Biosynthesis of Sesquiterpenes

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I. Introduction

Sesquiterpenes are a diverse group of terpenoid compounds containing a 15-carbon skeleton. Their structure diversification and pharmacologic activity make this group of exceptional interest.

Biosynthetically, their derivation along the lines of the Ruzicka hypothesis[1,2] is assured. Further elaboration of the primary precursor farnesol is frequent, and this skeletal profusity and the accompanying stereochemical problems have been the subject of substantial speculation,[3-6] albeit with the minimum of experimental support. Discussion of biogenesis per se is kept to a minimum in this work.

The biosynthetic work in this field has been the subject of only selective review,[7-38] and this contribution attempts to partially fill this void. Organization of this review is based on the number of carbocyclic rings contained in the sesquiterpene under consideration as summarized above. Farnesol, a precursor of all sesquiterpenes, is therefore treated first.

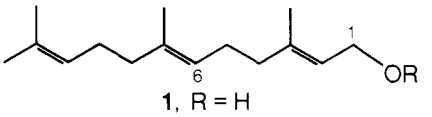
The literature is reviewed to the end of November 1974.

II. Biosynthesis of Acyclic Sesquiterpenes

A. Farnesol (1)

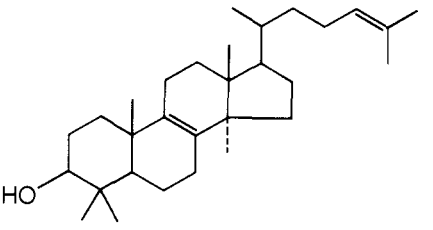
The biosynthesis of farnesol (**1**) is one of the most elegantly investigated areas in the whole of biosynthesis, and space will permit only a cursory examination of this area.

The details of the formation of farnesol (**1**) are important because of the subsequent tail-to-tail dimerization of farnesyl pyrophosphate (**2**), leading to the key triterpenes lanosterol (**3**) (in mammals) and cycloartenol (**4**) (in plants). Indeed much of the information on farnesol (**1**) biosynthesis is derived from information of the biosynthesis of cholesterol (**5**), with the clear implication that the farnesyl units are utilized in the biosynthetic scheme.



1, R = H

2, R = PP



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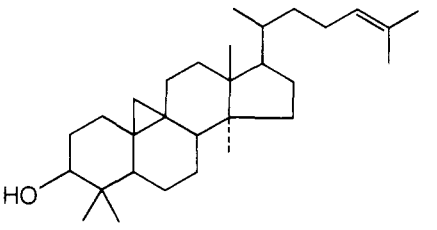

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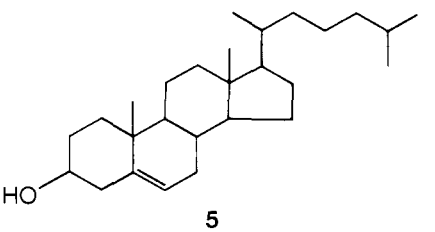

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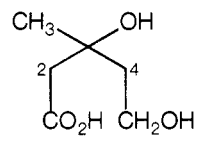
In order to both understand and appreciate the subsequent work on the formation of **1** and of the sesquiterpenes from **1**, we must reexamine some of the early work on the steroid nucleus.

The discovery of mevalonic acid (**6**) by Folkers[39-42] and Tamura[43] and its demonstration as an efficient precursor of cholesterol (**5**)[44] in rat liver homogenate gave early experimental support to the biogenetic ideas of Ruzicka and coworkers.[2] Subsequently, the specific precursor relationship of mevalonic acid (**6**) to farnesyl pyrophosphate (**2**) was demonstrated.[45]

At this time there was considerable speculation as to the origin of squalene (**7**).[46] However, it was found[46] that added farnesol



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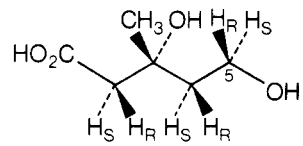

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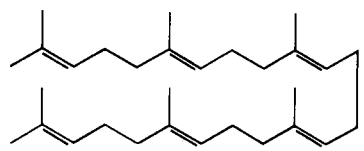

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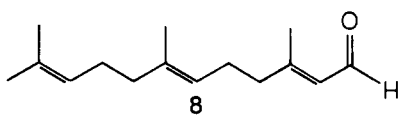

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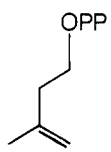
(**1**) and farnesal (**8**) considerably inhibited the incorporation of [1-^{14}C]acetate into the unsaponifiable fraction of rat liver homogenate, thus confirming the results of previous workers.[47,48] These results were followed to their logical conclusion by Lynen[45] who investigated the formation of squalene (**7**) from **1**. The results confirmed the suspicions of Isler[46] who regarded the union of two C_{15} units to be more likely than the union of three C_{10} units in the biosynthesis of squalene. Others were less successful with C_{15} precursors of squalene (**7**).[49,50]

Lynen also demonstrated[51] the existence, not only of an isopentenyl pyrophosphatase, but also a farnesylpyrophosphate synthetase which successfully converted [1-^{14}C]isopentenyl pyrophosphate (**9**)[52] into geranyl (**10**) and farnesyl (**2**) pyrophosphates. From rat liver Popjak[53,54] obtained an enzyme system which utilized [2-^{14}C]mevalonate (**6**) to produce a number of interesting allylic alcohols, including nerolidol (**11**) and *trans,trans*-farnesol (**1**) (60% of total activity). When [2-^{14}C]-5-phosphomevalonic acid (**12**)[55] was used, the level of incorporation in **1** and **11** was raised to 88%.[54,56]



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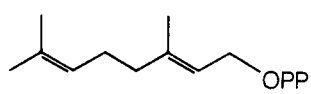

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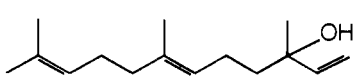

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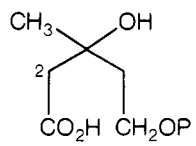

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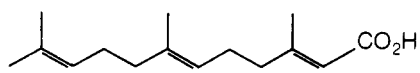

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Also isolated (artifact?) from both the rat liver enzyme[57] and liver alcohol dehydrogenase (LAD)[58] systems was *trans,trans*-farnesoic acid (**13**), suggested[57,59] and later disproved[60] as a precursor of squalene (**7**).

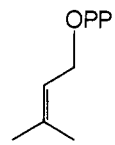
In order to understand the stereochemical formation of **1**, we must digress slightly and discuss relevant aspects of the formation of **7**. Much of this work was initially due to Bloch.[61-64]

Later, some of these results were invalidated,[65-70] and it was conclusively demonstrated that a single hydrogen is stereospecifically[71,72] replaced in the central bond of squalene.

Farnesol (**1**) is therefore produced without oxidation at C-5 of mevalonate (**6**), and the stereochemical integrity of the 2-carbon of **6** is maintained. Each unit of **6** does, however, lose a hydrogen atom from C-4 on conversion to **1**.

In order to investigate the stereospecific formation of the double bonds of squalene (**7**) (and therefore of *trans,trans*-farnesol (**1**)), mevalonic acids stereospecifically labeled at the pro-4*R* and pro-4*S* positions were used in the biosynthesis of squalene (**7**) in rat liver,[73,74] fungi,[75,76] and higher plants.[77-79] From *pro*-[4*R*-^{3}H]mevalonate (**6**) essentially all of the tritium was retained in both **1**[76,78] and **7**,[74] whereas from *pro*-[4*S*-^{3}H]-mevalonate (**6**) activity was lost. Therefore, in the formation of the trans double bonds, the pro-4*S* proton is specifically lost. Exactly the opposite situation obtains in the formation of rubber,[78,80,81] where specific retention of the pro-4*S* hydrogen of **6** occurs.

The enzyme isopentenylpyrophosphate isomerase catalyzes the conversion of isopentenyl pyrophosphate (**9**) to dimethylallyl pyrophosphate (**14**) (IPP and DMAPP, respectively). Cornforth has recently demonstrated[82] that the proton added from the medium in this isomerization approaches from the *re* side of the double bond in IPP. This suggests that formation of the double bond in DMAPP takes place by concerted addition–abstraction. Aspects of this and other work by Cornforth and his associates have recently been summarized.[83]



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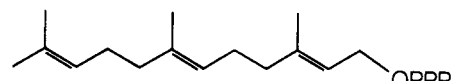

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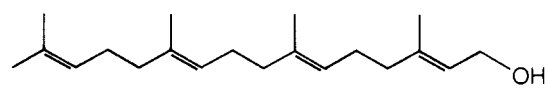
Further work on the biosynthesis of *trans,trans*-farnesol (**1**) from mevalonate (**6**) has concentrated on confirmation of its biosynthetic origin in various organisms and tissues.[76,78,84-87] In some instances partially purified enzyme systems were obtained which were responsible for the formation of **2** and even for the formation of *trans,trans*-farnesyl triphosphate (**15**).[88]

Juvenile hormones are probably the best known of the sesquiterpene-type compounds found in insects, and their biosynthesis will be covered in the next section. A number of other sesquiterpenes are also known to occur in insects, and, indeed, these are found to the exclusion of triterpene and steroid metabolites in many cases,[89,90] apparently because enzymes beyond squalene oxidase are not present or are of extremely low activity.[91] These systems are therefore useful for the isolation of enzyme systems responsible for early reactions in the biosynthetic sequence.

The enzyme responsible for the first step in the biosynthesis of farnesyl pyrophosphate (**2**) from **6**, mevalonic kinase, has been obtained from a number of organisms,[92-96] including the fly, *Sarcophaga bullata*.[97] Further work with *S. bullata*[98] demonstrated that along with *trans,trans*-farnesol (**1**) and *trans*-nerolidol (**11**), geranylgeraniol (**16**) was the most complex metabolite labeled by [2-^{14}C]mevalonate (**6**). Mevalonic kinase is



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inhibited in vitro by both geranyl pyrophosphate (**10**) and farnesyl pyrophosphate (**2**).[99,100]

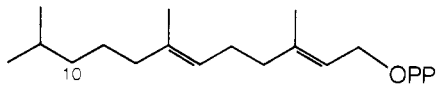
An enzyme system capable of synthesizing **2** was first obtained by Lynen and co-workers.[101] This synthetase catalyzed the isomerization of isopentenyl pyrophosphate (**9**) to dimethylallyl pyrophosphate (**14**), the formation of geranyl pyrophosphate (**10**) and the formation of farnesyl pyrophosphate (**2**). From *Micrococcus lysodeikticus,* Bloch's group[102] obtained an enzyme which catalyzed the formation of **2, 10,** and **16.** An enzyme system from pig liver was found[103] to catalyze only the synthesis of **2**, indicating that possibly as many as four enzyme systems were involved in these reactions (one isomerase and three synthetases).

Subsequent work[104] demonstrated an inadequacy in the monitoring of geranylpyrophosphate synthetase, and additional work with various systems derived from higher plants and mammalian organs has demonstrated that the same enzyme system (prenyl transferase) is responsible for the synthesis of both **10** and **2**.

Prenyl transferase (farnesylpyrophosphate synthetase) in various degrees of activity has been obtained from a number of sources including pig liver,[103-105] beef liver,[85,106] yeast,[51,107] *Andrographis paniculata,*[108] *Citrus sinensis,*[109] *Cucurbita pepo,*[110] *Gibberella fujikuroi,*[111] *Gossypium hirsutum,*[112] *Iris hollandica,*[113] *Micrococcus lysodeikticus,*[114-116] *Pinus radiata,*[109,117] and a *Pisum* sp.[118] The lack of homogeneity of these enzyme systems makes substrate and product specificity highly questionable in this area.

A technique has been described by which many of the intermediates between mevalonate (**6**) and farnesyl pyrophosphate (**1**) can be separated chromatographically.[119]

Farnesylpyrophosphate synthetase, as well as catalyzing the reaction of the natural substrates DMAPP (**14**) and geranyl pyrophosphate (**10**) with IPP (9), also catalyzes the reaction of a number of other (artificial) substrates. The work of Popjak initiated this important area with the demonstration of the enzymic formation of 10,11-dihydrofarnesyl pyrophosphate (**17**) from isopentenyl pyrophosphate and 6,7-dihydrogeranyl pyrophosphate (**18**). Because it appeared that the pyrophosphate unit of



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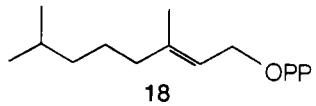

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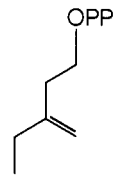
18 was bound to the enzyme,[122,123] it was considered that many other similar compounds would act as substrate for the enzyme. The simplest allylic substrate was shown to be DMAPP (**14**),[121] but several cis and trans higher homologues were also effective.[121]

Independently, a Japanese group,[124,125] using an enzyme system from pumpkin, confirmed these results and have since extended their work to the higher homologues of isopentenyl pyrophosphate,[126] geranyl pyrophosphate,[127] geranylgeranyl pyrophosphate,[128] and squalene pyrophosphate.[129] While many changes in the allylic moiety could be made before enzyme specificity limited the reaction, only one other isopentenyl derivative was active as a substrate,[126] indicating a much narrower specificity for this "half" of the reacting system. Interestingly, from the point of view of juvenile hormone biosynthesis, the second reactive substrate was 3-ethyl-3-butenyl pyrophosphate (**19**).

More recently there has been considerable widespread interest (ref 108, 109, 112, 117, 122, 130, 132) in another isomer of farnesol, namely *cis,trans*-farnesol (**20**). There had been speculation as to both the origin and subsequent utilization of



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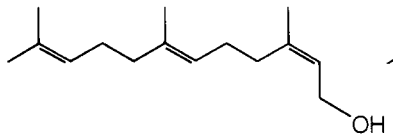
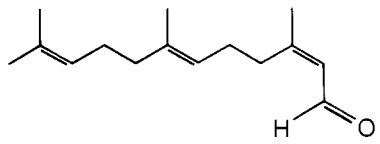
the 2-cis C_{10} and C_{15} pyrophosphates. These isomers are essentially unreactive in further condensation with IPP (**9**),[117] yet nerol (**21**) is a key precursor of the cyclic monoterpenes[133] and the iridoids,[134] and 2-*cis*-6-*trans*-farnesol (**20**) is, as we shall see, apparently an important intermediate in the formation of many polycyclic sesquiterpenes.



20 21

The possible importance of **20** in sesquiterpene biosynthesis was first recognized by Hendrickson[3] and extensively elaborated on by Ramage.[4] The compound was first identified in *Pinus radiata* seedlings,[117] and its formation from [1-^{3}H]geranyl pyrophosphate (**10**) was established. Apparently there was no isomerization of [1-^{3}H]-**2**.

In further work, cell-free extracts of orange flavedo[130] were used to examine the biosynthesis of the farnesols from **10.** The latter compound, derived from [4-^{14}C]-IPP (**9**), was a precursor not only of *trans,trans*-farnesol (**1**) but also of *cis,trans*-farnesol (**20**) and the corresponding aldehydes **8** and **22**.[135] Of the four



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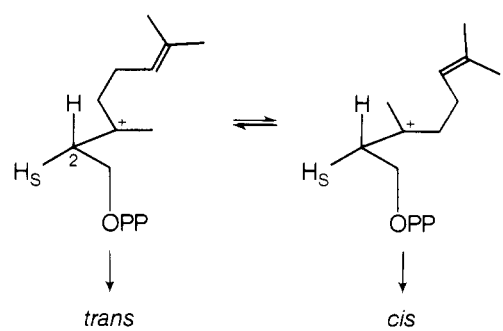
products formed, the trans compounds, **1** and **8**, were produced more rapidly and in greater quantity than the cis isomers, **20** and **22.** Again, isomerization of the farnesyl pyrophosphates was not observed, and a redox scheme was proposed involving the initial formation of both the *cis*- and *trans*-farnesyl pyrophosphates.

More sophisticated work with the farnesylpyrophosphate synthetase system of *Pinus radiata,*[109] *Citrus sinensis,*[109] and *Andrographis paniculata*[108] demonstrated that in the formation of both the 2-cis and 2-trans double bonds it was the pro-4*S* proton of **6** which was specifically eliminated.[108,109] Previous work had shown that the enzyme system has a low specificity with regard to the long-chain part of the allylic substrate,[120,121,123,124] and Cori and co-workers suggested that this may permit rotation of any intermediate carbonium ion about the 2,3 bond (Scheme I).

SCHEME I



trans *cis*

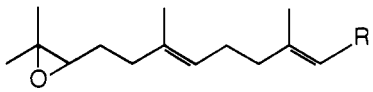
Alternatively, it was suggested[109] that there may be a specificity in the binding of IPP (**9**) to the enzyme, the double bond being either cis or trans to the alcohol function. Work by Overton,[108] however, supports a third possibility. When [5-$^3H_2$,2-^{14}C]mevalonate (**6**) was used as a precursor, *trans,trans*-farnesol (**1**) retained all ^3H activity, but *cis,trans*-farnesol (**20**) lost one-sixth of the total ^3H activity, indicating a loss of ^3H activity from C-1 aldehydes as intermediates in the 2,3 isomerization.

Interestingly, in the sequential labeling study of Cori,[130] the apparent order of synthesis was *trans,trans*-farnesol (**1**), *trans,trans*-farnesal (**8**), *cis,trans*-farnesal (**22**), and *cis,trans*-farnesol (**20**).

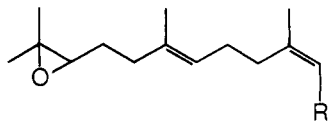
Overton[132] has extended this work and determined that the pro-1*S* hydrogen was lost in the oxidation of **1** to **20.** It was also demonstrated that a reverse reaction takes place in which **20** is converted to **1**, the stereochemistry of this reaction being exactly the opposite of the forward reaction in that the pro-1*R* hydrogen is specifically lost.

$$\textit{trans,trans}\text{-farnesol (1)} \underset{\text{pro-1}R\text{ lost}}{\overset{\text{pro-1}S\text{ lost}}{\rightleftharpoons}} \textit{cis,trans}\text{-farnesol} \quad (20)$$

During an examination of the metabolites of *trans*-10,11-epoxyfarnesol (**23**) in *Helminthosporium sativum,*[136] Suzuki and Marumo[136] found that one of the products was the corresponding cis,trans isomer **24.** When [1-$^2H_2$]-*trans,trans*-10,11-epoxyfarnesol (**23**) was used as a precursor, 42% of the deuterium was lost at C-1 in **24,** indicating the probable intermediacy of the trans (**25**) and cis (**26**) aldehydes in the isomerization process. When the trans,trans aldehyde **25** was used as a precursor, it too was incorporated into **24.** Similar results were also obtained with [1-$^2H_2$]-*trans,trans*-farnesol (**1**).



23, R = CH_2OH
25, R = CHO



24, R = CH_2OH
26, R = CHO

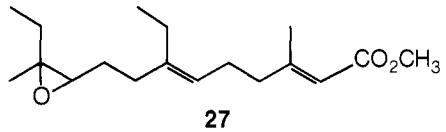
Arigoni and co-workers,[137] as part of their recent extensive work on sesquiterpene biosynthesis, have demonstrated that alternative routes *not* involving oxidation must also be considered for this isomerization. It was demonstrated, for example, that overall inversion at C-1 of farnesol (**1**) can occur involving nerolidol (**11**) as an intermediate. This nonoxidative process affords a *cis,trans*-farnesol (**20**) with opposite stereochemical labeling at C-1 to that in the original **1**. The consequences of this result in terms of evaluating the earlier "stereospecific" losses from C-1 are clear. The factors which influence stereospecific oxidation or an intact inversion at C-1 must be evaluated. Further discussion of the work of Arigoni in this area is deferred to the sections on the tricyclic sesquiterpenes.

Considerable further effort is required in this area, in particular in evaluating and monitoring the enzyme systems involved and their distribution.

B. Juvenile Hormone

Many aspects of the isolation, structure elucidation, and synthesis of the juvenile hormones (JH) have been discussed,[138-141] but for the purpose of this review we shall consider only three of these, JH I (**27**), JH II (**28**), and JH III (**29**).

The simplest of these compounds and the one most closely related to farnesol (**1**) is JH III, 10,11-epoxy methyl farnesoate (**29**), isolated from organ cultures of the tobacco hornworm moth,



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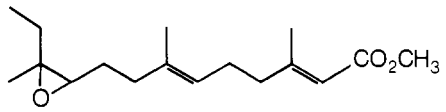

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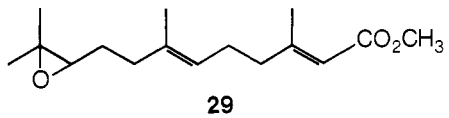
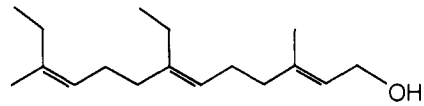


29

Manduca sexta,[142] and from the grasshopper, *Schistocera vaga.*[143] Preliminary work with simple precursors indicated that methionine labeled only the ester methyl group and that [2-^{14}C]acetate gave only a low incorporation, the specificity of which could not be determined.[143]

Similar problems had been reported earlier by Schmialek[144] who found that mevalonate labeled the sesquiterpenes rather than JH I (**27**) in *Samia cynthia.* Likewise, Roller and co-workers,[145] when studying the formation of JH I (**27**) and JH II (**28**) in *Hyalophora cecropia,* found that whereas methionine provided the ester methyl group for both compounds, it was not utilized in the formation of the carbon skeleton. The remaining carbon atoms were not labeled by farnesol (**1**), mevalonate (**6**), or propionate although each was metabolized. Acetate gave only low incorporation into JH I (**27**) and JH II (**28**), and the specificity could not be determined. These results led the authors to conclude that either mevalonate was not reaching the site of synthesis or the sesquiterpenoid-like chain of JH I (**27**) and JH II (**28**) may be derived via the fatty acid pathway.

Ajami and Riddiford recently reported[146] the first extensive experimental work on the mode of incorporation of precursors into JH I (**27**). Acetate, glucose, methionine, isoleucine, and valine were incorporated, but propionate, mevalonate (**6**), lysine, 4-methyl-*cis*-3-hexenol, 4-methyl-3-pentenol, farnesol (**1**), and bishomofarnesol (**30**) were not. On this basis it was suggested[146] that the common pathways of terpenoid or fatty acid biosynthesis were not operating.



