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INTEGRATED CONFORMATIONAL STUDIES OF CYCLOPEPTIDES

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The stereochemistry of carbon compounds whose foundation a century ago we owe to the genius of Van't Hoff and Le Bel, now includes conformational phenomena, the importance of which to the realm of bio-organic chemistry cannot be overestimated. This, naturally, refers also to the bio-organic chemistry of cyclopeptides, a class of compounds spectacular for the variegation of their biological functions, so that among their representatives one encounters hormones, different types of antibiotics, toxins and antitoxins etc. Since elucidation on the molecular level of the modes of action of cyclopeptides is a prerequisite to rational interference in the processes proceeding with their participation quite naturally it is a primary objective of the researchers in cyclopeptide chemistry.

Not so long ago the approaches to this objective were confined to synthesis of analogs of the biologically active compounds, comparison of their properties and thereby disclosure of the particular groupings responsible for manifestation of the given activity[1]. This could only result in very limited information about the molecular mechanism of cyclopeptide functioning since, paying little attention to the specific spatial relations of the reactive groups in the molecule, i.e. to the stereochemistry, one hardly could expect considerable gains in an area where the mutual interaction of such groups had a decisive effect on the properties of such polyfunctional compounds.

Further progress could thus be attained only by direct study of the conformational states of the cyclopeptide systems. Especially important were data covering wide ranges of environmental conditions shedding light on the dynamics of the conformational equilibria and the potentialities of the peptide molecule. For this reason studies of peptides in solution have definite advantage over the X-ray analysis of crystalline specimens, despite the unsurpassed resolving power of crystallographic methods.

Conformational studies of cyclopeptides, in particular the large subclass of depsipeptides, were initiated in our laboratory by its former head, M. M. Shemyakin in 1963. At that time there were only scattered data on the solution structures of such compounds. Even the question of whether it was possible in principle to obtain unambiguous results in this area by means of the existing methods had still to be answered. We strove, therefore, from the very beginning to utilize for this purpose the widest variety of physico-chemical methods of which each making an independent contribution to the general solution. Among the selected objects were the membrane active antibiotics valinomycin (*vide infra*), the enniatins[2-11] and gramicidin S,[12-14] the antitoxin antamanide[15,16] the neutral fungal metabolites sporidesmolides,[17] as well as various synthetic analogs of all these compounds. We also included in the study a large series of model cyclohexapeptides.[18-26]

As the study progressed there crystallized out an optimal way of applying the instrumental and theoretical methods wherein, in opposition to their non-discriminate use, which one still may encounter, they were integrated into a whole such that each is used in a logical sequence and only insofar as it will further the structural analysis.[27] Briefly, such an approach consists in the following.

Spectral investigations are most conveniently begun with CD or ORD measurements giving immediate knowledge of the conformational lability of the peptide and in certain cases even of the number of principal conformers and the conditions for their existence. This could be followed by NMR spectroscopy which from the line shapes and intensities, could yield the relative contents of the forms, their symmetry and the thermodynamical equilibrium parameters. Next would follow, say, an IR study of the intramolecular hydrogen bonding (IMHB) in non-polar solvents such as CCl_4, $CHCl_3$, C_7H_{16} etc and in the more polar media by means of the NH parameters of the NMR spectra, namely, the chemical shift, δ_{NH}, its temperature dependence $\Delta\delta/\Delta T$ and the rate of NH→ND exchange in labile-deuterium containing solvents. Simultaneously the vicinal proton spin–spin coupling constants of the NH–C^{α}H and C^{α}H–C^{β}H fragments

are determined in order to ascertain the range of possible ϕ[28] and χ[29] values.* At this point one can decide as to whether it is necessary or not to synthesize isotope-labeled analogs, not only for making possibly lacking assignments of the NMR signals but for determination of vicinal coupling constants such as $^3J_{^{15}NC'-C^{\alpha}H}$, $^3J_{^{15}NH-C^{\alpha}-^{13}C}$ etc which enrichment of the peptides with ^{15}N and ^{13}C isotopes makes possible and which could aid in elucidating Ψ angles.[31,32]

The combined results from all the above techniques serve as the outgoing point for theoretical conformational analysis, the dipole moments of the deduced structures then being compared with the experimental values. If a conclusive choice of structure cannot be made at this stage the course of further quests is dictated by the concrete properties of the substance undergoing study. Naturally the reliability of the conclusions is greatly enhanced if X-ray structural studies of the crystalline form as well as the solution studies are carried out.

The potentiality of the above outlined approach will now be illustrated in the following description of the study of valinomycin, the first large peptide molecule to have its solution spatial structure and that of its synthetic analogs elucidated. In this we shall make use of results published earlier,[3,7,27,33-37] reported at meetings[14,38-43] or not published heretofore.

The conformational states of valinomycin and its analogs

Valinomycin (Fig 1) was discovered in 1955 by Brockmann and Schmidt-Kastner[44] and synthesized in 1963 by Shemyakin and collaborators.[45,46] It soon after acquired a prime position among the "ionophores", substances forming complexes with alkali metal ions and enhancing their permeability to artificial and biological membranes.

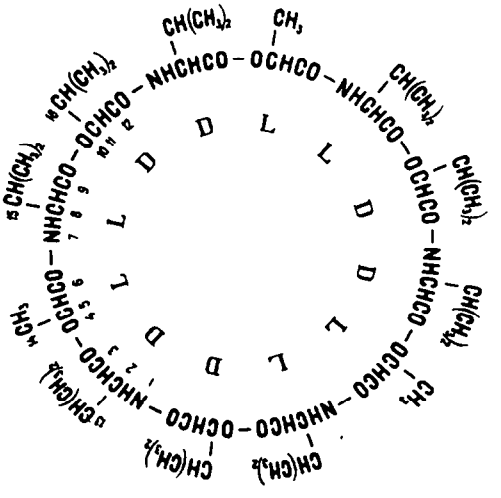


Fig 1. Valinomycin.

In solution valinomycin is capable of binding a wide variety of ions.[47] It displays an exceptionally high K/Na complexing selectivity (see Table 5), unequalled by any other alkali metal complexone. Valinomycin is capable of inducing alkali metal ion transport across the membranes of mitochondria, erythrocytes, chloroplasts and other biological objects as well as bilayers, bulk membranes and phospholipid micelles.†

In conformity with the stability of the complexes, valinomycin manifests a high K/Na permeability inducing ratio. For instance, in the presence of this antibiotic the ratio of the potassium and sodium fluxes across the mitochondrial membrane is ca. 10000:1.[53]

1. *Free valinomycin.* The 36-membered valinomycin ring is endowed with a wealth of conformational possibilities. Its spatial structure depends on the solvent species, as can be seen from the variability of the CD and ORD curves (Fig 2).

The relatively high δ_{NH} values in CCl_4 and $CDCl_3$ (7·76–7·90 ppm) (Fig 3 and Table 1) indicate that the NH hydrogens are participating in H-bonding. The low concentrational dependence of δ_{NH} is explained by the H-bonds being of the intramolecular type. This is also evidenced by IR spectra (see below). The NMR signals of the antibiotic show no signs of splitting even on cooling to −95°, which is strong support for the C_3 symmetry which corresponds to its formula. The nature of the conformational rearrangements accompanying augmentation of the solvent polarity can be followed by observing the NMR spectra of solutions in CCl_4–$(CD_3)_2SO$ and $CDCl_3$–$(CD_3)_2SO$ mixtures (Fig 4). Gradual addition of the $(CD_3)_2SO$ (up to ~40 mole%) shifts the N_7H signal more and more to lower field, whereas the N_1H signal undergoes a lesser shift to higher field. For a mixture of 30% $(CD_3)_2SO$ in CCl_4 the N_1H chemical shift is almost temperature independent whereas the N_7H signal, on the contrary displays very high temperature dependence. At the same time one can observe strong differentiation of the NH signals also with respect to their $^3J_{NH-C^{\alpha}H}$ constants. In pure dimethyl-sulfoxide the $\Delta\delta/\Delta T$ values of both NH signals are close to those characteristic for NH groups solvated by the solvent. Similar spectral parameters were observed for solutions of the antibiotic in 3:1 CH_3OH–H_2O (Table 1).

The above results make it possible to give the following characteristics of the conformational behaviour of free valinomycin. Depending upon the solvent valinomycin can basically assume three conformations which we have named *A*, *B* and *C* (Fig 5). Form *A* has a threefold axis and all the NH groups are participating in IMHB. Form *B* is

*For the conformational nomenclature of peptides see Ref 30.

†See Ref 47 and refs cited therein.

Table 1. NMR data on valinomycin and its Na^+, K^+ and Cs^+ complexes

Solvent	Chemical shifts (δ, ppm)[a]									Vicinal coupling constants, Hz				
	$CH-(CH_3)_2$ Val, HyIv	$CH-CH_3$ Lac	$CH-(CH_3)_2$ Val, HyIv	$C^\alpha H$ D-Val	$C^\alpha H$ L-Val	$C^\alpha H$ L-Lac	$C^\alpha H$ D-HyIv	NH D-Val	NH L-Val	$^3J_{C^\alpha H-C^\beta H}$[b] D-Val	$^3J_{C^\alpha H-C^\beta H}$[b] L-Val	$^3J_{C^\alpha H-C^\beta H}$[b] D-HyIv	$^3J_{NH-C^\alpha H}$[c] D-Val	$^3J_{NH-C^\alpha H}$[c] L-Val
CCl_4	0·94–1·15	1·45	2·31	3·97	3·89	5·21	4·95	7·90	7·76	10·0	10·0	2·9	8·5	6·6
$CDCl_3$	0·94–1·13	1·46	2·31	4·13	4·00	5·34	5·05	7·88	7·78	9·5	10·1	2·9	8·8	6·6
$CDCl_3$–CS_2 (1:1)	0·92–1·10	1·42	2·27	3·92; 4·02		5·24	4·97	7·85	7·73	—	—	3·9	8·8	6·8
CCl_4–$(CD_3)_2SO$ (3:1)	0·90–1·10	1·29	2·30	4·80	4·08	5·42	4·94	7·52 (0·65)	8·75 (11·0)	4·6	9·8	3·0	10·1	7·6
$(CD_3)_2SO$	0·92	1·29	2·20	4·35	4·35	5·11	4·82	7·88 (4·5)	8·40 (9·0)	—	—	3·8	8·7	8·1
CH_3OH–H_2O(3:1)	1·01	1·43	—	—	—	—	—	8·25	8·29	—	—	—	8·6	8·6
CD_3OD (CH_3OH)	1·02	1·41	2·27	4·29; 4·44		5·19	4·93	8·14 (11·9)	8·24 (10·8)	7·1; 7·76		4·4	9·2	8·9
$CD_3OD(CH_3OH)$ + KSCN	0·96–1·19	1·55	2·27	3·85; 3·87		4·97	4·68	8·30 (1·9);	8·38 (2·1)	10·9	10·9	3·8	5·6	5·6
CH_3OH–NaSCN	—	—	—	—	—	—	—	7·99 (6·6)	7·99 (6·6)	—	—	—	7·2	7·2
$CDCl_3$–CS_2–CH_3OH (1:1:1) + KSCN	0·96–1·20	1·58	2·22	—	—	—	—	8·28; 8·38		—	—	—	5·3	5·3
CH_3CN	0·92–1·08	1·40	—	4·11	4·13	5·28	5·01	7·61	7·58	8·7	9·8	3·8	8·4	7·5
CH_3CN + KSCN	0·91–1·16	1·52	—	3·80; 3·85		4·95	4·64	8·34; 8·42		11·0	11·0	3·6	5·2	5·2
CCl_4–CH_3CN (1:1)	0·95–1·13	1·45	—	3·97	4·01	5·32	5·04	7·78 (4·0)	7·70 (4·2)	10·4	10·4	3·2	8·6	8·0
CCl_4–CH_3CN (1:1) + KSCN	0·95–1·18	1·53	—	3·79; 3·84		4·89	4·57	8·30 (1·5);	8·37 (1·8)	10·7	10·7	3·8	5·4; 5·5	
CCl_4–CH_3CN (1:1) + NaSCN	0·97–1·15	1·51	—	4·07; 4·13		5·07	4·78	7·88 (0·9);	7·94 (0·9)	8·6	8·6	4·0	6·0	6·0
$CDCl_3$ + CsCl	0·95–1·20	1·55	2·25	3·81	3·81	5·08	4·72	7·89; 7·98		—	—	—	5·5	5·5

