Terpendole M, a Novel Indole−Diterpenoid Isolated from *Lolium perenne* Infected with the Endophytic Fungus *Neotyphodium lolii*

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Terpendole M (**1**), a novel indole−diterpenoid, was isolated from perennial ryegrass (*Lolium perenne*) infected with the endophytic fungus *Neotyphodium lolii*. It was identified as 14α-hydroxyterpendole C by NMR and mass spectral techniques. The known indole−diterpenoids paspaline (**3**) and 13-desoxypaxilline (**4**) were also isolated from perennial ryegrass for the first time. Terpendole M was less tremorgenic than terpendole C (**2**) in a standard mouse bioassay. These findings provide clues to the biogenesis of the lolitrem neurotoxins, and information on the structure−activity relationships within the indole−diterpenoids.

Keywords: *Terpendole; paxilline; paspaline; lolitrem; neurotoxin; tremor; Neotyphodium lolii; Acremonium lolii; Lolium perenne; ryegrass staggers*

INTRODUCTION

Ryegrass staggers is a neurotoxic disorder occurring in livestock grazing perennial ryegrass (*Lolium perenne* L.) infected with the endophytic fungus *Neotyphodium lolii* (Latch, Christensen & Samuels) Glenn, Bacon & Hanlin (formerly *Acremonium lolii*). Lolitrem B (**8**), an indole−diterpenoid produced by the endophyte (Miles et al., 1992; Penn et al., 1993), is believed to be the principle neurotoxin responsible for the condition (Gallagher et al., 1984), although it has become apparent that other indole−diterpenoids contribute significantly to the toxicity of perennial ryegrass (Miles et al., 1992, 1994; Munday-Finch et al., 1995, 1996b, 1997, 1998; Weedon and Mantle, 1987).

The lolitrems are part of a large family of fungal indole−diterpenoids, many of which are tremorgenic, that includes the penitrems, janthitrems, the paxilline analogues, the paspaline/emindole group, and the terpendoles. Although the lolitrems appear to be biosynthetically closely related to the other indole−diterpenoid groups (Mantle and Weedon, 1994; Munday-Finch et al., 1998), only lolitrems (Munday-Finch, 1997) and paxilline (Weedon and Mantle, 1987) have been isolated from perennial ryegrass; no members of other indole−diterpenoid toxin groups have been identified in this grass.

Here we report the isolation of the novel indole−diterpenoid terpendole M (**1**) as well as the first isolation of the known indole−diterpenoids paspaline (**3**) and 13-desoxypaxilline (**4**) from perennial ryegrass. We also report the relative tremorgenic activities of terpendoles C (**2**) and M (**1**) in a standard mouse bioassay.

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EXPERIMENTAL PROCEDURES

General. Mass spectra were obtained on a Kratos MS-80 RFA instrument, with a direct insertion probe. Flash chromatography (Still et al., 1978) was performed on silica gel (Merck, Art. 9385). The indole−diterpenoid content of fractions obtained during purification was assessed by analytical HPLC with a 4.6 mm × 25 cm Zorbax silica gel column (5 μm) and acetonitrile−dichloromethane (1:4) 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. Semipreparative HPLC purification was performed on an RCM-100 Radial Compression Separation System (Waters) fitted with a silica gel Radial-PAK cartridge (8 mm × 10 cm, 10 μm) (Waters), with acetonitrile−dichloromethane (1:9) as eluent (3.0 mL min^{-1}). Eluting compounds were detected at 230 or 280 nm with a Perkin-Elmer LC-85B spectrophotometric detector. The calculated structure of **1** (Figure 3) was minimized (MM2, Chem3D Pro 3.5, CambridgeSoft Corp., Cambridge, MA) from a structure based on that calculated for lolitrem B (**8**) by Ede et al. (1994). Authentic specimens of paspaline (**3**) and 13-desoxypaxilline (**4**) were available from previous work (Munday-Finch et al., 1996a). The tremorgenic activities of terpendole M (**1**) and terpendole C (**2**) were assessed at ca. 8 mg kg^{-1} after intraperitoneal injection (0.1 mL, 2.0 mg mL^{-1} solutions in 9:1 DMSO−water) into mice (female Swiss, weight 25 ± 5 g, 13−18 weeks old) (Miles et al., 1992). 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). The mice were monitored daily for any unusual behavior for 2 weeks after dosing. All animal manipulations were approved by animal ethics committees established under the Animal Protection (code of ethical conduct) Regulations Act, 1987 (New Zealand).

