Formation of *N*-(Carboxymethyl)fumonisin B_1, Following the Reaction of Fumonisin B_1 with Reducing Sugars

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The fumonisins are mycotoxins produced by fungi that contaminate primarily corn and are toxic through interruption of intracellular sphingolipid synthesis. Several reports have indicated that fumonisin B_1 concentrations decreased when heated in aqueous solutions of reducing sugars. The incubation of fumonisin B_1 with D-glucose resulted in the formation of *N*-(carboxymethyl)fumonisin B_1, which was characterized by NMR and electrospray mass spectroscopy. We determined the methylene carbon of the carboxymethyl group is derived from C1 on glucose, while the carbonyl carbon is derived from the C2 of glucose, using ^{13}C glucose. Apparently *N*-(carboxymethyl)fumonisin B_1 arises from Schiff's base formation, Amadori rearrangement to a β-ketoamine, and oxidation with molecular oxygen. *N*-(Carboxymethyl)fumonisin B_1 formation is favored by alkaline conditions (pH >7), requires molecular oxygen, and is catalyzed by several reducing sugars. *N*-(carboxymethyl)-fumonisin B_1 was detected in raw corn samples that contained fumonisin B_1 (0.5−1.4 ppm) at an average of 4% of the fumonisin B_1 levels.

Keywords: *Fumonisin B_1; fumonisins; N-(carboxymethyl)fumonisin B_1; detoxification; Amadori rearrangement; Maillard reaction; reducing sugars; mycotoxin*

INTRODUCTION

The fumonisins are a group of toxins produced by fungi primarily of the *Fusarium* genus, of which fumonisin B_1 (see Figure 1) is the most prevalent (Nelson et al., 1991, 1992; Thiel et al., 1991; Desjardins et al., 1994). These fungal toxins contaminate primarily corn worldwide (Nelson et al., 1991; Chulze et al., 1994; Shelby et al., 1994). Although the consumption of foods containing high levels of *Fusarium* contamination has been associated with an increased frequency of esophageal cancer (Sydenham et al., 1991; Rheeder et al., 1992), the participation of fumonisin B_1 as the etiological agent remains to be established.

Fumonisin B_1 is toxic to several species of animals. The ingestion of purified fumonisin B_1 or fumonisin B_1-contaminated feed induces equine leukoencephalomalacia (Butler, 1902; Kriek et al., 1981; Marasas et al., 1988; Kellerman et al., 1990; Wilson et al., 1992; Ross et al., 1994). Fumonisin B_1 induces fatal pulmonary edema in pigs (Harrison et al., 1990; Osweiler et al., 1992; Haschek et al., 1992, 1995), is hepatotoxic and nephrotoxic in rats and mice (Kriek et al., 1981; Harrison et al., 1990; Osweiler et al., 1992; Casteel et al., 1994), and is a tumor promoter in rats following initiation with alkylating agents (Gelderblom et al., 1992, 1994, 1995). For this reason, this compound is currently being tested for carcinogenicity under the National Toxicology Program.

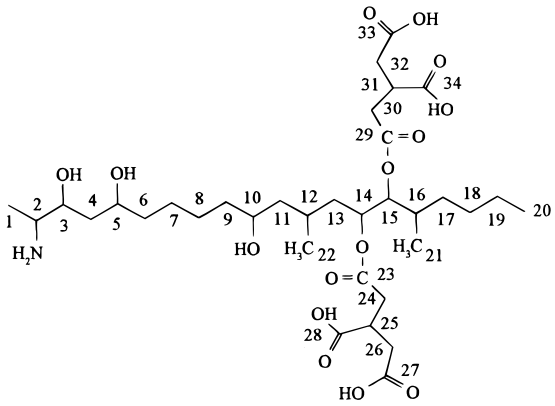

Figure 1. Structure of fumonisin B_1.

The mechanism of action of fumonisin B_1 is interruption of sphingolipid synthesis. Inhibition of ceramide synthase by fumonisin B_1 causes increased sphinganine levels and decreased ceramide and complex sphingolipid levels (Wang et al., 1991, 1992; Yoo et al., 1992). This interruption of sphingolipid synthesis results in apoptosis in the liver and kidney in rodents (Tolleson et al., 1996a; Bucci and Howard, 1996), and in human cells (Tolleson et al., 1996a,b, 1998). The increase in apoptosis in rodent livers and kidneys is accompanied by an increase in compensatory cell proliferation (Howard et al., 1996), probably explaining the tumor promoter properties of fumonisin B_1.

The contamination of corn with fumonisin B_1 in this country represents a possible health concern. Therefore, understanding the stability of fumonisin B_1 in foods is critical in estimating the extent of potential human exposure. Fumonisin B_1 is stable when exposed to 75 °C for 135 min or 125 °C for 5 min (Dupuy et al.,1993).

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Our own data (unpublished) have shown that fumonisin B_1 levels in rodent feed do not change as a result of autoclaving; however, Jackson et al. (1997) have shown that up to 43% of the fumonisin B_1 present in spiked corn meal is lost after baking at 175−200 °C for 20 min. In the same study, fumonisin B_1 content was decreased in a time and temperature dependent manner following the frying of corn to corn chips in soybean oil (Jackson et al., 1997). Fumonisin B_1 is hydrolyzed by strongly alkaline conditions to form the deesterified fumonisin B_1 backbone product, referred to as aminopentol. The fumonisins are hydrolyzed to the aminopentols during nixtamalization, the process of alkaline (calcium hydroxide) treatment of corn meal in tortilla preparation (Hendrich et al., 1993). Hydrolysis of the fumonisins, however, does not occur with 2% ammoniation of corn for 4 days (Norred et al., 1991), a process which is frequently used to detoxify aflatoxin-contaminated corn.

It has been reported that fumonisin B_1 reacts with reducing sugars to form a stable Schiff's base (Murphy et al., 1995; Lu et al., 1997). We sought to identify the product of the reaction between fumonisin B_1 and reducing sugars. We report the formation of *N*-(carboxymethyl)fumonisin B_1 as the principal reaction product following the heating of fumonisin B_1 with reducing sugars, and the presence of this fumonisin B_1 derivative in raw corn.

MATERIALS AND METHODS

Chemicals. Fumonisin B_1 is classified as a class 2B carcinogen (International Agency for Research on Cancer, 1993). Appropriate caution should be taken in the handling of fumonisin B_1 or any of its derivatives.

D-Glucose was from Gibco-BRL (Grand Island, NY). D-Galactose, D-fructose, sucrose, D-trehalose, maltose, sedoheptulose anhydride, D-xylulose, D-ribose, 2-deoxy-D-ribose, D-ribulose, bis-Tris, bicine, HEPES, ammonium acetate, sodium potassium tartrate, cupric sulfate, and ammonium formate were from Sigma Chemical Co. (St. Louis, MO). [1,2,3,4,5,6,6-$^2H_7$]D-glucose (98 atom %) and [U-$^{13}C_6$]D-glucose (99+ atom %), were from Cambridge Isotope Laboratories (Andover, MA). Formic acid, [1-^{13}C]D-glucose (99 atom %), and [2-^{13}C]D-glucose (99 atom %) were from Aldrich Chem. Co. (Milwaukee, WI). ^2H_2O was from MSD Isotopes (Montreal, Canada). HPLC grade methanol and acetonitrile were from J. T. Baker (Phillipsburg, NJ). *myo*-Inositol was from Calbiochem (La Jolla, CA).

Fumonisin B_1 was isolated from cultures of *Fusarium proliferatum*, was purified as the ammonium salt, and was provided by R. Epply (Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC).

Incubation of Fumonisin B_1 with Sugars. Incubations of fumonisin B_1 with sugars were conducted in 13 × 100 mm borosilicate culture tubes (Fisher Chemical Co.) capped with a tight stopper (Milan Instruments, Columbus, OH). Unless otherwise specified, the incubations contained 50 mM potassium phosphate, pH 7.5, 20 mM sugar, and 0.25 mM fumonisin B_1 in a total volume of 1 mL. The tubes were incubated at 74−78 °C in a block heater (Fisher Scientific, Pittsburgh, PA) overnight (14−18 h). Incubations at 34, 50, and 94 °C were conducted in water baths. All reactions were stored at 2−4 °C until analyzed.

