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Recombinant Squalene Synthase. A Mechanism for the Rearrangement of Presqualene Diphosphate to Squalene

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Abstract: Squalene synthase (SQase) catalyzes the condensation of two molecules of farnesyl diphosphate (**FPP**) to form presqualene diphosphate (**PSPP**) and the subsequent rearrangement and NADPH-dependent reduction of **PSPP** to squalene (**SQ**). These reactions are the first committed steps in cholesterol biosynthesis. When recombinant SQase was incubated with **FPP** in the presence of dihydroNADPH ($NADPH_3$, an unreactive analogue lacking the 5,6-double bond in the nicotinamide ring), three products were formed: dehydrosqualene (**DSQ**), a C_{30} analogue of phytoene; 10(*S*)-hydroxysqualene **(HSQ)**, a hydroxy analogue of squalene; and rillingol (**ROH**), a cyclopropylcarbinyl alcohol formed by addition of water to the tertiary cyclopropylcarbinyl cation previously proposed as an intermediate in the rearrangement of **PSPP** to **SQ** (Poulter, C. D. *Acc. Chem. Res.* **1990**, *23*, 70−77). The structure and absolute stereochemistry of the tertiary cyclopropylcarbinyl alcohol were established by synthesis using two independent routes. Isolation of **ROH** from the enzyme-catalyzed reaction provides strong evidence for a cyclopropylcarbinyl−cyclopropylcarbinyl rearrangement in the biosynthesis of squalene. By comparing the SQase-catalyzed solvolysis of **PSPP** in the absence of $NADPH_3$ to the reaction in the presence of $NADPH_3$, it is apparent that the binding of the cofactor analogue substantially enhances the ability of SQase to control the regio- and stereochemistry of the rearrangements of **PSPP**.

Squalene synthase catalyzes the synthesis of **PSPP**[1] from **FPP** and its subsequent rearrangement to squalene. These reactions are the first committed steps in sterol biosynthesis. Rilling discovered that **PSPP** accumulated in incubations that did not contain NADPH and was then converted to **SQ** when NADPH was subsequently added.[2] After the structure of **PSPP** was firmly established,[3,4] several groups proposed mechanisms for the conversion of **PSPP** to **SQ**[4−8] based on the rearrangements of cyclopropylcarbinyl cations.[9−17] Model studies designed to mimic the enzymatic reaction gave mixed results. Although several important features predicted by the mechanistic proposals were duplicated by the model reactions, the regioselectivity of the c1′−2−3 → 1′−1 rearrangement required to convert the presqualene skeleton to **SQ** was poor, giving only trace quantities of 1′−1 products.[18−20]

Mechanistic studies with SQase have been hampered by the unavailability of purified enzyme. A soluble recombinant form of SQase lacking a putative membrane-spanning α-helix at its C-terminus[21] catalyzed reactions similar to those of the wild-type membrane-bound protein.[22−24] In an incubation with NADPH, **FPP** was rapidly converted to **PSPP**, which was then rapidly processed to **SQ**. However, when NADPH was not added to the buffer, **PSPP** accumulated and was then slowly

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(1) All of the abbreviations used except those listed here were defined in the preceding article (Jarsfter et al., see ref 27): FPPase, farnesyl diphosphate synthase; $NADPH_3$, 5,6-dihydro reduced nicotinamide adenine dinucleotide phosphate; NMO, *N*-methylmorpholine *N*-oxide; **ROH**, rillingol; TPAP, tetrapropylammonium perruthenate.
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converted to a mixture of triterpenes with 1′−1 and 1′−3 fusions between the farnesyl residues,[25−27] reminiscent of the structures seen in earlier model studies.[18−20] These reactions were stereoselective, and the absolute stereochemistries of the triterpene products corresponded to those of the analogous biosynthetic transformations that give the 1′−1 and 1′−3 isoprenoids found in nature.[27] In addition, the proportion of 1′−1 products was substantially higher in the SQase-catalyzed reaction than in the model studies.

SQase binds its substrates in an ordered manner.[24] Two molecules of **FPP** add to the enzyme, followed by NADPH, to generate an E·**FPP**·**FPP**·NADPH complex. Under normal conditions, **PSPP** is synthesized from **FPP** and then converted directly to **SQ** without dissociating from the enzyme. NADPH plays an important role in catalysis beyond serving as a reductant. Without the cofactor, the rate of **PSPP** synthesis decreases substantially.[27] Although SQase can trigger the reaction that normally leads to **SQ** from **PSPP**, the enzyme can no longer control the regiochemistry of the ensuing rearrangement, and substantial amounts of 1′−3 products are formed along with 1′−1 structures.[27] Also, there is an accompanying reduction in stereocontrol. We now report studies in which SQase was incubated with **FPP** and 5,6-dihydroNADPH ($NADPH_3$), an unreactive analogue of the normal cofactor. When the NADPH binding site was occupied by $NADPH_3$, no 1′−3 products were seen. In addition, a new cyclopropylcarbinyl triterpene alcohol with a c1′−1−2 fusion of farnesyl units was formed along with the 1′−1 compounds. This new triterpene alcohol provides direct evidence for a c1′−2−3 → c1′−1−2 cyclopropylcarbinyl−cyclopropylcarbinyl cationic rearrangement within the active site of SQase during the biosynthesis of 1′−1 isoprenoids.

Results

Studies with $NADPH_3$. We used $NADPH_3$ to determine the effect of occupying the NADPH-binding site on the product distribution from the SQase-catalyzed rearrangement of **PSPP**. $NADPH_3$ was prepared by a partial catalytic hydrogenation of $NADP^+$ according to the procedure of Dave et al.[30] A steady-state kinetic analysis indicated that the analogue was a competitive inhibitor against NADPH, $K_I = 45\ \mu M$. A comparison of K_I with K_M^{NADPH} ($\sim 100\ \mu M$) suggests that the affinity of the analogue for the NADPH site is similar to that of the normal cofactor.[24]

Incubation of recombinant SQase with saturating levels of **FPP** and $NADPH_3$ resulted in the rapid formation of **PSPP**, $k_{cat} \approx 0.2\ s^{-1}$, followed by a much slower consumption of **PSPP**, $k_{cat} \approx 0.002\ s^{-1}$. Similar results were found for incubations in the absence of NADPH.[27] After 1.5 h, the incubation mixture was extracted with methyl *tert*-butyl ether (MTBE). Analysis of the mixture by HPLC revealed three products. Two of the compounds, **DSQ** (23%) and **HSQ** (55%), were seen previously in incubations where $NADPH_3$ was not included in the incubation buffer.[27] The third compound, rillingol (**ROH**, 17%), which was not previously observed, eluted between **HSQ** and the 1′−3 triterpene hydroxybotryococcene (**HBO**) (see Figure 1). The new product gave a molecular ion at *m*/*z* 426.3865, corresponding to a triterpene alcohol that is isomeric with **HSQ** and **HBO**. The ^1H NMR spectrum of the alcohol had a three-proton resonance at 1.14 ppm for a tertiary methyl group, resonances for four cyclopropyl protons, and a vinyl proton that appeared as a doublet at 4.6 ppm. The chemical shifts and coupling patterns for the cyclopropyl and vinyl protons were reminiscent of those reported for rothrockene, an irregular monoterpene with a c1′−1−2 structure.[28] The mechanism originally proposed by Rilling et al.[4] for the conversion of **PSPP** to **SQ** involved rearrangement of a c1′−2−3 presqualene cation to its c1′−1−2 isomer. The structure proposed for **ROH** (see Figure 2) is the product expected from capture of the c1′−1−2 cation at the carbinyl carbon by water. **ROH** has the same carbon skeleton as "compound X", originally proposed by Rilling as the structure for **PSPP**,[2] and has previously been synthesized as a racemic mixture of diastereomers.[29]

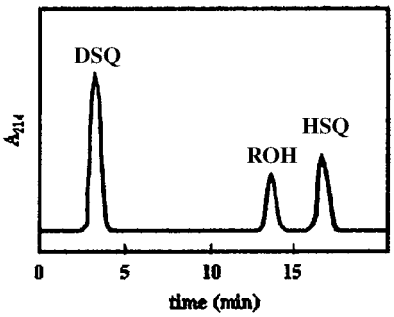

Figure 1. HPLC trace of products from incubation of SQase with **FPP** and $NADPH_3$. Analysis on a normal-phase Ranin microsorb MV silica gel column with isocratic elution using 1:19 (v/v) MTBE/hexanes at 1 mL/min.

