Biosynthesis of Flavonoid and Terphenyl Metabolites by the Fungus *Aspergillus candidus*

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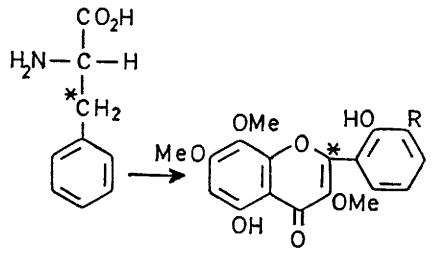
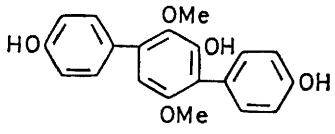
Summary Chlorflavonin and two accompanying phenylalanine-derived metabolites, characterized from spectral evidence as dechlorochlorflavonin and 1,4-dimethoxy-2,4',4''-trihydroxy-*p*-terphenyl, are biosynthesized *de novo* by *Aspergillus candidus*.

FLAVONOIDS are generally accepted to be characteristic metabolites of higher plants,[1] and most accounts of their formation by micro-organisms have been either poorly documented[2] or subsequently found to be in error.[3] A recent report on the isolation of the antibiotic chlorflavonin from *Aspergillus candidus* established its flavonoid structure (Ia), but did not distinguish between two possible origins: *de novo* synthesis or bioconversion of a flavonoid constituent in the corn steep nutrient. These alternatives have been tested with isotopically labelled substrates.

High (153%) specific incorporation of [*U*-^{14}C]-D-glucose was observed. Exclusive labelling *via* methionine of the *O*-methyl groups is unlikely since [*Me*-^{14}C]-L-methionine was a less efficient (32·7%) precursor than [*U*-^{14}C]-D-glucose and both [*U*-^{14}C] and [3-^{14}C]-L-phenylalanine labelled the antibiotic (27·6 and 18·5% respectively). Degradation of chlorflavonin with alkali and decarboxylation of the recovered 3-chlorosalicylic acid[4] established that label from [3-^{14}C]-L-phenylalanine was located exclusively in C-3. We conclude that this unique chlorinated flavonoid is a true fungal metabolite.



(Ia) R = Cl
(Ib) R = H



(II)

We have isolated from cultures of *A. candidus* two additional metabolites biosynthetically labelled by [*U*-^{14}C]-L-phenylalanine. One of these, $C_{18}H_{16}O_7$, m.p. 208° is probably dechlorochlorflavonin, (Ib) although an alternative structure with 5-hydroxy-6,7-dimethoxy substitution in ring A has not been excluded; λ_{max} (EtOH) 252 (inflex.), 265, 304 (inflex.), and 346 nm (log ϵ, 4·32, 4·41, 3·82 and 3·88); M^+ at *m/e* 344·0898, τ (60 MHz; $CDCl_3$; Me_4Si) −2·2 (1H, s, exchanges with D_2O, chelated OH), 1·97br (1H, s, exchanges with D_2O, unchelated OH), 2·15—3·1 (4H, complex m, ABCD system, ArH), 3·55 (1H, s, ArH), 6·04 (3H, s, OMe), and 6·13 (6H, s, OMe). Radioactivity from ^{14}C-labelled (Ib) was incorporated irreversibly into (Ia).

The other metabolite, $C_{20}H_{18}O_5$, m.p. 239° (decomp.), showed λ_{max} (EtOH) 225 (inflex.) and 275 nm (log ϵ, 4·06 and 4·06); M^+ at *m/e* 338·1168 with fragment ions at *m/e* 245 ($M - C_6H_5O)^+$ and 154 ($M - C_6H_5O - C_6H_3O)^+$. The n.m.r. spectrum [100 MHz; $(CD_3)_2CO$; with Me_4Si] showed temperature-dependent hydroxy-signals at τ 1·63br (1H, s), 1·83br (1H, s), and 2·58 (1H, s); aromatic protons at τ 2·5 (2H, d, *J* 8·7 Hz) and 3·08 (2H, d, *J* 8·7 Hz) comprising one AA'BB' system, and at 2·76 (2H, d, *J* 8·8 Hz) and 3·17 (2H, d, *J* 8·8 Hz) comprising a second AA'BB' system. An aromatic proton at τ 3·53 (1H, s) was weakly coupled (*J* 0·5 Hz) to an aromatic methoxy-group at τ 6·32 (3H, s, *J* 0·5 Hz) but was not coupled to a second aromatic methoxy-group at τ 6·63 (3H, s). The evidence is consistent with structure (II) for this metabolite. *p*-Terphenyl derivatives are rare in microfungi, the only other example being volucrisporin, a pigment of the Hyphomycete *Volucrispora aurantiaca* which is known to be derived biosynthetically from phenylalanine.[5]

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