^{1}H and ^{13}C N.m.r. Spectral Assignment Studies of Terretonin, a Toxic Meroterpenoid Metabolite of *Aspergillus terreus*

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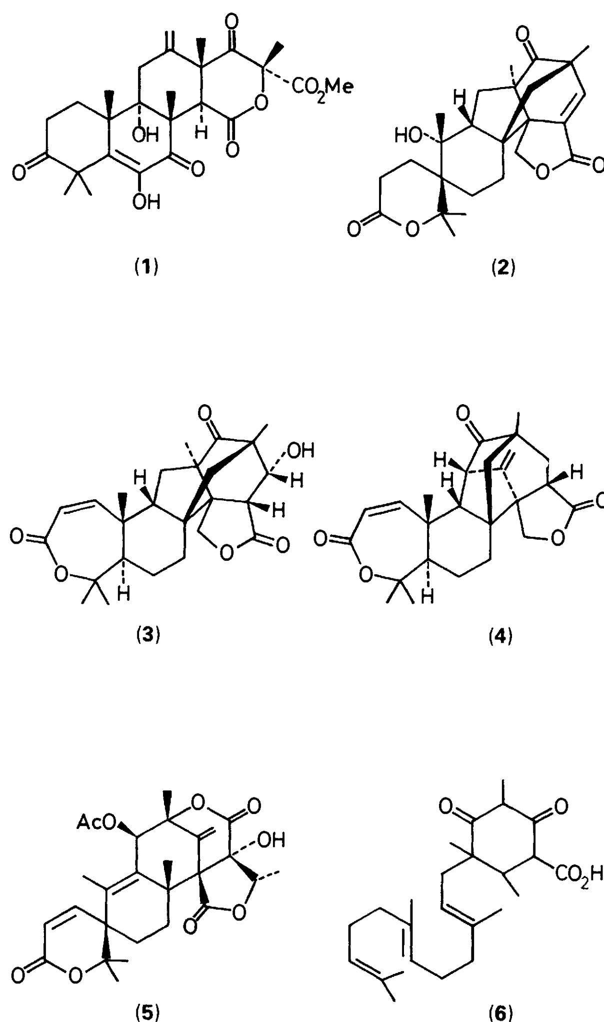
Complete assignments of all the resonances in the ^{1}H and ^{13}C n.m.r. spectra of terretonin have been made using a variety of one-dimensional and two-dimensional correlation methods. Nuclear Overhauser enhancements and spin-spin couplings observed in the ^{1}H n.m.r. spectrum indicate that the molecule adopts a conformation in solution similar to that observed in the crystal structure.

Terretonin (1),[1] andibenin B (2),[2] andilesin A (3),[3] anditomin (4)[4] and austin (5)[5] comprise a group of metabolites of diverse structures which have been isolated from different species of *Aspergillus*. They have all been shown to be biosynthesized by a mixed polyketide-terpenoid (meroterpenoid) pathway, in which the key step is *C*-alkylation of the tetraketide-derived 3,5-dimethylorsellinic acid by farnesyl pyrophosphate. The resulting common intermediate (6) then undergoes cyclisation followed by varying degrees of rearrangement and oxidative modification of both the terpenoid- and polyketide-derived entities to produce the variety of metabolites observed.[6] We have been engaged in an extensive study of the biosynthesis of these metabolites making use of precursors labelled with the stable isotopes ^{13}C, ^{2}H, and ^{18}O, and n.m.r. spectroscopy to establish the mode of incorporation of these precursors. An essential prerequisite of such studies is the rigorous assignment of the ^{1}H and ^{13}C n.m.r. spectra of the metabolites to be studied, and in compounds such as (1)—(5) this represents a non-trivial problem. Complete assignment of the ^{1}H and ^{13}C n.m.r. spectra for austin (5) has been reported,[7] but not for the remaining metabolites in this group. This paper describes the complete assignment of the ^{1}H and ^{13}C n.m.r. spectra of terretonin (1) without relying on spectral comparisons with model compounds, as a prelude to extensive isotopic labelling studies which are described elsewhere.



Terretonin was originally isolated in very low yields from a toxigenic strain of *Aspergillus terreus* (NRRL 6273) grown on a medium of shredded wheat in mycological broth supplemented with sucrose and yeast extract.[1] Isolation of terretonin from this medium required a protracted work up procedure. However, small quantities of terretonin were isolated along with smaller quantities of a second metabolite of molecular formula, $C_{29}H_{34}O_9$. The spectroscopic properties of this compound established its identity with territrem B (7) a tremorgenic metabolite isolated from rice cultures of a Taiwanese strain of *A. terreus*. Subsequent investigations showed that terretonin could be more conveniently isolated from liquid cultures using a medium based on malt extract broth. This medium has been used for all subsequent work.

Although some tentative resonance assignments in both the ^{1}H and ^{13}C n.m.r. spectra of terretonin were made during the initial structural studies,[1] these were not sufficiently complete for biosynthetic work. The complete assignments of the ^{1}H and ^{13}C n.m.r. spectra of terretonin cannot, however, be carried out entirely independently. In this study, spectra were obtained using deuteriochloroform as solvent unless otherwise stated.

