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STRUCTURE OF AF-TOXIN II, ONE OF THE HOST-SPECIFIC TOXINS PRODUCED BY ALTERNARIA ALTERNATA STRAWBERRY PATHOTYPE

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Abstract: AF-toxin II was a mixture of three stereo-isomers , whose structures were determined as 2a, 2b and 2c by means of chemical and spectroscopic methods.

Host specificity of plant pathogens is a most intriguing problem in the field of plant pathology.[1] A distinct pathotype of Alternaria alternata (Fries) Keissler, the cause of Alternaria black spot disease of strawberry, is pathogenic to only one cultivar of strawberry, Morioka-16, and to limited cultivars of Japanese pear including cultivar Nijisseiki.[2] This pathogen produces three host-specific toxins, AF-toxin I, II and III, in both the culture filtrates and the spore-germinated fluids with the same host-selectivity as the producing fungus.[3, 4] These toxins expressed different biological activities on the hosts as shown in Table 1. Now we report the isolation and structure determination of AF-toxin II.

Table 1. Host specific toxicity of AF-toxin I, II and III

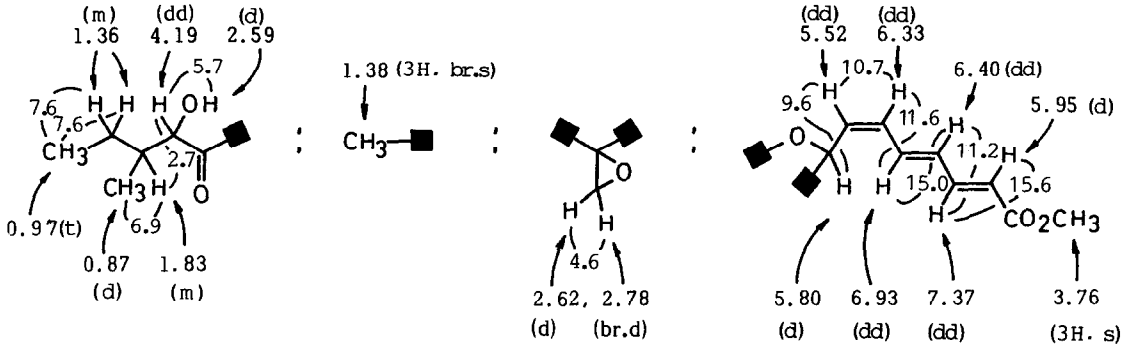
Toxin	Strawberry (cv. Morioka-16)	Japanese pear (cv. Nijisseiki)
AF-toxin I (1)	+	+
AF-toxin II (2)	-	+
AF-toxin III (3)	+	±

+ : Sensitive to toxin, - : Insensitive to toxin

Crude AF-toxin II (2) previously reported[3] was further purified by HPLC (ODS-5μ; MeOH-H_2O-AcOH=35:65:1) to give an colorless oil; single peak on HPLC (ODS-5μ or silica gel-3μ), FAB-MS: m/z 347$(M+Na)^+$; UV: λ_{max} 287 nm(MeOH). The minimum concentration of pure AF-

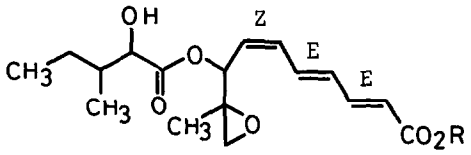
toxin II (2) to give obvious toxic effects to Japanese pear, cv. Nijisseiki, was estimated to be 10 ng/ml. Leaves of strawberry, cv. Morioka-16, suffered no toxin-induced damage at 48 hr after treatment with the toxin at 10 μg/ml. 200 MHz ^{1}H-NMR spectrum of 2 in CD_3OD pointed out AF-toxin II to be a mixture of isomers. Although these isomers could not be separated by TLC or HPLC, we could obtain three kinds of pure monomethyl esters 4a, 4b and 4c by treatment of 2 with diazomethane in MeOH at 25 °C followed by TLC and HPLC separation. The ratio of 4a:4b:4c was 7:3:1.

