

Chemical Transformation of Hydrolyzed Fumonisin B₁ to Hydrolyzed Fumonisin B₂

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The fumonisins, a series of sphingosine-analog mycotoxins produced by the ubiquitous corn contaminant *Fusarium moniliforme*, are an important food safety concern because they are tumor promoters with sufficient chemical stability to persist through normal food processing. Removing propane-1,2,3-tricarboxylic acid side chains produces hydrolyzed fumonisins (HF), including HFB₁ and HFB₂, which retain biological activity. Detection of hydrolyzed fumonisins in food products has created a need for efficient methods to prepare them for use as analytical standards. In the present study conversion of the more abundant HFB₁ to HFB₂ was accomplished by selectively protecting the hydroxyl groups to be retained as the bis(acetonide), deoxygenating the remaining free hydroxyl, and removing the blocking groups. Deoxygenation was accomplished by two methods: (i) free radical-initiated homolytic cleavage of the *O*-phenoxythiocarbonyl ester in the presence of tributyltin hydride and (ii) LiAlH₄ reduction of the tosylate ester.

Keywords: Fumonisin; *Fusarium moniliforme*; hydrolyzed fumonisin B₂; mycotoxins; partial synthesis; deoxygenation

The fungus *Fusarium moniliforme* J. Sheldon is the major ear-rot fungus in temperate zones and a ubiquitous pathogen of stored corn worldwide (Marasas *et al.*, 1984). *F. moniliforme* produces a variety of mycotoxins including the fumonisins, a series of substituted long-chain alkylamines structurally analogous to sphingosine (Riley *et al.*, 1993; Shier, 1992), which are esterified with two propane-1,2,3-tricarboxylic acid (PTCA) moieties (Bezuidenhout *et al.*, 1988; Shier *et al.*, 1995). The most abundant member of the series is fumonisin B₁ (1) (FB₁), which typically represents 70% or more of the fumonisins produced by most isolates of *F. moniliforme* in field samples and in culture (Ross *et al.*, 1992). FB₁ has been shown to cause leukoencephalomalacia in horses (Marasas *et al.*, 1988), pulmonary edema in swine (Harrison *et al.*, 1990), hepatoma in rats (Gelderblom *et al.*, 1991), and cytotoxicity in certain differentiated mammalian cell types in culture (Shier *et al.*, 1991; Norred *et al.*, 1992). Fumonisin B₂ (2) (FB₂), which differs in structure from FB₁ in that it lacks a hydroxyl group on C-10 (Bezuidenhout *et al.*, 1988; Harmange *et al.*, 1994) (see Figure 1), is produced in substantially lower amounts by almost all isolates of *F. moniliforme* (Ross *et al.*, 1992). The PTCA side chains on FB₁ and FB₂ can be removed by alkaline hydrolysis to yield the corresponding backbones, which will be referred to herein as hydrolyzed fumonisins, HFB₁ (3) and HFB₂ (4), respectively. They have sometimes been referred to as aminopentols AP₁, AP₂ (Bezuidenhout *et al.*, 1988), and AP₃, but the term is a misnomer for AP₂ and AP₃, which are actually aminotetrols. Not only have HFB₁ and HFB₂ been shown to retain biological activity in mammalian cell culture and phytotoxicity bioassays despite loss of about half the molecular weight during conversion from the parent toxins, they also exhibited a broader spectrum of activity in mammalian cell culture bioassays. That is, they were toxic to undifferentiated cell lines

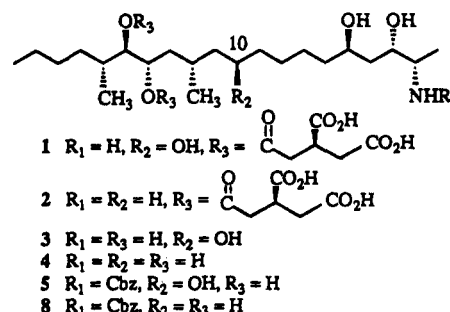


Figure 1. Chemical structures of fumonisin B₁ (1), fumonisin B₂ (2), hydrolyzed fumonisin B₁ (3), hydrolyzed fumonisin B₂ (4), the *N*-benzyloxycarbonyl derivative of hydrolyzed fumonisin B₁ (5), and the *N*-benzyloxycarbonyl derivative of hydrolyzed fumonisin B₂ (8).

