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SYNTHESES OF CYCLOTETRADEPSIPEPTIDES, AM-TOXIN I AND ITS ANALOGS[1)]

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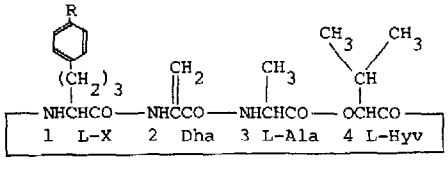
For the synthesis of a cyclotetradepsipeptide corresponding to the sequence of AM-Toxin I (1a), [O-Me-L-Tyr^1]-AM-Toxin (1b) was selected as a model peptide in a preliminary study Synthesis of 1b was attempted through six different routes, and one (the route D) afforded the 1b in a good yield Synthesis of a peptide corresponding to 1a was achieved according to the route D, and the peptide obtained was identical with natural Toxin I in regard to TLC, UV, MS and biological activity. The 1b and other analogs showed extremely weak activity.

AM-Toxins are host specific phytotoxic metabolites produced by *Alternaria mali*. The structure of AM-Toxin I was elucidated in two laboratories,[2,3)] and the II and III in Fukami's laboratory [2)] We attempted to establish the synthetic confirmation of the structure of AM-Toxin I and to clarify the relationship of the structure-activity on natural AM-Toxins through the syntheses of some analogs Since O-methyl-L-tyrosine (Tyr(Me))[4)] has been more easily available than L-Amv, which is a component of 1a, we selected [Tyr(Me)^1]-AM-Toxin (1b) as a model peptide for these purposes In the previous paper,[5)] we synthesized protodestruxin, which is a cyclohexadepsipeptide belongs to the class involves AM-Toxins, by several routes, and we found that



	R	X
AM-Toxin I (1a)	OCH_3	Amv
AM-Toxin II	H	Apv
AM-Toxin III	OH	Ahv

the cyclization by intramolecular ester-bond formation did not afford the desired product Therefore, a route *via* H-Hyv-Tyr(Me)-Ser-Ala-OH was excluded in the present study

H-Tyr(Me)-Dha-Ala-Hyv-OSu (2)
H-Ala-Hyv-Tyr(Me)-Ser(Tos)-N_3 (3)
H-Ala-Hyv-Tyr(Me)-Dha-N_3 (4)
H-Ser-Ala-Hyv-Tyr(Me)-OSu (5b)
H-Ala-Hyv-Tyr(Me)-Ser-OSu (6)
H-Tyr(Me)-Ser-Ala-Hyv-OSu (7)

The synthesis of 1b was designed by six different routes using the intermediates (2-4, 5b, 6-7) [6] The route A was a design of cyclization of 2 Boc-Tyr(Me)-Ser-OMe (8) (mp 72°, $[\alpha]_D^{20}$+23 2° $CHCl_3$) was obtained from Boc-Tyr(Me)-OH and H-Ser-OMe HCl by the mixed anhydride method in yield of 72% [7] Boc-Tyr(Me)-Ser(Tos)-OMe derived from 8 was converted to Boc-Tyr(Me)-Dha-OH (9) by the action of NaOH [8] Boc-Tyr(Me)-Dha-Ala-Hyv-OH (10) was prepared from 9 and H-Ala-Hyv—OH HCl by the mixed anhydride method, and 10 was converted to 2 CF_3COOH salt by an usual procedure [9] However, the cyclization[9] of 2 did not produce 1b In the route B, Boc-Ala-Hyv-Tyr(Me)-Ser(Tos)-NHNHBoc (11) was changed to H-tetrapeptide-$NHNH_2$ $2CF_3COOH$ (12), and 12 was converted to 3 In the route C, Boc-Ala-Hyv-Tyr(Me)-Dha-NHNHBoc (13) derived from 11 by the action of Et_2NH[8] was converted to 4 However, either 3 nor 4 produced [Tyr(Me)^1,Ser(Tos)^2]-AM-Toxin (14b) or 1b In the routes D-F, the linear intermediates (5b, 6-7) different each other in the sequence of components were considered In the synthesis of protodestruxin,[5] we observed that the insertion of a hydroxy acid apart from N- and C-terminus is one factor to give the cyclopeptide in good yield Therefore, we tried to cyclize 5b as the route D at first