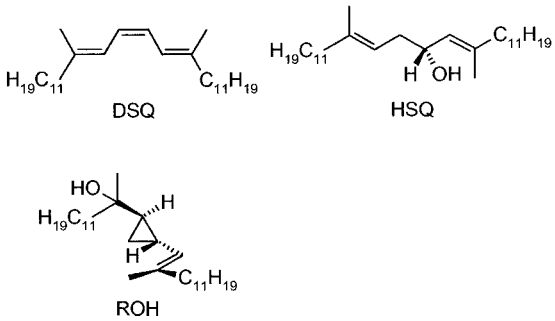

Figure 2. Products from incubation of SQase with **FPP** and $NADPH_3$.

Absolute Stereochemistry of Rillingol. On the basis of the absolute stereochemistry of **PSPP** and the suprafacial stereoselectivity for 1,2-bond migrations during cyclopropylcarbinyl−cyclopropylcarbinyl rearrangements, we predicted that the chiral cyclopropane carbons in **ROH** should have a 1*S*,2*R* configuration.[30] This prediction was verified, and the absolute stereochemistry of the carbinyl center was established by the two independent syntheses of **ROH** shown in Schemes 1 and 2. The synthesis presented in Scheme 1 established the absolute stereochemistry of the cyclopropane ring, while that in Scheme 2 established the absolute stereochemistry of the carbinyl carbon. Together, they unambigiously determine the structure of **ROH** produced by the SQase-catalyzed rearrangement of **PSPP**.

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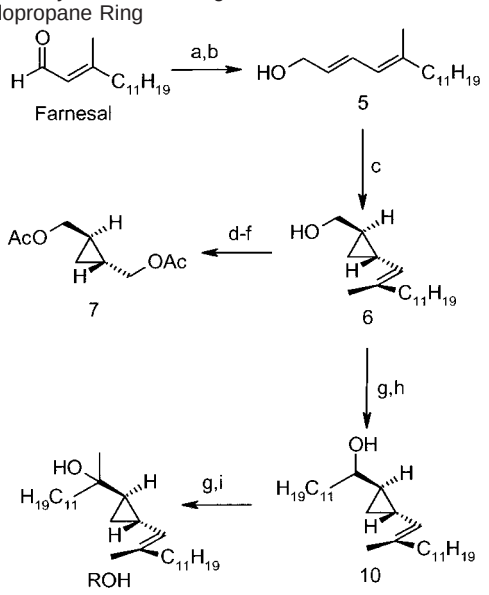
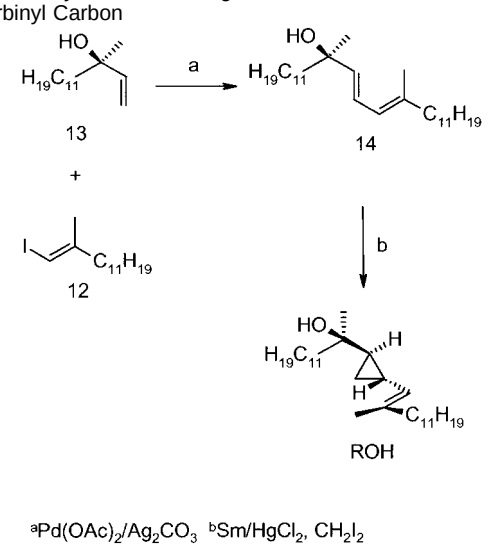
Scheme 1. Synthesis of Rillingol: Absolute Stereochemistry of the Cyclopropane Ring



Scheme 2. Synthesis of Rillingol: Absolute Stereochemistry of the Carbinyl Carbon



(a) The Cyclopropane Ring. In the synthesis described in Scheme 1, farnesal was converted to *trans*-allylic alcohol **5** in high yield by a Horner−Wadsworth−Emmons condensation, followed by reduction of the resulting ethyl ester (**4**). Alcohol **5** was then complexed with (D)-dioxaboralane[31] and added to Furukawa's reagent[32] to give disubstituted cyclopropylcarbinol **6**. The enantioselectivity of the cyclopropanation was determined by converting **6** to the corresponding Mosher's ester.[33] A ^{19}F NMR spectrum of the ester had two peaks for the trifluoromethyl resonance in a 9:1 ratio. The primary cyclopropylcarbinol was oxidized with TPAP/NMO[34] to cyclopropyl aldehyde **8**, which was treated with homogeranylmagnesium iodide[35] to give diastereomeric secondary cyclopropylcarbinols **10**. The mixture of diastereomers was oxidized with TPAP/NMO, and the resulting ketone (**11**) was treated with methylmagnesium bromide to furnish **ROH** as a 1:6 mixture of diastereomers. Although the diastereomers did not resolve during flash chromatography,[29] we were able to separate and purify the compounds by HPLC. When the mixture of diastereomers was coinjected with **ROH** from the enzymatic reaction, the alcohol produced by SQase coeluted with the minor diastereomer obtained by synthesis. In addition, the ^1H NMR and mass spectra of the minor diastereomer and the enzyme-derived material were identical.

The absolute stereochemistry of the cyclopropane ring in **6** was established as shown in Scheme 1. Cyclopropyl diacetate **7** was obtained from **6** by ozonolysis, reduction of the ozonides with $LiAlH_4$, and treatment of the resulting alcohols with acetic anhydride. A comparison of the optical rotation of **7**, $[\alpha]^{23}_D$ −14.1°, with the literature value[36] for (*R*,*R*)-diacetate, $[\alpha]^{23}_D$ −17.7°, served to establish its stereochemistry and the 2*R*,3*S* stereochemistry of primary alcohol **6**.

(b) The Carbinyl Carbon. The absolute stereochemistry of the carbinyl carbon in **ROH** was established by a second synthesis (see Scheme 2). (*R*)-Nerolidol (**12**) was prepared from farnesol in a 19:1 er by the procedure of Yasuda et al.[37] Vinyl iodide **12** was prepared from geranyl bromide[24] by the procedure of Zheng et al.[38] and coupled to **13** with palladium(II) acetate and Ag_2CO_3.[39] The trans disubstituted double bond in alcohol **14** was then regioselectively cyclopropanated using $Sm/HgCl_2/CH_2I_2$[40] to give **ROH**. The cyclopropanation reaction produced a single diastereomer in 29% yield after purification by HPLC. The ^1H and ^{13}C NMR spectra of the tertiary cyclopropylcarbinyl alcohol obtained by this route were identical to those for the minor product obtained in Scheme 1 and **ROH** from the enzymatic reaction. Hence, **ROH** is the 10*R*,11*R*,12*S* stereoisomer.

Absolute Stereochemistry of HSQ. The absolute configuration and optical purity of **HSQ** isolated from an incubation when $NADPH_3$ was included in the buffer were determined as described in the preceding paper.[27] The Mosher's ester derivative of **HSQ** obtained by treatment of the alcohol with (*S*)-Mosher's chloride gave a single peak that had the same retention time as an authentic sample of the Moshers ester from (*R*)-**HSQ** on a Chiracel OD column. The ester was degraded with ozone, the ozonide was reduced to the corresponding diol with $NaBH_4$, and the hydroxyl groups were esterified with (*S*)-Mosher's chloride. The tris-Mosher ester gave a single peak that comigrated with a sample of tris ester prepared from (*R*)-1,2,4-butanetriol on normal-phase HPLC. Thus, **HSQ** isolated from the incubation of SQase with **FPP** and $NADPH_3$ is the *R* enantiomer. We estimate that at least 1% of the *S* enantiomer would have been detected in the HPLC traces of the Mosher's esters of **HSQ** and (*R*)-1,2,4-butanetriol.

