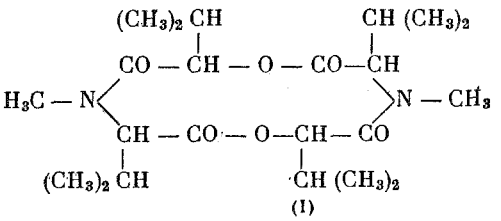
DEPSIPEPTIDES

COMMUNICATION 7. STRUCTURE OF ENNIATIN B*

M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov

Institute for the Chemistry of Natural Products, Academy of Sciences, USSR
Translated from Izvestiya Akademii Nauk SSSR, No. 12, 2154-2161, December, 1962
Original article submitted April 19, 1962

In 1947 Plattner and co-workers [2-4] isolated two new antibiotics from the mycelium of some species of Fusarium. They were named enniatin A ($C_{24}H_{42}N_2O_6$; m.p. 121-122°; $[\alpha]_D^{20}$ − 92°; c = 1 in chloroform) and enniatin B ($C_{22}H_{38}N_2O_6$; m.p. 173-175°; $[\alpha]_D^{21}$ − 108°; c = 0.6 in chloroform), and were found to be highly active against mycobacteria. On the basis of the results of acid and alkaline hydrolysis, one of these, enniatin B, was considered to have the structure (I) [5]:

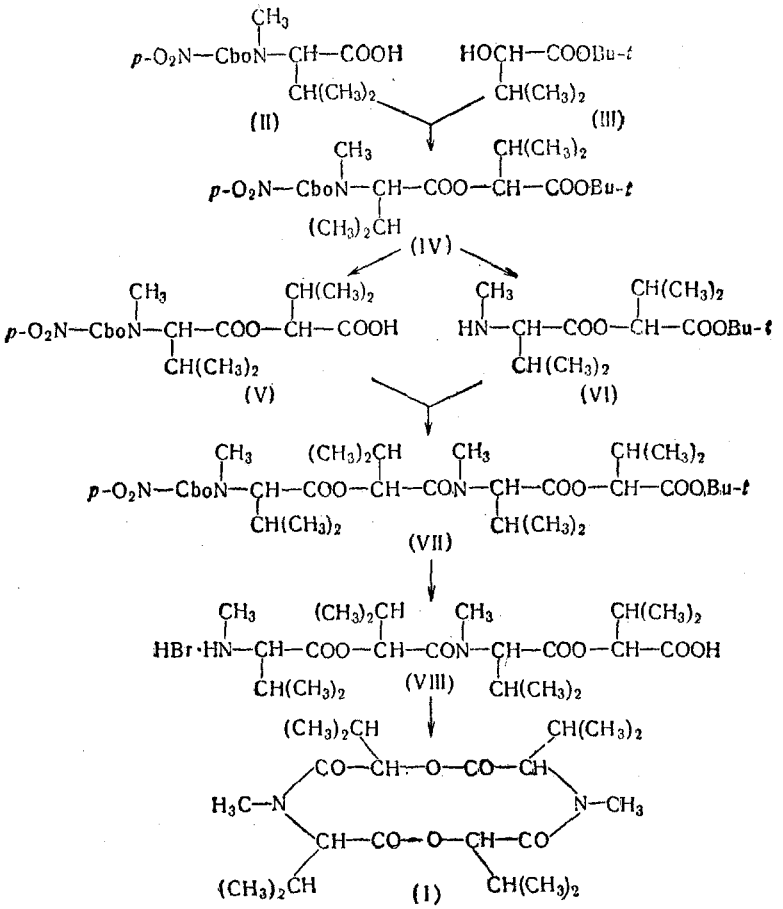


(I)

In the same year Cook and co-workers [6] isolated five related antibiotics from the culture fluids of some species of Fusarium, and one of these, lateritiin-1 ($C_{26}H_{46}N_2O_7$; m.p. 121-122°; $[\alpha]_D^{20}$ − 95.6°; c = 1 in ethanol), was found to be identical in properties to enniatin A. Later, however, it was shown [7, 8] that in the cleavage of lateritiin-1 the same compounds are formed as in the cleavage of anniatin B. These results did not allow us to regard formula (I) for enniatin B as finally proved.

