Aspochracin, a New Insecticidal Metabolite of
Aspergillus ochraceus

Part II. Synthesis of Hexahydroaspochracin

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Hexahydroaspochracin (II) derived on hydrogenation of aspochracin (I), an insecticidal metabolite of *Aspergillus ochraceus*, was synthesized by cyclization of N-methyl-L-valyl-N-methyl-L-alanyl- α -caprylyl-L-ornithine (XIV). In addition to II, isohexahydroaspochracin (XV) was isolated from the reaction mixture.

As described in the preceding report,¹¹ we have elucidated the structure of aspochracin, a new insecticidal peptide isolated from *Aspergillus ochraceus* Wilhelm, as I on the basis of the degradation experiments conducted mainly on hexahydroaspochracin (II), the hydrogenation product of I. Here we wish to report in details the total synthesis of II which was carried out in order to confirm the validity of the structure I.

At first the synthesis of II was attempted according to the scheme shown in Fig. 1. N-Methyl-L-alanine methyl ester (III) was condensed with carbobenzoxy-N-methyl-L-valine (IV) by the dicyclohexylcarbodiimide (DCCI) method to afford carbobenzoxy-N-methyl-L-valyl-N-methyl-L-alanine methyl ester (V). Hydrogenolysis of V on palladium in methanol containing hydrochloric acid gave N-methyl-L-valyl-N-methyl-L-alanine methyl

$$\text{I; R}=\text{CH}_3\text{-CH=CH-CH=CH-CH=CH-}$$

II; R=CH₃-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-

FIG. 1. Synthetic Scheme (A) of Hexahydroasporcracin.

Z=Carbobenzyloxy; DCCI=Dicyclohexylcarbodiimide.

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FIG. 2. Synthetic Scheme (B) of Hexahydroaspochracin.

Z=Carbobenzy; DCCI=Dicyclohexylcarbodiimide.

ester hydrochloride (VI). Contrary to the expectation, however, treatment of VI with triethylamine at room temperature easily caused cyclization resulting in the formation of a diketopiperazine, N-methyl-L-valyl-N-methyl-L-alanine anhydride, and did not give the desired free base VII.

Then another synthetic scheme was devised as illustrated in Fig. 2, leading to the success in the preparation of II. The protected dipeptide ester V obtained in the former process was hydrolyzed with methanolic sodium hydroxide to give carbobenzy-N-methyl-L-valyl-N-methyl-L-alanine (VIII). On another side, δ -carbobenzy-L-ornithine was acylated with caprylyl chloride in the presence of sodium hydroxide to yield δ -carbobenzy- α -caprylyl-L-ornithine (IX), which was treated with diazomethane. The protected methyl ester X thus obtained was hydrogenolyzed on palladium in methanol containing hydrochloric acid to give α -caprylyl-L-ornithine methyl ester hydrochloride (XI). Attempt to isolate the free base from XI on treatment with triethylamine at room temperature was

unsuccessful, resulting in the formation of a cyclic compound, 3-caprylamino-2-piperidone.²⁾ However, when XI treated with triethylamine at -5°C was immediately coupled with the protected dipeptide VIII by the DCCI method, the expected carbobenzy-N-methyl-L-valyl-N-methyl-L-alanyl- α -caprylyl-L-ornithine methyl ester (XIII) was afforded in a high yield.* The possibility for the occurrence of racemization was expected during the coupling process in N-methyl-L-alanine residue constituting the C-terminal of VIII. As a matter of fact, the NMR measurement indicated that XII was a mixture of diastereoisomers, which were inseparable from each other into optically pure forms.

XII, as a mixture, was hydrolyzed with methanolic sodium hydroxide and the resulting protected tripeptide XIII was catalytically hydrogenolyzed to give N-methyl-L-valyl-N-methyl-L-alanyl- α -caprylyl-L-ornithine (XIV). The free peptide XIV thus obtained was treated with a half equivalent of DCCI to afford the corresponding anhydride, which was cyclized, without isolation, to the desired product in dilute pyridine solution. On successive application to silicic acid column and thin layer chromatography, the product was separated into two components showing positive coloration with the chlorine-*o*-tolidine-potassium iodide reagent.^{3,4)}

The compound with the lower *R_f* value was obtained as crystals melting at 137°C in 3.5% yield and proved complete identity with the authentic hexahydroaspochracin in the mixed melting points, IR (Fig. 3), mass and NMR spectra and optical rotation. Another compound (XV) named isohexahydroaspochracin was obtained as a viscous oil in 1.6%

* Coupling of VI with IX in the similar procedure was unsuccessful.

2) E. Fischer and G. Zemplien, *Ber.*, **42**, 4878 (1909).

