Isolation and Characterization of a New Fumonisin from Liquid Cultures of Fusarium moniliforme

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ISOLATION AND CHARACTERIZATION OF A NEW FUMONISIN FROM LIQUID CULTURES OF *FUSARIUM MONILIFORME*[1]

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ABSTRACT.—A new fumonisin has been isolated from liquid cultures of *Fusarium moniliforme*. The new compound was separated from fumonisin B_1 by preparative hplc and characterized by liquid sims, gc-ms analysis of TMSi and TFA derivatives, and ^1H and ^{13}C nmr. The new compound, fumonisin C_1 [**2**] lacked the amino-end terminal-methyl group of fumonisin B_1 and is the diester of 13,14-propane-1,2,3-tricarboxylic acid and 1-amino-11,15-dimethyl-2,4,9,13,14-pentahydroxynonadecane.

Fumonisins are a recently discovered class of fungal mycotoxins implicated in a number of animal diseases (1–3). Epidemological studies have indicated that fumonisins may cause human esophageal cancer (4). Fumonisins are produced by several species of Fusaria within section Liseola, but the most copious producers are found within the species *Fusarium moniliforme* Sheldon (5). Fumonisin B_1 [**1**] was first characterized in 1988 (6) and typically accounts for 70% of the total fumonisins produced. Three other fumonisins, (A_1, A_2, and B_2) were reported by Bezuidenhout *et al.* (6). The A-series fumonisins are acetylated on the amino group while the B-fumonisins have a free amine. The A-fumonisins have, so far, not been reported by other investigators and may be artifacts of the isolation procedure employed by Bezuidenhout *et al.* (6). Fumonisin B_2 is produced in lesser quantities than **1** and usually accounts for 20–30% of the total fumonisins. In 1992, Plattner *et al.* (7) reported a third fumonisin, B_3, which is a structural isomer of B_2. In this paper we report the isolation of a new fumonisin designated C_1 [**2**].

Preparative hplc of liquid culture extracts of *F. moniliforme* M-2326 under three mobile phase conditions afforded 21 mg of **2**. Compound **2** was analyzed by liquid sims, ms/ms, hydrolysis followed by gc-ms (9), and ^1H and ^{13}C nmr. A mol wt of 707 was determined by fabms in a glycerol matrix ($[M+H]^+$ 708). The mol wt of **1** is 721, yielding a difference of 14 daltons between **1** and **2**, indicating loss of a methyl group. A comparsion of ms/ms spectra of **1** and **2** showed that the backbone of **2** was 14 daltons less than **1**, suggesting a methyl group was lost on the fumonisin backbone. Hydrolysis of **1** and **2** in base was followed by derivatization to form trifluoroacetates (TFA) and trimethylsilyl (TMSi) derivatives which were analyzed by electron ionization gc-ms (9). The mol wt observed as the molecular anion in negative cims of the TFA derivative of **2** was 967, compared to 981 for **1**, and again indicated the loss of a methyl group as suggested by the liquid sims spectrum. In eims the TFA derivative of **1** showed an intense fragment at *m/z* 140 which arises from cleavage between C-2 and C-3. In the eims of the TFA derivative of **2** this

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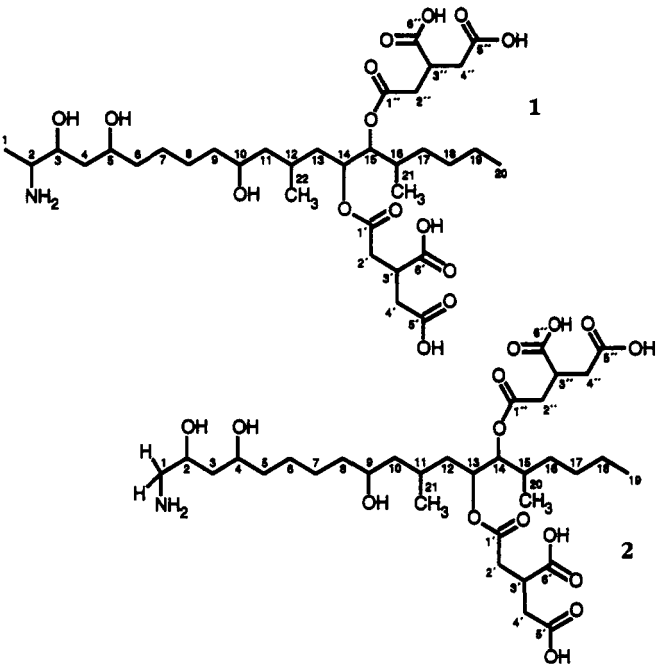

signal shifted to *m/z* 126, indicating that loss of the methyl group occurred on the amino-end of the fumonisin backbone. Similarly in the spectra of the TMSi derivatives the signal from cleavage between C-2 and C-3 of **1** was observed at *m/z* 116 in **1**, and the signal from cleavage between C-1 and C-2 in **2** was at *m/z* 102. These data together strongly suggest that the difference between **1** and **2** is that the amino terminal carbon in **1** is absent from **2**. The ^{13}C- (Table 1) and ^{1}H-nmr data (data not shown) provide further evidence for the structural assignment of **2**. The chemical shifts of **1** (7) compared to **2** show that signal for C-1 is absent in the spectrum of **2**. The shift for the amino-bearing carbon in compound **2** moved upfield 8 ppm as expected. The ^{1}H-nmr spectrum of **2** is similar to that of **1** except that the doublet for C-1 at 1.3 ppm is missing in **2**. Thus, the structure of **2** is consistent with the ms and nmr data presented.

