The Biosynthesis of Glauconic Acid[1]

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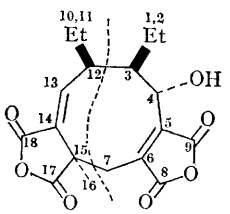
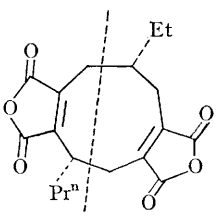
DURING the work on the nonadride group of fungal metabolites, exemplified by glauconic acid[2] (I) and byssochlamic acid[3] (II), it was postulated[4] that (I) and (II) were formed in the mould from the coupling of two C_9-units of identical carbon skeleton [see dotted lines in (I) and (II)]. Furthermore the C_9-unit (VI) could result from the condensation of an hexanoyl derivative (III) with oxalacetic acid (IV) to give the substituted citric acid (V) which by decarboxylation and elimination of water would generate the C_9-unit (VI).



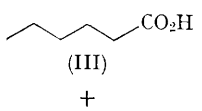
(I)



(II)

In order to obtain some evidence for this scheme we have investigated the biosynthesis of glauconic acid (I) chosen because of the ease with which it can be degraded *via* its pyrolytic fission[4,5] to glauconin (VII) and diethylacraldehyde (VIII). The further degradations of (VII) and (VIII) are summarised in Figure 1 and are based on published methods.[6] The acetic and propionic acids[7] were degraded stepwise to carbon dioxide.[8]

When a culture of *Penicillium purpurogenum* was grown in the presence of [1-^{14}C]acetate for 5 weeks, labelled glauconic acid (9·4% incorporation) was obtained and degradation gave the distribution of activity shown in (IX) (glauconic acid = 100) for the C_9-unit. Similar feeding of [2-^{14}C] acetate (13·2% incorporation) gave the results summarised in (X). Before discussing these results it is necessary to consider the degradation



(III)

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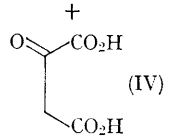
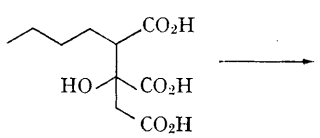
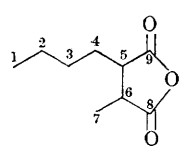


(IV)



(V)



(VI)

[1] We wish to thank Professor D. H. R. Barton, F.R.S., for his advice and encouragement during this work.
[2] D. H. R. Barton, L. M. Jackman, L. Rodriguez-Hahn, and J. K. Sutherland, *J. Chem. Soc.*, 1965, 1772.
[3] J. E. Baldwin, D. H. R. Barton, and J. K. Sutherland, *J. Chem. Soc.*, 1965, 1767.
[4] D. H. R. Barton and J. K. Sutherland, *J. Chem. Soc.*, 1965, 1769.
[5] N. Wijkman, *Annalen*, 1931, **485**, 61.
[6] (*a*) H. Sutter and N. Wijkman, *Annalen*, 1935, **519**, 97.
(*b*) D. H. R. Barton, H. P. Faro, E. P. Serebryakov, and N. F. Woolsey, *J. Chem. Soc.*, 1965, 2438.
(*c*) T. Curtius, *J. prakt. Chem.*, 1895, **52**, (2), 222.
(*d*) F. Pregl, "Quantitative Organic Microanalysis", J. and A. Churchill, Ltd., London, 1951, p. 206.
[7] The propionic acid must be formed in equal yield from each of the chains since 2 × activity $CH_3CH_2CO_2H$ = activity (VIII) − activity C-9.
[8] E. F. Phares, *Arch. Biochem. Biophys.*, 1951, **33**, 173.

scheme since, because of the symmetry of glauconin (VII) and the degradation of both chains of (VIII) to propionic acid, the activity values quoted for all of the carbons except C-4 and C-13 are averages for pairs of carbon atoms originally at different

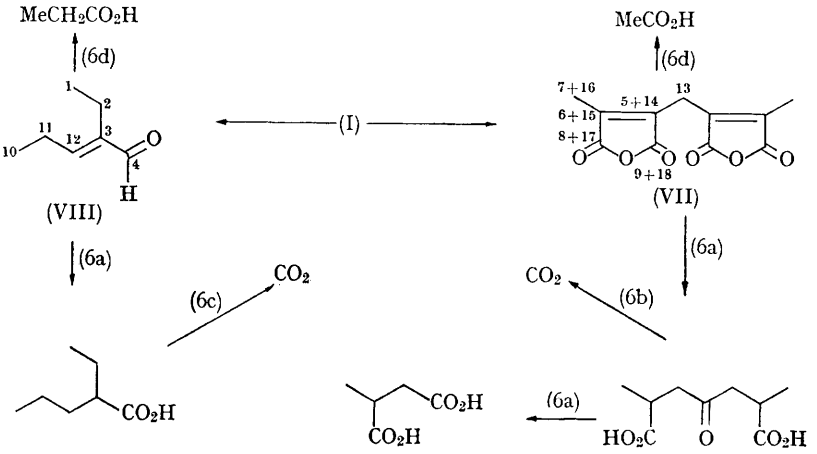
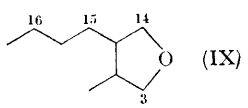
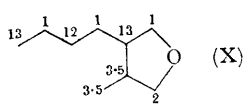


