# Preparation and Isolation of the Partially Hydrolyzed Moiety of Fumonisin $B_1$

Eric W. Sydenham,*,† Pieter G. Thiel,† Gordon S. Shephard,† Klaus R. Koch,‡ and Thérèse Hutton§

Programme on Mycotoxins and Experimental Carcinogenesis, Medical Research Council, P.O. Box 19070, Tygerberg 7505, South Africa, Department of Chemistry, University of Cape Town, Private Bag, Rondebosch 7700, South Africa, and Fisons Instruments VG Organic, Tudor Road, Altrincham, Cheshire WA14 5RZ, United Kingdom

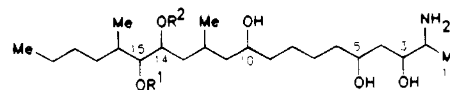
The natural occurrence in corn of carcinogenic mycotoxins, the fumonisins, has prompted the development of potential decontamination procedures. Chemical treatment of fumonisin $B_1$ ($FB_1$)-contaminated corn with calcium hydroxide [$Ca(OH)_2$] results in the base hydrolysis of $FB_1$ (the major naturally occurring fumonisin analogue) to yield its corresponding aminopentol ($AP_1$) and tricarballylic acid (TCA) moieties. Complete hydrolysis proceeds in a sequential reaction involving the removal of one TCA group and the formation of a partially hydrolyzed moiety ($PH_1$), which exists as an equilibrium mixture of the two possible monoesters. $PH_1$ was prepared by the treatment of *Fusarium moniliforme* culture material with $Ca(OH)_2$ and subsequently isolated and purified using chromatographic methods. $PH_1$ was also prepared, using similar methods, from pure $FB_1$. The identity of the $PH_1$ moiety was determined by liquid chromatography–electrospray mass spectrometry.

**Keywords:** *Fumonisin $B_1$; alkaline hydrolysis; partially hydrolyzed fumonisin $B_1$; isolation*

## INTRODUCTION

Fumonisin $B_1$ ($FB_1$) (Figure 1) is the diester of propane-1,2,3-tricarboxylic acid [tricarballylic acid (TCA)] and 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane, in which the C-14 and C-15 hydroxyl groups are esterified with one of the terminal carboxy groups of TCA (Bezuidenhout et al., 1988). Although several other fumonisin analogues have been characterized (Bezuidenhout et al., 1988; Gelderblom et al., 1988; Cawood et al., 1991), $FB_1$ remains the most abundant in fungal cultures and in naturally contaminated corn-based foods and feeds (Ross et al., 1992; Sydenham et al., 1991, 1992a, 1993; Thiel et al., 1991b, 1992). $FB_1$ is produced by several related *Fusarium* species that are known to be fungal contaminants of corn and other human and animal dietary staples (Thiel et al., 1991a; Nelson et al., 1992) and has been shown to be hepatocarcinogenic to rats (Gelderblom et al., 1992) and to induce leukoencephalomalacia in horses (Kellerman et al., 1990) and pulmonary edema in swine (Harrison et al., 1990).

Consumption of corn-based feeds contaminated with total fumonisin levels of $\geq 10$ and $\geq 100$ $\mu$g/g has been suggested as potentially harmful to horses and swine, respectively (Marasas et al., 1993). In commercially available corn and/or corn-based human foods, total fumonisin levels have been found to range from <0.05 to 4.7 $\mu$g/g (Sydenham et al., 1991, 1993). Significantly higher fumonisin levels, of up to 140 (Rheeder et al., 1992) and 155 $\mu$g/g (Chu and Li 1994), have, however, been recorded in selected fractions of home-grown corn harvested in rural areas of the Transkei, southern Africa, and China, respectively, where high incidence rates of human esophageal cancer have been reported (Marasas et al., 1988; Chu and Li, 1994). On the basis of the existing hazard assessment data together with the fumonisin levels thus far reported in human foods and animal feeds, the International Agency for Research on Cancer (IARC) recently designated "toxins derived from *F. moniliforme*" as possibly carcinogenic to humans (Vainio et al., 1993).




[1] $R^1$ AND $R^2$ = $CO\text{-}CH_2\text{-}CH(CO_2H)\text{-}CH_2\text{-}CO_2H$
[2] $R^1$ AND $R^2$ = H

**Figure 1.** Chemical structures of [**1**] fumonisin $B_1$ and [**2**] the aminopentol moiety ($AP_1$).

Investigations have been undertaken aimed at the development of procedures for the removal of fumonisins from contaminated corn. In one potential approach, Sydenham et al. (1995) reported that significant reductions in $FB_1$ levels could be achieved following treatment of $FB_1$-contaminated corn with a saturated solution of calcium hydroxide [$Ca(OH)_2$]. Such treatment resulted in the base hydrolysis of $FB_1$ to yield its aminopentol ($AP_1$) (Figure 1) and TCA moieties, the bulk of which were recovered in the easily separable aqueous $Ca(OH)_2$ fraction (Sydenham et al., 1995). Additional studies have subsequently shown that the complete base hydrolysis of $FB_1$ proceeds in a sequential manner involving the initial removal of a single TCA group and the formation of a partially hydrolyzed moiety containing only one ester group. The isolation and identification of this compound are the subject of this paper.

