Mechanism of Formation of Natural Cyclopropanes: Synthesis of Postulated Intermediates in Presqualene and Chrysanthemyl Alcohol Biosynthesis

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The α-unsaturated esters (**15**), (**28**), and (**33**) have been used in deconjugative additions to ketones, to provide the β,γ-hydroxy esters (**17**), (**18**), (**29**), (**30**), and (**37**) and hence the unsaturated diols (**22**), (**23**), and (**38**). These diols have been postulated as precursors to the natural cyclopropanes chrysanthemyl alcohol and presqualene, through homoallylic participation. Experiments with cell-free extract of *Artemisia annua* foliage show that neither diol (**22**) nor (**23**) is converted to chrysanthemyl alcohol. Limited experiments with an homogenate of *Rhizopus arrhizus* provide little support for the intervention of the diol (**38**) in squalene biosynthesis.

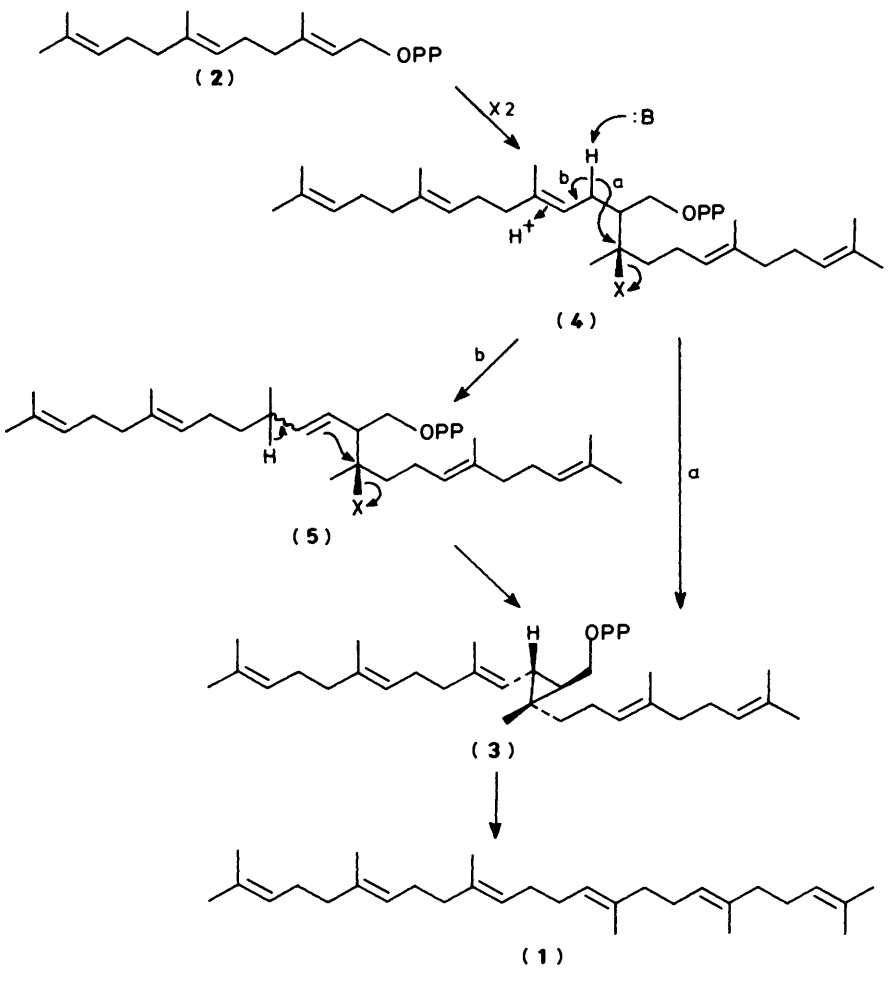
A considerable body of work has delineated the biosynthesis, from mevalonate, of squalene (**1**), the precursor to all steroids and triterpenoids. The post-farnesyl pyrophosphate (**2**) stages are the least well understood. The cyclopropyl carbinol (**3**), presqualene,[1] has been established as an intermediate, being transformed into squalene in the presence of NADPH. Squalene synthetase deprived of the reducing cofactor accumulates compound (**3**), whose constitution rests on structural investigations[2] and total synthesis.[3] Evidence that presqualene is an obligate intermediate has been advanced.[4] A C_{40} prenylogue, prephytoene, holds a parallel role in carotenoid biosynthesis.[3a,5]

The remarkable coupling of two farnesyl pyrophosphate molecules to form presqualene has provoked various speculations on the mechanism.[2c,6,7,8] A dimer (**4**) figures in all such proposals, possibly as a discrete entity, and the parent alcohol (**4**; X = OH), bifarnesol, has very recently been synthesised.[9] The (**4**) ⟶ (**3**) conversion has been viewed as 1,3-elimination[2c,6] of HX (path a) from (**4**; X = OPP or enzyme attached function) or as a two-step process (path b) in which double bond migration precedes homoallylic displacement.[7,8] In path b, originally proposed by van Tamelen and Schwartz, compound (**5**; X = OPP) becomes a second intermediate in the (**2**) ⟶ (**1**) transformation, i.e. 'pre-presqualene.' A synthesis of 'pre-presqualene' would open the way to evaluate its role, if any, in squalene biosynthesis. This paper reports such a synthesis, and certain biological experimentation.

The biosynthesis of irregular monoterpenes such as chrysanthemyl alcohol (**6**), lavandulol (**7**), artemisia alcohol (**8**), and santolina triene (**9**) has been widely discussed over a long period. Experimental work *in vivo* has been hampered by the unyielding biological systems involved, and with many negative results, no clearly agreed view on the biogenetic connections has emerged. This area is too extensive to review here. However, it is worthy of note that structural parallels exist between bifarnesol (**4**) and lavanduladiol (**10**), and between presqualene (**3**) and chrysanthemyl alcohol (**6**), *i.e.* these are pairs of prenylogues. Thus a plausible mechanistic parallel may be drawn between the modes of formation of (**3**) and (**6**), with a homoallylic alcohol (**11**), analagous to pre-presqualene, as a key intermediate. This suggestion has been recently supported by Goldschmidt, Crammer, and Ikan[10] who claimed to have observed the formation of the cyclopropane ester (**12**), albeit in low yield, among other esters, on acid treatment of the hydroxy ester (**13**). The reverse reaction (**12**) ⟶ (**13**) was also noted. Divergence between the C_{10} and C_{30} series would follow the cyclopropane stage with (**6**) giving rise to the artemisia and santolina series *e.g.* (**8**) and (**9**), rather than the squalene prenylogue (**14**) which is not found in Nature, to our knowledge. Various chemically induced conversions of chrysanthemyl alcohol to other irregular monoterpenoid skeletons have been observed,[11] some of which may have biomimetic significance. The availability of the diol (**11**) would afford another opportunity to test the possibility of homoallylic participation in the biosynthesis of natural cyclopropanes.

