PERKIN 1 COMMUNICATION

Synthesis of (2*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate, a key intermediate in the biosynthesis of isoprenoids

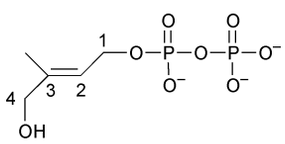
Jane L. Ward and Michael H. Beale *

IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, UK BS41 9AF. E-mail: mike.beale@bbsrc.ac.uk

Received (in Cambridge, UK) 21st January 2002, Accepted 19th February 2002
First published as an Advance Article on the web 26th February 2002

(2*E*)-4-Hydroxy-3-methylbut-2-enyl diphosphate, (HMB-PP) has been synthesised by regioselective hydroxylation and diphosphorylation of dimethylallyl alcohol.

(2*E*)-4-Hydroxy-3-methylbut-2-enyl diphosphate (HMBPP), **1**,[1] is a natural product with potent immunomodulatory activity, that has been recently identified in *Escherichia coli*.[2,3] This compound is produced by the enzyme encoded by the *GcpE* (*IspG*) gene[3] and is a key intermediate in the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (Scheme 1) leading to



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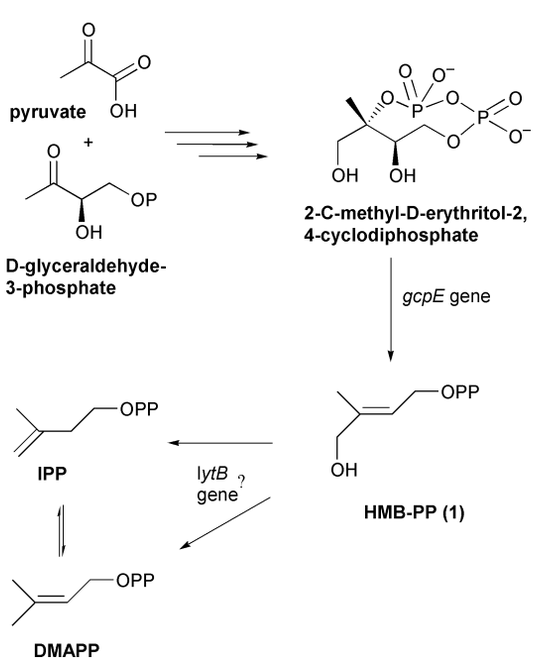
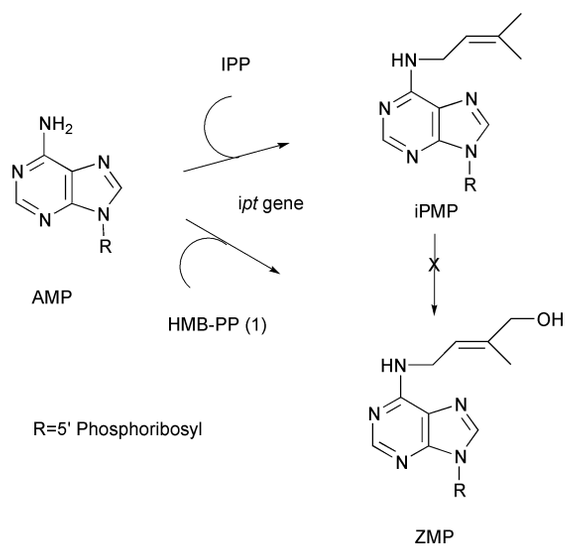
isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the universal isoprenoid precursors.[4–6] In plants, this pathway operates in chloroplasts, providing precursors for the biosynthesis of monoterpenoids, diterpenoids and carotenoids. The biosynthesis of cytokinins, the important regulators of plant growth and development, has traditionally been considered to proceed *via* a condensation of adenosine-5′-monophosphate and DMAPP to form isopentenyladenosine-5′-monophosphate (iPMP) catalysed by the enzyme encoded by the *ipt* gene.[7,8] The zeatin type cytokinins (*e.g.* ZMP, Scheme 2) were thought to arise from subsequent hydroxylation of the dimethylallyl moiety of iPMP. However, recent isotope-labelling studies with transgenic Arabidopsis plants expressing the *ipt* gene from *Agrobacterium tumefacians* have suggested that **1** may be an alternate substrate for the ipt protein leading directly to the zeatin-type cytokinins (Scheme 2).[9]



