Biosynthesis of Sclerin: Incorporation Studies with Advanced Precursors

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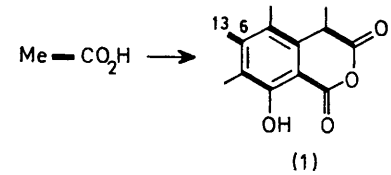
Summary Evidence is presented that sclerin, a metabolite of *Sclerotinia sclerotiorum*, is derived *via* novel structural reorganisation of sclerotinin A, one of its co-metabolites.

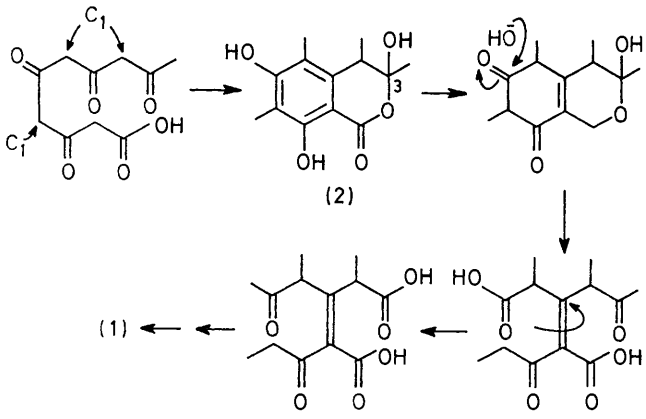
SCLERIN (**1**),[1] a metabolite of the fungus *Sclerotinia sclerotiorum*, acts as a growth promoter in plants[2] and has been shown to be acetate-derived.[3] We have recently studied the incorporation of [1,2-$^{13}C_2$] acetate into (**1**);[4] five acetate units are incorporated intact as shown in Scheme 1.



(1)

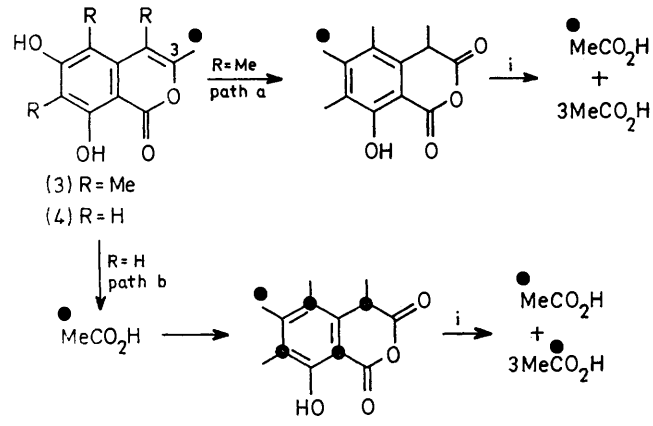
SCHEME 1

Similar results were obtained by Holker and Cox[5] and by Yamazaki *et al.*[6] These authors concluded that the biosynthesis might involve condensation of two separately formed polyketone chains. However, we suggested that sclerin might be formed by novel structural reorganisation of one of its co-metabolites, sclerotinin A (**2**). Our suggestion (Scheme 2) is supported by the report[6] that only one starter unit [C(6) + C(13)] is incorporated into the nucleus of sclerin.



SCHEME 2

We have tested our proposal by carrying out incorporation experiments with the potential advanced precursors (**2**), (**3**), and (**4**), labelled in each case with ^{14}C in the methyl group attached to C(3). The results, given in the Table, show that activity was incorporated into sclerin from all three compounds; the metabolite was recrystallised to constant activity from two separate solvents. The specificity of incorporation was tested by a Kuhn–Roth oxidation followed by Schmidt degradation of the resultant acetic acid. For comparison, sclerin labelled with [2-^{14}C]acetate was subjected to the same degradation. In this case (Table, expt. 1), the molar activity of the derived acetic acid was 20% that of the metabolite and the ratio of the activities at C(1) and C(2) was 3:1. This result is consistent with acetic acid being produced with equal efficiency from all four *C*-methyl groups in the Kuhn–Roth degradation (see Scheme 3). Significantly, the same result was obtained with the sclerin derived from 6,8-dihydroxy-3-methylisocoumarin (**4**) (Table, expt. 2). We conclude therefore that this compound is degraded to [2-^{14}C]acetate prior to incorporation (Scheme 3, path b); a similar result was obtained in our earlier investigation of terrein biosynthesis.[7]



SCHEME 3. i, Kuhn–Roth oxidation. ● = ^{14}C.

In contrast, degradation of sclerin derived from 6,8-dihydroxy-3,4,5,7-tetramethylisocoumarin (**3**) and from sclerotinin A (**2**) gave acetic acid with a molar activity which was 25% that of the metabolite, consistent with a specific incorporation (Scheme 3, path a). This conclusion was confirmed in expt. 4 (Table) by Schmidt degradation of the acetic acid to methylamine; this was found to contain all the activity of the acetic acid. The relatively low incorporations of these advanced precursors may be attributed to the low permeability of the fungal cell to these compounds.

TABLE. Incorporation of advanced precursors into sclerin

Expt.	Precursor	% Incorporation into sclerin	% Molar activity in acetic acid[a] ($C_1 + C_2$)	% Molar activity[b] [$C_1/(C_1+C_2)$]
(1)	[2-^{14}C]Acetate	1·34	18·6	27·1
(2)	[9-^{14}C]-6,8-Dihydroxy-3-methylisocoumarin	0·69	19·1	26·1
(3)	Sclerotinin A ([9-^{14}C]-3,4-Dihydro-3,6,8-trihydroxy-3,4,5,7-tetramethylisocoumarin) (**2**)	0·01	25·9	—
(4)	[9-^{14}C]-6,8-Dihydroxy-3,4,5,7-tetramethylisocoumarin (**3**)	0·045	25·6	99·2

[a] Counted as *p*-bromophenacyl derivative. [b] Obtained by Schmidt degradation; methylamine counted as *p*-bromo-*N*-methylbenzoylamide.

The specific incorporations of (**2**) and (**3**) (which may be interconvertible prior to incorporation) are consistent with formation of the sclerin nucleus by rearrangement of the aryl ring of a conventional polyketide. This remarkable transformation may take place by non-oxidative cleavage of the aryl ring as shown in Scheme 2.

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