Facile and Stereospecific Synthesis of (*S*)- and (*R*)-[2-^{2}H]Isopentenyl Pyrophosphates[1]

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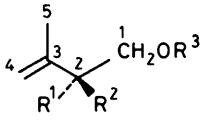
(*S*)- and (*R*)-[2-^{2}H]Isopentenyl† pyrophosphates, which are useful precursors for stereochemical studies on the biosynthesis of isoprenoids, were chemically synthesized in five steps *via* (*S*)- and (*R*)-3-methyl-2,3-epoxybutan-1-ols starting with 3,3-dimethylallyl alcohol, in 18% and 15% overall yields, respectively.

During the course of our work on the unusual elimination of the pro-4*S* hydrogen atom of mevalonic acid (MVA) in the process of the *Z*-prenyl chain elongation in the biosynthesis of the polyprenols by higher plants,[2] we required (*S*)- and (*R*)-[2-^{2}H]-isopentenyl pyrophosphates (IPPs) (**1**) and (**2**) for elucidation of the stereochemistry of hydrogen elimination in the enzymic formation of *E*- and *Z*-prenyl chains of the polyprenols. No chemical synthesis of stereospecifically deuteriated IPPs (**1**) and (**2**) has yet been developed, although (*S*)- and (*R*)-[2-^{3}H]IPPs have been enzymically synthesized from (4*R*)- and (4*S*)-[4-^{3}H]-mevalonic acids (MVAs), respectively.[3–5] Previous attempts to synthesize compounds (**1**) and (**2**) in multigram quantities failed due to troublesome procedures for the preparation of the enzyme system and the synthesis of stereospecifically deuteriated MVA. We therefore developed a facile and stereospecific chemical synthesis of compounds (**1**) and (**2**), and here describe the results.

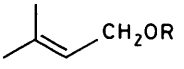


(**1**) R^1 = H, R^2 = ^{2}H, R^3 = $P_2O_6(NH_4)_3$

(**2**) R^1 = ^{2}H, R^2 = H, R^3 = $P_2O_6(NH_4)_3$

(**7**) R^1 = H, R^2 = ^{2}H, R^3 = Ts

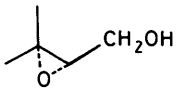
(**11**) R^1 = ^{2}H, R^2 = H, R^3 = Ts



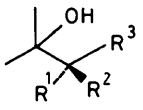
(**3**) R = H

(**12**) R = Ts

(**15**) R = $P_2O_6(NH_4)_3$



(**4**)



(**5**) R^1 = H, R^2 = ^{2}H, R^3 = CH_2OH

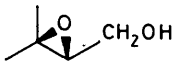
(**6**) R^1 = H, R^2 = ^{2}H, R^3 = CH_2OTs

(**9**) R^1 = ^{2}H, R^2 = H, R^3 = CH_2OH

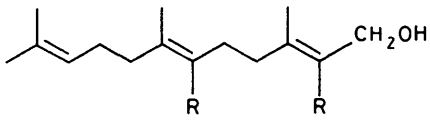
(**10**) R^1 = ^{2}H, R^2 = H, R^3 = CH_2OTs

(**13**) R^1 = H, R^2 = ^{2}H, R^3 = CHO

(**14**) R^1 = ^{2}H, R^2 = H, R^3 = CHO



(**8**)



(**16**) R = ^{2}H

(**17**) R = H

† In this paper isopentenyl refers to the 3-methylbut-3-enyl radical.

Compounds (**1**) and (**2**) were synthesized from 3,3-dimethylallyl alcohol (**3**) by a five-step procedure; (i) asymmetric epoxi-

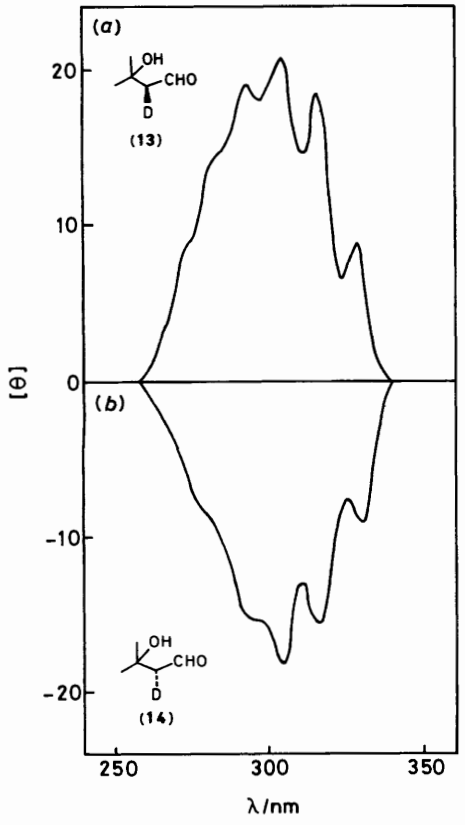


Figure. Circular dichroism curves of compounds (**13**) and (**14**) in diethyl ether

