# STEREOCHEMICAL ASPECTS OF SESQUITERPENE BIOSYNTHESIS

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## ABSTRACT

The biosynthesis of the two antipodal forms of longifolene and of three sesquiterpenes related to different stereochemical variations of a basic cadalane type has been investigated in detail. It was shown that in each case formation of the prototype involves a stereospecific 1,3-hydride migration; the identity of the migrating H-atom is correlated with the eventual configuration of the carbon centre from which it departed and with the size of the first formed ring. On this basis a detailed stereochemical model has been developed for the formation of the relevant compounds.

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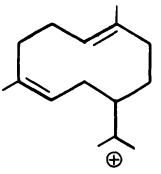
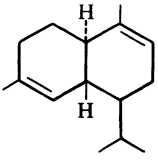
From a biogenetic point of view the sub-class of mevalonoid compounds known as sesquiterpenes poses a number of intriguing and largely unsolved problems. As members of the terpene family, sesquiterpenes are, of course, expected to conform to the tenets of the classical Ruzicka hypothesis[1], namely that formation of their polycyclic carbon frameworks proceeds by cyclization of aliphatic precursors; however, Nature has been particularly prodigal in this area in playing variations on a known theme[2], and it seems that almost every conceivable structural possibility, including some which would have defied the ingenuity of even the most daring organic chemist, has been put into practice by living organisms. In a general sense, the gross structural relationship between the cyclic prototypes and their precursors is fairly well understood, if only seldom backed by appropriate experiments; yet, despite considerable speculation[3], there remains a remarkable lack of knowledge concerning the precise identity of the cyclizing precursors and the intimate nature of their transformations. Neither has it proved possible so far to develop a simple and unifying stereochemical concept of sesquiterpene biosynthesis akin to the one which found wide acceptance and has now been verified in a number of cases for the formation of the higher homologues, the di- and triterpenes[4].

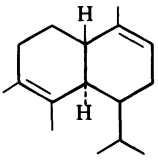
Some of the ambiguities specific to this area of biosynthesis are illustrated in *Figure 1*. Thus, it is believed (although by no means proved) that the carbonium ion **9** from the cyclization of all-*trans* farnesyl-PP provides a reasonable entry into the germacrane and, hence, into the eudesmane group of sesquiterpenes (cf. **7** with respect to **6** and **8**), whereas its *cis–trans* counterpart **1** is made responsible for the formation of members of the



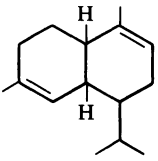
1



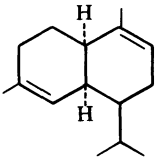
2 α-Cadinene



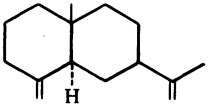
3 α-Bulgarene



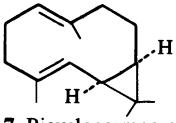
4 α-Muurolene



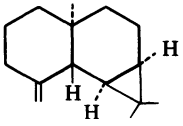
5 α-Amorphene



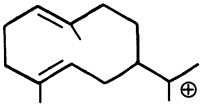
6 β-Selinene



7 Bicyclogermacrene



8 γ-Maaliene

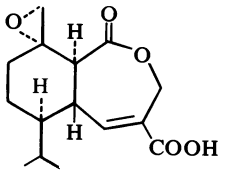


9

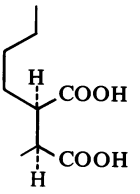
*Figure 1*

cadalene group exemplified by **2–5**. Some regularities and their possible biogenetic implications have been noted, e.g. the fact that a common stereochemistry in the two sets is almost invariably maintained at the carbon bearing the $C_3$ side chain in components of vascular plants[5]; even so, the existence of the four diastereomeric hydrocarbons **2–5** must be considered baffling from a biogenetic standpoint. As an added complication, it now appears on the basis of recent work[6] that liverworts and lower vegetal forms, as well as marine organisms, specialize in the production of sesquiterpenes belonging to an antipodal set.

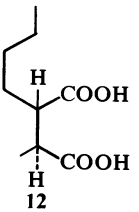
A welcome opportunity for approaching experimentally some of the biosynthetic puzzles posed by cadalane-type compounds arose from our structural studies on avocettin, a metabolite of the fungus imperfectus *Anthostoma avocetta*, first isolated in a pure form by Dr H. P. Sigg and his colleagues at Sandoz, Basel. The structure of avocettin was settled as in **10** (*Figure 2*) by chemical degradation[7], the only remaining ambiguity being the relative stereochemistry of the oxirane ring. Inspection of this structure, more specifically the presence in it of six different terminal carbon atoms, suggested at once a possible derivation from a bicyclic sesquiterpene precursor through oxidative processes (*vide infra*). The structure of two by-products of avocettin fermentation, **11** and **12**[8], was at first taken as addi-




**10** Avocettin




**11**




**12**

ex *Anthostoma avocetta*

*Figure 2*

tional indirect evidence for the operation of an extended oxidative catabolism of preformed terpene intermediates in this fungus. Indeed, both **11** and **12** acquired significant radioactivity upon feeding of 2-[$^{14}$C]mevalonate to the fungal culture; however, subsequent degradation of the labelled specimens soon revealed that incorporation of label had occurred exclusively via a prior fragmentation of the precursor to methyl-labelled acetate and that formation of the two acids is best explained through condensation of an appropriate derivative of hexanoic acid with a $C_3$ fragment from an intermediate of the citric acid cycle. While precedents for such a mode of biosynthesis are well established[9], our results emphasize once again the essential need for unambiguous location of label in biosynthetic tracer work.

In spite of the deceptive outcome of this ancillary investigation, it was felt that the original concept for avocettin biosynthesis warranted further consideration. As illustrated in *Figure 3*, formation of the metabolite can be interpreted as the outcome of an extensive oxidative attack on (−)-γ-cadinene, **18**; in this event the known absolute stereochemistry of avocettin demands that the hydrocarbon **18** belong to the 'antipodal' set of compounds, which is in good keeping with its fungal origin. Ignoring for the moment stereochemical details, the most economical but by no means unique biosynthetic route from the alleged aliphatic precursor **13** to the hydrocarbon **18** leads through the carbonium ions **14** and **17**. Three different mechanistic paths are available in principle for the necessary conversion of **14** into **17**: (1) a double 1,2 hydride shift involving the additional ionic species **15** as a discrete intermediate; (2) insertion of the ionic centre of **14** into the allylic methylene group with loss of a proton and formation of a stable cyclopropane intermediate, **16**, followed by cleavage of the cyclopropane ring in **16** through addition of an external proton; (3) a direct conversion involving a 1,3 transfer of a hydride ion. Path (1) is reminiscent of the well-documented consecutive hydride migrations in the biosynthesis of lanosterol[10] and β-amyrin[11]; path (2) finds a formal analogy in the formation of presqualenol-PP[12], whereas no biochemical precedent for path (3) was available at the time our investigation was started.

To put this general scheme on a firm basis and in order to pave the way for further studies of mechanistic details, we decided to check at first on the role of the aliphatic precursor. As indicated in *Figure 4*, a sample of all-*trans* farnesyl-PP labelled with tritium on the internal double bond (cf. **20**) was made available from the tetrahydropyranyl ether of pure *trans*-geraniol, **19**, in several steps by adaptation of known methods[13]. Feeding of this material

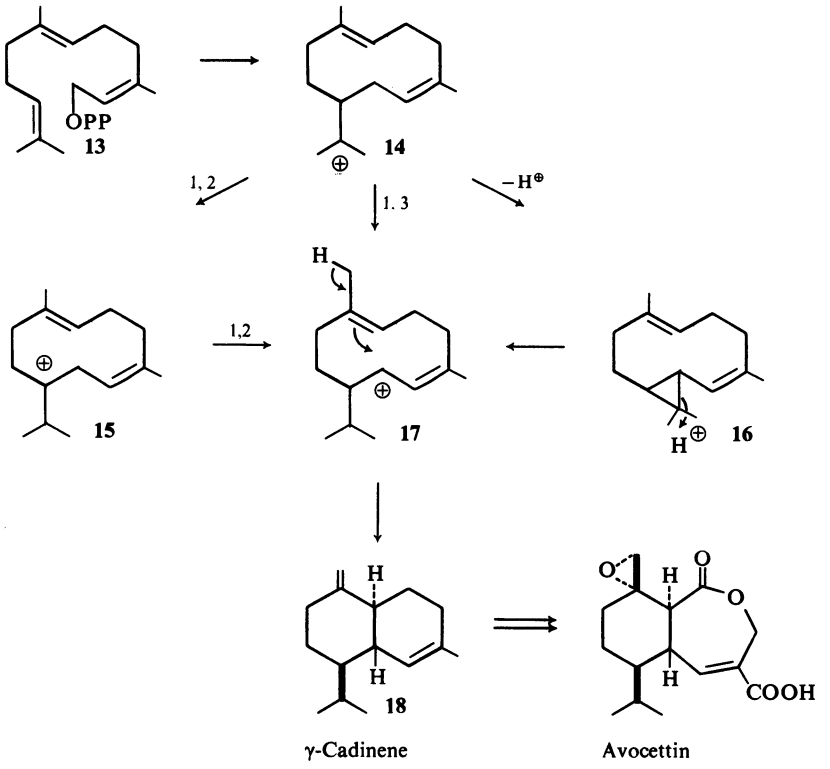



*Figure 3*

to a shaking culture of *A. avocetta* led to a 0.12 per cent incorporation of tritium into avocettin, **21**. The labelled material lost the bulk (more than 80 per cent) of its tritium content upon base-catalysed conversion of the corresponding methyl ester into the α,β-unsaturated lactone **22**, thus showing that the label was localized essentially at C-1 and that incorporation of the precursor had occurred almost entirely, if not exclusively, without prior degradation to smaller fragments.