On the basis of previously developed methods for the synthesis of optically active linear and cyclic depsipeptides [9, 10] we undertook the synthesis of the cyclotetradepsipeptide (I), which has the structure attributed by Plattner to enniatin B. On condensing N-(p-nitrobenzyloxycarbonyl)-N-methyl-L-valine (II) with t-butyl D-α-hydroxyisovalerate [t-butyl D-2-hydroxy-3-methylbutyrate] (III) by the method of mixed anhydrides (benzenesulfonyl chloride in pyridine) we obtained t-butyl N-(p-nitrobenzyloxycarbonyl)-N-methyl-L-valyl-D-α-hydroxyisovalerate (IV) in 80% yield. Boiling of this in benzene in presence of p-toluenesulfonic acid led to the p-nitrobenzyloxycarbonyl acid (V); on the other hand, by the hydrogenolysis of (IV) in presence of a palladium catalyst the amino ester (VI) was formed. For the linking of the fragments (V) and (VI) with an amide linkage we used the acid chloride method: by the action of phosphorus pentachloride on the p-nitrobenzyloxycarbonyl acid (V) we obtained its acid chloride, the condensation of which with the amino ester (VI) in presence of triethylamine led to the tetradepsipeptide (VII) in 90% yield; when dicyclohexylcarbodiimide was used, the yield of (VII) was 60%.

*For preliminary communication see [1].

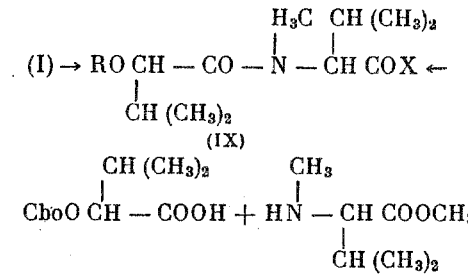


The simultaneous removal of the C- and N-protective groups from the tetradepsipeptide (VII) was effected by the action of hydrogen bromide in glacial acetic acid, as a result of which we obtained a 70% yield of N-methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovaleric acid hydrobromide (VIII)*. On the cyclization of this compound by the method of mixed anhydrides (ClCOOEt in tetrahydrofuran) we obtained the cyclotetradepsipeptide (I) in 17% yield. When the acid chloride method was used for this purpose ($SOCl_2$; Et_3N in benzene), the yield of the cyclotetradepsipeptide (I) was 60%, and in this case we isolated simultaneously, in about 2% yield, the stereoisomeric cyclotetradepsipeptide (Ia), in which one of the N-methylvaline residue has the D-configuration**.

In its physical, biological, and, to some extent, chemical properties the product (I) differed substantially from enniatin B. The structure of the cyclodepsipeptide (I) was established as follows. Determination of the molecular weight by three different methods gave the values 424 (cryoscopy), 417 (isothermal distillation), and 460 (thermoelectric method), in agreement with the formula (I). In the acid hydrolysis of the cyclodepsipeptide (I) we isolated D-α-hydroxyisovaleric acid (80% yield) and N-methyl-L-valine (90% yield). In the hydrolysis of (I) with barium hydroxide and subsequent treatment of the hydrolysis products with diazomethane we obtained D-α-hydroxyisovaleryl-N-methyl-L-valine methyl ester (IXa), and from this we prepared the amide (IXb) and the hydrazide (IXc). The methyl ester (IXa) was prepared also by a confirmatory synthesis by the acid chloride method starting from O-(benzyloxycarbonyl)-D-α-hydroxyisovaleric acid (X) and N-methyl-L-valine methyl ester hydrochloride (XI) with subsequent hydrogenolysis of the ester (IXd). The products (IXa), (IXb), and (IXc) were found to be identical with the previously described [5, 11] degradation products of enniatin B.

*The free tetradepsipeptide (VIIIa) can be isolated from the hydrobromide (VIII) by the action of silver carbonate in aqueous solution.

**The formation of the cyclotetradepsipeptide (Ia) was associated with the presence of a little D-isomer impurity in the original N-(p-nitrobenzyloxycarbonyl)-N-methyl-L-valine (II).



(IXa): R = H, X = OCH_3; (IXb): R = H, X = NH_2; (IXc): R = H, X = $NHNH_2$; (IXd): R = Cbo, X = OCH_3

The structure of the cyclotetradepsipeptide (I) was confirmed by its infrared spectrum. The substance showed no activity against Mycobacterium phlei at concentrations of up to 150 γ/ml, whereas enniatin B is active at a concentration of 3 γ/ml [4].

