Tremorgenic Mycotoxins from *Penicillium crustosum*: Isolation of Penitrems A—F and the Structure Elucidation and Absolute Configuration of Penitrem A[1]

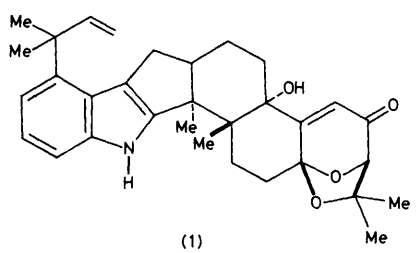
Amelia E. de Jesus, Pieter S. Steyn,* Fanie R. van Heerden, Robert Vleggaar, and Philippus L. Wessels
National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, Republic of South Africa
William E. Hull
Bruker Analytische Messtechnik, Silberstreifen, D-7512 Rheinstetten-Fo, Federal Republic of Germany

The isolation and characteristics of six tremorgenic mycotoxins, penitrems A—F from cultures of *Penicillium crustosum* are reported. The assignment of structure (2) to penitrem A is based on a detailed study of its high-field ^1H and ^{13}C n.m.r. spectra. The conformation and relative configuration of penitrem A was deduced from the observed proton–proton nuclear Overhauser effects (n.O.e.) and the magnitude of the proton–proton coupling constants. The chirality of C-25 was determined as *S* by the ' partial resolution ' method of Horeau and penitrem A must therefore have the (12*R*, 14*S*, 15*R*, 18*S*, 19*R*, 22*S*, 23*S*, 24*R*, 25*S*, 26*R*, 28*S*, 31*R*, 32*S*) absolute configuration.

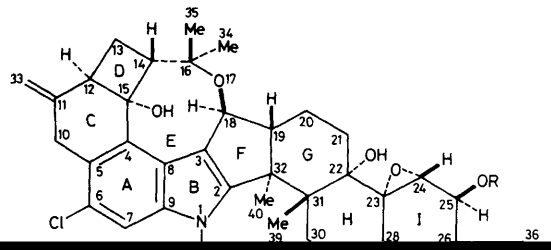
The current awareness of the importance of mycotoxins as environmental contaminants has led to the discovery of numerous complex substances which elicit a diverse spectrum of biological effects in experimental animals. An increasing number of newly discovered toxins induces a neurotoxicity syndrome in vertebrate animals which is characterized by sustained tremors, limb weakness, ataxia, and convulsions.

In 1964 Wilson described the isolation of a tremorgen, aflatrem (1)[2] from cultures of *Aspergillus flavus*, the structure of which was only recently elucidated by *X*-ray crystallography.[3] Penitrem A, $C_{37}H_{44}ClNO_6$, a member of the group of tremorgenic fungal metabolites, now known as penitrems (but initially called tremortins) was first isolated by Wilson *et al.*[4] from *Penicillium cyclopium*. Hou *et al.*[5] subsequently isolated penitrem A, as well as penitrem B, $C_{37}H_{45}NO_5$ and penitrem C (no formula given) from *P. palitans*.

In an early survey of the genus *Penicillium*, Ciegler and Pitt[6] found that production of the penitrems was confined to certain species of the subsection Fasciculata, section Asymmetrica and specifically to *P. crustosum*, *P. cyclopium*, *P. granulatum* and *P. palitans*. Recently Pitt,[7] however, concluded



(1)



