Use of Long Range ^{1}H–^{13}C Couplings in Structure Determination: Stellatin, a Novel Dihydroisocoumarin from *Aspergillus variecolor*

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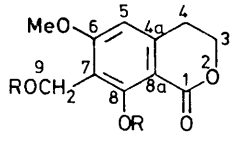
Summary Stellatin, a novel phenolic metabolite of *Aspergillus variecolor* is shown, by examination of its chemical and spectroscopic properties, in particular by analysis of the fully ^{1}H-coupled ^{13}C n.m.r. spectrum, to be 3,4-dihydro-8-hydroxy-7-hydroxymethyl-6-methoxyisocoumarin.

THE mycelium of *Aspergillus variecolor* (syn. *A. stellatus*) is a rich source of xanthone and related benzophenone pigments;[1] the common fungal products, terrein and kojic acid,[2] and recently a group of novel terpenoid metabolites[3] have been isolated from the culture liquors. The isolation of a novel, optically inactive phenolic metabolite, stellatin, from the culture filtrates of *A. variecolor* (C.M.I. 75219) is

now reported, and from examination of its chemical and spectroscopic properties, the dihydroisocoumarin structure (**1**) is established.

Stellatin, $C_{11}H_{12}O_5$, m.p. 126—128 °C, showed λ_{max} (MeOH) 265 and 300 nm (ϵ 16,800 and 5900); λ_{max} (MeOH–KOH) 338 nm (ϵ 6000), [*cf.* 3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin,[4] λ_{max} (EtOH) 267 and 302 nm) (ϵ 14,800 and 6000)]; and ν_{max} 3580, 3200(br), 1670, 1629, and 1588 cm^{-1}. The ^1H n.m.r. spectrum indicated the presence of an $ArCH_2CH_2OCO$-unit (δ 3·02 and 4·53, both 2H, t, J 7 Hz), an aromatic proton (δ 6·32), an aromatic hydroxymethyl (δ 4·76), a methoxy (δ 3·90), and two exchangeable protons (δ 2·38 and 11·50). Treatment of stellatin with acetic anhydride in pyridine gave the diacetate (**2**), $C_{15}H_{16}O_7$, m.p. 171—172 °C, which



(1) R = H
(2) R = COMe

showed ν_{max} 1775, 1722, and 1615 cm^{-1}. The ^1H n.m.r. spectrum of (**2**) confirmed the presence of a phenolic acetate (δ 2·39) and the acetylation of the hydroxymethy-group (δ 2·03 and 5·16, 3H and 2H singlets, respectively), and the downfield shift of the aromatic proton, to δ 6·68, suggested the presence of a phenol with a free *para*-position.[5] The *ortho*-relationship between the phenolic hydroxy and lactone carbonyl groups was shown by the presence of the low-field exchangeable proton in the ^1H n.m.r. spectrum of stellatin and the shift of the lactone carbonyl absorption from 1670 to 1722 cm^{-1} on acetylation.

The precise arrangement of substituents on the aromatic ring was unambiguously defined by analysis of the ^1H–^{13}C couplings observed in the fully ^1H-coupled ^{13}C n.m.r. spectrum of (**1**). The C-5 resonance, 101·2 p.p.m., appeared as a doublet of triplets (J 162 and 3 Hz), due to coupling to 5-*H* and 4-CH_2, respectively, collapsing to a simple doublet on selective low-power irradiation of 4-CH_2. This irradiation also changed the quintet (J 6 Hz) at 141·1 p.p.m. to a triplet. Irradiation of the 3-CH_2 also caused the quintet to collapse to a triplet so this resonance must be assigned to C-4a showing equal 2- and 3-bond couplings to the lactone ring methylene protons. The C-8 resonance appeared as a quartet (J 4 Hz) at 161·4 p.p.m. owing to coupling to the chelated phenolic proton and 9-CH_2, changed to a triplet on addition of D_2O, and further sharpened to a singlet on irradiation of 9-CH_2. This irradiation also sharpened the multiplet at 114·9 p.p.m. due to C-7 to a doublet (J 4 Hz) the residual coupling being the expected 3-bond coupling to the aromatic proton.[6] Addition of D_2O also caused the multiplet due to C-8a at 102·4 p.p.m. to sharpen, and irradiation of 4-CH_2 sharpened it further to a doublet (J 7 Hz), the residual coupling again being a 3-bond coupling to the aromatic proton. C-6 appeared as a broad unresolved resonance at 163·0 p.p.m. which sharpened on irradiation of either 9-CH_2 or OCH_3. The remaining 1, 3, 4, 9, and OMe carbon atoms, were readily assigned to the resonances at 169·3, 67·5, 28·0, 55·9, and 53·9 p.p.m., respectively. These observations can only be accommodated by structure (**1**) for stellatin.

Although dihydroisocoumarins are comparatively common fungal metabolites,[7] stellatin is unique in being unsubstituted at both C-3 and C-4. The overall structure is consistent with a polyketide origin. However, if stellatin is a tetraketide, C-3 must be derived by introduction of a methyl group from the C_1-pool on the methyl carbon of the chain-initiating acetate unit. Alternatively, if it is of pentaketide origin, the methyl carbon of the chain-initiating acetate must be lost. Neither of these processes have any firmly established precedent in polyketide biosynthesis.

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