J. Soc. Cosmet. Chem. **22** 231-248 (1971) © *1971 Society of Cosmetic Chemists of Great Britain*

The biogenesis of terpenoid essential oils

G. P. MOSS*

Presented on 20th April 1970 at the Symposium on "Perfumery", organised by the British Society of Perfumers and the Society of Cosmetic Chemists of Great Britain, at Eastbourne, Sussex.

Synopsis—The BIOSYNTHESIS of mono- and SESQUITERPENOIDS is reviewed with especial reference to the ESSENTIAL OILS.

INTRODUCTION

Although there has been considerable speculation (1-3) on the detailed routes by which plants produce terpenoid essential oils there has been relatively little direct study of this problem. This arises in part from some of the technical difficulties inherent in this research. With the present knowledge concerning the biosynthesis of selected terpenoids it is possible to rationalise most terpenoid structures in biosynthetic terms. However, it cannot be emphasised too strongly that there is no substitute for direct experimental proof. Although the biogenesis of a particular terpenoid usually appears obvious, the plant may have evolved a less direct, or even totally different approach to the synthesis of the compound.

Terpenoid biosynthesis may be conveniently considered in two stages – the formation of the universal acyclic precursors and related compounds and the formation of cyclic terpenoids. The acyclic precursors have been studied in considerable detail (4). Geranyl pyrophosphate and farnesyl pyrophosphate are then the precursors of all the numerous mono- and

*Department of Chemistry, Queen Mary College, London, E.1.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

sesquiterpenoid skeleta, and corresponding oxygenated compounds. The experimental evidence for this generalisation is considered below.

Biosynthesis of the acyclic precursors

Since the nineteenth century (5) it has been recognised that terpenes appear to be constructed from a common building unit, isoprene. Although without any chemical basis the "isoprene rule" has proved a valuable, but empirical, aid to structure determination. With the advent of isotopic labelling techniques, and hence direct experimental study of biosynthesis, the rule is now shown to be based on a fundamental biosynthetic principle. The "biogenetic isoprene rule" not only suggests (2) that all terpenoid substances are derived from a few selected acyclic precursors, but also

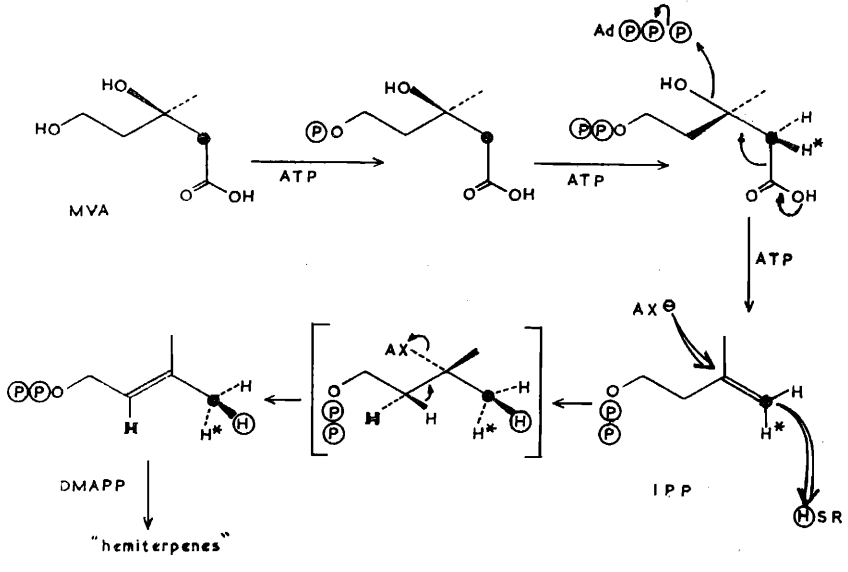

Figure 1

explains the increasing number of compounds which, although of terpenoid origin, do not fit the classical isoprene rule.

Isoprene itself is not involved. In 1956 a growth factor for *Lactobacillus acidophilus*, mevalonic acid, was discovered (6). It was realised that, though a C_6 compound, it was a true isoprenoid precursor. Detailed studies by Popják and Cornforth (4) on the biosynthesis of cholesterol, and the

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

corresponding acyclic precursor, squalene, from mevalonic acid showed the complete stereospecificity of every step in the biosynthesis. Their conclusions are summarised in *Figs. 1* and *2*.

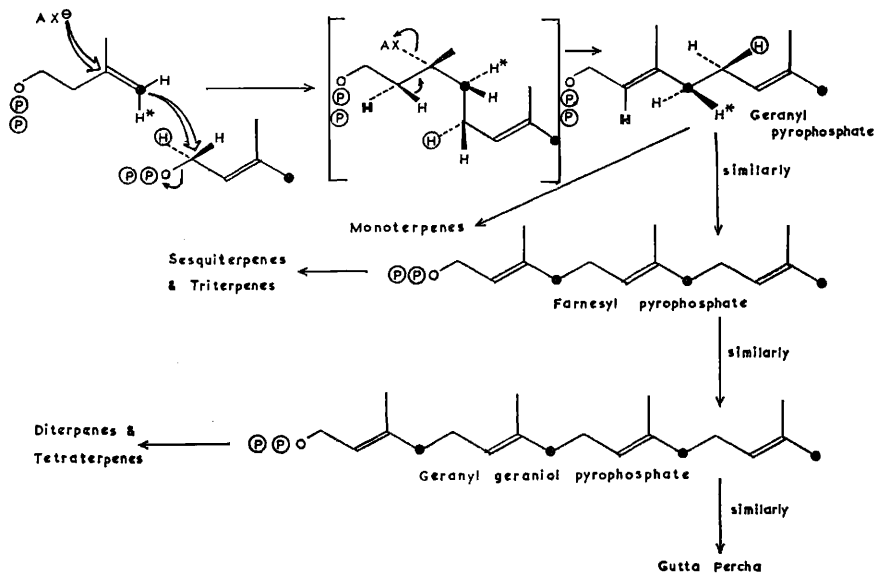

Figure 2

The initial stages (*Fig. 1*) involve phosphorylation of the primary alcohol group of mevalonic acid (MVA), followed by an eliminative decarboxylation to isopentenyl pyrophosphate (IPP) This compound is then isomerised to dimethylallyl pyrophosphate (DMAPP). Successive units of isopentenyl pyrophosphate are then added to dimethylallyl pyrophosphate to give (*Fig. 2*) a series of prenyl pyrophosphates, of which geranyl pyrophosphate and farnesyl pyrophosphate are the key precursors of the mono- and sesquiterpenoids respectively. The latter precursor is also used in the biosynthesis of the hydrocarbon squalene, from which all triterpenoids and steroids are derived. It is of note that at each stage where a prochiral (7) methylene group is involved the enzyme is completely stereospecific with respect to the proton utilised.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

Acyclic terpenoids

Although geranyl pyrophosphate and farnesyl pyrophosphate are key intermediates they have rarely been detected in nature. However the corresponding free alcohols are more frequently encountered, not only in the all *trans* form, but also the *cis* isomers, nerol and 2-*cis*-farnesol and the rearranged isomers linalool and nerolidol. It seems probable that the corresponding isomeric pyrophosphates are readily interconverted by intramolecular allylic rearrangements (*Fig. 3*). The enzymic formation of nerolidol from farnesyl pyrophosphate was demonstrated by Popják (8).

