THE BIOSYNTHESIS OF SOME SESQUITERPENOIDS

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Abstract - The determination of the labelling patterns of the
sesquiterpenoids,dihydrobotrydial and alliacolide,when biosynthesized
from ^{13}C and ^2H labelled acetate and mevalonate is described.

INTRODUCTION

The rationalization of sesquiterpenoid structures in terms of the biogenetic
isoprene rule[1] was one of the masterpieces of the speculative era of biogenesis.
Subsequently with the advent of appropriate experimental techniques, the
sesquiterpenoids have proved to be a fruitful field for biosynthetic studies.
The variety of cyclic carbon skeleta that can be generated[2] utilizing the
pyrophosphate leaving group and the three double bonds of farnesyl pyrophosphate
have provided the stimulus for many investigations notably in the laboratories
of Professor Arigoni.[3] Our studies[4] have concentrated on defining the manner
of folding of farnesyl pyrophosphate in fungal sesquiter penoids, with the
elucidation of the stereochemical fate of mevalonoid hydrogen atoms and in
trying to relate groups with common primary cyclizations.

RESULTS AND DISCUSSION

The investigations to be reported here concern two sesquiterpenoids each with
a novel carbon skeleton.   The first, dihydrobotrydial [1][5] is produced by the
fungus, Botrytis cinerea, the noble mould of grapes and a serious pathogen of
lettuce and tomatoes.   The second is alliacolide [2][6] which is a metabolite
of the wood-rotting fungus, Marasmius alliaceus.   Comparison of both structures

R = COCH$_3$

1

2

shows that they do not strictly obey the isoprene rule. Indeed they both have
two possible starting points for the farnesyl pyrophosphate chain together with
several possible ways of folding that chain including possible skeletal
rearrangements.   They both have secondary methyl groups, which in terpenoid
biosynthesis are good, although not invariable, markers of hydrogen rearrange-
ments.   Stable isotopes with n.m.r. methods of analysis,[7] now play an
important role in biosynthetic studies and I want to illustrate these methods
here.   We can consider each problem in three stages:- firstly the origin of
the carbon skeleton,secondly the fate of the mevalonoid hydrogen atoms and
thirdly, the oxidative processes.

Dihydrobotrydial

Farnesyl pyrophosphate was incorporated into dihydrobotrydial to the extent of
0.33% thereby establishing its sesquiterpenoid origin. By dissecting out
possible isoprene units, several possible ways (fig.1 a - d) of forming the
carbon skeleton can be envisaged.

figure   1
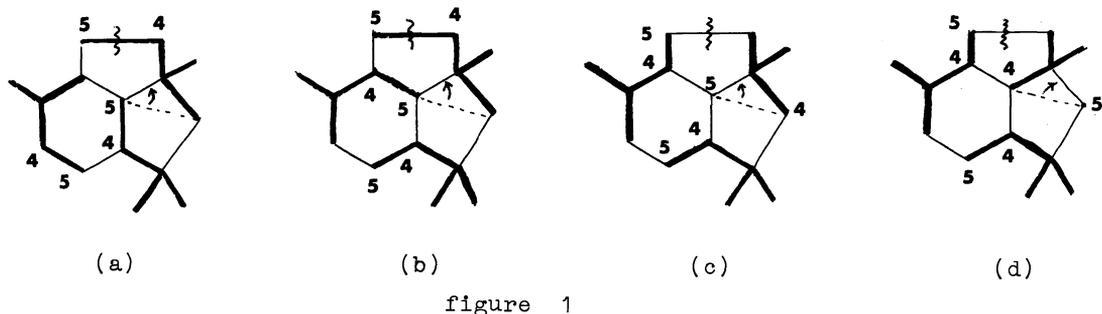
The carbon skeleton of farnesyl pyrophosphate will be labelled from [1-^{13}C]-
acetate as shown in fig. 2(a) whilst [1,2-^{13}C$_2$]-acetate will generate the
coupling pattern as shown in fig. 2(b) provided it is fed at such a dilution
that individual molecules of farnesyl pyrophosphate are not biosynthesized
from more than one labelled acetate unit (vide infra). [4,5-^{13}C$_2$]-Mevalonate
generates the much simpler coupling pattern (fig. 2(c)).

