Resolution of Presqualene and Prephytoene Alcohols

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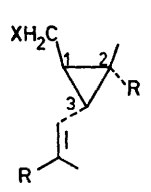
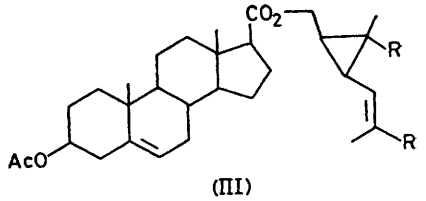
Summary Synthetic presqualene and prephytoene alcohols have been resolved through their etienic acid derivatives; the biologically active prephytoene alcohol (as its pyrophosphate) has the (1*R*,2*R*,3*R*) absolute configuration.

THE stereospecific head-to-head condensation of two molecules of farnesyl pyrophosphate during the biosynthesis of squalene[1] and geranylgeranyl pyrophosphate in phytoene[2,3] biosynthesis proceeds through cyclopropyl carbinyl intermediates presqualene pyrophosphate (Ib) and prephytoene pyrophosphate (IIb), respectively. The absolute configuration of natural (+)-presqualene alcohol was shown to be (1*R*,2*R*,3*R*).[4] Although natural prephytoene alcohol has been suggested[1e] to have the same absolute configuration as presqualene alcohol, this has not received experimental support. In 1972, Porter and his co-workers reported the isolation of 'prelycopersene pyrophosphate' from a tomato enzyme system.[3] This intermediate exhibited a positive optical rotatory dispersion curve analogous to presqualene pyrophosphate. However, the results of Rilling[2b] strongly suggest that Porter's intermediate was a product of squalene synthetase and not of the carotogenic enzyme responsible for the head-to-head dimerization of geranylgeranyl pyrophosphate.





(III)

(Ia); R = $C_{11}H_{19}$, X = OH
(Ib); R = $C_{11}H_{19}$, X = OPP
(IIa); R = $C_{15}H_{27}$, X = OH
(IIb); R = $C_{15}H_{27}$, X = OPP

a; R = $C_{11}H_{19}$
b; R = $C_{15}H_{27}$

We now report the resolution[5] of synthetic presqualene and prephytoene alcohols through their etienic acid derivatives (IIIa and IIIb) and the demonstration that the biologically active prephytoene alcohol (as its pyrophosphate) exhibits a positive optical rotatory dispersion curve and that its absolute configuration is (1*R*,2*R*,3*R*).

(±)-Presqualene and (±)-prephytoene alcohols were synthesized as previously described.[1a,2a] Treatment of the alcohols with readily available 3β-acetoxy-17β-chloroformyl androst-5-ene[6] in dry pyridine gave presqualene

TABLE. Optical rotatory dispersion data[a]

Wavelength λ/nm	(+)-Prephytoene *c* 0·0037 [α]	(−)-Prephytoene *c* 0·0035 [α]	(+)-Presqualene *c* 0·0010 [α]	(−)-Presqualene *c* 0·0011 [α]
365 Hg	149	−143	193	−206
436 Hg	90	−83	103	−110
546 Hg	45	−46	48	−52
578 Hg	39	−40	48	−48
589 Na	38	−37	47	−48

[a] Rotations were measured at 20·0 °C in $CHCl_3$ using a Perkin Elmer Model 241 Polarimeter; deviations < 10% of the reported values were observed.

etienate (IIIa) and prephytoene etienate (IIIb) as a mixture of diastereomers. Presqualene etienate, $[\alpha]_D^{20}$ — 14·5° in $CHCl_3$, and prephytoene etienate, $[\alpha]_D^{20}$ — 6·4° in $CHCl_3$, gave satisfactory elemental analyses. The diastereomers were separated by high pressure liquid chromatography on μ-Porasil (Waters Assoc. 3·9 mm I.D. × 30 cm; eluent CH_2Cl_2; 1 ml min^{-1}). The pure diastereomers of presqualene and prephytoene yielded identical n.m.r. data except for a characteristic octet of an ABX system for the diastereotopic cyclopropyl carbinyl protons that each showed.

The individual diastereomers of (IIIa) and (IIIb) were reduced with $LiAlH_4$ to give the enantiomeric alcohols in 85—90% yield. The o.r.d. data for the enantiomeric alcohols are given in the Table.

The (+)-enantiomers of presqualene alcohol and prephytoene alcohol showed a normal positive o.r.d. curve, identical to natural presqualene alcohol[7] thus establishing the absolute configuration of (+)-prephytoene as (1*R*,2*R*,3*R*).

For biological studies each of the enantiomeric alcohols was tritiated at the cyclopropyl carbinyl position by reduction of the corresponding aldehyde with tritium labelled $NaBH_4$. The labelled alcohols were then converted into the corresponding pyrophosphates. Only the pyrophosphates derived from (+)-presqualene alcohol and (+)-prephytoene alcohol proved to be biologically active.†

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† (+)-Presqualene pyrophosphate was utilised by yeast subcellular particles and (+)-prephytoene was utilised by phycomyces Blakesleeanus strains C_9 and C_5.

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