Biogenetic Studies in *Mentha* × *piperita*. 1. Deuterium-Labeled Monoterpene Ketones: Synthesis and Stereoselective Analysis

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Pulegone, menthone, and isomenthone isotopomers are synthesized as regioselectively deuterated d_5- and d_8-stereoisomers. Deuterium-labeled menthone and isomenthone enantiomers are analyzed using enantioselective multidimensional gas chromatography/mass spectrometry. The deuterated stereoisomers of menthone and isomenthone are separated from the unlabeled menthone and isomenthone on a glass capillary column, coated with 50% octakis(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin in OV 1701vi as the chiral stationary phase. These deuterium-labeled monoterpene ketones are proved to be highly valuable substrates in biosynthetic studies of terpenoid compounds.

Keywords: *Deuterium-labeled monoterpene ketones; stereoselective synthesis; enantioselective multidimensional gas chromatography/mass spectrometry (enantio-MDGC/MS)*

INTRODUCTION

Recently, feeding experiments with deuterium-labeled monoterpenes were found to be most efficient tools for in vivo biogenetic studies on plants (Wüst et al., 1996, 1998). Extending such investigations to *Mentha* species, deuterium-labeled oxygenated *p*-menthanes are expected to serve as suitable precursors.

In this paper different syntheses of deuterium-labeled pulegones, menthones, and isomenthones are reported. The deuterium-labeled menthone and isomenthone stereoisomers (Figure 1) are separated from their unlabeled analogues using enantioselective multidimensional gas chromatography/mass spectrometry (enantio-MDGC/MS).

MATERIALS AND METHODS

Enantioselective Gas Chromatography (Enantio-GC). An HP 5890 Series II gas chromatograph, equipped with a duran glass capillary (10 m × 0.25 mm i.d.) coated with 30% heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in SE 52 (film thickness = 0.5 μm), was used for the enantioseparation of d_5-pulegone. Conditions were as follows: carrier gas, hydrogen at 50 kPa; split, 30 mL/min; injector temperature, 280 °C; detector, FID, 240 °C; oven temperature, 80 °C, raised at 2 °C/min to 200 °C (30 min isothermal).

Gas Chromatography/Mass Spectrometry (GC/MS). The GC/MS analysis of the synthesized monoterpenes was carried out with a Fisons Instruments GC 8065, coupled to a Fisons Instruments MD 800 mass spectrometer, equipped with a BPX-5 fused silica column (50 m × 0.25 mm i.d., film thickness = 0.25 μm; SGE-Analytic, Weiterstadt, Germany; column A) or with a fused silica capillary coated with SE 52 (30 m × 0.25 mm i.d., film thickness = 0.5 μm; column B). Conditions were as follows: carrier gas, helium, column A 107 kPa, column B 70 kPa; split, 30 mL/min; injector temperature, 230 °C; oven temperature, 40 °C (5 min isothermal) raised at 2.5 °C/min to 250 °C (30 min isothermal); ion source temperature, 200 °C; interface temperature, 250 °C; mass range, 40−250 amu; EI, 70 eV. The molecular ion (M^+) and the fragmentation ions were given as *m*/*z* with relative peak intensities to the base peak (percent).

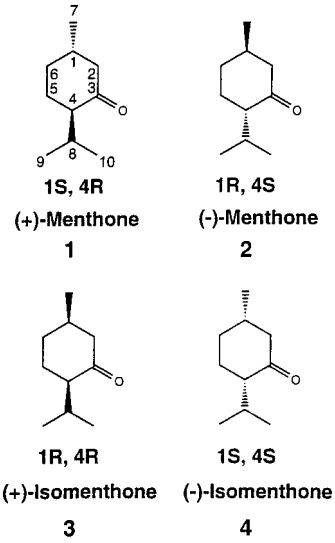

Figure 1. Enantiomers of menthone (**1**, **2**) and isomenthone (**3**, **4**).

Enantio-MDGC/MS. The enantio-MDGC/MS analyses were performed with a Siemens SiChromat 2, equipped with two independent column oven programs and a live-T-switching device. The main column was coupled to the transfer line of a Finnigan MAT ITD 800, using an open split interface. GC conditions were as follows: precolumn, duran glass capillary (30 m × 0.23 mm i.d.) coated with a 0.23 μm film of SE 52; carrier gas, hydrogen at 120 kPa; split, 8 mL/min; injector temperature, 220 °C; detector, FID, 250 °C; oven temperature, 50 °C (5 min isothermal) raised at 5 °C/min to 250 °C (15 min isothermal); cut times, 24.5−28.5 min; main column, duran glass capillary (30 m × 0.23 mm i.d.) coated with a 0.23 μm film of 50% octakis(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin in OV 1701vi (Schmarr, 1992); carrier gas, hydrogen at 100 kPa; oven temperature, 60 °C (5 min isothermal) raised at 2 °C/min to 100 °C (0 min isothermal) and then at 1 °C/min to 135 °C; detector, ITD 800; transfer line, 250 °C; open split interface, 250 °C; helium sweeping flow, 1 mL/min; ion trap manifold, 230 °C; EI 70 eV.

