PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Terpenoid Biosynthesis and the Stereochemistry of Enzyme-Catalysed Allylic Addition-Elimination Reactions

David E. Cane, Christopher Abell, Paul H. M. Harrison, Brian R. Hubbard, Charles T. Kane, Rene Lattman, John S. Oliver and Steven W. Weiner

Phil. Trans. R. Soc. Lond. B 1991 **332**, 123-129
doi: 10.1098/rstb.1991.0040

Terpenoid biosynthesis and the stereochemistry of enzyme-catalysed allylic addition–elimination reactions

DAVID E. CANE, CHRISTOPHER ABELL, PAUL H. M. HARRISON, BRIAN R. HUBBARD, CHARLES T. KANE, RENE LATTMAN, JOHN S. OLIVER AND STEVEN W. WEINER

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

SUMMARY

Allylic addition–elimination reactions are widely used in the enzyme-catalysed formation of terpenoid metabolites. It has earlier been shown that the isoprenoid chain elongation reaction catalysed by farnesyl pyrophosphate synthase involving successive condensations of dimethylallyl pyrophosphate (DMAPP) and geranyl pyrophosphate (GPP) with isopentenyl pyrophosphate (IPP) corresponds to such an $S_{E'}$ reaction with net syn stereochemistry for the sequential electrophilic addition and proton elimination steps. Studies of the enzymic cyclization of farnesyl pyrophosphate (FPP) to pentalenene have now established the stereochemical course of two additional biological $S_{E'}$ reactions. Incubation of both (9*R*)- and (9*S*)-[9-^{3}H, 4,8-^{14}]FPP with pentalenene synthase and analysis of the resulting labelled pentalenene has revealed that H-9*re* of FPP becomes H-8 of pentalenene, while H-9*si* undergoes net intramolecular transfer to the adjacent carbon, becoming H-1*re* (H-1α) of pentalenene, as confirmed by subsequent experiments with [10-^{2}H, 11-^{13}C]FPP. These results correspond to net anti-stereochemistry in the intramolecular allylic addition–elimination reaction. The stereochemical course of a second $S_{E'}$ reaction has now been examined by analogous incubations of (4*S*, 8*S*)-[4,8-^{3}H, 4,8-^{14}C]FPP and (4*R*,8R)-[4,8-^{3}H, 4,8-^{14}C]FPP with pentalenene synthase. Determination of the distribution of label in the derived pentalenenes showed stereospecific loss of the original H-8*si* proton. Analysis of the plausible conformation of the presumed reaction intermediates revealed that the stereochemical course of the latter reaction cannot properly be described as either syn or anti, since cyclization and subsequent double bond formation require significant internal motions to allow proper overlap of the scissile C–H bond with the developing carbocation.

INTRODUCTION

Cyclic sesquiterpenes are widely distributed plant and microbial metabolites. Although so far some 200 different sesquiterpene carbon skeletons have been identified, it is believed that all of these are derived from a single acyclic precursor, farnesyl pyrophosphate (FPP) (**1**) by variations of a common cyclization mechanism (Ruzicka *et al.* 1953; Ruzicka 1959; Ruzicka 1963). According to the currently accepted picture, ionization of the allylic pyrophosphate ester is followed by electrophilic attack of the resulting allylic cation on one of the remaining two double bonds of the substrate (figure 1). The resulting cationic intermediates can undergo further electrophilic reactions, including chemically well-precedented rearrangements, with eventual quenching of the positive charge by deprotonation or capture of an external nucleophile, such as water. Support for these schemes has come not only from classical biosynthetic precursor-product experiments with intact organisms (Cane 1981; Croteau 1981), but from detailed studies of a small group of sesquiterpene synthases and related monoterpene cyclases (Cane 1990; Croteau 1987).

One of the most fundamental bond-forming motifs in the biosynthesis of cyclic terpenes is the generation of a new carbon–carbon bond by the addition of a carbenium ion to a double bond, followed by loss of one of the original allylic protons with formation of a new double bond (Cane 1980) (figure 2). In principle, this transformation can occur with net suprafacial or antarafacial stereochemistry, depending on whether the allylic C–H bond undergoing cleavage is syn or anti to the face of the double bond undergoing electrophilic attack. The prototype of this electrophilic allylic addition–elimination sequence, formally an $S_{E'}$ transformation, is the terpenoid chain-elongation reaction carried out by the family enzymes known as prenyl transferases (Poulter & Rilling 1981) (figure 3). For example, farnesyl pyrophosphate synthase catalyses the condensation of dimethylallyl pyrophosphate (DMAPP) (**2**) with two equivalents of isopentenyl pyrophosphate (IPP) (**3**). Ionization of DMAPP yields an allylic cation which forms a new C–C bond by electrophilic attack on C-4 of the co-substrate IPP. Subsequent loss of one of the C-2 protons generates the intermediate allylic pyrophosphate, geranyl pyrophosphate (GPP) (**4**), which itself undergoes an

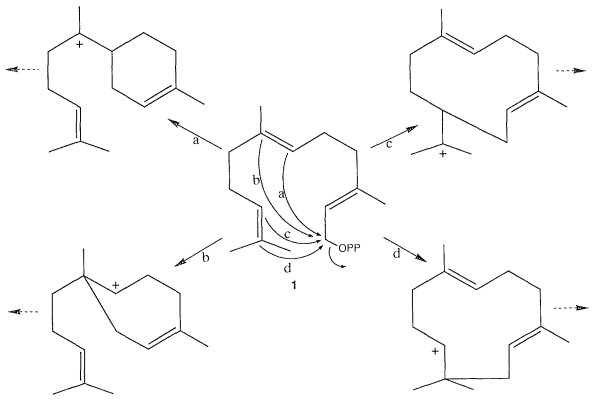

Figure 1. Cyclization of farnesyl pyrophosphate to cyclic sesquiterpenes.

