Biosynthesis of Chlorflavonin in *Aspergillus candidus*: A Novel Fungal Route to Flavonoids

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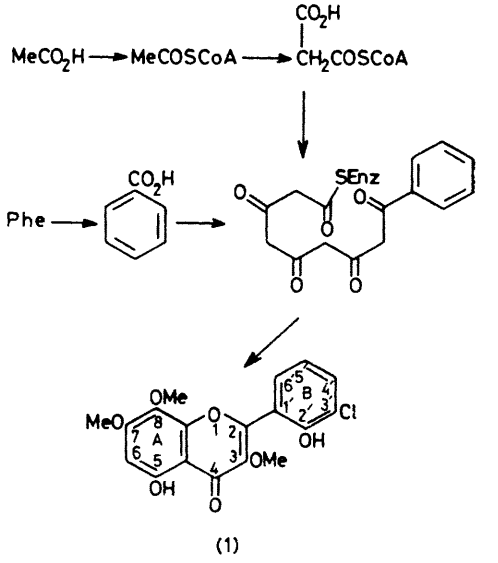
Summary The distribution of radioactivity in chlorflavonin from cultures of *Aspergillus candidus* supplemented with [1-^{14}C]acetate, [α-^{14}C]cinnamate, and [7-^{14}C]benzoate indicates that this flavonoid antibiotic is biosynthesized from one C_6C_1 and four C_2 precursors.

AMONG the large class of flavonoid natural products chlorflavonin (**1**) and the congeneric dechloro-compound are unique in their fungal origin.[1,2] As with the flavonoids of higher plants,[3] phenylalanine is a biosynthetic precursor and chemical degradation of the product labelled from L-[β-^{14}C]-phenylalanine located all of the radioactivity at C-2.[2] [1-^{14}C]Acetate, a specific precursor of ring A of the plant flavonoids, is incorporated less efficiently than L-[β-^{14}C]-phenylalanine into chlorflavonin (specific incorporation 2·5 *vs.* 9·9%). Cinnamate is a known intermediate on the route from phenylalanine to the plant flavonoids. Radioactivity from [α-^{14}C]- and [β-^{14}C]-cinnamate was incorporated into chlorflavonin with an efficiency similar to that of [1-^{14}C]acetate.

To ascertain whether *A. candidus* possesses a novel biosynthetic pathway, samples of chlorflavonin derived from [1-^{14}C]acetate, [α-^{14}C]cinnamate, and [β-^{14}C]cinnamate were degraded with alkali[2] and the distribution of radioactivity into 4,5-dimethoxyresorcinol and 3-chlorosalicylic acid, derived from rings A and B, respectively, was measured. The Table shows that the label from [1-^{14}C]-acetate was not present exclusively in ring A and that [α-^{14}C]cinnamate labelled ring A heavily. The results can be accounted for if C-3 and C-4, as well as ring A originate from acetate. Thus the distribution of the label from [α-^{14}C]cinnamate is due to its prior metabolism to [^{14}C]-acetate; the reduced incorporation efficiency of acetate compared with phenylalanine is attributed to stronger metabolic competition for the precursor.



(1)

SCHEME. Proposed route for the biosynthesis of chlorflavonin.

TABLE. Distribution (%) of radioactivity in the degradation products of chlorflavonin.

Precursor	4,5-Dimethoxy-resorcinol	3-Chloro-salicylic acid	Barium carbonate[a]
L-[β-^{14}C]Phenyl-alanine	Nil	102	102
[β-^{14}C]Cinnamate	Nil	103	102
[α-^{14}C]Cinnamate	48·2	3·7	—
[7-^{14}C]Benzoate	0·13	97·6	94·0
[1-^{14}C]Acetate	66·5	1·35	—

[a] Obtained by decarboxylation of 3-chlorosalicylic acid.

Specific incorporation of [7-^{14}C]benzoate into chlorflavonin (Table) is consistent with such a pathway. In a parallel experiment no radioactivity was incorporated from [7-^{14}C]salicylic acid, suggesting the route in the Scheme as the most likely for the biosynthesis of flavonoids in *A. candidus*. Here, as in the pathways used to make certain quinonoid secondary metabolites[4] plants and fungi seem to have discovered independent solutions to a biosynthetic problem.

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