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Cyclodepsipeptides as Chemical Tools for Studying Ionic Transport Through Membranes*

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Summary. This paper reports a study of the chemistry of valinomycin, enniatins and related membrane-active depsipeptides that increase alkali metal ion permeability of model and biological membranes. The antimicrobial activity of these compounds and their effect on membranes has been correlated with their cation-complexing ability. The complexing reaction has been studied by spectropolarimetric and conductimetric methods. Nuclear magnetic resonance, optical rotatory dispersion, and infrared spectrophotometric studies have revealed the coexistence of conformers of the cyclodepsipeptides in solution and have led to elucidation of the spatial structure of valinomycin, enniatin B and their K^+ complexes. The effect of the conformational properties of the cyclodepsipeptides on their complexation efficiency and selectivity, surface-active properties and behavior towards phospholipid monolayers, bimolecular phospholipid membranes and a number of biological membrane systems has been ascertained. The studies have clearly shown the feasibility of using cyclodepsipeptides with predetermined structural and conformational parameters as chemical tools for membrane studies. It is suggested that the principle of conformation-dependent cation binding through ion-dipole interactions may possibly lie at the basis of the mode of action of systems governing the natural ion permeability in biological membranes.

The use of various naturally occurring and synthetic compounds capable of specifically modifying the properties of biological membranes (e.g., their permeability, excitability, the activity of their enzymatic constituents) as chemical tools for probing into the elementary acts of a given membrane process, has become one of the principal approaches to the physicochemical basis of membrane functioning. Among such membrane-active compounds of particular importance are peptides, depsipeptides and depsides that specifically increase the alkali metal ion flow through artificial and biological membranes (*see*, for instance, [12, 13, 18, 24]).

* For preliminary communications, *see* Refs. [9, 19, 20, 27, 29].

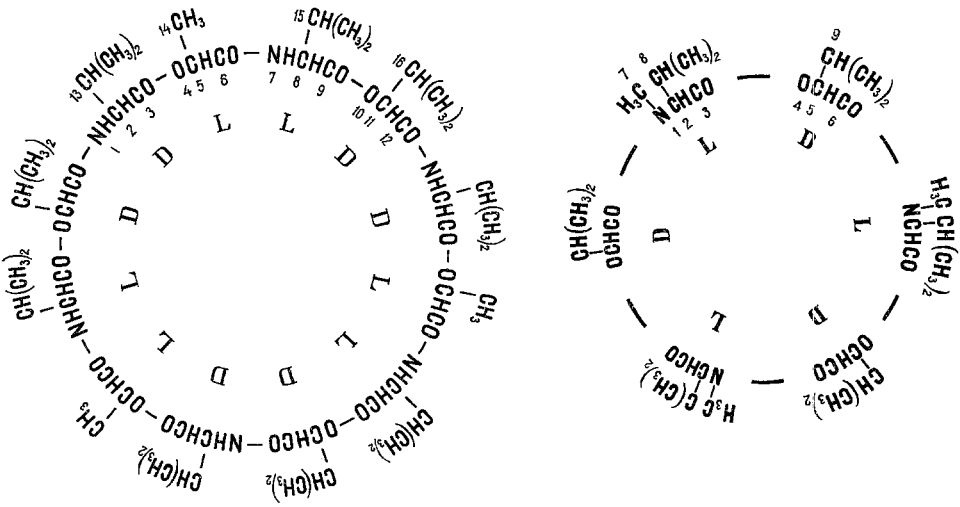

Fig. 1. Structure of valinomycin and enniatin B

In recent years, we have been pursuing the study from various aspects of cyclodepsipeptide antibiotics, namely, valinomycin (1), the enniatins (15, 16, 25) and their analogs (Fig. 1; *see* also Tables 1 and 2), selectively increasing the alkali metal ion permeability of plasmatic and mitochondrial membranes [5–7, 22, 23, 33], a property which has made them popular for the study of multifarious membrane systems.

Our very first investigations of synthetic enniatin and valinomycin analogs had already revealed a rather close relation between the influence of these compounds on the membrane permeability and their antimicrobial activity [20, 31]. As the work progressed, it became increasingly evident that the cause of such a dramatic effect of the cyclodepsipeptides on membranes lies in the unique molecular structure of these compounds which confers on them the ability to complex with alkali metal cations in lipophilic media. We therefore undertook an extensive study of the structure-membrane activity relation of depsipeptides with the aim of obtaining data on the origin of their induced ion flux specificity and interaction with membranes. Such information could be expected to increase our knowledge of the intimate mechanism underlying the augmentation of ion permeability (carrier mechanism, relay transport or specific modification of the membrane structure). The accumulation of such data would also bring us a significant step forward in our understanding of the molecular basis of naturally occurring ion permeability in biological membranes. Our approach was based on detailed correlation of the structural and conformational para-

meters of the cyclodepsipeptides with, in particular, their physicochemical complexing properties in different media, with their behavior toward the most varied types of artificial and biological membranes and with their antibiotic activity.

Materials and Methods

Valinomycin used for the physicochemical and biological studies was prepared biosynthetically by MacDonald's procedure [14] and was identical to the synthetic product [25, 26]. All valinomycin analogs (2 – 14) and the enniatin cyclodepsipeptides (15 – 28) (Tables 1 and 2) were prepared by total synthesis using the general methods of cyclodepsipeptide synthesis we had developed earlier [25, 26, 28, 32]. The cyclopeptide (29) was prepared by methylating cyclo-(L-alanylglycyl)$_3$ [10] according to Lederer [2].

Nuclear magnetic resonance (NMR) spectra were run on a JEOL JNM-4H-100 instrument operating at 100 MHz. Chemical shifts were measured with an accuracy of ± 0.01 ppm from TMS as internal reference and spin-spin coupling constants of ± 0.1 Hz.

Infrared (IR) spectra were taken on a Zeiss UR-10 spectrometer at 25 °C, using 4×10^{-4} to 4×10^{-2} M solutions.

Dipole moment measurements were carried out at 25 °C by the beat method with an accuracy of ± 0.1 D.

Optical rotatory dispersion (ORD) curves were obtained on a Cary 60 spectropolarimeter at room temperature, using $1 \cdot 10^{-4}$ to $1 \cdot 10^{-3}$ M solutions. The stability constants were calculated according to the formula

$$K = \frac{\alpha}{(1-\alpha)(B_0 - A_0\,\alpha)},$$

where A_0 is the initial cyclodepsipeptide concentration, B_0 that of the inorganic salt, and α the degree of complexation as determined from the relation

$$\alpha = \frac{[\Phi]_{CDP} - [\Phi]_{obs.}}{[\Phi]_{CDP} - [\Phi]_{CDP \cdot M^+}},$$

where $[\Phi]_{CDP}$ and $[\Phi]_{CDP \cdot M^+}$ are the molecular rotations of the initial cyclodepsipeptide (CDP) and of its complex, and $[\Phi]_{obs.}$ is the observed rotation of their equilibrium mixture at the selected wavelength. The accuracy of this method was ± 30 %.

Conductimetric measurements were made at 25 °C using an AC-bridge operating at a frequency of 2 KHz. The electrodes were platinum discs 18 mm in diameter and 2 mm apart, with glass-coated backs. Solutions employed were of a concentration of 1.5×10^{-4} to 3.0×10^{-4} M. The stability constants in this case were determined by the formula

$$K = \frac{\frac{\Delta\kappa}{\delta}}{\left(B_0 - \frac{\Delta\kappa}{\delta}\right)\left(A_0 - \frac{\Delta\kappa}{\delta}\right)},$$

where $\Delta\kappa$ is the decrease in specific conductivity of the alcoholic solution of the salt on addition of cyclodepsipeptide, and

$$\delta = \frac{\lambda_{M^+} - \lambda_{CDP \cdot M^+}}{1000}.$$

(λ_{M^+} and $\lambda_{CDP \cdot M^+}$ are the free and complexed ion mobilities, respectively). Constants were determined with an accuracy of ± 40 %.

Monomolecular layers were prepared in a $24 \times 10 \times 0.8$-cm Teflon trough with a movable barrier of the same material. Triple-distilled water was used, the last distillation being done with potassium permanganate. The surface tension was determined by Wilhelmy's method [4] employing a torsion balance and a 2×0.5-cm platinum plate. The depsipeptide monolayers were prepared by micropipetting out their petroleum ether solutions in the aqueous surface. The phospholipid monolayers were prepared similarly from 3:1 hexane: ethanol solution. Adsorption monolayers were obtained by adding to the water less than 0.1 ml of a 10^{-5} M solution of the depsipeptide in ethanol. Egg lecithin was isolated by Dawson's method [3]. DL-Dipalmitoyl lecithin was synthesized according to the method of Molotkovskii, Lazurkina and Bergelson [17].