Nuclear Magnetic Resonance Spectroscopy. One- and two-dimensional ^{1}H (400.13 MHz) and ^{13}C (100.26 MHz) NMR spectra of terpendole M (**1**) were obtained with a Bruker DRX-400 spectrometer fitted with a 5 mm dual inverse probe, as described previously (Munday-Finch et al., 1997). HMBC and HMQC spectra (phase-sensitive) were obtained with gradient selection, the NOESY spectrum (phase-sensitive) was acquired with a mixing time of 0.5 s, and the ROESY spectrum (phase

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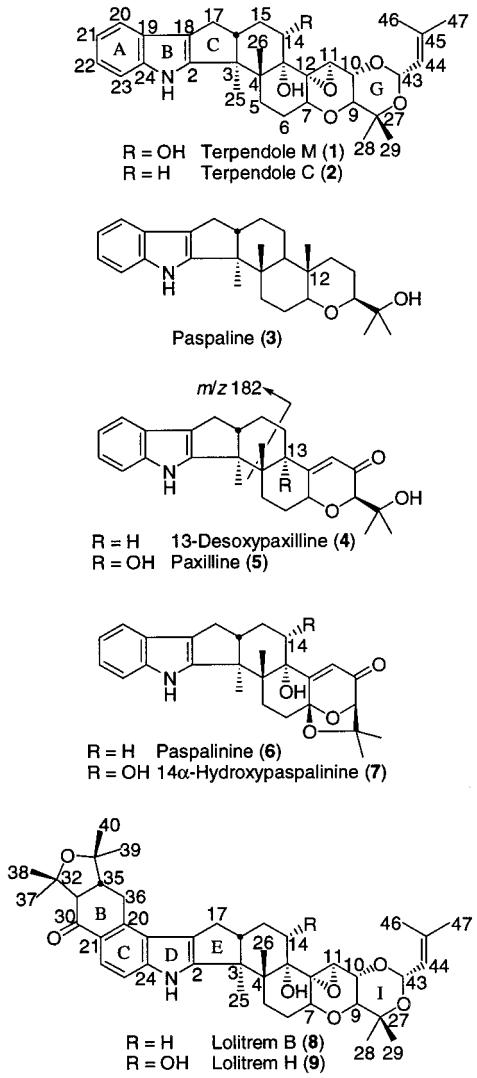

Figure 1. Structures of terpendole M (**1**), terpendole C (**2**), paspaline (**3**), 13-desoxypaxilline (**4**), paxilline (**5**), paspalinine (**6**), 14α-hydroxypaspalinine (**7**), lolitrem B (**8**), and lolitrem H (**9**). Atom numbering for terpendoles (**1** and **2**) follows that for lolitrems (e.g., **8** and **9**) to facilitate comparison of NMR data.

sensitive) was acquired with a spinlock time of 0.25 s. The ^{1}H (300.13 MHz) NMR spectra of paspaline (**3**) and 13-desoxypaxilline (**4**) were determined with a Bruker AC-300 spectrometer fitted with a 5 mm dual probe. The ^{1}H NMR spectrum of **2** was obtained (at 300 MHz) in order to check its purity prior to dosing as part of a previous structure−activity study (Munday-Finch et al., 1997). NMR spectra were obtained from deuteriochloroform ($CDCl_3$) solutions, and chemical shifts are reported relative to TMS, based on $CDCl_3$ $\delta^{13}C = 77.1$ and $CHCl_3$ δ $^1H = 7.26$.

Fractionation of the Ryegrass Extract. During purification of lolitrem B (**8**) (Miles et al., 1994), a fraction was retained that eluted just prior to **8** during the first flash column chromatography step. HPLC analysis of this fraction revealed the presence of compounds that coeluted with paspaline (**3**) and 13-desoxypaxilline (**4**) (Figure 2). This fraction was purified by flash column (4 × 20 cm) chromatography with acetonitrile−dichloromethane (1:19, 750 mL; 1:9, 750 mL; 1:4, 500 mL) as eluents, and fractions of 250 mL were collected.

