Pergamon

Tetrahedron Letters, Vol. 36, No. 10, pp. 1571-1574, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)00090-9

Complete Structures of the Sphingosine Analog Mycotoxins Fumonisin B_1 and AAL Toxin T_A: Absolute Configuration of the Side Chains

W. Thomas Shier*[1], Hamed K. Abbas[2] and Farid A. Badria[1]

[1]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455, USA
[2]Southern Weed Science Laboratory, Agricultural Research Service, United States Department of Agriculture, Stoneville, MS 38776, USA

Abstract: Fumonisin B_1 and AAL toxin T_A are sphingosine-analog mycotoxins characterized by propane-1,2,3-tricarboxylic acid side chains esterified to alkylamine backbones. The absolute configuration of all stereogenic centers in the backbones is known. Using chiral gas chromatography methodology we have determined the absolute configuration at C-3' in the side chains to be S, thereby completing structure determination of both toxins.

Fumonisin B_1[1] (FB_1) (**1a**) (Fig. 1) and AAL toxin T_A[2] (**2a**) (Fig. 2) are mycotoxins produced by two agriculturally important plant pathogens, *Fusarium moniliforme* and *Alternaria alternata*. FB_1 is of particular concern because of the ubiquitous occurrence of the fungus on maize, and because it is a putative environmental tumor promoter with sufficient chemical stability to persist through most types of normal food processing.[3,4] The 2D-structure of AAL toxin T_A was determined by Bottini *et al.*[2] and that of FB_1 by Bezuidenhout *et al.*[1] who showed that the toxins consist of one (AAL toxin T_A) or two (FB_1) propane-1,2,3-tricarboxylic acid (PTCA) side chains esterified to alkylamine backbones that are structurally analogous to sphingosine,[5] a putative intracellular regulatory lipid.[6] The absolute configuration at each stereogenic center in the FB_1 backbone has been determined by Hoye *et al.*[7] using a combination of NMR and chiral gas chromatography methods. The same relative configurations were established for the C-1 to C-5 fragment of FB_1 by ApSimon *et al.*[8,9] and by Pock *et al.*[10] based on NMR analysis of 2,3-carbamate and oxazoline derivatives, respectively; and for the C-10 to C-16 fragment of FB_1 by Blackwell *et al.*[11] using NMR analysis of a 10,14-cyclic ether derivative of the FB_1 backbone. The absolute configuration at each stereogenic center was established in AAL toxin T_A backbone by Boyle *et al.*,[12] and in fumonisin B_2 by Harmange *et al.*,[13] using a combination of synthetic and NMR methods. The only unknown structural feature of these toxins is the absolute configuration at C-3' of the side chain PTCA. The PTCA side chains are of particular interest in light of the observation[14] that hydrolyzed fumonisins (aminopentols) not only retain biological activity despite

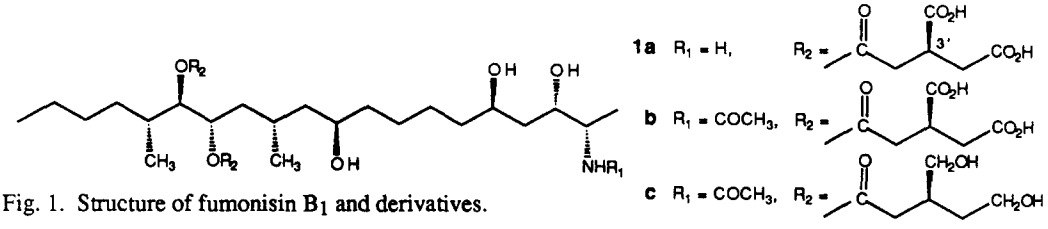


Fig. 1. Structure of fumonisin B_1 and derivatives.

