CONFORMATIONAL STATES OF VALINOMYCIN AND ITS COMPLEXES WITH ALKALI-METAL CATIONS IN SOLUTIONS

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In recent years, great attention has been attracted by macrocyclic compounds forming stable complexes with alkali-metal cations and specifically affecting the transport of cations through biological and artificial membranes. Among substances of this type a central position is occupied by the depsipeptide antibiotic valinomycin (Fig. 1), discovered in 1955 by Brockmann et al. [1] and synthesized in 1963 by Shemyakin et al. [2, 3].

Valinomycin possesses the capacity for stimulating in exceptionally low concentrations the transport of alkali-metal cations through mitochondria [4-7], erythrocytes [6, 8-10], chloroplasts [11], and other biological membrane systems [7, 12], and also through various two-layer [6, 7, 9, 10, 13] and bulk membranes [7, 10, 14] and phospholipid micelles [6, 7, 15, 16]. An important feature of the action of valinomycin is the high selectivity of the cationic permeability induced by it: for example, the ratio of the fluxes of potassium and sodium through the membranes of mitochondria in the presence of valinomycin is 10,000 : 1 [4].

The influence of valinomycin on ionic transport through membranes is closely connected with its capacity for selectively forming stable complexes with potassium cations. In view of the decisive role of conformational factors in the functioning of metal-complexing macrocycles [17-19], we have investigated the spatial structure of valinomycin under various conditions. The work was performed with the aid of a wide selection of physicochemical and computational methods giving independent and mutually supplementary information.

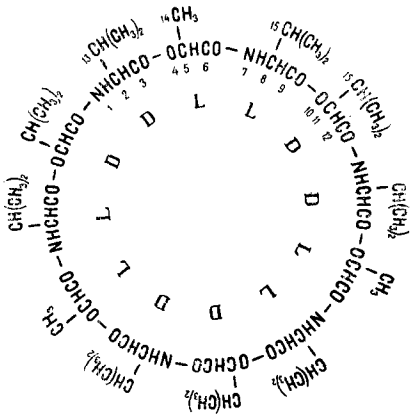

Fig. 1. Valinomycin.

For the physicochemical investigations we used a biosynthetic preparation obtained by MacDonald's improved method [20]. The optical rotatory dispersion (ORD) curves were recorded on a Cary-60 spectropolarimeter at concentrations of the solutions of $(0.5\text{-}1) \cdot 10^{-3}$ M at 23-26°C in cells 0.01-2 cm thick. The values of the molecular rotation [Φ] are given without correction for the refractive index of the solvent.

The IR spectra were recorded on a UR-10 instrument with LiF and NaCl prisms. In the case of measurements in CCl_4, the thickness of the cell was 10 mm at 3500-1600 cm^{-1} and 0.1 mm at 1600-1000 cm^{-1}, the concentrations of valinomycin being $4 \cdot 10^{-4}$ and $4 \cdot 10^{-2}$ M. On measurements in $CHCl_3$, the thickness of the cell was 20 mm at 3500-1600 cm^{-1} and the concentrations $0.86 \cdot 10^{-4}$ M. On

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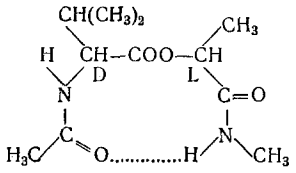
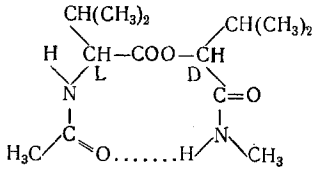
TABLE 1. Optimum Conformations of the Methylamide of N-Acetyl-D-valyl-L-lactic Acid (1)



Type of conformation	Geometrical parameters: D-Val Φ_1	Ψ_1	X_1	L-Lac Φ_2	Ψ_2	Energy
$\overline{p-r}$	239	67	194	107	200	0
$\overline{r-b}$	135	118	169	85	223	1,8
$\overline{r-r}$	128	150	163	113	148	2,5
$\overline{b-l}$	130	280	185	233	219	2,6
$\overline{l-l}$	233	221	186	232	213	2,5

TABLE 2. Optimum Conformations of the Methylamide of N-Acetyl-L-valyl-D-α-hydroxyisovaleric Acid (2)



Type of conformation	Geometrical parameters: L-Val Φ_3	Ψ_3	X_3	D-Hyiv Φ_4	Ψ_4	X_4	Energy
$\overline{b-l}$	119	287	175	257	205	64	0
$\overline{l-p}$	226	244	190	280	138	68	2,4
$\overline{l-l}$	237	186	196	250	207	61	2,4
$\overline{r-r}$	123	143	175	128	146	165	6,0
$\overline{p-r}$	235	58	193	125	149	170	6,0

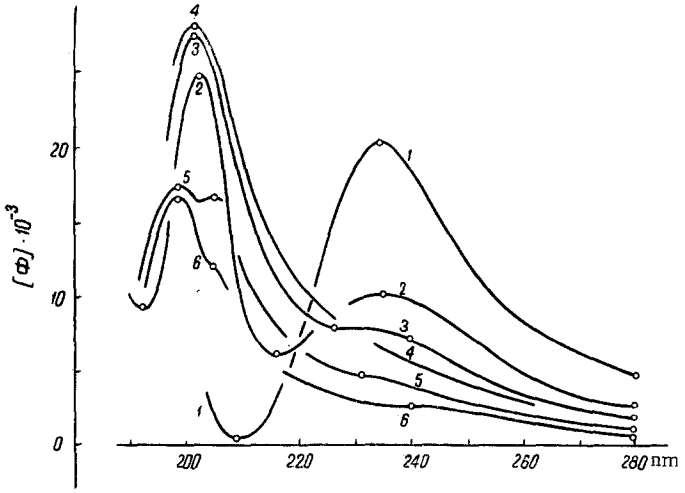


Fig. 2. ORD curves of valinomycin in various solvents: 1) heptane–dioxane (10 : 1); 2) heptane–ethanol (3 : 1); 3) ethanol; 4) acetonitrile; 5) trifluoroethanol; 6) water–trifluoroethanol (3 : 1).

measurements in CCl_4-CH_3CN (2 : 1), the layer thickness was 2 mm and the concentration of valinomycin $(0.5-1)\cdot 10^{-3}$ M; to obtain the complexes with K^+, Rb^+, and Cs^+ a three- to sevenfold excess of the corresponding thiocyanate was added to the solution. In all cases, the absorption of the solvents did not exceed 60%.

The dipole moment of valinomycin was measured in an instrument working on the beat principle at a frequency of 1 MHz; calculation was performed by Hedestrand's method [21].

The ^1H NMR spectra were taken on a JNM 4H-100 instrument with a working frequency of 100 MHz and with stabilization of the resonance conditions with respect to one sample. Tetramethylsilane was used as internal standard. The chemical shifts were determined with an accuracy of ± 0.005 ppm and the spin–spin coupling constants with an accuracy of ± 0.1 Hz. The temperature was measured by a copper-constantan thermocouple with an accuracy of ± 2°C by means of the thermal attachment to the JNM 4H-100 instrument.

In measurements of the NMR spectra of valinomycin in solutions containing CD_3OD, the exchange NH → ND takes place. Consequently, the parameters of the signals from the NH groups were determined

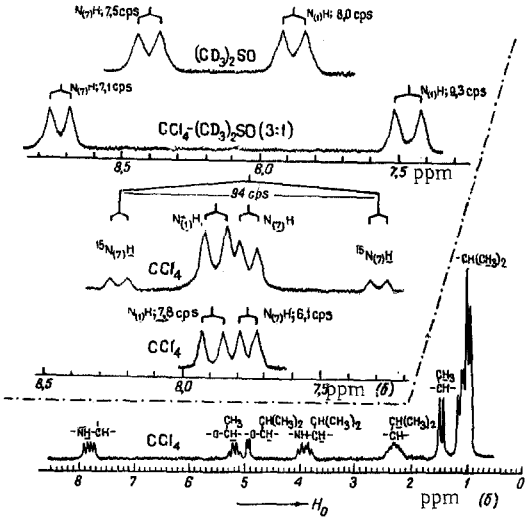


Fig. 3. NMR spectra of valinomycin and its [^{15}N]-L-Val analog in CCl_4, $(CD_3)_2SO$, and $CCl_4-(CD_3)_2SO$ (3 : 1).

in CH_3OH. To measure the spectrum of the Cs^+ complex in $CDCl_3$ a solution of equimolar amounts of valinomycin and CsCl in CH_3OH was evaporated to dryness, and the residue was dissolved in $CDCl_3$. The optimum conformations of the fragments of valinomycin were calculated by the method developed previously [22] including the minimization of the potential energy of the system with respect to the angles $\Phi_{C^\alpha-N}$, $\Phi_{C^\alpha-O}$, $\Psi_{C^\alpha-C'}$ and $X_{C^\alpha-C^\beta}$, using the nomenclature proposed by Edsall et al. in 1966 [23]. Possible values of Φ and Ψ (in degrees) for the various forms of valinomycin were found by a comparison of the conformational charts (see Figs. 8-10), the data of Tables 1 and 2, and an analysis of molecular models. For the values selected in this way the distance between the ends of the dodecadepsipeptide chain was calculated with the standard parameters of the amide and ester groups [24, 25]. The mean coordinates Φ and Ψ for which the distances between the ends of the chain do not exceed 2 Å are given in the text; possible deviations amount to 10-15°. The calculation of the dipole moments from the values of the coordinates Φ and Ψ was effected by the successive summation of the vectors of the dipole moments of the amide and ester groups along the depsipeptide chain; we had determined their magnitude and direction previously [24, 25].

