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STRUCTURE OF ASPOCHRACIN, AN INSECTICIDAL METABOLITE OF ASPERGILLUS OCHRACEUS

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Some of the pathogenic fungi causing silkworm muscardines are known to excrete toxins, of which destruxins A and B, insecticidal depsipeptides produced by Metarrhizium anisopliae, have been extensively studied[1]. Further investigation on metabolites of muscardine fungi led us to the isolation of a new toxic peptide named aspochracin from culture medium of Aspergillus ochraceus WILHELM.

Ethyl acetate extract from the culture filtrate of Asp. ochraceus was chromatographed on a silica gel column. The eluate with a solvent system of benzene-ethyl acetate-acetone (45:40:15) was further purified by a silica gel column using a solvent system of chloroform-acetone (98:2). Thus, aspochracin (I) was obtained as a pale yellow powder, which was confirmed to be homogeneous with thin layer chromatography (t.l.c.) on silica gel. Injection of I to silkworm larvae at a dose of 15 γ/g caused immediate paralysis followed by death.

I has the following physico-chemical properties: $[\alpha]_D^{23}$ -76.0° (c 1, in MeOH); ν_{max}^{Nujol} 3300, 1630, 1520 cm^{-1}; λ_{max}^{MeOH} 297 mμ (ε 29500). Molecular formula of I was not deduced from elementary analysis because of its instability at room temperature even under atmosphere of nitrogen. When I was hydrolysed with 6N HCl at 105 °C for 16 hrs., three amino acids were detected with paper chromatography (p.p.c.) and paper electrophoresis. Then, they were isolated and identified as N-methyl-L-alanine, N-methyl-L-valine and L-ornithine, respectively. However, no fragments originated in the chromophore of I could be isolated from the hydrolysate.

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On catalytic hydrogenation with PtO_2 in ethanol, I consumed three moles of hydrogen to yield crystalline hexahydroaspochracin (II), $C_{23}H_{42}O_4N_4$ (Found: C,62.61 ; H,9.72; N,12.67%. Calcd.: C,62.98; H,9.65; N,12.78%. Molecular ion peak in high resolution mass spectrum, 438.315; Calcd., 438.321); m.p. 137°C; $[\alpha]_D^{23}$ -101.0° (c 2.5 in MeOH); ν_{max}^{Nujol} 3350, 1640, 1510 cm^{-1}; u.v., end-absorption. Hydrolysis of II with 6N HCl at 105°C for 16 hrs. followed by ether extraction gave an acid, which was identified as caprylic acid by gas chromatography of its methyl ester. Besides, the amino acids mentioned above were obtained from the aqueous residue. In the nmr spectrum of II, all signals are attributable to the three amino acids and caprylic acid. Therefore, the strong u.v. absorption of I mentioned earlier should be due to an octatrienecarboxamide. This is rationalized by the nmr spectral data of I shown in Table I. The signals of $=CHCH_3$ at 1.82 d and olefinic protons (6H at 5.7 to 7.4) in the spectrum of I are changed to the signals due to caprylic acid ($-CH_2CH_3$ at 0.89 t, $(CH_2)_4$ at 1.27 s, and $-COCH_2-$ at 2.18 t) in II. No great difference is observed in the signals attributable to the amino acids.

TABLE I[2)]

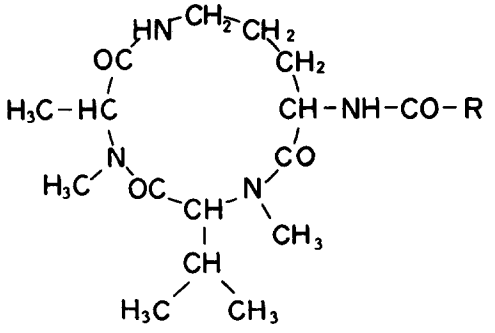
	(I)	(II)
$-CH(CH_3)_2$	0.92 d, 0.75 d (J=6.4)	0.91 d, 0.74 d (J=6.7)
$-CH_2CH_3$	---	0.89 t (J=6.5)
$-(CH_2)_4-$	---	1.27 s
$-NCH(CO)CH_3$	1.51 d (J=7.4)	1.50 d (J=7.0)
$=CHCH_3$	1.82 d (J=6.2)	---
$-COCH_2(CH_2)_4-$	---	2.18 t (J=7.0)
2 NCH_3	2.97 s, 3.05 s	2.94 s, 3.04 s
$-NCH(CO)CH_3$	4.62 q (J=7.4)	4.58 q (J=7.0)
$-NHCH(CO)CH_2-$	5.01 m (J=8.0, 7.2)	4.90 m (J=8.0, 7.2)
$-NCH(CO)CH-$	5.11 d (J=10.7)	5.09 d (J=10.5)
$-CH_2NH(CO)-$	6.0~6.5	5.75 m
$-NHCH(CO)CH_2-$	5.7~7.4	6.39 d (J=7.0)
$-(CH=CH)_3-$	5.7~7.4	---

