Metabolites of The Higher Fungi. Part 21. 3-Methyl-3,4-dihydroisocoumarins and Related Compounds from the Ascomycete Family Xylariaceae

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Culture solutions of members of the ascomycete family Xylariaceae produce a series of 5-substituted 8-hydroxy-3-methyl-3,4-dihydroisocoumarins as major metabolites. Ramulosin, iso-ochracein, and mellein are produced by three *Hypoxylon* species and 4-hydroxy iso-ochracein by one species. The antibiotic pyrenophorin is produced by *Nummularia broomeiana*. The synthesis of 7-formylmellein from mellein is described and the products from the alkaline rearrangement of 5-formylmellein are studied spectroscopically.

The xylariaceous fungi form one of the many families of the Ascomycetes; this family is an assemblage of genera with obscure but apparently ancient common ancestry. The family consists of a central core of genera, namely *Hypoxylon*, *Xylaria*, *Rosellinia*, *Poronia*, *Podosodaria*, *Hycocopra*, *Daldinia*, *Nummularia*, *Kretzschmaria*, *Camillea*, and *Penzigia*.[1] Within the very large genus *Hypoxylon*, further subclassifications based on morpholigical features have been proposed, and of these, that proposed by Miller [2] is generally accepted; this subdivides the genus into the sub-sections hypoxylon, papillata, annulata, applanata, and primo-cinerea.

Chemically the xylariaceous fungi have been little investigated. The fruiting bodies of *Daldinia concentrica* have been shown to contain the dihydroxyperylene quinone (1) which is derived from the chromagen (2);[3,4] the same fungus in culture produces the naphthalene derivatives (3) and (4), the dihydroxy ketones (5) and (6), the chromanone (7), and the chromone (8).[5] The colouring matter of the fruiting bodies of *H. fragiforme* consists of a mixture of related mitirubins (9)—(12) [6] and the same fungus is reported to produce iso-ochracein (13) in culture.[7] *Rosellinia necatrix* has been shown to produce rosellinic acid (15),[8] the diketopiperazines (16) and (17),[9] and cytochalasin E (18),[10] and we have recently shown that members of *Hypoxylon*, subsection primo-cinerea, *viz. H. serpens*, *H. serpens* (Barrons strain), and *H. chestersii* produce the butyrolactones (19), (20),[11] and (21),[12] and the allene (22),[13] respectively.

As part of a systematic survey of the metabolites of the xylariaceous fungi we now report the isolation of a series of dihydroisocoumarin derivatives, *viz.* mellein (23) and the 5-methyl- (24), 5-formyl- (25), 5-carboxy- (26), 5-methoxycarbonyl- (27), 5-hydroxymethyl- (28), and 6-methoxy-5-methyl- (29) analogues. Also produced are the metabolically related phthalides iso-ochracein (13) and 4-hydroxyiso-ochracein (14), the chromanone (7), and ramulosin (30). In addition to some of these compounds, the fungus *Nummularia broomeiana* also produces the antibiotic pyrenophorin (31). The species producing these compounds are set out in the Table.

We have confirmed that *H. fragiforme*, the type species of the sub-section hypoxylon (previously described as *H. coccineum*,[7] does produce iso-ochracein (13). However, the quantity varies with the strain. In all the strains examined, mellein (23) is also produced, and in one strain of American origin, mellein replaced iso-ochracein as the major metabolite.

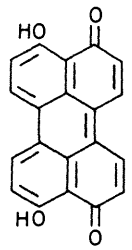
The closely related, smaller spored species *H. howeianum* can be distinguished from *H. fragiforme* by the production of the two additional metabolites ramulosin (30), and 4-hydroxyiso-ochracein (14). Ramulosin appears between mellein and iso-ochracein on silica-gel t.l.c. plates in the solvent system used, and is readily identified by the yellow colour produced with a diazotised *p*-nitroaniline spray; with this reagent both mellein and iso-ochracein produce an orange colouration, and 4-hydroxyiso-ochracein is detectable as a colourless, opaque spot. Ramulosin has previously been identified as a metabolite of the wood-pulp-inhabiting fungus *Pestalotia ramulosa*.[14,15] Two other species of the Miller sub-classification hypoxylon, *H. haematostroma* and *H. venustuissimum*, also produce mellein, iso-ochracein, and ramulosin but not 4-hydroxyiso-ochracein.

4-Hydroxyiso-ochracein (14), $C_{10}H_{10}O_4$, m.p. 218 °C, yields a purple Fe^{3+} colouration, and the presence of two hydroxy groups was established by the formation of a diacetate and a dimethyl ether. A five-membered lactone ring was shown to be present by the change in the i.r. $\nu_{max.}$ from 1 712 cm^{-1} in the parent to 1 765 and 1 768 cm^{-1} in the acetate and methyl ether, respectively. The *para* location of the second hydroxy group with respect to the first was shown by the compound's failure to produce a methylenedioxy derivative and by the ^1H n.m.r. spectrum which showed splitting of the methylene protons of the ethyl group into two separate multiplet signals at δ 1.82 and 2.24 due to the proximity of the hydroxy group. The multiplet signals arise from the two non-equivalent mutually coupled prochiral methylene protons which are each coupled to the methyl protons and unequally coupled to the chiral methyne proton. The same two methylene protons of iso-ochracein are almost superimposed and produce a broad multiplet at δ 1.62—2.38. It is also significant that the i.r. lactone absorption of iso-ochracein (1 735 cm^{-1}) is much higher than that of the 4-hydroxy analogue (1 712 cm^{-1}).

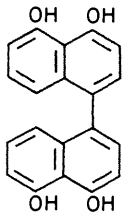
No other species of *Hypoxylon* examined to date produce the above metabolites but instead produce varying proportions of 5-methylmellein (24) and/or other 5-substituted analogues.

In order to establish a possible chemotaxonomic relationship between the genus *Hypoxylon* and other xylariaceous fungi, the metabolites of several members of the relatively small genus *Nummularia* were examined. *N. discreta* (Schweinitz) Tulasne is the causative agent of nailhead blister on apple and examination of the malt-culture medium of this fungus indicated the presence of two major metabolites. The minor component of the mixture proved to be 5-methylmellein (24) and the major component 5-formylmellein (25).