## EXPERIMENTAL PROCEDURES

**1. Culture Material and Analytical Standards.** Corn-based culture material of *F. moniliforme* MRC 826 was

* Author to whom correspondence should be addressed [fax (+27)-21-938-0260; e-mail esydenha@eagle.mrc.ac.za].

† Medical Research Council.

‡ University of Cape Town.

§ Fisons Instruments VG Organic.

0021-8561/95/1443-2400$09.00/0 © 1995 American Chemical Society

**Table 1. HPLC Conditions for the Separation of $FB_1$ and Its Hydrolysis Products**

| | preliminary conditions for the separation of $FB_1$ and $AP_1$ | optimized conditions for the separation of $FB_1$, $PH_1$, and $AP_1$ |
|---|---|---|
| HPLC column | | |
| source | Phenomenex | Phenomenex |
| dimensions | 25 cm × 4.6 mm i.d. | 7.5 cm × 4.6 mm i.d. |
| packing | Ultracarb ODS 30 | Ultramex $C_{18}$ |
| particle size | 5 $\mu$m | 3 $\mu$m |
| mobile phase | | |
| MeOH | 80% | 67% |
| 0.1 M $NaH_2PO_4$ | 20% | 33% |
| pH | 3.3 (adjusted)[a] | 6.0 |
| flow rate | 1 mL/min | 1 mL/min |

[a] Adjusted by the addition of orthophosphoric acid.

prepared as previously described (Thiel et al., 1991a). $FB_1$ was isolated and characterized as described by Cawood et al. (1991).

**2. Preliminary Studies.** Corn-based culture material of *F. moniliforme* MRC 826 (10 g) was treated with 0.1 M $Ca(OH)_2$ (200 mL). The solution was stirred constantly, and 1 mL aliquots were removed hourly over a period of 9 h. The aliquots were transferred to plastic microfuge tubes and centrifuged (Beckman Microfuge) for 1 min prior to high-performance liquid chromatography (HPLC) analyses of the supernatants.

$FB_1$ (10 $\mu$g) was dissolved in a saturated solution of 0.1 M $Ca(OH)_2$ (400 $\mu$L) and incubated for a period of 5 h at 23 °C. An aliquot of the resultant solution was derivatized with *o*-phthaldialdehyde (OPA) and analyzed by HPLC using the optimized conditions detailed in Table 1.

**3. Isolation of the Partially Hydrolyzed Moiety ($PH_1$) of $FB_1$ from Corn Culture Material of *F. moniliforme*.** *(a) Initial Extraction of $PH_1$.* Thirty grams of culture material of *F. moniliforme* MRC 826 was treated with 600 mL of 0.1 M $Ca(OH)_2$. The solution was mixed slowly by magnetic stirring, for 4.5 h at 23 °C, and then centrifuged at 500*g* for 10 min. The supernatant was filtered, under vacuum, through a Whatman No. 4 filter paper and the eluate acidified to a pH value of 2.7 by the addition of 5 M hydrochloric acid.

*(b) Preliminary Purification of $PH_1$.* Amberlite XAD-2 resin, previously washed sequentially with diethyl ether (400 mL), methanol (400 mL), and distilled water (400 mL), was used to prepare a 22 cm chromatographic bed in water, in a large chromatography column (50 × 3 cm i.d.). The pH-adjusted eluate (from part *a*) was passed through the column at a flow rate of *ca.* 10 mL/min. The column was then washed with distilled water (400 mL), followed by methanol/water (25:75; 400 mL), and eluted with methanol (800 mL). The methanol eluate was collected and the solvent removed by rotary evaporation, under vacuum at 50 °C.

*(c) Silica Gel Column Chromatography.* A chromatography column (50 × 3 cm i.d.) containing Kieselgel 60 (0.063–0.2 mm diameter, Merck, Darmstadt, Germany) was prepared in chloroform/methanol/acetic acid/water (55:36:8:1, solvent 1) to give a chromatographic bed of 42 cm. The residue from the Amberlite column was dissolved in solvent 1 (10 mL) and applied to the top of the Kieselgel column. The column was subsequently eluted at a flow rate of *ca.* 10 mL/min with solvent 1, and 20 mL fractions were collected. Each fraction was screened by thin layer chromatography (TLC), and where necessary, the results were confirmed by HPLC, using the optimized conditions given in Table 1.