Stereochemistry of the Conversion of FPP to DSQ. The stereochemistry of the double bonds in the central conjugated

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triene of **DSQ** was determined to be *E*,*Z*,*E* by UV spectroscopy and described in the preceding paper.[27] The stereochemistry for removal of a proton from C(1) of each of the molecules of **FPP** incorporated into **DSQ** was determined by mass spectrometry for samples of **DSQ** obtained from (*R*)- and (*S*)-[1-^{2}H]**FPP**.[27] **DSQ** produced from (*R*)-[1-^{2}H]**FPP** had a molecular ion at *m*/*z* 410, indicating the retention of two deuterium atoms, whereas the material from (*S*)-[1-^{2}H]**FPP** had a molecular ion at *m*/*z* 408, indicating that both deuterium atoms had been lost. These results are similar to those reported when **FPP** was incubated without NADPH.

SQ produced from the incubation of SQase with (*R*)-[1-^{2}H]-**FPP** and NADPH had a molecular ion at *m*/*z* 412, while material from an incubation with (*S*)-[1-^{2}H]**FPP** gave **SQ** with a molecular ion at *m*/*z* 411. These results are consistent with earlier stereochemical studies of squalene biosynthesis by Cornforth and Popjak,[41] where a *si*-hydrogen is lost from C(1) of one of the molecules of **FPP** during the formation of **PSPP**.

Effects of Solvent and Divalent Metal Ions. In the preceding paper we found that methanol competed with water as a nucleophile in the substitution reaction that produced **HSQ**.[27] Under similar conditions in buffer containing 20% methanol and $NADPH_3$ in addition to the normal components, only 2% of the **HSQ** methyl ether was formed. This observation is consistent with our proposal that the products are formed from the SQase·**PSPP**·$NADPH_3$ complex, and apparently binding of $NADPH_3$ either excludes some of the methanol from the active site or interferes with addition of methanol to the cationic intermediates. We also reported that the mixture of products formed upon the SQase-catalyzed solvolysis reaction was sensitive to the divalent metal.[27] In contrast, we found no change in the distribution of products when Mg^{2+} was replaced by Mn^{2+} for incubations in the presence of $NADPH_3$.

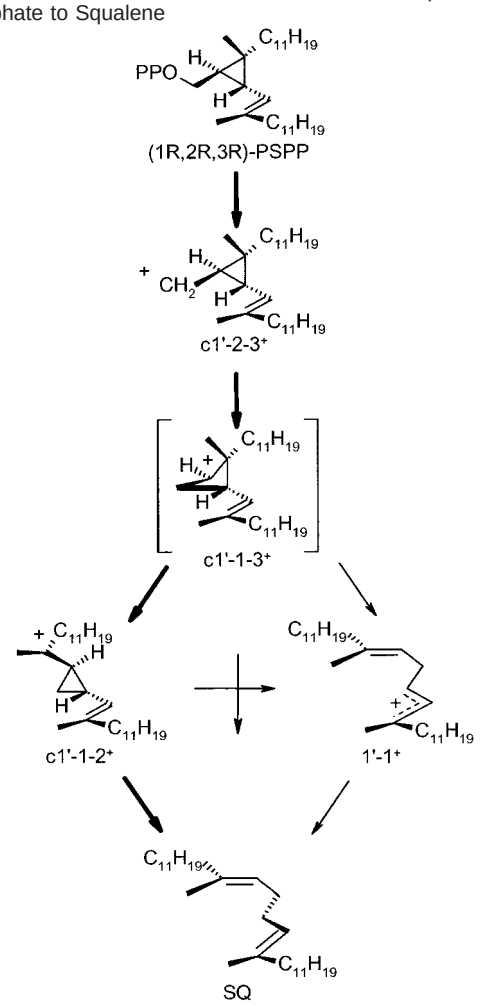
Discussion

The steps leading from **PSPP** to **SQ** have been a topic of speculation ever since **PSPP** was discovered as an intermediate in the 1′−1 coupling of **FPP**. Salient features of the mechanisms that have been proposed are summarized in Scheme 3. All involve formation of a primary c1′−2−3 cyclopropylcarbinyl cation from **PSPP**, followed by rearrangements that eventually lead to the 1′−1 system.[4−8,42,43]

We now have data that allow us to distinguish among these proposals. Isolation of **ROH** from incubations of **FPP** with SQase in the presence of $NADPH_3$ provides direct evidence for a cyclopropylcarbinyl−cyclopropylcarbinyl rearrangement of c1′−2−3$^+$ to c1′−1−2$^+$. These rearrangements are known to be highly stereoselective[9−12] and can be envisioned as the net result of two consecutive suprafacial [1,2] rearrangements where the C(2)−C(1′) bond in the primary c1′−1−2$^+$ cation rearranges to form the c1′−1−3$^+$ cyclobutyl isomer, followed by rearrangement of the C(1)−C(3′) bond to give the tertiary c1′−1−2$^+$ cation. Cyclopropylcarbinyl−cyclopropylcarbinyl rearrangements can proceed through cyclobutyl cations as discrete intermediates. However, the putative cyclobutyl species in the c1′−2−3$^+$ to c1′−1−2$^+$ rearrangement could not be trapped during model studies, suggesting that the c1′−1−3 cation might be the transition state that links c1′−2−3$^+$ and c1′−1−2$^+$.[8] The 11*R* and 12*S* stereocenters in **ROH** are exactly what one expects for the c1′−2−3$^+$ → c1′−1−2$^+$ rearrangement that converts (1*R*,2*R*,3*R*)-**PSPP** to **ROH**.[8] The 10*R* stereocenter in **ROH** is generated by addition of water to the *re* face of the tertiary cation. One would not anticipate a stereoelectronic preference for this reaction, and presumably the direction of addition to the trigonal center in c1′−1−2$^+$ is imposed by structural features in the catalytic site of the enzyme. Finally, formation of (12*R*)-**HSQ** from c1′−1−2$^+$ is the predicted result from addition of water to C(1′) of the tertiary cyclopropylcarbinyl cation with inversion. With no compelling evidence requiring 1′−1$^+$ as a precursor of c1′−1−2 or 1′−1 products, we view its inclusion in the mechanism for formation of **SQ** from **PSPP** as an unnecessary embellishment.

The active site of SQase is designed to selectively promote the c1′−2−3 → c1′−1−2 rearrangement over other possible reactions of c1′−2−3$^+$. In model studies, rearrangement of the primary c1′−2−3 cation to its allylic 1′−3 isomer was highly favored where $k_{c1'-1-2}/k_{1'-3} \approx 4 \times 10^{-4}$. When SQase catalyzed the "solvolysis" of **PSPP** in the absence of NADPH, $k_{c1'-1-2}/k_{1'-3} = 5.2$, and the ratio increased to >200 when $NADPH_3$ was bound in the active site along with **PSPP**. These rate ratios correspond to differences in transition-state energies leading from c1′−2−3$^+$ to c1′−1−2$^+$ or 1′−3$^+$, where $\Delta G^{\ddagger}_{c1'-2-3} - \Delta G^{\ddagger}_{1'-3} \approx 4.7$ kcal/mol in the model system, −1.0 kcal/mol

Scheme 3. Mechanisms for the Conversion of Presqualene Diphosphate to Squalene



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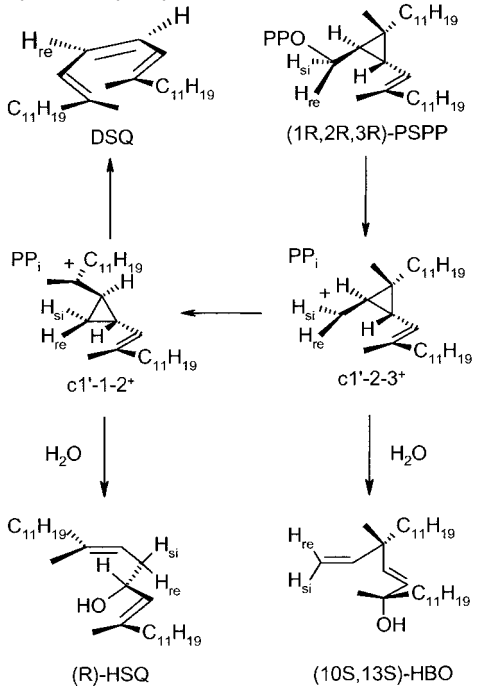
Scheme 4. Stereochemistry for Formation of Hydroxybotryococcene, Hydroxysqualene, and Dehydrosqualene from Presqualene Diphosphate



for the enzyme catalyzed "solvolysis" in the absence of NADPH, and > −3.2 kcal/mol when $NADPH_3$ is bound! Thus, SQase selectively catalyzes the c1′−2−3^+→ c1′−1−2^+ rearrangement by lowering the barrier of the cyclopropylcarbinyl−cyclopropylcarbinyl rearrangement by over 7.9 kcal/mol relative to the barrier for the accompanying c1′−2−3^+ → c1′−3^+ rearrangement. Thus, we propose the pathway in Scheme 3 delineated by heavy arrows.

