Studies in Terpenoid Biosynthesis. Part 25.[1] The Fate of the Mevalonoid Hydrogen Atoms in the Biosynthesis of the Sesquiterpenoid, Dihydrobotrydial

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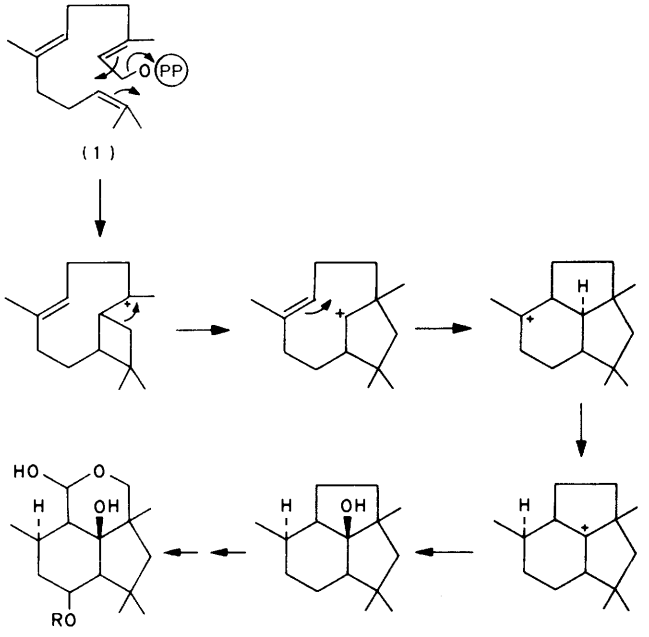
The ^{1}H n.m.r. spectrum of dihydrobotrydial and its ethyl ether were assigned. The number of mevalonoid labels that were incorporated into dihydrobotrydial by the fungus, *Botrytis cinerea*, were determined from ^{3}H : ^{14}C ratio studies whilst the location of the labels was defined by ^{2}H n.m.r. methods. A 1,3-hydrogen shift occurs during the cyclization and ^{18}O studies show that the 9-hydroxy-group arises from water.

THE sesquiterpenoid metabolites[2-5] of *Botrytis cinerea* contain a number of features of biogenetic interest. Biosynthetic studies with ^{13}C-labelled substrates have defined[1] the way in which farnesyl pyrophosphate (1) is folded to generate the carbon skeleton of the major sesquiterpenoid, dihydrobotrydial (2). This has led to the biogenetic proposals which are embodied in Scheme 1.



(1)

(2) R = Ac

SCHEME 1 Biosynthesis of dihydrobotrydial

A hydrogen migration to C-2 of dihydrobotrydial and the cleavage of the C(4)–C(5) bond of farnesyl pyrophosphate with the formation of the C(10)–C(15) hemiacetal of dihydrobotrydial (2) are implicit in this scheme. The fate of the mevalonoid hydrogen atoms has provided[6] mechanistic and stereochemical information on these steps. The location of the labels, which was determined by a combination of ^{3}H–^{14}C radio-isotopic and ^{2}H–^{13}C n.m.r. methods, forms the subject of this paper.

A thorough assignment of the ^{1}H n.m.r. spectrum (see Table 1) is a prerequisite for the application of ^{2}H n.m.r. methods in biosynthesis. The ethyl ether (3) prepared from dihydrobotrydial (2) with ethanol and silver nitrate and a trace of acetic acid, proved a suitable substrate for this analysis. Spin-decoupling studies were conducted at 360 MHz where the signals are well resolved. Irradiation of the sextet (J 4.6, 11, and 11 Hz)

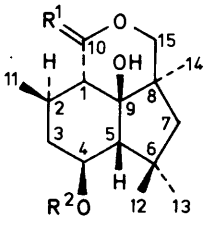
TABLE 1

^{1}H N.m.r. spectra of some derivatives of dihydrobotrydial determined in $CDCl_3$ at 360 MHz

