JOURNAL OF NATURAL PRODUCTS

Volume 59, Number 11 November 1996

Full Papers

Isolation and Characterization of Two New Type C Fumonisins Produced by *Fusarium oxysporum*

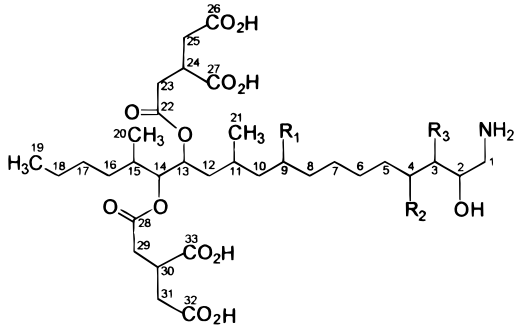
Jeong-Ah Seo, Jin-Cheol Kim, and Yin-Won Lee*

Department of Agricultural Biology and Research Center for New Biomaterials in Agriculture, College of Agriculture and Life Sciences, Seoul National University, Suwon 441-744, Republic of Korea

Received February 12, 1996[⊗]

Two new fumonisins, hydroxylated fumonisin C_1 (**1**) and fumonisin C_3 (**3**), were isolated from wheat cultures of *Fusarium oxysporum* together with known fumonisin C_1 (**2**) and C_4 (**4**). Compound **1** is structurally similar to **2** except for the presence of an additional hydroxy group at C-3. Compound **3** is similar in structure to fumonisin B_3 except that the C-1 terminal methyl group is missing.

Fumonisins are structurally related toxins originally isolated from cultures of *Fusarium moniliforme*,[1] and they have been implicated in a number of animal diseases. Fumonisin B_1, normally the predominant fumonisin, is known to cause leukoencephalomalacia in horses[2,3] and pulmonary edema in pigs[4] and to be hepatotoxic and hepatocarcinogenic to rats.[5] Fumonisin levels in food are statistically associated with an increased risk of human esophageal cancer in the Transkei, South Africa.[6,7] Nine fumonisins have been isolated and characterized. Three of these, fumonisin B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3), appear to be the major fumonisins produced in nature, while fumonisins B_4 (FB_4), A_1 (FA_1), and A_2 (FA_2) are produced in relatively minor quantities.[1] The A-series of fumonisins are acetylated on the amino group, while the B-series have a free amine. Two C-series fumonisins, C_1 (**2**) and C_4 (**4**), were reported from cultures of *F. moniliforme*;[8,9] **2** and **4** correspond in structure to FB_1 and FB_4 with the C-1 terminal methyl group missing, respectively. Recently, fumonisin AK_1 (FAK_1) was isolated from cultures of *F. proliferatum*.[10] FAK_1 differs from FB_1 in that one tricarballylic acid functionality at the C-15 position of the eicosane backbone is replaced by a ketone and the amino group is acetylated. In this paper, we describe the isolation and structural characterization of two new type C fumonisins, hydroxylated fumonisin C_1 (**1**) and fumonisin C_3 (**3**), produced by *F. oxysporum*, Tuberculariaceae.



1: $R_1 = R_2 = R_3 = OH$
2: $R_1 = R_2 = OH$, $R_3 = H$
3: $R_1 = OH$, $R_2 = R_3 = H$
4: $R_1 = R_2 = R_3 = H$

* To whom correspondence should be addressed. Phone: 82-331-2902443. Fax: 82-331-2945881. E-mail: lee2443@plaza.snu.ac.kr.
[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1996.

S0163-3864(96)00234-0 CCC: $12.00

Table 1. ^{13}C NMR Shifts for **1**−**4** in CD_3OD[a]

