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# Isolation and NMR Characterization of Fumonisin $B_2$ and a New Fumonisin $B_6$ from *Aspergillus niger*

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A new fumonisin, fumonisin $B_6$ (**1**), has been isolated by cation-exchange and reverse-phase chromatography, together with fumonisin $B_2$ (**2**), from stationary cultures of the fungus *Aspergillus niger* NRRL 326. Analysis of mass spectrometric and NMR data determined that $FB_6$ is a positional isomer of $FB_1$ and iso-$FB_1$, having hydroxyl functions at C3, C4, and C5. Analysis of the NMR data for $FB_2$ showed very similar chemical shift values when compared to an authentic *Fusarium* $FB_2$ standard, strongly indicating identical molecules despite that an absolute stereochemical assignment of $FB_2$ from *A. niger* was not possible.

**KEYWORDS: Fumonisins; *Aspergillus niger*; mycotoxins; food safety; NMR**

## INTRODUCTION

Recently, our group was the first to report the production of the mycotoxin fumonisin $B_2$ ($FB_2$) from *Aspergillus niger* NRRL 3122, using HPLC−MS (*1*). The finding did not come as a great surprise, since others (*2*, *3*) had already reported a fumonisin-like gene cluster in the *A. niger* genome of two different strains of the species. Later, we also reported $FB_4$ from *A. niger*, generally produced in amounts around 10−25% of the $FB_2$ (*4*, *5*). Until these findings, fumonisin production has only been previously reported in certain species within the *Gibberella fujikuroi* and *Fusarium oxysporum* species complexes, which are common fungal contaminants of maize-based foods and feeds (*6*−*9*). The first fumonisins were described by Gelderblom et al. (*9*) from cultures of *F. verticillioides*. Since then, more than 25 fumonisins have been identified by NMR (*6*) and a further 25 have been putatively identified by LC−$MS^n$ (*10*). The most abundant and most toxic fumonisins are the B-series analogues (*11*−*13*), which contain a terminal 2-amino-3-hydroxy motif on an eicosane backbone and two hydroxy groups esterified with tricarboxylic acids (TCA). In most Fusaria, $FB_1$ is predominant, usually representing some 70% of the total fumonisin content. $FB_2$ and $FB_3$ usually account for up to 15−25% and 3−8%, respectively, while $FB_4$ is normally present in insignificant amounts (*14*−*16*).

The recent documentation of fumonisin B-series production by *A. niger* has raised the question of whether fumonisins might be an overlooked health risk due to the ubiquitous presence of *A. niger* on a wide range of food stuffs not usually contaminated with *Fusarium* species (*17*). This includes grapes and thereby raisins and wine, peanuts, coffee, tea, and several other products (*1*). Moreover, one could be concerned that industrial products such as enzymes and fine chemicals could be contaminated with traces of fumonisins because of the wide application of *A. niger* as "workhorse" in industrial fermentations (*18*−*21*).

Our recent LC−HRMS based investigations of fumonisin production by *A. niger* and other black Aspergilli suggested that besides $FB_2$ and $FB_4$ *A. niger* can also produce an additional third fumonisin with the same elementel composition as $FB_1$ and iso-$FB_1$ but with a different retention time than the two latter compounds. The present study reports the isolation and characterization of this novel compound, which we named fumonisin $B_6$. In addition, we isolated and characterized $FB_2$ from *A. niger* by NMR in order to further validate that $FB_2$ from *A. niger* is indeed identical to that of $FB_2$ previously reported from several *Fusarium* species.

## MATERIALS AND METHODS

**Fungal Incubation.** In a 10 L glass vessel, 2 L of still culture of 55% rice meal and 45% corn steep liquor (RC) medium was prepared according to Bullermann (*22*) but with a reduced amount of agar (1.5 g/L). It was inoculated with a spore suspension from 7 d old ex-type cultures of *A. niger* NRRL 326, ex-tannin-gallic acid fermentation (=IBT 27876 = ATCC 16888 = CBS 554.65) from CYA. After incubation for 14 d in darkness at 25 °C, the mycelia and media were homogenized with 2 L of MeCN/$H_2O$ (50/50, v/v) and shaken overnight. After filtration the extract was concentrated on a rotary evaporator and freeze-dried.

