Relative and Absolute Configuration of the Fumonisin B_1 Backbone

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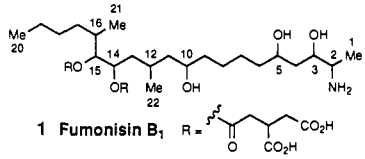
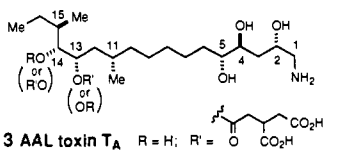
Fumonisin B_1 (FB_1, **1**)[1] is a potent toxin produced by *Fusarium moniliforme*, a ubiquitous mold found in corn, sorghum, and other grains.[2] It is the most prevalent member of a family of structurally related toxins that are responsible for diseases such as equine leukoencephalomalacia and porcine 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 =

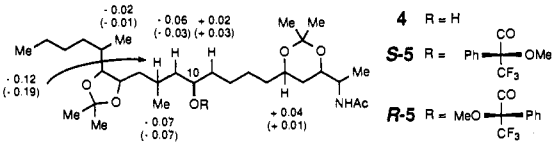
2 FB_1-AP R = H

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' =

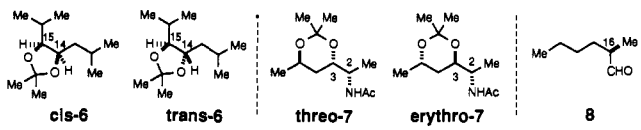
Sequential peracetylation of **2** (Ac_2O, py), penta-ester cleavage (K_2CO_3, MeOH), and bis-acetonide formation (DMP, CSA, acetone) efficiently produced the acetamide **4**, which contained a single free carbinol center at C(10). Preparation of the methoxy-(trifluoromethyl)phenylacetate (MTPA) esters (*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.



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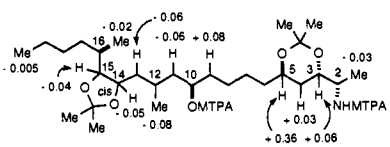
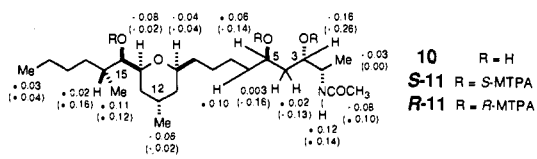
Determination of absolute configuration at the methyl-bearing C(16) was achieved through sodium periodate cleavage of the amino pentol **2**, which released (*R*)-2-methylhexanal (**8**). The configuration of this fragment was deduced by comparative, *in situ* GC analysis of the $NaIO_4$ reaction mixture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

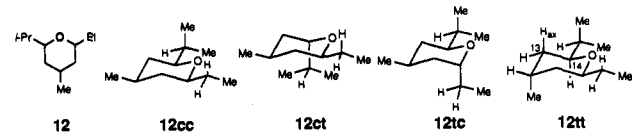
Determination of absolute configuration at the amino-bearing C(2) was achieved by a different derivatization sequence of FB_1-AP (**2**). Selective Cbz protection of the primary amine (Cbz-Cl, Na_2CO_3, H_2O), bis-acetonide formation (DMP, CSA, acetone), Cbz removal (H_2, Pd/C, EtOH), and MTPA formation[9b] 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]



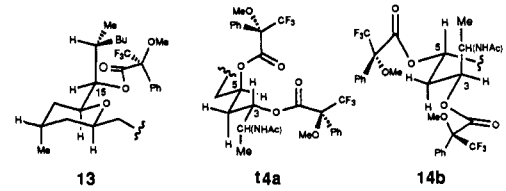
One final sequence addressed the remaining issues of the absolute configurations at C(12) and at C(14) or C(15). Bis-acetonide **4** was converted to the C(10) mesylate (MsCl, Et_3N, DMAP, CH_2Cl_2), the acetonides were removed (MeOH, Dowex), and pyran **10** was generated (NaH, THF),[15] presumably 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



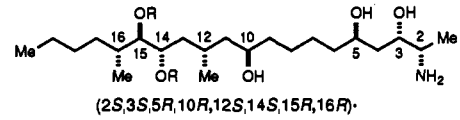
10 and **11** spectral properties indicated that substituents at C(10) and C(14) were oriented cis to one another. That is, H(10) was a broad multiplet with ΣJ's = 29–30 Hz, and H(14) was a ddd with $J_{H(14)/H(15)}$ = 4.0–5.5 Hz, $J_{H(14)/H(13eq)}$ = 2.0 Hz, and $J_{H(14)/H(13ax)}$ = 11.5–12 Hz, indicating 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]



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.



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.



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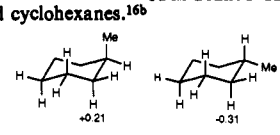
(14) A sample enriched in (*S*)-**8** was prepared by methylation of the (*S*)-phenylalaninol-derived Evans auxiliary $Me(CH_2)_4COX_P$, $LiBH_4$ reduction, and PCC oxidation. The retention time of (*S*)-**8** was 23.6 min under conditions in which the racemic sample showed base-line-resolved peaks at 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]



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