(a) ● label from [1-^{13}C]acetate

(b) ▬ coupling from [1,2-^{13}C$_2$] acetate

(c) ▬ coupling from [4,5-^{13}C$_2$] mevalonate
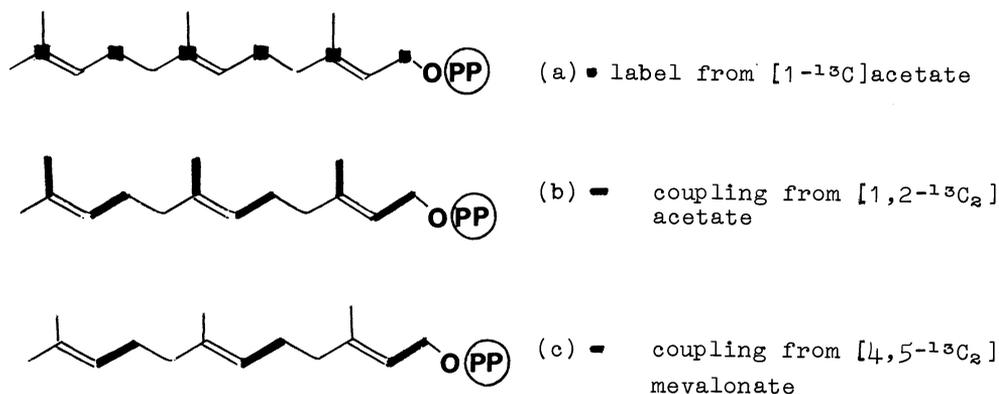
figure 2

Both [1,2-^{13}C$_2$]-acetate and [4,5-^{13}C$_2$]-mevalonate were fed to Botrytis
cinerea. Analysis of the ^{13}C n.m.r. spectra of the resultant dihydrobotrydial
revealed coupling patterns which were consistent with either foldings (c) or
(d). These foldings were distinguished by the use of [1-^{13}C]-acetate. In the
biosynthesis of polyketides, small (in intensity) couplings have sometimes been
observed between acete units.   These arise by the multiple-labelling of the
polyketide chain.   Whilst studying the biosynthesis of the sesquiterpenoid
trichothecin by Trichothecium roseum, we showed[8] that feeding large quantities
of acetate inhibited the formation of endogenous acetate by, for example,
glycolysis.   Hence if a pulse of [1-^{13}C]-acetate is fed to the fungus, a
situation arises in which, for a short time, the farnesyl pyrophosphate is
made primarily from exogenous material and is multiply-labelled. If a bond is
then created in a subsequent cyclization or rearrangement between two C-1
labelled centres, a new ^{13}C-^{13}C coupling is observed. Folding (c) will
generate a coupling between C-8 and C-9 in dihydrobotrydial whilst folding (d)
willgenerate couplings between C-6,C-7 and C-8.   In practice the latter set
of induced couplings were observed. This leads us to propose the following
biosynthetic pathway (figure 3) which is reminiscent of that leading to the
sesquiterpenoid hydrocarbon, caryophyllene.[9]
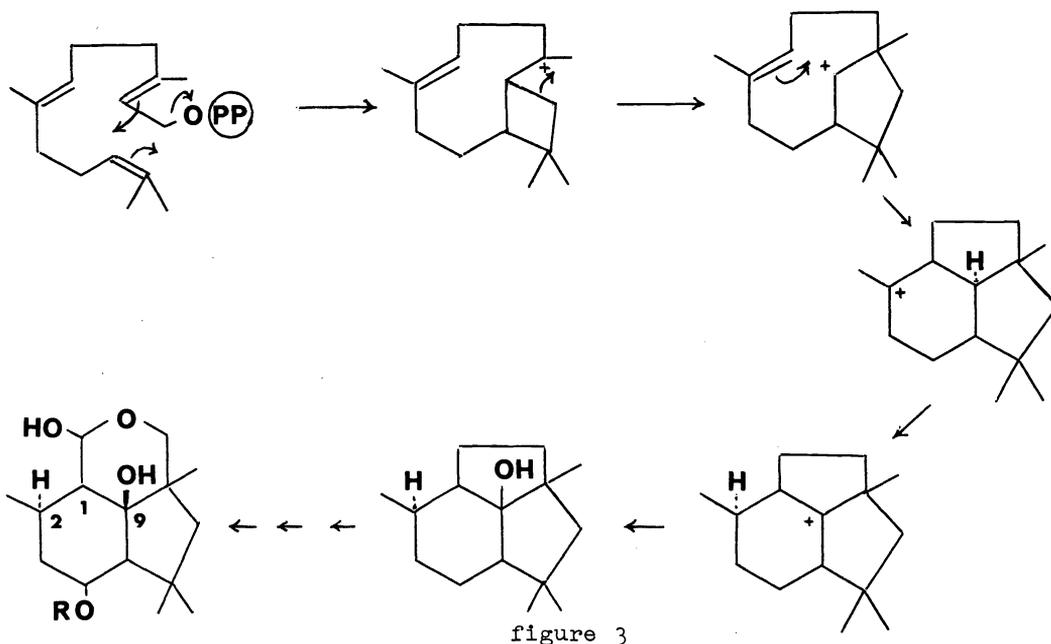
figure 3

