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Biogenetic Studies in *Syringa vulgaris* L.: Synthesis and Bioconversion of Deuterium-Labeled Precursors into Lilac Aldehydes and Lilac Alcohols

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Syringa vulgaris L. inflorescences were fed with aqueous solutions of regioselectively deuterated compounds assumed to be precursors of lilac aldehyde and lilac alcohol, respectively. Volatiles were extracted by stir bar sorptive extraction (SBSE) and analyzed using enantioselective multidimensional gas chromatography/mass spectrometry (enantio-MDGC/MS); deuterium-labeled lilac aldehydes and lilac alcohols were separated from unlabeled stereoisomers on a fused silica capillary column, coated with heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DIME-β-CD) (30%) in SE 52 (70%), as the chiral stationary phase. Feeding experiments with [5,5-$^2H_2$]mevalonic acid lactone **22** and [5,5-$^2H_2$]deoxy-D-xylose **23** indicate that the novel mevalonate independent 1-deoxy-D-xylose 5-phosphate/2*C*-methyl-D-erythritol 4-phosphate pathway is the dominant metabolic route for biosynthesis in lilac flowers. Additionally, bioconversion of deuterium-labeled d_5-(*R/S*)-linalool **3**, d_6-(*R*)-linalool **21**, d_5-(*R/S*)-8-hydroxylinalool **6**, d_5-(*R/S*)-8-oxolinalool **7**, d_5-lilac aldehydes **8**−**11** and d_5-lilac alcohols **12**−**15** into lilac during in vivo feeding experiments was investigated and the metabolic pathway is discussed. Incubation of petals with an aqueous solution of deuterated d_5-(*R/S*)-linalool **3** indicates an autonomic terpene biosynthesis of lilac flavor compounds in the flower petals of lilac.

KEYWORDS: ***Syringa vulgaris* L. biosynthesis; stir bar sorptive extraction (SBSE); enantioselective multidimensional gas chromatography/mass spectrometry (enantio-MDGC/MS); deuterium-labeled monoterpenes**

INTRODUCTION

From the earliest to present times, fragrance compositions of lilac are important constituents in perfumery. Until now there is no commercially available lilac flower oil, although many attempts have been made to produce satisfactory lilac concentrates (*1*, *2*). Because no natural lilac flower oil is being produced, synthetic lilac compounds are used in perfumery. The common lilac (*Syringa vulgaris* L., Oleaceae) originates from southeastern Europe and Asia and has been cultivated in many parts of Europe (*1*). Lilac aldehydes **8**−**11** and lilac alcohols **12**−**15** have been described as characteristic monoterpenes in *S. vulgaris* L. flowers with a desirable influence on the lilac odor quality (*3*). In previous studies it has been reported that four diastereoisomers of 2-[(5′-methyl-5′vinyl)tetrahydrofuran-2′-yl]propanal and -propanol were assigned as the genuine structures of lilac aldehydes and lilac alcohols (**Figure 1**) (*3*−*5*), but little recent research has been carried out on the biosynthetic origin of these monoterpenes in *Syringa* species. With the extension of such investigations to *S. vulgaris* L., deuterium-labeled linalool **3**, **21**, 8-hydroxylinalool **6**, and 8-oxolinalool **7** are expected to be suitable precursors.

Volatile monoterpenes play an important role as attractants for pollinating insects such as bee and wasp species (*6*). Various studies in *Rosa* (*7*), *Jasminium*, and *Gardenia* species (*8*, *9*) show that monoterpenes are glycosylated and accumulated in flower buds. Glycoconjugation allows better storage within plant vacuoles and protects plants from any toxicity of the free aglycon (*6*). In a hypothetical mechanism of volatile monoterpene formation Watanabe et al. (*8*, *10*) postulated that monoterpenes are biosynthesized and glycosylated in the leaves and then transported via the phloem to the flowers, where they are accumulated in the flower buds. The glycosylated compounds are then enzymatically hydrolyzed and transformed into volatile compounds during flower opening. Recently different authors (*12*−*16*) suggested the direct emission of monoterpenes from floral tissue after the de novo biosynthesis. Pichersky et al. (*17*, *19*, *20*) confirmed the localization and emission of methyl benzolate and methyleugenol genes in *Clarika*, snap dragon flower. A direct incorporation of deuterium-labeled precursor to phenylethanol in rose flower head has also been reported.

In addition to the feeding experiments that were carried out to clarify the metabolism of linalool and linalool derivatives leading to the formation of lilac alcohol, we also investigated the biosynthetic origin of isopentenyl diphosphate (IPP) and

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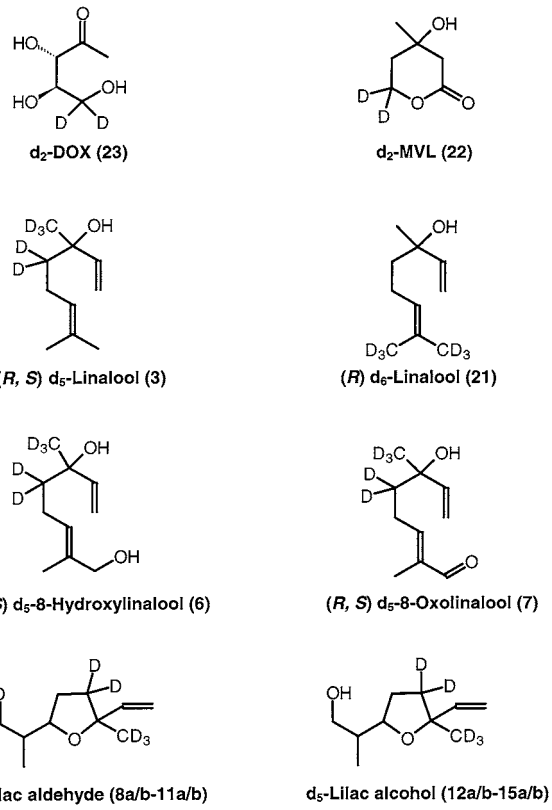

Figure 1. Deuterium-labeled precursors for biogenetic studies in *S. vulgaris* L.

dimethylallyl diphosphate (DMAPP), the two basic precursors of monoterpenoids (*21*−*23*). Besides the classical mevalonate route, a mevalonate-independent pathway for the formation of IPP and DMAPP was recently discovered in bacteria, algae, and plants. Therefore, the de novo biosynthesis of monoterpenes in *S. vulgaris* L. was investigated by in vivo feeding experiments using deuterium-labeled deoxy-D-xylose **23** and mevalonic acid lactone **22** as precursors.

