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The chemistry of fumonisins and related compounds. Fumonisins from *Fusarium moniliforme*: Chemistry, structure and biosynthesis

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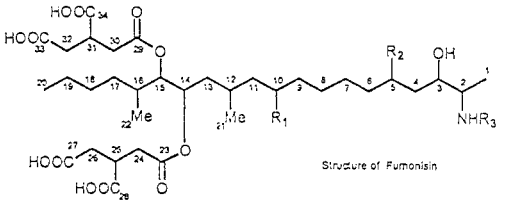
Abstract: Recent work on the biosynthesis and stereochemical determinations of the fumonisin structure and the determination of related compounds is presented.

The pathogenic nature of certain species of fungi to plants has been observed virtually since the beginning of agriculture. These plant pathogens often produce metabolites that show toxic effects when they are ingested. In particular, toxigenic *Fusarium* species are now recognized to be a major agricultural problem. The extensive research carried out over the past two decades has revealed a large number of toxic *Fusarium* secondary metabolites (1, 2, 3).

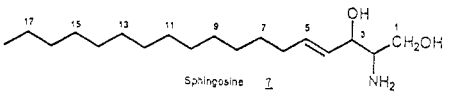
Fusarium moniliforme (Sheldon) is a prevalent mould on corn, sorghum, and other grains throughout the world and has been shown to be toxic and carcinogenic for animals both as a contaminant of grains and as a culture isolate (4, 5). Consumption of *F. moniliforme*-contaminated corn has been correlated with human esophageal cancer in areas of southern Africa, China, and other countries (6). This mould contains a number of toxins, a group of which (termed fumonisins) (6, 7, 8) are thought to be mainly responsible for these diseases. Purified fumonisin B_1 has been shown to cause equine leukoencephalomalacia (ELEM) (9, 10, 11), porcine pulmonary edema (12), and hepatotoxicity and liver tumors in rats (4, 13).

Structural studies were first published in 1988, 1, 2, 3, 4 (6, 14). The closely related fumonisins B_3, 5, and very recently the first member of 'C' series 6 has been described (15, see also 16).



Structure of Fumonisin

		R_1	R_2	R_3
Fumonisin B_1	1	OH	OH	H
Fumonisin B_2	2	H	OH	H
Fumonisin B_3	5	OH	H	H
Fumonisin A_1	3	OH	OH	$COCH_3$
Fumonisin A_2	4	H	OH	$COCH_3$
Fumonisin C_1	6	C-1, H replaces		CH_3



Sphingosine 7

Biosynthetic Studies

In our laboratories a method for the production of specifically enriched fumonisins was developed (17) involving the addition of enriched acetate to liquid cultures of *Fusarium moniliforme* in shake flasks. In addition stable isotope labelling was accomplished by feeding methionine, glutamate and chorine. The location of labels from these experiments is outlined in Fig. 1. These data seems to indicate a polyketide origin for the backbone of fumonisins.

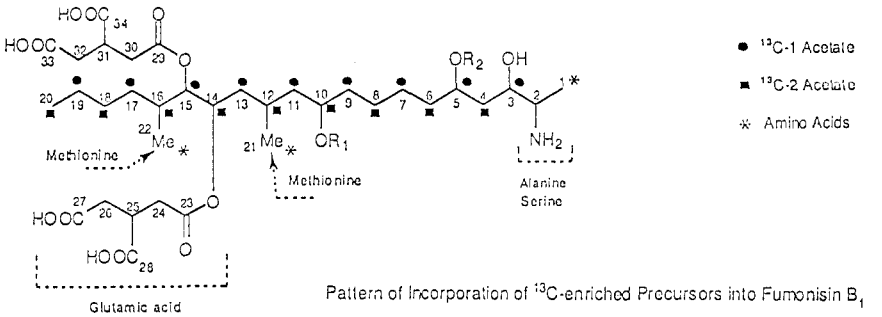


FIG. 1

The similarity between the structures of fumonisins and sphingosine, 7, suggests a biological interplay that could explain the physiological effects of these mycotoxins. Fumonisin B_1 inhibits the incorporation of serine into sphingosine, reducing the concentration of sphingosine (18), consequently causing the accumulation of its precursor, sphinganine, up to toxic levels and reducing the transformation of sphingosine to ceramide.

