RELATIONSHIP BETWEEN THE STRUCTURE AND PROPERTIES OF CYCLODEPSIPEPTIDES OF THE VALINOMYCIN SERIES

I. TOPOCHEMICAL ANALOGS

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We have previously performed the detailed physicochemical investigation of the depsipeptide antibiotic valinomycin (I) [1-3], the main representative of a group of biologically active compounds ("ionophores") that form stable complexes with alkali-metal ions and induce the permeability of biological and artificial membranes with respect to these ions. The conformational states of valinomycin in various media and on complex formation with alkali-metal cations have been established. The results obtained showed a high sensitivity of the spatial structure of valinomycin to the external conditions and enabled the causes of the high efficiency and the unique K/Na selectivity of complex formation to be discovered. In order to develop these investigations, it appeared desirable to study the influence of a change in the primary structure of the antibiotic on the spatial structure of the analogs formed, the stability of their complexes, and their membrane activity. The characteristics found will permit a deeper understanding of the nature of the interactions responsible for the functioning of valinomycin.

The present paper gives the results of an investigation of topochemical analogs of valinomycin* (II)-(VII).

$[\underline{(\text{D-Val-L-Lac-L-Val-D-Hylv})_3}]$
(I), valinomycin

$[\underline{(\text{D-Val-D-Hylv-L-Val-L-Lac})_3}]$
(II), retrovalinomycin

$[\underline{(\text{D-Hylv-L-Ala-L-Hylv-D-Val})_3}]$
(III), pseudovalinomycin

$[\underline{(\text{D-Hylv-D-Val-L-Hylv-L-Ala})_3}]$
(IV), pseudoretrovalinomycin

$[\underline{(\text{L-Val-L-Hylv-D-Val-D-Lac})_3}]$
(V), retroenantiovalinomycin

$[\underline{(\text{L-Hylv-D-Ala-D-Hylv-L-Val})_3}]$
(VI), pseudoenantiovalinomycin

$[\underline{(\text{L-Hylv-L-Val-D-Hylv-D-Ala})_3}]$
(VII), pseudoretroenantiovalinomycin

*Analogs obtained by the modification of the initial molecule as a whole: by reversing the direction of acylation (retro analogs), by replacing amide links by ester links and ester links by amide links ("pseudo" analogs), by changing the configurations of all the asymmetric centers (enantio analogs), and also by combining these modifications [4].

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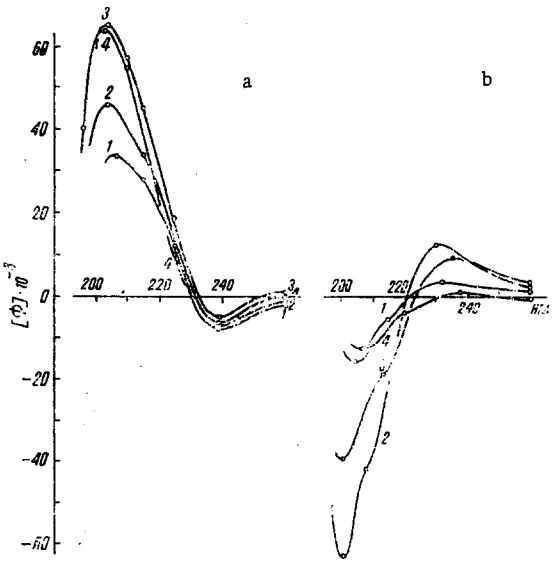


Fig. 1. ORD curves of retrovalinomycin (a) and pseudovalinomycin (b): 1a) heptane–dioxane (6 : 1); 1b) heptane–dioxane (5 : 1); 2) ethanol; 3) acetonitrile; 4) water–trifluoroethanol (2 : 1).

It has been shown previously that compounds (II, III, V, and VI), in contrast to valinomycin, do not form complexes with potassium ions in ethanolic solutions and have no appreciable effect on the permeability of biological and artificial membranes, nor do they possess antimicrobial activity [5]. On the other hand, compounds (IV) and (VII) give extremely stable potassium complexes with a high membrane and antimicrobial activity. To explain the reasons for such different behaviors of the analogs mentioned, we investigated the conformational states of compounds (II-IV). The conclusions obtained for them are, naturally, valid for the corresponding enantiomers (V-VII). During the work we used the methods and means developed in a study of the spatial structure of valinomycin [1-3].

Let us first consider compounds (II) and (III). Their ORD curves are given in Fig. 1, their IR spectra in Fig. 2, and their NMR spectra in Figs. 3-5 and in Tables 1 and 2. The ORD curves of these analogs in all solvents differ substantially in intensity, form, and position of the extrema from the corresponding curves for valinomycin (see Figs. 1 and 8a), which shows conformational rearrangements accompanying the changes in the primary structure of the antibiotic. In the IR spectra of compounds (II) and (III) taken in CCl_4 and $CHCl_3$, attention is attracted by the considerably greater intensity, as compared with valinomycin, of the bands of free NH groups (3400-3450 cm^{-1}) and the higher frequency of the band of the bound NH groups [3334 and 3376 cm^{-1} in (II) and 3330 cm^{-1} in (III) as compared with 3313 cm^{-1} in valinomycin (I)]. It follows from this that, in contrast to valinomycin, compounds (II) and (III) in nonpolar solvents lack conformations with strong intramolecular hydrogen bonds (intraHBs) and forms with free NH groups or NH groups forming weak intraHBs predominate.* The latter are easily destroyed on the addition of polar solvents, as can be seen from the extremely pronounced dependence of the chemical shifts of all the NH signals on the composition of the medium [concentration of $(CD_3)_2SO$ in $CDCl_3$, see Fig. 4]. The high values of $\Delta\delta/\Delta T$ for solutions in $(CD_3)_2SO$ ($6.0 \cdot 10^{-3}$ to $9.2 \cdot 10^{-3}$ ppm/deg, see Fig. 5) also show the absence of intraHBs under these conditions.

