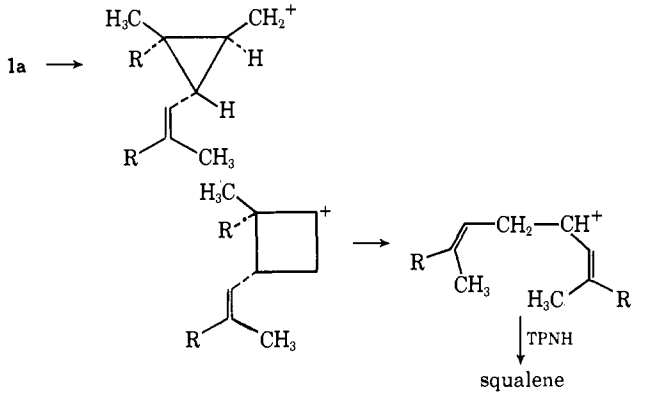
propylcarbinyl, cyclobutyl, and allylcarbinyl cations generated in solvolyses or deaminations.[10] This mechanism should be formulated as proceeding through equilibrating bicyclobutonium ions;[11] for simplicity it is depicted in the following scheme as proceeding through classical ions.



Acknowledgment. Acknowledgment is made to the Syntex Corporation, E. I. du Pont de Nemours and Co., to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and to the National Institutes of Health (GM 08321 and Research Career Development Award 2-K3-6M-6354) for partial support of this research.

(10) (a) R. H. Mazur, W. N. White, D. A. Semenov, C. C. Lee, M. S. Silver, and J. D. Roberts, *J. Amer. Chem. Soc.*, **81**, 4390 (1959); (b) K. B. Wiberg, A. H. Hess, Jr., and A. J. Ashe, III, in "Carbonium Ions," Vol. III, G. A. Olah and P. v. R. Schleyer, Ed., Wiley-Interscience, New York, N. Y., 1970, in press.

(11) G. A. Olah, D. P. Kelly, C. L. Jeuell, and R. D. Porter, *J. Amer. Chem. Soc.*, **92**, 2544 (1970).

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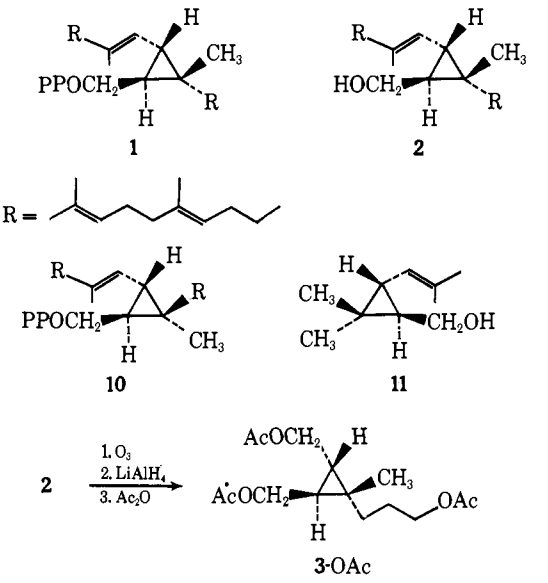
Studies on the Mechanism of Squalene Biosynthesis. Presqualene Pyrophosphate, Stereochemistry and a Mechanism for Its Conversion to Squalene

Sir:

In a previous publication[1] we presented the results of our studies leading to the gross structure of presqualene pyrophosphate, a biological precursor to squalene. We now wish to report chemical and physical evidence in support of **1** for the stereochemistry of this intermediate and to suggest a rational mechanism for the stereospecific biosynthesis of squalene[2] from farnesyl pyrophosphate.