Stereochemical Studies

Fumonisin B_1 has 10 chiral centres, hence structural representations shown so far cover 1024 isomers! Any understanding of the structure-activity relationship involved in the physiological activity of fumonisins requires a clear delineation of the absolute stereochemistry at all sites. Attempts to prepare suitable crystalline derivatives of these compounds have failed to date and we have therefore undertaken a systematic study of the chemistry of the fumonisins with the goal of determining the stereochemistry at all sites by derivatization, transformation or degradation followed by the use of the available array of physical methods.

The first experiments were targeted to positions 2, 3, and 5 and are summarized in Fig. 2 below.

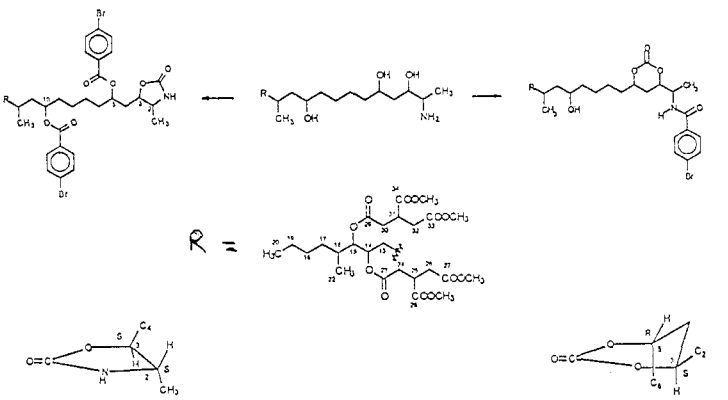


FIG. 2

The relative configuration of carbons 3 and 5 arise from the ^1H-^1H coupling constants in the derived carbonates and that of positions 2 and 3 from the ^1H-^1H coupling constants measured for derived cyclic carbamates.

Combining the relative configurations demonstrated for position 2, 3, and 5 then gives the relative configuration of fumonisin B_1 at these positions as shown in Fig. 3. No information is yet available concerning the absolute configuration at these sites.

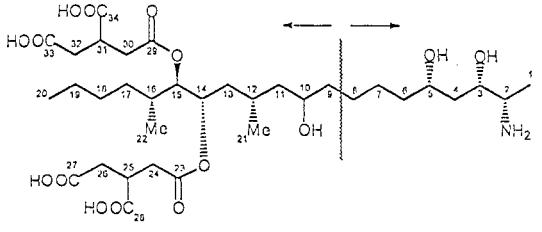


Figure 3. Relative stereochemistry at C2 to C5 and at C12 to C16 in Fumonisin B_1 tetramethyl ester.

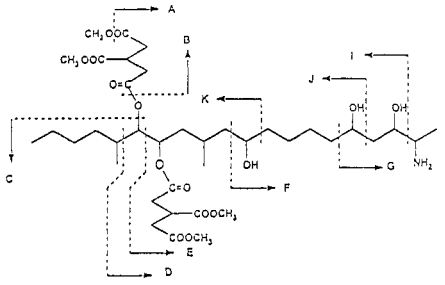
(Note: these are separate determinations as indicated by the dividing line. No implication of the stereochemical relations between both halves is proposed).

Next the stereochemistry of positions 12, 14, 15, and 16 was probed. To date our preliminary data based on coupling constants and nuclear Overhauser effect suggests that the relative stereochemistry shown in Fig. 3 pertains. It is stressed that this is preliminary data only since the possibility of a conformationally mobile chain embodying the section C12-16 cannot be completely eliminated. Our ongoing experiments at present are aimed at the preparation of more rigid molecules susceptible to confirmation of this relative stereochemistry. Further experiments are incomplete but are centred on cleavage of the fumonisin chain at 3 sites (C9-10, C10-11 and at C14-15).

Detection, Structure Determination and the Identification of Fumonisin Congeners

The most sensitive method for fumonisin detection and determination is mass spectrometry. By methylation of fumonisins and fumonisin containing culture fractions, followed by LC/Particle Beam Mass Spectrometry both chemical ionization and electron ionization (EI) spectra may be obtained.

The EI spectra are particularly revealing by the appearance of a series of systematic fragments as shown in Fig. 4 (23). The appearance of these fragments has been most valuable in the detection of a variety of fumonisin analogues. Ongoing work is now focused on the isolation of these material for full spectroscopic and chemical confirmation and in sufficient amounts for physiological assays.



FB1.Mc4

FIG. 4

Conclusion

The Ottawa-based fumonisin program is able to provide sufficient quantities of known fumonisins for physiological and chemical studies. Substantial progress has been made in the delineation of the biosynthetic steps leading to these compounds and the relative stereochemistry at 7 of the 10 chiral centres has been determined.

Acknowledgements

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