Fractions containing $PH_1$ (11–20) were combined and evaporated to dryness at 50 °C. The residue was dissolved in 15 mL of ethyl acetate/acetic acid/water (12:6:1, solvent 2), which was then applied to the top of a second Kieselgel 60 column (of similar dimensions to that of the first), prepared in solvent 2. The column was eluted with solvent 2 (750 mL), with 20 mL fractions being monitored for the presence of $PH_1$. However, under the prevailing conditions, $PH_1$ failed to elute (probably due to the size of the chromatography column). The eluate was therefore discarded, and the $PH_1$ was stripped from the column with solvent 1 (1000 mL) at a flow rate of *ca.* 10 mL/min, the eluate being collected and evaporated to dryness under vacuum at 50 °C.

The residue from the second Kieselgel column was redissolved in solvent 2 (5 mL) and applied to a third Kieselgel column (15 × 3 cm i.d.) prepared in solvent 2 to give a 10 cm chromatographic bed. The column was eluted with solvent 2 at a flow rate of *ca.* 5 mL/min, and fractions (15 mL) were collected and screened for $PH_1$ by TLC and HPLC. Those fractions shown to contain $PH_1$ (16–40) were combined and concentrated to dryness.

*(d) Minicolumn Purification of $PH_1$.* A short column (5 cm × 13 mm i.d.) was prepared by adding together the sorbent contents of six Bond-Elut strong anion exchange (SAX) cartridges (500 mg capacity; Varian, Harbor City, CA). The column was packed in methanol (20 mL) and washed with distilled water (40 mL). The residue from the third Kieselgel column was dissolved in methanol/water (75:25; 20 mL), and the pH of the solution was adjusted to pH 6.2 by the addition of 1 M sodium hydroxide. The pH-adjusted solution was applied to the SAX column and washed with methanol (30 mL) at a flow rate of *ca.* 2 mL/min. The eluates (including the application solution) were collected, combined, and dried by rotary evaporation.

The dried residue from the SAX minicolumn was dissolved in water (20 mL) and acidified to pH 2.9 by the addition of 3 M hydrochloric acid. This solution was applied to a second minicolumn (5 cm × 13 mm i.d.) packed with 1 g of reversed-phase $C_{18}$ end-capped (ec) material (Chromabond $C_{18}$ec, Macherey-Nagel, Düren, Germany), which had been conditioned with methanol (10 mL) followed by distilled water (10 mL). Following application, the column was washed with water (40 mL) followed by methanol/water (25:75; 20 mL). The purified $PH_1$ was eluted with methanol/water (50:50; 40 mL), which was collected and evaporated to dryness under vacuum at 50 °C. During application and elution of the minicolumn, the flow rate was maintained at *ca.* 2 mL/min.

**4. Preparation of $PH_1$ Moiety from $FB_1$.** $FB_1$ (5 mg) was dissolved in 0.1 M $Ca(OH)_2$ (40 mL) and stirred at *ca.* 23 °C for 4.5 h. The solution was then acidified to pH 6.2 with 2 M hydrochloric acid and applied to a short chromatographic column (5 cm × 13 mm) packed with 2 g of SAX media (prepared as in section 3d). The column was washed with methanol (40 mL) at a flow rate of *ca.* 2 mL/min, and the combined eluates (including the application solution) were collected and further acidified to pH 2.9. This solution was applied to a Chromabond $C_{18}$ec cartridge, which was then washed with distilled water (40 mL). The $PH_1$ and $AP_1$ moieties were eluted with methanol/water (40:60) at a flow rate of *ca.* 2 mL/min, with 2 mL fractions being collected and screened by HPLC, using the optimized conditions cited in Table 1.

**5. Thin Layer Chromatography (TLC).** During the isolation procedure, fractions collected from several chromatographic columns were screened for the presence of $PH_1$ by normal phase TLC using silica gel 60 TLC plates (Merck). The plates were developed in solvent 1 (section 3c), dried, and sprayed with a 0.5% *p*-anisaldehyde solution in methanol/acetic acid/sulfuric acid (85:10:5). The plates were then heated to 110 °C for 10 min and visually assessed.

**6. High-Performance Liquid Chromatography (HPLC).** Fractions were analyzed by HPLC as their OPA derivatives, prepared as previously described (Sydenham et al., 1992b). Separations were performed using the preliminary and optimized HPLC conditions cited in Table 1. Chromatographic peaks were detected using a Hewlett-Packard 1046A fluorescence detector, with excitation and emission wavelengths of 335 and 445 nm, respectively.