Some resonances in the ^{1}H n.m.r. spectrum (Figure 1) can be unambiguously assigned from their chemical shifts and peak areas. The singlets at δ 3.53 and 3.75 clearly arise from 14-H and the *O*-methyl protons respectively and the broad signals at δ 1.8 and 6.2, which disappear on shaking the solution with deuterium oxide, arise from the OH groups bonded to C-9 and C-6 respectively. Chemical shifts and couplings allow separation of the remaining resonances into the following groups: (*a*) the multiplets W to Z from the protons bonded to C-1 and C-2, (*b*) the doublets at δ 2.26 and 2.94 from the protons bonded to C-11, (*c*) the singlets A to F from the 6 C-methyl groups, with the

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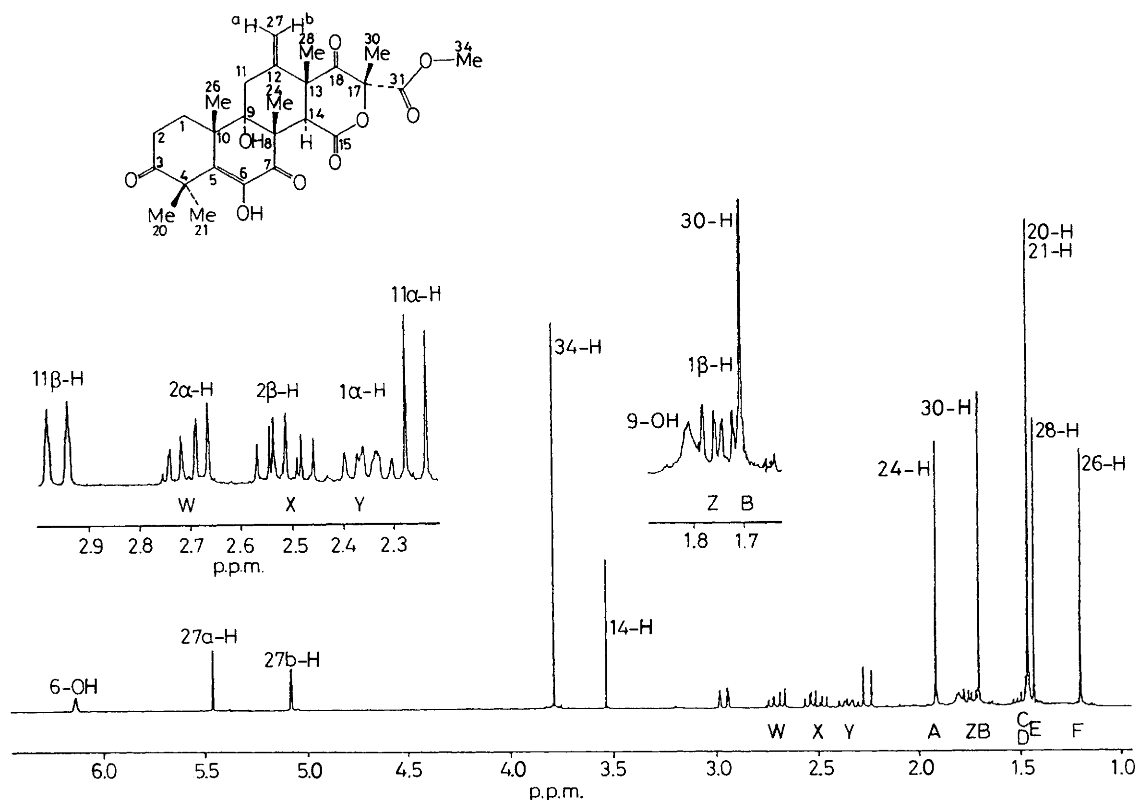
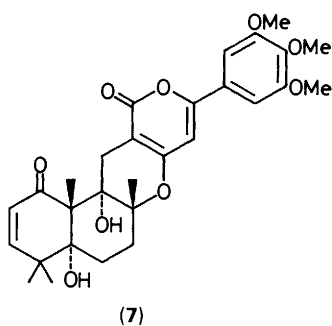


Figure 1. 360.13 MHz ^{1}H N.m.r. spectrum of terretonin in deuteriochloroform



(7)

integral trace indicating that two singlets, C and D, coincide, (*d*) the broad singlets at δ 5.06 and 5.42 from the alkene protons.

The coupling constants observed for the protons bonded to C-1 and C-2 are obtainable directly from the spectrum and are consistent with a twist boat conformation for ring A (see Figure 2) as is found in the solid state.[1] Decoupling multiplet Z removes the 8.2 and 13.5 Hz couplings from multiplets X and Y and leaves multiplet W intact. The absence of coupling between protons W and Z indicates[9] that these are sited on adjacent carbon atoms with a dihedral angle of *ca.* 90°, thus occupying pseudo-equatorial positions. Of these 2α-H is expected to have the higher chemical shift being closer to the carbonyl group and therefore gives rise to multiplet W. Thus multiplet Z corresponds to 1β-H. The 19.2 and 13.5 Hz geminal couplings show that multiplets X and Y correspond respectively to 2β-H and 1α-H which occupy pseudo-axial positions and show a mutual coupling of 11.1 Hz. The high chemical shift of the 1α-H resonance is explained by its spatial proximity to the axial 9-hydroxy group. These assignments were confirmed and the remaining proton assignments established largely by homonuclear n.O.e. measurements.[10]