Before proceeding any further, we had to remove a remaining point of ambiguity. In principle there are still two foldings of the farnesol chain which can be invoked in order to arrive at the bicyclic intermediate **18** and, hence, at avocettin; these are shown as (A) and (B) in *Figure 5*, the two representations being meant exclusively to convey information about possible correspondences of carbon atoms in precursor and product rather than to imply stereochemistry. Folding (B), improbable as it may seem on mechanistic grounds, can hardly be dismissed *a priori*, and its participation has in fact been implied by experiments on the biosynthesis of gossypol[14], a compound from cotton-wood formed by dimerization of sesquiterpene units of the cadalane type. The two modes of folding can be differentiated experimentally, since they predict alternate locations of label from 2-[$^{14}$C]mevalolactone in the ε-lactone ring of avocettin (black dots in A and B). Accordingly, a radioactive sample of the metabolite biosynthesized from the appropriate

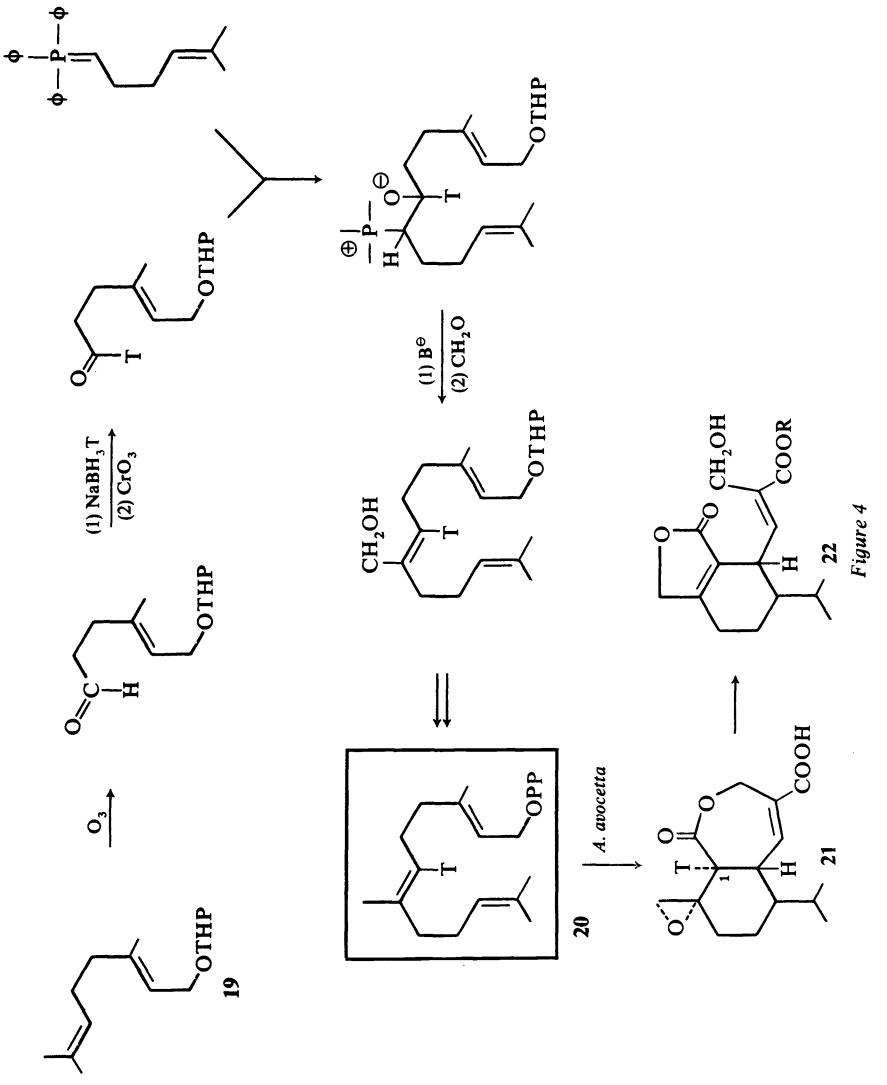

OTHP
19
$O_3$
(1) $NaBH_3T$
(2) $CrO_3$
OTHP
T
OTHP
(1) $B^{\ominus}$
(2) $CH_2O$
$CH_2OH$
OTHP
T
OPP
T
20
A. avocetta
O
O
T
1
O
COOH
H
21
$CH_2OH$
COOR
O
H
22

*Figure 4*

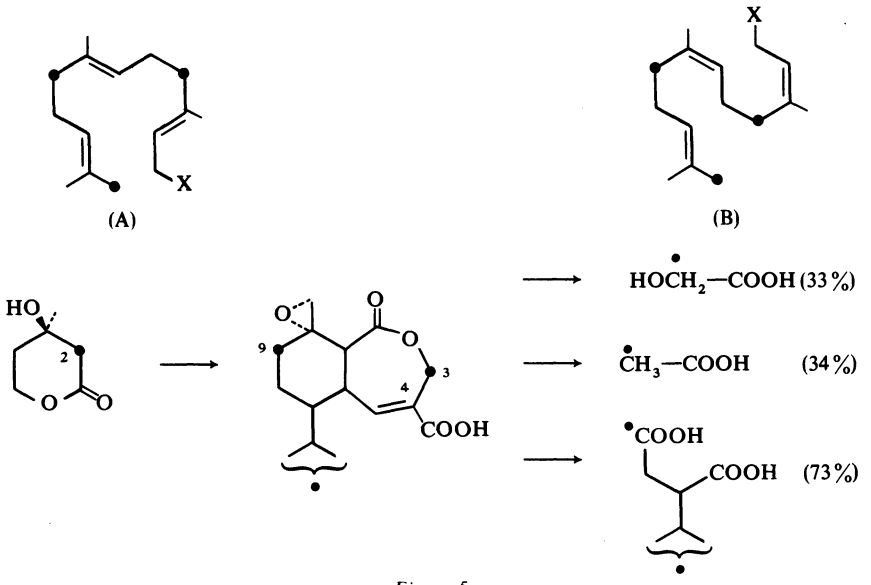



*Figure 5*

precursor was degraded in a multistep sequence to glycolic acid containing the C-3 and C-4 atoms of the starting material and shown by subsequent oxidative cleavage to carry a third of the original radioactivity in the hydroxymethyl group. The cyclizing farnesol chain must therefore fold as in (A). Corroboratory evidence for the general validity of the proposed scheme was obtained through Kuhn–Roth oxidation of the labelled avocettin to a specimen of acetic acid containing the expected amount of label in its methyl group; in addition, the isopropyl succinic acid from a drastic oxidation of avocettin with $HNO_3$ gave radioactivity values consistent, by difference, with the location of the residual activity at C-9 of the metabolite.

Having settled the origin of critical atoms in the carbon framework of avocettin and established its relationship to an aliphatic $C_{15}$-precursor, we now felt justified in taking avocettin as a *bona fide* model for the biosynthesis of a cadalane-type sesquiterpene and were in a position to attempt a first differentiation between the mechanistic possibilities outlined in *Figure 3*. Towards this end we elected to follow the fate of tritium atoms from (4*R*)-[$^3H_1$]mevalolactone in the course of avocettin biosynthesis (*Figure 6*). Accepting all-*trans* farnesyl-PP, **23**, as the first $C_{15}$-intermediate, retention of one label equivalent can be predicted at each of its three double bonds[15]. Label from the internal double bond should appear at C-1 of avocettin; as for the other two positions, the allylic double bond may, but need not, lose its label in the elusive isomerization which is a prerequisite for the generation of the *cis* double bond in avocettin, while the location of the third tritium atom is critically dependent on the mechanisms for the further evolution of the first-formed carbonium ion. Using a standard of 2-[$^{14}$C]mevalolactone for internal calibration, it was found that three equivalents are actually retained in avocettin following incorporation of the tritiated precursor.

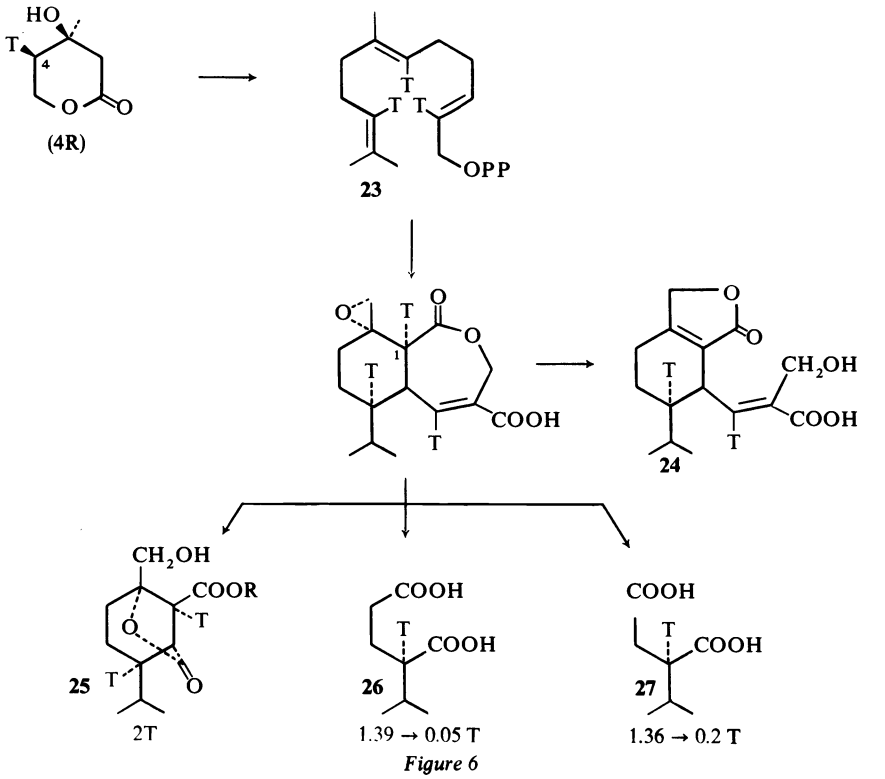



*Figure 6*

One of these was easily located at C-1 by conversion to the known unsaturated lactone **24**. The residual activity of the *trisnor*-lactone **25** from the oxidation with $HNO_3$ provided evidence for the presence of a second tritium equivalent on the double bond of the starting material. From the same oxidation mixture it was also possible to isolate small amounts of labelled and optically pure (*R*)-isopropyl glutaric acid, **26**, and (*R*)-isopropyl succinic acid, **27**, both of which lost the bulk of their tritium content (arrows under the formulae) upon racemization with concentrated HCl at 150°. The latter result localizes in an unambiguous way the remaining tritium equivalent at the carbon centre bearing the isopropyl side chain and thus eliminates a path involving a 1,2 hydride shift.