The molecular formula of 4a [UV: λ_{max}(MeOH) 292 nm; IR: ν_{max}($CHCl_3$) 3690, 1725, 1709 cm^{-1}] was determined to be $C_{18}H_{26}O_6$ from its high resolution MS spectrum [338.1755 (M^+)]. EI-MS spectra [338 (M^+)] of 4b and 4c indicated these were isomers of 4a. 500 MHz ^{1}H-NMR spectrum of 4a [δ($CDCl_3$) 0.87(3H, d, J=6.9 Hz), 0.97(3H, t, J=7.6 Hz), 1.36(1H, m), 1.38(3H, br.s), 1.83(1H, m), 2.59(1H, d, J=5.7 Hz), 2.62(1H, d, J=4.6 Hz), 2.78(1H, d, J=4.6 Hz), 3.76(3H,s), 4.19(1H, dd, J=2.7, 5.7 Hz), 5.52(1H, dd, J=9.6, 10.7 Hz), 5.80(1H, d, J=9.6Hz), 5.95(1H, d, J=15.6 Hz), 6.33(1H, dd, J=10.7, 11.6 Hz), 6.40(1H, dd, J=11.2, 15.0 Hz), 6.93(1H, dd, J=11.6, 15.0 Hz), 7.37(1H, dd, J=11.2, 15.6 Hz)] suggested the presence of 2-hydroxy-3-methyl-pentanoyl moiety (A), an isolated C-CH_3 (B), a terminal epoxide (C), and a (2E,4E,6Z)-trienoic acid methyl ester (D). A long range coupling between C-CH_3 (B, 1.38 ppm) and one of the methylene protons of epoxide (C, 2.78 ppm) was observed. Components A and D were certified by the decoupling experiments and IR spectrum (two carbonyl group). Furthermore, the 1H double doublet at 4.19 ppm [-CH(OH)-] in the ^{1}H-NMR spectrum of 4a was changed to a doublet by addition of D_2O, and acetylation of 4a gave mono-acetate 5a[5] [$C_{20}H_{28}O_7$, MS: m/z 380 (M^+); NMR: δ($CDCl_3$) 5.01(1H, d)]. Consequently the structure of major methyl ester of AF-toxin II was concluded to be 4a.

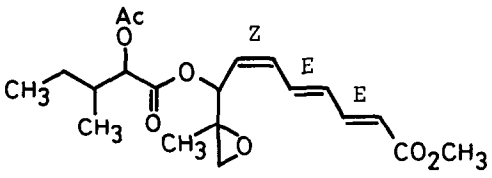


Spectroscopic data of other methyl esters 4b[6] and 4c[7] were very similar to those of 4a except their olefinic proton signals of the trienoic part in the ^{1}H-NMR spectra. The structures were determined to be (2E,4Z,6E)- and (2E,4E,6E)-trienoic esters for 4b and 4c, respectively, from the coupling constants of olefinic protons and decoupling experiments. Methyl esters 4a and 4b were also toxic but 4c was less toxic (≤ 10%) to the leaves of Nijisseiki pear.

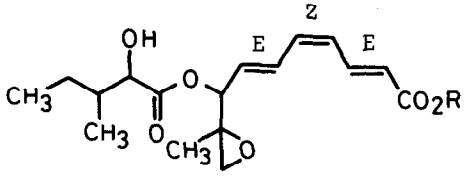
Since 4a, 4b and 4c were isomers obtained by methylation of AF-toxin II (2) with diazomethane, and since ^{1}H-NMR spectrum of 2 indicated that it is a mixture, AF-toxin II was concluded to be a mixture of stereoisomers 2a, 2b and 2c which we named AF-toxin IIa, IIb and IIc, respectively.



