SYNTHESIS OF NEW ANALOGS OF VALINOMYCIN AND THEIR PROPERTIES. I.

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Among the synthetic analogs of valinomycin (1) used in the study of the relationship between structure and biological function in the series of membrane-active valinomycin depsipeptides [1-6] it is possible to distinguish four main groups: analogs differing from valinomycin by the size of the ring; analogs with various related side chains; analogs with a changed configuration of one or more of the amino acid or (and) hydroxy acid residues; and analogs obtained by the replacement of the ester or amide groups of valinomycin by amide, N-methylamide, or ester groups. The investigation of the analogs mentioned yielded important information on the mechanism of the biological action of valinomycin, and also information permitting a deeper understanding of the role of the individual structural elements of the antibiotic in complex-formation with ions of alkali metals and in reaction with membranes [7-10].

The present paper gives the results of a further study of this question. For this purpose we have synthesized a series of new analogs of valinomycin (2)-(6) and have determined their antimicrobial activities and measured the stability constants of the complexes of these analogs with sodium and potassium ions in ethanol.

cyclo [-(D.Val-L.Lac-L, Val-D. Hylv)$_3$-]*-cyclo (-A$_3$-) (1)
cyclo [-(L. Val-L. Lac-D. Val-L. Hylv)$_3$-] (2)
cyclo [-(L. Val-L. Hylv-L. Val-D. Hylv)$_3$-] (3)
cyclo [-D. Val-L. Lac—L. Val-D. Lac-A$_2$-] (4)
cyclo [-D. Hylv-L.Lac-L. Val-D. Hylv-A$_2$-] (5)
cyclo [-(D. N-MeVal-L. Lac-L. N-MeVal-D. Hylv)$_3$-] (6)

Compound (2), belonging to the third group of analogs, is a derivative of valinomycin or, more accurately, of enantiovalinomycin – cyclo-(L-Val – D-Lac – D-Val – L-Hylv)$_3$ in which the configurations of all three lactic acid residues have been changed. Its synthesis completed the preparation of a series of diasteromers of valinomycin with the reversed configuration of three identical acid residues. The remaining members of this series, differing from valinomycin by the substitutions 3D-Val → 3L-Val, 3L-Val → 3D-Val, and 3D-Hylv → 3L-Hylv have been described in [1]. Compound (3), which is an analog of the so-called "meso"-Hylv-valinomycin – cyclo-(D-Val – L-Hylv – L-Val – D-Hylv)$_3$ – contains valine residues only of the L configuration. The results of a study of analog (3) together with two compounds obtained previously (D-Val → L-Val and 2D-Val → 2L-Val [4]) permits the determination of the influence of successive changes in the configurations of the amino-acid residues in the "meso"-Hylv-valinomycin series. Compound (4), belonging to the second group of analogs, differs from valinomycin by the substitution D-Hylv → D-Lac. In its structure it is intermediate between valinomycin (1) and "meso"-Lac-valinomycin – cyclo-(D-Val – L-Lac – L-Val – D-Lac)$_3$ – the properties of which are of considerable interest from the point of view of an investigation of the mechanism of the induction of the ionic permeability of membranes by valinomycin cyclodepsipeptides [8]. Analog (5) of the fourth group was obtained by replacing one amide bond by an ester bond, and analog (6) by replacing all the amide bonds with the N-methylamide bonds. Since in the complex of valinomycin with the potassium ion all six amide groups of the antibiotic participate in the formation of the system of intramolecular

*Lac=—O—CH(CH$_3$)—CO—; Hylv=—O—CH(CH(CH$_3$)$_2$)—CO—; A=D.Val-L. Lac-L. Val-D.Hylv

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TABLE 1. Yields and Physicochemical Properties of the Cyclodepsipeptides (CDPs)*