The tritium values for **26** and **27** are significantly and reproducibly higher than expected; as a consequence, the sum of the three localized labels is larger than the value for the starting material, a situation which clearly deserves some comment. Bearing in mind that all the tritium values are expressed in a relative way with reference to an internal $^{14}C$ standard (the label from 2-[$^{14}C$]mevalolactone) and that for practical purposes a given molecule will at no time carry simultaneously a $^{3}H$ and a $^{14}C$ label, the paradox can be clarified in the simple and very reasonable assumption that enolization of a carbonyl group is a rate-determining condition for attack of substrate by $HNO_3$. Because of the sizeable isotope effect to be

expected for such a step, further degradation of $^{14}C$-labelled molecules (and of non-labelled molecules as well) will proceed faster than for molecules carrying tritium at the critical α-position, thus leading to an increment in the $^{3}H/^{14}C$ ratios. In other words, tritium atoms located in a strategic position can to some extent protect the intermediate from further attack by the oxidizing reagent!

A definitive proof for the mechanism which gives rise to the formation of the isopropyl group of avocettin was obtained in experiments with mevalonate precursors bearing tritium at the C-5 position (*Figure 7*). The availability of optically pure samples of both (3*R*,5*R*)-5-[$^{3}H$]mevalonate (from the enzymic reduction[16] of optically pure (3*R*)-mevaldic acid[17]) and its (5*S*)-isomer[17] (corresponding to $^{3}H = H_A$ and $H_B$ in *Figure 7*) proved of great help in this context. The two precursors were fed in separate runs in admixture with the usual $^{14}C$ standard and the radiolabelled avocettin degraded as before with $HNO_3$. In addition to the three compounds **25–27**, a further oxidation product, the nitroacetal **28**, was included in the analysis. The results of the two sets of experiments proved nicely complementary (cf. *Figure 7*, in which the framed values are again abnormally high as a by now

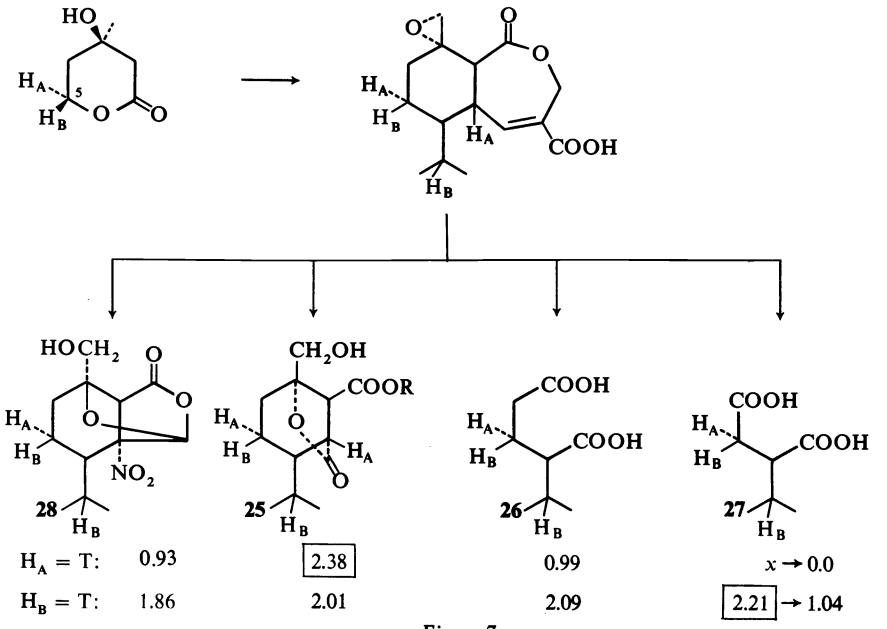



*Figure 7*

expected consequence of the previously discussed isotope effect) and provided a convincing demonstration that (a) the (3*R*)-isomer of mevalonate is a specific precursor of avocettin, (b) no loss of tritium occurs from the terminal carbon atom of the farnesol precursor in the course of the biosynthesis, (c) saturation of the isopropyl side chain occurs via a 1,3 hydride shift and—last, not least—(d) migration into the side chain involves specifically the $H_B$ hydrogen, $H_A$ being retained at the ring junction.

In order to integrate all of these facts into a more detailed scheme of biosynthesis, we must first focus attention on the double bond isomerization process required by the demonstrated relationship between all-*trans* farnesyl-PP and avocettin. Recent work by different groups[18], notably by Overton and his colleagues, has indicated that free all-*trans* farnesol can be equilibrated in biological systems with the *cis* isomer through formation and interconversion of the corresponding aldehydes. We had independently uncovered a similar situation for the *trans–cis* conversion of the lower pair of homologues, geraniol, **30**, and nerol, **33**, in *Menyanthes trifoliata* (*Figure 8*)[19]. Briefly, it was found with the help of suitably labelled substrates

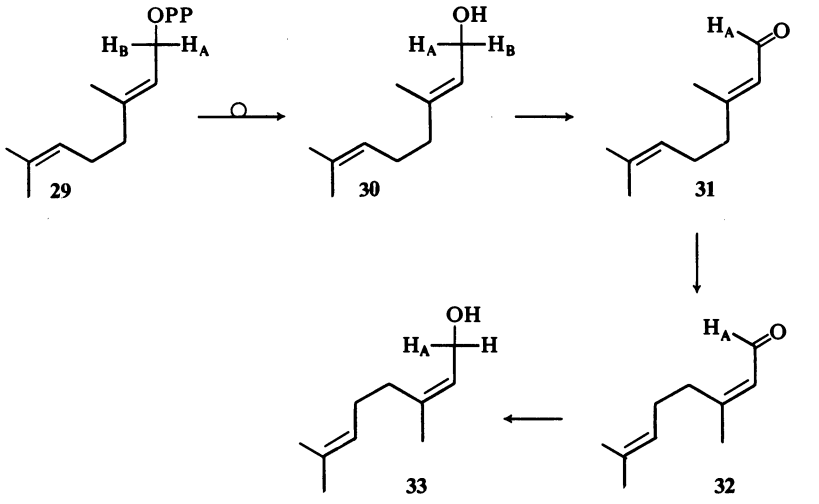



*Figure 8*

that conversion of geranyl-PP, **29**, to the free alcohol **30** occurs with inversion of configuration at the relevant carbon centre and, hence, presumably through cleavage of the allylic C—O bond. The free geraniol is then converted to nerol, **33**, with stereospecific loss of $H_B$ and reduction of the alleged intermediate **32** to restore the original steric arrangement of $H_A$ at C-1. The compulsory requirement of such schemes, namely that a proton from the terminal group of the aliphatic precursor be lost to the medium before the isomerization proper, is, however, not fulfilled in avocettin biosynthesis, and, hence, one has to conclude that in this case *cis*-farnesol is not acting as an intermediate; if it is, it must owe its formation to a different set of events. The first alternative can be expanded as outlined in *Figure 9*. Besides setting up the correct configuration at the chiral centre bearing the $C_3$ side chain, the first cyclization is expected to cause inversion of the stereochemical situation at the carbon atom undergoing the nucleophilic substitution. Following the required 1,3 shift of the $H_B$ atom, the process is terminated through extrusion of a proton to give the s-*cis* diene **34**. This has enough conformational mobility to equilibrate with the s-*trans* form **35**. Addition of an external proton then triggers the second cyclization step, leading to the desired bicyclic hydrocarbon with a *cis* double bond. In this way the

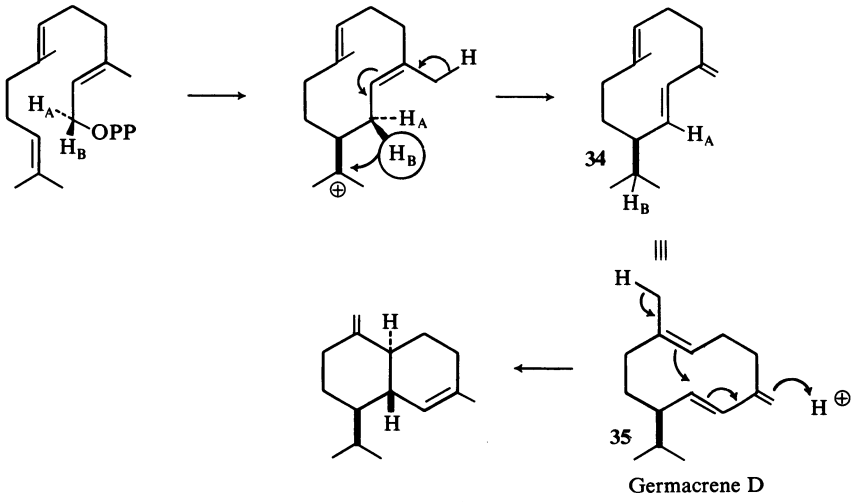



*Figure 9*

mechanistically intriguing *trans–cis* isomerization of the critical double bond is traced back to a readily acceptable conformational change around the single bond of a diene system in an intermediate, the antipode of which is known to occur in higher plants[20]. Inspection of models and the somewhat idealized perspective view of *Figure 10* indicates the existence of a strainless conformation of the carbonium ion which allows a fine alignment of the empty orbital in the side chain, the critical C—H σ bond and the π orbitals of the adjacent double bond, thus meeting in an ideal way the stereoelectronic requirements for an assisted 1,3 shift.

