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SYNTHESIS OF A CYCLOHEXADEPSIPEPTIDE, PROTODESTRUXIN

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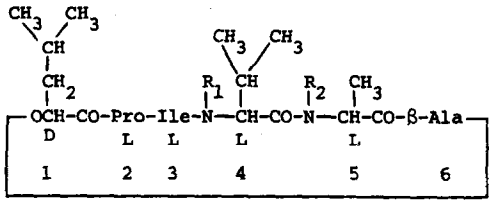
Synthesis of a cyclohexadepsipeptide corresponding to the sequence of protodestruxin was attempted through five different routes, and the three routes afforded the same cyclodepsipeptide. Properties of the synthetic peptide were identical with those of natural protodestruxin.

Destruxins are insecticidal cyclodepsipeptides isolated from a culture filtrate of Metarrhizium anisopliae, and the structures of destruxins A (1966), B (1964), C (1970), D (1970), and desmethyldestruxin B (1970) were elucidated.[1] The structures of destruxin B group are shown in this paper such as 1. Hassall classifies cyclic depsipeptide into three groups[2] in which the group 2 involves compounds with irregular array of amino acids and hydroxy acids as destruxins and monamycin.

We attempted to develop a convenient synthetic method of cyclodepsipeptide classified as the group 2 by Hassall though syntheses of several cyclodepsipeptides in the group 2 had been reported,[3] and selected [L-Val^4,L-Ala^5]-destruxin B (1) as a model peptide for the purpose. Though 1 had been a material unknown in nature until the last stage of our synthetic study,[4] a natural peptide was isolated from a culture filtrate, its structure was proposed to be 1 (1972),[5] and it has been termed as protodestruxin. Then we wanted to examine the identity of synthetic 1 with the natural peptide.

Synthesis of 1 was attempted by five different routes. The route 1 was an attempt of intramolecular ester-bond formation between D-Hyc[6] and β-Ala residues in H-D-Hyc-Pro-Ile-Val-Ala-β-Ala-OH (2). Though we could synthesize benzoylserine ethyl ester in good yield by gentle



	R_1	R_2
destruxin B	CH_3	CH_3
desmethyldestruxin B	H	CH_3
[L-Val^4,L-Ala^5]-destruxin B (1) or protodestruxin	H	H

heating of benzoylserine and ethanol with the presence of benzene and catalytic amount of TosOH,[7] the heating of crystalline 2[6] and TosOH with a solvent of benzene, CCl_4 or DMF did not afford the desired cyclopeptide. In the route 2, 2 was treated with one half equivalent of DCCI as reported,[3] however the desired cyclopeptide could not be isolated. In the literature,[3] a cyclodepsipeptide corresponding to destruxin B was synthesized from H-D-Hyc-Pro-Ile-MeVal-MeAla-β-Ala-OH and DCCI.

Since it is generally accepted that intramolecular peptide-bond formation of a linear depsipeptide gives a cyclodepsipeptide more easily than the intramolecular ester-bond formation, we carried out the cyclization reaction of H-Pro-Ile-Val-Ala-β-Ala-D-Hyc-OH (3) as follows. In the route 3, crystalline 3 was treated with $SOCl_2$, the solution was evaporated, and the residue was dissolved in hexane. To the solution was added a mixture of triethylamine and benzene. The solvent was evaporated, and the residue was dissolved in a mixture of dioxane and water (2:1 by vol). The solution was passed through a column of Dowex 50X8 (H^+ form), the effluent was evaporated, and the resulting solid was collected by filtration with the aid of water. The product was reprecipitated from methanol-ether-petroleum ether; yield of 1 from 3, 18%; mp 260-265° dec; $[\alpha]_D^{20°}$ 135° (c 1, MeOH); R_f^a (TLC with $CHCl_3$:MeOH=5:1 by vol) 0.50, R_f^b (TLC with AcOEt:MeOH=10:1 by vol) 0.74. In TLC, the synthetic 1 showed several very faint spots beside a main, but elemental analyses of the 1 were agreed with the calculated values.