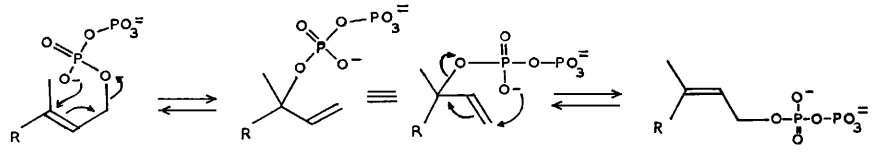

Figure 3

Recent work with rose petals by Francis (9) showed that although the free alcohols are formed from [2-^{14}C] mevalonic acid, most of the radioactivity is incorporated into the corresponding monoterpenoid glucosides.

Many terpenoids possess an acyclic carbon skeleton which is clearly derived by simple elimination, oxidation and/or reduction of the precursor (*Fig. 4*). Myrcene biosynthesis (10) in *Santolina*, and citral and citronellal in *Eucalyptus* (11), as well as ants (12), has been briefly studied. The ses-

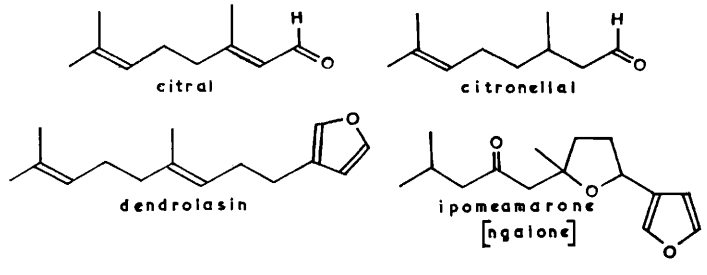

Figure 4

quiterpenoids dendrolasin (13) and ipomeamarone (14) were studied in ants. and sweet potatoes infected with *Ceratocystis fimbriata*, respectively.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

These compounds are representative of several essential oil components such as perillene, ngaione and elsholtzione. Other examples of acyclic terpenoids examined are the antibiotic mycelianamide (15), the alkaloid thiobinupharidin (16), and the bismonoterpenoid foliamenthin (17).

Cyclic monoterpenoids

The terpenoid compounds most frequently encountered in essential oils are based on the menthane skeleton or derived from further cyclisation of such a system (e.g. pinene). In order to generate the cyclohexane ring of menthane the C-1 position of the acyclic precursor must be joined to C-6. It seems probable that part of the driving force for this reaction is derived from the elimination of the pyrophosphate group from either neryl or linaloyl pyrophosphate (*Fig. 5*). (The *trans* double bond of geranyl pyrophosphate prevents C-1 from approaching C-6.) Recent work supports the involvement of linaloyl pyrophosphate (18, 19).

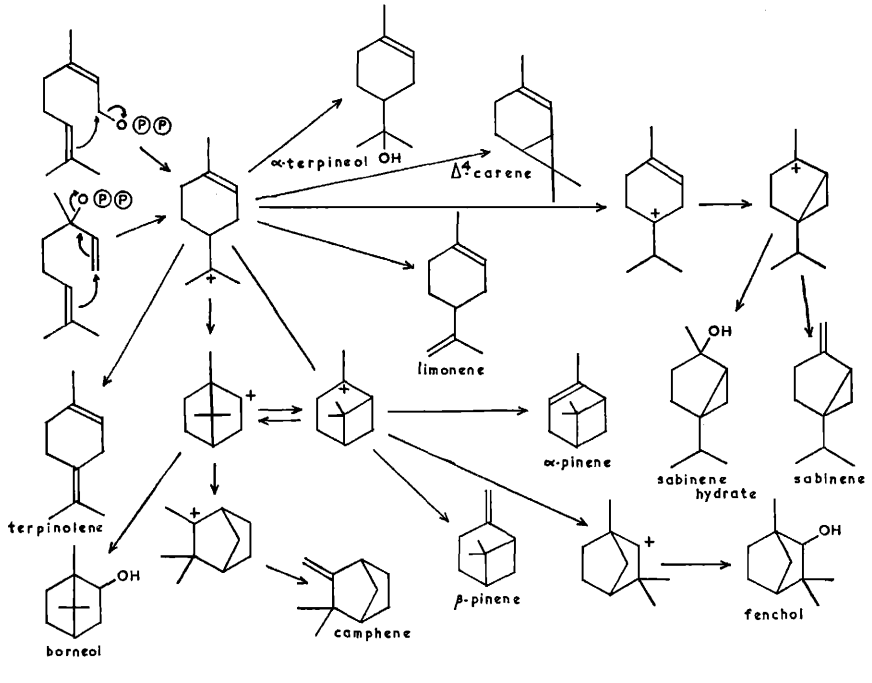

Figure 5

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

The carbonium ion, or biological equivalent, from the cyclisation may undergo many alternative processes depending on the plant or conditions. Simple hydration gives α-terpineol (19, 20) or an elimination of a proton may give Δ^4-carene, limonene (21-24), or terpinolene. If the remaining double bond is involved in cyclisation the new "ion" may give borneol, α-pinene (19, 23-27), or β-pinene (10, 23, 24, 27) or rearrange further to give camphene or fenchol. Alternatively a rearrangement of the "carbonium ion" by a 1,2-hydride shift gives a system which could give sabinene (19, 24, 28), or sabinene hydrate (24). Thus seven skeletal types may be generated from the one "carbonium ion" by elimination of a proton or hydration with or without rearrangement. However, of course, individual plants may only use one or a few of these processes.

Experimental work on the biosynthesis of cyclic monoterpenoids (*Fig. 5*) is limited. Present evidence suggests (19, 27) that many of the reactions, including some of the further oxidation processes, may be reversible. The further metabolism of these basic skeletal types may involve a combination of processes such as reduction and oxidation to give the plethora of monoterpenoid structures. For example α-terpineol may be considered (*Fig. 6*) to cyclise to 1,8-cineol. However, although there is good evidence for the terpenoid origin of 1,8-cineol with the expected labelling from the incorporation of [1-^{14}C]geranyl pyrophosphate (29), when Arigoni (30) studied the utilisation of [2-^{14}C]mevalonic acid he found complete randomisation. Presumably the precursor had been degraded to CO_2 by the plant before the precursor could reach the site of the relevant enzymes.