The last stereochemical detail to which we addressed our attention concerns the retention of biogenetic identity in the methyl groups of the isopropyl unit. Loss or maintenance of this identity is critically dependent on the conformational mobility of the $C_3$ side chain in the ionic species of *Figure 10*,

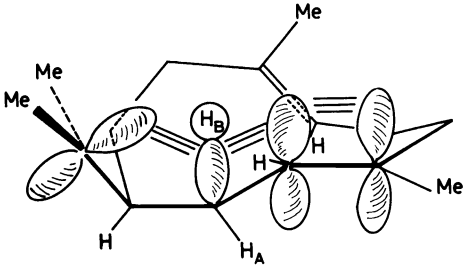



*Figure 10*

and knowledge of the actual situation would help in casting additional light on the mechanism of the unusual hydride transfer reaction. Differentiation between the two diastereotopic methyl groups of avocettin and its derivatives is fairly simple on a n.m.r. spectroscopic basis, even though a specific assignment of the relevant signals cannot be made. This, however,

provided no help for the envisaged tracer work, and we had to devise a new approach for a chemical differentiation of the two groups, which is summarized in *Figure 11*. The anhydride of α-isopropyl glutaric acid, **36**, was converted in three steps to the amine **37**. Irradiation of the corresponding *N*-chloro derivative with u.v. light in $CF_3COOH$ gave after treatment with

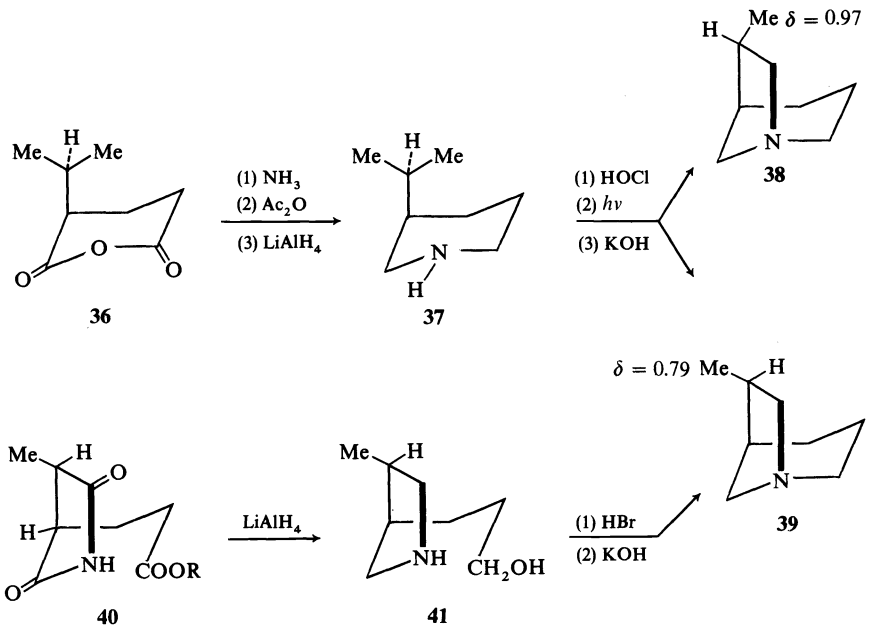



*Figure 11*

base a 3:1 mixture of bicyclic tertiary amines which was separated into the pure components, **38** and **39**, by preparative g.l.c. The presumed exo arrangement of the residual methyl group in the preponderant isomer **39** was settled unambiguously through an independent synthesis of the same compound using the known *trans*-dihydro-haematinimide, **40**[21], as the starting material.

Operating on this secure background, we could now carry on with the critical experiment (*Figure 12*). A sample of avocettin biosynthesized from 2-[$^{14}$C]mevalonate was degraded with $HNO_3$ to α-isopropyl glutaric acid and the latter, after dilution with excess cold racemic carrier material, converted to the exo and endo amines **39** and **38** along the lines indicated above. Kuhn–Roth oxidation of **38** and **39** gave samples of acetic acid containing respectively 85 and 15 per cent of the activity expected for one label equivalent. Clearly, the biogenetic identity of the two methyl groups is retained to a large degree*. The above experiments locate the label in the (pro-*S*)-methyl group of the side chain (cf. black dots in *Figure 12*). In the

* The observed small amount of label randomization presumably reflects a corresponding degree of racemization of the labelled **36** during formation of the anhydride ring.

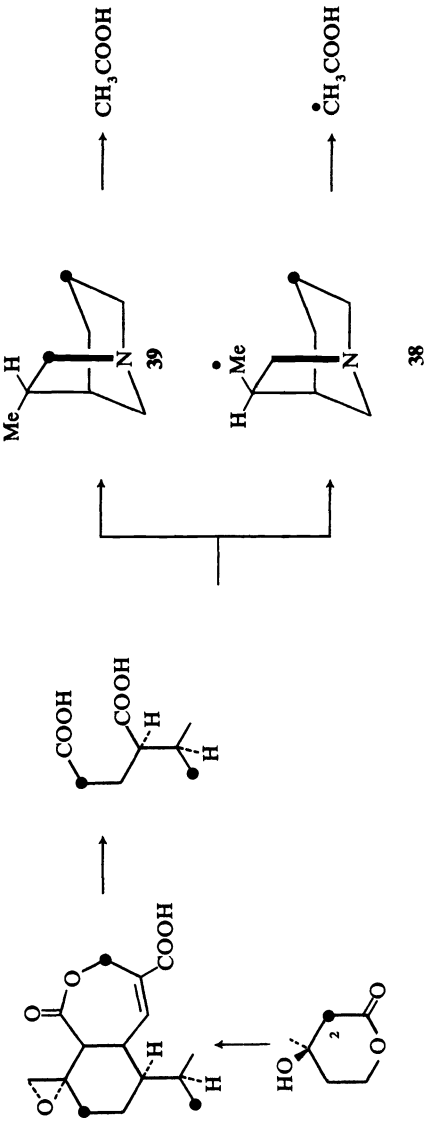



*Figure 12*

aliphatic precursor only the *E*-methyl group of the isopropylidene moiety is labelled in this experiment; in view of this and of the known configuration of avocettin, the over-all addition to the double bond can now be formulated as a *cis* process (*Figure 13*); as a consequence, transfer of the hydride ion

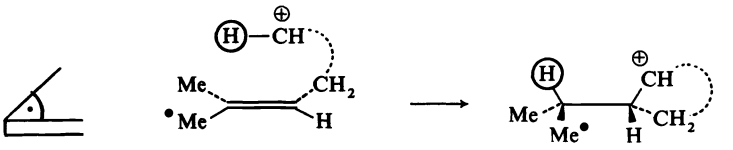
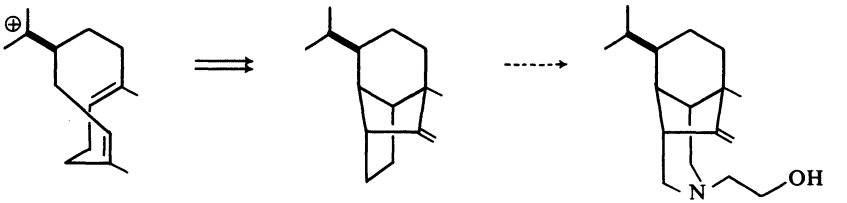


*Figure 13*

must be faster than rotation of the charged side chain unit in the ionic species of *Figure 10.*



**42** **43** Sativene **44** Victoxinine

ex *Helminthosporium sativum* and *H. victoriae*

*Figure 14*

At this point we can leave temporarily the *Avocetta* system and turn to a consideration of a related biosynthetic problem in another fungal species. In a previous investigation[22] we had succeeded in establishing structure **44** for victoxinine, a phytotoxic principle from *Helminthosporium sativum* and *H. victoriae.* Formation of **44** can be visualized as proceeding from (−)-sativene, **43**[23], with which it co-occurs in the two moulds, through cleavage of the two-carbon bridge and insertion of an ethanolamine unit. Sativene, in turn, is thought to be generated from the by now familiar ion **42** in a reaction sequence which will be considered in greater detail at a later stage. Further analysis of the sesquiterpene metabolites from the two *Helminthosporium* species yielded an impressive array of compounds, most of which shared a sativene or seco-sativene framework. Some of these are portrayed in *Figure 15*, with broken arrows indicating a plausible order of formation from the parent compound **43**. This situation can be advantageously exploited in biosynthetic tracer work, since the availability of such a variety of interrelated structures obviates the need for subsequent lengthy degradation by providing a simple entry into parts of the sativene molecule otherwise accessible only through considerable chemical effort. To exemplify, the presence of a tritium label in the isopropyl chain of **43** can be monitored by differential radioactivity measurements on the pairs **45**/**48** and **47**/**50**, the retention of the biogenetic identity in the two secondary methyl groups can be assayed by selective degradation of the isopropenyl chain of **49**, and oxidative cleavage of **51** as well as hydrolysis of **52** provide a simple