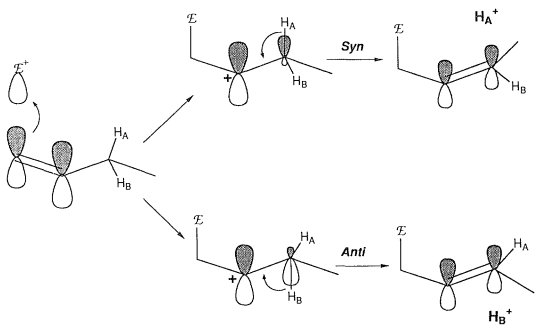

Figure 2. Syn and anti electrophilic allylic addition–elimination ($S_{E'}$) reactions.

analogous condensation–elimination with IPP to yield farnesyl pyrophosphate (**l**). Analogous chain-elongation reactions account for the formation of polyisoprenoids ranging in length from four to several thousand isoprenoid units.

The stereochemical course of the farnesyl pyrophosphate synthase reaction was established nearly 25 years ago by Cornforth & Popjak as part of their landmark studies of cholesterol biosynthesis (Donninger & Popjak 1966; Cornforth *et al.* 1966*a*, *b*). In an elegant series of experiments, these investigators showed that displacement of the pyrophosphate moiety from C-1 of both allylic substrates, DMAPP and GPP, takes place with net inversion of configuration (figure 3). They also showed that electrophilic attack by each allylic cation takes place exclusively on the *re*-face of the IPP double bond coupled with stereospecific loss of the 2*re* proton (H_D in figure 3), corresponding to net syn or suprafacial stereochemistry for the $S_{E'}$ reaction. In a related series of experiments, it was also found that formation of the *cis* double bonds of the polyisoprenoid rubber involved loss of the 2*si* proton of IPP. These results were interpreted as reflecting an alternative folding of the IPP substrate at the active site of the prenyl transferase with preservation of the overall syn stereochemistry of the allylic addition–elimination reaction. Remarkably, recent studies have shown that the inherent prenyl transferase activity of *Hevea* latex is indistinguishable from farnesyl pyrophosphate synthase in chain length specificity and stereochemical preference (Light *et al.* 1989; Dennis & Light 1989; Dennis *et al.* 1989). Instead, the ability of the rubber prenyl transferase to mediate the formation of polymeric *cis*-isoprenoids is modulated by a low molecular mass protein designated as rubber elongation factor.

The prenyl chain-elongation reaction is the intermolecular counterpart of the intramolecular cyclization reactions catalysed by sesquiterpene and monoterpene synthases. For example, the first step in the enzymic conversion of farnesyl pyrophosphate to aristolochene (**5**) is believed to be an electrophilic attack at C-10 of the distal double bond followed by loss of a proton from the C-12 (*cis*) methyl group of FPP to generate the enzyme-bound intermediate, germacrene A (**6**) (Cane *et al.* 1989*b*; Cane *et al.* 1990*a*) figure 4). Similarly, the last step in the conversion of farnesyl pyrophosphate to β-*trans*-bergamotene (**7**) is thought to be cyclization of the derived bisabolyl cation **8** by attack on the cyclohexene double bond and loss of a proton from the attached methyl group (Cane *et al.* 1989*a*) (figure 5).

For the past few years we have been studying the enzymic cyclization of farnesyl pyrophosphate to the tricyclic sesquiterpene pentalenene (**9**), the parent hydrocarbon of the pentalenolactone family of antibiotics (Cane & Tillman 1983; Cane *et al.* 1984; Cane *et al.* 1990*b*). This intriguing transformation, which is catalysed by a soluble, monomeric enzyme of M_r 43 ± 1.5 kilodaltons (kD) isolated from *Streptomyces*

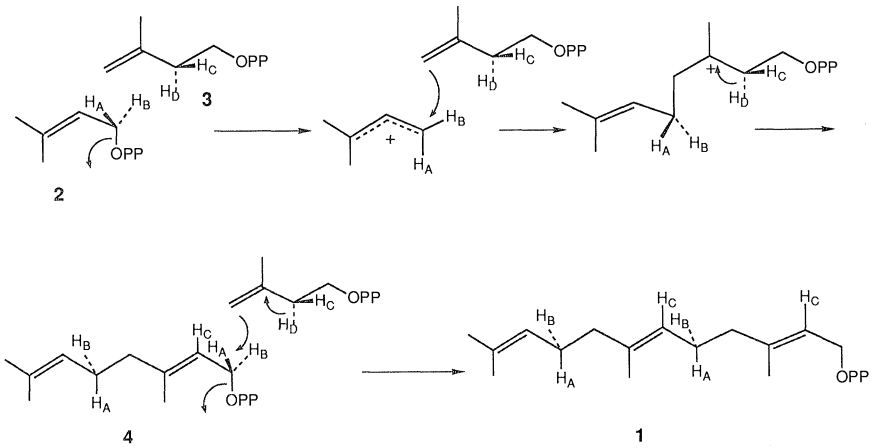

Figure 3. Formation of farnesyl pyrophosphate (**l**) by stepwise condensation of DMAPP (**2**) with IPP (**3**) catalysed by FPP synthase.