[a] The values in parentheses are for $\Delta\delta/\Delta T \cdot 10^3$, ppm/C°

[b] For $^3J_{C^\alpha H-C^\beta H}$ (L–Lac) the values range from 6·2 to 7·1 Hz

[c] Corrected for substituent electronegativities ($J_{corr} = 1{\cdot}09\, J_{exp}$)

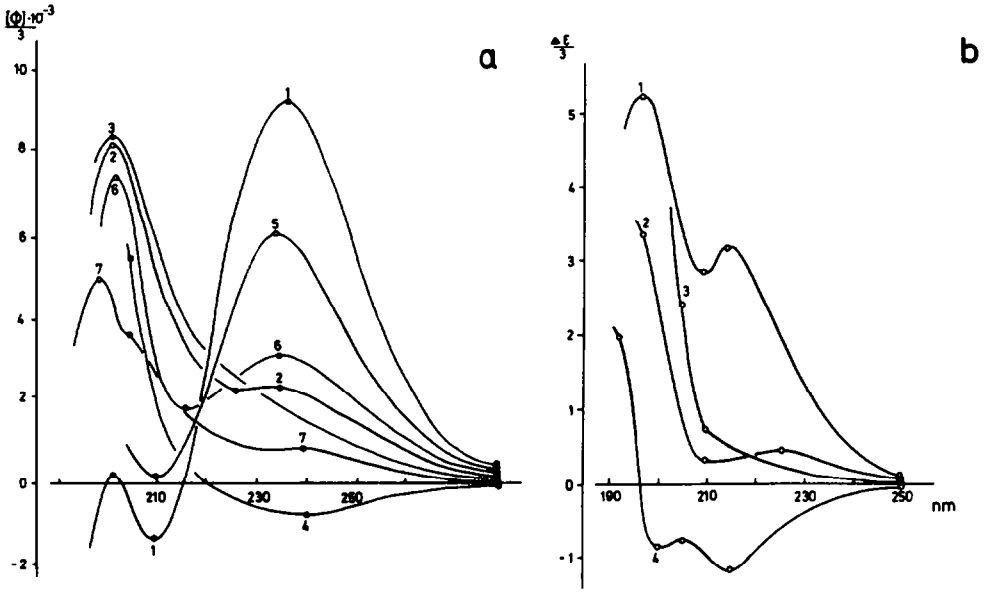


Fig 2. ORD (a) and CD (b) curves of valinomycin. 1 heptane; 2 ethanol; 3 acetonitrile; 4 water-ethanol (1 : 1); 5 dioxane-heptane (10 : 1); 6 ethanol-heptane (1 : 3); 7 trifluoroethanol-water (1 : 2).

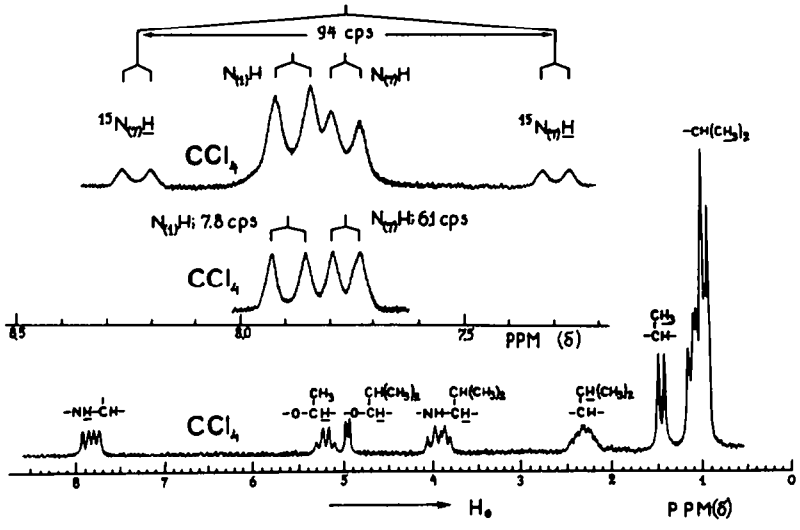


Fig 3. NMR spectrum of valinomycin in carbon tetrachloride. Assignments of the NH signals were made with the aid of the ^{15}N-L-Val-valinomycin spectrum shown in the upper part.

predominant in solvents of medium polarity; it retains the three more stable H-bonds formed by the N_iH atoms. Form *C* is predominant in polar solvents and contains no IMHB. Apparently it has no fixed structure, comprising an equilibrium mixture of a large number of equi-energetic conformers.

In a study of relaxation processes by means of ultrasonic absorption, Grell *et al.*[49] found other forms apparently intermediate between *A*, *B* and *C* and possessing five, four, two or one IMHB.

Further refinement of the valinomycin *A* structure can conveniently be initiated with examination of the IR data for solutions in non-polar solvents (n-C_7H_{16}, CCl_4, $CHCl_3$) (Fig 6). The amide *A* region displays a prominent $\nu_{NH} = 3307$–$3317\ cm^{-1}$ band evidence of formation of strong IMHB and a weaker band due to free or weakly hydrogen

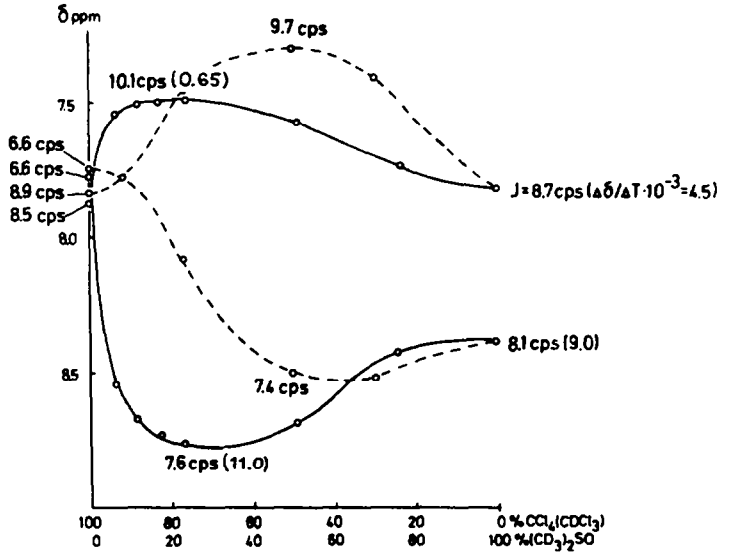


Fig 4. Chemical shifts (δ, ppm) of the NH signals of valinomycin in CCl_4-$(CD_3)_2SO$ (solid lines) and $CDCl_3$–$(CD_3)_2SO$ (dashed lines) mixtures; the figures are for $^3J_{NH-CH}$, Hz and (in parentheses) $\Delta\delta/\Delta T \cdot 10^{-3}$, ppm/°C.

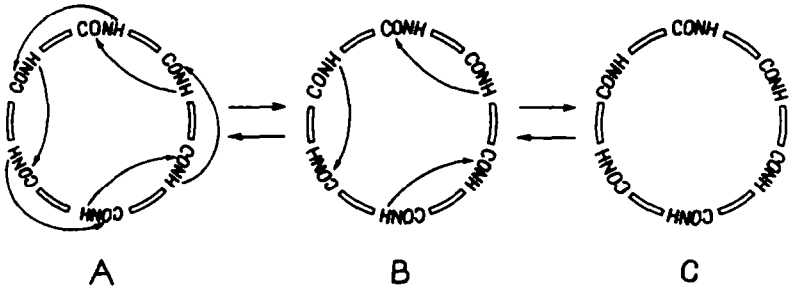


Fig 5. $A \rightleftarrows B \rightleftarrows C$ equilibrium of valinomycin.