dation of compound (**3**), (ii) epoxide opening with $LiAl^2H_4$, (iii) selective toluene-*p*-sulphonylation, (iv) regiospecific dehydration, and (v) pyrophosphorylation, as follows. Following the method of Sharpless,[6] epoxidation of 3,3-dimethylallyl alcohol (**3**) using (+)-diethyl tartrate as a chiral source yielded (*S*)-3-methyl-2,3-epoxybutan-1-ol (**4**) (55% yield after distillation). The optical purity of the epoxide (**4**) was found to be in >89% enantiomeric excess (e.e.) by comparison with literature values.[7] Reduction of the epoxide (**4**) with $LiAl^2H_4$ gave (*R*)-[2-^{2}H]-3-methylbutane-1,3-diol (**5**) in 82% yield (99% incorporation of ^{2}H). Toluene-*p*-sulphonylation of the diol (**5**) with toluene-*p*-sulphonyl chloride in dry pyridine gave the (*R*)-monotoluene-*p*-sulphonate (**6**) in 68% yield which was dehydrated with methanesulphonyl chloride and triethylamine to give the (*S*)-isopentenyl toluene-*p*-sulphonate (**7**) in 71% yield. Pyrophosphorylation[8] of the compound (**7**) gave (*S*)-[2-^{2}H]IPP (**1**) in 83% yield. The overall yield of compound (**1**) from compound (**3**) was 18%.

In the preparation of (*R*)-[2-^{2}H]IPP (**2**), (*R*)-3-methyl-2,3-epoxybutan-1-ol (**8**) (>88% e.e.) was obtained from compound (**3**) by Sharpless oxidation using unnatural (−)-diethyl tartrate as a chiral source. The epoxy alcohol (**8**) was converted into (*R*)-[2-^{2}H]IPP (**2**) *via* compounds (**9**), (**10**), and (**11**), successively, in a manner similar to that described above. The overall yield of compound (**2**) from compound (**3**) was 15%.

Although the dehydrated products derived from compounds (**6**) and (**10**) were carefully analysed by means of h.p.l.c. (Radial Pak B10) and t.l.c. (Merck precoated silica gel $60F_{254}$ plate), the internal alkene, 3,3-dimethylallyl toluene-*p*-sulphonate (**12**), was not detected. Furthermore, the ^{1}H n.m.r. spectra of the dehydrated products exhibited no signals due to the internal alkene (**12**), thus confirming that the dehydration of compounds (**6**) and (**10**) forms specifically the isopentenyl toluene-*p*-sulphonates (**7**) and (**11**), respectively.

The absolute configuration at C-2 of the diol (**5**) was determined on the basis of a positive Cotton effect in the circular dichroism spectrum of the aldehyde (**13**), which was obtained from the diol (**5**) by oxidation with pyridinium chlorochromate. The positive Cotton effect associated with the $n \longrightarrow \pi^*$ transition of the carbonyl group is shown in the Figure (*a*). On the other hand, the aldehyde (**14**) from the diol (**9**) derived from the epoxide (**8**) showed a negative Cotton effect in its c.d. spectrum, as shown in the Figure (*b*). It has been shown that (*R*)-α-deuteriated aldehydes show a negative Cotton effect,[9] thus, the absolute configurations of the aldehydes (**13**) and (**14**) were determined to be *S* and *R*, respectively. Accordingly, the absolute configurations of the deuteriated IPPs (**1**) and (**2**) should be *S* and *R*, respectively.

The absolute configurations of (**1**) and (**2**) were further examined by feeding experiments using pig liver farnesyl pyrophosphate (FPP) synthetase, as follows. When compound (**1**) and dimethylallyl pyrophosphate (DMAPP) (**15**) were used as precursors, (*E,E*)-farnesol (**16**), thus enzymically synthesized, exhibited the molecular ion peak at *m/z* 224; this clearly indicates the retention of the deuterium atom of compound (**1**) in the biological formation of (*E,E*)-farnesol. When compound (**2**) and DMAPP (**15**) were used as precursors, on the other hand, the mass spectrum of the enzymically synthesized (*E,E*)-farnesol (**17**) was the same as that of authentic farnesol, indicating the loss of the deuterium atom of compound (**2**). According to Cornforth's stereochemical picture for the biosynthesis of isoprenoids,[10–12] the pro-2*S* hydrogen of IPP is retained and the pro-2*R* hydrogen is eliminated during the formation of *E*-prenyl chain in the biosynthesis of farnesol with the pig liver enzyme, thus the absolute configurations assigned to (**1**) and (**2**) as described above were established to be correct.

