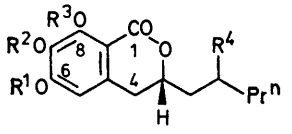
Metabolic Products of *Fusarium larvarum* Fuckel. The Fusarentins and the Absolute Configuration of Monocerin

By **John Frederick Grove** * and **Michael Pople**, ARC, Unit of Invertebrate Chemistry and Physiology, University of Sussex, Falmer, Brighton, East Sussex BN1 9RQ

The known fungal metabolites (+)-mellein and monocerin, and the new natural products 7-*O*-demethylmonocerin, fusarentin 6-methyl ether, fusarentin 6,7-dimethyl ether, and fusarentin 6,8-dimethyl ether have been isolated from two strains of the entomogenous fungus *Fusarium larvarum* grown in surface culture on a synthetic medium. Fusarentin is shown to be (3*S*)-3,4-dihydro-6,7,8-trihydroxy-3-(2-hydroxy-n-pentyl)isocoumarin and has been related to monocerin, which has the (3a*R*, 9b*R*) absolute configuration.

Two cultures (numbers 26 and 27 in our collection of entomogenous fungi) classified as *Fusarium larvarum*, a pathogen of insects, particularly of the balsam woolly aphid *Adelges piceae*,[1] have been examined [2] for the production in surface culture on Raulin–Thom medium



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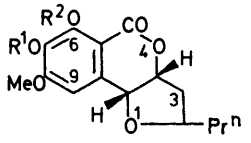

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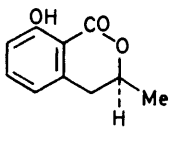

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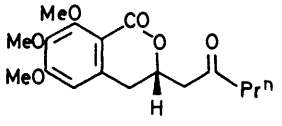

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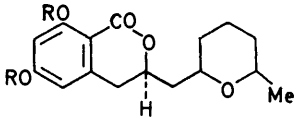

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of insecticidal secondary metabolic products. In this paper we describe the isolation of these products and the determination of their structure. The compounds mainly responsible for the insecticidal activity of solvent extracts of the fermentation broth are identified as the known fungal metabolic products monocerin (2; R^1 = Me, R^2 = H),[3] from both strains, and (+)-mellein (3),[4] from strain 27, together with two new natural products, fusarentin 6-methyl ether and fusarentin 6,7-dimethyl ether, from both strains. The minor products, 7-*O*-demethylmonocerin (2; $R^1 = R^2$ = H) and fusarentin 6,8-dimethyl ether, were obtained from strain 27 but in insufficient amounts for an accurate assessment of their insecticidal activity.[2] Two new substances without insectidical activity [2] were also isolated, as minor products, from strain 26.

RESULTS AND DISCUSSION

Yields were 5—15 mg l^{-1} for the major components, monocerin and the fusarentin ethers, and 1—2 mg l^{-1} for the minor components. Monocerin and the fusarentin ethers were also produced, but in very low yield, when strain 26 was cultured on glucose–ammonium nitrate medium.

After the removal of tartaric acid, the neutral portions of the crude fermentation products,[2] were subjected to column chromatography, giving seven fractions which were further purified by preparative t.l.c. The compositions of the products isolated from these fractions, in order of elution by benzene–methanol, were, from strain 26: $C_{16}H_{20}O_6$, $[\alpha]_D$ +28°, identified as monocerin (2; R^1 = Me, R^2 = H); $C_{16}H_{22}O_6$; $C_7H_8O_4$, obtained on one occasion only; $C_{15}H_{20}O_6$; and $C_{14}H_{18}O_6$.