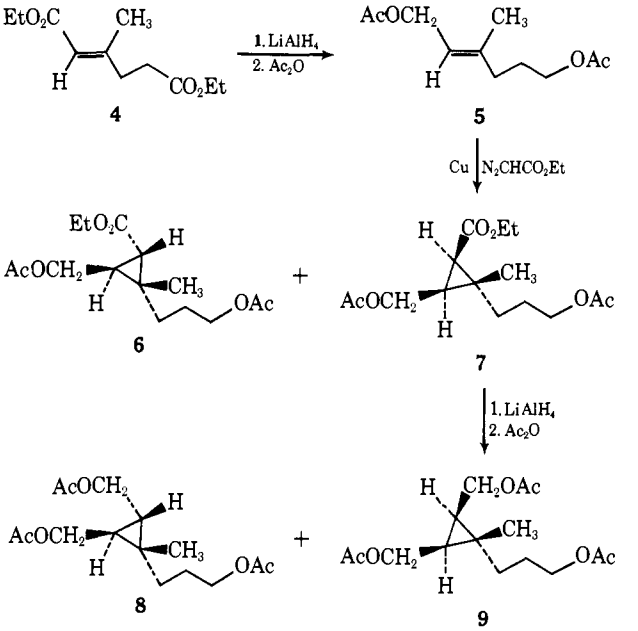
The relative stereochemistry of presqualene pyrophosphate was studied by a combination of synthetic and degradative investigations. Since the unresolved stereochemistry of the intermediate resides in the location of the substituents about the cyclopropane ring



and since the difficulties of chemical synthesis could be considerably reduced by the isolation of the cyclopropyl portion of the natural product by degradation, we undertook the synthesis of the triacetate, **3**-OAc, anticipated to be derived from the product of ozonolysis of presqualene alcohol (**2**).

The synthesis of **3**-OAc is outlined in Scheme I.

Scheme I



With a slight modification of Wadsworth and Emmons original procedure,[3] 1,4-dicarbethoxy-2-methylbutene-1 was prepared in 91% yield as a 60:40 trans–cis mixture and the trans isomer **4**, isolated in better than 90% purity by distillation (96° (1.2 mm)). The trans stereochemistry of **4** was assigned by a comparison of the chemical shift of the olefinic methyl groups of the two isomers.[4] The methyl resonance of the trans isomer occurs as a doublet (J = 1.5 Hz) at δ 2.17 while the methyl group of the cis isomer has its doublet at δ 1.91. $LiAlH_4$ reduction of **4** gave in high yield,

(1) W. W. Epstein and H. C. Rilling, *J. Biol. Chem.*, **245**, 4597 (1970).

(2) (a) G. Popjak and J. W. Cornforth, *Biochem. J.*, **101**, 553 (1966); (b) J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, G. Ryback, and G. J. Schroepfer, *Proc. Roy. Soc., Ser. B*, **163**, 436 (1965); (c) J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popjak, *ibid., Ser. B*, **163**, 492 (1965).

(3) W. S. Wadsworth, Jr., and W. D. Emmons, *J. Amer. Chem. Soc.*, **83**, 1733 (1961).

(4) J. W. K. Burrell, R. F. Garwood, L. M. Jackman, E. Oskay, and B. C. L. Weedon, *J. Chem. Soc. C*, 2144 (1966).