The structure of the cyclotetradepsipeptide (Ia) was established in the same way as that of the compound (I). In the determination of its molecular weight we obtained values of 435 (isothermal distillation) and 460 (thermoelectric method), and as a result of complete hydrolysis we isolated D-α-hydroxyisovaleric acid and racemic N-methylvaline in high yields.

Hence, the formula (I) does not correspond to enniatin B, and the question of its structure remains open. At the present time we are occupied in verifying the structures of other natural cyclodepsipeptides, in the first place enniatin A, a formula for which was proposed by Plattner in 1948 [12].

EXPERIMENTAL

N-(p-Nitrobenzyloxycarbonyl)-N-methyl-L-valine (II). This was prepared by the resolution of the racemate with the aid of L-threo-2-amino-1-p-nitrophenyl-1,3-propanediol; constants: m.p. 83.5-84° (from 1:1 mixture of benzene and hexane); $[\alpha]_D^{20}$ − 84° (c = 1.0 in benzene) [13].

O-(Benzyloxycarbonyl)-D-α-hydroxyisovaleric Acid. 188 g of benzyl chloroformate was added with stirring at − 20° for one hour to a solution of 118 g of D-α-hydroxyisovaleric acid [14] in 800 ml of dry pyridine. The mixture was stirred for two hours at 0° and left overnight; 200 ml of water was added, and pyridine was vacuum-distilled off. The residue was dissolved in 1200 ml of ether, washed with two 200-ml portions of 2 N HCl and with water, and extracted with six 100 ml portions of saturated $NaHCO_3$ solution. The extract was acidified to Congo Red with concentrated hydrochloric acid, the oil that separated was extracted with three 300 ml portions of ether, and the ethereal solution was dried with magnesium sulfate and evaporated. We obtained 207 g (82%) of O-(benzyloxycarbonyl)-D-α-hydroxyisovaleric acid, m.p. 57-58° (from petroleum ether); $[\alpha]_D^{20}$ + 9.7° (c = 0.6 in benzene). Found: C 61.93; H 6.34%. $C_{13}H_{16}O_5$. Calculated: C 61.89; H 6.39%.

t-Butyl D-α-Hydroxyisovalerate (III). A mixture of 126 g of O-(benzyloxycarbonyl)-D-α-hydroxyisovaleric acid, 1 liter of methylene chloride, and 25 g of p-toluenesulfonic acid was saturated with isobutene until the volume had increased to 500 ml. The solution was left for 70 hours at 20°, diluted with 2 liters of ether, washed with 10% Na_2CO_3 solution and with water, dried with magnesium sulfate, and vacuum-evaporated. The resulting t-butyl O-(benzyloxycarbonyl)-D-α-hydroxyisovalerate (142 g; 92%) was dissolved in 800 ml of absolute alcohol and hydrogenated in a stream of hydrogen (20°, 40 h) in presence of 3 g of palladium oxide. The catalyst was filtered off, the filtrate was vacuum-evaporated, 500 ml of ether was added, and the ethereal solution was washed with saturated $NaHCO_3$ solution, dried with magnesium sulfate, and evaporated. Vacuum distillation of the residue gave 56 g (64%) of the t-butyl ester (III); b.p. 42-43° (0.5 mm); m.p. 30-31° (from hexane); $[\alpha]_D^{20}$ + 2.9° (c = 0.8 in benzene). Found: C 61.70; H 10.48%. $C_9H_{18}O_3$. Calculated: C 62.04; H 10.41%.

t-Butyl N-(p-Nitrobenzyloxycarbonyl)-N-methyl-L-valyl-D-α-hydroxyisovalerate (IV). 25.7 g (18.6 ml) of benzenesulfonyl chloride was added with stirring at 0° for ten minutes to a solution of 47.4 g of N-(p-nitrobenzyloxycarbonyl)-N-methyl-L-valine (II) in 130 ml of dry pyridine. After 15 minutes a solution of 23.1 g of the t-butyl ester (III) in 20 ml of dry pyridine was added, and the mixture was stirred for two hours at 0° and then for three hours at room temperature; the mixture was poured into water and extracted with two 300 ml portions of ether. The extract was washed with two 200 ml portions of 1 N H_2SO_4, with four 100 ml portions of saturated $NaHCO_3$ solution, and with water; it was dried with magnesium sulfate and evaporated. The residual oil (54.7 g) was chromatographed on a column of

neutral alumina. After elution with benzene we obtained 49.4 g (80%) of the ester (IV) as a yellow oil; $[\alpha]_D^{20} - 50°$ (c 1.3 in benzene). Found: C 59.52; H 7.54; N 6.07%. $C_{23}H_{34}O_8N_2$. Calculated: C 59.21; H 7.35; N 6.01%.