The stereochemistries for formation of **DSQ**, **HSQ**, **HBO**, and **ROH** are summarized in Scheme 4. A detailed analysis of these reactions provides important insights about the conformation of **PSPP** in the active site of the enzyme and the mechanism for formation of the products. During synthesis of squalene, the C(1) methylene in **PSPP** is inverted during the c1′−2−3 to 1′−1 rearrangement.[30,41] This requires that the C(1) oxygen and the C(2)−C(1′) bonds in **PSPP** be antiperiplanar in the E·**PSPP**·NADPH complex in order to accommodate the mandatory suprafacial [1,2] bond migration of the C(1′)−C(2) bond from C(2) to C(1) during rearrangement of c1′−2−3^+ to c1′−1−2^+. Both **SQ** and **HSQ** are formed with inversion at C(1′), again in accord with the stereoelectronic requirements for addition of nucleophiles to the cyclopropane carbons in cyclopropylcarbinyl cations. Thus, the nucleophile (NADPH or water) must be located at the backside of C(1′) in c1′−1−2^+ after c1′−2−3^+ → c1′−3^+. Interestingly, this particular topology would require the nucleophile to add to C(1′) in c1′−2−3^+ with retention of configuration, a stereoelectronically unfavorable orientation. Finally, the *Z* double bond between C(1′) and C(2′) **HBO**[27] requires that the dihedral angle between the C(1′)−H and C(2′)−H bonds in the E·**PSPP** complex be greater than 90° and probably near 180°, assuming that **SQ** and **HBO** are both derived from c1′−2−3^+, as suggested by the regioselective formation of **HSQ** and **HBO**, to the exclusion of their respective allylic isomers iso**HBO** and **isoHSQ**, when SQase is incubated with **FPP** in the absence of NADPH.[27]

On the basis of the stereochemical analysis presented above, it is straightforward to envision how botryococcene synthase, which generates a 1′−3 structure from **PSPP**, might have evolved from squalene synthase by mutations that repositioned NADPH slightly so that the hydrogen atom transferred from the cofactor is moved from near C(1′), where a stereoelectronic barrier prevents addition to c1′−2−3^+ with retention of configuration, to near C(3′), where addition to the *si* face of the double bond is allowed.

The stereochemical analysis of the reactions catalyzed by SQase also has important mechanistic implications for the c1′−2−3^+ → 1′−3^+ rearrangement. In an unconstrained model system, the transition state for c1′−2−3^+ → 1′−3^+ was ∼4.7 kcal/mol below that for c1′−2−3^+ → c1′−1−2^+. To achieve the observed level of regioselectivity seen during the biosynthesis of **SQ**, the transition state for the c1′−2−3^+ → c1′−1−2^+ rearrangement must be selectively stabilized by >7.8 kcal/mol in the SQase·$NADPH_3$·c1′−2−3^+·PP_i complex. Assuming that **PSPP** is bound in the conformation shown in Scheme 4, the negatively charged pyrophosphate anion is located near the bond between C(2) and C(3) in the cyclopropane ring after the C(1)−oxygen bond in bound **PSPP** is ruptured. We propose that regioselectivity in the rearrangement is achieved through electrostatic interactions between positively and negatively charged partners in the c1′−2−3^+·PP_i and c1′−1−2^+·PP_i intimate ion pairs. These interactions should facilitate c1′−2−3^+ → c1′−1−2^+ relative to the other reactions seen in model studies or in the enzyme-catalyzed reaction in the absence of NADPH.

The stereochemistry for elimination of a proton from C(1) in c1′−1−2^+ to form the 1′−1 double bond in (*Z*)-**DSQ** is identical to that seen during biosynthesis of (*Z*)-phytoene from prephytoene diphosphate.[44] Interestingly, the other C(1) proton is lost during biosynthesis of (*E*)-phytoene.[44] All of these results can be easily explained if one assumes that the elimination of the proton removed from C(1′) in the corresponding c1′−1−2 cation proceeds through a least motion transition state to give the respective *Z* and *E* double bonds.

Several aspects of the reactions catalyzed by SQase and PHase suggest that the two proteins share a common ancestor. The cyclopropane rings in **PSPP** and **PPPP** have identical 1*R*,2*R*,3*R* absolute stereochemistries, and the two enzymes catalyze identical c1′−2−3 → 1′−1 cyclopropylcarbinyl−cyclopropylcarbinyl rearrangements. When deprived of NADPH, **DSQ**, a C_{30} analogue of phytoene, is one of the major products synthesized by SQase. In addition to these chemical similarities, multiple alignments of amino acid sequences for squalene and phytoene synthases from widely diverged organisms indicate that the enzymes are genetically related. Table 1 lists conserved amino acids in three different regions that are found in both enzymes. When the conserved regions are mapped onto the recently published X-ray structure of human SQase,[45] all three cluster around the putative active site of the enzyme, as illustrated in Figure 3. In addition, SQase consists of α-helicies arranged in an "isoprenoid fold" [46] similar to the one first reported for farnesyl diphosphate synthase (FPPase),[47] suggesting a familial link between the cyclopropanation enzymes and

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Table 1. Conserved Regions[a] in Squalene[b] and Phytoene Synthases[c]

	Region I	Region II	Region III
Squalene Synthase	YLxlRalDtxED	YchyvAGlVG	GlfLQktNIiRdYxED
Phytoene Synthase	YxxxRxxDxxxD	YxxxvAxxxG	GxAxQxtNixRDxxeD
Consensus	YxxxRxxDxxxD	YxxxvAxxxG	GxxxQxtNixRdxxeD

[a] Uppercase designates conserved amino acids with no more than one mismatch in the sequence alignments. Lowercase designates conserved amino acids with no more than three mismatches in the sequence alignments. Amino acids in blue denote aspartate/glutamate-rich putative diphosphate binding motifs. [b] Based on 19 sequences. [c] Based on 27 sequences. Includes one sequence for a dehydrosqualene synthase.

