Cyclic Peptides. XV. Synthesis of [4-α-Hydroxyalanine]AM-toxin II

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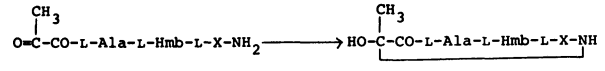
Synopsis. An AM-toxin II analog, *cyclo*(–L-Ala[1]–L-Hmb[2]–L-App[3]–Hyala[4]–), with an α-hydroxyalanine (Hyala) residue in place of dehydroalanine in position 4 was synthesized from pyruvoyl–L-Ala–L-Hmb–L-App–amide (Hmb, 2-hydroxy-3-methylbutanoic acid; App, 2-amino-5-phenylpentanoic acid) by intramolecular condensation between the pyruvoyl and carbamoyl groups. The synthetic peptide did not show the toxic activity of AM-toxin II.

The cyclotetradepsipeptide AM-toxins are phytotoxic metabolites of *Alternalia mali* causing necrosis on apple leaves. The structure of AM-toxin II[1] was shown to be *cyclo*(–L-Ala[1]–L-Hmb[2]–L-App[3]–ΔAla[4]–).[2] Previously, we attempted the synthesis of their analogs with L-Phe or L-Tyr residue replacing the uncommon aromatic amino acid in position 3 by the intramolecular condensation of pyruvoyl–L-Ala–L-Hmb–L-Phe–NH_2 (**1b**) or –L-Tyr–NH_2 (**1c**).[3] The product was, however, identified to be cyclotetrapeptide **2b** or **2c** containing a Hyala residue instead of ΔAla expected (Fig. 1), and did not show the activity of AM-toxins. The inactivity of **2b** or **2c** is not attributed to the replacement of ΔAla by Hyala, because [L-Phe[3]]AM-toxin[4] and [L-Tyr-(Me)[3]]AM-toxin[5] were also almost inactive.

In order to examine the influence of substitution of ΔAla by Hyala in position 4 on the activity, we have synthesized [Hyala[4]]AM-toxin II (**2a**). The Hyala residue was expected to contribute toward the toxic activity, because it might be a possible precursor of



1a, X=App **2a**, X=App
1b, X=Phe **2b**, X=Phe
1c, X=Tyr **2c**, X=Tyr

Fig. 1. Intramolecular condensation of pyruvoyl-tripeptide amides.

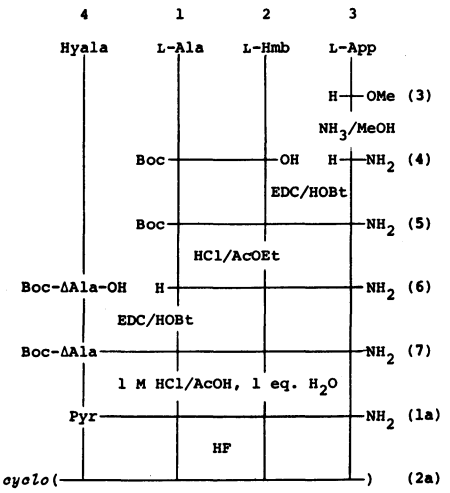


Fig. 2. Synthetic route for [Hyala[4]]AM-toxin II (**1a**).

ΔAla, and an AM-toxin I analog with L-Ser[4], the structural isomer of Hyala, possessed a recognizable activity.[6]

Figure 2 shows the reaction route for the synthesis. The tripeptide, Boc–L-Ala–L-Hmb–L-App–NH_2 (**5**), was prepared by coupling of Boc–L-Ala–L-Hmb–OH and L-App amide (**4**) by the EDC method.[7] Removal of the Boc group of **5** followed by coupling with Boc–ΔAla–OH afforded tetrapeptide amide **7**, which was converted to pyruvoyl-tripeptide amide **1a** by the treatment with HCl in AcOH in the presence of an equivalent amount of water. Treatment of **1a** with HF cyclized the peptide intramolecularly with the formation of Hyala residue to give the desired peptide **2a**. Dimer and polymer formation was not observed in this reaction. After purification by chromatography with silicic acid, the homogeneity and identity of the product were established by thin-layer chromatography, mass spectroscopy and ^{1}H-NMR spectroscopy. The singlet peak assingned for the α-OH of Hyala residue in the NMR data suggests that the Hyala is of a single optical configuration of either L or D.

Bioassay showed that, even at high concentration, **2a** did not show the toxic activity for the induction of necrosis on apple leaves. Thus, replacement of the ΔAla residue by Hyala eliminated the biological activity. The possible conversion of serine residue to ΔAla in the biological system was suggested from the previous results that [L-Ala[4]]AM-toxin I was inactive[4] while [L-Ser[4]]analog was active.[6] The present data indicates that there is no possibility of such a conversion of the Hyala residue to ΔAla in the biological system. The chemical conversion of the Hyala residue to ΔAla is under investigation.

Experimental

Thin layer chromatography was carried out on silica gel G (Merck) with the following solvent systems: R_f^1, $CHCl_3$–MeOH (9 : 1); R_f^2, $CHCl_3$–MeOH–AcOH (85 : 10 : 5); R_f^3, *n*-BuOH–AcOH– pyridine–H_2O (4 : 1 : 1 : 2). Optical rotations were measured with a Union polarimeter PM-201, Mass spectra were taken on a Hitachi RMS-4 mass spectrometer and NMR spectra on a JEOL LNM PS-100 spectrometer.

H–L-App–OMe·HCl (3·HCl). This compound was obtained by the treatment of H–L-App–OH[8] with MeOH and $SOCl_2$ according to the procedure described in the literature;[9] yield, 96%; mp 115 °C;$[\alpha]_D^{25}$ +21.5° (*c* 2, EtOH); R_f^1 0.23; R_f^2 0.54.