The $C_{15}H_{20}O_6$ and $C_{16}H_{22}O_6$ compounds, which contained one and two OMe groups respectively, were closely related, methylation giving the same trimethyl ether (1; $R^1 = R^2 = R^3$ = Me, R^4 = OH). The u.v. absorption, $\lambda_{max.}$ 274 and 308 nm, of the $C_{16}H_{22}O_6$ compound, taken in conjunction with the i.r. evidence ($\nu_{max.}$ 1 650 cm^{-1}), suggested a dihydroisocoumarin chromophore carrying an 8-hydroxy-substituent [positive iron(III) reaction]. The n.m.r. spectra (see the Table)

Chemical shifts (τ) for protons in fusarentin methyl ethers and derivatives

Compound	5-H	4-H [a]	3-H [b]	2′-H [b]	Me [c]	OMe	OH
(1; R^1 = Me, $R^2 = R^3$ = H, R^4 = OH)	3.70	7.10	5.13	5.90	9.05	6.04	−0.85, 4.56, 8.2
(1; $R^1 = R^2$ = Me, R^3 = H, R^4 = OH)	3.72	7.12	5.15	5.95	9.05	6.10, 6.12	−1.0, 8.2
(1; $R^1 = R^3$ = Me, R^2 = H, R^4 = OH)	3.52	7.15	5.20	5.95	9.05	6.04, 6.06	4.18, 8.2
(1; $R^1 = R^2 = R^3$ = Me, R^4 = OAc)	3.54	7.15	5.52	4.90	9.10	6.02, 6.10, 6.12	
(4) [d]	3.55	7.15	5.15		9.10	6.04, 6.12, 6.16	

[a] AB part of ABX system with $J_{AB} > \Delta\nu$, unsuited to first-order interpretation. [b] Multiplet. [c] Triplet. [d] Also 1′-H, τ 6.9 and 7.3 (dd, *J* 6, 8, and 17 Hz); 3′-H, τ 7.56 (t); and 4′-H, 8.40 (m).

showed only one aromatic ring proton (τ 3.7). The sixth oxygen atom was present in a secondary alcohol group since acetylation of the trimethyl ether, $\nu_{max.}$ 3 420 cm^{-1}, gave a monoacetate (1; $R^1 = R^2 = R^3 = Me$, $R^4 =$ OAc) with no OH absorption, and caused a one-proton multiplet to move downfield from τ 5.9 to 4.9. A second one-proton multiplet at τ 5.1, coupled to benzylic protons at τ 7.1, indicated substitution at the 3-position of the dihydroisocoumarin ring. The location of the secondary alcohol group in this side-chain was determined by oxidation, with chromic oxide–sulphuric acid, of the trimethyl ether (1; $R^1 = R^2 = R^3 = Me$, $R^4 =$ OH) to the ketone (4). The signals from all the side-chain protons of this derivative were well separated in the n.m.r. spectrum. It was consistent only with structure (4) which was confirmed by suitable double-irradiation experiments.

Confirmation of structure (1) and particularly of the substitution pattern of the aromatic ring was obtained by the isolation, albeit in low yield, of the $C_{16}H_{22}O_6$ compound as one of the products of the hydrogenolysis of monocerin in the presence of a reduced platinum oxide catalyst. It follows that the $C_{16}H_{22}O_6$ compound has the structure (1; $R^1 = R^2 = Me$, $R^3 = H$, $R^4 = OH$). The $C_{15}H_{20}O_6$ compound was shown to have the structure (1; $R^1 = Me$, $R^2 = R^3 = H$, $R^4 = OH$), with *vic*-hydroxy substituents, as suggested by the intense green colour obtained with iron(III) chloride, and by the preparation of the derivative (1; $R^1 = Me$, $R^2R^3 = Ph_2C$, $R^4 = Cl$) by reaction with dichlorodiphenylmethane.

The trivial name fusarentin was assigned to the unmethylated structure (1; $R^1 = R^2 = R^3 = H$, $R^4 =$ OH). The natural products are thus the 6-methyl and 6,7-dimethyl ethers of fusarentin.

