The Biosynthesis of Fungal Metabolites. Part VII.[1] Production and Biosynthesis of 4,7-Dimethoxy-5-methylcoumarin in *Aspergillus variecolor*

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4,7-Dimethoxy-5-methylcoumarin (I) (4,7-dimethoxy-5-methylchromen-2-one) is shown to be a metabolite of *Aspergillus variecolor*, strain IMI 53749. Incorporation of [2-^{14}C]acetate into this natural product shows that it is derived on the β-ketide pathway. A synthesis of compound (I) is reported.

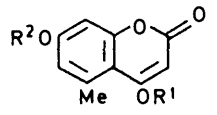
DURING an investigation [1] into the xanthone and dibenzoxepin metabolites of a number of variant strains of *A. variecolor*, a biogenetically unrelated metabolite, $C_{12}H_{12}O_4$, was isolated from strain IMI 53749. Initially this was produced in relatively large quantities together with arugosin C [1,2] and minor amounts of arugosins A and B.[1,3] In later cultures it was formed in decreasing quantities and finally production ceased altogether. At the same time the amount of arugosin C also decreased and was replaced by a mixture of shamixanthone,[1,4] epiisoshamixanthone,[1] 25-*O*-methylarugosin A,[1] arugosin D,[1] and sterigmatocystin.[1,5] Fortunately in the early stages of the investigation we were able to produce a sufficient quantity of the new metabolite for identification and to carry out a study of the incorporation of [2-^{14}C]acetate.

The ^{1}H n.m.r. spectrum of the new compound showed two *meta*-coupled aromatic protons [τ 3·34 and 3·42 (*J* 3 Hz)], a singlet vinyl proton (τ 4·48), two methoxy-groups (τ 6·10 and 6·20), and an aromatic methyl substituent (τ 7·41). The u.v. spectrum [$\lambda_{max.}$ 226, 288, 306, and 317 nm (ε 9000, 11,600, 14,700, and 12,700)] was closely similar to that of kotanin (IV), a recently characterised bicoumarin which occurs with the corresponding demethyl compound (V) in the related organism *A. clavatus*.[6] It therefore seemed likely that the new compound was the corresponding 4,7-dimethoxy-5-methylcoumarin (I).

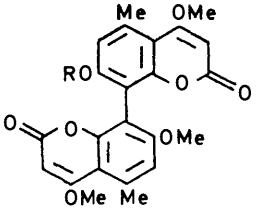
This structure was readily confirmed by synthesis. 4′,6′-Dimethoxy-2′-methylacetophenone (VI),[7] was monodemethylated with boron trichloride, a convenient reagent for the specific demethylation of *ortho*-methoxycarbonyl compounds,[8] to give the 6′-hydroxy-compound

[1] Part VI, K. K. Chexal, J. S. E. Holker, and T. J. Simpson, preceding paper.
[2] J. A. Ballantine, V. Ferrito, C. H. Hassall, and M. L. Jenkins, *J.C.S. Perkin I*, 1973, 1825.
[3] J. A. Ballantine, D. J. Francis, C. H. Hassall, and J. L. C. Wright, *J. Chem. Soc.* (*C*), 1970, 1175.
[4] K. K. Chexal, C. Fouweather, J. S. E. Holker, and T. J. Simpson, *J.C.S. Perkin I*, 1974, 1584.
[5] E. Bullock, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 1962, 4179.
[6] G. Büchi, D. H. Klaubert, R. C. Shank, S. M. Weinreb, and G. N. Wogan, *J. Org. Chem.*, 1971, **36**, 1143; G. Büchi, Y. Kitaura, S. S. Yuan, H. E. Wright, J. Clardy, A. L. Demain, T. Glinsukon, N. Hunt, and G. N. Wogan, *J. Amer. Chem. Soc.*, 1973, **95**, 5423.
[7] K. Hoesch, *Ber.*, 1915, **48**, 1122.
[8] F. M. Dean, J. Goodchild, L. E. Houghton, J. A. Martin, R. B. Morton, B. Parton, A. W. Price, and N. Somvichien, *Tetrahedron Letters*, 1966, 4153.