This synthetic method is favourable for the large-scale preparation of stereospecifically labelled IPP, because the sequence is simple and the reaction time at each step is relatively short. In addition, $LiAl^3H_4$, instead of the deuteride can be used in the sequence to yield the stereospecifically tritiated IPP.

Experimental

^{1}H and ^{13}C N.m.r. spectra were recorded on Hitachi R-600 FT n.m.r. (60 MHz) and Hitachi R-42 FT n.m.r. spectrometers, respectively; chemical shifts are given in δ (p.p.m.) downfield from internal $SiMe_4$ and [2,3-$^2H_4$]-3-(trimethylsilyl)propionic acid sodium salt (TSP) for a [^{2}H]chloroform solution and a ^2H_2O solution, respectively. Electron impact (e.i.) mass spectra were obtained on a Shimadzu QP-1000 spectrometer operating with an ionization potential of 70 eV. Chemical ionization (c.i.) mass spectra were recorded on a Shimadzu QP-1000 spectrometer using isobutane as the carrier gas. Optical rotation was measured with a JASCO DIP-360 digital polarimeter, using a 1.0-dm cell. C.d. spectra were recorded on a JASCO J-40 spectropolarimeter. G.l.c. was carried out on a 2 m column packed with 2% OV-17 or 15% DEGS on Chromosorb W (AW-DMCS; 80—100 mesh) with N_2 gas. H.p.l.c. analysis was performed on a Waters Radial-pak B10 column with a flow rate of 0.6 ml min^{-1} and the column effluent was monitored at 261 nm.

(S)-3-*Methyl*-2,3-*epoxybutan*-1-*ol* (**4**).—Titanium tetraisopropoxide (11.9 ml, 40 mmol), (+)-diethyl tartrate (7.0 ml, 40 mmol), 3,3-dimethylallyl alcohol (**3**) (3.4 g, 40 mmol), and t-butanoic acid (3.0M) in toluene (Aldrich) (26.9 ml, 80 mmol) was added to dichloromethane (400 ml) at −20 °C (solid CO_2–CCl_4 bath), successively, and the mixture was maintained at the same temperature for 24 h. After addition of Me_2S (9.9 g), the mixture was stirred for 45 min at −20 °C and poured into saturated NaF solution (800 ml) with vigorous stirring. After having

been stirred for 14 h, the mixture was saturated with NaCl and filtered through a Celite pad. The aqueous layer was extracted with dichloromethane (× 3), the combined organic phase was dried over Na_2SO_4 and concentrated to give an oily residue (9.5 g). This residue was distilled under reduced pressure to give the epoxy alcohol (**4**) (2.22 g, 55%) as a colourless oil: b.p. 58—60 °C (7 mmHg) and $[\alpha]_D^{25}$ −20.1 ± 0.9° (*c* 0.42 in $CHCl_3$) {lit.,[7] $[\alpha]_D^{25}$ +21.0° (*c* 1.71 in $CHCl_3$) for the *R*-isomer; >93% e.e.}; n_D^{25} 1.427; d_4^{25} 0.9912; 99.8% pure on g.l.c.; $\nu_{max.}$(neat) 3 420 (OH) and 1 250 cm^{-1} (epoxy); $\delta_H(CDCl_3)$ 1.32 and 1.35 (each 3 H, s, 2 × Me), 2.54 (1 H, br t, OH, disappeared in D_2O), 2.88—3.06 (1 H, m, 2-H), and 3.57—4.02 (2 H, m, 1-H); *m/z* (e.i.) 85 (5%) and 59 (100); *m/z* (c.i.) 103 (M^+ + H, 16%), 102 (M^+, 12), and 85 (100).

