DEPSIPEPTIDES

COMMUNICATION 45. STRUCTURE AND SYNTHESIS OF VALINOMYCIN

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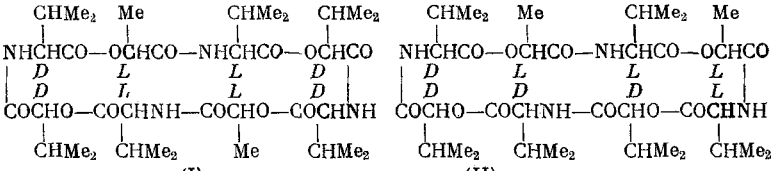
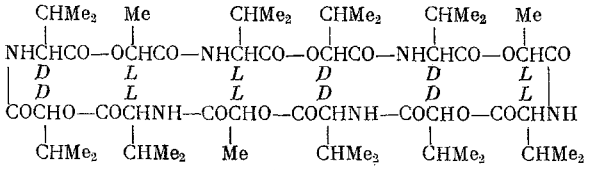
For the antibiotic valinomycin, which has a cyclodepsipeptide structure, Brockmann [1] proposed the two possible formulas (I) and (II). We have shown previously [2] that the formula (I) does not correspond to the structure of valinomycin because the synthesized cyclooctadepsipeptide (I) differs greatly in its properties from the natural compound. In the present work to check the correctness of the formula (II) we synthesized the compound corresponding to this formula, but the compound obtained was also not identical to valinomycin, which compelled us to reject the formula (II) also.



(I) (II)

It appeared most probable that valinomycin is a cyclopolymer homolog of one of the above compounds. We have found, in fact, that the molecular weight of a sample of the antibiotic kindly provided by W. Teuber, determined by the thermoelectric method in ethyl acetate, is 1070 ± 30. Simultaneously, by the sedimentation method Brockmann and co-workers obtained the value of 1149 ± 50 for valinomycin [3]. The figures indicate that valinomycin is probably a cyclododecadepsipeptide (mol.wt. 1111.4). The latter could correspond to at least four different formulas, one which, (III), was regarded by us as most closely corresponding to the structure of the antibiotic on the basis of data obtained previously in the hydrolysis of valinomycin [1].



(III)

The correctness of this hypothesis was confirmed by the synthesis of valinomycin. The compound (III) which we obtained proved to be identical to the antibiotic valinomycin in its physical, chemical, and biological properties [4]. The synthesis of the compounds (II) and (III) was effected by the methods developed previously in our laboratory, which consist in the gradual building up of the depsipeptide chain by the creation first of ester and then amide links with subsequent cyclization of the linear depsipeptides obtained [5, 6]. In all cases the ester link was formed by the method of mixed anhydrides (benzene sulfonyl chloride in pyridine) and the amide link—by the acid chloride method (in benzene in presence of triethylamine). The cyclization of the linear depsipeptides was effected by the acid chloride method under high-dilution conditions in benzene in presence of triethylamine. We must mention that in the cyclization process we again encountered the phenomenon which we discovered previously—the doubling of the depsipeptide molecules [7, 8]. Thus, in the synthesis of the cyclooctadepsipeptide (II) from the corresponding linear octadepsidpeptide we also obtained a cyclic homolog of twice the molecular weight—the cyclohexadecadepsipeptide (IV)

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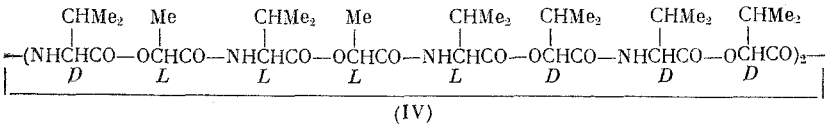
Compound	Yield, %	M.p. or B.p., °C	$[\alpha]_D^{20}$	Molecular Formula	Found, % C	Found, % H	Calculated, % C	Calculated, % H
VI	70	46—47 (hexane)	—9,5° (c 2, C_2H_5OH)	$C_{20}H_{29}NO_6$	63,51	7,57	63,30	7,70
VII	72	Oil*	—52,4° (c 2, C_2H_5OH)	$C_{20}H_{29}NO_6$	63,41	7,62	63,30	7,70
VIII	57	92—93 (0,5 mm)	—26,5° (c 2, C_2H_5OH)	$C_{12}H_{23}NO_4$	58,30	9,22	58,75	9,45
IX	85	oil	+4,6° (c 2, C_2H_5OH)	$C_{16}H_{21}NO_6$	59,26	6,38	59,43	6,55

