Combined Synthetic/CD Strategy for the Stereochemical Assignment of the Tricarballylic Acid Side Chains of Fumonisin B_1

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The circular dichroism (CD) exciton chirality method was employed for the stereochemical assignment of the tricarballylic acid (TCA) side chains of fumonisin B_1 **1a** (FB_1). Using 2-naphthoate for chromophoric derivatization of the reduced TCA moieties, the absolute configuration was shown to be *R*. For additional confirmation, an optically active dihydroxy-*tert*-butanoate **2** related to the TCA group of fumonisin B_1 was synthesized to serve as a model compound.

Introduction

The fumonisins are a family of recently discovered mycotoxins produced by *Fusarium moniliforme* and widely spread among corn throughout the world.[1] Since they are classified by the International Agency for Research on Cancer (IARC) into group 2B as possibly carcinogenic substances[2] and an NTP long-term feeding study provided clear evidence for the carcinogenic activities of fumonisin B_1,[3] the interest in the toxicological properties of the fumonisins is increasing. Aiding the effort of understanding the mode of action, the knowledge of their relative and absolute configuration is a valuable contribution. Several approaches from various groups[4] led to the stereochemical assignment of an 2*S*,3*S*,5*R*,-10*R*,12*S*,14*S*,15*R*,16*R* configuration in the FB_1 backbone (**1a**). However, contradictory information existed regarding the stereochemistry at the chiral center in the tricarballylic acid (TCA) side chains of FB_1. Both *R* and *S* configurations were postulated,[5] determined via NMR studies and chiral gaschromatography of the methylesters, respectively. Only recently, another approach was published which supports the *R* configuration.[6] In all cases, the work implied large-scale syntheses and comparison of the NMR or GC−MS data of the various possible isomers. In our previous studies, we have successfully employed the circular dichroism (CD) spectroscopy for the stereochemical assignment of the amino terminus of FB_1 and FB_3, thus establishing the nonempirical exciton chirality method as a very convenient, elegant and reliable way for the structure elucidation of fumonisins.[4d] It was therefore our intention to develop a similar approach for the configurational assignment of the stereogenic centers in the TCA side chains of FB_1 via CD spectroscopy. The result reported here was additionally ensured by comparison with the synthesized compound **2** which served as a model compound.

Results and Discussion

The CD exciton chirality method is based on the through-space coupling of two or more chromophores either already existing in the molecule or introduced by derivatization of functional groups. This interaction gives rise to characteristic bisignate CD curves, the signs of which are defined by the absolute sense of twist between the coupled chromophoric electric transition moments reflecting their spatial arrangement in a nonempirical manner.[7] For the configurational assignment of the TCA side chains of FB_1 we used the strategy outlined in Scheme 1. To enhance the solubility in organic solvent the first step was the complete acetylation of the molecule with acetic anhydride. The resulting *N*-acetyl triacetate bis-anhydride **1b** was subjected to partial hydrolysis in aqueous THF to yield the free carboxylic groups again and was then selectively reduced with BH_3 in THF to give **1c**.[5b]

The NMR and MS data proved the successful formation of the *N*-acetyl triacetyl tetraol **1c**. Chromophoric derivatization using the 2-naphthoylimidazole **3** and DBU as a catalytic base yielded the tetranaphthoate **1d** (Scheme 1), the CD and UV spectra of which are depicted in Figure 1A.

The typically split CD curve is characterized by a positive first Cotton effect (CE) at 243 nm ($\Delta\epsilon$ +19.1) and a second negative CE at 229 nm ($\Delta\epsilon$ −20.7) with an overall amplitude A of +39.8, thus establishing a positive chirality between the ^1B_b bands of the two naphthoate chromophores.[8] As the CD effect is depending on the conformation, the preferred minimum energy conformations for **1d** both for the *S* and the *R* configuration were

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(1) Marasas, W. F. O. *Adv. Exp. Med. Biol.* **1996**, *392*, 1.

(2) International Agency for Research on Cancer. *Some naturally occurring substances: Food items and constituents, heterocyclic amines and mycotoxins*; IARC Monographs on the Evaluation of Carcinogenic risks to Humans, Vol. 56; IARC: Lyon, 1993.

(3) NTP study, NIH Publication No. 99-3955, US Department of Health and Human Services, National Institutes of Health, 1999.

