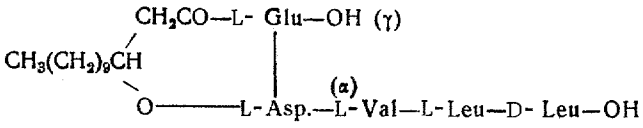
UDC 547.9

STRUCTURE OF THE ANTIBIOTIC ESPERINE

Yu. A. Ovchinnikov, V. T. Ivanov, P. V. Kostetskii, and M. M. Shemyakin

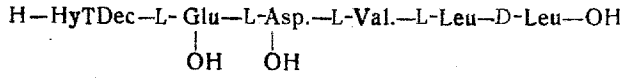
Khimiya Prirodnykh Soedinenii, Vol. 4, No. 4, pp. 236-244, 1968

In 1959, the Japanese workers Ito and Ogawa proposed for the antibiotic esperine (mp 238° C, $[\alpha]_D^{15}$ −24°, c 0.66, in CH_3OH), which they had isolated previously from the culture liquid of certain strains of Bacillus mesentericus [1, 2], the structure of a cyclolinear hexadepsipeptide (1) [3]. However, since the question of the configuration of the β-hydroxytridecanoic acid (H-HyTDec-OH) residue remained unresolved, the antibiotic could have either structure (1a) or (1b).



(1a): L-HyTDec; (1b): D-HyTDec.

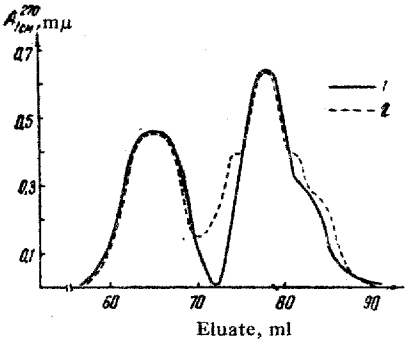
In the course of investigation in the chemistry of the depsipeptides we have effected the synthesis of the diastereoisomeric hydroxyacylpentapeptides (2a) and (2b) [4, 5]. According to the Japanese authors [3], one of these compounds should correspond to the product of the alkaline hydrolysis of esperine—esperinic acid:



(2a): L-HyTDec; (2b): D-HyTDec.

However, the properties of the hydroxyacylpeptides (2a) and (2b) that we have synthesized differ sharply from those of esperinic acid. Consequently, the latter could not possess the structure given to it.

The results obtained, of course, threw doubt upon the correctness of the structure of esperine itself (1). At the same time, it was impossible to exclude the possibility that esperine has the structure (1), but on alkaline hydrolysis a tricarboxylic acid differing in structure from the hydroxyacylpentapeptide (2a) or (2b) (for example, because of the transformation of the esperine molecule with the formation of the corresponding linear β- and γ-peptides of aspartic and glutamic acids by an alkali-catalyzed rearrangement). Consequently, for a definitive resolution of the problem of the structure of the antibiotic, we undertook a total synthesis of the depsipeptides (Ia) and (Ib). Depsipeptide structures of type (1) have not been found in nature and have not been obtained synthetically, in view of which their synthesis offered an independent interest, since it permitted the study of the physical and chemical features of these strained cyclotridepsipeptide systems.



Results of gel filtration on Sephadex LH-20 (1.7 × 150 cm) (elution with dioxane at the rate of 4.5 ml/hr): 1) 3 mg of the products of the cyclization of the hydrochloride (14b); 2) mixture (3 mg) of the products of of the cyclization of the hydrochloride (14b), 0.8 mg of the p-nitrobenzyl ester of L-benzyloxytridecanoyl-L-γ-benzylglutamyl-L-β-benzyl-aspartyl-L-valyl-L-leucyl-D-leucine [5] (mol. wt. 1205) and 0.4 mg of (15a) (mol. wt. 1007).