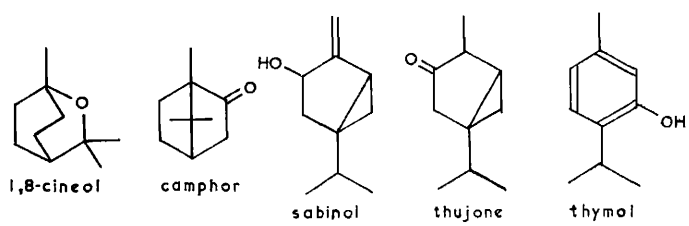

Figure 6

Another problem was encountered by Banthorpe (28, 31) when he studied camphor, sabinol and thujone biosynthesis (*Fig. 6*). Apart from a small amount of randomisation he found that up to 87% of the label was at one of the two positions instead of 50%. This result may be interpreted by postulating a pool of unlabelled dimethylallyl pyrophosphate in the plant

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

which dilutes the precursor so that in the biosynthesis of geranyl pyrophosphate (*Figs. 1* and *2*) from [2-^{14}C] mevalonic acid C-4 will be more radioactive than C-8. A similar reason may explain the results of Sandermann (21, 32) when he studied limonene (*Fig. 5*) and pulegone biosynthesis (*Fig. 7*), and possibly his results with α-pinene (26) and thujone (33). In the other cases

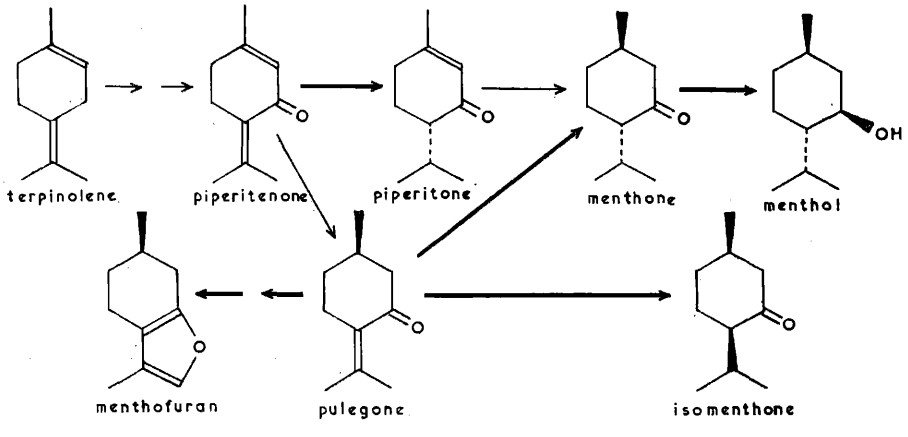

Figure 7

where the labelling pattern has been examined [carvone (22) and thymol (34)] the two positions are not clearly distinguished.

In the essential oil field probably the most studied system is *Mentha piperita*. Unfortunately the experimental problem necessitates much of the work being conducted with $^{14}CO_2$. However, this precursor does allow the study of the variation in concentration, specific activity, and total activity, with time (24, 35-37). Their conclusions are summarised in *Fig. 7*. A limited amount of work supports some of the steps, with the incorporation of piperitenone (37), pulegone (37, 38), and menthone (36). The general scheme seems to involve allylic oxidation to piperitenone, presumably via piperitenol, followed by successive reduction of the double bonds and finally the ketone function (or oxidation of pulegone to menthofuran).

Several other monoterpenoid skeleta are encountered in essential oils.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

The cyclopentanyl monoterpenoids (*Fig. 8*) have recently been extensively studied, especially since the discovery that loganin is the C_{10} precursor of

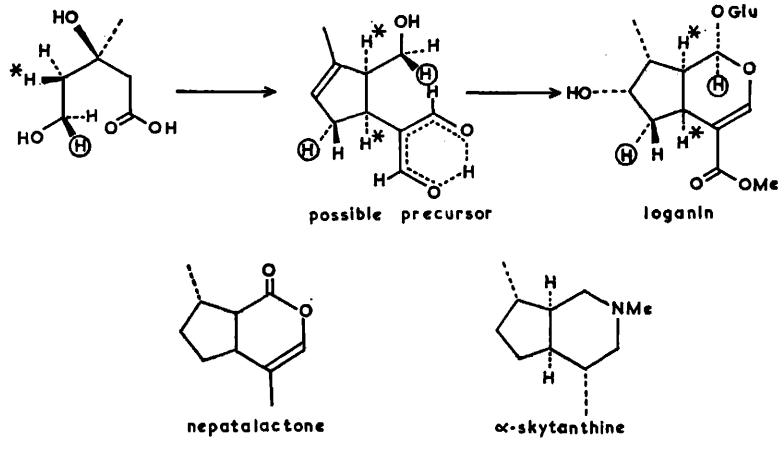

Figure 8

the indole alkaloids (39). Many of these monoterpenoids examined, including loganin, appear (40) to have involved an intermediate in their biosynthesis, such as the precursor shown (*Fig. 8*), where the two terminal aldehyde carbon atoms are equilibrated. Hence with [2-^{14}C]mevalonic acid approximately 50% of the radioactivity is in the ring position and 25% at each of the oxidised methyl groups. Recent work by Eisenbraun (41) showed that this effect depends on the age of the plant. More mature plants did not equilibrate the two terminal carbon atoms of skytanthine. He also obtained (42) similar results with the essential oil terpenoid, nepatalactone (*Fig. 8*).

Due to the stimulus of indole alkaloid biosynthesis many details of loganin biosynthesis are known. By studying the incorporation of [2-^{14}C, 3R, 4R-$^{3}H_1$]mevalonic acid and the 4S isomer, Battersby (43) and Coscia (44) showed that the two 4R protons were incorporated into the ring junction positions (H*). Even more interesting results were obtained by Arigoni (45) who showed that *both* [3R, 5R-$^{3}H_1$]mevalonic acid and [1S-$^{3}H_1$]geraniol were incorporated giving a radioactive acetal hydrogen atom. These results mean that *trans*-geranyl pyrophosphate is converted into geraniol in the plant with inversion of stereochemistry (*Fig. 8*).

There are a group of monoterpenoids which are not obviously derived from geranyl pyrophosphate (e.g. lavandulol). They have not been ade-

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

quately studied. Attempts to label artemisia ketone failed (10). However chrysanthemum carboxylic acid was labelled as expected (46) but this result provided little information on how the skeleton was constructed. Several mechanisms have been postulated (47).

