Relative and Absolute Configuration of the Fumonisin B_1 Backbone

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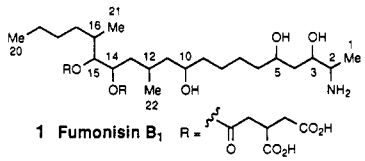
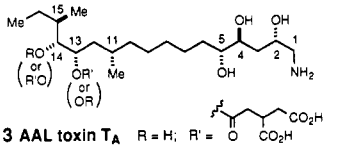
Fumonisin B_1 (FB_1, **1**)[1] is a potent toxin produced by *Fusarium moniliforme*, a ubiquitous mold found in corn, sorghum, and other grains.[2] It is the most prevalent member of a family of structurally related toxins that are responsible for diseases such as equine leukoencephalomalacia and porcine pulmonary edema and that cause hepatotoxicity and liver tumors in rats. Human consumption of *F. moniliforme*-contaminated corn has been associated with esophageal cancer in a number of epidemiological studies from geographically diverse countries. FB_1 interferes with sphingosine biosynthesis in rat liver hepatocytes primarily through inhibition of ceramide synthase.[3] FB_1 contains two ester side chains derived from propane-1,2,3-tricarboxylic acid. Saponification of those esters (1 N KOH, reflux, 24 h)[4] gives the aminopentol backbone **2** (FB_1-AP). This "hydrolyzed fumonisin" retains biological activity[5a] and has been shown to be produced by certain types of food processing.[5b]



1 Fumonisin B_1 R = CO_2H, CO_2H

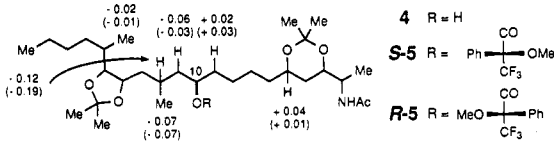
2 FB_1-AP R = H

The AAL toxins are phytotoxins produced by *Alternaria alternata* f. sp. *lycopersici* and are structurally related to the fumonisins. Kishi and co-workers have recently unraveled all stereochemical features of the backbone of the AAL toxin T_A, as shown in **3**.[6] In the course of that work a stereostructure for fumonisin B_2 (10-deoxy-FB_1) was also proposed. Their strategy involved independent synthesis and spectroscopic comparison of all of the diastereoisomers corresponding to the individual halves of **3**. We have independently solved the FB_1 stereostructure by a quite different and complementary strategy. Thus, from an ~200 mg sample of FB_1-AP (**2**) and by the series of derivatization and degradation studies described here, we assign the relative and absolute configuration of the eight stereogenic centers in the backbone of **1**.[7]



3 AAL toxin T_A R = H; R' = CO_2H, CO_2H

Sequential peracetylation of **2** (Ac_2O, py), penta-ester cleavage (K_2CO_3, MeOH), and bis-acetonide formation (DMP, CSA, acetone) efficiently produced the acetamide **4**, which contained a single free carbinol center at C(10). Preparation of the methoxy-(trifluoromethyl)phenylacetate (MTPA) esters (*S*)-**5** and (*R*)-**5** permitted Mosher analysis[8] that established the *R* configuration at C(10). Thus, the ^1H NMR $\Delta\delta$ ($\equiv\delta_S - \delta_R$) values for various protons in the spectra in $CDCl_3$ and C_6D_6 (in parentheses) of the diastereomeric esters **5** are positive at C(9) and C(5) and negative at C(11), C(12), C(21), and C(22). The modified Mosher ester analysis[9] relies on the reinforcing nature of multiple $\Delta\delta$ values.



4 R = H

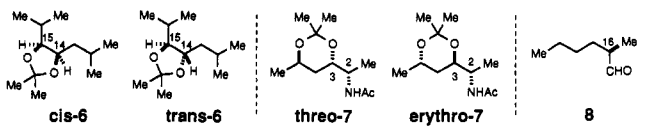
S-5 R = Ph, CO, OMe, CF_3

R-5 R = MeO, CO, Ph, CF_3

The relative configuration between C(3) and C(5) was initially assigned as 1,3-anti on the basis of the nearly identical chemical shifts of the diastereotopic protons H(4a) (δ 1.75) and H(4b) (δ 1.72) in the peracetylated derivative of **2**. Further support for the C(3)/C(5) relationship was deduced by Rychnovsky analysis at the stage of the bis-acetonide **4**. The ketal carbon in the six-membered dioxane ring of **4** appears at δ 100.4, consistent with its 4,6-trans disubstitution.[10]

