Pergamon

Tetrahedron Letters 41 (2000) 4885–4889

TETRAHEDRON LETTERS

Synthesis of tritium labelled 2-*C*-methyl-D-erythritol, a useful substrate for the elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis

Jean-François Hoeffler, Catherine Grosdemange-Billiard and Michel Rohmer*

Université Louis Pasteur/CNRS, Institut Le Bel, 4 rue Blaise Pascal, 67070 Strasbourg Cedex, France

Received 11 April 2000; accepted 14 April 2000

Abstract

Free 2-*C*-methyl-D-erythritol is utilised for isoprenoid biosynthesis by *Escherichia coli* mutants lacking the two first enzymes of the methylerythritol phosphate pathway, the deoxyxylulose phosphate synthase and isomero-reductase. For feeding experiments, this tetrol was synthesised with an overall 43% yield from readily available 1,2-*O*-isopropylidene-α-D-xylofuranose, including the possibility of tritium labelling.

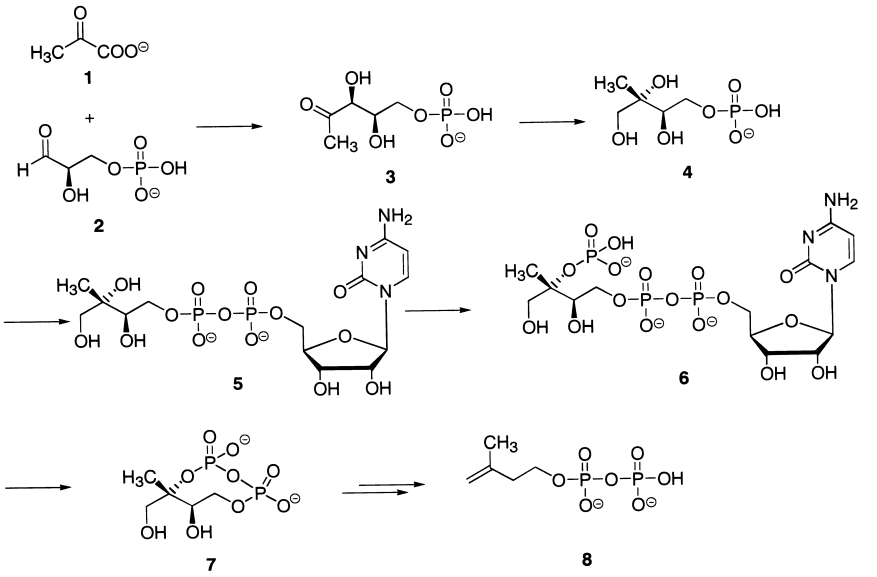
Keywords: biosynthesis; isoprenoids; 2-*C*-methyl-D-erythritol.

For many years, it was generally accepted that isopentenyl diphosphate **8** (IPP), the universal precursor for all isoprenoids, was synthesised through the well-known mevalonate pathway.[1] From in vivo feeding experiments with ^{13}C labelled precursors, IPP was however shown to originate in several eubacteria,[2] in unicellular algae[3] and in all plant plastids[4] from 2-*C*-methyl-D-erythritol 4-phosphate (MEP) (Scheme 1).[5] The initial step of this novel pathway is the formation of 1-deoxy-D-xylulose 5-phosphate **3** (DXP) by condensation of pyruvate **1** and D-glyceraldehyde 3-phosphate **2** mediated by the 1-deoxy-D-xylulose synthase.[6] An intramolecular rearrangement of DXP followed by a reduction, catalysed by the DXP isomero-reductase, affords MEP **4**.[7] If no other role as that of isoprenoid precursor is found for MEP, the reaction leading to the formation of this hemiterpene most probably represents the first committed step of this pathway. Further steps include the formations of methylerythritol 4-diphosphocytidine **5**, its 2-phosphate **6** and methylerythritol 2,4-cyclodiphosphate **7**.[8]

Deuterium labelled isotopomers of free methylerythritol **17** proved useful tools for the elucidation of this pathway. Indeed, free 2-*C*-methyl-D-erythritol (ME) is incorporated by the wild type *Escherichia coli* and supports the growth of mutants lacking the DXP synthase and/or

* Corresponding author. Fax: +33 (0)3 88 41 61 01; e-mail: mirohmer@chimie.u-strasbg.fr