(Abbas *et al.*, 1993) as well as to the differentiated cell lines which are sensitive to FB₁ and FB₂ (Shier *et al.*, 1991). This observation has prompted the suggestion that hydrolyzed fumonisins may be active metabolites in some systems (Shier, 1992; Abbas *et al.*, 1993). Hydrolyzed fumonisins are also of interest as potential intermediates in the biosynthesis of fumonisins in *F. moniliforme*.

Hopmans and Murphy (1993) observed that HFB₁ contaminated a variety of corn-derived processed food products including tortilla chips and canned yellow corn. They attributed the increased toxicity observed when *Fusarium proliferatum*-contaminated corn-based diet was exposed to high pH and heat (Hendrich *et al.*, 1993) to the production of hydrolyzed fumonisins, which appeared to be more toxic than intact fumonisins in rats. It is apparent from these observations that many of the processed food products which have been examined for only fumonisin content should be re-examined to determine if the processing that reduced fumonisin content (Sydenham *et al.*, 1991) served only to produce hydrolyzed fumonisins of equal or greater toxicity. This situation has created a demand for HFB₁ and HFB₂ for use as analytical standards, as well as for mechanism

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of action and biosynthesis studies. A convenient method for the production of HFB₁ has been described (Shier and Abbas, 1992). Therefore, we have developed the following method for the partial synthesis of HFB₂ (4) from the more abundant HFB₁ (3).

EXPERIMENTAL PROCEDURES

Materials. Pentafluorophenyl chlorothionoformate (PFT), tributyltin hydride (*n*-Bu₃SnH), benzyl chloroformate, dimethoxypropane (DMP), and camphorsulfonic acid (CSA) were purchased from Aldrich Chemical Co., Milwaukee, WI. 2,2'-Azobis(2-methylpropionitrile) [azobis(isobutyronitrile), AIBN] was obtained from Janssen Chimica/Spectrum Chemical Manufacturing Corp., New Brunswick, NJ. Unless otherwise indicated, all other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Instrumental Analysis. Nuclear magnetic resonance (NMR) spectra were determined in CD₃OD or CDCl₃ on a 300 MHz GE NMR Instruments Co. Model Omega HR spectrometer. Signals were assigned by chemical shift, by comparison with previously reported data, and by distortionless enhancement by polarization transfer (DEPT). Negative ion fast atom bombardment mass spectra (FAB-MS) were obtained by T. Krick, Department of Biochemistry, College of Biological Sciences, University of Minnesota, on a Kratos model MS-25 mass spectrometer equipped with a Kratos model FAB ion source using glycerol or thioglycerol as the matrix. HPLC analysis was carried out on a Shimadzu CR 501 ChromatoPac, with Shimadzu PF-535 fluorescence detector, excitation 333 nm, emission 444 nm.

Preparation of Compounds. *N*-Benzyloxycarbonyl-HFB₁ (5). HFB₁ (3) (25 mg, 62 μmol) was dissolved in 150 μL of water containing Na₂CO₃ (10 mg, 94 μmol). Benzyl chloroformate (13 μL, 90 μmol) was added slowly with continuous stirring for 15 min at room temperature. The reaction mixture was cooled in an ice bath and acidified with ~2 mL of concentrated HCl. The precipitation of product was allowed to proceed in an ice bath for 2 h. The solid was collected by filtration, washed with ice water, and chromatographed on a silica gel microcolumn (15 × 0.8 cm, eluted with 9:1 CHCl₃/EtOH) to produce 18.4 mg (34 μmol, 55% yield) of compound 5 as a yellow oily liquid: *R*_f = 0.5 (CHCl₃/MeOH 9:1, silica); FAB-MS 540 [M + 1]⁺; ¹H NMR (300 MHz, CDCl₃) δ 0.81 (3H, d, 6.0 Hz, H-21), 0.89 (3H, t, 7.5 Hz, H-20), 0.96 (3H, d, 6.0 Hz, H-22), 1.17 (3H, d, 7.0 Hz, H-1), 3.37 (1H, dd, 3.0, 9 Hz, H-15), 3.6–3.59 (2H, m, H-5 and H-10), 3.68 (1H, m, H-2), 3.81 (1H, ddd, 3.0, 5.5, 11.0 Hz, H-14), 3.87 (H-3, t, 7.8 Hz, H-3), 5.03 (1H, d, 12 Hz, NHCO₂CH₂H₅Ph), 5.1 (1H, d, 11.6 Hz, NHCO₂CH₂H₅Ph), 5.5 (1H, d, 7.8 Hz, -NH), 7.24–7.42 (5H, m; NHCO₂CH₂H₅Ph).

