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Communication

Studies on the Host-Specific Pathotoxins Produced in Minor Amounts by *Helminthosporium maydis*, race T

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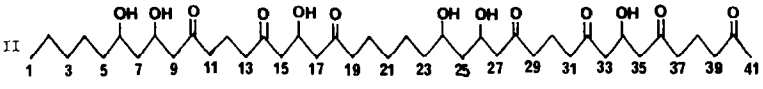
The fungal pathogen of corn, *Helminthosporium maydis*, race T, produces a host-specific toxin that causes typical damage in corn with Taxas male sterile cytoplasm (susceptible) but not corn with normal fertile cytoplasm (resistant). The pathotoxin appears to be a complex of C_{37} to C_{45} moieties, which are obtained by charcoal (Norit A) adsorption of toxin from culture filtrates and 70% aqueous acetone homogenates of mycelium, followed by elution and precipitation with solvents (*1–3*). Individual components separated by tlc have the same specific biological toxicity and specificity (*4*). The major active components (Bands 1, 2) are straight C_{41} polyketopolyalcohols (*1–3*), shown as structures I and II.

Band 1-toxin



Band 2-toxin

This report presents evidence on the structures of two minor toxins with C_{39} chain lengths. Knowledge of their structure may be helpful in understanding the relationship of structure to specific toxicity exhibited on a number of processes in susceptible corn (*4*).

Field-desorption mass spectra (Fig. 1) of the toxin complex shows several clusters of ion peaks. The two main peaks at *m/e* 791 and 793 correspond to empirical formulas of $C_{41}H_{68}O_{13}Na$ (confirmed by hr-fd-ms (*3*)) and $C_{41}H_{70}O_{13}Na$ and account for 19 and 26% of the total intensity of the prominent peaks of the

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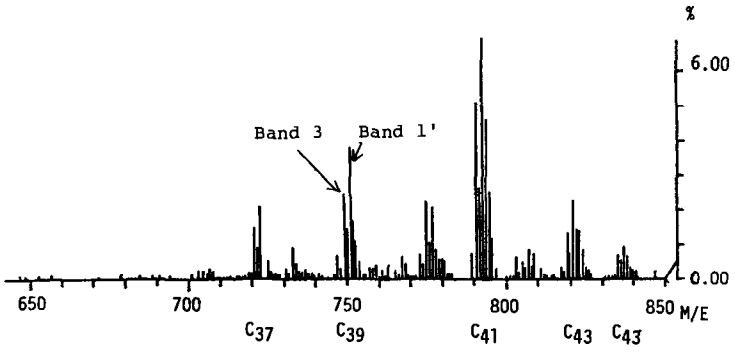


FIG. 1. Field desorption–mass spectrometry spectrum of toxin complex.

spectrum. These peaks are attributed to the sodium complexes of Bands 1 and 2 toxins (*1*) and their dehydrated forms $(M - 18)^+$ were observed at *m/e* 773 and 775, respectively. Eight minor peaks with their dehydrated ions were observed in the range of *m/e* 700 to 850, and their presumptive formulas based on consideration of the previously presented structures of C_{41} toxins, are as follows; *m/e* 721 (6% of the total sum of the intensities of prominent peaks of the spectrum), $C_{37}H_{62}O_{12}Na$; 723 (8%), $C_{37}H_{64}O_{12}Na$; 751 (9%), $C_{39}H_{66}O_{12}Na$; 753 (14%), $C_{39}H_{68}O_{12}Na$; 819 (5%), $C_{43}H_{72}O_{13}Na$; 821 (8%), $C_{43}H_{74}O_{13}Na$; 835 (3%), $C_{43}H_{72}O_{14}Na$; 837 (4%), $C_{43}H_{74}O_{14}Na$. The results are consistent with the distribution of hydrocarbons prepared by elimination of oxygen from other samples of the pathotoxin complex (*1*).

The marked similarity in the ir and nmr spectra of the toxin complex and purified C_{41} toxins isolated by tlc also suggest that the minor components share a number of common functions with the C_{41} toxins. The ^{1}H-nmr spectrum of the acetylated toxin complex, for example, has three quintets at δ5.48, 5.22, and 4.90 ppm (J = 6 Hz), which appear identical to those of individually acetylated Bands 1 and 2 toxins (*1*) and assigned to hydroxymethine (–CHOH–) groups.

Chemical modification of the toxin complex likewise yielded results in accord with those obtained with the C_{41} components. The complex was oxidized with Jones CrO_3 reagent in acetone in order to convert hydroxyl groups to ketones and thus form β-diketo units. Refluxing with KOH to cause cleavage of the latter resulted in the formation of glutaric, pimelic, and β-hydroxyoctanoic acids as the main acidic products, as shown by gc–ms comparisons with authentic specimens. The isolation of only these two dibasic acids from CrO_3 oxidation of the toxin complex, as occurred with isolated C_{41} components (*2, 3*), reinforces the supposition of ζ-dioxo or ζ-oxyoxo and δ-dioxo groups in all moieties of the complex. There were no detectable amounts of similar acids in hydrolysates from unmodified toxin complex.