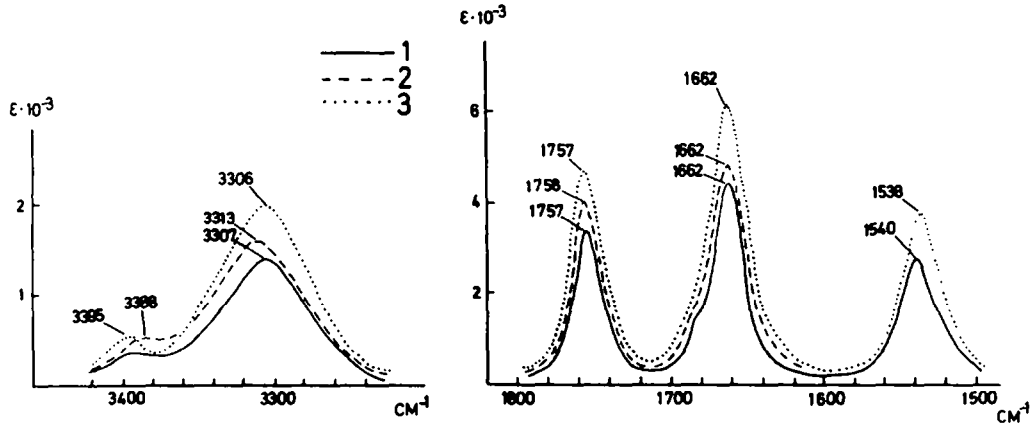


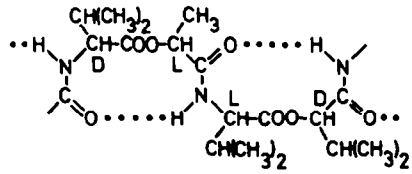
Fig 6. IR spectra of valinomycin in heptane (1), carbon tetrachloride (2) and chloroform (3).

bonded NH (ν_{NH} = 3388–3395 cm^{-1}). Separation of the bands, calculation of their integral intensities and determination of the number of NH groups by a procedure described[21] showed these groups to be in a 5:1 ratio.* This result is in agreement with the participation in the equilibrium of ~ 70% of form *A* and ~ 30% of form *B*. It is also possible that form *B* is absent under the conditions of measurement, the high frequency band corresponding to form *A* in which the NH groups alternate in the formation of the weak IMHB. In such cases the "migration" frequency of the weakened H-bond must greatly exceed the NMR frequency even on cooling because of the homogeneity of the NMR spectra. The frequency of the additional IR band varies in the valinomycin analogs within the limits 3360–3422 cm^{-1}, i.e. in some cases it is too low for a free NH group,[21] so that it cannot correspond to form *B*. In other words the data on the valinomycin analogs turn the scales in favor of the second of the aforementioned possibilities.

In the carbonyl region there is a high symmetric ν_{CO} band (1775–1757 cm^{-1}), corresponding to non-IMHB forming ester carbonyls. The amide carbonyl region displays a strong amide II peak (1538–1540 cm^{-1}) indicating the amide bonds to have a *trans* configuration, a strong amide I band at 1662 cm^{-1} and a much weaker band at 1675 cm^{-1} contingent on the former. The relative intensities of the latter two bands correspond approximately to the intensity ratios of the 3300–3400 cm^{-1} NH bands. It follows, therefore, that all the IMHB of valinomycin are formed by the amide CO and NH groups.

Analysis of molecular models shows that for *trans* amide and ester bonds only one possibility exists for the formation of six IMHB that would be in accord with the above data. It is shown schematically on Figs 5 and 7. In this conformation the depsipeptide chain of valinomycin is closed into a system of six fused 10-membered rings stabilized by 4→1 type IMHB so as to form a "bracelet" 8 Å in diameter and 4 Å high. Such a structure can be in two different forms (A_1 and A_2, Fig 7), differing in the chirality of the ring system and orientation of the side chains. Conformation A_1 can be most easily distinguished from A_2 as follows: if the bracelet is so positioned that the lactic acid residues are in the upper part then in conformation A_1 acylation follows a clockwise and in A_2 a counterclockwise course. The amide bonds and asymmetric carbon atoms in forms A_1 and A_2 are fixed, whereas the ester carbonyls can point inwards (i.e. in the direction of the symmetry axis) or outwards (i.e. away from the symmetry axis). As a result each of the forms A_1 and A_2 can be in two types of conformations, differing in the mutual orientation of the carbonyl groups; they are listed in Table 2.

The next step in analysis made use of the optimal conformations of model compounds calculated by Popov *et al.*[34,50] Below in Fig 24 are produced the conformational maps of compounds simulating the amino and hydroxy acid fragments of valinomycin and in Table 3—data on two protected didepsipeptides simulating its 10-membered H-bonded rings:



The fragment maps show four principal energy minima located in the R, B, P and L regions; additional minima N and S on the Ac–L–Lac–OMe map lie close to the B and L regions. The didepsipeptide conformational types represented in Table 3 are accordingly designated as P–R, B–L, R–B, L–P etc. (the overhead bracket stands for a IMHB). The calculation showed that the parameters of the individual amino and hydroxy acid residues in the

*One could not *a priori* exclude the possibility that the minor band in the amide *A* region is due to Fermi resonance of the NH stretch vibrations with the carbonyl stretch overtones or with their combinations. This, however, was not confirmed by a study of the valinomycin analogs, from which it followed that the intensity of the band in question reflects the degree of steric strain in the H-bonded structure of peptide skeleton. For instance, the IR spectrum of hexadecavalinomycin is practically the same as that of the antibiotic, except for the absence of the high frequency band (Fig 17).

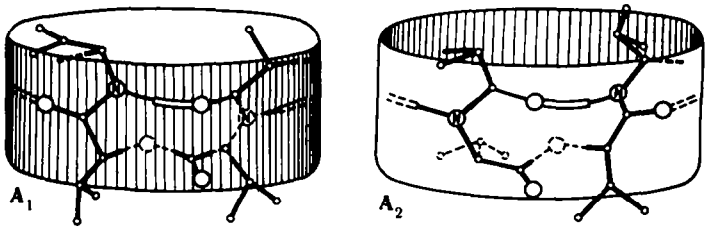


Fig 7. Schematic representation of the A_1 and A_2 forms of valinomycin.

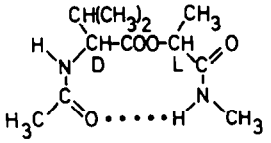
Table 2. Possible conformations of the A forms of valinomycin

	Conformation	Orientation of the CO groups D–Val	Orientation of the CO groups L–Val	Relative energy kcal/mole
Form A_1	[(R–B–L–P)$_3$]	Out	Out	12·8
	[(B–L–L–P)$_3$]	In	Out	15·0
	[(R–B–L–P)$_3$]	Out	In	25·2
	[(B–L–P–R)$_3$]	In	In	27·6
Form A_2	[(L–P–R–B)$_3$]	Out	Out	>60
	[(P–R–R–B)$_3$]	In	Out	>40
	[(L–P–B–L)$_3$]	Out	In	>18
	[(P–R–B–L)$_3$]	In	In	0

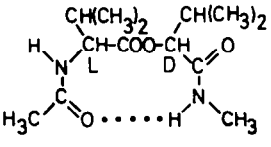
optimal didepsipeptide conformations correspond to the most advantageous regions of the corresponding conformational maps. The IMHB energy for the optimal structures represented in Table 3 lies within the limits of 3·8–4·0 kcal/mole and the bridged N··O length is 2·8–2·9 Å. The formation of IMHB considerably narrows the regions of permissible ϕ–ψ values of the didepsipeptides. For instance, no IMHB could form beyond the region $\phi_{Val} \pm 75°$.

The data of Table 3 enable one to calculate the energies of the different bracelet confromations of valinomycin. The results presented in Table 2 show that the most advantageous from the standpoint of energy of the individual fragments is the conformation [(P–R–B–L)$_3$] belonging to the A_2 type. Following it, but far behind energetically are [(R–B–L–P)$_3$] and [(B–L–L–P)$_3$]. The other conformations are of little probability, because of their excessive energy. The aforementioned range of the ϕ_{Val} angles corresponds to 0–6·5 Hz for $^3J_{NH-CH}$ values of A_2 and 0–9·5 Hz for A_1. Experimental values (NMR data for solutions in $CDCl_3$ and CCl_4) amount to 6·6–8·8 Hz, whence it follows that form A_2 cannot be predominant in non-polar solvents. In other words, A_2 with "all in" ester carbonyls, the most advantageous from the point of view of nearest neighbour interactions, is in fact not realized. The reason for this is the transannular destabilizing interactions, among wich first of all are the electrostatic repuslions of the six carbonyl groups all pointing towards the center of the molecule. For the same reasons, of the two remaining conformations A_1 "all out" and A_1 "three out, three in", the latter must be considered to be less probable. It follows, therefore, that the predominant conformation of

Table 3. Optimal conformations of the model depsipeptides



Conformation	Geometrical parameters D–Val ϕ°	D–Val ψ°	D–Val χ°	L–Lac ϕ°	L–Lac ψ°	Relative energy, kcal/mole
P–R	59	−113	−166	−73	20	0
R–B	−45	−62	169	−95	43	1·8
R–R	−52	−30	163	−67	−32	2·5
B–L	−50	100	−175	53	39	2·6
L–L	53	41	−174	52	33	2·5



Conformation	Geometrical parameters D–Val ϕ°	D–Val ψ°	D–Val χ°	D–HyIv ϕ°	D–HyIv ψ°	D–HyIv χ°	Relative energy, kcal/mole
B–L	−61	107	175	77	25	64	0
L–P	46	64	−170	100	−42	68	2·4
L–L	57	6	−164	70	27	61	2·4
R–R	−57	−37	175	−52	−34	165	6·0
P–R	55	−122	−167	−55	−31	170	6·0

valinomycin in nonpolar solvents is $\overline{(R\text{–}B\text{–}L\text{–}P)_3}$ with six outwardly pointing ester carbonyls. In conformity with the calculated results (see the χ values in Table 3) the $C^{\alpha}H$ and $C^{\beta}H$ protons of the amino acid residues are under such circumstances in *trans* conformation ($^3J_{C^{\alpha}H\text{–}C^{\beta}H} = 9{\cdot}5\text{–}10{\cdot}1$ Hz in CCl_4 and $CDCl_3$) and of the α-hydroxyisovaleric acid residues in the *gauche* conformation ($^3J_{C^{\alpha}H\text{–}C^{\beta}H} = 2{\cdot}9$ Hz). The spatial structure found for valinomycin is represented in Fig 8. The dipole moment calculated for the symmetric conformation $\overline{(R\text{–}B\text{–}L\text{–}P)_3}$ is 0–0·7 D, whereas the experimental value is significantly higher ($3{\cdot}5 \pm 0{\cdot}1$ D in CCl_4). This is apparently due to the presence of a weakened IMHB.

Form *B* stabilized by only three IMHB, between the lactyl carbonyl and the D-valyl NH groups, is more flexible. Its structure was determined from NMR spectral data aided by theoretical conformational analysis. Two energy structures were found to be in accord with the experimental $^3J_{NH\text{–}CH}$ constants determined for a solution in 3:1 CCl_4–$(CD_3)_2SO$ (Fig 4, Table 1). In one of them (Figs 9a and 9b) the D-Val isopropyl groups, whereas in the other the L-valyl isopropyl groups are spatially neared (Fig. 9c). Since in form *B* the steric hindrances can be detected in the D-valyl side chains (judging from the $^3J_{C^{\alpha}H\text{–}C^{\beta}H} = 4{\cdot}6$ Hz constants they are forced into the *gauche* conformation) it is only natural that the first structure should be assigned to this form. One can discern in this structure a hydrophobic "nucleus" comprising the aliphatic D-Val and L-Lac side chains which is encircled by the depsipeptide chain with its polar groupings. The IMHB-stabilized 10-membered rings thereby turn out to be at the periphery lending to the molecule the form of a propeller.