The differences noted between valinomycin and the analogs (II) and (III) are not unexpected, since on using such principles of the transformation of peptides and depsipeptides as the reversal of the direction of acylation or the replacement of CONH→COO by COO→CONH the system of intraHBs is unavoidably affected which, naturally, is reflected in the conformational characteristics and properties of the analogs formed.

*The considerably stronger magnetic shield of the NH protons of analogs (II) and (III) (δ = 6.35-6.87 ppm) as compared with valinomycin (δ = 7.78 and 7.88 ppm [3]) and of pseudoretrovalinomycin (δ = 7.75 and 7.78 ppm, see Table 1), is also apparently explained by their feeble participation in hydrogen bonds.

TABLE 1. Chemical Shifts (δ, ppm) of the Signals of the Protons of Compounds (II, III, and IV) and also of the K^+ Complex of (IV) in $CDCl_3$ (34°C) and of the NH Signals in $(CD_3)_2SO$ (40°C)

Compound	Fragment											
	$(CH_3)_2CH-$	CH_3-CH-	$CH(CH_3)_2$ | $-HN-CH-$	$CH(CH_3)_2$ | $-O-CH-$	$CH_3-O-CH-$	$CH_2-NH-CH-$	$CH(CH_3)_2$ | $-NH-CH-$	CH_3 | $-NH-CH-$		$CH(CH_3)_2$ | $-NH-CH-$	
								$CDCl_3$	$(CD_3)_2SO$	$CDCl_3$	$(CD_3)_2SO$
Retrovalinomycin (II)	~0,94	1,50	~2,26	5,00	4,96	—	4,45; 4,54	—	—	6,49; 6,87	8,11; 7,95
Pseudovalinomycin (III)	~0,94	1,43	~2,24	4,91; 4,96	—	4,58	4,47	6,79	8,01 or 8,04	6,35	8,04 or 8,01
Pseudoretrovalinomycin (IV)	~0,94	1,39	~2,30	5,01; 5,14	—	4,37	4,03	7,75	8,07	7,78	7,94
K^+ complex of pseudoretrovalinomycin	~0,96	1,42	~2,21	4,57; 4,57	—	4,25	3,78	8,18	—	8,16	—

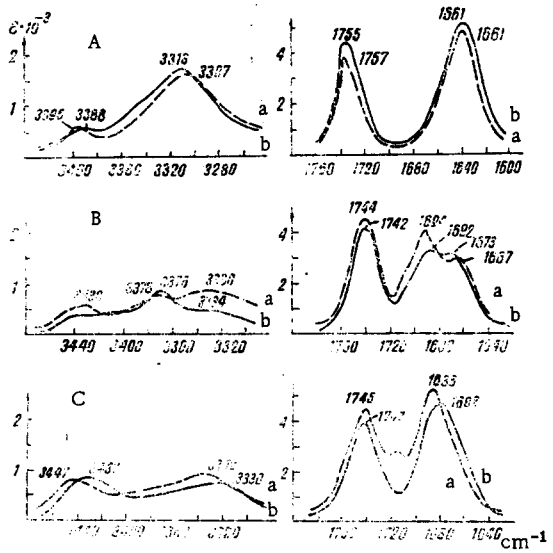


Fig. 2. IR spectra of valinomycin (A), retrovalinomycin (B) and pseudovalinomycin (C) in CCl_4 (a) and $CHCl_3$ (b).

For example, if in the initial peptide (A) a CO group forms an intraHB with an NH group located at a definite distance in the direction of acylation, in the retro isomer (B) the conditions for the formation of an intraHB between the same groups change. In particular, the distance between them along the chain increases. Although under these conditions the possibility appears of the formation of an intraHB having similar geometric parameters with an NH group which, in the initial compound (A), was located the same number of amino-acid residues away contrary to the direction of acylation, in the general case (different R's) this new intraHB is nonequivalent to the initial one because of the different surroundings. So far as concerns substitutions of the type COO⇌CONH, in spite of the stereoelectronic similarity of amide and ester groups, which is expressed in the closeness of their valence angles, the lengths of the bonds, and the distribution of the charges on the atoms (see, for example, [7]), there are also substantial differences between these two groups: the main one is the absence from ester groups of hydrogen atoms capable of acting as electron acceptors for the formation of H bonds. Consequently, in the substitutions CONH→COO the H bonds in which the NH groups participate are destroyed and, conversely, in the substitutions COO→CONH the possibility of the formation of new H bonds appears. It follows from what has been said that conformational analysis has an important place in investigations of the properties of biologically active peptides and their topochemical analogs. This hypothesis has been confirmed completely in the course of the present work.

