Note

Synthesis of a Lactam Analog of AM-Toxin I

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AM-toxins are host-specific phytotoxic metabolites produced by *Alternaria mali*, which cause leaf spot disease in apple.¹⁾ AM-toxin I has the structure shown in Fig. 1, and AM-toxins II and III have similar structures, but instead of L-2-amino-5-(*p*-methoxyphenyl)-pentanoic acid (L-Amp) in I, AM-toxins II and III contain L-2-amino-5-phenylpentanoic and L-2-amino-5-(*p*-hydroxyphenyl)-pentanoic acid, respectively. We have previously synthesized AM-toxins I, II, III, and their analogs and determined necrotic activities on apple leaf with these toxins.^{2~4)} A characteristic feature of the structure of AM-toxins is the occurrence of a lactone bond in their cyclic back-

bone. Other phytotoxic cyclic tetrapeptides such as ten-toxin⁵⁾ and chlamydocin⁶⁾ are exclusively composed of lactam linkages. Therefore, we tried to synthesize AM-toxin I analogs containing L-valine in place of L-2-hydroxy-3-methylbutanoic acid (L-Hmb). As the minimum toxic concentrations of AM-toxins I, II and III were reported to be 0.002, 0.02, and 0.02 µg/ml, respectively, we tried to synthesize a lactam analog of the most toxic AM-toxin I and to evaluate the effect of substitution of an amide bond for an ester bond in AM-toxins.

Synthesis of peptides. As a preliminary study for synthesizing the desired [L-Val²]-AM-toxin I (**6a**), a simplified analog, *cyclo*-L-Ala¹-L-Val²-L-Tyr(Me)³-ΔAla⁴- (**6b**), was prepared, because *O*-methyl-L-tyrosine (L-Tyr(Me)) was easily derived by the methylation of L-tyrosine. To prepare the ΔAla residue, we used the Hofmann degradation of D-2,3-diaminopropionic acid (D-Dap) according to the successful synthesis of AM-toxin III.⁴⁾ In order to obtain a cyclic monomer in good yield at the cyclization step, the D-amino acid residue was placed at the *N*-terminus of a cyclization precursor, because in the case of the AM-toxin III synthesis, cyclization of the precursor having a β-benzoyloxycarbonyl-D-Dap (D-Dap(Z)) residue at the *N*-terminus yielded the cyclic monomer exclusively.⁴⁾ Boc-D-Dap (Z)-L-Ala-L-Val-L-Tyr(Me)-OH (**1b**) was prepared by stepwise elongation from L-Tyr(Me) toward the amino end using Boc-amino acid *N*-hydroxysuccinimide (HONSu) ester and following by removal of the Boc-group with HCl in dioxane. Compound **1b** was converted to the corresponding HONSu ester, Boc-D-Dap(Z)-L-Ala-L-Val-L-Tyr(Me)-ONSu (**2b**), using HONSu and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, and **2b** was treated with trifluoroacetic acid (TFA) to give H-D-Dap(Z)-L-Ala-L-Val-L-Tyr(Me)-ONSu trifluoroacetate (**3b**·TFA). Cyclization of **3b** unexpectedly yielded a mixture of the cyclic monomer and dimer in the ratio of 35:45, but the insoluble monomer, *cyclo*-(L-Ala-L-Val-L-Tyr(Me)-D-Dap(Z)-) (**4b**), was easily separated by washing with *N,N*-dimethylformamide in a yield of 28%. Substitution of the amide bond for the ester bond resulted in a marked difference in the composition of the product.

The Z group in **4b** was removed by treating HBr in AcOH (the poor solubility of **4b**, even in *N,N*-dimethylformamide, precluded catalytic hydrogenolysis of the Z group). The hydrobromide, *cyclo*-(L-Ala-L-Val-L-Tyr(Me)-D-Dap(Z)-)·HBr (**5b**), was treated with CH₃I and KHCO₃ in MeOH at room temperature for 1 day to give the desired cyclic monomer, *cyclo*-(L-Ala-L-Val-L-Tyr(Me)-ΔAla-) (**6b**). Crude **6b** was purified by silica gel column chromatography to give pure **6b** (25% from **4b**; mp 259~260°C (dec.); MW, 416 (calcd. 416.2) (MW was determined on a Nihondenshi JMS-01SG-2 mass spectrometer). Compound **6a**, *cyclo*-(L-Ala-L-Val-L-Amp-ΔAla-), was synthesized in the same manner as for **6b**;

Peptide	X	Y	n	W
AM-Toxin I	O	Hmb	3	Amp
[L-Val ²]- AM-Toxin I (6a)	NH	Val	3	Amp
[L-Val ² , L-Tyr(Me) ³]- AM-Toxin I (6b)	NH	Val	1	Tyr(Me)

FIG 1. Structures of AM-Toxin I and Its Lactam Analogs.

yield of **6a** from *cyclo*-(L-Ala-L-Val-L-Amp-D-Dap(Z)-), 27%; mp 220~223°C (dec.); MW 444 (calcd. 444.2). Compounds **6a** and **6b** showed one spot each on TLC with several solvent systems and gave satisfactory results in elemental analysis.

Study by nuclear magnetic resonance spectroscopy. There are several differences between the ^1H -NMR spectra (in DMSO- d_6) of **6a** and those of AM-toxin I. The signals of the amide proton of the ΔAla residue in the lactam analog shifted downfield from 9 ppm to 10.4 ppm. Two sets of signals with similar intensity were observed for the aromatic protons and β,γ -protons in the aliphatic side chains at 29°C. These spectra turned to one set of the signals at 100°C. These observations indicated that the lactam analog exists as a mixture of two conformers with the similar populations. It should be noted that AM-toxin II is composed of two conformers with very unequal populations.⁷⁾

Necrotic activity on apple leaf. The minimum toxic concentration of the analog **6a** was 0.1 $\mu\text{g}/\text{ml}$, whereas that of AM-toxin I was 0.002 $\mu\text{g}/\text{ml}$. This means that the lactone ring in AM-toxin I is not indispensable for activity but is replaced by a lactam bond and accomplished with considerable loss of activity. This replacement simultaneously caused a marked change in conformer populations. The loss of activity might have resulted from a

decrease in the population of the biologically active conformer. Precise study on the conformation of **6a** and **6b** is in progress.

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