Conversion of the same toxin complex used for Fig. 1 to hydrocarbons by removal of all oxygen groups are carried out by the method used for C_{41} toxins (*2, 3*). The toxin complex (45 mg) was hydrogenated to a polyalcohol mixture by catalytic hydrogenation. The resultant mixed polyalcohols were converted to iodinated hydrocarbons by treatment with hydriodic acid and red phosphorous,

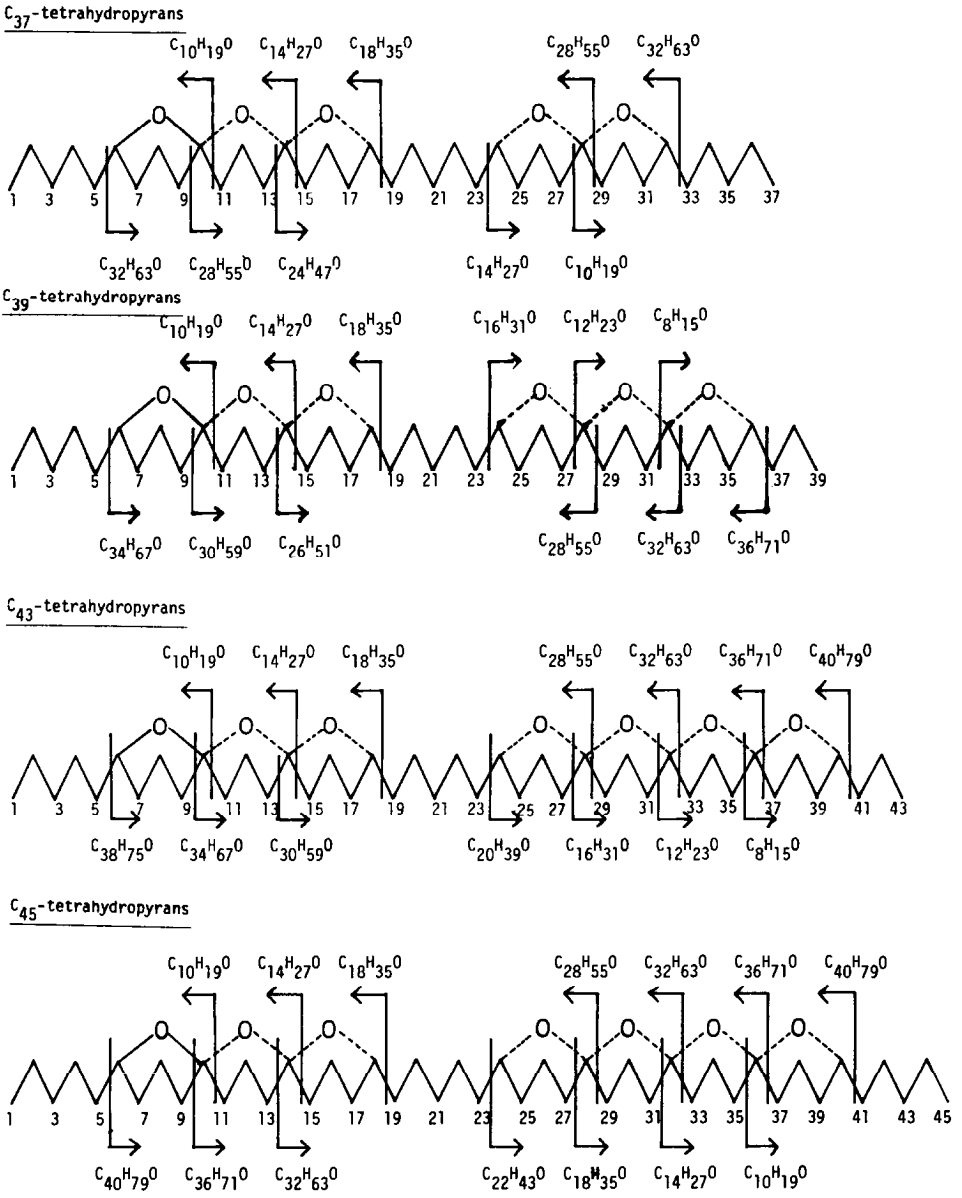


FIG. 2. Schematic of fragmentation of tetrahydropyran compounds.

and the iodides were dehalogenated with $LiAlH_4$ in tetrahydrofuran. These procedures were repeated, and the products eluted from silica gel columns with *n*-hexane yielded two fractions. The first eluate gave a series of hydrocarbons (12.8 mg) which were characterized by gc–ms as *n*-$C_{37}H_{76}$, *n*-$C_{39}H_{80}$, *n*-$C_{41}H_{84}$, *n*-$C_{43}H_{88}$ and $C_{45}H_{92}$. The C_{45} species may or may not belong to the normal hydrocarbons, unlike the others, as seen from its fragmentation pattern.

The mixture obtained from the second eluate consisted of oxygenous products,

TABLE 1

LOW AND HIGH-RESOLUTION MASS SPECTROSCOPY OF TMS[a] ETHERS FROM TOXIN BANDS

Band	Mass and relative abundance						
	M-18	M-18-90	M-18-90 × 2	M-18-90 × 3	M-18-90 × 4	M-18-90 × 5	M-18-90 × 6
3	1068(13)	978(30)	888(52)	798(95)	708(100)[b]	618(95)	528[c]
1′	1142(14)	1052(30)	962(50)	872(76)	782(69)	692(96)	602(100)[b]
5	1114(9)	1024(15)	934(28)	844(48)	754(50)	664(100)[b]	574(46)
					M^+	$(M - 18)^+$	$(M - 18 - 90)^+$
3		$C_{54}H_{106}O_{12}Si_5$	Calcd		1086.6531	1068.6425	978.5924
			Obs		1086.6530	1068.6425	978.5871
1′		$C_{57}H_{116}O_{12}Si_6$	Calcd		1160.7083	1142.6977	1052.6476
					ND	1142.6977	1052.6474

[a] $(CH_3)_3SiOH$ (MW = 90).

[b] For masses above 600.

[c] Observed, but expected isotopic masses not obvious.

which was fortunate because they served in identifying the precise positions of oxygen in the original toxins. Five peaks were observed in the gc analysis of the above mixture. Each gc peak was analyzed by chemical ionization gc–ms (isobutane), and showed the $(M + 1)^+$ ion at m/e 535($C_{37}H_{74}O$ + H), 563 ($C_{39}H_{78}O$ + H), 591 ($C_{41}H_{82}O$ + H), 619 ($C_{43}H_{86}O$ + H), and 647 ($C_{45}H_{90}O$ + H) respectively. The peak compounds were considered to be mixtures containing a single tetrahydropyran group on their respective carbon chains, as was the case for C_{41} toxins. By high-resolution gc–ms, each of the above monooxygen products gave three to seven pairs of prominent peaks, which were interpreted as the result of cleavage of the molecule at either side of the tetrahydropyran ring. The details of analysis of ms data are summarized in Fig. 2. We presume, in analogy with the above results, that ζ-dioxo or ζ-oxyoxo function are located between the C_{18} to C_{24} carbons, in common with the C_{41} toxins (*2*).