The required compounds in both series were prepared using a simple strategy based on α-hydroxyalkylation of anions generated from α-unsaturated esters.[12] Thus treatment of the ester (**15**) with bis(trimethylsilyl)amidolithium at −78 °C in the presence of hexamethylphosphoramide, gave the anion (**16**). This was trapped with acetone to yield the *E* and *Z* esters (**17**) and (**18**) (50%, based on the ester utilised). Since the mass spectra of these products were dominated by fragmentation to acetone, the methylthiomethyl ethers (**19**) were prepared for characterisation, by reaction with dimethyl sulphoxide–acetic anhydride;[13] the thioacetals gave appropriate molecular ions. Analysis of the mixture of (**17**) and (**18**) by ^{1}H n.m.r. spectroscopy, using the europium shift reagent Eu(fod)$_3$ showed a preponderance (85%) of the *Z*-isomer (**18**). Increasing the reaction temperature raised the proportion of *E*-ester, *e.g.* to 43% at −25 °C, while lowering the overall yield (protonation of the anion increased at the expense of addition). In one experiment at −84 °C, 100% *Z*-form was obtained. These results suggest that the *Z*-form of enolate (**10**) is kinetically preferred. A similar preference for *Z*-isomers in deconjugation processes has been observed.[14]

Since stereoisomers (**17**) and (**18**) proved difficult to separate, the ester (**15**) was deprotonated at −25 °C and reprotonated to yield the β,γ-unsaturated esters (**20**) and (**21**), which were readily separated using a silver nitrate impregnated silica column. The separate esters were then deprotonated at −84 °C; protonation afforded the starting compounds without stereomutation, and quenching of the anions with acetone also proceeded with retention of stereochemistry to yield the separate isomers (**17**) and (**18**), in 35% yield on a 0.5 mmol scale (suitable for radiochemical work). Lithium aluminium hydride reduction of esters (**17**) and (**18**) then gave the *E*- and *Z*-diols (**22**) and (**23**), with the expected ^{1}H n.m.r. coupling constants. Employing [2-^{14}C]acetone in the reaction sequence then provided [^{14}C]-labelled specimens of (**22**) and (**23**). Both the *E*- and *Z*-hydroxy esters (**17**) and (**18**) were smoothly dehydrated by thionyl chloride in benzene to the 4*E*- and 4*Z*-diene esters, (**24**) and (**25**), with complete retention of geometry (product composition analysed by g.l.c.). Thus the 4,5-double bond does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(2)
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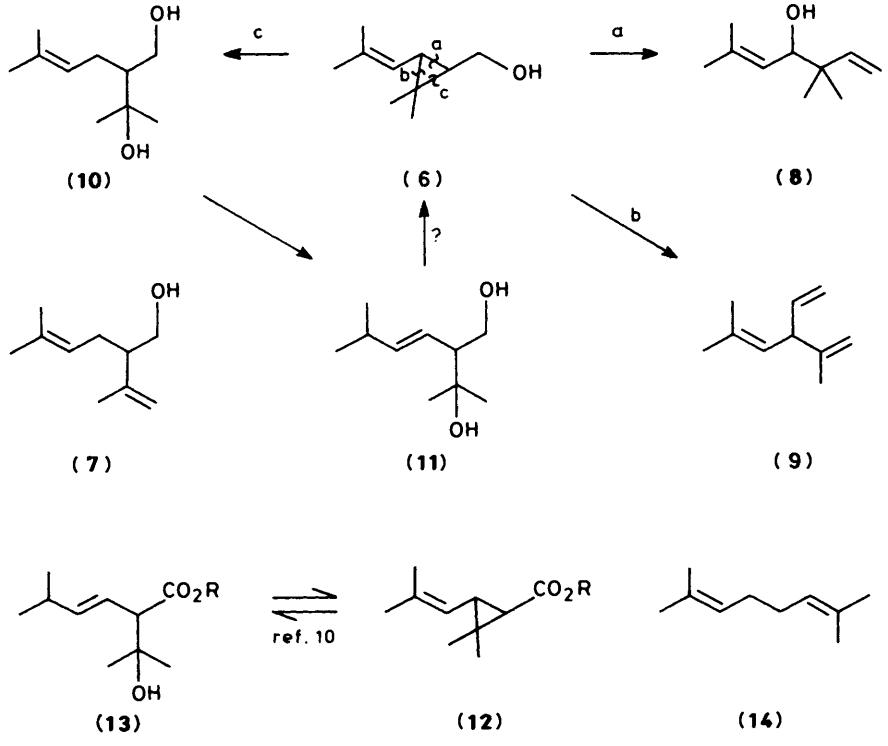
OH
c
a
OH
OH
(10)
(6)
(8)
?
b
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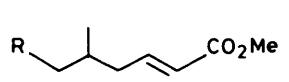

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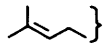
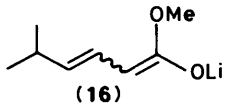
(28) R = 



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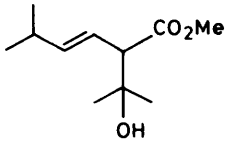

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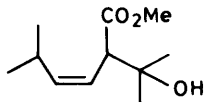

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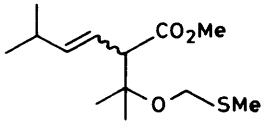

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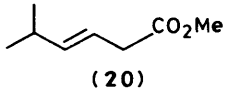

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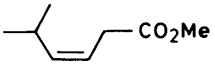

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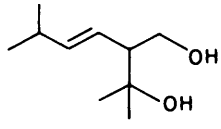

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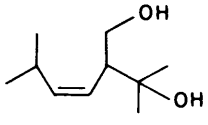

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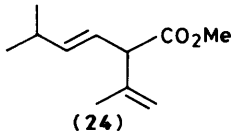

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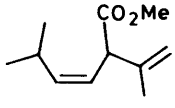

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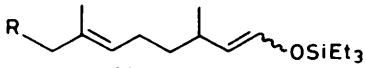

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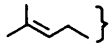
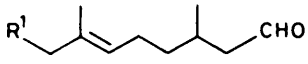
(31) R = 



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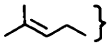
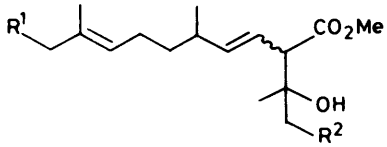
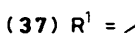
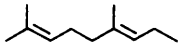
(32) R = 



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

(30) $R^1 = H$; R^2 = 

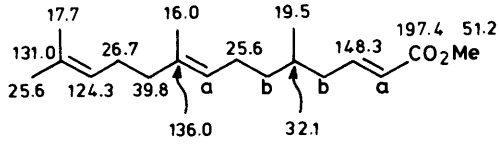
(37) R^1 =  ; R^2 = 

not participate in this reaction. However, in view of the regiospecificity (Hofmann) it is unlikely that the reaction involves a carbocation.

The applicability of these methods to higher prenylogues was then examined before turning to the C_{30} series. Thus (2*EZ*)-citral was reduced with triethylsilane,[15] using Wilkinson's catalyst to the *EZ*-silyl enol ether (**26**). Hydrolysis to dihydrocitral (**27**) was followed by reaction with methoxycarbonylmethylenetriphenylphosphorane to provide the 2*E*-ester (**28**). The ester (**28**) was deprotonated with bistrimethylsilylamido lithium at −78 °C and the resulting anions allowed to react first with acetone, and then with 6-methylhept-5-en-2-one, to form the hydroxy esters (**29**) and (**30**) (42%; 70% on ester utilised) respectively.

