THE BIOSYNTHESIS OF MONOTERPENES

D. V. BANTHORPE,* B. V. CHARLWOOD,† AND M. J. O. FRANCIS‡

Christopher Ingold Laboratories, University College, London, England, Department of Pharmacognosy, The School of Pharmacy, University of London, London, England, and Nuffield Department of Orthopaedic Surgery, University of Oxford, Nuffield Orthopaedic Centre, Oxford, England

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Contents

I. Introduction

Monoterpenes can be formally derived by condensation of two isoprene or isopentane units. They are predominantly products of the secondary metabolism of plants, although specialized classes occur in some animals and microorganisms, and are usually isolated from the oils obtained by steam distillation or solvent extraction of leaves, fruit, some heartwoods, and, rarely, roots and bark. In favorable cases they occur to the extent of several per cent of the wet weight of the tissue. Conjugated nondistillable forms, *e.g.*, terpene-β-D-glucosides, are also frequently found especially in the floral organs. As regards structure, the situation is of a wide variation on the theme of a few fundamental carbon skeletons. Good surveys of sources, isolation methods, the techniques of elucidating structure and stereochemistry, and physical properties are available.[1-4]

Modern methods of separation and structure determination and the advent of radioisotope techniques have led to a very rapid advance in knowledge of the routes of biosynthesis of this class and the other types of terpenoids over the past 15 years. Several reviews, of differing completeness, have outlined the routes to terpenoids and steroids[5-11] in general and monoterpenes in particular.[12,13] One important conclusion that emerges is the accuracy with which chemical theory can predict the course of the biochemical processes. Enzymes exploit the innate reactivity of their substrates, and the biosynthetic routes can be dissected into unit steps such as elimination, electrophilic addition, and Wagner–Meerwein rearrangement that are controlled by the stereoelectronic factors known to operate in nonbiological systems. Even the reactivity of apparently nonactivated atoms can usually be rationalized in terms of conformational and electronic changes imposed by postulated substrate–enzyme or substrate–cofactor linkages. The well-established patterns found can be used to assess feasible structures for novel terpenoids and to design "biogenetic-type" syntheses.

This review surveys the literature to April 1971 and attempts a more comprehensive coverage of the biosynthesis and metabolism of monoterpenes than has hitherto been made. Optical isomerism and absolute configurations of the compounds discussed are ignored unless of some particular

* Author to whom correspondence should be addressed at University College.
† University of London.
‡ University of Oxford.

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(2) D. A. Whiting and S. H. Harper in "Chemistry of Carbon Compounds," Vol. IIB, 2nd ed, S. Coffey, Ed., Elsevier, Amsterdam, 1968, p 153.
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(4) D. D. Davies, J. Giovanelli, and T. Ap Rees, "Plant Biochemistry," Blackwell, Oxford, 1964, p 309.
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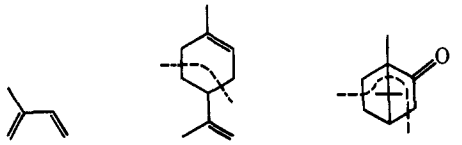
(13) W. D. Loomis in "Terpenoids in Plants," J. B. Pridham, Ed., Academic Press, London and New York, 1967, p 59.

significance. A useful distinction[14] between the terms biogenesis and biosynthesis—that are often used as synonyms— is adopted: the former is applied to hypothetical schemes, unbacked by experimental evidence, used to rationalize the formation of natural products, whereas the latter refers to schemes for which there is some experimental justification.

II. Biosynthetic Pathways

A. ISOPRENE RULE

The earliest attempt to rationalize the pattern of structures of the monoterpenes was the rule proposed by Wallach in 1887 who envisaged such compounds to be constructed from isoprene units **1**. Some 30 years later, Robinson extended



1 **2** **3**

this "isoprene rule" by pointing out that in monoterpenes, and such higher terpenes as were then known, the units were almost invariably linked in a head-to-tail fashion as shown for limonene (**2**) and camphor (**3**). However, many higher terpenes and a few monoterpenes were later found not to obey this amended rule, and Ruzicka and his collaborators proposed[15,16] a "biogenetic isoprene rule." This generalization, which is now universally accepted, states that naturally occurring terpenoids are derived either directly or by way of predictable stereospecific cyclizations, rearrangements, and dimerizations from acyclic C-10, C-15, C-20, and C-30 precursors—geraniol, farnesol, geranylgeraniol, and squalene, respectively. This rule implies a common pathway of biosynthesis for the whole family and proposals for "irregular" biogenetic routes must be treated with reservations.

Although isoprene has been formed on pyrolytic decomposition of some monoterpenes, it is not found in plants, and many speculations were made as to the nature of the "active isoprene" of the condensing unit ranging from apiose to tiglic acid. The C-5 unit was also postulated to arise from degradation of carbohydrates, proteins, amino acids, and many other classes of plant metabolites or by elaboration of acetic acid, ethyl acetoacetate, or acetone. These early views have been well summarized.[8,17,18] Many C-10 compounds have been implicated as progenitors of monoterpenes including citral,[19] geraniol,[20] nerol,[21] limonene,[22] linalool,[23] ocimene,[24] and others.[25-28] All of these speculations were unbacked by experimental evidence of any kind.

B. EXPERIMENTAL METHODS

The earliest work on the origin and interconversion of monoterpenes involved study of their occurrence in various families of plants and their relative daily and seasonal abundances throughout the growth cycle.[8] Such methods sometimes enabled correct deduction of the interrelationships to be made but often led to wildly unrealistic conclusions. Modern knowledge of the biochemical construction of the C-5 unit, its condensation, and the subsequent modification of the product has been gathered over the past 2 decades as a result of radioisotope studies of the synthesis of steroids in both cell-free systems and tissue slices from liver, and in cell-free systems from yeast, as well as a few investigations using intact higher plants. A common pattern of synthesis for steroids and terpenes emerged from this work. In particular, the steps leading to the construction of the C-10 parent were extensively studied using fractions of crude homogenates that could sustain part or all of the reaction sequence; the cofactor requirements (*e.g.*, for ATP, coenzyme A, NADPH, metal ions, etc.) were investigated, and purified preparations of certain enzymes were obtained.

Confusing and sometimes conflicting results often arose in these early tracer studies, and although most of these can now be understood as arising from complex interconversions of added precursors, it is important to appreciate the limitations of the feeding and radiochemical techniques that were, and still are, used. In this section *in vivo* experiments are particularly considered, but much of the discussion applies to the *in vitro* studies described in section VII; the latter, however, present additional problems.

Carbon dioxide is the only radioactive precursor that can be fed to plants under physiological conditions; all others must perturb the system and may evoke unnatural patterns of metabolism during the period after feeding and until any excess of additive has been degraded and eliminated by salvage mechanisms.[29] Growth chambers, such as have been designed for other phytochemical experiments,[30,31] can be used for such studies, but this use of carbon dioxide as a precursor has limited application and compounds further along the biogenetic pathway are normally fed.

The most common method of introducing such presumed precursors is *via* the stems of cut shoots, to cut petioles,[32] or to shoots or intact plants by means of a cotton wick,[33,34] but other methods are by injection into bulbs,[35] by spraying onto leaves,[36] by introduction onto a leaf surface and sealing with silicone oil,[37] by feeding to leaf disks cut out with a cork borer,[38] or by incorporating into the roots followed by

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repotting of the plant.[38] Mevalonic acid (*cf.* section II.C) was, however, not taken up into certain plants when the last method was used.[39] Water-insoluble substances may often be emulsified[40] using methods that have been used in *in vitro* systems[41-48] or can be dispersed by sonification.[44] The addition of ATP was found to increase the percentage incorporation in certain cases.[45] When microorganisms were used, the methods were usually more straightforward, the additive being directly introduced into the culture medium or enclosed in a capsule that permitted controlled release by diffusion.[46]

After feeding, plant oils are recovered by conventional steam distillation or solvent extraction, by briefly heating the leaves,[47] by direct injection of the plant tissue into a gas chromatograph,[48] or by microdissection of the oil glands.[49]

The use of intact plant tissue suffers from the limitation that lack of, or low, incorporation of tracer may merely imply that the additive failed, or had difficulty, in reaching the sites of biosynthesis. Such factors may well vary from species to species and may depend on the physiological state of the vascular system, but the influence of such parameters has been almost entirely ignored. In addition, particular precursors such as phosphate esters may be cleaved by the phosphatases that appear ubiquitous in plants,[50] and the resulting alcohols may be oxidized or otherwise modified.

Although feeding experiments of the types outlined above may indicate a possible biosynthetic pathway, additional criteria are necessary to decide whether the pathway is obligatory or an artifact. These matters have been excellently reviewed.[51,52] The following set of working rules represent an ideal experimental design.

(i) Labeled precursors should be fed at the highest possible specific radioactivities and at the lowest feasible concentrations (the specific activities of commercially available compounds labeled with carbon-14 or hydrogen-3 are unlikely to be sufficiently high to cause tissue damage). Under these conditions, in the absence of specific compartmentation and other effects to be discussed in section VI.C, obligate precursors should be efficiently incorporated into end products, and compounds that are absent in controls should not be synthesized.

(ii) Biogenetic schemes should account for groups of concurrently formed products and should result in an experimentally verifiable pattern of tracer in products formed from precursors labeled at specific positions. The position of all the tracer in the product should be located by specific and unambiguous chemical degradations, and passage of tracer into interrelated groups of compounds should follow the time course predicted.

(iii) Proposed intermediates should be characterized, if their stability permits, and postulated enzymic activities should be detected.

(iv) The sequential synthesis of related compounds during the growing season of higher plants should be consistent with biogenetic speculations. In addition, for microorganisms, the use of species with blocked biosynthetic pathways (caused by either mutation, spontaneous or induced, or the application of growth inhibitors) should lead to the expected products.

(v) Isolated products containing tracer must be rigorously purified, preferably by recrystallization to constant specific radioactivity, as contamination with traces of more heavily labeled impurities can invalidate any conclusions. This simple and obvious precaution has often been ignored, especially for products produced in cell-free systems (*cf.* section VII). It is not sufficient to collect fractions from gas–liquid or thin-layer chromatographic separations and to assume that radiochemically pure compounds have been obtained. Nor is it valid to scan chromatographic plates or papers with automatic counters and to assign relative radioactivities to the components of the chromatograms. The last procedure is especially dangerous as phytoene (which is colorless at the levels usually encountered) and β-carotene run with R_f values typical of monoterpenes in many commonly used elution systems.[40] These compounds, which are usually more heavily labeled in feeding experiments using tracer-containing carbon dioxide, acetate, or mevalonic acid than are the monoterpenes, also "tail" on most chromatographic systems and are almost always contaminants of monoterpenes that have been isolated by the solvent extraction of the plant tissue.

These criteria and precautions have rarely been applied to any one example, but their existence emphasizes that it is *not* sufficient to isolate a radioactive product after feeding a presumed precursor and to claim to have characterized a normal pathway of metabolism, as has often been done in the work to be described. For example, recovery of a labeled product after feeding a plant with [^{14}C]mevalonic acid is no evidence that the product is a terpene. Although direct pathways for the scrambling of the tracer of the physiologically active (+) isomer into the acetate pool by the reverse of the route of synthesis appear to be very uncommon, if not unknown, other degradative routes may be available for this and for the physiologically inactive (−) isomer that co-occurs in the racemic [^{14}C]mevalonic acid that is commercially available. Labeled terpenes that are formed from (+)-mevalonic acid can also be degraded by similar routes.[40,53] Consequently tracer originating from either isomer of the precursor may be incorporated into a wide class of compounds such as carbohydrates, proteins, alkaloids, and polyketides as well as terpenoids.

C. FORMATION OF GERANYL PYROPHOSPHATE

The biosynthesis of geranyl pyrophosphate (GPP, **10**) from acetate in liver and yeast systems has been elucidated, and the enzymes implicated in the accepted pathways have been

(39) K. J. Treharne, E. I. Mercer, and T. W. Goodwin, *Biochem. J.*, **99**, 239 (1966).
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(41) G. V. Vahouny, S. Weersing, and C. R. Treadwell, *Arch. Biochem. Biophys.*, 107, 7 (1964).
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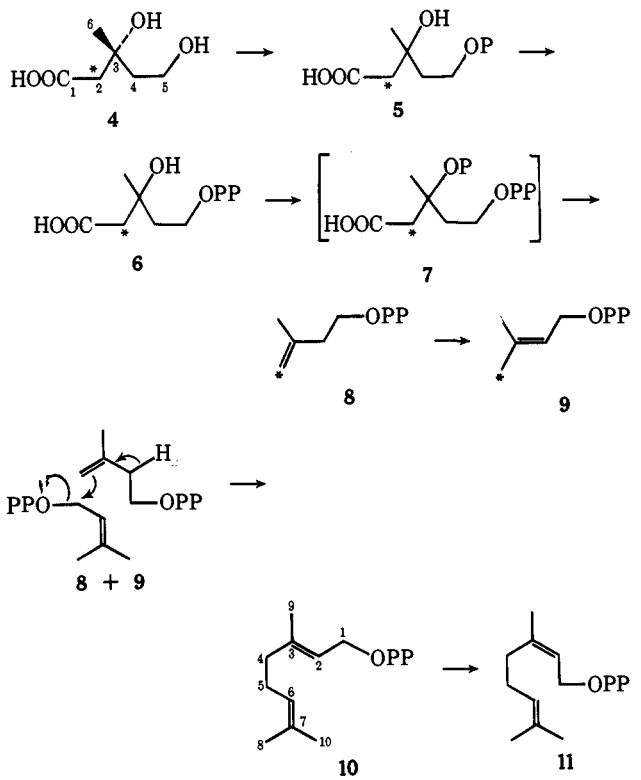
(53) J. W. Dicks and J. Friend, *Phytochemistry*, 7, 1933 (1968).

demonstrated to occur, and in some cases have been isolated, from various plant sources. Reviews on the subject are available[6,7,10,11,54] and Scheme I (where P and PP represent

Scheme I



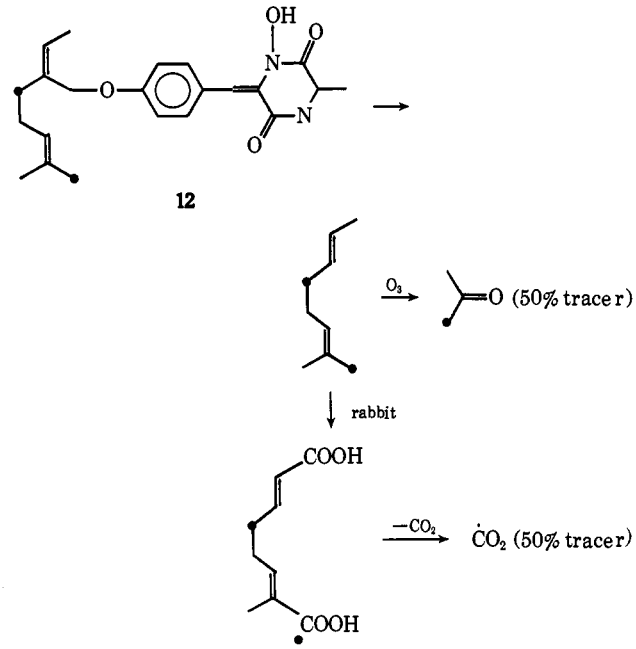
phosphate and pyrophosphate groups, respectively) summarizes the situation.

Acetoacetyl coenzyme A, formed by self-condensation of acetyl coenzyme A in the presence of β-ketoacylthiolase, condenses with another molecule of acetyl coenzyme A to form β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) that is irreversibly converted by a specific reductase (possibly with the intermediate formation of a hemithioacetal adduct formed from HMG and the thiol group of an enzyme) into (*R*)-(+)-mevalonic acid (MVA, **4**). This product is sequentially phosphorylated to 5-phosphomevalonic acid (MVAP, **5**) and 5-pyrophosphomevalonic acid (MVAPP, **6**), and the latter is converted into isopentenyl pyrophosphate (IPP, **8**), probably with the intermediate formation of the triphospho ester **7**. IPP and 3,3-dimethylallyl pyrophosphate (DMAPP, **9**) are joint substrates for prenyl transferase that catalyzes the formation of GPP and possibly neryl pyrophosphate (NPP, **11**) in what can be formally regarded as a coupled SN2–E2 process. These products are the parents of the acyclic and alicyclic monoterpenes, respectively.

It is generally accepted that this biogenetic route is also followed in higher plants, although alternative pathways have been proposed that involve the formation of HMG from malonyl coenzyme A[55–57] and from leucine.[58] The latter route has been suggested to be important in plants, but there is little evidence on this point.

MVA is converted into the two C-5 units such that the starred carbon in **4** becomes trans to the ester group in DMAPP. This nonequivalence of the *gem*-dimethyl groups has been proved by detailed degradation studies on higher terpenes biosynthesized from [2-^{14}C]MVA (for numbering of skeleton, see **4**) and by an ingenious degradation of the monoterpenoid part of mycelianamide (**12**) formed by *Penicillium griseofulvum* from the same precursor. In the critical step of the degradation of the last compound, that part of the molecule that had been labeled from MVA was fed to a rabbit, and the stereospecifically oxidized product in which half the tracer resided on a particular carboxylic acid group was recovered[59,60] from the animal's urine (Scheme II).

Scheme II



IPP and DMAPP may be regarded jointly as "active isoprene," the biochemical equivalent of the isoprene unit. Chain extension of GPP or NPP by sequential addition of IPP can lead to the whole family of terpenoids. The enzymes involved in the formation of GPP from IPP and DMAPP have been investigated[61,62] and the stereochemical details most elegantly worked out.[63,64] Of special interest are the

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(55) J. D. Brodie, G. Wasson, and J. W. Porter, *J. Biol. Chem.*, **239**, 1346 (1964).

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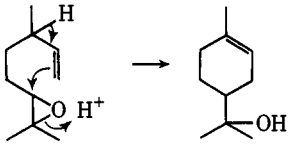
characterization of enzymes from rubber latex that convert MVA into IPP in over 30% yield[65] and similar but less efficient systems from mammalian liver.[66] An isomerase from rat liver that converts IPP into DMAPP has also been studied in some detail.[67] These enzyme systems have proved very useful for the synthesis of ^{14}C-labeled substrates for feeding experiments.

III. Acyclic Compounds and Cyclohexane Derivatives

A. HYPOTHESES

Plausible biogenetic routes to monoterpenes may readily be drawn up, but a surprisingly small number of meaningful tracer investigations have been carried out. Although it seems reasonable to assume that a plant which can synthesize a presumed precursor will use this to elaborate simple derivatives, this is not necessarily so. A chastening example arises in alkaloid biosynthesis where it has been shown that different plant species are able to construct the pyridine ring in at least three totally different ways.[68-70] Nevertheless, except for modifications that will be considered shortly, it is generally and very reasonably believed that acyclic monoterpenes are derived from GPP and NPP while cyclic compounds are formed from the latter ester; GPP cannot directly cyclize because of the constraint caused by the trans-substituted double bond.[71]

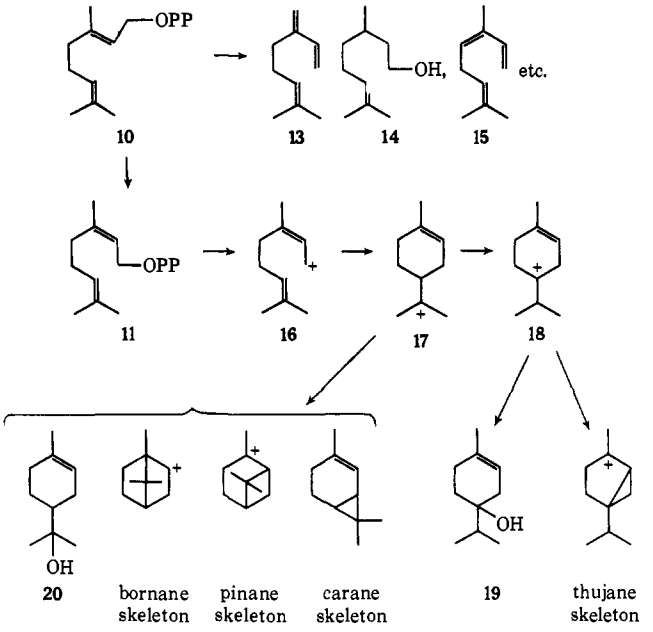
The proposals of Ruzicka and his coworkers[15] for the pattern of monoterpene biogenesis are outlined in Scheme III. Several of the intermediates are formally represented as carbonium ions, but structurally equivalent species such as alcohols, phosphate esters, terpene glycosides, or sulfonium salts, either free or bonded to proteins, may be the reactants *in vivo*. The scheme is extremely attractive; the formation of acyclics such as myrcene (**13**), citronellol (**14**) or *cis*-ocimene (**15**) from GPP has many *in vitro* analogies, and monocyclization of the ion **16** formed from NPP to give α-terpineol (**20**) or terpinen-4-ol (**19**) is also chemically reasonable, although the biochemical details are open to conjecture. For the latter process, either epoxides (which have been isolated from several essential oils[72]) or sulfonium compounds formed with a thiol group of an enzyme[73] may be involved as outlined in eq 1 and 2. Both of these types of intermediates



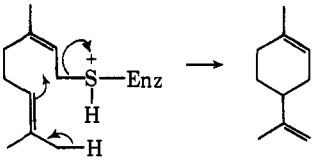
(1)

are known to be implicated in the formation of rings in higher terpenoids,[11] and interesting model systems for the

Scheme III



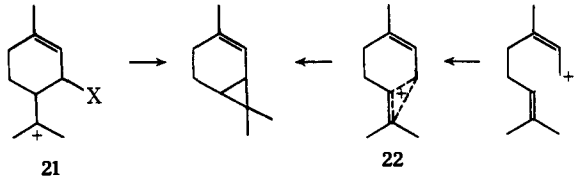
synthesis of monoterpenes *in vitro* using sulfonium ylides have been developed.[74-76] The elucidation of the importance



(2)

(if any) of such routes in the plant must await the advent of suitable cell-free systems.

Bicyclic skeletons of the pinane and bornane series are (according to Ruzicka's scheme) derived by internal additions of positive centers to double bonds within monocyclic frameworks in a direction governed either by electronic factors (Markovnikov addition) or by steric factors. Hydride shift within the ion **17** followed by cyclization of **18** gives rise to the thujane skeleton, and that of the caranes arises from an internal electrophilic substitution at the allylic position of the former carbonium ion. This latter reaction, as given, is biochemically improbable, and an internal displacement in an intermediate such as **21** (X = ester?) or the intermediacy of a nonclassical ion **22** has been suggested,[10] but both proposals beg the question. A recent study[77] of the mechanism



of decomposition of certain unsaturated epoxides suggests that eq 3 is feasible and the mechanism could be modified to

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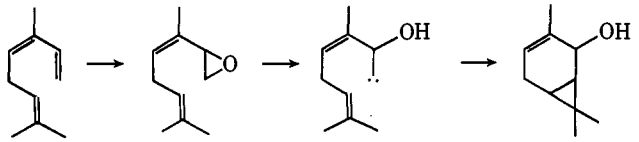
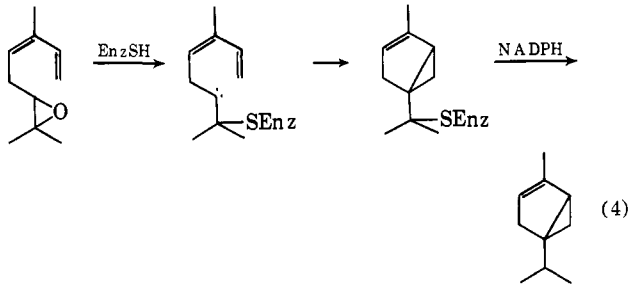
form other bicyclic monoterpenes directly from acyclic precursors; *cf.* eq 4. The generation of the intermediate



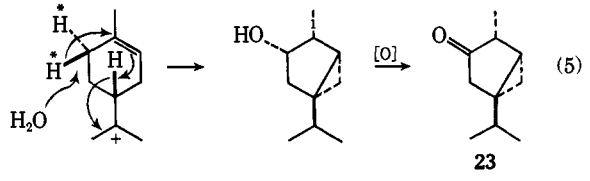
(3)



(4)

carbenes, or their formal equivalents, may be possible at the enzyme surface where water and other potential scavengers may be locally excluded. No evidence is available to assess these hypotheses.

It would seem probable that the mono- and bicyclizations that are usually formally represented as proceeding through different ions formed in sequence, *cf.* Scheme III, are actually concerted addition–rearrangement processes similar to those involved[11] in the folding and cyclization of sesqui-, di-, and triterpenes. Thus thujone (**23**) can be envisaged as resulting from the sequence in eq 5 (the starred hydrogens are labeled

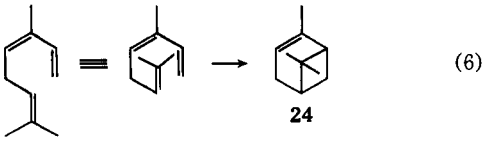


(5)

23

by incorporation of (2*R*)- or (2*S*)-[$^3H_1$]MVA) in which the required trans specificity of rearrangement occurred; some evidence for such a process will be outlined in section III.D.

Other schemes for bicyclization *in vivo* have been proposed. Radical-induced cyclizations (presumably photochemically promoted) of *cis*-ocimene or myrcene to form α-pinene (**24**) or β-pinene (**25**) (eq 6 and 7) have been suggested as alternatives to the formally ionic routes.[15,78] Also direct bicyclizations of straight-chain precursors, or even direct condensation of IPP and DMAPP, to form bicyclic products were argued[79] to be thermodynamically more favorable than the stepwise sequence of Ruzicka's scheme. On this view, monocyclics such as limonene and the other menthadienes arise from cleavage of initially formed bicyclic compounds.[79,80]



(6)

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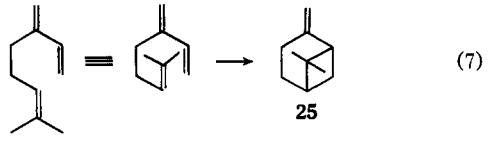
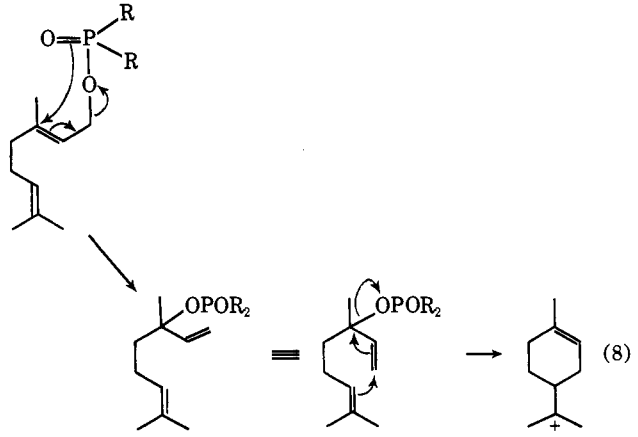


(7)

25

Although photochemically induced bicyclizations of the types in eq 6 and 7 have been demonstrated *in vitro*,[81,82] no evidence has been put forward to support such reactions *in vivo*. A detailed investigation of the time dependence of incorporation of tracer into the monoterpenes of *Tanacetum vulgare* (tansy) has ruled out similar direct bicyclization in the biosynthesis of thujone, and the precursor relationships were in complete agreement with Ruzicka's original ionic mechanisms.[40] In any event, any intermediate radicals would almost certainly have to be protein-bonded to account for the strict stereospecificity of cyclization, and the distinction between heterolytic and homolytic processes would become difficult to maintain.

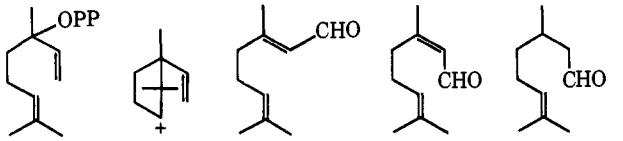
Studies with model systems have led to deductions concerning monoterpene biosynthesis. Solvolyses of mono-, pyro- and diphenyl phosphate esters of nerol led to monocyclic monoterpenes, whereas open-chain products either entirely or predominantly resulted from similar reactions of the corresponding geranyl derivatives.[83–85] The conversion of GPP into NPP *in vivo* followed by cyclization was suggested to be unlikely on the basis of these results,[13] and direct coupling of IPP and DMAPP to form NPP was considered probable, but such extrapolations from *in vitro* situations are clearly inconsequential. A more plausible suggestion was that cyclization of the geranyl esters *in vitro*, and by inference *in vivo*, proceeded through the formation of linaloyl esters **26**; *cf.* eq 8. These latter esters were not detected after solvolysis of geranyl and neryl diphenyl phosphates but such products were expected to cyclize with great ease. It was pointed out[85] that acid hydrolysis of linaloyl esters generated formally the same carbonium ion as that formed from neryl esters and hence linaloyl pyrophosphate (LPP, **27**) could be the progenitor of cyclic monoterpenes. The proposal has been followed up (*cf.* section VI.B), and the synthesis of LPP by direct condensation of MVAPP and DMAPP with



(8)

26

(78) R. L. Burwell, *J. Amer. Chem. Soc.*, **73**, 4461 (1951).
(79) R. M. Gascoigne, *J. Chem. Soc.*, 876 (1958).
(80) H. Rothbaecher, *Pharmazie*, **23**, 389 (1968).
(81) R. C. Cookson, *Quart. Rev., Chem. Soc.*, **22**, 423 (1968).
(82) K. J. Crowley, *Tetrahedron Lett.*, 2863 (1965).
(83) W. Rittersdorf, *Angew. Chem., Int. Ed. Engl.*, 4, 444 (1965).
(84) R. C. Haley, J. A. Miller, and H. C. S. Wood, *J. Chem. Soc. C*, 264 (1969).
(85) W. Rittersdorf and F. Cramer, *Tetrahedron*, 24, 43 (1968).



