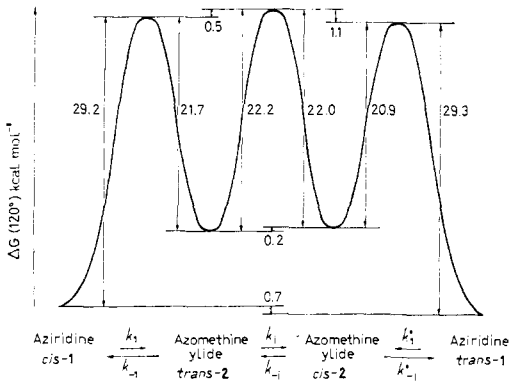

Figure 1. Energy profile for the thermal equilibration of the cis–trans isomeric dimethyl 1-(*p*-methoxyphenyl)aziridine-2,3-dicarboxylates (**1**) *via* the azomethine ylides **2** at 120°.

markable depth. The height of the barrier for the cyclization of **2** → **1** discloses a deep-seated change of the bond system. The geometrical isomerization *trans*-**2** ⇌ *cis*-**2** demands $\Delta G^{\ddagger}(120°)$ = 22.2 or 22.0 kcal mol^{-1}, respectively. If *trans*-**2** ⇌ *cis*-**2** takes place by rotation around a NC bond, the energy barrier gives a rough measure of the resonance energy of the heteroallyl anion system found in **2**. The *cis*- and *trans*-azomethine ylides **2** virtually do not differ in their energy levels; hence, there must be other reasons for the differing 1,3-dipolar activities of the ylides.[6]

Nevertheless, the net gain of converting a π into a σ bond in the change of **2** → **1** is exothermic by 8 kcal mol^{-1}. That explains the failure of spectroscopic means to detect the azomethine ylide **2** in thermal equilibrium with **1**. For each molecule of the 1,3-dipole **2** there are 30,000 aziridine molecules at 120°, while the ratio at 25° amounts to even 1:50 million.

The nitrones, considered as azomethine oxides, are structural relatives of azomethine ylides. Cis–trans isomerization of *C*-cyano-*C*,*N*-diphenylnitrone shows $\Delta G^{\ddagger}(120°)$ = 27.5 kcal mol^{-1}.[7] In contrast to the azomethine ylides, the azomethine oxides are thermodynamically favored as against the cyclic oxaziridines. The ring scission of 2-*tert*-butyl-3-phenyloxaziridine to form the nitrone occurs with $\Delta G^{\ddagger}(120°)$ = 29.2 kcal mol^{-1}.[8]

(6) R. Huisgen, W. Scheer, H. Mäder, and E. Brunn, *Angew. Chem., Int. Ed. Engl.*, **8**, 604 (1969).
(7) Calculated from the data given by K. Koyano and I. Tanaka, *J. Phys. Chem.*, **69**, 2545 (1965).
(8) Calculated from the data given by M. F. Hawthorne and R. D. Strahm, *J. Org. Chem.*, **22**, 1263 (1957).

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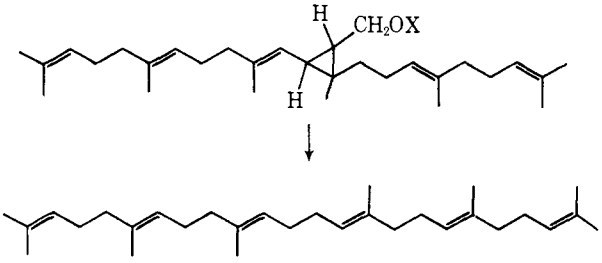
Received January 18, 1971

Mechanism of Presqualene Pyrophosphate–Squalene Biosynthesis

Sir:

None of the numerous, diverse speculations concerning the mechanism of squalene biogenesis published during the 1960's[1] anticipated the role of "presqualene pyrophosphate," a natural product shown by Rilling to be an intermediate in the biosynthesis of squalene from farnesyl pyrophosphate[2] and assigned structure **1** by Rilling and Epstein.[3] In view of the recently accomplished unequivocal synthesis by Altman of cyclopropylcarbinol possessing structure **2** and the demonstration that the synthetic material was well incorporated into squalene,[4] detailed consideration of

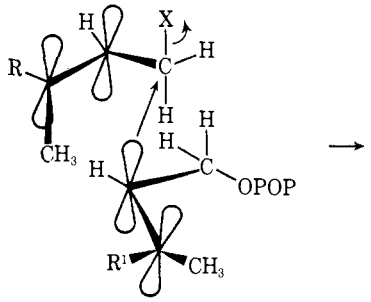


1, X = POP
2, X = H

the bioorganic chemistry of squalene synthesis seems desirable at this time.