Detection of Fumonisin B_1 Derivatives. Fumonisin B_1 and other compounds were separated using reversed-phase high-pressure liquid chromatography (HPLC) and detected using an evaporative light-scattering detector. Up to 25 μL aliquots of the reaction mixtures were injected onto a Varian 5000 HPLC (Varian Instruments, Walnut Creek, CA) using a Rheodyne 7125 HPLC injector (Rheodyne Inc., Cotati, CA). A Phenomenex UltraCarb ODS-30 5 μm HPLC column (4.6 × 150 mm; Phenomenex, Torrance, CA) was the stationary phase. The mobile phase was delivered at 1 mL/min and consisted of the following gradient: 0−12 min, 26% acetonitrile in 25 mM formic acid; 12−20 min, linear change to 70% acetonitrile in 25 mM formic acid; 20−30 min, 70% acetonitrile in 25 mM formic acid. The detector was an evaporative light-scattering detector (Sedex 55, SEDERE, Alfortville, France) with nitrogen (99+% pure, 1.9 bar pressure) as the nebulizer gas at 41 °C. Fumonisin B_1 and derivatives were quantified on the basis of peak height.

Classification of Reducing Sugars. The sugars were classified as reducing or nonreducing on the basis of the ability to reduce Fehling's solution (Atack, 1957), which consists of 280 mM $CuSO_4$ mixed with an equal volume of 1.23 M sodium potassium tartrate and 3.33 M NaOH. Approximately 50 mg of sugar in 1 mL of water was added to a 13 × 100 mm test tube containing 250 μL of Fehling's solution, and the solution was heated to 70 °C for 5 min. The sugars were qualitatively evaluated on their ability to produce a positive Fehling's test (red/orange cuprous oxide precipitate) and graded as nonreducing (−, blue solution), marginally reducing (±, slight orange solution), mildly reducing (+, red/orange solution), or strongly reducing (++, considerable red/orange precipitate).

Preparation and Isolation of *N*-(Carboxymethyl)fumonisin B1 for Spectral Analysis. *N*-(Carboxymethyl)-fumonisin B_1 was generated at 78 °C in 20 mL volumes containing 2 mM fumonisin B_1, 50 mM potassium phosphate, pH 7.5, and 150 mM of either D-glucose, [1,2,3,4,5,6,6-$^2H_7$]D-glucose, [U-$^{13}C_6$]D-glucose, [1-^{13}C]D-glucose, or [2-^{13}C]D-glucose. The conversion of fumonisin B_1 to *N*-(carboxymethyl)fumonisin B_1 was followed by HPLC/light-scattering detection until either the fumonisin B_1 was depleted or the decrease in fumonisin B_1 had ceased.

The solutions containing *N*-(carboxymethyl)fumonisin B_1 were adjusted to between pH 3 and pH 4 using 500 mM HCOOH, the pH verified with pH indicator sticks (ColorpHast, MC/B Manufacturing Chemists, Inc., Cincinnati, OH), and 3 mL was added to an Oasis cartridge (60 mg column, Waters Associates, Medford, MA). The cartridge had been prewashed with 3 mL of methanol and equilibrated with 6 mL of 25 mM HCOOH. Following the addition of the sample, the cartridge was washed with 2 mL of 25 mM HCOOH, and fumonisin B_1 was eluted with 2 mL of acetonitrile:25 mM HCOOH (1:3). *N*-(Carboxymethyl)fumonisin B_1 was eluted with 2 mL of acetonitrile:25 mM HCOOH (1:1) and dried in vacuo. The *N*-(carboxymethyl)fumonisin B_1 from each Oasis column was dissolved in a minimal amount of water, combined, dried in vacuo, and stored at 2−4 °C until analyzed.

Spectroscopic Methods. A Platform II single quadrupole mass spectrometer (Micromass, Altrincham, U.K.) equipped with an atmospheric pressure ionization (API) source and electrospray interface (ES) was used for the mass spectrometry analyses (ES-MS). The LC column effluent was diverted from the mass spectrometer for the first 8 min, then split 1:4, and delivered into the ion source (150 °C) through the ES capillary (3.3 kV) using nitrogen as the nebulizing and drying gas. Positive ions were acquired in either full scan (*m/z* range of 100−1000) or selected-ion monitoring mode (dwell time 0.3 s, span 0.02 u, and interchannel delay time 0.03 s). In full-scan studies the sampling cone-skimmer potential was varied between 20 and 70 V to produce in-source collision induced dissociation (CID). Since the injected samples contained phosphate salts, the HPLC effluent during the first 8 min of each run was diverted from the ES interface. The spectra were processed using MassLynx software (Micromass).

NMR spectra were obtained using a Bruker AM 500 Spectrometer (Bruker Instruments, Billerica, MA). *N*-(Carboxymethyl)fumonisin B_1 (≈10 mg) was dissolved in approximately 1 mL of ^2H_2O, centrifuged at 800*g* for 10 min, and the solution transferred to a 5 mm NMR tube. Proton spectra were obtained at 500.13 MHz, and the chemical shifts were established on the basis of the residual $^1H^2HO$ resonance.

The *N*-(carboxymethyl)fumonisin B_1 samples generated from the incubation of 250 μM fumonisin B_1 with 20 mM of either [U-$^{13}C_6$]D-glucose, [1-^{13}C]D-glucose, or [2-^{13}C]D-glucose were

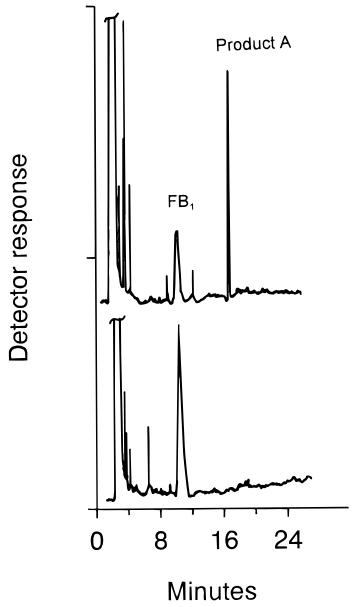

Figure 2. HPLC detection of fumonisin B_1 and products. HPLC analysis of the incubation of fumonisin B_1 with (upper trace) or without (lower trace) D-glucose. Fumonisin B_1 (250 μM) was incubated overnight at 78 °C with 20 mM D-glucose in 50 mM potassium phosphate buffer, pH 7.5. A 25 μL aliquot was injected onto a Phenomenex UltraCarb ODS 5 μm HPLC column (4.3 × 150 mm) and eluted at 1 mL/min with a mobile phase consisting of the following: 0−12 min, 26% acetonitrile in 25 mM formic acid; 12−20 min, linear change to 70% acetonitrile in 25 mM formic acid; 20−32 min, 70% acetonitrile in 25 mM formic acid. The compounds were detected using an evaporative light-scattering detector with 1.9 bar nitrogen as the nebulizer gas at 41 °C. FB_1 refers to fumonisin B_1 and product A to the compound eluting from the HPLC column at 16.5 min.

dissolved in approximately 600 μL of 1:9 ^2H_2O:H_2O, centrifuged at 800*g* for 10 min, and transferred to 5 mm NMR tubes.

Detection of *N*-(Carboxymethyl)fumonisin B_1 in Corn. Whole kernel corn was obtained from four different sources and ground to ≈150 μm using a commercial coffee grinder (Glen Mills S500, Glen Mills, NY). Triplicate 2 g samples of the corn were extracted with 10 mL of acetonitrile:25 mM HCOOH (1:1) for 1 h in 20 mL glass vials using continuous rotation (60 rpm, GlasCol RD-50 rotator, Terre Haute, IN). After standing for 30 min, 6 mL of the extract was clarified by centrifugation at 1000*g* for 10 min and the clear extract dried to approximately 3 mL in vacuo. The solution was vigorously mixed following the addition of 2 mL of $CHCl_3$, and the phases were separated by centrifugation at 1000*g* for 10 min. The aqueous layer (3 mL) was removed and added to a 60 mg Oasis cartridge that had been prewashed with 3 mL of methanol and equilibrated with 6 mL of 25 mM HCOOH. The column was washed with 4 mL of 25 mM HCOOH and eluted with 2 mL of 1:3 and 2 mL of 1:1 acetonitrile:25 mM HCOOH. These latter two eluates were combined and dried in vacuo for HPLC/ES-MS analysis of fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1.