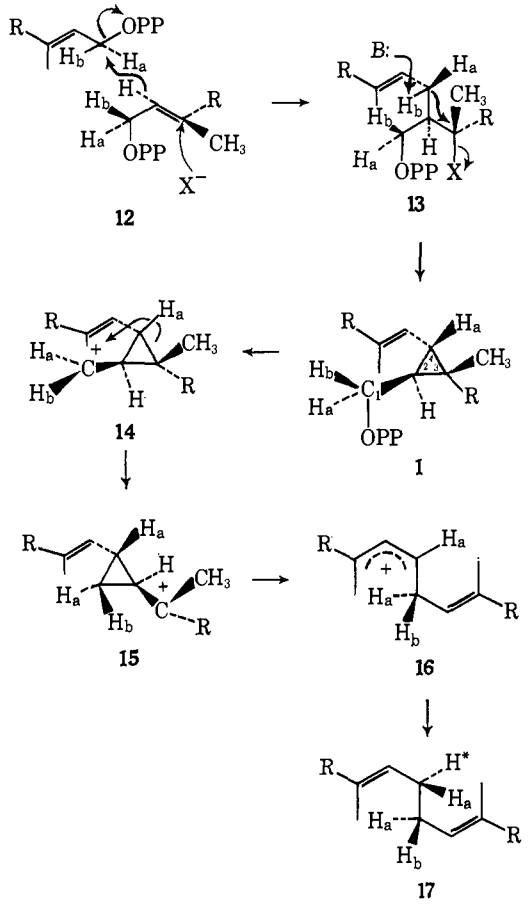
3-methyl-*trans*-2-hexene-1,3-diol (bp 95–99° (0.25 mm)) (**5**-OH), which was converted to the diacetate **5** (bp 74–76° (0.25 mm)), before construction of the cyclopropane ring. Treatment of **5** with ethyl diazoacetate in the presence of copper powder[5] gave two major products in 36% yield which were assigned structures **6** and **7**. Isomers **6** and **7** were isolated by preparative glc and the relative stereochemistry was determined by a comparison of the nmr of the two compounds.[6] In the case, **6**, where the cyclopropylmethyl is trans to the carboxyethyl group, the resonance singlet occurs at δ 1.23 while in the corresponding cis case, **7**, the methyl singlet is at δ 1.18. Reduction of **6** and **7** by $LiAlH_4$ followed by acetylation gave **8** and **9**, respectively, in high yield.

Treatment of [^3H]presqualene pyrophosphate synthesized from farnesyl-1 [^3H]pyrophosphate with $LiAlH_4$ yields presqualene alcohol with an unrearranged skeleton.[1] The radioactive alcohol was then ozonized, the resulting ozonide was reduced by $LiAlH_4$, and the triol was acetylated. Glc comparison of the radioactive material derived from the natural product with **8** and **9** using five different liquid phases showed **8** to have the same retention times as the radioactive compound derived from presqualene pyrophosphate. These chromatographic systems clearly resolved **8** and **9**. These results fully confirm the presence of the cyclopropane ring in **1** and, although structure **10** is also consistent with these data, the synthetic work of Altman[7] removes this possibility.

The absolute configuration of **2** was assigned by comparing the circular dichroism spectrum of **2** ($[\alpha]^{27}D$ +55°) with the model compound, (1*R*,2*R*)-*trans*-chrysanthemyl alcohol (**11**). Alcohol **11** ($[\alpha]^{27}D$ +46°) was prepared by $LiAlH_4$ reduction of known methyl (1*R*,2*R*)-*trans*-chrysanthemate.[9] Presqualene alcohol (**2**) showed a plain positive CD curve while **11** showed a similar but negative curve indicating that the absolute orientation of **1** was opposite to the known configuration of **11** or that indicated for **1**.

The formation of **1** from farnesyl pyrophosphate (**12**) can be interpreted as a process similar in many respects to the well-understood prenyl transfer[2a,10] reactions for aliphatic head-to-tail terpene biosynthesis. Rather than activation of an isopentenyl pyrophosphate by an electron-donating X group, we suggest activation of C_2 of farnesyl pyrophosphate and nucleophilic attack at C_1 of a second farnesyl pyrophosphate, displacing the pyrophosphate ion with inversion (Scheme II). The overall reaction then involves a trans addition of one farnesyl group and of X to the C_2–C_3 double bond of the second farnesyl moiety. The second step in prenyl transfer is a trans 1,2 elimination of X and an H. By analogy the second step in presqualene pyrophosphate formation is a trans 1,3 (W) elimination[11] of H_b and X to form **1**. Although there

Scheme II. Proposed Route for the Biosynthesis of Squalene from Farnesyl Pyrophosphate



are two modes of trans addition and thus two of elimination, the absolute orientation of **1** allows only the path shown.

