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SYNTHESES OF AM-TOXIN I ANALOGS CONTAINING A LOWER OR HIGHER HOMOLOG OF L-2-AMINO-5-(*p*-METHOXYPHENYL)PENTANOIC ACID

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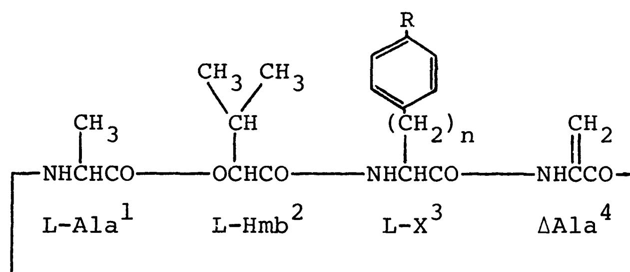
Two analogs of AM-toxin I (cyclic tetradepsipeptide), [L-2-amino-4-(*p*-methoxyphenyl)butanoic acid^3]-AM-toxin I and [L-2-amino-6-(*p*-methoxyphenyl)hexanoic acid^3]-AM-toxin I, were synthesized by the conventional method of peptide synthesis. Their weak necrotic activities on apple leaf indicated high importance of the side-chain length of L-2-amino-5-(*p*-methoxyphenyl)pentanoic acid residue at position 3 in AM-toxin I.

AM-Toxins are host-specific phytotoxic metabolites produced by *Alternaria mali*, which cause spot disease on apple leaf.[1] The structures of AM-toxin I (1c), II and III are shown in Fig. 1 and were confirmed by chemical syntheses through the conventional method.[2,3] In the study of the relationship between structure and activity in these toxins using their analogs, we have found some important factors for inducing necrotic activity: the presence of the ΔAla[4] residue and the specific ring conformation. Furthermore, the substitution of an L-Tyr(Me) residue for L-Amp[4] caused great decrease in activity.[5] This result may suggest the biological importance of the side-chain length at position 3 in AM-toxins. We, therefore, were interested in investigating systematically and precisely the influence of the side-chain length at position 3 on the activity. We selected two analogs, [L-Amb^3]-AM-toxin I (1a)[4] and [L-Amh^3]-AM-toxin I (1b),[4] as the synthetic targets, because AM-toxin I (1c) shows the highest activity among natural AM-toxins. Moreover, the synthesis of 1c was tried again, as its yield by the previous method was unsatisfactory.[2]



Peptide	n	R	X
AM-Toxin I (1c)	3	OCH_3	Amp
AM-Toxin II	3	H	App
AM-Toxin III	3	OH	Ahp
[L-Amb^3]-AM-Toxin I (1a)	2	OCH_3	Amb
[L-Amh^3]-AM-Toxin I (1b)	4	OCH_3	Amh
[L-Tyr(Me)^3]-AM-Toxin I (1d)	1	OCH_3	Tyr(Me)

Fig. 1. Structure of AM-toxin I-III and analogs of AM-toxin I.

According to our successful synthesis of AM-toxin III,[3)] the ΔAla residue was formed by Hofmann degradation of A_2pr[4)] residue.[6)] D-Amino acid residue was placed at the *N*-terminus and the ester bond at the center of a precursor-linear peptide in order to obtain cyclic monomer in good yield. L-Amb and L-Amh were prepared from the corresponding acetyl-DL-amino acids by the use of acylase.[7,8)] Figure 2 shows the scheme for the synthesis of 1a. Boc-L-Ala-L-Hmb-ONSu[3)] was coupled with L-Amb to afford Boc-L-Ala-L-Hmb-L-Amb-OH (2a). After deprotection of Boc group in 2a with HCl in dioxane, the hydrochloride (3a·HCl) was coupled with Boc-D-A_2pr(Z)-ONSu to afford Boc-D-A_2pr(Z)-L-Ala-L-Hmb-L-Amb-OH (4a). The acid (4a) was converted to the corresponding active ester trifluoroacetate (5a·TFA) by treatment with HONSu[4)] and EDC[4)] followed by removal of Boc group. Cyclization of 5a·TFA (3 mM) in pyridine gave *cyclo*(-L-Ala-L-Hmb-L-Amb-D-A_2pr(Z)-) (6a) in yield of 53%. The presence of dimer could not be detected in the product. After removal of Z group in 6a by catalytic hydrogenation, treatment of the hydrogenated product with CH_3I and $KHCO_3$ in EtOAc for 6 d at room temperature and then heating at 60-70°C for 2 h gave the desired 1a. Crude 1a was purified by silica gel column chromatography using $CHCl_3$-acetone (4:1) followed by recrystallization from EtOAc-ether to give pure 1a (28%); mp 216-218°C (decomp); MW, 431 (calcd. 431.2) (MW was determined on a Nihondenshi mass spectrometer JMS-01SG-2). Compounds 1b and 1c were synthesized in the same

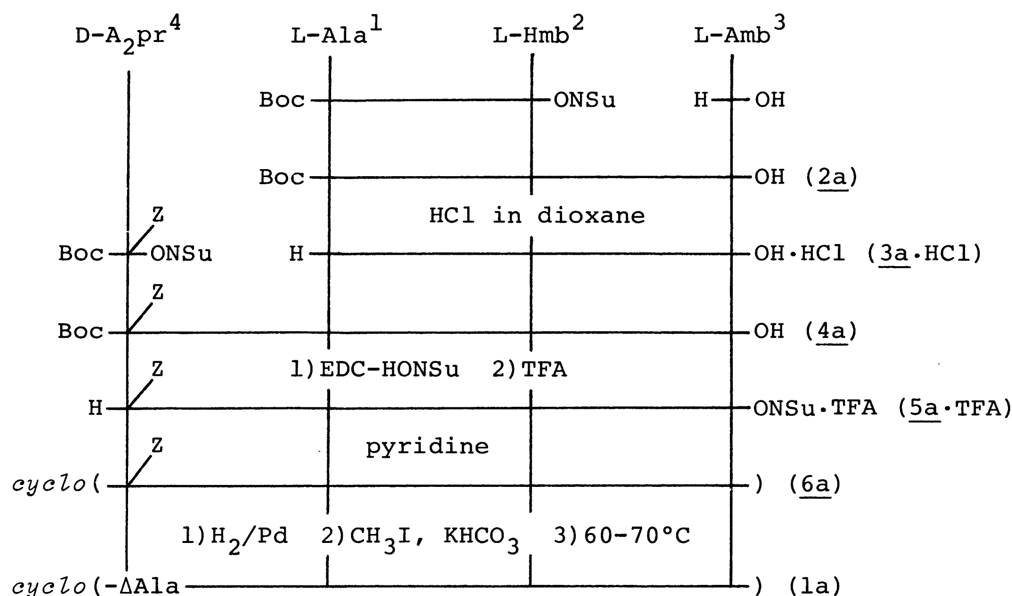


Fig. 2. Synthesis of [L-Amb^3]-AM-toxin I (1a).

manner; yield of 1b, 33%; mp 168-170°C; MW, 459 (459.2); yield of 1c, 26%; mp 210-213°C (decomp); MW, 445 (445.2). All compounds showed satisfactory results in elemental analysis and gave single spots on TLC using several solvent systems.

Synthetic 1c showed the same ^{1}H-NMR (in DMSO-d_6) and UV (in MeOH) spectra as those of natural AM-toxin I. Spectra of ^{1}H-NMR, UV and CD (in MeOH) of 1a and 1b were similar to those of 1c, indicating that the essential conformation was maintained in these analogs. However, minimum toxicities of 1a and 1b on apple leaves (Indo) were 0.1 and 1 μg/ml, respectively, whereas the activity of 1c or natural AM-toxin I was 0.001 μg/ml. Furthermore, the activity of 1d was 10 μg/ml. These results suggest that the aromatic ring in the L-Amp residue in AM-toxin I favorably interacts with a possible counterpart of the plant.

A similar cyclotetrapeptide, tentoxin, interacts with chloroplast-coupling factor 1 in a sensitive plant and inhibits the photosynthesis of the plant.[9)] In the case of 1a and 1b, shortening or elongation of the side chain in the L-Amp residue by only one methylene group reduces the chance of suitable positioning of the aromatic ring to cause grest decrease in the activity. Moreover, elimination of two methylene groups as shown in 1d scarcely allows the toxic interaction of the compound with the apple leaf. It is noteworthy that the exact side-chain length at position 3 must be an important factor for induction of necrotic activity, in consideration of the fact that a proteolytic enzyme trypsin strictly requires a Lys

or Arg residue at the susceptible site in a substrate, and the difference in the side-chain length by only one methylene causes great decrease in the suscepbitility.[10,11)]

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4) Abbreviations: ΔAla, α,β-dehydroalanine; Amb, 2-amino-4-(*p*-methoxyphenyl)butanoic acid; Amh, 2-amino-6-(*p*-methoxyphenyl)hexanoic acid; Amp, 2-amino-5-(*p*-methoxyphenyl)pentanoic acid; A_2pr, 2,3-diaminopropionic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Hmb, 2-hydroxy-3-methylbutanoic acid; HONSu, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid; Tyr(Me), *O*-methyltyrosine.
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