Synthesis of 4- and 5-Regioselectively Deuteriated Geranyl Diphosphates

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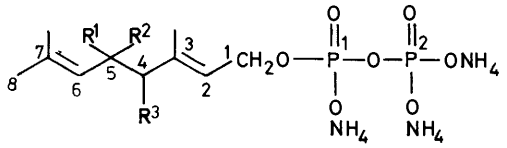
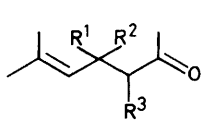
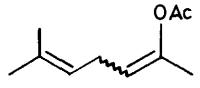
[4-^{2}H]- and [5-$^2H_2$]-Geranyl† diphosphates have been synthesized from 6-methylhept-5-en-2-one and 3-methylbut-2-enoic acid with the regioselective synthesis of 6-methylhepta-2,5-dien-2-yl acetate and the acetoacetic ester synthesis of 6-methyl[4-$^2H_2$]hept-5-en-2-one as the key steps, respectively.

In the course of studies on the cyclization mechanism of acyclic allylic diphosphates in the biosynthesis of cyclic monoterpenoids having a bornane skeleton *e.g. endo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (borneol), in higher plants,[1] we needed deuteriated geranyl diphosphate (GPP) in order to determine the activity of the enzyme responsible for the cyclization of GPP to borneol. Although a chemical synthesis of [1-^{2}H]GPP has been reported,[2] this deuteriated GPP would be unsuitable for the study, since on enzymic cyclization it is expected to afford borneol deuteriated at the 3-position, a compound likely to give deuterium-containing ion peaks in the mass spectrum with poor intensity.[3,4] [4-^{2}H]GPP (**1**) and [5-$^2H_2$]GPP (**2**) are more desirable precursors since when subjected to enzymic cyclization they should yield borneols deuteriated at the 6- and 5-positions, respectively; the latter are expected to give base peaks, due to the deuterium-containing ion, in their mass spectra.[3,4] The synthesis of these compounds was, therefore, undertaken.



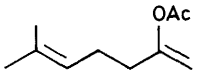
(**1**) $R^1 = R^2 = H$, $R^3 = D$
(**2**) $R^1 = R^2 = D$, $R^3 = H$





(**6**)

(**3**) $R^1 = R^2 = R^3 = H$
(**4**) $R^1 = R^2 = H, R^3 = D$
(**5**) $R^1 = R^2 = D, R^3 = H$



(**7**)

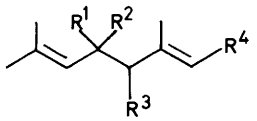
Results and Discussion

[4-^{2}H]GPP (**1**) was synthesized starting from 6-methylhept-5-en-2-one (**3**). Applying the method[5] for the preparation of enol acetates from heptan-2-one, the methylheptenone (**3**) was converted into a mixture of (*Z*)- and (*E*)-6-methylhepta-2,5-dien-2-yl acetates (**6**) in 30% yield by treatment with isopropenyl acetate and toluene-*p*-sulphonic acid (*p*-TsOH), followed by equilibration with *p*-TsOH at 110 °C of the mixture of (*Z*)- and (*E*)-enol acetates (**6**) and isomer (**7**) having a terminal methylene. Attempts to generate the enolate of the heptenone (**3**) with Pr^i_2NMgBr[6] followed by quenching with deuterium oxide or trapping as the trimethylsilyl enol ether, gave either intractable mixtures or the recovery of unchanged starting material. Applying the method[7] for the preparation of 2-methyl[2-^{2}H]cyclopentanone from 1-acetoxy-2-methylcyclopentene, the mixture of (*Z*)- and (*E*)-enol acetates (**6**) was treated with methyl-lithium and then quenched with deuterium oxide to give 6-methyl[3-^{2}H]hept-5-en-2-one (**4**) in 20% yield after purification by silica gel chromatography. The deuterium content of the deuteriated 6-methylheptenone (**4**) was found to be 92% by mass spectral analysis. The presence of deuterium solely at the 3-position of compound (**4**) was confirmed by a decrease in the intensity of the signal at δ 2.44 assigned 3-H. Deuteriation solely at the 3-position was further confirmed by a shift of the fragment ion peak at *m/z* 83 [$(CH_3)_2C{=}CHCH_2CH_2^+$] of the undeuteriated sample to *m/z* 84 in the mass spectrum of the deuteriated compound (**4**), while the fragment ion peaks at *m/z* 43 (CH_3CO^+) and 69 [$(CH_3)_2C{=}CHCH_2^+$] were unaltered. These findings demonstrate the regioselective introduction of deuterium into the 3-position of 6-methylhept-5-en-2-one.