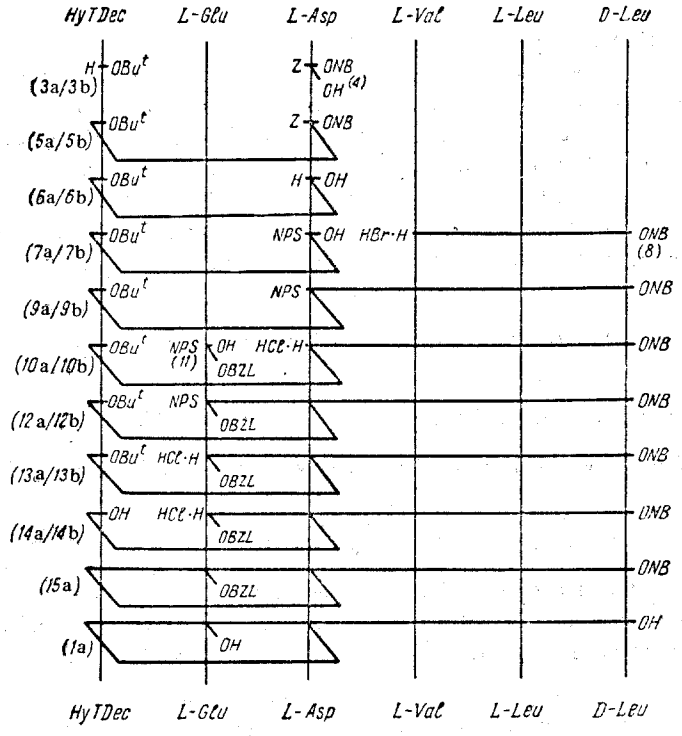
We decided on a plan of the synthesis in which the ester bond was created in the first stage and then the linear hexadepsipeptide containing a β-hydroxy acid residue at the C-end was built up and then, finally, cyclization through an amide bond was carried out between the carboxy group of the β-hydroxytridecanoic acid and the amino group of the glutamic acid residue. This made it possible to use the extremely effective acid-chloride method in the concluding stage of the synthesis (scheme)*.

By condensing the α-p-nitrobenzyl ester of carbobenzoxy-L-aspartic acid (4) [6] with the tert-butyl ester of L- or D-β-hydroxytridecanoic acid (3a) or (3b)** by the mixed-anhydride method (benzenesulfonyl chloride in pyridine) we obtained the corresponding triesters (5a) and (5b), the hydrogenolysis of which in the presence of a Pd catalyst led to the amino esters (6a) and (6b). The latter were converted by treatment with o-nitrobenzenesulfenyl chloride into the corresponding o-nitrobenzenesulfenyl derivatives (7a) and (7b).

*In the scheme, the index "a" denotes compounds containing the L-β-hydroxytridecanoic (HyTDec) residue and the index "b" compounds containing the D-β-hydroxytridecanoic acid residue.

**The esters (3a) and (3b) were obtained by the reaction of the silver salts of the corresponding β-hydroxy acids with tert-butyl bromide.

These derivatives were condensed by the carbodiimide method with the previously-synthesized [4, 5] tripeptide ester (8). The removal of the N-protective group from the pentadepsipeptides (9a) and (9b) formed the hydrochlorides of the amino esters (10a) and (10b), which were then condensed with the γ-benzyl ester of o-nitrobenzenesulfenyl-L-glutamic acid (11) [7] with the aid of N,N'-dicyclohexylcarbodiimide. Then from the hexadepsipeptides (12a) and (12b), we successively split off under the appropriate conditions the o-nitrobenzenesulfenyl protective groups, giving the amino esters (13a) and (13b), and then the tert-butyl protective groups, giving the hexadepsipeptides (14a) and (14b).



The cyclization of compound (14a) by the acid chloride method under conditions of high dilution in benzene solution led to the corresponding cyclodepsipeptide (15a) with a yield of 30%. However, in attempts to cyclize the stereoisomeric depsipeptide (14b) by various methods (acid-chloride method, and using dicyclohexylcarbodiimide or carbonyldiimidazole), it was impossible to isolate the cyclic reaction product. The results of the filtration through Sephadex LH-20 of a dioxane solution of the reaction products after cyclization by the acid chloride method (figure) showed that in addition to the linear hexadepsipeptide the mixture contained a considerable amount of high-molecular-weight compounds and products of the decomposition of the initial acid chloride. The cause of such different behaviors of the depsipeptides (14a) and (14b) in cyclization is apparently conformational factors determining the preferred conformation of the initial linear compounds and the degree of strain of the rings formed (see [8]).

