Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 1549–1556

TETRAHEDRON:
ASYMMETRY

Assigning the absolute configuration of fumonisins by the circular dichroism exciton chirality method

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Received 26 February 1998; accepted 24 March 1998

Abstract

Fumonisins are mycotoxins produced by *Fusarium moniliforme* (Sheldon) and other related fungi that are common contaminants of corn and other grains throughout the world. The circular dichroism (CD) exciton chirality method was applied to determine the absolute configuration of the terminal part of the backbone of fumonisins. Using the *p*-dimethylaminobenzoate chromophore, the structure of FB_1 was confirmed to be 2*S*, 3*S* and 5*R*, while that of FB_3 is described for the first time to be 2*S* and 3*S*. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Fumonisins represent a new class of mycotoxins produced by *Fusarium moniliforme* (Sheldon), a prevalent mould on corn, sorghum, and other grains throughout the world.[1] The fungus is associated particularly with two animal diseases, equine leukoencephalomalacia[2] and porcine pulmonary oedema.[3] Moreover, consumption of food contaminated with *Fusarium moniliforme* seems to be implicated in the aetiology of human oesophageal cancer in the Transkei region of South Africa.[4] The toxicological properties of fumonisin B_1 **1**, the most abundant isomer, have been investigated carefully and it has proved to be a cancer promoter and to be hepatotoxic and -carcinogenic to rats.[5,6] With respect to the toxicological effects of fumonisins on humans, research is still necessary in order to understand the mechanism of action. Fumonisins are sphinganine **3** analogues (Fig. 1) and they have been shown to be potent competitive inhibitors of ceramide synthase, a key enzyme in the sphingolipid metabolism, resulting in an accumulation of sphinganine and a complete blockage of the *de novo* sphingolipid biosynthesis.[7] Ceramide synthase inhibition has been characterized in vitro with liver and brain microsomes, as well as in intact mammalian cells in culture.[7]

In order to understand the enzymatic recognition of fumonisins as ceramide synthase inhibitors, the knowledge of the absolute configuration plays a decisive role. In the case of fumonisins, due to the

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PII: S0957-4166(98)00123-2

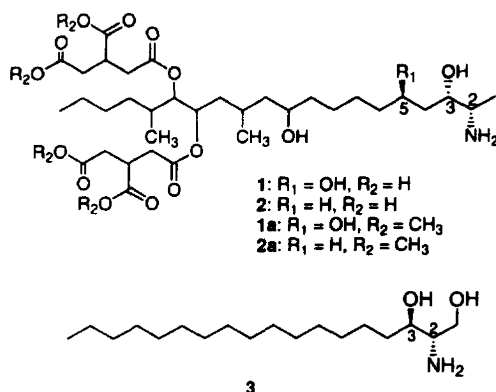
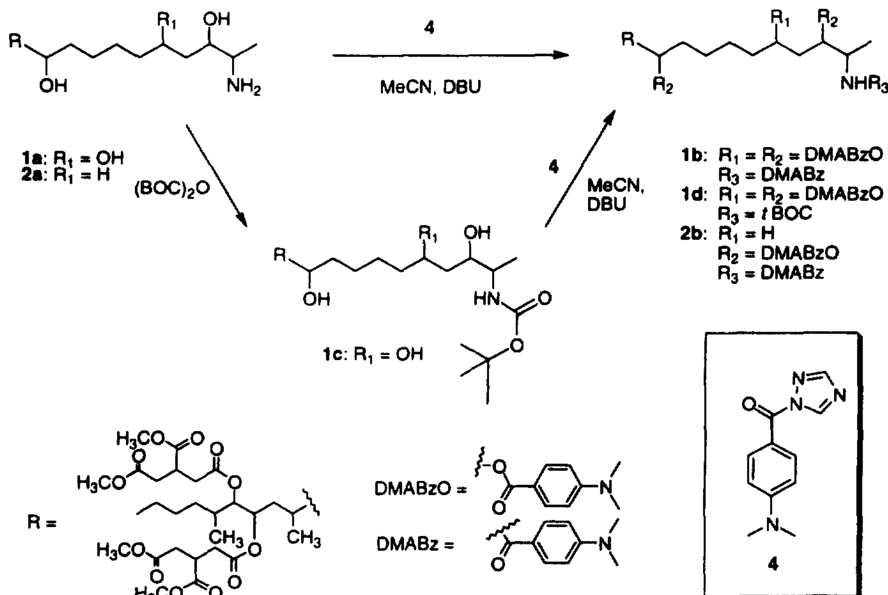


