CHEMISTRY LETTERS, pp. 1411-1414, 1977. Published by the Chemical Society of Japan

SYNTHESES OF CYCLOTETRADEPSIPEPTIDES, AM-TOXIN II AND ITS ANALOG

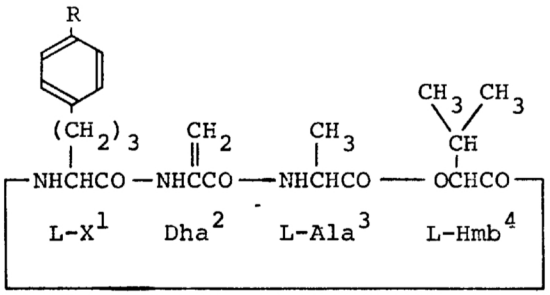
Yasuyuki SHIMOHIGASHI, Sannamu LEE, Tetsuo KATO, and Nobuo IZUMIYA*
Laboratory of Biochemistry, Faculty of Science 33, Kyushu University,
Higashi-ku, Fukuoka 812

Tamio UENO and Hiroshi FUKAMI
Pesticide Research Institute, College of Agriculture, Kyoto University,
Sakyo-ku, Kyoto 606

Synthesis of a cyclotetradepsipeptide corresponding to the sequence of AM-toxin II (2a) was achieved, using methanesulfonyl chloride containing sulfur dioxide in the final step of dehydration. The peptide obtained was identical with natural 2a in regard to mp, TLC, UV, MS, ORD, crystal form and biological activity. An analog [L-Phe^1]-AM-toxin (2b), simultaneously synthesized, showed extremely weak activity.

AM-toxins are host-specific phytotoxins produced by *Alternaria mali* causing necrosis on apple leaves.[1] Previously,[2,3] we reported the syntheses of AM-toxin I (1) and its several analogs and clarified the relationships of the structure-activity of 1. Here we describe the synthetic confirmation of the structure of AM-toxin II (2a) and elucidation of the relationships of the structure-activity of 2a with respect to the influence of the side chain of App^1 in 2a through the synthesis of [Phe^1]-AM-toxin (2b).[4] Previous results showed that cyclic tetradepsipeptides with one ester bond were obtained in high yields by cyclization of linear precursors in which a hydroxy acid residue occupied the third position from the N-terminus[2] and a D-amino acid residue was in the N-terminus.[3] Therefore, we selected the sequence of H-D-Ser-Ala-Hmb-App(or Phe)-ONSu (10) as a linear depsipeptide precursor in this study.

In the syntheses of 1 and its analogs,[2] the conversion of a L-Ser residue into a Dha residue in cyclodepsipeptides was achieved by a tosylation and a



		R	X
AM-toxin I	(1)	OCH_3	Amp
AM-toxin II	(2a)	H	App
AM-toxin III		OH	Ahp

subsequent elimination reaction with Et_2NH.[2] In the present study, however, the tosylation reaction of cyclotetradepsipeptide (11) (Fig. 1) was difficult to progress and, furthermore, tosylated 11 was quite stable in the treatment of bases such as Et_2NH or piperazine. Vigorous conditions such as the use of a large excess of bases or boiling in pyridine[5] made tosylated 11 decompose. The use of phenylmethanesulfonyl chloride[6] instead of TosCl gave the same result. Methyl chlorosulfite[7] hardly reacted with 11, but methanesulfonyl chloride containing sulfur dioxide ($MsCl/SO_2$)[8] gave methanesulfonylated 11, [D-Ser(Ms)^2]-AM-toxin (12). Compound 12 was converted into cyclotetradepsipeptide (2) containing a Dha residue by the treatment with Et_2NH. Since MsCl did not react with 11 without SO_2, SO_2 seems necessary as a catalyst. These results suggest that the difference in behavior to form sulfonic esters between cyclotetradepsipeptides containing D-Ser and L-Ser may be attributed to the difference of their conformations,[3] and that the difficulty in the elimination of tosyl and phenylmethanesulfonyl groups may be due to the steric hindrance of their bulky phenyl groups preventing the access of the bases.

