Communications to the Editor

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SYNTHESIS OF THE HOST-SPECIFIC TOXINS AF-TOXIN II_c AND AK-TOXIN CONGENERS AND THEIR STEREOSTRUCTURE-TOXICITY RELATIONSHIP

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AF-toxin II_c and AK-toxin-related compounds were synthesized from Vitamin C as a chiral material. In both toxins, C_8(R) and C_9(S)-configurations at the two chiral centers of the trienoic acid moiety are indispensable for the toxic effect.

KEYWORDS- host-specific toxin; *Alternaria kikuchiana* Tanaka; *Alternaria alternata*; Vitamin C; toxicity; acylation; epoxidation; Wadsworth-Emmons reaction; absolute configuration

AF- and AK-toxins are host-specific to Japanese pear, *Pyrus serotina* Rehder *var. culta*. They are produced by *Alternaria alternata* and *A. kikuchiana* Tanaka, respectively. Their structures have been described by the Nagoya and Kyoto groups.[1, 2] The structures of AK-toxin I (1) and II (2) were firmly established by X-ray crystallographic analysis, while the stereostructures of AF-toxins remain to be elucidated. We are aiming at the synthesis of these toxins and their congeners in order to investigate the relationship between their stereostructures and toxicities. Here we report the synthesis in optically active form of a compound (3) whose entire structure corresponds to the AF-toxin II_c described by the Nagoya group.[2] We also report the synthesis of AK-toxin congeners and their toxicities to leaves of the host-plant.

The ester (4), obtained from Vitamin C as a chiral material in the same way as its ethyl ester,[3] was reduced with diisobutylaluminium hydride (DIBAH) to give the alcohol in 95% yield. This was smoothly converted into the aldehyde (6) in 92% yield by manganese dioxide oxidation. Wadsworth-Emmons reaction of (6) with methyl dimethylphosphonoacetate and sodium hydride gave the diene-ester (7) in good yield. The *trans* geometry of the newly formed double bond is confirmed by its ^{1}H-NMR spectrum (C_α-H, J=15Hz). The same reaction sequence on the diene-ester (7) (reduction with DIBAH, oxidation with manganese dioxide, and Wadsworth-Emmons reaction) gave the triene-ester (8) in 68% overall yield. Oxidation of each olefinic ester [(4), (7), and (8)] with m-chloroperbenzoic acid gave two stereoisomeric epoxides in almost 1:1 ratio in each case [(9) and (10) from (4); (11) and (12) from (7); (13) and (14) from (8)]. The carbon bearing methyl and epoxide oxygen in each oxide had the S-configuration

in (9), (11), and (13), and the R-configuration in (10), (12), and (14). This was indicated by the fact the first three epoxides had AB-type quartets assigned to the methylene protons of the epoxide group similar to the same protons in AK-toxins, while (10), (12), and (14) had a singlet due to the same type of methylene protons. An experiment with the ethyl-ester analogue of (9) using the nuclear Overhauser effect further confirmed this postulated stereochemistry.[3)]

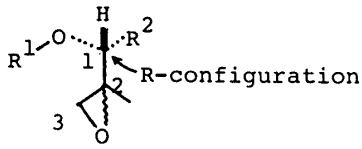
Treatment of all the esters (9)-(14) with tetrabutylammonium fluoride gave the hydroxy-esters (15)-(20). Acylation of these hydroxy-esters with N-acetyl-(L)-phenylalanine and dicyclohexylcarbodiimide in the presence of 4-pyrrolidino-pyridine in methylene chloride[4)] afforded two stereoisomeric esters in each case. Thus (15) gave the N-acetyl-(L)-phenylalanine ester (21) and the (D)-ester (22) in 1:1 ratio. This indicates that, in all cases, N-acetyl-(L)-alanine was completely racemized during the course of acylation. All the compounds synthesized are listed in the Table.[5)] The stereochemistry of the part of the amino acid in the esters was confirmed by an L- and D-amino acid determination experiment[6)] with the hydrolysates obtained from the esters with 6-N hydrochloric acid.

