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STRUCTURE OF THE HOST-SPECIFIC PATHOTOXINS PRODUCED BY PHYLLOSTICTA MAYDIS

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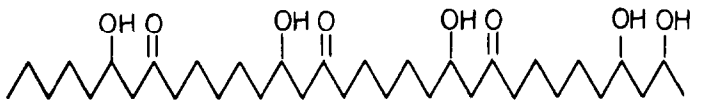
Abstract Linear polyketol structures of two host-specific pathotoxins from Phyllosticta maydis (PM-toxin B and PM-toxin C) were elucidated by MS fragmentation patterns of the toxins and their derivatives, and by NMR analysis

The fungus, Phyllosticta maydis, a pathogen of corn, produces a series of 10-12 chemically analogous pathotoxins (PM-toxins) which specifically damage only corn carrying Texas-male-sterile cytoplasm. In biological activity, these toxins closely resemble T-toxin (from Helminthosporium maydis, race T) in specificity, toxicity, and qualitative effects on susceptible corn, but have no effects on corn with normal cytoplasm T-toxin consists of a series of linear polyketols of C_{35} to C_{45} chain length (1, 2, 3) A C_{39} component of T-toxin is illustrated below

We report the isolation and structural elucidation of two major components (PM-toxin B and PM-toxin C) of the PM-toxin complex They were isolated and purified by procedures developed for T-toxin (1) Like T-toxin, these components are linear polyketols, but have shorter chain lengths and fewer oxygen-containing functional groups.



PM-toxin B is a colorless powder, MP 97-98 C; λ_{max}^{MeOH}= 275 nm (ε=138), $[\alpha]_D^{27}$= -10 (MeOH, c=0 14); IR ν_{max}^{KBr}= 3400 (OH), 2930, 2850 (CH_2, CH_3), 1709 (C=O), 1465, 1402 ("active" CH_2) Complete reduction to hydrocarbon by the method of Cope et al (4) and analysis by GC-MS indicated a C_{33} linear alkane product PM-toxin C also is a colorless powder, MP 108-109 C;

λ_{max}^{MeOH}= 276 nm (ε=130), $[\alpha]_D^{27}$= -6 (MeOH, c=0.13), IR same as for PM-toxin B. Complete reduction to a hydrocarbon followed by GC-MS indicated the presence of a C_{35} linear alkane. Fast-atom-bombardment MS (FAB) provided m/z 587 $(M+H)^{+\cdot}$, while FAB- and FD-MS of the sodium complex of PM-toxin B yielded m/z 609$(M+Na)^{+\cdot}$ corresponding to MW = 586 and, based on analogy with T-toxin (1, 2), a purported empirical formula of $C_{33}H_{62}O_8$ for the free toxin. For PM-toxin C, m/z was 631 $(M+H)^{+\cdot}$ for the free toxin, while FAB- and FD-MS of the sodium complex of PM-toxin C yielded m/z 653$(M+Na)^{+\cdot}$, corresponding to MW = 630 and a purported empirical formula of $C_{35}H_{66}O_9$

The IR spectra of PM-toxin B and PM-toxin C closely resemble the IR spectrum of T-toxin or its isolated components. In addition, the ^1H NMR spectra of acetylated PM-toxins were also very

