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BIOSYNTHESIS OF SCLERIN

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The structure (1) of sclerin (I), a physiologically active metabolite produced by Sclerotinia fungus (2), poses an interesting problem in the biosynthesis of acetogenins. It contains three adjacent methyl groups on the phenol ring and biogenetic routes A or A' would be thought out in order to accomodate to the postulated alkylation (3) of a polyketide at the methylene site. These routes, however, involve rather unfamiliar way of cyclization as a derivative of five acetyl units. On the other hand, the occurrence of sclerotinin A (II) and B (III) as co-metabolites of sclerin (4) tempts to presume the pathway (b), including more common cyclization. In fact II was recently found in the metabolites of Penicillium citrinum (5), a fungus producing citrinin (V), for which the biogenetic scheme (b') is demonstrated (6). But the presumption of path (b) requires the acceptance of unusual alkylation of the polyketide at the ketonic site (7). It should be also noted that the routes postulated so far include the rupture of one carbon atom, a situation against biogenetic economy.

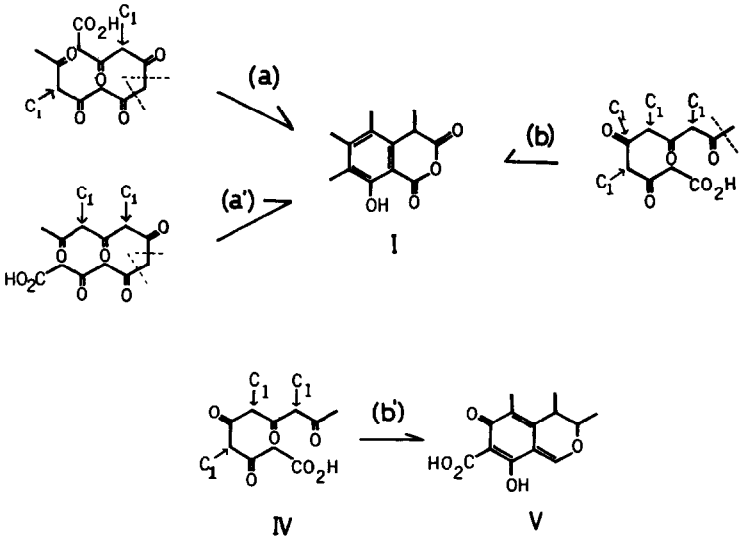

FIG. I

The present paper deals with the result of tracer study to clarify these points. Sodium acetate-1-^{14}C, -2-^{14}C and sodium formate-^{14}C was administered at the 4th day of culture of Sclerotinia sclerotiorum (Lib.) de Bary on 5% bran extract, added with 0.3% $CaCO_3$. The cultures were incubated with shaking at 25° for further 4 days and harvested. The isolated sclerin showed the incorporation ratios 0.9%, 2.3% and 1.2% respectively. The labelled sclerin thus obtained was, after appropriate dilution, degraded as outlined in FIG. II. The decarboxylation of VI was carried out at

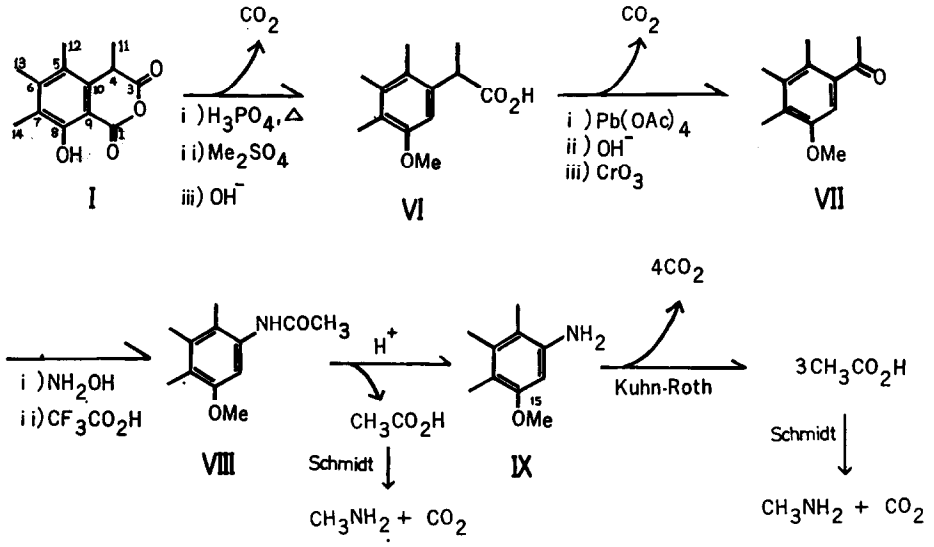

FIG. II

60° by means of lead tetraacetate in acetic acid solution (8). Since this reagent give no appreciable amounts of carbon dioxide in acetic acid solution except at the temperature near its boiling point (9), lead tetraacetate method can be used as a substitute of Schmidt reaction in the tracer study for secondary or tertiary acids which are easily decarboxylated as in the present case. It has an advantage that strongly acidic or dehydrating or sulphonating condition could be avoided. The radioactivity of the product was measured as $BaCO_3$ at infinite thickness, after the oxidation by Van Slyke method (10) for the products other than carbon dioxide, by a gas flow counter. The obtained values are listed in TABLE I.