The second phase of the problem involved the stereochemical fate of the mevalonoid hydrogen atoms. [2-^{3}H$_2$,2-^{14}C]-, [2-(R)-2-^{3}H,2-^{14}C]-,[4-(R)-4-^{3}H, 2-^{14}C]-, [5-^{3}H$_2$,2-^{14}C]- and [5-(R)-5-^{3}H,2-^{14}C]-Mevalonates were each fed to the fungus and the atom ratios obtained in the product are given in table 1

Table 1   Incorporation of Mevalonates into Dihydrobotrydial (1)

Mevalonate	2-^{3}H,2-^{14}C	4-(R)-4-^{3}H,2-^{14}C	5-^{3}H,2-^{14}C
^{3}H:^{14}C atom ratio in (1)	4.9:3 (0.14%)		3.9:3 (0.52%)
^{3}H:^{14}C atom ratio from stereospecific(R) MVA in (1)	2.86:3(1.28%)	2.9:3 (0.2%)	3.3:3 (0.7%)

incorporation (%)

All three 4(R)-4-^{3}H mevalonoid hydrogen labels were incorporated. In dihydrobotrydial oneof the centres (C-9) labelled by C-4 of mevalonate, is fully substituted and hence the corresponding hydrogen atom has been involved in a rearrangement. In the proposed biosynthetic scheme (fig.3), the rearrangement leads to a hydrogen atom appearing at C-2 thus generating the secondary methyl group. The rearrangement could take the form of two 1,2-shifts (H-9 → C-1; H-1 → C-2) or a direct 1,3-shift (H-9 → C-2)(see figure 4):

pathway (a)

