Pergamon

Tetrahedron Letters, Vol. 35, No. 37, pp. 6819-6822, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01477-9

Relative and Absolute Stereochemistry of the Fumonisin B_2 Backbone

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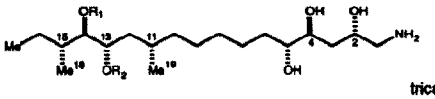
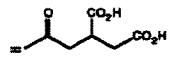
Abstract: The relative and absolute stereochemistry of the backbone of fumonisin B_2 is established to be **7a**.

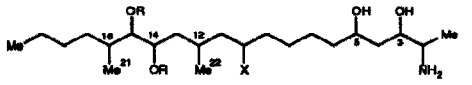
Fumonisins are mycotoxins produced by the corn pathogen *Fusarium moniliforme*.[1] They are known to be carcinogenic and directly linked to human esophageal cancer. Coupled with its contamination in some commercially based corn products, this biological activity has drawn worldwide attention to the fumonisin family of mycotoxins.[2] Many comparisons have been made between fumonisins and AAL toxins produced by the tomato fungus *Alternaria alternata* f. sp. *lycopersici*.[3] In particular, both families exhibit cross-bioactivity and have been shown to inhibit sphingolipid biosynthesis.[2,4] Fumonisins and AAL toxins bear striking structural similarity. Their gross structures have been determined, but their relative and absolute stereochemistry remains unknown.[1,3] We have recently established the relative and absolute stereochemistry of the backbone of AAL toxin T_A by using a stepwise approach: (1) determination of the relative stereochemistry of the left and right halves independently, (2) differentiation of the two possible diastereomers, corresponding to **4a** and **6a** in the fumonisin B_2 series, by the ^1H NMR spectra in the presence of an achiral shift reagent, and (3) determination of the absolute stereochemistry of the amino alcohol, corresponding to **4a** vs. **7a** in the fumonisin B_2 series, by the ^1H NMR spectra in the presence of a chiral shift reagent.[5]



AAL-toxin T_A :
a mixture of R_1=TCA / R_2=H
and R_1=H / R_2=TCA

tricarballylic acid (TCA)



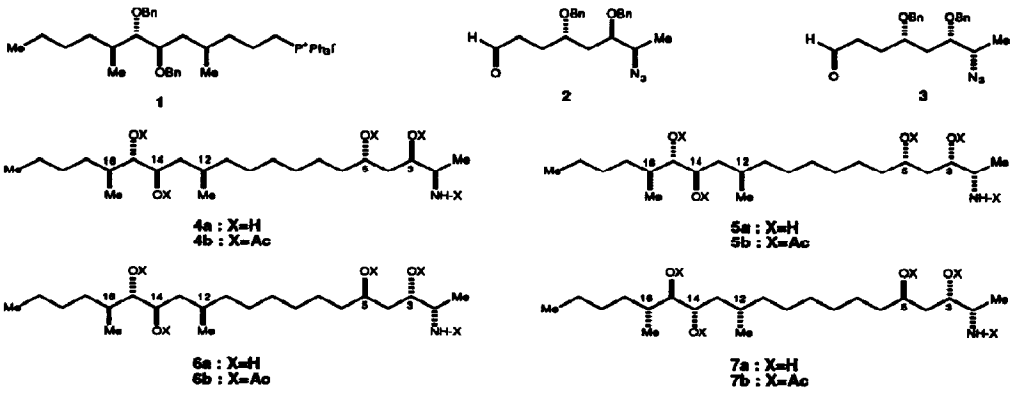


fumonisin B_1 : X=OH, R=TCA
fumonisin B_2 : X=H, R=TCA

The striking similarity in their gross structures may suggest that fumonisins and AAL toxins are biosynthesized via related pathways, yielding the same stereochemical array on their backbones. Indeed, the ^1H NMR characteristics reported for the C12-C16 moiety of N-acetyl fumonisin B_1 methyl ester[1] compare amazingly well with those for the corresponding portion of the peracetate prepared from the amino alcohol of AAL toxin T_A. This fact convincingly argues that the stereochemistry of the left half of fumonisins relates to that of AAL toxins. Then, it is tempting to suggest that the stereochemistry at the C3 and C5 positions of fumonisin B_1 or B_2 corresponds to that at the C2 and C4 positions of AAL toxin T_A. Comparing the ^{13}C NMR data of the C1-C4 portion of N-acetyl fumonisin B_1 methyl ester with the acetates derived from 2-aminotetradeca-5,7-dien-3-ols,[6] the relative stereochemistry at the C2 and C3 positions appears to be syn. On the basis of these considerations and

assumptions, we have suggested the stereochemistry of the backbone of fumonisin B_2 to be **7a**.[5] Extending the stepwise approach used for AAL toxin T_A to the present case, we present the proof for the suggested structure.