I. Conformational States of Free Valinomycin

As can be seen from Fig. 2, the ORD curves of valinomycin measured in various solvents differ substantially from one another. Thus, the curves taken in heptane–dioxane (10 : 1) (curve 1 in Fig. 2) show a Cotton effect at ~220 nm which disappears on passing to ethanol or acetonitrile. A further increase in the polarity of the solvent is accompanied by a considerable fall in optical activity. The results obtained show the existence of a conformational equilibrium which is displaced with a change in the nature of the solvent.

Fundamental information on the structure of valinomycin can be obtained from the NMR spectra taken in various solvents (Fig. 3, Tables 3 and 4). In the first place, attention is attracted by the correspondence of the number and nature of the signals with the structural formula of the antibiotic. For example, in the weak-field region there are two doublets from the $N_{(1)}H$ and $N_{(7)}H$ residues of D- and L-valine the assignment of which proved possible as the result of a study of the spectra of the ^{15}N-labeled analog of valinomycin specially synthesized for this purpose [27], in which one L-valine residue was replaced by a [^{15}N]-L-valine residue. In the region of the signals from the protons attached to the C^α atoms and those of the side chains the groups of signals from the residues of D- and L-valine and L-lactic and D-α-hydroxyisovaleric acids can easily be seen. The "homogeneity" of the NMR spectra observed shows the absence of any appreciable cis-trans isomerism whatever of the amide and ester groups in valinomycin, the energy barriers of which are extremely high (9-20 kcal/mole [28]) and correspond to coalescence temperatures of 40-100°C. The nature of the spectra does not change significantly on cooling [to −95°C in CS_2-CDCl_3 (1 : 1)], from which it follows that at least in nonpolar solvents the amino and hydroxy acid residues of the same type in valinomycin are equivalent, i.e., the molecule of the antibiotic possesses a third-order (C_3) axis of symmetry in correspondence with its chemical formula. In this respect, valinomycin differs from the cyclodepsipeptide antibiotics of the enniatin group [29, 30] and the cyclohexapeptide $\overline{[(L-Ala-Gly)_3]}$ [31] which, although constructed of three chemically identical fragments, assume a conformation deprived of elements of symmetry. The signals from the $N_{(1)}H$ and $N_{(7)}H$ protons differ insignificantly with respect to their chemical shifts in CCl_4 solution (δ 7.88 and 7.75 ppm, respectively). However, with the gradual addition of $(CD_3)_2SO$ (up to 20 mole %) the second of them shifts significantly into the weaker-field region (by 1.00 ppm), while the first undergoes a weaker shift in the upfield direction (by 0.41 ppm). The further addition of $(CD_3)_2SO$ (up to 40 mole %) does not affect the relative positions of the signals from the NH groups with a still further increase in the concentration of $(CD_3)_2SO$, and also when the solution is heated, they approach one another again (Fig. 4). It is important that in this process a symbatic change in the spin–spin coupling constants of the protons in the $N_{(1)}H-C_{(2)}H$ and $N_{(7)}H-C_{(8)}H$ fragments is also observed:

TABLE 3. Chemical Shifts (δ, ppm) of the Protons of Valinomycin and Its Complexes with Na^+, K^+, and Cs^+

Medium	Concn., M	Fragment								
		CH_3 CH_3 \ / $C_{(13,15,16)}$	$C_{(5)}-CH_3$	$HC_{(13,15,16)}$	$-C_{(2)}H$	$-C_{(8)}H$	$-C_{(5)}H$	$-C_{(11)}H$	$>N_{(1)}H^*$	$>N_{(7)}H^*$
CCl_4	0,071	0,94—1,15	1,45	2,31	3,97	3,89	5,21	4,95	7,90	7,76
$CDCl_3$	0,089	0,94—1,13	1,46	2,31	4,13	4,00	5,34	5,05	7,88	7,78
$CDCl_3$—CS_2 (1 : 1)	0,030	0,92—1,10	1,42	2,27	3,92; 4,02		5,24	4,97	7,85	7,73
CCl_4—$(CD_3)_2SO$ (3 : 1)	0,045	0,90—1,10	1,29	2,30	4,80	4,08	5,42	4,94	7,52 (0,65)	8,75 (11,0)
$(CD_3)_2SO$	0,073	0,92	1,29	2,20	4,35	4,35	5,11	4,82	7,88	8,40
CH_3OH—H_2O (3 : 1)	0,030	1,01	1,43	—	—	—	—	—	8,25	8,29
CD_3OD (CH_3OH)	0,063	1,02	1,41	2,27	4,29; 4,44		5,19	4,93	8,14	8,24
CD_3OD+KSCN (CH_3OH+KSCN)	0,063	0,96—1,19	1,55	2,27	3,85; 3,87		4,97	4,68	8,30; 8,38	
$CDCl_3$—CS_2—CH_3OH (1 : 1 : 1)+KSCN	0,030	0,96—1,20	1,58	2,22	—	—	—	—	8,29; 8,38	
CH_3CN	0,041	0,92—1,08	1,40	—	4,11	4,13	5,28	5,01	7,61	7,58
CH_3CN+KSCN	0,041	0,91—1,16	1,52	—	3,80; 3,85		4,95	4,64	8,34; 8,42	
CCl_4—CH_3CN (1 : 1)	0,030	0,95—1,13	1,45	—	3,97	4,01	5,32	5,04	7,78 (4,0)	7,70 (4,2)
CCl_4—CH_3CN (1 : 1)+KSCN	0,030	0,95—1,18	1,53	—	3,79; 3,84		4,89	4,57	8,30 (1,5);	8,37 (1,8)
CCl_4—CH_3CN (1 : 1)+NaSCN	0,030	0,97—1,15	1,51	—	4,07; 4,13		5,07	4,78	7,88 (0,9);	7,94 (0,9)
$CDCl_3$+CsCl	0,030	0,95—1,20	1,55	2,25	3,81	3,81	5,08	4,72	7,89; 7,98	

* The values of $\Delta\delta/\Delta T \cdot 10^3$, ppm/deg, are given in brackets.

TABLE 4. Vicinal Spin–Spin Coupling Constants (^3J) of the Protons of Valinomycin and Its Complexes with Na^+, K^+, and Cs^+, Hz*

Medium	Fragment					
	$-\overset{\vert}{C}_{(5)}H-C_{(14)}H_3$	$-\overset{\vert}{C}_{(2)}H-\overset{\vert}{C}_{(13)}H$	$-\overset{\vert}{C}_{(8)}H-\overset{\vert}{C}_{(15)}H$	$-\overset{\vert}{C}_{(11)}H-\overset{\vert}{C}_{(16)}H$	$-N_{(1)}H-C_{(2)}H$†	$-N_{(7)}H-C_{(8)}H$†
CCl_4	6,8	10,0	10,0	2,9	7,8 (8,5)	6,1 (6,6)
$CDCl_3$	6,8	9,5	10,1	2,9	8,1 (8,8)	6,1 (6,6)
$CDCl_3-CS_2$ (1 : 1)	6,5	—	—	3,9	8,1 (8,8)	6,3 (6,8)
$CCl_4-(CD_3)_2O$ (3 : 1)	6,4	4,6	9,8	3,0	9,3 (10,1)	7,0 (7,6)
$(CD_3)_2SO$	6,2	—	—	3,8	8,0 (8,7)	7,5 (8,1)
CH_3OH-H_2O (3 : 1)	6,5	—	—	—	7,9 (8,6)	7,9 (8,6)
CD_3OD (CH_3OH)	7,0	7,1 ; 7,6		4,4	8,5 (9,2)	8,2 (8,9)
CD_3OD+KSCN (CH_3OH+KSCN)	7,1	10,9	10,9	3,8	5,2 (5,6)	5,2 (5,6)
$CDCl_3-CS_2-CH_3OH$ (1 : 1 : 1)+KSCN	6,5	—	—	—	4,9 (5,3)	4,9 (5,3)
CH_3CN	7,0	8,7	9,8	3,8	7,7 (8,4)	6,9 (7,5)
CH_3CN+KSCN	7,0	11,0	11,0	3,6	4,8 (5,2)	4,8 (5,2)
CCl_4-CH_3CN (1 : 1)	6,9	10,4	10,4	3,2	7,9 (8,6)	7,4 (8,0)
CCl_4-CH_3CN (1 : 1)+KSCN	7,0	10,7	10,7	3,8	5,0 (5,4) ; 5,1 (5,5)	
CCl_4-CH_3CN (1 : 1)+NaSCN	6,9	8,6	8,6	4,0	5,5 (6,0)	5,5 (6,0)
$CDCl_3$+CsCl	—	—	—	—	5,1 (5,5)	5,1 (5,5)

* The concentrations of the solutions are given in Table 3.

† The values of the $^3J_{NH-CH}$ constants taking into account the correction for the electronegativity of the substituents [26] are given in brackets.

