be expected to deactivate rapidly to the $^1\Delta$ on collision.[6]

On continued irradiation the $^3\Sigma$ concentration decreases with time, presumably due to irreversible reactions of O_2 with the sensitizer. The $^1\Delta$ also decreases with the total pressure but more slowly. It is possible that at the lower pressures the excited naphthalenes are not completely scavenged by the $^3\Sigma$ to produce $^1\Delta$. At quite low O_2 pressures, ~0.01 mm, the $^1\Delta$ concentrations can be higher than that of the $^3\Sigma$. We have obtained O_2–IV mixtures in which 70% of the total O_2 is converted to $^1\Delta$. Such an inverted population can occur because we are not producing the $^1\Delta$ by direct absorption of light by the $^3\Sigma$ but rather through an intermediate excited aromatic.

One significant point is that the energy transfer we are observing in the above experiments is essentially that postulated by Khan, *et al.*, to explain how significant concentrations of $^1\Delta$ could arise in an atmosphere which contained some aromatic compounds.[7] Direct excitation from the $^3\Sigma$ would not be feasible because of the low transition probabilities. The apparently high efficiencies of the transfer even at low O_2 pressures make the process particularly likely. It would thus appear that substantial amounts of $^1\Delta$ might be available in a polluted atmosphere and could serve as a reactant in subsequent oxidations.

The following compounds have also been found to be sensitizers. The figures given are percentages of $^3\Sigma$ converted to $^1\Delta$: quinoxaline, 3.5%; 1-naphthaldehyde, 2.5; biphenyl, 2.4; 1-acetonaphthone, 0.9; 1-chloronaphthalene, 0.4; phenanthrene, 0.4; anthracene, 0.3; 2-acetonaphthone, 0.2; dibenzfuran, 0.2; anthraquinone, 0.1; phenanthridine, 0.1.

(6) S. J. Arnold, M. Kubo, and E. A. Ogryzlo, *Progr. Reaction Kinetics*, in press.
(7) A. U. Khan, J. N. Pitts, Jr., and E. B. Smith, *Environ. Sci. Technol.*, **1**, 656 (1967). We are indebted to Drs. R. W. Murray and P. R. Story for bringing this reference to our attention.
(8) Bell Telephone Laboratories, Inc.
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Studies on the Mechanism of Squalene Biosynthesis. Presqualene, a Pyrophosphorylated Precursor to Squalene

Sir:

Recently an intermediate in the biosynthesis of squalene from farnesyl pyrophosphate was isolated, and, on the basis of isotopic evidence, a tentative structure was proposed.[1] This structure has since been shown to be incorrect by Corey and Ortiz de Montellano,[2] who synthesized the compound and found it to be inactive in squalene synthesis. We now report chemical and spectroscopic evidence on this intermediate and on some of the derivatives which permits a revised formulation of its structure (I).

This intermediate was enzymatically prepared from farnesyl-1,1-t_2 pyrophosphate.[1,3] Butanol extracts of the incubation mixtures were concentrated and chromatographed on Dowex-1.[3] Fractions containing the intermediate were combined and, after the addition of NH_4OH, lyophilized. The residue was dissolved in benzene and precipitated with ammoniacal methanol. Thin-layer chromatography showed one major radioactive component with traces of nonradioactive contaminants.