The hydrogenolysis of the cyclic diester (15a) in the presence of a Pd catalyst gave the cyclodepsipeptide (1a), which forms one of the possible structures proposed for the antibiotic esperine. The structures of the cyclodepsipeptides (15a) and (1a) were shown by a determination of their molecular weights by the thermoelectric method (in dioxane) and by isothermal distillation (in chloroform) and by the results of titration (for 1a), and were also confirmed by their IR and NMR spectra.

Although the cyclodepsipeptide (1a) had properties (mp 238–241° C; $[\alpha]_D^{20}$ −30°; c 1 in CH_3OH) similar to those of esperine, it was completely devoid of antimicrobial activity. Its direct comparison with a sample of natural esperine kindly given to us (2 mg) by Dr. Ito showed that these compounds differed fundamentally from one another (ORD curves, chromatographic behavior in a thin layer of silica, solubility, etc.). Thus, formula (1a) proposed by the Japanese workers does not correspond to the structure of esperine.

We also established that the alkaline hydrolysis of compound (1a) under various conditions (1 N NaOH, 100 g-eq or 0.05 N NaOH, 3 g-eq) leads to the corresponding acid (2a) with a high yield, i.e., the saponification of the cyclohexadepsipeptide (1a) is not accompanied by intramolecular rearrangements involving the ω-carboxy groups in the peptide chain. Consequently, it seems to us unlikely that esperine corresponds to structure (1b) with a D-β-hydroxytridecanoic acid residue, either, since the acid (2b) diastereoisomeric with the acid (2a) differs from the product of the alkaline hydrolysis of esperine—esperinic acid. The same fact permitted the conclusion that esperine is not a cyclopolymer homolog of the depsipeptide (1a) or (1b) with a larger ring.

Thus, the data given above permit the conclusion that formulas (1a) and (1b) proposed by the Japanese workers do not correspond to esperine, and the question of its structure must be considered open.

Experimental

All the melting points are uncorrected. The purity of the compounds obtained was checked by paper or thin-layer chromatography on alumina, silica, or silica gel. The optical rotatory dispersion was measured on a Jasco ord/Uv-5 instrument at 20° C with a cell thickness of 2 mm on solutions in ethanol with concentrations of 0.03–0.5%.

tert-Butyl L-β-hydroxytridecanoate (3a). With stirring, 100 ml of a 1 N aqueous solution of caustic potash and 100 ml of a 1 N solution of silver nitrate were added to a solution of 23 g (0.1 mole) of L-β-hydroxytridecanoic acid [4, 5] in 20 ml of methanol. The precipitate that deposited was carefully washed with distilled water until Cl ions were absent from the filtrate and was dried in the dark in a vacuum desiccator over calcium chloride. The silver salt so obtained (32 g, 95%) was suspended in 300 ml of dry benzene, 27 g (0.2 mole) of tert-butyl bromide was added to the suspension, and the mixture was stirred at 20° C for 24 hr. The solid matter was filtered off, the filtrate was evaporated in vacuum, the residue was treated with hexane, the unchanged L-β-hydroxytridecanoic acid (10 g) was separated off, and the filtrate was chromatographed on neutral alumina in the benzene–hexane system (gradient elution). Two main fractions were isolated from the eluate.

The first fraction (3 g, 16%) consisted of tert-butyl-L-β-tert-butyloxytridecanoate; $[\alpha]_D^{20}$ +5° (c 5; chloroform, n_D^{20} 1.4425.

Found, %: 74.04; H 12.30. Calculated for $C_{21}H_{42}O_3$, %: C 73.95; H 12.38.