carbon	1	2	3	4	carbon	1	2	3	4
1	44.5	46.6	46.1	46.1	18	23.9	23.9	23.8	23.8
2	70.9	70.0	69.9	68.8	19	14.4	14.4	14.4	14.4
3	76.0	43.1	39.2	39.0	20	16.0	16.0	15.9	16.0
4	69.0	66.3	30.6[b]	30.7[c]	21	20.7	20.8	20.6	20.9
5	37.6	37.9	30.5[b]	30.5[c]	22	173.4[e]	173.5[e]	173.3[e]	173.1[e]
6	26.9	26.7	26.7	27.5[d]	23	36.8[f]	37.0[f]	36.6[f]	36.3[f]
7	26.8	26.6	26.2	26.3	24	39.5[g]	39.7[g]	39.0[g]	38.9[g]
8	39.2	39.0	39.0	30.5[c]	25	37.3[h]	37.6[h]	36.8[h]	36.7[h]
9	70.0	68.6	68.7	30.5[c]	26	178.6[i]	178.7[i]	177.7[i]	177.5[i]
10	44.0	44.6	44.5	30.1[c]	27	178.0[i]	178.2[i]	177.3[i]	177.3[i]
11	26.9	26.9	26.9	20.9[d]	28	173.2[e]	173.3[e]	173.1[e]	173.0[e]
12	34.8	34.9	35.8	35.9	29	36.7[f]	36.8[f]	36.6[f]	36.1[f]
13	72.9	72.8	73.0	73.0	30	39.5[g]	39.6[g]	39.0[g]	38.9[g]
14	78.7	78.8	78.7	78.8	31	37.1[h]	37.2[h]	36.8[h]	36.6[h]
15	34.6	34.9	34.8	34.9	32	176.9[i]	176.7[i]	176.0[i]	175.9[i]
16	33.0	33.0	33.0	33.0	33	176.5[i]	176.7[i]	175.7[i]	175.6[i]
17	29.6	29.7	29.5	29.6					

[a] Chemical shifts in ppm from CD_3OD (49.0 ppm) signal. [b−i] Shift assignments with identical superscripts may be interchanged.

Results and Discussion

Compounds **1**−**4** were isolated from wheat cultures of *F. oxysporum* by Si gel column chromatography and preparative HPLC. The purified compounds were analyzed by FABMS and NMR spectrometry. The molecular weight of each of the four toxins was determined by FABMS: 724 ($[M + H]^+$) for **1**, 708 ($[M + H]^+$) for **2**, 692 ($[M + H]^+$) for **3**, and 676 ($[M + H]^+$) for **4**. The difference of 16 Da among the four compounds indicated the loss of a hydroxy group, successively. Ion signals at *m/z* 708 (**2**) and *m/z* 676 (**4**) are consistent with the protonated molecular ions of **2** and **4** reported previously,[8,9] and the masses of protonated **1** (*m/z* 724) and **3** (*m/z* 692) suggested that **1** and **3** contained four and two hydroxy groups, respectively. FAB mass spectra of the four toxins exhibited two abundant fragments corresponding to $(M + H - 176)^+$ and $(M + H - 352)^+$ which are indicative of successive losses of two tricarballylic groups. The spectra also contained evidences of the number of hydroxy groups as indicated by the presence of a succession of fragments attributed to $(M + H - 352 - nH_2O)^+$, where *n* is an integer that ranges from 1 to the number of hydroxy groups.

The NMR data (^1H, ^{13}C, $^1H-^1H$ DQF COSY experiments) of **2** and **4** agreed with the results of the previously published reports[8,9] and formed the basis for the assignment of structures of **1** and **3**. The ^{13}C NMR data of the four toxins are presented in Table 1. The positions of the hydroxy groups were assigned on the basis of the $^1H-^1H$ DQF COSY experiments.

In the $^1H-^1H$ DQF COSY spectrum of **1**, a downfield proton (δ 3.88) shown by H-2 was correlated to two downfield protons H-1_a (δ 3.00)[11] and H-1_b (δ 3.28) and another downfield proton H-3 (δ 3.36), indicating that the first and second hydroxy groups of **1** are at C-2 and C-3, respectively. The C-1 protons were assigned easily because they do not couple to any signal in the upfield region. The methine proton at C-3 was also correlated to a downfield proton (H-4, δ 3.79), providing evidence that the third hydroxy group is at C-4. The presence of another hydroxy group at C-9 was deduced through comparison of the $^1H-^1H$ DQF COSY spectrum of **1** to that of **2**; in $^1H-^1H$ DQF COSY spectra of both compounds, a downfield proton H-9 (δ 3.63) coupled with four upfield protons (H-8_a, H-8_b, H-10_a, and H-10_b) of methylene region. Thus, the structure of **1** was elucidated as 1-amino-11,15-dimethyl-2,3,4,9,13,14-hexahydroxynonadecane that is esterified at both the 13 and 14 positions with propane-1,2,3-tricarboxylic acid.

