

## Synthesis of $[10,10,10\text{-}^2\text{H}_3]$ Geranyl Diphosphate

Shunsuke Izumi, Mie Aihara, Yoshikazu Hiraga,  
Toshifumi Hirata, and Takayuki Suga\*

Department of Chemistry, Faculty of Science,  
Hiroshima University, Higashisenda-Machi,  
Naka-ku, Hiroshima 730, Japan

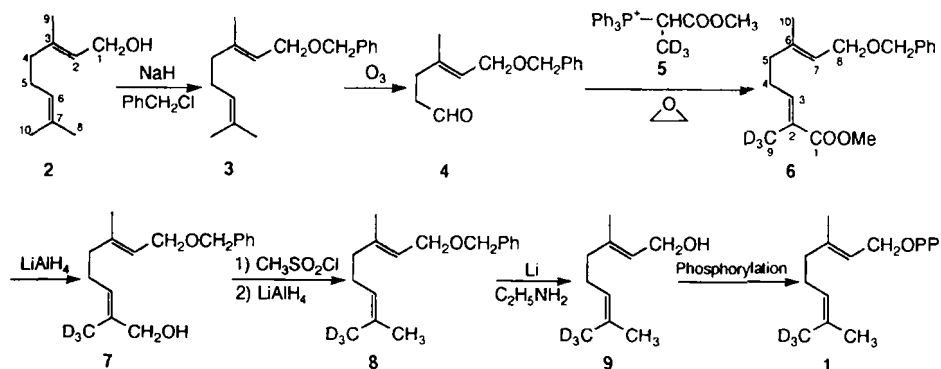
### SUMMARY

$[10,10,10\text{-}^2\text{H}_3]$ -3,7-Dimethyl-2(E),6-octadienyl diphosphate ( $[10,10,10\text{-}^2\text{H}_3]$ geranyl diphosphate) was synthesized from 6-(benzyloxy)-4-methyl-4(E)-hexenal by regioselective deuteration involving a modified Wittig reaction as the key step.

**Key words:**  $[10,10,10\text{-}^2\text{H}_3]$ geranyl diphosphate, Wittig reaction

### INTRODUCTION

In the course of studies on the biosynthesis of limonene,<sup>1–3)</sup> we found that geranyl diphosphate is converted into (4S)-(-)-limonene with an enzyme preparation from *Mentha spicata* (spearmint). In order to elucidate the regioselectivity of the hydrogen elimination from the methyl group at 8- or 10-position of geranyl diphosphate during the formation of the 8(9)-double bond of the limonene, we needed regioselectively deuterated geranyl diphosphate.  $[8,8,8\text{-}^2\text{H}_3]$ Geraniol was already synthesized,<sup>4)</sup> but  $[10,10,10\text{-}^2\text{H}_3]$ geranyl diphosphate 1 ( $[10,10,10\text{-}^2\text{H}_3]$ -3,7-dimethyl-2(E),6-octadienyl diphosphate) had not yet been synthesized. We



Scheme 1. Synthesis of  $[10,10,10\text{-}^2\text{H}_3]$ geranyl diphosphate (1).

now have established its synthesis involving a modified Wittig reaction as a key step.

#### RESULTS AND DISCUSSION

The synthesis of  $[10,10,10\text{-}^2\text{H}_3]$ geranyl diphosphate (1) is outlined in Scheme 1. The double bond at the 6-position of 3,7-dimethyl-2(E),6-octadienyl benzyl ether (geranyl benzyl ether (3)) was selectively ozonized in dichloromethane at  $-78\text{ }^\circ\text{C}$ <sup>5)</sup> to give 6-(benzyloxy)-4-methyl-4(E)-hexenal (4). A modified Wittig reaction of the aldehyde (4) with [1-(methoxycarbonyl)(2,2,2- $^2\text{H}_3$ )ethyl]-triphenyl phosphonium iodide (5) gave methyl (2E,6E)-8-benzyloxy-2,6-dimethyl(9,9,9- $^2\text{H}_3$ )octa-2,6-dienoate (6) in 68.9 % yield. The key step in the synthesis of 1 is the use of the deuterated triphenyl phosphonium iodide which prepared from methoxycarbonylmethylene triphenylphosphorane and iodomethane- $\text{d}_3$ . Reduction of 6 with  $\text{LiAlH}_4$  gave an alcohol (7) in 98 % yield. The alcohol (7) was subjected to mesylation, and the resulting mesylate was immediately reduced with  $\text{LiAlH}_4$  to give  $[10,10,10\text{-}^2\text{H}_3]$ -3,7-dimethyl-2(E),6-octadienyl benzyl ether ( $[10,10,10\text{-}^2\text{H}_3]$ geranyl benzyl ether (8)). The ether (8) was treated with Li in  $\text{C}_2\text{H}_5\text{NH}_2$  solution to give  $[10,10,10\text{-}^2\text{H}_3]$ geraniol (9).  $^2\text{H}$  and  $^1\text{H}$  NMR spectral analysis of 9 indicated that the  $^2\text{H}$ -label is located in the methyl group (6