**Preparative Chromatography.** The raw extract, 9.3 g, was initially subjected to flash chromatography on a 135 g, 50 $\mu$m $C_{18}$ material (Phenomenex, Torrance, CA) which was eluted with $H_2O$−MeOH mixtures in steps of 10%, starting from 10% MeOH. The 70% MeOH fraction (163 mg) was further enriched for fumonisins on a 1000 mg, 33 $\mu$m Strata X-C mixed-mode RP-cation-exchange material (Phenomenex), which was washed with MeCN and eluted with MeCN−$H_2O$ (containing 4% $NH_4OH$) in steps of 20% from 4% $NH_4OH$. The fumonisin-containing fraction (20% MeCN, 19 mg), was finally subjected

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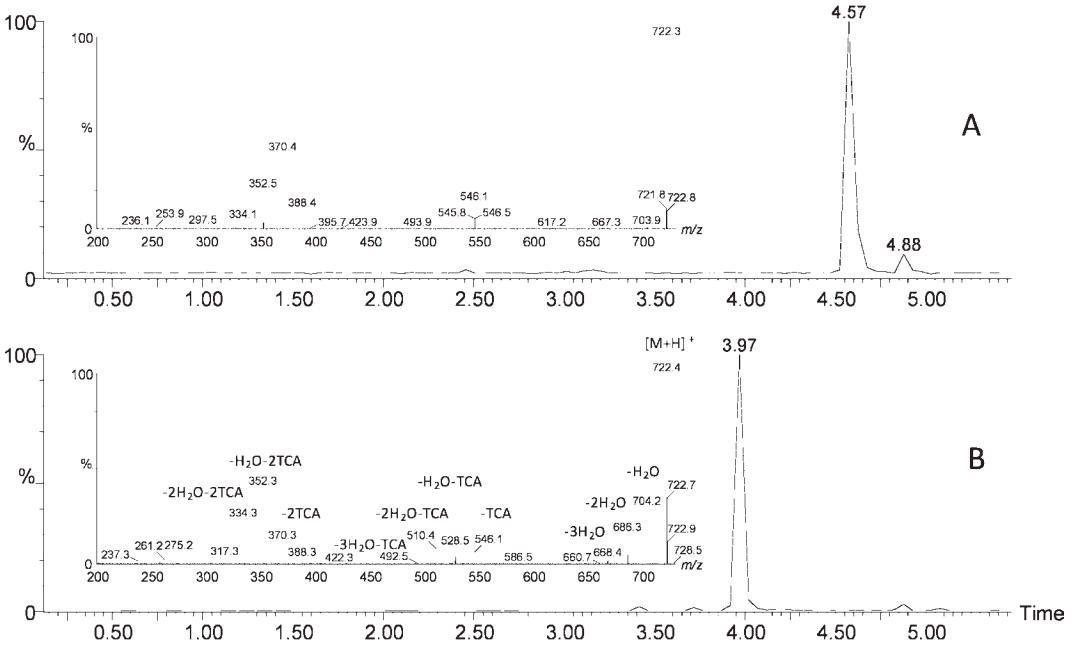


**Figure 1.** Daughter-ion chromatogram (*m/z* 722) showing $FB_6$ (4.57 min) and $FB_1$ (retention time = 3.97 min) and the corresponding daughter-ion spectra (25 eV) from the compound produced by *A. niger* ($FB_6$) sample (**A**) and an authentic $FB_1$ standard (**B**).

to RP separation on a 250 mm × 10 mm i.d., 5 $\mu$m Luna $C_{18}$ (II) column (Phenomenex) using an MeCN−$H_2O$ gradient (starting at 30% MeCN + 50 ppm TFA increasing to 70% over 15 min). Fractions were collected automatically every 20 s and pooled for each chromatographic run after analysis of the content of each fraction by LC−MS had shown which fractions contained fumonisin $B_6$ (1.8 mg) and fumonisin $B_2$ (4.2 mg).

