Metabolites of the Higher Fungi. Part 26.[1] Cubensic Acid, 3,7,11,15-Tetrahydroxy-18-(hydroxymethyl)-2,4,6,10,14,16,20-heptamethyldocosa-4*E*,8*E*,12*E*,16*E*-tetraenoic acid, a Novel Polysubstituted C_{22} Fatty Acid from the Fungus *Xylaria cubensis* (Mont.) Fr. with Substituents and Substitution Pattern Similar to the Macrolide Antibiotics

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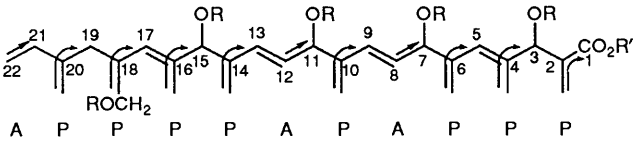
Cubensic acid, 3,7,11,15-tetrahydroxy-18-(hydroxymethyl)-2,4,6,10,14,16,20-heptamethyldocosa-4*E*,8*E*,12*E*,16*E*-tetraenoic acid, has been isolated from the mycelium and culture medium of *Xylaria cubensis*. Cytochalasin D is an additional metabolite isolated from the culture medium. The structure of cubensic acid has been determined by a combination of spectroscopic and chemical methods. A possible biosynthetic link between cubensic acid and certain macrolide antibiotics is suggested.

In part 22 of this series[2] we described the isolation of a hexenyl(methyl)succinic acid from the culture medium of a number of *Xylaria* species; there was no evidence of any significant additional metabolites in the culture media of these species. The genus *Xylaria* contains a large and undetermined number of species, most of them inhabiting tropical and sub-tropical regions. Their exact physiological role is unknown but most of them produce a white rot in wood. Recently we have been able to examine a number of species collected from the Amazon rain forest and we now report the isolation of a unique polysubstituted C_{22} fatty acid from the fungus *Xylaria cubensis*.

Xylaria cubensis,[3,4] collected in the Peruvian Amazon rain forest, was grown for eight weeks on 3% malt extract to yield initially a colourless mycelium, which later turned brittle and black on the underside and which supported copious phototropic, unbranched, white-tipped xylaria-type fruiting bodies. Solvent extraction of the culture medium and chromatography of the extract produced virtually pure cytochalasin D as the only major metabolite. This is the first report of the occurrence of a cytochalasin as a metabolite of a *Xylaria* species. A minor polar component, which gave a bright blue colouration on silica gel when sprayed with acetic acid–anisaldehyde–sulphuric acid and heated at 100–120 °C for 2–3 min, could not be eluted from the column. Since large quantities of cytochalasins constitute the mycelium of *Hypoxylon terricola*,[5] it was thought possible that cytochalasin D might occur as the major metabolite in the mycelium of *Xylaria cubensis*. Cytochalasin D was not isolated. Instead, a colourless solid was obtained which we name cubensic acid; this compound gave the characteristic blue colouration with the spray reagent.



1 R = R′ = H
2 R = Ac, R′ = H
3 R = H, R′ = Me
4 R = Ac, R′ = Me

Cubensic acid **1**, $C_{30}H_{52}O_7$, m.p. 196–198 °C; *m/z* (FAB) $(M + Na)^+$ 547, was obtained by direct crystallisation of the mycelium extract. There was no M^+ peak in either the electron impact (EI) or chemical ionisation (CI) mass spectrum. With a mixture of acetic anhydride and pyridine, cubensic acid **1** yields a pentaacetate **2**, $C_{40}H_{62}O_{12}$, but with acetic anhydride and sulphuric acid it is decomposed with the development of a transient pink, blue, green and finally brown colouration. Cubensic acid **1** also decomposes on refluxing with aq. sulphuric acid for 10 min. A monomethyl ester, methyl cubensate, $C_{31}H_{54}O_7$ **3**, is formed on treatment with diazomethane, and which on treatment with acetic anhydride and pyridine yields methyl cubensate pentaacetate **4**, $C_{41}H_{64}O_{12}$, FAB $(M + Na)^+$ 771. Direct crystallisation of cubensic acid **1** leads to much loss and the compound is best isolated as the methyl ester prepared by the action of diazomethane on the crude solid obtained by evaporation of the chloroform extract of the mycelium. The ^1H NMR spectra of compounds **1**, **3** and **4** are shown in Fig. 1.

The ^{13}C NMR spectrum of cubensic acid **1** mistakenly suggests a molecule containing only 28 carbon atoms (Table 1). The true carbon content was established from the spectrum of the methyl ester **3**, which is virtually identical with that of the acid **1** except for an additional methoxy resonance at δ_C 51.50 and the resolution of the resonances at δ_C 136.16 and 11.72 in acid **1** into four, at δ_C 135.62, 136.64 and 11.53, 11.72. Apart from the methoxy resonance the thirty other resonances in the spectrum of the ester **3** comprise three quaternary, sixteen methine, three methylene and eight methyl carbons. The signal at δ_C 178.6 can be assigned to the carboxy group, and the eight signals between δ_C 131 and 138 to six unsaturated methine and two unsaturated quaternary carbons indicating the presence of four olefinic double bonds. In the δ_C 66–84 region, five resonances indicate the presence of one methylene and four methine carbons bonded to oxygen, and between δ_C 30–40 eight resonances are associated with six alkyl methine and two methylene carbons. The eight resonances between δ_C 11–19 constitute eight methyl groups.

The ^{13}C–^1H COSY† spectrum of ester **3** confirms the presence of six methyl groups between δ 0.8–1.5 in the ^1H NMR spectrum but the latter is insufficiently resolved both at 270 and 400 MHz to permit elucidation of the multiplicity of the couplings. A ^1H *J*-resolved spectrum of ester **3** (Fig. 2) reveals a highfield triplet at δ 0.85, *J* 7.5 Hz and doublets at δ 0.86, 0.93, 0.99, 1.03 and 1.07, *J* 7.0 Hz, indicating an ethyl and five methyl-methine subunits. Two remaining methyl

† Heteronuclear chemical-shift correlation.

