699. *Studies in Relation to Biosynthesis. Part XXX.* Rotiorin, Monascin, and Rubropunctatin.*

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The biogenesis of the mould metabolites rotiorin (II), monascin (III), and rubropunctatin (IV) has been examined by using $Me\cdot{}^{14}CO_2H$ and $H\cdot{}^{14}CO_2H$. In agreement with prediction they arise in part from both sources. The extent of introduction of label from $Me\cdot{}^{14}CO_2H$ into different parts of the molecule does not vary significantly.

The mould metabolites sclerotiorin [1] (I), rotiorin [2,3] (II), monascin [4] (III), and rubropunctatin [3] (IV) form a biogenetic family which may include other compounds, notably citrinin [5] (V). On the basis of structure analysis, supported by tracer work [6] with sclerotiorin (I) and citrinin (V), the substances (II), (III), and (IV) should have a main skeleton built up by the polyketide route with methyl groups introduced by *C*-methylation.[7] In the compounds (I)—(IV) two chains must be present, the extra one varying from a simple OAc (in I) to an acetoacetyl which has undergone ring-closure (in II) and an n-hexanoylacetyl residue which has undergone similar ring-closure (in III and IV). Other oxidations, and reductions and dehydrations, are also necessary. The most interesting reaction which must be postulated is the biochemical reduction of the terminal carboxyl group of the main polyketide chain (probably as its coenzyme-A ester) to the oxidation level of an aldehyde (I, II, IV, V) or an alcohol (III). A similar reduction also appears to occur in citromycetin.[6]

In view of the presence of two distinct chains [8] it was of particular interest to examine the extent of incorporation of $Me\cdot{}^{14}CO_2H$ into both; such incorporation has been shown [6] to occur to the same extent in the acetyl group and the main chain of sclerotiorin (I).

Rotiorin.—Penicillium multicolor was fed with sodium [^{14}C]acetate or [^{14}C]formate, and the labelled rotiorin was extracted and purified by standard methods.[2] The expected labelling pattern is shown in (II) (● = radioactivity from $Me\cdot{}^{14}CO_2H$ acid; ■ = radioactivity from the C_1-pool, contributed by [^{14}C]formic acid).

Kuhn–Roth oxidation of [^{14}C]formate-derived rotiorin (r.m.a. 554) gave acetic acid, the activities of whose methyl and carbonyl group were examined separately by the method of Cornforth *et al.*[9] (r.m.a. 108·8 and 1·0, respectively). Therefore, as expected, the radioactivity is almost all in the methyl group; the calculated r.m.a. is 111 on the assumption of equal yield from each *C*-methyl in the oxidation. Alkaline hydrolysis [2] gave acetaldehyde of unknown source, which from its low activity (r.m.a. 3·6) cannot contain "introduced" methyl groups, together with 4,6-dimethylocta-2,4-dienoic acid [2] (r.m.a. 35·6) (calc., 37·1). Rotioramine (VI) derived from this rotiorin, contained as expected all the activity (r.m.a. 563), and on decarboxylation and aromatisation to aporotioramine [2] (VII) the carbon dioxide was almost inactive (r.m.a. 0·8).

[^{14}C]Acetate-derived rotiorin (r.m.a. 177), which should have the label-distribution

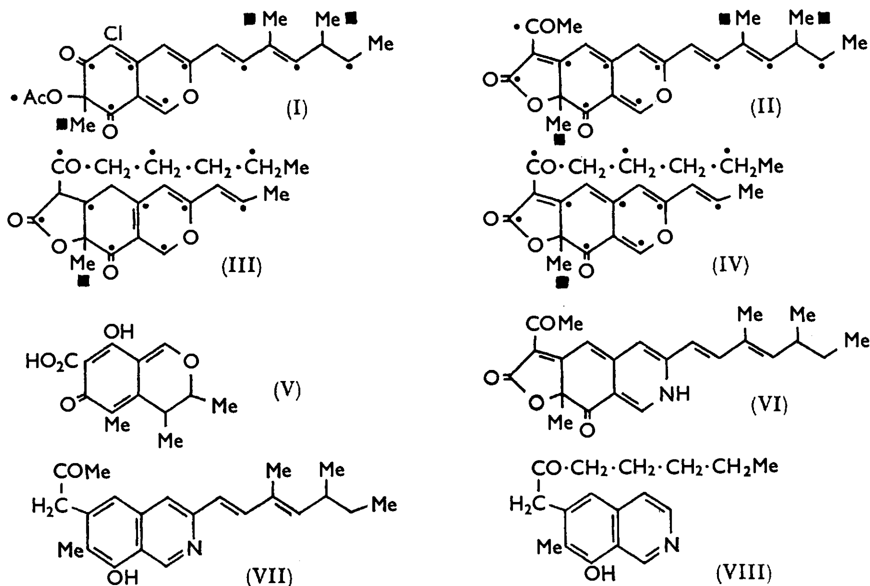
* Part XXIX, *J.*, 1962, 1502.

