Studies in Terpenoid Biosynthesis. Part 18.[1] Biosynthesis of Culmorin

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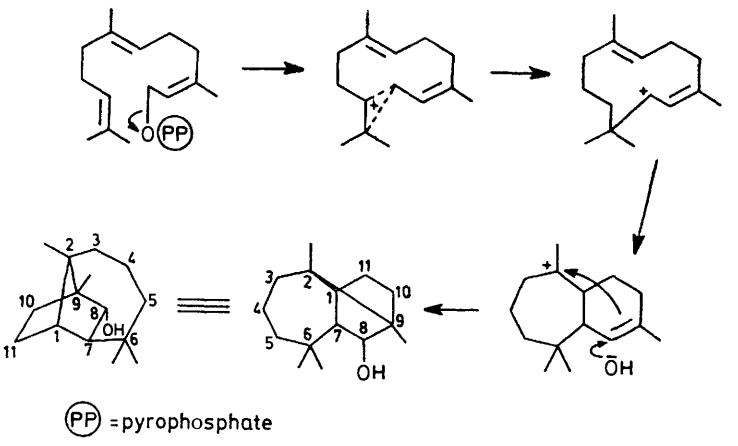
The labelling pattern has been partially determined for the sesquiterpenoid culmorin when biosynthesized from [2-$^3H_2$,2-^{14}C]-, (2*S*)-[2-^3H,2-^{14}C]-, (4*R*)-[4-^3H,2-^{14}C]-, [5-$^3H_2$,2-^{14}C]-, and (5*R*)-[5-^3H,2-^{14}C]-mevalonic acid by *Fusarium culmorum*. The results are discussed in terms of the cyclization of farnesyl pyrophosphate.

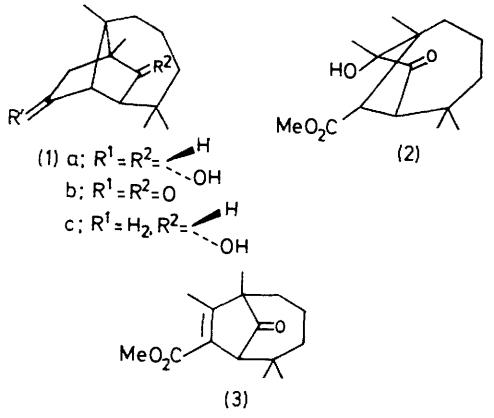
CULMORIN (1a) [2,3] is a tricyclic sesquiterpenoid metabolite of the fungus *Fusarium culmorum*. Although it is a member of the longiborneol series, it is enantiomeric to those which hitherto have been isolated from higher plants.[4] The biogenetic scheme shown in Scheme 1 has been proposed [5] for the sesquiterpenoids of this class. This Scheme implies that mevalonoid hydrogen, the location of which is known in all-*trans*-farnesyl pyrophosphate,[6] will be retained in the final cyclic metabolite. Although the 2,3-double bond of farnesol must eventually take up a *cis* geometry for the formation of the polycyclic system, the primary cyclization may take place with either the 2-*cis*- or the 2-*trans*-isomer of farnesyl pyrophosphate, *i.e.* the double bond isomerization may either precede or succeed the primary cyclization. In the formation of 2-*cis*-farnesol [7] and trichodiene,[8] the parent hydrocarbon of the trichothecenes, there is a loss of a 5-*pro-S* mevalonoid hydrogen atom from C-1 of farnesol, whereas this hydrogen atom is retained in