The bi-ionic potentials and resistance of phospholipid membranes were measured in a cell with a Teflon diaphragm (with a 2.5-mm aperture). The transmembrane potential was measured by an Orion TR-1501 electrometer using silver/silver chloride electrodes. The phospholipid bilayers were prepared from a solution of 20 mg of purified egg lecithin, and 5 mg of cholesterol in 1 ml of n-decane. The unmodified bilayers in 0.1 M KCl solution had a resistance of 1 to 3×10^7 ohm $\cdot$ cm^2 (25 °C).

The antimicrobial activity of the cyclodepsipeptides was determined by the serial dilution method; the substance undergoing the test was dissolved in dimethylformamide or methanol and diluted to the respective concentration with distilled water (for details, *see* [31]). *Streptococcus faecalis* was cultivated for 18 hr at 37 °C in a medium containing 5 g peptone, 10 g yeast autolysate, 1 g $MgSO_4 \cdot 7H_2O$, 7.7 g KH_2PO_4, and 2.56 g K_2HPO_4 in 1 liter of distilled water. The cells were removed by centrifugation (2,500 rev/min, 10 min) and washed twice with 0.001 M KCl. The same medium inoculated with 1,000 cells/ml (18 hr, 37 °C) was used for the test on growth inhibition of *S. faecalis* by the cyclodepsipeptides.

Results and Discussion

Data on the antimicrobial activity of some of the valinomycin and enniatin analogs we have synthesized, and on their complexation with alkali metal ions and ability to induce K^+ sorption in mitochondria are presented in Tables 1 and 2 (*see* also [15, 30–32]). The results obtained show that maximum antimicrobial activity among the cyclodepsipeptides investigated is displayed by compounds of definite ring size, (namely, 36 members in the case of valinomycin analogs and 18 members in the case of the enniatins) and of given pattern of amino and hydroxy acid residues, an essential part being played also by their nature and their configuration (*see* below for further details). This bears evidence of the importance of conformational factors in the manifestation of antimicrobial activity by the cyclodepsipeptides. It was also established that such activity is intimately associated with the efficiency and selectivity of the alkali metal ion binding by the cyclodepsipeptides (*see* Tables 1 and 2). Armed with these preliminary results, we then carried out a more detailed study of the problems.

Two independent methods, optical and conductimetric, were used for measuring complexing constants. Optical rotatory dispersion (ORD) and

Table 1. *Antimicrobial activity and complexation with K^+ of valinomycin and its analogs*

Analog no.	Compound[a]	Antimicrobial activity (minimal growth-inhibiting concentration, γ/ml)				Complexation[b] (stability constant, $K \cdot 10^{-5}$ liter/mole, EtOH, 25 °C)
		Staphylococcus aureus UV-3	*Sarcina lutea*	*Mycobacterium phlei*	*Candida albicans*	
1	$\lfloor$(D-Val-L-Lac-L-Val-D-HyIv)$_3\rfloor$ Valinomycin	0.8	1.5	0.3	0.8	20
2	D-Val→D-Ala	0.7	1.5	0.4	0.5	1.0[c]
3	D-Val→D-Leu; L-Val→L-Leu	8	22	1	8	4.5
4	L-Val→L-HyIv	Inactive	Inactive	Inactive	Inactive	0.025
5	L-Val→L-MeVal	Inactive	Inactive	Inactive	Inactive	0
6	L-Lac→L-Ala	2	2	2	2	2.2
7	$\lfloor$(D-Val-L-Lac-L-Val-D-HyIv)$_2\rfloor$	Inactive	Inactive	Inactive	Inactive	0
8	$\lfloor$(D-Val-L-Lac-L-Val-D-HyIv)$_4\rfloor$	Inactive	Inactive	Inactive	Inactive	0.001
9	$\lfloor$(D-Val-L-Lac-L-Val-D-Lac)$_3\rfloor$	Inactive	Inactive	Inactive	Inactive	23
10	$\lfloor$(D-Val-L-HyIv-L-Val-D-HyIv)$_3\rfloor$	Inactive	Inactive	Inactive	Inactive	4.0[c]
11	$\lfloor$(L-Val-L-Lac-L-Val-D-HyIv)$_3\rfloor$	Inactive	Inactive	Inactive	Inactive	0
12	$\lfloor$(D-Val-L-Lac-D-Val-D-HyIv)$_3\rfloor$	Inactive	Inactive	Inactive	Inactive	0
13	$\lfloor$(D-Val-L-Lac-L-Val-L-HyIv)$_3\rfloor$	Inactive	Inactive	Inactive	Inactive	0
14	$\lfloor$(L-Val-D-Lac-D-Val-L-HyIv)$_3\rfloor$ Enantio-valinomycin	0.8	1.5	0.3	0.8	20

[a] The standard symbols and abbreviations for amino acids are used in the table; HyIv and Lac designate α-hydroxyisovaleric and lactic acid residues, respectively. The arrows indicate substitution of one amino or hydroxy acid for another.

[b] Measured conductimetrically (corrected value).

[c] Preliminary data.

Table 2. *Biological activity and complexation of enniatins and their analogs*

Analog no.	Compound	Antimicrobial activity (minimal growth-inhibiting concentration, γ/ml)				Relative K^+ transport induction in mitochondria	Complexation [a] (stability constant, $K \cdot 10^{-3}$ liter/mole, EtOH, 25 °C)	
		M. phlei	*S. aureus* 209 *P*	*S. lutea*	*C. albicans*		Na^+	K^+
15	$\lceil$(L-MeIle-D-HyIv)$_3\rfloor$ Enniatin A [b]	1.5	2–3	2	4.5–6	47	2.9	9.8
16	$\lceil$(L-MeVal-D-HyIv)$_3\rfloor$ Enniatin B	9–12	18	18	9–12	100	2.6	6.5
17	L-MeVal→L-MeLeu	9	18	9–12	18	–	2.2	5.0
18	2L-MeVal→2L-MeLeu	9	9	9	37	–	1.7	5.1
19	L-MeVal→D-MeVal	Inactive	Inactive	Inactive	Inactive	–	0.7	1.3
20	D-HyIv→L-HyIv	Inactive	Inactive	Inactive	Inactive	–	<0.1	0.6
21	$\lceil$(L-MeVal-D-HyIv)$_2\rfloor$	Inactive	Inactive	Inactive	Inactive	0	0	0
22	$\lceil$(L-MeVal-D-HyIv)$_4\rfloor$	18–25	>50	3–4.5	>50	18	1.0	2.4
23	$\lceil$(L-MeVal-D-HyIv)$_6\rfloor$	Inactive	Inactive	Inactive	Inactive	–	0	0
24	$\lceil$L-Val-D-HyIv)$_3\rfloor$ (Tri-N-desmethyl)-enniatin B	Inactive	Inactive	Inactive	Inactive	–	2.5	2.6
25	$\lceil$(L-MeLeu-D-HyIv)$_3\rfloor$ Enniatin C	Inactive	Inactive	Inactive	Inactive	12	2.5	5.5
26	Enantio-enniatin A	1.5	2–3	2	4.5–6	–	2.9	9.8
27	Enantio-enniatin B	9–12	18	18	9–12	–	2.6	6.5
28	Enantio-enniatin C	Inactive	Inactive	Inactive	Inactive	–	2.5	5.5
29	$\lceil$(L-MeAla-Sar)$_3\rfloor$	Inactive	Inactive	Inactive	Inactive	–	0.4	<0.2

[a] Measured spectropolarimetrically (CD).

[b] Reinvestigation of "false" enniatin A

$\lceil$(D-MeVal-L-HyMeVal)$_3\rfloor$ (HyMeVal = -OCH(CH$_3$)C$_2$H$_5$CO-)

[20,29] has shown it to possess practically the same biological activity, ORD curve and complexation capacity as enniatin A.

circular dichroism (CD) studies have shown that the enniatin cyclodepsipeptides form complexes in solution with a variety of cations (Li^+, K^+, Na^+, Rb^+, Cs^+, Zn^{++}, Mg^{++}, Ca^{++}, etc.). In the majority of cases, the ORD and CD curves of the initial compound and the corresponding complexes were found to differ sharply as can be seen, for example, in the complexing of K^+ by enniatin B (16) and (tri-N-desmethyl)-enniatin B (24) in alcohol (Fig. 2a, c). Complexation-induced changes in the dispersion curves are not so much the result of changes in the electronic characteristics of the chromophores as of the conformational changes in the molecule. That this is so can be seen from the fact that the ORD curves of the initial compound and of the complex are sometimes very similar (Fig. 2b). Valinomycin and its closely related analogs have an equal number of the same type of asymmetric centers with opposite configurations, the apparent high symmetry of these compounds nearing them to the meso-form [*compare* compounds (9) and (10) in Table 1]. These compounds and their complexes therefore possess very weak optical rotatory properties, which often hinders quantitative spectropolarimetric evaluation of their complexing properties.