[1] Dean, Staunton, and Whalley, *J.*, 1959, 3004.
[2] Jackman, Robertson, Travers, and Whalley, *J.*, 1958, 1825.
[3] Haws, Holker, Kelly, Powell, and Robertson, *J.*, 1959, 3598; Haws and Holker, *J.*, 1961, 3820.
[4] Fielding, Holker, Jones, Powell, Richmond, Robertson, and Whalley, *J.*, 1961, 4579.
[5] Johnson, Robertson, and Whalley, *J.*, 1950, 2971.
[6] Birch, Fitton, Pride, Smith, and Whalley, *J.*, 1958, 4576; Alexander and Schwenk, *J. Amer. Chem. Soc.*, 1957, **79**, 4554; Schwenk, Alexander, Gold, and Stevens, *J Biol. Chem.*, 1958, **233**, 1211.
[7] Birch, Elliott, and Penfold, *Austral. J. Chem.*, 1954, **7**, 169; Birch, *Fortschr. Chem. org. Naturstoffe*, 1957, **16**, 186.
[8] Whalley, "Recent Developments in the Chemistry of Natural Phenolic Compounds," ed. Ollis, Pergamon, London, 1961, p. 20.
[9] Cornforth, Hunter, and Popjak, *Biochem. J.*, 1953, **54**, 597.

shown in (II), with formate-derived methyl groups practically inactive, was submitted to Kuhn–Roth oxidation and the resulting acetic acid (89% of 5 mol.) degraded by the Schmidt method. The methylamine was measured as *N*-methyl-2,4-dinitroaniline (r.m.a. 0·4; calc., 0), and the carbon dioxide had r.m.a. 7·7 (calc., 7·1, for two-fifths of a label). Derived aporotioramine (VII) has, as expected, lost one active atom (r.m.a. 161) which is in the carbon dioxide simultaneously produced (r.m.a. 18·8; calc., 17·7). The 4,6-dimethylocta-2,4-dienoic acid obtained by hydrolysis of this rotiorin had r.m.a. 67·8 (calc., 70·8 for four labels), together with acetaldehyde of r.m.a. 18·8. The source of the latter is unknown, but it must come from an undiluted acetic acid unit. Since rubropunctatin (IV) does not give rise to hexanal the acetaldehyde is unlikely to arise from the acetyl side-chain, and from the results above it does not contain an " introduced " methyl group. The most likely source therefore is the potential CHO of the pyran ring and the attached carbon atom, the reactions involved being hydrolysis and reverse-aldol fission.[10]

The r.m.a. of the dimethyloctadienoic acid from acetate-derived rotiorin is rather low, and that of the carbon dioxide derived from the lactone rather high, but the discrepancies are only marginally above the expected maximum counting errors (~3%).

Monascin.—Monascin (III) and rubropunctatin (IV) are obviously closely related but it is convenient to consider them separately.



[^{14}C]Formate-derived monascin (III) contains only one introduced *C*-methyl group, and the total activity of the molecule should be found in the methyl of the acetic acid produced by Kuhn–Roth oxidation. In fact, degrading this acid by the Schmidt technique showed all of the activity in the resulting methylamine, measured as *N*-methyl-2,4-dinitroaniline (r.m.a. 197). Since the active acid is diluted with 2 mol. of inactive acid, the r.m.a. contribution of the introduced methyl group must be 591, which compares with r.m.a. 566 for the initial monascin. The rather high value is probably due to differential yields in the oxidation of different methyl groups, although the overall yield is quite good (83% of 3 mol.).

[10] Cf. Travers, Ph.D. Thesis, Liverpool, 1958.

