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STRUCTURES OF PHYTOTOXINS, AV-TOXINS C, D AND E, PRODUCED BY ZONATE LEAF SPOT FUNGUS OF MULBERRY

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Summary: The structures of phytotoxins, AV-toxins C, D and E, produced by zonate leaf spot fungus of mulberry, were characterized.

Zonate leaf spot fungus newly found on mulberry, Morus alba L., in Japan since 1978 was identified as Acrospermum viticola Ikata[1]. The disease caused by this fungus has spread more and more in mulberry fields in a part of Western Japan. Many new host plants, being 28 species belonging to 24 genera of 16 families, for this fungus were recorded through the field survey around mulberry plantations[2]. It has also been revealed that this fungous disease distributes in South East Asia, Africa, South America and U.S.A., mainly in the subtropical and tropical zones.

We have now isolated the phytotoxins, tentatively named AV-toxins C and D, from the cultivation of A.viticola and, AV-toxin E from the part of the disease leaves.

The present paper deals with the structures of these phytotoxins, AV-toxins C, D and E.

AV-toxin C (1), dark red needles (MeOH), mp 185-188°C, showed absorptions due to the NH_2 and aromatic ring at 3300 and 1590 cm^{-1}, respectively, in the IR spectrum. The EI-MS exhibited a molecular ion at m/z 212. The ^{1}H-NMR spectrum suggested the presence of one 1,2-disubstituted aromatic ring (1H, dd, J=8,2 Hz, at δ 7.50, 1H, td, J=8,2 Hz, at δ 7.48, 1H, td, J=8,2 Hz, at δ 7.41 and 1H, dd, J=8,2 Hz, at δ 7.71) and two olefinic protons (1H, s, at δ 6.37 and 1H, s, at δ 6.40). The ^{13}C-NMR spectrum (Table I) showed total 12 sp^2 carbons which imply a disubstitited aromatic ring, an α,ß, α',ß'-unsaturated ketone system and a -C=N function group. The above evidence indicated that 1 possesses a phenoxazine framework, being reminiscent of questiomycin A[3], before-obtained as an antibiotic from Streptomyces species by Anzai et al. The ^{13}C-NMR, ^{1}H-NMR, MS and IR spectra of 1 were identical with those[4,5] of questiomycin A.

AV-toxin D (2), brown yellow plates (MeOH), mp 148-150°C, showed absorptions at 3008 and 1648 cm^{-1} due to the aromatic C-H streching and carbonyl group in the IR spectrum. The elementary analysis of 2 afforded a molecular formula $C_{14}H_{11}NO_4$, with which the M^+ (m/z 257) on the FD-MS was coincident. The ^{1}H-NMR spectrum suggested the presence of a 1,2-disubstituted benzene ring (1H, dd, J=8,2 Hz, at δ 7.33, 1H, td, J=8,2 Hz, at δ 7.53, 1H, td, J=8,2 Hz, at δ 7.38 and 1H, dd, J=8,2 Hz, at δ 7.90), an olefinic proton (s, at δ 6.23) and two methoxyl groups (both s, at δ 4.12 and 4.14). From the above evidence, 2 was supposed to be an analogous compound to 1. The ^{13}C-NMR spectrum of 2 showed total 14 carbon signals and those could be unambiguously assigned by the aid of ^{13}C-^{1}H COSY and long range COSY spectra as shown in Table I. While signals due to

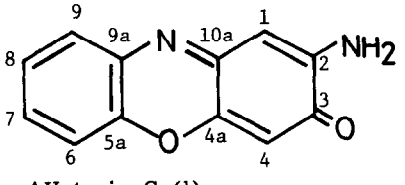
the A-ring were good in accordance with each other in comparing of the ^{13}C-NMR spectrum of 2 with that of 1, a significant change in signals was observed on the C-ring, namely it was an increase of one oxygen bearing carbon signal and appearance of two methoxyl signals which resonated at lower field than δ 60 ppm by suffered a steric compression. Consequently, the structure of 2 should be represented as shown in the formula having the methoxyl groups substituted at C-1 and -2.

AV-toxin E (3), red needles (MeOH), mp 160-162°C. The elementary analysis and FD-MS provided a molecular formula, $C_{15}H_{13}NO_5$. The ^{13}C-NMR spectrum of 3 showed signals due to newly bearing one more methoxyl group and the respective shifts by -3.7, +29.6 and -11.0 ppm at C-3, -4 and -4a compared with those of 2, suggesting that a methoxyl group attached to the C-4. Therefore, the structure of 3 could be represented as shown in the formula.

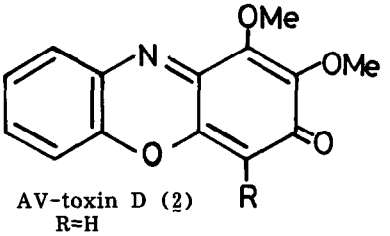
The above phytotoxins, AV-toxins C, D and E were active in a concentration of ca. 10 μg/ml by the soaking method.

Table I. ^{13}C-NMR Spectral Data of AV-toxins C (1), D (2) and E (3).

	1	2	3	
C-1	103.3 (d)	145.1 (s)	144.0 (s)	(Solvents:
2	148.8 (s)	145.9 (s)	144.9 (s)	DMSO-d_6 for 1,
3	180.1 (s)	181.8 (s)	178.1 (s)	$CDCl_3$ for 2 and 3)
4	98.3 (d)	104.7 (d)	134.3 (s)	
4a	147.2 (s)	147.3 (s)	136.3 (s)	
5a	141.8 (s)	143.5 (s)	143.5 (s)	
6	115.8 (d)	116.0 (d)	116.0 (d)	
7	127.9 (d)	132.2 (d)	132.0 (d)	
8	128.7 (d)	125.3 (d)	125.2 (d)	
9	125.1 (d)	130.3 (d)	130.4 (d)	
9a	133.6 (s)	132.7 (s)	132.7 (s)	
10a	148.1 (s)	147.8 (s)	145.7 (s)	
1-OMe		62.3 (q)	62.4 (q)	
2-OMe		61.2 (q)	61.3 (q)	
3-OMe			60.9 (q)	



AV-toxin C (1)



AV-toxin D (2)
R=H

AV-toxin E (3)
R=OMe

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