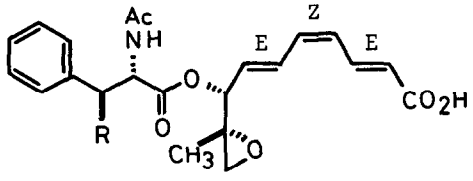
2a AF-toxin IIa : R = H
4a R = CH_3



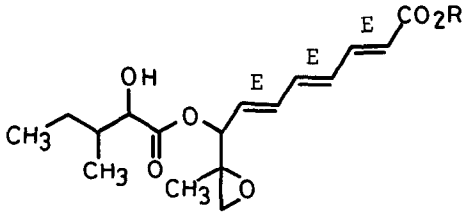
5a



2b AF-toxin IIb : R = H
4b R = CH_3



6 AK-toxin I : R = CH_3
7 AK-toxin II : R = H



2c AF-toxin IIc : R= H
4c R = CH_3

We assume that AF-toxin IIa (2a) is the original toxin produced by A. alternata strawberry pathotype and AF-toxin IIb and IIc (2a and 2c) are its isomerization products because the ratio of 4a:4b:4c obtained from freshly harvested culture filtrates was 9:1:1. The structure of AF-toxin IIb (2b) is similar to those of AK-toxin I (6) and II (7)[8,9] produced by A. alternata Japanese pear pathotype, causing black spot of Japanese pear. Interestingly, the (2E,4Z,6E)-trienoic acid structure found in AK-toxin series was found in the minor component (2b) in our AF-toxin series. Further studies on the stereochemistry of AF-toxin II and the structures of AF-toxin I and III are now in progress.

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(5) 5a; MS: m/z 380 (M^+); ^{1}H-NMR($CDCl_3$): δ (ppm) 0.92(3H, t), 0.95(3H, d), 1.34(3H, br.s), 1.2-1.8(2H, m), 2.01(1H, m), 2.13(3H, s), 2.58(1H, d), 2.74(1H, d), 3.74(3H, s), 5.01 (1H, d), 5.51(1H, dd), 5.70(1H, d), 5.94(1H, d), 6.30(1H, dd), 6.37(1H, dd), 6.89(1H, dd), 7.35(1H, dd).

(6) 4b; MS: m/z 338 (M^+); ^{1}H-NMR($CDCl_3$): δ (ppm) 0.86(3H, d, J=6.9 Hz), 0.98(3H, t, J=7.5 Hz), 1.36(2H, m), 1.37(3H, br.s), 1.84(1H, m), 2.60(1H, d, J=5.6 Hz), 2.63(1H, d, J= 4.8), 3.77(1H, s), 4.24(1H, dd, J=2.6, 5.6 Hz), 5.40(1H, d, J=7.5 Hz), 5.80(1H, dd, J= 11.4, 15.3 Hz), 5.94(1H, dd, J=11.9, 15.3 Hz), 6.16(1H, dd, J=11.4, 11.5Hz), 6.28(1H, dd, J=11.5, 11.9 Hz), 6.91(1H, dd, J=11.4, 15.3Hz), 7.70(1H, dd, J=11.9, 15.3Hz).

(7) 4c; MS: m/z 338 (M^+); ^{1}H-NMR($CDCl_3$): δ (ppm) 0.87(3H, d, J=6.9 Hz), 0.97(3H, t, J=7.5 Hz), 1.36(3H, br.s), 1.36(2H, m), 1.83(1H, m), 2.60(1H, d, J=5.8 Hz), 2.62(1H, d, J= 4.8 Hz), 2.78(1H, d, J=4.8 Hz), 3.75(3H, s), 4.21(1H, dd, J=2.8, 5.8 Hz), 5.34(1H, d, J=7.4 Hz), 5.80(1H, dd, J=7.4, 14.9 Hz), 5.94(1H, d, J=15.2 Hz), 6.39(1H, dd, J=11.2, 14.4 Hz), 6.43(1H, dd, J=7.4, 14.9 Hz), 6.53(1H, dd, J=10.7, 14.4 Hz), 7.29(1H, dd, J= 11.2, 15.2 Hz).

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