

Cyclic Peptides. XV. Synthesis of [4- α -Hydroxyalanine]AM-toxin IIKosaku NODA,* Junko NAKASHIMA, Sannamu LEE,[†] and Nobuo IZUMIYA[†]

Laboratory of Biochemistry, Fukuoka Women's University, Kasumigaoka, Higashi-ku, Fukuoka 813

[†]Laboratory of Biochemistry, Faculty of Science, Kyushu University, 33, Hakozaki, Higashi-ku, Fukuoka 812

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Synopsis. An AM-toxin II analog, *cyclo*(-L-Ala¹-L-Hmb²-L-App³-Hyla⁴-), with an α -hydroxyalanine (Hyla) 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 cyclotetrapeptide AM-toxins are phytotoxic metabolites of *Alternaria mali* causing necrosis on apple leaves. The structure of AM-toxin II¹⁾ was shown to be *cyclo*(-L-Ala¹-L-Hmb²-L-App³- Δ Ala⁴-).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-NH₂ (**1b**) or -L-Tyr-NH₂ (**1c**).³⁾ The product was, however, identified to be cyclotetrapeptide **2b** or **2c** containing a Hyla residue instead of Δ Ala 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 Δ Ala by Hyla, because [L-Phe³]AM-toxin⁴⁾ and [L-Tyr(Me)³]AM-toxin⁵⁾ were also almost inactive.

In order to examine the influence of substitution of Δ Ala by Hyla in position 4 on the activity, we have synthesized [Hyla⁴]AM-toxin II (**2a**). The Hyla residue was expected to contribute toward the toxic activity, because it might be a possible precursor of

Δ Ala, and an AM-toxin I analog with L-Ser⁴, the structural isomer of Hyla, possessed a recognizable activity.⁶⁾

Figure 2 shows the reaction route for the synthesis. The tripeptide, Boc-L-Ala-L-Hmb-L-App-NH₂ (**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- Δ Ala-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 Hyla 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 ¹H-NMR spectroscopy. The singlet peak assigned for the α -OH of Hyla residue in the NMR data suggests that the Hyla 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 Δ Ala residue by Hyla eliminated the biological activity. The possible conversion of serine residue to Δ Ala in the biological system was suggested from the previous results that [L-Ala⁴]AM-toxin I was inactive⁴⁾ while [L-Ser⁴]analog was active.⁶⁾ The present data indicates that there is no possibility of such a conversion of the Hyla residue to Δ Ala in the biological system. The chemical conversion of the Hyla residue to Δ Ala is under investigation.

Experimental

Thin layer chromatography was carried out on silica gel G (Merck) with the following solvent systems: R_f^1 , CHCl₃-MeOH (9 : 1); R_f^2 , CHCl₃-MeOH-AcOH (85 : 10 : 5); R_f^3 , *n*-BuOH-AcOH-pyridine-H₂O (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-OH⁸⁾ with MeOH and SOCl₂ according to the procedure described in the literature;⁹⁾ yield, 96%; mp 115 °C; $[\alpha]_D^{25} + 21.5^\circ$ (*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 C₁₂H₁₈N₂O₂Cl: C, 59.13; H, 7.44; N, 5.75%.

H-L-App-NH₂·HCl (**4·HCl**). This compound was prepared from **3·HCl** by the treatment with saturated NH₃ in MeOH according to the literature;¹⁰⁾ yield, 82%; mp 200 °C; $[\alpha]_D^{25} + 17.0^\circ$ (*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 C₁₁H₁₇N₂OCl: C, 57.76; H, 7.49; N, 12.25%.

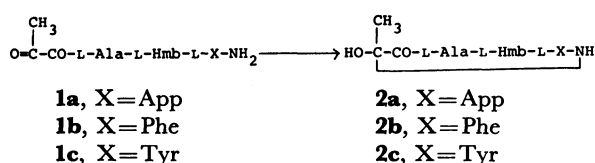


Fig. 1. Intramolecular condensation of pyruvoyl-tripeptide amides.

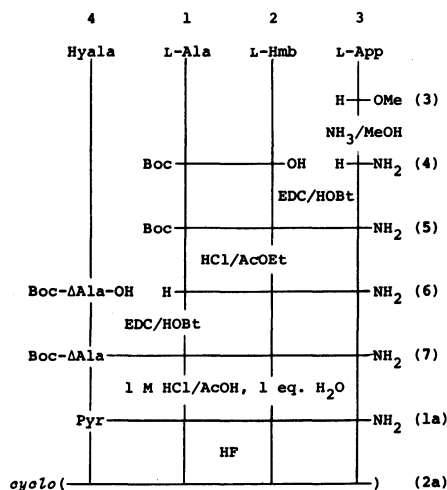


Fig. 2. Synthetic route for [Hyla⁴]AM-toxin II (**1a**).

Boc-L-Ala-L-Hmb-L-App-NH₂ (**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 NEt₃ (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% NaHCO₃ and 10% citric acid, dried (Na₂SO₄), 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 C₂₄H₃₇N₃O₆: C, 62.18; H, 8.05; N, 9.07%.

Boc-ΔAla-L-Ala-L-Hmb-L-App-NH₂ (**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-NH₂ (**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 NEt₃ (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% NaHCO₃ and saturated NaCl, dried (Na₂SO₄), 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 C₂₇H₄₀N₄O₇: C, 60.88; H, 7.57; N, 10.52%.

Pyr-L-Ala-L-Hmb-L-App-NH₂ (**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 CHCl₃ and the solution chromatographed on

silicic acid (Mallinckrodt, 100 mesh) using a column (1.2 cm × 10 cm) and a solvent system of CHCl₃-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, HyalaCH₃), 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 C₂₂H₃₁N₃O₆: 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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