SYNTHESIS OF NEW VALINOMYCIN ANALOGS. II.

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UDC 547.96

The detailed physicochemical study of the membrane-active antibiotic valinomycin has enabled the reason for the high efficiency and unique K/Na selectivity of complex formation [1].

It has been established that complex formation takes place mainly through an ion–dipole interaction of the ester carbonyl group with a cation located in the molecular cavity, the spatial structure of the complex being rigidly fixed by a system of six intramolecular hydrogen bonds. Since the amides and N-methylamides can also be ligands in complexes of macrocylic compounds with alkali-metal ions [2, 3], analogs of valinomycin obtained by the replacement of ester groups by N-methylamide groups must be expected to be capable of forming analogous complexes (in such substitutions, the CO and NH groups necessary for the formation of hydrogen bonds are retained and the ligand carbonyl groups are present).

The present paper describes the synthesis of the analogs of valinomycin in which one, two and three ester groups of the molecule of the antibiotic have been replaced successively by N-methylamide groups (compounds 2-9, Table 1) and the determination of their antimicrobial activity and of the stability constants of the complexes of these compounds with sodium and potassium ions in ethanol:

cyclo [-(*D*. Val-L. Lac-L. Val-D. Hylv)$_3$-] (1)

cyclo [-(*D*. Val-L. N-MeAla-L. Val-D. Hylv) (A_2)-*] (2)

cyclo [-(D. Val-L. Lac-L. Val-D. N-MeVal) (A_2)-] (3)

cyclo [-(D. Val-L. N-MeAla-L. Val-D. Hylv)$_2$A-] (4)

cyclo [-(D. Val-L. Lac-L. Val-D. N-MeVal)$_2$A-] (5)

cyclo [-(*D*-. Val-L. N-MeAla-L. Val-D. Hylv)$_3$-] (6)

cyclo [-(*D*-. Val-L. Lac-L. Val-D. N-MeVal)$_3$-] (7)

cyclo [-(D. Val-L. N-MeAla-L. Val-D. Hylv)$_2$-] (8)

cyclo [-(D. Val-L. Lac-L. Val-D. N-MeVal)$_2$-] (9)