0040-4039/00/$ - see front matter
PII: S0040-4039(00)00726-7

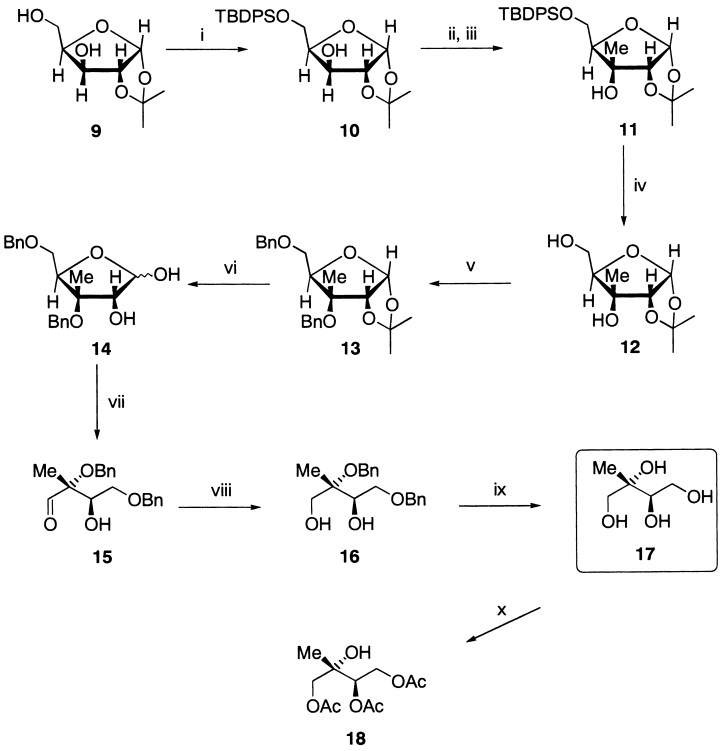


Scheme 1. 2-*C*-Methyl-D-erythritol 4-phosphate pathway for isoprenoid biosynthesis

the isomero-reductase.[9,10c] For the identification of further intermediates of this pathway, tritium labelled ME was required. Former syntheses of methylerythritol or of its 4-phosphate were either not adapted for obtaining such labelled material and/or were not fully enantioselective.[10] A simple method, yielding optically pure ME and allowing simple tritium introduction at the last but one step was adapted from our previous synthesis of MEP.[11]

2-*C*-Methyl-D-erythritol was synthesised in eight steps from the commercially available 1,2-*O*-isopropylidene-α-D-xylofuranose in an overall yield of 43% (Scheme 2). The synthetic strategy involved the addition of an alkyl group on the less hindered face of the carbohydrate ring.[11] In fact, addition of a Grignard reagent on the 3-oxo groups of protected 1,2-*O*-isopropylidene-α-D-xylofuranose affords the *ribo*-configured isomer as major product, the presence of the 1,2-*O*-isopropylidene group on the α face of the ulose directing the addition of Grignard reagents to proceed on the β-face of the furanose.[12]

The first step was the selective protection of the primary alcohol **9** at 0°C with the *t*-butyldiphenylchlorosilane in dichloromethane to give the monoprotected 5-*O*-*t*-butyldiphenylsilyl-1,2-*O*-isopropylidene-α-D-xylofuranose **10** in 88% yield. Oxidation of the secondary alcohol **10** was performed in THF using the Swern oxidation modified by Ireland.[13] In these conditions, the methyl group was directly introduced by addition of methylmagnesium chloride without isolation of the ketone to provide the branched-chain carbohydrate as a single diastereomer with the desired configuration in 95% yield. After removal of the *O*-silyl protective group with tetrabutylammonium fluoride, the protection of the two hydroxy functionalities required a protecting group showing high stability under acidic conditions and easily removed at the last step without purification of methylerythritol. Benzyl groups were chosen for this reason. Benzylation was performed with 83% yield using a suspension of NaH in dry DMSO and benzyl bromide.[14] The isopropylidene group was removed in acidic conditions, and the resulting mixture of the two anomers of hemicetal **14** was oxidised with sodium metaperiodate into the aldehyde **15**. Reduction of **15** with sodium borohydride afforded 2,4-*O*-dibenzyl-2-*C*-methyl-D-erythritol.[15,16] Finally, the