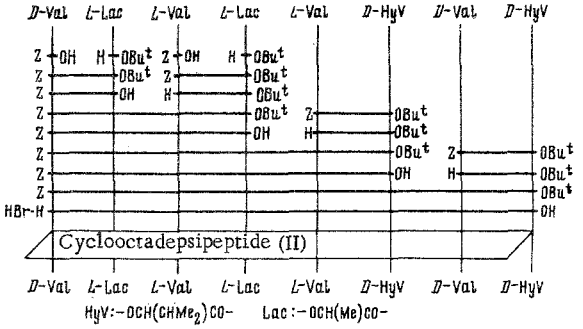
*Here and below, all the noncrystallizing and nondistilling depsipeptides, apart from acids, were purified by chromatography on neutral alumina in benzene-ethyl acetate (gradient elution).



(IV)

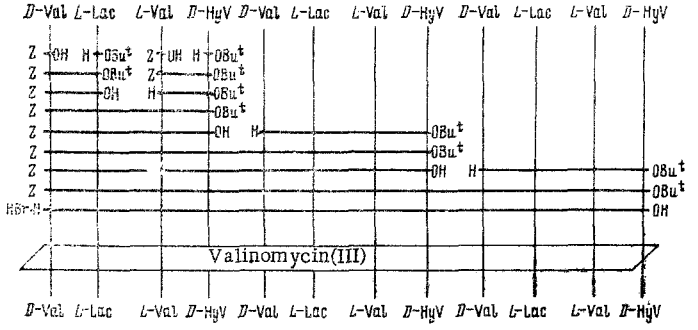
In the case of (II) the linear chain was built up by the successive grafting of the corresponding didepsi peptides in accordance with Scheme 1.

Scheme 1



In the preparation of the linear dodecadepsipeptide necessary for the synthesis of valinomycin we chose a route based on the twofold addition of the corresponding tetradepsipeptide (Scheme 2).

Scheme 2



*Here and below the system of abbreviations recommended by the Fifth European Peptide Symposium (Oxford, 1962) is used; new abbreviations used in this paper are explained in the text.

EXPERIMENTAL

O-Acetyl-L-lactic Acid. 15.5 ml of acetyl chloride was added in the course of 30 min with vigorous stirring to a suspension of 60.4 g of finely ground L-threo-2-amino-1-p-nitrophenyl-1, 3-propanediol L-lactate salt [6] at 18-20°, and the mixture was then stirred further for 10-12 h. The precipitate of the amine hydrochloride was filtered off, solvent was driven off, and the residue was vacuum-distilled. We obtained 18.5 g (70%) of O-acetyl-L-lactic acid; b.p. 137-138° (15 mm); n_D^{20} 1.4250; $[\alpha]\ D^{20}$ −43.2° (s 2, C_6H_6). Found %: C 45.56; H 6.15. $C_5H_8O_4$. Calculated %: C 45.45; H 6.10.

t-Butyl L-Lactate (V). A solution of 26.4 g of O-acetyl-L-lactic acid and 1.2 ml of concentrated sulfuric acid in 200 ml of methylene chloride was saturated with isobutene at 0-5°until the volume had increased by 150 ml, and the mixture was then left for 40 h at 18-20°. The solution was diluted with ether, washed with 10% sodium carbonate solution and water, and dried with $MgSO_4$. Solvent was vacuum-ditilled off, and we obtained t-butyl O-acetyl-L-lactate (33.8 g, 90%); this was dissolved in 17 ml of water, and in the course of one hour 95 ml of 2 N NaOH was added with stirring at 0-5°. The mixture was stirred for four hours, after which it was extracted with ether, and the extract was washed with water and dried with $MgSO_4$. Solvent was driven off, and the residue was vacuum-distilled. We obtained 19.8 g (68%) of the ester (V), m.p. 42° (petroleum ether); $[\alpha]\ D^{20}$ −10.5° (s 2, C_6H_6). Found %: C 57.55; H 9.50. $C_7H_{14}O_3$. Calculated %: C 57.51; H 9.65.