The products isolated from strain 27 were: $C_{10}H_{10}O_3$, $[\alpha]_D$ +97°, identified as (+)-mellein (3); monocerin; fusarentin 6,7-dimethyl ether; $C_{15}H_{18}O_6$; fusarentin 6-methyl ether; and $C_{16}H_{22}O_6$. The spectroscopic properties of the $C_{15}H_{18}O_6$ compound, which had only one OMe group, were very similar to those of monocerin; and methylation of both compounds with methyl iodide gave the same trimethyl ether (2; $R^1 = R^2 = Me$). It gave the same intense green colour with iron(III) chloride given by fusarentin 6-methyl ether and coupled with its chromatographic behaviour on borate-impregnated silica, is accordingly assigned the 7-*O*-demethylmonocerin structure (2; $R^1 = R^2 = H$), with vicinal OH groups. The $C_{16}H_{22}O_6$ compound, eluted after fusarentin 6-methyl ether, gave no colour with iron(III) chloride and had the spectroscopic properties ($\lambda_{max.}$ 274 and 312 nm; $\nu_{max.}$ 1 690 cm^{-1}; n.m.r., see Table) consistent with those of a fusarentin *n*,8-dimethyl ether. Partial demethylation with boron trichloride in dichloromethane afforded fusarentin 6-methyl ether, indicating that the $C_{16}H_{22}O_6$ compound was the 6,8-dimethyl ether (1; $R^1 = R^3 =$ Me, $R^2 = H$, $R^4 = OH$).

The fusarentins are closely related to the asperentins (5; R = H or Me),[5] insecticidal metabolic products of an entomogenous strain of *Aspergillus flavus*. Monocerin has previously only been obtained from *Drechslera monoceras* (= *Helminthosporium monoceras*), and (+)-mellein, the less common of the naturally occurring enantiomers, from *Apiospora camptospora* [6] and *Cercospora taiwanensis*.[7] With the exception of monocerin and its relatives,[3] microbial 6,7,8-trioxygenated isocoumarins have previously been obtained only from *Streptomyces* sp.[8–10]

The absolute configuration of the fusarentin ethers at position 3 was shown to be (*S*), as in structure (1), by c.d. measurements which gave curves of opposite sign at 270 nm to those obtained with the asparentins.[5] (+)-(*S*)-Mellein has a 3α-hydrogen (3). *F. larvarum* thus produces two 3-substituted dihydroisocoumarins with opposite absolute configuration at this centre. This contrasts with the *Aspergillus ochraceus* fermentation [11] where the absolute configuration of the mellein produced is the same as that of the ochratoxin co-metabolities.

It follows from the relationship between fusarentin and monocerin that the latter has the (3a*R*, 9b*R*) absolute configuration. From a study of molecular models the smaller of the two (6 and 1.5 Hz) coupling constants [3] for the 3a,3-hydrogens in monocerin is assigned to 3aβ,3α (dihedral angle close to 90°), and, in consequence, the multiplets at τ 7.8 (*J* 14, 6, and 1.5 Hz) and 7.4 (*J* 14, 9, and 6 Hz) are assigned to the 3α and 3β hydrogens respectively. The larger (9 Hz) of the two 3-H,2-H-coupling constants can then reasonably be assigned to hydrogens in a *cis* relationship, *i.e.* to 3β-2β. It is highly probable therefore that C-2 in monocerin has the (*S*)-configuration and, assuming retention of configuration during hydrogenolysis, the corresponding centres in the fusarentin ethers also have the (*S*)-configuration, but a formal proof of these assignments is still required.

Although the $C_{14}H_{18}O_6$ substance had the same composition as fusarentin, it gave no colour with iron(III) chloride and the u.v. and i.r. spectra indicated a different ring system. Insufficient material was available for more extensive characterisation.

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage apparatus and are corrected. Unless stated otherwise, i.r. spectra were determined for mulls in Nujol and u.v. spectra, optical rotations, and c.d. measurements for solutions in methanol. N.m.r. spectra were obtained in deuteriochloroform at 100 MHz with tetramethylsilane as internal standard. Molecular weights and compositions were obtained from mass spectra recorded with a Varian CH5D (double-focusing) mass spectrometer coupled to a Varian 620L computer. Merck silica gel HF_{254} was used in t.l.c. and the R_F values quoted are for chloroform–methanol (95 : 5). In preparative t.l.c. silica layers (40 × 20 × 0.075 cm) were developed in this solvent. Merck silica gel 7734 was used in column chromatography. Light petroleum had b.p. 60—80 °C.

Isolation of F. larvarum *Metabolites from Strain* 26.—(*a*) *Raulin–Thom medium.*—The brown paste (1.24 g) obtained

by extraction of the culture filtrate (6.3 l) at the natural pH (3.2) with ethyl acetate [2] was redissolved in ethyl acetate and extracted with sodium hydrogencarbonate. The acid fraction, on recovery, was identified as (+)-tartaric acid.