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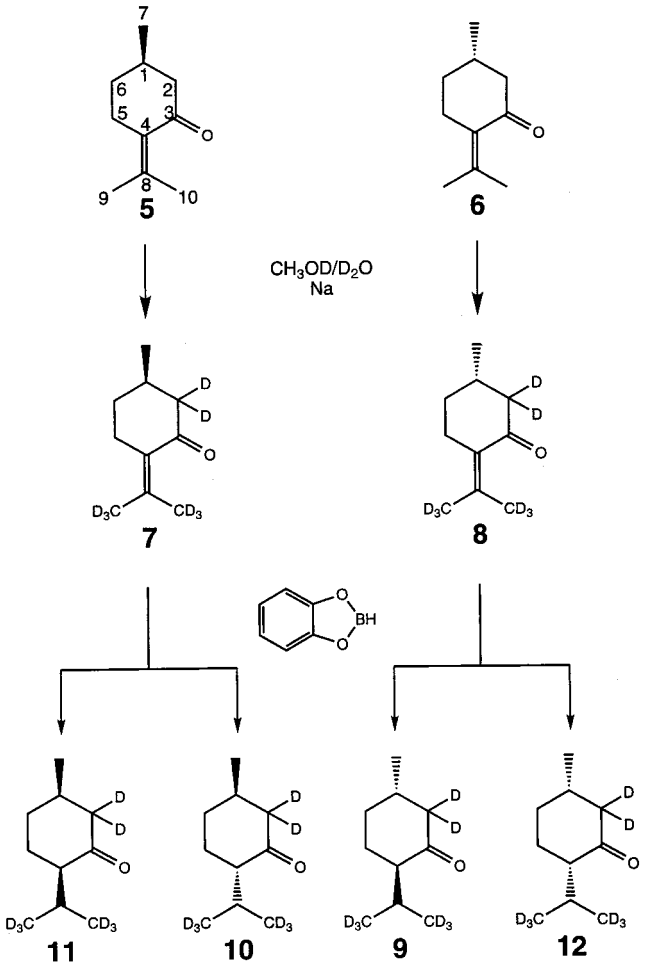
Scheme 1. Synthesis of the d_8-Labeled Precursor Pulegone and d_8-Labeled *p*-Menthan-3-one Reference Compounds, Starting from Pulegone Enantiomers



^{1}H NMR. A Bruker ARX 300, 300 MHz, was employed for recording the ^{1}H NMR spectra. $CDCl_3$ was used as solvent; abbrevations: s = singlet, d = doublet, m = multiplet, J = spin−spin coupling constant (Hz), a = axial, e = equatorial. The terpene nomenclature, given in Scheme 1 and Figure 1, was used for assignment.

Synthesis of d_8-Labeled Pulegone. *Synthesis of 5-(R)-2-[1-[$^2H_3$]Methyl[2,2,2-$^2H_3$]ethylidene]-5-methyl[6,6-$^2H_2$]-cyclohexanone [d_8-(R)-Pulegone, **7**] and 5-(S)-2-[1-[$^2H_3$]Methyl-[2,2,2-$^2H_3$]ethylidene]-5-methyl[6,6-$^2H_2$]cyclohexanone [d_8-(S)-Pulegone, **8**].* The method of Gibson (1983) was applied to pulegone. Three millimoles (470 mg) of **5** (Fluka, Deisenhofen, Germany) was added to a solution containing 2 mL of CH_3-OD, 0.9 mL of D_2O, and 1.3 mmol (30 mg) of sodium metal. The mixture was heated to reflux for 1.5 h, cooled, diluted with diethyl ether, and extracted twice with water, once with brine. After drying over sodium sulfate, the diethyl ether was removed in a vacuum. The product was purified by flash chromatography. Chromatographic conditions were as follows: column diameter, 20 mm; silica gel, 30−60 μm (Baker 7024-01); eluent, pentane/diethyl ether 10:1 (v/v). After removal of the eluent, 1.2 mmol (190 mg) of **7** was obtained; 1.8 mmol (280 mg) of **6** (Fluka), 1.2 mL of CH_3OD, 2.5 mL of D_2O, and 0.8 mmol (18 mg) of sodium metal lead to 0.4 mmol (64 mg) of **8** after purification. MS (**7**) 160 (82, M^+), 159 (23), 142 (34), 117 (53), 114 (64), 88 (59), 82 (100), 70 (50); ^{1}H NMR (**7**) δ 1.00 (d, 3H, 7-H, J = 6.5 Hz), 1.33 (m, 1H, 6a-H), 2.25 (m, 1H, 5a-H), 2.71 (dd, 1H, 5e-H), 1.84−2.00 (m, 1H, 6e-H). Traces of the isopropylidene protons were found at δ 1.79 (9-H) and 1.84−2.00 (10-H). Traces of the 2-H protons were found at δ 1.84−2.00 (Tori et al., 1975; Bambrige et al., 1995).