CDP	Yield, %	M	Mp, °C	$[\alpha]_D^{20}$, deg	Stability constant of the K^+ complex (liter/mole, KCl, ethanol, 25°C)
1	25	1110	190° (dibutyl ester)	+32,8 (c 0,2; benzene)	$2 \cdot 10^6$
2	32	1110	Amorphous	−51 (c 0,2; chloroform)	—
3	48	1194	Amorphous	−28,3 (c 0.2; chloroform)	$1,5 \cdot 10^8$
4	42	1082	168, 188-189 (hexane)†	+30 (c 0,2; ethanol)	$3 \cdot 10^5$
5	28	1111	Amorphous	−4,5 (c 0,2; chloroform)	$4,2 \cdot 10^4$
6	60	1188	266-268 (acetone)	−35 (c 0,25; chloroform)	—

*In view of the inadequate solubility of compounds (2) and (6), the stability constants for the K^+ complexes were not determined.
†The first figure is the softening temperature after which the substance solidifies again, and the second figure is the melting point.

TABLE 2. Yields and Constants of the Intermediate Linear Depsipeptides

No.	Compound	Yield, %	Mp, °C	$[\alpha]_D^{20}$, deg
7	Z-D·Nylv-L·Lac-OBu^t	81	Oil	−4,3 (c 0,6; benzene)
8	Z-L·Val-L·Lac-OH [1]	—	—	—
9	Z-L·Val-L·Hylv-OH [4]	—	—	—
10	Z-D·Val-L·Lac-OH [1,3]	—	—	—
11	Z-L·Val-D·Hylv-OH [4]	—	—	—
12	H-D·Val-L·Hylv-OBu^t [1]	—	—	—
13	H-L·Val-D·Hylv-OBu^t [3,4]	—	—	—
14	H-L·Val-D·Lac-OBu^t [1]	—	—	—
15	H-D·Val-L·Lac-OBu^t [3]	—	—	—
16	H-D·Hylv-L·Lac-OBu^t	85	Oil	−34 (c 0,5; benzene)
17	Z-D·Val-L·Lac-L·Val-D·Lac-OBu^t [1]			
18	Z-L·Val-L·Lac-D·Val-L·Hylv-OBu^t	83	Oil	−27 (c 0,5; ethanol)
19	Z-L·Val-L·Hylv-L·Val-D·Hylv-OBu^t [4]			
20	Z-L·Val-D·Hylv-D·Val-L·Lac-OBu^t	87	70—71° (hexane)	−5,5 (c 0,2; ethanol)
21	Z-D·Val-L·Lac-L·Val-D·Hylv-OBu^t [1,3]			
22	Z-L·Val-D·Hylv-D·Hylv-L·Lac-OBu^t	88	Oil	−8 (c 0,2; ethanol)
23	Z-D·Val-L·Lac-L·Val-D·Lac-OH [1]			
24	Z-L·Val-L·Lac-D·Val-L·Hylv-OH	91	Amorph.	−17,5 (c 0,2; ethanol)
25	Z-L·Val-L·Hylv-L·Val-D·Hylv-OH [4]			
26	Z-L·Val-D·Hylv-D·Val-L·Lac-OH	89	110—111 (hexane)	−2 (c 0,2; ethanol)
27	H-L·Val-L·Lac-D·Val-L·Hylv-OBu^t	74	Oil	−5 (c 0,2; ethanol)
28	H-L·Val-L·Hylv-L·Val-D·Hylv-OBu^t [4]			
29	H-L·Val-D·Hylv-D·Val-L·Lac-OBu^t	85	Oil	−23 (c 0,2; ethanol)
30	H-D·Val-L·Lac-L·Val-D·Hylv-OBu^t [1,3,13]			
31	H-L·Val-D·Hylv-D·Hylv-L·Lac-OBu^t	79	Oil	+15 (c 0,2; benzene)
32	Z-D·Val-L·Lac-L·Val-D·Lac-(A)-OBu^t	83		−6,3 (c 0,2; ethanol)
33	Z-(L·Val-L·Lac-D·Val-L·Hylv)$_2$-OBu^t	80		−33 (c 0,2; ethanol)
34	Z-(L·Val-L·Hylv-L·Val-D·Hylv)$_2$-OBu^t [4]			
35	Z-(L·Val-D·Hylv-D·Val-L·Lac)$_2$-OBu^t	83		−6 (c 0,2; ethanol)
36	Z-D·Val-L·Lac-L·Val-D·-Lac-(A)-OH	80		−10 (c 0,2; ethanol)
37	Z-(L·Val-L·Lac-D·Val-L·Hylv)$_2$-OH	78		−30 (c 0,2; ethanol)
38	Z-(L·Val-L·Hylv-L·Val-D·Hylv)$_2$-OH [4]			
39	Z-(L·Val·D·Hylv-D·Val-L·Lac)$_2$-OH	90		−2 (c 0,2; ethanol)
40	Z-(L·Val-L·Lac-D·Val-L·Hylv)$_3$-OBu^t	91	Amorphous	−38 (c 0,2; ethanol)
41	Z-(L·Val-L·Hylv-L·Val-D·Hylv)$_3$-OBu^t	85		−35 (c 0,2; ethanol)
42	Z-D·Val-L·Lac-L·Val-D·Lac-(A)$_2$-OBu^t	91		−4 (c 0,3; ethanol)
43	Z-(B)$_2$-L·Val-D·Hylv-D·Hylv-L·Lac-OBu^t*	92		−5 (c 0,2; ethanol)
44	HBr·H-(L·Val-L·Lac-D·Val-L·Hylv)$_3$-OH	82		−22,5 (c 0,45; ethanol)
45	HBr·H-(L·Val-L·Hylv-L·Val-D·Hylv)$_3$-OH	80		−31 (c 0,2; ethanol)
46	HBr·H-D·Val-L·Lac-L·Val-D·Lac-(A)$_2$-OH	82		−20 (c 0,2; ethanol)
47	HBr·H-(B)$_2$-L·Val-D·Hylv-D·Hylv-L·Lac-OH	79		−29 (c 0,3; ethanol)