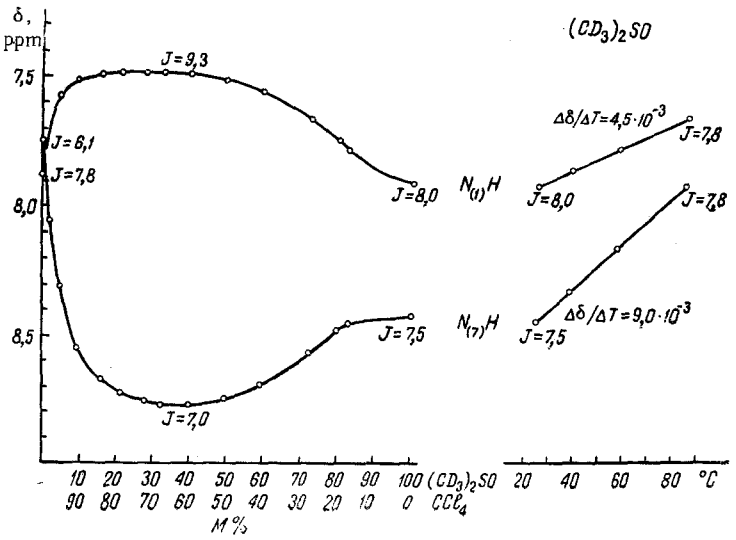


Fig. 4. Dependence of the chemical shifts of the NH protons and the $^3J_{NH-CH}$ constants on the composition of the $CCl_4-(CD_3)_2SO$ mixtures and the temperature.

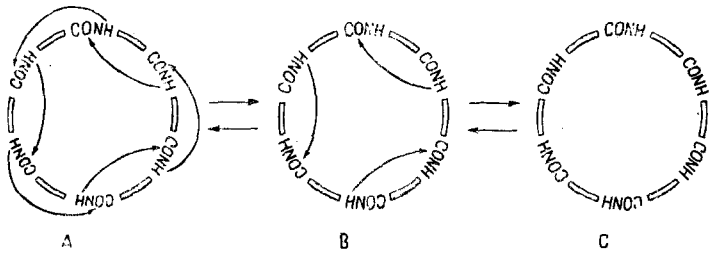


Fig. 5. Schematic illustration of the equilibrium of forms A, B, and C of valinomycin.

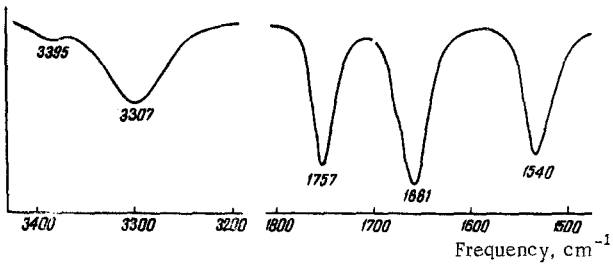


Fig. 6. IR spectrum of valinomycin in CCl_4.

in the region of the extrema of the chemical shifts the differentiation of the NH groups with respect to the constants $^3J_{NH-CH}$* is enhanced [transition from 8.5 and 6.6 Hz in CCl_4 to 10.1 and 7.6 Hz in $CCl_4-(CD_3)_2SO$ (3 : 1)], and a further increase in the concentration of $(CD_3)_2SO$ up to 100% is accompanied by an approach of the constants to one another (8.7 and 8.1 Hz) until they coincide completely (8.5 Hz) when the solution is heated to 68°C. Analogous $^3J_{NH-CH}$ constants (8.6 Hz) are observed in CH_3OH-H_2O (3 : 1) solution (see Table 4).

The facts given above permit the assumption that the three different types of conformations (Fig. 5) which we have called A, B, and C can be realized in valinomycin, depending on the nature of the solvent: in nonpolar solvents one type dominates (form A with an ORD curve of type 1 in Fig. 2), in which all the NH groups form intramolecular hydrogen bonds (IMHB) (see the details of the IR spectra below). In solvents of medium polarity [$CCl_4-(CD_3)_2SO$ (3 : 1), ethanol, and acetonitrile] only the three most stable IMHBs formed by the $N_{(1)}H$ groups are retained (form B with curves of types 3 and 4 in Fig. 2). In addition to the values of δ_{NH} at 30°C, this is also convincingly shown by the unusually strong dependence of $\delta_{N_{(7)}H}$ on the temperature ($\Delta\delta/\Delta T = 11.0 \cdot 10^{-3}$ ppm/deg) in solution in $CCl_4-(CD_3)_2SO$ (3 : 1) and the absence of a tem-

*In contrast to Figs. 3, 4, 15, and 17, the values of the $^3J_{NH-CH}$ constants are given in the text with a correction for the electronegativity of the substituents [26, 32] (see Table 4).

TABLE 5. Features of the IR Spectra of Valinomycin in $CHCl_3$ Solution*

ν_{N-H}, cm^{-1}	$A \cdot 10^{-4}$	$A_1^R \cdot 10^{-4}$	$n^R = \frac{A^R}{A_1^R}$	$A^W \cdot 10^{-4}$	$A_1^W \cdot 10^{-4}$	$n^W = \frac{A^W}{A_1^W}$
3388	2,06	2,22	0,93	0,86	1,40	0,61
3313	24,94	5,00	4,99	11,95	2,87	4,16

* A^R and A^W are the integral intensities of the bands expressed in $mole^{-1} \cdot liter \cdot cm^{-2}$ and calculated by Ramsay's method [36] and also by that of Wilson and Wells with the cutting off of the base line [37]; A_1^R and A_1^W are the integral intensities of the bands and the corresponding frequencies corresponding to one NH group and determined from the correlation curves of the dependences of A_1^R and A_1^W on ν_{N-H} [33]; n^R and n^W are the mean numbers of different groups in an equilibrium mixture of forms A and B of valinomycin determined by the two methods.

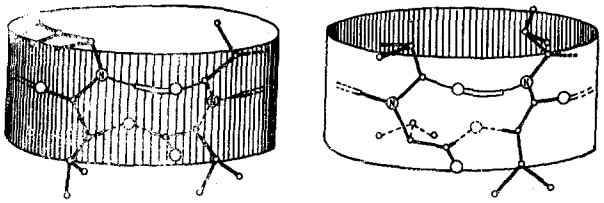


Fig. 7. The A_1 and A_2 forms of valinomycin.

TABLE 6. Possible Conformations of Form A of Valinomycin

	Type of conformation	Orient. of CO groups: D-valine	Orient. of CO groups: L-valine	Relative energy, kcal/mole
Form A_1	$[(\overline{r-b-l-p})_3]$	Outside	Outside	12,8
	$[(\overline{b-l-l-p})_3]$	Inside	"	15,0
	$[(\overline{r-b-p-r})_3]$	Outside	Inside	25,2
	$[(\overline{b-l-p-r})_3]$	Inside	"	27,6
Form A_2	$[(\overline{l-p-r-b})_3]$	Outside	Outside	>60
	$[(\overline{p-r-r-b})_3]$	Inside	"	>40
	$[(\overline{l-p-b-l})_3]$	Outside	Inside	>18
	$[(\overline{p-r-b-l})_3]$	Inside	"	0

perature dependence for $\delta_{N_{(1)}H}$ ($\Delta\delta/\Delta T = 0.65 \cdot 10^{-3}$ ppm/deg).* Finally, in polar solvents [$(CD_3)_2SO$, CH_3OH-H_2O (3:1), CF_3CH_2OH, $CF_3CH_2OH-H_2O$(1:2)], particularly at high temperatures, form C containing no IMHBs predominates.

Form A of Valinomycin. It is desirable to begin a consideration of form A of valinomycin with the features of the IR spectra taken in solutions in nonpolar organic solvents (CCl_4, Fig. 6, and $CHCl_3$, Fig. 15) which are not H-bond donors or acceptors for amide or ester groups.† In the region of the stretching vibrations of NH groups (3300-3500 cm^{-1}) there is a strong band from NH groups participating in IMHBs (3307-3313 cm^{-1}) and a considerably less intense band (3388-3395 cm^{-1}) from practically free NH groups. The separation of the bands, the calculation of their integral intensity (A), and the determination of the number of NH groups corresponding to each band by a method that we have developed [33] show that the ratio of the number of bound and practically free NH groups in $CHCl_3$ is ~5:1 (Table 5), which corresponds to the presence of ~70% of form A and ~30% of form B. A similar ratio of forms A and B is found for solutions in CCl_4 (judging from the similarity of the IR spectra in $CHCl_3$ and CCl_4).

Further, in the region of the stretching vibrations of the carbonyl groups there is a symmetrical

* As has been shown by Urry and Ohnishi [34, 35], the chemical shifts of the signals of peptide NH groups participating in IMHBs depend on the temperature more feebly than the signals of solvated NH groups.
† The IR spectra of valinomycin in CCl_4 at 20-25°C do not depend on the concentration in the range from $4.1 \cdot 10^{-2}$ to $4.0 \cdot 10^{-4}$ M, which shows the absence of intermolecular association; these results are confirmed by measurements of the molecular weight by the thermoelectric method in various solvents over a wide range of concentrations.