N-Benzyloxycarbonyl-HFB₁ Bis(acetonide) (6). Compound 5 (12 mg, 22 μmol) was dissolved in a mixture of 300 μL of acetone and 300 μL of DMP, mixed with a catalytic amount of CSA, and stirred at room temperature for 16 h. The solvent was evaporated under reduced pressure to produce 15 mg of crude mixture, which was chromatographed on a silica gel microcolumn (15 × 0.8 cm, hexanes/EtOAc 2:1) to produce 11 mg (17.8 μmol, 81% yield) of compound 6 as a yellowish powder: *R*_f = 0.25 (hexanes/EtOAc 2:1, silica); FAB-MS 620 [M + 1]⁺; ¹H NMR (300 MHz, CDCl₃) δ 0.88 (3H, t, 7.4 Hz, H-20), 0.97 (3H, t, 6.0 Hz, H-21), 0.98 (3H, d, 7.0 Hz, H-22), 1.13 (3H, d, 7.0 Hz, H-1), 3.62–3.68 (2H, m, H-5 and H-10), 3.71 (1H, dd, 5.0, 10.0 Hz, H-15), 3.87 (H-3, t, 7.8 Hz, H-3), 3.89 (1H, m, H-2), 4.1 (1H, ddd, 2.5, 3.5, 5.5 Hz, H-14), 5.03 (1H, d, 12 Hz, NHCO₂CH₂H₅Ph), 5.1 (1H, d, 11.6 Hz, NHCO₂CH₂H₅Ph), 5.3 (1H, d, 8.9 Hz, -NH), 7.3–7.8 (5H, m, NHCO₂CH₂H₅Ph).

N-Benzyloxycarbonyl-HFB₂ (8) by Free Radical-Initiated Homolytic Deoxygenation of 6. Compound 6 (11.5 mg, 18.6 μmol) was dissolved in 3 mL of CH₂Cl₂ and 1 mL of pyridine with addition of 30 μL of PFT and stirring for 16 h at room temperature. The solvent was evaporated to produce 20 mg of crude thionocarbonyl ester, which was dissolved in 5 mL of toluene containing 40 μL of *n*-Bu₃SnH and 2 mg of AIBN. The

mixture was heated under reflux in nitrogen at 85 °C for 3 h and then evaporated under reduced pressure. The brown oily residue was applied to a silica gel column (18 × 1 cm) and eluted with 50 mL each of petroleum ether, CHCl₃, EtOAc, and finally MeOH. The methanolic fraction was concentrated under reduced pressure and treated with 1 N HCl at room temperature to remove the acetonide blocking groups to produce 9.6 mg (18.4 μmol, 98% yield) of compound 8 as a yellowish powder: *R*_f = 0.63 (hexanes/EtOAc 3:1, silica); FAB-MS, 524 [M + 1]⁺; ¹H NMR (300 MHz, CDCl₃) δ 0.81 (3H, d, 6.2 Hz, H-21), 0.89 (3H, t, 7.5 Hz, H-20), 0.96 (3H, d, 6.1 Hz, H-22), 1.04 (3H, d, 7.0 Hz, H-1), 3.37 (1H, dd, 3.0, 9.0 Hz, H-15), 3.59 (1H, m, H-5), 3.87 (H-3, m, H-3), 3.81 (1H, ddd, 3.0, 5.5, 11.0 Hz, H-14), 4.01 (1H, m, H-2), 5.03 (1H, d, 12 Hz, NHCO₂CH₂H₅Ph), 5.1 (1H, d, 11.6 Hz, NHCO₂CH₂H₅Ph), 5.3 (1H, d, 7.8 Hz, -NH), 7.24–7.42 (5H, m, NHCO₂CH₂H₅Ph).

