Structure Elucidation of the Fumonisins, Mycotoxins from *Fusarium moniliforme*

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The structures of the fumonisins, a family of structurally related mycotoxins isolated from cultures of *Fusarium moniliforme*, were elucidated by mass spectrometry and ^1H and ^{13}C n.m.r. spectroscopy as the diester of propane-1,2,3-tricarboxylic acid and either 2-acetylamino- or 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyicosane as well as in each case the C-10 deoxy analogue; in all cases both the C-14 and C-15 hydroxy groups are involved in ester formation with the terminal carboxy group of propane-1,2,3-tricarboxylic acid.

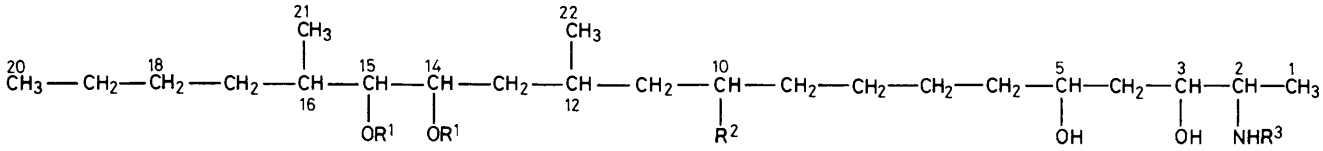
The fungus *Fusarium moniliforme* Sheldon occurs world-wide on a variety of plant hosts and is one of the most important ear rot pathogens of maize (*Zea mays* L.).[1] An isolate of *F. moniliforme* (strain MRC 826) obtained from maize in an area of the Transkei with a high oesaphageal cancer rate[2] was found to be not only highly toxic to experimental animals and able to induce leukoencephalomalacia in horses,[3] but also hepatocarcinogenic to rats,[4] and mutagenic to *Salmonella typhimurium*.[5] Although *F. moniliforme* (strain MRC 826) produces the mutagen fusarin C,[6] this compound is apparently not involved in the carcinogenicity of the fungus. In continuation of our efforts towards solving this important problem, we now report on the isolation and structure elucidation of a family of related mycotoxins, the fumonisins, from *F. moniliforme*.

Extraction of cultures of *F. moniliforme* (strain MRC826) grown on sterilized maize with aqueous methanol gave an extract which was active in rat liver histopathological and cancer initiation/promotion bioassays.[7] Subsequently, extensive fractionation of this extract, using macroreticular polystyrene resin (XAD-2), Sephadex LH-20 and reversed phase silica gel chromatography, and guided by the assays mentioned, resulted in the isolation of a mixture of fumonisin A_1 (**1**) and A_2 (**2**), as well as fumonisin B_1 (**3**) and B_2 (**4**). Treatment of the mixture of fumonisin A_1 and A_2 with an excess of diazomethane, followed by chromatography on silica gel (chloroform–methanol, 92 : 8 v/v) gave colourless oils of the tetramethyl ester derivatives (**5**) and (**6**), respectively.

Liquid secondary ion mass spectrometry (l.s.i.–m.s.) of tetramethylfumonisin A_1 (**5**), $\nu_{max.}$ ($CHCl_3$) 1730 (ester CO) and 1650 (amide CO) cm^{-1}, gave the protonated molecular ion as *m/z* 820 $(M + H)^{+\cdot}$, supported by an ion at *m/z* 842 (*M*

+ Na)$^{+\bullet}$. The multiplicities of the different resonances in the ^{13}C n.m.r. spectrum of (**5**) were determined by generating the proton-decoupled CH, CH_2, and CH_3 subspectra using the DEPT pulse sequence.[8] These data (see Table 1) suggested the empirical formula $C_{40}H_{69}NO_{16}$ for (**5**) which is consistent with the accurate mass measurement of the ion of highest mass in the electron impact (e.i.) mass spectrum at *m/z* 770.4321 ($C_{39}H_{64}NO_{14}$) $[M-(H_2O + CH_3O^{\bullet})]^+$. The presence of three hydroxy groups in (**5**) was indicated by the formation of a tris(trimethylsilyl) derivative (M^+ 1035, $C_{49}H_{93}NO_{16}Si_3$) on treatment with *N*-methyl-*N*-trimethylsilyl trifluoroacetamide and a triacetate (M^+ 945, $C_{46}H_{75}NO_{19}$) on acetylation. Basic hydrolysis with 0.05 M methanolic potassium hydroxide for 16 h at room temperature gave a neutral product (**7**), $C_{24}H_{49}NO_6$, and a salt which on acidification and methylation with an excess of diazomethane was converted into a product identical (g.c.–m.s.) to trimethylpropane-1,2,3-tricarboxylate.