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More recently, however, Schooley and co-workers[147] have studied more carefully the biosynthesis of JH II (**28**) and JH III (**29**) in *Manduca sexta* and have confirmed their terpenoid origin. An elegant scheme for the degradation of JH II (**28**) and JH III (**29**) was evolved whereby each compound was broken down to essentially three fragments. Each of these fragments could be derivatized to a crystalline compound suitable for work with the minute amount of material available. The degradation scheme used by Schooley is shown in Scheme II, and particular attention should be paid to the three fragments obtained as crystalline products, **31, 32,** and **33.**

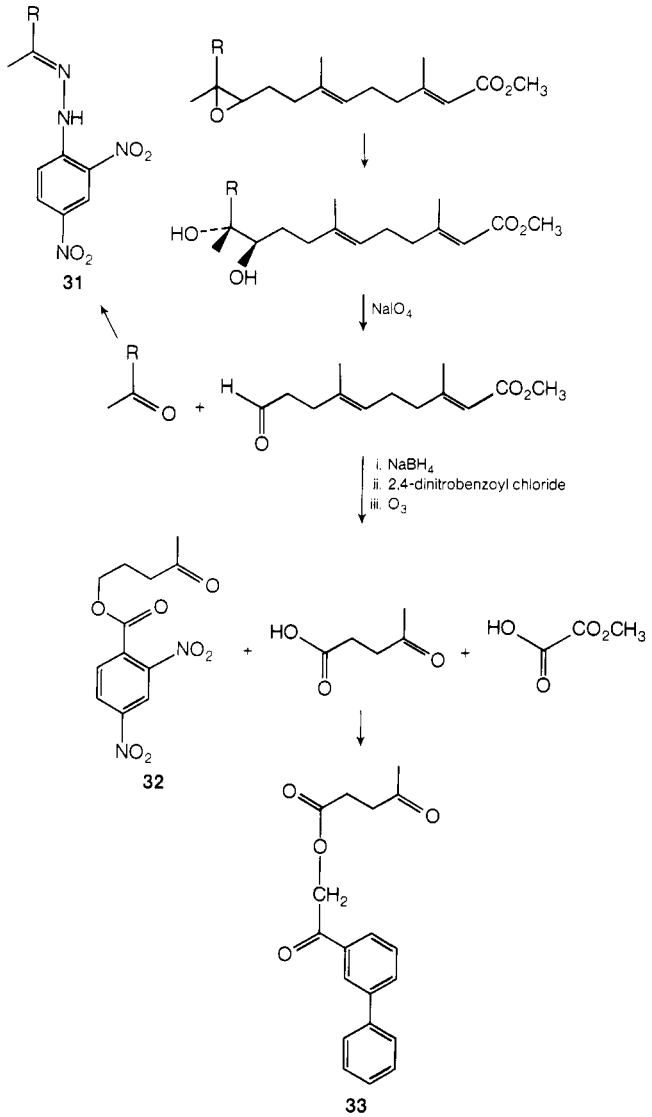
Table I summarizes the incorporation and degradation of JH II (**28**) and JH III (**29**) after feeding acetate, propionate, and mevalonate (**6**). These results confirm for the first time the biosynthesis of the juvenile hormone skeleton by the isoprenoid pathway, there being a specific incorporation of mevalonate (**6**) into JH II (**28**) and JH III (**29**).

The results are in agreement with the theory of juvenile hormone biosynthesis propounded by Schooley[142,147] and mentioned briefly by others.[145] This theory differs drastically from the previous theories which have proposed the addition of one

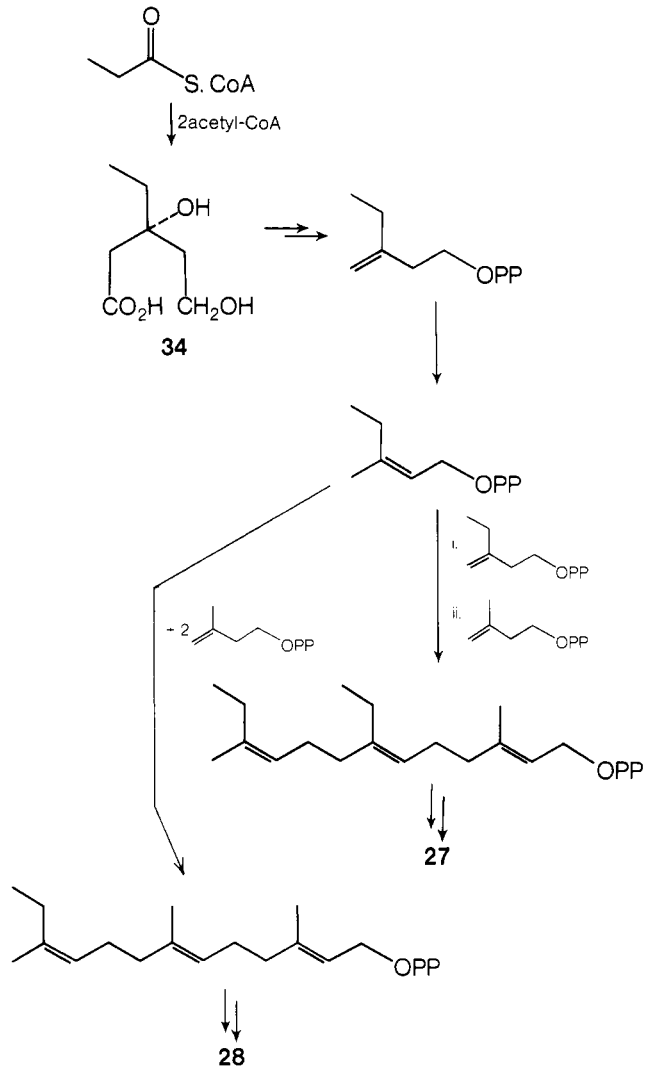
TABLE I. Degradation of JH II (28) and JH III (29)[147]

Compound	Predicted total no. labeled atoms	Fragment 31 Found	31 Predicted	32 Found	32 Predicted	33 Found	33 Predicted
JH II (28)							
[2-^{14}C] Acetate	8	0.97	1	3.14	3	2.87	3
[2-^{14}C] Mevalonate	2	0.02	0	0.97	1	1.05	1
[2-^{14}C] Propionate	1	0.98	1	0.05	0	0.05	0
[1-^{14}C] Propionate	1	0.90	1		0		0
JH III (29)							
[2-^{14}C] Acetate	9	1.93	2	3.07	3	3.23	3
[2-^{14}C] Mevalonate	3	1.14	1	1.05	1	1.03	1

SCHEME II

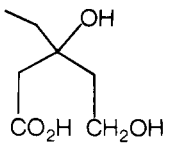


SCHEME III



or two single carbon units to a preformed 15-carbon chain. In Schooley's theory JH II (**28**) is derived from one homoisoprenoid unit and two isoprenoid units, the former unit [homomevalonic acid (**34**)] being produced from propionate and two acetates rather than from three acetates. The data in Table I indicate the specific incorporation of propionate into the starting unit, and the specific incorporation of mevalonate (**6**) *only* into the units derived from IPP (**9**). There is therefore no extension of mevalonate (**6**) by single carbon units. The probable biosynthetic route to JH I (**27**) and JH II (**28**) is therefore as shown in Scheme III.

Additional work has demonstrated the possible intermediacy of a number of the acidic metabolites. Bishomofarnesoic acid (**35**) and farnesoic acid (**13**) were incorporated into JH I (**27**) and JH III (**29**), respectively, in *Schistocerca gregarid.*[148] 10-Epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoic acid (**36**) was incorporated to the extent of 10% into JH I (**27**).[149]



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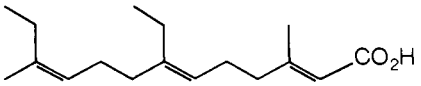

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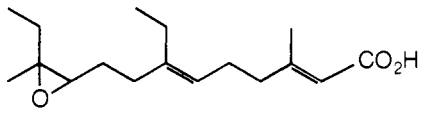

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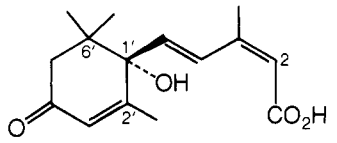
III. Biosynthesis of Monocyclic Sesquiterpenes

A. Abscisic Acid

The structure[150-158] of abscisic acid (**37**), the widely occurring[159] plant growth regulator, suggests two possible routes of formation: (i) oxidative cleavage of a carotenoid derivative or (ii) direct biosynthesis from **1**. The importance of **37** in the regulatory processes in higher plants has given rise to a flurry of activity on biosynthesis and metabolism. This work has been summarized at various stages.[160-163]



37

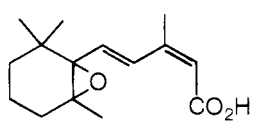
In 1967 Milborrow[159] demonstrated the presence of **37** in a number of ripening fruits. Later, avocado, strawberry, banana, and tomato were shown by Noddle and Robinson[164] to contain **37,** albeit in minute quantities. Each of these fruits was demonstrated[164] to give labeled abscisic acid (**37**) after feeding [2-^{14}C]mevalonate (**6**) to the unripe fruit. The quantity of **37** produced did not permit degradative studies.

These early results were quickly followed by a more discriminating experiment using doubly labeled **6**. Abscisic acid (**37**) contains a terminal cis double bond, and theoretically there are two reasonable pathways for the formation of this group. These pathways may potentially be distinguished by the utility of pro-4*R* and pro-4*S* labeled mevalonates. Thus we may imagine that initial formation of a terminal cis double bond would take place with loss of the pro-4*R* proton, whereas isomerization of an initially formed trans double bond would lead to loss of the pro-4*S* proton and possibly a subsequent loss of the pro-4*R* proton in the isomerization step.

When [4*R*-^{3}H]- and [4*S*-^{3}H]mevalonates were separately fed to avocado fruit,[165] two-thirds and zero tritium labeling were observed, respectively. The terminal cis double bond is therefore produced indirectly at some stage from a trans double bond with retention of tritium during the isomerization. It should be noted that the 4*R* proton of mevalonate (**6**), retained at C-5 in **1**, is lost upon cyclization to **37.** Although the stereochemical information is important, exactly the same result would be observed if abscisic acid (**37**) were produced by degradation of a carotenoid.

When [^{14}C]phytoene was applied to avocado fruit, label was incorporated into the carotenoids but not into **37**.[163] Abscisic acid (**37**) is therefore not produced from degradation of a carotenoid.

Compound **38** also acts as a plant growth inhibitor, and Milborrow and co-workers[166] examined the possible intermediacy of **38** in the formation of abscisic acid (**37**). When the 2-cis

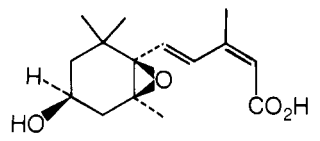


38

isomer of **38** was used as a precursor of abscisic acid (**37**) in green tomato fruit, a 1.8% incorporation was observed after 48 h. The corresponding 2-trans isomer gave only 0.04% incorporation.

It has been observed that, when wilting is induced in wheat, there is a very rapid increase (25-fold) in abscisic acid (**37**) content.[167] This short time period is therefore potentially very useful for the study of abscisic acid (**37**) biosynthesis, depending upon whether abscisic acid is released from a bound state or if it is synthesized de novo at this time. When [2-^{3}H]mevalonate was used as a precursor, nine times as much activity appeared in the **37** from wilted wheat as in that from normal wheat seedlings, suggesting that much of the abscisic acid (**37**) produced during wilting is synthesized de novo. When the [2-^{14}C]epoxide (**38**) was used as a precursor of **37** in wilted wheat, incorporation was observed. If this compound is an intermediate on the route to **37,** one of the further steps is formation of a hydroxy group at C-1′. Using the epoxide (**38**) specifically labeled at the epoxide oxygen with ^{18}O, Milborrow showed[168] that essentially all (97%) of the heavy isotope label was retained upon conversion to **37;** hence only one of the enantiomers of the epoxide is converted to **37.**

In addition to **37,** [2-^{14}C]-**38** also gave rise to another labeled product, the structure of which was elucidated to be 1′,2′-epi-2-*cis*-xanthoxin acid (**39**), in which the C-1′ stereochemistry is opposite to that observed in **37.** Labeled **39** was not a precursor of **37.** However, the opposite 1′,2′ epimer, (+)-2-*cis*-xanthoxin acid (**40**), was shown to be a precursor of **37** in tomato shoots.[168]



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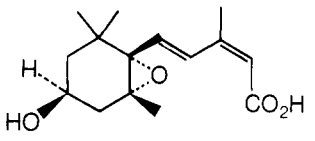

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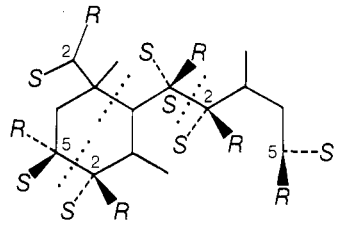
Attempts to show the presence of **40** in avocado fruit failed to give detectable activity by dilution analysis; **40** may therefore not be a normal intermediate in the biosynthesis of **37,** or the reactivity of **40** may prevent a measurable pool size forming.

The nonmetabolite (**39**) arises because the labeled epoxide (**38**) originally fed was a synthetic, racemic mixture of 1′ epimers. Thus only one of the epimers was carried through to **37.**

An important point about both the epoxide **40** and **37** itself is that each compound contains a C-4,5 double bond. The availability of the six stereospecifically labeled mevalonates permits an examination of the double bond forming processes in detail.

Structure **41** indicates the sites of labeling and stereochemistry from 2- and 5-labeled (**6**) (prior to junction of the isoprene fragments) in the abscisic acid skeleton. Clearly in abscisic acid



41

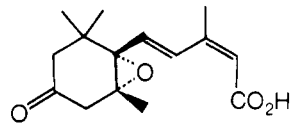
(**37**) itself, a number of these labels are completely lost, e.g., at C-1 and C-4′; indeed only one of the six C-5 hydrogens of mevalonate (**6**) should be retained in **37.**

The situation regarding the retention of labels from a stereospecifically labeled C-2 in **6** is more complex. It depends not only upon the stereospecificity of elimination at C-4,5 and C-2′,3′, but also upon the presence of isopentenyl isomerase which tends to isomerize any stereospecifically labeled **6**, resulting in an apparent loss of the 2*R*-^{3}H and retention of the 2*S*-^{3}H. The intricacies of the results cannot be discussed, there being sufficient space only to discuss the conclusions from these experiments.

From [2*R*-^{3}H]mevalonate (**6**) three tritium atoms were retained in **37**,[169] and degradative work indicated that these tritium atoms were specifically retained at the expected positions, in

particular at C-4 and C-3′. From [2*S*-^{3}H]mevalonate (**6**), only one tritium was retained in **37**. When [5*S*-^{3}H]mevalonate (**6**) was used as a precursor, one tritium was specifically retained at C-5 in **37**.[169] There is therefore an overall trans elimination of hydrogen at C-4,5 in the formation of the C-4,5 double bond. This result complements the work of Goodwin and co-workers[170-172] on the formation of the corresponding double bonds in the carotenoids.

Chemical evidence suggests that the 1′,2′-*cis*- and *trans*-diols are moderately unstable, thus possibly precluding their isolation from natural sources. However, Taylor and Burden[154] have shown that 2-*cis*-4′-oxoxanthoxin acid (**42**) is a very labile



42

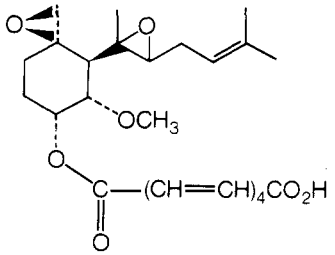
compound, rearranging to **37** under mild conditions, suggesting that possibly dehydrogenation at C-4′ occurs prior to ring cleavage of the epoxide.

More recent work by Milborrow[173] has involved the elaboration of cell-free systems producing **37**. Because the rate of synthesis of **37** in ripening avocado is similar whether the mesocarp slices are turgid or wilted,[174] Milborrow considered that the biosynthesis of **37** from mevalonate (**6**) might be increased by damaging the chloroplasts. Extensive work with lysed chloroplasts, investigating the importance of various cofactors and time, indicated that maximal incorporation of **6** into **37** was observed with a complete medium after 17 h. It was also demonstrated that unlike the situation in wheat leaves,[163] **37** does not inhibit its own biosynthesis in avocado. Compounds which inhibit carotenoid biosynthesis[175] did not inhibit abscisic acid (**37**) biosynthesis.

At this point it is not worthwhile considering an overall scheme for the biosynthesis of abscisic acid (**37**), even bearing in mind the stereochemical limitations imposed by the elegant work of Milborrow and associates. It is still not certain that farnesol (**1**) is a true intermediate since there is no record of this experiment having been attempted. Indeed it is true to say that details of the early steps in the scheme are known only very scantily. Thus, still to be investigated are (i) the point at which C-1 oxidation of farnesol occurs; (ii) the intermediacy of a compound having a 1′2′ double bond; (iii) the point at which the 4,5 double bond is introduced; (iv) the mechanism of introduction of the 4,5 bond; and (v) the mechanism of introduction of the 1′2′ double bond. Clearly there is much further work needed before the intricacies of abscisic acid (**37**) biosynthesis are understood.

B. Fumagillin (43)

The carbon skeleton of fumagillin (**43**), an antibiotic from *Aspergillus fumigatus*,[176] is not classically isoprenoid, and initial attempts were therefore aimed at establishing the terpenoid nature of this molecule.



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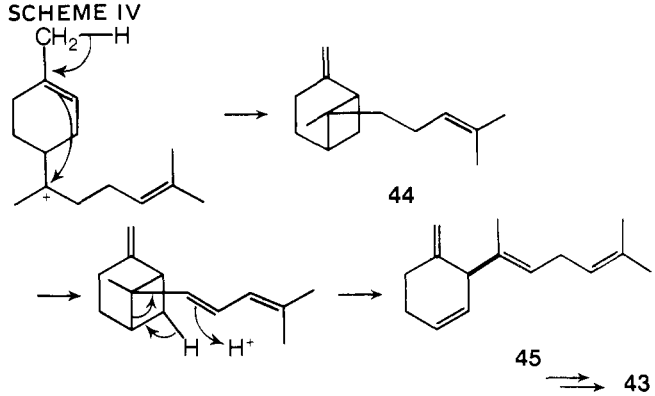
[1-^{14}C]- and [2-^{14}C]acetates gave good incorporations[177] into fumagillin (**43**), although, upon hydrolysis of the sebacic acid

SCHEME IV



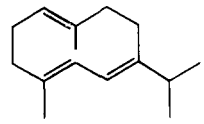
unit, almost half of the activity was lost. The remaining unit was shown by degradation to be derived from acetate in a specific manner, although the complete degradation pattern was not determined.

When mevalonate (**6**) was used as a precursor, a higher incorporation than with acetate was observed, and degradation indicated that one-third of the activity was found to be specifically in the terminal dimethyl group.[176]

A novel mechanism was proposed by Birch[177] to account for the biosynthesis of fumagillin (**43**) and related compounds (Scheme IV).[35] Cyclization of *cis,trans*-farnesol (**20**) in a manner similar to monoterpene cyclization, and subsequent formation of a four-membered ring, gives **44**. Introduction of a second double bond in the side chain and subsequent rearrangement would give rise to **45** which is at the same oxidation state as fumagillin (**43**).

C. Germacrene C (46)

The thermal instability of many of the simple germacrene derivatives, relative to the elemene derivatives, precluded their isolation for many years. Thus germacrene C (**46**), one of the



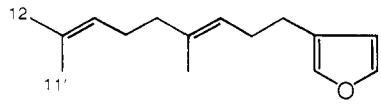
46

simplest sesquiterpenes, eluded isolation in substantial quantities until 1969,[178] when it was found to be the main constituent of the oil of the seed of *Kadsura japonica*.

Subsequent work on the biosynthesis of germacrene C (**46**) by the same workers demonstrated its formation as expected from mevalonate (**6**).[179] A supernatant of the homogenized seeds of *K. japonica* catalyzed the conversion of [2-^{14}C]mevalonate (**6**) into germacrene C (**46**) and *trans,trans*-farnesol (**1**). The latter (as the pyrophosphate **2**) was also an effective precursor of **46**. No further work has been reported.

D. Dendrolasin

Dendrolasin (**47**), a simple sesquiterpene derivative containing a furan ring, occurs in the ant, *Lasius filiginosus*,[180] and a number of higher plants.[181,182] In 1969, Schmid and co-workers[183]



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confirmed the terpenoid derivation of dendrolasin (**47**). Incorporation into dendrolasin (**47**) of [2-^{14}C]mevalonate (**6**) was invariably poor in spite of considerable effort.

Degradation of the labeled dendrolasin (**47**) after feeding [2-^{14}C]mevalonate (**6**), [1-^{14}C]acetate, and [U-^{14}C]glucose

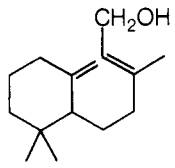
TABLE II. Degradation of Dendrolasin (47)

Precursor	% total activity in C-11, -11′, and -12
[U-^{14}C] Glucose	20
[1-^{14}C] Acetate	15
[2-^{14}C] Mevalonate	32

indicated that incorporation was specific for the former two precursors and uniform for the labeled glucose (Table II). No further work has been reported, although a discussion of the many related furan derivatives in *Myoporium deserti* from a biogenetic point of view has appeared.[184]

E. *trans*-γ-Monocyclofarnesol (48)

From the disrupted cells of the leaf-spot fungus *Helminthosporium siccans*, a compound was isolated which was demonstrated to be a novel sesquiterpene alcohol, *trans*-γ-monocyclofarnesol (**48**).[185] Further work demonstrated that in a cell-free



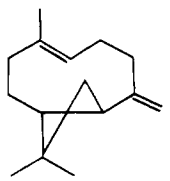
48

system of *H. siccans*, 25–50% of the total activity from [2-^{14}C]mevalonate (**6**) was located in **48**.[185] Farnesol (**1**) was not found in this incubation, and the labeled *trans*-γ-monocyclofarnesol (**48**) was not used as a precursor of the more elaborate metabolites of *H. siccans*.

IV. Biosynthesis of Bicyclic Sesquiterpenes

A. Caryophyllene (49)

Caryophyllene (**49**), a bicyclic sesquiterpene, contains a novel 11-membered ring and is the major sesquiterpene of peppermint, *Mentha piperita*.[186,187] The earliest work on the formation of caryophyllene (**49**) was, however, carried out using the catnip plant, *Nepeta cataria*.[188] Waller and co-workers demonstrated the incorporation of [2-^{14}C]mevalonate (**6**), but no degradative results were reported.



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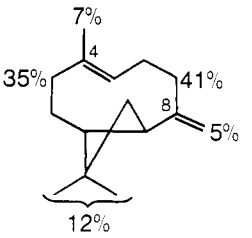
Loomis and Croteau,[187] as part of their work on the physiology of mono- and sesquiterpene biosynthesis,[189] have also investigated the formation of **49**. When [2-^{14}C]mevalonate (**6**) was fed to peppermint plants through the cut stem, incorporation into **49** was about 0.22%, rising to this level after 6 h and decreasing rapidly, demonstrating a quite rapid turnover of this metabolite. Later work[190] demonstrated that in the presence of added sucrose, the level of mevalonate (**6**) incorporation was doubled after the same period of time, suggesting that sucrose may be an energy source at the site of biosynthesis.

Chemical degradation[187] of the radioactive caryophyllene (**49**) yielded activities at the carbon atoms as shown in **50**. Two interesting observations may be deduced from these results. The first is that the IPP-derived portions of caryophyllene (**49**) incorporate label more extensively than that derived from DMAPP (**14**). The second is that there is some loss of specificity from the label originally at C-2 of **6** into the C-4 methyl and the C-8 methylene, indicating the presence of an IPP isomerase.



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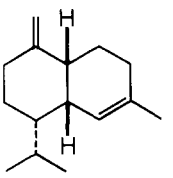
B. γ-Muurolene (51)

γ-Muurolene (**51**) co-occurs with caryophyllene (**49**) in *Mentha piperita*. Concurrently with their work on **49**, Loomis and Croteau[187] also examined the formation of **51** from [2-^{14}C]mevalonate (**6**). After reaching a maximal incorporation of 0.1% after 4.5 h, the level decreased rapidly, again indicating active metabolism of **51**. With added sucrose the level of incorporation rose to 0.4%. No degradations were reported.



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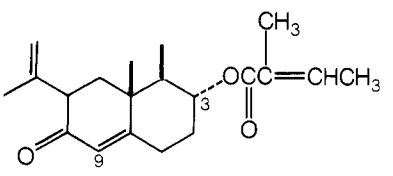


52

C. Petasin (52)

Petasin (**52**) and related compounds from *Petasites hybridus*[191] are members of the eremophilane series of sesquiterpenes, and again the skeleton cannot be derived by direct cyclization of farnesol (**1**).

Initial work with [2-^{14}C]mevalonate (**6**) in this plant succeeded in labeling mainly the sterols and triterpene alcohols,[192] but subsequent work[193,194] improved the specific incorporation into **52**.

The level of incorporation was raised again when an enzyme inhibitor suppressing triterpene biosynthesis was used.[193] At a dose level of 100 ppm, the enzyme inhibitor tris(2-diethylaminoethyl) phosphate trihydrochloride (SK and F 7997-A_3) improved the incorporation into petasin (**57**) fivefold with concomitant reduction of incorporation into the phytosterols.