Since chemical-shift information is frequently unreliable in distinguishing the remaining resonances in the above groups, a series of homonuclear n.O.e. difference spectra (Figure 3) was obtained as the spatial relationships of many of the protons was known. To avoid ambiguities arising from the overlap of the broad OH resonance at δ 1.8 with the 1β-H resonance and resonance B, n.O.e. measurements were made on solutions previously shaken with deuterium oxide. The fused ring systems in the molecules provide a fairly rigid framework in which 11β-H and the methyl groups at the ring junctions all occupy axial positions on the same side of the molecule, and where some small degree of flexibility is possible in the A and D rings only (Figure 4). Irradiation of resonances A, E, and F, but not B, C, and D, all increase the intensity of the doublet at δ 2.94. Therefore resonances A, E, and F arise from the axial methyl groups and the doublet at δ 2.94 from 11β-H. Therefore the doublet at δ 2.26 arises from 11α-H. Irradiation of resonance A also enhances the resonances E and F and irradiation of each of these separately enhances resonance A but not each other. Resonance A therefore corresponds to the 8-methyl group (24-H). Irradiation of resonance F but not E, enhances the multiplet assigned above to 2β-H indicating that resonance F arises from the 10-methyl group (26-H) and confirming that 2β-H occupies a pseudo-axial position. No enhancements of resonances A, E, or F were observed on irradiating resonances B, C, or D or *vice versa* indicating that neither of the methyl groups bonded β to C-4 and C-17 was likely to occupy a pseudo axial position

Figure 2. Three dimensional view of the A-ring of terretonin

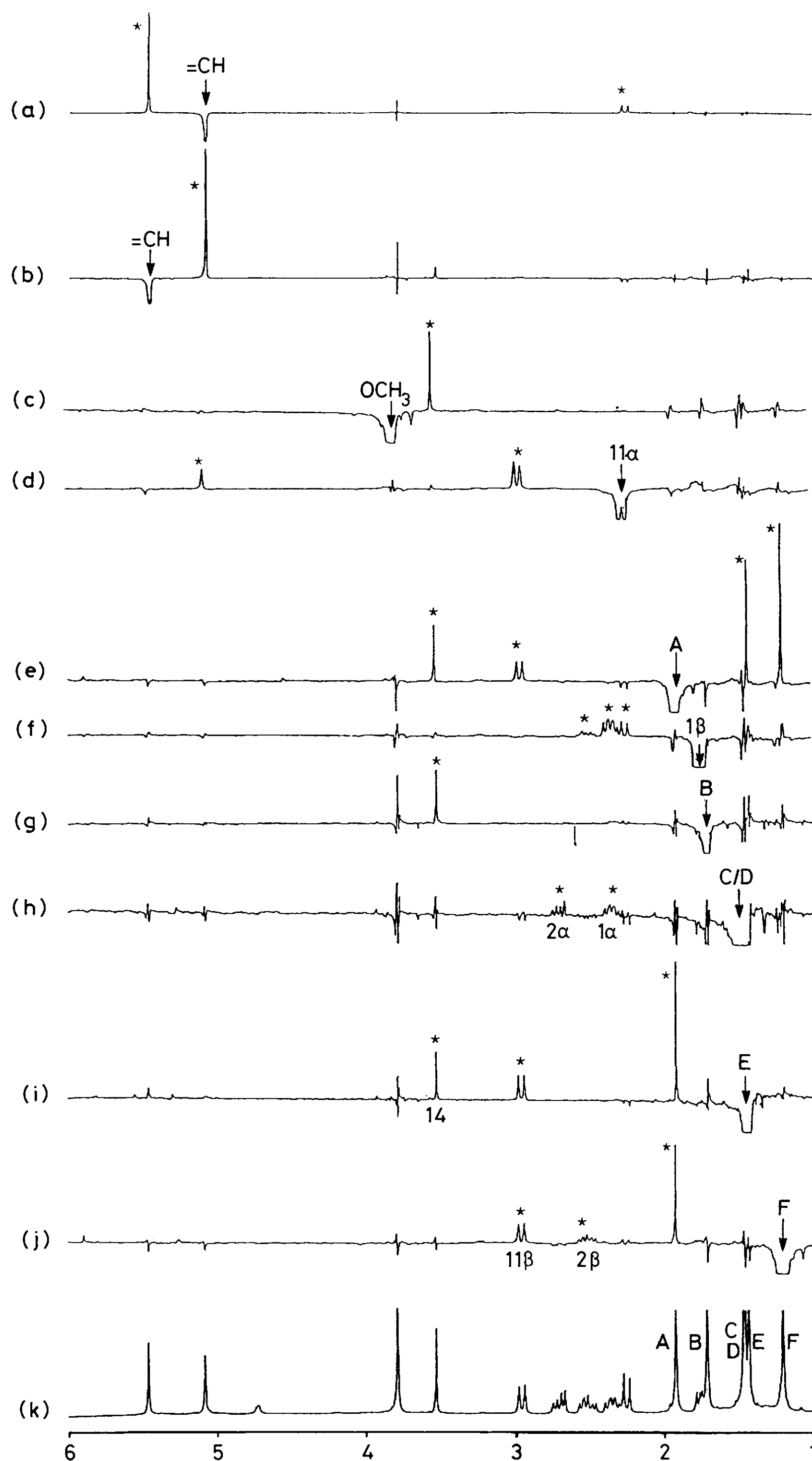


Figure 3. (a)—(j) Homonuclear proton n.O.e. difference spectra with irradiation at positions indicated ↓; enhancements indicated *. (k) Control proton spectrum