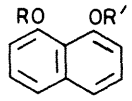
5-Formylmellein (25), $C_{11}H_{10}O_4$, m.p. 127 °C; $\nu_{max.}$ 1 672—1 690 cm^{-1} (br), gives a weak orange colouration on t.l.c. plates with diazotised *p*-nitroaniline, forms a 2,4-dinitrophenylhydrazone, and gives a red-violet Fe^{3+} colouration. Attempted methylation with dimethyl sulphate gave a mixture



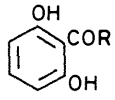
(1)



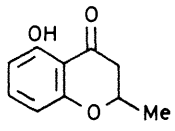
(2)



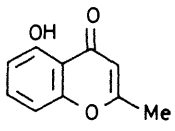
(3) R = H, R′ = Me
(4) R = Me, R′ = Me



(5) R = Pr
(6) R = Me



(7)



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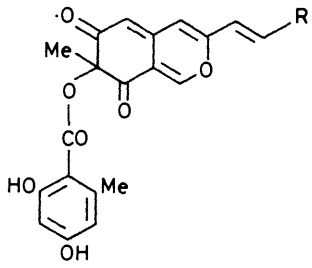
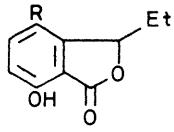
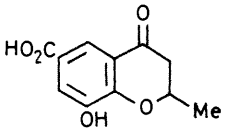


(9) R = Me
(10) R = CH_2OH
(11) R = CH_2OCOMe
(12) R = CO_2H



(13) R = H
(14) R = OH



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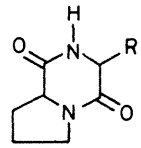
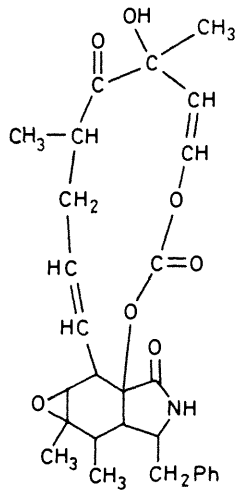


(16) R = $(CH_3)_2CH$ (prolyl valyl anhydride)
(17) R = CH_2Ph (prolyl phenylalanyl anhydride)



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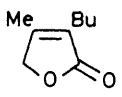

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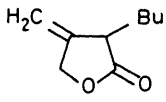

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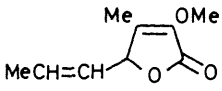
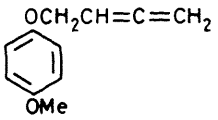


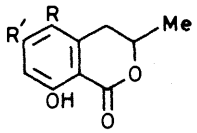
(21)

of products but a monomethyl ether, $\nu_{max.}$ 1 695 and 1 723 cm^{-1}, was obtained by reaction with iodomethane and silver oxide in dimethylformamide. The ^1H n.m.r. ($CDCl_3$) spectrum of (25) with absorptions at δ 1.59 (d, *J* 7 Hz), 3.03 (1 H, dd, *J* 11.5 and 18.5 Hz), 3.96 (1 H, dd, *J* 3.5 and 18.5 Hz), 4.76 (1 H, m), 7.11 (1 H, d, *J* 8 Hz), 7.99 (1 H, d, *J* 8 Hz), 10.12 (1 H, s), and 12.08 (1 H, s) showed the compound to be a formyl-substituted mellein, and the presence of two distinct single-proton methylene signals arising from two prochiral non-equivalent mutually coupled protons coupled unequally to the chiral methyne proton and forming part of an AMX system strongly suggested a 5-formyl derivative. This was confirmed by the formation of the hemiacetal (32) by dissolution in alkali and precipitation with acid. This type of ring closure is typical of δ-hydroxy aldehydes but does not appear to have been reported for aromatic systems bearing *ortho*-substituted formyl and 2-hydroxypropyl substituents. The structure is confirmed by the loss of the aldehyde signal at (δ 10.12) in the ^1H n.m.r. spectrum of (25).

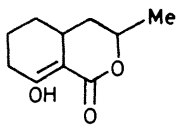
The ^1H n.m.r. spectrum of the acetic acid-recrystallised material showed it to be a mixture of two anomers; one with well defined absorptions at δ 6.77 (1 H, d, *J* 7 Hz), 7.18 (1 H, d, *J* 7 Hz), 5.71 (1 H, s), 4.18 (1 H, m), 2.74 (2 H, m), and 1.24 (3 H, d, *J* 7 Hz), and for the other at δ 6.81 (1 H, d, *J* 7 Hz), 7.07 (1 H, d, *J* 7 Hz), 5.89 (1 H, s), 4.18 (1 H, m), 2.74 (2 H, m), and 1.38 (2 H, d, *J* 7 Hz). In dimethyl sulphoxide the intensities of the anomeric proton signals at δ 5.71 and 5.89 indicated a *ca.* 2 : 3 component ratio. Addition of 1 drop of D_2O to the mixture caused a marked simplification of the spectrum (Figure). The change was slow and could be followed to completion during 45 min. During this time the anomeric proton absorption at δ 5.71 increased in intensity with a corresponding fall in the intensity of the absorption at δ 5.89. After 45 min the spectrum was that of the single isomer showing absorption of the anomeric proton at δ 5.71; the aromatic region was also simplified to a double doublet and the methyl signal a simple doublet at δ 1.24. The reduction in the intensity of the most deshielded anomeric proton and the most deshielded methyl protons indicates an H equatorial, CH_3 axial arrangement for the major component of the mixture. The



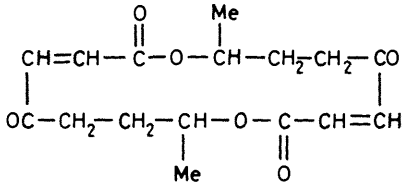
(22)



(23) R = H, R′ = H
(24) R = Me, R′ = H
(25) R = CHO, R′ = H
(26) R = CO_2H, R′ = H
(27) R = CO_2Me, R′ = H
(28) R = CH_2OH, R′ = H
(29) R = Me, R′ = OMe



(30)



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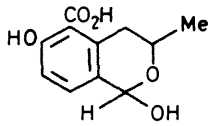


(32)

most stable structure in the presence of D_2O is that with an H axial and CH_3 equatorial arrangement. The methyl acetal, which was readily prepared by refluxing the hemiacetal with methanol for a short time, showed a similar isomerisation with D_2O but in this case the epimerisation was complete within 4 min and the most stable anomer in the absence of D_2O is that in which the methoxy group is equatorial. The methylated compound is again a mixture and shows two anomeric proton signals, one of low intensity at δ 5.45 and the other of high intensity at δ 5.39; the corresponding methoxy signals occur at δ 3.4 (high intensity) and δ 3.21 (low intensity). Addition of one drop of D_2O causes a reversal of the intensities within 4 min. Significantly, in the methyl acetal the aromatic doublets of each isomer absorb in the same position and there is not the multiplicity of signals in the mixture as is observed in the hemiacetal. Similarly, the methyl signals of each anomer absorb at the same position and there is no apparent change in these regions of the spectrum during the isomerisation.

The direct introduction of substituents into the aromatic ring of 3,4-dihydroisocoumarins has been little investigated; most substituted compounds are usually prepared by the cyclisation of appropriately substituted aromatic compounds. One exception is found in the synthesis of ochratoxin where a chloromethyl group has been introduced at C-7 of 5-chloro-8-hydroxy-3-methyl-3,4-dihydroisocoumarin by the use of chloromethyl methyl ether and $TiCl_4$.[16] The availability of mellein during this work prompted us to investigate the possible synthesis of 5-formylmellein from this compound.

