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# TETRAHEDRON REPORT

# CONFORMATIONAL STATES AND BIOLOGICAL ACTIVITY OF CYCLIC PEPTIDES

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## 1. INTRODUCTION

There is no dearth of literature on the structural,[1-6] synthetic (both artificial[2-7] and biological[2,8-11]) or functional[2,3,12] aspects of cyclopeptide chemistry. Cyclopeptides, well known for their biological importance as antibiotics, toxins, hormones, ion transport regulators, etc. are the objects of intensive research and thus always in need of periodic stock taking, which is one of the justifications for this review. Yet another and, perhaps, even more important reason is the very strong dependence of the physico-chemical and biological properties of these compounds on the spatial arrangement of their constituent atoms and whereas it is this aspect, namely the structure-function relation, which has received either only cursory consideration,[4-6,13] or has been confined to a very limited number of objects.[14] The authors have thus set themselves the task of a more comprehensive treatment of the structure-function relation of cyclic peptides and have at the same time included available data on new members of this class, even though they may as yet have been investigated only to a limited degree.

One of the difficulties in the approach to the cyclopeptide conformation (the knowledge of which is an obvious prerequisite to the understanding of their behaviour *in vitro* and *in vivo* and to the prediction on the properties of new representatives) is that, in spite of being more rigid than their linear counterparts, they still possess flexibility to the extent that their spatial structures are environment-dependent, such that in solution they exist as a complex equilibrium of interconverting forms. This necessitates the study of their three-dimensional structure (as of peptides in general) under the most varied conditions and in both solution and the crystalline state. Only by this means can one expect to obtain the key to the why's and wherefore's of their biological function. It is this which defines the scope and limitations of the present review, in which we consider present day methods for describing peptide conformation and also the techniques used in their study.

## 2. CONFORMATIONAL NOMENCLATURE OF PEPTIDES

The spatial structures of peptides may, obviously, be represented by the three-dimensional coordinates of their constituent atoms. This provides a precise description without recourse to any additional assumptions. At the the same time the regular structure of the peptide chain and certain stereochemical properties of the amino acid residues and the amide groups provide a simpler and more convenient way of describing such structures, namely by the angles about the bonds of the peptide chain.

In the early fifties, from analysis of available X-ray data, Pauling and Corey[15] arrived at the conclusion, that the amide bonds of peptides have a planar *cis*- or *trans*-configuration, and that bond angles and bond lengths in the backbone depend very little on its size or on the nature of the side chains. The parameters of the *trans*-amide bond were reported by Pauling and Corey in 1953 and those of the much less frequent *cis*-amide bond by Ramachandran and Venkatachalam[16] in 1968.

Subsequent investigations[17,18] have confirmed Pauling and Corey's conclusions, which served as the starting point for the rational conformational nomenclature of peptides.†[19]

Since in agreement with Pauling and Corey, bond angles and bond lengths in a "peptide unit"‡ are equal for all peptides, the conformation of a peptide backbone requires for its definition only three independent parameters; viz., the rotation angles $\phi$, $\psi$ and $\omega$ around the N–C–$^{\alpha}$, C$^{\alpha}$–C′– and C′–N– bonds, respectively. As zero points ($\phi = \psi = \omega = 0°$) for the angles, the angles of the fully extended form with the amide bond *trans*- and the C$^{\alpha}$–C′ and C$^{\alpha}$–N bonds *cis*- to the N–H and C′–O bonds (Fig. 1a) have been adopted. The rotations are measured in the clock-wise direction when looking from the N- to the C-terminus of the respective bond in the backbone. Similar designations were assumed for depsipeptides (Fig. 1b). In 1970 the I.U.P.A.C. commission proposed a new nomenclature[22] and it is this which is used in the present review. The latter differs from the former by a 180° rotation of the 0° point of all three angles $\phi$, $\psi$ and $\omega$ and by the angles being measured either clockwise (positive values from 0° to 180°) or counter clockwise (negative values from 0° to 180°). The new rotation angles are related to the old ones as follows: $(\phi, \psi, \omega)_{new} = (\phi, \psi, \omega)_{old} - 180°$.

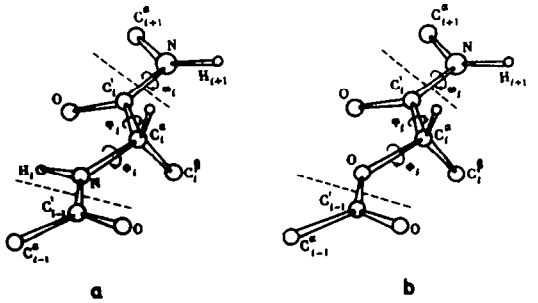


Fig. 1. Torsional angles in peptides (a) and depsipeptides (b).

†Deviations of amide groups from planarity, although not large, were recently proved to be of a general nature.[20-22]

‡The "peptide unit" is C$^{\alpha}$–C′(=O)–N(H)–C$^{\alpha}$; the "amino acid residue" is that in the chemical sense: –NH–C$^{\alpha}$HR–C′O–.

Side chain atoms are designated in alphabetical order by the indices $\alpha$, $\beta$, $\gamma$, $\delta$..., $C^{\alpha}-X^{\beta}$, $X^{\beta}-X^{\gamma}$, $X^{\gamma}-X^{\delta}$... (X = C, O or S) bonds—by numbers 1, 2, 3 and the rotation angles around these bonds—as $\chi_1$, $\chi_2$, $\chi_3$. As illustration Fig. 2 shows the Newman projections of amino acid side chain fragments for $\chi_1 = 0°$.

Important structural elements of peptides and depsipeptides are intramolecular H-bonds of the type NH···OC between the amide NH and the amide and ester carbonyls. A H-bond between the NH of an amino acid residue of sequence number $m$ and CO of a residue of the sequence number $n$ is designated as $m-n$ or $m \rightarrow n$. The resulting structures are shown in Fig. 3. Sites with $4 \rightarrow 1$ H- bonds frequently found in cyclopeptides are usually referred to as "$\beta$-bends", "$\beta$-turns", "$\beta$-loops" or "hairpin bends".

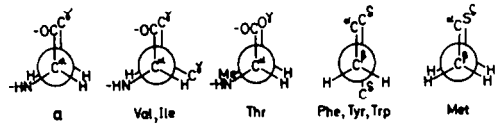



Fig. 2. Conformations of the amino acid side chains with $\chi_1 = 0°$ (a—fragment of the amino acid residue with no branching at $C^{\beta}$).

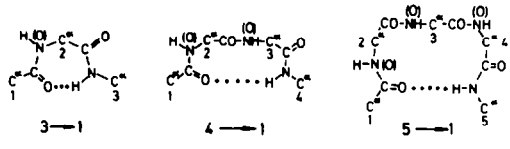



Fig. 3. Peptide (depsipeptide) fragments containing intramolecular H-bonds.

### 3. METHODS FOR INVESTIGATING THE CONFORMATIONAL STATES OF CYCLOPEPTIDES

There are two major ways of studying the three-dimensional structure of peptides. The first is X-ray analysis, which while yielding the most exact information about the three-dimensional structure in the crystal form leaves unanswered questions concerning conformational equilibria in solution and structural interconversion dynamics, and how these are related to the biological function. Such questions may be resolved by an alternate approach, first applied to depsipeptide antibiotics. This approach consists of the composite use of physicochemical methods—NMR, ORD, IR and UV spectroscopy, dipole moment measurements, etc. together with theoretical analysis. In this way, at the price of the exactitude of the X-ray method with respect to which the composite approach yet lags behind, many important factors influencing peptide conformation such as the nature of the solvent or the effect of temperature etc. can be investigated and, moreover, the comparative study of a large series of compounds may be performed. It goes without saying that the most reliable and complete information will be obtained when solution studies are coupled with X-ray analysis.

1. *X-ray analysis.* The X-ray method was the prevailing technique for studying the three-dimensional structure of peptides in the early stages of the problem.[17,18] We have mentioned its use in elucidating the geometrical parameters of the amide bond, the hydrogen NH···OC bond and the bond angles and bond lengths of the $C^{\alpha}$ atoms. This was followed by a long period of sporadic investigations in this field yielding on an average, only a single structure per year, mainly of derivatives of glycine-containing di- and tripeptides. The recent progress in the experimental and computational techniques of the X-ray method, especially direct ways for phase determinations, has been a strong impetus to the diffraction studies. Beginning in 1969, the crystalline structures of a variety of cyclopeptides, including such important biological compounds as actinomycin $C_1$ and valinomycin have been determined in this way.

2. *UV spectra, circular diochroism (CD) and optical rotatory dispersion (ORD).* As there is no observable correlation between the electronic spectra and spatial structure of peptides, this technique is not very informative for these compounds in the structural sense. Only when several amide chromophores with similar absorption peaks in the $\pi \rightarrow \pi^*$ region are in close proximity do they take part in the so-called exciton interaction resulting in a splitting of the absorption band and change in its intensity. Such effects are well known for polyamino acids in the $\alpha$-helical (hypochromicity) and $\beta$-conformations (hyperchromicity);[23] cyclopeptides have barely been touched.[24,25]

Difference UV spectroscopy, a well-known method in protein chemistry for investigating the solvent accessibility to protein aromatic groups, has not yet been applied to structural problems of cyclopeptides.

On the other hand, CD and ORD measurements provide a wealth of information on the conformational states of peptides because the resultant curves are a sensitive function of the relative orientation of chromophoric groups and their neighbours.

All optically active peptides display chiroptical effects at 180–205 nm and 210–240 nm, corresponding to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively. When the compounds in a stable coformation contain several spatially close peptide chromophores, the $\pi \rightarrow \pi^*$ transition band splits into two components of opposite sign.[26-32]

Several papers have discussed the possibility of the $n \rightarrow \sigma^*$ transition in the unshared electron pair of the carbonyl oxygen being reflected in the peptide ORD and CD curves.[31-37] A pertinant band has been found between the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ bands in the electronic spectra of amides in the gaseous phase.[38-40] However, there is no unequivocal evidence of any $n \rightarrow \sigma^*$ chiroptical effects in peptides. Clearly if they exist at all, they should only slightly contribute to the total CD or ORD spectra.

For a long time, the most favoured method of interpreting the CD and ORD spectra of peptides was by comparing them with the respective curves of polyamino acids in the $\alpha$-helical, $\beta$-, and so-called "random coil" conformations (see, e.g.[41]). This method, however, cannot be considered reliable, as the above three forms are by no means representative of the variety of conformational states peptides can assume, each state with its characteristic optical activity. Besides, the CD and ORD curves typical of $\alpha$-helices or $\beta$-structures may also be displayed by other conformations. A very good illustration of this is gramicidin S which while having no amino acid residues with $\alpha$-helical $\phi$ and $\psi$ parameters (see Section 6) exhibits CD and ORD curves almost identical with those for a $\alpha$-helix.[25,42-47]

Unfortunately, we do not have at our disposal direct methods for analysing CD and ORD data, that is methods for determining all the peptides conformational states that accord with the experimental CD and ORD curves. However, the reverse problem, namely, calculation of the optical activity of a peptide in a given conformation, has

now found an approximate solution.[28-30,48-50] In some cases it has even served as a criterion for the adequacy of a model chosen by other means.[51-55] Also noteworthy is the fact that the sign of the $n \rightarrow \pi^*$ effect for compounds with an isolated peptide chromophore obeys the quadrant rule.[56-58]

Peptides with Phe, Tyr, His or Trp residues as a rule give chiroptical effects arising from the induced asymmetry of their aromatic chromophores. Recently, attempts have been made to estimate the contribution by tyrosine chromophores to the chiroptical effect for varying $\phi$, $\psi$ and $\omega$ angles.[59,60]

Generally speaking, the CD and ORD curves have very limited value for the direct determination of three-dimensional peptide structures. They can, however, give unequivocal evidence as to the persistence or alteration of the conformational states or alteration of the environment, in other words they furnish information on the rigidity of a given peptide system. The CD and ORD techniques also can provide evidence of the conformational consequences of intentional or natural structural perturbations in a series of related compounds. The high sensitivity of the method, its simplicity, the possibility of carrying out the investigation under a variety of conditions and the ready availability of equipment has made the use of ORD and CD practically universal in conformational studies of peptides.

3. *IR and Raman spectroscopy.* Like CD and ORD, IR spectra of unknown peptides are usually compared with those for the canonical forms of poly-$\alpha$-amino acids. For example, the intensive bands at 1650 $cm^{-1}$ in the amide I region are believed to be characteristic of $\alpha$-helical fragments, the 1656 $cm^{-1}$ bands—of random sites, and the 1631 $cm^{-1}$ bands—of $\beta$-structure. Moreover, parallel $\beta$-structures are expected to have weaker bands at 1645 $cm^{-1}$ and antiparallel, at 1685 $cm^{-1}$.[61] Similar assignments although not so distinct have been proposed for the amide II–amide VII regions.[62,63] The conclusions made on this basis must be regarded as conjectures and cannot be referred to any particular site of the peptide chain.

IR spectroscopy is the only spectral method permitting reliable discrimination between *cis*- and *trans*-secondary amide bonds. The amide II frequencies of the two configurations differ by 100 $cm^{-1}$, no overlap in the absorptions taking place (1420–1460 $cm^{-1}$ for *cis* and 1480–1575 $cm^{-1}$ for *trans* amides[61-63]). Therefore the presence of 1550 $cm^{-1}$ bands is indicative of *trans*-peptide bonds, and their absence, of all *cis*-amide bonds. The amide II bands of *cis*-amides overlap the $CH_2$ scissoring bonds and cannot, therefore, be used for determining amide bond configurations in peptides.

The NH stretching frequency is very sensitive to participation of this group in H-bonding, whereby valuable information about the presence or absence of intramolecular H-bonds can be gleaned by studying progressively more dilute solutions of the cyclopeptides in non-proton-accepting solvents (such as heptane, $CCl_4$ or $CHCl_3$). Bands at 3420–3480 $cm^{-1}$ indicate the presence of free NH groups, and bands at 3300–3380 $cm^{-1}$ of H-bonded NH groups. Bands in the ~3400 $cm^{-1}$ region should be interpreted with precaution, as they may indicate the existence of both free and weakly H- bonded NH groups.† It should be mentioned that the integral intensity of the amide A bands can be used for estimating the relative amounts of the different types of NH groups in the molecule.[65]

Recently, theoretical computations have been made of the IR frequencies of peptides in relation to their spatial structure. Naturally, they concern only the simplest models.[66-68]

The development of laser Raman spectroscopy has stimulated its use as supplement to the IR spectral data.[69-73]. While there has as yet appeared no major work using laser Raman spectroscopy to explore the stereochemistry of peptides, no doubt this method should have great possibilities, particularly if one bears in mind that contrary to IR it may be used for aqueous solution.

4. *NMR spectroscopy.* This method, the development of which at present is in full swing, has provided numerous possibilities for the study of three-dimensional cyclopeptide structures. The general pattern of the spectrum, i.e. the number and intensity of signals gives information on the number of conformers participating in the equilibrium, their relative content and symmetry and the temperature dependence of the line shapes on the kinetic parameters of the conformational equilibrium. As the $C^{\alpha}$ protons are spatially close to the amide or ester groups situated on either side of the chain, their chemical shifts strongly depend on the orientation of the respective carbonyl groups, i.e. on the $\phi$ and $\psi$ angles. Aromatic groups with their characteristic magnetic anisotropy also can strongly affect the chemical shifts of closely situated protons.

The NH signals have proved to be highly sensitive to the peptide conformational states. When involved in intramolecular H- bonding these groups are blocked from the medium resulting in a low rate of hydrogen–deuterium exchange in labile deuterium containing solvents ($D_2O$, $CD_3OD$, $CD_3COOD$, etc.). On this basis the following approach has been developed for study of intramolecular H-bonding in peptides:

(i) Measurement of the temperature dependence of NH chemical shifts ($\delta$) in proton accepting solvents (for example, dimethyl sulphoxide or methanol). High $\Delta\delta/\Delta T$ values of $(6\text{–}12) \times 10^{-3}$ ppm/°C correspond to solvated NH groups whereas low values, $(0\text{–}2) \times 10^{-3}$ ppm/°C—to the NH groups involved in intramolecular H-bonding. Intermediate $\Delta\delta/\Delta T$ values should be interpreted with caution as this parameter depends not only on the accessibility of the NH group to the solvent, but also on the temperature stability of the H-bonds and on the orientation of neighbouring magnetically anisotropic side chains which may change on heating. Moreover, intermediate $\Delta\delta/\Delta T$ values may be indicative of a conformational equilibrium of several forms differing in the number and/or position of the H-bonds.

(ii) Determination of the solvent dependence of the NH chemical shift. Weak changes in $\delta$ indicate the presence of stable intramolecular H-bonds.[74]

(iii) Determination of the NH → ND exchange rate from the rate of intensity decrease of the respective signals. Sharp differences in exchange rates (by at least one order of magnitude in the half-life ($\tau_{1/2}$) of the NH group) will normally allow immediate straightforward assignment of free and H-bonded NH groups. For smaller differences additional information is required since the deuterium exchange rate depends not only on the presence of intramolecular H-bonds but also on other factors (steric shielding, the electronic structures of the various amide groups, etc.).

The peptide NH protons are spin–spin coupled to the

†For example, see. Ref. 64.

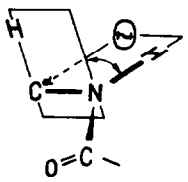


Fig. 4. Dihedral angle $\theta$ between the H–N–C$^\alpha$ and N–C$^\alpha$–H planes.

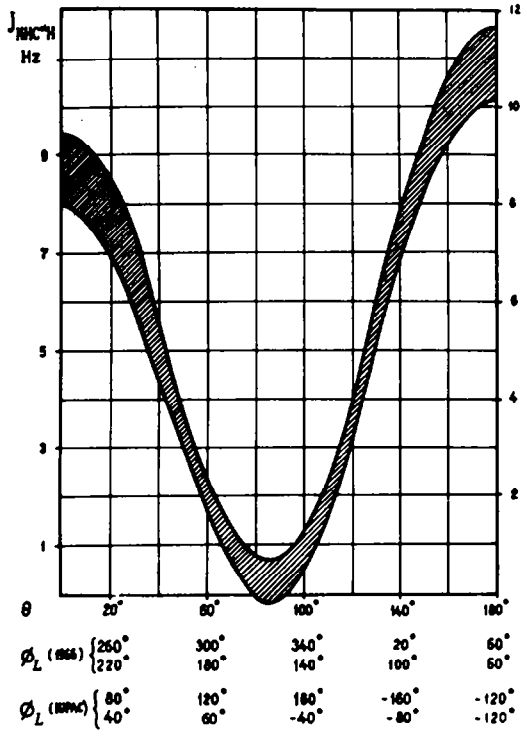



Fig. 5. Dependence of the vicinal coupling constant $^3J$(H–NC$^\alpha$–H) on the dihedral angles $\theta$ and $\phi$.