Synthesis of d_5-Labeled Pulegone. *Synthesis of 6-Methyl-5-[1,1,1,3,3-$^2H_5$]hepten-2-one, **14**.* Compound **13** (10.8 mmol, 1.36 g) was dissolved in a solution of 7 mmol (160 mg) of sodium metal in 80 mL of CH_3OD. This solution was stirred for 72 h at room temperature. The CH_3OD was removed by distillation over a Vigreux column, and 8 mL of D_2O was added to the residue. This solution was extracted three times with diethyl ether, and the combined diethyl ether extract was washed twice with water and dried over sodium sulfate to give 8 mmol (1.05 g) of crude **14** after removal of the solvent. MS 131 (5, M^+), 113 (95), 95 (60), 69 (60), 46 (100).

*Synthesis of (Z)-3,7-[9,9,9-$^2H_3$]Dimethyl-2,6-[4,4-$^2H_2$]ethyl Octadienoate (d_5-Ethyl Nerate, **15a**), and (E)-3,7-[9,9,9-$^2H_3$]-Dimethyl-2,6-[4,4-$^2H_2$]ethyl Octadienoate (d_5-Ethyl Geranate, **15b**).* An NaH suspension (ω = 60%, 420 mg, 10.5 mmol of NaH) was washed three times with pentane under nitrogen atmosphere. After addition of 9 mmol (1.6 g) of triethylphosphonoacetate (TEPA), the mixture was heated under reflux for 1 h. Eight millimoles (1.05 g) of **14** was dissolved in 6 mL of dry diethyl ether and added to the solution, which was then refluxed for 6 h. Thereafter, 10 mL of H_2O was carefully added, and the aqueous phase was washed twice with diethyl ether. The combined diethyl ether solutions were washed with brine and dried over sodium sulfate. Yield, 6.3 mmol (1.27 g) of crude **15a**/**15b**; diastereomeric ratio, **15a** (29%), **15b** (71%), proved by GC/MS; MS (**15a**) 201 (1, M^+), 200 (1), 156 (7), 133 (22), 127 (29), 105 (36), 87 (36), 69 (100).

*Synthesis of (R/S)-3,7-[9,9,9-$^2H_3$]Dimethyl-6-[4,4-$^2H_2$]ethyl Octenoate (d_5-Ethyl Citronellate, **16**).* The reduction procedure is deduced as reported by Semmelhack and Stauffer (1975). Compound **15a**/**15b** (1.8 mmol, 360 mg), dissolved in 8 mL of dry THF, was added to a suspension of 20.1 mmol (2.88 g) of copper bromide and 16.7 mmol (4.8 mg) of sodium dihydridobis(2-methoxyethoxy)aluminate (RedAl) in 30 mL of dry THF at −20 °C under nitrogen atmosphere. The solution was stirred at −20 °C for 1 h and for 1 h at room temperature; subsequently, 8 mL of H_2O was added carefully. After the addition of diethyl ether (30 mL), the slurry was filtered. The diethyl ether phase was extracted with saturated ammonium chloride solution, water, and brine and dried over sodium sulfate. Yield, 1.3 mmol (0.25 g) of crude **16**; MS 203 (1, M^+), 202 (1), 157 (20), 113 (19), 111 (21), 98 (29), 84 (34), 69 (100).

*Synthesis of (R/S)-3,7-[9,9,9-$^2H_3$]Dimethyl-6-[4,4-$^2H_2$]octenol (d_5-Citronellol, **17**).* The reduction was carried out according to the method of Málek (1988). RedAl (4.3 mmol, 1,3 mg) was reacted with a solution of **16** in 5 mL of dry diethyl ether under nitrogen atmosphere at 0 °C. The solution was stirred 2 h at room temperature before 1.5 mL of H_2O was added. The solution was filtered through a hydrophobic filter, yielding 0.8 mmol (0.13 g) of crude **17**. MS 142 (7), 99 (13), 84 (49), 69 (100), 56 (22).

