# Biosynthesis of Citrinin: Incorporation Studies with Advanced Precursors

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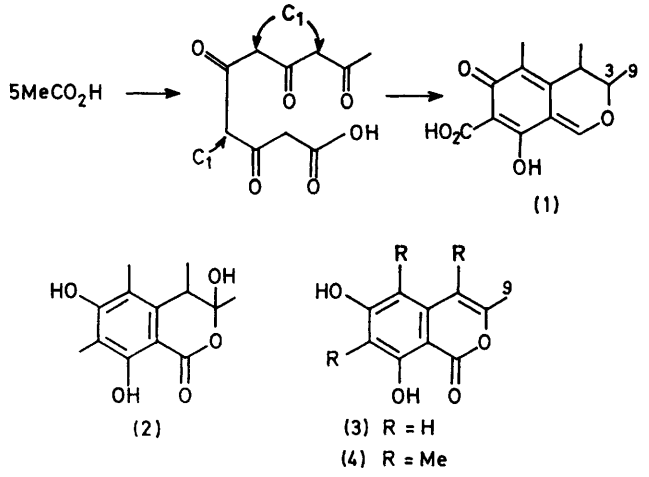
*Summary* 6,8-Dihydroxy-3,4,5,7-tetramethylisocoumarin is incorporated specifically into citrinin by *Penicillium citrinum*.

THE antibiotic fungal metabolite citrinin (**1**) is derived from five acetate units and three $C_1$-units as shown in the Scheme.[1] A possible intermediate in its biosynthesis is sclerotinin A (**2**) which has been isolated from a mutant of *Penicillium citrinum*, a fungus which usually produces citrinin.[2]




SCHEME

We have carried out incorporation experiments with the possible advanced precursors (**3**) and (**4**); the latter compound may be interconvertible with sclerotinin A under the conditions of the incubation. Both compounds were labelled with $^{14}C$ at C(9) and were administered to intact surface cultures of *P. citrinum*.

Citrinin incorporated 0·7% of the activity from the isocoumarin (**3**). The specificity of labelling was checked by a minor variation of the degradation used by Rodig *et al.*[3] which produces acetophenone in which the acetyl group is derived from C(3) together with its attached methyl. The molar activity of the acetophenone was only 20% of that of the metabolite, from which we conclude that (**3**) is not a true precursor but is degraded to [2-$^{14}C$]acetate prior to incorporation.

The trimethylated analogue (**4**) also gave a significant incorporation (0·05%). In this case the specificity of the labelling in the citrinin was checked by Kuhn–Roth oxidation followed by a Schmidt degradation of the derived acetic acid. The results of this experiment and a control incorporation study using [2-$^{14}C$] acetate are given in the Table. They show that activity is incorporated specifically from (**4**) which is therefore converted directly into citrinin without prior degradation to acetate.[4] In spite of this specific incorporation, further work described in the following communication has shown that the isocoumarin

TABLE. Incorporation experiments

| Expt. | Precursor | % Molar activity of acetic acid[a] | % Molar activity of methylamine[b] |
|---|---|---|---|
| 1 | [9-$^{14}C$]-(**4**) | 28·1 | 28·0 |
| 2 | [2-$^{14}C$]Acetate | 22·2 | 7·3 |

[a] Counted as *p*-bromophenacyl derivative. [b] Counted as *p*-bromo-*N*-methyl benzoylamide.

(**4**) is not an obligatory intermediate on the normal biosynthetic pathway but that it must be converted *in situ* to a true biosynthetic intermediate such as sclerotinin A (**2**).

From the failure of (**3**) to give a specific incorporation we infer that at least one, and possibly all three, of the $C_1$-units are added prior to aromatisation of the polyketone chain; previous work on the timing of the introduction of the methyl groups has produced inconclusive results.[1,3] Similar conclusions have been reached recently concerning the timing of the methylation steps in the biosynthesis of ascochitine.[5]

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