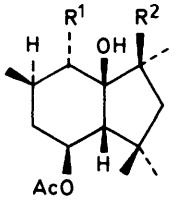
	Compound			
Proton	(2)	(3)	(7)	(4)
1	1.65	1.59	2.76	1.56
2	1.82	1.81	2.07	1.75
3	1.08	1.05	1.09	1.08
	2.09	2.01	2.03	1.91
4	5.11	5.07	5.02	3.95
5	1.92	1.90	2.00	1.58
7	1.15	1.09	1.56	1.08
	1.90	1.85	2.32	1.85
10	5.35	4.92	9.85	4.92
11	0.98	0.94	0.87	0.96
12	1.25	1.23	1.34	1.23
13	1.10	1.09	1.06	1.31
14	1.28	1.28	1.28	1.27
15	3.27	3.18		3.18
	4.22	3.97		3.97
AcO	2.00	2.00	2.02	
CH_3CH_2O		1.20	3.68 (OMe)	1.20
CH_3CH_2O		3.43		3.43
		3.78		3.78

at δ 5.07, which was readily assigned to H-4,[2] caused a doublet (J 11 Hz) at δ 1.90 (5-H) to collapse to a singlet; it also perturbed a multiplet, partly obscured by the acetoxy-signal, at δ 2.01 (3-H), and converted a quartet (J 11 Hz) at δ 1.05 (3-H) into a triplet. Irradiation at δ 2.01 removed the geminal coupling from the signal at δ 1.05, and reduced the sextet at δ 5.07 to a quartet and a multiplet at δ 1.81 (2-H) to an octet. Irradiation at δ 1.90 (5-H) caused the sextet at δ 5.07 to collapse to a double doublet (J 11 and 4.6 Hz). Irradiation at δ 1.59 (1-H) also caused the collapse of the multiplet at δ 1.81 (2-H). Irradiation at δ 1.85 (7-H) caused a doublet at δ 1.09 to collapse to a singlet, a coupling confirmed by the converse experiment. The ^{13}C n.m.r. spectra of the ethyl ether (3) and the corresponding 4-alcohol (4) were readily assigned (see Experimental section) by comparison with the assignments for dihydrobotrydial and its derivatives.[1] A similar series of decoupling experi-

ments at 220 MHz with dihydrobotrydial (2) confirmed these coupling patterns and also established that the methyl group doublet (δ 0.98, J 6.3 Hz, 11-H) was coupled to the multiplet at δ 1.82 (2-H). A Birdsall-Feeney [7] plot of the methyl group ^{13}C SFORD couplings enabled the remaining methyl group signals to be assigned. These showed that the proton signal at δ_H 0.98 was related to the carbon signal δ_0 20.1 assigned to C-11, the signal at δ_H 1.10 was related to δ_0 27.2 (C-13), δ_H 1.25 was related to δ_0 35.8 (C-12), and δ_H 1.28 was related to δ_0 25.3 (C-14). The proximity of the 15β-hydrogen atom to the 9-hydroxy-group and the C-10 oxygen atom provided a basis for the distinction between the C-15 diastereotopic hydrogen atoms. The lower field (δ 4.22) proton resonance showed a greater pyridine solvent shift ($\Delta\delta_{CDCl_3,C_5D_5N}$ 0.33 p.p.m. *versus* 0.05 p.p.m. for the 3.27 resonance) and was thus assigned to the 15β-hydrogen atom.



(2) R^1 = α-H, β-OH; R^2 = Ac
(3) R^1 = α-H, β-OEt; R^2 = Ac
(4) R^1 = α-H, β-OEt; R^2 = H
(8) R^1 = O; R^2 = Ac



(5) $R^1 = R^2 = CH_2OH$
(6) R^1 = CHO; $R^2 = CO_2H$
(7) R^1 = CHO; $R^2 = CO_2Me$
(9) $R^1 = CO_2Me$; $R^2 = CH_2OH$
(10) $R^1 = R^2$ = CHO

[2-$^3H_2$,2-^{14}C]-, [2(*R*)-2-^{3}H, 2-^{14}C]-, [4(*R*)-4-^{3}H,2-^{14}C]-, [5-$^3H_2$, 2-^{14}C]- and [5(*R*)-5-^{3}H, 2-^{14}C]-Mevalonates were each fed to *Botrytis cinerea*. The atom ratios in the resultant dihydrobotrydial (2) and botrydial (10) are given in Table 2. Since the dialdehyde botrydial, is rather unstable, in some instances it was reduced to the acetoxy-triol (5) with sodium borohydride before being counted. Subsequently [2-$^2H_2$]-, [4-$^2H_2$]-, and [5-$^2H_2$]-mevalonates were also fed to *B. cinerea*. The ^2H n.m.r. labelling patterns are given in Table 3 along with the assignments.

