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Biosynthesis of Head-to-Head Terpenes. Carbonium Ion Rearrangements Which Lead to Head-to-Head Terpenes†

C. Dale Poulter,* Oliver J. Muscio, and Robyn J. Goodfellow

ABSTRACT: Hydrolysis of (1*S*,2*R*)-2-[*trans*-2′-(2′′-methylpropenyl)cyclopropyl]propan-2-yl *p*-nitrobenzoate and (1*S*,3*R*)-*trans*-2,2-dimethyl-3-(2′-methylpropenyl)cyclobutyl *p*-toluenesulfonate gave 2-[*trans*-2′-(2′′-methylpropenyl)cyclopropyl]-propan-2-ol, *trans*-2,7-dimethyl-3,6-octadien-2-ol and (*S*)-2,7-dimethyl-2,6-octadien-4-ol. The three alcohols were also obtained by hydrolysis of *trans*-2,7-dimethyl-3,6-octadien-2-yl 3,5-dinitrobenzoate. The chemical properties of the carbonium ion intermediates are discussed in terms of the product and stereochemical studies. Biosynthesis of head-to-head terpenes is compared to the chemical results and a biosynthetic mechanism is presented.

Synthesis of the higher terpenes in the sterol and carotenoid classes requires head-to-head condensation of two head-to-tail polyprenyl pyrophosphates. In squalene (sterol) synthesis the overall process is reductive, while in phytoene (carotenoid) synthesis it is not[1] (see Scheme I). With the discovery of cyclopropylcarbinyl pyrophosphates as intermediates in these two pathways (Epstein and Rilling, 1970; Altman *et al.*, 1972), the transformations can be considered in terms of two distinct steps. The first is the formation of the intermediate **2** by stereospecific insertion of C_1 of a molecule of **1** into the C_2–C_3 double bond of a second **1** (Popjak *et al.*, 1973). The second is the stereospecific rearrangement of the cylopropylcarbinyl intermediates to squalene (**3**) or phytoene (**4**) by rupture of the $C_{1'}$–$C_{2'}$ and $C_{1'}$–$C_{3'}$ cyclopropane bonds followed by bonding

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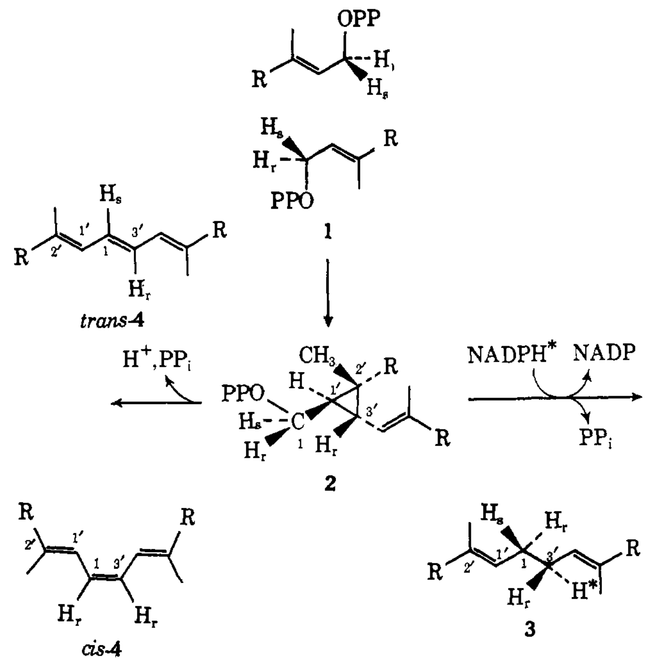
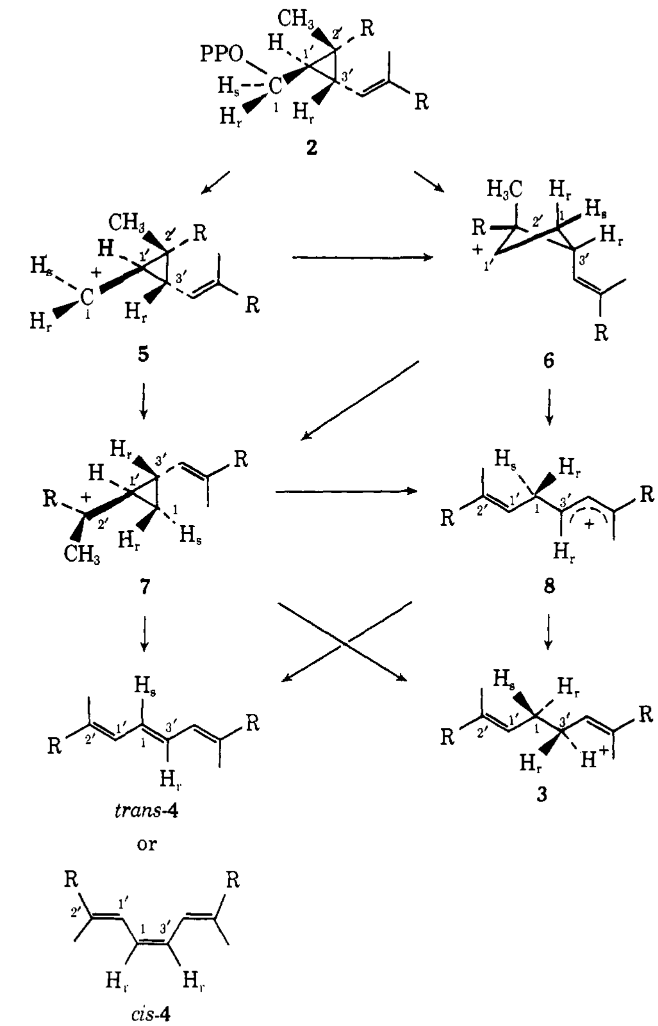
[1] This aspect has been discussed by Gregonis and Rilling (1974). We will treat phytoene as the first isolatable intermediate; however, an extension of the arguments for squalene to lycopersene is trivial, assuming identical stereochemistry.

SCHEME I



SCHEME II



between C_1 and $C_{3'}$ (Cornforth *et al.*, 1966; Grob and Butler, 1956).

Several mechanisms for the second reaction have been proposed. Most involve carbonium ion intermediates[2] and are summarized in Scheme II (Rilling *et al.*, 1971; Altman *et al.*, 1971; van Tamelen and Schwartz, 1971; Coates and Robinson, 1972). Chemical studies have demonstrated that reactivities of cyclopropylcarbinyl derivatives and rearrangements of the resulting cations are strongly dependent on substitution patterns and stereoelectronic considerations (Richie, 1972; Wiberg *et al.*, 1972). The factors which govern the course of the chemical reactions should be the same as those which influence the biochemical rearrangements. However, vinyl-substituted cations analogous to the structures shown in Scheme II had not been very well studied when the mechanisms for head-to-head coupling were formulated. Consequently we have undertaken an investigation of the cationic rearrangements of systems (R = CH_3) which are close analogs of intermediates expected from presqualene (R = $C_{11}H_{19}$) and prephytoene (R = $C_{16}H_{27}$) pyrophosphates. It is our belief that shortening the alkyl substituents about the cyclopropane and cyclobutane rings will not alter the chemistry of the vinyl-substituted cyclopropylcarbinyl core. We now report work concerned with cyclobutyl (**6**), cyclopropylcarbinyl (**7**), and allylic (**8**) intermediates which helps clarify the nature of the complex rearrangements and the stereochemistry for capture of **7** at $C_{3'}$ by a nucleophile. A preliminary account of this research has been published (Poulter *et al.*, 1972b).