After publication of our studies similar results as to the solution conformations of valinomycin based on more limited experimental material were ob-

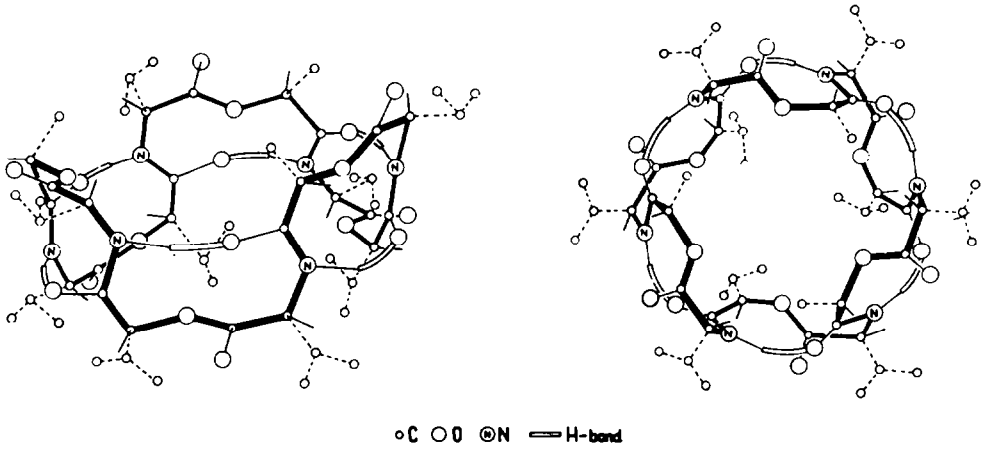


Fig 8. Conformation of valinomycin in non-polar solvents (a—side view; b—view along the C_3 axis). D-Val: ϕ – 40°, ψ – 70°; L-Lac: ϕ – 100°, ψ 40°; L-Val: ϕ 25°, ψ 70°; D-HyIv: ϕ 100°, ψ – 30°.

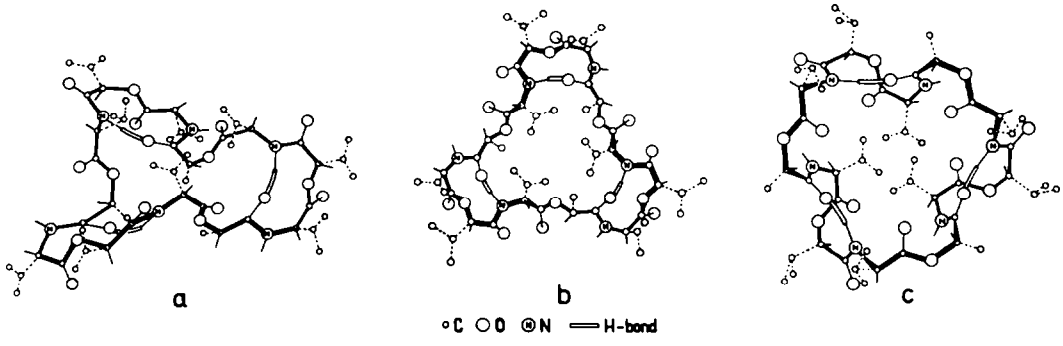


Fig 9. a and b—two views of the propeller conformation of valinomycin ($\overline{(L\text{–}B\text{–}L\text{–}P)_3}$ type (D-Val: ϕ120°, ψ85°; L-Lac: ϕ –60°, ψ120°; L-Val: ϕ50°, ψ65°; D-HyIv: ϕ100°, ψ –40°); c – $\overline{(P\text{–}R\text{–}L\text{–}L)_3}$ conformation of valinomycin (D-Val: ϕ120°, ψ – 130°; L-Lac: ϕ –60°, ψ120°; L-Val: ϕ50°, ψ65°; D-HyIv: ϕ100°, ψ –40°).

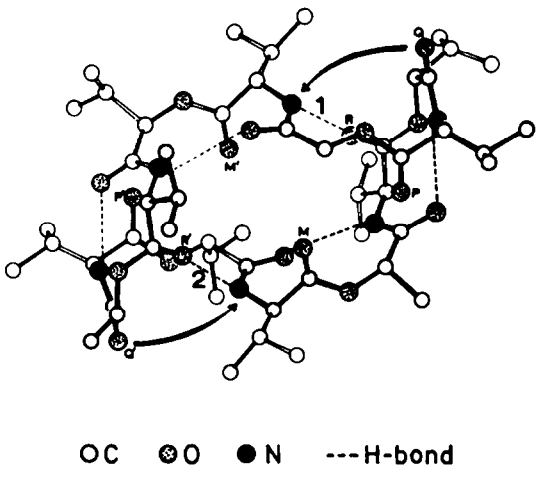


Fig 10. Conformation of crystalline valinomycin. M and M', P and P', R and R' are ester carbonyl oxygens related by the pseudo symmetry center; Q and Q' are the amide oxygens not participating in hydrogen bonding. 1 and 2—H-bonds of the 5→1 type. Arrows mark the direction of Q and Q' displacement on rearrangement to the complexed form.

tained.[51,52] It is not without interest to compare our data with those obtained by an X-ray analysis of crystalline valinomycin (Fig 10).[53] The molecule has here a pseudosymmetry center, but lacks the C_3 axis. As in form *A*, it has six IMHB, of which four are of the type 4→1 between amide CO and NH groups. However, the other two are of the type 5→1, involving the ester carbonyls and serving as closures for 13-membered rings. In general the crystalline valinomycin conformation can be relegated to a distorted A_2 "all in" structure, in which electrostatic interactions shift the ester carbonyls somewhat towards the bracelet's periphery. Such rotation modifies the IMHB system, the CO and NH groups forming the 5→1 bonds assuming positions at considerable angles to each other and in different planes. Thus by a certain weakening of the H-bonds the valinomycin molecule is able to retain the optimal conformations of the individual amino and hydroxy acid residues.

There is evidence to indicate that the crystalline conformation is not present to any considerable extent in non-polar solvents, although it has precipitated from n-octane. For instance the $^3J_{NH-CH}$ values and dipole moments calculated for this conformation are far from the experimental values.[52] Also considerable differences are revealed in the IR spectra of the crystalline and dissolved specimens (*cf* Figs 6 and 11). Thus, in solution the ester carbonyls display a single symmetrical band speaking against the presence of two H-bonded and four free CO groups whereas with the crystalline specimens, as one should expect the band is split.

Doubtless from the standpoint of non-bonding interactions the A_1 "all out" conformation is less advantageous then is the crystalline conformation the stability differences of the two IMHB should cause no considerable changes in the energy ratio and of the two forms. Why then should the first seemingly less advantageous, form be preferred in solution? The question has been partly answered in the above explanation of the causes for the instability of the A_2 "all in" form in non-polar solvents. Essentially, it is that in form A_1 "all out" the ester carbonyl oxygens are maximally apart, i.e. the conformation is more advantageous from the standpoint of electrostatic interactions than is the crystalline conformation in which these atoms are nearer to each other. Hence in non-polar media, where the Coulomb interaction is at a maximum, it is the first structure which is preferred. In the crystal, the medium has a higher effective dielectric constant thereby weakening the Coulomb interaction and making A_2 "all in" the preferred form. Hence if the IMHB were not destroyed in the polar solvents one could have expected the A_2 "all in" form to be present there. A significant amount of the "crystallinic" conformation might be present in solvents of medium polarity that are weak donors and/or acceptors of protons (for instance in tetrahydrofuran or acetonitrile).

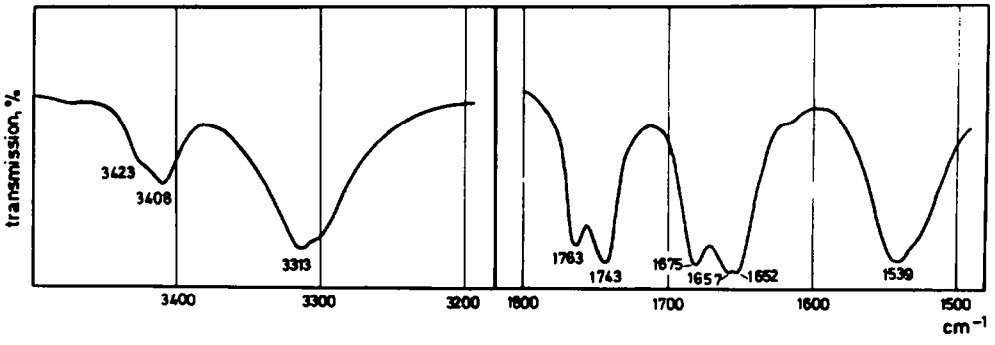


Fig 11. IR spectrum of crystalline valinomycin (nujol mull).