HFB₂ (4) by Hydrogenolysis of 8. The *N*-Cbz group of 8 (9.8 mg, 18.8 μmol) was removed by dissolving in 3 mL of MeOH and shaking with hydrogen at 45 psi over 50 mg of 10% Pd-C to produce 7.2 mg (18.5 μmol, 98% yield) of 4 with an overall yield of 43%. To facilitate analysis by NMR, compound 4 (5 mg) was peracetylated by dissolving in 500 μL of pyridine and 500 μL of Ac₂O and stirring at room temperature for 16 h. The excess Ac₂O and pyridine were removed under reduced pressure, and the residue was chromatographed on a silica gel microcolumn (15 × 0.8 cm, EtOAc/hexane, 2:1) to provide 4.6 mg of *N*-acetyl pentaester of 4 as a white powder: *R*_f = 0.46 (hexane/EtOAc 1:2, silica); FAB-MS 658 [M + 1]⁺; ¹H NMR (300 MHz, CDCl₃) δ 0.88 (3H, t, 6.8 Hz, H-20), 0.95 (3H, d, 6.9 Hz, H-21), 0.99 (3H, d, 6.4 Hz, H-22), 1.12 (3H, d, 6.9 Hz, H-1), 1.23 (3H, m, H-19), 1.53–1.68 (2H, m, H-11), 1.73 (1H, m, H-12), 4.01 (1H, m, H-2), 4.85 (1H, dd, 3.6, 4.2 Hz, H-15), 4.86 (1H, m, H-5), 4.75–5.01 (1H, m, H-3), 5.17 (1H, ddd, 3.8, 3.8, 10.9 Hz, H-14). ¹³C NMR, DEPT experiments (75 MHz, CDCl₃) δ 18.2 (q, C-1), 48.1 (d, C-2), 69.8 (d, C-3), 40.6 (t, C-4), 68.9 (d, C-5), 38.3 (t, C-6), 25.6 (t, C-7), 27.2 (t, C-8), 29.9 (t, C-9), 30.7 (t, C-10), 35.2 (t, C-11), 31.3 (t, C-12), 40.9 (t, C-13), 70.7 (d, C-14), 81.1 (d, C-15), 35.7 (t, C-16), 31.6 (t, C-17), 30.5 (t, C-18), 24.1 (q, C-19), 14.4 (q, C-20), 16.2.3 (q, C-21), 21.3 (q, C-22), 169.6 (-CONH), 173.8, 170.9, 170.8, and 170.4 were assigned for four acetate carbonyl groups on carbons 3, 5, 14, and 15. ¹³C NMR, DEPT experiments (75 MHz, CDCl₃), for *N*-acetyl pentaester of HFB₁ δ 18.3 (q, C-1), 48.2 (d, C-2), 69.6 (d, C-3), 40.4 (t, C-4), 71.9 (d, C-5), 36.1 (t, C-6), 25.6 (t, C-7), 25.4 (t, C-8), 35.3 (t, C-9), 70.1 (d, C-10), 34.1 (t, C-11), 25.2 (t, C-12), 35.4 (t, C-13), 70.8 (d, C-14), 77.4 (d, C-15), 33.6 (t, C-16), 31.84 (t, C-17), 28.5 (t, C-18), 22.7 (q, C-19), 13.9 (q, C-20), 15.4 (q, C-21), 20.4 (q, C-22), 169.8 (-CONH), 173.7, 170.7, 170.5, 170.6, and 170.5 were assigned for five acetate carbonyl groups on carbons 3, 5, 10, 14, and 15.

10-O-Tosyl-N-benzyloxycarbonyl-HFB₁ Bis(acetonide) (7). Compound 6 (5 mg, 8.1 μmol) was dissolved in 2 mL of pyridine containing catalytic amounts of DMAP. The mixture was kept at -5 °C, and 3.2 mg of *p*-toluenesulfonyl chloride (16.8 μmol) was added in portions to the reaction mixture while stirring to produce a reddish precipitate. The mixture was allowed to stand overnight, and then 2 mL of water was added; the organic layer was separated, dried over anhydrous MgSO₄, and purified on a silica gel column (10 × 0.4 cm, hexanes/EtOAc, 1:1.5). The product was subjected to hydrogenolysis at 45 psi over 50 mg of 10% Pd-C to afford compound 7 obtained as 4 mg (6.3 μmol, 78% yield) of yellowish powder: *R*_f = 0.63 (hexanes/EtOAc 3:1, silica); FAB-MS 640 [M + 1]⁺; ¹H NMR (300 MHz, CDCl₃) δ 0.91 (3H, t, 7.0 Hz, H-20), 0.97 (3H, d, 6.3 Hz, H-21), 0.98 (3H, d, 7.0 Hz, H-22), 1.15 (3H, d, 7.5 Hz, H-1), 2.3 (3H, s, CH₃PhSO₂-R), 3.68 (1H, dd, 5.0, 10.0 Hz, H-15), 3.69 (1H, m, H-5), 3.72 (H-3, m, H-3), 3.99 (1H, m, H-2), 4.01 (1H, ddd, 3.0, 5.5, 12.1 Hz, H-14), 7.1 (2H, d, 8.2 Hz, CH₃PhSO₂-R), 7.9 (2H, d, 7.9 Hz, CH₃PhSO₂-R).

