The Biosynthesis of Glauconic Acid: C_9 Precursors

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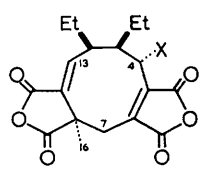
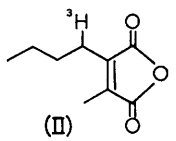
IN a previous Communication,[1] evidence was presented supporting a biosynthetic pathway for the mould metabolite glauconic acid[2] (I) *via* dimerisation of a C_9 unit of carbon skeleton (II). This evidence rested on the interpretation of labelling patterns obtained using small labelled precursors involved in the general cell metabolism. To gain more insight into this unique dimersiation C_9 precursors were prepared and fed.

Acid-catalysed ethanolysis of the enol acetate (III) with [*O*–^{3}H]-ethanol gave ethyl [3-^{3}H]-2-oxohexanoate which, in a Reformatsky reaction with zinc and ethyl α-bromopropionate, yielded the expected malic ester. Dehydration of the ester with phosphorus oxychloride and pyridine resulted in a mixture of the corresponding maleic, fumaric, and itaconic esters which on alkaline hydrolysis yielded the anhydride (II) and the related fumaric acid. On refluxing with acetic anhydride the mixture was converted into the pure anhydride (II). Addition of (II) to a growing culture of *Penicillium purpurogenum* gave labelled glauconic acid (0·25% incorporation). The presence of equal labelling at C-4 and C-13 was supported by the pyrolysis[2] of (I) to glauconin (IV) and diethyl acraldehyde (V) with identical molar activities, indicating that dimersation had indeed occurred.

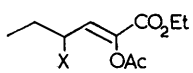
The other precursor (VI) was prepared starting again from the enol acetate (III) which reacted with *N*-bromosuccinimide to give the bromo-ester (VII). We were unable to convert (VII) directly into the ketone (X) but the same result was achieved by the reduction with zinc in acetic acid to give the βγ-unsaturated ester* (VIII), followed by ethanolysis to the alcohol (IX), oxidized with manganese dioxide to the ketone



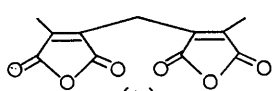
(I : X = OH)
(XIV : X = H)



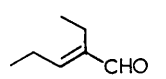
(II)



(III : X = H)
(VII : X = Br)



(IV)



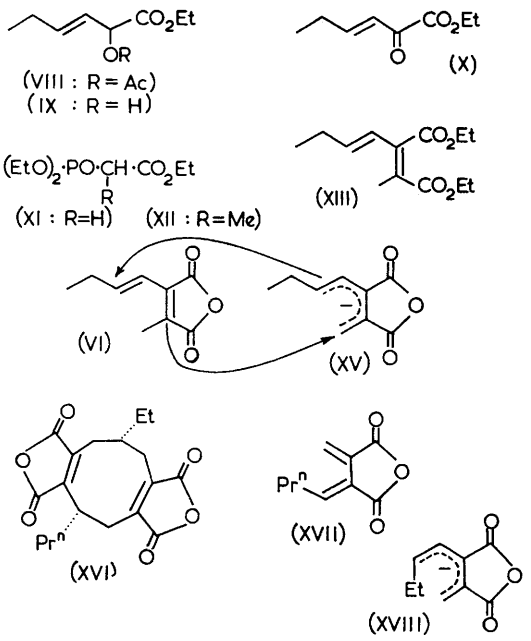
(V)

(X). Alkylation[3] of the phosphonate (XI) with [^{14}C]methyl iodide and sodium ethoxide in ethanol gave the homologue (XII), the anion of which condensed with (X) in a Wittig-type reaction[4] to yield stereospecifically† the maleic ester (XIII). Alkaline hydrolysis of (XIII) gave

* This is a general reaction of γ-bromo-αβ-unsaturated esters.

† The general reaction of α-keto-esters with the phosphonate anions derived from α-bromo-esters gives maleic, but not fumaric, esters.

the anhydride (VI) labelled in the methyl group. All the compounds prepared had the appropriate spectroscopic properties, (VI) showed ν_{max} (liq.) 1840, 1780, 1670 cm.$^{-1}$, λ_{max} (hexane) 303 mμ (ϵ 12,110), Me singlet at τ 7·95, and two *trans*-vinyl protons, one a doublet at τ 3·9 (J = 16 c./sec.) the other a pair of triplets at τ 2·9 (J = 16 and 6 c./sec.). When (VI) was fed to the growing mould 51·5% of the activity was incorporated into glauconic acid (I). Degradation of the derived glauconin (IV) showed 97·5% of this activity to be present at C-7 and C-16. An additional 4% of the activity originally present in (VI) appeared in glaucanic acid (XIV) and in agreement with glaucanic being a precursor of glauconic the molar activity of the former was 1·4 times that of (I). We have also shown that generally labelled glaucanic acid can be incorporated into glauconic acid. There seems little doubt that anhydride (VI) is the C_9 unit which dimerises to form glaucanic acid (XIV) and mechanistically this can be represented as an electrocyclic addition of the *cisoid* diene (VI) (4π-electrons) and the anion derived from it (XV) (6π-electrons), an allowed process by the Woodward–Hoffman rules.[5] Protonation of the anion formed would then give (XIV). Furthermore, reaction by an *endo*-transition state would give the stereochemistry observed for glaucanic acid. The biosynthetically related byssochlamic acid[6] (XVI) would then arise from a similar addition of (XVII) to the derived anion (XVIII), an isomer of (XV), thus explaining the different absolute configurations of the ethyl groups in (XIV) and (XVI). Some support for these ideas has come from treating the labelled anhydride (VI) with sodium hydride in dimethylformamide. T.l.c. of the reaction mixture gave a spot corresponding to glaucanic acid, and isotope dilution analysis indicated a yield of 1·8%. Work is now in progress to identify this compound unambiguously.



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[4] W. D. Emmons and W. S. Wadsworth, *J. Amer. Chem. Soc.*, 1961, **83**, 1773.
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