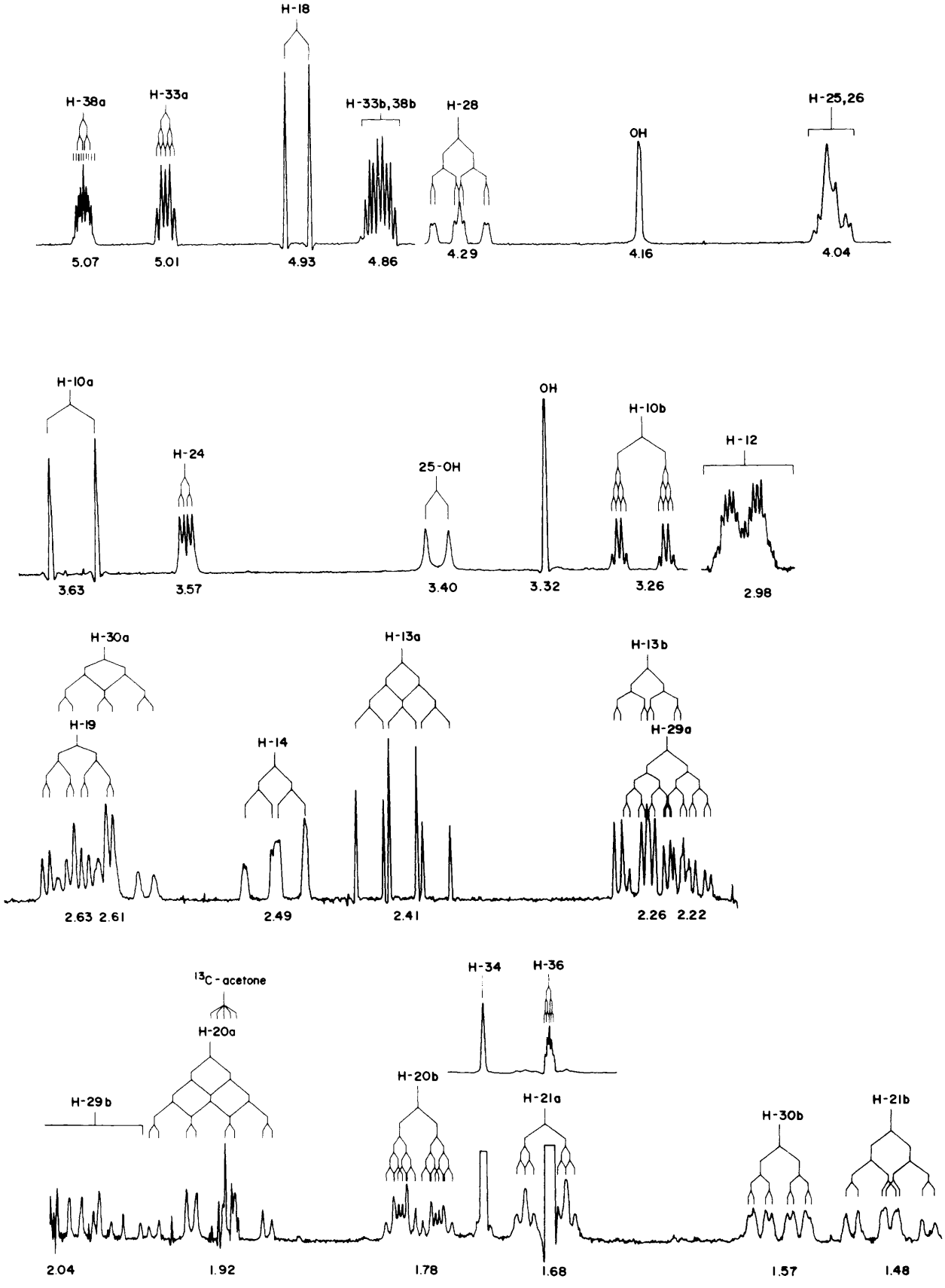
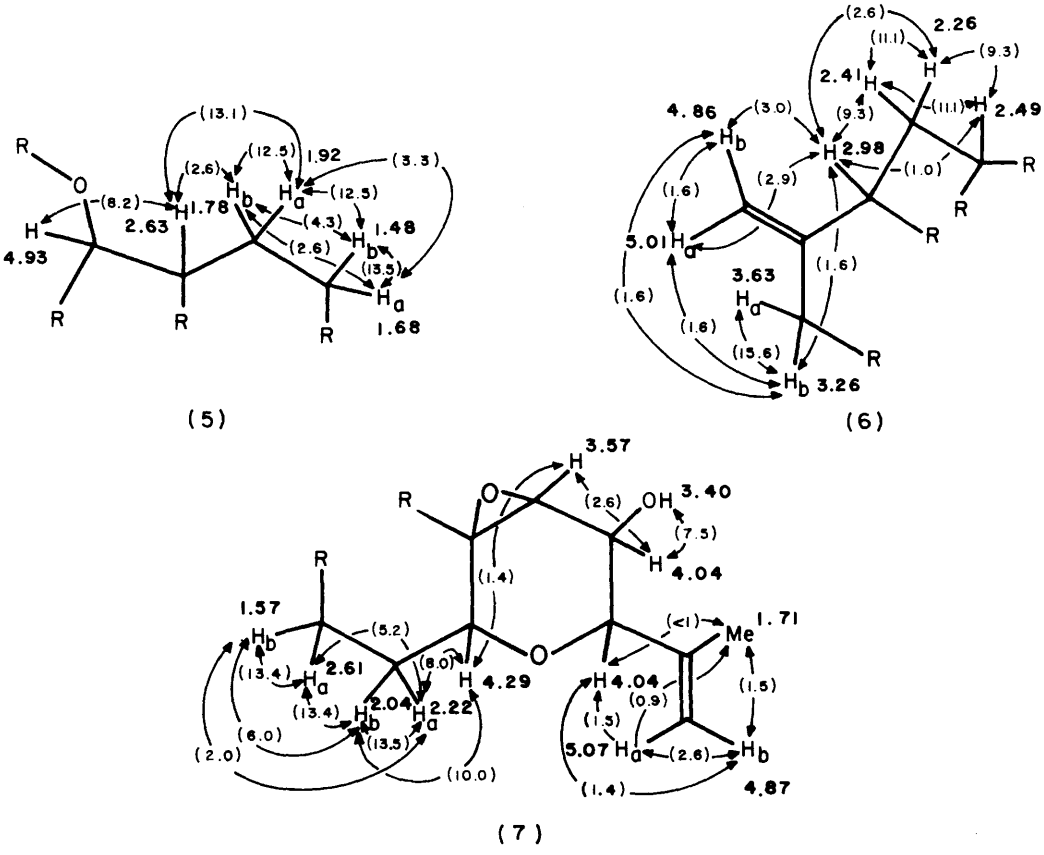


Figure 1. The analysed ^{1}H n.m.r. spectrum of penitrem A recorded at 500 MHz.



