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SYNTHESES OF AM-TOXIN III AND ITS ANALOGS USING THE HOFMANN DEGRADATION

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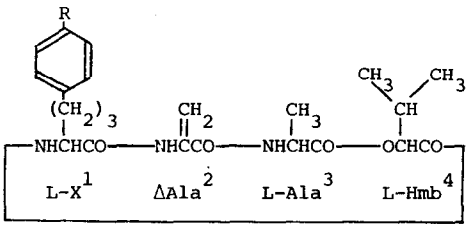
Abstract: Syntheses of cyclotetradepsipeptides, AM-toxin III and two analogs, were achieved, in which the Hofmann degradation method was used in order to derive a dehydroalanine residue from a 2,3-diaminopropionic acid residue in cyclodepsipeptide precursors.

AM-Toxins (Fig. 1) are host-specific phytotoxic metabolites produced by *Alternaria mali*, which cause leaf spot disease of apple.[1-3] Previously, we reported the confirmation of the structure of AM-toxin I and II by their chemical syntheses.[4,5] The structure of AM-toxin III was determined only by its mass and NMR spectra, and other physicochemical properties and chemical nature could not be elucidated because of lack of the sample.[3] Thus we were interested in confirming the structure of AM-toxin III (1c) and further in providing the pure product by its chemical synthesis.

In the syntheses of AM-toxin I and II, a dehydroalanine (ΔAla) residue was formed by β-elimination of a Ser residue in a cyclic depsipeptide precursor.[4,5] However this method is not applicable to the synthesis of 1c because it contains a reactive phenol group in position 1. Recently Nomoto *et al.* reported a new method converting a peptide containing 2,3-diaminopropionic acid (Dap) into a ΔAla-peptide using the Hofmann degradation.[6] They suggested that a phenol



	R	X
AM-toxin I	OCH_3	Amp
AM-toxin II	H	App
AM-toxin III (1c)	OH	Ahp

Fig. 1. Structure of AM-toxin congeners.

group was not affected during this reaction, and therefore we intended to synthesize 1c using the Hofmann degradation. To evaluate the applicability of this method to the synthesis of AM-toxin III, we attempted to synthesize an analog, [L-Tyr(Me)[1]]-AM-toxin (1a) (Tyr(Me), *O*-methyltyrosine),[7] as a model peptide.

A cyclic tetradepsipeptide precursor (9a) of 1a containing a D-Dap(Z) residue (Z, benzyloxycarbonyl) was synthesized through the route shown in Fig. 2. Previously we observed that a cyclic monomer related to AM-toxin could be obtained exclusively and in good yield from a linear precursor containing a D-amino acid at the *N*-terminus and an L-hydroxy acid at the third position from the *N*-terminus.[5] Thus we selected a linear depsipeptide having a sequence of H-D-Dap(Z)-L-Ala-L-Hmb-L-Tyr(Me)-ONSu (Hmb, 2-hydroxy-3-methylbutanoic acid; ONSu, *N*-hydroxysuccinimide ester) for the synthesis of 1a. Boc-L-Ala-L-Hmb-ONSu (3) (Boc, *t*-butyloxycarbonyl) prepared from Boc-L-Ala-L-Hmb-OH (2)[7] was coupled with H-L-Tyr(Me)-OH.[8] The acid (4a) was deprotected by the action of HCl in dioxane to give the desired H-L-Ala-L-Hmb-L-Tyr(Me)-OH·HCl (5a·HCl) (83%). Boc-D-Dap(Z)-L-Ala-L-Hmb-L-Tyr(Me)-OH (6a) (82%) was synthesized by the coupling of Boc-D-Dap(Z)-ONSu with 5a·HCl. Compound 6a in DMF was treated with *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, and the solution was evaporated. Boc-D-Dap(Z)-L-Ala-L-Hmb-L-Tyr(Me)-ONSu (7a) was collected by filtration with the aid of cold water. Boc group of 7a was removed by the treatment with trifluoroacetic acid, and the trifluoroacetate (8a·TFA) was subjected to cyclization in pyridine at the concentration of 3×10^{-3} M. After the solution had

