Large-Scale Isolation of Lolitrem B and Structure Determination of Lolitrem E

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A method for isolation of hundreds of milligrams of lolitrem B is reported. Lolitrem E, the most abundant of the minor lolitrems, was isolated from side fractions and its structure determined by mass spectrometry and one- and two-dimensional NMR techniques. Lolitrem E differs from lolitrem B in being an ether, rather than an acetal, derivative of lolitriol. Evidence is presented that the compound previously isolated by Gallagher and co-workers, and assigned the structure of lolitrem C, was in fact lolitrem E. Lolitrem E, which is not tremorgenic in mice, is proposed as a biosynthetic precursor of lolitrem B.

INTRODUCTION

Research into ryegrass staggers (RGS) has been hindered by the limited availability of lolitrem B (1) (see Figure 1), the tremorgenic indole–diterpene believed to be the causative agent of this disease (Gallagher et al., 1984). The lolitrems are present in perennial ryegrass (*Lolium perenne* L.) infected with the endophytic fungus *Acremonium lolii* L. but are only produced in minute quantities by *A. lolii* when grown in culture (Miles et al., 1992). We therefore set out to develop an efficient method for isolating large quantities of lolitrem B from *A. lolii*-infected ryegrass seed.

In addition to lolitrem B, the relative stereochemistry of which has only recently been determined (Ede et al., 1994), endophyte-infected ryegrass was reported to contain smaller amounts of lolitrems A, C, and D, collectively known as the minor lolitrems (Gallagher et al., 1982). The minor lolitrems have been characterized by mass spectrometry (Gallagher et al., 1981, 1982), and a structure was proposed for lolitrem C on the basis of unpublished ^{1}H NMR data (Gallagher et al., 1984). Because little else was known about the structures of the minor lolitrems and their possible contributions to RGS, fractions rich in minor lolitrems obtained during the purification of lolitrem B were set aside. From these fractions we isolated the most abundant of the minor lolitrems, which we name lolitrem E.

We now report a method for the large-scale isolation of lolitrem B and for the isolation and structure determination of lolitrem E (2) (see Figure 1) using one- and two-dimensional ^{1}H and ^{13}C NMR techniques. Unlike lolitrem B, which is an indole–diterpene acetal, lolitrem E is an indole–diterpene ether. The "lolitrem C" previously isolated by Gallagher et al. (1984), and assigned the structure 44,45-dihydrololitrem B (3) (see Figure 1), is shown to have been lolitrem E (2).

EXPERIMENTAL PROCEDURES

General. Flash chromatography (Still et al., 1978) was performed on silica gel (Merck, art. 9385). Mass spectra were obtained on a Kratos MS-80 RFA, using a direct insertion probe. Estimates of lolitrem B content were obtained by HPLC, according to the method of Gallagher et al. (1985), using a 4.6 mm × 25 cm 5-μm Zorbax silica column with acetonitrile–dichloromethane (1:4, 3:17, or 1:9, as appropriate) as eluent (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 connected in series. The tremorgenic activity of lolitrem E was determined as described previously (Miles et al., 1992).

Nuclear Magnetic Resonance Spectroscopy. One- and two-dimensional ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained from a saturated deuteriopyridine (C_5D_5N) solution using a Bruker AC-300 instrument fitted with a standard 5-mm probe head. Chemical shifts are reported relative to TMS. ^{1}H NMR spectra were obtained at 300, 315, 330, and 350 K. Two-dimensional COSY spectra were obtained at 300 and 350 K. ^{1}H NMR decoupling experiments were performed at 300 K with a decoupling power of 15 L. ^{13}C NMR signal multiplicities (s, d, t, or q) were determined using the DEPT sequence. ^{13}C NMR spectra and the inverse mode two-dimensional HMBC spectrum were determined at 350 K. NOE difference experiments were performed at 300 K with an irradiation power of 40 L. NOE difference spectra were obtained by subtracting an off-resonance control FID from that of an irradiated FID and Fourier transforming the resulting difference FID.