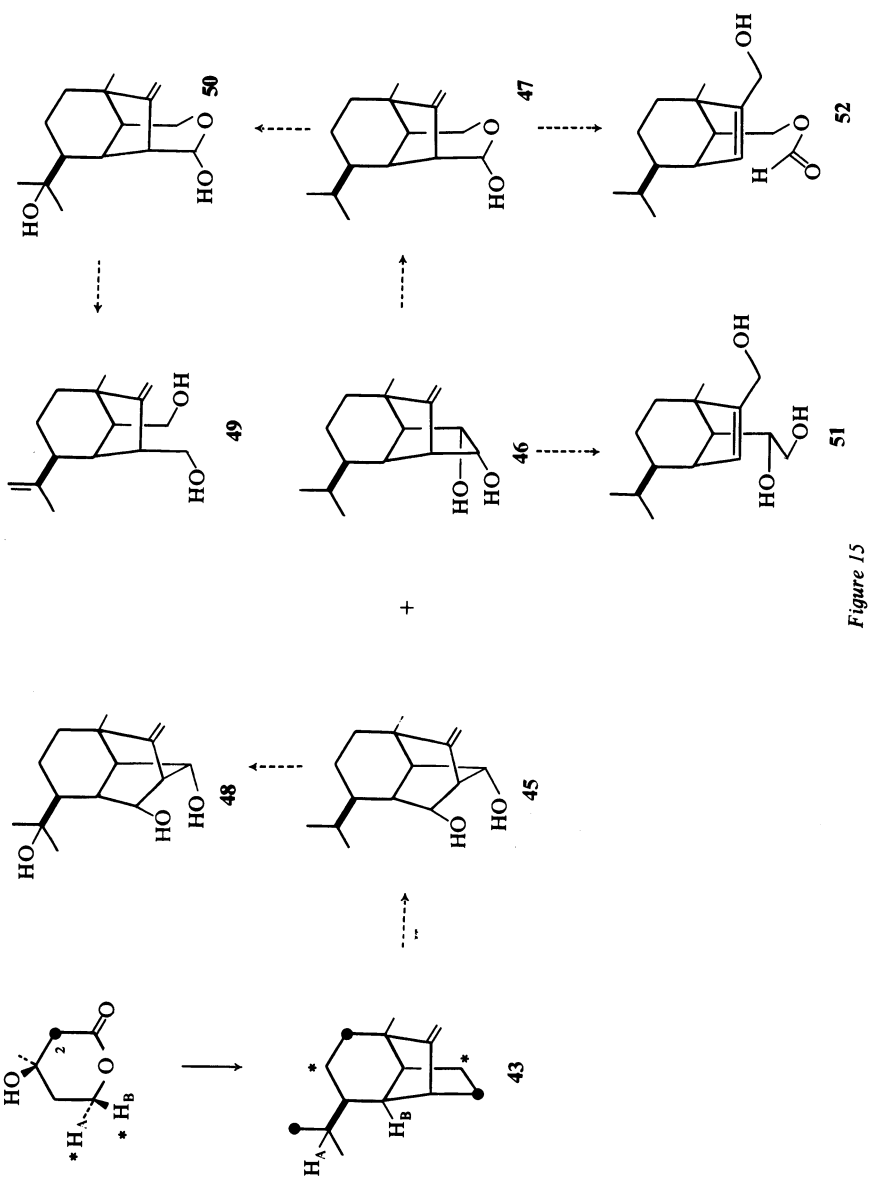


*Figure 15*

means for carving out one of the two carbon atoms in the $C_2$ bridge. Starting with appropriately labelled samples of mevalonate and working along these lines, it was therefore a relatively simple matter to secure a number of details in the biosynthesis of sativene. As it turns out, all of the hydrogen atoms from C-5 of the precursor are retained in the formation of **43**; again, a stereospecific 1,3 hydride shift can be detected, but, in contrast to the avocettin case, the migrating hydrogen belongs this time to the $H_A$ type. The specific derivation of the terminal methylene group in the side chain of **49** from C-2 of the precursor demonstrates that biogenetic identity of the secondary methyl is preserved in the parent compound **43**. Moreover, it can be shown that in the $C_2$ bridge of **43** label from C-2 of mevalonate is restricted to the dotted rather than to the starred position*.

The pivotal importance of the latter finding for a subsequent discussion can best be realized by consideration of the scheme in *Figure 16*. As indicated, there are in fact two different routes for reaching the desired hydrocarbon from the aliphatic $C_{15}$-precursor. Using the hypothetical ionic species **53** as a starting point, formation of sativene can proceed in a straightforward manner through the intermediacy of **54** and **55**. However, one of the intermediate ions, **55**, can also be generated in a plausible way from an antipodal conformation of the precursor, **56**, via the tricyclic ions **57** and **58**, the latter of which can merge into **55** by a 1,2 migration of the three-carbon bridge. In the same way copacamphene, a stereoisomer of sativene, can be derived either directly from **56** or from **53** by the longer route. Inspection of **59**, a molecular species at the midpoint between **55** and **58**, in which the numbering of the carbon atoms refers to their expected derivation from mevalolactone via **53**, makes it clear that in experiments with this precursor the only way in which the two paths can be eventually distinguished is by analysis of the origin of the two carbon atoms in the lower bridge. Knowledge of the actual chiral conformation of the cyclizing precursor is, of course, essential for a proper evaluation of the results; for this reason it was important to learn from the experiments cited above that in both *H. sativum* and *H. victoriae* sativene is in fact biosynthesized by the shorter of the two routes of *Figure 16*.

The possibility of crossing over from one path to the other through the rearrangement **55** → **58** is less far-fetched than one might believe. Indeed, sativene and copacamphene have been interconverted *in vitro* by this route in the presence of Lewis acids[25]. In addition, an interesting biochemical counterpart of this process seems to be realized in *H. victoriae*. Two further metabolites of this fungus were suspected on the basis of chemical and spectroscopic evidence to possess structures **61** and **62**, related to the copacamphene set. Eventually these structures were verified through their partial synthesis from the epoxy compound **60** in a process which clearly involves a 1,2 migration of the three-carbon bridge. There can be little doubt that this correlation mimics very closely a late stage in the biochemical formation of the two compounds; these, therefore, represent an interesting instance of copacamphene derivatives formed biologically by the sativene route.

* For a previous cursory biosynthetic investigation of another metabolite related to **43**, cf. ref. 24.

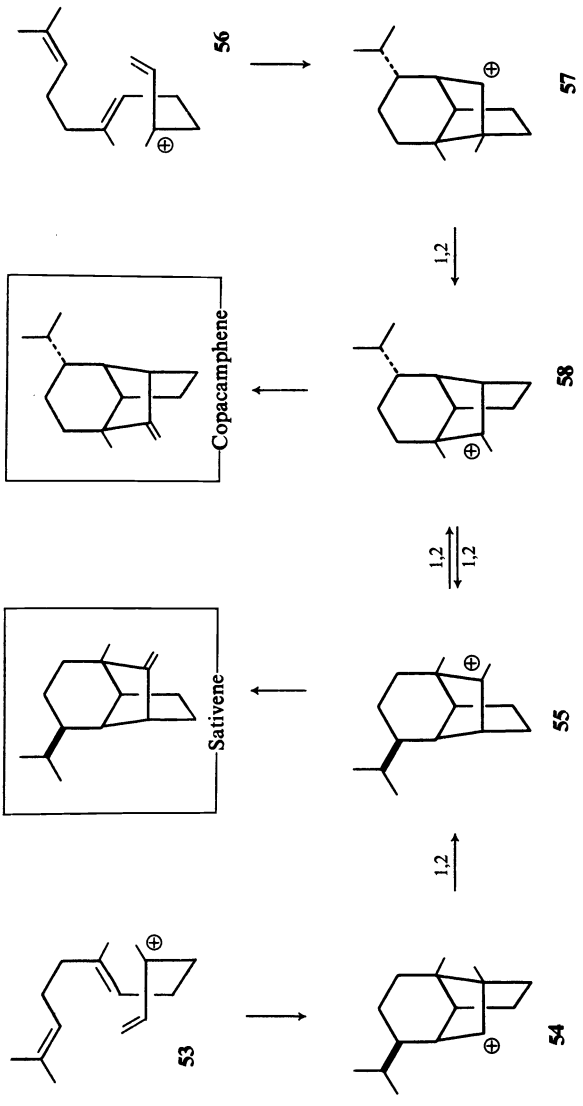



*Figure 16*

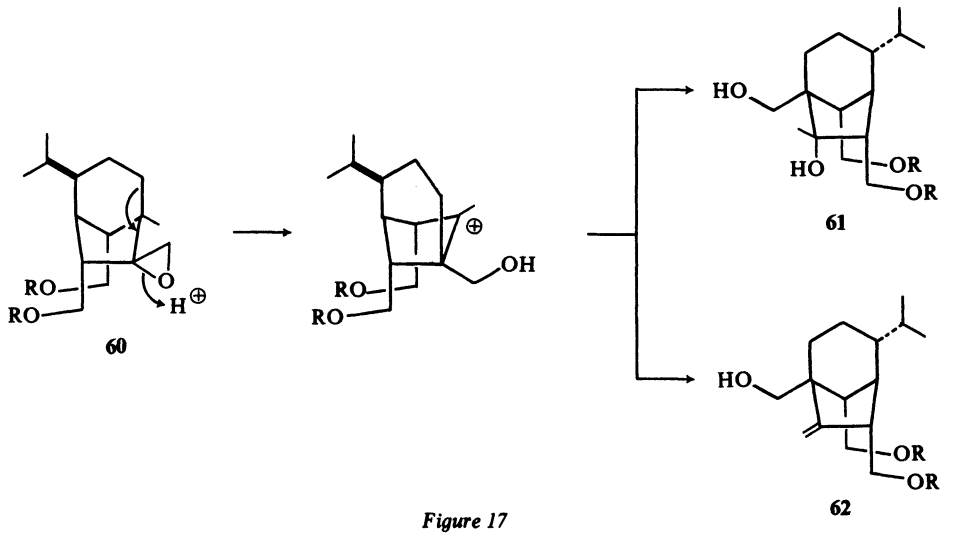



*Figure 17*

Mechanistic and stereochemical information on the direct route to the copa-system can be obtained by studies on the biosynthesis of dendrobine **67**, an alkaloid from the orchid *Dendrobium nobile* (*Figure 18*). This compound belongs to the picrotoxane group of sesquiterpenes which includes tutin, **65**, and coriamyrtin, **66**, and previous work with *Coriaria japonica*[26] had demon-