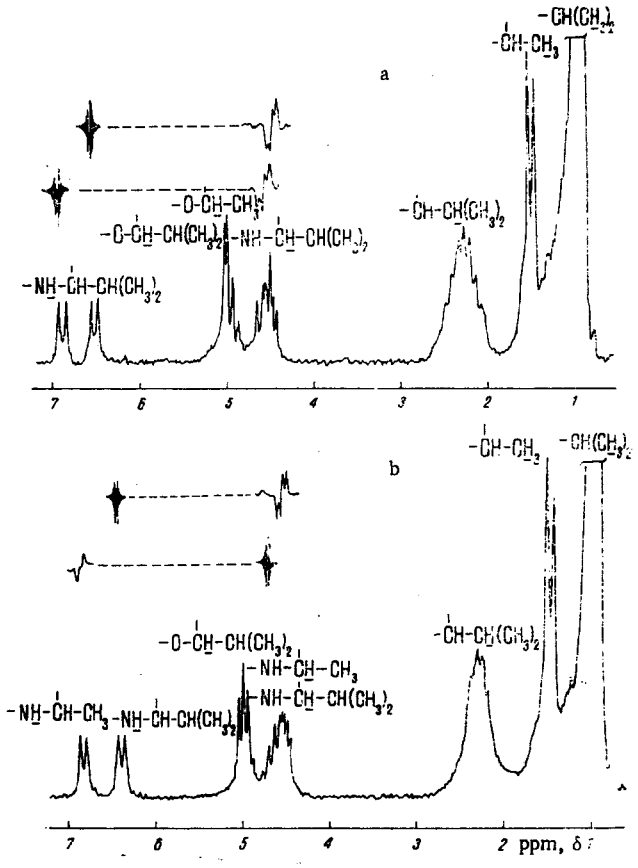
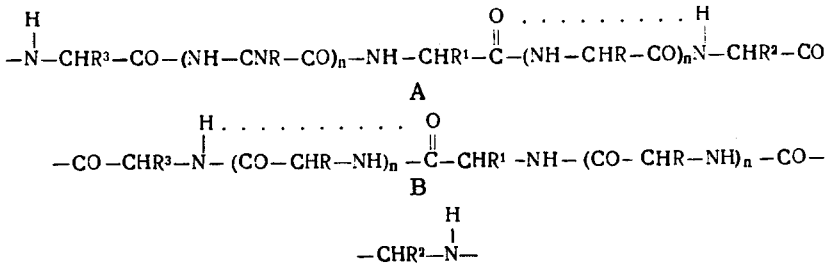


Fig. 3. NMR spectra of retrovalinomycin (II) (a), and pseudovalinomycin (III) (b) in $CDCl_3$ (34°C). In the top part of this figure, and also in Figs. 3b, 11, and 12, are given the INDOR spectra, which show spin-spin coupling of the protons of the NH–CH groups.



By analogy with valinomycin, for compounds (II) and (III) in pyridine it is possible to imagine the formation of a closed system of intraHBs of the 4→1 type formed by the amide CO and NH groups and stabilizing six condensed ten-membered rings (so-called "bracelet" [1-3] or "cylindrical" [8] conformation); the fragments of such structures are shown in Fig. 6. However, while in valinomycin the ten-membered rings are formed by amino- and hydroxy-acid residues with different configurations (D-Val and L-Lac, L-Val and D-HyIv), in the analogs (II) and (III) residues of the same configuration are included in the ring [D-Val and D-HyIv, L-Val and L-Lac in (II); D-Val and D-HyIv, L-Ala and L-HyIv in (III)]. As will be shown below, this, at first sight, insignificant difference leads to a marked increase in the energy of the system which completely destabilizes the bracelet type of conformation in compounds (II) and (III).

Formally, eight variants of bracelet conformations are possible for the analogs considered (Tables 4 and 5), these being analogous to those considered previously [3] for valinomycin. In an evaluation of the relative energies of these forms we made use of the calculation figures [9] for a series of compounds modeling the amino- and hydroxy-acid residues of the valinomycin depsipeptides (Fig. 7, Table 3). It can be

TABLE 2. Vicinal Spin-Spin Coupling Constants (^3J, Hz*) of the Protons of Compounds (II, III, and IV) and also of the K^+ Complex of (IV) in $CDCl_3$ (34°C) and of the Protons in the NH–CH Fragments in $(CD_3)_2SO$ (40°C)

Compound	Fragments						
	$CH_3-\underline{NH}-\underline{CH}-$	$CH(CH_3)_2-O-\underline{CH}-$	$CH(CH_3)_2-\underline{NH}-\underline{CH}-$	CH_3 $-\underline{NH}-\underline{CH}-$		$CH(CH_3)_2$ $-\underline{NH}-\underline{CH}-$	
				$CDCl_3$	$(CD_3)_2SO$	$CDCl_3$	$(CD_3)_2SO$
Retrovalinomycin (II)	7,5	3,1	4,0; 5,7	—	—	7,5 (8,2); 8,7 (9,5)	8,8 (9,6); 9,0 (9,8)
Pseudovalinomycin (III)	7,0	5,0; 5,0	4,8	7,2 (7,8)	7,9 (8,6) or 6,4 (7,0)	7,4 (8,1)	6,4 (7,0) 7,9 (8,6)
Pseudoretrovalinomycin (IV)	7,0	3,2; 3,8	9,8	6,4 (7,0)	6,6 (7,2)	7,6 (8,3)	7,1 (7,7)
K^+ complex of pseudoretrovalinomycin (IV)	7,8	3,6; 3,6	10,8	4,8 (5,2)	—	4,3 (4,7)	—

*In parentheses are given the values of $^3J_{NH-CH}$ corrected for the electronegativity of the substituents [6].