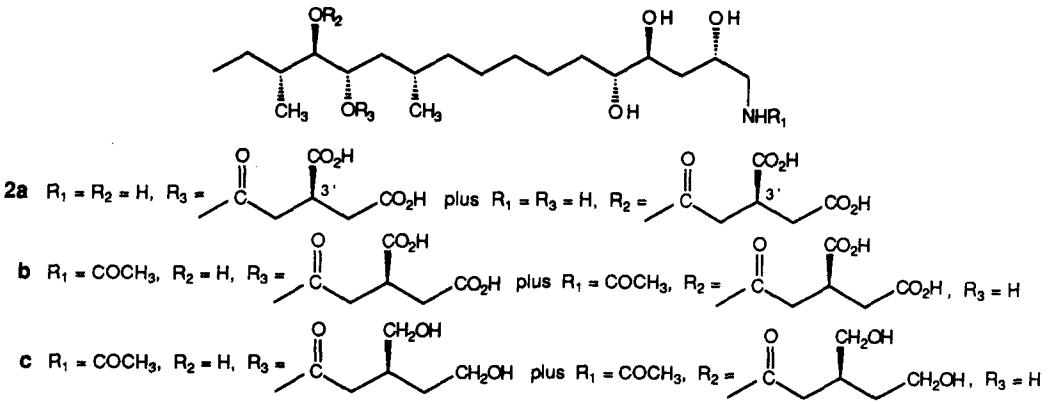
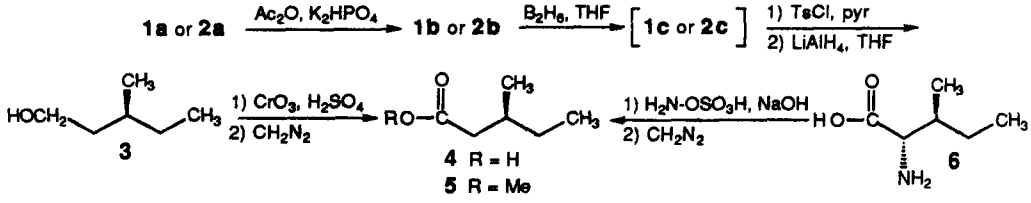


Fig. 2. Structure of AAL toxin T_A and derivatives.

having lost about half their molecular weight, but also have a broader spectrum of activity than the intact toxins. This observation has led to the suggestion that the producing fungi may enzymatically add PTCA moieties to the toxin backbone as a detoxification mechanism. To better understand the nature of the side chains we have used chiral gas chromatography column methodology to show that the absolute configuration at C-3' is *S* in both FB_1 and AAL toxin T_A.

The same approach was used to determine the absolute configuration at C-3' of the side chains of the two toxins, specifically conversion (Fig. 3) of the side chains to a derivative resolvable on a chiral gas chromatography column. Since free PTCA is symmetrical, it is necessary to differentiate the free carboxyl groups from the esterified carboxyl prior to separation from the backbone. Selective reduction of free carboxyl groups with diborane in THF[15] was chosen in preference to borohydride salt reduction of the ester linkage, because diborane reduction yields derivatives that do not have an asymmetric proton alpha to a free carboxyl group, and hence would not be expected to racemize under mild alkaline conditions. Reduction of the toxins[16,17] by diborane in THF was facilitated by conversion[18] to the N-acetyl derivatives (**1b** and **2b**), which increased solubility in THF. In order to minimize the possibility that the side chains might be lost by intramolecular transesterification, the predicted products **1c** and **2c** were protected from acid, and immediately tosylated with tosyl chloride in pyridine and reduced with $LiAlH_4$ in THF. The side chains, released as alcohol **3**, were extracted into ether using saturated citric acid solution to suppress extraction of the alkylamine backbone, and examined by gas chromatography on a Chiraldex GT-A column.[19] However, neither the corresponding racemic alcohol (3-methyl-1-pentanol) nor its acetate was resolved into two peaks on the column[19]. The released side chain alcohols **3** were subjected to Jones oxidation[20] to yield the carboxylic acids



4, which were examined by gas chromatography on a Chiraldex GT-A column.[21] Again, the corresponding racemic free carboxylic acid (3-methylvaleric acid) was not resolved, but the methyl ester[21] gave baseline resolution.[22] Authentic (*S*)-3-methylvaleric acid methyl ester (**5**) was prepared from L-isoleucine (**6**) by treatment with hydroxylamine-O-sulfonic acid in alkali to yield **4**,[23] which was methylated with diazomethane to yield **5**.[24] The *S* configuration of the stereogenic centers in L-isoleucine [(2*S*,3*S*)-2-amino-3-methylpentanoic acid] are known from the x-ray crystallographic studies of Trommel and Bijvoet.[25] The side chain acids **4** released from the toxins were methylated with diazomethane to yield **5**. The side chain derivatives released from both FB_1 and AAL toxin T_A eluted with the retention time of (*S*)-**5**.[22]

These observations complete the 3-D structure determinations for both FB_1 and AAL toxin T_A.