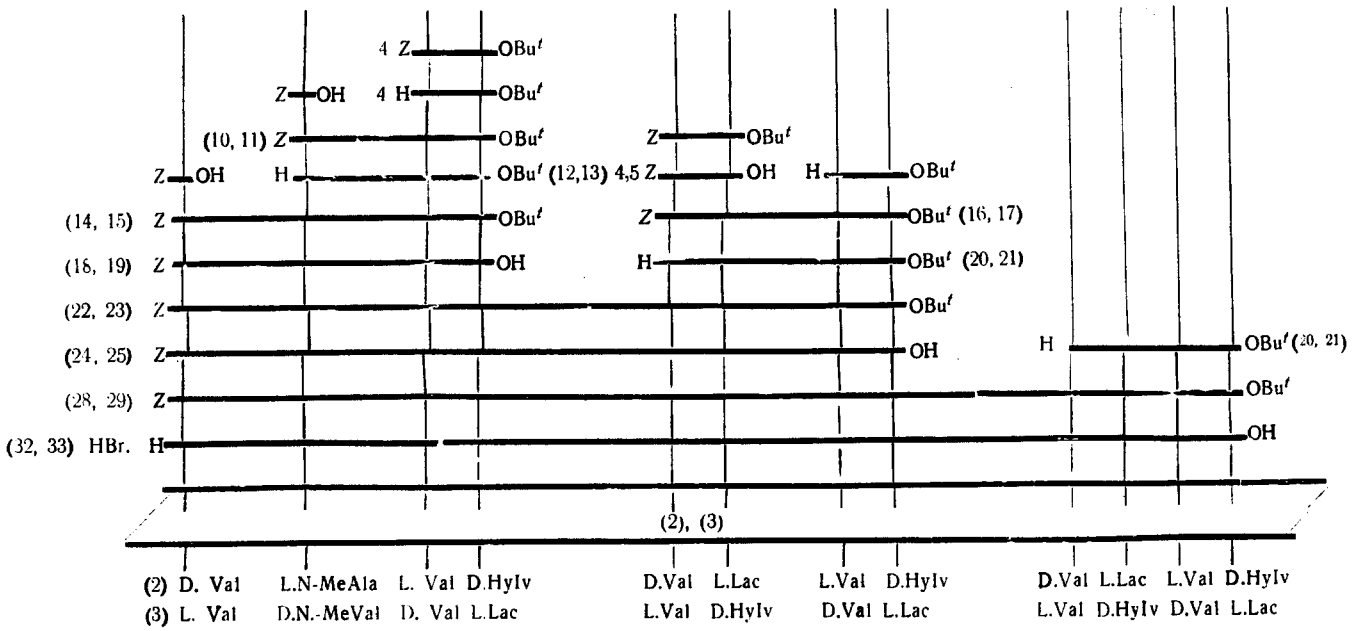
* A-(D. Val-L. Lac-L. Val-D. Hylv)-

Analogs 2 and 2 were synthesized by Scheme 1, analogs 4 and 5 by Scheme 2, and analogs 6-9 by Scheme 3. For general information on their synthesis, see Communication I. The synthesis of the linear dodecadepsipeptides corresponding to compounds 6 and 7 could not be performed because of the pronounced resinification of the reaction mixture accompanying the acyl chloride condensation of the tetradepsipeptides. For the synthesis of these cyclodepsipeptides we used the hydrobromides of the corresponding linear tetradepsipeptides, the molecules of which had previously been subjected to doubling and tripling under the conditions of the cyclization reaction (see Scheme 3).

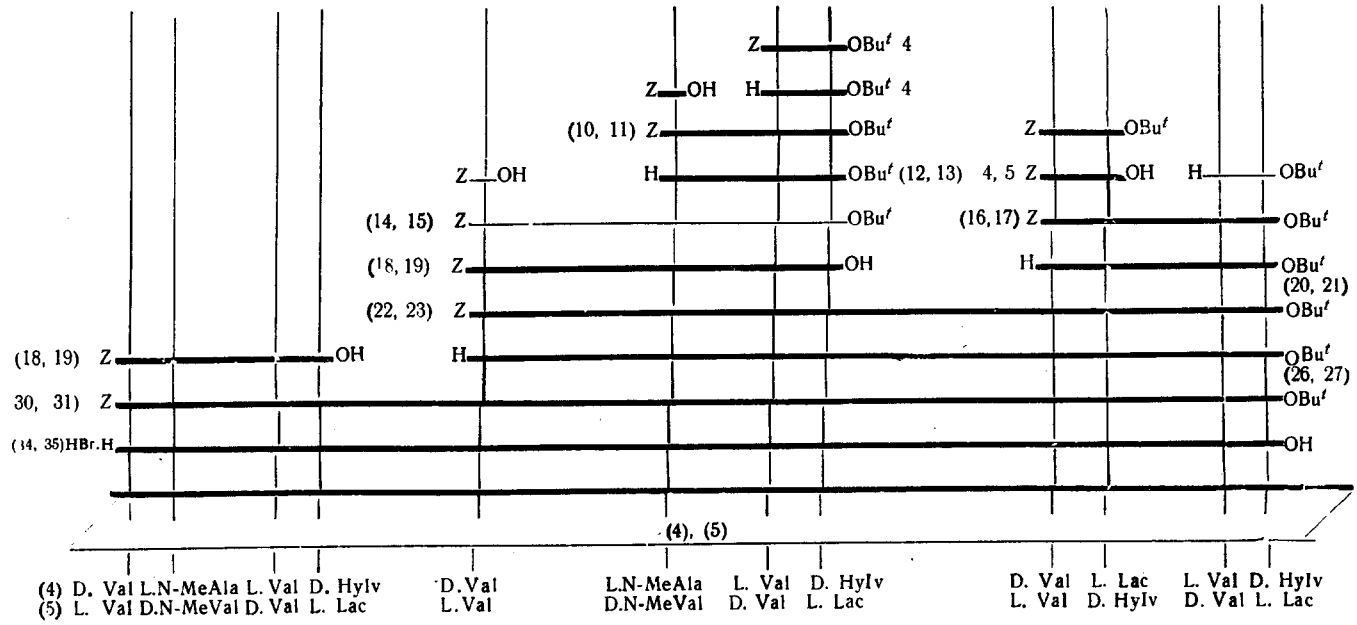
It can be seen from Tables 1 and 2 that all the cyclodecadepsipeptides obtained are distinguished by high stability constants of the complexes and show biological activity. Attention is attracted by the exceptionally high stability of the K^+ complex of analog 6. The results of measurements of the stability constants in various media (Table 3) show that its complex with K^+ possesses a "record" stability among the valinomycin cyclodepsipeptides, far exceeding the antibiotic in this respect. The question of the relationship between the primary structures of compounds 2-9 and the stability of their complexes will be considered in our subsequent communications on investigations of their spatial structures.

