Cyclic Peptides. XXII.[1] Synthesis of [2-Amino-2,3-dehydrobutanoic Acid[4]]AM-Toxin I

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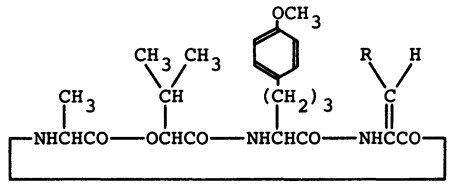
Synopsis. To investigate the role of the side chain of the 2,3-dehydroalanine (ΔAla) residue at position 4 in a cyclic tetradepsipeptide phytotoxin AM-toxin I on necrotic activity for apple leaf, [2-amino-2,3-dehydrobutanoic acid[4]] AM-toxin I was synthesized by two different routes. This analog showed no toxic activity, indicating that the side chain of the ΔAla residue can not be handled without loss of the activity.

AM-toxin I is a host-specific phytotoxic metabolite produced by *Alternaria mali*, which causes spot disease on apple leaf. The structure of AM-toxin I is a cyclic tetradepsipeptide containing a ΔAla[2] residue as shown in Fig. 1.[3] We have synthesized various analogs of AM-toxin I in order to study the relationship between structure and function (host-specific toxicity) of the toxin. According to our previous results,[4-8] for the induction of the full toxic activity, AM-toxin I requires factors such as specific ring conformation, the presence of the L-Hmb[2] and L-Amp[3] residues, and a double bond in the ΔAla[4] residue. However, significance of bulkiness of the side chain in the ΔAla[4] residue has not yet been explored. Thus, we synthesized an AM-toxin I analog (**1**) containing Δ^{Z}Abu which has a methyl group instead of a hydrogen atom in ΔAla (Fig. 1) and examined the activity of **1**.

Two synthetic routes for the cyclic peptide (**1**) are shown in Fig. 2. According to the successful synthesis of AM-toxin III and AM-toxin I analogs[6-9] using a D-amino acid residue as the precursor of ΔAla at the *N*-terminal of linear intermediates, a linear tetradepsipeptide, H-D-Thr-L-Ala-L-Hmb-L-Amp-ONSu (**6**), was selected as the precursor for the cyclization reaction. Lee et al.[10] reported that an L-Thr residue was converted to a Δ^{Z}Abu residue by the Photaki method[11] and Δ^{E}Abu was prepared similarly from an L-aThr residue, indicating that the D-Thr residue can be similarly converted to the Δ^{Z}Abu residue after cyclization. On the other hand, Ueda et al.[12] reported that cyclization of a linear tetradepsipeptide containing an L-Ser(Bzl) residue as the precursor of ΔAla residue at the *N*-terminal gave a desired cyclic tetradepsipeptide in high yield comparable to that of cyclization of a precursor containing a D-Ser residue.[13] Therefore, we also synthesized H-L-Thr(Bzl)-L-Ala-L-Hmb-L-Amp-ONSu (**7**) as a precursor of the cyclic peptide. Boc-D-Thr(or L-Thr(Bzl))-ONSu was coupled with H-L-Ala-L-Hmb-L-Amp-OH[6] to give Boc-D-Thr (or L-Thr(Bzl))-L-Ala-L-Hmb-L-Amp-OH (**2** or **3**).



	R	X
AM-toxin I	H	ΔAla
[Δ^{Z}Abu[4]]AM-toxin I (**1**)	CH_3	Δ^{Z}Abu

Fig. 1. Structure of AM-toxin I and [Δ^{Z}Abu[4]]AM-toxin I.

