Structure and Biosynthesis of the Penitrems A–F, Six Novel Tremorgenic Mycotoxins from *Penicillium crustosum*

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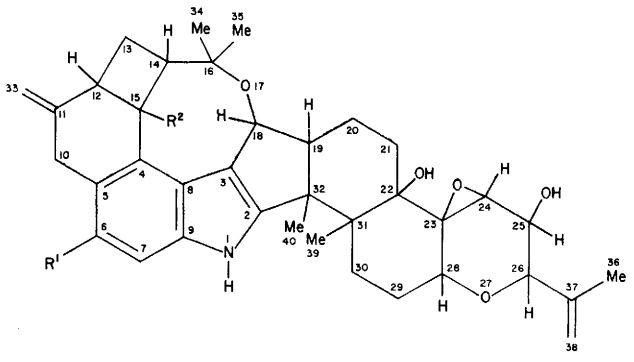
Summary The structures of six new mycotoxins, penitrems A—F, isolated from cultures of *Penicillium crustosum* are deduced from their ^{13}C and ^{1}H n.m.r. spectra and from biosynthetic results.

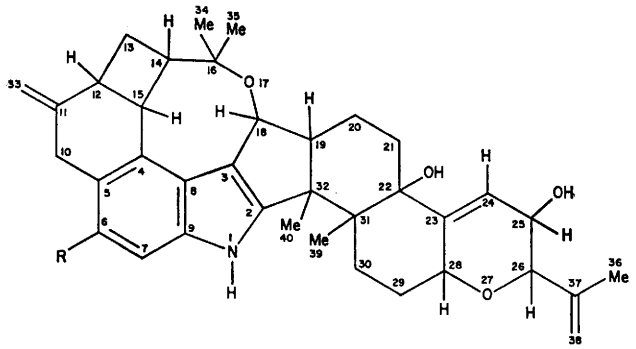
PENITREM A, $C_{37}H_{44}NO_6Cl$ (**1**), m.p. 237—239 °C was first isolated in 1968 by Wilson *et al.*,[1] whereas penitrem B, $C_{37}H_{45}NO_5$ and penitrem C (no formula given) were subsequently obtained from *Penicillium palitans* by Hou *et al.*[2] Pitt[3] recently concluded that all of the isolates involved in the production of penitrem A belong to *Penicillium crustosum*. Penitrem A elicits sustained tremors, discoordination, and convulsions in laboratory and farm animals. Pharmacological and biochemical studies indicated that this tremor-causing agent interferes with amino-acid neurotransmitter-release mechanisms.[4] None of the structures of the members of the penitrem group has been elucidated because of the molecular complexity, the instability, and insufficient amounts of materials. Herein we report the isolation and structural elucidation of the penitrems A—F. Structure (**1**) is proposed for penitrem A based mainly on the interpretation of high-field ^{1}H and ^{13}C n.m.r. spectra (Bruker WH-400 and WM-500 n.m.r. spectrometers, recorded in [$^{2}H_6$]acetone) in conjunction with biosynthetic reasoning.



Penitrem A; (**1**) R^1 = Cl, R^2 = OH
Penitrem B; (**2**) R^1 = R^2 = H
Penitrem E; (**3**) R^1 = H, R^2 = OH
Penitrem F; (**4**) R^1 = Cl, R^2 = H

A penitrem-producing isolate, Sol-7, was grown for 8 d at 25 °C in a stationary culture in 400 Erlenmeyer flasks (500 ml), each containing 100 ml of Czapek medium enriched with 2% yeast extract. The mycelial mats were recovered and homogenized in a Waring blender in acetone. The homogenates were filtered, dried, and subjected to solvent

partition and column chromatography to give penitrem A (**1**), $C_{37}H_{44}NO_6Cl$ (1300 mg); penitrem B (**2**), $C_{37}H_{45}NO_5$ (170 mg); penitrem C (**5**), $C_{37}H_{44}NO_4Cl$ (60 mg); penitrem D (**6**), $C_{37}H_{45}NO_4$ (300 mg); penitrem E (**3**), $C_{37}H_{45}NO_6$ (100 mg); and penitrem F (**4**), $C_{37}H_{44}NO_5Cl$ (150 mg). The penitrems were resolved on silica thin layer chromatoplates in the solvents hexane–ethyl acetate (6:4 v/v) (A) and dichloromethane–ethyl acetate (9:1 v/v) (B) and detected by



Penitrem C; (**5**) R = Cl
Penitrem D; (**6**) R = H

spraying with 1% cerium sulphate in 6N H_2SO_4. Using solvent A, the penitrems A, B, C, D, E, and F appear at R_F 0·32, 0·36, 0·22, 0·22, 0·28, and 0·36, respectively; using solvent B, the penitrems B and F appear at R_F 0·46 and 0·50, whereas the penitrems C and D appear at R_F 0·32 and 0·29, respectively.