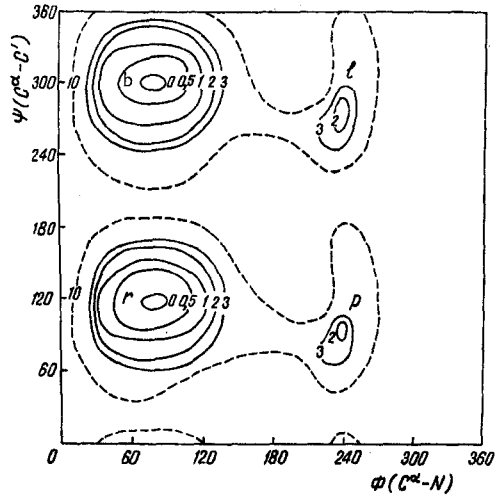


Fig. 8. Conformational chart of the methyl ester of N-acetyl-L-valine.

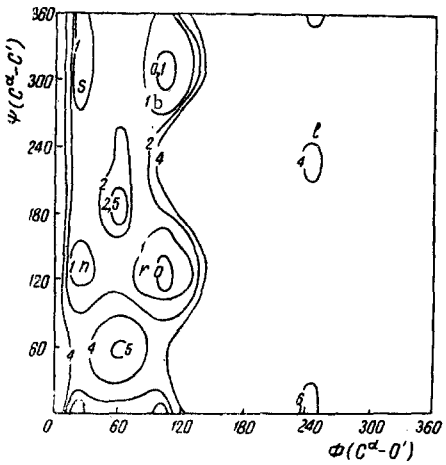


Fig. 9. Conformational chart of the methylamide of O-acetyl-L-lactic acid.

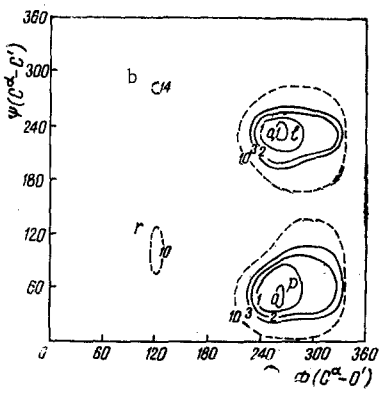


Fig. 10. Conformational chart of the methylamide of O-acetyl-D-α-hydroxyisovaleric acid.

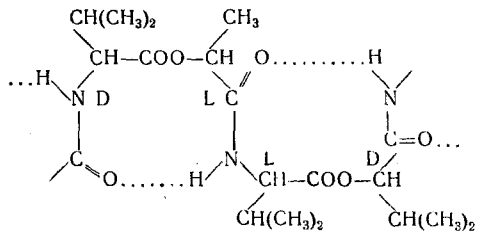
band at 1755-1757 cm^{-1} corresponding to ester carbonyl groups not participating in the formation of IMHBs. In the region of amide carbonyl groups there is a strong amide II band (1540 cm^{-1} in CCl_4), which shows the trans configuration of the amide bonds, a strong amide I band at 1661 cm^{-1}, and a considerably weaker band overlapping it at ~1675 cm^{-1}. The ratio of the intensities of the two latter bands corresponds approximately to the ratio of the intensities of the bands of the bound and free NH groups in the 3300-3400 cm^{-1} region. Consequently, all the IMHBs of valinomycin are formed by the CO and NH groups of the amide links of the antibiotic.

A conformational analysis of the valinomycin molecule* shows that there is only one possibility for the formation of six IMHBs in form A satisfying the facts given above; this is shown schematically in Fig. 5. In the conformation obtained in this way, the depsipeptide chain of the valinomycin molecule consists of a closed system of six condensed ten-membered rings stabilized by IMHBs of the 4→1† type and forming a "bracelet" with a diameter of ~8 Å and a height of ~4 Å. Under these conditions, two different structures of this type are possible (A_1 and A_2, Fig. 7), differing by the chirality of the cyclic system and the orientation of the side chains. Conformations of type A_1 differ most simply from A_2 in the following way: if the lactic acid residues are arranged in the top part of the bracelet, in the A_1 conformations acylation takes place clockwise and in the A_2 conformations anticlockwise. In the A_1 and A_2 forms the positions of the amide bonds and of the asymmetric carbon atoms are fixed, and the carbonyl groups of the ester bonds can be oriented "inside" the bracelet (i.e., in the direction of the axis of symmetry) or "outside" it (i.e. in the direction away from the axis of symmetry). In accordance with this, within the limits of each of forms A_1 and A_2, four types of conformations are possible which differ by the mutual orientation of the ester carbonyl groups; these are listed in Table 6.

To establish which of the eight conformations listed is actually realized in solution, their relative energies must be evaluated. For this purpose, we have made a theoretical analysis and have calculated the optimum conformations of the two protected didepsipeptides Ac-D-Val-L-Lac-NHMe (1) and Ac-L-Val-D-HyIv-NHMe (2) containing IMHBs of the 4→1 type and modeling the most important structural elements of forms A and B of valinomycin – their ten-membered rings stabilized by TMHBs.

*In the analysis, the trans configuration of the ester bonds was adopted since cis ester bonds are realized only in strained lactams having less than 11 atoms in the ring [38].

†For the nomenclature of IMHBs in peptides, see [49].



For this we used the results of a calculation of the conformational charts of Ac-L-Val-OMe, Ac-L-Lac-NHMe, and Ac-D-HyIv-NHMe, modeling the amino acid and hydroxy acid fragments of valinomycin (the chart for Ac-D-Val-OMe is symmetrical with the chart for the L isomer relative to the center of the chart, $\Phi = \Psi = 180°$) [39].

As can be seen from Figs. 8-10, on the charts of the fragments there are four main energy minima located in regions r, b, p, and *l*; the additional minima n and s on the chart of Ac-L-Lac-OMe are close to the b and *l* regions. In accordance with this, for the various conformational types of depsipeptide fragments with 4→1 IMHBs we have adopted the symbols $\overbrace{p-r}$, $\overbrace{b-l}$, $\overbrace{r-b}$, $\overbrace{l-p}$, etc.*; analogous symbols are also used for the various forms of valinomycin.

The relative energies of the optimum forms of the didepsipeptide (1) and (2) and the values of Φ and Ψ corresponding to them that were found in the course of calculation are given in Tables 1 and 2. It has been shown that the parameters of the individual amino acid and hydroxy acid residues in the optimum conformations (1) and (2) correspond to the energetically most favorable regions of the corresponding conformational charts; the most favorable in all cases is the gauche orientation of the protons in the $C^{\alpha}H-C^{\beta}H$ fragments of the α-hydroxyisovaleric acid residues and the trans orientation of the analogous protons of valine. Calculation also showed that the $\overbrace{l-p}$ form for (1) and the $\overbrace{r-b}$ form for (2) have no local minima in view of the high energy of the p region for Ac-L-Lac-OMe and the b region for Ac-D-HyIv-OMe.

The energy of the IMHBs of the optimum structures obtained is between 3.8 and 4.0 kcal/mole, and their length (N...O distance) is 2.9-2.8 Å. The formation of a 4→1 IMHB places substantial limitations on the region of permitted coordinates Φ and Ψ of the didepsipeptides. For example, comparatively small deviations of Φ_1 from the optimum values ($105 > \Phi_1 > 255$) makes the formation of an IMHB impossible. Furthermore, in the region permitted from the point of view of the retention of the IMHB the variation of Φ and Ψ within the limits of the potential depressions of the amino acid and hydroxy acid fragments does not lead to a sharp increase in the potential energy of the system.

The figures given in Tables 1 and 2 permit an evaluation of the energies of the various conformations possible for form A of valinomycin on the assumption that the closure of the dodecadepsipeptide ring changes the geometrical parameters and relative energies of the didepsipeptide fragments from their optimum values only insignificantly. This assumption is based on the dominating role of short-range interactions in the formation of the spatial structure of the cyclodepsipeptides which we have shown for the case of a large number of compounds [24, 30, 40]. The results given in Table 6 show that the most favorable conformation from the point of view of the energy of the individual fragments is $[\overbrace{p-r-b-l}_3]$ belonging to type A_2. Following this, and considerably inferior in energy, are the conformations $[\overbrace{r-b-l-p}_3]$ and $[\overbrace{b-l-l-p}_3]$ (type A_1). On the basis of the theoretical analysis, the other conformations must be regarded as unlikely, since they possess far higher energies.

The choice between the three conformations mentioned was made by an analysis of the $^3J_{NH-CH}$ constants measured from the NMR spectra and their correspondence with the calculated figures. Since in CCl_4 and $CHCl_3$ solutions, containing, according to the IR spectra, ~30% of form B and ~70% of form A (see above), $^3J_{N(1)H-C(2)H} = 8.5$-8.8 Hz and $^3J_{N(7)H-C(8)H} = 6.6$ Hz and in form B $^3J_{N(1)H-C(2)H} = 10.1$ Hz and $^3J_{N(7)H-C(8)H} = 7.6$ Hz [measurements in $CCl_4-(CD_3)_2SO$ (3 : 1)], it is easy to see that in the pure form A the fragment $N_{(1)}H-C_{(2)}H$ corresponds to $^3J = 7.8$-8.2 Hz and the fragment $N_{(7)}H-C_{(8)}H$ corresponds to

* The first and second letters show the regions of the realization of the parameters Φ and Ψ of the amino acid and hydroxy acid fragments; the brace placed above denotes an IMHB.