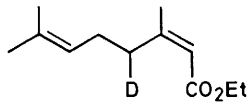
Wittig reaction of the deuteriated 6-methylheptenone (**4**) with the sodium salt of ethyl diethylphosphonoacetate gave a mixture of ethyl (*E*)- and (*Z*)-[4-^{2}H]geranates (**8**) and (**14**) which was then subjected to preparative t.l.c. to give pure (*E*)-geranate (**8**) in 62% yield. The deuterium content of the latter was found to be 62% by mass spectral analysis. The loss of deuterium may be attributed to the hydrogen exchange of the deuterium at the α-position of the heptenone (**4**) during the Wittig reaction. Reduction of the geranate (**8**) with lithium aluminium hydride gave [4-^{2}H]geraniol (**9**) in 55% yield. Following the method described in the literature,[8] the deuteriated geraniol (**9**) was converted into [4-^{2}H]geranyl chloride (**10**) and then phosphorylated to give [4-^{2}H]GPP (**1**) which was isolated as a triammonium salt in 89% yield. The overall yield of compound (**1**) from compound (**3**) was 1.8%. The ^{2}H n.m.r. spectrum of compound (**1**) showed a signal at δ 2.11 which is assigned to the deuterium at the 4-position. The deuterium content of compound (**1**) was established as 61% on the basis of a mass spectral analysis of [4-^{2}H]geraniol which was obtained from compound (**1**) by enzymic hydrolysis with alkaline phosphatase.

[5-$^2H_2$]GPP (**2**) was synthesized starting from 3-methylbut-

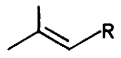
† In this paper geranyl refers to (*E*)-3,7-dimethylocta-2,6-dienyl radical.



(8) $R^1 = R^2 = H$, $R^3 = D$, $R^4 = CO_2Et$
(9) $R^1 = R^2 = H$, $R^3 = D$, $R^4 = CH_2OH$
(10) $R^1 = R^2 = H$, $R^3 = D$, $R^4 = CH_2Cl$
(11) $R^1 = R^2 = D$, $R^3 = H$, $R^4 = CO_2Et$
(12) $R^1 = R^2 = D$, $R^3 = H$, $R^4 = CH_2OH$
(13) $R^1 = R^2 = D$, $R^3 = H$, $R^4 = CH_2Cl$



(14)



(15) $R = CO_2H$
(16) $R = CD_2OH$
(17) $R = CD_2Br$

2-enoic acid (**15**). Reduction of the acid (**15**) with lithium aluminium deuteride yielded 3-methyl[1-$^2H_2$]but-2-en-1-ol (**16**), which was then converted into 1-bromo-3-methyl[1-$^2H_2$]-but-2-ene (**17**) by treatment with *N*-bromosuccinimide and dimethyl sulphide in a method developed for the preparation of allylic bromides from the corresponding alcohols.[9] Condensation of the bromide (**17**) with ethyl acetoacetate followed by hydrolysis and decarboxylation gave 6-methyl[4-$^2H_2$]hept-5-en-2-one (**5**), the yield of which from the methylbutenoic acid (**15**) was 48%. The deuterium content of compound (**5**) was determined as 99% by mass spectral analysis. The dideuteriated compound (**5**) was converted into the triammonium salt of [5-$^2H_2$]GPP (**2**) *via* compounds (**11**), (**12**), and (**13**), successively, in a manner similar to that described for the conversion of the deuteriated 6-methylheptenone (**4**) into [4-^{2}H]GPP (**1**). The overall yield of compound (**2**) from the methylbutenoic acid (**15**) was 8.4%. The ^{2}H n.m.r. spectrum of compound (**2**) showed a signal at δ 2.18 assigned to the deuterium at the 5-position. The deuterium content of compound (**2**) was established as 81% on the basis of mass spectral analysis of [5-$^2H_2$]geraniol which was obtained from compound (**2**) by enzymic hydrolysis with alkaline phosphatase.