3M-H_2SO_4. Using solvent A, the penitrems A—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 yield of the various penitrems in extracts can be quantitated by an h.p.l.c. method which utilizes reversed-phase column chromatography with aqueous methanol as mobile phase.[10]

Penitrem A (2), is a colourless crystalline substance, m.p. 237—239 °C. Absorptions at $\lambda_{max.}$ 295 and 233 nm (ε 11 600 and 37 000, respectively) in the u.v. spectrum pointed to the presence of a substituted indole moiety in the molecule. This supposition was supported by its acid lability and by feeding experiments with different labelled tryptophans. In separate experiments (2*S*)-[3-^{14}C]tryptophan and (2*RS*)-[*benzene-ring-U*-^{14}C]tryptophan were added to cultures of *P. crustosum*. The latter precursor gave an eight-fold higher incorporation (0.16%) of radio-activity into penitrem A than did the side-chain labelled tryptophan. The indole part of tryptophan thus contributes the aromatic nucleus of the penitrems.

The i.r. spectrum of penitrem A was structurally uninformative. Absorptions at 3 580 and 3 475 cm^{-1} were assigned to OH and NH groups, respectively. The presence of at least one hydroxy-group in the molecule was confirmed by acetylation to give 25-*O*-acetylpenitrem A (3). Catalytic hydrogenation of penitrem A afforded the tetrahydro-derivative (4). The n.m.r. properties of both these compounds are discussed in conjunction with those of penitrem A.

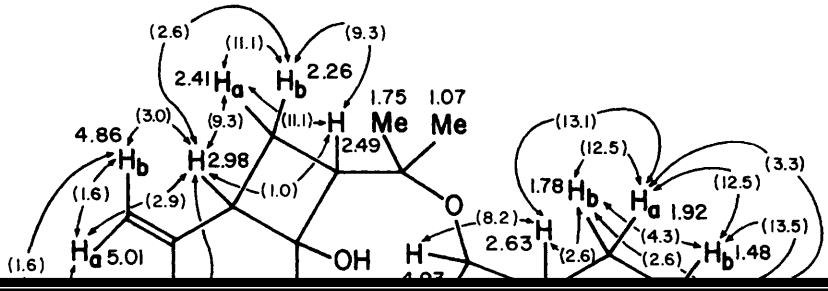
The intensities of four of the signals [δ 10.03 (s), 4.16 (s), 3.40 (d, *J* 7.5 Hz), and 3.32 (s)] in the 500 MHz ^{1}H n.m.r. spectrum of penitrem A decreased upon addition of deuterium oxide to the sample as well as through saturation transfer upon irradiation of the water resonance at δ 2.84. The signal at δ 10.03 was assigned to the NH proton of the indole moiety [11] whereas the remaining signals were attributed to hydroxy-protons. The one-proton aromatic singlet at δ 7.24 was assigned to 7-H. Five three-proton signals at δ 1.75, 1.71, 1.40, 1.22, and 1.07 were assigned to tertiary methyl groups. The signal at δ 1.71 showed broadening, which was resolved in the resolution enhanced spectrum, due to long-range couplings. The remainder of the ^{1}H n.m.r. spectrum exhibited extensive fine structure (Figure 1). First-order analyses of these multiplets yielded the values of the proton chemical shifts and proton–proton coupling constants. From the values of the coupling constants, as corroborated by extensive ^{1}H-{^{1}H} homonuclear decoupling experiments, three fragments A, B, and C of the penitrem A molecule could be constituted as shown in (5)—(7).

Fragment A (5). The doublet at δ 4.93 (*J* 8.2 Hz), assigned to 18-H served as the starting point in the analysis of this spin system. The chemical shift value indicated that this proton is located on an oxygen-bearing carbon atom in close proximity to an aromatic system.[12] In addition the value of the coupling constant suggests a possible *trans* relationship with its only neighbour, 19-H. The geminal and vicinal proton–proton coupling constants for the 19-H, 20-H, and 21-H protons are indicative of the protons of a six-membered ring in a chair conformation.[13]

Fragment B (6). The terminus of the second spin system, containing eight protons, is formed by a doublet at δ 3.63 (*J* 15.6 Hz) which was assigned to 10-H_a. The appreciable chemical shift difference (0.37 p.p.m.) between the geminal protons, 10-H_a and 10-H_b as well as the value of the coupling constant (*J* 15.6 Hz) implied that these protons might be situated in close proximity to an aromatic system. The chemical shifts and coupling constants of the protons resonating at δ 5.01 and δ4.86, 33-H_a and 33-H_b, (as well as those at δ 5.07 and δ 4.87, see below) are characteristic of the protons of an

exocyclic methylene group. The values of the coupling constants between these protons and those resonating at δ 3.26 (10-H_b) and δ2.98 (12-H) are in the range reported for