FIGURE 1

positions in glauconic acid (I). However, the pairs of atoms whose average activities are determined are at equivalent positions in the postulated C_9 precursor and if the two halves of



(IX)



(X)

the molecule arise from a single C_9-unit the average values will correspond to the true ones. The activities of the pair C-4 and C-13 have been determined separately and have been found to be identical within experimental error, which, in conjunction with some experiments on larger metabolites,[9] supports the common origin of the two C_9-units. Turning now to (IX) and (X) it is apparent that the labelling of the C_6-chain accords with its derivation from one acetate and two malonate units to give hexanoic acid by the well established route of fatty acid biosynthesis.[10] In the C_3-chain C-8 can be derived from both the carboxyl and methyl[11] of acetate while C-6 and C-7 are exclusively formed from the methyl of acetate by a head-to-head coupling probably involving a symmetrical intermediate. In particular the patterns in (IX) and (X) exclude schemes in which the final condensation leading to (VI) is between C-4 and -5, C-5 and -9, C-6 and -7, or C-6 and -8 and firmly point to this condensation occurring between C-5 and C-6.

Since all of the carbons of the C_3-chain appear in glauconin a rough indication of increased (or decreased) incorporation of activity into this part can be gauged from the ratio of activity of glauconin (VII) to the acraldehyde (VIII). The ratios found in various feeding experiments are shown in the Table. From the acetate results it follows that activity is more rapidly incorporated into the C_6-chain in line with the proposed biosynthesis. [1-^{14}C]Hexanoate was fed to see if it was specifically incorporated, but, as expected, the results are complicated by breakdown to C_2-units and resynthesis from these.[12] However, the ratios tabulated support some specific incorporation, and degradation showed 34% of the total activity at C-9 as opposed to 28% in the acetate, derived samples.

[9] Unpublished experiments.
[10] F. Lynen, *Fed. Proc.*, 1961, **20**, 941.
[11] The route whereby activity is incorporated into this carbon is obscure as it seems to be too high to be due to scrambling of acetate *via* the citric acid cycle.
[12] D. I. Crandall and S. Gurin, *J. Biol. Chem.*, 1949, **181**, 829.

Both [2-^{14}C]pyruvate and [2-^{14}C]glucose (which when metabolised by the Embden–Meyerhof pathway[13] will give [2-^{14}C]pyruvate) are good precursors for [1-^{14}C]acetate[14] which would give the known labelling pattern if this was the only route for incorporation of activity. However, the ratios in the Table indicated another mechanism of incorporation into the C_3-chain. Degradation of the [2-^{14}C]glucose-derived glauconic acid showed 8% of the total activity at C-6 and 1·5%

TABLE

Precursor	Feeding Time (days)	Activity (VII) / (Activity (VIII))
[1-^{14}C]Acetate	35	1·13
,,	5	1·13
,,	1	0·99
[2-^{14}C]Acetate	35	0·88
,,	35	0·90
,,	1	0·76
[1-^{14}C]Hexanoate	35	1·25
,,	1	1·47
[2-^{14}C]Pyruvate	11	1·69
[2-^{14}C]Glucose	14	1·58
[2,3,-^{14}C]Succinate	35	4·63

at C-7 whereas virtually none was found at these positions in the [1-^{14}C]acetate-derived material. This shows that pyruvate can be incorporated without degradation to acetate or by passing through a *symmetrical* C_4-unit. The foregoing results do not distinguish between a C_3- or a C_4-unit as the immediate precursor of the C_3-chain, but this can be resolved by feeding [2,3-^{14}C]-succinate which, if degraded to acetate or pyruvate before incorporation, would give results similar to these obtained before. The Table indicates that the succinate is incorporated efficiently into the C_3-chain and degradation showed 55% of the total activity to be at C-6 and -7 in contrast to the 14% in the case of [2-^{14}C]acetate and (calculated) 19% with pyruvate. This is strong evidence that the immediate precursor of the C_3-chain is a C_4-compound.

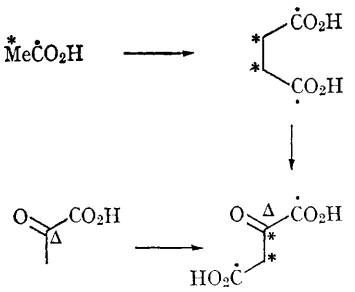


FIGURE 2

The two main routes by which oxaloacetic acid can be synthesised in living organisms appear to be *via* the Krebs cycle by an (overall) head-to-head coupling of acetate and by the carboxylation of pyruvate.[15] Our results (summarised in Figure 2) are completely consistent with oxaloacetic acid, alone of the C_4-acids of the citric acid cycle, being the precursor of the C_3-chain.

Glauconic acid, then, appears to result from a diverging citric acid cycle in which hexanoate can, to some extent, replace acetate in the citric acid condensation.

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[13] See J. S. Frutton and S. Simmonds, "General Biochemistry", 2nd Edn., John Wiley and Sons, Inc., 1958, p. 467.
[14] I. C. Gunsalus, in W. D. McElroy and B. Glass, eds., "The Mechanism of Enzyme Action," Johns Hopkins Press, Baltimore, 1954.
[15] See H. L. Komberg, *Angew. Chem. Internat. Edn.*, 1965, **4**, 558.