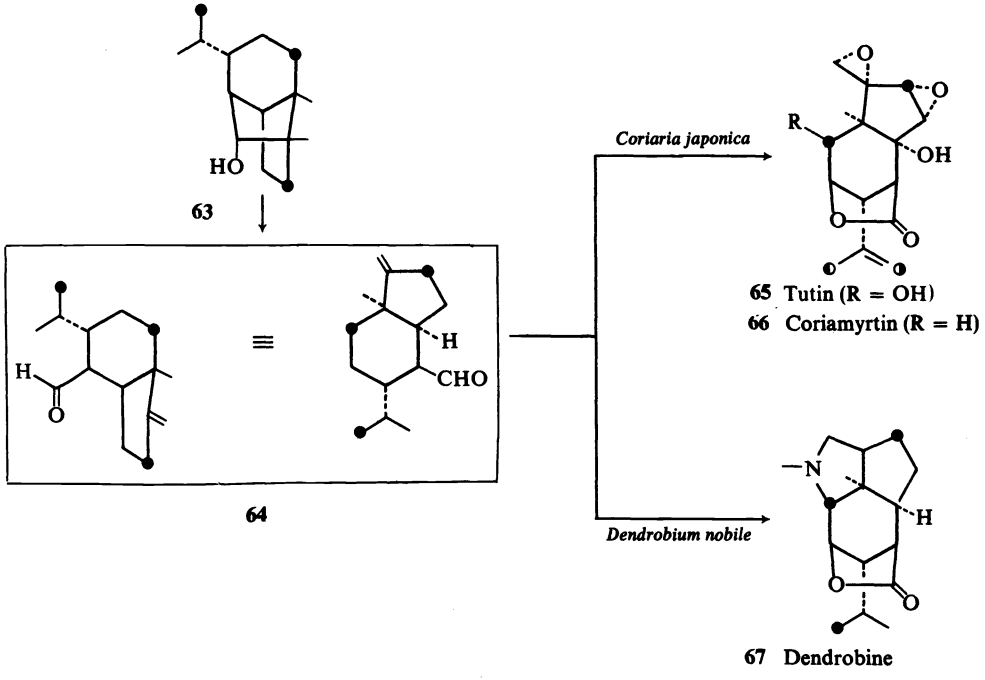
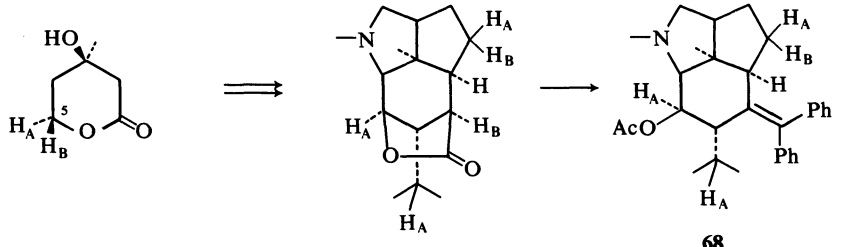



*Figure 18*

strated a direct relationship between these two bitter principles and copaborneol, **63**, probably via **64**. Moreover, the known[27] distribution of label from 2-[$^{14}$C]mevalonate in the cyclopentane ring of **65** ensures that the intermediate **63** is generated as a 'true' copa derivative, i.e. by the shorter of two possible routes. A complete loss of identity in the end groups of the isopropenyl side chain of **65** and **66** has been noted[27,28], and is likely to represent the unexpected outcome of a late and non-stereospecific dehydrogenation step. Whereas further studies on the mode of formation of the saturated side chain of the precursor **63** are partially thwarted in the case of tutin by the subsequent introduction of the double bond (however, cf. ref. 29), this disadvantage is not present in the case of dendrobine, which retains the side chain in an intact form. The mevalonoid origin of dendrobine had been reported[30] and quite recently[31] evidence has been presented that biosynthesis of the alkaloid involves a 1,3 hydride shift. We have now complemented this work by feeding to the plant optically pure (3*R*,5*S*)-5-[$^{3}$H]mevalonate ($H_B$ = $^{3}$H in *Figure 19*) and showing by degradation to



**67** **68**

*Figure 19*

**68** that one of the two tritium equivalents retained in the labelled dendrobine is located at the position α to the carbonyl group. Hence, the migrating hydrogen belongs in this case to the $H_A$ type.

As the fourth and last example of our studies in this area, we shall now consider the biosynthesis of longifolene (*Figure 20*). The (+)-form of this hydrocarbon, **71**, is known to occur in higher plants[32], mainly *Gymnospermae*, while the presence of its antipode, **74**, has been detected in liverworts[33]. We have now been able to show that the rare (−)-form co-occurs with (−)-sativene in both *H. sativum* and *H. victoriae*. Longifolene can be considered as a ring-expanded version of sativene; accordingly, its formation from an aliphatic precursor must involve a first cyclization to an eleven- rather than a ten-membered ring (cf. **69**). From here on, the proposed biogenetic scheme follows very closely the one which has been verified for sativene. Here again one may note that access to each of the enantiomers **71** and **74** is open in two ways which can intersect by a 1,2 migration of the $C_4$-bridge (**70** → **73**). Reasonable incorporations of activity (0.1 to 0.2 per cent) from radiolabelled mevalonates into (+)-longifolene could be achieved using cuttings of a young *Pinus ponderosa* tree (*Figure 21*). For the subsequent degradation work we were in the fortunate position of resorting to an exceptionally large body of known transformations. To gain an entry into

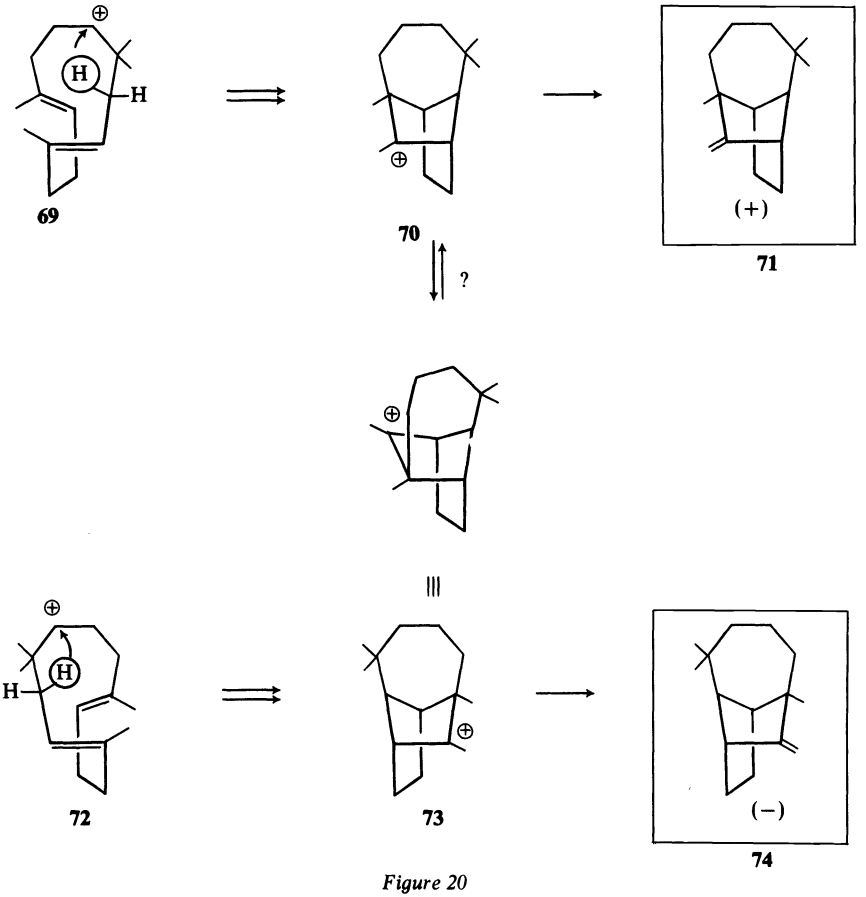



*Figure 20*

the four-carbon bridge, **71** was converted with acid into isolongifolene **75**[34]. In a separate investigation[35] we had succeeded in showing that this remarkably deep-seated rearrangement follows the mechanism first proposed by Berson *et al.*[36], and independent confirmation for this has now been provided by Sukh Dev and his colleagues[37]. In the present context it is sufficient to note that the large bridge of **75** corresponds entirely to the one of the starting material. Oxidation of **75** with bichromate afforded the enone **76** and the saturated ketone **77**[37]; the former could be used to assay the presence of tritium at C 4 and, through its base-catalysed enolization, at C 3 as well, while conversion of **77** to a phenyl carbinol followed by drastic oxidation allowed isolation of C-5 as the carbonyl group of benzoic acid. In a second degradation sequence **71** was converted by hydroboration under equilibrating conditions to **78**, and thence via **79** to the keto acid **80**[38], enolization of which enabled us to detect the presence of tritium at the C-10 methylene group. In this way it was possible to ascertain that (+)-longifolene is assembled in *P. ponderosa* from three mevalonate units along the shorter of the two possible routes. All of the hydrogen atoms from the