N-(p-Nitrobenzyloxycarbonyl)-N-methyl-L-valyl-D-α-hydroxyisovaleric Acid (V). A mixture of 23 g of the t-butyl ester (IV), 2 g of p-toluenesulfonic acid, and 100 ml of benzene was boiled for 90 minutes and diluted with 400 ml of ether. The ethereal solution was washed with water and extracted with four 100 ml portions of saturated $NaHCO_3$ solution. The extract was acidified to Congo Red with concentrated hydrochloric acid, and the oil that separated was extracted with three 100 ml portions of ether. The ethereal solution was dried with magnesium sulfate and evaporated. We obtained 16.2 g (80%) of the p-nitrobenzyloxycarbonyl acid (V), m.p. 83-84° (from 1:1 mixture of benzene and hexane); $[\alpha]_D^{20} - 86°$ (c = 0.6 in benzene). Found: C 55.86; H 6.59; N 7.02%. $C_{19}H_{26}O_8N_2$. Calculated: C 55.60; H 6.39; N 6.83%.

t-Butyl N-Methyl-L-valyl-D-α-hydroxyisovalerate (VI). 20.8 g of the ester (IV) was dissolved in 250 ml of methanol containing 5 ml of glacial acetic acid and was hydrogenated in presence of 0.4 g of palladium oxide until the theoretical amount of hydrogen had been absorbed (4 liters, 20°, 3 h)*. The catalyst was filtered off, the filtrate was vacuum-evaporated at 25-30°, and the residue was dissolved in ether. The ethereal solution was washed with 5% Na_2CO_3 solution, dried with magnesium sulfate, and evaporated. Vacuum fractionation of the residue gave 9 g (70%) of the amino ester (VI); b.p. 86.89° (0.15 mm); $[\alpha]_D^{20} + 22°$ (c = 0.7 in benzene). Found: C 63.01; H 10.05; N 4.73%. $C_{15}H_{29}O_4N$. Calculated: C 62.68; H 10.17; N 4.87%.

t-Butyl N-(p-Nitrobenzyloxycarbonyl)-N-methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovalerate (VII). a) 5.2 g of finely ground phosphorus pentachloride was added to a cooled (0°) solution of 6.6 g of the p-nitrobenzyloxycarbonyl acid (V) in 50 ml of dry ether. The mixture was stirred for two hours at 0°, and the solution was decanted from unchanged PCl_5 and vacuum-evaporated. The residue, which consisted of the acid chloride of the p-nitrobenzyloxycarbonyl acid (V), was dissolved in 20 ml of dry tetrahydrofuran, and the resulting solution was added (− 40°, 1 h) to a solution of 4.02 g of the amino ester (VI) and 1.6 g of triethylamine in 30 ml of dry tetrahydrofuran. The mixture was stirred for one hour, left overnight, and diluted with 200 ml of ether. It was then washed with two 40 ml portions of 1 N H_2SO_4, with water, and with three 50 ml portions of saturated $NaHCO_3$ solution. It was dried with magnesium sulfate and evaporated. The residue was chromatographed on neutral alumina; after elution with a 3:1 mixture of benzene and ethyl acetate we obtained 8.79 g (93 %) of the tetradepsipeptide (VII) as a yellow oil; $[\alpha]_D^{20} - 97°$ (c = 0.5 in benzene). Found: C 59.97; H 7.82; N 6.21%. $C_{34}H_{53}O_{11}N_3$. Calculated: C 60.08; H 7.86; N 6.18%.

b) 134 mg of dicyclohexylcarbodiimide was added to a solution of 282 mg of the p-nitrobenzyloxycarbonyl acid (V) in 2 ml of methylene chloride, and then with ice-cooling and stirring 197 mg of the amino ester (VI) in 2 ml of methylene chloride was added. The mixture was left for 20 hours at 20°, the precipitate was filtered off, and the filtrate was diluted with 20 ml of ether and washed with two 5 ml portions of 1 N H_2SO_4, with water, and with three 10 ml portions of saturated $NaHCO_3$ solution; the solution was then dried with magnesium sulfate and evaporated. After chromatography of the residue on neutral alumina we obtained 270 mg (60%) of the tetradepsipeptide (VII) as an oil; $[\alpha]_D^{20} - 97°$ (c = 0.5 in benzene).

