Synthesis and Tremorgenicity of Paxitriols and Lolitriol: Possible Biosynthetic Precursors of Lolitrem B

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The syntheses of lolitriol and of α- and β-paxitriol are reported. Full assignment of ^1H and ^{13}C NMR resonances of the paxilline and lolitrem derivatives was achieved by 2D NMR methods. The tremorgenic activities of paxitriols, lolitriol, paxilline, and lolitrem B were determined in mice. Possible roles for paxitriols and lolitriol in the biosynthesis of lolitrem B and in the ryegrass staggers syndrome are considered.

INTRODUCTION

The lolitrems (Gallagher et al., 1981, 1982), of which the main component (Gallagher et al., 1984) is lolitrem B (**1**), are indole–diterpene neurotoxins that have been isolated from perennial ryegrass (*Lolium perenne* L.). These lipophilic compounds are thought to be the principal causative agents of ryegrass staggers, a nervous disorder of livestock grazing ryegrass-dominant pastures. The occurrence of lolitrems is associated with the presence in the ryegrass of the endophytic fungus *Acremonium lolii* Latch (Gallagher et al., 1982).

Although lolitrem B is structurally related to the indole–diterpene fungal tremorgens (see Figure 1), only very recently has the production of lolitrem B (along with lolitriol) by cultures of *A. lolii* been demonstrated and even then only at low levels (M. E. di Menna and J. M. Sprosen, Ruakura Agricultural Centre, Hamilton, personal communication). However, such cultures also produce much larger amounts of another tremorgenic indole–diterpene mycotoxin, paxilline (**2a**) (Weedon and Mantle, 1987). That paxilline co-occurs with lolitrem B in endophyte-infected ryegrass (Weedon and Mantle, 1987) and that every atom in paxilline can be located by spatial observation at an equivalent position in the lolitrem B molecule suggest that lolitrem B is derived from a proximate biosynthetic precursor of paxilline, if not from paxilline itself. Conversion of **2a** to **1** requires the addition of just three structural features, viz. (i) the addition of an isoprene unit to the two appropriate and favorably disposed oxygen atoms (on C-10, C-27), (ii) the epoxidation of the olefinic double bond, and (iii) the diprenylation of the aromatic nucleus to form the bicyclic terpenoid moiety.

We now report the hydrolysis of the acetal moiety of lolitrem B to afford **3**, which we name lolitriol. The presence of lolitriol has since been demonstrated in extracts of endophyte-infected ryegrass leaf and seed (authors, unpublished observations) and in *A. lolii* cultures (di Menna and Sprosen, personal communication). Reduction of paxilline afforded the two diastereoisomeric allylic alcohols, which we name α- (**2b**) and β-paxitriol (**2c**). Because of its stereochemistry (assigned by ^1H NMR) at C-10, **2b** may be a more direct precursor than paxilline of lolitrem B. In addition, **2b,c** and **3** are potential mammalian metabolites of **2a** and **1**, respectively. Unexpectedly, neither **2b,c** nor **3** was tremorgenic in mice, a result that requires a revision of current theories [e.g., Selala et al. (1989)] on the structure–activity relationships within the indole–diterpenes.

The ^1H and ^{13}C NMR spectra of **1**, **2a–c**, and **3** were determined in the same solvent to allow direct comparison of resonances among the compounds, and a numbering system was chosen so as to facilitate this. Complete or nearly complete assignment of resonances was possible for all of the compounds using 2D methods. Previous assignments for the methylene carbons of **2a** were found to be in error.