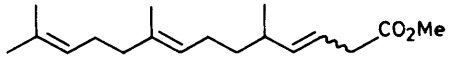
Both pairs of hydroxy esters [*EZ*-(**29**) and *EZ*-(**30**)], C_{15} and C_{20} prenylogues of the lavandula series, were resolved into their geometric isomers on silver nitrate loaded silica.

For 'pre-presqualene', (2*EZ*, 6*E*)-farnesol was oxidised to farnesal and converted to the ester (**33**) by way of the enol ether (**31**) and dihydrofarnesal (**32**). The 2*E*,8*E* stereochemistry of (**33**) was checked by ^{13}C n.m.r. (5*EZ*)-Geranylacetone was separated, on a silver nitrate–silica column, into the isomers (**35**) and (**36**) whose stereochemistry was established by ^{13}C n.m.r. spectroscopy. The carbanion from the ester (**33**) was generated



(**33**) a 122.1, 124.3

b 36.7, 39.8

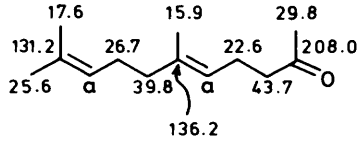


(**34**)

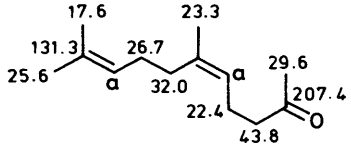
as before:* quenching with water generated the deconjugated esters (**34**), and reaction with (5*E*)-geranylacetone provided the desired C_{30} series ester (**37**), as a mixture of stereoisomers. Reduction of this mixture with lithium aluminium hydride then gave the stereoisomers of the diol (**38**). Compounds (**37**) and (**38**) had appropriate ^1H n.m.r. and mass spectra, and the hydroxy ester gave a methylthiomethyl ether derivative that was also characterised. Reduction of (**37**) with [^3H]lithium aluminium hydride gave a tritiated specimen of (**38**). The hydride reduction was complicated by some concomitant retro-aldol reaction of (**37**) (Lewis acid catalysed), so that the alcohols arising from the reduction of compounds (**33**) and (**35**) were also found in the product mixture. Diol (**38**) could be separated by t.l.c. into two sets of stereoisomers (probably the *E*- and the *Z*-racemates). It was planned to test, in the first instance, a mixture of stereoisomers in biosynthetic experiments since it was of course not known which, if any, stereoisomer would be transformed by squalene synthetase. However we did attempt to resolve the ester (**33**), with a view to providing two groups of stereoisomers of (**38**) clearly epimeric at one centre, for suitable control experiments. To this end we prepared esters (**39**) (from menthol), (**40**) [using an alcohol prepared from di-*O*-ethyl-(+)-mandelate], and (**41**) (from ergosterol); however in no case could the two diastereoisomers be separated by h.p.l.c.

To test the status of the diols (**22**) and (**23**) as precursors of chrysanthemyl alcohol we chose to employ a cell-free system

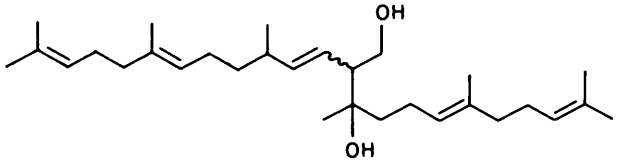
* The use of lithium di-isopropylamide lead to markedly lower yields.



(35) α 122.8,124.3



(36) α 123.7,124.3



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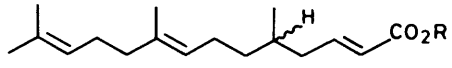
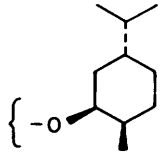
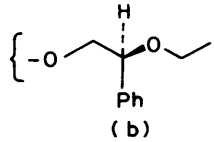


(39) R = (a)
(40) R = (b)
(41) R = (c)



(a)



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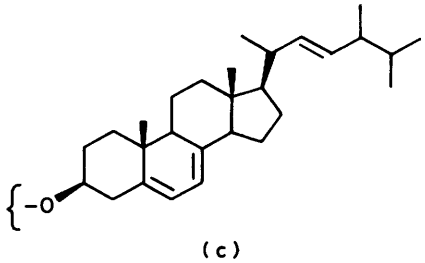

(c)

prepared from the foliage of *Artemisia annua* plants, using the methods developed by Banthorpe, Doonan, and Gutowski. These workers, in an important paper,[16] have shown that a crude homogenate from *A. annua* will convert isopentenyl pyrophosphate, dimethylallylpyrophosphate, and dimethylvinylcarbinol (separately) into artemisia alcohol (**8**) and artemisia ketone, and also into lavandulol (**7**) and *trans*-chrysanthemyl alcohol (**6**). Further, the homogenate interconverts *cis*- and *trans*-chrysanthemyl alcohols and transforms both of these into artemisia alcohol and ketone (in the presence NAD and NADP). Thus the enzymes and cofactors necessary to form *trans*-chrysanthemyl alcohol, and ring cleave it to artemisia alcohol, are present; and since free alcohols are metabolised it is likely that the system will conduct phosphorylation (ATP supplied). This system appeared very suitable to test the possible role of the homoallylic diols (**22**) and (**23**) in the formation of *trans*-chrysanthemyl alcohol.

Thus fresh foliage of *A. annua* was ground in buffer with polyclar AT, L-cysteine, diethyl dithiocarbamate, and EDTA; the filtered product was centrifuged and the supernatant was chromatographed on Sephadex-G10. The first protein band was used in subsequent incubations, with suitable cofactors: phosphatase and apyrase were then employed to cleave any phosphates formed. For simplicity we chose to isolate only the artemisia ketone product; after dilution with carrier, the ketone was reduced and the (±)-alcohol (**8**) converted to its 3,5-dinitrobenzoate ester for crystallisation to constant activity. In a test run, [^{14}C]isopentenyl pyrophosphate was efficiently incorporated (9%) into artemisia ketone in this system, in broad agreement with previous results.[15] However, neither (**22**) nor (**23**) (both sufficiently water soluble) was transformed into artemisia ketone; incorporations $> 10^{-2}$% would certainly have been detected. Thus neither was converted into *trans*-chrysanthemyl alcohol and there is no support from these experiments for a homoallylic mechanism for cyclopropane formation in this system.