*Synthesis of 5-(R/S)-2-Isopropylidene-5-[$^2H_3$]methyl-[4,4-$^2H_2$]cyclohexanone (d_5-Pulegone, **19**).* The method of Corey et al. (1976) was used in a modified cleanup procedure. Compound **17** (0.8 mmol, 0,13 g) was added to a suspension of 2.5 mmol (0.53 g) of pyridinium chlorochromate (PCC) in 3 mL of dry methylene chloride. After 36 h of stirring, pentane/diethyl ether 1:1 (v:v) was added and the suspension filtered (Celite). The solution was washed with 10% HCl, 10% $NaHCO_3$, and H_2O and dried over sodium sulfate. The crude product, d_5-isopulegone (**18**) (0.5 mmol, 82 mg), was dissolved in 3 mL of ethanolic sodium hydroxide (0.14 mol/L). The solution was refluxed for 1 h, subsequently diluted with water, and extracted with diethyl ether. The organic solution was washed with 10% HCl, washed twice with H_2O, and dried over sodium sulfate. Purification was done by preparative thin-layer chromatography (TLC); chromatographic conditions were as follows: silica gel 60 F 254 (Merck); mobil phase pentane/diethyl ether 10:1 (v/v); detection, UV 254 nm; R_f 0,4; 0.02 mmol (3 mg) of pure **19** was obtained. MS (**18**) 157 (16, M^+), 156 (13), 126 (51), 111 (99), 94 (63), 68 (100), 67 (98), 53 (46). MS (**19**) 157 (15, M^+), 156 (34), 141 (12), 111 (44), 85 (100), 67 (47). ^{1}H NMR δ 1.79 (s, 3H, 9-H), 1.99 (s, 3H, 10-H), 1.9−2.1 (m, 2H,

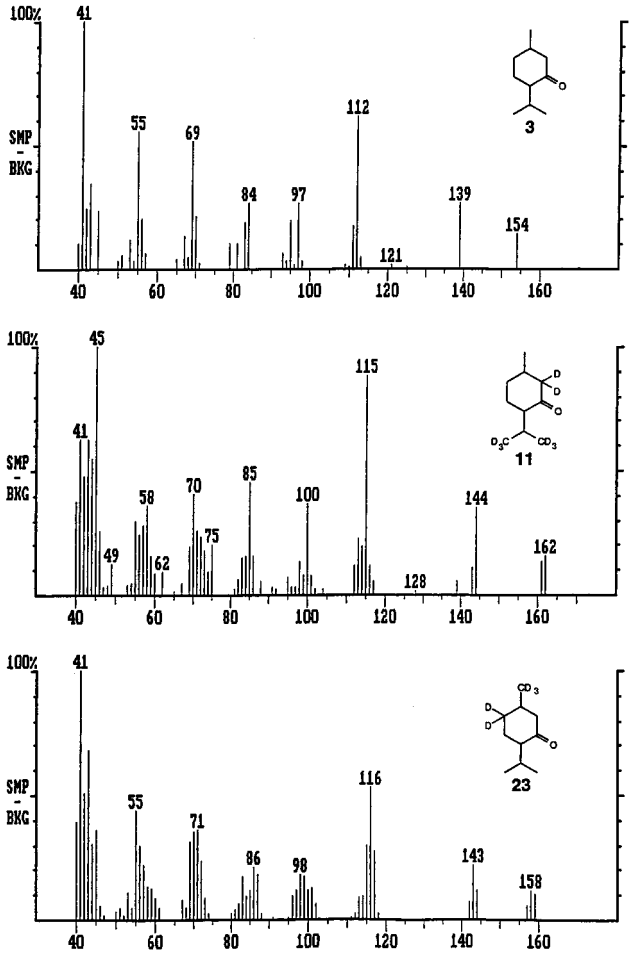


Figure 2. Mass spectra of genuine isomenthone (**3**), d_8-isomenthone (**11**), and d_5-isomenthone (**23**).

1-H, 2a-H), 2.22 (d, 1H, 5a-H, J = 15.5 Hz), 2.51 (dd, 1H, 2e-H, $J_{1,2e}$ = 3.5 Hz, $J_{2e,2a}$ = 14.0 Hz), 2.71 (d, 1H, 5e-H, J = 15.5 Hz). Traces of 7-H were found at δ 1.0, of 6a-H at δ 1.3, and of 6e-H at δ 1.8.