2. *Complexes of valinomycin with alkali metal ions.* The CD and ORD curves of the K^+-valinomycin complex are independent of the anion species (F^-, Cl^-, Br^-, NO_3^-, NCS^- and $C_{12}H_{25}SO_3^-$) and vary little with the solvent (C_2H_5OH, CF_3CH_2OH, CH_3CN or C_2H_5OH–n–C_7H_{16}, 1:1). In $CHCl_3$ the amide I region of the IR spectra of this complex is like that of the free antibiotic; the 3395 cm^{-1} band disappears and the ester CO band undergoes a 16 cm^{-1} shift to longer wavelengths (Fig 12). Similar IR spectra of the K^+ complex have been obtained in a CCl_4–CH_3CN (2:1) mixture (see below, Fig 15). The IMHB system is also retained in more polar solvents, as one can see from the low $\Delta\delta/\Delta T$ values for the NH signals of the K^+ complex in CCl_4–CH_3CN (1:1) and in CH_3OH ($\sim 1{\cdot}8 \cdot 10^{-3}$ ppm/°C). The above facts speak in favor of rigidity of the bracelet structure of the complex where all the ester carbonyls are involved in ion-dipole interaction with the cation. Such a structure can be of the [(B–L–P–R)$_3$] or [(P–R–B–L)$_3$] types (i.e. A_1 or A_2 "all in"). The considerably higher energy of the former (by 28 kcal/mole, see Table 2) allows it to be excluded from further consideration and thus to conclusively select A_2 "all in" as the structure of the complex (Fig 13). A characteristic sign of complex formation is the relatively low $^3J_{NH-CH}$ values in the NMR spectra (5·2–5·6 Hz), indicating *gauche* orientation for the NH and C$^\alpha$H protons. As in the form *A* of free valinomycin the valyl side chains in the K^+ complex are *trans* ($^3J_{C^\alpha H-C^\beta H}$ = 10·7–11·0 Hz) and in the hydroxyisovaleryl side chains *gauche* ($^3J_{C^\alpha H-C^\beta H}$ = 3·6–3·8 Hz). The same kind of structure for the K^+ complex in solutions has been proposed by Urry *et al.*[51,54,55] and by Patel[56] on the basis of the NMR spectra and for the crystalline complex (valinomycin·K^+). $AuCl_4^-$ by Pinkerton *et al.* on the basis of X-ray data.[57]

The O atoms of the ester carbonyls are arranged about the cation to form the apices of a triangular antiprism;[7] the diameter of the complex's molecular cavity is ~ 2·8 Å. Thereby, whereas, in the absence of cations such a conformation, the most advan-

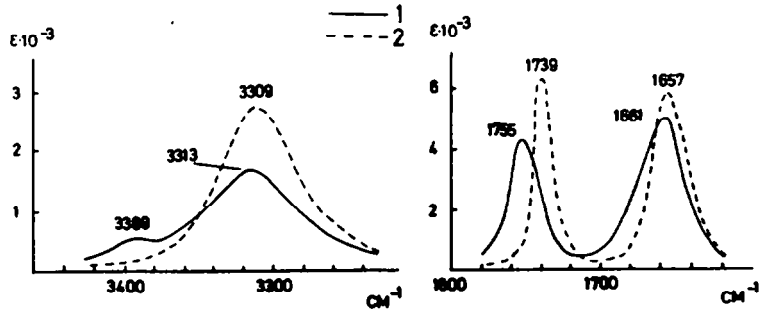


Fig 12. IR spectra of valinomycin (1) and its K^+ complex (2) in chloroform.

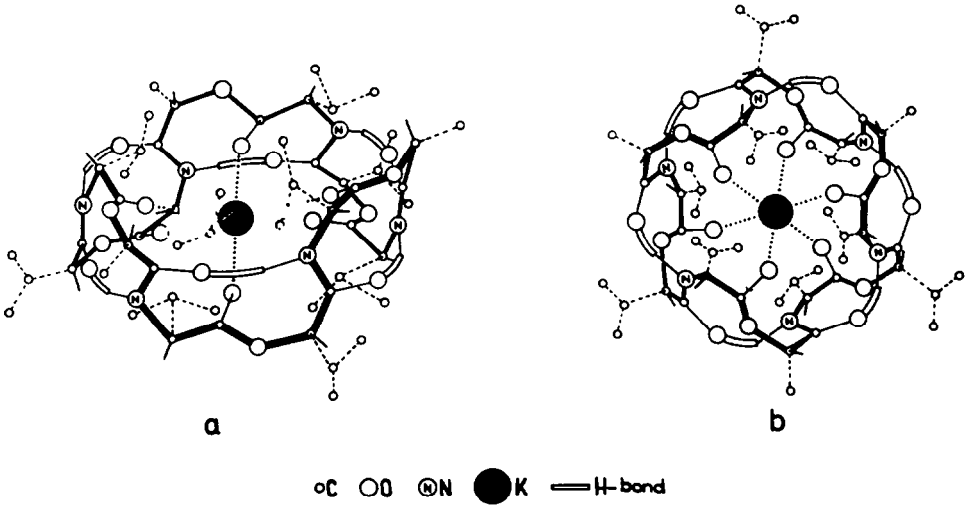


Fig 13. Conformation of the K^+ complex of valinomycin (a—side view, b—view along the C_3 axis). D-Val: ϕ60°, ψ −120°; L-Lac: ϕ −70° ψ0°; L-Val: ϕ −60°, ψ120°: D-HyIv: ϕ75°, ψ20°.

tageous from the standpoint of nonbonded interactions, is destabilized as we have seen above by repulsion of the ester carbonyls, the presence of a positive charge in the center of the cavity not only eliminates the electrostatic repulsion, but turns out to be an additional stabilizing factor.

A distinguishing feature of the complex conformation is the effective screening of the central cation by ester groups, the IMHB system and the pendant valylic isopropyl groups, the hydroxy acid side chains project out from the "bracelet" external surface, screening the IMHB from the solvent. The lipophilic nature of the molecular surface of the complex cation explains its high solubility in neutral organic solvents and plays a very essential part in the functioning of valinomycin in membranes.

A comparison of the free and complex conformations of the valinomycin shows that complexing is accompanied by substantial conformational rearrangements. First of all is the opposite chirality of the system of amide groups in the complex relative to the "non-polar" conformation as well as the opposite orientations of their ester carbonyls (Figs 8 and 13). Interconversion of the two structures is impossible without the breaking some of the H-bonds. Consequently, form *B* may be an intermediate in the complexing reaction in non-polar solvents or in membranes (Fig 14).

It was similarly shown that in relatively non-polar media (CCl_4–CH_3CN, 2:1 or 1:1) the Na^+, Rb^+ and Cs^+ complexes of valinomycin differ little with respect to layout of the depsipeptide chain. At the same time each has its own specificities, manifested clearly in the IR spectra (Fig 15). The Rb^+ complex reveals a small (*ca* 9 cm^{-1}) short wave shift of the NH stretch frequencies and a fall in its intensity indicating augmentation of the CO···NH distance and a decrease in the IMHB energy. The tendency towards a weakening of the IMHB's with increasing cation size is particularly striking in the Cs^+ complex ($\Delta\nu = 23\ cm^{-1}$). With the Na^+ complex the ester CO band is non-homogeneous, revealing non-equivalency of the carbonyls. Most likely owing to its small size the sodium ion is not situated in the middle of the cavity but closer to its periphery, so that different ester groups interact with the ion with different strength. The structure of the Rb^+ and Cs^+ complexes is independent of the solvent, but that of the sodium complex assumes a quite different conformation in the more polar solvents. Evidence of this is, for instance, the change in shape of the CD curve[49] and in the $\Delta\delta/\Delta T$ values we have found for the solution in CH_3OH ($6{\cdot}6 \cdot 10^{-3}$ ppm/°C.) showing that this solvent ruptures at least some of the IMHB.

Despite the incompleteness of the structural work on the Na^+ complex the available data make it

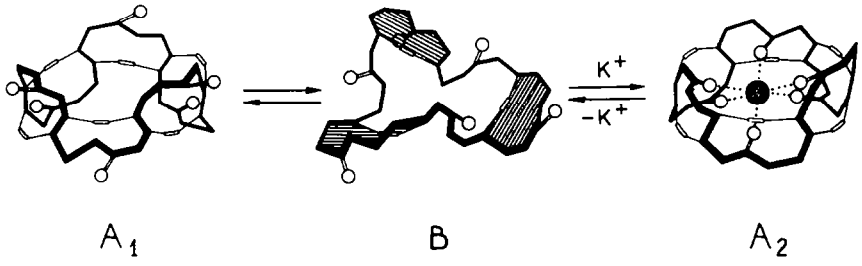


Fig 14. Conformational equilibrium of valinomycin in the presence of K^+ ions.

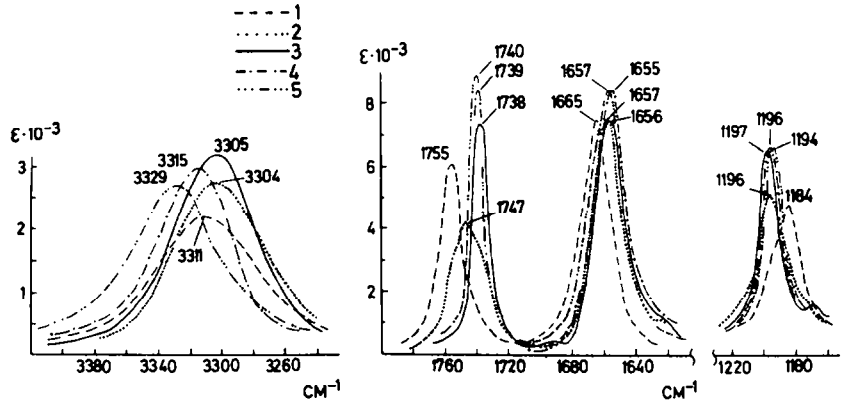


Fig 15. IR spectra of valinomycin (1) and its complexes with Na^+ (2), K^+(3), Rb^+(4) and Cs^+(5) in CCl_4–CH_3CN (2:1) mixture.

possible to explain the reason for the exceptionally high K/Na selectivity of the valinomycin complexing reaction. Owing to the conformational characteristics of the antibiotic's depsipeptide chain the ester groups cannot approach each other sufficiently so that all the carbonyl groups could simultaneously make good contact with the sodium ion and compensate for the increase in desolvation energy accompanying the K^+ to Na^+ transition (Table 4) by equivalent increase in efficiency of the ion-dipole interaction. The small stability increase of the Rb^+ complex and stability decrease of the Cs^+ complex are apparently due to steric hindrances appearing in the interaction of the bulky cations with the rigid system of carbonyl oxygens.

