Novel Structure Elucidation of AAL Toxin T_A Backbone

Craig D. Boyle, Jean-Christophe Harmange, and Yoshito Kishi*

Department of Chemistry
Harvard University
Cambridge, Massachusetts 02138

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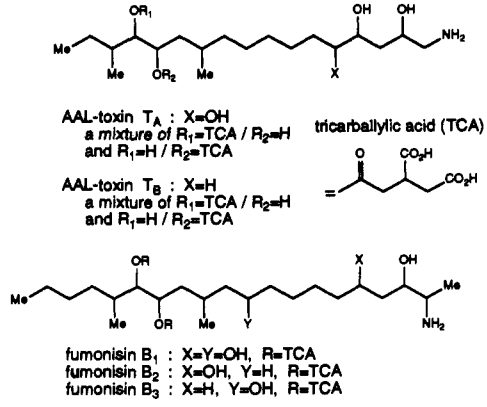
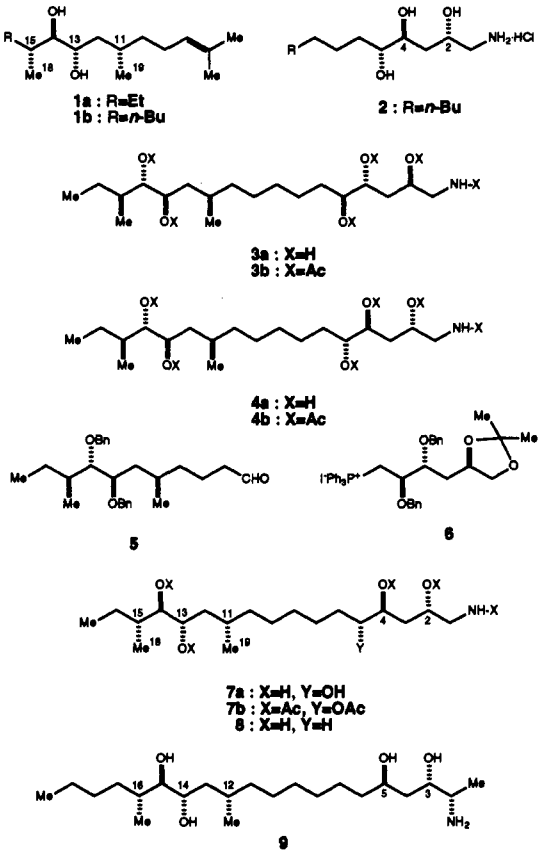
AAL toxins, isolated from *Alternaria alternata* f. sp. *lycopersici*, are host-specific phytotoxins responsible for the stem canker disease of tomato.[1] Fumonisins, found in the corn fungus *Fusarium moniliforme*, are known to exhibit a variety of biological activities.[2] Notably, they have recently received attention due to their presence in corn products and their association with esophageal cancer.[3] Both classes of natural products exhibit cross-bioactivity and have been shown to inhibit sphingolipid biosynthesis.[3,4] AAL toxins and fumonisins bear striking structural similarity: they possess similar amino alcohol backbones as well as a unique tricarballylic acid moiety. The gross structures of AAL toxins and fumonisins have been determined, but their relative and absolute configurations remain unknown.[1,2] Complete structure elucidation of these toxins should provide insight on their role in biology at the molecular level. In this communication, we report the relative and absolute stereochemistry for the amino alcohol **7a** derived from AAL toxin T_A.[5]