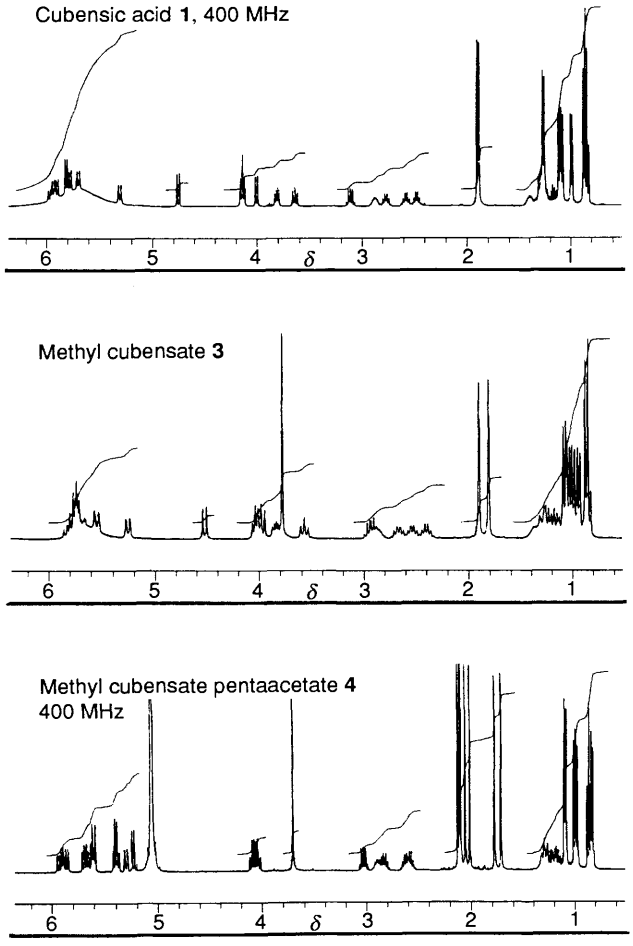


Fig. 1 ^{1}H NMR spectra of cubensic acid **1**, methyl cubensate **3** and methyl cubensate pentaacetate **4**

groups appear as doublets at δ 1.81 and 1.90, J 1.0 Hz, indicative of allylic coupling and the presence of two MeC=CH subunits.

Acetylation to form the acetates **2** and **4** is confirmed by the appearance in their ^{13}C NMR spectra, of five additional ester carbonyl signals between δ_C 169 and 171 and establishes the presence of five hydroxy groups in the acid **1**. A comparison of the ^{1}H NMR spectra of compounds **3** and **4** (Fig. 1) in the region δ 3.5–5.6 reveals, in the ester **3**, that two one-proton doublets at δ 3.97 and 4.53 and a two-proton multiplet at δ 4.04 move downfield by $\Delta\delta$ 1.20, 1.05 and 1.40, respectively, indicating the presence of four secondary hydroxy groups. A pair of doublet of doublets at δ 3.59 and 3.82 in the spectrum of ester **3**, each comprising a single proton, and found by ^{1}H–^{1}H DQF-COSY* and ^{13}C–^{1}H COSY spectra to be due to a methylene signal, move downfield to δ 4.07, confirming the presence of a primary hydroxy group.

Acid **1** was examined using ^{1}H–^{1}H DQF-COSY (Fig. 3) and 1-D spin-decoupling experiments to determine connectivities but this resulted only in partial success; it was possible to identify structural subunits (A–E), but it proved impossible to determine with certainty how these might be interconnected.

Subunit A.—Inspection of the ^{1}H–^{1}H DQF-COSY spectrum of acid **1** (Fig. 3) reveals that the primary alcohol methylene protons at δ 3.55 and 3.84 are coupled to a proton multiplet at δ 2.92; this in turn is coupled to an olefinic doublet at

* Double quantum-filtered COSY.

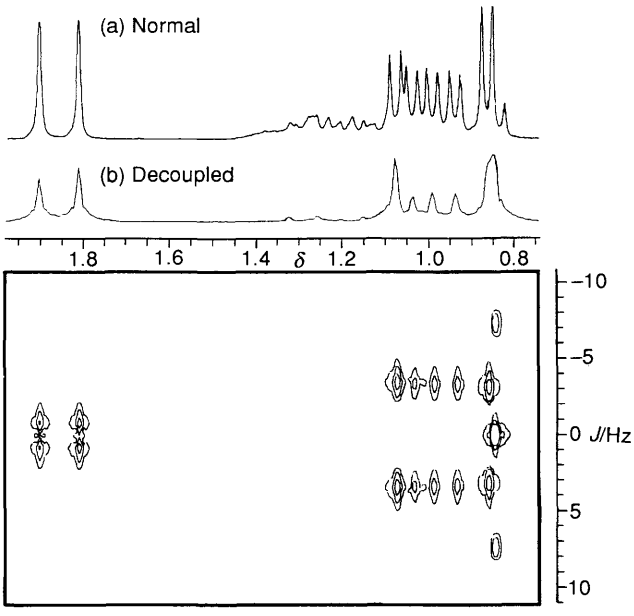


Fig. 2 1H J-Resolved spectrum of methyl cubensate **3**. (a) High-resolution ^{1}H NMR spectrum. (b) Low-resolution proton-decoupled ^{1}H NMR spectrum.

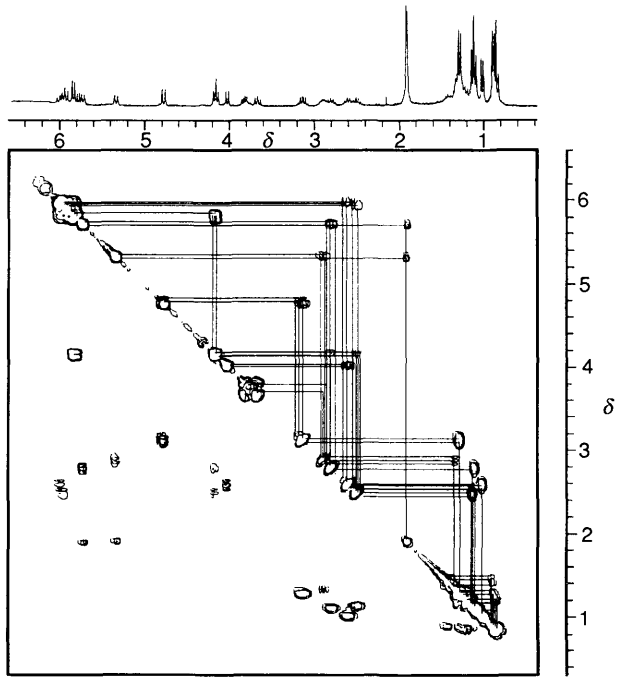


Fig. 3 ^{1}H–^{1}H DQF-COSY spectrum of cubensic acid **1**

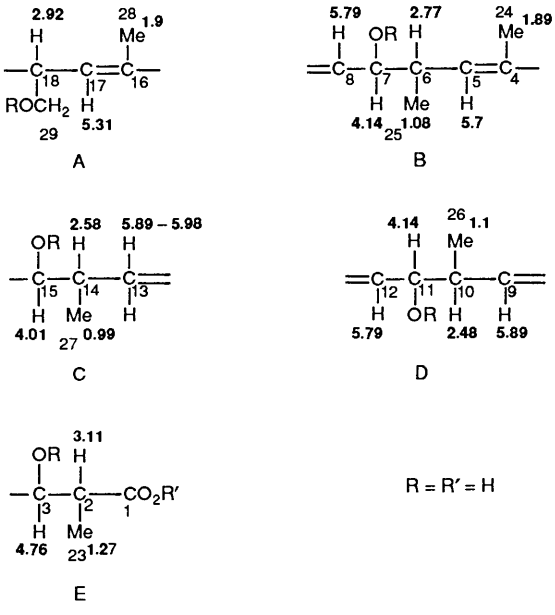
δ 5.31, which is coupled to the lowest field allylic methyl at δ 1.9.