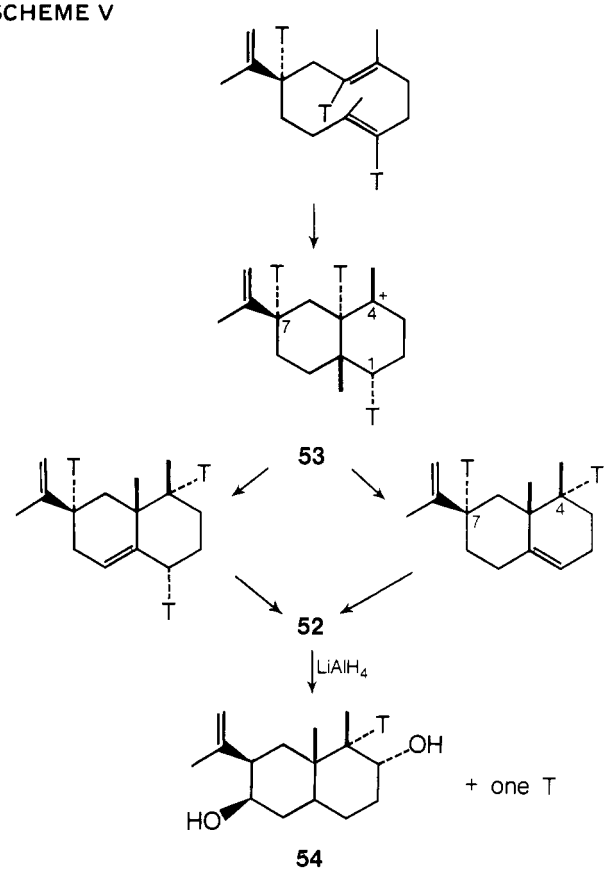
It was suggested[186] that the eremophilane group of sesquiterpenoids arises from the eudesmane skeleton by shift of the methyl group at C-10 to C-5 with sequential loss of the C-1α (or C-9α) proton. Thorough degradation of the product after [2-^{14}C]mevalonate (**6**)[194] indicated that one-third of the label was in the isopropylidene group, a further third was specifically at C-3, and the final third of the activity was specifically at C-9.

If Scheme V is correct for the formation of the eremophilane skeleton, then [4*R*-^{3}H]mevalonate (**6**) should provide the 1α, 5α and 7α hydrogens in an intermediate eudesmane (**53**), and subsequent rearrangement should lead to an eremophilene labeled at 4α and 7α. Alternatively, if the 9α hydrogen were eliminated, one would expect complete retention of all tritium into petasin (**52**).

When this experiment was conducted,[194] it was found that specific labeling of C-4α occurred, supporting the hypothesis that methyl migration takes place with simultaneous 1,2-hydride shift. Reduction of petasin (**52**) with $LiAlH_4$ gave the diol (**54**) which contained only two tritium atoms. Because positions 1 and 7 of petasin (**52**) are base labile, however, partial exchange may have occurred.

Lawton has proposed[195] an alternative scheme for the biosynthesis of the eremophilane skeleton involving spiro intermediates.

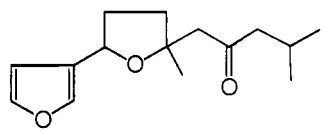
SCHEME V



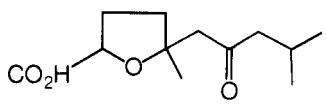
D. Ipomeamarone (55)

When the root of the sweet potato (*Ipomoea batatas*) is infected with the black rot fungus, *Ceratocystis fimbriata*, the former produces a novel sesquiterpene[196] derivative ipomeamarone (**55**),[197] which acts as a potent antifungal agent against the invading fungus.[198]

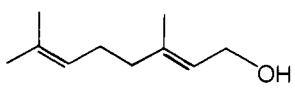
The amount of **55** produced under these conditions is an amazing 2%,[197] after quite short time periods. Preliminary feeding experiments with [2-^{14}C]acetate[199,200] and [2-^{14}C]-mevalonate (**6**)[199] gave poor incorporations. Subsequently[201] much higher incorporations were obtained, 11–16% for [2-^{14}C]acetate and 48% for [2-^{14}C]mevalonate (**6**) under slightly different conditions. Degradation to afford ipomeanic acid (**56**) indicated that all of the activity was retained from mevalonate (**6**) and 73% of the activity from acetate.



55



56



57

Pyruvate and citrate[202] were considerably poorer precursors of **55**. In theory 7/9 of the activity from [2-^{14}C]acetate should be retained in ipomeanic acid (**56**). The slightly (~5%) lower activity observed may indicate some unsymmetrical incorporation of acetate into farnesol (**1**). Further attempts to isolate small fragments failed, but the sesquiterpenoid nature of **55** was clearly established.

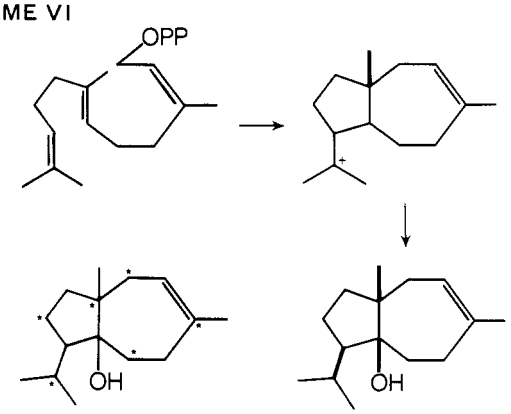
Added farnesol (**1**), nerolidol (**11**), or geraniol (**57**) reduced the incorporation of [2-^{14}C]acetate although the level of ipomeamarone (**55**) was markedly increased.[119] [2-^{14}C]Farnesol was evaluated as a precursor of **55** in a time-course study.[203] After 6 h 4.8% of the activity was located in **55**, and this maximized to 6.8% after 21 h. The specificity of the label was not determined.

Ethanol is rarely used as a biosynthetic precursor, but when [2-^{14}C]ethanol was used as a precursor of **55**, it proved to be more readily incorporated than acetate.[204] Pyrazole, a potent inhibitor of alcohol dehydrogenase,[206] drastically decreased the level of incorporation of ethanol, suggesting that the route of ethanol incorporation was via acetaldehyde and subsequently acetyl-CoA.

E. Carotol (58)

Carotol, one of the principal sesquiterpene alcohols of the seeds of the carrot, *Daucus carota*,[206] has a probable biogenesis from *cis,trans*-farnesol (**20**) as shown in Scheme VI.[207] Only very

SCHEME VI



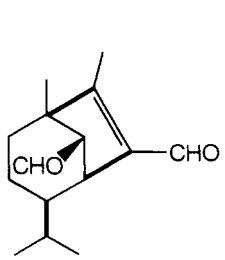
59 58

preliminary work has been reported. [1-^{14}C]Acetate should give rise to **58** labeled as in **59**; degradation of acetate-labeled carotol (**58**) afforded the carbon atoms indicated as acetic acid, and these were found to contain 16% of the total activity as expected.[207]

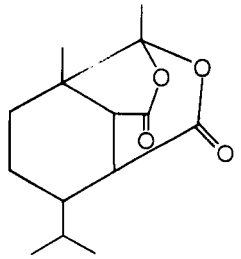
Thus although the sesquiterpene nature of **58** is established, the mechanism of cyclization and the specificity of any hydride shifts in carotol (**58**) biosynthesis have not been determined.

F. Helminthosporal (60)

Helminthosporium sativum is the fungus responsible for the seedling blight, root rot, and leaf spot of a number of cereals but most importantly (economically) of barley. The most important compound obtained from this fungus is the toxin helminthosporal (**60**).[208] Although this compound is an artifact of the basic work-up procedure,[209] we shall consider it here as a natural product.



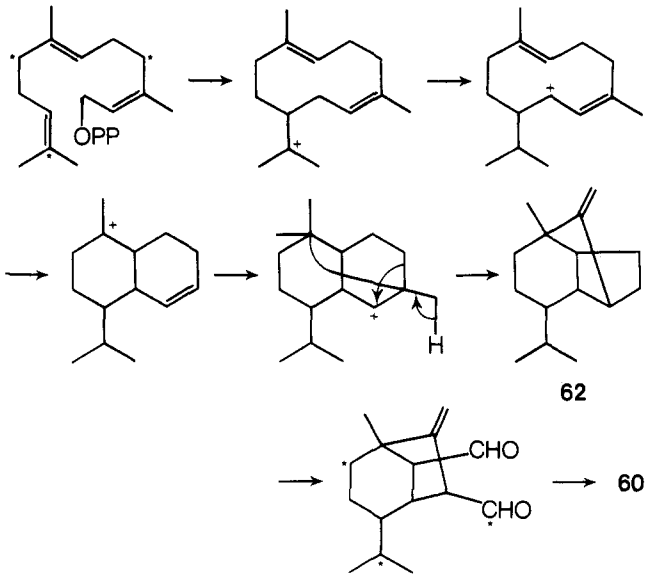
60



61

Only one experiment has been reported and that some years ago. De Mayo and co-workers,[210] following up their isolation and structure elucidation studies, also examined the biosynthesis of helminthosporal (**60**) from [2-^{14}C]mevalonate (**6**). Incorpo-

SCHEME VII

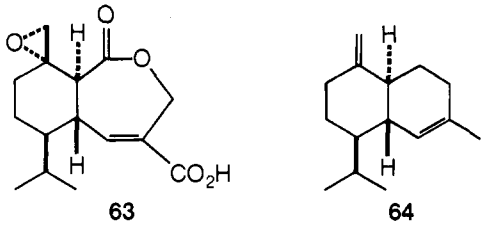


ration was low, but oxidation and subsequent ozonolysis produced a dilactone (**61**) retaining 62% of the initial activity of **60.** In this degradation one carbon atom, the exocyclic carbon of the α,β-unsaturated aldehyde, was specifically lost.

Scheme VII depicts a route for helminthosporal (**60**) biosynthesis proposed by De Mayo et al.[210] involving a germacrene intermediate which subsequently cyclizes to a tricyclic compound (**62**). This intermediate, sativene, was subsequently isolated from *H. sativum,*[211] thereby adding some credence to its possible biosynthetic utility in the formation of **60.** Recent work by Arigoni on the biosynthesis of sativene (**62**) is discussed later.

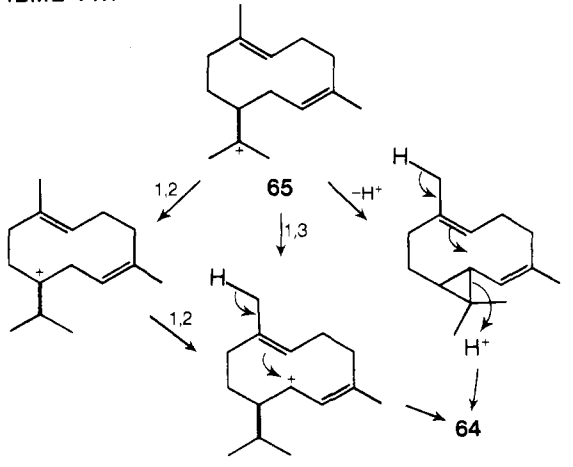
G. Avocettin (63)

Avocettin (**63**), from *Anthostoma avocetta,* may be thought of as the end product of extensive oxidative attack on *ent*-γ-cadinene (**64**). Three possibilities for the formation of **64** from

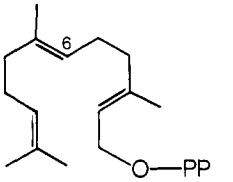


a germacrene cation such as **65** are shown in Scheme VIII. The work on the biosynthesis of avocettin (**63**), in particular that relating to the conversion of *trans,trans*-farnesol (**1**) to *ent*-γ-cadinene (**64**), forms part of an important and elegant study by Arigoni* and co-workers.[137]

SCHEME VIII



[6-^{3}H]Farnesol (**1**) was used initially as a precursor of avocettin (**63**). A 0.12% incorporation was observed and the label was at the ring junction position. [2-^{14}C]Mevalonate (**6**) was incorporated into avocettin (**63**), and degradation indicated labeling to be at C-3, C-9, and in the isopropyl side chain. The mode of cyclization of **1** is therefore as illustrated in **66,** i.e., from 6-*trans*-farnesol. Distribution of label was fairly even for the active sites [*cf.* caryophyllene (**49**)].



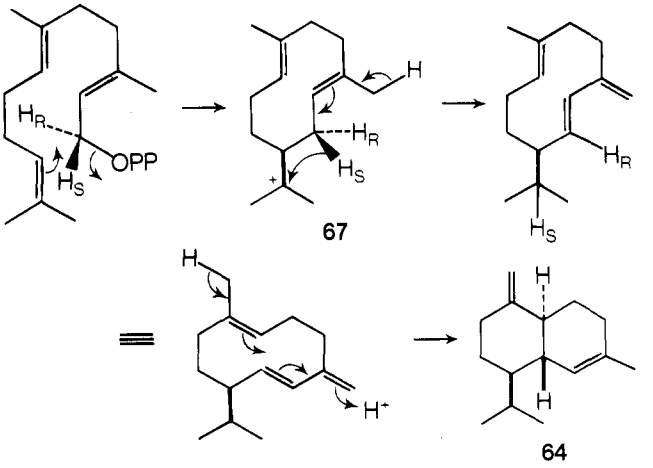
In order to investigate the formation of γ-cadinene (**64**) more carefully, [4*R*-^{3}H]mevalonate (**6**) was used in admixture with [2-^{14}C]-**6**. Intact incorporation was observed, and degradation indicated that one tritium was located at the carbon bearing the isopropyl group. Any route to **64** involving a 1,2-hydride shift can therefore be discounted.

The mechanism of formation of the isopropyl group of avocettin (**63**) was then investigated with [5*R*-^{3}H]- and [5*S*-^{3}H]-mevalonates (**6**). Degradation of the labeled avocettin (**63**) indicated that *no* loss of tritium occurred from C-1 of the farnesyl precursor and that formation of the isopropyl chain involved the stereospecific migration of the pro-5*S* proton of **6**.

In the biosynthesis of *cis,trans*-farnesol (**20**) from **1**, one of the hydrogens on C-1 was lost (see earlier discussion). Since this was not observed in the biosynthesis of **63,** some alternative mechanism must be postulated to account for the formation of **64,** which by its existence contains a cis double bond at the positions derived from C-2,3 of farnesol.

In addition, any mechanism must also take into account the stereospecific shift of the pro-5*S* of **6**. Scheme IX illustrates the rationalization of these results by Arigoni.[137]

SCHEME IX



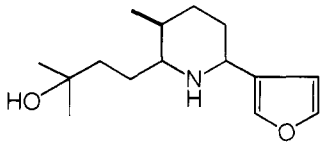
Further work by Arigoni[137] involved an examination of the stereochemical origin of the methyl groups in the isopropyl side chain. In the biosynthesis of **1**, [2-^{14}C]mevalonate (**6**) labels only the (*Z*)-methyl group in the isopropylidene residue. A brilliant degradation of avocettin (**63**) succeeded in chemically isolating the methyl groups of the isopropyl side chain and demonstrated

* The author is deeply indebted to Professor D. Arigoni, who provided a preprint of his symposium lecture delivered at the 9th IUPAC meeting, Ottawa, Canada, June 1974.

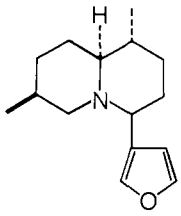
the integrity of these groups. Thus the initial ring closure and subsequent hydrogen migration to the isopropyl chain occurs faster than rotation of the side chain in **67.** The explanation of these results is deferred to a later point.

H. Alkaloids of *Nuphar*

The genus of aquatic plants, *Nuphar,* contains a number of alkaloids containing both a piperidine and furan ring.[212] Two typical examples are nupharamine (**68**) and deoxynupharidine (**69**). In both cases derivation from a linear sesquiterpene can be discerned.



68



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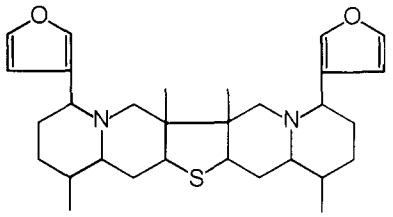
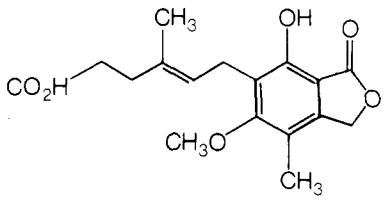


70

The only reported study of the biosynthesis of this group of compounds is by Schutte and co-workers who made a preliminary study of the formation of the dimeric compound, thiobinupharidine (**70**), in *Nuphar luteum.*[213] Cadaverine was not a direct precursor of the piperidine rings, whereas [2-^{14}C]- and [3,4-^{14}C]mevalonates (**6**) were each specifically incorporated. The derivation from a 15-carbon precursor remains to be demonstrated, since at the time of the biosynthetic work the correct structure of thiobinupharidine (**70**) was not known.

I. Mycophenolic Acid (71)

Mycophenolic acid (**71**) from *Penicillium brevi-compactum*[214] does not appear to contain any unit derived from a terpenoid precursor. The nucleus has the standard substitution for derivation from acetate, save for an additional nuclear methyl group, and the side chain contains seven, rather than five or ten, carbon atoms.



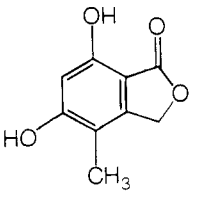
71

Early work on the biosynthesis of mycophenolic acid (**71**) demonstrated that methionine was a precursor of both the nuclear and *O*-methyl groups.[215,216] The methyl group on the side chain is therefore derived from a source other than methionine.

It was Birch[217-219] who first demonstrated that the seven-carbon unit was derived from mevalonate (**6**) rather than acetate. [1-^{14}C]Acetate labeled the side chain *and* the nucleus of **71**,[216-218] but [2-^{14}C]mevalonate (**6**) was incorporated only into the side chain.[217,218] Although the specificity of labeling within this unit was not determined, it was felt at the time that the origin of each carbon atom in the molecule was defined.

This area remained dormant for many years, but the recent work of Money, and more particularly Canonica and their respective co-workers, has illuminated the steps on the biosynthetic pathway quite elegantly.

Canonica considered that nuclear methylation was an early step in the sequence and proved this theory in two separate series of experiments. In the first of these, a variety of phthalide derivatives were used as potential precursors. Only one, **72,** was incorporated to any great extent (36.5%).[220] The corresponding compound lacking the 4-methyl group was not incorporated into **71,** but did give rise to a new metabolite, **73,** also lacking the 4-methyl group. When the incubation was interrupted after feeding [^{14}C]methionine, **72** was isolated radioactivity labeled to the extent of 3% of the total activity.[221]



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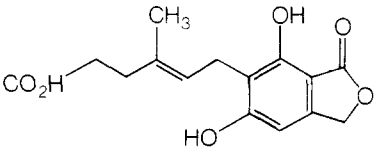
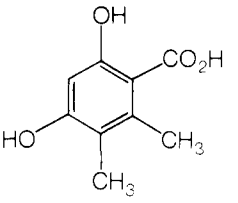


73

Both Money[222] and Canonica[221] have investigated more carefully the point at which nuclear methylation occurs. Whereas 4,6-dihydroxy 2,3-dimethylbenzoic acid (**74**) was a precursor, orsellinic acid (**75**), a postulated precursor,[223] was not. On this basis it was suggested[221,222] that C-methylation occurs at the stage of an enzyme-bound polyketide.



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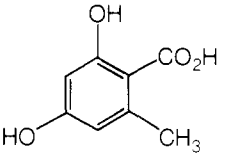
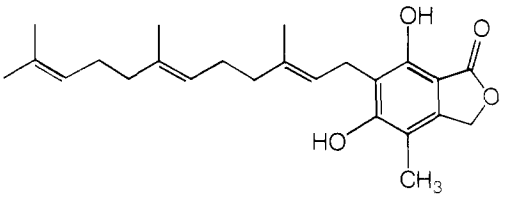
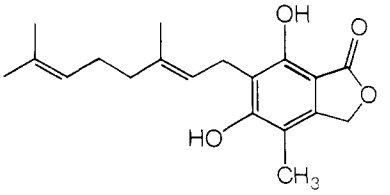


75

It had been speculated[224,225] that the side chain of mycophenolic acid was derived from a geranyl unit. However, when the phthalide derivative (**72**) was added to the medium, a new metabolite (**76**) containing a C_{15} side chain was isolated.[221] Reintroduction of **76,** specifically labeled at the nuclear methyl group, gave **71** containing 33.6% of the total activity.[221] The corresponding geranyl derivative (**77**) was incorporated to a much lesser extent, and labeled geraniol (**57**) was not a precursor.[222]



76

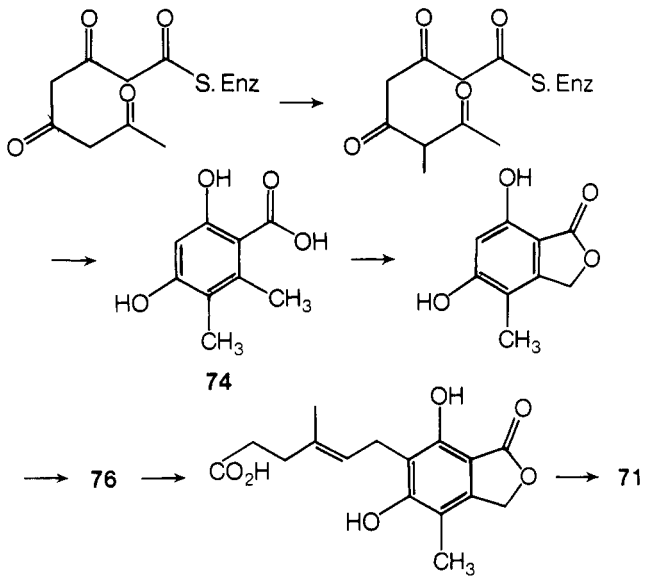


77

The seven-carbon side chain is therefore derived not from a C_{10} unit, but from a C_{15} farnesyl, unit. Oxidation of the side chain probably precedes O-methylation.[226]

A reasonable route for the biosynthesis of mycophenolic acid (**71**) is shown in Scheme X.

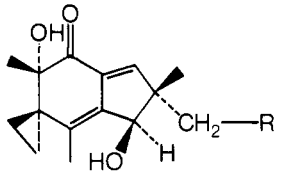
SCHEME X



V. Biosynthesis of Tricyclic Sesquiterpenes

A. The Illudins, Hirsutic Acid, and the Coriolins

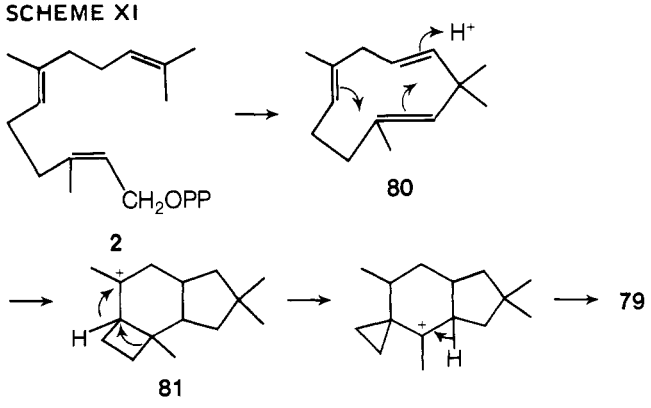
The fungal metabolites illudin S (**78**)[227-230] and illudin M (**79**),[227-230] from the Basidomycetes *Clitocybe illudens*[227] and *Lampteromyces japonicus*,[228] exhibit antibacterial,[231,232] toxic[231,232] and antitumor[230] activity. Initial biosynthetic work



78, R = OH
79, R = H

with [2-^{14}C]mevalonate (**6**) demonstrated incorporation into **78** and **79**,[230] and it was suggested that the humulene skeleton (**80**) was an important intermediate as shown in Scheme XI.