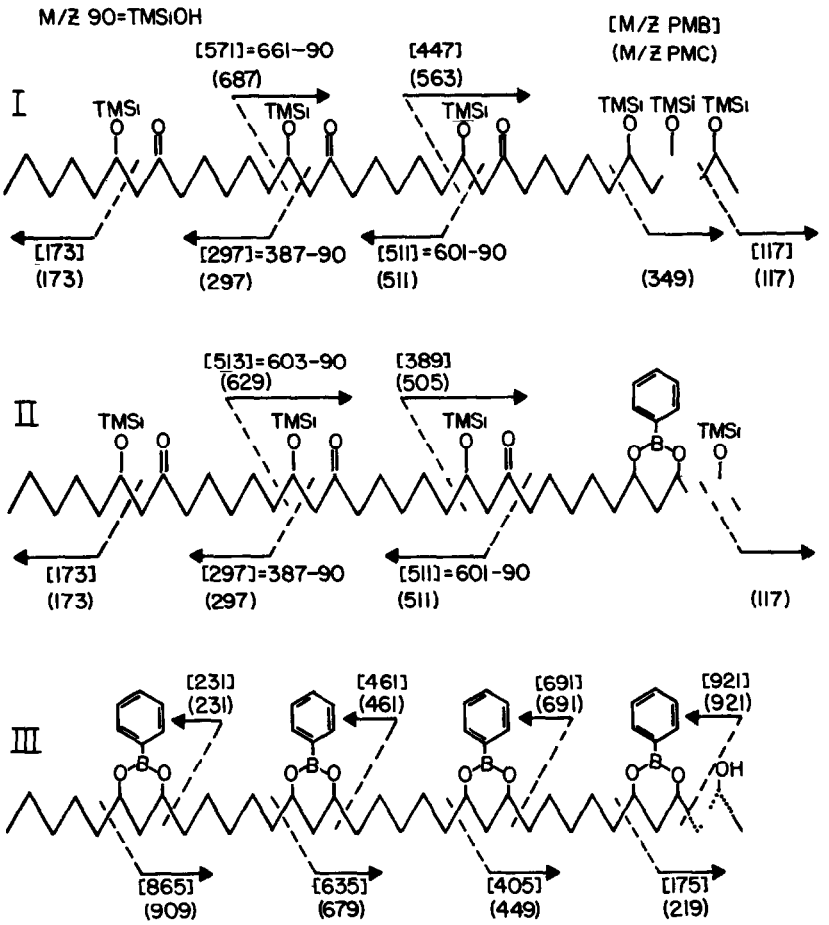


Figure 1 Fragmentation patterns of PM-toxins B and C derivatives. I, Trimethyl silyl ethers; II, TMSi ethers of phenyl borate esters; III, phenyl borate esters of $NaBH_4$ reduced toxins PM-toxin C contains the dotted portions of the structures, while PM-toxin B does not Masses derived from PM-toxin B are indicated in brackets and masses for PM-toxin C are in parentheses.

similar to those of acetylated T-toxin components, although the spectra indicated that the PM-toxins were different. For example, acetylated PM-toxins B and C showed chemical shifts at δ4 93 and 5 22 ppm corresponding to signals at 4.90 and 5.22 ppm for two methine protons of acetylated secondary alcohols of T-toxin, but lacked a third signal for similar methine protons at δ5.48 ppm which occurs in T-toxin (1). Accordingly, PM-toxins B and C were derivatized and then analyzed by EI-MS. The trimethylsilyl ether of PM-toxin B produced a parent ion at m/z 946 corresponding to $C_{33}H_{57}O_8 \cdot 5(C_3H_9Si)$, and a series of diagnostic ions corresponding to the successive loss of five C_3H_9SiOH. PM-toxin C yielded a molecular ion m/z 1062 ($C_{35}H_{60}O_9 \cdot 6(C_3H_9Si)$) with a series of six ions characteristic for successive loss of six C_3H_9SiOH. Phenyl borate esters were prepared and then reacted with trimethylchlorosilane. Parent ions of m/z 888 ($C_{48}H_{59}O_8Si_3B$) and m/z 1004 ($C_{55}H_{101}O_9Si_4B$) were observed for PM-toxin B and PM-toxin C, respectively, indicating each compound contained one β-dioxy function Carbonyl groups of underivatized PM-toxin B and PM-toxin C were reduced with either $NaBH_4$ or $NaBD_4$ and then phenyl borate esters were prepared Derivatives of the $NaBH_4$ reduced toxin yielded m/z 936 ($C_{57}H_{80}O_8B_4$) for PM-toxin B and m/z 980 ($C_{59}H_{84}O_9B_4$) for PM-toxin C. When the reduction was performed with $NaBD_4$, the derivatives of PM-toxins B and C exhibited molecular ions of m/z 939 and 983, respectively The addition of three hydrogen or deuterium atoms, coupled with the presence of 4 phenyl