Subunit B.—The other allylic methyl, at δ 1.89, is coupled to an olefinic proton doublet at δ 5.7; this is linked to a methine multiplet at δ 2.77, which is coupled to a secondary alcohol methine proton in the two-proton 'triplet' at δ 4.14 and to a methyl doublet at δ 1.08. The signal at δ 4.14 is also coupled to a two-proton olefinic methine doublet of doublets at δ 5.79 (J 15.3 and 7.57 Hz), suggesting *trans* coupling to another olefinic methine proton, which lies in the two-proton multiplet at δ 5.89–5.98. Whilst the exact

Table 1 ^{13}C and ^{1}H NMR chemical shifts (δ relative to Me_4Si in $CDCl_3$,[a] C_5D_5N[b]) (multiplicity + J-values)

	Cubensic acid **1**		Methyl cubensate pentaacetate **4**			Methyl cubensate **3**	
Position	δ_C[b]	δ_H[b]	δ_C[a]	δ_C[b]	δ_H[b]	δ_C[b]	δ_H[b]
1	178.58		174.34	174.71		176.61	
2	44.67	3.11 (dq, 9.5, 6.8)	41.91	42.17	3.03 (dq, 10.3, 7.1)	43.97	2.93 (dq, 9.9, 7.0)
3	81.29	4.76 (d, 9.5)	80.65	81.24	5.60 (d, 10.3)	81.27	4.52 (d, 9.9)
4	136.16		131.25	131.81		135.62	
5	132.92	5.70 (d, 9.5)	133.63	134.23	5.61 (d, 10.1)	133.58	5.55 (d, 9.5)
6	38.75	2.74–2.80 (m)	36.47	36.87	2.83 (m)	38.55	2.66 (ps)
7	77.78*	4.14 (t, 7.5)	77.40	77.90	5.39 (t, 7.3)	78.01*	4.02 (dd, 7.7, 6.4)
8	133.33**	5.80 (dd, 15.4, 7.6)	127.51	128.28	5.68 (dd, 15.4, 7.6)	133.66**	5.68 (m)*
9	136.92	5.95 (dd, 15.4, 8.3)	136.03	136.68	5.92 (dd, 15.4, 7.5)	136.64	5.81 (dd, 15.0, 8.7)
10	43.72	2.48 (ps)	40.71	41.13	2.58 (ps)	43.74	2.40 (ps)
11	77.60*	4.14 (t, 7.5)	77.27	77.70	5.41 (t, 7.3)	77.81*	4.02 (dd, 7.7, 6.4)
12	133.03**	5.80 (dd, 15.4, 7.6)	127.32	128.03	5.65 (dd, 15.5, 7.6)	133.12**	5.78 (m)*
13	136.16	5.91 (dd, 13.2, 8.3)	136.61	137.27	5.88 (dd, 15.5, 8.0)	137.32	5.81 (dd, 15.0, 8.7)
14	40.75	2.58 (ps)	38.74	39.17	2.62 (ps)	40.78	2.53 (ps)
15	83.12	4.01 (d, 8.8)	81.94	82.25	5.23 (d, 8.8)	83.3	3.96 (d, 9.5)
16	138.41		134.29	134.80		138.33	
17	131.45	5.31 (d, 9.8)	130.93	131.37	5.30 (d, 10.0)	131.81	5.25 (d, 10.1)
18	39.01	2.84–2.91 (br m)	35.00	35.38	2.90 (br m)	38.91	2.88 (br m)
19	38.96	1.23–1.34 (m)	38.45	38.63	1.20–1.30 (m)	38.79	1.12 + 1.27 (m)
20	32.41	1.40 (m)	32.01	32.25	1.20–1.34 (m)	32.41	1.40 (m)
21	30.92	1.18 (pp)	30.40	30.66	1.11–1.24 (m)***	30.91	1.23 (m)
22	11.72	0.85 (t, 7.3)	11.50	11.66	0.85 (t, 7.3)	11.72	0.85 (t, 7.5)
23†	15.25	1.27 (d, 7.1)	16.67	14.09	1.08 (d, 7.1)	14.71	1.07 (d, 7.0)
24†	11.07	1.89 (d, 1.0)	11.47	11.55	1.71 (d, 1.2)	10.73	1.81 (d, 1.0)
25†	17.65	1.09 (d, 6.8)	16.14	16.43	1.00 (d, 6.8)	17.48	0.99 (d, 6.8)
26†	17.46	1.11 (d, 6.8)	15.88	16.17	1.09 (d, 6.8)	17.84	1.03 (d, 6.8)
27†	18.02	0.99 (d, 6.6)	13.86	16.88	0.97 (d, 6.8)	18.02	0.93 (d, 6.8)
28†	11.72	1.90 (d, 1.0)	12.48	12.59	1.78 (d, 1.2)	11.53	1.90 (d, 1.0)
29a†	66.89	3.64 (dd, 10.3, 8.0)	67.52	67.73	4.10 (dd, 10.7, 7.3)	66.93	3.57 (dd, 9.5, 9.2)
29b†		3.81 (dd, 10.3, 5.6)			4.40 (dd, 10.7, 6.6)		3.84 (dd, 9.5, 4.8)
30†	19.06	0.87 (d, 6.6)	18.87	18.92	0.82 (d, 6.6)	19.05	0.86 (d, 7.0)
			*Me*O 51.77	51.77	3.71 (s) MeO	51.50	3.78 (s)
6 × OH		5.0–6.4 (br)	*Me*CO_2 20.78–21.04	20.77–21.05	2.01–2.13, 5s		
			Me*C*O_2 168.98–170.72	169.36–170.71			

* May be interchanged. ** May be interchanged. *** Two distinct single-proton multiplets at 1.18–1.24 and 1.11–1.17. † Carbons 23–30 are shown in subunits F–K. ps Pseudosextuplet. pp Pseudopentuplet.



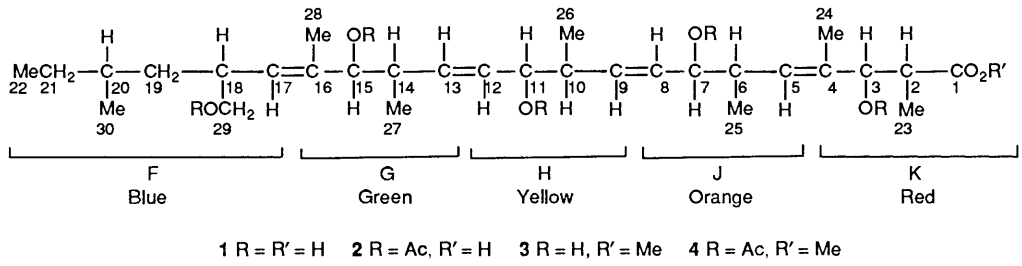
connectivity pathways from the latter are indistinct, it is possible to elucidate two spin systems emanating from it.