Isolation of Lolitrem B. Lolitrems were extracted from ground endophyte-infected perennial ryegrass seed (*L. perenne* L. cv. MacMR) (0.6 kg), containing lolitrem B (ca. 10 mg kg^{-1}, 0.001%), by Soxhlet extraction with petroleum spirit (35–60 °C) for 11 h. A bank of five extractors was used, with fresh seed being introduced twice daily, allowing the extraction of 30 kg of seed in 5 days. After concentration in vacuo, the filtered petroleum extract (4 L) was partitioned against ethanol–water (4:1; 4 L). The alcoholic extract was diluted by addition of water (4 L) and extracted with dichloromethane (2 × 2 L), the extract was dried ($MgSO_4$), and the solvent was removed in vacuo. The resulting brown oil (80 g) contained lolitrem B (ca. 206 mg, 0.3%). This oil was dissolved in petroleum spirit (4 L) and extracted with acetonitrile–water (13:7; 1 × 4 L), and the petroleum fraction was discarded. The aqueous acetonitrile extract was then washed with petroleum spirit (2 L), diluted by addition of water (4 L), and extracted with dichloromethane (2 × 4 L). The extract was dried ($MgSO_4$) and the solvent removed in vacuo to give a brown oil (12 g) containing ca. 219 mg (1.8%) of 1.

This oil was applied in the minimum volume of dichloromethane to a flash column (7 × 9 cm, packed as a slurry in petroleum spirit). The column was eluted first with petroleum spirit (0.5 L), then with petroleum spirit–dichloromethane (1:1, 0.5 L) and dichloromethane (1 L), and then with a stepwise gradient of acetonitrile–dichloromethane (1:19, 2 L; 1:9, 1 L; 3:17, 1 L; 1:4, 1 L; 2:3, 1 L; 3:2, 1 L), and 0.5-L fractions were collected.

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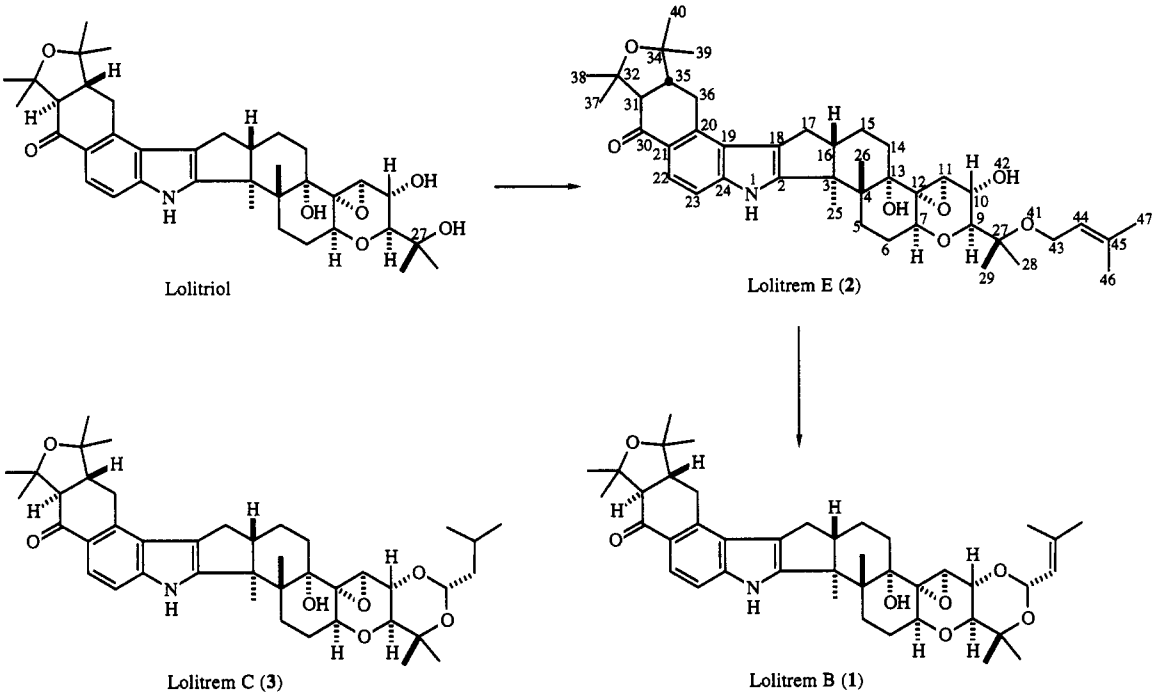

Figure 1. Structures of lolitriol, lolitrem B (1), lolitrem E (2), and lolitrem C (3), showing the possible biosynthetic route from lolitriol to lolitrem B involving lolitrem E as an intermediate.