27 28 29 30 31

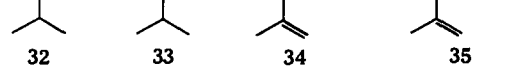
concomitant decarboxylation has been suggested[86] without, however, any supporting evidence.

The abundance of linalool and the decrease in its concentration relative to that of limonene during the maturation of citrus fruits was held[87] to indicate that LPP rather than NPP was the precursor of cyclic monoterpenes in these species; an additional speculation was that LPP was directly formed by combination of IPP and DMAPP. A feasible route for the bicyclization of LPP and related compounds could involve the formation of **28**, but derivatives with this skeleton are not found to accompany bornane derivatives in the appropriate plants, whereas compounds with the conventional cyclohexane skeletons are widely distributed along with bornyl compounds.

B. NONTRACER STUDIES

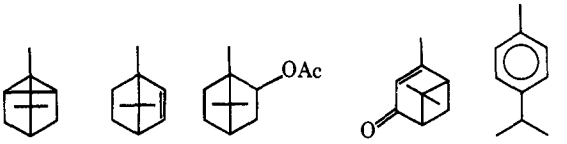
Scattered investigations of the interrelationships of monoterpenes as revealed by comparisons of the structures of the compounds obtained from particular species or genera of plants predated many of the above-described schemes. The reasonable assumption was made that terpenes that were closely related in structure would be related biogenetically.

A study[88] of the composition of the oil from both young and old leaves of particular specimens of *Mentha* species throughout the growing season led to a proposed sequence of interconversions. Earlier, citral a (**29**), citral b (**30**), citronellal (**31**), and linalool had been isolated from lemongrass oil, and their biogenetic dependence, which with the advantage of hindsight appears obvious, was outlined.[89] Further examples of such studies have been reviewed.[5,17] Especially interesting are the discussions of the relationship between ascaridole (**32**) and α-terpinene (**33**) in chenopodium oil[90,91] and the deduction of limonene (**2**) to be the precursor of carvone (**34**) with the intermediacy of carveol (**35**) in *Carum carvi* (caraway).[92]

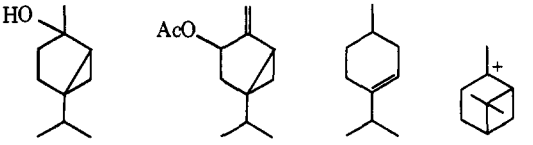


32 33 34 35

Examples of this type of approach still appear. The biogenetic relationship between tricyclene (**36**), bornylene (**37**), and bornyl acetate (**38**) has been deduced from their cooccurrence in oil from siberian pine needles;[93] and detailed studies of the oil from *Rosemarinus officinalis* (rosemary) has confirmed[94,95] the plausible ideas that α-pinene (**24**) is the precursor of verbenone (**39**) and that aromatics such as



36 37 38 39 40



41 42 43 44

p-cymene (**40**) are formed from 4-thujanol (**41**) and terpinen-4-ol (**19**).

A more general approach was the investigation of the frequency of occurrence of monoterpenes in different genera, with the assumption that the most commonly occurring would be directly related to the first-formed C-10 compound.[96] Initial correlations led to the selection of geraniol, nerol, citral, and linalool as possible parent compounds; and further study of the distribution of the last named led to it being chosen as the most likely candidate. These qualitative correlations were the forerunners of rigorous statistical approaches to the problems of chemotaxonomy that will be discussed in section VI.E.

The only direct methods of study that predated the use of radioisotopes were some preliminary investigations in which citronellal, geraniol, and certain other monoterpenes were sealed into holes bored in *Ficus retusa* trees.[97] Comparison of the oil collected several months later with that from controls showed that several rearrangements, oxidations, and degradations had taken place, but it was not possible to decide whether normal metabolic pathways or merely salvage mechanisms had been characterized.

C. TRACER STUDIES (GENERAL)

Detailed knowledge of the biosynthetic processes could come only with the advent of radioisotopes as a mechanistic tool. Reasonable analogs were quickly drawn between the well-understood mechanisms of formation of steroids in animals or microorganisms and those of monoterpenes in plants. The latter pathway is often considered to be an offshoot of the former, and although the direct evidence for this is actually very slender,[13] this hypothesis is probably very close to the truth. The paucity of evidence arises from the oft-demonstrated finding that uptake of MVA into the monoterpenes of whole plants or intact plant tissues is generally extremely low.[13] Incorporations are usually in the range 0.01–0.1% of the applied tracer (these figures are for conventional monoterpenes; incorporations into methylcyclopentanoids (*cf.* section IV) are often considerably higher), and sometimes no

(86) Y. Fujita, *Kagaku No Ryoiki*, 16, 227 (1962); *Chem. Abstr.*, 58, 12817 (1963).
(87) J. A. Attaway, A. P. Pieringer, and L. J. Barabas, *Phytochemistry*, 6, 25 (1967).
(88) R. H. Reitsema, *J. Amer. Pharm. Assoc., Sci. Ed.*, 47, 267 (1958).
(89) P. Rovesti, *Parfumerie*, 47, 61 (1955); *Chem. Abstr.*, 49, 16359 (1955).
(90) A. J. Halpern, *J. Amer. Pharm. Assoc., Sci. Ed.*, 40, 68 (1951).
(91) G. O. Schenck and K. Ziegler, *Naturwissenschaften*, 32, 157 (1944).
(92) W. Sandermann, *J. Prakt. Chem.*, 151, 160 (1938).
(93) F. Porsch and H. Farnow, *Dragoco Rep. Ger. Ed.*, 194 (1962).
(94) R. Granger, J. Passet, G. Arbousset, and J. P. Girard, *C. R. Acad. Sci.*, 270, 209 (1970).
(95) R. Granger, J. Passet, and M. C. Pinede, *ibid.*, 267, 1886 (1968).
(96) Y. Fujita, *Kagaku (Tokyo)*, 20, 514 (1950).
(97) Y. Fujita, *Ogawa Perfume Times*, No. 202, 502 (1951); quoted in ref 8.

significant incorporations can be demonstrated over periods of several days after feeding the labeled precursor. Two schools of thought exist on the last topic: one believes that incorporation over relatively short terms (*ca.* 5 hr) are significant as an indication of normal metabolic processes whereas longer periods allow the tracer to pass "through" the compounds of interest; the other believes that incorporations at these short-term periods only reveal perturbations in the normally occurring pathways triggered by the excess of additive. The situation is unresolved.

These low incorporations would undoubtedly lead to the conclusion that MVA was not a precursor of terpenoids (or of phytosterols where similarly low incorporations are the rule) if there was any evidence whatsoever for an alternative pathway. Except for an uncorroborated report (which will be discussed in section III.D) that 3,3-dimethylacrylic acid is a very efficient precursor for pulegone in a *Mentha* species, no such support has been forthcoming. Uptake of [^{14}C]acetate into terpenoids is also low, but this is understandable, for whereas MVA has only one known biochemical function, acetate is widely incorporated into metabolites other than terpenoids, some of which are in a state of rapid turnover.

The low incorporation of MVA is probably not due to poor translocation from the site of injection to the sites of synthesis, as in several species the additive is rapidly transported throughout the plant,[29,98,99] but several plausible explanations of the phenomenon may be advanced.

First, MVA may not readily penetrate to the intracellular sites of terpenoid synthesis; similar phenomena have been shown to account for low incorporations of MVA and other precursors into steroids and pigments formed in chloroplasts[100] (*cf.* section VI.C).

Secondly, such [^{14}C]MVA as can thus penetrate may not be able to intervene in the biosynthetic pathway. MVA never occurs *in vivo* as the free acid or lactone in significant quantities but is probably enzyme bonded, as are certain intermediates[53] in its synthesis in pigeon liver tissue. Whether extraneously added MVA would be acted upon by a synthetase system, which would be probably particulate or membrane-bonded to form a "conveyor belt" between entering C-2 units and departing C-5 or C-10 units, will depend on the position of equilibrium between free and enzyme-bonded MVA. This could well vary in different species or with the physiological state of tissues of the same species. Analogies for these speculations can be found in the action of the different fatty acid synthetase systems.[101,102]

Thirdly, MVA that does enter the "conveyor belt" system may be shunted into physiologically important steroids and carotenoids rather than into the monoterpene pool.

Fourthly, if MVA is indeed an obligatory intermediate, its intracellular concentration must be very low, and so its addition in large quantities, including the unphysiological *S* isomer, during the feeding experiments, may well lead to inhibition by feedback mechanisms of enzymes involved in terpenoid synthesis.

Fifthly, the unavoidable introduction of large quantities of MVA results in degradation of the additive to products that may also act as enzyme inhibitors. Such degradations are well demonstrated both by the observations that sabinyl acetate (**42**) biosynthesized from [2-^{14}C]MVA by *Juniperus sabina* contained 30-fold more radioactivity in the acetate group than in the terpenoid moiety,[103] and by the extensive labeling of the sugar residues in iridoids formed from the same precursor; *cf.* section IV. In these examples the labeled acetate was not significantly incorporated into the terpene skeleton which was almost exclusively labeled by incorporation of undegraded MVA; this implies that the site of construction of the terpene skeleton differs from the sites of salvage degradations and of esterification.

In support of these rationalizations, there is excellent evidence for utilization of the MVA pathway in the biosynthesis of monoterpenes in petals. The internal anatomy of these differs from leaves, and the first and second factors mentioned above may be now less important than in the latter tissue, or even absent. Within 1 hr of feeding the flower head of a hybrid tea rose with [2-^{14}C]MVA, up to 11% of the applied tracer (from racemic precursor) had been incorporated into geraniol, nerol, and citronellol and their β-glucosides; the sugar moiety was unlabeled.[104-106] The unprecedentedly large uptake and the specificity of the position of incorporation of label (*cf.* section III.D) were very strong support for the accepted pathway. In another study, a relatively high (*ca.* 1.4% over 24 hr) incorporation of [2-^{14}C]MVA into the total terpene alcohols of the petals of a hybrid of *Rosa damascena* was reported, although the position of labeling was not determined.[107,108] Similar large uptakes and specific incorporations of tracer from MVA had previously been obtained in the biosynthesis of irregular monoterpenes and iridoids in flower heads of other species (see sections IV and V).

Uptake of tracer from [2-^{14}C]MVA into monoterpenes produced by several species of plants[13] and a few insects[109] have been recorded; *e.g.*, α-pinene and myrcene produced by *Pinus attenuata* and *Santolina chaemocyparissus*, respectively, were thus labeled,[110,111] as were citral and citronellal formed in some species of ants.[112] However, in none of these examples was the position of labeling ascertained. Scattered studies have also demonstrated the gross incorporation of tracer from other precursors[8,11,13] including [3-^{14}C]linalool.[113] Often tracer from glucose or glycine was more rapidly transferred to the terpene pool than that from either acetate or MVA; this is probably attributable to the more efficient translocation to, and penetration of, the biosynthetic sites by the former additives, followed by degradation and incorporation of the labeled fragments. [^{14}C] amino acids, fatty acids, and sugars as well as the obligate precursors

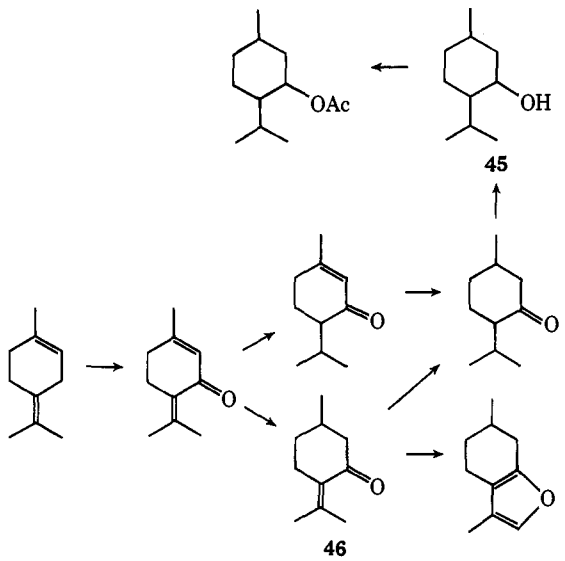
(98) A. G. Horodysky, G. R. Waller, and E. J. Eisenbraun, *J. Biol. Chem.*, **244**, 3110 (1969).
(99) F. E. Regnier, G. R. Waller, E. J. Eisenbraun, and H. Auda, *Phytochemistry*, 7, 221 (1968).
(100) L. J. Rogers, S. P. J. Shah, and T. W. Goodwin in "Biochemistry of Chloroplasts," Vol. 2, T. W. Goodwin, Ed., Academic Press, London, 1967, p 283.
(101) S. J. Wakil and J. Ganguly, *J. Amer. Chem. Soc.*, **81**, 2597 (1959).
(102) F. Lynen, *Fed. Proc., Fed. Amer. Soc., Exp. Biol.*, 20, 941 (1961).
(103) D. V. Banthorpe and K. W. Turnbull, *Chem. Commun.*, 177 (1966).
(104) M. J. O. Francis and C. Allcock, *Biochem. J.*, **113**, 38P (1969).
(105) M. J. O. Francis and C. Allcock, *Phytochemistry*, **8**, 1339 (1969).
(106) M. J. O. Francis and M. O'Connell, *ibid.*, **8**, 1705 (1969).
(107) A. R. Guseva and V. A. Paseshnichenko, *Biokhimiya*, **31**, 988 (1966).
(108) V. A. Paseshnichenko and A. R. Guseva, *ibid.*, **32**, 1020 (1967).
(109) J. Meinwald, G. M. Happ, J. Labows, and T. Eisner, *Science*, **151**, 79 (1966).
(110) R. G. Stanley, *Nature (London)*, **182**, 738 (1958).
(111) G. R. Waller, G. M. Frost, D. Burleson, D. Brannon, and L. H. Zalkow, *Phytochemistry*, 7, 213 (1968).
(112) G. M. Happ and J. Meinwald, *J. Amer. Chem. Soc.*, **87**, 2507 (1965).
(113) J. A. Attaway and B. S. Buslig, *Phytochemistry*, **8**, 1671 (1969).

were fed to *Humulus lupulus* (hops), but only tracer from glucose (0.08% in 6 hr), acetate (0.02% in 24 hr), and, to a still lesser extent, alanine was significantly incorporated into myrcene. No uptake of tracer from MVA could be detected under the same conditions.[13] Isolated needles of *Pinus radiata* incorporated sodium [^{32}P]phosphate into ATP, MVAPP, and IPP and [2-^{14}C]MVA into the last two. The latter precursor was also incorporated into MVAP by a cell-free extract of the seedlings.[114]

Studies using [^{14}C]carbon dioxide can be used to define unperturbed metabolic pathways *in vivo*, and very significant results have accrued from the use of this approach. In many experiments incorporation of tracer into only a few monoterpenes has been studied at a small number of time intervals after onset of feeding, and the relative incorporations have been assigned by the semiqualitative method of autoradiography, but more quantitative studies of the time incorporation into several compounds are also available. The monoterpenes of *Mentha piperita* (peppermint) were rapidly labeled after exposure to [^{14}C]carbon dioxide but not after feeding [2-^{14}C]-MVA although the latter was an efficient precursor of carotenoids.[115] This was attributed to the cutinization of the wall of the stem cell of the oil gland (*cf.* section VI.A) causing restriction of migration of hydrophilic substances with more than five carbon atoms into the secretary cells; but an alternative suggestion, which took into consideration the rapid evolution of labeled carbon dioxide that was observed after feeding [2-^{14}C]MVA to other species, was that MVA was in general degraded *in situ* into carbon dioxide which was fixed into monoterpenes.[116] However, later studies on *Mentha piperita* have shown that MVA can indeed be significantly incorporated into monoterpenes,[117,118] and measurements of the time course of incorporation of [^{14}C]acetate, [^{14}C]carbon dioxide, and [2-^{14}C]MVA into compounds of this species and *Tanacetum vulgare*, both in the light and dark, have ruled out incorporation occurring through degradation of MVA.[40,118] A particularly direct demonstration of the unimportance of the latter route was the negligible incorporation of tracer from [1-^{14}C]MVA into the monoterpenes of *Artemisia annua* under conditions where tracer from [2-^{14}C]MVA was significantly incorporated. Extensive *in situ* release of labeled carbon dioxide from the former additive must have occurred and 14% of the radioactivity was lost as such to the atmosphere, but less than 1% of the tracer was so released after uptake of [2-^{14}C]MVA under the same conditions.[29] This work again demonstrates that the sites of degradation of additives and of synthesis of MVA and thence of terpenoids are quite different. The observation that incorporations of tracer from [2-^{14}C]MVA occurred specifically into the expected positions of many terpene skeletons (*cf.* section III.D) also proves that degradation of MVA to carbon dioxide and incorporation of this cannot be a general route under these feeding conditions.

Although the use of [^{14}C]carbon dioxide cannot distinguish between possible mechanisms for the construction of monoterpenes, it can reveal the precursor relationships between the terpenoids of a particular species of plant. Typically some 0.2% incorporation of applied radioactivity occurs within 6 hr, and small but significant labeling of the monoterpene pool occurs within 15 min of exposure to the tracer-containing atmosphere. Scheme IV (in which optical and

Scheme IV



stereoisomerism is ignored) is a consensus of opinion on the pathways deduced from such studies on *Mentha piperita* and certain closely related species,[80,88,115,116,119–123] although some differences of detail exist. Generally, the initially labeled monoterpenes were unsaturated relative to the later-formed products, but an invariable sequence of formation of the components need not always take place, and different steps may have differing importance depending on the physiological state of the plants.[118,119,121] Several of the proposed steps were directly demonstrated by isolation of labeled monoterpenes from plants exposed to tracer and feeding them back to leaves or leaf slices; and reactions unrelated to the scheme such as the conversion of [^{14}C]limonene or α-pinene into unidentified (presumably terpenoid) metabolites, and of menth-3-ene (**43**) into menthol (**45**) and pulegone (**46**), were tentatively (*cf.* section II.B) identified by autoradiography.[120] Later studies on the profiles of tracer incorporation revealed that limonene is probably a precursor of 3-oxomenthanes such as carvone in *Mentha piperita*.[124]

Determination of the time courses of incorporation of [2-^{14}C]MVA and [2-^{14}C]malonate into camphene and linalool produced by *Monarda punctata*,[125] of [1-^{14}C]acetate into geraniol, α-terpineol, terpinen-4-ol, α-pinene, thujone, and sabinene in *Tanacetum vulgare*[40] and of [^{14}C]carbon dioxide into limonene, α- and β-pinenes, myrcene, and menthane derivatives in *Mentha piperita*,[123] have also allowed

(114) P. Valenzuela, E. Beytia, O. Cori, and A. Yudelevich, *Arch. Biochem. Biophys.*, **113**, 536 (1966).
(115) J. Battaile and W. D. Loomis, *Biochim. Biophys. Acta*, **51**, 545 (1961).
(116) R. G. Battu and H. W. Youngken, *Lloydia*, **29**, 360 (1966).
(117) F. W. Hefendehl, *Planta Med.*, **14**, 66 (1966).
(118) F. W. Hefendehl, E. W. Underhill, and E. von Rudloff, *Phytochemistry*, 6, 823 (1967).
(119) A. J. Burbott and W. D. Loomis, *Plant Physiol.*, 42, 20 (1967).
(120) R. H. Reitsema, F. J. Cramer, N. J. Scully, and W. Chorney, *J. Pharm. Sci.*, 50, 18 (1961).
(121) F. W. Hefendehl, *Planta Med.*, 10, 241 (1962).
(122) H. Rothbaecher and H. Heltmann, *Pharmazie*, 23, 387 (1968).
(123) F. H. L. van Os, *Bull. Soc. Pharm. Strasbourg*, 7, 49 (1964).
(124) F. W. Hefendehl, *Planta Med.*, **15**, 121 (1967).
(125) R. W. Scora and J. D. Mann, *Lloydia*, 30, 236 (1967).

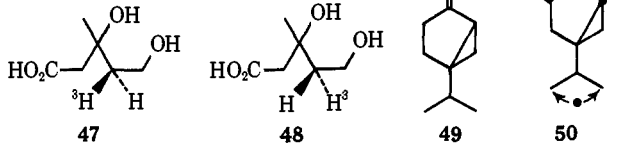
the sequence of synthesis of the various components to be assigned, and routes consistent with Ruzicka's proposals (Scheme III) have been inferred. An interesting observation[126] was that β-pinene biosynthesized from [^{14}C]carbon dioxide by needles of *Pinus radiata* consistently throughout the time course of incorporation had a greater specific radioactivity than the α-pinene concomitantly synthesized. Although the more stable α-isomer would be expected to be formed more readily from a common intermediate such as **44**, the (+)-α- and (−)-β-pinenes that are produced have different absolute configurations, and thus must be formed by entirely different pathways perhaps involving enzymes of opposite specificities.

Although the work summarized in this section undoubtedly reveals real trends, it must be emphasized that the details may often be invalidated by faulty experimental procedures. Several workers have measured the radioactivities of spots from thin layer chromatograms or of cuts from gas chromatographic separations with no attempts at further purification; consequently contamination by unsuspected impurities may well have taken place. The percentage incorporations of various precursors are collected in section VIII. The efficiency of incorporation must depend on the species, the tissue used, the environmental conditions, and the physiological state of the material, and apparent inconsistencies which are probably attributable to such factors have been discussed.[40]

D. TRACER STUDIES (SPECIFIC LABELING)

More subtle information than that previously described may be obtained by use of precursors specifically labeled with tracer at particular positions followed by degradation of the purified products of biosynthesis to locate the site of tracer.

A fundamental question is the possible independence of routes to GPP and NPP, the parents of acyclic and higher terpenoids and of cyclic monoterpenes, respectively. NPP could be formed either by direct coupling of IPP with DMAPP, or by the isomerization of preformed GPP (or LPP). The former scheme is attractive in implying a special route in plants (which generally accumulate monoterpenes) that is absent in animals (in which such compounds are rarely found), but experiments using (4*R*)-[4-^{3}H$_1$]MVA (**47**) and its (4*S*) isomer (**48**) have ruled out this pathway in two species of



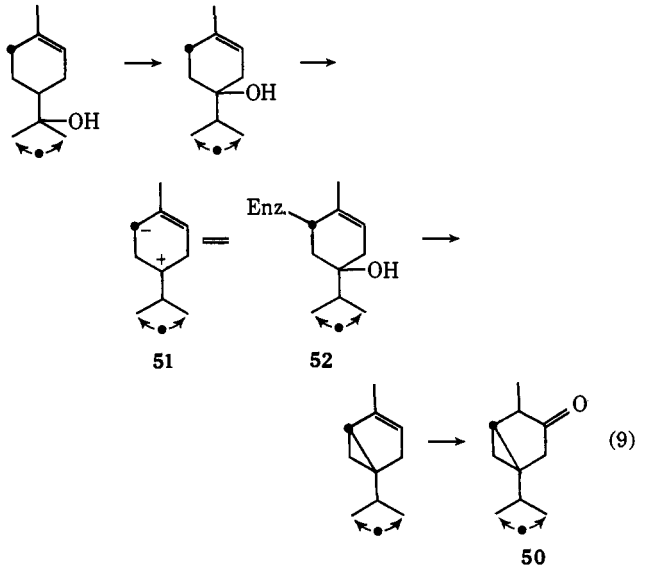
47 **48** **49** **50**

higher plants.[127,128] Incorporation of each isomer in turn in admixture with [2-^{14}C]MVA (to act as a marker) into a hybrid tea rose and isolation of geraniol and nerol both free and bonded as β-glucosides showed that the 4*S* hydrogen was stereospecifically lost in all cases; thus when the 4*R* and 4*S* isomers of MVA were fed together with [2-^{14}C]MVA such that the hydrogen-3/carbon-14 ratios were 1.99 and 1.25, respectively, geraniol and nerol were recovered from their β-glucosides in which the ratios were 1.97 and 0.05, and 2.01 and 0.09, respectively. A similar pattern was obtained from α-pinene produced by *Pinus attenuata*. Direct condensation of the C-5 compound to form NPP would have resulted in the loss of the 4*R* hydrogen.[64] These results also extend to higher plants the conclusions derived from studies of the formation of steroids in yeast and mammalian tissue[64] that the 4*S* hydrogen of MVA was lost in the isomerization of IPP into DMAPP. NPP is thus apparently formed in plants from GPP. Possible mechanisms for the conversion involve either an unusual type of isomerase,[127] reduction to citronellol and subsequent oxidation using enzyme activities similar to those characterized in other contexts,[129] or dephosphorylation and oxidation to citral a that can readily equilibrate with citral b at neutral pH and be subsequently reduced.[130] The last route has been found to be sustained by acetone powders prepared from various plant tissues.[130] In animals, geraniol or GPP formed *en route* to steroids may rarely be released from an enzyme surface, and so no opportunity for conversion into nerol and the subsequent accumulation of a monoterpene pool may arise.

Doubly labeled MVA has also been utilized to investigate the mechanism of bicyclization of thujone. If the concerted route in eq 5 is followed and (2*R*)-[^{3}H$_1$]- and (2*S*)-[^{3}H$_1$]MVA are fed to the plant in separate experiments, in one case the tracer will be retained in that part of the skeleton derived from IPP, although it will be transferred to the adjacent carbon atom, whereas in the other the tracer will not migrate and and will be lost in the final oxidation step. This difference has been demonstrated[131] in experiments using *Tanacetum vulgare*.

Almost all other studies using specifically labeled precursors have utilized [2-^{14}C]MVA. Incorporation of this into thujone (**23**), the epimeric isothujone, sabinene (**49**), and sabinyl acetate (**42**) produced variously by *Thuja occidentalis*, *Thuja plicata*, *Tanacetum vulgare*, and *Juniperus sabina* gave the unexpected result that tracer was located almost exclusively in that part of the skeleton derived from IPP. In thujone, tracer was located at the carbonyl carbon atom in accordance with Ruzicka's hypothesis, but over 99% of that incorporated was thus situated and the "bottom" half of the molecule that, on the basis of the accepted theory should have been derived from DMAPP, was essentially unlabeled. The situation for the other related terpenes was similar.[132] This labeling pattern differed entirely from a previous report[133,134] that thujone biosynthesized from the same precursor by *Thuja occidentalis* contained tracer almost equally divided between the carbon atoms shown in **50**. This last conclusion, which was based on a very incomplete degradation of the substrate, led to the proposal of the formation of a novel zwitterion (**51**) from α-terpineol which reacted as in eq 9. For such a scheme, **52**, which is a biosynthetic equivalent of **51**, would be a more realistic intermediate. Either this work is in error or the discrepancy with the more detailed later investigation must be ascribed to a seasonal effect.[103] The latter explanation would require that in the earlier work [^{14}C]IPP had been largely converted into the more stable DMAPP which would have

(126) P. Valenzuela, O. Cori, and A. Yudelevich, *Phytochemistry*, **5**, 1005 (1966).
(127) M. J. O. Francis, D. V. Banthorpe, and G. N. J. Le Patourel, *Nature (London)*, **228**, 1005 (1970).
(128) G. N. J. Le Patourel, Ph.D. Thesis, University of London, 1971.
(129) V. H. Potty and J. M. Bruemmer, *Phytochemistry*, **9**, 1001 (1970).
(130) P. Dunphy, personal communication.
(131) J. Mann, Ph.D. Thesis, University of London, 1970.
(132) D. V. Banthorpe, J. Mann, and K. W. Turnbull, *J. Chem. Soc. C*, 2689 (1970).
(133) W. Sandermann and W. Schweers, *Tetrahedron Lett.*, 257 (1962).
(134) W. Sandermann, W. Schweers, K. Bruns, H. Stockmann, and O. Beinhoff, *Bull. Nat. Inst. Sci., India*, **37**, 168 (1968).

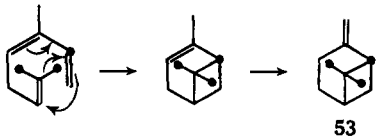


51 52 50 (9)

condensed with unlabeled IPP derived from a metabolic pool to form thujone with essentially all the tracer residing on the *gem*-dimethyl groups and negligible amounts in the ring; this possibility was not excluded by the degradation scheme that was applied. However, the "asymmetric" labeling pattern was obtained with plant material of different ages under different feeding conditions.[132]

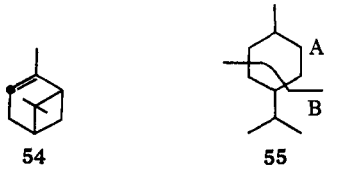
Nevertheless, there is evidence from work on the biosynthesis of the cyclopentane monoterpenes verbenalin[98] and skytanthine[135,136] that enzyme levels and pool sizes may vary during the growing season of a plant, and different mechanisms to form the same product may also occur under different conditions. Much more work is needed before such phenomena are accepted as general, but the possibility of their occurrence must be allowed.

A zwitterion analogous to **51** was also proposed to account for the presumed tracer pattern obtained after an incomplete degradation of β-pinene biosynthesized from [2-^{14}C]MVA by *Pinus nigra*,[137] and this pattern was also rationalized[10] by formation of the bicyclic skeleton by an internal Diels–Alder condensation of *cis*-ocimene (eq 10). However, the conclusions



53 (10)

may again be erroneous, since α-pinene biosynthesized from [2-^{14}C]MVA by *Pinus attenuata* has[128] an asymmetric labeling pattern similar to that found for thujone, and the tracer in the part of the molecule derived from IPP was located as in **54**,



54 55

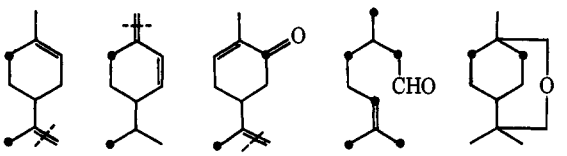
which is consistent with Ruzicka' suggestions (*cf.* Scheme III), whereas the pattern in **53** would be expected from cyclization of the zwitterion or its structural equivalent.