Fig. 1. Structures of FB_1 **1**, FB_3 **2**, FB_1Me_4 **1a**, FB_3Me_4 **2a** and sphinganine **3**

multitude of stereogenic centers, the determination of the absolute configuration emerges as a complex task. So far, the complete topological structures, ascertained by the synthesis of analogues and extensive NMR studies, have been reported only for fumonisins B_1 and B_2.[8,9] With respect to the remaining isomers, fumonisins of the 'A' and 'C' series and the recently discovered 'P' series containing a hydroxypyridinium moiety,[10] a more rapid and convenient method, especially useful on the microscale, would be desirable.

Here we report the determination of the absolute configuration of fumonisins by the circular dichroism (CD) exciton chirality method using the *p*-dimethylaminobenzoate chromophore **4** (Scheme 1). Since the intervention in the sphingolipid metabolism represents an important toxicological mechanism of FB_1, the stereochemistry of the sphinganine-analogue terminal part of the backbone bearing the hydroxy and amino groups is crucial for the mode of action as an enzyme inhibitor.[11] We therefore focused on this part of the molecule and can report the successful utilization of the exciton chirality method for assigning the absolute configuration of C2, C3 and C5 in FB_1 and of C2 and C3 in FB_3, respectively.



Scheme 1. Boc-protection and chromophoric derivatization of the fumonisin methyl esters **1a** and **2a**

2. Results and discussion

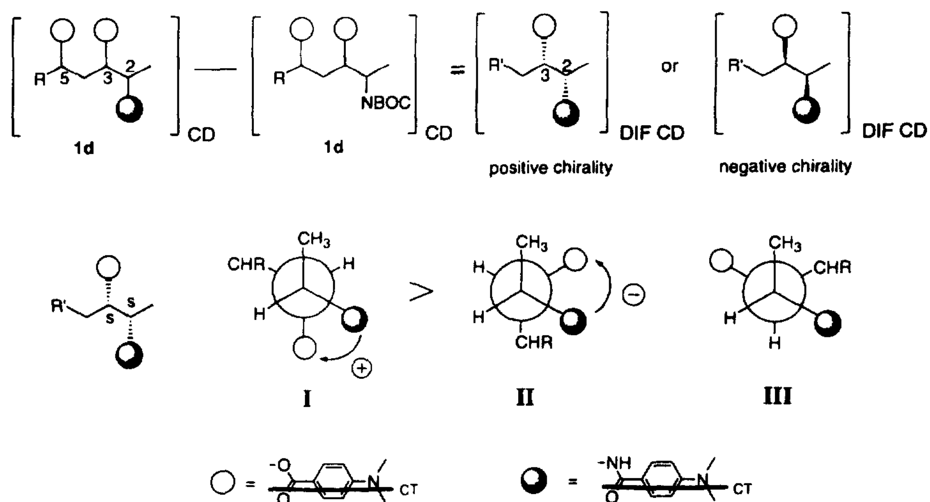
The circular dichroism exciton chirality method as a nonempirical microscale procedure to determine the absolute configuration has a wide scope of application, including natural products.[12] The method is based on the through-space interaction of two or more chromophores giving rise to bisignate CD curves. The signs of these are defined nonempirically by the absolute sense of twist between the coupled chromophoric electric transition dipole moments, which depends on the spatial arrangement of the functional groups bearing the chromophores.[12] A crucial aspect of the exciton chirality method is the additivity relation. When more than two identical chromophores interact through space, the amplitude A of the resulting CD curves can be approximated by the summation of each interacting basis pair (pair-wise additivity rule).[13,14] The principle of pairwise additivity was extensively studied with *p*-bromobenzoates of sugars[14] and was recently applied to the stereochemical assignment of natural products.[15] One of the most commonly used chromophores for hydroxy groups is *p*-methoxycinnamate.[16] However, since the *p*-methoxycinnamate group is light sensitive and undergoes cis/trans isomerization, we decided to apply the *p*-dimethylaminobenzoate, a chromophore more stable while having a comparably strong extinction coefficient (λ_{max} 309 nm, ε 30,400).[17]

