RELATIONSHIP BETWEEN STRUCTURE, STABILITY OF POTASSIUM COMPLEXES, AND ANTIMICROBIAL ACTIVITY IN A SERIES OF ANALOGS OF VALINOMYCIN

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As has been shown previously [1-3], the macrocyclic cyclodepsipeptides (CDPs), which increase the cationic permeability of artificial and biological membranes, form complexes with alkali-metal ions. Such compounds include, in particular, the antibiotic valinomycin (compound 1, Table 1), which has found wide use in the study of various biochemical processes connected with the transport of ions through membranes. The functioning of valinomycin in membranes is determined to a considerable extent by conformational factors [1-3]. In a detailed physicochemical investigation of valinomycin in solutions, the presence of a complex conformational equilibrium in it which is displaced as a function of the properties of the medium and the presence of alkali-metal ions was established [3, 4]. In developing these investigations, it was desirable to continue work begun previously on the influence of structural features of the analogs of valinomycin on their conformation and, as a consequence, on their capacity for complex formation [2, 3], and also their membrane and biological activities [5, 6]. The present paper considers the question of the stability of the complexes of 41 analogs of valinomycin with potassium and sodium ions, and also the question of the antimicrobial activity of these analogs.

The stability constants of the complexes and the free energies of complex formation of the analogs of valinomycin (compounds 2-42) derived from them can be judged from the figures of Table 1, and their antimicrobial activities can be seen from Table 2.

We have investigated rings having 12 members (compounds 2-5), 24 members (compounds 6 and 7), 48 members (compound 8), and 36 members (compounds 9-42). The latter differ from valinomycin by the fact that individual amide bonds in them were replaced by ester bonds (compound 9), and N-methylamide and ester bonds were replaced by amide bonds (compounds 10 and 11 and compounds 12-15, respectively). In addition, compounds with a changed configuration of the individual hydroxy- and amino-acid residues (compounds 16-22) and with changed side chains of these residues (compounds 23-24), and also analogs of the meso compound 32 differing in the configuration of the valine and α-hydroxyisovaleric acid residues (35-42) were studied. Not one of the analogs considered, apart from 14 and 15, reacted with sodium ions in a manner detectable by the conductometric method.

As shown previously [2, 5], and as follows from Tables 1 and 2, for the formation by a compound of stable complexes with alkali-metal ions, and also for their display of antimicrobial activity a large role is played by the size of the CDPs. Compounds forming the most stable complexes with K^+ and exerting the strongest antimicrobial action have a 36-membered ring (for example, compounds 13-15, 23, 26). CDPs with 12- and 24-membered rings (compounds 2-7) have neither the capacity for forming stable complexes with potassion ions nor antibiotic activity. A CDP with a 48-membered ring (compound 8) shows a feeble complex-forming capacity and possesses no antimicrobial action. Among the 36-membered analogs of

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TABLE 1. Stability Constants of the K^+ Complexes of Valinomycin Analogs and the Free Energies of Their Formation*