Experimental Section

Materials. Analytical reagent grade acetone and practical grade *p*-dioxane were used without further purification. Pyridine and 2,6-lutidine were distilled from barium oxide, and stored over molecular seives under a nitrogen atmosphere.

p-Toluenesulfonyl chloride was purified by the method of Pelletier (1953), mp 67.8–69.2°. *p*-Nitrobenzoyl chloride was dissolved in pentane, insoluble *p*-nitrobenzoic acid removed by filtration, and the pure acid chloride recrystallized from the filtrate, mp 71.8–73.5°.

Technical grade dinitrobenzoyl chloride was used without further purification.

Preparation of Compounds. The syntheses of alcohols 2-[*trans*-2′-(2′′-methylpropenyl)cyclopropyl]propan-2-ol (**9**-OH) and *trans*-2,2-dimethyl-3-(2′-methylpropenyl)cyclobutanol (**10**-OH), resolution of intermediates, and determination of their optical purities and absolute configuration are described elsewhere (C. D. Poulter *et al.*, submitted for publication).

Preparation of 2-[trans-2′-(2′′-Methylpropenyl)cyclopropyl]-propan-2-yl p-Nitrobenzoate (**9**-*OpNB*). In a typical preparation, 0.382 g (2.48×10^{-3} mol) of **9**-OH was dissolved in 2.1 ml of dry pyridine, and 0.690 g (3.72×10^{-3} mol) of *p*-nitrobenzoyl chloride was added. The resulting mixture was stirred magnetically overnight. An additional 1.0 ml of pyridine was added to aid stirring, and the mixture was stirred an additional 4 hr. Work-up was carried out by dilution with 25 ml of pentane, removal of insoluble material by filtration, and evaporation of solvent under reduced pressure. This sequence was repeated until no further insoluble material precipitated upon addition of pentane. Three cycles were usually sufficient. (No odor of unreacted **9**-OH was present.) The product was a yellow-orange low-melting solid, formed in quantitative yield: nmr (δ, $CHCl_3$) 0.4–1.6 (4, m, H at $C_{1'}$, $C_{2'}$, and $C_{3'}$), 1.65 (6, s, H at C_1 and C_3), 1.77 (6, m, CH_3's

[2] For purposes of clarity we will represent the cations with charge localized structures; however, it must be remembered that extensive delocalization occurs in these systems.

at $C_{2''}$), 4.85 (1, d of m, H at $C_{1''}$, $J_{2',1''} = 9$ Hz), and 8.53 ppm (4, m, aromatic H).

Preparation of trans-2,2-Dimethyl-3-(2'-methylpropenyl)-cyclobutyl p-Toluenesulfonate (**10**-*OTs*). Reaction of **10**-OH with a 15% excess of *p*-toluenesulfonyl chloride by the above procedure, followed by removal of all volatiles by high-vacuum bulb-to-bulb distillation, afforded **10**-OTs in only 22% yield. The remainder had evidently eliminated to volatile olefins. Shorter reaction times, even with larger excesses of the acid chloride, allowed better material recovery, but with large amounts of unreacted **10**-OH remaining.

Better results were obtained by using 2,6-lutidine instead of pyridine. In 1.0 ml of 2,6-lutidine was dissolved 0.190 g (1.23×10^{-3} mol) of **10**-OH, and 0.47 g (2.5×10^{-3} mol) of *p*-toluenesulfonyl chloride. The resulting mixture was allowed to stir overnight at ambient temperature. Work-up as in the preparation of **9**-OpNB gave **10**-OTs in good yield. The **10**-OTs so prepared was used as obtained without further purification to avoid decomposition.

Preparation of trans-2,7-Dimethyl-3,6-octadien-2-yl Dinitrobenzoate (**11**-*ODNB*). By the same procedure as used for the preparation of **9**-OpNB, 0.0388 g (2.52×10^{-4} mol) of **11**-OH which had been purified by glpc (Carbowax 20M, 140°) was dissolved in 0.3 ml of pyridine and allowed to react with 0.115 g (5.0×10^{-4} mol) of dinitrobenzoyl chloride. Benzene was used in the work-up instead of pentane. The yield of product, a syrupy oil, was 0.077 g (88%).

Hydrolyses of **9**-*OpNB*, **10**-*OTs, and* **11**-*ODNB*. Hydrolyses were carried out in three media (all percentages v/v): (a) 80% acetone–water with 2,6-lutidine in threefold molar excess over substrate; (b) 50% dioxane–water with 2,6-lutidine; (c) 50% dioxane–water which was *ca.* 0.5 M in sodium hydroxide. Hydrolyses under conditions (a) were carried out at substrate concentrations of approximately 0.1 M, while substrate concentrations in hydrolyses (b) and (c) were approximately 0.065 M.

Hydrolyses (b) and (c) were run in parallel, and solutions were made up by dissolving the substrate in the proper quantity of dioxane, dividing the resulting solution into two parts, and adding an equal volume of 1 M NaOH solution to one (c), while adding a threefold excess of lutidine to the other (b), followed by an equal volume of water.

Work-up was the same in all cases, except as noted below for procedure c. The mixtures were diluted with about a threefold volume of ether, and anhydrous potassium carbonate was added until no more dissolved. The organic layer was decanted from the aqueous layer, and extracted with cold 1 M hydrochloric acid to remove the pyridine or lutidine (not necessary in procedure c). This was followed by washing in series with saturated solutions of sodium chloride, sodium bicarbonate, and sodium chloride. Drying of the organic phase was accomplished by filtration through sodium sulfate and the use of molecular seives. Solvent was removed under reduced pressure and the residue distilled bulb-to-bulb under high vacuum.

The isomeric product alcohols were isolated by preparative gas chromatography (Carbowax 20M, 140°). Control experiments with alcohols **9**-OH, **11**-OH, and **12**-OH, a equal molar amount of *p*-nitrobenzoic acid, and a threefold molar excess of 2,6-lutidine in 80% acetone–water at 50° for 24 hr indicated that the products were stable to reaction conditions. We also reinjected materials collected by preparative glpc to test their stability to collection conditions. We found that care must be exercised to keep the columns and injector port free of acidic material.

Acid-Catalyzed Hydrolysis of **9**-*OH*. A 240-mg portion of **9**-OH was dissolved in a mixture of 0.5 ml of 8.8×10^{-3} N perchloric acid and 2.0 ml of dioxane and allowed to stand at 40° for 42 hr. Work-up gave 228 mg of a light yellow oil which was mostly (>97%) comprised of **11**-OH and **12**-OH.