3. *Valinomycin analogs and their complexes.* Over 80 analogs in the valinomycin series have been prepared and their antimicrobial action and Na^+ and K^+-complexes stability investigated.[52] Some of the pertinent data are summarized in Table 5. In our laboratory we have elucidated the conformational states of most of these analogs in order to

Table 4. Influence of the ion size on the solvation energy and stability of the valinomycin complexes

Parameter	Na^+	K^+	Rb^+	Cs^+
Radius Å	0·98	1·33	1·48	1·65
Disolvation energy (EtOH, 25° Kcal/mole)[61]	89·0	72·5	66·5	58·5
Stability constant of the complex (K, 1/mole, EtOH, 25°)	< 50	$2{\cdot}0 \times 10^6$	$2{\cdot}6 \times 10^6$	$6{\cdot}5 \times 10^5$
Free energy of complex formation ($-\Delta F = RT\ln K$, kcal/mole)	< 2·3	8·6	8·8	7·9

Table 5. Stability of valinomycin analogs

No.	Compound[a]	Stability constant of the potassium complex (K, 1/mole, EtOH, 25°)[b]	Free energy of complex formation ($-\Delta F$ = kcal/mole
1	c[-(D-Val-L-Lac-L-Val-D-HyIv)$_3$-] c(-A_3-), valinomycin	2 000 000	8·6
2	c(-A_2-)	< 5[c]	< 1·0
3	c(-A_4-)	100	2·7
4	c(-D-Val-L-Lac-*L-Ala*-D-HyIv-A_2-)	3 000 000	8·9
5	c[-(D-Val-L-Lac-*L-Ala*-D-HyIv)$_2$-A-]	200 000	7·3
6	c[-(D-Val-L-Lac-*L-Ala*-D-HyIv)$_3$-]	20 000	5·9
7	c(-*D-Ala*-L-Lac-Val-D-HyIv-A_2-)	2 000 000	8·6
8	c[-(*D-Ala*-L-Lac-L-Val-D-HyIv)$_2$-A-]	88 000	6·8
9	c[-(D-Val-L-Lac-L-Val-*D-Lac*)$_3$-] meso-Lac-valinomycin	2 300 000	8·7
10	c[-(D-Val-*L-HyIv*-L-Val-D-HyIv)$_3$-] meso-HyIv-valinomycin	> 10^7	> 9·6
11	c[-(D-Val-*L-HyIv-L-Ala*-D-HyIv)$_3$-]	2 900 000	8·9
12	c(-*L-Val*-L-Lac-L-Val-D-HyIv-A_2-)	11 000	5·6
13	c(-D-Val-*D-Lac*-L-Val-D-HyIv-A_2-)	75 000	6·7
14	c(-D-Val-L-Lac-*D-Val*-D-HyIv-A_2-)	4 400	5·0
15	c(-D-Val-L-Lac-L-Val-*L-HyIv*-A_2-)	100	2·7
16	c[-(*L-Val*-L-Lac-*D-Val*-D-HyIv)$_3$-]	< 50	< 2·3
17	c[-(*L-Val-L-HyIv-D-Ala*-D-HyIv)$_3$-]	< 50	< 2·3
18	c(-D-Val-L-Lac-*L-HyIv*-D-HyIv-A_2-)	2 500	4·7
19	c(-D-Val-L-Lac-*L-MeVal*-D-HyIv-A_2-)	< 50	< 2·3
20	c(-*D-HyIv*-L-Lac-L-Val-D-HyIv-A_2-)	42 000	6·3
21	c(-D-Val-*L-Ala*-L-Val-D-HyIv-A_2-)	300 000	7·5
22	c[-(D-Val-*L-Ala*-L-Val-D-HyIv)$_2$-A-]	220 000	7·3
23	c(-D-Val-L-Lac-L-Val-*D-Val*-A_2-)	5 200	5·1
24	c[-(D-Val-L-*MeAla*-L-Val-D-HyIv)$_3$-]	> 10^7	> 9·6

[a] c = cyclo. Modified residues are underlined

[b] The stability constants of the sodium complexes are below 50 1/mole. Exceptions are compound 21 (K_{Na^+} = 100 l/mole, $-\Delta F$ = 2·7 kcal/mole), compound 22 (K_{Na^+} = 600 l/mole, $-\Delta F$ = 3·8 kcal/mole) and compound 24 (K_{Na^+} = 200 l/mole, $-\Delta F$ = 3·2 kcal/mole).

[c] The stability constant of the Na^+ complex is *ca* 10 1/mole ($-\Delta F \approx 1{\cdot}3$ kcal/mole)

obtain a deeper understanding of the intramolecular interactions in these compounds and also the part played by the individual valinomycin fragments in forming the spatial structure of this antibiotic and determining its membrane-affecting properties. The present paper will touch only upon the principal conclusions and the more interesting experimental results of this investigation.

Hexadecavalinomycin (3) consisting of four tetradepsipeptide fragments, -D-Val-L-Lac-L-Val-D-HyIv-, instead of the three in valinomycin is very similar to the latter in its conformational potentialities. It has been shown to possess the A_1 "all out" bracelet conformation in non-polar solvents, the propeller conformation in $CDCl_3$–$(CD_3)_2SO$ mixtures, the non-ordered conformation in polar media and A_2 "all in" conformation in its complexes with Na^+, K^+ and Cs^+ ions (Fig 16). In contrast to valinomycin, analog (3) displays no weak band in the amide A region of the IR spectra (Fig 17) and thus has no weakened "migrating" hydrogen bond. Such a concept is supported by the agreement between the experimental dipole moment (2·6D in CCl_4) and the value (2·7D) calculated on the basis of the ϕ–ψ values given in the legend to Fig 16.

The O atom ligands in the complex form a square antiprism. The hexadecavalinomycin molecular cavity (which according to molecular models is 4–5 Å in diameter) is too large to provide for efficient interaction of all the ester carbonyls with an alkali metal ion much like the valinomycin cavity is too large for effective complexing of the Na^+ ion. The cation thus "rools about" in the cavity, interacting at any moment only with part of the carbonyl groups and thereby giving rise to the heterogeneity in the ester carbonyl bands shown in Fig 17b. The interaction of Cs^+ with hexadecavalinomycin is closer to that observed for the interaction of valinomycin with K^+, as manifested in the larger shift of the ester band in CCl_4–CH_3CN (1:1) (Fig 17b) and its symmetric nature in $CHCl_3$ (Fig 17a). From the above said one can easily understand why the hexadecavalinomycin complexes are of relatively low stability.[37,47] Contrary to valinomycin the cations are here apparently not completely desolvated. The large size of the hexadecavalinomycin cavity is manifested also in its unique ability to transport across membranes also bulky organic cations such as the trimethylammonium ion and (choline ·H)$^+$.[60]

Octavalinomycin (2) assumes the bracelet conformation in CCl_4 and $CHCl_3$ but with the IMHB weakened because of unsuitable geometric factors (the C=O and NH bonds are at an angle approaching 90°, see Fig 18a). The weakening of the H-bonds is manifested in the IR spectra by shift in the ν_{NH} frequencies to 3350 cm^{-1} (Fig. 19a, *cf.* Figs 6 and 17) and in the NMR spectra (in $CDCl_3$) by a high field shift of the NH signal to 6·93 ppm (as compared with δ_{NH} = 7·75–7·88 ppm in valinomycin and hexadecavalinomycin). Despite accompanying steric strains analog 2 does form complexes with Na^+ and K^+, but in accordance with the cavity size gives preference to Na^+ (Table 5). No signs were detected of its forming a complex with Rb^+. Judging from the IR carbonyl region (Fig 19b), in the K^+ complex the ligands belong to the ester groups, i.e. the complex rests on the same structural principle as that of valinomycin (Fig 20a). In the Na^+ complex, an active part is played by the amide carbonyls, as well as the ester carbonyls. At the same time there the H-bonds are weaker than those of the K^+ complex (shift of the NH bands from

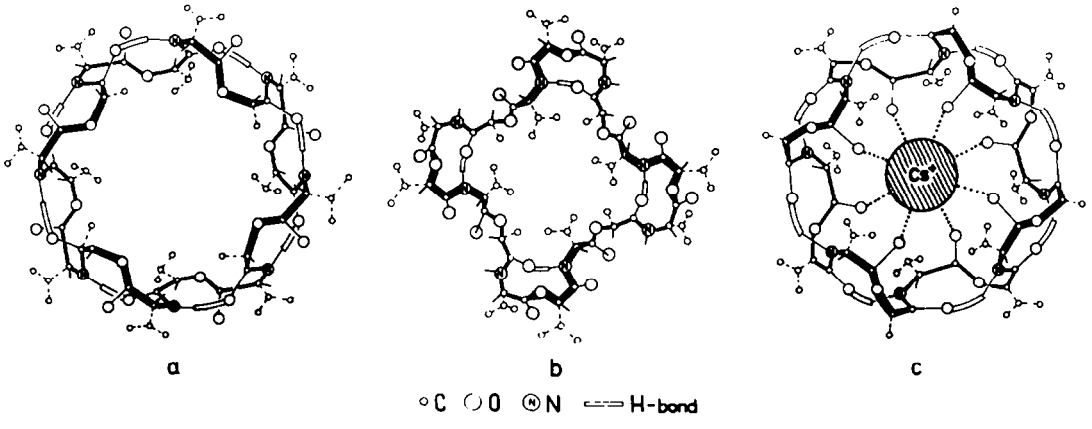


Fig 16. Conformation of hexadecavalinomycin (a) in non-polar solvents (D-Val: ϕ −40°, ψ −90°; L-Lac: ϕ −120°, ψ60°; L-Val: ϕ30°, ψ90°; D-HyIv: ϕ120°, ψ −60°), (b) in solvents of medium polarity (D-Val: ϕ 80°, ψ 90°; L-Lac: ϕ −60°, ψ 60°; L-Val: ϕ40°, ψ 90°; D-HyIv: ϕ 120°, ψ −40°), and (c) in complexes with alkali metal ions (D-Val: ϕ60°, ψ −100°; L-Lac: ϕ −30°, ψ −60°; L-Val: ϕ −70°, ψ100°; D-HyIv: ϕ30°, ψ60°.

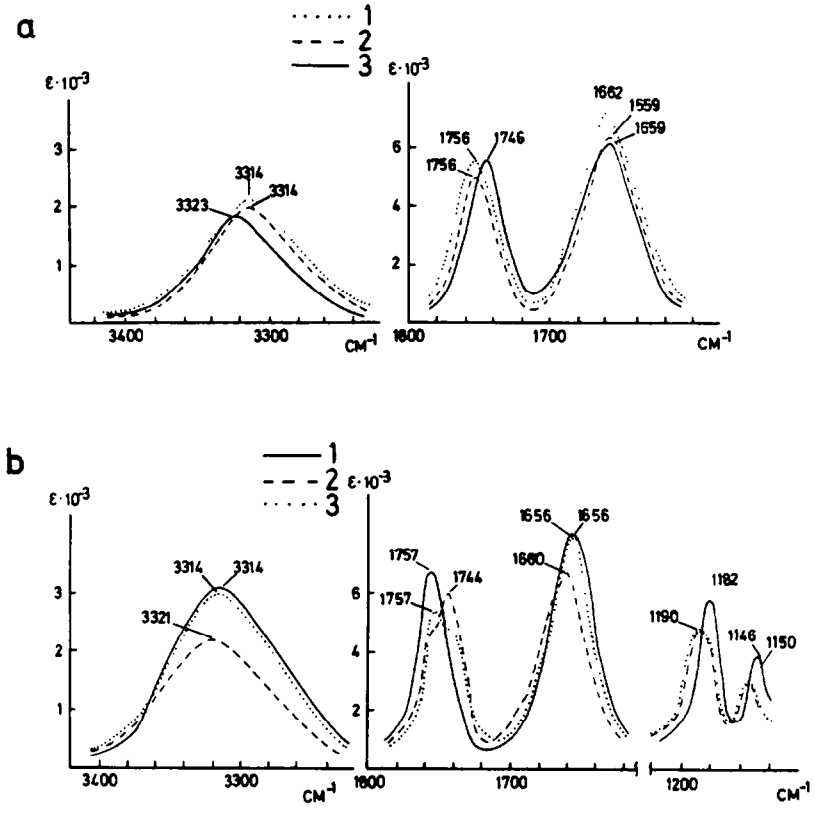


Fig 17. IR spectra of hexadecavalinomycin in CCl_4 (a3), $CHCl_3$(a2) and CCl_4–CH_3CN (2:1) (b1), of its K^+ complex in CCl_4–CH_3CN (2:1) (b3) and of the Cs^+ complex in $CHCl_3$ (a1) and in CCl_4–CH_3CN (2:1) (b2).