The now ready availability of [4-^{2}H]GPP (**1**) and [5-$^2H_2$]-GPP (**2**) is expected to facilitate studies on the activity of bornyl diphosphate synthetase in the cyclization of the C_{10} prenyl chain in the biosynthesis of bornane derivatives.

Experimental

N.m.r. spectra were obtained on the following instruments: Hitachi R-600 FT n.m.r. spectrometer (^{1}H, 60 MHz); JEOL GSX 270 (^{1}H, 270 MHz; ^{13}C, 67.94 MHz); JEOL GSX 500 (^{2}H, 76.65 MHz; ^{13}C, 125.65 MHz; ^{31}P, 202.35 MHz). Chemical shifts of ^{1}H and ^{13}C n.m.r. spectra are given in δ (p.p.m.) downfield from internal $SiMe_4$ and 3-(trimethylsilyl)[2,3-$^2H_4$]-propionic acid sodium salt (TSP) for a [^{2}H]chloroform solution and a ^2H_2O solution, respectively. Chemical shifts of ^{31}P n.m.r. spectra are given in δ (p.p.m.) downfield from external phosphoric acid for an H_2O solution and a ^2H_2O solution. Chemical shifts of ^{2}H n.m.r. spectra are given in δ (p.p.m.) using ^2H_2O as an internal standard (δ 4.78) for an H_2O solution. G.l.c. was carried out on a glass column (3 mm × 2 m) packed with 2% OV-17 or 15% DEGS on Chromosorb W (AW-DMCS; 80—100 mesh) with N_2 gas. Electron impact mass spectra were obtained on a Shimadzu QP-1000 spectrometer operating with an ionization potential of 70 eV. The deuterium content of deuteriated compounds was determined by subtracting the natural isotopic abundance from the observed isotopic abundances of the deuteriated compounds as described in the literature.[10]

Materials. 6-Methylhept-5-en-2-one (**3**) was commercially available and distilled just prior to use [b.p. 59 °C (12 mmHg); 99.9% pure on g.l.c.; n_D^{25} 1.4370; d_4^{25} 0.8483]. 3-Methylbut-2-enoic acid (**15**) was commercially available (m.p. 69 °C) and used without purification.

(E)- *and* (Z)-6-*Methylhepta*-2,5-*dien*-2-*yl Acetates* (**6**).—A mixture of the heptenone (**3**) (15.2 g, 120 mmol), isopropenyl acetate (23.0 g, 230 mmol), and *p*-TsOH·H_2O (133 mg, 0.70 mmol) was refluxed for 22 h and then heated for 4 h with slow removal of the acetone so generated by distillation. The reaction mixture was cooled and then poured into a separating funnel containing saturated aqueous $NaHCO_3$ and hexane at 0—5 °C. The aqueous layer was separated and extracted with hexane (× 2) and the combined organic layers were then dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product (21.0 g). This on column chromatography on silica gel using hexane–EtOAc (9:1) as eluant gave a mixture of compounds (**6**) and (**7**) (15.3 g). The ratio of (**6**) to (**7**) was found to be 63:37 on the basis of a comparison of the integral value of the ^{1}H n.m.r. signal at δ 1.85, assigned to the 1-methyl protons of (*E*)- and (*Z*)-acetates (**6**), with that of the signal at δ 4.67, assigned to the terminal methylene protons at 1-position of compound (**7**). When the mixture of compounds (**6**) and (**7**) (12.7 g, 76 mmol) with *p*-TsOH·H_2O (58 mg, 0.34 mmol) was heated at 110 °C for 39 h the ^{1}H n.m.r. signal at δ 4.67 assigned to the terminal methylene protons of compound (**7**) disappeared. The mixture was poured into a separating funnel containing saturated aqueous $NaHCO_3$ and hexane at 0—5 °C. The aqueous layer was separated and extracted with hexane (× 2) and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give a residue (11.5 g). Column chromatography of the residue on silica gel using hexane–EtOAc (9:1) as eluant gave a mixture of the (*E*)- and (*Z*)-acetates (**6**) (5.05 g, 30%); $\nu_{max.}$(neat) 1 750 cm^{-1} ($OCOCH_3$); $\delta_H(CDCl_3)$ 1.60 [3 H, s, $(CH_3)_2C$=], 1.66 [3 H, s, $(CH_3)_2C$=], 1.85 [3 H, d, *J* 1 Hz, CH_3(OAc)C=], 2.07 (0.9 H, s, CH_3CO), 2.11 (2.1 H, s, CH_3CO), 2.50—2.90 (2 H, m, CH_2), and 4.85—5.30 (2 H, m, C=CH); *m/z* 168 (M^+).