pathway (b)
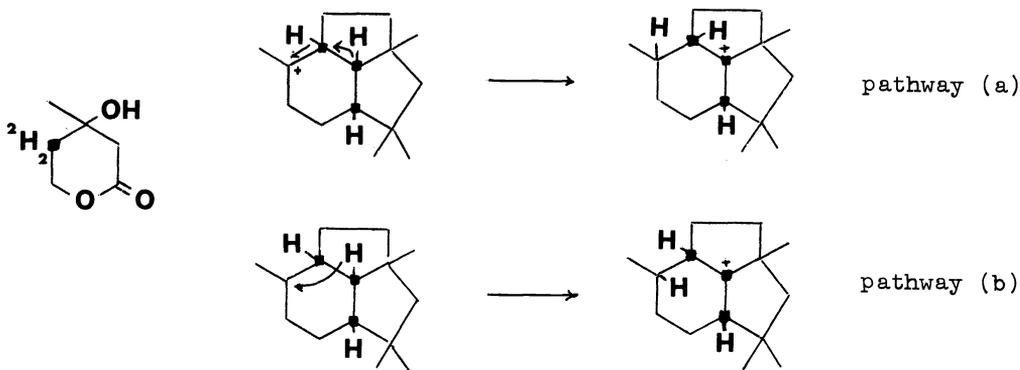
figure 4

These have been distinguished by both ^{13}C and ^{2}H n.m.r. methods. $[4-^{2}H_{2},4-^{13}C]$
Mevalonic acid was prepared so that each carbon-13 label also bore a deuterium
atom.   The mevalonate was fed at such a dilution to <u>Botrytis</u> <u>cinerea</u> that the
individual molecules of dihydrobotrydial contained only one labelled unit.   In
pathway (a) two deuterium atoms become detached from their carbon-13 partners
whilst in pathway (b) only one atom becomes detached.   This has consequences
in both the carbon and deuterium n.m.r. spectra.   In the proton noise decoupled
^{13}C n.m.r. spectrum, the $^{1}H:^{13}C$ couplings are removed and the relaxation mode
of the carbon-13 is also modified.   Both these effects lead to an enhancement
of the signal. If deuterium with a spin of 1, is attached to the carbon atom,
not only does the $^{2}H:^{13}C$ coupling remain in the proton noise decoupled spectrum
but also the nuclearOverhauser enhancement is absent.   Hence when deuterium
is attached to a carbon-13 any biosynthetic enrichment shown by signal increase
over the natural abundance spectrum, is quenched. Pathway (a) moves two
deuterium atoms away from their carbon-13 partners and thus two out of the three
enrichments will be revealed and only one will be quenched.   On the other hand
pathway (b) moves only one deuterium atom and hence only one enrichment will be
revealed, that of the tertiary alcohol C-9.   Since carbon-13 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 spectrum. Pathway (a) destroys two couplings
and will afford two singlets and one doublet in the deuterium spectrum whilst
pathway (b) gives one singlet and two doublets.   Furthermore since the proton
n.m.r. spectrum has been assigned, the deuterium chemical shifts indicates the
sites of the labels.   The carbon-13 n.m.r. results supported pathway (b) with
only C-9 showing an enrichment.   The deuterium n.m.r. spectrum of botryaloic
acid ester (3), a relative of dihydrobotrydial and also produced by the fungus,
was more clearly resolved. The ^{2}H-1 signal and the ^{2}H-5 signal appeared as
doublets (J 19.5 and21 Hz) whilst the ^{2}H-2 signal appeared as a singlet each
superimposed on the natural abundance spectrum.   Hence there is a 1,3-hydride
shift in dihydrobotrydial biosynthesis which is stereochemically much more
acceptable producingan inversion of configuration at C-9, i.e. the hydroxyl
group lies on the opposite face of the molecule to H-2 (see figure 5).
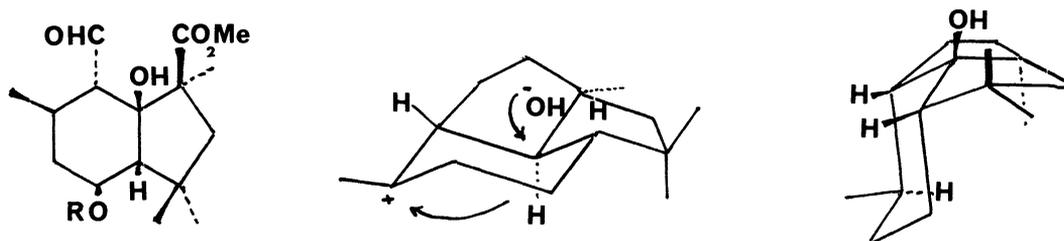
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figure 5

