The Mevalonoid Origin of the Hydrogen Atoms of Culmorin

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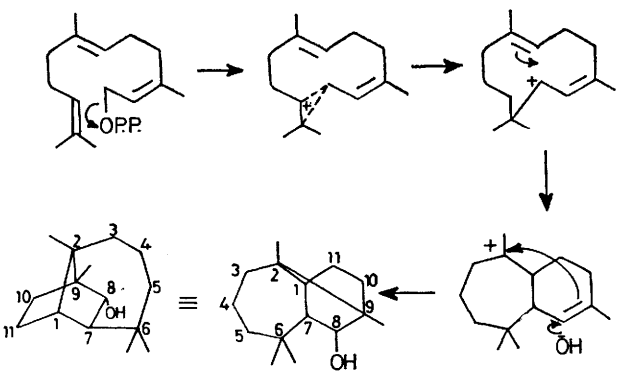
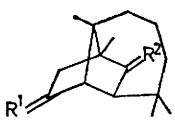
Summary The labelling pattern of the sesquiterpenoid culmorin from [2-$^3H_2$,2-^{14}C]-, [4(*R*)-4-^{3}H,2-^{14}C]-, and [5-$^3H_2$,2-^{14}C]-mevalonate has been partially determined to define the manner of folding of the farnesyl pyrophosphate precursor.

THE tricyclic sesquiterpenoid metabolite of *Fusarium culmorum*, culmorin (**1**, $R^1 = R^2 =$ )[1] is a member of the longiborneol series. Biogenetic proposals have suggested[2] that the skeleton may be formed from a 2-*cis*-farnesyl pyrophosphate as shown in the Scheme.

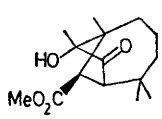


SCHEME

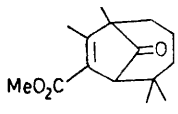
We now report some evidence concerning the fate of the mevalonoid hydrogen atoms in this biosynthesis. [2-$^3H_2$,2-^{14}C]-, [4(*R*)-4-^{3}H,2-^{14}C]- and [5-$^3H_2$,2-^{14}C]-mevalonic



(1)



(2)



(3)

acid (50—80 μCi ^{14}C) were incubated with two-day old cultures of *F. culmorum* and culmorin was isolated after a further 14 days. In each case the culmorin was oxidized to the diketone (**1**, $R^1 = R^2 = O$) (Table). The C(1), C(7) and C(10) proton resonances in the diketone have been assigned.[1] After 16 h treatment with NaOD–D_2O at room temperature, 55% of a $C_{15}H_{22}{}^2H_2O_2$ species (*m/e* 236) was obtained. This lacked the *exo*-C(10) proton resonance (δ 2·28 *J* 18 Hz) whilst the *endo*-proton resonance (δ 1·94 *J* 18 Hz) collapsed

TABLE

The incorporation of the [2-$^3H_2$,2-^{14}C]-, [4(*R*)-4-^3H,2-^{14}C]- and [5-$^3H_2$,2-^{14}C]-mevalonates into culmorin.

	[2-$^3H_2$,2-^{14}C] $^3H:^{14}C$	Atom ratio	[4(*R*)-4-^3H,2-^{14}C] $^3H:^{14}C$	Atom ratio	[5-$^3H_2$,2-^{14}C] $^3H:^{14}C$	Atom ratio
Mevalonate	8·92:1	6:3	6·17:1	3:3	12·35:1	6:3
Culmorin	8·37:1	5·63:3	6·19:1	3·03:3	10·53:1	5·12:3
% Incorporation	0·23		1·9		0·02	
Culmorin Diketone	8·11:1	5·45:3	4·05:1	1·96:3	8·17:1	3·97:3

to a diminished broad multiplet partly obscured by the C(7) proton resonance. After 60 h the diketone contained 92% of a $^2H_3$ species. The C(7) proton resonance as well as both of the C(10) proton resonances were now absent. The culmorin diketone from the [2-$^3H_2$,2-^{14}C] mevalonate experiment was treated with 2N NaOH for 60 h. The diketone then had a $^3H:^{14}C$ ratio of 6·99:1 which corresponded to an atom ratio of 3·93:3 (*i.e.* to the loss of 1·52 atoms of tritium). Further degradation of the diketone to the *nor*-hydroxy-ester (**2**)[1] gave $^{14}CO_2$. The ester had lost one third of the ^{14}C specific activity of the starting material and had a $^3H:^{14}C$ ratio of 8·20:1 corresponding to an atom ratio of 3·67:2. This degradation located a [2-^{14}C]- and a [2-$^3H_2$]-mevalonoid label at C(10). Non-integral atom ratios have been observed in other [2-$^3H_2$,2-^{14}C] mevalonate experiments and have been ascribed to the effect of prenyl isomerase.[3]

The culmorin diketone from the [4(*R*)-4-^3H,2-^{14}C] mevalonate experiment revealed the loss of a ^3H label from C(8). On further degradation to the *nor*-hydroxy-ester (**2**), the ^{14}C specific activity dropped by one third and the $^3H:^{14}C$ ratio changed to 6·01:1 corresponding to an atom ratio of 1·95:2. The alcohol was treated with thionyl chloride and the resultant crude mixture of olefins was treated with base to afford (**3**).[1] This compound had a $^3H:^{14}C$ ratio of 3·25:1 corresponding to an atom ratio of 1·05:2 thus locating [4(*R*)-4-^3H]-mevalonoid labels at C(1) and C(8). The oxidation of the culmorin from the [5-$^3H_2$,2-^{14}C]-mevalonate experiment to the diketone (**1**, $R^1 = R^2 = O$) with the loss of one label served to locate a [5-$^3H_2$]-mevalonoid label at C(11).

Thus the labelling pattern associated with C(1), C(8), C(10) and C(11) is in accord with the proposed folding of farnesyl pyrophosphate. Although during the secondary cyclizations, the C(2) double bond must adopt a *cis*-configuration, the number of [5-$^3H_2$]-mevalonoid hydrogen atoms which are retained suggests that all-*trans*-farnesyl pyrophosphate may act as the primary substrate for cyclization.[4] Since the skeletal hydrogen atoms are mevalonoid, the location of the extra [5-^3H]-label is probably at C(5) arising by a rearrangement from C(7) perhaps during the isomerization and secondary cyclizations.[2]

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[1] J. N. Ashley, B. C. Hobbs, and H. Raistrick, *Biochem. J.*, 1937, **31**, 385; D. H. R. Barton and N. H. Werstiuk, *Chem. Comm.*, 1967, 30, *J. Chem. Soc.* (*C*), 1968, 148.
[2] W. Parker, J. S. Roberts, and R. Ramage, *Quart. Rev.*, 1967, **21**, 331.
[3] P. W. Holloway and G. Popjak, *Biochem. J.*, 1968, **106**, 835.
[4] R. Evans, A. Holtom, and J. R. Hanson, *J.C.S. Chem. Comm.*, 1973, 465.