Scheme 1



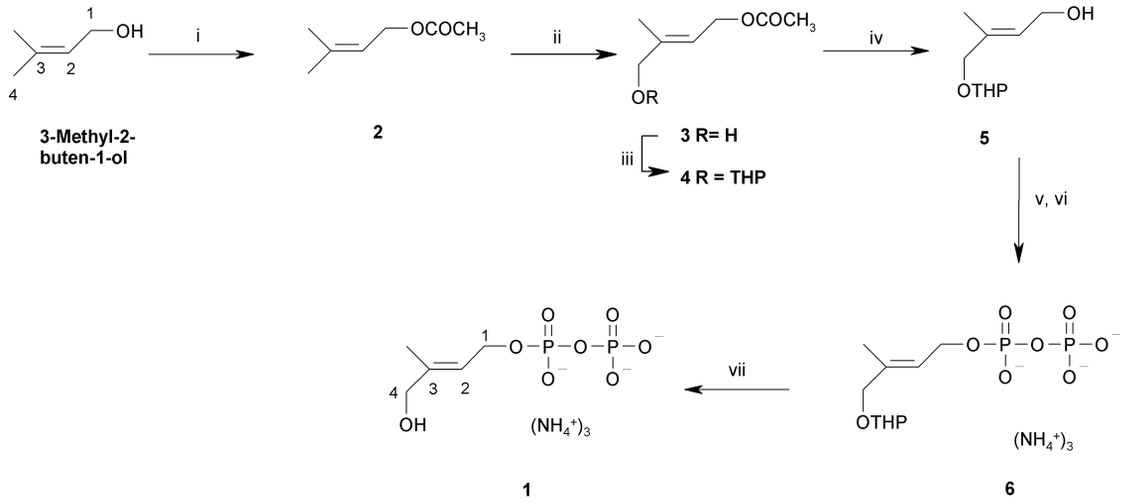
Scheme 2

There is an obvious need for a source of **1** for biochemical studies of the MEP pathway and of zeatin biosynthesis and for further study of its antigenicity. In this paper we describe the first synthesis of HMBPP **1**. The synthesis starts from commercially available 3-methylbut-2-en-1-ol (dimethylallyl alcohol) and is shown in Scheme 3. Although the key step in the synthesis is the regiospecific hydroxylation of the 4-carbon, an important factor in the route adopted was to fulfil the requirement for an effective protecting group strategy that differentiated between the C-1 and C-4 hydroxy groups.

The 1-alcohol function of starting material was converted to the acetate by treatment with pyridine–acetic anhydride to afford **2** in 99% yield. Selective allylic hydroxylation at C-4 of **2** was achieved using selenium dioxide and *tert*-butyl hydroperoxide. This reaction yielded a product with two components (TLC) that were separable by flash chromatography. The less polar product (24% yield) contained a mixture of compounds, amongst which the 4-aldehyde was observed, by ^{1}H NMR. The more polar fraction was identified as the desired 4-hydroxy compound **3** (25% yield). The regiochemistry of the reaction was determined by a 1D-gradient NOE NMR experiment (1D-GOESY),[10] which demonstrated that the 3-methyl group and 2-H atom were *trans*-orientated (no NOE), while the 4-hydrogens and 2-H were *cis*-orientated (strong NOE). The oxidation reaction was regiospecific and there was no indication (^{1}H- and ^{13}C-NMR) of the formation of any of the corresponding *cis*-orientated alcohol.

Ready supply of **3** allowed us to develop a strategy of protection of the new C-4 hydroxy function, to allow deprotection and diphosphorylation at the C-1-position. The 4-hydroxy

 DOI: 10.1039/b200712f



Scheme 3 *Reagents and conditions*; i, Ac_2O–pyridine; ii, SeO_2, t-BuOOH, CH_2Cl_2; iii, dihydropyran, CH_2Cl_2, cat. PPTS; iv, K_2CO_3, MeOH–H_2 (3 : 5); v, *N*-chlorosuccinimide, Me_2S, CH_2Cl_2, 0 °C; vi, tris(tetrabutylammonium)hydrogen diphosphate, $(NH_4)_2CO_3$, MeCN; vii, CH_3COOH, $(NH_4)_2CO_3$.