In order to clarify these suppositions, separation of the minor toxin components was attempted. The toxin complex was fractionated using silica gel preparative tlc plate with chloroform–methanol (9:1) as the solvent (*3*). The developed tlc was visualized by spraying with water or exposing to iodine vapor. Components at R_f 0.42 (Band 1′-toxin) and R_f 0.46 (Band 3-toxin) were eluted with hot methanol. This procedure was repeated two to three times. Band 1′-toxin is a colorless powder of mp 121–122°C. Its molecular formula has been determined to be $C_{39}H_{68}O_{12}$ by high-resolution ms of its hexa-TMS derivative (*1*). Band 3-toxin, colorless powder, melts at 134–135°C. Its molecular formula has been determined to be $C_{39}H_{66}O_{12}$ by high-resolution ms of its penta-TMS derivative (*1*). The low- and high-resolution ms of their TMS derivatives (Table 1) were in accord with the results of fd–ms of Bands 1′- and 3-toxins. Their ir spectra are not distinguishable from the spectrum of toxin complex. The two isolated toxins were oxidized and analyzed by the same procedures used for the toxin complex and also yielded glutaric, pimelic, and β-hydroxyoctanoic acids. The ^{1}H-nmr spectrum of Band 1′-toxin acetate, further purified by silica gel column and hplc (Lichrospher SI 100, 8 mm × 25 cm; CH_2Cl_2–isopropanol, 98:2), suggested that the Band 1′-toxin acetate

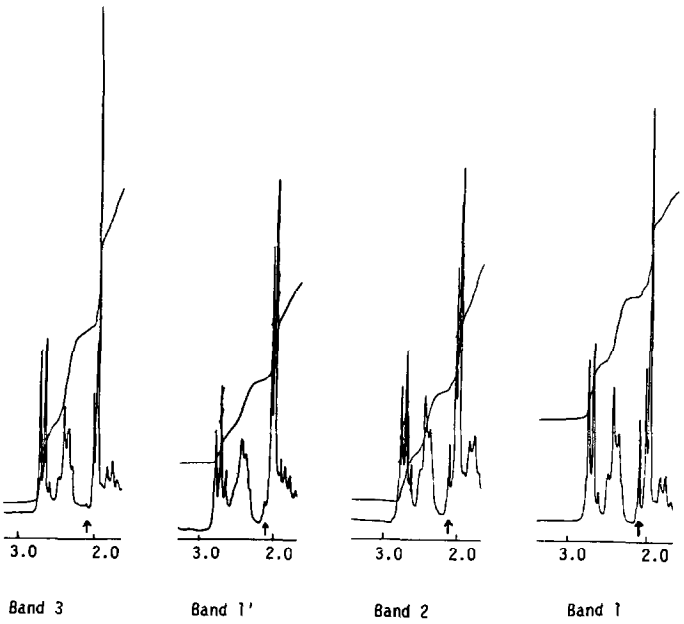


FIG. 3. ^{1}H-nmr (δ2.0 to 3.0 ppm) of Bands 1-, 2-, 1'-, and 3-toxin acetates (arrow; δ2.12 ppm) (100 MHz).

contains many partial structures common with Band 2-toxin acetate (*2, 3*). Three pairs of protons were observed as quintets at δ5.48 (2H), 5.22 (2H), and 4.90 (2H) ppm as was the case for Band 2-toxin acetate (*1*). The results of decoupling studies also showed that the immediate chemical environments of these protons are essentially identical with the three pairs of quintets of Band 2-toxin acetate.

A significant difference between the spectra of Bands 1'- and 2-toxin acetates was the absence of a signal at 2.12 ppm in the former (as shown in Fig. 3), ascribed to the terminal methyl ketone in Band 2-toxin (structure **II**). In addition, Band 1'-toxin acetate integrated for two methyl groups at δ0.91 ppm rather than one methyl as with Band 2-toxin acetate. Scheme of structural assignment of Band 1'-toxin is summarized in Fig. 4.

Figure 5 summarizes the observed and calculated assignments resulting from ^{13}C-nmr spectra of Band 1'-toxin acetate. The chemical shifts presented previously for Band 2-toxin acetate are in good agreement, except for signals corresponding to a terminal butyryl moiety ($CH_3CH_2CH_2CO$–, 13.6, 17.1, and 45.3 ppm) which was not present in the C_{41} toxins (*1, 3*). These new assignments were based on a comparison with the chemical shifts of 2-pentanone (*5*).

On the other hand, Band 3-toxin is similar to Band 1-toxin in that it appears to contain five hydroxyls rather than six as in the case for Bands 1'- and 2-toxins. The important parallelism between Bands 3- and 1-toxins lies in signals at δ5.48 (3H), 5.22 (1H), and 4.90 (1H) ppm in the ^{1}H-nmr spectra for the acetates of both compounds. Like Band 1'-toxin, Band 3-toxin did not show a signal at δ2.12 ppm (Fig. 3) and the signal at δ0.91 ppm integrated for two methyls. Scheme of structural assignment of Band 3-toxin is summarized in Fig. 6.