*Separation of d_5-Ethyl Nerate **15a** and d_5-Ethyl Geranate **15b***. Cyclic preparative HPLC was employed for the separation. Chromatographic conditions were as follows: Sep Tech TM pump, 70 mL/min; Knauer variable wavelength monitor 210 nm (system: Cyclomat, Merck, Darmstadt, Germany); column, LiChrospher Si 60 (self-packed; Merck), 250 mm × 50 mm i.d., 12 μm particle size; eluent, pentane/diethyl ether 40:1 (v/v). After separation of 0.82 g of **15a**/**15b**, 1.1 mmol (219 mg) of pure **15a** and 2.4 mmol (479 mg) of pure **15b** were obtained.

*Synthesis of (Z)-3,7-[9,9,9-$^2H_3$)Dimethyl-2,6-[4,4-$^2H_2$]octadienol (d_5-Nerol, **24a**) and (E)-3,7-[9,9,9-$^2H_3$)Dimethyl-2,6-[4,4-$^2H_2$]-octadienol (d_5-Geraniol, **24b**) [According to the Method of Málek (1988)]*. RedAl (3.7 mmol, 0.75 g) was added to a stirred, ice-cooled solution of 1.1 mmol (217 mg) of **15a** in 4 mL of dry diethyl ether under nitrogen atmosphere. After removal of the ice bath, the solution was stirred for 1 h at room temperature. Water (5 mL) was added carefully, and the suspension was diluted with diethyl ether and filtered. Yield, 0.7 mmol (110 mg) of crude **24a**; 2.4 mmol (479 mg) of **15b** yielded 1.8 mmol (285 mg) of crude **24b**. MS (**24a**) 141 (4), 126 (5), 97 (26), 85 (12), 69 (100), 53 (12).

Synthesis of (R)-2,2′-Bis(diphenylphosphino)-1,1′-binaphthyl)ruthenium Diacetate [(R)-BINAP] (Kitamura et al., 1992). Ruthenium(II) chloride-1,5-cyclooctadiene complex (0.17 mmol/47.3 mg) and (*R*)-2,2′-diphenylphosphino-1,1′-binaphthalene (0.18 mmol/112.1 mg) were dissolved under nitrogen atmosphere in 7 mL of dry DMF. After heating at 160 °C for 20 min, the solution was cooled to room temperature; 3.5 mL of a degassed methanol solution of sodium acetate (364 mg, 29.9 mmol) was added and stirred for 5 min. Water (3.5 mL) and dry toluene (1.7 mL) were added, and the resulting layers were mixed by vigorous stirring. The aqueous solution was extracted with two portions of 2 mL of dry toluene. The combined toluene solutions were extracted four times with 0.4 mL of water. All of these extractions were done under nitrogen atmosphere. The solvent was removed under reduced pressure. The resulting crude (*R*)-BINAP was dissolved in 5 mL of dry methanol, and this solution was used without further purification.