Compound **3** contained one less hydroxy group than **2**, and the two hydroxy groups were determined to be on C-2 and C-9 by comparing the $^1H-^1H$ DQF COSY spectrum of **3** with that of **2**. The $^1H-^1H$ DQF COSY spectrum of **3** showed that the C-4 hydroxy group of **2** was replaced by hydrogen atom in **3**. This new fumonisin (**3**) corresponds in structure to FB_3 except that the C-1 terminal group is missing.

Fumonisin B_1 and B_2 were also produced by the isolate as minor metabolites when the culture extract was analyzed with TLC. This is the first report that C-type fumonisins were produced by *F. oxysporum*, although B-type fumonisins were produced by *F. oxysporum*.[12]

Experimental Section

General Experimental Procedures. FAB mass spectra were collected on a JEOL JMS-AX 505 mass spectrometer with a glycerol matrix and Ar as the bombarding gas. ^1H and ^{13}C NMR and $^1H-^1H$ DQF COSY spectra were recorded in CD_3OD on a JEOL λ400 spectrometer, and spectra were referenced to TMS (^1H) or to solvent signals (^{13}C).

Fungal Material. The fungal isolate was *F. oxysporum* (strain CAR), which had been isolated from carnation by us in Korea. This isolate caused wilt disease to carnation. The stock culture of the isolate was single spore isolated, maintained on moist autoclaved soil, and stored at −15 °C. Erlenmeyer flasks (1 L), each containing 200 g of wheat and 120 mL of distilled water, were autoclaved for 1 h at 121 °C twice within a 24-h interval. The wheat was inoculated with mycelium plugs from a 5-day-old potato dextrose agar plate of the fungus. The flasks were incubated for 4 weeks at 25 °C. The mycelial mass and substrate were dispersed onto a screen-bottom tray and allowed to air dry in a ventilated hood. When dry, this inoculated substrate was ground to the consistency of flour.

Extraction and Isolation. Solid culture material (1 kg) was extracted three times with MeOH−H_2O (3:1) (totalling 7 L) and filtered through a Buchner funnel. The filtrate was concentrated to dryness and redissolved in 85 mL of H_2O. The aqueous phase was chromatographed on an Amberlite XAD-2 column (55 × 600 mm, 1 kg, 20−60 mesh; Sigma Chemical Co.). The column was successively eluted with MeOH−H_2O (1:3, 1 L), MeOH−H_2O (1:1, 1 L), MeOH−H_2O (3:1, 1 L), and MeOH (1 L). The fractions were analyzed by analytical TLC with $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1) as the developing solvent system. Fumonisins were visualized by spraying the TLC plate with 0.5% *p*-anisaldehyde solution in MeOH−H_2SO_4−HOAc (85:5:10) and heating it at 110 °C for 10 min. Compounds **1**−**4** were eluted with MeOH−H_2O (3:1) and MeOH fractions, which were combined and concentrated to dryness. The residue (36.37 g) was dissolved in a minimal volume of $CHCl_3$−MeOH−HOAc (6:3:1) and loaded onto a Si gel column (30 × 600 mm, 300 g, 70−230 mesh, E. Merck). The column was eluted with $CHCl_3$−MeOH−HOAc (6:3:1), and the eluate was collected in 15-mL fractions with a fraction collector. The fractions were monitored by TLC and reduced to two fractions called F1 and F2. The F1

fraction (1.2 g) was fractionated on a Si gel column (20 × 600 mm, 90 g, 230−400 mesh, E. Merck) with EtOAc−HOAc−hexane−H_2O (6:2:2:1) as the mobile phase. Fractions (10 mL) were collected and analyzed by TLC as described above. The fractions containing a single component of either **3** or **4** were combined separately and concentrated to dryness. The F2 fraction (2.0 g) was separated on a preparative μ Bondapak C_{18} column (25 × 100 mm, 10 μm, 125 Å, Millipore Co.) using a gradient mobile phase of MeOH−H_2O−HOAc (20:80:1) to MeOH−HOAc (100:1) at a flow rate of 10 mL min^{-1}. Fractions (20 mL) were collected, and those containing a single component of either **1** or **2** were combined separately and taken to dryness. Final purification of each of the four fumonisins prior to characterization was performed on a strong-anion exchange (SAX) cartridge (Sep-Pak Accell Plus QMA, 35 mL, Millipore Co.). Each compound was dissolved in 35 mL of MeOH−H_2O (3:1) and applied to a SAX cartridge, which was successively washed with MeOH−H_2O (3:1, 35 mL) and MeOH (35 mL) and eluted with 1% HOAc in MeOH (50 mL). This procedure yielded 90 mg of **1**, 50 mg of **2**, 350 mg of **3**, and 250 mg of **4**.