consistent with the view that the molecular conformation adopted in the solid state[1] is maintained in solution. Particularly unexpected were enhancements observed for the axial 14-H resonance on irradiating the resonances of each of the axial methyl groups bonded to C-8 and C-13. These methyl groups are located on the opposite side of the ring system from 14-H and their protons never approach closer than 3.5 Å to 14-H. In the absence of the knowledge of the molecular structure, these observations would have almost certainly been taken as evidence for a *cis* fusion between the C and D rings. This emphasises that utmost care must be taken when making stereochemical deductions from n.O.e. results. These enhancements probably arise because the α-face of the molecule is remarkably devoid of protons and apart from, on occasion, the mobile methoxy group, the protons of these two axial methyl groups are closer than any others to 14-H. Enhancement of the 14-H resonance was also observed on irradiating the methoxy proton resonance and, again unexpectedly, on irradiating resonance B. This suggests that resonance B arises from the 17-methyl protons (30-H) since these are closer to 14-H than the

protons of the 4-methyl groups. Irradiation of the superimposed resonances C and D enhances the intensity of the resonances assigned above to 1α-H and 2α-H indicating that at least one of resonances C and D corresponds to the α-methyl group bonded to C-4 and confirming the proton assignments and the conformation of the A-ring. The enhancements obtained are summarised in Figure 4. Since n.O.e.s are frequently observed between protons of geminal methyl groups, the absence of any n.O.e. on irradiating resonance B and observing the resonances C and D or *vice versa* supports the suggestion that resonances C and D arise from the protons of both methyl groups bonded to C-4. Confirmation of the assignments of the resonance of the methyl groups bonded to carbons C-4 and C-17 by double resonance methods are described later. Irradiation of the 1β-H resonance gave intensity increases for the resonances assigned to 11α-H (6%), 2β-H (2%), and 1α-H (2%). Irradiation of the 11α-H resonance gave intensity increases for the 11β-H resonance (4%) as expected and for the alkene resonance at δ 5.06 (2%) indicating that this alkene resonance arises from 27a-H. This was confirmed by irradiating this alkene resonance which gave intensity increases for the resonance (25%) of the other alkene proton, 27b-H, and the 11α-H resonance (5%) together with a small negative enhancement (−0.5%) of the 11β-H resonance due to the 3-spin effect.[11] Conversely irradiation of the 27b-H resonance gave an intensity increase for 27a-H (21%) and a negative (−1%) three-spin enhancement for 11α-H.

The classification of the resonances in the broad-band ^{1}H decoupled carbon-13 n.m.r. spectra (Figure 5) due to methyl, methylene, methine, or quaternary carbon was made by reference to spectra obtained by the DEPT technique.[12] Only three resonances may be confidently assigned from these spectra alone. The signal at 44.4 p.p.m. arises from C-14 since there is only one CH group in the molecule. Similarly their chemical shifts allow the assignment of the resonances at 53.5 and 116.7 p.p.m. respectively to the methoxy and alkene methylene groups

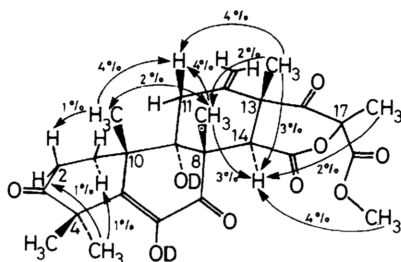


Figure 4. Three-dimensional view of the terretonin molecule with observed homonuclear n.O.e. results. Enhancements (%) are obtained for those nuclei at the arrow heads on irradiating those at the arrow tails.

in the molecule. From these spectra the other resonances may be separated as follows. The resonances below 25 p.p.m., two of which coincide, arise from the six *C*-methyl groups, and those between 25 and 35 p.p.m. arise from the alicyclic methylene groups. The remaining resonances do not appear in the DEPT spectra and arise from the quarternary carbons which fall into five groups (*a*) unfunctionalised C-4, C-8, C-10, and C-13 in the range 40—55 p.p.m., (b) oxygen bearing C-9 and C-17 in the range 75—90 p.p.m., (c) alkene C-5, C-6, and C-12 in the range 130—140 p.p.m., (d) ester carbonyl C-15 and C-31 around 167 p.p.m., and (*e*) ketone carbonyl C-3, C-7, and C-18 above 190 p.p.m.

The resonances of the two hydroxylated carbon atoms, C-6 and C-9, were identified by comparison of ^{13}C n.m.r. spectra obtained before and after exchange with 50:50 mixture of D_2O and H_2O. Since proton-deuteron exchange in this case occurs sufficiently slowly on the n.m.r. time-scale, this results in an observable, isotopically-induced splitting in the resonances of carbon atoms bearing hydroxy groups.[13] To avoid obscuring the terretonin resonance at 77.5 p.p.m. with the solvent resonance of $CDCl_3$, these spectra were obtained using CD_2Cl_2–$(CD_3)_2SO$ (70:30) as solvent. This had a negligible effect (<1 p.p.m.) on the terretonin resonances. The addition of the D_2O–H_2O mixture affected only the resonances at 77.5 and 138.5 p.p.m. In each case the resonances were broadened and the relative line heights were roughly halved, but at the resolution employed, the expected splittings could not be seen. Taken in conjunction with the chemical shifts, this observation allows the assignment of these two resonances to C-9 and C-6 respectively. Therefore, the resonance at 85.3 p.p.m. must be assigned to the remaining non-protonated, oxygen-bearing, C-17.