Compound No.	Compound	Stab. const. of K^+ compl. (K, l/mole, E-OH, 25°C)	Free energy of complex-form. with K^+ ($-\Delta f = RT \ln K$, kcal/mole)
1	[(D.Val-L.Lac-L.Val-D.HyIv)$_3$] [9]	2 200 000	8.6
2	[(D.Val-L.HyIv-L.Val-D.HyIv)] [6]	<50	<2.3
3	[(D.Val-L.Lac-L.Val-L.Lac)] [6]	<50	<2.3
4	[(D.Val-L.Lac-L.Val-L.HyIv)] [6]	<50	<2.3
5	[(L.Val-L.HyIv-L.Val-L.HyIv)] [6]	<50	<2.3
6	[(D.Val-L.HyIv-L.Val-D.HyIv)$_2$] [6]	<50	<2.3
7	[(D.Val-L.Lac-L.Val-D.HyIv)$_2$] [10]	<50	<2.3
8	[(D.Val-L.Lac-L.Val-D.HyIv)$_4$] [6]	50—100	2.3—2.7
9	[(D.Val-L.Lac-L.HyIv-D.HyIv)(A)$_2$]† [11]	2500	4.7
10	[(D.Val-L.Lac-L.N-MeVal-D.HyIv)(A)$_2$] [11]	<50	<2.3
11	[(D.N-MeVal-L.Lac-L.N-MeVal-D.HyIv)$_3$] [12]	<50	<2.3
12	[(D.Val-L.Lac-L.Val-D.Val)(A)$_2$] [11]	5200	5.1
13	[(D.Val-L.Val-L.Val-D.HyIv)(A)$_2$] [11]	100 000	6.9
14	[(D.Val-L.Ala-L.Val-D.HyIv)(A)$_2$] [11]	300 000	7.5
15	[(D.Val-L.Ala-L.Val-D.HyIv)$_2$(A)] [11]	220 000	7.3
16	[(L.Val-L.Lac-L.Val-D.HyIv)(A)$_2$] [6]	11 000	5.5
17	[(D.Val-L.Lac-D.Val-D.HyIv)(A)$_2$] [6]	4400	5
18	[(D.Val-L.Lac-D.Val-D.HyIv)$_3$] [6]	<50	<2.3
19	[(L.Val-L.Lac-L.Val-D.HyIv)$_3$] [6]	<50	<2.3
20	[(D.Val-D.Lac-L.Val-D.HyIv)(A)$_2$] [6]	75 000	6.7
21	[(D.Val-L.Lac-L.Val-L.HyIv)(A)$_2$] [6]	100—150	2.7
22	[(D.Val-L.Lac-L.Val-L.HyIv)$_3$] [6]	<50	<2.3
23	[(D.Ala-L.Lac-L.Val-D.HyIv)(A)$_2$] [6]	2 000 000	8.6
24	[(D.Ala-L.Lac-L.Val-D.HyIv)$_2$(A)] [11]	88 000	6.8
25	[(D.Ala-L.Lac-L.Val-D.HyIv)$_3$] [11]	160	3.0
26	[(D.Val-L.Lac-L.Ala-D.HyIv)(A)$_2$] [11]	3 000 000	8.9
27	[(D.Val-L.Lac-L.Ala-D.HyIv)$_2$(A)] [11]	200 000	7.3
28	[(D.Val-L.Lac-L.Ala-D.HyIv)$_3$] [11]	20 000	5.9
29	[(D.Leu-L.Lac-L.Leu-D.HyIv)(A)$_2$] [11]	130 000	7.0
30	[(D.Ala-L.Lac-L.Ala-D.HyIv)(A)$_2$] [6]	100 000	6.9
31	[(D.Val-L.HyIv-L.Val-D.HyIv)(A)$_2$] [6]	400 000	7.7
32	[(D.Val-L.HyIv-L.Val-D.HyIv)$_3$] [6]	370 000	7.6
33	[(D.Val-L.Lac-L.Val-D.Lac)$_3$] [11]	2 300 000	8.7
34	[(D.Ala-L.HyIv-L.Ala-D.HyIv)$_3$] [13]	220 000	7.3
35	[(L.Val-L.HyIv-L.Val-D.HyIv)(B)$_2$]‡ [13]	1 000 000	8.3
36	[(L.Val-L.HyIv-L.Val-D.HyIv)$_2$(B)] [13]	11 000	5.5
37	[(L.Val-L.HyIv-L.Val-D.HyIv)-(D.Val-L.HyIv-D.Val-D.HyIv)(B)] [13]	10 000	5.5
38	[(L.Val-L.HyIv-D.Val-D.HyIv)(B)$_2$] [13]	10 000	5.5
39	[(D.Val-L.HyIv-L.Val-L.HyIv)(B)$_2$] [13]	1 500	4.4
40	[(D.Val-L.HyIv-L.Val-L.HyIv)$_2$(B)] [13]	<50	<2.3
41	[(D.Val-L.HyIv-L.Val-L.HyIv)-(D.Val-D.HyIv-L.Val-D.HyIv)(B)] [13]	180	3.3
42	[(D.Val-D.HyIv-L.Val-L.HyIv)(B)$_2$] [13]	<50	<2.3

*The stability constants of the complexes of the cyclodepsipeptides with Na^+ and K^+ were determined by the conductometric method [7]. The stability constants of the Na^+ complexes of compounds 1-42 are lower than 50 liters/mole (with the exception of compound 14, for which K_{Na^+}=100 liters/mole, Δf=2.7 kcal/mole, and compound 15, which has K_{Na^+}=600 liters/mole, Δf=3.8 kcal/mole [8]).

†A = (D. Val-L. Lac-L. Val-D. HyIv).

‡B = (D. Val-L. HyIv-L. Val-D. HyIv).

