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Synthesis of (*E*)-4-Hydroxydimethylallyl Diphosphate. An Intermediate in the Methyl Erythritol Phosphate Branch of the Isoprenoid Pathway

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Abstract: The syntheses of (*E*)-1-hydroxy-2-methyl-2-buten-4-yl diphosphate ((*E*)-4-hydroxydimethylallyl diphosphate, HDMAPP), an intermediate in the methyl erythritol phosphate pathway, and (*E*)-[4-^{2}H]HDMAPP were accomplished in two steps from (*E*)-4-chloro-2-methyl-2-butenal. The synthetic route is easily adaptable for the facile incorporation of tritium at C-4 of the diphosphate.

Isoprenoid compounds constitute one of the largest and most diverse groups of natural products, with greater than 35 000 identified members to date.[1] These molecules are derived from the simple five-carbon precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Until recently, IPP and DMAPP were believed to originate from acetate by the mevalonate pathway.[2] However, pioneering studies by Rohmer[3] and Arigoni[4] revealed an alternative pathway that operates in plant chloroplasts, algae, and bacteria.[5] As outlined in Figure 1, pyuvate and glyceraldehyde 3-phosphate are condensed in a thiamine diphosphate-dependent reaction catalyzed by 1-deoxy-D-xylulose 5-phosphate (DXP) synthase to give DXP.[6] The branched skeleton typical of isoprenoid compounds is then constructed from the linear deoxysugar by a reversible rearrangement−NADPH-dependent reduction catalyzed by 2-*C*-methyl-D-erythritol 4-phosphate (MEP) synthase.[7] MEP is converted to 2-*C*-methyl-D-erythritol-2,4-cylcodiphosphate (cMEPP) by three successive enzymes, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDPME) synthase,[8] 4-diphosphocytidyl-2-*C*-methyl-D-erythritol-2-phosphate (CDPME) kinase,[9] and cMEPP synthase.[10] Recently, Wolff et al.[11] reported that the cyclic diphosphate is converted to (*E*)-1-hydroxy-2-methyl-2-buten-4-yl diphosphate ((*E*)-4-hydroxydimethylallyl diphosphate, HDMAPP) by the protein encoded by the *Escherichia coli gcpE* gene and established the structure of HDMAPP by synthesis. We now report a short synthesis of HDMAPP that accommodates the introduction of an isotopic tag in the penultimate step.

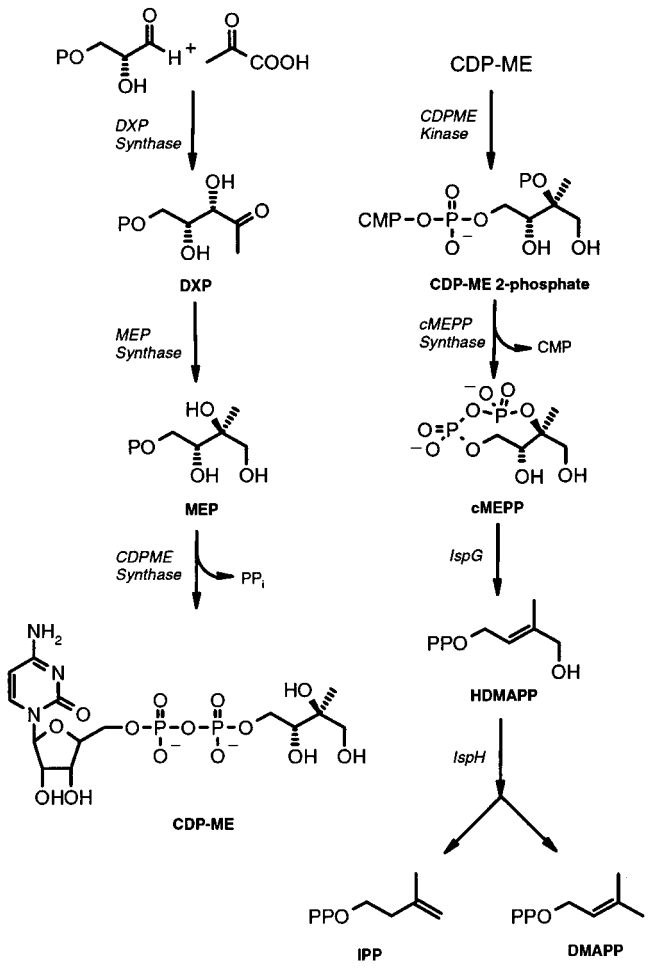

FIGURE 1. Methyl erythritol phosphate (MEP) pathway for isoprenoid biosynthesis.