The u.v. characteristics of penitrem A [λ_{max}(MeOH) 295 and 233 nm (ϵ 11,600 and 37,000)] indicated a substituted indole nucleus. This supposition was confirmed by the ^{13}C n.m.r. parameters of penitrem A (Table) and by feeding (2*S*)-[3-^{14}C]tryptophan and (2*RS*)-[*benzene-ring-U*-^{14}C]-

TABLE. ^{13}C N.m.r. (100·62 MHz) data for penitrem A (**1**).

Carbon atom	δ_C [a]	^1J(CH)/Hz	^1J(CC)/Hz
2	154·32s		
3	120·62s		
4	133·26s		
5	125·77s		
6	124·54s		
7	111·84d	163·6	
8	121·97s		
9	139·70s		
10	35·05t	126·0 131·6	(40·3[c])
11	149·44s		73·9 (40·3[c])
12	47·00d	137·1	29·9
13	24·67t	135·9	29·9
14	52·69d	129·5	33·6
15	80·99s		33·6
16	76·07s		39·1
18	72·43d	146·8	39·7
19	58·77d	125·3	39·7
20	18·56t	127·1	(34·8[c])
21	30·59t	b	37·8
22	78·22s		39·1
23	66·09s		(29·9[c])
24	61·91d	179·4	(29·9[c])
25	66·29d	144·0	39·3[d]
26	74·64d	142·2	39·6[d]
28	71·98d	148·0	37·2
29	28·88t	128·3	37·8
30	26·90t	128·3	(33·6[c])
31	43·54s[e]		36·6
32	50·07s[e]		34·8
33	107·08t	155·6	73·9
34	20·32q	128·3	39·7
35	31·06q	125·8	(40·3[c])
36	19·70q	126·3	(42·7[c])
37	143·23s		73·2 (42·7[c])
38	111·63t	156·0	73·2
39	18·97q	126·4	36·0
40	21·35q	127·6	34·8

[a] Relative to Me_4Si. Solvent [$^2H_6$]acetone. [b] Not obtained owing to overlap of signals. [c] (C,C) coupling constants due to multiple labelling. [d] AB spin system at 25·2 MHz. [e] One transition obscured.

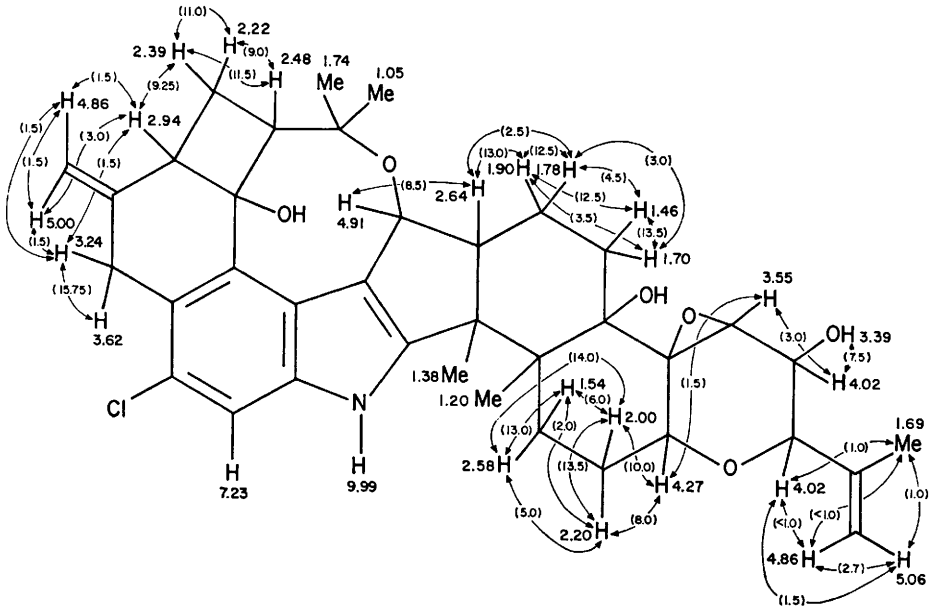


FIGURE 1. ^1H N.m.r. spectral data and proton–proton coupling constants for penitrem A.

tryptophan to cultures of the fungus. The latter precursor gave a higher (eight-fold) incorporation (0·16%) of radioactivity into penitrem A than did the side-chain labelled tryptophan, thereby establishing that the indole part of tryptophan contributed the aromatic nucleus of penitrem A.

Several structural units were recognized from a detailed analysis of the resolution-enhanced ^1H n.m.r. spectra of penitrem A. Extensive proton-decoupling experiments facilitated the unique assignments. The results derived from the ^1H n.m.r. studies are shown in Figure 1.

The assignment of the natural abundance ^{13}C n.m.r. spectrum of penitrem A (**1**) was derived from single frequency n.O.e., proton-noise decoupled (p.n.d.), single-frequency off-resonance proton-decoupled,[5] selective proton decoupled, and selective population-inversion experiments,[6] as well as from the known chemical shifts,[7] proton–carbon[8] and carbon–carbon coupling constants,[9] and deuterium exchange experiments. The ^{13}C n.m.r. data of penitrem A are collated in the Table.