**7. Mass Spectrometry.** The mass spectra of selected (purified) fractions were recorded using a VG platform mass spectrometer (Fisons Instruments VG Organic, Altrincham, U.K.), configured for electrospray mass spectrometry. Solvated samples, dissolved in methanol/water (40:60) (approximately 200 ng per component), were introduced by flow injection, using a Pharmacia LC pump operated at 10 $\mu$L/min. To reduce component fragmentation, the mass spectra (collected over the range *m/z* 200–700) were obtained using low cone voltage (33 V) and source temperature (60 °C) settings.

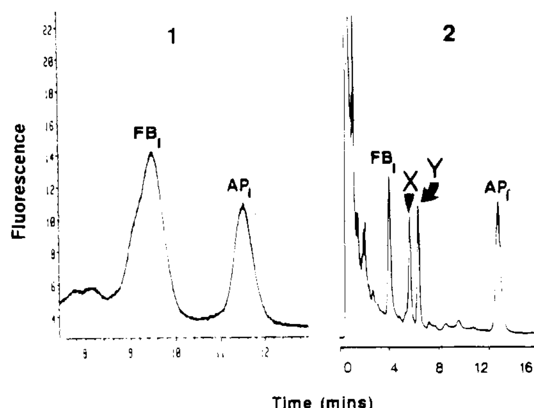



**Figure 2.** HPLC separations of the OPA derivatives of (**1**) a purified aqueous $Ca(OH)_2$ extract following the treatment of fumonisin-contaminated corn and (**2**) the same extract using optimized chromatographic parameters (Table 1) ($FB_1$ = fumonisin $B_1$; $AP_1$ = aminopentol moiety derived from $FB_1$; **X** and **Y** = unknown compounds).

## RESULTS AND DISCUSSION

Sydenham et al. (1995) reported that, under certain conditions, treatment of $FB_1$-contaminated corn with a saturated solution of $Ca(OH)_2$ resulted in the transfer of the majority of $FB_1$ from the solid matrix to the easily separable aqueous $Ca(OH)_2$ phase. The $FB_1$ recovered in the aqueous phase was present predominantly as the fully hydrolyzed $AP_1$ moiety. The HPLC chromatograms obtained for the aqueous $Ca(OH)_2$ fractions following treatment, using the preliminary HPLC conditions in Table 1, indicated that the peak corresponding to $FB_1$ was not symmetrical, suggesting the likely presence of a coeluting component (Figure 2.1). Optimization of the chromatographic conditions (Table 1) followed by reanalysis of the fraction illustrated that the peak previously identified as $FB_1$ could be resolved into three peaks, two of which were unknown and which were subsequently designated compounds **X** and **Y**, respectively (Figure 2.2). Similar peaks were observed for a $FB_1$ standard, incubated in a 0.1 M (saturated) solution of $Ca(OH)_2$ (data not shown).

The progression of the hydrolysis of $FB_1$ in culture material of *F. moniliforme*, incubated at room temperature in 0.1 M $Ca(OH)_2$, was monitored by HPLC using chromatographic conditions similar to those used for Figure 2.2. Following an incubation period of 1 h, it was possible to observe the presence of two minor chromatographic peaks eluting between 6 and 8 min (which corresponded to those compounds designated **X** and **Y**, Figure 2.2) and a smaller peak with a retention time (rt) similar to that observed for the $AP_1$ moiety. Following an incubation period of between 4 and 5 h, the peak areas (and presumably concentrations) of compounds **X** and **Y** increased with a corresponding decrease in the area of the $FB_1$ peak, to such an extent that they were larger than the peak areas of either the $FB_1$ or $AP_1$ moieties. Thereafter, an increase in the area of the peak corresponding to $AP_1$ was accompanied by ever decreasing areas for the two peaks corresponding to compounds **X** and **Y**. The results suggested that these latter compounds may be related intermediate hydrolysis products formed during the base hydrolysis of $FB_1$.

In a subsequent study, compounds **X** and **Y** were coisolated and purified from culture material of *F. moniliforme*, incubated in the presence of 0.1 M $Ca(OH)_2$. During the isolation, the presence of the two compounds was monitored primarily by TLC, where they were observed as independent brown/purple spots. The resultant purified extract (a combination of compounds **X** and **Y**) was subjected to electrospray mass spectrometry (Figure 3). A single strong protonated molecular ion $[MH]^+$ was observed at *m/z* 564.3, which corresponds to a molecular formula of $C_{28}H_{53}NO_{10}$. The data could be interpreted as being representative of a fumonisin-like compound in which $FB_1$ has lost one of its two TCA groups, suggesting that the mixture (**X** and **Y**) consists of the partial hydrolysis products ($PH_1$) of $FB_1$. Ions at *m/z* 586.3 and 602.3 correspond to $[MNa]^+$ and $[MK]^+$, respectively.