M. M. Shemyakin Institute of the Chemistry of Natural Compounds of the Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 278-286, May-June, 1974. Original article submitted April 13, 1974.

Scheme 1



Scheme 2



Scheme 3

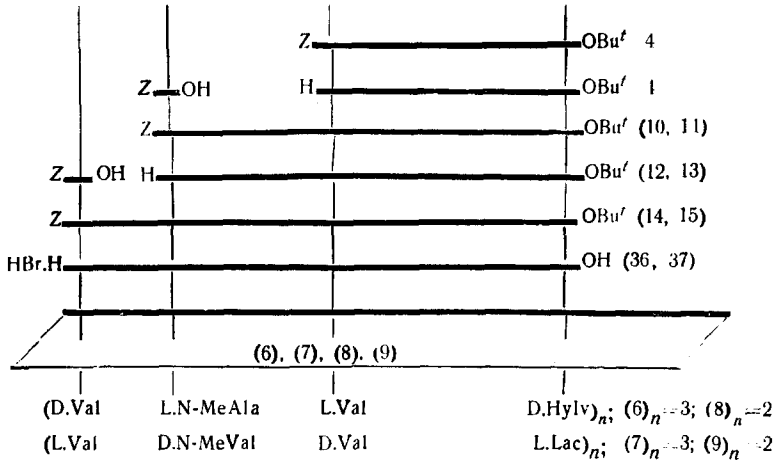


TABLE 1. Yields and Physicochemical Properties of the Cyclodepsipeptides (CDP's)

CPD	Yield, %	Mol. wt.*	mp, °C	$[\alpha]_D^{20}$, deg	Stability constant of the K^+ complex (liter/mole, KCl, ethanol, 25°C)
1	25	1110	190(dibutyl ether)	+32,8 (*c* 0,2; benzene)	$2 \cdot 10^6$
2	30	1123	203—204(heptane)	−6 (*c* 0,1; ethanol)	$5{,}5 \cdot 10^5$
3	25	1123	167—169(hexane)	+31 (*c* 0,1; ethanol)	$5 \cdot 10^6$
4	12	1136	Amorphous	−32 (*c* 0,1; ethanol)	$\sim 10^3$
5	17	1136	202—204(cyclohexane)	+38 (*c* 0,1; ethanol)	$3{,}1 \cdot 10^3$
6	8	1149	197—199(heptane)	−17 (*c* 0,1; ethanol)	$> 10^7$
7†	5	1149	208—209(benzene)		
8	25	766	228—229 (heptane)	+51 (*c* 0,1; ethanol)	<50
9	28	766	219—220 (ethanol)	+190 (*c* 0,1; ethanol)	<50

*The molecular weights of the CDP's were determined mass-spectrometrically.

†In view of the inadequate solubility of compound 7, the stability complex of its K^+ complex was not determined.

TABLE 2. Antimicrobial Activity of Valinomycin and Its Analogs*

Compound	Minimum concentration (ml) suppressing growth					
	Staph. aureus 209 P, concn. of K^+ in the medium (nM)		Staph. aureus UV-3	Str. faecalis	Sarcina lutea	Bac. mycoides
	5	100				
1	>25	0,2	0,1—0,2	0,2—0,3	0,1—0,2	>25
2	>10	0,2	0,1	0,1	0,2—0,4	>10
3	>10	2	1	2	4	>10
4	>20	15	7,5—10	7,5—10	7,5	>20
5	>20	>20	>20	>20	>20	>20
6	>20	0,8	0,4—0,8	0,1—0,2	0,2—0,4	>20
8	>20	>20	>20	>20	>20	>20
9	>20	>20	>20	>20	>20	>20