TABLE 2. Antimicrobial Activity of the Valinomycin Analogs

Compound No.	Minimum concentration suppressing growth, γ/ml									
	Staph. aureus 209-R	Staph. aureus UV-3*	Strept. faecalis	Sarcina lutea	Bac. mycoides	Bac. subtilis	E. coli B.	Mycob. phlei	Candida albicans	Sacch. cerevisiae
1	>50	0.1—0.2	0.2—0.3	0.1—0.2	>50	>50	>50	0.3	0.2—0.4	0.2—0.3
2	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
3	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
5	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
6	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
7	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
8	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
9	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
10	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
11	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
12	>25	1	2	1.5	>25	>25	>25	1—2	2—3	3
13	>50	0.5—0.7	0.7	0.5—1	>50	>50	>50	0.4	10—12†	10—12†
14	4.5	0.1	0.3	0.1—0.2	>50	>50	>50	0.2—0.4	4.5	4
15	2—4	2	4—6	2	12	>50	>50	2	12—18	2
16	>50	2	3—4	1—2	>50	>50	>50	1—2	3—5	4.5
17	>50	1—2	4.5	1—2	>50	>50	>50	1—2	4.5	4.5
18	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
19	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
20	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
21	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
22	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
23	>50	0.1—0.2	0.2	0.1—0.2	>50	>50	>50	0.4	0.2—0.3	0.3
24	>50	0.5—0.7	0.7	0.7	>50	>50	>50	0.5—1	1—1.5	1.5
25	>50	18	37	37	>50	>50	>50	18	37	37
26	>50	0.3—0.5	0.3	0.1—0.2	>50	>50	>50	0,2	0.2—0.5	0.3—0.5
27	>50	0,5	1—1.5	0.1—0.2	>50	>50	>50	1	1.5—2	1.5—2
28	>50	1—1.5	4.5—6	2	>50	>50	>50	4.5	4.5	4.5
29	>50	2	3—4	1—1,5	>50	>50	>50	1—2	3—4	3—4
30	>50	2	3—4.5	1.5—2	>50	>50	>50	1.5	4	3—4
31	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
32	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
33‡	>25	2	>25	2—3	>25	>25	>25	>25	>25	>25
34	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
35	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
36	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
37	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
38	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
39	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
40	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
41	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
42	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25

* The Staph. aureus mutant UV-3 was obtained from Prof. F. F. Gauze of the Institute for the Search for New Antibiotics of the Academy of Medical Sciences of the USSR. In the opinion of American workers [14], this culture is a Corynebacterium sp.

† In this case, not suppression but retardation of the growth of the microbes was found.

‡ By the method described (see Experimental section) for determining antimicrobial activity it was possible to detect activity in compound 33, which was previously [2, 3, 5] considered to be inactive.

valinomycin, compound 9, obtained by replacing one of the amide groups by an ester group, gives a fairly stable complex with K^+, but this analog possesses no antimicrobial action. However, when one amide bond is replaced by a N-methylamide bond (compound 10), the compound proves to be quite incapable of forming a K^+ complex and has no biological activity. The completely N-methylated analog (11), of course, likewise is not a complexone for potassium ions and is biologically inactive. The valinomycin analogs obtained by replacing one residue of D-α-hydroxyisovaleric acid by a D-valine residue (compound 12), by replacing one L-lactic acid residue by a L-valine residue (compound 13) or a L-alanine residue (compound 14), or by replacing two L-lactic acid residues by two L-alanine residues (compound 15) retain their capacity for forming stable complexes with K^+ and exhibit a high antimicrobial activity while, as can be seen from Table 1, in contrast to the other analogs, compounds 14 and 15 form complexes not only with K^+ but also with Na^+ and have a broader spectrum of antimicrobial action (see Table 2).

So far as concerns the influence on the complex-forming capacity and antimicrobial action of the configuration of the amino- and hydroxy-acid residues, with a change in the valinomycin molecule (the configuration of one amino-acid residue) (compounds 16 and 17) the stability constant of the potassium complex falls by 2-3 orders of magnitude; the biological activity falls by a factor of approximately 10. Analogs containing valine residues with only the D or only the L configuration (compounds 18 and 19) were completely devoid of both these properties. A change in the configuration of the hydroxy-acid residues (compounds 20-22) also leads to a considerable fall in the stability of the complexes of these compounds with K^+; the change in the configuration of α-hydroxyisovaleric acid affects the stability of the potassium complex to a far greater extent than a change in the configuration of the lactic acid. For example, with a change in the configuration of one lactic acid residue (compound 20), the stability constants of the complex fall by only two orders of magnitude, while with a change in the configuration of one α-hydroxyisovaleric acid residue (compound 21) it falls by 4 orders of magnitude. With a change in the configuration of all three residues of α-hydroxyisovaleric acid (compound 22) the compound becomes completely devoid of complex-forming capacity. A change in the configuration of the hydroxy-acid residues is reflected to a still greater extent on the antimicrobial activity of the compound: a change in the configuration of both lactic acid and α-hydroxyisovaleric acid residues (compounds 20-22) leads to the complete loss of antibiotic activity.

