Stable Isotope Studies of the Biosynthesis of the Sesquiterpenoid Dihydrobotrydial

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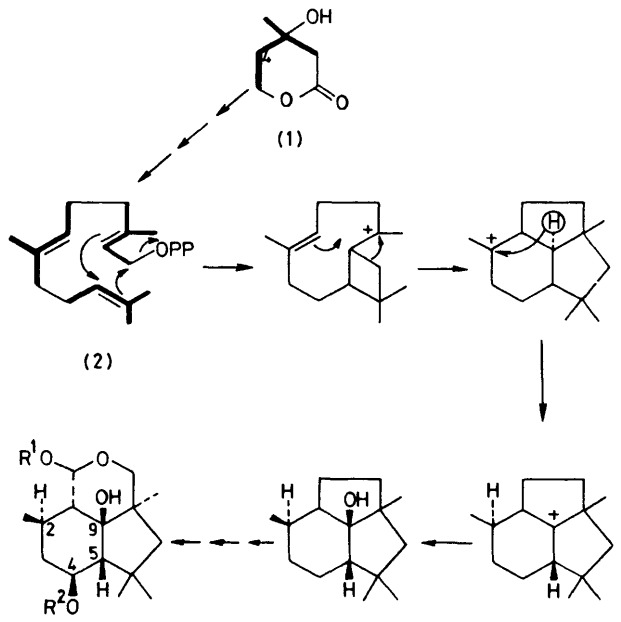
Summary The pattern of incorporation of [4-^{2}H,4-^{13}C] mevalonic acid and $H_2{}^{18}O$ into dihydrobotrydial has confirmed that there is a 1,3-hydride shift in the biosynthesis of this metabolite and that there is a discharge of the C(9) carbocation by hydration.

^{13}C N.M.R. studies have shown[1] that the sesquiterpenoid phytotoxic metabolites of *Botrytis cinerea* such as dihydrobotrydial (**3**)[2] are formed by folding farnesyl pyrophosphate (**2**) as in the Scheme. Recently we used the ^{2}H n.m.r. spectrum of the methyl ester of botryaloic acid, a co-metabolite of dihydrobotrydial derived biosynthetically from [4-$^2H_2$,4-^{13}C] mevalonate (**1**), to establish that a 1,3-hydrogen shift from C(9) to C(2) occurs during the biosynthesis. This led to a proposed biosynthetic pathway for dihydrobotrydial (**3**) as shown in the remainder of the Scheme. We have now been able to examine this further by using both the ^{2}H and ^{13}C n.m.r. spectra of the ethyl



(3) $R^1 = H, R^2 = Ac$
(4) $R^1 = Et, R^2 = Ac$
(5) $R^1 = Et, R^2 = H$

SCHEME. PP = pyrophosphate.

ethers (**4**) and (**5**), derived chemically from biosynthetically enriched dihydrobotrydial. Careful spin decoupling studies at 360 MHz enabled the well resolved ^1H n.m.r. signals of the acetate (**4**), and hence of the corresponding 4-alcohol (**5**), to be assigned. The dihydrobotrydial from a second experiment with [4-$^2H_2$,4-^{13}C]mevalonate (**1**) was converted into the ethyl ether (**4**) by treatment with ethanol in chloroform containing a trace of toluene-*p*-sulphonic acid and then the acetoxy-group was hydrolysed with methanolic potassium carbonate to afford (**5**). The ^2H n.m.r. spectrum comprised a singlet at δ 1·7 corresponding to H(2) and doublets (*J ca.* 20 Hz) centred on δ 1·5 corresponding to H(1) and H(5). The ^{13}C n.m.r. spectrum of the alcohol contained small triplet signals at δ 63·2 (*J* 20 Hz), 0·6 p.p.m. upfield from the signal at δ 63·8 assigned to C(5), and at δ 54·1 (*J* 19 Hz), 0·5 p.p.m. upfield from the signal at δ 54·9 assigned to C(1). The signal at δ 82·4, assigned to C(9) was enriched. Thus the [4-^2H,4-^{13}C]mevalonate labels at C(1) and C(5) have remained attached whilst the [4-^2H]-mevalonoid label, originally at C(9), has migrated to C(2) as required by the biosynthetic scheme.

Implicit in this scheme is the fact that the oxygen atom of the tertiary alcohol at C(9) originates from water. In a normal hydroxylation it would come from aerial oxygen. The discharge of a 'carbocation' in biosynthesis by hydration, although a feature of a number of other sesquiterpenoid biosynthetic schemes, has rarely been established experimentally.[4] We have now confirmed the origin of this oxygen atom. *B. cinerea* was grown on a $H_2{}^{18}O$ medium (*ca.* 12% ^{18}O). The dihydrobotrydial (**3**) was isolated and in order to remove any possible ambiguity concerning the origin of the hemi-acetal oxygen, it was converted into the ethyl ether (**4**). Although the ethyl ether does not show a molecular ion in the electron-impact mass spectrum, it shows ions corresponding to $(M - EtO)^+$ and $(M - EtOH)^+$ ($C_{17}H_{27}O_4$ requires 295·191, found 295·190; $C_{17}H_{26}O_4$ requires 294·183, found 294·182). The labelled material contained ions at 297 and 296 a.m.u. ($C_{17}H_{26}{}^{16}O_3{}^{18}O$ requires 296·187, found 296·187). The relative intensities of these ions were measured (see Table)

TABLE. Incorporation of ^{18}O into dihydrobotrydial ethyl ether.[a]

Ion/a.m.u.	Mean intensity[b] Unlabelled sample	Labelled sample
294	74	72·04 $(M-EtOH)^+$
295	100·0[c]	100·0 $(M-EtO)^+$
296	16·90	29·61
297	1·35	19·71

[a] ^{18}O content based on the $(M-EtOH)^+$ ion = 15·47%; ^{18}O content based on the $(M-EtO)^+$ ion = 15·5%. [b] Mean of 31 determinations, we thank Mr. A. Greenway for these. [c] Normalized.

revealing an enrichment with ^{18}O. The ethyl ether also shows ions at 262 and 263 a.m.u. corresponding to the loss of H_2O and $CH_3CO_2(H)$. Comparison of the ions of 262—265 a.m.u. between the labelled and unlabelled samples showed no significant incorporation of ^{18}O confirming the anticipated location of the label at C(9). The stereochemistry of the oxygen atom at C(9) is *trans* to the hydrogen atom at C(2) and this would be in accord with an inversion of configuration at C(9) as a consequence of this rearrangement and hydration.

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[4] For a recent example see: D. E. Cane, R. Iyengar, and M. S. Shiao, *J. Am. Chem. Soc.*, 1981, **103**, 914.