The procedure of Miyakado et al.[12] was used to convert pyruvic aldehyde dimethyl acetal **1** and vinylmagnesium bromide to (*E*)-4-chloro-2-methyl-2-butenal **(3)**, as outlined in Scheme 1. Both ^{1}H NMR and ^{13}C NMR spectra indicated that only one double bond isomer was present, and the NMR data were in full agreement with those

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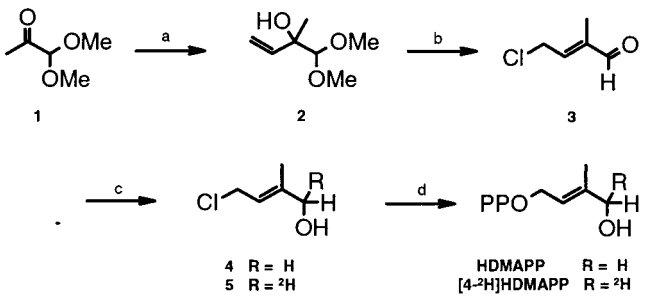
SCHEME 1[a]



[a] Reagents and conditions: (a) 1.0 M vinylmagnesium bromide in THF, THF, −20 °C; (b) cat. $CuCl_2$, NaCl, concd HCl, benzene 0 → 50 °C, overnight, 25% (two steps); (c) $NaBH(D)_4$, THF, −20 °C, 80%; (d) tris(tetra-*n*-butylammonium) hydrogen pyrophosphate, MeCN, Dowex (NH_4^+), 83%.

reported by Miyakado et al. The (*E*)-geometry of the double bond was originally established by Miyakado et al. by using **3** in a total synthesis of tricholin, a natural product isolated from *Trichocline incana* that contains the (*E*)-4-hydroxydimethylallyl[13] moiety in the side chain of the furocoumarin. Wolff et al. confirmed the (*E*)-geometry of synthetic HDMAPP from nuclear Overhauser effects. Reduction of aldehyde **3** with $NaBH_4$[12] or NaB^2H_4 gave **4** and [1-^{2}H]-**5**. (*E*)-4-Chloro-2-methyl-2-buten-1-ol **(4)** and [1-^{2}H]-**5** were readily converted to the corresponding diphosphates by displacement of chloride with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate. The tetrabutylammonium counterion was replaced by cation-exchange over Dowex (ammonium form) and purification of the crude ammonium diphosphates over cellulose gave HDMAPP[11] and [4-^{2}H]-HDMAPP.

The (*E*)-chloroaldehyde is a versatile intermediate. The compound is sufficiently stable to be purified by chromatography, and reduction with commercially available NaB^2H_4 or NaB^3H_4 allows one to easily attach an isotopic label to C-1 in the penultimate step of the synthesis. The overall yield for reduction of chloroaldehyde **3** to chloro alcohol **4** and the subsequent phosphorylation to give HDMAPP, with purification of the intermediate olefin, were 66%. This procedure should be readily amenable to the synthesis of tritium labeled HDMAPP by modifying the procedure to omit purification of [1-^{3}H]-**4** by flash chromatography in order to minimize handling volatile radioactive materials before the phosphorylation.