We pursued the following strategy. The first step represented the methylation of the carboxyl functions in the tricarballylic side chains of FB_1 **1** with diazomethane, since the carboxyl groups would otherwise interfere with the chromophoric derivatization reaction. The methyl ester **1a** (Fig. 1) was submitted to derivatization with *p*-dimethylaminobenzoyltriazole **4** to obtain the chromophoric derivative **1b** (Scheme 1). In an additional approach, Boc-protection of the amino function of **1a** gave rise to **1c**, which was subjected to *p*-dimethylaminobenzoyltriazole **4** derivatization to yield **1d** (Scheme 1). The final products **1b** and **1d** were purified by preparative TLC on silica gel and were used for subsequent UV and CD[18] measurements. The CD spectrum of **1b** is determined by the interaction between the intramolecular charge transfer (CT) transitions (Scheme 2) of the chromophores at C2, C3 and C5. The benzoate at C10 has no appreciable influence due to the long spatial distance. The CD spectrum of **1d** reflects the coupling between the two interacting transition dipoles of the chromophores at C3 and C5 only, as the amino function at C2 is Boc-protected and bears no benzoate. Unambiguous assignment of the configuration at C2 and C3 is possible by calculating the difference CD spectrum (Scheme 2).

2.1. *Determination of the absolute configuration of chiral centers C2, C3 and C5 in FB_1*

The CD spectrum of the N-Boc-protected chromophoric derivative **1d** (Fig. 2(a), solid line) shows a strong negative split CD band with a negative first Cotton effect (CE) at 318 nm (−45.9) and a positive second CE at 291 nm (+18.6), amplitude A of −64.5, unequivocally establishing a negative chirality between the chromophoric substituents at C3 and C5. The strong CD effect indicates an anti configuration[20] and the negative sense of twist allows the stereochemical assignment of C3 to be *S* and C5 to be *R*. The CD curve of **1b** (Fig. 2(a), dashed line) is also characterized by a strong CD couplet, also showing a negative chirality with extrema at 319 nm (−35.5) and 293 nm (+12.0), but with a smaller amplitude A of −47.5.

Figure 2(b) shows the difference CD spectrum, obtained by subtracting the spectrum of **1d** from the spectrum of **1b**. As can be seen from Scheme 2, the difference CD spectrum represents only the interaction between the *p*-dimethylaminobenzoate chromophores attached to the NH_2-group in position 2 and the OH-group in position 3, exhibiting a positive first (315 nm, $\Delta\varepsilon$=+9.6) and a negative second (287 nm, $\Delta\varepsilon$=−7.0) Cotton effect, thus establishing a positive chirality. With the relative configuration known to be syn,[21] the absolute configuration was assigned to be 2*S* and 3*S* (Scheme 2). The relatively



Scheme 2. Correlations of the absolute configuration at C2 and C3 with the possible difference CD Cotton effects and Newman projections of the three staggered conformers (I–III) around the C2–C3 bond (—— transition dipole moment)