TABLE 3. Relative Energies of the Optimum conformations of Model Compounds, kcal/mole [9]

Compound	Conformation			
	r	*l*	*b*	*p*
Ac-L-Lac-NHMe	0	4,5	0,1	5,9
Ac-L-Hyiv-NHMe	0,1	8	0	14
Ac-L-Ala-OMe	0	1,2	0,2	1,0
Ac-L-Val-OMe	0	1,9	0,1	1,9

seen from Tables 4 and 5 that from the point of view of the short-range interaction energy conformations of type A_1, $[\overline{(r-b-l-p)_3}]$ are the most suitable for compounds (II) and (III).* After these, considerably inferior from the energy point of view, come the A_2 conformations $[\overline{(p-r-b-l)_3}]$ and, for compound (II) also A_1 $[\overline{(b-l-l-p)_3}]$; the remaining structures have very high energies and are therefore unlikely. An analysis of molecular models shows that in the conformations of type A_1 of compounds (II) and (III) all 12 side chains are oriented in the direction of the axis of symmetry, which leads to serious steric hindrance, to a weakening of the intraHBs, and to a sharp increase in the energy of the system. So far as concerns the form A_2, $[\overline{(p-r-b-l)_3}]$, in which all the ester carbonyl groups are oriented within the molecule as has been shown for the case of valinomycin, in the absence of cations such conformations are destabilized as the result of the transannular electrostatic interaction of the negatively charged oxygen atoms. Thus, steric or electrostatic interactions make the formation of the bracelet system of intraHBs impossible for compounds (II) and (III) in solutions.

A comparison of the A_2, "all intra" structure of valinomycin [3] and of the analogs (II) and (III) (Tables 4 and 5) shows that the latter are less stable because of steric interactions by 9-12 kcal/mole, i.e., by a magnitude exceeding the free energy of formation of the K^+ complex of valinomycin in ethanol (8.6 kcal/mole [2]). This explains the incapability of the analogs (II) and (III) for complexing potassium ions.

The relatively (in comparison with valinomycin) high optical activity (see Figs. 1 and 8) and similarity of the ORD curves (isolated extrema at 230-240, ~215, and 205 nm, see Fig. 1) permit the assumption that

*Structures A_1 and A_2 have been described in the previous paper [3]; the letters b, *l*, p, and r show the region of realization of the conformational parameters Φ and Ψ [10] corresponding to the amino- and hydroxy-acid fragments (see Fig. 7); the bracket at the top denotes an intraHB.

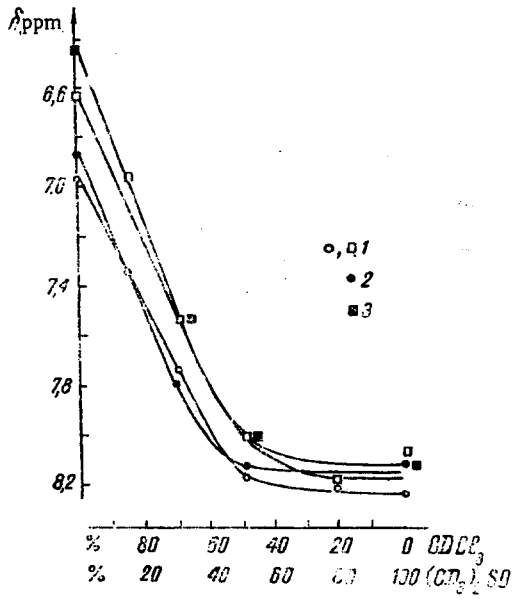


Fig. 4

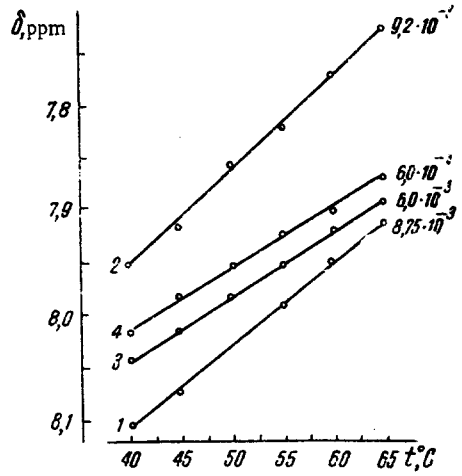


Fig. 5

Fig. 4. Dependence of the chemical shifts (δ) of the NH signals on the composition of $CDCl_3-(CD_3)_2SO$ mixtures: 1) valine NH groups of retrovalinomycin; 2) alanine NH groups of pseudovalinomycin: 3) valine NH groups of pseudovalinomycin.

Fig. 5. Dependence of the chemical shifts (δ) of the NH signals on the temperature in $(CD_3)_2SO$: 1, 2) retrovalinomycin; 3, 4) pseudovalinomycin.

in solvents of moderate polarity (such as ethanol) similar conformations predominate in the case of retrovalinomycin (II) and pseudovalinomycin (III) [more accurately: retrovalinomycin (II) and pseudoenantiovalinomycin (VI), taking into account the opposite signs of the Cotton effects in the curves of the analogs (II) and (III)], the parameters of these conformations, in view of the absence of intraHBs, being determined by nonvalence and electrostatic interactions. A considerable proportion of the conformers of this type apparently also exists in nonpolar solvents [judging from the retention of the usual form of the ORD curves and also from the high values of the dipole moments (7.9 and 8.8 D, respectively) of compounds (II) and (III) in chloroform]. The comparatively low values of the spin-spin coupling constants of the $C^{\alpha}H-C^{\beta}H$ protons of the valine residues of compounds (II) and (III) (4.0, 4.8, and 5.7 Hz, see Table 2*) show the preferential formation of the gauche rotamers, the formation of which, as has been reported previously [3], is connected with steric hindrance in the region of the valine isopropyl groups. Together with the high optical activity, this result forms one more argument in favor of the existence of specific forms of compounds (II) and (III) in solutions, since in unordered conformations the appearance of these interactions is unlikely. The values of the constants $^3J_{NHCH}$ in $CDCl_3$ and $(CD_3)_2SO$ [8.2-9.8 Hz for (II) and 7.0-8.6 Hz for (III), see Table 2] agree with the formation in the L-amino-acid residues of the most favorable conformations of type b or r (Φ −130 to −80°) (Fig. 7a, c) and in the D residues, respectively, *l* or p (Φ 80 to 130°) (Fig. 7b). Since compounds (II) and (III) possess no biological activity, no more detailed analysis of their structures was performed.