In order to rationalize the formation of presqualene, the following individual steps are proposed. Initially, a new σ bond is formed through interaction of the allylic pyrophosphate units present in two farnesyl pyrophosphate molecules, specifically involving SN2 displacement by the π bond in one center, of the pyrophosphate anion in the second (**3**). In this process, attack of external or internal pyrophosphate would be expected to occur in such a way as to maintain the stereochemical relationship of the substituents on the original olefinic center. Once formed, the new pyrophosphate **4** is subjected to the action of an isomerase which produces the disubstituted olefin **5**. Such a process, although thermodynamically unfavorable, is precedented by the observed biological interconversion of isopentenyl pyrophosphate and dimethylallyl pyrophosphate.[5] The resulting homoallylic system is subject to chemically well-precedented cyclopropane ring closure, which in this case is accompanied by proton elimination to establish a trans-trisubstituted olefinic bond at the original site, prior to isomerase action. It should be emphasized at this point that in the entire mechanistic sequence, only *one* of the four original C-1 hydrogens present in the two starting farnesyl pyrophosphates will have been lost, in conformance with the biochemical findings.[1a,b] The established stereochemistry,[4] de-

(1) (a) G. Popják, DeW. S. Goodman, J. W. Cornforth, R. H. Cornforth, and R. Ryhage, *J. Biol. Chem.*, **236**, 1934 (1961); (b) J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popják, *Proc. Roy. Soc., Ser. B*, **163**, 492 (1966); (c) G. Krishna, H. W. Whitlock, Jr., D. H. Feldbruegge, and J. W. Porter, *Arch. Biochem. Biophys.*, **114**, 200 (1966); (d) J. E. Baldwin, R. E. Hackler, and D. P. Kelly, *J. Amer. Chem. Soc.*, **90**, 4758 (1968); (e) G. E. Risinger and H. D. Durst, *Tetrahedron Lett.*, 3133 (1968); (f) B. M. Trost and R. LaRochelle, *ibid.*, 3327 (1968); G. M. Blackburn, W. D. Ollis, C. Smith, and I. O. Sutherland, *Chem. Commun.*, 99 (1969).
(2) H. C. Rilling, *J. Biol. Chem.*, **241**, 3233 (1966).
(3) (a) H. C. Rilling and W. W. Epstein, *J. Amer. Chem. Soc.*, **91**, 1041 (1969); (b) W. W. Epstein and H. C. Rilling, *J. Biol. Chem.*, **18**, 4597 (1970). The mechanistic considerations described by these authors differ in several important respects from those presented in this contribution.
(4) L. J. Altman, R. C. Kowerski, and H. C. Rilling, *J. Amer. Chem. Soc.*, **93**, 1782 (1971).
(5) B. W. Agranoff, H. Eggerer, U. Henning, and F. Lynen, *ibid.*, **81**, 1254 (1959); *J. Biol. Chem.*, **235**, 326 (1960). The isomerization reaction could well proceed by a thiol addition–elimination sequence. In that case, the observed inhibition of squalene biosynthesis by thiols[1c] would find an explanation.



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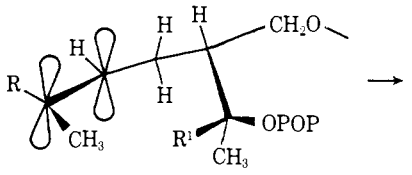

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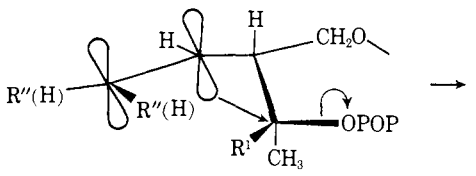