Chloromethylation of mellein using molar quantities of chloromethyl methyl ether and $TiCl_4$ led to low yields of the 5,7-bis(chloromethyl) compound (33) with the bulk of the mellein remaining unchanged. By the use of a considerable excess of chloromethyl methyl ether, the yield of the disubstituted compound approached 70%. There was no evidence for the formation of any mono-substituted 5- or 7-derivative. Attempts to formylate mellein directly by the Vilsmeier synthesis failed. Both dichloromethoxymethane and methyl orthoformate in the presence of $TiCl_4$ or $AlCl_3$, respectively, are active formylating agents for phenols [17] and their use suggested a possible direct route to the desired aldehyde. There was no reaction of mellein with methyl orthoformate in the presence of $AlCl_3$. However, with an excess of dichloromethoxymethane and $TiCl_4$ a 30% yield of 7-formylmellein (34) was produced. This was readily identified from its ^1H n.m.r. spectrum which, unlike that of the 5-formyl isomer (25), shows two identical 4-methylene protons split into a simple doublet as seen in mellein.

Two other species of the genus *Nummularia*, *viz.* *N. broomeiana* and *Biscogniauxia simplicior*,[18] also produce 5-formylmellein in small quantities. *N. broomeiana* also produces a second metabolite, $C_{16}H_{20}O_6$, m.p. 176 °C; M^+ 308, which gives a positive 2,4-dinitrophenylhydrazine reaction. The ^1H n.m.r. spectrum showed the apparent presence of only ten protons with absorptions at δ 1.30 (3 H, d, *J* 8 Hz), 2.08 (2 H, q, *J* 8 Hz), 2.62 (2 H, q, *J* 8 Hz), 5.00 (1 H, q, *J* 8 Hz), 6.96 (1 H, d, *J* 16 Hz), and 6.48 (1 H, d, *J* 16 Hz) and indicated a dimeric molecule composed of two identical halves and consistent with the published data for the antibiotic macrolide pyrenophorin (31).[19–22] This was confirmed by the ^{13}C spectrum with absorptions at δ_C 19.6 (q, CH_3), 32.1 (t, CH_2), 37.2 (t, CH_2), 72.2 (d, OCH), 131.4 (t, CH), 139.7 (t, CH), 165 (s, C=O), and 199.7 p.p.m. (s, C=O). The olefinic triplets presumably arise from long-range coupling across two bonds. The structure is further confirmed by the formation of a bis-2,4-dinitrophenylhydrazone (M^+ 668) which shows an ester carbonyl absorption band in the i.r. at 1 725 cm^{-1}.

The splitting of the 4-methylene protons observed in the ^1H n.m.r. spectrum of 5-formylmellein is also seen in 5-methoxycarbonylmellein (27), isolated from culture solutions of *H. mammatum*; this fungus is responsible for canker disease of quaking aspen in the U.S.A. and some regions of Europe. 5-Methoxycarbonylmellein (27), $C_{12}H_{12}O_5$, m.p. 65 °C; $\nu_{max.}$ 1 715 and 1 675 cm^{-1} is the major metabolite of this fungus and replaces the 5-methylmellein produced by most of the other species of the genus *Hypoxylon*. The carbonyl band at 1 675 cm^{-1} shifts to 1 720 cm^{-1} in both the monoacetate and monomethyl ether and the broad chelated hydroxyl absorption at 3 300 cm^{-1} in the parent is absent in these derivatives. The ^1H n.m.r. spectrum showed a three-proton singlet at δ 3.96 and proof that this was due to an ester function situated at C-5 was obtained by alkaline hydrolysis which produced a mixture of two acids (26) and (35) resulting from ester hydrolysis, ring opening, and relactonisation between the alcohol and the two different *ortho* carboxy groups. The two isomers were readily separated by chromatography and the faster running 5-carboxy isomer yields a less pronounced Fe^{3+} colouration. The same two isomers are also produced by silver oxide oxidation of 5-formylmellein in alkaline solution. The unambiguous assignment of structure to these two compounds by spectroscopy was not possible since both showed similar i.r. carbonyl bands at 1 665 and 1 695 cm^{-1} and also similar ^1H n.m.r. spectra. A structural assignment was obtained by methylation with diazomethane during 20 min when compound (35) gave a quantitative yield of the methoxymethyl ester and compound (26) formed only the methyl ester (27). The formation of the former product is surprising since salicylic acid rapidly gives only the methyl ester with diazomethane. In the i.r. spectrum the dimethylated product shows carbonyl absorptions at 1 722 and 1 715 cm^{-1} and, for the monomethylated product, signals at 1 715 and 1 675 cm^{-1}. The carboxylic acid (26) is also produced in very

Table. Isolation of metabolites from *Hypoxylon* and *Numularia* species [a]

Species	Sub-section	Mellein (23)	Iso-ochracein (13)	Ramulosin (30)	4-Hydroxyiso-ochracein (14)	5-Methylmellein (24)	5-Formylmellein (25)	5-Methoxycarbonylmellein (27)	6-Methoxy-5-methylmellein (29)	5-Hydroxymethylmellein (28)	5-Carboxymellein (26)	2-Methyl-5-hydroxychroman-4-one (7)
Hypoxylon fragiforme	hypoxylon	×	×									× [b]
H. howeianum	hypoxylon	×	×	×	×							
H. haematostroma	hypoxylon	×	×	×								
H. venustuissimum	hypoxylon	×	×	×								
H. jecorinum	hypoxylon					×						
H. rubiginosum	hypoxylon					×						
H. agrillaceum	hypoxylon					×						
H. cohaerens	papillata											
H. multiforme	papillata											
H. investiens	papillata											
H. rutilum	papillata					×						
H. confluens	primo-cinerea					×						
H. udum	primo-cinerea											
H. mammatum	primo-cinerea					×		×			×	× [b]
H. illitum	primo-cinerea					×				×	×	
H. deustum	primo-cinerea	×										
H. chestersii	primo-cinerea					×						
H. serpens	primo-cinerea					×						
H. truncatum	annulata					×						
H. stigium	annulata					×						
H. grenadense	applanata					×						
H. atropunctatum	applanata					×			×			
H. mediterraneum	applanata					×						
H. tinctor	applanata					×						
H. punctulatum	applanata											
H. microplacum	applanata					×						
Numularia discreta						×	×				×	
N. broomiana						×	×					
N. simplicior												
N. dennisii						×						

[a] All compounds were crystallised and identified by m.p., mixed m.p., and i.r. and ^{1}H n.m.r. spectroscopy. [b] From one strain only.

low yield by *H. mammatum*, and also by *H. illitum* and *N. discreta*.

The major isocoumarin produced by *H. illitum* is the alcohol (28), $C_{11}H_{12}O_4$, $\nu_{max.}$ 1 670 cm^{-1}; m.p. 164 °C, which is readily identified from its ^{1}H n.m.r. spectrum in which the $ArCH_2$-$CH(CH_3)O$ system produces a twelve-line ABX system. The AB signal at δ 3.2 is further downfield than the corresponding signal in 5-methylmellein (δ 2.8), indicative of the deshielding effect of the hydroxymethyl group at C-5.