Complexation can be conveniently studied by the decrease in electroconductivity of alcoholic solutions of alkali metal chlorides on addition of cyclodepsipeptides brought about by the low mobility of the complex cation. This method permits the calculation of a variety of parameters of the complexes. Table 3 presents the mean values of the stability constants of the complexes of a number of cyclodepsipeptides with various univalent cations, the calculated free energies of complexation and also the limiting mobilities of the complex ions and their effective Stokes' radii.

The combination of optical and conductimetric methods makes it possible to follow the course of the complexing reaction in different media and under different conditions (temperature, concentration, solvent, etc.) and to calculate the thermodynamic parameters of the reaction and stability constants of the complexes.

Of considerable interest is the molecular structure of the complexes. They are equimolar as can be seen by data on both the complexing equilibria in solution and elementary analysis of the KCNS complexes of enniatins B and C and valinomycin [29, 34]. The results of IR, NMR and other studies led to the conclusion that the cyclodepsipeptide molecule binds the cation by ion-dipole interaction with the ester and amide groupings.

It should be stressed that although an ion-dipole interaction of this type is not a novelty, the valinomycin, enniatins and related molecules are unique in the high efficiency and ionic selectivity of the complexing reaction. It was therefore only natural to expect the complexing capacity in this case

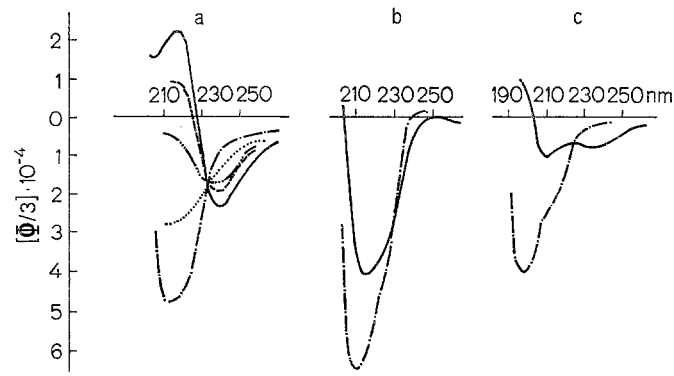

Fig. 2. ORD curves of enniatin B (*a*), enniatin C (*b*), (tri-N-desmethyl)-enniatin B (*c*), and their K^+ complexes in ethanol ([CDP] = 1.5×10^{-4} M) — — —[KCl] = 0.75×10^{-4} M; —···—···[KCl] = 2.25×10^{-4} M; ···· [KCl] = 4.5×10^{-4} M; —·—· [KCl] = 75×10^{-4} M

Table 3. *Conductimetric data on the complexation of the depsipeptides with ions in alcohol solutions*

Compound	Cation	K, liter/mole	$-\Delta F$, kcal/mole	Λ, $cm^2/ohm \cdot mole$	Stokes' radius, A
Valinomycin (I)	Na^+	0	—	—	—
	K^+	2.0×10^6	8.63	13.15	5.68
	Rb^+	2.6×10^6	8.78	13.90	5.38
	Cs^+	6.5×10^5	7.96	14.22	5.25
(9)	K^+	2.3×10^6	8.71	14.95	5.00
Enniatin B (16)	Na^+	1.3×10^3	4.30	16.00	4.80
	K^+	3.7×10^3	4.90	14.50	5.15
	Rb^+	4.0×10^3	4.95	14.50	5.15
	Cs^+	2.2×10^3	4.55	14.50	5.15
(22)	Na^+	7.0×10^2	3.90	12.10	6.16
	K^+	2.2×10^3	4.55	14.60	5.12
(Tri-N-desmethyl)-	Na^+	2.5×10^3	4.65	13.20	5.67
enniatin B (24)	K^+	2.6×10^3	4.66	14.50	5.15
Enniatin C (25)	Na^+	2.5×10^3	4.65	15.80	4.75
	K^+	5.5×10^3	5.15	14.40	5.20
	Rb^+	7.5×10^3	5.30	14.40	5.20
	Cs^+	4.1×10^3	4.96	14.40	5.20

to be highly conformation-specific since binding of a cation with a given radius, charge and coordination number by polar groups far removed along the chain will depend on both the number of such groups and their location and orientation in the depsipeptide molecule. We therefore undertook a study of the conformational states of the cyclodepsipeptides and their complexes in solution on the example of valinomycin and enniatin B with the aid of various physicochemical methods.

The ORD curves of valinomycin in different solvents differ significantly from each other, indicating the existence of various conformers in equilibrium, the latter being shifted with change in polarity of the medium (Fig. 3). Further information on the nature of the equilibrium was obtained from the NMR spectra of valinomycin in CCl_4 and $(CD_3)_2SO$ solution (Fig. 4, *cf.* [8]). These spectra clearly show the presence of two doublets corresponding to two types of NH protons, quite close to each other (δ 7.90 and 7.76 ppm) in CCl_4 but more removed due to a 0.6 ppm downfield shift of low-field signal, whereas the high-field signal remains practically constant in $(CD_3)_2SO$. From this it can be assumed that in nonpolar media (CCl_4), valinomycin prefers a conformation wherein all NH groups form intramolecular hydrogen bonds, whereas in more polar solvents, part of the NH protons (belonging to the L-valine residues) pass over from intramolecular hydrogen bonding to bonding with the solvent molecules.

This assumption is fully confirmed by the IR spectra of valinomycin in dilute $CHCl_3$ (or CCl_4) solution (Fig. 5). These spectra display a strong band at 3313 cm^{-1} attributable to the intramolecular, hydrogen-bonded, NH stretching mode and a much weaker one at 3388 cm^{-1} that is characteristic of the free NH group. Also in the CO stretching range there is a symmetric band at 1755 cm^{-1} to the non-hydrogen-bonded ester carbonyl. The amide carbonyls give rise to an asymmetric band at 1661 cm^{-1} (amide I). These data show that in low-polarity solvents there are two coexisting forms of valinomycin; in one, A, strongly predominant, all the amide groupings participate in the formation of six intramolecular hydrogen bonds, in the other, B, only three groups are hydrogen-bonded (Fig. 6).

Of the possible ways of forming intramolecular hydrogen bonds in valinomycin, analysis shows that all six amide groups can simultaneously participate in such bonding only if valinomycin is in conformation A. The hydrogen bonds forming a system of six condensed ten-membered rings resembling a "bracelet" about 8 A in diameter and 4 A in height lend considerable rigidity to this conformation.

Further refinement of the valinomycin conformation in non-polar media was made on the basis of the NMR data. From analysis of the spin-spin coupling constants based on the recently found stereochemical dependence ([1], Fig. 7), it follows that one group of protons of the NH-CH fragments is gauche-oriented ($^3J_{N_{(7)}H-C_{(8)}H} = 6.7$ Hz, corrected value, CCl_4), whereas the other ($^3J_{N_{(1)}H-C_{(2)}H} = 8.4$ Hz, corrected value, CCl_4) is cis-oriented. This made possible determination of the orientation of all six ester and amide carbonyls as three within and three without the ring. Finally the value of the $^3J_{C_\alpha H-C_\beta H}$ constant showed the protons in the $C_\alpha H - C_\beta H$

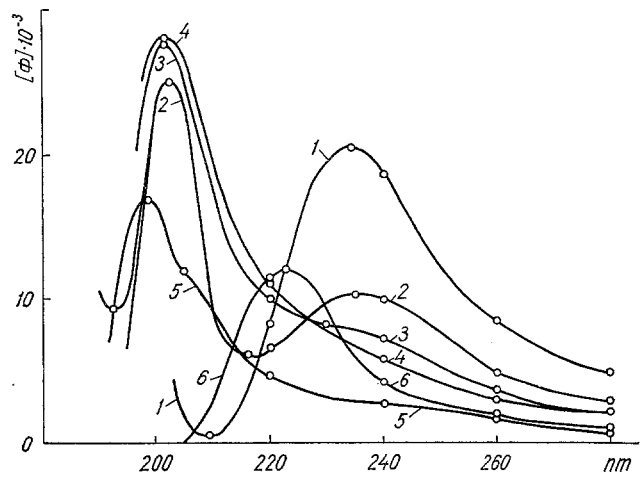

Fig. 3. ORD curves of valinomycin (curves *1*—*5*) and its K^+ complex (curve *6*). [CDP] = 0.5 to 1×10^{-3} mole/liter; [KBr]/[CDP] = 5. *1* Heptane-dioxane (10:1), *2* Heptane-ethanol (3:1), *3* Ethanol, *4* Acetonitril, *5* Trifluoroethanol-water (1:2), *6* + Ethanol + KBr

