

a secondary or tertiary hydroxyl in the sidechain) it is possible to surmise that they arise by epoxidation or peroxidation of the sidechain of plastoquinone, possibly by molecular oxygen generated by photosynthesis with ensuing rearrangement, reduction and esterification.

Regulation of biosynthesis

Plastoquinone, phylloquinone and α -tocopherol are present in much lower amounts in etiolated tissue compared with green tissue, and the increase in these compounds on illumination is concomitant with the formation of the thylakoid membrane. This is perhaps the major regulatory level in biosynthesis of chloroplastic quinones and chromanols. Evidence for light-dependent synthesis of phytyl plastoquinone and plastoquinone in isolated chloroplasts was provided by experiments of Hutson & Threlfall (1980b), whereas formation of 2-methyl-6-phytylbenzoquinone was found to be light-independent (Soll *et al.*, 1980). The variable effects may well depend upon the levels of ATP and NADPH in the system.

Tocopherols and plastoquinone increase in concentration throughout the life of the plant, when α -tocopherol in young seedlings may be only 10 μ g/g wet wt. and rises to over 1 mg/g wet wt. in mature green leaves. There is a correlation between the low tocopherol level in fast-growing plants and the high tocopherol level in slow-growing plants (Booth & Hobson-Frohock, 1961) and since it is difficult to show tocopherol or plastoquinone biosynthesis from radioactive precursors in mature leaves, slow rates of degradation may be more likely than uncontrolled synthesis.

Finally, although biosynthesis of phylloquinone, plastoquinone and tocopherols can be shown to occur in isolated chloroplasts (Soll *et al.*, 1980; Schultz *et al.*, 1981) it is not likely that control is exercised solely from within the chloroplast. Most chloroplastic proteins are coded for by nuclear genes and are transported by a post-translational mechanism to the chloroplast envelope (Ellis, 1981). Thus, not only may there be control of metabolites passing into the chloroplast but there may

be control of biosynthetic enzymes at the nuclear level. If a feedback mechanism operates to control the level of thylakoid membrane components (which are bound to the lipid components of the membrane) then it must be a quite complicated regulation mechanism.

- Bentley, R. & Meganathan, R. (1982) *Microbiol. Rev.* **46**, 241–280
 Booth, V. H. & Hobson-Frohock, A. (1961) *J. Sci. Food Agric.* **12**, 251–258
 Burnett, A. R. & Thomson, R. H. (1968) *J. Chem. Soc.* 857–860
 Campbell, I. M. (1969) *Tetrahedron Lett.* 4777–4780
 Dansette, P. & Azerad, R. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1090–1095
 Ellis, R. J. (1981) *Annu. Rev. Plant Physiol.* **32**, 111–137
 Etman-Gervais, C., Tendille, C. & Polonsky, J. (1977) *Nouvo Chimento* **1**, 323–325
 Fiedler, E., Soll, J. & Schultz, G. (1982) *Planta* **155**, 511–515
 Hutson, K. G. & Threlfall, D. R. (1980a) *Phytochemistry* **19**, 535–537
 Hutson, K. G. & Threlfall, D. R. (1980b) *Biochim. Biophys. Acta* **632**, 630–648
 Janiszowska, W. & Pennock, J. F. (1976) *Vitam. Horm.* **34**, 77–105
 McKenna, M., Henninger, M. D. & Crane, F. L. (1964) *Nature (London)* **203**, 524–525
 Pennock, J. F. & Threlfall, D. R. (1983) in *Biosynthesis of Isoprenoid Compounds* (Porter, J. W. & Spurgeon, S. L., eds.), vol. 2, pp. 191–303, Wiley, New York
 Schoch, S., Lempert, V. & Rudiger, W. (1977) *Z. Pflanzephysiol.* **83**, 427–436
 Schultz, G., Ellerbrock, B. H. & Soll, J. (1981) *Eur. J. Biochem.* **117**, 329–332
 Soll, J. & Schultz, G. (1980) *Phytochemistry* **19**, 215–218
 Soll, J. & Schultz, G. (1981) *Biochem. Biophys. Res. Commun.* **99**, 907–912
 Soll, J., Douce, R. & Schultz, G. (1980) *FEBS Lett.* **112**, 243–246
 Threlfall, D. R. (1971) *Vitam. Horm.* **29**, 153–200
 Threlfall, D. R. & Whistance, G. R. (1977) *Phytochemistry* **16**, 1903–1907
 Welburn, A. R. (1968) *Phytochemistry* **7**, 1523–1528
 Whistance, G. R. & Threlfall, D. R. (1970) *Phytochemistry* **9**, 213–219
 Young, I. G. (1975) *Biochemistry* **14**, 399–406

Cell-free studies of monoterpene and sesquiterpene biosynthesis

DAVID E. CANE

Department of Chemistry, Brown University, Providence, RI 02912, U.S.A.