Fractions containing lolitrem B (by HPLC) were concentrated in vacuo to give a brown oil (2.9 g) containing ca. 200 mg (7%) of 1.

This material was purified by flash chromatography (24 × 4 cm column) using acetonitrile–dichloromethane (3:47, 3.25 L, then with 2:23) to afford a yellow solid (0.9 g) containing ca. 225 mg (25%) of 1. The solid was further purified by flash chromatography (21 × 4 cm column) using acetonitrile–dichloromethane first at 3:97 (0.8 L) and then at 3:47. Fractions containing 1 were concentrated in vacuo to give a pale yellow solid (0.32 g) containing ca. 199 mg (62%) of 1.

Crystallization from acetonitrile–dichloromethane gave a colorless solid containing 1 (47 mg) and 2 (7 mg). The mixture was reacted with acetic anhydride–pyridine (1:1, 1 mL) and 4-(*N*,*N*-dimethylamino)pyridine (10 mg) for 24 h to acetylate 2. The mixture was dissolved in dichloromethane (50 mL), washed with water (2 × 50 mL), dried ($MgSO_4$), and eluted from a flash column (8 × 2.5 cm) with acetonitrile–dichloromethane (3:17). The residue was crystallized from acetonitrile–dichloromethane to give 1 (26 mg).

The mother liquor from the first crystallization afforded a second crop of colorless crystals containing 1 (80 mg).

From the resulting mother liquor was obtained a third crop, as a pale yellow solid, containing 1 (31 mg) along with lolitrem F. Recrystallization of this material gave a cream-colored solid containing 1 (27 mg).

The lolitrem B from the first, second, and third crops was then combined. Recrystallization gave 1 as a colorless solid (125 mg, 42% yield), identical in every respect to the material isolated by Gallagher et al. (1984).

Isolation of Lolitrem E. Fractions enriched in minor lolitrems were obtained during the purification of lolitrem B (above). Repeated flash chromatography, using acetonitrile–dichloromethane (as above), was used to isolate lolitrem E (2)—the most abundant minor lolitrem (see Figure 2). Eluted fractions were monitored by HPLC (Figure 2), and fractions enriched in lolitrem E were pooled for further purification, while fractions rich in other minor lolitrems were set aside for later study. After four flash chromatography purifications, the crude lolitrem E was dissolved in the minimum of dichloromethane and an equal volume of petroleum spirit was added, precipitating most of the lolitrem E; the bulk of the other lolitrems remained in the mother liquor (see text for discussion of solubility properties). The precipitate was treated with dilute acidic ethanol to convert contaminating lolitrems into lolitriol (lolitrem E is

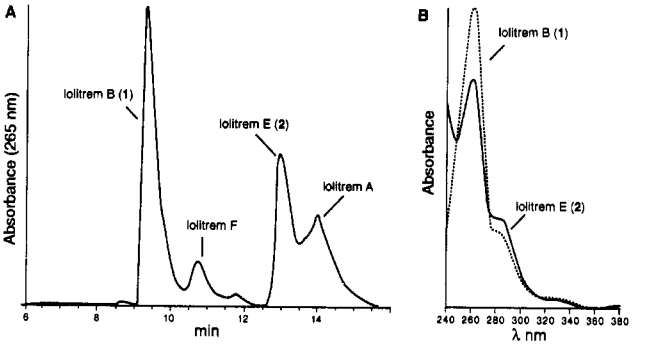

Figure 2. (A) HPLC chromatogram of a minor lolitrems fraction, with dichloromethane–acetonitrile (9:1) as the eluent. (B) UV absorbance spectra of 1 and 2, obtained from the chromatogram using a diode array detector.

