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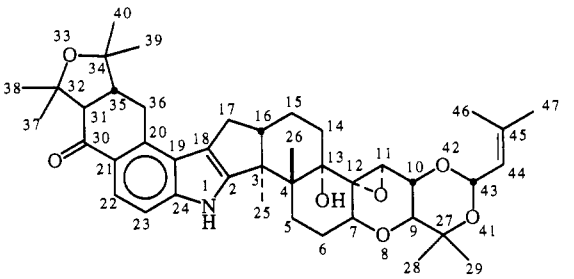
SHORT COMMUNICATIONS

Relative Stereochemistry of the A/B Rings of the Tremorgenic Mycotoxin Lolitrem B

INTRODUCTION

The lolitrems are neurotoxic indole–diterpenes (Gallagher et al., 1981, 1982) isolated from perennial ryegrass (*Lolium perenne* L.). These compounds, which are produced by the endophytic fungus *Acremonium lolii* L. (Gallagher et al., 1982, 1984), are the principal causative agents of ryegrass staggers, a nervous disorder of livestock grazing ryegrass-dominant pastures (Gallagher et al., 1984; Miles et al., 1992). The structures of the lolitrems that have been previously reported (Miles et al., 1992; Gallagher et al., 1984) have lacked any definitive determination of the configuration of the A/B ring junction. While Gallagher et al. (1984) reported the stereochemistry of the A/B ring junction of the major neurotoxin lolitrem B (**1**) to be trans, based on the observed vicinal coupling constant for H-31 and H-35 of 14.3 Hz, the configuration relative to rings E–I was not accessible from NMR data.

We now report the elucidation of the configuration of the A/B ring junction of **1** based on an NMR and molecular modeling investigation of 2β,18β-dihydrololitrem C (**2**). NOE NMR experiments on **2** indicate that the configuration of the A/B ring junction of **1** and **2** is as shown in Figure 1.



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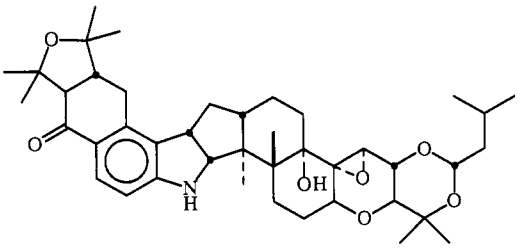

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Figure 1. Structures of lolitrem B (**1**) and 2β,18β-dihydrololitrem C (**2**).

EXPERIMENTAL PROCEDURES

General. Flash chromatography was performed on silica gel (E. Merck 9385) using acetonitrile–dichloromethane (1:9) as eluent. Mass spectra were obtained on a Kratos MS-80 RFA by direct insertion probe. HPLC analysis, based on the method of Gallagher et al. (1985), was performed on a 4.6-mm × 25-cm 5-μm Zorbax silica column with acetonitrile-dichloromethane eluent (1:4; 1.8 mL min^{-1}). Eluting compounds were detected with a Shimadzu RF-530 fluorescence spectromonitor (excitation at 268 nm, emission detection at 440 nm) and a Hewlett-Packard 1040M diode-array UV detector in series.

Synthesis of 2. Compound **1** (12 mg, 0.0175 mmol) was dissolved in methanol–water (19:1; 5 mL). Potassium acetate (5 mg) and a catalytic amount of 5% Pd on charcoal was added, and the reaction was magnetically stirred under a balloon full of hydrogen. The progress of the reaction was monitored by HPLC analysis of aliquots taken at 30-min intervals. After 1.5 h, the solution was filtered to remove the catalyst, diluted with water (150 mL), and extracted with dichloromethane (2 × 50 mL). The extract was dried ($MgSO_4$), concentrated *in vacuo*, and then purified by flash chromatography to give **2** (7 mg). Qualitative UV data from the HPLC diode-array detector: 253 (m), 338 (vs) nm. For NMR data, see Tables 1 and 2; for high-resolution EI-MS data, see text.