The only disubstituted mellein derivative (29) encountered during this work was obtained from a freshly isolated strain. of *H. atropunctatum* grown on potato dextrose–yeast medium. Initial culturing of the fungus produced compound (29) as the only metabolite but repeated subculturing resulted in a change of metabolism and the production of inseparable mixtures of this metabolite and 5-methylmellein. 6-Methoxy-5-methylmellein (29), $C_{12}H_{14}O_4$, M^+ 222; m.p. 121 °C; $\nu_{max.}$ 1 668 cm^{-1}, gives the typical violet Fe^{3+} colouration shown by the compounds described above and a shift of the lactone carbonyl i.r. absorption to 1 722 cm^{-1} on acetylation. The ^{1}H n.m.r. spectrum is similar to that of methylmellein but shows only one aromatic proton and an additional methoxy group at δ 3.93. During the course of this work, de Alvarenga [23] reported the isolation of this compound and several of the isocoumarins described in this paper from wood samples. The compounds were believed to be of fungal origin and produced by unknown fungi contaminating the wood during storage. In view of our findings it is not unlikely that these compounds originated from xylariaceous fungi and most probably from those of the genus *Hypoxylon* which are common inhabitants of tropical regions.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer 681 spectrophotometer, u.v. spectra on a Unicam SP 800 spectrophotometer, ^{1}H n.m.r. spectra on a JEOL JNM-MH-100 spectrometer (with tetramethylsilane as internal standard), mass spectra on an AEI MS902 spectrometer and optical rotations on a Perkin-Elmer

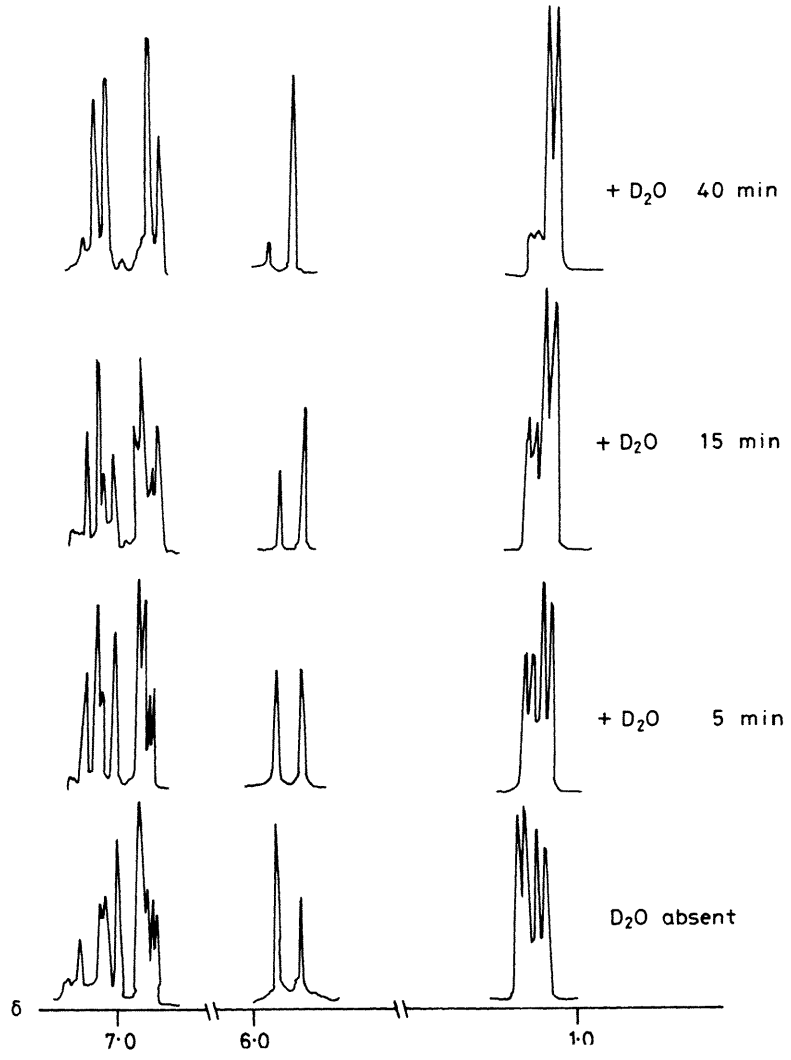
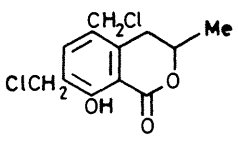
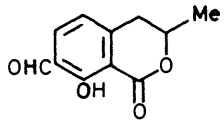


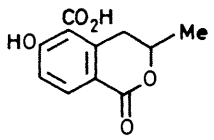
Figure. ^{1}H N.m.r. spectrum of the isochroman (32) in $(CD_3)_2SO$ with added D_2O



(33)



(34)



(35)

141 polarimeter. All t.l.c., preparative layer (p.l.c.), and column chromatography was done on Merck Kieselgel PF 256 + 366; p.l.c. was performed on silica gel (16 g) on 20 × 20 cm glass plates. Extracts were dried over Na_2SO_4.

Isolation of Mellein (23), *Iso-ochracein* (13), 4-*Hydroxyiso-ochracein* (14), *and Ramulosin* (30) *from* Hypoxylon howieanum.—*Hypoxylon howieanum* [Strain 525, or 11 (I) 38] was cultured for eight weeks on a malt solution (4% Boots). A dark green mycelium and solution were produced. The filtered medium (44 l) was extracted continuously with diethyl ether for 50 h and the dried extract was evaporated. The dark brown residual gum (14.2 g) was triturated with chloroform; filtration gave a crystalline solid (870 mg) which was washed with hot light petroleum (b.p. 60—80 °C) and recrystallised from ethyl acetate to yield lustrous plates of 4-*hydroxyiso-ochracein* (14) (270 mg), $[\alpha]_D^{22}$ −94° (*c* 0.7 in C_2H_5OH), m.p. 218—219 °C (Found: C, 61.5; H, 5.0. $C_{10}H_{10}O_4$ requires C, 61.8; H, 5.2%); *m/z* 194 (M^+); $\nu_{max.}$ (KBr) 1 712 cm^{-1}; $\lambda_{max.}$ (EtOH) 237 and 327 nm (log ε 3.78 and 3.62); δ_H ([$^2H_6$]-acetone) 8.46 (1 H, s), 7.82 (1 H, s), 7.02 (1 H, d, *J* 8 Hz), 6.72 (1 H, d, *J* 8 Hz), 5.46 (1 H, dd, *J* 3.5 and 7 Hz), 2.24 (1 H, m), 1.8 (1 H, m), and 0.9 (3 H, t, *J* 9 Hz).