**Analytical LC−MS.** LC−DAD-HRMS was performed as described by Frisvad et al. (*1*) or by LC−tandem MS (MS/MS) on an Agilent 1100 liquid chromatograph (Waldbronn, Germany) coupled to a Quattro Ultima triple mass spectrometer (Micromass, Manchester, U.K.) with an ESI source. The separation was performed on a 50 mm × 2 mm i.d., 3 $\mu$m Gemini $C_6$-phenyl (Phenomenex) column fitted with a security guard system using a linear gradient starting from 20% MeCN in $H_2O$ (both with 20 mM formic acid) to 55% MeCN for 6 min at a flow rate of 300 $\mu$L/min. MS/MS was performed using nitrogen as collision gas and making daughter ion scans of the protonated molecular ions (*m/z* 722 and 706), using fragmentation potentials from 15 to 50 V. Solvents were HPLC grade, and all other chemicals were analytical grade unless otherwise stated. Fumonisin standards ($FB_1$ and $FB_2$ mixture 50 $\mu$g/mL) used for MS analysis were acquired from Biopure (Tulln, Austria). $FB_3$ was from Chiron (Trondheim, Norway). $FB_2$ used for NMR analysis was from Alexis Biochemicals (Lausen, Switzerland).

**NMR Spectroscopy.** NMR spectra were recorded on a Bruker Avance 800 MHz spectrometer with a 5 mm TCI cryoprobe at the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules. The 2D DQF-COSY, NOESY, gHSQC, gH2BC, and gHMBC spectra were acquired using standard pulse sequences. The NMR data used for the structural assignment of the fumonisins were acquired in $CD_3OD$ ($\delta_H$ 3.31 ppm and $\delta_C$ 49.15 ppm).

## RESULTS AND DISCUSSION

In the current study, the ex-type culture of *A. niger* was used, as it has been found to be a strong producer strain of fumonisins (*1*). With no published protocols for purification of fumonisins from *A. niger*, we chose to use mixed-mode RP cation-exchange for purification. This was done because *A. niger* produces high amounts of acidic naphtho-$\gamma$-pyrones and organic acids, which would likely also bind to a strong anion-exchanger (SAX), which is the usual way to purify fumonisins (*23*).

Analytical LC−MS was used to compare the retention times and daughter-ion spectra of the purified *A. niger* fumonisin analogues to those of authentic standards of $FB_1$, $FB_2$, and $FB_3$. The daughter-ion spectra of the $FB_2$ standard and $FB_2$ from *A. niger*, eluting at the same time, were identical and consistent with previously reported ESI-MS/MS data (*24*−*26*), with losses of $H_2O$ and TCA groups from the alkyl backbone dominating the spectra. This confirmed the presence of $FB_2$ or an isomer thereof in the *A. niger* extract. LC−HRMS analyses also showed a peak with the same elemental composition as $FB_1$, however, eluting slightly later (**Figure 1**). LC−MS/MS also confirmed this and showed that it eluted 0.6 min after the authentic standard of $FB_1$ and 0.15 min before $FB_2$ on the $C_6$-phenyl column. Significant differences in the daughter-ion spectra clearly showed that *A. niger* produces an $FB_1$ analogue and not $FB_1$ (**Figure 1**). The same was observed in the crude *A. niger* extract; thus, the $FB_1$ analogue was not an artifact of the purification process but proved to be the novel analogue fumonisin $B_6$ ($FB_6$). $FB_6$ displayed significantly less abundant losses of $H_2O$ than $FB_1$, which indicates the presence of adjacent hydroxyl groups blocking the hydrogens from detaching, thereby allowing the $H_2O$ loss. This is in agreement with our previous findings during our studies of the fragmentation patterns of 474 microbial metabolites using LC−MS $ESI^+$ and $ESI^-$ MS (*27*), where we have never observed $H_2O$ losses unless a $\beta$-hydrogen could be lost because of double bond formation.