Synopsis

Allylic pyrophosphates have been recognized for many years as the key precursors of cyclic monoterpenes and sesquiterpenes. Recent work in our laboratories using cell-free enzyme systems from both micro-organisms and higher plants has helped to clarify many of the key mechanistic details of the rearrangement and cyclization of the primary allylic pyrophosphates, geranyl and farnesyl pyrophosphate, to monoterpene and sesquiterpene metabolites, respectively.

Introduction

Within the last decade the study of natural products biosynthesis has undergone a revolution in experimental methodology. The widespread application of stable-isotope n.m.r. has resulted in a dramatic increase in the rate at which biosynthetic problems can be solved while the parallel development of multiple isotope labelling techniques has provided an enormously powerful tool for tracing the metabolic fate of chemical bonds during the course of biosynthetic transformations. At the same time, there has been a steady growth in the use of cell-free experimental systems as attention has been turned from work with intact cells or whole organisms in order

to focus on the individual enzymes of secondary metabolism. As a result of the development of cell-free methodology, significant advances have recently been made in the understanding of the biochemistry of indole alkaloid, β -lactam, porphyrin, and isoprenoid biosynthesis (Cane, 1983). Much of our own recent work has been concerned with the metabolism of allylic pyrophosphates, the substrates for the ubiquitous and fascinating cyclases which catalyse the central synthetic transformations of the terpenoid biosynthetic pathway (Cane, 1980). Using cell-free extracts of both plants and micro-organisms, we have been examining the mechanisms by which the universal acyclic precursors, geranyl and farnesyl pyrophosphate, are converted to cyclic monoterpenes (Croteau, 1981) and sesquiterpenes (Cane, 1981), respectively. The results of some of our more recent investigations are described below.

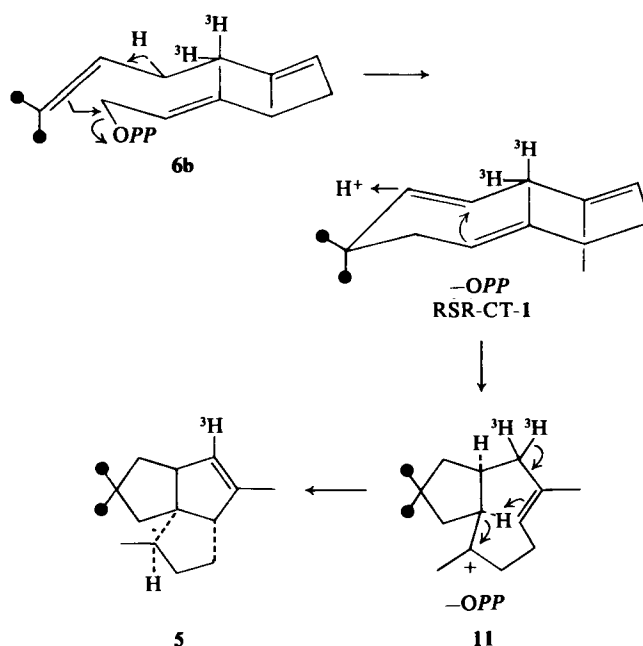
Since 1974 we have been investigating the biosynthesis of a family of sesquiterpenes characterized by the presence of one or more, frequently fused, cyclopentane rings, and formally derivable from the commonly occurring 11-membered ring triene humulene (1). Among these metabolites, the antibiotic pentalenolactone (2) attracted our attention, not only by virtue of its unusual structure, but also due to its production by a variety of prokaryotic *Streptomyces* species (Scheme 1). The vast majority of cyclic terpenoids, by contrast, are restricted to eukaryotic microorganisms and higher plants. As a result of incorporation experiments with ^{13}C -labelled precursors, we were

eventually able to demonstrate the mevalonoid origin of pentalenolactone and to propose a plausible pathway for its formation (Cane *et al.*, 1981a). While these studies were being carried out parallel investigations by Seto and his collaborators (Seto *et al.*, 1978a,b) led to the isolation of a series of co-metabolites of pentalenolactone representing possible intermediates in the biosynthetic pathway. In our own laboratories we have isolated two additional representatives of this group of compounds, pentalenolactones E (3) (Cane & Rossi, 1979) and F (4) (Tillman & Cane, 1983). Of greatest interest, however, was the isolation by Seto of a tricyclic sesquiterpene, pentalenene (5), the parent hydrocarbon of the pentalenolactone family of metabolites (Seto & Yonehara, 1980). In fact, the identical hydrocarbon, in racemic form, had already been prepared by Matsumoto and Shirahama in the course of a comprehensive study of biogenetically modelled cyclizations of protoilludyl and other humulene-derived cations (Ohfuné *et al.*, 1976). Indeed, using a variation of the latter synthesis, we have prepared specifically tritiated pentalenene and confirmed the postulated role of 5 as a precursor of the more oxidized pentalenenes by feeding experiments with intact cells followed by appropriate degradations (Cane & Tillman, 1983).