Acknowledgements. This work was supported by grant 93-37201-9561 awarded by NRICGP/USDA. We thank T. Krick for obtaining FAB-MS spectra, and Dr. T. R. Hoye for valuable discussions.

REFERENCES AND NOTES

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**, 743-745.
2. Bottini, A. T.; Gilchrist, D. G. *Tetrahedron Lett.* **1981**, *22*, 2719-2722; Bottini, A. T.; Bowen, J. R.; Gilchrist, D. G. *Tetrahedron Lett.* **1981**, *22*, 2723-2726.
3. Riley, R. T.; Norred, W. P.; Bacon, C. W. *Annu. Rev. Nutr.* **1993**, *13*, 167-189.
4. Scott, P. M. *Int. J. Food Micro.* **1993**, *18*, 257-270.
5. Shier, W. T. *J. Toxicol. Toxin Rev.* **1992**, *11*, 241-257.
6. Merrill, A. H.; Hannun, Y. A.; Bell, R. M. *Advances in Lipid Res.* **1993**, *25*, 1-24.
7. Hoye, T. R.; Jiménez, J. I.; Shier, W. T. *J. Amer. Chem. Soc.* **1994**, *116*, 9409-9410.
8. ApSimon, J. W.; Blackwell, B. A.; Edwards, O. E.; Fruchier, A. *Tetrahedron Lett.* **1994**, *35*, 7703-7706.
9. ApSimon, J. W.; Blackwell, B. A.; Edwards, O. E.; Fruchier, A.; Miller, J. D.; Savard, M.; Young, J. C. *Pure & Appl. Chem.* **1994**, *66*, 2315-2318.
10. Pock, G. K.; Powell, R. G.; Plattner, R. D.; Weisleder, D. *Tetrahedron Lett.* **1994**, *35*, 7707-7710.
11. Blackwell, B. A.; Edwards, O. E.; ApSimon, J. W.; Fruchier, A. *Tetrahedron Lett.* **1994**, *35*, in press.
12. a) Boyle, C. D.; Harmange, J.-C.; Kishi, Y. *J. Amer. Chem. Soc.* **1994**, *116*, 4995-4996. b) For assignment of the configuration at C(1) - C(5) in AAL toxin T_A see Oikawa, H.; Matsuda, I.; Ichihara, A.; Kohmoto, K. *Tetrahedron Lett.* **1994**, *35*, 1223-1226.
13. Harmange, J.-C.; Boyle, C. D.; Kishi, Y. *Tetrahedron Lett.* **1994**, *35*, 6819-6822.
14. Abbas, H. K.; Gelderblom, W. C. A.; Cawood, M. E.; Shier, W. T. *Toxicon*, **1993**, *31*, 345-353.
15. Yoon, N. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem.* **1973**, *38*, 2786-2792.
16. FB_1 was prepared by the method of Vesonder, R. F.; Peterson, R.; Plattner, R.; Weisleder, D. *Mycotoxin Res.* **1990**, *6*, 85-88, except that additional purification of the final product was achieved by preparative tlc on silica gel using the solvent system $CHCl_3$:MeOH:H_2O:HOAc 55:36:8:1. FAB-MS: 722 [M + 1].
17. AAL toxin T_A was prepared by the method of Abbas, H. K.; Vesonder, R. F. *Toxicon* **1993**, *31*, 355-358, except that the MeOH eluate from Amberlite XAD-2 was purified by normal phase chromatography on a silica gel column eluted with $CHCl_3$:MeOH 2:1 containing increasing amounts of water, followed by reverse phase chromatography of the combined toxin-containing fractions on octadecyl-functionalized silica gel eluted with 10% v/v aqueous MeOH, followed by preparative tlc on silical gel using the solvent system $CHCl_3$:MeOH:HOAc 9:2:1. FAB-MS: 522 [M + 1], 544 [M + Na].
18. The N-acetyl derivatives **1b** and **2b** were prepared by a modification of the method of Pan, S. C.; Dutcher, J. D. *Anal. Chem.* **1956**, *28*, 836-838. Specifically, 1% wt/v solutions were mixed with an equal volume of 3M K_2HPO_4, then with one volume of Ac_2O; after 10 min the mixture was diluted 100-fold with water and applied to a mini-column of Amberlite XAD-2, which was washed with water and eluted with MeOH. For **1b**: FAB-MS 764 [M + 1]; the ^{1}H-NMR (500 MHz) was in agreement with published spectra[1]. For **2b**: FAB-MS 564 [M + 1], 586 [M + Na], 608 [M + 2Na - 1]; the ^{1}H-NMR (500 MHz) was in agreement with published spectra given in Caldas, E. D.; Jones, A. D.; Ward, B.; Winter, C. K.; Gilchrist, D. G. *J. Agric. Food Chem.* **1994**, *42*, 327-333.