C$^\alpha$H protons, the value of the vicinal $^3J$(H–NC$^\alpha$–H) constant depending on the dihedral angle $\theta$ between the H–N–C$^\alpha$ and N–C$^\alpha$–H planes (Fig. 4). Several versions of the $^3J$(H–NC$^\alpha$–H) vs. $\theta'$ dependence have been suggested, the curve shown in Fig. 5 making the best fit with the experimental data.[75]† With the aid of the curve shown in Fig. 6, one may readily obtain the angular dependence of the total $^3J$(H–NC$\langle^{H}_{H}$) constant of the glycine residue (Fig. 6).[75] Thus, determination of the $^3J$(H–NC$^\alpha$–H) constants from NMR spectra permits one to confine possible $\theta$ values to a certain region. It should be noted that the most definite results are obtained for conformationally rigid structures, i.e. when the region of possible $\theta$ values may be found directly from the curves in Figs. 5 or 6. When several forms are in equilibrium, straightforward interpretation is possible only for $^3J$(H–NC$^\alpha$–H) > 9·5 Hz and < 3·0 Hz, values corresponding, to $\theta \sim 180°$ and $\theta \sim 90°$, respectively, whereas the region 3 Hz < $^3J$(H–NC$^\alpha$–H) < 9·5 Hz requires additional information. Judging from quantum-chemical calculations, the $^3J$(H–NC$^\alpha$–H) constant also depends on $\psi$, albeit much less strongly.[76]

From an analogous stereochemical dependence, one may determine the proton–proton orientations in C$^\alpha$H–C$^\beta$H fragments, to obtain the side chain conformations of the amino acid and hydroxy acid residues. In the case of a single proton at each atom, two of the three most probable rotamers about the C$^\alpha$–C$^\beta$ bond, (*gauche*-rotamers *a* and *b* in Fig. 7) correspond to $^3J$(H–C$^\alpha$C$^\beta$–H) ~ 3 Hz, whereas the third (*trans*-rotamer *c*) corresponds to $^3J$(H–C$^\alpha$C$^\beta$–H) ~ 12 Hz.[77] The analysis of the C$^\alpha$H–C$^\beta$H signals is more complicated although here, too, one can make use of NMR spectroscopy for determining the C$^\alpha$–C$^\beta$ rotamer populations.‡

As $^{13}$C-NMR spectroscopy develops into a full-fledged technique for conformational analysis, its application to peptides has great possibilities. Although our knowledge of the dependence of the $^{13}$C spectral parameters (chemical shifts, relaxation times) on the spatial structure is still very limited, it is already beginning to make its contributions to the composite solution techniques. For instance, it is the only method discriminating between *cis*- and *trans*-X–Pro bonds (see below the data on antamanide, Section 7).

Apparently the most straightforward information about the spatial structure of a peptide from the NMR spectra can be obtained from the vicinal couplings of $^{13}$C or $^{15}$N, as well as $^1$H, which together yield all the torsional ($\phi$, $\psi$, $\omega$ and $\chi$) angles of a peptide fragment.[79–83,277,278] For example the

†And references quoted.

‡For example see Refs 78 and 79.

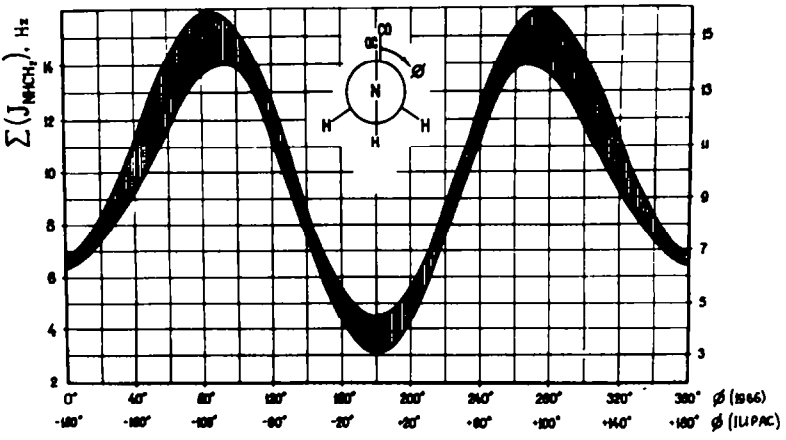



Fig. 6. Dependence of the total $^3J\left(\text{H–NC}^\alpha\langle^{H}_{H}\right)$ coupling constant on angle $\phi$.

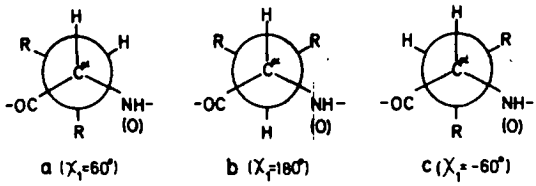



Fig. 7. $C^{\alpha}$-$C^{\beta}$ rotational isomers.

refinement of the valinomycin conformation in a non-polar solvent (see Section 4.1), has been based on the established correlation between the dihedral $\theta$ angle and the $^3J(^{13}C'-NC^{\alpha}-H)$ constant (Fig. 8).[83]

The major problem to be resolved before $^{15}N$ and $^{13}C$ spectroscopy can become routine techniques for peptide conformational studies is the development of synthetic methods for the selective enrichment of the peptide with the particular isotope.

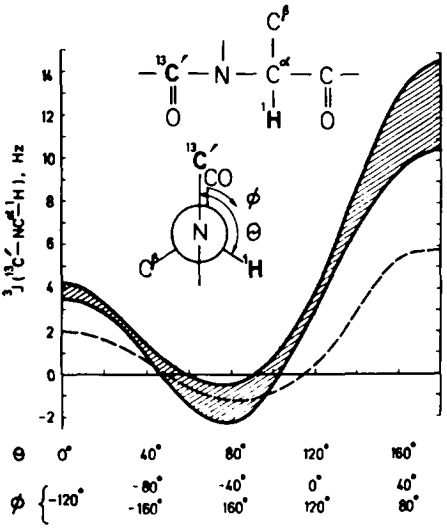



Fig. 8. Dependence of the $^3J(^{13}C-NC^{\alpha}-^1H)$ coupling constant on angles $\theta$ and $\phi$.

5. *Theoretical conformational analysis of peptides.* In order to visualize the three-dimensional structure of a peptide from experimental data, one usually requires a conformational analysis of its molecule. The starting point for such analysis is examination of molecular models, from which a rough idea can be formed of the structural types not at variance with the physico-chemical (mainly spectral) data. This is followed by an estimate of the interactions between the atoms and groups in the possible structures. Attempts to predict the oligopeptide structure from mere analysis of molecular models without recourse to experimental material are usually unsuccessful.

Computational methods have found wide application in spatial structural studies. The methods may be classified into two groups: *semi*-empirical[84-89] and quantum-chemical.[90] The two approaches usually give similar results, although, as a rule, the former provides better agreement with experiment.[91-93]

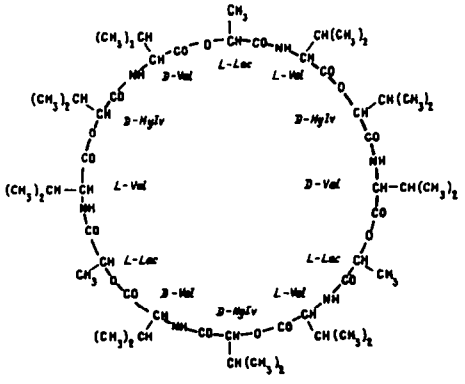
It is, certainly, very tempting to search for a means of determining the optimal conformations of oligopeptides based solely on their primary structure. However, even small oligopeptides may present such numbers of energetically similar conformers to choose from, that the radical solution of such a problem, i.e. determination of the "global" minimum of potential energy, is at present practically impossible. Another obstacle is that the error in estimating the energies of the various solution conformations by present day methods is at least of the same order of magnitude as the energy difference between the possible optimal forms. Finally, such calculations take into account only the enthalpy of a given conformation, usually neglecting the entropy factor.

Whatever the difficulties and shortcomings of the computational methods, a rational combination of the theoretical and experimental techniques will, on agreement of the two approaches, give greater confidence in the results obtained. In the search for preferable conformations among those derived from molecular models, one begins with the conformational maps; viz., two-dimensional diagrams describing the potential energy of the amino and hydroxy acid residues (-NX-CHR-CO- and -O-CHR-CO-) or the model compounds (such as Ac-NX-CHR-CONYMe) as function of the $\phi$ and $\psi$ angles, assuming bond lengths, bond angles and the angle $\omega$ (0° or 180°) as constant. The energy of the more complex peptide fragments or of the whole peptide is approximated as the sum of the respective fragment energies (obtained from the conformational maps). The resultant values can then be refined by minimizing the total energy of the system with respect to a number of variables such as all the $\phi$, $\psi$, $\omega$ and $\chi$ angles and the $C^{\alpha}$ bond angles. The dipole moments of the preferable cyclopeptide conformers are then computed and their values compared with the experimental, agreement between the experimental and theoretical results serving as criterion for the validity of the particular structure. Conclusions stemming from energy calculation of the entire molecule rather than of the constituent fragments are, of course more reliable. In general the procedure is cumbersome and requires the expenditure of much computer time; such investigation are therefore not numerous, but doubtless have considerable potentiality.

## 4. ANTIBIOTIC-IONOPHORES

A systematic treatment will now be given of the conformational states of biologically active cyclopeptides classified according to their biological function. First depsipeptides of the valinomycin, enniatin and monamycin groups will be considered, all of which bind metal ions (as a rule of the alkali or alkaline earth group) in solution or on membrane surfaces. The resultant lipophilic complexes can freely penetrate the lipid layers of biomembranes, effecting thereby passive cation transport (along the electrical field gradient or the cation concentration gradient) across the membrane. The wealth of accumulated material on these compounds has been discussed in a recent monograph[14] and in *Tetrahedron*.[94] We shall therefore limit ourselves here to a short discussion of the problem, paying special attention to new data and new trends.

1. *Valinomycin and its analogs.* Valinomycin is the most important of the antibiotic-ionophores. At rather low concentrations ($\leq 10^{-8}$ M) it selectively induces the potassium permeability of a wide variety of biological membranes; the ionophoric properties of valinomycin are apparently responsible for its bacteriostatic action. Valinomycin is known to form more or less stable equimolar complexes with $K^+$, $Rb^+$ and $Cs^+$ in neutral solvents, the stability of such complexes decreasing with increase in solvent polarity. The $K^+$ complex is $10^4$ – $10^3$ times more stable than the $Na^+$ complex, the K/Na




complexing selectivity of valinomycin being the highest among the known alkali metal complexones.[14,94,†]

Valinomycin with its 36-membered ring possesses a wealth of conformational possibilities and elucidation of its three-dimensional structure has required considerable effort. Despite that, it was the first peptide molecule of biological importance whose spatial structure was established without recourse to X-ray analysis, providing thereby a dramatic demonstration of the effectiveness of the composite use of a variety of physical chemical methods in structural work. The data obtained was also helpful in shedding light on the structure-function relation of valinomycin, which in turn has led to synthesis of certain of its membrane-affecting analogs with unique properties.

The spectral methods have revealed that in solution valinomycin is an equilibrium of three major conformers (A, B and C, Fig. 9).[94–103] Form A which is predominant in heptane, $CCl_4$ or $CHCl_3$ has all its NH groups engaged in intramolecular H-bonding with the amide carbonyls; form B, predominant in medium polar solvents, has three H-bonds with participation of the D-valyl NH groups; whereas form C, which is typical of the form in polar media, especially at elevated temperatures, has the NH groups H-bonded to the solvent. Relaxation studies by ultrasonic absorption techniques have revealed the existence of certain other forms, possessing five, four, two or one H-bonds[104,105,139] apparently intermediate between A, B and C.

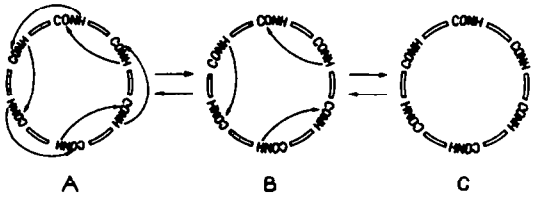



Fig. 9. A ⇄ B ⇄ C equilibrium of valinomycin (schematically).

In A the depsipeptide chain forms an H-bonded system of six condensed 10-membered cycles, in which each amide carbonyl is 4→1 H-bonded with the NH of the neighbouring (in the direction of acylation) amide group. Thus, in non-polar media the valinomycin molecule acquires a compact bracelet-like conformation with an internal diameter of ~8 Å and height of ~4°. In principle, form A has a dual way of chain folding ($A_1$ and $A_2$, Fig. 10)

†And references quoted.

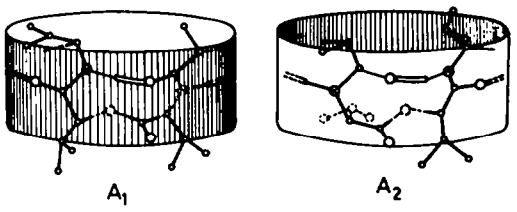



Fig. 10. Principal structure of $A_1$ and $A_2$ forms of valinomycin.

with opposite chiralities of the cyclic system and differently oriented side chains. Within each of these $A_1$ and $A_2$ forms, four conformations are possible each differing in orientation of the ester carbonyls. As can be seen in Fig. 10, if the L-lactic acid residues are located in the upper part of the molecule, acylation is clockwise in the $A_1$ conformation and counter clockwise in the $A_2$ conformation.

The $A_1$ structures have *cis* NH–CH protons ($\theta \approx 0$) whereas they are *gauche* ($\theta \sim 120°$) in $A_2$ (Figs. 4 and 5). In view of the high $^3J(H–NC^\alpha–H)$ values constants the first structural type of valinomycin was at first considered predominant in non-polar solvents.[94–103,107] However, very recent studies have shown $^3J(^{13}C–NC^\alpha–H)$ constants of valinomycin in $CH_3Cl$ to be small (<3·5 Hz),[106] from which it follows that, after all, the NH–CH protons must be *gauche* (see Fig. 8) and that in non-polar solvents valinomycin is preferably in the $A_2$ conformation. Here one of the H-bonds is weakened, the weakened site circulating about the ring with high frequency.[94] The accompanying distortion of the $C_3$ symmetry of the $A_2$ structure causes twisting of the NH–CH bonds which apparently explains the above mentioned high $^3J(H–NC^\alpha–H)$ values.

The triple H-bond-stabilized B form is more flexible than A (Fig. 11). In the former one can discern a hydrophobic "nucleus" of D-Val and L-Lac side chains about which are situated the depsipeptide chain and its polar groupings; the 10-membered H-bond-stabilized rings are on the molecular periphery lending form B the resemblance of a "propeller".

Form C, lacking intramolecular H-bonds, apparently has no fixed structure but is rather an equilibrium mixture of a large number of energetically similar conformers.[14,97,103]

It is of interest to compare the aforementioned findings with the X-ray data on crystalline valinomycin. The crystals investigated were of two modifications, triclinic

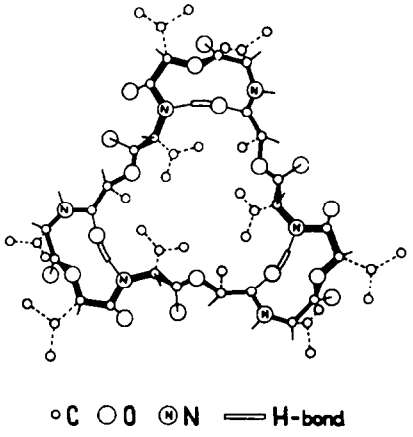



Fig. 11. Propeller-like conformation of valinomycin.

form octane,[110] acetone[110] and aqueous ethanol[109] solutions and monoclinic form octane.[108,109]

Their conformations differ little, belonging to the same structural type shown in Fig. 12. They have no $C_3$ axis but do possess a center of pseudosymmetry. They have six H-bonds as in form A, but of these only four of the 4→1 type linking CO and NH groups, whereas two are of 5→1 type with participating ester carbonyls that close 13-membered rings.

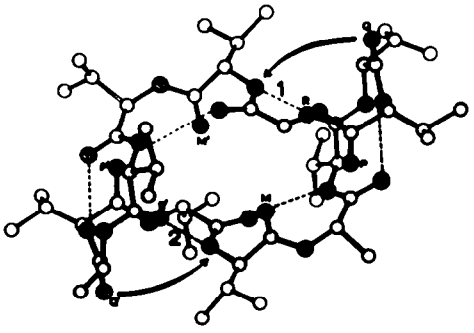



Fig. 12. Conformation of crystalline valinomycin. M and M', P and P', R and R' are ester carbonyl oxygens related by the *pseudo* symmetry center; Q and Q' are the amide oxygens not participating in hydrogen bonding. 1 and 2-H-bonds of the 5→1 type. Arrows mark the direction of Q and Q' displacement after the crystalline confromer rearrangement into the complexed form.

The H-bonding of the two ester carbonyls is clearly manifested by a splitting of the IR[94] and Raman[70,71] bands at 1760 $cm^{-1}$ in crystalline valinomycin. No such splitting occurs in solution. The above results are the main grounds on which rests the proof of the spatial difference structures of valinomycin in the crystalline state and when dissolved in non-polar solvents. At the same time these different states have similar $\phi$, $\psi$ parameters, the crystalline conformation of valinomycin resembling a distorted $A_2$ structure, wherein the ester carbonyls are somewhat displaced from bracelet the periphery by electrostatic repulsion. Apparently the interconversion barriers between these forms are not very high.

A structural study of valinomycin complexes with alkali metal ions is of decisive importance for the understanding of its complexing behaviour and membrane affecting properties. In all solvents[94,97,107,111-113] and in the crystalline state[114,115] valinomycin·$K^+$ acquires a bracelet $A_2$ conformation with inwardly oriented ester carbonyls binding the unsolvated cation in the cavity they form (Fig. 13).

The complex conformation has the following characteristic features: (i) effective shielding of the central cation by ester groups, the H-bond system and the pendant valylic isopropyls; (ii) projection of the hydroxy acid residue side chains from the "bracelet" backbone, thus protecting the H-bonded system from solvent action; (iii) a lipophilic exterior lending high solubility to the complex in neutral organic solvents, apparently an essential factor in the membrane-affecting properties of valinomycin.

The valinomycin complexes with $Rb^+$ and $Cs^+$ are basically the same as the $K^+$ complexes regardless of the solvent. The increase in size of the molecular cavity required for accomodating the former is attained by small

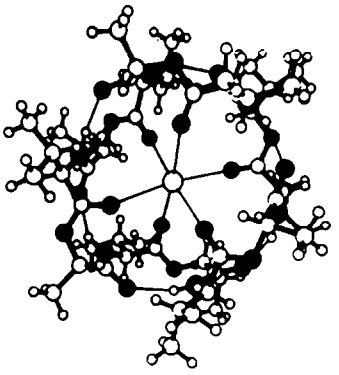



Fig. 13. Conformation of the crystalline (valinomycin·$K^+$)·0·5$I_3$·0·5$I_3$ complex. D-Val: $\phi$ 58°, $\psi$ 131°; L-Lac: $\phi$ 71°, $\psi$ − 18°; L-Val: $\phi$ − 59°, $\psi$ 132°; D-Hylv: $\phi$ 82°, $\psi$ 2°, all $\omega$ angles ~ 180°.

changes in the rotational angles and elongation of the H-bonds, as revealed by IR spectra.[97]

The structure of the $Na^+$ complex is solvent-dependent. In non-polar media the folding of the depsipeptide chain resembles that of the $K^+$ complex, and the cation is interacting effectively only with part of the ester carbonyls.[97] In methanol the $Na^+$ complex has a quite different conformation, whose parameters have not yet been elucidated.[94,116] The fact that the ester carbonyls, constituents of the rigid bracelet system, cannot approach one another near enough to ensure close contact of the sodium ion simultaneously with all these groups of course, has a direct bearing on the extremely high K/Na complexing selectivity of valinomycin. Thus, owing to its conformational peculiarities, the valinomycin molecule cannot compensate for the increase in desolvation energy accompanying the change of $K^+$ for $Na^+$ by an equivalent increase in effectiveness of the ion-dipole interaction.