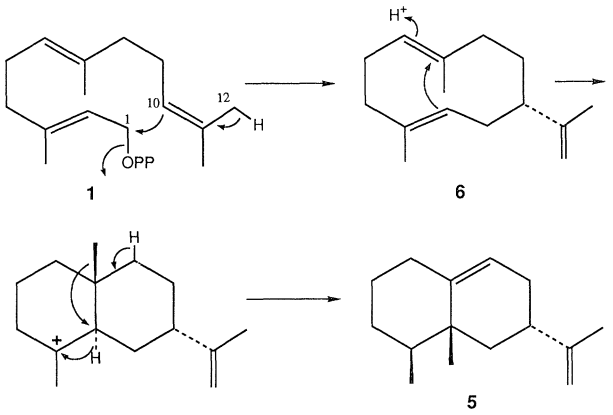

Figure 4. Enzymic conversion of FPP (**1**) to aristolochene (**5**), illustrating $S_{E'}$ reaction in the cyclization of FPP to germacrene A (**6**).

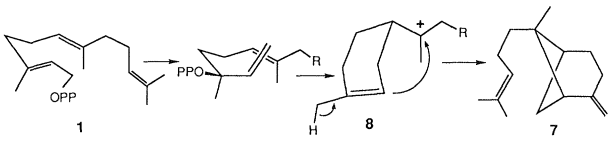

Figure 5. Enzymic conversion of FPP (**1**) to β-*trans*-bergamotene (**7**) involving $S_{E'}$ cyclization of bisabolyl cation **8** to **7**.

UC5319, involves two formal $S_{E'}$ reactions (figure 6). In the first, ionization of FPP and electrophilic attack of the resulting cation on C-11 of the distal double bond is thought to generate the humulyl cation **10** which undergoes deprotonation to form the intermediate humulene (**11**). Following reprotonation and further cyclization, the derived protoilludyl cation **12** can undergo a hydride shift, setting the stage for a second $S_{E'}$ reaction. In the course of the latter transformation, transannular attack on the remaining *trans* double bond in **13** and subsequent deprotonation yields pentalenene.

Incubation of [9-^{3}H]FPP (**1**, H_A=H_B=T) with pentalenene synthase and determination of the distribution of isotopic label in the derived pentalenene had shown that the product retained the bulk of the original tritium, with half at the expected position C-

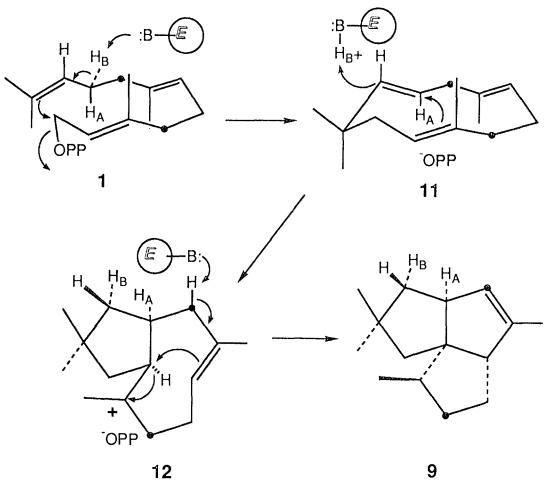

Figure 7. Enzymic cyclization of (9*R*)- and (9*S*)-[9-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=T, H_B=H; and **1**, H_A=H, H_B=T) to pentalenene (**9**).

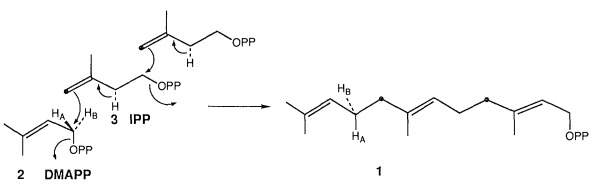

Figure 8. Enzymic synthesis of (9*R*)- and (9*S*)-[9-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=T, H_B=H; and **1**, H_A=H, H_B=T) from (1*R*)- and (1*S*)-[1-^{3}H]DMAPP (**2**, H_A=T, H_B=H; and **2**, H_A=H, H_B=T) and [4-^{14}C]IPP (**3**).