3) R. H. Mazur, B. W. Ellis and P. S. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).

4) O. J. Schmid, *J. Chromatog.*, **3**, 499 (1960).

FIG. 3. IR Spectra of Hexahydroaspochracin (II).
— Authentic, --- Synthesis.

FIG. 4. IR Spectrum of Isohexahydroaspochracin (XV).

yield. The molecular ion peak in the mass spectrum of XV was observed at m/e 438, and the IR (Fig. 4) and NMR spectra were quite similar to those of II, though distinct optical activity was not recognized in XV. Paper chromatogram of the hydrolysate of XV with hydrochloric acid revealed the formation of three amino acids together with a fatty acid which are the components of II. Accordingly XV has been proved to be an isomer of II. Since the occurrence of racemization in the N-methyl-L-alanine residue has been indicated during the process from VIII to XII, it is presumable that the structural difference between II and XV originates in the configuration of this amino acid in the both molecules. However, due to the minute

quantity of XV, further examination on this problem was discontinued.

Anyhow, the structure of hexahydroaspochracin assigned in the preceding paper was unequivocally established through this experiment and, in consequence, the structure of aspochracin has been ultimately determined.

EXPERIMENTAL

Melting points were uncorrected. IR measurements were made with JASCO IR-S KCl and Koken DS 301 spectrophotometers, and NMR spectra were measured with a JNM-4H-100 spectrometer. Mass spectra were recorded with a JEOL JMS-OISG spectrometer. Optical rotations were measured with a DIP-S polarimeter.

N-Methyl-L-alanine methyl ester hydrochloride. N-Methyl-L-alanine⁵⁾ (3 g) was added to an ice-cooled solution of 5 ml of thionyl chloride in 100 ml of methanol. After refluxing for 3 hr, the reaction mixture was evaporated to dryness under reduced pressure to yield 3.5 g of the ester hydrochloride.

Carbobenzoxy-N-methyl-L-valyl-N-methyl-L-alanine methyl ester (V). N-Methyl-L-alanine methyl ester hydrochloride (1.3 g) was dissolved in 10 ml of acetonitrile, to which 0.8 g of triethylamine was added with ice-cooling and stirring to give the ester III. After removal of insoluble matters, the filtrate was poured to a solution of 2.1 g of carbobenzoxy-N-methyl-L-valine (IV)^{5,6)} and 1.6 g of dicyclohexylcarbodiimide in 20 ml of acetonitrile with stirring at 0°C. The mixture was stirred for an additional 1 hr at 0°C and left to stand overnight at 4°C. Dicyclohexylurea was removed by filtration, and the filtrate was evaporated under reduced pressure to afford a residual oil, which was dissolved in 50 ml of ethyl acetate. The solution was washed successively with saturated aqueous sodium bicarbonate, 1 N hydrochloric acid and water, and dried over anhyd. sodium sulfate. Evaporation of the solvent under reduced pressure yielded 2.7 g of a syrup. The syrup was dissolved in 5 ml of benzene and applied to a column packed with 30 g silicic acid (Mallinckrodt). Elution with benzene-chloroform (3:2, v/v) afforded 1.8 g of V as a colorless oil. *Anal.* Found: C, 62.60; H, 7.93; N, 7.93. Calcd. for $C_{19}H_{28}O_5N_2$: C, 62.62; H, 7.74; N, 7.69%. $[\alpha]_D^{23} = -139.6^\circ$ ($c=5$, MeOH). IR $\nu_{\max}^{\text{Film}}$ cm^{-1} : 1746, 1690, 1649, 773, 702.

N-Methyl-L-valyl-N-methyl-L-alanine methyl ester hydrochloride (VI). V (500 mg) was dissolved in 30 ml of methanol containing 0.1 ml of conc. hydrochloric acid and subjected to hydrogenolysis on 500 mg of palladium-charcoal at ordinary pressure and room temperature for 2 hr. After removal of the catalyst, the filtrate was evaporated under reduced pressure to give 400 mg of VI. Recrystallization from chloroform-ethyl acetate afforded colorless needles, mp 183~185°C. *Anal.* Found: C, 49.43; H, 8.52; N, 10.28; Cl, 13.16. Calcd. for $C_{11}H_{23}O_3N_2Cl$: C, 49.52; H, 8.69; N, 10.50; Cl, 13.29. $[\alpha]_D^{23} = -40.5^\circ$ ($c=5$, MeOH). IR $\nu_{\max}^{\text{NaIol}}$ cm^{-1} : 1740, 1640, 1215, 1102.

