Use of ^{2}H–^{13}C N.M.R. Coupling Patterns in Terpenoid Biosynthesis

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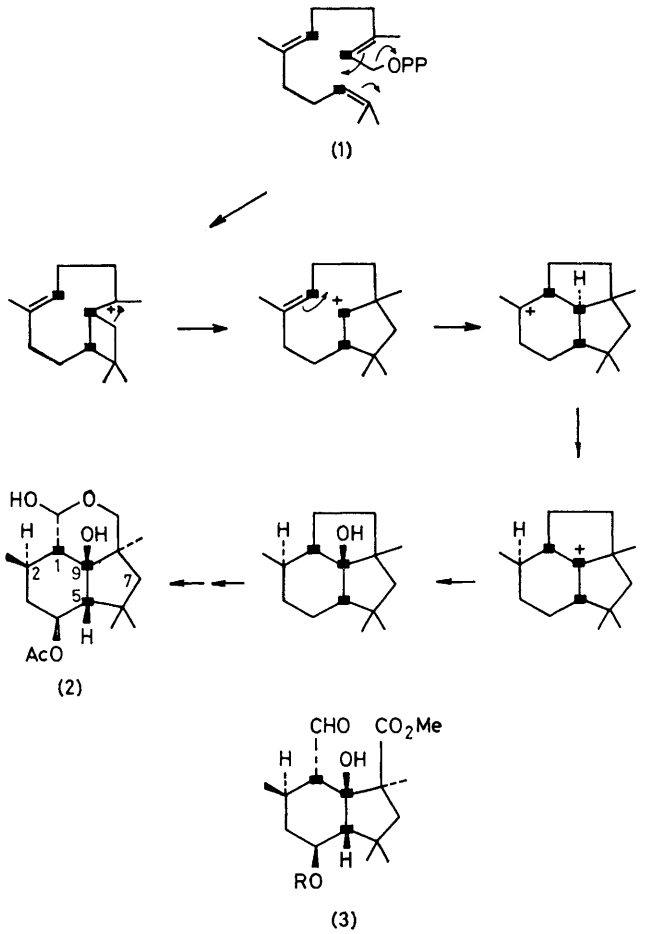
Summary The heteronuclear ^{2}H–^{13}C n.m.r. coupling pattern of methyl botryaloate, a relative of dihydrobotrydial, derived from [4-$^{2}H_{2}$,4-^{13}C]mevalonic acid, has been used to distinguish between two possible pathways for a biosynthetic rearrangement; a 1:3 hydrogen shift occurs.

DIHYDROBOTRYDIAL (**2**)[1] is one of a group[2] of sesquiterpenoid metabolites of the plant pathogen *Botrytis cinerea*. Our previous studies[3] on the biosynthesis of this compound utilizing [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate, and [4,5-$^{13}C_2$]-mevalonate have defined the way in which farnesyl pyrophosphate (**1**) undergoes cyclization to generate this skeleton. We have also described[4] the application of ^{2}H n.m.r. spectroscopy to the determination of the stereochemistry of a label at C-15 in dihydrobotrydial biosynthesis. We now report on the use of ^{2}H–^{13}C coupling patterns in elucidating the course of a hydrogen rearrangement during this biosynthesis.

(4*R*)-[4-^{3}H,2-^{14}C]Mevalonic acid (^{3}H : ^{14}C, 6·53 : 1) was fed to the fungus *Botrytis cinerea*. Dihydrobotrydial (**2**) had an incorporation of 0·2% and a ^{3}H : ^{14}C ratio of 6·31 : 1 representing an incorporation of 2·9 atoms ^{3}H/mol. However C-9 which is derived from C-4 of mevalonate is fully substituted, bearing a hydroxy-group rather than a hydrogen atom. Consequently a hydrogen migration has occurred. The most likely terminus for the rearrangement appeared to be C-2.

A rearrangement to C-2 could occur either by two consecutive 1 : 2 shifts (H-9 → C-1 and H-1 → C-2) comparable to rearrangements which have been found in steroid biosynthesis, or it could occur by a direct 1 : 3 shift (H-9 → C-2). The location of the (4*R*)-mevalonoid hydrogen atoms and the distinction between these rearrangements was provided by feeding [4-$^{2}H_2$,4-^{13}C]mevalonic acid to *Botrytis cinerea*. If two 1 : 2-shifts occur, two deuterium atoms will become separated from their carbon-13 partners and, provided there is sufficient dilution by endogenous material, only one of the three ^{2}H–^{13}C couplings will remain. On the other hand a 1 : 3-shift will separate only one ^{2}H–^{13}C pair leaving two couplings. Since deuterium has a spin of 1 and carbon-13 a spin of ½, these couplings are more easily observed in the ^{2}H n.m.r. spectrum. The H-1 (δ 2·76), H-2 (δ 2·07), and H-5 (δ 2·00) signals were clearly distinguished in the 360 MHz ^{1}H n.m.r. spectrum of methyl botryaloate (**3**). Botryaloic acid is a relative of dihydrobotrydial which is found in the same fermentations. The ^{2}H n.m.r. spectrum (determined at 55·3 MHz) revealed the ^{2}H signals corresponding to H-1 and H-5 as doublets (*J* 19·5 and 21 Hz) and that for H-2 as a singlet superimposed on the natural abundance spectrum. Thus a 1 : 3-rearrangement has occurred.

Taken with our earlier work this leads to the biosynthetic scheme in which the preliminary steps resemble the formation of caryophyllene. The 1 : 3-shift produces the necessary inversion of configuration at C-9. Dihydrobotrydial thus joins the increasing group of terpenoid substances in which a secondary methyl group marks the terminus of a hydrogen rearrangement. ^{2}H–^{13}C Coupling patterns may have some potential in unravelling biosynthetic rearrangements since, as in the present example, they can be used to define both the starting point and the terminus of the rearrangements.



■ sites derived from the C-4 of mevalonic acid; PP = pyrophosphate.

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[1] H.-W. Fehlhaber, R. Geipal, H.-J. Mercker, R. Tschesche, and K. Welmar, *Chem. Ber.*, 1974, **107**, 1720; H. J. Linder and B. von Grosse, *ibid.*, p. 3332.
[2] O. Cuevas and J. R. Hanson, *Phytochemistry*, 1977, **16**, 1061; A. P. W. Bradshaw and J. R. Hanson, *J. Chem. Soc., Perkin Trans.* 1, 1980, 741.
[3] J. R. Hanson and R. Nyfeler, *J. Chem. Soc., Chem. Commun.*, 1976, 72; A. P. W. Bradshaw, J. R. Hanson, and M. Siverns, *ibid.*, 1977, 819.
[4] A. P. W. Bradshaw and J. R. Hanson, *J. Chem. Soc., Chem. Commun.*, 1979, 924.