EXPERIMENTAL

FUNGAL CULTURES AND CONDITIONS.—Liquid cultures of *F. moniliforme* M-2326 (provided by P.E. Nelson of the Fusarium Research Center, Pennsylvania State University) were prepared using the medium of Clouse *et al.* (8). Levels of production of **1** were typically in the range of 150–250 mg per liter.

PHYSICAL ANALYSES.—Mass spectra were collected on a Finnigan TSQ-700. Liquid sims was performed with an Ion Tech fast-atom gun with xenon as the primary atom beam with an energy of approximately 8 KeV. ^{1}H- and ^{13}C-nmr spectra were determined in D_2O on a Bruker WM-300 instrument.

ISOLATION.—Fungal mycelia were removed by filtration, and 4 liters of the crude culture material was separated on preparative reversed-phase hplc using a C_{18} column (47×300 mm), 50 ml·min^{-1} flow rate, and a gradient from 100% H_2O to 100% MeOH. Fractions of 100 ml were collected, and one fraction, eluting at a solvent composition of approximately MeOH-H_2O (60:40), contained 88% of the injected fumonisin B_1. This fraction was taken to dryness and chromatographed on a preparative C_{18} column (41×300 mm) using an isocratic mobile phase of 0.05 M NaH_2PO_4 adjusted to pH 3.35 with H_3PO_4-MeCN (72:28) at a flow rate of 25 ml·min^{-1}. Hplc analysis of the fractions indicated a second component on the front side of **1**. The combined fractions containing **1** and the unknown component were rechromatographed with the same conditions described above except that the mobile phase composition was changed to 0.05 M NaH_2PO_4 (pH 3.35)/MeCN (74:26). Fractions (50 ml) were collected, and those containing the

Table 1. ^{13}C nmr Shifts for Fumonisins B_1 [**1**] and C_1 [**2**][a].

Carbon	Compound	
	1	**2**
C-1	17.7	47.5
C-2	55.5	71.2
C-3	71.8	43.6
C-4	42.2	67.4
C-5	69.9	39.7
C-6	39.5	27.6
C-7	27.7	27.5
C-8	27.6	39.7
C-9	39.8	69.8
C-10	71.4	45.2
C-11	45.1	27.9
C-12	27.7	37.6
C-13	37.5	74.7
C-14	74.7	80.5
C-15	80.6	35.8
C-16	35.9	34.0
C-17	34.3	30.5
C-18	30.8	24.9
C-19	25.1	15.9
C-20	16.3	17.4
C-21	17.3	22.2
C-22	22.4	—
C-1′	175.2[b]	175.4[b]
C-2′	37.8[c]	38.4[c]
C-3′	40.0[d]	41.0[d]
C-4′	38.1[c]	39.0[c]
C-5′	179.6[e]	180.4[e]
C-6′	179.1[e]	180.0[e]
C-1″	175.1[b]	175.4[b]
C-2″	37.8[c]	38.4[c]
C-3″	39.9[d]	41.0[d]
C-4″	38.0[c]	38.9[c]
C-5″	178.0[e]	179.0[e]
C-6″	177.6[e]	178.6[e]

[a]Chemical shifts in ppm from TMS.

[b–e]Shift assignments with identical superscripts may be interchanged.

unknown component were combined, taken to dryness, and desalted through a C_{18} mini-column. This procedure yielded 21 mg of **2**.

MASS SPECTRAL DATA.—*Ms/Ms of* m/z *708 from fabms of* **2**.—708 (parent), 690 (25), 532 (7), 514 (5), 374 (6), 356 (30), 338 (100), 320 (55).

TFA derivative of hydrolyzed **1** *(mol wt 981).*—754 (0.1), 640 (2), 639 (2), 582 (1), 557 (1.5), 556 (1.5), 542 (3), 526 (1.8), 428 (1.5), 264 (2), 211 (10), 180 (20), 140 (100), 97 (40), 55 (23), 43 (18).

TFA derivative of hydrolyzed **2** *(mol wt 967).*—740 (0.1), 626 (6), 625 (6), 568 (2), 542 (3), 541 (3), 528 (7), 512 (6), 414 (3), 264 (4), 211 (20), 180 (60), 126 (59), 97 (100), 55 (66), 43 (43).

TMSi derivative of hydrolyzed **1** *(mol wt 837).*—$[M-187]^+$ 650 (20), 578 (7), 560 (3), 488 (2), 187 (100), 145 (38), 143 (39), 116 (100), 73 (77), 44 (16).

TMSi derivative of hydrolyzed **2** *(mol wt 823).*—$[M-187]^+$ 636 (12), 564 (1), 546 (4), 474 (3), 187 (85), 145 (30), 143 (41), 102 (100), 73 (76), 44 (10).

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