(4) (a) ApSimon, J. W.; Blackwell, B. A.; Edwards, O. E.; Fruchier, A. *Tetrahedron Lett.* **1994**, *35*, 7703. (b) Blackwell, B. A.; Edwards, O. E.; ApSimon, J. W.; Fruchier, A. *Tetrahedron Lett.* **1995**, *36*, 1973. (c) Hoye, T. R.; Jimenez, J. I.; Shier, W. T. *J. Am. Chem. Soc.* **1994**, *116*, 9409. (d) Hartl, M.; Humpf, H.-U. *Tetrahedron: Asymmetry* **1998**, *9*, 1549.

(5) (a) Boyle, C. D.; Kishi, Y. *Tetrahedron Lett.* **1995**, *36*, 5695. (b) Shier, W. T.; Abbas, H. K.; Badria, F. A. *Tetrahedron Lett.* **1995**, *36*, 1571.

(6) Edwards, O. E.; Blackwell, B. A.; Driega, A. B.; Bensimon, C.; ApSimon, J. W. *Tetrahedron Lett.* **1999**, *40*, 4515.

(7) (a) Harada, N., Nakanishi, K. *Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, 1983. (b) Nakanishi, K., Berova, N. In *Circular Dichroism-Principles and Applications*; Nakanishi, K., Berova, N., Woody, R. W., Eds.; VCH: New York, 1994; pp 361−398.

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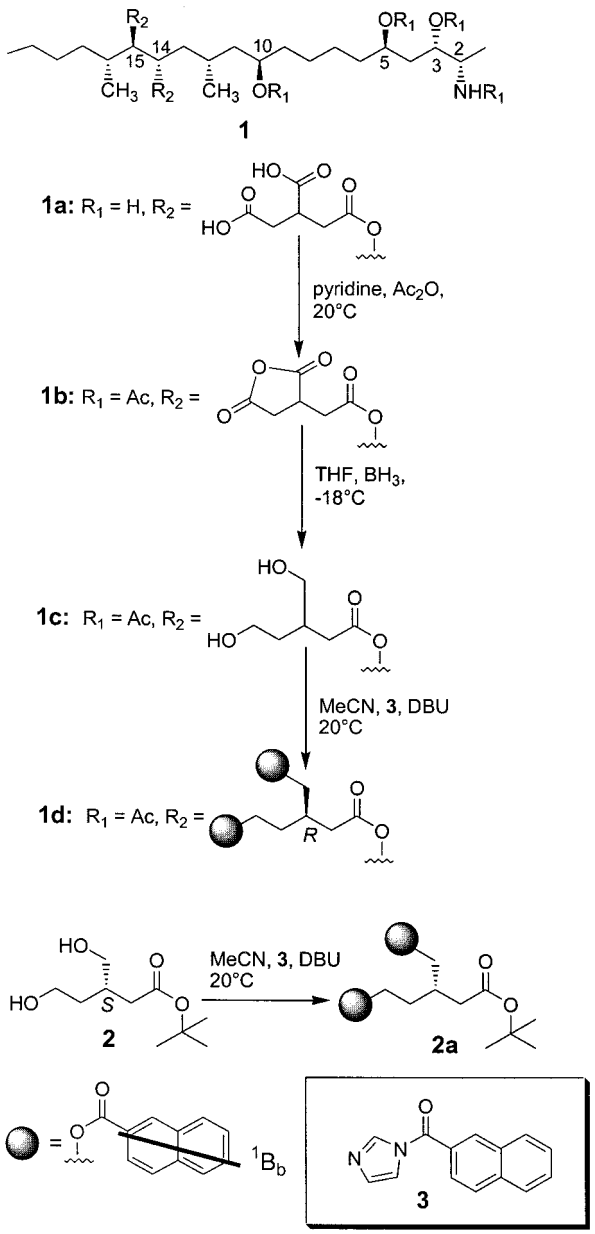
Scheme 1. Synthesis of Chromophoric Derivatives 1d and 2a



determined by computer calculation using MacroModel 5.0 with the modified Allinger MM2 force field.[9] Figure 2 shows the predominant low energy conformation of (*R*)-**1d** and illustrates the resulting first Cotton effect, being positive for an *R* configuration as the transition moments of the two chromophores are oriented in a clockwise sense. Correlation with the experimental data unambiguously establishes the stereochemistry of the TCA moieties of FB_1 to be *R*.