Compound	Minimum concentration (ml) suppressing growth					
	Bac. subtilis, concn. of K^+ in the medium (nM)		E. coli	Micob. phlei	Candida albicans	Sacch. cereviseae
	5	100				
1	>25	1—2	>25	0,3	0,2—0,4	0,2—0,4
2	>10	2	>10	0,3—0,4	0,4—0,8	0,4—0,8
3	>10	10—20	>10	4—6	2—4	2—4
4	>20	>20	>20	15	>20	>20
5	>20	>20	>20	>20	>20	>20
6	>20	1	>20	0,2—0,4	0,2—0,4	0,2—0,4
8	>20	>20	>20	>20	>20	>20
9	>20	>20	>20	>20	>20	>20

*In view of the inadequate solubility of compound 7, its antimicrobial activity was not determined.

TABLE 3. Stability Constants (liter/mole) of the K^+ Complexes of Valinomycin (1) and Its Analog 6 in Various Solvents*

Compound	Solvent		
	C_2H_5OH	42 mol. % C_2H_5OH + 58 mol. % CH_3OH	32 mol. % C_2H_5OH + 68 mol. % CH_3OH
1	$2.0 \cdot 10^6$	500	100
6	$\cdot 10^7$	11 000	3900

*Experimental results due to G. G. Malenkov.

EXPERIMENTAL

For general information on the experimental work, see Communication I and Table 4.

1. tert-Butyl Esters of N-Benzyloxycarbonyltridepsipeptides (10) and (11). With stirring, a solution of 0.01 mole of dicyclohexyldicarbodiimide in 10 ml of methylene chloride was added to a solution of 0.01 mole of the tert-butyl ester of D-valyl-L-lactic acid [4] and 0.01 mole of N-benzyloxycarbonyl-N-methyl-D-valine in 30 ml of methylene chloride cooled to −10°C. The reaction mixture was stirred at −10°C for another 1 h and was left at 20°C for 12 h. The precipitate of dicyclohexylurea that deposited was filtered off, and the filtrate was washed with 5% hydrochloric acid, with water, with saturated $NaHCO_3$ solution and with water again, and was dried with $MgSO_4$. The solvent was distilled off and the residue was chromatographed on silicic acid, and compound 10 was isolated by gradient elution (benzene−ethyl acetate).

The protected tridepsipeptide (11) was obtained under similar conditions from N-benzyloxycarbonyl-N-methyl-L-alanine and the tert-butyl ester of L-valyl-D-α-hydroxyisovaleric acid [4].

2. The tert-Butyl Esters of the Tridepsipeptides (12) and (13). Compound (10) (0.02 mole) was dissolved in 100 ml of methanol containing 0.02 mole of citric acid and 1 g of palladium black and hydrogenated with the passage of a current of hydrogen. After the end of hydrogenation the catalyst was filtered off and the filtrate was evaporated to dryness; the residue was dissolved in a 5% aqueous solution of citric acid.