HFB₂ (4) by LiAlH₄ Reduction of 7. Compound 7 (4.5 mg, 7.0 μmol) was dissolved in anhydrous ether and kept at 0 °C under nitrogen while an excess of LiAlH₄ was added cautiously in portions with stirring to produce a slurry mixture which was allowed to rise to room temperature overnight. The reaction mixture was quenched with a saturated solution of sodium potassium tartarate (Russel salt) until the slurry

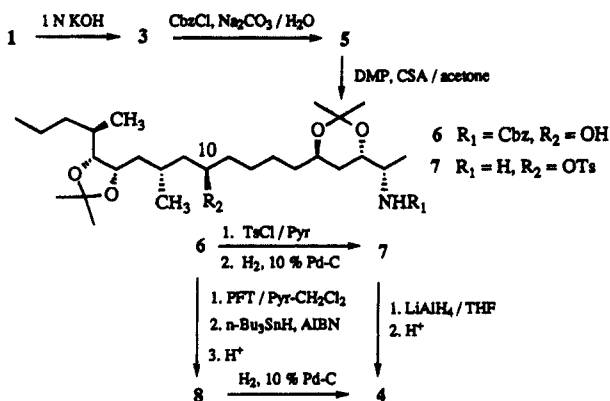


Figure 2. Synthesis of hydrolyzed fumonisin B₂ (4).

became clear. The mixture was stirred for an additional 1 h and extracted three times with 10 mL aliquots of EtOAc; the extracts were combined and dried over anhydrous MgSO₄, and the solvent was evaporated under reduced pressure. The residue was purified on a silica gel column (10 × 0.4 cm, CHCl₃/MeOH/HAC 6:3:1) to produce 2.6 mg of 4 (6.7 μmol, 96% yield) as a pure white powder with an overall yield of 34%: *R*_f = 0.56 (CHCl₃/MeOH/NH₄OH 30:15:1); FAB-MS 390 [M + 1]⁺; ¹H and ¹³C NMR are the same as described above for HFB₂ prepared by free radical-initiated homolytic deoxygenation of 6.

Comparison of Semisynthetic HFB₂ (4) to Authentic HFB₂. Authentic HFB₂ was prepared from FB₂ produced by *F. moniliforme* JW#1 grown on corn cultures and isolated and purified according to the method of Cawood *et al.* (1991). FB₂ was hydrolyzed and purified according to the method of Bezuidenhout *et al.* (1988). HPLC analysis was carried out by following a modification of the methods of Ueno *et al.* (1993) and Hopmans and Murphy (1993) using as mobile phase MeOH/0.1 M NaH₂PO₄ (80:20), pH 3.35, and a flow rate of 1.2 mL/min on an Econosil C₁₈, 5 μm, 250 × 4.6 mm column after precolumn derivatization with *o*-phthalaldehyde (OPA). Authentic HFB₂ and HFB₁ were peracetylated as described above for semisynthetic HFB₂ and characterized by ¹H and ¹³C NMR under the same conditions.

RESULTS AND DISCUSSION

Our strategy for the conversion of HFB₁ to HFB₂ was to selectively protect the hydroxyl groups at C-3, C-5, C-14, and C-15 of HFB₁ as the bis(acetonide) and then deoxygenate the remaining free OH at C-10. Figure 2 depicts the synthesis of HFB₂. The starting material, HFB₁ (3), was prepared according to the method of Shier and Abbas (1992) and converted to the *N*-Cbz-bis(acetonide) derivative 6 according to the method of Hoyer *et al.* (1994). Deoxygenation of 6 at the single free carbinol center at C-10 was accomplished by two methods: the free radical-initiated homolytic deoxygenation method of Robins *et al.* (1983) and LiAlH₄ reduction of the C-10 tosylate after removal of the *N*-Cbz blocking group.