The second fraction (8 g, 50%) was tert-butyl L-hydroxytridecanoate (3a); $[\alpha]_D^{20}$ +14.5° (c 7; chloroform); n_D^{20} 1.4478.

Found, %: C 71.20; H 12.07. Calculated for $C_{17}H_{34}O_3$, %: C 71.28; H 11.96.

tert-Butyl D-β-hydroxytridecanoate (3b). Under the conditions of the preceding experiment, 23 g (0.1 mole) of D-β-hydroxytridecanoic acid [4, 5] and 27.4 g (0.2 mole) of tert-butyl bromide gave 3.2 g (17%) of tert-butyl D-β-tert-butyloxytridecanoate in the form of an oil with $[\alpha]_D^{20}$ −4.5° (c 5; chloroform), n_D^{20} 1.4415.

Found, %: C 74.18; H 12.14. Calculated for $C_{21}H_{42}O_3$, %: C 73.95; H 12.38 and also 9 g (56%) of tert-butyl-D-β-hydroxytridecanoate (3b), $[\alpha]_D^{20}$ −14.5° (c 7; chloroform), n_D^{20} 1.4478.

Found, %: C 71.04; H 11.85. Calculated for $C_{17}H_{34}O_3$, %: C 71.28; H 11.96.

tert-Butyl carbobenzoxy-L-(α-p-nitrobenzyl)-β-asparagyl-L-β-oxytridecanoate (5a). With stirring (−15° C, 3 min) 1.77 g (10 mM) of benzenesulfonyl chloride was added to a solution of 4.01 g (10 mM) of the α-p-nitrobenzyl ester of carbobenzoxy-L-aspartic acid (4) [6] in 10 ml of absolute tetrahydrofuran and 10 ml of dry pyridine. After 25 min (−30° C), a solution of 1.43 g (5 mM) of tert-butyl L-β-hydroxytridecanoate in 2 ml of tetrahydrofuran was added, and the mixture was stirred at −20° C for 1 hr, at 0° C for 6 hr, and at 20° C for 3 hr, and was poured into 20 ml of 1 N hydrochloric acid and extracted with ethyl acetate. The extract was washed with water, dried with sodium sulfate, and evaporated in vacuum. The residue was treated with ether, the solution was filtered, and the filtrate was concentrated in vacuum. After the addition of petroleum ether, the crystals that deposited were separated off and recrystallized from methanol. This gave 2.6 g (78%) of the tert-butyl ester (5a) with mp 70–71° C $[\alpha]_D^{20}$ + 7.6° (c 5; chloroform).

Found, %: C 64.64; H 7.51; N 4.28. Calculated for $C_{36}H_{50}H_2O_{10}$, %: C 64.46; H 7.51; N 4.18.

tert-Butyl carbobenzoxy-L-(α-p-nitrobenzyl)-β-asparagyl-D-β-oxytridecanoate (5b). Under the conditions of the preceding experiment, 4.01 g (10 mM) of the α-p-nitrobenzyl ester of carbobenzoxy-L-aspartic acid (4) and 1.43 g (5 mM) of the tert-butyl ester (3b) gave (after chromatography on neutral Al_2O_3 in the petroleum ether–benzene system with gradient elution) 2.3 g (69%) of the tert-butyl ester (5b) in the form of an oil; $[\alpha]_D^{20}$ +6.9° (c 5; chloroform).

Found, %: C 64.42; H 7.58; N 4.20. Calculated for $C_{36}H_{50}N_2O_{10}$, %: C 64.46; H 7.51; N 4.18.

tert-Butyl L-β-asparagyl-L-β-oxytridecanoate (6a). In 15 ml of methanol containing 0.06 ml (1 mM) of glacial acetic acid, in the presence of a palladium catalyst, 670 mg (1 mM) of the carbobenzoxy triester (5a) was hydrogenated until the theoretical amount of hydrogen had been absorbed (4 hr). The catalyst was filtered off, the filtrate was evaporated in vacuum, and the residue was dried in a vacuum desiccator over phosphorus pentoxide (40° C, 24 hr). After recrystallization from dimethylformamide, 320 mg (80%) of the tert-butyl ester (6a) was obtained with mp 209–210° C, $[\alpha]_D^{20}$ −7.5° (c 2; methanol).