We then turned to the C_{30} series. We noted, during the course of our work, that Weete and Campbell[17] had reported a cell-free extract from the mycelium of *Rhizopus arrhizus* which was able to convert mevalonic acid into squalene, with absolute incorporations in the range 20—40%. This system appeared ideal for testing our C_{30} diol (**38**) as a squalene precursor. Professor Weete* agreed to carry out these experiments and we supplied two chromatographic fractions of the [^{3}H]-diol stereoisomers. These were separately incubated with the homogenate, using a blank control experiment and a parallel [^{14}C]mevalonic acid (MVA) incubation for comparison. After 1 h, both fractions were incorporated into squalene to 0.16%, after correcting for results in the blank experiment, while the MVA was incorporated to the extent of 11%. These results are clearly not encouraging since (*a*) the incorporation of diol (**38**) is much lower than that of MVA, (*b*) both fractions, presumably of different stereoisomers, give the same result, and (*c*) the squalene activity was not obtained through crystallisation of a derivative and the activity recorded may be high, despite the use of a control. Lower incorporations (0.10%) were obtained after 3 h incubations, suggesting further metabolism of squalene. However these experiments are not unequivocal since (*a*) stereoisomers are present in unknown proportions, (*b*) water solubility problems may not have been overcome by detergent (Tween 20), (*c*) inhibition from excess diol may have arisen, and (*d*) the extract may not have been able to effect phosphorylation of the diol in the necessary way. For these reasons the experiments should be regarded as preliminary, but nevertheless as they stand they offer very little support to the view that a stereoisomer of the diol (**38**) is an intermediate in squalene biosynthesis.

Experimental

N.m.r. spectra were measured at 100 MHz using tetramethylsilane as an internal standard; observed couplings (*J*) are given in Hz. 'Eu(fod)$_3$' is tris(heptafluoro-2,2-dimethyloctane-3,5-dionato)europium. Mass spectra were recorded using electron

* We are very grateful to Professor J. D. Weete, Department of Botany and Microbiology, Auburn University, Alabama, U.S.A., for organising these experiments.

impact ionisation. Isobutane was used in chemical ionisation measurements (c.i.m.s.). 'Extracted into ether' signifies extraction of aqueous solutions with diethyl ether, washing of the extracts with water, and drying of extracts over 'anhydrous' magnesium or sodium sulphate. 'Evaporation' refers to evaporation under reduced pressure. Silica gel HF_{254} was used in thin layer chromatography (t.l.c.); in preparative t.l.c. layers *ca.* 1 mm. thick were employed, pre-eluted with methanol. 'Light petroleum' refers to the fraction b.p. 40—60 °C.

(3E)- *and* (3Z)-*Methyl 5-Methylhex-3-enoate.*—(*a*) In general, lithium bis(trimethylsilyl)amide (LBTMSA) solutions were generated as follows. Hexamethyldisilazane (1 mol equiv.) was treated with butyl-lithium (0.99 mol equiv.) in hexane, at room temperature, for 30 min. The hexane was evaporated off under reduced pressure and the white crystalline residue was dissolved in anhydrous tetrahydrofuran (1.5 cm^3 per mmol). The solution was cooled to *ca.* −20 °C and hexamethylphosphoramide (1 mol equiv.) was added.

(*b*) (2*E*)-Methyl 5-methylhex-2-enoate (0.9 g, 6.3 mmol), (prepared from isovaleraldehyde and methoxycarbonylmethylene triphenylphosphorane in dichloromethane, at room temperature for 18 h) was added to a solution of LBTMSA (9.3 mmol) at −78 °C. The reaction temperature was raised to −25 °C, and the deep yellow solution maintained at −25 °C for 45 min. Saturated aqueous ammonium chloride was then added, and the mixture was extracted with ether. The extracts yielded a mixture of the title isomers which were separated by column chromatography on silica gel G impregnated with silver nitrate (30%), eluting with hexane–benzene (1:1), and monitoring by g.l.c. The compound eluted first was (3E)-*methyl 5-methylhex-3-enoate* (**20**) (230 mg), one peak (R_t 18.5 min.) on g.l.c. (10% silicone oil on diatomite; 85 °C; 40 cm^3 min^{-1}); $\nu_{max.}$(film) 2 950, 1 730, 1 465, 1 440, 1 320, 1 260, 1 160, 1 025, and 980 cm^{-1}: δ($CDCl_3$) 5.56 (2 H, m, 3-, 4-H), 3.75 (3 H, s, OMe), 3.06, (2 H, d, *J* 5, 2-H_2), 2.5—2.1 (1 H, m, 5-H), and 1.02 (6 H, d, *J* 6, 5-Me_2); $J_{3,4}$ 16 was measured from a spectrum shifted by Eu(fod)$_3$: *m/z* 260 (M^+). The second compound eluted was (3Z)-*methyl 5-methylhex-3-enoate* (**21**) (270 mg), one peak (R_t 16 min) on g.l.c. (conditions above); $\nu_{max.}$(film) 2 950, 1 730, 1 465, 1 440, 1 335, 1 260, 1 170, and 1 030 cm^{-1}: δ($CDCl_3$) 5.42 (2 H, m, 3-, 4-H), 3.70 (3 H, s, OMe), 3.10 (2 H, d, *J* 6, 2-H_2), 2.7—2.3 (1 H, m, 5-H), and 0.96 (6 H, d, *J* 6, 5-Me_2); $J_{3,4}$ 12 was measured from a spectrum shifted by Eu(fod)$_3$: *m/z* 260 (M^+).

(4Z)-2-*Hydroxy*-3-*methoxycarbonyl*-2,6-*dimethylhept*-4-*ene.*—(*a*) (*Z*)-Methyl 5-methylhex-3-enoate (67 mg, 0.47 mmol) was added to a solution, at −78 °C, of LBTMSA (0.47 mmol), and the mixture was stirred at this temperature for 1 h. Acetone (27 mg, 0.46 mmol) in tetrahydrofuran (0.25 cm^3) was added and stirring was continued at −78 °C for 1.5 h before quenching with saturated aqueous ammonium chloride. The product was extracted with ether, and was purified by preparative t.l.c., eluting with hexane–ether (3:1), to yield the *title hydroxy ester* (**18**) as an oil (27 mg, 29%); [Found: (M^+ − C_3H_6O), 142.098. $C_8H_{14}O_2$ (M − C_3H_6O) requires 142.099]: $\nu_{max.}$(film) 3 500, 2 960, 1 725, and 970 cm^{-1}; δ($CDCl_3$) 5.46 (2 H, m, 4-, 5-H), 3.72 (3 H, s, OMe), 3.38 (1 H, m, 3-H), 3.3 (1 H, br s, OH), 2.8—2.4 (1 H, m, 6-H), 1.26 (3 H, s, 2-Me), 1.18 (3 H, s, 2-Me), and 0.96 (6 H, d, *J* 6, 6-Me_2); $J_{3,4} = J_{5,6}$ = 12 were measured from a spectrum shifted by Eu(fod)$_3$.

(*b*) In a separate experiment the carbanion from (*Z*)-methyl 5-methylhex-3-enoate, generated as above, was quenched with aqueous ammonium chloride. G.l.c. analysis of the product showed that the starting *Z*-ester was recovered, not detectably contaminated by the *E*-isomer.

(*c*) Experiment (*a*) was repeated on the same scale, but using [2-^{14}C]acetone (50 μCi), to yield (*Z*)-[2-^{14}C]-2-hydroxy-3-methoxycarbonyl 2,6-dimethylhept-4-ene (39.2 mg, 46%; 20.7 μCi, radiochemical yield, 41%).