Asymmetric labeling such that the tracer very predominantly resides in the moiety A of the terpene skeleton **55** is also found for (+)- and (−)-camphor (separately) biosynthesized from [2-^{14}C]MVA by *Salvia leucophylla*, *Artemisia californica*, and *Chrysanthemum balsamita*[138,139] and is inferred from results of an incomplete degradation of pulegone formed from the same precursor by *Mentha pulegium*.[29] An analogous pattern occurs for the sesquiterpenes tutin and coriamyrtin formed in other plant species,[140] and the situation may be general for mono and higher terpenes formed from MVA fed under these conditions. *A priori* there is no reason to assume that the specific activities of the moieties A and B in **55** should be the same, as IPP and DMAPP could well be diluted to differing extents by inactive precursors present in pools, but the pronounced asymmetry that is observed is contrary to preconceptions.[10,13]

The phenomenon may be rationalized in terms of the operation, perhaps in concert, of several factors. First, a pool of DMAPP in the plant may exist which can react with IPP generated from exogenous MVA before the labeled IPP can be isomerized to DMAPP. An unstable compound that was possibly 3,3-dimethylallyl alcohol was reported[141] in the leaf oil of *Tanacetum vulgare*, but a detailed examination of this species in which the major monoterpenes are asymmetrically labeled and also of certain *Chrysanthemum* species[131] gave no evidence for a pool of this or of a related C-5 compound. Secondly, DMAPP may not be of direct mevalonoid origin; a possible route for its formation by degradation of monoterpenes using reactions that have been characterized as occurring in microorganisms has been outlined,[12] and tracer studies on the fate of monoterpenes injected into *Tanacetum vulgare* have shown[40] that enzymic pathways for such degradations to form C-2 or C-5 fragments are available. However, 3,3-dimethylacrylic acid, leucine, or valine, which are possible candidates as precursors of DMAPP by this route, were not significantly incorporated into thujone by *Tanacetum vulgare*.[131,142] Thirdly, compartmentation effects similar to those believed to occur for pigment and phytosterol biosynthesis in plastids[39] may intervene. This could permit [^{14}C]IPP to penetrate an intracellular membrane that is impermeable to both MVA and DMAPP and to reach a site where reaction with endogenous DMAPP could take place. Fourthly, the excess of MVA unavoidably used may inhibit IPP–isomerase; this explanation is consistent with the association of low incorporations of MVA with asymmetric labeling, whereas high incorporations (in petals; see later) give a symmetrical pattern whereby that part of the skeleton derived from DMAPP is equivalently labeled. The use of a wide range of concentrations of applied MVA does not significantly affect either the percentage incorporation of [2-^{14}C]MVA into *Tanacetum vulgare* or the labeling pattern,[132] but studies of the possible inhibition of IPP–isomerase by MVA, MVAP, and the other intermediates of monoterpenes synthesis would be extremely worthwhile.

(135) H. Auda, H. R. Juneja, E. J. Eisenbraun, G. R. Waller, W. R. Kays, and H. H. Appel, *J. Amer. Chem. Soc.*, **89**, 2476 (1967).
(136) A. G. Horodysky, Ph.D. Thesis, Oklahoma State University, 1967.
(137) W. Sandermann and W. Schweers, *Tetrahedron Lett.*, 259 (1962).
(138) D. V. Banthorpe and D. Baxendale, *Chem. Commun.*, 1553 (1968).
(139) D. V. Banthorpe and D. Baxendale, *J. Chem. Soc. C*, 2694 (1970).
(140) M. Biollaz and D. Arigoni, *Chem. Commun.*, 633 (1969).
(141) E. von Rudloff and E. W. Underhill, *Phytochemistry*, **4**, 11 (1965).
(142) B. V. Charlwood, unpublished results.

The remaining studies of position-specific labeling of monoterpenes formed in leaf tissue have usually been interpreted in terms of equivalent labeling of the moieties derived from IPP and DMAPP, but such conclusions are based on either incomplete or ambiguous degradation schemes and the results do not always exclude asymmetric labeling. Studies on the formation from [2-^{14}C]MVA of limonene in *Pinus pinea*,[143] β-phellandrene in *Pinus contorta*,[144] and carvone from *Anethum graveolens*[145] led to the conclusion that the tracer patterns were as in **56** to **58**, but these proposals rested entirely

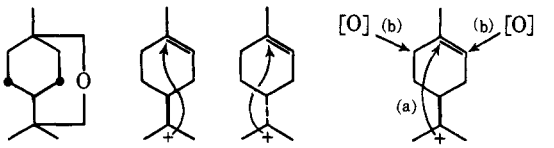


56 **57** **58** **59** **60**

on the demonstrations that cleavage at the double bonds as shown liberated formaldehyde containing no tracer. These results are consistent with the proposed patterns of tracer, but they do not prove, as was claimed, that the *gem*-dimethyls derived from DMAPP and the C_4 and C_5 atoms derived from IPP were not equivalently labeled with tracer, nor do they rule out asymmetric labeling similar to that found in the thujane derivatives. In addition, when the position of tracer is not located and biosynthetic deductions are made on the basis of of the cleavage of nonradioactive fragments from a presumed radioactive substrate, the possibility is always present that the tracer could have been wholly or partly due to an unsuspected impurity.

Labeling of that part of the molecules of carvone and limonene derived from DMAPP was claimed when *Carum carvi* was fed with [2-^{14}C]MVA, but few details are available.[146] The methyl and methylene groups in the side chain of these compounds were not equivalently labeled, and this is consistent with the view that the presumed precursor **17** (Scheme III) is not an open carbonium ion which would have resulted in the two groups becoming radiochemically equivalent. The analogous *gem*-dimethyl groups have been found to become equivalent in the biosynthesis of nepetalacetone (section IV).

The incorporation of [2-^{14}C]acetate into citronellal and [2-^{14}C]MVA into 1,8-cineole in branches of *Eucalyptus citriodora* and *E. globulus*, respectively, has been investigated.[147] Although very extensive randomization of tracer occurred, the former product was claimed to show the expected pattern of labeling, **59**. The activity in 1,8-cineole was demonstrated to be at the C_2 and C_6 positions (*cf.* **60**), and the balance was presumed to be in the *gem*-dimethyl groups, although this was not directly demonstrated. Another study of the formation of 1,8-cineole in the same species with the same precursor reported complete randomization of tracer in product;[148] as glucose was concomitantly heavily labeled it is likely that the precursor was degraded to carbon dioxide and tracer was thence incorporated into the monoterpene. [1-^{14}C]GPP was incorporated[149] into 1,8-cineole in about 0.2% yield by *Rosemarinus officinalis* without appreciable degradation to yield the symmetrically labeled product **61**. A very important



61 **62** **63** **64**

investigation[150] of the formation of citral from [1-^{14}C]IPP and [2-^{14}C]MVA in *Eucalyptus steigeriana* reported relatively high (*ca.* 2.6 and 1.6%) incorporations. Although complete degradations were not carried through, the former precursor appeared to be incorporated almost equivalently into the two C-5 moieties, whereas the latter predominantly entered that part of the molecule derived from IPP. This difference may reflect the operation of the selective permeability or enzymic-inhibition effects that have been discussed previously.

Partial degradations of menthol[151] and thymol[152] biosynthesized from [1-^{14}C]acetate by *Mentha arvensis* led to the expected patterns with little randomization such as would have occurred if the tracer on acetate had been scrambled through the incorporation of the latter into the Krebs cycle.

Some monoterpenes, *e.g.*, linalool, α-pinene, and camphor, occur either as practically pure optical isomers or as mixtures that occasionally are racemic.[153] Enzyme systems producing each enantiomer must exist and must sometimes co-occur in the same species. The only tracer study of such enantiomers (occurring in different species) was of camphor[138,139] which showed each isomer to be formally derived by cyclization of the cation **17** (Scheme III) formed from the corresponding optical isomer of α-terpineol; *cf.* **62** and **63**. The formation of both isomers of camphor from a particular enantiomer of α-terpineol by cyclization and oxidation at C_2 and C_6, respectively (*cf.* **64**), was ruled out by the tracer pattern observed from incorporation of [2-^{14}C]MVA. (+)-α-Pinene commonly occurs in admixture with (−)-β-pinene of opposite absolute configuration, but, whereas the former is rarely optically pure, the latter is invariably so. No appropriate tracer studies have been carried out on this system, but evidence[154] from the extent of co-occurrence of the pinenes suggests two pathways to the α isomer, only one of which involves the intermediacy of **17**. The validity of this type of argument is difficult to assess.

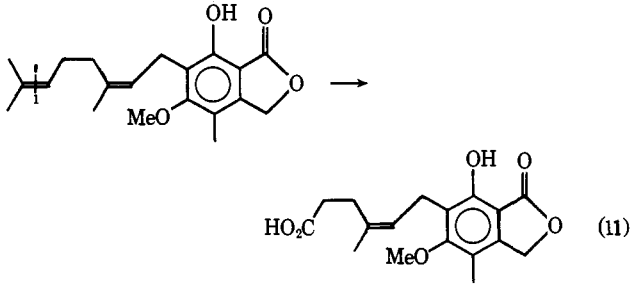
A few attempts (see before) have been made to determine the effectiveness of 3,3-dimethylacrylic acid as a precursor for monoterpenes. The [1-^{14}C] acid was claimed to be incorporated without degradation into pulegone formed in *Mentha pulegium*,[155,156] but the conclusion must be treated with reserve for the degradation scheme was incomplete, the products were neither well characterized nor satisfactorily purified, and the

(143) W. Sandermann and K. Bruns, *Naturwissenschaften*, **49**, 258 (1962).
(144) K. Bruns, quoted by G. Weissmann in "Comparative Phytochemistry," T. Swain, Ed., Academic Press, London, 1966, p 97.
(145) W. Sandermann and K. Bruns, *Planta Med.*, **13**, 364 (1965).
(146) M. Souchek and J. Vrkoc, quoted in ref 11, p 168.
(147) A. J. Birch, D. Boulter, R. I. Fryer, P. J. Tomson, and J. L. Willis, *Tetrahedron Lett.*, No. 3, 1 (1959).
(148) D. Arigoni, quoted in ref 13, p 65.
(149) B. Achilladelis and J. R. Hanson, *Phytochemistry*, **7**, 1317 (1968).
(150) L. P. Neethling, H. G. Reiber, and C. O. Chichester, *Nat. Conf. Nucl. Energy, Appl. Isotopes Radiat., Proc.*, 451 (1963).
(151) H. Mitsuhashi, K. Kaneko, S. Eguchi, and M. Otsu, *Yakugaku Zasshi*, **80**, 268 (1960); *Chem. Abstr.*, **54**, 21343 (1960).
(152) M. Yamazaki, T. Usui, and S. Shibata, *Chem. Pharm. Bull.*, **11**, 363 (1963); *Chem. Abstr.*, **59**, 1966 (1963).
(153) V. Plouvier, *Phytochemistry*, **5**, 955 (1966).
(154) E. Zavarin, *ibid.*, **9**, 1049 (1970).
(155) W. Sandermann and H. Stockmann, *Naturwissenschaften*, **43**, 580 (1956).
(156) W. Sandermann and H. Stockmann, *Chem. Ber.*, **91**, 930 (1958).

unprecedently high incorporation for this type of feeding experiment (7.8%) suggests that a tracer-containing impurity could have been present in the pulegone that was isolated. In contrast, the acid was found to be some 10,000-fold less efficient than MVA as a precursor for unspecified isoprenoids in germinating peas[157] and was extensively degraded to acetate before incorporation into monoterpenoids by fungi.[59,60] Incorporation of [2-^{14}C]MVA into *Mentha pulegium* results in pulegone with over 90% of the tracer in the moiety derived from IPP.[29] Attempts to feed geranic acid failed as the additive was toxic at dilutions of 1 in 10,000.[155,156]

In contrast to the patterns of asymmetric labeling repeatedly found in leaves, the high incorporations of [2-^{14}C]MVA into geraniol and nerol and their β-glucosides in rose petals[127] and into pyrethrins in flower heads of *Chrysanthemum cinerariaefolium* (see section V)[158,159] result in almost equal labeling of the parts derived from IPP and DMAPP. Consequently, the synthetic sites may be different in petals and in stem or leaf tissue; in particular, the latter types of tissue contain discrete oil glands on the surface (*cf.* section VI.A) that differ from the internal secreting cells of petals, and these different types of cells may not be similarly permeable to biosynthetic precursors.

The fungal product myceliamide (**12**) has been shown to possess a labeling pattern resulting from equilibration of tracer from MVA between the C-5 units.[59,60] This implies that fungi may not possess the intracellular complexity of structure that causes compartmentation in leaf tissue. In a parallel study,[60] mycophenolic acid (**65**) produced by *Penicillium brevi-*



(11)

65

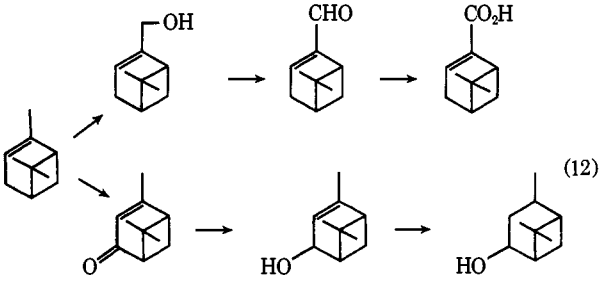
compactum was labeled by both [1-^{14}C]acetate and [2-^{14}C]-MVA, and oxidative cleavage of a C-10 side chain as in eq 11 was inferred.[160,161] In accordance with this scheme, acetone and mycophenolic acid in approximately equimolar quantities and with approximately equal specific radioactivities were isolated[162] from the culture medium after incubation of the fungus with [2-^{14}C]MVA, but cleavage of a C-15 side chain has been recently suggested.[163,164] The modified route is in accordance with the lack of incorporation of [^{14}C]geraniol into the metabolite and the isolation from the culture medium of products with C-15, but not C-10, side chains.

E. MODIFYING REACTIONS

The hydrocarbons, alcohols, etc., that are believed to be biosynthesized as described in the previous sections can be modified in numerous ways to give the whole spectrum of monoterpenoid products. Although most of these modifications may be readily rationalized on chemical grounds, there is seldom much evidence for their sequence or even direction, although some notions can be gleaned from studies of the frequency of occurrence and stereochemical relationships of co-occurring compounds, and of seasonal changes in composition of the oils. Tracer studies using "early" precursors such as carbon dioxide and MVA do not in general provide sufficient detail to define later steps, and few studies have involved feeding of precursors further along the pathway. There is certainly considerable scope for studies of the interconversions of [^{14}C]-labeled monoterpenes *in vivo*, although such additives, could extensively perturb the normal pathways if fed in gross amounts.

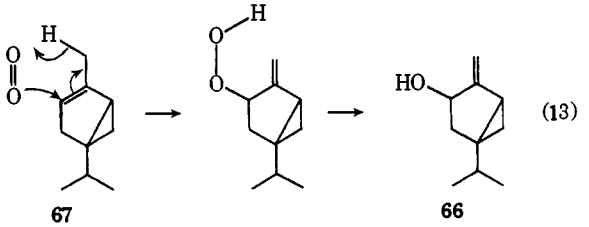
Modifying reactions may be catalyzed by commonly occurring enzymes of low specificity toward substrates, or some may be nonenzymic reactions linked, for example, to photochemical processes occurring in the chloroplasts. The oil glands are excellently situated to benefit from the latter process, although their heavily cutinized outer walls would provide considerable shielding.

The presumed sequence of oxidative modification may usually be rationalized by schemes with many *in vitro* analogies. Thus γ oxidation of α-pinene may lead to myrtenyl or verbenyl derivatives that co-occur in particular plants,[5] as in eq 12. Another route is the "ene" reaction[165] that readily oc-



(12)

curs on photosensitization to give a product with a rearranged double bond; such a nonenzymic process could lead to the formation of sabinol (**66**) from α-thujene (**67**) (eq 13)



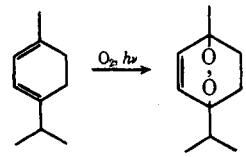
(13)

67 **66**

in an almost stereospecific fashion since model systems show that photoxidation of this substrate in the presence of various organic dyes gives over 90% yields of that epimeric hydroperoxide formed by approach of oxygen from the least hindered side of the skeleton.[166] The hydroperoxides would be rapidly broken down by the peroxidases that appear ubiqui-

(157) D. J. Baisted and W. R. Nes, *J. Biol. Chem.*, 238, 1947 (1963).
(158) P. J. Godin, H. S. Inglis, M. Snarey, and E. M. Thain, *J. Chem. Soc.*, 5878 (1963).
(159) M. P. Crowley, P. J. Godin, H. S. Inglis, M. Snarey, and E. M. Thain, *Biochem. Biophys. Acta*, 60, 312 (1962).
(160) A. J. Birch and J. J. Wright, *Aust. J. Chem.*, 22, 2635 (1969).
(161) A. J. Birch, *Science*, 150, 202 (1964).
(162) A. J. Birch and R. W. Rickards, quoted by W. B. Whalley in ref 8, p 1047.
(163) C. T. Bedford, J. C. Fairlie, P. Knittel, T. Money, and G. T. Phillips, *Chem. Commun.*, 323 (1971).
(164) L. Canonica, W. Kroszczynski, B. M. Ranzi, B. Rindone, and C. Scolastico, *ibid.*, 257 (1971).
(165) H. M. R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, 8, 556 (1969).
(166) G. Ohloff, E. Uhde, A. F. Thomas, and E. Sz. Kovats, *Tetrahedron*, 22, 309 (1966).

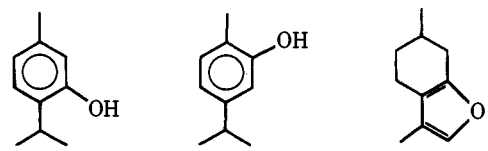
tous in photosynthesizing tissue to form alcohols. A direct Diels–Alder-type addition of oxygen to α-terpinene (**33**) may form ascaridol (**32**) in *Chenopodium anthelminticum* (eq 14);



33 **32** (14)

again an *in vitro* analogy is available[167] using chlorophyll as the photosensitizer.

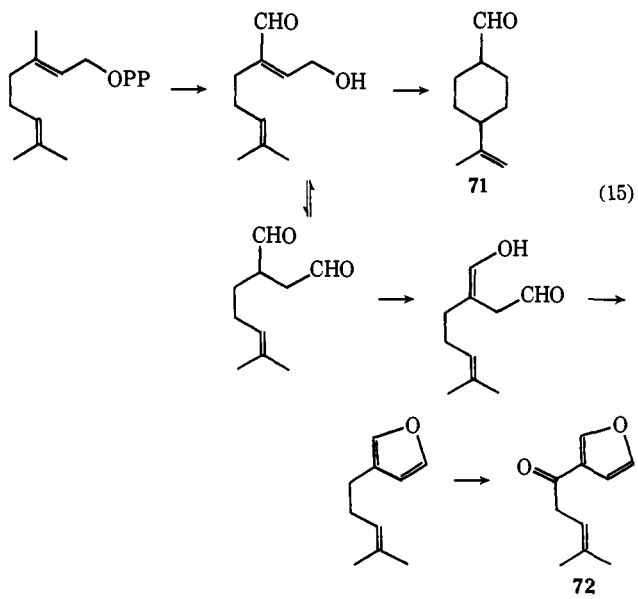
Oxygenated terpenoids and terpene hydrocarbons usually predominate in the oils from angiosperms and gymnosperms, respectively, and the routes to the two classes of compounds from early common precursors have been suggested to be in competition rather than oxidation being sequential to the formation of hydrocarbons.[168] Dehydrogenations, such as the conversion of menthadienes into *p*-cymene (**40**) and the formation of considerable quantities of thymol (**68**) and carvacrol (**69**) in *Thymus vulgaris* and related species, are believed to



68 **69** **70**

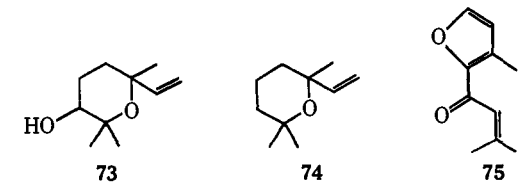
be nonenzymatic.[169] Oil from *Monarda fistularia* (wild bergamot) contains limonene together with a range of aromatics (*p*-cymene, carvacrol, thymoquinone, thymohydroquinone, and dihydrothymoquinone[3]) that are probably formed in a similar manner.

Formation of a furan ring is exemplified in the co-occurrence of menthofuran (**70**) with pulegone in *Mentha* species[3] and is further illustrated by the proposed sequence[3,170,171] to perillaldehyde (**71**) and β-dehydroperilla ketone (**72**) (eq 15).

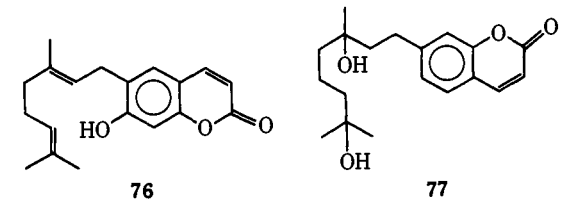


71 **72** (15)

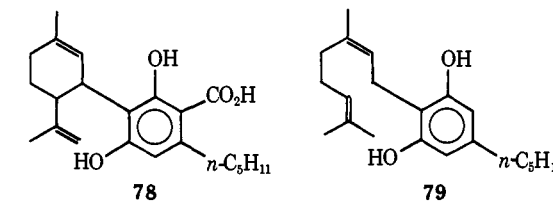
Other examples of oxygen heterocyclics are epoxydihydrolinalool (**73**),[172] dehydroelshotzione (**75**),[178] and pyrans such as **74**.[174] Nitrogen heterocyclics will be discussed in section IV.



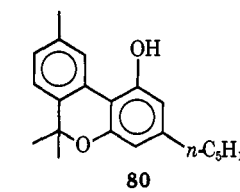
73 **74** **75**



76 **77**



78 **79**



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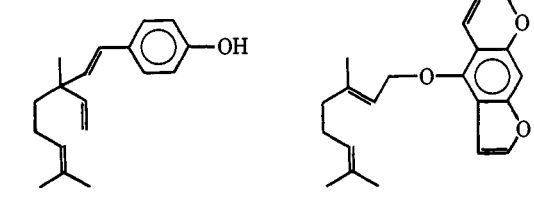


81 **82**

GPP can alkylate acetate-derived phenolic rings to give a variety of terpene derivatives, but it is uncertain whether the electrophilic attack occurs at the polyketide stage or after cyclization.[10,175,176] Well-characterized examples are ostruthin (**76**), marmin (**77**), in which the terpene moiety is hydrated, the cannabis compounds cannabidiolic acid (**78**) and cannabigerol (**79**), and the aromatized end product cannabinol (**80**). Bakuchiol (**81**)[177] is derived from a different type of condensa-

(167) G. O. Schenck, *Angew. Chem.*, **64**, 12 (1952).
(168) R. Mayer, *Z. Chem.*, **1**, 161 (1961).
(169) R. Granger, J. Passet, and R. Verdier, *C. R. Acad. Sci.*, **258**, 5539 (1964).
(170) R. Aneja, S. K. Mukerjee, and T. R. Seshadri, *Tetrahedron*, 4, 256 (1958).
(171) T. Ueda and Y. Fujita, *Chem. Ind. (London)*, 1618 (1962).
(172) Y. R. Naves, D. Lamporsky, and P. Ochsner, *Bull. Soc. Chim. Fr.*, 645 (1961).
(173) Y. R. Naves and P. Ochsner, *Helv. Chim. Acta*, **43**, 406 (1960).
(174) H. Strickler and E. Sz. Kovats, *ibid.*, **49**, 2055 (1966).
(175) D. H. G. Crout, *Top. Carbocyclic Chem.*, **1**, 63 (1969).
(176) T. A. Geissman and D. H. G. Crout, "Organic Chemistry of Secondary Plant Metabolism," Freeman, Cooper & Co., San Francisco, Calif., 1969.
(177) G. Mehta, U. Ramdas Nayak, and S. Dev, *Tetrahedron Lett.*, 4561 (1966).

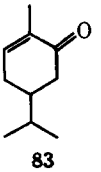
tion and many other conjugates of monoterpenes have been isolated[8,175] including ethers such as bergamottin (**82**).

Cyclopentane monoterpenes frequently occur as glucosides (*cf.* section IV), but only recently have such derivatives of the more common terpene classes been isolated and unambiguously characterized from rose petals and other sources.[104-106] The β-D-glucosides of geraniol, nerol, and citronellol thus found could be derived from GPP *via* the usual uridine–diphosphoglucose donor systems and could thus provide a means whereby a monoterpene pool is formed by diverting material *en route* to higher terpenes. Hydrolysis of GPP and transglucosylation of the free alcohol is probably less effective, partly because of the low solubility of the latter in the tissue fluids and partly because of rapid rephosphorylation. Geranyl-β-D-glucoside is, however, readily soluble in both organic and aqueous phases and could represent a transportable form of monoterpenes, or indeed of glucose. The glucose moiety could also act as a protecting group that stabilizes an otherwise highly reactive compound such as α-terpineol. This may be the role of such compounds in iridoid synthesis (see section IV.A).

The generality of occurrence of terpene-β-glycosides is at present uncertain, for only very small amounts could be detected in the leaves or petals of *Chysanthemum sinese* and *Tanacetum vulgare*.[181] Recently geraniol and other monoterpenes have been found as esters of fatty acids in rose petals.[180]

F. ARTIFACTS

Many of the trace components of essential oils that are detected in profusion by gas chromatography at high resolution and sensitivity could well arise during the isolation procedures. Such oils are often steam distilled out of iron vessels under conditions where organic acids can be liberated from the plant material, and cyclizations of aldehydes and other monoterpenes may occur.[178] Thus the pH can fall as low as 2.8 during such extraction of the oil from *Pelargonium graveolens*.[38] Well-established examples[1,2] of secondary products that were once believed to be natural components of the appropriate oils are carvotanacetone (**83**) that is readily formed from thujone in Thuja oils by rearrangement at below 100°, and sylvestrene, a mixture of **84** and **85** also known as carvestrene,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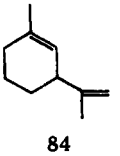
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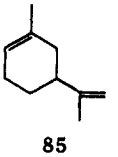

85


86

that was isolated as the dihydrochloride from pine oils, but is now known to be formed by rearrangement of car-3-ene (**86**).

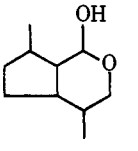
Oils that have been obtained from plant material which has been gathered and stored, often in the sun, may also contain products of photolysis, oxidation, and other chemical modification. Such contaminants are often extremely important as regards odor and flavor for commercial use but have no biosynthetic relevance.

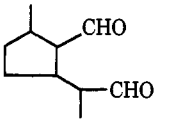
For biochemical investigations, the oils should be preferably extracted by freezing the plant material and grinding at liquid nitrogen temperature with appropriate solvents.[40,179] Only by such a procedure can secondary transformations be eliminated and, in particular, can the action of phosphatases be inhibited. The latter are active[180] even at −30° and cleave phosphate esters to give the free alcohols characteristic of isolated plant oils. The distribution of terpene alcohols between free and esterified forms *in vivo* is not known in any particular oil, but considerable amounts of the latter and other bonded forms (*e.g.*, glucosides and esters) are probably present.

IV. Cyclopentane Derivatives

A. GENERAL

Iridoids are a family of compounds based on carbon skeleton **87** that can be regarded as being formed by cyclization of **88**.


87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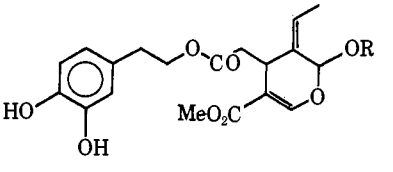
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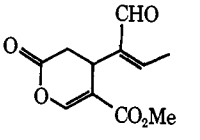
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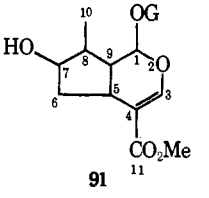
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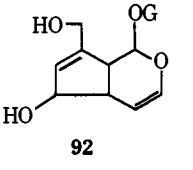
92

G = β-D-glucose

They were originally isolated from the defensive secretions of Iridomyrmex, a genus of ant,[181,182] but are now known to be widely distributed in higher plants usually, but not invariably, as the β-D-glucosides. Several hundred iridoids and related compounds have been isolated [183-185] from leaf, seed, fruit, bark, and root tissue of dicotyledons. This widespread distribution in plant tissues may be a consequence of the water solubility endowed by the sugar residue and contrasts with the storage and retention in specialized oil glands (*cf.* section VI.A) of the largely water-insoluble monoterpenes of the types considered previously. Few systematic studies of chemotaxonomy have been made[184] although a simple field test is available[186] to detect iridoids.

(178) D. A. Baines, R. A. Jones, T. C. Webb, and J. Campio-Smith, *Tetrahedron*, **26**, 4901 (1970).