**NMR Based Structural Investigations.** To further validate that the $FB_2$ purified from *A. niger* is identical to that produced by *Fusarium*, 1D and 2D homo- and heteronuclear NMR spectra of the purified *A. niger* fumonisin $FB_2$ were carefully inspected and compared to those of an authentic standard of $FB_2$. The NMR data obtained in methanol-$d_4$ displayed comparable chemical shifts for the 34 expected $^{13}C$ NMR chemical shift values except for the resonances originating from the two TCA side chains and their sites of attachment to the fumonisin backbone. In this region, deviations in the $^{13}C$ NMR chemical shifts of between −1.8 to +0.3 ppm were observed. These results could be explained by a difference in the structural fold of the $FB_2$ between the two samples, likely to be caused by differences in pH and thereby overall level of protonation of the four carboxylic acid functionalities. The fact that $FB_2$ does have a unique globular

**Table 1.** NMR Data for Fumonisin $B_6$, $B_1$, and Iso-$B_1$

| | fumonisin $B_6$ | | | | fumonisin $B_1$ (*16*) | | fumonisin iso-$B_1$ (*29*) | |
|---|---|---|---|---|---|---|---|---|
| ID | H (ppm) | C (ppm) | HMBC[c] | H2BC[c] | H (ppm) | C (ppm) | H (ppm) | C (ppm) |
| 1 | 1.34 (d, 7.0) | 16.3 | 2, 3 | 2 | 1.27 (d, 6.7) | 16.0 | 1.30 (d, 6.8) | 14.2 |
| 2 | 3.67 (qd, 2.0, 7.0) | 50.0 | 1, 3 | 1 | 3.14 (dq, 6.7, 6.8) | 53.7 | 3.43 (dq, 4.3) | 51.8 |
| 3 | 3.64 (dd, 2.0, 7.1) | 71.8 | 1, 4 | | 3.74 (ddd, 9.6, 6.8, 3.2) | 70.3 | 3.55 (dd, 4.0) | 73.2 |
| 4 | 3.46 (dd, 1.8, 7.1) | 74.9 | 2, 5 | | 1.55 (m) | 41.7 | 3.65 (m) | 72.2 |
| 5 | 3.71 (ddd, 1.8, 5.1, 8) | 71.4 | | | 3.84 (m) | 68.4 | 1.45 | 34.2 |
| | | | | | | | 1.55 | |
| 6 | 1.53 (m) | 34.8 | | | 1.40−1.60 | 39.1 | 1.34 (br m) | 30.6 |
| | 1.59 (m) | | | | | | | |
| 7[b] | 1.26−1.37 | 30.6 | 4, 8 | | 1.30−1.50 | 26.6 | 1.38 | 26.3 |
| | | | | | | | 1.54 | |
| 8[b] | 1.26−1.37 | 30.6 | | | 1.30−1.50 | 26.7 | 1.32 | 26.5 |
| | | | | | | | 1.52 | |
| 9[b] | 1.26−1.37 | 30.6 | 10 | | 1.40−1.60 | 39.0 | 1.4 | 38.9 |
| 10 | 1.25 | 27.5 | 9 | | 3.62 (m) | 69.8 | 3.62 (m) | 70.0 |
| 11 | 1.04 (m) | 36.1 | | | 1.15 (m) | 44.6 | 1.08 | 44.5 |
| | 1.40 (m) | | | | 1.45 (m) | | 1.5 | |
| 12 | 1.50 (m) | 29.9 | 15 | 21 | 1.81 (m) | 26.9 | 1.83 (m) | 27.0 |
| 13 | 1.43 (m) | 36.1 | 14, 16, 21 | 12 | 1.55 (m) | 36.6 | 1.45 | 37.0 |
| | 1.62 (m) | | | 14 | 1.70 (m) | | 1.66 | |
| 14 | 5.18 (ddd,∼3, ∼4, 10.9)[a] | 72.8 | | | 5.16 (ddd, 10.8, 3.7, 2.4) | 72.8 | 5.16 (ddd, 11, 3, 4) | 72.7 |
| 15 | 4.92 (dd, 3.5, 8.3) | 78.7 | 13, 14, 16, 17, 22, 29 | | 4.94 (dd, 8.1, 3.7) | 78.7 | 4.94 (dd, 4, 8) | 78.9 |
| 16 | 1.70 (m) | 34.7 | 15 | 15, 22 | 1.70 (m) | 34.8 | 1.70 (m) | 34.9 |
| 17 | 1.09 (m) | 32.9 | 16, 22 | | 1.07 (m) | 32.9 | 1.08 | 32.9 |
| | 1.44 (m) | | | | 1.44(m) | | 1.43 | |
| 18 | 1.18 (m) | 29.5 | | | 1.18 (m) | 29.5 | 1.18 | 29.8 |
| | 1.13 (m) | | | | 1.33 (m) | | 1.33 | |
| 19 | 1.31 (m) | 23.7 | 17, 20 | 20 | 1.10 (m) | 23.9 | 1.22 | 23.9 |
| | 1.26 (m) | | | | 1.40 (m) | | 1.38 | |
| 20 | 0.89 (t, 7.0) | 14.2 | 18, 19 | | 0.89 (t, 7.1) | 14.4 | 0.89 (t, 7) | 14.4 |
| 21 | 0.92 (d, 6.7) | 20.8 | 12, 13 | 12 | 0.96 (d, 6.7) | 20.8 | 0.96 (d, 6.7) | 20.8 |
| 22 | 0.94 (d, 6.9) | 15.7 | 14, 16 | 16 | 0.94 (d, 6.8) | 16.0 | 0.93 (d, 6.8) | 16.1 |
| 23 | | 172.9 | | | | 173.5 | | 173.5 |
| 24 | 2.51 (dd, 6.7, 17.0) | 36.3 | 23, 25, 26, 28 | | 2.45 (dd, 16.7, 7.4) | 37.1 | 2.45 | 37.3 |
| | 2.72 (dd, 7.2, 17.0) | | 25, 26, 27 | | 2.71 (dd, 16.7, 7.4) | | 2.71 | |
| 25 | 3.15 (m) | 38.7 | 23, 24, 28 | | 3.10−3.20 (m) | 39.6 | 3.14 | 39.7 |
| 26 | 2.55 (dd, 5.9, 17.0) | 36.3 | 24, 25, 27 | | 2.50 (dd, 16.5, 5.2) | 37.8 | 2.48 | 38.0 |
| | 2.68 (dd, 7.5, 16.8) | | 25, 27 | | 2.66 (dd, 16.5, 8.3) | | 2.64 | |
| 27 | | 175.4 | | | | 177.4 | | 177.2 |
| 28 | | 177.1 | | | | 178.5 | | 178.7 |
| 29 | | 172.9 | | | | 173.0 | | 173.2 |
| 30 | 2.64 (dd, 6.3, 16.7) | 36.3 | 29, 31, 32, 34 | | 2.60 (dd, 16.6, 6.4) | 36.9 | 2.59 | 37.0 |
| | 2.80 (dd, 7.2, 16.9) | | 29, 31, 32, 34 | | 2.79 (dd, 16.6, 7.2) | | 2.79 | |
| 31 | 3.16 (m) | 38.7 | 29, 30, 34 | | 3.10−3.20 (m) | 39.7 | 3.14 | 39.6 |
| 32 | 2.58 (dd, 6.2, 16.9) | 36.1 | 30, 31, 33 | | 2.52 (dd, 16.5, 6.0) | 37.5 | 2.51 | 37.7 |
| | 2.74 (dd, 7.2, 16.9) | | 31, 33 | | 2.72 (16.5, 7.5) | | 2.7 | |
| 33 | | 175.2 | | | | 177.0 | | 176.7 |
| 34 | | 176.8 | | | | 178.0 | | 178.2 |