Subunit C.—One olefinic proton is coupled to a methine sextet at δ 2.58; this is linked to a methyl doublet at δ 0.99 and to a secondary alcohol methine proton doublet at δ 4.01.

Subunit D.—The other olefinic proton is coupled to a methine sextet at δ 2.48; this is connected to a methyl doublet at δ 1.1 and to a secondary alcohol methine proton in the 'triplet' at δ 4.14, which is coupled into the two-proton olefinic doublet of doublets at δ 5.79.

Subunit E.—This was obtained by examining the secondary alcohol methine doublet at δ 4.76; this is connected to the methine multiplet at δ 3.11, which is coupled to a methyl doublet at δ 1.27. The lowfield position of the methine multiplet at δ 3.11 *vis-à-vis* the other methine multiplets suggests that it is adjacent to the carboxylic acid group.

This leaves an ethyl, a methyl, a methylene and a methine group to sequence, a task which proved impossible even after careful examination of an enlargement of the δ 0.6–1.6 region of the ^{1}H–^{1}H DQF-COSY spectrum. The low solubility of cubensic acid **1** or the methyl ester **3** in C_5D_5N precluded the use of 2D-INADEQUATE. Although methyl cubensate pentaacetate **4** shows acceptable solubility, several attempts to obtain a 2D-INADEQUATE spectrum proved futile. A literature search revealed that low-viscosity solvents such as $CDCl_3$ or $(CD)_3CO$ give better results for 2D-INADEQUATE experiments[5] than do viscous solvents such as C_5D_5N. The ^{13}C NMR spectrum of compound **4** in $CDCl_3$ is virtually identical with that in C_5D_5N, all resonances being shifted upfield by $\Delta\delta$ 0.0–0.6 (Table 1), thus enabling results obtained



1 R = R′ = H **2** R = Ac, R′ = H **3** R = H, R′ = Me **4** R = Ac, R′ = Me

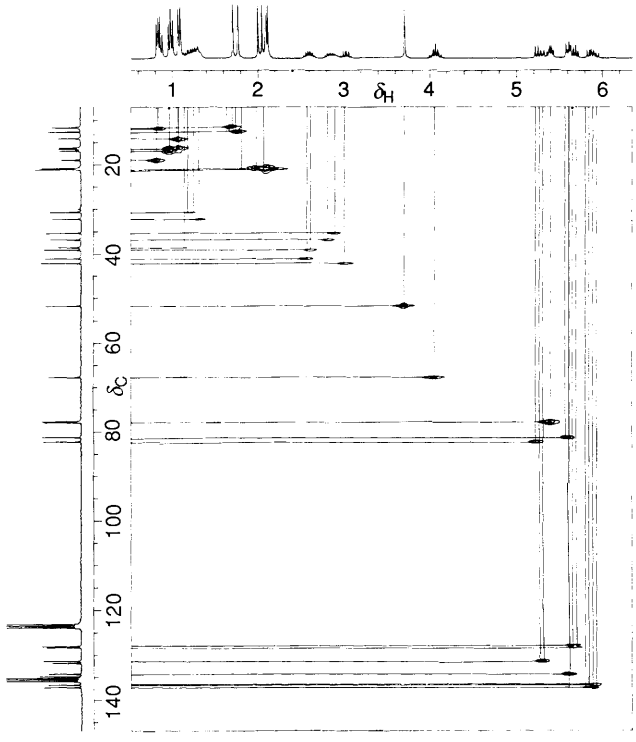


Fig. 5 ^{13}C–^{1}H COSY spectrum of methyl cubensate pentaacetate **4**

in $CDCl_3$ to be compared with those in C_5D_5N. A 2D-INADEQUATE spectrum of compound **4** in $CDCl_3$ (Fig. 4) enabled us to identify the five spin systems outlined in blue, green, yellow, orange and red: *i.e.* subunits F, G, H, J and K, respectively. However as has been reported by other workers,[6] connectivities were lost or became indistinguishable in the olefinic region.

The olefinic connectivities C(4)–C(5), C(8)–C(9), C(12)–C(13) and C(16)–C(17) in compound **4** were established by utilising data from the 2D-INADEQUATE, ^{1}H–^{1}H DQF-COSY, ^{13}C–^{1}H COSY [$^{1}J_{C-H}$] and COLOC* [$^{n}J_{C-H}$] spectra of compound **4**. The C(4)–C(5) linkage was identified by focussing attention on the terminal fragment (F) and the quaternary olefinic resonance at δ_C 131.25 (C-4), which the 2D-INADEQUATE spectrum shows is linked to a methyl carbon at δ_C 11.47 (C-24). ^{13}C–^{1}H COSY [$^{1}J_{C-H}$] (Fig. 5) correlated C-24 with 24-H_3 at δ 1.71, and ^{1}H–^{1}H COSY revealed allylic coupling (J 1.2 Hz) of the latter with a single-proton doublet at δ 5.61 (5-H). ^{13}C–^{1}H COSY [$^{1}J_{C-H}$] correlated this with C-5 at δ_C 134.23 and established the C(4)–C(5) linkage. In an analogous manner the C(16)–C(17) linkage was confirmed. The 2D-INADEQUATE spectrum indicated that the quaternary olefinic carbon at δ_C 134.29 (C-16) is joined to a methyl carbon at δ_C 12.48 (C-28). ^{13}C–^{1}H COSY [$^{1}J_{C-H}$] linked C-28 with 28-H_3 at δ 1.78, which in the ^{1}H–^{1}H COSY displayed allylic coupling (J 1.2 Hz) with the methine proton doublet at δ 5.3 (17-H) of fragment K. ^{13}C–^{1}H COSY [$^{1}J_{C-H}$] (Fig. 5) correlated C-17 at δ_C 131.4 with the single-proton doublet for 17-H at δ 5.3 and established the C(16)–C(17) linkage.

These conclusions were substantiated by a COLOC experiment[7,8] with Δ^1 88.00 ms and Δ^2 44.00 ms, equivalent to J 5 Hz (Fig. 6a), which clearly showed $^{3}J_{C-H}$ long-range correlations between the protons of the methyl groups on C-4 and C-16 with carbons C-5 and C-17, respectively, as well as $^{2}J_{C-H}$ correlations with C-4 and C-16, respectively, and further confirmed the C(4)–C(5) and C(16)–C(17) linkages.

