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SYNTHESIS OF "PRE-PRESQUALENE', A PREDICATED INTERMEDIATE IN PRESQUALENE BIOSYNTHESIS, AND OF PRENYLOGUES.

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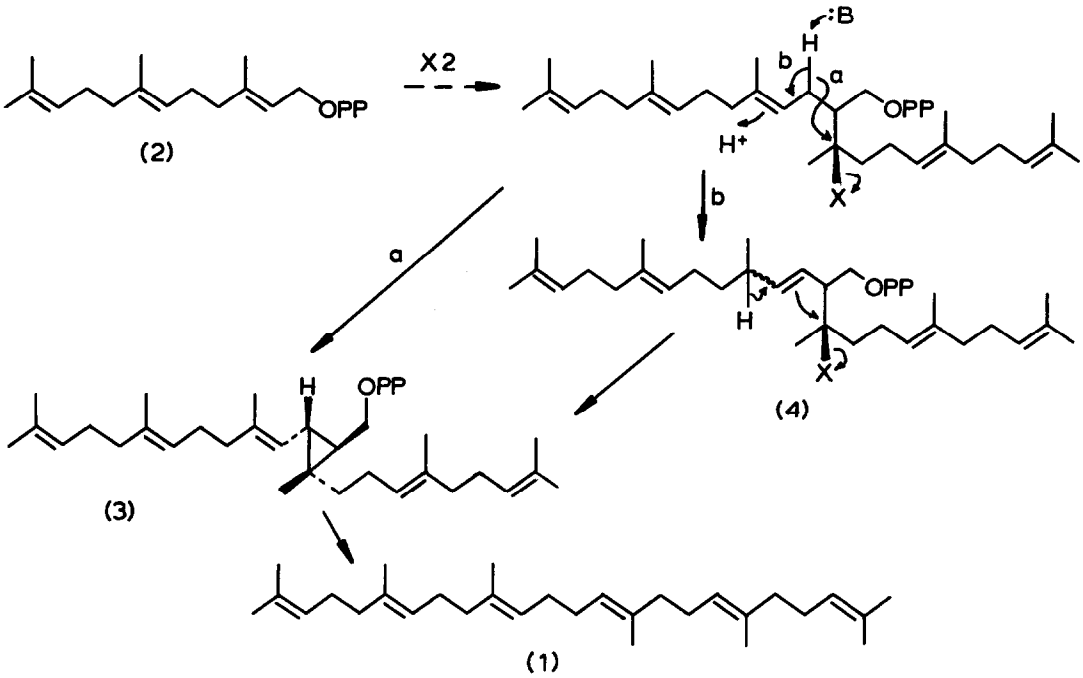
Summary: The synthesis is reported, using deconjugative addition, of the E- and Z- diols (15 g) and (16 g), postulated intermediates[8] in presqualene biosynthesis.

The classical work of Cornforth, Popják, and their collaborators has revealed the biosynthesis of squalene (1) from mevalonate in remarkable detail. A stage of great mechanistic interest is the tail-tail coupling of two farnesyl pyrophosphate (2) units to give presqualene[1] (3), which is converted to (1) in the presence of NADPH. Squalene synthetase systems deprived of the reducing cofactor accumulate cyclopropane (3) whose constitution rests on structural investigations[2] and total syntheses[3]. Since an analogue of (3) inhibits squalene, but not presqualene, biosynthesis, presqualene is shown to be an obligatory intermediate.[4] A C_{40} prenylogue, prephytoene, holds a similar role in carotenoid biosynthesis.[3a,5]

Various mechanisms for the formation of presqualene from (2) have been discussed {originally in terms of the 1S,2S,3S-structure,[6] but following stereochemical revision,[7] in terms of the enantiomer shown, (3)}. Two groups of proposals may be distinguished; those, Scheme 1, path (a), in which the cyclopropane is formed by 1,3-elimination[2c,6] (between an allylic proton and a group X, either pyrophosphate or an enzyme-attached function) or those, path b, where double bond migration is postulated to allow a cyclopropane to be formed by homoallylic displacement.[8,9] In path b, with X=OPP (as expounded by van Tamelen and Schwarz[8]) compound (4) becomes a discrete intermediate, which we refer to here as 'pre-presqualene'. With the ultimate aim of testing the possible role of 'pre-presqualene' in squalene biosynthesis, we have synthesised the corresponding diols (15 g) and (16 g) and several prenylogues.

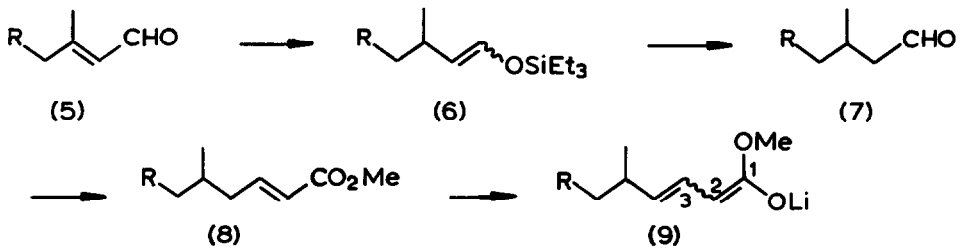
We set out to use a simple synthetic strategy employing α-hydroxyalkylation of anions (9) generated from α-unsaturated esters[10] (8). Thus treatment of ester (8a) with 1 mol. equiv. of bistrimethylsilylamidolithium at -78°, with HMPA, gave (9a) which on quenching with acetone yielded the hydroxyesters (13d + 14d) (36%; recovered ester 28%). The products were separated by p.l.c. and the stereochemistry established by ^{1}H.m.r. using europium shift reagent to separate overlapping signals. At -78°, the reaction gave 85% Z-hydroxyester (14d) with 15% (13d); increasing the reaction temperature raised the proportion of E-hydroxyester, e.g. to 43% at -25°; but overall yields declined, with