EXPERIMENTAL PROCEDURES

NMR spectra were obtained at 300 (^1H) or 75 MHz (^{13}C) on a Bruker AC-300 instrument using a 5-mm probe. Chemical shifts are reported relative to internal TMS. Two-dimensional experiments were performed using absolute value mode (COSY, double-quantum-filtered COSY, and long-range ^{13}C–^1H correlated spectra) or phase-sensitive mode (^1J ^{13}C–^1H correlated and NOESY) experiments. UV spectra were obtained on a Shimadzu UV-160A in 1-cm quartz cells. Thin layer and flash chromatography was performed on silica gel (Merck, Art. 5554 and 9385, respectively). Mass spectra were obtained on a Kratos MS-80 RFA, by direct insertion probe. Lolitrem B was available from previous studies (Gallagher et al., 1984). Paxilline was produced according to the method of Ibba et al. (1987).

Reduction of Paxilline (2a). To a stirred solution of **2a** (120.3 mg, 0.276 mmol) in methanol (10 mL) was added sodium borohydride (26.1 mg, 0.687 mmol). After 85 min, no starting material could be detected by TLC (acetone–dichloromethane 1:3), and the reaction was poured into water (50 mL). After addition of HCl (5 mL; 1 M), products were extracted with dichloromethane (3 × 20 mL) and dried (Na_2SO_4) overnight at 4 °C in the dark. Removal of solvent in vacuo afforded a pale yellow oil. Addition of dichloromethane (ca. 1 mL) resulted initially in dissolution of the product, followed by precipitation. After standing at 4 °C for 30 min, solvent was removed by decantation. The off-white crystals were then washed with fresh solvent (3 × 1 mL) to afford pure **2c** (60.8 mg; 50%). The washings were purified by flash chromatography (acetone–dichloromethane 3:17) to give pure **2b** (the less mobile diastereoisomer) (16.0 mg; 13%) and **2c** (25.7 mg; 21%) and mixed fractions [overall yield 112.4 mg (93%)] as colorless solids. Both **2b** and **2c** decomposed without melting at 250–300 °C.

α-Paxitriol (2b). NMR data for the less mobile compound (by TLC) indicated a trans relationship between H-9$_\alpha$ and H-10$_\beta$ (J = 8.7 Hz), establishing α-OH at C-10. EI-MS m/z 437.2587 (M^+, 437.2557 for $C_{27}H_{35}NO_4$, 55%), 422 (47), 419 (42), 404 (28), 343 (15), 328 (18), 208 (9), 182 (100), 169 (54), 230 (56); UV λ_{max}^{MeCN} nm (log ε) 231 (4.53), 281 (3.91) nm. ^1H and ^{13}C NMR data are in Table I.

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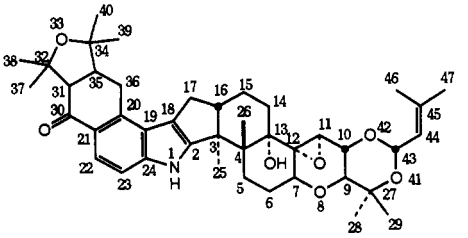
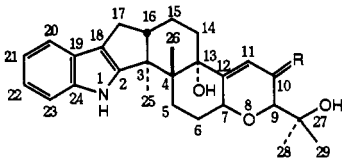
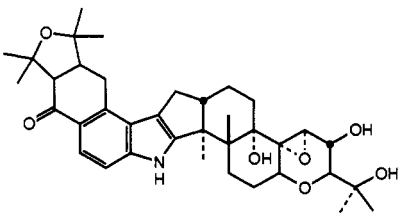


(1)



(2a) R = O
(2b) R = H, α-OH
(2c) R = H, β-OH



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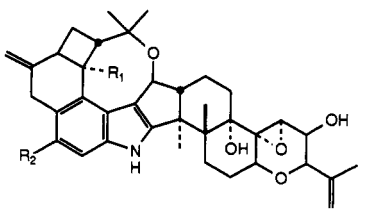
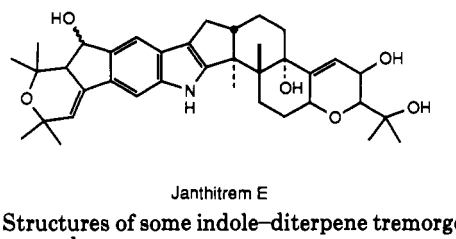


Penitrem A R_1 = OH, R_2 = Cl
Penitrem B R_1 = R_2 = H



Janthitrem E

Figure 1. Structures of some indole–diterpene tremorgens and related compounds.