1.60) which is cis to the chain at C(6) of 9. Following the method described in the literature,<sup>6)</sup> the deuterated geraniol was chlorinated and then phosphorylated to give [10,10,10-<sup>2</sup>H<sub>3</sub>]geranyl diphosphate (1) in 47 % yield. The deuterium enrichment at the 10-position of 1 was found to be 99.4 % on the basis of a mass spectral analysis of [10,10,10-<sup>2</sup>H<sub>3</sub>]geraniol, which was obtained from 1 by enzymatic hydrolysis with alkaline phosphatase. Thus, the synthesis of [10,10,10-<sup>2</sup>H<sub>3</sub>]geranyl diphosphate (1) from 6-(benzyloxy)-4-methyl-4(E)-hexenal (4) was achieved by use of [1-(methoxycarbonyl)(2,2,2-<sup>2</sup>H<sub>3</sub>)ethyl]triphenyl phosphonium iodide (5) as a modified Wittig reagent in 20.1 % of the overall yield.

#### EXPERIMENTAL

GLC was carried out on a glass column (3 mm × 2 m) packed with 2 % OV-17 on Chromosorb W (AW-DMCS; 80-100 mesh). Electron impact mass spectra were obtained on a Shimadzu QP-1000 spectrometer operating with an ionization potential of 70 eV. The deuterium enrichment of the deuterated product was determined by the mass spectrum of the deuterated compound as described in the literature.<sup>7)</sup> NMR spectra were obtained on the following instruments: JEOL GSX 270 (<sup>1</sup>H, 270 MHz; <sup>13</sup>C, 67.94 MHz); JEOL GSX 500 (<sup>31</sup>P 202.35 MHz).

6-(Benzyloxy)-4-methyl-4(E)-hexenal (4). According to the method described in reference 5, geraniol (2) (8.32 g, 53.9 mmol) was benzylated with 4.21 g (105 mmol) of NaH and 13.2 g (104 mmol) of benzylchloride, and the resulting benzylate (3) was then treated with O<sub>3</sub> in dichloromethane at -78 °C to afford, after work up and purification, 3.73 g (17.1 mmol) of 4 as a pale yellow liquid: IR (neat) 3010, 2700 (C-H), and 1718 cm<sup>-1</sup> (C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ=9.77 (s, 1H), 7.33 (m, 5H), 5.42 (t, 1H, J=6.8 Hz), 4.49 (s, 2H), 4.02 (d, 2H, J=6.8 Hz) 2.60—2.34 (m, 4H), and 1.66 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ=201.8, 138.3, 138.1, 128.4, 127.8, 127.6, 121.9, 72.2, 66.4, 41.8, 31.5, and 16.6.

[1-(Methoxycarbonyl)(2,2,2-<sup>2</sup>H<sub>3</sub>)ethyl]triphenyl phosphonium iodide (5). According to the method described in reference 8, a suspension of methoxycarbonylmethylene triphenylphosphorane (1.78 g, 5.34 mmol) and iodomethane-d<sub>3</sub> (1.00g, 6.90 mmol) in dry ethyl acetate (30 ml) was refluxed for 2 h. After filtration, the residue was washed with warm ethyl acetate and then dried. A deuterated Wittig reagent 5 (2.22 g, 4.63 mmol) was given as a white powder: mp 137—140 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ=8.02—7.66 (m, 15H), 6.76 (d, 1H, J=14.2 Hz), and 1.58 (s, 3H).