Molecular Modeling. Structure calculation was performed on a Silicon Graphics IRIS Indigo XS24 workstation running Macromodel V3.5X (Mohamadi et al., 1990). The minimizations were performed *in vacuo* with the MM2* force field and the Polak–Ribiere conjugate gradient minimizer and were deemed to have converged when the gradient reached 0.01 kJ mol^{-1} $Å^{-1}$.

Nuclear Magnetic Resonance Spectroscopy. One- and two-dimensional ^1H and ^{13}C NMR spectra were obtained from a deuteriochloroform solution using a Bruker AC-300 instrument

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Table 1. ^1H and ^{13}C NMR Chemical Shifts (δ) of 2β,18β-Dihydrololitrem C(2) in $CDCl_3$

	^{13}C	^1H (α,β)		^{13}C	^1H (α,β)
C-2	66.6 (d)	4.24	C-24	156.1 (s)	
C-3	51.9 (s)		C-25	10.9 (q)	0.91
C-4	42.8 (s)		C-26	18.2 (q)	1.12
C-5	27.6 (t)	2.52, 1.03	C-27	74.1 (s)	
C-6	27.8 (t)	2.14, 1.65	C-28	28.3 (q)	1.25
C-7	71.4 (d)	4.23	C-29	16.7 (q)	1.22
C-9	71.4 (d)	3.50	C-30	194.4 (s)	
C-10	71.1 (d)	3.82	C-31	59.6 (d)	2.67
C-11	61.7 (d)	3.59	C-32	80.0 (s)	
C-12	68.1 (s)		C-34	79.1 (s)	
C-13	78.3 (s)		C-35	49.3 (d)	2.52
C-14	28.9 (t)	1.43, 1.26	C-36	28.5 (t)	2.64, 2.73
C-15	21.0 (t)	1.63, 1.53	C-37	30.7 (q)	1.50
C-16	42.2 (d)	1.89	C-38	25.1 (q)	1.27
C-17	34.5 (t)	1.56, 2.15	C-39	25.0 (q)	1.21
C-18	44.5 (d)	3.80	C-40	29.3 (q)	1.34
C-19	128.5 (s)		C-43	95.1 (d)	4.90
C-20	124.1 (s)		C-44	43.3 (t)	1.49
C-21	139.6 (s)		C-45	24.0 (d)	1.79
C-22	129.6 (d)	7.84	C-46	22.8 (q)[a]	0.89[a]
C-23	105.5 (d)	6.38	C-47	22.7 (q)[a]	0.92[a]

[a] Assignments interchangeable.

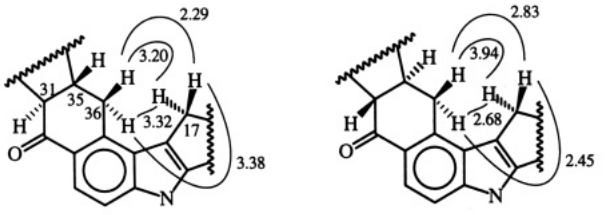

Figure 2. MM2* calculated internuclear distances (Å) for the two possible diastereoisomers of lolitrem B (1).

operating at 300.13 and 75.47 MHz, respectively. Chemical shifts are reported relative to internal TMS. ^{13}C NMR signal multiplicities (s, d, t, or q) were determined using the DEPT sequence. NOE difference experiments were performed with sequential irradiation of each line in the multiplets using the NOEMULT method of Kinns and Sanders (1984). Difference spectra were obtained by subtracting an off-resonance control FID from that of the irradiated FID and Fourier transforming the resulting difference FID. Two-dimensional double-quantum filtered COSY and inverse mode heteronuclear multiple-bond correlation (HMBC) spectra were determined in the absolute value mode. ^{13}C–^1H correlated spectra (including inverse-mode HMQC spectra) were determined in the phase-sensitive mode.