The known reactions of cyclopropylcarbinyl derivatives under solvolytic conditions[12] suggest that pyrophosphate **1** may be converted to squalene **17** by a series of cationic rearrangements.[13] Ample precedent exists for each of the steps shown in Scheme II between **1** and **17**.[15] Ionization of **1** is expected to proceed with the stereochemistry indicated in Scheme II by utilizing the C_2–C_4 bonding cyclopropane electrons. Several examples demonstrate that the relative orientation of the cyclopropane ring and the leaving group is important during ionization[12,16] and that substituents at C_3 and C_4 which stabilize positive charge induce stereospecificity at C_1.[12,16a,17] Preliminary experimental evidence indicates that the C_4 vinyl substituent is more effective in stabilizing the transition state than the alkyl substituents at C_3.[18] A high barrier to rota-

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(6) J. L. Pierre and P. Arnaud, *Bull. Soc. Chim. Fr.*, 1040 (1966).
(7) L. J. Altman, R. C. Kowerski, and H. C. Rilling, *J. Amer. Chem. Soc.*, 93, 1782 (1971). In addition to the above synthesis, Professor L. Crombie[8] has prepared a synthetic sample of 11 which as the pyrophosphate can be enzymatically converted to squalene.
(8) Personal communication.
(9) We wish to thank McLaughlin, Gormly, King Co. for a generous sample of (1*R*,2*R*)-*trans*-chrysanthemyl chloride.
(10) J. W. Cornforth, *Angew. Chem., Int. Ed. Engl.*, 7, 903 (1968).
(11) A. Nickon and N. H. Werstiuk, *J. Amer. Chem. Soc.*, 89, 3915 (1967).
(12) C. D. Poulter, E. C. Friedrich, and S. Winstein, *ibid.*, 92, 4274 (1970), and references therein.
(13) Cyclopropylcarbinyl cations have been suggested as intermediates in the biosynthesis of other non-head-to-tail isoprenoids.[14]
(14) (a) R. B. Bates and P. K. Paknikar, *Tetrahedron Lett.*, 1453 (1965); (b) R. B. Bates and D. Feld, *ibid.*, 4875 (1967); (c) L. Crombie, R. P. Houghton, and D. K. Woods, *ibid.*, 4553 (1967).
(15) For simplicity, delocalized cyclopropylcarbinyl cationic intermediates are represented by classical structures. The rearrangement of **14** to **15** may accompany ionization.
(16) (a) C. D. Poulter and S. Winstein, *J. Amer. Chem. Soc.*, 92, 4282 (1970); (b) B. R. Ree and J. C. Martin, *ibid.*, 92, 1660 (1970); (c) P. von R. Schleyer and Y. Buss, *ibid.*, 91, 5880 (1969).
(17) (a) M. Gasic, D. Whalen, B. Johnson, and S. Winstein, *ibid.*, 89, 6382 (1967); (b) D. Whalen, M. Gasic, B. Johnson, H. Jones, and S. Winstein, *ibid.*, 89, 6384 (1967).
(18) Preliminary kinetic data support this statement: C. D. Poulter, C. J. Spillner, and S. Moesinger, unpublished results.

tion about the C_1–C_2 bond in **14** will retain the specific orientation of H_a and H_b.[12,16,19] The cyclopropylcarbinyl–cyclopropylcarbinyl rearrangement should then occur with expected inversion of configuration at C_1 and C_4.[20,21] Model studies indicate that cation **15** rearranges to allylic ion **16**[22] followed by stereospecific hydride transfer to C_4 from NADPH*[2b] to give squalene.

Most of the stereospecific steps shown in Scheme II do not require special orientation by an enzyme, although the efficiency of the overall transformation obviously depends on enzyme catalysis at several points. It is also interesting to note that models indicate the entire sequence of molecular rearrangements shown in Scheme II can take place with little movement of the long isoprenoid side chains.