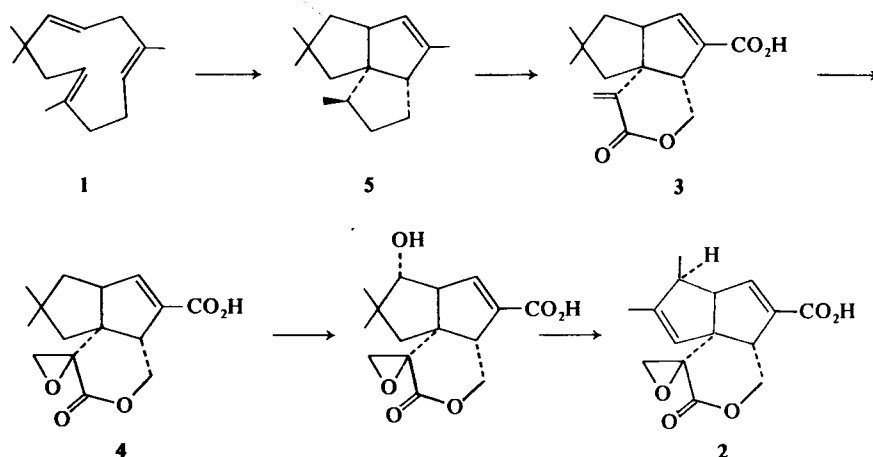
In the course of our early work with ^{13}C -labelled precursors we were repeatedly frustrated by the failure of many of the more common isoprenoid precursors to give rise to labelled metabolites, presumably due to extracellular or intracellular permeability barriers, a problem more often encountered in work with higher plants than with micro-organisms. We were therefore attracted to the possibility of using cell-free extracts in order to study directly the conversion of the presumed precursor, *trans,trans*-farnesyl pyrophosphate, to pentalenene itself (Scheme 2). Since the expected level of enzyme-catalysed turnover would be well below the ordinary limits of ^{13}C -n.m.r. detection, we were obliged to resort to traditional methods of radioisotopic labelling in order to assure the rigorous determination of the eventual isotopic distribution in the sesquiterpene product. The requisite sample of *trans,trans*-[8- ^3H]farnesol (6a) was prepared by coupling of 8-chloro[8- ^3H]geranylbenzyl ether with the lithio anion of dimethylallylphenyl sulphone, followed by reductive cleavage with lithium in ethylamine. The tritiated farnesol was mixed with [12,13- ^{14}C]farnesol as internal standard and a portion of the resulting mixture was converted to the corresponding diphenylurethane before recrystallization to constant radioactivity ($^3\text{H}/^{14}\text{C}$ atom ratio 2:2). The remainder of the farnesol was used to prepare the required pyrophosphate ester (6b).

After considerable experimentation we eventually found that an active cell-free preparation could be obtained from a 60h culture of *Streptomyces* UC5319 by rapid stirring of the

harvested cells in pH 7.2 phosphate buffer with glass beads in the presence of suitable stabilizers or protecting agents such as a combination of dithiothreitol, EDTA, and glycerol. The 34 000 g supernatant was immediately incubated with [8- ^3H ,12,13- ^{14}C]farnesyl pyrophosphate and MgCl_2 for 1.5h at 30°C. After addition of acetone to quench the reaction, the mixture was extracted with pentane. Synthetic ($\pm$)-pentalenene was added to the pentane extract as carrier and the mixture was purified by preparative t.l.c. on silica gel. Based on the activity of the recovered pentalenene, the rate of formation of 5 was estimated to be approx. 0.03 nmol/h per mg of protein. In a series of control experiments the rate of pentalenene formation was found to be proportional to enzyme concentration, while a boiled control exhibited no significant formation of pentalenene. The extremely low specific activity of the cell-free extract is typical of such crude preparations of terpenoid cyclases, as was the pronounced instability (50% loss of activity after 4h at 4°C) and contamination by competing phosphatase/pyrophosphatase activities.



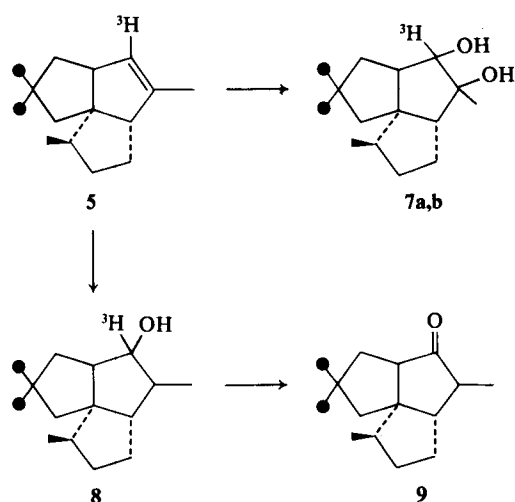
Scheme 2. Conversion of [8- ^3H ,12,13- ^{14}C] farnesyl pyrophosphate to pentalenene