Regarding the question of their enantiomeric purity, earlier works have demonstrated that only one configuration exists for the tricarballylic acid units both at C-14 and C-15.[5a,6]

Though the exciton chirality method is an approved and nonempirical method for the establishment of absolute configurations, a comparison with the CD effects of appropriate model compounds to verify results is common practice, especially with acyclic substances lacking a rigid conformation. Thus, we decided to synthesize the diol **2** as a model compound, for which Boyle and Kishi[10] already provided a stereoselective reaction pathway. Briefly, the route employed a modified Hanessian procedure for the asymmetric Michael addition of a chiral allylphosphonamide to *tert*-butyl sorbate.[11] Ozonolysis and reduction with $NaBH_4$ resulted in the formation of **2** (Scheme 1). Due to the limited availability of enantiomerically pure starting material, we only synthesized the *S*-configurated compound. (*S*)-**2** was then derivatized using 2-naphthoylimidazole to give the bischromophoric derivative **2a** in quantitative yield (Scheme 1). (*S*)-**2a** revealed a negative split CD curve with extrema at 229 nm ($\Delta\epsilon$ +18.3) and 242 nm ($\Delta\epsilon$ −25.6), amplitude A of −43.9 (Figure 1B). Due to the modifications of the tricarballylic side chain carried out for FB_1, a direct comparison of the CD spectra of both **1d** and the model compound **2a** was possible. Since the synthetically derived *S*-configured moiety **2a** shows a mirror image CD, this is an additional proof of the *R* configuration in the TCA side chains of FB_1 and in good agreement with the molecular modeling results. Furthermore, in the preferred minimum energy conformation of (*S*)-**2a**, as determined by computer calculations using MacroModel 5.0,[9] the chromophoric transition moments show a counterclockwise orientation, predicting a negative chirality, which matches exactly the experimental observation.

Theoretically, if the interactions between the C-14 and C-15 side chains are negligible and the two side chains are homochiral, the exciton CD intensity of **1d** should be twice of that of **2a**. From the observed CD amplitudes of **1d** ($A = +39.8$) and **2a** ($A = -43.9$) we can conclude that there are weak CD interactions between the two side chains. However, this weak interaction between the two side chains will not influence the overall positive exciton coupling of each individual side chain for several reasons: (i) the absolute configuration of C-14 and C-15 is anti[4c] (see Scheme 1), and it is known from literature data that 1,2-anti diols bearing chromophoric groups give only very weak or no exciton coupling CD effects;[7] (ii) our computer calculations[9] showed that the two chromophores in each side chain are oriented in a clockwise sense (Figure 2) resulting in a positive CD couplet. Furthermore, the two side chains are oriented in that way that exciton coupling between the side chains would be expected to be very weak due to the long distance and/or the unfavorable alignment of the chromophores.

Though obtained by a completely different approach employing the circular dichroism exciton chirality method, our findings along with recent data[5a,6] provide clear evidence for the absolute configuration of the tricarballylic acid moieties in FB_1 to be *R*.

Experimental Section

General Aspects. All solvents used in reactions were of gradient grade or freshly distilled. MeCN, THF, and CH_2Cl_2 were dried over molecular sieves prior to use. FB_1 was

(8) The direction of the ^1B_b transition moment of the 2-naphthoate chromophore was determined by: Dong, J. G.; Akritopoulou-Zanze, I.; Guo, J.; Berova, N.; Nakanishi, K.; Harada, N. *Enantiomer* **1997**, *2*, 397.

(9) Calculations were carried out with MM2 force field in $CHCl_3$; Monte Carlo conformational search was used; at least 1000 conformers were searched for each simulation.

(10) Boyle, C. D.; Kishi, Y. *Tetrahedron Lett.* **1995**, *36*, 4579.

(11) Hanessian, S.; Gomtsyan, A.; Payne, A.; Hervé, Y.; Beaudoin, S. *J. Org. Chem.* **1993**, *58*, 5032.