[a] Only approximate *J* coupling constants (in Hz) due to overlap in the region. [b] Ambigous assignment due to total overlap in the region. [c] HMBC and H2BC connectivities are from the assigned proton to the indicated carbon atom(s).

folded structure has previously been addressed by Beier and Stanker (*28*). In order to ensure that the two samples had been subjected to the same purification protocol, the standard $FB_2$ sample was run over a cation exchange column, in the same way as the last purification step for the isolated *A. niger* $FB_2$ sample. This proved to be very important, since the spectra obtained for $FB_2$ from *Fusarium* after this procedure were now identical to those from *A. niger* $FB_2$, confirming that only minor changes in the sample preparation (e.g., pH) can have a large effect on the structural fold of the fumonisin molecules and thereby spectroscopic output. Comparisons of the *J* coupling constants for the protons in the amino terminal part of the $FB_2$ (C2−C5) and the attactments of the TCA side chains (C14 and C15), from *A. niger* and *Fusarium*, showed that they are of the same size, proving that the relative stereochemistry for these parts of the $FB_2$ backbone is the same for *Aspergillus* and *Fusarium*. On the other hand the stereochemistry at positions C12 and C16 could not be elucidated on the basis of the data available because of chemical shift overlaps. The optical rotation of $FB_2$ from *A. niger* ($[\alpha]_D^{20}$ −11.36 (*c* 1 mg/mL, MeOH)) was similar to that known from *Fusarium*; however, with several stereocenters in the molecule this is not a direct proof of identical stereochemistry for $FB_2$ originating from the two different genera.