Methyl (2E,6E)-8-benzyloxy-2,6-dimethyl(9,9,9-<sup>2</sup>H<sub>3</sub>) octa-2,6-dienoate (6). The aldehyde (4) (2.65 g, 5.54 mmol) and the deuterated triphenylphosphonium iodide (5) (1.03 g, 4.71 mmol) were suspended in 6 ml of benzene-oxirane (1:1 v/v) and left in a sealed tube for 20 h at 25 °C. After heating for a further 2 h at 70 °C, the tube was opened. The oxirane was removed under a gentle stream of N<sub>2</sub>, and then the residue was taken up in Et<sub>2</sub>O-pentane (1:1 v/v) and filtered through 5 g of SiO<sub>2</sub> to afford a crude product. GLC indicated that the crude product was composed of 98 % of 6 and 2 % of the (2Z,6E)-isomer. Purification by chromatography on a SiO<sub>2</sub> column with hexane-EtOAc (95:5 v/v) gave 946 mg (68.9 %) of 6: IR (neat) 2250, 2220, 2090 (C-D), 1710 (C=O), 1645 (C=C), and 1060 cm<sup>-1</sup> (C-O); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ=7.35—7.26 (m, 5H), 6.74 (t, 1H, J=6.8 Hz), 5.42 (t, 1H, J=6.8 Hz), 4.50 (s, 2H), 4.02 (d, 2H, J=6.8 Hz), 3.71 (s, 3H), 2.36—2.13 (m, 4H), and 1.66 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ=168.5, 141.7, 139.1, 138.5, 128.3, 127.8, 127.5, 121.7, 72.1, 66.4, 51.6, 38.1, 26.9, and 16.4; m/z 200(M<sup>+</sup>-C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>), 91(100). The deuterium enrichment of 6 was found to be 99 % by mass spectral analysis.

(2E,6E)-8-Benzyloxy-2,6-dimethyl(9,9,9-<sup>2</sup>H<sub>3</sub>)octa-2,6-dienol (7). A soln. of 1.94 g (6.66 mmol) of 6 in 7 ml of ether was slowly added to a suspension of 0.50 g (13.1 mmol) of LiAlH<sub>4</sub> in 8 ml of ether at 0 °C. After stirring at 25 °C, the product was

isolated and purified by column chromatography on a SiO<sub>2</sub> (hexane-EtOAc 8:2 v/v) to afford 1.72 g (98.2 %) of 7: IR (neat) 3400 (O-H), 2930, 2860 (C-H), 2180, 2090 (C-D), and 1662 cm<sup>-1</sup> (C=C); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ=7.35—7.27 (m, 5H), 5.40—5.38 (m, 2H), 4.50 (s, 2H), 4.02 (d, 2H, J=6.8 Hz), 3.97 (s, 2H), 2.19—2.04 (m, 4H), and 1.66 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ=139.9, 138.5, 135.0, 128.3, 127.8, 127.5, 125.6, 121.1, 72.1, 68.9, 66.5, 39.1, 25.7, and 16.4; m/z 263(M<sup>+</sup>), 91(100). The deuterium enrichment of 7 was found to be 99 % by mass spectral analysis.

[10,10,10-<sup>2</sup>H<sub>3</sub>]-3,7-dimethyl-2(E),6-octadienyl benzyl ether (8). According to reference 4, the alcohol 7 (615 mg, 2.33 mmol) was subjected to mesylation with 0.5 ml (6.46 mmol) of CH<sub>3</sub>SO<sub>2</sub>Cl, and the mesylate was immediately reduced with 174 mg (4.58 mmol) of LiAlH<sub>4</sub> to give 461 mg (1.86 mmol) of 8 as a colorless liquid: IR (neat) 2930, 2880 (C-H), 2220, 2190, 2055 (C-D), and 1064 cm<sup>-1</sup> (C-O); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ=7.35—7.25 (m, 5H), 5.40 (t, 1H, J=6.8 Hz), 5.10 (m, 1H), 4.50 (s, 2H), 4.02 (d, 2H, J=6.8 Hz), 2.14—2.00 (m, 4H), 1.68 (s, 3H) and 1.64 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ=140.5, 138.7, 131.3, 128.3, 127.8, 127.5, 124.1, 121.0, 71.9, 66.6, 40.0, 26.4, 25.7, and 16.4; m/z 247(M<sup>+</sup>) and 91 (100). The deuterium enrichment of 8 was found to be 99 % by mass spectral analysis.