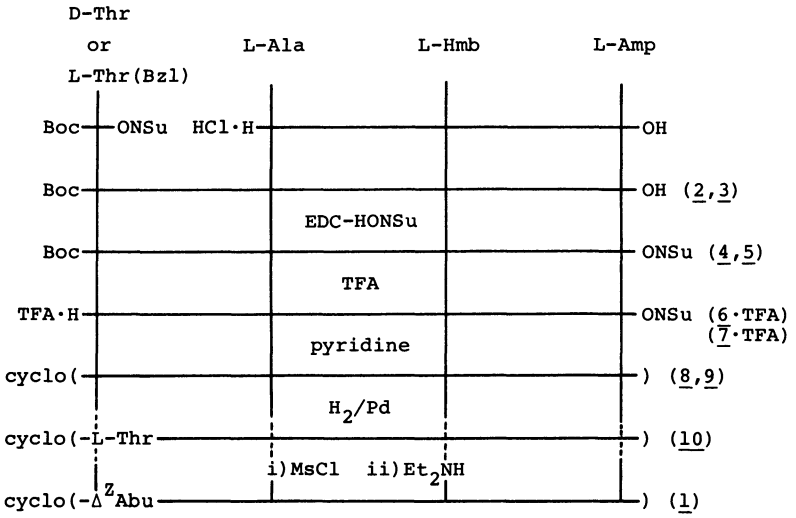


Fig. 2. Synthesis of [Δ^{Z}Abu[4]]AM-toxin I. H_2/Pd procedure was applied to L-Thr(Bzl) derivative (**9**).

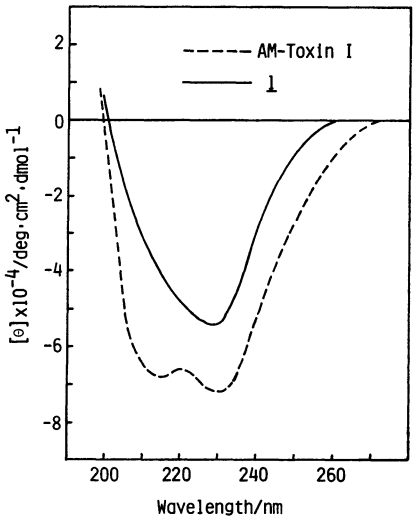


Fig. 3. CD spectra of AM-toxin I and [Δ^ZAbu^4]AM-toxin I in MeOH.

After the conversion of **2** and **3** to the corresponding active ester trifluoroacetates (**6**·TFA and **7**·TFA), their cyclizations at a concentration of 3 mM in pyridine gave cyclic peptides (**8** and **9**) in yields of 47 and 33%, respectively. The benzyl group of **9** was removed by catalytic hydrogenation. Both compounds **8** and **10** were treated with methanesulfonyl chloride and followed by diethylamine to give desired **1**. This was purified by silica-gel column chromatography followed by recrystallization. Although the yield in cyclization of **6** (D-Thr derivative) was higher than that of **7** (L-Thr(Bzl) derivative), compound **9** was converted to **1** in higher yield (16%) compared with **8** (7.2%). Noticeable difference was not observed between the total yields of **1** from **2** and **3**. Configuration of ΔAbu^4 in **1** was identified as Z form by means of ^{1}H NMR chemical shift of methyl proton in ΔAbu (1.7 ppm) compared with that reported by Lee et al.[10] (1.73 ppm for Z form and 2.05 ppm for E form).

CD spectra of **1** and AM-toxin I in methanol are shown in Fig. 3. Although AM-toxin I showed two negative ellipticity minima at 210 and 230 nm, **1** showed only a minimum at 230 nm. This result indicates that the conformation of **1** slightly differs from that of AM-toxin I.

Although minimum toxic activity of AM-toxin I for the induction of necrosis on an apple leaf (Indo) was 0.001 $\mu g\,ml^{-1}$, **1** did not show any toxicity at the concentration of 100 $\mu g\,ml^{-1}$. These results indicate that increasing the bulkiness of the side chain in the ΔAla^4 residue may result in alteration of the conformation of analog **1** and cause loss of the activity. This inactivity may also be due to the result that the unsaturated side chain of the ΔAbu residue in **1** can not react with a receptive substance in an apple leaf differing from the ΔAla residue in AM-toxin I. Combined with the results of no or very little activity of [L- or D-Ala^4]AM-toxin I,[4] the ΔAla residue in this toxin is certainly essential to induce the activity. Phytotoxic tentoxin is a cyclic tetrapeptide containing a *N*-Me-ΔPhe residue[14] and an antibacterial heptapeptide TL-119 contains a Δ^ZAbu residue in its cyclic tetrapeptide part.[15] Since the Δ^ZAbu residue in **1** can not be used as a substitute for the ΔAla residue in AM-toxin I, roles of the dehydroamino acid residues in these peptides on interactions with their receptive substances seem to be different from that of the ΔAla residue in AM-toxin I.

Experimental

Thin layer chromatography was carried out on Merck silica gel G with the following solvent systems: 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, $CHCl_3$–acetone (1:1). Optical rotation was measured with a Union high sensitivity polarimeter PM-71. Mass spectra were taken on a Nihondenshi mass spectrometer Model JMS-01SG-2 with a direct inlet system operating at 75 eV, CD on a JASCO J-40A spectropolarimeter, and ^{1}H NMR on a Jeol JNM-FX90Q.

Boc-D-Thr-L-Ala-L-Hmb-L-Amp-OH (2). To a solution of H-L-Ala-L-Hmb-L-Amp-OH·HCl[6] (410 mg, 0.95 mmol) and Et_3N (0.27 ml, 1.9 mmol) in a mixture (10 ml) of H_2O and dioxane (1:1) was added Boc-D-Thr-ONSu (250 mg, 0.8 mmol). 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 product was obtained as an oil: Yield, 340 mg (72%); R_f^1=0.57.

Boc-L-Thr(Bzl)-L-Ala-L-Hmb-L-Amp-OH (3). Compound **3** was prepared from Boc-L-Thr(Bzl)-ONSu (610 mg, 1.5 mmol) and H-L-Ala-L-Hmb-L-Amp-OH·HCl[6] (780 mg, 1.8 mmol) as described for **2**. Compound **3** was crystallized from EtOAc–ether–petroleum ether: Yield, 800 mg (77%); mp 85—87 °C; $[\alpha]_D^{20}$ −7.2° (*c* 0.5, DMF); R_f^1=0.49. Found: C, 62.17; H, 7.50; N, 6.13%. Calcd for $C_{36}H_{51}N_3O_{10}\cdot 1/2H_2O$: C, 62.23; H, 7.54; N, 6.05%.

Boc-D-Thr-L-Ala-L-Hmb-L-Amp-ONSu (4). To a chilled solution of **2** (300 mg, 0.5 mmol) and HONSu (115 mg, 1.0 mmol) in DMF (3 ml) was added EDC·HCl (192 mg, 1.0 mmol). The mixture was stirred at 0 °C for 24 h. After evaporation, the residue was solidified by the addition of cold water and dried in vacuo: Yield, 320 mg (92%); R_f^1= 0.71. This was used to the next step without purification.

Boc-L-Thr(Bzl)-L-Ala-L-Hmb-L-Amp-ONSu (5). Compound **5** was prepared from **3** (690 mg, 1.0 mmol) as described for **4**: Yield, 770 mg (98%); R_f^2=0.70.

***cyclo*(-L-Ala-L-Hmb-L-Amp-D-Thr-) (8).** A solution of **4** (320 mg, 0.45 mmol) in TFA (2 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-Thr-L-Ala-L-Hmb-L-Amp-ONSu·TFA (**6**·TFA). This was dissolved in DMF (5 ml) and the solution was added dropwise into pyridine (130 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 water, and washed with 10% citric acid and water. This was recrystallized from DMF-EtOAc–ether; yield, 100 mg (47%); mp 242—244 °C (decomp); MS *m/z* 477 (M^+); R_f^1=0.76, R_f^2= 0.47. Found: C, 59.77; H, 7.38; N, 8.65%. Calcd for $C_{24}H_{35}N_3O_7\cdot 1/3H_2O$: C, 59.61; H, 7.36; N, 8.69%.

***cyclo*(-L-Ala-L-Hmb-L-Amp-L-Thr(Bzl)-) (9).** Compound **9** was prepared from **5** (780 mg, 1.0 mmol) as described for **8**. This was recrystallized from DMF-EtOAc: Yield, 190 mg (33%); mp 208—210 °C (decomp); MS *m/z* 567 (M^+); R_f^2=0.72, R_f^3=0.33. Found: C, 64.66; H, 7.14; N, 7.43%. Calcd for $C_{31}H_{41}N_3O_7\cdot 1/2H_2O$: C, 64.57; H, 7.34; N, 7.29%.

