Biosynthesis of Epoxydon with [^{13}C]Acetate

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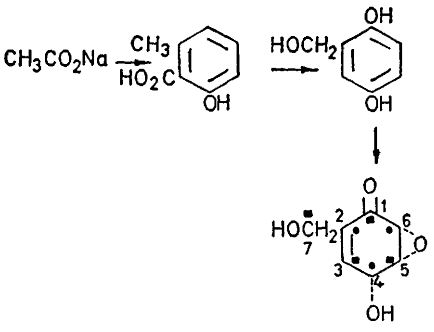
Summary The tetraketide origin of epoxydon produced by *Phyllosticta sp.* is established by ^{13}C n.m.r. measurements on labelled epoxydons obtained from sodium [1-^{13}C]- and [2-^{13}C]-acetate.

Epoxydon,[1,2] a major phytotoxic and antitumour metabolite produced by *Phyllosticta sp.*,[1] was postulated to be formed *via* a tetraketide intermediate in the organism, because *Phyllosticta sp.* also gives 6-methylsalicylic acid and gentisyl alcohol which are biosynthesized from acetate and malonate.[3,4] This hypothesis was confirmed by the present structural assignment of the ^{13}C-labelled metabolites (Ia) and (Ib) and by the fact that ^{14}C-labelled gentisyl alcohol was incorporated into epoxydon with 25% efficiency by the mycelial mats from 14 day old cultures.[5] The proton-decoupled natural abundance ^{13}C n.m.r. spectrum of epoxydon in MeOH is given in the Table. Peaks at δ 63·1 (p.p.m.) and 69·1 were ascribed to C-4 and C-7 respectively from a



Epoxydon
(Ia): from [2-^{13}C]AcONa (■)
(Ib): from [1-^{13}C]AcONa (•)

Table. ^{13}C N.m.r. data for ^{13}C-labelled epoxydon

	Shift δ_C[a]	[2-^{13}C]acetate[b]	[1-^{13}C]acetate[c]
C-1	−68·1	−68·5	—
C-2	−8·0	—	−6·9
C-3	−14·7	−14·8	—
C-4	63·1	—	62·8
C-5	73·6	73·6	—
C-6	74·5	—	74·5
C-7	69·3	69·2	—

[a] Natural abundance; *ca.* 120 mg per 1·0 ml of MeOH with 1034 scans (3611 Hz) at 400 s/scan. [b] Epoxydon from sodium [2-^{13}C]acetate; *ca.* 60 mg per 1·0 ml of MeOH with 56 scans (4000 Hz) at 100 s/scan. [c] Epoxydon from sodium [1-^{13}C]-acetate; *ca.* 40 mg per 1·0 ml of MeOH with 1208 scans (4000 Hz) at 40 s/scan.

Chemical shifts were measured relative to the ^{13}C signal of methanol and converted to p.p.m. from benzene using the relationship δ_C (benzene) = δ_C (MeOH) + 79·0 p.p.m.

continuous wave (CW) decoupled spectrum which gives a doublet for the former and triplet in the latter. Peaks at δ 73·6 and 74·5 were assigned to C-6 and C-5 respectively *via* off-resonance CW decoupling.[6] Thus the ^{1}H spectrum of epoxydon shows 6-H (δ 3·45) separated further from the irradiating field than 5-H (δ 3·90). In the CW spectrum of epoxydon, the epoxy-carbon atoms C-6 and C-5 appear as doublets and the assignments are based on $J_r = 21$ Hz as observed for C-6 relative to $J_r = 16$ Hz for C-5 according to Ernst's equation.[7]

The ^{13}C n.m.r. spectra (b and c in the Table) of the epoxydons (Ia) and (Ib) enriched in ^{13}C (88 and 86%, respectively) biosynthesized from sodium [1-^{13}C]- and [2-^{13}C]-acetate (400 mg) clearly demonstrated that C-1, C-3, C-5, and C-7 of epoxydon were derived from the methyl carbon of the acetate and C-2, C-4, and C-6 from the carboxy-carbon. The ^{1}H–^{13}C satellite bands for 3-H in (Ia) (J_{C-H} 164 Hz) and 6-H in (Ib) (J_{C-H} 191 Hz) in the ^{1}H n.m.r. spectra (90 MHz) support this labelling pattern.

This labelling pattern confirms the polyketide origin of epoxydon and related compounds.[5,8]

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