The chloroform solution from the above separation was evaporated and the resulting gum was dissolved in benzene–ethyl formate–formic acid (75 : 25 : 1) (20 ml) and applied to a column of silica gel (33 × 2.5 cm). Elution of the column with the same solvent system gave, after evaporation, three major fractions. (i) An oil which crystallised overnight and which was recrystallised from light petroleum (b.p. 80—100 °C) to yield plates of mellein (23) (570 mg), m.p. 58 °C; $[\alpha]_D^{22}$ −100° (*c* 1 in $CHCl_3$) (Found: C, 67.3; H, 5.7. Calc. for $C_{10}H_{10}O_3$: C,

67.4; H, 5.7%); *m/z* 178 (M^+); $\nu_{max.}$ ($CHCl_3$) 1 675 cm^{-1}; $\lambda_{max.}$ (EtOH) 246 and 314 nm (log ε 3.81 and 3.60); δ_H ($CDCl_3$) 11.1 (1 H, s), 7.6 (1 H, t, *J* 8 Hz), 7.04 (1 H, d, *J* 8 Hz), 6.84 (1 H, d, *J* Hz), 4.82 (1 H, m), 2.96 (2 H, d), and 1.55 (3 H, d); (ii) a solid which was recrystallised from light petroleum (b.p. 80—100 °C) to yield needles of ramulosin (30) (30 mg), m.p. 120 °C (lit.,[14,15] 120 °C) (Found: C, 65.6; H, 7.6. Calc. for $C_{10}H_{14}O_3$: C, 65.9; H, 7.7%); *m/z* 182 (M^+); $\nu_{max.}$ ($CHCl_3$) 1 650 cm^{-1}; $\lambda_{max.}$ (EtOH) 265 nm (log ε 4.01); δ_H ($CDCl_3$) 13.1 (1 H, s), 4.3 (1 H, m), 2.4 (4 H, m), 1.9 (4 H, m), and 1.4 (3 H, d); and (iii) an oil which slowly crystallised and which was recrystallised from light petroleum (b.p. 80—100 °C) to yield *iso-ochracein* (13) (3 g), m.p. 79 °C; $[\alpha]_D^{20}$ −63.5° (*c* 1 in $CHCl_3$) (Found: C, 67.3; H, 5.7. $C_{10}H_{10}O_3$ requires C, 67.4; H, 5.6%); *m/z* 178 (M^+); $\nu_{max.}$ ($CHCl_3$) 1 735 cm^{-1}; $\lambda_{max.}$ (EtOH) 214, 234, and 300 nm (log ε 4.26, 3.85, and 3.66); δ_H ($CDCl_3$) 7.5 (1 H, t, *J* 9 Hz), 6.88 (2 H, d, *J* 8 Hz), 5.4 (1 H, t), 1.85 (2 H, m), and 0.98 (3 H, t).

One strain of *H. howeianum*, (11.I.45), gave an additional metabolite which was incompletely separated from mellein in the above solvent system. Separation using the solvent system toluene–ethyl acetate–formic acid (90 : 10 : 1) gave a first fraction which, on evaporation, yielded a yellow oil which crystallised slowly. Recrystallisation from light petroleum (b.p. 60—80 °C) gave plates of 2-methyl-5-hydroxychroman-4-one (7) (20 mg), m.p. 30 °C (lit.,[5] 30—33 °C) (Found: C, 65.6; H, 7.6. Calc. for $C_{10}H_{10}O_3$: C, 65.9; H, 7.7%); $\lambda_{max.}$ (EtOH) 270 and 347 nm (log ε 3.97 and 3.49); $\nu_{max.}$ ($CHCl_3$) 1 645 cm^{-1}; δ_H ($CDCl_3$) 7.41 (1 H, t, *J* 8 Hz), 6.50 (2 H, dd, *J* 8 Hz), 4.62 (1 H, m), 2.7 (2 H, d, *J* 6 Hz), and 1.48 (3 H, d, *J* 6 Hz). A red colouration was produced by (7) on SiO_2 with diazotised *p*-nitroaniline spray.

4,7-*Diacetoxy*-3-*ethylphthalide*.—A mixture of 4-hydroxy-iso-ochracein (14) (60 mg), acetic anhydride (3 ml), and pyridine (3 drops) was heated on a water-bath for 30 min. The cooled mixture was poured onto ice and the resulting aqueous solution was extracted with diethyl ether. Evaporation of the dried extract gave a solid which was crystallised from light petroleum (b.p. 60—80 °C) as needles of 4,7-*diacetoxy*-3-*ethylphthalide* (56 mg), m.p. 85 °C (Found: C, 60.3; H, 5.0. $C_{14}H_{14}O_6$ requires C, 60.4; H, 5.1%); $\nu_{max.}$ ($CHCl_3$) 1 791, 1 777, and 1 765 cm^{-1}; δ_H ($CDCl_3$) 2.35 (3 H, s) and 2.28 (3 H, s).

Similarly, iso-ochracein (13) (100 mg) gave an oil which was dissolved in light petroleum (b.p. 60—80 °C). The solution was cooled at 5 °C for 7 d to yield needles of 4-O-*acetyliso-ochracein* (68 mg), m.p. 55 °C (Found: C, 65.4; H, 5.5. $C_{12}H_{12}O_4$ requires C, 65.4; H, 5.5%); $\nu_{max.}$ (KBr) 1 771 and 1 759 cm^{-1}; δ_H ($CDCl_3$) 2.34 (3 H, s).

3-*Ethyl*-4,7-*dimethoxyphthalide*.—A mixture of 4-hydroxy-iso-ochracein (14) (60 mg), dimethyl sulphate (0.5 ml), potassium carbonate (2 g), and dry acetone (10 ml) was refluxed for 16 h. The mixture was cooled and filtered and the filtrate was evaporated. Recrystallisation of the residue from light petroleum (b.p. 80—100 °C) gave needles of 3-*ethyl*-4,7-*dimethoxyphthalide*, m.p. 156 °C (Found: C, 64.8; H, 6.4. $C_{12}H_{14}O_4$ requires C, 64.85; H, 6.35%); $\nu_{max.}$ ($CHCl_3$) ($CHCl_3$) 1 768 cm^{-1}; δ_H ($CDCl_3$) 7.0 (1 H, d, *J* 8 Hz), 6.79 (1 H, d, *J* 8 Hz), 5.26 (1 H, dd, *J* 7 and 3.5 Hz), 3.9 (3 H, s), 3.82 (3 H, s), 2.16 (1 H, m, *J* 7, 7, and 3.5 Hz), 1.74 (1 H, m, *J* 7 and 7 Hz), and 0.9 (3 H, t, *J* 7 Hz).

5-*Formylmellein* (25) *and* 5-*Methylmellein* (24) *from* Nummularia discreta.—The fungus (Strain 495) was grown for 8 weeks on malt extract medium (4% Boots). The sparse colourless mycelium was filtered off and the dark brown medium (8 l) was extracted with diethyl ether (3 l). Evaporation of the dried extract gave a brown solid residue (1.7 g) which was dissolved in light petroleum (b.p. 60—80 °C)–acetone (9 : 1) and the solution was applied to a column of silica gel (30 × 2.5 cm). Elution of the column with the same solvent system gave a first fraction which, after evaporation of the solvent, yielded an oil which crystallised overnight and which was recrystallised from light petroleum (b.p. 80—100 °C) to yield 5-methylmellein (24) (72 mg), m.p. 131—133 °C (lit.,[24] 126—127 °C); $[\alpha]_D^{20}$ −115° (*c* 1 in $CHCl_3$) (Found: C, 68.8; H, 6.1. Calc. for $C_{11}H_{12}O_3$: C, 68.8; H, 6.2%); *m/z* 192 (M^+); $\nu_{max.}$ ($CHCl_3$) 1 680 cm^{-1}; $\lambda_{max.}$ (EtOH) 247 and 323 nm (log ε 3.64 and 3.43); δ_H ($CDCl_3$) 11.14 (1 H, s), 7.4 (1 H, d, *J* 8.4 Hz), 6.9 (1 H, d, *J* 8.4 Hz), 4.65 (1 H, m), 2.8 (2 H, m), 2.2 (3 H, s), and 1.4 (3 H, d).