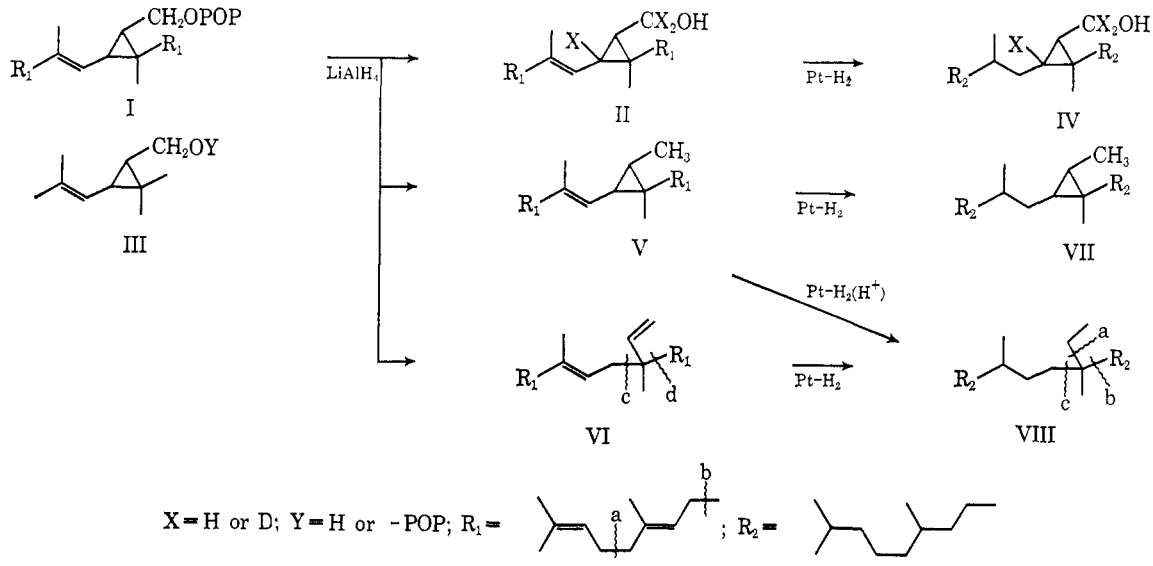
This compound was stable to strong base and labile to dilute acid and could be enzymatically converted to squalene in the presence of NADPH. The nmr spectrum[4] of I had, as does squalene, resonances at δ 5.3 (broad multiplet, olefinic H), 2.0 (methylene envelope), and 1.6 and 1.69 (incompletely resolved methyl singlets). In addition, there as a δ 4.07 multiplet (CH_2O) and a 1.16 broad singlet (at least 5 H) presumably due to the cyclopropyl methyl overlapped by the complex splitting of the cyclopropyl hydrogens. The later resonances are in reasonable agreement with the nmr spectrum of a model compound, III (Y = POP).

Since I is an unstable and nonvolatile pyrophosphate ester, most of the structural work was performed on reduction products from I. Treatment with $LiAlH_4$ gave a mixture of hydrocarbons (25%) and an alcohol (25%), II (X = H), whose homogeneity was established as follows. Chromatography of the alcohol on a 1 × 20 cm column of silica gel G with benzene as a solvent revealed a single radioactive component. This material was found to be homogeneous by tlc (cyclohexane–ethyl acetate 70:30, R_f 0.50) and by glpc on 3% SE 30 and 3% OV-1 with retention times of 1.47 and 1.78 relative to squalene. Acetylation of II yielded a single product as evidenced by tlc (R_f 0, hexane; 0.35, benzene; and 0.66, cyclohexane–ethyl acetate 70:30). Glpc of II-acetate showed a major component with retention times of 1.90 and 1.86 relative to squalene on OV-1 and SE-30. Substantial decomposition of II-acetate occurred during glpc.

The following experiment established that rearrangements had not occurred during the formation of II. Tritiated II was mixed with farnesol as a carrier and pyrophosphorylated,[5] and the products were separated by ion-exchange chromatography. The yield of II-pyrophosphate was 14% while that of farnesyl pyrophosphate was 25%. The II-pyrophosphate had chromatographic properties (ion exchange and tlc) identical with those of I and could be enzymatically converted to squalene in 65% yield.

The nmr spectrum of II (X = H) was similar to that of I except the signal due to the methylenoxy hydrogens was shifted to δ 3.82 and the cyclopropyl methyl singlet was relatively sharp at δ 1.15 with other ill-defined absorption at δ 0.9 and 1.26, presumably due to the cyclopropyl H and to CH_2 adjacent to cyclopropane. The

(1) H. C. Rilling, *J. Biol. Chem.*, **241**, 3233 (1966).
(2) E. J. Corey and P. Ortiz de Montellano, *Tetrahedron Letters*, 5113 (1968). We wish to thank Professor Corey for communicating these results to us prior to publication.
(3) S. Sofer and H. C. Rilling, *J. Lipid Res.*, in press.
(4) A Varian A-56/60 spectrometer was used in conjunction with a C-1024 time-averaging computer. Because of the small amounts of material available, each sample was scanned a minimum of 300 times. Deuteriochloroform was used as the solvent. The nmr spectra of *cis*- and *trans*-III (Y = H) were obtained from Professor R. B. Bates, whom we wish to thank.
(5) A. H. Kandutsch, H. Paulus, E. Levin, and K. Bloch, *J. Biol. Chem.*, **239**, 2507 (1964).