RESULTS

Detection and Mass Spectrometric Characterization of *N*-(Carboxymethyl)fumonisin B_1. Fumonisin B_1 eluted from an UltraCarb ODS column at approximately 11 min when the mobile phase was the acetonitrile and formic acid gradient described in Materials and Methods. Incubation at 78 °C overnight at pH 7.5 did not result in any loss of fumonisin B_1 (Figure 2, lower trace). Since an evaporative light-scattering detector was used, salts and buffers were detected,

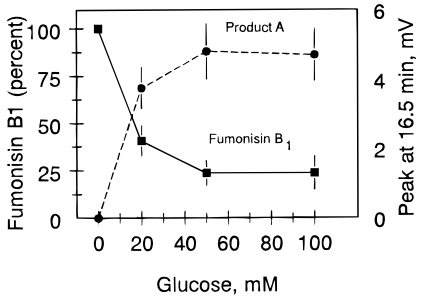

Figure 3. Effect of D-glucose on loss of fumonisin B_1. The loss of fumonisin B_1 and appearance of product A [*N*-(carboxymethyl)fumonisin B_1] following incubation with D-glucose at 78 °C. Fumonisin B_1 (250 μM) was incubated overnight at 78 °C in 50 mM potassium phosphate, pH 7.5, with 0, 20, 50, or 100 mM D-glucose. Fumonisin B_1 and product A (Figure 2) were quantified using HPLC with evaporative light-scattering detection. The fumonisin B_1 is presented as the peak height relative to the fumonisin B_1 incubated without glucose. The amount of *N*-(carboxymethyl)fumonisin B_1 is presented as the peak height in millivolts. The data are presented as the mean and range of duplicate analyses in a representative experiment.

explaining the additional peaks in the chromatogram between 1.5 and 8 min (Figure 2). The overnight incubation of 250 μM fumonisin B_1 with 20 mM glucose at pH 7.5 and 78 °C resulted in a decrease in the fumonisin B_1 peak (Figure 2, upper trace) and the appearance of several new peaks between 3 and 4 min (identity undetermined) and one peak at 16.5 min (product A, Figure 2). The decrease in fumonisin B_1 was dependent on the concentration of the glucose, as shown in Figure 3. This loss of fumonisin B_1 was accompanied by a concomitant increase in product A. Increasing the glucose concentration above 50 mM (glucose:fumonisin B_1, 200:1) did not result in further decreases in fumonisin B_1 or increases in product A.

Structural information on the peaks eluting from the UltraCarb ODS column (Figure 2) was acquired using ES-MS. The compound eluting at 11 min had a retention time identical to authentic fumonisin B_1, a molecular mass of 721 Da (M + H, m/z = 722), and a fragmentation pattern consistent with fumonisin B_1 (Figure 4, upper two panels). The fragmentation of fumonisin B_1 by CID includes the loss of three water molecules to form three ions with m/z 704.4, 686.5, and 668.4, as expected from the presence of the hydroxyl groups at C3, C5, and C10 (Figure 4, Table 1). In addition, the loss of one of the tricarballylic acid groups ($C_6H_8O_6$, 176 Da) from the parent ion and each of the dehydrated fragment ions results in the four fragments at m/z 546.4, 528.4, 510.4, and 492.4. Similarly, the loss of both tricarballylic acid groups ($C_{12}H_{16}O_{12}$, 352 Da) results in the formation of four fragment ions at m/z 370.5, 352.4, 334.4, and 316.3.

Product A had a molecular weight of 779 Da (M + H, m/z = 780.3) when analyzed with a sample cone-skimmer voltage of 20 V (Figure 4, Table 1). Increasing the cone-skimmer voltage to 70 V results in further CID fragmentation of the molecule, and detection of monosodium (m/z 802.3, M + H + Na) and monopotassium (m/z 818.2, M + H + K) adducts. As expected from a fumonisin B_1 derivative, product A underwent three successive water losses to m/z 762.4, 744.4, and 726.4. This indicated the hydroxyl groups at C3, C5, or C10 remained intact upon reaction with D-glucose. Furthermore, the observed losses of one (m/z 604.3, 586.4, 568.2, and 550.2) and two (m/z 428.4, 410.3, 392.3, and 374.3)

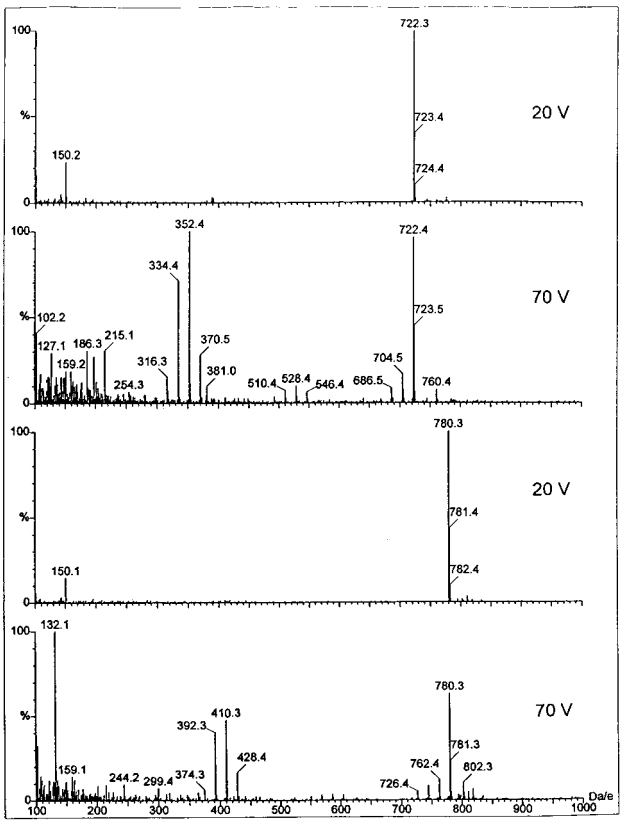

Figure 4. Mass spectral analysis of *N*-(carboxymethyl)-fumonisin B_1. Fumonisin B_1 (250 μM) and 20 mM D-glucose were incubated overnight at 78 °C in 50 mM potassium phosphate, pH 7.5. The incubate was subjected to HPLC separation on an UltraCarb ODS column and analyzed by electrospray-mass spectrometry with a scanning range of *m/z* 100−1000. The mass fragmentation patterns (sampling cone-skimmer potentials at 20 or 70 V) are presented for fumonisin B_1 (upper two panels) and product A of Figure 2 (lower two panels).

tricarballylic acid groups from the parent ion and each of the three dehydrated fragment ions implied that substitution had not occurred on a carboxyl group (Figure 4 and Table 1). As a result, the primary amino group at C2 was deduced to be the site of substitution.

The increased mass of product A was consistent with the addition of $C_2H_3O_2$ (59 Da), presumably a $-CH_2$-COOH group, to yield *N*-(carboxymethyl)fumonisin B_1. The presence of an intense fragment at *m/z* 132.1 (C_5H_9-NO_3; Figure 4, lower panel), presumably arising from cleavage of the C3−C4 bond to yield a stabilized N-substituted ion, further substantiates this interpretation. However, it was not clear how many carbon and hydrogen atoms from the glucose were incorporated into the new compound. Therefore, fumonisin B_1 was incubated with [$^2H_7$]D-glucose or [$^{13}C_6$]D-glucose, as described for D-glucose. Additionally, a similar incubation was conducted with [$^2H_7$]D-glucose, using ^2H_2O as the solvent. The results of the HPLC separation and evaporative light-scattering analyses of these incubations are shown in Figure 5. The incubation of fumonisin B_1 with D-glucose (Figure 5a) and [$^{13}C_6$]D-glucose (Figure 5b) resulted in essentially equivalent HPLC profiles. The incubation of fumonisin B_1 with [$^2H_7$]D-glucose resulted in a substantial decrease in the extent of the reaction (Figure 5c). This trend was even more pronounced when fumonisin B_1 was incubated with [$^2H_7$]-D-glucose in ^2H_2O, where product A was not detected

Table 1. Mass Spectrometric Analysis of *N*-(Carboxymethyl)fumonisin B_1 Generated from the Incubation of Fumonisin B_1 with D-Glucose, [^{13}C]D-Glucose, or [^{2}H]D-Glucose

	(M + H) water loss				(M + H − tricarballylic acid) water loss				[M + H − 2(tricarballylic acids)] water loss				
compound	0	×1	×2	×3	0	×1	×2	×3	0	×1	×2	×3	$C_5H_{10}NO_3$[a]
fumonisin B_1	722.4	704.4	686.5	668.4	546.4	528.4	510.4	492.4	370.5	352.4	334.4	316.3	*b*
N-(carboxymethyl)fumonisin B_1 from D-glucose	780.4	762.4	744.4	726.4	604.3	586.4	568.2	550.2	428.4	410.4	392.4	374.4	132.1
N-(carboxymethyl)fumonisin B_1 from [U-^{13}C]glucose	782.4	764.4	746.4	728.4	606.4	588.4	570.4	552.4	430.4	412.4	394.4	376.4	134.2
N-(carboxymethyl)fumonisin B_1 from [$^2H_7$]glucose	781.4	763.4	745.4	727.4	605.4	587.4	569.4	551.4	429.4	411.4	693.4	375.4	133.2
N-(carboxymethyl)fumonisin B_1 from [$^2H_7$]glucose in ^2H_2O	782.4	764.4	746.4	728.4	*c*	*c*	*c*	*c*	430.4	412.4	394.4	376.3	134.2

[a] Fragment following cleavage between C3 and C4 [*N*-(carboxymethyl)-2-aminopropane-1-aldehyde]. [b] This fragment was not detected in fumonisin B_1 since it would have a *m/z* (73; C_3H_7O) less than the lower limit of the experiment (*m/z* 100). [c] Due to limited amount of compound, insufficient signal was present to detect this fragment.