The spatial structure of valinomycin has provided the basis for an insight into the properties of its numerous synthetic analogs, in particular the stabilities of their $K^+$ complexes.[14,94] Thus, with increase in ring size, the conformational potentialities of the depsipeptide skeleton are retained, but at the same time there is a considerable increase in the dimensions of the molecular cavity.[117] In line with this, cyclo[-(D-Val-L-Lac-L-Val-D-Hylv)$_4$-] (so called hexadecavalinomycin) can bind and carry across membranes such bulky cations as trimethylammonium and (choline·H)$^+$.[118] Changes in the size of the side chains have a relatively weak effect on the complex stability, but tell markedly on the screening of the bound cation from solvent action and also on the surface active properties of both the free and complexed molecules.[119,120] Reversal of the amino and hydroxy acid configurations give rise to steric strain, thereby weakening the complex, the effect being particularly strong in the case of the diastereomers differing in the configuration of the hydroxyisovaleric acid residues (Hylv).[121,122] Substitutions such as CONH→COO→CONMe impair the H-bonding system, causing a fall in the complex stability and in the antimicrobial activity.[123] Contrariwise, the substitution COO→CONH→CONMe, which does not affect the original system of H-bonds and retains the cation-binding carbonyls yields effective complexones and ionophores.[124]

The basic principles of the valinomycin action on membrane systems may be considered as reliably established; namely, the antibiotic sorbed on the mem-

brane surface binds $K^+$ cations from the aqueous phase by a heterogeneous complexing reaction, after which the complex cation diffuses to the other side of the membrane. However, precise details of the behaviour of valinomycin are still insufficiently clear to explain all its properties.

Thus, there seems to be no united opinion as to the number of ionophore molecules participating in the act of ion transport, some authors giving preference to the single carrier model[125-127] while others are in favour of a relay mechanism.[14,128]

In this connection, the recent discovery, that valinomycin has the ability to form, under certain conditions (excess ionophore at its sufficiently high absolute concentration) 2:1 "sandwich" complexes, as well as the equimolar ones,[129] is of great interest. According to the UV and CD spectra both depsipeptide molecules in the 2:1 "sandwich" are in the bracelet conformation, and on this basis the structure of the former can, in principle, be depicted as in Fig. 14. Such structures may quite possibly arise as intermediates in the relay transfer of the cation or as ion carriers during the functioning of valinomycin in membranes. The possibility of "sandwich" formation by valinomycin seems to be the more reasonable if it be taken into account that certain indirect evidence indicates this antibiotic to be not merely "floating" randomly about in the membrane (Fig. 15a), but rather forming clusters of two (Fig. 15b) or more molecules, already prepared for the non-stoichiometric complexing process.[129,130]

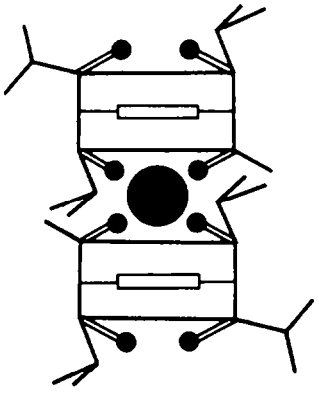


Fig. 14. Proposed structure of the (valinomycin)$_2$·$K^+$ sandwich.

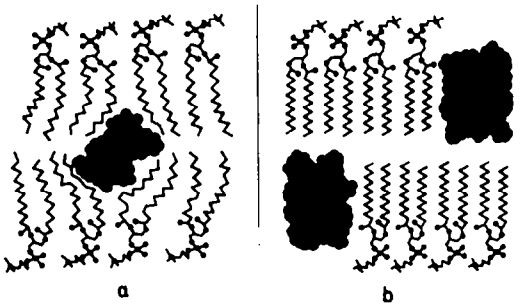
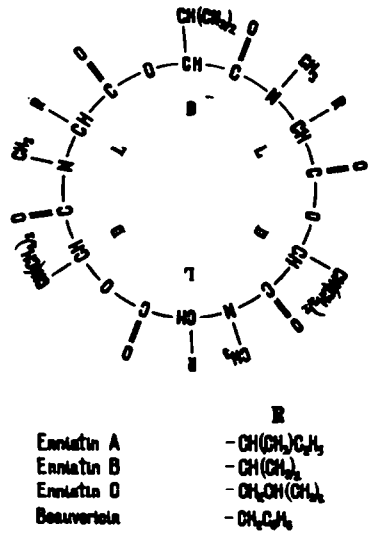


Fig. 15. Valinomycin "floating" in dimiristoyl bilayer (a) and a form of stacks (b).

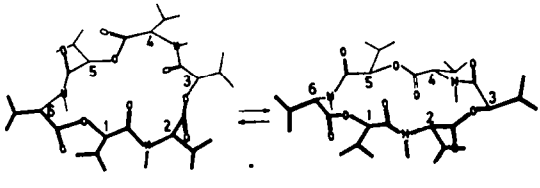
2. *Enniatins A, B, C, beauvericin and their analogs.* In organic solvents enniathins complex a wide range of cations. They rather indiscriminatly form 1:1 complexes with alkali metals, ammonium, alkaline earth metals and some transition metals ($Ag^+$, $Tl^+$, $Mn^{2+}$, $Zn^{2+}$, $Cd^{2+}$).[14,132],† Besides the 1:1 complexes, enniatins B and C and beauvericin form complexes with a macrocycle:cation ratio of 2:1 (possibly 3:2) displaying a notable K over Na selectivity.[129,131,132]



| | R |
|---|---|
| Enniatin A | $-CH(CH_3)C_2H_5$ |
| Enniatin B | $-CH(CH_3)_2$ |
| Enniatin C | $-CH_2CH(CH_3)_2$ |
| Beauvericin | $-CH_2C_6H_5$ |

Enniatin antibiotics may assume two basic conformational forms: *N* which is predominant in non-polar media (heptane, dioxan) and *P* which is predominant in polar media (water, trifluoroethanol).[96,132-135] According to IR and NMR data, both forms have *trans* N-methylamide bonds.[132] The dispersion of ultrasonic absorption indicates that form *N* is a rapidly interconverting mixture of two conformers[104,105,139] of which one, in accordance with the NMR spectra becomes insignificant on cooling.[132] Theoretical analysis of the ennniatins revealed five possible low energy non-symmetric conformations ($N_1$–$N_5$)[136] and one symmetric conformation ($P$)[136,138] for all of which the $\phi$ and $\psi$ parameters have been computed. Estimation of the possible $C^\alpha H$ and $CH_3$–N proton shifts for the $N_1$–$N_5$ conformations show that the low temperature experimental values can be ascribed only to structure $N_3$; the $N_3 \rightleftarrows P$ equilibrium is shown in Fig. 16. The second structure was assigned to $N_1$ on the basis of energy and dipole moment considerations.[132,137]

Form *P* is preferable to $N_3$ or $N_1$ from the point of view of non-bonded interactions, but it is destabilized by electrostatic repulsion of the carbonyl oxygens. Hence solvation of the carbonyl groups with resultant decrease in dipolar repulsion is the driving force of the $N \rightarrow P$ shift with increasing solvent polarity.[137]



| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $\phi$ | 89 | -118 | 154 | 73 | -58 | -133 |
| $\psi$ | -109 | 92 | 32 | -55 | -92 | 140 |

N

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $\phi$ | 74 | -103 | 74 | -103 | 74 | -103 |
| $\psi$ | -138 | 171 | -136 | 171 | -136 | 171 |

P

Fig. 16. $N_3 \rightleftarrows P$ equilibrium of enniatin B.

†And references quoted.

The same function can be performed by metal cations entrapped in the enniatin molecular cavity; judging by the CD curves, enniatins also assume the *P* conformation in complexes with the ions of alkali, alkaline earth and other metals.[132] The crystalline complex (enniatin B)·KNCS (Fig. 17) resembling a charged disk with a lipophilic circumference, has been studied by X-ray analysis.[140]

Enniatin complexes with a 2:1 macrocycle:cation ratio are believed to be in the form of "sandwiches", in which the most probable ligands are the amide carbonyls (Fig. 18a). In 3:2 complexes ligand functions are assumed by both the amide and ester carbonyls (Fig. 18b). The cation is protected from the anion and the medium more effectively in the non-equimolar complexes than in the equimolar ones. This explains why, despite their apparent low stability, the former make the major contribution to the enniatin-induced cation permeability of artificial lipid membranes.[128,131]

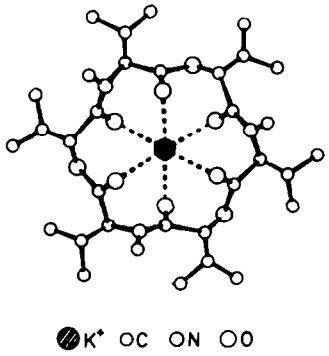
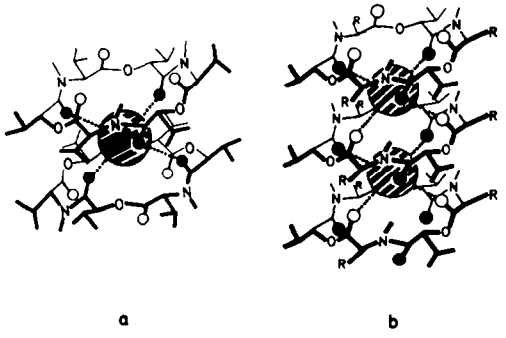



Fig. 17. Conformation of the crystalline (enniatin B)·KNCS complex. L-Val: $\phi$ −60°, $\psi$ 120°; D-HyIv: $\phi$ 60°, $\psi$ −120°; all $\omega$ angles ~ 180°.



a b

Fig. 18. Proposed structures for 2:1 (a) and 3:2 (b) complexes of enniatins.

The structure of the crystalline beauvericin (Bv) complex with barium picrate ($BaPic_2$) of the composition $(Bv{\cdot}Ba{\cdot}Pic_3{\cdot}Ba{\cdot}Bv)^+{\cdot}Pic^-$[141] (Fig. 19) is very unique. Its center is occupied by two barium ions held together in the form of a very short bridge (interatomic distance 4·13 Å), by three picrate anions extending radially outward like a three-bladed propeller, each picrate contributing two oxygen atoms for coordination with each barium ion. The two beauvericin molecules are located at opposite ends of the propeller shaft; each molecule contributes three amide oxygens, the MePhe benzene rings providing the packing to enclose the anions. It is still too early to say whether such a structure is the functioning form of the enniatin ionophores, although it does have a number of features in

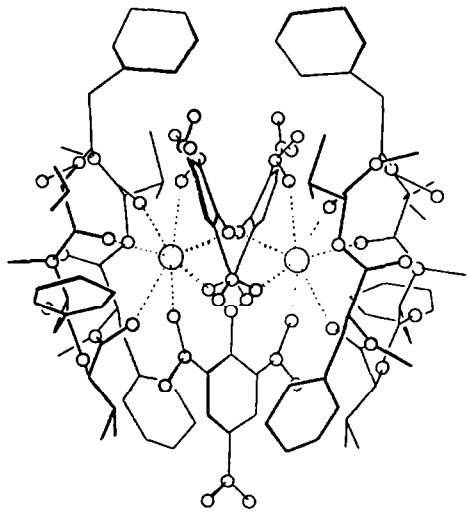


Fig. 19. Structure of the crystalline $(Bv{\cdot}Ba{\cdot}Pic_3{\cdot}Ba{\cdot}Bv)^+{\cdot}Pic^-$ complex.

common with the structure represented in Fig. 18a (the cation at the periphery of the ionophore interacts predominantly with the amide ligands, providing an extra argument in favour of such a conformation). Morever, the results obtained stress the fact that despite the wealth of indirect evidence that in solutions of 1:1 complexes the cation is incorporated into the molecular cavity no vigorous proof of this has as yet been forwarded and the same authors[141] doubt the conclusions of the X-ray analysis of the enniatin·$K^+$ complex,[140] which had not been carried through to the stage of determining the atomic coordinates.

The relation between primary structure and metal binding properties among the enniatin B analogs differing in ring size, asymmetric center configurations side chains and ligand groupings has been treated by Orchinnikov *et al.*[14] and Mikhaleva *et al.*[135] The general conclusion resulting from the treatment can be summarized as follows: the flexibility of these compounds and the absence of H-bonds are responsible for a comparatively weak correlation between complex stability, cyclopeptide structure and cation species; in other words, for a relatively low structural and cation specificity of complexation. The ionophorous properties of the enniatin analogs are still insufficiently clarified.

3. *Monamycins.* Monamycins A–I are 18-membered cyclic depsipeptides comprising five amino acid and one hydroxy acid residues.[142–145] Difficultly soluble complexes

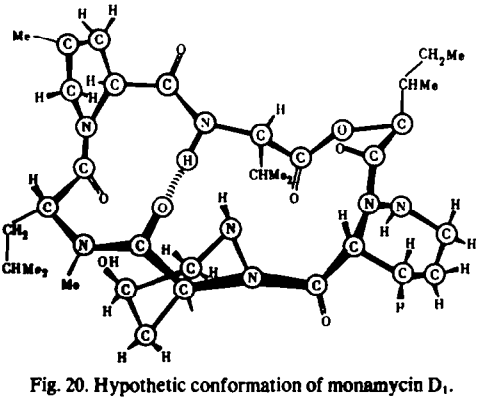
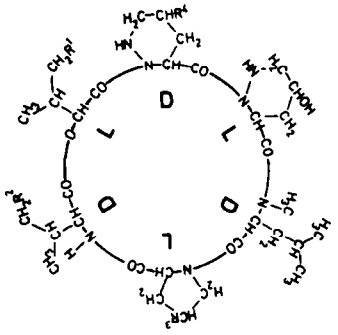


Fig. 20. Hypothetic conformation of monamycin $D_1$.

of monamycins with sodium, potassium, rubidium, cesium, silver and barium salts have been reported to form in aqueous ethanol,[146,147] a fact which justifies consideration of the monamycins in the section devoted to ionophores.



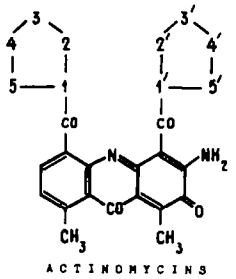
| Monamycin | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| A | H | H | Me | H |
| $B_1$ | H | H | Me | H |
| $B_2$ | H | Me | H | H |
| $B_3$ | Me | H | H | H |
| C | Me | H | Me | H |
| $D_1$ | Me | H | Me | H |
| $D_2$ | H | Me | Me | H |
| E | Me | Me | Me | H |
| F | Me | Me | Me | H |
| $G_1$ | H | H | Me | Cl |
| $G_2$ | H | Me | H | Cl |
| $H_1$ | Me | H | H | Cl |
| $H_2$ | Me | H | Me | Cl |
| I | H | Me | Me | Cl |
| | Me | Me | Me | Cl |

In a review[13] dealing with the conformations of cyclic and "cylindrical" peptides, Hassall and Thomas suggested a possible structure for monamycin $D_1$ (Fig. 20), based on IR and NMR data. In this structure the single peptide NH forms a 4→1 H-bond.

Like the enniatin antibiotics, monamycins have alternating LDLDLD amino acid and hydroxy acid residues. Such structural similarity prompts the suggestion that the complexes of both groups are also built on identical principles.

## 5. ACTINOMYCINS

Actinomycins are a group of rather toxic chromopeptides manifesting both antimicrobial and antitumor properties. All of them contain the same phenoxazone chromophore group (3 - amino - 4,5 - dicarboxy - 1,8 - dimethyl - 2 - phenoxazone, which has been named actinomycin) and various cyclopeptide moieties some of which may have very similar structures.[1,148,149,157]

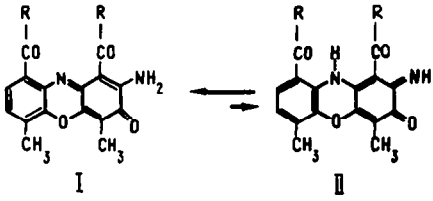


ACTINOMYCINS

1,1' = Thr or MeThr[152]
2,2' = D-Val, D-aIle or D-Leu[154]
3,3' = Pro, 4-oxo-5-methylproline, 4-allo-hydroxy-5-methylproline, cis-5-methylproline[151,152,156], pipecolic acid, 4-oxopipecolic acid or 4-hydroxy-pipecolic acid[155]
4,4' = Sar or Gly[153]
5,5' = MeVal, MeAla or MeAla[151,152,156]

†And references quoted.

Luminescence data in various solvents has revealed[150] that the actinomycin chromophore exists as a tautomeric equilibrium of two forms, a major (I) and a minor (II).



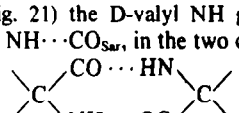
I ⇌ II

Actinomycins with two identical cyclopeptide groups are referred to as the *iso*-series, and those with differing groups—as the *aniso*-series.[158] Actinomycin $C_1$ (frequently referred to as actinomycin D) is the most readily available member of this group (1 = 1' L-Thr, 2 = 2' = D-Val, 3 = 3' = L-Pro, 4 = 4' = Sar, 5 = 5' = L-MeVal).

The mode of action of actinomycins involves formation of highly stable complexes with DNA, which precludes the latter from performing its biological function.[149],†

Investigation of the three-dimensional structure of actinomycins entails the problem of the mutual orientation of the chromophore and the lactone rings[159] as well as the usual determination of the $\phi$, $\psi$ and $\omega$ parameters.

NMR studies of actinomycin $C_1$ carried out independently by several teams[160-165] have shown the lactone rings to have similar spatial structures, observed differences in the chemical shifts being due to the asymmetry of the chromophore. A low rate of deuterium exchange bears evidence of the D-Val NH groups participation in intramolecular H-bonding. Further structural knowledge from the NMR experiments came from the spin coupling constants yielding the conformations of the NH–CH and $C^{\alpha}H$–$C^{\beta}H$ fragments.

Theoretical analysis[166,167] showed that there are only three low energy conformations of actinomycin $C_1$ which conform to experimental data. Earlier suggested structures based on examination of molecular models[162,163,168-171] proved to be erroneous. In the first of the actual conformations (Fig. 21) the D-valyl NH groups form intracyclic H-bonds $NH\cdots CO_{Sar}$, in the two others, the bonds are intercyclic,  (Figs. 22 and 23). All the structures contain *cis* tertiary amide

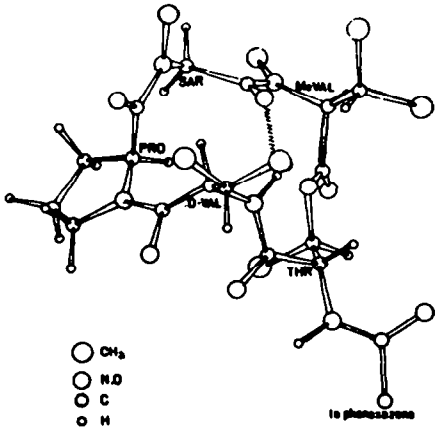



Fig. 21. Computed conformation of the lactone ring in actinomycin $C_1$ with intracyclic H-bond $NH_{Val}\cdots OC_{Sar}$. $\phi_1 - 150°$ (−90°), $\psi_1$ 179°, $\omega_1 - 180°$, $\chi_1$ 152°; $\phi_2 - 50°$, $\psi_2 - 92°$, $\omega_2 - 180°$; $\phi_3 - 68°$, $\psi_3$ 157°, $\omega_3$ 9°; $\phi_4 - 86°$, $\psi_4 - 164°$, $\omega_4$ 6°; $\phi_5$ 67°, $\psi_5 - 76°$, $\omega_5 - 178°$.