6-*Methyl*[3-^{2}H]*hept*-5-*en*-2-*one* (**4**).—The mixture (**6**) (2.71 g, 16 mmol) was added dropwise to methyl-lithium in diethyl ether (1.0M; 34 ml) with stirring at 0 °C. This solution was further stirred at room temperature for 30 min and then added to a mixture of deuterium oxide (30 ml, 1.7 mol) and acetic anhydride (334 mg, 3.3 mmol). This suspension, after being stirred at room temperature for 5 min, was partitioned between diethyl ether and saturated aqueous Na_2CO_3. The organic layer was then separated, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified on a silica gel column with hexane–EtOAc (9:1) to give the deuteriated methylheptenone (**4**) (407 mg, 20%): 91% pure on g.l.c.; $\nu_{max.}$(neat) 2 150 (CD) and 1 710 cm^{-1} (C=O); $\delta_H(CDCl_3)$ 1.61 [3 H, s, $(CH_3)_2C$=], 1.66 [3 H, s, $(CH_3)_2C$=], 2.10 (3 H, s, CH_3CO), 2.32 (2 H, t, *J* 6 Hz, CH_2CH=C), 2.44 (1 H, br, COCHD, partially overlapping with the signal at δ 2.32), and 5.03 (1 H, m, C=CH); *m/z* 128 (0.7%), 127 (M^+, 3), 126 (0.3), 84 [$(CH_3)_2C$=$CHCH_2CHD^+$, 8], 69 [$(CH_3)_2C$=$CHCH_2^+$, 36], and 43 (CH_3CO^+, 100). The deuterium content of the deuteriated compound (**4**) was found to be 92% by mass spectral analysis.

Ethyl (E)-3,7-*Dimethyl*[4-^{2}H]*octa*-2,6-*dienoate* (**8**) (*Ethyl Geranate*).—A solution of the deuteriated 6-methylheptenone (**4**) (393 mg, 3.1 mmol) in dry 1,2-dimethoxyethane (8 ml) was added to a solution of ethyl diethylphosphonoacetate (1.65 g, 7.3 mmol) in dry 1,2-dimethoxyethane (12 ml) which had previously been cooled and allowed to react with sodium hydride (60% suspension in oil; 301 mg, 7.5 mmol). The mixture was stirred at room temperature under an atmosphere of nitrogen for 23 h after which the cooled reaction mixture was diluted with water (17 ml) and extracted with diethyl ether. The extract was dried ($MgSO_4$), and evaporated under reduced pressure to give an oil, which was subjected to preparative t.l.c. on a silica-gel plate [Kieselgel GF_{254}; 0.75 mm thick; hexane–diethyl ether (49:1)] to afford the geranate (**8**) (376 mg, 62%); 97% pure on g.l.c.; $\nu_{max.}$(neat) 2 150 (CD), 1 705 (C=O), and 1 640 cm^{-1} (C=C); $\delta_H(CDCl_3)$ 1.24 (3 H, t, *J* 7 Hz, CH_3CH_2O), 1.57 [3 H, s, $(CH_3)_2C$=], 1.65 [3 H, s, $(CH_3)_2C$=], 2.12 [6 H, br s, =$C(CH_3)CHDCH_2$), 4.10 (2 H, q, *J* 7 Hz, CH_3CH_2O), 5.03 (1 H, m, C=C*H*CH_2), and 5.60 (1 H, br s, C=CHCO); *m/z* 197 (M^+, 0.5%), 196 (0.3), 124 ($M^+ - CO_2Et$, 14), and 69 (100). The deuterium content of the geranate (**8**) was found to be 62% by mass spectral analysis.

