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SYNTHESES OF CYCLOTETRAPEPTIDES, AM-TOXIN ANALOGS, CONTAINING α-HYDROXYALANINE

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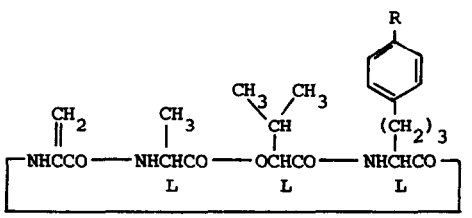
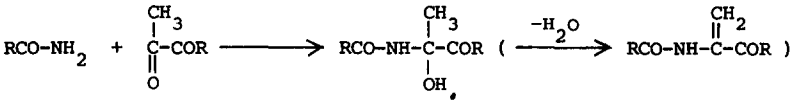
Summary: Condensation of amide with α-keto acid to yield a α-hydroxyalanine (α-Hyala) or dehydroalanine residue was applied to syntheses of analogs of AM-toxins, cyclotetradepsipeptides *Cyclo*(-α-Hyala-L-Ala-L-Val-L-Phe-), *cyclo*(-α-Hyala-L-Ala-L-Hmb-L-Phe-) and *cyclo*(-α-Hyala-L-Ala-L-Hmb-L-Tyr-) (Hmb, 2-hydroxy-3-methylbutanoic acid) were obtained from the corresponding pyruvyl-tripeptide amides in good yields by the treatment of anhydrous hydrogen fluoride.

AM-Toxins are host-specific phytotoxic metabolites produced by *Alternaria mali*, and cause veinal necrosis on apple leaves.[1] The primary structures of three congeners, AM-toxins I, II and III, were determined to be cyclotetradepsipeptide.[2,3] In previous papers,[4-7] we reported the syntheses of AM-toxins I, II and their several analogs, and partly clarified the structure-activity relationship of the toxins. The synthetic methods used for AM-toxins I and II, in which dehydroalanine (ΔAla) residue was formed by β-elimination of Ser residue, can



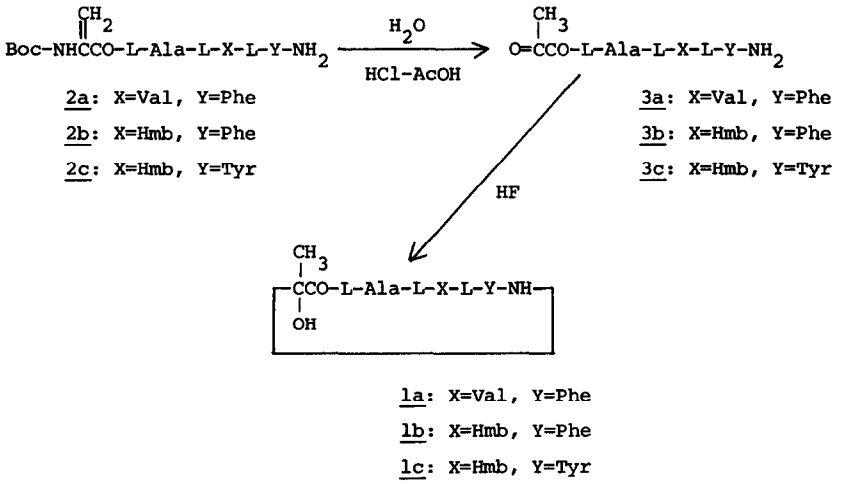
	R
AM-Toxin I :	OCH_3
AM-Toxin II :	H
AM-Toxin III :	OH

hardly be utilized for AM-toxin III because it contains a free hydroxy group. Therefore, we tried to apply the condensation of amide with α-keto acid (Scheme 1). The synthesis of peptides



Scheme 1. Condensation of acylamide with α-keto acid.

through the condensation has been reported in the case of the synthesis of 2,5-piperadinediones.[8] Gross and Morell observed that the reversible reaction of degradation-formation of α,β-unsaturated amino acids takes place in the peptide antibiotics, nisin and subtilin.[9] Recently, Noda and Gross obtained a cyclic hexapeptide containing α-Hyala or ΔAla residue through the intramolecular condensation of pyruvyl-pentapeptide amide with *C*-terminal sequence of luteinizing hormone-releasing hormone by the action of anhydrous HF.[10] This paper describes the syntheses of AM-toxin analogs (1a, 1b and 1c) by the intramolecular condensation of pyruvyl-tripeptide amides (Scheme 2). As the first step of the experiments, three Boc-ΔAla-tripeptide amides, namely Boc-ΔAla-L-Ala-L-Val-L-Phe-NH_2 (2a), Boc-ΔAla-L-Ala-L-Hmb-L-Phe-NH_2 (2b) and Boc-ΔAla-L-Ala-L-Hmb-L-Tyr-NH_2 (2c), were prepared by the conventional method, and converted to the corresponding pyruvyl-tripeptide amides, namely Pyr-L-Ala-L-Val-L-Phe-NH_2 (3a), Pyr-L-Ala-L-Hmb-L-Phe-NH_2 (3b) and Pyr-L-Ala-L-Hmb-L-Tyr-NH_2 (3c) (Pyr, pyruvyl). Then, each of 3a-3c was treated with anhydrous HF to yield a cyclic peptide.