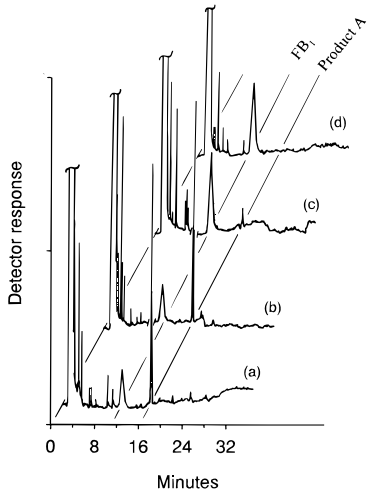

Figure 5. Effects of labeled D-glucose on reaction with fumonisin B_1. HPLC analysis of the incubation of fumonisin B_1 with D-glucose (a), [U-$^{13}C_6$]D-glucose (b), [1,2,3,4,5,6,6-$^2H_7$]-D-glucose (c), or [1,2,3,4,5,6,6-$^2H_7$]D-glucose in ^2H_2O (per-deutero conditions; d). Fumonisin B_1 (250 μM) was incubated overnight with 20 mM carbohydrate at 78 °C with 50 mM potassium phosphate buffer, pH 7.5. A 25 μL aliquot of each incubation was injected onto a Phenomenex UltraCarb ODS 5 μm HPLC column (4.3 × 150 mm), and the compounds separated with an acetonitrile:25 mM HCOOH gradient as described in the text and Figure 2. The compounds were detected using an evaporative light-scattering detector with 1.9 bar nitrogen as the nebulizer gas at 41 °C. FB_1 refers to fumonisin B_1.

(Figure 5d). Therefore, the reaction mixtures containing [$^2H_7$]D-glucose (in both ^1H_2O and ^2H_2O) were kept at 78 °C for an additional 6 days, to obtain sufficient product A for mass spectral analysis.

The incubations of fumonisin B_1 with D-glucose, [$^2H_7$]-D-glucose, or [$^{13}C_6$]D-glucose were subjected to HPLC separation and ES-MS analysis, and the results are shown in Figure 6 and summarized in Table 1. Product A resulting from the incubation of fumonisin B_1 with D-glucose (panel a) showed a protonated molecule of *m/z* 780.4 with characteristic water losses to *m/z* 762.4, 744.4, and 726.4. Major fragments were also detected at *m/z* 410.4, 392.4, and 374.4 corresponding to dehydrated fragment ions following the loss of both tricarballylic groups. When fumonisin B_1 was incubated with [$^{13}C_6$]D-glucose (Figure 6b and Table 1), product A had a protonated molecule of *m/z* 782.4, characteristic water losses to *m/z* 764.4, 746.4, and 728.4, and additional fragment ions at *m/z* 430.4, 412.4, 394.4, 376.4, and 134.2. Comparison of the parent and fragment ions obtained from D-glucose indicates a systematic increase of 2 Da in all ions, which is consistent with incorporation of two carbon atoms from glucose into product A.

The ES-MS spectra of the *N*-(carboxymethyl)fumonisin B_1 resulting from incubation of fumonisin B_1 with [$^2H_7$]D-glucose is shown in Figure 6c. The masses of the parent and fragment ions are increased by 1 Da when compared to the mass fragments of *N*-(carboxymethyl)-fumonisin B_1 isolated from incubation of fumonisin B_1 with D-glucose (Figure 6a). This indicates that one proton from the glucose is incorporated into *N*-(carboxymethyl)fumonisin B_1.

Further insight into the formation of *N*-(carboxymethyl)fumonisin B_1 was gained by conducting the reaction of fumonisin B_1 and [$^2H_7$]D-glucose in ^2H_2O (Figure 6d and Table 1). Under these conditions, the parent and all the fragment ions reflected an increase

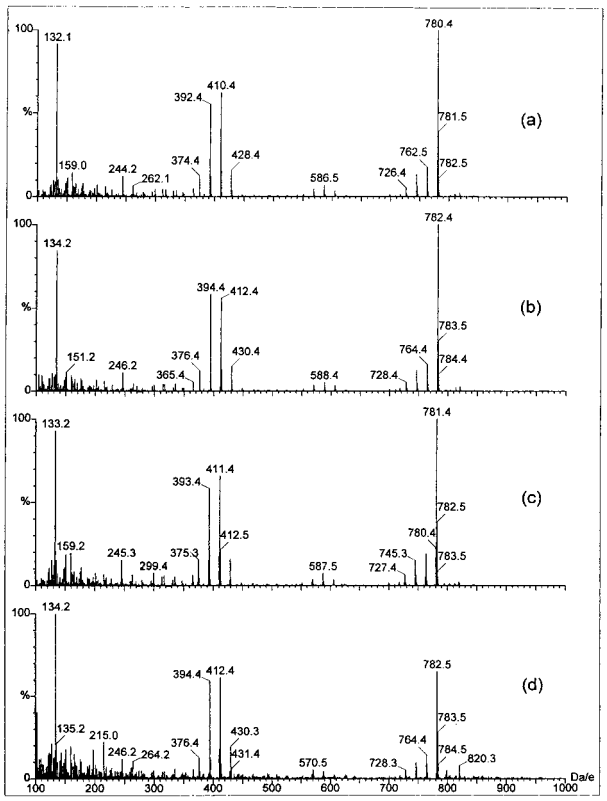

Figure 6. Mass spectroscopic analysis of *N*-(carboxymethyl)-fumonisin B_1. Fumonisin B_1 (250 μM) was incubated overnight at 78 °C in 50 mM potassium phosphate, pH 7.5 with D-glucose (a), [U-$^{13}C_6$]D-glucose (b), [1,2,3,4,5,6,6-$^2H_7$]D-glucose (c), or [1,2,3,4,5,6,6-$^2H_7$]D-glucose in ^2H_2O (per-deutero conditions; d). An aliquot from each incubation was subjected to HPLC separation on the UltraCarb ODS column and analyzed by electrospray-mass spectrometry using a scanning range of *m/z* 100−1000. The fragmentation patterns are shown for the peak eluting at 16.5 min from the HPLC column with a sampling cone-skimmer potential of 70 V.

of 2 Da compared to the product obtained from D-glucose (Figure 6a). This indicates that one of the nonexchangeable protons incorporated into *N*-(carboxymethyl)fumonisin B_1 must have originated in the solvent.

NMR Characterization of *N*-(Carboxymethyl)-fumonisin B_1. The ^1H NMR spectrum of *N*-(carboxymethyl)fumonisin B_1 is shown in Figure 7. All of the 50 nonexchangeable fumonisin B_1 protons were present. The two downfield protons at 4.9 and 5.1 ppm were assigned, respectively, to carbons C14 and C15, the sites of attachment of the tricarballylic acid groups. The methine protons at C10, C3, and C5, the sites of attachment of the hydroxyl groups, were also present at 3.6, 3.7, and 3.8 ppm. The fact that all these resonances are virtually identical to those of the corresponding protons in fumonisin B_1 (Bezuidenhout et al., 1988; Savard and Blackwell, 1994) strongly supports the conclusion that substitution did not occur through an oxygen atom. With the exception of the methine proton at 3.2 ppm, which is slightly shifted downfield (~0.06 ppm), all the remaining proton resonances of fumonisin B_1 (2 at 3.0−3.1 ppm, 8 at 2.2−2.7 ppm, and 34 at 0.8−1.8 ppm) are essentially unchanged in the product. The resonance at 3.2 ppm is conceivably due to the proton at C2, which is consistent with substitution through the amino group. The only significant difference between the proton NMR spectra of fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1 is the presence in the