Scheme 1. Pentalenolactone (2) and biogenetically related metabolites

The specificity of the enzyme-catalysed cyclization was confirmed by a chemical degradation sequence which established the presence of the tritium at the expected site, C-7, of pentalenene. Thus, following dilution with additional carrier pentalenene, a portion of the labelled sample was converted to a diastereomeric mixture of *cis*-6,7-diols (**7**) by treatment with OsO_4 (Scheme 3). The individual diols were separated by fractional recrystallization and individually recrystallized to constant specific activity and isotope ratio. Both solid derivatives showed the expected loss of half the tritium activity ($^3\text{H}/^{14}\text{C}$ atom ratio 0.9:2). Conversion of the remainder of the labelled pentalenene to the corresponding 7-hydroxypentalenane (**8**) by hydroboration-oxidation followed by pyridinium chlorochromate oxidation gave pentalen-7-one (**9**) which had lost all tritium activity, as expected.

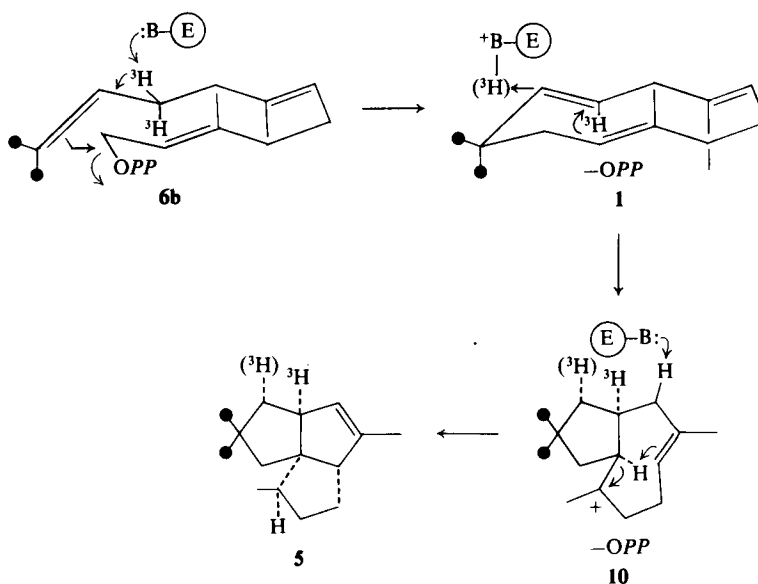
The observed distribution of isotope in the enzymically generated pentalenene may be explained by initial cyclization of



Scheme 3. Degradation of pentalenene derived from [8- ^3H ,12,13- ^{14}C]farnesyl pyrophosphate

farnesyl pyrophosphate (**6b**), folded as illustrated in Scheme 2, to give humulene (**1**). From our earlier work with ^{13}C -labelled precursors we know that electrophilic attack by C-1 of the farnesyl moiety takes place on the *si*-face of the distal 10,11-double bond. Protonation at C-10 of the newly generated *trans*-9,10-double bond of humulene, followed by intramolecular electrophilic attack at C-2 will generate the cation **10**, which may undergo a hydride migration and further cyclization with loss of one of the hydrogen atoms originally at C-8 of the farnesyl precursor. From the relative and absolute configuration of the pentalenene itself it can be inferred that the intermediate humulene is folded in the illustrated *RSR-CT* conformation.

The apparent correlation between the postulated stereochemistry of farnesyl pyrophosphate ring closure and that of the subsequent humulene cyclization is consistent with our earlier reported observations on the formation of dimethylcyclopentane sesquiterpenes, and suggests that both cyclization processes may take place at a single active site (Cane & Nachbar, 1978). With the availability of an active pentalenene synthetase, we are now able to test this proposition. In the absence of a homogeneous synthetase a number of experimental approaches are being explored. Circumstantial evidence that humulene does not accumulate during the course of the enzymic cyclization of farnesyl pyrophosphate was obtained by quenching a reaction after 15 min and adding inactive carrier humulene to the incubation mixture before extraction with pentane. The reisolated humulene, purified as the corresponding Ag^+ complex, was devoid of activity. However, a more rigorous test of the single enzyme hypothesis is possible. If formation and cyclization of humulene takes place at a common active site, some fraction of the protons which are initially abstracted from C-9 of farnesyl pyrophosphate may be returned to C-10 of the intermediate humulene, provided there is not significant exchange of the transiently generated conjugate acid of the enzyme base with the external medium (Scheme 4). Since the active site would presumably be well insulated from the external environment in order to protect the transiently generated carbocations, there is, in fact, a reasonable chance of at least some internal proton return. An alternative hydride shift mechanism interconverting the 9- and 10-humulyl cations would transfer all of the relevant protons. Such a mechanism would, of course,