MATERIALS AND METHODS

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 MD800 mass spectrometer, equipped with a 30 m × 0.25 mm i.d., 0.23 μm fused silica capillary coated with SE 52. Conditions were as follows: carrier gas, helium, 65 kPa; split, 20 mL/min; injector temperature, 230 °C; oven temperature, 40 °C (5 min isothermal) raised at 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 are given as *m*/*z* with relative peak intensities to the base peak (percent).

Enantio-MDGC/MS. The enantio-MDGC/MS analyses were performed with a Siemens SiChromat 2-8, 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 ITD800, using an open split interface. GC conditions were as follows: precolumn, 30 m × 0.23 mm i.d., 0.23 μm, fused silica capillary coated with SE 52; carrier gas, helium at 155 kPa; split, 20 mL/min; injector temperature, 220 °C; detector, FID, 250 °C; oven temperature, 60 °C (10 min isothermal) raised at 3 °C/min to 250 °C (20 min isothermal); cut times, 31.5−33.0 min for lilac aldehyde and 34.5−37.5 min for lilac alcohol; main column, 30 m × 0.23 mm i.d., 0.23 μm, fused silica capillary coated with heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DIME-β-CD) in SE 52; carrier gas, helium at 102 kPa; oven temperature, 60 °C (30 min isothermal) raised at 1 °C/min to 200 °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.

Enantio-SBSE-MDGC/MS. The previously reported system was employed for simultaneous enantioselective analysis of the volatile monoterpenes (*24*).

^{1}H NMR. A Bruker ARX 300, 300 MHz, was employed for recording the ^{1}H NMR spectra. $CDCl_3$ was used as solvent. Abbreviations: s, singlet; d, doublet; m, multiplet; J = spin−spin coupling constant (hertz).

Synthesis of d_5-Labeled (*R*/*S*)-Linalool. *Synthesis of 6-Methyl-5-[1,1,1,3,3-$^2H_5$]hepten-2-one (**2**).* The method of Keinan et al. (*25*) was applied. 6-Methyl-5-hepten-2-one **1** (3.37 mmol, 425 mg) was dissolved in a solution of 2.17 mmol (50 mg) of sodium in 25 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 1 mL of D_2O was added to the residue. This solution was extracted three times with diethyl ether, and the combined diethyl ether extracts were washed twice with water and dried over sodium sulfate to give 1.22 mmol (181.4 mg) of crude **2** after removal of the solvent: MS, *m*/*z* 131 (4, M^+), 113 (89), 95 (16), 69 (100), 56 (57); ^{1}H NMR δ 1.60 (s, 3H, 7-H), 1.66 (s, 3H, 8-H), 2.21 (m, 2H, 4-H), 5.05 (m, 1H, 5-H).

*Synthesis of 3,7-[10,10,10-$^2H_3$]Dimethyl-1,6-octadien[4,4-$^2H_2$]-3-ol [d_5-(R/S)-Linalool (**3**)].* The Grignard reaction was carried out according to the method of Keinan et al. (*25*). A solution of 1.5 mmol (197 mg) of vinylmagnesium bromide in dry THF was cooled in an ice bath, and compound **2** (1.38 mmol, 181.4 mg) dissolved in 10 mL of anhydrous diethyl ether was added with vigorous stirring. When the addition was complete, the mixture was stirred for 1 h and then allowed to stand for 12 h. A saturated solution of ammonium chloride was then added to decompose the magnesium complex formed. The organic layer was dried over anhydrous sodium sulfate, solvents were removed, and the residue was purified by flash chromatography. Chromatographic conditions were as follows: silica gel 60 (Merck); mobile phase, diethyl ether/petroleum ether 1:3 (v/v). 28.9 mg of pure **3** was obtained: MS, *m*/*z* 159 (2, M^+), 141 (44), 126 (85), 98 (100), 85 (90), 69 (95); ^{1}H NMR δ 1.55 (s, 3H, 7-H), 1.68 (s, 3H, 8-H), 2.02−2.05 (m, 2H, 5-H), 5.03−5.24 (m, 3H, 1-H, 2-H), 5.88−5.95 (m, 1H, 6-H).

Synthesis of d_5-Labeled (*R*/*S*)-8-Hydroxylinalool. *Synthesis of (R/S)-3-Acetoxy-3,7[10,10,10-$^2H_3$]dimethyl-[4,4-$^2H_2$]-1,6-octadiene (**4**).* The acetylation was carried out according to the method of Vidari et al. (*26*). To a solution of 1.47 mmol (234.3 mg) of **3** in 25.3 mmol (2 mL) of pyridine were added 2.14 mmol (218 mg) of Ac_2O and 0.053 mmol (6.5 mg) of DMAP. The mixture was stirred at 90 °C for 44 h and then quenched by adding CH_3OH and aqueous $NaHSO_4$. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were dried over sodium sulfate. The residue was purified by flash chromatography on silica gel 60 (Merck) eluted with a mobile phase of pentane/diethyl ether 7:3 (v/v). After removal of the eluent, 272.8 mg (97%) of **4** was obtained: MS, *m*/*z* 201 (1, M^+), 159 (4), 141 (42), 126 (85), 98 (100), 85 (91), 69 (98).