(R)-[2-^{2}H]-3-*Methylbutane*-1,3-*diol* (**5**).—A solution of epoxide (**4**) (6.13 g, 60 mmol) in dry diethyl ether (50 ml) was added dropwise to a stirred suspension of $LiAl^2H_4$ (2.0 g) in dry diethyl ether. The mixture was refluxed for 16 h, treated with saturated Na_2SO_4 solution (5 ml), and stirred for a further 1 h. The white solid was filtered off and the filtrate was dried over Na_2SO_4; the white solid was then extracted with a 20 ml portion of $CHCl_3$ (× 3). The combined organic phase was concentrated to give the diol (**5**) (5.17 g, 82%) as a colourless oil; $[\alpha]_D^{25}$ −0.1 ± 0.1° (*c* 3.55 in $CHCl_3$); n_D^{25} 1.436; d_4^{25} 0.9923; 99.8% pure on g.l.c.; $\nu_{max.}$(neat) 3 370 (OH) and 2 170 cm^{-1} (CD); $\delta_H(CDCl_3)$ 1.27 (6 H, s, 2 × Me), 1.70 (1 H, m, 2-H), 3.65 (2 H, br, 2 × OH, disappeared in D_2O), and 3.85 (2 H, d, *J* 6 Hz, 1-H); *m/z* (e.i.) 90 (M^+ − Me, 10%), 72 (18), 59 (95), and 43 (100); *m/z* (c.i.) 106 (M^+ + H, 4%), 105 (M^+, 2), 88 (M^+ − H_2O + H, 65), and 70 (M^+ − $2H_2O$ + H, 100). The deuterium content was determined to be 99% by e.i.m.s. spectrometry.

Oxidation of (R)-[2-^{2}H]-3-*Methylbutane*-1,3-*diol* (**5**).—Compound (**5**) (110 mg) in dry dichloromethane (2 ml) was added to a slurry of pyridinium chlorochromate (340 mg) in dry dichloromethane (2 ml), and the mixture was stirred at room temperature for 3 h. Dry diethyl ether (10 ml) was then added to the reaction mixture, and the resulting slurry was stirred for another hour. The slurry was passed through a column packed with 5 g of Florisil, and the eluate was concentrated under reduced pressure. The condensate was purified on a silica gel column with diethyl ether–hexane (1:1) to give (*S*)-[2-^{2}H]-3-hydroxy-3-methylbutanal (**13**) (43 mg, 40%) as a colourless oil; $\nu_{max.}$(neat) 3 400 (OH), 2 180 (CD), and 1 708 cm^{-1} (C=O); $\delta_H(CDCl_3)$ 1.34 (6 H, s, 2 × Me), 2.60 (1 H, d, *J* 2 Hz, 2-H), and 9.83 (1 H, d, *J* 2 Hz, 1-H); *m/z* (e.i.) 88 (M^+ − Me, 8%), 59 (49), 57 (21), and 43 (100); *m/z* (c.i.) 104 (M^+ + H, 4%) and 86 (M^+ − H_2O + H, 100). This methylbutanal was immediately used for c.d. measurements.

(R)-[2-^{2}H]-3-*Hydroxy*-3-*methylbutyl Toluene*-p-*sulphonate* (**6**).—A solution of toluene-*p*-sulphonyl chloride (14.1 g, 74 mmol) in dry pyridine (5 ml) was added dropwise to a stirred solution of compound (**5**) (5.17 g, 49 mmol) in the same solvent (5 ml) at 0 °C under a nitrogen atmosphere. The solution was stirred at 0 °C for 20 min and left at 4 °C for 2 days, and then poured onto ice. The resulting mixture was extracted with ether (× 2), and the combined organic phase washed successively with cold 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions and then dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave a colourless oil (9.86 g). This oil was chromatographed on a silica gel column, using EtOAc–hexane (1:4) as eluant to give the pure monotoluene-*p*-sulphonate (**6**) (8.70 g, 68%) as a colourless oil; $[\alpha]_D^{25}$ −0.13 ± 0.02° (neat); n_D^{25} 1.504; d_4^{25} 1.1684; $\lambda_{max.}$(EtOH) 273 (log ε 2.38), 267 (2.46), 261 (2.51), 255 (2.48), and 249 nm (2.31); $\nu_{max.}$(neat) 3 540—3 420 (OH), 2 170 (CD), 1 598, and 1 495 cm^{-1} (Ar); $\delta_H(CDCl_3)$ 1.21 (6 H, s, 2 × Me), 1.74 (1 H, br s, OH, disappeared in D_2O), 1.83 (1 H, m, 2-H), 2.45 (3 H, s, ArMe), 4.21 (2 H, d, *J* 7 Hz, 1-H), and 7.27—7.87 (4 H, m, ArH); *m/z* (e.i.) 259 (M^+, 0.1%), 244 (M^+ − Me, 9), 230 (5), 172 (69), 155 (21), 91 (66), 72 (82), 60 (100), and 45 (78); *m/z* (c.i.) 260 (M^+ + H, 4%) and 242 (M^+ − H_2O + H, 100). The e.i.m.s. spectrum indicated that little or no deuterium was lost during the toluene-*p*-sulphonylation of (**5**). The monotoluene-*p*-sulphonate (**6**) showed a single peak on analytical h.p.l.c. which was eluted with EtOAc–hexane (1:9).