The column, on further elution with the solvent system benzene–ethyl formate–formic acid (75 : 25 : 1), yielded a second fraction which, on evaporation of the solvent, gave a solid which was recrystallised from ethanol as needles of 5-formylmellein (25) (780 mg), m.p. 127 °C, $[\alpha]_D^{20}$ −180° (*c* 1 in $CHCl_3$) (Found: C, 64.0; H, 4.6. Calc. for $C_{11}H_{10}O_4$: C, 64.1; H, 4.8%); *m/z* 206 (M^+); $\nu_{max.}$ ($CHCl_3$) 1 695 and 1 680 cm^{-1}; $\lambda_{max.}$ (EtOH) 276 and 314 (log ε 4.15 and 3.58). 2,4-*Dinitrophenylhydrazone*, orange needles from acetic acid, m.p. >300 °C (Found: N, 15.0. $C_{17}H_{14}N_4O_7$ requires N, 14.5%).

5-*Formylmellein Methyl Ether*.—A mixture of 5-formylmellein (25) (206 mg), DMF (10 ml), iodomethane (426 mg), and silver oxide (462 mg) was stirred for 24 h at room temperature. The mixture was filtered and the filtrate was evaporated. The residue was digested for 2—3 min with hot ethanol and the mixture was then filtered. Evaporation of the filtrate gave a solid which was recrystallised from ethyl acetate–light petroleum (b.p. 80—100 °C) as needles (82 mg) of 5-*formyl*-8-*methoxymellein*, m.p. 133—135 °C (Found: C, 65.3; H, 5.6. $C_{12}H_{12}O_4$ requires C, 65.4; H, 5.5%); $\nu_{max.}$ 1 695 and 1 723 cm^{-1}; δ_H ($CDCl_3$) 1.54 (3 H, d, *J* 7 Hz), 2.91 (1 H, dd, *J* 11.5 Hz), 3.86 (1 H, dd, *J* 3.5 and 18.5 Hz), 4.06 (3 H, s), 4.5 (1 H, m), 7.0 (1 H, d, *J* 8 Hz), 7.9 (1 H, d, *J* 8 Hz), and 10.01 (1 H, s). The compound develops a strong pink colouration on exposure to light.

1,6-*Dihydroxy*-3-*methylisochroman*-5-*carboxylic Acid* (32).—A suspension of 5-formylmellein (25) (200 mg) in sodium hydroxide solution (2 ml; 2M) was set aside for 2 h. Dissolution occurred within 15 min. After 2 h the mixture was acidified with hydrochloric acid (2M) and the precipitate was filtered off. The solution was extracted with diethyl ether and the extract was dried (Na_2SO_4) and evaporated. The combined residue and precipitate (170 mg) was recrystallised from acetic acid to yield needles of 6-*dihydroxy*-3-*methylisochroman*-5-*carboxylic acid*, m.p. 135 °C (Found: C, 59.1; H, 5.1. $C_{11}H_{12}O_5$ requires C, 58.9; H, 5.4%); $\nu_{max.}$ 3 650—2 400 and 1 665 cm^{-1}.

6-*Hydroxy*-1-*methoxy*-3-*methylisochroman*-5-*carboxylic Acid*.—A solution of the above isochroman (100 mg) in methanol (3 ml) was refluxed during 15 min. The solution was evaporated under reduced pressure and the crystalline residue (52 mg) was recrystallised from nitromethane to yield needles of 6-*hydroxy*-1-*methoxy*-3-*methylisochroman*-5-*carboxylic acid*, m.p. 149—152 °C (Found: C, 60.2; H, 5.8. $C_{12}H_{14}O_5$ requires C, 60.5; H, 5.9%); $\nu_{max.}$ (KBr) 3 700—2 500 and 1 668 cm^{-1}.

8-*Hydroxy*-3-*methyl*-3,4-*dihydroisocoumarin*-5-*carboxylic Acid* (5-*Carboxymellein*) (26) *and* 6-*Hydroxy*-3-*methyl*-3,4-*dihydroisocoumarin*-5-*carboxylic Acid* (35) *from* 5-*Formylmellein*.—A mixture of 5-formylmellein (100 mg) and silver

nitrate (200 mg), in an aqueous solution of sodium hydroxide (8 ml; 2M) was refluxed for 1 h. The solution was cooled and filtered, and the precipitate was washed with water. The combined filtrate and washings were acidified with hydrochloric acid and extracted with ethyl acetate (3 × 5 ml). After being washed with water and dried (Na_2SO_4), the solution was evaporated to yield a pale brown crystalline solid which was separated into two components by preparative layer chromatograhy (p.l.c.) in the solvent system benzene–ethyl acetate–acetic acid (50 : 48 : 1) (detected by violet Fe^{3+} reaction). The upper band gave a crystalline solid which yielded needles of 8-hydroxy-3-methyl-3,4-dihydroisocoumarin-4-carboxylic acid (5-carboxymellein) (26) (35 mg), m.p. 245 °C (from nitromethane), identical by m.p., mixed m.p., and i.r. and ^1H n.m.r. spectroscopy with the product isolated from *H. illitum* (see below), and the product obtained by alkaline hydrolysis of 5-methoxycarbonylmellein (see below). Treatment during 15 min with an excess of ethereal diazomethane gave 5-methoxycarbonylmellein (27), m.p. 66 °C, identical by m.p., mixed m.p., and i.r. and ^1H n.m.r. spectroscopy with the product isolated from *H. mammatum* (see below). The lower component gave a solid which yielded needles of 6-hydroxy-3-methyl-3,4-dihydroisocoumarin-5-carboxylic acid (35) (30 mg), m.p. 209 °C (from nitromethane) identical by m.p., mixed m.p., and i.r. and ^1H n.m.r. spectroscopy with the product obtained by the alkaline hydrolysis of 5-methoxycarbonylmellein (see below). Treatment of this product during 15 min with an excess of ethereal diazomethane gave needles of *methyl 6-methoxy-3-methyl-3,4-dihydroisocoumarin-5-carboxylate*, m.p. 114 °C (Found: C, 62.5; H, 5.4. $C_{13}H_{14}O_5$ requires C, 62.4; H, 5.6%); $\nu_{max.}$ 1 715 and 1 722 cm^{-1}; δ ([$^2H_6$]acetone) 1.4 (3 H, d, *J* 7 Hz), 2.8 (2 H, m), 3.8 (3 H, s), 3.88 (3 H, s), 4.6 (1 H, m), 7.08 (1 H, d, *J* 8 Hz), and 7.96 (1 H, d, *J* 8 Hz).