Initial studies were carried out with the free radical-initiated homolytic deoxygenation method of Robins *et al.* (1983), which was of particular interest because it could in principle also be used for the partial synthesis of FB₂ from FB₁ by deoxygenation at C-10 of the *N*-Cbz-3,5-acetonide tetramethyl ester derivative of FB₁. The method converted 6 to HFB₂ (4), but the product was contaminated with tin-containing impurities that were difficult to remove. Attempts to prepare FB₂ from FB₁ by deoxygenating the *N*-Cbz-3,5-acetonide tetramethyl ester derivative of FB₁ yielded intractable mixtures.

In the second method, deoxygenation at C-10 of 6 was accomplished by LiAlH₄ reduction of the tosylate ester.

Prior to LiAlH₄ reduction, the *N*-Cbz blocking group was removed by hydrogenolysis to give 7, to prevent it from being reduced to the *N*-methyl derivative. LiAlH₄ reduction of 7, followed by hydrolysis of the bis(acetonide) blocking groups during acid workup, proceeded smoothly to give HFB₂ (4) in higher purity than the free radical-initiated homolytic deoxygenation method.

HFB₂ produced semisynthetically from HFB₁ exhibited the same properties as authentic HFB₂ prepared by hydrolysis of FB₂ (Bezuidenhout *et al.*, 1988), including (i) migration on normal phase TLC (*R*_f = 0.56 on silica gel in CHCl₃/MeOH/concentrated NH₄OH 30:15:1), (ii) retention time (7.39 min) on reversed phase HPLC (Ueno *et al.*, 1993; Hopmans and Murphy, 1993), (iii) FAB-MS, and (iv) ¹H and ¹³C NMR. The FAB-MS spectra of semisynthetic and authentic 4 both contained the major peak at *m/z* 390, which was assigned to [M + H]⁺. Semisynthetic and authentic compound 4 were also characterized by ¹H and ¹³C NMR of both unmodified and peracetylated (Ac₂O/pyridine) material, the latter of which gave better resolved, more easily interpreted spectra. Specifically, DEPT (Distortionless Enhancement by Polarization Transfer) experiments in ¹³C NMR showed that one oxygenated methine carbon (CH–O) at 70.1 ppm in the spectrum of the starting material HFB₁ was replaced by a methylene carbon at 30.4 ppm in the spectrum of the HFB₂ produced from it.

Both HPLC retention time and peak splitting patterns in ¹H NMR spectra are sensitive to stereochemistry in molecules with more than one stereogenic center; the observation of identical retention time and ¹H NMR spectra implies the same stereochemistry in semisynthetic and authentic HFB₂ and, hence, the same absolute configurations at the stereogenic centers common to HFB₁ and HFB₂. This observation confirms by an independent method all relative and absolute configuration assignments made for HFB₂ by Harmange *et al.* (1994).

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LITERATURE CITED

- Abbas, H. K.; Gelderblom, W. C. A.; Cawood, M. E.; Shier, W. T. Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme*, in jimsonweed (*Datura stramonium* L.) and mammalian cell cultures. *Toxicon* **1993**, *31*, 345–353.
- Bezuidenhout, S. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Horak, R. M.; Marasas, W. F. O.; Spiteller, G.; Vleggaar, R. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *J. Chem. Soc., Chem. Commun.* **1988**, 743–745.
- Cawood, M. E.; Gelderblom, W. C. A.; Vleggaar, R.; Behrend, Y.; Theil, P. G.; Marasas, M. F. O. Isolation of the fumonisins—a quantitative approach. *J. Agric. Food Chem.* **1991**, *39*, 1958–1962.
- Gelderblom, W. C. A.; Kreik, N. P. J.; Marasas, W. F. O.; Theil, P. G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B₁, in rats. *Carcinogenesis* **1991**, *12*, 1247–1251.
- Harmange, J.-C.; Boyle, C. D.; Kishi, Y. Relative and absolute stereochemistry of the fumonisin B₂ backbone. *Tetrahedron Lett.* **1994**, *35*, 6819–6822.
- Harrison, L. R.; Colvin, B. M.; Greene, J. T.; Newman, L. E.; Cole, R. J. Pulmonary edema and hydrothorax in swine produced by fumonisin B₁, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* **1990**, *2*, 217–221.