group was protected as the tetrahydropyranyl(THP) ether **4**, prepared in the standard way using dihydropyran in 84% yield. Removal of the 1-acetate group from **4** by treatment with potassium carbonate in methanol–water (3 : 5) afforded the 1-hydroxy analogue **5** in 72% yield. Diphosphorylation and isolation of the protected diphosphate **6** was carried out according to the methods of Davisson *et al.*[11,12] *via* the 1-chloride. The purified product yield was low (14%) but this is not atypical of this two step conversion and lengthy purification procedure.[11,12] The THP group was removed by treatment with acetic acid. The target (2*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate **1** was obtained as the ammonium salt after purification by cellulose chromatography.[11] NMR spectroscopy (D_2O–NH_4OD) confirmed loss of the –OTHP related proton signals, accompanied by coalescence of the 4-H_2 signal to a 2H singlet at δ 4.03. ^{31}P NMR (D_2O–NH_4OD) showed two peaks corresponding to the diphosphate at δ −6.85 and δ −9.80. The stereochemistry around the double bond in **1** was reconfirmed at this final stage by performing a second 1D-gradient NOE NMR experiment. Reciprocal nuclear Overhauser enhancements were observed from 2-H (multiplet at δ 5.66) to both the 4-H_2 singlet at δ 4.03 and the 1-H_2 triplet at δ 4.56. Similarly, reciprocal enhancements were observed from the 3-Me signal (δ 1.72) to both 4-H_2 at δ 4.03 and 1-H_2 at δ 4.56. Significantly, no NOE effect was observed between the 3-Me (δ 1.72) and 2-H (δ 5.66) signals. This confirms that the 3-Me and 2-H reside in a *trans* arrangement across the double bond. Absence of NOE between 1-H_2 and 4-H_2 supported this assignment and therefore **1** is confirmed as having the *E* stereochemistry. Comparison of the ^{1}H and ^{13}C NMR data with that reported[2,3] for the natural product, showed slight chemical shift differences, attributable to use of different solvents (D_2O–NH_4OD *versus* D_2O[2] or CD_3OD–D_2O[3]) but were otherwise in agreement. Negative ion ESI MS-MS analysis of the neutralised ammonium salt was in total agreement with data published[2] for the natural compound. Ions of note are the deprotonated molecular ion at *m*/*z* 261 with a daughter ion at 243 ($-H_2O$), and those at 177 ($H_3P_2O_7^-$) and 159 (base peak, $H_2P_2O_6^-$).

In conclusion, the establishment of this synthesis of HMBPP now provides a means to investigate further the pivotal role of this compound in the MEP pathway, and in particular the function of the *lytB* gene, its role in cytokinin biosynthesis and its pharmacological properties.

Experimental

General techniques were as previously described.[13] Tris(tetra-*n*-butylammonium) hydrogen pyrophosphate was prepared by the method of Davisson *et al.*[10] ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer, in $CDCl_3$, with tetramethylsilane as an internal reference. For diphosphate analogues NMR analysis was carried out in D_2O, adjusted to pH 8 by the addition of NH_4OD. ^{1}H- chemical shifts are reported as δ values using sodium 3-(trimethylsilyl)propane-1-sulfonate as an internal reference. ^{31}P-NMR spectroscopy was carried out in D_2O–NH_4OD and was externally referenced to phosphoric acid (δ 0.0). HR-MS analyses were performed on a Kratos MS80RFA mass spectrometer by direct insertion probe. ESI-MS-MS was carried out on an LCQ mass spectrometer (Thermoquest) using negative ion mode and HR-ESI-TOF MS analysis was carried out on a Micromass Q-TOF spectrometer.

3-Methylbut-2-enyl acetate 2

3-Methylbut-2-en-1-ol (20 g) in pyridine (50 mL) and acetic anhydride (100 mL) was stirred at room temperature for 2.5 h. The solution was then poured into water (250 mL), acidified to pH 3 with c.HCl and extracted into diethyl ether (2 × 200 mL) to afford the crude acetate **2** (29.5 g, 99%) as a colourless oil, which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 1.71(s, 3-CH_3), 1.77(s, 4-H_3), 2.06(s, –OCOCH_3), 4.58(d, J = 7 Hz, 1-H_2), 5.37(m, 2-H). HR-MS (EI) *m*/*z* 128.0851(2%, M^+, $C_7H_{12}O_2$ requires 128.0837), 86(12%), 71(18), 68(100), 60(6), 53(39).