Altogether, the presented data point toward identical stereochemistry of the two $FB_2$ molecules; however, a genetic comparison of the fumonisin gene cluster in the two different genera revealed several differences in the placement and orientation of the different involved synthase genes (*2*). This could lead to structural differences in the polyketide synthases and thereby the final stereochemistry of fumonisins produced by *A. niger*,

| | $M_r$ | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| $FB_1$ | 721.3884 | H | OH | OH |
| *Iso*-$FB_1$ | 721.3884 | OH | H | OH |
| $FB_2$ (**2**) | 705.3935 | H | OH | H |
| $FB_3$ | 705.3935 | H | H | OH |
| $FB_4$ | 689.3986 | H | H | H |
| $FB_6$ (**1**) | 721.3884 | OH | OH | H |

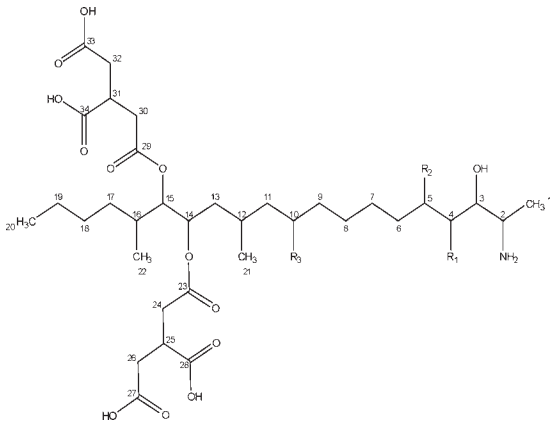

**Figure 2.** Fumonisin structures and numbering scheme.

which is why we conclude that further structural studies such as X-ray analysis need to be performed to establish the absolute stereochemistry of $FB_2$ from *A. niger*.

NMR studies of $FB_6$ substantiated the MS analyses, indicating a new fumonisin of the B type. Unambiguous assignments of the proton and carbon resonances of $FB_6$ could be obtained for most of the resonances. However, spectral overlap hampered some specific assignments in the alkane-like part of the molecule. The assignment was compared to literature values for fumonisins (*16*, *29*). A series of 2D DQF-COSY, HSQC, HMBC, and H2BC experiments established partial assignment of the backbone as well as the TCA side chains present in the structure. Thirty-four carbon resonances could be identified comprising four $CH_3$ groups, 14 $CH_2$ groups, 10 CH groups, and 6 carboxyl carbon resonances (**Table 1**). The two TCA side chains bound to the backbone structure could be assigned as two separate spin systems C23−C28 and C29−C34 (**Figure 2**) with only minor differences in their chemical shifts. The attachment of the C29−C34 TCA side chain to the backbone structure could be confirmed by the presence of an HMBC correlation between H15 and C29, whereas the position of the C23−C28 TCA side chain is based on the chemical shift of H14/C14 and comparison with the assignment obtained for $FB_1$ and iso-$FB_1$. Unambiguous assignments of the fragments C1−C6 and C10−C20 were obtained. In **Table 1**, a comparison of the chemical shifts of $FB_6$ to $FB_1$ and iso-$FB_1$ can be seen. Comparing the resonance assignment of $FB_6$ to $FB_1$ and iso-$FB_1$ showed only minor deviations between the structures except at the positions of OH attachment in the backbone structure. Where $FB_1$ and iso-$FB_1$ have OH groups attached to C3, C5, and C10 and to C3, C4, and C10, respectively, the new fumonisin $FB_6$ has OH groups at C3, C4, and C5 (**Figure 2**). The chemical shift values of 1.25 ppm for H10 and 27.5 ppm for C10, DQF-COSY connectivities from H 11 to H 10 and HMBC correlations from H 9 to C10 and from H 10 to C9, confirm that no OH group is bound at this position due to the large upfield shift of the resonances when compared to $FB_1$, where H 10 and C10 resonate at 3.62 and 69.8 ppm, respectively. In the amino terminal portion of the backbone structure, DQF-COSY connectivities can be traced along the chain from H3 at 3.64 ppm, H4 at 3.46 ppm, and H5 at 3.71 ppm to H6 at 1.53/1.59 ppm. In addition, HMBC correlations can be seen from H4 to C2/C5 and from H3 to C4, confirming the presence of the third OH group at C4 in the structure. The chemical shift obtained for H4/C4 of 3.46 ppm/74.9 ppm compared to 3.65 ppm/72.2 ppm as seen in iso-$FB_1$ and equivalently for H5/C5 of 3.71 ppm/71.4 ppm compared to 3.84 ppm/68.4 ppm for $FB_1$ is in agreement with the proposed structure for $FB_6$.