Hydrolysis of (1*S*,2*R*)-**9**-*OpNB* (*Method a*). Preparation of **9**-OpNB was carried out as described using *p*-nitrobenzoate prepared from (1*S*,2*R*)-**9**-OH, 54% optically pure. Two components comprised more than 99% of the product mixture after hydrolysis. They were separated by glpc (Carbowax 20M, 140°). The first eluted major alcohol was *trans*-2,7-dimethyl-3,6-octadien-2-ol (**11**-OH): ir (CCl_4) 970 cm^{-1}; nmr (δ, CCl_4) 1.22 (6, s, CH_3's at C_2), 1.63 (6, d, CH_3's at C_7), 2.00 (1, broad s, hydroxyl group), 2.59 (2, m, H at C_5), 5.2 (1, broad triplet, H at C_6), and 5.43 ppm (2, m, H at C_3 and C_4). The second major alcohol to elute was 2,7-dimethyl-2,6-octadien-4-ol: $[\alpha]^{25}_D$ −7.37° (*c* 1.61, $CHCl_3$); nmr (δ, CCl_4) 1.68–1.77 (12, CH_3's at C_2 and C_7), 2.18 (2, broad triplet, H at C_5, $J \simeq 7$ Hz), 4.1–4.5 (1, m, H at C_4), and 4.0–5.4 ppm (2, m, H at C_3 and C_6).

The remainder of the product alcohols were acetylated with acetyl chloride and pyridine, and the product acetates were subjected to ozonolysis, reductive work-up with lithium aluminum hydride, and acetylation with acetic anhydride (Donninger and Popjak, 1966). The procedure was altered by the use of a mixture of acetic anhydride, pyridine, and acetyl chloride (2:2:1.5 by volume) as the acetylation reagent, instead of acetic anhydride alone. Preparative gas chromatography (Carbowax 20M, 187°) gave 1,3,4-triacetoxybutane (**13**), $[\alpha]^{25}_D$ −3.13° (*c* 0.80, $CHCl_3$). An nmr spectrum of the triacetate matched that found for **13** independently synthesized from (*S*)-malic acid.

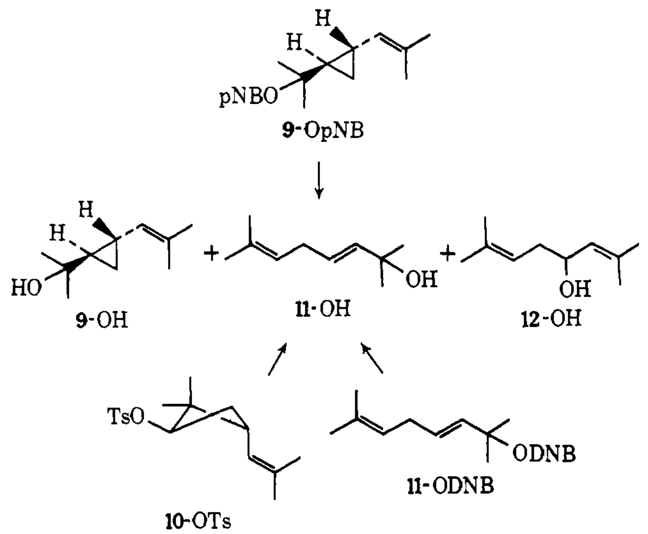
Preparation of (*S*)-*1,3,4-Triacetoxybutane* (**13**). To a new 50-ml erlenmeyer flask equipped with a Teflon stir bar, and cooled in an ice bath, was added 200 mg (1.49 mmol) of (*S*)-malic acid, mp 103–104°, $[\alpha]^{25}_D$ −1.53° (*c* 9.68, H_2O), 93% optically pure, and 10 ml of ether. To a second 50-ml flask cooled in an ice bath was added 900 mg (8.9 mmol) of *N*-methylnitrourea in 10 ml of ether. To this stirred solution was added 3.0 ml of 50% potassium hydroxide solution. After 5 min the yellow ether layer was decanted into the flask containing the malic acid. The KOH–urea mixture was extracted with two 10-ml portions of ether, each stirring 5 min, and the combined CH_2N_2–acid flask was allowed to stand at room temperature overnight. The solution was dried over $MgSO_4$ and solvent removed at reduced pressure, yielding dimethyl maleate as a colorless oil: nmr (δ, CCl_4) 2.52 (2, d, H at C_3, $J_{2,3} = 6$ Hz), 3.47 and 3.55 (6, s, CH_3's), 3.65 (1, broad, OH), and 4.29 ppm (1, t, H at C_2).

The crude dimethyl maleate was reduced with 200 mg of $LiAlH_4$; work-up and acetylation as described above for the ozonolysis of the solvolysis products yielded **13**: $[\alpha]^{25}_D$ −20.9° (*c* 1.84, $CHCl_3$); nmr (δ, CCl_4), 1.88 (2, q, H at C_3, $J = 7$ Hz), 2.02 and 2.07 (9, s, acetoxy CH_3's), 4.2 (4, m, H at C_1 and C_4), and 5.17 ppm (1, m, H at C_2).

Hydrolysis of **9**-*OpNB* (*Methods b and c*). The preparation of **9**-OpNB was carried out as before, using (1*R*,2*S*)-**9**-OH, 13.4% optically pure. The resulting *p*-nitrobenzoate was dissolved in 20 ml of dioxane, and divided into fractions of 10.8 and 9.2 ml, which were used in solvolyses procedures b and c, respectively. Work-up and preparative glpc yielded **12**-OH; $[\alpha]^{25}_D$ +3.20° (*c* 3.16, $CHCl_3$) method b, and $[\alpha]^{25}_D$ +3.44° (*c* 3.55, $CHCl_3$) method c.

Hydrolysis of **10**-*OTs* (*Method a*). The **10**-OTs obtained from 0.190 g (1.23×10^{-3} mol) of (1*S*,3*R*)-**10**-OH, $[\alpha]^{25}_D$

SCHEME III



$+6.73°$ (c 8.95, $CHCl_3$), 91% optically pure, was solvolyzed by method a in 10 ml of 80% aqueous acetone overnight at room temperature. Preparative glpc of the solvolysis products yielded **12**-OH, $[\alpha]_D^{25}$ $-9.83°$ (c 3.27, $CHCl_3$). Analytical glpc of the collected **12**-OH showed the presence of 11% unreacted **10**-OH, and 4% **11**-OH, resulting in a computed specific rotation for pure **12**-OH of $[\alpha]_D^{25}$ $-12.6°$.