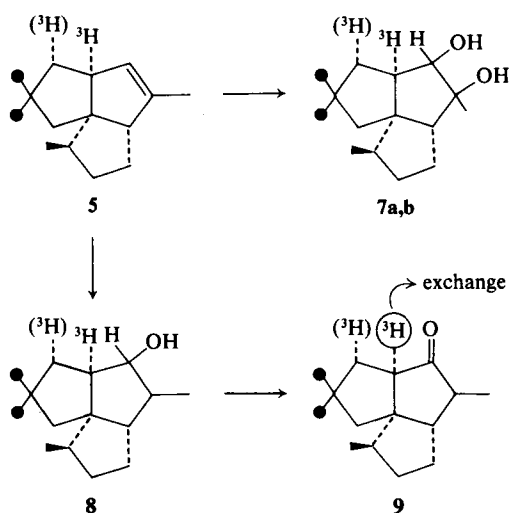
Scheme 4. Conversion of [9- ^3H ,12,13- ^{14}C]farnesyl pyrophosphate to pentalenene

require a single cyclization enzyme. Accordingly we have now prepared [9- $^3\text{H}_2$,12,13- ^{14}C]farnesyl pyrophosphate ($^3\text{H}/^{14}\text{C}$ atom ratio 2:2) and incubated this substrate with pentalenene synthetase. The recrystallized sample of diol A (**7**), obtained by treatment of the recovered pentalenene with OsO_4 (Scheme 5) was found to retain more than one equivalent of tritium, based on the observed $^3\text{H}/^{14}\text{C}$ ratio (atom ratio 1.7:1). An equivalent of tritium was shown to be located at C-8, as expected, by exchange of the derived pentalen-7-one (**9**) with NaO^2H in dioxane/ $^2\text{H}_2\text{O}$ (final $^3\text{H}/^{14}\text{C}$ atom ratio 0.8:1). Experiments are now in progress to establish definitively the site of the remaining tritium label which is expected to be found at H-1 α . In the meantime, this preliminary data is consistent only with the operation of a single enzyme mediating pentalenene formation, as originally predicted on purely stereochemical grounds. It might also be noted that the observed retention of a non-integral

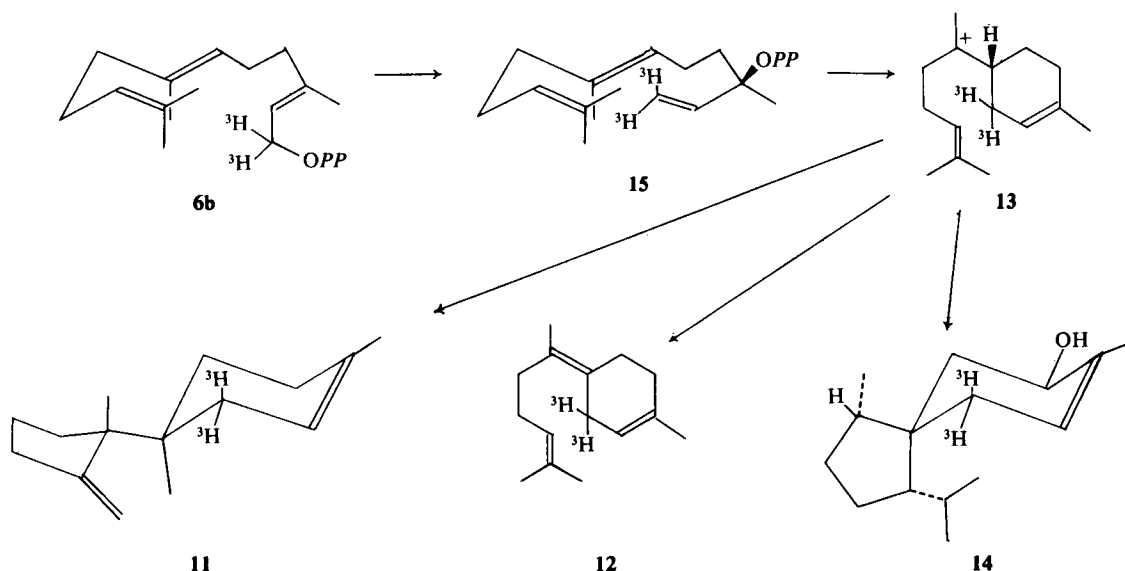
quantity of tritium is more consistent with a partially efficient proton transfer step than with the alternative hydride shift mechanism, although a definitive distinction between these two mechanisms must await the results of further experimentation.

A large and varied group of sesquiterpenes are believed to be derived by initial electrophilic attack of the C-1 carbinyl carbon of farnesyl pyrophosphate on the central, 6,7-double bond (Cane, 1981). Until very recently there had been considerable disagreement about the precise mechanism by which the *trans*-2,3-double bond of the allylic pyrophosphate precursor might be isomerized in the course of the generation of the necessary *cis*-cyclohexene ring of the product. The evidence bearing on the various isomerization-cyclization theories was reviewed in 1980 and need not be considered here (Cane, 1980). In the interval, however, the experimental picture has become a good deal clearer. For example, cell-free studies of the conversion of farnesyl pyrophosphate to the sesquiterpene hydrocarbons trichodiene (**11**) (Cane *et al.*, 1981*b*) and bisabolene (**12**) (Anastasis *et al.*, 1982) have firmly established that in each case both of the hydrogen atoms attached to C-1 of the allylic pyrophosphate are retained in the cyclized product, thereby ruling out all previously suggested redox mechanisms for the double bond isomerization (Scheme 6). These conclusions were also in accord with the results of an independent study of the biosynthesis of a third bisabolyl cation (**13**)-derived metabolite, coccinol (**14**) (Gottfredsen, 1978). Although the weight of evidence is consistent with the intermediacy of the tertiary allylic pyrophosphate, nerolidyl pyrophosphate (**15**), the role of this metabolite has yet to be explicitly demonstrated.

