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MOLECULAR STRUCTURE AND MECHANISMS OF ACTION OF CYCLIC AND LINEAR ION TRANSPORT ANTIBIOTICS

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As a direct result of the vision, determination, and magnetic personality of Yuri Ovchinnikov a collaboration between the Shemyakin Institute of Bioorganic Chemistry and the Medical Foundation of Buffalo was begun in the early 1970's. The collaboration generated valuable insight into the structural basis for the capture, transport, and release of ions by ion transport antibiotics and the basis for the ion selectivity of these compounds. The collaboration produced dozens of joint publications on the structure and function of cyclic and linear ion transport antibiotics, fostered fruitful exchange visits between scientists in the two Research Institutes and has been a major source of creativity in my scientific career and those of many of my colleagues in Buffalo. This review summarizes major accomplishments of the collaboration.

The transport of ions in and out of living cells is vital to many physiological processes. There is ample evidence that under normal circumstances ions are transported through channels created by proteins embedded in membranes [1]. Ionophores are antibiotics which induce ion transport across living and model membranes. They differ widely in ion specificity and in their apparent mode of action and include both polypeptides and polyethers as either linear or cyclic compounds.Most ionophores act as shuttle carriers or they form channels through membranes [2]. The shuttle carriers consist of cyclic or linear compounds, contain oxygen atoms in the form of ether linkages, hydroxy groups or carbonyl groups that are capable of providing a coordination sphere for a metal ion. In some cases more than one molecule is required to coordinate the metal ion leading to the formation of "sandwich" complexes. Following the coordination of an ion, minor conformational changes produce a species with a hydrophobic exterior which is capable of traversing

Abbreviation according to IUPAC-IUB Commission (Eur. J. Biochem. 1984. V. 138. P. 9—37) are used through out. Additional abbreviations: Lac — lactic acid, Hyi — α-hydroxyisovaleric acid.

the membrane. Release of the ion at the other side of the membrane completes the process [3]. The Shemyakin Institute of Bioorganic Chemistry established a major program in the synthesis of ionophores, the study of their transport properties, and the analysis of their conformations by NMR techniques. Because of the complexity of many of these structures they had a significant degrees of conformational flexibility and additional information on their three-dimensional structures was sought by X-ray crystallographic techniques. Scientists of the Shemyakin Institute were able to prepare diffraction quality crystals of many ion transport antibiotics that were a challenge to contemporary methods of X-ray crystal structure determination. Yuri Ovchinnikov recognized the need to gain expertise in the theory and practice of X-ray crystallography.

In the 1960's determination of noncentrosymmetric crystal structures of 40 or more nonhydrogen atoms continued to present an impossible challenge to most crystallographers. At the Medical Foundation of Buffalo, Herbert Hauptman was attempting to develop more powerful mathematical techniques that would make such structure determinations routine. Valinomycin, a compound composed of seventy-eight nonhydrogen atoms, was a widely studied ionophore. Since crystals of excellent diffracting quality of valinomycin could be grown easily, it was an ideal candidate to test Hauptman's methods of phase determination. The determination of the crystal structure of valinomycin in 1972 [4] was significant for at least two reasons. It was the largest all light atom (C, N, O) structure solved by direct phasing methods, and the conformation of valinomycin observed in the solid state had never been previously proposed or predicted. The structure contained a hydrogen bonding pattern not previously characterized, and it provided a plausible model for ion capture [4]. Shortly after the results of this study were published in 1972, during a visit to the United States, Yuri Ovchinnikov came to Buffalo to meet Herbert Hauptman and propose the beginning of collaborative studies between the Medical Foundation of Buffalo and the Shemyakin Institute.

Valinomycin

Valinomycin a cyclic dodecadepsipeptide, is highly selective for potassium ions [5]. The alternating amino acid, hydroxy acid sequence, which has the potential for possessing a three-fold rotation axis, is *cyclo*[-(*D*-Val-*L*-Lac-*L*-Val-*D*-Hyi)3-]. Infrared, NMR, Raman, and X-ray crystallographic studies have shown that the potassium ion complex exists in the solid state and in solution as a bracelet-shaped molecule (Fig. 1) [6–13]. The potassium ion lies in the center of the bracelet and is octahedrally coordinated by the carbonyl oxygens of the amino acid residues. The complex exhibits nearly perfect three-fold symmetry (Fig. 1). The conformation is stabilized by six 4→1 intramolecular hydrogen bonds [12,13]. These hydrogen bonds, Type II, when *L*-Val and *L*-Lac are at the corners of the β-bend, occur between the amide protons and the carbonyl oxygen atoms of the hydroxy acid residues. The overall shape of the complex is such that the oxygens and potassium ion are buried in the central portion of the complex while the exterior surface consists of a

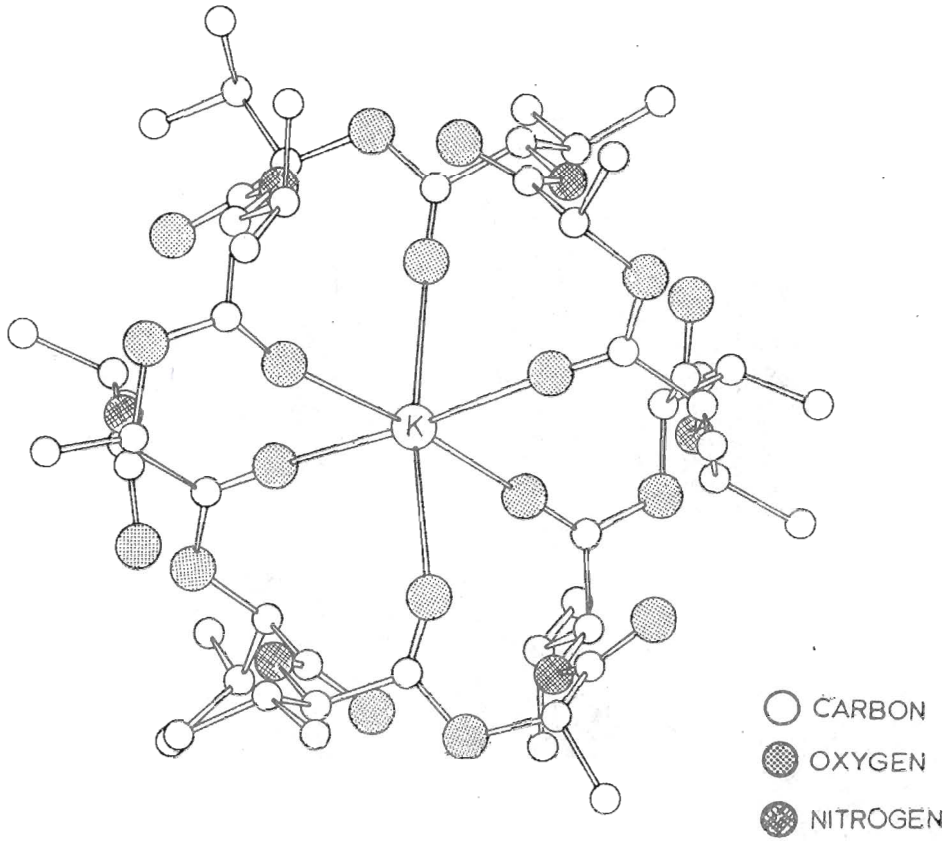

Fig. 1. The observed structure of the potassium complex of valinomycin

hydrophobic environment made up of the isopropyl and methyl side chains of the various residues.