Results

Products. Hydrolysis of 2-[*trans*-2′-(2′′-methylpropenyl)-cyclopropyl]propan-2-yl *p*-nitrobenzoate (**9**-OpNB), *trans*-2,2-dimethyl-3-(2′-methylpropenyl)cyclobutyl *p*-toluenesulfonate (**10**-OTs), and *trans*-2,7-dimethyl-3,6-octadien-2-yl 3,5-dinitrobenzoate (**11**-ODNB) in the presence of a threefold molar excess of 2,6-lutidine gave the compounds shown in Scheme III. After recovery, the three products, **9**-OH, **11**-OH, and 2,7-dimethyl-2,6-octadien-4-ol (**12**-OH), accounted for more than 95% of the substrate, and the percentages of each listed in Table I are normalized to 100% (also see Coates and Robinson, 1972). The only noticeable minor products had very short glpc retention times characteristic of hydrocarbons, presumably elimination products, and were only found in trace amounts. Control experiments ensured that all of the products were stable to the reaction conditions and analytical procedures. Allylic alcohols **11**-OH and **12**-OH were also obtained in high yield by treating cyclopropylcarbinol **9**-OH with perchloric acid (8.8×10^{-3} N) in 80% dioxane–water. Identification of **9**-OH is based on coinjections with an authentic sample on two 500 ft × 0.03 in. open tubular columns (Carbowax 20M and 95% OV-101-5% IGEPAL). The structures of **11**-OH and **12**-OH were assigned from their respective nmr and ir spectra (see Experimental Section). No cyclobutyl alcohol **10**-OH was found under conditions in which 0.5% could have detected.

Hydrolysis of **9**-OpNB and **10**-OTs in 80% acetone–water gave identical ratios of tertiary allylic alcohol **11**-OH and its secondary isomer **12**-OH. One explanation of this result requires that all of both substrates funnel through a common intermediate during hydrolysis. A logical choice is **7** (R = CH_3) since the tertiary cyclopropylcarbinyl cation is more stable than secondary cyclobutyl cation **6** (Richie, 1972; Wiberg *et al.*, 1972). In this situation rearrangement to the more stable species is extremely rapid or concerted with ionization (Wiberg *et al.*, 1972).

The ratio of **11**-OH and **12**-OH from hydrolysis of the allylic derivative **11**-ODNB differed from that for **9**-OpNB and **10**-OTs. At least a portion of the products from **11**-ODNB came *via* nucleophilic attack on tertiary cyclopropylcarbinyl cation **7** since **9**-OH was found among the hydrolysis products. Also, as seen in Table I, the ratio of allylic alcohols **11**-OH and **12**-OH from hydrolysis of **9**-ODNB was sensitive to the water content of the solvent, with an increase in the concentration of water yielding more of the secondary alcohol. In addition, introduction of a more reactive nucleophile, hydroxide, gave a modest increase in the relative proportion of **12**-OH during hydrolysis of **9**-OpNB in 50% dioxane–water.

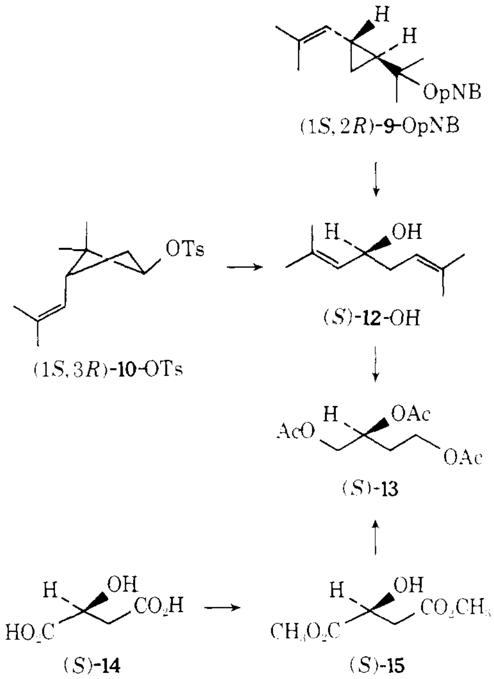
Widely different reactivities of the three model compounds necessitated changes in leaving group, temperature, or solvent in order to obtain convenient rates of hydrolysis. However, the observed variations in product ratios during the reactions cannot be attributed to these alterations. Hydrolysis of **9**-OpNB and **10**-OTs with *p*-nitrobenzoate and *p*-toluenesulfonate leaving groups, respectively, gave identical ratios of **11**-OH to **12**-OH. Changing the reaction temperature of **9**-OpNB from 23 to 50° also produced no change in the composition of the product mixture. Finally, the similarity in product ratios found between cyclopropylcarbinyl **9**-OpNB and cyclobutyl **10**-OTs in 80% acetone–water compared to differences seen for **9**-OpNB and allylic **11**-ODNB in 50% dioxane–water argues against ion-pair phenomena as a factor in changing the relative proportions of **11**-OH and **12**-OH. One would expect the effects of ion pairs on product distribution to decrease as the water content of the solvent increased. Thus, we are drawn to the conclusion that partial equilibration between cyclopropylcarbinyl cation **7** and allylic cation **8** is responsible for the variations seen in the ratio of **11**-OH to **12**-OH as a function of solvent.

TABLE I: Products from Hydrolysis of **9**-OpNB, **10**-OTs, and **11**-ODNB.

Substrate	Solvent	Products[a]			% Inversion of **12**-OH
		9-OH	**11**-OH	**12**-OH	
9-OpNB	80% (v/v) acetone–water, 2,6-lutidine, 23°	0.3	63	37	26
	80% (v/v) acetone–water, 2,6-lutidine, 50°	0.3	63	37	
	50% (v/v) dioxane–water, 2,6-lutidine, 23°	0.3	58	42	44
	50% (v/v) dioxane–water, ~0.5 M NaOH, 23°	12[b]	44 (50)[c]	44 (50)[c]	48
10-OTs	80% (v/v) acetone–water, 2,6-lutidine, 23°	0.3	63	37	26
11-ODNB	50% (v/v) dioxane–water, 2,6-lutidine, 55°	0.3	68	33	

[a] Normalized to ~100%; in all cases yields were >95%. No **10**-OH (<0.1%) could be seen. [b] Likely due mostly to base-catalyzed ester hydrolysis. [c] Normalized, assuming <1% of **9**-OH.

SCHEME IV



Stereochemistry. Hydrolysis of (1*S*,2*R*)-2-[*trans*-2′-(2′′-methylpropenyl)cyclopropyl]propan-2-yl *p*-nitrobenzoate((1*S*,2*R*)-**9**-OpNB) which was 54% optically pure (77% 1*S*,2*R* and 23% 1*R*,2*S*) gave optically active secondary allylic alcohol **12-OH**. The absolute configuration and optical purity of **12-OH** were established by the sequence of reactions shown in Scheme IV. In a separate set of reactions, (*S*)-malic acid, 93% optically pure, was esterified with diazomethane, and the resulting dimethyl ester reduced with lithium aluminum hydride. Acetylation of the triol afforded (*S*)-**13** of known absolute configuration and optical purity. By comparing triacetates from both sources, 2,7-dimethyl-2,6-octadien-4-ol formed during hydrolysis of (1*S*,2*R*)-**9**-OpNB was found to be predominately the *S* enantiomer. The reaction proceeded with 26% net inversion of configuration at C_4. Hydrolysis of cyclobutyl tosylate (1*S*,3*R*)-**10**-OTs, 91% optically pure, under similar conditions also gave (*S*)-**12**-OH, again with 26% net inversion at C_4. We feel that it would be unlikely to obtain identical product distributions and stereoselectivities for **9**-OpNB and **10**-OTs unless, as previously mentioned, cyclobutyl cation **6** rearranged to cyclopropylcarbinyl cation **7** *prior* to reaction with solvent.