Coupling constant analysis within the bis-acetonide **4** was also informative. Protons H(14) and H(15) on the dioxolane ring are coupled with J = 5.0 Hz. The cis- and trans-disubstituted, truncated model compounds *cis*-**6** and *trans*-**6** were subjected to Monte Carlo conformational analysis using the MM2 force field as implemented in Macromodel.[11] The weighted average of the coupling constants across the Boltzmann distribution of all conformers within 3.0 kcal of the global minimum for *cis*-**6** and *trans*-**6** were 4.7 and 8.9 Hz, respectively, indicative of a C(14)/C(15) 1,2-anti (or erythro) relationship.

By a similar strategy, the relative configuration between C(2) and C(3) was tentatively assigned as 1,2-syn (or threo). $J_{H(2)/H(3)}$ was measured as 2.5 Hz in acetamide **4**. Model compounds *threo*-**7** and *erythro*-**7** were subjected to Monte Carlo analysis by MM2 both in the gas phase and using $CHCl_3$ solvation.[12] The calculated, Boltzmann-weighted J's for *threo*-**7** ranged from 1.2 to 1.3 Hz, a closer fit to the observed value of 2.5 Hz than the range of 6.0–6.4 Hz calculated for *erythro*-**7**. This conclusion is consistent with that suggested by Kishi for C(2)/C(3)/C(5) of FB_1[6] based on comparison of ^{13}C NMR data of *N*-acetyl-FB_1 methyl ester[1] with appropriate model compounds[13] of established relative configuration.



cis-6 trans-6 threo-7 erythro-7 8

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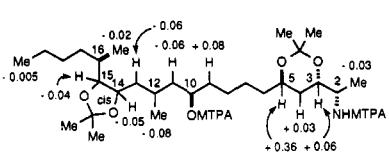
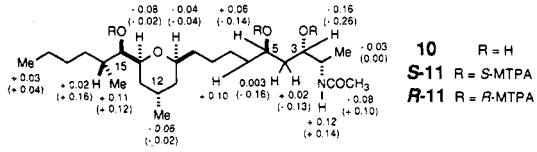
Determination of absolute configuration at the methyl-bearing C(16) was achieved through sodium periodate cleavage of the amino pentol **2**, which released (*R*)-2-methylhexanal (**8**). The configuration of this fragment was deduced by comparative, *in situ* GC analysis of the $NaIO_4$ reaction mixture on a Chiraldex GT-A column (trifluoroacetyl-γ-cyclodextrin) with authentic racemic and enantiomerically enriched samples of **8**.[14]

Determination of absolute configuration at the amino-bearing C(2) was achieved by a different derivatization sequence of FB_1-AP (**2**). Selective Cbz protection of the primary amine (Cbz-Cl, Na_2CO_3, H_2O), bis-acetonide formation (DMP, CSA, acetone), Cbz removal (H_2, Pd/C, EtOH), and MTPA formation[9b] gave both *S* and *R* Mosher amide esters (*S*)-**9** and (*R*)-**9**. The *R* configuration at C(10) was reconfirmed by identification of a set of local Δδ's similar to that obtained for **5**. The configuration at C(2) was assigned as *S* on the basis of the ^1H NMR Δδ values for the spectra in $CDCl_3$ as indicated in structure **9** [i.e., negative at C(1) and positive at C(3), C(4), and C(5)].[9c]



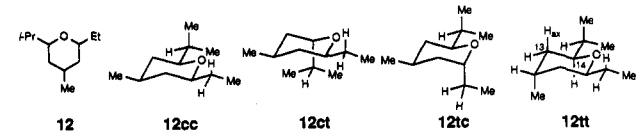
S-9 S-MTPA
R-9 R-MTPA

One final sequence addressed the remaining issues of the absolute configurations at C(12) and at C(14) or C(15). Bis-acetonide **4** was converted to the C(10) mesylate (MsCl, Et_3N, DMAP, CH_2Cl_2), the acetonides were removed (MeOH, Dowex), and pyran **10** was generated (NaH, THF),[15] presumably with inversion of configuration at C(10). Finally, the tris-(*S*)- and tris-(*R*)-MTPA esters **11** were made. In each of the three pyrans