The proton–proton connectivity pattern of tetramethyl fumonisin A_1 (**5**) was determined by two-dimensional (2D) (^{1}H, ^{1}H) correlation spectroscopy using the COSY-45 pulse sequence,[9] and defined two distinct structural units, *i.e.* C-1—C-5 and C-10—C-17. The involvement of both the C-14 and C-15 oxygen atoms in ester linkages with the terminal



(**1**) $R^1 = COCH_2CH(CO_2H)CH_2CO_2H$, $R^2 = OH$, $R^3 = C^{23}OC^{24}H_3$

(**2**) $R^1 = COCH_2CH(CO_2H)CH_2CO_2H$, $R^2 = H$, $R^3 = COCH_3$

(**3**) $R^1 = COCH_2CH(CO_2H)CH_2CO_2H$, $R^2 = OH$, $R^3 = H$

(**4**) $R^1 = COCH_2CH(CO_2H)CH_2CO_2H$, $R^2 = R^3 = H$

(**5**) $R^1 = COCH_2CH(CO_2CH_3)CH_2CO_2CH_3$, $R^2 = OH$, $R^3 = COCH_3$

(**6**) $R^1 = COCH_2CH(CO_2CH_3)CH_2CO_2CH_3$, $R^2 = H$, $R^3 = COCH_3$

(**7**) $R_1 = H$, $R^2 = OH$, $R^3 = COCH_3$

Table 1. N.m.r. data for tetramethylfumonisin A_1 (**5**).[a]

Carbon atom	δ_C[b]	δ_H[c,j]	*J*(HH)/Hz
1	18.03 Q	1.139 d	6.8
2	49.72 D	3.902 m	
3	71.49 D	3.79 m	
4	40.22 T	1.56	
		1.43	
5	68.83 D	3.82	
6	38.55 T[d]		
7	25.63 T[e]		
8	25.48 T[e]		
9	37.22 T[d]		
10	68.78 D	3.56	
11	43.08 T	1.37	
		0.96	
12	25.17 D	1.75	
13	35.39 T	1.64	
		1.28	
14	71.24 D	5.137 ddd	11.3, 3.3, 2.3
15	77.79 D	4.867 dd	8.8, 3.3
16	33.64 D	1.58	
17	31.84 T	1.31	
		0.98	
18	28.48 T	1.23	
		1.08	
19	22.73 T	~1.2	
20	13.92 Q	0.829 t	7.1
21	15.36 Q	0.872 d	6.8
22	20.36 Q	0.912 d	6.6
23	170.48 S	—	
24	23.32 Q	1.949 s	

C-15 ester: $R^1 = C^{25}OC^{26}H_2C^{27}H(C^{30}O_2C^{31}H_3)C^{28}H_2C^{29}O_2C^{32}H_3$

Carbon atom	δ_C[b]	δ_H[c]	*J*(HH)/Hz
25	170.88 S	—	
26	35.27 T[h]	2.780 dd[h]	16.7, 6.7
		2.603 dd	16.8, 6.4
27	37.28 D[f]	3.22 m	
28	35.10 T[h]	2.732 dd[h]	16.6, 7.1
		2.583 dd	16.7, 6.5
29	173.41 S	—	
30	171.69 S[g]	—	
31	52.31 Q[i]	3.664 s[i]	
32	51.82 Q[i]	3.645 s[i]	

C-14 ester: $R^1 = C^{33}OC^{34}H_2C^{35}H(C^{38}O_2C^{39}H_3)C^{36}H_2C^{37}O_2C^{40}H_3$

Carbon atom	δ_C[b]	δ_H[c]	*J*(HH)/Hz
33	171.42 S	—	
34	35.10 T[h]	2.672 dd	16.8, 8.1
		2.400 dd	16.8, 5.9
35	37.19 D[f]	3.22 m	
36	34.93 T[h]	2.647 dd[h]	16.7, 6.5
		2.583 dd	16.7, 6.5
37	173.93 S	—	
38	171.66 S[g]	—	
39	52.15 Q[i]	3.645 s[i]	
40	51.80 Q[i]	3.634 s[i]	

[a] Recorded on a Bruker WM-500 spectrometer. [b] Relative to $CDCl_3$ at δ_C 77.00. Chemical shifts of the proton-bearing carbon atoms were correlated with specific proton resonances.[9,11] [c] Relative to $CDCl_3$ at δ_H 7.24. [d—i] May be interchanged. [j] The NH proton appears as a doublet at δ_H 6.071 [*J*(NH, 2-H) 8.7 Hz].

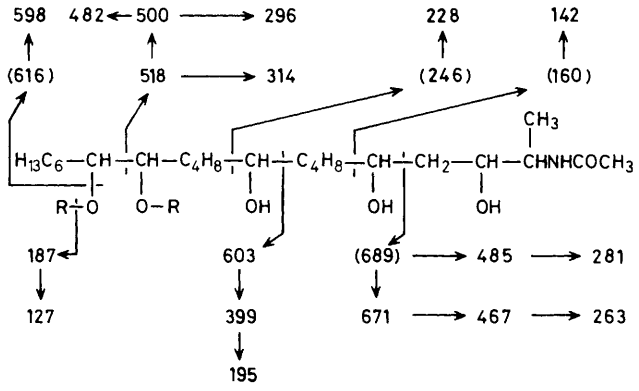


Figure 1. Fragmentation pattern observed in the e.i. mass spectrum of (**5**).