(E)-3,7-*Dimethyl*[4-^{2}H]*octa*-2,6-*dien*-1-*ol* (**9**) (*Geraniol*).—Compound (**8**) (360 mg, 1.8 mmol) dissolved in dry diethyl ether (5 ml) was added slowly to lithium aluminium hydride (72.1 mg, 1.9 mmol) suspended in dry diethyl ether (3 ml) at −78 °C. After being stirred for 1 h at −78 °C, the suspension was allowed to warm to room temperature and stirred for a further 1 h. The excess of hydride was quenched with ethyl acetate and the mixture, after addition of 1M Na_2CO_3 (10 ml), was extracted with diethyl ether (× 3). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give the deuteriated geraniol (**9**) (192 mg, 55%): 80% pure on g.l.c.; $\nu_{max.}$(neat) 3 375 (OH), 2 150 (CD), and 1 660 cm^{-1} (C=C); $\delta_H(CDCl_3)$ 1.29 (1 H, s, OH), 1.60 (3 H, s, CH_3), 1.66 (6 H, s, CH_3 × 2), 2.01 (3 H, br s, $CHDCH_2$), 4.09 (2 H, d, *J* 7 Hz, CH_2O), 5.05 [1 H, m, $(CH_3)_2C$=C*H*], and 5.38 (1 H, t, *J* 7 Hz, C=C*H*CH_2O); *m/z* 155 (M^+, 0.4%), 154 (0.2), 124 ($M^+ - CH_2OH$, 6), and 69 (100). The deuterium content of the deuteriated geraniol (**9**) was found to be 62% by mass spectral analysis.

(E)-3,7-*Dimethyl*[4-^{2}H]*octa*-2,6-*dienyl Diphosphate* (**1**) (*Geranyl Diphosphate*).—Following the method reported in the literature,[8] [4-^{2}H]geraniol (**9**) (192 mg, 1.0 mmol) was treated with *N*-chlorosuccinimide (176 mg, 1.3 mmol) and dimethyl sulphide (0.11 ml, 1.4 mmol) to give [4-^{2}H]geranyl chloride (**10**). The chloride was treated with tristetrabutylammonium hydrogen diphosphate (2.42 g, 2.69 mmol). The resulting material was converted into the ammonium form with cation exchange resin and purified on a cellulose column with isopropyl alcohol–acetonitrile-0.1M NH_4HCO_3 (2:1:1) to give the diphosphate ester (**1**) (326 mg, 89% yield as a triammonium salt): $\delta_H(D_2O)$ 1.64 (3 H, s, CH_3), 1.70 (3 H, s, CH_3), 1.73 (3 H, s, CH_3), 2.13 (3 H, m, $CHDCH_2$), 4.49 (2 H, dd, *J* 6.6 Hz, $J_{H,P}$ 6.6 Hz, CH_2O), 5.23 [1 H, t, *J* 6.8 Hz, $(CH_3)_2C$=C*H*], and 5.47 (1 H, t, *J* 6.6 Hz, C=C*H*CH_2O); $\delta_C(D_2O)$ 18.39 (3-Me), 18.42 (3-Me of undeuteriated species), 19.78 (7-Me), 27.64 (C-8), 28.35 (C-5), 28.42 (C-5 of undeuteriated species), 41.25 (t, $J_{C,^2H}$ 19 Hz, C-4), 41.61 (C-4 of undeuteriated species), 65.44 (d, $J_{C,P}$ 5.2 Hz, C-1), 122.75 (d, $J_{C,P}$ 9.4 Hz, C-2), 127.00 (C-6), 136.56 (C-7), 145.51 (C-3), and 145.53 (C-3 of undeuteriated species); $\delta_P(D_2O)$ −10.46 (1 P, d, $J_{P,P}$ 22.0 Hz, P-1), and −7.67 (1 P, d, $J_{P,P}$ 22.0 Hz, P-2); $\delta_D(H_2O)$ 2.11 (br s, CHD).

Analysis of the deuteriated GPP (**1**) by t.l.c. on silica gel with isopropyl alcohol–19% NH_3 (3:2) showed a single spot (R_F 0.24). The spot was visualized by spraying with vanillin–H_2SO_4 (1:134 w/w) followed by heating on a hot-plate. Enzymic hydrolysis of the deuteriated GPP (**1**) with alkaline phosphatase yielded [4-^{2}H]geraniol: *m/z* 155 (M^+, 0.4%), 154 (0.2), 124 ($M^+ - CH_2OH$, 4), and 69 (100). The deuterium content of the geraniol was found to be 61% by mass spectral analysis.