   The 2-(R)-2-^{3}H- and 5-(R)-5-^{3}H-mevalonate results provided some information
on the formation of the hemi-acetal ring.   Oxidation of the dihydrobotrydial
to the corresponding lactone showed that the C-10 hydrogen atom was derived
from the pro-5(R) position of mevalonic acid whilst degradation to the 10,15-
dicarboxylic acid ester showed that C-15 bore only one 2-^{3}H - mevalonoid label
and that this was a pro-2(R)-hydrogen atom.   The distinction between the two
 enantiotopic hydrogen atoms at C-15 was made using deuterium labelling.   The
two hydrogen atoms may be distinguished in the ^{1}H n.m.r. spectrum by solvent
and shift reagent studies.   [2-$^{2}H_{3}$]-Acetate labels the C-2 position of MVA and
 hence C-15 in dihydrobotrydial.   Examination of the deuterium n.m.r. spectrum
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 protons on the
same face of the molecule as the two hydroxyl groups.   The stereochemistry of
labelling of farnesyl pyrophosphate at C-4 and C-5 from pro-2(R)- and pro-5(R)
 mevalonate is known.   Consequently the formation of the hemi-acetal ring
proceeds with overall retention of configuration at both centres (see figure 6).
Although a number of mechanisms may be proposed to account for this, the
dialdehyde, botrydial (4) was incorporated into dihydrobotrydial (1) to the
extent of 32% whereas the reverse reaction proceeded in 1.09% yield. Thus the
cleavage of the 10:15 bond in the formation of the hemi-acetal probably occurs
through the dialdehyde which in turn could be formed via a trans-15α,10β-glycol.
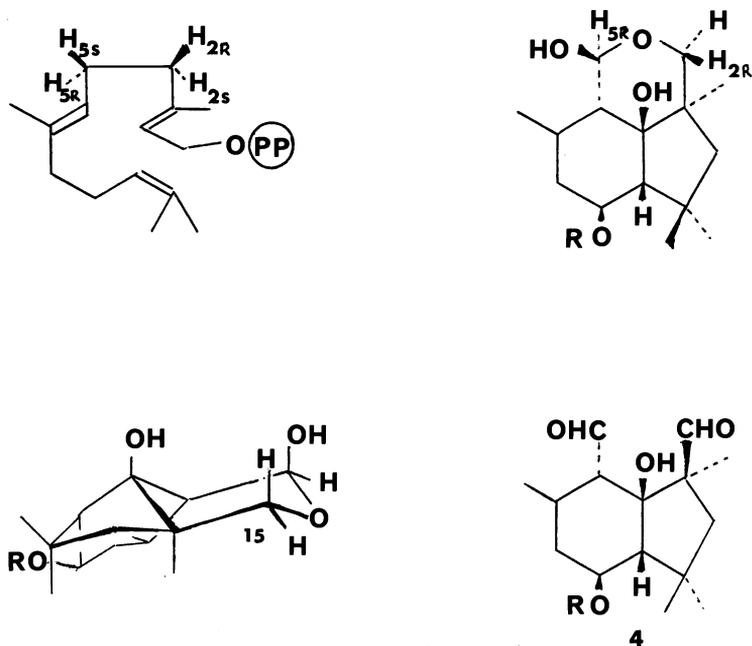
figure 6

The oxidation of a trans-glycol would lead to a dialdehyde. The subsequent reduction of the aldhyde at C-15 proceeds with the re-stereospecificity which is typical of a microbial dehydrogenase. A similar cleavage may occur in the formation of another sesquiterpenoid fungal metabolite, helminthosporal.

Alliacolide

The crystallographic data for the second fungal metabolite, alliacolide (2), did not permit the assignment of an absolute configuration. Degradation and circular dichroism measurements on the following compounds (see figure 7) have enabled us to define this.
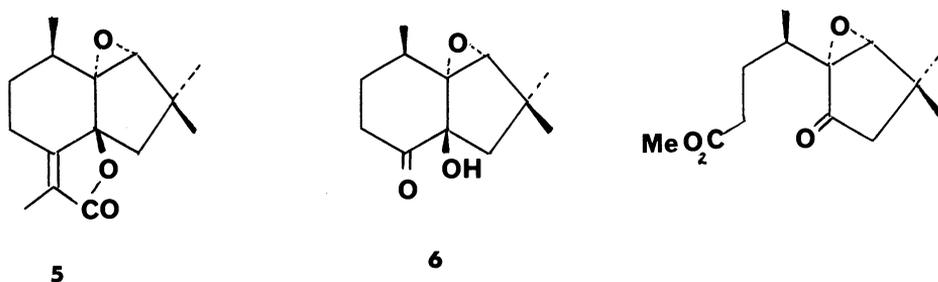
figure 7

Examination of the culture broth of <u>Marasmius</u> <u>alliaceus</u> has led to the isolation of the following metabolites (see figure 8). Careful examination of the [13]C n.m.r. spectra of these and of a number of derivatives facilitated the assignment of the carbon-13 resonances whilst shift reagent and high field (360 MHz) n.m.r. studies permitted the assignment of the proton resonances of alliacolide.