(179) E. Schratz and T. Wahlig, *Planta Med.*, **13**, 218 (1965).
(180) R. L. Bieleski, *Anal. Biochem.*, **9**, 431 (1964).
(181) G. W. K. Cavill, *Rev. Pure Appl. Chem.*, **10**, 169 (1960).
(182) L. H. Briggs, B. F. Cain, P. W. Le Quesne, and J. N. Shoolery, *Tetrahedron Lett.*, 69 (1963).
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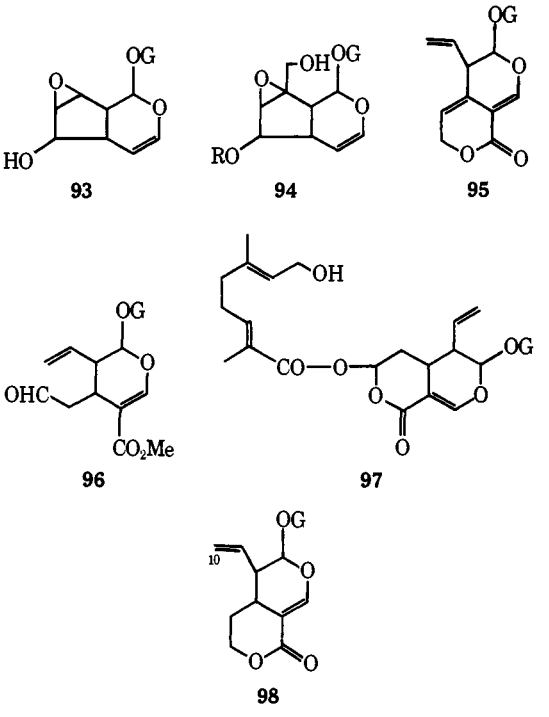
(184) R. Hegnauer, *ibid.*, **41**, 577 (1966).
(185) J. M. Bobbitt and K. P. Segebarth in "Cyclopentanoid Terpene Derivatives," W. I. Taylor and A. R. Battersby, Ed., Marcel Dekker, New York, N. Y., 1969, p 1.
(186) J. H. Wieffering, *Phytochemistry*, **5**, 1053 (1966).

A decade ago it was suggested[187,188] that tetrahydropyran–methylcyclopentane monoterpenes of this, then unusual, type were possible biogenetic precursors of the indole alkaloids; similar proposals[189] were made for the formation of oleuropeine (**89**) and elenolide (**90**). More recent work has amply confirmed these speculations, and there is little doubt that loganin (**91**), or a closely related compound, does fulfill these roles.[190,191] Most of the biosynthetic studies on the iridoids have been concerned with their function as intermediates *en route* to indole alkaloids, and it is only recently that these monoterpenes have begun to be studied in their own right. This weighting of interest is apparent in most of the many reviews on the subject,[11,12,190–201] the most recent of which are especially comprehensive.[201a,201b]

Loganin is also an intermediate in the biosynthesis of other iridoids and of secoiridoids formed by rearrangement and functionalization of the skeleton **87**.[190,193] Its aglucone is unstable[202–209] and the sugar moiety may play a solubilizing, transport-facilitating, and, very importantly, protective role. In particular it may protect the C_1-linked hydroxyl group (for numbering of ring see **91**; alternative systems are sometimes used) from oxidation until the appropriate stage in the biosynthetic scheme when the sugar residue is cleaved off.

The fused bicyclic system of loganin accounts for eight of the ten carbon atoms derived from the acyclic monoterpene precursor. One of the remaining carbons is absent in some compounds that co-occur with, and are undoubtedly related to, the iridoids, although there is no formal biosynthetic demonstration for these relationships save in the case of aucubin (**92**).[210] Unedoside (**93**)[211] is the only compound so far characterized that has lost both peripheral carbons; none have been reported which have lost the C_{10}



G = β-D-glucose

methyl group but not the C_{11} carboxy group, whereas in contrast several families of compounds have lost the latter group but retained the former, *e.g.*, aucubin (**92**) and catalposide (**94**, R = *p*-hydroxybenzoyl).[212,213] Secoiridoids such as gentiopicroside (**95**)[214–222] may be derived from loganin or a closely related compound by cleavage of the C_7–C_8 bond yielding initially, in the case of loganin itself,[189,222,223] secologanin (**96**).[224,225] The isolation of compounds such as foliamenthin (**97**),[224,226,227] sweroside (**98**),[228] and ipecoside (**99**),[224,229,230] as well as biosynthetic studies, provide further evidence that these groups of compounds are biogenetically

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(188) E. Wenkert, *J. Amer. Chem. Soc.*, **84**, 98 (1962).
(189) H. C. Beyerman, L. A. Van Dijck, J. Levisalles, A. Malera, and W. L. C. Veer, *Bull. Soc. Chim. Fr.*, 1812 (1961).
(190) A. R. Battersby in "Natural Substances Formed Biologically from Mevalonic Acid," T. W. Goodwin, Ed., Academic Press, London, 1970, p 157.
(191) A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Martin, and A. O. Plunkett, *Chem. Commun.*, 890 (1966).
(192) W. I. Taylor, *Science*, **153**, 954 (1966).
(193) A. R. Battersby, *Pure Appl. Chem.*, **14**, 117 (1967).
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(195) R. Ramage, *Annu. Rep. Chem. Soc. (London)*, **64B**, 511 (1967).
(196) R. Ramage, *ibid.*, **65B**, 577 (1968).
(197) I. D. Spencer in "Comprehensive Biochemistry," Vol. 20, M. Florkin and E. N. Stotz, Ed., Elsevier, Amsterdam, 1968, p 231.
(198) E. Leete, *Advan. Enzymology*, **32**, 373 (1969).
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(200) J. Staunton, *Annu. Rept. Chem. Soc. (London)*, **66B**, 467 (1969).
(201) J. P. Beuge, *Bull. Soc. Chim. Fr.*, 2545 (1969.)
(201a) A. I. Scott, *Accounts Chem. Res.*, **3**, 151 (1970).
(201b) A. R. Battersby, Specialist Periodical Reports, No. 6, The Chemical Society, London, 1971, Vol. 1, p 31.
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(204) S. Brechbühler-Bader, C. J. Coscia, P. Loew, Ch. von Szczepanski, and D. Arigoni, *Chem. Commun.*, 136 (1968).
(205) A. R. Battersby, R. S. Kapil, and R. Southgate, *ibid.*, 131 (1968).
(206) A. R. Battersby, E. S. Hall, and R. Southgate, *J. Chem. Soc. C*, 721 (1969).
(207) H. Inouye, T. Yoshida, S. Tobita, and M. Okigawa, *Tetrahedron*, **26**, 3905 (1970).
(208) P. J. Lentz and M. G. Rossmann, *Chem. Commun.*, 1269 (1969).
(209) G. Buchi, J. A. Carlson, J. E. Powell, and L. F. Tietze, *J. Amer. Chem. Soc.*, **92**, 2165 (1970).
(210) J. E. S. Hueni, H. Hiltebrand, H. Schmid, D. Groger, S. Johne, and K. Mothes, *Experientia*, 22, 656 (1966); see also ref 214.
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(214) H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *ibid.*, 2351 (1969).
(215) H. Inouye, T. Yoshida, Y. Nakamura, and S. Tobita, *ibid.*, 4429 (1968).
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(217) C. J. Coscia and R. Guarnaccia, *J. Amer. Chem. Soc.*, **89**, 1280 (1967).
(218) C. J. Coscia, L. Botta, and R. Guarnaccia, *Arch. Biochim. Biophys.*, **136**, 498 (1970).
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(221) R. Guarnaccia, L. Botta, K. M. Madyastha, and C. J. Coscia, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **29**, 3621 (1970).
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(225) A. R. Battersby, A. R. Burnett, and P. G. Parsons, *Chem. Commun.*, 1282 (1968).
(226) A. R. Battersby, A. R. Burnett, G. D. Knowles, and P. G. Parsons, *ibid.*, 1277 (1968).
(227) P. Loew, Ch. von Szczepanski, C. J. Coscia, and D. Arigoni, *ibid.*, 1276 (1968).
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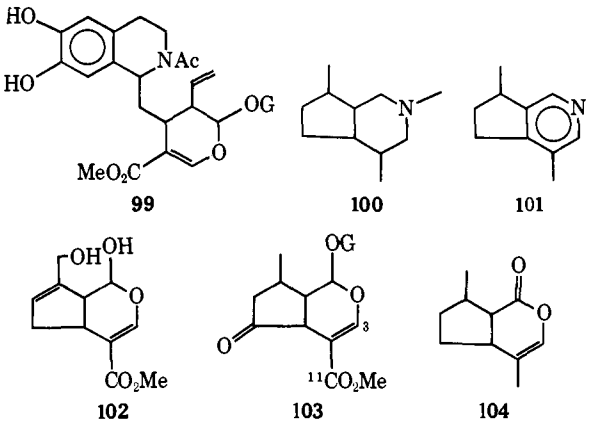
related. Other relatives are the alkaloids β-skytanthine (**100**)[231,232] and actinidine (**101**).[233,234] Most of these compounds occur as their glucosides, but in addition to those described, genipin (**102**)[235] and a few others[236] appear to be



99 **100** **101** **102** **103** **104**

G = β-D-glucose

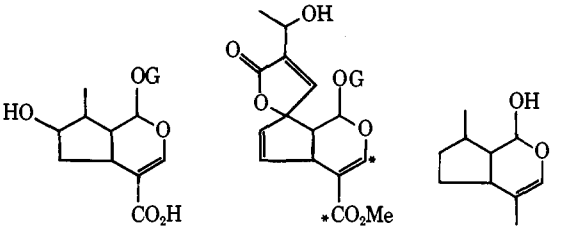
present in plant tissues as their aglucones. A diglucoside[237] and a thioester[238] are among interesting iridoids that have recently been characterized.

All the biosynthetic studies (save one in part[239]) on this group of compounds have depended on investigation of the fate in intact plant tissue of specifically labeled and carefully chosen precursors, and these have often been supplemented by the isolation of suspected intermediates from the tissue.[190] Only a few plant species have been investigated, especially young (*ca.* 3 months old) shoots of *Vinca rosea* or *Catharanthus roseus.*

Whereas the broad outlines of the biosynthetic pathways have undoubtedly been unveiled, some of the minor details may be species or even tissue specific. For example, differences in labeling pattern between the same compound found in the leaves and flowers may occur (*cf.* section III.D). Generally the influence of this, and of other physiological parameters, on biosynthetic routes has been ignored, but studies on the formation of verbenalin (**103**), β-skytanthine (**100**), and nepetalactone (**104**) have demonstrated the critical importance these factors may have on labeling patterns.[98,135,136] The same substrate may also be an effective precursor of a particular iridoid in one plant species but not in another; for example, whole and sliced rhizomes of *Menyanthes trifoliata* did not incorporate [2-^{14}C]MVA into loganin,[204] whereas in *Vinca rosea* the additive was an efficient and specific precursor.[240] Data based on several different experimental approaches or procedures are thus desirable for investigation of any one species.

B. LOGANIN AND LOGANIC ACID

The compounds from which tracer has been incorporated[190,193] into loganin (**91**) and loganic acid (**105**) are listed in section



105 **106** **107**

G = β-D-glucose

VIII.B, and there is little doubt that MVA and GPP or NPP are precursors in the plants that have been investigated.

Experiments using the 4*R* and 4*S* isomers of [2-^{14}C,4-^{3}H$_1$]MVA have confirmed that the stereospecificity of formation of the two double bonds of geraniol used in loganin formation is similar to that found in terpene synthesis in general (*cf.* section III.D), and that direct condensation of IPP with DMAPP to give nerol rather than geraniol directly also does not occur in this class of compounds.[218,241] Geraniol, GPP, or some other derivative such as the enzyme-bound intermediate previously discussed, appears to be an obligatory precursor. The use of (1*R*)- and (1*S*)-[2-^{14}C,1-^{3}H$_1$]GPP has demonstrated that conversion of the C_1 carbon into an aldehydic or equivalent oxidation level is also stereospecific,[190,241] and the hydrogens at C_2 and C_6 of geraniol (**10**) are retained during its transformation into loganin.[190] However, if saturation of the C_2/C_3 double bond of geraniol is a prerequisite for the formation of loganin, then both reduction and subsequent removal of the added proton occur in a stereospecific fashion.[190,241]

The occurrence of foliamenthin (**97**) and related compounds also suggests that oxidation of the isopropylidene group in geraniol is essential for its conversion into loganin.[190] However, evidence from the incorporation of doubly labeled MVA into indole alkaloids suggests that incorporation of the intact propylidene unit of geraniol takes place. Such findings are now reconciled by our knowledge that oxidation occurs at both C_9 and C_{10} of geraniol (Scheme V) and that equilibration of these two carbons of geraniol occurs during the biosynthesis of loganin and related compounds from geraniol. Thus early studies[242] on the biosynthesis of plumieride (**106**)[243,244] proved that during its formation from geraniol the C_9 and C_{10} atoms of the latter became biosynthetically equivalent, for (see later) ~25% of the label present was located at the starred atoms in **106** when [2-^{14}C]MVA was used as a precursor. A similar pattern in loganin (**91**) was obtained with the same precursor[190] and with [3-^{14}C]MVA, and analogous results have been reported for all the iridoids, secoiridoids, and indole alkaloids that have been studied.[245] To account for the pattern in plumieride, iridodial (or irodial) (**107**) was proposed as an intermediate,[242] but this compound is not a precursor of loganin or vindoline (**108**) in *Vinca rosea.*[246] The equilibration

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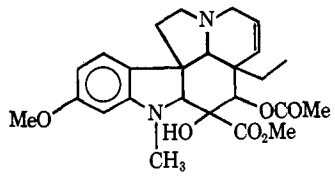
(239) A. A. Qureshi and A. I. Scott, *Chem. Commun.*, 948 (1968).
(240) P. Loew and D. Arigoni, *ibid.*, 137 (1968).
(241) A. R. Battersby, J. C. Byrne, R. S. Kapil, J. A. Martin, T. G. Payne, D. Arigoni, and P. Loew, *ibid.*, 951 (1968).
(242) D. A. Yeowell and H. Schmid, *Experientia*, **20**, 250 (1964).
(243) O. Halpern and H. Schmid, *Helv. Chim. Acta*, **41**, 1109 (1958).
(244) G. Albers-Schonberg and H. Schmid, *ibid.*, **44**, 1447 (1961).
(245) S. Escher, P. Loew, and D. Arigoni, *Chem. Commun.*, 823 (1970).



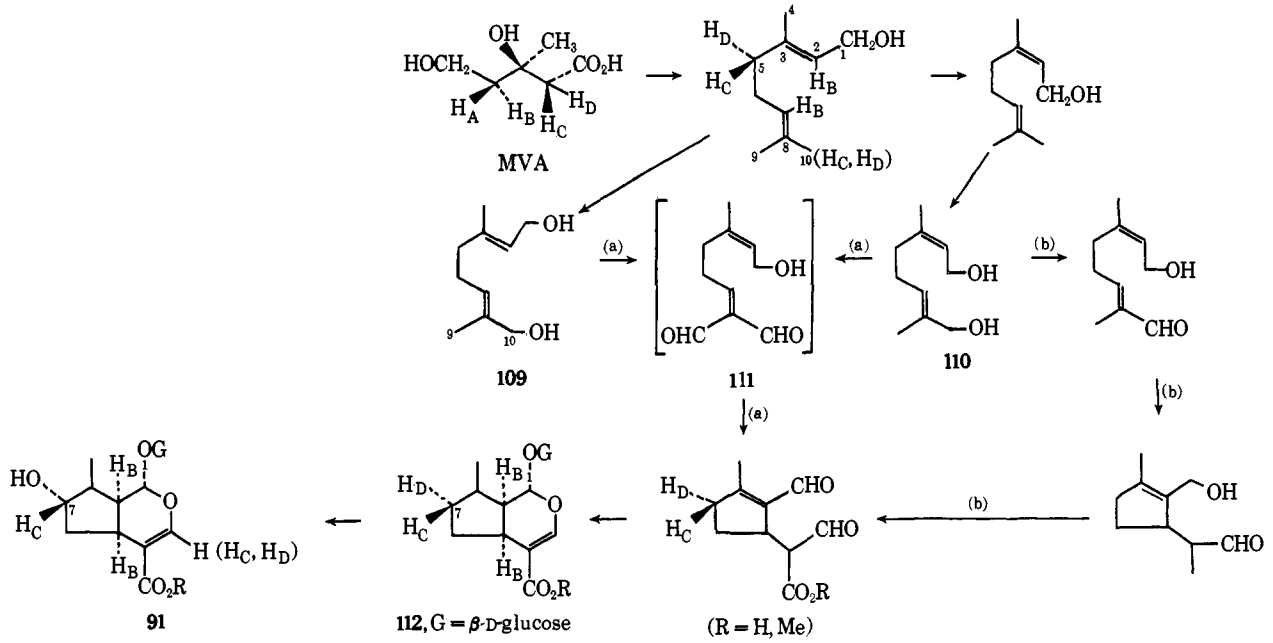
108

of carbon atoms equivalent to C_9 and C_{10} of geraniol may, however, not always be complete and can vary with the physiological condition of the plant used.[135,136] However, the point was made that asymmetric labeling of the part of the molecule derived from IPP, such as was common for the monoterpenes described in the previous section, is not as widespread a phenomenon for these cyclopentane derivatives.

10-Hydroxygeraniol (**109**) and 10-hydroxynerol (**110**) (using the accepted numbering) have recently been shown to be precursors of loganin and of the indole alkaloids,[245,247] and a reasonable route for loganin biosynthesis can be summarized as in Scheme V.[248-250] Complete randomization of ^{14}C label from C_9 and C_{10} of **109** was observed. Several related monoterpenes, linalool, citronellol, and citral, were not significantly incorporated. These results suggest that a further step after **109** and **110** in the biosynthesis of iridoids involves attack on C_9 of **109** or **110** (or of the corresponding aldehydes) to give a hypothetical species such as **111** (route a, Scheme V). It is not known whether C_9 or C_{10} is oxidized first or if indeed there is a specific order. 10-Hydroxynerol was a more efficient precursor than its isomer, and this suggests that the immediate precursors of the iridoids and indole alkaloids (see section IV.D) have the cis double bond at C_2 and C_3 that is expected on stereochemical grounds. The rate of isomerization of this double bond may play an important role in diverting GPP from its alternative function as a precursor of higher terpenoids. It is also possible that cyclization may proceed prior to further oxidation at C_9 of 10-hydroxynerol (route b, Scheme V).

The only other intermediates that have been demonstrated between geraniol and loganin or loganic acid are deoxyloganin and deoxyloganic acid, respectively (**112**, R = Me, H), and both have been shown to be specific precursors of loganin.[214,248] Deoxyloganin occurs together with loganin in *Vinca rosea* and in *Strychnos nux vomica*.[190,248] Neither the aglucone of deoxyloganin nor the isomers with the double bonds at the C_6/C_7 or C_7/C_8 positions were incorporated into the final product. The final stage of loganin biosynthesis is therefore envisaged as hydroxylation of deoxyloganin at C_7, which data on loganic acid biosynthesis suggest is stereospecific[218] like other biological hydroxylations.[251] Both deoxyloganin and loganic acid occur in *Vinca rosea*,[247,250] and a cell-free system from this plant can convert the acid into loganin;[252,253] thus a dual pathway is suggested in which methylation can occur at different points. Similar, and more complicated metabolic grids have been observed in the biosynthesis of other terpenoids, especially carotenoids,[254] and others will be mentioned shortly.

Scheme V[a]



[a] H_A, H_B, H_C, and H_D refer to the 4*S*, 4*R*, 2*R*, and 2*S* hydrogens, respectively, of MVA.

(246) R. M. Bowman and E. Leete, *Phytochemistry*, **8**, 1003 (1969).
(247) A. R. Battersby, S. H. Brown, and T. G. Payne, *Chem. Commun.*, 827 (1970).
(248) A. R. Battersby, A. R. Burnett, and P. G. Parsons, *ibid.*, 826 (1970).
(249) C. J. Coscia and R. Guarnaccia, *ibid.*, 138 (1968).
(250) R. Guarnaccia, L. Botta, and C. J. Coscia, *J. Amer. Chem. Soc.*, **92**, 6098 (1970).
(251) H. Levy, P. Talalay, and B. Vennesland, *Progr. Stereochem.*, **3**, 344 (1962).
(252) R. Guarnaccia, L. Botta, and C. J. Coscia, *J. Amer. Chem. Soc.*, **91**, 204 (1969).
(253) K. M. Madyastha, P. Guarnaccia, and C. J. Coscia, *FEBS (Fed. Eur. Biochem. Soc.)*, **14**, 175 (1971).
(254) J. D. Bu'Lock, "The Biosynthesis of Natural Products," McGraw Hill, London, 1965.

Recent work on loganic acid and gentiopicroside[217,218,249,250,252] biosynthesized from ^{14}C and ^{3}H doubly labeled isomers of MVA and geraniol has confirmed the formation of geraniol and hence of the cyclopentane derivatives from MVA. The stoichiometry of both the decarboxylation of MVAPP to give IPP and of the addition of IPP to DMAPP to give GPP is similar to that reported previously for other terpenoids and steroids.[63,64] Deviations from the expected $^{14}C/^{3}H$ ratio of activities of C_7 of loganic acid were found that were similar to those reported in steroid synthesis.[255,256] Such results have been accounted for by the relatively slow rate of removal of DMAPP by prenyl transferase as compared to the rate of establishing the equilibrium between IPP and DMAPP by IPP isomerase.[257] Conversion of DMAPP into IPP in the latter equilibration would result in a partial loss of asymmetry of the ^{3}H, ^{1}H pair at C_2 of IPP.

No preferential labeling of the two isoprene units of loganic acid was observed. However, such patterns can occur at the monoterpene level; formation of menthiafolin, a hydroxylated isomer of **97**, from [2-^{14}C]geraniol gave a product in which the two C-10 moieties were labeled in the ratio of 3:1.[226] This finding suggests that either the monoterpene or its constituent units may be synthesized in different pools which may correspond to intra- and extrachloroplastic sites of synthesis (both of which sites contain terpene synthesizing enzymes).[258] The pools may be connected at the monoterpene–glucoside level as these compounds are water soluble. However, the stage at which glucose is coupled to a monoterpene remains unknown; present evidence suggests that it is not the final step in loganin or iridoid biosynthesis.[248]

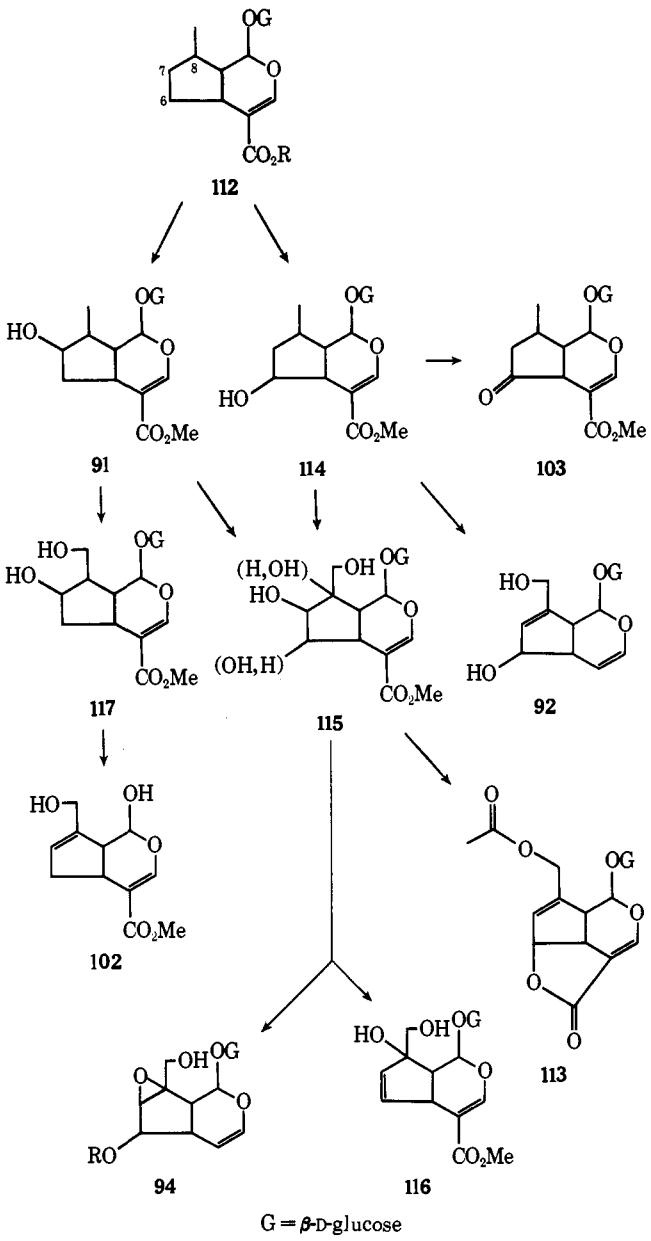
The above findings indicate that iridoids may pass through several intra- and extracellular compartments during biosynthesis, and the distribution of iridoids in all types of plant tissues may provide further evidence for such tortuous pathways. The changes in labeling pattern at C_3 and C_{11} of certain iridoids and related compounds dependent on the age of the plant material may also be related to the need for the biosynthetic scheme to occur at several distinct sites. Indeed, the observed $^{14}C/^{3}H$ isotope ratios at C_7 of loganic acid biosynthesized from 4*R* and 4*S* isomers of [2-^{14}C,-4-$^{3}H_1$]MVA that have been discussed above may be the result of incomplete randomization at the two positions, since the expected isotope ratios were calculated on the assumption of the complete biosynthetic equivalence of these two positions. However, the pattern of randomization between C_3 and C_{11} of loganic acid formed from [2-^{14}C]MVA did not vary with the age of the *Vinca rosea* specimen that was used.[250]

C. OTHER IRIDOIDS AND RELATED COMPOUNDS

The biosynthesis of some members of one family of iridoids, most of which have been mentioned in the preceding discussion, is outlined in Scheme VI. Percentage incorporation of the various precursors are given in section VIII.B.

Deoxyloganic acid (**112**, R = H) is an efficient precursor[214] for asperuloside (**113**),[259] aucubin (**102**), and verbenalin (**103**)

Scheme VI



as well as loganin. Early work[210] showed that [2-^{14}C]MVA was a specific precursor of verbenalin in *Verbena officinalis*, but not of aucubin in *Verbascum thapsus*. The incorporation of tracer into the latter was very low and was randomly distributed with appreciable radioactivity appearing in the glucose moiety. Similar labeling of the sugar occurred on biosynthesis of plumieride[242] from [2-^{14}C]MVA and of loganic acid from HMG,[250] and such observations emphasize the imperative need for determination of specific labeling patterns when presumed precursors are fed and compounds possibly derived from them are isolated.

Verbenalin may be biosynthesized directly from 7-deoxyloganin, but it is usually found that **114** or a close relative is a parent of both verbenalin and aucubin as shown in Scheme VI,[194] although the biochemical details are wanting. [2-^{14}C]-MVA was found to be a specific precursor of verbenalin in *Verbena officinalis*,[98,136] and differences occurred in the

(255) A. R. H. Smith, L. J. Goad, and T. W. Goodwin, *Chem. Commun.*, 926 (1968).
(256) T. Bimpson, L. J. Goad, and T. W. Goodwin, *ibid.*, 297 (1969).
(257) P. W. Holloway and G. Popjak, *Biochem. J.*, **106**, 835 (1968).
(258) S. P. J. Shah and L. J. Rogers, *ibid.*, 114, 395 (1969).
(259) J. Grimshaw, *Chem. Ind. (London)*, 403 (1961).

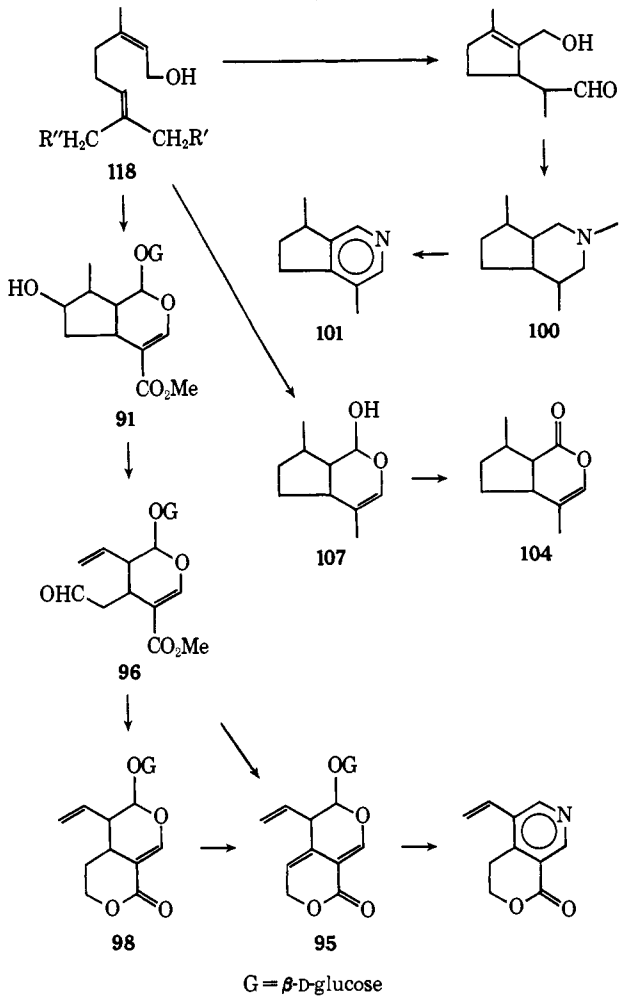
labeling of the product after feeding 1-2- or 4-month-old plants; in the young plants complete randomization of label between C_3 and C_{11} had occurred (27% of total in C_3 and 23% in C_{11} of the expected total in these two positions of 50% of that incorporated). In older plants little randomization took place (42% in C_3 and 8% in C_{11}). These differences, as mentioned before, have implications for all work on terpene biosynthesis and may either reflect differences in pool sizes or may indicate that the actual pathway of biosynthesis varies with age. The actual patterns of randomization here, and in similar experiments on the formation of β-skytanthine (**100**),[135,231,232,260] although varying in extent, are similar to those found in the biosynthesis of loganin and indole alkaloids from [2-^{14}C]MVA.