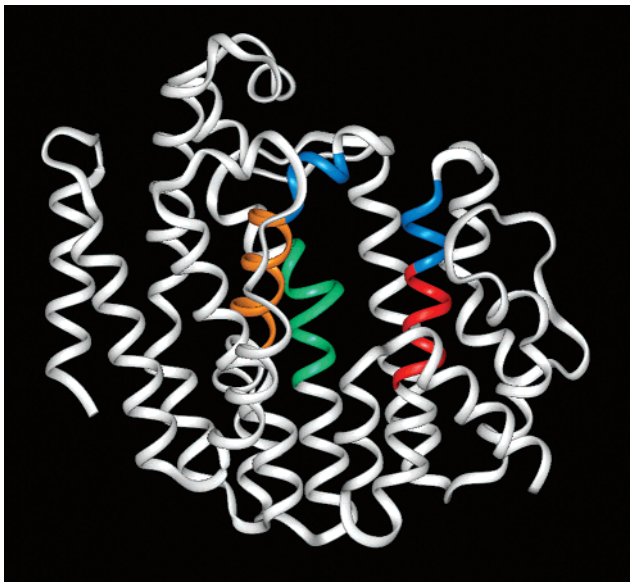

Figure 3. Ribbon diagram of human SQase showing the "isoprenoid fold" of the protein. The first (red), second (green), and third (gold) conserved regions common to SQase and PHase cluster around the putative active site, and the aspartate/glutamate-rich regions are shown in blue.

the chain elongation enzymes in the isoprenoid pathway. Also, the aspartate/glutamate-rich motifs in regions 1 and 2 map onto the highly conserved aspartate-rich sequences that bind the diphosphate residues or the substrates for FPPase. Finally, the recently published sequence for chrysanthemyl diphosphate synthase, an enzyme that catalyzes the cyclopropanation of two molecules of dimethylallyl diphosphate, is strikingly similar to FPPase.[48]

In conclusion, the biosynthesis of 1′−1 structures from c1′−2−3 cyclopropylcarbinyl diphosphates in the sterol and carotenoid pathways proceeds via c1′−2−3$^+$ and c1′−1−2$^+$ carbocationic intermediates. The key step in these reactions is a c1′−2−3$^+$ → c1′−1−2$^+$ cyclopropylcarbinyl−cyclopropylcarbinyl rearrangement. Without catalysis, the rearrangement is a minor reaction channel for the c1′−2−3 cation, where the preferred reaction is a c1′−2−3$^+$→ c1′−3$^+$ rearrangement. However, within the active site of the enzymes, the transition state for the cyclopropylcarbinyl−cyclopropylcarbinyl rearrangement is stabilized by more than 7.9 kcal/mol relative to the competing 1′−3 rearrangement. We suggest that squalene synthase and phytoene are able to synthesize their respective 1′−1 products with a high degree of regioselectivity by relying on electrostatic interactions between PP_i and the cyclopropylcarbinyl cations.

Experimental Section[49]

1,4,5,6-TetrahydroNADPH ($NADPH_3$). NADP (NH_4^+ salt, 100 mg, 0.12 mmol), 100 mg (5% Pd, 0.05 mmol) of Pd on $BaCO_3$, and 13 mg (0.11 mmol) of NH_4SO_4 were dissolved in 15 mL of H_2O. The solution with outgassed with N_2 before H_2 was added, and the progress of the reaction was followed by UV until the ratio of A_{260}/A_{290} reached ~1.2. The reaction mixture was passed through a 0.45 μm filter and concentrated by lyophilization. The residue was resuspended in 50 mM NH_4HCO_3 and chromatographed on a 100 mL DEA-sephacyl ion-exchange column using 100 mL of 100 mM NH_4HCO_3, a 300 mL linear gradient to 300 mM NH_4HCO_3, and 300 mM NH_4HCO_3 until the $NADPH_3$ eluted. The purest fractions, judged by $A_{260}/A_{290} = 1.2$, were pooled and lyophilized to give a white solid (75 mg, 0.073 mmol based on A_{290}, 61%). $NADPH_3$ was stored at −80 °C as the ammonium salt: UV $\lambda_{max} = 263$ ($\epsilon = 16\,500$ M^{-1} cm^{-1}) and 291 nm ($\epsilon = 13\,750$ M^{-1} cm^{-1}); ^{1}H NMR (D_2O) δ 8.44 (s, 1H), 8.21 and 8.18 (s, 1H), 7.22 (s, 1H,), 6.17 (d, 1H, $J = 5$ Hz), 6.16 (d, 1H, $J = 5$ Hz), 4.93 (dt, 1H, $J = 6.6$, 5 Hz), 4.56 (t, 1H, $J = 5$ Hz), 4.34 (m, 1H), 4.23−4.12 (m, 4H), 3.97−3.75 (m, 4H), 3.01 (ddd, 2H, $J = 6.5$, 6.5, 4.2 Hz), 1.96 (dd, 2H, $J = 6.5$, 6.5 Hz), 1.75−1.52 (m, 2H); HRMS (−) FAB calcd for $C_{21}H_{31}P_3N_7O_{17}$ (M − 1) 746.0991, found 746.0973.

SQase Assay. SQase (SA = 2.0 μmol min^{-1} mg^{-1}) was assayed by the procedure of Zhang and Poulter.[25] In a typical assay, [1-^{3}H]**FPP** (100 μM, 7.5 μCi ^{3}H/μM) was incubated with 200 ng of SQase in 200 μL of 50 mM MOPS buffer, pH 7.2, containing 20 mM $MgCl_2$, 1 mM DTT, 1 mg/mL BSA, and 2% (v/v) Tween 80. Reactions were initiated by the addition of SQase. A 4 × 4 matrix of varied concentrations of NADPH and $NADPH_3$ at fixed concentrations of **FPP** and enzyme was used to determine mode of inhibition by $NADPH_3$ and the inhibition constant.

Analysis of Products from Incubations of FPP with SQase in the Presence of $NADPH_3$. (a) Analytical Scale. Using conditions similar to those described for incubation of SQase with FPP in the absence of NADPH,[27] **FPP** (300 μM) was incubated with 1 mg (22 μM) of SQase in 50 mM MOPS buffer, pH 7.2, containing 700 μM $NADPH_3$ and 10 mM $MgCl_2$. The extracts were dried, concentrated, and analyzed by normal-phase HPLC.

(b) Preparative Scale. Typically, 5 mg (11.4 μmol) of **FPP** and 12 mg (0.3 μmol) of SQase were incubated at 30 °C in 12 mL of 50 mM MOPS buffer, pH 7.2, containing 10 mM $MgCl_2$ and 700 μM $NADPH_3$. The reaction was worked-up as previously described to give the following products in order of elution:

Dehydrosqualene (DSQ, **1**),[27] 4.0 min, 23%.

10-Hydroxysqualene (HSQ, **2**),[27] 28.5 min, 55%.

Rillingol (ROH, **3**), 24 min, 22%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.15 (t, 1H, $J = 6.3$ Hz), 5.11−5.07 (m, 3H), 4.61 (d, 1H, $J = 9.3$ Hz), 2.15−1.95 (m, 16H), 1.73 (s, 3H), 1.68 (s, 6H), 1.62 (s, 3H), 1.59 (s, 3H), 1.47−1.41 (m, 1H), 1.13 (bs, 1H), 1.12 (s, 3H), 0.85−0.79 (m, 2H), 0.42−0.39 (m, 1H); LRMS (EI, 70 eV) *m/z* 426 (M, 3),

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(48) Rivera, S.; Swedlund, B. D.; King, G. J.; Bell, R. N.; Hussey, C. E., Jr; Shattuck-Eidens, D. M.; Wrobel, W. M.; Peiser, G. D.; Poulter, C. D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4373−4378.

(49) General experimental conditions are described in ref 27.

408 (M − H_2O, 19), 271 (33), 149 (42), 81 (55), 69 (C_5H_9 100); HRMS (EI, 70 eV) for $C_{30}H_{50}O$ calcd 426.3861, found 426.3865.

Ethyl (*E*,*E*,*E*)-5,9,13-Trimethyl-2,4,8,12-tetradecatetraenoate (4). NaH (2.15 g, 53.5 mmol, 60% in mineral oil) was washed twice with 10 mL of pentane and suspended in 180 mL of dry toluene. The flask was cooled to 0 °C, and 9.39 g (53.5 mmol) of triethyl phosphonoacetate was added dropwise by syringe. The mixture was stirred for 50 min at 0 °C before 9.8 g (44.6 mmol) of farnesal was added dropwise. The reaction was stirred for an additional 2.5 h at 0 °C and was quenched by the addition of 20 mL of saturated NH_4Cl solution. The organic layer was separated, and the aqueous layer was washed 3 times with 25 mL of ether. The combined organic layers were dried and concentrated. The residue was chromatographed on silica (1:19 (v/v) ethyl acetate/hexane) to yield 10.61 g (82%) of a colorless oil: ^{1}H NMR δ 7.61 (dd, 1H, J = 15, 12 Hz), 5.99 (d, 1H, J = 12 Hz), 5.78 (d, 1H, J = 15 Hz), 5.05−5.13 (m, 2H), 4.20 (q, 2H, J = 7 Hz), 2.17 (m, 4H), 1.94−2.12 (m, 4H), 1.9 (s, 3H), 1.68 (s, 3H), 1.61 (s, 6H), 1.30 (t, 3H, J = 7 Hz); ^{13}C NMR δ 167.1, 149.8, 141.0, 135.8, 131.4, 124.2, 123.3, 123.2, 118.8, 60.1, 40.2, 39.7, 26.7, 26.2, 25.7, 17.7, 17.4, 16.0, 14.34; MS *m*/*z* 290 (M^+, 1.3), 245 (3.9) 154 (65), 137 (13), 69 (100); HRMS calcd for $C_{19}H_{30}O_2$ 290.2245, found 290.2254.