β-Paxitriol (2c). NMR data for the more mobile compound (by TLC) indicated a cis relationship between H-9$_\alpha$ and H-10$_\alpha$ (J = 1.7 Hz), establishing β-OH at C-10. EI-MS m/z 437.2558 (M$^+$, 437.2557 for $C_{27}H_{35}NO_4$, 69%) 422 (52), 419 (15), 343 (18), 328 (18), 208 (17), 182 (100), 169 (52), 130 (57); UV λ_{max}^{MeCN} (log ε) 231 (4.58), 280 (3.94) nm. ^{1}H and ^{13}C NMR data are in Table I.

Hydrolysis of Lolitrem B (1). Lolitrem B (19.0 mg; 85% purity by HPLC) was stirred at 40 °C with ethanol (50 mL) to which concentrated HCl (1 mL) was added. After 1 h, examination of the now clear solution by TLC (methanol–dichloromethane 1:19) showed complete conversion of 1 into 3. The solution was diluted with a large excess of $NaHCO_3$ (1 M), and products were extracted with dichloromethane (2 × 50 mL). Drying (Na_2SO_4) and removal of solvent in vacuo afforded a colorless solid (18 mg). This was purified by flash chromatography (methanol–chloroform 6:94) to afford 3 as a pale yellow solid, 14.8 mg (100%): mp 322–5 °C; EI-MS m/z 620 (17%), 619.3528 (M$^+$, 619.3496 for $C_{37}H_{49}NO_7$, 43), 604 (41), 471 (55), 456 (44), 348 (100), 335 (44); UV λ_{max}^{MeCN} 263 (log ε 4.72), 290 (shoulder) nm. ^{1}H and ^{13}C NMR data are in Table II.

Table I. ^{1}H and ^{13}C NMR Assignments for Paxilline, α-Paxitriol, and β-Paxitriol in $CDCl_3$–DMSO (2:1) at 300 K

	paxilline			α-paxitriol			β-paxitriol		
atom	^{13}C	^{1}Hα	^{1}Hβ	^{13}C	^{1}Hα	^{1}Hβ	^{13}C	^{1}Hα	^{1}Hβ
2	152.6			153.5			153.4		
3	50.4			50.5			50.5		
4	42.6			42.5			42.4		
5	26.4	2.55	1.81	26.8	2.57	1.69	26.8	2.61	1.74
6	28.5	2.26	1.80	28.3	2.01	1.65	28.3	2.19	1.91
7	71.7	4.87		73.3	4.60		74.1	4.61	
9	83.0	3.69		81.7	3.03		80.4	3.06	
10	198.8			64.5		4.28	63.3	4.19	
11	119.3	5.80		120.2	5.35		117.5	5.64	
12	169.6			144.5			147.9		
13	75.9			75.8			76.2		
14	32.9	1.72	1.87	33.5	1.69	1.87	33.7	1.69	1.94
15	20.9	2.02	1.69	21.2	2.06	1.67	21.2	2.03	1.64
16	49.5		2.78	49.7		2.80	49.8		2.82
17	27.0	2.37	2.64	27.1	2.36	2.62	27.1	2.39	2.67
18	115.3			115.1			115.2		
19	124.6			124.7			124.7		
20	117.7	7.29		117.6	7.28		117.6	7.33	
21	118.5	6.93		118.4	6.92		118.5	6.96	
22	118.8	6.91		119.1	6.91		119.2	6.94	
23	111.8	7.28		111.7	7.27		111.8	7.32	
24	139.9			139.9			139.9		
25	16.2	1.29		16.3	1.29		16.3	1.33	
26	18.9	0.96		19.5	0.95		19.6	1.04	
27	72.6			72.4			72.0		
28	24.7	1.22		24.2	1.22		26.7	1.29	
29	26.3	1.24		27.8	1.26		27.1	1.30	

