Penitremones A–C, *Penicillium* metabolites containing an oxidised penitrem carbon skeleton giving insight into structure–tremorgenic relationships

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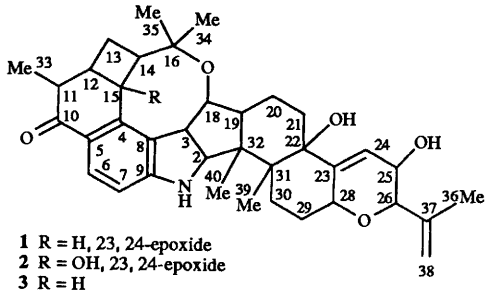
A new group of microbial metabolites, designated penitremones A–C, have been characterised by mass spectrometry and ^1H and ^{13}C NMR spectroscopy as 10-keto, 11,33-dihydro-variants of the penitrem indole-isoprenoid skeleton. The principal metabolite penitremone A, produced with penitrem A by a *Penicillium* sp., is an isomer of penitrem E and was also similarly tremorgenic. Reduction of the carbonyl of penitremones A and B with $NaHB_4$ altered tremorgenic activity, thereby indicating potential for new insights into structure–activity relationships in tremorgenic mycotoxins, particularly with respect to neurological disorders in agricultural ruminants caused by the related keto-alkaloid lolitrem B.

Penitrem mycotoxins are a group of structurally related compounds differing by chlorine, hydroxy and epoxide substituents on a common indole-isoprenoid carbon skeleton.[1] They are metabolites of several *Penicillium* spp., most notably the common soil-borne *P.crustosum*.[2] Our attention has been drawn by J. C. Frisvad, Technical University of Denmark, to a *Penicillium* isolate that does not conform to a described species but that readily produces penitrem A in culture as shown by HPLC analysis with diode array detection.[3]

The occurrence of penitrem A has been confirmed, but, in addition, the fungus elaborated four other compounds seen on thin layer chromatograms by their green or cerise coloration after treatment with $FeCl_3$ in butanol. The most abundant of these, isolated by preparative HPLC, and analysed by EI mass spectrometry, showed a molecular ion, *m/z* 599, the accurate mass of which equated to that of the elemental composition of penitrem E ($C_{37}H_{45}NO_6$). In TLC, the compound gave the green penitrem colour with $FeCl_3$. It had a slightly lower R_F value than penitrem E (R_F 0.55), for which it could easily be mistaken since it occurred as a co-metabolite with penitrem A. The IR spectrum showed a carbonyl signal at 1652 cm^{-1}. The UV spectrum (λ_{max}/nm 260 and 288) was significantly different from that of penitrem E (λ_{max}/nm 220 and 286).[1] In the ^1H NMR spectrum a notable feature, not evident in the spectrum of other indole-isoprenoid fungal metabolites, was a methyl doublet signal (δ 1.11, *J*/Hz 5.7) which collapsed to a singlet when a single proton signal, centred at δ 2.77, was irradiated. Resolution of the ^{13}C spectrum by DEPT showed that the compound **1** possessed 6 methyl carbons, 6 methylene carbons, 12 methine carbons, and 12 quaternary carbons including a carbonyl signal at 200.22 ppm. Comparison with the data for penitrem E[4] readily allowed assignment of most of the carbon signals (Table 2) except for the absence in **1** of the CH_2 at both C-10 and C-33, and the quaternary carbon signals for C-11 and C-15. Instead, a new methyl carbon (12.74 ppm) linked to a new CH (45.94 ppm), as indicated by $^1H/^1H$ decoupling experiments, are assigned to C-33 and C-11, respectively. The new carbonyl signal is assigned to C-10, and compensates for the change from a hydroxy at C-15 in penitrem E to a CH, thereby completing the carbon signal assignments for **1**. These were confirmed in part by ^1H NMR spectral data (Table 1) which conformed to literature values for the diterpenoid moiety.[1] In addition, penitrem-like proton signals for C-12, C-13, C-14, C-34 and C-35 were apparent. Shifts of two



1 R = H, 23, 24-epoxide
2 R = OH, 23, 24-epoxide
3 R = H

Fig. 1 General structure for penitrem-10-ones

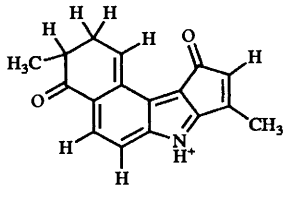
aromatic coupled protons [δ 7.17 (*J*/Hz 7.6) and δ 7.36 (*J*/Hz 7.5)] were close to the penitrem values for those at C-7 and C-6. Proton signals for C-10 in penitrem E were absent from **1**, as was the C-15 hydroxy signal.