(*E*,*E*,*E*)-5,9,13-Trimethyltetradeca-2,4,8,11-tetraenol (5). A solution of 8.3 g (28.6 mmol) of **4** and 200 mL of CH_2Cl_2 was cooled to −78 °C before 68.6 mL (68.6 mmol, 1 M in hexanes) of DIBALH was added dropwise. The mixture was stirred at −78 °C for 1.5 h before addition of 5 mL of saturated NH_4Cl solution. The layers were separated, the aqueous layer was extracted with ether, and the combined organic layers were washed with brine, dried, and concentrated. Purification of the residue by flash chromatography (1:9 (v/v) ethyl acetate/hexane) yielded 7.02 g (99%) of a colorless oil: ^{1}H NMR δ 6.48 (dd, 1H, J = 15, 11 Hz), 6.86 (d, 1H, J = 11 Hz), 5.74 (dt, 1H, J = 15, 6 Hz), 5.06−5.16 (m, 2H), 4.15−4.23 (t, 2H, J = 6 Hz), 1.94−2.18 (m, 8H), 1.78 (s, 3H), 1.69 (s, 3H), 1.61 (s, 6H), 1.56 (br s, 1H); ^{13}C NMR δ 140.0, 135.5, 131.4, 129.5, 128.5, 124.5, 124.0, 123.9, 64.0, 40.1, 40.0, 27.0, 26.7, 26.0, 17.9, 16.9, 16.2; FTIR (film, cm^{-1}) 3356 (br), 2919 (s), 2825 (m), 1742 (m), 1654 (w); MS *m*/*z* 248 (M^+, 4.9), 232 (1.2), 137 (24.3), 69 (100.0); HRMS calcd for $C_{17}H_{28}O$ 248.2133, found 248.2120.

(2*R*,3*S*)-Cyclopropylcarbinol 6. A solution of 1.60 g (6.4 mmol) of **5** and 2.05 g (7.6 mmol) of d-dioxaborolane in 10 mL of CH_2Cl_2 was stirred for 10 min. In parallel, 8.32 mL (8.32 mmol, 1 M in hexanes) of $ZnEt_2$ and 1.4 mL (16.0 mmol) of CH_2I_2 were added to 20 mL of CH_2Cl_2 at −42 °C, and the mixture was stirred for 5 min. The contents of the first flask were slowly transferred via cannula into the zinc solution at −42 °C. The mixture was slowly warmed to room temperature and stirred for 8 h before addition of 5 mL of saturated NH_4Cl solution. The layers were separated, the aqueous layer was extracted with three 25 mL portions of ether, and the combined organic layers were washed with brine, dried, and concentrated. The residue was purified by flash chromatography (1:9 (v/v) ethyl acetate/hexanes) to give 1.28 g (77%) of a colorless oil: $[\alpha]^{23}_D$ −35.6° (*c* 2.5, EtOH); ^{1}H NMR δ 5.06−5.14 (m, 2H), 4.63 (d, 1H, J = 9 Hz), 3.44−3.60 (m, 2H), 1.94−2.14 (m, 8H), 1.74 (s, 3H), 1.69 (s, 3H), 1.60 (s, 6H), 1.45 (t, 1H, J = 6 Hz), 1.33−1.44 (m, 1H), 1.02−1.14 (m, 1H), 0.63−0.71 (dt, 1H, J = 6, 5 Hz), 0.51−0.58 (dt, 1H, J = 6, 5 Hz); ^{13}C NMR δ 135.2, 135.0, 131.5, 126.7, 124.5, 124.2, 67.0, 40.0, 39.7, 27.0, 26.8, 26.0, 23.2, 17.9, 16.8, 16.3, 16.3, 12.1; FTIR (film, cm^{-1}) 3336 (br), 2924 (s), 2855 (m), 1652 (m), 1559 (w); MS *m*/*z* 262 (M^+, 28), 244 (1.5), 177 (53), 107 (89), 69 (100); HRMS calcd for $C_{18}H_{30}O$ 262.2289, found 262.2296.

(*R*,*R*)-Cyclopropyl Diacetate 7. A solution of 295 mg (1.13 mmol) of **6** in 10 mL of CH_2Cl_2 was cooled to −78 °C, and ozone was added until a blue color persisted for 2 min. Excess ozone was removed by bubbling nitrogen gas through the solution. The solvent was removed in vacuo. Ether (15 mL) and 0.5 g (13.1 mmol) of $LiAlH_4$ were added, and the suspension was stirred at room temperature for 13 h before 1 mL of brine was added. Ethyl acetate (10 mL) was added, and the material was filtered through 6 g of Celite. The eluent was concentrated and dissolved in 10 mL of CH_2Cl_2, and 2 mL (14.3 mmol) of Et_3N was added at room temperature. After the mixture was stirred for 5 min, DMAP (10 mg, 0.1 equiv) was added, followed by 1 mL (10.6 mmol) of acetic anhydride. The solution was stirred for 4.5 h at room temperature before 1 mL of brine was added. The mixture was diluted with ether (5 mL), and the organic layer was separated, dried, and concentrated. The residue was purified by flash chromatography (1:9 to 100 (v/v) ethyl acetate/hexanes) to give 62 mg (29%) of a colorless oil: $[\alpha]^{23}_D$ −14.1° (*c* 2.1, EtOH); ^{1}H NMR δ 3.93 (d, 4H, J = 7 Hz), 2.06 (s, 6H), 1.08−1.18 (m, 2H), 0.61 (dd, 2H, J = 7, 7 Hz); ^{13}C NMR δ 171.0, 67.4, 21.0, 16.1, 8.9; MS (CI) *m*/*z* 187 (M^+ + 1, 8.6), 163 (3), 127 (100), 67 (31); HRMS calcd for $C_9H_{15}O_4$ 187.0969, found 187.0939.

(2*R*,3*S*)-Cyclopropyl Aldehyde 8. A solution of 1.28 g (4.88 mmol) of cyclopropyl trienol **6**, 30 mL of CH_2Cl_2, and 3 mL of CH_3CN over 1.2 g of crushed 4 Å activated molecular sieves was cooled to 0 °C before 1.05 g (9.0 mmol) of NMO and 85 mg (0.24 mmol, 5 mol %) of TPAP were added. The suspension was stirred at 0 °C for 10 min, warmed to room temperature, and stirred for an additional 2 h. The material was filtered through 50 g of silica, dried, and concentrated. The residue was purified by flash chromatography (toluene) to give 1.12 g (89%) of a colorless oil: $[\alpha]^{23}_D$ −61.1° (neat); ^{1}H NMR δ 9.19 (d, 1H, J = 5 Hz), 5.05−5.15 (m, 2H), 4.67 (d, 1H, J = 9 Hz), 2.16−2.36 (m, 1H), 1.93−2.12 (m, 8H), 1.78−1.86 (m, 1H), 1.74 (s, 3H), 1.70 (s, 3H), 1.62 (s, 3H), 1.60 (s, 3H), 1.54 (dt, 1H, J = 5, 9 Hz), 1.10 (ddd, 1H, J = 5, 7, 8 Hz); ^{13}C NMR δ 200.3, 138.7, 135.8, 131.9, 124.2, 123.8, 123.5, 40.0, 39.7, 32.2, 27.0, 26.6, 26.0, 22.5, 18.0, 17.0, 16.3, 16.2; FTIR (film, cm^{-1}) 2918 (s), 2855 (s), 2724 (m) 1709 (s); MS *m*/*z* 260 (5), 136 (14), 123 (11), 69 (100); HRMS calcd for $C_{18}H_{28}O$ 260.2133, found 260.2153.