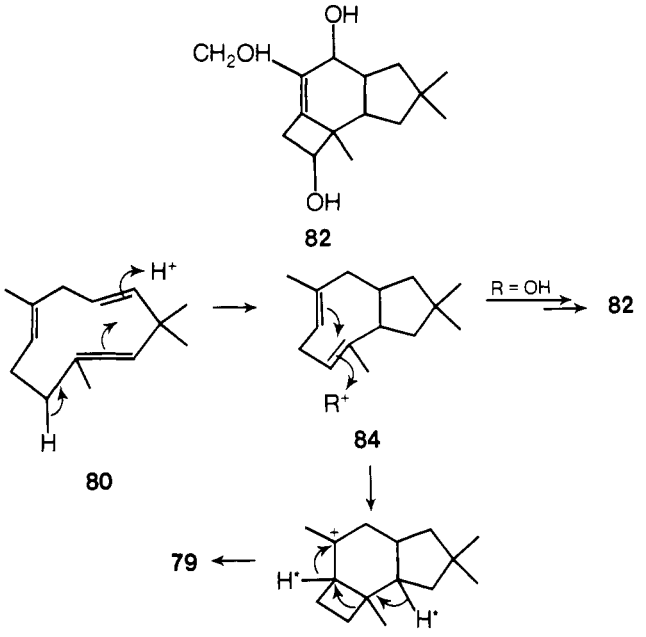
SCHEME XI



A compound illudol, with the skeleton **81** was isolated from *C. illudens*,[231] but its structure was not determined to be **82** until 1967.[233] Preliminary biosynthetic work[233] indicated that [2-^{14}C]mevalonate (**6**) was a precursor.

More recently, Hanson and co-workers[234] have investigated the biosynthesis of this group of compounds more carefully. Using [4*R*-^{3}H,2-^{14}C]mevalonate (**6**), it was demonstrated that only one tritium was specifically retained in the formation of illudin M (**79**), confirming the proton losses outlined in Scheme XI. However, degradation of illudin M (**79**) after feeding [2-$^{3}H_2$,2-^{14}C]mevalonate (**6**) indicated the presence of only three

SCHEME XII

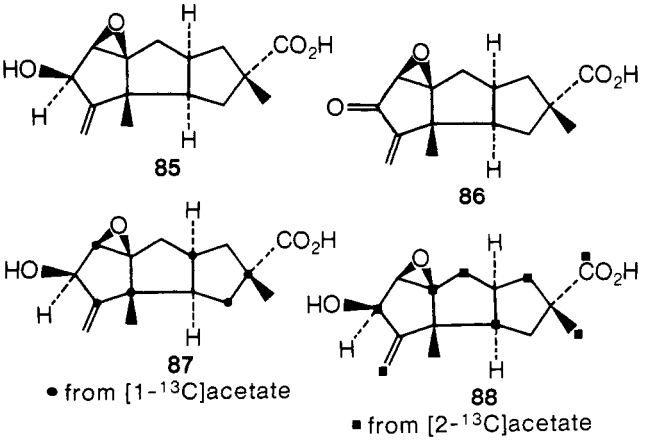


H* protons from [4*R*-^{3}H]mevalonate

(rather than four) tritium atoms at the indicated positions. The ^{3}H:^{14}C ratio dropped from 9.6:1 in **6** to 4.7:1 in illudin M (**79**), with a specific incorporation of 0.06%.[234]

Clearly an alternative mechanism is involved, and it was suggested[234] that the tricyclic intermediate **83** (rearranging as shown) is not produced directly from the humulene skeleton, but that a bicyclic intermediate of the type **84** is involved. The mode of formation of this intermediate would involve loss of a proton derived from C-2 of **6** as shown in Scheme XII. The invocation of a bicyclic intermediate would also allow a rationalization of the formation of illudol (**82**).

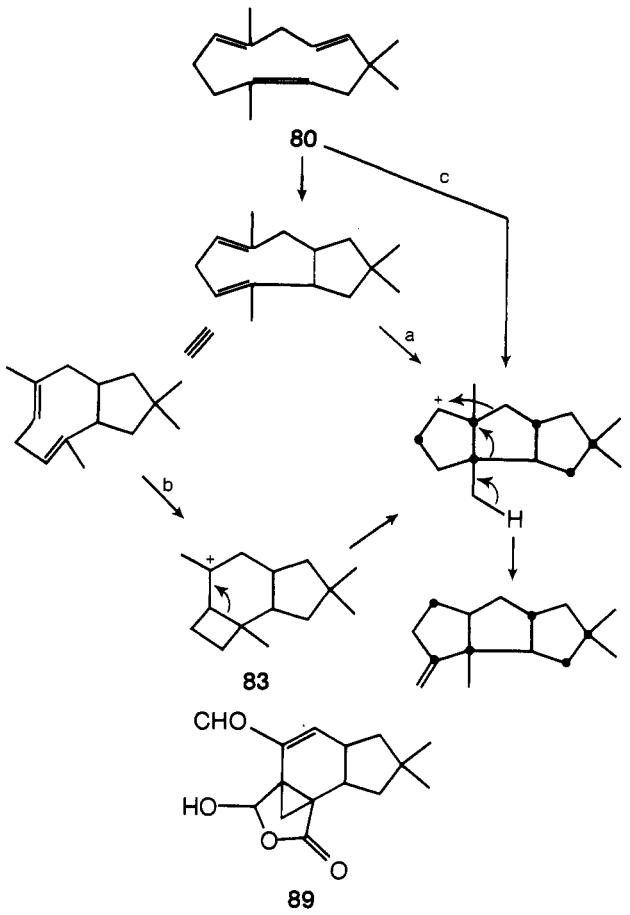
CMR spectroscopy has recently been used in a variety of biosynthetic studies,[235] and this technique has been applied to an evaluation of the possible biosynthetic routes to hirsutic acid (**85**) and complicatic acid (**86**) in *Stereum complicatum*.[236] In particular, the utilization of [1-^{13}C]- and [2-^{13}C]acetates as precursors of hirsutic acid (**85**) was studied.[237] From [1-^{13}C]-acetate carbon atoms 1, 3, 4, 6, 9, and 11 were specifically enriched whereas [2-^{13}C]acetate specifically enriched carbon atoms 2, 5, 7, 8, 10, 12, 13, 14, and 15 as shown in **87** and **88**, respectively.



• from [1-^{13}C]acetate

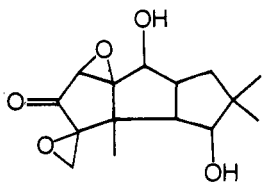
▪ from [2-^{13}C]acetate

The observed pattern of labeling clearly demonstrated that two 1,2-shifts occurred, and three schemes were postulated to explain the rearrangement from the humulene derivative **80**.[237] One of these (route a) was a modification of a route originally



proposed by Scott,[238] and another involved an intermediate (**83**) previously deemed important in the formation of illudin M (**79**), and possibly marasmic acid (**89**) biosynthesis. It remains to determine which of these routes is the correct one.

The coriolins are a group of novel antitumor antibiotics from *Coriolus consors*,[239] bearing a close structural relationship to hirsutic acid (**85**) and are typified by **90**. As with the biosynthesis



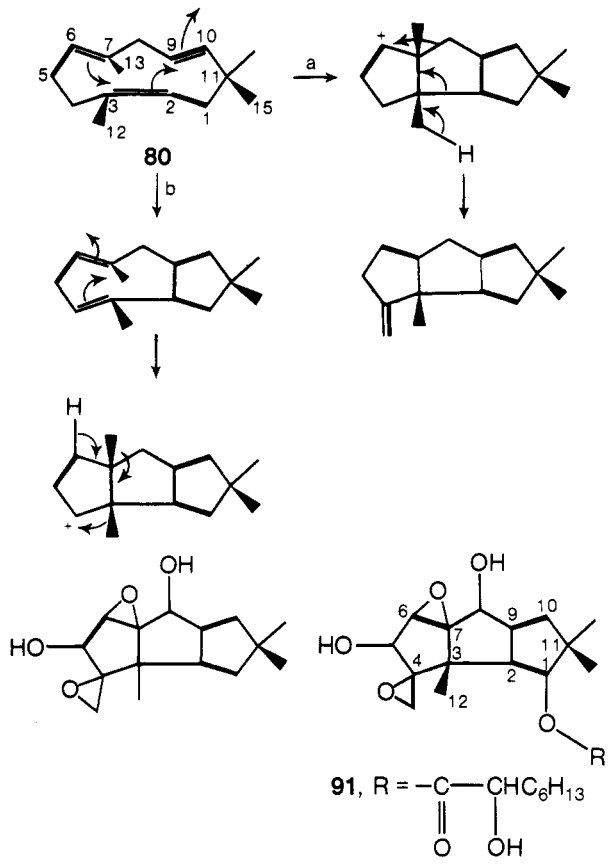
90

of hirsutic acid (**85**) referred to previously, ^{13}C NMR spectroscopy was utilized in order to gain additional information concerning the biosynthetic route. The key to the method used in this case is the observation of $^{13}C-^{13}C$ coupling constants when an intact [1,2-$^{13}C_2$]acetate moiety is incorporated. If the acetate C–C bond is broken, the coupling is irretrievably lost. In this way the migration of a methyl group, for example, can be easily monitored. As with all ^{13}C NMR work, however, the assignment of each carbon resonance in the spectrum must be made.

[1,2-$^{13}C_2$]Acetate was therefore used as a precursor of 5-dihydrocoriolin C (**91**) in *Coriolus consors*.[240] From the ^{13}C NMR spectrum it was deduced that six couplings were present in the labeled **91** out of the 24 possible C–C couplings. On the basis of previous assignments, these $^{13}C-^{13}C$ couplings were found to be as follows: 1–2, 3–12, 4–13, 6–7, 9–10 and 11–15.

This single experiment served to distinguish between three possible pathways which the previous ^{13}C labeling work with hirsutic acid (**85**)[237] had not been able to do. The only scheme compatible with these results is route a illustrated in Scheme XIII, from humulene (**80**), in which intact $^{13}C-^{13}C$ couplings are

SCHEME XIII



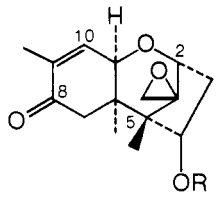
marked with heavy lines (stereochemistry not implied). In order to give some idea of the theory behind this expermment, an alternative scheme (route b) is also included which gives a product with different $^{13}C-^{13}C$ labeling. Of particular note in this case are the lack of $^{13}C-^{13}C$ couplings at 3–12 and 4–13 which are lost at the stage of the double methyl migration. Since these couplings are observed in **91**, route b is excluded from consideration.

Also used as precursors were [1-^{13}C]acetate, [2-^{13}C]acetate, and [2-^{13}C]mevalonate. The latter gave specific enrichment at C-5, C-8, and C-14, indicating that the stereospecific integrity of DMAPP (**14**) was again maintained upon further elaboration to more complex cyclized products.

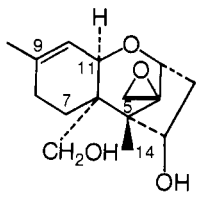
B. Trichothecin (92) and Helicobasidin (96)

The fungus *Trichothecium roseum* produces a number of metabolites of which trichothecin (**92**) is the best known.[35] The correct structure for trichothecin (**92**) was first proposed by Godtfredsen and Vangedal,[245] and biosynthetically it is one of the best studied of the sesquiterpenes. From a number of related fungi, including *Myrothecium roridum*,[241-244] *M. verrucaria*,[243] and *Trichoderma viride*,[245] closely related compounds such as verrucarol (**93**),[241-244,246] trichodermol (**94**),[245-247] and roridin (**95**)[243] were isolated.

Helicobasidin, one of the two major metabolites of *Helicobasidium mompa*,[248] was shown to have structure **96** by Nishikawa and co-workers.[249] Although **92** and **96** have quite different carbon skeleta, their biosyntheses are, as we shall see, quite closely related. If we consider that formation of the quinone ring of helicobasidin (**96**) is a final step, we can imagine a precursor having the carbon skeleton depicted in **97**. If we also break the pyran ring in trichothecin (**92**) and remove all oxygenation, the skeleton **98** is obtained. Of these, only **97** obeys the isoprene rule, the other being related either by a 1,3-methyl shift or two 1,2-methyl shifts. We can naively predict, therefore, that the



92, R = COCH=CHCH$_3$



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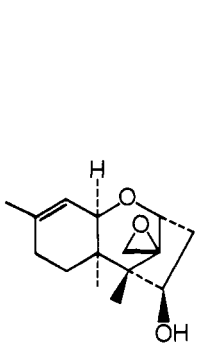

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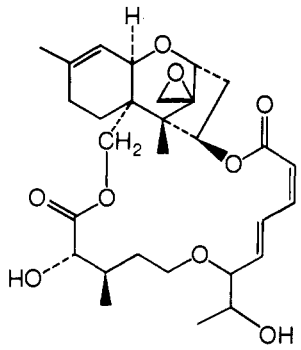

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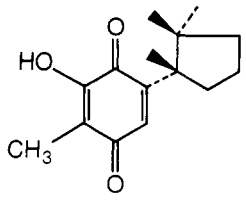

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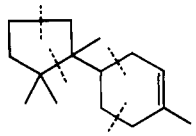

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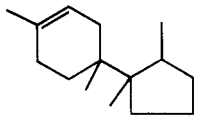
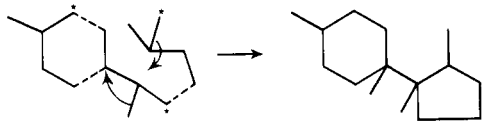


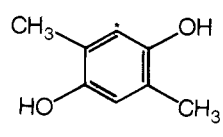
98

skeleton contained in **97** may well be a precursor of the tricothecane skeleton (**98**).



98

Initial work on the biosynthesis of trichothecin (**92**) was reported some years ago by Jones and co-workers.[250,251] [2-^{14}C]Mevalonate (**6**) was incorporated to the extent of 0.5% and extensive degradation indicated that one label was located in the six-membered ring fragment **99**, and two labels in the cyclopentenone fragment **100**. [1-^{14}C]Acetate gave rise to trichothecin (**92**), containing 95% of the label in the crotonate moiety.



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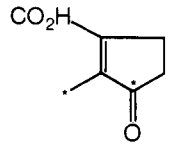
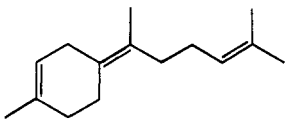


100

The crude scheme suggested for trichothecin (**92**) biosynthesis[251] was refined by Ruzicka[252] and Godtfredsen and Vangedal.[245] Each scheme involved a bisabolene derivative such as **101** as the key intermediate, and the latter scheme[245] also accounts for the stereochemistry of **92** via a boat conformation of the side chain in the cyclization reaction. There are two possible modes of cyclization of farnesol to give the bisabolene intermediate **101**, depending upon the stereochemical nature of the central double bond of farnesol. At that point there was no information available as to which isomer was the preferred precursor.



101

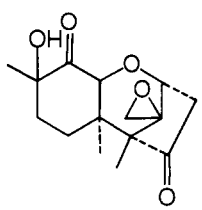
Polish workers have investigated the biosynthesis of verrucarol (**93**) using [1-^{14}C]- and [2-^{14}C]acetates and [2-^{14}C]-mevalonate (**6**).[253] Degradation of the product derived from [1-^{14}C]acetate and [2-^{14}C]mevalonate (**6**) indicated that the labeling was specific.

Similar work was reported by Bentley and Chen[254] and Nishikawa et al.[255] on helicobasidin (**96**) in *H. mompa*. [1-^{14}C]Acetate was incorporated to the extent of 0.9% and [2-^{14}C]-6 to the extent of 0.04%.[254] Degradation of the labeled helicobasidin (**96**) indicated[254,255] that each precursor was used specifically in accordance with a scheme from **2**, although the various hypotheses[249,256] could not be distinguished by this method.

In 1967 Hanson published his first results in this area.[257,258] Farnesyl pyrophosphate (**2**) was shown to be a good (1.5%) precursor of trichothecin (**92**) in *T. roseum*. This was the first incorporation of an intact C-15 unit into a cyclic sesquiterpene derivative and was the first of a very successful series of experiments by the Sussex group. Simultaneously, Nozoe and co-workers[260] have studied in detail the biosynthesis of helicobasicin (**96**).

As we have seen previously, it is the pro-4*S* proton of mevalonate (**6**) which is lost in the formation of *trans,trans*-farnesol (**1**). Thus from [4*R*-^{3}H]mevalonate we would expect complete retention of tritium in farnesol (**1**), loss of one tritium upon incorporation into trichodermol (**94**) and trichothecin (**92**), and loss of two tritiums on incorporation into helicobasicin (**96**). Thus trichothecin (**92**) and trichodermol (**94**) should be labeled at C-2 and C-10, and helicobasidin (**96**) at C-2.

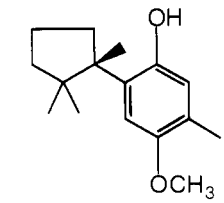
Hanson[259] demonstrated that when [4*R*-^{3}H]mevalonate (**6**) was used as a precursor of trichothecin (**92**) in *T. roseum*, and of trichodermol (**94**) in *Trichoderma* sp., two out of three tritium labels were retained. Degradation of trichodermol (**94**) indicated that the ketol **102** retained only half of the tritium activity of **94**, a result apparently in conflict with the earlier work of Jones and Lowe.[251]



102

When [4*R*-^{3}H]mevalonate (**6**) was used as a precursor of helicobasidin (**96**) in *H. mompa*,[260] an interesting result was observed. Instead of two tritium atoms being lost, two of three were *retained*. Evidence from chemical conversion indicated that the tritium originally of C-8 of farnesol (**1**) is somehow transferred to the five-membered ring, for **103**, a cometabolite of **96**, retained all tritium activity. Because of this observation, Nozoe[260] postulated that a γ-bisabolene derivative such as **101** was *not* involved as a biosynthetic intermediate.

In order to investigate the mode of cyclization of the farnesyl chain, [1-^{3}H$_2$]- and [2-^{3}H]farnesyl pyrophosphates (**2**) (each used in admixture with [2-^{14}C]-**2**) were used as precursors of



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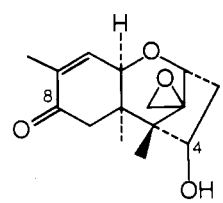

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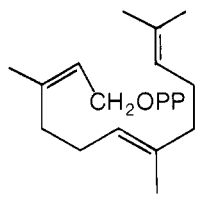

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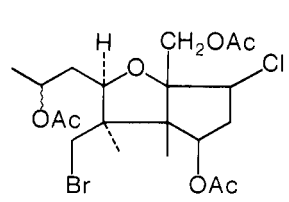


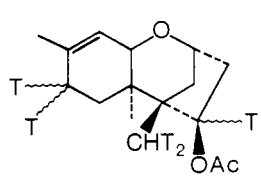
106

trichothecolone (**104**) in *T. roseum*.[259,261] With [1-$^3H_2$]farnesyl pyrophosphate (**2**) one tritium label was lost in trichothecolone (**104**), but no label was lost from [2-^{3}H]-**2**. On this basis it was suggested[259-261] that *cis,trans*-farnesyl pyrophosphate, cyclizing as in **105**, was the initial intermediate.

Similar work using different precursors has also been reported by Nozoe and Machida.[262] [4*R*-^{3}H,2-^{14}C]Mevalonate (**6**) retained two tritium atoms (in agreement with Hanson's work[259]) upon incorporation into trichothecolone (**104**). The radioactive trichothecolone (**104**) was then degraded by a series of steps in which the carbon atom at C-8 was specifically removed. This compound, **106**, was shown to have a ^{3}H:^{14}C atomic ratio of 1.78:2; i.e., the carbon at C-8 was specifically derived from [2-^{14}C]mevalonate (**6**), confirming the result of Hanson.[259,261]

Additional evidence for the farnesol cyclization came from experiments with [2-$^3H_2$]mevalonate (**6**). When trichodermin (**107**) from *Trichoderma sporulosum* and trichothecolone (**104**) from *T. roseum* were isolated after feeding [2-$^3H_2$]mevalonate (**6**), five and four tritium labels were incorporated, respectively.[261,263] The methyl groups of the three mevalonate units therefore retain their individuality.

When [2*R*-^{3}H]mevalonate (**6**) was used as a precursor, the isolated trichodermol (**94**) contained nearly two tritium labels.[261,263] This is an expected result if hydroxylation at C-4 proceeds specifically with removal of the pro-2*R* proton (of **6**) at this position. This observed specificity was not checked with [2*S*-^{3}H]mevalonate (**6**). The retention of four tritium labels into trichothecolone after feeding [2-$^3H_2$]mevalonate (**6**) is an unexpected result.[261,263] One label was demonstrated by oxidation to be at C-4. The location of one of the other labels was suggested after the multiply labeled **107** (obtained from [2*R*-^{3}H]-**6**) lost only one tritium upon incorporation into trichothecolone (**104**) and trichothecin (**92**). A tritium must therefore have migrated from C-8 to C-7 during formation of **92**, possibly via an NIH shift type mechanism on the epoxide **108**.



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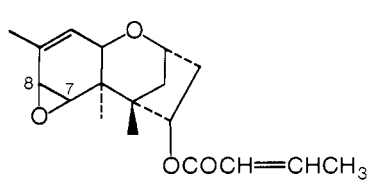


108

When [5-$^3H_2$,2-^{14}C]mevalonate (**6**) was used,[261,264] only four of the expected five tritium labels were observed in tricothecin (**92**). The assumption (not checked) was that one tritium was lost from C-7 of trichothecin (**92**) at some stage.

Similar results were obtained by Hanson.[265] [2-$^3H_2$,2-^{14}C]-Mevalonate (**6**) was transformed into helicobasidin (**96**) in *H. mompa* with retention of four tritium labels, and when [5-$^3H_2$,2-^{14}C]mevalonate (**6**) was used two tritium atoms were retained.

Tamm and co-workers[266] investigated the use of [2-$^3H_2$]-mevalonate (**6**) as a precursor of verrucarol (**93**), and one of these was shown (by oxidation) to be at C-4. No tritium was found at C-10 but two tritiums were located at C-8. A bisabolene intermediate was suggested.[266] When the individual pro-[2-^{3}H] isomers were used as precursors, it was demonstrated that [2*S*-^{3}H]mevalonate (**6**) gave rise to a tritium at C-4, indicating again that hydroxylation at C-4 was stereospecific.

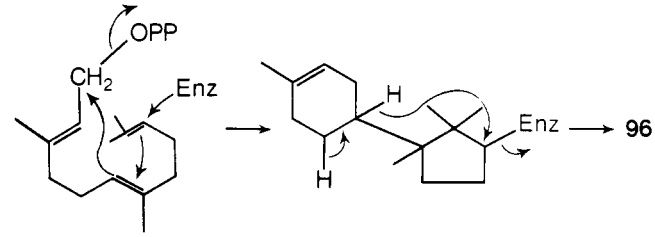
Tamm and co-workers[267] also examined the biosynthesis of verrucarol (**93**) from farnesyl pyrophosphate (**2**). A specific incorporation of 0.22% into verrucarol (**93**) was observed when [6-^{3}H,12,13-^{14}C]-*trans,trans*-farnesyl pyrophosphate (**2**) was used. There was no loss of tritium, and degradation indicated that the tritium was probably located at C-2 of verrucarol (**93**). Thus a specific migration of tritium occurs from C-6 to C-2 after formation of the cyclic system. The biosynthesis of verrucarol (**93**), therefore, mirrors that of trichothecin (**92**) in this respect.