So far as concerns pseudoretrovalinomycin (IV), in spite of the fairly complex transformations of the initial molecule of valinomycin, this compound proved to be more similar in its properties to valinomycin than compounds (II) and (III). As can be seen in Fig. 6, in order to pass from valinomycin to pseudoretrovalinomycin it is sufficient merely for the methyl groups of the L-lactic acid residues and the isopropyl groups of the L-valine residues to change places (3 L-Lac →3 L-HyIv, 3 L-Val →3 L-Ala). If the monotypical nature of the conformational maps of the corresponding model compounds is considered (Fig. 7a and c, d and e), the far-reaching similarity of the behavior of valinomycin and its analog (IV) and, in the first place, the stability of the K^+ complexes, and also the high membrane and antimicrobial activity, become understandable.

*In the text, the values of the constants $^3J_{NH-CH}$ taking into account the corrections for the electronegativity of the substituents are given.

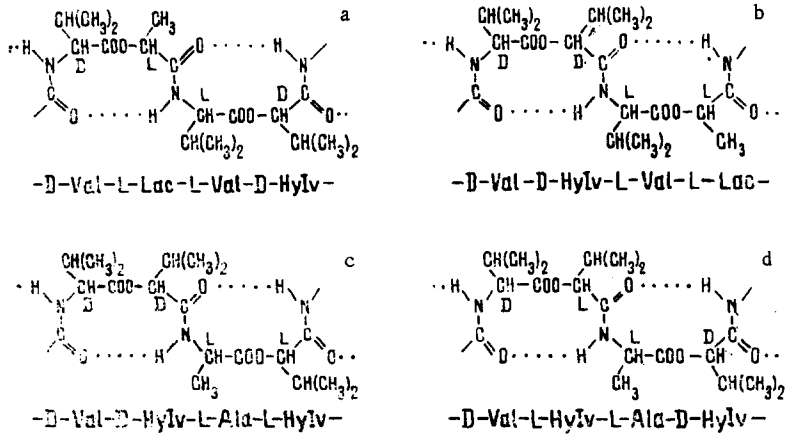


Fig. 6. Fragments of the bracelet system of valinomycin (a), retrovalinomycin (b), pseudovalinomycin (c) and pseudoretrovalinomycin (d).

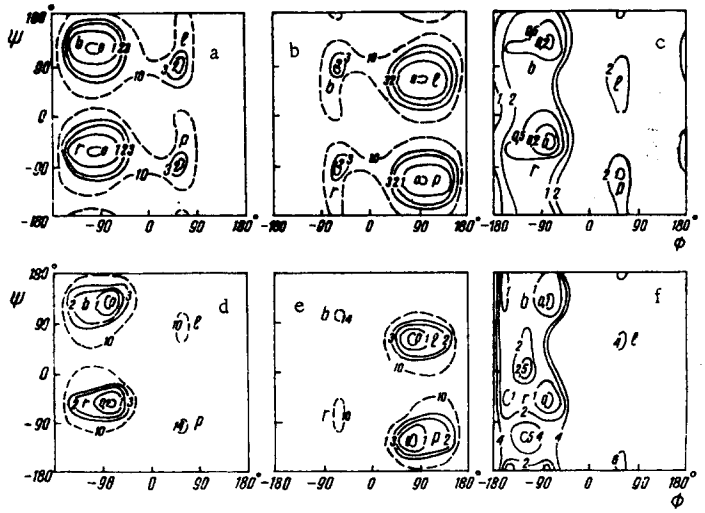


Fig. 7. Conformational maps of derivatives of amino-acid (a-c) and hydroxy-acid (d-f) derivatives modeling fragments of compounds (II-IV): a) Ac-L-Val-OMe; b) Ac-D-Val-OMe; c) Ac-L-Ala-OMe; d) Ac-L-HyIv-NHMe; e) Ac-D-HyIv-NHMe; f) Ac-L-Lac-NHMe.

The conformational states of pseudoretrovalinomycin (IV) and its K^+ complex were investigated by means of ORD curves (see Fig. 8) and IR (Fig. 9) and NMR (Figs. 10-12, Tables 1 and 2) spectra. As in the case of valinomycin, the spatial structure of the analog (IV) is extremely sensitive to the surrounding medium and changes markedly on passing from nonpolar to polar solvents (judging, for example, from the ORD curves). The IR spectra of the analog (IV) in CCl_4 and $CHCl_3$ are extremely similar to the spectra of valinomycin taken under the same conditions (compare Figs. 2 and 9) and show the predominance of bracelet conformations. The $^3J_{NH-CH}$ constants found from the NMR spectra (7.0 and 8.3 Hz, Table 2) show an orientation intermediate between the cis and the gauche orientations of the NH–C$^\alpha$H fragments, i.e., the realization of the form A_1 (see [3]); as also in valinomycin, it must be assigned the energetically most favorable conformation of the $[\overline{(r-b-l-p)_3}]$ type. The higher dipole moment of the analog (IV) in CCl_4 than of valinomycin (6.0 D as compared with 3.5 D [1, 3]) is probably due to greater differences in the orientation with respect to the axis of symmetry of the carbonyl groups of the alanine and valine residues in the analog (IV) than of the L- and D-valine carbonyl groups in valinomycin.