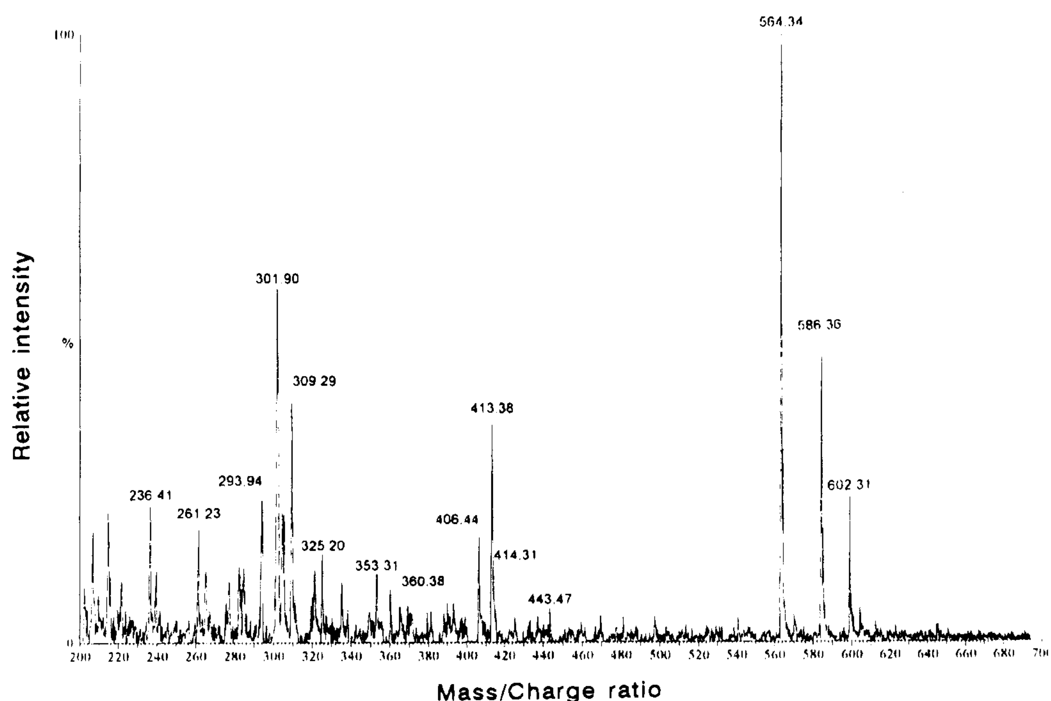



**Figure 3.** Continuum electrospray mass spectrum of the mixture of compounds **X** and **Y**, isolated and purified from $Ca(OH)_2$-treated culture material of *F. moniliforme*.