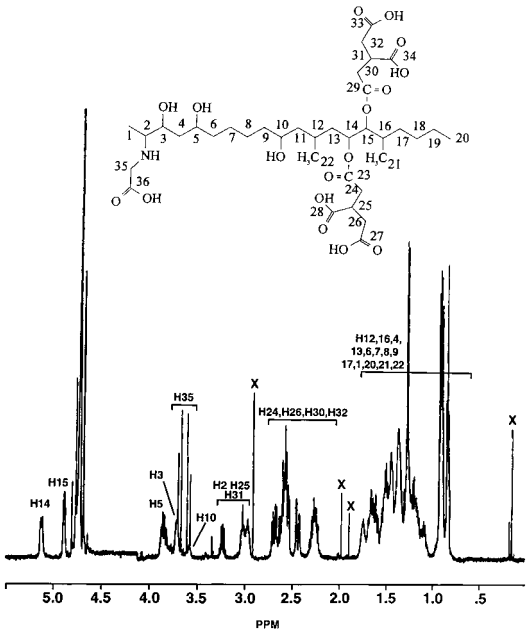

Figure 7. ^{1}H NMR spectrum of *N*-(carboxymethyl)fumonisin B_1. Fumonisin B_1 (250 μM) was incubated with 20 mM D-glucose overnight at 78 °C in 50 mM potassium phosphate buffer, pH 7.5, and purified using Oasis cartridges as described in Materials and Methods. The *N*-(carboxymethyl)fumonisin B_1 was dissolved in ~600 μL ^2H_2O, and the spectrum was obtained.

latter of two mutually coupled doublets, centered at ~3.6 ppm, each accounting for 1 proton. The magnitude of the coupling constant (16.3 Hz) is indicative of geminal coupling, which implies that the two doublets stem from two diastereotopic methylene protons. In view of the evidence discussed above, the methylene group must, therefore, be bonded to the nitrogen. This is consistent with *N*-(carboxymethyl)fumonisin B_1.

^{13}C NMR was used for further confirmation of the structure of *N*-(carboxymethyl)fumonisin B_1. Fumonisin B_1 was incubated with [$^{13}C_6$]D-glucose and the *N*-(carboxymethyl)fumonisin B_1 purified and subjected to ^{13}C NMR analysis following reconstitution in 9:1 H_2O:^2H_2O (Figure 8). Two ^{13}C enriched carbons were present with resonances at 46.0 and 171.2 ppm. Both signals were split into doublets, with a mutual coupling constant of 52.8 Hz. The magnitude of this constant is fully consistent with a one bond C−C coupling between an sp^3 and carbonyl carbon (Breitmeier and Voelter, 1987). Furthermore, the signal at 171.2 ppm is within the region (160−180 ppm) of the fumonisin B_1 carbonyl carbons (C23, C27−29, C33, and C34) (Bezuidenhout et al., 1988; Savard and Blackwell, 1994) also found in natural ^{13}C abundance in Figure 8. The signal at 46.0 ppm is shifted downfield compared to the methine and methylene carbons of the tricarballylic groups of fumonisin B_1 (C24−26 and C30−32) (Bezuidenhout et al., 1988; Savard and Blackwell, 1994), which are all adjacent to a carbonyl carbon. This indicates additional deshielding by an adjacent electron withdrawing group on the carbon resonating at 46.0 ppm, as expected from substitution at the C2 amino group. Taken together, the ^{13}C NMR data support the identification of the fumonisin B_1 derivative as *N*-(carboxymethyl)fumonisin B_1, in which the two additional carbons originated from D-glucose.

N-(Carboxymethyl)fumonisin B_1 was isolated following the incubation of fumonisin B_1 with [1-^{13}C]D-glucose or [2-^{13}C]D-glucose. We were able to establish (spectra not shown) that the methylene carbon on *N*-(carboxymethyl)fumonisin B_1 arises from the C1 of glucose and the carbonyl carbon was originally the C2 position of glucose.

Effect of Reaction Conditions on Formation of *N*-(Carboxymethyl)fumonisin B_1. Conditions favoring the formation of *N*-(carboxymethyl)fumonisin B_1 were investigated. *N*-(Carboxymethyl)fumonisin B_1 was formed when fumonisin B_1 was incubated with D-glucose overnight at 78 °C in 50 mM potassium phosphate or sodium phosphate at pH 7.5 but did not form when the buffer was replaced with equimolar ammonium formate, ammonium acetate, bis-Tris, Tris-HCl, bicine, or HEPES at pH 7.5 (data not presented).

As shown in Figure 9, the formation of *N*-(carboxymethyl)fumonisin B_1 required alkaline pH. Fumonisin B_1 (250 μM) was stable at pH 4.8−8.9 overnight at 78 °C in the absence of glucose (open circles and dashed line, Figure 9). In the presence of glucose (20 mM), no loss of fumonisin B1 was detected at pH 4.8 or 6 (closed circles and solid line, Figure 9); however, loss of fumonisin B_1 occurred when the pH was increased to 7 and was not significantly increased with an increase in the pH to 8.9 (closed circles, Figure 9). This pH dependent decrease in fumonisin B_1 in the presence of glucose was accompanied by a concomitant increase in the formation of *N*-(carboxymethyl)fumonisin B_1 (closed squares, Figure 9).

The effect of temperature on the reaction of fumonisin B_1 with D-glucose, and the subsequent formation of *N*-(carboxymethyl)fumonisin B_1 is shown in Figure 10. There was no loss of fumonisin B_1 when the overnight incubations were conducted at 4−37 °C; however, as the temperature of the incubation was increased to 50, 78, or 94 °C, there was a corresponding decrease in fumonisin B_1 (open circles) and an increase in *N*-(carboxymethyl)fumonisin B_1 (closed circles). Although the fumonisin B_1 concentration decreased by 85% at 94 °C, the amount of *N*-(carboxymethyl)fumonisin B_1 that was produced was less than that at 78 °C.

The decrease in the formation of *N*-(carboxymethyl)-fumonisin B_1 at 94 °C when compared to 78 °C was further investigated (Figure 11). The decrease in fumonisin B_1 at 78 °C was first-order ($r = 0.999$) and had a half-life of approximately 15.5 h. The decrease in fumonisin B_1 at 94 °C was likewise first-order ($r = 0.991$) with a half-life of 8.3 h. Although the increased temperature resulted in an increased rate of loss of fumonisin B_1, this resulted in approximately half the formation of *N*-(carboxymethyl)fumonisin B_1 at the higher temperature when compared to 78 °C. These results are consistent with the data presented in Figure 10, where decreased *N*-(carboxymethyl)fumonisin B_1 formation was detected at 94 °C. Incubation of the fumonisin B_1 with D-glucose at 94 °C resulted in the formation of additional peaks that eluted following the *N*-(carboxymethyl)fumonisin B_1 peak. The characterization of the compounds responsible for these peaks is in progress.

The relationship between the ability of a sugar to reduce Fehling's solution and the ability of that sugar to react with fumonisin B_1 and form *N*-(carboxymethyl)-fumonisin B_1 was determined (Figure 12). Two of the sugars tested (sucrose and sedoheptulose, Figure 12) were nonreducing sugars, and following overnight incubations, we found no depletion of fumonisin B_1 and