8, the remainder having been transferred to C-1 (Cane *et al.* 1984) (figure 7). Although formation of humulene requires loss of one of the original C-9 protons of FPP, this same proton is returned in the subsequent deprotonation step, without apparent exchange with the medium. To determine the stereochemical course of the allylic addition–elimination reaction, we required samples of FPP stereospecifically tritiated at C-9. The individual samples of (9*R*)- and (9*S*)-[9-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=T, H_B=H; and **1**, H_A=H, H_B=T) could be prepared by using avian prenyl transferase to catalyse the condensation of (1*R*)- and (1*S*)-[1-^{3}H]DMAPP (**2**, H_A=T, H_B=H; and **2**,

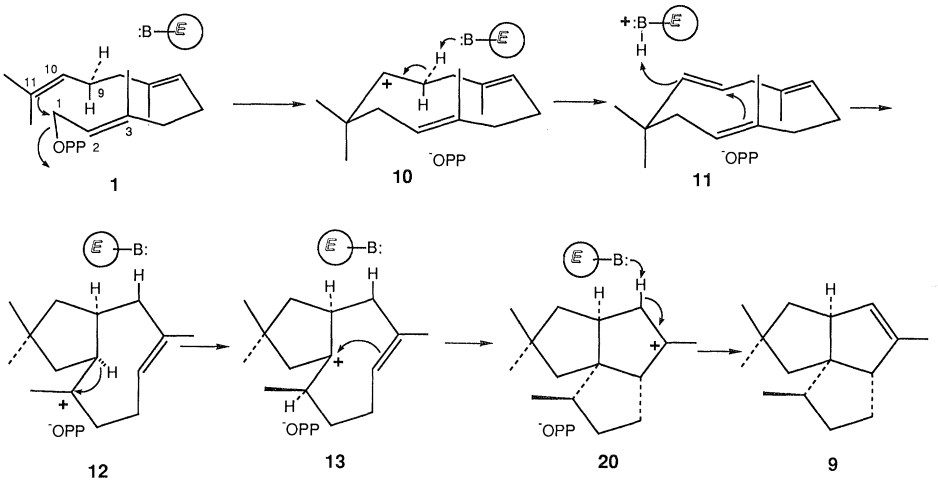

Figure 6. Enzymic cyclization of FPP (**1**) to pentalenene (**9**). The conversion of **1** to humulene (**11**).

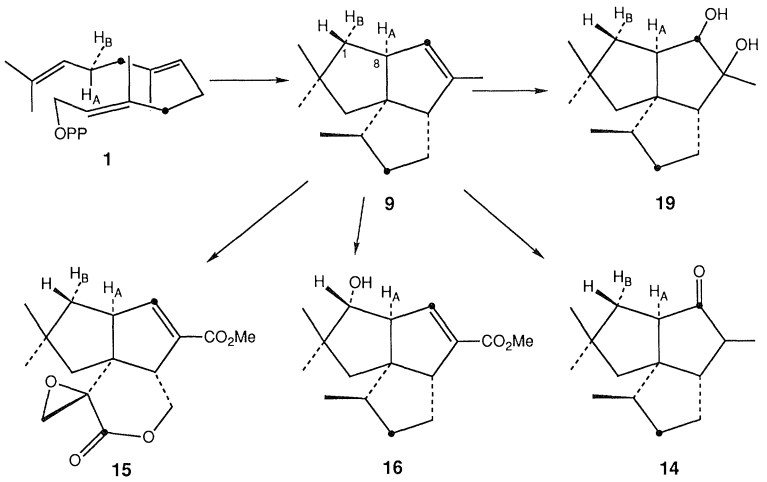

Figure 9. Chemical and microbial degradation of pentalenene (**9**) derived from (9*R*)- and (9*S*)-[9-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=T, H_B=H; and **1**, H_A=H, H^B=T).

H_A=H, H_B=T), respectively, with [4-^{14}C]IPP (**3**) (figure 8). Incubation of the resulting stereospecifically labelled FPP samples with pentalenene synthase gave labelled pentalenene, which was analysed by a combination of chemical and microbial degradation methods (Cane *et al.* 1990*b*) (figure 9). Thus treatment of pentalenene derived from (9*R*)-[9-^{3}H, 4,8-^{14}C]FPP with diborane followed by oxidation with PCC gave the ketone **14** (H_A=T, H_B=H) which lost all tritium upon base catalysed exchange. In a complementary series of experiments, the tritium corresponding to H-9*si* of FPP was located at C-1 of pentalenene by refeeding labelled pentalenene derived from (9*S*)-[9-^{3}H, 4,8-^{14}C]FPP to growing cultures of *Streptomyces* UC5319. Whereas the ^{3}H:^{14}C ratio of the resulting epipentalenolactone F methyl ester (**15**, H_A=H, H_B=T) was unchanged with respect to the original FPP and derived pentalenene, the corresponding sample of pentalenic acid methyl ester (**16**) was devoid of tritium. Since earlier experiments involving [^{13}C]-labelled substrates had already established that the initial electrophilic attack takes place on the si face of the 10,11-double bond of FPP, the allylic addition–elimination must take place with net anti-stereochemistry, in contrast to the demonstrated syn stereochemistry of the prototype prenyl transferase reaction. The observed stereochemistry of the pentalenene synthase-catalysed reaction suggests that the folding of the substrate prevents access of the enzymic base to the H-9*re* proton (figure 7).