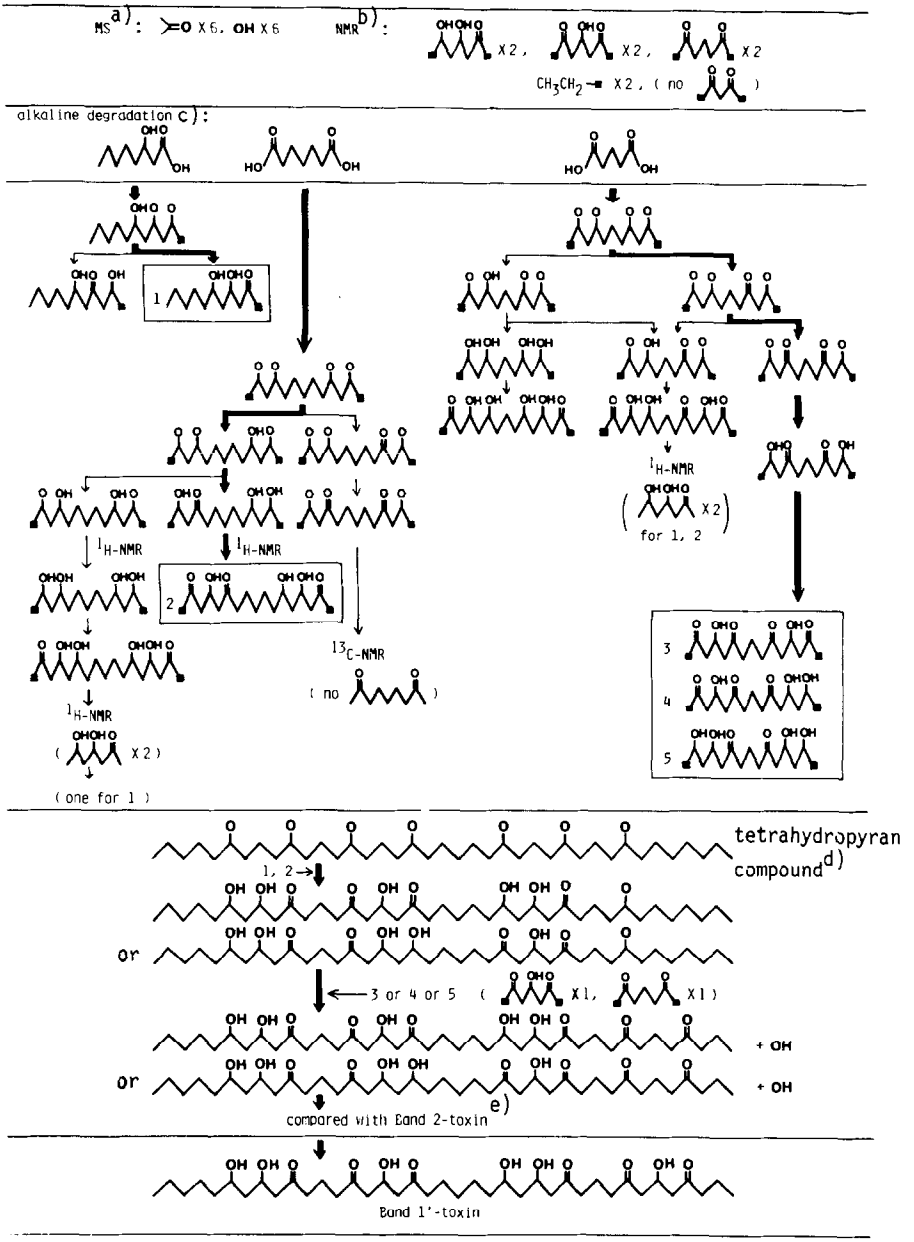
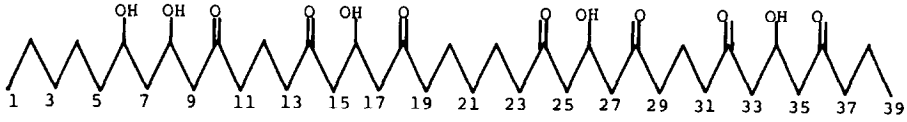


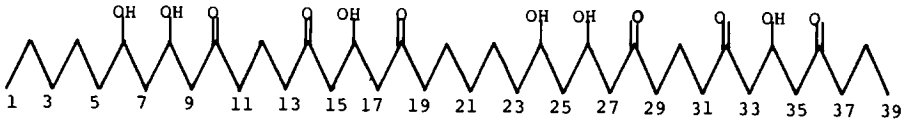
FIG. 4. Scheme of structural assignment for Band 1'-toxin. (a) Partial structures obtained from ms data. (b) Partial structures obtained from proton nmr data. (c) Alkaline degradation products from oxidized toxin (see text). (d) Positions of oxygen atoms on carbon chain were determined by hr–gc–ms on the tetrahydropyran compounds from toxin (see text). (e) Yield of glutaric acid in alkaline degradation was almost the same (6% for Band 1', 7% for Band 2); position of β, δ-dioxyoxo was estimated from Band 2-toxin. ➡Possible assignments; → inadequate assignments.

III



Band 3-toxin

IV



Band 1'-toxin

The proposed structures of Bands 3- and 1'-toxins (**III** and **IV**), based on the present results, suggest that the compounds are biogenetic intermediates in the production of the Bands 1- and 2-toxins, and also suggest that the methyl ketone function found in the major C_{41} toxins is not indispensable for biological activity, since Band 3 is as toxic and specific as Bands 1- and 2-toxins (*4*).

EXPERIMENTAL

Melting points were measured in a Yanaco microscope hot plate and uncorrected. Infrared spectra were obtained with a Shimadzu IR-27 and JASCO IRA-1 with films on KBr pellets or disks. Ultraviolet spectra in methanol were obtained with Shimadzu double-beam spectrometer uv 200 instrument. Low-resolution mass spectra were obtained with a Hitachi RMU-6M(G) mass spectrometer with a direct inlet system or a gas chromatographic inlet system using OV-1 column (4 mm × 50 cm). High-resolution mass spectra were obtained with and AEI-MS 5076, a JEOL JMS-D300, or Hitachi M-80. Nuclear magnetic resonance spectra were obtained with a JEOL JNM-PMX 60 (60 MHz), a JEOL FX-100 (100 MHz), or a JEOL FX-90Q (90 MHz). Thin-layer chromatography was carried out on Merck silica gel 60 F_{254} (0.25 mm for detection, 0.5 mm for preparative). Gas chromatographic analysis was done with a Shimadzu GC-4B (OV-1, 4 mm × 50 cm).