Scheme 2. Syntheses of AM-toxin analogs by the intramolecular condensation of pyruvyl-tripeptide amides.

Synthesis of 2a was performed in a stepwise manner from its *C*-terminus. Boc-L-Val-L-Phe-OMe (4) (mp 104-106°, $[\alpha]_D^{25}$ -26° in EtOH) was prepared by the coupling of Boc-L-Val-OH and H-L-Phe-OMe·HCl using 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (WSCI) in yield of 74%. Removal of the Boc-group of 4 with 2 M HCl in ethyl acetate and coupling with Boc-L-Ala-OH using WSCI led to Boc-L-Ala-L-Val-L-Phe-OMe (5) (86%, mp 140-141°, $[\alpha]_D^{25}$ -42.5° in EtOH). The protected tripeptide 5 was converted to Boc-L-Ala-L-Val-L-Phe-NH_2 (6) (mp 210°, $[\alpha]_D^{25}$ -26° in EtOH) by the action of saturated ammonia in MeOH in yield of 95%. Action on 6 with HCl in ethyl acetate and coupling with Boc-ΔAla-OH, which was prepared from Boc-L-Ser(Tos)-OMe by the action of NaOH,[11] using WSCI afforded 2a (49%, mp 185-187°, $[\alpha]_D^{25}$ -7.5° in EtOH).

Syntheses of 2b and 2c were initiated with Boc-L-Ala-L-Hmb-OH,[5] which was coupled with H-L-Phe-NH_2·HCl or H-L-Tyr-NH_2·HCl by WSCI to give Boc-L-Ala-L-Hmb-L-Phe-NH_2 (7) (55%, mp 120-122°, $[\alpha]_D^{25}$ -57° in EtOH) and Boc-L-Ala-L-Hmb-L-Tyr-NH_2 (8) (43%, mp 144-145°, $[\alpha]_D^{25}$ -57° in EtOH), respectively. Removal of the Boc-group of 7 or 8 and coupling with Boc-ΔAla-OH by WSCI led to 2b (68%, mp 99-100°, $[\alpha]_D^{25}$ -36° in EtOH) and 2c (40%, mp 130-133°, $[\alpha]_D^{25}$ +32° in EtOH).

The ΔAla-peptide amide 2a was converted to 3a by the treatment with 1 M HCl in AcOH in the presence of an equivalent amount of water,[12] and, without further purification, 3a was allowed to react with anhydrous HF for 1 h at 0°. After evaporation *in vacuo*, the residual solid was collected with the aid of ether. The compound obtained showed a major spot (R_f 0.57) and a minor spot (R_f 0.05) on silica gel TLC with the solvent system of chloroform-MeOH-AcOH (85:10:5). After purification by column chromatography using silicic acid and the solvent system of chloroform-dioxane (1:1), a single product was obtained in high yield (85%) (R_f 0.57, mp 199-202°, $[\alpha]_D^{25}$ -14.5° in DMF). By chemical and physical analyses, the compound was identified to be *cyclo*(-α-Hyala-L-Ala-L-Val-L-Phe-) (1a): *anal* ($C_{20}H_{28}O_5N_4$) C,H,N; mol wt[13] 404 (calcd 404); ^{1}NMR[14] (DMSO) δ 2.32 (s, CH_3), δ 7.04 (s, C^{α}-OH), δ 7.30 (s, NH) (signals arising only from α-Hyala residue are given). No dimer was detected. The treatment of 2a with anhydrous HF afforded the same product 1a (78%). Although the α-Hyala residue in 1a involves an asymmetric carbon atom, the α-Hyala residue was determined to have a single optical configuration of either L or D from the NMR data on 1.

The cyclic peptides 1b and 1c were also obtained from 3b and 3c, respectively, by the same procedure described above. Peptide 1b: yield, 74%; mp 167-169°; $[\alpha]_D^{25}$ -48° in EtOH; *anal* ($C_{20}H_{27}O_6N_3$) C,H,N; mol wt 405 (calcd 405); ^{1}NMR (DMSO) δ 2.36 (s, CH_3), δ 7.07 (s, C^{α}-OH), δ 7.30 (s, NH). Peptide 1c: yield, 77%; mp 151-153°; $[\alpha]_D^{25}$ -26.5° in EtOH; *anal* ($C_{20}H_{27}O_7N_3$) C,H,N;

mol wt 421 (calcd 421); ^{1}NMR (DMSO) δ 2.35 (s, CH_3), δ 7.07 (s, C^{α}-OH), δ 7.26 (s, NH).

None of the synthetic analogs, 1a, 1b and 1c, showed the toxic activity for induction of necrosis on apple leaves. The present results, however, provide a facile method for cyclization of peptides, which gives only monomer in high yield containing an interesting amino acid residue, α-hydroxyalanine, with a definite optical configuration.

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13) Mol wt was determined on Hitachi RMS-4 mass spectrometer.

14) Recorded on JEOL JNM PS-100 spectrometer.

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