Found, C 62.70; H 9.64; N 3.49. Calculated for $C_{21}H_{37}NO_6$, %: C 62.97; H 9.56; N 3.50.

tert-Butyl L-β-asparagyl-D-β-oxytridecanoate (6b). By the method of the preceding experiment, 670 mg (1 mM) of the carbobenzoxy triester (5b) gave 310 mg (78%) of the tert-butyl ester (6b) with mp 201–202° C (from dimethyl

formamide, $[\alpha]_D^{20}$ −12° (c 2; methanol).

Found, %: C 63.01; H 9.63; N 3.44. Calculated for $C_{21}H_{37}NO_6$, %: C 62.97; H 9.56; N 3.50.

tert-Butyl o-nitrobenzenesulfenyl-L-β-asparagyl-L-β-oxytridecanoate (7a). With stirring, in small portions, 63 mg (0.34 mM) of o-nitrobenzenesulfenyl chloride and, simultaneously, 0.63 ml of a 1 N solution of Na_2CO_3 were added to a suspension of 120 mg (0.3 mM) of the tert-butyl ester (6a) in 5 ml of dimethylformamide. The mixture was stirred at 20° C for 30 min, diluted with ethyl acetate, washed with 1 N H_2SO_4 and water, dried with sodium sulfate, and evaporated. The residue was chromatographed on silica in the benzene–ethyl acetate (10 : 1) system. This yielded 125 mg (75%) of the tert-butyl ester (7a) in the form of a yellow oil, $[\alpha]_D^{20}$ −12.5° (c 1; chloroform).

Found, %: C 58.31; H 7.64; N 5.02; S 5.67. Calculated for $C_{27}H_{12}N_2O_8S$, %: C 58.46; H 7.63; N 5.06; S 5.78.

tert-Butyl o-nitrobenzenesulfenyl-L-β-asparagyl-D-β-oxytridecanoate (7b). Under the conditions of the preceding experiment, 120 mg (0.3 mM) of the tert-butyl ester (6b) and 63 mg (0.34 mM) of o-nitrobenzenesulfenyl chloride yielded 120 mg (73%) of the tert-butyl ester (7b) in the form of a yellow oil; $[\alpha]_D^{20}$ −15° (c 1.6; chloroform).

Found, %: C 58.52; H 7.63; N 5.12; S 5.71. Calculated for $C_{27}H_{42}N_2H_8S$, %: C 58.46; H 7.63; N 5.06; S 5.78.

The protected pentadepsipeptides (9a) and (9b). A. With stirring, 184 mg (0.33 mM) of the hydrobromide of the p-nitrobenzyl ester of L-valyl-L-leucyl-D-leucine (8) [4, 5] and 33 mg (0.33 mM) of triethylamine were added to a solution of 166 mg (0.3 mM) of the tert-butyl ester (7a) in 3 ml of chloroform. The mixture was cooled to 0° C, treated with 68 mg (0.33 mM) of N, N'-dicyclohexylcarbodiimide, and stirred at 0° C for 2 hr and at 20° C for 12 hr. The precipitate was filtered off and the filtrate was evaporated in vacuum; the solid residue was dissolved in 5 ml of ethyl acetate and the solution was kept at 0° C for 2 hr. The precipitate of N, N'-dicyclohexylurea that deposited was filtered off, the filtrate was concentrated in vacuum, and the residue was chromatographed on silica in the benzene–ethyl acetate (4 : 1) system. This gave 180 mg (59%) of the pentadepsipeptide (9a) with mp 155–156° C (from methanol); $[\alpha]_D^{20}$ −26.5° (c 1.7; chloroform).

Found, %: C 60.46; H 7.74; N 8.26; S 3.24. Calculated for $C_{51}H_{78}N_6O_{13}S$, %: C 60.37; H 7.75; N 8.28; S 3.16.