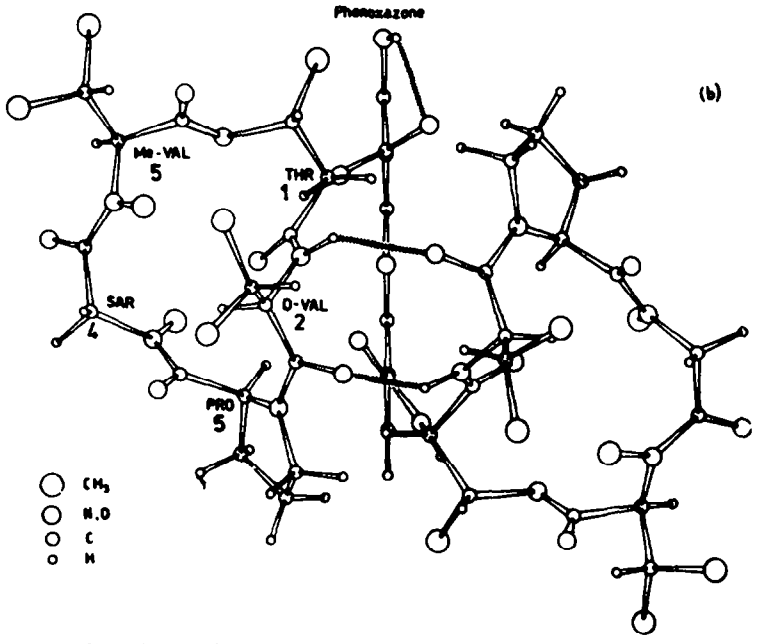



Fig. 22. Computed conformation of actinomycin $C_1$ with intercyclic H-bonds and two *cis*-amide bonds D-Val-L-Pro. $\phi_1$ $-153°(-71°)$, $\psi_1$ 158°, $\omega_1$ $-180°$, $\chi_1$ 64°; $\phi_2$ 101°, $\psi_2$ $-117°$, $\omega_2$ 166°; $\phi_3$ $-68°$, $\psi_3$ 154°, $\omega_3$ 30°; $\phi_4$ 128°, $\psi_4$ $-106°$, $\omega_4$ $-175°$; $\phi_5$ $-121°$, $\psi_5$ 59°, $\omega_5$ $-162°$.

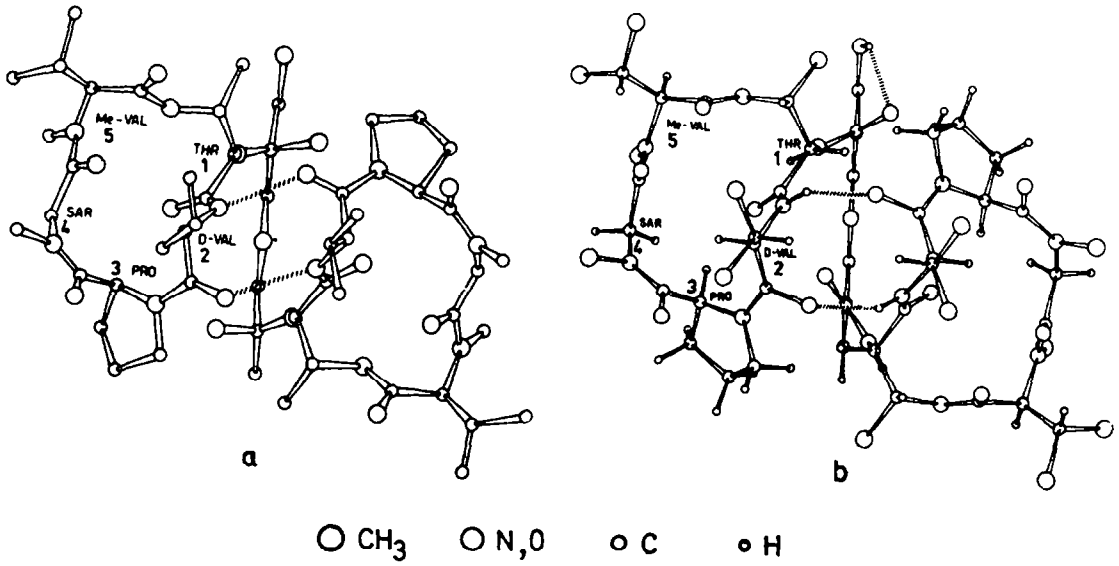



Fig. 23. Conformation of actinomycin $C_1$ with intercyclic H-bonds and four *cis*-amide bonds (D-Val-L-Pro and L-Pro-Sar). a—the X-ray structure. b—computed structure: $\phi_1$ $-153°$ $(-71°)$, $\psi_1$ 158°, $\omega_1$ $-180°$, $\chi_1$ 60°; $\phi_2$ 80°, $\psi_2$ $-114°$, $\omega_2$ 174°; $\phi_3$ $-69°$, $\psi_3$ 151°, $\omega_3$ 6°; $\phi_4$ $-108°$, $\psi_4$ 172°, $\omega_4$ $-6°$; $\phi_5$ 55°, $\psi_5$ 95°, $\omega_5$ $-172°$.

groups, namely, D-Val-L-Pro and L-Pro-Sar in Figs. 21 and 23 and D-Val-L-Pro in Fig. 22.

An important step forward not only in elucidation of the spatial structure of actinomycin $C_1$, but also in explaining the specificity of its interaction with nucleotides was made when the X-ray analysis of the deoxyguanosine complex of the antibiotic[172-174] was carried out. The crystalline conformation has a pseudo twofold symmetry axis and almost exactly coincides with one of the calculated forms (Fig. 23); the small differences (for example, in the neighbourhood of the $NH_{Thr}$-bonded carbonyl groups) may be ascribed to complexing of the heteroring.[175,176] In the complexed state actinomycin $C_1$ is bound to two nucleoside molecules such that one heterocyclic base is on either side of the chromophore, giving rise to numerous hydrophobic contacts ("sandwich" packing). The specific binding of guanosine is due to strong H-bonding of the threonine carbonyls by the guanine $NH_2$ groups and the bonding, albeit weaker, between the guanine $N_{(3)}$ atom and the threonine NH (Fig. 24).

The structure of the crystalline complex has served as a basis for a model depicting the interaction of actinomycins with the DNA double helix. According to this model the phenoxazone chromophore is intercalated between the base paired dinucleotide sequences, $G_pC$, such that

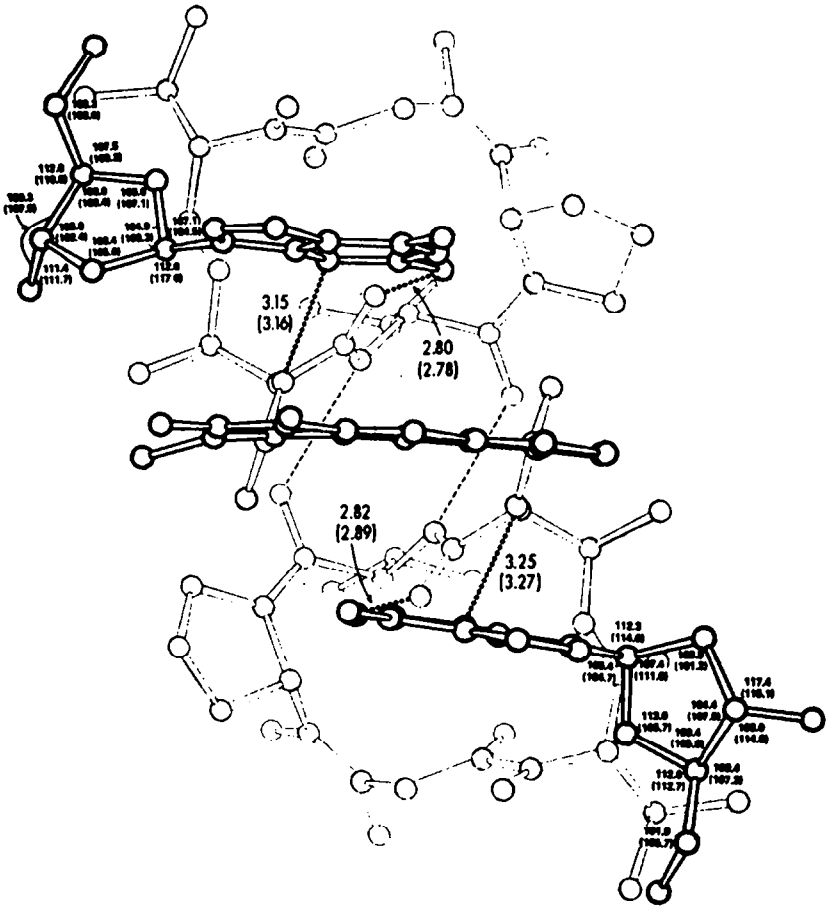



Fig. 24. Conformation of the crystalline complex (actinomycin $C_1$). (deoxyguanosine)$_2$. Figures at the respective bonds are bond angles and distances obtained from the light-atom analysis, values in parentheses obtained from heavy-atom analysis.

the peptide rings fall into a narrow groove, becoming attached to the DNA by specific H-bonds and hydrophobic contacts.[173] The model is in accord with the latest results of studies on actinomycin complexes with DNA and oligonucleotides in solution by spectrophotometry,[177] NMR[178–184] and low angle X-ray scattering.[185] However, data on the structural details of the DNA complexes with actinomycins are still indirect and further work is required before an unequivocal answer to the problem will be obtained. In this connection, Lackner's[186,187] results show slow interconversion of two conformers in the synthetic pentapeptide–lactone "halves" of actinomycin $C_1$, apparently differing in configuration of the tertiary amide bond, and also the results of Ascoli *et al.*,[188,189] show that conformational transitions in actinomycin D can be induced by solvents with *gem*-diol groups (hexafluoroacetone hydrate, chloral hydrate). In the presence of such solvents the conformational equilibrium is shifted from forms with intramolecular intercyclic H-bonds to such with the D-valine residues specifically solvated by the solvent. As one can see from Fig. 25, this transition is accompanied by a 180° rotation of the lactone rings with respect to the plane of the chromophore and the formation of an approximately enantiomeric structure, resulting in the experimentally observed sign reversal of the chiroptical effects.

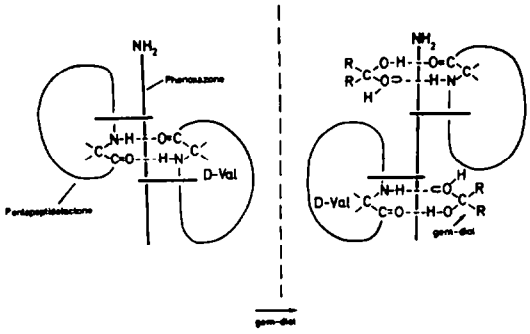


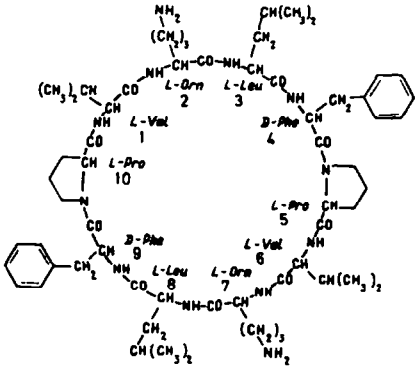

Fig. 25. Schematic representation of conformational transition in actinomycin $C_1$ induced by *gem*-diols.

A more or less complete analysis of the relation between the primary structure, conformations and biological action of the numerous naturally occurring actinomycin antibiotics and their synthetic analogs is yet to appear. However, initial steps in this direction have already been taken.[190] The data on actinomycin D and its mono (1=1′=L-Thr, 2=2′=D-Val, 3=3′=L-Pro, (4,4′) = (Sar, Gly), 5=5′=L-MeVal) and tetra-N-desmethyl (1=1′=L-Thr, 2=2′=D-Val, 3=3′=L-Pro, 4=4′=Gly, 5=5′=L-Val) analogs demonstrates what this could reveal. The antimicrobial potency of mono-N-desmethyl derivative is 1/10 to 1/50 that of actinomycin D[153] and the tetra-N-desmethyl derivative is in general inactive.[191] Investigation of the CD and ORD curves have revealed "*gem*-diol" patterns in the first compound in a larger range of solvents than for actinomycin D so that it must be more prone to assume

the type b conformation shown on Fig. 25, whereas the second prefers the latter conformation in all solvents investigated. Apparently here the correlation between the extent of N-desmethylation, the spatial structure and the biological activity has the following explanation: when the N-methyl groups are replaced by amide groups the *cis* configuration of the pentapeptide-lactone rings becomes sterically unfavourable, leading to conformational reforming of the ring and destabilization of the intercyclic H-bonds of conformers a on Fig. 25 essential for effective interaction with DNA.

### 6. GRAMICIDIN S AND TYROCIDINES A-E

Gramicidin S has for many years been one of the most popular subjects in the conformational study of peptides. Its relatively simple primary structure and rather high biological activity present unique possibilities for exploration into the secondary structure of the polypeptide chain and its relationship to biological function. Gramicidin S



Gramicidin S

is also convenient for verifying the adequacy and reliability of many methods for investigating peptide and protein systems. Despite its relative simplicity, over 12 structures have been proposed for the antibiotic since 1953, based on analysis of molecular models,[170,171] *semi*-empirical calculations,[192-197] X-ray diffraction studies,[198,199] cyclization reactions,[200,201] UV and ORD/CD data[28,42-47,202] and IR,[47,202,203] NMR-$^1$H[47,204-208,215] and NMR-$^{13}$C[209-214] spectroscopy. Recent findings[47,192,206,208] have given support to one of the early models, viz., the "pleated sheet" structure suggested by Hodgkin and Oughton in 1957[198] and by Schwyzer in 1958.[200,201]

The "pleated sheet" (Fig. 26), an extremely rigid conformation is retained in solvents of a wide range of polarity. The compact antiparallel folding of the gramicidin S backbone precludes the existence of a molecular cavity, explaining the non-complexation with alkali metal ions in contrast with its cyclopeptide counterpart, antamanide (see Section 7).

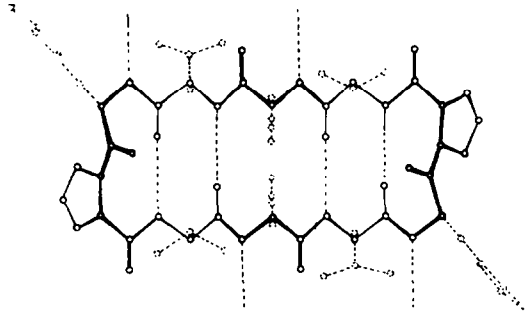
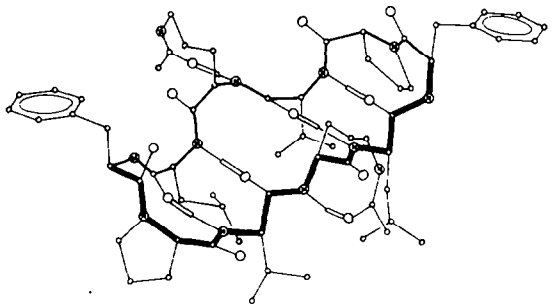


Fig. 26. "Pleated sheet" conformation of gramicidin S.

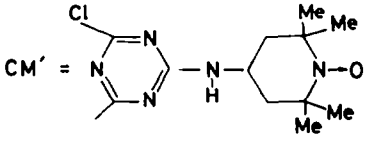


o-C ● - N O-O ═ - H- bond

Fig. 27. Preferred conformation of N,N-diacetylgramicidin S in non-polar solvents.

In non-polar media, the side chains of the Orn$^2$ and Orn$^7$ residues or of their derivatives may form H-bonds with the peptide skeleton, as is the case, for example, with N,N'-diacetylgramicidin S (Fig. 27).[47] In methanol the side chains of all residues (excepting proline) are rather mobile, as evidenced from $^{13}$C spin-lattice relaxation measurements.[209] The unidirectional orientation of the ornithine side chains is an important feature of the gramicidin S structure, permitting their proximity in space so as to interact simultaneously with $Cu^{2+}$ (after formation of a Schiff's base with salicyclic aldehyde).[216,217] The same orientation of the Cys$^2$ and Cys$^7$ side chains in [Cys,$^2$ Cys$^7$] gramicidin S, promotes oxidation to the disulfide. The resulting bicyclic derivative has the same backbone conformation as the antibiotic itself.[218,219]

The average distance between the $\delta$-amino groups of the ornithine residues was estimated by an ESR study of iminoxy spin labels (SL) incorporated into the Orn$^2$ and Orn$^7$ side chains.[220] It was shown that in ethanol the spin



labels collide at room temperature with an activation energy of *ca.* 5 kcal/mole (A conformation in Fig. 28); in chloroform the collision frequency is much lower, as conformers of type C are realized. The average distance between the labels in both solvents (conformations B and C) measured at −196°C was ~12 Å, which corresponds to about 8–10 Å between the free ornithine amino groups of the antibiotic.

The presence of charged groups in the second and seventh amino acid residues are known to be essential for the antimicrobial activity of gramicidin S. Conformational studies prompt the conclusion that spatial proximity of these groups should be another important factor. To verify this, a series of gramicidin S analogs was studied, in which the majority or all of the functional and hydrophobic groups were retained but the stereochemical parameters were altered.[221-225] The results, presented in Table 1, show that, in fact, good correlation exists between the antimicrobial activity and the ability of the Orn side chains to achieve spatial proximity.

It has been shown that gramicidin S solubilizes lecithin

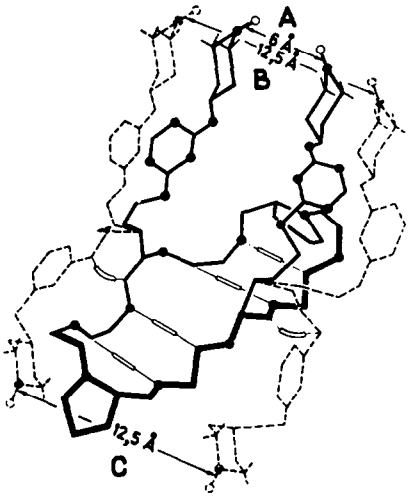



Fig. 28. Spin-labelled gramicidin S with different orientation of ornithine side chains.

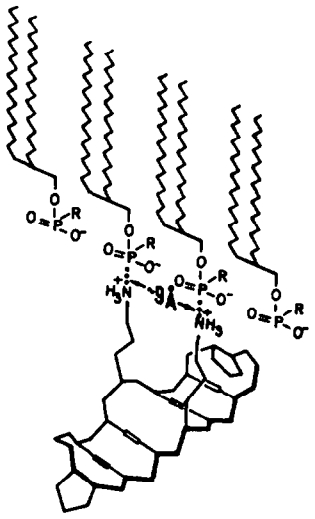



Fig. 29. Schematic representation of the interaction of gramicidin S with a lecithin membrane (R = $CH_2CH_2NMe_3OH$).

liposomes, the protonated amino groups of the antibiotic forming salt linkages with the phosphate groups of the lipid.[226,227] The same type of interaction is likely between gramicidin S and the lipoprotein complex of bacterial membranes because the completely identical (qualitative and quantitative) activity of *enantio*-gramicidin S or that of [Gly$^5$, Gly$^{10}$] gramicidin S with its enantiomer (Table 1) testify to the absence of specific protein receptors.

It is noteworthy that, judging from the area of 55–75 Å$^2$ per phospholipid molecule,† the above-mentioned average distance between the $NH_3^+$ groups in gramicidin S (8–10 Å) must be the same as that between the phosphate groups in lipid monolayers (7–9 Å). Such similarity and the decrease in activity with increase of $NH_3^+ \cdots NH_3^+$ distance (Table 1) indicates that an important step in the functioning of gramicidin S is the simultaneous salt linking to two neighbouring phospholipid molecules (Fig. 29). The effectiveness of this reaction is due to the rigidity of the spatial arrangement of the antibiotic.

In the light of these findings it is not difficult to explain the correlation between the intensity of the chiroptical effects at 220 nm in the ORD curves of the gramicidin S analogs with altered side chains and their antibiotic activity as reported by Kato *et al.*[229] It should be remembered that the extremely intensive $n \rightarrow \pi^*$ chiroptical effect is a distinguishing feature of the "pleated sheet" conformation. In linear peptides of the gramicidin S series,[230–236] and in 15- and 20-membered sesqui- and digramicidins S,[237,238] and δ-amino groups of the ornithine residues seem to be capable of attaining spatial proximity, which accounts for their retention of some antimicrobial activity.

