Synthesis of Presqualene Alcohol

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Summary Synthesis of Rilling's suggested structure (IIIb) for presqualene alcohol is described: one stereoisomer, as its pyrophosphate (IIId), is reported to be microsomally converted into squalene.

THE manner in which two farnesyl residues unite, tail to tail, in the biosynthetic process which leads to squalene has remained an unsolved problem in terpene chemistry.[1] Requirements for an acceptable process are defined by the known chirality, and the stereospecific loss of one hydrogen from C-1 of one farnesyl residue.[2] Rilling[3] has isolated an intermediate lying between farnesyl pyrophosphate and squalene, and the same, or a similar, intermediate has been obtained by Popják and colleagues[4] who formulated it as a glycol pyrophosphate (I). An earlier suggestion (II)[3] has been disproved by synthesis[5] and revised to (IIId)[6]. We now report a synthesis of Rilling's presqualene alcohol (IIIb).

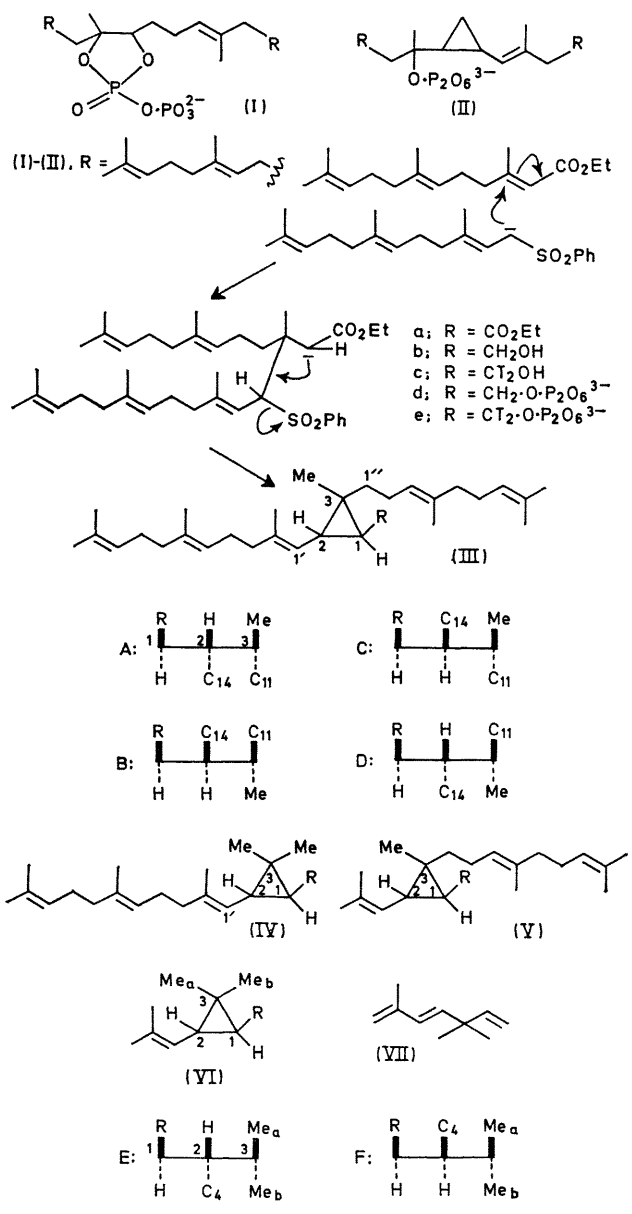
A sulphone addition–elimination approach[7] was first studied by the synthesis of (IVa) and (Va). Phenyl *trans*-farnesyl sulphone condensed with ethyl 3-methylbutenoate in dimethylformamide–potassium t-butoxide to give (IVa) (33%). This formed one spot on t.l.c. and had $M^+ C_{22}H_{36}O_2$. The ester absorbed three mols. of hydrogen over a platinum catalyst to give the saturated cyclopropane ester $M^+ C_{22}H_{42}O_2$. In the n.m.r. (IVa) showed resonances for three olefinic protons τ 4·8—5·2 (one of these, 1′-H, a doublet 5·15, J 8 Hz), four vinyl methyls 8·31, 8·35, and 8·42 (two), and cyclopropane *gem*-dimethyls at 8·76 and 8·88 [ethyl *trans*-chrysanthemate (VIaE), 8·76, 8·88; *cf.* ref. 8] as expected for a 1,2-*trans*-configuration. Close examination, however, suggests 10—20% of 1,2-*cis*-isomer may be present. Ethyl *E*-2,*E*-6-farnesoate and phenyl 3-methylbut-2-enyl sulphone similarly gave (Va) (50%), $M^+ C_{22}H_{36}O_2$, ν_{max} 1719 cm^{-1}. The n.m.r. data showed three olefinic protons, five vinyl-methyl resonances, and resonances for the single cyclopropane methyl at τ 8·79 and 8·90, indicating that (Va) was a mixture of 1,3-*cis*- and 1,3-*trans*-isomers. In agreement, t.l.c. [silica gel HF 254, ether (1)–light petroleum b.p. 40—60° (20)], resolved the product into two components. The alcohol mixture, (Vb) $M^+ C_{20}H_{34}O$ was formed from (Va) by reduction with lithium aluminium hydride and could be resolved into two components by t.l.c. (C-3-methyls τ 8·89 and 8·97). Similarly (IVa) gave (IVb), $M^+ C_{20}H_{34}O$, ν_{max} 3400 cm^{-1}, with n.m.r. resonances for three olefinic protons 4·8—5·3 (1′-H, d., 5·19, J 8); CH_2OH, 6·30, dd, J 6 and 11, and 6·58, dd, J 8, 11; cyclopropane *gem*-dimethyls 8·86, 8·96; cyclopropane 1-hydrogen *ca.* 9·2. With this information in hand, the problem of presqualene alcohol synthesis was examined. Phenyl *E*-2,*E*-6-farnesyl sulphone condensed, as above, with ethyl *E*-2,*E*-6-farnesoate to give (IIIa) (44%), separated by preparative-layer chromatography [silica gel HF 254, ether (1)–light petrol (4)] into low-R_F (LR_F ester, 45%) and high-R_F (HR_F ester, 55%) components. Each had ν_{max} 1720 cm^{-1}, $M^+ C_{32}H_{52}O_2$, and their mass spectra were almost indistinguishable, showing the expected losses of OEt, C_5H_9, $C_{10}H_{17}$, *etc.* In the n.m.r. the HR_F ester showed seven vinyl-methyl resonances at τ 8·31, 8·35, and 8·43, five olefinic protons [about four protons as a broad multiplet at 4·95, and about one at C-1′, a broad doublet at 5·14, J 8 Hz] and a cyclopropane 3-methyl resonance at 8·89. The LR_F ester also showed seven vinyl-methyls, τ 8·31, 8·36 and 8·43, five olefinic protons [about 4H at 4·95, br.m., and the 1′H (about 1H) at 5·11, br. d. J 8 Hz] with a cyclopropane 3-methyl at 8·80. On reduction with lithium aluminium hydride the HR_F ester gives HR_F alcohol, and the LR_F ester gives LR_F alcohol, the two alcohol preparations (IIIb) being chromatographically separable by t.l.c. Each had $M^+ C_{30}H_{50}O$ with the expected series of fragment losses, H_2O, CH_2OH, C_5H_9, $C_{10}H_{17}$, ($H_2O + C_5H_9$), ($H_2O + C_{10}H_{17}$), in their virtually identical mass spectra. The alcohols showed the following n.m.r. features (HR_F with LR_F in parentheses): seven vinyl methyls 8·35, 8·43 (8·34, 8·42), five olefinic protons [four as a multiplet 5·00 (5·00) and one, at 1′, d. 5·17, J 8 Hz (5·12, J 8 Hz)], CH_2OH multiplet centred at 6·45 (6·28 J_{gem} 11, J_{vic} 6; 6·61 J_{gem} 11, J_{vic} 8), CH_2 envelope 8·0 (8·0), satd. 1″ 8·7m (8·7m), cyclopropane 3-methyl 8·97 (8·87).