*B = L-Val − D-Hylv − D-Val − L-Lac.

TABLE 3. Antimicrobial Activity of Valinomycin and Its Analogs*

Compound	Minimum concentration (γ/ml) suppressing the growth of											
	Staph. aureus 209 P		Staph. aureus UV-3†	Staph. faecalis	Sarcina lutea	Bac. mycoides	Bac. subtilis		E. coli	Micob. phlei‡	Candida albicans	Sacch. cerevisseae
	concn. of K^+ in the medium (mM)						concn. of K^+ in the medium (mM)					
	5	100					5	100				
1	>25	0,2	0,1—0,2	0,2—0,3	0,1—0,2	>25	>25	1—2	>25	0,3	0,2—0,4	0,2—0,4
3	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
4	>5	0,1	0,1	0,2—0,3	0,1	>5	>5	1—2	>5	0,3	0,3—0,4	0,2—0,4
5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5

*In view of the inadequate solubility of compounds (2) and (6), their antimicrobial action was not determined.

†A mutant of Staph. aureus obtained from Prof. G. F. Gauze, Institute for the Search for New Antibiotics of the Academy of Medical Sciences of the USSR. In the opinion of American workers [13], this culture is a Corynebacterium sp.

‡The seed material for this culture was prepared by O. O. Makeeva's method [14].

hydrogen bonds, when one amide bond is replaced by an ester bond in compound (5), as in the case of the analogous derivative L-Val → L-Hylv [3, 6], such a system of bonds is disturbed and the stability of the complexes of these compounds must be weakened. In compound (6), indeed, which contains no NH groups at all, the formation of such bonds is quite impossible. The synthesis of the valinomycin analogs was performed in accordance with the scheme shown on page 241.

