A Facile Synthesis of (*S*)- and (*R*)-[6-^{2}H]Carvones and (*S*)- and (*R*)-[2-^{2}H]Limonenes

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SUMMARY

(*S*)- and (*R*)-[6-^{2}H]Carvones and (*S*)- and (*R*)-[2-^{2}H]limonenes, non-radioactive tracers for the studies on the biosynthesis and metabolism of monoterpenoids in higher plants, were synthesized from (*R*)- and (*S*)-carvones by regioselective deuteration *via* a convenient route with highly enantiomeric purity.

Key words: (*S*)- and (*R*)-[6-^{2}H]carvones, (*S*)- and (*R*)-[2-^{2}H]limonenes

INTRODUCTION

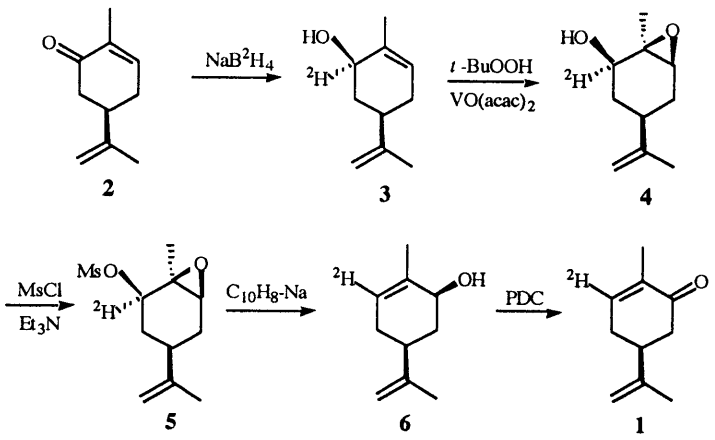
In the course of studies on the biotransformation of exogenous substrates by plant cell cultures, we found that (*S*)-limonene was enantioselectively converted into carvone with the cultured cells of *Nicotiana tabacum* and that carvone was further converted into dihydrocarvone [1-4]. In order to elucidate the stereochemistry and mechanism in the biotransformation of limonene to carvone and dihydrocarvone with the cultured cells and/or its enzyme preparation, we needed regioselectively deuterated (*S*)- and (*R*)-limonene and carvone. In the present work, we show procedures for the synthesis of (*S*)- and (*R*)-[6-^{2}H]carvones and (*S*)- and (*R*)-[2-^{2}H]limonenes with high enantiomeric purity.

RESULTS AND DISCUSSION

The synthesis of (*S*)-(+)-[6-^{2}H]carvone (**1**) is outlined in Scheme 1. (*R*)-(–)-Carvone (**2**) was reduced with NaB^2H_4 to give *cis*-[2-^{2}H]carveol (**3**) (68% yield), *trans*-[2-^{2}H]carveol (11%),

CCC 0362–4803/97/040285–06$17.50

Received 27 August 1996
Revised 5 December 1996

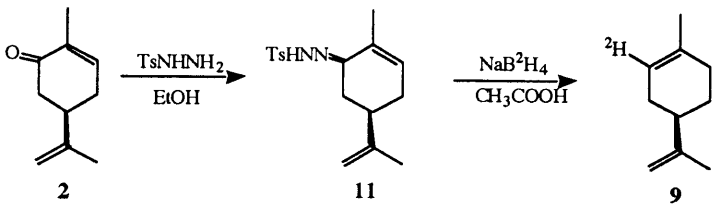


Scheme 1. Synthesis of (*S*)-[6-^{2}H]carvone (1).

and [^{2}H]dihydrocarveol (21%). The deuterated carveol (**3**) was oxidized to epoxyalcohol (**4**) with *t*-BuOOH and $VO(acac)_2$ in benzene in 60% yield, and **4** was mesylated, without purification, with mesyl chloride-triethylamine in CH_2Cl_2. The mesylate (**5**) obtained was converted into (*S*)-(+)-*cis*-[6-^{2}H]carveol (**6**) by the method of Nozaki and co-workers [5] with sodium-naphthalene in THF in 66% yield. Then **6** was converted to (*S*)-(+)-[6-^{2}H]carvone (**1**) by oxidation with pyridinium dichromate (PDC) in CH_2Cl_2 in 82% yield [6]. The intensity of the molecular ion peak in the mass spectrum of **1** indicated that 96% of the molecules of carvone were labeled with deuterium. In the ^{1}H NMR spectrum of the deuteriated carvone (**1**), no ^{1}H signal at δ 6.80 due to C(6)-H of carvone was observed. The optical purity of **1** was found to be 96% e.e. by comparison of the specific optical rotation of **1** with the reported data [7].