Altogether, the analysis of these data proves $FB_6$, with hydroxyl functionalities at C3, C4, and C5, to be a structural isomer of $FB_1$ and iso-$FB_1$. As can be seen in **Figure 2** $FB_6$ and iso-$FB_1$ have a hydroxyl at C4, which is rare for the B-series fumonisins and only previously seen for iso-$FB_1$ (*29*). As is the case for $FB_2$, the absolute stereochemistry of $FB_6$ still needs to be resolved, which is work that is ongoing in our laboratory. Analysis of several *A. niger* strains in our laboratory (results not shown) has shown that the frequencies of production of fumonisins in *A. niger* on average are 100% ($FB_2$), 10−25% ($FB_4$), and 5−10% ($FB_6$). Testing of the toxicological properties of not only $FB_2$ and $FB_6$ but also $FB_4$, including investigations of the different conformations that we have seen for these *A. niger* fumonisins, is also in progress in our laboratory.

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## LITERATURE CITED

(1) Frisvad, J. C.; Smedsgaard, J.; Samson, R. A.; Larsen, T. O.; Thrane, U. Fumonisin $B_2$ production by *Aspergillus niger*. *J. Agric. Food Chem.* **2007**, *55*, 9727–9732.

(2) Baker, S. E. *Aspergillus niger genomics*: past, present and into the future. *Med. Mycol.* **2006**, *44*, S17–S21.

(3) Pel, H. J.; de Winde, J. H.; Archer, D. B.; Dyer, P. S.; Hofmann, G.; Schaap, P. J.; et al. Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nat. Biotechnol.* **2007**, *25*, 221–231.

(4) Nielsen, K. F.; Mogensen, J.; Johansen, M.; Larsen, T. O.; Frisvad, J. C. Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. *Anal. Bioanal. Chem.* **2009**, *395*, 1225–1242.

(5) Noonim, P.; Mahakarnchanakul, W.; Nielsen, K. F.; Frisvad, J. C.; Samson, R. A. Fumonisin $B_2$ production by *Aspergillus niger* from Thai coffee beans. *Food Addit. Contam., Part A* **2009**, *26*, 94–100.

(6) Rheeder, J. P.; Marasas, W. F. O.; Vismer, H. F. Production of fumonisin analogs by *Fusarium* species. *Appl. Environ. Microbiol.* **2002**, *68*, 2101–2105.

(7) Paepens, C.; De Saeger, S.; Van Poucke, C.; Dumoulin, F.; Van Calenbergh, S.; Van Peteghem, C. Development of a liquid chromatography/tandem mass spectrometry method for the quantification of fumonisin B-1, B-2 and B-3 in cornflakes. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2021–2029.

(8) Ross, P. F.; Nelson, P. E.; Richard, J. L.; Osweiler, G. D.; Rice, L. G.; Plattner, R. D.; Wilson, T. M. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary-edema syndrome in swine. *Appl. Environ. Microbiol.* **1990**, *56*, 3225–3226.