There are a number of biogenetic schemes that may lead to the formation of the carbon skeleton. [1-^{13}C]- and [1,2-[13]C$_2$]-Acetate feeding experiments gave the enricnment and coupling pattern shown in figure 9, suggesting that the structure is made up of the combination of isoprene units indicated in figure 9. Significantly the carbonyl group of the lactone ring (C-13) is coupled to carbon-11, i.e. it must originate from the methyl group of mevalonic acid. In an isoprenoid chain, the methyl group of mevalonate remains a pendant group.
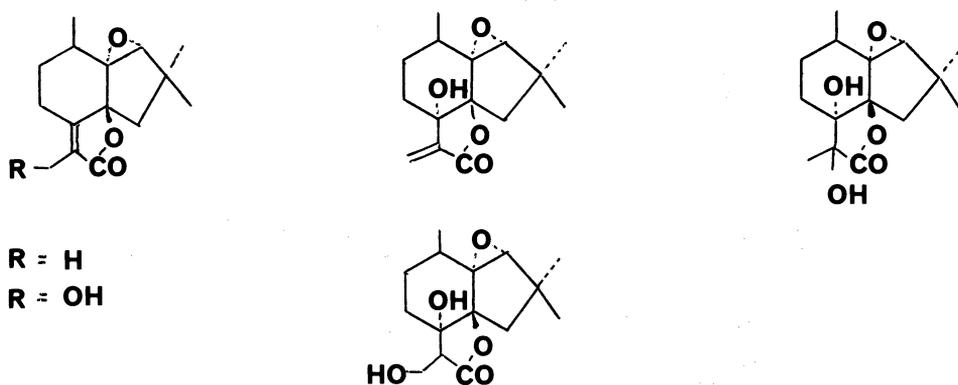
figure 8

Consequently the lactone ring may not have its origin in the cleavage of the isoprene chain.   This result could even suggest that the isopropyl group rather than the gem-dimethyl group forms the starting point of the isoprene chain.
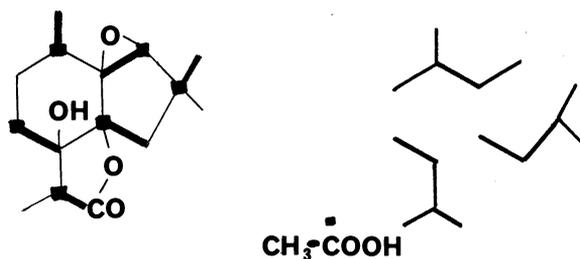
figure 9

The numbers of mevalonoid hydrogen atoms which were incorporated, were established by conventional $^3H:^{14}C$ ratio studies which are summarized in table 2.

Table 2   Incorporation of Mevalonates into Alliacolide (2)

Mevalonate	$2-^3H_2,2-^{14}C$	$4(R)-4-^3H,2-^{14}C$	$5-^3H_2,2-^{14}C$
$^3H:^{14}C$ atom ratio in (2)	5.2:3	1.9:3	3.96:3
$^3H:^{14}C$ atom ratio in (5), obtained by degradation.	5.3:3	1.9:3	4:3
incorporation(%) into (2)	1.29	0.99	1.59

Two of the three centres (C-4 and C-9) which would be expected to be labelled by $4(R)-4-^3H$-mevalonate are full substituted and hence to account for the incorporation of two labels, one pro-$4(R)$-mevalonoid hydrogen atom must be involved in a rearrangement. Secondly one of the centres (C-5) labelled by a 5-mevalonate is fully-substituted and another (C-8) bears only one hydrogen atom.   To account for the incorporation of the fourth ($5-^3H$)-mevalonoid label, it must also be involved in a rearrangement. Simple dehydration to dehydro-alliacolide (5) showed, suprisingly, that there was no mevalonoid label at C-11 whilst degradation to the hydroxy-ketone (6) established that C-12 bore two (2-^3H)-mevalonoid hydrogen labels. However the remaining sites of label were determined using (2-$^2H_3$)-acetate and the (2-$^2H_2$)- ,(4-$^2H_2$)- and (5-$^2H_2$)-mevalonates with deuterium ñ.m.r. spectroscopy. Although it was possible to assign the proton resonances of alliacolide, they were not sufficiently well resolved for satisfactory deuterium n.m.r. studies except in the case of the