Formation of 10-hydroxyloganin (**117**) from loganin initiates a hypothetical route to genipin (**102**)[235] and further hydroxylations at C_6 and/or C_8 as well as at C_7 and C_{10} could yield a group of compounds **115** that could in turn give, by decarboxylation, dehydrogenation, and other well-established reactions, products such as asperuloside (**113**), catalposide (**94**, R = *p*-hydroxybenzoyl), and monotropeine methyl ester (**116**). The order in which the presumed hydroxylations at C_6, C_7, C_8, or C_{10} occurs, the extent of these reactions, and subsequent modification of these oxygen centers would provide plausible biogenetic routes to many iridoids not shown in the scheme. The existence of these pathways remains speculative although the occurrence of metabolic grids at this level of iridoid synthesis is likely.[254] Another metabolic grid may also exist at an earlier stage, for the order of hydroxylation of the *gem*-dimethyls of geraniol or nerol may account both in part for the randomization of label in the carbon atoms of iridoids derived from these methyls and may govern whether the iridoids, the analogous alkaloids, or such reduced species as iridodial (**107**) and nepetalactone (**104**) are formed.

Another pattern of biosynthesis is shown in Scheme VII. MVA, deoxyloganin, and both loganin and loganic acid are precursors of the secoiridoid gentiopicroside (**95**)[214-220,259,262] which may be more immediately derived from secologanin (**96**).[190,224,225] Sweroside (**98**) is also a known precursor of gentiopicroside, as detailed by feeding experiments,[228] and is itself probably formed from secologanin by an intramolecular transesterification either before or after reduction of the aldehyde group at C_7 or of an equivalent compound.[190,218] Further work on the biosynthesis of ipecoside (**99**) has demonstrated that cleavage of loganin (**91**) to secologanin (**96**) occurs *via* a mechanism which leaves the proton at C_9 unaffected.[262a] As expected, disacetyl ipecoside (but not its isomer), the condensation product of dopamine with secologanin, is also a precursor of ipecoside.

The biosynthesis of β-skytanthine (**100**) from MVA has been studied in detail,[135,261,263] and it was confirmed that this compound is biogenetically related to the iridoids, as is also the pyridine alkaloid actinidine (**101**).[233,234,260,264] The

Scheme VII

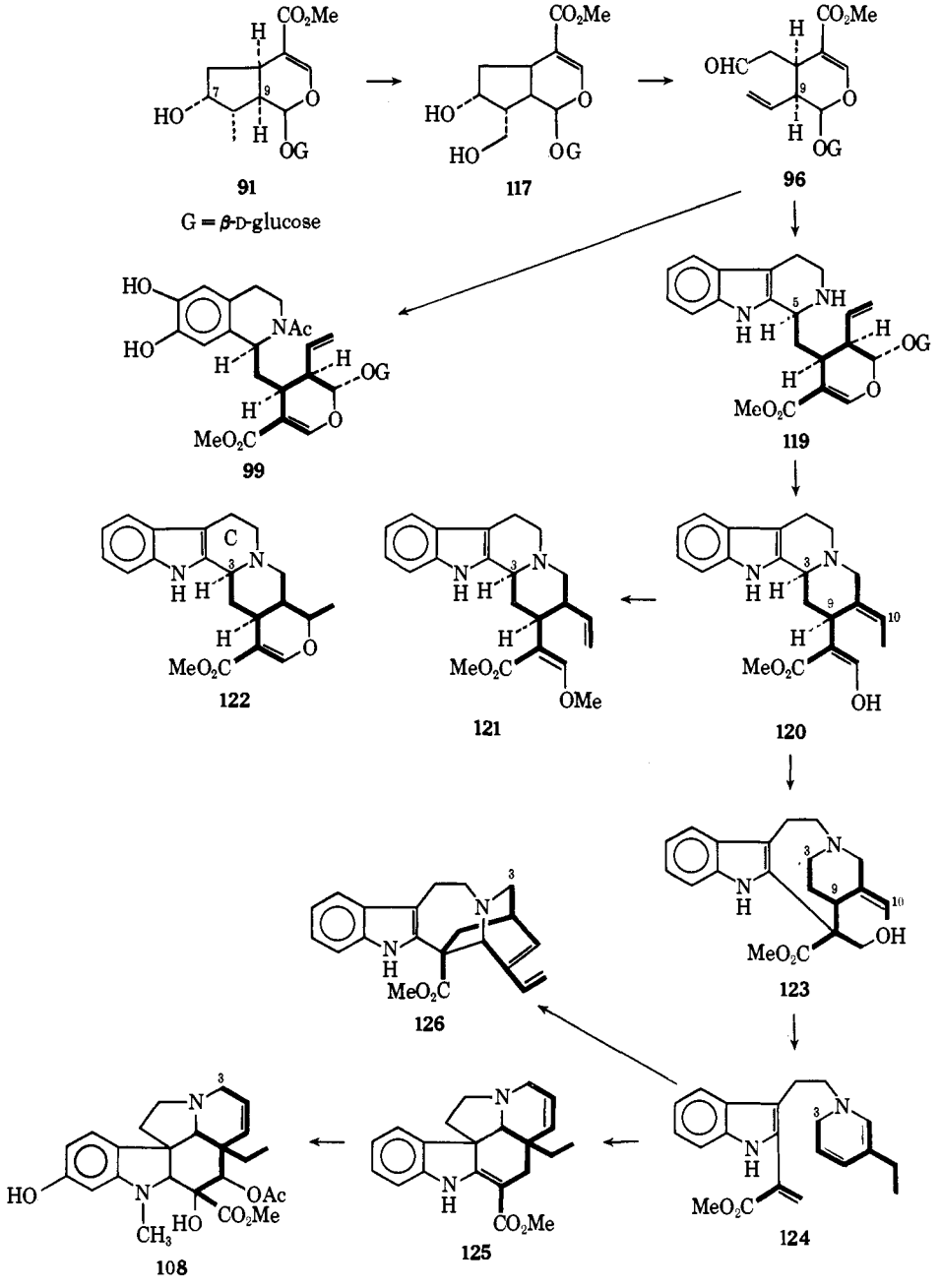


labeling pattern of nepetalactone (**104**) biosynthesized from [2-^{14}C]MVA by *Nepeta cataria*[99,265,266] suggests that some randomization of label occurs at the C-5 (IPP-DMAPP) as well as at the C-10 (monoterpene) stage of biosynthesis. The work confirms the suggested[267] monoterpene nature of the compound which was indicated by preliminary tracer studies.[268] The observed labeling pattern would not be in accord with the proposed mechanism of IPP-isomerase[67,269] which certainly applies in the biosynthesis of loganin and loganic acid.[190,218,241] Evidence for the catabolism of MVA was obtained in these experiments on *Nepeta cataria* and the observed randomization of label may result from this effect which was probably brought into prominence by the prolonged periods of incubation that were used in the feeding procedures.

Various biogenetic schemes for β-skytanthine, actinidine, and nepetalactone have been outlined,[11] and pathways to other iridoids have been proposed.[242] In all these schemes

(260) R. Wildner, E. Le Men, and J. Wiesner, in ref 185, p 271.
(261) C. G. Casinovi and G. Giovannozzi-Sermanni, IUPAC Meeting, London, 1968, Abstract Ab. 3, p 285.
(262) H. G. Floss, U. Mothes, and A. Rettig, *Z. Naturforsch. B*, **19**, 1106 (1964).
(262a) A. R. Battersby and R. J. Parry, *Chem. Commun.*, 901 (1971).
(263) C. G. Casinovi, G. Giovannozzi-Sermanni, and G. B. Marini-Bettolo, *Gazz. Chim. Ital.*, 94, 1356 (1965).
(264) H. Auda, G. R. Waller, and E. J. Eisenbraun, *J. Biol. Chem.*, **242**, 4157 (1967).
(265) G. W. K. Cavill in ref 185, p 202.
(266) R. B. Bates, E. J. Eisenbraun, and S. M. McElvain, *J. Amer. Chem. Soc.*, **80**, 3420 (1958).
(267) E. Leete, *Science*, 147, 1000 (1965).
(268) F. E. Regnier, E. J. Eisenbraun, and G. R. Waller, Abstracts, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, p C166.
(269) D. H. Shah, W. W. Cleland, and J. W. Porter, *J. Biol. Chim.*, 240, 1946 (1965).

Scheme VIII



iridodial is thought to be a key intermediate, but the recent demonstration that the C_8 and C_{10} atoms of nerol must be oxidized at an early stage en route to these compounds may rule this out.[245,247] Furthermore, iridodial is not a precursor for loganin or vindoline.[246]

Schemes V–VII, which are based on recent detailed discussions,[11,198] summarize most of the speculations made in this section and include many steps for which there is evidence from feeding experiments. The nature of the latter experiments and the incorporations of tracer are gathered in section VIII.B. The major problems of the mechanism of the closure of the cyclopentane ring and of the order in which the oxidation steps occur are still uncertain.

D. INDOLE ALKALOIDS

Scheme VIII summarizes our present knowledge of the formation of the indole alkaloids from loganin and later precursors. The terpenoid moieties in the alkaloids are outlined with heavier lines.

The indole part of these compounds was shown to be derived from tryptophan or tryptamine,[225,270–273] but the

(270) E. Leete, *J. Amer. Chem. Soc.*, 82, 6338 (1960).
(271) E. Leete, *Tetrahedron*, 14, 35 (1961).
(272) J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall, V. R. Nelson, and D. C. Wigfield, *J. Amer. Chem. Soc.*, 90, 3566 (1968).
(273) E. Leete, A. Ahmad, and I. Kompis, *ibid.*, 87, 4168 (1965).

first experiments designed to confirm the suggestion[187,188] that the remaining nine or ten carbon atoms were of terpenoid origin were inconclusive.[274] However, recent work has clearly shown[190] that ^{14}C-labeled MVA, geraniol, and loganin are efficient precursors of the nontryptophan part of the molecule in many members of this class. As with the iridoids, current ideas on the routes involved are based almost entirely on experiments which trace the metabolic fate of added presumed precursors, and these data are summarized in section VIII.B. In most cases, the postulated intermediate has also been isolated from the plants under investigation. The presence of structurally related compounds such as ipecoside (**99**),[224,229,230] foliamenthin (**97**),[226,227] vincoside (**119**) and its isomer isovincoside (or strictosidine),[275-277] derivatives of secamine,[278-282] and many others[283] provide indirect evidence for the accepted pathways, although the secamines may be artifacts of isolation.[281]

The nature of the postulated monoterpenoid precursor was demonstrated by the incorporation[193,284] of *O*-[^{3}H]methylloganin into representatives of the three main structural types of indole alkaloids: ajmalicine (**122**) and corynantheine (**121**) (Corynantheine type), catharanthine (**126**) (Iboga type), and vindoline (**108**) (Aspidosperma type). The related iridoids monotropeine methyl ester (**116**), verbenalin (**103**), and genipin (**102**) were not incorporated. The incorporation results with loganin could not therefore be attributed to transfer of the *O*-methyl group. [8-^{14}C]- or [2-^{14}C]loganin, as well as various tritiated forms of this compound, were also specifically incorporated.[240,285]

The next established precursor of the class was secologanin (**96**).[190,224,241] The route of formation of this compound from loganin is at present obscure although it is generally believed that 10-hydroxyloganin may be an intermediate as outlined in Scheme VIII; the isolation of many 10-hydroxylated compounds such as genipin (**102**) demonstrates that the C_{10} methyl group can be hydroxylated *in vivo*. 10-Hydroxyloganin should readily be cleaved to secologanin,[190,224,241] particularly if the exocyclic hydroxyl was first converted into a good leaving group such as phosphate or pyrophosphate. Secologanin has been shown to condense in *vitro* with tryptamine to form vincoside (**119**) and isomeric compounds,[227] and the reaction also occurs *in vivo*.[190,224,225,276,283]

Sweroside (**98**), which is closely related to secologanin, is also an excellent precursor of vindoline and is incorporated in 11% yield,[228] but recent evidence suggests that this and its hydroxy derivative swertiamarin are probably on a branch of the biosynthetic pathway leading from secologanin but not proceeding directly to the indole alkaloids.[190,218,241] Gentiopicroside cannot be a direct precursor of the indole alkaloids since it loses a C_5 hydrogen when biosynthesized from loganin, whereas the indole alkaloids lose a C_9 hydrogen.[218,241]

Vincoside (**119**) seems to be[225,286] the precursor of most indole alkaloids, being initially converted into geissoschizine (**120**,) and corynantheine aldehyde (**121**, \/OMe = \/OH), and present evidence suggests that the rearrangement of the C-10 monoterpene skeleton to give the three classes of indole alkaloids takes place after formation of this parent compound. Isovincoside does not appear to be a natural precursor.[191,253,277]

Current investigation suggests that the three main classes of indole alkaloid are formed in the order: Corynantheine, Aspidosperma, and Iboga types.[201,239,287] A novel approach to the problem has been introduced[239] by following the formation of different alkaloids during the germination of seeds of *Vinca rosea*. Saturation of the non-indolic bond of vincoside destroyed its ability to act as precursor of the class,[224,286] and, furthermore the hydrogen at C_5 of vincoside is retained in all three classes of alkaloid. Geissoschizine may also be a precursor of all classes and has been isolated from *Vinca rosea*,[286] whereas corynantheine aldehyde is not, and is only a precursor compound of its own class, *e.g.*, corynantheine (**121**). In feeding experiments, geissoschizine is specifically incorporated into catharanthine (**126**), coronaridine (a dihydro derivative of **126**), vindoline (**108**), and also the strychnos-group alkaloid akuammicine.[283,288,289] An isomer of stemmadenine (**123**) was also isolated which was converted by base into akuammicine.[289]

Stemmadenine (**123**) may be related to the intermediates, the secamines (**124**), and tabersonine (**125**), which rearrange to give Iboga- and Aspidosperma-type compounds.[287,290] 16,17-Dihydrosecodin-17-ol that is isolated from *Rhazya orientalis*,[282] similar secodines,[278,279] and also an alkaloid isolated from *Tabernamontana cumminsii*[291] may also be related to this intermediate. In *Vinca rosea*, tabersonine (**125**) is a precursor of catharanthine (**126**) and of vindoline (**108**).[272,290]

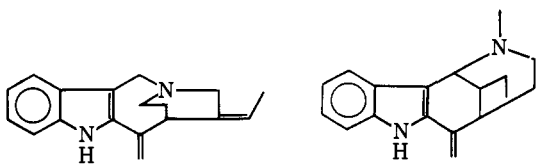
Further evidence for the pathway in Scheme VIII is provided by the location in the alkaloids of the tritium from C_7 of loganin (**91**) which is incorporated without loss.[291a] No migration of hydrogen occurs from the carbon corresponding to C_7 of loganin and C_5 of vincoside (**119**)—the C_3 of the alkaloids—during all the subsequent rearrangements (Scheme VIII: **119** → **120** → **122**, and **119** → **120** → **123** → **124** → **126**, **108**).

These last steps in the biosynthesis (Scheme VIII) are supported by the reported conversions *in vitro* of the Aspidosperma-type alkaloid tabersonine (**125**) into the Iboga-type compound catharanthine (**126**) and of stemmadenine (**123**) into tabersonine and catharanthine;[287,292,293] but other

(274) A. R. Battersby, R. Binks, W. Lawrie, G. V. Parry, and B. R. Webster, *Proc. Chem. Soc., London*, 369 (1963).
(275) G. N. Smith, *Chem. Commun.*, 912 (1968).
(276) R. T. Brown, G. N. Smith, and K. S. J. Stapleford, *Tetrahedron Lett.*, 4349 (1968).
(277) A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc. C*, 1193 (1969).
(278) G. A. Cordell, G. F. Smith, and G. N. Smith, *Chem. Commun.*, 189 (1970).
(279) R. T. Brown, G. F. Smith, K. S. J. Stapleford, and D. A. Taylor, *ibid.*, 190 (1970).
(280) D. A. Evans, G. F. Smith, G. N. Smith, and K. S. J. Stapleford, *ibid.*, 859 (1968).
(281) G. A. Cordell, G. F. Smith, and G. N. Smith, *ibid.*, 191 (1970).
(282) A. R. Battersby and A. K. Bhatnagar, *ibid.*, 193 (1970).
(283) A. I. Scott, P. C. Cherry, and A. A. Qureshi, *J. Amer. Chem. Soc.*, **91**, 4932 (1969).
(284) A. R. Battersby, R. T. Brown, R. S. Kapil, A. O. Plunkett, and J. B. Taylor, *Chem. Commun.*, 46 (1966).
(285) A. R. Battersby, R. S. Kapil, J. A. Martin, and L. Mo, *ibid.*, 133 (1968).
(286) A. R. Battersby, A. R. Burnett, E. S. Hall, and P. G. Parsons, *ibid.*, 1582 (1968).
(287) A. A. Qureshi and A. I. Scott, *ibid.*, 945 (1968).
(288) A. R. Battersby and E. S. Hall, *ibid.*, 793 (1969).
(289) A. I. Scott and A. A. Qureshi, *J. Amer. Chem. Soc.*, **91**, 5874 (1969).
(290) J. P. Kutney, C. Ehret, V. R. Nelson, and D. C. Wigfield, *ibid.*, **90**, 5929 (1968).
(291) P. A. Crooks, B. Robinson, and G. F. Smith, *Chem. Commun.*, 1210 (1968).
(291a) A. R. Battersby and K. H. Gibson, *ibid.*, 902 (1971).
(292) A. I. Scott and P. C. Cherry, *J. Amer. Chem. Soc.*, **91**, 5872 (1969).
(293) A. A. Qureshi and A. I. Scott, *Chem. Commun.*, 947 (1968).

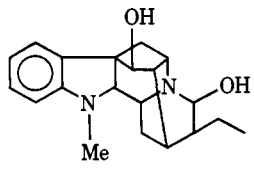
workers have unfortunately been unable to repeat these experiments.[294]

Recently the biosynthesis of apparicine (**127**) and uleine (**128**) has been studied.[295,296] These are structurally unusual



127

128

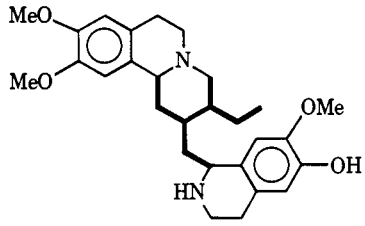


129

in having only a single carbon atom in the link between the indole ring and the non-indolic nitrogen atom. The α-carbon atom of the side chain of the precursor tryptophan is lost and the β-carbon atom is retained. Tryptophan, however, was only incorporated into apparicine. The fission of the side chain must have occurred at a late biosynthetic stage as stemmadenine is incorporated.

The origin of the MVA inferred to be a precursor of indole alkaloids has also been investigated. [2-^{14}C]Glycine is incorporated specifically into the C-10 unit of ajmaline (**129**) found in *Rauwolfia serpentina* whereas [2-^{14}C]acetate is not.[297] [2-^{14}C]Glycine is also a specific precursor of certain other alkaloids but [2-^{14}C]acetate is not, whereas the reverse occurred for the formation of β-sitosterol.[298,299] These results were interpreted in terms of recent studies which show that MVA may be synthesized both within and without chloroplasts.[258] However, [2-^{14}C]glycine is not always a specific precursor of the indole alkaloids as was suggested[297] since it is not incorporated specifically into ajmalicine (**122**) by *Vinca rosea*.[300]

The monoterpene nature of the C-9/C-10 unit of cephaeline, in heavy lines, in **130** has been confirmed, though surprisingly disacetyl ipecoside rather than its isomer disacetyl isoipecoside is a precursor,[262a] and various other alkaloids related to those of the indole class have also been shown to be biosynthesized from iridoids. [2-^{14}C]MVA, [2-^{14}C]- and [3-^{14}C]geraniol, [10-^{14}C]sweroside, and [7-^{3}H]loganin are specific precursors of quinine (**131**);[300-303] [2-^{14}C]MVA and [10-^{14}C]sweroside are of reserpinine (**132**);[303,304] and [2-^{14}C]MVA and [2-^{14}C]-geraniol are of strychnine (**133**).[305]



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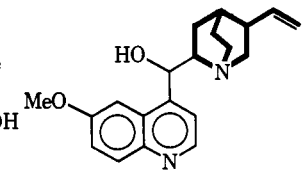

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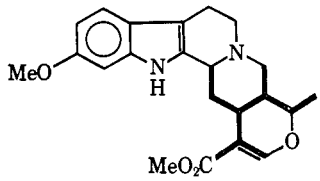

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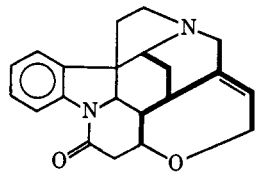
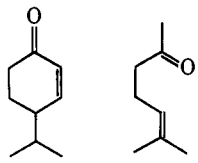


133

V. Irregular Structures

Two classes of compounds can be grouped under this heading: first, degraded monoterpenes that contain less than ten carbon atoms; and, secondly, compounds that apparently break the isoprene rule, in its simpler statements,[306] in containing C-5 units that are not linked head to tail.

The first class presents no biogenetic problem. An early example[307] was cryptone (**134**), which is almost certainly



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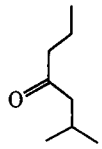

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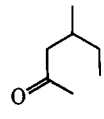
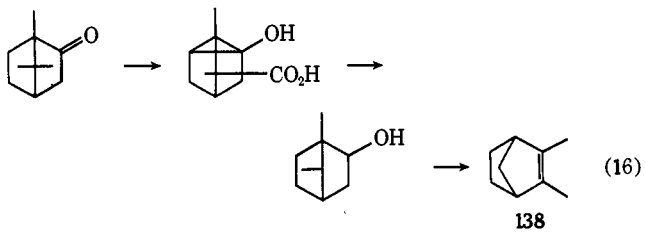


137

formed *in vivo* from β-phellandrene (**57**) with which it co-occurs;[308] others are[309] the arthropod defensive substances **135** to **137**, the origin of which can be reasonably deduced, although no tracer studies have been carried out. Santene (**138**) is believed to be formed by eq 16 and all the presumed



(16)

138

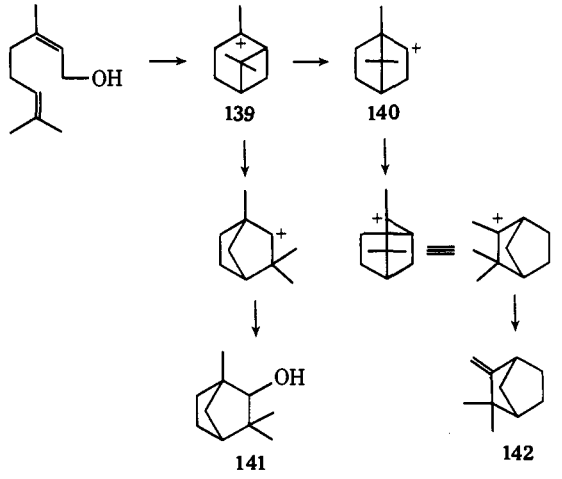
intermediates have been identified as co-occurring in sandalwood oils.[3] The oils of *Pinus jeffreyi* and *Pinus sabiniana* consist predominantly (greater than 95% w/w) of *n*-heptane, but as [2-^{14}C]HMG was not incorporated into this compound, it was concluded[310] to be of polyketide rather than of mevalonoid origin. Such conclusions are questionable in view of the negligible incorporations of MVA and biogenetically related compounds into many products that are of undoubted

(294) R. T. Brown, J. S. Hill, G. F. Smith, K. S. J. Stapleford, J. Poisson, M. Muquet, and N. Kunesch, *Chem. Commun.*, 1475 (1969).
(295) J. P. Kutney, V. R. Nelson, and D. C. Wigfield, *J. Amer. Chem. Soc.*, **91**, 4278 (1969).
(296) J. P. Kutney, V. R. Nelson, and D. C. Wigfield, *ibid.*, **91**, 4279 (1969).
(297) A. K. Garg and J. R. Gear, *Chem. Commun.*, 1447 (1969).
(298) J. R. Gear and A. K. Garg, *Tetrahedron Lett.*, 141 (1968).
(299) A. K. Garg and J. R. Gear, *ibid.*, 4377 (1969).
(300) J. P. Kutney, J. F. Beck, V. R. Nelson, K. L. Stuart, and A. J. Bose, *J. Amer. Chem. Soc.*, **92**, 2174 (1970).
(301) E. Leete and J. N. Wemple, *ibid.*, **88**, 4743 (1966).
(302) H. Inouye, S. Ueda, and Y. Takeda, *Tetrahedron Lett.*, 407 (1969).
(303) A. R. Battersby and E. S. Hall, *Chem. Commun.*, 194 (1970).
(304) H. Goeggel and D. Arigoni, *ibid.*, 538 (1965).
(305) Ch. Schlatter, E. E. Waldner, H. Schmid, W. Maier, and D. Groger, *Helv. Chim. Acta*, **52**, 776 (1969).
(306) R. Robinson, *Annu. Rept. Progr. Chem. (London)*, 20, 100 (1923).
(307) R. S. Cahn, A. R. Penfold, and J. L. Simonsen, *J. Chem. Soc.*, 1366 (1931).
(308) P. A. Berry, A. K. Macbeth, and T. B. Swanson, *ibid.*, 1448 (1937).
(309) J. Weatherstone, *Quart. Rev., Chem. Soc.*, **21**, 287 (1967).
(310) W. Sandermann, W. Schweers, and O. Beinhoff, *Chem. Ber.*, **93**, 2266 (1960).

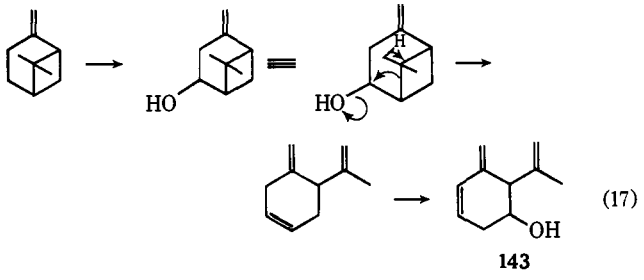
mevalonoid origin. In this context, it is interesting that leucine was incorporated in over 80% yield into amyl alcohol and its acetate in disks of banana fruit and in yeast,[311,312] and this amino acid may be a precursor of certain unusual "terpenoids."

Some of the irregularly linked C-10 compounds of the second class are very probably formed by well-established rearrangements of precursors biosynthesized with conventional head-to-tail linking of the C-5 units, and thus come within the province of the operation of the biogenetic isoprene rule. Examples are fenchane derivatives such as fenchol (**141**) derived from the ion **139** (Scheme IX) and isocamphane

Scheme IX



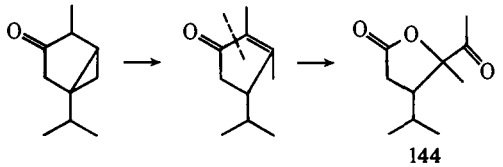
derivatives such as camphene (**142**) derived from **140** by a similar Wagner–Meerwein shift. A more unusual type of rearrangement[313] (eq 17) gives carquejol (**143**) which occurs



(17)

143

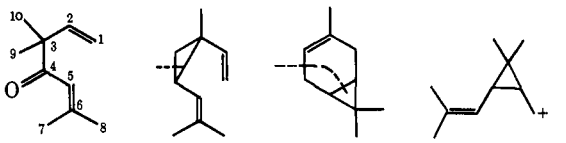
in the oil of the same name[314] and is the only known naturally occurring σ-menthane derivative. Another speculative proposal is the derivation of **144** from thujone (eq 18).



(18)

144

One of the most discussed compounds of this class is artemisia ketone **145**.[315] A novel route for its biosynthesis



145 146 147 148

was implied by the discovery that [2-^{14}C]MVA was not detectably incorporated into the compound formed by *Santolina chamaecyparissus* under conditions where the regularly constructed and co-occurring monoterpenes were significantly labeled.[111] These observations have been confirmed, but the same precursor was found to be normally incorporated into artemisia ketone produced by *Artemisia annua* such that the position of label allowed delineation of the route of synthesis.[316] On degradation about 92% of the incorporated tracer was deduced to be at C_9 and C_{10} and only about 8% was located at C_7 and C_8 (these pairs of atoms were not distinguished by the degradation scheme); thus asymmetric labeling occurred, although not to such an extreme as in the monoterpenes previously discussed.

