of the two molecules. *N*-Acetylmuramic acid is not a rigid molecule, and the configuration considered was not the most stable, but one which could occur on the active site of the enzyme. Penicillin, in contrast, has a rigid configuration across both rings in the nucleus, and there is restricted rotation about the peptide bond; the amide linkage is rigid, but rotation is possible between carbon-9 and carbon-10 of the side chain. The binding of an *N*-acetylmuramic acid residue to an active center of an enzyme will depend primarily on ionic and hydrogen bonding, and the antibiotic could therefore bind with part of the same active center for the following reasons.

1) All but one of the hydrogen bonding groups in penicillin are exposed on one face of the molecule which closely resembles part of the face of the *N*-acetylmuramic acid molecule.

2) Both molecules are strongly acidic with pK_a values of 2.6 to 2.7.

3) The nitrogen atom in the thiazolidine and the oxygen atom in *N*-acetylmuramic acid occupy identical positions in the two molecules, and the nitrogen atom can form a hydrogen bond in a similar direction to one of the two hydrogen bonds that can be formed by the oxygen atom.

4) The carbonyl groups in penicillin and *N*-acetylmuramic acid arise in slightly different directions, but the two oxygen atoms occupy identical positions in the two molecules, and each can form a hydrogen bond in a common direction.

5) There are no large groups in penicillin which protrude beyond the face

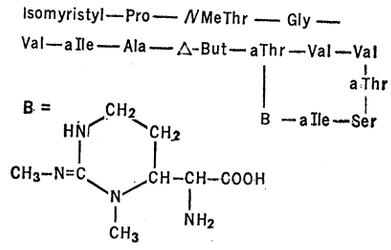

Fig. 6. Stendomycin.

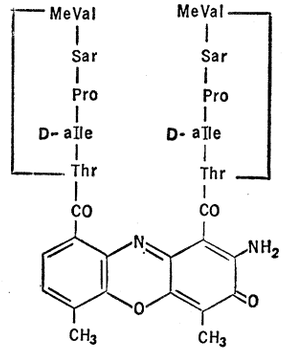

Fig. 7. Actinomycin C_3.

of the molecule and which could interfere with binding of the antibiotic at the active center. The side chain is curled back, and rotation about this position cannot bring the phenyl ring near the reacting face of the molecule.

Strominger *et al.* (*34*) have concluded from their laboratory studies that penicillin inhibits formation of peptide bonds in the D-alanyl-D-alanine portion of the muramic acid of the cell wall. In working with models of the dipeptide and the penicillin molecule they noted that the reactive CO-N bond in the β-lactam ring of penicillin and the CO-N bond in the peptide (D-alanyl-D-alanine) are in exactly the same position. Strominger postulated that the transpeptidase involved in peptide chain formation first reacts with the end of the chain (the D-alanyl-D-alanine end) to form an acyl-enzyme intermediate, with the elimination of D-alanine. Then the amino end of the glycine chain probably reacts with the activated intermediate to form the cross-bridge. If penicillin is an analog of the end of the chain, it would also react with the transpeptidase. The CO-N bond (the analog of the peptide bond in D-alanyl-D-alanine) would acylate the enzyme and thereby inactivate it. It seems evident that modification of penicillins by introducing different side chains does not affect the conformation of the thiazolidine ring structure and, consequently, the major advantage of such modifications comes

Table 4. Practical applications of peptide antibiotics.