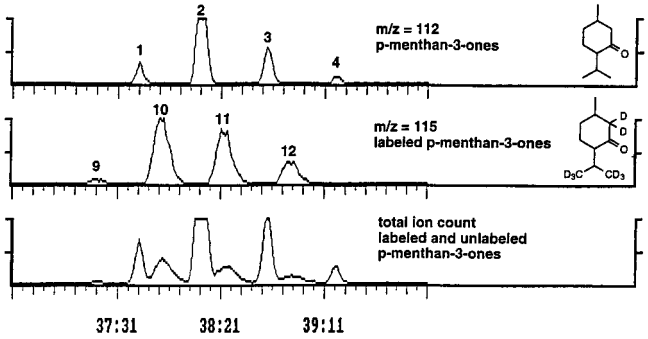


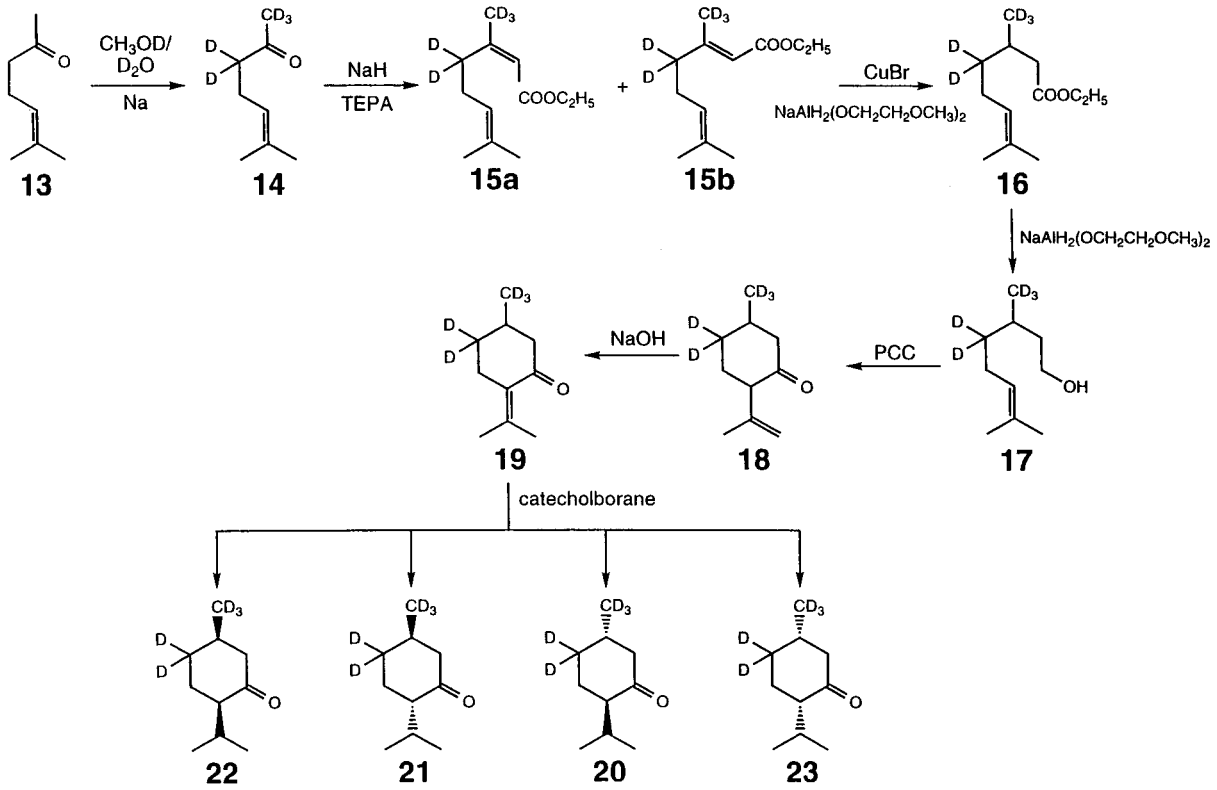
Figure 3. Enantio-MDGC/MS analysis of a standard mixture of unlabeled and labeled d_8-menthone and d_8-isomenthone (main column chromatogram).

*Synthesis of (R)-3,7-[9,9,9-$^2H_3$]Dimethyl-6-[4,4-$^2H_2$]octenol [(R)-d_5-Citronellol, **17a**)] and (S)-3,7-[9,9,9-$^2H_3$]Dimethyl-6-[4,4-$^2H_2$]octenol [(S)-d_5-Citronellol, **17b**] According to the Modified Procedure of Takaya et al. (1987).* Compound **24a** (0.7 mmol, 110 mg) was dissolved in a nitrogen atmosphere in 50 mL of dry methanol. (*R*)-BINAP in methanol (2 mL) was added. The solution, in a stainless steel autoclave, was stirred at room temperature for 94 h at 30 bar of hydrogen. The methanol was removed under reduced pressure, and pentane was added. After filtration through Celite 545, the solvent was removed, giving 0.7 mmol (112 mg) of crude **17a**. Compound **24b** (1.8 mmol, 285 mg) was dissolved in 2 mL of dry methanol. The complete solution of (*R*)-BINAP in 5 mL of dry methanol was added. After stirring at 30 bar of hydrogen at room temperature for 66 h and working up as described above, 0.7 mmol (111 mg) of crude **17b** was obtained.

*Synthesis of 5-(R)-2-Isopropylidene-5-[$^2H_3$]methyl-[4,4-$^2H_2$]-cyclohexanone d_5(R)-Pulegone, **19a**) and 5-(S)-2-Isopropylidene-5-[$^2H_3$]methyl-[4,4-$^2H_2$]cyclohexanone d_5(S)-Pulegone, **19b**).* Starting from 0.7 mmol (112 mg) of **17a**, 0.15 mmol (1 mg) of pure **19** was obtained after purification by TLC. The enantiomeric distribution **19a**:**19b** was 27:73 (enantio-GC). **17b** (0.7 mmol, 111 mg) yielded 0.07 mmol (10.7 mg) of pure **19b**. The enantiomeric distribution **19b**:**19a** was found by enantio-GC to be 92:8. ^{1}H NMR (**19b**) δ 1.79 (s, 3H, 9-H), 1.99 (s, 3H, 10-H), 1.9−2.1 (m, 2H, 1-H, 2a-H), 2.25 (d, 1H, 5a-H, J = 15.5 Hz), 2.50 (dd, 1H, 2e-H, $J_{1,2e}$ = 3.5 Hz, $J_{2e,2a}$ = 14.0 Hz), 2.71 (d, 1H, 5e-H, J = 15.5 Hz). Traces of 7-H at δ 1.0, of 6a-H at δ 1.3, and of 6e-H at δ 1.8 were found.