In a related series of experiments we have also determined the stereochemical course of the reprotonation reaction in which the original H-9*si* proton of FPP undergoes net intramolecular transfer of H-1 of pentalenene (Cane *et al.* 1990*b*) (figure 10). Thus incubation of [10-^{2}H, 11-^{13}C]FPP with pentalenene synthase and analysis of the derived labelled pentalenene by a combination of ^{13}C and ^{2}H nuclear magnetic resonance (NMR) spectroscopy established that the deuterium atom from C-10 of FPP occupied exclusively the H-1*si* (H-1β) position in **9**, indicating that protonation occurred on the 10 *re* face of the C-9,10 double bond of the intermediate humulene.

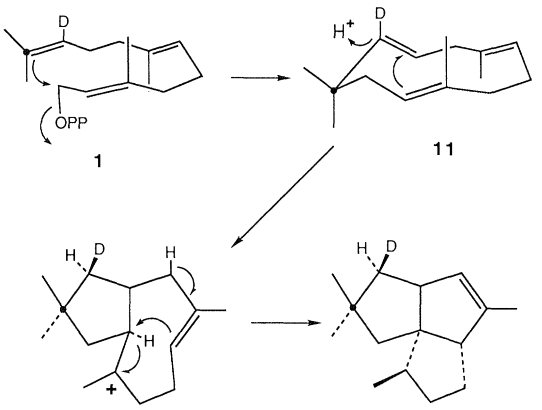

Figure 10. Cyclization of [10-^{2}H, 11-^{13}C]FPP (**1**) to pentalenene (**9**).

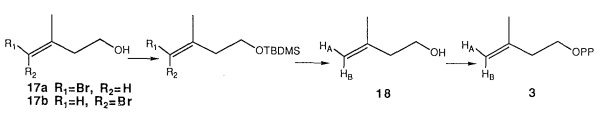

Figure 11. Preparation of (*E*)- and (*X*)-[4-^{3}H]isopentenyl pyrophosphate (**3**, H_A=T, H_B=H; and **3**, H_A=H, H_B=T) from (*E*)- and (*X*)-bromoisopentenols (**17a** and **17b**).

We have earlier shown that the formation of pentalenene involves loss of one of the two hydrogen atoms originally attached to C-8 of farnesyl pyrophosphate, presumably in the final step of the overall cyclization reaction (Cane & Tillman 1983). To establish the stereochemical course of this second $S_{E'}$ process, we turned once again to the prenyl transferase reaction as a means of preparing the requisite samples of FPP, in this case stereospecifically labelled with tritium at C-8. To this end, (*E*)- and (*Z*)-bromoisopentenols (**17a** and **17b**), prepared by literature methods (Cornforth *et al.* 1966*b*), were separately protected as the *t*-butyldimethylsilyl (TBDMS) ethers and metallated by treatment with *t*-butyllithium (Ito *et al.* 1987; Cane *et al.* 1990*a*) (figure 11). The resulting vinyllithium intermediates were each quenched with

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

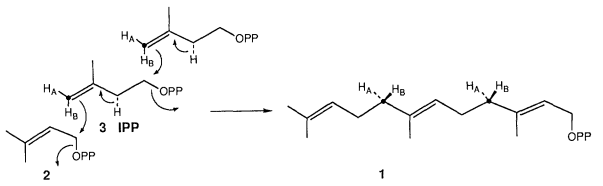

Figure 12. Enzymic synthesis of (4*S*, 8*S*)-[4,8-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=T, H_B=H) and (4*R*, 8*R*)-[4,8-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=H, H_B=T) from stereospecifically tritiated IPP (**3**).

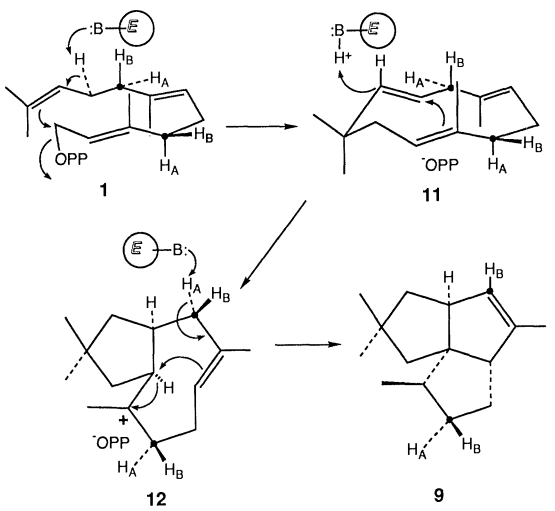

Figure 13. Cyclization of (4*S*, 8*S*)-[4,8-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=T, H_B=H) and (4*R*, 8*R*)-[4,8-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=H, H_B=T) to pentalenene (**9**).