RESULTS AND DISCUSSION

A molecular modeling investigation of the two possible diastereoisomers of 1 resulting from the two possible A/B ring junction stereochemistries indicated that an NOE difference experiment should resolve the ambiguity. The 31α,35β isomer would be expected to show a strong NOE between H-36β and H-17β, as the internuclear distance

Table 2. Long-Range ^{13}C–^1H Correlations Determined for the Methyl Group Protons of 2β,18β-Dihydrololitrem C(2)

^1H signal, δ	correlated ^{13}C signals, δ
0.89 (H-46)[a]	22.7 (C-47), 24.0 (C-45), 43.3 (C-44)
0.91 (H-25)	42.2 (C-16), 42.8 (C-4), 51.9 (C-3), 66.6 (C-2)
0.92 (H-47)[a]	22.8 (C-46), 24.0 (C-45), 43.3 (C-44)
1.12 (H-26)	27.6 (C-5), 42.8 (C-4), 51.9 (C-3), 78.3 (C-13)
1.21 (H-39)	29.3 (C-40), 49.3 (C-35), 79.1 (C-34)
1.22 (H-29)	28.3 (C-28), 71.4 (C-9), 74.1 (C-27)
1.25 (H-28)	16.7 (C-29), 71.4 (C-9), 74.1 (C-27)
1.27 (H-38)	30.7 (C-37), 59.6 (C-31), 80.0 (C-32)
1.34 (H-40)	25.0 (C-39), 49.3 (C-35), 79.1 (C-34)
1.50 (H-37)	25.1 (C-38), 59.6 (C-31), 80.0 (C-32)

[a] Assignments interchangeable.

H-36βH–17β was calculated to be 2.29 Å (see Figure 2). In contrast, the 31β,35α isomer would not be expected to show such a strong NOE, with a calculated internuclear distance of 2.83 Å. In practice, however, such an NOE investigation was not viable because irradiation at the only available H-36 chemical shift (δ 3.43 in $CDCl_3$) cannot lead to discrimination of the enhancements of H-35 (δ2.66) and the other H-36 (δ2.96) from those of H-17 (δ 2.62 and 2.92). The situation was not improved by using benzene-d_6 as solvent because there remained considerable spectral overlap in the region 2.5–3.0 ppm.

As part of an investigation of the structures of the minor lolitrem constituents (to be reported separately), we subjected lolitrem B to catalytic hydrogenation (5% Pd on charcoal) in an attempt to generate lolitrem C (44,45-dihydrololitrem B) and isolated a compound of molecular formula $C_{42}H_{59}NO_7$ (high-resolution EI-MS M^+ = 689.4251, calcd m/z = 689.4291), corresponding to the addition of four hydrogens to lolitrem B. The UV spectrum indicated that modification of the previously indolic chromophore had taken place. The ^1H and ^{13}C NMR data are given in Table 1. It was readily apparent from the ^{13}C NMR spectrum of this compound that hydrogenation of both the C-44–C-45 and C-2–C-18 double bonds of 1 had occurred to give 2β,18β-dihydrololitrem C (2). The alkene carbon signals at δ 152.77 (C-2), 118.58 (C-18), 121.96 (C-44), and 139.54 (C-45) in 1 (Gallagher et al., 1984) had disappeared and were replaced by the corresponding peaks at δ 66.6, 44.5, 43.3, and 24.0, respectively, in 2. In the ^1H NMR spectrum of 2, the new signals from H-2 (d, δ 4.24) and H-18 (δ 3.80) were shown by a COSY experiment to be overlapping those of H-7 and H-10, respectively. Also apparent were the upfield-shifted methyl resonances H-46 and H-47 (δ 0.89 and 0.92). That cis-addition of hydrogen across the indole double bond had occurred on the β face of 1 was shown by the observation of an NOE between H-2 and H-26 in an NOE difference experiment and a ^4J trans-1,3-diaxial coupling between H-2 and H-25 in the absolute value double-quantum-filtered COSY experiment. Further evidence of the β face addition of hydrogen was seen