Discussion

Rearrangements of Cations **6**, **7**, *and* **8**. We are now in a position to consider many of the ambiguities found in Scheme II. All of the evidence obtained with cyclobutyl *p*-toluenesulfonate **10**-OTs and cyclopropylcarbinyl *p*-nitrobenzoate **9**-OpNB suggests a concerted migration of the $C_{2'}$–$C_{3'}$ cyclobutane bond from $C_{2'}$ to $C_{1'}$ during ionization or complete rearrangement of cyclobutyl cation **6** to tertiary cyclopropylcarbinyl cation **7** prior to reaction with solvent. Otherwise, as previously mentioned, one would not expect to find identical product distributions and stereoselectivities upon hydrolysis of the two substrates, especially when one considers the changes found for hydrolysis of **9**-OpNB as a function of solvent composition. We can also rule out direct rearrangement of the cyclobutyl *p*-toluenesulfonate to allylic cation **8**, bypassing cyclopropylcarbinyl cation **7**, since optically active secondary allylic alcohol **12-OH** is obtained from hydrolysis of (1*S*,3*R*)-**10**-OTs.

The question of whether the cyclobutyl species **6** is an intermediate in the rearrangement of primary cyclopropylcarbinyl cation **5** to its tertiary isomer **7** remains unanswered, as does this aspect of the mechanism for all cyclopropylcarbinyl interconversions (Richie, 1972; Wiberg *et al.*, 1972; Gajewski and Oberdier, 1972; Poulter and Winstein, 1972). However, the overall stereochemistry of either path (**5** → **7** or **5** → **6** → **7**) should be the same. Our results indicate that if cyclobutyl cation **6** were an intermediate, its rearrangement to **7** would be so rapid that it would not be trapped by a nucleophile. Nevertheless, for the sake of generality we will show the biogenetic rearrangements with **6** as an intermediate.

Variations in product distributions and stereoselectivities found during hydrolysis of **9**-OpNB and **11**-ODNB in different solvents (Table I) are attributed to partial equilibration of the cyclopropylcarbinyl (**7**) and allylic (**8**) cations. The observation that capture of the two cationic species proceeds at comparable rates for hydroxide and water implies that the rate constant for reaction with solvent must be approaching a diffusion-controlled limit, otherwise capture by hydroxide would be much more rapid (Ritchie, 1972). For rearrangement to be competitive with capture by water, but with equilibration between **7** and **8** still incomplete, the barrier between the two cations *in both directions* must be *ca.* 4 kcal/mol.[3]

The regioselectivities[4] for nucleophilic attack at $C_{3'}$ and $C_{2'}$ are different for cyclopropylcarbinyl cation **7** and allylic cation **8**. The exact regioselectivities of the individual cations toward water or hydroxide cannot be determined since we do not know the extent of their equilibration or the position of equilibrium. If one assumes that rearrangement of **7** to **8** is irreversible and reaction between solvent and $C_{3'}$ of the cyclopropylcarbinyl cation is stereospecific, the ratios of **12-OH** to **11-OH** from **7** and **8** are 1.38 and 0.43, respectively. Since **7** and **8** do interconvert, our numbers represent a lower limit for **7** and an upper limit for **8**.

The partial inversion of configuration at $C_{3'}$ in allylic alcohol **12-OH** requires some retention of chirality before reaction with solvent. We prefer to explain the results by stereospecific solvent attack at $C_{3'}$ in cyclopropylcarbinyl cation **7** accompanied by partial equilibration with allylic cation **8**, although stereoselective attack on **7** accompanied by partial equilibration is possible. Relevant to this is the stereospecific inversion of configuration at the cyclopropane carbons observed during solvolysis of alkyl-substituted systems which cannot racemize by means of a cyclopropylcarbinyl to allyl isomerization (Whalen *et al.*, 1967; C. D. Poulter and C. J. Spillner, unpublished results).

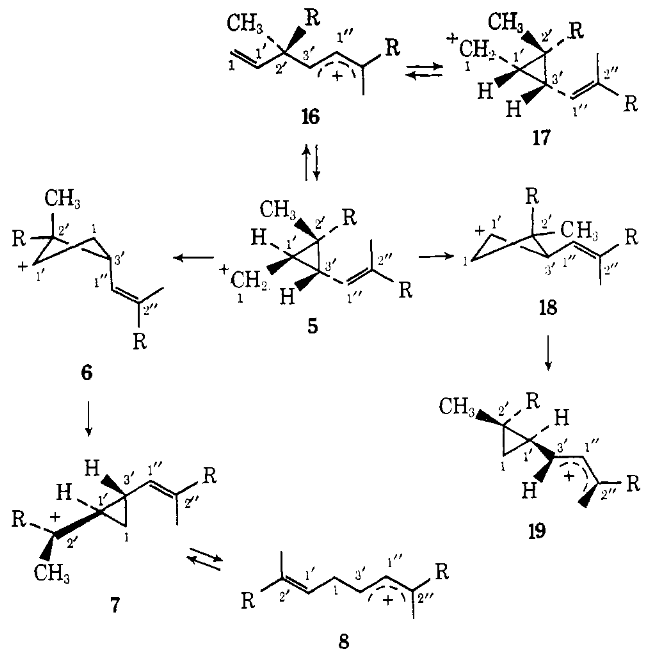
Rearrangements of **5**. The rearrangements reported for carbonium ions derived from the primary cyclopropylcarbinyl system **2** (R = CH_3) are summarized in Scheme V (Poulter, 1973; Poulter *et al.*, 1972a,b; Coates and Robinson, 1972; Trost *et al.*, 1971; Sasaki *et al.*, 1972). The relative stabilities of the cations which are shown can be estimated by using data from several sources.

Since we are concerned with isomeric cations which have similar substitution patterns, our estimates should be reasonably accurate. Alkyl substituents at the trigonal carbon atom bonded to the cyclopropane ring or in the cyclobutane ring are

[3] Calculated activation energies for the following rate constants suggest similar energies for **7** and **8**: 10^9 (5.2 kcal/mol), 10^{10} (3.8 kcal/mol), and 10^{11} (2.4 kcal/mol).

[4] For a definition, see Hassner (1968).

SCHEME V



more influential than the corresponding substituents at other atoms. Primary cyclopropylcarbinyl and secondary cyclobutyl cations are comparable in stability but are less stable than isomeric tertiary cyclopropylcarbinyl systems (Richie, 1972; Wiberg *et al.*, 1972). In addition we found that tertiary cyclopropylcarbinyl cation **7** and allylic cation **8** have similar stabilities. However, cyclopropyl-substituted vinyl cation **19** should be much more stable than any of the other species shown in Scheme V (Richie, 1972). Thus the intermediates can be listed in order of increasing stability as follows: **17** $\approx$ **6** $\approx$ **18** $\approx$ **5** $<$ **16** $\approx$ **8** $\approx$ **7** $<$ **19**. These considerations indicate that the enzymatic synthesis of squalene and phytoene does not follow the sequence of rearrangements judged to be thermodynamically most favorable.