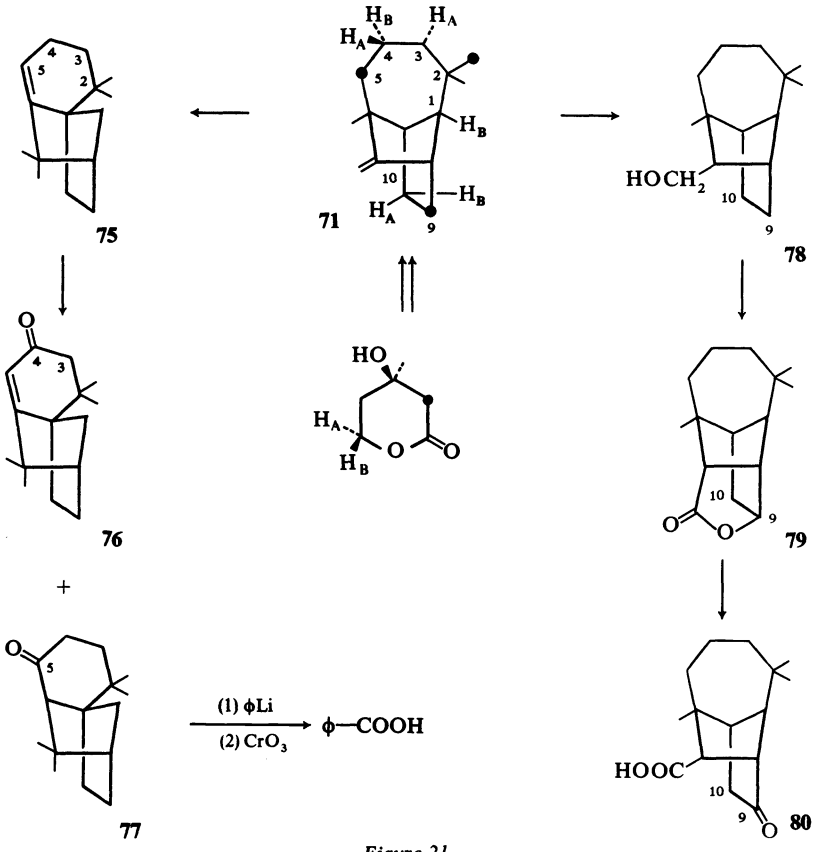



*Figure 21*

C-5 position of the precursor are retained in the biosynthesis, two of them appearing at C-4 and two others at C-10; the fifth hydrogen has moved to C-3 by a 1,3 shift, and it stems specifically from $H_A$ of the precursor, whence it follows that an $H_B$ hydrogen is retained at the bridgehead position.

In contrast to the previous cases, in which the stereochemistry of the first electrophilic attack is immediately apparent from the configuration of the generated chiral centre, the point of attachment of the isopropyl chain, no such guiding element is left in the longifolene molecule; the problem can be solved here only by detecting the steric location of the migrated hydrogen at C-3, its migration terminus (*Figure 22*). To this aim the starting material was converted by hydroboration to a separable mixture of the primary and secondary alcohols **81** and **82**, according to the directions worked out in Ourisson's group[39]. In the formation of **82** the endo hydrogen atom at C-3 jumps across the eight-membered ring to generate the secondary methyl group and therefore subsequent oxidation of **82** to the ketone **83** must involve specific removal of a proton originally located at the exo position of the starting material. When this sequence of reactions was applied to a sample of ( +) longifolene biosynthesized from (3*R*,5*R*)-5-[$^3$H]mevalonate

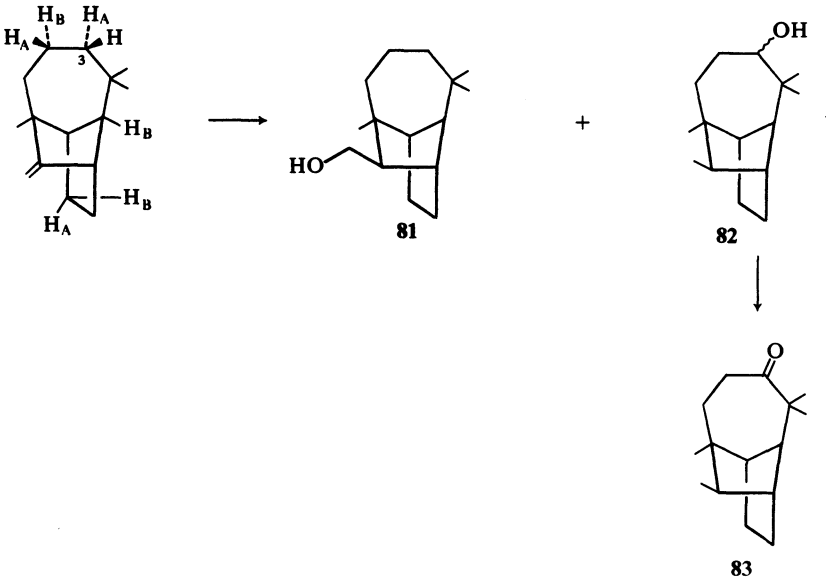



*Figure 22*

one third of the total tritium content was lost upon conversion to **83**. It follows that the migrated $H_A$-atom occupies an exo position at C-3 and this, in turn, uniquely defines the si-direction of attack on the relevant double bond during generation of the cycloundecane ion **69**.

The relevant data from all of these studies are summarized in *Figure 23* using the parent prototypes for purpose of better comparison. Though a complete set of experimental values on the mode of formation of (−)-longifolene, **88**, in *H. sativum* is not yet available, the results expected on the

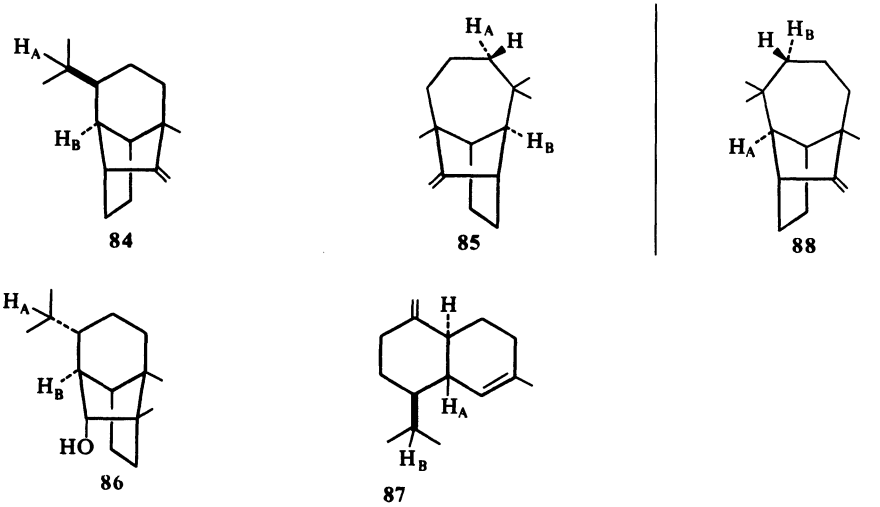



*Figure 23*

basis of its antipodal relationship to **85** have been included in the figure for the sake of convenience. In each of the cases investigated a 1,3 hydride shift has been detected, but additional salient features emerge from their confrontation: (a) hydrogen atoms of different origin undergo the 1,3 migration in the biosynthesis of **84** and **88**, a pair of co-occurring, configurationally related compounds, formation of which is initiated by attack on the same face of the relevant isopropylidene group in the aliphatic precursor (b) in the biosynthesis of cadalenes and their further cyclization products the origin of the migrating hydrogen atom does not correlate with a given steric arrangement of the resulting isopropyl side chain (cf. **84** versus **86**), but rather with the eventual configuration of the carbon centre from which it departed (cf. **84** and **86** versus **87**). This finding is of great importance, as it permits one to rule out a common germacrane-type intermediate, ionic or not, in the formation of **84** and **87**, two compounds which share the same configuration at the centre bearing the isopropyl group.

Can then a common basis be found for the biosynthesis of these compounds and, if so, what are the factors governing diversification? To find a reasonable answer to these questions we must leave the safe ground of experimentation and reconsider briefly the tantalizing problem of the formation and possible intermediacy of *cis* farnesyl-PP. A mechanism compatible with the necessary limitation that no proton loss is allowed in the generation of this compound can be formulated as in *Figure 24*. Starting with a fixed conformation of the *trans* isomer, **89a**, it involves a first suprafacial anionotropic rearrangement to (*R*)-nerolidyl-PP, which, following simple conformational change (**90a** → **90b**), is then converted to the desired material **91a** through a second rearrangement of the same type. A similar route can be traced from the antipodal conformation **89b** and takes to the same product through (*S*)-nerolidyl-PP, **92a** and **92b**. It will be seen that, regardless of which path is actually followed, the *trans–cis* isomerization of the double bond always results in an inversion of the original steric arrangement of the $H_A$- and $H_B$-atoms.