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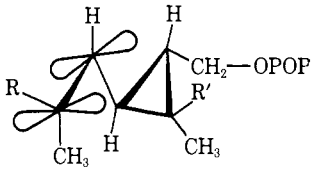
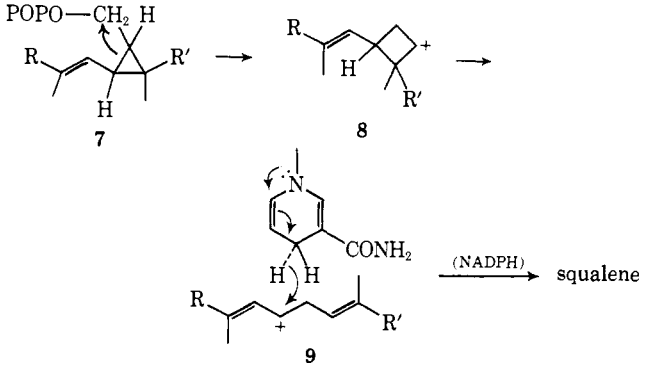


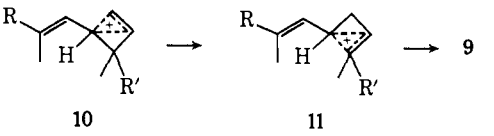
6

picted in **6**, would result from (1) SN2 displacement within the homoallyl framework and (2) development of the thermodynamically more stable stereochemical arrangement at the chiral center bearing the π bond. Similar mechanistic considerations are applicable to the biogenesis of chrysanthemumcarboxylic acid and pyrethric acid, both shown to be derived from mevalonic acid.[6]

Formation of squalene from presqualene pyrophosphate undoubtedly is achieved by chemically acceptable and probably anchimerically assisted overall ring opening within the cyclopropylcarbinyl pyrophosphate system. In the case at hand, the bond involved in the initial ring expansion should be that one which permits stabilization through allylic participation (**7**). This process, involving SN2 attack at the pyrophosphate center,[7] could thus simply afford cyclobutylcarbonium ion **8**, which collapses to allylcarbonium ion **9** (or the corresponding pyrophosphate), susceptible to stereospecific NADPH reduction to squalene,[7] probably in concert with the rearrangement process. Alternatively, initial loss of pyrophosphate, as in **7**, could result in formation of bicyclobutonium ion **10**, which through bond rehybridization could isomerize to bicyclobutonium ion **11**. Once formed, the latter undergoes



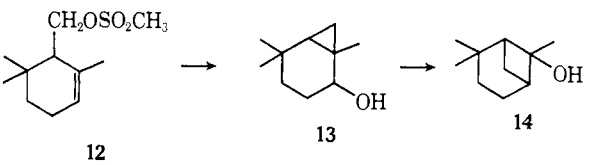
7 8 9



10 11

ring opening to the aforementioned allylcarbonium ion (or equivalent) and thence to squalene. Either version explains the observed (1) stereospecific replacement of lost proton (*vide supra*) by NADPH hydrogen, and (2) inversion of configuration at C-1 of the original farnesyl unit not involved in the aforementioned hydrogen exchange.[7]

Although there exist various nonenzymic precedents for the homoallylcyclopropylcarbinyl–cyclobutylcarbonium ion transformations presented above,[8] an instructive, parallel series, also in the terpene family, has been uncovered. Cyclogeranyl mesylate (**12**), mp 27–28°, on being heated at 50° for 45 min in aqueous acetone over solid $CaCO_3$, is converted mainly to the



12 13 14

cyclopropylcarbinol (**13**): mp 34–36°; nmr (60 MHz) δ 0.3–0.8 (complex multiplet), 0.9, 1.03, and 1.13 (3 CH_3's, all singlets), 3.73 (J = 5.5 Hz) (HC–O–, triplet).[9] On being treated with methanesulfonic acid in aqueous acetone, most of the cyclopropylcarbinol is slowly converted during the course of days to an isomer, mp 61–63°. The new product is unchanged by MnO_2 or CrO_3 in acetone–water–sulfuric acid and exhibits nmr signals at δ 0.85, 0.92, 1.33 (3 CH_3's, all singlets), 1.5–2.0 (complex series of multiplets), properties consistent with structure **14**.[10] It is noteworthy that these homoallylcyclopropylcarbinyl–cyclobutyl conversions are not the concurrent phenomena observed in scrambling solvolysis reactions,[8] but constitute a *selective and directed stepwise sequence*, similar to the proposed biochemical homoallyl (**5**) → cyclopropylcarbinyl (**6**) → cyclobutyl (**8**) sequence. Whereas operation of the biological series of reactions depends heavily on enzymic regulation, thermodynamic–kinetic control in the nonenzymic counterparts must be related to such variables as the type of leaving group, pH, and the nature

(6) (a) M. D. Crowley, P. J. Godin, H. S. Inglis, M. Snarey, and E. M. Thain, *Biochim. Biophys. Acta*, **60**, 312 (1962); (b) P. J. Godin, H. S. Inglis, M. Snarey, and E. M. Thain, *J. Chem. Soc.*, 5878 (1963).