Experimental Section

(*E*)-1-Hydroxy-2-methyl-2-buten-4-yl Diphosphate ((*E*)-4-Hydroxydimethylallyl Diphosphate, HDMAPP). Tris(tetra-*n*-butylammonium) hydrogen pyrophosphate[14] (2.0 g) was dried by azeotropic removal of water by rotary evaporation of dry acetonitrile (2 × 5 mL) at 40 °C. (*E*)-4-Chloro-2-methyl-2-buten-1-ol (40 mg, 0.33 mmol) in 200 μL of dry acetonitrile was added via syringe to a stirred solution of 634 mg (0.663 mmol) tris(tetra-*n*-butylammonium) hydrogen pyrophosphate in 1 mL dry acetonitrile at room temperature. After being stirred for 2 h, the suspension was concentrated by rotary evaporation. The residue was dissolved in 1 mL of cation-exchange buffer (49:1 (v/v) 25 mM NH_4HCO_3 (pH 8.0)/2-propanol) and passed over 120 mequiv of Dowex 50WX8-200 cation-exchange resin (ammonium form) preequilibrated with two column volumes of the same buffer. The diphosphate was eluted with two column volumes (120 mL) of the same buffer, flash frozen, and lyophilized.[15] The residue was resuspended in 1 mL of a 50 mM ammonium bicarbonate solution to which 1 mL of 2-propanol and a few drops of acetonitrile were added. After vortexing and centrifugation at 2000 rpm for 2 min, the supernatant was decanted. This procedure was repeated three times, and the supernatants were combined, concentrated, frozen, and lyophilized. The residue was resuspended in 1 mL of 53:47 (v/v) 2-propanol/50 mM NH_4HCO_3 (pH 8.0) and loaded onto a cellulose column (4 × 15 cm) preequilibrated with the same buffer. Fractions containing HDMAPP were pooled, frozen, and lyophilized to give 86 mg (83%) of a white solid: R_f 0.47 (53:47 (v/v) 2-propanol/50 mM NH_4HCO_3 (pH 8.0)); ^{1}H NMR (300 MHz, D_2O) δ 5.62 (dt, 1 H, J = 6.8, 0.98 Hz), 4.50 (t, 2 H, J = 7.3 Hz), 3.99 (s, 2 H), 1.68 (s, 3 H); ^{13}C NMR (75 MHz, D_2O/CD_3OD) δ 140.63, 121.76 (d, J = 7.5 Hz), 67.56, 63.38 (d, J = 5.0 Hz), 14.08; ^{31}P NMR (121 MHz, D_2O) δ −10.5 (d, J = 20.8 Hz), −8.65 (d, J = 21.4 Hz); HRMS (FAB, M − H) calcd for $C_5H_{11}P_2O_8$ 260.9929, found 260.9924.

(*E*)-4-Chloro-1-deuterio-2-methyl-2-buten-1-ol ([1-^{2}H]-5). A solution (100 mg, 0.84 mmol) of **3** in 0.25 mL of THF was added via syringe to 35 mg (0.84 mmol) of NaB^2H_4 in 0.5 mL of THF at −20 °C. The mixture was allowed to stir for 5 h at the same temperature before ice-cold MeOH was added. The reaction mixture was filtered through a plug of silica with diethyl ether, and solvent was removed by rotary evaporation. The residue was purified by flash silica chromatography (7:3 hexanes/ether) to yield 82 mg (80%) of a pale yellow oil: R_f 0.17 (7:3 hexanes/ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.74 (tt, 1 H, J = 8.0, 1.5 Hz), 4.14 (d, 2 H, J = 8.0 Hz), 4.06 (br s, 1 H), 1.76 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.41, 120.57, 67.35 (t, J = 21.9 Hz), 40.38, 13.68.

(*E*)-1-Hydroxy-1-deuterio-2-methyl-2-buten-4-yl Diphosphate ((*E*)-[4-^{2}H]-4-Hydroxydimethylallyl Diphosphate, [4-^{2}H]-HDMAPP). Following the procedure described for **HDMAPP**, 40 mg (0.33 mmol) of **5** was converted to 80 mg (83%) of **[4-^{2}H]-HDMAPP**: R_f 0.47 (53:47 (v/v) 2-propanol/50 mM NH_4HCO_3 (pH 8.0)); ^{1}H NMR (300 MHz, D_2O) δ 5.64 (t, 1 H, J = 6.8 Hz), 4.51 (t, 2 H, J = 7.1 Hz), 3.98 (br s, 1 H), 1.69 (s, 3H); ^{13}C NMR (75 MHz, D_2O/CD_3OD) 140.37, 122.11 (d, J = 7.6 Hz), 67.25 (t, J = 21.9 Hz), 63.15 (d, J = 5.0 Hz), 14.10; ^{31}P NMR (121 MHz, D_2O) δ −10.25 (d, 1 P, J = 22.0 Hz), −6.53 (d, 1 P, J = 22.6 Hz); HRMS (FAB, M − H) calcd for $C_5DH_{10}P_2O_8$ 261.9992, found 261.9982.

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Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra for **HDMAPP** and **[4-^{2}H]HDMAPP**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) In IUPAC nomenclature, the correct numbering scheme of the carbon framework has the methyl group at the C-2 position and the hydroxyl at C-1. The common name given to the isoprenoid precursor, HDMAPP, places the methyl group at the C-3 position and the hydroxyl moiety at C-4.

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