carboxy groups of the two propane-1,2,3-tricarboxylate moieties (as indicated by the ^1H and ^{13}C n.m.r. data)[10] and the location of the methyl groups in (**5**) followed from the two- and three-bond (^{13}C, ^1H) connectivity pattern (Table 2) determined in a chemical shift correlation experiment adjusted to detect correlations *via* long-range (^{13}C, ^1H) couplings (^nJ 5 Hz).[9,11]

The fragmentation pattern for compound (**5**) (see Figure 1) in the electron impact mass spectrum was of vital importance in determining the linkage of the C-1—C-5 and C-10—C-17 structural fragments by a C_4H_8 group and placing a C_6H_{13} substituent at C-15. The base peak in the mass spectrum occurs at *m/z* 187 ($C_8H_{11}O_5$) and is associated with the cleavage of the ester moiety as indicated. The loss of 204 mass units from a number of fragment ions throughout the spectrum is due to the loss of a dimethyl propane-1,2,3-tricarboxylate group. The location of the hydroxy groups in (**5**) as indicated by the n.m.r. data is consistent with the fragment ions at *m/z* 671, 603, 228, and 142 and was confirmed by the fragmentation pattern of the tris(trimethylsilyl) derivative.

Tetramethylfumonisin A_2 (**6**), $C_{40}H_{69}NO_{15}$, contains one oxygen atom less than tetramethylfumonisin A_1 (**5**). The protonated molecular ion of (**6**) appears at *m/z* 804 $(M + H)^{+\cdot}$ in the l.s.i. mass spectrum and the fragment ion which arises from the molecular ion in the e.i. spectrum through loss of (H_2O + $CH_3O^{\cdot}$) appears at *m/z* 754 ($C_{39}H_{64}NO_{13}$). A comparison of the fragmentation patterns of compounds (**6**) and (**5**) shows that it is the C-10 hydroxy group of (**5**) which is replaced by a hydrogen atom in (**6**) as the bond cleavage which generates the fragment ions *m/z* 603 → 399 → 195 in the spectrum of (**5**) does not occur. In addition a number of bond cleavages common to both compounds generate fragment ions of 16 mass units less for tetramethylfumonisin A_2 (**6**).

L.s.i. mass spectrometry of fumonisin B_1 (**3**) ($C_{34}H_{59}NO_{15}$) gave the protonated molecular ion as *m/z* 722 whereas that of fumonisin B_2 (**4**) ($C_{34}H_{59}NO_{14}$) appears at *m/z* 706. In each case ions are also present at *m/z* values corresponding to $(M + Na)^{+\cdot}$. A comparison of the ^{13}C n.m.r. data of these two compounds† indicates that once again it is the C-10 hydroxy group of fumonisin B_1 (**3**) (δ_C 67.17) which is replaced by a hydrogen atom in fumonisin B_2 (**4**). In addition, the ^1H and ^{13}C resonances of the atoms of the *N*-acetyl group present in the fumonisin A metabolites, *e.g.* (**5**): δ_H 1.949; δ_C 170.48, and 23.32, are absent from the spectra of the fumonisin B compounds.

Table 2. Two- and three-bond (^1H, ^{13}C) connectivity pattern for tetramethylfumonisin A_1 (**5**).

δ_H	Correlation signals δ_C/p.p.m.
0.829 (20-H)	22.73 T (C-19), 28.48 T (C-18)
0.872 (21-H)	31.84 T (C-17), 33.64 D (C-16), 77.79 D (C-15)
0.912 (22-H)	25.17 D (C-12), 35.39 T (C-13)
1.139 (1-H)	49.72 D (C-2), 71.49 D (C-3)
1.949 (24-H)	170.48 S (C-23)
4.867 (15-H)	33.64 D (C-16), 71.24 D (C-14), 170.88 S (C-25)
5.137 (14-H)	77.79 D (C-15), 171.42 S (C-33)

It is of interest to note that a related mono-ester of propane-1,2,3-tricarboxylic acid and 1-amino-11,15-dimethyl-2,4,5,13,14-pentahydroxyheptadecane is a host-specific phytotoxin produced by *Alternaria alternata* (Fr.) Keissler f. sp. *lycopersici*, the causal agent of stem canker disease of tomato.[10,12]

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† (**3**): δ_C (CD_3SOCD_3) 175.91S, 175.60S, 174.31S, 173.97S, 171.45S (C-25), 171.39S (C-33), 76.48D (C-15), 70.35D (C-14), 68.42D (C-13), 67.17D (C-10), 66.01D (C-5), 51.69D (C-2), 43.28T (C-11), 40.72T (C-4), 38.17D (C-27 and C-35), 38.19T, 38.07 (2 × T), 37.80T (C-26), 36.54T (C-28), 36.38T (C-34), 35.72T (C-36), 32.96D (C-16), 30.96T (C-17), 28.08T (C-18), 25.43T, 25.28T, 25.20D (C-12), 22.22T (C-19), 20.19Q (C-22), 15.57Q (C-1), 15.51Q (C-21), 13.86Q (C-20).