(Z)-2-*Hydroxy*-3-*hydroxymethyl*-2,6-*dimethylhept*-4-*ene.*—(*a*) The *Z*-hydroxy ester (**18**), (45 mg, 0.22 mmol) in dry ether (1.5 cm^3) was added to a stirred suspension of lithium aluminium hydride (30 mg, 0.79 mmol) in dry ether (1 cm^3). The mixture was stirred at room temperature for 5 h before being cooled and quenched with water. Dilute sulphuric acid was added, and the solution was extracted with ether. The product from extraction was purified by preparative t.l.c. to yield the *title diol* (**23**), (18 mg, 46%); [Found: *m/z* 173 (c.i.), 157.121 (electron impact). $C_{10}H_{20}O_3$ requires 172, $C_9H_{17}O_2$ (M^+ − Me) requires 157.123]: $\nu_{max.}$(film) 3 400, 2 970, 960, and 880 cm^{-1}: δ($CDCl_3$) 5.48 (1 H, dd, *J* 10,10) and 5.11 (1 H, dd, *J* 10,10) (4-, 5-H), 3.80 (2 H, m, CH_2OH), 2.9—2.3 (4 H, m, 3-, 6-H, 2 × OH), 1.28 (6 H, s, 2-Me_2), and 0.98 (6 H, d, *J* 7, 6-Me_2).

(*b*) A parallel experiment using the [2-^{14}C]ester gave *Z*-[2-^{14}C]-2-hydroxy-3-hydroxymethyl-2,6-dimethylhept-4-ene (63%; radiochemical yield from [2-^{14}C]acetone, 27%).

(E)-2-*Hydroxy*-3-*methoxycarbonyl*-2,6-*dimethylhept*-4-*ene.*—(*a*) (*E*)-Methyl 5-methylhex-3-enoate (66 mg, 0.47 mmol) was added to a solution of LBTMSA (0.47 mmol) at −78 °C. After the reaction had been stirred at this temperature for 1 h, acetone (26 mg, 0.47 mmol) in tetrahydrofuran (0.25 cm^3) was added. The reaction was allowed to proceed at −78 °C for 15 h before being quenched with aqueous ammonium chloride. The product was extracted with ether and was purified by preparative t.l.c. [hexane–ether (3:1)] to yield the *title hydroxy ester* (**17**), (31 mg, 35%) as an oil; [Found: (M^+ − $C_8H_{14}O_2$) 142.098. (M − C_3H_6O) requires 142.099]: $\nu_{max.}$(film) 3 450, 2 950, 1 720, 980, 905, and 850 cm^{-1}: δ($CDCl_3$) 5.60 (2 H, m, 4-, 5-H), 3.75 (3 H, s, OMe), 3.30 (1 H, d, *J* 11, 3-H), 3.1 (1 H, br s, OH), 2.5—2.2 (1 H, m, 6-H), 1.24 (3 H, s, 2-Me), 1.20 (3 H, s, 2-Me), and 1.00 (6 H, d, *J* 6, 6-Me_2); $J_{3,4}$ 11, $J_{4,5}$ 16 and $J_{5,6}$ 7 were measured from a spectrum shifted with Eu(fod)$_3$.

(*b*) In a parallel experiment using [2-^{14}C]acetone (50 μCi) (*E*)-[2-^{14}C]-2-hydroxy-3-methoxycarbonyl-2,6-dimethylhept-4-ene (6.5 mg, 7.5%; 2.87 μCi) was prepared.

(E)-2-*Hydroxy*-3-*hydroxymethyl*-2,6-*dimethylhept*-4-*ene.*—(*a*) The *E*-hydroxy ester (**17**) (33 mg, 0.16 mmol) was stirred for 5 h in ether (2.5 cm^3) with lithium aluminium hydride (25 mg, 0.66 mmol), at ambient temperature. After treating the mixture with dilute sulphuric acid, the organic products were collected in ether and were purified by t.l.c. to yield the *title diol* (**22**), (14 mg, 50%) [Found: *m/z* 173 (c.i.), 157.121 (e.i.)]: $\nu_{max.}$(film) 3 400, 2 980, 980, 955, and 880 cm^{-1}; δ($CDCl_3$) 5.60 (1 H, dd, *J* 7,16) and 5.20 (1 H, dd, *J* 10,16) (4-, 5-H), 3.70 (2 H, m, CH_2OH), 2.8—2.0 (4 H, m, 3-, 6-H, 2 × OH), 1.22 (6 H, s, 2-Me_2), and 1.00 (6 H, d, *J* 7, 6-Me_2).

(*b*) A parallel experiment using the [2-^{14}C]ester gave (*E*)-[2-^{14}C]-2-hydroxy-3-hydroxymethyl-2,6-dimethylhept-4-ene (43%; radiochemical yield from [2-^{14}C]acetone 2.8%).

(EZ)-3-*Methoxycarbonyl*-2,6-*dimethyl*-2-*methylthiomethoxyhept*-4-*ene.*—(*EZ*)-2-Hydroxy-3-methoxycarbonyl-2,6-dimethylhept-4-ene (59 mg, 0.3 mmol) in dry dimethyl sulphoxide (1.5 cm^3) was treated with acetic anhydride (1.5 cm^3) and the solution was set aside at ambient temperature for 42 h when it was diluted with ether. The mixture was thoroughly washed with aqueous sodium carbonate and then water. Evaporation of the dried organic phase gave a residue purified by t.l.c. (chloroform) to yield the *title thioacetal* (**19**) as an oil (30%); (Found: M^+, 260.146. $C_{13}H_{24}O_3S$ requires M^+, 260.145); δ($CDCl_3$) 5.40 (2 H, m, 4-, 5-H), 4.54 (2 H, s, OCH_2S), 3.66 (4 H,

3-H, OMe), 2.8—2.3 (1 H, m, 6-H), 2.14 (3 H, s, SMe), 1.3 (6 H, 2-Me_2), and 0.92 (6 H, 6-Me_2).

(E)- *and* (Z)- 3-*Methoxycarbonyl*-2,6-*dimethylhepta*-1,4-*diene*.—(*a*) (*E*)-2-Hydroxy-3-methoxycarbonyl-2,6-dimethylhept-4-ene (42 mg, 0.21 mmol) in ether (1.5 cm^3) was added to thionyl chloride (550 mg) in ether (1.5 cm^3). The solution was set aside at ambient temperature for 89 h, then cooled to 0 °C, and treated with aqueous sodium carbonate. The aqueous layer was extracted with ether, and the combined ether extracts were dried and evaporated. The residue was purified by t.l.c. to yield the *title* E-*diene* (**24**), (19 mg, 50%) as an oil, showing on g.l.c. single peak (R_t 26 min; 10% Apiezon L; 86 °C; 40 cm^3 min^{-1}) (Found: M^+, 182.130. $C_{11}H_{18}O_2$ requires M^+, 182.131): δ($CDCl_3$) 5.7—5.3 (2 H, m, 4-, 5-H), 4.92 (2 H, br s, 1-H_2), 3.74 (4 H, 3-H, OMe), 2.3—2.0 (1 H, m, 6-H), 1.78 (3 H, br s, 2-Me), and 1.0 (6 H, d, *J* 8, 6-Me_2); $J_{4,5}$ 16 was measured in Eu(fod)$_3$ studies. G.l.c. examination of the whole organic reaction product showed none of the *Z*-isomer (see below).