Cyclo-N-methyl-L-valyl-N-methyl-L-alanine anhydride. VI (200 mg) was dissolved in 10 ml of chloroform, to which 0.1 g of triethylamine was added at 0°C with stirring. After separation of insoluble matters, the filtrate was evaporated to dryness to give 140 mg of the anhydride. Recrystallization from ethyl acetate afforded colorless prisms, mp 148°C. *Anal.* Found: C, 60.63; H, 9.07; N, 13.95. Calcd. for $C_{10}H_{18}O_2N_2$: C, 60.58; H, 9.15; N, 14.13%. $[\alpha]_D^{23} + 13.5^\circ$ ($c=5$, MeOH). M^+ in the mass spectrum: 198. IR $\nu_{\max}^{\text{NaIol}}$ cm^{-1} : 1652, 1260, 1179, 1050. NMR (in $CDCl_3$) δ : 1.02 (3H, d), 1.17 (3H, d), 1.58 (3H, d), 2.94 (3H, s), 2.97 (3H, s), 2.20 (1H, m), 3.71 (1H, d), 3.91 (1H, q).

Carbobenzoxy-N-methyl-L-valyl-N-methyl-L-alanine (VIII). V (750 mg) was added to 8 ml of 1 N methanolic sodium hydroxide and the mixture was left to stand for 48 hr at 37°C. After neutralization with dilute hydrochloric acid, the solvent was removed under reduced pressure. Twenty ml of saturated aqueous sodium bicarbonate was added to the residue and the resulting solution was shaken with two 20 ml-portions of ether. The aqueous solution was acidified with dilute hydrochloric acid and extracted with three 20 ml-portions of ethyl acetate. The combined extracts were dried over anhyd. sodium sulfate and evaporated under reduced pressure to give 720 mg of oily VIII. IR $\nu_{\max}^{\text{Film}}$ cm^{-1} : 1740, 1685, 1643, 772, 700.

α -Caprylyl- δ -carbobenzoxy-L-ornithine (IX). Caprylyl chloride (0.65 g) and 8 ml of 2 N sodium hydroxide were simultaneously added to an ice-cooled solution of 1 g of δ -carbobenzoxy-L-ornithine⁷⁾ in 7 ml of 2 N sodium hydroxide during 15 min with stirring, and the stirring was continued for an additional 30 min. The solution was shaken with two 10 ml-portions of ether, acidified with dilute hydrochloric acid and extracted with three 20 ml-portions of ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give 1.3 g of IX. Recrystallization from ethyl acetate-hexane afforded colorless needles, mp 97~98°C. *Anal.* Found: C, 64.00; H, 8.11; N, 7.23. Calcd. for $C_{21}H_{32}O_5N_2$: C, 64.26; H, 8.22; N, 7.23%. IR

* Chemical shifts are expressed in δ (ppm) down field from tetramethylsilane as an internal standard. The abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet; b, broad.

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5) P. Quitt, J. Hellerbach and K. Vogler, *Helv. Chim. Acta*, **46**, 327 (1963).

6) P. A. Plattner, K. Vogler, R. O. Studer, P. Quitt and W. Keller-Schierlein, *ibid.*, **46**, 927 (1963).

$\nu_{\max}^{\text{Nujol}}$ cm^{-1} : 3400, 3260, 1693, 1550, 1525, 762, 703.
 $[\alpha]_{\text{D}}^{25} -2.4^{\circ}$ ($c=5.0$, MeOH).

α -Caprylyl- δ -carbobenzoxy-L-ornithine methyl ester (X). IX (500 mg) was treated with 20 ml of ethereal diazomethane, and the solvent was evaporated to give 520 mg of crystalline X, mp $73\sim 74^{\circ}\text{C}$. IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1} : 3320, 1750, 1689, 1650, 1270, 748, 700.

α -Caprylyl-L-ornithine methyl ester hydrochloride (XI). X (520 mg) was dissolved in 30 ml of methanol containing 0.1 ml of conc. hydrochloric acid and hydrogenolyzed on the same condition as in the case of VI. Recrystallization of the product from chloroform-ethyl acetate afforded XI as colorless needles, mp $122\sim 123^{\circ}\text{C}$. Anal. Found: C, 54.42; H, 9.58; N, 9.12. Calcd. for $\text{C}_{14}\text{H}_{29}\text{O}_3\text{N}_2$: C, 54.44; H, 9.46; N, 9.07%. $[\alpha]_{\text{D}}^{25} -23.8$ ($c=5$, MeOH). IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1} : 3320, 1768, 1652, 1535, 1200, 1170.