$^1H/^1H$ decoupling experiments demonstrated connectivity between signals at δ 4.87 and 5.06 and reciprocal interaction between signals at δ 1.10 and 2.77, 2.63 and 4.86, and 2.21 and 4.29. Couplings were also demonstrated between signals assigned to 11-H and 12-H, 12-H and 13-H_2, 13-H and 13'-H, 12-H and 15-H, 15-H and 14-H, and 13'-H and 14-H, thereby contributing to the assignments in Table 1. The mass spectrum of **1** generally followed the pattern of penitrems A and E.[5] The fragment ions *m/z* 530 and 264, rationalised by accurate mass measurement in consequence of losses first of C_5H_9 and then also of $C_{15}H_{22}O_4$, are broadly consistent with a conserved penitrem-like structure of the diterpenoid and location of the different elemental arrangement of **1** in the aromatic region. However, scission of the diterpenoid is slightly different from that in penitrems since an additional CH_2 is lost. Link scanning (B/E constant) of the molecular and fragment ions of penitrem A confirmed the principal electron impact fragmentation patterns previously reported,[5] particularly concerning the sequence of losses of 18 mass units (water), 86 m.u. ($C_5H_{10}O$), and 251 m.u. ($C_{14}H_{19}O_4$) representing ~80% of the diterpenoid moiety (Scheme 1). Notably, loss of 251 m.u. occurs from the odd-electron ion *m/z* 547 which is derived by loss of $C_5H_{10}O$ from the molecular ion. However, loss of $C_5H_{10}O$ does not occur in **1**. Instead, the analogous fragmentation occurs from $M^+ - C_5H_9$, the even-electron ion *m/z* 530. Loss of the odd-electron neutral $C_{14}H_{19}O_4$ would thus be improbable, but loss of an even-electron neutral much

Table 1 ^{1}H NMR data for *Penicillium* indole-diterpenoid metabolites in $(CD_3)_2CO$