Isolation of Bands 1'- and 3-toxins (3)

H. maydis, race T, freshly isolated from the leaf lesions, was inoculated into the bottles containing 800 ml of modified Fries medium and grown under 12,000 lux of continuous fluorescent light (*6*). Mycelium obtained by filtration of cultures through Miracloth was homogenized for 15 min in 1 liter of 70% aqueous acetone. The culture filtrates were treated with 3% (w/v) Norit A for 2 hr with stirring. After recovery of the Norit A and washing with water, it was added to the acetone homogenate and stirred for another 2 hr. The recovered Norit A was washed several times with acetone. Toxin was desorbed by three 12-hr extractions with $CHCl_3$–MeOH (95 : 5), one-quarter to one-fifth of the volume of the filtrate. The

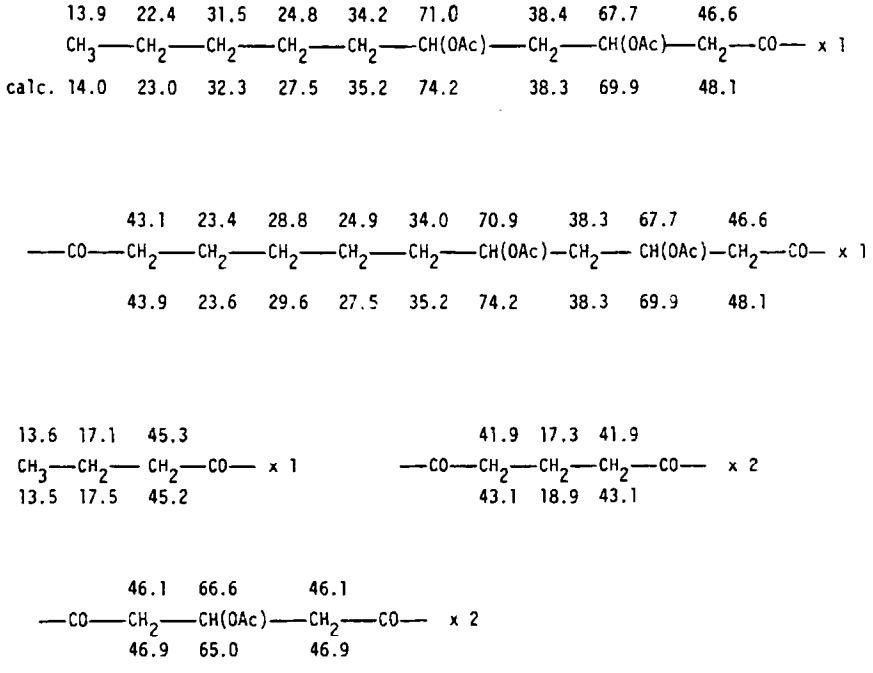


FIG. 5. ^{13}C-nmr assignments for Band 1′-toxin acetate.

use of pure chloroform in desorption resulted in less pigmented preparation but was less efficient. Purer preparations can also be obtained by treatment of the original culture filtrate with 3% (w/v) animal charcoal, desorption of toxin with 80% acetone homogenate of the mycelium, followed by treatment of the acetone with Norit A. The yield of toxin is lower by this procedure because the adsorption on animal charcoal is not complete.

The slightly yellow $CHCl_3$–MeOH eluates were combined and evaporated to dryness under vacuum. Temperatures during drying did not exceed 45°C. The red oil was dissolved in a minimum volume of $CHCl_3$ and kept at 2–4°C overnight. The resulting colorless precipitate was collected on a sintered glass filter and washed with cold $CHCl_3$. The precipitate was dissolved in hot methanol and allowed to stand at 2–4°C overnight followed by collection of the precipitate and drying under vacuum. Yield of precipitate was 50 to 300 mg from 10 liters culture. The precipitate of the toxin complex (295 mg) was fractionated using silica gel preparative tlc plate with chloroform–methanol (9 : 1) as a solvent. The developed tlc was visualized by spraying with water or exposing to iodine vapor (*3*). Bands 1′-toxin (R_f 0.42) and 3-toxin (R_f 0.46) were scraped off and eluted with hot methanol. This procedure was repeated two to three times. The yields of purified toxins were 14.5 mg for Band 1′-toxin (mp 121–122°C) and 14.9 mg for Band 3-toxin (mp 134–135°C).

Physicochemical Properties of Band 3-Toxin and Band 1′-Toxin

Band 1′-toxin: UV λ_{max}^{MeOH} nm (ϵ) = 274 (131), $[\alpha]_D^{25}$ +6° (*c* 0.28, MeOH). IR ν_{max}^{KBr} cm^{-1}; 3420 (νO—H), 2940, 2860 (νC—H), 1712 (νC=O), 1400 (active methy-