N-Methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D α-hydroxyisovaleric Acid Hydrobromide (VIII). A solution of 7.3 g of the tetradepsipeptide (VII) in 40 ml of a 35% solution of hydrogen bromide in glacial acetic acid was left for nine hours at 20° and vacuum-evaporated. The residue was dissolved in 200 ml of ether, the ethereal solution was extracted with three 100 ml portions of water, and the aqueous solution was vacuum-evaporated; dry toluene was added to the residue and was then vacuum-distilled off; this operation was repeated twice. The amorphous residue was dissolved in 20 ml of ethyl acetate and left overnight at 0°; the precipitated crystals were filtered off and recrystallized from ethyl acetate. We obtained 3.95 g (70%) of the hydrobromide (VIII); m.p. 90-93°; $[\alpha]_D^{20} - 11.5°$ (c = 0.5 in alcohol). Mol.wt. found, 534 (titration with 0.1 N NaOH). $C_{22}H_{41}O_7N_2Br$. Mol. wt. calculated, 525.5.

N-Methyl-L-valyl-D-α-hydroxyisovaleryl-N-methyl-L-valyl-D-α-hydroxyisovaleric Acid (VIIIa). 2 g of freshly precipitated silver carbonate was added to a solution of 2 g of the hydrobromide (VIII) in 30 ml of water, and the mixture was shaken for two hours. The precipitate of silver salts was filtered off, the filtrate was saturated with hydrogen sulfide and boiled for ten minutes, and the precipitate of silver sulfide was filtered off; the filtrate was vacuum-evaporated, and the residue was dried over P_2O_5. We obtained 1.6 g (95%) of the tetradepsipeptide (VIIIa) as an amorphous powder; $[\alpha]_D^{20} - 19.7°$ (c = 0.8 in water). Found: C 59.29; H 9.13; N 6.40%. $C_{22}H_{40}O_7N_2$. Calculated: C 59.43; H 9.07; N 6.30%.

*The carbon dioxide liberated was absorbed with 4 N NaOH.

Cyclization of the Tetradepsipeptide (VIII). a) A solution of 7.1 g of the hydrobromide (VIII) in 30 ml of thionyl chloride was left for 30 minutes at 20°, excess of thionyl chloride was distilled off, and dry toluene was added to the residue and vacuum-distilled off, and then toluene was added once more and again evaporated. The acid chloride formed was dissolved in 400 ml of dry benzene, and simultaneous dropwise addition was made with stirring (at 20° over a period of 11 hours) of this solution and a solution of 3.02 g of triethylamine in 400 ml of dry benzene to 2 liters of dry benzene. The mixture was left overnight, 10 ml of triethylamine was added, and after two hours the reaction mixture was evaporated to dryness. The residue was dissolved in ether, and the ethereal solution was washed with 1 N H_2SO_4, with water, with saturated $NaHCO_3$ solution, and again with water; it was dried with magnesium sulfate and evaporated. The semicrystalline residue was chromatographed on a column of neutral alumina; on elution with benzene and then with a mixture of benzene and ethyl acetate (with progressive increase in the ethyl acetate concentration from 3% to 30%) two fractions were isolated.

Fraction I was the cyclotetradepsipeptide (I); yield 3.4 g (59%); m.p. 228-229° (from heptane); $[\alpha]_D^{20}$ + 4.8° (c = 0.9 in chloroform): ν 1194, 1653, and 1754 cm^{-1}. Found: C 61,81; N 8.98; N 6.71 %; mol. wt. 424 (cryoscopy in benzene), 417 (isothermal distillation in acetone), 460 (thermoelectric method in butyl acetate). $C_{22}H_{38}O_6N_2$. Calculated: C 61.94; H 8.98; N 6.57%; mol. wt. 426.5. Fraction II was the cyclotetradepsipeptide (Ia); yield 0.1 g (1.8%); m.p. 143.5-144° (from hexane); $[\alpha]_D^{20}$ + 219° (c = 0.7 in chloroform); ν 1191, 1655, and 1755 cm^{-1}. Found: C 62.01; H 8.96; N 6.60%; mol. wt. 435 (isothermal distillation in acetone), 460 (thermoelectric method in butyl acetate). $C_{22}H_{38}O_6N_2$. Calculated: C 61.94; H 8.98; N 6.57%; mol. wt. 426.5.