Cyclic sesquiterpenoids

The biogenesis of sesquiterpenoids has been discussed (3) in detail. Four basic "carbonium ions" may be postulated (*Figs. 9* and *10*), derived from either *cis*- or *trans*-farnesyl pyrophosphate (or nerolidyl pyrophosphate). These are derived by displacement of the pyrophosphate group with cycli-

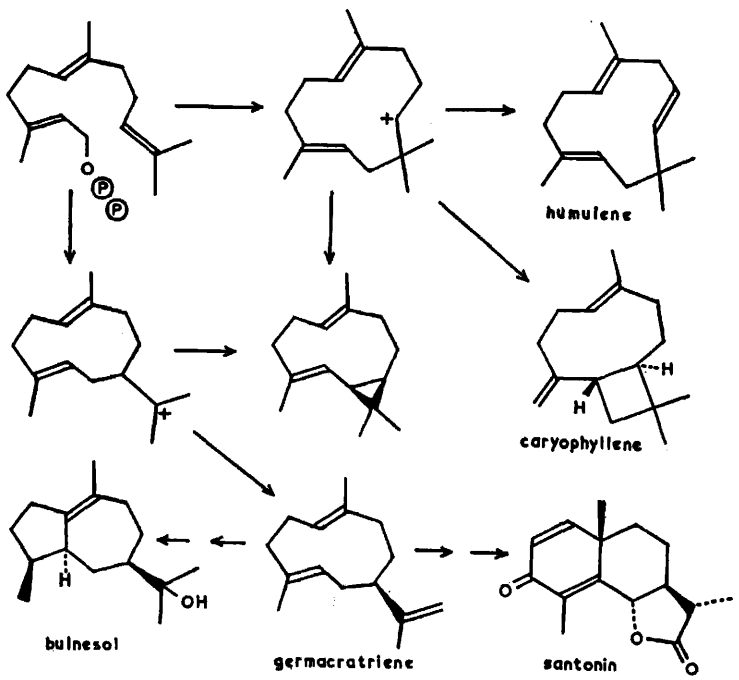

Figure 9

sation involving one of the double bonds. Steric requirements restrict the formation of 10- or 11-membered rings from all *trans*-farnesyl pyrophosphate and 6- or 7-membered rings from the 2-*cis* isomer.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

Elimination of a proton from the 11-membered ring "ion" (*Fig. 9*) will give all *trans*-humulene present in several essential oils. Alternatively further cyclisation will give caryophyllene. This sesquiterpenoid has been briefly studied (48). Humulene is presumably the precursor of the tricyclic fungal metabolites illudol (49), illudin (50) and marasmic acid (51).

The 10-membered ring "carbonium ion" from *trans*-farnesyl pyrophosphate on elimination of a proton would give germacratriene. Although a

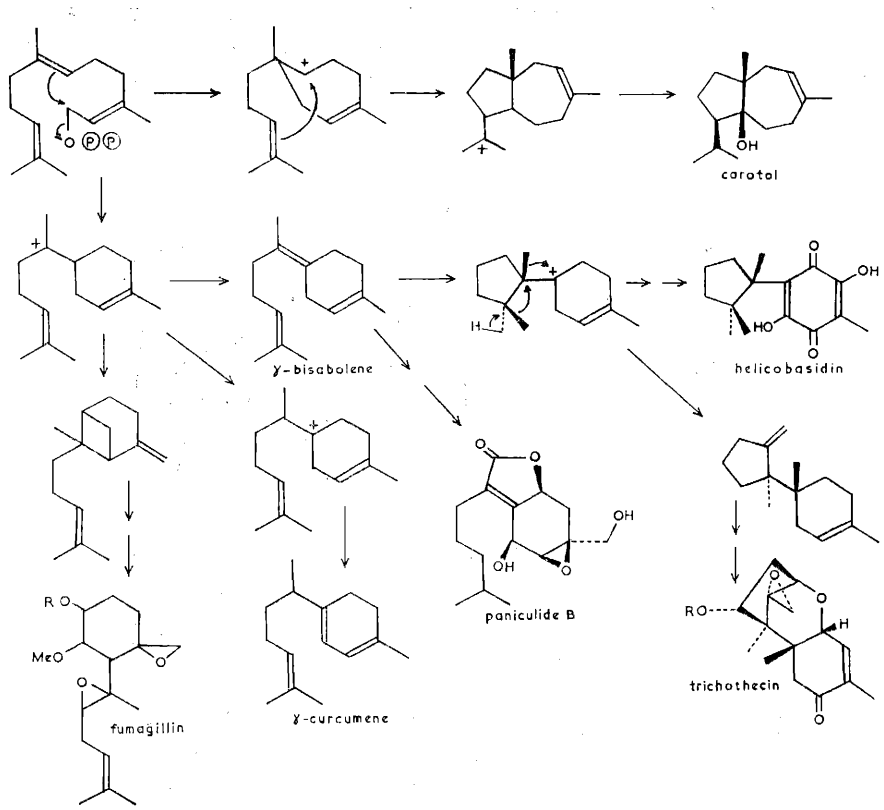

Figure 10

cyclodecadiene compound is clearly the precursor of a large group of sesquiterpenoids, santonin is the only example studied (52). This compound is the only sesquiterpenoid where details of the intermediate sesquiterpenoids have also been investigated. Probably germacratriene (or germacradienol formed by hydration of the "carbonium ion") is the precursor of all the eudesmane (e.g. santonin), and guaiane (e.g. bulnesol) sesquiter-

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

penoids, and their related rearrangement products. Several germacrane derived sesquiterpenoids have a cyclopropane ring which may be derived from either "carbonium ion" by loss of the adjacent allylic proton (*Fig. 9*).

Whenever a sesquiterpenoid contains a *cis*-double bond derived from the acyclic precursor it is most probably derived from 2-*cis*-farnesyl pyrophosphate. Although some biogenetic schemes postulate the involvement of 6-*cis*-farnesyl pyrophosphate there is no evidence for its occurrence

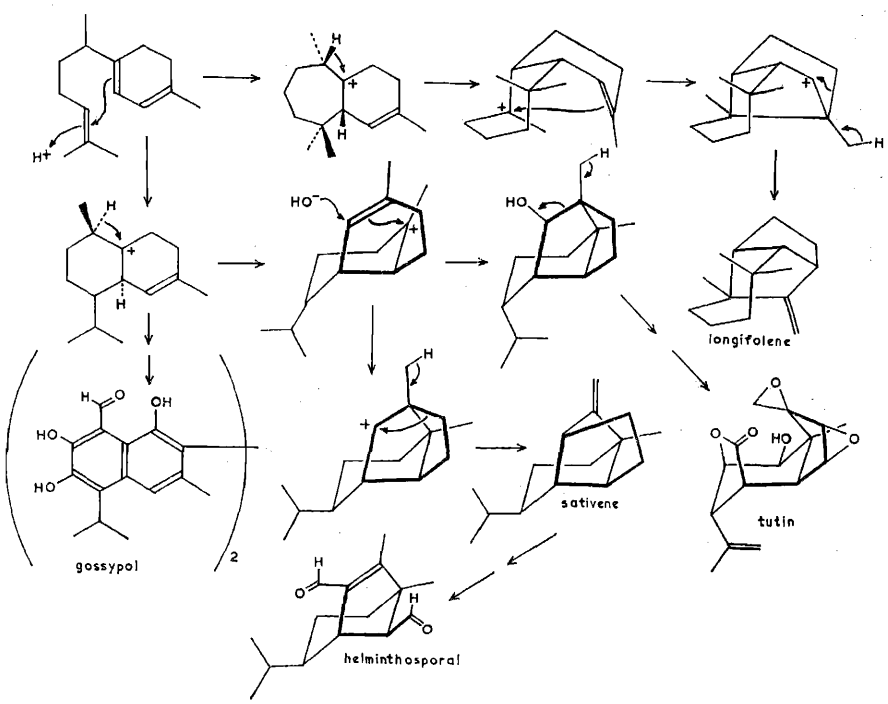

Figure 11

in nature. Initial cyclisation of 2-*cis*-farnesyl pyrophosphate will generate a 6- or 7-membered ring "carbonium ion" (*Fig. 10*). Few examples derived from the 7-membered ring system are known. One of these is carotol (53) which is probably derived by further cyclisation followed by a hydride shift and hydration at the ring junction.