Hanson[268] followed up Nozoe's[260] observation that a hydrogen shift was occurring in the biosynthesis of trichothecin (**92**) and helicobasidin (**96**).

When [4*R*-^{3}H]mevalonate (**6**) was used as a precursor of helicobasidin (**96**) in *H. mompa*, two of the three tritium atoms were retained.[265-268] In order to investigate whether the tritium from the central prenyl unit was retained, [2-^{3}H,2-^{14}C]geranyl pyrophosphate (**10**) was used. There was no change in the ^{3}H:^{14}C ratio and consequently the 6-proton of **1** migrates during the biosynthesis of **96**. Similar work[261,168] demonstrated that this migration also takes place in the biosynthesis of trichothecin (**92**) and trichodermol (**94**).

On this basis Hanson[265,268] suggested that the cyclization of *cis,trans*-farnesyl pyrophosphate (**105**) may be concerted with attack of an enzyme at C-10 initiating cyclization. The hydride from C-6 of farnesol then migrates to C-10 with concomitant displacement of the enzyme as shown in Scheme XIV.

SCHEME XIV

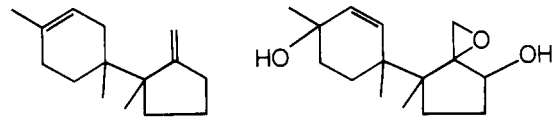


Forrester and Money[269] investigated the utility of a number of bisabolene derivatives and a monocyclofarnesol in the biosynthesis of trichothecin (**92**). In each case low incorporations and extensive degradation were observed. The intervention of a bisabolene intermediate in the biosynthesis of trichothecin (**92**) and helicobasidin (**96**) is mitigated both by this work and the work of Hanson and Nozoe.

Substantial progress has also been made in the isolation of various advanced intermediates and their subsequent utilization in the biosynthesis of the major metabolites. In 1970 Nozoe's group isolated from *Trichothecium roseum* a number of new trichothecin-type compounds of considerable biogenetic significance. Among these were trichodiene (**109**)[270,271] and trichodiol (**110**).[271] The relationship of **109** and **110** to the more elaborate derivatives is clear, for trichodiol (**110**) needs only to cyclize at C-11 to afford the trichothecin nucleus **111**. These precursor relationships have been substantiated by subsequent work.

Tritium-labeled trichodiene (**109**) was fed to *T. roseum*,[272] and a 3.2% incorporation was observed into trichothecolone (**104**) after base hydrolysis. Also labeled in this study were 12,13-epoxytrichothec-9-ene (**111**) and trichodiol A (**112**). Since

trichodiol A (**112**) is an artifact produced[271] by saponification of trichodiol (**110**), this may be regarded as incorporation (2%) of trichodiene (**109**) into trichodiol (**110**).



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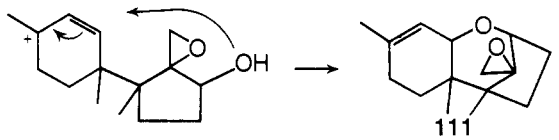
110



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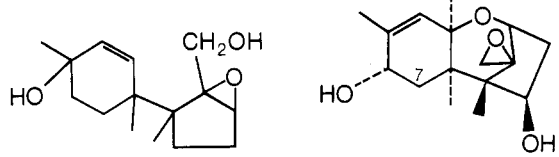


112

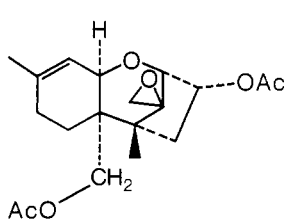
113

The incorporation of trichodiene (**109**) into **111** prompted a search for this compound in the culture broth. Although only a small quantity of material was obtained, chemical interrelation with trichodiol (**110**) confirmed the structure of the new metabolite to be **111.**

Thus there remain only two hydroxylations which occur to afford the fully oxygenated trichothecane nucleus, one at C-8 and the other at C-4, the latter having been demonstrated to be stereospecific.[261,263] The product of these two hydroxylations, trichothecadiol (**113**), was isolated from *T. roseum.*[262] Isolation of a biogenetically reasonable metabolite does not prove intermediacy, however, and the results of feeding experiments in this area should be interesting. It should be remembered that Hanson[261,263] has demonstrated that tritium migrates from C-8 to C-7 in trichothecin (**92**) and has postulated an epoxide intermediate.

However, some of these results are controvertible. When [2*R*-^{3}H,2-^{14}C]- and [2*S*-^{3}H,2-^{14}C]mevalonates (**6**) were used as precursors of trichothecin (**92**), the results[273] were not as expected from the data based on the work with [2-$^3H_2$]mevalonate. From the 2*R*-labeled mevalonate (**6**), only one tritium (at C-14) was retained; from the 2*S*-labeled mevalonate (**6**), two tritium atoms were retained, and one of these was located at C-4α (the other at C-14?). This is in contrast to Hanson's work[261,263] which suggested that a tritium migration from C-8 to C-7 had taken place. The need to involve NIH shift in the rearrangement of a 7,8-epoxide to a 7-ketone is therefore questioned. The stereospecificity of hydroxylation at C-4 with removal of a pro-2*R* hydrogen from **6** is, however, confirmed.

Calonectrin (**114**), which has no oxygenation at either C-4 or C-8, was also investigated with the stereospecifically labeled [2-^{3}H]mevalonates (**6**). Both pro-2*R* and pro-2*S*-^{3}H mevalonates (**6**) showed incorporation of three tritium labels, and one of these was established to be at C-8.[278]



114

However, calonectrin (**114**) is oxygenated at C-3, a position which should be labeled by the 5-carbon of **6**. When **114** was isolated after incorporation with [5*R*-^{3}H]mevalonate (**6**), two tritium atoms were retained.[273] In the formation of trichothecin, three tritiums were retained, and one of these was at C-7.[273]

Several interpretations of these results are possible, but the most straightforward can be presented as follows. In the biosynthesis of the trichothecane skeleton, the 5-carbon of mevalonate (**6**) should label positions 3, 7, and 11. Carbon 7 which in calonectrin (**114**) does not have exchangeable hydrogens should retain tritium. Since the tritium at C-7 in trichothecolone (**104**) was exchanged, C-7 contains a label from pro-5*R*-H **6** in both **92** and **114.** The stereochemistry of C-11 is the same in both **92** and **114,** and possibly accounts for the location of a second label. That trichothecolone (**104**) retains the third tritium atom, probably at C-3, indicates that in calonectrin (**114**) a stereospecific hydroxylation has again occurred, this time with specific loss of the pro-5*R* proton of **6**.

An interesting observation can be made on the basis of these experiments. Nozoe's isolation of trichodiol (**110**),[271] although not demonstrating intermediacy of this precise system, suggests at least that in the formation of the pyran ring attack probably takes place by a 2-hydroxy group on a 10,11-double bond. Since the pro-5*R* proton is apparently retained at C-11, the formation of the 10,11-double bond must proceed stereospecifically with loss of the 5*S* proton. This may occur by formation of an enzyme bound intermediate at C-9α, which then undergoes stereospecific attack by the hydroxyl group at C-11 in trichodiol (**110**) with displacement of the enzyme. If this were the case, calonectrin (**114**) and trichothecin (**92**) should retain only two tritium labels after incorporation of [5*S*-^{3}H]mevalonate (**6**). This experiment has not yet been reported.

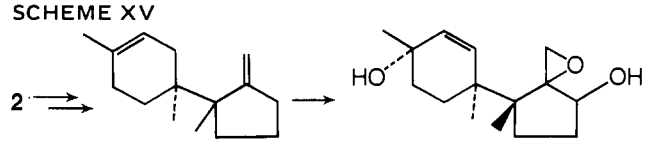
In order to establish conclusively the location of the label from the 2-carbon of mevalonate, [2-^{13}C]mevalonate (**6**) was used as a precursor of helicobasidin (**96**) in *H. mompa.*[274] Prototropic tautomerization is expected to distribute the label equally between C-8 and C-10, and this was observed. Carbon atoms 4 and 12 were also enriched equally, thus confirming the specificity but giving no information as to the mode of cyclization of farnesol.

Additional experiments with [1-^{13}C]- and [2-^{13}C]acetates gave the expected specific enrichments of carbons 1, 3, 5, 7, 9, and 11 from [1-^{13}C]acetate and of carbons 2, 4, 6, 8, 10, 12, 13, 14, and 15 from [2-^{13}C]acetate.[274]

Similar work on the biosynthesis of the trichothecane skeleton gave different results.[275] Initially the optimum incorporation time was determined using [2-^{14}C]mevalonate (**6**). [2-^{13}C]Mevalonate (**6**) (containing a small quantity of [2-^{14}C]mevalonate (**6**) as tracer) was then used, and after 5 days trichothecin (**92**) was isolated. The hydrolysis product trichothecolone (**104**) was examined by ^{13}C NMR. Only three carbons, C-4, C-8, and C-14, showed any enrichment, and all of these were enriched to the extent of 0.35%. That C-8 rather than C-10 is specifically enriched by C-2 of mevalonate (**6**) confirms the mode of cyclization of farnesyl pyrophosphate (**2**) determined previously.[259-261,271]

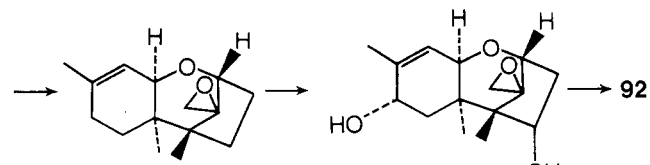
When *trans,trans*-farnesyl pyrophosphate (**2**) was used as

SCHEME XV



109

110



111

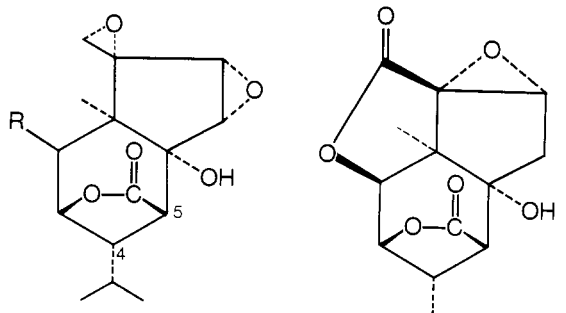
113

a precursor of trichodiene (**109**), one label from C-1 of **2** was specifically lost, supporting the possible involvement of *cis,trans*-farnesol (**20**).

The probable sequence of the late stages in the biosynthesis of **92** is outlined in Scheme XV. Some of the factors and requirements for trichothecin (**92**) biosynthesis in *T. roseum*[276-280] have recently been established.

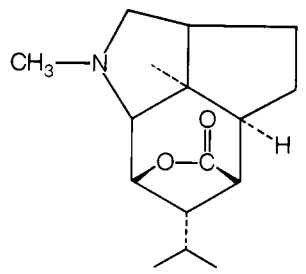
C. Picrotoxinin (117) and Dendrobine (118)

Four closely related sesquiterpenes from widely differing origins are part of a structurally interesting group which has been studied biosynthetically in a number of laboratories. The compounds involved are coriamyrtin (**115**), tutin (**116**), picrotoxinin (**117**), and the alkaloid dendrobine (**118**). Picrotoxinin (**117**) is



115, R = H
116, R = OH

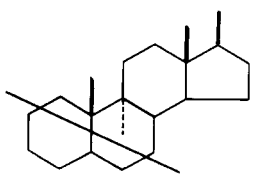
117



118

part of the complex picrotoxin, found in several species of the Menispermaceae,[281] and has the structure indicated.[282,283] Coriamyrtin, which occurs in a number of *Coriaria* species,[281] was shown to have the structure **115**.[284] Tutin has the structure[285,286] and absolute configuration given in **116**[287] and has been chemically correlated with coriamyrtin (**115**).[288] It cooccurs with coriamyrtin (**115**) in *Coriaria japonica*[281] and has also been isolated from *Hyenancha globosa* of the Euphorbiaceae.[289] Dendrobine, from the Chinese drug "Chiu Shi Hu" (*Dendrobium nobile*),[290] was shown to have the structure and absolute configuration **118**.[292]

Biogenetically, it was suggested by Conroy[292,293] that picrotoxinin was derived by the oxidative partial removal of the A and B rings of a 17-methylandrostane derivative (**119**). Cross,

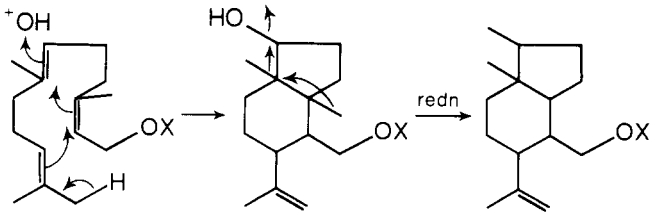


119

however, considered[294] that these compounds could be derived from farnesol (**1**) by cyclization and two 1,2-methyl migrations (Scheme XVI).

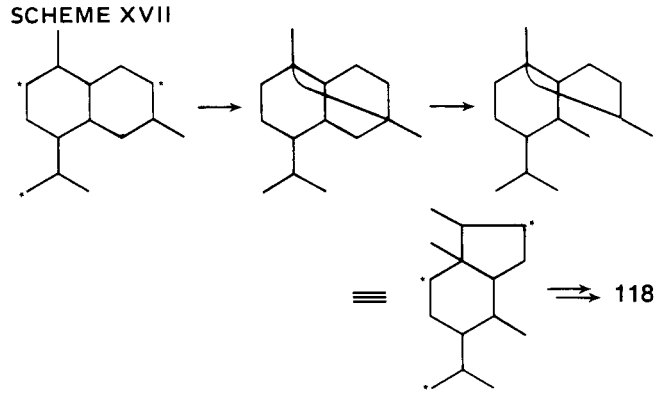
It was not until 1966 that the first biosynthetic results appeared, when Yamazaki and co-workers[295] reported preliminary experiments on the biosynthesis of dendrobine (**118**) in *Dendrobium nobile*. A total incorporation from [2-^{14}C]mevalonate (**6**) of 0.012% was observed, and degradation indicated that this

SCHEME XVI

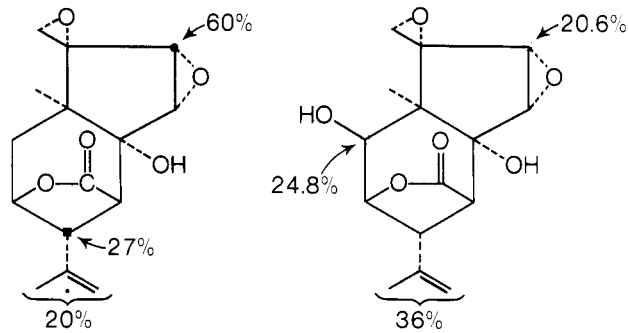


incorporation was specific. On the basis of their results, these workers suggested that dendrobine (**118**) was derived from a cadalane intermediate in which cyclization and ring cleavage subsequently occurred to give the picrotoxinin skeleton which was then aminated (Scheme XVII).

SCHEME XVII



Independent work by the groups of Arigoni[296] and Jommi[297] afforded considerable information on the biosynthesis of coriamyrtin (**115**) and tutin (**116**). [2-^{14}C]- and [4-^{14}C]mevalonates (**6**) gave only low incorporation into **115** and **116** in *C. japonica*,[296] but elegant degradation succeeded in establishing the labeling in **115** as shown. Double-labeling studies of tutin (**116**) in *C. japonica*,[297] with subsequent careful degradation studies, gave a specific incorporation (for ^{3}H) as shown in **120.** Two



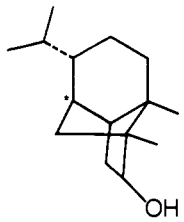
115
• from [2-^{14}C]mevalonate
▪ from [4-^{14}C]mevalonate

120
from [2-^{3}H$_2$]mevalonate

important points emerge from these results: (i) incorporation of mevalonate (**6**) into DMAPP (**13**) is lower than predicted, and (ii) labeling of C-9 and 10 in the isopentenyl unit was essentially equal. The latter observation is rare in terpenoid derivatives other than the iridoids.[134]

With these results in hand, copaborneol (**121**), the major constituent of *Pinus sylvestris* oil,[298,299] was implicated in the biosynthesis of tutin (**116**) and related compounds by both Arigoni and Jommi, and when copaborneol (**121**), specifically tritiated as shown, was used as a precursor of tutin (**116**), 90% of the activity was located at C-5 in **116**.[300]

The intermediate nature of copaborneol (**121**) in the biosynthetic scheme confirms the earlier work on the cyclization of farnesol (**1**) and excludes the 1,2-methyl shift hypothesis com-



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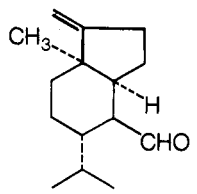
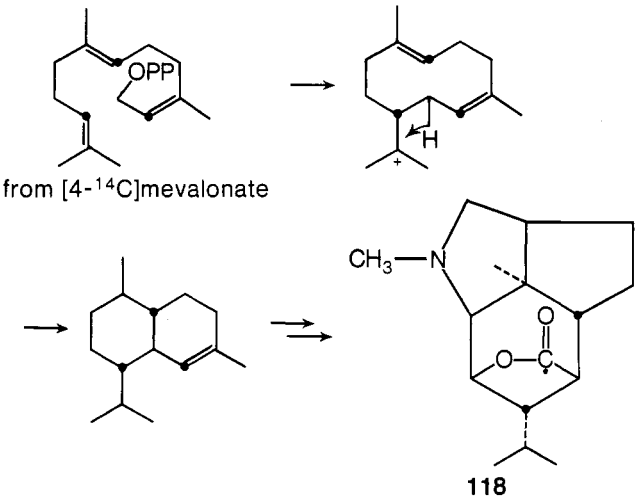


122

pletely. One may speculate that as with the ring cleavage of the iridoids to the secoiridoids,[134] hydroxylation of the methyl group is the next step followed by fragmentation to give an olefinic aldehyde such as **122,** which is subsequently oxidized. The importance either of **122** or of the ring cleavage reaction in the formation of tutin (**116**) remains to be demonstrated.

Some details are known of the stereospecificity of the formation of the monocyclic and bicyclic precursors of both tutin (**116**) and dendrobine (**118**); this has involved clarification of the steps between *cis,trans*-farnesol (**20**) and copaborneol (**121**).

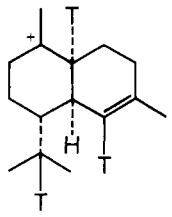
In order to distinguish between the available cyclization schemes, [4-^{14}C]mevalonate (**6**) was used as a precursor of dendrobine (**118**).[301] Degradative work proved to be quite difficult, the aim being to specifically obtain the carbonyl carbon of dendrobine. Eventually this carbon atom, isolated as benzophenone, was shown to contain one-third of the total activity originally present in **118.** *cis,trans*-Farnesol (**20**) is therefore a key intermediate in the formation of dendrobine (**118**).



118

The initial cyclization of **20** produces a compound with a cation at C-11 which subsequently rearranges to a cation at C-1 of a muurolane derivative.[4] The use of [4*R*-^{3}H]mevalonate (**6**) serves to distinguish some of the possible mechanisms of hydrogen migration involved in this transformation. A double 1,2-hydride shift and cyclization would give a muurolane derivative labeled as in **123.** Alternatively, a 1,3-hydride shift or the intermediate formation of a cyclopropane would leave the tritium originally at C-10 in farnesol (**1**) intact, giving the muurolane derivative labeled as in **124.** The first mechanism would afford a tutin (**116**) molecule devoid of activity, since C-8 would be labeled and subsequently oxidized. This mechanism was eliminated when tutin (**116**) was found to specifically retain a single tritium at C-4.[302]

In order to investigate the possibility of a 1,3-hydride shift, C-1 of farnesol (**1**) must be labeled, and this carbon atom is derived from C-5 of **6.** If the 1,3-hydride shift hypothesis were correct, labeling of the muurolane derivative from [5-^{3}H$_2$]mevalonate (**6**) should be as in **125.** Dendrobine would therefore be labeled as shown in **126;** i.e., five tritium atoms would be retained including one specifically at C-8. Indeed, it was found that only one-sixth of the tritium was lost in **118.**[303] Degradation indicated



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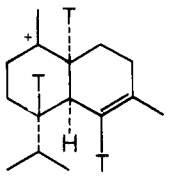

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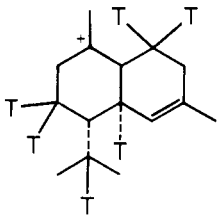

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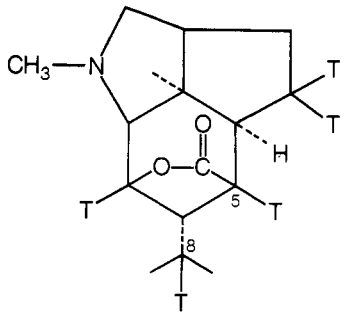


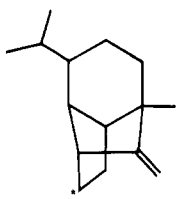
126

specific tritium labeling at C-8 and at C-5[303] in accordance with the 1,3-hydride shift hypothesis. The remaining activity was specifically located at C-3 and C-11.[304] This result also eliminated the possible intermediacy of a cyclopropane derivative.[305,306]

That both hydrogens from C-1 of *trans,trans*-farnesol (**1**) are retained in dendrobine (**118**) also indicates a nonoxidative isomerization of **1** to *cis,trans*-farnesol (**20**). The significance of this result will be discussed subsequently. It is important to note at this point that Arigoni has recently demonstrated that the pro-5*R* proton of **6** specifically migrates from the carbon derived from C-1 of farnesol (**1**) to C-8 of dendrobine (**118**).

D. Sativene (62)

In addition to the previously discussed work on avocettin (**63**) and dendrobine (**118**) biosynthesis, Arigoni and co-workers have also examined the corresponding stages in the formation of a number of other ring systems. One of these is the compound sativene (**62**), which, like avocettin (**63**), may be derived from



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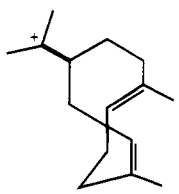
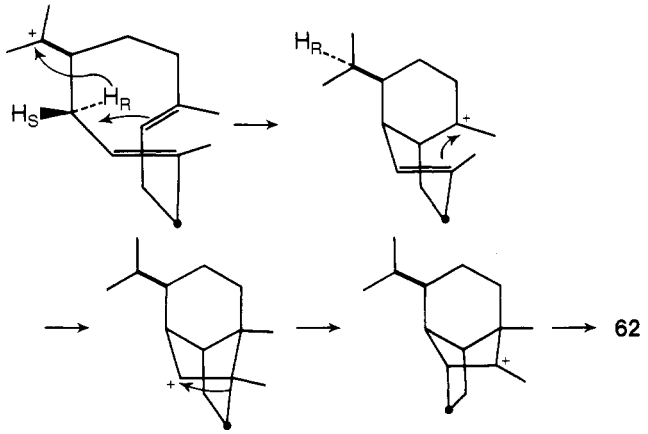


65

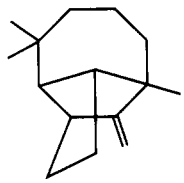
SCHEME XVIII



the cation **65**. Having assured the sesquiterpene nature of sativene (**62**), degradation and examination of a number of closely related metabolites established that all of the C-5 protons of mevalonate (**6**) are retained in sativene (**62**). Unlike the situation with avocettin (**63**), but analogous to the data for dendrobine (**118**), it is the pro-5*R* proton which migrates to the isopropyl side chain.[137] Two further stereochemical points were also established. The isopropyl side chain retains the biogenetic identity of the methyl group (compare tutin (**116**) biosynthesis), and the starred carbon of **62** is also specifically derived from C-2 of **6**.[137] On the basis of these results Arigoni suggested Scheme XVIII for the biosynthesis of sativene (**62**).