(*R*)-(−)-[6-^{2}H]Carvone (**7**) was also synthesized from (*S*)-(+)-carvone (**8**) in 23% overall yield by similar procedures as above.

Next, deuterated limonene was prepared as follows. Reduction of the mesylate of *cis*-[2-^{2}H]carveol (**3**) with reducing reagents, such as $LiAlH_4$ and $LiEt_3BH$, gave a deuterated limonene. However, the reaction product was found to be a mixture of (*R*)-[2-^{2}H]limonene (**9**) and (*S*)-[6-^{2}H]limonene (**10**) in the ratio of 3:2. The hydride displacement of the C-2 mesyl group of



Scheme 2. Synthesis of (*R*)-[2-^{2}H]limonene (9).

allylic mesylate might prove unsuccessful due to competing hydride addition to C-6 by S_N2' addition processes [8,9]. Therefore, optically pure (*R*)-(+)-[2-^{2}H]limonene (**9**) was prepared by reduction [10] of the tosylhydrazone of carvone with sodium borodeuteride as shown in Scheme 2. (*R*)-(−)-Carvone (**2**) was converted to tosylhydrazone (**11**) in 95% yield. Reduction of **11** with NaB^2H_4-CH_3COOH gave (*R*)-(+)-[2-^{2}H]limonene (**9**) (96% deuterium-enrichment) in 90% yield. ^{1}H and ^{2}H NMR measurements showed that only the 2-position of limonene was deuterated. (*S*)-(-)-[2-^{2}H]Limonene (**10**) was prepared from (*S*)-(+)-carvone in about 85% overall yield by a similar procedure as above.

EXPERIMENTAL

General Methods. — NMR spectra were recorded on JEOL GSX 270 spectrometer (^{1}H, 270 MHz; ^{2}H, 41.5 MHz) with Me_4Si as internal standard. TLC was performed on 0.25 mm thick silica gel plates (Merck silica gel 60 F254). GLC analysis was performed on a Shimadzu GC-14A instrument equipped with FID and a capillary column (0.25 mm × 30 m) coated with OV-17. GC-MS spectra were recorded on a Shimadzu QP-1000 spectrometer operating with an EI mode at 70 eV.

(*R*)-*cis*-[2-^{2}H]Carveol (3). — To a solution of (*R*)-(-)-carvone (18.1 g, 120 mmol) ($[\alpha]_D^{25}$ -59.0° (neat)) in EtOH (270 ml), NaB^2H_4 (2.5 g, 60 mmol) was added by portions and stirred at 10 °C for 1 h. The reaction mixture was extracted with pentane, washed with sat. brine, dried, and concentrated *in vacuo*. The acquired products (18.1 g) were a mixture of *cis*-carveol (68%), *trans*-carveol (11%), and dihydrocarveol (21%). The crude products were subjected to silica gel column chromatography (9:1 hexane-EtOAc) to give (*R*)-*cis*-[2-^{2}H]carveol (**3**): ^{1}H NMR ($CDCl_3$) δ 1.48 (m, 1H, 5-Ha), 1.74 (s, 6H, 1- and 8-Me), 1.80-2.15 (m, 4H, 3-H_2, 4-H and 5-Hb), 4.70 (m, 2H, C=CH_2), and 5.40 (s, 1H, 6-H); *m/z* (rel. intensity) 153 (M^+, 1), 138 (M^+-CH_3, 13), 135 (M^+-H_2O, 73), 120 (43), 110 (35), and 85 (100).

(*S*)-*cis*-[2-^{2}H]1,6-Epoxycarveol (4).—A solution of 70% aqueous *tert*-butyl-hydroperoxide (7.9 g, 61 mmol) in benzene (70 ml) was added dropwise to a mixture of **3** (5.5 g, 36 mmol) and vanadium oxyacetylacetonate dissolved in benzene at 0 °C. After stirring at 50 °C for 6 h, the reaction mixture was cooled to room temperature and poured into sat'd sodium sulfite solution. This solution was extracted with ether, washed with sat'd brine, dried, and evaporated.