A key intermediate postulated in the biosynthesis of many sesqui-

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

terpenoids is γ-bisabolene derived directly from the 6-membered ring "ion". The only example of this skeleton examined is paniculide B (54). However, a number of compounds derived from further cyclisation have been examined, in particular trichothecin (55), helicobasidin (56) and lagopodine B (57). Other products derived from the 6-membered ring "ion" involve either further cyclisation (fumagillin (58), or a 1,2-hydride shift followed by elimination of a proton to give γ-curcumene (or β-curcumene).

A large group of sesquiterpenoid skeleta may be considered as derivatives of γ-curcumene (*Fig. 11*) but may also be derived by several other routes (3). The only example of the cadinene group examined is gossypol (59), the dimer derived by phenol oxidation of a naphthalenoid precursor. More complex cyclisation processes are involved in the biosynthesis of longifolene (60), helminthosporal (61), dendrobine (62), coriamyrtin (63) and tutin (63). The last three sesquiterpenoids have the same carbon skeleton. Arigoni (63) found that the three positions labelled by [2-^{14}C]mevalonic acid were not equally radioactive. This result implies a pool of geranyl pyrophosphate (cf. camphor, etc. above).

The important plant hormone abscisic acid appears to be a sesquiter-

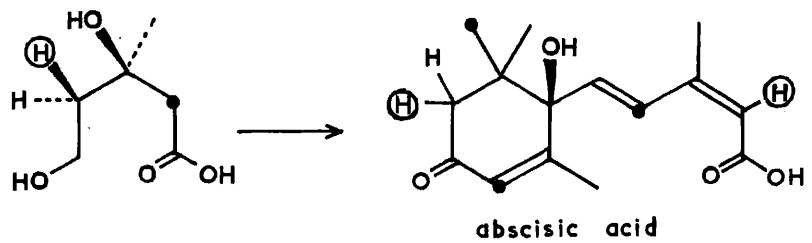

Figure 12

penoid. Biosynthetic results (65) support its terpenoid origin but do not distinguish between the direct route, and degradation of a carotenoid (66). Incorporation of [2-^{14}C, 3R, 4R-$^{3}H_1$] mevalonic acid shows (67) that an all-*trans*-farnesyl pyrophosphate was first formed in the biosynthesis (*Fig. 12*).

(Received: 3rd January 1970)

REFERENCES

(1) Clayton, R. B. Biosynthesis of sterols, steroids and terpenoids. *Quart. Rev.* **19** 168, 201 (1965). Nicholas, H. J. Biogenesis of naturally occurring materials. *in* Bernfeld, P. *The biogenesis of natural compounds* 2nd Edn. (1967) (Pergamon Press, Oxford); Pridham,

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

T. B. *Terpenoids in plants* (1967) (Academic Press); Rickards, J. H. and Hendrickson, J. B. *The biosynthesis of steroids, terpenes and acetogenins* (1964) (Benjamin); Teisseire, P. *Recherches* **17** 77 (1969).

(2) Ruzicka, L. Isoprene rule and the biogenesis of terpenic compounds. *Experientia* **9** 357 (1953); Perspektiven der Biogenese und der Chemie der Terpene. *Pure Appl. Chem.* **6** 493 (1963).

(3) Parker, W., Roberts, J. S. and Ramage, R. Sesquiterpene biogenesis. *Quart. Rev.* **21** 331 (1967).

(4) Popják, G. and Cornforth, J. W. Substrate stereochemistry in squalene biosynthesis. *Biochem. J.* **101** 553 (1966).

(5) Ruzicka, L. History of the isoprene rule. *Proc. Chem. Soc.* 341 (1959).

(6) Tavormina, P. A., Gibbs, M. H. and Huff, J. W. The utilization of β-hydroxy-β-methyl-δ-valerolactone in cholesterol biosynthesis. *J. Am. Chem. Soc.* **78** 4498 (1956); Tavormina, P. A. and Gibbs, M. H. The metabolism of β, γ-dihydroxy-β-methyl valeric acid by liver homogenates. *J. Am. Chem. Soc.* **78** 6210 (1956).

(7) Hanson, K. R. Applications of the sequence rule. I. Naming the paired ligands g,g at a tetrahedral atom Xggij. II. Naming the two faces of a trigonal atom Yghi. *J. Am. Chem. Soc.* **88** 2731 (1966).

(8) Popják, G. The biosynthesis of derivatives of allylic alcohols from [2-^{14}C] mevalonate in liver enzyme preparations and their relation to synthesis of squalene. *Tetrahedron Letters* 19 (1959 No. 19); see also Goodman, D. S. and Popják, G. Studies on the biosynthesis of cholesterol, XII. Synthesis of allyl pyrophosphate from mevalonate and their conversion into squalene with liver enzymes. *J. Lipid Res.* **1** 286 (1960).

(9) Francis, M. J. O. and O'Connell, M. The incorporation of mevalonic acid into rose petal monoterpenes. *Phytochem.* **8** 1705 (1969); see also Pasechnichenko, V. A. and Guseva, A. R. Essential oil biosynthesis by isolated rose petals. *Biochem. U.S.S.R.* **32** 1020 (1967); Guseva, A. R. and Pasechnichenko, V. A. Essential oil alcohols biosynthesis from a *Rosa damascena* × *R. Gallica* hybrid. *Biochem. U.S.S.R.* **31** 988 (1966).

(10) Waller, G. R., Frost, G. M., Burleson, D., Brannon, D. and Zalkow, L. H. Biosynthesis of monoterpenoids by *Santolina chamaecyparissus* L. *Phytochem.* **7** 213 (1968).

(11) Birch, A. J., Boulter, D., Fryer, R. I., Thomson, P. J. and Willis, J. L. The biosynthesis of citronellal and of cineole in *Eucalyptus. Tetrahedron Letters* 1 (1959) No. 3; Neethling, L. P., Reiber, H. G. and Chichester, C. O. Biosynthesis of citral in *Eucalyptus staigeriana. Proc. Nat. Conf. Nucl. Energy, Pretoria*, 451 (1963).

(12) Happ, G. M. and Meinwald, J. Biosynthesis of arthropod secretions. I. Monoterpene synthesis in an ant (*Acanthomyops claviger*). *J. Am. Chem. Soc.* **87** 2507 (1965).