E. Longifolene (127)

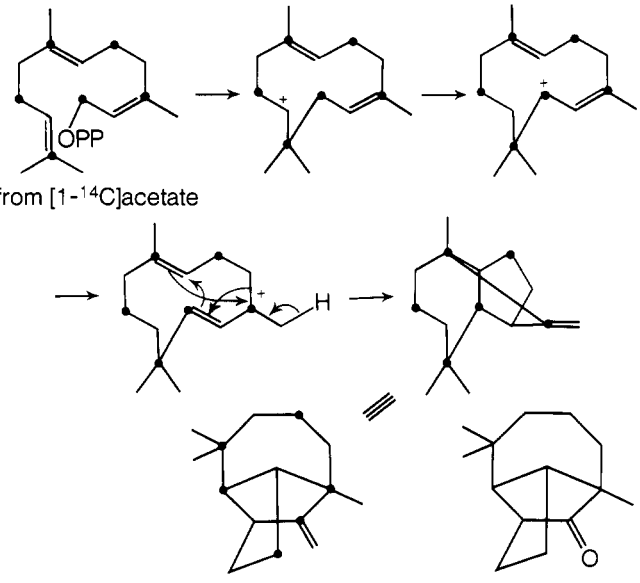
Longifolene (**127**), a novel tricyclic sesquiterpene occurring in a number of *Pinus* species,[307] was one of the first sesquiterpenes to be studied biosynthetically. Until recently though, only one result[308,309] had been reported with this compound, and this same 13 years ago.



127

Ourisson[310] and Hendrickson[3] suggested schemes from *cis,trans*-farnesol (**20**) involving a 1,2-shift of the 3,4 bond to 2,4, possibly with simultaneous formation of a 3,7 bond as shown in Scheme XIX. On this basis [1-^{14}C]acetate should label longifolene as shown in **128.**

SCHEME XIX

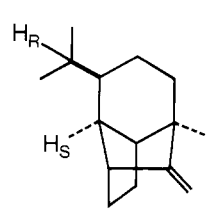


128 **129**

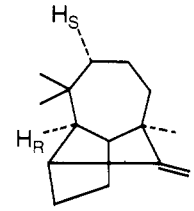
Degradation of longifolene (**127**) to the ketone **129** after feeding [1-^{14}C]acetate, and comparison of activities, indicated that none of the original activity had been lost.[308,309] Specific incorporation of acetate was deduced.

(−)-Sativene (**130**) and (−)-longifolene (**131**) co-occur in *Helminthosporium sativum* and *H. victoriae*,[137] and it is not difficult to imagine that the latter stages in the biosynthesis of these two metabolites would be extremely similar, the structural differences being due to a difference in the initial cyclization. In the case of longifolene (**131**), an 11-membered ring is formed, and for sativene (**130**) a 10-membered ring.

As with sativene (**130**), there are two possible routes for the



130, (−)-sativene

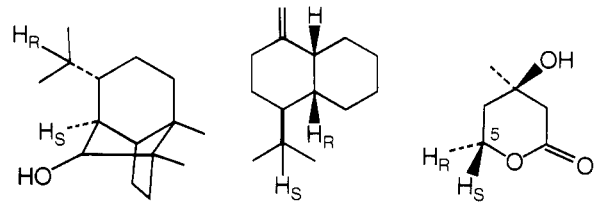


131, (−)-longifolene

formation of (−)-longifolene (**131**) depending on the enantiomeric nature of the initially formed 11-membered ring compound. Establishment of the specific derivation of the two carbon bridge of (+)-longifolene (**132**) in *Pinus ponderosa*[137] indicates that formation is direct and does not involve a process which would equilibrate the label in the two-carbon bridges. The stereospecific nature of a 1,3-hydrogen shift to C-3 of (+)-longifolene (**132**) was established.[137] The migrating hydrogen was shown to be that derived from the pro-5*R* position in mevalonate (**6**), and to be located in an exo orientation.

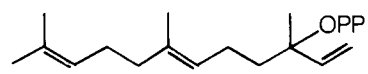
In the antipodal compound (−)-longifolene (**131**), therefore, we can presume (though it is not established) that the migrating hydrogen is derived from the pro-5*S* position in mevalonate (**6**).

The data for the four compounds investigated by Arigoni and co-workers[137] are summarized below and have led to a new theory concerning the formation and utilization of germacrene derivatives. The importance (and beauty) of these results cannot be overestimated. They do, however, require considerable explanation, some of it speculative. The facts to be explained may be delineated as follows: (i) although the absolute stereochemistries of (−)-sativene (**130**) and (−)-longifolene (**131**) are the same, different hydrogen atoms from C-5 of **6** migrate during ring formation; (ii) copaborneol (**121**) and (−)-sativene contain isopropyl groups of opposite absolute stereochemistry yet the migrating hydrogen is in each case the same; and (iii) the cadalene derivative (**64**), however, even though it contains an isopropyl group of the same antipodal nature as that in (−)-sativene (**130**), is produced by migration of a different hydrogen atom. A common germacrene-type precursor is therefore eliminated in the formation of (−)-sativene (**130**) and the cadalene derivative (**64**).



121 **64** **6**

A detailed explanation of these results is beyond the scope of this review. Central, however, is a rationalization of the formation of *cis,trans*-farnesol (**20**) from *trans,trans*-farnesol (**1**) *not* involving an oxidation–reduction reaction sequence, but rather nerolidyl pyrophosphate (**133**), where an inversion of the C-1 protons of farnesol (**1**) can occur. Previous work had shown that this can be a nonenzymic process.[311]

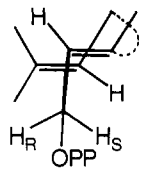


133

Three parameters were suggested by Arigoni as being important in rationalizing the observed stereochemical preferences for hydrogen migration: (i) the face of attack on the isopropylidene double bond (*re* or *si*); (ii) the conformation of the cyclizing chain to the olefinic hydrogen of the isopropylidene group (*syn* or *anti*); and (iii) the intermediacy of *cis*- or *trans*-farnesyl pyrophosphates.

The development of this theory precludes an unequivocal

description of the parameters involved in dendrobine (**118**) and avocettin (**63**) biosynthesis, but does permit the conclusion that it is *cis,trans*-farnesylpyrophosphate (**105**) which is involved in the biosynthesis of (−)-sativene (**130**) and (−)-longifolene (**131**). The initial cyclization then takes place on the *re* face of the isopropylidene group from an anti conformation as shown in **134.**



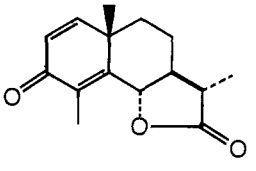
134 *re, anti, cis*

These results demonstrate that although we may consider apparently similar molecules to be biogenetically derived by common precursors or common routes, Nature almost invariably deludes the organic chemist into a false sense of security, i.e., that we have knowledge of the elaborate processes involved by deductive reasoning without doing the appropriate experimental work.

F. Santonin (135)

Santonin (**135**), one of the classic sesquiterpene lactone derivatives, has been studied only cursorily with respect to its biosynthesis, Barton[312] having investigated the late stages.

Of the advanced compounds used as precursors, only two were incorporated, **136** and **137.** Compounds such as **138** were not effectively utilized, suggesting that lactone formation precedes oxidation of ring A. However, [2-^{14}C]mevalonate (**6**) and [1-^{3}H]-*trans,trans*-farnesyl pyrophosphate (**2**) were not incorporated, so that there are clearly some problems in any interpretation of these results.



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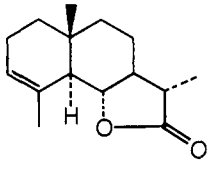

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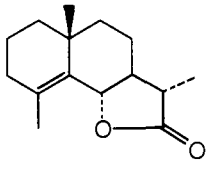

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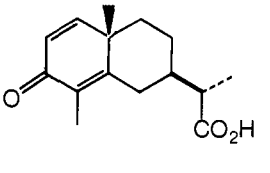
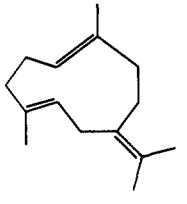


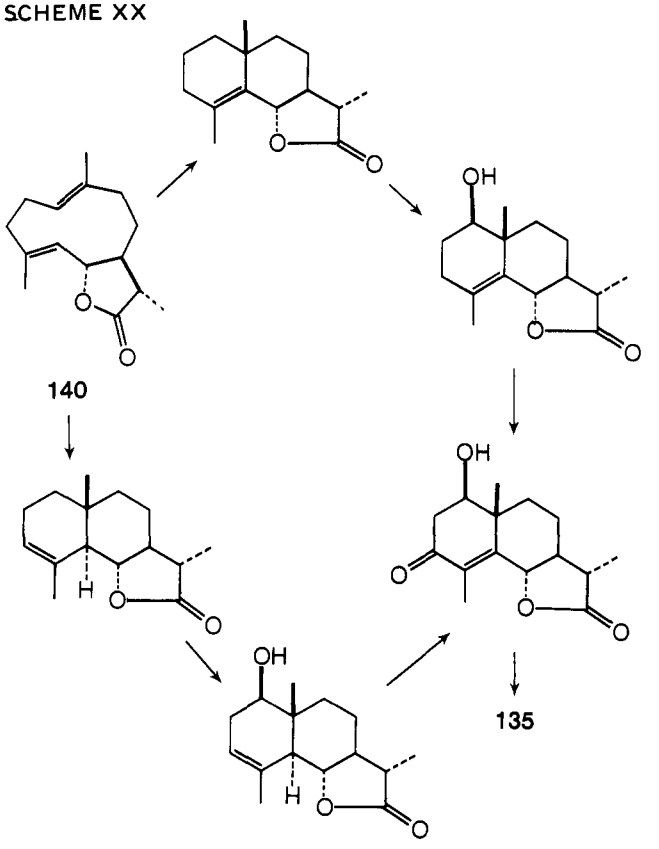
138

Barton also investigated the stages immediately after the cyclization of farnesol, but with no success.[312] For example, germacrene B (**139**) was not incorporated. Although incorporation data on a number of eudesmenolide derivatives indicated a possible pathway involving 11,12-dihydrocostunolide (**140**) and 11,12-dihydrosantamarin (**141**) as key intermediates (see Scheme XX), the low yields and poor incorporation of potentially excellent precursors make these results difficult to interpret.



139

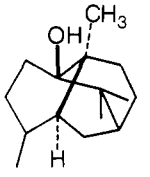
SCHEME XX



140 **141**

G. Sesquiterpenes of *Pogostemon cablin*

Although no extensive data are available, a preliminary study of the formation of some of the sesquiterpenes of *Pogostemon cablin* oil has been carried out.[313] Four components of the oil were studied: patchouli alcohol (**142**), α-bulnesene (**143**), α-guaiene (**144**), and a mixture of α-patchoulene (**145**) and caryophyllene (**49**). Surprisingly, neither [2-^{14}C]acetate nor [2-^{14}C]mevalonate (**6**) was incorporated, whereas [U-^{14}C]sucrose and glucose were moderately well incorporated into this fraction. Incorporation was rapid as was subsequent metabolism of the labeled materials. Specific terpenoid precursors were apparently unable to reach the site of sesquiterpene synthesis in the leaf disks.



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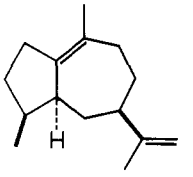

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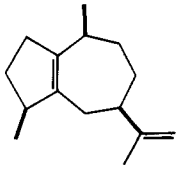

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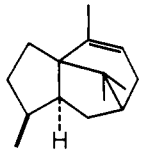

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VI. Biosynthesis of Tetracyclic Sesquiterpenes

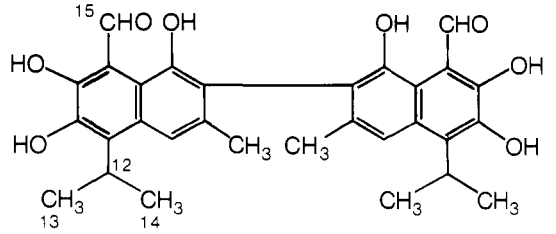
A. Gossypol (146)

The yellow, toxic, phenolic compound gossypol occurs in the resin glands of the seeds,[314] tap-root bark,[314] and roots[315] of the cotton plant *Gossypium hirsutum.* The structure of gossypol

(**146**) was first determined by Adams,[316] but its biosynthesis remained obscure until 1962. Initial work[315] demonstrated that the excised root tissue produced moderate amounts (1.0%) of gossypol (**146**) under aseptic conditions.

Feeding experiments with [1-^{14}C]- and [2-^{14}C]acetates,[317] and subsequent degradation of the labeled gossypol (**146**), demonstrated specific labeling at carbons 12 (from [2-^{14}C]-acetate) and 13(14) and 15 (from [1-^{14}C]acetate). This is to be expected on the basis of an isoprenoid rather than an acetate pathway. Confirmation of the isoprenoid pathway came from the incorporation (21.9%) of [2-^{14}C]mevalonate (**6**)[317] into gossypol (**146**).

Subsequent work involved the isolation of an enzyme system capable of synthesizing **146** and degradation of the labeled **146.** A key compound in the degradation scheme was apogossypol hexamethyl ether (**147**) in which both C-15 formyl groups have been removed. This compound, **147,** exhibited the same ra-



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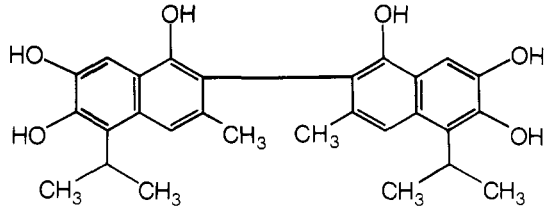

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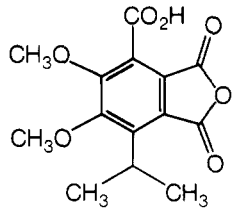
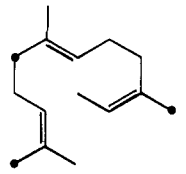


148



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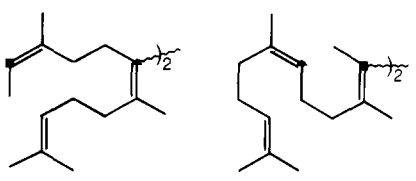
dioactivity as gossypol (**146**), thereby confirming the specific incorporation of [2-^{14}C]-**6**. Similarly, labeled gossic acid (**148**) retained all the activity of the starting material, thus eliminating a very common mode of cyclization involving *cis,trans*-farnesol (**20**) as shown in **149,** which would have led to a loss of one-third of the label in gossic acid (**148**). The intact incorporation of a farnesyl unit was demonstrated by this experiment.

The two remaining possible modes of cyclization of **150** and **151** were investigated using geranyl (**57**), neryl (**21**), and farnesyl (**1**) derivatives. The theory behind this simple but elegant series of experiments is shown in **150** and **151.**

If the mode of cyclization were as in **150,** [2-^{14}C]geraniol (**57**)



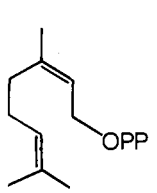
150 **151**

▲, label from [2-^{14}C]geraniol (**57**) or nerol (**21**)
■, label from [2-^{14}C]farnesol

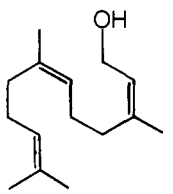
should lead to unlabeled gossic acid (**148**), and [2-^{14}C]farnesol (**1**) should give labeled gossic acid (**148**). If cyclization (**151**) were correct, the exact reverse situation should be observed.

In practice, gossic acid (**148**) was labeled from [2-^{14}C]geraniol (**57**) but not from [2-^{14}C]farnesol (**1**), thereby supporting the mode of cyclization shown in **151.**

In addition,[318] all four isomers of farnesol (as the pyrophosphates) were used as precursors. Only the two 2-cis isomers were incorporated and the cis,cis isomer was by far the best precursor. Neryl pyrophosphate (**152**) was a much better precursor than geranyl pyrophosphate (**10**). These results are also in agreement with the mode of cyclization (**151**), which requires a *cis,cis*-farnesol (**153**).



152

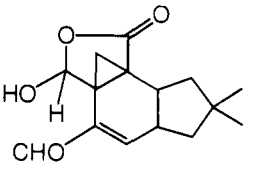


153

Various aspects of the physiological role and dynamics of gossypol (**146**) formation have been studied.[319,320]

B. Marasmic Acid (89)

The structure of the antibacterial compound marasmic acid from the mold *Marasmus conigenus* was deduced by De Mayo and co-workers to be **89.**[321] These workers also investigated the biogenesis of this extremely novel terpenoid derivative. [2-^{14}C]Mevalonate (**6**) was incorporated into marasmic acid (**89**) to the extent of 0.3% and Kuhn–Roth degradation indicated that marasmic acid (**89**) had one-third of the label at the geminal dimethyl group.



89

Although no details of the biosynthetic scheme have been elucidated, De Mayo has indicated that one can write a biogenetic scheme for the illudin group of compounds and marasmic acid involving a common precursor as shown in Scheme XXI.[321]

SCHEME XXI

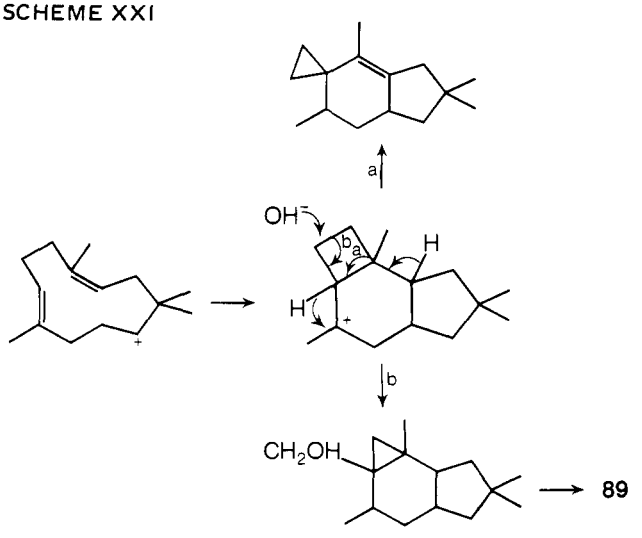


TABLE III. Incorporation Data on the Biosynthesis of Sesquiterpenes

Compound Precursor	Organism	Incorporation	Degradative result	Ref
Abscisic Acid				
[2-^{14}C]Mevalonate	*Fragaria* cv. Talisman	NG		164
	Lycopersicon esculentum	0.6		164
	Musa sapientum	NG		164
	Persea gratissima	0.5, 0.4		164, 190
[2-^{3}H]Mevalonate	*Triticum vulgare*	0.0013	Nonwilted	166
		0.0012	Wilted, 5 h	166
		0.05	Wilted, 24 h	166
[2-$^{3}H_2$]Mevalonate	*P. gratissima*	0.0022	Intact chloroplast, 2 h	173
		0.005	Lysed chloroplast, 2 h	173
		0.0027	Intact chloroplast, 17 h	173
		0.014	Lysed chloroplast, 17 h	173
		0.022	Intact chloroplast, 17 h ABA added	173
[2*R*-^{3}H,2-^{14}C]Mevalonate	*P. gratissima*	0.03	Slight ^{3}H lost	169
[2*R*-^{3}H,2-^{14}C]Mevalonate	*P. gratissima*	0.1	Two ^{3}H lost	169
[4*R*-^{3}H,2-^{14}C]Mevalonate	*P. gratissima*	0.076	One ^{3}H lost	165
[4*S*-^{3}H,2-^{14}C]Mevalonate	*P. gratissima*	0.06	All ^{3}H lost	165
[5*S*-^{3}H,2-^{14}C]Mevalonate	*P. gratissima*	0.36	One ^{3}H retained	169
5-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)-3-methyl[2-^{14}C]penta-2-*cis*-4-*trans*-dienoic acid	*L. esculentum*	3.6		166
	T. vulgare	1.12	None wilted	166
		3.42	Wilted, 5 h	166
		36	Wilted, 24 h	166
[2-^{14}C,1′,2′-^{18}O]Epoxide	*T. vulgare*	NG		166
5-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)-3-methyl[2-^{14}C]penta-2-*cis*,4-*trans*-dienoic acid	*L. esculentum*	0.042		166
5-(1,2-Dihydroxy-2,6,6-trimethylcyclohexyl)-3-methyl[2-^{14}C]penta-2-*cis*,4-*trans*-dienoic acid	*L. esculentum*	0.017		166
5-(1,2-Dihydroxy-2,6,6-trimethylcyclohexyl)-3-methyl[2-^{14}C]penta-2-*trans*-4-*trans*-dienoic acid	*L. esculentum*	0		166
[2-^{14}C]-2-*cis*-1′,2′-*epi*-Xanthoxin acid	*L. esculentum*	0		168
[2-^{14}C]-2-*cis*-Xanthoxin acid	*L. esculentum*	2.8		168
[^{14}C]Phytoene	*Persea gratissima*	0		163
Avocettin				
[2-^{14}C]Mevalonate	*Anthostoma avocetta*	NG	Specific	137
[4*R*-^{3}H,2-^{14}C]Mevalonate	*A. avocetta*	NG	Incorpn intact	137
[5*R*-^{3}H,2-^{14}C]Mevalonate	*A. avocetta*	NG	Incorpn intact	137
[5*S*-^{3}H,2-^{14}C]Mevalonate	*A. avocetta*	NG	Incorpn intact, one ^{3}H migrates	137
[6-^{3}H]-*trans*,*trans*-Farnesyl pyrophosphate	*A. avocetta*	0.12	Specific	137
Calonectrin				
[2*R*-^{3}H,2-^{14}C]Mevalonate	*Calonectria nivalis*	1.1	Incorpn intact	273
[2*S*-^{3}H,2-^{14}C]Mevalonate	*C. nivalis*	0.9	Incorpn intact	273
[5*R*-^{3}H]Mevalonate	*C. nivalis*	NG	Two ^{3}H retained	273
Carotol				
[1-^{14}C]Acetate	*Daucus carota*	NG	Specific	207
Carophyllene				
[2-^{14}C]Mevalonate	*Mentha piperata*	0.22		187
		0.42	Sucrose added	190
	Nepeta cataria	0.03		188
Coriamyrtin				
[2-^{14}C]Mevalonate	*Coriaria japonica*	0.004	Specific	296
		NG	Specific	296
Dendrobine				
[2-^{14}C]Mevalonate	*Dendrobium nobile*	0.012	Specific	295
		NG		301
[4-^{14}C]Mevalonate	*D. nobile*	0.96	Specific	301
[5-$^{3}H_2$,2-^{14}C]Mevalonate	*D. nobile*	1.4	One-sixth ^{3}H lost	303

TABLE III (Continued)