It proved impossible to establish with any certainty the C(8)–C(9) and C(12)–C(13) connectivities by either ^{1}H–^{1}H DQF-COSY or spin–spin decoupling experiments due to the narrow chemical-shift range and multiplicity of the signals. Connectivities were established definitively using COLOC with Δ^1 44 ms and Δ^2 22 ms, equivalent to J 10 Hz (Fig. 6b). The spectrum shows $^{1}J_{C-H}$ and $^{2}J_{C-H}$ correlations between the various protons and carbons as indicated, permitting unambigous assignment of the C(8)–C(9) and C(12)–C(13) linkages and the correct orientation of fragment H. The COLOC technique proved most useful, and by the application of two different sets of values of Δ^1 and Δ^2, equivalent to J 5 and 10 Hz, it was possible to check many of the connectivities established by the 2D-INADEQUATE experiment.

^{1}H J-resolved NMR spectroscopy showed, in ester **3**, the presence of 4 doublet of doublet systems (J 15.0 and 7.0 Hz), centred at δ 5.7, 5.72, 5.8 and 5.84, indicating (*E*) stereochemistry about the C(8)–C(9) and C(12)–C(13) double bonds. NOE Difference spectroscopy established the stereochemistry of the C(4)–C(5) and C(16)–C(17) linkages in compound **4** (Fig. 7). Irradiation of the methyl signal at δ 1.71 (24-H_3) resulted in enhancements of the methine multiplets at δ 2.83 (6-H) and 3.03 (2-H), whilst irradiation of the methyl signal at δ 1.78 (28-H_3) resulted in enhancements of the methine multiplets at δ 2.60 (14-H) and 2.89 (18-H), respectively. Therefore the stereochemistry of the C(4)–C(5) and C(16)–C(17) linkages must be (*E*) in both instances (Fig. 8).

The ^{13}C NMR resonances for methyl cubensate pentaacetate **4** (Table 1) were assigned unambiguously using the 2D-INADEQUATE and COLOC data. The ^{1}H NMR assignments (Table 1) were established subsequently by using a combination of ^{13}C–^{1}H COSY, ^{1}H–^{1}H COSY and ^{1}H J-resolved experiments in conjunction with 1-D NMR data and spin-decoupling experiments.

Having established the structure of methyl cubensate pentaacetate **4** and hence cubensic acid **1**, the total assignments of the ^{1}H and ^{13}C NMR spectra of acid **1** and ester **3** (Table 1) were accomplished using ^{1}H–^{1}H DQF COSY, ^{1}H J-resolved, ^{13}C–^{1}H COSY [$^{1}J_{C-H}$] and ^{13}C–^{1}H FLOCK[9] [$^{n}J_{C-H}$, n = 2 or 3] experiments in conjunction with 1-D NMR data and spin-decoupling experiments.

With the exceptions of C-4 and C-16, it was possible to assign definitively the proton and carbon resonances of cubensic acid **1** by using the ^{1}H–^{1}H DQF COSY and ^{13}C–^{1}H COSY spectra

* ^{1}H–^{13}C heteronuclear correlation *via* long-range coupling.