Conversion of Lolitriol (3) into Lolitrem B (1). Lolitriol (1 mg) was dissolved in $CHCl_3$–DMF (25:3; 280 μL). To the stirred solution was added 3-methyl-2-butenal dimethyl acetal [prepared from 3-methyl-2-butenal (Aldrich) according to the method of Bandaranayake (1971)] (50 μL) and pyridinium toluene-4-sulfonate (0.5 mg). The reaction was followed by TLC (chloroform–methanol 19:1), which showed complete conversion to lolitrem B after 4 h. The identity of the product was confirmed by HPLC analysis (Gallagher et al., 1985) and by EI-MS after purification by flash chromatography.

Tremorgen Bioassay. The procedure of Gallagher and Hawkes (1986) was followed with minor changes. Compounds tested were administered intraperitoneally (ip) by injection into mice (female Balb/c, weight 25 ± 5 g, 13–18 weeks old) as solutions in DMSO–water (9:1; 100 μL). Mice were assessed for tremor score at 15-min intervals for the first hour, then approximately hourly for the next 4–5 h, and where necessary at 21 h after injection. Tremor score was assessed by observing the severity of spontaneous resting tremor and tremor induced by forcing the mouse to balance on an outstretched finger and rated 1–5 according to the scale of Gallagher and Hawkes (1985, 1986).

RESULTS AND DISCUSSION

The production of lolitrem B by *A. lolii* in culture (di Menna and Sprosen, personal communication) proves it to be a mycotoxin. Lolitriol is a likely biosynthetic precursor of lolitrem B, since it requires only the addition of the acetal moiety to form 1. That this is chemically feasible is demonstrated by the ready formation of 1 from 3. The co-occurrence of 3 with 1 in ryegrass (authors, unpublished observations) and in *A. lolii* cultures also supports this contention, although it is possible that the

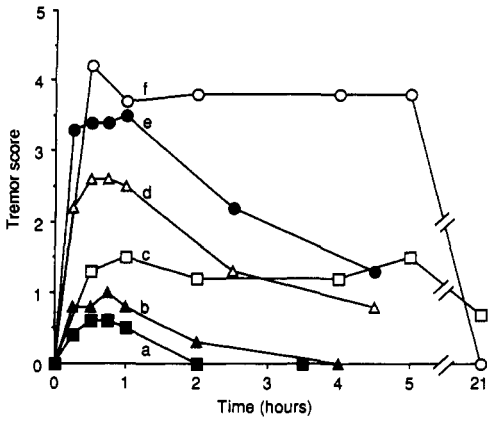


Figure 2. Mean tremor score vs time postinjection for groups of mice dosed with (a) control (n = 6 mice), (b) paxilline at 4 mg kg^{-1} (n = 3), (c) lolitrem B at 1 mg kg^{-1} (n = 3), (d) paxilline at 6 mg kg^{-1} (n = 6), (e) paxilline at 8 mg kg^{-1} (n = 6), and (f), paxilline at 80 mg kg^{-1} (n = 3).

lolitriol present is due to hydrolysis of **1** in vivo. It is unlikely that **3** is produced from **1** during the extraction procedure, since Gallagher et al. (1985) recovered up to 98.7% of lolitrem B from spiked grass using the same extraction protocol. Researchers should, however, be aware that lolitrem B is readily hydrolyzed; we observed the partial conversion of **1** into **3** and 3-methyl-2-butenal after prolonged standing in moist $CDCl_3$–DMSO (2:1). Furthermore, the drying of grass at 50 and 90 °C reduced its lolitrem B content to ca. 83 and 57%, respectively, of that of freeze-dried controls (J. M. Sprosen, personal communication). This reduction in lolitrem B content may be due in part to its hydrolysis to **3**. The mildest possible conditions for drying samples for lolitrem B analysis should be used, and contact of samples or extracts with aqueous or alcoholic acid solutions should be avoided.