Scheme 2. Synthesis of 2-*C*-methyl-D-erythritol **17**. Reagents: (i) TBDPSCl, DMAP, TEA, CH_2Cl_2 (88%); (ii) $(COCl)_2$, DMSO, TEA, THF, –78 to –35°C and (iii) CH_3MgCl, –10°C (95%); (iv) $(n\text{-}Bu)_4NF$, THF (98%); (v) NaH, BnBr, DMSO, 60°C (83%); (vi) TFA:H_2O, 9:1, –10°C (78%); (vii) $NaIO_4$, MeOH:H_2O, 1:1 (90%); (viii) $NaBH_4$, isopropanol (92%); (ix) H_2, Pd/C, EtOH (98%); (x) Ac_2O, pyridine, overnight, rt (95%)

diol **16** was debenzylated using standard hydrogenation to give free 2-*C*-methyl-D-erythritol **17**, which was characterised as free tetrol or via its triacetate **18**.

This synthesis provides enantiopure methylerythritol **17** without tedious isolation and purification protocols. It also allows easy tritium labelling by reduction of the aldehyde **15** with commercially available tritium labelled sodium borohydride. The tritium labelled dibenzyl ether of methylerythritol **16** was purified by silica gel chromatography, and no further purification of the free 2-*C*-methyl-D-[1-^{3}H]erythritol **17** was required after hydrogenolysis of the benzyl groups.[17]

Acknowledgements

We thank Mr. J.-D. Sauer and R. Huber for NMR and MS measurements. This investigation was supported by a grant to M.R. from the 'Institut Universitaire de France'.

References

1. (a) Spurgeon, S. L.; Porter, J. W. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W.; Spurgeon, S. L., Eds.; John Wiley and Sons: New York, 1981; Vol. 1, pp. 1–53. (b) Qureshi, N.; Porter, J. W. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W.; Spurgeon, S. L., Eds.; John Wiley and Sons: New York, 1981; Vol. 1, pp. 47–94.