(*b*) A similar experiment using the *Z*-hydroxy ester afforded only the *title* Z-*diene* (**25**) (58%) also an oil, one peak on g.l.c. (R_t 23 min), with conditions as above; (Found: M^+, 182.130); ($CDCl_3$) 5.64—5.28 (2 H, m, 4-, 5-H), 4.9 (2 H, br s, 1-H_2), 4.04 (1 H, d, *J* 8, 3-H), 3.76 (3 H, s, OMe), 2.8—2.3 (1 H, m, 6-H), 1.80 (3 H, br s, 2-Me), 0.92 and 0.90 (both 3 H, d, *J* 8, 6-Me_2); $J_{4,5}$ 10 was measured in Eu(fod)$_3$ studies. None of the *E*-isomer could be detected in the organic reaction product.

Preparation of a Monoterpene-synthesising Homogenate from Artemisia annua *foliage*.[16]—Foliage (7 g) from young *A. annua* plants (*ca.* 18″ high) was frozen with liquid nitrogen, mixed with sand, and ground to a fine powder during 15 min. Polycar AT (*ca.* 2 vols.) was added with tris-acetate buffer (pH 7.01; 40 cm^3) containing L-cysteine hydrochloride (70 mg), diethyl dithiocarbamate (86 mg), and ethylenediamine tetra-acetic acid disodium salt (13.5 mg). The mixture was set aside at 0 °C for 90 min when it was quickly filtered through glass wool. The filtrate was centrifuged at 17 000 r.p.m. for 15 min. The yellowish green supernatant was passed down a column (Sephadex G-10, 50 g), at 4 °C, eluting with tris-acetate buffer (pH 7.01) containing L-cysteine hydrochloride (1.75 mg cm^{-3}). The eluate was cooled to 0 °C, and the clear yellow fraction was used immediately.

Incubation of [1-^{14}C]*Isopentenyl Pyrophosphate with an Homogenate of* A. annua.—[1-^{14}C]Isopentenyl pyrophosphate (ammonium salt: 1.6 × 10^5 d.p.m.) in maleate buffer (0.2 cm^3; pH 6.62) was added to a sample (1 cm^3) of the homogenate prepared as in the preceding experiment and maleate buffer (1 cm^3) containing ATP (3.4 mg); NADP (4.4 mg), NAD (3.96 mg), dithiothreitol (0.86 mg), manganese chloride (1.2 mg) and magnesium chloride (1.0 mg). The solution was maintained at 31 °C for 2 h, when alkaline buffer (1 cm^3; sodium carbonate–sodium hydrogen carbonate; pH 10.4) containing apyrase (10 mg), phosphatase (15 mg), and magnesium chloride (6 mg), and the incubation continued at 33 °C for 3.5 h. Chloroform (0.05 cm^3) and ethanol (0.05 cm^3) were then added and the solution was stored at 0 °C overnight. The thawed solution was then saturated with sodium chloride and it was extracted with ether (4 × 1.5 cm^3) using centrifugation to break emulsions. Artemisia ketone* (90 mg) was added as a carrier to the combined organic extracts. The organic solution was dried and treated with excess sodium dihydrobis(2-methoxyethoxy) aluminate in toluene at 0 °C for 30 min and then at room temperature for 60 min. The product was treated with dilute hydrochloric acid at 0 °C; the organic layer was removed and the aqueous layer extracted with ether. The combined organic solutions were washed with aqueous sodium carbonate (10 cm^3), dried, and evaporated. The residue oil (100 mg) in pyridine (4 cm^3) was treated with 3,5-dinitrobenzoyl chloride (350 mg) at room temperature for 15 h. The mixture was poured into 2M-hydrochloric acid at 0 °C and after 15 min the organic products were collected in ether. The ethereal solution was washed sequentially with dilute hydrochloric acid, aqueous sodium carbonate, and water. The dried solution yielded an oil after evaporation from which was isolated (±)-artemisia alcohol 3,5-dinitrobenzoate by preparative t.l.c. The ester was recrystallised from dry light petroleum to m.p. 92—92.5 °C and to constant radioactivity, 70 d.p.m. mg^{-1}.

* = 3,3,6-trimethylhepta-1,5-dien-4-one.

Incubation of (E)- *and* (Z)-2-*Hydroxy*-3-*hydroxymethyl*-2,6-*dimethylhept*-4-*ene with an Homogenate of* A. annua.—The *E*- and *Z*-diols (**22**) and (**23**) were separately dissolved in maleate buffer pH 6.62, with a little Tween 20. Samples (0.4 cm^3) of the solutions of the *E*-diol (1.04 × 10^6 d.p.m.) and the *Z*-diol (1.52 × 10^7 d.p.m.) were separately incubated with homogenate (5 cm^3) of *Artemisia annua* foliage prepared as in the previous experiment. The incubation, addition of artemisia ketone carrier, and isolation of (+)-artemisia alcohol 3,5-dinotrobenzoate were executed as before; repeated crystallisation of this ester, from both diols, afforded samples with no detectable radioactivity.

(1E,6E)-*and*(1Z,6E)-3,7,11-*Trimethyl*-1-*triethylsiloxydodeca*-1,6,10-*triene*.—(2*EZ*,6*E*)-Farnesal (4.4 g, 20 mmol) was added to a suspension of tris(triphenylphosphine)rhodium chloride (100 mg) stirred under nitrogen at 50 °C in triethylsilane (freshly distilled, 3.5 g). After 3 h under these conditions light petroleum (12 cm^3) was added to the mixture which was then filtered through a short alumina column. The filtrate was evaporated. The residue was chromatographed on silica (160 g) eluting with light petroleum–benzene (9:1); the major band yielded a mixture (90%) of the 1*E*- and 1*Z*-isomers of the title compound (**31**). In preparative work the mixture was hydrolysed as below. For characterisation a small sample was separated by preparative t.l.c. on silica with light petroleum–benzene (9:1) into the *title* (1Z,6E)-*enol ether*, R_F 0.65 (Found: M^+, 336.286. $C_{21}H_{40}OSi$ requires *M*, 336.285); δ($CDCl_3$) 6.18 (1 H, d, *J* 6, 1-H), 5.16 (2 H, m, 6-, 10-H), 4.22 (1 H, dd, *J* 6,9, 2-H), 1.68 (3 H, s, CMe) and 1.60 (6 H, s, 2 × CMe); and the *title* (1E,6E)-*enol ether*, R_F 0.48 (Found: M^+, 336.286); δ($CDCl_3$) 6.24 (1 H, d, *J* 12, 1-H), 5.14 (2 H, m, 6-, 10-H), 4.89 (1 H, dd, *J* 10, 12, 2-H), 1.72 (3 H, s, CMe) and 1.64 (6 H, s, 2 × CMe). The 1*Z*-isomer was predominant in the mixture (1*Z*:1*E* = 3.4:1). In a parallel preparation citral was reduced to (1*EZ*)-3,7-dimethyl-1-triethylsilyloxyocta-1,6-diene[15] (**26**) (87%) (Found: M^+, 268.221. Calc. for $C_{16}H_{32}OSi$ M^+, 268.222).