On the other hand, acylation of the hydroxy-esters (19) and (20) with the (2S,3S)-2-siloxy-3-methyl-pentanoic acid (33) gave the esters (38) and (39), respectively, in good yield without racemization of the siloxy-acid (33). Preparation of the siloxy-acid (33) was accomplished as follow. Treatment of isoleucine with sodium nitrite in acetic acid followed by esterification of the acetate (34) with benzyl alcohol and p-toluenesulphonic acid yielded a mixture of acetoxy-ester (35) and hydroxy-ester (36) in 31 and 34% yield, respectively. The former gave the latter when hydrolyzed with lithium carbonate in aqueous methanol. Treatment of the hydroxy-ester (36) with t-butyldiphenylsilyl chloride and imidazole in dimethylformamide gave the siloxy-ester (37). Hydrogenation of (37) with palladium on carbon in ethanol furnished the required acid (33) ($[\alpha]_D^{26}$ -21.7° (c=1.04 in ethanol)).

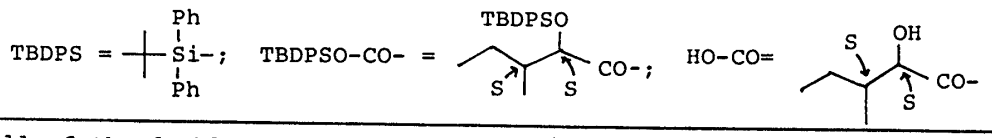
Desilylation of the esters (38) and (39) gave the hydroxy-esters (40) [^{1}H-NMR ($CDCl_3$) δ: 7.29 (1H, dd, J=16.0, 11.5Hz), 6.52 (1H, dd, J=14.9, 11.0Hz), 6.43 (1H, ddd, J=14.5, 11.0, 1.0Hz), 6.36 (1H, dd, J=14.5, 11.5Hz), 5.94 (1H, d, J=16.0Hz), 5.80 (1H, dd, J=14.5, 7.5Hz), 5.35 (1H, dd, J=7.5, 1.0Hz), 3.75 (3H, s), 2.80 (1H, d, J=4.8Hz), 2.64 (1H, d, J=4.8Hz), 1.35 (3H, s), 1.30 (3H, m), 1.00 (3H, d, J=6.5Hz), and 0.90 (3H, t, J=7.0Hz)], and (41). On the other hand, mild alkaline hydrolysis of the esters (38) and (39) (1.2 molar equivalent of sodium hydroxide in aqueous ethanol at room temperature) gave the acids (42) and (43), desilylation of which afforded the hydroxy-acid (44) and (45) in good yield. The latter (45) was not toxic but the former was toxic to leaves of the host-plant. This suggests that the structure of AF-toxin II_c is (44).

As shown in the Table, the C_1-(R) and C_2-(S)-configuration of the hydroxy-acid moiety, including the oxide ring corresponding to the C_8- and C_9-carbon of the toxins, are indispensable. Also in the AF-toxin series the hydroxyl group in α-hydroxy-β-methyl-pentanoic acid moiety is required for toxicity.