b) To a solution of 22 mg of ethyl chloroformate in 2 liters of dry tetrahydrofuran simultaneous addition was made with stirring (0°, 6 h) of a solution of 109 mg of ethyl chloroformate in 200 ml of dry tetrahydrofuran and a solution of the triethylamine salt of the tetradepsipeptide (VIIIa) prepared from 526 mg of the hydrobromide (VIII) and 200 mg of dry tetrahydrofuran. The mixture was stirred for 30 hours at 20°, and then 0.5 g of triethylamine was added and the mixture was stirred further for three hours, vacuum-evaporated, and treated as described for Expt (a) on the cyclization of the tetradepsipeptide. We obtained 73 mg (17%) of the cyclotetradepsipeptide (I), identical to the sample obtained in the preceding experiment.

Acid Hydrolysis of the Cyclotetradepsipeptide (I). A solution of 190 mg of the cyclotetradepsipeptide (I) in 5 ml of concentrated hydrochloric acid was heated in a sealed tube at 100° for 20 hours. The mixture was diluted with 5 ml of water and extracted with ten 5 ml portions of ether; the ethereal solution was washed with five 5 ml portions of saturated $NaHCO_3$ solution, dried with magnesium sulfate, and evaporated; there remained 106 mg of unchanged cyclodepsipeptide (I).

The aqueous solution was vacuum-evaporated to dryness, and the residue was dissolved in 10 ml of water; 200 mg of silver carbonate was added, and the mixture was shaken for two hours and then treated as described in the experiment on the preparation of (VIIIa). After two vacuum sublimations we obtained 46 mg (89%) of N-methyl-L-valine; m.p. 278° (sublimes); $[\alpha]_D^{20}$ + 28.5° (c = 0.85 in 5 N HCl); cf. [5]. The bicarbonate extract was acidified to Congo Red with concentrated hydrochloric acid, saturated with ammonium sulfate, and extracted with ten 5 ml portions of ether. The ethereal solution was dried with magnesium sulfate and evaporated; after vacuum sublimation of the residue we obtained 38 mg (80%) of D-α-hydroxyisovaleric acid; m.p. 67-68°; $[\alpha]_D^{20}$ − 13.5° (c = 1.2 in acetic acid); $[\alpha]_D^{20}$ − 19° (c = 1.0 in chloroform); cf. [8].

Alkaline Hydrolysis of the Cyclotetradepsipeptide (I). 5 ml of 0.35 N $Ba(OH)_2$ was added to a solution of 295 mg of the cyclodepsipeptide (I) in 10 ml of methanol, and the mixture was left for 30 hours at 35°, diluted with water, extracted with ether, acidified to Congo Red with concentrated hydrochloric acid, and again extracted with ether (ten portions of 10 ml). The ethereal solution obtained by the extraction of the acid solution was washed with water and dried, and at 0° ethereal diazomethane was added until a stable yellow color persisted. After one hour the excess of diazomethane was decomposed with acetic acid, and the ethereal solution was washed with saturated $NaHCO_3$ solution, dried with magnesium sulfate, and evaporated. We obtained 330 mg (97%) of D-α-hydroxyisovaleryl-N-methyl-L-valine methyl ester (IXa); m.p. 68.5-69° (from hexane); $[\alpha]_D^{20}$ − 132° (c = 1.5 in chloroform). The substance was identical to that described in the account of the experiment on the preparation of (IXa).

Complete Hydrolysis of the Cyclotetradepsipeptide (Ia). 1.6 ml of 0.35 N $Ba(OH)_2$ was added to a solution of 83 mg of the cyclodepsipeptide (Ia) in 5 ml of methanol, and the mixture was left at 35° for 30 hours. The solution was diluted with water, extracted with ether, acidified with concentrated hydrochloric acid, and again carefully extracted with ether; the ethereal extract obtained in the second case was dried with magnesium sulfate and evaporated. 7 ml of 20% hydrochloric acid was added to the residue, and the mixture was boiled for 15 hours, diluted with 7 ml

of water, and treated as described for the experiment on the acid hydrolysis of the cyclotetradepsipeptide. We obtained 32 mg (70%) of D-α-hydroxyisovaleric acid; m.p. 67-68°; $[\alpha]_D^{20}$ − 14° (c = 1.0 in acetic acid) and 40 mg (71%) of racemic N-methylvaline, m.p. 295-299° (sublimes).