- Hendrich, S.; Miller, K. A.; Wilson, T. M.; Murphy, P. A. Toxicity of *Fusarium proliferatum*-fermented nixtamalized corn-based diets fed to rats: effect of nutritional status. *J. Agric. Food Chem.* **1993**, *41*, 1649–1654.
- Hopmans, E. C.; Murphy, P. A. Detection of fumonisins B₁, B₂, and B₃ and hydrolyzed fumonisin B₁ in corn-containing foods. *J. Agric. Food Chem.* **1993**, *41*, 1655–1658.
- Hoye, T. R.; Jimenez, J. I.; Shier, W. T. Relative and absolute configuration of fumonisin B₁ backbone. *J. Am. Chem. Soc.* **1994**, *116*, 9409–9410.
- Marasas, W. F. O.; Nelson, P. E.; Toussoun, T. A. *Toxigenic Fusarium Species*; Pennsylvania State University Press: University Park, PA, 1984.
- Marasas, W. F. O.; Kellerman, T. S.; Gelderblom, W. C. A.; Coetzee, J. A. W.; Theil, P. G.; van der Lugt, J. J. Leukoencephalomalacia in a horse induced by fumonisin B₁ isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res.* **1988**, *55*, 197–203.
- Norred, W. P.; Wang, E.; Bacon, C. W.; Riley, R. T.; Merrill, A. H., Jr. In vitro toxicology of fumonisins and the mechanistic implications. *Mycopathologia* **1992**, *117*, 73–78.
- Riley, R. T.; Norred, W. P.; Bacon, C. W. Fungal toxins in foods: recent concerns. *Annu. Rev. Nutr.* **1993**, *13*, 167–189.
- Robins, M. J.; Wilson, J. S.; Hansske, F. Nucleic acid related compounds. 42. A general procedure for the efficient deoxygenation of secondary alcohols. Regiospecific and stereoselective conversion of ribonucleosides to 2'-deoxynucleosides. *J. Am. Chem. Soc.* **1983**, *105*, 4059–4065.
- Ross, P. F.; Rice, L. G.; Osweiler, G. D.; Nelson, P. E.; Richard, J. L.; Wilson, T. M. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. *Mycopathologia* **1992**, *117*, 109–114.
- Shier, W. T. Sphingosine analogs: an emerging new class of toxins that includes the fumonisins. *J. Toxicol.-Toxin Rev.* **1992**, *11*, 241–257.
- Shier, W. T.; Abbas, H. K. A simple procedure for the preparation of aminopentols (fumonisin hydrolysis products AP₁ and AP₂) from *Fusarium moniliforme* on solid media. *Abstracts of the 106th Association of Official Analytical Chemists Meeting*, Cincinnati, OH; AOAC International: Arlington, VA, 1992; p 237.
- Shier, W. T.; Abbas, H. K.; Mirocha, C. J. Toxicity of the mycotoxins fumonisins B₁ and B₂ and *Alternaria alternata* f. sp. *lycopersici* toxin (AAL) in cultured mammalian cells. *Mycopathologia* **1991**, *116*, 97–104.
- Shier, W. T.; Abbas, H. K.; Badria, F. A. Complete structures of the sphingosine analog mycotoxins fumonisin B₁ and AAL toxin T_A: absolute configuration of the side chains. *Tetrahedron Lett.* **1995**, *36*, 1571–1574.
- Sydenham, E. W.; Shephard, G. S.; Theil, P. G.; Marasas, W. F. O.; Stockenstrom, S. Fumonisin contamination of commercial corn-based foodstuffs. *J. Agric. Food Chem.* **1991**, *39*, 2014–2018.
- Ueno, Y.; Aoyama, S.; Sugiura, Y.; Wang, D.-S.; Lee, U.-S.; Hirooka, E. Y.; Hara, S.; Karki, T.; Chen, G.; Yu, S.-Z. A limited survey of fumonisins in corn and corn-based products in Asian countries. *Mycotoxin Res.* **1993**, *9*, 27–34.

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