TABLE 4. Relative Energies and Stereochemical Characteristics of the Bracelet Forms of Retrovalinomycin (II)

Type	Conformation	Orientation of the ester CO groups		Orientation of the side groups	Relative energy, kcal/mole
		L-Val	D-Val		
A_1	$[(\overline{r-b-l-p})_3]$	Outwards	Outwards	All inwards	0,3
	$[(\overline{b-l-l-p})_3]$	Inwards	"	" "	13,8
	$[(\overline{r-b-p-r})_3]$	Outwards	Inwards	" "	24,6
	$[(\overline{b-l-p-r})_3]$	Inwards	"	" "	38,1
A_2	$[(\overline{l-p-r-b})_3]$	Outwards	Outwards	All outwards	71,1
	$[(\overline{p-r-r-b})_3]$	Inwards	"	" "	53,4
	$[(\overline{l-p-b-l})_3]$	Outwards	Inwards	" "	29,4
	$[(\overline{p-r-b-l})_3]$	Inwards	"	" "	11,7

TABLE 5. Relative Energies and Stereochemical Characteristics of the Bracelet Forms of Pseudovalinomycin (III)

Type	Conformation	Orientation of the ester CO groups		Orientation of the side groups	Relative energy, kcal/mole
		L-Ala	D-Val		
A_1	$[(\overline{r-b-l-p})_3]$	Outwards	Outwards	All inwards	0
	$[(\overline{b-l-l-p})_3]$	Inwards	"	" "	24,6
	$[(\overline{r-b-p-r})_3]$	Outwards	Inwards	" "	24,3
	$[(\overline{b-l-p-r})_3]$	Inwards	"	" "	48,9
A_2	$[(\overline{l-p-r-b})_3]$	Outwards	Outwards	All outwards	93,3
	$[(\overline{p-r-r-b})_3]$	Inwards	"	" "	51
	$[(\overline{l-p-b-l})_3]$	Outwards	Inwards	" "	51,6
	$[(\overline{p-r-b-l})_3]$	Inwards	"	" "	9,3

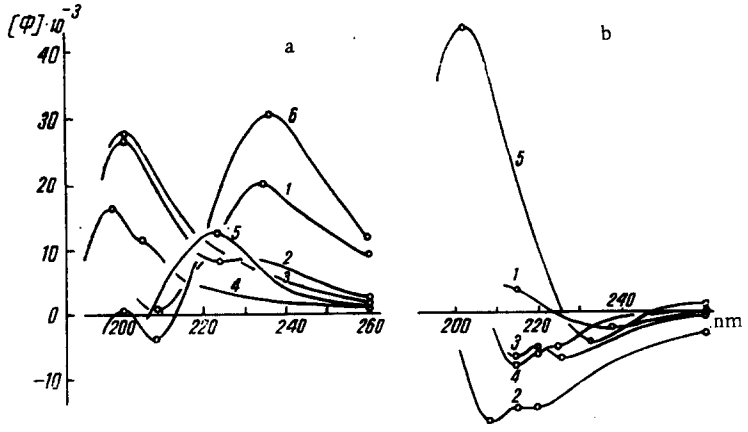


Fig. 8. ORD curves of valinomycin (1a-4a and 6a), pseudoretrovalinomycin (1b-4b), and their K^+ complexes (5a and 5b): 1) heptane–dioxane (10:1); 2) ethanol; 3) acetonitrile; 4) water–tetrafluoroethanol (2:1); 5) $1 \cdot 10^{-2}$ M KCl in ethanol; 6) heptane.

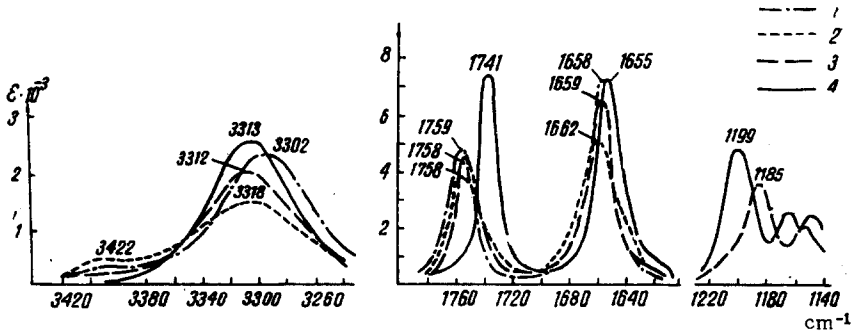


Fig. 9. IR spectra of pseudoretrovalinomycin in CCl_4 (1), $CHCl_3$ (2), CCl_4-CH_3CN (2 : 1) (3), and of its K^+ complex in CCl_4-CH_3CN (2 : 1) (4).