The most striking feature of the chemistry of primary cyclopropylcarbinyl cation **5** with regard to biosynthesis of head-to-head terpenes is the inefficient rearrangement of **5** to **7**. More than 98% of the hydrolysis products of ten carbon models for **2** arise from **5** and its allylic isomer **16**. Only trace quantities of allylic alcohols **11**-OH and **12**-OH which come from cations **7** and **8** are found (Poulter, 1973), along with similar amounts of products resulting from cyclopropyl-substituted allylic cation **19**. Solvolysis of derivatives of **2** or precursors to **16** in solvents where the cationic intermediates are longer-lived gives a larger proportion of products derived from rearranged cations **7**, **8**, and **19** (Poulter, 1973; Trost *et al.*, 1971). However, under these conditions, the product mixtures are complex.

Regiospecific rearrangement of primary cyclopropylcarbinyl cation **5** to cyclobutyl cation **6** appears to be the major obstacle to obtaining the proper head-to-head carbon skeleton. We have just shown that once **6** is formed it quickly rearranges to tertiary cyclopropylcarbinyl cation **7** and that more than 99% of the hydrolysis products have a head-to-head carbon skeleton. If a carbonium ion intermediate similar to **5** is involved in the biosynthetic reactions of presqualene and prephytoene pyrophosphates, the pathways shown in Scheme V which compete with rearrangement of **5** to **6** (**5** $\rightarrow$ **16**, **5** $\rightarrow$ **18**, and reaction of **5** with hydride or elimination of a proton from **5**) must be eliminated.

Stereochemistry. Although squalene and phytoene have high degrees of symmetry, their syntheses from cyclopropylcarbinyl precursors are stereospecific. We will first consider squalene and return later to unresolved aspects concerning phytoene. For squalene three stereochemical considerations are pertinent: inversion of configuration of C_4, inversion of configuration of $C_{3'}$, and generation of the trisubstituted $C_{1'}$–$C_{2'}$ double bond with an *E* configuration.[5]

The bonding of C_1 to other atoms is altered at two points, during ionization of pyrophosphate ester **2** and during migration of the $C_{1'}$–$C_{3'}$ cyclopropane bond in **5** when the primary cyclopropylcarbinyl cation rearranges to the isomeric cyclobutyl cation **6**. Cyclopropylcarbinyl cations prefer the bisected conformations shown for **5** and **7** in Scheme II (Richie, 1972; Wiberg *et al.*, 1972; Buss *et al.*, 1971). Therefore loss of the pyrophosphate group in **2** must occur from one of two limiting conformations which differ greatly in their topology. The orientation of the diastereotopic protons at C_1 with respect to the cyclopropane ring in **5** is locked during ionization because of the large barrier to rotation about the C_1–$C_{1'}$ bond. Stereoelectronic considerations dictate that subsequent rearrangement of cyclopropylcarbinyl species **5** to its cyclobutyl (**6**) or tertiary cyclopropylcarbinyl (**7**) isomers occurs with the stereochemistry shown in Scheme II (Richie, 1972; Wiberg *et al.*, 1972). Thus, observed inversion of C_1 is a consquence of two separate events: the ionization of **2** from the conformer in which the C_1–oxygen bond is trans to the $C_{1'}$–$C_{3'}$ cyclopropane bond and the stereospecific migration of the $C_{1'}$–$C_{3'}$ bond.

The configuration of $C_{3'}$ is determined by the stereochemistry of hydride transfer from NADPH to the final cationic intermediate. Biochemical studies indicate that this step must occur with inversion of configuration of tertiary cyclopropylcarbinyl cation **7**, if it is the immediate precursor of squalene, in accord with chemical properties of the cation. Although allylic cation **8** has no stereochemical bias for reaction with a nucleophile, there are numerous examples of asymmetric enzymatic reductions of trigonal carbon atoms by NADPH. We will explain the roles of cations **7** and **8** in the biosynthetic transformations later.

Another stereochemical consideration is the configuration of the C_1'–C_2' double bond, which is the same for squalene and both isomers of phytoene. The stereochemical arguments presented for C_1 during ring expansion of primary cyclopropylcarbinyl cation **5** to cyclobutyl cation **6** also apply to the ring contraction of **6** to tertiary cation **7** (or rearrangement of **5** directly to **7**) and will result in a cis orientation between $C_{2'}$ and the cyclopropane ring of the tertiary cation. Rotation about the $C_{1'}$–$C_{2'}$ bond has a large activation energy and should not occur prior to hydride transfer. If **7** isomerizes to allylic cation **8**, the configuration is locked even more tightly. Recyclization of **8** will simply regenerate **7**.

Finally, two isomers of phytoene, cis and trans with respect to the C_{15}–C_{16} double bond, are known (Gregonis and Rilling, 1974). During biosynthesis of the cis isomer both *pro-R* hydrogen atoms at C_1 of geranylgeranyl PP are retained (see Scheme II) while one *pro-R* and one *pro-S* are retained in *trans*-phytoene (Gregonis and Rilling, 1974). One interpretation of these results is a direct proton elimination from tertiary cyclo-

[5] The terms cis and trans can be confusing when discussing trisubstituted double bonds. An *E* configuration refers to a double bond in which the two substituents of highest priority (Cahn–Ingold–Prelog Convention) are trans and a *Z* configuration in which they are cis.

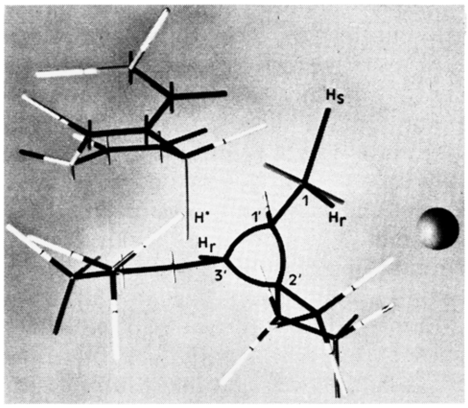


FIGURE 1: Ion pair between primary cyclopropylcarbinyl cation **5** and pyrophosphate.

