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Relative Configuration of the C-1 to C-5 Fragment of Fumonisin B_1

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Abstract: Synthesis of the 2,3-carbamate (**2**) and the 3,5-carbonate-N-p-bromobenzoate (**4**) derivatives of fumonisin B_1 have been made in an initial study of the configuration of fumonisins. These have been used to determine the relative configuration of the C(1)-C(5) fragment.

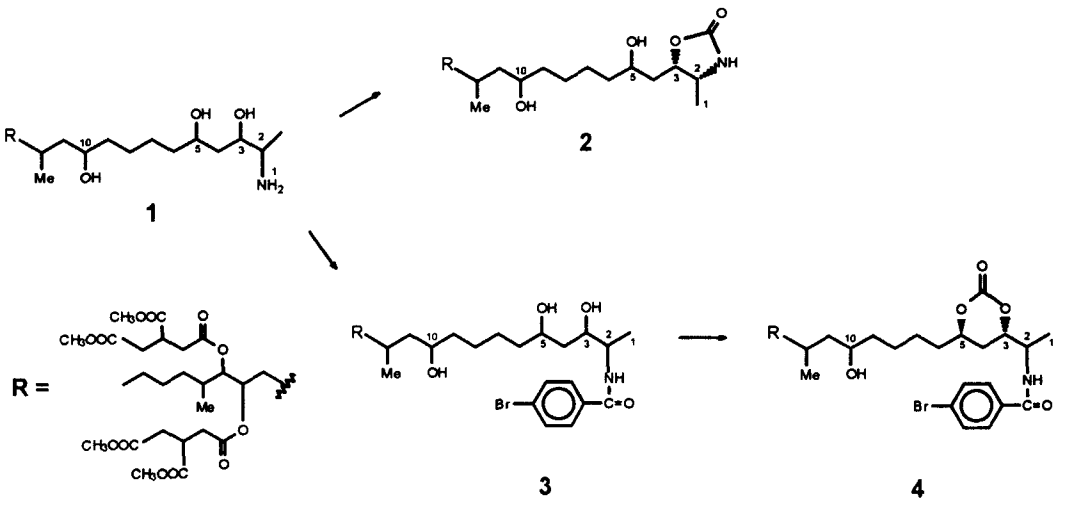
Fusarium moniliforme (Sheldon) is a prevalent mould on corn, sorghum and other grains throughout the world and has been shown to be toxic and carcinogenic for animals both as a contaminant of grains and as a pure culture.[1,2] This fungus contains a number of toxins, a group of which called fumonisins are thought to be mainly responsible for these diseases. Purified fumonisin B_1 (FB_1) has been shown to cause equine leukoencephalomalacia,[3] porcine pulmonary edema[4] and to promote tumour formation in rats.[5] FB_1 has also been shown to be a potent inhibitor of ceramide synthetase,[6] thus blocking sphingolipid biosynthesis. This activity must be related to the stereochemical arrangement of the ten chiral centres in FB_1. With the hope of obtaining a crystalline compound for X-ray analysis, a number of derivatives of FB_1 containing semi-rigid units were prepared. These failed to crystallize, but enabled assignment of the relative stereochemistry at carbons 2,3 and 5 using NMR spectroscopy.

Results. FB_1 tetramethyl ester (FB_1 Me_4, **1**) was converted to the 2,3-carbamate (**2**) using triphosgene and triethylamine in benzene (25% yield). ir ν_{max} (CH_2Cl_2) 3600, 3530, 3445, and 1735 cm^{-1}. FABMS,[7] m/z 804 (M+1). ^1H and ^{13}C NMR;[8] see Tables 1 and 3.

The N-p-bromobenzoyl derivative **3** of compound **1** was prepared in 70% yield using p-bromobenzoyl chloride in a tetrahydrofuran/triethylamine mixture at 0°C. ir ν_{max} (CH_2Cl_2) 3600, 3520, 3425, 1735 (str), 1665 (med), and 1591 (wk) cm^{-1}. FABMS, m/z 960 (M+1). ^1H and ^{13}C NMR; see Tables 1 and 3.

The 3,5-carbonate **4** was prepared from **3** using phosgene in pyridine at 0°C (added $Cr(OAc)_3$ catalysed the reaction and prevented side reactions). Recycling twice was required to bring the yield to 60% based on unrecovered **3**. ir ν_{max} (CH_2Cl_2) 3520, 3420, 1738, 1655 cm^{-1}. FABMS, m/z 986 (M+1). ^1H and ^{13}C NMR; see Tables 1 and 3.

Discussion. Selected NMR data for compounds **1** to **4** are given in Tables 1 and 3, and are compared to the parent compound, FB_1. Complete assignments have been made for all protons and carbons of **1-4**. These will be published elsewhere.[9] Chemical shifts varied little from those of FB_1, except at the sites of substitution. The presence of the N-p-bromobenzoate ester induced a relatively large 1.4 ppm downfield shift of the H-2 resonance of **3**, whereas the formation of the carbamate ring induced a 0.8-0.9 ppm downfield shift. The introduction of the 3,5-carbonate ring in **4** caused only a 0.6 ppm shift in the relevant proton peaks.