Synthesis of the Deuterium-Labeled Menthone and Isomenthone Isomers (9−12). *Synthesis of (2S,5R)-2-[1-[$^2H_3$]-Methyl-[2,2,2-$^2H_3$]ethyl]-5-methyl-[6,6-$^2H_2$]cyclohexanone [d_8-(2S,5R)-Menthone, **10**]; (2R,5R)-2-[1-[$^2H_3$]Methyl-[2,2,2-$^2H_3$]-ethyl]-5-methyl-[6,6-$^2H_2$]cyclohexanone [d_8-(2R,5R)-Isomenthone, **11**]; (2R,5S)-2-[1-[$^2H_3$]Methyl-[2,2,2-$^2H_3$]ethyl]-5-methyl-[6,6-$^2H_2$]cyclohexanone [d_8-(2R,5S)-Menthone, **9**], and (2S,5S)-2-[1-[$^2H_3$]Methyl-[2,2,2-$^2H_3$]ethyl]-5-methyl-[6,6-$^2H_2$]cyclohexanone [d_8-(2S,5S)-Isomenthone, **12**].* The method described by Evans and Fu (1990) was modified as follows. Compound **7** (1.8 mmol, 280 mg) was dissolved in 2.5 mL of dry THF under nitrogen. Catecholborane (1.9 mmol, 225 mg) was added at 0 °C. The solution was stirred for 1 h at 25 °C, quenched by the addition of 6 mL of acetone and 1 mL of saturated ammonium chloride solution. The diastereomeric products were purified and separated (two times) by column chromatography [silica gel 60; 0.063−0.200 mm particle size; eluent, pentane/diethyl ether 6:1 (v:v)]. Compounds **10** (0.12 mmol, 20 mg) and **11** (0.02

Scheme 2. Synthesis of the d_5-Labeled Precursor Pulegone and d_5-Labeled *p*-Menthan-3-one Reference Compounds, Starting from d_5-6-Methyl-5-hepten-2-one



mmol, 4 mg) were obtained. Compounds **9** and **12** were obtained when the procedure was begun from **8**. MS (**10**) 162 (5, M^+), 161 (23), 144 (26), 143 (36), 115 (100), 114 (70), 100 (29), 85 (29), 70 (59), 70 (60); ^{1}H NMR (**10**) δ 1.02 (d, 3H, 7-H, J = 6.5 Hz), 1.3−1.5 (m, 2H), 1.8−2.2 (m, 7 H). Traces of the isopropyl proton signals were seen at δ 0.83 and 0.89. Traces were also seen at δ 2.34.

*Synthesis of (2R/S,5R/S)-2-Isopropyl-5-[$^2H_3$]methyl-(4,4-$^2H_2$)cyclohexanone (**20**−**23**).* Compounds **20**−**23** were synthesized from **19** as described above. MS (**21**, **22**) 159 (4, M^+), 158 (12), 143 (17), 117 (33), 116 (100), 115 (78), 99 (53), 83 (60), 70 (93).

RESULTS AND DISCUSSION

Stable isotope labeling is known to be a suitable technique in monitoring the metabolic pathway of biologically active compounds, using mass selective detection. During our investigations on the biosynthesis of monoterpenoids in plants, deuterium-labeled precursors were needed. For this purpose deuterium was introduced into the precusor pulegone in such a way that the base peak [*m*/*z* 112, McLafferty rearrangement (Wilhalm and Thomas, 1965)] of the unlabeled menthone or isomenthone was shifted to higher *m*/*z* ratios [*m*/*z* 115 (d_8), *m*/*z* 116 (d_5)] in the labeled menthone or isomenthone, as seen from Figure 2.

In addition, isotopic effects were observed in enantio-MDGC/MS analysis. Thus, labeled *p*-menthan-3-ones as well as unlabeled *p*-menthan-3-ones were differentiated not only by their mass spectra but also by their elution behavior (Figure 3).