In the route D, oily Boc-Ala-Hyv-OBzl (15) (yield, 100%) was prepared by CDI method, and 15 was converted to oily Boc-Ala-Hyv-OH (16) (77%) by hydrogenation Boc-Ala-Hyv-Tyr(Me)-OBzl (17) (62%) was prepared by the mixed anhydride method, 17 was converted to oily H-tripeptide-OBzl HCl (18) (99%), and Boc-Ser-Ala-Hyv-Tyr(Me)-OBzl (19) was prepared from Boc-Ser-OH and 18 by WSCI The 19 (42%) was obtained by silica gel column chromatography (solvent, $CHCl_3$ AcOEt=3 1 by vol) and Boc-Ser-Ala-Hyv-Tyr(Me)-OH (20b) (90%, mp 96°) was prepared from 19 by hydrogenation The 20b (0 5 mmol) in DMF was treated with HOSu (0 75 mmol) and WSCI HCl (0 75 mmol), the solution was evaporated, and Boc-Ser-Ala-Hyv-Tyr(Me)-OSu (21) was collected by filtration with the aid of water The 21 was dissolved in CF_3COOH (3 ml), evaporated, and 5b CF_3COOH was collected with the aid of ether The 5b CF_3COOH dissolved in DMF was treated with pyridine (170 ml) at room temperature for 1 day, the solution was evaporated, and the solid was collected with the aid of water The solid dissolved in AcOEt was washed with 10% citric acid, and the solution was dried over Na_2SO_4 and evaporated The powder (22b) obtained showed major two spots in TLC The 22b

was treated with AcOEt and the less soluble component (23b) collected was recrystallized from dioxane-ether, yield of pure 23b ([Tyr(Me)^1,Ser^2]-AM-Toxin) from 20b, 32%, mp 256°, R_f^a (TLC with $CHCl_3$ MeOH=5 1 by vol) 0 60, mol wt, 435[10] (calcd, 435) The more soluble component (24b) was purified by Sephadex LH-20 column chromatography (solvent, dioxane), yield of pure 24b (*cyclo*(-Tyr(Me)-Ser-Ala-Hyv-)$_2$), 11%, mp 238°, R_f^a 0 64, mol wt, 870[10] (calcd, 870) The ratio of 23b 24b in 22b was about 3 2 Pure 23b (0 08 mmol) in pyridine (1 ml) was treated with TosCl (0 16 mmol),[8] evaporated, and [Tyr(Me)^1,Ser(Tos)^2]-AM-Toxin (14b) was collected with the aid of water and washed with a mixture of ether and petroleum ether (1 1) The 14b (0 07 mmol) in DMF was treated with Et_2NH (0 14 mmol) at room temperature for 6 hr, evaporated, the solid of crude Dha-derivative (25b) was collected with the aid of water and purified by silica gel column chromatography ($CHCl_3$ AcOEt=7 3) The 1b obtained was recrystallized from $CHCl_3$-peptroleum ether, yield of 1b from 23b, 16%, mp 193°, R_f^b (TLC with $CHCl_3$ AcOEt=7 3)0 16, mol wt, 417[10] (calcd, 417)

We wished a good luck that other intermediate (6 or 7) produces only monomer (23b) In the route E, the same procedure as described in the route D was applied for 6 derived from Boc-Ala-Hyv-Tyr(Me)-Ser-OH (mp 105°) TLC of the crude product, which was obtained by cyclization of 6, showed that the formation of monomer (23b) was very low and the ratio of 23b 24b was about 5 95 (this 24b contained polycyclic peptides) In the route F, Boc-Tyr(Me)-Ser-Ala-Hyv-OH (mp 98°) was converted to 7, and the result of cyclization of 7 was very similar to that shown in the route E, the ratio of 23b 24b was also about 5 95 It is difficult to explain why only 5b was converted efficiently into monomer (23b), but we suppose that the conformation of 5b in the solvent is fit for the formation of the cyclic monomer (we are now carrying out the studies on ORD and CD of 1a, 1b, 5b and the related compounds)