[1] Part 17, J. R. Hanson, R. Evans, and R. Nyfeler, *J.C.S. Perkin I*, 1976, 1214.
[2] J. N. Ashley, B. C. Hobbs, and H. Raistrick, *Biochem. J.*, 1937, **31**, 385.
[3] D. H. R. Barton and N. H. Werstiuk, *Chem. Comm.*, 1967, 30; *J. Chem. Soc.* (*C*), 1968, 148.
[4] R. Nayak and Sukh Dev, *Tetrahedron*, 1960, **81**, 42.
[5] For reviews, see W. Parker, J. S. Roberts, and R. Ramage, *Quart. Rev.*, 1967, **21**, 331; G. Rucker, *Angew. Chem. Internat. Edn.*, 1973, **12**, 793.
[6] J. W. Cornforth and G. Popjak, *Biochem. J.*, 1966, **101**, 553.
[7] K. H. Overton and F. M. Roberts, *J.C.S. Chem. Comm.*, 1973, 378; R. Evans, A. M. Holtom, and J. R. Hanson, *ibid.*, p. 465.
[8] R. Evans and J. R. Hanson, *J.C.S. Perkin I*, 1976, 326.



SCHEME 1

sesquiterpenoids such as cyclonerodiol and cyclonerotriol which may be derived without the geometrical isomerization.[9] Culmorin co-occurs [10] with both trichothecenes



and cyclonerotriol in *Fusarium culmorum*. Thus the fate of mevalonoid hydrogen in this metabolite might shed some light on the order of these events. [11]

work.[2] [2-$^3H_2$,2-^{14}C]-, (2*S*)-[2-^3H,2-^{14}C]-, (4*R*)-[4-^3H,2-^{14}C]-, [5-$^3H_2$,2-^{14}C]-, and (5*R*)-[5-^3H,2-^{14}C]-mevalonic acids were separately incubated with two-to-four-day-old cultures of *F.culmorum*. The culmorin (1a) was isolated after a further 14 days' growth. The results are tabulated.[11]

The labelling pattern of culmorin at C-1, C-7, C-8, C-10, and C-11 was then established by the following degradations. In each case the culmorin was oxidized [3] to the diketone (1b). The labelling of C-1, C-7, and C-10 was then defined by exchange reactions. The C-1, C-7, and C-10 proton resonances in this compound have been assigned.[3] After treatment for 16 h with 2N-sodium deuteroxide at room temperature, a sample containing 55% of a $C_{15}H_{22}{}^2H_2O_2$ species (M^+ 236) was obtained. The n.m.r. spectrum of this sample lacked the *exo*-C-10 proton resonance (δ 2.28, *J* 18 Hz) and the *endo*-C-10 proton signal (δ 1.94, *J* 18 Hz) had collapsed 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 (δ 1.83)

Incorporation of mevalonates into culmorin

	[2-$^3H_2$,2-^{14}C]	(2*S*)-[2-^3H,2-^{14}C]	(4*R*)-[4-^3H,2-^{14}C]	[5-$^3H_2$,2-^{14}C]	(5*R*)-[5-^3H,2-^{14}C]
Mevalonate ^3H : ^{14}C	8.92 : 1	4.38 : 1	6.17 : 1	12.35 : 1	6.56 : 1
Atom ratio	6 : 3	3 : 3	3 : 3	6 : 3	3 : 3
μCi ^{14}C fed	66	60	51	80	108
Culmorin ^3H : ^{14}C	8.37 : 1	3.70 : 1	6.19 : 1	10.53 : 1	4.63 : 1
Atom ratio	5.63 : 3	2.53 : 3	3.03 : 3	5.12 : 3	2.1 : 3
% Incorporation	0.23	4.3	1.9	0.02	0.34
Culmorin diketone ^3H : ^{14}C	8.11 : 1	3.68 : 1	4.05 : 1	8.17 : 1	4.52 : 1
Atom ratio	5.45 : 3	2.98 : 3 *	1.96 : 3	3.97 : 3	2.0 : 3
^3H : ^{14}C after exchange	6.01 : 1	3.59 : 1			2.41 : 1
Atom ratio	4.0 : 3	2.91 : 3			1.1 : 3
Degree of deuteriation	7% $^2H_2$ 93% $^2H_3$	69.3% $^2H_1$ 30.7% $^2H_2$			3% $^2H_2$ 97% $^2H_3$

* Atom ratio based on a value for culmorin of 3 : 3 to allow for losses due to prenyl isomerase.