The compounds of interest, which HPLC showed to be in the third fraction, were further purified by flash column (2 × 20 cm) chromatography with acetonitrile−dichloromethane (1:19, 600 mL; 1:4, 200 mL) as eluents and fractions of 18 mL were collected. Analysis by HPLC indicated that fractions 13−16 contained predominantly 13-desoxypaxilline (**4**) and that fractions 17−36 contained paspaline (**3**).

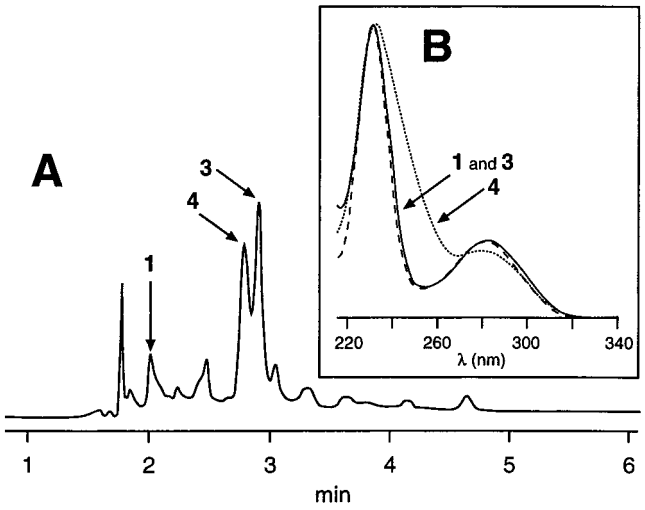

Figure 2. (A) Analytical HPLC chromatogram of the crude fraction containing terpendole M with (B) normalized UV absorbance spectra of terpendole M (**1**), paspaline (**3**), and 13-desoxypaxilline (**4**) obtained from the chromatogram by means of a diode array detector.

Isolation of Terpendole M (1) and 13-Desoxypaxilline (4). Unidentified sterols that precipitated from the 13-desoxypaxilline fraction (fractions 13−16, above) during concentration were discarded. The mother liquor was purified by flash column (1 × 20 cm) chromatography with acetonitrile−dichloromethane (1:49, 250 mL; 3:97, 100 mL; 1:9, 100 mL) as eluents, and fractions of ca. 10 mL were collected. Fractions containing compounds of interest were identified by HPLC, combined, and further purified by flash column (1 × 20 cm) chromatography with ethyl acetate−dichloromethane (1:9) as eluent and fractions of ca. 10 mL collected. HPLC revealed the presence of two major compounds, one of which was in fractions 1−5 (**1**) and the other in fractions 6−15 (**4**).

The crude **1** was purified by semipreparative HPLC to provide semipure terpendole M. Crystallization of this material from acetonitrile gave terpendole M (**1**) as a colorless solid (2.4 mg). EI-MS *m*/*z* 535.2951 (M^+, 535.2934 for $C_{32}H_{41}NO_6$, 100%), 520 (48), 182 (28), 169 (19), 83 (14), 55 (18), 41 (22). ^{1}H NMR included δ 1.74 (3H, d, J = 1.3 Hz, H-46), 1.75 (3H, d, J = 1.4 Hz, H-47), 2.45 (1H, dd, J = 12.9, 10.3 Hz, H-17α), 2.73 (1H, dd, J = 12.9, 6.0 Hz, H-17β), 3.60 (1H, d, J = 9.4 Hz, H-9α), 3.89 (1H, dd, J = 9.4, 6.1 Hz, H-14β), 3.98 (1H, d, J = 9.4 Hz, H-10β), 4.48 (1H, br t, J = 8.8 Hz, H-7α), 5.54 (1H, d, J = 6.6 Hz, H-43β). ^{1}H and ^{13}C NMR assignments for **1** are reported in Table 1. For HPLC retention and UV absorbance data, see Figure 2.

The crude **4** (fractions 6−15, above) was purified by semipreparative HPLC to give **4** contaminated by minor impurities and a second fraction containing paspaline (**3**). Final purification of **4** was achieved on the analytical HPLC system to give 13-desoxypaxilline (**4**) as a colorless solid. This material coeluted with authentic **4** upon HPLC analysis and possessed UV-absorbance (Figure 2) and ^{1}H NMR spectra identical to those of authentic **4**.

