Biosynthesis of Capsidiol in Sweet Peppers (*Capsicum frutescens*) Infected with Fungi: Evidence for Methyl Group Migration from ^{13}C Nuclear Magnetic Resonance Spectroscopy

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Summary The mode of incorporation of [1,2-$^{13}C_2$]-acetate into capsidiol (I) during fungus-induced biosynthesis supports the hypothesis that the angular methyl group arises by migration from C(10).

The biosynthesis of eremophilane-type sesquiterpenoids is generally assumed to proceed *via* methyl group migration, as originally postulated by Robinson.[1] An alternative proposal, with particular reference to the stereoisomeric nootkatane (valencane) skeleton, involves a sequence of spiro-rearrangements.[2] The results of a study of the biosynthesis of petasin[3] were consistent with the classical mechanism, but did not rule out the more recent suggestion. We now report an experimental distinction between the two routes based on the use of [1,2-$^{13}C_2$]-acetate.[4] An adequate degree of incorporation has been achieved by application of the finding by Stoessl *et al.*[5] that the production of the phytoalexin, capsidiol (I)[6,7] (a 4-*epi*-eremophilane derivative) by *Capsicum frutescens* L. is greatly enhanced by infection of the fruit with fungi.[†]

Semi-ripe or ripe capsicums were supplied by the West of Scotland College of Agriculture, Auchincruive. Whole fruits were inoculated with *Monilinia fructicola* as described by Stoessl *et al.*[5] Preliminary experiments with *RS*-[2-^{14}C]-mevalonolactone and with sodium [2-^{14}C]-acetate, introduced in the same manner as the spore suspensions, showed

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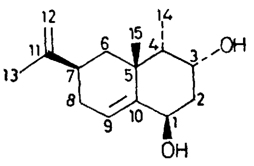
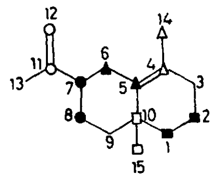
[†] After the inception of this work, a report appeared showing good incorporation of ^{14}C-labelled acetate and mevalonate, in the latter case, up to 8·5% based on *RS*-precursor, into the norsesquiterpenoid, rishitin, in potato tuber slices inoculated with *Phytophthora infestans*.[8]

incorporation levels of 1–3%. These results suggested that incubation with [1,2-^{13}C$_2$]-acetate should yield capsidiol suitably labelled for study by n.m.r. spectrometry.



(I)

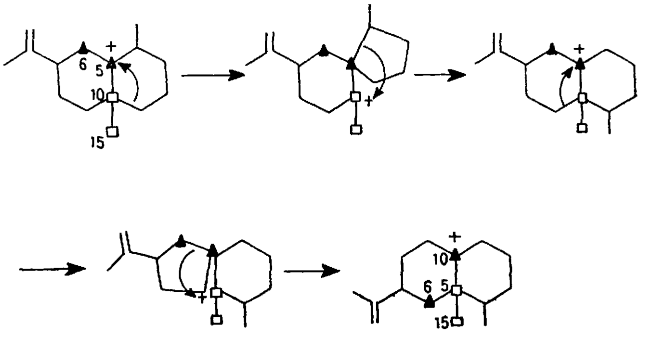


(II)

Capsidiol (51 mg) was isolated from 103 peppers after incubation with fungal spores in the presence of sodium [1,2-^{13}C$_2$]-acetate (>90% ^{13}C, 1 or 2 mg per fruit). Purity was confirmed by t.l.c., g.l.c. and g.c.–m.s. of the diol. Mass spectrometry indicated that *ca.* 5% of the molecules were doubly labelled. The measured ^{13}C n.m.r. chemical shifts for capsidiol agreed closely with those reported.[7]

Of the six intact C$_2$ units expected to be incorporated into a eudesmane-type intermediate (II), one must be cleaved during conversion to the eremophilane or capsidane skeleton. The remaining five units were indicated in the enriched capsidiol by carbon satellite signals with the following coupling constants: $J_{1,2}$ 35 ± 1; $J_{4,14}$ 36; $J_{5,6}$ 35; $J_{7,8}$ 33; $J_{11,12}$ 72 Hz. The relative intensities showed no evidence of gross differences in the degree of enrichment at these different sites. Accordingly, the almost complete absence of coupling between C(5) and C(15) indicated that these atoms were derived from separate acetate units, in agreement with the migration of a methyl group from C(10) to C(5). In a rearrangement *via* spiro-intermediates (*cf.* Scheme), the atoms becoming C(5) and C(15) would have remained paired, while C(6) would have become isolated, instead of being strongly coupled to C(5) as observed. Our data rule out the occurrence of spiro-rearrangement, except possibly as a very minor pathway.

Two additional inferences can be drawn. (i) The identity of the carbon atom derived from C(2) of mevalonate is maintained at the terminal position [C(13)] in harmony with the postulated cyclisation of farnesyl pyrophosphate. (ii) Similarly, C(9) appears to derive solely from C(2) of mevalonate, as expected; there is no indication of coupling.



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The remaining atom [C(3) of capsidiol] that would be expected to originate from C(2) of mevalonate, though affording mainly a singlet, also gave rise to a minor pair of satellite signals; the question whether these denote coupling to C(2) or to C(4) is under investigation.‡ C(3)–C(4) coupling might imply the intermediacy of an 'abnormal' isopentenyl pyrophosphate unit in which part of the terminal olefinic carbon is derived from C(3′) of mevalonate. Such an apparent 'exchange' of carbon between the methyl and methylene groups has been previously noted in sesquiterpenoid biosynthesis, and the possible existence of a *cis*-isopentenyl pyrophosphate isomerase has been considered.[10]

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‡ The application of homonuclear decoupling should be informative in this respect.[9]

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