(2*E*)-4-Hydroxy-3-methylbut-2-enyl acetate 3

3-Methylbut-2-enyl acetate **2** (29.5 g) in dichloromethane (200 mL) was treated with selenium dioxide (19.4 g) and *tert*-butyl hydroperoxide (74 mL). The solution was stirred under nitrogen at room temperature overnight. Dilute HCl (pH 3) was added and the organic layer was collected and washed with water, dried ($MgSO_4$) and evaporated to afford the crude oxidised product. Flash chromatography using hexane–EtOAc mixtures afforded a mixture containing the 4-aldehyde [δ 9.46-(s, 4-CHO), 6.52(m, 2-H), 4.92(dd, J = 6 and 1 Hz, 1-H_2)] contaminated with *tert*-butyl hydroperoxide containing residues (8.01 g, 24%) followed by the desired (2*E*)-4-hydroxy-3-methylbut-2-enyl acetate **3** (8.51 g, 25%) as a colourless oil. ^{1}H NMR ($CDCl_3$) δ 1.71(s, 3-CH_3), 2.06(s, –OCOCH_3), 4.02(s, 4-H_2), 4.65(d, J = 7 Hz, 1-H_2), 5.60(m, 2-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0(q, 3-CH_3), 21.1(q, –OCO*C*H_3), 60.9(t, 1-C), 67.3(t, 4-C), 118.4(d, 2-C), 141.3(s, 3-C), 171.6(s, –O*C*OCH$_3$). HR MS (EI) *m*/*z* 144.0793(2%, M^+, $C_7H_{12}O_3$ requires 144.0786), 129(37%), 119(12), 103(30), 101(100), 86(25), 71(15), 59(89).

(2*E*)-4-Tetrahydropyranyloxy-3-methylbut-2-enyl acetate 4

(2*E*)-4-Hydroxy-3-methylbut-2-enyl acetate **3** (4.5 g) in dichloromethane (100 mL) was treated with dihydropyran

(5.7 mL) and PPTS (15 mg). The resultant solution was stirred overnight under nitrogen. Removal of the solvent under reduced pressure afforded the crude product which upon flash chromatography with hexane–ethyl acetate mixtures gave the clean THP derivative **4** (6.01 g, 84%). ^{1}H NMR ($CDCl_3$) δ 1.50–1.70(5H, m, 2′-H, 3′-H_2, 4′-H_2), 1.77(s, 3-CH_3), 1.88(m, 2′-H), 2.06(s, –OCOCH_3), 3.50(m, 5′-H), 3.85(m, 5′-H), 3.91(d, J = 12 Hz, 4-H), 4.16(d, J = 12 Hz, 4-H), 4.63(m, 1′-H), 4.65(d, J = 7 Hz, 1-H_2), 5.63(m, 2-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5(q, 3-CH_3), 20.1(t), 21.3(q, –OCOCH_3), 25.7(t), 30.9(t), 60.7(t, 1-C), 62.4(t, 5′-C), 71.8(t, 4-C), 98.1(d, 1′-C), 120.4(d, 2-C), 138.5(s, 3-C), 171.3(s, COCH$_3$). EI-MS m/z 213(1%, M − 15), 117(12), 101(22), 85(100).

4-Tetrahydropyranyloxy-3-methylbut-2-en-1-ol 5

4-Tetrahydropyranyloxy-3-methylbut-2-enyl acetate **4** (6 g) in methanol–water (3 : 5, 80 mL) was treated with potassium carbonate (4 g). The reaction was stirred at room temperature for 2 h whereupon a more polar product was observed by TLC. The methanol was removed by evaporation and the resultant residue partitioned between water and ethyl acetate. The organic layer was separated, dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the crude product (4.5 g). Flash chromatography using hexane–ethyl acetate mixtures afforded the pure alcohol **5** (3.5 g, 72%). ^{1}H NMR ($CDCl_3$) δ 1.50–1.70(5H, m, 2′-H, 3′-H_2, 4′-H_2), 1.71(s, 3-CH_3), 1.88(m, 2′-H), 3.50(m, 5′-H), 3.85(m, 5′-H), 3.90(d, J = 12 Hz, 4-H), 4.11(d, J = 12 Hz, 4-H), 4.19(d, J = 7 Hz, 1-H_2), 4.65(t, J = 3.5 Hz, 1′-H), 5.67(m, 2-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3(q, 3-CH_3), 19.4(t), 25.7(t), 30.8(t), 58.8(t, 1-C), 62.3(t, 5′-C), 72.4(t, 4-C), 97.9(d, 1′-C), 126.5(d, 2-C), 134.8(s, 3-C). HR-MS (EI) m/z 168.1139[1%, M^+ − 18, $C_{10}H_{16}O_2$ (M − H_2O) requires 168.1150], 141(1%), 116(3), 101(27), 85(100), 67(18), 55(22).