Irradiation at this frequency changes the patterns observed at δ 5.07 (38-H_a), δ 4.87 (38-H_b), δ 3.57 (24-H) and δ 1.71 (protons of the C-36 methyl group). In addition a small

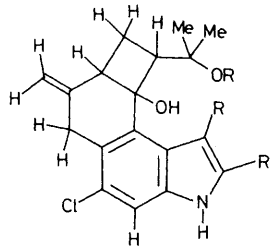

(2.6)
(11.1)
(9.3)
2.41 Ha
Hb 2.26
1.75
1.07
(3.0)
(9.3)
(11.1)
H
Me
Me
4.86
Hb
H 2.98
2.49
(1.6)
(2.9)
(1.0)
O
(13.1)
(12.5)
(3.3)
1.78 Hb
Ha 1.92
(12.5)
(8.2)
H
2.63
(4.3)
(13.5)
(1.6)
Ha 5.01
OH
H
(2.6)
(2.6)
Hb 1.48

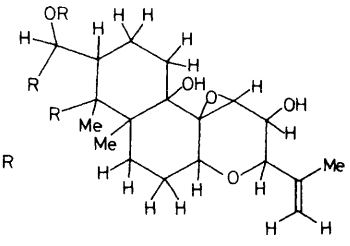
2 Hz).[23] The value of ^2J(C,H) can, however, increase if an electronegative group is located on the carbon atom.[23] The above remaining three resonances can therefore be assigned to C-5, C-8, and C-6, respectively with the chlorine atom located at C-6. In the ^{1}H n.m.r. spectrum of penitrem E (≡ 6-dechloropenitrem A) signals due to two *ortho* aromatic protons are observed.[24]

The observed deuterium isotope shifts (Table) for C-9 (δ 139.73, Δδ −0.148 p.p.m.) and the carbon atom resonating at δ 154.36 (Δδ −0.157 p.p.m.) are of the same magnitude. The deuterium isotope shift of C-9 results from substitution of the indole N–H proton by deuterium and is thus a two-bond effect. The signal at δ 154.36 is therefore assigned to C-2. Irradiation of the 1-H resonance (δ 10.03) affected the C-2, C-9, and C-8 resonances as well as the signal at δ 120.64 thereby assigning it to C-3, the only remaining sp^2 carbon atom three or less bonds removed from 1-H. The remaining aromatic carbon resonance at δ 133.29 must by elimination be assigned to C-4. The two- and three-bond deuterium isotope shifts (see Table) support the above assignments, although the isotope shift observed for C-4 is not explicable by substitution of 1-H with deuterium (but see below). At this stage of the structural analysis we have unambiguously assigned the carbon atoms of the indole ring and defined the substitution pattern of the indole moiety. We now have to assemble the penitrem A structure by making further interconnections between specific carbon atoms of the indole ring and the different fragments A (5), B (6), and C (7).

As the chemical shift and geminal coupling constant [^2J(H,H) 15.6 Hz] of 10-H_a (δ_H 3.63) and 10-H_b (δ_H 3.26) indicated that these protons are probably located in close proximity to an aromatic system, we decided to utilize the long-range (C,H) couplings of these protons. Selective irradiation of the 10-H_b proton (δ_H 3.26) transitions in a ^{13}C-{^{1}H} SPI experiment affected the resonances due to C-5, C-6, and C-4. Four-bond (C,H) couplings are normally small (*ca.* 1 Hz)[25] and the irradiating power used in these experiments (γH_2 = 5 Hz) precludes their detection. The 10-H_b proton therefore is two and/or three bonds removed from C-5, C-6, and C-4 and consequently the C-10 carbon atom of fragment B (6) must be linked to C-5 of the indole ring.

Selective decoupling of 12-H in fragment B (6) changed the resonances assigned to C-33, C-14, C-10 and the quaternary carbon signal at δ 81.01. The chemical-shift value indicates that this carbon atom (C-15) carries an oxygen substituent while the observed deuterium isotope shift (Δδ −0.118 p.p.m.) verified the nature of the oxygen function as a hydroxy-group. Three-bond deuterium isotope shifts were also observed for C-12 (δΔ −0.063 p.p.m.) and C-4 (Δδ −0.030 p.p.m.). The isotope shift for C-4 was mentioned before and must result from the replacement of the proton of the 15-hydroxy-group with a deuterium. This in turn confirms that C-15 is bonded to C-4 of the indole ring. The chemical shift of C-13 (δ 24.67) and the directly bonded (C,H) coupling constants of C-12 (^1J 137.1 Hz), C-13 (^1J 135.9 Hz), and C-14 (^1J 129.5 Hz) are similar to those observed for cyclobutane (δ 23.1; ^1J 134 Hz).[26] The carbon atoms C-12, C-13, C-14, and C-15 are therefore present as a cyclobutane moiety. The proton resonation of the C-14 proton which changed both the C-34 and C-35 methyl carbon resonances to a quartet of quartets. The chemical shift of the quaternary carbon resonance at δ 76.09 (C-16) is indicative of the presence of an oxygen-bearing carbon atom. Since no deuterium isotope shift was observed for this resonance it must be concluded that the oxygen atom is present as an ether function.