(S)-[2-^{2}H]-3-*Methylbut*-3-*enyl Toluene*-p-*sulphonate* (**7**) (*Isopentenyl Toluene*-p-*sulphonate*).—A solution of compound (**6**) (3.21 g) in dry ether (100 ml) containing methanesulphonyl chloride (3.0 g) was cooled to 0 °C. Triethylamine (20 g) was added dropwise to the solution over 1 h with vigorous stirring at 0 °C. Stirring was continued at room temperature for 1 h. The mixture was filtered and the filtrate was evaporated to dryness. The crude product was purified by silica gel chromatography with EtOAc–hexane (1:9) as eluant to give the title compound (**7**) (2.12 g, 71%) as a colourless oil; $[\alpha]_D^{25}$ −0.47 ± 0.03° (*c* 13.8 in $CHCl_3$); n_D^{25} 1.511; d_4^{25} 1.1304; $\lambda_{max.}$(EtOH) 273 (log ε 2.39), 267 (2.47), 261 (2.52), 255 (2.49), and 249 nm (2.30); $\nu_{max.}$(neat) 3 090, 1 650, and 898 (C=CH_2), 2 150 (CD), 1 600, and 1 495 cm^{-1} (Ar); $\delta_H(CDCl_3)$ 1.66 (3 H, s, Me), 2.31 (1 H, m, 2-H), 2.45 (3 H, s, ArMe), 4.14 (2 H, d, *J* 7 Hz, 1-H), 4.68 and 4.79 (2 H, m, 4-H), and 7.27—7.88 (4 H, m, ArH); *m/z* (e.i.) 155 (17%), 91 (59), 69 (100), and 68 (61); *m/z* (c.i.) 242 (M^+ + H, 15%) and 89 (100). On analytical h.p.l.c. the isopentenyl toluene-*p*-sulphonate (**7**) which was eluted with EtOAc–hexane (1:19), diethyl ether–hexane (1:9), and CH_2Cl_2–hexane (1:19) showed a single peak.

(S)-[2-^{2}H]-3-*Methylbut*-3-*enyl Pyrophosphate* (**1**).—Pyrophosphorylation of compound (**7**) (1.10 g, 4.6 mmol) with tris-tetrabutylammonium hydrogen pyrophosphate (12.1 g) was carried out following the method reported previously.[8] Cellulose column chromatography of the reaction mixture gave the pure pyrophosphate (**1**) (1.128 g, 83%) as a highly hygroscopic solid; $[\alpha]_D^{25}$ −0.19 ± 0.05° (*c* 8.18 in H_2O); $\nu_{max.}$(KBr) 3 600—2 600 (NH), 2 180 (CD), 1 645 (C=C), and 1 195 cm^{-1} (P=O); $\delta_H(D_2O)$ 1.79 (3 H, s, Me), 2.38 (1 H, t, *J* 7 Hz, 2-H), and 4.06 (2 H, t, *J* 6.5 Hz, 1-H). The signals of the terminal alkene protons, expected to be present at *ca.* δ_H 4.8, overlapped with the solvent signal (δ_H 4.75—4.90); $\delta_C(D_2O)$ 146.9 (C-3), 114.7 (C-4), 67.5 (d, $J_{^{13}C,P}$ 3 Hz, C-1), 40.6 (dd, $J_{^{13}C,^2H}$ 20 Hz, $J_{^{13}C,P}$ 7 Hz, C-2), and 24.7 (C-5). The IPP (**1**) showed a single spot on analytical t.l.c. (Merck precoated cellulose plate). The spot was visualized according to the procedures reported previously.[8]

Feeding Experiments.—A mixture of $MgCl_2$ (5 mmol), 1,4-dithiothreitol (4 mmol), KF (5 mmol), iodoacetamide (5 mmol), compound (**15**) (170 μmol), compound (**1**) or (**2**) (170 μmol), and pig liver farnesyl-pyrophosphate synthetase[13] (100 mg) in Tris-HCl buffer solution (20 mM, 500 ml; pH 7.7) was incubated at 37 °C for 24 h and then treated with alkaline phosphatase as usual. The reaction mixture was extracted with hexane, and the extract subjected to g.l.c.–m.s. analysis. The farnesols (**16**) and (**17**) synthesized enzymically were estimated at 0.50 mg and 0.61 mg, respectively, by analytical g.l.c.

Acknowledgements

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