Fig. 1 shows the reaction route for the synthesis.[4] L-App was prepared

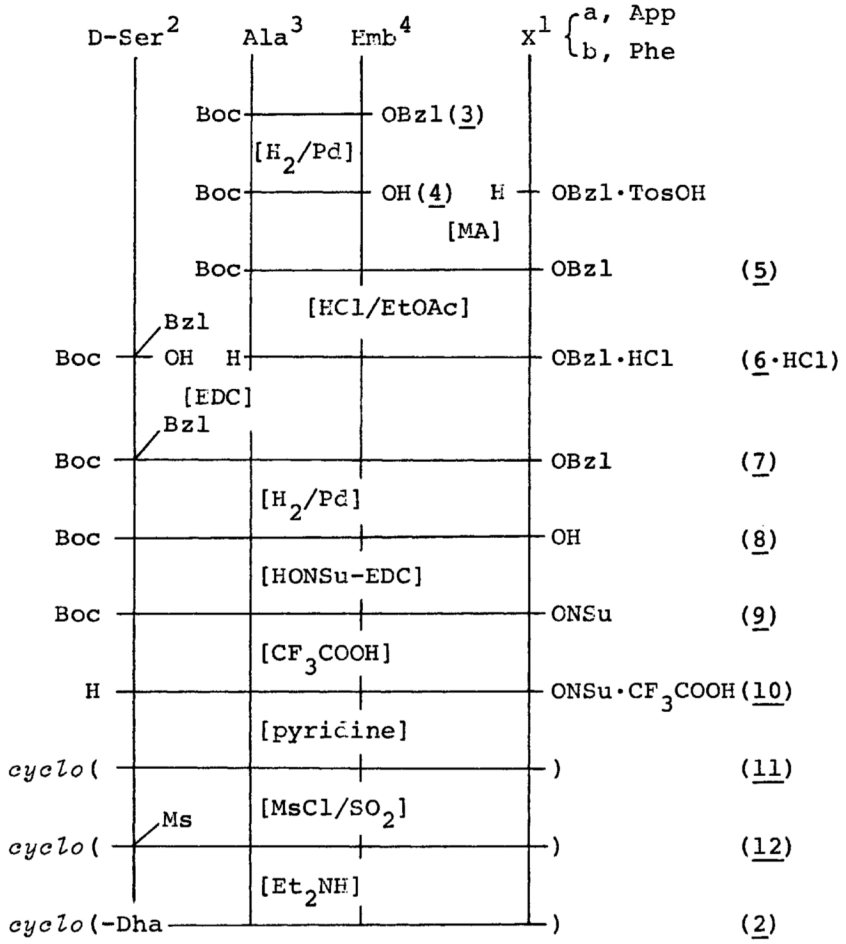


Fig. 1. Synthetic route for AM-toxin II (2a) and [Phe^1]-AM-toxin (2b).

by the resolution of Ac-DL-App-OH with acylase.[9] Oily Boc-Ala-Hmb-OBzl (3) (yield, 100%) was prepared by CDI method, and 3 was converted to oily Boc-Ala-Hmb-OH (4) (94%) by hydrogenation. Boc-Ala-Hmb-App-OBzl (5a) (69%) was prepared by MA method, and 5a was converted to oily H-tripeptide-OBzl·HCl (6a·HCl) (100%). Boc-D-Ser(Bzl)-Ala-Hmb-App-OBzl (7a) (74%) was prepared from Boc-D-Ser(Bzl)-OH and 6a by EDC, and was obtained purely by silica gel column chromatography (solvent, $CHCl_3$:EtOAc = 3:1 by vol). Boc-D-Ser-Ala-Hmb-App-OH (8a) (81%, mp 120-121°C) was prepared from 7a by hydrogenation and subsequent silica gel chromatography ($CHCl_3$:acetone:AcOH = 75:25:2). Compound 8a (0.5 mmol) in DMF was treated with HONSu (0.75 mmol) and EDC·HCl (0.6 mmol), the solution was evaporated, and Boc-D-Ser-Ala-Hmb-App-ONSu (9a) was collected by filtration with the aid of water. Compound 9a was dissolved in CF_3COOH (2 ml), the solution was evaporated, and 10a was collected with the aid of ether. Compound 10a dissolved in DMF was treated with pyridine (165 ml) at room temperature for 2 days, the solution was evaporated, and the solid was collected with the aid of water. The solid was washed with 10% citric acid, and suspended in EtOAc (10 ml) for 1 day under stirring. After filtration and a subsequent recrystallization from DMF-EtOAc-ether pure 11a ([D-Ser^2]-AM-toxin II) was obtained: yield from 8a, 50%; mp 260-261°C; $[\alpha]_{250}^{20}$ -6400° (*c* 0.01, CF_3COOH); R_f^a (TLC with $CHCl_3$:MeOH:AcOH = 95:5:1) 0.40; mol wt, 433 [10] (calcd, 433). Compound 11a (0.15 mmol) in pyridine (1 ml) was treated with MsCl (0.75 mmol) containing SO_2 (4.9% by wt), the solution was evaporated, and [D-Ser(Ms)^2]-AM-toxin II (12a) was collected with the aid of water; mol wt of 12a, 511 (calcd, 511); R_f^a 0.74. Compound 12a (0.13 mmol) in DMF was treated with Et_2NH (0.26 mmol) at room temperature for 4.5 hr, the solution was evaporated, and the solid containing 2a was collected with the aid of water. The solid was suspended in EtOAc (2 ml) for 1 hr under stirring, filtered, and the filtrate was purified by silica gel column chromatography ($CHCl_3$:EtOAc = 1:1). Compound 2a obtained was recrystallized from EtOAc-ether; yield from 11a, 3.7%; mp 212-213°C (reported value, 213-214°C)[3]; R_f^a 0.38, R_f^b (TLC with $CHCl_3$:EtOAc = 1:1) 0.36; mol wt 415 (calcd, 415). In addition to having the same R_f in TLC with several solvent systems, synthetic 2a and natural AM-toxin II showed the same mass spectra, UV pattern, ORD pattern and crystal form in needles.