(8)

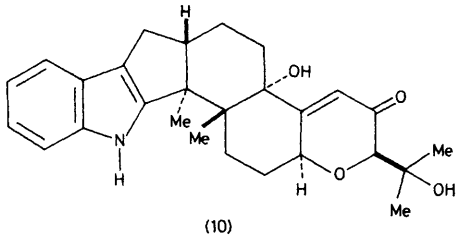

(9)

On the basis of the ^{1}H and ^{13}C n.m.r. data presented so far the partial structure as shown in fragment D (8) is proposed for penitrem A.

The presence of the oxiran moiety in fragment C (7) as postulated on the basis of ^{1}H n.m.r. data (see above) was readily confirmed by ^{13}C n.m.r. spectroscopy. The directly bonded (C,H) coupling constant of 179.4 Hz observed for the resonance centred at δ 61.91, and which had been correlated with the chemical shift of 24-H (δ_H 3.57), is diagnostic for a proton-bearing oxiran carbon atom.[27] The resonance at δ 66.11 was assigned to the quaternary carbon atom (C-23) of the oxiran moiety on the basis of its chemical-shift value and the change observed in this ^{13}C resonance when the protons resonating at δ_H 4.04 (25-H/26-H) were selectively irradiated.

Upon selective irradiation at δ_H 2.63, the resonance position of 19-H and 30-H_a, (C,H) couplings of 5.8 and 7.6 Hz are removed from the methyl carbon signals at δ 21.35 and 18.98, respectively. These (C,H) couplings must be over three bonds since the corresponding proton resonances of these tertiary methyl groups are singlets. In the ^{1}H n.m.r. spectrum the protons 18-H and 30-H_b are *inter alia* also affected upon irradiation at δ_H 2.63. As a result of this experiment a two-carbon unit consisting of a methyl group located on a quaternary carbon atom must be located at both C-30 of fragment C and at C-19 of fragment A. The two quaternary carbon atoms concerned resonate at δ 43.55 and 50.08, and must be contiguous since a one-bond (C,C) coupling of 37.2 Hz is observed for these signals in the p.n.d. ^{13}C n.m.r. spectrum of penitrem A derived from [1-^{13}C]acetate.[28] Furthermore, in the p.n.d. ^{13}C n.m.r. spectrum of penitrem A derived from [1,2-^{13}C]-acetate the resonance at δ 43.55 exhibits a one-bond (C,C)-coupling (^1J 36.0 Hz) with that at δ 18.98; a one-bond (C,C)-coupling (^1J 34.8 Hz) is similarly observed for the resonances at δ 50.08 and 21.35.[28] The above results define the mode of linkage between C-19 in fragment A (5) and C-30 in fragment C (7).

We are now left with only one unaccounted for quaternary carbon atom. The chemical shift of this carbon atom, δ 78.24 as well as the two-bond deuterium isotope shift (δΔ −0.112



(10)

resonance. In each case the C-2 resonance (δ 154.36) changed to a triplet with ^3J(C,H) 4.2 Hz. This result taken in conjunction with the chemical shift of the C-18 proton (δ_H 4.93) necessitates the linkage of C-18 with C-3 of the indole nucleus and, in addition, 19-H must be three-bonds removed from C-2.

On the basis of the evidence presented, penitrem A is assigned the structure (2). The assignment of the resonances in the ^{13}C n.m.r. spectrum of 25-*O*-acetylpenitrem A (3) and tetrahydropenitrem A (4) is given in the Table and follows from the