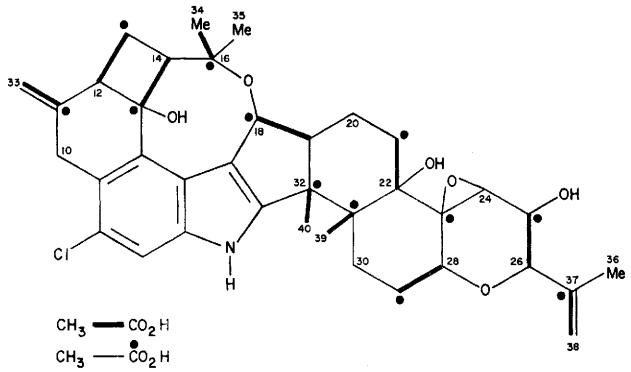


FIGURE 2. Labelling pattern of penitrem A derived from [1-^{13}C]- and [1,2-^{13}C]-acetate labelling experiments.

The p.n.d. ^{13}C n.m.r. spectrum of [1-^{13}C]acetate-derived penitrem A showed enhancement of 12 carbon atoms, *viz.* C(11), C(13), C(15), C(16), C(18), C(21), C(23), C(25), C(29), C(31), C(32), and C(37). The measured carbon–carbon coupling constants (Table) in the p.n.d. ^{13}C n.m.r. spectrum of [1,2-^{13}C]acetate-derived penitrem A are in accordance with the following intact acetate units: C(11)–C(33), C(12)–C(13), C(14)–C(15), C(16)–C(34), C(18)–C(19), C(21)–C(22), C(25)–C(26), C(28)–C(29), C(31)–C(39), C(32)–C(40), and C(37)–C(38). It is of interest to note that the following carbon atoms showed carbon–carbon coupling because of multiple labelling within a particular isoprene unit *viz.* C(10), C(11), C(20), C(23), C(24), C(30), C(35), C(36), and C(37). It is evident that six isoprene units contribute to the non-indole part of penitrem A; see Figure 2. In the course of the biosynthesis a single carbon atom, originally present as a methyl group of an isoprene unit, is lost from the diterpenoid precursor at C(23). The distribution of labels in the diterpenoid part of the penitrems indicates a similar origin to that proposed for paxilline and paspalicine.[10]

The ^{13}C n.m.r. spectra of the penitrems B—F, 25-*O*-acetylpenitrem A, and tetrahydropenitrem A were recorded at 125·76 MHz. The relevant data conform to the proposed structures.

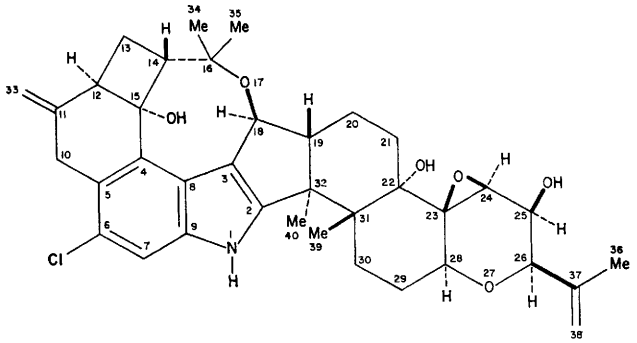


FIGURE 3. Proposed relative stereochemistry for penitrem A.

The indicated relative configuration of penitrem A (Figure 3) is based on proton–proton coupling, construction of Dreiding molecular models, and the relative configuration of paxilline as determined by *X*-ray crystallography.[11]

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[1] B. J. Wilson, C. H. Wilson, and A. W. Hayes, *Nature*, 1968, **220**, 77.
[2] C. T. Hou, A. Ciegler, and C. W. Hesseltine, *Can. J. Microbiol.*, 1971, **17**, 599.
[3] J. I. Pitt, *Mycologia*, 1979, **71**, 1166.
[4] P. J. Norris, C. C. T. Smith, J. de Belleroche, H. F. Bradford, P. G. Mantle, A. J. Thomas, and R. H. C. Penny, *J. Neurochem.*, 1980, **34**, 33, and references cited therein.
[5] K. G. R. Pachler, *J. Magn. Reson.*, 1972, **7**, 442.
[6] K. G. R. Pachler and P. L. Wessels, *J. Magn. Reson.*, 1977, **28**, 53.
[7] J. T. Clerc, E. Pretsch, and S. Sternhell, '^{13}C Kernresonanzspektroskopie,' Akademische Verlagsgesellschaft, Frankfurt am Main, 1973.
[8] J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972, Ch. 10, p. 331.
[9] V. Wray, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1979, **13**, 177.
[10] M. Yamazaki, 'The Biosynthesis of Mycotoxins,' ed. P. S. Steyn, Academic Press, New York, 1980, p. 210.
[11] J. P. Springer, J. Clardy, J. M. Wells, R. J. Cole, and J. W. Kirksey, *Tetrahedron Lett.*, 1975, 2531.