*Synthesis of (R/S)-2,6-[10,10,10-$^2H_3$]Dimethyl-[5,5-$^2H_2$]-6-acetoxy-2,7-octadienal (**5**).* The method of Wakayama et al. (*3*) was used. Compound **4** (1.36 mmol, 272.8 mg) and selenium dioxide (1.4 mmol, 0.155 g) were dissolved in 5 mL of dioxane/ethanol 9:1 (v/v), and the solution was heated at 80 °C for 5 h. After filtration, the solvent was removed under reduced pressure. The residue was treated with diethyl ether/petroleum ether 1:1 (v/v), and after removal of the solvent, the residue was purified by flash chromatography on silica gel 60 (Merck), with a mobile phase of petroleum ether/diethyl ether 3:2 (v/v), affording 196 mg of compound **5**: MS, *m*/*z* 215 (1, M^+), 155 (11), 137 (7), 126 (14), 82 (100), 74 (95).

*Synthesis of (R/S)-2,6-[10,10,10-$^2H_3$]Dimethyl-[5,5-$^2H_2$]-2,7-octadienol [d_5-(R/S)-8-Hydroxylinalool (**6**)].* Compound **5** (0.91 mmol, 196 mg) was dissolved in 20 mL of dry diethyl ether, and 38.5 mg (1 mmol) of lithium aluminum hydride was added in small portions. After 60 min of stirring at room temperature, water was added and the reaction mixture was acidified with sulfuric acid and extracted with a mixed solvent of diethyl ether/petroleum ether 1:1 (v/v). The organic layer was dried over sodium sulfate. After removal of the solvent, the residue was subjected to flash chromatography on silica gel 60 (Merck), eluted

with petroleum ether/diethyl ether 1:4 (v/v). After removal of the eluent, 39.4 mg of **6** was obtained: MS, *m*/*z* 175 (1, M^+), 157 (2), 139 (11), 126 (7), 98 (14), 74 (100), 84 (65); ^{1}H NMR δ 1.18−1.23 (m, 2-H, 8-H), 1.66 (s, 3H, 9-H), 2.04−2.07 (m, 2H, 5-H), 5.05−5.44 (m, 3H, 1-H, 2-H), 5.87−5.96 (m, 1H, 6-H).

Synthesis of d_5-Labeled (*R*/*S*)-8-Oxolinalool. *Synthesis of (R/S)-2,6-[10,10,10-$^2H_3$]Dimethyl-[5,5-$^2H_2$]-2,7-octadienal [d_5-(R/S)-8-Oxolinalool (7)].* The method of Corey et al. (*27*) was used. Compound **6** (0.08 mmol, 15 mg) was added to a suspension of 0.25 mmol (53 mg) of pyridinium chlorochromate in 5 mL of dry methylene chloride. After 36 h of stirring, pentane/diethyl ether 1:1 (v/v) was added and the suspension was filtered over Celite. The solution was washed with 10% HCl, 10% $NaHCO_3$, and H_2O and dried over sodium sulfate. After removal of the solvent, the residue was purified with flash chromatography on silica gel 60 (Merck), eluted with petroleum ether/diethyl ether 1:4 (v/v). After removal of the eluent, 0.02 mmol (4 mg) of **7** was obtained: MS, *m*/*z* 173 (1, M^+), 155 (5), 137 (4), 126 (5), 74 (100), 85 (22).

Synthesis of d_5-Labeled (*R*/*S*)-Lilac Aldehyde. *Synthesis of (R/S)-2-[(5′-Methyl-5′-vinyl)- [4′,4′-$^2H_2$][8′,8′,8′-$^2H_3$]tetrahydrofuran-2′-yl]-propanal [d_5-Lilac Aldehyde (**8a/b**−**11a/b**)].* In dry methanol (30 mL) catalytic amounts (∼10 mg) of sodium were dissolved, and compound **5** (1.29 mmol, 223 mg) was added dropwise. After 48 h of stirring under an argon atmosphere at room temperature, the reaction was stopped by adding acidic Dowex (H^+-form) until the solution was neutralized. The residue was filtered, and the organic layer was dried over sodium sulfate. The solvent was removed with a Vigreux column, and the residue was purified with preparative thin-layer chromatography (TLC) on silica gel 60 (Merck), mobile phase petroleum ether/diethyl ether 1:4 (v/v), affording 4 mg of compound **8a/b**−**11a/b**: MS, *m*/*z* 173 (1, M^+), 155 (5), 116 (27), 97 (43), 55 (100), 74 (27).

Synthesis of d_5-Labeled (*R*/*S*)-Lilac Alcohol. *Synthesis of (R/S)-2-[(5′-methyl-5′-vinyl)-[4′,4′-$^2H_2$][8′,8′,8′-$^2H_3$]tetrahydrofuran-2′-yl]propanol [d_5-Lilac Alcohol (**12a/b**−**15a/b**)].* Compound **8a/b**−**11a/b** (0.058 mmol, 10.0 mg) was dissolved in 10 mL of dry ether, and 3 mg (0.078 mmol) of lithium aluminum hydride was added in small portions. After 2 h of stirring at room temperature, water was added and the reaction mixture was acidified with sulfuric acid and extracted with a mixed solvent of diethyl ether/petroleum ether 1:1 (v/v). The organic layer was dried over sodium sulfate. After removal of the solvent, the residue was subjected to TLC on silica gel 60 (Merck), mobile phase petroleum ether/diethyl ether 1:1 (v/v). After removal of the eluent, 0.02 mmol (3.5 mg) of **12a/b**−**15a/b** was obtained: MS, *m*/*z* 175 (1, M^+), 157 (9), 116 (29), 96 (55), 74 (23), 55 (100).

Synthesis of d_6-Labeled (*R*)-Linalool. *Synthesis of (R)-3-Acetoxy-3,7-dimethyl-1,6-octadiene (**17**).* Compound **17** was synthesized analogously to the synthesis of compound **4** described above, using (*R*)-linalool **16** as the starting material, yielding 11.36 g of **17**: MS, *m*/*z* 177 (1, M^+), 154 (4), 136 (28), 121 (89), 107 (41), 93 (100), 80 (91), 69 (85).