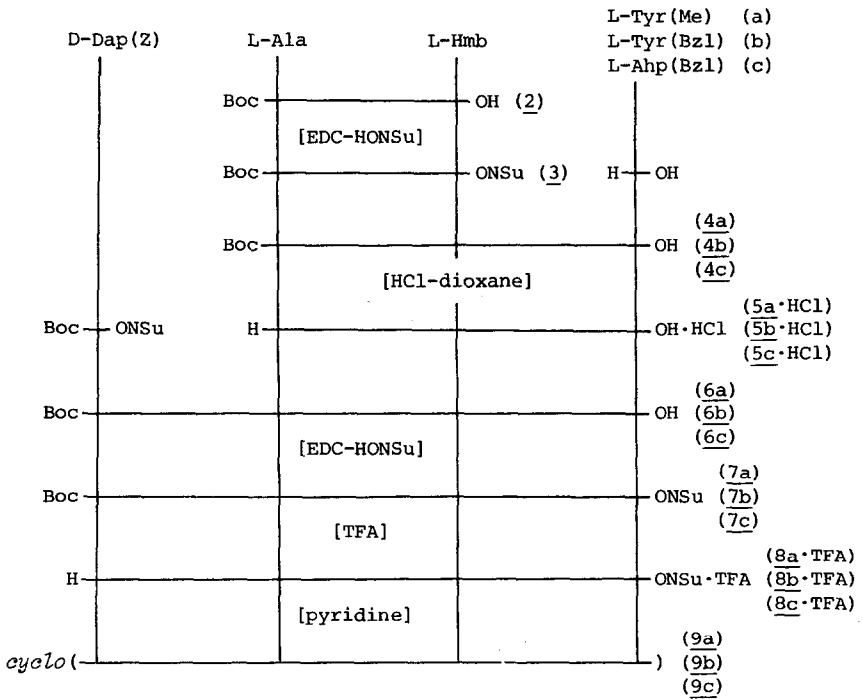


Fig. 2. Synthetic route of cyclotetradepsipeptide precursors (9a, 9b and 9c) of AM-toxin.

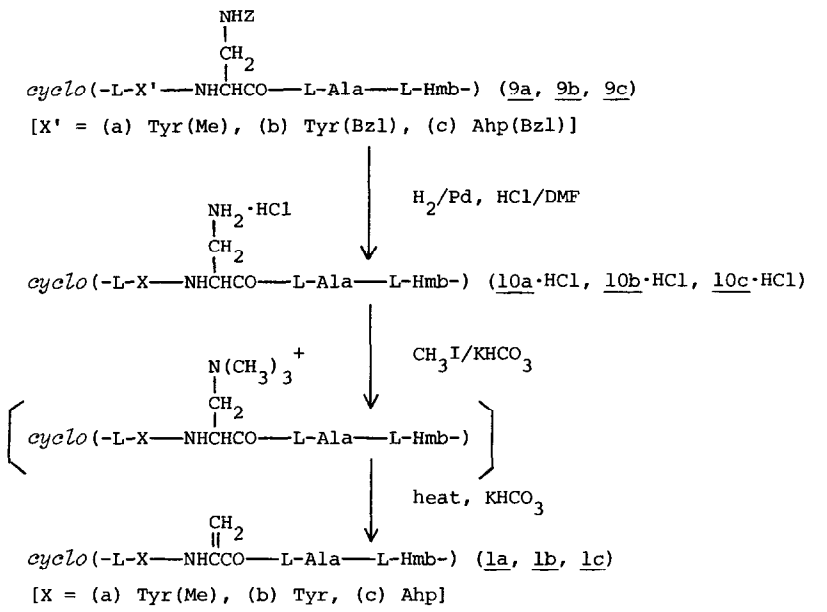


Fig. 3. Syntheses of AM-toxin III and its analogs.

been stirred at room temperature for 2 d, the solvent was evaporated. The residue was easily purified by washing with DMF because compound 9a was sparingly soluble to any solvent.