Compound Precursor	Organism	Incorporation	Degradative result	Ref
[5*S*-^{3}H,2-^{14}C]Mevalonate	*D. nobile*	NG	Two ^{3}H retained, one ^{3}H migrates	137
Dendrolasin				
[U-^{14}C]Glucose	*Lasius fuliginosus*	0.15	Uniform labeling	183
[1-^{14}C]Acetate	*L. fuliginosus*	0.02	Specific	183
[2-^{14}C]Acetate	*L. fuliginosus*	0.001	Specific	183
Deoxyhelicobasidin				
[4*R*-^{3}H,2-^{14}C]-Diacetoxyscirpenol	*Helicobasidium mompa*	0.7	Two ^{3}H retained	260
[2-^{14}C]Mevalonate	*Fusarium equiseti*	1.0		327
Dihydrocoriolin C				
[1-^{13}C]Acetate	*Coriolus consors*	NG	Specific	240
[2-^{13}C]Acetate	*C. consors*	NG	Specific	240
[1,2-$^{13}C_2$]Acetate	*C. consors*	NG	Six C–C couplings retained	240
[2-^{13}C]Mevalonate	*C. consors*	NG	Specific	240
trans,trans-10,11-Epoxyfarnesol				
[1-^{2}H]-*trans,trans*-10,11-Epoxyfarnesal	*Helminthosporium sativum*	31	Enzyme	136
cis,trans-10,11-Epoxyfarnesol				
[1-$^{2}H_2$]-*trans,trans*-10,11-Epoxyfarnesol	*H. sativum*	NG	Enzyme, 42% of ^{2}H	136
[1-^{2}H]-*trans,trans*-10,11-Epoxyfarnesol	*H. sativum*	17	Enzyme	136
trans,trans-Farnesal				
[1-^{3}H]Geranyl pyrophosphate + IPP	*Citrus sinensis*	NG	Enzyme	130
[2-^{14}C]Mevalonate	*Samia cynthia*	NG		144
[1,2-$^{14}C_2$]Farnesol	*Tenebrio molitor*	NG		328
cis,trans-Farnesal				
[1-^{3}H]Geranyl pyrophosphate + IPP	*C. sinensis*	NG	Enzyme	130
trans,trans-Farnesol				
[2-^{14}C]Acetate	*Hyalophora cecropia*	0.004		145
		0.07		119
[2-^{14}C] Mevalonate	*Andrographis paniculata*	NG	Enzyme	132
	Beef liver	NG		85, 106
	Gibberella fujikuroi	NG	Enzyme	111
	Iris hollandica	NG	Enzyme	113
	Nicotiana tabacum	NG		86, 87
	Phytophthora cactorum	NG		76
	Pig liver	NG	Enzyme	329
	Pinus radiata	0.4		117
	Pisum sativum	0.2		118
	Rat liver	NG	Enzyme	53, 54, 84
	Saccharomyces cereviseae	NG		45, 70
	Samia cynthia	NG		144
	Sarcophaga bullata	0.26		98
	Samia cynthia	NG		144
	Sarcophage bullata	0.26		98
[2-^{14}C] Mevalonate, 5-phospho-	Rat liver	NG		54, 56, 67
[5-^{3}H] Mevalonate	*H. cecropia*	0.53		145
[2*R*-^{3}H, 2-^{14}C] Mevalonate	Pig liver	NG	Enzyme	82
[2*S*-^{3}H, 2-^{14}C] Mevalonate	Pig liver	NG	Enzyme	82
[4*R*-^{3}H, 2-^{14}C] Mevalonate	*A. paniculata*	NG	Incorpn intact	108
	C. sinensis	0.1	Incorpn intact	109
	Hevea brasiliensis	NG	Incorpn intact	78
	P. cactorum	NG	Incorpn intact	76
	P. radiata	0.55	Enzyme, incorpn intact	109
[4*S*-^{3}H,2-^{14}C] Mevalonate	*A. paniculata*	NG	All ^{3}H lost	108
	C. sinensis	0.07	All ^{3}H lost	109
	H. brasiliensis	NG	All ^{3}H lost	78
	P. cactorum	NG	All ^{3}H lost	76
	P. radiata	1.4	All ^{3}H lost	109
[5-$^{2}H_2$,2-^{14}C] Mevalonate	Rat liver	NG	Enzyme	67
		NG	Enzyme	68, 156

TABLE III (Continued)

Compound Precursor	Organism	Incorporation	Degradative result	Ref
		NG	Enzyme, one ^2H lost	66
[5-$^3H_2$,2-^{14}C] Mevalonate	*A. paniculata*	8.5	Enzyme	108
	Pig liver	NG	Enzyme	131
[5-^3H,4-^{14}C] Mevalonate	Pig liver	NG	Enzyme	69
[1-^{14}C] Isopentenyl pyrophosphate	*Gossypium hirsutum*	1.56	Enzyme	112
	G. hirsutum	1.31	Enzyme, iodoacetamide	112
	Micrococcus lysodeikticus			114, 115
	Pig liver	NG		105
	S. cereviseae			51
[1-^{14}C] Isopentenyl pyrophosphate + DMAPP	*G. hirsutum*	NG	Enzyme	110
[1-^{14}C] Isopentenyl pyrophosphate + geranyl pyrophosphate		NG	Enzyme	110
	G. hirsutum	0.7	Enzyme	112
[1-^{14}C] Isopentenyl pyrophosphate + neryl pyrophosphate	*G. hirsutum*	0	Enzyme	112
[4-^{14}C] Isopentenyl pyrophosphate	Pig Liver	NG	Enzyme	103, 120
[4-^{14}C] Isopentenyl pyrophosphate + DMAPP	Pig Liver	NG	Enzyme	104
[1,4-^{14}C] Isopentenyl pyrophosphate + geranyl pyrophosphate	Pig liver	NG	Enzyme	122, 123
[1-^3H] Geranyl pyrophosphate	*P. radiata*		Enzyme	117
[1-^3H] Geranyl pyrophosphate + isopentenyl pyrophosphate	*C. sinensis*	NG	Enzyme	130
[1-^3H] Neryl pyrophosphate	*P. radiata*	0	Enzyme	117
[1-^3H] Neryl pyrophosphate + isopentenyl pyrophosphate	*C. sinensis*	0	Enzyme	130
[1*R*-^3H,4,8,12-$^{14}C_3$]-*trans,trans*-Farnesol	*A. paniculata*	NG	Incorpn intact	132
[1*R*-^3H,4,8,12-$^{14}C_3$]-*cis,trans*-Farnesol	*A. paniculata*	NG	All ^3H lost	132
[1*S*-^3H,4,8,12-$^{14}C_3$]-*trans,trans*-Farnesol	*A. paniculata*	NG	Incorpn intact	132
[1*S*-^3H,4,8,12-$^{14}C_3$]-*cis,trans*-Farnesol	*A. paniculata*	NG	Incorpn intact	132
[1,1,5,5,9,9-$^3H_6$,4,8,12-$^{14}C_3$]-*trans,trans*-Farnesyl pyrophosphate	*Trichotecium roseum*	2.1	Enzyme, incorpn intact	113
cis,trans-Farnesol				
[2-^{14}C] Mevalonate	*P. radiata*	0.1	Enzyme	117
[4*R*-^3H,2-^{14}C] Mevalonate	*A. paniculata*	NG	Incorpn intact	108
	C. sinensis	0.08	Enzyme, incorpn intact	109
	P. radiata	0.2	Enzyme, incorpn intact	109
[4*S*-^3H,2-^{14}C] Mevalonate	*A. paniculata*	NG	All ^3H lost	108
	C. sinensis	0	Enzyme	109
	P. radiata	0.27	Enzyme, all ^3H lost	109
[5-$^3H_2$,2-^{14}C] Mevalonate	*A. paniculata*	1.5	Enzyme, one-sixth of ^3H label lost	108
[1-^{14}C] Isopentenyl pyrophosphate + geranyl pyrophosphate	*G. hirsutum*	3.2	Enzyme	112
[1-^{14}C] Isopentenyl pyrophosphate + neryl pyrophosphate	*G. hirsutum*	4.4	Enzyme	112
[1,4-$^{14}C_2$] Isopentenyl pyrophosphate + DMAPP	Pig liver	0		122
[1,4-$^{14}C_2$] Isopentenyl pyrophosphate + geranyl pyrophosphate	Pig liver	0	Enzyme	122

TABLE III (Continued)

Compound Precursor	Organism	Incorporation	Degradative result	Ref
[1-^{3}H]Geranyl pyrophosphate	*P. radiata*			117
[1-^{3}H]Geranyl pyrophosphate + isopentenyl pyrophosphate	*C. sinensis*	NG	Enzyme	130
[1-^{3}H]Neryl pyrophosphate	*P. radiata*	0		117
[1-$^{2}H_2$]-*trans,trans*-Farnesol	*H. sativum*	NG	Enzyme, 37% of ^{2}H lost	136
[1*R*-^{3}H,4,8,12-$^{14}C_3$]-*trans,trans*-Farnesol	*A. paniculata*	NG	Incorpn intact	132
[1*R*-^{3}H,4,8,12-$^{14}C_3$]-*cis,trans*-Farnesol	*A. paniculata*	NG	Incorpn intact	132
[1*S*-^{3}H,4,8,12-$^{14}C_3$]-*trans,trans*-Farnesol	*A. paniculata*	NG	All ^{3}H lost	132
[1*S*-^{3}H,4,8,12-$^{14}C_3$]-*cis,trans*-Farnesol	*A. paniculata*	NG	Incorpn intact	132
[1-^{3}H]-*trans,trans*-Farnesyl pyrophosphate	*P. radiata*	0	Enzyme	117
[1,1,5,5,9,9-$^{3}H_6$,4,8,12-$^{14}C_3$]-*trans,trans*-Farnesyl pyrophosphate	*T. roseum*	0.4	Enzyme, one-sixth of label lost	168
cis,cis-Farnesol				
[1-^{14}C]Isopentenyl pyrophosphate + geranyl pyrophosphate	*G. hirsutum*	0.14	Enzyme	112
[1-^{14}C]Isopentenyl pyrophosphate + neryl pyrophosphate	*G. hirsutum*	5.2	Enzyme	112
trans,trans-Farnesoic acid				
[^{14}C]HCO_3	*Pseudomonas citronellolis*	NG	Enzyme	330
[2-^{14}C]Mevalonate	Rat liver	NG	Enzyme	59, 60
	T. molitor	NG		57
trans,trans-Farnesol	Rat liver	17	Enzyme	60
[1,2-$^{14}C_2$]-*trans,trans*-Farnesol	*T. molitor*	NG		328
trans,trans-Nerolidol				
[2-^{14}C]Mevalonate	*N. tabacum*	NG		87
	P. radiata	0.1	Enzyme	117
	Pisum sativum	0.06	Enzyme	118
	Rat liver	NG	Enzyme	53, 54
	S. bullata	0.06		98
[2-^{14}C]Mevalonate, 5-phospho-	Rat liver	NG	Enzyme	54, 56
[1-^{14}C]Isopentenyl pyrophosphate + geranyl pyrophosphate	*G. hirsutum*	0.8	Enzyme	112
[1-^{14}C]Isopentenyl pyrophosphate + neryl pyrophosphate	*G. hirsutum*	0	Enzyme	112
[4-^{14}C]Isopentenyl pyrophosphate	Pig liver	NG	Enzyme	120
[4-^{14}C]Isopentenyl pyrophosphate + DMAPP	Pig liver	NG	Enzyme	104
[1,4-$^{14}C_2$]Isopentenyl pyrophosphate + geranyl pyrophosphate	Pig liver	NG	Enzyme	122
[1-^{3}H]Farnesyl pyrophosphate	*P. radiata*	NG	Enzyme	311
6-Farnesyl-5,7-dihydroxy-4-methylphthalide				
[Me-^{14}C]Methionine	*Penicillium brevicompactum*	1.8		221
[7-^{14}C]-5,7-Dihydroxy-4-methylphthalide	*P. brevicompactum*	9		221

TABLE III (Continued)

Compound Precursor	Organism	Incorporation	Degradative result	Ref
Fumagillin				
[1-^{14}C]Acetate	*Aspergillus fumigatus*	0.21	Specific	177
[2-^{14}C]Acetate	*A. fumigatus*	0.2	Specific	177
[2-^{14}C]Mevalonate	*A. fumigatus*	1.6	Specific	177
Germacrene				
[2-^{14}C]Mevalonate	*Kadsura japonica*	NG		179
trans,trans-Farnesyl pyrophosphate	*K. japonica*	NG		179
Gossypol				
[1-^{14}C]Acetate	*G. hirsutum*	2.1	Specific	317
[2-^{14}C]Acetate	*G. hirsutum*	NG	Specific	317
[2-^{14}C]Mevalonate	*G. hirsutum*	22		317
		2.2		318
		1.43	Specific	318
[2-^{14}C]Geranyl pyrophosphate	*G. hirsutum*	1.45	Specific	318
[2-^{14}C]Neryl pyrophosphate	*G. hirsutum*	2.96	Specific	318
[2-^{14}C]-*trans,trans*-Farnesyl pyrophosphate	*G. hirsutum*	0.21		318
[2-^{14}C]-*cis,trans*-Farnesyl pyrophosphate	*G. hirsutum*	0.23		318
[2-^{14}C]-*trans,cis*-Farnesyl pyrophosphate	*G. hirsutum*	1.16	Specific	318
[2-^{14}C]-*cis-cis*-Farnesyl pyrophosphate	*G. hirsutum*	5.5		230
Helicobasidin				
[1-^{14}C]Acetate	*Helicobasidium mompa*	0.9	Specific	254
[2-^{14}C]Acetate	*H. mompa*	0.05	Specific	255
[1-^{13}C]Acetate	*H. mompa*	NG	Specific	274
[2-^{13}C]Acetate	*H. mompa*	NG	Specific	274
[2-^{14}C]Ethyl malonate	*H. mompa*	0.07		255
[2-^{14}C]Mevalonate	*H. mompa*	0.004	Specific	254
		0.11	Specific	255
[2-^{13}C]Mevalonate	*H. mompa*	NG	Specific	274
[2-$^{3}H_2$,2-^{14}C]Mevalonate	*H. mompa*	NG	Four ^{3}H retained	265
[4*R*-^{3}H,2-^{14}C]-Mevalonate	*H. mompa*	1.7	Two ^{3}H retained	260
		NG	Two ^{3}H retained	265, 268
[5-$^{3}H_2$,2-^{14}C]Mevalonate	*H. mompa*	NG	Two ^{3}H retained	265
[2-^{3}H,2-^{14}C]Geranyl pyrophosphate	*H. mompa*	NG	Incorpn intact	265, 268
Helminthosporal				
[2-^{14}C]Mevalonate	*H. sativum*	NG	Specific	210
Hirsutic acid				
[1-^{13}C]Acetate	*Stereum complicatum*	NG	Specific	237
[2-^{13}C]Acetate	*S. complicatum*	NG	Specific	237
Illudol M				
[2-^{14}C]Mevalonate	*Clitocybe illudens*	NG		230
[2-$^{3}H_2$,2-^{14}C]-Mevalonate	*C. illudens*	0.06, 0.03	Three ^{3}H lost	234
[4*R*-^{3}H,2-^{14}C]-Mevalonate	*C. illudens*	0.1	Two ^{3}H lost	234
Illudin S				
[2-^{14}C]Mevalonate	*C. illudens*	NG		230
Illudol				
[2-^{14}C]Mevalonate	*C. illudens*	NG		40
Ipomeamarone				
[2-^{14}C]Acetate	*Ipomoea batatas*	0.22	Specific	199
		5.45		

TABLE III (Continued)

Compound Precursor	Organism	Incorporation	Degradative result	Ref
		11.6	Specific	119
		2.52	10^{-3} M geraniol added	201
		3.71	10^{-3} M nerolidol added	119
		4.41	10^{-3} M farnesol added	119
		7.0		119
		6.2		202
		4.6	After 2 h	204
[2-^{14}C]Ethanol	*I. batatas*	5.9	After 2 h	204
[3-^{14}C]Pyruvate	*I. batatas*	1.7		202
[2,4-$^{14}C_2$]Citrate	*I. batatas*	1.0		202
[2-^{14}C]Mevalonate	*I. batatas*	0.44	Specific	199
		48	Specific	201
[2-^{14}C]Farnesol	*I. batatas*	6.8		203
Isopetasol[a]				
[2-^{14}C]Mevalonate	*Petasites hybridus*	0.01	Specific	194
Juvenile Hormone I				
Methionine	*H. cecropia*	0		146
[^{3}H]Methionine	*H. cecropia*	0.09		145
[^{14}C]Methionine	*H. cecropia*	0.014		145
	Schistocerca vaga	0	In vitro	143
Isoleucine	*H. cecropia*	0		146
Valine	*H. cecropia*	0		146
Lysine	*H. cecropia*	0		146
Glucose	*H. cecropia*	0		146
Acetate	*H. cecropia*	NG		146
[1-^{14}C]Acetate	*H. cecropia*	0		145
[2-^{14}C]Acetate	*H. cecropia*	0.001		145
	S. vaga	0	In vitro	143
Propionate	*H. cecropia*	0		146
[1-^{14}C]Propionate	*H. cecropia*	0		145
Mevalonate	*H. cecropia*	0		146
	H. cecropia	0		144
[2-^{14}C]Mevalonate	*Samia cynthia*	0		145
[5-^{3}H]Mevalonate	*H. cecropia*	0		146
4-Methyl-3-pentenol	*H. cecropia*	0	146	
4-Methyl-*cis*-3-hexenol	*H. cecropia*	0		146
Farnesol	*H. cecropia*	0		146
[2-^{14}C]Farnesol	*H. cecropia*	0		145
Bishomofarnesol	*H. cecropia*	0		146
10-Epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoic acid	*H. cecropia*	10		149
Juvenile Hormone II				
[^{14}C]Methionine	*S. vaga*	0	In vitro	143
[2-^{14}C]Acetate	*Manduca sexta*	0.11	In vitro, specific	147
[1-^{14}C]Propionate	*M. sexta*	0.05	In vitro, specific	147
[2-^{14}C]Propionate	*M. sexta*	1.23	In vitro, specific	147
[2-^{14}C]Mevalonate	*M. sexta*	0.16	In vitro, specific	147
Juvenile Hormone III				
[^{14}C]Methionine	*Gastrimargus africanus*	NG	In vitro	143
	M. sexta	71	In vitro	142
	S. vaga	NG	In vitro	143
[2-^{14}C]Acetate	*G. africanus*	NG	In vitro	146
	M. sexta	0.04	In vitro, specific	146
	S. vaga	NG	In vitro	143
[1-^{14}C]Propionate	*M. sexta*	0	In vitro	146
[2-^{14}C]Propionate	*M. sexta*	0.21	In vitro, nonspecific	146
[2-^{14}C]Mevalonate	*M. sexta*	0.51	In vitro, specific	146
(+)-Longifolene				
[1-^{14}C]Acetate	*Pinus longifolia*	0.07	Specific	308, 309
[2-^{14}C]Mevalonate	*Pinus ponderosa*	0.1	Specific	137
[5*R*-^{3}H,2-^{14}C]Mevalonate	*P. ponderosa*	NG	Incorpn intact, one ^{3}H migrated	137
[5*S*-^{3}H,2-^{14}C]Mevalonate	*P. ponderosa*	NG	Incorpn intact	137

TABLE III (Continued)

Compound Precursor	Organism	Incorporation	Degradative result	Ref
Marasmic acid				
[2-^{14}C]Mevalonate	*Marasmius conigenus*	0.3	Specific	24
trans-γ-Monocyclofarnesol				
[2-^{14}C]Mevalonate	*Helminthosporium siccans*	25–50		185
γ-Muurolene				
[2-^{14}C]Mevalonate	*Mentha piperata*	0.1		187
		0.4	Sucrose added	190
Mycophenolic acid				
[^{14}C]Methionine	*Pinicillium brevicompactum*	77	O- and nuclear methyl	215, 216
	P. brevicompactum	10	?	221
[$^{2}H_3$]Methionine	*P. brevicompactum*	NG		326
[^{14}C]Acetic acid	*P. brevicompactum*	0.4	Random	216–218
[1-^{14}C]-3-Methyl-2-butenoic acid	*P. brevicompactum*	0.49	Random	218
[2-^{14}C]Mevalonic acid	*P. brevicompactum*	1.5	Side-chain labeled	218
[1-$^{3}H_2$]Geraniol	*P. brevicompactum*	low	Nonspecific	222
[1-^{14}C]Geraniol	*P. brevicompactum*	low	Nonspecific	222
[1-$^{3}H_2$]Geranyl pyrophosphate	*P. brevicompactum*	low	Nonspecific	222
[1-^{14}C]-4,6-Dihydroxy-2-methylbenzoic acid	*P. brevicompactum*	0		221, 222
[6-^{14}C]-4,6-Dihydroxy-2-methylbenzoic acid	*P. brevicompactum*	0.3		221, 226
[1-^{14}C]-4,6-Dihydroxy-2,3-dimethylbenzoic acid	*P. brevicompactum*	83.5		221, 226
[1-^{14}C]-4,6-Dihydroxy-2,3-dimethylbenzoic acid	*P. brevicompactum*	11.5	Specific	222
[7-^{14}C]-5,7-Dihydroxyphthalide	*P. brevicompactum*	0.15		220, 226
[7-^{14}C]-5,7-Dihydroxy-4-methylphthalide	*P. brevicompactum*	36.5		220, 226
		6.2		221
[7-^{14}C]-7-Hydroxy-5-methoxy-4-methylphthalide	*P. brevicompactum*	3.8		220, 226
[7-^{14}C]-6-Geranyl-5,7-dihydroxy-4-methylphthalide	*P. brevicompactum*	33.6		220, 226
[7-^{14}C]-6-Farnesyl-5,7-dihydroxy-4-methylphthalide	*P. brevicompactum*	33.6		221, 226
[7-^{14}C]-*O*-Demethylmycophenolic acid	*P. brevicompactum*	78.3		221
Nobiline				
[2-^{14}C]Mevalonate	*Dendrobium nobile*	0.71		301
Petasin				
[2-^{14}C]Mevalonate	*Petasites hybridus*	0.01–0:027	SK and F 7997-A_3 added	193
Roridin A				
[2-$^{3}H_2$,2-^{14}C]Mevalonate	*Myrothecium verrucaria*	3.0	Four ^{3}H retained	266
[5-^{14}C]Mevalonate	*M. verrucaria*	2.2	Specific	266
[6-^{3}H,12,13-$^{14}C_2$]-Farnesyl pyrophosphate	*M. roridum*	0.22		267
Santonin				
[2-^{14}C]Mevalonate	*Artemisia maritima*	0		312
[1-^{3}H]Farnesol	*A. maritima*	0.0003		312
[1-^{3}H]Farnesyl pyrophosphate	*A. maritima*	0		312
[8-^{3}H]Germacrene	*A. maritima*	0		312
[11-^{3}H]-1,2-Dihydrosantonin	*A. maritima*	0		312
[11-^{3}H]-5a,6β,11β-Eudesm-3-en-6,13-olide	*A. maritima*	0.03		312
[11-^{3}H]-6β,11β-Eudesm-4-en-6,13-olide	*A. maritima*	0		312

TABLE III (Continued)