Three [4(*R*)-4-^{3}H]mevalonoid hydrogen labels, one from each isoprene unit, were retained in the dihydrobotrydial. However one of the centres (C-9) which is labelled by C-4 of mevalonate,[1] is fully substituted and hence the corresponding hydrogen atom has been involved in a rearrangement. In terpenoid biosynthesis a secondary methyl group is often formed as a consequence of a rearrangement of a hydrogen atom. The biogenetic scheme envisages 2-H arising as a consequence of such a rearrangement. This rearrangement could take the form either of two 1,2-shifts [9-H ⟶ C-1; 1-H ⟶ C-2; pathway (*a*)] or a direct 1,3-shift [9-H ⟶ C-2; pathway (*b*)] (see Scheme 2). These pathways have been distinguished by both ^{13}C and ^2H n.m.r. methods.

TABLE 2

Incorporation of [^{3}H, ^{14}C]mevalonates into dihydrobotrydial and botrydial

	Mevalonate	
	[2-$^3H_2$, 2-^{14}C]-	[5-$^3H_2$, 2-^{14}C]-
^{3}H : ^{14}C ratio fed	7.52 : 1	22.52 : 1
Dihydrobotrydial (2)—		
^{3}H : ^{14}C ratio found	6.14 : 1	14.68 : 1
Incorporation of ^{14}C, %	0.14	0.52
^{3}H : ^{14}C atom ratio	4.9 : 3	3.9 : 3
Botrydial (10)—		
^{3}H : ^{14}C ratio found		14.70 [a]
Incorporation of ^{14}C, %		0.08
^{3}H : ^{14}C atom ratio		3.92 : 3

	Stereospecifically (*R*) labelled mevalonate		
	[2(*R*)-2-$^3H_2$, 2-^{14}C]	[4(*R*)-4-$^3H_2$, 2-^{14}C]-	[5(*R*)-5-$^3H_2$, 2-^{14}C]-
^{3}H : ^{14}C ratio fed	12.90 : 1	6.53 : 1	2.61 : 1
Dihydrobotrydial (2)—			
^{3}H : ^{14}C ratio found	12.30 : 1	6.31 : 1	2.89 : 1
Incorporation of ^{14}C, %	1.28	0.2	0.71
^{3}H : ^{14}C atom ratio	2.86 : 3	2.9 : 3	3.3 : 3
Botrydial (10)—			
^{3}H : ^{14}C ratio found	12.66 : 1 [a]	6.65 : 1	3.07 : 1 [a]
Incorporation of ^{14}C, %	0.02	0.13	0.02
^{3}H : ^{14}C atom ratio	2.94 : 3	3.06 : 3	3.5 : 3

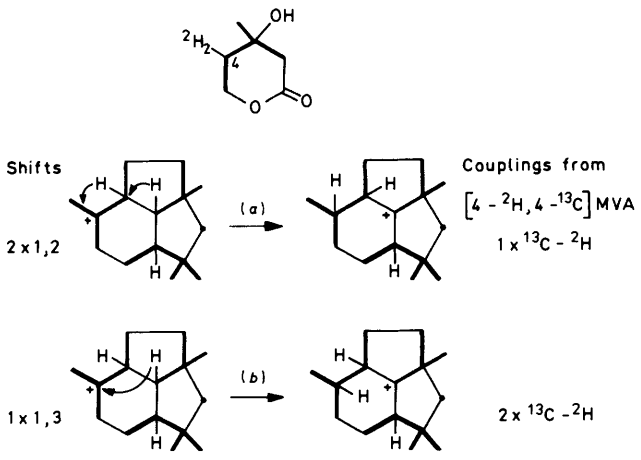
[a] As acetoxy-triol (5).

TABLE 3

[^{2}H]Mevalonoid labelling patterns determined by ^2H n.m.r.