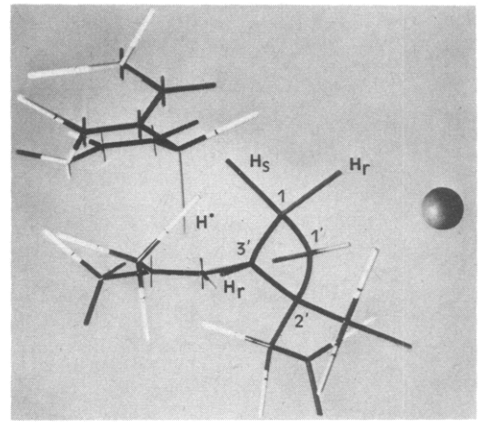
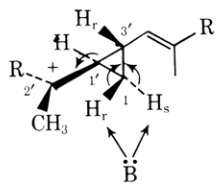


FIGURE 2: Ion pair between cyclobutyl cation **6** and pyrophosphate.

propylcarbinyl cation **7**. The new double bond between C_1 and $C_{1'}$ will be cis if H_s is removed and trans if H_r is removed.



Interestingly, only a slight change in the position of the base (B:) which assists in proton removal is necessary for evolution of a cis synthetase from the trans system or *vise versa.*

A Mechanism for Biosynthesis of Head-to-Head Terpenes from Cyclopropylcarbinyl Precursors. Without assistance from an enzyme, the solvolytic properties of vinyl-substituted cyclopropylcarbinyl cations are not compatible with the biosynthesis of head-to-head terpenes. The greatest difficulty occurs at the rearrangement of primary cyclopropylcarbinyl cation **5** to cyclobutyl cation **6** (or the direct rearrangement of **5** to **7**). Other than that, the regio- and stereoselectivities of the ten carbon models adequately mimic their proposed biological counterparts and require only minimal constraints to become regio- and stereospecific. Thus, the enzyme must selectively force the rearrangement of the $C_{1'}$–$C_{3'}$ cyclopropane bond in **5** from $C_{1'}$ to C_1. Other rearrangements must be suppressed, especially since **7** and **8** are not the most stable cations to which **5** can isomerize.

Coates and Robinson (1972) have suggested that for squalene the proper rearrangement is forced by an enzyme–substrate complex which is oriented prior to ionization so that the plane of the π orbitals of the adjacent double bond is perpendicular to the $C_{1'}$–$C_{3'}$ cyclopropane bond. In this orientation it was proposed that cyclopropylcarbinyl pyrophosphate **2** would rearrange to cyclobutyl cation **6** during ionization by a concerted migration of the $C_{1'}$–$C_{3'}$ cyclopropane bond. However, if the double bond and cyclopropane ring are held out of conjugation, one would expect **2** to behave like an alkyl-substituted system with a slight amount of inductive destabilization from the nonconjugated double bond. In this case, a concerted rearrangement to **6** should not compete favorably with direct ionization to **5** (Richie, 1972). Ionization of **2** from a twisted conformation to a twisted conformer of **5**, followed by rearrangement to **6**, does not provide a satisfactory explanation. The twisted primary cyclopropylcarbinyl cation should be free to rearrange to cyclobutyl cation **18** by migration of the $C_{1'}$–$C_{2'}$ cyclopropane bond as well as to **6** by migration of the $C_{1'}$–$C_{3'}$ bond (C. D. Poulter and C. J. Spillner, unpublished results).

We wish to present a mechanism for biosynthesis of squalene and phytoene from the corresponding cyclopropylcarbinyl pyrophosphates which takes advantage of the chemical properties of the cationic intermediates **5**, **6**, and **7**. The biosynthesis of squalene will be used to illustrate the mechanism and then phytoene will be considered as an extension. In view of the rapidity with which cationic rearrangements occur, we suggest a process in which the substrate and the coenzyme are bound before the reaction begins. The order in which squalene synthetase binds substrates and releases products is known (Beytia *et al.*, 1973) and is compatible with our mechanism.

Presqualene pyrophosphate must be oriented in the active site such that the $C_{2'}$–$C_{3'}$ cyclopropane bond is trans to the C_1–oxygen bond in order to accommodate the expected stereoselectivity for C_1. Selection of a particular conformer of **2** results from the topology of the active site. Interestingly, the enzyme is specific for the conformer that has at least a ninefold kinetic advantage over all others if charge delocalization into the $C_{1''}$–$C_{2''}$ double bond occurs during ionization (Poulter, 1972). Ionization of cyclopropylcarbinyl pyrophosphates would be triggered, even in a nonpolar environment, by neutralization of the negative charge in the pyrophosphate dianion of **2**.

Heterolysis of the C_1–oxygen bond in the bound substrate should give an intimate ion pair. The relative orientations of the resulting primary cyclopropylcarbinyl cation, NADPH, and an oxygen atom of the pyrophosphate group (represented as a sphere) are shown in Figure 1. If the relative positions of NADPH and the two ionic fragments are maintained throughout rearrangement of **5** to **7**, the negatively charged pyrophosphate could be used as a template for directing the head-to-head rearrangement.

An analysis of the ion pair shown in Figure 1 reveals that it has three properties which are important for the ensuing rearrangements. If cyclopropylcarbinyl cation **5** opens up to allylic isomer **16** by rupture of the $C_{1'}$–$C_{3'}$ cyclopropane bond (see Scheme V), a considerable separation of positive and negative centers must occur. Crude estimates, based on an electrostatic model (Ritchie, 1972), indicate that the cyclopropylcarbinyl to allyl isomerization would be endothermic by 8–15 kcal/mol in an ion pair relative to a free ion.[6] If isomerization to the allylic cation is blocked, the location of

[6] The large range of values results from various combinations of estimates of charge distribution and dielectric constant.

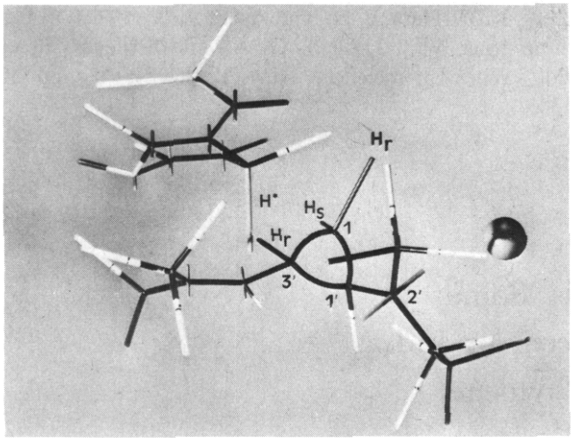


FIGURE 3: Ion pair between tertiary cyclopropylcarbinyl cation **7** and pyrophosphate.

NADPH precludes transfer of hydride to $C_{3'}$ in the cyclopropane ring of **5** with inversion of configuration. There should be a substantial stereoelectronic barrier to attack at $C_{3'}$ with retention which could prevent reaction of cation **5** with NADPH. The interaction between **5** and pyrophosphate must be strong enough to block rearrangement of the cyclopropylcarbinyl cation to allylic species **16**. The stereoelectronic barrier to reaction with NADPH no longer exists in the allylic cation and hydride transfer to $C_{3'}$ would result. Finally, of the intimate ion pairs resulting from the two possible 1,2-bond migrations in **5**, cyclobutyl cation **6** maintains close proximity of positive and negative centers while rearrangement to the other cyclobutyl species (**18**) results in charge separation. Thus, by use of an ion-pair mechanism, the enzyme forces the isomerization of **5** to **6** by blocking other alternatives.