Several cyclodepsipeptides such as valinomycin[8] had been synthesized by the method in the route 3. On the other hand, cyclopeptides had been obtained by cyclization of corresponding linear peptide active ester. For example, a homodetic cyclodecapeptide, antamanide, was synthesized from the corresponding linear decapeptide with HOSu and DCCI.[9] However, we could not isolate 1 from 3 with HOSu and WSCI.

We have been synthesized many analogs of gramicidin S *via* cyclization of a linear decapeptide p-nitrophenyl ester.[10] Recently we developed a convenient procedure to synthesize them through OSu ester in an excellent yield.[11] In this study, this procedure was applied for synthesis of 1 as the route 4. Crystalline Boc-Pro-Ile-Val-Ala-β-Ala-D-Hyc-OH (4) was converted to

the corresponding OSu ester (5), and 5 was changed to H-depsipeptide-OSu·TFA salt (6). The 6 was treated with pyridine as described in later section (the route 5); yield of 1 from 4, 22%; mp 262-265° dec; $[\alpha]_D^{20°}$ 131° (c 1, MeOH); R_f^a 0.50, R_f^b 0.74.

In the route 5, the procedure[10] was applied for an another material, namely Boc-Ile-Val-Ala-β-Ala-D-Hyc-Pro-OH (7). Z-Val-Ala-OEt (8), mp 167°, was prepared in 81% yield from Z-Val-OH and H-Ala-OEt·HCl by the mixed anhydride method, and 8 was converted to oily H-Val-Ala-OEt·HCl (9) in 94% yield by hydrogenation. Condensation of Boc-Ile-OH with 9 by the mixed anhydride method gave Boc-Ile-Val-Ala-OEt (10) in 81% yield with mp 144°. The 10 was treated with hydrazine to afford Boc-Ile-Val-Ala-$NHNH_2$ (11) in 78% yield with mp 194°. Boc-β-Ala-D-Hyc-OBzl (12) was prepared in 36% yield with mp 58° from Boc-β-Ala-OH and oily H-D-Hyc-OBzl by the method using $C_6H_5SO_2Cl$,[12] and 12 was converted to oily Boc-β-Ala-D-Hyc-OH (13) in 80% yield by hydrogenation. Condensation of 13 with H-Pro-OBzl·HCl by the mixed anhydride method afforded oily Boc-β-Ala-D-Hyc-Pro-OBzl (14) in 76% yield, and 14 was converted to oily H-β-Ala-D-Hyc-Pro-OBzl·HCl (15) in 98% yield by treatment with HCl in AcOEt. The azide derived from 11 with isoamyl nitrite[13] was condensed with 15 to afford oily Boc-Ile-Val-Ala-β-Ala-D-Hyc-Pro-OBzl (16) in 76% yield. Hydrogenation of 16 yielded crystalline 7, 91%, mp 150°, $[\alpha]_D^{20°}$ -58.8° (c 1, MeOH).

The cyclization reaction was carried out as follows. The 7 (0.6 mmol) in DMF was treated with HOSu (0.9 mmol) and WSCI·HCl (0.9 mmol) at room temperature for 2 days, the solution was evaporated, and Boc-Ile-Val-Ala-β-Ala-D-Hyc-Pro-OSu (17) was collected by filtration with the aid of water. The 17 was dissolved in TFA (4 ml) at 0° for 20 min, the solution was evaporated, and H-peptide-OSu·TFA salt (18) was collected with the aid of ether. The 18 dissolved in DMF (20 ml) was treated with pyridine (200 ml) at room temperature for 1 day, the solution was evaporated, and the resulting solid was collected with the aid of water. The solid dissolved in a mixture of dioxane and water (3:2) was passed through a Dowex 50X8 column, and the effluent was evaporated. The resulting solid was collected with the aid of water and recrystallized from methanol-ether to afford beatiful needle crystals; yield of 1 from 7, 46%; mp 263-265° dec; $[\alpha]_D^{20°}$ 132° (c 1, MeOH); R_f^a 0.50, R_f^b 0.74 (Calcd for $C_{28}H_{47}O_7N_5 \cdot H_2O$: C, 57.61; H, 8.46; N, 12.00; mol wt, 584. Found: C, 57.77; H, 8.31; N, 12.04; mol wt, 587[14]). It is difficult to explain why 7 gave 1 in better yield than 4, but we suppose that the insertion of a hydroxy acid (D-Hyc) residue as 7 apart from N- or C-terminus is one factor to give a cyclodepsipeptide in an excellent yield.