7-Formylmellein (34) *from Mellein.*—To a solution of mellein (172 mg) in dichloromethoxymethane (3 ml) cooled to 0 °C was added $TiCl_4$ (0.3 ml). The mixture was set aside for 5 min and an excess of hydrochloric acid (10%) was then added. The mixture was extracted with diethyl ether, the extract was washed in turn with water, aqueous sodium hydrogen carbonate, and water, dried, and evaporated. The gummy residue was separated by p.l.c. on silica gel in the solvent system benzene–ethyl formate–formic acid (75 : 25 : 1). Two major components were detected by an orange colouration with diazotised *p*-nitroaniline. The upper component was isolated as needles of unchanged mellein (30 mg), m.p. 58 °C (from light petroleum).

The lower component (82 mg) was isolated and then re-separated by p.l.c. in the solvent system benzene–ethyl formate–formic acid (90 : 10 : 1) to yield needles of *7-formylmellein* (34) (61 mg), m.p. 125 °C (from ethanol) (Found: C, 64.0; H, 4.8. $C_{11}H_{10}O_4$ requires C, 64.1; H, 4.8%); $\nu_{max.}$ 1 700 and 1 660 cm^{-1}; δ ($CDCl_3$) 11.72 (1 H, s), 10.4 (1 H, s), 7.92 (1 H, d, *J* 8 Hz), 6.76 (1 H, d, *J* 8 Hz), 4.78 (1 H, m), 2.97 (2 H, d, *J* 6 Hz), and 1.53 (3 H, d, *J* 6 Hz).

5,7-Bis(chloromethyl)-8-hydroxy-3-methyl-3,4-dihydroisocoumarin (33) *from Mellein.*—$TiCl_4$ (1.3 ml) was added dropwise during 5 min to a solution of mellein (0.48 g) in chloromethyl methyl ether (10 ml) cooled to 0 °C. The red solution was stirred for 1 h at 0 °C and was then allowed to attain room temperature. The mixture was warmed at 60 °C for 3 h and evaporated under reduced pressure to remove excess of chloromethyl methyl ether. Hydrochloric acid (10 ml; 2M) was added to the residue. The mixture was extracted with ethyl acetate and the extract was washed in turn with aqueous sodium hydrogen carbonate and water, and was then dried (Na_2SO_4). Evaporation of the solvent gave a pale yellow solid. Purification by p.l.c. in the solvent system benzene–ethyl formate–formic acid (75 : 25 : 1) gave *5,7-bis(chloromethyl)-8-hydroxy-3-methyl-3,4-dihydroisocoumarin* (33) as needles (0.42 g), m.p. 86 °C (from light petroleum, b.p. 60—80 °C) (Found: C, 52.5; H, 4.5; Cl, 25.3. $C_{12}H_{12}Cl_2O_3$ requires C, 52.4; H, 4.4; Cl, 25.8%); δ ($CHCl_3$) 11.41 (1 H, s), 7.49 (1 H, s), 4.56 (2 H, s), 4.47 (2 H, s), 4.34—4.48 (1 H, m), 2.7—3.2 (2 H, m), and 1.51 (3 H, d, *J* 7 Hz).

Isolation of Pyrenophorin (31) *from* Nummularia broomeiana.—*N. broomeiana* was cultured on a malt-extract medium for eight weeks. The solution (22 l) was filtered from the almost colourless mycelium and extracted with ethyl acetate (3 × 1.5 l). Evaporation of the dried (Na_2SO_4) extract yielded a brown gum (0.7 g). Separation by p.l.c. in the solvent system benzene–acetone (9 : 1) gave three components: (a) an upper component (orange–salmon *p*-nitroaniline reaction) which yielded a pale yellow oil which was crystallised from light petroleum (b.p. 80—100 °C) as plates of 5-methylmellein (24) (15 mg), m.p. and mixed m.p. with an authentic sample 131 °C; (b) an intermediate component (orange dinitrophenylhydrazine reaction) which yielded needles of *5-formylmellein* (25) (56 mg), m.p. and mixed m.p. with an authentic sample 127 °C (from ethanol); and (c) a lower component (orange 2,4-dinitrophenylhydrazine reaction) which yielded a crystalline solid which was recrystallised from ethanol as needles of pyrenophorin (31) (27 mg), m.p. 175—176 °C (lit.,[19] 175 °C) (Found: C, 62.0; H, 6.5. Calc. for $C_{16}H_{20}O_6$: C, 62.3; H, 6.5%); *m/z* 308 (M^+); $\nu_{max.}$ ($CHCl_3$) 1 725 and 1 704 cm^{-1}.

5-Methoxycarbonylmellein (27) *from* Hypoxylon mammatum.—*H. mammatum* (C.B.S.) was cultured for eight weeks in Thompson bottles (1 l) on malt solution (4% Boots). The pale brown medium (46 l) was extracted with diethyl ether and the dried extract (Na_2SO_4) was evaporated to yield a viscous brown oil (10.1 g) which slowly solidified. The gummy solid (1.4 g) was dissolved in the solvent system benzene–ethyl formate–formic acid (75 : 25 : 1) and applied to a column of silica gel (40 × 2 cm). Elution of the column with the same solvent system, and evaporation of the first fraction, gave a yellow oil (494 mg) which crystallised slowly. Recrystallisation from light petroleum (b.p. 80—100 °C) yielded needles of *5-methoxycarbonylmellein*, m.p. 65—66 °C, $[\alpha]_D^{22}$ −163° (*c* 1 in $CHCl_3$) (Found: M^+, 236.0691; C, 61.2; H, 5.1. $C_{12}H_{12}O_5$ requires *M*, 236.068 46; C, 61.0; H, 5.1%); $\nu_{max.}$ ($CHCl_3$) 1 715 and 1 675 cm^{-1}; $\lambda_{max.}$ (EtOH) 226, 250, and 307 nm (log ε 4.40, 3.93, and 3.52); δ ($CDCl_3$) 11.87 (1 H, s), 8.15 (1 H, d, *J* 7 Hz), 6.95 (1 H, d, *J* 7 Hz), 4.7 (1 H, m), 3.96 (1 H, m), 3.90 (3 H, s), 3.0 (1 H, m), and 1.55 (3 H, d, *J* 6 Hz).

5-Methoxycarbonylmellein Acetate.—A solution of 5-methoxycarbonylmellein (27) (100 mg) in acetic anhydride (1 ml) containing pyridine (1 drop) was heated on a water-bath for 1 h and was then set aside for 16 h. The solution was poured into water and the brown oil extracted with diethyl ether. The extract was washed in turn with saturated aqueous sodium hydrogencarbonate and water and was then dried (Na_2SO_4). Evaporation of the diethyl ether gave a solid which was recrystallised from ethanol as plates of *5-methoxycarbonylmellein acetate* (60 mg), m.p. 93 °C (Found: C, 60.6; H, 5.3. $C_{14}H_{14}O_6$ requires C, 60.4; H, 5.0%); *m/z* 278 (M^+); $\nu_{max.}$ ($CHCl_3$) 1 767, 1 720, and 1 715 cm^{-1}.