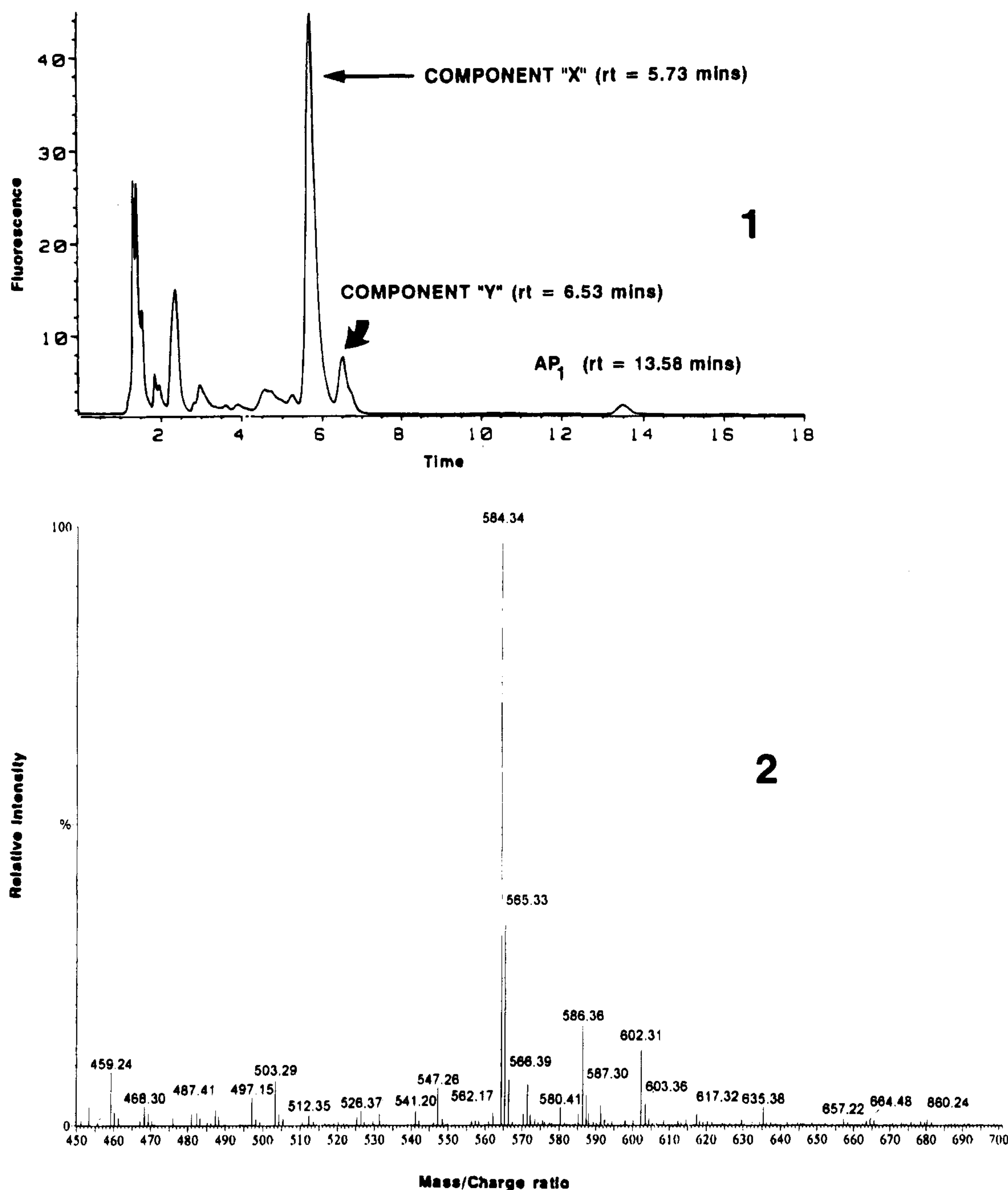



**Figure 4.** (1) HPLC chromatogram of a fraction primarily containing compound **X**, as its OPA derivative and (2) the partial centroid electrospray mass spectrum of the same fraction ($AP_1$ = aminopentol moiety derived from fumonisin $B_1$).

Other major ions present in the mass spectrum included *m/z* 301.9, 309.3, 406.4, and 413.4 (Figure 3). Ion *m/z* 406.4 would correspond to a molecular formula of $C_{22}H_{47}NO_{15}$, consistent with the loss of both TCA side chain moieties present in $FB_1$, to yield the $AP_1$ moiety. However, the low cone voltage and source temperature settings, used for the generation of mass spectra, were selected to reduce possible fragmentation. Therefore, the presence of the *m/z* 406.4 ion can not be positively ascribed to the fragmentation of $PH_1$. Consideration of the structures of $FB_1$, $AP_1$, and $PH_1$ did not result in the identification of possible fragments that could fully explain the presence of the other major ions. However, ions at *m/z* 301.9 and 413.4 were subsequently found to be contaminants, present in an aqueous solution of the $Ca(OH)_2$ used for the chemical treatment of the culture material. Figure 3 illustrates the electrospray mass spectrum obtained for a mixture of the two compounds **X** and **Y**. Conclusive proof of the identity of these compounds was sought by treating pure $FB_1$ with $Ca(OH)_2$, followed by isolation and identification of the individual compounds **X** and **Y**. Figure 4.1 shows the HPLC chromatogram of a fraction collected during the reversed-phase isolation of $Ca(OH)_2$-treated $FB_1$, as its OPA derivative. The major peak, corresponding to component **X**, eluted at a rt of 5.73 min. A smaller peak, corresponding to component **Y**, eluted at a rt of 6.53 min together with a minor peak, corresponding to $AP_1$, eluting at 13.58 min (Figure 4.1). The partial mass spectrum (from *m/z* 450 to 700) obtained for this fraction, corresponding to component **X**, is illustrated in Figure 4.2. The protonated molecular ion was observed at *m/z* 564.3, which confirmed that component **X** was the partially hydrolyzed moiety of $FB_1$. The $[MNa]^+$ and $[MK]^+$ ions may also be seen at *m/z* 586.3 and 602.3, respectively. An identical series of ions were recorded in the electrospray mass spectrum of a fraction containing component **Y** (results not shown).