We examined a number of cultures of *Fusarium culmorum* before, through the courtesy of Dr. J. F. Grove, obtaining a strain from which we were able to isolate culmorin. However the yields which we obtained never approached the levels described in the original

was clear, 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-sodium hydroxide for 60 h, after which it had a tritium to

[9] R. Evans, J. R. Hanson, and R. Nyfeler, *J.C.S. Chem. Comm.* 1975, 814.

[10] D. Gardner, A. T. Glen, and W. B. Turner, *J.C.S. Perkin I*, 1972, 2576; M. M. Blight and J. F. Grove, *ibid.*, 1974, 691.

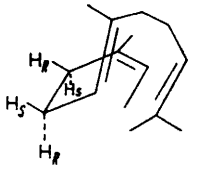
[11] Preliminary communications, J. R. Hanson, and R. Nyfeler, *J.C.S. Chem. Comm.*, 1975, 171, 824.

carbon-14 atom ratio of 3.93 : 3, corresponding to the loss of 1.52 atoms of tritium. The stereochemistry of labelling at C-10 was established by utilizing the difference in rates of exchange of the *exo*- and *endo*-protons.[12] The diketone from the (2*S*)-[2-^{3}H,2-^{14}C]mevalonic acid feed was treated with 2N-sodium deuteroxide for 1.5 h. The product contained 69.3% $^2H_1$ and 30.7% $^2H_2$ species and its n.m.r. spectrum lacked the *exo*-C-10 proton resonance. However the product retained the (2*S*)-[2-^{3}H]mevalonoid label. More prolonged treatment (16 h) led to species containing 29.0% $^2H_2$ and 71.0% $^2H_3$ species and to losses of the (2*S*) label (^{3}H : ^{14}C ratio 2.81 : 1; atom ratio 2.2 : 3). Thus the *endo*-C-10 proton of culmorin originates from the 2-*pro*-*S* position of mevalonate. The diketone was degraded by oxidation with potassium t-butoxide and oxygen and subsequent methylation to afford the 10-nor-hydroxy-ester (2).[3] This degradation afforded $^{14}CO_2$, and the hydroxy-ester had 67.2% of the ^{14}C specific activity of the starting diketone. The ^{3}H : ^{14}C ratio of 8.20 : 1 corresponded to an atom ratio of 3.67 : 2. This degradation served to locate [2-^{14}C]- and [2-^{3}H]-mevalonoid labels at C-10.

Study of the culmorin diketone from the (4*R*)-[4-^{3}H, 2-^{14}C]mevalonate experiment revealed the loss of a ^{3}H label from C-8. On further degradation to the 10-nor-hydroxy-ester (2), the ^{14}C specific activity again dropped by one-third and the ^{3}H : ^{14}C ratio changed to 6.01 : 1 (atom ratio 1.95 : 2). In this compound C-1 has become a potentially enolizable position. However we could not effect exchange with either sodium methoxide or potassium t-butoxide. Therefore the alcohol was treated with thionyl chloride and the resultant crude mixture of olefins was then treated with sodium methoxide to afford the unsaturated ester (3).[3] This degradation product had a ^{3}H : ^{14}C ratio of 3.25 : 1 which corresponded to an atom ratio of 1.05 : 2. Hence there were (4*R*)-[4-^{3}H]-mevalonoid labels at C-1 and C-8.

On oxidation of the culmorin from the [5-$^3H_2$, 2-^{14}C]-mevalonate feed to the diketone, there was a loss of one label. However when (5*R*)-[5-^{3}H,2-^{14}C]mevalonic acid was fed to *F.culmorin*, the resultant culmorin retained only two of the possible three labels and there was no change in the ^{3}H : ^{14}C ratio on oxidation to the diketone. Hence the C-11 hydroxy-group has displaced a 5-*pro*-*R* mevalonoid hydrogen atom. When the diketone was treated with 1.5N-sodium deuteroxide for 3 days, the product contained 3% $^2H_2$ and 97% $^2H_3$ species and the C-10 and C-7 proton resonances had disappeared from the n.m.r. spectrum. In this exchange reaction, there was a loss of a (5*R*)-[5-^{3}H]mevalonoid label from C-7.