Name	Antimicrobial spectrum	Some clinical uses	Other uses
Bacitracins	Gram+ bacteria	Topical preparations	Animal feed supplement
Cactinomycin	Antitumor Gram+ bacteria	Cancer therapy	
Capreomycins	Gram+ bacteria Mycobacteria	Tuberculosis	
Cephalosporins	Gram+ and gram— bacteria	Systemic infections	
Dactinomycin	Antitumor Gram+ bacteria	Cancer therapy	
Gramicidin	Gram+ bacteria	Topical preparations	
Gramicidin S (J)	Gram+ bacteria	Topical preparations (Japan)	
Mikamycins	Gram+ bacteria		Animal feed supplement (Japan)
Nisins	Some streptococci		Food preservative (England)
Penicillins	Gram+ bacteria	Systemic infections	
Polymyxins (including colistin)	Gram— bacteria	Urinary tract infections	
Pristinamycins	Gram+ bacteria	Systemic infections (France)	
Staphylomycins	Gram+ bacteria	Systemic infections (Europe)	
Stendomycins	Antifungal		Was used for plant pathogens
Thiostrepton	Gram+ bacteria		Bovine mastitis treatment
Tyrothricin	Gram+ and gram— bacteria	Topical preparations	

from protecting the β-lactam structure of penicillin from enzymatic attack in body fluids.

Actinomycin (Fig. 7) offers an excellent example for the studies of the relationship of structure to site of action. The chemistry of the actinomycins was examined by Brockmann (*35*) and by Todd, Johnson, and associates (summarized in *36*), and the structure proposed on the basis of chemical degradation included a phenoxazine chromophore joined to two pentapeptide rings. Bachmann and Müller (*37*), using x-ray diffraction techniques, concluded that a decapeptide-dilactone-chromophore configuration was unable to fulfill the requirements of the hexagonal (rhombohedral) space groups observed. Review of their data prompted M. F. Perutz (*38*) to conclude that actinomycin does appear to possess a pseudo-twofold axis of symmetry in the plane of the phenoxazine ring, and since actinomycin is a basically asymmetric molecule, the choice between the structure with two pentapeptide rings and that with the decapeptide-dilactone cannot be made from considerations of space group symmetry alone, but requires detailed x-ray analysis. Further studies by Palmer *et al.* (*39*) substantiated the idea that the pentapeptide model must have the two rings packed to either side of the chromophore rather than lying in the chromophore plane.

Although this somewhat inconclusive study seems to cast doubts on the validity of the pentapeptide ring-chromophore structure, the several elegant chemical syntheses of actinomycins by Brockmann *et al.* (*22*) provide convincing proof of the correctness of this structure.

While the x-ray experts have been trying to determine the shape of the molecule, the biochemists have not been inactive. Reich (*40*) found that the formation of complexes between actinomycin and DNA [first reported by Kirk (*41*)] is the result of a highly specific interaction which occurs in intact cells and under controlled experimental conditions in vitro. The functional groups of actinomycin which are indispensable for biological activity and for complex formation with DNA are the chromophore amino group, an unreduced quinoidal oxygen, and the intact, cyclic pentapeptide lactones. The requirement for the amino group and the quinoidal oxygen are shown by the disappearance of biological activity which accompanies modification of these structural elements.

Changes in the structure which result in loss of DNA binding power include (i) replacement of the two cyclic pentapeptides of actinomycin with a single neutral decapeptide, for example, gramicidin S (*23*); (ii) replacement of the COOH-terminal L-*N*-methylvaline of the cyclic pentapeptides with L-valine; (iii) conversion of the pentapeptide lactones to carboxylic acids (mono- or di-acids); and (iv) replacement of the pentapeptide sequences by related hexapeptide sequences (*23*). These results are consistent with the hypothesis that certain functional groups of the peptides interact in some way with specific counterparts on the surface of the DNA helix, and the stereochemically reactive configuration is determined by the structure of the peptide lactones.