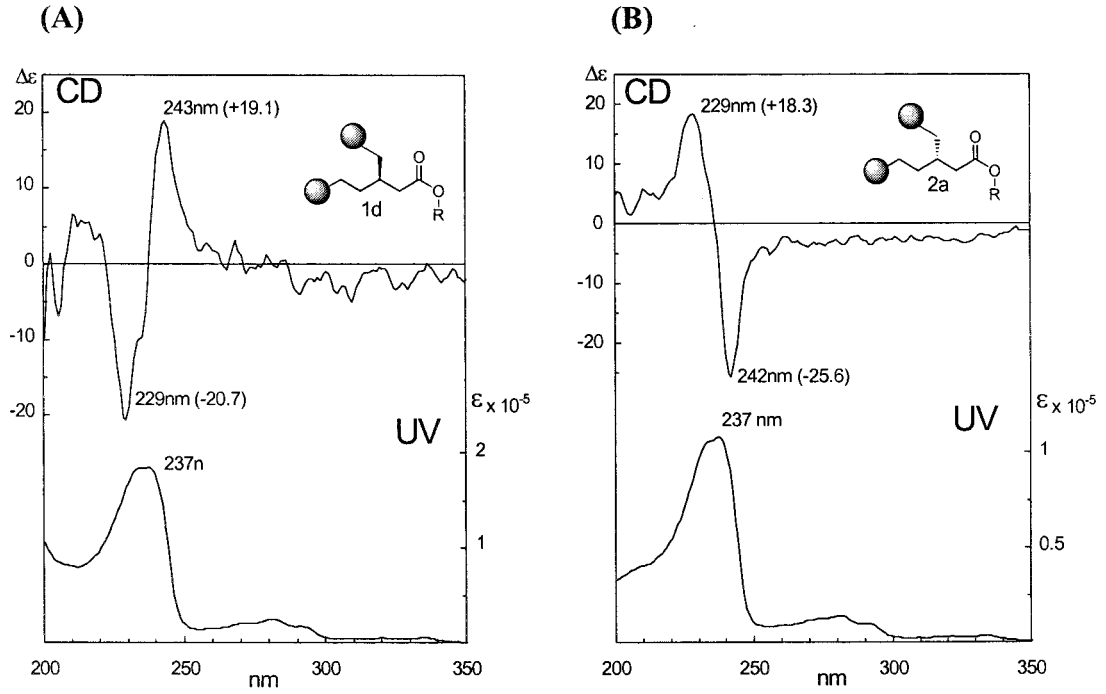
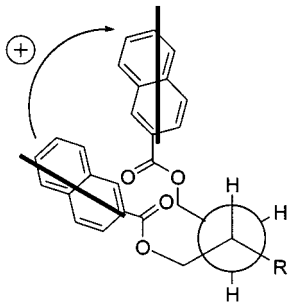


Figure 1. UV and CD spectra of **1d** (A) and **2a** (B) in acetonitrile.



***R*-1d**

Figure 2. Preferred conformation and the predicted sign of the first Cotton effect of *R*-**1d**, as determined by computer calculation using MacroModel 5.0[9] (bars indicate the location of the ^1B_b transition moments).

purchased from Alexis Biochemicals (Grünberg, Germany). (1*R*,2*R*)-Diaminomethylcyclohexane, serving as starting material for the synthesis of **2**, was from Acros Organics (Schwerte, Germany), and 2-naphthoylimidazole for chromophoric derivatizations was supplied by Fluka (Neu-Ulm, Germany).

^{1}H NMR spectra were recorded at 400 or 600 MHz in $CDCl_3$. Chemical shifts are relative to $CHCl_3$ ($\delta = 7.26$ ppm); coupling constants (*J*) are given in hertz (Hz). UV−vis and CD spectra were recorded as acetonitrile solutions in a 1-cm cell on a Shimadzu UV-2101 PC-spectrometer (Shimadzu, Kyoto, Japan) and a JASCO J-600 spectropolarimeter (Jasco, Gross-Umstadt, Germany), respectively. Mass spectra were measured via loop injection on a Finnigan TSQ 7000 triple stage quadrupole mass spectrometer with ESI interface (Finnigan MAT, Bremen, Germany). HPLC analyses were performed using a Knauer Maxi Star pump and a Knauer variable-wavelength monitor UV detector. For chromatographic separations an Eurospher 100 C18 column (250 × 4 mm i.d., Knauer, Berlin, Germany) was employed.

Preparation of 1b. To a solution of FB_1 **1a** (4 mg, 5.6 μmol) in 200 μL of dry pyridine was added 200 μL of acetic anhydride, and the mixture was stirred at room temperature overnight. After addition of 200 μL of CH_2Cl_2, the pyridine was removed by extraction with a 7% $CuSO_4$ solution, the organic layer was dried over $NaSO_4$, and the solvent was gently evaporated in a nitrogen stream, yield >90%. ESI-MS: *m/z* 854 $[M + H]^+$, 876 $[M + Na]^+$.