The LR_F alcohol (IIIb), on hydrogenation (Pt) gave the expected cyclopropane alcohol $M^+ C_{30}H_{60}O$, together with the corresponding hydrogenolysis product, the cyclopropane $M^+ C_{30}H_{60}$: both had the expected mass spectra. Natural

presqualene alcohol† had the same mass spectrum as LR_F alcohol and t.l.c. comparison in three solvent systems showed the two to be indistinguishable: HR_F alcohol, although closely similar in mass spectrum, was readily distinguishable by t.l.c. On g.l.c. (3% OV-1, 190°) however, it was apparent that LR_F alcohol consisted of two just resolvable components, LR_Fl (40% approx.) and LR_Fh (60%) of low and high g.l.c. retention times, respectively. The latter co-chromatographed on the g.l.c. system with natural presqualene alcohol. By reduction of LR_F ester (IIIa) with lithium aluminium tritiide, the LR_F alcohol was made in tritiated form (IIIc). Its pyrophosphate (IIIe) co-chromatographed with presqualene pyrophosphate on t.l.c. and ion-exchange chromatography. The pyrophosphate LR_F (IIIe), when incubated with yeast microsomes and NADPH, yielded radioactive squalene in 63% of the theoretical yield for a (±)-precursor. Identity of the product was verified by combining it with authentic squalene and crystallising the squalene thiourea adduct to constant activity. The synthetic LR_Fh (IIIb) thus appears to be identical with natural presqualene alcohol, in agreement with Rilling's suggestion.[6] Our HR_F alcohol could be similarly resolved into two components HR_Fl (35% approx.) and HR_Fh (65%), both different from presqualene alcohol. Preparation of HR_F in tritiated form (IIIc), conversion into (IIIe), and incubation with yeast microsomes as above gave 0·4% theoretical conversion into squalene. This is probably due to contamination by LR_Fh, and there is g.l.c. evidence of contamination at the retention time of the latter.

Comparative studies of the LR_F and HR_F esters and alcohols,‡ using *cis*- and *trans*-chrysanthemic esters and alcohols together with (IV) and (V) as models, and concentrating particularly on the chemical shifts of the cyclopropane 3-methyl groups, suggest that the components of LR_F alcohol are (IIIbA) and (IIIbB). The HR_F alcohol consists of (IIIbC) and (IIIbD).

Further work is required on the mechanism by which (IIId) is converted into squalene. Both *cis*- and *trans*-chrysanthemyl alcohol (VIb-E and -F) give (VII) as a substantial product when treated with acid catalyst in benzene, with thionyl chloride, or with toluene-*p*-sulphonyl chloride in pyridine,[9] suggesting that a directly analogous carbonium-ion decomposition is not involved in the natural process.



(*Received, December 30th*, 1970; *Com.* 2257.)

† Supplied by Professor H. C. Rilling, University of Utah. The pyrophosphorylation, enzymic examination and g.l.c. of our tritiated alcohols was carried out by Professor Rilling, to whom we express our warm appreciation. He has informed us that whilst testing our presqualene alcohols, a further specimen, prepared in a different way, was received from Dr. L. J. Altman (Stanford University) and has been found to be converted enzymically, by his procedure, into squalene in 68% of the theoretical yield.

‡ A more detailed study of this problem, using solvent shifts, and lanthanide shift reagents, is in progress.

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