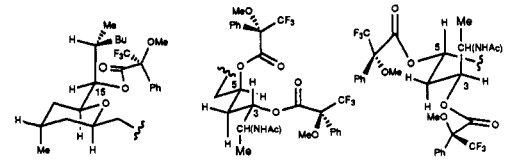
10 R = H
S-11 R = S-MTPA
R-11 R = R-MTPA

10 and **11** spectral properties indicated that substituents at C(10) and C(14) were oriented cis to one another. That is, H(10) was a broad multiplet with ΣJ's = 29–30 Hz, and H(14) was a ddd with $J_{H(14)/H(15)}$ = 4.0–5.5 Hz, $J_{H(14)/H(13eq)}$ = 2.0 Hz, and $J_{H(14)/H(13ax)}$ = 11.5–12 Hz, indicating that both protons were axially oriented. Further support for the relative configuration between C(10) and C(14) came from multiconformation searching of the four possible diastereomers of the model pyrans **12**. Not surprisingly, the low-energy set of conformations in each case was dominated by various side-chain rotamers of ring conformers **12cc**, **12ct**, **12tc**, and **12tt**. The axial proton $H(13)_{ax}$ in, e.g., **10** is a ddd with $J_{H(13ax)/H(13eq)}$ = 13.5 Hz, $J_{H(13ax)/H(14)}$ = 12 Hz, and $J_{H(13ax)/H(12)}$ = 5.5 Hz. The smallest of these coupling constants indicates that the C(12) methyl group is axial on the pyran ring. Only diastereomer **12tt** is consistent with the observed multiplicities for H(14) and H(13ax).[16]



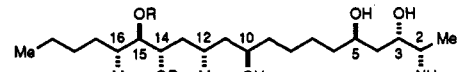
12 **12cc** **12ct** **12tc** **12tt**

Finally, the ^1H NMR Δδ's for spectra in $CDCl_3$ (and C_6D_6) of the tris-MTPA esters **11** are consistent with the partial structures **13** and **14**, which arbitrarily are shown for the (*S*)-MTPA esters. The 15*R*, 5*R*, and 3*S* configurations indicated by this analysis are identical to and, therefore, serve to reinforce those already deduced for the same stereocenters by the various analyses presented above.



13 **14a** **14b**

On the basis of the collection of arguments just presented, we assign the 2*S*,3*S*,5*R*,10*R*,12*S*,14*S*,15*R*,16*R* configuration to the stereogenic carbons in the backbone of fumonisin B_1 and the derived FB_1-AP as indicated in structures **15** and **16**, respectively. The conclusions reached in this work are entirely consistent with those proposed by Kishi[6] for the FB_2 stereostructure and for the C(1)–C(4) portion of FB_1 as well as with those deduced by Oikawa et al. for C(1)–C(5) of the AAL toxin.[17] Fumonisin B_1 (**15**) and AAL toxin T_A (**3**)[6] have identical configurations at all common, backbone stereocenters.



(2*S*,3*S*,5*R*,10*R*,12*S*,14*S*,15*R*,16*R*)-
15 Fumonisin B_1 R = $COCH_2CH(CO_2H)CH_2CO_2H$
16 FB_1-AP R = H

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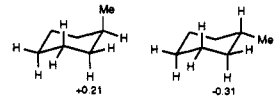
(14) A sample enriched in (*S*)-**8** was prepared by methylation of the (*S*)-phenylalaninol-derived Evans auxiliary $Me(CH_2)_4COX_P$, $LiBH_4$ reduction, and PCC oxidation. The retention time of (*S*)-**8** was 23.6 min under conditions in which the racemic sample showed base-line-resolved peaks at 23.7 and 24.4 min. *In situ* analysis of the periodate cleavage reaction mixture showed a peak at t_R = 24.6 min.
(15) Some pyran **10** was also formed spontaneously under the acetonide removal reaction conditions.
(16) (a) The unusual circumstance that the chemical shift of the axial proton at C(13) (δ = 1.79) is further *downfield* than its equatorial, geminal partner (δ 1.26, from COSY) is consistent with the methyl chemical shift effects summarized in **i** and **ii** as deduced in Grant's ^2H NMR study of a series of many methylated cyclohexanes.[16b]



i **ii**

We find this tool to be extremely useful in the chemical shift analysis of conformationally defined systems, rigid or not. (b) Grant, D. M.; Dalling, D. K.; Curtis, J. *J. Org. Chem.* **1986**, *51*, 136.
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