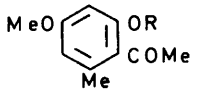
(VII). Treatment of this with ethyl chloroformate gave the ethoxycarbonyl derivative (VIII), which was converted into 4-hydroxy-7-methoxy-5-methylcoumarin (II) with potassium t-butoxide under the same conditions as those used in the synthesis of kotanin.[6] Methylation of 4-hydroxycoumarins with dimethyl sulphate gives only



(I) $R^1 = R^2 = Me$
(II) $R^1 = H, R^2 = Me$
(III) $R^1 = R^2 = H$



(IV) R = Me
(V) R = H



(VI) R = Me
(VII) R = H
(VIII) $R = CO_2Et$

the corresponding 4-methoxycoumarins, whereas use of diazomethane gives both this type of compound and the isomeric 2-methoxychromone.[6] The 4-hydroxycoumarin (II) was methylated with dimethyl sulphate to give 4,7-dimethoxy-5-methylcoumarin (I), identical with the natural product.

Since the completion of this work, siderin, a metabolite of *Sideritis canariensis* Ait. and *S. romana* L. (Labiatae), originally thought to be 6,7-dimethoxy-4-methylcoumarin,[9] has been shown to be 4,7-dimethoxy-5-methylcoumarin.[10] This compound has been synthesised by methylation of the corresponding 4,6-dihydroxy-compound (III), obtained by condensation of orcinol with malonic acid.

Formally, the metabolite (I) could be considered to be derived biogenetically from acetate and malonate on the β-ketide pathway with introduction of the two *O*-methyl groups from the C_1 pool, or on the shikimate–prephenate pathway with introduction of the *C*-methyl group from the C_1 pool. In fungi, the operation of the β-ketide pathway seemed more likely but it has been pointed out that in the cyclisation of a poly-β-ketide intermediate the uncyclised residues from the methyl ends of the chains are never shorter than the residues from the carbonyl ends.[11] Thus, a β-ketide origin for compound (I) would require an exceptional cyclisation of the pentaketide intermediate. Hence, the biogenetic origin of this compound in *A. variecolor* was worthy of investigation.

[2-^{14}C]Acetate was incorporated by *A. variecolor* into the metabolite (I) with 0·75% overall efficiency. Kuhn–Roth oxidation of the labelled metabolite gave acetic acid, which was isolated and counted as the *p*-bromophenylacyl ester. This contained 25·8% of the total activity of the starting material. Even incorporation of acetate into a pentaketide-derived compound would require 20% activity in the acetic acid. However, since this degradation involves the isolation of the starting acetate it is perhaps not surprising that it has a high activity, especially as this particular feeding experiment involved rather large amounts of acetate precursor. It would have been desirable to investigate the distribution of label in other parts of the molecule but lack of material precluded any further degradations. However, we feel that the result justifies the conclusion that the fungal metabolite is derived on the β-ketide pathway.

It would be interesting to see if siderin is produced on the same pathway in higher plants.

EXPERIMENTAL

Unless otherwise stated, i.r. spectra were measured with a Perkin-Elmer 257 instrument for KBr discs, u.v. spectra with a Unicam SP 800 instrument for solutions in ethanol, and ^{1}H n.m.r. spectra with a Varian HA-100 instrument for solutions in deuteriochloroform containing tetramethylsilane as internal standard. Radioactivity measurements were made by liquid scintillation counting [Packard 3003 Tricarb Scintillation Spectrometer and Butyl-PBD (CIBA) scintillator solution]. Counting efficiencies were determined with [^{14}C]hexadecane as internal standard. M.p.s were determined with a Kofler hot-stage instrument.

4,7-Dimethoxy-5-methylcoumarin (I) *from* A. variecolor (IMI 53749).—This organism was grown in flat vessels each containing Czapek-Dox medium (500 ml) as previously described.[1,4] The dried mycelium was exhaustively extracted with light petroleum (b.p. 60—80°) and the solution evaporated. The residue (1·1 g l^{-1}) was dissolved in the minimum volume of hot methanol and the crude product separated on cooling. Recrystallisation from hot methanol gave 4,7-dimethoxy-5-methylcoumarin (I) (4,7-dimethoxy-5-methylchromen-2-one) as needles (100 mg l^{-1}), m.p. 194—195° (lit.,[10] 194—195°), $\nu_{max.}$ 1725, 1625, and 1612 cm^{-1} (Found: C, 65·3; H, 5·5. Calc. for $C_{12}H_{12}O_4$: C, 65·4; H, 5·5%). The methanolic mother liquors were used in the isolations of the xanthones and dibenzoxepins previously described.[1]