Isolation of Paspaline (3). The fraction containing paspaline, obtained during semipreparative HPLC purification of **4** from fractions 6−15 (above), was further purified by semipreparative HPLC. Paspaline (**3**) was obtained as a colorless solid which coeluted with authentic **3** upon HPLC analysis and exhibited UV-absorbance (Figure 2) and ^{1}H NMR spectra identical to those of authentic **3**.

RESULTS AND DISCUSSION

Structure Elucidation of Terpendole M (1). The UV-absorbance spectrum of terpendole M (**1**) was almost identical to those reported for paspaline (**3**) (Figure 2)

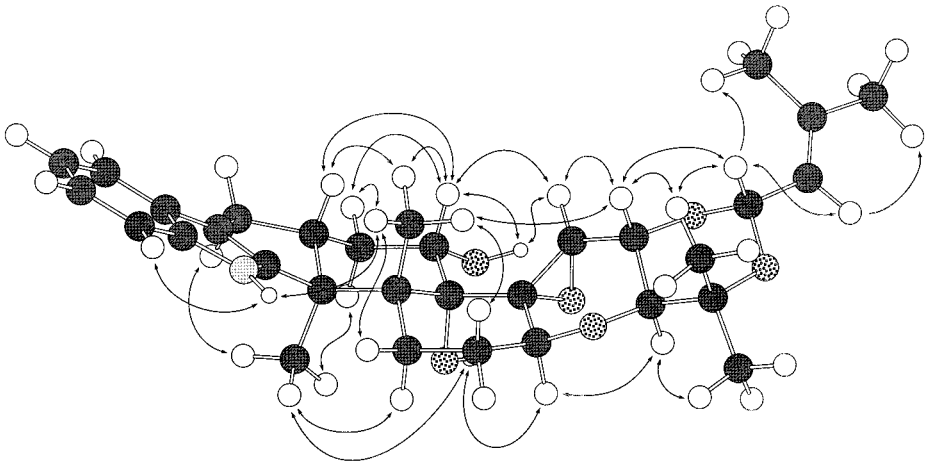

Figure 3. Selected correlations observed in the NOESY and ROESY NMR spectra of terpendole M (**1**).

and paxilline (Munday-Finch et al., 1996a), suggesting that they contained similar chromophores (rings A−B). The mass spectrum of terpendole M exhibited a molecular ion of *m*/*z* 535.2951, consistent with a molecular formula of $C_{32}H_{41}NO_6$ and corresponding to that required for a hydroxylated analogue of terpendole C (**2**) (Huang et al., 1995a). The mass spectrum of **1** also included a prominent fragment ion of *m*/z 182 (Figure 1), attributable to cleavage in ring D across the C-3−C-4 and C-14−C-15 bonds of an indole−diterpenoid (Mantle et al., 1990). These results are consistent with **1** being an analogue of terpendole C (**2**) containing a hydroxyl group in rings D−F.

The ^{1}H NMR spectrum of **1** contained four quaternary aliphatic (δ 1.12, 1.28, 2 × 1.30) and two olefinic (δ 1.74, 1.75) methyl resonances, the chemical shifts of which corresponded closely with those of the methyl groups on rings C−G of terpendole C (**2**) (i.e., H-25, H-26, H-28, H-29, H-46, and H-47). Also readily recognized were resonances attributable to H-7, H-9, H-10, H-11, H-43, H-44, H-20, H-21, H-22, and H-23 of a terpendole C analogue. The ^{1}H spectrum of **1** was characterized by a doublet of doublets at 3.89 ppm which was not observed in the spectrum of **2** (Huang et al., 1995a). The chemical shift of this signal and its coupling constants (J = 9.4, 6.1 Hz) were consistent with the presence of an axially oriented proton attached to an oxygenated carbon and adjacent to two other protons. These results indicated **1** to be an analogue of terpendole C (**2**) which had been hydroxylated at either C-5 or C-14.

The COSY spectrum of **1** demonstrated that the H-6 protons (2.37 and 1.77 ppm) were coupled to two mutually coupled H-5 protons (2.77 and 1.37 ppm) as well as to H-7 (4.48 ppm), thereby locating the hydroxyl group at C-14. Confirming this was the observation of a COSY correlation between the signal at 3.89 ppm and the H-15 protons (1.89 ppm). Further evidence was provided by the HMBC spectrum of **1** (Table 2), which showed that H-15 (1.89 ppm) correlated to two oxygenated carbons (C-13 (77.3 ppm) and C-14 (70.5 ppm)) as well as to C-16 (46.0 ppm) and C-3 (50.7 ppm). A ROESY correlation (Figure 3) between H-26 (1.12 ppm) and the signal at 3.89 ppm located H-14 on the β-face. Thus, terpendole M (**1**) was identified as 14α-hydroxy-**2** (Figure 1).