In the preparation of the intermediate linear depsipeptides, for protecting the amino and hydroxy groups we used the benzyloxycarbonyl group, and for protecting the carboxy groups we used the tert-butyl group. The building up of the depipeptide chain was effected by first creating ester bonds between N- or O-protected amino or hydroxy acids and esters of hydroxy acids (mixed anhydride method using benzene sulfonyl chloride) and then forming the amide bonds by the acid chloride method. The cyclization of the linear depsipeptides was also performed by the acid chloride method under conditions of high dilution. Compound (6) was obtained by the direct methylation of valinomycin using Lederer's method [11]. The yields and the molecular weights of the cyclodepsipeptides measured mass spectrometrically, the stability constants of the potassium complexes in ethanolic solutions determined by the conductometric method [7], and other physicochemical constants are given in Table 1; the yields and constants of the intermediate linear depsipeptides are given in Table 2, and the results of the determination of the antimicrobial activities of compounds (2-6) in Table 3. Like the valinomycin analogs obtained previously [2, 6, 7], in the series of compounds (2-6) a definite correlation is observed between the stabilities of the complexes and their antimicrobial activities. Compound (4) with the highest stability constant of the complex had the greatest activity, and the weaker complexones (3) and (5) show no antimicrobial activity. Unfortunately, compounds (2) and (6) possess insufficient solubility in ethanol and water to study their complex-forming and antimicrobial properties.

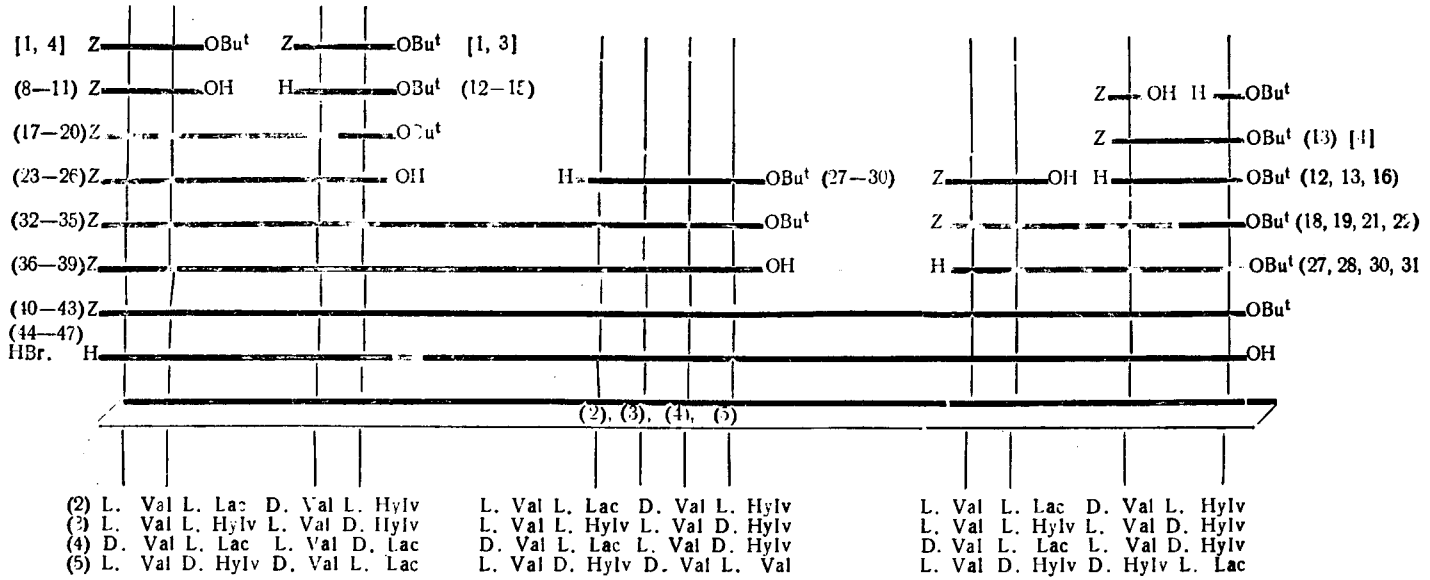
The question of the relationship between the primary structures of compounds (2-6) and the stabilities of their complexes will be considered in our subsequent communications on the study of their spatial structure.

