216. *Studies in Relation to Biosynthesis. Part XXXVII.* Some Structures Derived from Acetic Acid by Two Pathways.*

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Auroglaucin (II) and fuscin (VIII) both arise completely from " acetate," the polyketide and terpene pathways both being involved. The extent of incorporation of $Me\cdot{}^{14}CO_2H$ into the two portions of the molecule is different.

Activated acetic acid in the form of acetyl coenzyme-A, reversibly convertible into malonyl coenzyme-A, has been shown [1] to act as the building unit in the biosynthesis of fats, steroids, terpenes, and polyketides. Some molecules contain several portions derived ultimately from acetic acid, but by different routes, and it is of general interest to examine details of their biosyntheses. We report some results on the incorporation of [^{14}C]-labelled precursors into auroglaucin (II) and fuscin (VIII), both of which, by inspection, should arise by a combination of the terpene and polyketide routes.

Auroglaucin.—This substance (II) is produced by *Aspergillus novus* [2] (*A. pseudoglaucus* Blochwitz NRRL 46) in admixture with flavoglaucin, which contains a saturated C_7 chain. From the difficulties encountered in purifying the latter substance, we suspect that compounds with intermediate unsaturation may also be present. Inspection of the formula

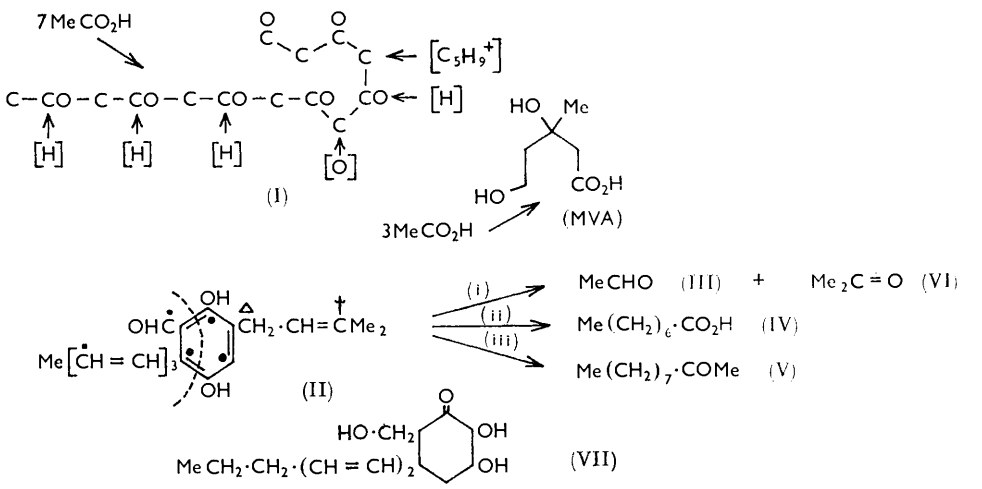
* Part XXXVI, Birch, Holzapfel, Rickards, Djerassi, Suzuki, Westley, Dutcher, and Thomas, *Tetrahedron Letters*, 1964, 1485.

[1] " Biogenesis of Natural Compounds," ed. Bernfeld, Pergamon, Oxford, 1963.

[2] Raistrick, Robinson, and Todd, *J.*, 1937, 80; Cruickshank, Raistrick, and Robinson, *J.*, 1938, 2056.

led to the hypothesis that it is biosynthetically derived as in (I). Support for the idea was obtained by degradation (Scheme A), using previously published methods,[2,3] of the

Scheme A §



Reagents: i, O_3; ii, Pd–H_2, H_2O_2; iii, Pd–H_2, Zn–HAc, H_2O_2.

§ For meaning of symbols see Birch, Schofield, and Smith, *Chem. and Ind.*, 1958, 1321.

substance obtained by incubation of the organism with Me·$^{14}CO_2H$ or with 3,5-dihydroxy-3-methyl[2-^{14}C]pentanoic acid δ-lactone ([2-^{14}C]MVAL). Radioactive assay data are given in Table 1.

TABLE 1.

Incorporation experiments, and r.m.a. values ($\times 10^{-3}$) of the compounds shown in Scheme A.

Substrate	Incorporation (%)	R.m.a. values (II)	(III)	(IV)	(V)	(VI)
Me·$^{14}CO_2H$ * (*a*)	1·9	5·55	0·566	2·24	2·82	0·43
Me·$^{14}CO_2H$ * (*b*)	0·8	5·55	0·419	—	—	0·482
[2-^{14}C]MVAL	3·8	2·97	0·003	—	—	2·93

* Runs (*a*) and (*b*) represent separate incorporations in which the ratios of labelling in the polyketide and terpene portions differed significantly and were, therefore, affected by the conditions of the fermentation.