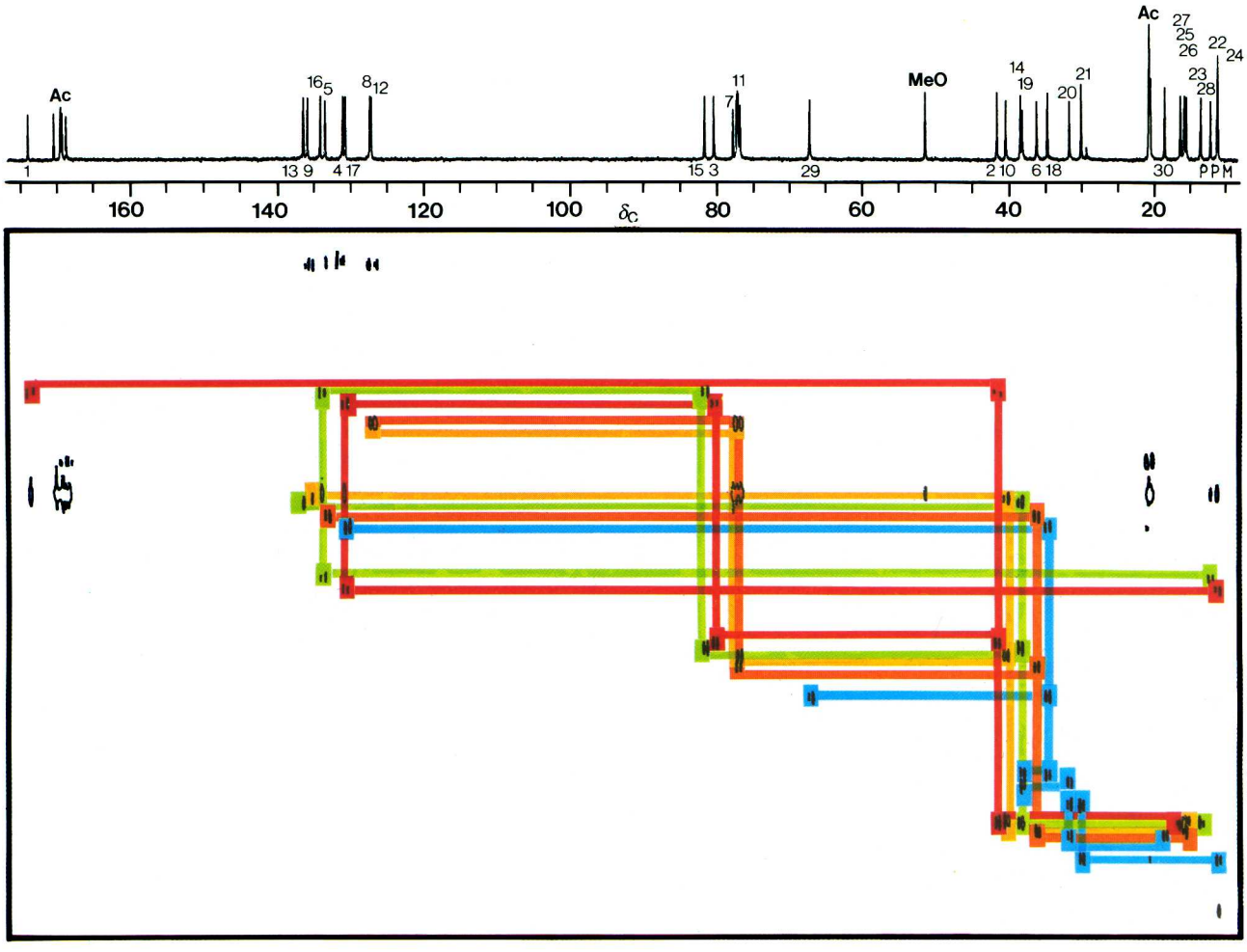


Fig. 4 2D-INADEQUATE ^{13}C NMR spectrum of methyl cubensate pentaacetate **4**

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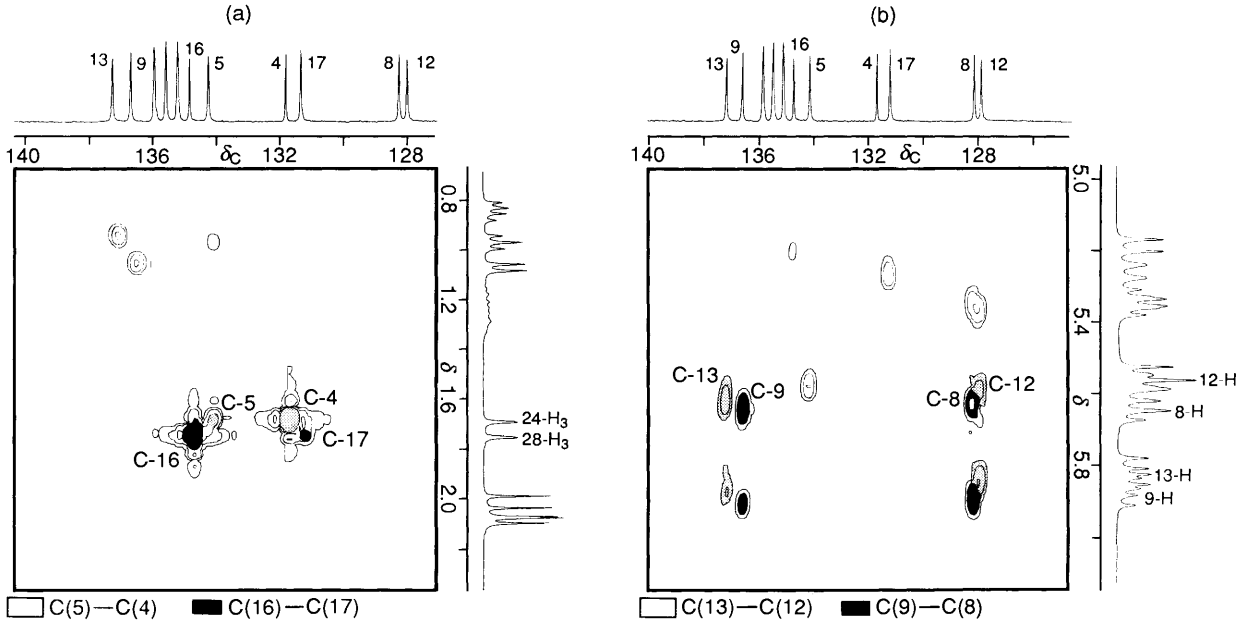


Figs. 6a and 6b $^{13}C-^{1}H$ COLOC spectra of methyl cubensate pentaacetate **4**

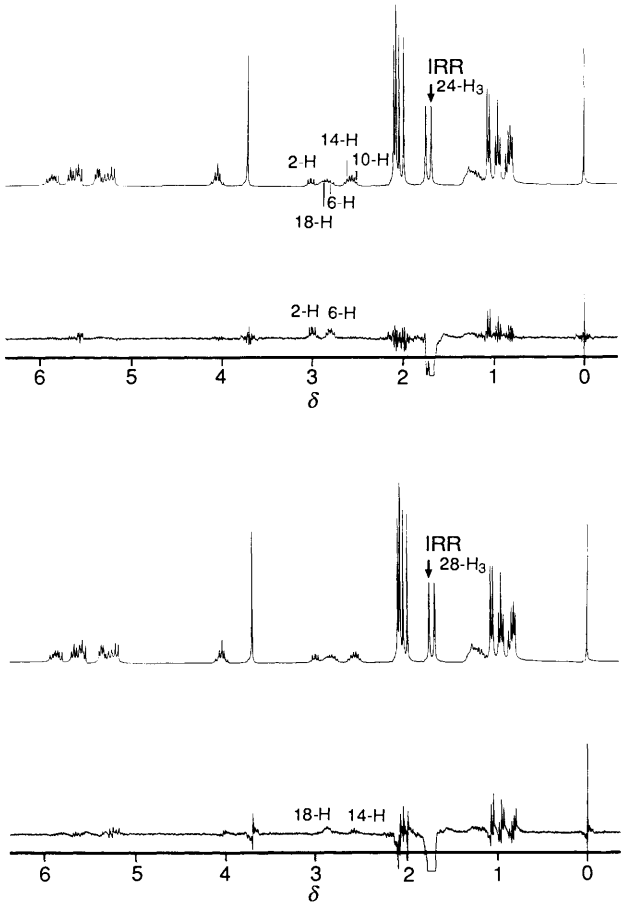


Fig. 7 NOE Difference spectra of methyl cubensate pentaacetate **4**

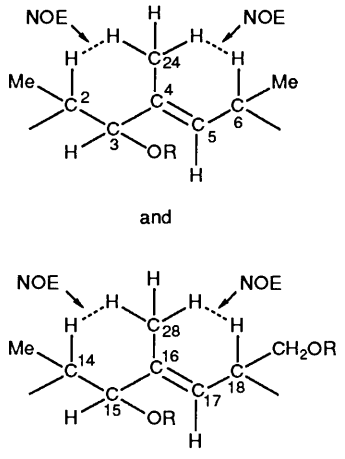
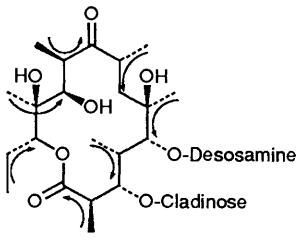


Fig. 8 NOE Interactions of 24-H_3 and 28-H_3

in association with 1-D ^{1}H and ^{13}C NMR data. Spin-decoupling was used as appropriate to establish J_{H-H} coupling constants. The ^{13}C NMR resonances of C-4 and C-16 were identified and those of C-5 and C-17 confirmed by using the FLOCK pulse sequence[9] with Δ^1 86.5 and Δ^2 46.5 ms, respectively; 28-H_3 at δ 1.90 showed correlations to C-16 and C-17 at δ 138.41 and 131.45, respectively, whilst 24-H_3 at δ 1.89 showed correlations to C-4 and C-5 at δ 136.16 and 132.92, respectively. Analogous studies confirmed the assignments for ester **3**. The FLOCK pulse sequence is reported to offer greater sensitivity than COLOC for the detection of $^{2}J_{C-H}$ and $^{3}J_{C-H}$ couplings as it effectively cancels modulations due to $^{1}J_{C-H}$ couplings that are normally present in COLOC spectra.