TABLE 4. Yields and Constants of the Linear Depsipeptides

No.	Compound	Yield, %	mp, °C	$[\alpha]_D^{20}$, deg
10	Z-D.N-MeVal-D.Val-L.Lac-OBu^t	81	Oil	+58 (c 0,1; ethanol)
11	Z-L.N-MeAla-L.Val-D.Hylv-OBu^t	77		−73 (c 0,1; ethanol)
12	H-D.N-MeVal-D.Val-L.Lac-OBu^t	70		+ 3,5 (c 0,2; ethanol)
13	H-L.N-MeAla-L.Val-D.Hylv-OBu^t	85		12 (c 0,1; ethanol)
14	Z-L.Val-D.N-MeVal-D.Val-L.Lac-OBu^t	80		+52 (c 0,1; ethanol)
15	Z-D.Val-L.N-MeAla-L.Val-D.Hylv-OBu^t	85		−37 (c 0,1; ethanol)
16	Z-L.Val-D.Hylv-D.Val-L.Lac-OBu^t	87	70 71 (hexane)	5,5 (c 0,2; ethanol)
17	Z-D.Val-L.Lac-L.Val-D.Hylv-OBu^t [4]	92	Amorph-	+60 (c 0,1; ethanol)
18	Z-L.Val-D.N-MeVal-D.Val-L.Lac-OH	90	ous	+ 1,5 (c 0,2; ethanol)
19	Z-D.Val-L.N-MeAla-L.Val-D.Hylv-OH	85	Oil	23 (c 0,2; ethanol)
20	H-L.Val-D.Hylv-D.Val-L.Lac-OBu^t			
21	H-D.Val-L.Lac-L.Val-D.Hylv-OBu^t [4]	80		+35 (c 0,1; ethanol)
22	Z-L.Val-D.N-MeVal-D.Val L.Lac-(B)*-OBu^t	77		−13 (c 0,1; ethanol)
23	Z-D.Val-L.N-MeAla-L.Val-D.Hylv-(A)-OBu^t	85		−17 (c 0,1; ethanol)
24	Z-L.Val-D.N-MeVal-D.Val-L.Lac-(B)-OH	80		−27 (c 0,1; ethanol)
25	Z-D.Val-L.N-MeAla-L.Val-D.Hylv-(A)-OH	68		+31 (c 0,1; ethanol)
26	H-L.Val-D.N-MeVal-D.Val-L.Lac-(B)-OBu^t	70		−14 (c 0,1; ethanol)
27	H-D.Val-L.N-MeAla-L.Val-D.Hylv-(A)-OBu^t	90		+ 9 (c 0,1; ethanol)
28	Z-L.Val-D.N-MeVal-D.Val-L.Lac-$(B)_2$-OBu^t	89		−18 (c 0,1; ethanol)
29	Z-D.Val-L.N-MeAla-L.Val-D.Hylv-$(A)_2$-OBu^t	80	Amorph-	+30 (c 0,1; ethanol)
30	Z-(L.Val-D.N-MeVal-D.Val-L.Lac)$_2$-(B)-OBu^t	83	ous	-14 (c 0,1; ethanol)
31	Z-(D.Val-L.N-MeAla-L.Val-D.Hylv)$_2$-(A)-OBu^t	90		+15 (c 0,2; ethanol)
32	HBr·H-L.Val-D.N-MeVal-D.Val-L.Lac-$(B)_2$-OH	95		−20 (c 0,2; ethanol)
33	HBr·H-D.Val-L.N-MeAla-L.Val-D.Hylv-$(A)_2$-OH	92		+18 (c 0,1; ethanol)
34	HBr·H-(L.Val-D.N-MeVal-D.Val-L.Lac)$_2$-(B)-OH	70		5 (c 0,1; ethanol)
35	HBr·H-(D.Val-L.N-MeAla-L.Val-D.Hylv)$_2$-(A)-OH	82		140 (c 0,1; ethanol)
36	HBr·H-D.Val-L.N-MeAla-L.Val-D.Hylv-OH	80		+68 (c 0,1; ethanol)
37	HBr·H-L.Val-D.N-MeVal-D.Val-L.Lac- H			

*L.Val-D.Hylv-D.Val-L.Lac

The solution was washed with ether and, with ice-water cooling, was neutralized with sodium bicarbonate. The compound (12) that separated out in the form of an oil was extracted with ether. The ethereal extract was washed with water and dried with magnesium sulfate. After the distillation of the solvent, compound (12) remained.

Compound (13) was obtained similarly from the tridepsipeptide (11).

3. The tert-butyl esters of N-benzyloxycarbonyltetradepsipeptides (14) and (15) were obtained by condensing the tert-butyl esters of the tridepsipeptides (12) and (13) with N-benzyloxycarbonylvaline of the appropriate configuration under the conditions of Experiment 1.

4. The N-benzyloxycarbonyltetradepsipeptide tert-butyl ester (16) was obtained by condensing N-benzyloxycarbonyl-L-valyl-D-α-hydroxyisovaleryl chloride [3] and tert-butyl D-valyl-L-lactate under the conditions of Experiment 3 (Communication I).