While there has been agreement on the conformation of the complexed form of valinomycin, this has not been the case for the uncomplexed form. On the basis of spectral studies [7–9, 14], many different conformations have been postulated to exist in solution. Three of the models proposed to explain solution spectra are illustrated in Fig. 2. X-Ray crystallographic studies have been reported for four crystal forms of uncomplexed valinomycin [4, 15–18].

In the first of these determinations [4, 15, 16] the valinomycin molecule was found to take up a conformation that had not been previously suspected (Fig. 3). Instead of having pseudo-three fold symmetry, the molecule has only a pseudo-center of inversion if the differences in the side chains are ignored. Furthermore, instead of being stabilized by six 4→1 intramolecular hydrogen bonds, the molecule has only four such bonds. In addition, there are a pair of hydrogen bonds of a type never observed before, a 5→1 hydrogen bond. These two hydrogen bonds have the effect of elongating the molecule and place several oxygen atoms on the

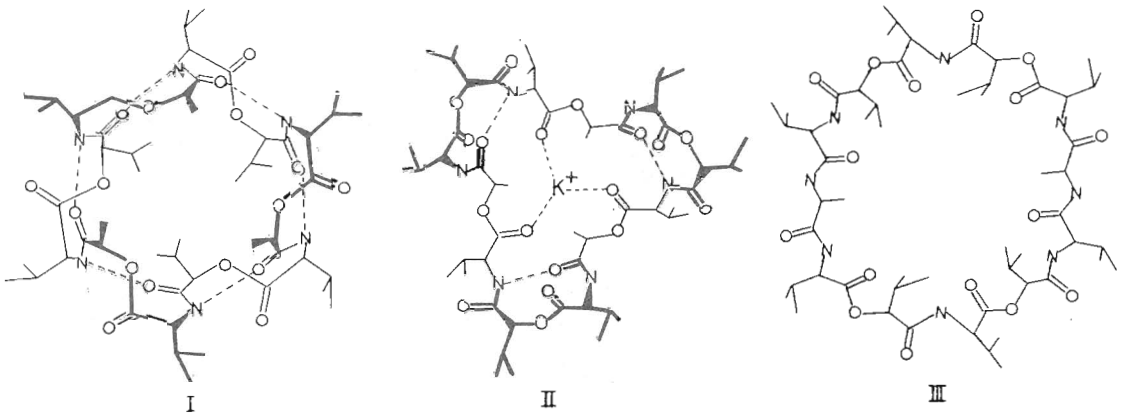

Fig. 2. Conformation of uncomplexed valinomycin proposed to satisfy solution state spectra: nonpolar solvents (I) octahedral cage with valine oxygens pointing outwards, slightly polar solvents (II) trigonal propeller, and the flexible bracelet conformer (III) that is proposed to exist in 4:1 dioxane-water above 30°C