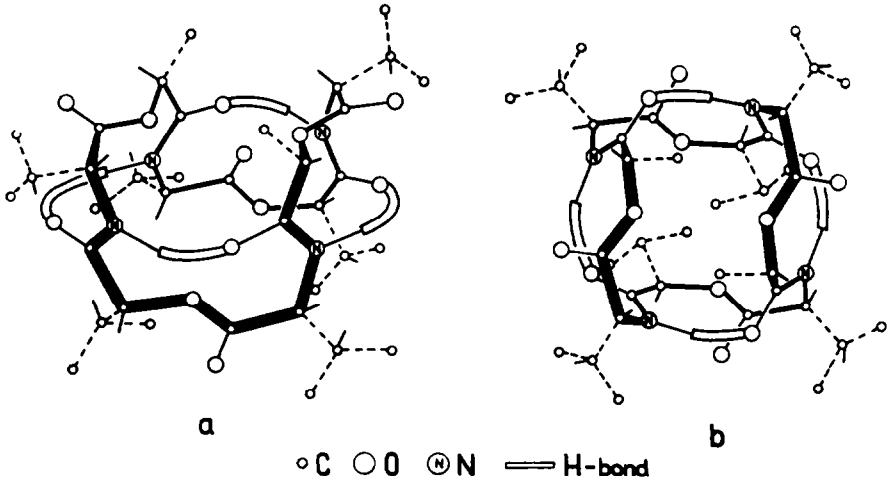


Fig 18. Conformation of octavalinomycin in non-polar solvents (a—side view, b—view along the C_2 axis). D-Val: ϕ −60°, ψ −60°; L-Lac: ϕ −90°, ψ60°; L-Val: ϕ60°, ψ30°; D-HyIv: ϕ120°, ψ −30°.

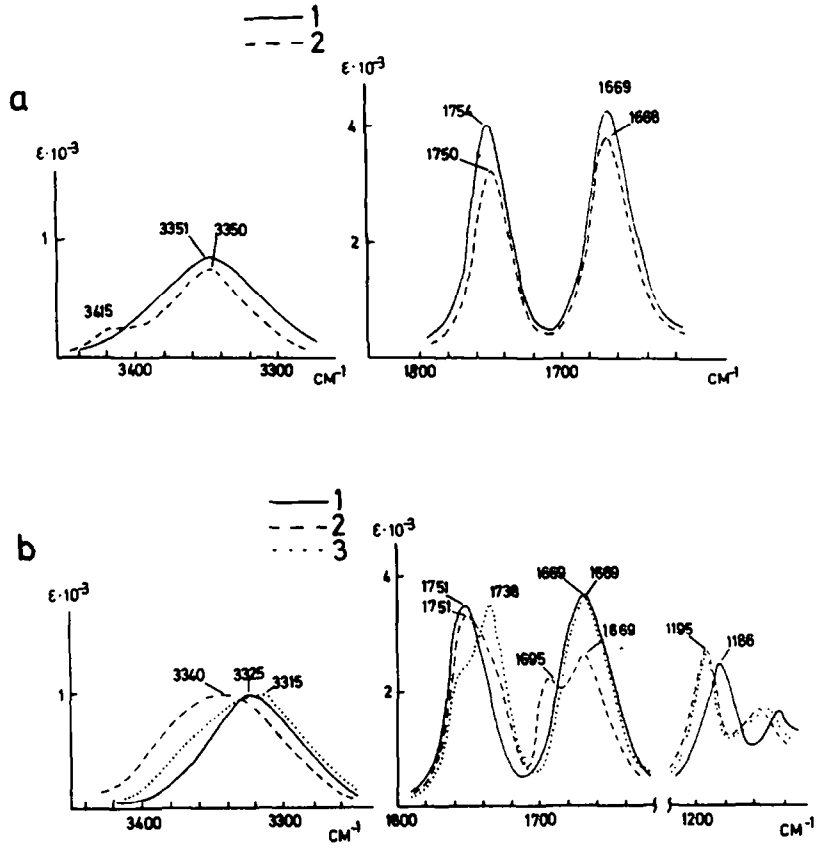
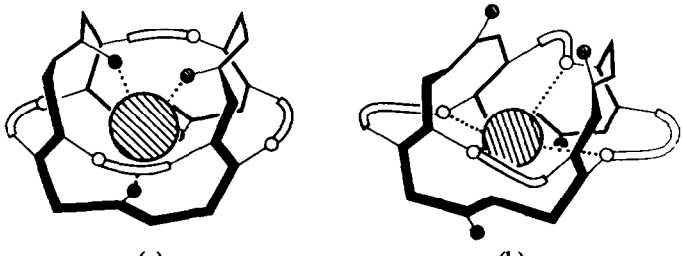


Fig 19. IR spectra of octavalinomycin in CCl_4 (a1), $CHCl_3$(a2) and CCl_4–CH_3CN(2:1) (b1) and of its complexes with Na^+ (b2) and K^+ (b3) in CCl_4–CH_3CN (2:1).



(a) (b)

Fig 20. Conformation of the K^+ complex of octavalinomycin (a) and the hypothetical conformation of its Na^+ complex with all amide groups interacting with the cation (b).

3315 cm^{-1} to 3340 cm^{-1} Fig 19b). Fig 20b shows the conformation wherein the cation is interacting only with the amide carbonyls. In the case of the Na^+ complex there is most likely an equilibrium mixture of the conformers shown in Figs 20a and 20b together with intermediate structures. On the example of octavalinomycin one can readily note a greater inclination of the amide groups then the ester groups to interact with the smaller ions (Na^+, but not K^+), apparently owing to specificities of their electronic structures.[60]

Spectral studies of the analogs (**4–11**) have shown that modification of the valinomycin side chains does not lead to appearance of new conformations but does affect the relative stability of forms *A*, *B* and *C*. For instance, meso-HyIv-valinomycin (**10**) assumes the more stable *A* form than does the naturally occurring antibiotic (absence of the minor band in the amide *A* region and ν_{NH} = 3294–3298 cm^{-1} as compared with 3307–3313 cm^{-1} in valinomycin; in the NMR spectra δ_{NH} = 8·12 ppm as compared to 7·78–7·88 ppm in the present an-

tibiotic). In the case of analogs (**6** and **11**) with L-valine residues substituted by L-alanine residues, the characteristics of the propeller conformation are much less pronounced than in the case of valinomycin. The sensitivity of the propeller conformation to the size of the side chains indicates that they apparently screen its IMHB from the solvent (as one can see from Fig. 9b they are oriented pseudo-axially to the plane of the H-bonded 10-membered rings). Similar modification of the D-valine side chains (compounds 7 and 8) does not affect the stability of form *B*, since these chains are oriented inwards and do not participate in screening of the H-bonds.

All the analogs (**4–11**) are good complexones (Table 5); the $\phi-\psi$ parameters of their K^+ complexes are close to those of valinomycin. At the same time, the decrease in bulk of the amino acid side chains in the analogs (**4–8** and **11**) lowers the effectiveness by which the central ion is screened from the solvent action. The asymmetry of the amide and ester chromophoric system induced by such "one sided" solvation is the cause for augmentation of the optical activity relative to valinomycin. As could have been expected, solvation "from below" (Figs 21b and 21d) and "from above" (Fig 21c) induces opposite chiralities of the depsipeptide skeleton leading to antipodal ORD curves on Fig 22. The weakened cation screening in the analog (**11**) complex is also manifested in membranes, where it discloses a higher surface activity than the K^+ complex of valinomycin.[42]

The layout of the depsipeptide skeleton in the K^+ complexes of the valinomycin diastereomers (**12–15**) is approximately the same as in the initial antibiotic; reversal of the configuration therefore reduces to orientational changes in the corresponding side chains as schematically represented on Fig 23. The energetic consequences of configurational change in the amino and hydroxy acid residues of

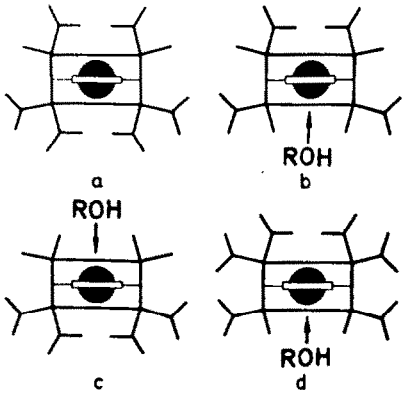


Fig 21. Schematic representation of the K^+ complexes of valinomycin (a), compounds (**5** and **6**) (b), (**8**) (c) and (**11**) (d). The sites of probable access of solvent are indicated by arrows.

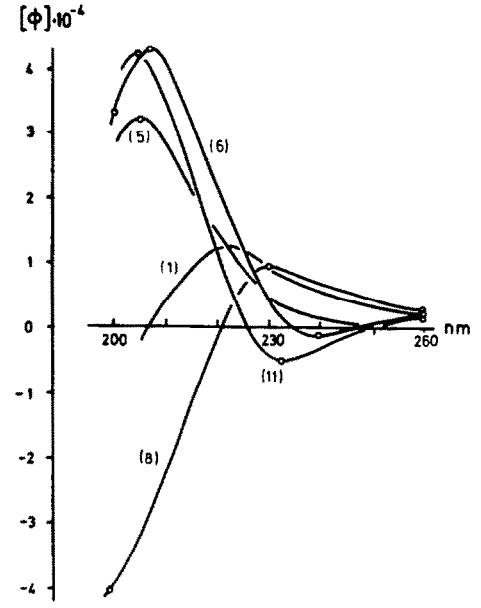


Fig 22. ORD curves of the K^+ complexes of valinomycin (**1**) and compounds (**5**), (**6**), (**8**) and (**11**) in EtOH.

