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Cyclic Peptides. XXIII.[1] Synthesis of Retro-Enantio-AM-Toxin I

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Synopsis. To characterize a receptive substance in an apple leaf of phytotoxic AM-toxin I, retro-enantiomer of the toxin was synthesized by the solution method. This analog showed no toxic activity, indicating that the specific backbone conformation of the toxin is essential for the activity.

AM-toxins are host-specific phytotoxic metabolites produced by *Alternaria mali*, which cause spot disease on apple leaves. The structure of AM-toxin I is a cyclic tetradepsipeptide as shown in Fig. 1.[2] We have synthesized various analogs of AM-toxin I and examined their necrotic activities on apple leaves in order to study the relationships between structure and activity of the toxin. We have found that the following factors are important for the induction of the full toxic activity: i) the specific ring conformation,[3] ii) bulkiness of the isopropyl side chain of the L-Hmb[4] residue at position 2,[5] iii) the exact side chain length of the L-Amp residue at position 3,[6] iv) the presence of the ΔAla residue at position 4.[7] The size of side chain of the L-Ala residue at position 1 is not critical to the toxic activity.[8] Thus, AM-toxin I holds a unique structure which can not be easily handled by chemical modifications without loss of the activity. Previously, we illustrated a scheme on interaction between AM-toxin I and a possible receptive substance in the sensitive apple leaf.[6] In this connection, we attempted to design a compound which could characterize the receptive substance.

The cycloretro-enantiomer is a cycloisomer which has unique topochemical relations to the parent cyclic peptide as follows: both are equivalent in the spatial arrangement of various side chains, but reversed in the chirality of each center and the direction of the amide bonds. As the first step of receptor study, we tried to synthesize retro-enantio-AM-toxin I (**1**, Fig. 1) and examined its necrotic activity on an apple leaf. Compared with the sequence of AM-toxin I, the retro-enantio-AM-toxin I (**1**) should have inversed chirality of each center and reversed direction of amide and ester bonds. Therefore, the retro-enantiomer contains D-Val and D-Lac instead of L-Hmb and L-Ala, respectively, in AM-toxin I. Topochemical arrangement of each side chain in **1** is equivalent to that in AM-toxin I.

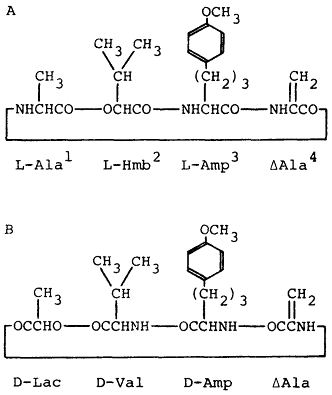

Fig. 1. Structure of AM-toxin I (A) and its retro-enantiomer (**1**, B).

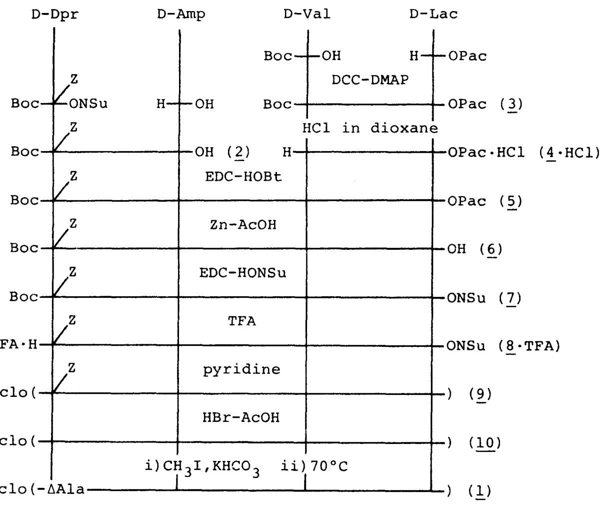

Fig. 2. Synthesis of retro-enantio-AM-toxin I.