(13) Waldner, E. E., Schlatter, Ch. and Schmid, H. Zur Biosynthese des Dendrolasins, eines Inhaltsstoffes der Ameise *Lasius fuliginosus LATR. Helv. Chim. Acta*, **52** 15 (1969).

(14) Oguni, I., Oshima, K., Imaseki, H. and Uritani, I. Biochemical studies on the terpene metabolism in sweet potato root tissue with blackrot. *Agri. Biol. Chem.* **33** 50 (1969) and refs. therein.

(15) Birch, A. J., Kocór, M., Sheppard, N. and Winter, J. Studies in relation to biosynthesis. Part XXIX. The terpenoid chain of mycelianamide. *J. Chem. Soc.* 1502 (1962).

(16) Schütte, H. R. and Lehfeldt, J. Biosynthese von *Nuphar luteum* alkaloids. *Arch. Pharm.* **298** 461 (1965).

(17) Battersby, A. R., Burnett, A. R., Knowles, G. D. and Parsons, P. G. Seco-cyclopentane glucosides from *Menyanthes trifoliata:* foliamenthin, di-hydrofoliamenthin, and menthiafolin. *Chem. Comm.* 1277 (1963); Loew, P., Szczepanski, Ch. V., Coscia, C. J. and Arigoni, D. The structure and biosynthesis of foliamenthin. *Chem. Comm.* 1276 (1968).

(18) Attaway, J. A. and Buslig, B. S. The metabolism of linalool in *Citrus* plants. *Phytochem.* **8** 1671 (1969); Potty, V. H., Moshonas, M. G. and Bruemmer, J. H. Cyclisation of linalool by enzyme preparation from orange. *Arch. Biochem.* **138** 350 (1970); see however Cori, O. Terpene biosynthesis: utilisation of neryl pyrophosphate by an enzyme system from *Pinus radiata* seedlings. *Arch. Biochem. Biophys.* **135** 416 (1969); Escher, S., Loew, P. and Arigoni, D. The role of hydroxygeraniol and hydroxynerol in the biosynthesis of loganin and indole alkaloids. *Chem. Comm.* 823 (1970); Battersby, A. R., Brown, S. H. and Payne, T. G. Biosynthesis of loganin and the indole alkaloids from hydroxygeraniol-hydroxynerol. *Chem. Comm.* 827 (1970).

(19) Banthorpe, D. V. and Wirz-Justice, A. Terpene biosynthesis. Part I. Preliminary tracer

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

studies on terpenoids and chlorophyll of *Tanecetum vulgare* L. *J. Chem. Soc.C* 541 (1969).

(20) Beytía, E., Valenzuela, P. and Cori, O. Terpene biosynthesis: formation of nerol, geraniol, and other prenols by an enzyme system from *Pinus radiata* seedlings. *Arch. Biochem. Biophys.* **129** 346 (1969).

(21) Sandermann, W. and Bruns, K. Über die Biogenese des Limonens in *Pinus pinea* L. *Naturwiss*, **49** 258 (1962).

(22) Sandermann, W. and Bruns, K. Biogenese von carvon in *Anethum graveoleus* L. *Planta Med.* **13** 364 (1965).

(23) Valenzuela, P., Cori, O. and Yudelevich, A. Occurrence of monoterpenes in *Pinus radiata* and utilization of labelled CO_2 and mevalonic acid. *Phytochem.* **5** 1005 (1966).

(24) Hefendehl, F. W. Beitrage zur Biogenese ätherischer Öle, die Rolle der Kohlenwasserstoffe bei der Biogenese sauerstoffhaltiger Monoterpene. *Planta Med.* **15** 121 (1967).

(25) Stanley, R. G. Terpene formation in pine from mevalonic acid. *Nature*, **182** 738 (1958).

(26) Sandermann, W. and Schweers, W. Über die Biogenese von α-pinen in *Pinus nigra austriaca. Tetrahedron Letters*, 257 (1962).

(27) Schweers, W. Über die weitere umwandlung von α-pinen in *Pinus ponderosa. Tetrahedron Letters*, 4425 (1968).

(28) Banthorpe, D. V. and Turnbull, K. W. The biosynthesis of thujane derivatives in higher plants. *Chem. Comm.* 177 (1966).

(29) Achilladelis, B. and Hanson, J. R. Studies in terpenoid biosynthesis. III. The incorporation of geranyl pyrophosphate into cineole. *Phytochem.* **7** 1317 (1968); see also (11).

(30) Arigoni, D. quoted by Loomis, W. D. in Pridham, J. B. *Terpenoids in Plants* 65 (1967). (Academic Press, London and New York).

(31) Banthorpe, D. V. and Baxendale, D. The biosynthesis (+)- and (—)-camphor. *Chem. Comm.* 1553 (1968).

(32) Sandermann, W. and Stockmann, H. Über die Biogenese von Pulegon. *Chem. Ber.* **91** 930 (1958).

(33) Sandermann, W. and Schweers, W. Über die Biogenese von Thujon in *Thuja occidentalis. Tetrahedron Letters*, 259 (1962).

(34) Yamazaki, M., Usui, T. and Shibata, S. Biogenesis of plant products. II. Biogenesis of thymol. *Chem. Pharm. Bull.* **11** 363 (1963).

(35) Battu, R. G. and Youngken, H. W. Jr. Biogenesis of terpenoids in *Mentha piperita*. I. Monoterpenes. *Lloydia* **29** 360 (1966); Burbott, A. J. and Loomis, W. D. Evidence for metabolic turnover of monoterpenes in peppermint. *Plant Physiol.* **44** 173 (1969); Hefendehl, F. W. Der Einbau von Mevalonsäure in die Terpene des ätherischen Öls von *Mentha piperita. Planta Med.* **14** 66 (1966); Hefendehl, F. W., Underhill, E. W. and von Rudloff, E. The biosynthesis of the oxygenated monoterpenes in mint. *Phytochem.* **6** 823 (1967).

(36) Reitsema, R. H., Cramer, F. J., Scully, N. J. and Chorney, W. Essential oil synthesis in mint. *J. Pharm. Sci.* **50** 18 (1961).

(37) Battaile, J. and Loomis, W. D. Biosynthesis of terpenes. II. The site and sequence of terpene formation in peppermint. *Biochim. Biophys. Acta*, **51** 545 (1961).

(38) Battaile, J., Burbott, A. J. and Loomis, W. D. Monoterpene interconversions: metabolism of pulegone by a cell-free system from *Mentha piperita. Phytochem.* **7** 1159 (1968).

(39) Bégué, J. P. Acquisitions récentes en biosynthèse des alcoloïdes indoliques. *Bull. Soc. Chim. France*, 2545 (1969); Leete, E. Biosynthesis of quinine and related alkaloids. *Acc. Chem. Res.* **2** 59 (1969).