Oxidation, with alkaline permanganate,[3] of [^{14}C]acetate-derived monascin (r.m.a. 284) gave hexanoic acid, measured as the 4-bromophenacyl ester (r.m.a. 82) (calc., 85 for three labels). Ozonolysis of the monascin [3] gave acetaldehyde, measured as the 2,4-dinitrophenylhydrazone (r.m.a. 29) (calc., 28 for one label). Reductive fission of the lactone-carbonyl group [3] in the labelled monascin produced carbon dioxide (r.m.a. 30), also close to the theoretical r.m.a. of 28, although again this carbon is slightly high in activity (compare rotiorin above).

Rubropunctatin.—Ozonolysis of the [^{14}C]formate-derived rubropunctatin (r.m.a. 1735) gave acetaldehyde [4] (r.m.a. 25·6) which is relatively inactive, showing that incorporation of the activity into the acetate-derived portion of the molecule is low. Aporubropunctatamine [4] (VIII) also had about the same r.m.a. (1744) as the starting material, and the carbon dioxide from the lactone group, produced simultaneously, was practically inactive (r.m.a. 2). Because of the obvious structural relation to monascin, and of shortage of material, no other degradations were attempted.

[^{14}C]Acetate-derived rubropunctatin (r.m.a. 892) gave rise on oxidation [4] to hexanoic acid measured as the *p*-bromophenacyl ester (r.m.a. 267) (calc., 268 for three labels). Kuhn–Roth oxidation and degradation of the acetic acid gave r.m.a. 57 for the carbon dioxide (calc., 59) and r.m.a. 2 for the *N*-methyl-2,4-dinitroaniline (calc., 0). Carbon dioxide from degradation [4] to aporubropunctatamine (VIII) had r.m.a. 92 (calc., 89), and acetaldehyde from ozonolysis, measured as the 2,4-dinitrophenylhydrazone, had r.m.a. 94 (calc., 89). There is again no notable difference between the two chains in regard to incorporation of activity.

There are quite large differences in labelling of terpene and polyketide portions of the same molecule in auroglaucin,[11] fuscin,[12] and mycophenolic acid,[13] despite the fact that these both come ultimately from acetic acid. It can be concluded, therefore, that the two chains of sclerotiorin, rotiorin, monascin, and rubropunctatin arise by very similar routes which are probably almost identical in type so far as assembly of the activated acetic acid units is concerned. The results emphasise the biogenetic relation of polyketides to straight-chain fatty acids, and show that a fairly closely concerted assembly of the major units must occur without accumulation of pools of intermediates (cf. refs. 7, 8, 14).

C-Methylation appears to occur directly on an isolated ethylene group in ergosterol and eburicoic acid,[15] so that this is a conceivable process in an introduction of the *C*-methyl residues into the unsaturated side-chain of intermediates in the cases of sclerotiorin (I) and rotiorin (II). However, in these and in all other cases so far examined, such as citrinin, the methyl group is found on a carbon of the polyketide chain originally derived from methyl of acetic acid. There seems no reason for such selectivity if a double bond is involved, and the process may well occur at an intermediate stage where a β-polyketone chain still exists; it is then also mechanistically more acceptable.

Experimental

Apparatus and general conditions for tracer measurement were the same as in previous Parts of the series. Me·$^{14}CO_2$Na (100 μc) or H·$^{14}CO_2$Na (100 μc) was fed to the appropriate organisms [2–4] under the conditions described for sclerotiorin; [1] the incorporations were 1—3% and 15—20%, respectively. The compounds were isolated by the methods described,[2–4] final purification being by dilution with pure unlabelled material and crystallisation to constant radioactivity.

The degradations were carried out as described in the literature; [2–4] Kuhn–Roth oxidations

[11] Birch, Schofield, and Smith, *Chem. and Ind.*, 1958, 1321.
[12] Birch, Ryan, and Smith, unpublished work.
[13] Birch, *Chem. Weekblad*, 1960, **56**. 597.
[14] Birch, Simonsen Lecture, 1961, *Proc. Chem. Soc.*, 1962, 3; Lynen and Tada, *Angew. Chem.*, 1961, **73**, 513.
[15] Alexander, Gold, and Schwenk, *J. Amer. Chem. Soc.*, 1957, **79**, 2967, 4554; Dauben, Ban, and Richards, *J. Amer. Chem. Soc.*, 1957, **79**, 968, 1000.

and Schmidt degradations were carried out by the methods previously used in this series (*e.g.*, ref. 6). For reductive fission of monascin [3] a control examination for carbon dioxide was made on an aliquot portion of the zinc dust.

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