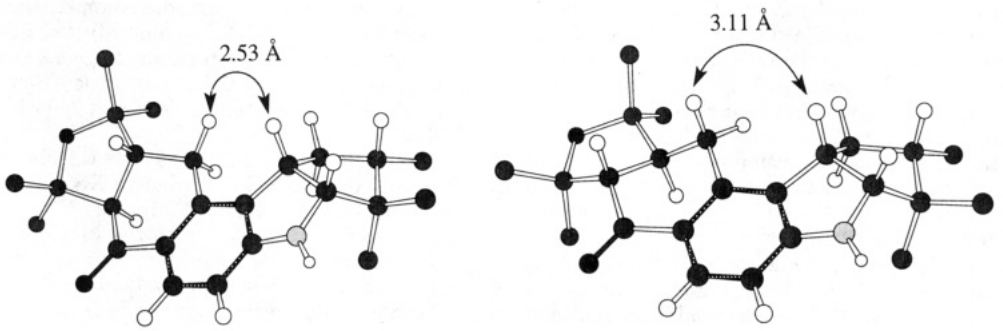

Figure 3. MM2* calculated structures of 31β,35α-2 (right) and 31α,35β-2 (left). The expected internuclear distances (Å) between H-18 and H-36β are shown for both diastereoisomers.

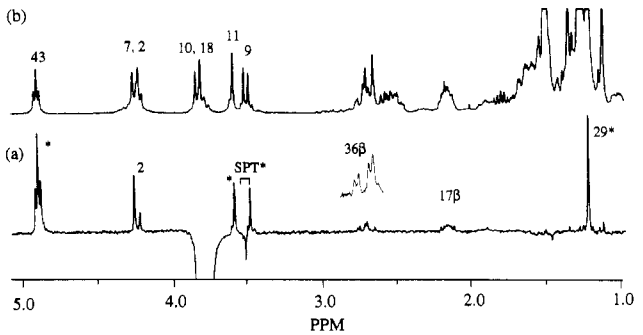

Figure 4. (a) NOE difference spectrum obtained by irradiation at the frequency of H-18 and H-10. Peaks marked with an asterisk are those originating from irradiation of H-10; (b) ^{1}H NMR spectrum of **2**.

in the upfield shift of H-25 to δ 0.91. Examination of a molecular model showed that the fold induced at the D/E ring junction moved the C-25 protons into the shielding region of ring C. Final confirmation of all ^{1}H and ^{13}C chemical shift assignments was obtained from the $^2J_{^{13}C-^1H}$ and $^3J_{^{13}C-^1H}$ correlations observed in the inverse mode HMBC experiment (see Table 2).

An unexpected result of the hydrogenation of the indole double bond was that the position of one of the H-36 resonances of lolitrem B (δ 3.43) had shifted a considerable distance upfield in the ^{1}H NMR spectrum of **2**, to δ 2.73. A change had also occurred in the H-17α/H-17β proton chemical shifts owing to their conversion from being allylic in **1** to aliphatic in **2**. These chemical shift changes prompted us to undertake a molecular modeling investigation of **2**, with a view to determining if there might be any NOE which could be useful in the determination of the configuration of the A/B rings of **1**.

Rings A–E of the MM2*-calculated structures of the two possible diastereoisomers of **2** are shown in Figure 3. The MM2* calculations indicated that the 31α,35β diastereoisomer should show an NOE between H-18 and H-36β, whereas this NOE ought not to be present in 31β,35α-**2**. Irradiation of H-18 led to the expected enhancement of H-17β (see Figure 4) and of an additional signal at δ 2.73 (dd, $^3J_{36\beta,36\alpha}$ = 15.9 Hz, $^3J_{36\beta,35}$ = 3.9 Hz). This signal was assigned to H-36β based on its chemical shift, multiplicity, and its observed correlation with C-36 at δ 28.5 in the ^{13}C–^{1}H chemical shift correlation experiment. Because the resonances of H-18 and H-10 overlapped, other enhancements were also observed associated with rings H and I. The observed NOE between H-18 and H-36β is thus unambiguous evidence that the A/B ring junction configuration of **2** is 31α,35β, and it follows that the configuration of the A/B ring junction of lolitrem B is also 31α,35β.

There is strong evidence that the lolitrems are derived from the indole–diterpenoid paxillines by simple isoprenylation reactions (Weedon and Mantle, 1987; Miles et al., 1992). The absolute configuration of paxilline is established by its direct biosynthetic incorporation into penitrem A (Mantle and Penn, 1989), the absolute stereochemistry of which is known (De Jesus et al., 1983). Since there is an exact correspondence between rings C–G of **1** and rings A–E of paxilline, it follows that the absolute configuration of lolitrem B is as shown in Figure 1.

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