Having unambiguously identified the resonances arising from C-14 and C-17, the proton resonance assignments described earlier for the methyl groups bonded to C-4, C-10, C-13, and C-17 were confirmed from ^{13}C n.m.r. spectra obtained with single frequency proton irradiation at a sufficiently low power level to remove 2-bond and 3-bond proton-carbon couplings but leave the one bond couplings intact.[7,14] Irradiation of proton resonance B, assigned to 30-H, resulted *inter alia* in a sharp singlet for the C-17 resonance which appears as a quartet (J 4 Hz) in the absence of proton irradiation. This confirmed the assignment of resonance B and also, therefore, the assignment of the six-proton resonance C/D to the protons of the two methyl groups bonded to C-4. Irradiation of proton resonance E, assigned to 28-H, simplified the long-range coupling pattern of the C-14 resonance confirming the assignment of resonance E and also, therefore, of resonance F to 26-H.

Since the proton resonance assignments of the six methyl groups were now established without doubt the corresponding carbon-13 resonance assignments were made from a two-

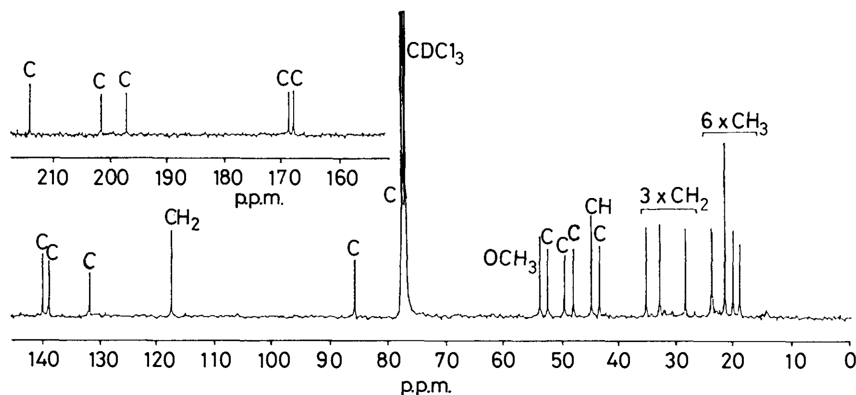


Figure 5. 90.56 MHz Carbon-13 n.m.r. spectrum of terretonin in deuteriochloroform. Resonance classification obtained from DEPT spectra (not illustrated)

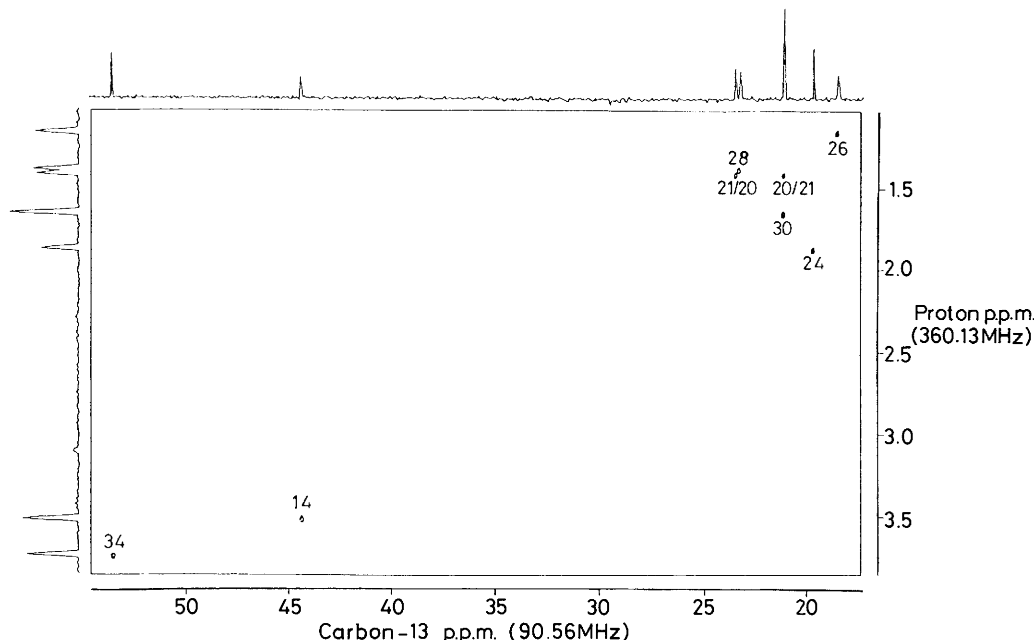


Figure 6. Contour plot of two dimensional one-bond C–H chemical shift correlation spectrum of terretonin.

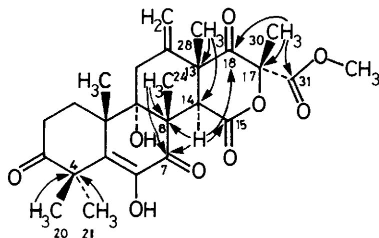


Figure 7. Long-range C–H couplings established from carbon-13 n.m.r. spectra obtained with selective low-power proton decoupling

dimensional carbon-hydrogen correlation experiment[15] optimized for one-bond couplings. The contour plot (Figure 6) shows, interestingly, that the coincident resonances in the carbon-13 spectrum do not arise from the same two methyl groups which give coincident resonances in the proton spectrum. There is, unfortunately, no way in which the resonances arising from C-20 and C-21 can be distinguished; however this point is not crucial for the interpretation of biosynthetic experiments.

Although the assignments of the resonances arising from C-14 and C-34 were also confirmed in this two-dimensional experiment, correlation signals for the aliphatic methylene groups could not be detected above the noise level. The methylene carbon resonances were assigned by the method of Birdsall, Birdsall, and Feeney[16] in which a series of ^{13}C n.m.r. spectra were obtained with single frequency proton irradiation set at 0.7 p.p.m. intervals over the range 0.0—4.2 p.p.m., and the residual reduced one-bond carbon-hydrogen coupling constants plotted against the irradiation frequency. Intersection of the straight lines obtained showed that the carbon resonances at 27.9, 32.5, and 34.7 p.p.m. correlated respectively with the resonances arising from the protons bonded to C-1, C-2, and C-11.