N-Methyl-L-valine Methyl Ester Hydrochloride (XI). A solution of 12.5 g of N-methyl-L-valine in 1 liter of methanol was saturated with hydrogen chloride and, after 24 hours, vacuum-evaporated; the residue was dissolved in 1 liter of methanol, again saturated with HCl, and left for one day; the operation was repeated yet again. The solid residue remaining after evaporation was carefully vacuum-dried over P_2O_5, and we isolated 17.4 g (100%) of the hydrochloride (XI); m.p. 140-141° (from acetone); $[\alpha]_D^{20}$ + 17.5° (c = 1.0 in water). Found: C 45.96; H 8.87; N 7.46; Cl 19.70%. $C_7H_{16}O_2NCl$. Calculated: C 46.25; H 8.87; N 7.71; Cl 19.51%.

O-(Benzyloxycarbonyl)-D-α-hydroxyisovaleryl-N-methyl-L-valine Methyl Ester (IXd). At 0° 12.5 g of phosphorus pentachloride was added to a solution of 10.1 g of O-(benzyloxycarbonyl)-D-α-hydroxyisovaleric acid in 50 ml of dry ether, and the mixture was stirred at this temperature for two hours. The solution was decanted from the precipitate and vacuum-evaporated; the residue of O-(benzyloxycarbonyl)-D-α-hydroxyvaleryl chloride was dissolved in 50 ml of dry tetrahydrofuran, and the resulting solution was added with stirring (− 40°, 2 h) to a solution of 6 g of the hydrochloride (XI) and 9.8 ml of triethylamine in 50 ml of dry tetrahydrofuran. The mixture was stirred for two hours at 0°, left overnight, and then diluted with 300 ml of ether; the ethereal solution was washed with water, with 1 N H_2SO_4, and with saturated $NaHCO_3$ solution; it was dried with magnesium sulfate and evaporated. We obtained 11.5 g (99%) of the methyl ester (IXd); m.p. 73.5-74° (from hexane); $[\alpha]_D^{20}$ − 83° (c = 1.4 in alcohol). Found: C 63.04; H 7.63; N 3.51%. $C_{20}H_{29}O_6N$. Calculated: C 63.30; H 7.70; N 3.69%.

D-α-Hydroxyisovaleryl-N-methyl-L-valine Methyl Ester (IXa). 6.2 g of the methyl ester (IXd) was dissolved in 100 ml of ethanol and hydrogenated in a stream of hydrogen (20°, 24 h) in presence of 120 mg of palladium oxide with stirring with a magnetic stirrer. We obtained 4 g (100%) of the methyl ester (IXa); m.p. 68.5-69° (from hexane); $[\alpha]_D^{20}$ − 138° (c = 1.3 in chloroform); cf. [5].

Amide of D-α-Hydroxyisovaleryl-N-methyl-L-valine (IXb). A solution of 1 g of the methyl ester (IXa) in 20 ml of methanol was saturated with ammonia and left for 40 hours at 20°. The mixture was evaporated to dryness, and the residue was recrystallized from a 1:1 mixture of benzene and hexane. We obtained 780 mg (83%) of the amide (IXb); m.p. 109-110°; $[\alpha]_D^{20}$ − 169° (c = 1.3 in alcohol); cf. [11].

Hydrazide of D-α-Hydroxyisovaleryl-N-methyl-L-valine (IXc). A mixture of 1 g of the methyl ester (IXa), 0.3 g of hydrazine hydrate, and 7 ml of ethanol was boiled for six hours and then evaporated to dryness; the residue was recrystallized from a 1:1 mixture of benzene and hexane. We obtained 850 mg (85%) of the hydrazide (IXc); m.p. 142-144°; $[\alpha]_D^{20}$ − 163° (c = 1.0 in alcohol); cf. [5].

SUMMARY

1. The cyclotetradepsipeptide (I), whose structure was attributed by Plattner to the antibiotic enniatin B, was synthesized.

2. The compound obtained differs greatly in its properties from the natural antibiotic, and therefore enniatin B does not have the structure (I).

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