Partial hydrolysis of II with 4N $Ba(OH)_2$ at 105°C for 16 hrs. gave peptides A and B, which were isolated through preparative p.p.c. (n-BuOH-AcOH-H_2O, 4:1:1) and subsequent t.l.c. on silica gel (iso-PrOH-H_2O, 3:2). The results obtained by acid hydrolysis, DNP degradation and hydrazinolysis of the peptides are shown in Table II.

TABLE II

	Amino acid constitution	N-Terminal	C-Terminal
Peptide A	N-Me-ala, N-Me-val, orn, caprylic acid	α-DNP-orn	N-Me-ala
Peptide B	N-Me-ala, N-Me-val, orn	di-DNP-orn	N-Me-ala

As the possible structure for hexahydroaspochracin, only II shown below can be deduced from these results, and consequently aspochracin should have the structure I, which is the first natural cyclotripeptide. The assignment was further confirmed by the synthesis of II.



Aspochracin (I);

R = CH_3-CH=CH-CH=CH-CH=CH-CH=CH-

Hexahydroaspochracin (II);

R = $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2$-

N-Methyl-L-alanine methyl ester was condensed with carbobenzoxy-N-methyl-L-valine by the dicyclohexylcarbodiimide (DCCI) method in acetonitrile. The crude product was chromatographed on a silica gel column, which was eluted with a solvent system of benzene-chloroform (60:40) to give an oily carbobenzoxy-N-methyl-L-valyl-N-methyl-L-alanine methyl ester (III) in 52% yield ($[\alpha]_D^{23}$ -139.6°, c 5.0 in MeOH). Hydrolysis of the ester function in III with methanolic sodium hydroxide at 37°C for 48 hrs. afforded an oily acid (IV) in 98% yield.

On another side, preparation of δ-carbobenzoxy-α-caprylyl-L-ornithine (V) was attempted in the following way. δ-Carbobenzoxy-L-ornithine was treated with caprylyl chloride in 2N NaOH at 0°C for 30 min. Methyl ester of V thus obtained was hydrogenolyzed with Pd-C in methanol containing HCl to yield crystalline α-

caprylyl-L-ornithine methyl ester monohydrochloride in 96% yield. Treatment of the salt with triethylamine in chloroform at -5°C gave the free base, which was coupled with IV by the DCCI method to produce carbobenzoxy-N-methyl-L-valyl-N-methyl-L-alanyl-α-caprylyl-L-ornithine methyl ester (VI), $[\alpha]_D^{23}$ -96.5° (c 5.0 in MeOH), in 90% yield. The ester function of VI was hydrolysed with methanolic sodium hydroxide at 37°C for 48 hrs., and then the carbobenzoxy residue of the resulting acid was removed by catalytic hydrogenation in the usual way. The free peptide base thus obtained was, without isolation, treated with a half equivalent of DCCI in acetonitrile to afford the anhydride, which was cyclized to the desired cyclotripeptide in a diluted pyridine solution at 85°C for 4 hrs. in rather poor yield. This compound showed complete identity with II in the mixed melting points and the infrared spectra.

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References

(1) S. Tamura, S. Kuyama, Y. Kodaira and S. Higashikawa, Agr. Biol. Chem., 28, 137 (1964); S. Kuyama and S. Tamura, ibid., 29, 168 (1965); A. Suzuki, S. Kuyama, Y. Kodaira and S. Tamura, ibid., 30, 517 (1966).

(2) Nmr spectra were recorded at 100 Mc. in $CDCl_3$ solution; chemical shifts are expressed in δ (ppm) down field from TMS as internal standard, coupling constants in cps. The abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet. Assignments of the signals were confirmed by double irradiations.