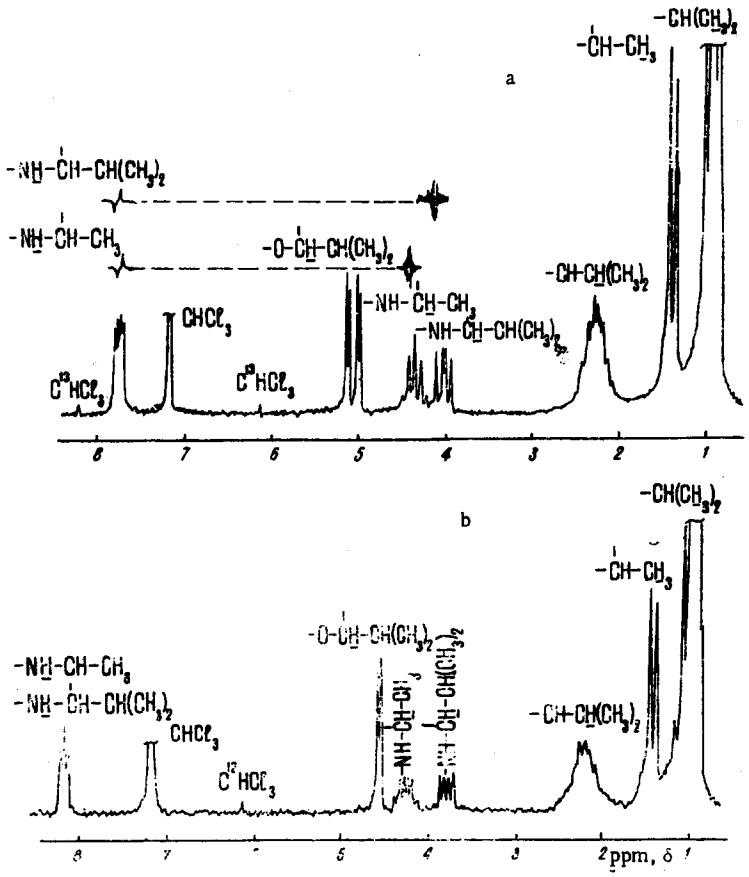


Fig. 10. NMR spectra of pseudoretrovalinomycin (a) in $CDCl_3$ (34°C) and of the K^+ complex of pseudoretrovalinomycin (b) in $CDCl_3$.

The K^+ complex of pseudoretrovalinomycin scarcely differs from the valinomycin complex with respect to the parameters Φ and Ψ, since it retains the system of intraHBs, the ester groups interact with the cation to the same extent [symmetrical band of the stretching vibrations of ester CO groups at 1741 cm^{-1} in the IR spectra (see Fig. 9)], and the values of $^3J_{NH-CH}$ (4.7 and 5.2 Hz, see Table 2) are close to the corresponding constants found for the K^+ complex of valinomycin (5.2-5.6 Hz [1, 3]).

However, in spite of the general features mentioned, a more detailed physicochemical investigation of the analog (IV) showed a number of significant differences between it and valinomycin which have been responsible for the fruitfulness of its use in the study of the mechanism of the functioning of cyclodepsipeptides of the valinomycin group on membranes [11]. In particular, a characteristic property of valino-

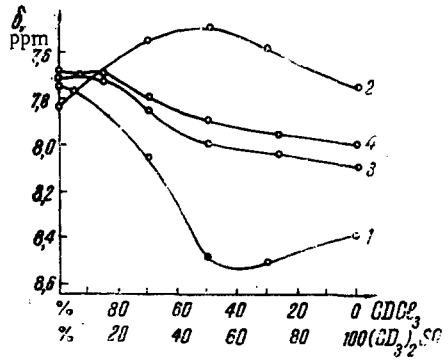


Fig. 11. Dependence of the chemical shifts (δ) of the NH protons of valinomycin (1, 2 – the L- and D-valine NH groups, respectively) and pseudoretrovalinomycin (3, 4 – the alanine and valine NH groups, respectively) on the composition of mixtures of $CDCl_3$ and $(CD_3)_2SO$.

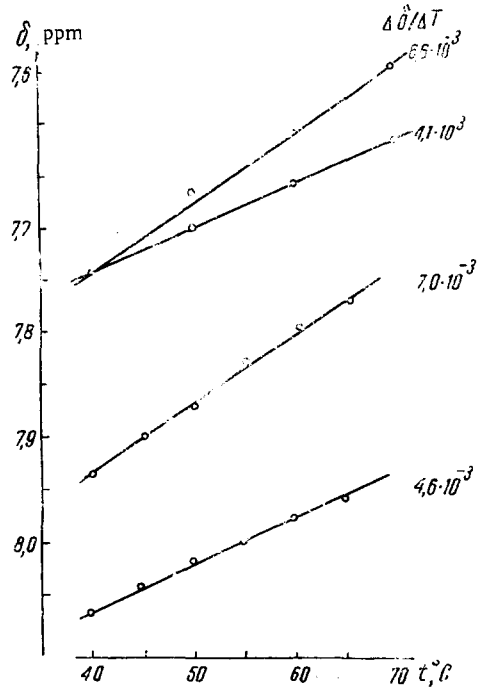


Fig. 12. Dependence of the chemical shifts (δ) of the signals of the NH protons of pseudoretrovalinomycin on the temperature in $CDCl_3-(CD_3)_2SO$ (85 : 15) [1) Ala, 2) Val] and in $(CD_3)_2SO$ [2) Ala, 4) Val].

mycin is its capacity for adopting, over a wide range of conditions, the "propeller" conformation (form B) stabilized by three intraHBs [3]; the conclusion that this exists is based to a considerable extent on the sharp differentiation of the signals from the protons of the NH groups with respect to their chemical shifts, the temperature dependences of the chemical shifts, and the $^3J_{NH-CH}$ constants in mixtures of CCl_4 and $(CD_3)_2SO$ [5] and of $CDCl_3-(CD_3)_2SO$ (see Fig. 11). In the case of the analog (IV), however, the addition of $(CD_3)_2SO$ to its solution in $CDCl_3$ leads only to a gradual and approximately uniform cleavage of the intraHBs, judging from the closeness of the values of δ_{NH}, $\Delta\delta/\Delta T$, and $^3J_{NH-CH}$ for the L-Ala and D-Val residues in all the solvent ratios studied (see Figs. 11 and 12 and Table 2). The absence of specific conformations of compound (IV) in solvents of medium polarity is also in harmony with the considerably weaker, as compared with valinomycin, optical activity of the analog in ethanol and acetonitrile (see Fig. 8). Apparently, for the realization of propeller conformations in cyclodepsipeptides of the valinomycin group the presence of lactic acid residues is essential, and their replacement by α-hydroxyisovaleric acid residues, the voluminous isopropyl groups of which prove to be oriented within the molecule, leads to steric hindrance and to the destabilization of the B form. If it is assumed that the high surface activity of valinomycin is connected with structural features of form B (the presence of a hydrophobic nucleus surrounded by polar groups), the reduced stability of monolayers of the analog (IV) at a boundary of separation between air and water and its lower tendency to penetrate into condensed lecithin monolayers become understandable.