The neutral fraction, a brown oil (480 mg), in benzene–methanol (20 : 1, 20 ml) was chromatographed on a column of silica gel (30 g, 19 × 1.8 cm) made up in benzene. The following fractions, all gums, were obtained on elution of the column with benzene–methanol (composition in parentheses): (*i*) 54 mg (200 : 1, 200 ml); (*ii*) diffuse yellow band, 215 mg (100 : 1, 500 ml); (*iii*) dark band, 108 mg (50 : 1, 500 ml); (*iv*) 12 mg (20 : 1, 100 ml); (*v*) 39 mg (20 : 1, 200 ml); (*vi*) 11 mg (20 : 1, 200 ml); and (*vii*) 6 mg (20 : 1, 100 ml). Fraction (*i*) had no specific u.v. absorption and was discarded.

Fraction (*ii*) was purified by preparative t.l.c. giving two bands from which gummy material of R_F 0.80 (30 mg) and R_F 0.50 (88 mg) was recovered in the usual way. The gum of R_F 0.80 crystallised at low temperature from ether–light petroleum (b.p. 40—60 °C) in prisms, m.p. 65—70 °C, $[\alpha]_D^{20}$ +28° (Found: C, 61.9; H, 6.3%; *M*, 308.123 8. Calc. for $C_{16}H_{20}O_6$: C, 62.3; H, 6.5%; *M*, 308.1260); identified as monocerin (2; R^1 = Me, R^2 = H) (lit.,[3] m.p. 64—66 °C, $[\alpha]_D^{24}$ +53°) by comparison of the i.r. spectrum with that of an authentic specimen.

The methyl ether (2; R^1 = R^2 = Me), prepared with methyl iodide as described below, was an oil,[3] R_F 0.62, $\nu_{max.}$ 1 720 and 1 598 cm^{-1} (Found: *M*, 322. Calc. for $C_{17}H_{22}O_6$: *M*, 322).

The gum of R_F 0.50 crystallised from benzene–light petroleum in needles, m.p. 99 °C; $\nu_{max.}$ 3 460, 3 390, 1 650, 1 635, 1 615, and 1 575 cm^{-1}: or prisms, m.p. 103 °C; $\nu_{max.}$ 3 460, 3 370, 1 662, 1 645, 1 620, and 1 578 cm^{-1}; $[\alpha]_D^{20}$ −29°, $[\alpha]_{578}^{20}$ −32°, $[\alpha]_{546}^{20}$ −44°, $[\alpha]_{436}^{20}$ −85°, and $[\alpha]_{365}^{20}$ −182°; it was 3,4-*dihydro*-8-*hydroxy*-3-(2-*hydroxy*-*n*-*pentyl*)-6,7-*dimethoxyisocoumarin* (*fusarentin* 6,7-*dimethyl ether*) (1; R^1 = R^2 = Me, R^3 = H, R^4 = OH) (Found: C, 61.7; H, 7.1%; *M*, 310.140 3. $C_{16}H_{22}O_6$ requires C, 61.9; H, 7.1%; *M*, 310.141 6); $\lambda_{max.}$ 274 and 308 nm (log ε 4.13 and 3.51), c.d. λ 310, 270, 243, and 205 nm (Δε −0.42, −2.55, +1.56, and −4.84 respectively). In ethanol it gave a purple colour with iron(III) chloride.