Hydroxylated fumonisin C_1 (**1**) was obtained as a colorless liquid: R_f = 0.26, $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1); positive FABMS *m/z* 724 $[M + H]^+$ (100), 706 (6), 688 (4), 548 (9), 372 (12), 354 (6), 336 (4); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.14 (1H, dt, J = 11.0, 3.0 Hz, H-13), 4.95 (1H, dd, J = 8.3, 3.2 Hz, H-14), 3.88 (1H, td, J = 8.0, 3.4 Hz, H-2), 3.79 (1H, td, J = 7.8, 3.4 Hz, H-4), 3.63 (1H, m, H-9), 3.36 (1H, H-3), 3.28 (1H, dd, J = 13.4, 3.4 Hz, H-1_b), 3.15 (2 × 1H, m, H-24 and H-30), 3.00 (1H, dd, J = 12.7, 8.3 Hz, H-1_a), 2.78 (1H, dd, J = 16.6, 7.1 Hz), 2.74 (1H, dd, J = 11.8, 3.9 Hz), 2.69 (1H, dd, J = 7.1, 4.3 Hz), 2.63 (1H, dd, J = 7.6, 4.9 Hz), 2.57 (1H, dd, J = 13.9, 6.6 Hz), 2.56 (1H, dd, J = 11.2, 5.4 Hz), 2.50 (1H, dd, J = 11.7, 6.1 Hz), 2.46 (1H, dd, J = 10.5, 6.3 Hz), 1.81 (1H, m, H-11), 1.69 (1H, H-15), 1.64 (1H, H-12_b), 1.35−1.57 (14H, m), 1.31 (1H, m, H-17_a), 1.16 (1H, H-10_a), 1.09 (1H, H-16_a), 0.96 (3H, d, J = 6.3 Hz, Me-21), 0.94 (3H, d, J = 6.8 Hz, Me-20), 0.90 (3H, t, J = 7.1 Hz, Me-19); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1.

Fumonisin C_1 (**2**) was obtained as a colorless liquid: R_f = 0.29, $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1); positive FABMS *m/z* 708 $[M + H]^+$ (100), 690 (6), 672 (3), 532 (7), 356 (12), 338 (12), 320 (10); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.14 (1H, dt, J = 11.3, 3.7 Hz, H-13), 4.93 (1H, dd, J = 8.3, 3.9 Hz, H-14), 4.00 (1H, m, H-2), 3.79 (1H, m, H-4), 3.66 (1H, m, H-9), 3.14 (2 × 1H, m, H-24 and H-30), 3.03 (1H, dd, J = 13.0, 3.4 Hz, H-1_b), 2.80 (1H, dd, J = 12.9, 7.6 Hz), 2.76 (1H, H-1_a), 2.72 (1H, dd, J = 7.6, 3.0 Hz), 2.69−2.66 (3 × 1H, m), 2.53−2.41 (3 × 1H, m), 1.81 (1H, m, H-11), 1.67 (1H, H-15), 1.58 (1H, H-12_b), 1.35−1.54 (16H, m), 1.30 (1H, m, H-17_a), 1.16 (1H, H-10_a), 1.11 (1H, H-16_a), 0.95 (3H, d, J = 6.3 Hz, Me-21), 0.93 (3H, d, J = 6.8 Hz, Me-20), 0.88 (3H, t, J = 7.1 Hz, Me-19); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1.