(7) G. Popják, J. Edmond, K. Clifford, and V. Williams, *J. Biol. Chem.*, **244**, 1897 (1969).

(8) (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) for a review, see E. M. Kosower, "An Introduction to Physical Organic Chemistry," Wiley, New York, N. Y., 1968, pp 84–143.

(9) The corresponding liquid cyclopropyl ketone exhibited ir and nmr properties which would be expected for the assigned structure.

(10) Satisfactory elemental analyses were secured on all substances described.

of the medium. These factors, which roughly indicate the extent of enzymic assistance needed in the biological processes, and the attendant results, demonstrate the feasibility and reasonableness of an overall biosynthetic pathway that, at first sight, might seem highly unusual, if not improbable.

Acknowledgment. The author is grateful to Professor L. J. Altman for discussion and for information on the chemistry of presqualene alcohol prior to its publication, and to the National Science Foundation (GP 7187) for financial support.

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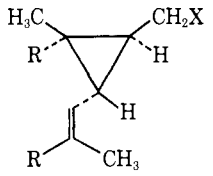
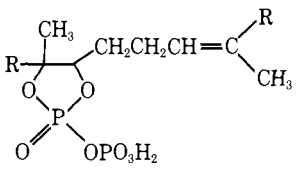
Synthesis and Conversion of Presqualene Alcohol to Squalene

Sir:

The course of the biosynthesis of squalene from farnesyl pyrophosphate has remained unsolved[1] in the course of recent years. Rilling and Epstein isolated[2] an intermediate from TPNH-starved yeast subcellular particles and assigned[3] its structure as **1a**. Apparently the same intermediate was isolated by Popjak, *et al.*, who assigned[4] structure **2**. We wish to report an unambiguous synthesis of presqualene alcohol (**1b**), and the successful conversion of **1a**, prepared from **1b**, to squalene by yeast subcellular particles.



1a, X = OPP
1b, X = OH



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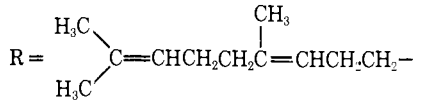
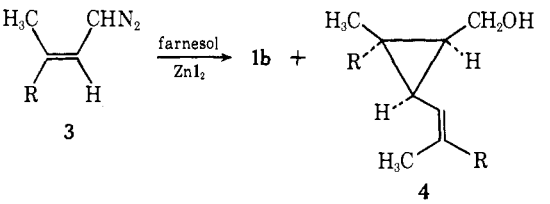


The synthesis was accomplished by the addition of the allylic diazo compound[5] **3** to a solution of *trans,trans*-farnesol and zinc iodide[6] in ether at 0°. A crude mixture containing **1b** and an isomer to which we assign structure **4** in an approximately 70:30 ratio was obtained in 25% overall yield. The separation



3 **4**

of isomers was accomplished by preparative layer chromatography (silica gel–HF; 20:80 ether–CCl_4) with relative R_f's of **1b** as 0.32 and **4** as 0.45. Both compounds showed similar mass spectra (70 eV, direct inlet) with molecular ions at *m/e* 426 and major high molecular weight fragment ions at *m/e* 339, 357, 395, and 408.

The nmr spectrum of **1b**[7] ($CDCl_3$) showed resonances at τ 8.85 (s, cyclopropyl methyl), 8.40 and 8.32 (21 H, s, allylic methyls), 8.00 (16 H, broad, allylic methylenes), 6.40 (2 H, AB of an ABX pattern, J_{AB} = 11, J_{AX} = 6, J_{BX} = 8 Hz, $\Delta\gamma_{AB}$ = 0.38 ppm, CH_2OH), and 4.8 (5 H, broad, vinyllic protons) whereas that of **4** ($CDCl_3$) showed resonances at τ 8.98 (s, cyclopropyl methyl), 8.43 and 8.35 (21 H, s, allylic methyls), 8.00 (16 H, broad, allylic methylenes), 6.40 (2 H, d, J = 7 Hz, CH_2OH), and 4.96 (5 H, broad, vinylic protons). In addition, both isomers showed additional unresolved resonances between τ 8.5 and 9.2 (cyclopropyl protons).