B. Similarly, 166 mg (0.3 mM) of the tert-butyl ester (7b) and 184 mg (0.33 mM) of the hydrobromide (8) gave 175 mg (58%) of the pentadepsipeptide (9b) with mp 134–135° C (from methanol), $[\alpha]_D^{20}$ −39.5° (c 1.7; chloroform).

Found, %: C 60.43; H 7.79; N 8.14; S 3.29. Calculated for $C_{51}H_{78}N_6O_{13}$, %: C 60.37; H 7.75; N 8.28; S 3.16.

Hydrochlorides of the pentadepsipeptides (10a) and (10b). A. A solution of 203 mg (0.2 mM) of the pentadepsipeptide (9a) in 0.5 ml of chloroform was treated with stirring (0° C) with 1.6 ml of a 0.3 N solution of hydrogen chloride (0.5 mM) in chloroform. The mixture was stirred at room temperature for another 15 min and was evaporated in vacuum at 20° C, and the residue was treated with 5 ml of petroleum ether. After filtration, 170 mg (95%) of the hydrochloride (10a) was obtained in the form of a colorless amorphous powder; decomp. p. 133–137° C, $[\alpha]_D^{20}$ −18° (c 1; chloroform).

Found, %: Cl 3.79; mol. wt. 878 (titration with 0.01 N NaOH). Calculated for $C_{45}H_{76}ClN_5O_{11}$, %: Cl 4.06; mol. wt. 898.5.

B. Similarly, 203 g (0.2 mM) of the pentadepsipeptide (9b) yielded 172 mg (96%) of the hydrochloride (10b) in the form of an amorphous powder with decomp. p. 130–134° C, $[\alpha]_D^{20}$ −18° (c 1.3; chloroform).

Found, %: Cl 3.82; mol. wt. 880 (titration 0.01 N NaOH). Calculated for $C_{45}H_{76}ClN_5O_{11}$, %: Cl 4.06; mol. wt. 898.5.

The protected linear hexadepsipeptides (12a) and (12b). A. With stirring, 188 mg (0.33 mM) of the dicyclohexylammonium salt of the γ-benzyl ester of o-nitrobenzenesulfenyl-L-glutamic acid (11) [7] was added to a solution of 270 mg (0.3 mM) of the hydrochloride (10a) in 3 ml of chloroform. The mixture was cooled to 0° C, 68 mg (0.33 mM) of N, N'-dicyclohexylcarbodiimide was added and it was stirred at 0° C for 2 hr and then at 20° C for 12 hr. It was diluted with ethyl acetate and after 30 min it was filtered and the filtrate was evaporated. The residue was recrystallized from methanol to give 230 mg (62%) of the hexadepsipeptide (12a) with mp 196–197° C, $[\alpha]_D^{20}$ −27° (c 0.6, chloroform).

Found, %: C 61.34; H 7.40; N 7.70; S 2.67. Calculated for $C_{63}H_{91}N_7O_{16}S$, %: C 61.31; H 7.43; N 7.77; S 2.60.

B. Under the conditions of the preceding experiment, 270 mg (0.3 mM) of the hydrochloride (10b) and 188 mg (0.33 mM) of the dicyclohexylammonium salt (11) yielded 250 mg (68%) of the hexadepsipeptide (12b) with mp 198° C (from methanol), $[\alpha]_D^{20}$ −50° (c 0.6; chloroform).

Found, %: C 61.30; H 7.40; N 7.63; S 2.65. Calculated for $C_{63}H_{91}N_7O_{16}S$, %: C 61.31; H 7.43; N 7.77; S 2.60.

Hydrochlorides of the linear hexadepsipeptides (13a) and (13b). A. Under the conditions for the synthesis of substances (10a) and (10b), 246 mg (0.2 mM) of the protected hexadepsipeptide (12a) yielded 215 mg (96%) of the hydro-

chloride of the hexadepsipeptide (13a) in the form of a colorless amorphous powder with decomp. p. 135–139° C, $[\alpha]_D^{20}$ –25° (c 1.4; chloroform).

