Structure, Synthesis, and Biosynthesis of Fumonisin B₁ and Related Compounds

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The absolute stereochemical description of fumonisin B₁ (FB₁) and presumably of its congeners is now secure. In this article I summarize studies leading to this conclusion and outline the biosynthetic and synthetic studies of FB₁. Key words: absolute configuration, fumonisin biosynthesis, fumonisin B₁, fumonisins, fumonisin synthesis, mycotoxins. — Environ Health Perspect 109(suppl 2):245–249 (2001).
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are derived from molecular oxygen for the backbone and from water for the tricarballylic acids that esterify positions 15 and 16 (Structure 1). These observations are entirely consistent with the biosynthesis proposals described.

**Stereochemistry of FB1**

Examination of the structure of FB1 shows that the molecule contains 10 stereocenters, providing a possible 1,024 different stereochanical structures (1). The presence of a single unique high-resolution nuclear magnetic resonance (1H NMR) spectrum for FB1 derived from different species of *Fusarium* and isolated by different methods suggests that only one of these 1,024 structures describes FB1. Because fumonisins do not crystallize, several groups have been involved in determining the stereochemistry through the synthesis of derivatives and NMR analysis thereof (1–8).

To date, all have come to the same conclusions using different derivatization schemes.

Hoping to obtain a crystalline compound for X-ray analysis, we prepared several derivatives containing “bulky” functions and semi-rigid units in our laboratories. All of the derivatives failed to crystallize, but their spectral properties revealed the relative stereochemistry for portions of the FB1 backbone. The synthetic strategy is summarized in Figure 1. Generally the derivatives were formed from tetramethyl FB1 [FB1(CH₃)₄], where the carboxylic groups have been methylated so that organic solvents could be used in the purification steps and so as not to involve the tricarballylic ester functions.

FB1(CH₃)₄ (Structure 1 with COOMe for COOH) was converted to the 2,3-carbamate (2) using phosgene and triethylamine in benzene. The 3,5-carbonate (5) was prepared using phosgene in pyridine after protecting the amide function with N-p-bromobenzoate.

We used these two compounds to determine the relative stereochemistry of the C-1 to C-5 fragment of FB1 as shown in Structure 1, using coupling constants and nuclear Overhauser effects (nOe). A parallel study (7) confirmed these results.

Compound 7 was obtained by taking advantage of the adventitious formation of chloro-compound 6 (see Figure 1), which on treatment with potassium hydroxide in ethanol provided the pyran 7.

Analysis of nOe data defines the relative configuration of the 6-membered ring of Structure 7 as shown in Figure 2. Enhancements were observed between H-10, H-12, and H-15, but not between H-10 and H-14, thus indicating these protons to be trans. We determined the relative stereochemistry of the C-10 to C-16 moiety by comparing the NMR parameters of FB1 to those of the 10,14-cyclic ether derivative of

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**Structure 1. FB1.**

**Structure 2. Variety of FB1-related congeners.**

**Structure 3. Incorporation of acetate in FB1.**
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N-p-bromobenzoate FB₁ aminopentanol (Structure 7).

With the relative configuration of two portions of the molecule thus defined, the absolute stereochemistry at any one position must be determined to define the absolute stereochemistry at each asymmetric center. Mosher’s method (18) was applied directly to FB₁ to form the α-methoxy-α-trifluoromethylphenylacetyl (MPTA) amide derivatives in a method similar to that of Hoye et al. (8), except that in this case positions C-3 and C-5 were underivatized. FB₁(CH₃)₄ was treated with MPTA chloride in tetrahydrofuran (THF) for 30 min and the amides purified on silica using 10% methanol in chloroform. The S-amide was prepared using the R-MPTA chloride, and the R-amide was prepared using the S-MPTA chloride. NMR analysis and comparison of the magnitude and sign of the differences in the proton chemical shifts between the two amides (18) determined that the absolute configuration at C-2 was S (as shown in Structure 1), identical to that of the naturally occurring amino acids. L-alanine is incorporated with retention of configuration into FB₁.

The resultant absolute configuration of the FB₁ backbone is therefore as shown in Structure 1. The connection between C-10 and C-5 was provided by the hexanoic acid derivative of Hoye et al. (8). This also agrees with the studies of Harmange et al. on FB₂ (19) and shows that the fumonisins have the same absolute configuration as the AAL toxins as determined by Boyle et al. (20). The configuration of the side chains was suggested to be S (22) and most recently argued to be R in studies on FB₂ by Kishi and colleagues (22,23).