The following conclusions may be drawn from this work. First the loss of (4*R*)-[4-^{3}H]- and [5-^{3}H]-mevalonoid labels on oxidation of culmorin to the diketone and of a [2-^{14}C]mevalonoid label on degradation to the nor-hydroxy-ester (2) defines the manner of folding of the farnesyl pyrophosphate as (4) rather than (5). Secondly, the stereochemical relationship between the 2-*pro*-*S* and 5-*pro*-*R* labels is that which would be expected from the folding (4) and with hydroxylation at C-11 proceeding normally with retention of configuration. Thirdly, the culmorin was shown to incorporate five of the possible six [5-^{3}H]mevalonoid labels, *i.e.* one more than would be expected. This could be accommodated if, as in other recently studied cases [13] in which the primary cyclization is between the terminal pyrophosphate and the distal double bond, there is a hydrogen migration from C-1 of farnesyl pyrophosphate. Owing to shortage of material and the relative inaccessibility of the likely centre, we have been unable to locate the site to which the hydrogen



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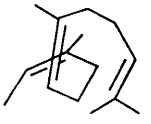
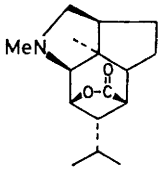
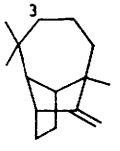


(5)



(6)



(7)

atom has migrated. However since the skeletal hydrogen atoms are mevalonoid and there are no unexpected losses, this is probably C-5. The function of these rearrangements in sesquiterpenoid biosynthesis is to transfer a cationic centre from C-10 or C-11 (the distal end) of farnesyl pyrophosphate back to C-1 to initiate further isomerization and secondary cyclizations of the 10- or 11-membered ring.[14] In the biosynthesis of dendrobine (6), in which a 10-membered primary cyclic intermediate is implicated, the 1-*pro*-*R* hydrogen atom of farnesol is transferred.[13]

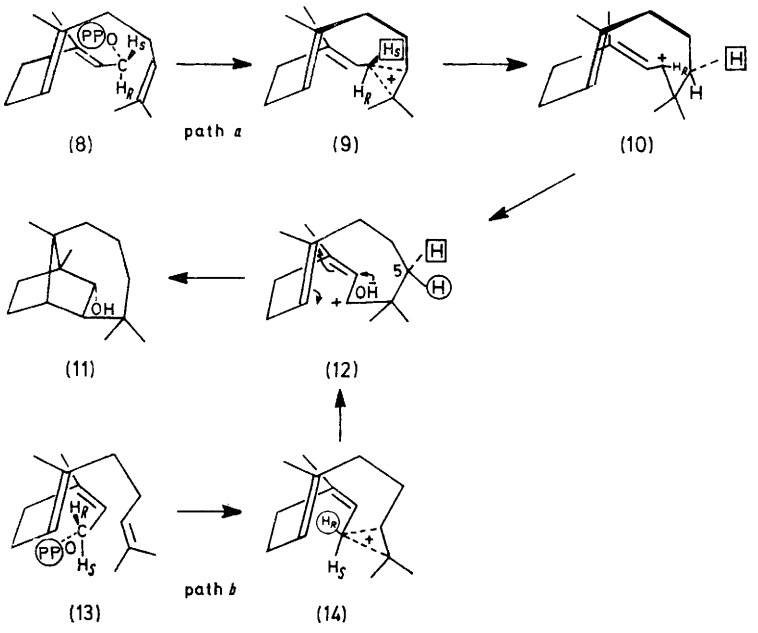
Two distinct cyclization pathways may be envisaged leading to culmorin.[14,15] The first (*a*) involves an ' anti ' coiling of the chain and cyclization of *trans*-farnesyl pyrophosphate (8) on the ' *si* ' face of the distal double bond to give a cyclopropyl cation (9), followed by a hydrogen migration to give an allylic cation (10). The *trans*-double bond may then undergo isomerization to afford the *cis*-isomer (12), thus permitting the formation of the tricyclic system (11). If a 5-*pro*-*S* mevalonoid hydrogen atom (indicated by a square) migrates under these circumstances, it will take up the *pro*-*S* stereochemistry at C-5 in culmorin (see Scheme 2). An alternative pathway (*b*) involves prior isomerization of the

[12] A. F. Thomas, R. A. Schneider, and J. Meinwald, *J. Amer Chem. Soc.*, 1967, **89**, 68.