Mevalonate	^2H N.m.r. signal	Relative integral	^1H N.m.r. signal	Assignment
[2-$^2H_2$]-				
Dihydrobotrydial (2)	1.17	3.5	1.06 and 1.25	H-3 and H-12
	1.95	1	2.01	H-3
	4.1	1	4.17	H-15
Ethyl ether (3)	1.03	1	1.08	H-3
	1.2	2	1.23	H-12
	2.0	0.9	2.09	H-3
	3.9	0.8	3.96	H-15
[4-$^2H_2$]-				
Dihydrobotrydial (2)	1.7	broad	1.65 and 1.82	H-1 and H-2
	1.9		1.92	H-5
[5-$^2H_2$]-				
Dihydrobotrydial (2)	1.1	1.1	1.15	H-7
	1.9	1	1.90	H-7
	5.0	0.85	5.11	H-4
	5.2	1	5.35	H-10

[4-$^2H_2$, 4-^{13}C]Mevalonic acid was prepared so that each ^{13}C- label also bore a deuterium label. Sodium [2-^{13}C]-acetate was converted into ethyl [2-^{13}C]acetate and this was then condensed [8] with 1,1-dimethoxybutan-3-one to afford ethyl 5,5-dimethoxy-3-hydroxy-3-methyl[2-^{13}C]pentanoate. This was equilibrated with sodium methoxide in [^{2}H]methanol to afford methyl 5,5-dimethoxy-3-hydroxy-3-methyl[2-$^2H_2$, 2-^{13}C]pentanoate.



SCHEME 2 Possible hydrogen rearrangements in dihydrobotrydial biosynthesis and their ^{2}H : ^{13}C n.m.r. consequences

The ester was reduced with lithium aluminium hydride, the acetal hydrolysed with dilute hydrochloric acid, and the aldehyde oxidized with bromine water to afford the required mevalonate.

In pathway (*a*) two [4-$^2H_2$]mevalonoid deuterium atoms become detached from their ^{13}C partners whilst in pathway (*b*) only one atom becomes detached. This has consequences in both the ^{13}C and ^{2}H n.m.r. spectra which yield complementary pieces of information. If deuterium with a spin of 1 is attached to a ^{13}C carbon atom not only does the ^{2}H–^{13}C coupling remain in the ^{13}C proton noise decoupled spectrum but also the nuclear Overhauser enhancement is absent. Hence when deuterium remains attached to ^{13}C from a substrate, any biosynthetic enrichment of a metabolite, which might be shown by an increase in signal over the natural abundance spectrum, is quenched. Pathway (*a*) moves two deuterium atoms away from their ^{13}C partners and thus two out of the three enrichments will be revealed and only one will be quenched. In contrast, pathway (*b*) moves only one deuterium atom and hence only one enrichment will be revealed, that of the tertiary alcohol, C-9. Since ^{13}C has a spin of $\frac{1}{2}$ whilst deuterium has a spin of 1, any ^{13}C—^{2}H heteronuclear coupling is more easily observed in the deuterium n.m.r. spectrum. Pathway (*a*) destroys two couplings and will afford two singlets and one doublet in the ^{2}H n.m.r. spectrum whilst pathway (*b*) gives one singlet and two doublets. Finally the ^{2}H chemical shift of the singlets is indicative of the site to which the rearrangement has occurred.

Two feeding experiments to *B. cinerea* were carried out. The first experiment gave botryaloic acid (6) as the major metabolite. This was isolated as its methyl ester (7). Spin decoupling studies at 220 MHz enabled the ^{1}H n.m.r. spectrum of the ester (7) to be fully assigned (see Table 1). In particular the 1-H, 2-H, and 5-H signals were clearly identified at δ 2.76, 2.07 and 2.00 respectively. In the ^{2}H n.m.r. spectrum, determined at 55.3 MHz, the 1-^{2}H and 5-^{2}H signals appeared as doublets, (δ 2.7 and 2.00, *J* 19.5 and 21 Hz respectively) whilst the 2-^{2}H signal appeared as a singlet (δ 2.00). These signals were superimposed on the natural abundance spectrum. In the second experiment, the dihydrobotrydial (2) was converted into its ethyl ether (3) and then the acetoxy-group was hydrolysed by methanolic potassium carbonate to afford the alcohol (4). The ^{2}H n.m.r. spectrum comprised a singlet at δ 1.7 corresponding to 2-H and doublets (*J* 20 Hz) centred on the 1-H and 5-H signals (δ 1.5). The ^{13}C n.m.r. spectrum of the alcohol contained small triplet signals at δ_C 63.2 (*J* 20 Hz), 0.6 p.p.m. upfield from the signal at δ_C 63.8 assigned to C-5 and at δ_C 54.1 (*J* 19 Hz) 0.5 p.p.m. upfield from the signal at δ_C 54.9 assigned to C-1. The signal at δ_C 82.4, assigned to C-9, was enriched. Comparable deuterium isotope shifts in ^{13}C n.m.r. spectra have been observed [9] in other biosynthetic studies. These results show that the [4-^{2}H,4-^{13}C]-mevalonoid labels at C-1 and C-5 have remained attached whilst the [4-^{2}H]mevalonoid label originally at C-9 has migrated to C-2, as required by the biosynthetic scheme.