There are seven chiral centers on the amino alcohol **7a**. We planned to assign its stereochemistry in the following stepwise approach. In the first phase, we assumed that **7a** consisted of two distinct halves, exhibiting characteristic spectroscopic properties *independent* from the remote stereocenters on the other half of the molecule. Thus, the assignment of the relative stereochemistry of **7a** could be reduced to determining the relative stereochemistry of the left and right halves separately. For this purpose, all eight diastereomers possible for **1a,b**, representing the left half of **7a**, were synthesized from (*S*)-(−)-citronellal and subjected to ^{1}H NMR studies.[6] As hoped, all eight compounds had different spectroscopic properties, and the ^{1}H NMR spectra of **1a,b** were virtually superimposable on the appropriate regions of the spectrum of **7a**.[7] This not only established **1a,b** as having the same relative stereochemistry as **7a** but also demonstrated our proposal that the remote right half of **7a** had no effect on the spectroscopic properties of the left half of the molecule. Similarly, all four diastereomers possible for **2**, representing the right half of **7a**, were synthesized from D-mannose or D-glucose.[6] ^{1}H NMR studies established that the stereoisomer **2** represented the relative stereochemistry of the right half of **7a**.[8] As with the left half, **2** had virtually identical ^{1}H NMR data for its protons common with **7a**. These results established the relative stereochemistry of **7a** to be represented by the diastereomer **3a** or **4a**.



The second phase of this work was to synthesize **3a** and **4a** and to develop a method to distinguish them. Thus, **3a** and **4a** were synthesized from **5**[9] and **6** or its antipode[10] via Wittig olefination, followed by four steps of functional group transfor-

(1) (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. Based on the values of spin coupling constants, the relative stereochemistry at C2, C4, and C5 was suggested to be *R*, *R*, and *R* or its antipode. The numbering of all compounds in this communication will correspond to those of AAL toxin T_A, cf. **7**. One exception is **9**, which is numbered as its parent fumonisin.

(2) 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.

(3) See *Mycopathologia*, **1992**, *117*, 1–124 for 18 reviews regarding various aspects of biological activity of fumonisins and AAL toxins.

(4) Merrill, A. H., Jr.; Wang, E.; Gilchrist, D. G.; Riley, R. T. *Adv. Lipid Res.* **1993**, *26*, 215.

(5) AAL toxin T_A used for these studies was purchased from the South African Research Council, Tygerberg, South Africa. For the conversion of AAL toxin T_A to **7a**, see ref 1a.

(6) Synthetic schemes and ^{1}H NMR data for compounds **1** and **2** and their diastereomers are included as supplementary material. **1a** and **1b** gave identical ^{1}H NMR data for the C11–C15 region.

(7) ^{1}H NMR (500 MHz, CD_3OD) of **1a,b**: δ 0.89 (3H, d, J = 6.8 Hz; H-18), 0.95 (3H, d, J = 6.8 Hz; H-19), 3.18 (1H, dd, J = 5.8, 5.9 Hz; H-14), 3.62 (1H, ddd, J = 2.2, 6.0, 10.2 Hz; H-13). **7a**: 0.89 (3H, d, J = 6.9 Hz; H-18), 0.94 (3H, d, J = 6.8 Hz; H-19), 3.19 (1H, dd, J = 5.9, 5.9 Hz; H-14), 3.63 (1H, ddd, J = 2.1, 5.4, 9.6 Hz; H-13).

(8) ^{1}H NMR (500 MHz, D_2O) of **2**: δ 1.42 (1H, ddd, J = 3.0, 10.8, 14.6 Hz; H-3), 1.55 (1H, ddd, J = 2.1, 9.9, 14.6 Hz; H-3′), 2.82 (1H, dd, J = 2.9, 13.1 Hz; H-1), 3.04 (1H, dd, J = 9.9, 13.1 Hz; H-1′), 3.47 (1H, ddd, J = 3.1, 4.4, 8.7 Hz; H-5), 3.64 (1H, ddd, J = 2.1, 4.4, 10.8 Hz; H-4), 3.94 (1H, dddd, J = 2.9, 3.0, 9.9, 9.9 Hz; H-2). **7a**·HCl: 1.43 (1H, ddd, J = 3.0, 10.5, 14.6 Hz; H-3), 1.56 (1H, ddd, J = 2.0, 10.0, 14.6 Hz; H-3′), 2.82 (1H, dd, J = 2.9, 13.1 Hz; H-1), 3.05 (1H, dd, J = 9.9, 13.1 Hz; H-1′), 3.48 (1H, ddd, J = 3.0, 4.3, 8.7 Hz; H-5), 3.65 (1H, ddd, J = 2.0, 4.3, 10.5 Hz; H-4), 3.95 (1H, dddd, J = 2.9, 3.0, 9.9, 10.0 Hz; H-2).