Found, %: Cl 3.25; mol. wt. 1105 (titration with 0.01 N NaOH). Calculated for $C_{57}H_{89}ClN_6O_{14}$, %: Cl 3.17; mol. wt. 1117.5.

B. Similarly, 246 mg (0.2 mM) of the linear hexadepsipeptide (12b) yielded 218 mg (98%) of the hydrochloride (13b) in the form of an amorphous powder with decomp. p. 133–138° C, $[\alpha]_D^{20}$ –28° (c 2.1; chloroform).

Found, %: Cl 3.35; mol. wt. 1100 (titration with 0.01 N NaOH). Calculated for $C_{57}H_{89}ClN_6O_{11}$, %: Cl 3.17; mol. wt. 1117.5.

Hydrochlorides of the linear hexadepsipeptides (14a) and (14b). A. After 30 min (20° C), a solution of 224 mg (0.2 mM) of the hydrochloride (12a) in 0.9 ml of trifluoroacetic acid was evaporated in vacuum, and the residue was dried (40° C, 12 hr) in vacuum over granulated caustic potash. This gave 212 mg (99%) of the hexadepsipeptide hydrochloride (14a) in the form of a colorless amorphous powder with decomp. p. 184–188° C, $[\alpha]_D^{20}$ –21° (c 0.7; chloroform).

Found, %: Cl 3.54; mol. wt. 1030 (titration with 0.01 N NaOH). Calculated for $C_{53}H_{81}ClN_6O_{14}$, %: Cl 3.36; mol. wt. 1061.5.

B. Under similar conditions, 224 mg (0.2 mM) of the hydrochloride (13b) yielded 210 mg (99%) of the hexadepsipeptide hydrochloride (14b) in the form of an amorphous powder, decomp. p. 183–186° C, $[\alpha]_D^{20}$ –23° (c 2.2; chloroform).

Found, %: Cl 3.48; mol. wt. 1020 (titration with 0.01 N NaOH). Calculated for $C_{53}H_{81}ClN_6O_{14}$, %: Cl 3.36; mol. wt. 1061.5.

Cyclization of the linear hexadepsipeptides (14a) and (14b). A. A solution of 212 mg (0.2 mM) of the hydrochloride (14a) in 1 ml of freshly-distilled thionyl chloride was stirred at 20° C for 30 min and was then evaporated in vacuum. 220 mg of the corresponding hydrochloride was obtained in the form of an amorphous powder.

Found, %: Cl 6.53. Calculated for $C_{53}H_{80}Cl_2N_6O_{14}$, %: Cl 6.37.

A solution of 220 mg (0.2 mM) of this hydrochloride in 40 ml of dry methylene chloride and a solution of 60 mg (0.6 mM) of triethylamine in 40 ml of absolute benzene were added simultaneously in drops (20° C, 8 hr) with a vigorous stirring to 250 ml of absolute benzene. The mixture was stirred at 20° C for another 48 hr and was evaporated in vacuum. The residue was dissolved in 1 ml of chloroform and was chromatographed on silica in the benzene–ethyl acetate (3:2) system. This yielded 60 mg (30%) of the protected cyclohexadepsipeptide (15a) with mp 244–245° C (from ethyl acetate), $[\alpha]_D^{20}$ –9.5° (c 1.8; chloroform).

Found, %: C 63.02; H 7.79; N 8.35; mol. wt. 960 (thermoelectric method, in dioxane), 980 (isothermal distillation, in chloroform). Calculated for $C_{53}H_{78}N_6O_{13}$, %: C 63.23; H 7.81; N 8.35; mol. wt. 1007).