EXPERIMENTAL

The melting points are not corrected. The individualities of the compounds obtained were checked by thin-layer chromatography on Silufol and Eastman plates. The results of the elementary analysis of the compounds corresponded to the calculated C, H, and N contents.

1. tert-Butyl Ester of O-Benzyloxycarbonyl-D-α-hydroxyisovaleryl-L-lactic Acid (7). With stirring, 0.02 mole of benzenesulfonyl chloride was added (5-6 min at 0°C) to a solution of 0.02 mole of O-benzyloxycarbonyl-D-α-hydroxyisovaleric acid in 15 ml of anhydrous pyridine, and after 6-7 min 0.02 mole of tert-butyl L-lactate in 10 ml of anhydrous pyridine was also added. The mixture was stirred at 0°C for 2 h and at 20°C for 2 h and was then poured into 100 ml of cold (0 to +5°C) water, and the oil that separated out

Scheme



(2) L. Val L. Lac D. Val L. Hylv
(3) L. Val L. Hylv L. Val D. Hylv
(4) D. Val L. Lac L. Val D. Lac
(5) L. Val D. Hylv D. Val L. Lac

L. Val L. Lac D. Val L. Hylv
L. Val L. Hylv L. Val D. Hylv
D. Val L. Lac L. Val D. Hylv
L. Val D. Hylv D. Val L. Val

L. Val L. Lac D. Val L. Hylv
L. Val L. Hylv L. Val D. Hylv
D. Val L. Lac L. Val D. Hylv
L. Val D. Hylv D. Hylv L. Lac

was extracted with ether. The ethereal solution was washed with 10% hydrochloric acid, water, saturated sodium bicarbonate solution, and water and was dried with magnesium sulfate. After the solvent had been distilled off, compound (7) was obtained.

2. tert-Butyl Ester of D-α-Hydroxyisovaleryl-L-lactic Acid (16). A solution of 0.02 mole of compound (7) in 50 ml of methanol was hydrogenated in the presence of 1 g of palladium black in a current of hydrogen. After the end of hydrogenation, the catalyst was filtered off, the solvent was evaporated off, and the residue was distilled in vacuum; a fraction containing compound (16) distilled over at 84-85°C/1.5 mm.

3. tert-Butyl Esters of the N-Benzyloxycarbonyltetradepsipeptides (17-22). A solution of 0.01 mole of a N-benzyloxycarbonylaminoacyloxy acid (one of compounds 8-11) in 15 ml of $SOCl_2$ was kept at 30-35°C for 30 min, and the excess of $SOCl_2$ was carefully distilled off in vacuum. The resulting acid chloride was dissolved in 40 ml of absolute benzene and, with stirring and cooling (+3 to +5°C) it was added dropwise simultaneously with a solution of 0.015 mole of dry triethylamine in 40 ml of absolute benzene to a solution of 0.01 mole of the tert-butyl ester of an aminoacyloxy or a hydroxyacyloxy acid (one of compounds 12-16) in 30 ml of absolute benzene. The reaction solution was stirred at +3-5°C for another 30 min and at 20°C for 2 h and was then washed with 5% hydrochloric acid, water, saturated sodium bicarbonate solution, and water again, and was dried with magnesium sulfate. The benzene solution was evaporated in vacuum to dryness, and the residue was chromatographed on a column of alumina (activity grade II), the tetradepsipeptides (17-22) being isolated by gradient elution (benzene-ethyl acetate).