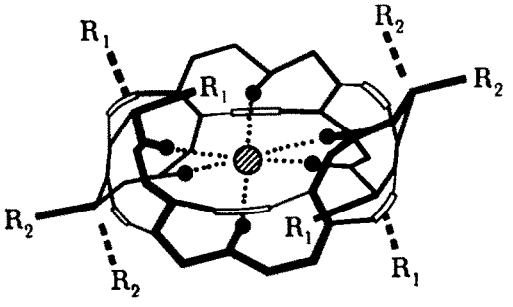


Fig 23. Schematic representation of the configurational changes in the amino (R_1) and hydroxy (R_2) acid residues of the valinomycin $\cdot K^+$ complex (the dashed lines show the side chain orientations after change of configuration).

the valinomycin complex can be estimated to a first approximation by comparison of the conformational maps of the initial residues and their enantiomers (Fig 24). It can be readily seen that in all cases configurational inversion is accompanied by increased energy of the system. Calculations have shown[50] that the energy of the D-HyIv residues undergoes a stronger increase than that of D(L)–Val or L–Lac. It thus becomes understandable why the complexes of compounds (**12–15**) are less stable than those of valinomycin (Table 5) and why the lowest value is for complex **15**. Compounds **16** and **17** obtained from valinomycin by configurational inversion of the six residues are naturally entirely devoid of complexone properties.

In the substitution CONH→COO (compounds **18** and **20**, Fig 25a) all ester ligands are retained but

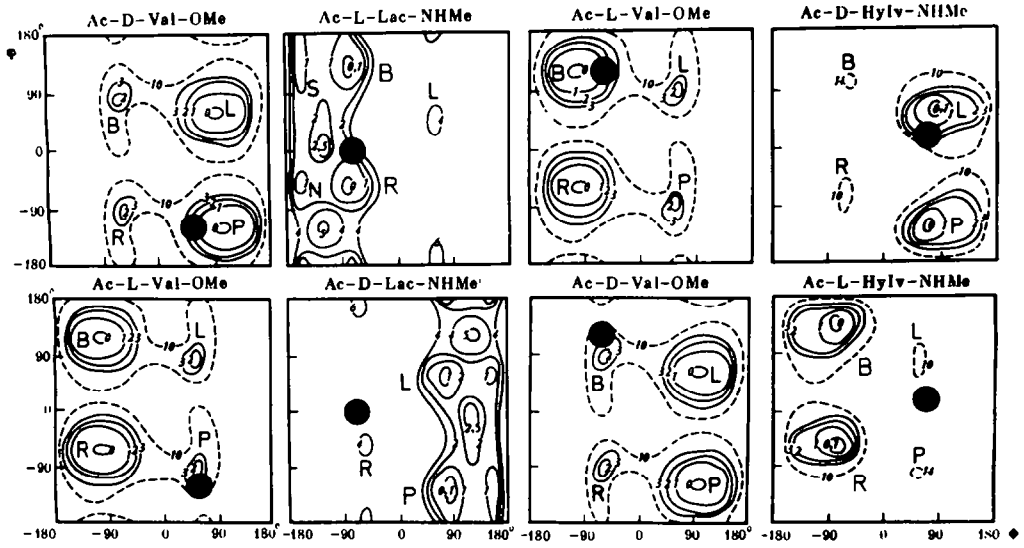


Fig 24. Conformational maps of the amino and hydroxy acid derivatives modeling the fragments of valinomycin and its diastereomers. The circles indicate the $\phi - \psi$ coordinates realized in the K^+ complexes of valinomycin and its diastereomers.

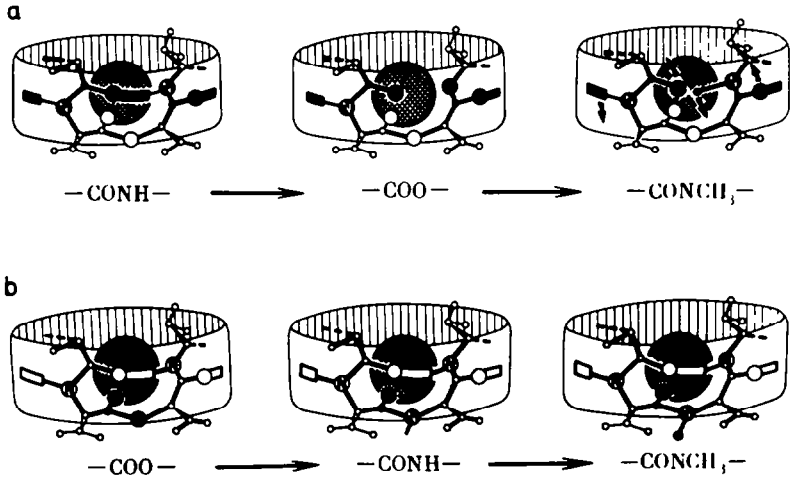


Fig 25. Schematic representations of the substitution of amide by ester or N-methylamide groups (a) and ester by amide or N-methylamide groups (b) in the valinomycin · K^+ complex (the dark circles indicate atoms participating in the exchange).

one IMHB is lost. Correspondingly, the free energy of complexing is reduced from 8·6 kcal/mole to 6·3 and 4·7 kcal/mole, i.e. by a value approximating the energy of an IMHB (~3·9 kcal/mole, see p. 7). When the substitution is CONH→CONMe not only is a IMHB lost, but the other IMHB are formed less readily because of steric hindrance. Therefore compound **19** is a still weaker complexone than **18**. The substitution COO→CONH (Fig 25b) leads to analogs (**21**–**23**) retaining a considerable amount of the K^+ binding capacity. Here the IMHB system is not affected, and part of the ester groups are replaced by amide groups, the presence of which explains the enhanced stability of the Na^+ complexes of compounds **21** and **22** (*cf* data on octavalinomycin).

Of particular interest is analog (**24**) in which the three ester groups have been replaced by N-methylamide groups. The complexes of compound **24** display all the characteristic sings of conformation A_2 "all in": relatively low $^3J_{NH-CH}$ values (4·6–6·5 Hz), an intensive symmetrical bound-NH band ($\nu_{NH} = 3301\ cm^{-1}$) and long-wave shift of the ν_{CO} frequencies of the ligand amide and ester CO

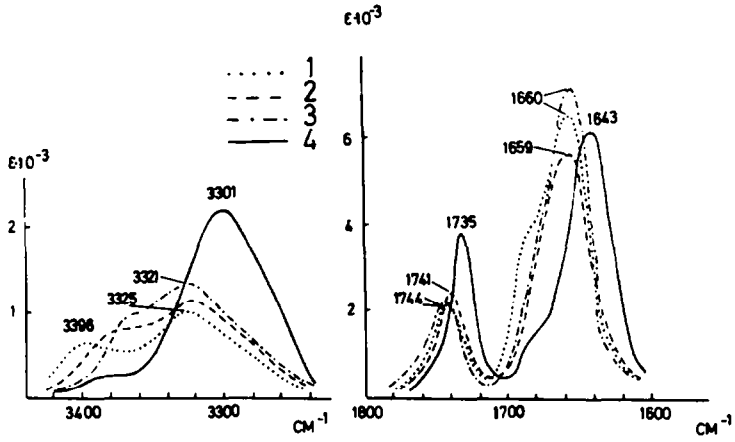
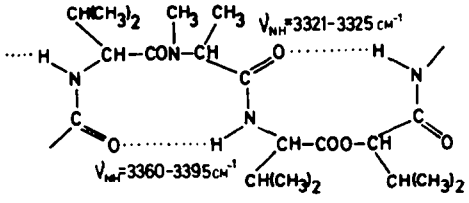


Fig 26. IR spectra of compound (24) in heptane (1), carbon tetrachloride (2) and chloroform (3) and of its K^+ complex in chloroform (4).

groups (Fig 26). In non-polar solvents, as with valinomycin, the A_1 "all out" form is predominant. However, judging from the presence of intensive $\nu_{NH} = 3360–3395\ cm^{-1}$ bands (Fig 26) the H-bonds of the modified 10-membered rings are greatly weakened.



-D-Val-L-MeAla-L-Val-D-Hyiv-

The propeller conformation of compound **24** is sterically forbidden and in general is not realized. Its CD curves display no fundamental changes even on passing from heptane to aqueous solution (Fig 27). In other words the free compound (**24**) is conformationally much more limited than valinomycin, which thus leads to an increase in energy of the left hand side of the system

$$\text{macrocycle} + K^+ \rightleftarrows (\text{macrocycle} \cdot K^+)$$

and increase in the free energy of complexation. As a result, the (**24**) $\cdot K^+$ complex has an exceptionally high stability constant greatly exceeding that of the complexes of valinomycin and of all its analogs (Table 6).

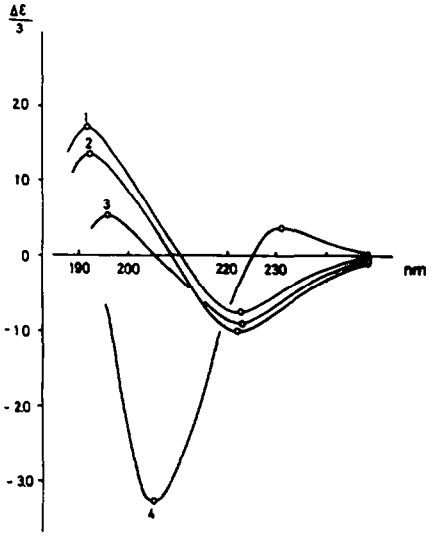


Fig 27. CD curves of compound (**24**) in heptane (1), ethanol (2) and water-ethanol (2:3) (3) and of its K^+ complex in ethanol (4).

It thus appears that elucidation of the laws determining the formation of the spatial structure

Table 6. Stability constants (K, 1/mole) of the potassium complexes of valinomycin (1) and analogue (**24**) in different solvents at 25°

Compound	MeOH	EtOH	42 mol% EtOH + 58 mol% H_2O	32 mol% EtOH + 68 mol% H_2O	H_2O (extrapolated values)
1	27 000	2 000 000	500	< 100	0·25
24	7 000 000	> 10 000 000	11 000	3900	100

of valinomycin under various conditions enables one to explain and predict the behaviour of its analogs differing in the side chains and polar groups, in the asymmetric center configurations etc. As a result we have the unique ability in peptide chemistry to carry out the predetermined synthesis of analogs of a comparatively complex biologically active compound with given physico-chemical and biological properties.

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