spectrum of II (X = H) was consistent with that of the model compound, III (Y = H). The mass spectrum[6] of II (X = H) contained a low-intensity molecular ion peak at *m/e* 426 ($C_{30}H_{50}O$) and a peak at *m/e* 408 (M − H_2O). The observed loss of 31 mass units (M − CH_2OH) was also found with the model compound III (Y = H) which, in addition to the nmr evidence, confirmed a primary alcohol. Other important peaks at *m/e* 357, 339, 289, and 271 can be attributed to allylic cleavages at a and b before and after the loss of water. The spectrum below *m/e* 200 closely resembles that of squalene. The mass spectrum of II (X = D) (obtained by isolation and reduction of I enzymatically prepared from farnesyl-1,1-d_2 pyrophosphate) was consistent with the assigned structure (M^+ at *m/e* 429 *vs.* 426, M − H_2O at *m/e* 411 *vs.* 408, M − CD_2OH at *m/e* 396 *vs.* 397, M − C_5H_9 at *m/e* 360 *vs.* 357). This definitely establishes the nature of the precursor of the pyrophosphate function in I.

Hydrogenation of II (X = H or D) in ethyl acetate gave only a decahydro product, IV, retaining the cyclopropyl ring as expected from similar reduction of III (Y = H). The mass spectrum of IV (or deuterated IV) shows a molecular ion, *m/e* 436 (439), and significant peaks at *m/e* 419 (422), M − OH; 420 (423), M − H_2O; and 405 (406), M − CH_2OH (CD_2OH), consistent with the assigned structure and model studies on III (Y = H).

The hydrocarbon fraction from $LiAlH_4$ treatment of I could be separated into three components by glpc. Structures V and VI were assigned to the two major compounds on the basis of hydrogenation and mass spectral data. In addition to the M^+ ion at *m/e* 410, V ($C_{30}H_{50}$) gave fragments at the expected allylic positions a and b similar to squalene. The absence of a $C_{15}H_{25}$ loss, observed with squalene, is consistent with V, retaining a cyclopropyl ring. Ring-opened VI ($C_{30}H_{50}$), in common with V, fragmented to give loss of C_5H_9 (a) and $C_{10}H_{17}$ (b) and also $C_{15}H_{25}$ (c) (*m/e* 205) like squalene and, in addition, loss of $C_{11}H_{19}$ (d) consistent with structure VI. Again the low-mass part of the spectra (below 200 mass units) closely resembled that of squalene. Hydrogenation of the hydrocarbon mixture in 10% acetic acid in ethyl acetate gave, in about equal amounts, two products. After glpc separation, mass spectra suggested one of the hydrogenated compounds to be VII, a $C_{30}H_{60}$ compound, retaining one degree of unsaturation, and the other, VIII, a fully saturated $C_{30}H_{62}$ material. If the hydrocarbon mixture was hydrogenated in the presence of strong mineral acid, VIII was formed at the expense of VII. The mass spectrum of VIII showed, in addition to the parent ion at *m/e* 422, a loss of C_2H_5 (a), indicating the cyclopropane ring had opened at the least substituted position. The other prominent, high-mass cleavages (b and c) occurred about the tetrasubstituted position.

The chemistry and spectra of this intermediate in squalene biosynthesis and its derivatives suggest structure I for this compound, for which we propose the name "presqualene." This structure meets the rigid requirements of the isotopic and stereochemical data of Popják, Cornforth, and their collaborators.[7] The data presented provide little evidence concerning the stereochemistry about the cyclopropane ring.

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(6) The mass spectra of II, III, and IV were obtained using a direct inlet technique on a C.E.C. Model 110B instrument. We are grateful to Mr. Leonard Wojcik for determining these spectra. Other mass spectra were obtained through the courtesy of Professor C. C. Sweeley, whose assistance we greatly appreciate.

(7) G. Popják and J. W. Cornforth, *Biochem. J.*, **101**, 553 (1966); J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, G. Ryback, and G. J. Schroepfer, *Proc. Roy. Soc.* (London), **B163**, 436 (1966); J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popjak, *ibid.*, **B163**, 492 (1966).

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