***cyclo*(-L-Ala-L-Hmb-L-Amp-Δ^ZAbu-) ([Δ^ZAbu^4]AM-toxin I, 1) from D-Thr Derivative (8).** To a chilled solution of **8** (67 mg, 0.14 mmol) in pyridine (3 ml) was added MsCl (0.055 ml, 0.7 mmol). After 24 h at 4°C, the solution was evaporated. The residue was solidified by the addition of cold water and washed with ether–petroleum ether (1:1) to give a powder of *cyclo*(-L-Ala-L-Hmb-L-Amp-D-Thr(Ms)-) (**11**): Yield, 69 mg (89%); R_f^2=0.82. This structure was confirmed by ^{1}H NMR spectrum. To a solution of **11** in DMF (1 ml) was added Et_2NH (0.026 ml, 0.26 mmol) at room temperature. The mixture was allowed to stand for 24 h at room temperature. After evaporation, the residue was solidified by the addition of cold water. This crude product (57 mg) was applied to a silica-gel column (1.1×27 cm) and eluted with $CHCl_3$–CH_3CN (1:1). The fraction was pooled and evaporated. The residue was recrystallized from EtOAc–petroleum ether: Yield, 4.6 mg (7.2%); mp 220—222°C (decomp); MS *m/z* 459 (M^+); R_f^2=0.38, R_f^4=0.34. Found: C, 62.50; H, 7.17; N, 9.21%. Calcd for $C_{24}H_{33}N_3O_6$: C, 62.72; H, 7.24; N, 9.14%.

[Δ^ZAbu^4]AM-toxin I (1) from L-Thr(Bzl) Derivative (9): Compound **9** (100 mg, 0.18 mmol) in DMF (25 ml) was hydrogenated for 4 h in the presence of Pd-black. After removal of the catalyst, the filtrate was evaporated and the residue was solidified by the addition of ether to give a powder of *cyclo*(-L-Ala-L-Hmb-L-Amp-L-Thr-) (**10**): Yield, 86 mg (100%); R_f^1=0.50. Compound **10** was treated with MsCl and Et_2NH, and then purified with a silica-gel column to give **1**; Yield, 13 mg (16%). This was identified to that obtained from **8** by mp, TLC and ^{1}H NMR spectrum.

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References

1) Part XXI of this series: H. Mihara, K. Ikesue, S. Lee, H. Aoyagi, T. Kato, T. Ueno, and N. Izumiya, *Int. J. Peptide Protein Res.*, in press.

2) Abbreviations: aThr, allothreonine; Abu, 2-aminobutanic acid; Amp, 2-amino-5-(*p*-methoxyphenyl)-pentanoic acid; Bzl, benzyl; Δ, 2, 3-dehydro; DMF, *N,N*-dimethylformamide; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; Hmb, 2-hydroxy-3-methylbutanoic acid; HONSu, *N*-hydroxysuccinimide; MsCl, methanesulfonyl chloride; TFA, trifluoroacetic acid.

3) T. Ueno, T. Nakashima, Y. Hayashi, and H. Fukami, *Agric. Biol. Chem.*, **39**, 1115 (1975).

4) Y. Shimohigashi, S. Lee, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Jpn*, **51**, 584 (1978).

5) T. Higashijima, Y. Shimohigashi, T. Kato, N. Izumiya, T. Ueno, and T. Miyazawa, *Biopolymers*, **22**, 1167 (1983).

6) H. Mihara, H. Aoyagi, S. Lee, M. Waki, T. Kato, and N. Izumiya, *Int. J. Peptide Protein Res.*, **23**, 447 (1984).

7) H. Aoyagi, H. Mihara, S. Lee, T. Kato, T. Ueno, and N. Izumiya, *Int. J. Peptide Protein Res.*, **25**, 144 (1985).

8) H. Mihara, K. Ikesue, S. Lee, H. Aoyagi, T. Kato, T. Ueno, and N. Izumiya, *Int. J. Peptide Protein Res.*, in press.

9) T. Kanmera and N. Izumiya, *Int. J. Peptide Protein Res.*, **19**, 79 (1982).

10) S. Lee, T. Kanmera, H. Aoyagi, and N. Izumiya, *Int. J. Peptide Protein Res.*, **13**, 207 (1979).

11) I. Photaki, *J. Am. Chem. Soc.*, **85**, 1123 (1963).

12) K. Ueda, M. Kondo, M. Waki, and N. Izumiya, "Peptide Chemistry 1984," ed by N. Izumiya, Protein Research Foundation, Osaka (1985), pp. 157—160.

13) Y. Shimohigashi and N. Izumiya, *Int. J. Peptide Protein Res.*, **12**, 7 (1978).

14) G. E. Templeton, *Microb. Toxins*, **8**, 160 (1972).

15) J. Shoji, H. Hinoo, Y. Wakisaka, K. Koizumi, and M. Mayama, *J. Antibiot.*, **28**, 126 (1975).