5-Methoxycarbonylmellein Methyl Ether.—To a refluxing mixture of 5-methoxycarbonylmellein (27) (110 mg) and anhydrous potassium carbonate (2 g) in dry acetone (10 ml)

was added a mixture of dimethyl sulphate (200 mg) in dry acetone (3 ml) during 45 min. The mixture was refluxed for 27 h and then filtered, and the residue was washed with diethyl ether. The ethereal solution was washed with water, dried (Na_2SO_4), and combined with the acetone solution. The combined solutions were evaporated and the residue was recrystallised from light petroleum (b.p. 80—100 °C) to yield needles of *5-methoxycarbonylmellein methyl ether* (118 mg), m.p. 164—166 °C (Found: C, 61.6; H, 5.8. $C_{13}H_{14}O_5$ requires C, 62.4; H, 5.6%); $\nu_{max.}$ 1 720 and 1 715 cm^{-1}.

Hydrolysis of 5-Methoxycarbonylmellein.—5-Methoxycarbonylmellein (27) (165 mg) was dissolved in sodium hydroxide solution (4 ml; 2M) and the mixture was set aside for 20 min. The solution was then acidified with hydrochloric acid (2M) and the precipitate was extracted into diethyl ether. The extract was washed with water, dried (Na_2SO_4), and evaporated to yield a crystalline solid which was separated into two components by p.l.c. in the solvent system benzene–ethyl acetate–acetic acid (50 : 49 : 1). The upper component yielded needles of 5-carboxymellein (65 mg), m.p. 245 °C (from nitromethane), identical by m.p., mixed m.p., and i.r. and ^1H n.m.r. spectroscopy with the product isolated from *H. illitum*. The lower band yielded needles of *6-hydroxy-3-methyl-3,4-dihydroisocoumarin-5-carboxylic acid* (50 mg), m.p. 209 °C (from nitromethane) (Found: C, 59.2; H, 4.3. $C_{11}H_{10}O_5$ requires C, 59.5; H, 4.5%); $\nu_{max.}$ ($CHCl_3$) 1 695 and 1 655 cm^{-1}; δ ([$^2H_6$]acetone) 11.8 (1 H, s), 8.4—7.6 (1 H, br), 8.18 (1 H, d, *J* 8 Hz), 6.94 (1 H, d, *J* 8 Hz), 4.6 (1 H, m), 3.75 (1 H, m), 3.15 (1 H, m), and 1.45 (3 H, *J* 7 Hz).

5-Methylmellein (24), *5-Hydroxymethylmellain* (28), *and 5-Carboxymellein* (26) *from* Hypoxylon illitum.—*H. illitum* was cultured on a malt solution (4% Boots) for eight weeks. The colourless mycelium was filtered off and the pale brown medium (35 l) was extracted with diethyl ether. The extract was dried (Na_2SO_4) and evaporated. The brown gum (4.6 g) was dissolved in the solvent system benzene–acetone (9 : 1) and applied to a column of silica gel (35 × 2.5 cm) and eluted with the same solvent system. The first fraction yielded, on evaporation, an oil that crystallised slowly to yield needles of 5-methylmellein (24) (350 mg), m.p. 131—133 °C. The second fraction yielded a crystalline solid (47 mg) which was washed with hot light petroleum (b.p. 80—100 °C). The cooled washings produced further needles of 5-methylmellein (7 mg). The residual solid was recrystallised from benzene as needles of *5-hydroxymethylmellein* (28) (39 mg), m.p. 163—164 °C (Found: C, 63.4; H, 6.7. $C_{11}H_{12}O_4$ requires C, 63.8; H, 5.8%); *m/z* 208 (M^+); $\nu_{max.}$ (KBr) 1 670 cm^{-1}; $\lambda_{max.}$ (EtOH) 214, 242, and 342 nm (log ε 3.93, 3.32, and 3.64), δ ([$^2H_6$]acetone) 11.15 (1 H, s), 7.52 (1 H, d, *J* 8 Hz), 6.96 (1 H, d, *J* 8 Hz), 4.88 (2 H, s), 4.67 (1 H, m), 3.2 (2 H, m), 2.2 (1 H, s), 1.5 (3 H, d). The third fraction gave a crystalline solid which was recrystallised from nitromethane or ethyl acetate to yield needles of 5-carboxymellein (26) (12 mg), m.p. 245—246 °C (lit.[23] 247—249 °C) (Found: C, 59.4; H, 4.5. Calc. for $C_{11}H_{10}O_5$: C, 59.5; H, 4.5%); *m/z* 222 (M^+); $\nu_{max.}$ (KBr) 2 980, 2 930, 1 695, and 1 655 cm^{-1}; $\lambda_{max.}$ (EtOH) 226, 244, and 314 nm (log ε 4.16, 3.74, and 3.23); δ ([$^2H_6$]acetone) 11.6 (2 H, br s), 7.96 (1 H, d, *J* 8 Hz), 6.86 (1 H, d, *J* 8 Hz), 4.6 (1 H, m), 3.7 (1 H, q), 2.9 (1 H, m), and 1.46 (3 H, d, *J* 6 Hz).

Isolation of 6-Methoxy-5-methylmellein (29) *from* Hypoxylon atropunctatum.—The fungus (Strain 496) was cultured on a potato–dextrose–yeast medium for eight weeks. The dark brown medium (10 l) was separated from the dark green mycelium and was extracted continuously with diethyl ether (48 h). The extract was dried (Na_2SO_4) and evaporated. The dark green gum (0.44 g) was dissolved in the solvent system benzene–ethyl formate–formic acid (75 : 25 : 1) and applied to a column of silica gel (50 × 1.5 cm). The column was eluted with the same solvent to yield a fast running fraction which, on evaporation, yielded a brown solid. Recrystallisation from light petroleum (b.p. 60—80 °C) gave needles of 6-methoxy-5-methylmellein (39 mg), m.p. 121 °C (lit.,[23] 118—119 °C); $[\alpha]_D^{21}$ −99° (*c* 1 in $CHCl_3$) (Found: M^+ 222.0892; C, 64.8; H, 6.4. Calc. for $C_{12}H_{14}O_4$: *M*, 222.0891; C, 64.85; H, 6.35%); $\lambda_{max.}$ (EtOH) 266 and 310 nm (log ε 4.10 and 3.78); $\nu_{max.}$ ($CHCl_3$) 1 668 cm^{-1}; δ ($CDCl_3$) 11.6 (1 H, s), 6.39 (1 H, s), 4.62 (1 H, m), 3.93 (3 H, s), 2.80 (2 H, m), 2.10 (3 H, s), and 1.56 (3 H, d).

Subsequent cultures of this species on potato dextrose–yeast media produced mixtures of 5-methylmellein and the above metabolite which could not be separated either by chromatography or by fractional crystallisation. Similar mixtures were also produced when the fungus was grown on Czapek–Dox medium.

The strains of fungi examined in this investigation are retained in the H. J. S. Whalley collection, Dept. of Biology, Liverpool Polytechnic, Liverpool L3 3AF.

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