Acknowledgments. The technical assistance of Mrs. E. K. Davis is gratefully acknowledged. We also wish to thank the Research Corporation, the Petroleum Research Fund (1694-G1), administered by the American Chemical Society, the National Science Foundation, and the National Institutes of Health (GM 08 321 and Research Career Development Award 2-K3-GM-6354).

(19) D. S. Kabakoff and E. Namanworth, *J. Amer. Chem. Soc.*, **92**, 3234 (1970).

(20) (a) K. B. Wiberg and G. Szeimies, *ibid.*, **91**, 571 (1970); (b) J. E. Baldwin and W. D. Foglesong, *ibid.*, **90**, 4303 (1968).

(21) The possibility of a puckered cyclobutyl cation intervening between **14** and **15** cannot be ruled out:[16a] C. D. Poulter and S. Winstein, *ibid.*, **91**, 3650 (1969).

(22) Hydride attack of **16** at C_4 should proceed with inversion,[12,16a] giving the wrong absolute configuration. We have preliminary evidence that a delicate balance exists between vinyl-substituted cyclopropylcarbinyl cations similar to **14** and **15** and their allylic isomers. Thus, the rearrangement **14** → **15** → **16** is quite plausible: C. D. Poulter and S. Moesinger, unpublished results.

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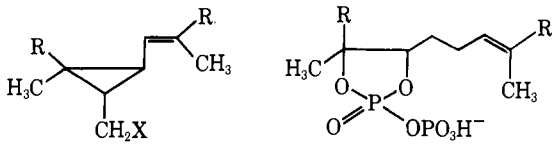
Stereoselective Total Synthesis of (±)-Presqualene Alcohol

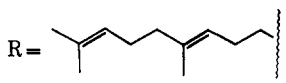
Sir:

The mechanism of the enzymatic coupling of two molecules of farnesyl pyrophosphate to squalene has been a subject of considerable interest and conjecture.[1–5] The recent discovery[2a] of a C-30 intermediate (presqualene) in the biosynthetic process has, owing to the possible mechanistic implications, focused attention upon the structure of this new triterpene. Structural investigations with enzymatically produced material have led to two independent proposals, **1a**[2b,c] and **2**,[3b] for the constitution of presqualene. We wish to report a stereoselective total synthesis of racemic presqualene alcohol[2] which confirms structure **1b** for this dephosphorylated derivative of presqualene, and in particular defines the relative stereochemistry about the cyclopropane ring.[6,7]



1a, X = OPP
b, X = OH

2



(homogeranyl)

Copper-catalyzed decomposition (cupric acetylacetonate or copper powder in refluxing toluene) of *trans,trans*-farnesyl diazoacetate (**4**, ν_{max} 2100 cm^{-1}),[8a,b] prepared in 76% yield from *trans,trans*-farnesol (**3**)[9] by reaction with glyoxalyl chloride tosylhydrazone and triethylamine in methylene chloride,[10] affords the cyclopropyl lactone **5** (ν_{max} 1775 cm^{-1}, M^+ 262)[8a,b] in about 20% yield after purification by column chromatography and hydrolysis–relactonization (dicyclohexylcarbodiimide in methylene chloride). The γ-lactone ring must be cis fused to the three-membered ring and, in view of the stereospecificity of intermolecular copper-catalyzed diazo ester cycloadditions,[11] the trans relationship between the side chain and the oxymethylene group should be retained; hence the stereochemistry of **5** is assigned. The corresponding hydroxy acid **6a** (mp 58.5–60.5°)[8b] was esterified with diazomethane and then oxidized to the *cis*-aldehyde ester **7** [69%; ν_{max} 1730, 1700 cm^{-1}; δ 9.58 (1 H, d, J = 6 Hz); 1.52 (3 H, s)],[8a,b] with the chromium trioxide–dipyridine complex in methylene chloride.[12] Exposure of **7** to 5% sodium hydroxide in aqueous methanol (1:1) at room temperature effects first rapid ester hydrolysis followed by a slower ($t_{1/2}$ ~ 2 hr) epimerization of the aldehyde group; reesterification with diazomethane gives the more stable *trans*-aldehyde ester **8** [92%; ν_{max} 1730, 1700; δ 9.56 (1 H, apparent t, J ~ 1 Hz), 1.32 (3 H, s)].[8a–c,13,13a]