(40) Yeowell, D. A. and Schmid, H. Zur Biosynthese des Plumierids. *Experientia* **20** 250 (1964); Hüni, J. E. S., Hittebrand, H., Schmid, H., Gröger, D., Johne, S. and Mothes, K. Zur Biosynthese des Verbenalins and Aucubins. *Experientia* **22** 656 (1966); see also Inouye, H., Ueda, S. and Nakemura, Y. Zur Biosynthese der bitteren Glucoside der Gentianazeen, des Gentiopicrosids, des Swertiamarins and Swerosids. *Tetrahedron Letters* 3221 (1967); Coscia, C. J. and Guarnaccia, R. Biosynthesis of gentiopicroside, a novel monoterpene. *J. Am. Chem. Soc.* **89** 1280 (1967); Gröger, D. and Simchen, P. Uber den Einbau von Loganin in Gentiopikroside. Z. *Naturf.* **24b** 356 (1969); Coscia, C. J., Guarnaccia, R. and Botta, L. Monoterpene biosynthesis. I. Occurrence and mevalonoid origin of gentiopicroside and loganic acid in *Swertia caroliniensis. Biochem.* **8** 5036 (1969).

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

(41) Auda, H., Juneja, H. R., Eisenbraun, E. J., Waller, G. R., Kays, W. R. and Appel, H. H. Biosynthesis of methylcyclopentane monoterpenes. I. *Skytanthus alkaloids*. *J. Am. Chem. Soc.* **89** 2476 (1967); Horodysky, A. G., Waller, G. R. and Eisenbraun, E. J. Biosynthesis of methylcyclopentane monoterpenes. IV. Verbenalin. *J. Biol. Chem.* **244** 3110 (1969).

(42) Regnier, F. E., Waller, G. R., Eisenbraun, E. J. and Auda, H. The biosynthesis of methylcyclopentane monoterpenes. II. Nepetalactone. *Phytochem.* **7** 221 (1968); see also Meinwald, J., Happ, G. M., Labows, J. and Eisner, T. Cyclopentanoid terpene biosynthesis in a phasmid insect and in catmint. *Science*, **151** 79 (1966).

(43) Battersby, A. R., Byrne, J. C., Kapil, R. S., Martin, J. A., Payne, T. G., Arigoni, D. and Loew, P. The mechanism of indole alkaloid biosynthesis. *Chem. Comm.*, **951** (1968).

(44) Guarnaccia, R., Botta, L. and Coscia, C. J. Mechanism of indole alkaloid biosynthesis. *J. Am. Chem. Soc.* **91** 204 (1969); Coscia, C. J., Botta, L. and Guarnaccia, R. On the mechanism of iridoid and secoiridoid monoterpene biosynthesis. *Arch. Biochem. Biophys.* **136** 498 (1970).

(45) Arigoni, D. Chemical Society Simonsen Lecture (12th November, 1969).

(46) Crowley, M. P., Godin, P. J., Inglis, H. S., Snarey, M. and Thain, E. M. The biosynthesis of the "pyrethrins". I. The incorporation of ^{14}C-labelled compounds into the flowers of *Chrysanthemum cinerariaefolium* and the biosynthesis of chrysanthemum monocarboxylic acid. *Biochim. Biophys. Acta* **60** 312 (1962).

(47) Mukherji, S. M. Biogenesis of 3,4-coupling of isoprenoids. *J. Ind. Chem. Soc.* **41** 309 (1964); Bates, R. B. and Paknikar, S. K. Terpenoids. IX. Biogenesis of some monoterpenoids not derived from a geranyl precursor. *Tetrahedron Letters* 1453 (1965).

(48) Regnier, F. E., Eisenbraun, E. J., Waller, G. R. and Auda, H. The biosynthesis of nepetalactone and caryophyllene. *Abs. Am. Chem. Soc. Meeting*, **150** 116C (1965).

(49) McMorris, T. C., Nair, M. S. R. and Anchel, M. The structure of illudol, a sesquiterpenoid triol from *Clitocybe illudens*. *J. Am. Chem. Soc.* **89** 4562 (1967).

(50) McMorris, T. C. and Anchel, M. Fungal metabolites. The structure of the novel sesquiterpenoids illudin-S and -M. *J. Am. Chem. Soc.* **87** 1594 (1965).

(51) Dugan, J. J., de Mayo, P., Nisbet, M., Robinson, J. R. and Anchel, M. Terpenoids. XIV. The constitution and biogenesis of marasmic acid. *J. Am. Chem. Soc.* **88** 2838 (1966).

(52) Barton, D. H. R., Moss, G. P. and Whittle, J. A. Investigations on the biosynthesis of steroids and terpenoids. Part I. A preliminary study of the biosynthesis of santonin. *J. Chem. Soc.C*, 1813 (1968).

(53) Souček, M. Terpenes CXLVIII. Biosynthesis of carotol in *Daucus carota*. A contribution to configuration of carotol and daucol. *Collection Czech. Chem. Commun.* **27** 2929 (1962).

(54) Allison, A. J., Butcher, D. N., Connolly, J. D. and Overton, K. H. Paniculides A, B, and C, bisabolenoid lactones from tissue cultures of *Andrographis paniculata*. *Chem. Comm.* 1493 (1968).

(55) Jones, E. R. H. and Lowe, G. The biogenesis of trichothecin. *J. Chem. Soc.* 3959 (1960); see also for verrucarin Kocór, M. and Siewiński, A. Biosynthesis of verrucarol. *Bull. Acad. Polon. Sci. Ser. Sci. Chem.* **14** 341 (1966); Achilladelis, B., Adams, P. M. and Hanson, J. R. The biosynthesis of the sesquiterpenoid tricothecane antibiotics. *Chem. Comm.* 511 (1970).

(56) Natori, S., Inouye, Y. and Nishikawa, H. The structures of mompain and deoxyhelicobasidin and the biosynthesis of helicobasidin, quinonoid metabolites of *Helicobasidium mompa* Tanaka. *Chem. Pharm. Bull. Tokyo* **15** 380 (1967); Bentley, R. and Chen, D. Helicobasidin: a fungal benzoquinone of isoprenoid origin. *Phytochem.* **8** 2171 (1969).

(57) Bollinger, P. Über die Konstitution und Konfiguration der Lagopodine A, B and C. Thesis No. 3595, E.T.H., Zürich (1965).

(58) Birch, A. J. and Hussain, S. F. Studies in relation to biosynthesis. Part XXXVIII. A preliminary study of fumagillin. *J. Chem. Soc.C* 1473 (1969).

(59) Heinstein, P. F., Smith, F. H. and Tove, S. B. Biosynthesis of C^{14}-labelled gossypol. *J. Biol. Chem.* **237** 2643 (1962); *Fed. Proc.* **23** 425 (1964)

(60) Sandermann, W and Bruns, K. Über die Biogenese von Longifolen in *Pinus longifolia* Roxb. *Chem. Ber.* **95** 1863 (1962).