*Synthesis of (R)-3-Acetoxy-6,7-epoxy-3,7-dimethyl-1-octene (**18**).* The synthesis was performed analogously to the method of Manning et al. (*28*). Compound **17** (57.87 mmol, 11.36 g) was stirred at 0 °C in 150 mL of methylene chloride. Then 23.72 g of 42% *m*-chloroperoxybenzoic acid in methylene chloride was added dropwise. After stirring for 1 min, the mixture was filtered and washed with aqueous $NaHCO_3$, water, and brine and then dried. The solvent was removed under reduced pressure, and the residue was purified by Kugelrohr distillation at 125 °C (0.3 mbar) to give 10.39 g (84.6%) of **18**: MS, *m*/*z* 212 (1, M^+), 155 (2), 137 (6), 94 (48), 79 (98), 71 (100); ^{1}H NMR δ 1.26 (s, 3H, 8-H), 1.29 (s, 3H, 9-H), 1.54 (s, 3H, 10-H), 2.00 (s, 3H, 12-H), 5.11−5.18 (m, 2H, 1-H), 5.87−6.01 (m, 1H, 6-H).

*Synthesis of (R)-4-Acetoxy-4-methyl-5-hexenal (**19**).* The method of Manning et al. (*28*) was used. Compound **18** (14.2 mmol, 3 g) was dissolved in 15 mL of dry diethyl ether, and powdered periodic acid (14.2 mmol, 3.23 mg) was added portionwise under vigorous stirring. The mixture was stirred for 30 min, and the organic layer was separated and washed with water, aqueous $NaHCO_3$, and brine. After drying, the organic layer was removed under reduced pressure, and the residue was purified by Kugelrohr distillation at 125−130 °C (0.3 mbar) to give 0.91 g (41.3%) of **19**: MS, *m*/*z* 170 (1, M^+), 113 (23), 84 (38), 71 (100).

*Synthesis of (R)-3-Acetoxy-3,7-dimethyl-[8,8,8,9,9,9-$^2H_6$]-1,6-octadiene (**20**).* The synthesis was performed analogously to the method of Puyn et al. (*29*) with a modified cleanup procedure. Isopropyl [1,1,1,3,3,3-$^2H_6$][2 -$^2H_1$]triphenylphosphoniumbromide (2.06 g, 524 mmol), synthesized according to the method of Puyn et al., was dissolved in 30 mL of THF and cooled at 0 °C as 3.8 mL of 1.6 M *n*-butyllithium (5.24 mmol) in hexane was added. After stirring for 1 h at room temperature, the solution was cooled at 0 °C, and 0.9 g (5.24 mmol) of **19** was added and stirring was continued for 3 h. After addition of 2 mL of methanol, the mixture was concentrated under reduced pressure, and the residue was extracted with diethyl ether/petroleum ether 1:1 (v/v). The solvent was removed under reduced pressure, and the residue was purified by Kugelrohr distillation at 120 °C (0.1 mbar) to give 0.409 g (38.7%) of **20**: MS, *m*/*z* 202 (1, M^+), 160 (2), 142 (11), 124 (28), 93 (100), 71 (38); ^{1}H NMR δ 1.58 (s, 3H, 10-H), 1.95 (s, 3H, 12-H), 5.05−5.17 (m, 3H, 1-H, 2-H), 5.91−6.01 (m, 1H, 6-H).

*Synthesis of (R)-3,7-Dimethyl-1,6-octadien[8,8,8,9,9,9-$^2H_6$]-3-ol [d_6-(R)-Linalool (**21**)].* Compound **21** was synthesized analogously to the synthesis of compound **6** described above. The crude product was purified by flash chromatography on silica gel 60 (Merck), mobile phase petroleum ether/diethyl ether 3:1 (v/v), to give 0.198 g (56.7%) of **21**: MS, *m*/*z* 160 (1, M^+), 142 (7), 124 (15), 93 (86), 89 (16), 75 (52), 71 (100); ^{1}H NMR δ 1.55 (s, 3H, 10-H), 1.97−2.04 (m, 1H, 2-H), 5.03−5.25 (m, 2H, 1-H), 5.86−5.96 (m, 1H, 6-H).

[5,5-$^2H_2$]Mevalonic Acid Lactone (22). [5,5-$^2H_2$]Mevalonic acid lactone synthesis was performed analogously to the method of Simpson et al. (*30*).

[5,5-$^2H_2$]-1-Deoxy-D-xylose (23). [5,5−$^2H_2$]-1-Deoxy-D-xylose was prepared according to the method of Jux and Boland (*31*).

Plant Material. During the flowering period, cuttings from *S. vulgaris* L. were made from wild and domesticated lilac bushes with differently colored flowers. The inflorescences were 10−15 cm high, containing 5 cm long pedicles (∼10 g of plant material).

Feeding Experiments. The deuterium-labeled precursors d_6-(*R*)-linalool **21**, d_5-(*R*/*S*)-linalool **3**, d_5-(*R*/*S*)-8-hydroxylinalool **6**, d_5-(*R*/*S*)-8-oxolinalool **7**, d_5-lilac aldehydes **8**−**11**, and d_5-lilac alcohols **12**−**15**, respectively, were dissolved in distilled water containing 0.1 mg/mL Tween 20 to give a solution of 0.1 mg/mL of the monoterpene in each case. In the case of d_2-mevalonic acid lactone **22** and d_2-1-deoxy-D-xylose **23**, respectively, 2 mg/mL of the precursor was dissolved in distilled water. The inflorescences were cut off and put into the feeding solutions. In each case, blank experiments were carried out with distilled water containing 0.1 mg/mL Tween 20. During a period of 48 h, 3 μmol of the precursor was taken up by the plant. In addition, d_5-(*R*/*S*)-linalool **3** was fed through a cut stem into green leaves (∼10 g of plant material). During 48 h, 7 μmol of d_5-(*R*/*S*)-linalool **3** was taken up by the leaves. Furthermore, lilac flower petals were isolated and incubated directly with a solution containing 0.1 mg/mL d_5-(*R*/*S*)-linalool **3** and 0.1 mg/mL Tween 20.