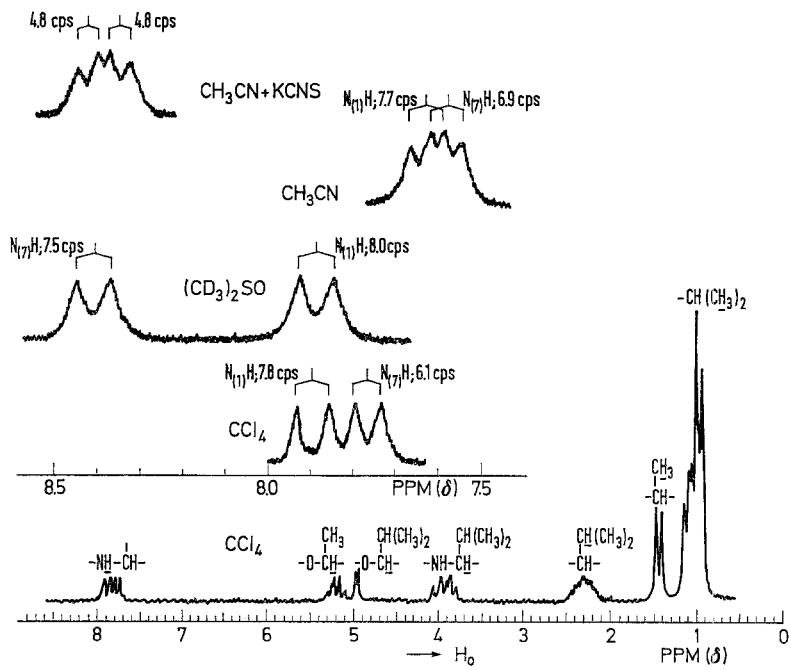

Fig. 4. NMR spectra of valinomycin and its K^+ complex. The $^3J_{NH-CH}$ constants should be corrected for the electronegativity of the substituents (+0.6 Hz) [1]. The assignment of the NH proton signals was confirmed by the the NMR-^{1}H spectra of valinomycin one of whose L-valine residue was enriched with ^{15}N

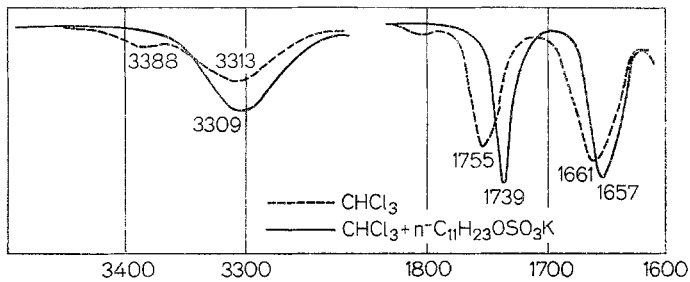

Fig. 5. IR spectra of valinomycin and its K^+ complex in $CHCl_3$

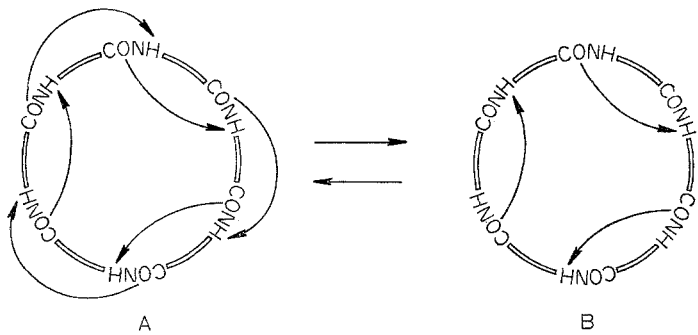

Fig. 6. A ⇄ B equilibrium of valinomycin

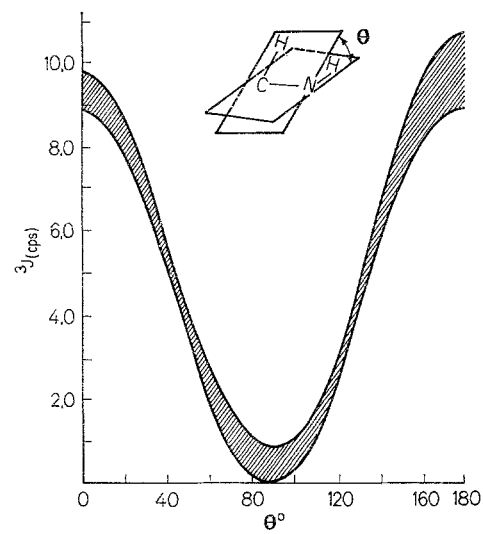

Fig. 7. Stereochemical dependence of $^3J_{NH-CH}$ coupling constant

fragment to be preferentially trans-oriented in the amino acid (10 Hz), and gauche-oriented in the hydroxy acid [2.9 Hz(HyIv) and 6.8 Hz(Lac)] residues.

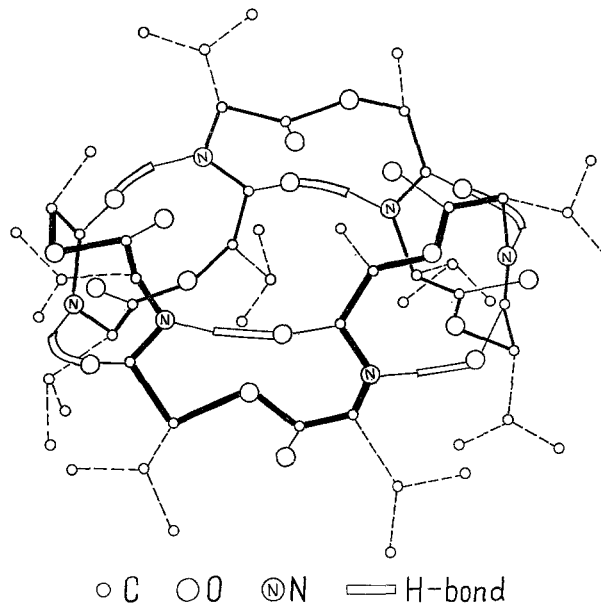

Fig. 8. Conformation of valinomycin in nonpolar solvents

All these data served as evidence that the bracelet conformation presented in Fig. 8 is the one preferred in nonpolar media. The dipole moment calculated for this conformation is 2.5 ± 1.5 D, which is in good agreement with the experimental value of 3.5 ± 0.1 D (in CCl_4), especially if one takes into account the existence of a certain amount of the less symmetric form B.

Analogous physicochemical studies made it possible to determine the conformation of the K^+ complex in solution. The IR spectrum of this complex (Fig. 5) displays no free NH stretching band, and the ester carbonyl frequency is shifted to the longer wavelength region by about 15 cm^{-1} with simultaneous narrowing of the band (as compared with valinomycin). This indicated retention of the hydrogen bond-stabilized framework in the K^+ complex of valinomycin, wherein all the ester carbonyls become involved in ion-dipole interaction with K^+. Complexation is accompanied by conformational reforming of the molecule in which the three ester carbonyls are now also oriented within the ring to form a hexadentate system of oxygen atoms about the cation. The ORD curve of the K^+ complex of valinomycin differs sharply from the ORD curves of both the *A* and *B* forms (*see* Fig. 3). Moreover, the NMR spectra of the complex in all solvents investigated (*see* Fig. 4) showed almost identical spin-spin coupling constants equal to 5.4 Hz for all six NH-CH fragments, a sign of the gauche orientation of these protons in all cases. These results are in complete agreement with

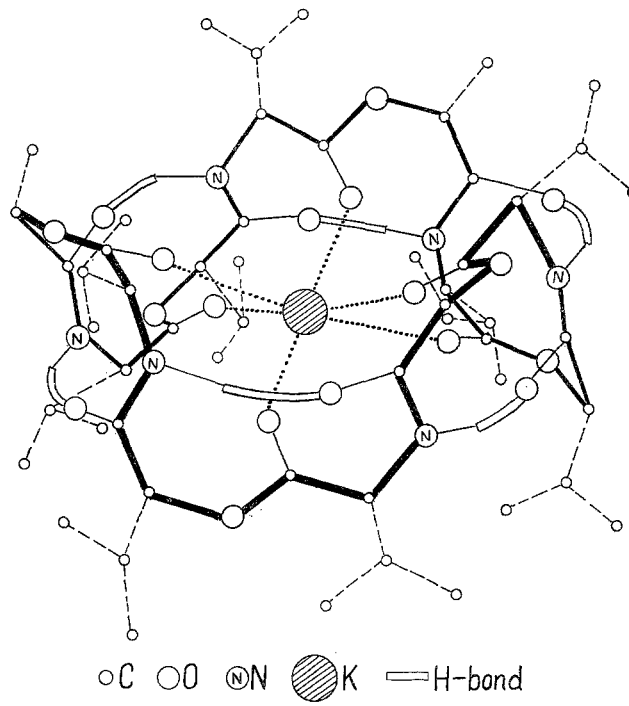

Fig. 9. Conformation of the K^+ complex of valinomycin

the rigid symmetric conformation of the K^+ complex of valinomycin presented in Fig. 9, a characteristic feature of which is effective screening of the K^+ atom and the system of hydrogen bonds by the hydrophobic peripheral side chains from solvent action.