stable to acid hydrolysis, see text) using the method of Miles et al. (1992). The residue was then treated briefly (10 min) with $NaBH_4$ (ca. 100 mg in 5 mL of ethanol) to reduce contaminating ketosterols (known to be present by GC–MS and ^1H NMR) to diols. Lolitrem E was then easily separated from the more polar lolitriol and dihydroxysterols by flash chromatography to give 2 as a colorless solid, which decomposed without melting above 150 °C. Qualitatively, the UV absorbance spectrum (see Figure 2) was identical to that reported by Gallagher et al. (1985) for lolitrem B: EI-MS *m/z* 688 (21%), 687.4101 (M^+, 687.4135 for $C_{42}H_{57}NO_7$, 34), 619 (18), 604 (18), 586 (14), 472 (19), 471 (30), 468 (15), 457 (16), 456 (30), 349 (29), 348 (100), 336 (15), 335 (34), 278 (14), 277 (18); ^1H NMR (C_5D_5N, 300 K) δ 1.34 (3H, s, H-40), 1.38 (3H, s, H-29), 1.40 (3H, s, H-28), 1.45 (3H, s, H-26), 1.49 (3H, s, H-39), 1.54 (3H, s, H-38), 1.58 (3H, s, H-47), 1.59 (3H, s, H-46), 1.69 (3H, s, H-37), 1.75 (3H, s, H-25), 2.95 (1H, d, J = 14.1 Hz, H-31), 3.59 (1H, dd, J = 15.6, 4.1 Hz, H-36), 3.93 (1H, d, J = 9.6 Hz, H-9), 3.96 (1H, br s, H-11), 4.06 (2H, br d, J = 7.2 Hz, H-43), 4.36 (1H, d, J = 8.6 Hz, H-10), 4.63 (1H, t, J = 8.5 Hz, H-7), 5.36 (1H, t, J = 6.6 Hz, H-44), 5.65 (1H, m, 10-OH), 6.37, (1H, s, 13-OH); ^{13}C NMR (C_5D_5N, 350 K) δ data are reported in Table 1.

RESULTS AND DISCUSSION

Ryegrass staggers research has hitherto been hindered by the limited availability of lolitrem B (1), the major neurotoxin believed to be the causative agent of RGS. For

Table 1. ^1H and ^{13}C NMR Assignments (δ, C_5D_5N at 350 K) of Lolitriol[a] and Lolitrem E

	lolitriol	lolitrem E			lolitriol	lolitrem E	
	^{13}C	^{13}C	^1H		^{13}C	^{13}C	^1H
2	154.6	154.7		24	143.3	143.5	
3	51.5[b]	51.5		25	16.4	16.5	1.70
4	42.8	43.1		26	18.6	18.7	1.43
5	27.1	26.9		27	72.5	78.7	
6	29.0	28.7		28	24.7	23.9	1.42
7	72.1	72.1		29	28.3	20.5	1.40
9	77.5	76.2		30	196.4	196.5	
10	68.4	67.8		31	60.6	60.5	
11	64.7	64.5		32	79.6	79.7	
12	69.5	69.3		34	79.2	79.3	
13	77.3	77.6		35	50.6	50.5	
14	30.2	30.1		36	28.8	28.7	
15	21.4	21.2		37	30.9	31.0	1.70
16	50.9	50.7		38	25.4	25.5	1.55
17	29.7	29.8		39	29.5	29.6	1.51
18	118.2	118.5		40	25.0	25.1	1.37
19	125.9	126.3		43		58.9	
20	124.6	124.9		44		122.5	
21	137.4	137.4		45		135.9	
22	120.0	119.8		46		25.4	1.66
23	111.2	111.0		47		17.7	1.64

[a] From Miles et al. (1992). [b] Value corrected from typographical error in Miles et al. (1992).

example, resistance to RGS is a heritable trait (Campbell, 1986), and selective breeding would therefore increase tolerance to the disease. It should be possible to identify sires resistant to RGS by using a performance testing procedure analogous to that developed by Towers et al. (1983) for improving tolerance to facial eczema, another mycotoxicosis showing heritable resistance. Such a procedure would, however, require supplies of 1 far beyond the capacity of previous methods of isolation. The procedure described here yielded 125 mg of 1 from a cycle of extraction and purification that took 2 weeks; it is therefore realistic to contemplate isolation of gram quantities of 1. In addition to allowing identification of resistant animals, such quantities of 1 should permit the metabolic fate of the toxin to be investigated.