Most of the ^{1}H NMR assignments of **1** could be made on the basis of COSY and ^{1}H NMR experiments. The ^{1}H NMR assignments of **1** (Table 1) were confirmed, and the relative stereochemistry defined, by correlations observed in NOESY and ROESY spectra of **1** (Figure 3). Because H-28 and H-29 both resonated at 1.30 ppm, C-28 (28.3 ppm) and C-29 (16.6 ppm) were assigned by comparison with spectral data for lolitrems A, B, and F (Miles et al., 1994; Munday-Finch et al., 1995, 1996b).

The relative stereochemistry deduced for **1** from NMR data is in accord with those determined for analogous indole−diterpenoids, such as paxilline (Springer et al., 1975), paspaline (Springer and Clardy, 1980), paspalinine (Gallagher et al., 1980), and terpendoles D (Huang et al., 1995a) and E (Tomoda et al., 1995), by X-ray crystallography. The NMR assignments obtained for **1** were generally in close agreement with those of **2** (Huang et al., 1995a), with the exception of resonances of atoms in the vicinity of the 14-OH group and for four apparent errors in the reported NMR assignments of **2** (Table 1).

Identification of 13-Desoxypaxilline (4) and Paspaline (3). The identities of **3** and **4**, isolated from *L. perenne*, were confirmed by the observation that they gave UV-absorbance and ^{1}H NMR spectra identical to those of, and upon HPLC coeluted with, authentic specimens of **3** and **4** isolated from *Penicillium paxilli* by Munday-Finch et al. (1996a).

Tremorgenic Activities. The tremorgenic activity of terpendole M (**1**) was much lower than that of the known tremorgen (Munday-Finch et al., 1997) terpendole C (**2**) in a standard mouse bioassay (Figure 5). However, the carrier (DMSO−water, 9:1) alone does not induce tremors (or other behavioral changes) (Miles et al., 1992; Munday-Finch et al., 1995, 1997), and the time-course of the tremors induced by **1** closely paralleled that induced by **2**, indicating that **1** is weakly tremorgenic. However, the scarcity of **1** prevented evaluation of its relative tremorgenic potency by testing at higher dose rates. No behavioral changes, apart from tremors, were observed in any of the dosed mice.

Thus, it appears that 14α-hydroxylation of terpendole C reduced, but did not abolish, tremorgenic activity. This marked reduction in the tremorgenic activity may be due to interference by the 14α-OH group with the binding of **1** to the tremorgen receptor(s). It is interesting, therefore, to note that the presence of a 13α-OH is required for tremorgenic activity in the paspalinine (**6**) system (Cole, 1976, cited by Cole et al., (1977)), and molecular modeling indicates that the 14α-OH of **1** is

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts ($CDCl_3$) for Terpendole M (1), Terpendole C (2),[a] and Lolitrem B (8)[b]