2. (a) Flesch, G.; Rohmer, M. *Eur. J. Biochem.* **1988**, *175*, 405–411. (b) Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. *Biochem. J.* **1993**, *295*, 517–524. (c) Rohmer, M.; Seemann, M.; Horbach, S.; Bringer-Meyer, S.; Sahm, H. *J. Am. Chem. Soc.* **1996**, *118*, 2564–2566.
3. (a) Schwender, J.; Seemann, M.; Lichtenthaler, H. K.; Rohmer, M. *Biochem. J.* **1996**, *316*, 73–80. (b) Disch, A.; Schwender, J.; Müller, C.; Lichtenthaler, H. K.; Rohmer, M. *Biochem. J.* **1998**, *333*, 381–388.
4. (a) Schwarz, M.; Arigoni, D. In *Comprehensive Natural Product Chemistry*; Cane, D. E., Ed.; Pergamon Press: Oxford, UK, 1999; Vol. 2, pp. 367–399. (b) Eisenreich, W.; Menhard, B.; Hylands, P. J.; Zenk, M. H.; Bacher, A. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6431–6436. (c) Lichtenthaler, H. K.; Schwender, J.; Disch, A.; Rohmer, M. *FEBS Lett.* **1997**, *400*, 271–274.
5. (a) Eisenreich, W.; Schwarz, M.; Cartayrade, A.; Arigoni, D.; Zenk, M. H.; Bacher, A. *Chem. Biol.* **1998**, *5*, R 221–R 233. (b) Rohmer, M. *Nat. Prod. Rep.* **1999**, *16*, 565–574 and references cited therein.
6. (a) Sprenger, G. A.; Schörken, U.; Wiegert, T.; Grolle, S.; de Graaf, A. A.; Taylor, S. V.; Begley, T. P.; Bringer-Meyer, S.; Sahm, H. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12857–12862. (b) Lange, B. M.; Wildung, M. R.; McCaskill, D.; Croteau, R. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2100–2104. (c) Lois, L. M.; Campos, N.; Rosa Putra, S.; Danielsen, K.; Rohmer, M.; Boronat, A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2105–2110.
7. (a) Takahashi, S.; Kuzuyama, T.; Watanabe, W.; Seto, H. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9879–9884. (b) Lange, B. M.; Croteau, R. *Arch. Biochem. Biophys.* **1999**, *365*, 170–174. (c) Schwender, J.; Müller, C.; Zeidler, J.; Lichtenthaler, H. K. *FEBS Lett.* **1999**, *455*, 140–144.
8. (a) Rohdich, F.; Wungsintaweekul, J.; Fellermeier, M.; Sagner, S.; Herz, S.; Kis, K.; Eisenreich, W.; Bacher, A.; Zenk, M. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11758–11763. (b) Kuzuyama, T.; Takagi, M.; Kaneda, K.; Dairi, T. W.; Seto, H. *Tetrahedron Lett.* **2000**, *41*, 703–706. (c) Lüttgen, H.; Rohdich, F.; Herz, S.; Wungsintaweekul, J.; Hecht, S.; Schuhr, C. A.; Fellermeier, M.; Sagner, S.; Zenk, M. H.; Bacher, A.; Eisenreich, N. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1062–1067. (d) Herz, S.; Wungsintaweekul, J.; Schuhr, C. A.; Hecht, S.; Lüttgen, H.; Fellermeier, M.; Sagner, S.; Zenk, M. H.; Bacher, A.; Eisenreich, N.; Rohdich, F. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2486–2490.
9. Charon, L.; Hoeffler, J. F.; Pale-Grosdemange, C.; Rohmer, M. *Biochem. J.* **2000**, *346*, 737–742.
10. (a) Anthonsen, T.; Hagen, S.; Kazi, M. A.; Shah, S. W.; Tagar, S. *Acta Chem. Scand.* **1976**, *B30*, 91–92. (b) Anthonsen, T.; Hagen, S.; Sallam, M. A. E. *Phytochemistry* **1980**, *19*, 2375–2377. (c) Duvold, T.; Cali, P.; Bravo, J. M.; Rohmer, M. *Tetrahedron Lett.* **1997**, *38*, 6181–6184. (d) Koppisch, A. T.; Blagg, S. J.; Poulter, D. *Org. Lett.* **2000**, *2*, 215–217. (e) Kis, K.; Wungsintaweekul, J.; Eisenreich, W.; Zenk, M.; Bacher, A. *J. Org. Chem.* **2000**, *65*, 587–592.
11. Hoeffler, J. F.; Pale-Grosdemange, C.; Rohmer, M. *Tetrahedron* **2000**, *56*, 1485–1489.
12. (a) Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423–3433. (b) Nakatani, K.; Arai, K.; Terashima, S. *Tetrahedron* **1993**, *49*, 1901–1912.
13. Ireland, R. E.; Norbeck, D. W. *J. Org. Chem.* **1985**, *50*, 2198–2200.
14. Funabashi, M.; Yamazaki, S.; Yoshimura, J. *Carbohydr. Res.* **1975**, *44*, 275–283.
15. Most analytical and experimental techniques were previously described.[11] Only the two last key steps leading to tritium labelled ME were described in detail. High-resolution mass spectrometry was performed on a ZAB-HF spectrometer with an acceleration potential of 8 keV using polyethylene glycol and a solution of sodium iodide in glycerol as matrix. The radioactivity was detected on TLC plates using a linear radioactivity detector BERTHOLD LB 2832. Liquid scintillation counting was performed on a Packard TRI-CARB 4000 series counter with an external standard allowing quenching corrections. All intermediates as well as the final 2-*C*-methyl-D-erythritol **16** were found to be pure by the usual criteria (^{1}H, ^{13}C NMR, melting point for the crystalline products and TLC).
16. 2,4-*O*-Dibenzyl-2-*C*-methyl-D-[1-^{3}H]erythritol **16**: To an ice-cooled solution of the aldehyde **15** (510 mg, 1.62 mmol, 1 equiv.) in isopropanol (4 ml) was added sodium borohydride (43 mg, 1.13 mmol, 0.7 equiv.). Stirring was continued at room temperature, and after 2 h the reaction mixture was diluted with water (5 ml), treated with 0.1N HCl and extracted with $CHCl_3$ (4×20 ml). The combined extracts were dried, filtered, concentrated, and the residue afforded after flash chromatography 2,4-*O*-dibenzyl-2-*C*-methyl-D-erythritol **16** as colourless crystals (472 mg, 92%, mp 71–72°C, R_f = 0.26, ethyl acetate/cyclohexane, 70:30). ^{1}H NMR ($CDCl_3$): δ = 1.25 (3H, s, CH_3); 2.58 (1H, t, $J_{1,OH}$ = 6.5 Hz, OH); 2.84 (1H, d, $J_{3,OH}$ = 3.9 Hz, OH); 3.60 (1H, dd, $J_{4a,4b}$ = 9.9 Hz, $J_{3,4a}$ = 7.0 Hz, 4-H_a); 3.72 (2H, d, $J_{1,OH}$ = 6.5 Hz, 1-H); 3.79 (1H, dd, $J_{4a,4b}$ = 9.9 Hz, $J_{3,4b}$ = 3.5 Hz, 4-H_b); 4.02 (1H, ddd, $J_{3,4a}$ = 7.0 Hz, $J_{3,OH}$ = 3.9 Hz, $J_{3,4b}$ = 3.5 Hz, 3-H); 4.51 (2H, d, J = 12.3 Hz, CH_2Ph); 4.61 (2H, d, J = 12.3 Hz, CH_2Ph); 7.25–7.38 (10H, m). ^{13}C NMR ($CDCl_3$): δ = 16.22 (CH_3); 64.18 (CH_2); 65.10 (CH_2); 70.60 (CH_2); 73.33 (CH); 73.52 (CH_2);