tritiated trifluoroacetic and deprotected to yield the individual (*E*)- and (*Z*)-[4-^{3}H]isopentenols (**18**, H_A=T, H_B=H; and **18**, H_A=H, H_B=T). NMR analysis of the corresponding deuterated isopentenols confirmed the essentially complete stereospecificity of the isotopic labelling procedure. Tosylation and displacement with tris(tetrabutylammonium) pyrophosphate gave (*E*)- and (*Z*)-[4-^{3}H]isopentenyl pyrophosphate (**3**, H_A=T, H_B=H; and **3**, H_A=H, H_B=T), respectively (Davisson *et al.* 1986). The individual tritiated IPP samples, mixed with [4-^{14}C]IPP were then incubated with DMAPP and avian prenyl transferase to generate the desired samples of (4*S*, 8*S*)-[4,8-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=T, H_B=H) and (4*R*, 8*R*)-[4,8-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=H, H_B=T) (figure 12). Incubation of (4*S*, 8*S*)-[4,8-^{3}H, 4,8-^{14}C]FPP with pentalenene synthase gave pentalenene (**9**, H_A=T, H_B=H), which had lost half of the original tritium, based on comparison of the ^{3}H:^{14}C ratio of the derived crystalline diols **19** with the corresponding isotope ratio of the diphenylurethane derivative of the original farnesol (figure 14). Hydroboration–oxidation of **9** in the manner described above gave the derived ketone **14** (H_A=T, H_B=H) without further loss of tritium. In the complementary series of experiments, cyclization of (4*R*, 8*R*)-[4,8-^{3}H, 4,8-^{14}C]FPP gave pentalenene of unchanged ^{3}H:^{14}C ratio, which upon conversion to the ketone **14** (H_A=H, H_B=T) lost the expected one equivalent of tritium. These results establish that the second allylic addition–elimination reaction catalysed by pentalenene synthase involves exclusive loss of the H-8*si* proton of farnesyl pyrophosphate.

Intriguingly, the latter $S_{E'}$ transformation involves neither simple syn nor anti stereochemistry, but rather initial electrophilic attack *orthogonal* to the C–H bond to be broken (figure 15). Based on the absolute configuration of the eventually formed pentalenene, it is clear that cyclization of the bicyclic protoilludane intermediate **13** involves electrophilic attack on the *si* (inner) face of the *trans*-6,7-double bond. Considering the presumed conformation of the latter intermediate, it is evident that the p-orbital at C-7 is essentially staggered with respect to the adjacent pair of allylic protons. As the new C–C bond is formed, the attached methyl group at the now positively charged C-7 carbon atom must rotate outwards. Remarkably, although a rotation of approximately only 30° would suffice for proper overlap of the vacant p-orbital with the adjacent β-proton (originally H-8*re* of FPP), the demonstrated loss of H-8*si* of FPP implies a rotation of some 90°. Interestingly, the latter rotation brings the lobe of the p-orbital originally on the *si* face of the 6,7-double bond (i.e. syn to the newly formed C–C bond) into overlap with the C–H bond undergoing cleavage. In the latter sense, therefore, the allylic addition–elimination sequence may be considered to take place with net syn stereochemistry, although in the most likely reactive conformation, both protons are originally anti to the attacking electrophile!

Taken together, the above results show that there is no stereochemical imperative governing biochemical $S_{E'}$ reactions. The overall stereochemistry of such electrophilic allylic addition–elimination reactions is most likely the result of the relative arrangement at the active site of the electrophile and the base which eventually quenches the positive charge. For example, IPP isomerase catalyses the prototropic rearrangement of IPP to DMAPP (figure 16). In contrast to the prenyl transferase reaction, protonation at C-4 and loss of the H-2*re* (H_A) proton of IPP have been shown to take place with net anti stereochemistry (Poulter & Rilling 1981). Interestingly, in recent experiments with cloned IPP isomerase, Poulter has reported that equilibration of IPP and DMAPP in deuterated buffer results in slow exchange not only of H-2*si* (H_B) but of all six allylic methyl protons as well (Street *et al.* 1990). It is proposed that the overall stereochemical course of the allylic addition–elimination reaction is invariant, but that the observed scrambling results from binding of different conformations of the substrate at the active site of the isomerase. Significantly, this relaxation in binding specificity would have no observable consequence on product structure or geometry, and would be unrecognizable in the absence of isotopic labelling. Poulter, based on an earlier suggestion of Popjak, has also proposed that, for the prenyl transferase-catalysed condensation reaction, the base is in fact the inorganic pyrophosphate counter ion which is released in the initial ionization step, thereby accounting for the net syn stereochemistry of isoprenoid chain elongation (Poulter & Rilling 1978). Indeed, consistent with this hypothesis, Poulter has shown that incubation of

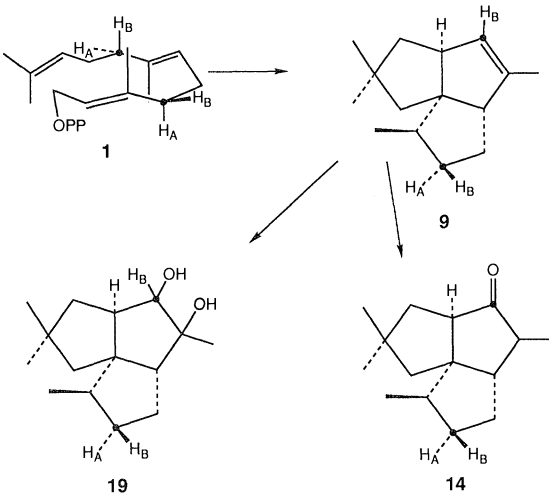

Figure 14. Determination of the distribution of tritium in pentalenene (**9**) derived from (4*S*,8*S*)-[4,8-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=T, H_B=H) and (4*R*,8*R*)-[4,8-^{3}H, 4,8-^{14}C]FPP (**1**, H_A=H, H_B=T).

suitably designed bisubstrate analogs with FPP synthase results in formation of cyclic products (Davisson *et al.* 1985).