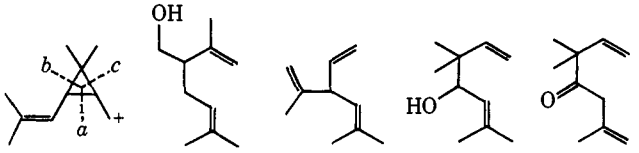
A variety of mechanisms has been proposed, all unbacked by any experimental evidence, for the biogenesis of this compound; these are (a) ring opening of a cyclopropane intermediate **146** derived from linalool,[317] (b) fission of a carane skeleton **147**,[318] (c) Stevens rearrangement of a sulfonium ylide derived from condensation of two molecules of DMAPP,[319] (d) condensation of two units of 1,1-dimethylallyl pyrophosphate,[320] (e) vague speculations about an origin from a cationic intermediate common to linalool and menthol,[321] and (f) the intermediacy of the chrysanthemyl ion **148** or its biogenetic equivalent.[322]

The observed pattern of incorporation of tracer was inconsistent with routes b, c, and d; *e.g.*, a direct condensation of two molecules of DMAPP would, unless specific compartmentation effects were evoked, lead to an equal distribution of tracer between the C_7, C_8, C_9, and C_{10} atoms. Also, when [2-^{14}C]geraniol was fed to *Artemisia annua*, considerable scrambling of tracer resulted in artemisia ketone;[316] each carbon now contained at least 6% of the tracer, although C_2 and C_4 were by far the most heavily labeled in accounting for over half of the total. This contrasts with the smooth incorporation of [1-^{14}C]GPP into cineole in a *Eucalyptus* species with negligible scrambling.[149] If routes a or e were operative, geraniol would reasonably be expected to be a more efficient precursor than MVA and would be incorporated with less randomization, whereas route f would require the additive to be degraded to C-1, C-2, or C-5 fragments that would be incorporated through formation of **148**. The tracer results seem better in accord with the last route, especially as the details of

(311) M. J. Myers, P. Issenberg, and E. L. Wick, *Phytochemistry*, **9**, 1693 (1970).
(312) J. F. Guymon, *Develop. Ind. Microbiol.*, 7, 88 (1966).
(313) F. Bohlmann and C. Zdero, *Tetrahedron Lett.*, 2418 (1969).
(314) Y. Naves, *Bull. Chem. Soc. Fr.*, 1871 (1959).
(315) J. R. Hanson, *Perfum. Essent. Oil Rec.*, **58**, 787 (1967).
(316) D. V. Banthorpe and B. V. Charlwood, *Nature, New Biol.*, **231**, 285 (1971).
(317) R. Robinson, "Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, p 14.
(318) G. R. Clemo, *Perfum. Essent. Oil Rec.*, 41, 435 (1950).
(319) B. M. Trost and R. Larochelle, *Tetrahedron Lett.*, 3327 (1968).
(320) L. Ruzicka, *Pure Appl. Chem.*, **6**, 493 (1963).
(321) P. Crabbé, *Rec. Chem. Progr., Kresge-Hooker Sci. Lib.*, 20, 189 (1959), quoted in ref 8.
(322) R. B. Bates and S. K. Paknikar, *Tetrahedron Lett.*, 1453 (1965).

mechanisms a and e seem biochemically unlikely, but nothing is known about the route to **148**.

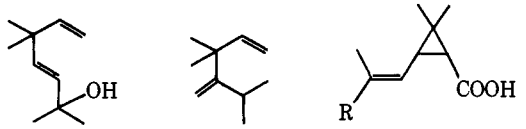
The intermediacy of **148** is also attractive in that the formation of other irregular monoterpenes can be accommodated within its framework. Fission at *a* in **149** leads to artemisia



149 **150** **151** **152** **153**

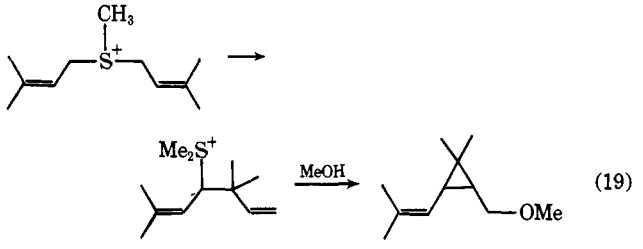
ketone, whereas fission at *b* gives the skeleton of lavandulol (**150**) that is found in lavender oil, and cleavage at *c* gives the skeleton of santolina triene (**151**) which, together with derivatives, co-occurs with artemisia ketone in certain plant oils.[323] In model systems, careful choice of reaction conditions has led to cleavage in turn at each one of the three positions of derivatives of **149**.[324,325]

Artemisia alcohol (**152**) and isoartemisia ketone (**153**) have also been isolated from natural sources,[326,327] although the latter may well have been formed by rearrangement of the normal ketone during extraction. The allylic isomer **154**



154 **155** **156**

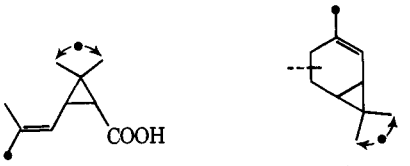
of artemisia alcohol may also be an artifact of isolation although it is reported to occur in other species;[328] originally[329] the incorrect structure **155** was proposed and claimed as an example of a novel mode of linkage of C-5 units. An interesting possibility is that artemisia alcohol is the biological precursor of the chrysanthemyl skeleton. Model experiments suggest[329a-d] that solvolysis of the sulfonium salt **156a** could lead to the cyclic structure (eq 19), but no bio-



156a (19)

chemical studies that have bearing on this view have been carried out.

The skeleton **148** is present in the chrysanthemic acid esters (**156**, R = H, CO_2Me) that occur as the insecticidal pyrethrins and cinerins in flowers of *Chrysanthemum cinerariaefolium*. [2-^{14}C]MVA was incorporated into the terpenoid moieties of these compounds formed in the flower heads in high (*ca.* 1.4%) yield,[158,159] and the tracer was equally distributed between two discernible C-5 units as in **157**. A possible rationalization of this pattern is cleavage of a carane skeleton (**158**) that is equally labeled in its moieties derived



157 **158**

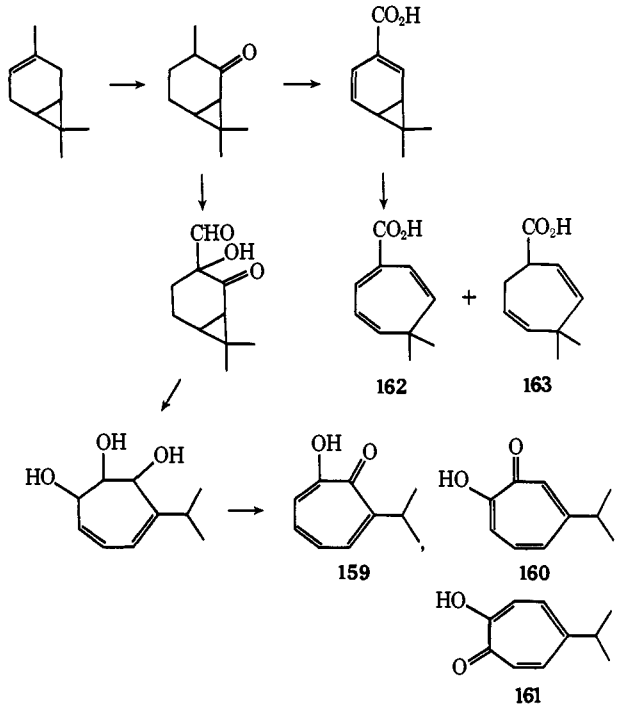
from IPP and DMAPP (such symmetry of labeling may be common in terpenes synthesized in petals (see section III.D); other suggestions are condensation of two DMAPP units[158,159] or α elimination from DMAPP to form a carbene (presumably stabilized by being enzymically bonded) that adds to the double bond of DMAPP.[176] No biochemical precedent for any of these reactions is available. At present the mode of formation of **148**, if this be an intermediate for the classes of compounds exemplified by artemisia ketone and the pyrethrins, is obscure, and the biogenetic isoprene rule may not apply to these compounds.

Certain compounds with seven-membered rings that are found in the heartwoods of gymnosperms are considered to be of isoprenoid origin,[330] but no tracer studies have been carried out. One suggestion is that these unusual structures may arise from a type of benzilic acid rearrangement whereby the six-membered ring of a menthane derivative is enlarged; the reverse ring contraction to form an isopropylbenzoic acid derivative has been demonstrated *in vitro*.[8] A more likely route to α-, β-, and γ-thujaplicins (**159-161**), thujic acid (**162**), shonanic acid (**163**), and related compounds is Scheme X,[331] wherein the biogenetic isoprene rule is obeyed as the class is elaborated from the carane framework. Nezukone (**164**) and the bicyclic ketone **165** may be intermediates.[332,333] An alternative hypothetical route is through the polyacetate pathway whereby three molecules of malonyl coenzyme A condense with DMA-CoA to form a branched C-11 polyketide that can cyclize, decarboxylate, and be functionalized. Related compounds with seven-membered carbocyclic rings which are formed by molds are known to be of polyketide origin,[176] but these do not have an isopropyl substituent on the ring. The occurrence of the latter group in the thujaplicins suggests their isoprenoid origin.

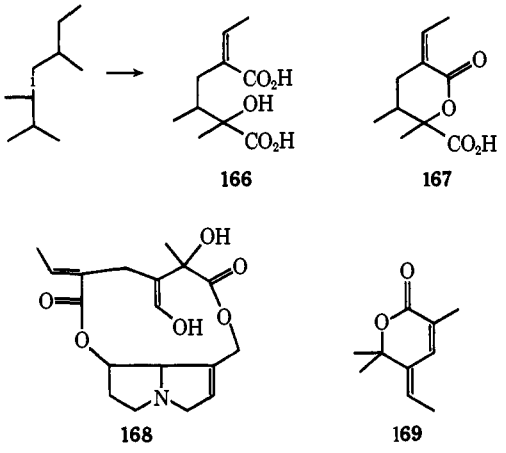
164 **165**

(323) A. F. Thomas and B. Willhalm, *Tetrahedron Lett.*, 3775 (1964).
(324) R. B. Bates and D. Feld, *ibid.*, 4875 (1967).
(325) L. Crombie, R. P. Houghton, and D. K. Woods, *ibid.*, 4553 (1967).
(326) T. Takemoto and T. Nakajima, *Yagugaku Zasshi*, 77, 1310 (1957); *Chem. Abstr.*, **52**, 4478 (1958).
(327) L. H. Zalkow, D. R. Brannon, and J. W. Uecke, *J. Org. Chem.*, **29**, 2786 (1964).
(328) B. Willhalm and A. F. Thomas, *Chem. Commun.*, 1380 (1969).
(329) S. Hayashi, K. Yano, and T. Matsuura, *Tetrahedron Lett.*, 6241 (1968).
(329a) G. M. Blackburn, W. D. Ollis, J. D. Plackett, C. Smith, and I. O. Sutherland, *Chem. Commun.*, 186 (1968).
(329b) J. E. Baldwin, R. E. Hackler, and D. P. Kelly, *ibid.*, 537 (1968).
(329c) J. E. Baldwin, R. E. Hackler, and D. P. Kelly, *ibid.*, 538 (1968).
(329d) J. E. Baldwin, R. E. Hackler, and D. P. Kelly, *J. Amer. Chem. Soc.*, **90**, 4758 (1968).
(330) A. J. Birch, *Annu. Rept. Progr. Chem. (London)*, 47, 177 (1950).
(331) H. Erdtman, *Progr. Org. Chem.*, **1**, 22 (1952).
(332) Y. Hirose, B. Tomita, and T. Nakatsuka, *Tetrahedron Lett.*, 5875 (1966).
(333) A. J. Birch and R. Keeton, *J. Chem. Soc. C*, 109 (1968).
(334) C. C. J. Culvenor and T. A. Geissman, *J. Amer. Chem. Soc.*, **83**, 1647 (1961).

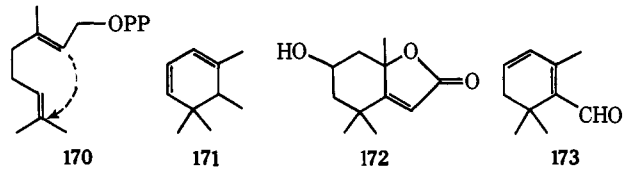
Scheme X



Many compounds that could be of part isoprenoid origin are known not to be of part mevalonoid origin.[176] Senecio alkaloids are most commonly derivatives of a dehydroxypyrrolizidine coupled with a C-10 dibasic acid to form a cyclic ester.[384] A typical senecic acid, apparently constructed from isoprene units, is **166**; its lactone is **167**; and a typical alkaloid is seneciphylline **168**. Evidence suggests that amino



acids, such as valine, leucine, isoleucine etc., which contain the isopentane skeleton, are precursors of **166**, whereas MVA is not.[335] Isoleucine was incorporated into **168** some 20 times more rapidly than acetate, and threonine was also an excellent precursor. Similar examples are available[176] and reasonable biogenetic speculations have been proposed for the formation of C-9 acids, found as units in certain alkaloids, that are considered to be degraded senecic acids.[336] These demonstrations of a nonmevalonoid pathway invalidated previous attempts to assign structures to senecic acids and senecio alkaloids on the basis of the isoprene rule. Another type of compound, **169**, that is considered,[337] although with no supporting tracer evidence, to be formed by condensation of two molecules of DMAPP, may be biosynthesized similarly from amino acids. Irregular structures in the sense of being derived from an unusual cyclization of (presumably) GPP (*cf.* **170**) are the isomeric pyronenes (*cf.* the α isomer **171**)



that occur in pine oils.[3,5] Related compounds are loliolide (**172**) from *Lolium perenne* (rye grass),[338] and safranal (**173**), the aglucone of picrocrocin.[8]

VI. Biological Aspects

A. SITES OF SYNTHESIS

Although monoterpenes must be almost ubiquitous in the plant kingdom as intermediates (perhaps enzyme-bound) for phytosterols and carotenoids, they only accumulate in chlorophyta, rhodophyta, gymnosperms, and angiosperms.[339]

Although there has been much speculation, the sites of synthesis of the components of essential oils are uncertain. It is known that the oils accumulate in specialized tissues—the oil glands—that are usually resin ducts or modified epidermal hairs,[340] and the actual sites of synthesis are usually supposed to be secretory cells associated with these glands,[11,13] although the ordinary parenchyma cells cannot be ruled out for this role. Practically nothing is known about the situation in the fungi.[341]

Several investigations have been made of this presumed synthetic apparatus of higher plants. Light and electron microscopy revealed[342-345] the oil glands of *Mentha piperita* to be modified epidermal hairs made up of either three- or ten-celled glandular trichomes containing one or eight presumed secretory cells, respectively. These aggregates were connected by a stalk comprising a single stem cell to a basal cell in the epidermis. As the exterior surface of the aggregate and the wall of the stalk cell were heavily cutinized, any additive would presumably have difficulty in entering the trichomes. The glands formed at an early stage of growth and were fully developed before the leaves were 1 mm long; they can be regarded as a bag of enzymes concerned with terpenoid metabolism with associated storage space. The inaccessible site of the oil glands is consistent with the deduction made from the observation of low incorporation of precursors (sections III.C and D) that

(335) D. H. G. Crout, M. H. Benn, H. Imaseri, and T. A. Geissman, *Phytochemistry*, **5**, 1 (1966).
(336) C. K. Atal, R. S. Sawhney, C. C. J. Culvenor, and L. W. Smith, *Tetrahedron Lett.*, 5605 (1968).
(337) F. Bohlmann and M. Grenz, *Tetrahedron Lett.*, 2413 (1969).
(338) R. Hodges and A. L. Porte, *Tetrahedron*, 20, 1463 (1964).
(339) G. Weissmann in "Comparative Phytochemistry," T. Swain, Ed., Academic Press, London, 1966, p 97.
(340) K. Paech, "Biochemie und Physiologie der Sekundären Pflanzenstoffe," Springer-Verlag, Berlin, 1950.
(341) E. Sprecher, *Planta Med.*, **11**, 119 (1963).
(342) K. J. Howe and F. C. Steward, *Cornell Univ. Agr. Exp. Sta. Mem.*, **379**, 11 (1962).
(343) F. Amelunxen, *Planta Med.*, **12**, 121 (1964).
(344) F. Amelunxen, *ibid.*, **13**, 457 (1965).
(345) G. Heinrich, *Protoplasma*, **69**, 15 (1970).

the site of monoterpene biosynthesis is isolated from the sites of primary metabolism. Oxygen may not be able readily to reach the site and monoterpenes may be produced as an adaption to an anaerobic environment akin to fermentation in yeast.[346]

Microscopic examination also revealed that the glands filled with oil at an early stage in the development of the leaf. Moreover, studies of tracer incorporation showed that *de novo* synthesis of mono- or sesquiterpenes occurred predominantly from exogenous precursors in juvenile glands, while only endogenous precursors were used when the glands were more mature; for example, no fixing of carbon dioxide into these terpenoids occurred in fully grown leaves of *Mentha piperita*.[15] However, under other conditions the oil content of this species did decline after flowering, perhaps due to the monoterpenes being catabolized (see section VI. H).[115,119]

Examination of sections of leaves of *Nepeta cataria* (catnip) at different stages of development[99] revealed glandular structures similar to those described above. These were found on both sides of the leaves, were multicellular, and rested between hairs arising from the cuticle or the epidermis. They originated from a single cell and developed a globular head on a stalk; the former contained the oil and the surrounding fragile membrane released the characteristic odor on being touched.

Sesquiterpenes, and presumably monoterpenes, of *Pogostemon cablin* (Patchouli) accumulated not only in modified epidermal hairs but also in specialized internal cells that developed with the leaf.[347] These cells contained degenerate chloroplasts in which starch grains were apparent and could be implicated in the fixation of carbon dioxide into terpenes. If the latter have to be converted into glucosides in order to be transported to or gain access to these storage sites,[347] trans-glucosidation may occur in the golgi bodies by an analogy with the sites of formation and secretion of glycoproteins.[348]

These, and other,[349-351] investigations revealed a similar pattern for the nature and occurrence of oil glands in several species. However, terpene synthesis may also take place in plastids,[345] and bundles of fibrous material that are believed to be connected with the process were found not only in the oil glands but throughout the leaf.[352]

Analysis of the contents of individual oil glands is feasible, especially by application of direct-injection techniques to gas–liquid chromatography. The mono- or sesquiterpenes in such glands of several species occurred in similar proportions to those in the oils extracted from whole plants;[347,351,353] but, in contrast, the oils extracted from different glands and from different tissues of the same *Mentha* species[354,355] were qualitatively different. The glands in these last experiments may have been in different stages of development, and the technique could be valuable for elucidating relationships between age of tissue and oil composition. Little oil is lost by evaporation from the glands; intact glands full of oil have been found in leaves kept frozen for several years,[18] and only about 10% loss of weight and no change of composition occurred when leaves of *Mentha piperita* or *Tanacetum vulgare* were dried and stored at room temperature for many months.[356,357] Specific primordial leaves in the stem apex usually contain the bulk of the oil.[347]

In contrast to the above, terpenes of *Pinus* species are apparently synthesized in glandular cells in the leaves and secreted for storage into resin ducts in the leaves or wood, where secondary transformations possibly occur.[358] The oil composition varies in different tissues of *Pinus maritima* or *Pinus sylvestris*;[359,360] woody parts and needles have varying proportions of monocyclics and bicyclics, although with increase of age the percentage of α-pinene increases in all tissues, and especially in the needles. The evolution of monoterpene biosynthesis may be related to either that of higher terpenes *via* GPP or of rubber *via* NPP, and the presence of the compounds in either discrete oil glands or in resin ducts may reflect this relationship.

Isolated flower heads can also synthesize monoterpenes, and in rose species, in particular, most of the essential oil is located there.[361] [2-^{14}C]MVA was a specific and highly efficient precursor of the monoterpenes in the petals of both hybrid tea roses and *Chrysanthemum cinerariaefolium* (see sections III.D and V). In the former species the maximum rates of accumulation of monoterpenes were found during the period when the flowers were unfurling;[104,105] in the latter species the period in which biosynthesis occurred depended on the ripeness and freshness of the ovules.[158,159] Rose petals possessed stalked cells on their upper epidermal surfaces that were presumably oil glands, and these surfaces also contained unusual intracellular inclusions that could have a connection with terpene biosynthesis. These inclusions resembled degraded chloroplasts.[362]

Citrus fruits metabolize monoterpenes,[113] and recently orange juice vesicles and flavedo have been shown to contain a set of enzymes for activating MVA and converting it into simple monoterpenoids (see section VII).

Monoterpenes do not seem to be formed or stored in roots. Grafting experiments on *Anethum graveolens*, *Carum carvi*, *Foeniculum vulgare*, *Mentha* species, and *Pelargonium graveolens*[12,363] showed that the composition of the essential oils was determined by the scion and was uninfluenced by the rhizome or root stock.

B. GENETIC CONTROL

The ability to produce particular monoterpenes, and by inference other terpenoids, is generally believed to be under fairly strict genetic control, although the quantitative composition of plant oils is often affected by environmental factors (see section VI.D).

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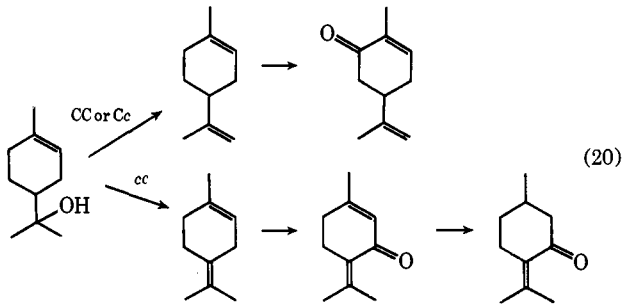
Analysis of the oil of various *Mentha* species[364,365] revealed three types, in each of which a mixture of related monoterpenes predominated. One type, exemplified by *Mentha citrata* (lemon mint), formed mainly acyclic products; a second type, including *Mentha spicata* (spearmint), mainly yielded 2-oxo-substituted menthanes; and the third type, such as *Mentha piperita* (peppermint), formed 3-oxo-menthanes. *Mentha crispa* mainly produced carvone and dihydrocarvone and fell in the second class, but after self-pollination the progeny produced oils of the second and third types (the latter containing pulegone and menthone) in the ratio of 3:1, and certain strains of *Mentha spicata* behaved similarly. This proportion is the Mendelian ratio for segregation of a single gene pair with the dominant allele producing carvone, and the gene that differentiates between the two types of oils may be the one determining the position of the double bond.[13] Presence of the genotype CC or Cc enables α-terpineol to be converted into limonene which, in turn, is oxidized by a relatively unspecific enzyme system that attacks the α position to the double bond and may not be genetically determined for this particular role. The genotype *cc*, in contrast, leads to formation of a menthadiene that is converted into pulegone and menthol (see eq 20). Re-



(20)

ductases, again probably unspecific as to their substrate, must be present that can convert pulegone into menthol but not carvone to the corresponding saturated alcohol, carvomenthol. Such enzyme systems can reduce substrates with an α,β-conjugated carbonyl group but cannot apparently react with hydrocarbons.

Similar genetic analysis[366] of *Mentha crispa* led to the conclusion that dominant and recessive alleles determined alternative routes of cyclization of a common antecedent ketone to give carvone or menthone. The lemon mints contain predominantly citral and limonene, and this simplicity of composition has been considered to imply that they are primitive members of the family,[367] but there are many problems inherent in inferring a phylogenetic sequence from such observations.[368,369] Another study of breeding experiments linked with analysis of oils[370,371] revealed that the conversion of menthone into menthol in *Mentha arvensis* was genetically controlled with the allele for menthol being dominant; this gene probably controlled the production of a menthol dehydrogenase. Again, such enzymes may not be specific to their terpenoid substrates; for example, purified liver alcohol dehydrogenase can oxidize geraniol and other terpene alcohols.

Comparable investigations have been carried out on the similarly commercially important *Pinus* species and again the monoterpene composition and hence the nature of the rearrangements characteristic of terpene metabolism in these species was shown to be genetically controlled.[372-375] In certain of these species, environmental factors such as site and climate had a small effect on the monoterpene pattern, although age differences were important; but clones (genotypically identical) plants of *Pinus monticola* grown in diverse situations showed negligible differences of levels of the different monoterpenes.[376]

Abies (fir) and *Cupressus* (cypress) species have also been studied;[377,378] and the monoterpene pattern has been used as a character in elucidating the distribution of introgressing species of *Salvia* (sage) and *Juniperus*, and hybrid indexes have been calculated.[379-381]

Morphologically identical members of a plant species may possess very different monoterpene compositions when grown in the same conditions. The classical examples are *Eucalyptus* species,[382] and more recently *Tanacetum vulgare* has been found to exist in "chemical" races or taxa that predominantly produce thujone, isothujone, camphor, umbellulone, or artemisia ketone.[383,384] *Pinus*,[385-388] *Abies*,[377] *Chrysanthemum*,[389] and other species[390-393] show similar behavior; for certain pines the monoterpene composition was altered almost as much by intraspecific selection and breeding as by interspecific hydridization.[394]

The importance of these chemical taxa in general cannot be assessed without laborious studies on a large number of individual plants rather than on oil obtained from combined batches, but the probability of the phenomenon being widespread undermines attempts[395] to use the composition of the oil as a chemotaxonomic character. The distribution of such populations at the intraspecific level is undoubtedly genetically

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controlled, but the driving force for selection of monoterpenes may be quite low, and stepwise or mosaic patterns, depending on the ease of gene exchange, may be easily developed.[393,396] Usually, oils with quantitative rather than qualitative differences in their composition resulted from this pooling of genetic information.[397,398]

C. COMPARTMENTATION EFFECTS

The inaccessibility of the sites of monoterpene synthesis in leaf tissue to obligate precursors has been noted in section III. The most detailed rationalization of the factors governing terpenoid synthesis at this level pertains to the formation of phytosterols and carotenoids in chloroplasts and evokes so-called "compartmentation effects."[399,400] Intracellular membranes were considered to be permeable only to certain precursors and the relevant enzyme systems for terpenoid synthesis were thought to be segregated within or without the chloroplast. Tracer experiments showed that MVA formed outside the chloroplast from acetyl coenzyme A that had been derived from the breakdown of sugars or fats could not penetrate the organelle. The chloroplastic terpenoids were formed by the usual, mevalonoid pathway, but the MVA used was derived from carbon dioxide that had been fixed *via* a sequence including glycine, serine, pyruvate, to acetyl coenzyme A.[258]

The position of monoterpenes in this scheme has never been considered, but similar effects may occur at the oil glands and may account for some of the apparent anomalies observed in the labeling patterns described in sections III.D and IV.

D. ENVIRONMENTAL EFFECTS

The influence of environment on the composition, usually as judged by the odor, of essential oils has long been apparent to commercial producers. Plants of nominally the same species give different oils when grown in different areas and in different harvests in the same area.[401,402] Chromatographic analyses have shown major variations in composition of numerous oils caused by alterations in climate and habitat,[403-414] and also by seasonal and diurnal effects.[415-418] Such variations are not, however, found in oils from mature specimens of many species,[141,379,419-421] although young plants may be so affected; consequently plant families or individual species must differ in their sensitivity to such factors. Two metabolically distinct pools of terpenes may exist, one of which is susceptible to outside influences (and which may be readily labeled with added precursors?) and the other which is more inert; the relative sizes of these pools could vary in different species and under different circumstances.

The only rigorously controlled experiments on environmental effects on monoterpene synthesis apply to *Mentha piperita* that was cultivated in growth chambers.[119,422] Although growth and flowering were influenced by the photoperiod, there was no true effect of this on monoterpene metabolism. The level of monoterpenes was controlled by a balance between photosynthesis in the light and utilization of the photosynthate in the dark. The first process was considered both to provide respiratory substrates for the oil glands and to maintain the respiratory coenzymes in a reduced condition which would, for example, promote reduction of pulegone to menthone. In the dark, the products of photosynthesis were used for respiration and growth, both of which processes occurred more rapidly at high temperatures. When the respiratory substrates were depleted, the respiratory coenzymes were believed to shift to the oxidized state and oxidation of the monoterpenes was promoted. As a consequence, warm nights caused the terpene pool to be oxidized and cool nights had the reverse effect. Studies on other species are urgently needed to see if these results, which indicate a dynamic terpene metabolism, are general.

In section VI.B, the influence of genetic factors on monoterpene composition was outlined. These factors must program for the existence of enzymes for specific cyclization, oxidations, reductions, etc., but the relative levels of these enzymes and hence the proportions of the final products must be influenced, in the cases discussed above, by environmental effects. The terpenoid products so formed could in turn effect the pattern of metabolism by acting as enzyme inducers or feed-back inhibitors. A good example of these modifying influences is provided by *Mentha piperita* var. Mitcham. This is the commercially grown peppermint variety and the stock is all vegatatively propagated from a mutant that arose at Mitcham; hybridization and intraspecific variations are thus absent, but the composition of the oil is well known to vary with the season and time of day, and with the age and physiological state of the plant.[415,423,424]

E. CHEMICAL TAXONOMY OF PLANTS

Only the more phylogenetically recent higher plants produce terpenoids other than those that are physiologically necessary. The mutations responsible for these apparently "luxury" products may have only been tolerated after a system for storage and development had been evolved. The distributions

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and levels of occurrence of secondary metabolites in general are beginning to be of some value in defining evolutionary and taxonomic pathways, and the approach has been applied to terpenoids.[425,426]

Such chemotaxonomy is based on the assumption that systematically related plants will show similar chemical characteristics. For such purposes, simply constructed compounds of widespread distribution are less valuable than more complex compounds formed in long reaction chains with the mediation of many enzymes specified by many different genes. However, the existence of environmental effects on monoterpene composition and of chemical taxa (section VI.B) complicates the issue. It was concluded[386] futile to attempt to devise a biochemical classification of the *Pinus* genus that would replace the existing botanical classification, but chemical characters were considered to be useful for clarifying relationships that were not disclosed by purely morphological factors. This conclusion appears sound. One guiding principle is that the terpenes present in the greatest quantity are probably those near the end of biosynthetic pathways, whereas those present in small amounts are often reactive compounds; thus if overall composition is taken as a taxonomic character attention is best focused on the main components, whereas if enzymic activity is to be considered, the minor components may be as significant.

Several studies on monoterpenes have chemotaxonomic importance. Computer correlations showed a significant linkage between monoterpene composition and classical morphology of *Salvia* (sage) and *Persea* species.[379,427] A more ambitious scheme developed a mathematical framework for utilizing the qualitative and quantitative co-occurrence of terpenoids as a tool for deducing biogenetic routes.[154] Routes to specific monoterpenes were claimed to be elucidated by this technique, but most conclusions were trivial in the light of information already gathered from tracer studies. One valuable observation, however, was that different plant species are often able to produce two or more terpenoids only in a fixed ratio; this implies genetic control of the formation of a particular precursor, such as a carbonium ion, that was in turn modified *in vivo* to give equilibrated products. A possible use of this type of statistical method is to suggest which of many feasible mechanisms could be easily studied by conventional tracer methods.