Fumonisin C_3 (**3**) was obtained as a colorless liquid: R_f = 0.36, $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1); positive FABMS *m/z* 692 $[M + H]^+$ (100), 674 (7), 516 (8), 340 (15), 322 (14), 304 (7); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.13 (1H, dt, J = 11.3, 2.9 Hz, H-13), 4.95 (1H, dd, J = 8.6, 2.9 Hz, H-14), 3.77 (1H, m, H-2), 3.61 (1H, m, H-9), 3.16 (2 × 1H, m, H-24 and H-30), 3.03 (1H, dd, J = 12.7, 2.7 Hz, H-1_b), 2.80 (1H, dd, J = 11.7, 7.3 Hz), 2.76 (1H, H-1_a), 2.74−2.64 (3 × 1H, m), 2.61−2.52 (3 × 1H, m), 2.48 (1H, dd, J = 16.6, 6.6 Hz), 1.79 (1H, m, H-11), 1.70 (1H, H-15), 1.66 (1H, H-12_b), 1.27−1.53 (19H, m), 1.17 (1H, H-10_a), 1.09 (1H, H-16_a), 0.95 (3H, d, J = 6.6 Hz, Me-21), 0.94 (3H, d, J = 6.6 Hz, Me-20), 0.88 (3H, t, J = 6.8 Hz, Me-19); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1.

Fumonisin C_4 (**4**) was obtained as a colorless liquid: R_f = 0.44, $CHCl_3$−MeOH−H_2O−HOAc (55:36:8:1); positive FABMS *m/z* 676 $[M + H]^+$ (100), 658 (3), 500 (5), 324 (30), 306 (11); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.17 (1H, dt, J = 11.1, 3.2 Hz, H-13), 4.92 (1H, dd, J = 8.5, 3.4 Hz, H-14), 3.76 (1H, m, H-2), 3.16 (2 × 1H, m, H-24 and H-30), 3.03 (1H, dd, J = 12.7, 2.9 Hz, H-1_b), 2.80 (1H, dd, J = 12.7, 7.1 Hz), 2.76 (1H, H-1_a), 2.74−2.64 (3 × 1H, m), 2.59 (1H, dd, J = 10.3, 6.4 Hz), 2.56−2.47 (3 × 1H, m), 1.71 (1H, H-15), 1.62 (1H, H-12_b), 1.14−1.54 (23H, m), 1.06 (1H, H-16_a), 0.93 (3H, d, J = 7.1 Hz, Me-21), 0.91 (3H, d, J = 6.4 Hz, Me-20), 0.89 (3H, t, J = 7.1 Hz, Me-19); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1.

Acknowledgements. This work was supported in part by a grant from the Research Center for New Biomaterials in Agriculture, Seoul National University.

References and Notes

(1) Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. J. *Appl. Environ. Microbiol.* **1988**, *54*, 1806−1811.
(2) Kellerman, T. S.; Coetzer, J. A. W.; Naude, T. W. *Plant poisoning and mycotoxicoses of livestock in southern Africa*; Oxford University Press: Cape Town: South Africa, 1988.
(3) Marasas, W. F. O.; Kellerman, T. S.; Gelderblom, W. C. A.; Coetzer, J. A. W.; Thiel, P. G.; van der Lugt, J. J. *Ondersterpoort J. Vet. Res.* **1988**, *55*, 197−204.
(4) Harrison, L. P.; Colvin, B. M.; Greene, J. T.; Newman, L. E.; Cole, J. R. *J. Vet. Diagnost. Invest.* **1990**, *2*, 217−221.
(5) Gelderblom, W. C. A.; Thiel, P. G.; Jaskiewicz, K.; Marasas, W. F. O. *Carcinogenesis* **1986**, *7*, 1899−1901.
(6) Cawood, M. E.; Gelderblom, W. C. A.; Vleggaar, R.; Thiel, P. G.; Marasas, W. F. O. *J. Agric. Food Chem.* **1991**, *39*, 1858−1862.
(7) Gelderblom, W. C. A.; Marasas, W. F. O.; Vleggaar, R.; Thiel, P. G.; Cawood, M. E. *Mycopathologia* **1992**, *117*, 11−16.
(8) Branham, B. E.; Plattner, R. D. *J. Nat. Prod.* **1993**, *56*, 1630−1633.
(9) Plattner, R. D. *Natural Toxins* **1995**, *3*, 294−298.
(10) Musser, S. M.; Eppley, R. M.; Mazzola, E. P.; Hadden, C. E.; Shockcor, J. P.; Crouch, R. C.; Martin, G. E. *J. Nat. Prod.* **1995**, *58*, 1392−1397.
(11) For the assignment of nonequivalent methylene protons, higher and lower field methylene protons were suffixed by subscript a and b, respectively.
(12) Abbas, H. K. *Plant Disease* **1995**, *79*, 968.

NP960234P