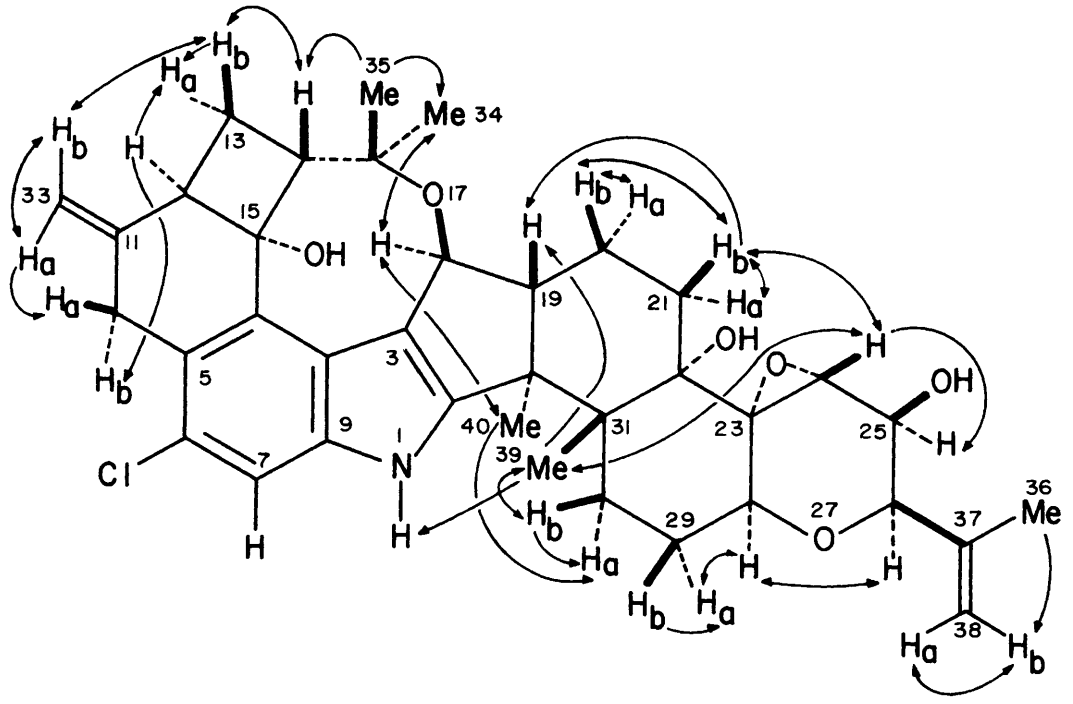


Figure 3. The n.O.e. connectivity pattern observed for penitrem A

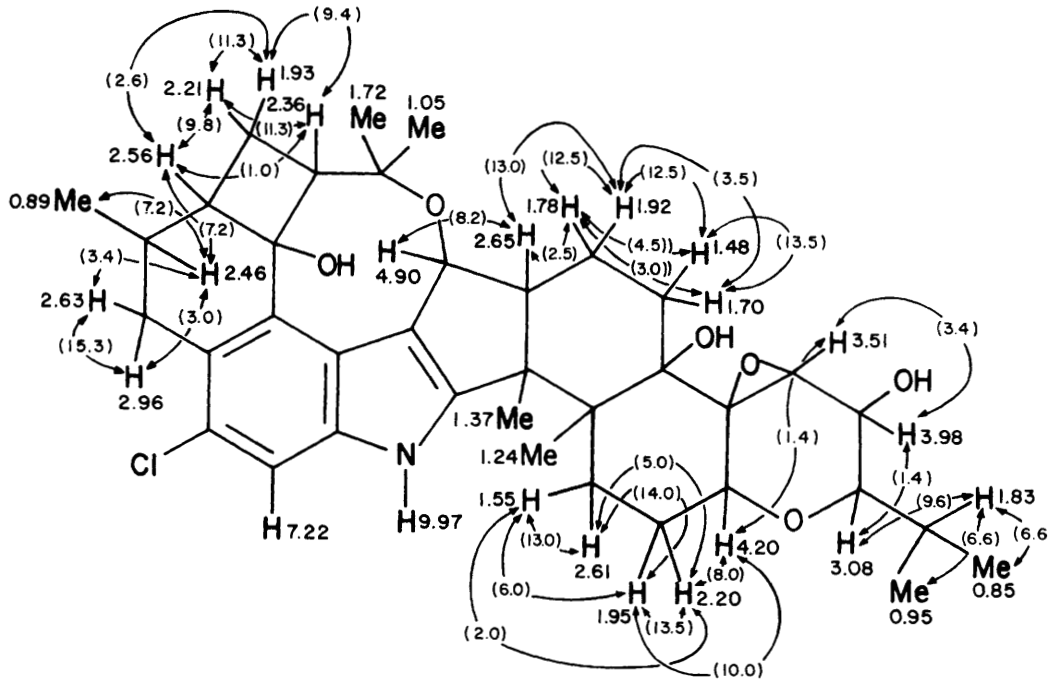


Figure 4. The ^{1}H n.m.r. spectral data and proton–proton coupling constants for 11,33,37,38-tetrahydropenitrem A

transfer of a hydrogen atom to the five-carbon fragment, gives an abundant ion at *m/z* 547. The loss of a 251 mass unit fragment ($C_{14}H_{19}O_4$) from both the *m/z* 615 ($M^+ - 18$) and *m/z* 547 ($M^+ - 86$) fragment is due to cleavage of the C(31)–C(32) and C(20)–C(21) bonds with transfer of a hydrogen atom to the indole-containing fragment and is the same loss which occurs in paspalinine and related compounds.

The conformation and relative configuration of penitrem A was deduced from the proton–proton and carbon–proton coupling constants as well as the proton–proton nuclear Overhauser effects (n.O.e.s).[34] The n.O.e.s between different protons were measured in the difference mode[35] with a decoupling power of 2 Hz and a n.O.e. generating delay of 3 s. The n.O.e. connectivity pattern is shown in Figure 3.