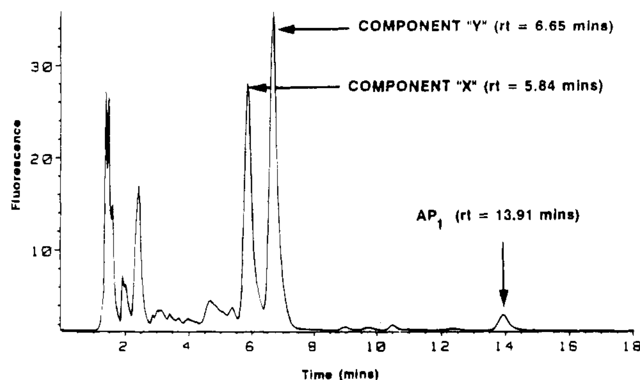



**Figure 5.** HPLC chromatogram of the OPA derivative of the purified partially hydrolyzed ($PH_1$) moiety shown in Figure 4.1, following incubation at 70 °C for 3 h.

An aqueous solution of the fraction corresponding to component **X**, shown in Figure 4.1, was subsequently subjected to incubation at 70 °C for 3 h. An HPLC chromatogram of the OPA-derivatized fraction is shown in Figure 5. Comparison of the HPLC chromatograms shown in Figures 4.1 and 5 clearly shows that the predominant peak observed in the former (rt = 5.73 min) was replaced by two peaks (rt = 5.84 and 6.65 min, respectively), while the small peak eluting at 13.91 min, corresponding to $AP_1$, was also present (Figure 5). The size and area of this latter peak were similar to that previously recorded in the fraction prior to incubation (Figure 4.1). These results indicated that incubation of component **X**, at an elevated temperature, resulted in the intramolecular *trans*-esterification of the monoester to a mixture of two forms (one the C-14 ester and the other the C-15 ester) of the 1,2-diol system but did not undergo further hydrolysis to $AP_1$. The process is analogous to the isomerization of the monoesters of glycerol, in which the acid moiety is transferred by an intramolecular mechanism to a vicinal hydroxyl group (Finar, 1967). Additional incubation of the fraction did not induce further chromatographic changes, suggesting that the mixture had attained equilibrium. The relative ratio of the two monoester forms illustrated in Figure 5 was identical to that observed for the original residue, isolated and purified from the culture material of *F. moniliforme*, the electrospray mass spectrum of which is shown in Figure 3.

Shephard et al. (1994) isolated $PH_1$ from the feces of a nonhuman primate that had been exposed to $FB_1$. Their studies indicated that $PH_1$ was an equilibrium mixture of two structural isomers of partially hydrolyzed $FB_1$. They concluded that, on the basis of $^{13}C$ nuclear magnetic resonance spectroscopy data, the $PH_1$ moiety showed two sets of signals in a ratio of 55:45 in favor of the ester formation with the C-14 hydroxy group (Shephard et al., 1994). The (partial) electrospray mass spectrum of the $PH_1$ isolated from the feces of the nonhuman primate was shown to be identical to that isolated from the $Ca(OH)_2$ treatment of $FB_1$ (Figure 4.2).

None of the electrospray mass spectral data generated for the partially hydrolyzed moieties of $FB_1$ indicated the presence of a calcium complex. The formation of a $FB_1$–calcium complex had been considered as a possible explanation for the catalytic influence that calcium appeared to exert on the base hydrolysis of $FB_1$ (Sydenham et al., 1995). It is entirely possible that the calcium ion may complex with, for instance, the TCA moiety of $FB_1$. Further investigations into the role of $Ca(OH)_2$ in the hydrolysis of $FB_1$ and the possible formation of a calcium–TCA complex are currently in progress.

In conclusion, this paper details a method for the qualitative preparation and isolation of the partially hydrolyzed moiety of $FB_1$. Given the fact that this moiety has been isolated from the feces of a $FB_1$-treated nonhuman primate (Shephard et al., 1994), and in extracts derived from the $Ca(OH)_2$ treatment of fumonisin-contaminated corn, the provision of an authentic $PH_1$ standard should be useful for future metabolic and other studies.

## LITERATURE CITED


Bezuidenhout, G. 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.; Thiel, P. G.; Marasas, W. F. O. Isolation of the fumonisin mycotoxins: a quantitative approach. *J. Agric. Food Chem.* **1991**, *39*, 1958–1962.

Chu, F. S.; Li, G. Y. Simultaneous occurrence of fumonisin $B_1$, and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Appl. Environ. Microbiol.* **1994**, *60*, 847–852.

Finar, I. L. In *Organic Chemistry*, 5th ed.; Longmans: London, 1967; Vol. 1, p 290.

Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. J. Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806–1811.

Gelderblom, W. C. A.; Marasas, W. F. O.; Vleggaar, R.; Thiel, P. G.; Cawood, M. E. Fumonisins: isolation, chemical characterization and biological effects. *Mycopathologia* **1992**, *117*, 11–16.