Cubensic acid is a 'free' fatty acid of unusually long chain length. Usually, fatty acids in plants, animals and fungi occur as esters in the form of fats and waxes and even then the occurrence of such compounds derived from acids of chain length greater than C_{20} is not common. In the case of fungi the occurrence of 'free' fatty acids of chain length greater than C_{18} is virtually unknown. Most of the examinations have been restricted to saponifiable material and their saponified products, thus the fat from the yeast *Saccharomyces cerevisiae* has yielded a mixture comprosing 1–2% of C_{20}–C_{30} fatty acids,[10,11] and more recently the fat from fifteen different basidiomycetes have given a wide range of fatty acids on saponification consisting of straight-chain C_{12}–C_{30} acids, a number of branched-chain C_{12}–C_{18} acids, and several unsaturated acids.[12] Unsaturated fatty acids from fungi occurring as fats have *Z* double bonds as is normal among plant fatty acids which derive from desaturation of the preformed



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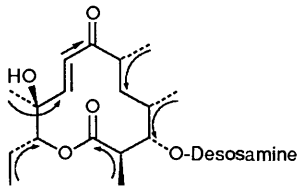

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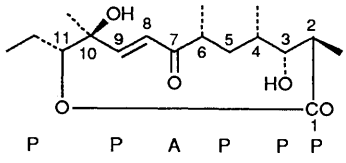

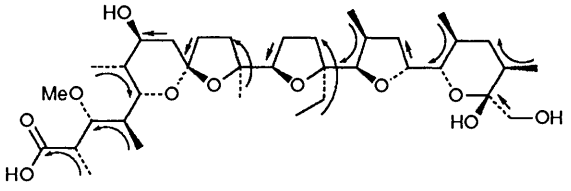

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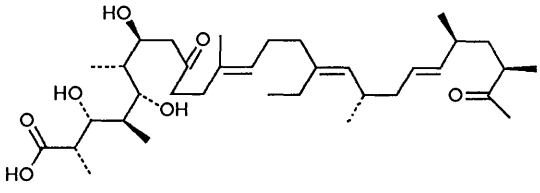

saturated compounds. This desaturation is carried a stage further in the formation of the 'free' polyacetylenic acids of both plant and fungal origin. The occurrence of only *E* double bonds in cubensic acid is unusual and implies their formation during the chain building rather than desaturation of the completed chain.

The alternating hydroxy–methyl substitution pattern in this acid is analogous to similar patterns found in the macrolide antibiotics as represented by erythromycin **5** and methymycin **6** and the polyether antibiotics as represented by monensin A **7**. The former are large branched-chain polyoxygenated lactones and the latter are large branched-chain polyoxygenated carboxylic acids. Both types of compounds are produced by a wide range of actinomycetes and are known to be derived by condensation of acetate, propionate and sometimes butyrate units.

Cubensic acid can be envisaged as being made up of a combination of eight propionate (P) and three acetate (A) units in the order APPPPAPAPPP. During the chain-building process the normal fatty acid synthetic sequence takes place in which each successive condensation with malonyl-CoA or methylmalonyl-CoA is followed by an enzymic sequence terminating with β-(keto acyl)-CoA reduction, dehydration or double-bond reduction. The exact sequence is dictated by the relevant chain-building enzyme. The dehydrations presumably occur during the chain building to yield double bonds at C-4, -8, -12, -16, -18 and -20; further reduction as in the formation of normal saturated acids occurs only at C-18 and C-20, *i.e.* relatively early in the chain-building process and reduction at C-18 is presumably preceded by a vinylic hydroxylation of the methyl group on C-18.

The macrolides and polyethers show a very variable building pattern in which common sequences can be identified among several of their members, and these have proved useful in establishing their possible common biogenetic origin. It is perhaps significant that the PAPPP sequence in the *final chain-building steps* of cubensic acid can be also identified in the twelve-membered macrocycle methymycin **6** and the shorter APPP sequence occurs in monensin A **7**; this points to a common origin and if so then the empirical stereochemical model developed by Celmer,[13,14] which has proved valuable in defining the stereochemistry of the macrolides,[15,16] may prove useful in defining the stereochemistry of cubensic acid and its yet hypothetic homologues.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected; IR spectra on a Perkin-Elmer 681 spectrophotometer; mass spectra (EI) on an AEI MS902 spectrometer and optical rotations on a Perkin-Elmer 141 polarimeter. Extracts were dried over Na_2SO_4.

^1H and ^{13}C NMR spectra (C_5D_5N solutions, unless specified otherwise, with tetramethylsilane as internal standard) were determined at 270 and 67.8 MHz, respectively, with a JEOL GX 270 spectrometer fitted with a dual 5 mm C/H probe. The 400 MHz ^1H NMR spectra were recorded on a JEOL GX 400 spectrometer. Standard, homodecoupled and NOE difference ^1H spectra were acquired with 32K data points over a spectrum width of 3001.2 Hz. Carbon-atom types were established in the ^{13}C NMR spectrum by employing a combination of broad-band proton-decoupled and distortionless enhancement by polarisation transfer (DEPT) experiments with 32K data points over a spectrum width of 17 605.6 Hz.

2-Dimensional spectra were acquired and processed using standard JEOL software; ^1H–^1H correlation by double quantum-filtered COSY (VDQFN), resolution 2.93 Hz in the *f*1 and *f*2 domains, PW1 = PW2 = π/2; ^1H–^1H *J*-resolved spectra (VJRESN), resolution *f*2 2.93 Hz and *f*1 0.1 Hz, pulse delay 1 s; [$^1J_{C-H}$] ^{13}C–^1H correlations (VCHSHF), resolution *f*2 17.19 Hz and *f*1 5.9 Hz, pulse delay 1, 2 or 3 s, J_{C-H} 140 Hz; [$^nJ_{C-H}$] ^{13}C–^1H correlations (VCOLOC), resolution *f*2 17.19 Hz and *f*1 5.9 Hz, pulse delay 1, 2 or 3 s, Δ^1 88.0 ms and Δ^2 44.0 ms or Δ^1 44.0 ms, Δ^2 22.0 ms; some [$^2J_{C-H}$ and $^3J_{C-H}$] ^{13}C–^1H correlations were established by using the FLOCK pulse sequence of Reynolds *et al*,[9] resolution *f*2 17.19 Hz and *f*1 5.9 Hz, pulse delay 1, 2 or 3 s, Δ^1 86.5 ms and Δ^2 46.5 ms; ^{13}C–^{13}C connectivities with 2D-INADEQUATE (V2DINA128), with compound **4** (400 mg) in $CDCl_3$ (0.6 cm^3), FREQ 12 787.7 Hz, CLFRQ 25 575.4 Hz, POINT 4096, CLPNT 128, SCANS 2816, PI3 4.55 ms, PD 1.0 s, *f*1 axis × 2 zero-filling. Exponential window function BF 4 Hz, T1 0, T2 10, T3 70, T4 100, CBF = CLRES, CT1 0, CT2 10, CT3 70, CT4 100, total acquisition time 6 days.