The synthetic route for retro-enantiomer (**1**) is shown in Fig. 2. In a preliminary study, according to the successful synthesis of AM-toxin I—III and AM-toxin analogs,[5–8] a linear tetradepsipeptide H–D-Amp–D-Val–D-Lac–D-Dpr(*Z*)–ONSu (**11**), in which the ester bond was placed at the center was selected for cyclization. Cyclization of **11**, however, gave the desired cyclic tetradepsipeptide **9** in a very low yield (4%). Main product of this reaction was a 2,5-piperazinedione *cyclo*(-D-Amp-D-Val-) identified with mass spectrum and elemental analysis. This may be due to preferential ability to form the 2,5-piperazinedione from H–D-Amp–D-Val–OR. In the previous studies,[5–8] such a by-product was not found in cyclization of linear tetradepsipeptide, e.g., H–D-Dpr(*Z*)–L-Ala–L-Hmb–L-Amp–ONSu.

Therefore, the sequence of a linear tetradepsipeptide as the precursor of **9** was changed to H–D-Dpr(*Z*)–D-Amp–D-Val–D-Lac–ONSu (**8**), in which the D-Lac residue was placed at the C-terminal to avoid the 2,5-piperazinedione formation, as shown in Fig. 2. Boc–D-Dpr(*Z*)–ONSu was coupled with D-Amp to give Boc–D-Dpr(*Z*)–D-Amp–OH (**2**). Boc–D-Val–D-Lac–OPac (**3**) was prepared from Boc–D-Val–OH and D-Lac–OPac with DCC-DMAP.[9] Boc group in **3** was removed with HCl followed by condensation with **2** to give Boc–tetradepsipeptide–OPac (**5**). After Pac group was removed with Zn-AcOH, the resulting acid (**6**) was

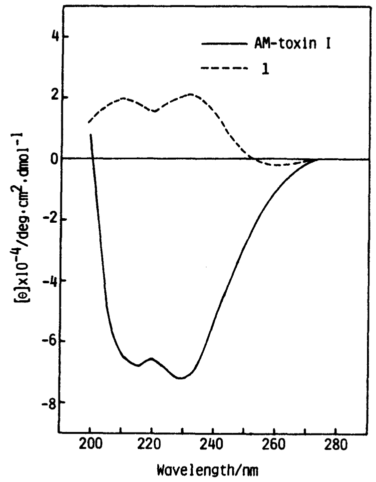

Fig. 3. CD spectra of AM-toxin I and its retro-enantiomer (**1**) in MeOH.

converted to the corresponding active ester (**7**) and its Boc group was removed with TFA to give the active ester trifluoroacetate (**8**·TFA). Cyclization of **8** at a concentration of 3 mM in pyridine gave the cyclic peptide **9** in a yield of 37% from **7**. After deprotection of Z group in **9** with HBr-AcOH, the resulting HBr salt was treated with CH_3I and $KHCO_3$[10] to give the desired **1**. The yield of **1** from **9** was 17% comparable to the yields in previous syntheses of AM-toxins.[5-8]

CD spectra of the retro-enantiomer **1** and AM-toxin I in MeOH are shown in Fig. 3. Although AM-toxin I showed double minima of negative molar ellipticities at 210 and 230 nm, **1** showed positive ones at similar wavelengths. The enantiomer of AM-toxin I, *cyclo*(-D-Ala-D-Hmb-D-Amp-ΔAla-), should show positive bands with the same ellipticities at the same wavelengths as AM-toxin I. When the spectrum of the retro-enantiomer **1** was compared with that of the enantiomer, wavelengths of minima were similar each other but ellipticities of **1** were much smaller than those of the enantiomer. These results indicate that the conformation of **1** differs from that of AM-toxin I which should have the same conformation as the enantiomer, or the content of the common conformation is low in **1**.

Minimum toxic activity of AM-toxin I for the induction of necrosis on an apple leaf (Indo) was 0.001 μg ml^{-1}, while the retro-enantiomer **1** did not show any toxicity at the high concentration up to 100 μg ml^{-1}. The retro-enantiomer **1** contains each side chain topo-equivalently arranged to that of AM-toxin I, but the chirality of each center and the direction of the amide and ester bonds in **1** were reversed. Combined with the previous results,[3,5-8] the presence of each side chain in AM-toxin I is very important, but the direction of the amide and ester bonds, i.e., backbone conformation, also is essential to induce the toxic activity. This specific conformation[3] of AM-toxin I may confirm the exact orientation of each side chain, and then can reveal the full toxic activity.