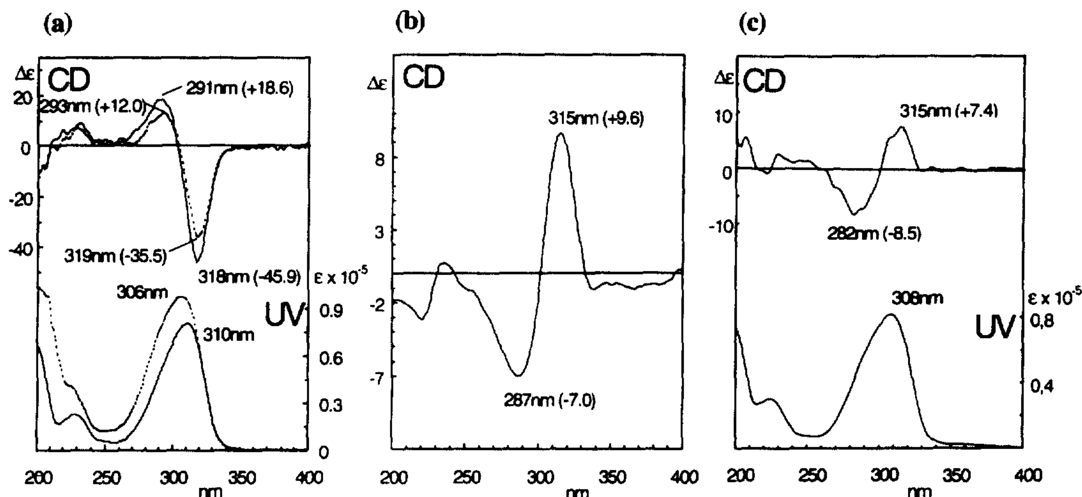


Fig. 2. (a) UV and CD spectra of **1b** (dashed line) and of **1d** (solid line) in MeCN; (b) difference CD spectrum (spectrum **1b**−spectrum **1d**); (c) UV and CD spectra of **2b** in MeCN[18]

weak CD effect (amplitude A=+16.6) reflects the population of the three staggered conformers **I–III**, which evoke contrary (**I, II**) or no (**III**) Cotton effects (Scheme 2).[20,22] Although the CD spectrum is dominated by the positive exciton chirality of the preferred rotamer **I**, the CD couplet is diminished by the negative exciton chirality of rotamer **II**, which decreases the overall CD amplitude.

2.2. Determination of the absolute configuration of stereogenic centers C2 and C3 in FB$_3$

In order to assign the absolute configuration for the stereogenic centers of the terminal part of the backbone in FB$_3$, an analogous strategy was applied. Transformation of **2** into the methyl ester **2a** (Fig. 1) was followed by chromophoric derivatization with **4** to afford **2b** (Scheme 1), which was purified by preparative TLC using silica plates. The UV and CD spectra of **2b** are shown in Fig. 2(c). The CD spectra of **2b** is characterized by a positive split CD curve with a positive first Cotton effect at 315 nm (+7.4),

a negative CE at 282 nm (−8.5) and an amplitude A of +15.9, representing a positive chirality between the chromophores at C2 and C3. Hence, the absolute configuration can be determined to be 2*S* and 3*S*, in analogy to FB_1. We assumed the substituents to be syn to each other, as the 1,2-anti configuration is reported to show almost no CD effect. In the 1,2-anti (erythro) case, the preferred conformation[19] leads to a dihedral angle of 180° between the chromophores resulting in a CD curve which is close to nil.[20,22] Moreover, Mori et al. applied a similar strategy to acyclic 1,2,4-triols and obtained comparable weak effects for the 1,2-benzoate derivatives.[23] Comparing the ^{13}C-NMR data of FB_3 with synthesized 2-amino-3-hydroxyoctadecanes of established relative configuration,[11] the relative stereochemistry of FB_3 at position C2 and C3 appears also to be syn.

FB_1 serves as a model compound as its relative and absolute configurations have already been determined by NMR structural studies. The observed CD effect resulting from exciton coupling between chromophores at C2 and C3, definitely syn to each other,[23] is of comparable magnitude, confirming the assumption of an 1,2-syn arrangement in the FB_3 case. From these data we can conclude that the absolute stereochemistry of the sphinganine-analogue terminal part of fumonisins FB_1 and FB_3 is the same (2*S*,3*S*).