Analysis of the oils of many *Artemisia*, *Tanacetum*, and *Chrysanthemum* species revealed that the first two genera were closely related on the basis of their monoterpene patterns,[357] and a particular member was assigned *Tanacetum vulgare* rather than the classification *Chrysanthemum vulgare* favored by several botanical authorities.

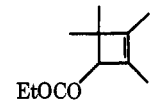
The discovery of the role of iridoids in the biosynthesis of indole alkaloids should lead to taxonomic studies of the distributions of the two classes of compounds.

F. OCCURRENCE IN ANIMALS

The great majority of animals do not accumulate monoterpenes although these compounds must be formed as intermediates for steroids. Such intermediates may be enzyme-bound at all times or the absence of an isomerase or other systems for the conversion of GPP into NPP or LPP could result in all the C-10 material passing on to higher terpenoids. Nevertheless, certain monoterpenoids, especially iridoids, have been characterized, particularly in *Insecta* and *Arthropoda*.[309,428]

Arthropod defensive secretions include citronellal, citral, limonene, and degraded terpenes (see section V), and the incorporation of [^{14}C]acetate and [2-^{14}C]MVA into the first two[112] suggests *de novo* synthesis rather than accumulation from dietary sources. Nepetalactone is also synthesized from the same precursors in stick insects.[109] Citronellal occurs in the scent glands of alligators,[429] citral is a trail pheromone for bees,[430] and iridoids and degraded monoterpenes act similarly or as sex attractants and are secreted by certain species of beetles or ants.[428]

Two exotic observations are noteworthy: males and females of the beetle *Dendroctonus frontalis* contain verbenone and verbenol, respectively;[431,432] and the sex attractant of the females of a cockroach species was believed to be a degraded monoterpene,[433] but more recent work has shown the compound possibly to have the structure **174**.[433a,b] The



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metabolic significance of these various compounds is completely obscure.

G. BIOLOGICAL SIGNIFICANCE

Few terpenoids play any known major biological role[9,434] although minor effects are legion. Specific monoterpenes are claimed to act as antimicrobial agents;[435] growth, heat, and transpiration regulators;[436] and as participants in photosynthesis.[436] Other haphazard functions are as tumor inhibitors;[436a] stimulators of carotenogenesis;[437] inhibitors of oxidative phosphorylation;[438] sex determinants for algae;[8] insect repellants;[439] insect phoromones;[428] feline and canine attractants;[440] antidiabetics;[441] and even stimulators of learning power of rats.[441]

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Many of these properties appear almost anecdotal and many must be coincidental to the terpene structure. Two additional roles that could endow significant survival value are those of repelling predators (a property based on the penetrating odor and taste of monoterpenes)[434] and of inhibiting the growth of competing grassland plants. The latter effect is well established both under controlled conditions and in the natural habitat[442-444] and could be most effective in those semiarid conditions in which oil-bearing plants thrive. Monoterpene glucosides could also play a role in the synthesis of plant cell walls similar to that of mannosyl-1-phosphoryl polyisoprenol in mannan synthesis in bacteria.[445]

Because of the wide variation in structure of monoterpenes, it is clearly unrealistic to seek out specific functions for each member; rather, any basic biological role must be associated either with the class as a whole or with a main group. *A priori* some significant biological function would be expected as otherwise the biosynthetic route would have been eliminated by natural selection.

The classical view has been that terpenoids in general, except for a few specific exceptions, are functionless anomalies, metabolic wastage that is the inert and slowly formed ballast of a prolific metabolic scheme.[3,5] However, recent evidence suggests that secondary metabolites in general, and monoterpenes in particular, are by no means inert. They are often rapidly synthesized even in young tissue, are rapidly metabolized, and may play a dynamic role in the plant's maintenance.

Two approaches are available to characterize any metabolic turnover. These are, first, the following of the time course of incorporation of labeled precursors into the various components of the oil; and, secondly, the elucidation of the fate of labeled monoterpenes that are fed to the plant. Except when [^{14}C]carbon dioxide is the precursor, both methods must perturb the system and may introduce unnatural pathways of metabolism which must be guarded against.

Many such feeding experiments in a variety of species, and sometimes using carbon dioxide at natural concentrations,[13,40,88,104-106,119,422,446] have now shown that tracer rapidly passed through the C-10 pool in a matter of hours without effecting its size. In contrast, the turnover can sometimes be slow; the half-life of [^{14}C]monoterpenes formed from administering [^{14}C]carbon dioxide to *Pinus sylvestris* in a growth chamber was about 6 months, and this was much slower than the rate of turnover of sugars, chlorophyll, and other constituents.[447,448]

Tracer administered in monoterpenes to intact plants or tissue slices was also transferred to other monoterpenes,[40,449-452] higher terpenes[453] or amino acids and sugars[40] in quantities of up to half of that applied. Early studies[401,454] on the diurnal fluctuation of the components of essential oils attributed these to evaporation and resinification, but degradation and resynthesis are a more likely explanation.[40]

Three theories have been proposed to account for the biological significance of terpenoids as a class.

The first is that the terpene pool maintains the respiratory coenzymes in a reduced form by acting as a substrate for metabolism to provide ATP when other sources have been depleted (see section VI.D).[119,422] Linked to this is a suggestion[40] that the monoterpenes provide a pool of material for the synthesis of physiologically important pigments. Monoterpenes would certainly be very good substrates for this role; monoterpenes would also be excellent sources of oxidizable substrate. The aerobic oxidation of menthone through pathways similar to those discovered for the microbial oxidation of geraniol could yield about 12 molecules of ATP per C-2 unit, in contrast to the 16 to 17 molecules of ATP per C-2 unit for oxidation of fatty acids and 10 to 11 molecules for oxidation of glucose.

The second proposal[434] is that the majority of terpenoids have no function but are side products of an evolving network from which the essential terpenoids—plant hormones, phytosterols, and carotenoids—are being selected. On this basis, all but the few essential terpenoids will disappear in the course of further evolution.

The third theory[254] directs attention to the activity of formation of secondary metabolites rather than the nature of the products. Plants and microorganisms are considered to produce terpenes during periods of dormancy of either the whole organism or of localized tissues, in order to maintain the appropriate enzyme systems in an active state. Particular enzyme networks define specific key intermediates, *e.g.*, GPP or NPP, that in turn are subject to relatively unspecific interconversions in a metabolic grid. The diversity of products is beneficial in preventing the accumulation of possible toxic or inhibitory products. This theory accounts for the occurrence of secondary metabolites in plants and microorganisms where dormancy is followed under favorable conditions by a regeneration of specific tissue or of growth.

All three theories probably account for aspects of the existing situation.

H. CATABOLISM

Very little is known of this subject and a wide field for study exists on mechanisms of breakdown of monoterpenes, especially in plants. The most detailed work has concerned microorganisms; the conversion of citronellal into citronellol, and of citral into geraniol by yeast, was early discovered,[455,456] as was the reduction of geraniol to citronellol.[457] More recently α-pinene was claimed to be converted by *Aspergillus niger* into verbenol, verbenone, and certain other monoterpenes,[458] but adequate controls to exclude nonenzymic autoxidation and rearrangement during incubation and work-up were not carried out.

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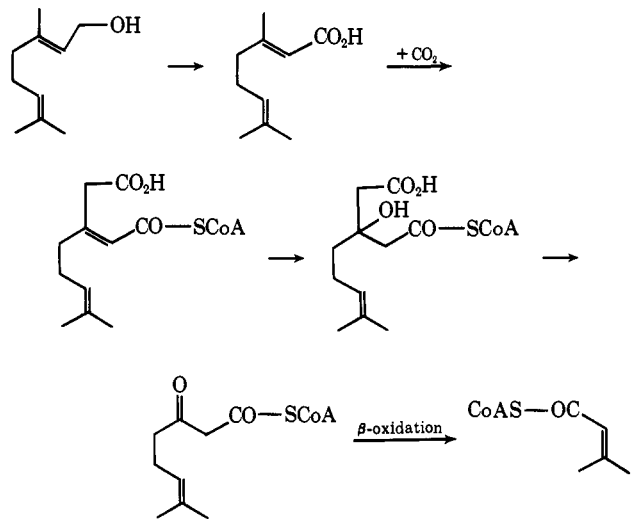
Such reactions preserve the C-10 skeleton, but degradations of α-pinene, limonene, carene, and other monoterpene hydrocarbons by *Aspergillus niger*[459,460] and soil pseudomonads[461] have been reported when the microorganisms were grown on the terpenes as sole carbon sources or in the presence of small amounts of glucose. The oxidative pathways have been outlined, and certain of the relevant enzyme systems have been obtained in cell-free sonicates. Very detailed and impressive studies of different paths of breakdown of camphor by similar pseudomonads have been reported[462-466] and microbiological transformations of, *inter alia*, fenchane[467,468] and bornane derivatives,[469,470] have been described. Of special interest is the route utilized for the degradation of geraniol to C-5 units and ultimately to carbon dioxide by species of bacteria isolated from the soil of pine woods (Scheme XI).[471]

Scheme XI



CoASH = coenzyme A

Mono and also higher terpenes may be thus degraded to give acetate, acetyl coenzyme A, and 3,3-dimethylallyl coenzyme A, all of which can be further broken down by well-known routes. Although such pathways may not operate in higher plants, this type of information provides clues as to the possible situation in these. Several bacterial species also degrade monoterpenes to carbon dioxide by pathways analogous to those used in the catabolism of leucine.[472]

Large quantities of monoterpenes are ingested by animals, but little is known of the fate of these compounds. Most of the information that is available dates from before the advent of the general use of radiochemical techniques and is almost wholly derived from analyses of the urine of man and of force-fed domestic animals.[473] The general salvage mechanism, discovered in outline by Emil Fischer at the start of the century, involves oxidation and sometimes subsequent formation of a β-D-glucuronide before excretion; *e.g.*, in man dietary limonene may be converted into "uroterpenol" (*p*-menth-1-ene-8,9-diol) which is excreted as the β-glucuronide,[474] and camphane diols and camphene are eliminated by rabbits as similar derivatives.[475] The bicyclic monoterpene skeletons are appreciably stable *in vivo* and oxidation occurs without cleavage of the ring structure.[11]

VII. In Vitro Systems

A. GENERAL

Most of the details of the biosynthesis of cholesterol and squalene were elucidated by studies on homogenates of liver tissue and yeast, although a few purified extracts of enzymes were prepared. Soluble extracts generally could support the route to GPP or farnesyl pyrophosphate, but after this stage the substrates become insufficiently soluble in water, and the appropriate enzyme systems were particulate or membrane-bound and were only solubilized with difficulty. A membrane-bound system with high spatial organization is highly efficient as the substrate can be circulated with none of the delays and risks attendant on dissociation from the catalytic surface, and the enzymes that are involved in the formation of the monoterpenes are probably thus bound *in vivo*, although they can exist in a functional state (albeit of probably very low efficiency) in solution.

A few studies have been made on cell-free systems from nonplant sources containing enzymes involved in monoterpene metabolism,[476] and an interesting study of the inhibitory effects of terpenyl pyrophosphates and their analogs on MVA-kinase from pig liver has appeared.[477] Most of the *in vitro* studies in higher plants have been directed at the biosynthesis of higher terpenes, and the route to the monoterpene intermediates has been neglected.[478-480] Nevertheless, studies on cell-free systems that can maintain monoterpene synthesis are now beginning to appear, and such systems, when optimally defined, have very real advantages compared with the methods using the whole plant or intact tissues. These are, first, an increased incorporation of the precursor; secondly, less formation of side products and degradation of the precursor; and, thirdly, absence of complications caused by compartmentation effects and the inaccessibility of the

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biosynthetic site. The first and second advantages are particularly favorable if a purified enzyme system with a limited range of synthetic capacities is used rather than the crude homogenates whose use is prevalent in the literature at present.

Isolation of enzymes or the preparation of cell-free systems from plants is much more difficult than the corresponding operations with animal tissue, and the resulting preparations of proteins are usually more unstable. The technical difficulties have been reviewed,[481-481b] and may be summarized as follows. (a) The cell walls of higher plants are more resistant to disruption than are those of animal cells. (b) Vacuoles of plants contain acidic material that can rapidly denature proteins when the cell is disrupted; this necessitates a precise choice of buffers and pH. (c) Maceration of plants liberates phenol oxidases that catalyze aerobic oxidation to form quinones that, in turn, denature proteins; this oxidation can be detected by a rapid darkening of the homogenate. These effects can be minimized by addition of reducing agents such as ascorbic acid or sodium thiosulfate, or by carrying out the extraction under nitrogen. (d) Plant phenolics are also liberated which deactivate proteins and may be oxidized as described above. These can be removed by complexing with insoluble additives such as polyvinylpyrrolidine,[482,483] or with a soluble form of the same polymer when particulate fractions such as mitochondria are to be isolated. (e) Maceration destroys compartmentation of enzymes and releases proteases, whose effects may be minimized by working at low temperatures or in certain cases[484] by adding oxidizing agents such as sodium bromate. (f) Maceration also releases pyrophosphatases and phosphatases from the specific cells in which they are stored[484a] that hydrolyze the intermediates of terpene synthesis to form alcohols which are normally not rephosphorylated in the cell-free extract. These enzymes may be removed by sucrose-gradient fractionation or inhibited by the addition of fluoride, citrate, molybdate, or other ions.[485-487] (g) Nonspecific interactions between proteins that occur in aqueous plant extracts are claimed to cause inactivation of certain plant enzymes during storage at low temperature; this effect is stated to be reduced by the addition of sugar or polyglycols.[488] (h) Adsorption of soluble protein on to cell walls or membranes during homogenization. This can be minimized by repeated homogenization and washing of the cell-wall fraction, or by a careful choice of buffer and incorporation of nonaqueous solvents into the extraction medium. The last technique has been applied with particular success to the isolation of chloroplasts[489] that maintain most of their photosynthesizing ability.

Although many of these difficulties can be overcome, the basic problem is almost certainly the particulate or membrane-bound nature of the enzymes *in vivo*, even for monoterpene metabolism. This organization may rarely survive extraction, let alone solubilization, and as a result very low (less than 1%) incorporations of precursors into monoterpenes are typically found. Recent work (see section VII.B) indicates that enzyme systems may be more easily extracted from fruits than from leaves. Little is known of the prospects of using petals. Soluble, particulate, microsomal, and broken or intact plastid fractions, used in various combinations and states of homogeneity, are employed in the studies summarized in the next section, but little work has been carried out on the location of the various enzymic activities *in vivo*.

B. CELL-FREE SYSTEMS

The best-studied enzyme of the sequence under consideration is MVA-kinase which converts MVA into MVAP. Homogenates containing this, and often MVA-phosphokinase, have been prepared from yeast[490] and liver,[11] algae,[491] and many higher plants,[126,492-500] and the cofactor requirements have been delimited, although no very pure preparations were achieved. These enzymic activities in callus tissue from cultures of *Kalanchoe crenata* greatly increased on greening.[496] MVA-kinase is essentially irreversible in its action, whereas MVA-phosphokinase is known to catalyze the back-reaction effectively in the presence of excess of ADP. Both enzymes require divalent metal ions, especially magnesium, for activation and have a pH optimum near 7.0. Geraniol kinase has been detected in homogenates from germinating *Pisum sativum* (peas)[501] or leaves of *Mentha piperita*;[502] and extracts which convert nerol, geraniol, and 3,3-dimethylallyl alcohol into their monophosphates have been prepared from *Mentha piperita*, *Pisum sativum*, *Humulus lupulus* (hops), and other species.[503] All these preparations required ATP and manganese ion, and they may contain the same or a closely related group of kinases. Most of these homogenates also contained phosphatases which had to be inhibited in order to get significant (*ca.* 1%) amounts of phosphorylation; such phosphatases appear to be ubiquitous in plant tissue,[11,50,504] and a balance between kinase and phosphatase activity may be important in controlling the rate and extent of terpenoid synthesis.

The first successful characterizations of enzymes of terpene biosynthesis in high plants were of conversions of MVA into MVAP, MVAPP, IPP, DMAPP, and GPP in extracts from

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Pisum seedlings,[497] but the total incorporation of tracer into products was only about 0.1%, and, as in most of the studies to be discussed, there was little real evidence that the presumed monoterpene was actually synthesized (see section II.B). Subsequently, isopentyl monophosphate and IPP were shown to be produced by the latex of *Hevea brasiliensis* (rubber);[505] and IPP–isomerase (which converts IPP into DMAPP) was shown to exist in pumpkins as two isoenzymes.[506] Enzymes yielding MVAP and MVAPP from MVA were also partially purified from orange juice vesicles.[498]

Later studies have demonstrated that orange juice vesicles contain the full complement of enzymes for stepwise transformation of MVA through IPP and DMAPP to LPP.[507] GPP and NPP were not detected in the incubation mixture, and LPP was proposed to be the parent of cyclic monoterpenes in this tissue; this suggestion had already been made on the basis of studies of the composition of the oil at different stages of maturity.[87] In most of this work the incorporations were less than 1%, often much lower, and the products were neither characterized nor purified to the criteria listed in section II.B. Enzymes that synthesized or reduced HMG-CoA or formed MVA from acetate could not be detected in these orange juice preparations, and it is possible that a novel but undefined route to MVA was here utilized.[508]

The characterization of cell-free systems that synthesize specific monoterpenes, rather than the vague terpenoid, or "prenyl" fraction oft-quoted in earlier studies, has now got under way. Such a system from needles of *Pinus radiata* converted [2-^{14}C]MVA into limonene although only in 0.05% yield, and no α- or β-pinene (the major components of the plant oil) was formed. ^{126}MVAP and MVAPP were also labeled from [2-^{14}C]MVA using extracts from the *Pinus* seedlings. A more recent preparation from the same source[509] led to tracer from [2-^{14}C]MVA being transferred to IPP, DMAPP, GPP, NPP, and an undefined "prenyl phosphate" fraction in total of 6–7%. The relative radioactivities in IPP and terpenylpyrophosphates could be altered by changes in the amounts of magnesium and manganese ions in the incubation medium, and this effect was similar to one reported in the synthesis of higher terpenoids in cell-free extracts from *Pisum* seedlings.[479,480] A proportion, usually about 1%, of the applied radioactivity was also protein-bound. This activity could be released by treatment with 1-butanol and comprised sesqui- and diterpenoids; again there is a precedent for such an effect now in a bacterial prenyl synthetase system.[510] Specific activities were not measured in these experiments using *Pinus* extracts and so no conclusions could be drawn as to the order of formation of GPP and NPP. The incorporation of tracer was increased by about one-third if soluble phospholipids were added to the incubation mixture;[511] this effect may be due to solubilization of precursors that had been dephosphorylated by endogenous phosphatases or to micelle formation creating a favorable environment for the enzymic reactions. The most recent study on extracts from *Pinus radiata* seedlings reported conversion of [^{14}C]NPP into α- and β-pinene in about 0.6% yield; this was the first example of a bicyclization carried out by a cell-free system.[512]

A few other cell-free systems have been developed that synthesize monoterpenes from C-5 or C-6 precursors. An orange preparation converted [2-^{14}C]MVA into linalool and certain menthane derivatives in about 1% total yield;[513] an extract of *Tanacetum vulgare* converted [2-^{14}C]MVA and [4-^{14}C]IPP into geraniol and nerol to a similar extent;[514] and a crude homogenate of *Nepeta cataria* was effective in incorporating [2-^{14}C]MVA into nepetalacetone and other iridoids.[99]

It is easier to prepare extracts that interconvert monoterpenes in good yields than to establish systems capable of efficient *de novo* synthesis from simpler precursors, perhaps because the enzymes carrying out the former class of reactions are not membrane-bound. One of the most interesting of the former type is a preparation from *Mentha piperita* leaves that converted [C^{14}]pulegone into tentatively identified menthone, isomenthone, and menthols in about 30% yield.[515] NADPH was a cofactor and successful operation required removal of endogenous substrate as well as an anaerobic atmosphere—precautions that have not been necessary in other studies. After storage for 1 day at −20°, over half the enzymic activity was lost, and one-third was lost in a similar period at −78°.

In other investigations, α-terpineol was converted into terpinen-4-ol (6%) and thujone (2%), and terpinen-4-ol was converted into α-terpineol (10%) in different fractions from homogenates of *Tanacetum vulgare*;[514] and a system from *Vinca rosea* esterified loganic acid to yield loganin.[253]

Considerable work has been carried out on the interconversion of monoterpenes in orange juice vesicles. [3-^{14}C]-Linalool was converted into $\Delta^{2,8}$-*p*-menthadien-1-ol in 25% yield,[513] and this reinforces the view that LPP is the immediate precursor of cyclic terpenes in this tissue. A geraniol dehydrogenase has been found in the same source and was partially purified and separated from conventional alcohol dehydrogenase;[129] this enzyme also oxidized nerol, citronellol, and farnesol but at slower rates than geraniol. Such oxidation may generally be carried out by relatively unspecific enzymes, for purified liver alcohol dehydrogenase can convert geraniol into citral[44] and carrot slices catalyze the reverse reaction.[450] Dehydrogenation of secondary terpene alcohols appears common *in vivo* since ketones are generally[516] present in higher concentration than their corresponding alcohols.

A limonene reductase has also been characterized from orange juice vesicles.[517] (+)-Limonene was converted in unstated yield into menth-1-ene in the presence of NADPH or NADH, whereas the (−) isomer was unaffected. The preparation proved specific for the reduction of the exocyclic double bond, but the significance of this enzyme is unclear as menthenes do not occur in citrus fruits.

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This last observation and several of the preceding point to the possibility that many terpene skeletons may be functionalized by the action of enzymes of low specificities that are mainly involved in a more primary metabolic role. Highly specific enzymes whose occurrence, under rigid genetic control, is restricted to particular tissues, may collaborate to construct the terpenoid skeleton and to introduce key substituents, but modifications to this basic pattern may occur on a complex metabolic grid maintained by relatively nonspecific enzymes or even by nonenzymic processes.

No plant enzyme associated with terpene metabolism has yet been isolated in a highly purified form and nothing is known about the mechanism of action of any of the enzymes that have been characterized, although reasonable speculations have often been made. These fields will surely be explored as techniques for developing highly active cell-free systems are discovered. Another fruitful field should be the study of the interrelationship, both at the cell-free and enzymic levels between the biosynthetic pathways and the overall physiological condition.

C. TISSUE CULTURES

Another type of approach, as yet almost unexplored, is the study of tissue cultures whereby root, leaf, or stem tissue are excised and grown in sterile media under chemically defined conditions that can permit growth, development, differentiation, or reversion to a primitive state. The techniques and limitations of the method have been reviewed.[518-522] Tissue culture could overcome many of the difficulties associated with studies on whole plants while avoiding the loss of organization inherent in the preparation of cell-free extracts, but a major drawback is the slow rate of growth of the tissue and the need for frequent subculture to maintain the cell lines.

Numerous studies have been made on the maintenance of plant cultures. In particular, *Mentha*, *Rosa*, and *Juniperus* species have been so cultivated[523-525] and methods of "continuous" and "shaken" cultures have been developed.[526] Nevertheless, progress has been very slow as specific culture media and conditions have usually to be discovered for each type of plant material and the finding of the optimum composition of the media, in particular, is largely a matter of trial and error.

The only work on monoterpene biosynthesis in culture appears to be a preliminary study on leaf and stem explants of *Tanacetum vulgare*.[527] [2-^{14}C]MVA was converted into DMAPP, PP, and certain unidentified terpene hydrocarbons, and the monoterpene pattern in the callus tissue was quite different from that in the intact leaf.

Tissue culture would seem to be ideal for studying the factors causing differentiation of plants and the interrelationships between general differentiation, development of chloroplasts and oil glands, and terpene biosynthesis. At the moment this is a virgin field.

Acknowledgment. We thank Dr. Katharine A. Charlwood for much help with the preparation of this review.

VIII. Appendix. Incorporation of Tracer into Monoterpenes

This section records all the main available data on the subject (Table I, pp 150–155). Experiments are classified into four groups, *viz.*

A. Significant incorporation of precursor into monoterpene was claimed, but the reference does not contain sufficient detail to quote the percentage incorporation.

B. The pattern of tracer in monoterpene was not studied, hence incorporation of precursor *via* prior degradation cannot be entirely ruled out.

C. The pattern of tracer in monoterpene was established to demonstrate with reasonable certainty that incorporation was not *via* prior degradation of precursor.

D. Incorporation of precursor was claimed to occur *via* prior degradation to smaller fragments.

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Table I

Monoterpene	*Precursor*	*Plant*	*% incorporation (where given)*
	A. Acyclic Compounds and Cyclohexane Derivatives		
Artemisia ketone	[1-^{14}C]Acetate	*Artemisia annua*	..(A, B)[29]
	[2-^{14}C]MVA	*Santolina chamaecyparissus*	0;[111] 0[316]
		A. annua	0.001–0.01 (C)[316]
	[2-^{14}C]Geraniol	*A. annua*	0.004 (D)[316]
Bornyl acetate	[2-^{14}C]MVA	*Monarda punctata*	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
Camphene	[2-^{14}C]MVA	*M. punctata*	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
	[^{3}H]GPP	*Tanacetum vulgare*	0.008 (B)[40]
Camphor	[^{14}C]Terpinen-4-ol	*T. vulgare*	0.5 (B)[40]
	[^{14}C]α-Terpineol	*T. vulgare*	..(A, B)[40]
(+)-Camphor	[2-^{14}C]MVA	*Artemisia californica*	0.002–0.032 (C)[138,139]
		Salvia leucophylla	0.004–0.02 (C)[138,139]
(−)-Camphor	[2-^{14}C]MVA	*Chrysanthemum balsamita*	0.04–0.2 (C)[138,139]
	[2-^{3}H]MVA	*C. balsamita*	..(A, B)[138]
Car-3-ene	$^{14}CO_2$	*Pinus syvestris*	..(A)[447]
	[^{14}C]α-Pinene	*Pinus ponderosa*	~3 (B)[449]
Carvone	[2-^{14}C]MVA	*Anethum graveolens*	0.002 (C)[145]
		Carum carvi	..(A, C)[146]
	[^{14}C]Limonene	*A. graveolens*	..(A, B)[a]
1,8-Cineole	$^{14}CO_2$	*Mentha piperita*	..(A)[124]
	[2-^{14}C]MVA	*M. punctata*	..(A, B)[125]
		Eucalyptus globulus	0.01 (D);[148] ..(A, C)[147]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
	[1-^{14}C]GPP	*Rosmarinus officinalis*	0.22 (C)[149]
Citral	[1-^{14}C]Acetate	*Acanthomyops claviger*[b]	..(A, B)[112]
	[2-^{14}C]Acetate	*A. claviger*[b]	..(A, B)[112]
	[1-^{14}C]MVA	*A. claviger*[b]	0[112]
	[2-^{14}C]MVA	*Eucalyptus steigeriana*	1.16–1.66 (C)[150]
		A. claviger[b]	..(A, B)[112]
	[1-^{14}C]IPP	*E. steigeriana*	1.98–2.58 (C)[150]
	[3-^{14}C]Linalool	*Citrus jambhiri*	..(A, B)[113]
		Citrus reticulata	..(A, B)[113]
Citronellal	[1-^{14}C]Acetate	*Eucalyptus citriodora*	..(A, D)[147]
		A. claviger[b]	0.07–0.10 (B)[112]
	[2-^{14}C]Acetate	*A. claviger*[b]	0.07–0.10 (B)[112]
	[1-^{14}C]MVA	*A. claviger*[b]	0[112]
	[2-^{14}C]MVA	*A. claviger*[b]	0.07–0.10 (B)[112]
p-Cymene	[2-^{14}C]MVA	*M. punctata*	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
Geranial	[2-^{14}C]MVA	*M. punctata*	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
Geraniol	[^{14}C]Sodium bicarbonate	*Lady Seaton Tea Rose*	<0.01 (B)[106]
	[1-^{14}C]Acetate	*Pelargonium graveolens*	0.02 (B)[40]
	[2-^{14}C]Acetate	*Lady Seaton Tea Rose*	1 (B)[106]
		Rosa damascena Mill. x Rosa gallica	0.29–1.2 (B)[107]
	[2-^{14}C]MVA	*Lady Seaton Tea Rose*	<1 (B)[104,106]
	(4*S*)-[2-^{14}C,4-^{3}H]MVA	*Lady Seaton Tea Rose*	2.5–5.0 (C, E)[127,c]
	(4*R*)-[2-^{14}C,4-^{3}H]MVA	*Lady Seaton Tea Rose*	2.5–5.0 (C, E)[127]
	[2-^{14}C]Cinnamate	*R. damascena x R. gallica*	0[107]
	[3-^{14}C]Linalool	*C. jambhiri*	0[113]
		C. reticulata	..(A, B)[113]
	[U-^{14}C]Phenylalanine	*R. damascena x R. gallica*	0.004–0.01 (B)[107]
	[U-^{14}C]Glucose	*R. damascena x R. gallica*	0[107]
	[U-^{14}C]Sucrose	*R. damascena x R. gallica*	0[107]
Geranyl β-D-glucoside	[^{14}C]Sodium bicarbonate	*Lady Seaton Tea Rose*	<0.01 (B)[106]
	[2-^{14}C]Acetate	*Lady Seaton Tea Rose*	1 (B)[106]
	[2-^{14}C]MVA	*Lady Seaton Tea Rose*	10–11 (B)[104,106]
	(4*S*)-[2-^{14}C,4-^{3}H]MVA	*Lady Seaton Tea Rose*	2.5–5.0 (C, E)[127]
	(4*R*)-[2-^{14}C,4-^{3}H]MVA	*Lady Seaton Tea Rose*	2.5–5.0 (C, E)[127]
n-Heptane	[1-^{14}C]Acetate	*Pinus jeffreyi*	..(A, C)[310]
	[2-^{14}C]Acetate	*P. jeffreyi*	..(A)[310]
	[2-^{14}C]MVA	*P. jeffreyi*	0[310]
Limonene	$^{14}CO_2$	*M. piperita*	..(A)[124]