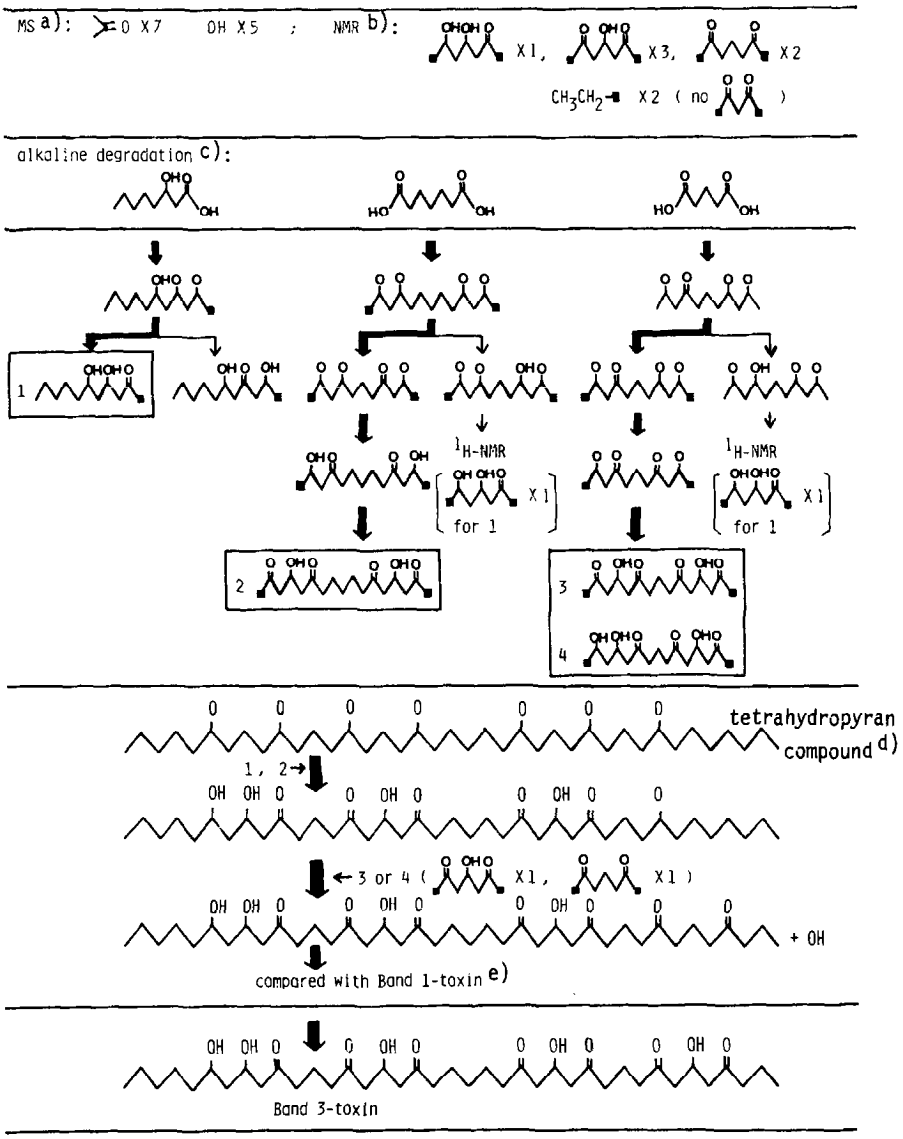


FIG. 6. Scheme of structural assignment for Band 3-toxin. (a) Partial structures obtained from ms data. (b) Partial structures obtained from proton nmr data. (c) Alkaline degradation products from oxidized toxin (see text). (d) Positions of oxygen atoms on carbon chain were determined by hr-gc-ms on the tetrahydropyran compounds from toxin (see text). (e) Yield of glutaric acid in alkaline degradation was almost the same (4% for Band 3, 5% for Band 1). ⟹ Possible assignments; → inadequate assignments.

lene), 1090, 1065, 1040 (νC—OH). Mass spectrometry of hexa-TMS derivative m/e, 1142.6977 $(M - 18)^+$, 1142.6977 calcd for $C_{57}H_{114}O_{11}Si_6$. Field desorption-mass spectrometry of sodium complex m/e 753 (M^+, 753 calcd for $C_{39}H_{68}O_{12}Na$).

Low-resolution ms of TMS derivatives (*3*) of Band 1'-toxin: m/e, 1142 (14%), 1052 (30), 962 (50), 872 (76), 782 (69), 692 (96), and 602 (100).

High-resolution ms of TMS derivatives of Band 1′-toxin: $(M-18)^+$ 1142.6977 (calcd 1142.6977), $(M-18-90)^+$ 1052.6474 (1052.6476).

Band 3-toxin: UV λ_{max}^{MeOH} nm (ϵ) = 278 (290), $[\alpha]_D^{25}$ +15.9° (*c* 0.15, MeOH). IR ν_{max}^{KBr} cm^{-1}; 3420 (νO—H), 2940, 2860 (νC—H), 1712 (νC=O), 1400 (active methylene), 1090, 1065, 1040 (νC—OH).Mass spectrometry of penta-TMS derivative (*1*) *m/e* M^+ 1086.6530, 1086.6531 calcd for $C_{54}H_{106}O_{12}Si_5$. Field desorption–mass spectrometry of sodium complex *m/e* 751 (M^+, 751 calcd for $C_{39}H_{66}O_{12} \cdot Na$).

Low-resolution ms of TMS derivatives of Band 3-toxin: *m/e* 1068 (13%), 978 (39), 888 (52), 798 (95), 708 (100), and 618 (95).

High-resolution ms of TMS derivatives of Band 3-toxin (*1*): M^+ 1086.6530 (calcd 1086.6531), $(M-18)^+$ 1068.6425 (1068.6425), and $(M-18-90)^+$ 978.5871 (978.5924).

Proton nmr of Toxin Complex, Band 3-Toxin and Band 1′-Toxin Acetates

δ5.47 (quintet, J = 6 Hz, 3H for Band 3-toxin and 2H for Band 1′-toxin), 5.21 (quintet, J = 6 Hz, 1H, 2H), 4.86 (quintet, J = 6 Hz, 1H, 2H), 2.74 (doublet, J = 6 Hz, 12H, 8H), 2.67 (doublet, J = 6 Hz, 2H 4H), 2.43 (triplet-like, J = 6 Hz, 14H, 12H), 2.04 (singlet, 3H, 6H), 2.00 (singlet, 3H, 6H), 1.98 (singlet, 9H, 6H), 1.79 (quartet-like, 8H, 10H), 1.54 (multiplet, 6H-8H, 6H-8H), 1.26 (multiplet, 8H, 10H), 0.91 (triplet, 3H, 3H), and 0.89 ppm (triplet, 3H, 3H).