One implication of this scheme is that the oxygen atom of the tertiary alcohol at C-9 originates from water rather than from air. Although the discharge of a ' carbocation ' in biosynthesis by hydration features in a number of sesquiterpenoid biosynthetic schemes, it has rarely been established experimentally (for a recent example see ref. 10). *B. cinerea* was grown in an H_2 ^{18}O medium (*ca.* 12% ^{18}O). The dihydrobotrydial (2) was isolated and in order to remove any possible ambiguity concerning the origin of the hemiacetal oxygen, it was converted into the ethyl ether (3). Like many derivatives of dihydrobotrydial, the ethyl ether does not have a significant molecular ion in the electron-impact mass spectrum. However it shows ions corresponding to $(M - EtO)^+$ and $(M - EtOH)^+$ (Found: $M^+ - EtO$, 295.190. $C_{17}H_{27}O_4$ requires $M - EtO$, 295.191. Found: $M^+ - EtOH$, 294.182. $C_{17}H_{26}O_4$ requires $M - EtOH$, 294.183). The labelled material contained ions at 297 and 296 a.m.u. (Found: $M^+ - EtOH$, 296.187. $C_{17}H_{26}{}^{16}O_3{}^{18}O$ requires $M - EtOH$, 296.187). The relative intensities of these ions were measured (see Table 4) and revealed a 15.5% enrichment. The difference between this figure and the estimated content of the initial ^{18}O probably arises because the culture was allowed to grow for 3 d to ensure that there was no contamination before the ^{18}O-labelled water was added to the medium and some evaporation may have occurred. The mass spectrum of the ethyl ether also showed ions at 262 and 263 a.m.u. corresponding to the loss of H_2O and $CH_3CO_2(H)$. Comparison of the ions in the mass

range 262—265 a.m.u. between the labelled and unlabelled samples showed no significant incorporation of thus confirming the anticipated location of the label at C-9.

The [2(*R*)-2-^{3}H, 2-^{14}C]- and [5(*R*)-5-^{3}H, 2-^{14}C]-mevalonate results provided some information on the formation of the hemiacetal ring of dihydrobotrydial. The metabolite retained four [5-^{3}H]mevalonoid hydrogen

TABLE 4

Incorporation of ^{18}O into dihydrobotrydial ethyl ether (3)

	Mean intensity [a]		
Ion (a.m.u.)	Unlabelled sample	Labelled sample	
294	74	72.04	$(M-EtOH)^+$
295	100.0 [b]	100.0 [b]	$(M-EtO)^+$
296	16.90	29.61	
297	1.35	19.71	

The ^{18}O content, based on the $(M-EtOH)^+$ ion was 15.47% whilst that based on the $(M-EtO)^+$ ion was 15.5%.

[a] Mean of 31 determinations. [b] Normalized.

atom labels including all three of the *pro*-5(*R*)-labels. Oxidation of the dihydrobotrydial (atom ratio 3.32 : 3) from the [5(*R*)-5-^{3}H, 2-^{14}C]mevalonate experiment to the C-10 lactone (8) (atom ratio 2.2 : 3) established that C-10 bore a *pro*-5(*R*)-label. However, oxidation of the [2-^{3}H]-labelled material (atom ratio 4.9 : 3) to the lactone (8) followed by hydrolysis and methylation afforded the hydroxy-ester (9). Further oxidation of C-15 and methylated to form the dimethyl ester (7) (atom ratio 3.85 : 3) established that C-15 bore only one [2-^{3}H]-mevalonoid label. Since all three *pro*-2(*R*)-mevalonoid labels were incorporated into dihydrobotrydial, it must be a *pro*-2(*R*)-mevalonoid hydrogen atom which remained at C-15.