Formula	Observed	Calculated
$C_{29}H_{59}O_5Si_3$	571.3708	571.3668
$C_{28}H_{55}O_4Si_2$	511 3590	511 3635
$C_{21}H_{47}O_4Si_3$	447.2820	447.2780
$C_{17}H_{33}O_2Si$	297.2266	297.2258
$C_9H_{21}OSi$	173.1354	173.1360
$C_5H_{13}OSi$	117.0729	117.0724

Table 1. High resolution MS data for fragments from TMSi ethers of PM-toxin B.

	NMR Assignments (^1H)	(^{13}C)
CH_3	0.87 t	14.1 q
CH_2	1 28*	22 6 t
CH_2	1.28*	31 7 t
CH_2	1.28*	25.2 t
CH_2	1.54*	34.2 t
CH-OAc	5.24	70.7 d
CH_2	2.54, 2 74**	47.2 t
C=O	--	207.7 s
CH_2	2.40	43 3 t
CH_2	1.54*	23.6 t
CH_2	1.28*	29.0 t
X2		
CH_2	1 28*	25.2 t
CH_2	1 54*	34 2 t
CH-OAc	5.24	70.4 d
CH_2	2.59, 2 74**	47.2 t
C=O	--	207.7 s
CH_2	2 40	43.3 t
CH_2	1.54*	23.6 t
CH_2	1.28*	29 0 t
CH_2	1.28*	25.2 t
CH_2	1.54*	34.2 t
CH-OAc	4.93	71.2 d
CH_2^+	1 73, 1.88**	38.9 t
$CH\text{-}OAc^+$	4.93	68.1 d^{++}
CH_2	1.73, 1.88**	40.0 t
CH-OAc	4 93	68.7 d^{++}
CH_3	1 22 d	20.2 q

Table 2. NMR assignments for PM-toxin B pentaacetate and PM-toxin C hexaacetate in $CDCl_3$.
* approximate ppm
+ only for PM-toxin C
** ABX type coupling
++ these assignments may be interchanged

borate groups after reduction, indicate that three β-oxyoxo functions as well as single β-dioxy function, are present in the underivatized compounds In addition, PM-toxin C must contain an additional hydroxyl group to acccount for the elements indicated by the empirical formulae of the derivatives The fragmentation patterns of the derivatives (Fig 1) provided evidence for the arrangement of the oxygen-containing functional groups along the hydrocarbon chain The assignments of empirical formulae for the TMSi derivatives of PM-toxins were justified by high resolution MS, as shown in Table 1 for PM-toxin B.

The hydroxyl groups of PM-toxin B and PM-toxin C were acetylated, and the 400 MHz ^{1}H NMR and 22.5 MHz ^{13}C NMR spectra were obtained in $CDCl_3$. The chemical shifts of the proton and carbon signals (ppm relative to TMS) were verified by selective proton decoupling (Table 2) In the ^{1}H NMR spectrum, many of the methylene proton signals overlapped and were thus impossible to distinguish. For these protons, an approximate shift (1.28* or 1 54*) is given In the ^{13}C spectrum, assignments of CH-OAc carbons were tentatively made by comparison with T-toxin (1). Signals for both PM-toxin B and PM-toxin C were identical except for the $-CH_2-CH(OAc)-$ group at the end of the molecule which is present in PM-toxin C but absent in PM-toxin B For this reason, only one set of shifts is given The observed shifts (Table 2) are in good agreement with the proposed structures

PM-toxins are unusual natural products with unique specificity for susceptible corn cultivars. Elucidation of the structural features of the other components of the native toxin may be useful in defining a mode of action The length of the carbon chains may indicate that interaction with cell membranes is necessary for their lethal effects As noted above, T-toxins have chain lengths that are slightly longer PM-toxins may have some advantages over T-toxin in mode of action studies because they are less complex structurally

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