Table I (Continued)

Monoterpene	*Precursor*	*Plant*	*% incorporation (where given)*
	[2-^{14}C]MVA	*A. graveolens*	0.011 (B)[145]
		C. carvi	..(A, C)[146]
		Pinus pinea	..(A, C)[a,134,143]
	[3-^{14}C]Linalool	*C. jambhiri*	..(A, B)[113]
		C. reticulata	..(A, B)[113]
Linalool	[2-^{14}C]MVA	*M. punctata*	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
trans-2,1-*p*-Menthadien-1-ol	[3-^{14}C]Linalool	*C. reticulata*	..(A, B)[113]
Menthofuran	$^{14}CO_2$	*M. piperita*	..(A);[115] 0.0072[118]
	[2-^{14}C]Acetate	*M. piperita*	..(A, D)[116]
	[2-^{14}C]MVA	*M. piperita*	..(A, D);[116] 0[115]
	[^{14}C]3,3-Dimethylacrylic acid	*M. piperita*	0[115]
	[^{14}C]Pulegone	*M. piperita*	..(A, B)[115]
Menthol	$^{14}CO_2$	*M. piperita*	..(A);[115] ..(A);[120] 0.0046[118]
	[1-^{14}C]Acetate	*Mentha arvensis*	..(A, C)[151]
	[2-^{14}C]Acetate	*M. piperita*	..(A, D)[116]
	[2-^{14}C]MVA	*M. piperita*	0;[115] ..(A, D)[116]
	[^{14}C]3,3-Dimethylacrylic acid	*M. piperita*	0[115]
	[^{14}C]Limonene	*M. piperita*	..(A, B)[120]
	[^{14}C]Menthone	*M. piperita*	..(A, B)[120]
	[^{14}C]α-Pinene	*M. piperita*	..(A, B)[120]
Menthone	$^{14}CO_2$	*M. piperita*	..(A);[115] ..(A);[120] 0.11[118]
	[2-^{14}C]Acetate	*M. piperita*	..(A, D)[116]
	[2-^{14}C]MVA	*M. piperita*	..(A, D);[116] 0[115]
	[^{14}C]3,3-Dimethylacrylic acid	*M. piperita*	0[115]
	[^{14}C]Limonene	*M. piperita*	..(A, B)[120]
	[^{14}C]α-Pinene	*M. piperita*	..(A, B)[120]
	[^{14}C]Pulegone	*M. piperita*	..(A, B)[115]
Isomenthone	$^{14}CO_2$	*M. piperita*	..(A, B);[124] 0.0029[118]
	[2-^{14}C]MVA	*M. punctata*	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
Menthyl acetate	[2-^{14}C]Acetate	*M. piperita*	..(A, D)[116]
	[2-^{14}C]MVA	*M. piperita*	..(A, D)[116]
Methylheptenone	[3-^{14}C]Linalool	*C. jambhiri*	..(A, B)[113]
Myrcene	[2-^{14}C]Acetate	*Humulus lupulus*	0.016 (B)[13]
	[2-^{14}C]MVA	*H. Lupulus*	0[13]
		Santolina chamaecyparissus	..(A, B)[111]
	[2-^{14}C]Malonate	*H. lupulus*	0[13]
	[2-^{14}C]Propionate	*H. lupulus*	0[13]
	[^{14}C]Geraniol	*H. lupulus*	0[13]
	[3-^{14}C]Linalool	*C. jambhiri*	..(A, B)[113]
		C. reticulata	..(A, B)[113]
	[2-^{14}C]Alanine	*H. lupulus*	..(A, B)[13]
	[G-^{14}C]Leucine	*H. lupulus*	0[13]
	[2-^{14}C]Leucine	*H. lupulus*	0[13]
	[G-^{14}C]Isoleucine	*H. lupulus*	0[13]
	[4-^{14}C]Valine	*H. lupulus*	0[13]
	[G-^{14}C]Glucose	*H. lupulus*	0.08 (B)[13]
Neral	[2-^{14}C]MVA	*M. punctata*	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
Nerol	(4*S*)-[2-^{14}C,4-^{3}H]MVA	*Lady Seaton Tea Rose*	2.5–5.0 (C, E)[127]
	(4*R*)-[2-^{14}C,4-^{3}H]MVA	*Lady Seaton Tea Rose*	2.5–5.0 (C, E)[127]
Neryl-β-D-glucoside	(4*S*)-[2-^{14}C,4-^{3}H]MVA	*Lady Seaton Tea Rose*	2.5–5.0 (C, E)[127]
	(4*R*)-[2-^{14}C,4-^{3}H]MVA	*Lady Seaton Tea Rose*	2.5–5.07 (C, E)[127]
cis-β-Ocimene	[3-^{14}C]Linalool	*C. jambhiri*	0[113]
Octanal	[3-^{14}C]Linalool	*C. reticulata*	..(A, B)[113]
β-Phellandrene	[2-^{14}C]MVA	*Pinus contorta*	..(A, C)[144]
α-Pinene	$^{14}CO_2$	*P. syvestris*	..(A)[447]
		Pinus radiata	Needles, 0.015[126]
			Stem, 0.005[126]
			Roots, 0.003[126]
		Mentha piperita	..(A)[124]
	[1-^{14}C]Acetate	*Pinus muricata*	~0.003 (B)[449]
		Pinus attenuata	0.004 (B)[40]

Table I (Continued)

Monoterpene	*Precursor*	*Plant*	*% incorporation (where given)*
	[2-^{14}C]Acetate	*P. attenuata*	0.01 (B)[110]
		T. vulgare	0.006 (B)[40]
	[2-^{14}C]MVA	*P. attenuata*	0.02 (B);[110] slices of shoot tips, 0.5 (B)[110]
		Pinus nigra austriaca	0.01 (C)[133,134]
		M. punctata	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
	[^{3}H]GPP	*T. vulgare*	0.007 (B)[40]
	[3-^{14}C]Linalool	*C. jambhiri*	0[113]
		C. reticulata	0[113]
	[^{14}C]Terpinen-4-ol	*T. vulgare*	0[40]
β-Pinene	$^{14}CO_2$	*P. radiata*	Needles, 0.035[126] Stem, 0.003[126] Roots, 0.0056[126]
		M. piperita	..(A)[124]
	[2-^{14}C]MVA	*S. chamaecyparissus*	..(A, B)[111]
	[3-^{14}C]Linalool	*C. jambhiri*	0[113]
		C. reticulata	..(A, B)[113]
	[^{14}C]α-Pinene	*P. ponderosa*	~2.5 (B)[449]
Piperitenone	$^{14}CO_2$	*M. piperita*	..(A)[115]
		Mentha pulegium	..(A)[115]
	[2-^{14}C]Acetate	*M. piperita*	..(A, B);[115] ..(A, D)[116]
	[2-^{14}C]MVA	*M. piperita*	0;[115] ..(A, D)[116]
	[^{14}C]3,3-Dimethylacrylic acid	*M. piperita*	0[115]
Piperitone	$^{14}CO_2$	*M. piperita*	..(A);[115] ..(A);[124] 0.0009[118]
	[2-^{14}C]Acetate	*M. piperita*	..(A, D)[116]
	[2-^{14}C]MVA	*M. piperita*	0;[115] ..(A, D)[116]
	[^{14}C]3,3-Dimethylacrylic acid	*M. piperita*	0[115]
	[^{14}C]Piperitenone	*M. piperita*	..(A, B)[115]
Pulegone	$^{14}CO_2$	*M. piperita*	..(A);[115] ..(A);[120] ..(A);[124] 0.0023[118]
		M. pulegium	..(A)[115]
	[2-^{14}C]Acetate	*M. piperita*	..(A, D)[116]
	[2-^{14}C]MVA	*M. piperita*	0;[115] ..(A, D)[116]
		M. pulegium	0.002 (C)[29]
	[^{14}C]3,3-Dimethylacrylic acid	*M. piperita*	0[115]
		M. pulegium	7.87 (C)[155,156]
	[^{14}C]Limonene	*M. piperita*	..(A, B)[120]
	[^{14}C]Menthone	*M. piperita*	..(A, B)[120]
	[^{14}C]Pinene	*M. piperita*	..(A, B)[120]
Pyrethric acid	[2-^{14}C]Acetate	*Chrysanthemum cinerariaefolium*	~0.1 (B)[159]
	[1-^{14}C]MVA	*C. cinerariaefolium*	0[159]
	[2-^{14}C]MVA	*C. cinerariaefolium*	~1.4 (C)[159]
Sabinene	$^{14}CO_2$	*M. piperita*	..(A)[124]
	[1-^{14}C]Acetate	*Juniperus sabina*	0.008 (B)[40]
	[2-^{14}C]Acetate	*T. vulgare*	0.05 (B)[40]
	[2-^{14}C]MVA	*J. sabina*	0.004–0.05 (B)[103,132]
		M. punctata	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
	[^{3}H]GPP	*T. vulgare*	0.03 (B)[40]
	[^{14}C]Linalool	*T. vulgare*	..(A, B)[40]
	[3-^{14}C]Linalool	*C. jambhiri*	0[113]
	[^{14}C]Sabinyl acetate	*T. vulgare*	..(A, B)[40]
	[^{14}C]Terpinen-4-ol	*T. vulgare*	..(A, B)[40]
	[^{14}C]α-Terpineol	*T. vulgare*	..(A, B)[40]
	[^{14}C]α-Thujene	*T. vulgare*	..(A, B)[40]
	[^{14}C]Thujone	*T. vulgare*	..(A, B)[40]
Sabinol	$^{14}CO_2$	*M. piperita*	..(A)[124]
	[2-^{14}C]MVA	*J. sabina*	0.004–0.05 (C)[103,132]
Sabinyl acetate	[1-^{14}C]Acetate	*J. sabina*	0.018 (B);[40] 0.004–0.05 (C)[103,132]
	[^{14}C]α-Terpineol	*T. vulgare*	..(A, B)[40]
α-Terpinene	[3-^{14}C]Linalool	*C. jambhiri*	0[113]
γ-Terpinene	[3-^{14}C]Linalool	*C. jambhiri*	0[113]
		C. reticulata	..(A, B)[113]
Terpinen-4-ol	[2-^{14}C]Acetate	*T. vulgare*	0.06 (B)[40]
	[^{3}H]GPP	*T. vulgare*	0.001 (B)[40]

Table I (Continued)

Monoterpene	*Precursor*	*Plant*	*% incorporation (where given)*
	[^{14}C]Linalool	*T. vulgare*	..(A, B)[40]
	[3-^{14}C]Linalool	*C. jambhiri*	..(A, B)[113]
		C. reticulata	..(A, B)[113]
	[^{14}C]Sabinene	*T. vulgare*	..(A, B)[40]
	[^{14}C]Sabinyl acetate	*T. vulgare*	..(A, B)[40]
	[^{14}C]α-Terpineol	*T. vulgare*	..(A, B)[40]
	[^{14}C]α-Thujene	*T. vulgare*	..(A, B)[40]
	[^{14}C]Thujone	*T. vulgare*	..(A, B)[40]
α-Terpineol	[2-^{14}C]MVA	*M. punctata*	..(A, B)[125]
	[2-^{14}C]Malonic acid	*M. punctata*	..(A, B)[125]
	[^{14}C]Linalool	*T. vulgare*	..(A, B)[40]
	[3-^{14}C]Linalool	*C. jambhiri*	..(A, B)[113,451]
		C. reticulata	..(A, B)[113]
	[^{14}C]Sabinene	*T. vulgare*	..(A, B)[40]
	[^{14}C]Sabinyl acetate	*T. vulgare*	..(A, B)[40]
	[^{14}C]Terpinen-4-ol	*T. vulgare*	0.5 (B)[40]
	[^{14}C]α-Thujene	*T. vulgare*	..(A, B)[40]
	[^{14}C]Thujone	*T. vulgare*	..(A, B)[40]
Terpinolene	[3-^{14}C]Linalool	*C. jambhiri*	0[113]
		C. reticulata	..(A, B)[113]
α-Thujene	[2-^{14}C]Acetate	*T. vulgare*	0.05 (B)[40]
	[^{14}C]Sabinene	*T. vulgare*	..(A, B)[40]
	[^{14}C]Terpinen-4-ol	*T. vulgare*	..(A, B);[40] 0[40]
	[^{14}C]α-Terpineol	*T. vulgare*	..(A, B)[40]
Thujol	[^{14}C]Terpinen-4-ol	*T. vulgare*	2.0 (B)[40]
	[^{14}C]α-Thujene	*T. vulgare*	..(A, B)[40]
Thujone	[1-^{14}C]Acetate	*Thuja occidentalis*	..(A, B)[134,137]
		Thuja plicata	0.007 (B)[40]
		T. vulgare	..(A, B)[29]
	[2-^{14}C]Acetate	*T. vulgare*	0.008 (B)[40]
	[2-^{14}C]MVA	*T. occidentalis*	..(A, C);[134,137] 0.004–0.05 (C)[103,132]
		T. plicata	0.004–0.05 (C)[103,132]
	[^{3}H]GPP	*T. vulgare*	0.003 (B)[40]
	[^{14}C]Linalool	*T. vulgare*	..(A, B)[40]
	[^{14}C]Sabinene	*T. vulgare*	..(A, B)[40]
	[^{14}C]Sabinyl acetate	*T. vulgare*	..(A, B)[40]
	[^{14}C]Terpinen-4-ol	*T. vulgare*	0.6 (B)[40]
	[^{14}C]α-Terpineol	*T. vulgare*	..(A, B)[40]
	[^{14}C]α-Thujene	*T. vulgare*	..(A, B)[40]
	[U-^{14}C]L-Leucine	*T. vulgare*	0[29]
	[U-^{14}C]L-Valine	*T. vulgare*	0[29]
Isothujone	[1-^{14}C]Acetate	*T. vulgare*	0.002 (B)[40]
	[2-^{14}C]Acetate	*T. vulgare*	0.008 (B)[40]
	[2-^{14}C]MVA	*J. sabina*	0.004–0.05 (C)[103,132]
		T. vulgare	0.004–0.05 (C)[103,132]
		T. occidentalis	0.004–0.05 (C)[103,132]
		T. plicata	0.004–0.05 (C)[103,132]
Thymol	[1-^{14}C]Acetate	*Orthodon japonicum*	~0.002 (C)[152]
	[2-^{14}C]MVA	*O. japonicum*	~0.002 (C)[152]
		B. Cyclopentane Derivatives	
		Loganin and Loganic Acid	
Loganin	[2-^{14}C]MVA	*Menyanthes trifoliata*	0[204]
	[3-^{14}C]MVA	*Vinca rosea*	..(A, C)[245]
	[2-^{14}C,4-^{3}H]MVA	*V. rosea*	..(A, C),[241] all ^{3}H retained
	[2-^{14}C]Geraniol	*M. trifoliata*	0.25 (C)[205,206]
	[3-^{14}C]Geraniol	*M. trifoliata*	0.1 (C)[204]
	[1-^{3}H$_2$]Geraniol	*V. rosea*	0.02 (C)[265]
		M. trifoliata	0.2 (C)[205]
	[2-^{14}C,1-^{3}H$_2$]Geraniol	*V. rosea*	..(A, C),[241] 50% ^{3}H retained
	[2-^{14}C,6-^{3}H$_2$]Geraniol	*V. rosea*	..(A, C)[206]
	[2-^{14}C]Geraniol + nerol	*M. trifoliata*	..(A, C)[285]
	[2-^{14}C,2-^{3}H]Geraniol + nerol	*V. rosea*	..(A, C),[241] all ^{3}H retained
	[9-^{14}C]10-Hydroxygeraniol	*V. rosea*	0.09 (C)[245]
	[1-^{3}H$_2$]10-Hydroxygeraniol	*V. rosea*	0.3 (C)[247]

Table I (Continued)

Monoterpene	*Precursor*	*Plant*	*% incorporation (where given)*
	[9-^{14}C]10-Hydroxynerol	*V. rosea*	0.16 (C)[245]
	[1-$^{3}H_2$]10-Hydroxynerol	*V. rosea*	0.31 (C)[247]
	[*O-methyl*-^{3}H]7-Deoxyloganin	*V. rosea*	6.4 (C)[248]
	[^{14}C]7-Deoxyloganic acid	*Lonicera japonica*	0.27 (B)[214]
	[^{14}C]Loganic acid	*Swertia caroliniensis*	..(A, C)[252,253]
		V. rosea	..(A, C)[252,253]
	[*methyl*-^{14}C]L-Methionine	*M. trifoliata*	1 (C)[204]
Loganic acid	[3-^{14}C]Hydroxymethylglutaryl CoA	*V. rosea*	..(A, D)[280]
	[2-^{14}C]MVA	*S. caroliniensis*	0.08–1.2 (C)[220,249]
		V. rosea	0.5 (C)[280]
	(4*R*)-[4-^{3}H]MVA	*S. caroliniensis*	1.2 (C)[218]
	(4*S*)-[4-^{3}H]MVA	*S. caroliniensis*	0.1 (C)[218]
	[2-^{14}C,2-$^{3}H_2$]MVA	*S. caroliniensis*	0.5–0.8 (C, E)[218,220]
	[1-^{14}C]GPP	*S. caroliniensis*	0.002 (C)[249]
	Iridoids, Secoiridoids, and Related Compounds		
Actinidine	[2-^{14}C]Acetate	*Actinidia polygama*	..(A, B)[264]
	[2-^{14}C]MVA	*A. polygama*	..(A, B)[264]
	[1-^{14}C]GPP	*A. polygama*	..(A, B)[264]
Asperuloside	[^{14}C]Loganin	*Daphniphyllum macropodum*	0.45 (B)[214]
	[^{14}C]7-Deoxyloganic acid	*D. macropodum*	0.60 (B)[214]
Aucubin	[2-^{14}C]MVA	*Verbascum thapsus*	0.018 (D)[210]
	[^{14}C]7-Deoxyloganic acid	*Aucuba japonica*	0.51 (B)[214]
Foliamenthin (and dihydrofoliamenthin)	[2-^{14}C]Geraniol	*M. trifoliata*	5 (B)[226]
	[4-^{14}C]Geraniol	*M. trifoliata*	2.5 (B, C)[227]
Gentiopicroside	[2-^{14}C]MVA	*S. caroliniensis*	0.02–0.11 (C)[217,220,249]
		Gentiana triflora	0.01–0.06 (C)[216]
	[4-$^{3}H_2$]MVA	*S. caroliniensis*	0.04–0.1 (C)[218,262]
	(2*R*)-[2-^{14}C,2-^{3}H]MVA	*S. caroliniensis*	0.1 (C)[218,262]
	[^{14}C]Loganin	*G. triflora*	4.5 (B)[214]
	[9-^{14}C]Loganin	*Swertia petiola*	3.2 (B)[219,228]
	[^{3}H]Loganic acid	*S. caroliniensis*	1.3–3.2 (C)[218,262]
	[10-^{14}C]Sweroside	*Gentiana scabra*	..(A, B)[228]
Ipecoside	[2-^{14}C]Geraniol	*Cephaelis ipecacuanha*	0.038 (C)[229]
	[*O-methyl*-^{3}H]Loganin	*C. ipecacuanha*	1.7 (C)[229]
Nepetalactone	[2-^{14}C]MVA	*Nepeta cataria*	~0.01 (C)[102,252,268]
Plumieride	[2-^{14}C]MVA	*Plumiera acutifolia*	1.03 (C)[242]
Secologanin	[7-^{3}H]Loganin	*V. rosea*	6 (C)[224,225]
	[*O-methyl*-^{3}H]Loganin	*M. trifoliata*	5.1 (C)[224]
β-Skytanthine	[2-^{14}C]MVA	*Skytanthus acutus*	0.56 (C)[136,263]
Sweroside	[2-^{14}C]MVA	*Swertia japonica*	0.06 (B)[216]
Verbenalin	[2-^{14}C]MVA	*Verbena officinalis*	0.2 (C)[98,135,210]
	[1-^{14}C]Geraniol	*V. officinalis*	0.009 (C)[98,135]
	[^{14}C]7-Deoxyloganic acid	*V. officinalis*	11 (B)[214]
	Indole Alkaloids		
Ajmalicine[d]	[5-^{14}C]MVA	*V. rosea*	..(A, C)[301]
	[2-^{14}C]Glycine	*V. rosea*	0.26 (D)[300]
Ajmaline	[1-^{3}H]Loganin	*Rauwolia serpentina*	0.04 (C)[285]
	[2-^{14}C]Glycine	*R. serpentina*	..(A, C)[297]
	[2-^{14}C]Tryptophan	*R. serpentina*	~0.1–0.25 (C)[270,271]
Akuammicine	[*Ar*-^{3}H]Geissoschizine	*V. rosea*	0.63 (C)[288]
Apparicine	[*Ar*-^{3}H]Stemmadenine	*Aspidosperma pyricollum*	0.55 (C)[296]
	[^{14}C]Tryptophan	*A. pyricollum*	..(A, C)[295]
Aspidosperma alkaloids	[3-^{14}C]Tryptophan	*Vinca minor*	~0.3 (B)[290]
1,2-Dihydroaspidospermine	[2-^{14}C]MVA	*Rhazya stricta*	0.15 (C)[193]
	[3-^{14}C]MVA	*R. stricta*	0.15 (C)[193]
	[4-^{14}C]MVA	*R. stricta*	..(A, C)[193]
Catharanthine[d]	[1-$^{3}H_2$]Geraniol	*V. rosea*	0.25 (C)[301]
	[1-$^{3}H_2$]Nerol	*V. rosea*	0.21 (C)[301]
	[*Ar*-^{3}H or *O-methyl*-^{3}H]Corynantheine aldehyde	*V. rosea*	0[241]
	[11-^{14}C]Tabersonine	*V. rosea*	0.8 (C)[293]
	[^{14}C]Tryptophan (F)[e]	*V. minor*	~0.1 (C)[272]
Coronaridine	[*Ar*-^{3}H]Geissoschizine	*V. rosea*	Seedlings, 1.53 (C)[283]
Corynantheine[d]	[*O-methyl*-^{3}H]Corynantheine aldehyde	*V. rosea*	Seedlings, 13 (C)[293]
Geissoschizine	[7-^{3}H]Loganin	*V. rosea*	1.3 (C)[288]

Table I (Continued)

Monoterpene	*Precursor*	*Plant*	*% incorporation (where given)*
16,17-Dihydrosecodin-17-ol	[*O-methyl*-^{3}H]Loganin	*V. rosea*	..(A, B)[282]
		Rhazya orientalis	0.013 (B)[282]
Serpentine[d]	[3-^{14}C]MVA	*V. rosea*	..(A, C)[f]
	[*Ar*-^{3}H]Ajmalicine	*V. rosea*	1.8 (C)[241]
	[*Ar*-^{3}H]Geissoschizine	*V. rosea*	0.58 (C)[288]
	[2-^{14}C]Tryptophan	*R. serpentina*	0.007 (C)[271]
Stemmadenine	[3-^{14}C]Tryptophan	*V. rosea*	0.8 (C)[239]
Tabersonine	[*O-methyl*-^{3}H]Catharanthine	*V. rosea*	<0.001 (C)[239]
	[*O-methyl*-^{3}H]Stemmadenine	*V. rosea*	0.27 (C)[239]
Uleine	[*Ar*-^{3}H]Stemmadenine	*A. pyricollum*	<0.0007 (D)[296]
Vincoside	[7-^{3}H]Loganin	*V. rosea*	1.5 (C)[225,286]
Isovincoside	[7-^{3}H]Loganin	*V. rosea*	1.5 (C)[225]
	[*O-methyl*-^{3}H]Loganin	*V. rosea*	5.2 (C)[276]
	[*Ar*-^{3}H]Tryptophan	*V. rosea*	1 (C)[276]
Vindoline	[2-^{14}C]MVA	*V. rosea*	0.02–0.5 (C);[193,284,306,g,h] 0.05 (B)[i]
	[3-^{14}C]MVA	*V. rosea*	0.5 (C);[193,i] ..(A, C)[301]
	[4-^{14}C]MVA	*V. rosea*	..(A, C)[193,301]
	[5-^{14}C]MVA	*V. rosea*	..(A, C)[241]
	[5-^{2}H$_2$]MVA	*V. rosea*	0.2 (C)[k]
	[^{14}C,^{3}H]MVA	*V. rosea*	..(A, C)[190,241]
	[2-^{14}C]Geraniol	*V. rosea*	0.1 (C)[j,k]
	[2-^{14}C]GPP	*V. rosea*	0.2 (C)[f]
	[3-^{14}C]Geraniol	*V. rosea*	..(A, C)[l]
	[1-^{2}H$_2$]Geraniol	*V. rosea*	0.8 (C)[k]
	[2-^{14}C,1-^{3}H$_2$]Geraniol	*V. rosea*	..(A, C)[190,241]
	[2-^{14}C]Geraniol + nerol	*V. rosea*	0.37 (C)[h]
	[9-^{14}C]10-Hydroxygeraniol	*V. rosea*	0.72 (C)[245]
	[9-^{14}C]10-Hydroxynerol	*V. rosea*	1.2 (C)[245]
	[1-^{3}H$_2$]10-Hydroxygeraniol + 10-hydroxynerol	*V. rosea*	0.25 (C)[247]
	[7-^{14}C]Iridodial	*V. rosea*	0.0005 (B, D)[246]
	[8-^{14}C]Loganin	*V. rosea*	0.2 (C)[240]
	[9-^{14}C]Loganin	*V. rosea*	0.23 (C)[285]
	[*O-methyl*-^{3}H]Loganin	*V. rosea*	0.5 (C)[190,191,193,284]
	[^{14}C,^{3}H]Loganin	*V. rosea*	..(A, C)[241,286]
	[*O-methyl*-^{3}H]7-Deoxyloganin	*V. rosea*	0.1–0.5 (C)[248]
	[*O-methyl*-^{3}H]7-Deoxyloganin aglucone	*V. rosea*	0[248]
	[*O-methyl*-^{3}H]7-Deoxyloganin Δ6-7 or Δ7-8	*V. rosea*	0[248]
	[*O-methyl*-^{3}H]Secologanin	*V. rosea*	~0.1 (C)[224,225,241,m]
	[10-^{14}C]Sweroside	*V. rosea*	11 (C)[228]
	[*Ar*-^{3}H]Ajmalicine	*V. rosea*	Seedlings, 0.1 (C)[293] Plants, 0.004 (C)[293]
	[*O-methyl*-^{3}H]Corynantheine aldehyde	*V. rosea*	Seedlings, 0.1 (C)[293] Plants, 0.003 (C)[241,293]
	[*O-methyl*-^{3}H,*Ar*-^{3}H]Geissoschizine	*V. rosea*	0.13 (C)[288]
	[*O-methyl*-^{3}H,11-^{14}C]Stemmadenine	*V. rosea*	0.3–1.8 (C)[239,293]
	[*O-methyl*-^{3}H,11-^{14}C]Tabersonine	*V. rosea*	1.1–4.8 (C)[239]
	[*O-methyl*-^{3}H]Vincoside	*V. rosea*	0.78 (C)[277,286]
	[*Ar*-^{3}H]Vincoside	*V. rosea*	0.39 (C)[225,277]
	[*O-methyl*-^{3}H]Vincoside (side chain vinyl group reduced)	*V. rosea*	0[277,286]
	[*O-methyl*-^{3}H]Isovincoside	*V. rosea*	0[277,286]
	[U-^{14}C]Shikimic acid	*V. rosea*	1.26–2.2 (C)[n,o]
	[^{14}C]Tryptophan	*V. rosea*	..(A, B, C)[225,270,271,273,290]
	[*Ar*-^{3}H]Tryptamine	*V. rosea*	0.39 (C)[277]

[a] W. Sandermann, quoted in ref 8, p 854. [b] A species of ant. [c] E = per cent incorporation based on ^{14}C tracer. [d] Further evidence for the biosynthesis of this alkaloid has been presented in the papers quoted in the section on vindoline. [e] F = other aryl precursors included in this reference. [f] A. R. Battersby, R. T. Brown, J. A. Knight, J. A. Martin, and A. O. Plunkett, *Chem. Commun.*, 346 (1966). [g] F. McCapra, T. Money, A. I. Scott, and I. G. Wright, *ibid.*, 537 (1965). [h] T. Money, I. G. Wright, F. McCapra, E. S. Hall, and A. I. Scott, *J. Amer. Chem. Soc.*, **90**, 4144 (1968). [i] T. Money, I. G. Wright, F. McCapra, and A. I. Scott, *Proc. Nat. Acad. Sci. U. S.*, **53**, 901 (1965). [j] P. Loew, H. Goeggel, and D. Arigoni, *Chem. Commun.*, 347 (1966). [k] E. S. Hall, F. McCapra, T. Money, K. Fukumoto, J. R. Hanson, B. S. Mootoo, G. T. Phillips, and A. I. Scott, *ibid.*, 348 (1966). [l] E. Leete and S. Ueda, *Tetrahedron Lett.*, 4915 (1966). [m] A. R. Battersby, A. R. Burnett, and P. G. Parsons, *Chem. Commun.*, 1280 (1968). [n] D. Groger, K. Stolle, and K. Mothes, *Z. Naturforsch. B*, **21**, 206 (1966). [o] K. Stolle, D. Groger, and K. Mothes, *Chem. Ind. (London)*, 2065 (1965).