(6E)-3,7,11-*Trimethyldodeca*-6,10-*dienal* [(E)-2,3-*Dihydrofarnesal*].—(1*EZ*,6*E*)-3,7,11-Trimethyl-1-triethylsiloxydodeca-1,6,10-triene (4.3 g), acetone (80 cm^3), methanol (130 cm^3) and 3% aqueous sodium hydrogen carbonate (10 cm^3) were stirred together at ambient temperature for 2.5 h. The solution was evaporated to a small volume, diluted with ether, and washed with water. Evaporation of the dried ethereal solution gave the title aldehyde, (2.86 g). As this compound proved labile it was used for the next reaction without further purification. A small sample was purified by preparative t.l.c. (silica–chloroform) to provide (6E)-2,3-*dihydrofarnesal* (**32**) as an oil which decomposed on storage at 0 °C; $\nu_{max.}$(film) 2 900, 2 850, 2 700, 1 710, 1 660, and 1 105 cm^{-1}; δ($CDCl_3$) 9.84 (1 H, m, 1-H), 5.16 (2 H, br t, 6-, 10-H), 1.70 (3 H, s, 12-H_3), 1.64 (6 H, s, 7-, 11-Me), and 0.98 (3 H, d, *J* 7, 3-Me). 2,3-Dihydrocitral

(Found: M^+, 154.134. Calc. for $C_{10}H_{18}O$: M^+ 154.136) was prepared from citral in a similar way.

(2E,8E)-*Methyl* 5,9,13-*Trimethyltetradeca*-2,8,12-*trienoate*.—(6*E*)-2,3-Dihydrofarnesal (2.81 g, 12.6 mmol) and methoxycarbonylmethylenetriphenylphosphorane (4.2 g, 13 mmol) were set aside in dichloromethane (50 cm^3) at ambient temperature for 20 h. The mixture was evaporated to dryness and the residue extracted with light petroleum (5 × 10 cm^3). The combined extracts were passed through a short alumina H column (light petroleum elution) and evaporated to yield an oil, which was purified on a silica column (120 g), using chloroform as the eluant, to yield the *title ester* (**33**), (2.36 g, 67%), b.p. 106 °C/0.1 mmHg; (Found: M^+, 278.224. $C_{18}H_{30}O_2$ requires M, 278.225); one peak on g.l.c. (10% OV-101; 176 °C); $\nu_{max.}$(film) 2 900, 2 840, 1 715, 1 650, 1 045, and 990 cm^{-1}; $\delta(CDCl_3)$ 7.22 (1 H, dt, J 8,16, 3-H), 6.02 (1 H, d, J 16, 2-H), 5.30 (2 H, br t, 8-, 12-H), 3.92 (3 H, s, OMe), 1.76 (3 H, s, 14-H_3), 1.70 (6 H, s, 9-, 13-Me), and 0.94 (3 H, d, J 7, 5-Me). (*E*)-Methyl 5,9-dimethyldeca-2,8-dienoate was prepared from 2,3-dihydrocitral in an analogous reaction (Found: M^+, 210.164. Calc. for $C_{13}H_{22}O_2$: M, 210.162).

Esters of (2E,8E)-5,9,13-*Trimethyltetradeca*-2,8,12-*trienoic Acid*.—(*a*) Methyl (2*E*,8*E*)-5,9,13-trimethyltetradeca-2,8,12-trienoate (0.8 g) was refluxed in methanol (10 cm^3) and water (30 cm^3) with sodium hydroxide (3.2 g), for 3 h. The cooled solution was acidified, and the organic products collected in ether, from which was obtained the title acid (0.5 g), 7.08 (1 H, m, 3-H), 5.81 (1 H, d, J 15, 2-H), 5.10 (2 H, m, 8-, 12-H), 1.68 (3 H, s) and 1.60 (6 H, s) (3 × CMe), and 0.91 (3 H, d, J 7, 5-Me). Without further purification the acid was converted to the corresponding acid chloride by treatment with oxalyl chloride in dry benzene at room temperature (1.5 h).

(*b*) The above acid chloride (72 mg) in dry benzene (1 cm^3) was added to a stirred mixture of alcohol (46 mg, prepared from (+)-mandelic acid by bis-*O*-ethylation followed by lithium aluminium hydride reduction) and silver cyanide and benzene (2 cm^3). The mixture was refluxed for 22 h, filtered through a short silica column, and evaporated. Preparative t.l.c. of the residue afforded the *ester* (**40**) (10 mg) as a mixture of diastereoisomers (Found: M^+, 412.298. $C_{27}H_{40}O_3$ requires M, 412.298); $\nu_{max.}(CHCl_3)$ 1 725 and 1 668 cm^{-1}; $\delta(CDCl_3)$ 7.44 (5 H, s, Ar*H*), 7.02 (1 H, m, C*H*=CHCO$_2$R), 5.90 (1 H, d, J 16, CH=C*H*CO$_2$R), 5.17 (2 H, m, R^{1}R^{2}C=C*H*R^3), 4.63 (1 H, m, PhC*H*OEt), 4.31 (2 H, m, R^{1}COCH_2R^2), 3.53 (2 H, q, J 8 Hz, OCH_2Me), 1.75(s), and 1.67(s).

(*c*) (−)-Menthol (56 mg) in dry tetrahydrofuran (5 cm^3) was treated at room temperature with butyl-lithium (0.3 cm^3, 1.48M). After 1.5 h the above acid chloride (104 mg) in tetrahydrofuran (4 cm^3) was added and the reaction mixture was refluxed for 3 h. The mixture was evaporated, and the residue was mixed with brine (20 cm^3) before extracting with ether (2 × 20 cm^3). The extracts afforded a yellow oil which was purified by preparative t.l.c. to provide the *menthyl ester* (**39**), (59 mg), as two diastereoisomers (Found: M^+, 402.351. $C_{27}H_{46}O_2$ requires M, 402.350); $\nu_{max.}$ 1 720 and 1 657 cm^{-1}; δ 6.94 (1 H, C*H*=CHCO$_2$R), 5.82 (1 H, J 16, CH=C*H*CO$_2$R), 5.11 (2 H, m, R^{1}R^{2}C=C*H*R^3), 4.78 (1 H, ddd, J 4,10,10 R^{1}CO$_2$C*H*R^{2}R^3), 1.61 (3 H, s) and 1.56 (6 H, s) (3 × vinyl Me), and 0.93 (broad, 3 × C-Me) and 0.76 (3 H, d). A similar preparation was carried out in which ergosterol replaced menthol, to yield the *ergosteryl ester* (**41**) (Found: M^+, 642.542. $C_{45}H_{70}O_2$ requires M, 642.537); $\nu_{max.}(CHCl_3)$ 1 725 and 1 655 cm^{-1}.