Harrison, L. R.; Colvin, B. M.; Greene, J. T.; Newman, L. E.; Cole, J. R., Jr. Pulmonary edema and hydrothorax in swine produced by fumonisin $B_1$, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* **1990**, *2*, 217–221.

Kellerman, T. S.; Marasas, W. F. O.; Thiel, P. G.; Gelderblom, W. C. A.; Cawood, M. E.; Coetzer, J. A. W. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin $B_1$. *Onderstepoort J. Vet. Res.* **1990**, *57*, 269–275.

Marasas, W. F. O.; Jaskiewicz, K.; Venter, F. S.; Van Schalkwyk, D. J. *Fusarium moniliforme* contamination of maize in oesophageal cancer areas in Transkei. *S. Afr. Med. J.* **1988**, *74*, 110–114.

Marasas, W. F. O.; Shephard, G. S.; Sydenham, E. W.; Thiel, P. G. World-wide contamination of maize with fumonisins: food-borne carcinogens produced by *Fusarium moniliforme*. In *Cereal Science and Technology —Impact on a Changing South Africa*; Taylor, J. R. N., Randall, P. G., Viljoen, J. H., Eds.; CSIR: Pretoria, 1993; pp 791–805.

Nelson, P. E.; Plattner, R. D.; Shackelford, D. D.; Desjardins, A. E. Fumonisin $B_1$ production by *Fusarium* species other than *F. moniliforme* in section Liseola and some related species. *Appl. Environ. Microbiol.* **1992**, *58*, 984–989.

Rheeder, J. P.; Marasas, W. F. O.; Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; Van Schalkwyk, D. J. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* **1992**, *82*, 353–357.

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* species. *Mycopathologia* **1992**, *117*, 109–114.

Shephard, G. S.; Thiel, P. G.; Sydenham, E. W.; Vleggaar, R.; Alberts, J. F. Determination of the mycotoxin fumonisin $B_1$ and identification of its partially hydrolysed metabolites in the faeces of non-human primates. *Food Chem. Toxicol.* **1994**, *32*, 23–29.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G.; Marasas, W. F. O.; Stockenström, S. Fumonisin contamination of commercial corn-based human foodstuffs. *J. Agric. Food Chem.* **1991**, *39*, 2014–2018.

Sydenham, E. W.; Marasas, W. F. O.; Shephard, G. S.; Thiel, P. G.; Hirooka, E. Y. Fumonisin contamination of Brazilian feeds associated with field outbreaks of confirmed and suspected animal mycotoxicoses. *J. Agric. Food Chem.* **1992a**, *40*, 994–997.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G. Liquid chromatographic determination of fumonisins $B_1$, $B_2$, and $B_3$ in foods and feeds. *J. AOAC Int.* **1992b**, *75*, 313–318.

Sydenham, E. W.; Shephard, G. S.; Gelderblom, W. C. A.; Thiel, P. G.; Marasas, W. F. O. Fumonisins: their implications for human and animal health. In *Proceedings of the UK Workshop on the Occurrence and Significance of Mycotoxins*; Scudamore, K. A., Ed.; MAFF: London, 1993; pp 42–48.

Sydenham, E. W.; Stockenström, S.; Thiel, P. G.; Shephard, G. S.; Koch, K. R.; Marasas, W. F. O. The potential of alkaline hydrolysis for the removal of fumonisins from contaminated corn. *J. Agric. Food Chem.* **1995**, *43*, 1198–1201.

Thiel, P. G.; Marasas, W. F. O.; Sydenham, E. W.; Shephard, G. S.; Gelderblom, W. C. A.; Nieuwenhuis, J. J. Survey of fumonisin production by *Fusarium* species. *Appl. Environ. Microbiol.* **1991a**, *57*, 1089–1093.

Thiel, P. G.; Shephard, G. S.; Sydenham, E. W.; Marasas, W. F. O.; Nelson, P. E.; Wilson, T. M. Levels of fumonisins $B_1$ and $B_2$ in feeds associated with confirmed cases of equine leukoencephalomalacia. *J. Agric. Food Chem.* **1991b**, *39*, 109–111.

Thiel, P. G.; Marasas, W. F. O.; Sydenham, E. W.; Shephard, G. S.; Gelderblom, W. C. A. The implications of naturally occurring levels of fumonisins in corn for human and animal health. *Mycopathologia* **1992**, *117*, 3–9.

Vainio, H.; Heseltine, E.; Wilbourn, J. Report on an IARC working group meeting on some naturally occurring substances. *Int. J. Cancer* **1993**, *53*, 535–537.

Received for review October 12, 1994. Revised manuscript received May 31, 1995. Accepted June 19, 1995.[⊗]

JF9405719

[⊗] Abstract published in *Advance ACS Abstracts*, August 1, 1995.