Position	Penitrem E[4]	Penitremone A **1**	Reduced penitremone A **5**	Penitremone B **2**	Penitremone C **3**	Penitrem D[4]	Penitrem A
	δ_H	δ_H	δ_H	δ_H	δ_H	δ_H	δ_H
1(NH)	9.82	10.31s	9.78s	10.35s	10.29s	9.96	10.05
6	6.70	7.36d	7.28d	7.33d	7.36d	6.70	—
7	7.09	7.17d	7.05d	7.24d	7.17d	7.02	7.22
10	3.22	—	4.20dd	—	—	*ca.* 3.2 omitted	3.24
10′	3.48	—	3.90d(OH)	—	—	3.39	3.62
11	—	2.77m	2.82m	2.77m	2.77m	—	—
12	2.95	2.27q	2.26m	2.24m	2.27m	3.13	Obscured
13	2.38	2.18m	2.49q	2.48m	2.18m	2.44	2.41
13′	2.23	1.90dd	1.82m	1.86m	*ca.* 1.90	2.27	2.26
14	2.47	*ca.* 2.8 (under solvent)	2.04m	*ca.* 2.8 (under solvent)	2.10m	2.11	2.49
15	3.96	3.82t	3.54d	3.56s	3.82t	3.86	4.18
18	4.94	4.86d	4.75d	5.01d	4.88d	4.79	4.91
19	2.63	2.63m	2.60m	2.64m	2.61m	2.61	2.63
20	1.93	1.93m	1.90q	1.94m	*ca.* 2.0	2.04	1.94
20′	1.78	1.73q	1.75m	1.82m	1.88q	1.88	1.78
21	1.68	1.63m	1.68m	1.74m	*ca.* 1.75	1.74	1.48
21′	1.48	1.50m	1.45m	1.51m	*ca.* 1.9	1.90	1.68
22(OH)	3.28	3.30s	3.30s	3.30s	3.35s	3.39	3.30
24	3.56	3.56s	3.55s	3.55m	5.75d	5.75	3.55
25	4.03	4.03s	4.02s	4.03s	3.90s	3.90	4.02
25′(OH)	3.36	3.38m	3.39d	3.38d	3.16d	3.12	3.40
26	4.04	4.03s	4.02s	4.03s	3.80s	3.80	4.02
28	4.29	4.29m	4.27dd	4.29t	4.65t	4.61	4.27
29	2.22	2.21m	2.21m	2.15m	*ca.* 2.07	2.07	2.22
29′	2.04	2.01m	2.03m	2.08m	*ca.* 1.83	1.83	2.04
30	2.61	2.63m	2.60m	2.61m	2.63m	2.63	2.61
30′	1.59	1.61m	1.61m	1.61m	1.61m	1.61	1.57
33	4.91	1.10d	1.11d	1.11d	1.10d	5.01	5.01
33′	4.78	—	—	—	—	4.82	4.85
34	1.75	1.55s	1.48s	1.77s	1.56s	1.54	1.73
35	1.05	1.15s	1.07s	1.10s	1.16s	1.10	1.05
36	1.70	1.70s	1.68s	1.70s	1.76s	1.77	1.69
38	5.07	5.06s	5.05s	5.06s	5.08d	5.09	5.06
38′	4.87	4.87s	4.85s	4.87s	4.86s	4.87	4.86
39	1.22	1.23s	1.21s	1.25s	1.02s	1.02	1.21
40	1.40	1.46s	1.39s	1.46s	1.49s	1.44	1.38

more likely. The observed loss of such, $C_{15}H_{22}O_4$, which also seems to require an H-shift from C-21 to C-31, would give the structure **I** for the prominent fragment ion *m/z* 264:



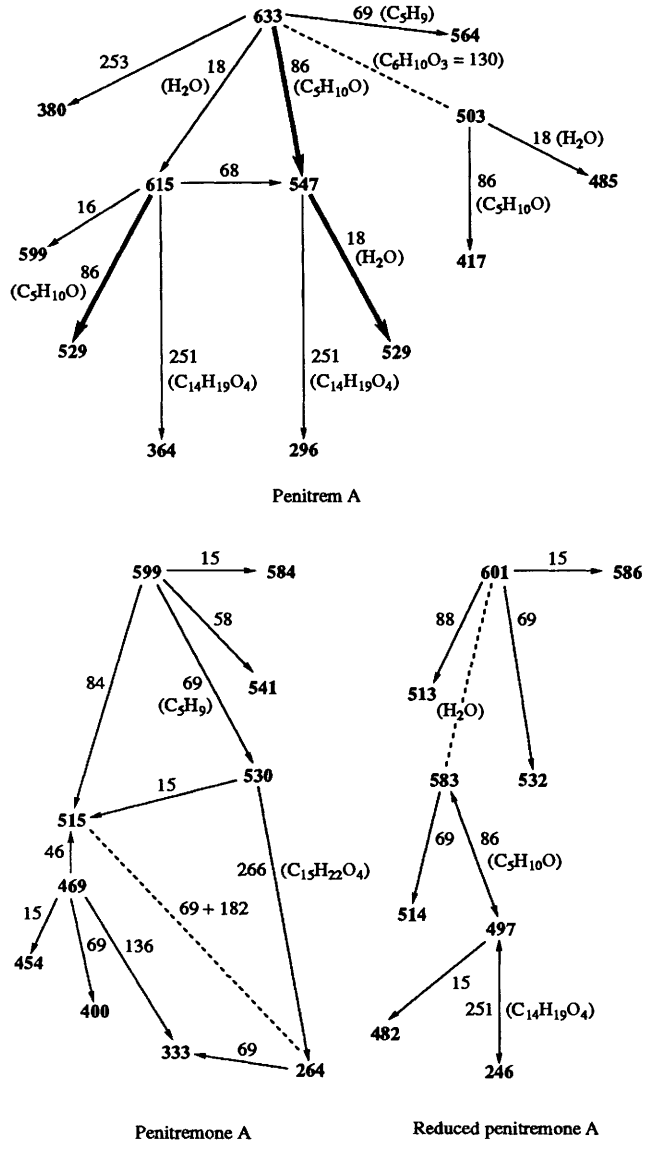
I

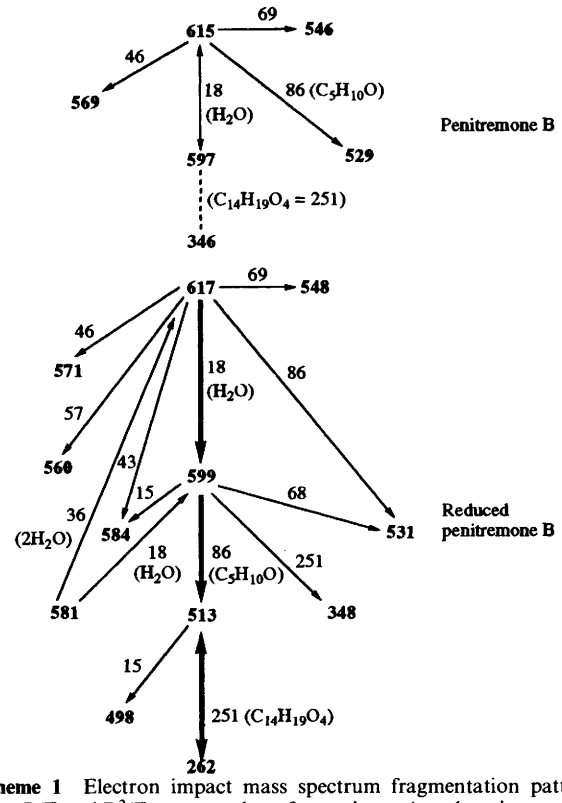
It is notable that link scanning demonstrated connectivity between the minor fragment ion *m/z* 333 with the molecular ion by summation of differences of 84, 46 and 136 mass units which equate to the 266 m.u. difference between *m/z* 264 and 530 attributed to the diterpenoid fragment $C_{15}H_{22}O_4$. This takes into account the ion *m/z* 469 which is the base peak in the electron impact mass spectrum. Consequently, since the basic penitrem structure is that of penitrem D,[1] compound **1** is 11,33-dihydro-23,24-epoxypenitrem-10-one and is designated penitremone A.

Compound **2** differed from **1** by addition of one oxygen atom as shown by accurate mass measurement of the molecular ion. It was notable that the important fragment ions *m/z* 597 and 529 were derived directly from the molecular ion by losses of H_2O and $C_5H_{10}O$, respectively (Scheme 1). The ion at *m/z* 346 differed from that at *m/z* 597 by $C_{14}H_{19}O_4$, consistent with penitrem-like losses from the molecular ion, first of H_2O and then of the penitrem diterpenoid fragment of 251 mass units. Location of the additional oxygen at C-15 was evident from the absence of the single proton at C-15 in **1** and the closer equivalence of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) to that of penitrem E on account of the hydroxy function being in common at C-15. Compound **2** demonstrated its more polar character than **1** on TLC, but similarly gave a green colour with $FeCl_3$. Compound **2** is 15-hydroxy-11,33-dihydro-23,24-epoxypenitrem-10-one and is designated penitremone B.

Compound **3** had a lower R_F value on TLC than **2** and also contrasted by its cerise colour with $FeCl_3$. The mass spectrum showed one oxygen atom less than **1**. It is deduced that the oxygen atom forming the epoxide in **1** and **2** is absent in **3**, with a concomitant $\Delta^{23,24}$. The proposed structure of **3** is supported by the close similarities of the ^{1}H and ^{13}C NMR spectra to those of **1**, since omission of the epoxide only changed the shifts of C-23 and C-24 to values comparable with those in the epoxide-deficient penitrem C (Table 2).[4] However, the C-10 and C-33 signals in **3** were clearly of the penitremone type. It is notable also that penitrem C gave a pink colour with $FeCl_3$ but had less polar behaviour in TLC. Compound **3** is 11,33-dihydropenitrem-10-one and is designated penitremone C.