(1) Reviews: R. B. Clayton, *Quart. Rev., Chem. Soc.*, **19**, 168 (1965); I. D. Franz and G. J. Schroepfer, *Annu. Rev. Biochem.*, 36, 691 (1967).

(2) (a) H. C. Rilling, *J. Biol. Chem.*, **241**, 3233 (1966); (b) H. C. Rilling and W. W. Epstein, *J. Amer. Chem. Soc.*, **91**, 1041 (1969); (c) W. W. Epstein and H. C. Rilling, *J. Biol. Chem.*, **245**, 4597 (1970).

(3) (a) J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popjak, *Proc. Roy. Soc., Ser. B*, **163**, 492 (1966); (b) G. Popjak, J. Edmond, K. Clifford, and V. Williams, *J. Biol. Chem.*, **244**, 1897 (1969).

(4) (a) G. Krishna, H. W. Whitlock, Jr., D. H. Feldgruegge, and J. W. Porter, *Arch. Biochem. Biophys.*, 114, 200 (1966); (b) G. E. Risinger and H. D. Durst, *Tetrahedron Lett.*, 3133 (1968).

(5) (a) B. M. Trost and R. LaRochelle, *ibid.*, 3327 (1968); (b) J. E. Baldwin, R. E. Hackler, and D. P. Kelly, *J. Amer. Chem. Soc.*, **90**, 4758 (1968); (c) G. M. Blackburn, W. D. Ollis, C. Smith, and I. O. Sutherland, *Chem. Commun.*, 99 (1969).

(6) The relative and absolute stereochemistry of (−)-**1b** has recently been established by degradative studies: H. C. Rilling, W. W. Epstein, and B. Larsen, *J. Amer. Chem. Soc.*, submitted for publication. We are grateful to Professor Epstein for advance disclosure of these results and a preprint of the manuscript.

(7) Two concurrent and independent syntheses of presqualene alcohol have been completed: L. J. Altman, R. C. Kowerski, and H. C. Rilling, *J. Amer. Chem. Soc.*, **93**, 1782 (1971); L. Crombie and coworkers, private communication from Professor Rilling.

(8) (a) This compound gave infrared and nmr spectra compatible with the structure shown. Only the key data are cited. (b) A satisfactory combustion analysis was obtained. (c) Elemental composition was verified by exact mass determination (with ±0.0003) either on the molecular ion, or indirectly by the metastable defocusing technique.

(9) R. B. Bates, D. M. Gale, and B. J. Gruner, *J. Org. Chem.*, **28**, 1086 (1963).

(10) H. O. House and C. J. Blankley, *ibid.*, **33**, 53 (1968).

(11) W. von E. Doering and T. Mole, *Tetrahedron*, **10**, 65 (1960).

(12) J. C. Collins, W. W. Hess, and F. J. Frank, *Tetrahedron Lett.*, 3363 (1968); R. Ratcliffe and R. Rodehorst, *J. Org. Chem.*, **35**, 4000 (1970).

(13) At equilibrium <5% of original cis isomer **7** remains. There seems little doubt that epimerization has occurred only at the aldehyde position, since cyclopropane carboxylate undergoes <10% exchange in 0.25 *M* sodium deuteroxide–deuterium oxide at 150° for 5 days: J. G. Atkinson, J. J. Csakvary, G. T. Herbert, and R. S. Stuart, *J. Amer. Chem. Soc.*, **90**, 498 (1968).