That both isomers had the same cis relationship of the cyclopropyl methyl group to the carbinyl alcohol was demonstrated by the relatively large downfield chemical shift of the cyclopropyl methyl resonance observed (0.17 ppm) upon oxidation of the alcohols to aldehydes. The addition of tris(dipivalomethanato)-europium[8] to a $CDCl_3$ solution of either **1b** or **4** allowed the determination of the coupling constant between the two cyclopropyl protons (**1b**, J = 5 Hz; **4**, J = 9 Hz), thus establishing the stereochemistry of **1b** and **4** as depicted.

Synthetic **1b** cochromatographed with **1b** prepared from natural **1a** from yeast on thin-layer chromatography (silica gel G; 70:30 cyclohexane–ethyl acetate) and on gas–liquid chromatography (3% OV-1, 200°).

For enzymatic conversion experiments, **1b** was oxidized to the aldehyde (CrO_3 in pyridine) which was reduced with $LiAl^3H_4$ to obtain the labeled alcohol. The tritiated alcohol was phosphorylated in the presence of farnesol as a carrier.[3] The pyrophosphate ester which was isolated by ion-exchange chromatography, cochromatographed with authentic presqualene pyrophosphate on thin-layer chromatography on buffered silica gel ^3H. When the synthetic ester was incubated with yeast subcellular particles, NADPH and $MgCl_2$, it was converted to a radioactive hydrocarbon, which was identified as squalene by cocrystallization with pure squalene as the thiourea adduct. The yield of squalene from synthetic **1a** was 34 or 68% of theoretical since **1a** is a *d,l* mixture.[9]

A possible mechanism for the biological conversion of presqualene pyrophosphate to squalene is based on the well-established equilibrium between cyclo-

(1) (a) G. Popjak, DeW. S. Goodman, J. W. Cornforth, R. H. Cornforth, and R. Ryhage, *J. Biol. Chem.*, **236**, 1934 (1961); (b) J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popjak, *Proc. Roy. Soc., Ser. B*, **163**, 492 (1966); (c) J. E. Baldwin, R. E. Hackler, and D. P. Kelly, *J. Amer. Chem. Soc.*, 90, 4758 (1968); (d) G. Krishna, H. W. Whitlock, Jr., D. H. Feldbruegge, and J. W. Porter, *Arch. Biochem. Biophys.*, **114**, 200 (1966).

(2) H. C. Rilling, *J. Biol. Chem.*, **241**, 3233 (1966).

(3) (a) H. C. Rilling and W. W. Epstein, *J. Amer. Chem. Soc.*, **91**, 1041 (1969); (b) W. W. Epstein and H. C. Rilling, *J. Biol. Chem.*, **18**, 4597 (1970).

(4) G. Popjak, J. Edmond, K. Clifford, and V. Williams, *ibid.*, **244**, 1897 (1969).

(5) (a) E. J. Corey and K. Achiwa, *Tetrahedron Lett.*, 3257 (1969); (b) R. M. Coates and R. M. Freidinger, *Tetrahedron*, **26**, 3487 (1970).

(6) (a) S. Winstein and J. Sonnenberg, *J. Amer. Chem. Soc.*, **83**, 3235 (1961); (b) G. Wittig and K. Schwarzenbach, *Justus Liebigs Ann. Chem.*, **650**, 1 (1961); (c) W. G. Dauben and G. H. Berezin, *J. Amer. Chem. Soc.*, **85**, 468 (1963); (d) S. H. Goh, L. E. Closs, and G. L. Closs, *J. Org. Chem.*, **34**, 25 (1969).

(7) *Anal.* Found: C, 84.35, 84.29; H, 11.69, 11.71.

(8) J. K. M. Sanders and D. H. Williams, *Chem. Commun.*, 422 (1970).

(9) Professor L. Crombie has independently and by a different procedure prepared a different mixture of isomers of presqualene alcohol. One of us (H. C. R.) has found one of the isomers to be identical with the natural product by the same procedures described in this communication.