Fraction (*iii*) was purified by preparative t.l.c. giving three bands from which gummy material of R_F 0.50 (5 mg), 0.28 (45 mg), and 0.18 (15 mg), was recovered. The material of R_F 0.50 was identified as fusarentin 6,7-dimethyl ether. The gum of R_F 0.28 (R_F of 0.05 on silica impregnated with 2% sodium tetraborate) crystallised from benzene in prisms, m.p. 137 °C; $\nu_{max.}$ 3 490, 3 250, 3 130, 1 650, 1 625, and 1 585 cm^{-1}; or needles, m.p. 75—78 °C (decomp.) (solvate); $\nu_{max.}$ 3 530, 3 330, 1 670, 1 630, and 1 585 cm^{-1}; it was 3,4-*dihydro*-7,8-*dihydroxy*-3-(2-*hydroxy*-*n*-*pentyl*)-6-*methoxyisocoumarin* (*fusarentin* 6-*methyl ether*) (1; R^1 = Me, R^2 = R^3 = H, R^4 = OH) (Found: C, 60.3; H, 6.85%; *M*, 296.124 4. $C_{15}H_{20}O_6$ requires C, 60.8; H, 6.8%; *M*, 296.126 0); $\lambda_{max.}$ 232, 277, and 320 nm (log ε 4.28, 4.09, and 3.44); $[\alpha]_D^{20}$ −30°, $[\alpha]_{578}^{20}$ −31°, $[\alpha]_{546}^{20}$ −39°, $[\alpha]_{436}^{20}$ −97°, and $[\alpha]_{365}^{20}$ −210°. In ethanol it gave an intense dark green colour with iron(III) chloride. The gum of R_F 0.18 was intractable.

Fractions (*iv*), (*v*), and (*vi*) were combined and submitted to preparative t.l.c. giving two bands, of R_F 0.27 (2 mg) which yielded fusarentin 6-methyl ether, and material of R_F 0.18 (2 mg). The material of R_F 0.18 crystallised from ethyl acetate–light petroleum in needles, m.p. 163—166 °C, of a *substance* (Found: *M*, 282.110 3. $C_{14}H_{18}O_6$ requires *M*, 282.110 3); $\lambda_{max.}$ 225, 247, *ca.* 305, 320, and *ca.* 335 nm; $\nu_{max.}$ 3 310(br), 3 060, 1 750, 1 715, 1 645, and 1 560 cm^{-1}, which gave no colouration with iron(III) chloride.

Fraction (*vii*) was purified by preparative t.l.c. giving an amorphous solid (2 mg) of R_F 0.12; $\lambda_{max.}$ 255 nm; m.p. 80—160 °C, which resisted further purification.

On one occasion preparative t.l.c. of fraction (*ii*) gave an additional band of R_F 0.35 yielding a gum (14 mg) which crystallised from ether in laths (8 mg), m.p. 140—143 °C, of a *substance* (Found: *M*, 156.042 9. $C_7H_8O_4$ requires *M*, 156.042 6); $\lambda_{max.}$ 288 nm (ε 12 500); $\nu_{max.}$ 3 250, 1 655w, 1 610, and 1 590 cm^{-1}, which gave a purple colour with iron(III) chloride.

(*b*) *Glucose–ammonium nitrate medium.* The gum (216 mg) obtained by extraction of the culture filtrate [2] (6 l) with ethyl acetate at pH 5.9 was subjected to preparative t.l.c. giving three bands yielding gums, $\lambda_{max.}$ 275 nm, R_F 0.75 (39 mg), monocerin (2 mg); 0.64 (1 mg), fusarentin 6,7-dimethyl ether; and 0.34 (5 mg), fusarentin-6-methyl ether (1 mg).

Isolation of F. larvarum *Metabolities from Strain* 27.—The neutral portion (675 mg) of the crude extract (1.7 g) from 7.5 l of culture filtrate was dissolved in benzene–methanol (50 : 1, 100 ml) and chromatographed on a column of silica gel (50 g, 27 × 2 cm) made up in benzene. The following fractions were obtained, eluting with benzene–methanol, as described above: (*i*) 37 mg (100 : 1, 300 ml); (*ii*) diffuse yellow band, 37 mg (50 : 1, 100 ml); (*iii*) brown band, 185 mg (50 : 1, 300 ml); (*iv*) interband, 83 mg (50 : 1, 300 ml); (*v*) dark band, 90 mg (50 : 1, 500 ml); (*vi*) interband, 35 mg (20 : 1, 300 ml); (*vii*) dark band, 33 mg (20 : 1, 300 ml).