The reason for the progressive increase of the $NH_3^+ \cdots NH_3^+$ distance and concomitant decrease in antimicrobial activity when the substitution CONH→COO is realized (depsipeptide analogs in Table 1) is the gradual destabilization of the "pleated sheet" structure due to decrease in H-bonding, as shown by spectroscopic methods.[225] With the substitutions L-Leu→L-MeLeu and 2 L-Leu→2 L-MeLeu[239] the conformational freedom attained through breakage of H-bonds is probably compensated by the inherent rigidity of the N-methylated peptide backbone,[53] which explains the high biological activity of the corresponding analogs.

Like gramicidin S, tyrocidines A–E are cyclic decapeptides,[240–254] the similarity being enhanced by their having a common peptide fragment (1–5) and a common "pleated

†For example see Ref. [228].

Table 1. Antimicrobial spectra and ESR data on gramicidin S derivatives[220–222]

| Compound | Minimal growth inhibiting concentration (γ/ml) | | | | Mean distance between free radicals in the spin labeled derivatives (Å, −196°, EtOH) |
|---|---|---|---|---|---|
| | *Staph. aureus* 209P | *Bac. subtilis* | *E. coli* | *Mycob. phlei* | |
| Gramicidin S | 0·5–1 | 0·5–1 | 2 | 2–4 | 12·5 |
| *Enantio*-gramicidin S | 0·5–1 | 0·5–1 | 2 | 2–4 | 12·5 |
| [Gly$^5$, Gly$^{10}$]gramicidin S | 18 | 9–18 | 9–18 | 12–18 | 15 |
| *Enantio*[Gly$^5$, Gly$^{10}$]gramicidin S | 18 | 9–18 | 9–18 | 12–18 | 15 |
| "All-L"-gramicidin S | 60–75 | 9–18 | 18 | 12–25 | 16·5 |
| *Retro*-gramicidin S | 60–75 | 9–18 | 18 | 18–25 | 18 |
| *Retro*[Gly$^5$, Gly$^{10}$]gramicidin S | >100 | 12–18 | 9–18 | 37–60 | 18 |
| [HyIv$^1$, HyIv$^6$]gramicidin S | 9 | 2 | 18 | 12 | 15 |
| [HyIv$^1$, HyIc$^3$, HyIv$^6$, HyIc$^8$] gramicidin S | >100 | >100 | >100 | >100 | >30 |

sheet" backbone (Fig. 30). Tyrocidines display a marked tendency to associate in aqueous media.[244,255-260]

It is still too early to consider the structure-function relationship in the tyrocidine series, for their mode of action is still controversial. Some consider these compounds to interact, as in the caselof gramicidin S, with the membrane phospholipids;[519] it should be noted, however, that interactions of the type shown in Fig. 29 which require the participation of two $NH_3^+$ groups of the antibiotic are possible only with tyrocidine associates, as the monomers contain only a single $NH_3^+$ grouping. Others consider tyrocidines to participate in more specific interactions, such as inhibition of sporulation in the producing organism[261] by forming a complex with its DNA.[520,521]

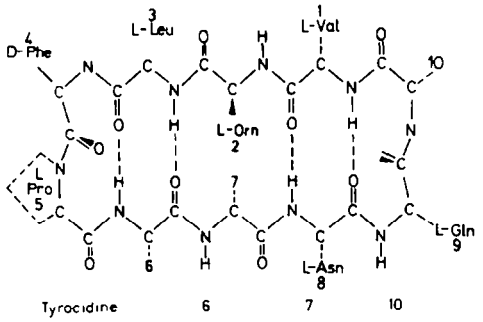
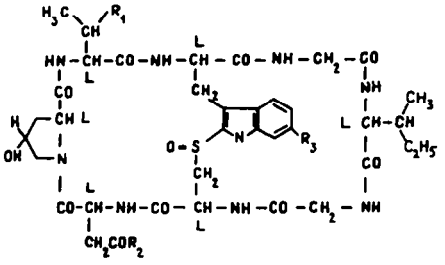



Fig. 30. "Pleated sheet" structure of tyrocidines A-E.

### 7. CYCLIC PEPTIDES FROM THE GREEN DEATHCAP TOADSTOOL AMANITA PHALLOIDES

Since the forties Th. Wieland's group has been intensively studying the active principles of the poisonous *Amanita* mushrooms. Their efforts have resulted in the

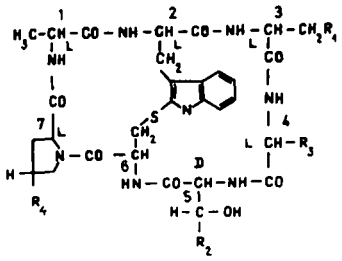


| Amatoxin | $R_1$ | $R_2$ | $R_3$ | References |
|---|---|---|---|---|
| α-Amanitin | $CH(OH)CH_2OH$ | NH | OH | 276 |
| β-Amanitin | $CH(OH)CH_2OH$ | OH | OH | 276 |
| γ-Amanitin | $CH(OH)CH_3$ | $NH_2$ | OH | 276 |
| ε-Amanitin | $CH(OH)CH_3$ | OH | OH | 271 |
| Amanin | $CH(OH)CH_2OH$ | OH | H | 272,273 |
| Amanullin | $C_2H_5$ | $NH_2$ | OH | 271 |

isolation and structural elucidation of the major components of the toxins.[262-270] These can be divided into two groups, namely, phallotoxins and amatoxins.

Phallotoxins are cyclic heptapeptides bridged by the side chains of the tryptophan and cysteine residues. Amatoxins have a larger ring (eight amino acid residues) with two additional oxygen functions in the bridge.

†And references quoted.



| Phallotoxin | $R_1$ | $R_2$ | $R_3$ | $R_4$ | References |
|---|---|---|---|---|---|
| Phalloidin | $C(OH)CH_2OH$, $CH_3$ | $CH_3$ | $CH_3$ | OH | 271 |
| Phalloin | $C(OH)CH_3$, $CH_3$ | $CH_3$ | $CH_3$ | OH | 262-264 |
| Phallisin | $C(OH)CH_2OH$, $CH_2OH$ | $CH_3$ | $CH_3$ | OH | 272,273 |
| Phallicidin | $C(OH)CH_2OH$, $CH_3$ | COOH | $CH(CH_3)_2$ | OH | 262-264 |
| Phallin B (tentatively) | $C(OH)CH_3$, $CH_3$ | $CH_3$ | $CH_2C_6H_5$ | H | 274 |
| Norphalloin | $CH_2CH_3$ | $CH_3$ | $CH_3$ | OH | 275 |

The toxins primarily affect the liver cells; phalloidin localizes in this organ immediately after administration of the poison and displays prolonged resistance to enzymatic degradation.[264,279-281]

Despite the quite similar structures, phallotoxins and amatoxins have different modes of action, a good illustration of the caution required in inferences by analogy. Phalloidin impairs the membranes of the liver cells, apparently binding to the (as yet hypothetic) actin-like protein constituents and thereby causing their aggregation into (experimentally found) filamentous structures, so that potassium ions and enzymes are released from the cell.[264,282-288,313] The amanitins have a much more specific action, becoming strongly bound to one of the DNA-dependent RNA-polymerases in the eukaryotes and thus suppressing its activity.[289] For this reason the amanitins are being widely used as a powerful tool in biochemical research.†[290-303]

NMR studies of this toxin revealed the bicyclic system to be rigid, the tryptophan NH group to be shielded and one of the two alanine methyls to resonate in an anomalously high field apparently because of proximity to the indole grouping. A structure in accord with these findings is shown in Fig. 31.[304,305] For phalloidin, amanitines and their derivatives certain correlations are observable between primary structure, toxicity and chiroptical properties,[306-308] but they are still of an empirical nature and are not linked to any specific structures.

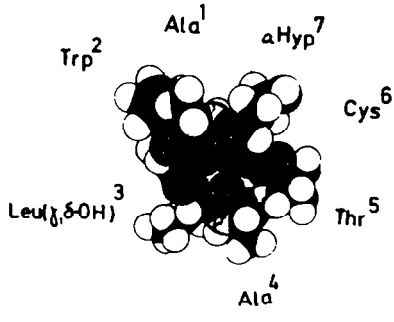
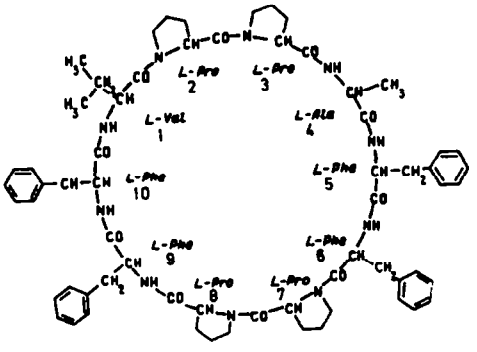



Fig. 31. Proposed conformation of phalloidin. $\phi_1 -80°$, $\psi_1 -60°$; $\phi_2 -120°$, $\psi_2$ 150°; $\phi_3 -70°$, $\psi_3 -70°\psi_3$ 180°; $\phi_4 -80°$, $\psi_4$ 150°; $\phi_5$ 90°, $\psi_5 -90°$; $\phi_6 -80°$, $\psi_6$ 150°; $\phi_7 -58°$, $\psi_7 -55°$; $\omega_{1-7}$ 180°.

Considerable interest has been attracted lately by the cyclic decapeptide antamanide, produced in small amounts by *Amanita phalloides* and counteracting the lethal effect of its toxins. Wieland *et al.* ascribe its antitoxicity to its specific ability to "tighten" the cellular membranes of liver, lowering their permeability to the toxin molecules.[264,281,309-313]



Antamanide

As ionophoric antibiotics antamanide and several of its synthetic analogs are capable of complexing alkali and alkaline earth metal ions,[311,312,314-316] the most stable complexes being with $Na^+$ and $Ca^{2+}$.

The sodium complexing ability of antamanide determines its potential value as a tool for selectively inducing $Na^+$ permeability in biomembranes. However, in comparison with valinomycin and the enniatins, it displays lower lipophilic properties and penetrates with difficulty phospholipid monolayers. This may account for the very poor ionophoric activity of antamanide.[98,315,317] A comparison of the complexing and antitoxic properties of antamanide and its analogs has led to the conclusion that the ability to complex $Na^+$ (or $Ca^{2+}$) ions is a necessary, but insufficient condition for the manifestation of biological activity in this series of compounds.† The reason for the interdependence of these two phenomena will be discussed in the closing paragraph of this section.

Considerable effort was made in elucidating the molecular structures of antamanide and its complexes in both the crystalline and molecular states. In solution antamanide is involved in a complex conformational equilibrium in which at least four forms (**A**, **B**, **C** and **D**) can be discerned.[98,102,318-325] In non-polar solvents (heptane-dioxan, 5:2, chloroform) one of the forms (**A**) which has a pseudo two-fold axis and an H-bond system involving all the six NH groups is predominant. When hydroxyl-containing solvents ($CH_3OH$, $C_2H_5OH$, $CF_3CH_2OH$, $H_2O$) are gradually added, the H-bonds formed with participation of the $Ala^4$ and $Phe^9$ NH groups are the first to break (form **B**) only then being followed by solvation of the remaining NH groups to give form **C**. The NMR spectra of a dimethylsulfoxide-containing solutions of [$Val^6$, $Ala^9$]antamanide, an analog stereochemically very close to the naturally occurring cyclopeptide revealed considerable amounts (up to 60%) of the conformers, differing from the **A**, **B** and **C** forms in the configurational set of the tertiary amide bonds (form **D**).[102,323,324]

On the basis of the spectral and computational data, several possible structures were suggested for antamanide in solution, containing both *trans*- and *cis*- X-Pro bonds.[320,323-327] As in the $Na^+$ complex of antamanide the Pro–Pro bonds are *cis*- (see below) and the amide configuration appears to be the same in the free and the complexed cyclopeptide,[328] such conformational types should evidently be preferable for the **A**, **B** and **C** forms of antamanide. The existence of two *cis* X-Pro linkages in most solvents is also supported by $^{13}C$-NMR spectroscopy.[325] The structural details of forms **A**–**D** still require further investigation.

On the basis of crystallographic,[329-331] solution[129,321,328,332] and theoretical[326] studies it can now be considered that under all conditions equimolar complexes of antamanide with $Li^+$, $Na^+$, $K^+$ and $Ca^{2+}$ assume a common type of saddle-like conformation with *cis* Pro–Pro amide bonds, the cation interacting with $CO^7$, $CO^3$, $CO^6$ and $CO^8$ amide carbonyl oxygens situated approximately in the apices of a square. This is illustrated in Fig. 32 by the crystalline structure of $Li^+$·antamanide.[329,330] The $CO^2$, $CO^4$, $CO^7$ and $CO^9$ carbonyls oriented towards the symmetry axis may also be involved in weak ion-dipole interaction. It is of interest that the $CO^3$ and $CO^8$ carbonyl ligands are at the same time proton acceptors involved in 4 → 1 H-bonding with $NH^1$ and $NH^6$. The antamanide cavity is markedly smaller than that of valinomycin. This and the peculiarities of the electronic structure of the amide ligands[118,333] account for the high Na/K selectivity of antamanide in the complex formation. Besides, antamanide has a much slower complexing rate than valinomycin, due to the high energy barrier for the conformational rearrangements characteristic of Pro-containing peptides.[334]

A distinguishing feature of the equimolar complexes of antamanide in comparison with those of enniatin B or valinomycin, is that only part of the ion solvent sheath in the former is supplanted by the ligands of the macrocycle. For instance, $Li^+$ bound to antamanide retains a solvate molecule of acetonitrile (Fig. 32), and $Na^+$ complexed with [$Phe^4$, $Val^6$]antamanide is in contact with a molecule of ethanol.[329,331] The relative accessibility of the cation to the environment in complexes of the antamanide cyclopeptides is also reflected in their ability to form contact ion pairs, as found on extracting an aqueous solution of sodium picrate with methylene chloride containing

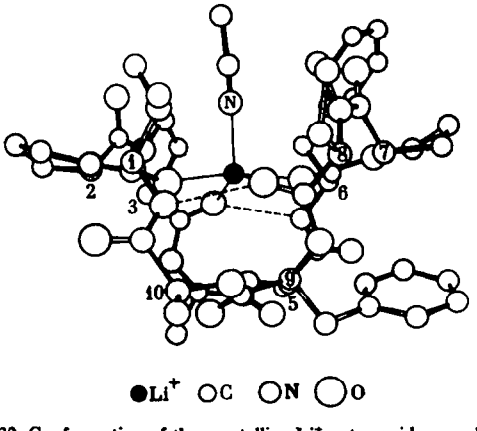



Fig. 32. Conformation of the crystalline $Li^+$·antamanide complex Some side chains are omitted for clarity. $\phi_1$ −115°, $\psi_1$ 138°, $\omega_1$ 178°; $\phi_2$ −65°, $\psi_2$ 139°, $\omega_2$ −3°; $\phi_3$ −33°, $\psi_3$ 147°, $\omega_3$ −173°; $\phi_4$ −67°, $\psi_4$ −14°, $\omega_4$ 176°; $\phi_5$ −84°, $\psi_5$ −6°, $\omega_5$ −178°; $\phi_6$ −123°, $\psi_6$ 139°, $\omega_6$ −171°; $\phi_7$ −74°, $\psi_7$ 144°, $\omega_7$ −3°; $\phi_8$ −69°, $\psi_8$ 144°, $\omega_8$ −176°; $\phi_9$ −78°, $\psi_9$ −78°, $\omega_9$ −15°; $\phi_{10}$ −88°, $\psi_{10}$ 7°, $\omega_{10}$ 173°.

†See pp. 35–41 in Ref. [14].

antamanide or perhydroantamanide (an analog with the phenyl groups replaced by cyclohexyls).[129]

Recent CD and NMR studies[129] revealed the ability of antamanide to form not only equimolar but also sandwich complexes with $Na^+$ and $Ca^{2+}$, thus providing further evidence of the unsaturated character of the metal ion's coordination sphere in the equimolar complex. The underlying structural principle proposed for the 2:1 complexes is shown in Fig. 33.

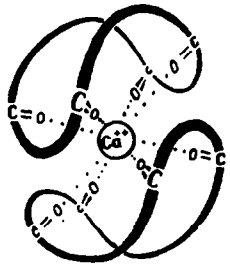


Fig. 33. Possible structure of antamanide sandwich complexes.

The results described lead to the following proposal[129] for the mode of action of antamanide the basic principle of which is schematically shown in Fig. 34. Binding the $Ca^{2+}$ or $Na^+$ ions sorbed on the membrane surface, antamanide "covers" quite considerable areas of the latter (250–300 Å$^2$ according to molecular models), thereby modifying its properties, including its permeability to the *Amanita phalloides* toxins. Since the antitoxic action of antamanide is stereospecific (*enantio*-antamanide is less potent by an order of magnitude than the natural antitoxin[335]), the protein components of the membrane apparently also participate in the binding process. The most probable type of interaction is stacking of the aromatic groups of the protein with the phenyl groups of antamanide, inasmuch as substitution of the latter by cyclohexyls as in perhydroantamanide, while practically without effect on the complex stability, causes complete loss of the antitoxic potency.[312] It is interesting that ionophoric properties concurrently appear so that like the naturally occurring ionophores perhydroantamanide inhibits microbial growth and stimulates the outflow of $Na^+$ or $K^+$ ions from lecithin liposomes; antamanide itself lacks these properties.[317] Thus the intimate study of the peoperties of two outwardly biologically very different classes of compounds (ionophores and antitoxins) has dramatically disclosed a very much related type of action.

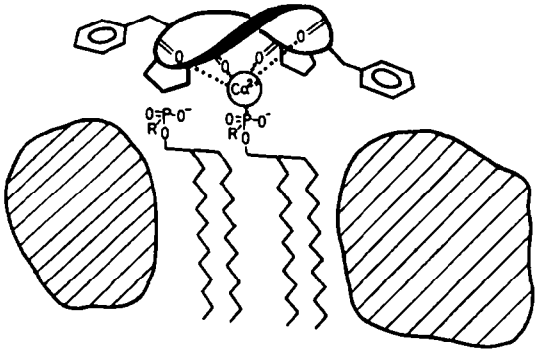


Fig. 34. Proposed principle of antamanide interaction with a biomembrane (hatched areas refer to protein globuls).

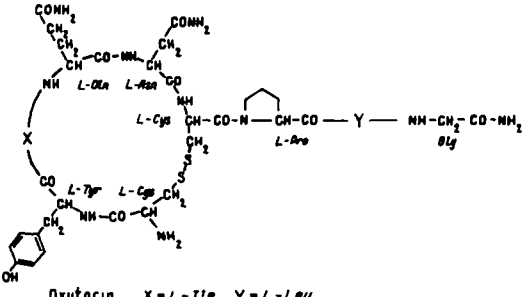
†See Refs. [348–366] for discussions.