[13] M. Biollaz and D. Arigoni, *Chem. Comm.*, 1969, 633; A. Corbella, P. Gariboldi, G. Jommi, and C. Scolastico, *ibid.*, p. 634; A. Corbella, P. Gariboldi, and G. Jommi, *ibid.*, 1973, 729; A. Corbella, P. Gariboldi, G. Jommi, and M. Sisti, *ibid.*, 1975, 228.

[14] J. B. Hendrickson, *Tetrahedron*, 1959, **7**, 82.

[15] (*a*) F. Dorn, P. Bernasconi, and D. Arigoni, *Chimia* (*Switz.*), 1975, **29**, 25; (*b*) D. Arigoni, *Pure Appl. Chem.*, 1975, **41**, 217.



SCHEME 2

farnesyl pyrophosphate to the 2-*cis*-isomer (13). A '*re*' face attack on the distal double bond would afford a maximum interaction between the 10,11-double bond and C-1. Subsequent hydrogen migration (of the circled atom) would then generate a *pro-R* stereochemistry at C-5 in culmorin. We favoured [11] the first pathway involving the direct cyclization of all-*trans*-farnesyl pyrophosphate because the formation of 2-*cis*-farnesol involved the loss of a C-1 hydrogen atom from farnesol and the introduction of an additional hydrogen from farnesol and the introduction of an additional hydrogen from NADPH. However Arigoni has determined [15] the location and stereochemistry to which the corresponding hydrogen atom has moved in *ent*-longifolene (7), a close structural relative of culmorin. In this case it is also a mevalonoid 5-*pro-S* hydrogen atom that has migrated, but it has taken up the *pro-R* stereochemistry at C-3 (longifolene numbering ≡C-5 of culmorin). Consequently Arigoni favours prior isomerization to the 2-*cis*-isomer of farnesyl pyrophosphate. This implies that there are two separate pathways, one for the trichothecenes with hydrogen loss and one for culmorin with hydrogen retention, for the formation of the 2-*cis*-isomer of farnesyl pyrophosphate in the same fungus. In order for a 5-*pro-S* mevalonoid hydrogen to migrate in this manner there must be a net inversion of configuration at C-1 in farnesyl pyrophosphate in the isomerization to the *cis*-isomer (*i.e.* the 5-*pro-S* mevalonoid hydrogen atom must occupy the 1-*pro-R* position in the 2-*cis*-isomer of farnesyl pyrophosphate).[15] The initial cyclization therefore warrants further study, possibly in a cell-free system in which earlier intermediates might be detected. So far we have been unable to detect (radio-t.l.c. scanning) *ent*-longiborneol (1c), which is a possible intermediate, in either mycelial or broth extracts of *F. culmorum*.

EXPERIMENTAL

General experimental details have been described previously.[16] Mass spectra were recorded on an A.E.I. MS30 instrument equipped with a DS 50 data system.