Biosynthesis of the Lolitrems. It seems highly likely that **1** and **3** are derived biosynthetically from a paxilline-like precursor, since every atom present in **2a** is present in an equivalent position in both **1** and **3** and with identical stereochemistry. The fact that **2b** also has the same oxidation state and stereochemistry at C-10 as do **1** and **3** suggests that **2b** might be an even closer biosynthetic precursor of **1** and **3** than is **2a**. We therefore propose the following biosynthetic scheme for lolitrem B:

$$\mathbf{2a} \rightarrow \mathbf{2b} \rightarrow \rightarrow \mathbf{3} \rightarrow \mathbf{1}$$

The feasibility of this scheme is supported by the recent isolation of the acetate of **2c** from *Penicillium crustosum* (Hosoe et al., 1990). In addition, Mantle and Penn have shown that radiolabeled **2a** is incorporaed into penitrems by *Penicillium janczewskii* (Mantle and Penn, 1989) and into janthitrem B by *Penicillium janthinellum*, while labeled **2a** and **2c**, but not **2b**, are incorporated into penitrems A and E by *P. janczewskii* (P. G. Mantle and J. Penn, Imperial College, London, personal communication). An understanding of the biosynthesis of **1** is important from a practical point of view. One strategy for eliminating ryegrass staggers is to develop strains of *A. lolii* endophyte that do not produce **1**. Such strains might indeed produce no **1** but might well produce **2a–c**, **3**, or other metabolites instead, depending upon the point at which lolitrem B biosynthesis has been blocked. The tremorgenicity and toxicity of compounds **1–3** are discussed below.

Tremorgenicity. Results of the tremorgenicity testing are presented in Figure 2. As expected, both **1** and **2a** were tremorgenic, with **1** being ca. 5 times more tremorgenic than **2a**. Paxilline is much more tremorgenic than was indicated by the results of Cole et al. (Cole et al., 1974; Cole and Cox, 1981), who reported only intermittent tremors with 35 mg kg^{-1} of **2a**. It should be noted, however, that Cole administered **2a** in corn oil rather than in DMSO–water as in the present study, and it is well-known that DMSO is an active penetrant and carrier of many compounds through skin and cellular membranes (David, 1972). In view of the relative tremorgenicities of **2a** and **1**, determined here under identical conditions for the first time, the potential contribution of paxilline to the ryegrass staggers syndrome can no longer be ignored. Lolitrem B is, however, likely to be the major contributor to ryegrass staggers, due to its prolonged tremorgenic action (at least when administered ip in mice); at 21 h postinjection, mice still exhibited significant tremors from 1 mg kg^{-1} of **1**, whereas even those given 80 mg kg^{-1} of **2a** did not (see Figure 2).

Surprisingly, **3** was nontremorgenic even at 20 mg kg^{-1}, making it at least 20 times less tremorgenic than its parent compound, **1**. Compounds **2b** and **2c** were not tremorgenic even at 100 mg kg^{-1}, making them at least 20 times less active than their parent compound, **2a**. Although nontremorgenic, **2b** and **2c** caused lethargy and rough coats at 100 mg kg^{-1}, and normal activities such as walking, rearing, and preening were greatly reduced for several hours relative to control animals. Behavior was normal by 24 h postinjection. Administration of **2c** and **3** (100 and 16 mg kg^{-1}, respectively) in 200 μL (rather than in 100 μL) of DMSO–water (9:1) proved lethal after an initial period of lethargy, whereas dosing with 200 μL of carrier caused initial lethargy but no deaths. Thus, it appears that indole–diterpene "tremorgens" bearing C-10 hydroxyl and C-9 2-propanol moieties may be toxic but nontremorgenic. This result is unprecedented for this class of compound, and the generality of this phenomenon requires further investigation. Penitrems A and E are both tremorgenic (Kyriakidis et al., 1981); both contain a C-10 β-OH, but both lack the C-27 OH present in compounds **2b,c** and **3**. Janthitrem E (de Jesus et al., 1984) is, however, a very close analogue of **2c** but to our knowledge has not been tested for tremorgenicity. In light of these results, current ideas about the structural features necessary for tremorgenic activity [e.g., Selala et al. (1989)] need to be revised.