In the meantime, dramatic progress has been made in the study of the enzymology of monoterpene biosynthesis by Professor Rodney Croteau and his collaborators at Washington State University (Croteau, 1981). Using cell-free preparations from a variety of plants known to be rich in essential oils, Croteau has made major contributions to our knowledge of the formation of the main classes of monocyclic and bicyclic terpenes, including p-menthanes, pinanes, bornanes, fenchanes, and thujanes. In all these cases, geranyl pyrophosphate has now been shown to undergo cyclization without loss of hydrogen from C-1 (Croteau & Felton, 1981). Moreover, the corresponding tertiary allylic pyrophosphate, linalyl pyrophosphate, serves as an alternative substrate for these cyclases.



Scheme 5. Degradation of pentalenene derived from [9- $^3\text{H}_2$,12,13- ^{14}C]farnesyl pyrophosphate

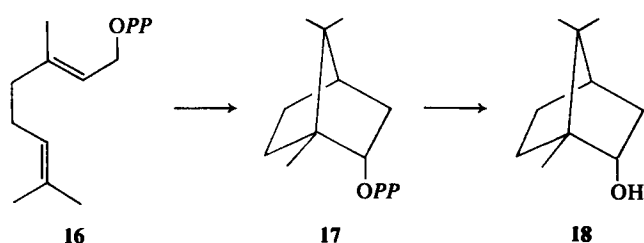


Scheme 6. Conversion of *trans,trans*-farnesyl pyrophosphate to trichodiene (**11**), bisabolene (**12**), and coccinol (**14**) with retention of both C-1 hydrogen atoms

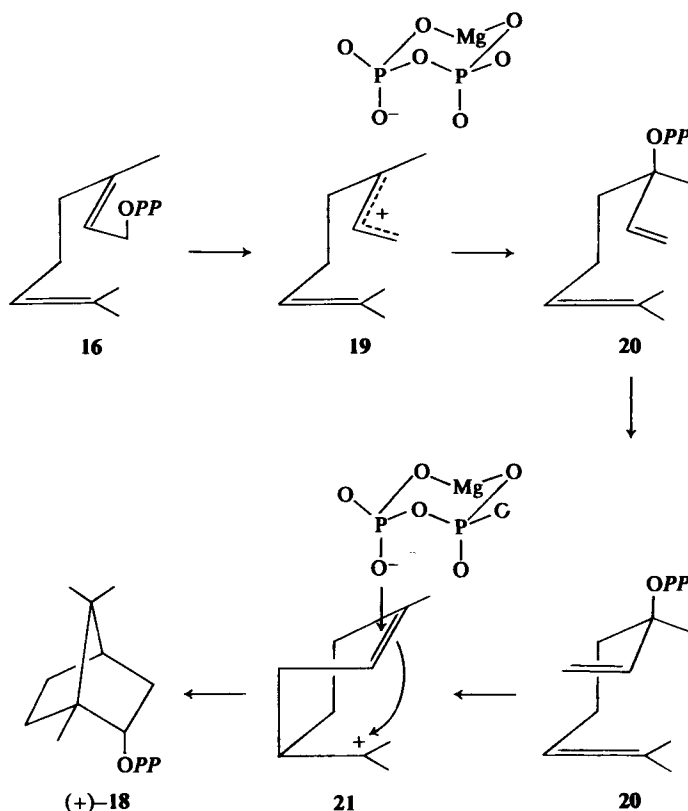
One particularly exciting observation reported by Croteau was the finding that an enzyme system from sage (*Salvia officinalis*) catalysed the conversion of geranyl pyrophosphate (**16**) to (+)-bornyl pyrophosphate (**17**), which is subsequently hydrolysed by a distinct pyrophosphatase to (+)-borneol (**18**) (Scheme 7) (Croteau & Karp, 1977). This discovery has in fact provided a unique opportunity to examine experimentally the role of the pyrophosphate moiety in the coupled isomerization-cyclization process. A reasonable model for the formation of (+)-bornyl pyrophosphate from geranyl pyrophosphate, based on closely related chemical models (Gotfredsen, 1977, 1978) and analogous to the mechanism which we have proposed for trichodiene formation is illustrated in Scheme 8 (Cane *et al.*, 1981b). Initial isomerization of geranyl pyrophosphate to its tertiary allylic isomer followed by 2,3-single bond rotation would generate linalyl pyrophosphate (**20**) in a *cisoid* con-

formation suitable for ionization and electrophilic attack on the 6,7-double bond. The transiently generated α -terpinyl cation (**21**), formally analogous to the bisabolyl intermediate (**13**) of sesquiterpene cyclizations, can further cyclize by attack on the newly formed cyclohexene double bond and capture of the resultant cation by the paired inorganic pyrophosphate ion. Of particular interest is the extent to which the pyrophosphate moiety becomes free of its cationic partner during the course of the cyclization. In collaboration with Professor Croteau we therefore undertook ^{18}O labelling studies designed to shed light on the role of the pyrophosphate group in the enzymic formation of bornyl pyrophosphate (Cane *et al.*, 1982).