Preparation of 1c. Prior to reduction, **1b** was treated at room temperature with aqueous THF to yield the free carboxyl groups. The solvent was removed and the resulting product dried in vacuo. The following reduction[12] was carried out under argon atmosphere. A solution of the product (4.9 mg, 5.5 μmol) in 100 μL of dry THF was cooled to −18 °C in an ice−salt bath, and 22 μL of a BH_3 solution (1 M in THF) was added dropwise with a syringe. The resulting clear reaction mixture was stirred well, and the ice−salt bath was allowed to equilibrate to room temperature during a 16-h time period. Excess hydride was destroyed by addition of 200 μL of water at 0 °C. The aqueous phase was saturated with potassium carbonate, the THF layer separated, and the water layer extracted with THF (3 × 500 μL). The combined organic extracts were washed with 500 μL of brine and dried over Na_2SO_4 and the solvent evaporated, yield 87%. ESI-MS: *m/z* 834 $[M + H]^+$, 856 $[M+Na]^+$.

Preparation of 2. The *S*-configurated compound was synthesized stereoselectively starting with (1*R*,2*R*)-diaminomethylcyclohexane as described by Boyle and Kishi.[10]

Procedure for the Preparation of Chromophoric Derivatives 1d and 2a. Product **1c** (4 mg, 4.8 μmol) or **2** (1.3 mg, 6.4 μmol) was transferred to dry, argon-flushed vials, and 2-naphthoylimidazole (23 μmol and 15.4 μmol respectively; 1.2 equivalents per OH group) was added. The vials were evacuated, flushed with argon, and sealed with a septum. Compounds were dissolved in 1 mL of dry MeCN, and DBU (3.6 and 4,6 μL; 1.2 equiv per OH group) as a catalytic base was added with a syringe. The mixtures were stirred at room temperature until no further loss of starting material could be detected by thin-layer chromatography. Product **2a** was purified using reversed-phase high-performance liquid chromatography (RP-HPLC; UV detection 237 nm) with an acetonitrile/water gradient, product **1d** was isolated via thin-layer chromatography (SiO_2, diethyl ether−dichloromethane 1:1) and further purified by isocratic RP-HPLC (UV detection 237 nm) using acetonitrile.

FB_1-*N*-acetyl-3,5,10-triacetyltetranaphthoyltetraol (1d). ESI-MS: *m/z* 1451 $[M + H]^+$, 1468 $[M + NH_4]^+$. ^{1}H NMR (600

(12) Yoon, N. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem.* **1973**, *38*, 2786.

MHz, $CDCl_3$): δ 0.78 (t, J = 6.46/6.96 Hz, 3H), 0.88 (dd, J = 7.06/6.96 Hz, 6H), 1.23 (d, J = 7.67 Hz, 3H), 2.05 (s, 12H), 2.37 (q, J = 7.67 Hz, 4H), 2.58 (dd, J = 6.56/5.85 Hz, 4H), 2.67 (m, 2H), 4.47−4.58 (m, 8H), 4.86 (m, 2H), 4.95 (m, 1H), 5.02 (m, 1H), 5.19 (brd, J = 10.60 Hz, 1H), 5.56 (brd, J = 9.29 Hz, 1H), 7.48 (dd, J = 7.07/7.26 Hz, 4H), 7.55 (dd, J = 7.16/7.07 Hz, 4H), 7.80 (m, 8H), 7.87 (dd, J = 8.18/8.07 Hz, 4H), 8.00 (m, 4H), 8.54 (s, 2H), 8.56 (s, 2H).

***tert*-Butyl 3,4-Bis[hydroxymethyl(naphthoyl)]butanoate (2a).** ESI-MS: m/z 530 $[M + NH_4]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.46 (s, 9H), 2.09 (q, J = 6.62/6.98 Hz, 2H), 2.54 (dd, J = 6.61/6.98 Hz, 2H), 2.67 (m, 1H), 4.49 (dd, J = 3.0/3.3 Hz, 2H), 4.56 (t, J = 6.6/6.3 Hz, 2H), 7.54 (t, J = 6.6 Hz, 2H), 7.60 (m, 2H), 7.86 (m, 4H), 7.92 (dd, J = 6.99/6.98 Hz, 2H), 8.05 (d, J = 8.46, 2H), 8.59 (s, 1H), 8.61 (s, 1H).

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