6′-Hydroxy-4′-methoxy-2′-methylacetophenone (VII).—4′,6′-Dimethoxy-2′-methylacetophenone (VI), m.p. 45—47° (lit.,[7] 48°) (1 g), in dichloromethane (20 ml) was stirred at 0° with a large excess of boron trichloride for 5 min and the mixture was then quenched in water (30 ml). The dichloromethane layer was separated, washed with water (2 × 10 ml), dried ($MgSO_4$), and evaporated to give a dark brown solid. This was crystallised from hexane giving long needles of the monomethyl ether (VII) (0·8 g), m.p. 78—79° (lit.,[7] 79°).

4-Hydroxy-7-methoxy-5-methylcoumarin (II).—Compound (VII) (0·7 g) and ethyl chloroformate (2·8 g) in pyridine (3 ml) were heated at 85° for 5 h under nitrogen. The cooled solution was poured into 2M-hydrochloric acid (20 ml) and the product isolated in chloroform (2 × 50 ml). Evaporation left the crude carbonate (VIII) (0·9 g), which was

[9] A. G. Gonzàles, B. M. Fraga, M. G. Hernandez, and J. G. Luis, *Phytochem.*, 1972, **11**, 2115.

[10] P. Venturella, A. Bellino, and F. Piozzi, *Tetrahedron Letters*, 1974, 979.

[11] W. B. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, p. 198.

dissolved in a solution of potassium t-butoxide in t-butyl alcohol (80 ml) [from potassium (1·0 g)] and heated under reflux for 4 h. After cooling, the solution was poured into 2M-hydrochloric acid (200 ml) and the resultant yellow solid collected. Crystallised from methanol, *4-hydroxy-7-methoxy-5-methylcoumarin* (II) (4-hydroxy-7-methoxy-5-methylchromen-2-one) formed needles (0·5 g), m.p. 264—265°, $\nu_{max.}$ 1690 and 1612 cm^{-1}, $\lambda_{max.}$ 234, 289, 309, and 319 nm (ε 8850, 10,800, 18,800, and 10,900), τ ($CDCl_3$–CF_3·CO_2H) 3·14 (ArH, d, *J* 3·5 Hz), 3·20 (ArH, d, *J* 3·5 Hz), 3·70 (3-H, s), 6·10 (OMe, s), and 7·24 (5-Me, s) (Found: C, 63·9; H, 4·8. $C_{11}H_{10}O_4$ requires C, 64·1; H, 4·9%).

4,7-Dimethoxy-5-methylcoumarin (I).—Prepared from compound (II) (410 mg) with dimethyl sulphate (700 mg) and anhydrous potassium carbonate (1 g) in 1,2-dimethoxyethane (100 ml), 4,7-dimethoxy-5-methylcoumarin separated from methanol in needles (250 mg), m.p. and mixed m.p. with the natural product, 194—195°. The samples had identical i.r., u.v., and n.m.r. spectra.

[^{14}C]-*4,7-Dimethoxy-4-methylcoumarin.*—*A. variecolor*, IMI 53749, was grown as previously described, except that on the seventh day sodium [2-^{14}C]acetate (100 mg; 0·50 μCi) in water was added to each of 5 flasks (500 ml culture fluid in each) under aseptic conditions. After a further 21 days the cultures were harvested and [^{14}C]-4,7-dimethoxy-4-methylcoumarin was isolated as previously described. The compound was purified by crystallisation from methanol to constant radioactivity (12 mg, and $3·73 \times 10^{-3}$ μCi per pan). The overall efficiency of incorporation was thus 0·75%.

[^{14}C]-p-*Bromophenacyl Acetate.*—[^{14}C]-4,7-Dimethoxy-4-methylcoumarin (52 mg; $3·73 \times 10^{-3}$ μCi $mmol^{-1}$) was subjected to Kuhn–Roth oxidation and the resultant [^{14}C]acetic acid was converted into its *p*-bromophenacyl ester, which was purified by preparative t.l.c. on silica gel GF (Merck), followed by crystallisation from light petroleum (b.p. 60—80°) to constant radioactivity (9 mg; $9·62 \times 10^{-4}$ μCi $mmol^{-1}$); m.p. 82—83°.

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