A fourth metabolite was recognised in TLC after $FeCl_3$ treatment, as a violet-coloured spot of lower R_F value than **3**.





Scheme 1 Electron impact mass spectrum fragmentation patterns, from B/E and B^2/E constant data, for penitrem A and penitremones A **1** and B **2** and their reduction products **5** and **6**. Bracketed elemental compositions after some mass difference values are derived from accurate mass measurements. Differences of 18, 69, 86 and 251 mass units elsewhere may be assumed to have the same composition as those that were measured accurately. Dotted lines with bracketed elemental composition indicate deduced interrelationships.

Mass spectrometry of a partially purified Sep-pak fraction showed an M^+ (*m/z* 571), the accurate mass of which gave the molecular formula $C_{37}H_{49}NO_4$ which indicated a deficit of one oxygen atom, but a gain of four protons, in comparison with **3**. Fragment ions were consistent with the predicted fragmentation of the tetrahydro derivative of penitrem D,[4] by analogy with tetrahydropenitrem A.[5] The metabolite was also not a penitremone because the *m/z* 248 fragment implies absence of the only oxygen atom in this region of penitremones.

Reduction of **1** by $NaHB_4$ was confirmed by mass spectrometry. The chirality at C-10 is unknown, although reduction of the carbonyl of the related compound paxilline under the same conditions yielded principally the β-hydroxy derivative.[6] However, only one product was evident in TLC and HPLC. The ^{13}C NMR spectrum of 10-hydroxy-**1** (**5**) was generally similar to that of **1**, but with a notable loss of the lowfield carbonyl signal and a new CH (DEPT) signal at 73.31 ppm assigned to C-10 (Table 2). Generally the ^1H NMR spectrum resembled that of **1**. Resolution of some complex signals was even improved, and $^1H/^1H$ decoupling experiments confirmed the protons at C-20, 24, 25 and 26 (Table 1), which is in accord with mass spectral fragmentation (Scheme 1). The fidelity of proton signal assignments in the region of C-10 of **5** was confirmed by $^1H/^1H$ decoupling experiments in CD_2Cl_2 by which reciprocal irradiation-response connectivities were demonstrated between 12-H and both 14-H and 15-H, and connectivities between 13-H and both 14-H and 15-H, and between 14-H and 13-H. Irradiation of the signal at δ 4.20 in $(CD_3)_2CO$ sharpened the doublet at δ 7.28, implying that the latter should be assigned to C-6. Assignment of the 6-H/7-H signals of penitremones A–C therefore followed this principle.

Reduction of **2** to give compound **6** was confirmed by mass spectrometry. Link scanning was particularly rewarding by revealing a comprehensive pattern of fragmentation interrelationships (Scheme 1).

The absence of a M^+–86 fragmentation in **1** may be associated with the absence of a C-15 hydroxy group, whereas in **2**, which has a C-15 hydroxy, an M^+–86 fragmentation occurs. However, loss of $C_5H_{10}O$ is a characteristic feature of the penitrems, irrespective of whether a C-15 hydroxy is present or not, and is the dominant fragmentation of the molecular ion. Link scanning of **5** failed to recognise the ion *m/z* 583 as a daughter of the molecular ion. Therefore, it is deduced that the *m/z* 583 ion arises from the product of thermal dehydration in the ion source, in effect penitrem B or an isomer, which then fragments in a way typical of penitrem B[5] and similar to the fragmentation of penitrem A shown in Scheme 1. Loss of 86 m.u. persisted in **2** after reduction though still not as the most prominent first fragmentation, as it is in penitrem A.