Isolation of Cubensic Acid **1**.—The fungus *Xylaria cubensis* was surface cultured for eight weeks at 23 °C in subdued daylight on 3% malt extract (20 dm^3) in Thompson bottles (2 dm^3 × 20), each containing 1 dm^3 of medium. The thick, brittle mycelium was filtered through muslin, air dried (162 g)

and extracted (Soxhlet, 16 h) with chloroform. Evaporation of the solvent yielded a thick orange-red gum (23.6 g), which was dissolved in hot ethanol (60 cm^3) and the mixture was set aside overnight to yield a waxy solid (17.2 g) after filtration and drying. A solution of this solid (1.0 g) in chloroform (25 cm^3) was extracted with aq. 1 mol dm^{-3} sodium carbonate (25 cm^3, ×3) and the combined extracts were acidified to yield a gelatinous precipitate, which was filtered off, washed with water, dried (0.43 g) and recrystallised from aq. ethanol (×2) to yield *cubensic acid* **1** (0.2 g) as aggregates of small hydrated needles, m.p. 196–198 °C (Found: C, 66.3; H, 9.9. $C_{30}H_{52}O_7 \cdot H_2O$ requires C, 66.4; H, 9.9%); *m/z* (FAB) $(M + Na)^+$ 547.3611; $[\alpha]_D^{23}$ + 62.5° (*c* 1.0 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3420 and 1722; ν_{max}($CHCl_3$)/cm^{-1} 3410, 3320sh and 1736.

Cubensic Acid Pentaacetate **2**.—A mixture of the acid **1** (38 mg), acetic anhydride (3 cm^3) and pyridine (3 drops) was set aside overnight and then poured into water. After 3 h at 5 °C the precipitate was filtered off and recrystallised from dil. acetic acid to yield the *pentaacetate* **2** as hydrated needles, m.p. 57–58 °C (Found: C, 64.1; H, 8.6. $C_{40}H_{62}O_{12} \cdot H_2O$ requires C, 63.7; H, 8.5%); $[\alpha]_D^{23}$ − 8° (*c* 1.0 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3460 and 1748; ν_{max}($CHCl_3$)/cm^{-1} 1734.

Methyl Cubensate **3**.—Crude cubensic acid **1** (5 g), isolated as above as the waxy solid by recrystallisation from ethanol (×1), was dissolved in diethyl ether (50 cm^3) and the mixture was treated with excess of an ethereal solution of diazomethane. Nitrogen was vigorously evolved and a solid was rapidly precipitated. The mixture was set aside overnight, then filtered and the solid was recrystallised from ethanol (×2) to yield needles (1.9 g) of *methyl cubensate* **3**, m.p. 208 °C (Found: C, 69.1; H, 10.3 $C_{31}H_{54}O_7$ requires C, 69.1; H, 10.1%); $[\alpha]_D^{22}$ + 75.3° (*c* 1.0 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3410, 3340sh, 1748 and 1719; ν_{max}($CHCl_3$)/cm^{-1} 3410, 3330sh and 1733.

Cubensic Acid by Hydrolysis of Methyl Ester.—The ester **3** (50 mg) was dissolved in a mixture of warm ethanol (5 cm^3) and aq. 1 mol dm^{-3} sodium hydroxide (2 cm^3). The mixture was set aside overnight, then water was added (13 cm^3) and the mixture was boiled at 100 °C to remove the alcohol. The cooled mixture was extracted with diethyl ether (×3) to remove any unchanged ester and the aq. solution was acidified at room temperature. The mixture, which if left produced a gel, was immediately extracted with diethyl ether, and the extract washed with water, dried and evaporated to yield a solid, which was recrystallised from alcohol to yield cubensic acid **1** (23 mg), identical in all respects with the sample obtained directly from the fungal extracts.

Methyl Cubensate Pentaacetate **4**.—A mixture of the methyl ester **3** (302 mg), acetic anhydride (4 cm^3) and pyridine (3 drops) was warmed at 100 °C to produce a clear solution. The mixture was set aside for three days at room temperature and was then poured into water. After 3 h at 5 °C the precipitate was filtered off and recrystallised from aq. acetic acid to yield needles of *methyl cubensate pentaacetate* **4**, m.p. 70–71 °C (Found: C, 65.7; H, 8.7. $C_{41}H_{64}O_{12}$ requires C, 65.7; H, 8.61%); $[\alpha]_D^{24}$ − 16.6° (*c* 1.0 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 1750.

Cytochalasin D from Xylaria cubensis.—The filtered culture medium (15 dm^3) was extracted in portions (3 dm^3) with ethyl acetate (3 × 1 dm^3). The combined extracts were dried and evaporated to yield a gummy yellow solid (3.3 g). Trituration with acetone (2 cm^3), filtration and recrystallisation of the residue from methanol gave cytochalasin D as needles (810 mg), m.p. 267–270 °C; *m/z* 507, and showing ^1H and ^{13}C NMR identical with an authentic sample isolated from *H. terricola*.[17]

Acknowledgements

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References

1 Part 25, R. L. Edwards, D. J. Maitland, J. P. Poyser and A. J. S. Whalley, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1939.
2 J. R. Anderson, R. L. Edwards and A. J. S. Whalley, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1481.
3 J. D. Rodgers, *Mycologia*, 1984, **76**, 912.
4 J. D. Rodgers, *Sydowia, Ann. Mycol. Ser.* II, 1985, **38**, 255.
5 JEOL Application Notes on Basic Two-Dimensional NMR Methods, Material for Users' Meetings, 1987, ch. 5, p. 58.
6 J. Buddrus and H. Bauer, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 625.
7 H. Kessler, C. Griesinger, J. Zarbock and H. R. Loosli, *J. Magn. Reson.*, 1984, **57**, 331.
8 H. Kessler, C. Griesinger and J. Lautz, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 444.
9 W. F. Reynolds, S. McLean and M. Perpick-Dumont, *Magn. Reson. Chem.*, 1989, **27**, 162.
10 J. W. Welch and L. Burlingame, *J. Bacteriol.*, 1973, **115**, 464.
11 J. Baraud, A. Maurice and C. Napias, *Bull. Soc. Chim. Biol.*, 1970, **52**, 421.
12 T. Rezanka and P. Mares, *J. Chromatogr.*, 1987, **409**, 390.
13 W. D. Celmer, *J. Am. Chem. Soc.*, 1965, **87**, 1799.
14 W. D. Celmer, *J. Am. Chem. Soc.*, 1965, **87**, 1801.
15 D. E. Cane, T. C. Liang, P. B. Taylor, C. Chang and C. C. Yang, *J. Am. Chem. Soc.*, 1986, **108**, 4957.
16 D. E. Cane, W. D. Celmer and J. W. Westley, *J. Am. Chem. Soc.*, 1983, **105**, 3594.
17 R. L. Edwards, D. J. Maitland and A. J. S. Whalley, *J. Chem. Soc., Perkin Trans. 1*, 1989, 57.

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