The distinction between the two enantiotopic hydrogen atoms at C-15 was made by deuterium labelling. The two hydrogen atoms resonate at δ 3.27 and 4.22 (J_{AB} 10.5 Hz) in dihydrobotrydial. The lower field signal was assigned (*vide supra*) to the 15β-proton. [2-$^{2}H_3$]-Acetate labels the C-2 position of mevalonate and hence C-15 in dihydrobotrydial. Examination of the labelled dihydrobotrydial derived from [2-$^{2}H_3$]acetate showed that the mevalonoid label was on the lower field (δ 4.20) of the two C-15 protons, *i.e.* it is an '*S*' centre in the labelled material. Subsequently the experiment was repeated with [2-$^{2}H_2$]mevalonate. The dihydrobotrydial (2) was isolated (see Table 3). The signal at δ 4.1 was enriched and there was no signal at δ 3.25. This signal (at δ 3.9) was also clearly apparent in the ethyl ether (3). The stereochemistry of labelling of farnesyl pyrophosphate at C-4 and C-5 by *pro*-2(*R*) and *pro*-5(*R*)-mevalonate is known [11] and is shown in Figure 1. Comparison of this with the labelling pattern of dihydrobotrydial shows that the formation of the hemiacetal ring has proceeded with overall retention of configuration at both centres. Insight into the formation of the hemiacetal ring comes from the observation [12] that the dialdehyde, botrydial (10) is incorporated into dihydrobotrydial (2) by *B. cinerea* to the extent of 32% whereas the reverse reaction proceeded in 1.09%.

Examination of the ^{2}H n.m.r. spectra of both dihydrobotrydial and the corresponding ethyl ether (Table 3) showed that the C-12 methyl group was labelled by [2-$^{2}H_2$]mevalonate. This methyl group is *cis* to the hydrogen atom at C-5 (labelled by [4-$^{2}H_2$]mevalonate) and would thus be expected to carry this label in the event of a normal isoprenoid cyclization.

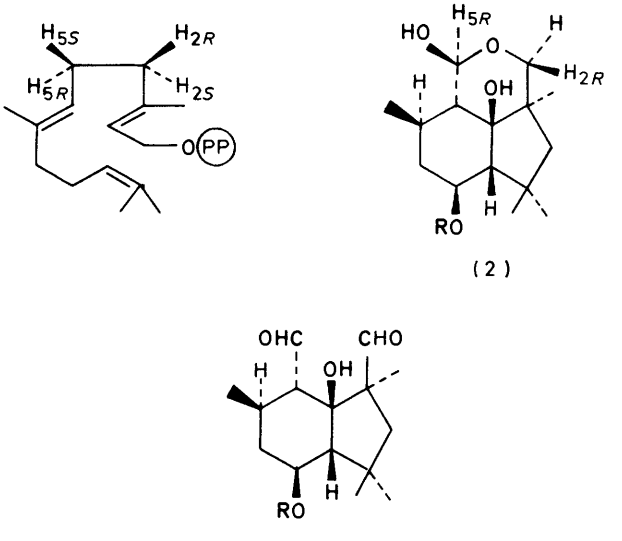


FIGURE 1 Formation of the hemiacetal ring of dihydrobotrydial

The following conclusions may be drawn from the labelled pattern. The fate of the mevalonoid hydrogen atoms is consistent with the folding of farnesyl pyrophosphate such that the terminal C-1 attacks the 're' face of the distal double bond with the formation of an eleven-membered ring [13] and the 6,7-bond of dihydrobotrydial. This system is the parent of humulene. However, further cyclization occurs with the formation of the 5,9-bond to afford a carbocation which could collapse to caryophyllene.[14] Rearrangement of the 9,7 bond to C-8 generates a C-9 carbocation and thence the formation of the tricyclic system (see Scheme 1). A comparable rearrangement has recently been proposed to acount for the formation of the silphenene sesquiterpenoids.[15] The relative stereochemistry of the hydrogen atom which migrates from C-9 to C-2 and the hydroxy-group (Figure 2) at C-9 are consistent with an anticipated inversion of configuration at this step. The mevalonoid labelling pattern at C-4 is as expected from a normal hydroxylation with retention of configuration. Since the dialdehyde, botrydial (10) is implicated in the formation of the hemiacetal ring and the labelling pattern at C-10 and C-15 indicates an overall retention of configuration, the microbial reduction of the C-15 aldehyde must have proceeded with the 're' stereospecificity. This is typical of a microbial dehydrogenase.[16] The dialdehyde could be formed by cleavage

of a vicinal glycol, which, assuming that hydroxylation has proceeded with the normal retention of configuration, would be a *trans*-15β,10α-glycol.