Fraction (*i*), after preparative t.l.c. in chloroform–methanol (98 : 2), crystallised from light petroleum in prisms (9 mg), m.p. 56—57 °C, $[\alpha]_D^{21}$ +97°, of mellein (3) (Found: *M*, 178. Calc. for $C_{10}H_{10}O_3$: *M*, 178); $\lambda_{max.}$ 246 and 314 nm (log ε 3.73 and 3.57) (lit.,[4] m.p. 52 °C, $[\alpha]_D^{25}$ +88°), identified by comparison of the i.r. spectrum ($\nu_{max.}$ 1 670, 1 620, and 1 580 cm^{-1}) with that of an authentic specimen.

Fraction (*ii*) yielded monocerin (8 mg), and fraction (*iii*), after preparative t.l.c., yielded monocerin (48 mg) and fusarentin 6,7-dimethyl ether (48 mg).

Fraction (*iv*) crystallised from methanol in rosettes of needles (7 mg), R_F 0.50, m.p. 172—175 °C, of 2,3,3a,9b-*tetrahydro*-6,7-*dihydroxy*-8-*methoxy*-2-*n*-*propylfuro*[3,2-b]-[3]*benzopyran*-5-*one* (7-O-*demethylmonocerin*) (2; R^1 = R^2 = H) (Found: *M*, 294.110 4. $C_{15}H_{18}O_6$ requires *M*, 294.110 3); $\lambda_{max.}$ 234, 279, and 320 nm; $\nu_{max.}$ 3 300(br), 1 670, 1 625, and 1 588 cm^{-1}. It gave an intense green colour with iron(III) chloride; R_F on borate-treated silica, 0.10.

The dimethyl ether, prepared with methyl iodide, as described below, was identical with monocenin methyl ether (2; R^1 = R^2 = Me) by comparison of the R_F and i.r. and mass spectra.

Fractions (*v*) and (*vi*) were combined and yielded fusarentin 6-methyl ether (11 mg). Fraction (*vii*) gave gums of R_F 0 28 (11 mg) and 0.22 (13 mg) which, after further preparative t.l.c., yielded, respectively, fusarentin 6-methyl ether and *fusarentin* 6,8-*dimethyl ether* (1; R^1 = R^3 = Me, R^2 = H, R^4 = OH) as a gum; $\nu_{max.}$ 3 320(br), 1 690, 1 610, and 1 507 cm^{-1}; $\lambda_{max.}$ 274 and 312 nm (Found:

M, 310.1410. $C_{16}H_{22}O_6$ requires *M*, 310.141 6). It gave no colouration with iron(III) chloride.

Partial Demethylation of Fusarentin 6,8-*Dimethyl Ether*.—The ether (6 mg) in dichloromethane (1 ml) at 0 °C was treated with boron trichloride (6 mg) in dichloromethane (0.1 ml). The solution was allowed to warm up to room temperature and was set aside for 2 h. After removal of the solvent *in vacuo*, the residue was triturated with warm water and extracted with ethyl acetate. Crystallisation of the recovered product from benzene afforded fusarentin 6-methyl ether, identified by its i.r. spectrum.

Methylation of Fusarentin Methyl Ethers.—(*a*) The 6,7-dimethyl ether (1; $R^1 = R^2 = Me$, $R^4 = H$, $R^4 = OH$) (7 mg) in acetone (2 ml) was heated under reflux for 6 h with methyl iodide (0.2 ml) in the presence of anhydrous potassium carbonate. Preparative t.l.c. of the gummy product (8 mg), obtained by working up in the usual way, showed two bands (R_F 0.58 and 0.39) from which the components were recovered by extraction with chloroform. The band of R_F 0.39 yielded the 6,7,8-*trimethyl ether* (1; $R^1 = R^2 = R^3 = Me$, $R^4 = OH$) as an oil (5 mg) (Found: C, 62.6; H, 7.4%; *M*, 324.157 3. $C_{17}H_{24}O_6$ requires C, 63.0; H, 7.5%; *M*, 324.157 3); $\nu_{max.}$ 3 420(br), 1 710, and 1 598 cm^{-1}.