Several quaternary carbon resonances were assigned from single frequency low-power proton decoupled ^{13}C n.m.r. spectra of the type described above. The long-range proton-carbon couplings thus established are shown in Figure 7. Irradiation of the 14-H resonance collapsed the doublet at 167.6 p.p.m. to a singlet identifying this carbon resonance as arising from C-15. The multiplet at 168.3 p.p.m. therefore arises from C-31. This was confirmed by collapse of this multiplet to a quartet on irradiating the 30-H resonance. The ketone carbonyl resonances were similarly distinguished. The fully proton coupled ^{13}C n.m.r. spectrum shows these resonances as broad singlets with barely discernible fine structure. The resonance at 196.8 p.p.m. sharpens markedly on irradiating each of the 14-H and 24-H resonances and that at 201.2 p.p.m. develops fine structure on irradiating each of the 14-H and 30-H resonances. From these observations the resonance at 196.8 p.p.m. is assigned to C-7 and that at 201.2 p.p.m. to C-18. Thus the resonance at 213.9 p.p.m. must arise from C-3.

Of the unfunctionalised quaternary carbon resonances, that at 52.2 p.p.m. sharpened from a broad multiplet to a sharp triplet on irradiating the 24-H resonance and an intensity increase was observed on irradiating the 14-H resonance. This carbon resonance was therefore allocated to C-8. Irradiating either the 28-H resonance or the coincident 20-H and 21-H resonances very markedly sharpened the resonance at 47.7 p.p.m. Since no carbon nucleus is within coupling or n.O.e. range of both these sets of protons, this effect is attributed to insufficiently selective irradiation of the proton resonances which are only 10 Hz apart. The sharpening was more pronounced for irradiation of the 20-H/21-H resonance suggesting that this carbon resonance arises from C-4. This was supported by the response of the quaternary resonance at 49.3 p.p.m. Although irradiation of these proton resonances again produced a discernible sharpening, the effect here was more pronounced for 28-H. Additionally, an intensity increase was observed on irradiating the 14-H resonance; hence this carbon-13 resonance arises from C-13.

These assignments were confirmed and the remaining resonances assigned from a second two-dimensional proton-carbon correlation experiment optimised for long-range couplings of around 5 Hz. The correlations observed are summarised with the contour plot (Figure 8). A clear correlation is obtained between the carbon resonance at 47.7 p.p.m. and the coincident proton resonances arising from 20-H and 21-H, confirming the assignment of this carbon resonance to C-4. Similarly the above assignments of the C-7, C-8, and C-17 resonances are confirmed by correlation with the 24-H, 24-H, and 30-H resonances respectively. The resonance arising from C-31 correlated with resonances of 30-H and 34-H and that from