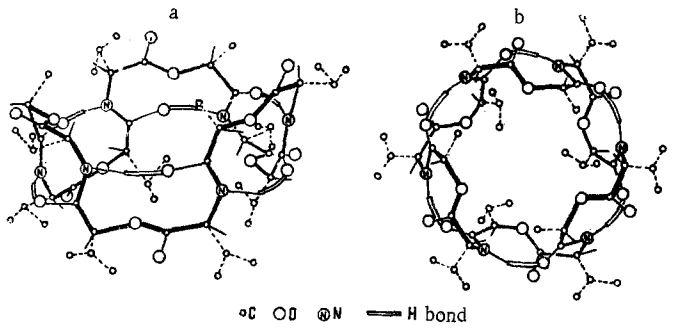


Fig. 11. Conformation of valinomycin in nonpolar solvents: a) side view; b) view along the C_3 axis.

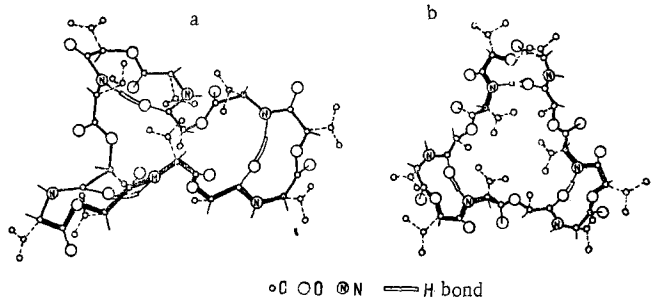


Fig. 12. Conformation of valinomycin in solvents of medium polarity: a) side view; b) view along the C_3 axis.

$^3J = 6.2$ Hz. In addition, it follows from a theoretical conformational analysis that the coordinate Φ_1 for the fragment-D-Val-L-Lac- of form A_2 can change within the range from 180 to 255° (⊛ 60-135°) and for the fragment -L-Val-D-Hyiv- between 105 and 180° (⊛ 135-60°). According to the stereochemical dependence of the $^3J_{NH-CH}$ constant that we have given previously [26, 32], the ranges of dihedral angles given correspond to constants of 0-6 Hz, i.e.,on the basis of the results of NMR spectroscopy the conformers of form A_2 are not dominating in solutions of nonpolar solvents. Conformations of type A_1, to which correspond $\Phi_1^{D\text{-Val}}$ 105-180° and $\Phi_1^{L\text{-Val}}$ 180-255°, i.e.,⊛ = 0-60° and $^3J_{NH-CH}$ 0-9 Hz ($^3J_{NH-CH}$ 7-9 Hz corresponding to the optimum values of Φ_1), on the other hand, are in full agreement with the experimental values of $^3J_{NH-CH}$.

Consequently, the structure most suitable from the point of view of short-range energies is not in fact realized in solutions of nonpolar solvents. The reason for this consists in the appearance in a cyclic structure of type $[(\overline{p-r-b-l})_3]$ of additional destabilizing interactions which must in the first place include the electrostatic repulsion of the six carbonyl groups turned to the inside of the molecule. In addition, a definite role is apparently played by the steric interaction of the isopropyl groups of the valine residues which, in all the conformers of type A_2, are directed towards the axis of symmetry.

Of the two remaining conformations, $[(\overline{r-b-l-p})_3]$ and $[\overline{(b-l}-\overline{l-p})_3]$, the second must be regarded as less likely because of destabilization (similar to the case of $[(\overline{p-r-b-l})_3]$) by the electrostatic interactions of the three carbonyl groups oriented towards the inside. In addition, the formation of the cyclic structure of valinomycin in the conformation $[(\overline{b-l-l-p})_3]$ requires a more substantial deviation of the coordinates Φ and Ψ from the optimum values than in the case of the conformation $[(\overline{r-b-l-p})_3]$.

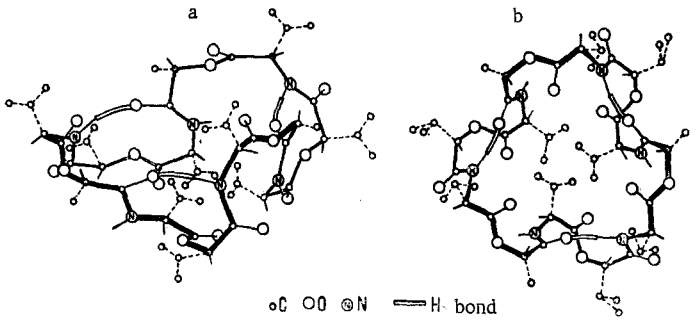


Fig. 13. Conformation of valinomycin of the $[(p-r-\overline{l-l})_3]$: a) side view; b) view along the C_3 axis.

Thus, the dominating conformation of valinomycin in nonpolar solvents is a structure of the type $[(\overline{r-b-l-p})_3]$ with six ester carbonyl groups directed outwards, which is characterized by the following coordinates Φ and Ψ:

	D-Val	*L*-Lac	*L*-Val	*D*-Hylv
Φ	140	80	205	280
Ψ	110	220	250	150.

In addition to this, the possibility of the realization in small amounts of the conformation $[(\overline{b-l-l-p})_3]$ which we have discussed previously [17, 41] on the basis of an analysis of molecular models, cannot be excluded.

	D-Val	*L*-Lac	*L*-Val	*D*-Hylv
Φ	145	235	205	250
Ψ	305	220	255	150

The orientation of the lateral isopropyl groups with respect to the main depsipeptide chain was determined from the $^3J_{C^{\alpha}H-C^{\beta}H}$ constants. It was found that in CCl_4 solutions the amino acid residues have the trans orientation of the $C^{\alpha}H$ and $C^{\beta}H$ protons [$^3J_{C_{(2)}H-C_{(13)}H} = {}^3J_{C_{(8)}H-C_{(15)}H} = 10.0$ Hz] and the hydroxy acids have the gauche orientation [$^3J_{C_{(11)}H-C_{(16)}H} = 2.9$ Hz]. This conclusion corresponds to the results of the calculation of the optimum conformations of the individual fragments of valinomycin (see Tables 1 and 2). The conformation of valinomycin found is shown in Fig. 11.

The dipole moment of the conformation $[(\overline{r-b-l-p})_3]$ is 0-0.7 D. The experimental value of the dipole moment (3.5 D in CCl_4) is in good agreement with the presence of ~70% of form A and ~30% of form B, which possesses a high dipole moment (7-9 D, see below), in the equilibrium mixture.

Form B of Valinomycin. Form B, stabilized by only three IMHBs between the CO groups of the lactic acid residues and the D-valinomycin NH groups, possesses a freer structure, which it has been possible to determine by the simultaneous use of NMR spectroscopy and theoretical conformational analysis. It can be seen from Table 2 that the $N_{(7)}H-C_{(8)}H$ fragment in form B must adopt the gauche conformation ($\overline{b-l}$ form) or the cis conformation (form $\overline{l-p}$ or $\overline{l-l}$).

The $^3J_{N_{(7)}H-C_{(8)}H}$ constants of 7.6 Hz found for form B (see above) show the realization of the second possibility, since the gauche rotameters of the NH–CH fragments are characterized by low $^3J_{NH-CH}$ constants (< 6 Hz). On the basis of the $^3J_{N_{(1)}H-C_{(2)}H}$ constant of 10.1 Hz, in the D-valine residues the NH and $C^{\alpha}H$ protons have the trans orientation, which corresponds to $\Phi_1 \sim 300°$, i.e.,the *l* and p conformations. The realization of any of these forms was also to be expected on the basis of the calculated figures, since the deepest and widest potential depressions in the corresponding conformational chart correspond to them (see Fig. 8). So far as concerns the L-lactic acid residue, for it no a priori choice can be made between the b, r, n, and s conformations. Conformational analysis shows that among all the possible combinations

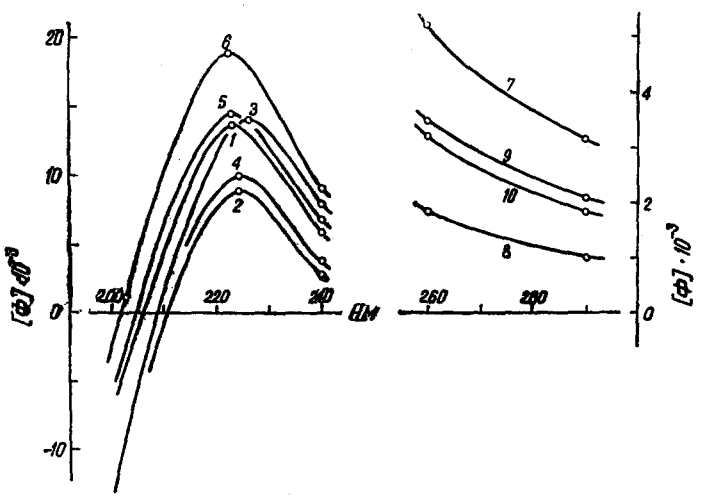


Fig. 14. ORD curves of complexes of valinomycin with alkali-metal cations: 1) ethanol + KCl; 2) ethanol + RbCl; 3) ethanol + CsCl; 4) trifluoroethanol + KCl; 5) acetonitrile + $C_{12}H_{25}SO_3K$; 6) heptane–ethanol (3 : 1) + KCl; 7) chloroform–acetonitrile (1 : 1); 8) chloroform–acetonitrile (1 : 1) + 5-20 equiv. of KSCN; 9) chloroform–acetonitrile (1 : 1) + 5 equiv. of NaSCN; 10) chloroform–acetonitrile (1 : 1) + 20 equiv. of NaSCN.