Fermentation Experiments.—*Fusarium culmorum* (U.I.C.P 34) [10] was grown in Roux bottles on a Raulin–Thom medium (150 ml per bottle) at 24 °C and harvested 2—3 weeks after inoculation. The mycelium was filtered and the filtrate was acidified to pH 2 with dilute hydrochloric acid, saturated with sodium chloride, and extracted (×3) with ethyl acetate. The extracts were washed with aqueous sodium hydrogen carbonate and aqueous sodium chloride, dried ($MgSO_4$), and evaporated, and the residual gum was chromatographed on alumina (Woelm neutral, grade III). The fractions containing culmorin were further purified by preparative layer chromatography on silica (Merck) in the solvent systems ethyl acetate–light petroleum (3 : 2) and chloroform–acetone–methanol (70 : 30 : 5). Culmorin (*ca.* 3 mg l^{-1}) crystallized from benzene or ethyl acetate–light petroleum as needles, m.p. 179—180°, $[\alpha]_D$ −13° (*c* 0.3) (lit.,[2] m.p. 173—175°, $[\alpha]_D$ −14°), identical (i.r. and t.l.c.) with an authentic sample. In the feeding experiments the labelled substrates (60—80 μCi ^{14}C) were dissolved in ethanol and distributed between 15—30 bottles as soon as growth was established. The fermentations were harvested after a further 14 days growth. The results are given in the Table.

Exchange Reactions.—Culmorin diketone, prepared as in ref. 3, had m.p. 102—103°, $[\alpha]_D$ −27° (*c* 0.1)(lit.,[3] m.p. 103—104°, $[\alpha]_D$ −29°). The diketone (23 mg) in dioxan (3 ml) was treated with sodium deuteroxide [from sodium (23 mg) in deuterium oxide (0.5 ml)] at room temperature for 16 h. The solution was acidified with 20% deuterochloric acid in deuterium oxide and extracted with ether. The extract was washed with deuterium oxide, dried, and evaporated. The residue was crystallized from light petroleum at −20 °C to afford culmorin diketone (15 mg), m.p. 102—104°, *m/e*

[16] J. R. Hanson, J. Hawker, and A. F. White, *J.C.S. Perkin I*, 1972, 1892.

234($^2H_0$, 5.7%), 235($^2H_1$, 27.8%), 236($^2H_2$, 55.6%), and 237($^2H_3$, 10.9%) (corrected for natural abundance of ^{13}C). The exchange reaction was repeated for a further 60 h to afford material showing *m/e* 236 ($^2H_2$, 6.5%), 237 ($^2H_3$, 92.7%), and 238 ($^2H_4$, 0.8%).

Degradation of Culmorin Diketone.—The diketone (10 mg) (from the (4*R*)-[4-^3H, 2-^{14}C]mevalonate experiment} (^3H : ^{14}C 4.06 : 1; specific activity 1.45 × 10^6 disint. min^{-1} $mmol^{-1}$) was converted [3] into the nor-hydroxy-ester (2) (7 mg), m.p. 134—136° (lit.,[3] 134—135°)(^3H : ^{14}C ratio 5.93 : 1, atom ratio 1.92 : 2, specific activity 0.984 × 10^6 disint. min^{-1} $mmol^{-1}$). After acidification of the potassium t-butoxide–t-butyl alcohol solution, a stream of nitrogen was bubbled through and thence into a solution of barium hydroxide.

The barium carbonate which was formed showed *ca.* 10 000 disint. min^{-1} (48% recovery of one centre). The nor-hydroxy-ester was treated with thionyl chloride in pyridine and then with sodium methoxide in methanol [3] to afford the unsaturated ester (3) (4 mg) as an oil (lit., [3] m.p. 45—47°), which was purified by repeated preparative layer chromatography in light petroleum–ethyl acetate (9 : 1). The oil had ^3H : ^{14}C 3.29 : 1, atom ratio 1.06 : 2, and was characterized by its mass spectrum. The same degradation was carried out on the diketone (12 mg)(^3H : ^{14}C 8.11 : 1, specific activity 5.29 × 10^5 disint min^{-1} $mmol^{-1}$) from the [2-$^3H_2$, 2-^{14}C]mevalonic acid experiment, to afford the nor-hydroxy-ester (2) (9 mg), m.p. 133—135°, (^3H : ^{14}C 8.26 : 1, atom ratio 3.7 : 2, specific activity 3.5 × 10^5 disint min^{-1} $mmol^{-1}$).

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[6/551 *Received*, 22*nd March*, 1976]