Cyclic peptide (1a) containing a ΔAla residue was prepared from 9a as shown in Fig. 3. Compound 9a was hydrogenated in DMF in the presence of Pd-black and HCl to afford desired *cyclo* (-D-Dap-L-Ala-L-Hmb-L-Tyr(Me)-)·HCl (10a·HCl). When AcOH was used instead of HCl or HCl was not added in DMF in this reaction, an unexpected product, which might be produced by an acyl migration from the α-amino to the β-amino group in a Dap residue, was obtained. The compound 10a·HCl was treated with CH_3I and $KHCO_3$ in DMF according to the literature.[6] However the formation of expected 1a could not be observed. Thus the conditions for the formation of 1a were modified as follows. To a suspension of 10a·HCl (150 μmol) in EtOAc (20 ml) were added CH_3I (0.5 ml) and $KHCO_3$ (150 mg) for three times at intervals of 2 d. After 6 d, the solvent and excess CH_3I were removed by evaporation. The residue containing trimethylated depsipeptide was heated in EtOAc at 60-70°C for 5 h to give the desired 1a. Crude 1a obtained was further purified by silica gel column chromatography with an eluant of $CHCl_3$-acetone (2:1), followed by recrystallization from acetone-ether-petroleum ether: yield, 24%; mp 224-227°C; R_f^a (TLC with benzene-ether-acetone, 1:1:1) 0.49; MW,[9] 417 (calcd 417.2); ^{1}H-NMR (DMSO-d_6), δ 3.70 (3H, s, -OC$\underline{H}_3$), δ 5.42 (2H, bs, -C=C$\underline{H}_2$), δ 9.13 (1H, s, -N$\underline{H}$-C=C). The same compound 1a was synthesized by a different method.[7]

On the basis of the succesfull synthesis of 1a by this method, we next synthesized another analog, [L-Tyr^1]-AM-toxin (1b) containing L-Tyr which is a lower homolog of L-2-amino-5-(*p*-hydroxyphenyl)pentanoic acid (L-Ahp) in AM-toxin III and holds a free phenol group. Peptide 1b (as 1/2 hydrate) was obtained in 18% yield from 9b; mp 208-210°C; R_f^2 0.36; MW, 403 (calcd 403.2); ^{1}H-NMR (DMSO-d_6), δ 5.41 (2H, bs, -C=C$\underline{H}_2$), δ 9.13 (2H, bs, -O$\underline{H}$ and -N$\underline{H}$-C=C). NMR spectrum of 1b

indicated that the phenolic hydroxyl group was not methylated during this Hofmann degradation.

Thus we applied this method for the synthesis of cyclodepsipeptide (1c) having a sequence of AM-toxin III. H-L-Ahp(Bzl)-OH (*O*-benzyl-L-Ahp) was prepared by the benzylation of Cu(II)-complex of H-L-Ahp-OH[10] with benzyl bromide, followed by the treatment with EDTA·2Na. Boc-D-Dap(Z)-L-Ala-L-Hmb-L-Ahp(Bzl)-OH (6c) was converted to H-D-Dap(Z)-L-Ala-L-Hmb-L-Ahp(Bzl)-ONSu·TFA (8c·TFA), and 8c·TFA was subjected to cyclization reaction as described for the synthesis of 1a. Crude 9c was purified by the reprecipitation from hot DMF: yield, 55%; mp 297-300°C (decomp); MW, 672 (calcd 672.3). Benzyloxycarbonyl and benzyl groups of 9c were removed by catalytic hydrogenation. The hydrochloride (10c·HCl) obtained was subjected to the Hofmann degradation, in the reaction the temperature of deamination was kept at 50°C to avoid possible side reactions. Crude 1c was purified by silica gel column chromatography with $CHCl_3$-acetone (2:1) and by recrystallization from hot acetone to give fine needles: yield, 20%; mp 224-227°C (reported value,[3] 228°C); R_f[a] 0.38; MW, 431 (calcd 431.2); ^{1}H-NMR (DMSO-d_6), δ 5.37 (1H, bs, -C=C$\underline{H}$), δ 5.41 (1H, s, C=C$\underline{H}$), δ 9.07 (1H, s, -N$\underline{H}$-C=C), δ 9.08 (1H, s, -O$\underline{H}$). *Anal*: C, 61.05; H, 6.97; N, 9.49% (calcd for $C_{22}H_{29}O_6N_3$: C, 61.24; H, 6.77; N, 9.74%). Synthetic 1c and natural AM-toxin III were identical in regard to mp, R_f on TLC, mass spectrum and crystal form.

Synthetic 1c and natural AM-toxin III showed the same minimum toxic activity of 0.1 μg/ml for the induction of necrosis on Indo apple leaves. On the other hand, [L-Tyr^1]-AM-toxin (1b) did not show the activity up to the level of 1 μg/ml. These results suggest that the length of methylene chain of the amino acid residue at position 1 in AM-toxin III is an important factor for revealing biological activity.

References and Notes

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