The residue was submitted to flash column chromatography (7:3 hexane-EtOAc) to give epoxyalcohol (**4**) as a pale yellow oil (2.52 g, 14.9 mmol): IR ν_{max} 3450 (OH) and 1645 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, 1H, *J*=12.2 Hz, 3-Ha), 1.45 (s, 3H, 1-Me), 1.57 (d, 1H, *J*=21.0 Hz, 3-Hb), 1.68 (s, 3H, 8-Me), 1.75 (t, 1H, *J*=12.7 Hz, 5-Ha), 2.01 (m, 2H, 4-H and 5-Hb), 3.16 (d, 1H, *J*= 4.8 Hz, 6-H), and 4.70 (m, 2H, $C=CH_2$); *m/z* (rel. intensity) 169 (M^+, 2), 154 (M^+-CH_3, 3), 151 (M^+-H_2O, 6), 136 (11), 125 (51), 110 (74), and 96 (100).

(*R*)-*cis*-[2-^{2}H]1,6-Epoxycarveol mesylate (5).— To a mixture of compound **4** (2.5 g, 15 mmol) and triethylamine (1.89 g, 18 mmol) dissolved in dichloromethane (25 ml), mesyl chloride (2.64 g, 22.5 mmol) was added dropwise at -15 °C. Stirring was continued for 1 h, and the reaction mixture was quenched in ice-cold water (30 ml). The organic phase was removed and the aqueous layer was extracted with ether. The combined organic solution was washed with sat'd brine, dried, and concentrated *in vacuo*. The reaction yielded almost quantatively the desired epoxy mesylate (**8**) (3.35 g, 13. 6 mmol): IR ν_{max} 1645 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 3H, 1-Me), 1.68 (s, 3H, 8-Me), 1.72-2.20 (m, 5H, 3-H_2, 4-H and 5-H_2), 3.09 (s, 3H, $MeSO_2$), 3.16 (d, 1H, *J*= 4.9Hz, 6-H), and 4.72 (m, 2H, $C=CH_2$).

***cis*-[6-^{2}H]Carveol (6).**— The epoxy mesylate (5 g, 20.3 mmol) was dropwised to sodium-naphthalene reagent in dry THF (0.3 mmol, 300 ml) at -78 °C. The sodium-naphthalene reagent was prepared in the usual manner. Stirring was continued for 1 h. The reaction was quenched by addition of ammonium chloride (40 g) and ice-cold water (100 ml). This mixture was extracted with ether and the organic layer was washed with sat'd brine, dried, and concentrated *in vacuo*. Silica gel column chromatography of the residue gave *cis*-[6-^{2}H]carveol (**6**) (1.5 g, 9.8 mmol, 48% yield): IR ν_{max} 3370 (OH) and 1645 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52 (m, 1H, 5-Ha), 1.76 (s, 6H, 1- and 8-Me), 1.95-2.25 (m, 4H, 3-H_2, 4-H and 5-Hb), 4.19 (bs, 1H, 2-H), and 4.75 (s, 2H, $C=CH_2$); *m/z* (rel. intensity) 153 (M^+, 2), 135 (M^+-H_2O, 72), 120 (40), 110 (52), and 85 (100).

(*S*)-[6-^{2}H]Carvone (1).— A solution of compound **6** (1.04 g, 6.5 mmol) and CH_2Cl_2 (2 ml) was dropwised to pyridinium dichromate (PDC) in CH_2Cl_2 (10 ml) at room temperature. After stirring for 16 h, this mixture was filtered and washed with ether. The filtrate was washed with sat. brine, dried and evaporated. Silica gel column chromatography of the residue gave (+)-[6-^{2}H]carvone (**1**) (832 mg, 5.5 mmol, 85% yield): IR ν_{max} 1670 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.75 (s, 3H, 8-Me), 1.78 (s, 3H, 1-Me), 2.21-2.75 (m, 5H, 3-H_2, 4-H and 5-H_2),

4.76 (s, 1H, 9-H_{cis}), and 4.80 (s, 1H, 9-H_{trans}); ^2H NMR ($CHCl_3$) δ 6.80 (s, 2-^2H); *m/z* (rel. intensity) 151 (M^+, 10), 136 (M^+-CH_3, 4), 109 (31), 94 (29), and 83 (100); [α]$_D^{25}$ +58° (*c* 1.0, EtOH) (lit. [7] , [α]$_D^{25}$ + 60.0° (neat)).