For the pentalenene synthase reaction, we have proposed that a single base is responsible for the sequential deprotonation–reprotonation–deprotonation events taking place at C-9,-10, and -8 of the farnesyl pyrophosphate substrate (figure 13). The above-described elucidation of the stereochemical course of the individual proton transfer processes is consistent with, but does not require, the action of a single enzymic base. For example, in removal of H-9α from the pentalenyl cation **20**, the C–H bond being broken is roughly parallel to the C-1–H-1α bond which has been generated in the earlier reprotonation step. Identification of the actual active site base should become possible once cloned pentalenene synthase is available.

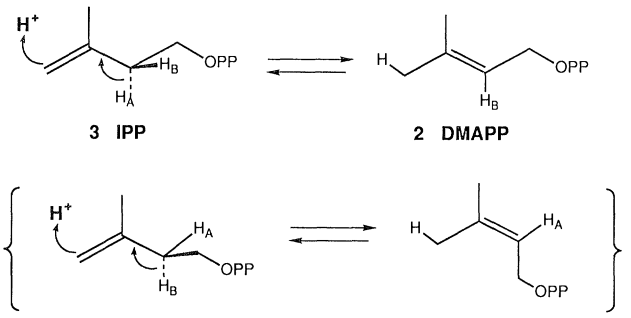

Figure 16. Isomerase catalysed interconversion of IPP and DMAPP by allylic proton addition–elimination with net anti-stereochemistry. The minor reaction leading to the same products but by anomalous binding of substrate is also shown.

This work was supported by a grant no. GM22172 from the U.S. National Institutes of Health.

REFERENCES

Cane, D. E. 1980 The stereochemistry of allylic pyrophosphate metabolism. *Tetrahedron* **36**, 1109.

Cane, D. E. 1981 The biosynthesis of sesquiterpenes. In *Biosynthesis of isoprenoid compounds* (ed. J. W. Porter & S. L. Spurgeon), vol. 1, pp. 283–374. New York: J. W. Wiley & Sons.

Cane, D. E. 1990 The enzymatic formation of sesquiterpenes. *Chem. Rev.* **9**, 1089.

Cane, D. E. & Tillman, A. M. 1983 Pentalenene biosynthesis and the enzymatic cyclization of farnesyl pyrophosphate. *J. Am. chem. Soc.* **105**, 122.

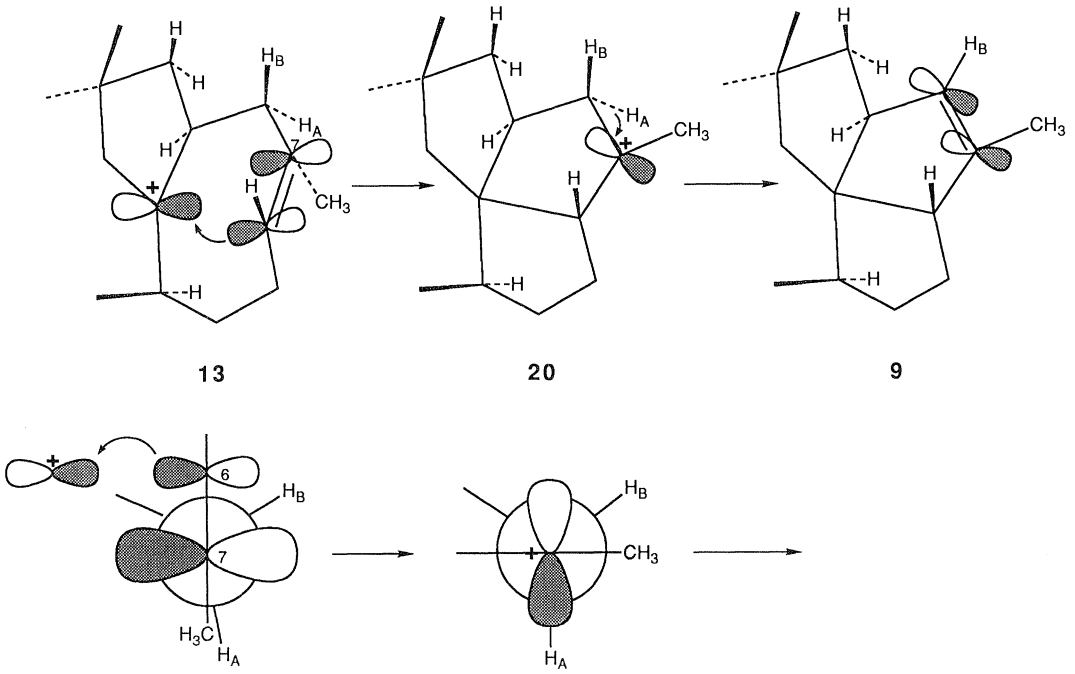

Figure 15. Stereochemical course of conversion of bicyclic cation **13** to pentalenene illustrating orthogonal relation between the direction of the initial electrophilic attack on the 6,7-double bond and the eventually broken C–H_A bond. The two Newman projections are along the 7,8 bond of **13** and **20**, respectively.