Experimental

Thin layer chromatography was carried out on Merck silica gel G with the following solvent system: R_f^1, $CHCl_3$-MeOH (5:1); R_f^2, $CHCl_3$-MeOH (9:1); R_f^3, $CHCl_3$-MeOH-AcOH (95:5:1); R_f^4, *n*-BuOH-AcOH-pyridine-H_2O (4:1:1:2). Optical rotations were measured with a Union high sensitivity polarimeter PM-71. Mass spectra were taken on a Nihon-denshi mass spectrometer Model JMS-01SG-2 with a direct inlet system operating at 75 eV, and CD on a JASCO J-40A spectropolarimeter.

Boc-D-Dpr(Z)-D-Amp-OH (2). To a solution of H-D-Amp-OH (810 mg, 3.3 mmol) and NEt_3 (0.46 ml, 3.3 mmol) in DMF (30 ml) was added a solution of Boc-D-Dpr(Z)-ONSu (1.3 g; 3.0 mmol) in DMF (5 ml). The mixture was stirred at room temperature overnight. After evaporation, 10% citric acid was added to the residue. The separated oil was extracted with EtOAc, and the solution was washed with water and dried over Na_2SO_4. After evaporation, the residue was recrystallized from EtOAc-ether-petroleum ether: yield, 1.2 g (73%); mp 141—143 °C; $[\alpha]_D^{20}$ +7.9° (*c* 1, DMF); R_f^1=0.57, R_f^3=0.46. Found: C, 61.78; H, 6.86; N, 7.71%. Calcd for $C_{28}H_{37}N_3O_8$: C, 61.86; H, 6.86; N, 7.73%.

Boc-D-Val-D-Lac-OPac (3). To a chilled solution of Boc-D-Val-OH (2.2 g, 10 mmol), D-Lac-OPac[11] (2.1 g, 10 mmol) and DMAP[9] (1.2 g, 10 mmol) in CH_2Cl_2 (30 ml) was added DCC (2.1 g, 10 mmol). The mixture was stirred at 0 °C for 3 h and at room temperature overnight. After evaporation, the residue was dissolved in EtOAc, washed with 10% citric acid, 4% $NaHCO_3$ and water, and dried over Na_2SO_4. After evaporation, **3** was obtained as an oil: yield, 3.4 g (83%); R_f^2=0.87, R_f^3=0.36.

H-D-Val-D-Lac-OPac·HCl (4·HCl). Compound **3** (2.9 g, 7.0 mmol) was dissolved in 4 M (1M=1 mol dm^{-3}) HCl in dioxane (20 ml). The solution was allowed to stand at room temperature for 2 h. After removal of the solvent, the evaporation was repeated several times by the addition of dioxane. The residue was crystallized by the addition of ether: yield, 2.0 g (92%); R_f^1=0.52.

Boc-D-Dpr(Z)-D-Amp-D-Val-D-Lac-OPac (5). To a chilled solution of **2** (1.2 g, 2.2 mmol), **4**·HCl (670 mg, 2.2 mmol), HOBt (450 mg, 3.3 mmol) and NEt_3 (0.31 ml, 2.2 mmol) in DMF (20 ml) was added EDC·HCl (630 mg, 3.3 mmol). The mixture was stirred at 0 °C overnight. After evaporation, the residue was dissolved in EtOAc, washed with 10% citric acid, 4% $NaHCO_3$ and water, and dried over Na_2SO_4. After evaporation, the residue was recrystallized from EtOAc-ether-petroleum ether: yield, 1.5 g (83%); mp 108—110 °C; $[\alpha]_D^{20}$ +35.1° (*c* 0.5, DMF); R_f^2=0.45, R_f^3=0.40. Found: C, 63.39; H, 6.79; N, 6.71%. Calcd for $C_{44}H_{56}N_4O_{12}$: C, 63.45; H, 6.78; N, 6.73%.