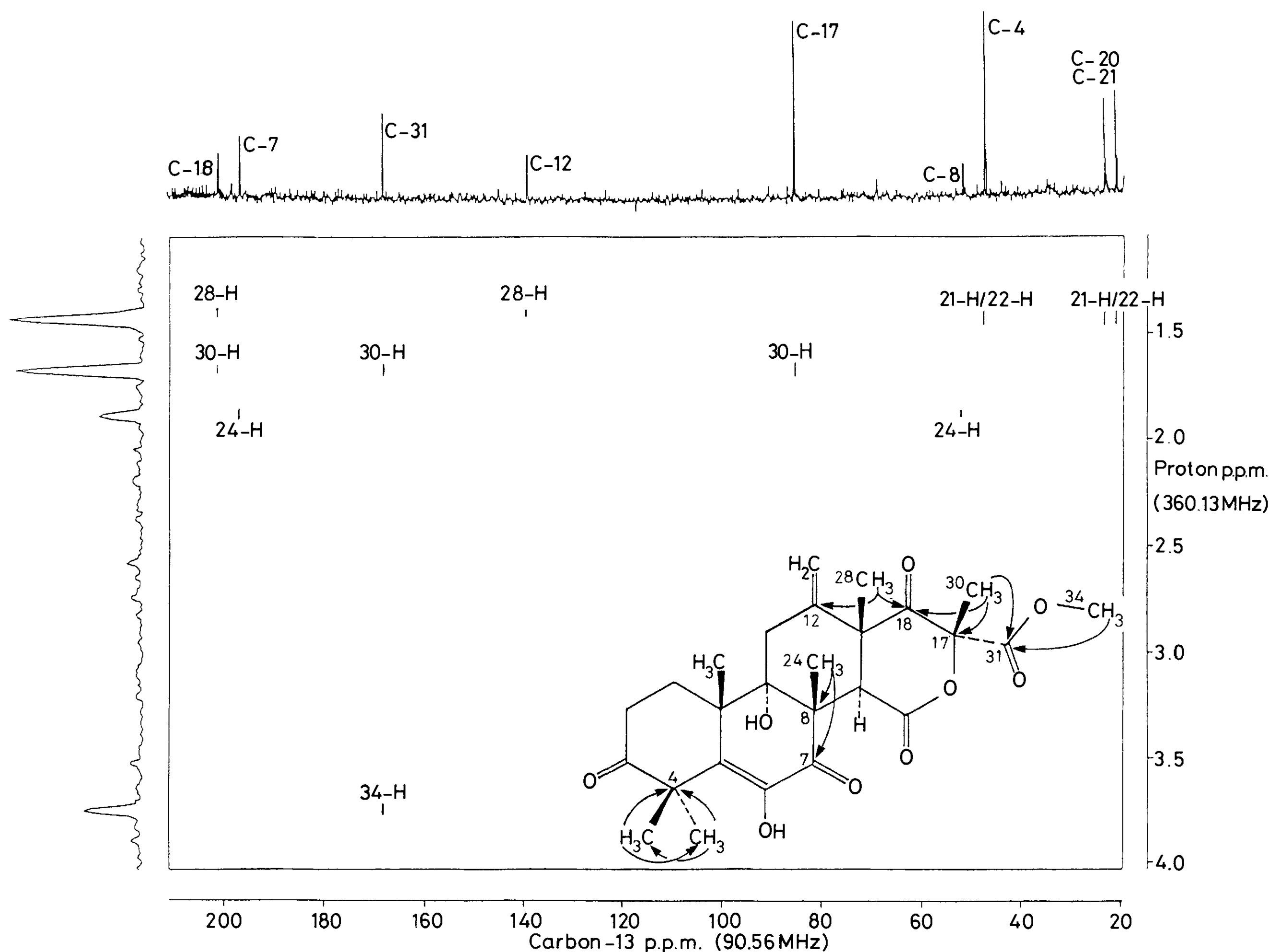


Figure 8. Contour plot of two-dimensional long-range C–H chemical-shift correlation spectrum of terretonin

C-18 with those of 28-H and 30-H. It is noteworthy that correlations are evident between the carbon of one geminal methyl group and the protons of the other. Correlation of the 28-H resonance with the alkene carbon resonance at 139.6 p.p.m. identifies this as arising from C-12 since the only other unidentified alkene carbon, C-5, is too far away to couple to 28-H. The resonance at 131.5 therefore corresponds to C-5 and this is in agreement with the assignment proposed on the basis of a ^{13}C n.m.r. spectrum of enriched terretonin obtained from [1,2-$^{13}C_2$]acetate in which this resonance showed a coupling of 84 Hz to the C-6 resonance. No unexplained correlations were observed in the long-range correlation experiment, although, due to the wide range of values found for long-range couplings, several correlations, *e.g.* those to 14-H were not detected. The remaining carbon resonance at 43.0 p.p.m. must therefore correspond to C-10.

This completes the assignments of the proton and carbon-13 n.m.r. spectra of terretonin which are summarised in the Table.

Experimental

N.m.r. Spectroscopy.—Spectra were recorded at 25 °C on a Bruker WH360 spectrometer operating at 360.13 MHz for protons and at 90.56 MHz for carbon-13 nuclei. Standard and homodecoupled proton spectra were acquired with 32 K data points over a spectrum width of 3 000 Hz (8.3 p.p.m.) and referenced to chloroform at δ 7.25. Proton n.O.e. spectra were obtained with 16 K data points over a spectral width of 2 400 Hz (6.7 p.p.m.). Secondary irradiation at 41 dB below 0.2 W was applied during a 9 s delay followed by spin excitation with a 90° pulse. Blocks of 32 scans preceded by 4 dummy scans were accumulated for each irradiation site to give a total of 640 scans per site. Multiplets were irradiated by the technique[17] in which the irradiation frequency was cycled through each of the multiplet line positions in turn. For the 1β-H multiplet only the three highest frequency lines were irradiated to avoid irradiating resonance B simultaneously. Two control spectra with irradiation at δ 0.1 and 4.2 were used. Line broadening of 1 Hz was applied before zero filling to 32 K and Fourier transformation. Subtraction of either control spectrum from any of the selectively irradiated spectra gave the same enhancements.

Carbon-13 spectra were obtained on a 120 mg sample dissolved in 2 ml of solvent with 32 K data points over a spectrum width of 20 000 Hz and referenced to deuteriochloroform at 76.9 p.p.m. or, where appropriate, [$^{2}H_2$] methylene chloride at 53.6 p.p.m. Selectively proton decoupled spectra were obtained using low decoupling power of 30 dB below 0.2 W.

Two-dimensional proton-carbon correlation spectra were obtained using the DEPT 2D pulse sequence[15] D1—(90H)—D2—(180H;90C)—D2—t1/2—(180C)—t1/2—(0H)—D2—AQ where D1 is a relaxation delay of 3 s, D2 is a fixed delay selected according to the coupling between the nuclei to be correlated, and t1 is the incremented delay. A 16-step phase cycle was used with proton broad-band decoupling applied only during the acquisition period AQ. For the one-bond correlation experiment (Figure 5) D2 was set at 0.0037 s, the other parameters being SW(carbon) = 4 386 Hz; 2 K data points;

Table Chemical shifts of protons and carbons and proton-proton coupling constants observed in the ^{1}H and ^{13}C n.m.r. spectra of terretonin

Position	C (p.p.m.)	H (p.p.m.)	J_{HH}/Hz
1α	27.9	2.30	13.5, 11.1, 8.7
1β		1.76	13.5, 8.2
2α	32.5	2.68	19.2, 8.7
2β		2.49	19.2, 11.1, 8.2
3	213.9		
4	47.7		
5	131.5		
6	138.5		
7	196.8		
8	52.2		
9	77.5		
10	43.0		
11α	34.7	2.26	14.3
11β		2.94	14.3
12	139.6		
13	49.3		
14	44.4	3.53	
15	167.6		
17	85.3		
18	201.2		
20/21	21.1/23.5	1.44	
24	19.6	1.89	
26	18.4	1.19	
27a	116.7	5.42	
27b		5.06	
28	23.3	1.41	
30	21.1	1.68	
31	168.3		
34	53.5	3.75	

