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Biosynthesis of Glauconic Acid from [2,3-^{13}C]Succinate

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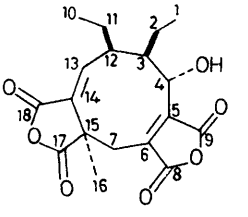
Summary The ^{13}C-n.m.r. spectrum of glauconic acid (I) enriched with [2,3-^{13}C]succinate shows only two pairs of ^{13}C–^{13}C couplings indicating incorporation into the oxalacetate-derived residue but no detectable incorporation into the β-ketide derived fragment *via* the expected degradation product [1,2-^{13}C]acetate.

It seems probable that the fungal metabolite glauconic acid (I) is biosynthesised by the dimerisation of a C_9 residue having the carbon skeleton (II) which is formed from the combination of a β-ketide derived hexanoate residue with a C_3 fragment generated from the C_4 Krebs' Cycle intermediate oxalacetate (Scheme).[1] In feeding experiments with [1- and 2-^{14}C]acetate, [1- and 2-^{14}C]glucose, [2-^{14}C]pyruvate, and [2,3-^{14}C]succinate radioactivity was found in both the β-ketide and oxalacetate derived portions of the molecule and it was only by comparisons of differential incorporations of the individual precursors into the two fragments that firm conclusions could be derived.[2] This problem of label randomisation *via* the Krebs' tricarboxylate cycle tends to complicate all biosynthetic studies involving feedings with succinate and related precursors.



(I)

A more direct approach to glauconic acid biosynthesis would involve the precursor [2,3-^{13}C]succinate when direct incorporation into the C_3 residue would be demonstrated by the appropriate ^{13}C—^{13}C couplings in the ^{13}C-n.m.r. spectrum of the enriched metabolite and any randomisation

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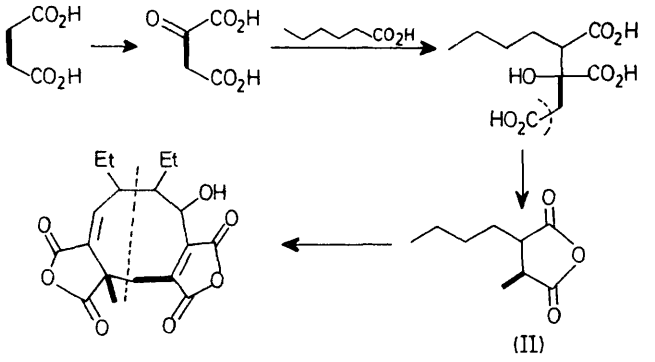
which occurred *via* [2,3-^{13}C]- oxalacetate and -pyruvate into [1,2-^{13}C]-acetate would be shown by similar couplings in the hexanoate residue.



SCHEME. Biosynthesis of glauconic acid with heavy bonds to denote couplings which arise from the precursor [2,3-^{13}C]-succinate.

In a preliminary study with the glauconic acid producing organism *Penicillium purpurogenum* (IMI 90178) it was found that succinate was tolerated in sufficient concentrations for a viable ^{13}C-experiment but that acetate was not. Accordingly [2,3-^{13}C]succinate (133 mg; prepared from 87·5% enriched [1,2-^{13}C]dibromoethane by reaction with sodium cyanide and subsequent hydrolysis, as previously described for the preparation of glutaric acid[3]) was enriched with [2,3-^{14}C]succinate (to give 0·89 μCi mmol^{-1}) and pulse fed (aliquot portions from day 2 to day 17) to a static culture of *P. purpurogenum* (100 ml on synthetic medium[4]). After a total of 23 days the culture was harvested and glauconic acid (190 mg; 0·017 μCi mmol^{-1} was isolated as previously described.[2]

TABLE

^{13}C-Chemical shifts and coupling constants of [2,3-^{13}C]succinate enriched glauconic acid (I)

Carbon atoms	δ/p.p.m.[a]	Carbon atoms	δ/p.p.m.[a]
1,10	12·6, 13·9	4	66·6
2,11	19·5, 27·5	5,14	130·3, 148·2
16	25·0 (*J* 33 Hz)	6	142·4 (*J* 48 Hz)
7	32·0 (*J* 48 Hz)	13	149·8
3,12	39·3, 52·9	8,9,18	164·0, 164·9, 165·6
15	48·3 (*J* 33 Hz)	17	174·8

[a] In $(CD_3)_2SO$ containing 0·1 M-Cr(acac)$_3$ at 110 °C; chemical shifts relative to Me_4Si.

The ^{13}C-n.m.r. spectrum of the enriched glauconic acid is summarised in the Table where assignments are based on expected values of chemical shifts, multiplicities in the off-resonance decoupled spectrum and observed ^{13}C–^{13}C couplings in the noise-decoupled spectrum. The spectra were determined at 110 °C in $(CD_3)_2SO$ when all the signals were sharp, whereas at room temperature some broad signals had been observed, presumably owing to slow conformational interconversions of the nine-membered ring.

The observed ^{13}C–^{13}C couplings in the spectrum of enriched glauconic acid, C(15)–C(16) and C(6)–C(7), are those required for direct incorporation of succinate into the C_3 residues (Scheme). The absence of couplings elsewhere in the spectrum indicates that there is negligible randomisation of label into the hexanoate residue *via* [1,2-^{13}C]acetate. Furthermore, the observed mean combined intensity of the satellite signals compared with that of the corresponding singlet (0·61 ± 0·07) is in close agreement with the figure (0·61 ± 0·02) calculated, as previously described,[5] from the dilution of ^{14}C-label, assuming incorporation into the C_3 residues only.

The specific incorporation of [2,3-^{13}C]succinate into the C_3- residues of glauconic acid is in direct contrast to earlier work[2] with tracer amounts of [2,3- ^{14}C]succinate where only 55% of the total incorporation occurred at C(6)–C(7) and C(15)–C(16). Furthermore, there is no evidence in the present work for any significant randomisation of label by conversion of [2,3- ^{13}C]- into [1,2- ^{13}C]succinate *via* one turn of the Krebs' cycle. A possible reason for this is operation of the 'enantiomeric' Krebs' cycle which would not randomise the label and this explanation has been advanced previously[2] to account for the distribution of ^{14}C-label in glauconic acid derived from [2-^{14}C] acetate. Although these results are not entirely clear they may be associated with the relatively large amounts of succinate used in the present work and the pulse feeding technique. In any case this experiment, which seems to be the first example of the use of [2,3-^{13}C]succinate, indicates the potential of the method in biosynthetic studies on metabolites which incorporate Krebs' cycle intermediates.

We thank Mrs. A. Lewis for the microbiological work and Mr. D. J. Birch for ^{13}C-spectral determinations.

(*Received*, 21*st May* **1976**; *Com.* 569.)

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