(9) **5** was synthesized from (*S*)-(−)-2-methyl-1-butanol and (*R*)-(−)-citronellyl bromide. The stereochemistry of a synthetic intermediate with all four stereocenters was confirmed by X-ray analysis. We thank Mr. Michael J. Scott in Prof. Holm's group at Harvard University for the analysis. The synthetic scheme and X-ray structure are included as supplementary material.

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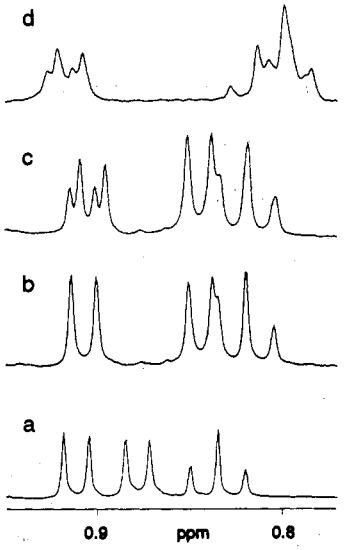

Figure 1. Methyl group region of ^{1}H NMR (500 MHz, $CDCl_3$). (a) A 2:1 mixture of **3b** or **4b** and **7b** without Eu(fod)$_3$ or (+)-Eu(hfc)$_3$. (b) A 2:1 mixture of **3b** and **7b** with 0.4 equiv of Eu(fod)$_3$. (c) A 2:1 mixture of **4b** and **7b** with 0.4 equiv Eu(fod)$_3$. (d) A 2:1 mixture of **3b** and **7b** with 1.0 equiv of (+)-Eu(hfc)$_3$.

mation.[11] The ^{1}H NMR spectra of **3a** and **4a** were found to be superimposable on each other, and furthermore on that of the amino alcohol obtained from AAL toxin T_A.[12] This was, of course, expected on the basis of our initial hypothesis and studies on the two separate halves **1** and **2**. However, we hoped the differentiation of **3a** from **4a** to be realized by observing the difference in their global structures. To this end, the peracetates **3b** and **4b** were prepared from **3a** and **4a**, respectively, and subjected to ^{1}H NMR studies. Once again, the peracetates **3b** and **4b** gave virtually identical ^{1}H NMR spectra. However, as illustrated in Figure 1, the differences were indeed detected in their ^{1}H NMR spectra in the presence of Eu(fod)$_3$. Critically, a 2:1 mixture of **3b** and AAL toxin T_A peracetate **7b** behaved as a single substance, whereas a 2:1 mixture of **4b** and AAL toxin T_A peracetate **7b** behaved as two chemically different substances. Thus, the amino alcohol **7a** derived from AAL toxin T_A must be represented by **3a** or its enantiomer.

The third phase of this work was to determine the absolute stereochemistry of **7a**. Among several possibilities,[13] a natural extension of the experiments outlined was to examine effects of a chiral shift reagent; a 2:1 mixture of the synthetic peracetate **3b** and AAL toxin T_A peracetate **7b** behaved as two chemically different substances (Figure 1), concluding the absolute configuration of the backbone of AAL toxin T_A to be **7a**. This conclusion was further supported from their optical rotations; both **7a·HCl** ($[\alpha]_D$ −18° (*c* 0.1, MeOH)) and peracetate **7b** ($[\alpha]_D$ −18° (*c* 0.1, $CHCl_3$)) exhibited the opposite sign of **3a·HCl** ($[\alpha]_D$ +11° (*c* 0.3, MeOH)) and **3b** ($[\alpha]_D$ +13° (*c* 0.1, $CHCl_3$)), respectively.[14]