Carbobenzoxy-N-methyl-L-valyl-N-methyl-L-alanyl- α -caprylyl-L-ornithine methyl ester (XIII). XI (190 mg) was dissolved in 2 ml of chloroform, to which 66 mg of triethylamine was added with stirring at -5°C . After 10 min, 230 mg of VIII and 140 mg of DCCI in 2 ml of acetonitrile were added to the mixture at -5°C . The stirring was continued for an additional 30 min at -5°C and, further, overnight at 4°C . After removal of resulting dicyclohexylurea, the filtrate was evaporated to a solid, which was dissolved in 30 ml of ethyl acetate. The solution was treated in the same manner as in the case of V. The residual syrup was dissolved in 3 ml of chloroform and applied to a column packed with 10 g of silicic acid. Elution with chloroform containing 2% methanol afforded 350 mg of XII as a colorless oil. Anal. Found: C, 63.66; H, 8.73; N, 9.55. Calcd. for $\text{C}_{32}\text{H}_{52}\text{O}_7\text{N}_4$: C, 63.55; H, 8.67; N, 9.27%. $[\alpha]_{\text{D}}^{25} -96.5^{\circ}$ ($c=5$, MeOH). IR $\nu_{\max}^{\text{Film}}$ cm^{-1} : 3200, 1745, 1690, 1645, 775, 702. NMR (in CCl_4) δ : 0.88 (9H, t), 1.26 (11H, bs), 1.53 (4H, b), 2.75 (3H, bs), 2.95 (3H, bs), 3.09 (3H, d), 5.05 (2H, bs).

Carbobenzoxy-N-methyl-L-valyl-N-methyl-L-alanyl- α -caprylyl-L-ornithine (XIII). XII (300 mg) was dissolved in 4 ml of 1N methanolic sodium hydroxide, and hydrolyzed in the manner similar to that for VIII. Oily XIII was obtained in a yield of 250 mg. IR $\nu_{\max}^{\text{Film}}$ cm^{-1} : 3320, 1723, 1690, 1665, 770, 698.

N-Methyl-L-valyl-N-methyl-L-alanyl- α -caprylyl-L-ornithine (XIV). XIII (180 mg) was dissolved in 20 ml of methanol and subjected to hydrogenolysis on 200 mg of palladium-charcoal in the same way as in the case of VI. The resulting oily XIV weighing 150 mg was subjected to the following cyclization reaction without purification.

Hexahydroaspochracin (II) and isohexahydroaspochracin (XV). Triethylamine (40 mg) was added to an ice-cooled solution of 150 mg of XIV in 2 ml of acetonitrile with stirring.* After 10 min, 38 mg of DCCI was added to the solution and the stirring was continued for an additional 1 hr at 0°C . After removal of insoluble matters, the filtrate was added dropwise to 300 ml of pyridine at 85°C over a period of 1.5 hr with vigorous stirring. The stirring was continued for an additional 2 hr at the same temperature. The solvent was removed to dryness and the residue was dissolved in 30 ml of ethyl acetate. The solution was washed successively with saturated aqueous sodium bicarbonate, 1N hydrochloric acid and water, and dried over anhyd. sodium sulfate. Evaporation of the solvent under reduced pressure gave 92 mg of a syrup. The syrup was dissolved in 1 ml of benzene and applied to a column prepared with 2 g of silicic acid. The eluate with benzene-ethyl acetate-acetone (6:3:1, v/v) afforded 12 mg of an oil, which was further applied to preparative thin layer chromatography using Silica gel (Merck) and a solvent system of chloroform-acetone (3:2, v/v). The zones at R_f 0.48~0.52 and 0.60~0.68 were extracted with 50 ml each of ethyl acetate and the solvents were evaporated under reduced pressure, respectively. The residue obtained from the lower R_f value zone was recrystallized from ethyl acetate-hexane to give 4.6 mg of II as colorless needles, mp 137°C . M^+ in mass spectrum, 438. $[\alpha]_{\text{D}}^{25} -89.81^{\circ}$ ($c=0.2$, MeOH). NMR (in CDCl_3) δ : 0.74 (3H, d), 0.89 (3H, t), 0.91 (3H, d), 1.27 (8H, s), 1.50 (3H, d), 2.94 (3H, s), 3.04 (3H, s).

An oily matter (3 mg) obtained from high R_f value zone was purified through repeated thin layer chromatography to give 2.1 mg of XV as a pure viscous oil. $[\alpha]_{\text{D}}^{25} -0.04^{\circ}$ ($c=0.2$, MeOH). M^+ in mass spectrum, 438. NMR (in CDCl_3) δ : 0.89 (3H, d), 0.94 (3H, t), 0.98 (3H, d), 1.28 (8H, s), 1.36 (3H, d), 3.11 (3H, s), 3.17 (3H, s).

* Triethylamine was added to neutralize the minute quantity of residual hydrochloric acid used for preparation of the catalyst in the preceding process.

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