[10,10,10-<sup>2</sup>H<sub>3</sub>]-3,7-dimethyl-2(E),6-octadienol (9). According to reference 4, the ether 8 (324 mg, 1.31 mmol) was treated with 272 mg of fresh Li wire in 10 ml C<sub>2</sub>H<sub>5</sub>NH<sub>2</sub> to give 0.162 g (1.03 mmol) of 9; IR (neat) 3340 (O-H), 2925 (C-H), 2210, 2180, 2050 (C-D), and 1662 cm<sup>-1</sup> (C=C); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ=5.42 (t, 1H, J=6.8 Hz), 5.09 (t, 1H, J=6.8 Hz), 4.15 (d, 2H, J=7.3 Hz), 2.12—2.04 (m, 4H) and 1.68 (s, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ=139.8, 131.7, 123.9, 123.3, 59.4, 39.6, 26.4, 25.6, and 16.3; m/z 157(M<sup>+</sup>), 139(M<sup>+</sup>-H<sub>2</sub>O), 72(100). The deuterium enrichment of 9 was found to be 99 % by mass spectral analysis.

[10,10,10-<sup>2</sup>H<sub>3</sub>]-3,7-dimethyl-2(E),6-octadienyl diphosphate (1).

Following the method reported in reference 6, [10,10,10-<sup>2</sup>H<sub>3</sub>]geraniol (9) (220 mg, 1.40 mmol) was treated with N-chlorosuccinimide (340 mg, 2.55 mmol) and dimethyl sulfide (0.19 ml, 2.59 mmol) resolved in dichloromethane (15 ml) to give [10,10,10-<sup>2</sup>H<sub>3</sub>]geranyl chloride. The chloride was treated with tris(tetra-n-butyl)ammonium hydrogen diphosphate (2.88 g, 3.19 mmol) resolved in acetonitrile (15 ml) for 2 h at 25 °C. The resulting material was converted into the ammonium form with cation exchange resin (50 g) and the ammonium salt was purified on a cellulose column (3 × 30 cm) with isopropyl alcohol-acetonitrile-0.1M NH<sub>4</sub>HCO<sub>3</sub> (2:1:1 v/v) to give the diphosphate ester as a triammonium salt. The triammonium salt was converted into the trilithium salt with lithium chloride. The diphosphate ester 1 (216 mg, 0.66 mmol) was given in 47.3 % yield as a trilithium salt: <sup>1</sup>H NMR (D<sub>2</sub>O) δ=5.49 (t, 1H, J=6.5 Hz), 5.24 (t, 1H, J=6.5 Hz), 4.49 (t, 2H, J=6.5 Hz), 2.19—2.11 (m, 4H), 1.74 (s, 3H), and 1.70 (s, 3H); <sup>13</sup>C NMR (D<sub>2</sub>O) δ=145.5, 136.5, 127.0, 122.7 (d, J<sub>C-P</sub>=8.6 Hz), 65.3 (d, J<sub>C-P</sub>=4.8 Hz), 41.5, 28.4, 27.6, and 18.4; <sup>31</sup>P NMR (D<sub>2</sub>O) δ=-5.46 (d, J=22.3 Hz) and -9.39 (d, J=22.3 Hz). To estimate deuterium enrichment of the diphosphate ester 1, 1 (5.0 mg) was hydrolyzed with suspension of alkaline phosphatase (0.1 mg, 14 U) in glycine buffer (5.0 ml, pH 8.5) at 37 °C for 2 h to afford [10,10,10-<sup>2</sup>H<sub>3</sub>]geraniol (9) (2.0 mg). The deuterium enrichment of the geraniol was found to be 99 % by the mass spectral analysis.

#### ACKNOWLEDGMENT

This work was supported in part by Grant-in-Aid for Scientific Research (No. 61430010) from the Ministry of Education, Science and Culture.

#### REFERENCES

- 1) Suga T., Hirata T., Aoki T., and Shishibori T. — *Phytochemistry* 25: 2769 (1986).

- 2) Suga T., Hirata T., Izumi S., Hiraga Y., and Okamoto K. — Chem. Lett: 115 (1988).
- 3) Suga T., Hirata T., Izumi S., Hiraga Y., Ito D. I., Okamoto K., and Katayama Y. — Proceedings of the 29th Symposium on the Chemistry of Natural Products, Sapporo, August, p. 185 (1989).
- 4) Fretz H. and Woggon W. D. — Helv. Chim. Acta 69: 1959 (1986).
- 5) Coates R. M., Ley D. A. and Cavender P. L. — J. Org. Chem. 43: 4915 (1978).
- 6) Davisson V. J., Woodside A. B., and Poulter C. D. — Methods in Enzymol. 110: 130 (1985).
- 7) K. Biemann — Mass Spectrometry, 1st ed., McGraw Hill, New York, p. 204 (1962).
- 8) Crombie L, Doherty C. F., and Pattenden G. — J. Chem. Soc: 1076 (1970).