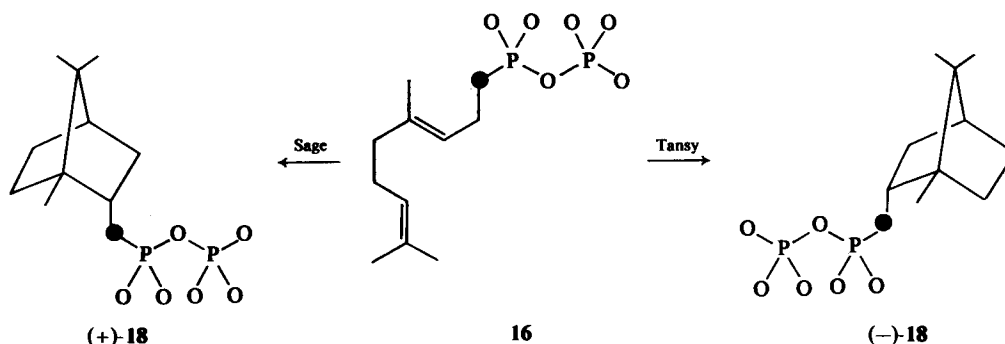
A sample of [$1-^{18}\text{O}$]geranyl pyrophosphate (67.9 atom% ^{18}O), containing [$8,9-^{14}\text{C}$]geranyl pyrophosphate as internal standard, was incubated in portions with (+)-bornyl pyrophosphate synthetase, obtained from the 105 000 g supernatants prepared from whole leaf homogenates of *Salvia officinalis*, as previously described. After 3 h at 30°C borneol and geraniol generated from the corresponding pyrophosphate esters by the action of endogenous phosphatases present in the crude cell homogenate were extracted into pentane and the concentrated extract was benzoylated. By a series of such incubations, requiring a period of several weeks, it was possible to accumulate sufficient (+)-bornyl benzoate (approx. 0.2 μmol) for analysis by mass spectrometry after extensive purification by h.p.l.c. The recovered (+)-[2- ^{18}O , 8,9- ^{14}C]bornyl benzoate was found to contain $52.4 \pm 1.4\%$ ^{18}O . This value could be further corrected for endogenous dilution by unlabelled intermediates, based on the measured decrease in ^{14}C specific activity of isolated bornyl benzoate, corresponding to a calculated ^{18}O enrichment of $72 \pm 3\%$. Since complete equilibration of the proximal pyrophosphate oxygen atoms would



Scheme 7. Conversion of geranyl pyrophosphate to bornyl pyrophosphate and borneol



Scheme 8. Mechanism of cyclization of geranyl pyrophosphate to (+)-bornyl pyrophosphate



Scheme 9. Lack of positional isotope exchange in the conversion of $[1\text{-}^{18}\text{O}]$ geranyl pyrophosphate to $(+)\text{-}$ and $(-)\text{-}$ bornyl pyrophosphates

have reduced the original ^{18}O enrichment of 67.9% to a value of 22.6% at C-2 of bornyl benzoate, isomerization–cyclization of $[1\text{-}^{18}\text{O}]$ geranyl pyrophosphate to bornyl pyrophosphate clearly involves no positional isotope exchange. This result implies a remarkably tight restriction on the motion of the pyrophosphate moiety during the cationic cyclization reaction. (Scheme 9). Control experiments confirmed that pyrophosphate ester hydrolysis took place with the normally observed P–O bond cleavage while parallel ^{32}P -labelling results, discussed elsewhere (Cane *et al.*, 1982), were fully consistent with the above experimental observations.

The availability of $(-)\text{-}$ bornyl pyrophosphate synthetase from *Tanacetum vulgare* (tansy) allowed the results obtained with the sage extract to be corroborated using an enzyme system which converted the same precursor, geranyl pyrophosphate, to a product of identical structure but enantiomerically opposite configuration. Indeed, analogous sets of incubations of $[1\text{-}^{18}\text{O}]$ geranyl pyrophosphate with the 105 000g supernatant of whole leaf homogenates of tansy, followed by mass spectrometric analysis of the recovered $(-)\text{-}$ bornyl benzoate, established that essentially 100% of the original ^{18}O isotope became attached to C-2 of the bornyl product.