	terpendole M (**1**)		terpendole C (**2**)		lolitrem B (**8**)	
atom	^{13}C	^{1}H[c]	^{13}C	^{1}H[c]	^{13}C	^{1}H[c]
2	150.9		151.7		152.8	
3	50.7		50.7		50.7	
4	43.5		42.4		42.4	
5	27.8	2.77, 1.37	27.4	2.72, 1.35	27.4	2.70, 1.36
6	28.8	2.37, 1.77	28.0	2.30, 1.80	28.0	2.27, 1.76
7	70.6	4.48	71.5	4.35	71.5	4.33
9	70.8	3.60	71.1	3.60	71.2	3.57
10	70.7	3.98	71.2	3.94	71.1	3.92
11	60.7	3.85	61.1	3.63	61.3	3.63
12	70.5		67.8		67.7	
13	77.3		78.1		78.1	
14	70.5	3.89	30.3	1.60, 1.44	30.3	1.56, 1.42
15	28.9	1.89, 1.89[h]	20.6	1.94, 1.65	20.5	1.95, 1.64
16	46.0	2.78	50.0	2.80	50.1	2.86
17	27.0	2.45, 2.73	27.2	2.43, 2.74	29.2	2.63, 2.94
18	117.9		117.6		118.6	
19	125.0		125.1		125.4	
20	118.7	7.43	118.5	7.45	123.9	
21	119.9	7.09	119.6	7.09[e]	137.0	
22	120.9	7.09	120.5	7.09[e]	120.2	7.87
23	111.5	7.30	111.4	7.32	110.4	7.22
24	139.9		139.7		142.0	
25	16.1	1.28	16.0	1.28	15.9	1.28
26	18.7	1.12	18.8[f]	1.14[f]	18.9	1.15
27	74.8		74.7		74.7	
28	28.3[i]	1.30	28.3	1.32	28.3	1.30
29	16.6[i]	1.30	16.6[f]	1.32[g]	16.6	1.30
30					196.5	
31					59.9	2.78
32					79.9	
34					79.3	
35					49.9	2.68
36					28.3	2.98, 3.44
37					30.6	1.54
38					25.1	1.32
39					25.0	1.26
40					29.3	1.39
43	92.7	5.54	92.6	5.56	92.7	5.54
44	121.9	5.30	122.0	5.33	122.0	5.30
45	139.9		139.6		139.5	
46	18.7	1.74	18.6	1.75	18.6	1.73
47	25.7	1.75	25.7	1.75	25.6	1.75
13-OH		3.35		nd[d]		nd
14-OH		3.63				
NH		7.72		7.79		8.00

[a] After Huang et al. (1995a). [b] After Munday-Finch et al. (1998). [c] Methylene protons are in the format "δ Hα, Hβ". [d] Not determined = nd. [e] Apparent typographical error. Correction based on terpendole M assignments and ^{1}H NMR of terpendole C (**2**). [f] Reassigned, based on lolitrem B (**8**) and terpendole M (**1**) assignments. [g] Reassigned, based on lolitrem B (**8**) and terpendole M (**1**) assignments, and ^{1}H NMR of terpendole C (**2**). [h] Center of overlapping multiplets; individual chemical shifts not determined. [i] Assignments by analogy with lolitrem B (**8**).

situated in close proximity to—and is capable of forming a hydrogen-bond with—the 13-OH group.

Tremorgen Biosynthesis. The available evidence indicates that indole−diterpenoid tremorgens are biosynthesized via paspaline (**3**) from geranylgeranyl pyrophosphate and tryptophan (Mantle and Weedon, 1994). Paspaline is then metabolized to simple indole−diterpenoids such as 13-desoxypaxilline (**4**) and paxilline (**5**) and further metabolized to produce more complex indole−diterpenoids such as the penitrems and janthitrems (Mantle and Weedon, 1994). Our isolation of **3** and **4**, and the earlier isolation of **5** (Weedon and Mantle, 1987), from *L. perenne* infected with *N. lolii* is entirely consistent with a similar pathway operating in the early stages of lolitrem biosynthesis.

Table 2. Long-Range ^2J and ^3J ^{13}C−^{1}H Correlations Observed in the Two-Dimensional HMBC NMR Spectrum of Terpendole M (1)

^{1}H signal (δ)	correlated ^{13}C signals (δ)
1.12 (H-26)	27.8 (C-5), 43.5 (C-4), 50.7 (C-3), 77.3 (C-13)
1.28 (H-25)	43.5 (C-4). 46.0 (C-16), 50.7 (C-3), 150.9 (C-2)
1.30 (H-28, H-29)	16.6 (C-29), 28.3 (C-28), 70.8 (C-9), 74.8 (C-27)
1.40 (H-5)	28.8 (C-6), 43.5 (C-4), 70.6 (C-7), 77.3 (C-13)
1.74 (H-46)	25.7 (C-47), 121.9 (C-44), 139.9 (C-45)
1.75 (H-47)	18.7 (C-46), 121.9 (C-44), 139.9 (C-45)
1.89 (H-15α, H-15β)	46.0 (C-16), 50.7 (C-3), 70.5 (C-14), 77.3 (C-13)
2.73 (H-17β)	46.0 (C-16), 50.7 (C-3), 117.9 (C-18), 150.9 (C-2)
7.72 (NH)	117.9 (C-18), 125.0 (C-19), 139.9 (C-24), 150.9 (C-2)