The results of decoupling studies:

[5.47 ppm] (coupling) [2.47 ppm]

[2.67 ppm] ---------- [5.21 ppm] ---- [1.79 ppm]

[1.79 ppm] ---------- [4.86 ppm] ---- [1.30 ppm]

[2.43 ppm] ---------- [1.79 ppm]

[2.38 ppm] ---------- [1.54 ppm]

[1.30 ppm] ---------- [0.91, 0.89 ppm]

Carbon-13 nmr of Toxin Complex and Band 1′-Toxin

Proton noise-decoupled spectra (off-resonance spectra): 13.6 (quartet), 13.9 (q), 17.1 (triplet), 17.3 (t), 21.0 (q), 21.1 (q), 22.4 (t), 23.4 (t), 24.8 (t), 24.9 (t), 28.8 (t), 31.5 (t), 34.0 (t), 34.2 (t), 38.3 (t), 38.4 (t), 41.9 (t), 43.1 (t), 45.3 (t), 46.1 (t), 46.6 (t), 66.6 (doublet), 67.7 (d), 70.9 (d), 71.0 (t), and singlets around 170 and 207 ppm.

The results of selective decoupling using single-frequency ^{13}C-{^1H} technique are shown below.

irr. (^1H)	obs. (^{13}C)
δ5.46 ppm	66.6, 67.7 (doublet to singlet)
4.87	67.7, 70.9, 71.0 (doublet to singlet)
2.75	46.1, 46.6 (triplet to singlet)
2.44	41.9, 43.1, 45.3 (triplet to singlet)
2.04	21.0, 21.1 (quartet to singlet, CH_3COO–)
1.78	17.1, 17.3, 38.3, 38.4 (triplet to singlet)

1.54	23.4, 24.8, 24.9, 34.0, 34.2 (triplet to singlet)
1.26	22.4, 28.8, 31.5 (triplet to singlet)
0.88	13.6, 13.9 (quartet to singlet)

Assignments of ^{13}C-nmr Spectrum of Band 1'-Toxin Acetate

The results of ^{1}H-nmr and ^{13}C-nmr of Band 1'-toxin acetate gave almost the same results as Band 2-toxin acetate except terminal butyryl moiety. Then the assignments of ^{13}C-nmr spectrum of Band 1'-toxin acetate was carried out in comparison with the results of Band 2-toxin acetate. From the results of single frequency ^{13}C-{^{1}H} technique, a doublet at 67.7 (2 carbons) and a triplet at 46.1 ppm (4 carbons) can be assigned to two β-oxy-δ-dioxo groups. Also triplets at 41.9 (4 carbons) and 17.3 ppm (2 carbons) can be assigned to two δ-dioxo groups. Band 2-toxin acetate gave a signal at 18.1 ppm which was expected for the terminal δ-dioxo moiety. Instead of this signal, Band 1'-toxin acetate gave a new signal at 17.1 ppm. In addition to this new signal, Band 1'-toxin acetate gave two new signals at 13.6 (quartet) and 45.3 (triplet) ppm. These results suggested that Band 1'-toxin acetate contained terminal butyryl moiety instead of terminal δ-dioxo moiety in Band 2-toxin acetate. From these comparison, the signals at 13.6, 17.1, and 45.3 ppm were assigned to terminal butyryl moiety. These result were also supported by the data of 2-pentanone (13.5, 17.5, and 45.2 ppm). A quartet at 13.9, triplets at 22.4, 31.5, 24.8, 34.2, 38.4, and 46.6, and doublets at 70.1 and 67.7 ppm were assigned for C_1 to C_9 carbons by comparison with Band 1- (a quartet at 14.0, triplets at 22.5, 31.5, 24.7, 34.1, 38.3, and 46.5, and doublets at 71.0 and 67.7 ppm), Band 2-toxin acetates (a quartet 14.0, triplets at 22.5, 31.5, 24.7, 34.1, 38.3, and 46.5, and doublets at 71.0 and 67.7 ppm), and calculated values (a quartet at 14.0, 23.0, 32.3, 27.5, 35.2, 38.3, and 48.1, doublets at 74.2 and 69.9 ppm) (*3*). Also, triplets at 43.1, 23.4, 28.8, 24.9, 34.0, 38.3, and 46.6, and doublets at 70.9 and 67.7 ppm were assigned to the carbons from C_{19} to C_{27} by comparison with Band 2-toxin acetate (triplets at 43.5, 23.6, 29.0, 25.1, 34.0, 38.3, and 46.5, and doublets at 70.9 and 67.7 ppm) and calculated values (triplets at 43.9, 23.6, 29.6, 27.5, 35.2, 38.3, and 48.1, and doublets at 74.2 and 69.9 ppm) (*3*).

Alkaline Degradation of Oxidized Toxin (3)

Band 1'-toxin (2 mg) was dissolved in 2 ml of warm acetone and 0.2 ml of Jones reagent (13.4 g of CrO_3 was dissolved in 1.2 ml of concentrated H_2SO_4 and diluted with distilled water to 10 ml) was added. The solution was stirred for 3 to 5 min at 40°C. The upper layer of acetone solution was collected by decantation and concentrated *in vacuo*. The residue was dissolved in 80% ethanol–20% water containing 3% KOH (5 ml) and refluxed for 18 hr. The reacted solution was concentrated and extracted with 10-ml portions of diethyl ether after acidification with dilute hydrochloric acid. The extract was dried with $MgSO_4$, concentrated *in vacuo* and treated with diazomethane in diethyl ether to form methyl esters. The products were analyzed by gc and gc–ms (OV-1). Glutaric (base peak at *m/e* 129), pimelic (base peak at *m/e* 115), and β-hydroxyoctanoic acids (base peak at *m/e* 103) were identified through comparison with authentic