TABLE I Percentage Distribution of Radioactivity*

	Acetate-1-^{14}C	Acetate-2-^{14}C	Formate-^{14}C
I	100% (100%)	100% (100%)	100% (100%)
CO_2(C-1)	20.1 (20.0)	1.1 (0.0)	0.4 (0.0)
VI	83.3 (80.0)	107.1 (100.0)	102.6 (100.0)
CO_2(C-3)	18.7 (20.0)	1.0 (0.0)	0.3 (0.0)
VII	62.5 (60.0)		
VIII	59.3 (60.0)	109.9 (100.0)	103.2 (100.0)
CH_3CO_2H(C-4,11)	0.1 (0.0)		
CO_2(C-4)		17.7 (20.0)	0.3 (0.0)
CH_3NH_2(C-11)		0.5 (0.0)	28.5 (33.3)
IX	58.7 (60.0)	83.1 (80.0)	67.4 (66.6)
CO_2(C-8,9,10,15)	28.8 (40.0)	25.3 (20.0)	10.6 (0.0)
CO_2(C-5,6,7)	19.8 (20.0)	17.0 (20.0)	1.2 (0.0)
CH_3NH_2(C-11,12,13)	0.0 (0.0)	34.5 (40.0)	62.7 (66.6)

The label pattern disclosed by the inspection of these results clearly indicates the validity of acetate-malonate pathway in general on sclerin biosynthesis. The number of the labelled carbon atoms in the nuclear methyl groups as shown by the analyses of acetic acid obtained by Kuhn-Roth oxidation of IX was 0, 1/3 and 2/3 respectively for the administration of sodium acetate-1-^{14}C, -2-^{14}C and sodium formate-^{14}C as substrates. The route (a) or (a') is obviously untenable for the explanation of these results (11). The remaining biosynthetic scheme which is consistent with the observed label pattern would be the path (c), representing condensation of two distinct polyketo chains. The biosynthesis in this way have been being established in an increasing number of examples — namely citromycetin (13), mollisin (14), rotiorin (15) and sulochrin (16). From the assumption of citrinin type intermediates (IV) for sclerotinin A (II) and B (III), it follows that two different ways of polyketo chain cyclization exist for the biosyntheses of two closely related substances, produced by the same fungus. It might be more rational to deduce that the branching of biosynthetic route in Sclerotinia is merely the result of the condensation of two common polyketide intermediate in two different directions as depicted [paths (c) and (d)]. There would be a possibility that the biosynthesis of acetogenins from the separate polyketide precursor is operative in wider range than postulated at present (e.g. citrinin) and re-investigations would be necessary for ambiguous cases. It may be pertinent for solution of the problem to determine the number of carbon atoms, derived from chain-initiating acetate unit.

* The values in parentheses denote the theoretical distribution.

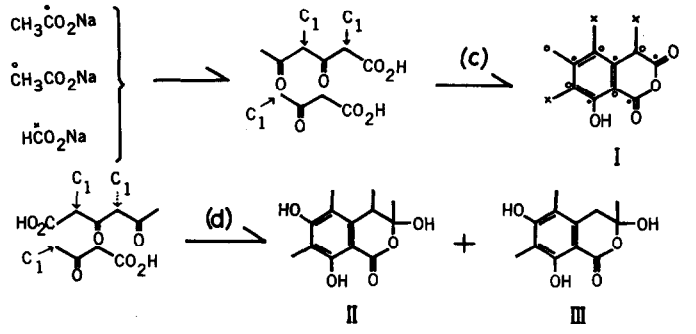

FIG.III

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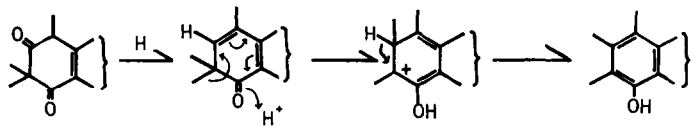
7. 1,2-Shift of a dimethylated intermediate like X or a novel mechanism of the carbonyl carbon methylation would be taken into account.



X

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