Compound **1** is at least similar to the insect antifeedant recently isolated from *Aspergillus sulphureus* sclerotia,[7] with which (courtesy of J. B. Gloer) it co-chromatographed in HPLC and had an identical UV spectrum. However, the chirality at C-11 in **1** is not known. Since the unusual 11-methylpenitrem-10-ones of *A. sulphureus* and the *Penicillium* sp. occur with either penitrem B or A, respectively, biosynthesis of the 11-methyl-

Table 2 ^{13}C NMR data for *Penicillium* indole-diterpenoid metabolites in $(CD_3)_2CO$

Carbon atom	Penitrem E[4]		Penitremone A **1**		Reduced penitremone A **5**	Penitremone B **2**	Penitremone C **3**		Penitrem D[4]
	Assignment	δ_C	DEPT	δ_C	δ_C	δ_C	DEPT	δ_C	δ_C
2	Q	153.48	Q	154.11	152.71	154.53	Q	154.60	153.49
3	Q	120.38	Q	121.28	119.66	122.04	Q	120.95	119.16
4	Q	131.52	Q	135.80	132.91	138.12	Q	135.74	128.80
5	Q	128.16	Q	126.02	128.66	125.52	Q	125.91	128.09
6	CH	120.34	CH	118.66	118.11	117.72	CH	118.54	120.91
7	CH	111.63	CH	110.65	109.44	111.61	CH	110.61	110.22
8	Q	122.75	Q	122.63	122.85	122.26	Q	122.50	123.18
9	Q	140.16	Q	142.46	139.44	143.26	Q	142.40	139.27
10	CH_2	38.11	Q	200.22	73.31	200.05	Q	200.30	38.75
11	Q	150.91	CH	45.94	44.47	45.73	CH	45.88	150.23
12	CH	47.41	CH	33.98	35.24	31.20	CH	33.92	35.04
13	CH_2	24.72	CH_2	28.33	27.42	26.88	CH_2	28.26	26.70
14	CH	52.78	CH	49.92	51.67	50.23	CH	49.88	52.36
15	Q	81.08	CH	35.15	35.11	76.47	CH	35.11	39.37
16	Q	76.09	Q	75.94	75.56	76.07	Q	75.90	75.44
18	CH	72.52	CH	72.08	72.13	72.57	CH	72.12	72.17
19	CH	58.86	CH	59.66	59.09	59.29	CH	59.36	58.87
20	CH_2	18.63	CH_2	18.53	18.57	18.56	CH_2	18.51	19.11
21	CH_2	Obscured	CH_2	30.44	30.54	30.57	CH_2	34.99	35.08
22	Q	78.28	Q	78.20	78.21	78.21	Q	77.32	77.48
23	Q	66.17	Q	66.06	66.14	66.09	Q	148.22	148.44
24	CH	61.95	CH	61.89	61.91	61.90	CH	119.59	119.59
25	CH	66.34	CH	66.27	66.28	66.27	CH	64.14	64.28
26	CH	74.69	CH	74.64	74.63	74.65	CH	74.28	74.40
28	CH	72.05	CH	71.93	71.99	71.96	CH	80.28	80.39
29	CH_2	28.94	CH_2	28.91	28.77	28.88	CH_2	29.10	29.24
30	CH_2	26.89	CH_2	26.86	26.81	26.81	CH_2	27.62	27.68
31	Q	43.58	Q	43.55	43.57	43.55	Q	43.59	43.71
32	Q	49.92	Q	50.30	49.71	50.51	Q	50.32	49.77
33	CH_2	105.47	CH_3	12.74	16.01	13.22	CH_3	12.74	105.83
34	CH_3	20.28	CH_3	18.81	18.68	20.62	CH_3	18.82	18.64
35	CH_3	31.10	CH_3	28.91	28.89	30.57	CH_3	28.89	28.81
36	CH_3	19.70	CH_3	19.67	19.68	19.68	CH_3	19.94	19.98
37	Q	143.31	Q	143.25	143.25	143.57	Q	143.81	143.92
38	CH_2	111.62	CH_2	111.61	111.59	111.95	CH_2	110.70	110.75
39	CH_3	18.99	CH_3	18.88	18.89	18.97	CH_3	19.67	20.11
40	CH_3	21.53	CH_3	21.19	21.21	21.54	CH_3	20.03	21.32

penitrems could be *via* dihydrogenation of Δ^{11-33}, the enzymes for which in different fungi may not necessarily have identical stereospecificities. The deduced occurrence of tetrahydropenitrem D in the *Penicillium* extract would also be consistent with dihydrogenase activity.