Isolation of Monoterpenes. Monoterpenes were extracted using the novel stir bar sorptive extraction (SBSE) method. Theory and practice have been described recently (*32*). Stir bars were obtained from Gerstel (Mühlheim, Germany) under the trade name Twister. Inflorescences, leaves, and flowers were homogenized with 1 mL of an aqueous 20% NaCl solution per gram of plant material and some sea sand. The homogenate was extracted for 60 min by SBSE, followed by extraction of the monoterpenes with 2 mL of pentane/diethyl ether 1:1 (v/v) from the poly(dimethylsiloxane) phase and concentration of the solution to ∼20 μL. Alternatively, the Twister was desorbed in the TDS system and subsequently analyzed by simultaneous enantioselective analysis of the volatile compounds (enantio-SBSE-MDGC/MS). In addition to this procedure, the volatile compounds of the homogenates were isolated using steam distillation (Deutsches Arzneibuch, DAB 9).

RESULTS AND DISCUSSION

Feeding experiments with aqueous solutions of deuterium-labeled monoterpenes have proven to be efficient methods for

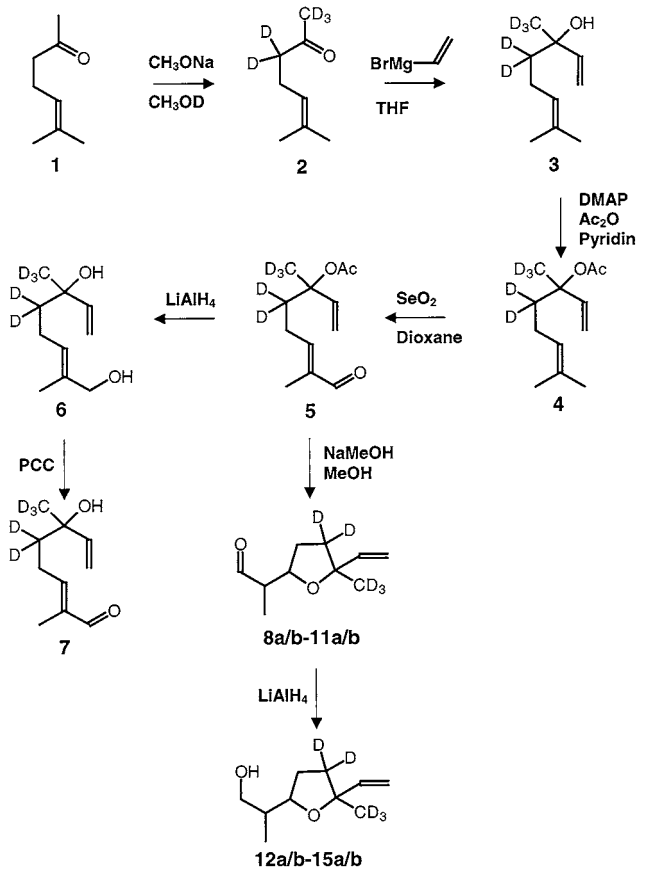

Figure 2. Synthesis of the precursors d_5-linalool, d_5-8-hydroxylinalool, d_5-8-oxolinalool, d_5-lilac aldehyde, and d_5-lilac alcohol.

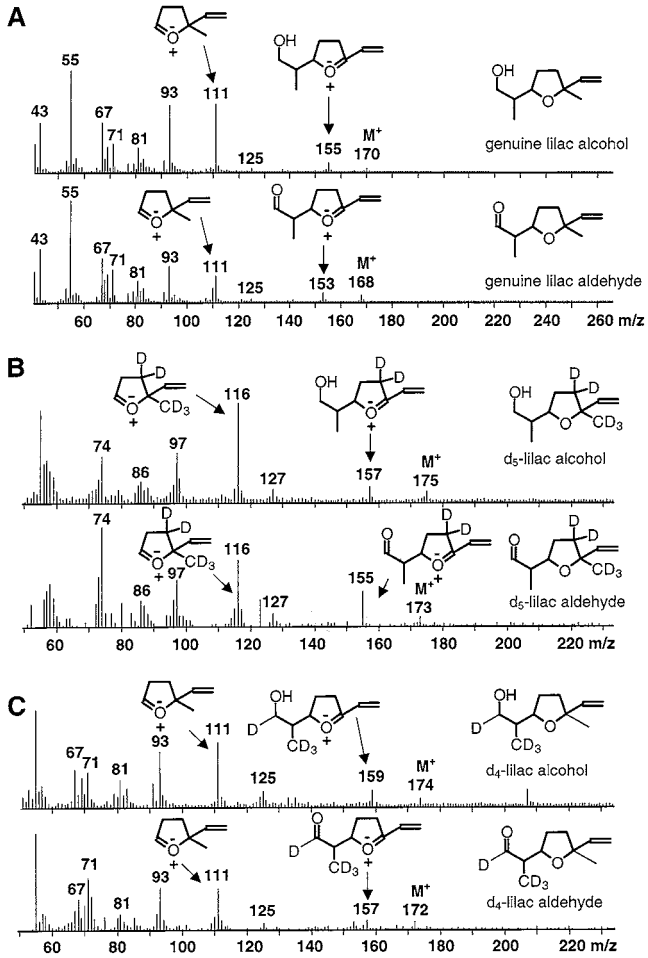

Figure 3. Mass spectra of (A) genuine lilac compounds, (B) d_5-labeled lilac compounds, and (C) d_4-labeled lilac compounds.