4. The N-Benzyloxycarbonyltetradepsipeptides (23-26). A solution of 0.01 mole of a tert-butyl ester of a N-benzyloxycarbonyltetradepsipeptide (one of compounds 17-20) in 30 ml of trifluoroacetic acid was kept at 20-25°C for 20 min, and the trifluoroacetic acid was carefully distilled off in vacuum. The residue was dissolved in ether and the ethereal solution was washed with water and extracted with saturated sodium bicarbonate solution. The bicarbonate solution was neutralized with 10% hydrochloric acid and the oil that separated out was extracted with ether. The ethereal extracts were washed with water until the acid reaction to Congo Red had disappeared and was dried with magnesium sulfate. After distillation of the ether, compounds (23-26) were obtained.

5. tert-Butyl Esters of the Tetradepsipeptides (27-31). A protected tetradepsipeptide (one of compounds 18-22) (0.02 mole) was dissolved in 100 ml of methanol containing 0.02 mole of citric acid and 1 g of palladium black and was hydrogenated by 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. The solution was washed with ether and, with ice-water cooling, it was neutralized with sodium bicarbonate. The oil that separated out was extracted with ether. The ethereal extract was washed with water and dried with magnesium sulfate. After the solvent had been distilled off, one of compounds (27-31) remained.

6. The tert-Butyl Esters of the N-Benzyloxycarbonyloctadepsipeptides (32-35) were obtained by condensing chlorides of N-benzyloxycarbonyltetradepsipeptides (23-26) with tert-butyl esters of the tetradepsipeptides (27-30) under the conditions of experiment 3.

7. The N-Benzyloxycarbonyloctadepsipeptides (36-39) were snythesized from the tert-butyl esters of the N-benzyloxycarbonyloctadepsipeptides (32-35) under the conditions of experiment 4.

8. The tert-Butyl Esters of the N-Benzyloxycarbonyldodecadepsipeptides (40-43) were obtained by condensing the chlorides of N-benzyloxycarbonyloctadepsipeptides (36-39) with the tert-butyl esters of tetradepsipeptides (27, 28, 30, 31) under the conditions of experiment 3.

9. Hydrobromides of Dodecadepsipeptides (Compounds 44-47). A tert-butyl ester of N-benzyloxycarbonyldodecadepsipeptide (40-43) (0.002 mole) was dissolved in the minimum amount of glacial acetic acid, and 15 ml of a 35% solution of hydrogen bromide in glacial acetic acid was added. The solution was left at 20°C for 1 h, and the solvent was carefully distilled off in vacuum. The residue was repeatedly washed with absolute ether and was dried in vacuum over P_2O_5. The corresponding compounds (44-47) were obtained.

10. The Cyclododecadepsipeptides (2-5). A solution of 0.001 mole of a hydrobromide of a linear dodecadepsipeptide (44-47) in 15 ml of $SOCl_2$ was kept at 30°C for 30 min, and the excess $SOCl_2$ was carefully distilled off. The resulting acid chloride was dissolved in 300 ml of absolute benzene and added with stirring (6 h at 20°C), simultaneously with a solution of 0.04 mole of triethylamine in 300 ml of absolute benzene, to 1500 ml of absolute benzene. The reaction mixture was stirred for another 2 h and was left at 20°C for 12 h; then it was evaporated in vacuum to a volume of 300 ml and was washed with 5% hydrochlo-

ric acid, water, saturated sodium bicarbonate solution, and water, and dried with magnesium sulfate. The solvent was distilled off and the residue was chromatographed on a column of neutral alumina (activity grade III), the corresponding cyclodecadepsipeptide being isolated by gradient elution (benzene–ethyl acetate).

11. The Cyclodecadepsipeptide (6). A suspension of 0.0004 mole of valinomycin, 0.014 mole of silver oxide, and 5 ml of methyl iodide in 10 ml of dimethylformamide in a sealed tube was stirred at 18-20°C for five days. Then the precipitate was filtered off and was washed with 20-25 ml of dimethylformamide. The filtrates were combined, diluted with 100 ml of chloroform, washed with 5% potassium cyanide solution and with water, and dried with magnesium sulfate. The solvent was distilled off to dryness and the residue was carefully washed with acetone. Compound (6) was isolated by reprecipitation from a saturated chloroform solution with acetone.