Hartl and Humpf have confirmed the absolute configuration of the amino terminus (C-1 → C-5) using the circular dichroism exciton method (24).

The disagreement that has surfaced over the stereochemistry of the two tricarballylic acid (TCA) esters present at positions C-14 and C-15 in this and related molecules led us to examine an independent route to determine the stereochemistry of the side chain acids.

We began by stabilizing the asymmetric centers in the TCA units of FB₁ by borane reduction of the free carboxyl groups, as was also done by Shier et al. (21). In practice, this required solubilization of FB₁ in THF, which was accomplished by conversion to the N-acetyl-O-triacetate bis-anhydride Structure 8a, using acetic anhydride. This was followed by partial hydrolysis in aqueous THF at room temperature to the acid Structure 8b, a compound that is the triacetate of the naturally occurring fumonisin A₁, (FA₁) first described by Bezuidenhout et al. (6). Reduction of Structure 8b using excess THF/BH₃ in THF

Figure 1. Synthesis of cyclic derivations of FB₁ tetramethyl ester.

Figure 2. NMR analysis of cyclic derivatives of FB₁.
gave the \(N\)-acetyl triacetyl tetraol Structure 8c, which is identical to compound 1C described by Shier et al. (21). Complete hydrolysis of Structure 8c using potassium hydroxide in aqueous methanol, followed by acidification gave a mixture rich in hydroxy \(\gamma\)-lactone (Structure 9) (R = H). Benzoylation of this mixture and separation on SiO\(_2\) using 1:1 ethyl acetate/hexane produced the benzoyloxy \(\gamma\)-lactone Structure 9 (R = COC\(_6\)H\(_5\)).

An authentic sample of optically active Structure 9 (R = COC\(_6\)H\(_5\)) was prepared from \(E\)-phenylitoconic acid (Structure 10) by asymmetric reduction (25) to \(S\) (–) benzylsuccinic acid Structure 11 ([\(\alpha\)]\(_D\)\(_{25}\) -27° (c 1.5, ethyl acetate)]. The absolute stereochemistry assigned to this (25,26) was confirmed by X-ray crystallography. Conversion of Structure 11 to the diol Structure 12 using borane-THF, then benzoylation and oxidation gave the dibenzoyloxy acid Structure 13. Alkaline hydrolysis followed by acidification gave the \(R\) (–) hydroxy \(\gamma\)-lactone Structure 9 (R = H) identified in all respects to the product obtained from FB1.

It follows that both the TCA units in FB1 have the R configuration illustrated in Structure 14. Thus, our conclusions agree with those of Boyle and Kishi (22). Our detailed NMR assignments for the carbons and hydrogens of the TCA units in FB1 (as well as FB2 and FB3) (2,18) agree well with those in the literature (13), making it improbable that two optical isomers of FB1 have been isolated. It is evident both from our work and that of Boyle and Kishi (22) that only one configuration exists for the TCA units at both the C-14 and C-15 positions in the backbone for all the fumonisins isolated to date. Moreover, it is interesting to observe that no fumonisin has yet been isolated with TCA units at any other position on the backbone, implying a biosynthetic preference for those sites. Comparison of the present results with previous biosynthetic studies (5,12) raise an intriguing question with respect to the biosynthetic origin of these TCA units. Studies using \(^{13}\)C-enriched glutamate have shown that the secondary carboxyl functions (C-28 and C-34) are derived from C-5 of l-glutamic acid, whereas studies with \(^{13}\)C-enriched acetate have shown that the unesterified four-carbon unit of the TCA unit (C-25, C-26, C-27, C-28, and C-31, C-32, C-33, C-34) is formed before the addition of a third acetate unit (leading to C-23, C-24 and C-29, C-30). The specific incorporation of glutamic acid suggests that the TCA units are derived from the Krebs acid cycle.

These results can be explained by three possible mechanisms: a) simple chiral esterification using TCA itself; b) esterification with cis-aconitate as in Structure 15, followed by chiral reduction of the double bonds; or c) esterification with the chiral intermediate 2R-35 isocitrate to give Structure 16, followed by deoxygenation at C-24 and C-30. In the latter case, the R configuration of the TCA units would arise, consistent with the
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The determination of the absolute stereochemistry of FB₁ has been achieved, providing a sound basis for a deeper understanding of the structural basis for the biologic effects of this class of mycotoxins.

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