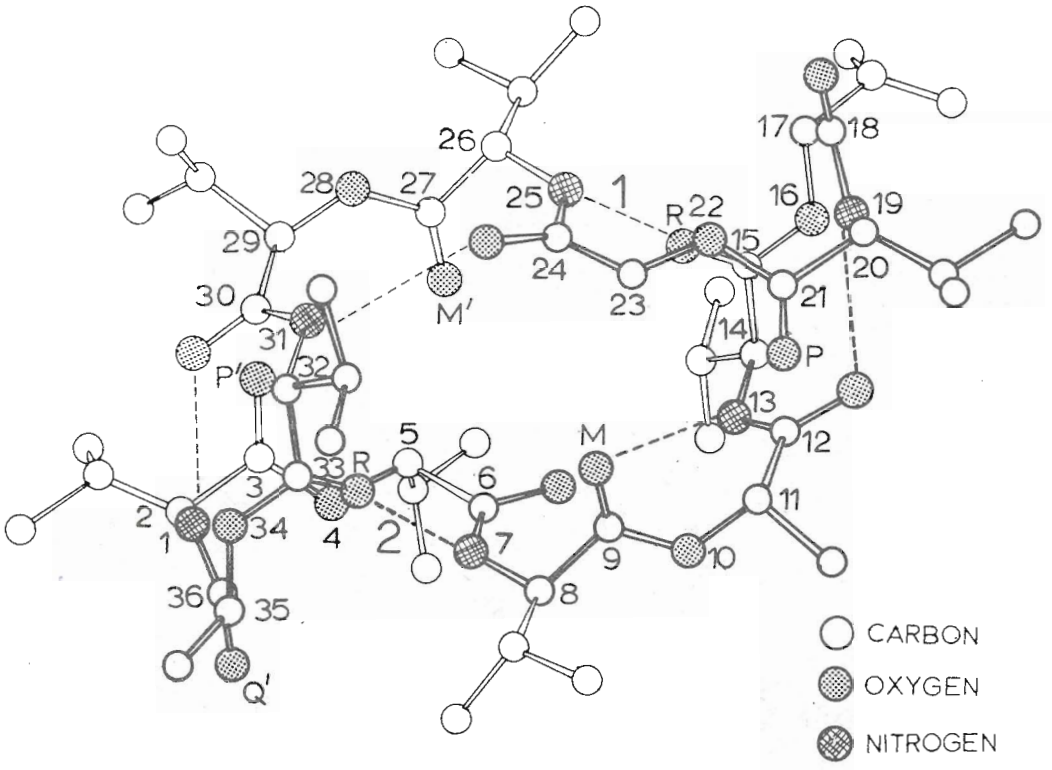

Fig. 3. The observed structure of uncomplexed valinomycin

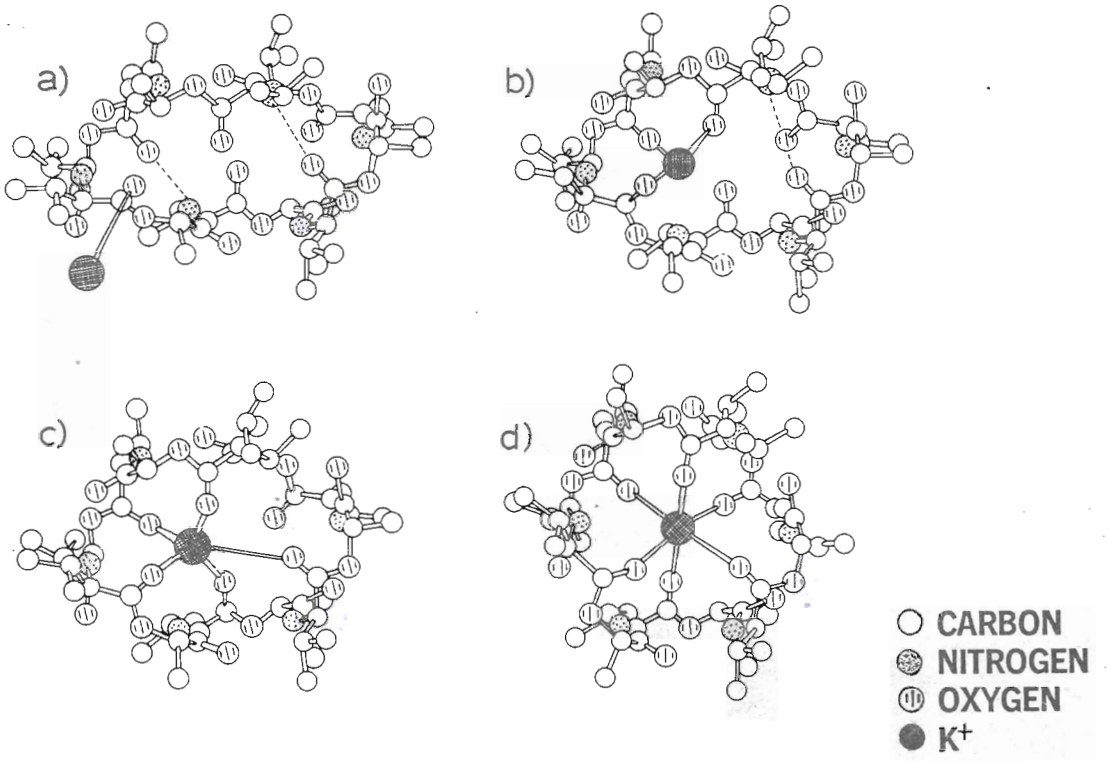

Fig. 4. The proposed steps of the complexation of potassium ion by valinomycin

surface of the molecule where they are ideally situated to initiate complexation to a potassium ion.

On the basis of the structures of the complexed and uncomplexed forms, we postulated a mechanism for ion capture [4, 15]. Because the conformation of the uncomplexed and complexed forms of valinomycin have pseudo-inversion centers, this center of symmetry was retained in the postulated intermediates. Shown in Fig. 4 are the computer-generated intermediates that illustrate the complexation of potassium ion by valinomycin. Four free ester carbonyls are in exposed positions at the surface of the uncomplexed molecule. One of these pairs of carbonyls may initiate complexation by displacing water molecules coordinated to a potassium ion (Fig. 4*a*). Once a loose complex is formed, the 5→1 type hydrogen bonds may be disrupted sequentially allowing the carbonyls to coordinate to the potassium (Fig. 4*b* and *c*). As the potassium ion is brought into full coordination (Fig. 4*d*), the molecule rounds out and the appropriate free carbonyl oxygens form the 4→1 hydrogen bonds observed in the complex. Further questions remain concerning the details of such a mechanism. There are two carbonyl oxygen atoms which can initiate the complexation process, one on either face. On one of these faces there are six isopropyl groups while on the other there are three methyl and three isopropyl groups. Since these two faces are different, the ease of access of potassium ions should differ for the two.

A second plausible model for the mechanism of ion capture by valinomycin is suggested by the conformation of the molecule observed in crystals obtained from dimethyl sulfoxide [17]. The structure is of type II (Fig. 2) and contains only three 4→1 hydrogen bonds. The disk shaped structure can be thought of as a flower having three petals. Ion complexation can be achieved by the closing of the flower petals around an ion as the solvent of coordination is replaced by coordination to carbonyl oxygen. When the "flower petals" close the cage structure is further stabilized by the formation of three additional 4→1 type hydrogen bonds.

When crystallized from aqueous dioxane, valinomycin molecules adopt two non-identical, octahedral, bracelet-shaped cage conformations in which water molecules complex in the ion binding cavity to deform the normal octahedral coordination geometry of the metal binding site in two distinct ways [18]. The crystallographically independent valinomycin molecules stack directly over one another and trap a dioxane molecule between them. The two faces of the cage structure differ. One face contains the isopropyl side chains of *L*-Val and *D*-Hyi residues whereas the other face, which contains the less bulky Lac residues, is slightly less crowded. In the crystals, hydrated valinomycin dimers come together with the valine-rich faces surrounding the dioxane (Fig. 5). Because these water filled stacked dimers have a length of approximately 20 Å, they suggest the possibility that valinomycin dimers might function as channel forming ionophores, and also illustrate how dimers of valinomycin might be designed as transport agents for small hydrophilic compounds.

Valinomycin Analogues

The structures of a series of valinomycin analogues are presented below. These structures include isoleucinomycin, which

differs from valinomycin by replacement of each *D*- and *L*-valine by *D*- and *L*-isoleucine residues, respectively; *meso*-valinomycin, which differs from valinomycin by replacement of *L*-Lac residues by *L*-α-hydroxyisovaleric acid residue, three variants on *meso*-valinomycin that differ in the chirality of two residues in each, and octaisoleucinomycin and hexadecaisoleucinomycin that have the four-residue template sequence repeated two and four times, respectively, rather than the three-fold repeat in the dodecadepsipeptide.

1.	Valinomycin	*cyclo*[-(*D*-Val-*L*-Lac-*L*-Val-*D*-Hyi)3-]
2.	Isoleucinomycin	*cyclo*[-*D*-Ile-*L*-Lac-*L*-Ile-*D*-Hyi)3-]
3.	*meso*-Valinomycin	*cyclo*[-(*D*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)3-]
4.	[*L*-Val[1],*D*-Val[3]]-*meso*-valinomycin	*cyclo*[-(*L*-Val-*L*-Hyi-*D*-Val-*D*-Hyi)-(*D*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)2-]
5.	[*L*-Val[1],*L*-Val[5]]-*meso*-valinomycin	*cyclo*[-(*L*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)2-(*D*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)-]
6.	[*D*-Hyi[2],*L*-Hyi[4]]-*meso*-valinomycin	*cyclo*[-(*D*-Val-*D*-Hyi-*L*-Val-*L*-Hyi)-(*D*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)2-]
7.	Octa-*meso*-valinomycin	*cyclo*[-(*D*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)2-]
8.	Octaisoleucinomycin	*cyclo*[-(*D*-Ile-*L*-Lac-*L*-Ile-*D*-Hyi)2-]
9.	Hexadecaisoleucinomycin	*cyclo*[-(*D*-Ile-*L*-Lac-*L*-Ile-*D*-Hyi)4-]

The crystal structure determination of isoleucinomycin, *cyclo*[-(*D*-Ile-*L*-Lac-*L*-Ile-*D*-Hyi)3-] [19], provides an example of a conformationally stable intermediate in the transition between the complexed and uncomplexed forms of valinomycin proposed in model I.The structure also provides a plausible answer to the question of which face of the molecule is most vulnerable to entry by potassium ions. The observed conformation of this molecule is shown in Fig. 6. One half of the molecule has the conformation and the intramolecular hydrogen bonding scheme of a molecule of the complexed form of valinomycin while the other half is similar to that of the uncomplexed form. Thus, there are five 4→1 intramolecular hydrogen bonds and one 5→1 hydrogen bond breaking the pseudo-symmetry observed in the parent forms.

In the isoleucinomycin structure the sole 5→1 hydrogen bond is closest to the *D*-Hyi-substituted face which consists primarily of hydrophobic side chains while three ester carbonyl oxygen atoms are in exposed positions on the *L*-Lac-substituted face. These three oxygen atoms are ideally positioned to coordinate an entering potassium ion during an intermediate stage of the complexation process. In fact, as shown in Fig. 7, there is a point in the plane of these three oxygens which is 2.78 Å from each of them (the average potassium-oxygen distance observed in the crystal structure of the complexed form of valinomycin). This would suggest that in complexing according to Model I, the complexing ion enters through the *L*-Lac substituted face, whereas in Model II this face is closed and the ion would approach from the other direction.

The substitution of *L*-Hyi for *L*-Lac in *meso*-valinomycin introduces the possibility of obtaining conformations that exhibit both true inversion symmetry and three-fold rotational symmetry (i.e. 3 or S_6 symmetry).

The structure of *meso*-valinomycin has been determined in two anhydrous crystal forms [20, 21] and one form obtained from aqueous

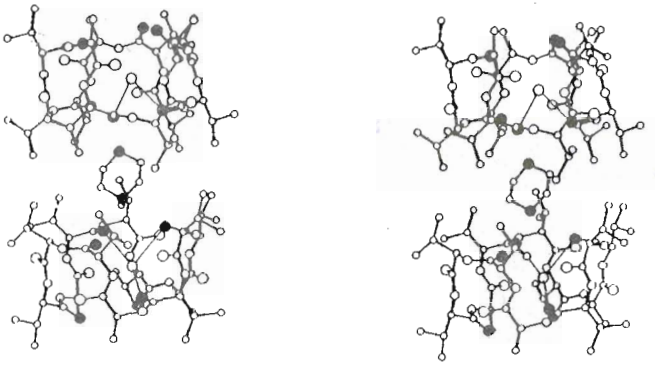

Fig. 5. Stereoview of one of the three dioxane molecules that is trapped between the two valinomycin hydrate cage complexes (the dioxane and valyl carbonyl oxygen atoms are shaded). Each oxygen atom of the dioxane makes contacts (3.36 ±12 Å) to the three *L*-valine carbonyl oxygen atoms that form an entrant face to the binding cavity of a neighboring complex. Ions and water molecules could enter or leave the cavity without disrupting the conformation of the ionophore. This arrangement of pairs of cages suggests that such an aggregate might form a channel and allow valinomycin to function as a channel as well as a shuttle carrier of ions

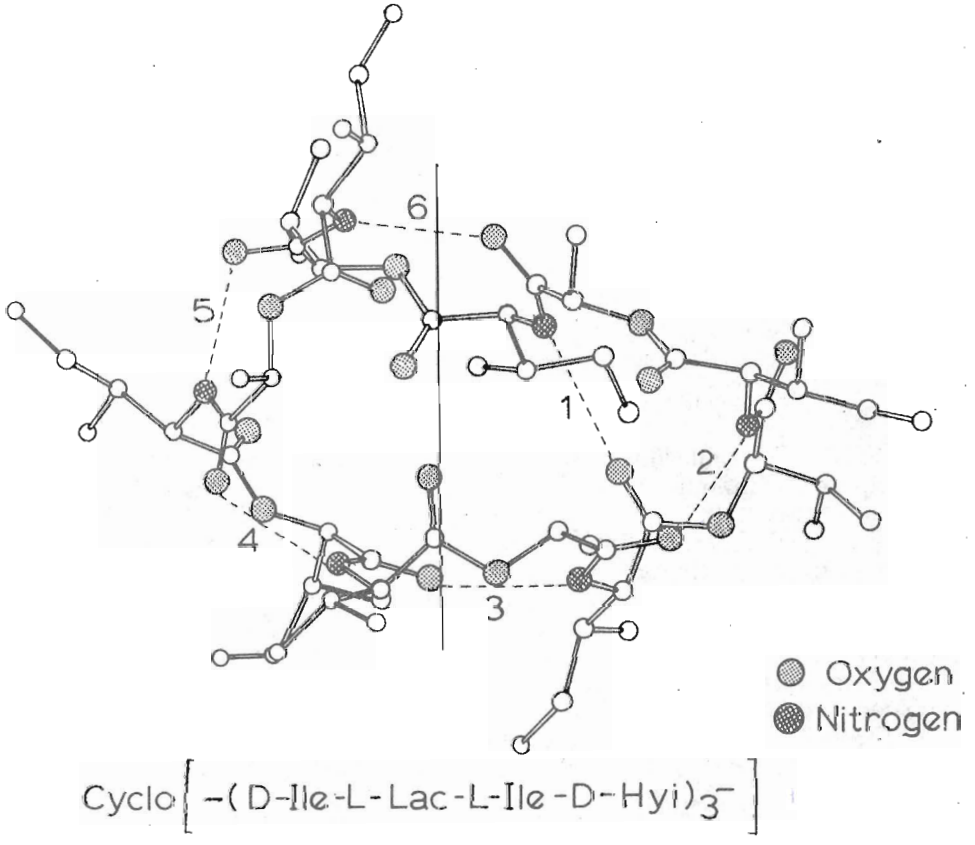

Fig. 6. The observed structure of isoleucinomycin. Dashed lines 2 through 5 are 4←1 hydrogen bonds; the dashed line labeled 1 is the 5←1 hydrogen bond

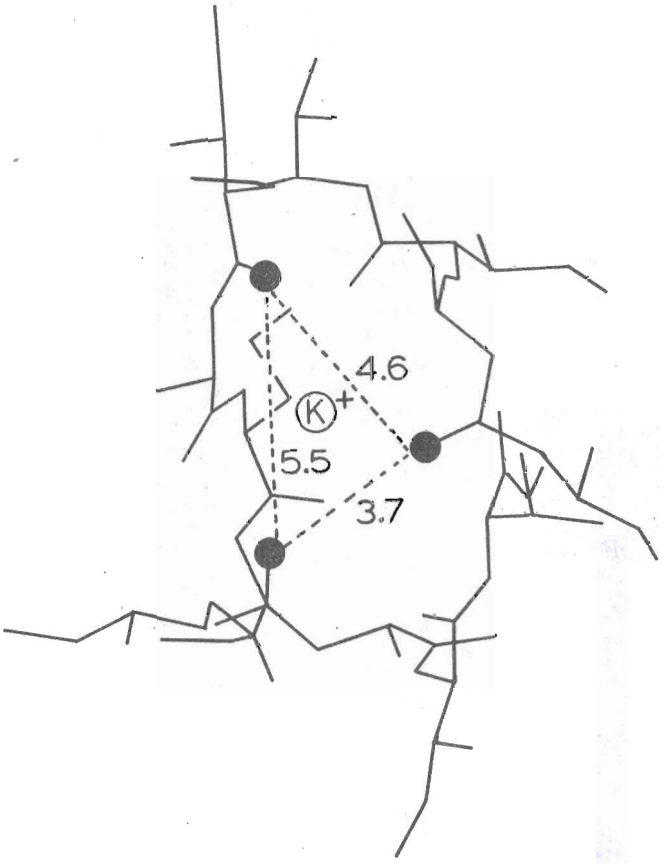

Fig. 7. The potential site of entry of a potassium ion illustrating the distances between the carbonyl oxygen atoms on the Lac face of isoleucinomycin

dioxane [22]. In all three structures the valinomycin molecules have crystallographic inversion centers, the bracelet form and six 4→1 hydrogen bonds similar to those found in the K^+ complexes of valinomycin. The substitution of *L*-Hyi- for *L*-Lac appears to stabilize this conformation. *meso*-Valinomycin has high-binding affinity for K^+ but is not as efficient at K^+ transport suggesting that the stabilization of the bracelet form may enhance binding and retard release of K^+ ions.

In crystals obtained from aqueous dioxane [22] the ion cavity is occupied by a disordered water molecule and dioxane molecules are sandwiched between translationally (and inversion) related molecules. This arrangement is similar to that found in valinomycin crystallized from aqueous dioxane (Fig. 5). Translationally related molecules of *meso*-valinomycin in the anhydrous triclinic form also form continuous channels lined by oxygen atoms but apparently unoccupied by ions or solvent.

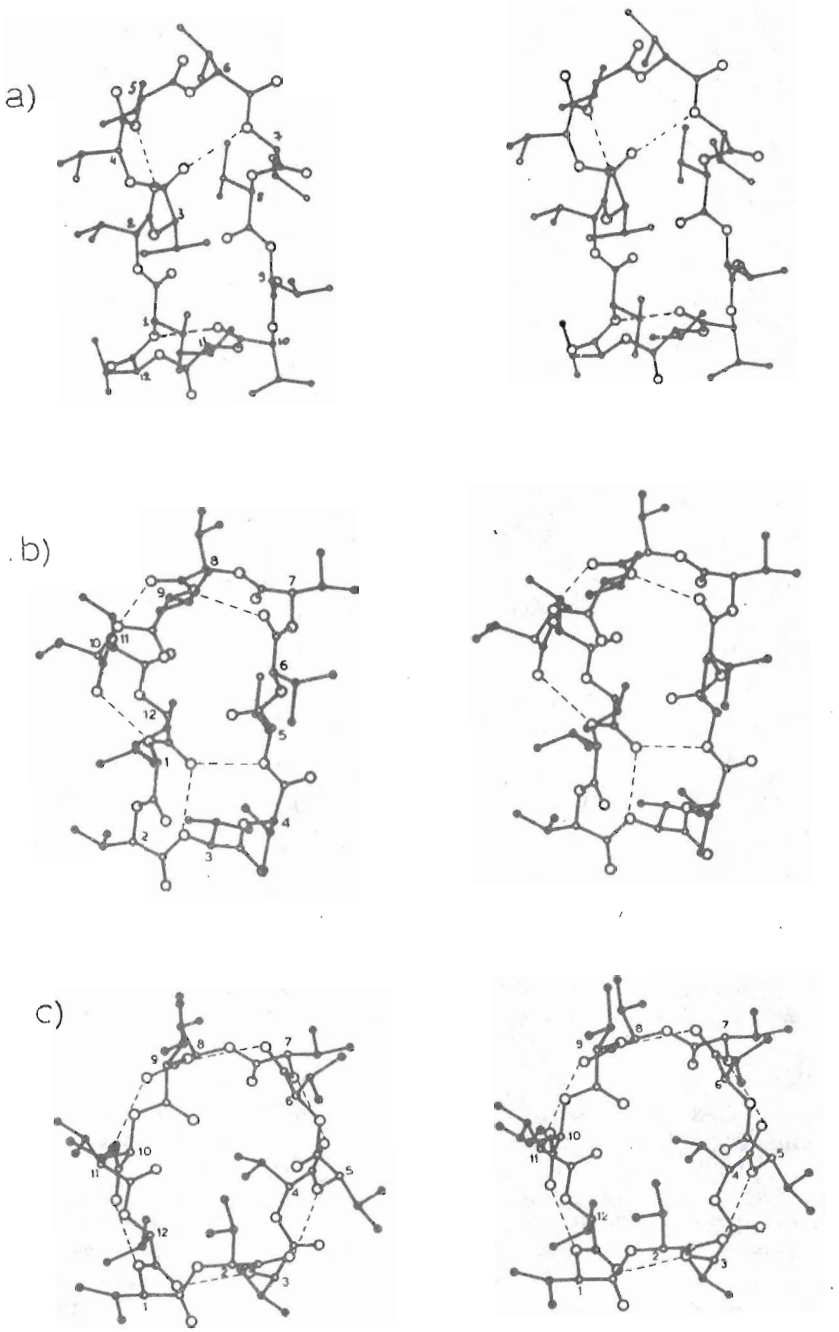

Fig. 8. Stereoviews of the structures of [*L*-Val^1-*D*-Val^3]- (*a*), [*L*-Val^1, *L*-Val^5]- (*B*) and [*D*-Hyi^2, *L*-Hyi^4]-*meso*-valinomycin (*C*)

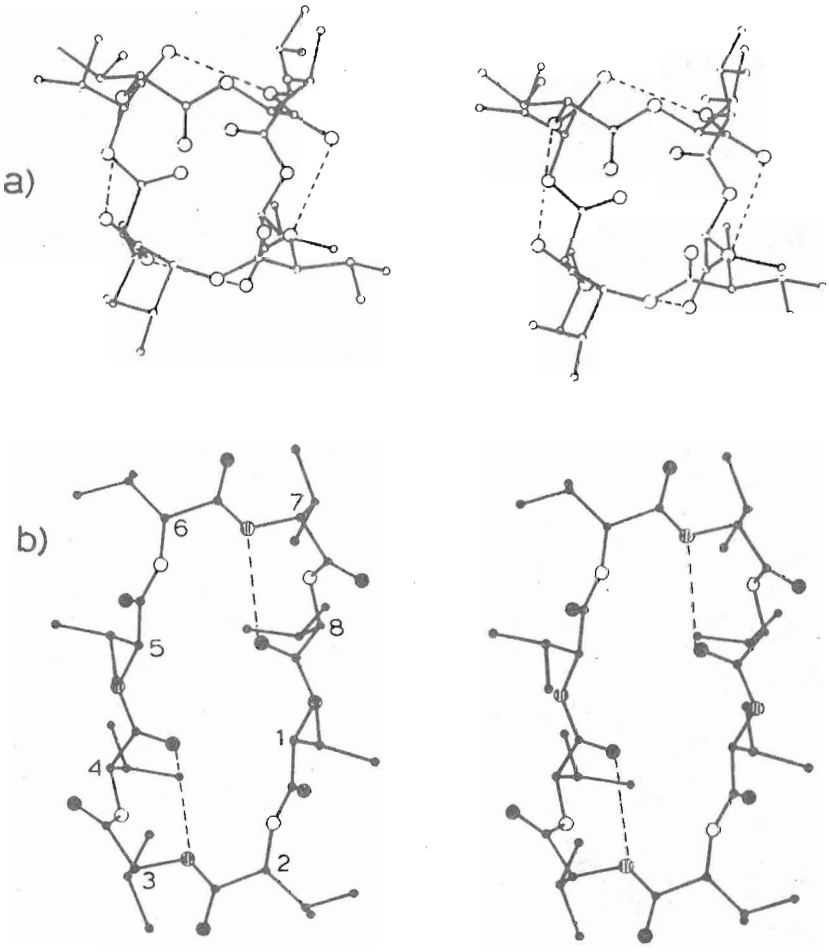

Fig. 9. Stereoviews of the conformation of *cyclo*(*D*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)$_2$-] observed in crystals grown from chloroform (*a*) and dioxane (*b*). Carbonyl oxygens are shown as closed circles, ether oxygens open circles, nitrogens as circles with cross-hatching, and H bonds as dashed lines

The effects of change of chirality in three different *meso*-valinomycin analogues altered in two of the twelve residues (formulas 4, 5, and 6) are illustrated in Fig. 8. Crystallographically observed structures of [*L*-Val^1,*D*-Val^3]- and [*L*-Val^1,*L*-Val^5]-*meso*-valinomycin [23, 24] have elongated structures with strong hydrogen bonds blocking ion access to their interiors. While the overall shapes of these two analogues are similar, their detailed structures are significantly different. There are only three intramolecular hydrogen bonds in [*L*-Val^1,*D*-Val^3]-*meso*-valinomycin and there are five intramolecular hydrogen bonds in [*L*-Val^1,*L*-Val^5]-*meso*-valinomycin. Although [*D*-Hyi^2, *L*-Hyi^4]-*meso*-valinomycin retains a bracelet shape and 6 intramolecular 4→1 hydrogen bonds in the solid state [25], the chiral changes on the α carbons of hydroxyisovaleryl residues 2 and 4 place valine side chains to block the entrant faces

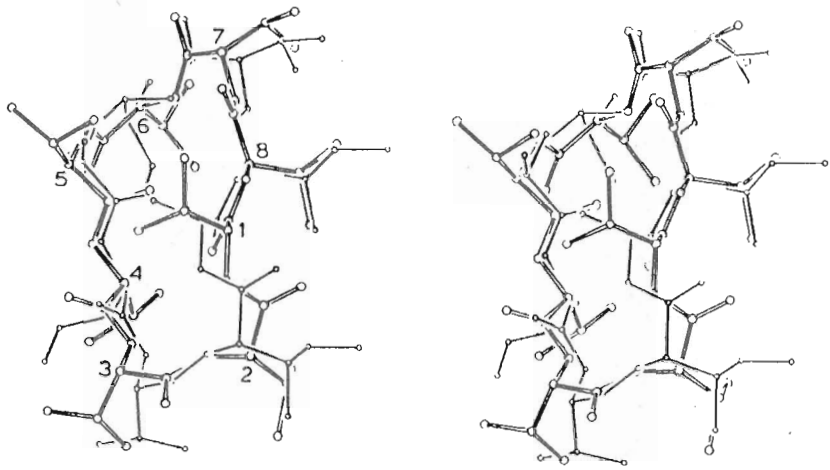

Fig. 10. Superposition of *cyclo*[-(*D*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)2-] (double line) with *cyclo*[-(*D*-Ile-*L*-Lac-*L*-Ile-*D*-Hyi)2-] (single line). The least-squares process minimized the separation between 24 atoms corresponding to the main-chain atoms of the molecules (rms = 0.69)

to the ion-binding site. This conformation is probably stabilized by the six hydrogen bonds but is not conductive to ion capture as evidenced by the negligible affinity of this analogue for ions. In contrast to this [*L*-Val^1,*L*-Val^5]-*meso*-valinomycin retains some affinity for K^+ ions (approximately 1/100 that of valinomycin).

The influence of ring size upon peptide conformation and ionophore properties has been explored by the synthesis and crystallographic analysis of various octadepsipeptides and hexadecadepsipeptides [26–31]. Only analogues based upon the repeat sequence in *meso*-valinomycin -(*D*-Val-*L*-Hyi-*L*-Val-*D*-Hyi)- and isoleucinomycin -(*D*-Ile-*L*-Lac-*L*-Ile-*D*-Hyi)- will be discussed here. The octapeptide dimer of the former sequence has been studied in crystal forms obtained from chloroform [27] and dioxane [28]. Crystals prepared from chloroform contain two independent molecules that are related by a pseudo-inversion center. These molecules have nearly identical bracelet structures possessing a pseudo two-fold axis and four 4→1 type hydrogen bonds (Fig. 9*a*). Crystals prepared from dioxane contain molecules that also have pseudo two-fold symmetry but a completely different rectangular structure and two unusual hydrogen bonds of a 3→4 type (Fig. 9*b*). Neither of the observed conformations provides sufficient space or suitably oriented carbonyl oxygen groups for ion capture and coordination, consistent with the undetectable levels of ion transfer noted for these octadepsipeptides. The crystallographically observed conformation of the octadepsipeptide based upon the isoleucinomycin sequence *cyclo*[-(*D*-Ile-*L*-Lac-*L*-Ile-*D*-Hyi)2-] is nearly identical in conforation to that of octamesovalinomycin in crystals grown from dioxane (Fig. 10).

While octaisoleucinomycin exhibit only weak association with Na^+ ions [32], hexadecaisoleucinomycin, *cyclo*[-(*D*-Ile-*L*-Lac-*L*-Ile-*D*-Hyi)4-] (HEXIL), complexes and transports larger cations, such as Cs^+ and tetramethyl ammonium ions [32]. The crystallographically

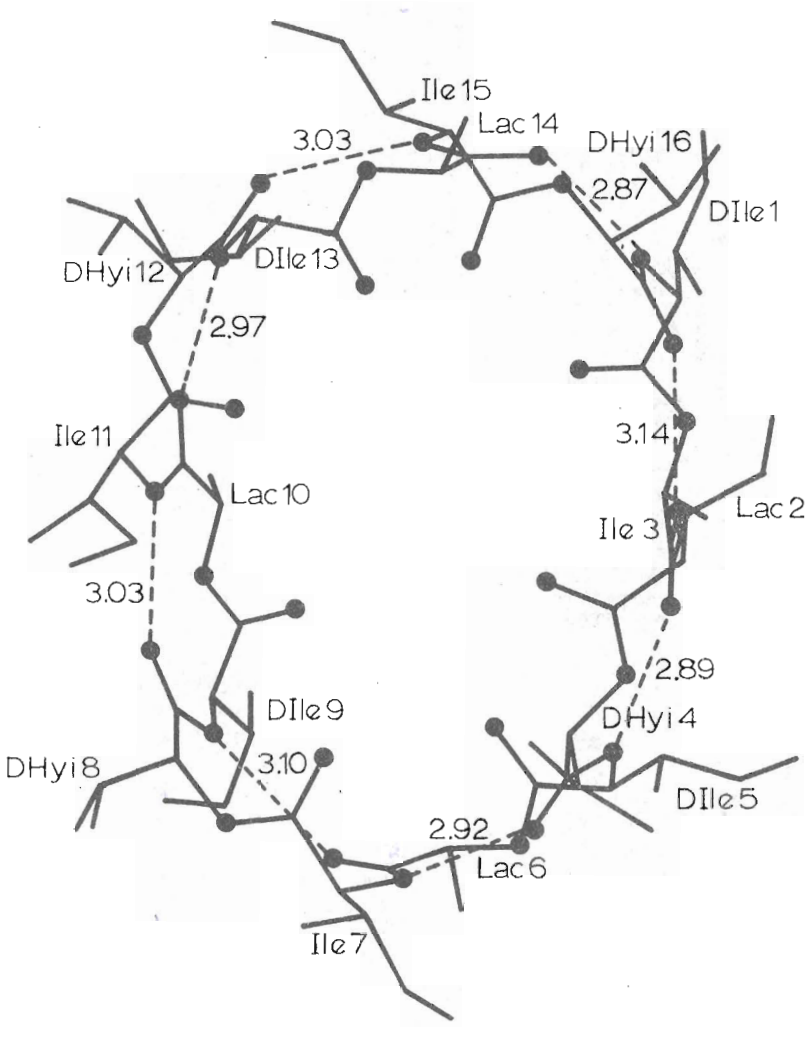

Fig. 11. Conformation of hexadecaisoleucinomycin (HEXIL) observed in the solid state

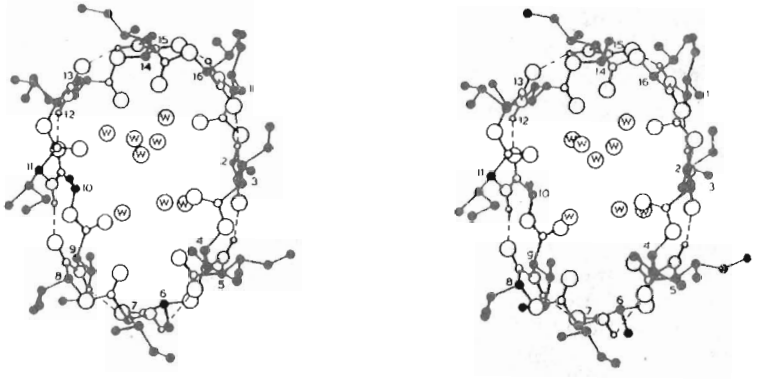

Fig. 12. Stereoview of the HEXIL structure

observed conformation of the HEXIL molecule is an asymmetric bracelet that is stabilized by eight intramolecular (4→1) hydrogen bonds (Fig. 11). The nitrogen atoms of the *D*-Ile and *L*-Ile residues are hydrogen bond donors to the carbonyl oxygen atoms of *L*-Lac and *D*-Hyi, respectively. The eight carbonyl oxygens of the *D*-Ile and *L*-Ile residues are directed toward the inside of the bracelet. These carbonyl oxygens form the boundaries of an elliptical cavity whose dimensions are approximately 7.5×5.5×4.0 Å.

Using molecular mechanics calculations to explore the conformational space of the HEXIL molecule, several low-energy forms [33] were found. Initial calculations using the backbone atoms and β carbons produced a dozen low-energy forms having eight hydrogen bonds of the 4→1 type and a twofold (or fourfold) symmetry axis. The molecular conformation corresponding to the lowest energy model is one in which the molecule has a rectangular shape with twofold symmetry and all eight of the *D*-Ile the *L*-Ile carbonyl oxygens are directed towards the outside of the ring. The next lowest energy conformation (+5.5 kcal/mol) closely approximates the crystallographically observed structure. All other model structures have calculated energies at least 13 kcal/mol higher than the minimum.

It is noteworthy that the calculated structures are biased by symmetry constraints that are not present in the crystal structure. The asymmetric shape seen in the crystal is probably stabilized by weak solvent interactions. Although molecules in solution will not be constrained to the crystallographically observed conformations, neither will they be constrained to a symmetric shape. It is likely that in solution there will be a flexing of the macro ring in which the crystallographically observed structure constitutes one local minimum energy conformation.

An examination of electron density difference maps calculated from the X-ray data indicated the presence of solvent molecules in the HEXIL cavities. The solvent structure forms channels that extend parallel to the axis through the stacks of bracelets. The density was modeled with seven full or partial occupancy water molecules (Fig. 12). The refined positions of these water molecules make contacts with the eight carbonyl oxygens that line the cavity and range from 2.98 to 3.50Å, but only four of these distances have lengths short enough for typical hydrogen bonds.

Gramicidin A

Gramicidin A is a linear pentadecapeptide isolated from *Bacillus brevis* [34] that has an alternating *D*- and *L*-amino acid structral sequence, HCO-*L*-Val-Gly-*L*-Ala-*D*-Leu-*L*-Ala-*D*-Val-*L*-Val-*D*-Val-(*L*-Trp-*D*-Leu)$_3$-*L*-Trp-NHCH$_2$CH$_2$OH. Gramicidin A is primarily active against Gram-positive bacteria [35]. In membranes, gramicidin A forms channels that are specific for monovalent cations [36]. Numerous models have been proposed for the structure of gramicidin A in various solvents, lipid bilayers and the solid state. These models have been based on NMR, CD, IR and Raman studies. Biochemical studies demonstrate that the active form of the molecule is a dimer [37]. Proposed models include a single-stranded, head-to-head $\beta^{6.3}$ helical dimer [38] and a double-stranded antiparallel $\beta^{5.6}$ helical dimer [37]. The peptide strands are hydrogen bonded in a β sheet

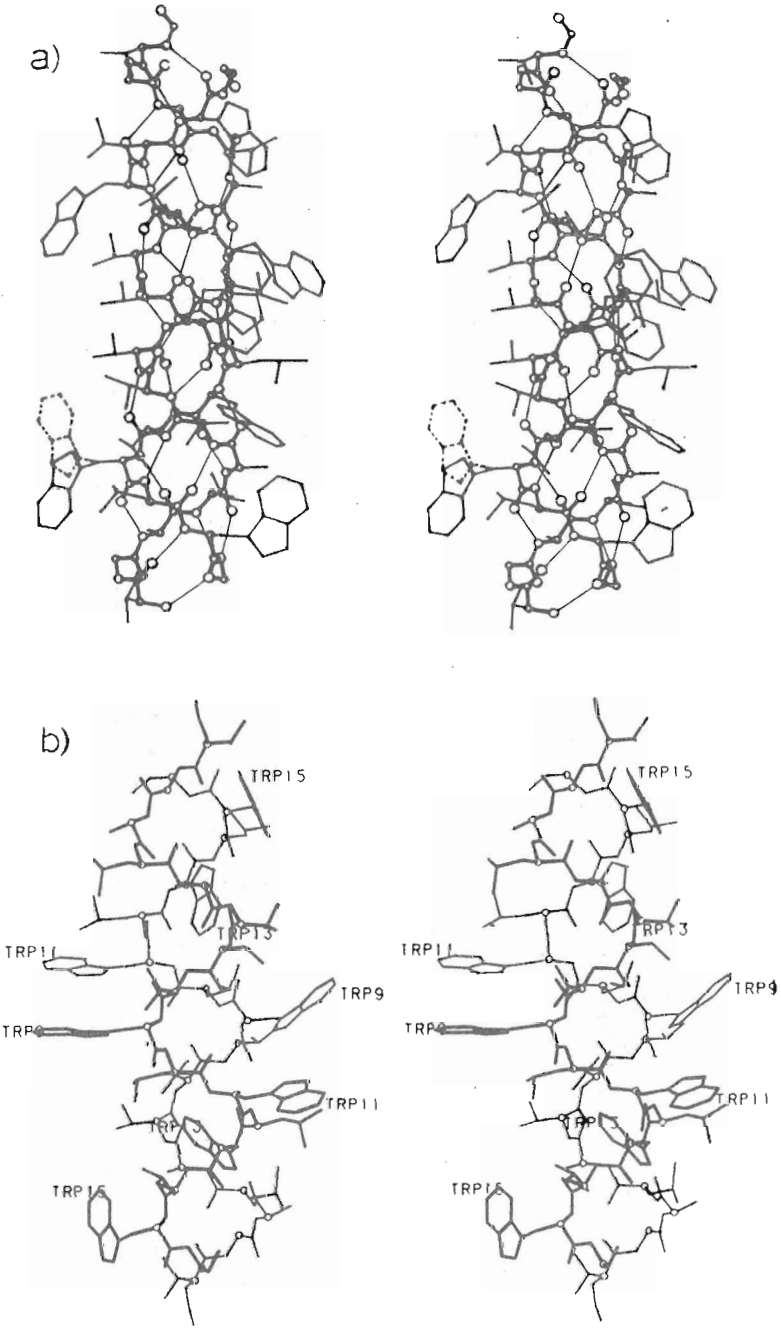

Fig. 13. Stereoviews of the antiparallel double-stranded $\beta^{5,6}$ channel helix of the uncomplexed gramicidin A molecular dimer observed in the orthorhombic (*a*) and monoclinic (*b*) crystal forms. One tryptophan is disordered as shown by dotted lines

pattern that is wrapped into a coil. Because of the alternating *L*- and *D*-configuration of the polypeptide, all the bulky side chains are on one face of the β-ribbon. Consequently, the ribbon can be coiled so that the side chain residues all lie on the outer surface.

The crystal structure of the cesium chloride/gramicidin A complex contains two independent, but structurally similar, molecular dimers. The peptide backbones of each dimer form a left-handed, antiparallel, double-stranded helix with a hydrogen-bonding pattern corresponding to an idealized $\beta^{7.2}$ helix but which has been twisted down to 6.4 residues per turn [39]. Each channel contains two cesium and three chloride ions. There is some evidence of distortion in the peptide backbone of the channel, with regard to the positioning of carbonyl groups in order to achieve coordination of the cesium ions. Uncomplexed gramicidin A forms crystals of exceptionally good quality. Two crystal forms characterized in 1949 were intensely studied in dozens of crystallographic laboratories. The crystal structure determination for the orthorhombic and monoclinic forms of gramicidin A were completed in 1988 [40] and 1991 [41], respectively. The orthorhombic form is the largest all light atom structure determination phased by direct methods to date.

The determination of the structure of the orthorhombic form of gramicidin A at 0.86 Å resolution was based upon intensity data gathered at 120 K using large well-formed crystals [40]. The excellent quality of the low-temperature data provides a structure rich in detail. A dimer of the pentadecapeptide forms the same type of double-stranded β-ribbon observed in the complexed form (Fig. 13). However, in the uncomplexed form the double-strand ribbon is wound more tightly to form a longer channel with a smaller diameter (Fig. 14). In the uncomplexed coil, the left-handed antiparallel double-stranded channel is 31 Å long, has 5.6 amino acids per turn and an approximate diameter of 4.85 Å. The tighter coiling is accomplished by a shift of the hydrogen bonds joining the edges of the double-stranded β-ribbon to an analogous interactive site two peptide units away. The idealized helix has a twofold axis normal to the helical axis at the center of the dimer that relates one molecular strand to the other. This diad axis is only approximate in the crystal structure dimer because of asymmetry in the crystalline environment of these molecules.

A double-stranded antiparallel helix of similar dimensions is observed in the monoclinic crystals. The two structures differ in the orientations of the tryptophan side chain residues, the association of the dimers in the crystal lattice and the topology of the internal channel. A pattern of bulges in the conformer found in the orthorhombic crystals appears to be correlated with the orientation of the tryptophan residues which in turn is correlated with the perpendicular stacking of indole side chains of adjacent dimers. Neither of the channels show evidence of ordered solvent molecules. The helical dimers in the orthorhombic crystal form contain three structurally similar pockets where the diameter exceeds 5.0 Å. A hypothetical potassium ion placed in the center of one of these pockets was found to make four keto oxygen and four peptide nitrogen contacts averaging 2.73 Å, with no oxygen-nitrogen contact less than 2.66 Å. These distances, although reasonable for an eight-coordinate potassium, would be quite unusual if the hydrogen-bonding pattern of the helix were not disrupted. Each of

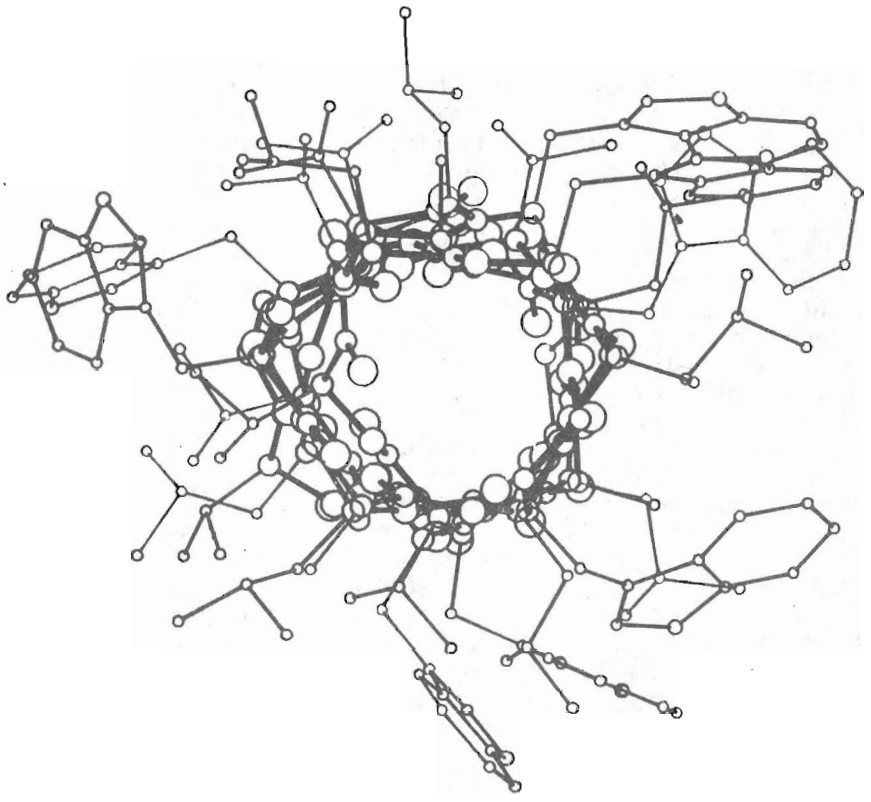

Fig. 14. A view down the double helical channel of gramicidin A molecules in the orthorhombic crystal form

the four oxygen atoms in the hypothetical coordination sphere is hydrogen bonded pairwise to one of the four peptide nitrogen atoms on the opposite strand of the helix. A potassium ion entering this pocket could form bonds to the four carbonyl oxygen atoms only at he expense of weakening or breaking the interstrand hydrogen bonds. While ion transport down an antiparallel $\beta^{5.6}$ helix is not inconceivable it appears more likely that this destabilization process might serve as a mechanism to transform the $\beta^{5.6}$ helix into the shorter double helix observed in the cesium complex and then into the more efficient ion-conducting single-stranded $\beta^{6.3}$ form.

The studies described here illustrate the power of combining biochemical and biophysical techniques in the study of physiologically important phenomena and could only have been achieved by bringing together the crystallographic expertise of the Medical Foundation of Buffalo and the biochemical talents of the staff of the Shemyakin Institute. The most important result of the collaboration that resulted from the meeting of Yuri Ovchinnikov and Herbert Hauptman has been the bridge of friendship, mutual understanding and respect that has been built between scientists in the two institutes. A bridge that has withstood the challenge of many political and economic upheavals since 1972.

"Yuri Ovchinnikov was one of those few scientists who recognized that, because of the possible disastrous consequences of modern day science and technology on civilization and the environment, it is essential that the individual scientist assume responsibility for the consequences of his work. He must do all in his power to insure that the fruits of his work be used to benefit humanity and not be used for destructive purposes. It is a great tragedy that he died at such a young age. The world needs more men and women having his insight and sense of responsibility" (Herbert Hauptman).

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