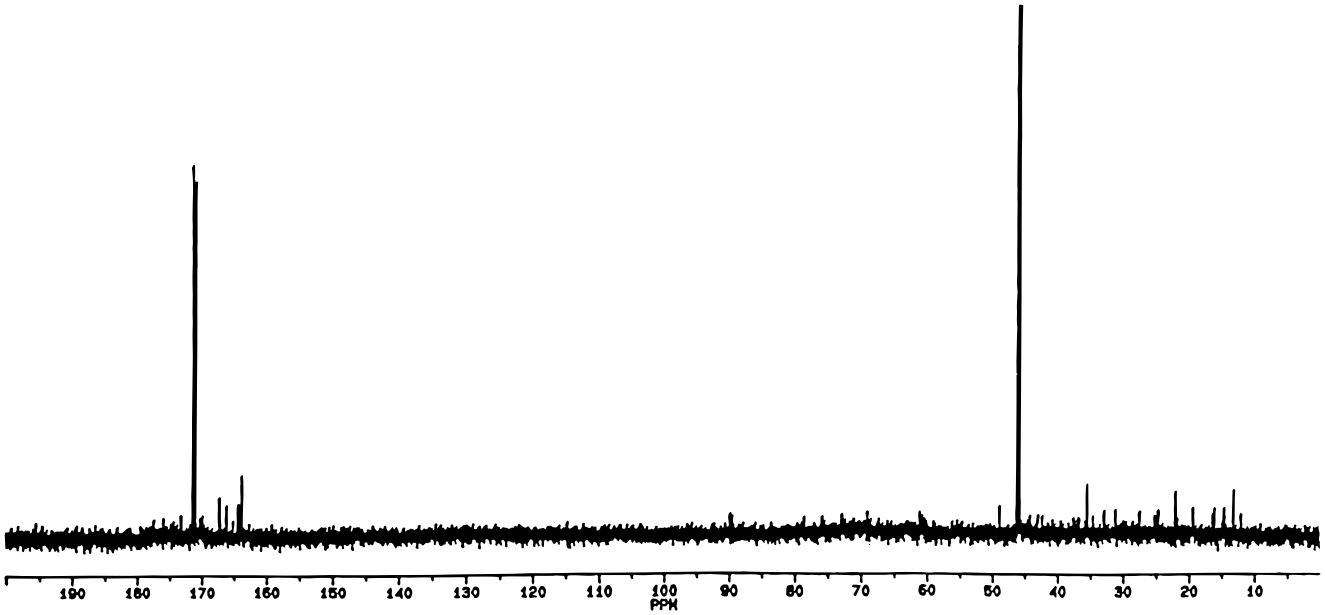

Figure 8. ^{13}C NMR spectrum of *N*-(carboxymethyl)fumonisin B_1. [^{13}C]*N*-(carboxymethyl)fumonisin B_1 was isolated following the incubation of fumonisin B_1 (250 μM) with 20 mM [U-$^{13}C_6$]D-glucose overnight at 78 °C in 50 mM potassium phosphate buffer, pH 7.5. The *N*-(carboxymethyl)fumonisin B_1 was purified using the Oasis cartridges as described in Materials and Methods and was dissolved in ~600 μL ^2H_2O:H_2O (1:9) and the spectrum obtained.

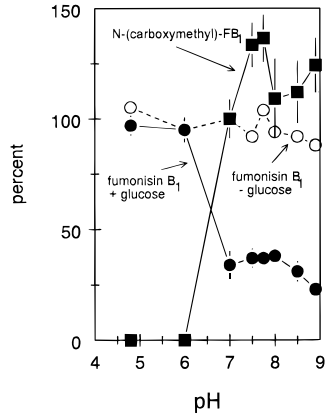

Figure 9. Effect of pH on formation of *N*-(carboxymethyl)-fumonisin B_1. Fumonisin B_1 (250 μM) was incubated with 20 mM D-glucose overnight at 78 °C in 50 mM potassium phosphate (pH 6−8.9) or 25 mM HCOOH (pH 4.8). Fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1 were quantified using HPLC with evaporative light-scattering detection. The fumonisin B_1 levels in the presence (closed circles, solid line) or absence (open circles, broken line) of D-glucose are normalized to the fumonisin B_1 concentration at pH 7 in the absence of D-glucose. *N*-(carboxymethyl)fumonisin B_1 levels (closed squares, solid line) are normalized to the level present at pH 7. The data are presented as the means and range of duplicate determinations for the solid symbols and single determinations for the open symbols.

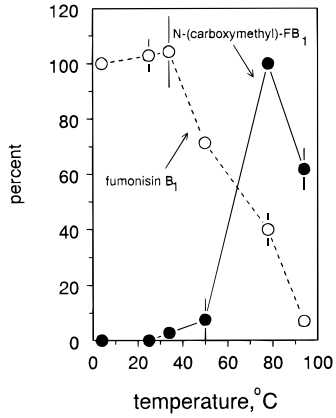

Figure 10. Effect of temperature on the formation of *N*-(carboxymethyl)fumonisin B_1. Fumonisin B_1 (250 μM) was incubated overnight with 20 mM D-glucose in 50 mM potassium phosphate, pH 7.5, at the indicated temperatures. Fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1 were quantified using HPLC with evaporative light-scattering detection. The levels of fumonisin B_1 (open circles, dashed line) were normalized to the amount present at 0 °C, and the levels of *N*-(carboxymethyl)fumonisin B_1 were normalized to the amount present at 78 °C (closed circles, solid line). The data are presented as the mean and range of duplicate determinations.

no formation of *N*-(carboxymethyl)fumonisin B_1. The reducing sugars 2-deoxy-D-ribose, maltose, D-glucose, and D-galactose reacted with fumonisin B_1 to different extents ranging from little reaction with 2-deoxy-D-ribose to extensive reactivity with D-galactose. In the case of maltose, D-glucose, and D-galactose, reaction with fumonisin B_1 produced *N*-(carboxymethyl)fumonisin B_1. Interestingly, when the incubation of D-glucose with fumonisin B_1 was conducted under argon, we found no depletion of fumonisin B_1 and no formation of *N*-(carboxymethyl)fumonisin B_1, indicating a role for atmospheric oxygen in the reaction. The incubation of fumonisin B_1 with the strongly reducing sugars D-ribulose, D-xylulose, and D-fructose resulted in greater than 75% loss of fumonisin B_1, but not as much formation of *N*-(carboxymethyl)fumonisin B_1 as with D-glucose or D-galactose. Although D-ribose was marginally active in reducing Fehling's solution, it caused a greater than 80% loss in fumonisin B_1 in an overnight incubation, and considerable formation of *N*-(carboxymethyl)fumonisin B_1. These results suggest that the ability of a sugar to induce *N*-(carboxymethyl)fumonisin B_1 formation is not directly related to its redox properties but may rather be associated with its propensity to undergo nucleophilic attack on the carbonyl carbon.

The inclusion of 1 mM EDTA or DTPA in the overnight incubation of fumonisin B_1 with D-glucose at pH 7.5 completely inhibited the reaction of fumonisin

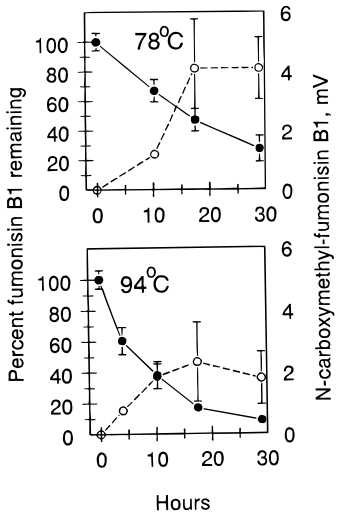

Figure 11. Effects of temperature (78 or 94 °C) on the loss of fumonisin B_1 and formation of *N*-(carboxymethyl)fumonisin B_1. Fumonisin B_1 (250 μM) was incubated overnight with 20 mM D-glucose in 50 mM potassium phosphate, pH 7.5, at 78 or 94 °C. Fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1 were quantified using HPLC with evaporative light-scattering detection. The levels of fumonisin B_1 (closed circles, solid line) were normalized to the amount present at 0 °C, and the levels of *N*-(carboxymethyl)fumonisin B_1 are presented as the peak height in millivolts (open circles, dashed line). The data are presented as the mean and range of triplicate determinations.

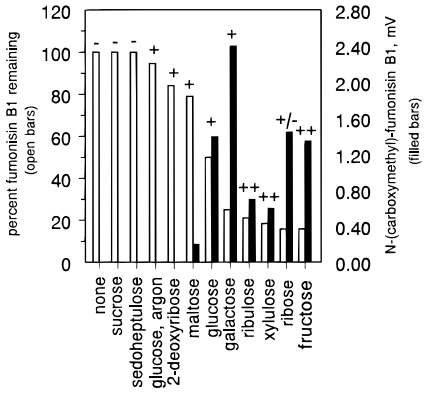

Figure 12. Effect of different sugars on the formation of *N*-(carboxymethyl)fumonisin B_1. Fumonisin B_1 (250 μM) was incubated overnight at 78 °C in 50 mM potassium phosphate, pH 7.5, with the following D-sugars at 20 mM: sucrose, sedoheptulose, glucose under argon, 2-deoxyribose, maltose, glucose, galactose, ribulose, xylulose, ribose, fructose. Fumonisin B_1 (open bars) and *N*-(carboxymethyl)fumonisin B_1 (filled bars) were quantified using HPLC with evaporative light-scattering detection. The ability of the sugars to reduce Fehling's solution is indicated as: "−", none; "±", marginally reducing; "+", mildly reducing; "++", strongly reducing.

B_1 with the sugar, thus eliminating the formation of *N*-(carboxymethyl)fumonisin B_1 (data not presented). This suggests that divalent transition metal ions participate in the oxidation of a sugar−fumonisin B_1 intermediate to *N*-(carboxymethyl)fumonisin B_1.