The preferential conformation of enniatin B and its K^+ complex was established in a similar way. The ORD curves of enniatin B in different solvents (Fig. 10) show that, like valinomycin, it exists as an equilibrium mixture of different forms, the point of equilibrium depending upon the polarity of the solvent. One form (NP) is predominant in nonpolar solvents (heptane); as the polarity is gradually increased, the conformational equilibrium is shifted until a new conformation (*P*) becomes predominant in the antibiotic. It is noteworthy that the conformation of the K^+ complex of enniatin B is the same (form *P*) as that of the noncomplexed compound in polar solvents, as can be seen from the similarity of the ORD curve of enniatin B in trifluoroethanol and the corresponding curve of the K^+ complex (Fig. 10a).

Considerable information concerning form (NP) of enniatin B could be obtained by comparison of its NMR spectra at various temperatures (Fig. 11).

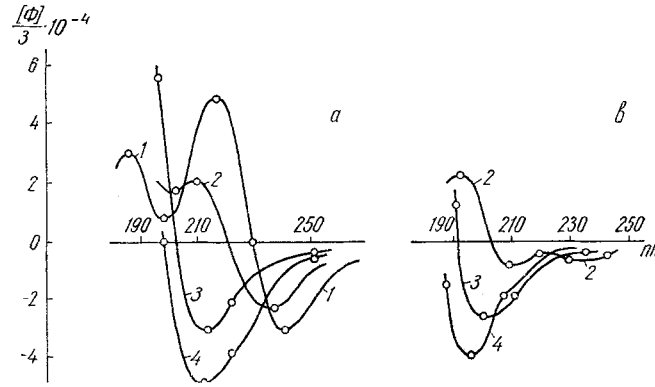

Fig. 10. ORD curves of enniatin B (*a*) and (tri-N-desmethyl)-enniatin B (*b*) in different solvents and their K^+ complexes in ethanol. *1* Heptane, *2* Ethanol, *3* Trifluoroethanol, *4* Ethanol + KCl; [KCl]/[CDP] = 50

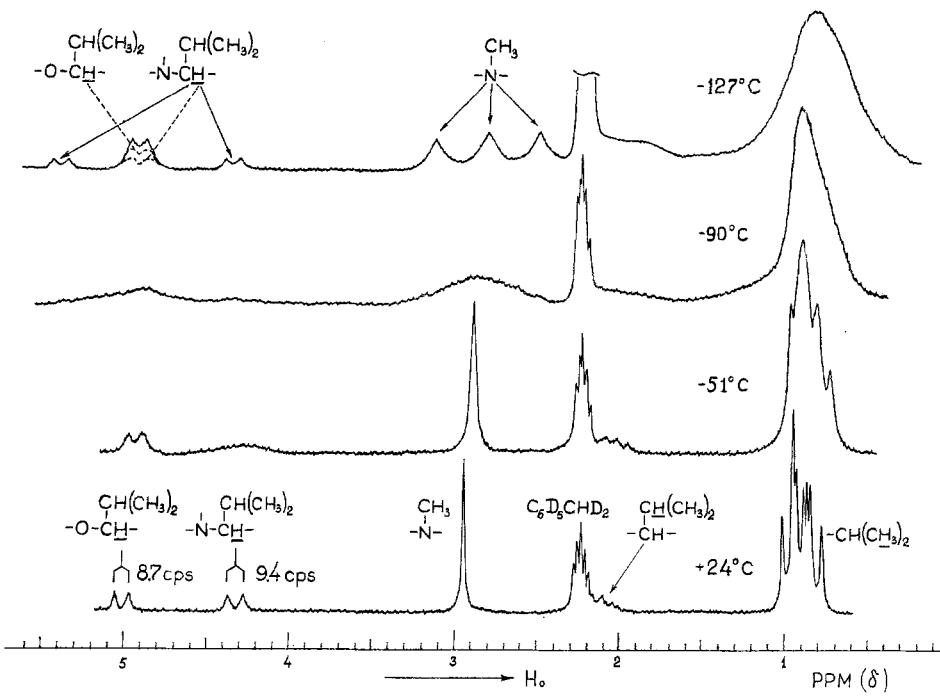

Fig. 11. NMR spectra of enniatin B in $CS_2 - CD_3C_6D_5$ (2:1) at different temperatures

On cooling a solution of enniatin B in $CS_2 - CD_3C_6D_5$ (2:1) mixture, its NMR spectra undergo considerable change. The main N-methyl signal now splits into three singlets of equal intensity; similarly the N-methyl-valyl α-proton doublets split into three equal doublets, the middle one coinciding

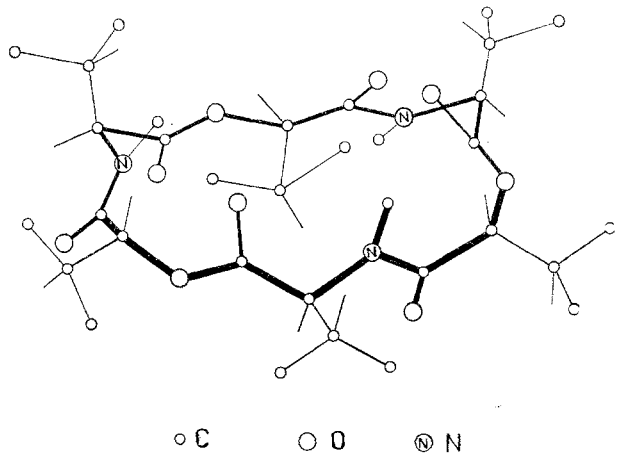

Fig. 12. Conformation of enniatin B in nonpolar solvents

with the α-proton doublets of the hydroxy acid. From this it follows that in the "non polar" conformation of enniatin B (NP), all three methylvaline fragments have differing spatial structures due to differences in rotation about the N–C and C–C single bonds (i.e., in the φ and ψ coordinates of the corresponding conformation charts). In other words, enniatin B which consists of three chemically identical units has a conformation (NP) in nonpolar media which lacks the elements of symmetry. This unusual structure followed from the conformational analysis of a number of simple molecules, modelling the amino and hydroxy acid residues of enniatin B. In particular, the conformational charts for O-acetyl-D-α-hydroxyisovaleryl-N-dimethyl amide and the methyl N-acetyl-N-methyl-L-valinate, calculated by an earlier described method [21], showed the most preferred conformation for the D-α-hydroxyisovaleryl fragment to be that with $\varphi \sim 300°$, $\psi \sim 60°$, whereas the L-N-methylvaline residue gives four isoenergetic minima (φ, ψ corresp. 61°, 270°; 56°, 104°; 241°, 266°; 238°, 90°). Analysis of molecular models of enniatin B demonstrated that only a single conformation shown in Fig. 12 of this cyclodepsipeptide is possible with the above rotational state of the hydroxy acid fragments and with the amino acid fragments in different permitted rotational states (φ, ψ corresp. 60°, 300°; 60°, 120°; 240°, 250°). This conformation was fully confirmed by the very good agreement between experimental (3.35 ± 0.1 D in CCl_4) and calculated (3.5 ± 0.5 D) values for the dipole moment of enniatin B. Characteristic features of this conformation are its compactness and the absence of a central cavity in the molecule.

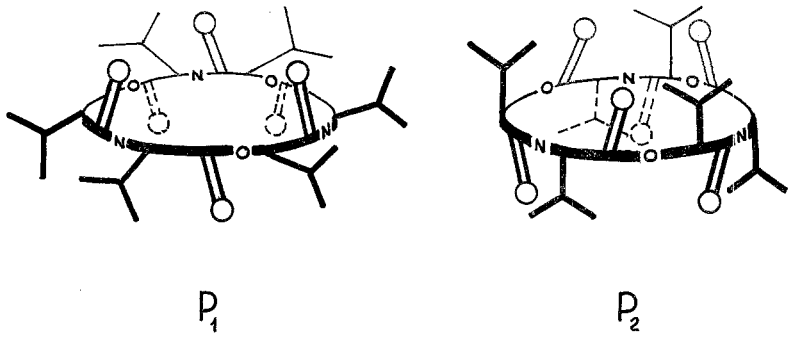

Fig. 13. Schematic representation of forms P_1 and P_2 of enniatin B

We have elucidated the conformation of the K^+ complex of enniatin B (similar to form *P* of the free enniatin B) in the following way. The formation of a complex of enniatin B with K^+ is accompanied by changes of the IR ester and amide bands, showing that all six carbonyls participate in the ion-dipole interaction. Obviously this interaction should be the decisive factor determining the spatial relations of both amide and ester carbonyls which are located on opposite sides of the plane of the ring and pointing to the cation in the center of the molecule.

This condition can be met by two different conformations of enniatin B, schematically represented in Fig. 13 and differing in the orientation of all the carbonyl and isopropyl groups. With the same "direction of acylation" in one of the conformations (P_1), the amide carbonyls are over the plane of the ring (whereas the ester carbonyls are under the plane), and all isopropyl groups are of pseudoequatorial orientation; in the other conformation (P_2), on the contrary, the amide carbonyls are under the plane of the ring (the ester carbonyls over the plane) and the isopropyl groups are in pseudoaxial position.