### 8. OXYTOCIN AND VASOPRESSINS

Oxytocin and vasopressins manifest a wide variety of physiological effects. At least some of these are due to augmentation of the cyclic adenosine-3',5'-monophosphate level through activation of the adenylcyclase system responsible for its synthesis from ATP. The primary act is, thus, most likely, directed upon the protein receptor.[336–340]

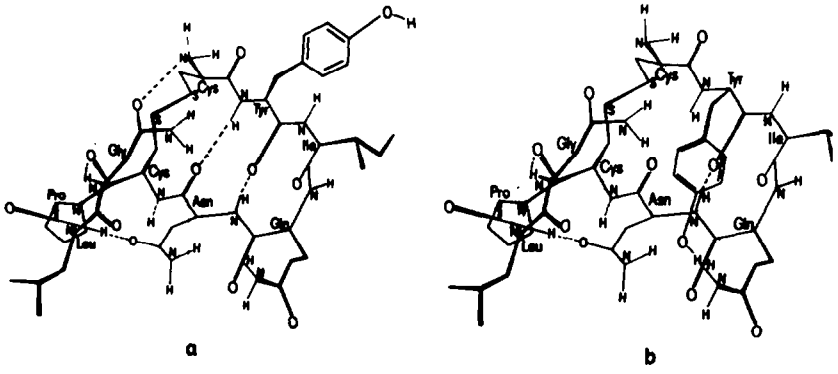
Oxytocin was the first peptide hormone to be synthesized,[341] and this was followed in the next two decades by synthesis of over two hundred analogs of the above two hormones. Now study of their structure-activity relation is in full swing. The data obtained heretofore are summarized in the monograph[2] and reviews.[4–7,342–344]

Theoretical analysis of the oxytocin conformation has revealed the possibility of numerous energetically similar conformers.[345–347] NMR-$^1$H studies of oxytocin, its deamino-, $^{15}$N- and deuterated analogs in various media have led to the tentative proposal of the formation of several intramolecular H-bonds (Fig. 35a).† The H-



Oxytocin, X = L-Ile, Y = L-Leu

Lys$^8$-Vasopressin, X = L-Phe, Y = L-Lys



a b

Fig. 35. Hypothetic conformations of oxytocin with five intramolecular H-bonds (a) and on binding with the receptor (b).

bonding tendency decreases on replacing the $CH_3OH$-$(CD_3)_2SO$ (7:3) or trifluoroethanol solvent by $(CD_3)_2SO$ and then by $D_2O$ ($H_2O$). Among the H-bonds shown in Fig. 35a two, $\alpha$-$NH_{Asn} \rightarrow CO_{Tyr}$ and $NH_{Gly} \rightarrow CO_{Cys}$, belong to the frequently encountered $4 \rightarrow 1$ type, whereas the others, $NH_{Cys} \rightarrow \alpha$-$CO_{Asn}$, $NH_{Leu} \rightarrow \beta$-$CO_{Asn}$ and $H_3N^+ \rightarrow CO_{Gly}$, have no analogies in oligopeptide systems. The last is quite poorly supported experimentally, while the most stable (judging from the temperature gradients and deuterium exchange rates) is the $\alpha$-$NH_{Asn} \rightarrow CO_{Tyr}$ bond. Walter *et al.*[353,367] believe that in the "biologically active" conformation, oxytocin has three out of the five aforementioned H-bonds (Fig. 35b). Moreover, the tyrosine hydroxyl is considered to be interacting with the $\omega$-amide functions of Asn or/and Gln. A major share in the stabilization of this structure is due to the asparagine residue which attaches to itself a considerable portion of the hormone molecule by covalent or H-bonding. Hence the exceptionally high sensitivity of the oxytocin potency to modifications in this position. According to the above authors, the Ile, Gln, Pro and Leu side chains are responsible for the interaction with the receptor.

The NMR-$^1H$[368] and NMR-$^{13}C$[369-375] studies of oxytocin and related disulfides, although interesting from a methodological standpoint, are not very revealing conformationally. However, measurements of $^{13}C$ spin-lattice relaxation times[376,377] have shown the C-terminal Gly residue and the oxytocin Ile, Gln and Leu side chains to have high flexibility in water and dimethyl sulfoxide.

X-ray analysis of the crystalline C-terminal tetrapeptide Cys(Bzl)-Pro-Leu-Gly-$NH_2$[378,379] revealed a $NH_{Gly} \rightarrow CO_{Cys}$ H-bond (Fig. 36a), but solutions of the same peptide contain a considerable fraction of conformers with a *cis* X-Pro bond absent in oxytocin itself.[380] As shown in Fig. 36b, the crystals of a shorter C-terminal peptide, Pro-Leu-Gly-$NH_2$ show the presence of a H-bond in a different position, namely, $NH_2 \rightarrow CO_{Pro}$.†[381] Also differing from the hormone in conformational characteristics are tocinamide and deaminotocinamide, analogs of the cyclic moiety of the hormone which lack the transannular H-bonds discussed above and in $(CD_3)_2SO$ probably have a new type of intramolecular H-bonding (Fig. 37).†[383] Hence the interaction between the cyclic and linear moieties of oxytocin seems to be an essential factor in the stabilization of its "biologically active" conformation.

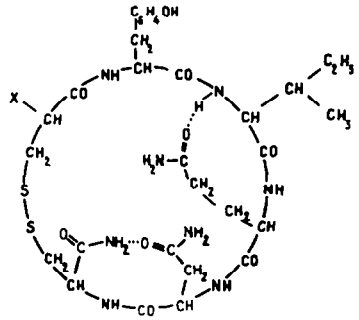


Fig. 37. Tocinamide (X=$NH_2$) and deamino tocinamide (X=H); dashed lines indicate the proposed H-bonds.

Several authors have assumed right-handed helicity of the C–S–S–C fragment,[384-386] but Donzel *et al.*[387] have pointed out that the left handed alternate cannot be excluded. The conformations of the $Lys^8$- and $Arg^8$-vasopressin should be similar to that of oxytocin although some differences do exist in their chemical shifts and $^3J(H\text{-}NC^{\alpha}\text{-}H)$ constants in dimethyl sulphoxide. On the other hand the peptide side chain of oxytocin has a tendency to fold over the cyclic framework, whereas in vasopressin it is more flexible owing to repulsion of the protonated amino groups.[355,356,358,388] This is supported by the higher rate of dialysis of oxytocin through a cellophane membrane.[389] An additional characteristic of $Lys^8$-vasopressin is the stacking of the aromatic rings in aqueous solutions.[353,390] The conformational properties of $Arg^8$-vasotocin seem to be intermediate between oxytocin and $Arg^8$-vasopressin.[388]

One may thus discern certain regularities in the spatial structures of oxytocin, $Lys^8$-vasopressin and related compounds. However, further experimental work and theoretical conformational analysis is needed to shed light on the various conformational details.

†For energy computations of the tripeptide see Ref. [382].

‡In the same work the H-bonds $\alpha$-$NH_{Asn} \rightarrow \beta$-$CO_{Gln}$ were postulated for oxytocin but this does not conform to the aforementioned flexibility of the Gln side chain.

### 9. OTHER NATURALLY OCCURRING CYCLOPEPTIDES OF UNKNOWN MOLECULAR TARGET

1. *Tentoxin.* This toxin inhibits chlorophyll synthesis and photophosphorylation,[391,392] and causes severe

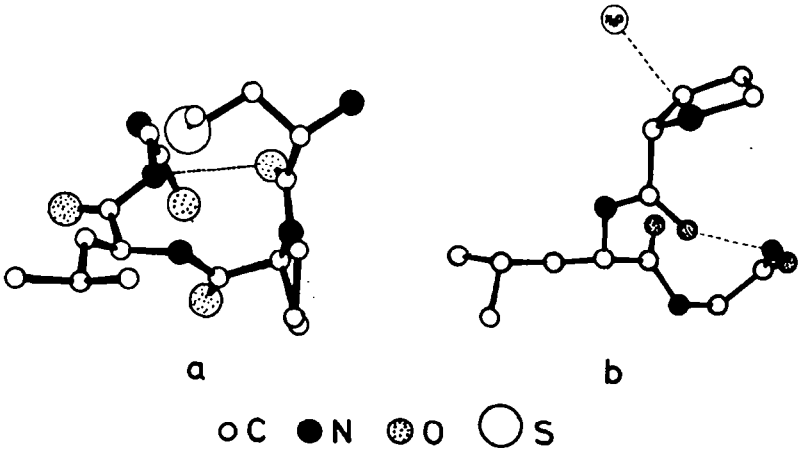



Fig. 36. Conformations of the crystalline Cys(Bzl)ProLeuGlyNH$_2$ (a), the phenyl group not shown and ProLeuGlyNH$_2$ (b).

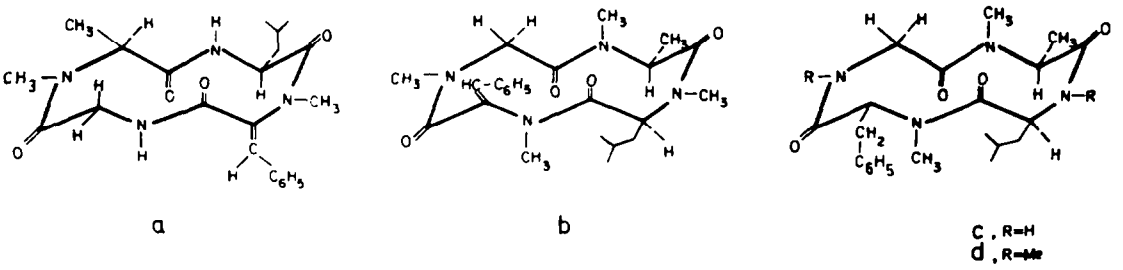


Fig. 38. Conformations of tentoxin (a), dihydrotentoxin (c), N,N-dimethyltentoxin (b) and N,N-dimethyldihydrotentoxin (d) in chloroform as deduced from NMR spectra.

chlorosis when applied to germinating seeds.[391,393,394] Determination of the structure of tentoxin and of its dihydro- and N-methylated derivatives by chemical and spectral methods led to contradictory results.[395-397] The formulas shown in Figs. 38 and 39 were substantiated by the X-ray analysis of dihydrotentoxin[398] and by the total synthesis of tentoxin.[399] A characteristic feature of the structures in Figs. 38 and 39 is the alternating *cis-trans-cis-trans* configuration of the amide bonds.[397,398] Two tertiary amide bonds in structures 38a, 38b and 38d are *cis* which is what one would have expected bearing in mind that this is also the case for other cyclic N-methylated tetrapeptides[400-403] and tetradepsipeptides.[404-406] On the other hand most unexpected are the *cis* configurations of the secondary amide bonds in dihydrotentoxin (Figs. 38c and 39). As yet this lacks an explanation.

2. *Serratamolide* is a 14-membered cyclotetradepsipeptide built of alternating L-serine and D-β-hydroxydecanoic acid residues.[407,408] Serratamolide does not complex alkali metal ions[14] and only weakly affects the $K^+$ permeability of lipid bilayers.[409] Although serratamolide induces some $K^+$ release and $H^+$ uptake in *Staphylococcus aureus*[409] it seems to owe its antimicrobial effect (lysis of the cellular membrane) mainly to its surfactant properties.[410]

Figure 40 shows the conformation of serratamolide (a) and its *meso*-analog (b) which is in accord with its IR and NMR data.[411] It has a twofold symmetry axis, all *trans* ester and amide groups, the serine hydroxyls form H-bonds with the carbonyls of the same residues; the compound in Fig. 40b is centrosymmetric.

The *bis*-cyclic derivative of serratamolide in Fig. 41 is believed to have four intercyclic H-bonds, forming the "wall" of a cylindric conformation,[412] a structure indirectly supported by X-ray analysis disclosing such

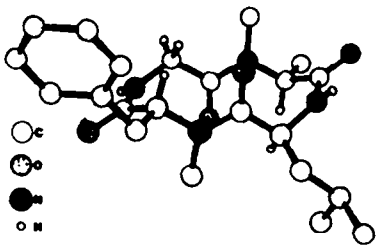


Fig. 39. Conformation of crystalline dihydrotentoxin.

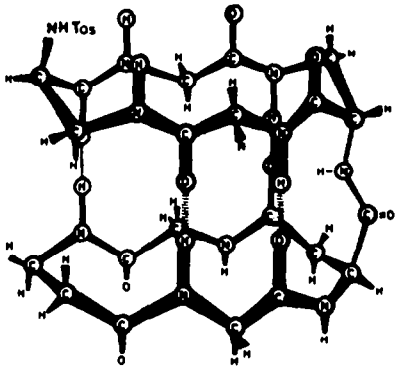



Fig. 41. Hypothetic conformation of "bis-serratamolide".

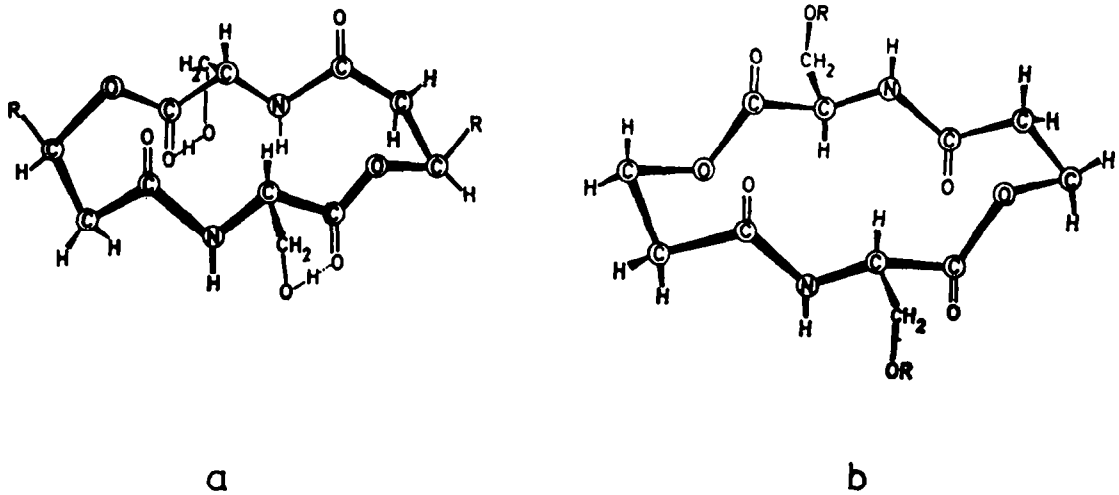
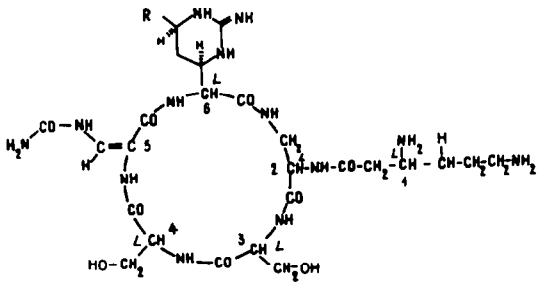


Fig. 40. Conformations proposed for serratamolide (a, R=$C_7H_{15}$) and its *meso*-analog (b, R=But) in chloroform.

intercyclic H-bonds in crystalline cyclo[-L-Ser(OBu$^t$)-β-Ala-Gly-L-β-Asp(OMe)-].[413]

3. *Viomycin and tuberactinomycin O.* Viomycin has found a certain restricted use in treating tuberculosis.[1,414] Its mode of action is apparently inhibition of protein synthesis at the stage of amino acid transfer from the tRNA-ribosome complex.[415,416] The X-ray structures of



R=H, tuberactinomycin O; R=OH, viomycin

these two antibiotics display similar spatial arrangements.[417-420] All amide bonds in the 16-membered ring are in *trans* planar configuration and there is a "β-loop" at 2-3-4-5 site; the extended side chain does not interact with the cyclic backbone (Fig. 42). The 4→1 H-bonded 10-membered ring is quite stable in aqueous solution, as follows from the low rate of NH→ND exchange in $D_2O$.[421]

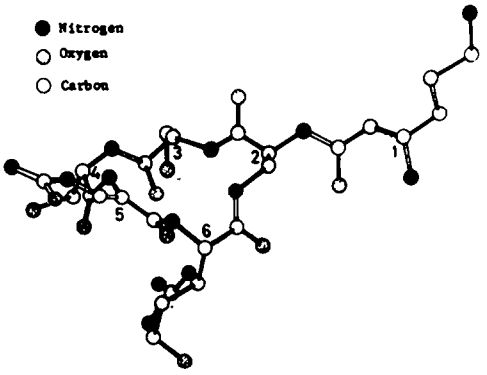
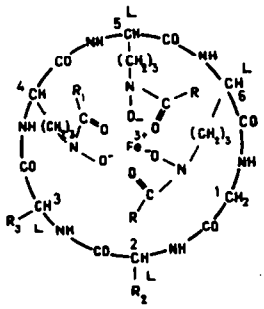



Fig. 42. Conformation of crystalline viomycin.

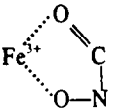
4. *Ferrichromes.* Trihydroxamate ferric complexes produced by various microorganisms are called siderochromes.[422] Some of them, are antimicrobial and these are usually known as sideromycins; other, inhibiting the antibiotic effect of the sideromycins and even stimulating microbial growth are known as sideramines. The biological role of sideramines is apparently associated with transport of iron ions in the cell.[423-426] The sideramines include a group of cyclic hexapeptides called ferrichromes as well as the linear compounds. The former have a large common fragment of three $N^\delta$-acylated $N^\delta$-hydroxyornithine residues, whose side chains serve as ligands in the $Fe^{3+}$ complex. Removal of the iron ions from the ferrichromes yields what are called desferri-ferrichromes, whose interaction with $Al^{3+}$ or $Ga^{3+}$ leads to the respective alumichromes or gallichromes.[427-429]

The three-dimensional structure of crystalline ferrichrome A has been established by X-ray analysis,[430,431] whereas desferri-ferrichromes, alumichromes and gallichromes have been studied in the molecular state by NMR spectroscopy;[427-429,432,433] the parent ferrichromes are not



| Compound | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Ferrichrome | $CH_3$ | H | H |
| Ferricrocin | $CH_3$ | $CH_2OH$ | H |
| Ferrichrysin | $CH_3$ | $CH_2OH$ | $CH_2OH$ |
| Ferrirhodin |  | $CH_2OH$ | $CH_2OH$ |
| Ferrirubin |  | $CH_2OH$ | $CH_2OH$ |
| Ferrichrome A |  | $CH_2OH$ | $CH_2OH$ |

amenable to the high resolution NMR technique because their paramagnetic ion causes considerable signal broadening. The rate of H→T exchange of the NH group has been studied for the case of ferrichrome, ferrichrome A and desferri-ferrichrome A.[522]

The peptide skeleton of crystalline ferrichrome A contains a 4→1 H-bond between the carbonyl of the $Ser^4$ and the NH of the $Orn^1$ residues (Fig. 43). Ferrichrome A also possesses an H-bond between the NH of $Orn^2$ and the NO oxygen of the latter's side chain. The ligand oxygens are at the apices of an octahedron surrounding the $Fe^{3+}$; the 5-membered  rings form a lefthand propeller (Fig. 44).

Ferrichromes, alumichromes and gallichromes evidently assume similar conformations in solution. Evi-

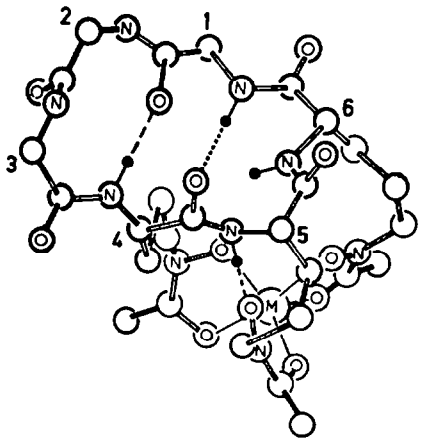


Fig. 43. Conformation of ferrichromes, alumichromes, and gallichromes. Dashed lines show the intramolecular H-bonds found in crystalline ferrichrome A ($\phi_1$ −145°, $\psi_1$ 20°; $\phi_2$ −77°, $\psi_2$ 131°; $\phi_3$ −105°, $\psi_3$ −175°; $\phi_4$ −163°, $\psi_4$ −6°; $\phi_5$ −57°, $\psi_5$ −149°; $\phi_6$ 82°, $\psi_6$ 178°; $\omega_{1-6}$ ~ 180°). Dotted line shows the additional H-bond found in solutions.