(61) de Mayo, P., Robinson, J. R., Spencer, E. Y. and White, R. W. The biogenesis of helminthosporal. *Experientia* **18** 359 (1962).

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

(62) Yamazaki, M., Matsuo, M. and Arai, K. Biosynthesis of dendrobine. *Chem. Pharm. Bull. Tokyo* **14** 1058 (1966).
(63) Biollaz, M. and Arigoni, D. Biosynthesis of coriamyrtin and tutin. *Chem. Comm.* 633 (1969).
(64) Corbella, A., Gariboldi, P., Jommi, G. and Scolastico, C. Biosynthesis of tutin. *Chem. Comm.* 634 (1969).
(65) Noddle, R. C. and Robinson, D. R. Biosynthesis of abscisic acid: incorporation of radioactivity from [2-^{14}C] mevalonic acid by intact fruit. *Biochem. J.* **112** 547 (1969)
(66) Taylor, H. F. and Smith, T. A. Production of plant growth inhibitors from xanthophylls: a possible source of dormin. *Nature* **215** 1513 (1967); Taylor, H. F. Carotenoids as possible precursors of abscisic acid in plants. *Soc. Chem. Ind. Monograph* **31** 22 (1968).
(67) Milborrow, B. V. Current research on abscisic acid. *Biochem. J.* **114** 1P (1969); Robinson, D. R. and Ryback, G. Incorporation of tritium from [(4R)-4-^{3}H] mevalonate into abscisic acid. *Biochem. J.* **113** 895 (1969).

DISCUSSION

DR. P. J. DUNPHY: In view of the probable necessity for a 2,3 (cis) double bond (nerol) or the isomeric linalool, as the precursor in the biosynthesis of cyclic monoterpenes is it possible that the generation of such double bonds proceeds by direct *cis* synthesis, as in *Hevea* (polycis) rubber rather than through the all-*trans* isomer geraniol?

THE LECTURER: On paper this certainly seems quite reasonable. The present evidence, however, suggests that all the monoterpenes and sesquiterpenes (and in fact diterpenes, triterpenes and carotenoids) are derived from an all-*trans* precursor. There is nothing inherently wrong with a *cis* double bond, but studies with stereospecifically labelled mevalonic acids so far completely eliminate that possibility. With [4R-^{3}H] mevalonic acid the tritium atom is retained when a *trans* double bond is generated. In the few cases where this has been studied the 4 R tritium atom is incorporated. There is no example of 4 S incorporation. *Fig. 12*, for instance, shows abscisic acid. The 4 R tritium atom of mevalonic acid is incorporated, not 4 S, although there is a *cis* double bond. Hence there must have been an isomerization of the double bond with retention of the vinyl proton in the biosynthesis.

DR. DUNPHY: Equally there are systems where you get direct *cis* synthesis and the epimeric proton is retained. *Hevea* rubber is a good example of this with a poly *cis* system.

THE LECTURER: This is the only one though.

DR. DUNPHY: In fact there is a mixed system as well. Many of the poly *iso*prenoid alcohols, which are poly *cis/trans* alcohols, appear to be generated in this way. The *trans* double bond is synthesised, as you would expect, with the retention of the 4 R proton of mevalonic acid, while with the *cis* double bond it is the other proton that is retained.

THE LECTURER: This is right. Polyprenols are long chain terpenoid alcohols, composed of 6–24 *iso*prene units. Most of them appear to contain only two or three *trans* double bonds, the remainder being *cis*. In their biosynthesis three or four (i.e. including the terminal double bond) *iso*prene units retain the 4 R proton of mevalonic acid and the remainder retain the 4 S proton. This evidence suggests that the plant

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

produces all *trans* farnesyl pyrophosphate, or all *trans* geranyl geranyl pyrophosphate, and then synthesises these higher *iso*prenoid compounds, by adding on *cis* isoprene units. This conclusion is somewhat speculative because the position of the tritium atoms along the chain has not been identified; however, it is certain that enzymes able to generate *cis* double bonds are present.

The biosynthesis of the indole alkaloids proceeds via the monoterpenoid loganin (*Fig. 8*). In this case the 4 R proton of mevalonic acid is retained by all *trans* geraniol, so that an isomerization to nerol must have occurred before cyclization to the cyclo—pentanyl monoterpenoid.

A MEMBER OF THE AUDIENCE: I was surprised when you were discussing dimethylallyl pyrophosphate and mentioned the stereo chemistry of the methylene carbon atom. This thrilled me quite considerably as I thought at least this part was elementary —and that one would not have expected any asymmetry at this carbon atom.

THE LECTURER: Most of the work I have described was conducted by Popják and Cornforth (4) who, in particular, were studying squalene biosynthesis. However, I am certain that their results can be applied to all terpenoids, and I think it came as a surprise to a lot of people that it was so stereo specific. At any stage where the enzyme has to select one of two atoms (where the organic chemist would say that there is nothing to choose between them) the enzyme in every respect appears to stereo specifically select only one of the two hydrogen atoms. Although they have not yet proved, as far as I am aware, that an "optically active methyl group" is generated (*Fig. 1*), I think there is every chance it will be found to be so. Certainly a number of other enzyme systems do involve "optically active methyl groups". An enzyme is made up of optically active α-amino acids, so that the enzyme is optically active in its own right. Hence it will normally generate an optically active product even though the starting material was optically inactive.

MR. D. E. BUTTERFIELD: Would you comment on possible practical applications of the knowledge of biogenesis for the future?

THE LECTURER: It is, of course, always very difficult to speculate in this way and one is almost bound to be caught out, but on the other hand I think one could make one or two comments. As I see essential oil chemistry developing, one is more and more going to require specific compounds. However, the plant certainly has the edge on the chemist in its ability to generate those trace components which give a particular fragrance or aroma. From the commercial point of view essential oils are an important source of complex molecules. In many cases the organic chemist can synthesise with great difficulty and in very small yield, a number of these compounds, but if we want to use them as a raw material it is necessary to use the natural source. Hence the problem is that if a particular compound is known to be produced by a plant in the remote Amazon jungle it may not be a useful commercial source. One then wants to consider where there might be a useful source of this particular compound. I think that this is where biosynthesis may have a part to play. The methods by which plants generate these complex molecules can often be related to our knowledge of plant taxonomy; one is complementary to the other. This suggests the botanical families or species which might be worth investigating if one is looking for a particular compound.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

Another area which is proving very important is the application of mono or sesquiterpenoids to pest control. The area of insect hormones and related compounds is certainly going to be a very important field in the future. Another group of interesting compounds is the cytotoxic sesquiterpenoids. These may have applications in cancer chemotherapy so that specific terpenoids need to be searched for. Abscisic acid (*Fig. 12*) is a very important plant hormone. There may be analogues of this compound as we do not know how universal this hormone is. Much of the present work relies on the very large specific rotation of abscissic acid so that it can be detected in very small quantities. However, there may well be analogues which are even more active biologically that have as yet to be found.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)