B. Under similar conditions 212 mg (0.2 mM) of the hydrochloride (14b) was cyclized. The residue after vacuum evaporation was dissolved in dioxane and filtered through Sephadex LH-20 (see figure). Two fractions were obtained. Fraction 1 (58–72 ml) corresponded to the products of the polymerization of the initial depsipeptide (14b), and fraction 2 (74–89 ml) to the initial compound and the products of its decomposition. Fraction 2 and the hydrochloride (14b) were chromatographed on silica gel in the chloroform–methanol (15:2) system; R_f 0.6 (revealed with ninhydrin). Under similar conditions the cyclic depsipeptide (15a) (mol. wt. 1007) is eluted in the range 80–84 ml and the nitrobenzyl ester of L-β-benzyloxytridecanoyl-L-γ-benzylglutamyl-L-β-benzyl-asparagyl-L-valyl-L-leucyl-D-leucine [4, 5] (mol. wt. 1205) in the range 72–75 ml.

Cyclodepsipeptide (1a). In the presence of a palladium catalyst, 101 mg (0.1 mM) of the protected cyclodepsipeptide (15a) in 3 ml of methanol containing 0.006 ml of glacial acetic acid was hydrogenated (20° C, 750 mm) in a current of hydrogen for 24 hr. The mixture was filtered, the filtrate was evaporated in vacuum, and the residue was dried (40° C, 24 hr) in a vacuum desiccator over phosphorus anhydride. This gave 78 mg (100%) of the cyclodepsipeptide (1a) with mp 238–241° C (from a mixture of acetone and hexane), $[\alpha]_D^{20}$ –30° (c 1; methanol).

Found, %: C 59.60; H 8.57; N 8.91; mol. wt. 860 (titration with 0.01 N NaOH). Calculated for $C_{39}H_{67}N_5O_{11}$, %: C 59.90; H 8.63; N 8.95; mol. wt. 782.

The cyclodepsipeptide (1a) and natural esperine were chromatographed on silica gel in the chloroform–acetic acid (3:1) system; R_f 0.6 and 0.65, respectively.

Measurement of the optical rotatory dispersion. A. The cyclodepsipeptide (1a): $[\alpha]_{266}$ –940°; $[\alpha]_{250}$ –2200°; $[\alpha]_{239}$ –3200° (trough); $[\alpha]_{215}$ +5500°.

B. Esperine: $[\alpha]_{266}$ –320°; $[\alpha]_{250}$ –480°; $[\alpha]_{231}$ –740° (trough); $[\alpha]_{213}$ +810°.

Alkaline hydrolysis of the cyclodepsipeptide (1a). A solution of 15.6 mg (0.02 mM) of the cyclodepsipeptide (1a) in 0.25 ml of ethanol was treated with 0.2 ml of a 0.4 N aqueous solution of caustic soda. The mixture was left at 20° C for 12 hr and was acidified with 1 N hydrochloric acid to pH 2, after which the precipitate that deposited was dried in a vacuum desiccator over phosphorus anhydride. This yielded 9 mg (56%) of the hydroxyacyl peptide (2a) with mp 179–180° C (from a mixture of acetone and hexane), $[\alpha]_D^{20}$ −29.5° (c 2; ethanol). The substance was identical with the compound that we have described previously [4, 5] in respect to its chromatographic behavior in a fixed thin layer of silica in chloroform–acetic acid (4 : 1) system, R_f 0.3.

Conclusions

It has been shown that the formula (1) proposed by the Japanese workers Ito and Ogawa does not correspond to the structure of the antibiotic esperine.

REFERENCES

1. Japanese patent 1145, 1951; C. A., 46, 11596, 1952.
2. Japanese patent 2497, 1951; C. A., 47, 690, 1953.
3. T. Ito and H. Ogawa, Bull. Agr. Chem. Soc. Japan, 23, 536, 1959.
4. Yu. A. Ovchinnikov, V. T. Ivanov, P. V. Kostetsky, and M. M. Shemyakin, Tetrah. Lett., 5285, 1966.
5. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, and P. V. Kostetskii, ZhOKh, 37, 2617, 1967.
6. E. Schröder and E. Klieger, Lieb. Ann., 673, 208, 1964.
7. L. Zervas and C. Hamalidis, J. Am. Chem. Soc., 87, 99, 1965.
8. Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, and M. M. Shemyakin, DAN SSSR, 153, 1342, 1963.

1 April 1967

Institute of the Chemistry of Natural Compounds AS USSR