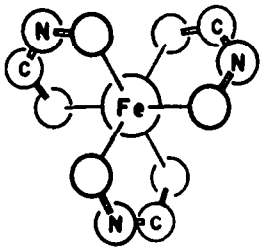


Fig. 44. Coordination of hydroxamate groups around the $Fe^{3+}$ ion in crystalline ferrichrome A.

dence for this among other facts is the agreement between the $\phi$ values represented in the footnote to Fig. 43 and those determined from the respective $^3J(H\text{-}NC^{\alpha}\text{-}H)$ constants. However, the low exchange rate of the NH protons in positions 1, 2, 3 and 4 and their low $\Delta\delta/\Delta T$ values indicate the presence of a third H-bond, namely $NH(Ser^4)\cdots CO(Orn^1)$ as shown in Fig. 43. In this conformation the cyclopeptide backbone is in the form of a "pleated sheet". The $Orn^3$ amide proton appears to be shielded from the solvent by the side chains of the substituted ornithine residues that owe their rigid position to participation in the complexing process. Judging from the chemical shifts and the deuterium exchange rates, consecutive Gly→Ser substitution, in other words passing from alumichrome to alumicrocin, and then to alumichrysin increases the strength of the H-bonding.

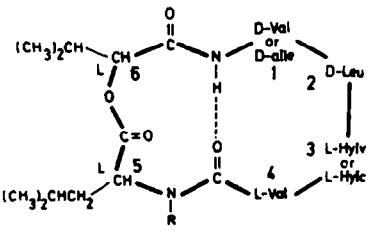
Removal of the metal ion removes the restriction imposed on the side chains of the ornithine residues, considerably augmenting the overall flexibility of the cyclopeptide. In desferri-ferrichromes the "pleated sheet" with its two transannular H-bonds is to be found only in organic solvents (dimethyl sulphoxide, dimethyl sulphoxide–chloroform mixture). In water, all the NH protons have a deuterium exchange rate several orders of magnitude higher in desferri-ferrichromes than in ferrichrome A, and the NH groups in the former do not differ in their $\Delta\delta/\Delta T$ values. Apparently under such conditions there exists an equilibrium mixture of several forms differing in the number and position of the intramolecular H-bonds.

5. *Sporidesmolides I–IV and related depsipeptides.* The spicules on the spores of the fungi *Pithomyces chartarum* consist of neutral lipophilic cyclodepsipeptides namely sporidesmolides I, II, III and IV, sporidesmolide I being the major component.†

In polar solvents (methanol, dimethyl sulphoxide) sporidesmolides I–IV possess a single intramolecular bond, most probably stabilizing the 4-5-6-1 "$\beta$-turn" (Fig. 45). In chloroform sporidesmolide I assumes a more compact, rigid structure in which all the NH groups form $CO\cdots NH$ H-bonds (Fig. 46); a similar conformation is assumed in chloroform by sporidesmolides II–IV.[434]

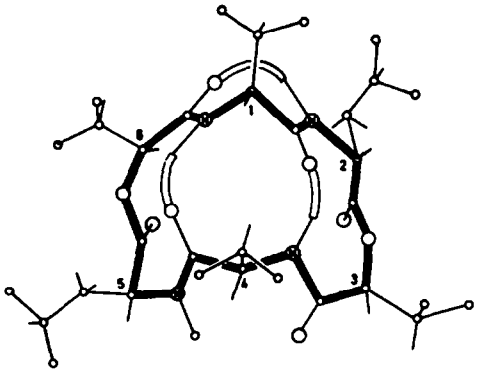
The biological role of sporidesmolides is unknown. They do not bind alkali metal ions in solution[14] nor inhibit bacterial growth.[436] The chemical inertness of sporidesmolides, their hydrophobicity and thermal stability (at temperatures above 250° and $10^{-3}$ mm pressure they sublime without degradation) lead to the surmise that perhaps they may play a part in protection of the spore surface. If so, the flexibility of the sporidesmolides could facilitate their interaction with the other surface components.

†See pp. 22–23 and cited references in Ref. [14].




| Sporidesmolide | 1 | 3 | R |
|---|---|---|---|
| I | D-Val | L-Hyiv | Me |
| II | D-alle | L-Hyiv | Me |
| III | D-Val | L-Hyiv | H |
| IV | D-Val | L-Hyic | Me |

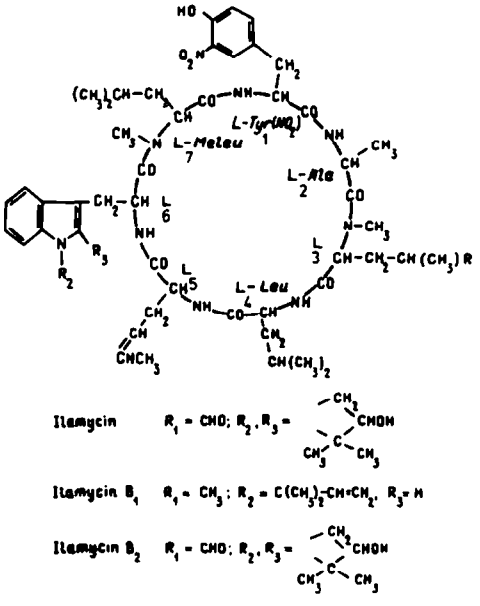
Fig. 45. $\beta$-turn in sporidesmolides I–IV found in polar solvents.



○C ○○ ⊗N ▭H-bond

Fig. 46. Conformation of sporidesmolide I in non-polar solvents, $\phi_1 -60°$, $\psi_1$ 60°; $\phi_2$ 80°, $\psi_2 -80° \pm 20°$; $\phi_3 -60° \pm 20°$, $\psi_3 -20°$; $\phi_4 -120°$, $\psi_4$ 90°; $\phi_5$ 40°, $\psi_5 -90° \pm 30°$; $\phi_6 -100° \pm 40°$, $\psi_6 -10° \pm 20°$, $\omega_{1-6} \sim 180°$.

The synthetic $C_2$-symmetric sporidesmolide analogs form exceptionally stable "pleated sheet" structures, as clearly follows from their IR, CD and NMR spectra.[435] Their conformations (Fig. 47) remain practically unchanged on passing from heptane to aqueous-ethanol mixtures, and the stability of their transannular H-bonds depends only slightly on the configurational pattern of the



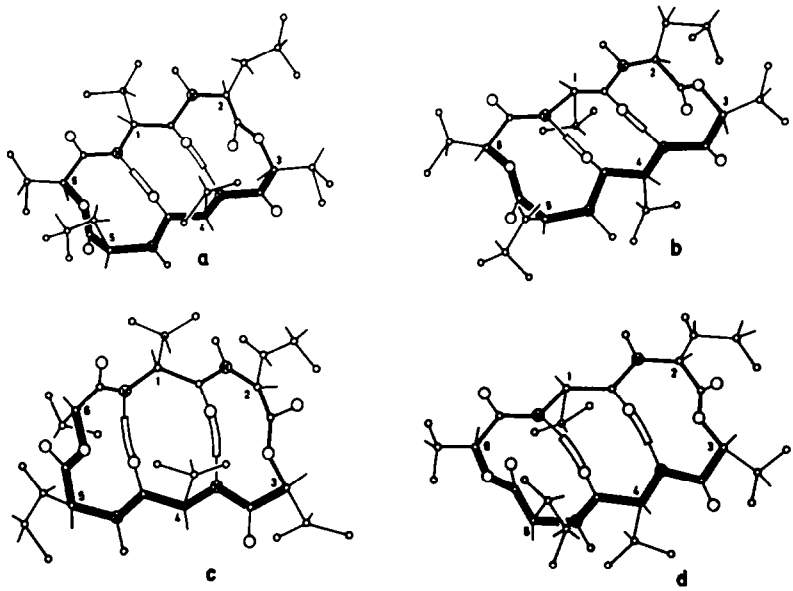



Fig. 47. Conformations of synthetic sporidesmolide analogs. a, cyclo[-(L-Val-L-MeLeu-L-HyIv)$_2$-]; b, cyclo[-(D-Val-L-MeLeu-L-HyIv)$_2$-]; c, cyclo[-(L-Val-L-MeIle-D-HyIv)$_2$-]; d, cyclo[-(D-Val-L-MeIle-D-HyIv)$_2$-].

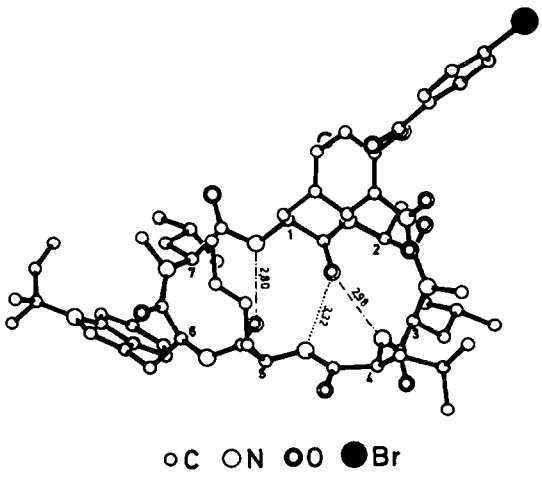
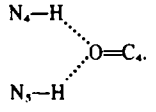


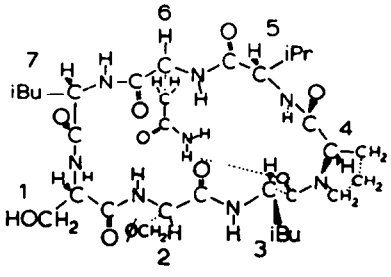

Fig. 48. Crystalline conformation of ilamycin $B_1$ p-bromobenzoate $\phi_1 - 156°$, $\psi_1$ 120°, $\omega_1 - 175°$; $\phi_2 - 61°$, $\psi_2$ 126°, $\omega_2 - 11°$; $\phi_3 - 121°$, $\psi_3$ 38°, $\omega_3 - 168°$; $\phi_4 - 123°$, $\psi_4$ 2°, $\omega_4$ 165°; $\phi_5 - 163°$, $\psi_5$ 168°, $\omega_5$ 171°; $\phi_6 - 86°$, $\psi_6$ 117°, $\omega_6$ 1°; $\phi_7 - 128°$, $\psi_7$ 99°, $\omega_7 - 179°$. Figures indicate the length of H-bonds, Å.

molecule. The behaviour of these compounds indicates that the presence of hydroxy acid residues in the peptide chain creates favourable conditions for the formation of "$\beta$-turns" a property which Nature has successfully made use of in the construction of the valinomycin molecule (see Section 4.1).

6. *Ilamycins* are antibiotics belonging to the few (see also evolidine, in the following section) homodetic cycloheptapeptides built solely of L-$\alpha$-amino acid residues.[437-441]

The conformation of the crystalline ilamycin $B_1$ derivative is shown in Fig. 48.[442] One can discern here two $4 \rightarrow 1$ intramolecular H-bonds, but the 10-membered rings they close differ from the usual $\beta$-turns by the *cis* N-methylamide bonds found in this antibiotic. Another, still more interesting structural peculiarity is that the carbonyl of its modified tyrosine residue simultaneously participates in two intramolecular H-bonds:



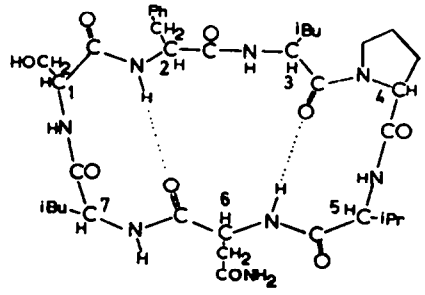
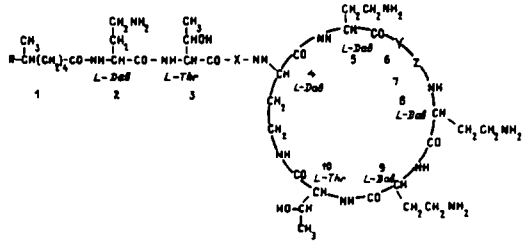




Fig. 49. H-bonds proposed for conformations I[454] ($\phi_1 - 110°$, $\psi_1$ 40°; $\phi_2 - 150°$, $\psi_2$ 160°; $\phi_3 - 60°$, $\psi_3$ 150°; $\phi_4 - 70°$, $\psi_4 - 50°$; $\phi_5 - 150°$, $\psi_5$ 80°; $\phi_6 - 170°$, $\psi_6$ 180°; $\phi_7 - 45°$, $\psi_7 - 40°$; $\omega_{1-3,5-7} \sim 180°$, $\omega_4 \sim 0°$), Ia[455] ($\phi_1 - 120°$, $\psi_1$ 60°; $\phi_2 - 150°$, $\psi_2$ 150°; $\phi_3 - 70°$, $\psi_3$ 120°; $\phi_4 - 60°$, $\psi_4 - 55°$; $\phi_5 - 90°$, $\psi_5$ 90°; $\phi_6 - 170°$, $\psi_6$ 150°; $\phi_7 - 60°$, $\psi_7 - 60°$; $\omega_{1-3,5-7} \sim 180°$, $\omega_4 \sim 0°$) and II[455] ($\phi_1$ 60°, $\psi_1$ 60°; $\phi_2 - 90°$, $\psi_2$ 120°; $\phi_3$ 180°, $\psi_3$ 120°; $\phi_4 - 60°$, $\psi_4 - 55°$; $\phi_5 - 90°$, $\psi_5$ 60°; $\phi_6 - 170°$, $\psi_6 - 60°$; $\phi_7 - 70°$, $\psi_7$ 150°; $\omega_{1-7} \sim 180°$) of evolidine.

Thus the existence of such bonds in cyclopeptides postulated earlier on the basis of the IR spectra of several model cyclohexapeptides[65] has now received direct confirmation.

The ilamycin $B_1$ NH NMR parameters in $(CD_3)_2SO$ are more or less in agreement with the H-bonding occurring in the crystal, although in solution the strongest H-bonds are due to $N_{(1)}H$ and $N_{(5)}H$, and in the crystal to $N_{(1)}H$ and $N_{(4)}H$.[443]

7. *Evolidine*, a cyclic heptapeptide of unknown biological function has been isolated from the leaves of *Evodin xanthoxyloides* (a tree growing in northern Australia).[444–446] It has been investigated by a number of chemical methods[447–450] and synthesized in several ways.[451–453] The NMR data of evolidine[454] are on the whole similar to those for ilamycin $B_1$, which should not be surprising as their primary structures strongly resemble



| Antibiotic | R | X | Y | Z | References |
|---|---|---|---|---|---|
| Polymyxin $A_1$ | $C_2H_5$ | D-Dab | D-Leu | L-Thr | 456 |
| Polymyxin $A_2$ | $CH_3$ | D-Dab | D-Leu | L-Thr | 456 |
| Polymyxin $B_1$ | $C_2H_5$ | D-Dab | D-Phe | L-Leu | 457 |
| Polymyxin $B_2$ | $CH_3$ | D-Dab | D-Phe | L-Leu | 458 |
| Polymyxin $D_1$ | $C_2H_5$ | D-Ser | D-Leu | L-Thr | 459,460 |
| Polymyxin $D_2$ | $CH_3$ | D-Ser | D-Leu | L-Thr | 460 |
| Polymyxin $E_1$ | $C_2H_5$ | L-Dab | D-Leu | L-Leu | 458,461,462 |
| Polymyxin $E_2$ | $CH_3$ | L-Dab | D-Leu | L-Leu | 462 |
| Circulin A | $C_2H_5$ | L-Dab | D-Leu | L-Ile | 463,464 |
| Circulin B | $CH_3$ | L-Dab | D-Leu | L-Ile | 465 |

each other. However quite different spatial structures have been suggested for evolidine (Fig. 49) between which no final choice can so far be made. Conformers I and Ia have a *cis* Leu-Pro amide bond.

8. *Polymyxins.* Heretofore the structures of ten antibiotics of this group have been established. All these compounds are strongly basic cyclolinear nonapeptides containing 5–6 $\alpha,\gamma$-diaminobutyric acid residues, the N-terminal amino acid being acylated by (+)-6-methyloctanoic (Ipel) or isooctanoic (Ioc) acid.

In the presence of polymyxins, the cell membranes of sensitive microorganisms no longer function as permeability barriers. From what is known about these antibiotics one may conclude that both ionic and hydrophobic forces are involved when they interact with the lipoprotein membrane complex.[466–472]

It was found from the $\Delta\delta/\Delta T$ values[112] and the rate of NH proton exchange that in aqueous solution polymyxins B, E and circulin[473–475] have 3–5 NH groups screened from the solvent. As a result a number of conformations have been proposed for these antibiotics, a common feature of which is the existence of "$\beta$-loops" (Figs. 50 and 51a). It should be noted, however, that a linear peptide L-Dab-L-Thr-L-Dab-L-Dab-D-Phe-L-Leu-L-Dab obtained from subtilopeptidase A[476] hydrolysis of polymyxin B has approximately the same deutero-exchange rates in water as the parent antibiotic.[477] The authors[477] therefore concluded that polymyxins apparently have no intramolcular H-bonds, nor any preferably ordered structures in aqueous solution. Obviously more work is required for resolution of this discrepancy.

9. *Bacitracin A* is a major component of a complex antibacterial mixture. Although known since 1945[1,2] its formula has been established only recently.[450,478,479]

Stone and Strominger[480] believe that the biological action of bacitracins[481] is the result of their complexing $C_{55}$-isoprenylpyrophosphate in the cell membrane with concomitant inhibition of the dephosphorylation reaction required for regeneration of the lipid carrier in biosynthesis of cell wall peptidoglucan. Bivalent metal ions ($Zn^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Co^{2+}$, $Cd^{2+}$, $Cu^{2+}$, $Mg^{2+}$ or $Ca^{2+}$) may also be involved in the complexing reaction, as the antibiotic is more active in the presence of salts of these metals.[482] Other mechanisms, involving transition metal

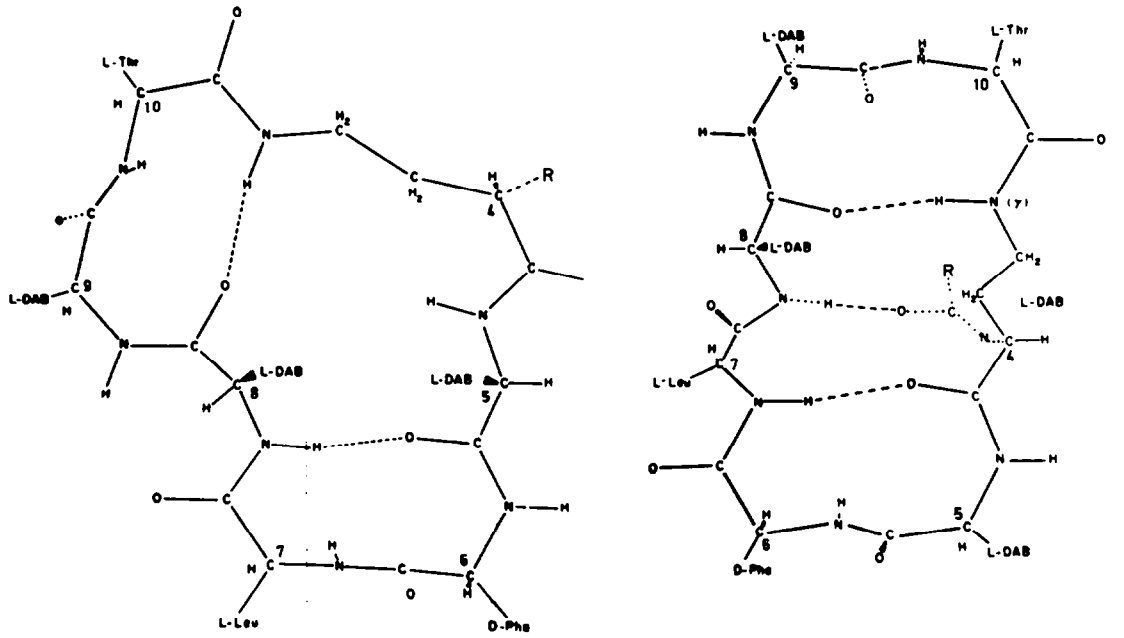


Fig. 50. Intramolecular H-bonds proposed by Urry[112] for the cyclic moiety of polymixin B (R stands for the linear moiety).