A rapid rearrangement of **6** to **7** (or perhaps **5** directly to **7**) is expected from our chemical studies. The resulting intimate ion pair is also situated for optimum interaction of charged centers. If electrostatic attraction is strong enough to suppress the cyclopropylcarbinyl to allyl isomerization for primary cation **5**, our experiments suggest the corresponding isomerization should also be eliminated for the tertiary isomer **7**. However, NADPH is now properly oriented to allow transfer of hydride to $C_{3'}$ with inversion of configuration. Our model studies indicate that the regioselectivity for reaction of **7** with NADPH should favor $C_{3'}$ over $C_{2''}$. However, the location of the hydride to be transfered with respect to $C_{3'}$ and $C_{2''}$ is the major factor in determining the regiospecificity and is undoubtedly set by the enzyme.

Ion-pair intermediates similar to those shown in Figures 1–3 can be used as the basis of a mechanism for the biosynthesis of *cis*- and *trans*-phytoene. One only need remove NADPH and insert a basic functional group to assist with removal of the appropriate proton from C_1 of cation **7**. Presumably the base is covalently bound to the enzyme. In both mechanisms the functions of the enzymes include proper alignment of the substrates, triggering ionization of the carbon–oxygen bond in **2**, and anchoring the cation and anion while rearrangement takes place.

There are a few other observations which are relevant to an ion-pair mechanism. Internal return between pyrophosphate and **5**, **6**, or **7** will not complicate the transformation since the primary cyclopropylcarbinyl and cyclobutyl pyrophosphates (**2** and **10**-OPP, respectively) are comparable in reactivity and about a factor of 10^6 less reactive than tertiary isomer **9**-OPP (Poulter *et al.*, 1972b). Whatever interaction is sufficient to trigger ionization of **2** should also ionize any of the isomeric pyrophosphates produced by internal return. Also the skeletal rearrangements must take place in a cavity of limited volume. If Figures 1–3 are superimposed, a maximum leeway in any direction of only 1.5 Å is necessary to accommodate the entire reaction sequence, which is no greater than the expansion necessary when squalene or both isomers of phytoene are obtained from cyclopropylcarbinyl pyrophosphates independent of mechanism.

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The Stereochemistry of *trans*-Phytoene Synthesis. Some Observations on Lycopersene as a Carotene Precursor and a Mechanism for the Synthesis of *cis*- and *trans*-Phytoene†

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ABSTRACT: *trans*-Phytoene biosynthesized by a *Mycobacterium sp.* has been shown to retain one *pro-S* and one *pro-R* hydrogen from C-1 of the two molecules of geranylgeranyl pyrophosphate that constitute this carotene. This complements the finding of Williams *et al.* [Williams, R. J. H., Britton, G., Charlton, J. M., and Goodwin, T. W. (1967), *Biochem. J. 104*, 767–777] who demonstrated that two *pro-R* hydrogens were retained in *cis*-phytoene. Although Barnes *et al.* [Barnes, F. J., Qureshi, A. A., Semmler, E. J., and Porter, J. W. (1973), *J. Biol. Chem. 248*, 2768–2773] have presented evidence that lycopersene is a precursor to phytoene, a stereochemical analysis of phytoene synthesis shows that lycopersene can be a precursor to *cis*-phytoene only if two special and unlikely requirements are met. These considerations make it unlikely that lycopersene is a carotene precursor. We propose a mechanism for the synthesis of *cis*- and *trans*-phytoene directly from prephytoene pyrophosphate.

The stereochemical aspects of bond formation in polyterpenoid biosynthesis have been studied extensively (Popjak and Cornforth, 1966; Goodwin, 1971). In an investigation concerning carotene biosynthesis, Williams *et al.* (1967) and Buggy *et al.* (1969) established that both *pro-S* hydrogens are lost from C-1 of geranylgeranyl pyrophosphate during its conversion to *cis*-phytoene. One would anticipate a retention of different hydrogens during the synthesis of the trans isomer if *cis*- and *trans*-phytoenes are synthesized from common intermediates by a similar mechanism. We have examined the stereochemistry of hydrogen retention during the synthesis of *trans*-phytoene from geranylgeranyl pyrophosphate in a bacterial system and have found that 1 *pro-R* and 1 *pro-S* hydrogen are retained during this transformation as predicted. These results in conjunction with those of Williams *et al.* (1967) and Buggy *et al.* (1969) lead us to postulate a consistent mechanism for *cis*- or *trans*-phytoene synthesis from prephytoene pyrophosphate.

A stereochemical analysis of phytoene synthesis reveals that lycopersene can be a precursor of *cis*-phytoene only if either of two requirements, which are considered unlikely, can be met. These and other considerations have led us to conclude that lycopersene is probably not a normal precursor to carotenes.

Materials and Methods

all-trans-Geranylgeraniol, a generous gift from Dr. L. J. Altman, was oxidized by MnO_2. The resulting aldehyde was then reduced with NaB^3H_4 to form [1-$^3H_2$]geranylgeraniol (75 Ci/mol). [1-^{3}H]Geranylgeranial, prepared from [1-$^3H_2$]-geranylgeraniol by MnO_2 oxidation, was stereoselectively reduced by NADH and liver alcohol dehydrogenase to yield (1*S*)-[1-^{3}H]geranylgeraniol (38 Ci/mol) (Donninger and Ryback, 1964). The pyrophosphate esters of the alcohols were prepared and isolated by methods previously described (Gregonis and Rilling, 1973). [4-^{14}C]Isopentenyl pyrophosphate (4.2 Ci/mol) was prepared by the method of Tchen (1963). [4-^{14}C]Geranylgeranyl pyrophosphate was enzymatically prepared from farnesyl pyrophosphate and [4-^{14}C]isopentenyl pyrophosphate. The enzyme used was derived from a photoinduced *Mycobacterium sp.* by ammonium sulfate precipitation (35%) of a 100,000*g* supernatant fraction. The ammonium sulfate was removed by dialysis against 0.05 M potassium phosphate–1 mM $MgCl_2$ (pH 7.4). The incubation mixture contained 11 mg of enzyme protein and 0.4 μM isopentenyl pyrophosphate, 1.6 μM *trans*-farnesyl pyrophosphate, 0.05 M potassium phosphate (pH 7.4), and 1 mM $MgCl_2$ in a volume of 4 ml. The [4-^{14}C]geranylgeranyl pyrophosphate was extracted into 1-butanol and purified by ion exchange chromatography (Gregonis and Rilling, 1973). For the experiments described, it was combined with (1*S*)-[1-^{3}H]-geranylgeranyl pyrophosphate. [1-$^3H_2$,4-^{14}C]Geranylgeranyl pyrophosphate was prepared from *trans*-farnesyl pyrophosphate and [1-$^3H_2$,4-^{14}C]isopentenyl pyrophosphate in the same manner. [^{3}H]NADPH was prepared by chemical reduction of NADP by [^{3}H]$NaBH_4$ as described by Chaykin (1965). The specific activity was 35 Ci/mol.

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