For the synthesis of natural Toxins, L-Amv ($[\alpha]_D^{20}$+33 5° 5N HCl DMF=1 1) and L-Apv ($[\alpha]_D^{20}$ +31 8°) were prepared by the resolution of Ac-DL-Amv and Ac-DL-Apv, respectively, with acylase I[11] The synthesis of 1a was carried out according to the same procedure as described in the route D. Boc-Ser-Ala-Hyv-Amv-OH (20a)(mp 85°) was converted to H-Ser-Ala-Hyv-Amv-OSu (5a), and 5a was subjected to the cyclization reaction as described for the synthesis of 23b The powder (22a) correspond to 22b was collected, and the ratio of [Ser^2]-AM-Toxin I (23a) *cyclo*(-Amv-Ser-Ala-Hyv-)$_2$ (24a) in 22a was about 1 1 After 22a was fractionated with AcOEt, the less soluble component (23a) was recrystallized from DMF-AcOEt-ether, yield of pure 23a from 20a, 18%, mp 195°, R_f^a 0 57, mol wt, 463[10] (calcd, 463) The more soluble component (24a) was recrystal-

lized from AcOEt yield of 24a from 20a, 9%, mp 225°, R_f^a 0 63, mol wt, 926[10] (calcd, 926) Purification of 23a and 24a was very difficult, even pure 23a was contaminated very slightly with 24a, and pure 24a with 23a Pure 23a was converted to crude Dha-derivative (25a), and 25a was treated with silica gel column chromatography ($CHCl_3$ AcOEt=1 1) A portion correspond to 1a was purified by preparative TLC ($CHCl_3$ AcOEt=7 3) The major spot afforded pure 1a, yield from 23a, 1 7%, R_f^a 0 65, R_f^b 0 16 The minor spot (R_f^b 0 20) afforded pure *cyclo*(-Amv-Dha-Ala -Hyv-)$_2$ (26a) in very small amount In addition to having the same R_f in TLC with several solvent systems, synthetic 1a and natural AM-Toxin I showed the same mass spectra and UV pattern

Both of synthetic 1a and natural Toxin I had the same minimum toxic activity of 2×10^{-3} µg/ml for induction of necrosis on apple leaves On the contrary, the dimer (26a), [Tyr(Me)^1]-Toxin (1b) and [Ser^2]-Toxin I (23a) showed extremely weak activity (the minimum activity was observed at 20-40 µg/ml) These results suggest that the ring size of natural Toxin I, and the presence of Dha and of side chain of Amv are important for the activity

Since the use of S-alkyl-cysteine residue has been proved effective to afford a peptide containing dehydroamino acid,[12] the synthesis of AM-Toxin II through H-Cys(Me)-Ala-Hyv-Apv-OSu are in progress in our laboratories We express our thanks to Drs Erhard Gross, Tetsuo Kato and Kosaku Noda for their advice

References and Notes

1) Presented at the 19th Symposium on the Chemistry of Natural Products, Hiroshima (Japan), Oct 26 (1975), S Lee, H Aoyagi, Y Shimohigashi, N Izumiya, T Ueno and H Fukami, Proceedings of the Symposium, p.342 (1975)
2) T. Ueno, T Nakashima, Y Hayashi and H Fukami, Agr Biol. Chem , 39, 1115, 2081 (1975).
3) T. Okuno, Y. Ishita, K Sawai and T. Matsumoto, Chemistry Lett., 635 (1974)
4) N Izumiya and A. Nagamatsu, Bull Chem Soc Japan, 25, 265 (1952)
5) S. Lee, N. Izumiya, A Suzuki and S. Tamura, Tetrahedron Lett., 883 (1975)
6) Amino acid and hydroxy acid symbols denote the L-configuration Some of other abreviations WSCI, 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide, CDI, carbonyldiimidazole
7) Satisfactory elemental analyses and chromatographic data were obtained for most crystalline compounds. For many compounds in this study, the description of data (yield, mp, $[\alpha]_D$ etc) was omitted.
8) I Photaki, J. Amer Chem. Soc , 85, 1123 (1963)
9) S Matsuura, H.Takiguchi, M.Waki and N Izumiya, Mem Fac Sci Kyushu Univ.C, 9, 277 (1975)
10) Mol wt was determined on Hitachi RMS-4 mass spectrometer
11) Y. Shimohigashi, S. Lee and N. Izumiya, the 33rd Meeting of Chem Soc Japan, Fukuoka (Japan), Oct 20 (1975), Abst. p 584 (1975).
12) D. H Rich *et al.*, Tetrahedron Lett., 4037 (1974), 211 (1975)