If the validity of this scheme is accepted we can then proceed to factorize the parameters which define the first cyclization step and the subsequent 1,3 hydride shift (*Figure 25*). These are: (1) the re- versus si-direction of attack on the isopropylidene double bond, (2) the *syn* versus *anti* conformation of the attacking segment of the cyclizing chain with respect to the olefinic hydrogen of the attacked double bond, and (3) the *trans* versus *cis* nature of the allylic double bond with a correspondingly fixed steric arrangement of the enantiotopic $H_A$- and $H_B$-atoms. The inclusion of Newman projections for (*R*)- and (*S*)-nerolidyl-PP in the scheme serves as a reminder of the fact that each of these structures can be converted to either of the farnesol derivatives with (crossed arrows) or without (vertical arrows) conformational change. Fixing these three parameters allows a clear-cut prediction as to which of the two heterotopic hydrogen atoms, $H_A$ or $H_B$, will undergo the 1,3 shift. In each of the cases investigated in our work both the origin of the migrating hydrogen and the face of the double bond which gets engaged in the cyclization have been firmly established. Working backwards from these data we can at last attempt to find out which com-

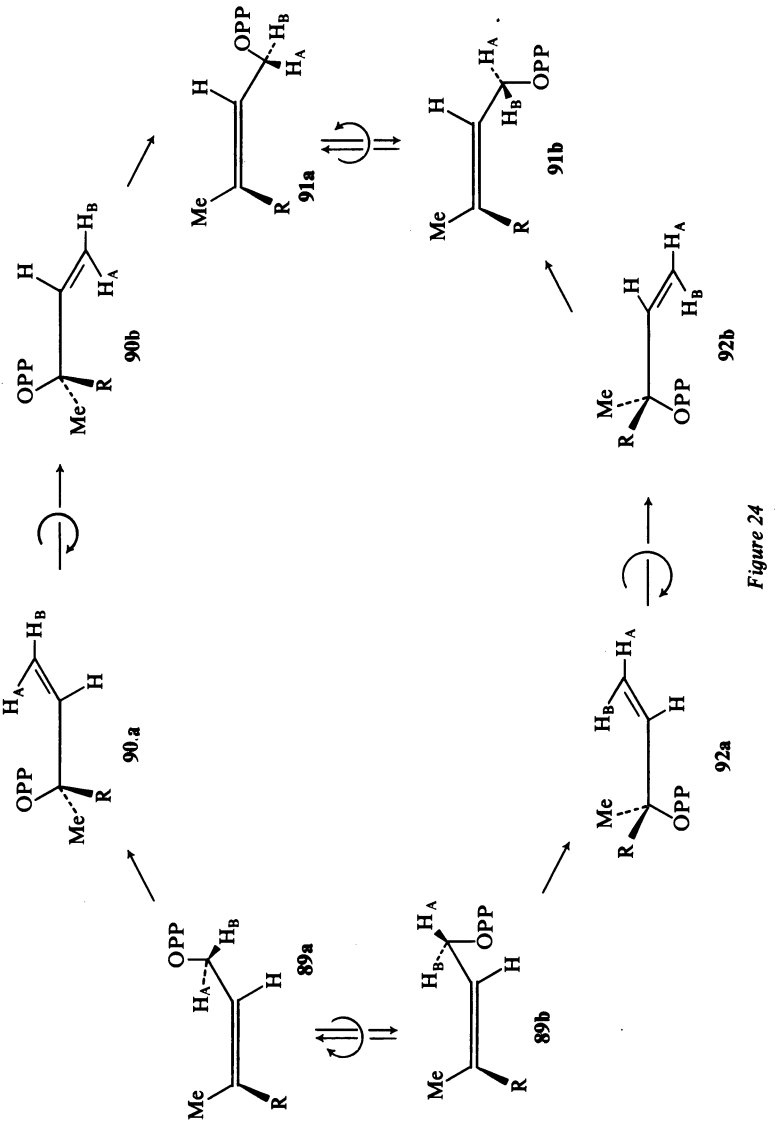



*Figure 24*

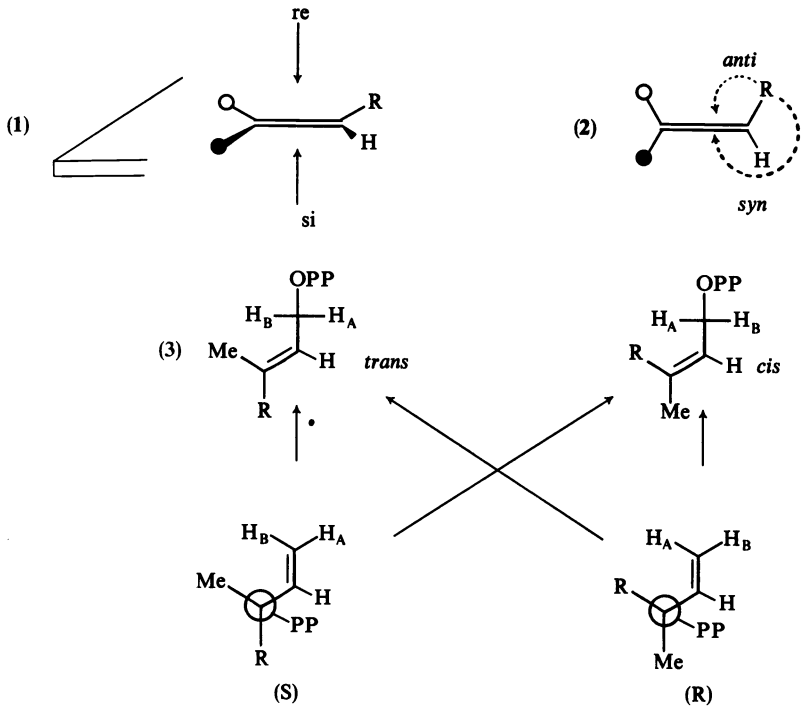



*Figure 25*

binations of the two remaining unknown parameters are still consistent with the experimental results. Within the framework of such an analysis the case of longifolene is now free of ambiguities. As indicated in B (*Figure 26*) for the (−)-antipode, the first cyclization must take place at the more substituted end on the re-face of the isopropylidene double bond from an *anti* conformation of a *cis* farnesyl-PP (which can be reached directly from (*S*)-nerolidyl-PP), and this enforces the subsequent migration of the $H_B$-atom. We note in passing that this arrangement defines the spatial location of seven out of the eleven members of the ring which is being closed. Formation of (−)-sativene is initiated essentially in the same manner (cf. A), with the only difference that bond formation now engages the less substituted end of the double bond, thus dictating specific migration of the $H_A$-atom. The marked similarities between A and B are reflected nicely in the co-occurrence of (−)-sativene and (−)-longifolene in *Helminthosporium* species[40] and of their antipodal counterparts in higher plants.[41] For the sake of completeness, we have included in *Figure 26* representations of the two arrangements C and D which can lead to dendrobine and avocettin starting from the same *cis* farnesol precursor; however, it must be emphasized that in each of these two cases the situation is not unequivocal and that an alternative involving a *trans* farnesol precursor (condensed in the squarebracketted symbols and discussed previously in detail for the case of avocettin) cannot be dismissed for the time being.

It will be interesting to learn in how far the concept developed herein

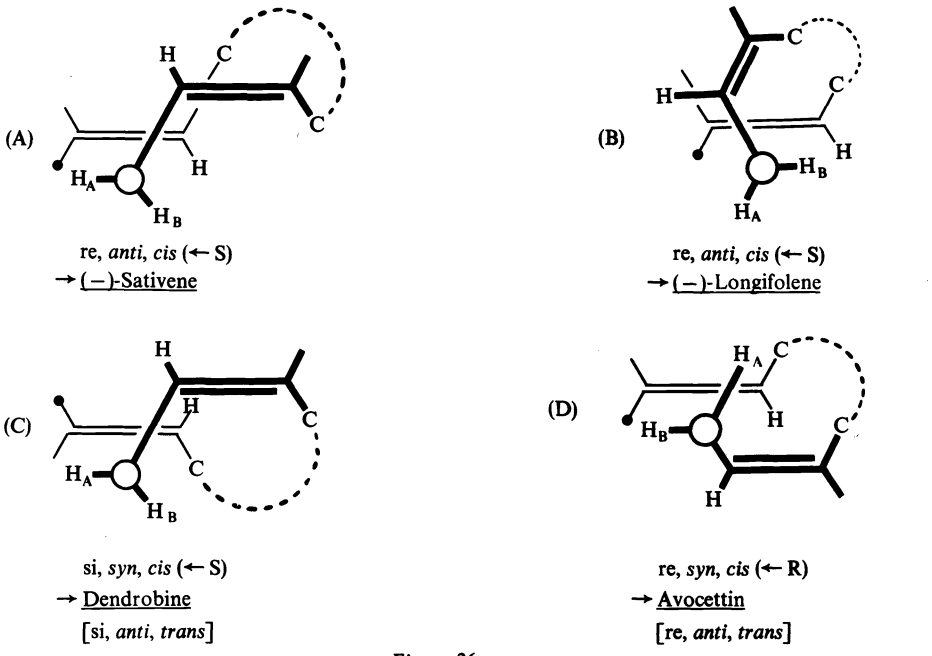



*Figure 26*

will stand the crucial test of further experimentation. At any rate, the picture which is slowly emerging indicates that in the biosynthesis of cadalane type and related sesquiterpenes the fate of the cyclizing precursor (and hence the nature of the cyclization product) is essentially imprinted on it by fixation of specific conformations on the appropriate enzyme surfaces. The larger stereodiversification which is observed in comparison with the higher homologues would then merely reflect the larger conformational mobility of ten- and eleven-membered rings with regard to their six-membered analogues.

## ACKNOWLEDGEMENT

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