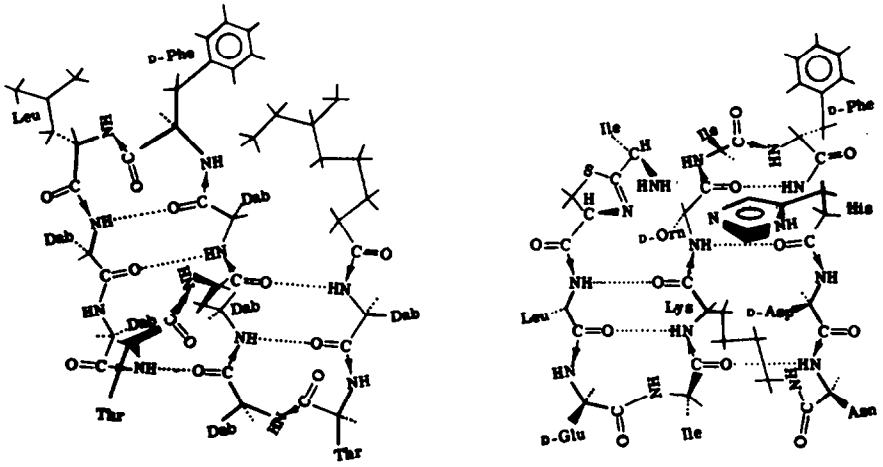
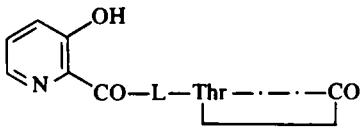


Fig. 51. Intramolecular H-bonds proposed by Chapman and Golden[475] for polymyxin B(a) and bacitracin (b).

ions, particularly $Mn^{2+}$, are currently being discussed in the literature.[482,483]

In the complex of bacitracin A with zinc ions, both the imidazole and thiazoline rings act as ligands, showing that they must be in close proximity. The ORD curves of the free and complexes antibiotic are similar, evidence of their having similar conformations.[484-485] It is also known that bacitracin has five amide protons capable of slow exchange with water.[479] On the basis of data on the polymyxin antibiotics and taking into account the findings on bacitracin A and oxytocin, Chapman and Golden[475] have postulated a general tendency for peptides of this type of fold over the cyclic backbone with formation of additional H-bonds. Figure 51b shows such a spatial structure of bacitracin A.

10. *Vernamycin B and patricin A.* A large group of peptide antibiotics inhibiting the functioning of ribosomes in protein biosynthesis[486-489] have the following common fragment:



They are thus heterodetic cyclopeptides (frequently called "peptide lactones") with an ester bond formed by the threonine hydroxyl and the C-terminal carboxyl; the threonine amino group is acylated by 3-hydroxypicolinic acid.[1-6,490]

The members of this group, vernamycin B and patricin A, have been subjected to NMR study in dimethyl sulphoxide and from the $\Delta\delta/\Delta T$ values and the deuterium exchange rates, Urry[112] has suggested that the latter of these two 19-membered cyclodepsipeptides has a system of intramolecular H-bonds; of the many such possible systems, one is shown in Fig. 52. Despite similarity of the primary structures the two antibiotics differ markedly in the NH deuterium exchange rates whence follows that their spatial structures must be different. We believe that such a conclusion requires more experimental grounding, particularly if one bears in mind that the deuterium exchange conditions for vernamycin B and patricin A are not identical.

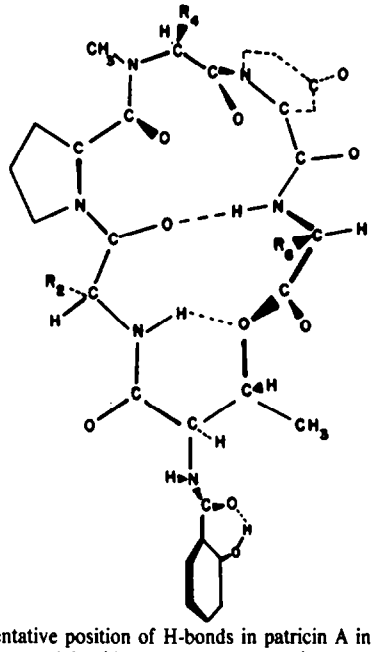


Fig. 52. Tentative position of H-bonds in patricin A in dimethyl sulphoxide ($R_2$=Et, $R_4$=Me, $R_6$=Ph).

11. *Stendomycin and telomycin.* In continuation of their studies on cyclopeptide antibiotics, Urry *et al.* have carried out a NMR investigation of stendomycin[491] and telomycin,[492,493] compounds in all likelihood among the most complicated peptidic subjects to have been explored by this method. Solvent screened NH groups were revealed, which, therefore, apparently are participating in intramolecular H-bonding, and, on the basis of the vicinal $^3J(H\text{-}NC^{\alpha}\text{-}H)$ constants showing the internal rotation of the NH–CH fragments to be restricted probable conformations of these compounds such as the types shown in Figs. 53 and 54 have been proposed. The linear fragment of the stendomycin molecule is possibly in a left handed helical conformation which if so, could explain the similarity of the Cd curves of stendomycin and the left-handed helix of poly-D-glutamic acid.[494]

Despite a number of common structural features for instance, formation of an ester bond by the threonyl hydroxyl and the C-terminal carboxyl these two antibio-

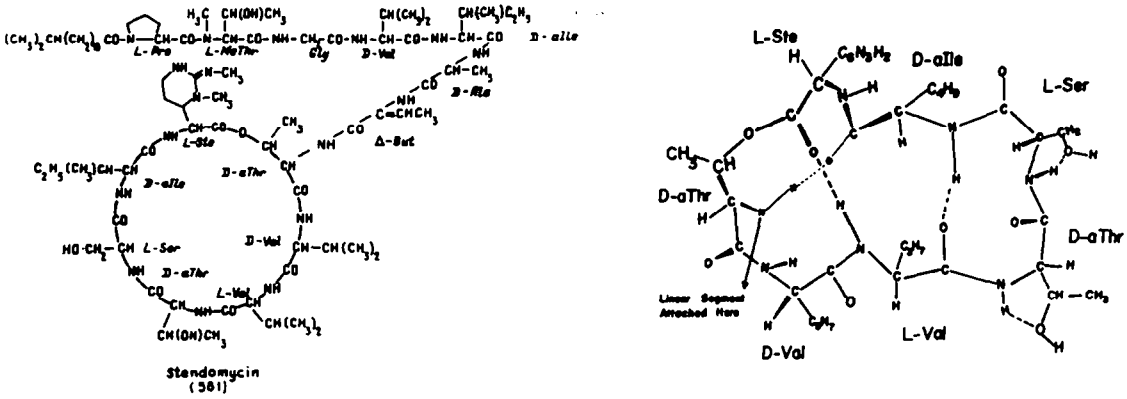



Fig. 53. Formula and proposed H-bonded system of stendomycin.

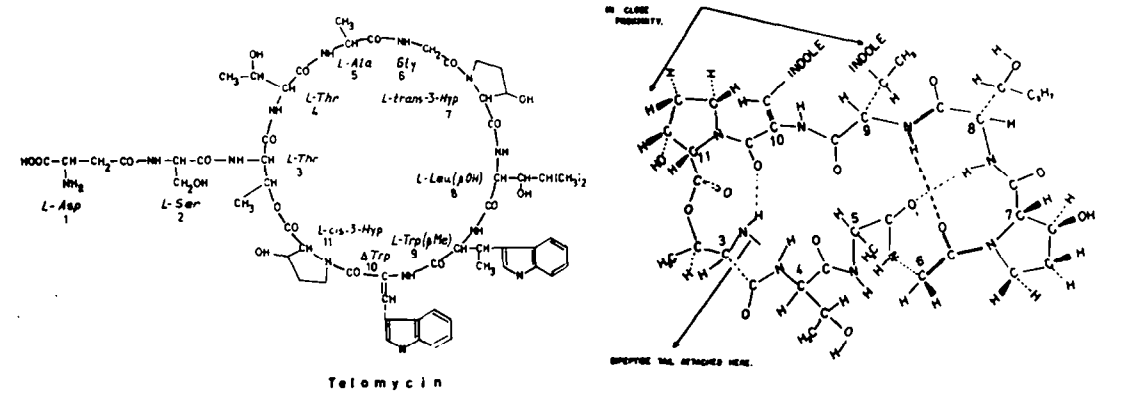



Fig. 54. Formula and proposed H-bonded system of telomycin.

tics apparently differ fundamentally in their mode of action. Thus, telomycin appears to damage cellular membranes,[495] whereas stendomycin, according to a brief note by Urry,[496] channels across the latter. How much effects are associated with the primary and spatial structure of the antibiotics is still one of the unsolved problems in this enticing field of bioorganic chemistry.

### 10. CYCLOPEPTIDES AS SUBSTRATES AND INHIBITORS OF PROTEOLYTIC ENZYMES

The stereochemistry of the peptide–enzyme interaction has now become one of the important objectives of conformational analysis. That the spatial arrangement of peptides markedly affect their substrate and inhibitor properties may be regarded as a truism. At the same time the detailed analysis of the structure-function relations of substances as flexible as linear peptides is not an easy matter. The more rigid cyclic peptides are much more amenable to study from such a standpoint (but see introductory remarks). Attempts to find proteolytic enzymes that could catalyze the hydrolysis of cyclopeptides have for long been unsuccessful. As illustration mention can be made of the resistance of Lys-containing diketopiperazines to the action of trypsin[497,498] and Phe-containing diketopiperazines and diketomorpholines to the action of α-chymotrypsin,[499] apparently because of the *cis*-configuration of their amide bonds. Gramicidin S[500] and tyrocidines[501] (see Section 6) do not undergo hydrolysis in the presence of the common proteases such as pepsin, α-chymotrypsin and papain, a fact apparently due to their containing D-amino acids. Oxytocin and Lys$^8$-vasopressin are resistant to the action of α-chymotrypsin, while their analogs with less constrained rings undergo cleavage of the peptide bonds in the cyclic moiety.[502] The cyclic pentapeptide cyclo(-Gly-Gly-L-Lys-Gly-L-Lys-) is trypsin-resistant,[503] the hexapeptide cyclo[-(Gly-L-Phe-L-Leu)$_2$-] is resistant to pepsin and cathepsin A and cyclo(-Gly-Gly-L-Phe-L-Phe-Gly-L-Lys-) (3) is resistant to pepsin;[504] the first two cyclopeptides do not inhibit reactions catalyzed by the respective enzymes.

Further study has shown that cyclic hexapeptides (1)–(3) may be hydrolyzed, although rather slowly, by trypsin and (8)- by α-chromotrypsin (Table 2). The effect of the conformational factors on the substrate properties of a series of cyclopeptides (4)–(7), containing the same L-Leu-L-Tyr region was very pronounced: the 18-membered cyclopeptides (4) and (5) cannot be hydrolyzed by α-chymotrypsin and pepsin, whereas the 24-membered (6) is a substrate for both enzymes; the 30-membered (7) is hydrolyzed still more rapidly. The differences in the binding energies of the four cyclopeptides to the active center of α-chymotrypsin are within an order of magnitude, as judged from the Michaelis constants, $K_M$, and inhibition constants, $K_I$ (Table 2); the higher hydrolysis rate is mainly due to increase in the rate constant, $k_{cat}$. The value of $k_{cat} = 9{\cdot}53\ sec^{-1}$ found for the decapeptide (7) is higher than any known rate constant for α-chymotryptichydrolysis of the peptide bond in oligopeptides.[506] Cyclo(-Gly$_4$-L-Leu-L-Tyr-) (4) in various solvents assumes the rigid "pleated sheet" structure, with H-bonds formed by oppositely situated glycine residues and the L-Leu-L-Tyr region, responsible for binding to pepsin or α-chymotrypsin, is in the form of a "β-turn" (Fig. 56c).[505] The L-Leu-L-Tyr region of cyclopeptide (5)

Table 2. Kinetic constants of the interaction of cyclopeptides with proteolytic enzymes

| N | Compound[a] | Rate constant $K_M$ (mM) | Michaelis constant $K_M$ (mM) | $k_{cat}/K_M$ ($M^{-1}.sec^{-1}$) | Inhibition constant $K_I$ (mM) | Ref. |
|---|---|---|---|---|---|---|
| | | | Trypsin | | | |
| 1 | cyclo(-$Gly_4$-*L-Lys-Gly*-) | ~0·04 | ~4·8[b] | ~0·8 | — | 506, 510 |
| 2 | cyclo[-(Gly-*L-Lys-Gly*)$_2$-] | 0·08 | 4·8 | 1·7 | — | 506, 508 |
| 3 | cyclo(-$Gly_2$-L-Phe-L-Phe-Gly-L-Lys-) | 0·043 | 10 | 4·3 | — | 504 |
| | | | Pepsin | | | |
| 3 | cyclo(-$Gly_2$-L-Phe-L-Phe-Gly-L-Lys-) | | No hydrolysis | | 10[c] | 504 |
| 4 | cyclo(-$Gly_4$-*L-Leu-L-Tyr*-) | | No hydrolysis | | — | 505, 507 |
| 5 | cyclo(-$Ave_2$-*L-Leu-L-Tyr*-)[d] | | No hydrolysis | | — | 505, 507 |
| 6 | cyclo(-$Gly_6$-*L-Leu-L-Tyr*-) | $1·5.10^{-4}$ | 1·6 ± 0·4 | 0·94 | — | |
| 7 | cyclo(-$Gly_8$-*L-Leu-L-Tyr*-) | $5·0.10^{-4}$ | 1·8 ± 0·2 | 0·28 | — | |
| | | | α-chymotrypsin | | | |
| 8 | cyclo[-(Gly-*L-Tyr-Gly*)$_2$-] | ~0·01 | ~10[e] | ~1 | — | 506, 509 |
| 4 | cyclo(-$Gly_3$-L-Leu-*L-Tyr-Gly*-) | — | — | <0·7 | 3·6 ± 0·3[f] | 505, 506 |
| 5 | cyclo(-Ava-L-Leu-*L-Tyr-Ava*-)[d] | | No hydrolysis | | 3·5 ± 0·4[f] | 505, 506 |
| 6 | cyclo(-$Gly_5$-L-Leu-*L-Tyr-Gly*-) | 0·54 | 15·4 | 35·1 | 8·5 ± 1·5[f] | 505, 506 |
| 7 | cyclo(-$Gly_7$-L-Leu-*L-Tyr-Gly*-) | 9·53 | 13·2 | 722 | 25 ± 5·6[f] | 505, 506 |

[a] In the italicized fragments the amide bond either undergoes or is expected to undergo splitting.
[b] Assumed by analogy with $K_M$ of compound (2).
[c] Obtained with $Gly_2$-$Phe_2$-Gly-Lys as a substrate.
[d] Ava - δ-amino valeric acid, $H_2N$–$CH_2$–$CH_2$–$CH_2$–$CH_2$–COOH.
[e] Assumed by analogy with other substrates, including compounds (6) and (7).
[f] Obtained with Ac-L-Tyr-OMe as a substrate.

is in a similar conformation. Cyclooctapeptide (6) and cyclodecapeptide (7) have much more flexible structures. The latter was suggested to have a conformation, in which the H-bonding system resembles that of gramicidin S (Fig. 55, see also Figs. 26–28). However, whereas the position of the H-bonds is fixed in the antibiotic due to the specific features of its primary structure it appears to be an equilibrium mixture of several different "pleated sheets" with the L-Leu and/or L-Tyr residues located in the extended moieties of the chain rather than in the "β-turn" (Fig. 56b).

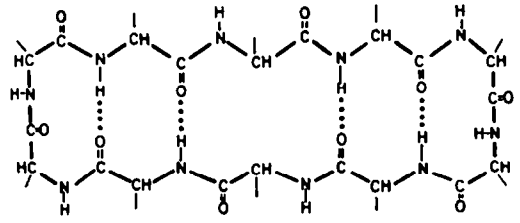


Fig. 55. H-bonds in the predominant conformations of cyclo(-$Gly_8$-L-Leu-L-Tyr-).

The above described data together with the X-ray picture of α-chymotrypsin,[511–515] its complexes with inhibitors[516] and also of the α-chymotryptic products of peptide chloromethyl ketones,[517] have led to a scheme for the binding of cyclopeptides to the active site of α-chymotrypsin. Figure 56a shows the active site of α-chymotrypsin, in which the imidazole ring of the His (57) residue is alkylated by Ac-L-Ala-L-Ala-L-Phe-$CH_2Cl$. If binding of the p-hydroxyphenyl group of cyclopeptides (4), (5), (6) and (7) in the "tosyl hole" is indispensable and the peptide chain should be located as shown in Fig. 56a Ac-L-Ala-L-Ala-L-Phe for the effective complex formation and subsequent catalysis of cyclopeptides, then one may understand why compounds (4) and (5) with the Tyr residue in position 3 of the "pleated sheet" cannot meet these two requirements simultaneously (Fig. 56c). Indeed, if the tyrosine side chain is attached in the "correct" manner the isobutyl group will be oriented somewhat differently from the side chain of the middle alanine residue so that the peptide chain as a whole cannot be in the arrangement shown in Fig. 56a. As a result, only one, out of the three, H-bonds between the oligopeptide and the enzyme, shown in Fig. 56a can be realised. Moreover,

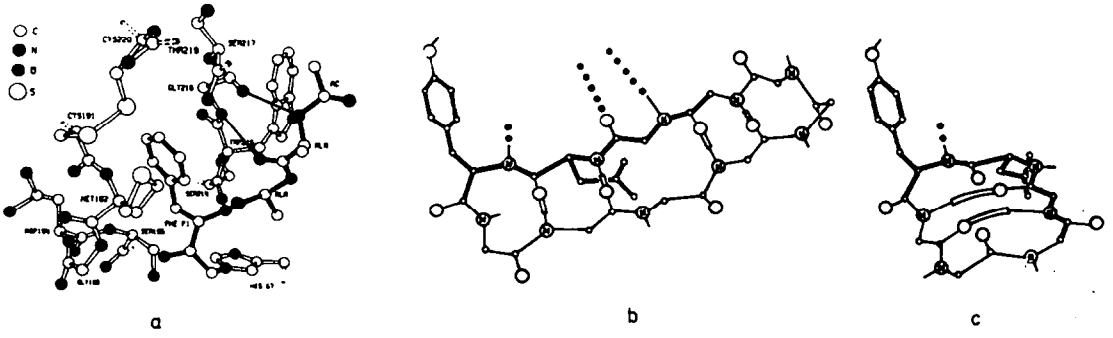


Fig. 56. The scheme of peptide binding of the active center at α-chymotrypsin. (a) Ac-L-Ala-L-Ala-L-Phe (according to [517]), (b) and (c) cyclopeptides (6) and (7) respectively.

the H-bonding of the Gly NH adjacent to tyrosine, restricts the mobility of the tyrosine carbonyl group, which may hinder subsequent formation of the acyl-enzyme.

On the other hand, cyclopeptide (7) fits "correctly" to the active site with respect to orientation of the Leu and Tyr side chains and its backbone conformation is such that all the necessary H-bonds can be realised (Fig. 56b). The high $k_{cat}$ value of cyclopeptide (7) is probably due to the fact that in this compound the "correct" (reactive) conformation of the peptide fragment responsible for binding is relatively rigid. In the structure shown in Fig. 56b, the position of the tyrosine residue corresponds to positions 2 and 5 in the cyclohexapeptide. Noteworthy from this standpoint is that the tyrosine residues in cyclo-[-(Gly-L-Tyr-Gly)$_2$-] (8) are located exactly in the same fashion;[518] and that this compound can be hydrolyzed by $\alpha$-chymotrypsin, although at a rather low rate[509] (Table 2).

Further study of the cyclic decapeptides may prove useful for better understanding of the intimate interactions of $\alpha$-chymotrypsin with polypeptides, its "natural" substrates.

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