19. Capillary gas chromatography was carried out on a Hewlett-Packard 5890 A series II gas chromatograph equipped with a Chiraldex GT-A 30 m x 0.32 mm (trifluoroacetyl-gamma-cyclodextrin bonded phase) and a flame ionization (FI) detector, and using He flow rate, 1.39 ml/min; injection temperature, 200 °C; and equilibration time, 5 min. Authentic 3-methyl-1-pentanol was purchased (Aldrich Chemical Co., Milwaukee, WI, USA) or prepared by $LiAlH_4$ reduction of 3-methylvaleric acid (Aldrich) in ether. Its acetate was prepared by treatment with acetic anhydride in pyridine. For the analysis of 3-methyl-1-pentanol instrumental parameters were as follows: initial oven temperature, 40 °C; heating rate, 2 °C/min; and final temperature, 130 °C. Under these conditions authentic 3-methyl-1-pentanol had a retention time of 9.96 min, and **3** from FB_1 and AAL toxin T_A had retention times of 10.00 and 10.01 min, respectively. **3** from FB_1 and AAL toxin T_A was further characterized by FAB-MS: 103 [M+H], and by ^{1}H-NMR (300 MHz) spectra which were the same as obtained for authentic 3-methyl-1-pentanol from Aldrich.
20. Brown, H. C.; Grag, C. P.; Liu, K. T. *J. Org. Chem.* **1971**, *36*, 387-390.
21. Capillary gas chromatography of 3-methylvaleric acid was carried out as described[15] using the following instrumental parameters: initial oven temperature, 90 °C; heating rate, 5 °C/min; and final temperature, 150 °C. Under these conditions authentic 3-methylvaleric acid had a retention time of 15.46 min, and **4** from FB_1 and AAL toxin T_A both had retention times of 15.46 min. **4** from FB_1 and AAL toxin T_A was further characterized by FAB-MS: 117 [M+H], and by ^{1}H-NMR (300 MHz) spectra which were the same as obtained for authentic **4** prepared from L-isoleucine.[23]
22. 3-Methylvaleric acid (200 mg) was dissolved in 500 µL of anhydrous ether and kept at 0 °C. Diazomethane was added dropwise with stirring until the reaction mixture retained a pale yellow color. After 1.5 h the solvent was evaporated and the product was identified by ^{1}H- and ^{13}C-NMR; FAB-MS: 131 [M + 1]. Capillary gas chromatography of 3-methylvaleric acid was carried out as described[15] using the following instrumental parameters: initial oven temperature, 40 °C; heating rate, 2 °C/min; and final temperature, 130 °C. Under these conditions 3-methylvaleric acid methyl ester had retention times of 12.18 and 12.84 min. Authentic (*S*)-3-methylvaleric acid methyl ester prepared from L-isoleucine had a retention time of 12.87 min. 3-Methylvaleric acid methyl ester, **5**, samples prepared from FB_1 and AAL toxin T_A had retention times of 12.81 and 12.87 min, respectively. **5** from FB_1 and AAL toxin T_A was further characterized by FAB-MS: 117 [M+H], and by ^{1}H-NMR (300 MHz) spectra which were the same as obtained for authentic **5** prepared from L-isoleucine.[23]
23. Doldouras, G. A.; Kollonitsch, J. *J. Amer. Chem. Soc.* **1978**, *100*, 341-342. **4** and **5** prepared from L-isoleucine were characterized by ^{1}H- and ^{13}C-NMR (300 MHz), FAB-MS, gas chromatography[22] and optical rotation. For **4**, $[a]_D$ = + 8.6 (c = 0.2, MeOH).
24. For **5**, $[a]_D$ = + 11.4 (c = 0.2, MeOH).
25. Trommel, J.; Bijvoet, J. M. *Acta Cryst.* **1954**, 7, 703-709.

(*Received in USA* 24 *October* 1994; *revised* 4 *January* 1995; *accepted* 9 *January* 1995)