3. Conclusions

In conclusion, the absolute configuration of the sphinganine-analogue part of the backbone of FB_1 was confirmed to be 2*S*, 3*S* and 5*R* and that of FB_3 was designated 2*S* and 3*S*. The absolute configuration at positions 2 and 3 in fumonisin FB_1 and FB_3 is different compared to sphingosin or sphinganin (2*S*,3*R*) and is therefore in agreement with the fact that fumonisins are inhibitors of the ceramide synthase, of which sphingosine would be the natural substrate. Furthermore, this work can serve as another demonstration for a successful application of the circular dichroism exciton chirality method, establishing it as an easy way to designate the absolute configuration of suitable molecules on the microscale.

4. Experimental

4.1. Chemicals

Fumonisin B_1 was purchased from Calbiochem–Novabiochem GmbH (Bad Soden, Germany). Fumonisin B_3 was kindly provided by Mary W. Trucksess (FDA, Washington, USA). Water and methanol, all of HPLC grade, and trifluoroacetic acid were from Merck (Darmstadt, Germany). Acetonitrile (absolute, over molecular sieve) was from Fluka (Neu–Ulm, Germany). TLC plates (silica gel, 60 F_{254}, 1 mm) were purchased from Merck (Darmstadt, Germany). All other chemicals were of reagent grade and either from Fluka (Neu–Ulm, Germany) or Aldrich (Steinheim, Germany).

4.2. General experimental procedures

^{1}H-NMR spectra were recorded on a Bruker 400 MHz instrument. Mass spectra were measured via loop injection on a Finnigan TSQ 7000 Triple Stage Quadrupole mass spectrometer with ESI interface (Finnigan MAT, Bremen, Germany). UV–vis and CD spectra were recorded as acetonitrile and chloroform solutions in a 1 cm cell on a Shimadzu UV-2101 PC spectrometer and a Jasco J-600 spectropolarimeter, respectively.

4.3. Preparation of p-dimethylaminobenzoyltriazole

A solution of *p*-dimethylaminobenzoic acid (2.0 mmol) and 1,1′-carbonyl-bis-(1,2,4-triazole) (2.2 mmol) in 4 ml dry MeCN was stirred overnight under an argon atmosphere at room temperature. After solvent evaporation, the residue was redissolved in 150 ml diethyl ether, extracted quickly with a 10% sodium hydrogencarbonate solution (3×10 ml) and with brine (1×10 ml). The organic layer was dried over sodium sulfate and after solvent evaporation, white crystals were obtained, yield 90%. ^{1}H-NMR ($CDCl_3$) δ 9.03 (s, 1H), 8.27 (d, *J*=9.2, 2H), 8.09 (s, 1H), 6.72 (d, *J*=9.2, 2H), 3.11 (s, 6H).

4.4. General procedure for the preparation of the methyl esters

To a solution of fumonisin (1 mg) in methanol (200 μl), freshly prepared diazomethane in ether was added dropwise until a yellow color persisted. The reaction mixture was evaporated to dryness and the tetramethyl esters were obtained as colorless oils, yields >90%.

4.4.1. Tetramethyl-FB_1 **1a**

^{1}H-NMR (CD_3OD) δ 3.86 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H). ESI-MS *m/z* 778 $[M+H]^+$. MS-MS (−35 eV) *m/z* 724 $[M+H-3H_2O]^+$, 556 $[M+H-C_8H_{12}O_6-H_2O]^+$, 352 $[M+H-2C_8H_{12}O_6-H_2O]^+$, 334 $[M+H-2C_8H_{12}O_6-2H_2O]^+$, 187 $[C_8H_{11}O_5]^+$.

4.4.2. Tetramethyl-FB_3 **2a**

^{1}H-NMR ($CDCl_3$) δ 3.59 (s, 3H), 3.64 (s, 3H), 3.65 (s, 3H), 3.68 (s, 3H), ESI-MS *m/z* 762 $[M+H]^+$.