The synthesis of 2b was carried out according to the same procedure as described above. Boc-D-Ser-Ala-Hmb-Phe-OH (8b) (mp 85°C) was converted to H-D-Ser-Ala-Hmb-Phe-ONSu (10b), and 10b was subjected to the cyclization reaction as described for 11a. Compound 11b was recrystallized from DMF-EtOAc-ether; yield from 8b, 53%; mp 262°C; $[\alpha]_{250}^{20}$ -6900° (*c* 0.01, CF_3COOH); R_f^a 0.35; mol wt, 405 (calcd, 405). Compound 11b was converted to methanesulfonyl derivative (12b) and 12b was treated with Et_2NH. Pure 2b was obtained by silica gel column chromatography ($CHCl_3$:EtOAc = 1:1) after the treatment with EtOAc; yield from 11b, 2.6%; mp 231-232°C; R_f^a 0.31; R_f^b 0.30; mol wt, 387 (calcd, 387).

Both of synthetic 2a and natural AM-toxin II had the same minimum toxic activity of 2×10^{-2} μg/ml for the induction of necrosis on apple leaves. On the contrary, 2b was inactive in a high concentration of 10 μg/ml. This result

clarified that the side chain of L-amino acid[1] in AM-toxins are very important for the activity.

In order to investigate the influence of the side chain length of L-amino acid[1] in AM-toxins for the activity, the syntheses of AM-toxin analogs with different side chains are in progress in this laboratory.

References and Notes

1) T. Ueno, T. Nakashima, Y. Hayashi, and H. Fukami, *Agric. Biol. Chem.*, 39, 1115, 2081 (1975).
2) S. Lee, H. Aoyagi, Y. Shimohigashi, N. Izumiya, T. Ueno, and H. Fukami, *Tetrahedron Lett.*, 843 (1976).
3) Y. Shimohigashi, S. Lee, T. Kato, N. Izumiya, T. Ueno, and H. Fukami, *Agric. Biol. Chem.*, 41, 1533 (1977).
4) Amino acid and hydroxy acid symbols denote the L-configuration except D-Ser. Satisfactory elemental analysis and chromatographic data were obtained for all crystalline compounds. Some of the abbreviations: CDI, carbonyldiimidazole; DMF, *N*,*N*-dimethylformamide; EDC, 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide; HONSu, *N*-hydroxysuccinimide; MA, mixed anhydride; MsCl, methanesulfonyl chloride; TosCl, *p*-toluenesulfonyl chloride; TosOH, *p*-toluenesulfonic acid; Boc-, *t*-butyloxycarbonyl.
5) O. Wintersteiner and M. Moore, *J. Am. Chem. Soc.*, 65, 1507 (1943).
6) H. Ako, C. H. Ryan, and R. J. Foster, *Biochem. Biophys. Res. Commun.*, 46, 1639 (1972).
7) E. M. Chamberlin, E. W. Tristram, T. Utne, and J. M. Chemerda, *J. Org. Chem.*, 25, 295 (1960).
8) G. G. Hazen and D. W. Rosenburg, *J. Org. Chem.*, 29, 1930 (1964).
9) Y. Shimohigashi, S. Lee, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 49, 3280 (1976).
10) Mol wt was determined on Hitachi RMS-4 mass spectrometer.

(Received September 9, 1977)