in vivo studies of biogenesis. For this purpose deuterium was introduced into the precursors (*R*/*S*)-linalool **3**, (*R*/*S*)-8-hydroxylinalool **6**, and (*R*/*S*)-8-oxolinalool **7** (**Figures 1** and **2**), so that the base peak of the corresponding unlabeled lilac alcohol and lilac aldehyde (*m*/*z* 111) was shifted to higher *m*/*z* ratios [*m*/*z* 116 (d_5)] (see **Figure 3A**,**B**). (*R*)-d_6-Linalool **21** is labeled at the isopropylidene group, so that the fragment *m*/*z* 155 of lilac alcohol was shifted to *m*/*z* 159 (d_4) and the fragment *m*/*z* 153 of lilac aldehyde to *m*/*z* 157 (d_4), as can be seen in **Figure 3C**. During cyclization, lilac aldehyde and lilac alcohol lose two deuterium atoms, yielding the d_4-labeled compounds.

In addition, inverse isotopic effects were observed during the enantio-MDGC/MS analysis (*33*). The deuterium-labeled lilac alcohol isotopomers showed a shift of their peak maximum of ~4 s compared with the unlabeled analytes.

The first enantioselective analysis of the eight stereoisomers of lilac aldehydes **8a/b**−**11a/b** and of lilac alcohols **12a/b**−**15a/b**, respectively, and the main column chromatogram obtained from Twister extraction of lilac flowers with the four genuine 5′-(*S*)-configured lilac aldehyde diastereoisomers **8a**, **9a**, **11b**, **10b** and **12b**, **13b**, **14a**, and **15b** as 5′-(*S*)-configured lilac alcohol diastereoisomers is shown in **Figure 4**. Six of the eight stereoisomers of lilac alcohol were well separated on this phase, only the stereoisomers **12a/b** remaining unresolved.

We were not able to assign the absolute configurations of the eight stereoisomers, but it is clear that the stereoisomers **8a**, **9a**, **11b**, and **10b** and **12b**, **13b**, **14a**, and **15b** were 5′-(*S*)-configured and their corresponding enantiomers **8b**, **9b**, **10a**, and **11a** and **12a**, **13a**, **14b**, and **15a** were 5′-(*R*)-configured. These assignments were concluded from an enantioselective synthesis of lilac aldehyde and lilac alcohol with (*R*)-linalool as starting material, which leads to the diastereoisomers **8b**, **9b**, **10a**, and **11a** and **12a**, **13a**, **14b**, and **15a** with 5′-(*R*) configuration. Further investigations, including X-ray structure analysis of derivatives, are in progress.

Due to the low concentration of some volatile monoterpenes in *S. vulgaris* L., SBSE was chosen as a highly sensitive isolation procedure of the target compounds lilac aldehydes and lilac alcohols (*32*). The volatile monoterpenes were extracted from the phase, and the concentrated solution was analyzed using GC/MS and enantio-MDGC/MS, which allows a selective and sensitive analysis of the chiral compounds. This method leads to the same results as the employed direct desorption of the Twister in the enantio-SBSE-MDGC/MS system and the analysis of the steam distilled solution of the volatile compounds. Only the sensitivities of the methods differ: Twister extraction or direct Twister desorption in the TDS system are more sensitive than direct steam distillation. In vivo feeding experiments with suitable precursors were used to differentiate between the classical cytosolic mevalonic acid pathway and the novel plastidic mevalonate-independent 1-deoxy-D-xylose 5-phosphate/2*C*-methyl-D-erythritol 4-phosphate pathway. To determine the biosynthetic origin of the basic building units, IPP and DMAPP [5,5-$^2H_2$]mevalonic acid lactone **22** and [5,5-$^2H_2$]-1-deoxy-D-xylose **23** were chosen for incorporation studies, which are frequently used for such purposes (*23*, *34*−*38*). In our in vivo feeding experiments aqueous solutions of the labeled precursors [5,5-$^2H_2$]-1-deoxy-D-xylose **23** and [5,5-$^2H_2$]mevalonic acid lactone **22** were administered to lilac inflorescences using

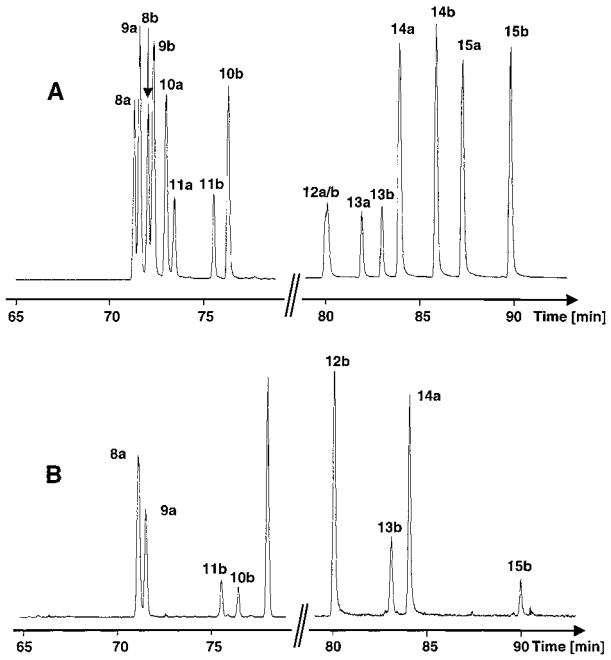

Figure 4. SBSE-enantio-MDGC/MS analysis of lilac aldehyde and lilac alcohol stereoisomers: (A) main column chromatogram of the reference solution, resulting in eight stereoisomers of lilac aldehyde and lilac alcohol, respectively; (B) main column chromatogram of the genuine lilac aldehyde **8a**, **9a**, **10b**, and **11b** and lilac alcohol **12b**, **13b**, **14a**, and **15b** stereoisomers in *S. vulgaris* L.

