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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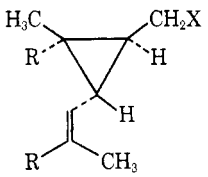
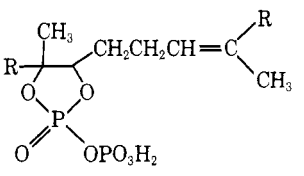
Synthesis and Conversion of Presqualene Alcohol to Squalene

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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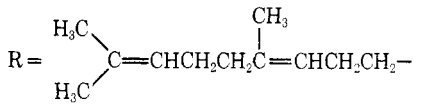
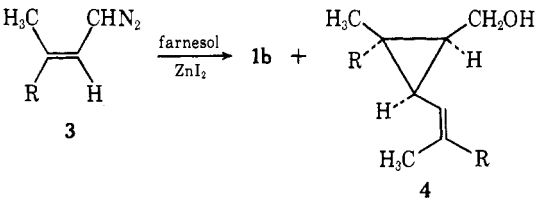


The synthesis was accomplished by the addition of the allylic diazo compound[5] **3** to a solution of *trans-trans*-farnesol and zinc iodide[6] in ether at 0°. A crude mixture containing **1b** and an isomer to which we assign structure **4** in an approximately 70:30 ratio was obtained in 25% overall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

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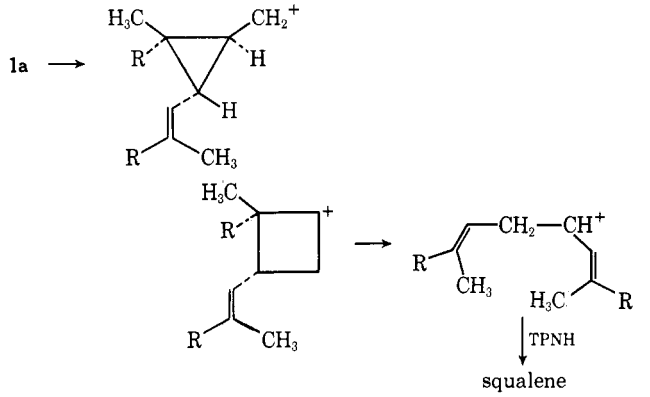
(7) *Anal.* Found: C, 84.35, 84.29; H, 11.69, 11.71.

(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.

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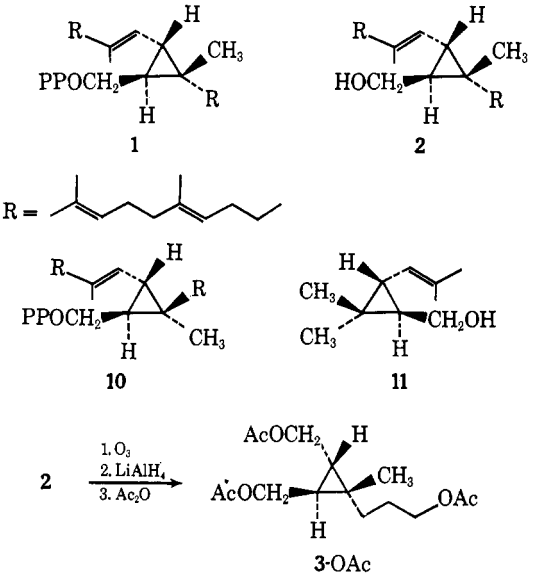
Studies on the Mechanism of Squalene Biosynthesis. Presqualene Pyrophosphate, Stereochemistry and a Mechanism for Its Conversion to Squalene

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.

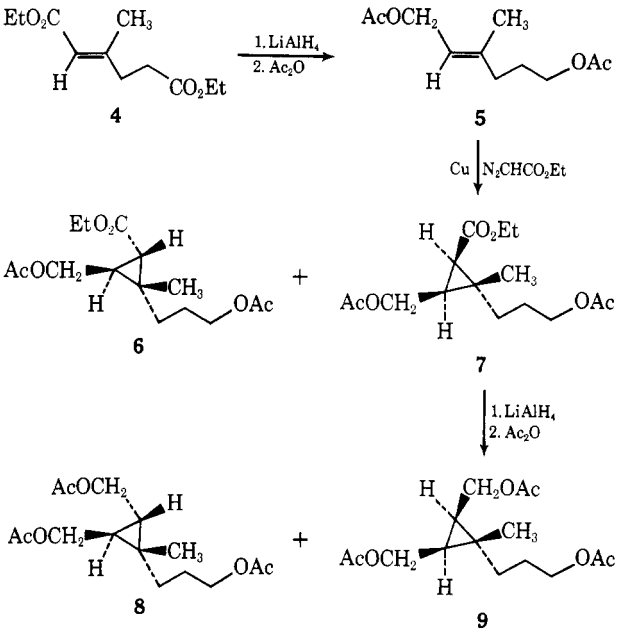
The relative stereochemistry of presqualene pyrophosphate was studied by a combination of synthetic and degradative investigations. Since the unresolved stereochemistry of the intermediate resides in the location of the substituents about the cyclopropane ring



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,

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(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).