4.4.3. Preparation of N-Boc-FB_1Me_4 **1c**

FB_1Me_4 (3.5 μmol) was dissolved in a 10% solution of triethylamine in methanol (10 μl), further diluted with additional methanol (100 μl) and di-*t*-butyldicarbonate $(Boc)_2O$ (7.0 μmol) was added with vigorous stirring. The mixture was then heated to 40–50°C for 15 min. Stirring was continued at room temperature for 30 min, the solvent evaporated under a nitrogen stream and the residue stirred for 10 min with ice-cold dilute hydrochloric acid (pH 2.15; 100 μl). The solution was extracted with ethyl acetate (5×200 μl) and the organic layer dried over sodium sulfate. Solvent evaporation resulted in an oily residue, which was characterized by ^{1}H-NMR and ESI-MS as the Boc-protected FB_1Me_4, yield 65%. ^{1}H-NMR (CD_3OD) δ 1.63 (s, 9H). ESI-MS *m/z* 878 $[M+H]^+$, 900 $[M+Na]^+$.

4.5. General procedure for the preparation of chromophoric derivatives

To a solution of the fumonisin tetramethyl esters (2.5 mg) in dry MeCN (1.25 ml) were added *p*-dimethylaminobenzoyltriazole (1.1 equiv. per functional group) and distilled DBU (1.2 equiv. per functional group). The mixture was stirred at room temperature for 48 h and the reaction was monitored by thin layer chromatography (silica gel, diethyl ether:dichloromethane (1:1), UV detection and *p*-anisaldehyde as spraying reagent). After vacuum concentration, the derivatives were purified via preparative TLC [silica gel, 2×diethyl ether:dichloromethane (1:1), 2×diethyl ether:acetone (8:2)]. The fluorescent bands were extracted with diethyl ether:dichloromethane (1:1) and subjected to HPLC–MS analysis.

*4.5.1. Tetrakis-(DMABz)-FB_1Me_4 **1b***

^{1}H-NMR ($CDCl_3$) δ 3.01 (3s, 24H), 3.59 (s, 3H), 3.64 (s, 3H), 3.65 (s, 3H), 3.68 (s, 3H), 4.40 (m, 1H), 4.90 (m, 1H), 5.12 (m, 3H), 5.29 (m, 1H), 6.58 (4d, *J*=8.5, 8H), 7.65 (d, *J*=8.5, 2H) 7.83 (3d, *J*=8.5, 6H). ESI-MS *m/z* 1365 $[M+H]^+$, 684 $[M+2H]^{2+}$. MS-MS (−10 eV) *m/z* 1219 $[M+H-C_9H_{10}NO]^+$, 1202 $[M+H-C_9H_{10}NO-H_2O]^+$, 1054 $[M+H-2C_9H_{10}NO-H_2O]^+$, 148 $[C_9H_{10}NO]^+$

*4.5.2. Tris-(DMABz)-N-Boc-FB_1Me_4 **1d***

^{1}H-NMR ($CDCl_3$) δ 1.57 (s), 3.01 (4s, 18H), 3.59 (s, 3H), 3.64 (s, 3H), 3.65 (s, 3H), 3.68 (s, 3H), 4.72 (m, 1H), 4.90 (m, 1H), 5.12 (m, 3H), 5.24 (m, 1H), 6.57 (3d, *J*=9.2, 6H), 7.83 (4d, *J*=9.2, 6H). ESI-MS *m/z* 1319 $[M+H]^+$, 660 $[M+2H]^{2+}$.

*4.5.3. Tris-(DMABz)-FB_3Me_4 **2b***

^{1}H-NMR ($CDCl_3$) δ 3.03 (4s, 18H), 3.61 (s, 3H), 3.66 (s, 3H), 3.67 (s, 3H), 3.69 (s, 3H), 4.43 (m, 1H), 4.90 (m, 1H), 5.14 (m, 3H), 6.39 (d, *J*=8.5, 2H), 6.66 (2d, *J*=5.5, 4H), 7.67 (d, *J*=8.8, 2H), 7.91 (3d, *J*=9.2, 4H). ESI-MS *m/z* 1203 $[M+H]^+$, 1225 $[M+Na]^+$.

Acknowledgements

We thank Mary W. Trucksess for supplying fumonisin standards. This study was supported by the Deutsche Forschungsgemeinschaft, Bonn (Hu 730/1-1) and the Fond der chemischen Industrie, Frankfurt.

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