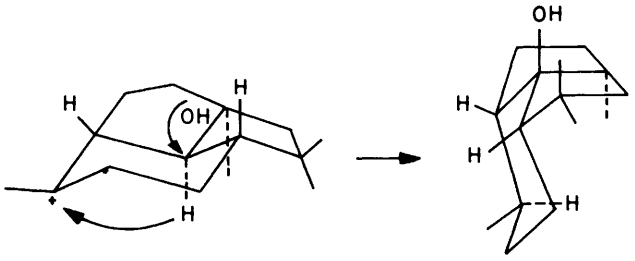


FIGURE 2 Stereochemistry of rearrangement in dihydrobotrydial biosynthesis

EXPERIMENTAL

General experimental details have been described previously.[17]

General Fermentation Conditions.—*Botrytis cinerea* (GCRI strain 23, obtained from the Glasshouse Crops Research Institute, Littlehampton, Sussex was grown on surface culture in Thompson bottles on a Czapek-Dox medium (750 ml) containing 0.1% yeast extract and 5% glucose. The ages of the cultures at the time of feeding and harvest are given in Table 5. The precursors were administered in ethanol or water. The mycelium was filtered off and the broth saturated with sodium chloride, acidified to pH 2 with dilute hydrochloric acid, and extracted with ethyl acetate. The extracts were separated into acidic and neutral fractions with aqueous sodium hydrogencarbonate. Dihydrobotrydial and botrydial were obtained from the neutral fractions by preparative layer chromatography on silica in chloroform–ethyl acetate–acetic acid (40 : 10 : 1) and identified by their m.p. and n.m.r. spectra.[2] The results from the various feeding experiments are given in Table 5.

Conversion of Botrydial into the Acetoxy-triol (5).—Botrydial (50 mg) in methanol (10 ml) was treated with sodium borohydride (50 mg) during 0.5 h. Dilute hydrochloric acid (1 ml) was added, the solution diluted with water and the product recovered in ethyl acetate and purified by p.l.c. on silica in the above solvent system. The acetoxy-triol (5) had m.p. 173—175.5 °C (lit.,[2] 175—176 °C) (Found: C, 65.0; H, 9.6. Calc. for $C_{17}H_{30}O_5$: C, 64.9; H, 9.6%).

Oxidation of Dihydrobotrydial to the Lactone (8).—Dihydrobotrydial (60 mg) in pyridine (5 ml) was treated with a solution of chromium trioxide in pyridine (1 ml) [prepared by carefully adding chromium trioxide (5 g) in water (3 ml) to pyridine (10 ml)] for 24 h. The solution was diluted with water and extracted with ether. The extract was washed with dilute hydrochloric acid, aqueous sodium hydrogencarbonate, and saturated aqueous sodium chloride, and then dried and evaporated. The lactone (8) was purified by p.l.c. on silica in the above solvent system. It crystallized as needles, m.p. 215—216 °C (lit.,[2] 217—219 °C) (Found: C, 65.8; H, 8.4. Calc. for $C_{17}H_{26}O_5$: C, 65.8; H, 8.4%).

Conversion of the Lactone (8) *into the Ester* (7).—The lactone (48 mg) in methanol (5 ml) was stirred overnight with 1M potassium carbonate (1 ml). The solution was cautiously acidified, concentrated *in vacuo* at room temperature and the product recovered in ether and methylated with diazomethane. The resultant hydroxy-ester (36 mg) in pyridine (1 ml) was oxidized with chromium trioxide (1 ml) as above to afford the 15-aldehyde (n.m.r.) and after autoxidation the 15-acid, which was methylated with diazomethane. The 10,15-dimethyl ester (7) was purified by chromatography on silica in 15% ethyl acetate–light petroleum and crystallized from light petroleum as needles (7.5 mg), m.p. 124—125 °C (lit.,[2] 125—126 °C).