Boc-D-Dpr(Z)-D-Amp-D-Val-D-Lac-OH (6). To a solution of **5** (1.5 g, 1.8 mmol) in 90% AcOH (20 ml) was added Zn powder (0.5 g) at room temperature. The mixture was stirred at room temperature for 8 h. The insoluble Zn was filtered off and the filtrate was evaporated. Ten per cent citric acid was added to the residue. The separated oil was extracted with EtOAc, washed with water, and dried over Na_2SO_4. After evaporation, the residue was recrystallized from EtOAc-ether-petroleum ether: yield, 1.17 g (91%); mp 115—118 °C; $[\alpha]_D^{20}$ +26.9° (*c* 0.5, DMF); R_f^1=0.55, R_f^3=0.33. Found: C, 60.64; H, 6.91; N, 7.61%. Calcd for $C_{36}H_{50}N_4O_{11}$: C, 60.49; H, 7.05; N, 7.84%.

Boc-D-Dpr(Z)-D-Amp-D-Val-D-Lac-ONSu (7). To a chilled solution of **6** (1.1 g, 1.5 mmol) and HONSu (345 mg, 3.0 mmol) in DMF (10 ml) was added EDC·HCl (575 mg, 3.0 mmol). The mixture was stirred at 0 °C overnight. After evaporation, the residual solid was collected with the aid of cold water and dried in vacuo: yield, 1.2 g (98%); R_f^2= 0.53. This was used to the next step without purification.

***Cyclo*(-D-Dpr(Z)-D-Amp-D-Val-D-Lac-) (9).** A solution of **7** (1.2 g, 1.5 mmol) in TFA (5 ml) was allowed to stand at 0 °C for 30 min. After evaporation, the residual oil was solidified by the addition of ether. The powder was washed several times with ether by decantation to give H-D-Dpr(*Z*)-D-Amp-D-Val-D-Lac-ONSu·TFA (**8**·TFA). The powder was dissolved in DMF (10 ml) and the solution was added dropwise into pyridine (500 ml) with stirring at room temperature. The stirring was continued for 3 d and the reaction mixture was evaporated. The residue was collected with the aid of cold water, and washed with 10% citric acid and water. This was recrystallized from EtOAc-ether: yield, 330 mg (37%); mp 224—226 °C (decomp); $[\alpha]_D^{20}$ +155° (*c* 0.3, DMF); MS *m/z* 596 (M^+); $R_f{}^2$=0.45, $R_f{}^3$=0.29. Found: C, 62.30; H, 6.87; N, 9.40%. Calcd for $C_{31}H_{40}N_4O_8$: C, 62.40; H, 6.76; N, 9.39%.

***Cyclo*(-ΔAla-D-Amp-D-Val-D-Lac-) (1).** Compound **9** (120 mg, 0.2 mmol) in 25% HBr in AcOH (5 ml) was allowed to stand at room temperature for 2 h. After evaporation, the residue was solidified by the addition of ether to give a powder of cyclo (-D-Dpr-D-Amp-D-Val-D-Lac-)·HBr (**10**·HBr). To a suspension of **10**·HBr in EtOAc (30 ml) were added CH_3I (2 ml) and $KHCO_3$ (400 mg), and the mixture was stirred in the dark at room temperature. The same amounts of CH_3I and $KHCO_3$ were added again 2 d later, the stirring being continued for 4 d. After removal of solvent and excess CH_3I in vacuo, the residue was suspended in EtOAc (30 ml) and the suspension was stirred at 70 °C for 2 h. Insoluble material was filtered off and the filtrate was evaporated to give a crude product. The crude product was purified by silica gel column (1.1×20 cm) chromatography using $CHCl_3$-acetone (4:1) followed by recrystallization from EtOAc-ether-petroleum ether: yield, 15 mg (17%); mp 158—160 °C; MS *m/z* 445 (M^+); $R_f{}^2$=0.48, $R_f{}^3$=0.36. Found: C, 61.83; H, 7.07; N, 9.01%. Calcd for $C_{23}H_{31}N_3O_6$: C, 62.01; H, 7.01; N, 9.43%.

Biological Assay. Biological assay on apple leaves (susceptible cultivar, Indo) was carried out as described previously.[6]

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4) Abbreviations: Amp, 2-amino-5-(*p*-methoxyphenyl)-pentanoic acid; ΔAla, 2,3-dehydroalanine; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Hmb, 2-hydroxy-3-methylbutanoic acid; HOBt, 1-hydroxybenzotriazole; HONSu, *N*-hydroxysuccinimide; Lac, lactic acid; -OPac, phenacyloxy; TFA, trifluoroacetic acid.

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