It is known [3] that a distinguishing feature of the molecular structure of the K^+ complex of valinomycin is the effective screening of the central cation from interaction with the solvent, and in this an important role is played by the valine isopropyl groups hanging over the aperture of the "cylinder" formed by the depsipeptide skeleton of the antibiotic (Fig. 13). A fundamentally different picture is observed for the K^+ complex of compound (IV): in the top of the complex above the cation instead of the valine isopropyl groups the alanine methyl groups, which are inferior to them in their shielding capacity, hang over the aperture. As a result of this, the prerequisites are created for ion-dipole interaction of the cation located in the central cavity with the solvents. The energy of this interaction apparently ensures the increased stability of the K^+ complex of the analog (IV) in comparison with valinomycin in monolayers and solutions, and also a tendency to an increase in the difference between the free energies of complex formation of the analog (IV) and of valinomycin with an increase in the polarity of the medium. The asymmetric solvation of the cation also explains the high optical activity of the analog (IV) (see Fig. 8). These facts permit the expectation for it of a higher, as compared with valinomycin, effective dipole moment in monolayers and also a higher surface activity; the latter hypothesis is in harmony with the results of an analysis of the conductivity of phospholipid two-layer membranes modified by these depsipeptides [11].

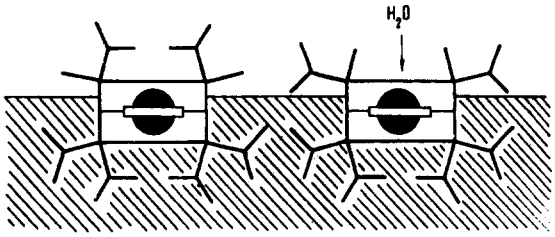


Fig. 13. Schematic illustration of the K^+ complex of valinomycin (on the left) and of pseudoretrovalinomycin (on the right) located at the surface of a membrane.

Thus, we have performed for the first time an analysis of the relationship between structure and function in a number of analogs of a biologically active compound of peptide nature based on a detailed investigation of their conformational parameters. The results obtained show the possibility of the directed synthesis of new valinomycin analogs with given physicochemical and biological properties, and also the promising nature of the topochemical principle of the transformation of peptide molecules.

EXPERIMENTAL

Before the physicochemical measurements, compounds (II-IV) were dried over P_2O_5 at 0.5 mm Hg and 50°C for 10 h. The ORD curves were measured on a Cary-60 spectropolarimeter using solutions with concentrations of $\sim 2 \cdot 10^{-3}$ M at a temperature of 23-26°C with a cell thickness of 0.01-1 cm. The IR spectra were recorded on a UR-10 instrument. In measurements in CCl_4 the cell thickness was 10 mm and the concentration of the solutions $(3\text{-}5) \cdot 10^{-4}$ M, in $CHCl_3$ 20 mm and $\sim 1 \cdot 10^{-4}$ M, and in CCl_4–CH_3CN (2 : 1) 2 mm and $(0.5\text{-}1) \cdot 10^{-3}$ M. To obtain the K^+ complex of (IV), a threefold excess of KNCS was added to the solution. The dielectric constants were measured on a Dipol' instrument working on the beat principle at a frequency of 1 MHz. The dipole moments were calculated by Hedestrand's method (see [12]).

The ^1H NMR spectra were measured on a Varian HA-100D instrument at concentrations of the solution of ~ 0.05 M. 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. The assignment of the signals of the protons with appreciable spin-spin coupling was performed by the INDOR method (see Figs. 3, 4, and 10) [13]. To obtain a solution of the K^+ complex of pseudoretrovalinomycin in $CDCl_3$, a weighed sample of the cyclodepsipeptide was dissolved in methanol containing two equivalents of KCl, the solution was evaporated to dryness, and the residue was treated with $CDCl_3$.

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SUMMARY

1. By means of physical methods (IR and NMR spectra, ORD curves, dipole moments) and a theoretical conformational analysis, a study has been made of the conformational states of three topochemical analogs of valinomycin: cyclo-(D-Val-D-HyIv-L-Val-L-Lac)$_3$ (II), cyclo-(D-HyIv-L-Ala-L-HyIv-D-Val)$_3$ (III), and cyclo-(D-HyIv-D-Val-L-HyIv-L-Ala)$_3$ (IV).

2. In compounds (II) and (III) steric interactions prevent the formation of "bracelet" conformations and the formation of complexes with alkali-metal ions.

3. In nonpolar media, compound (IV) assumes a "bracelet" conformation similar to that of valinomycin.

4. In view of steric hindrance, compound (IV) is incapable of adopting the "propeller" conformation that is characteristic for valinomycin, which leads to a reduced surface activity.

5. The conformation of the depsipeptide chain of the K^+ complex of compound (IV) is similar to the conformation of the K^+ complex of valinomycin. However, in it the cation is more feebly shielded from interaction with the solvent, which explains the higher stability and surface activity of the complex.

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