A model of the structure of actinomycin-DNA complexes based on x-ray and model-building studies was proposed by Hamilton *et al.* (*42*). According to this model, actinomycin is considered to be located in the minor groove of helical DNA, with which it can form up to seven hydrogen bonds. The geometry of three of these hydrogen bonds has been studied in detail and was found to be stereochemically satisfactory. The properties of the complex deduced from this model fit most of the known facts concerning the reaction of actinomycin with DNA and associated inhibition of DNA-dependent RNA synthesis. (i) The model accounts for the role of the functional groups of actinomycin known to be required for biological activity; reduction of the quinoidal oxygen would restrict the ability of the oxygen atom to function as a hydrogen bond acceptor and alterations of the amino group would eliminate one or both of the hydrogen bonds formed. Also, the lactones can be visualized as stabilizing the peptide chains in a conformation permitting the formation of four additional hydrogen bonds between the four peptide-NH groups and the phosphodiester oxygens of the DNA strand which are opposite to the strand containing the guanine which interacts with the chromophore. (ii) The model accounts for the structures in DNA on which complex formation is known to depend; only guanine can furnish the hydrogen in the DNA minor groove for which the quinoidal oxygen of actinomycin can serve as acceptor (provided the DNA is in the β conformation).

Even if the model accurately reflects some of the structural properties of the

actinomycin-DNA complex, the proposed interaction between deoxyguanosine in DNA and the actinomycin chromophore could not account for the binding energy corresponding to the observed dissociation constant of the complex. Thus it is probable that the interaction of actinomycin peptides with DNA makes a major contribution to the stability of the complex (*43*).

A somewhat different approach to the topological problem was taken by Shemyakin *et al.* who prepared the topological analogs of enniatin B (*44*) and gramicidin S (*45*)—that is, all L-amino acids replaced by D-isomers and vice versa. As both of the new compounds were as antibiotically active as the natural compounds, it is obvious that the shape of the molecule is more important than its amino acid composition.

The experimental studies with actinomycin, enniatin B, and gramicidin S, and to a lesser extent those with penicillin, emphasize the importance of knowing the topology of the molecule both in order to better understand the mechanism of action and to design chemically modified molecules which will have enhanced usefulness.

Practical Application of Peptide Antibiotics

Although some 225 peptide antibiotics have been described in the scientific and patent literature, only those listed in Table 4 have been sufficiently useful in treatment of infections or in agriculture to be manufactured and distributed on a commercial basis. All of these peptide antibiotics are potent antimicrobial agents and in some instances (the penicillins) are the therapeutic agent of choice for specific infections.

With the exception of the penicillins, cephalosporins, and thiostrepton, the commercially available peptide antibiotics are mixtures of closely related substances rather than single entities. However, as shown by biological studies carried out on individual members of any group, pure compounds do not show any advantages over the use of mixtures, at least insofar as efficacy, lowered incidence of side effects, or therapeutic elegance are concerned.

Relation of Peptide Antibiotics to Other Microbial Peptides

In our discussions so far mainly peptide antibiotics were mentioned. However, all the structural features commonly found in these compounds, such as cyclic nature, occurrence of D-amino acids, hypercyclization, and so forth, can be recognized in other microbial peptides as well. A number of microbial peptides with no apparent antibiotic activity are known, for example, the toxic principles of fungi like phalloidin (Fig. 8) from *Amanita phalloides* or ergotamine (Fig. 9), one of the numerous alkaloids found in ergot. It seems obvious that both antibiotic peptides and those without antibacterial activities were isolated because of biological properties, and the isolation procedure itself is always based on such properties. Probably a vast number of additional peptides are produced by microorganisms while only the few conspicuous ones are known. Some supporting evidence for this contention can be found in studies on the biogenesis of gramicidin S (*46*). Several smaller peptides with amino acid sequences related to those in the antibiotic were isolated from the cultures producing gramicidin S. It is very likely that a systematic search for peptides present in fermentation liquors or in microbial cells will yield a substantial number of new microbial peptides. If this assumption is correct, then the peptide antibiotics must be regarded simply as a class of microbial peptides, a class artificially separated on the basis of the principle followed during isolation.

While from a practical point of view the antibacterial activity and the mechanism of action of peptide antibiotics deserve considerable attention, in a more general perspective this activity is probably only incidental and rather trivial. In a way we "made" these peptide antibiotics, since they were isolated on the basis of antibiotic activity. In the same way one could "isolate" chemotherapeutic agents from any large collection of chemicals.