Comparison of the $^1H/^1H$ coupling constants of **2** and **4** is shown in Figure 1 and Table 2. Comparison of the carbonate coupling constants to those of the model compounds 2-oxo-1,3- dioxanes,[10] shows that the C-3 and the C-5 hydroxyl functions are trans in these cyclic compounds. The $J_{3,4}$ value of 10.3 Hz in **4** is somewhat larger than that observed in FB_1 (approximately 9 Hz) due to the fixed configuration induced by the ring. Analysis of the 2,3-carbamate spectrum shows that the C-2 amino function and the C-3 hydroxyl function are cis with respect to each other. The $J_{2,3}$ of 6.5 Hz indicates a dihedral angle of approximately 110° or 60° between H-2 and H-3, depending on the relative orientation between H-2 and the terminal methyl. The 110° orientation is confirmed by the observation of an nOe effect between CH_3-1 and H-3. An amino-lipid with the same terminal stereochemistry has recently been reported.[11]

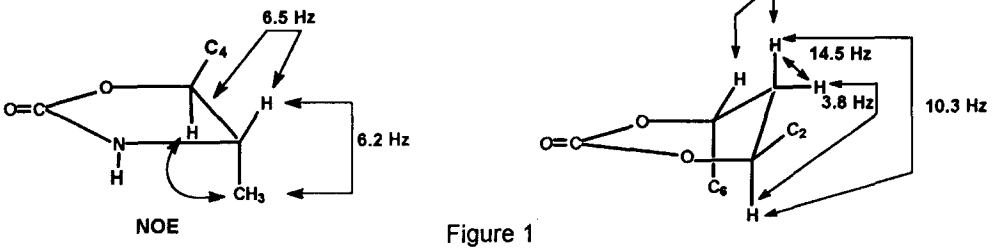


Figure 1

The ^{13}C data are also consistent with the proposed stereochemistry. The chemical shifts are virtually constant except for C-1 to C-5. Conformational changes due to the bulky p-bromobenzoate group are likely accountable for some of the induced shifts. The formation of the carbonate (**4**) induces a 12 ppm upfield shift of C-4 and smaller downfield shifts of C-3 and C-5, consistent with the model compounds, the 2-oxo-1,3-dioxanes.[10] The presence of the carbamate and carbonate rings is confirmed by the presence of a carbonyl resonance at 158.6 ppm (**2**) and 149.3 ppm (**4**).

TABLE 1. Selected Proton Chemical Shifts in $CDCl_3$* of Fumonisin Derivatives **1-4** (ppm from TMS).

δ	FB_1	**1**	**2**	**3**	**4**
NH		-	5.21	6.63	6.36
H-1	1.27	1.06	1.26	1.30	1.43
H-3	3.14	2.80	3.57	4.17	4.49
H-3	3.74	3.48	4.38	3.98	4.55
H-4a	~1.55	1.43	1.63	1.57	1.95
H-4b		-1.63	1.74	1.73	2.13
H-5	3.84	3.84	3.88	3.92	4.48
H-10	3.62	3.57	3.60	3.61	3.55
H-14	5.16	5.15	5.17	5.19	5.18
H-15	4.94	4.88	4.90	4.91	4.90

TABLE 2. $J_{H,H}$ Coupling Constants of Fumonisin Derivatives **1-4** (Hz)

$J_{H,H}$	FB_1	**1**	**2**	**3**	**4**
NH, H-2	-	-	-	8.7	9.0
H-1, H-2	6.7	6.5	6.2	6.8	7.0
H-2, H-3	6.8	-	6.5	3.1	2.2
H-3, H-4a	3.2	-	3.1	3.1	3.8
H-3, H-4b	9.6	-	9.7	9.4	10.3
H-4 (AB)	-	-	-	14.6	14.6
H-4a, H-5	-	-	-	7.5	3.6
H-4b, H-5	-	-	-	3.5	5.9

TABLE 3. Selected ^{13}C NMR Assignments of Fumonisin Derivatives **1-4** in $CDCl_3$*.

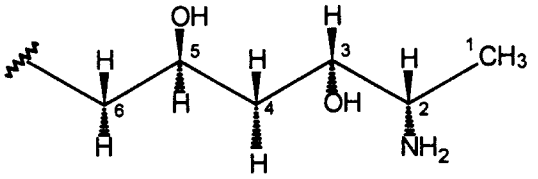
Position	FB_1	**1**	**2**	**3**	**4**
1	16.06	20.10	20.15	18.30	17.89
2	53.76	51.23	53.93	50.19	47.52
3	70.43	73.21	81.29	71.63	78.09
4	41.83	39.75	41.58	40.25	28.49
5	68.48	68.49	67.73	69.26	77.38
6	39.01	37.56	38.00	37.18	34.56
10	69.98	68.82	68.80	68.37	68.72
14	72.80	71.26	71.10	71.22	71.06
15	78.82	77.82	77.83	77.86	77.83

*FB_1 in CD_3OD.

While biosynthetic studies show that the C-1, C-2 terminus is derived from l-alanine,[12] this does not define the absolute stereochemistry of this portion of FB_1, since the assymetric centre may not be preserved during decarboxylation. The relative stereochemistry of this terminus of the FB_1 molecule is as displayed in Figure 2. Comparison of the NMR data for the cyclic derivatives to those for FB_1 indicates that the relative configuration is the same as determined from the derivatives, which suggests that the fumonisin backbone forms a more rigid structure in solution than might be expected from this lipid-like molecule. This relative stereochemistry (Figure 2)

is opposite that of sphingosine (2,3 functionalities are threo). A recent study of AAL toxin[13] reports the same relative stereochemistry with respect to FB_1 between the adjacent amino and hydroxyl substituents, but the opposite stereochemistry for the hydroxyl function analagous to C-5 of FB_1. In a parallel study, Poch et al.[14] have deduced the same relative conformation at C-2 and C-3.

Figure 2



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