For the basis of the discussion it is assumed that C-15 has the *R*-configuration, *i.e.* the hydroxy-group is located below the plane of the indole ring. The ring fusion between the six- and four-membered rings is through necessity *cis* and the C-12 proton is therefore *cis* to the C-15 hydroxy-group. The C-16 carbon atom can either be *cis* or *trans* to the C-15 hydroxy-group while the C-18 proton can be either above or below the plane of the indole ring. As a result three different stereoisomers can be formulated. An unambiguous configurational assignment is, however, possible. The n.O.e.s for the C-12, C-13, and C-14 protons indicate a *trans* relationship between 12-H and 14-H and the C-16 carbon atom must therefore be *cis* to the C-15 hydroxy-group. An appreciable n.O.e. is observed between 18-H and the methyl-group protons which resonate at δ_H 1.75 (34-H) as well as between 14-H and the protons of the methyl resonance at δ_H 1.07 (35-H). This result can be explained if 18-H and the C-15 hydroxy-group are both located below the plane of the indole ring since this arrangement will lead to the required stereochemical relationship for the C-35 methyl-group and the C-14 protons (*cis*) as well as the proton-bearing carbon atoms have been correlated with specific proton resonances (see above).

The n.O.e. observed between the C-40 protons and 18-H but not 19-H, show that rings F and G are *trans*-fused with 18-H *cis* to C-40. The *trans* configuration of 18-H and 19-H is based on the fact that no n.O.e. is observed between these two protons as well as the vicinal proton–proton coupling constant of 8.2 Hz.

The *J*(HH) values for the protons of rings G and H, and the n.O.e.s observed between 19-H and 21-H_b, 19-H and 39-H, and between 21-H_b and 20-H_b show that the two rings are *trans*-fused with both rings in a chair conformation. The n.O.e. connectivity pattern observed between 24-H and 39-H, and 21-H_b shows that rings H and I are *cis*-fused, *i.e.* the configuration of the epoxide ring is as shown in Figure 3. This result corrects an error in our earlier communication where the enantiomeric chirality for C-23 and C-24 is shown. The fact that an n.O.e. is observed between 39-H and 24-H but not for 39-H and 21-H_b, or 29-H_b indicates that appreciable flattening of the chair conformation of both rings G and H must occur.

When the irradiating frequency in an n.O.e. experiment is applied at δ_H 4.04 in the ^{1}H n.m.r. spectrum, n.O.e.s are observed for 28-H (from 26-H), 24-H (from 25-H) and for 36-H and 38-H_a (from 26-H and/or 25-H). These n.O.e.s in conjunction with the proton–proton coupling constants for the protons of ring I in 25-*O*-acetylpenitrem A (3) (see above) prove the relative configurations at the chiral centres in ring I: the C-25 hydroxy-group in penitrem A is *trans* to the epoxide ring and *cis* to C-37 while the C-26 and C-28 protons are *cis* in agreement with the finding that no n.O.e. is observed between 24-H and 28-H.

On the basis of the argument outlined above the relative configuration as shown in (2) *i.e.* 12*R*, 14*S*, 15*R*, 18*S*, 19*R*

cule with the proposed relative configuration as shown in (2), results in a fairly rigid structure with a number of interesting features.

The exocyclic methylene group, C-33 can be either above or below the plane of the indole ring. The n.O.e.s observed between 33-H_a and 10-H_a, and between 33-H_b and 13-H_b indicate that this group is located predominantly above the plane of the indole ring. The chemical shift difference observed for 10-H_a and 10-H_b is also explained by this conformation as 10-H_a is in the plane of the aromatic ring and is therefore deshielded. The C(11)–C(33) double bond also has a deshielding effect on the two C-10 protons as shown by the chemical shifts of these two protons in the ^{1}H n.m.r. spectrum

through a short silica-gel column with benzene–acetone (4 : 1, v/v) to give the mixture (4.3 g) of penitrems. Residual material on the column was eluted with benzene–acetone (1 : 4, v/v) to give the fraction containing crude roquefortine.[39] This material was purified by percolation through an aluminium oxide (activity II-III) column with chloroform–methanol (9 : 1, v/v) to yield, after crystallisation from aqueous methanol, roquefortine [39] (3.9 g), m.p. 196—200 °C, identical with authentic material.

The mixture of penitrems (4.3 g) was separated and purified by column chromatography on silica gel using benzene–acetone (85 : 15, v/v) to yield the six penitrems in the following order of descending R_f values: penitrems E, B, A, F, C, and D

methane mixture was extracted with 6M-sodium hydrogen carbonate (2 × 10 ml) and the organic phase washed with 6M-HCl (1 × 10 ml), water (2 × 10 ml), dried, and evaporated. The residual ester (120 mg) contained no starting material and had M^+, 779/781 ($C_{47}H_{54}ClNO_7$).

The combined sodium hydrogen carbonate extracts were acidified (6M-HCl) and extracted with dichloromethane to yield α-phenylbutyric acid (72 mg), $[\alpha]_D^{23}$ −7.5° (*c*, 3.60 in benzene) (theoretical $[\alpha]_D$ −24.3° [37]). The optical yield therefore was 30.8% (−), based on an esterification yield of 100%.

Acknowledgements

We thank Dr. R. J. Cole, National Peanut Research Laboratory, Dawson, Georgia for a strain of Sol-7, and Dr. J. I. Pitt, Division of Food Research, CSIRO, Australia for the identification of this strain as *Penicillium crustosum*.