($5-^2H_2$)-MVA labelled material. However a satisfactory set of ^2H n.m.r. spectra were obtained from the degradation product, dehydroalliacolide (5). The results are summarized in figure 10. The hydrogen atom adjacent to the methyl group at C-1 is derived from the pro-4(R) position of mevalonate whilst a ($5-^2H$)-mevalonoid label is attached to C-6. A small amount of scrambling of the ($2-^2H_2$)- and ($4-^2H_2$)-MVA labels was also apparent possibly due to degradation to acetate and re-synthesis. The absence of a mevalonoid label

label from $^2H_3$-acetate   label from ^2H-MVA
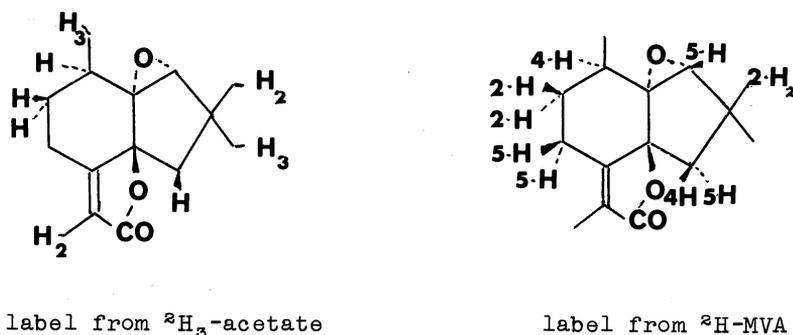
figure   10

from C-11 is accounted for by the fact that dehydroalliacolide (5) is a very effective (32.7%) precursor of alliacolide (2). One possible biosynthetic scheme which would accomodate these results, but is not proven by them, is shown in figure 11. Such a scheme suggests an interesting role and origin of the epoxide oxygen atom.
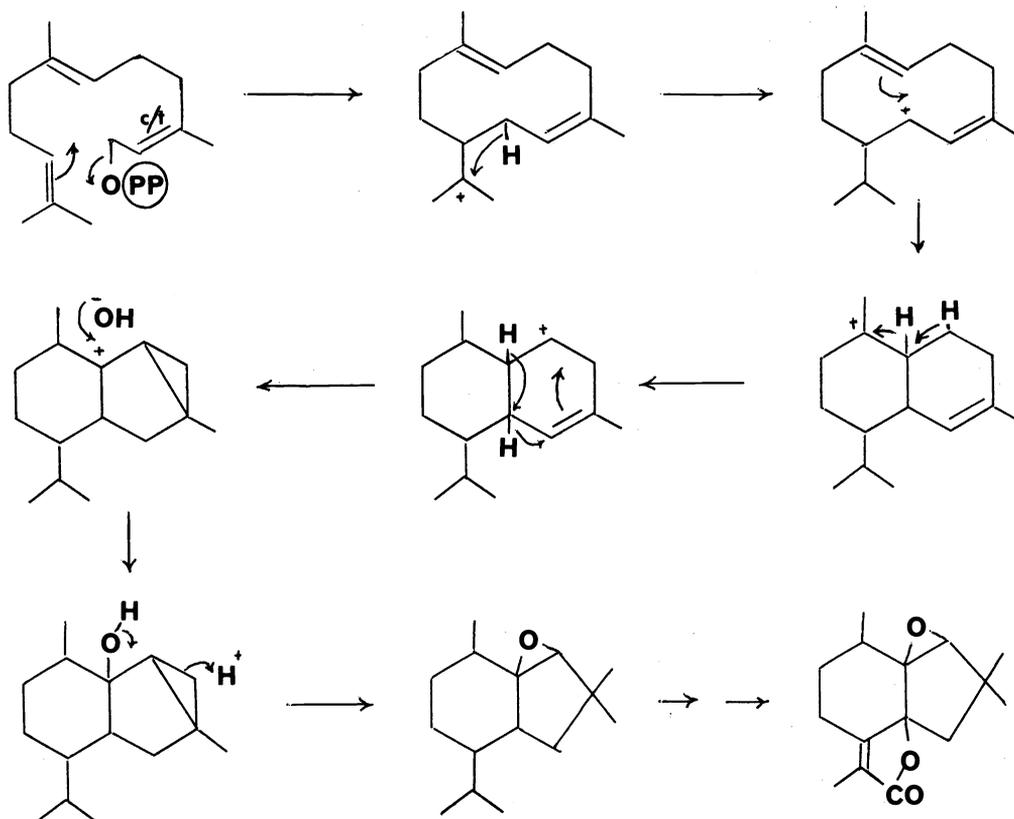
figure 11

In conclusion, although detailed sequence studies remain to be done on both these fungal metabolites, I hope that the work illustrates the power of modern n.m.r. methods in biosynthetic problems.

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