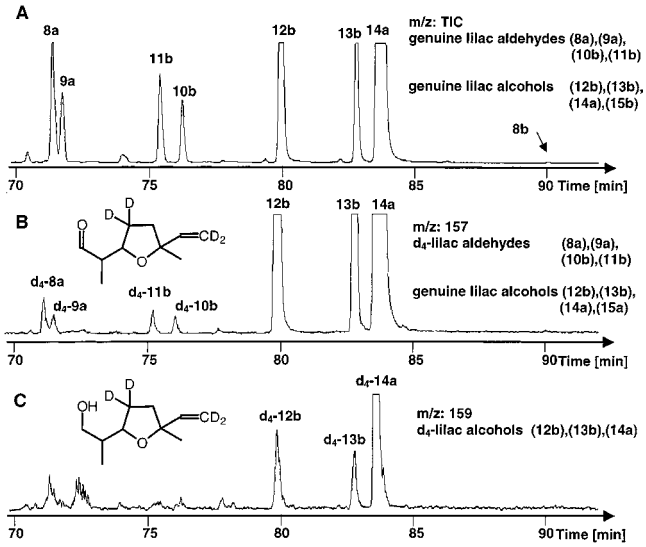

Figure 5. SBSE-enantio-MDGC/MS analysis of *S. vulgaris* L. fed with [5,5-$^2H_2$]-deoxy-D-xylose (DOX) **22** (main column chromatogram): (A) *m*/*z* TIC; (B) *m*/*z* 157; (C) *m*/*z* 159.

the cut stem method. **Figure 5** shows the main column chromatogram of lilac aldehydes and lilac alcohols obtained from the flower extract when [5,5-$^2H_2$]-1-deoxy-D-xylose **23** is administered. The four diastereoisomers (5′*S*)-[3′,3′,7′,7′-$^2H_4$]-lilac aldehyde and lilac alcohol are clearly detectable and well separated from the four genuine (5′*S*)-lilac aldehyde and lilac alcohol steroisomers, due to the inverse isotope effect in gas chromatography of deuterium-labeled compounds (*38*). Also, diastereoisomers of (5′*S*)-[3′,3′-$^2H_2$]lilac alcohols and (5′*S*)-[7′,7′-$^2H_2$]lilac alcohols are detectable in the mass spectra. The fragmentation patterns of the deuterium-labeled lilac alcohol and aldehyde isotopomers are in agreement with the incorporation of one or two units of labeled IPP/DMAPP that are generated from administered [5,5-$^2H_2$]-1-deoxy-D-xylose **23**, leading to d_2- and d_4-lilac alcohols, respectively. These results are in agreement with comparable studies of IPP incorporation from [5,5-$^2H_2$]-labeled 1-deoxyxylose **23** into linalool (*23*). When [5,5-$^2H_2$]mevalonic acid lactone **22** is administered to lilac inflorescences, incorporation into labeled lilac aldehydes and lilac alcohols was not detectable. This indicates that monoterpenes in *S. vulgaris* L. are not generated by the MVA pathway. The novel plasidic DOXP/MEP pathway is the dominant route for monoterpene biosynthesis in lilac inflorescences. Besides the feeding experiments that were carried out to clarify the biosynthetic origin of IPP/DMAPP, we also investigated the metabolism of labeled linalool and linalool derivatives leading to the formation of lilac alcohol. These in vivo feeding experiments were carried out with aqueous solutions of the labeled precursors (*R*/*S*)-[4,4,10,10,10-$^2H_5$]-linalool **3**, (*R*)-[8,8,8,9,9,9-$^2H_6$]linalool **21**, (*R*/*S*)-[5,5,10,10,10-$^2H_5$]-8-hydroxylinalool **6**, and (*R*/*S*)-[5,5,10,10,10-$^2H_5$]-8-oxolinalool **7**, administered to lilac influrescences using the cut stem method. **Figure 6** shows the enantioselective GC pattern of lilac aldehydes and lilac alcohols obtained from the flower extract when (*R*/*S*)-d_5-linalool **3** was administered. A significant incorporation of (*R*/*S*)-d_5-linalool **3** into lilac aldehydes and lilac alcohols is detectable for *m*/*z* 155 and 157, respectively. Surprisingly, (*R*/*S*)-d_5-linalool **3** was incorporated in all four diastereoisomeric enantiomeric pairs of lilac aldehyde and lilac alcohol. It is noteworthy that during incorporation of racemic deuterium-labeled linalool an enantiodiscrimination was observed. In particular, the enantiomeric pairs **13a**/**13b** and **10a**/**10b** show a discrimination in favor of the nongenuine 5′-(*R*)-configured enantiomers of **10a** and **13a**. Thus, it seems that the metabolism of linalool leading to the formation of lilac aldehyde and lilac alcohol exhibits a very low enantioselectivity. As a consequence, the enantiomeric purity of the genuine monoterpenes must result from a previous highly stereospecific enzymatic step. When enantiopure deuterium-labeled (*R*)-d_6-linalool **21** (R > 98%) was fed alternatively, only the four nongenuine 5′-(*R*)-configured lilac aldehyde and lilac alcohol stereoisomers were generated, corresponding to the absolute configuration at C-3 (*R*) of the precursor (**Figure 7**). Hence, a high enantiomeric purity of genuine linalool in *S. vulgaris* species with C-3 (*S*) configuration can be concluded, which leads to the genuine four 5′-(*S*)-configured diastereoisomers of both monoterpenes. Enantioselective analysis of genuine linalool in lilac confirms this supposition, as linalool shows a high enantiomeric purity in favor of the (*S*) enantiomer (>99%) (**Figure 8**). This is also in

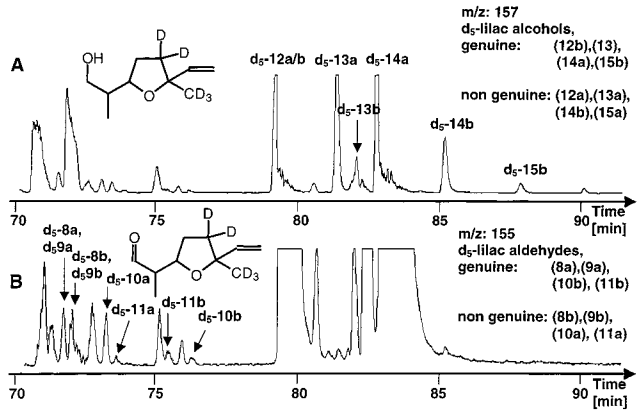

Figure 6. SBSE-enantio-MDGC/MS analysis of *S. vulgaris* L. fed with (*R*/*S*)-[4,4,10,10,10-$^2H_5$]-linalool **3** (main column chromatogram): (A) *m*/*z* 157; (B) *m*/*z* 155.