On the basis of the experimental evidence presented, the relative and absolute stereochemistry of the backbone of the AAL toxin T_A is represented as **7a**. Interestingly, the minor fraction AAL toxin T_B was isolated from the *A. alternata* f. sp. *lycopersici* fungus, and its gross structure was suggested to correspond to 5-deoxy-AAL toxin T_A.[1b] Considering the fact that both toxins co-occur in the same fungus and have similar gross structures, the stereochemistry of the backbone of AAL toxin T_B is likely to be **8**. It is also worthwhile to comment on the stereochemistry of the amino alcohol consisting of the backbone of fumonisins. Comparison of the ^{1}H NMR data of peracetate **7b**[15] with data reported for *N*-acetylfumonisin B_1 methyl ester[2] 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.[1] Comparing the ^{13}C NMR data of the C1–C4 portion of *N*-acetylfumonisin B_1 methyl ester[2] with those of the acetates derived from 2-aminotetradeca-5,7-dien-3-ols,[16] the relative stereochemistry at the C2 and C3 positions appears to be syn, suggesting that **9** is likely the amino alcohol consisting of fumonisin B_2. Further studies on the structure and synthesis of AAL toxins and fumonisins are in progress in our laboratories.

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Note Added in Proof: After we submitted this manuscript, Oikawa, Matsuda, Ichihara, and Kohmoto (*Tetrahedron Lett.* **1994**, *35*, 1223) reported the absolute configuration of the C1–C5 fragment of AAL toxin T_A.

Supplementary Material Available: Synthetic schemes and ^{1}H NMR tables for all diastereomers of **1** and **2**; synthetic schemes for **3**, **4**, **5**, and **6**; X-ray structure of a synthetic precursor of **5** including all four stereocenters; and ^{1}H NMR spectra of EuR_3 titration experiments (16 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) **6** and its antipode were synthesized from L- and D-mannose, respectively. The details of the synthesis are included as supplementary material.

(11) **3a** was synthesized from **5** and **6** in the following five steps: (1) **6**/*n*-BuLi/THF/HMPA/−78 °C, followed by addition of **5** and warming to −20 °C (60% yield); (2) *p*-TsOH/MeOH/room temperature (70%); (3) NaH/*p*-Ts-Imid/THF/5 °C (98%); (4) NaN_3/TBAI/DME/DMF/H_2O/$MeOCH_2CH_2OH$/120 °C (80%); (5) H_2 (1 atm)/Pd on C/*p*-TsOH/MeOH (87%).

(12) ^{1}H NMR spectra of **3a**, **4a**, and **7a** were taken in CD_3OD and were superimposable. ^{1}H NMR spectra of the HCl salts of these compounds were taken in D_2O and were also all superimposable. For a published ^{1}H NMR spectrum of **7a·HCl** in D_2O, see ref 1a.

(13) For example, IO_4^- oxidation of **7a**, followed by $NaBH_4$ reduction, should yield the degradation products preserving the C11 and C15 stereochemistry, respectively. Assignment of the absolute configuration for one of them should distinguish **7a** from **3a**. However, this was not tested because of the lack of material.

(14) The optical rotation of **7a·HCl** is reported in ref 1a: $[\alpha]_D$ −15° (*c* 2.7, H_2O).

(15) ^{1}H NMR (500 MHz, $CDCl_3$) of **7b**: δ 0.88 (3H, d, *J* = 6.5 Hz; H-18), 0.91 (3H, d, *J* = 6.8 Hz; H-19), 4.87 (1H, dd, *J* = 3.2, 8.8 Hz; H-14), 5.12 (1H, ddd, *J* = 2.8, 2.8, 10.9 Hz; H-13).

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