All the valinomycin analogs obtained by replacing the alkyl radicals of the amino- and hydroxy-acid residues by related radicals of different volume (compounds 23-34) form complexes with K^+. The stability of many of them is of the same order as that of the valinomycin complex (compounds 23, 26 and 33) or 1-2 orders of magnitude lower (compounds 24, 26-34), and only compound 25, containing three D-alanine residues in place of three D-valine residues, forms a complex with a stability constant four orders of magnitude lower than the valinomycin complex. A study of the biological activity of the analogs 23-34 has shown that compounds 23, 24, and 26-30, which possess a high complex-forming capacity, also possess a high antimicrobial activity, while compound 25, forming a potassium complex of low stability, also exhibits a feeble antimicrobial activity. However, some analogs having a high stability of the complexes with K^+ either exhibit no antimicrobial activity whatever (compounds 31, 32, and 34) or they possess a narrower spectrum of antimicrobial action (compound 33). All the analogs of the meso compound 32 differing from one another by the configurations of the valine and α-hydroxyisovaleric acid residues (compounds 35-42) are also devoid of antimicrobial activity. At the same time, many of them (for example, compounds 35-38) gave fairly stable complexes with potassium ions the stability constants of which are lower than that of valinomycin (by not more than two orders of magnitude).

At the present time there is much information according to which the biological action of valinomycin and other compounds with a depsipeptide structure is connected with the increase caused by them in the flows of potassium ions through biological membranes (see, for example, [15]), and, as has been stated above, CDPs that increase the potassium permeability of membranes form stable complexes with this cation. The results of the present work confirm the conclusion which we drew previously [2, 3] that compounds binding K^+ most effectively possess a higher antibiotic activity, as a rule. At the same time, among the compounds of the valinomycin group that form complexes with potassium ions, only some exhibit antimicrobial activity [2, 3]. This can be explained by the assumption that the rate of the transfer of K^+ through membranes stimulated by CDPs depends not only on the stability of the CDP complex but also on a number of other factors such as the surface-active properties of the CDPs or their complexes, the distribution coefficient between water and the membrane, etc. Furthermore, the capacity of a CDP for increasing the K^+ permeability of biological membranes is a necessary, but not the only condition for the exhibition by a compound of antimicrobial activity, which also depends upon other factors – in particular, on the nature and concentration of the ions in the medium. It must also be mentioned that many CDPs (for example, compounds 32 and 35-38) that form stable complexes with K^+ possess only a very low solubility under the conditions for determining their antibiological activity and it is not excluded that they may exhibit a biological effect only at concentrations lying beyond the limits of their solubility under the given conditions.

Thus, as we have stated previously [3, 5], for the appearance of complex-forming capacity and antimicrobial activity by compounds of the valinomycin group, a 36-membered ring is the optimum.

The replacement in the valinomycin molecule of amide links by ester or N-methylamide links deprives the compounds both of complex-forming capacity and of antibiotic activity. However, the replacement of ester links by amide links has little effect on the exhibition of these properties by the compound.

A change in the configuration of the amino acid residues with the retention by the compound of its complex-forming capacity and antimicrobial activity is permissible in a limited section of the molecule.

However, a change in the configuration of the hydroxy-acid residues deprives the compound of biological activity and leads to a pronounced fall or complete loss of the stability of its complex with K^+.

A change in the size of the radicals of the amino- and hydroxy-acid residues of the molecule of the antibiotic has little effect on the complex-forming capacity of the compound. So far as concerns the suppression of the growth of microorganisms a change in the radicals of the amino-acid residues is also little reflected in the manifestation of this activity by the compound, while with a change in the radicals of the hydroxy-acid residues, the biological activity falls more considerably.

The nature of the dependence of the complex-forming capacity and antimicrobial activity on the primary structure in the series of valinomycin analogs considered is in harmony with the hypothesis of a decisive importance of conformational factors for its manifestation of these properties. At the present time, the conformational states of the valinomycin analogs and their complexes are being studied under various conditions, and the results will be reported in special communications.

EXPERIMENTAL

The investigation was performed with valinomycin obtained by the biosynthetic method in MacDonald's improved variant [16]. The synthesis and characteristics of the valinomycin analogs have been described previously [6, 10-13]. The stabilities of the complexes of the CDPs with potassium and sodium ions were determined by the conductometric method [3, 7] in absolute ethanol at 25°C. The action of the compounds on the growth of microorganisms was determined by the method of serial dilutions of these CDPs in ethanol with the subsequent addition of the ethanolic solutions to a culture medium of the following composition: 10 g of glucose, 5 g of sodium chloride, 5 g of peptone, 30 mg of Hottinger's broth (760 mg-% of amine nitrogen); pH 7.0-7.02. The concentration of the solvent in the culture phase did not exceed 2% and the bacterial charge was 1000 cells/ml (an exception was formed by M. phlei, the seed material of which was prepared by O. O. Makeeva's method [17]).

The measurements of the stability constants of the complexes of the cyclopeptides with potassium and sodium ions were performed by N. A. Skobelev.

SUMMARY

1. The antimicrobial activity of valinomycin and its analogs and also their capacity for forming complexes with potassium and sodium ions in solution have been investigated.

2. It has been shown that the capacity of CDPs of the valinomycin series for forming complexes with potassium ions is a necessary, but not sufficient, condition for the manifestation of antimicrobial activity by these compounds.

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