The phosphonium salt **1**, corresponding to the left half, was synthesized from (S)-(-)-2-methyl-1-hexanol and (R)-(-)-citronellyl bromide.[7] For the right half, the diastereomeric azido aldehydes **2** and **3**, both bearing the C2-C3 syn configuration,[8] were synthesized from L-glutamic acid[9]---note that both **1** and **2/3** correspond to the antipode of **7a**, which is required for the absolute stereochemistry assignment (*vide infra*). The synthetic amino alcohol hydrochloride salts **4a·HCl** and **5a·HCl** were obtained via Wittig olefination (*n*-BuLi/THF/-78° C→r.t.) of **1** and **2** or **3**, followed by hydrogenation/hydrogenolysis (H_2/Pd on C/H^+/MeOH). The ^{1}NMR spectrum of the amino alcohol hydrochloride **7a·HCl**, derived from natural fumonisin B_2[10], was found to be clearly different from that of **5a·HCl**, but superimposable on that of **4a·HCl**. This experiment established that **1** and **2** represented the relative stereochemistry of the left and right halves, and that the relative stereochemistry of the backbone of fumonisin B_2 was represented by either **4a·HCl** or its diastereomer **6a·HCl**. Therefore, the HCl salt **6a·HCl** was synthesized from **1** and the enantiomer of **2**.[11] As expected, **4a·HCl** and **6a·HCl**, as well as **7a·HCl**, gave identical ^{1}H NMR spectra.[12]



In order to differentiate **4a** and **6a**, the corresponding pentaacetates **4b** and **6b** were synthesized. As before, both synthetic acetates gave identical ^{1}H NMR spectra, which were indistinguishable from that of the peracetate **7b** derived from natural fumonisin B_2.[13] However, **4b** and **6b** were found to exhibit different ^{1}H NMR responses to an achiral shift reagent, and **4b**'s responses coincided with those of **7b**; a 2:1 mixture of **6b** and **7b** behaved as two different compounds in the presence of Eu(fod)$_3$, but a 2:1 mixture of **4b** and **7b** acted as a single compound (Figures 1 and 2). This established **4a** to represent the relative stereochemistry of the backbone of fumonisin B_2.

Finally, using the same method as used for AAL toxin T_A, the absolute stereochemistry of the backbone of fumonisin B_2 was discovered to be **7a**; a 2:1 mixture of **4b** and **7b** behaved as two chemically different substances in the presence of (+)-Eu(hfc)$_3$ as depicted in Figure 2. In addition, the signs of optical rotation observed for **4b** ($[\alpha]_D$ +27° (*c* 0.12, $CHCl_3$)) and **7b** ($[\alpha]_D$ -29° (*c* 0.10, $CHCl_3$)) support this conclusion.