[1-$^2H_2$]-3-*Methylbut*-2-*en*-1-*ol* (**16**).—3-Methylbut-2-enoic acid (**15**) (1.61 g, 16 mmol) dissolved in dry diethyl ether (4 ml) was added dropwise to lithium aluminium deuteride (1.06 g, 25 mmol) suspended in dry diethyl ether (20 ml) at 0 °C. After the mixture had been refluxed for 2 h, the excess of hydride was quenched with 0.1M KOH (3 ml) and the mixture was extracted with diethyl ether (× 3). The combined extracts were washed with saturated aqueous K_2CO_3, dried (Na_2SO_4), and concentrated by distillation at atmospheric pressure to 1.5 ml. The residual solution of 3-methyl[1-$^2H_2$]but-2-en-1-ol (**16**) in diethyl ether was used for the next step without purification. A part of the diethyl ether solution of the butenol (**16**) was further concentrated and analyzed.

Compound (**16**): 89% pure on g.l.c.; $\nu_{max.}$(neat) 3 375 (OH), 2 200, 2 160, and 2 080 (CD), and 1 665 cm^{-1} (C=C); $\delta_H(CDCl_3)$ 1.54 (1 H, s, OH), 1.69 (3 H, s, CH_3), 1.74 (3 H, s, CH_3), and 5.41 (1 H, br s, C=CH); *m/z* 88 (M^+, 24%) and 73 ($M^+ - CH_3$, 100). The deuterium content of compound (**16**) was found to be 99% by mass spectral analysis.

1-*Bromo*-3-*methyl*[1-$^2H_2$]*but*-2-*ene* (**17**).—Dimethyl sulphide (2.13 ml, 29 mmol) was added dropwise to a solution of *N*-bromosuccinimide (4.31 g, 24 mmol) in dry dichloromethane (86 ml) at 0 °C under an atmosphere of nitrogen. After the mixture had been cooled to −20 °C with stirring the diethyl ether solution of the butenol (**16**) was added to it. The reaction mixture was then stirred at 0 °C for 3 h and poured into a cold, saturated brine. The aqueous layer was separated and extracted with pentane (× 3). The combined organic extracts were washed with a cold saturated brine, dried ($MgSO_4$), and concentrated to give 1-bromo-3-methyl[1-$^2H_2$]but-2-ene (**17**): *m/z* 152 and 150 (M^+, 2.6 and 2.9%) and 71 (M^+ − Br, 100). This bromomethylbutene (**17**) was used for the next step without purification.

6-*Methyl*[4-$^2H_2$]*hept*-5-*en*-2-*one* (**5**).—Ethyl acetoacetate (6.11 g, 47 mmol) was added to a mixture of sodium (0.915 g, 40 mmol) and absolute ethanol (60 ml). The allylic bromide (**17**) was then added dropwise to the stirred mixture at room temperature. The mixture was stirred at room temperature for 20 h after which 9M KOH (5 ml) was added. The reaction mixture was refluxed for 3 h, cooled, acidified with 2.5M H_2SO_4 (20 ml), diluted with water (90 ml), and extracted with diethyl ether (× 4). The combined extracts were washed with saturated brine (× 4), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified on a silica-gel column with hexane–EtOAc (7:1) to give the methylheptenone (**5**) [1.01 g, 48% yield from the acid (**15**)]: 97% pure on g.l.c.; $\nu_{max.}$(neat) 2 200, 2 160, and 2 100 (CD), 1 710 (C=O), and 1 620 cm^{-1} (C=C); $\delta_H(CDCl_3)$ 1.62 [3 H, s, $(CH_3)_2C$=], 1.66 [3 H, s, $(CH_3)_2C$=], 2.12 (3 H, s, CH_3CO), 2.41 (2 H, br s, CH_2CD_2), and 5.05 (1 H, br s, C=CH); *m/z* 128 (M^+, 11%), 113 ($M^+ - CH_3$, 33), 71 [$(CH_3)_2C$=$CHCD_2^+$, 38], and 43 (CH_3CO^+, 100). The deuterium content of compound (**5**) was found to be 99% by mass spectral analysis.

