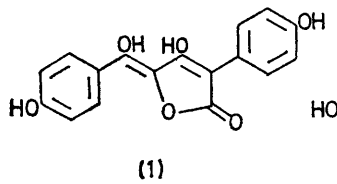
Specificities of Enzymatic Prenylation and Chromanation in the Biosynthesis of Aspulvinone Pigments in *Aspergillus terreus*

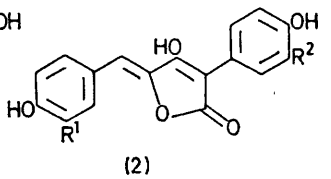
By DAVID W. KNIGHT and GERALD PATTENDEN*
(*Chemistry Department, The University, Nottingham* NG7 2RD)

Summary Unambiguous syntheses of key intermediates establish that the biosynthesis of aspulvinone (**6**) from (**1**) *via* (**3**) in *Aspergillus terreus* proceeds by selective mono-prenylation of (**1**) leading to intermediate (**2a**) and by selective 'chromanation' of (**3**) leading to intermediate (**4**).

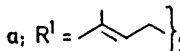
BIOSYNTHETIC experiments with a cell-free system from *Aspergillus terreus* have led to the suggestion that the aspulvinone pigment (**6**) originates from the 4,4'-dihydroxy compound (**1**) by step-wise enzymatic prenylation of the aryl rings leading to (**3**), followed by step-wise cyclisation ('chromanation').[1,2] The suggested pathway poses two

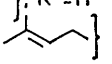


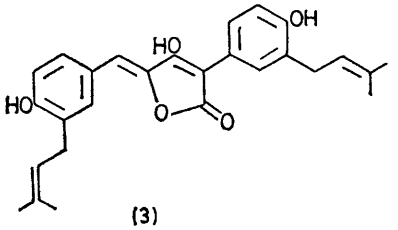
(1)



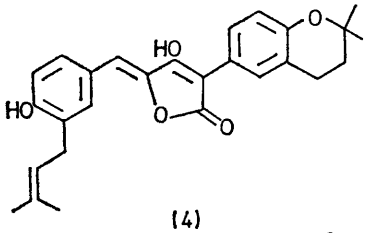
(2)

a; R^1 =  , R^2=H

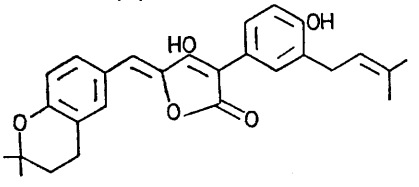
b; R^1=H, R^2= 



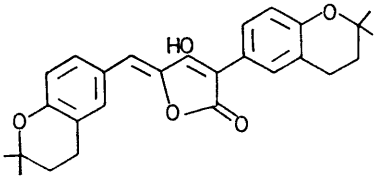
(3)



(4)

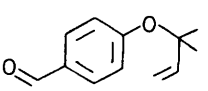


(5)

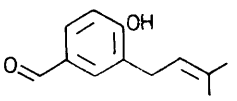


(6)

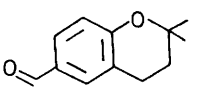
questions: (a) which aryl ring in (**1**) is first prenylated, and (b) is chroman (**4**) or chroman (**5**) the intermediate between (**3**) and (**6**)? A mono-prenylated aspulvinone, *viz.* (**2a**) or (**2b**), has recently been isolated along with (**3**) from young cultures of *A. terreus*, but these disappear rapidly as the culture grows older and are replaced by other aspulvinone pigments which include (**6**) and (**4**) or (**5**). Spectral data have failed to distinguish between (**2a**) and (**2b**) or (**4**) and (**5**). We have attempted to distinguish between these alternatives by effecting unambiguous syntheses of the unsymmetrical aspulvinones (**15**) and (**16**).



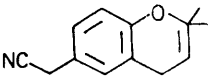
(7)



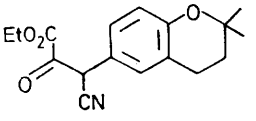
(8)



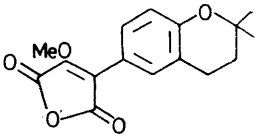
(9)



(10)

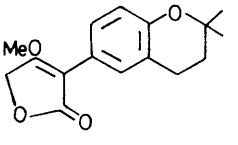


(11)

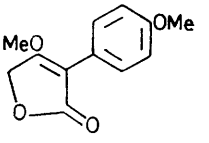


(12)