References

1 For a preliminary account see A. E. de Jesus, P. S. Steyn, F. R. van Heerden, R. Vleggaar, P. L. Wessels, and W. E. Hull, *J. Chem. Soc., Chem. Commun.*, 1981, 289.
2 B. J. Wilson and C. H. Wilson, *Science*, 1964, **144**, 177.
3 R. T. Gallagher, J. Clardy, and B. J. Wilson, *Tetrahedron Lett.*, 1980, **21**, 239.
4 B. J. Wilson, C. H. Wilson, and A. W. Hayes, *Nature*, 1968, **220**, 77.
5 C. T. Hou, A. Ciegler, and C. W. Hesseltine, *Can. J. Microbiol.*, 1971, **17**, 599.
6 A. Ciegler and J. I. Pitt, *Mycopathol. Mycol. Appl.*, 1970, **42**, 119.
7 J. I. Pitt, *Mycologia*, 1979, **71**, 1166.
8 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.
9 A. Ciegler, R. F. Vesonder, and R. J. Cole, *Adv. Chem. Ser.*, 1976, **149**, 163.
10 C. M. Maes, P. S. Steyn, and F. R. van Heerden, *J. Chromatog.*, 1982, **234**, 489.
11 P. J. Black and M. L. Hefferman, *Aust. J. Chem.*, 1965, **18**, 353.
12 Varian NMR Spectral Catalogue, Spectra 682 and 679.
13 E. Pretsch, T. Clerc, J. Seible, and W. Simon, 'Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden,' Springer-Verlag, Berlin, 1976.
14 H. Günther, 'NMR Spectroscopy,' Wiley, Chichester, 1980.
15 H. Booth, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1969, **5**, 149.
16 S. Sternhell, *Q. Rev.*, 1969, **23**, 236.
17 M. Barfield, A. M. Dean, C. J. Fallick, R. J. Spear, S. Sternhell, and P. Westerman, *J. Am. Chem. Soc.*, 1975, **97**, 1482.
18 K. G. R. Pachler and P. L. Wessels, *J. Magn. Reson.*, 1973, **12**, 337; 1977, **28**, 53.
19 R. A. Newmark and J. R. Hill, *Org. Magn. Reson.*, 1980, **13**, 40.
20 K. G. R. Pachler, P. L. Wessels, J. Dekker, J. J. Dekker, and T. G. Dekker, *Tetrahedron Lett.*, 1976, 3059.
21 R. G. Parker and J. D. Roberts, *J. Org. Chem.*, 1970, **35**, 996.
22 M. Begtrup, R. M. Cleramunt, and J. Elguero, *J. Chem. Soc., Perkin Trans. 2*, 1978, 99.
23 L. Ernst, V. Wray, V. A. Chertkov, and N. M. Sergeyev, *J. Magn. Reson.*, 1977, **25**, 123.
24 A. E. de Jesus, P. S. Steyn, F. R. van Heerden, R. Vleggaar, P. L. Wessels, and W. E. Hull, following paper.
25 P. E. Hansen, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1981, **14**, 175.
26 J. T. Clerc, E. Pretsch, and S. Sternhell, '^{13}C-Kernresonanzspektroskopie,' Akademische Verlagsgesellschaft, Frankfurt-am-Main, 1973.
27 J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1973.
28 A. E. de Jesus, C. P. Gorst-Allman, P. S. Steyn, F. R. van Heerden, R. Vleggaar, P. L. Wessels, and W. E. Hull, *J. Chem. Soc., Perkin Trans 1*, 1983, 1863.
29 J. P. Springer and J. Clardy, *Tetrahedron Lett.*, 1980, **21**, 231, and references cited therein.
30 R. T. Gallagher, J. Finer, J. Clardy, A. Leutwiler, F. Weibel, W. Acklin, and D. Arigoni, *Tetrahedron Lett.*, 1980, **21**, 235.
31 J. P. Springer, J. Clardy, J. M. Wells, R. J. Cole, and J. W. Kirksey, *Tetrahedron Lett.*, 1975, 2531.
32 R. J. Cole, J. W. Dorner, J. A. Lansden, R. H. Cox, C. Pape, B. Cunfer, S. S. Nicholson, and D. M. Bedell, *J. Agric. Food Chem.*, 1977, **25**, 1197.
33 P. A. Fellows, N. Kyriakidis, P. G. Mantle, and E. S. Waight, *Org. Mass. Spectrometry*, 1981, **16**, 403.
34 J. H. Noggle and R. E. Schirmer, 'The Nuclear Overhauser Effect,' Academic Press, New York, 1971.
35 M. L. Martin, J.-J. Delpuech, and G. J. Martin, 'Practical NMR Spectroscopy,' Heyden, London, 1980, p. 228.
36 A. Horeau, *Tetrahedron Lett.*, 1961, 506.
37 A. Horeau and J. K. Sutherland, *J. Chem. Soc. C*, 1966, 247.
38 W. Herz and H. B. Kagan, *J. Org. Chem.*, 1976, **32**, 216.
39 P. M. Scott, M.-A. Merrien, and J. Polonsky, *Experientia*, 1976, **32**, 140.

Received 25th August 1982; *Paper* 2/1481