TABLE

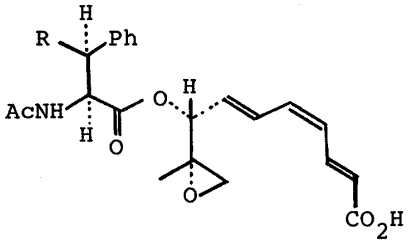


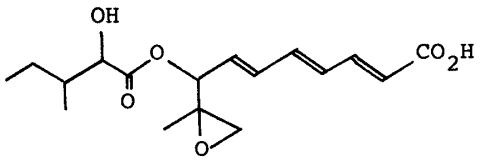
R^1	R^2	Configuration of C_2 and number of compounds	$[\alpha]_D°$ (c) (in EtOH)	Toxicity[7] to plant
TBDPS	$-CH=CH-CO_2Me$	S (9)	-13.1 (1.55)	
TBDPS	$-CH=CH-CO_2Me$	R (10)	-22.9 (1.86)	
TBDPS	$-(CH=CH)_2-CO_2Me$	S (11)	-74.6 (1.06)	
TBDPS	$-(CH=CH)_2-CO_2Me$	R (12)	-81.4 (1.05)	
TBDPS	$-(CH=CH)_3-CO_2Me$	S (13)	-60.0 (1.00)	
TBDPS	$-(CH=CH)_3-CO_2Me$	R (14)	-59.9 (0.94)	
H	$-CH=CH-CO_2Me$	S (15)	+68.2 (1.92)	
H	$-CH=CH-CO_2Me$	R (16)	+61.9 (1.34)	
H	$-(CH=CH)_2-CO_2Me$	S (17)	+76.1 (1.00)	
H	$-(CH=CH)_2-CO_2Me$	R (18)	+64.4 (1.26)	
H	$-(CH=CH)_3-CO_2Me$	S (19)	+68.0 (1.00)	
H	$-(CH=CH)_3-CO_2Me$	R (20)	+68.3 (1.20)	
Ac-(L)-Phe-	$-CH=CH-CO_2Me$	S (21)	+6.5 (0.28)	+
Ac-(D)-Phe-	$-CH=CH-CO_2Me$	S (22)	+27.2 (0.22)	+
Ac-(L)-Phe-	$-CH=CH-CO_2Me$	R (23)	+14.4 (0.54)	-
Ac-(D)-Phe-	$-CH=CH-CO_2Me$	R (24)	+35.5 (0.53)	-
Ac-(L)-Phe-	$-(CH=CH)_2-CO_2Me$	S (25)	+15.0 (1.00)	+
Ac-(D)-Phe-	$-(CH=CH)_2-CO_2Me$	S (26)	+13.4 (0.67)	+
Ac-(L)-Phe-	$-(CH=CH)_2-CO_2Me$	R (27)	+13.2 (1.13)	-
Ac-(D)-Phe-	$-(CH=CH)_2-CO_2Me$	R (28)	+21.9 (1.14)	-
Ac-(L)-Phe-	$-(CH=CH)_3-CO_2Me$	S (29)	+22.8 (0.90)	+
Ac-(D)-Phe-	$-(CH=CH)_3-CO_2Me$	S (30)	+16.3 (1.00)	+
Ac-(L)-Phe-	$-(CH=CH)_3-CO_2Me$	R (31)	+22.8 (1.20)	-
Ac-(D)-Phe-	$-(CH=CH)_3-CO_2Me$	R (32)	+21.0 (1.36)	-
TBDPSO-CO-	$-(CH=CH)_3-CO_2Me$	S (38)	-67.7 (0.98)	-
TBDPSO-CO-	$-(CH=CH)_3-CO_2Me$	R (39)	-39.0 (1.00)	-
HO-CO-	$-(CH=CH)_3-CO_2Me$	S (40)	+3.0 (0.67)	+
HO-CO-	$-(CH=CH)_3-CO_2Me$	R (41)	+7.8 (0.26)	-
TBDPSO-CO-	$-(CH=CH)_3-CO_2H$	S (42)	-40.4 (2.70)	-
TBDPSO-CO-	$-(CH=CH)_3-CO_2H$	R (43)	-50.5 (1.10)	-
HO-CO-	$-(CH=CH)_3-CO_2H$	S (44)	+6.5 (1.07)	+
HO-CO-	$-(CH=CH)_3-CO_2H$	R (45)	+8.4 (0.83)	-



All of the double bonds in this Table have the trans configuration.

Chart

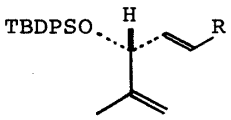


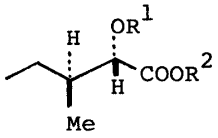


(3) without stereochemistry

(1) R=Me

(2) R=H





(4) R=CO_2Me

(5) R=CH_2OH

(6) R=CHO

(7) R= $-CH=CH-CO_2Me$

(8) R= $-(CH=CH)_2CO_2Me$

(33) R^1=TBDPS; R^2=H

(34) R^1=Ac; R^2=H

(35) R^1=Ac; R^2=CH_2Ph

(36) R^1=H; R^2=CH_2Ph

(37) R^1=TBDPS; R^2=CH_2Ph

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Optical purity (>99% enantiomeric excess) of the starting material, methyl (2R,3S)-2-hydroxy-3,4-isopropylidenedioxybutylate[3] is determined from ^1H-NMR spectrum of its Mosher's ester.
4) A. Hassner and V. Alexanian, Tetrahedron Lett., 1987, 4475.
5) We are deeply indebted to Professors H. Fukami, T. Ueno, and Dr. T. Nakashima (Kyoto University) for test of toxicities of our synthetic compounds.
6) W.H.P. Lewis and H. Harris, Nature, 215, 351 (1967).
7) Toxicities of the compounds in the Table were determined with 10^{-3} mol solution of each compound. The toxicity tended to increase with elongation of the conjugated carbon chain.

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