Compound Precursor	Organism	Incorporation	Degradative result	Ref
[2,11-^{3}H]-6-Deoxysantoninic acid	*A. maritima*	0		312
[11-^{3}H]-1,2-Dihydro-6-deoxysantoninic acid	*A. maritima*	0		312
[11,12-$^3H_2$]-11,12-Dihydrocostunolide	*A. maritima*	0.004		312
[11,12-$^3H_2$]-11,12-Dihydrosantamarin	*A. maritima*	0.006		312
[11-^{3}H]-1,2,4α,5α-Tetrahydrosantonin	*A. maritima*	0		312
[11-^{3}H]-1,2,4β,5α-Tetrahydrosantonin	*A. maritima*	0		312
Sativene				
[2-^{14}C]Mevalonate	*Helminthosporium victoriae*	NG	Specific	137
[5*R*-^{3}H,2-^{14}C]-Mevalonate	*H. victoriae*	NG	Incorpn intact, one ^{3}H	137
[5*S*-^{3}H,2-^{14}C]-Mevalonate	*H. victoriae*	NG	Incorpn intact	137
Sesquiterpene fraction of Patchouli oil				
[U-^{14}C]Glucose	*Pogostemon cablin*	0.14	Light	313
[U-^{14}C]Sucrose	*P. cablin*	0.14	Light, 45 h	313
		0.01	Light, 17 h	313
		0	Dark	313
[^{14}C]HCO_2	*P. cablin*	0.01		313
[2-^{14}C]Acetate	*P. cablin*	0	Light	313
		0	Dark	313
[2-^{14}C]Mevalonate	*P. cablin*	0		313
[U-^{14}C]Leucine	*P. cablin*			
Siccanochromene				
[Ar-^{3}H]Presiccanochromene	*Helminthosporium siccans*	25		325
[Ar-^{3}H]Epoxypresiccanochromene	*H. siccans*	70		325
Siccanochromenic acid fraction				
[2-^{14}C]Mevalonate	*H. siccans*	4		324
Thiobinupharidine				
[2-^{14}C]Mevalonate	*Nuphar luteum*	0.007	Specific	213
[3,4-$^{14}C_2$]Mevalonate	*N. luteum*	0.084	Specific	213
[1,5-$^{14}C_2$]Cadaverine	*N. luteum*	0.061	Random	213
Trichodermol				
[2-$^3H_2$,2-^{14}C]-Mevalonate	*Trichoderma sporulosum*		Five ^{3}H retained	263
[2*R*-^{3}H,2-^{14}C]-Mevalonate	*T. sporulosum*	0.05	Two ^{3}H retained	263, 273
[4*R*-^{3}H,2-^{14}C]-Mevalonate	*Trichoderma sp.*	NG	Two ^{3}H retained	259
		6.9	Two ^{3}H retained	261
[2-^{3}H,2-^{14}C]-Mevalonate	*T. polysporum*	NG	Incorpn intact	268
	T. sporulosum	NG	Incorpn intact	261, 268
[1-$^3H_2$,2-^{14}C]-Mevalonate	*T. sporulosum*	NG	One ^{3}H lost	261
Trichodiene				
[1,1,5,5,9,9,-$^3H_6$,4,8,12-$^{14}C_3$]-*trans,trans*-Farnesyl pyrophosphate	*Trichothecium roseum*	5.37	One-sixth tritium lost	131
Trichodiol				
[4*R*-^{3}H,2-^{14}C]-Mevalonate	*Trichothecium roseum*	0.04		262
[9-^{3}H]Trichodiene	*T. roseum*	2.0		272
Trichothecin				
[1-^{14}C]Acetate	*Trichothecium roseum*	0.2	95% act. in crotonate	250, 251
[2-^{14}C]Mevalonate	*T. roseum*	0.5	Specific	250, 251
[2-$^3H_2$,2-^{14}C]-Mevalonate	*T. roseum*	NG, 0.24	Four ^{3}H retained	261, 263
[2*R*-^{3}H,2-^{14}C]-Mevalonate	*T. roseum*	0.55	One ^{3}H retained	273

TABLE III (Continued)

Compound Precursor	Organism	Incorporation	Degradative result	Ref
[2*R*-^{3}H,2-^{14}C]-Mevalonate	*T. roseum*	0.55	Two ^{3}H retained	273
[4*R*-^{3}H,2-^{14}C]-Mevalonate	*T. roseum*	NG	Two ^{3}H retained	259
[5-$^3H_2$,2-^{14}C]-Mevalonate	*T. roseum*	0.8	Four ^{3}H retained	261
[5*R*-^{3}H,2-^{14}C]-Mevalonate	*T. roseum*	NG	Three ^{3}H retained	273
[2-^{3}H,2-^{14}C]Geranyl pyrophosphate	*T. roseum*	NG	Incorpn intact	261, 268
[1-^{14}C]Farnesyl pyrophosphate	*T. roseum*	1.51	Specific	257, 258
[1-$^3H_2$,2-^{14}C]Farnesyl pyrophosphate	*T. roseum*	NG	One ^{3}H retained	259
[2-^{3}H,2-^{14}C]Farnesyl pyrophosphate	*T. roseum*	NG	Incorpn intact	259
[9-^{14}C]-α-Bisabolol	*T. roseum*	0.008	Random	269
[9-^{14}C] β- and γ-bisabolene	*T. roseum*	0.003	Random	269
[4α,8α,8β,14α,14β-$^3H_5$]Trichodermol	*T. roseum*	NG	Three ^{3}H retained	263
Trichothecolone				
[2-$^3H_2$,2-^{14}C]-Mevalonate	*T. roseum*	0.9	Four ^{3}H retained	261
[4*R*-^{3}H,2-^{14}C]-Mevalonate	*T. roseum*	1.6	Two ^{3}H retained	261
		1.2	Two ^{3}H retained	262
[1-$^3H_2$,2-^{14}C]-Farnesyl pyrophosphate	*T. roseum*	NG	One ^{3}H retained	261
[2-^{3}H,2-^{14}C]-Farnesyl pyrophosphate	*T. roseum*	NG	Incorpn intact	261
12,13-Epoxytrichothec-9-ene				
[9-^{3}H]Trichodiene	*T. roseum*	0.6		272
Tutin				
[2-^{14}C]Mevalonate	*Coriaria japonica*	0.0004	Specific	296
		0.03	Specific	297
[4*R*-^{3}H,2-^{14}C]-Mevalonate	*C. japonica*	0.015	Two ^{3}H lost	302
[-^{3}H] Copaborneol	*C. japonica*	0.001	Specific, cut twig	300
		0.1	Specific, plant	300
Unnamed from *H. mompa*				
[4*R*-^{3}H,2-^{14}C]-Mevalonate	*H. mompa*	0.6	Incorpn tract	260
Verrucarol				
[1-^{14}C]Acetate	*M. roridum*	NG	Specific	253
[2-^{14}C]Acetate	*M. roridum*	NG	Random	253
[2-^{14}C]Mevalonate	*M. roridum*	1.0		244
		NG	Specific	253
[2-$^3H_2$,2-^{14}C]-Mevalonate	*M. verrucaria*	0.45	Approx, five ^{3}H retained	266
[2*R*-^{3}H,2-^{14}C]-Mevalonate	*M. verrucaria*	0.02	Two ^{3}H retained	266
[2*S*-^{3}H,2-^{14}C]-Mevalonate	*M. verrucaria*	0.33	Three ^{3}H retained	266
[5-^{14}C]Mevalonate	*M. verrucaria*	1.5	Specific	266
[6-^{3}H,12,13-$^{14}C_2$]-Farnesyl pyrophosphate	*M. roridum*	0.22	Incorpn intact	267
2-*cis*-Xanthoxin acid				
[2-^{14}C]Mevalonate	*P. gratissima*	0		168

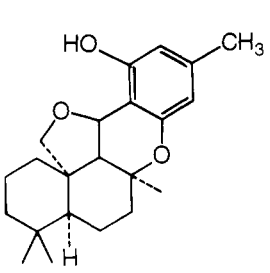
[a] Hydrolysis of petasin and isopetasin esters.

VII. Biosynthesis of Pentacyclic Sesquiterpenes

A. Siccanochromenes

As well as *trans*-γ-monocyclofarnesol (**48**) mentioned previously, *Helminthosporium siccans* also produces a novel group of chromene derivatives, the siccanochromenes[322] and the chroman derivative siccanin (**154**).[323]

Two intermediates were isolated (as their methyl esters) after [2-^{14}C]mevalonate (**6**) indicated that 4% of the total activity was in the carboxylic acid fraction.[324] These intermediates were shown to be presiccanochromenic acid (**155**) and siccanochromenic acid (**156**).



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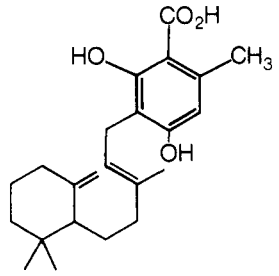

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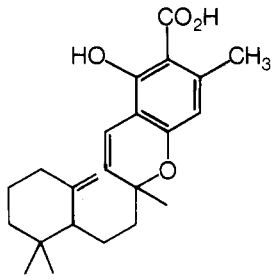

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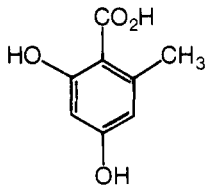
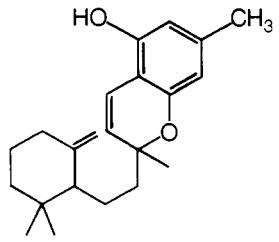


157

The formation of **156** apparently occurs by alkylation of orsellinic acid (**157**) with *trans*-γ-monocyclofarnesol (**48**). Evidence for the reaction sequence after **155** comes from the isolation[322] and feeding of the compounds **158** and **159**.[325] Tritiated **158** was incorporated into siccanin (**154**) to the extent of 25%, whereas 70% of the activity was in **154** when labeled **159** was a precursor.[325]



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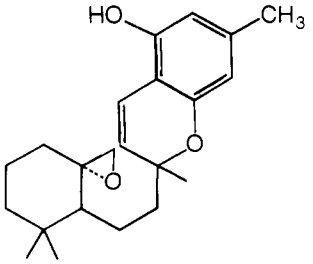

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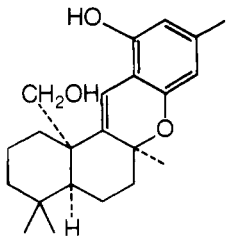


160

An additional intermediate (**160**) was also isolated,[324] so that we may formulate the sequence as orsellinic acid (**157**) + *trans*-γ-monocyclofarnesol (**48**) → **155** → **156** → **158** → **159** → **160** → siccanin (**154**).

VIII. Summary and Conclusions

Biosynthetic data for the sesquiterpenes are summarized in Table III.

The biosynthesis of sesquiterpenes, as with all biosynthetic work, is at an extremely exciting stage of development. The work of Arigoni[137] and the increasing use of ^{13}C NMR indicate that the challenges of deriving sophisticated biosynthetic schemes can be met with adequate effort and intellect. The wide range of pharmacologic activity exhibited by this group of compounds merits increasing attention to their precise biosynthetic derivation.

IX. Addendum

Since the completion of the main body of this review, a number of articles have appeared which bear directly on the biosynthesis of sesquiterpenes.

II.B. Juvenile Hormone

A recent review[331] has summarized the biosynthesis, secretion, and action of Cecropia juvenile hormone, and a full paper on the previously discussed work of Ajami and Riddiford has appeared.[332]

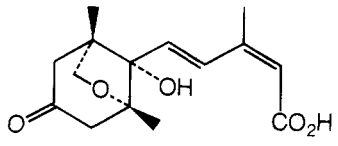
Peter and Dahm[333] have studied the biosynthesis of JH I (**27**) and JH II (**28**) using variously labeled propionates. [1-^{14}C]Propionate was specifically incorporated into only C-7 and C-11 of **27**. Propionate thus serves as a precursor of homomevalonate in agreement with the previous work of Schooley and coworkers.[147] Both [2-^{14}C]- and [3-^{14}C]propionates gave rise to extensive randomization of label.

As an extension of their original work,[147] Schooley and coworkers[334] have examined the importance of [5-$^{3}H_2$]homomevalonate as a precursor of JH I (**27**) and JH II (**28**). Degradation of the labeled JH II (**28**) as discussed previously indicated that the only site of the label was at the predicted position C-9.

III.A. Abscisic Acid

Milborrow,[335] continuing his work on the biosynthesis of abscisic acid (**37**), has elucidated the specificity of mevalonate in the 6′-methyl groups of abscisic acid (**37**) and phaseic acid (**161**). The latter compound occurs as a single isomer and is therefore admirable for such a study.

Degradation of **161** after feeding abscisic acid (**37**) biosynthesized from [2-^{14}C,2-$^{3}H_2$]mevalonate (**6**) demonstrated the *pro*-6′*R*-methyl group was specifically derived from C-2 of mevalonate.[335]



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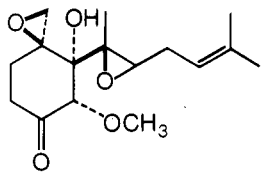
As a double check on the above work, the derivation of abscisic acid (**37**) from mevalonic acid specifically labeled at the methyl group was investigated. If C-2 and C-3′ of mevalonate retain their integrity throughout the biosynthetic process, the carbon atoms derived from C-3′ of mevalonate should appear as the pro-6′*S*, the C-2′, and the C-3 methyl groups of **37**. (+)-Abscisic acid (**37**) derived from [3′-^{14}C,3′-^{3}H]mevalonic acid in avocado showed no loss of label.[336] Treatment with base, however, increased the original ^{14}C:^{3}H ratio from 3:3 to 3:2. The exchange of ^{3}H at the C-2 methyl group was confirmed by modified Kuhn–Roth oxidation. Consequently, C-2 and C-3′ of mevalonate do retain their integrity in the formation of **37** and at no point become equivalent.

III.B. Ovalicin

Two groups have investigated the biosynthesis of ovalicin (**162**), a close relative of fumagillin (**43**).



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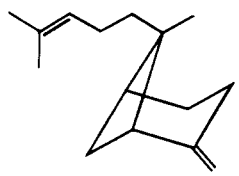
In the first study, conducted by Tanabe and Suzuki[337] [1,2-$^{13}C_2$]acetate was used as a precursor. Six intact acetate units were incorporated, thereby demonstrating that no methyl group migrations were involved in the biosynthesis.

Cane and Levin,[338] using [4-^{13}C]mevalonate (**6**) as a precursor showed that enrichment was specific at C-1, C-3, and C-10.

Very recently Cane and Levin[339] have extended their work on ovalicin (**162**) with the use of [3,4-$^{13}C_2$]mevalonate. [The author is very grateful to Dr. David Cane, Brown University, for a copy of a preprint of this paper prior to publication.] Carbons 1 and 6 of ovalicin (**162**), derived from C-4 and C-3, respectively, of the same mevalonate molecule, gave rise to a pair of doublets (J = 53 Hz). Carbon 7 derived from C-3 of mevalonate appears as an enhanced singlet uncoupled to the adjacent C-1.

Similarly C-10 and C-11, derived from carbon atoms 4 and 3 of mevalonate, respectively, appeared as doublets (J = 74 Hz) and must therefore be derived from the same molecule of mevalonate.

The data of Cane and Levin[338,339] complement those of Tanabe[337] and are additional evidence for the intermediacy of β-bergamotene (**44**) in the biosynthetic scheme, as originally suggested by Birch[177] for fumagillin (**43**).



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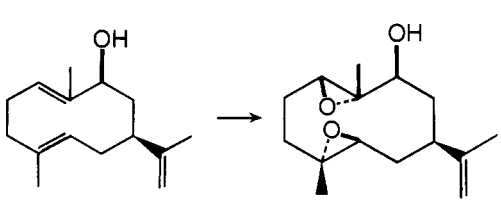
III.C. Ageratriol

The monocyclic sesquiterpenoid ageratriol (**163**) is the principal sesquiterpene of *Achillea ageratum*[340] and may be formulated as being derived from agerol ((+)-9β-hydroxygermacrene A) (**164**) which co-occurs with ageratriol.[341] [9-^{3}H]-Agerol (**164**) was administered[342] and a small specific incorporation was observed into both ageratriol (**163**) and agerol diepoxide (**165**), a postulated biosynthetic precursor. The latter compound labeled at C-13 was incorporated into ageratriol (**163**). Degradation of the labeled ageratriol (**163**) after feeding [2-^{14}C]- and [2-^{3}H]mevalonate indicated[342] that C-12 and C-13 become equivalent at some point in the biosynthetic process.



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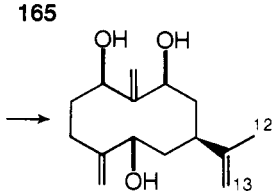

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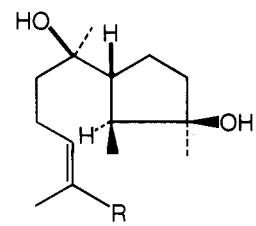
III.F. Cyclonerodiol and Cyclonerotriol

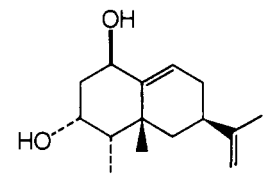
Cyclonerotriol (**166**) and the closely related cyclonerodiol (**167**) from *Fusarium culmorum*[343] have recently been the subject of study by Hanson and co-workers.[344]

Using [4,5-$^{13}C_2$]mevalonate the folding of the farnesyl chain was established since all three ^{13}C–^{13}C couplings remained. Three other precursors were also used. With [2-$^3H_2$,2-^{14}C]-mevalonate considerable tritium was removed by the action of a prenyl isomerase, but [4*R*-^{3}H,2-^{14}C]- and [5-$^3H_2$,2-^{14}C]-mevalonates were incorporated without loss of label.

all-trans-Farnesyl pyrophosphate (**2**) was a precursor of cyclonerodiol (**167**), which itself was a precursor (15% incorporation) of the triol. Nerolidol was not a precursor, indicating that an enzyme bound intermediate of some type is probably involved.



166, R = CH_2OH
167, R = CH_3



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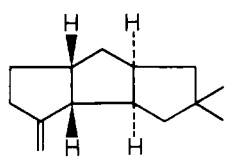
IV.C. Capsidiol

An additional member of the erimophilane group of sesquiterpenoids has been investigated in order to distinguish between the classical[186] and Lawtons spiromechanism for the valencane skeleton.[195] Capsidol (**168**) (a 4-epieremophilane) from *Capsicum frutescens* was labeled by [2-^{14}C]mevalonate and [2-^{14}C]acetate to the extent of 1–3%. In order to evaluate the possibility of a migration of a methyl group from C-10 to C-5, [1,2-$^{13}C_2$]acetate was used as a precursor.[345] Since no ^{13}C–^{13}C coupling was observed between C-5 and C-15, these carbon atoms must be derived from different acetate units; instead C-5 was coupled to C-6. This result is in agreement with a mechanism involving a methyl migration. If a spiro intermediate were involved, the C-5 and C-15 carbons would have remained paired.

V.A. Illudins and Coriolins

Matsumoto and co-workers[346] have synthesized equivalents of the cation **83**, a probable intermediate in the biosynthesis of the illudins.

From *Coriolus consors* Shibata and co-workers[347] have isolated hirsutene (**169**) (pre **91** in Scheme XIII), a postulated precursor of hirsutic acid, complicatic acid, and the coriolins. The structure of hirsutene was confirmed by synthesis.



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V.B. Trichothecin and Verrucarin

Tamm has published a series of papers on his extensive work on the biosynthesis of verrucarin and roridin.[348–351]

Verrucarin A and roridin A differ in the structure of their diacid components, but the sesquiterpene in both cases is verrucarol (**93**).

Using labeled [1-^{14}C]- and [2-^{14}C]acetate and [2-^{14}C,2-$^3H_2$]mevalonate, it was shown that the *cis,trans*-muconic acid unit derives from mevalonate.[348]

Previous work[261,263,273] had demonstrated that hydroxylation at C-4 of the trichothecane skeleton was stereospecific with an

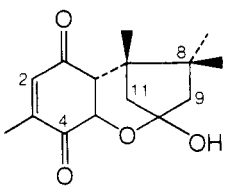
overall retention of configuration. This has now been confirmed for verrucanol using mevalonate stereospecifically tritiated at C-2. This experiment also confirmed the derivation of C-8 of the trichothecane skeleton from C-2 of mevalonate.

Tamm also investigated the mode of cyclization of trichodiene (**109**)[351] in the same way as Hanson.[273] Using [5*R*-^{3}H]mevalonate it was conclusively shown that verrucarol (**93**) retained three ^{3}H labels and that these were located at C-11, C-3, and probably C-7. That ^{3}H is retained at C-11 indicated a stereospecific loss of the pro-1*S* proton of farnesol in the formation of trichodiene (**109**). An entirely analogous result was obtained by Hanson and Evans[352] using a cell-free system from *Trichothecium roseum*. The precise mechanism of the formation of trichodiene remains to be determined particularly whether an oxidation–reduction is involved in the cis–trans isomerization of C-2,3.

Bisabolene derivatives were not incorporated into verrucarol (**93**).[351]

V.B. Lagopodin

Lagopodin B (**170**), a quinone antibiotic from *Coprinus lagopus*,[353] has also been the subject of study by Arigoni's group. [The author thanks Dr. David Cane, Brown University, for provision of relevant manuscripts and data prior to publication.] [2-^{14}C]Mevalonate was specifically incorporated into **170**,[354] degradation localizing a third of the activity at C-11, a further third at the C-8 α-methyl, and one-sixth each at C-2 and C-4. On this basis a scheme involving cuparene (**171**) as an intermediate was proposed.[354] Subsequently, a number of intermediates including cuparene (**171**), cuparenol (**172**), and cuparenone (**173**) were shown to be efficient precursors of **170**.[355] With the overall



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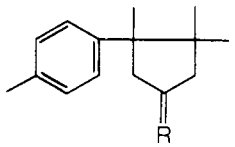
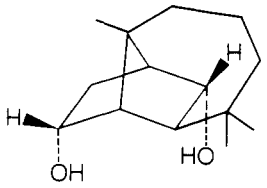


171, R = H_2
172, R = ~OH, H
173, R = =O



174

scheme evaluated, attention was turned to the mechanism of the second cyclization from a bisabolene-type compound to eventually give cuparene (**171**). [4*R*-^{3}H,2-^{14}C]Mevalonate was specifically incorporated into **170.** Degradative work indicated that a half tritium equivalent was at C-2, and that the remaining two equivalents of tritium were localized at C-9. In the cyclization, therefore, a 1,4-hydride shift from C-6 to C-9 occurs and a bisabolene intermediate ($\Delta^{6,7}$) is eliminated. The results are in agreement with those of previous workers[168,261,265-268] on the biosynthesis of helicobasidin (**96**) and trichodermin (**107**).

V.C. Dendrobine

Jommi and co-workers[356] have published additional details of the biosynthesis of dendrobine (**118**). Incorporation of [1-$^3H_2$]-2-*trans*-6-*trans*-farnesol (**1**) into dendrobine (**118**) in *Dendrobium nobile* and degradation indicated that 52% of the label was located at C-5 and 47% at C-8. The 2-cis isomer was not incorporated. [1*S*-^{3}H]-2-*trans*-6-*trans*-Farnesol (**1**) was incorporated into dendrobine (**118**), and 88% of the label was located at C-5. The hydride shift from C-1 of farnesol is therefore highly stereospecific with the 1-pro-*R* hydrogen being transferred.

Farnesal is therefore not an intermediate in the cis–trans isomerization of farnesol. These results are analogous to those of Arigoni[357] who used stereospecifically labeled mevalonate.

V.E. Culmorin

Another tricyclic sesquiterpene which has recently been investigated is culmorin (**174**), from *Fusarium culmorum*.[358] Hanson and Nyfeler[359] using [2-$^3H_2$]-, [4*R*-^{3}H]-, and [5-$^3H_2$]-mevalonate (administered with [2-^{14}C]mevalonate) incorporated them into culmorin (**174**). By degradation, the labeling pattern associated with C-1, C-8, C-10, and C-11 was formed to be in accord with a formation from farnesyl pyrophosphate. From [2*S*-^{3}H]mevalonate it was demonstrated[360] that the endo-C-10 proton of culmorin (**174**) originates from the 2*S* position of mevalonate. The total number of hydrogens derived from [5-$^3H_2$]mevalonate (five of six), however, indicated that a migration of [5-^{3}H] label had occurred, possibly from C-7 to C-5.[359]

Degradative work placed a pro-5*S*-mevalonoid hydrogen at C-11 and a pro-5*R*-mevalonoid hydrogen at C-7. It is C-7 which originates from C-1 of farnesol and consequently during the course of cyclization the pro-1*S* hydrogen of farnesyl pyrophosphate has migrated (probably to C-5).

This is in agreement with the work of Arigoni and co-workers[357,361] on the biosynthesis of sativene and longifolene. The stereochemistry of the loss of hydrogen from C-1 is therefore dependent upon the size of the initial ring formed. When C-1 of farnesyl pyrophosphate attacks the distal double bond, a cyclopropyl ion is formed, which subsequently collapses in one of two ways: (i) to a 10-membered ring with migration of the pro-1*R* hydrogen, e.g. dendrobine; (ii) to an 11-membered ring with migration of the pro-1*S* hydrogen, e.g., culmorin, sativene, longifolene. In either instance secondary reactions subsequently take place by reaction of the carbonium ion at C-1 of the farnesyl system.

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