(3EZ,8E)-*Methyl* 5,9,13-*Trimethyltetradeca*-3,8,12-*trienoate and Prenylogues*.—(*a*) A solution of LBTMSA (0.23 mmol) was generated as previously described and cooled to −62 °C. (2*E*,8*E*)-Methyl 5,9,13-trimethyltetradeca-2,8,12-trienoate (47.4 mg, 0.17 mmol) in tetrahydrofuran (0.25 cm^3) was added and the yellow solution was maintained at −62 °C for 4 h when it was quenched with aqueous ammonium chloride. The product was extracted with ether to afford an oil (39 mg) which after preparative t.l.c. yielded the *title ester* (**34**) (30%) as an oil, two peaks R_T 47 and 53 min on g.l.c. (10% OV-101; 35 cm^3 min^{-1} (Found: M^+, 278.224. $C_{18}H_{30}O_2$ requires M, 278.225); $\nu_{max.}$ 2 940, 2 860, 1 735, 1 720 infl., 1 030, and 980 cm^{-3}; $\delta(CDCl_3)$ 5.44 (2 H, m, 3-, 4-H), 5.10 (2H, br t, 8-, 12-H), 3.74 (3 H, s, OMe), 3.06 (2 H, m, 2-H_2), 1.70 (3 H, s, 14-H_3), 1.62 (6 H, s, 9-, 13-Me), and 0.96 (3 H, d, J 7, 5-Me). After addition of Eu(fod)$_3$ (21 mol %) the 3- and 4-H signals were resolved, and the 4-H signals of the 3*E*- and 3*Z*-isomers were separated: $J_{3,4}$ 16 and $J_{3,4}$ 11 were measured for the *E*- and *Z*-forms respectively. The *E*:*Z* ratio was 63:37. Deconjugation of methyl 5,9-dimethyldeca-2,8-dienoate was effected in similar fashion to yield (3EZ)-*methyl* 5,9-*dimethyldeca*-3,8-*dienoate*, b.p. 82—84 °C (oven)/1.6 mmHg, (Found: M^+, 210.162. $C_{13}H_{22}O_2$ requires M, 210.162); two peaks (3:2) on g.l.c. (OV-225, 115 °C).

(6E,12EZ,17E)-11-*Methoxycarbonyl*-2,6,10,14,18,22-*hexamethyl*-10-*hydroxytricosa*-2,6,12,17,21-*pentaene*.—(*a*) Commercial (5*EZ*)-geranylacetone (6,10-dimethylundeca-5,9-dien-2-one) was chromatographed on a silica column impregnated with silver nitrate (30% w/w), using benzene elution, and monitoring by g.l.c. (SCOT OV-17; 113 °C; 5 cm^3 min^{-1}). The 5*Z*-isomer was eluted first (R_t 34 min, above conditions) followed by the 5*E*-form (R_t 40 min) both >95% pure.

(*b*) A solution of LBTMSA (8.9 mmol) was prepared at −62 °C, as above and methyl (2*E*,8*E*)-5,9,13-trimethyltetradeca-2,8,12-trienoate (1.9 g, 6.8 mmol) was added to it. The solution was stirred at −62 °C for 4 h when (5*E*)-geranylacetone (1.3 g, 6.7 mmol) was added; these reaction conditions were maintained for 2 h. The products were isolated as in the previous experiment to yield an oil from which unchanged geranylacetone was distilled (b.p. 80—84 °C (oven)/0.1 mmHg). The residue was purified on a silica column (chloroform elution) to give the *title hydroxy ester* (**37**), (0.95 g, 30%) after distillation, b.p. 140—147 °C (oven)/0.1 mmHg; [Found: (M^+ + 1), 473 (c.i.), and m/z 278.223, 194.167. $C_{31}H_{52}O_3$ requires M, 472, (M − $C_{13}H_{22}O$) 278.225, (M − $C_{18}H_{30}O_2$) 194.167); $\delta(CDCl_3)$ 5.60 (2 H, m, 12-, 13-H), 5.18 (4 H, br t, 3-, 7-, 17-, 21-H), 3.78 (3 H, s, OMe), 3.48 (1 H, d, J 10, 11-H), 2.24—1.96 (12 H, br, 6 × vinyl CH_2), 1.72 (6 H, s, 1-H_3, 23-H_3), 1.64 (12 H, s, 2-, 6-, 18-, 22-Me), 1.25 (5 H, 10-Me, 9-H_2), and 1.00 (5 H, 14-Me, 15-H_2); m/z (c.i.m.s.) 473, 455, 279, 195, 177, 137, and 109. The compound formed a *methylthiomethyl ether* on treatment with dimethyl sulphoxide and acetic anhydride (see above), as an oil (Found: M^+, 532.593. $C_{33}H_{56}O_3S$ requires M, 532.395).

(*b*) The carbanion generated from methyl 5,9-dimethyldeca-3,8-dienoate (53 mg, 0.25 mmol) and LBTMSA (0.28 mmol) at −78 °C was trapped with acetone (18 mg, 0.31 mmol) over 45 min. Product isolation as above afforded (4*EZ*)-2-*hydroxy*-3-*methoxycarbonyl*-1,6,10-*trimethylundeca*-4,9-*diene* (**29**), (34%), b.p. 102—104 °C (oven)/1.6 mmHg; [Found: m/z 209.152 and 59.050. $C_{16}H_{28}O_3$ requires (M − C_3H_7O) 209.154, (M − $C_{13}H_{21}O_2$) 59.050]. The same carbanion was also treated with 6-methylhept-5-en-2-one to afford (8*EZ*)-6-*hydroxy*-7-*methoxycarbonyl*-2,6,10,14-*tetramethylpentadeca*-2,8,13-*triene* (**30**) (37%), b.p. 142—144 °C (oven)/1.4 mmHg; [Found: M^+, 337 (c.i.m.s.), and m/z 210.160, 126.104. $C_{21}H_{36}O_3$ requires M, 336, (M − $C_8H_{14}O$) 210.162, and (M − $C_{13}H_{22}O_2$) 126.105]. This compound formed a *methylthiomethyl ether* on treatment as above with dimethyl sulphoxide and acetic anhydride (Found: M^+, 396.269. $C_{23}H_{40}O_3S$ requires M, 396.270).

(6E,12EZ,17E)-10-*Hydroxy*-11-*hydroxymethyl*-2,6,10,14,18,22-*hexamethyltricosa*-2,6,12,17,21-*pentaene* ('*Pre-presqualene*').—To a stirred suspension of lithium aluminium hydride (20 mg, 0.53 mmol) in dry ether (1.5 cm^3) was added dropwise the ester (**37**) (105 mg, 0.22 mmol) in ether (1 cm^3). The mixture was stirred at room temperature for 3 h, quenched with water and acidified. The products were collected in ether in the standard way and purified by preparative t.l.c., to yield the *title diol* (**38**) (44.7 mg, 46%) as a mixture of stereoisomers, [Found: (M^+ + 1), 445 (c.i.m.s.), and *m/z* 426.387. $C_{30}H_{52}O_2$ requires *M*, 444 and ($M - H_2O$) 426.386]; $\nu_{max.}$(film) 3 340 cm^{-1}. T.l.c. on silica, using three elutions with chloroform gave two bands, 'high R_F' and 'low R_F' isomer sets. A parallel experiment using [$^3H_4$]lithium aluminium hydride gave [^{3}H]'pre-presqualene,' 'high R_F' isomers (3.8 mg, 122 μCi), and 'low R_F' isomers (8.1 mg, 5.03 μCi).

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