The required precusor d_8-pulegone was readily available using pulegone enantiomers (**5**, **6**) (Scheme 1). A comparison of the ^{1}H NMR of labeled and unlabeled pulegone indicated up to 95% labeling. The precursor (*R*/*S*)-d_5-pulegone (**19**) was synthesized, starting from 6-methyl-5-hepten-2-one (**13**) (Scheme 2).

The enantioselective generation of (*R*)-d_5-pulegone (**19a**) and (*S*)-d_5-pulegone (**19b**) was based on the enantioselective hydrogenation of d_5-nerol (**24a**) and d_5-geraniol (**24b**), respectively (Scheme 3). Note that in the Wittig−Horner−Emmons reaction there was a deuterium migration (Wilhalm and Thomas, 1967) from the C-1 and C-3-positions of the 6-methyl-5-[1,1,1,3,3-$^2H_5$]hepten-2-one (**14**) to the C-2-position of the generated esters **15a** and **15b**. Due to keto−enol tautomerism, the deuterium in the α-position to the carbonyl group was lost during the following reactions, as indicated by the ^{1}H NMR spectrum of **19**, where both the signals for the C-6-protons of pulegone are detected. Because of the migration in the Wittig−Horner−Emmons reaction some traces of unexchanged protons at C-4 and C-7 are found.

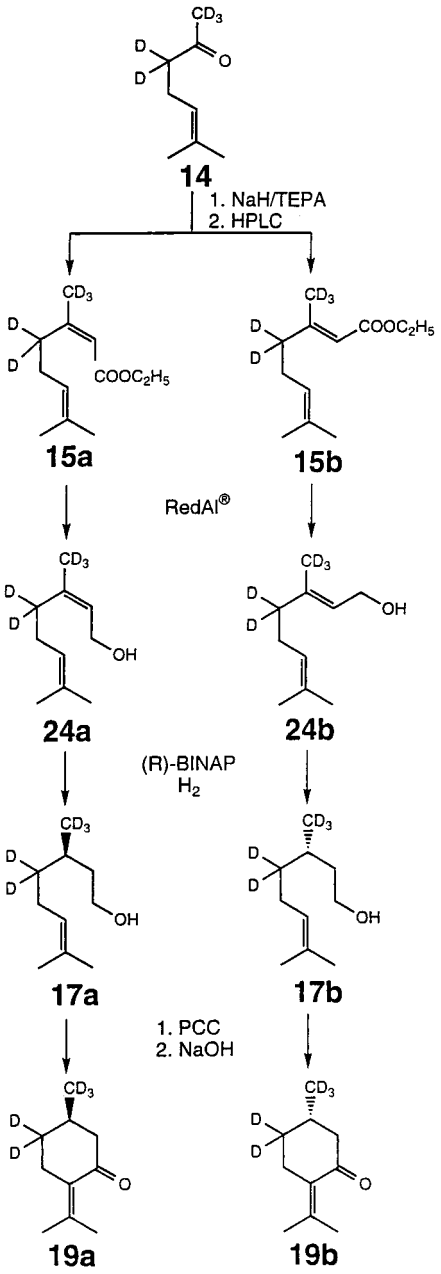
The required labeled reference compounds **9**−**12** and **20**−**23** were prepared by hydroboration with catecholborane. The base peak shift from *m*/*z* 112 to *m*/*z* 115 and a molecular ion at *m*/*z* 162 prove the formation of **9**−**12**. The base peak shift from *m*/*z* 112 to *m*/*z* 116/115 and a molecular ion at *m*/*z* 158/159 prove the formation of **20**−**23**, considering the loss of deuterium during the Wittig−Horner−Emmons reaction.

CONCLUSIONS

Deuterium-labeled pulegone, menthone, and isomenthone stereoisomers were prepared as suitable precursors for biogenetic studies, for example, on *Mentha* species. To realize these investigations, deuterium isotopomers of pulegone, menthone, and isomenthone were synthesized as regioselectively labeled d_5- and d_8-stereoisomers.

The separation and stereodifferentiation of labeled and unlabeled menthone and isomenthone were achieved

Scheme 3. Enantioselective Synthesis of d_5-Labeled Precursor Pulegone, Starting from 6-Methyl-5-hepten-2-one



using enantio-MDGC/MS. This is an indispensable prerequisite for biogenetic studies with regard to the stereoselectivity on the biosynthesis of menthone and isomenthone. The steroselectivity in the bioconversion of pulegone into menthone and isomenthone is reported in the following paper (Fuchs et al., 1999).

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