SW(proton) = 550 Hz; 256 FIDs each of 48 scans; t1 = 0.0009 s; θ = 45°. The data was processed using sine bell squared window in both dimensions with zero filling the F1 data from 256 W to 512 W. (Proton resolution 2.1 Hz/point; carbon-13 resolution 4.3 Hz/point). For the long-range correlation experiment (Figure 6) D2 was set at 0.1 s, the other parameters being SW(carbon) = 17 857 Hz; 4 K data points; SW(proton) = 550 Hz; 128 FIDs each of 128 scans; t1 = 0.0009 s; θ = 30°. The data was processed using a sine-bell squared window in both dimensions with zero filling of the F1 data from 128 W to 256 W. (Proton resolution, 4.2 Hz/point; carbon-13 resolution 8.7 Hz/point).

Production and Isolation of Terretonin.—Aspergillus terreus NRRL 6273 was stored in the dark at 4 °C under liquid paraffin on slopes of potato dextrose agar (Oxoid CM 139). Cultures for large-scale inoculations were prepared from these by growth, also on potato dextrose agar, in medical flats at 26 °C in constant light for 8—10 days. A spore suspension in distilled water was subsequently used to inoculate the production medium of malt-extract broth (Oxoid Malt-Extract, 3% w/v; Oxoid Mycological Peptone, 0.5% w/v; distilled water, to 100%) 200 ml of which was contained in each of the penicillin flasks employed. The fungus was allowed to grow for 14 days in constant light at 26 °C. A thick, brown, mycelial mat formed on the surface of the medium.

The growth medium was carefully decanted from the flasks and filtered through muslin to remove any stray pieces of mycelium. A little hot water, used to rinse the flasks and mycelium, was also filtered and added to the medium. The liquor was then extracted, at the harvest pH of *ca.* 7, with chloroform (4 × one-third of liquor volume). The extract was dried ($MgSO_4$) and reduced under reduced pressure to give a brown foam (typically 150 mg/l). This was subjected to preparative t.l.c., developing with an acetone–chloroform mixture (14:86), using a very light loading (20 mg per plate). Elution (chloroform) of the band at R_F = 0.4 gave terretonin (30–35 mg/l) appearing as a white foam on careful evaporation of the solvent. Recrystallisation from ethyl acetate gave, in small yield, white many-sided prisms, m.p. 261.5—264.5 °C (lit.,[1] m.p. 260—262 °C).

References

1 J. P. Springer, J. W. Dorner, R. J. Cole, and R. H. Cox, *J. Org. Chem.*, 1979, **44**, 4852.
2 A. W. Dunn, R. A. W. Johnstone, B. Sklarz, and T. J. King, *J. Chem. Soc., Chem. Commun.*, 1976, 270.
3 A. W. Dunn, R. A. W. Johnstone, B. Sklarz, L. Lessinger, and T. J. King, *J. Chem. Soc., Chem., Commun.*, 1978, 533.
4 T. J. Simpson and M. D. Walkinshaw, *J. Chem. Soc., Chem. Commun.*, 1981, 914.
5 K. K. Chexal, J. P. Springer, J. Clardy, R. J. Cole, J. W. Kirksey, J. W. Dorner, H. G. Cutler, and B. J. Strawler, *J. Am. Chem. Soc.*, 1976, **98**, 6748.
6 For preliminary reports see: C. R. McIntyre and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1981, 1043; C. R. McIntyre, T. J. Simpson, R. N. Moore, L. A. Trimble, and J. C. Vederas, *ibid*, 1984, 1498; C. R. McIntyre, F. E. Scott, T. J. Simpson, L. A. Trimble, and J. C. Vederas, *ibid*, 1986, 501; F. E. Scott, T. J. Simpson, L. A. Trimble, and J. C. Vederas, *ibid*, 1986, 214; T. J. Simpson, *Tetrahedron Lett.*, 1981, **22**, 3785.
7 T. J. Simpson, D. J. Stenzel, A. J. Bartlett, E. O'Brien, and J. S. E. Holker, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2687.
8 K. H. Ling, C. K. Yang, and F. T. Peng, *Appl. Environ. Microbiol.*, 1979, **37**, 355.
9 S. Sternhill, *Q. Rev.*, 1969, **23**, 245.
10 J. K. M. Saunders and J. D. Mersh, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1982, **15**, 353.
11 Ref. 10 p. 365.
12 D. T. Pegg, D. M. Doddrell, and M. R. Bendall, *J. Magn. Reson.*, 1983, **51**, 264.
13 C. P. Gorst-Allman, K. G. R. Pachler, P. S. Steyn, P. L. Wessels, and D. B. Scott, *J. Chem. Soc., Perkin Trans 1*, 1977, 2181; A. E. de Jesus, P. S. Steyn, R. Vleggar, and P. L. Wessels, *ibid.*, 1980, 52.
14 C. R. McIntyre and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1984, 704.
15 M. R. Bendall and D. T. Pegg, *J. Magn. Reson.*, 1983, **53**, 144.
16 B. Birdsall, N. J. M. Birdsall, and J. Feeney, *J. Chem. Soc.*, Chem. Commun., 1972, 316.
17 M. Kinns and J. K. M. Saunders, *J. Magn. Reson.*, 1984, **56**, 518.

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