The *acetate* (1; $R^1 = R^2 = R^3 = Me$, $R^4 = OAc$), prepared with acetic anhydride in pyridine during 18 h at room temperature, was a gum of R_F 0.62 (Found: C, 62.5; H, 7.2%; *M*, 366.167 9. $C_{19}H_{26}O_7$ requires C, 62.3; H, 7.1%; *M*, 366.167 9); $\nu_{max.}$ 1 730 and 1 600 cm^{-1}; $\lambda_{max.}$ 264 and 300 nm.

(*b*) The 6-methyl ether (1; $R^1 = Me$, $R^2 = R^3 = H$, $R^4 = OH$) (8 mg), treated as described above, gave the 6,7,8-trimethyl ether (1; $R^1 = R^2 = R^3 = Me$, $R^4 = OH$) (8 mg), identified by R_F and i.r. and mass spectra.

Oxidation of the Trimethyl Ether (1; $R^1 = R^2 = R^3 = Me$, $R^4 = OH$).—The ether (11 mg) in acetone (1 ml) at 0 °C was treated with 8N-chromic oxide–sulphuric acid (0.03 ml) during 20 min and then left to warm up to room temperature. The mixture was concentrated, and, after the addition of water, was extracted with ethyl acetate. The organic layer was washed with sodium hydrogencarbonate. Recovery of the neutral product gave 3,4-*dihydro*-6,7,8-*trimethoxy*-3-(2-*oxo*-*n*-*pentyl*)*isocoumarin* (4) (9 mg) which crystallised from benzene–light petroleum in waxy prisms, m.p. 80—82 °C (Found: C, 63.3; H, 7.0%; *M*, 322.141 0. $C_{17}H_{22}O_6$ requires C, 63.3; H, 6.9%; *M*, 322.141 6); $\nu_{max.}$ 1 705, 1 660w, and 1 592 cm^{-1}.

Hydrogenolysis of Monocerin.—Monocerin (10 mg) in acetic acid (2 ml) was hydrogenated in the presence of a reduced platinum oxide catalyst (6 mg) at room temperature during 3 h. There was a slow uptake of 1.2 mol of H_2. After filtration, the solvent was removed *in vacuo* at room temperature and the product was submitted to preparative t.l.c. A number of bands were seen in addition to monocerin, R_F 0.80 (7 mg recovered). The material recovered from a band of R_F 0.50 crystallised from benzene–light petroleum in rosettes of needles (1 mg), m.p. 98—100 °C, $[\alpha]_{578}^{20}$ −12°, $[\alpha]_{546}^{20}$ −32°, $[\alpha]_{436}^{20}$ −76°, and $[\alpha]_{365}^{26}$ −160°, of fusarentin 6,7-dimethyl ether, identified by the i.r. spectrum.

Reaction of Fusarentin 6-*Methyl Ether with Dichlorodiphenylmethane*.—The ether (3 mg) in toluene (2 ml) was heated under reflux with dichlorodiphenylmethane (6 mg) for 30 h in the presence of potassium carbonate (2 mg). The solution was filtered and the solvent removed *in vacuo*. Preparative t.l.c. of the residual gum in chloroform gave a band of R_F 0.67 which yielded 3-(2-*chloro*-*n*-*pentyl*)-3,4-*dihydro*-7,8-*diphenylmethylenedioxy*-6-*methoxyisocoumarin* (1; $R^1 = Me$, $R^2R^3 = Ph_2C$, $R^4 = Cl$) as an oil (6 mg) (Found: *M*, 478.153 6. $C_{28}H_{27}O_5Cl$ requires *M*, 478.154 7); $\lambda_{max.}$ 285 and 315 nm; $\nu_{max.}$ no OH, 1 715 cm^{-1}.

When the ether and dichlorodiphenylmethane were heated together at 100 °C for 2 h the product was 3-(2-*chloro*-*n*-*pentyl*)-3,4-*dihydro*-7,8-*dihydroxy*-6-*methoxyisocoumarin* (1; $R^1 = Me$, $R^2 = R^3 = H$, $R^4 = Cl$) an oil, R_F 0.50 (Found: *M*, 314.096 0. $C_{15}H_{19}O_5Cl$ requires *M*, 314.092 1); $\lambda_{max.}$ 273 and 310 nm; $\nu_{max.}$ 1 665 cm^{-1}.

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