The synthetic 1 was compared with natural protodestruxin in chemical and biological properties. In addition to having the same R_f in TLC, 1 and natural peptide had superimposal infrared and mass spectra. The both peptides had no insecticidal activity assayed by intrahemocoelic injection to fifth instar larvae of the silkworm, even at as high a concentration as 20 μg/g.[15] It would be noteworthy that the synthetic tridesmethyl enniatin B (natural enniatin B possesses three N-methylamino acids in a molecule) possessed almost no antibacterial activity.[16] The results obtained here confirm further the correctness of protodestruxin structure given as 1.

References and Notes

1) S. Tamura et al., Agr. Biol. Chem., 28, 137 (1964); ibid., 30, 517 (1966); ibid., 34, 813 (1970).

2) C.H. Hassall and W.A. Thomas, Chemistry in Britain, 7, 145 (1972).

3) e.g. S. Kuyama and S. Tamura, Agr. Biol. Chem., 29, 168 (1965).

4) Presented partly at the 10th Symposium on Peptide Chemistry, Sapporo (Japan), September 27 (1972); S. Lee and N. Izumiya, Proceedings of the Symposium, p.113 (1973).

5) A. Suzuki and S. Tamura, Agr. Biol. Chem., 36, 896 (1972).

6) Satisfactory elemental analyses and chromatographic data were obtained for all crystalline compounds. Boc-, t-butyloxycarbonyl; D-Hyc, D-α-hydroxyisocaproic acid; DCCI, dicyclohexylcarbodiimide; WSCI (water soluble carbodiimide), 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide; HOSu, N-hydroxysuccinimide; TosOH; p-toluenesulfonic acid; TFA, trifluoroacetic acid; DMF, dimethylformamide. Amino acid symbols denote the L-configuration.

7) T. Kato, S. Makisumi, M. Ohno and N. Izumiya, Nippon Kagaku Zasshi, 83, 1151 (1962).

8) M.M. Shemyakin, N.S. Aldanova, E.I. Vinogradova and M.Yu. Feigina, Tetrahedron Letters, 1921 (1963).

9) T. Wieland, C. Birr and F. Flor, Ann., 727, 130 (1969).

10) e.g. H. Takiguchi and N. Izumiya, Bull. Chem. Soc. Japan, 47, 221 (1974).

11) S. Matsuura, H. Takiguchi, M. Waki and N. Izumiya, Mem. Fac. Sci., Kyushu Univ., C, in press.

12) M.M. Shemyakin, Yu.A. Ovchinnikov, V.T. Ivanov and A.A. Kiryushkin, Tetrahedron, 19, 995 (1963).

13) J. Honzl and J. Rudinger, Collect. Czech. Chem. Commun., 26, 2333 (1961).

14) Mol wt was determined on a Hitachi Osmometer, type 115, using methanol as a solvent.

15) In the previous paper,[5] it was reported that natural protodestruxin possesses an insecticidal activity. However, it was found that natural material after extensive purification possesses no activity. We assume that the natural protodestruxin in the previous paper might contain a minor amount of other destruxins.

16) M.M. Shemyakin, Yu.A. Ovchinnikov and V.T. Ivanov, Angew. Chem. Internat. Edit., 8, 492 (1969).