Terpendoles are a large family of indole−diterpenoids which, along with emindole SB and **3** (but not **4** or **5**), have recently been isolated from *Albophoma yamanashiensis* (Huang et al., 1995a,b; Tomoda et al., 1995). Many of the terpendoles are analogous to known lolitrems, lacking only the lolitrem A/B ring (cf. terpendole C (**2**) and lolitrem B (**8**), Figure 1). The isolation of terpendole M (**1**) from *N. lolii*-infected *L. perenne* indicates that the endophyte is capable of assembling the right-hand end (rings H and I) of the lolitrem skeleton (e.g., **8**) regardless of whether the lolitrem A/B ring system has been assembled. Previous work (Munday-Finch et al., 1997, 1998) has similarly indicated that the endophyte can assemble the lolitrem A/B ring system regardless of whether the lolitrem G/H/I rings are in place. Thus, the biosynthetic machinery for building the lolitrem A/B rings of **8** appears to operate independently from that which assembles the G/H/I rings and is consistent with the metabolic grid shown in Figure 4. Lolitrem biosynthesis therefore appears to be modular and could proceed via terpendoles, and lolitrems may be formed without the intermediacy of paxilline (**5**) (Figure 4).

Staub et al. (1993) and Munday-Finch (1997) have also reported isolation of 14α-hydroxylated indole−diterpenoids (**7** and **9**, respectively) analogous to **1** (Figure 1), suggesting that 14α-hydroxylation is a normal step in indole−diterpenoid biosynthesis. Furthermore, the presence in *L. perenne* of terpendole M (**1**), which is very similar in structure to the lolitrems (e.g., **8** and **9**), indicates that other terpendoles (e.g., **2**) are likely to be present in endophyte-infected ryegrass. Because terpendoles C (**2**) (Munday-Finch et al., 1997) and M (**1**) are tremorgenic, and terpendole A (the 44,45-epoxide of **2**) would also be expected to be tremorgenic (based on information from lolitrem structure−activity relationships (Munday-Finch et al., 1995)), any terpendoles present could contribute, along with paxilline (**5**) and the lolitrems, to the toxicity of *L. perenne*.

Our findings provide useful information for plant breeders attempting to select nontoxic endophytes for insertion into *L. perenne*. Current methods involve screening of grass−endophyte combinations for their ability to produce lolitrem B (**8**). One of the difficulties with this approach is that of the four endophyte-infected grass species that are associated with staggers syndromes (*Echinopogon ovatus*, *L. perenne*, *Melica decumbens*, and *Poa huecu*), all produce indole−diterpenoids but only *L. perenne* produces lolitrems (Miles et al., 1998; Munday-Finch et al., 1998). Thus, some endophyte-grass combinations appear to be capable of pro-

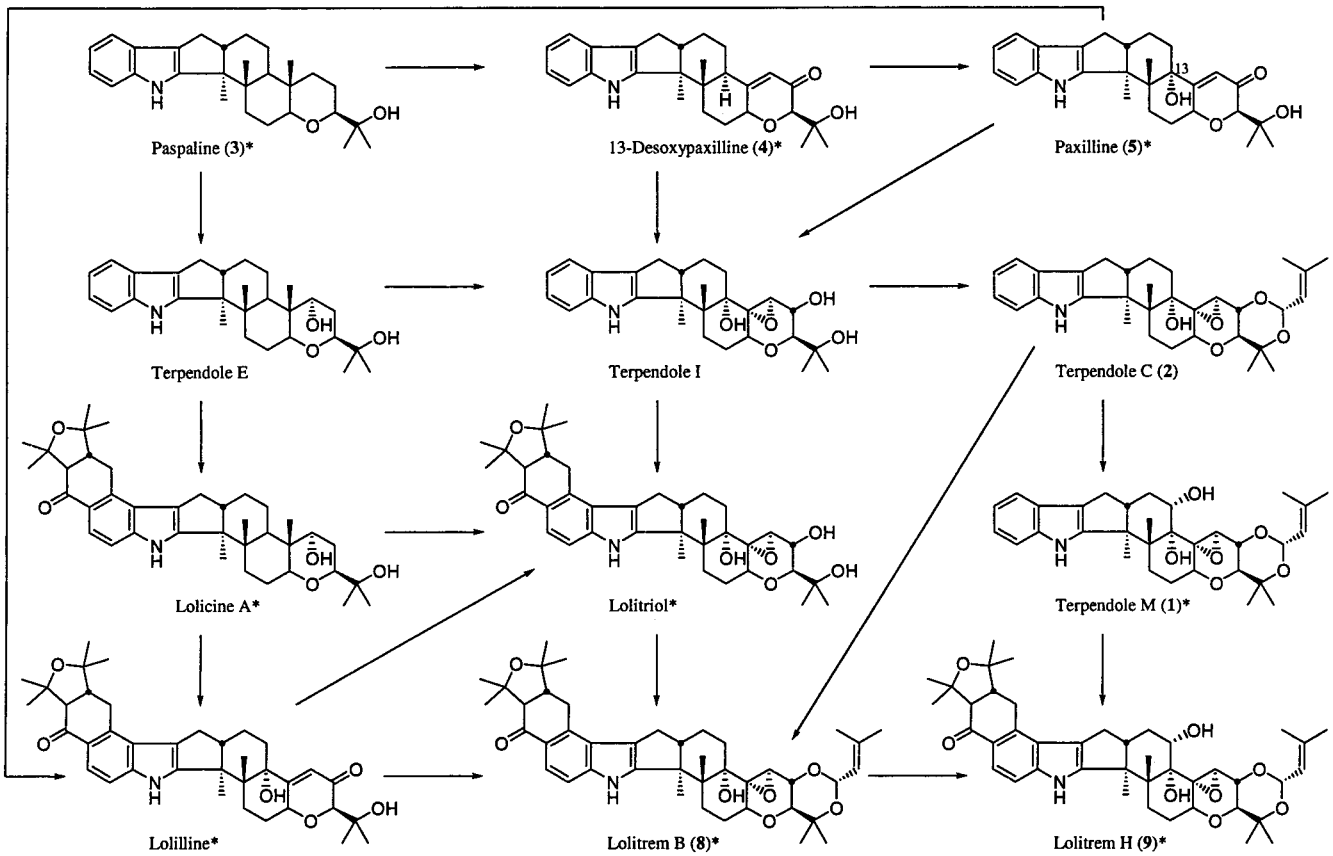

Figure 4. Possible biosynthetic grid for the lolitrems involving known paspaline, paxilline, and terpendole analogues. Compounds marked with an asterisk have been isolated from endophyte-infected *L. perenne.*

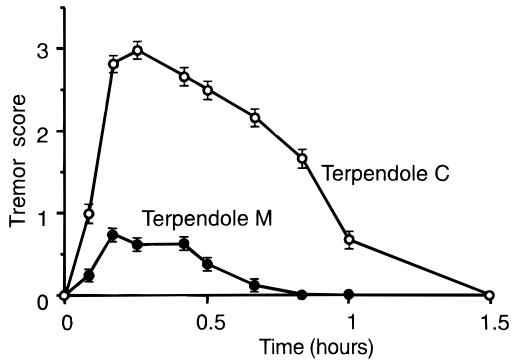

Figure 5. Tremorgenic activities of terpendole M (**1**) (●, 8 mg kg^{-1}, $n = 3$ mice) and terpendole C (**2**) (○, 8 mg kg^{-1}, $n = 4$). Error bars indicate the standard error of the mean.

ducing staggers-inducing alkaloids other than **8**, and endophyte selection criteria based on accumulation of **8** might not give the desired outcome (for example, see Fletcher et al., 1993).

A safer selection strategy might be based on what is known of indole−diterpenoid biosynthesis. Paspaline (**3**) appears to be the key intermediate in indole−diterpenoid (including lolitrem) biosynthesis; endophytes unable to produce **3** would be incapable of generating lolitrems, terpendoles, paxilline (**5**), or related tremorgens, regardless of the host grass into which they were inserted. A similar strategy, based on an ELISA that detects many simple paxilline analogues, has been used successfully to select mutants of *N. lolii* and *P. paxilli* which do not appear to produce indole−diterpenoids (Gurney et al., 1994; Young et al., 1998). This ELISA is, however, rather insensitive to paspaline (**3**) (Supporting Information for Miles et al. (1995)), and a more reliable outcome to screening might be obtained with an immunoassay designed to detect **3**.

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