NOTES

THE AMINO ACID SEQUENCE OF OCTAPEPTIN C_1 (333-25) (STUDIES ON ANTIBIOTICS FROM THE GENUS *BACILLUS*. XIX[1])

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The antibiotic previously named 333-25 is active against Gram-positive and Gram-negative bacteria, and produced by a strain of *Bacillus circulans*.[2] It is an acylpeptide closely related to antibiotic EM49.[3] The group name "octapeptin" was proposed for this group of antibiotics, and 333-25 was designated as octapeptin C_1.[4] The constituents of octapeptin C_1 have been reported to be Dab* (1D, 4L), Leu (2L), Phe (D) and β-hydroxy anteisononanoic acid.[5] Studies on the amino acid sequence described here indicated the structure of octapeptin C_1 as represented in Fig. 1.

2,4-Dinitrophenylated derivative of octapeptin C_1 was prepared in the usual way. Hydrolysis of tetra (DNP)-octapeptin C_1 released one mole of free Dab in addition to γ-DNP-Dab, suggesting a ring structure with a branched chain. The procedures used for hydrolysis and for automatic amino acid analysis, the better capable distinguishing α-DNP-Dab and γ-DNP-Dab, were described in our previous paper.[6]

Initial attempts at deacylation of octapeptin C_1 with the enzyme preparation, Polymyxin Acylase, by whose action we had previously succeeded in deacylating the acylpeptide antibiotics, cerexin A[7] and brevistin,[6] were unsuccessful. Since octapeptin C_1 was minimally soluble in the buffer solution used for the enzyme reaction, we used Tween 80 and carboxymethyl cellulose to disperse the antibiotic, but no improvement in enzymic susceptibility resulted. Moreover, N-succinyl octapeptin C_1, which was soluble in the buffer solution, was also unaffected by the acylase. Independently, deacylation of octapeptins A and B (EM49)[3] had been achieved by the degradative reaction dependent on the β-hydroxy group of the acyl residue that was developed for the fragmentation of peptides containing the serine or threonine residue. Therefore, we applied this reaction to octapeptin C_1. To a solution of tetra (DNP)-octapeptin C_1 (15 mg) in anhydrous dimethylsulfoxide (0.14 ml), pyridine (5 μl), trifluoroacetic acid (TFA) (3 μl) and dicyclohexylcarbodiimide (DCC) (53 mg) were added and the resulting mixture was allowed to stand for 20 hours at room temperature. The reaction mixture was diluted with ethyl acetate and the excess DCC destroyed by the addition of oxalic acid (40 mg). The precipitate of dicyclohexylurea (DCU) was removed by filtration and the solution was washed with dil. $NaHCO_3$ solution, water, 1 N HCl and saturated NaCl solution, and dried (Na_2SO_4). The residue obtained by evaporation of the solvent was triturated with benzene to give an intermediate ketone derivative as a yellow powder (19 mg). The ketone derivative was suspended in a mixture of acetic acid (0.9 ml), methanol (0.4 ml) and water (0.2 ml), and hydroxylamine hydrochloride (13 mg) was added. The mixture was heated at 85°C for 40 minutes. The resulting solution was diluted with ethyl acetate and the product was isolated by the same procedure as above. Tetra(DNP)-deacyl octapeptin C_1 was obtained as a yellow powder (11 mg).

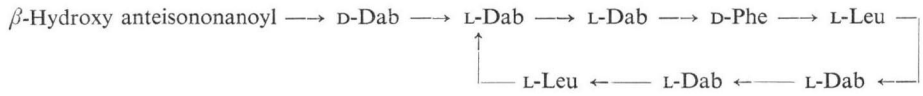
Successive EDMAN degradation was carried out with tetra(DNP)-deacyl octapeptin C_1 (3 mg) as described[6], with the following modifications: excess phenylisothiocyanate (PTC) was removed by extracting with cyclohexane twice and then with benzene twice, and PTC-amino acid was extracted with ethyl ether. Furthermore, the PTC-peptide in the 2nd step of the degradation reaction was heated at 40°C for 120 minutes in TFA and further heated at 80°C for 10 minutes in a mixture of acetonitrile - 2 N HCl (1 : 1). Formation of PTH-peptide from the PTC-peptide following opening of the peptide ring was anticipated at this step as in the case of octapeptins A and B (EM49).[3] The PTH-peptide, located on TLC using Silica gel GF with chloroform - methanol (9 : 1) (Rf *ca.* 0.46), was extracted with chloroform - methanol (1 : 1) (yield: 47% by amino acid analysis) and used for the next step. The results of the series of degradative reactions are

* Dab: 2,4-Diaminobutyric acid.

Table 1. EDMAN degradation of tetra (DNP)-deacyl octapeptin C_1.

	PTH-Amino acid	Amino acid found (in ratio)			
		Leu	Phe	γ-DNP-Dab	Dab
Original peptide		2.00	1.00	+	1.06
Step 1	γ-DNP-Dab	2.00	0.89	+	0.98
Step 2	—	2.00	1.18	+	0.00
Step 3	γ-DNP-Dab	2.00	0.98	+	0.00
Step 4	Phe	2.00	0.19	+	0.00
Step 5	Leu	1.00	0.12	+	0.00
Step 6	γ-DNP-Dab	1.00	0.13	+	0.00
Step 7	γ-DNP-Dab	1.00	0.12	trace	0.00
Step 8	Leu	—	—	—	—

Fig. 1. Structure of octopeptin C_1.



summarized in Table 1. These results clearly indicate the amino acid sequence of octapeptin C_1 to be as presented in Fig. 1. Moreover, when the residual peptide obtained at the first step in another experiment was reacted to give the 2,4-dinitrophenyl derivative and then hydrolyzed. One mole of α-DNP-Dab was found. This result provided additional evidence for the branching mode in the peptide ring.

The 2,4-dinitrophenyl derivative of tetra (DNP)-deacyl octapeptin C_1 (14 mg) was prepared and hydrolyzed. The hydrolyzate was extracted with ethyl acetate. From the ethyl acetate extract, the bis (DNP) derivative of the N-terminal Dab was isolated (1.07 mg) by TLC in the same manner as previously.[6] The optical rotation; $[M]_D^{25.0}+296\pm46.8°$ (*c* 0.102, acetic acid), proved it to be D-form.[3] The residual aqueous solution, containing the other four Dab residues, was concentrated to dryness and the 2,4-dinitrophenyl derivative prepared. The bis(DNP)-Dab isolated was proven to be the L-form by optical rotation: $[M]_D^{24.0}-298\pm31.1°$ (*c* 0.154, acetic acid).

Thus, the amino acids, their sequence and their chilarities were established to give the total structure of octapeptin C_1. While, methods used were essentially identical to those used in elucidating the structures of octapeptins A and B (EM49)[3], the procedure followed in the EDMAN degradation differed. It should be noted that different D-amino acid residues are present at the same positions in the amino acid sequences of these related antibiotics.

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