Conversion of Dihydrobotrydial into the Ethyl Ether (3).—Dihydrobotrydial (400 mg) in ethanol (10 ml) was treated with silver nitrate (118 mg) dissolved in the minimum of water and acetic acid (6 drops) overnight. The ethanol was evaporated and water was added; the product was recovered in ethyl acetate and purified by chromatography

TABLE 5

Feeding experiments to *Botrytis cinerea*

Mevalonate precursor	Quantity (μC ^{14}C)	Vol. of fermentation (l)	Period of incubation day—day	Mass of (2) isolated (mg)	Incorporation (enrichment)
2-^{3}H, 2-^{14}C	17.3	1.5	3—8	62	0.14
2(*R*)-2-^{3}H, 2-^{14}C	20.2	1.5	2—8	116	1.28
				11 [a]	0.02
4(*R*)-4-^{3}H, 2-^{14}C	50	2.25	4—8	115	0.2
				80	0.13
5-^{3}H, 2-^{14}C	12.05	3	4—8	195	0.52
				31 [a]	0.08
5(*R*)-5-^{3}H, 2-^{14}C	1.28	0.75	4—7	20	0.71
				3	0.02
CD_3CO_2D	3 g, 31.8 μC	4.5	4—7	227	0.43 (3.1%)
2-$^3H_2$, 2-^{14}C	320 mg, 16 μC	6	3,4,5,6,7—10	607	2.3 (0.97%)
4-$^3H_2$, 4-^{13}C, 2-^{14}C	470 mg, 25 μC	11	4—7	638	0.56 (0.33%)
				708 [b]	
4-$^3H_2$, 4-^{13}C, 2-^{14}C	250 mg, 25 μC	6	4,5,6,7—10	307	0.80 (0.52)
4-$^3H_2$	300 mg	6	4,5,6,7—10	220	—
5-$^3H_2$, 2-^{14}C	400 mg, 50 μC	6	3,4,5,6,7—10	215	0.66 (0.98)
^{18}O H_2O	1 g (64%)	4 ml	3—7	0.5 mg	(see Table 4)

[a] Botrydial. [b] Botryaloic acid methyl ester.

on silica in ethyl acetate–chloroform (1 : 4). The *ethyl ether* (3) (285 mg) crystallized from pentane at −20 °C as needles, m.p. 92—93 °C (Found: C, 67.3; H, 9.4. $C_{19}H_{32}O_5$ requires C, 67.0; H, 9.5%), $\nu_{max.}$ 3 480 and 1 720 cm^{-1}; δ_C 15.0 (q, CH_3CH_2O), 20.1 (q, C-11), 21.4 (q, OAc), 25.4 (q, C-14), 27.3 (q, C-13), 28.6 (d, C-2), 35.6 (q, C-12), 38.8 (s, C-6), 40.0 (t, C-3), 45.6 (s, C-8), 50.4 (t, C-7), 54.8 (d, C-1), 59.4 (d, C-5), 62.9 (t, CH_3CH_2O), 67.5 (t, C-15), 72.7 (d, C-4), 82.6 (s, C-9), 97.2 (d, C-10), and 170.4 (s, OAc; *m/z* 322 (*M* − 18, 0.5%), 295 (4), 263 (1), 262 (2), 235 (10), 219 (10), 206 (10), 179 (20), 109 (35), 96 (90), 55 (50), and 43 (100).

Hydrolysis of the Ethyl Ether (3).—The above ethyl ether (255 mg) was heated under reflux with methanolic 1M potassium carbonate (20 ml; methanol–water, 1 : 1) for 2 h. The methanol was removed under reduced pressure and the product recovered in ethyl acetate and purified by chromatography on silica (chloroform–ethyl acetate 4 : 1). The *alcohol* (4) (183 mg) crystallized from diethyl ether–light petroleum as needles, m.p. 123—126 °C, (Found: C, 68.8; H, 9.9. $C_{17}H_{30}O_4$ requires C, 68.4; H, 10.1%), $\nu_{max.}$ 3 500br cm^{-1}; δ_C 14.7 (q, CH_3CH_2O), 19.9 (q, C-11), 24.8 (q, C-14), 27.3 (q, C-13), 28.5 (d, C-2), 35.7 (q, C-12), 38.4 (s, C-6), 44.6 (t, C-3) 45.2 (s, C-8), 50.1 (t, C-7), 54.6 (d, C-1), 62.6 (t, CH_3CH_2O), 63.8 (d, C-5), 67.2 (t, C-15), 69.3 (d, C-4), 82.5 (s, C-9), and 96.9 (d, C-10).

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