The severely restricted motion of the transiently generated inorganic pyrophosphate moiety is all the more striking when one takes into account the transient generation of an α -terpinyl cation–pyrophosphate anion pair in which the charge separation must be at least 0.3 nm. It is not yet clear whether the observed lack of positional isotope exchange is due to the inherently strong electrostatic attraction between the inorganic pyrophosphate– Mg^{2+} complex and the paired carbocations or whether the apparent restriction of the motion of the pyrophosphate moiety is imposed by the cyclase enzyme itself. In contrast to these results, in a related study of the role of the pyrophosphate moiety in allylic pyrophosphate isomerizations we observed the equilibration of the proximal phosphate oxygens during the enzymic conversion of farnesyl to nerolidyl pyrophosphate (Cane *et al.*, 1981c). In the latter case, however, the observed oxygen scrambling is presumably due to the reversible conversion of the allylic pyrophosphate isomers at the enzyme active site. On the other hand, Poulter has recently reported that $[1\text{-}^{18}\text{O}]$ geranyl pyrophosphate reisolated from incubations with prenyl transferase has not undergone detectable scrambling, in spite of strong evidence for the generation of allylic cations at the enzyme active site (Mash *et al.*, 1981).

Within the last 5 years, enzyme-level studies have provided a wealth of mechanistic and stereochemical information about isoprenoid biosynthetic transformations which was previously inaccessible using traditional whole-cell feeding experiments.

Nonetheless, the use of cell-free systems to study terpenoid biosynthetic pathways is still in its relative infancy. Some 60 years after Ruzicka first proposed the Isoprene Rule, the study of the fascinating cyclases which lie at the heart of isoprenoid biogenetic theory is only just beginning.

The work described in this report would not have been possible without the energetic and enthusiastic assistance of the dedicated coworkers whose names may be found in the list of references. Financial support was provided by NIH grants GM22172 and GM30301. The author was a Fellow of the Alfred P. Sloan Foundation (1978–1982) and holds a National Institutes of Health Research Career Development Award (1978–1983).

- Anastasis, P., Freer, I., Gilmore, C., Mackie, H., Overton, K. & Swanson, S. (1982) *J. Chem. Soc. Chem. Commun.* 268–270
- Cane, D. E. (1980) *Tetrahedron* **36**, 1109–1159
- Cane, D. E. (1981) in *Biosynthesis of Isoprenoid Compounds* (Porter, J. W. & Spurgeon, S. L., eds.), pp. 283–374, John Wiley, New York
- Cane, D. E. (1983) in *The Impact of Enzyme Chemistry* (Suckling, C. J., ed.), Chapman & Hall, London, in the press
- Cane, D. E. & Nachbar, R. B. (1978) *J. Am. Chem. Soc.* **100**, 3208–3212 (see also correction **101**, 1908)
- Cane, D. E. & Rossi, T. (1979) *Tetrahedron Lett.* 2973–2974
- Cane, D. E. & Tillman, A. M. (1983) *J. Am. Chem. Soc.* **105**, 122–124
- Cane, D. E., Rossie, T., Tillman, A. M. & Pachlatko, J. P. (1981a) *J. Am. Chem. Soc.* **103**, 1838–1843
- Cane, D. E., Swanson, S. & Murthy, P. P. N. (1981b) *J. Am. Chem. Soc.* **103**, 2136–2138
- Cane, D. E., Iyengar, R. & Shiao, M.-S. (1981c) *J. Am. Chem. Soc.* **103**, 914–931
- Cane, D. E., Saito, A., Croteau, R., Shaskus, J. & Felton, M. (1982) *J. Am. Chem. Soc.* **104**, 5831–5833
- Croteau, R. & Karp, F. (1977) *Arch. Biochem. Biophys.* **184**, 77–86
- Croteau, R. (1981) in *Biosynthesis of Isoprenoid Compounds* (Porter, J. W. & Spurgeon, S. L., eds.), pp. 225–282, John Wiley, New York
- Croteau, R. & Felton, M. (1981) *Arch. Biochem. Biophys.* **207**, 460–464
- Gotfredsen, S., Obrecht, J. P. & Arigoni, D. (1977) *Chimia* **31**(2), 62
- Gotfredsen, S. E. (1978) *Dissertation*, ETH Zurich, no. 6243
- Mash, E. A., Gurria, G. M. & Poulter, C. D. (1981) *J. Am. Chem. Soc.* **103**, 3927–3929
- Ohfuné, Y., Shirahama, H. & Matsumoto, T. (1976) *Tetrahedron Lett.* 2869–2872
- Seto, H., Sasaki, T., Yonehara, H. & Uzawa, J. (1978a) *Tetrahedron Lett.* 923–926
- Seto, H., Sasaki, T., Uzawa, J., Takeuchi, S. & Yonehara, H. (1978b) *Tetrahedron Lett.* 4411–4412
- Seto, H. & Yonehara, H. (1980) *J. Antibiot.* **33**, 92–93
- Tillman, A. M. & Cane, D. E. (1983) *J. Antibiot.* **36**, 170–172