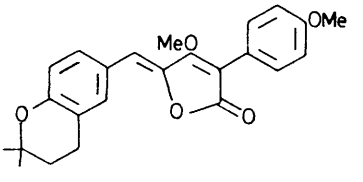
4-Hydroxybenzaldehyde was first converted into the 3-prenylated derivative (**8**), following conversion into the allyl ether (**7**) and Claisen rearrangement.[3] Cyclisation in acid (10% H_2SO_4, 100 °C) led next to the chroman (**9**) which was converted into the nitrile (**10**) *via* the corresponding benzyl alcohol and benzyl chloride. Condensation between the anion produced from (**10**) and diethyl oxalate then gave the pyruvate (**11**), which, by methylation followed by acid hydrolysis gave rise to the anhydride (**12**), yellow needles, m.p. 141—142 °C, ν_{max} 1836, 1761, and 1642 cm^{-1}. Regio-



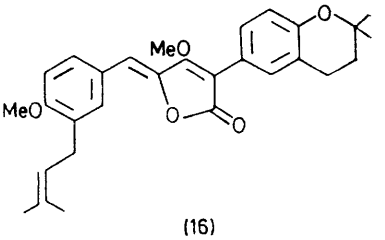
(13)



(14)



(15)



(16)

selective reduction of (**12**) with $LiAlH_4$ (1 h, −60 °C, then 1 h, −30 to 0 °C) then led to the key intermediate butenolide (**13**). In a closely similar sequence the butenolide (**14**) was prepared from 4-hydroxybenzaldehyde.[4]

Metallation of the butenolide (**14**) at −78 °C in tetrahydrofuran, using lithium *N*-cyclohexyl-*N*-isopropylamide, followed by treatment with the chroman-aldehyde (**9**) and dehydration of the intermediate carbinol gave the aspulvinone (**15**), yellow-green plates, m.p. 168—169 °C, ν_{max} 1748 cm^{-1}, τ 2·48—2·74 (4H, m), 3·15 (2H, d, *J* 9 Hz), 3·31 (1H, d, *J* 9 Hz), 3·88 (1H), 6·2 (OMe), 7·21 (2H, t, *J* 7 Hz), 8·19 (2H, t, *J* 7 Hz), and 8·66 (Me_2). This aspulvinone was identical (m.p., i.r., ^1H n.m.r.) with the compound derived from a mono-prenylated metabolite, m.p. 183—185 °C found in young cultures of *A. terreus*,[2] following acid cyclisation to the corresponding chroman and methylation. In a similar sequence the chroman-butenolide (**13**) and the methyl ether derivative of (**8**) led to the aspulvinone (**16**), m.p. 106—108 °C, ν_{max} 1755 cm^{-1}, τ 2·27 (1H, dd, *J* 9 and 2 Hz), 2·51 (1H, d, *J* 2 Hz), 2·73 (1H, d, *J* 9 Hz), 2·8 (1H, dd, *J* 9 and 2 Hz), 3·13 (1H, d, *J* 9 Hz), 3·21 (1H, d, *J* 9 Hz), 3·79 (1H), 4·7 (1H, t, *J* 6·5), 6·14 (3H), 6·16 (3H), 6·67 (2H, d, *J* 6·5 Hz), 7·19 (2H, t, *J* 7 Hz), 8·18 (2H, t, *J* 7 Hz), 8·25 (6H), and 8·65 (6H), identical with the dimethyl derivative of the mono-chromanated intermediate between (**3**) and (**6**) found in *A. terreus*.† It follows that the initially-formed prenylated metabolite in the biosynthesis of aspulvinone (**6**) is represented by (**2a**), and that chroman (**4**), not (**5**) is the most likely immediate precursor of (**6**).

We thank Dr. S. Seto of Tohoku University, Japan for exchange of information, and the S.R.C. for a studentship (to D.W.K.)

(*Received*, 20*th May* 1976; *Com.* 568.)

† The present study establishes that the structure earlier assigned to this metabolite is incorrect.[5]

[1] N. Ojima, K. Ogura, and S. Seto, *J.C.S. Chem. Comm.*, 1975, 717.
[2] N. Ojima, I. Takahashi, K. Ogura, and S. Seto, *Tetrahedron Letters*, 1976, 1013.
[3] *Cf.* K. Kyogoku, H. Hatayama, S. Yokomori, T. Seki, and I. Tanaka, *Agric. Biol. Chem.*, 1975, **39**, 133, 667.
[4] *Cf.* D. W. Knight and G. Pattenden,[1] *J.C.S. Chem. Comm.*, 1975, 876; M. J. Begley, D. W. Knight, and G. Pattenden, *Tetrahedron Letters*, 1975, 4279.
[5] N. Ojima, T. Takenaka, and S. Seto, *Phytochemistry*, 1975, **14**, 573; *cf.* also M. J. Begley, D. W. Knight, and G. Pattenden, *Tetrahedron Letters*, 1976, 131.