Protected Didepsipeptides. t-Butyl (benzyloxycarbonyl)-D-valyl-L-lactate (VI), t-butyl (benzyloxycarbonyl)-L-valyl-L-lactate (VII), t-butyl L-valyl-L-lactate (VIII), and (benzyloxycarbonyl)-D-valyl-L-lactic acid (IX) were prepared by the methods described in [6]; their constants and yields are given in the table.

t-Butyl (benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovalerate (X). 3.23 g of (IX) was dissolved in 6 ml of $SOCl_2$, the solution was kept at 30° for 30 min, excess of $SOCl_2$ was carefully vacuum-distilled off, and the acid chloride which remained was dissolved in 30 ml of dry benzene. One half of this solution was added with cooling to 0° and stirring to a solution of 2.73 g of t-butyl L-valyl-D-α-hydroxyisovalerate [9] and 0.75 ml of dry triethylamine in 20 ml of dry benzene; then we added a further 0.75 ml of triethylamine and the second half of the acid chloride solution. The mixture was stirred for two hours at 18-20°, the solution was washed with 5% HCl, water, saturated $NaHCO_3$ solution, and again water and was dried with $MgSO_4$, and solvent was driven off. We obtained 5.3 g (92%) of (X) as an oil; $[\alpha]\ D^{20}$ −1.5° (s 2, C_2H_5OH). Found %: C 62.10; H 8.05. $C_{30}H_{46}N_2O_9$. Calculated %: C 62.26; H 8.01.

t-Butyl (Benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-L-lactate (XI) was prepared as an oil under the conditions of the preceding experiment from (IX) and (VIII) in 83% yield: $[\alpha]\ D^{20}$ −47° (s 1.5, C_2H_5OH). Found %: C 60.98; H 7.84. $C_{28}H_{42}N_2O_9$. Calculated %: C 61.07; H 7.68.

t-Butyl D-Valyl-L-lactoyl-L-valyl-D-α-hydroxyisobalerate (XII). 5.7 g of (X) was hydrogenated in 80 ml of methanol (20°, 760 mm) in presence of 0.4 g of palladium black until the theoretical amount of hydrogen had been absorbed (about two hours). The carbon dioxide liberated in the hydrogenation was absorbed in 1N NaOH. Catalyst was filtered off, solvent was extracted with 1 M tartaric acid solution. The acid extract was neutralized with dry $NaHCO_3$, the oil liberated was extracted with ether and the ethereal solution was washed with water and dried with $MgSO_4$. After the removal of solvent we obtained 3.7 g (77%) of the ester (XII), m.p. 97-98° (ether); $[\alpha]\ D^{20}$ −32.6° (s1, C_2H_5OH). Found %: C 59.21; H 9.17. $C_{22}H_{40}N_2O_7$. Calculated %: C 59.43; H 9.07.

(Benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleric Acid (XIII). 5.7 g of (X) was dissolved in 12 ml of trifluoroacetic acid, the solution was left at 18-20° for 20 min, and solvent was driven off in a vacuum at 30-35°. The residue was dissolved in ether, and the solution was extracted repeatedly with saturated sodium bicarbonate solution. The extract was acidified with 10% HCl, and the oil which separated was extracted with ether. The ethereal solution was washed with water until neutral to Congo Red and dried with $MgSO_4$. Solvent was vacuum-distilled off. We obtained 4.5 g (87%) of the acid (XIII) as an amorphous powder; $[\alpha]\ D^{20}$ −12° (s 1, C_2H_5OH). Found %: C 59.50; H 7.25; mol. wt. 537 (titration). $C_{26}H_{38}N_2O_9$. Calculated %: C 59.75; H 7.31; mol. wt. 522.5.

(Benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-L-lactic Acid (XIV) was prepared as an amorphous powder from (XI) under the conditions of the preceding experiment in 82% yield;

[α] D^{20} −21.5° (s 1.8, C_2H_5OH). Found %: C 58.42; H 7.06; mol.wt. 515 (titration). $C_{24}H_{34}N_2O_9$. Calculated %: C 58.29; H 6. 93; mol. wt. 494.

t-Butyl(Benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovalerate (XV) was prepared by the acid chloride method under the conditions of the preparation of (X) from (XIV) and t-butyl L-valyl-D-α-hydroxyisovalerate [9] in 95% yield; [α] D^{20} +27° (s 2, C_2H_5OH). Found %: C 61.04; H 8.07. $C_{38}H_{59}N_3O_{12}$. Calculated %: C 60.86; H 7.93.

(Benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleric Acid (XVI) was prepared from (XV) under the conditions of the synthesis of (XIII) as an amorphous powder in 93% yield; [α]D^{20}−34° (s 1.8, C_2H_5OH). Found %: C 58.96; H 7.54; mol. wt. 660 (titration). $C_{34}H_{51}N_3O_{12}$. Calculated %: C 58.86; H 7.41; mol. wt. 693.

t-Butyl (benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-D-α-hydroxyisovalerate (XVII) was prepared by the acid chloride method under the conditions of the synthesis of (X) from (XVI) and t-butyl-D-valyl-D-α-hydroxyisovalerate [6] as an amorphous powder in 95% yield; [α] D^{20}−1.4° (s 2, C_2H_5OH). Found %: C 60.55; H 8.07. $C_{48}H_{76}N_4O_{15}$. Calculated %: C 60.73; H 8.07.

t-Butyl (Benzyloxycarbonyl)D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovalerate (XVIII) was prepared as an amorphous powder under the conditions of the synthesis of (X) from (XII) and (XIII) in 90% yield; [α] D^{20} 0° (s 2, C_2H_5OH). Found %: C 60.62; H 8.18. $C_{48}H_{76}N_4O_{15}$. Calculated %: C 60.73; H 8.07.

(Benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleric Acid (XIX) was prepared from (XVIII) as an amorphous powder under the conditions of the synthesis of (XIII) in 84% yield; [α]D^{20}−3.5° (s 1.5, C_2H_5OH). Found %: C 59.36; H 7.60; mol. wt. 894 (titration). $C_{44}H_{68}N_4O_{15}$. Calculated %: C 59.17; H 7.68; mol wt. 893.

t-Butyl(Benzyloxycarbonyl)-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-Lactoyl-L-valyl-D-α-hydroxyisovalerate (XX) was prepared under the conditions of the synthesis of (X) from (XIX) and (XII) in 87% yield; [α] D^{20}+1.5° (s 1.5, C_2H_5OH). Found %: C 60.21; H 8.21. $C_{66}H_{106}N_6O_{21}$ Calculated %: C 60.07; H 8.09.

D-Valyl-L-lactoyl-L-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-D-α-hydroxyisovaleric Acid Hydrobromine (XXI). 3 g of the protected octadepsipeptide (XVII) was dissolved in 50 ml of methanol and hydrogenated in presence of 0.15 g of palladium black until the theoretical amount of hydrogen had been absorbed (about two hours). Catalyst was filtered off, the filtrate was evaporated to dryness, and the residue was dissolved in 15 ml of a 30% solution of HBr in glacial acetic acid. The solution was left for one hour at room temperature, solvent was driven off, and the residue was washed carefully with dry ether and vacuum-dried over P_2O_5. We obtained 2.4 g (90%) of the hydrobromide (XXI) as an amorphous powder; [α] D^{20}−26.5° (s 1, C_2H_5OH). Found %: Br 9.80; mol. wt. 820 (titration). $C_{36}H_{63}BrN_4O_{13}$. Calculated %: Br 9.52; mol. wt. 839.8.