Whereas **1** was shown to have approximately 3-fold less tremorgenic activity than the potent tremorgen penitrem A,[8] though similar to that of penitrem E,[9] **5** was inactive at a similar dose. In contrast, **2** was not tremorgenic at a dose up to 3 times that used for **1**. Compound **6** was similarly not tremorgenic at a dose of 0.2 mg, but, when the dose was increased 10-fold, marked tremor was evident. These findings point for the first time to the biodynamic significance of substituents of the penitrem carbon skeleton in the vicinity of C-10 with respect to tremorgenic activity. This may extend to the structurally related tremorgenic mycotoxin lolitrem B,[10] the principal cause of ryegrass staggers, a neurological disorder of agricultural ruminants, particularly important in New Zealand, for which there are as yet only theoretical long-term prospects of control.[11]

Experimental

Production and isolation of metabolites

Penicillium sp. (IBT 13163) was grown stationary at 27 °C in 1 dm^3 Erlenmeyer flasks containing Czapek-Dox/yeast extract (0.5%) broth (300 cm^3). After 16 d the mycelium was homogenised in acetone and the filtrate evaporated to dryness. Total penitrems, determined spectrophotometrically at 633 nm after incubation at 70 °C in acidic methanol,[12] was 16 mg from the mycelium (*ca.* 1 g) grown on 100 cm^3 of medium. Penitrem-like compounds were analysed by TLC (SIL G_{254}, Camlab) in chloroform–acetone (19:1), and chromatograms sprayed with 3% $FeCl_3$ in butanol and warmed to develop colours in the green or red range. Penitremone A was the principal penitrem. Small-scale preparative separations used silica gel Sep-pak cartridges (Millipore). On a larger scale, metabolites were purified by flash chromatography through a silica gel 60 (Merck; 230–400 mesh) column (5 × 30 cm) using the same solvent, and by HPLC through a Dynamax C18 reversed-phase column (2 × 30 cm) with MeOH–H_2O (4:1) at 11 cm^3 min^{-1} and detection at λ/nm 230 or 260. Generally, relative retentions on the reversed-phase column mirrored the relative TLC mobilities, except that penitrem A was unexpectedly eluted before **1**. The following penitrem and penitremone metabolites were isolated as white amorphous solids.

Penitrem A (20 mg), λ_{max}/nm (MeOH) 233 and 295 (ε/dm^3 mol^{-1} cm^{-1} 37 000 and 11 600[1]) (Found: M^+, 633.2859. $C_{37}H_{44}ClNO_6$ requires *M*, 633.2857), major fragment ions at *m*/*z* 547 and 296; δ_H(selected data complementary to that in Table 1) 2.97 (1 H, m, 12-H), 3.25 (1 H, s, 10-βH), 3.60 (1 H, s, 10-αH), 4.18 (1 H, s, 15-H), 4.86 (1 H, s, 33-βH), 5.00 (1 H, s, 33-αH) and 7.22 (1 H, s, 7-H); δ_C(selected data complementary to

that in Table 2) 34.99 (C-10), 46.92 (C-12), 80.92 (C-15), 107.03 (C-33), 111.77 (C-7), 124.43 (C-6), 125.69 (C-5), 133.20 (C-4) and 149.40 (C-11); TLC R_F 0.6.

Penitremone A **1** (30 mg), λ_{max}(MeOH)/nm 260 and 288 (ε/dm^3 mol^{-1} cm^{-1} 41 000 and 12 500) (Found: M$^+$, 599.3231. $C_{37}H_{45}NO_6$ requires *M*, 599.3247); major fragment ions at *m/z* 530.2458 ($C_{32}H_{36}NO_6$ requires *M*, 530.2543), 469.2624 ($C_{31}H_{35}NO_3$ requires *M*, 469.2617) and 264.1005 ($C_{17}H_{14}NO_2$ requires *M*, 264.1025); TLC R_F 0.47.