We extracted triplicate 2 g samples of corn from four different sources as described in Materials and Methods in order to determine if *N*-(carboxymethyl)fumonisin B_1 is present in raw corn. The fumonisin B_1 content in the samples had been previously quantified and ranged from 0.5 to 1.4 ppm. As shown in Figure 13 for a representative sample, both fumonisin B_1 (13.7 min) and *N*-(carboxymethyl)fumonisin B_1 (16.2 min) were detected using HPLC/ES-MS with selected ion monitoring

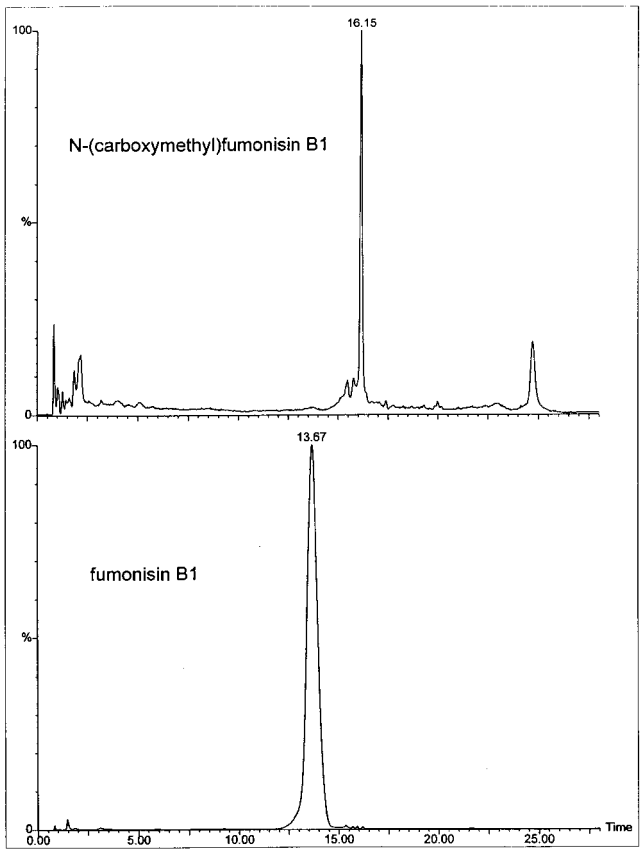

Figure 13. Detection of *N*-(carboxymethyl)fumonisin B_1 in corn. The presence of fumonisin B_1 and *N*-(carboxymethyl)-fumonisin B_1 was determined in raw corn. Corn samples were extracted with 25 mM HCOOH:acetonitrile (1:1), concentrated in vacuo and the oils extracted with chloroform, and the aqueous mixture was applied to an Oasis column and eluted with 25 mM HCOOH and acetonitrile as described in Materials and Methods. The fractions containing fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1 were combined, dried in vacuo, and reconstituted in 100 μL of 25 mM HCOOH:acetonitrile (26:74). Aliquots of 10 μL each were analyzed by HPLC/ES-MS with selected ion monitoring for the protonated molecules for fumonisin B_1 (*m/z* 722) and *N*-(carboxymethyl)fumonisin B_1 (*m/z* 780). The analysis of a representative sample is shown. Fumonisin B_1 eluted at 13.67 min with a peak area of 3.78×10^6, while *N*-(carboxymethyl)fumonisin B_1 eluted at 16.15 min with a peak area of 2.37×10^4.

at *m/z* 722 and 780 for fumonisin B_1 and *N*-(carboxymethyl)fumonisin B_1, respectively. The ratio of the responsiveness of ES-MS to fumonisin B_1 versus *N*-(carboxymethyl)fumonisin B_1 is 8.3:1 (data not presented). Since the extraction efficiencies for the two compounds are approximately equal (data not presented), the *N*-(carboxymethyl)fumonisin B_1 levels in the corn samples were 1.8 ± 0.5, 3.1 ± 0.5, 5.2 ± 0.4, and 5.5 ± 1.6% of the fumonisin B_1 levels.

DISCUSSION

We have shown that the incubation of fumonisin B_1 with several reducing sugars results in the formation of *N*-(carboxymethyl)fumonisin B_1, which was identified on the basis of NMR and mass spectrometric analyses. In the ^{1}H NMR analysis of *N*-(carboxymethyl)fumonisin B_1, integration of the proton resonances indicated the presence of the nonsolvent exchangeable fumonisin B_1 protons (Figure 7). This suggested the substitution was on a heteroatom. In addition, the presence of two

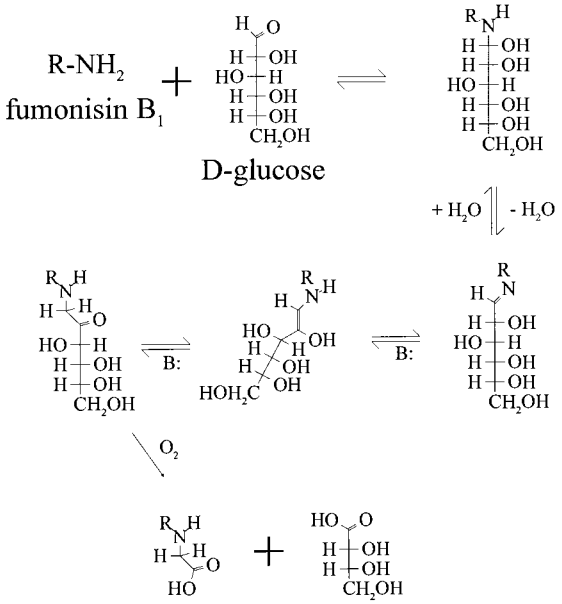

Figure 14. Scheme for the formation of *N*-(carboxymethyl)-fumonisin B_1.

mutually coupled one proton doublets (J_{gem} = 16.3 Hz) at 3.2 ppm was clearly indicative of two deshielded diastereotopic methylene protons. The mass spectrometric analysis of *N*-(carboxymethyl)fumonisin B_1 indicated that the substitution increased the mass of fumonisin B_1 by 58 Da (Figure 4), which among other possibilities, is consistent with addition of a carboxymethyl moiety. The mass spectral data also indicated that substitution did not occur on an oxygen atom. In fact, the fragmentation pattern was essentially identical to that of fumonisin B_1, with three successive losses of 18 Da (water molecules) as well as one and two losses of 176 Da (tricarballylic acid groups). Moreover, all the fragment ions had an increased mass of 58 Da compared to the corresponding fragments in fumonisin B_1 (Figures 4 and 6), which demonstrates that substitution must have occurred on the C2 amino group.

Three lines of evidence were used to conclude that the 58 Da addition to fumonisin B_1 was an *N*-carboxymethyl group. First, the inclusion of [$^{13}C_6$]D-glucose instead of D-glucose in the reaction with fumonisin B_1 resulted in an increase in the molecular weight of the derivative from 779 to 781 Da (Figure 6), suggesting two carbons from the glucose were present in the product. Second, ^{13}C NMR analysis indicated that the two carbons derived from [$^{13}C_6$]D-glucose were mutually coupled, with a typical one bond C−C coupling constant, and had chemical shifts consistent with a deshielded methylene and a carbonyl carbon (Figure 8). Using [1-^{13}C]D-glucose and [2-^{13}C]D-glucose (not shown), we were able to demonstrate that the methylene and carbonyl carbons originated, respectively, from the C1 and C2 carbons of glucose. Finally, the fragmentation of *N*-(carboxymethyl)fumonisin B_1 yielded an intense ion with *m*/*z* 132 (Figures 4 and 6), which is consistent with fission of the C3−C4 bond to yield an N-substituted ion stabilized by the hydroxyl group on C3.

On the basis of precedence in the literature, the initial reaction of fumonisin B_1 with reducing sugars is evidently the formation of a Schiff base (Yaylayan and Huyghues-Despointes, 1994), as shown for glucose in Figure 14. The formation of this fumonisin B_1 Schiff base has been suggested in other reports (Murphy et al., 1995; Lu et al., 1997). The formation of a Schiff base between an aliphatic primary amine and a reducing sugar has been reported for protein amines and reducing sugars and is commonly known as the Maillard reaction (Maillard, 1912. For reviews see: Danehy, 1985; O'Brian and Morrissey, 1989; Ledl and Schleicher, 1990; Yaylayan and Huyghues-Despointes, 1994). These amino acid-derived Schiff bases undergo two successive tautomerizations to form more stable β-ketoamines (Figure 14). This rearrangement is referred to as an Amadori rearrangement with aldoses (Amadori, 1931), and Heyns rearrangement with ketoses (Heyns and Noack, 1962).