The new isolation method is a considerable advance on that previously used by Gallagher and co-workers, which yielded 69 mg of 1 from 36 kg of seed (containing 13 mg kg^{-1} of 1) in a procedure taking several months (A. D. Hawkes, personal communication). The use of Soxhlet extraction allowed extraction of large quantities of seed into relatively small volumes of solvent. Extraction of 1 with petroleum spirit was found to be somewhat less efficient than with chloroform–methanol mixtures but greatly reduced the mass of the resulting extract. The aqueous acetonitrile–petroleum spirit partitioning step, when applied to the defatted extract, resulted in a 7-fold increase in the concentration of 1. As a consequence of these procedures, the concentration of 1 in the extract prior to chromatography was 1800 times that in the seed, and the mass of the extract had been reduced to a manageable level (12 g). Repeated flash chromatography efficiently increased the concentration of 1 in the extract to a level such that purification by crystallization became feasible. Lolitrem B was found to have low solubility in acetonitrile; crystallization of 1 could be achieved by concentrating acetonitrile–dichloromethane solutions on a rotary evaporator until the solution became cloudy and then allowing the solution to stand. The first crop of 1 was often contaminated with the much less soluble 2, but acetylation of the mixture converted 2 into its much more soluble acetate, which was easily removed from 1 by recrystallization. Alternatively, 2 could be separated from

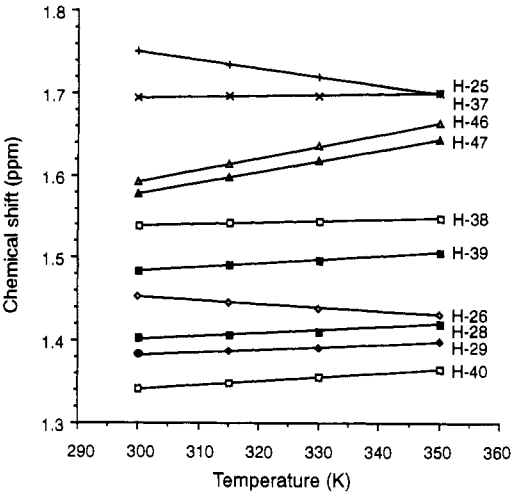

Figure 3. Temperature dependence of the methyl group chemical shifts of lolitrem E (2).

1 by acylation with glutaric anhydride followed by chromatographic purification.

Lolitrem E (2) was the second most abundant lolitrem (17% of 1 by HPLC) present in the ryegrass seed. Preliminary experiments indicated that 2, like 1, was resistant to reduction by $NaBH_4$ but, in contrast to the other lolitrems, was resitant to acidic hydrolysis—indicating the absence of the acid-labile acetal moiety that is present in 1. These characteristics, as well as its solubility properties (see below), were used to advantage during the purification of 2.

Because 2 was essentially insoluble in chloroform, ^1H NMR spectral data (including COSY and NOE difference spectra) were determined at 300 K using a saturated solution in C_5D_5N (ca. 1 mg in 0.4 mL). As greater solubility was achieved at 350 K, the ^{13}C NMR and the inverse mode two-dimensional HMBC NMR spectra were acquired at this temperature. At 350 K the chemical shifts of some of the methyl group protons (see Table 1) differed significantly from those determined at 300 K (see Experimental Procedures). Figure 3 depicts the temperature dependency of the methyl group chemical shifts. With the exception of the centrally located H-25 and H-26 methyl groups, methyl group chemical shifts increased with increasing temperature.

The ^1H NMR spectrum of 2 included 10 tertiary methyl group signals, together with proton multiplets attributable to the majority of low-field signals observed for 1 (Gallagher et al., 1984) and lolitriol (Miles et al., 1992). The ^1H NMR spectrum of 2 was also characterized by the presence of a two-proton doublet (4.06 ppm, J = 7.2 Hz) and the absence of a signal attributable to an H-43 acetal proton such as is present in 1. Decoupling experiments, and cross peaks observed in the two-dimensional COSY spectrum of 2, demonstrated that the two-proton signal at 4.06 ppm was strongly coupled to a triplet-like methine proton signal at 5.36 ppm and was weakly coupled to the pair of methyl group signals at 1.58 and 1.59 ppm. These observations indicated the presence in 2 of a five-carbon isoprene group, i.e., a $CH_2CH{=}C(CH_3)_2$ group.

Thus, it appeared that 2 was a derivative of lolitriol containing an additional five-carbon unit attached to either the 27-OH or the 10-OH. Two hydroxyl proton resonances (5.65 and 6.37 ppm) were observed in the ^1H NMR spectrum of 2 at 300 K (but not at 350 K). Cross peaks attributable to a weak ^5J coupling between the H-26 protons (i.e., the 4β-methyl group) and the 13α-OH (6.37 ppm) and a ^3J coupling between H-10β (4.36 ppm) and

Table 2. ^2J and ^3J ^1H–^{13}C HMBC Signal Correlations Determined for the Methyl Group Protons of Lolitrem E in C_5D_5N at 350 K

^1H signal, δ	^2J and ^3J correlated ^{13}C signals, δ
1.37 (H-40)	29.6 (C-39), 50.5 (C-35), 79.3 (C-34)
1.40 (H-29)	23.9 (C-28), 76.2 (C-9), 78.7 (C-27)
1.42 (H-28)	20.5 (C-29), 76.2 (C-9), 78.7 (C-27)
1.43 (H-26)	26.9 (C-5), 43.1 (C-4), 51.5 (C-3), 77.6 (C-13)
1.51 (H-39)	25.1 (C-40), 50.5 (C-35), 79.3 (C-34)
1.55 (H-38)	31.0 (C-37), 60.5 (C-31), 79.7 (C-32)
1.64 (H-47)	25.4 (C-46), 122.5 (C-44), 135.9 (C-45)
1.66 (H-46)	17.7 (C-47), 122.5 (C-44), 135.9 (C-45)
1.70 (H-37)	25.5 (C-38), 60.5 (C-31), 79.7 (C-32)
1.70 (H-25)	43.1 (C-4), 50.7 (C-16), 51.5 (C-3), 154.7 (C-2)

the 10-OH (5.65 ppm) appeared in the COSY spectrum of **2** at 300 K. It therefore follows that the $CH_2CH{=}C(CH_3)_2$ group is attached via the 27-OH group of lolitriol, rather than via the 10-OH. That this is so is supported by the observed facile acetylation of **2**, indicating the presence of a primary or secondary hydroxyl group in its structure.

Ring A methyl resonances were assigned by the presence in the COSY spectrum of **2** of cross peaks attributable to the existence of ^4J couplings between the H-37 and H-38 and the H-39 and H-40 protons, analogous to those observed by Miles et al. (1992) for lolitrem B and lolitriol.

In accord with the assignment of structure **2** to lolitrem E, its ^{13}C NMR spectrum comprised 15 quaternary (one of which was concealed under a solvent resonance—see below), 10 methine, 7 methylene, and 10 methyl carbon resonances. Although the C-45 (quaternary) resonance was not adequately defined in the conventional ^{13}C NMR spectrum, the resonant frequency of this carbon was revealed in an inverse mode two-dimensional HMBC experiment in which both the H-46 and H-47 (the olefinic methyl group) protons exhibited correlation with C-44 (122.5 ppm) and C-45 (135.9 ppm). Similarly, each of the other methyl proton resonances exhibited ^2J and ^3J correlation cross peaks (see Table 2) consistent with the ^1H and ^{13}C NMR assignments given in Table 1.

A feature of the ^{13}C NMR spectrum of **2** was the occurrence of a methylene carbon signal at 58.9 ppm, consistent with the presence of an oxygenated methylene group. While the majority of the other carbon resonances of lolitrem E corresponded closely with those we have reported for lolitriol (Miles et al., 1992), it is notable that C-27 experiences a downfield shift to resonate at 78.7 ppm, whereas the C-28 and C-29 methyl group carbons experience upfield shifts to resonate at 23.9 and 20.5 ppm, respectively. In contrast, the C-9 resonance of lolitriol experiences an upfield shift of only 1.3 ppm, while the C-10, and C-11 resonances of lolitrem E differ by less than 1 ppm from those determined for lolitriol (see Table 1).

The location of the $OCH_2CH{=}C(CH_3)_2$ group at C-27 was confirmed by NOE difference experiments performed at 300 K. Irradiation at 4.06 ppm (H-43) resulted in enhancements at 1.40 (H-28), 1.38 (H-29), 5.36 (H-44), and 1.59 ppm (H-46), whereas irradiation at 5.36 ppm (H-44) resulted in an enhancement at 1.58 ppm (H-47). Irradiation at 4.63 ppm (H-7) resulted in enhancements at 2.35 (H-6α), 3.93 (H-9), and 6.37 ppm (13α-OH), thereby confirming the configuration of **2** at C-9 to be the same as that observed for **1**. Similarly, the $J_{\text{H-9}\alpha\text{–H-10}\beta}$ coupling constant (9.6 Hz) exhibited by **2** is comparable to that of lolitrem B (9.5 Hz) (Gallagher et al., 1984) and α-paxitriol (8.7 Hz) (Miles et al., 1992). In contrast, β-paxitriol—in which the hydroxyl at H-10 is α-oriented—exhibits a $J_{\text{H-9}\alpha\text{–H-10}\alpha}$ coupling constant of 1.7 Hz (Miles et al., 1992).

Irradiation of H-29 (1.38 ppm) predominantly enhanced H-10 (4.36 ppm) and H-43 (4.06 ppm), whereas irradiation of H-28 (1.40 ppm) predominantly enhanced H-9 (3.93 ppm) and H-43 (4.06 ppm). These observations are consistent with the conclusion that in C_5D_5N solution there is restricted rotation about the C-9–C-27 bond, and consequently, C-29 and C-28 are oriented pseudoaxially and pseudoequatorially, respectively, with respect to ring H (see Figure 1).

The determination of the structure of lolitrem E as **2** revealed the presence of an ether-linked, rather than an acetal-linked, isoprene unit such as is present in **1**. This feature is responsible for the resistance of **2** to acidic hydrolysis. The low solubility of **2** in common organic solvents may be associated with the presence of the 10-OH group; **2** acetate was much more soluble than **2** in dichloromethane, and we have previously noted (Miles et al., 1992) that while paxilline and lolitrem B are soluble in $CDCl_3$, their respective derivatives paxitriol and lolitriol—both of which contain a 10-OH group—are virtually insoluble in this solvent.

Lolitrem E is the second most abundant lolitrem, being present at about 17% the concentration of lolitrem B. If **2** were tremorgenic, its contribution to RGS could be substantial because, unlike **1**, it would be unaffected by the acidic conditions encountered in the abomasum. When mice were dosed ip with **2** (2 mg kg^{-1}), no tremors could be detected. Under the same conditions, 1 mg kg^{-1} of **1** gave an easily detected tremorgenic response. Lolitrem E is therefore much less tremorgenic than lolitrem B. Since **1** and **2** are identical in rings A–H, it is clear that the structure–activity relationship within the indole–diterpene "tremorgens" is more complex than was previously thought. This is supported by the recent discovery (Miles et al., 1992; Penn et al., 1992) of several indole–diterpenes with unexpectedly low tremorgenic activity. A systematic study of the factors influencing both the tremorgenic potency and the duration of action of the indole–diterpenes could provide much needed structure–activity information about this class of mycotoxins.

The similarity of **2** to **1**, and to lolitriol, suggests a possible role for **2** in the biosynthesis of **1**. Addition of an isoprene unit to the oxygen at C-27 of lolitriol would give **2**. Conversion of **2** into **1** requires allylic oxidation (hydroxylation) of **2** at C-43 accompanied by cyclization with loss of H_2O. This sequence of events is depicted in Figure 1.

The molecular weight of lolitrem C, to which structure **3** was assigned by Gallagher et al. (1984), is the same as that for **2** (687). The "lolitrem C" studied by these workers was, however, virtually insoluble in $CDCl_3$, and they used deuteriopyridine as the solvent in their ^1H NMR study of this compound (R. Vleggaar, personal communication). Although Gallagher et al. (1984) proposed that their sample of lolitrem C had the structure 44,45-dihydrololitrem B (**3**), a compound with this structure would be expected to be freely soluble in $CDCl_3$; indeed, a semisynthetic sample of **3** prepared by us (Ede et al., 1994) was readily soluble in this solvent. Furthermore, HPLC analysis of a specimen labeled "lolitrem C", available at Ruakura from the earlier work of Gallagher and co-workers, showed that it coeluted with **2** but not with **3**. It therefore seems likely that the sample of lolitrem C studied by Gallagher et al. (1984) and assigned the structure of **3** on the basis of ^1H NMR and high-resolution mass spectrometry was actually a sample of lolitrem E (**2**). However, the name lolitrem C is now strongly associated in the literature with **3**. To avoid confusion, we propose that **3** retain the trivial name lolitrem C and that **2** be designated lolitrem E.

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