Found: C, 59.08; H, 7.39; N, 5.83%. Calcd for $C_{12}H_{18}NO_2Cl$: C, 59.13; H, 7.44; N, 5.75%.

H–L-App–NH_2·HCl (4·HCl). This compound was prepared from **3**·HCl by the treatment with saturated NH_3 in MeOH according to the literature;[10] yield, 82%; mp 200 °C; $[\alpha]_D^{25}$ +17.0° (*c* 1, EtOH); R_f^2 0.05; R_f^3 0.67.

Found: C, 57.70; H, 7.55; N, 12.28%. Calcd for $C_{11}H_{17}N_2OCl$: C, 57.76; H, 7.49; N, 12.25%.

Boc-L-Ala-L-Hmb-L-App-NH_2 (5). Boc-L-Ala-L-Hmb-OH[6] (1.45 g, 5 mmol), **4·HCl** (1.14 g, 5 mmol), HOBt (1.35 g, 10 mmol) and NEt_3 (0.70 ml, 5 mmol) was dissolved in DMF (10 ml). To the solution, EDC·HCl (1.15 g, 6 mmol) was added at 0 °C. The mixture was stirred for 1 h at 0 °C and overnight at room temperature, and evaporated *in vacuo*. The residue was treated with a mixture of water and AcOEt. The organic layer was washed with 4% $NaHCO_3$ and 10% citric acid, dried (Na_2SO_4), and evaporated. The product was recrystallized from AcOEt–ether–petroleum ether; yield, 1.67 g (72%); mp 94—95 °C; $[\alpha]_D^{25}$ −40.0° (*c* 1, EtOH); R_f^1 0.40; R_f^2 0.63.

Found: C, 62.33; H, 8.16; N, 9.17%. Calcd for $C_{24}H_{37}N_3O_6$: C, 62.18; H, 8.05; N, 9.07%.

Boc-ΔAla-L-Ala-L-Hmb-L-App-NH_2 (7). Compound **5** (963 mg, 2.1 mmol) was treated with 2 M HCl in AcOEt (25 ml) for 1 h at room temperature, and the mixture was concentrated *in vacuo*, the concentration being repeated twice after addition of AcOEt. The solid residue was washed with ether by means of decantation, and dried in a desiccator. The hydrochloride of H-L-Ala-L-Hmb-L-App-NH_2 (**6·HCl**) was obtained as a powder. To the solution of **6·HCl**, Boc-ΔAla-OH[3] (374 mg, 2 mmol), HOBt (338 mg, 2.5 mmol) and NEt_3 (0.28 ml, 2 mmol) in DMF (10 ml) was added EDC·HCl (383 mg, 2 mmol). The mixture was stirred for 1 h at 0 °C and overnight at room temperature, and evaporated. The residue was treated with a mixture of 20% NaCl and AcOEt. The organic layer was washed with 4% $NaHCO_3$ and saturated NaCl, dried (Na_2SO_4), and evaporated. The product was recrystallized from MeOH–ether–petroleum ether; yield, 643 mg (60%); mp 95—97 °C; $[\alpha]_D^{25}$ −21.0° (*c* 1, EtOH); R_f^1 0.41; R_f^2 0.64.

Found: C, 60.68; H, 7.62; N, 10.47%. Calcd for $C_{27}H_{40}N_4O_7$: C, 60.88; H, 7.57; N, 10.52%.

Pyr-L-Ala-L-Hmb-L-App-NH_2 (1a). Compound **7** (533 mg, 1 mmol) was dissolved in 1 M HCl in AcOH (5 ml) containing an equivalent of water (18 μl). After being kept for 10 min at 60 °C, the solution was evaporated. The residual solid was collected with the aid of ether; yield, 422 mg (96%). This compound was used in the cyclization reaction without further purification.

cyclo(-L-Ala-L-Hmb-L-App-Hyala-) (2a). A solution of **1a** (217 mg, 0.5 mmol) in anhydrous HF (5 ml) was stirred for 1 h at 0 °C. After HF was evaporated, the residual solid was dissolved in $CHCl_3$ and the solution chromatographed on silicic acid (Mallinckrodt, 100 mesh) using a column (1.2 cm × 10 cm) and a solvent system of $CHCl_3$–AcOEt (1 : 1). The main fractions (6—16 ml) were collected and evaporated. The residue was collected with the aid of ether; yield, 160 mg (74%); mp 124—126 °C; $[\alpha]_D^{25}$ −44.0° (*c* 1, DMF); R_f^1 0.39; R_f^2 0.47. NMR (signals arising only from Hyala residue are given) (DMSO-d_6) δ=2.38 (3H, s, HyalaCH$_3$), 7.04 (1H, s, HyalaOH), 7.35 (1H, s, HyalaNH).

Found: C, 60.77; H, 7.31; N, 9.63%; *m/e* 433. Calcd for $C_{22}H_{31}N_3O_6$: C, 60.95; H, 7.21; N, 9.69; M^+, 433.

Biological Assay. Biological assays on apple leaves (susceptible cultivar, Indo) were carried out as described previously.[6] Product **2a** did not show the toxicity at concentration up to 100 μg/ml, whereas synthetic or natural AM-toxin II showed at 0.02 μg/ml.

References

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2) Abbreviations given by the IUPAC-IUB Commission, *J. Biol. Chem.*, **247**, 977 (1972), have been used throughout. Additional abbreviations: ΔAla, dehydroalanine; App, 2-amino-5-phenylpentanoic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Hmb, 2-hydroxy-3-methylbutanoic acid; HOBt, 1-hydroxybenzotriazole; Hyala, α-hydroxyalanine; Pyr, pyruvoyl; Tyr(Me), *O*-methyltyrosine.

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