Ahmed et al. (1986) reported the incubation of the model Amadori compound N^{α}-formyl-N^{ϵ}-fructoselysine at physiological pH and temperature in the presence of oxygen resulted in the formation of N^{ϵ}-(carboxymethyl)-lysine as the principal degradation product. N^{ϵ}-(Carboxymethyl)lysine formation increased with increasing temperature, alkalinity, oxygen pressure, or phosphate concentration and was inhibited by chelators and oxygen radical scavengers (Ahmed et al., 1986). Many similarities exist in the reactions leading to the formation of *N*-(carboxymethyl)fumonisin B_1 and N^{ϵ}-(carboxymethyl)lysine. These include dependence of carboxymethyl product formation on oxygen, increased formation with increasing alkalinity, and increased formation with increasing temperature.

In an effort to understand the mechanism for *N*-(carboxymethyl)fumonisin B_1 formation, fumonisin B_1 was incubated with [$^2H_7$]D-glucose in the presence of ^1H_2O and ^2H_2O. When the incubation was conducted with [$^2H_7$]D-glucose in ^1H_2O, the parent ion of *N*-(carboxymethyl)fumonisin B_1 increased by 1 Da, whereas an increase of 2 Da was obtained from the incubation with [$^2H_7$]D-glucose in ^2H_2O (Table 1). Under our HPLC/ES-MS conditions, any protons on carboxylic acid groups would exchange with ^1H during the HPLC separation. Therefore, we can conclude that one of the two protons on the methylene carbon of the carboxymethyl substituent originated in the D-glucose, while the other was donated by the solvent. As shown in Figure 14, the Schiff's base formed upon initial attack of fumonisin B_1 on the carbonyl carbon of glucose will tautomerize with loss of the C2 proton on the D-glucose. Subsequent tautomerization to the β-ketoamine will then involve incorporation of one proton from the solvent. Assuming that the tautomerization steps will be rate-determining, this mechanism is fully consistent with the observed isotope effects on the reaction kinetics (Figure 5).

The role of metals or phosphate buffers in the formation of *N*-(carboxymethyl)fumonisin B_1 is not clear at this time. The addition of chelators to the reaction of fumonisin B_1 with D-glucose in phosphate buffers eliminated the formation of *N*-(carboxymethyl)fumonisin B_1 (data not shown). It is likely that the role of the divalent metal ions is in the oxidation of the β-ketoamine with molecular oxygen to form *N*-(carboxymethyl)fumonisin B_1. Studies are in progress to address these issues. Additionally, the apparent dependence of *N*-(carboxymethyl)fumonisin B_1 formation on phosphate buffers is likewise unclear at this time and is the subject of continued studies; however, sufficient metals and phosphates must be present in corn to catalyze the reaction since *N*-(carboxymethyl)fumonisin B_1 was detected in corn naturally contaminated with fumonisin B_1.

Several lines of evidence support the conclusion that

N-(carboxymethyl)fumonisin B_1 is the principal product from the reaction of fumonisin B_1 with reducing sugars. First, the removal of oxygen from the incubation of fumonisin B_1 with D-glucose (Figure 12) resulted in no formation of *N*-(carboxymethyl)fumonisin B_1 and no loss of fumonisin B_1. We would have seen a loss of fumonisin B_1 if either stable intermediates of *N*-(carboxymethyl)-fumonisin B_1 were formed or alternate pathways were present. Second, when generating *N*-(carboxymethyl)-fumonisin B_1 for spectroscopic or other studies, essentially stoichiometric recovery of *N*-(carboxymethyl)-fumonisin B_1 was achieved with incubations of fumonisin B_1 with either D-glucose or [^{13}C]D-glucose (data not presented). Third, the incubation of fumonisin B_1 with D-glucose at 94 °C resulted in the formation of *N*-(carboxymethyl)fumonisin B_1 and several additional derivatives of *N*-(carboxymethyl)fumonisin B_1. Finally, the overnight incubation of [^{14}C]fumonisin B_1 (0.25 mM) with D-glucose (20 mM) at pH 7.4 and 78 °C resulted in the formation of essentially [^{14}C]*N*-(carboxymethyl)-fumonisin B_1 (data not presented). Therefore, it would appear that *N*-(carboxymethyl)fumonisin B_1 is the principal product of the reaction of fumonisin B_1 with reducing sugars.

Since the fumonisins are produced by fungi that grow on corn and other foods, the exposure of humans to these mycotoxins is inevitable; however, specific measures can be taken to avoid or reduce exposure. Since the pathogenic growth of *Fusaria* fungi on corn is in response to drought or other forms of plant stress, minimizing plant stress through specific agricultural methods would reduce fungal growth and human exposure to fumonisins. Alternatively, *Fusaria*-resistant plants could be developed, reducing the exposure to fumonisin-contaminated corn.

Once an allotment of corn is contaminated, few options for fumonisin removal are available. Unlike aflatoxin, fumonisins are resistant to ammoniation (Norred et al., 1991) and are evidently stable at sterilization temperatures (Dupuy et al., 1993; unpublished). The treatment of corn meal with alkali to form maza meal in the preparation of tortillas results in the alkali dependent hydrolysis of the two tricarballylic groups at C14 and C15 on fumonisin B_1 to form hydrolyzed fumonisin B_1 (also referred to as the aminopentol). At present, there are conflicting reports in the literature on the relative toxicity of hydrolyzed fumonisin B_1 when compared to fumonisin B_1 (Hendrich et al., 1993; Voss et al., 1996).

Several reports have suggested that the heating of fumonisin B_1 in an aqueous solution with reducing sugars results in the formation of a fumonisin B_1−sugar adduct that does not contain a primary amine and is less toxic than fumonisin B_1 (Murphy et al., 1995; Hopmans et al., 1997; Lu et al., 1997). A fumonisin−fructose adduct was generated by the incubation of 125 μM fumonisin B_1 with 100 mM fructose (Hopmans et al., 1997) or 725 μM fumonisin B_1 with 1 M fructose (Lu et al., 1997) in 50 mM potassium phosphate, pH 7, at 80 °C for 2 days. This fumonisin−fructose adduct was quantified as hydrolyzed fumonisin B_1 following incubation in 2 N KOH for 2 h at 100 °C (Lu et al., 1997). Our results demonstrate that the incubation of fumonisin B_1 with D-fructose results in the formation of *N*-(carboxymethyl)fumonisin B_1 (Figure 12). Incubation of purified *N*-(carboxymethyl)fumonisin B_1 with 2 N KOH for 2 h at 100 °C resulted in the formation of the *N*-carboxymethyl derivative of hydrolyzed fumonisin B_1 (*m*/*z* 464) with essentially no formation of hydrolyzed fumonisin B_1 (*m*/*z* 406) (data not presented). Therefore, the chemical nature of the fumonisin−fructose adduct reported in Lu et al. (1997) is not consistent with *N*-(carboxymethyl)fumonisin B_1, a secondary amine that would not have been detected with *o*-phthaldialdehyde.

Humans will be exposed to *N*-(carboxymethyl)fumonisin B_1 as a result of corn consumption. We have demonstrated that *N*-(carboxymethyl)fumonisin B_1 is present in raw corn samples that contained 0.5 to 1.4 ppm fumonisin B_1 (Figure 13). The *N*-(carboxymethyl)-fumonisin B_1 was present at an average of approximately 4% of the levels of fumonisin B_1. Since the corn samples were from raw corn, it seems likely that the *N*-(carboxymethyl)fumonisin B_1 must have formed in the kernels. Studies are in progress to determine if cooking fumonisin B_1-contaminated corn in the presence of reducing sugars will result in increased formation of *N*-(carboxymethyl)fumonisin B_1; however, the demonstration that many reducing sugars will be involved in the formation of *N*-(carboxymethyl)fumonisin B_1 (Figure 12) suggests that fumonisin B_1 could be removed from foods through heating in the presence of reducing sugars.

The addition of the carboxymethyl group to the C2 amine of fumonisin B_1 will preclude its detection with some commonly used analytical techniques for fumonisin B_1 quantification. Analytical methods that use *o*-phthaldialdehyde derivatization require the C2 primary amine and will not detect *N*-(carboxymethyl)-fumonisin B_1. Other methods that include antibody-based detection or mass spectral detection (Churchwell et al., 1997) will detect *N*-(carboxymethyl)fumonisin B_1.

The impact of *N*-(carboxymethyl)fumonisin B_1 consumption is not known at this time. The inhibition of ceramide synthase by fumonisin B_1 derivatives is decreased by derivatization of the C2 amine group (Norred et al., 1997). Therefore, substitution with a carboxymethyl group should reduce binding of *N*-(carboxymethyl)fumonisin B_1 to ceramide synthase. Studies are in progress to determine the comparative toxicity of *N*-(carboxymethyl)fumonisin B_1 and fumonisin B_1 in vitro and in vivo.

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