78.37 (quaternary C); 127.38, 127.48, 127.77, 128.40, 137.77 and 138.79 (aromatic C). HRMS (FAB$^+$): $(M+Na)^+$ calculated for $C_{19}H_{24}O_4Na$ 339.1572, found 339.1577. To an ice-cooled suspension of tritium labelled sodium borohydride (100 mCi, 263 mCi/mmol, 0.38 mmol, 1 equiv., Isotopchim, Ganagobie-Peyruis, France) in isopropanol (200 μl) was added a solution of aldehyde **15** in isopropanol (300 μl) (360 mg, 0.76 mmol, 2 equiv.). Stirring was continued at room temperature, and after 24 h the reaction mixture was diluted with methanol. The solvents were removed in vacuo and the residue was purified by TLC to afford the 2,4-*O*-dibenzyl-2-*C*-methyl-D-[1-^{3}H]erythritol **16** (220 mg, 67 mCi, 91%, $R_f = 0.26$, ethyl acetate:cyclohexane, 70:30).

17. 2-*C*-Methyl-D-[1-^{3}H]erythritol **17**: The diol **16** (400 mg, 1.26 mmol) was hydrogenated over 10% Pd/C (40 mg) in EtOH (20 ml) for 40 h at room temperature under atmospheric pressure. The mixture was filtered, and the filtrate concentrated to give 2-*C*-methyl-D-erythritol **17** as a colourless oil (165 mg, 98%, $R_f = 0.30$, chloroform:methanol, 80:20) which was not further purified. ^{1}H NMR (D_2O): $\delta = 0.91$ (3H, s, CH_3); 3.24 (1H, d, $J_{1a,1b} = 11.8$ Hz, 1-H_a); 3.36 (1H, dd, $J_{4a,4b} = 10.5$ Hz, $J_{3,4a} = 8.4$ Hz, 4-H_a); 3.37 (1H, d, $J_{1a,1b} = 11.8$ Hz, 1-H_b); 3.44 (1H, dd, $J_{3,4a} = 8.4$ Hz, $J_{3,4b} = 1.5$ Hz, 3-H); 3.61 (1H, dd, $J_{4a,4b} = 10.3$ Hz, $J_{3,4b} = 1.5$ Hz, 4-H_b); ^{13}C NMR (D_2O): $\delta = 17.53$ (CH_3, C-5); 61.08 (CH_2); 65.39 (CH_2); 73.16 (CH, C-3); 74.03 (quaternary C, C-2). HRMS (FAB$^+$): $(M+Na)^+$ calculated for $C_5H_{12}O_4Na$ 159.0633, found 159.0633. 2,4-*O*-dibenzyl-2-*C*-methyl-D-[1-^{3}H]erythritol **16** (220 mg, 67 mCi) obtained after the reduction of aldehyde **15** was hydrogenated over 10% Pd/C (10 mg) in EtOH (2 ml) for 40 h at room temperature and under atmospheric pressure. The mixture was filtered, and the filtrate concentrated to give 2-*C*-methyl-D-[1-^{3}H]erythritol **17** (98 mg, 98%, 67 mCi, 92mCi/mmol, $R_f = 0.30$, chloroform:methanol, 80:20), which was not further purified.