Homogeranyl Iodide (9). A solution of 2.85 g (16.9 mmol) of homogeraniol[35] and 100 mL of CH_2Cl_2 was cooled to −42 °C before 3.28 g (25.5 mmol) of diisopropylethylamine was added, followed by 100 mg of DMAP. The solution was stirred for 5 min before 1.63 mL (20.4 mmol) of methyl sulfonyl chloride was added. The solution was stirred for 2 h at −42 °C and at 0 °C for an additional 2.5 h. Five milliliters of water was added, and the organic layer was separated, dried, and concentrated. The yellow oil was added to a solution of 19 g (119 mmol) of NaI in 150 mL of acetone. After the mixture was stirred at reflux for 36 h, 20 mL of water was added. The layers were separated, and the aqueous layer was washed with ether. The combined organic layers were dried and concentrated. The residue was purified by flash chromatography (hexanes) to give 3.65 g (78%) of a colorless oil: ^{1}H NMR δ 5.06−5.16 (m, 2H), 3.11 (t, 2H, J = 7 Hz), 2.59 (q, 2H, J = 7 Hz), 1.96−2.17 (m, 4H), 1.69 (s, 3H), 1.61 (s, 3H); ^{13}C NMR δ 138.0, 131.5, 124.0, 123.0, 39.6, 32.4, 26.5, 25.8, 17.8, 16.3, 6.1; MS *m*/*z* 278 (M^+, 9.7), 149 (22), 69 (100); HRMS calcd for $C_{11}H_{19}I$ 278.0526, found 278.0491.

(10*R*/*S*,11*R*,12*S*)-Cyclopropylcarbinol 10. A suspension of 2.5 g (9.0 mmol) of homogeranyl iodide (**9**), 4 mL of ether, and 400 mg (16.4 mmol) of magnesium turnings was heated at reflux for 6 h, during which time 25 mg (1.0 mmol) of magnesium was added every hour. The Grignard reagent was added by cannula to a solution of 782 mg (3.0 mmol) of cyclopropyl aldehyde **8** in 5 mL of ether at −42 °C. The solution was stirred at −42 °C for 1 h and allowed to warm to room temperature over the next 1.5 h before addition of 2 mL of saturated NH_4Cl solution. The layers were separated, and the aqueous layer was washed with ether. The combined organic layers were dried and concentrated. The residue was purified by flash chromatography (1:9 (v/v) ethyl acetate/hexanes)

Diastereomer 1 was isolated as a colorless oil, 430 mg (35%), R_f = 0.34: $[\alpha]^{23}_D$ −29.3° (*c* 5.8, EtOH); ^{1}H NMR δ 5.04−5.18 (m, 4H), 4.60 (d, 1H, J = 9 Hz), 3.03 (q, 1H, J = 8 Hz), 1.92−2.18 (m, 16H), 1.74 (s, 3H), 1.68 (s, 6H), 1.60 (s, 12H), 1.50 (br s, 1H), 1.36−1.49

(m, 1H), 0.80−0.94 (m, 1H), 0.65 (dt, 5 Hz, $J = 9$ Hz), 0.52 (dt, 1H, 5 Hz, $J = 9$ Hz); ^{13}C NMR δ 135.6, 135.0, 134.5, 131.4, 131.3, 126.8, 124.4, 124.3, 124.1, 124.1,75.4, 39.7, 39.5, 37.0, 27.3, 26.8, 26.6, 26.5, 25.7, 24.2, 17.7, 16.6, 16.0, 15.7, 11.7; FTIR (film, cm^{-1}) 3385 (br), 2968 (s), 2854 (s), 1668 (w); MS *m*/*z* 412 (M$^+$, 3.0), 394 (2.3), 257 (4.3), 137 (17.4), 69 (100); HRMS calcd for $C_{29}H_{48}O$ 412.3693, found 412.3681.

Diastereomer 2 was isolated as a colorless oil, 620 mg (50%), $R_f = 0.25$: $[\alpha]^{23}_D$ −29.4° (*c* 6.8, EtOH); ^{1}H NMR δ 5.04−5.18 (m, 4H), 4.60 (d, 1H, $J = 9$ Hz), 3.05 (q, 1H, $J = 7$ Hz), 1.93−2.18 (m, 16H), 1.72 (s, 3H), 1.69 (s, 6H), 1.61 (s, 12H), 1.49 (br s, 1H), 1.33−1.43 (m, 1H), 0.8−0.91 (m, 1H), 0.73 (dt, 1H, $J = 8$, 5 Hz), 0.52 (dt, 1H, $J = 8$, 5 Hz); ^{13}C NMR δ 135.6, 135.0, 134.5, 131.3, 131.2, 126.6, 124.3, 124.2, 124.0, 124.0, 75.5, 39.7, 39.5, 37.4, 27.3, 26.8, 26.7, 26.6, 25.7, 24.3, 17.7, 16.5, 16.0, 16.0, 16.0, 16.0, 11.6; FTIR (film, cm^{-1}) 3362 (br), 2922 (s), 2856 (s), 1668 (w); MS *m*/*z* 412 (M$^+$, 6.8), 394 (3.8), 257 (7.5), 137 (17.4), 69 (100); HRMS calcd for $C_{29}H_{48}O$ 412.3693, found 412.3666.

(11*R*,12*S*)-Cyclopropyl Ketone 11. A suspension of 913 mg (2.22 mmol) of a mixture of secondary alcohol diastereomers (**10**), 20 mL of CH_2Cl_2, and 1 g of finely ground 4 Å molecular sieves was cooled to 0 °C before 400 mg (3.42 mmol) of NMO and 40 mg (0.11 mmol, 0.05 equiv) of TPAP were added. The mixture was warmed to room temperature and stirred for 3.5 h, poured over a plug of silica, and eluted with ethyl acetate. The eluent was concentrated to give 870 mg (96%) of a colorless oil: $[\alpha]^{23}_D$ −141.2° (*c* 5.2, EtOH); ^{1}H NMR δ 5.03−5.14 (m, 4H), 4.62 (d, 1H, $J = 9$ Hz), 2.54−2.61 (m, 2H), 2.29 (q, 2H, $J = 7$ Hz), 1.92−2.12 (m, 13H), 1.87 (ddd, 1H, $J = 4$, 5, 8 Hz), 1.70 (s, 3H), 1.68 (s, 6H), 1.59 (br s, 12H), 1.45 (ddd, 1H, $J = 4$, 5, 8 Hz), 0.89 (ddd, 1H, $J = 4$, 6, 8 Hz); ^{13}C NMR δ 209.3, 137.5, 136.2, 135.2, 131.4, 131.3, 124.9, 124.3, 124.2, 123.9, 122.7, 43.8, 39.7, 39.7, 39.4, 29.9, 26.8, 26.7, 26.4, 25.7, 24.9, 22.7, 18.4, 17.7, 16.7, 16.0, 16.0; FTIR (film, cm^{-1}) 2918 (s), 2854 (s), 1695 (s); MS *m*/*z* 410 (M$^+$, 1.6), 341 (3.4), 193 (8.0), 149 (8.7), 137 (14.5), 123 (47.3), 81 (45.7), 69 (100); HRMS calcd for $C_{29}H_{46}O$ 410.3537, found 410.3533.