Penitremone B **2** (15 mg), λ_{max}(MeOH)/nm 260 and 288 (Found: M$^+$, 615.3185. $C_{37}H_{45}NO_7$ requires *M*, 615.3196); major fragment ions at *m/z* 597.3084 ($C_{37}H_{43}NO_6$ requires *M*, 597.3090), 529.2510 ($C_{32}H_{35}NO_6$ requires *M*, 529.2464), 485.2530 ($C_{31}H_{35}NO_4$ requires *M*, 485.2566), 467.2441 ($C_{31}H_{33}NO_3$ requires *M*, 467.2460), 399.1847 ($C_{26}H_{25}NO_3$ requires *M*, 399.1834), 346.1786 ($C_{23}H_{24}NO_2$ requires *M*, 346.1807), 278.1085 ($C_{18}H_{16}NO_2$ requires *M*, 278.1181) and 69.0694 (C_5H_9 requires *M*, 69.0704); TLC R_F 0.33.

Penitremone C **3** (5 mg), λ_{max}(MeOH)/nm 270 (Found: M$^+$, 583.3274. $C_{37}H_{45}NO_5$ requires *M*, 583.3298); major fragment ion at *m/z* 565.3201 ($C_{37}H_{43}NO_4$ requires *M*, 565.3192); TLC R_F 0.27.

Compound **4** (Found: M$^+$, 571.3644. $C_{37}H_{49}NO_4$ requires *M*, 571.3662); major fragment ions at *m/z* 553.3551 ($C_{37}H_{47}NO_3$ requires *M*, 553.3556) 485, 467, 332, 316 and 248; TLC R_F 0.23.

The above compounds were isolated from mycelium of dry weight estimated at *ca.* 15 g.

Reduction of penitremones A and B

Compounds **1** (15 mg) and **2** (8 mg) were reduced in 0.4 mol dm^{-3} cerium chloride in methanol (3 cm^3) by treatment with an excess of $NaBH_4$ for 10 min.[6] Water (10 cm^3) was added to the mixture which was then extracted with diethyl ether (2 × 10 cm^3); the combined extracts were evaporated to dryness. Efficient transformation of **1** or **2** into a more polar compound was shown by TLC: 23,24-*epoxy*-10-*hydroxy*-11,33-*dihydropenitrem* **5** (5 mg) λ_{max}(MeOH)/nm 230 and 280 (Found: M$^+$, 601.3337. $C_{37}H_{47}NO_6$ requires *M*, 601.3403); major fragment ions at *m/z* 583.3331 ($C_{37}H_{45}NO_5$ requires *M*, 583.3298), 497.2542 ($C_{32}H_{35}NO_4$ requires *M*, 497.2567) and 246.1216 ($C_{18}H_{16}N$ requires *M*, 246.1283); TLC R_F 0.2; 23,24-*epoxy*-10,15-*dihydroxy*-11,33-*dihydropenitrem* **6** (5 mg) (Found: M$^+$, 617.3301. $C_{37}H_{47}NO_7$ requires *M*, 617.3353); major fragment ions at *m/z* 599.3218 ($C_{37}H_{45}NO_6$ requires *M*, 599.3247), 581.3112 ($C_{37}H_{43}NO_5$ requires *M*, 581.3141), 513.2518 ($C_{32}H_{35}NO_5$ requires *M*, 513.2515), 262.1223 ($C_{18}H_{16}NO$ requires *M*, 262.1232) and 69.0704 (C_5H_9 requires *M*, 69.0704), TLC R_F 0.13.

Tremorgenic bioassay

Albino mice (25 g) were given, by intraperitoneal injection, compounds **1** (0.14 mg), **2** (0.2 mg or 0.4 mg), **5** (0.16 mg) or **6** (0.2 mg or 2.0 mg) dissolved in ethanol–polyethylene glycol 400–water (15:12.5:72.5; 0.3 cm^3).

NMR measurements were made under standard conditions at 500 MHz (^{1}H) or 125.8 Hz (^{13}C) on a Bruker AM-500. Mass spectra were obtained from a VG autospec Q spectrometer at 70 eV and probe temperature 200–220 °C. For linked scans, the collecting gas (helium) was introduced to reduce the intensity of the molecular ion by 50%. IR and UV spectra were recorded on Perkin–Elmer 1600 and Varian CARY 210 spectrometers, respectively.

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