D-Valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleric Acid Hydrobromide (XXII) was prepared from the protected dodecadepsipeptide (XX) under the conditions of the preceding experiment as an amorphous powder in 91% yield; [α] D^{20}−16.6° (s 1, C_2H_5OH). Found %: Br 6.84; mol. wt. 1200 (titration). $C_{54}H_{93}BrN_6O_{19}$. Calculated %: Br 6.61; mol. wt. 1210.4.

Cyclo (D-valyl-L-lactoyl-L-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-D-α-hydroxyisovaleryl) (II) and Cyclo (D-valyl-L-lactoyl-L-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl-D-valyl-D-α-hydroxyisovaleryl)$_2$ (IV). 1.2 g of the hydrobromide (XXI) was dissolved in 10 ml of $SOCl_2$, the solution was left for 30 min at 30°, excess of $SOCl_2$ was vacuum-distilled off, dry benzene was added to the residue, and the mixture was again vacuum-evaporated at not above 30-35°. The acid chloride formed was dissolved in 250 ml of dry benzene and this solution and a solution of 1.5 ml of triethylamine in 250 ml of dry benzene were added simultaneously dropwise in the course of nine hours to 1500 ml of dry benzene at 20°. The mixture was left overnight benzene was vacuum-distilled off down to a volume of 200 ml, and the solution was washed with 5% HCl,

saturated $NaHCO_3$ solution, and water and was dried with $MgSO_4$. Solvent was driven off, and the residue (about 1g) was chromatographed on a column of neutral alumina (activity II). By gradient elution in benzene -ethyl acetate we obtained two fractions.

Fractions I was the cyclooctadepsipeptide (II), 90 mg (8%); m.p. 221-222° (benzene-ether); [α] D^{20} +2° (s 1, $CHCl_3$). Found %: C 58.37; H 8.03; mol. wt. 762; 750 (thermoelectric method in chloroform). $C_{36}H_{60}N_4O_{12}$. Calculated %: C 58.36; H 8.16; mol. wt. 740.8.

Fraction II was the cyclohexadecadepsipeptide (IV), 220 mg (20%); m.p. 260-261° (alcohol); [α] D^{20} −23° (s 2, $CHCl_3$). Found %: C 58.76; H 8.13; mol. wt. 1420, 1445. $C_{72}H_{120}N_8O_{24}$. Calculated %: C 58.36; H 8.16; mol. wt. 1481.7.

Cyclo (D-valyl-L-lactoyl-L-valyl-D- α-hydroxyisovaleryl)$_3$, Valinomycin (III). Under the conditions of the preceding experiment from 1.2 g of the hydrobromide (XXII) we obtained an acid chloride, which was dissolved in 250 ml of dry benzene and added (20°, nine hours) simultaneously with a solution of 0.6 ml of triethylamine in 250 ml of dry benzene of 1 liter of dry benzene. The reaction mixture was treated as in the preceding experiment. We obtained 0.9 g of an amorphous substance, which was chromatographed on a column of neutral alumina (activity II). By gradient elution in benzene-ethyl acetate we isolated 270 mg (24.5%) of the cyclododecadepsipeptide (III); m.p. 187-188° (dibutyl ether); [α] D^{20} +32.8° (s 1, C_6H_6). Found %: C 58.45; H 8.20; mol. wt. 1086. $C_{54}H_{90}N_2O_{18}$. Calculated %: C 58.36; H 8.16; mol. wt. 1111.4.

The substance melted without depression in admixture with a sample of natural valinomycin; its biological activity was identical to that of the natural antibiotic (0.75 γ/ml against Candida albicans and 4 γ/ml against Mycobacterium phlei).

CONCLUSIONS

1. The octadepsipeptide cyclo (D-valyl-L-lactoyl-L-valyl-L-lactoyl-L-valyl-D-α-hydroisovaleryl-D-valyl-D-α-hydroxyisovaleryl) (II) was synthesized. The formula (II) does not correspond to the structure of the antibiotic valinomycin.

2. Valinomycin is the dodecadepsipeptide cyclo (D-valyl-L-lactoyl-L-valyl-D-α-hydroxyisovaleryl)$_3$, and its synthesis was effected by the cyclization of the corresponding linear dodecapdepsipeptide.

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