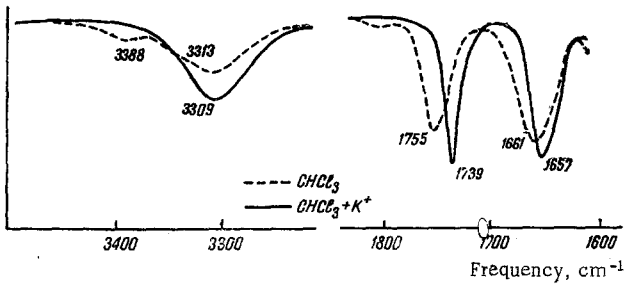


Fig. 15. IR spectra of valinomycin and its K^+ complex in $CHCl_3$.

of the permitted forms of the individual fragments listed above the cyclization condition is satisfied by only two structures of form B, which are shown in Figs. 12 and 13, and belong to the types $[\underline{(p-r-\overline{l-l})_3}]$ and $[\underline{(l-b-\overline{l-p})_3}]$:

		D-Val	L-Lac	L-Val	D-Hylv
$pr\,\overline{l\,l}$	Φ	300	125	230	250
	Ψ	50	170	190	210
$lb\,\overline{l\,p}$	Φ	300	120	230	280
	Ψ	265	300	245	140

According to an analysis of the fragments of valinomycin, the two forms have equal energies, but in the first case the closure of the cyclic structure requires a somewhat greater deviation of the coordinates Φ and Ψ from the optimum values than for the second form. Moreover, attention is attracted by the substantially differing dipole moments of these two forms: 1.5-3.5 D for the first and 7-9 D for the second. Of these, only the second value satisfies the dipole moment of 3.5 D found experimentally for a solution of valinomycin in CCl_4 (equilibrium mixture of ~70% of form A and ~30% of form B), if the small dipole moment of form A (0-0.7 D) is taken into account. It was possible to make a final choice between structures $[\underline{(p-r-\overline{l-l})_3}]$ and $[\underline{(l-b-\overline{l-p})_3}]$ by comparing the features of their conformations with the NMR spectra. It can be seen from a consideration of molecular models that in the conformation $[\underline{(p-r-\overline{l-l})_3}]$ the isopropyl groups of the L-valine residues are oriented within the molecule and are spatially close, while in the structure $[\underline{(l-b-\overline{l-p})_3}]$, on the other hand, the D-valine side groups are close (see Figs. 12 and 13). Since, in the NMR spectra of form B, spatial hindrance of the D-valine isopropyl groups forced in CCl_4–$(CD_3)_2SO$ (3 : 1) solution to adopt the gauche conformation [$^3J_{C_{(2)}H-C_{(13)}H}$ = 4.6 Hz] can be clearly seen, and the protons of the $C^{\alpha}H-C^{\beta}H$ fragments in the L-valine residues occupy the trans conformation that is most favorable from the point of view of short-range interactions [$^3J_{C_{(8)}H-C_{(15)}H}$ = 9.8 Hz], a structure of type $[\underline{(l-b-\overline{l-p})_3}]$ may be regarded as proved for form B.

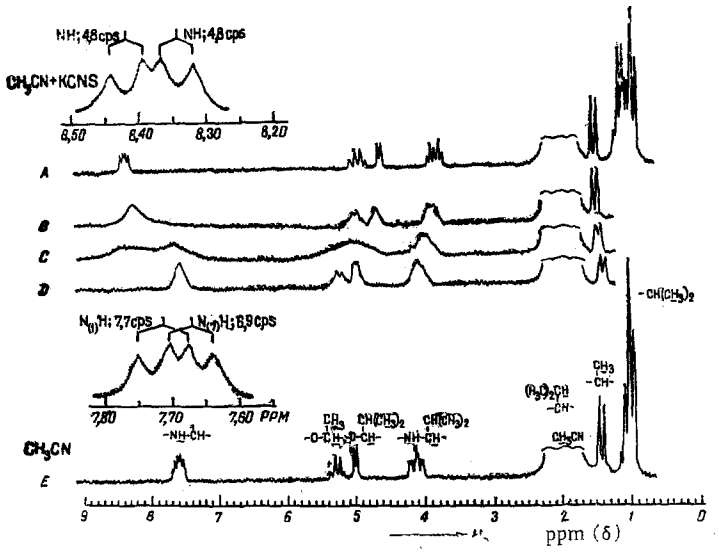


Fig. 16. NMR spectra of valinomycin (E), its K^+ complex (A), and the equilibrium mixtures in CH_3CN at various molar ratios of valinomycin and KSCN. B) 1:0.73; C) 1:0.43; D) 1:0.14.

In the proposed structure it is possible to separate the hydrophobic "nucleus" formed by the aliphatic side groups of the D-valine and lactic acid residues around which the depsipeptide chain with its polar groups is arranged. Under these circumstances, the depsipeptide rings stabilized by IMHBs are located on the periphery of the molecule, imparting to form B an external similarity to the "propeller" conformation of the trisalicylides [42].

On the basis of the NMR spectra of valinomycin in $(CD_3)_2SO$ solution, Urry and Ohnishi [34, 35] proposed a "pore" structure for valinomycin externally resembling the $[\underline{(l-b-\overline{l-p})_3}]$ conformation but sharply differing from it by the orientation of the carbonyl groups and lateral substituents. In our opinion, this structure, apparently belonging to the $[\underline{(b-r-\overline{p-b})_3}]$ type, is unlikely since it possesses an extremely high energy. Furthermore, a type b conformation of the D-valine residues with its cis orientation of the $N_{(1)}H-C_{(2)}H$ protons contradicts the constant $^3J_{N_{(1)}H-C_{(2)}H} = 10.1$ Hz that we have found for form B.

Form C of valinomycin, containing no IMHBs, possibly has no fixed structure and consists of an equilibrium mixture of a large number of conformers of similar energies. This is indicated, in particular, by the considerable lowering of the intensity of the Cotton effects in the ORD curves of valinomycin on passing to polar solvents (curves 5 and 6 in Fig. 2).

On the basis of the principle of the maximum hydrophobic interactions, Warner proposed for valinomycin in aqueous solutions a peculiar conformation in which there is a marked separation of the hydrophobic and polar parts of the molecule [43]. Since no other nonvalent interactions were taken into account (not to mention experimental results), Warner's purely speculative structure appears unlikely.

II. Conformations of Complexes of Valinomycin with Alkali-Metal Cations

An important part of our work consists of the results of an investigation of complexes of valinomycin with various alkali-metal cations, since their formation is a necessary condition for the functioning of valinomycin in artificial and biological membranes. As an instrument for the study of biochemical processes, valinomycin is most frequently used for the selective induction of the potassium permeability of biological membranes. Consequently, we studied the K^+ complex of valinomycin in most detail.

K^+ Complex of Valinomycin. The ORD curves of the K^+ complex differ substantially from the curves of the free antibiotic, vary only slightly with a change of solvent [CF_3CH_2OH, CH_3CN, EtOH, EtOH–C_7H_{16}

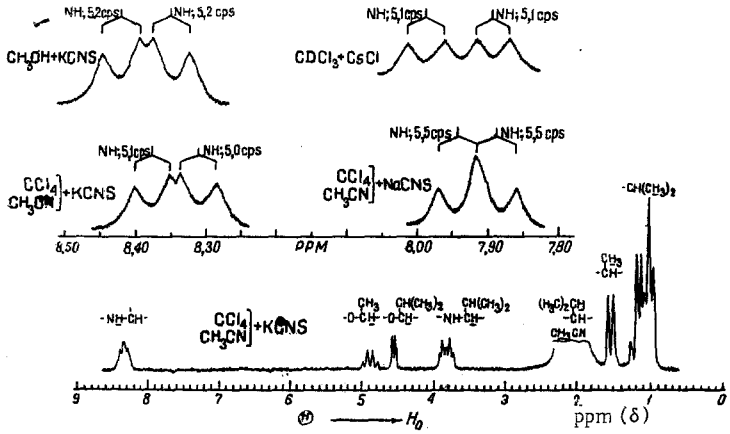


Fig. 17. NMR spectrum of the K^+ complex of valinomycin in CCl_4-CH_3CN (1:1) and the region of the NH signals of the complexes with Na^+, K^+, and Cs^+.

(1:1) (Fig. 14)], and do not depend on the nature of the anion (F^-, Cl^-, Br^-, NO_3^-, NCS^-, $C_{12}H_{25}SO_3^-$). It follows from this that in contrast to free valinomycin the conformation of the K^+ complex is characterized by considerable rigidity.