TABLE 2

THE RESULTS OF HR–GC–MS OF TETRAHYDROPYRAN COMPOUNDS

	Observed	Calculated		Observed	Calculated
C_{37}-Tetrahydropyrans					
$C_{10}H_{19}O$	155.1456	155.1436	$C_{32}H_{63}O$	463.4897	463.4876
$C_{14}H_{27}O$	211.2079	211.2062	$C_{28}H_{55}O$	407.4265	407.4252
$C_{18}H_{35}O$	267.2696	267.2688	$C_{24}H_{47}O$	351.3626	351.3626
C_{39}-Tetrahydropyrans					
$C_{10}H_{19}O$	155.1467	155.1436	$C_{34}H_{67}O$	491.5194	491.5192
$C_{14}H_{27}O$	211.2017	211.2026	$C_{30}H_{59}O$	435.4600	435.4563
$C_{18}H_{35}O$	267.2698	267.2688	$C_{26}H_{51}O$	379.3966	379.3938
$C_{28}H_{55}O$	407.4278	427.4253	$C_{16}H_{31}O$	239.2410	239.2375
$C_{32}H_{63}O$	463.4879	463.4876	$C_{12}H_{23}O$	183.1720	183.1749
$C_{36}H_{71}O$	519.5476	519.5501	$C_8H_{15}O$	127.1112	127.1123
C_{43}-Tetrahydropyrans					
$C_{10}H_{19}O$	155.1452	155.1436	$C_{38}H_{75}O$	547.5815	547.5818
$C_{14}H_{27}O$	211.2063	211.2062	$C_{34}H_{67}O$	491.5157	491.5188
$C_{18}H_{35}O$	267.2729	267.2688	$C_{30}H_{59}O$	435.4545	435.4563
$C_{28}H_{55}O$	407.4296	407.4250	$C_{20}H_{39}O$	295.3028	295.3006
$C_{32}H_{63}O$	463.4853	463.4876	$C_{16}H_{31}O$	239.2357	239.2373
$C_{36}H_{71}O$	519.5507	519.5501	$C_{12}H_{32}O$	183.1786	183.1749
$C_{40}H_{79}O$	575.6172	575.6131	$C_8H_{15}O$	NC[a]	127.1123
C_{45}-Tetrahydropyrans					
$C_{10}H_{19}O$	155.1472	155.1436	$C_{40}H_{79}O$	575.6162	575.6131
$C_{14}H_{27}O$	211.2091	211.2062	$C_{36}H_{71}O$	519.5516	519.5501
$C_{18}H_{35}O$	267.2688	267.2688	$C_{32}H_{63}O$	463.4876	463.4876
$C_{28}H_{55}O$	407.4283	407.4252	$C_{22}H_{43}O$	323.3333	323.3313

[a] NC, observed in low-resolution MS but not calculated in hr–ms.

specimens. The yield of above acids were about 6, 8, and 11%, respectively. Band 3-toxin gave the same products as the Band 1′-toxin, i.e., glutaric (about 4% yield), pimelic (12%), and β-hydroxyoctanoic acid (12%).

Conversion of the Toxin to Hydrocarbon (3)

The toxin complex (45 mg) was reduced using Adams platinum oxide (60 mg) in methanol–acetic acid (9 : 1, 20 ml) for 5 days until keto groups disappeared. The catalyst was separated and the solvent evaporated. The oily residue (48 mg) and red phosphorus (60 mg) were heated under reflux for 24 hr. in excess constant boiling hydriodic acid (5 ml) and *n*-heptane (3 ml). Water (5 ml) was added to the mixture, followed by extraction with 20-ml portions of chloroform (three times). The combined extracts were washed with 2% sodium thiosulfate solution (5 ml), then with water (10 ml, three times), and dried over magnesium sulfate. Removal of solvent gave an iodine-containing oil which was dissolved in tetrahydrofuran (5 ml) and refluxed overnight with lithium aluminum hydride (70 mg). Workup was

accomplished by successive addition of water (70 μl), 15% sodium hydroxide solution (70 μl), and water (210 μl). The precipitate was washed with chloroform (10 ml, three times). Then the tetrahydrofuran and the chloroform solution were combined and, after evaporation of the solvents, the residue was rehalogenated using above method (red phosphorus and hydriodic acid) without *n*-heptane. Then the product was dehalogenated with lithium aluminum hydride as above. The product was hydrogenated using Adams platinum oxide in *n*-hexane–ethyl acetate (2 : 1, 20 ml) for 1 hr. The catalyst was separated and the solvent evaporated. Then the product was separated using silica gel-column chromatography. The first eluate of *n*-hexane contained hydrocarbons (12.8 mg). The second eluate of *n*-hexane was a complex of tetrahydropyran compounds (6.4 mg). The results of hr–gc–ms of tetrahydropyran compounds are summarized in Table 2.

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REFERENCES

1. Y. KONO AND J. M. DALY, *Bioorg. Chem.* **8**, 391 (1979).
2. Y. KONO, S. TAKEUCHI, A. KAWARADA, J. M. DALY, AND H. W. KNOCHE, *Tetrahedron Lett.* 1537 (1980).
3. Y. KONO, S. TAKEUCHI, A. KAWARADA, J. M. DALY, AND H. W. KNOCHE, *Agr. Biol. Chem.* **44**, 2613 (1980).
4. G. PAYNE, H. W. KNOCHE, Y. KONO, AND J. M. DALY, *Physiol. Plant Path.* **16**, 227 (1980).
5. J. B. STOTHERS, "Carbon-13 NMR Spectroscopy." Academic Press, New York, 1972.
6. B. S. BHULLAR AND J. M. DALY, *Canad. J. Bot.* **53**, 1796 (1975).