The true role of the peptide antibiotics in the life processes of the producing cell is unknown. The speculation that they are agents of a chemical warfare between different strains is rather farfetched and completely unproven. The fact that the majority of these compounds are cyclic shows a similarity to functional proteins, such as the enzymes or proteins that play a role in the transfer of oxygen, and so forth. A well-defined architecture is suggested by some of their physical properties such as aggregation (*47*), complex formation with metals (*48*), and even more by results from studies on their optical rotatory dispersion (*49*). This architecture serves as an indication of functionality, because the "holes" or "clefts" easily discernible on molecular models

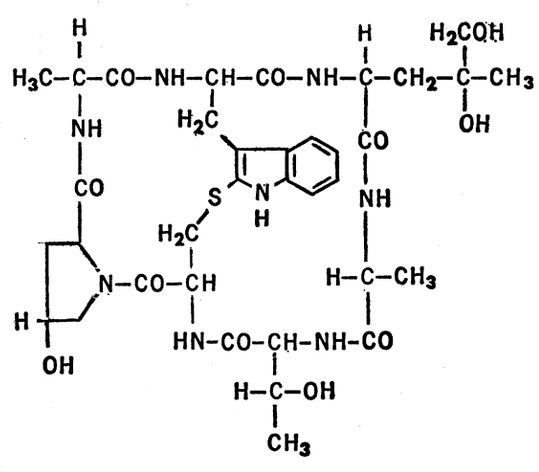

Fig. 8 (left). Phalloidin.

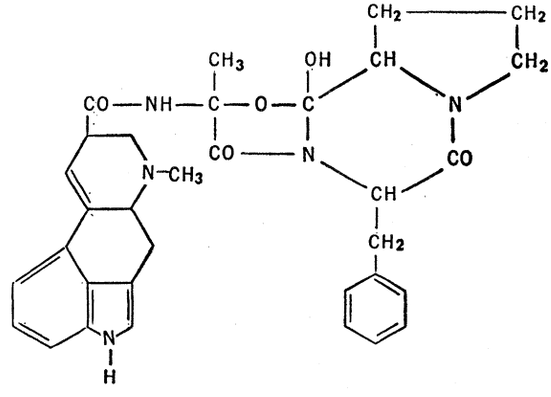

Fig. 9 (above). Ergotamine.

of microbial peptides could accept small "substrates," perhaps metal ions (*50*) or anions (*51*). The peptide envelope could help in the transportation of these ions through membranes, and so forth. Tentative and speculative as these concepts may be, one still cannot dispense entirely with the idea of functionality. It would be more difficult to assume that a large number of compounds with striking similarities could be formed in widely different organisms and that none of these compounds have any function.

If the functionality of microbial peptides is accepted, then it follows directly that they evolved at a period when the principle of L-amino acids had already been established, but the production of proteins (via nucleic acid coded synthesis on the ribosomes) had not been yet realized. The functions performed by microbial peptides could perhaps be taken over by the more elaborate proteins, in which case the microbial peptides are "fossils" (*52*) from an early stage in the evolution of life. The fact that proteins play a role in the biosynthesis of microbial peptides has not been overlooked. The structure of microbial peptides, while well defined, also shows some degree of freedom; they are between the statistically formed polymers and the entirely nonstatistical proteins.

A broad study of microbial peptides, both with and without antibacterial activity, should give us a better insight and a better understanding of the mysterious process of the emergence of life and its transformation to its presently known form.

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31. Abbreviations are: a, allo isomer of specified acid; Ala, alanine; Asp, aspartate; Δ-But, dehydrobutyrine; Cys, cysteine; Dab, diaminobutyric acid; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Moa, methyloctanoic acid; Orn, ornithine; Phe, phenylalanine; Pro, proline; Sar, sarcosine; Ser, serine; Thr, threonine; Try, tryptophan; Val, avline.
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