In the IR spectra of valinomycin taken in $CHCl_3$ solution saturated with potassium dodecyl sulfate, the amide I region does not differ appreciably from that in pure $CHCl_3$, the band at 3395 cm^{-1} disappears, and the band of the stretching vibrations of the ester carbonyl groups shifts in the long-wave direction by 16 cm^{-1}, with a simultaneous contraction (Fig. 15). Similar IR spectra of the K^+ complex are obtained in CCl_4-CH_3CN (2:1) (see Fig. 20). These results indicate the absence of conformations of type B with three IMHBs in a solution of the complex and a shift in the conformational equilibrium in the direction of the bracelet conformation of type A in which all the ester groups take part in ion–dipole interaction with the cation.

The NMR spectra of the K^+ complex of valinomycin in CH_3OH (CD_3OD), CH_3CN, and CCl_4-CH_3CN (1:1) are extremely similar to one another and differ substantially from the spectra of the free antibiotic in the same solvents (Figs. 16 and 17; Table 3). The chemical shift of the signals from the NH protons scarcely changes when solutions in CH_3OH and CCl_4-CH_3CN (1:1) are heated, which shows the retention of the bracelet system of IMHBs in these solvents. The cooling to −95°C of solutions of the K^+ complex in $CS_2-CDCl_3-CH_3OH$ (1:1:1) does not lead to any appreciable change in the nature of the spectra (apart from the normal equilibrium broadening of all the signals), which shows the symmetry of the complex conformation and the absence of appreciable amounts of any other conformations apart from the dominating one in solution. It is important that the $^3J_{N_{(1)}H-C_{(2)}H}$ constants of the K^+ complex are the same in all the solutions studied and are practically independent of the solvent, varying only between 5.2 and 5.6 Hz. Such values of the constants correspond to the gauche orientation of the NH and $C^{\alpha}H$ protons.

A comparison of the experimental results with those of the conformational analysis of form A (see Table 6) leads to the conclusion that in the formation of the complexes the valinomycin must occupy a conformation of type $[(\overline{b-l-p-r})_3]$ or $(\overline{p-r-b-l})_3]$, since only in these two forms is the gauche orientation of the protons in all the NH–CH fragments realized ($\Phi \sim 120°$ for the L-valine residues and $\sim 240°$ for those of D-valine) and all the ester carbonyl groups are oriented within the molecule, thereby ensuring an effective ion–dipole interaction with the cation located in its center. A comparison of the energies of these two forms shows that the $[(\overline{p-r-b-l})_3]$ conformation is more favorable (by ~28 kcal/mole, see Table 6) than the $[(\overline{b-l-p-r})_3]$ form, which permits the $[(\overline{b-l-p-r})_3]$ form to be excluded from further consideration and the structure shown in Fig. 18 to be regarded as proved for the K^+ complex of valinomycin in solutions. To it correspond the following coordinates Φ and Ψ:

	D-Val	*L*-Lac	*L*-Val	*D*-Hyiv
Φ	240	110	120	255
Ψ	60	180	300	200

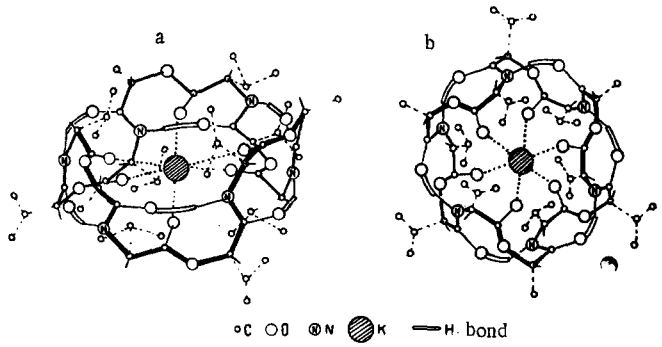


Fig. 18. Conformation of the K^+ complex of valinomycin: a) side view; b) view along the C_3 axis.

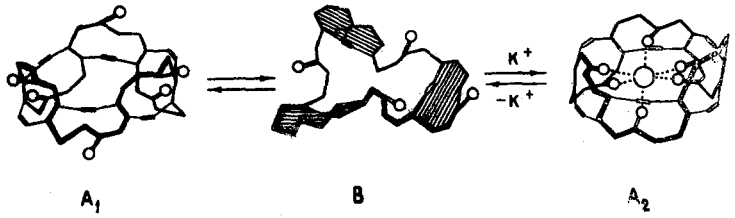


Fig. 19. Nature of the conformational equilibrium of valinomycin in the presence of potassium salts.

As in form A of free valinomycin, the side chains of the valine residues in the K^+ complex occupy the trans conformation ($^3J_{C^\alpha H-C^\beta H}$ = 10.7-11.0 Hz), and those in the hydroxy acid residues occupy the gauche conformation ($^3J_{C^\alpha H-C^\beta H}$ = 3.6-3.8 Hz).

A similar structure without the parameters Φ and Ψ being given* has been proposed for the K^+ complex of valinomycin by Urry and Ohnishi [35, 44] on the basis of the small temperature gradients of the chemical shifts of the NH protons in CH_3OH solution and the assumption of an interaction of all the ester groups with the central cation. The choice between the conformations of types $[(\overline{b-l}-\overline{p-r})_3]$ and $[(\overline{p-r}-\overline{b-l})_3]$ was made as a result of the assumption of the existence of an analogy (even though an extremely remote one) between the marginal sections of the antiparallel β structures found in proteins [45] with the IMHB-stabilized fragments of the depsipeptide chain of valinomycin. The authors mentioned also considered that the $[(\overline{p-r}-\overline{b-l})_3]$ structure had been found in an x-ray structural analysis of the crystalline complex valinomycin · $KAuCl_4$ [4].

Thus, regardless of the nature of the solvent, in the formation of complexes with K^+ valinomycin occupies a rigid conformation in which the ester carbonyl groups form a cavity with a diameter of 2.7-3.2 Å. At the same time, while in the absence of cations, this conformation, which is most favorable from the point of view of nonvalent interactions, is destabilized, as was shown above, by the electrostatic interaction of the spatially adjacent oxygen atoms; in the structure of the complex the presence of the positive charge in the center of the cavity not only eliminates the electrostatic repulsion but is a positive stabilizing factor.

A characteristic feature of complex formation is the effective screening of the central cation by the ester groups, the IMHB system, and the overhanging isopropyl groups of the valine residues†; the side groups of the hydroxy acid residues project from the outside of the "bracelet", screening the IMHB system

*In a review [35], Urry erroneously ascribed to the NH−C^αH fragments of the K^+ complex of valinomycin a dihedral angle Θ of ~30°, corresponding to $\Phi_1 \sim 150$ and $\Phi_3 \sim 210$.

†The steric hindrance arising under these conditions, which can be well seen from a consideration of molecular models, served as a basis for the hypothesis of the preferred nature of the conformation $[\overline{b-l-p-r})_3]$ expressed in our earlier papers [17, 41].

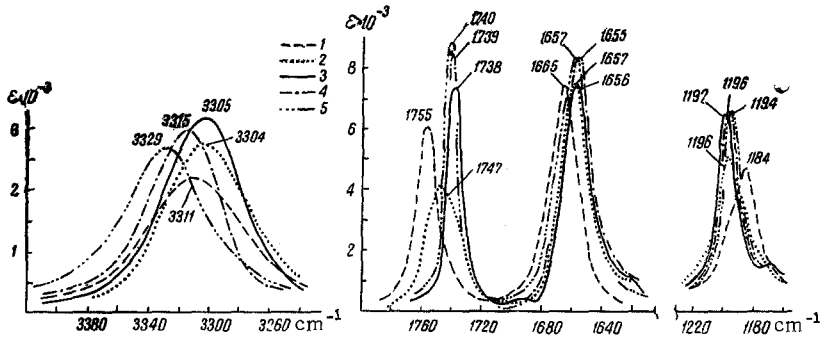


Fig. 20. IR spectra of valinomycin (1) and its complexes with Na^+ (2), K^+ (3), Rb^+ (4), and Cs^+ (5) in CCl_4-CH_3CN (2:1).

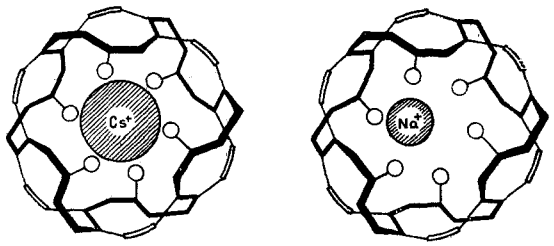


Fig. 21. Schematic illustration of the complexes of valinomycin with Na^+ and Cs^+.

from the action of the solvent (see Fig. 18). The lipophilic nature of the molecular surface of the complex cation explains the high solubility of the complex in neutral organic solvents and, apparently, is extremely important for the functioning of valinomycin in membranes (see [17]).