(2*E*)-4-Tetrahydropyranyloxy-3-methylbut-2-enyl diphosphate 6

N-Chlorosuccinimide (2.67 g) in dry dichloromethane (100 mL) was cooled to 0 °C. Dimethyl sulfide (2.24 mL) was added followed by 4-tetrahydropyranyloxy-3-methylbut-2-en-1-ol **5** (3.4 g). After stirring for 1 h at 0 °C, the temperature was then raised to room temperature and the mixture was stirred for a further 15 min. The mixture was diluted with saturated brine. The organic layer was separated, dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the crude chloride, which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 1.50–1.70(5H, m, 2′-H, 3′-H_2, 4′-H_2), 1.79(s, 3-CH_3), 1.88(m, 2′-H), 3.50(m, 5′-H), 3.85(m, 5′-H), 3.90(d, J = 12 Hz, 4H), 4.11(d, J = 7 Hz, 1-H_2), 4.14(d, J = 12 Hz, 4-H), 4.62(t, J = 3.5 Hz, 1′-H), 5.71(m, 2-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3(q, 3-CH_3), 19.7(t), 25.8(t), 30.9(t), 40.6(t, 1-C), 62.5(t, 5′-C), 71.7(t, 4-C), 98.2(d, 1′-C), 122.1(d, 2-C), 139.0(s, 3-C). The chloride was dissolved in acetonitrile (50 mL) and treated with tris(tetrabutylammonium)hydrogen diphosphate (34.7 g). The resultant solution was stirred under nitrogen overnight. The solvent was removed under reduced pressure to give the crude diphosphate. Purification by ion exchange chromatography (Dowex 50W-8X, NH_4^+ form) eluted with 1 : 49 isopropanol–aqueous ammonium bicarbonate (25 mM) and lyophilisation, followed by chromatography on cellulose (Whatman CF11) eluted with 9 : 5 : 6 isopropanol–acetonitrile–ammonium bicarbonate (100 mM) and final lyophilisation gave the diphosphate **6** (1.02 g, 14%) as a white solid. ^{1}H NMR (D_2O–NH_4OD) δ 1.50–1.70(5H, m, 2′-H, 3′-H_2, 4′-H_2), 1.73(s, 3-CH_3), 1.77(m, 2′-H), 3.58(m, 5′-H), 3.92(m, 5′-H and 1′-H), 4.02(d, J = 12 Hz, 4-H), 4.16(d, J = 12 Hz, 4-H), 4.53(t, J = 7 Hz, 1-H_2), 5.71(m, 2-H); ^{13}C NMR (D_2O–NH_4OD) δ 13.9(q, 3-CH_3), 19.5(t), 24.9(t), 30.4(t), 62.4(t, 1-C), 63.7(t, 5′-C), 73.0(t, 4-C), 98.9(d, 1′-C), 124.4(d, 2-C), 136.7(s, 3-C). ^{31}P NMR (D_2O–NH_4OD): δ = −6.10, −9.88.

(2*E*)-4-Hydroxy-3-methylbut-2-enyl diphosphate 1

(2*E*)-4-Tetrahydropyranyloxy-3-methylbut-2-enyl diphosphate **6** (100 mg) was treated with acetic acid and left at room temperature overnight. The mixture was then neutralised by the addition of sodium hydrogen carbonate solution. Cellulose chromatography as above followed by lyophilization afforded the pure ammonium salt of (2*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate **1** (40 mg, 51%) as a white solid. ^{1}H NMR (D_2O–NH_4OD) δ 1.72(s, 3-CH_3), 4.03(s, 4-H_2), 4.56(t, J = 7 Hz, 1-H_2), 5.66(m, 2-H); ^{13}C NMR (D_2O–NH_4OD): δ = 13.4(q, 3-CH_3), 62.9(t, 1-C), 66.9(t, 4-C), 121.0(d, 2-C), 140.4(s, 3-C); ^{31}P NMR (D_2O–NH_4OD): δ = −6.85, −9.80. ESI-TOF-MS (−ve) m/z 260.9913 (M − H), $C_5H_{12}O_8P_2$ requires M − H = 260.9929; 242.9844 (M − H − H_2O), $C_5H_{12}O_8P_2$ − H − H_2O requires 242.9824. ESI-MS-MS (−ve) 261(1%), 243(56), 177(3), 163(9), 159(100), 97(9), 79(6).

Acknowledgements

IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. We thank Mervyn Lewis and Nathan Hawkins for MS analysis.

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1 Synonyms also in use for **1** are (2*E*)-1-hydroxy-2-methylbut-2-enyl 4-diphosphate and 4-hydroxydimethylallyl diphosphate (HO-DMAPP).
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