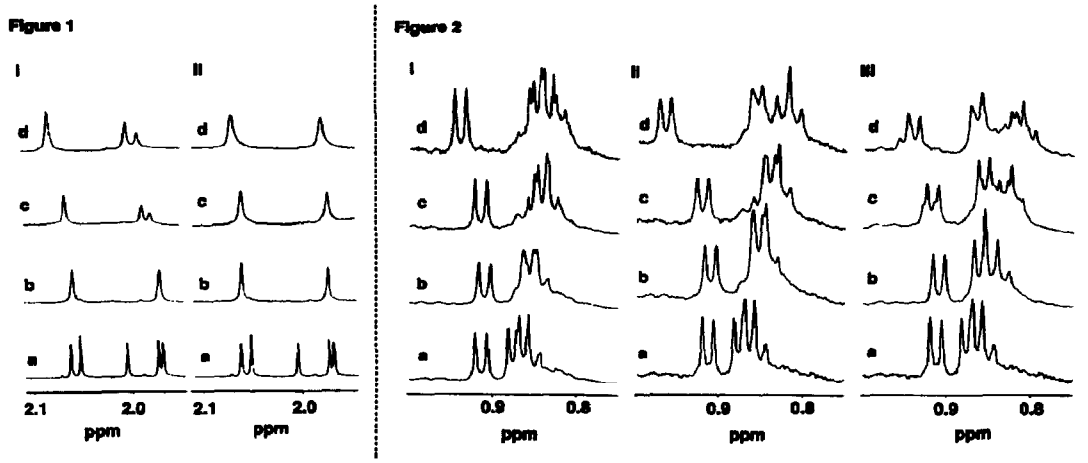


Figure 1. Acetate group region of ^{1}H NMR (500 MHz, $CDCl_3$). I: Eu(fod)3 titration of a 2:1 mixture of **6b** and **7b**. II: Eu(fod)3 titration of a 2:1 mixture of **4b** and **7b**. (a) 0 eq. Eu(fod)3. (b) ca. 0.2 eq. Eu(fod)3. (c) ca. 0.6 eq. Eu(fod)3. (d) ca. 0.8 eq. Eu(fod)3. *Note:* Upon addition of ca. 0.2 eq. Eu(fod)3, three out of the five acetate peaks broadened significantly and shifted downfield beyond 2.1 ppm.

Figure 2. Methyl group region of ^{1}H NMR (500 MHz, $CDCl_3$). I: Eu(fod)3 titration of a 2:1 mixture of **6b** and **7b**. II: Eu(fod)3 titration of a 2:1 mixture of **4b** and **7b**. III: (+)-Eu(hfc)3 titration of a 2:1 mixture of **4b** and **7b**. (a) 0 eq. EuR3. (b) ca. 0.4 eq. EuR3. (c) ca. 0.8 eq. EuR3. (d) ca. 1.2 eq. EuR3.

Further studies on the stereochemistry of the tricarballylic acid moiety of fumonisins and AAL toxins are in progress in our laboratories.

Acknowledgment. Financial support from the National Institutes of Health (NS 12108) and the National Science Foundation (CHE 89-09762) is gratefully acknowledged. J. C. H. thanks Groupe de Recherche Servier, Neuilly sur Seine, France, for a postdoctoral fellowship.

References and Footnotes:

1. Bezuidenhout, S. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Horak, R. M.; Marasas, W. F. O.; Spiteller, G.; Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1988**, 743.

2. For 18 reviews regarding various aspects of biological activity of fumonisins and AAL toxins, see *Mycopathologia*, **1992**, *117*, 1-124 .

3. (a) Bottini, A. T.; Gilchrist, D. G. *Tetrahedron Lett.* **1981**, *22*, 2719. (b) Bottini, A. T.; Bowen, J. R.; Gilchrist, D. G. *Tetrahedron Lett.* **1981**, *22*, 2723.

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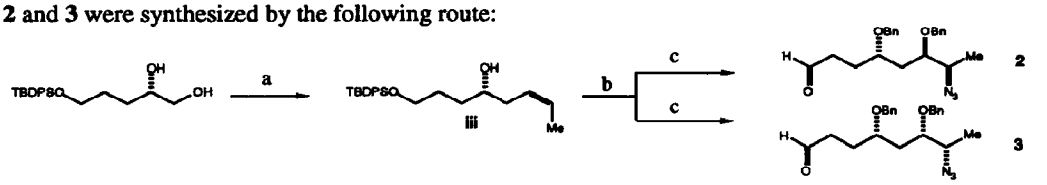
6. (a) Gulavita, N. K.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 366. (b) Mori, K.; Matsuda, H. *Liebigs Ann. Chem.* **1992**, 131.

7. 1 was synthesized by the route used to make a similar intermediate for AAL toxin[5], using (S)-(-)-2-methyl-1-hexanol instead of (S)-(-)-2-methyl-1-butanol. (S)-(-)-2-Methyl-1-hexanol was prepared from the procedure given in Kato, M.; Mori, K. *Agric. Biol. Chem.* **1985**, *49*, 2479.