From a comparison of the conformations of free valinomycin and its complex it can be seen that complex formation is accompanied by serious conformational rearrangements of the depsipeptide chain. In this respect, attention is attracted in the first place by the opposite orientation of the ester carbonyl groups in form A of free valinomycin ("all external") and in the complex ("all internal"). Furthermore, the opposite chiralities of the system of amide groupings and IMHBs in the "nonpolar" conformation of valinomycin [$[(\overline{r-b-l-p})_3]$, type A_1] and in the complex [$[(\overline{p-r-b-l})_3]$, type A_2] must be noted. The mutual transition of these two forms is not possible without the rupture of at least three IMHBs. Consequently, form B (Fig. 19) is apparently an intermediate stage in the formation of the complexes in nonpolar media. It is not excluded that the comparatively low rate of migration of potassium between valinomycin molecules, leading to a considerable broadening of the signals of the NMR spectra under the conditions of incomplete complex formation observed by Haynes, Kowalsky, and Pressman [46], is partially connected with the energy barriers of these conformational rearrangements (see also Fig. 16).

Complexes of Valinomycin with Na^+, Rb^+, and Cs^+. Interesting results have been obtained in a study of the interaction of valinomycin with sodium, rubidium, and cesium salts. We have shown previously [17, 18] that valinomycin scarcely increases the ohmic resistance of ethanolic solutions of sodium chloride. The absence of complex formation with Na^+ in ethanol also followed from measurements of the ORD curves, since the curves in ethanol and a 2.1 M solution of NaCl in ethanol (40-fold excess of salt) do not differ significantly. These results correlate well with the absence of any effect whatever in the action of valinomycin on model phospholipid membranes in the presence of sodium salts. However, it has recently been shown that under definite conditions valinomycin is capable of inducing the transfer of sodium ions into an organic phase [7] and their extraction from phospholipid micelles [16], which suggested the possible formation of complexes with Na^+ in nonpolar media. In actual fact, the ORD curves of valinomycin in $CH_3CN-CHCl_3$ (1:1) containing a 20-fold excess of sodium thiocyanate undergo a considerable reduction in intensity as compared with the pure solvent and approximate to the curve of the K^+ complex, which indicates an interaction with Na^+ (see Fig. 14).

The NMR spectra of valinomycin in CCl_4-CH_3CN (1:1) saturated with sodium thiocyanate not only enable the formation of an Na^+ complex to be established from the change in the parameters of the spectrum as compared with free valinomycin but also demonstrate that the Na^+ and K^+ complexes have similar structures. This is shown in the first place by the weak dependence of the chemical shifts of the NH signals on the temperature ($\Delta\delta/\Delta T = 0.9 \cdot 10^{-3}$ ppm/deg for both NH signals), reflecting the retention of the

TABLE 7. Influence of the Dimensions of the Cations on the Stability of Their Complexes with Valinomycin

Cation	Na^+	K^+	Rb^+	Cs^+
Radius, Å	0,98	1.33	1,48	1,65
Desolvation energy [47] (EtOH, 25°C, kcal/mole)	89,0	72,5	66,5	58,5
Stability constant of the complex ($K \cdot 10^{-5}$ liter/mole, EtOH, 25°C) [48]	<0,001	20	26	6,5
Free energy of complex-formation ($-\Delta F = RT \ln K$, kcal/mole)	<2,7	8,6	8,8	7,9

bracelet system of IMHBs in the Na^+ complex.* In addition to this, attention is attracted by the lowering of the $^3J_{NH-CH}$ constants from 8.6 and 8.0 Hz in CCl_4-CH_3CN (1:1) to 6.0 Hz when valinomycin reacts with Na^+ (see Fig. 17). Since one cannot exclude the possibility of incomplete complex formation under the experimental conditions, the true $^3J_{NH-CH}$ constants of the Na^+ complex may be still lower, i.e., practically coincident with the corresponding constants of the K^+ complex in the same solvent (5.4 and 5.5 Hz).

The formation of a Na^+ complex of valinomycin is also shown in the IR spectra taken in $CH_3CN-CCl_4$ (1:2) solution. Under these conditions the most characteristic regions are those of the stretching vibrations of the ester C=O and C–O groups (Fig. 20). For example, in the formation of complexes with K^+, the band at 1755 cm^{-1}, both in CCl_4 and $CHCl_3$ solutions, is shifted in the low-frequency direction to 1739 cm^{-1} and the band at 1184 cm^{-1} in the high-frequency direction to 1195 cm^{-1}. The opposite nature of these shifts agrees well with the participation of the ester groups in an ion–dipole interaction in which the length of the C=O double bond must increase somewhat and, in contrast, that of the ordinary C–O bond must decrease. The addition of a 20-fold excess of NaSCN to a solution of valinomycin is also accompanied by a shift in frequency from 1184 to 1196 cm^{-1}. The band at 1755 cm^{-1} not only shifts by ~9 cm^{-1} but also becomes asymmetrical, which shows the nonequivalence of the ester groups in the Na^+ complex. The most probable explanation of this fact is that in view of its small size the sodium cation is shifted from the center of the internal cavity of valinomycin to its edges, thereby creating the conditions for ion–dipole interactions of different efficiencies with the different ester groups (Fig. 21).

The complexes of valinomycin with Rb^+ and Cs^+ are extremely similar in structure to the potassium complex, as follows from the similarity of the ORD curves (see Fig. 14) and the closeness of the $^3J_{NH-CH}$ constants (see Table 4 and Fig. 17). In addition to this, the complexes with Rb^+ and Cs^+ have their individual features, which are clearly shown in the IR spectra (see Fig. 20) and NMR spectra. Thus, the complex with Rb^+ exhibits a small short-wave shift of the band of the stretching vibrations of the NH groups (by 9 cm^{-1}) and a lowering of its intensity, which shows an increase in the O...N distance and a reduction in the energy of the IMHBs. The tendency to the weakening of the IMHB system with an increase in dimensions of the cation is shown particularly clearly in the complex of valinomycin with Cs^+ ($\Delta\nu$ 23 cm^{-1}), since in this case the dimensions of the cation (d=3.3 Å) already exceed the "normal" dimensions of the internal cavity of valinomycin (2.7-3.2 Å). Apparently, the weakening of the IMHB system also explains the upfield displacement of the chemical shifts of the NH protons in the cesium complex as compared with the potassium complex observed by American authors [46] in $CHCl_3$ solutions.

Since the dimensions of the internal cavity of valinomycin in "complex" conformation are sufficient for the insertion of Na^+ and K^+ into it without any steric strain whatever, it is not surprising that the free energies of complex formation and the stability constants of the complexes correlate well with the solvation energies of the ions (Table 7). A slight increase in the stability of the complexes with Rb^+ and a decrease in the stability of those with Cs^+ are explained by the appearance of steric hindrance in the interaction of voluminous cations with the fixed octahedral system of carbonyl oxygen atoms.

Thus, in the course of the present investigation we have established the conformational states of valinomycin in different media and on complex formation with alkali-metal cations. For this purpose we used a methodological device not previously used for the study of peptide systems and consisting of the simultaneous application of a broad selection of physicochemical methods in combination with theoretical conformational analysis.

* The values of $\Delta\delta/\Delta T$ in the same solvent for free valinomycin are $4.0 \cdot 10^{-3}$ and $4.2 \cdot 10^{-3}$ ppm/deg and for the K^+ complex $1.5 \cdot 10^{-3}$ and $1.8 \cdot 10^{-3}$ ppm/deg.

First, by spectroscopic methods, the existence of a conformational equilibrium was established, its nature was determined, and the system of IMHBs was localized for each of the dominating forms. Then the most suitable forms of the antibiotic were determined by calculating the energies of the various conformations of the IMHB-stabilized fragments of valinomycin, the choice between these being made by a comparison of the dipole moments and $^3J_{NH-CH}$ constants calculated for them with the experimental values.

In nonpolar solvents, valinomycin adopts a characteristic "bracelet" conformation stabilized by six IMHBs of the 4→1 type with all the ester carbonyl groups directed away from the center of the molecule. In solvents of medium polarity, a "propeller" conformation is realized in which three ten-membered rings stabilized by IMHBs are grouped around a hydrophobic nucleus. Passage to polar solvents leads to the breakage of the IMHBs, as a result of which a large number of forms of similar energies begin to participate in the conformational equilibrium.

A distinctive feature of the complexes of valinomycin with alkali-metal cations is the existence of an internal cavity with a diameter of 2.7-3.3 Å in which the cation is retained by ion–dipole interactions with the oxygen atoms of the inwardly turned carbonyl groups. Under these conditions, the cation is effectively screened from interaction with the solvent and with the anion by the side groups of the amino acid and hydroxy acid residues and by the "bracelet" system of IMHBs. An important property of the valinomycin complexes is the hydrophobic nature of their molecular surface, leading to a high solubility of the complexes in neutral organic solvents. In the complexes with K^+, Rb^+, and Cs^+, the cation is located in the center of the cavity, but on reaction with Na^+, in view of its small size, the cation is displaced from the center of the cavity to its edges. The increase in the size of the cation on passing from K^+ to Rb^+ and Cs^+ is accompanied by an increase in the diameter of the internal cavity through an elongation of the H bonds and a lowering of their energy.

The results obtained show the high sensitivity of the spatial structure of valinomycin to the external conditions and enable the causes of the unique efficiency and specificity of its complex formation to be understood. The results of this work may be regarded as basic for a study of the molecular mechanism of the biological action of valinomycin. Furthermore, they permit the directed search for new analogs of valinomycin with given properties to be approached on a qualitatively new level.

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