(9) 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.* **1991**, *57*, 1089–1093.
(10) Bartok, T.; Szecsi, A.; Szekeres, A.; Mesterhazy, A.; Bartok, M. Detection of new fumonisin mycotoxins and fumonisin-like compounds by reversed-phase high-performance liquid chromatography/electrospray ionization ion trap mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2447–2462.
(11) Bezuidenhout, S. C.; Gelderblom, W. C. A.; Gorstallman, 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**, *11*, 743–745.
(12) Gelderblom, W. C. A.; Cawood, M. E.; Snyman, S. D.; Vleggaar, R.; Marasas, W. F. O. Structure−activity-relationships of fumonisins in short-term carcinogenesis and cytotoxicity assays. *Food Chem. Toxicol.* **1993**, *31*, 407–414.
(13) Gutleb, A. C.; Morrison, E.; Murk, A. J. Cytotoxicity assays for mycotoxins produced by *Fusarium* strains: a review. *Environ. Toxicol. Pharmacol.* **2002**, *11*, 309–320.
(14) Musser, S. M.; Plattner, R. D. Fumonisin composition in cultures of *Fusarium moniliforme*, *Fusarium proliferatum*, and *Fusarium nygami*. *J. Agric. Food Chem.* **1997**, *45*, 1169–1173.
(15) 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. *Mycopathology* **1992**, *117*, 3–9.
(16) Miller, J., Trenholm, H. L., Eds. *Mycotoxins in Grain: Compounds Other Than Aflatoxin*; Eagan Press: St. Paul, MN, 1994.
(17) Bouras, N.; Mathieu, F.; Coppel, Y.; Strelkov, S. E.; Lebrihi, A. Occurrence of naphtho-gamma-pyrones- and ochratoxin A-producing fungi in French grapes and characterization of new naphtho-gamma-pyrone polyketide (aurasperone G) isolated from *Aspergillus niger* C-433. *J. Agric. Food Chem.* **2007**, *55*, 8920–8927.
(18) Schuster, E.; Dunn-Coleman, N.; Frisvad, J. C.; Van Dijck, P. W. On the safety of *Aspergillus niger* — a review. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 426–435.
(19) Blumenthal, C. Z. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*: Justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. *Regul. Toxicol. Pharmacol.* **2004**, *39*, 214–228.
(20) Goldberg, I.; Rokem, J. S.; Pines, O. Organic acids: old metabolites, new themes. *J. Chem. Technol. Biotechnol.* **2006**, *81*, 1601–1611.
(21) Karaffa, L.; Kubicek, C. P. *Aspergillus niger* citric acid accumulation: do we understand this well working black box? *Appl. Microbiol. Biotechnol.* **2003**, *61*, 189–196.
(22) Bullermann, L. B. A screening medium and method to detect several mycotoxins in mold cultures. *J. Milk Food Technol.* **1974**, *37*, 1–3.
(23) Hinojo, M. J.; Medina, A.; Valle-Algarra, F. M.; Gimeno-Adelantado, J. V.; Jimenez, M.; Mateo, R. Fumonisin production in rice cultures of *Fusarium verticillioides* under different incubation conditions using an optimized analytical method. *Food Microbiol.* **2006**, *23*, 119–127.
(24) Josephs, J. Detection and characterization of fumonisin mycotoxins by liquid chromatography electrospray ionization using ion trap and triple quadrupole mass spectrometry. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1333–1344.
(25) Sewram, V.; Mshicileli, N.; Shephard, G. S.; Vismer, H. F.; Rheeder, J. P.; Lee, Y. W.; Leslie, J. F.; Marasas, W. F. O. Production of fumonisin B and C analogues by several *Fusarium* species. *J. Agric. Food Chem.* **2005**, *53*, 4861–4866.
(26) Caldas, E. D.; Jones, A. D.; Winter, C. K.; Ward, B.; Gilchrist, D. G. Electrospray-ionization mass-spectrometry of sphinganine analog mycotoxins. *Anal. Chem.* **1995**, *67*, 196–207.
(27) Nielsen, K. F.; Smedsgaard, J. Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography−UV−mass spectrometry methodology. *J. Chromatogr., A* **2003**, *1002*, 111–136.
(28) Beier, R. C.; Stanker, L. H. Molecular models for the stereochemical structures of fumonisin $B_1$ and $B_2$. *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 1–8.
(29) MacKenzie, S. E.; Savard, M. E.; Blackwell, B. A.; Miller, J. D.; ApSimon, J. W. Isolation of a new fumonisin from *Fusarium moniliforme* grown in liquid culture. *J. Nat. Prod.* **1998**, *61*, 367–369.

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