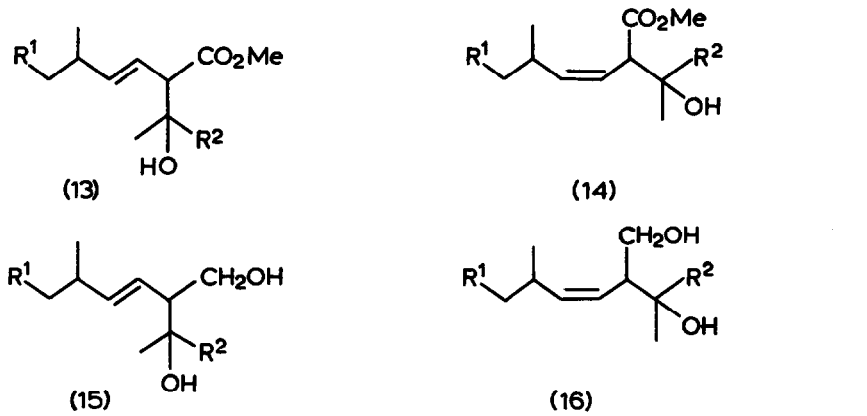


Scheme 1

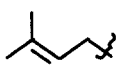
acetone acting as proton source instead of as carbon electrophile. Most conveniently, anion (9a) was generated at -25° and protonated ; clean deconjugation was observed, and the isomers (11a), (12a) separated on a silver nitrate loaded silica column. Deprotonation of (11a) and (12a) separately at -84° followed by trapping with acetone proceeded with complete retention of stereochemistry to afford the separate isomers (13d), 14d) each in ca. 35% yield. These experiments suggest that 3-Z anion (9a) is the kinetically-preferred product from deprotonation of (8a). Lithium aluminium hydride reduction of (13d) and (14d) then yielded the corresponding diols (15d) and (16d) (isomeric with lavanduladiol). It is worth noting that, if 'pre-presqualene' (4) is a precursor to presqualene, then the diols (15d), (16d) could be intermediates in the biosynthesis of chrysanthemyl alcohol.

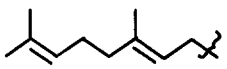
The prenylogues (8b) and (8c) of ester (8a) were then prepared from 2-E,Z-citral (5b) and 2-E,Z-6E-farnesal (5c) respectively, through reduction with triethylsilane using Wilkinson's catalyst to the enol silyl ethers (6b) and (6c),[12] readily hydrolysed to aldehydes (7b) and (7c) from which the desired esters were generated using carboxymethylenetriphenylphosphorane. The anion (9b) produced by deprotonation with bistrimethylsilylamido lithium[13] was reacted with acetone at -78° to give (13e) and 14e) (C_{15} prenylogues of the lavandulyl series), and with 6-methylhept-5-en-2-one to form (13f) and (14f) (C_{20} series; 42% yield, 70% on recovered ester).[14] Both pairs of



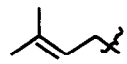


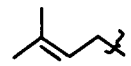
(a) R = H

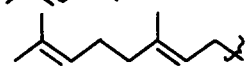
(b) R = 

(c) R = 

(d) $R^1 = R^2 = H$

(e) R^1 =  $R^2 = H$

(f) $R^1 = R^2$ = 

(g) $R^1 = R^2$ = 

hydroxyesters were separable into their geometric isomers on silver nitrate impregnated silica. Anion (9c) was protonated to the deconjugated esters (11c) and (12c), separable in the same way. For initial biochemical experiments to test the status of (4; X=OPP) in squalene biosynthesis a mixture of all stereoisomers of (15 g) and (16 g) was required. Thus (9c) was condensed at -78° with E-geranylacetone (10c). The resulting esters (13 g) and (14 g), ca. 40% trans, 60% cis) formed methylthiomethyl ethers[14] useful for characterisation. Lithium aluminium hydride reduction then provided the desired diols (15 g) and (16 g): the double bond isomers were separable by t.l.c. The diols form only monotrimethylsilyl ethers on treating with bistrimethylsilyltrifluoroacetamide at 50° (1 h). Finally, tritiated diols were obtained by reduction

of (13 g) and (14 g) with lithium aluminium tritiide. Further experiments to prepare the bispyrophosphates of (15 g) and (16 g), and to further resolve their stereoisomers, are planned, so that decisive biological investigations can be carried out.

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12. The geometric isomers of (6c) could be separated, and their stereochemistry established by n.m.r.

13. Anions (9b) and (9c) were readily generated using lithium diisopropylamide (LDA) with HMPA; however on attempted reaction with ketones, considerable proton transfer ensued resulting in recovery of deconjugated ester and the product of ketone self-condensation.

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