Antimicrobial Activities of the Cyclodepsipeptides. The effect of the cyclodepsipeptides on the growth of microorganisms was determined by the method of test-tube serial dilutions of solutions of compounds under investigation in ethanol with the subsequent transfer of the ethanolic solutions into a culture medium of the following composition: 10 g of glucose, 5 g of NaCl, 5 g of peptone, 30 ml of Hottinguer's bouillon (760 mg of amine nitrogen) in 1 liter, pH 7.01-7.02 (total concentration of K^+ in the medium 5 mM). The concentration of the solvent in the culture liquid did not exceed 2%, and the bacterial load 1000 cells/ml. Since we have shown previously [12] that the effect of valinomycin depends on the concentration of potassium ions in the medium and the growth of Staph. aureus 209-P and of Bac. subtilis are sensitive to the antibiotic in experiments with media containing not less than 10 mM of K^+, the antibacterial effect of the compounds investigated in relation to these microorganisms was determined additionally on a medium of similar composition in which the total concentration of K^+ was brought to 100 mM by the addition of KCl.

CONCLUSIONS

1. The synthesis of five valinomycin analogs differing by the configurations of the amino-acid and hydroxy-acid residues and also by the volumes of the radicals of the hydroxy-acid residues has been effected.

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

3. The antimicrobial activities of the compounds obtained have been studied and a correlation has been found between the degree of activity and stability of the complexes with K^+.

LITERATURE CITED

1. M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, Yu. B. Shvetsov, and L. A. Fonina, Zh. Obshch. Khim., 36, 1391 (1966).
2. V. T. Ivanov, I. A. Laine, I. D. Ryabova, and Yu. A. Ovchinnikov, Khim. Prirodn. Soedin., 744 (1970).
3. L. A. Fonina, A. A. Sanasaryan, and E. I. Vinogradova, Khim. Prirodn. Soedin., 69 (1971).
4. A. A. Sanasaryan, L. A. Fonina, Yu. B. Shvetsov, and E. I. Vinogradova, Khim. Prirodn. Soedin., 81 (1971).
5. M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, N. F. Loginova, I. D. Ryabova, and I. A. Pavlenko, Exper., 21, 548 (1965).
6. M. M. Shemyakin, E. I. Vinogradova, I. D. Ryabova, L. A. Fonina, and A. A. Sanasaryan, Khim. Prirodn. Soedin., 241 (1973).
7. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, E. I. Vinogradova, A. M. Shkrob, G. G. Malenkov, A. V. Evstratov, I. A. Laine, E. I. Melnik, and I. D. Ryabova, J. Membrane Biol., 1, 402 (1969).
8. E. I. Mel'nik, A. V. Babakov, and A. M. Shkrob, Symposium on "The Biophysics of Membranes," Palanga (1971) [in Russian], Kaunas, Part 1 (1971), p. 599.
9. Yu. A. Ovchinnikov, Pure Appl. Chem., 2, 123 (1971).
10. V. T. Ivanov, I. A. Laine, Yu. A. Ovchinnikov, I. I. Chervin, and G. I. Yakovlev, Khim. Prirodn. Soedin., 248 (1973).
11. B. C. Das, S. D. Gero, and E. Lederer, Biochem. Biophys. Res. Commun., 29, 211 (1967).
12. G. A. Gorneva, I. D. Ryabova, and G. Faizova, Symposium on "The Biophysics of Membranes," Palanga (1971) [in Russian], Kaunas, Part 1 (1971), p. 318.
13. F. L. Garrity, B. Detrick, and E. R. Kennedy, J. Bacteriology, 97, 557 (1969).
14. O. O. Makeeva, in: Methods of Experimental Chemotherapy (Tuberculosis) [in Russian], Moscow (1959), p. 148.