Ethyl [5-$^2H_2$]*Geranate* (**11**).—Following the procedure for compound (**8**), Wittig reaction of the methylheptenone (**5**) (521 mg, 3.9 mmol) with the sodium salt of ethyl diethylphosphonoacetate gave ethyl geranate (**11**) (574 mg, 68%): 92% pure on g.l.c.; $\nu_{max.}$(neat) 2 210, 2 170, and 2 110 (CD), 1 710 (C=O), and 1 650 cm^{-1} (C=C); $\delta_H(CDCl_3)$ 1.27 (3 H, t, *J* 7 Hz, CH_3CH_2O), 1.61 [3 H, s, $(CH_3)_2C$=], 1.69 [3 H, s, $(CH_3)_2C$=], 2.14 [5 H, br s, =$C(CH_3)CH_2CD_2$], 4.15 (2 H, q, *J* 7 Hz,

CH_3CH_2O), 5.07 (1 H, br, C=$CHCD_2$), and 5.66 (1 H, br s, C=CHCO); *m/z* 198 (M^+, 4%), 183 ($M^+ - CH_3$, 1), 125 ($M^+ - CO_2Et$, 47), and 71 [$(CH_3)_2C$=$CHCD_2^+$, 100]. The deuterium content of compound (**11**) was found to be 81% by mass spectral analysis.

[5-$^2H_2$]*Geraniol* (**12**).—Compound (**11**) (574 mg, 2.7 mmol) was converted into deuteriated geraniol (**12**) by reduction with lithium aluminium hydride (111 mg, 2.9 mmol) in the same way as that described for the preparation of (**9**). Purification by chromatography on a silica-gel column with hexane–EtOAc (7:1) gave the dideuteriated geraniol (**12**) (257 mg, 57%): 93% pure on g.l.c.; $\nu_{max.}$(neat) 3 340 (OH), 2 190, 2 160, and 2 100 (CD), and 1 665 cm^{-1} (C=C); $\delta_H(CDCl_3)$ 1.18 (1 H, br s, OH), 1.60 (3 H, s, CH_3), 1.67 (6 H, s, $CH_3 \times 2$), 2.03 (2 H, br s, CH_2CD_2), 4.11 (2 H, d, *J* 7 Hz, CH_2O), 5.06 (1 H, br s, =$CHCD_2$), and 5.38 (1 H, t, *J* 7 Hz, =C*H*CH_2); *m/z* 156 (M^+, 2%), 141 ($M^+ - CH_3$, 4), 125 ($M^+ - CH_2OH$, 14), and 71 [$(CH_3)_2C$=$CHCD_2^+$, 100]. The deuterium content of compound (**12**) was found to be 81% by mass spectral analysis.

[5-$^2H_2$]*Geranyl Diphosphate* (**2**).—Following the manner described for compound (**1**), compound (**12**) (201 mg, 1.2 mmol) was phosphorylated to give the pure diphosphate (**2**) (197 mg, 45% yield as a triammonium salt): $\delta_H(D_2O)$ 1.64 (3 H, s, CH_3), 1.70 (3 H, s, CH_3), 1.73 (3 H, s, CH_3), 2.09 (2 H, s, CH_2CD_2), 4.49 (2 H, dd, *J* 6.6 Hz, $J_{H,P}$ 6.6 Hz, CH_2O), 5.22 (1 H, s, =$CHCD_2$), and 5.47 (1 H, t, *J* 6.6 Hz, =C*H*CH_2O); $\delta_C(D_2O)$ 18.43 (3-Me), 19.78 (7-Me), 27.65 (C-8), 28.45 (C-5 of undeuteriated species), 41.44 (C-4), 41.61 (C-4 of undeuteriated species), 65.51 (d, $J_{C,P}$ 3.9 Hz, C-1), 122.69 (d, $J_{C,P}$ 9.8 Hz, C-2), 126.91 (C-6), 136.61 (C-7), and 145.60 (C-3); $\delta_P(H_2O)$ −10.38 (1 P, d, $J_{P,P}$ 19.5 Hz, P-1) and −8.13 (1 P, br, P-2); $\delta_D(H_2O)$ 2.18 (s, CD_2).

Analysis of compound (**2**) by t.l.c. on silica gel [as for (**1**)] showed a single spot (R_F 0.24). Enzymic hydrolysis of compound (**2**) with alkaline phosphatase yielded [5-$^2H_2$]geraniol: *m/z* 156 (M^+, 1%), 125 ($M^+ - CH_2OH$, 11), and 71 [$(CH_3)_2C$=$CHCD_2^+$, 100]. The deuterium content of the geraniol was found to be 81% by mass spectral analysis.

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