Acetaldehyde and acetone were assayed as the 2,4-dinitrophenylhydrazones, separated by chromatography, decan-2-one as the semicarbazone, and octanoic acid as the *p*-bromophenacyl ester. The relative molecular activity (r.m.a.) of the acetaldehyde was one quarter of that of the octanoic acid (IV) and one fifth of that of the decanone (V) which arises from the portion to the left of the dotted line in (II) after reduction of CHO to Me.[3,4] These results are strongly in favour of the presence of the polyketide labelling pattern shown in the C_7-chain, the aromatic nucleus, and the CHO of (II), with labels equal. Assuming that, in the ring and in the terpene portion of the molecule, the positions of the labels are those normally encountered as the result of incorporation of Me·$^{14}CO_2H$, the label on the whole terpene unit should be r.m.a. 1·609. The activity per labelled position is clearly different in the terpene and polyketide parts of the molecule. It is rather surprising that the r.m.a. of (VI), the acetone derived from the terpene unit, is only 0·43, making that on the CH_2 of the unit 1·179, that is, the labels in the terpene portion are also unequal. This is

[3] Quilico, *Gazzetta*, 1953, **83**, 776.
[4] Birch and Kocor, *J.*, 1960, 866.

probably a genuine difference, but must be regarded with some reserve since it is a calculated and not directly observed value.

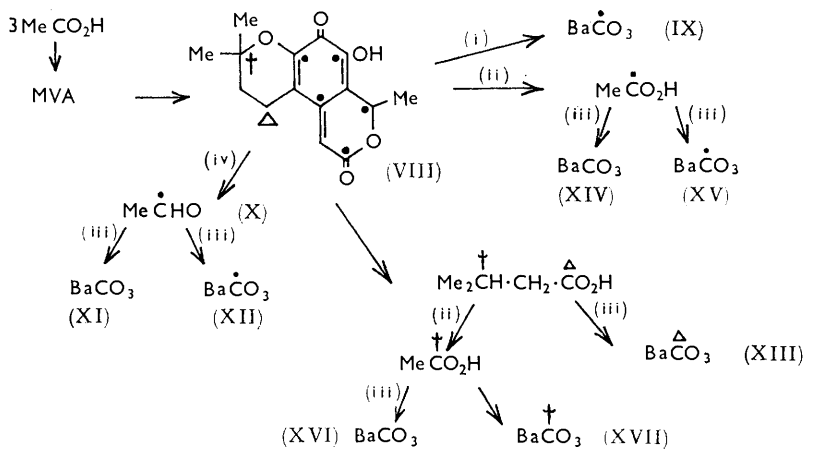
Degradations of the material derived from [2-^{14}C]MVAL showed clearly that the C_5-unit is incorporated by the usual terpene route, since the acetone obtained by ozonolysis contained almost all the radioactivity of the molecule. The inactivity of the acetaldehyde also proves that mevalonic acid (MVA) is not biochemically converted into acetic acid in this organism.

Incorporation of [^{14}C]formic acid into auroglaucin was low (~0·1%) and was extensively randomised since the resulting substance (r.m.a. 13,500), on Kuhn–Roth oxidation and degradation of the acetic acid, showed the r.m.a. of the carboxyl group of the latter to be 190 and of the methyl group to be 780. No specific incorporation of a C_1-unit therefore is involved in the biosynthesis.

The compound palitantin (VII) contains the same nucleus as auroglaucin, apart from the terpene unit, and may be biogenetically related, since it is also derived from acetic acid.[4]

Fuscin.—This substance (VIII) produced by *Oidiodendron fuscum*,[5] evidently contains an " introduced " isoprene unit, and it was for this reason that we were led to interpret the degradation evidence in terms of structure (VIII),[6] preparatory to biogenetic investigation. From inspection of the formula, two views might be held as to the origin of the rest of the molecule, depending on the biogenetic significance attached to the positions of the nuclear oxygen atoms.[7] One possible route is from a C_6–C_3 unit, less a terminal carbon atom, and hence from prephenic acid together with one acetate and one terpene unit. The other (Scheme B) involves a terpene unit and a polyketide.

Scheme B



Reagents: (i) Decarboxylation; (ii) Kuhn–Roth oxidation; (iii) Initial Schmidt degradation; (iv) NaOH.

Radioactive fuscin was obtained by incubating *O. fuscum* with Me·$^{14}CO_2Na$, and it was degraded by literature reactions.[5] The results are set out in Table 2 using the same conventions as above.

The acetaldehyde (X) obtained by alkaline hydrolysis and assayed as the 2,4-dinitrophenylhydrazone, had approximately the same r.m.a. as the barium carbonate (IX) produced by decarboxylation of the lactone group. Kuhn–Roth oxidation of the acetaldehyde 2,4-dinitrophenylhydrazone and degradation of the resulting acetic acid showed that the acetaldehyde was labelled only on the carbonyl group. Fusion of the labelled

[5] Birkinshaw, Bracken, Michael, and Raistrick, *Biochem. J.*, 1951, **48**, 67.
[6] Birch, *Chem. and Ind.*, 1955, 682; Barton and Hendrickson, *ibid.*, p. 682.
[7] *Chem. Soc. Special Publ.*, 1956, No. 5, 25.

fuscin with alkali gave a mixture of acetic and isovaleric acids, identified by paper chromatography, which were separated by counter-current distribution between chloroform and water. Schmidt degradation and Kuhn–Roth oxidation of the isovaleric acid gave the

TABLE 2.

Incorporation experiments, and r.m.a. values ($\times 10^{-3}$) of the compounds shown in Scheme B.

		R.m.a. values									
Substrate	Incorporation (%)	(VIII)	(IX)	(X)	(XI)	(XII)	(XIII)	(XIV)	(XV)	(XVI)	(XVII)
Me·$^{14}CO_2H$...	1	832	51·7	52	0	51·5	415	—	—	0	162
[2-^{14}C]MVAL	3	365	—		—	—	—	90	0	—	—

r.m.a. of the 1- and 3-carbon atoms, and showed the methyl groups to be inactive. Assuming that the labels in the terpene unit are in the usual positions, the total r.m.a. was 57·7 and the quinomethine (polyketide) portion of the molecule, by difference, had the r.m.a. contribution of 25·5, which accords with the five labels shown in (VII) [calculated from (XII), 25·75, and from (IX), 25·85].

It is notable that the labels in the terpene unit appear to be unequal in contrast to the position with steroids, triterpenes, mycophenolic acid, and mycelianamide. That the C_5-unit is introduced by the usual terpene route was shown by incorporation of [2-^{14}C]-MVAL; Kuhn–Roth oxidation of the product gave acetic acid containing in its methyl group one quarter of the r.m.a. of the original molecule, the carboxyl being inactive. This result is in accord with the expectation of an approximately equal yield from the two *C*-methyl sources and with all the label present in one of the terpene methyl groups.

The extent of incorporation of label into the polyketide and terpene portions of the molecule is not the same; this feature will be discussed in more detail in connection with work on mycophenolic acid.[8]

EXPERIMENTAL

The tracer methods, apparatus, and conventions are those previously described.

[^{14}C]*Auroglaucin.*—*Aspergillus pseudoglaucus* Blachwitz NRRL 46 was grown on a modified Czapek–Dox medium as previously described.[2] Labelled substrates were added after 14 days and the labelled metabolites isolated after a further 14—18 days' growth. Degradations were carried out by the published methods.[2,3] The octanoic acid was measured as the *p*-bromophenacyl ester, m. p. 66—67° (from ethanol), and the decan-2-one as its semicarbazone, m. p. 121—122° (from ethanol). The acetone and acetaldehyde 2,4-dinitrophenylhydrazones were obtained in the standard manner after ozonolysis in methylene chloride and were separated on bentonite–kieselguhr (3 : 1) with ethanol (6%) in ether. The first eluate contained acetaldehyde 2,4-dinitrophenylhydrazone, m. p. 167—169°, and the second the acetone derivative, m. p. 122—124°. In all cases the compounds were crystallised to constant activity.

[^{14}C]*Fuscin.*—*Oidiodendron fuscum* Robaks was cultured as previously described.[5] Labelled substrates were added after 28 days and the labelled metabolites isolated after a further 21 days. Degradations were carried out as previously described.[5] The alkali fusion of fuscin was carried out as described for dihydrofuscin except that it was not done under nitrogen. That the only volatile acid products were acetic and isovaleric acids was checked by paper chromatography. The acids were separated by 30 transfers from water to chloroform, the isovaleric acid being in the first six tubes. The purity was again checked by chromatography. Degradation of the acids was carried out by Schmidt decarboxylation, and also by standard Kuhn–Roth oxidation in the case of the isovaleric acid (yield of acetic acid 83%).

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[8] Birch, Cassera, Fryer, Ryan, and Smith, unpublished work.