(*R*)-(+)-[2-^{2}H]Limonene (9). — A solution of (*R*)-(–)-carvone (500 mg, 3.3 mmol) and *p*-toluenesulfonylhydrazide (744 mg, 4.0 mmol) in ethanol (2 ml) were refluxed for 3 h. Usual work-up of the reaction mixture gave a crude tosylhydrazone (**11**) (980 mg), which was recrystallized from ethanol to give pale yellow crystals; mp 164-167°C. To a solution of the tosylhydrazone (**11**) (200 mg, 0.63 mmol) in glacial acetic acid (3 ml), NaB^2H_4 (42 mg, 1.0 mmol) was added slowly in ice bath. The solution was stirred at room temperature for 1 h and at 70°C for 1.5 h. The mixture was poured into crushed ice, made basic with aqueous NaOH, and then extracted with pentane. After evaporation of the solvent, the crude product was subjected to column chromatography on silica gel to give (*R*)-(+)-[2-^{2}H]limonene (**9**) (77 mg, 90% yield): ^1H NMR ($CDCl_3$) δ 1.47 (m, 1H, 5-H_{cis}), 1.65 (s, 3H, 1-Me), 1.73 (s, 3H, 8-Me), 1.79 (m, 1H, 5-H_{trans}), 1.90 (m, 1H, 6-H_{trans}), 1.95 (m, 1H, 6-H_{cis}), 2.05-2.09 (m, 2H, 3-H_2), 2.10 (m, 1H, 4-H), and 4.70 (m, 2H, C=CH_2); ^2H NMR ($CHCl_3$) δ 5.40 (bs, 2-^2H).

Reduction of [^{2}H]carveol mesylate. — [^{2}H]Labeled carveol (**3**) was converted to the mesyl derivative with mesyl chloride-triethylamine in CH_2Cl_2. Reduction of the crude mesylate was performed as follows:

a) Reduction of mesylate by $LiAlH_4$-THF.— A mixture of crude mesylate and $LiAlH_4$ in dry THF was refluxed at 66 °C for 5 h. The reaction mixture was extracted with ether to give a crude product, which was a mixture of [2-^{2}H]limonene (**9**) and [6-^{2}H]limonene (**10**) (58% and 42%, respectively): ^1H NMR ($CDCl_3$) δ 1.47 (m, 1H, 5-H_{cis}), 1.65 (s, 3H, 1-Me), 1.73 (s, 3H, 8-Me), 1.79 (m, 1H, 5-H_{trans}), 1.90 (m, 1H, 6-H_{trans}), 1.95 (m, 0.4H intensity, 6-H_{cis}), 2.05-2.09 (m, 2H, 3-H_2), 2.10 (m, 1H, 4-H), 4.70 (m, 2H, C=CH_2), and 5.40 (s, 0.6H intensity, 2-H); ^2H NMR ($CHCl_3$) δ 5.4 (bs, 2-^2H) and 2.0 (bs, 6-$^2H_{cis}$); *m/z* (rel. intensity) 137 (M^+, 11), 122 (M^+-CH_3, 17), 94 (51), 80 (23), and 68 (100).

b) Reduction of mesylate by $LiEt_3BH$-THF.— To a mixture of crude mesylate (273 mg) and dry THF (5 ml), $LiEt_3BH$ (2.37 ml, 2.37 mmol) in THF (5 ml) was added dropwise in ice bath. The mixture was stirred at 21°C for 18 h. Excess hydride was decomposed with water and the organoborane was oxidized with 1 ml of 3 M NaOH and 1 ml of 30% H_2O_2. The reaction mixture was extracted with pentane to give a crude product, which was composed of [2-^{2}H]limonene (**9**) and [6-^{2}H]limonene (**10**) (60% and 40%, respectively).

ACKNOWLEDGMENTS

The ^{1}H and ^{2}H NMR measurements were made using JEOL GSX 270 spectrometer at Instrument Center for Chemical Analysis, Hiroshima University.

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