(10*R*/*S*,11*R*,12*S*)-Rillingol (ROH). A solution of 100 mg (0.24 mmol) of cyclopropyl ketone **11** in 4 mL of ether was cooled to −42 °C before 400 μL (1.2 mmol, 5 equiv) of methylmagnesium bromide (3.0 M in diethyl ether) was added dropwise. The mixture was stirred at −42 °C for 2 h before 1 mL of saturated NH_4Cl solution was added. The layers were separated, and the organic layer was washed with brine, dried, and concentrated. The residue was purified by flash chromatography (1:9 ethyl acetate/hexanes) to give 102 mg (99%) of a mixture of diastereomers (6:1 ratio). The diastereomers were separated by HPLC on silica using a linear gradient (1:5 to 1:1.5, *t*BME in hexanes).

The major diastereomer was isolated as a colorless oil: $[\alpha]^{23}_D$ −8° (*c* 0.8, EtOH); ^{1}H NMR (500 MHz) δ 5.06−5.17 (m, 4H), 4.61 (d, 1H, $J = 9$ Hz), 1.94−2.16 (m, 16H), 1.73 (s, 3H), 1.68 (s, 6H), 1.60 (s, 9H), 1.59 (s, 3H), 1.50−1.57 (m, 1H), 1.17 (s, 3H), 1.04 (br s, 1H), 0.81 (ddd, 1H, $J = 9$, 6, 5 Hz), 0.74 (ddd, 1H, $J = 9$, 4, 6 Hz), 0.38 (ddd, 1H, $J = 9$, 5, 4 Hz); ^{13}C NMR δ 135.4, 135.0, 134.0, 131.5, 131.3, 127.6, 124.5, 124.4, 124.3, 124.2, 71.5, 43.1, 39.8, 39.6, 30.3, 27.1, 26.8, 26.7, 26.7, 25.8, 22.7, 17.7, 16.6, 16.1, 16.0, 16.0, 13.7, 9.5; FTIR (film, cm^{-1}) 3474 (br), 2922 (s), 2854 (s); MS *m*/*z* 426 (M$^+$, 0.3), 408 (1.3), 339 (1.4), 175 (4.7), 137 (16.4), 109 (17.9), 69 (100); HRMS calcd for $C_{30}H_{50}O$ 426.3849, found 426.3879.

The minor diastereomer was a colorless oil, $[\alpha]^{23}_D$ −9° (*c* 0.3, EtOH), whose ^{1}H NMR spectrum was identical to that of **ROH** isolated from the incubation of FPP with SQase: ^{1}H NMR (500 MHz) δ 5.06−5.18 (m, 4H), 4.61 (d, 1H, $J = 9$ Hz), 1.94−2.16 (m, 16H), 1.73 (s, 3H), 1.68 (s, 6H), 1.62 (s, 3H), 1.60 (s, 6H), 1.59 (s, 3H), 1.41−1.48 (ddd, 1H, $J = 13$, 9, 5 Hz), 1.16 (br s, 1H), 1.14 (s, 3H), 0.78−0.86 (m, 2H), 0.38−0.44 (m, 1H); ^{13}C NMR δ 135.7, 135.2, 134.3, 131.7, 131.6, 127.8, 124.7, 124.6, 124.5, 124.4, 71.4, 43.4, 40.0, 39.8, 30.6, 27.0, 26.9, 26.8, 26.4, 26.0, 23.0, 18.0, 16.8, 16.3, 16.3, 13.9, 10.1; FTIR (film, cm^{-1}) 3474 (br), 2922 (s), 2854 (s); MS *m*/*z* 426 (0.5), 408 (1.7), 339 (1.6), 137 (13.6), 109 (15.5), 69 (100); HRMS calcd for $C_{30}H_{50}O$ 426.3849, found 426.3865.

Alcohol 14. A mixture of 400 mg (1.4 mmol) of iodotriene **12**, 932 mg (4.2 mmol) of (*R*)-nerolidol (**13**), 16 mg (0.072 mmol, 0.05 equiv) of Pd(II) acetate, 390 mg (1.4 mmol) of Ag_2CO_3, and 5 mL of DMF was heated at 65 °C for 2 h. The material was cooled to room temperature and concentrated in vacuo. The residue was diluted with 15 mL of ether and filtered through 2.0 g of Celite. The eluent was concentrated, and the residue was purified by flash chromatography (1:9 ethyl acetate/hexanes) to give 440 mg (76%) of a colorless oil: $[\alpha]^{23}_D$ −8.0° (*c* 1.7, EtOH); ^{1}H NMR δ 6.43 (dd, 1H, $J = 15$, 11 Hz), 5.82 (d, 1H, $J = 11$ Hz), 5.63 (d, 1H, $J = 15$ Hz), 5.02−5.18 (m, 4H), 1.92−2.15 (m, 16H), 1.76 (s, 3H), 1.66 (s, 6H), 1.58 (s, 12H), 1.29 (s, 3H); ^{13}C NMR δ 138.6, 137.8, 135.5, 135.3, 131.4, 131.3, 124.3, 124.3, 124.2, 124.1, 123.9, 123.8, 73.4, 42.6, 40.0, 39.8, 28.4, 28.3, 26.8, 26.7, 26.5, 25.8, 23.0, 22.8, 17.8, 16.8, 16.1, 16.1; FTIR (film, cm^{-1}) 3727 (br), 2917 (s), 2850 (s), 1653 (w); MS *m*/*z* 412 (M$^+$, 4.0), 395 (81.7), 257 (27.4), 149 (48.7), 69 (100); HRMS calcd for $C_{29}H_{48}O$ 412.3705, found 412.3736.

(10*R*,11*R*,12*S*)-Rillingol (ROH). A suspension of 482 mg (3.2 mmol) of Sm^0 and 80 mg (0.30 mmol) of $HgCl_2$ in 5 mL of THF was stirred at room temperature for 15 min before 100 mg (0.25 mmol) of alcohol **14** was added. The suspension was cooled to 0 °C, and 540 mg of CH_2I_2 was added. The mixture was stirred at 0 °C for 2 h, warmed to room temperature, and stirred for an additional 5 h. Saturated K_2CO_3 solution (2 mL) and 10 mL of ether were added. The layers were separated, and the aqueous layer was washed three times with 8 mL of ether. The combined organic layers were washed with brine, dried, and concentrated. The residue was purified by flash chromatography (1:49 (v/v) ethyl acetate/hexanes) to give 61 mg of a mixture that contained starting material. Additional purification by HPLC (18:82 (v/v) MTBE/hexanes) gave 29 mg (29%) of a colorless oil: $[\alpha]^{23}_D$ −9.7° (*c* 1.2, EtOH); ^{1}H NMR (500 MHz) δ 5.06−5.18 (m, 4H), 4.61 (d, 1H, $J = 9$ Hz), 1.94−2.16 (m, 16H), 1.73 (s, 3H), 1.68 (s, 6H), 1.62 (s, 3H), 1.60 (s, 6H), 1.59 (s, 3H), 1.41−1.48 (ddd, 1H, $J = 13$, 9, 5 Hz), 1.16 (br s, 1H), 1.12 (s, 3H), 0.78−0.86 (m, 2H), 0.38−0.44 (m, 1H); ^{13}C NMR δ 135.7, 135.2, 134.3, 131.7, 131.6, 127.8, 124.7, 124.6, 124.5, 124.4, 71.4, 43.4, 40.0, 39.8, 30.6, 27.0, 26.9, 26.8, 26.4, 26.0, 23.0, 18.0, 16.8, 16.3, 16.3, 13.9, 10.1; FTIR (film, cm^{-1}) 3474 (br), 2922 (s), 2854 (s); MS *m*/*z* 426 (1.2), 408 (2.4), 339 (4.3), 137 (25.7), 109 (26.6), 69 (100); HRMS calcd for $C_{30}H_{50}O$ 426.3849, found 426.3888.

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Supporting Information Available: Three tables showing a list of proteins used in multiple sequence alignments, pairwise percent identities among squalene synthases, and pairwise identities among phytoene synthases and a dehydrosqualene synthase; four figures showing amino acid sequence alignments for 19 squalene synthases, a phylogenetic analysis of squalene synthases based on amino acid sequences, amino acid sequence alignments for 26 phytoene synthases and a dehydrosqualene synthase, and a phylogenetic analysis of phytoene synthases and a dehydrosqualene synthase based on amino acid sequences (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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