Reduction of ketones by ruminal and mammalian metabolism is commonplace, so conversion of **2a** into **2b** and/or **2c** may well take place after ingestion of paxilline, while acid- or enzyme-catalyzed acetal hydrolysis of **1** during digestion would result in its conversion to **3**. Thus, the two tremorgenic compounds (**1** and **2a**) known to be associated with *A. lolii*-infected ryegrass may well be partially converted to toxic but nontremorgenic compounds during ingestion by livestock. This, and the finding that substantial quantities of **3** are present in the ryegrass itself, suggests a possible role for **2b,c** and **3** in the ill-thrift of livestock associated with ryegrass staggers (Fletcher, 1986; Prestidge et al., 1991). The possibility of antagonistic or synergistic effects involving compounds **1–3** requires further investigation.

NMR Signal Assignments. Hitherto a variety of solvents have been used to determine the ^{1}H and ^{13}C NMR of indole–diterpene tremorgenic mycotoxins. While $CDCl_3$ is the solvent of choice for low polarity compounds, a more polar solvent is required for the less soluble triol analogues prepared in this study. We have found that the triols are adequately soluble in $CDCl_3$–DMSO (2:1) at temperatures in the region 300–315 K. Tables I and II list the ^{13}C and ^{1}H NMR assignments established in a series of one- and

Table II. ^1H and ^{13}C NMR Assignments for Lolitriol and Lolitrem B

	lolitriol[a]			lolitriol[b]			lolitrem B[c]		
atom	^{13}C	$^1H\alpha$	$^1H\beta$	^{13}C	$^1H\alpha$	$^1H\beta$	^{13}C	$^1H\alpha$	$^1H\beta$
2	154.6			154.9			153.8		
3	50.5			50.5			50.4		
4	42.8			42.1			42.2		
5	27.1	2.97	1.89	25.8	2.56	1.68	25.8	2.54	1.72
6	29.0	2.34	1.97	28.1	2.23	1.68	28.4	2.22	1.73
7	72.1	4.60		71.0	4.18		71.1	4.33	
9	77.5	3.91		75.9	3.31		70.9	3.51	
10	68.4		4.39	67.0		3.90	70.8		3.94
11	64.7		3.92	63.5		3.45	59.6		3.50
12	69.5			68.8			67.3		
13	77.3			76.8			76.8		
14	30.2	1.66	1.99	29.2	1.66	1.38	29.0	1.66	1.36
15	21.4	2.25	1.67	21.4	1.91	1.58	20.4	1.91	1.58
16	50.9		3.09	50.0		2.80	49.9		2.79
17	29.7	2.76	3.06	28.9	2.55	2.91	28.8	2.57	2.90
18	118.2			117.2			117.0		
19	125.9			124.8			124.7		
20	124.6			123.5			123.4		
21	137.4			136.6			136.5		
22	120.0	8.26		118.6	7.67		118.6	7.66	
23	111.2	7.49		110.7	7.27		110.6	7.24	
24	143.3			142.4			142.3		
25	16.4	1.69		15.9	1.24		15.9	1.24	
26	18.6	1.42		18.2	1.07		18.3	1.09	
27	72.5			72.2			74.3		
28	24.7	1.50		24.0	1.16		28.3	1.22	
29	28.3	1.52		27.8	1.18		16.6	1.27	
30	196.4			196.2			196.0		
31	60.6	2.94		59.6	2.76		59.4	2.76	
32	79.6			79.3			79.3		
34	79.2			79.1			79.0		
35	50.6	2.78		49.7	2.60		49.6	2.61	
36	28.8	3.05	3.61	28.0	2.93	3.42	27.9	2.95	3.42
37	30.9	1.69		30.5	1.45		30.5	1.45	
38	25.4	1.54		25.0	1.24		25.0	1.23	
39	29.5	1.50		29.2	1.33		29.3	1.34	
40	25.0	1.36		24.9	1.23		24.9	1.22	
43							92.3	5.50	
44							122.3	5.18	
45							138.1		
46							25.4	1.71	
47							18.4	1.70	

[a] In C_5D_5N at 350 K. [b] In $CDCl_3$–DMSO (2:1) at 315 K. [c] In $CDCl_3$–DMSO (2:1) at 300 K.

two-dimensional NMR experiments for paxilline and the paxitriol isomers and for lolitriol and lolitrem B, respectively, in this solvent. The NMR spectra of lolitriol were also determined in C_5D_5N at 350 K. The atom numbering scheme used in Table I is that originally proposed by Springer et al. (1975) for paxilline; an extension of this numbering scheme is used in Table II for lolitriol and lolitrem B, such that atoms 1–29 are numbered in common.

Paxilline and Paxitriol Isomers. Most previous tabulations of ^{13}C NMR data for paxilline, including a recent 500-MHz study (Mantle et al., 1990), indicate (Nozawa et al., 1989; Mantle et al., 1990; Cole et al., 1977) that the lowest field methylene resonance (32.9 ppm in $CDCl_3$) arises from C-17, while interchangeable assignments are suggested for C-5 and C-6 and for C-14 and C-15. Even at high fields (Mantle et al., 1990; Gallagher et al., 1984) considerable signal overlap occurs in the ^1H NMR spectra of tremorgenic indole–diterpenes such as paxilline and lolitrem B, rendering it difficult, if not impossible, to achieve a complete assignment of the ^1H NMR resonances via a first-order analysis of coupling constants. Notwithstanding the use of a medium-field instrument (300 MHz), we were able to achieve complete assignments for the paxilline and lolitrem analogues prepared in this study via a series of one- and two-dimensional NMR experiments.

The manner in which complete assignment was achieved is illustrated here for some of the resonances of paxilline. First, ^1H NMR resonances were correlated with those of nearby protons in COSY and double quantum filtered COSY (DQFCOSY) experiments using acquisition and processing conditions which allowed for the detection of both short-range (^2J and ^3J) and long-range (^4J and ^5J) couplings. Thus, there appeared in the DQFCOSY spectrum of paxilline at the resonance frequency of H-7α (4.8 ppm) cross peaks arising from the coupling of this proton with H-6α, H-6β, H-9α, and H-11 (2.26, 1.80, 3.69, and 5.80 ppm, respectively). The protons of one of the tertiary methyl groups (H-26, 0.96 ppm) exhibited a cross peak at the resonance frequency of H-5α (2.66 ppm). It is now well established (Wilkins et al., 1989a,b) that in the five- or six-membered ring systems of steroids and triterpenes tertiary methyl groups exhibit small long-range couplings (resolved in COSY and DQFCOSY, but not in conventional ^1H NMR spectra) with methylene protons oriented 1,2-trans with respect to the methyl group. In paxilline such a relationship exists only for the 4β-methyl group (i.e., C-26) and H-5α.

Second, ^1H and ^{13}C resonance connectivities were correlated in a phase-sensitive XH-correlated 2D experiment, the digital resolution of which (1 Hz in the ^1H dimension) was sufficient to distinguish axial–axial couplings (typically 10–12 Hz) from axial–equatorial or equatorial–equatorial couplings (typically 3–5 Hz) (Wilkins et al., 1989a,b). Additionally, ^1H and ^{13}C resonances were correlated in an absolute value XH-correlated experiment optimized for the detection of ^1J and longer range ^2J and ^3J couplings in a single experiment. For example, H-25 (1.29 ppm) exhibited cross peaks at the resonance frequencies of C-3, C-4, C-5, C-13, and C-26 (50.4, 42.6, 26.4, 75.9, and 18.9 ppm, respectively).

Finally, the phase-sensitive 2D NOESY spectrum of paxilline included cross peaks which independently verified the majority of the assignments elucidated in the COSY and XH-correlated spectra. For example, H-11 (5.80 ppm) exhibited correlations with H-14α (1.72 ppm) and H-14β (1.87 ppm). Similarly, H-25 (1.29 ppm) exhibited correlations with H-17α (2.37 ppm), H-15α (2.02 ppm), and H-5α (2.66 ppm), while H-26 (0.96 ppm) exhibited correlations with H-16β (2.78 ppm) and H-6β (1.80 ppm). In conjunction with the COSY and XH-correlated data these observations led to the methylene carbon assignments presented in Table I. It is notable that the highest field methylene carbon resonance (32.8 ppm) arises from C-15 rather than from C-17 as proposed elsewhere (Mantle et al., 1990; Nozawa et al., 1989; Cole et al., 1977). This is in accord with the assignments for the janthitrems (de Jesus et al., 1984) and lolitrem B (Gallagher et al., 1984) and with Hosoe et al.'s (1990) assignments for paxilline (although our assignments for C-5, C-6, and C-17 do not agree with theirs). The generally accepted ^{13}C assignments for the methylene carbons of paspaline and related compounds (Steyn and Vleggaar, 1985) are clearly incorrect and in need of revision.

The complete assignment of the ^1H and ^{13}C NMR resonances of the paxitriol isomers was achieved in a manner analogous to that described for paxilline. While none of the methylene carbons of **2a–c** are particularly sensitive to the nature of the C-10 substituent, it is notable that the C-6 methylene protons are surprisingly sensitive to variation in the C-10 substituent. In β-paxitriol the presence of a 10β-hydroxyl group results in H-6β (1.91

ppm) exhibiting a downfield shift of 0.26 ppm relative to the same proton in α-paxitriol (1.65 ppm). This appears to be due to the absence of axially inclined β-face protons at C-7, C-9, C-11, and C-12.

Lolitriol and Lolitrem B. The assignments established in the present study for lolitriol and lolitrem B (see Table II) are generally comparable with those reported by Gallagher et al. (1984) for lolitrem B in $CDCl_3$. We were, however, able to more fully assign the methyl group resonances since the DQFCOSY spectra of lolitriol and lolitrem B included cross peaks arising from mutual ^4J couplings between H-28 and H-29, H-37 and H-38, and H-39 and H40, respectively. Additionally, in the C_5D_5N DQFCOSY spectrum of lolitriol, H-26 and H-37 exhibited cross peaks at the resonance frequencies of H-5α and H-31, respectively. Although the latter ^4J coupling is indicative of a trans relationship between H-31 and H-37, the relative and absolute configuration at C-31 and C-35 remains uncertain (Gallagher et al., 1984).

In the case of lolitrem B, irradiation of H-29 (1.27 ppm, i.e., the axial C-27 methyl group) resulted in NOEs at H-10β and H-43β, thereby confirming Gallagher et al.'s (1984) deduction that rings H and I were trans-fused with the C-10 oxygen function equatorially (α-face) inclined, and their assigned configuration at C-43. We were also able to verify Gallagher et al.'s (1984) conclusion that H-23 was long range coupled to one of the C-36 protons, since the COSY spectrum (optimized for long-range couplings) included a weak cross peak at the resonance frequencies of H-23 (7.24 ppm) and one of the C-36 protons (2.95 ppm). Mutual NOE effects were observed between H-23 and the NH proton on irradiation of these protons in separate NOE difference experiments.

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