Cane, D. E., Abell, C. & Tillman, A. M. 1984 Pentalenene biosynthesis and the enzymatic cyclization of farnesyl pyrophosphate. Proof that the cyclization is catalysed by a single enzyme. *Bioorg. Chem.* **12**, 312.

Cane, D. E., McIlwaine, D. B. & Harrison, P. H. M. 1989*a* Bergamotene biosynthesis and the enzymatic cyclization of farnesyl pyrophosphate. *J. Am. chem. Soc.* **111**, 1152.

Cane, D. E., Prabhakaran, P. C., Salaski, E. J., Harrison, P. H. M., Noguchi, H. & Rawlings, B. J. 1989*b* Aristolochene biosynthesis and enzymatic cyclization of farnesyl pyrophosphate. *J. Am. chem. Soc.* **111**, 8914.

Cane, D. E., Prabhakaran, P. C., Oliver, J. S. & McIlwaine, D. B. 1990a Aristolochene biosynthesis. Stereochemistry of the deprotonation steps in the enzymatic cyclization of farnesyl pyrophosphate. *J. Am. chem. Soc.* **112**, 3209.

Cane, D. E., Oliver, J. S., Harrison, P. H. M., Abell, C., Hubbard B. R., Kane, C. T. & Lattman, R. 1990*b* The biosynthesis of pentalenene and pentalenolactone. *J. Am. chem. Soc.* **112**, 4513.

Cornforth, J. W., Cornforth, R. H., Donninger, C. & Popjak, G. 1966*a* Studies on the Biosynthesis of Cholesterol. XIX. Steric Course of Hydrogen Eliminations and of C–C Bond Formations in Squalene Biosynthesis. *Proc. R. Soc. Lond.* B **163**, 492.

Cornforth, J. W., Cornforth, R. H., Popjak, G. & Yengoyan, L. 1966*b* Studies on the Biosynthesis of Cholesterol. XX. Steric Course of Decarboxylation of 5-Pyrophosphomevalonate and of the Carbon to Carbon Bond Formation in the Biosynthesis of Farnesyl Pyrophosphate. *J. biol. Chem.* **241**, 3970.

Croteau, R. 1981 Biosynthesis of monoterpenes. In *Biosynthesis of isoprenoid compounds* (ed. J. W. Porter & S. L. Spurgeon), vol. 1, pp. 225–282. New York: Wiley.

Croteau, R. 1987 Biosynthesis and catabolism of monoterpenoids. *Chem. Rev.* **87**, 929.

Davisson, V. J., Neal, T. R. & Poulter, C. D. 1985 Farnesylpyrophosphate synthetase. A case for common electrophilic mechanisms for prenyltransferases and terpene cyclases. *J. Am. chem. Soc.* **107**, 5277.

Davisson, V. J., Woodside, A. B., Neal, T. R., Stremler, K. E., Muehlbacher, M. & Poulter, C. D. 1986 Phosphorylation of isoprenoid alcohols. *J. org. Chem.* **51**, 4768.

Dennis, M. S. & Light, D. R. 1989 Rubber elongation factor from *Hevea brasiliensis*. *J. biol. Chem.* **264**, 18608.

Dennis, M. S., Henzel, W. J., Bell, J., Kohr, W. & Light, D. R. 1989 Amino acid sequence of rubber elongation factor protein associated with rubber particles in *Hevea* latex. *J. biol. Chem.* **264**, 18618.

Donninger, C. & Popjak, G. 1966 Studies on the biosynthesis of cholesterol. XVIII. The stereospecificity of mevaldate reductase and the biosynthesis of asymmetrically labelled farnesyl pyrophosphate. *Proc. R. Soc. Lond.* B **163**, 465.

Ito, M., Kobayashi, M., Koyama, T. & Ogura, K. 1987 Stereochemical analysis of prenyltransferase reactions leading to (*Z*)- and (*E*)-polyprenyl chains. *Biochemistry* **26**, 4745.

Light, D. R., Lazarus, R. A. & Dennis, M. S. 1989 Rubber elongation by farnesyl pyrophosphate synthases involves a novel switch in enzyme stereospecificity. *J. biol. Chem.* **264**, 18598.

Poulter, C. D. & Rilling, H. C. 1978 The Prenyl Transfer reaction. Enzymatic and mechanistic studies of the 1′-4 coupling reaction in the terpene biosynthetic pathway. *Acc. chem. Res.* **11**, 307.

Poulter, C. D. & Rilling, H. C. 1981 Prenyl transferases and isomerase. In *Biosynthesis of isoprenoid compounds* (ed. J. W. Porter & S. L. Spurgeon), vol. 1, pp. 161–224. New York: Wiley.

Ruzicka, L., Eschenmoser, A. & Heusser, H. 1953 The isoprene rule and the biogenesis of terpenic compounds. *Experientia* **9**, 357.

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

Ruzicka, L. 1963 Perspektiven der biogenese und der chemie der terpene. *Pure appl. Chem.* **6**, 493.

Street, I. P., Christensen, D. J. & Poulter, C. D. 1990 Hydrogen exchange during the enzyme-catalysed isomerization of isopentenyl diphosphate and dimethylallyl diphosphate. *J. Am. chem. Soc.* **112**, 8577.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES