of many of this type, is shown in Figure 18. The reactions can also lead to completely stereospecific introduction of deuterium into "allylic" and other positions (Birch and Thompson, 1973). This could be useful for introducing labeling which is stereospecifically needed in a terpene derivative.

To close on a completely terpenoid note, α-phellandrene, which is the first natural substance I ever examined (Birch, 1937), produces the two stereosiomeric complexes, which are shown in Figure 19 (Birch and Thompson, 1973), without racemization. The action of acid, as in all such cases, results in equilibration to the thermodynamically more stable isomer, in this instance with a 2-isopropyl group. Removal of iron by the use of cupric chloride (Birch and Chauncy, 1973) regenerates α-phellandrene or leads to the new diene shown from the appropriate complex.

Since only cisoid dienes can complex with $Fe(CO)_3$, and since double bond migrations occur during complexing, the process offers opportunities to obtain finally thermodynamically less stable from the more stable transoid diene structures.

LITERATURE CITED

Alder, K., Rickert, H. F., *Ber.* **70,** 1354 (1937).
Birch, A. J., *J. Proc. Roy. Soc. N. S. W.* **71,** 54 (1937).
Birch, A. J., *J. Chem. Soc.* 809 (1945).
Birch, A. J., *Discuss. Faraday Soc.* **2,** 246 (1947).
Birch, A. J., *J. Chem. Soc.* 367 (1950a).
Birch, A. J., *J. Chem. Soc.* 1552 (1950b).
Birch, A. J., *J. Chem. Soc.* 2325 (1950c).
Birch, A. J., Brown, J. M., Stansfield, F., *J. Chem. Soc.* 5343 (1964a).
Birch, A. J., Brown, J. M., Subba Rao, G., *J. Chem. Soc.* 3309 (1964b).
Birch, A. J., Butler, D. N., Siddall, J. B., *J. Chem. Soc.* 2932 (1964c).
Birch, A. J., Butler, D. N., Siddall, J. B., *J. Chem. Soc.* 2941 (1964d).
Birch, A. J., Chauncy, B., unpublished work, 1973.
Birch, A. J., Cross, P. E., Lewis, J., White, D. A., Wild, S. B., *J. Chem. Soc. A* 332 (1968).
Birch, A. J., Dastur, K. P., *J. Chem. Soc.* in press (1973a).
Birch, A. J., Dastur, K. P., unpublished work, 1973b.
Birch, A. J., Diekman, J., Dastur, K. P., unpublished work, 1973.
Birch, A. J., Graves, J. M. H., Subba Rao, G., *J. Chem. Soc.* 5137 (1965).
Birch, A. J., Haas, M., *J. Chem. Soc. C* 2465 (1971).
Birch, A. J., Hill, J. S., *J. Chem. Soc. C* 419 (1966a).
Birch, A. J., Hill, J. S., *J. Chem. Soc. C* 2323 (1966b).
Birch, A. J., Hill, J. S., *J. Chem. Soc. C* 125 (1967).
Birch, A. J., Hutchinson, E. G., *J. Chem. Soc. C* 3671 (1971).
Birch, A. J., Hutchinson, E. G., Subba Rao, G., *J. Chem. Soc. C* 637 (1971).
Birch, A. J., Keeton, R., *J. Chem. Soc. C* 109 (1968).
Birch, A. J., Keeton, R., *Aust. J. Chem.* **24,** 331 (1971).
Birch, A. J., Macdonald, P. L., Powell, V. H., *J. Chem. Soc. C* 1470 (1970).
Birch, A. J., Mukherji, S. M., *J. Chem. Soc.* 2531 (1949).
Birch, A. J., Powell, V. H., *Tetrahedron Lett.* 3467 (1970).
Birch, A. J., Robinson, R., *J. Chem. Soc.* 501 (1943).
Birch, A. J., Smith, H., *J. Chem. Soc.* 1883 (1951).
Birch, A. J., Smith, M., *Proc. Chem. Soc.* 356 (1962).
Birch, A. J., Subba Rao, G., *J. Chem. Soc.* 5139 (1965).
Birch, A. J., Subba Rao, G., *Tetrahedron Suppl.* 7, 391 (1966).
Birch, A. J., Subba Rao, G., *Advan. Org. Chem.* **8,** 1 (1972).
Birch, A. J., Thompson, D., unpublished work, 1973.
Birch, A. J., Wright, J. J., *Aust. J. Chem.* **22,** 2635 (1969).
Catchpole, A. G., Hughes, E. D., Ingold, C. K., *J. Chem. Soc.* 8 (1948).
Kharasch, M., Tawney, P. O., *J. Amer. Chem. Soc.* **63,** 2308 (1941).
Rogers, N. A. J., University of Lancaster, England, personal communication, 1969.

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Model Studies in Terpene Biosynthesis

C. Dale Poulter

The carbonium ion rearrangements which are thought to lead from the cyclopropylcarbinyl pyrophosphates, presqualene and prephytoene pyrophosphate, to head-to-head terpenes are discussed. Ten carbon model compounds were used for solvolysis studies. Hydrolysis of *N*-methyl-4-(chrysanthemyloxy)pyridinium iodide gave artemisia triene, santolina triene, yomogi alcohol, santolina alcohol, artemisia alcohol, chrysanthemol, *trans*-2,7-dimethyl-3,6-octadien-2-ol, 2,7-dimethyl-2,6-octadien-4-ol, and *trans*-2,7-dimethyl-4,5-octadien-2-ol. Hydrolysis of *trans*-2,2-dimethyl-3-(2′-methylpropenyl)cyclobutyl tosylate and 2-[*trans*-2′-(2″-methylpropenyl)cyclopropyl]propan-2-yl *p*-nitrobenzoate gave 2-[*trans*-2′-(2″-methylpropenyl)cyclopropyl]propan-2-ol, *trans*-2,7-dimethyl-3,6-octadien-2-ol, and 2,7-dimethyl-2,6-octadien-4-ol. The properties of the carbonium ion intermediates are discussed in terms of product and stereochemical studies. Biosynthesis of head-to head terpenes is compared to the chemical results and a biosynthetic mechanism is proposed.

It is a pleasure for me to participate in this symposium as a way to express my gratitude to Bill Dauben as a colleague and a teacher. I am sure that all of you know that his scientific contributions span a wide range of chemical problems. Inevitably all of his students are exposed in depth to topics in such seemingly diverse areas as natural products, synthesis, carbonium ions, and photochemistry. This was a valuable experience for which I am particularly grateful.

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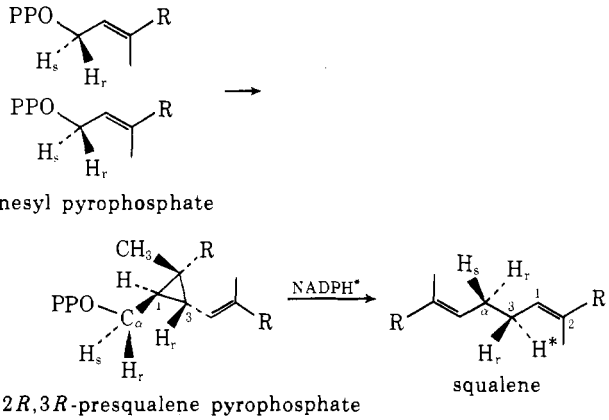
I would like to discuss the biosynthesis of a special class of terpenes—those in which two regular head-to-tail fragments have been joined by a head-to-head coupling. One example of a compound in this family is squalene, a C_{30} intermediate in the biosynthetic pathway to sterols, and another is phytoene, a C_{40} compound which has been implicated in biosynthesis of carotenoids. The symmetry of both molecules suggests that they were formed by joining two identical terpenoid units. These observations have been verified in experiments which clearly indicate that biosyntheses of squalene[1] and phytoene[2] require the coupling of two molecules of farnesyl pyrophosphate and geranylgeranyl pyrophosphate, respectively.

The mechanistic details surrounding these transformations were the subject of considerable speculation through the late sixties.[3] In 1966 Rilling[4] reported that an intermediate, named presqualene pyrophosphate, could be isolated from a squalene-synthesizing yeast preparation when the system was deprived of reduced nicotinamide adenine dinucleotide phosphate (NADPH). In the presence of NADPH, the intermediate was rapidly converted to squalene by the yeast enzyme system. The structure of presqualene pyrophosphate was first suggested by Epstein and Rilling in 1969[5] and was subsequently verified by synthesis,[6a-c] degradation,[6d,e] and spectroscopy.[6e,f] Earlier this year, Popjak[7] established the absolute configuration of the intermediate as 1*R*,2*R*,3*R* (see Scheme I).

Scheme I. Biosynthesis of Squalene from Farnesyl Pyrophosphate

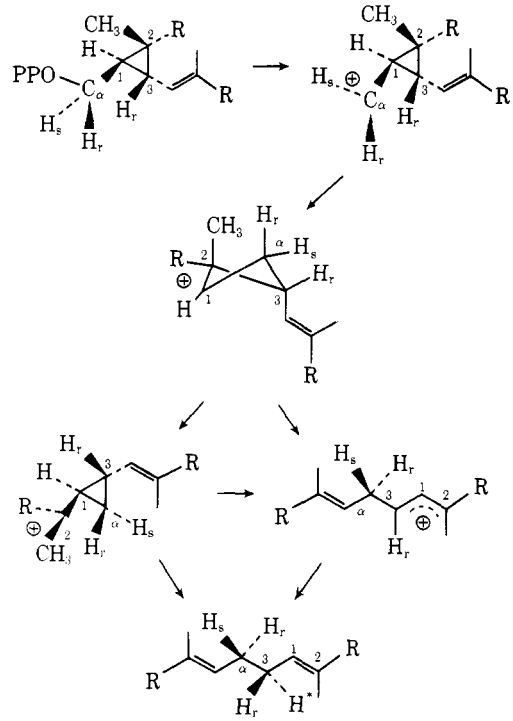


A similar cyclopropylcarbinyl compound, prephytoene pyrophosphate, is converted to the head-to-head terpene phytoene by cell-free preparations of certain photosynthetic bacteria[8] and tomatoes.[9] The absolute configuration of prephytoene pyrophosphate isolated from a tomato enzyme system has also been reported to be 1*R*,2*R*,3*R*.[9] Recently, Porter and coworkers[9,10] claimed that prephytoene pyrophosphate is converted first to lycopersene, a C_{40} analog of squalene, which is subsequently dehydrogenated to give other carotenoids. Regardless of whether phytoene or lycopersene is the carotenoid precursor, the biosynthetic similarity between the C_{30} and C_{40} head-to-head terpenes is obvious. Squalene and lycopersene must gain hydride to form the saturated central carbon–carbon bond, while the intermediate which produces phytoene must lose a proton.

From this point on I will concentrate on the C_{30} system because of the wealth of information concerning biosynthesis of squalene. However, the comments are directly applicable to biosynthesis of C_{40} head-to-head terpenes as well. The rearrangements by which the cyclopropylcarbinyl skeleton of presqualene pyrophosphate is converted to squalene are quite complicated (Scheme I). The net result of the transformation is cleavage of the C_1–C_3 and C_2–C_3 cyclopropane bonds and bonding between C_3 and C_α. In order to accommodate the stereochemical results of Popjak and Cornforth,[1,11] C_α and C_3 are both inverted during the reaction. In this scheme the retention of configuration seen for the carbon of farnesyl pyrophosphate which ultimately accepts a hydrogen from NADPH (C_3) is the result of two inversions—one during biosynthesis of presqualene pyrophosphate and another when hydrogen is transferred from NADPH.

There are logical chemical mechanisms for the rearrangement sequence by means of carbonium ion intermediates[6a,b,d,12] (Scheme II). Certainly the pyrophosphate functionality is an excellent leaving group and the resulting cyclopropylcarbinyl cation is in a class of intermediates which are well known to organic chemists. If one assumes a carbonium ion mechanism, the first step is ionization of the carbon–oxygen bond to give a primary cyclopropylcarbinyl cation. A subsequent 1,2 migration of the C_1–C_3 bond produces an isomeric cyclobutyl cation. At this juncture several possibilities arise. A second 1,2 migration will generate a tertiary cyclopropylcarbinyl cation. Capture of this intermediate at C_3 by hydride from NADPH gives squalene directly. Alternatively, rearrangement of the tertiary cyclopropylcarbinyl cation to its allylic isomer by rupture of the C_1–C_3 cyclopropane bond, followed by hydride capture at C_3, affords squalene. It is also possible that the cyclobutyl cationic intermediate rearranges directly to the allylic cation by cleavage of the C_2–C_3 bond and is then captured by hydride.

Scheme II. Possible Routes to Squalene from Presqualene Pyrophosphate



Since the stereochemistry for biosynthesis of squalene from presqualene pyrophosphate can be inferred from Popjak and Cornforth's work,[1,11] any viable mechanism must be stereochemically compatible. A carbonium ion mechanism fulfills this requirement if one carefully analyzes each step.

The first point of concern is the carbinyl carbon (C_α) whose bonding to other atoms is altered during ionization and the initial 1,2 bond migration. Two properties of cyclopropylcarbinyl cations are important in this regard. The interaction between C_α and the cyclopropane ring is sufficiently large so that rotation about the C_α–C_1 bond should not occur[13] after ionization. Stereoelectronic arguments and ample experimental evidence dictate that the 1,2 migration proceeds to give H_R and H_S in the indicated positions.[14] Thus, the stereochemical results of Popjak and Cornforth demand that ionization occur from the conformer in which the carbon–oxygen bond is *anti* to the C_1–C_3 cyclopropane bond. When discussing this point in an earlier publication,[12a] we reasoned that one might expect a significant kinetic advantage for this conformer *vs.* the one in which the C_1–C_2 cyclopropane bond is anti. The former orientation should permit charge delocalization into the double bond at the transition state for ionization with a consequent rate enhancement.

The stereochemistry at C_2 must be fixed with the methyl group endo to the cyclopropane ring during the second 1,2 bond migration. This prediction follows from the stereochemistry of presqualene pyrophosphate and arguments just presented for C_α, again in accordance with theory and experiment.[14]

Capture of the final carbonium ion intermediate by hydride must also be stereospecific. If the tertiary cyclopropylcarbinyl cation is the immediate precursor of squalene, the final step must proceed with inversion at C_3, again in agreement with expected stereoelectronic bias observed in other cyclopropylcarbinyl systems.[15] Obviously, the allylic cation has no stereochemical preference for capture by hydride. However, there are numerous examples of stereospecific reductions of trigonal carbon atoms which require NADPH, such as reduction of aldehydes to primary alcohols by yeast alcohol dehydrogenase.[16]

Two years ago when we first suggested a detailed chemical basis for biosynthesis of squalene from presqualene pyrophosphate,[6d] most of the available experimental data concerned alkyl-substituted cyclopropylcarbinyl systems. In fact, very little was known about their vinyl-substituted counterparts, chemically or biochemically. As a first step toward understanding this important and often misunderstood biosynthetic transformation, we decided to study the behavior of likely cationic intermediates under standard solvolysis conditions. Of course, the answers obtained in this way relate to "traditional" chemical behavior. Yet, in view of a proliferation of chemically based mechanisms in this area we felt it was absolutely necessary to establish the chemical feasibility of our proposals.

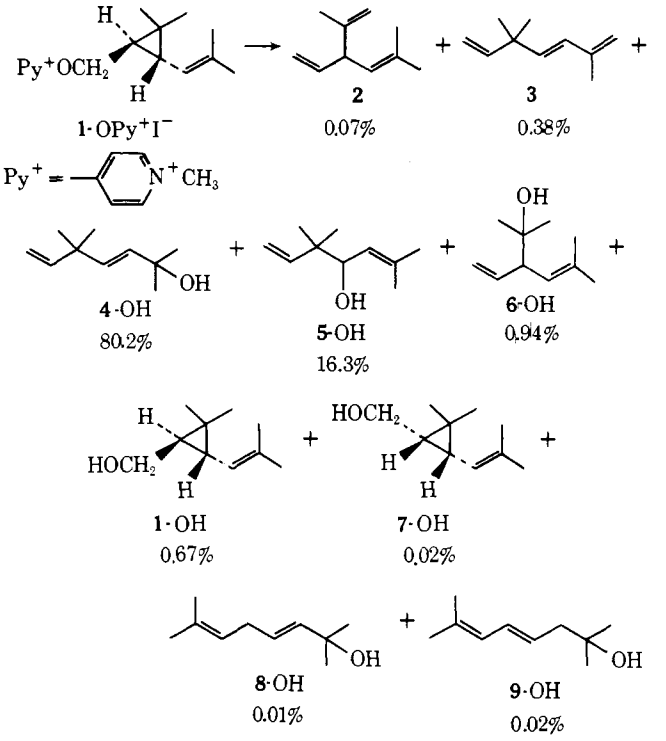
Biosynthesis of terpenes also has another interesting facet. The biological transformations often parallel the chemical transformations of the substrate. The parallelism has been cited often and has, on occasion, been suggested as a major factor in the evolutionary success of terpene biosynthetic pathways.[17] This is certainly a logical hypothesis, especially for transformations in which the substrate undergoes extensive molecular rearrangements. In such instances the steric and stereoelectronic properties of a molecule have a profound influence on its chemistry. However, one should not expect, *a priori*, an exact parallel between chemical and biochemical behavior. Obviously, the environment provided by the active site of an enzyme differs from that in which standard chemical behavior is assessed, and few chemical processes, especially rearrangements of terpenes, give a single product. Still, the chemical properties of a substrate should be important considerations in formulating a biosynthetic mechanism.

Our approach to the chemical aspects of biosynthesis of squalene involved entering the sequence of rearrangements directly from covalent precursors for each skeletal isomer. By a careful examination of the products, we hoped to determine the efficiency of each step in the absence of an enzyme or any constraints which could be construed to simulate enzymic control. We also wanted to determine the stereochemistry of C_α and C_3 during the chemical transformations. Our product studies are now nearing completion, as are stereochemical studies of C_α during ionization and C_3 during capture by a nucleophile.

Ten carbon models were selected for our work in which the homogeranyl substituents (R) were replaced by methyl groups. This substitution does not alter the basic electronic properties of the cyclopropylcarbinyl core. The substitution alleviates considerable synthetic problems and eliminates any side reactions between the positively charged centers and the double bonds in the side chains.

While the monoterpene analog of presqualene alcohol is not known to occur naturally, it can be obtained easily by reduction of the methyl ester of 1*R*,3*R*-chrysanthemic acid. We selected the *N*-methylpyridinium derivative since the leaving group, *N*-methyl-4-pyridone, is neutral and complications arising from internal return are eliminated.[18] Hydrolysis of *N*-methyl-4-(1*R*,3*R*)-chrysanthemyloxy]pyridinium iodide (1-OPy + I^-) gave the products shown in Scheme III. The structures of yomogi alcohol (4-OH) and artemisia alcohol (5-OH) were determined by comparisons of ir, nmr, and mass spectra with those of authentic samples. The structures of the less abundant

Scheme III. Products from Hydrolysis of *N*-Methyl-4-(chrysanthemyloxy)pyridinium Iodide



components were established by glpc–mass spectroscopy and coinjection of authentic samples using two 500-ft open tubular columns. Thus far, we have been able to identify components which are formed in as little as 0.01% yield.

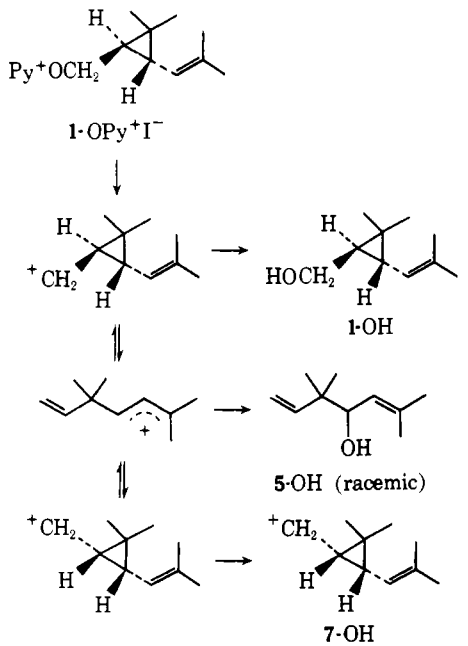
The products can be divided into four skeletal types, three of which have been found to occur naturally. Both santolina triene (2) and santolina alcohol (6-OH) are known monoterpenes as are artemisia triene (3), artemisia alcohol (5-OH), and yomogi alcohol (4-OH). Both *trans*- and *cis*-chrysanthemol (1-OH and 7-OH, respectively) are found among the hydrolysis products of the pure trans isomer. Two head-to-head monoterpenes, *trans*-2,7-dimethyl-3,6-octadien-2-ol (8-OH) and *trans*-2,7-dimethyl-4,6-octadien-2-ol (9-OH), were also identified. The structures of several minor components remain to be conclusively established.

The yields of head-to-head monoterpenes 8-OH and 9-OH from the chrysanthemyl system were disappointingly low. When the solvent was changed to acetic acid, the relative proportion of minor substitution products increased relative to yomogi and artemisia acetates. However, the isomeric head-to-head derivatives were still formed in relatively small amounts.

A likely cause for the lack of extensive rearrangement was identified right away. Hydrolysis at 25° of the pyridinium salt prepared from 1*R*,3*R*-chrysanthemol which was 97% optically pure gave mostly racemic artemisia alcohol. When the experiment was repeated with the corresponding 3,5-dinitrobenzoate ester at 100°, the results were similar. Artemisia alcohol (5-OH) obtained from natural sources has a sizable rotation and we found 96% racemization of configuration at C_3. The loss of stereochemistry indicates that most of the reaction passes through an intermediate which has a plane of symmetry. Certainly, racemization is not due to internal return to a derivative of 5-OH and reionization since 1-OPy$^+$I$^-$ gives a neutral leaving group, which should not be prone to internal return.

If the cyclopropylcarbinyl cation rearranged to an isomeric allylic cation by rupture of the C_1–C_3 cyclopropane bond, artemisia alcohol formed by nucleophilic capture at C_3 must be racemic (Scheme IV). In addition, our results

Scheme IV. Cyclopropylcarbinyl to Allyl Rearrangements during Hydrolysis of *N*-Methyl-4-(1*R*,3*R*)-chrysanthemyloxy]pyridinium Iodide



in water demand that the rearrangement must be extremely rapid or concerted with ionization. However, the rearrangement must also be reversible. Finding some *cis*-chrysanthemol demonstrates that the allylic cation can reclose to a *cis*-cyclopropylcarbinyl isomer. Since the trans cation is certainly more stable than its cis isomer and the allylic ion is common to both, the initial cyclopropylcarbinyl to allyl rearrangement must be reversible.

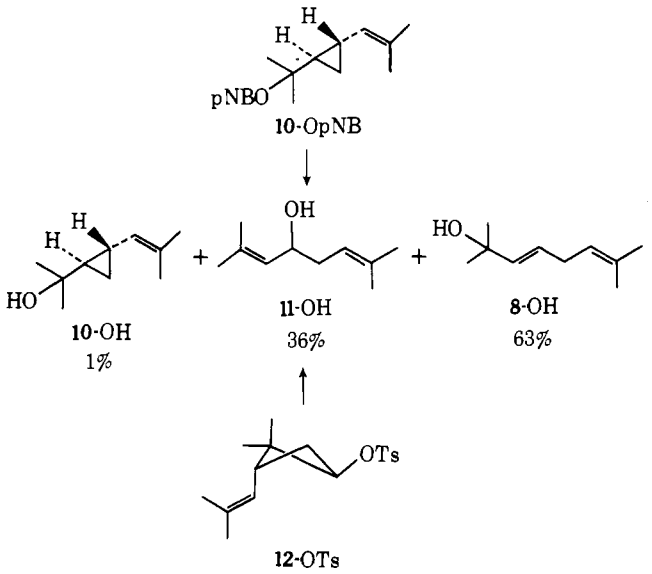
We cannot conclusively establish the position of the equilibrium under our reaction conditions. Yet, the almost complete racemization of artemisia alcohol and the overwhelming predominance of products logically derived from the allylic cation strongly suggest that it is more stable. Obviously the cyclopropylcarbinyl to allyl rearrangement represents a dead-end path as far as products with santolina, chrysanthemyl, or head-to-head skeletons are concerned. An enzyme that promotes head-to-head rearrangement must suppress this rearrangement or block capture of the allylic cation prior to reclosure and rearrangement.

The picture improves considerably when the rearrangement sequence is entered after the initial 1,2 bond migration. Hydrolysis of the C_{10} cyclobutyl tosylate or the tertiary cyclopropylcarbinyl *p*-nitrobenzoate under identical conditions gave identical product mixtures,[19] consisting of 1% tertiary cyclopropylcarbinol (**10-OH**), 36% of a secondary head-to-head alcohol analogous to squalene (**11-OH**), and 63% of a tertiary head-to-head alcohol (**8-OH**) (Scheme V). The first-order rate constants in 80% acetone–water for the tosylate and *p*-nitrobenzoate were 3.27×10^{-4} sec^{-1} and 4.97×10^{-4} sec^{-1}, respectively. Since tosylates are approximately 10^6 times more reactive than *p*-nitrobenzoates, the tertiary cyclopropylcarbinyl cation is much easier to generate than its cyclobutyl isomer, as expected.

In view of the identity of products we thought it likely that the immediate precursor of the substitution products was the same in both cases. Since the tertiary cyclopropylcarbinyl cation is considerably more stable than its cyclobutyl isomer, the likely candidates are reduced to the 3° cation, a head-to-head allylic isomer, or both.

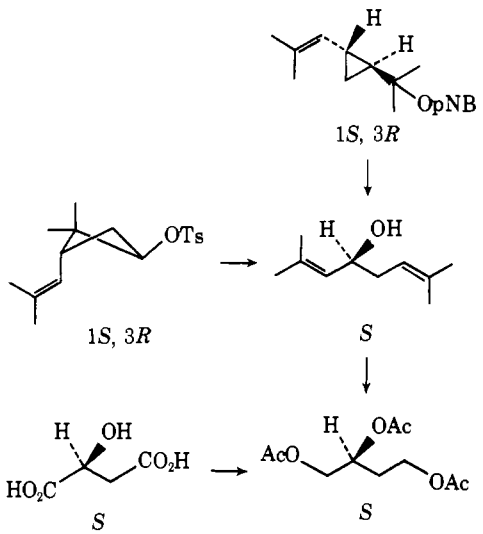
Hydrolysis of 1*S*,1*R*-**10**-OpNB and 1*S*,3*R*-**12**-OTs gave *S*-2,7-dimethyl-2,6-octadien-4-ol with 26% inversion of configuration. The configuration of the secondary alcohol was related to *S*-malic acid of known absolute configuration

Scheme V. Hydrolysis of Cyclobutyl and Tertiary Cyclopropylcarbinyl Models



and optical purity by the sequence of reactions shown in Scheme VI. Unfortunately a rather poor yield of triacetate was obtained from the ozonolysis–reduction–acetylation sequence. While we are certain of the directions of rotation, we feel that the magnitudes are still somewhat questionable and are repeating these experiments in order to confirm our results.

Scheme VI. Hydrolysis of Optically Active Cyclobutyl and Tertiary Cyclopropylcarbinyl Models

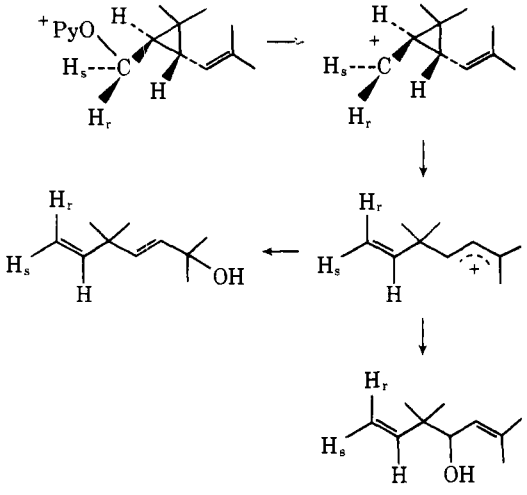


We can now rule out a direct cyclobutyl to allyl isomerization during the head-to-head rearrangement sequence, since a chiral center must be attacked by nucleophile to give the *S* secondary head-to-head alcohol 11-OH. Assuming that 26% inversion is correct, two obvious limiting situations could obtain. If attack at the asymmetric carbon (C_3 of the tertiary cyclopropylcarbinyl cation) is a stereospecific inversion, 74% racemization (presumably by a cyclopropylcarbinyl to allyl equilibration) precedes attack by nucleophile. Alternatively, attack at the asymmetric carbon could be stereoselective with 63% inversion and 37% retention of configuration. The former choice is more satisfying for two reasons. A reversible isomerization between primary cyclopropylcarbinyl and allyl cations has already been demonstrated in the chrysanthemyl system. Also, attack with retention of configuration implies front-side attack on a nonclassical structure. In this regard, we recently found the homoallylic alcohol obtained by hy-

drolysis of optically active *trans*-2-methylcyclopropylcarbinyl mesylate to be completely inverted.[20]

Now I would like to turn to another stereochemical problem, the stereochemistry of C_α of presqualene pyrophosphate during ionization. I alluded earlier to the two limiting orientations at C_α when carbon–oxygen bond rupture is underway and the large barrier to rotation about the C_α–C_1 bond once ionization is complete. Rapid rearrangement of the chrysanthemyl cation to its artemisyl isomer further locks the orientation of the substituents on C_α since it is now part of a carbon–carbon double bond. If one follows the pro-*R* and pro-*S* protons at C_α through the rearrangement sequence shown in Scheme VII, it is immediately evident that ionization of the indicated conformer will give yomogi and artemisia alcohols with the pro-*R* proton trans and pro-*S* proton cis to the vicinal hydrogen attached to C_1. Therefore, the preferred orientation for carbon–oxygen bond cleavage can be deduced by replacing the pro-*S* hydrogen with deuterium in 1*R*,3*R*-chrysanthemol.

Scheme VII. Hydrolysis of *N*-Methyl-4-[(αS,1R,3R)-chrysanthemyloxy]pyridinium Iodide-d_1



When the hydrolysis was performed with the appropriately labeled pyridinium iodide, we obtained the results for yomogi alcohol shown in Figure 1.[21] Similar spectra were found for artemisia alcohol. The high field portion of the olefinic region, part C, clearly shows one-half of two AB quartets of unequal intensity (85 and 15%). The more intense doublet has a coupling constant of 17.4 Hz and the less intense doublet has a 10.6 Hz coupling, indicative of trans and cis vicinal olefinic couplings, respectively. The low field portion of the olefinic region was undecipherable, part A, until a deuterium-decoupled spectrum was obtained as shown in part B. At that point the low field portions of both AB quartets were easily identified from intensity and coupling patterns. The small peak in the center of the low field doublets is due to a small amount of dideuterated chrysanthemol which was carried through our synthetic sequence.

It is clear that the major quartet corresponds to a structure in which the protons are trans on the monosubstituted double bond, with the minor quartet arising from the two protons in a cis orientation. After correcting for 5% of the αS,1S,3S diastereomer in the starting pyridinium iodide and tracing the stereochemistry through Scheme VII, it is evident that the conformer shown is more reactive, as we originally predicted.

We are definitely seeing an electronic effect of the vinyl substituent at C_3. When the double bond is reduced catalytically, the stereoselectivity for the ionization step drops from a ratio of 9:1 to only 1.4:1. Simple reduction of the double bond should not alter the steric environment of the leaving group.

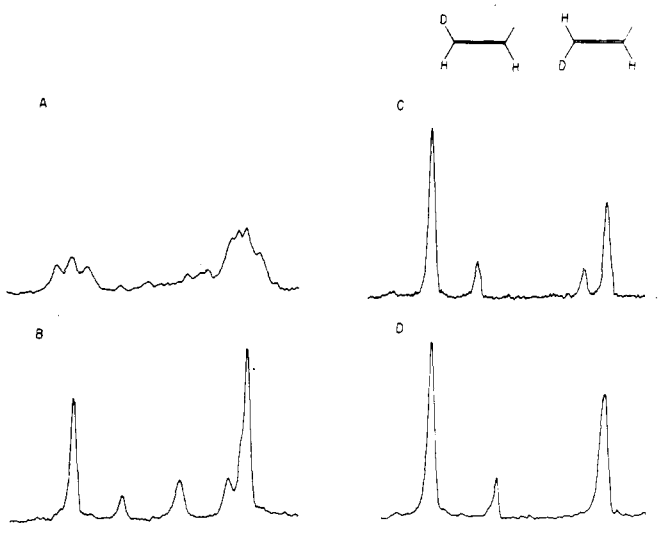
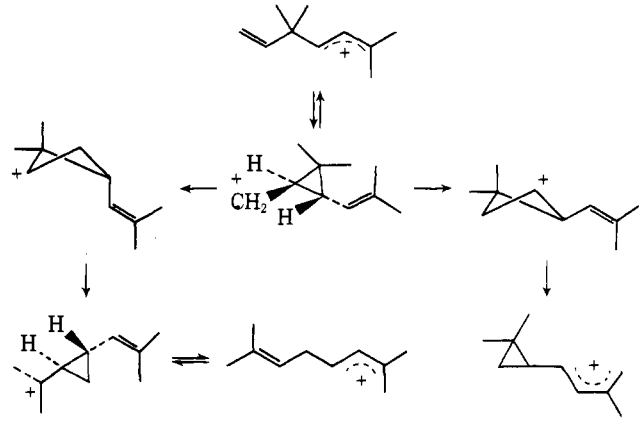


Figure 1. Nmr spectrum of vinyl group in yomogi alcohol-d_1. (A) ^{1}H, 100 MHz, low field; (B) ^{1}H, 100 MHz, low field, ^{2}H decoupled; (C) ^{1}H, 60 MHz, high field; (D) ^{1}H, 100 MHz, high field.

At this point I would like to summarize the chemistry of our model for the presqualene system as shown in Scheme VIII. The initial ionization is highly stereoselective in accord with the biosynthetic transformation. However, prior to the initial 1,2 bond migration, a chemical disaster strikes. We see some head-to-head products but the rearrangement is inefficient. The major competitive pathway is rupture of the C_1–C_3 cyclopropane bond, which produces the allylic isomer of chrysanthemyl cation. As previously mentioned, the allylic cation must revert to the chrysanthemyl system prior to any further "productive" rearrangement. A portion of the chrysanthemyl cations are trapped by nucleophile, another pesky side reaction, before 1,2 bond migration occurs.

Scheme VIII. Rearrangements of the Chrysanthemyl Cation



Also, I have been talking about a 1,2 migration of the C_1–C_3 cyclopropane bond. What if the C_1–C_2 bond migrates? *trans*-2,7-Dimethyl-4,6-octadien-2-ol is the logical end result of this migration. The resulting isomeric cyclobutyl cation should rearrange to a very stable cyclopropyl-substituted allylic cation. We have independently demonstrated that the tertiary diene alcohol 9-OH results when water attacks this cation at the disubstituted cyclopropyl carbon atom. It is interesting to note that the alcohol thus produced also has a head-to-head carbon skeleton. However, labeling studies have definitely ruled out this pathway during biosynthesis of squalene. Each of the previously mentioned "side reactions" occurs more readily than the desired migration of the C_1–C_3 cyclopropane bond.

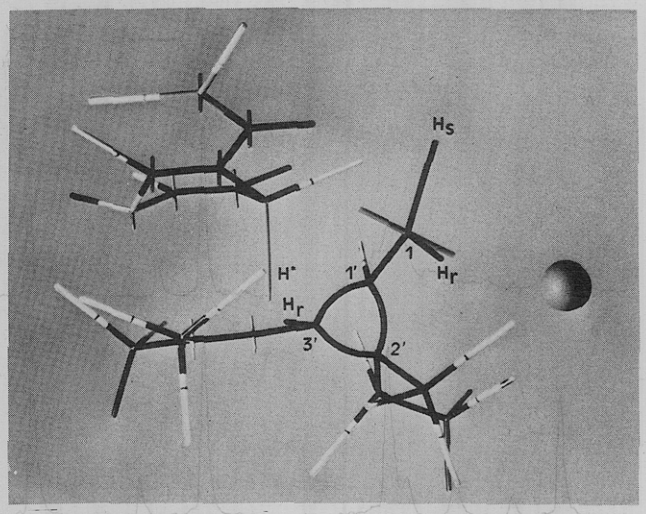


Figure 2. Presqualene cation.

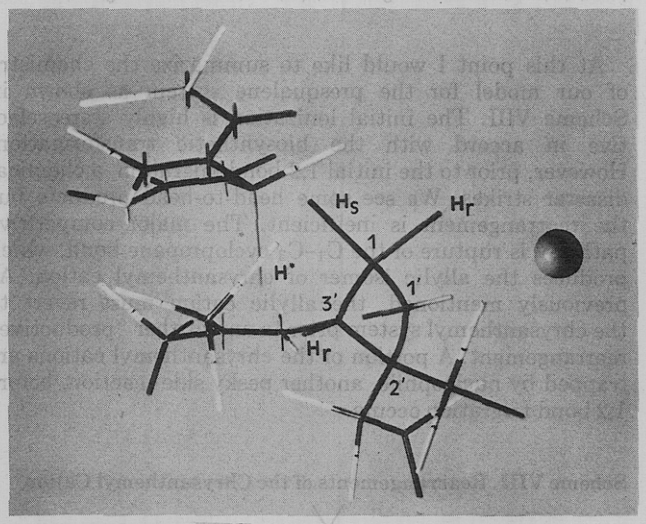


Figure 3. Cyclobutyl cation.

After the initial 1,2 migration, the system gathers itself together. The cyclobutyl cation rapidly rearranges to the expected tertiary cyclopropylcarbinyl cation. The final step, capture of the tertiary cation by a nucleophile, competes with equilibration to an isomeric cyclopropylcarbinyl cation, and the final capture proceeds with inversion of configuration. In summary of all of the aspects thus far examined, only the initial 1,2 bond migration cannot be simulated efficiently without an enzyme.

How could an enzyme avert the chemical disaster which faces the primary cyclopropylcarbinyl cation? Whatever the solution, three problems must be circumvented: the cyclopropylcarbinyl to allyl rearrangement; nucleophilic capture before rearrangement is complete; and migration of the C_1–C_2 cyclopropane bond. At the present time I only know of one suggestion in the literature which offers a solution to the problems just mentioned. Coates and Robinson[19b] proposed that the substrate–enzyme complex prior to ionization is oriented so that the plane of the π orbitals of the adjacent double bond is perpendicular to the C_1–C_3–cyclopropane bond. If this geometry is maintained after ionization, the cyclopropylcarbinyl to allyl isomerization is effectively blocked. However, there are some problems with this approach. Any kinetic advantage due to the double bond is lost. In fact, the rate may be significantly retarded inductively. There is still no obvious way to prevent premature capture by nucleophile or competitive migration of the C_1–C_2 cyclopropane bond.

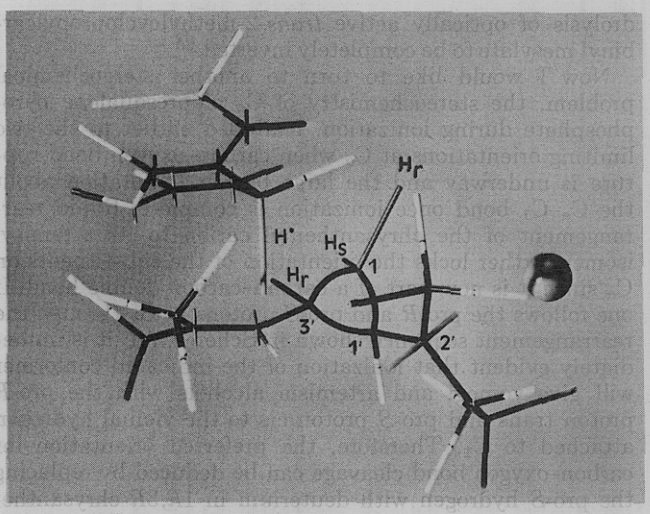


Figure 4. Tertiary cyclopropylcarbinyl cation.

I would like to offer another possibility which does not require an especially rigid enzyme–substrate complex. In view of the rapidity with which cationic rearrangements occur, it is likely that all of the components required for the enzyme-catalyzed reaction are in their proper places prior to ionization. Ionization could easily be triggered by charge neutralization of the pyrophosphate group.

My suggestion only requires that from this point on, three components—carbonium ion, pyrophosphate, and NADPH—are in close proximity and that their relative locations do not change. In this configuration, ionization generates the specificially oriented, intimate ion pair shown in Figure 2. This structure has three distinct and necessary chemical advantages. The undesired cyclopropylcarbinyl to allyl isomerization should be suppressed since the rearrangement results in a considerable separation of positive and negative centers. Very crude estimates of the energy required for charge separation range from 8 to 15 kcal/mol, depending on charge distribution in the cations and dielectric constants. Assuming that the cyclopropane ring remains intact and nucleophilic capture must occur with inversion at C_3, the location of NADPH precludes premature capture of the primary cyclopropylcarbinyl cation. Of the two 1,2 rearrangements possible, migration of the C_1–C_3 cyclopropane bond maintains a close proximity of positive and negative centers in the new intimate ion pair, as shown in Figure 3, while migration of the C_1–C_2 bond again separates centers of unlike charges. Thus, rearrangement of the primary cation which leads to squalene should be selected at the expense of the three competitive reactions. The intimate ion pair produced by the second 1,2 bond migration (Figure 4) also has the optimum geometry for interaction of unlike charges. At this point NADPH is properly oriented to capture the tertiary cation, with inversion of configuration to give squalene with the proper stereochemistry.

What about internal return? Since the primary cyclopropylcarbinyl and cyclobutyl pyrophosphates should be of comparable reactivity and the tertiary system is much more reactive, whatever triggers the initial reaction should certainly trigger ionization of any pyrophosphate formed by internal return.

I am proposing that a complex sequence of rearrangements occurs in a cavity of limited volume, the active site. If the structures in Figures 2–4 are superimposed, a maximum leeway of only 1.5 Å is necessary to accommodate the intermediates, which is no greater than the expansion of the squalene skeleton over the presqualene skeleton independent of mechanism.

In effect, this mechanism uses the negatively charged pyrophosphate as a template to direct the cationic rearrangement. These suggestions and the orientations de-

manded in Figures 2, 3, and 4 are consistent with Porter's recent kinetic data with a purified component of squalene synthetase.[22] The NADPH binds first, followed by presqualene pyrophosphate. The final products of the enzymatic transformation depart in the order pyrophosphate, squalene, and finally NADP. Since members of the artemisia family of monoterpenes are formed with such chemical facility, it is reasonable to expect that on occasion members of this family exist among higher terpenes. I know of two such examples, a tetramethyl derivative (C_{34}) isolated from the green alga *Botryococcus braunii*[23] and a diterpene found in bergamot oil.[24] Interestingly, bergamot oil also contains the C_{20} analog of squalene.

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REFERENCES

(1) G. Popjak and J. W. Cornforth, *Biochem. J.*, **101,** 553 (1966).
(2) (a) T. C. Lee and C. D. Chichester, *Phytochemistry,* **8,** 603 (1969); (b) D. V. Shah, D. H. Feldbruegge, A. R. Houser, and J. W. Porter, *Arch. Biochem. Biophys.,* **127,** 124 (1968).
(3) (a) G. Popjak, D. W. S. Goodman, J. W. Cornforth, R. H. Cornforth, and R. Ryhage, *J. Biol. Chem.,* **236,** 1934 (1961); (b) J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popjak, *Proc. Roy. Soc. Ser. B,* **163,** 492 (1966); (c) G. M. Blackburn, W. D. Ollis, C. Smith, and I. O. Sutherland, *Chem. Commun.,* 99 (1966); (d) J. E. Baldwin, R. E. Hackler, and D. P. Kelly, *J. Amer. Chem. Soc.,* **90,** 4758 (1968).
(4) H. C. Rilling, *J. Biol. Chem.,* **241,** 3233 (1966).
(5) H. C. Rilling and W. W. Epstein, *J. Amer. Chem. Soc.,* **91,** 1041 (1969).
(6) (a) L. J. Altman, R. C. Kowerski, and H. C. Rilling, *J. Amer. Chem. Soc.,* **93,** 1782 (1971); (b) R. M. Coates and W. H. Robinson, *J. Amer. Chem. Soc.,* **93,** 1785 (1971); (c) R. V. M. Campbell, L. Crombie, and G. Pattenden, *Chem. Commun.,* 218 (1971); (d) H. C. Rilling, C. D. Poulter, W. W. Epstein, and B. Larsen, *J. Amer. Chem. Soc.,* **93,** 1783 (1971); (e) J. Edmond, G. Popjak, S. M. Wong, and V. P. Williams, *J. Biol. Chem.,* **246,** 6254 (1971); (f) W. W. Epstein and H. C. Rilling, *J. Biol. Chem.,* **245,** 4597 (1970).
(7) G. Popjak, J. Edmond, and S. M. Wong, *J. Amer. Chem. Soc.,* **95,** 2713 (1973).
(8) (a) L. J. Altman, L. Ash, R. C. Kowerski, W. W. Epstein, B. Larsen, H. C. Rilling, F. Muscio, and D. E. Gregonis, *J. Amer. Chem. Soc.,* **94,** 3257 (1972); (b) L. Crombie, D. A. R. Findley, and D. A. Whiting, *Chem. Commun.,* 1045 (1972).
(9) F. J. Barnes, A. A. Qureshi, E. J. Semmler, and J. W. Porter, *J. Biol. Chem.,* **248,** 2768 (1973).
(10) A. A. Qureshi, F. J. Barnes, E. J. Semmler, and J. W. Porter, *J. Biol. Chem.,* **248,** 2755 (1973).
(11) (a) G. Popjak, "6th International Congress of Biochemistry," New York, N. Y., abstract, p 545; (b) C. Donninger and G. Popjak, *Biochem. J.,* **91,** 10 (1964); (c) C. Donninger and G. Popjak, *Biochem. J.,* **91,** 11 (1964).
(12) E. E. van Tamelen and M. A. Schwartz, *J. Amer. Chem. Soc.,* **93,** 1780 (1971).
(13) (a) D. S. Kabakoff and E. Namanworth, *J. Amer. Chem. Soc.,* **92,** 3234 (1970); (b) L. Radom, J. A. Pople, V. Buss, and P. v. R. Schleyer, *J. Amer. Chem. Soc.,* **92,** 6380 (1970); (c) W. J. Hehre and P. C. Hiberty, *J. Amer. Chem. Soc.,* **94,** 5917 (1972); (d) W. C. Danen, *J. Amer. Chem. Soc.,* **94,** 4835 (1972).
(14) (a) K. B. Wiberg and G. Szeimies, *J. Amer. Chem. Soc.,* **91,** 571 (1970); (b) J. E. Baldwin and W. D. Foglesong, *J. Amer. Chem. Soc.,* **90,** 4303 (1968); (c) G. A. Olah, C. L. Jeuell, D. P. Kelly, and R. D. Porter, *J. Amer. Chem. Soc.,* **94,** 146 (1972).
(15) (a) C. D. Poulter and S. Winstein, *J. Amer. Chem. Soc.,* **92,** 4282 (1970); (b) C. D. Poulter, E. C. Friedrich, and S. Winstein, *J. Amer. Chem. Soc.,* **92,** 4274 (1970); (c) M. Gasic, D. Whalen, B. Johnson, and S. Winstein, *J. Amer. Chem. Soc.,* **89,** 6382 (1967); (d) D. Whalen, M. Gasic, B. Johnson, H. Jones, and S. Winstein, *J. Amer. Chem. Soc.,* **89,** 6384 (1967).
(16) V. E. Althouse, D. M. Feigl, W. A. Sanderson, and H. S. Mosher, *J. Amer. Chem. Soc.,* **88,** 3595 (1966).
(17) R. B. Clayton, "Aspects of Terpenoid Chemistry and Biochemistry," T. W. Goodwin, Ed., Academic Press, New York, N. Y., 1971, p 16.
(18) C. D. Poulter, S. G. Moesinger, and W. W. Epstein, *Tetrahedron Lett.,* 67 (1962).
(19) (a) C. D. Poulter, O. J. Muscio, C. J. Spillner, and R. J. Goodfellow, *J. Amer. Chem. Soc.,* **94,** 5921 (1972); (b) R. M. Coates and W. H. Robinson, *J. Amer. Chem. Soc.,* **94,** 5920 (1972).
(20) C. D. Poulter and C. J. Spillner, unpublished results.
(21) C. D. Poulter, *J. Amer. Chem. Soc.,* **94,** 5516 (1972).
(22) E. Beytia, A. A. Qureshi, and J. W. Porter, *J. Biol. Chem.,* **248,** 1856 (1973).
(23) R. E. Cox, A. L. Burlingame, D. M. Wilson, G. Eglington, and J. R. Maxwell, *J. Chem. Soc. Chem. Commun.,* 284 (1973).
(24) M. Soucek, V. Herout, and F. Sorm, *Collect. Czech. Chem. Commun.,* **26,** 2551 (1961).

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