Biosynthesis of Metabolites of *Periconia macrospinosa* from [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]-Acetate

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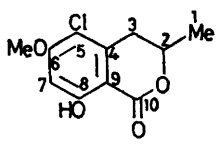
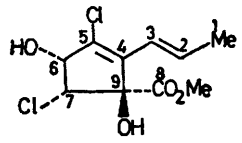
Summary The ^{13}C-n.m.r. spectra of methyl 2-allyl-3,5-dichloro-1,4-dihydroxycyclopent-2-enoate (II), enriched with [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]-acetate respectively by the fungus *Periconia macrospinosa* suggest that it is biosynthesised from an aromatic precursor, structurally related to the β-ketide derived co-metabolite, 5-chloro-8-hydroxy-6-methoxy-3-methyl-3,4-dihydroisocoumarin (I).

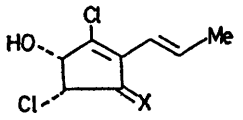
IT has been suggested that the cyclopentene derivative (II) is biosynthesised in *Periconia macrospinosa* by contraction of the aromatic ring in a precursor, structurally related to the co-occurring dihydroisocoumarin (I).[1,2] We now report results on the biosynthesis of these compounds using singly and doubly labelled ^{13}C-acetate.



(I)



(II)



(III); X = α-CH_2OH, β-OH
(IV); X = O

Cultures of *Periconia macrospinosa*, strain IMI 24411, were supplemented with [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]-acetate (90%) to give metabolites (I) and (II) enriched with approximately 1·6 and 1·3% respectively excess ^{13}C-abundance at each labelled position. This was established by comparison of the satellite and natural abundance resonance intensities in the proton-noise-decoupled (p.n.d.) ^{13}C-n.m.r. spectra of the doubly labelled metabolites.

Structure analysis of the dihydroisocoumarin (I) suggests its penta-β-ketide origin (Scheme). This was established by the p.n.d. ^{13}C-n.m.r. spectrum of the [1,2-^{13}C]-acetate enriched sample which showed five pairs of coupled ^{13}C-satellites superimposed on the natural abundance spectrum. Hence, compound (I) contains five intact acetate residues.[3] The complete spectral assignment (Table) followed from literature values,[4] the off-resonance natural abundance spectrum, and the values of individual ^{13}C–^{13}C coupling constants. The spectra of the [1-^{13}C]- and [2-^{13}C]-acetate derived samples showed the anticipated enhancements of individual ^{13}C-resonance intensities, as required by structure (V).

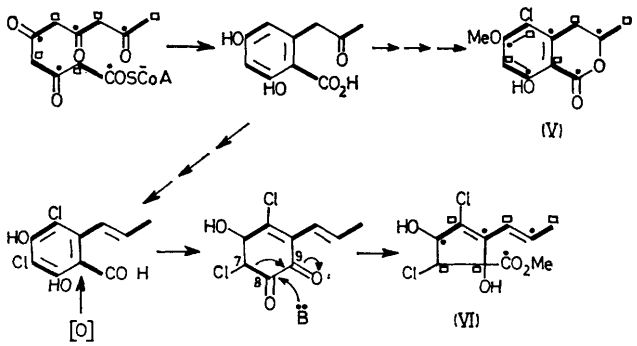
The p.n.d. ^{13}C-n.m.r. spectrum of the biogenetically more interesting cyclopentene (II), derived from [1,2-^{13}C]-acetate, showed three pairs of ^{13}C–^{13}C couplings indicating three intact acetate residues only in this metabolite. The complete spectral assignment (Table) followed from these couplings, literature values,[4] and the off-resonance decoupled natural abundance spectrum. The p.n.d. spectra of the [1-^{13}C]- and [2-^{13}C]-acetate derived samples showed

TABLE

^{13}C-Chemical shifts and coupling constants of [1,2-^{13}C]-acetate enriched samples of compounds (I) and (II).

Carbon	δ/p.p.m.[a]	^1J(^{13}C–^{13}C)/Hz
Compound (I)		
1	20·8	39
3	32·5	43
MeO	56·4	—
2	75·0	39
7	99·1	71
9	101·9	70
5	111·5	76
4	137·7	43
6	161·1	76
8	163·1	71
10	169·1	70
Compound (II)		
1	19·4	43
MeO	54·4	—
7	66·0	—
6	75·5	48
9	87·2	—
3	120·5	60
5	133·0	48
2	134·2	43
4	136·9	60
8	171·9	—

[a] Relative to internal Me_4Si.



(V)

(VI)

SCHEME. □ = Atom derived from C-2 of acetate, ● = atom from C-1 of acetate; intact acetate residues shown by heavy bonds.

sufficient enhancements of individual resonance intensities to establish the biogenetic origin of each carbon atom, as shown in (VI), except C-8† where the relative weakness of the signal due to lack of NOE and large T_1 precluded a satisfactory decision. This problem was resolved by chemical degradation of compound (II) ($10{\cdot}42 \times 10^{-3}$ μCi mmol^{-1}) derived from [1-^{14}C]-acetate. Reduction of this with borohydride gave the triol (III) ($10{\cdot}37 \times 10^{-3}$ μCi mmol^{-1}) which was oxidised with sodium metaperiodate giving the hydroxy-ketone (IV) ($8{\cdot}10 \times 10^{-3}$ μCi mmol^{-1}) together with formaldehyde, isolated as the dimethone ($2{\cdot}39 \times 10^{-3}$ μCi mmol^{-1}). Hence, the individual atoms in the carbon skeleton of compound (II) are all derived from acetate with three intact residues, as shown in (VI).

These results provided strong evidence that the cyclopentene (II) is biosynthesised from a β-ketide derived aromatic precursor, related to compound (I), by ring contraction involving fission of the 7—8 bond. The previous postulate[2] had involved fission of the 4—9 bond. A possible biogenetic sequence is illustrated in the Scheme.

Other naturally occurring cyclopentene derivatives, *e.g.* terrein[5] and pyrethrolone,[6] may be similarly derived, although in the former case ^{14}C-acetate studies have suggested an alternative ring contraction.[5]

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† The numbering system adopted for metabolites (I) and (II) conforms with the order of assembly of acetate units in the β-ketide precursor.

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[6] For review see S. H. Harper, *Ann. Reports*, 1948, **45**, 162.