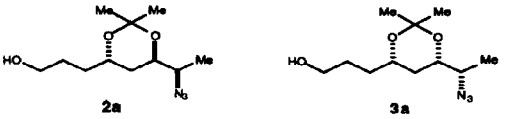
8. Inspection of ref. 6 shows that when the C2 amine and C3 hydroxyl group are syn, the C1 methyl of the peracetate derivative has a ^{13}C NMR chemical shift of ~18 ppm. However, when C2 and C3 are anti, the chemical shift is ~14 ppm. Fumonisin B_2 peracetate **7b** and model compounds **i** and **ii** all have a C1 methyl chemical shift at ~18 ppm in their ^{13}C NMR spectra.

9. **2** and **3** were synthesized by the following route:



Reagents and conditions: (a) (1) NaH, THF, then Ts-imid. (2) cis-propenylmagnesium bromide, CuI, THF. (b) (1) NaH, BnBr, TBAI, THF/DMF. (2) MCPBA, CH_2Cl_2. (3) NaN_3, TBAI, DMF/$HOCH_2CH_2OCH_3$/DME/H_2O. (4) NaH, BnBr, TBAI, THF/DMF, then separation of diastereomers via SiO_2 column chromatography. (c) (1) TBAF, THF. (2) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N.

The starting diol was synthesized in 6 steps from L-glutamic acid by a modified Larcheveque procedure: Larcheveque, M.; Lalande, J. *Tetrahedron* **1984**, *40*, 1061. The stereochemistry of **2** and **3** was determined by comparing the ^{13}C NMR data of derivatives **2a** and **3a** as reported by Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511.



10. The authentic amino alcohol was prepared from natural fumonisin B_2, purchased from the South African Research Council, Tygerberg, South Africa. Thus, fumonisin B_2 was treated with boiling NaOH to afford the free amine alcohol **7a**. Subsequent treatment with conc. HCl in MeOH provided the HCl salt **7a·HCl**, and treatment with acetic anhydride and pyridine gave the acetate **7b**.

11. The enantiomer of **2** was synthesized from **iii** via Mitsunobu inversion, followed by the sequence given in ref. 9.

12. ^1H NMR (500 MHz, D_2O) of **4a·HCl**, **6a·HCl** and **7a·HCl**: δ 0.76 (3H, t, *J* = 7.0 Hz), 0.77 (3H, d, *J* = 6.7 Hz), 0.80 (3H, d, *J* = 6.7 Hz), 0.98 (2H, m), 1.0-1.6 (22H, m), 1.18 (3H, d, *J* = 6.7 Hz), 3.13 (1H, m), 3.20 (1H, dd, *J* = 5.2, 6.4 Hz), 3.66 (2H, m), 3.74 (1H, m). **5a·HCl:** 0.77 (3H, t, *J* = 7.0 Hz), 0.78 (3H, d, *J* = 6.7 Hz), 0.82 (3H, d, *J* = 6.7 Hz), 0.99 (2H, m), 1.0-1.7 (22H, m), 1.19 (3H, d, *J* = 6.8 Hz), 3.20 (2H, m), 3.63 (1H, ddd, *J* = 3.4, 7.2, 10.2 Hz), 3.68 (1H, ddd, *J* = 1.8, 5.1, 10.3 Hz), 3.78 (1H, m).

13. ^1H NMR (500 MHz, $CDCl_3$) of **4b, 6b and 7b**: δ 0.86 (3H, t, *J* = 7.1 Hz), 0.88 (3H, d, *J* = 6.4 Hz), 0.91 (3H, d, *J* = 6.8 Hz), 1.0-1.8 (24H, m), 1.08 (3H, d, *J* = 6.8 Hz), 1.97 (3H, s), 1.97 (3H, s), 2.00 (3H, s), 2.05 (3H, s), 2.06 (3H, s), 4.13 (1H, m), 4.86 (1H, m), 4.88 (1H, dd, *J* = 3.3, 8.5 Hz), 4.95 (1H, m), 5.12 (1H, ddd, *J* = 2.7, 3.1, 10.8 Hz), 5.53 (1H, d, *J* = 9.3 Hz).

(*Received in USA* 3 *June* 1994; *revised* 21 *July* 1994; *accepted* 26 *July* 1994)