Although it could already be said *a priori* that the P_1 conformation would be the logical choice between the two, more rigorous grounds for the selection were obtained from a study of the complexes of (tri-N-desmethyl)-enniatin B (24) with various univalent cations. The use of this analog to determine the conformation of the antibiotic itself was justified by the fact that according to the ORD curves (Fig. 10a, b), the two cyclodepsipeptides assume the same conformations on both dissolution in polar solvents and complexation.

In a comparison of the complexes of enniatin B or its tri-N-desmethyl analog with cations of varying size, one should expect that such complexes would differ in the size of the internal cavity formed by the oxygen atoms

of the carbonyls participating in the ion-dipole interaction. Obviously the effective size of the cavity for a given ring size is determined by orientation of the carbonyl groups, which in turn depends upon the cation radius. With small cations, the closest distance between them would be with the carbonyls drawn into the center of the molecule. As the size of the cation is increased, all carbonyls are pushed more and more outward in order to accomodate the cation, so that the ensuing conformational changes could be likened to the opening of a flower bud.

It can be seen from Fig. 14 that such a change in orientation of the carbonyl group inevitably leads to simultaneous rotation of the CONH plane, the ultimate result of which would be change in the mutual arrangement in space of the N–H and C_α–H bonds as determined by the dihedral angle Θ. Analysis of molecular models shows that twisting of the carbonyl groups from the center to the periphery should increase the dihedral angle Θ, so that with the proviso of retention of the ion-dipole interaction, in the case of the P_1 conformation, the angle should increase approximately from 130 to 160°, and in the case of P_2 from 10 to 40°. This is a change which, from the angular dependence of the coupling constant $J=f(\Theta)$ (Fig. 7), should be reflected in the NMR spectra by an increase of $^3J_{NH-CH}$ in the case of P_1 and, on the contrary, by a decrease of one in the case of P_2 (*see* Fig. 14 & Table 4).

In fact, the NMR spectra of the complexes of (tri-N-desmethyl)-enniatin B with Li^+, Na^+, K^+ and Cs^+ displayed monotonous increase in the $^3J_{NH-CH}$ constant from 5.1 to 8.4 Hz (Table 4), unequivocally showing that complexes of (tri-N-desmethyl)-enniatin B and, consequently, enniatin B are preferentially in the P_1 conformation. Further, from an analysis of the $^3J_{C_\alpha H-C_\beta H}$ coupling constant of the enniatin B-K^+ complex, it follows that the $C_\alpha H-C_\beta H$ protons of valine residues are trans ($^3J_{C_\alpha H-C_\beta H}=9.8$ Hz), whereas in the case of α-hydroxyisovaleryl residues the isopropyl groups are apparently of gauche orientation ($^3J_{C_\alpha H-C_\beta H}=6.5$ Hz). The above findings lead to the conformation of the K^+ complex of enniatin B as depicted on Fig. 15.[1]

Characteristic features of this conformation are its planarity and the compact arrangement of the functional groups about the cation as well as its relative lability.

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[1] A similar ("flat disc" conformation) has been ascribed to the K^+ complex of enniatin B by Mueller and Rudin [18] on the basis of general considerations; according to a private communication by Dunitz this conformation is also characteristic of the crystal (from X-ray analysis).

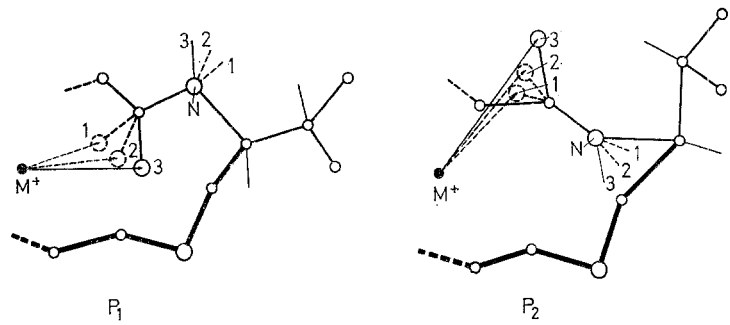

Fig. 14. Effect of the size of the complexed cation on orientation of amide group (*1* Li^+; *2* K^+; *3* Cs^+) in P_1 and P_2 conformations of (tri-N-desmethyl)-enniatin B (*see* Table 4)

Table 4. $^3J_{NH-CH}$ *Values of (tri-N-desmethyl)-enniatin B complexes with monovalent cations*

Cation	$M^+ \ldots O$ Distance $(r_{M^+} + r_0)$[a]	$^3J_{NH-CH^2}$, Hz		
		Calculated		Observed[b]
		P_1 form	P_2 form	
Li^+	1.2	3.2	7.9	5.1
K^+	1.7	5.8	7.0	7.9
Cs^+	2.1	7.6	6.1	8.4

[a] N. V. Belov's and G. B. Bokii's system of radii is used (*see*, for instance, G. B. Bokii, Introduction to Cristallochemistry, Publishing House of the Moscow State University, Moscow, 1954).

[b] Corrected values (*see* footnote to Fig. 4).

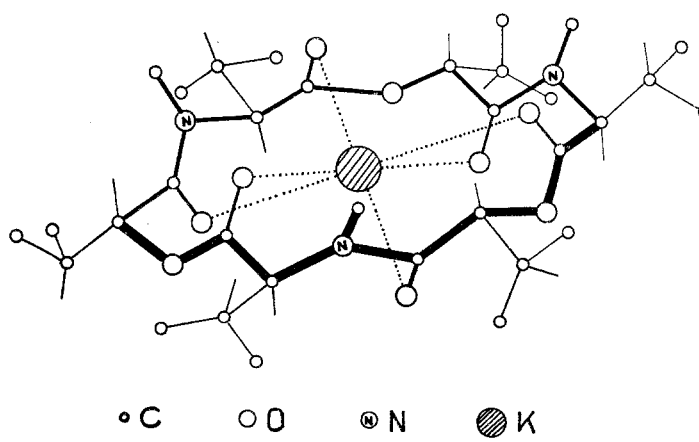

Fig. 15. Conformation of the K^+ complex of enniatin B

Comparison of the structures of the K^+ complexes of valinomycin (Fig. 9) and enniatin B (Fig. 15) makes comprehensible not only the general principles of cation binding by the cyclodepsipeptides but also the causes for the different efficiencies and selectivities of the complexing reaction.

First, it should be noted that the highly stable compact conformation of valinomycin makes the effective size of the inner cavity in this 36-membered molecule the same as that of the 18-membered enniatin (*cf.* Figs. 9 & 15). Moreover, the specifities of this conformation are such as to make for similar arrangement of the oxygen atoms about the cation (trigonal antiprism) in both depsipeptides. It is this which is mainly responsible for the similar cation binding capacities of valinomycin and enniatin.

Now it also becomes clear why individual amino and hydroxy acid residues in the molecule of valinomycin can be replaced by related compounds without loss of its complexing ability, but only up to the point when such replacement does not interfere with the bracelet conformation (analogs 2 and 3). Such exchange is "forbidden" if it disrupts the system of intramolecular hydrogen bonds, as can be seen by the complete disappearance of the complexing and biological activity on replacement of an amino acid by a hydroxy acid or an N-methyl-amino acid residue (analogs 4 and 5).

Naturally, configurational changes of the residues in the cyclodepsipeptide (analogs 11, 12 and 13) always accompanied by conformational changes also cause the disappearance of complexing properties and accordingly lead to complete loss of biological activity. At the same time, the enantiomers (14), (26), (27) and (28), conformationally identical with valinomycin and enniatins A, B and C, not only display the same complexing capacity but also similar antibiotic activity.

Structural comparison of the valinomycin- and enniatin-K^+ complexes shows that the ester carbonyl bond axes are differently oriented toward the cation in the two compounds, and consequently differences must exist in their cation-carbonyl interactions. In all probability, herein lies one of the reasons for the considerably greater K^+ binding efficiency of valinomycin than enniatin B, although in both molecules six carbonyl groups are participating in the ion-dipole interaction. However, it is to be noted that the stability constants of the cyclodepsipeptide complexes (and consequently also the ion selectivities of the cyclodepsipeptides) depend not only upon the ion's interaction with the structural elements of these molecules but also to a considerable extent upon solvation energy of both their polar groups and the free cations. Hence, the greater stability of the valinomycin-K^+ complex as compared to the enniatin-K^+ complex is

possibly due to a more effective shielding of the bound cation and the amide and ester groups by its hydrophobic side chains.

As already mentioned, contrary to the rigid, hydrogen bond-stabilized conformation of the valinomycin molecule, enniatin is characterized by a much higher conformational lability. Therefore, in its complexing with Na^+ and K^+, the higher solvation energy of Na^+ can be compensated by a more effective ion-dipole interaction caused by a rotation of the carbonyl groups, shortening the oxygen-cation distances and straightening the angles between the C=O and ion-dipole $M^+ \ldots O$ bonds (*see* Fig. 14). All this should equalize the free energies of formation of enniatin B complexes with Na^+ and K^+, i.e., lower the ion selectivity. On the contrary, the rigid structure of valinomycin debars such conformational transitions which explains the exceptional K^+/Na^+ selectivity of this depsipeptide.

As for the solvent effect on the complexing properties of the cyclodepsipeptides, one should expect a decrease in stability of the complexes with increasing polarity of the medium and, consequently, solvation energy of the free cation. In fact, in alcoholic solutions the stability constant of valinomycin decreases from 10^5 to 10^2 liter/mole (Fig. 16) as the water content is increased to 60 mole%; in pure water, no complexation can be detected at all. The solvent dependence of the complexing selectivity can be clearly seen on the example of enniatin C which loses its ability to discriminate between K^+ and Na^+ on transition from alcohol to a 1:2 alcohol-heptane mixture (Table 5).

A prerequisite for biological activity of cyclodepsipeptide is the ability to form complexes. Indeed, as one can see from the data in Tables 1 and 2, all biologically active analogs of valinomycin and the enniatins manifest this ability, and the relation between the biological and complexing properties is often not only of a qualitative but also of a quantitative nature. However, the ability to form complexes is not sufficient condition for manifestation of antimicrobial activity since the meso-analogs (9) and (10) of valinomycin and (tri-N-desmethyl)-enniatin B (24) form complexes of stability commensurate with those of the corresponding antibiotics and yet are practically inactive against microorganisms.

From all this follows that when the environment (solvent, temperature, etc.) is the same, the conformational characteristics of the cyclodepsipeptides are the decisive factor in the efficiency and ionic selectivity of the complexing reaction. At the same time, the findings also provide a clue to the understanding of the relation between the structure of the depsipeptides and their complexing and biological properties, which makes possible the rational search for analogs with predetermined membrane effects.

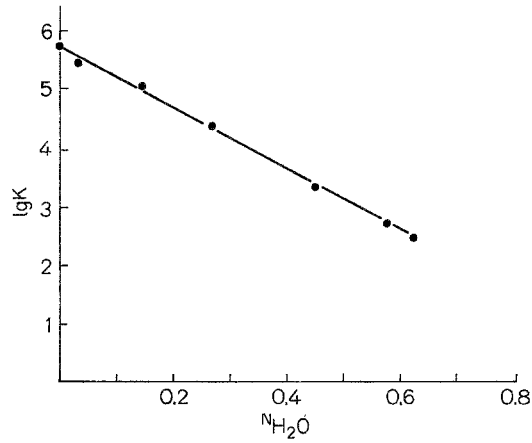

Fig. 16. Dependence of stability constant of K^+-valinomycin complex on the H_2O concentration in aqueous ethanol solution. N_{H_2O} mole fraction of water

Table 5. *Effect of the solvent polarity on the degree of complexation* (α)[a] *of enniatin C with* K^+ *and* Na^+ [b]

Cation	α in EtOH	$\frac{\alpha_{K^+}}{\alpha_{Na^+}}$	α in EtOH – C_7H_{16} (1:2)	$\frac{\alpha_{K^+}}{\alpha_{Na^+}}$
K^+	0.66	2.5	0.70	1.0
Na^+	0.26		0.71	

[a] Measured spectropolarimetrically (ORD).
[b] $[K^+]=[Na^+]=[\text{cyclodepsipeptide}]=2\times10^{-4}$ mole/liter; 25 °C.

Obviously, differences in biological activities of the cyclodepsipeptides are the result of differences not only in their complexing properties but also in the nature of their interaction with membranes. In view of this, noteworthy is the aforementioned observation that complex stability diminishes with increase in medium polarity and that apparently no complexes exist in water. It is thus quite plausible to assume that the cyclodepsipeptides "capture" the cations only after entering the lipophilic membrane. We have therefore undertaken a study of the behavior of the cyclodepsipeptides on model membrane systems, namely, monolayers at the water-air interface and bilayer membranes.

The behavior of the substances in question in membranes is dictated by the presence in the former of hydrophobic regions implementing their entrance into the lipophilic membrane regions and of polar groups imparting to the substance the ability to concentrate in the lipid-water interface.

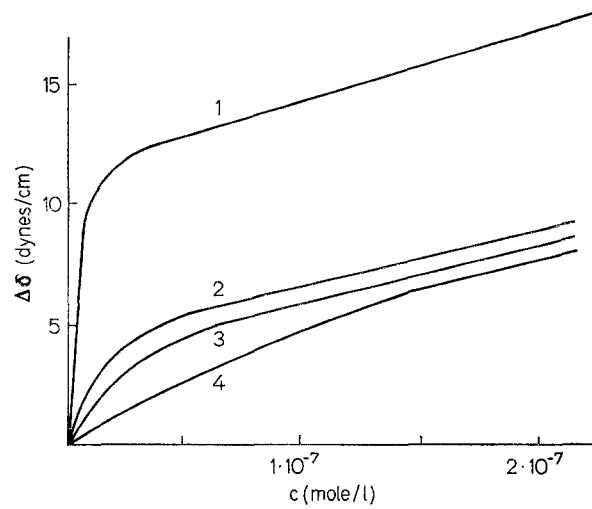

Fig. 17. Surface tension of aqueous solutions of cyclodepsipeptides. *1* Valinomycin; *2* Enniatin B; *3* Enniatin A; *4* Enniatin C. $\Delta\sigma$ decrease of the surface tension of the pure water. *c* depsipeptide concentration

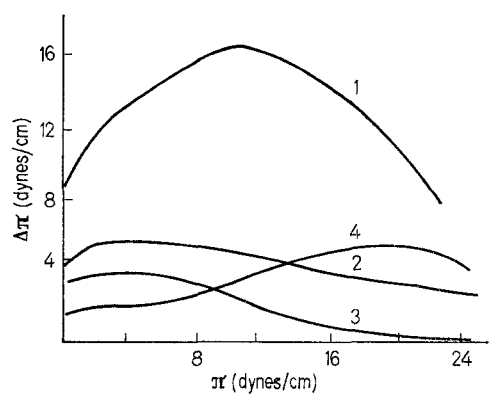

Fig. 18. Penetration of cyclodepsipeptides into egg lecithin monolayers. *1* Valinomycin; *2* Enniatin A; *3* Enniatin C; *4* (Tri-N-desmethyl)-enniatin B. π initial surface pressure of lecithin monolayer; $\Delta\pi$ increase of the surface pressure in the presence of depsipeptide. Depsipeptide concentration 3×10^{-8} M

For example, the bracelet conformation A (Fig. 8) of valinomycin predominant in nonpolar media should lend it surface-active properties owing to the variously oriented carbonyls in the "upper" and "lower" parts of the molecules; in fact, valinomycin forms stable surface layers with high collapse pressures (Fig. 17, curve *1*; Fig. 19, curve *3*). On the other hand, valinomycin was found to be capable of actively penetrating lecithin monolayers (maximum concentration of the cyclodepsipeptide in the monolayers occurs at an initial monolayer tension of 10 to 13 dynes/cm; Fig. 18). If

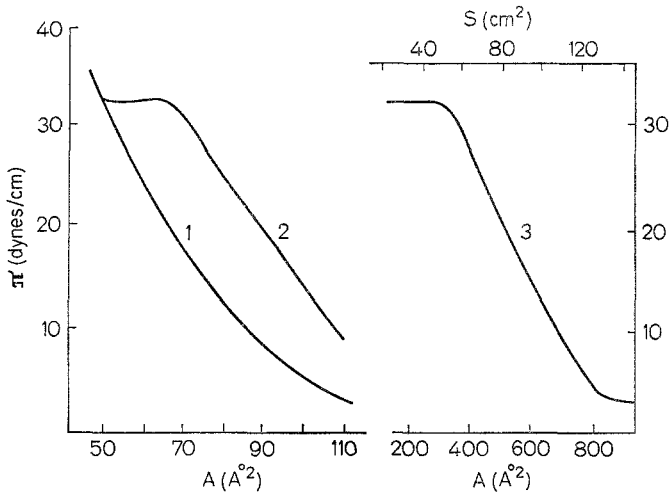

Fig. 19. Compression of DL-dipalmitoyl lecithin and valinomycin monolayers. *1* Dipalmitoyl lecithin on pure water; *2* Dipalmitoyl lecithin on aqueous solution of valinomycin (3×10^{-8} mole/liter); *3* Valinomycin. *A* area per one molecule of lecithin (*1, 2*) and valinomycin (*3*); *S* surface area of valinomycin monolayer under compression (for spread monolayer with initial surface concentration 1.7×10^{-11} mole/cm^2 or monolayer adsorbed on the surface of 3×10^{-8} M valinomycin solution)

the surface tension of the lecithin monolayers exceeds the collapse pressure of the neat valinomycin monolayers, cyclodepsipeptide does not enter into the lecithin layer (Fig. 19). This shows clearly that penetration of valinomycin into lecithin monolayers is due to the surface-active properties of this compound rather than to specific interaction with the lecithin molecules. From this follows the inference that on contact with phospholipid membranes the valinomycin molecules accumulate mainly on their surface. Very likely this is the reason for the usually observed ability of valinomycin in the absence of K^+ to inhibit the secondary blackening of bilayer phospholipid membranes. In the presence of K^+, the valinomycin molecules undergo conformational changes with accompanying loss of amphiphilicity (*see* Figs. 8 & 9). These complex cations are lipophilic and should accumulate in the central nonpolar region of the membrane. In other words, the binding of K^+ should promote the passage of valinomycin from surface to the inner regions of the membrane.

In contrast to valinomycin, the enniatins possess comparatively low surface activity and are less prone to accumulate in the lecithin monolayers (Figs. 17 & 18). One should therefore expect the enniatins to manifest a lower tendency than valinomycin to enter into phospholipid membranes. Their penetration into phospholipid membranes is apparently due to inter-

Table 6. *Action of cyclodepsipeptides on egg lecithin bilayer membranes*[a]

Analog no.	Compound	Concentration, M × 10^{-6}	Membrane resistance, Mohm × cm^2		Transmembrane potential[b], mV
			0.1 M KCl	0.1 M NaCl	
1	$\overline{\text{(D-Val-L-Lac-L-Val-D-HyIv)}_3}$ Valinomycin	0.1	0.0012	32	140
5	L-Val→L-MeVal	0.1	15	30	10
9	$\overline{\text{(D-Val-L-Lac-L-Val-D-Lac)}_3}$	0.1	0.008	10	130
10	$\overline{\text{(D-Val-L-HyIv-L-Val-D-HyIv)}_3}$	0.1	0.004	30	130
16	$\overline{\text{(L-MeVal-D-HyIv)}_3}$ Enniatin B	1	0.09	54	85
24	$\overline{\text{(L-Val-D-HyIv)}_3}$	1	25	30	10
25	$\overline{\text{(L-MeLeu-D-HyIv)}_3}$ Enniatin C	1	0.1	0.5	45
28	$\overline{\text{(D-MeLeu-L-HyIv)}_3}$	1	0.3	0.8	40
30[c]	$\overline{\text{(D-MeVal-L-Lac-L-MeVal-D-HyIv)}_3}$	0.1	20	40	10
—	No cyclodepsipeptides	—	20±15	35±15	≤10

[a] The membranes were formed in water solution containing the cyclodepsipeptide.
[b] The potential difference was measured on a membrane separating 0.1 M NaCl and 0.1 M KCl + 0.1 M NaCl solutions.
[c] The compound has no complexing properties and antimicrobial activity.

action with the lecithin molecules rather than to specific surface-active properties. This is evidenced by retention of their ability to penetrate the lecithin monolayers at pressures exceeding the maximum pressure of the neat enniatin monolayer (*see* Figs. 17 & 18).

A study of the behavior of valinomycin and enniatin cyclodepsipeptides in bimolecular phospholipid membranes has shown that complex formation is a necessary condition for their augmenting the permeability of artificial membranes; for a number of active cyclodepsipeptides, the strength of this effect and its cationic selectivity correlates satisfactorily with the stability of the complexes and the selectivity of complexation in alcoholic solutions (Table 6; *cf.* Tables 1 & 2).

In comparing the properties of various valinomycin and enniatin analogs, one finds compounds capable of complexation, active with respect to bimolecular phospholipid membranes but practically devoid of antibiotic activity. In this respect, of considerable interest is enniatin C (25), highly similar to enniatin B not only in the efficiency and ionic selectivity of the

complexing reaction in alcohol but also in the conformation of the complexes (Table 2; Fig. 2). Both compounds induce uptake of K^+ by mitochondria, but enniatin C is quite inactive against the microorganisms. Apparently the inactivity of enniatin C is associated with its low K^+/Na^+ discriminating ability in little polar media (*see* Table 5), in accord with very low K^+/Na^+ selectivity in the effect of this compound on bimolecular phospholipid membranes (Table 6). This illustrates the essential role played by selectivity of complex formation in the nonpolar phase of the membrane in the specificity of the induced permeability.

The relation between the ionic selectivity of enniatin C and its biological activity was studied on *S. faecalis*. It has recently been shown that growth inhibition of *S. faecalis* in the presence of valinomycin is apparently due to exchange of intracellular K^+ by H^+ [5]. We have confirmed this assumption, showing that growth inhibition occurs if K^+-H^+ exchange is induced in a K^+-enriched medium at pH 6.5 by the combined action of valinomycin (or enniatin B) and dinitrophenol (*see* [6]), although under the experimental conditions neither of these compounds is active when taken individually. One could assume that the insensitivity of *S. faecalis* to enniatin C is due to its increasing the permeability towards Na^+ as well as K^+, and since the former is present in much larger amounts than H^+, the exchange process should be predominantly of K^+ by Na^+ instead of K^+ by H^+. If this is so, increase of proton permeability of the plasmatic membrane should increase the proton flux and thus shift the process in favor of K^+-H^+ exchange. Indeed, in the presence of dinitrophenol, enniatin C inhibits the growth of *S. faecalis*, its activity under these conditions even exceeding that of enniatin B (Table 7).

In some cases, no rational explanation has been found for the absence of antibiotic activity of cyclodepsipeptides that form complexes. In particular, this pertains to the so-called meso-analogs (9) and (10) of valinomycin mentioned above, which not only selectively increase the K^+ permeability of model membranes but also (according to our preliminary results) effectively induce K^+ absorption by rat liver mitochondria. The peculiar feature of the structure of these compounds (whose stereochemistry and properties we are presently investigating in detail) is that the "upper" and "lower" regions of the molecule in the bracelet conformation (*see* Fig. 8) are identical. Moreover, the conditions of conformational equilibrium for the groups in these regions of the molecule in one case, compound (9), approach those existing in the "upper" half of the valinomycin molecule; in the other, compound (10), they approach those existing in the "lower" half of the molecule. This circumstance apparently has little bearing on

Table 7. *Growth inhibition of S. faecalis in a potassium-enriched medium* (0.086 M) *in the simultaneous presence of cyclo depsipeptide and dinitrophenol (DNP)*[a]

Concentration of DNP, M	Minimal growth-inhibiting concentration, γ/ml		
	Valinomycin	Enniatin B	Enniatin C
5×10^{-4}	0.05 – 0.1	3 – 4	2
2.5×10^{-4}	0.1 – 0.2	9	9
1.25×10^{-4}	0.2 – 0.4	18	18
0.6×10^{-4}	0.5 – 0.6	18	> 25
0.3×10^{-4}	1	18	> 25
0	> 25	18	> 25

[a] Minimal *S. faecalis* growth inhibiting-concentration of DNP is 1×10^{-3} M.

the stability and lipophilicity of the complexes, but should have a considerable influence on the conformational equilibrium of the free cyclodepsipeptide and, thereby, on its surface-active properties and on the nature of its interaction with the membrane. In a certain sense, it may be said that these two analogs with symmetric arrangement of the side chains in the bracelet conformation are even more convenient starting points than valinomycin itself for studying structure-activity and structure-conformation relations.

Conclusion

The study of macrocyclic depsipeptides has led to the discovery of a new mechanism by which peptidic systems devoid of ionizable functional groups can selectively bind alkali metal ions. This mechanism rests on the interaction with the ion of an orderly arranged system of carbonyls belonging to amide or ester groups, so that both the efficiency and the selectivity of the complexing reaction is highly dependent on the conformational parameters of the molecule. Since these compounds are structurally similar to the peptide chains of proteins, it could be conjectured that such a structure is also possessed by ion-exchange sites in biological membranes responsible for their ionic selectivity. If this is so, then one might wonder if the permeability of biological membranes might not be governed by conformational changes in such ion-exchange sites. On the other hand, it is also quite possible that conformational transitions incurred by the formation of complexes with alkali metal ions could play an essential part in the functioning of both membranes and other biological structures (in particular, certain enzymes). From this point of view, of interest is the role of

Na^+ and K^+ in phosphorylation and dephosphorylation reactions of the K^+, Na^+-dependent transport membrane ATPase [11, 16].

We still do not know if alkali metal ion transport through biological membranes is effected by constituents with valinomycin-like functions. However, highly significant is the fact that the ionic permeability induced by cyclodepsipeptides in bimolecular phospholipid membranes correlates well in magnitude and selectivity with the permeability induced by these substances in biological membranes. Hence, the possibility of synthesizing cyclodepsipeptides with predetermined conformational properties and therefore with given ionic selectivity followed by activity-selecting tests on model membranes may lead to a set of valuable chemical tools for modifying at will the properties of biological membranes.

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