5. The N-benzyloxycarbonyltetradepsipeptides (compounds 18 and 19) were obtained from compounds 14 and 15 under the conditions of Experiment 4 (Communication I).

6. The tetradepsipeptide tert-butyl ester (20) was obtained by the hydrogenolysis of the tert-butyl ester of the N-benzyloxycarbonyltetradepsipeptide (16) under the conditions of Experiment 2.

7. The N-benzyloxycarbonyloctadepsipeptides tert-butyl esters (22 and 23) were obtained by the condensation of the acid chloride derivatives of the N-benzyloxycarbonyltetradepsipeptides (18 and 19) with the tetradepsipeptide tert-butyl esters (20 and 21) under the conditions of Experiment 3 (Communication I).

8. The N-benzyloxycarbonyloctadepsipeptides (24 and 25) were obtained from N-benzyloxycarbonyl-octadepsipeptide tert-butyl esters (22 and 23) under the conditions of Experiment 4 (Communication I).

9. The octadepsipeptide tert-butyl esters (26, 27) were obtained by the hydrogenolysis of the N-benzyl-oxycarbonyloctadepsipeptide tert-butyl esters (22) and (23) under the conditions of Experiment 2.

10. The N-benzyloxycarbonyldodecapepsipeptides tert-Butyl esters (28-31). Compounds 28 and 29 were obtained by condensing the acid chloride derivatives of the N-benzyloxycarbonyloctadepsipeptides (24 and 25) with the tetradepsipeptide tert-butyl esters (20 and 21), and compounds 30 and 31 by the condensation of the acid chloride derivatives of the N-benzyloxycarbonyltetradepsipeptides (18) and (19) with the octadepsipeptide tert-butyl esters (26) and (27) under the conditions of Experiment 3 (Communication I).

11. The dodecadepsipeptide hydrobromides (32-35) and the tetradepsipeptides hydrobromides (36, 37) were obtained from compounds (14, 15, 28-31) under the conditions of Experiment 9 (Communication I).

12. The cyclododecapepsipeptides (2-5) were obtained by the cyclization of the acid chloride derivatives of compounds (32-35) under the conditions of Experiment 10 (Communication I).

13. The Cyclododeca- and Cyclooctadecadepsipeptides (6-9). A solution of 0.002 mole of the tetradepsipeptide hydrobromide (36) in 10 ml of $SOCl_2$ was kept at 20°C for 30 min, and the excess of $SOCl_2$ was carefully distilled off in vacuum. The residual acid chloride was distilled in 200 ml of absolute dioxane, and the solution was added with stirring (4 h at 20°C) simultaneously with a solution of 0.004 mole of triethylamine in 200 ml of absolute benzene to 400 ml of absolute benzene. The reaction mixture was kept at 20°C for 24 h and was then washed with 5% hydrochloric acid, water, saturated sodium bicarbonate solution, and water again, dried with magnesium sulfate, and evaporated to dryness. The purification and primary separation of the mixture of cyclohomologs obtained was performed by column chromatography on silica gel (Silikagel L.); gradient elution in the benzene–ethyl acetate system yielded a mixture of compounds 6 and 8. Compounds 6 and 8 were separated finally by gel filtration and columns containing Sephadex LH-20 in methanol.

The cyclization of the hydrobromide (37) was performed under similar conditions. The mixture of cyclodepsipeptides (7) and (9) obtained was dissolved in 20 ml of boiling ethanol, and the insoluble matter was filtered off. After 48 h, the filtrate deposited the cyclododecadepsipeptide (7). The mother solution was evaporated to dryness. The residue was chromatographed on columns of neutral alumina (activity g grade III), the cyclooctadepsipeptide (9) being isolated by gradient elution (benzene–ethyl acetate).

The antimicrobial activities of the cyclodepsipeptides (see Table 2) were determined as described in Communication I. The stability constants of the complexes were measured by the conductometric method [2].

CONCLUSIONS

1. The synthesis of eight cycloocta- and cyclododecapepsipeptide analogs of valinomycin containing different numbers of ester, amide, and N-methyl amide bonds has been effected.

2. The stability constants of the complexes of these analogs with potassium ions in ethanolic solution has been determined.

3. The antimicrobial activity of the compounds obtained has been studied.

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