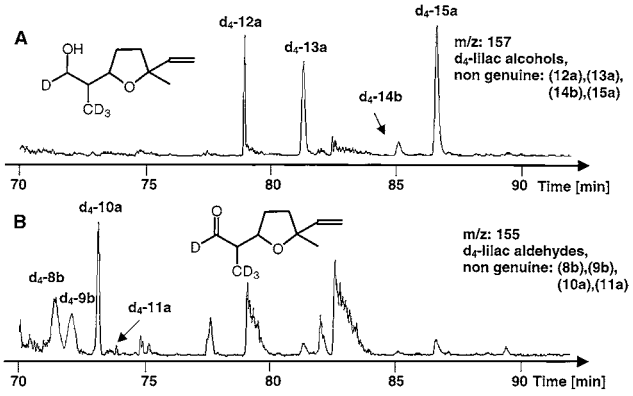

Figure 7. SBSE-enantio-MDGC/MS analysis of *S. vulgaris* L. fed with (*R*)-[8,8,8,9,9,9-$^2H_6$]-linalool **21** (main column chromatogram): (A) *m/z* 157; (B) *m/z* 155.

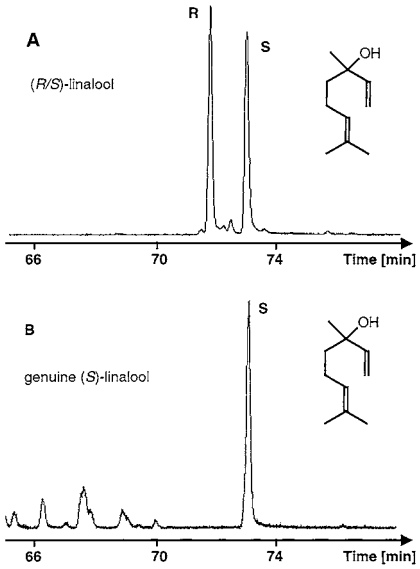

Figure 8. Enantio-MDGC/MS analysis of linalool: (A) main column chromatogram of a reference solution of (*R/S*)-linalool; (B) main column chromatogram of genuine linalool in *S. vulgaris* L.

agreement with the incorporation of deuterium-labeled 1-deoxy-D-xylose **23** into the corresponding genuine 5′-(*S*)-configured lilac aldehydes and lilac alcohols with high enantiomeric purity.

When racemic (*R/S*)-[5,5,10,10,10-$^2H_5$]-8-hydroxylinalool **6** was administered to lilac inflorescences, deuterated lilac aldehydes and lilac alcohols were clearly detectable. Enantioselective analysis of the deuterated monoterpenes shows that the four genuine and the four nongenuine lilac aldehyde stereoisomers were generated. Feeding experiments with (*R/S*)-[5,5,10,10,10-$^2H_5$]-8-oxolinalool **7** led to the same results. The deuterated monoterpene was incorporated into the four diastereoisomeric pairs of lilac aldehyde and lilac alcohol. Thus, the metabolism of this monoterpenol proceeds with remarkably low substrate specifity. When the eight stereoisomers of deuterium-labeled lilac alcohols **12**−**15** were administered to lilac inflorescences using the cut stem method, no incorporation into lilac aldehydes could be detected. Feeding experiments with the eight stereoisomers of deuterated d_5-lilac aldehydes **8**−**11** showed a significant incorporation into the eight deuterated lilac alcohol stereoisomers. Hence, it follows that lilac aldehyde is the biogenetic precursor of lilac alcohol. Considering these results, the biogenetic pathway of the four lilac alcohol stereoisomers starts with the biogenesis of IPP and DMAPP by the DOXP/MEP pathway, which leads to enantiopure (*S*)-linalool. (*S*)-Linalool must be enzymatically hydroxylated leading to 8-hydroxylinalool, which is subsequently oxidized to 8-oxolinalool. Cyclization of 8-oxolinalool leads to the four 5′-(*S*)-configured diasteroisomers of lilac aldehyde, which must be enzymatically reduced to the corresponding 5′-(*S*)-configured lilac alcohol stereoisomers. The biogenetic pathway is summarized in **Figure 9**. In addition to these investigations of the biogenetic pathways of lilac aldehydes and lilac alcohols, our interest was focused upon the location of biosynthesis in *S. vulgaris* L. To investigate the localization of the biosynthesis of monoterpenes in lilac, feeding experiments with detached flower petals, which were directly incubated in solutions of d_5-(*R/S*)-linalool **3** and experiments with green leaves using the cut stem method were carried out. It is noteworthy that the incubation experiment with flower petal leads to the same results as the feeding experiments with inflorescences as discussed previously, namely, enantiodiscrimination in favor of some nongenuine 5′-(*R*)-configured enantiomers. When d_5-(*R/S*)-linalool **3** was administered to green leaves using the cut stem method, no deuterated lilac aldehydes and lilac alcohols were detectable, although the genuine monoterpenes could be observed in green leaves. However, glycosylated monoterpenes cannot be detected with our method, but it is obvious that flower inflorescences and flower petals of *S. vulgaris* L. are capable of de novo biosynthesis of volatile monoterpenes.

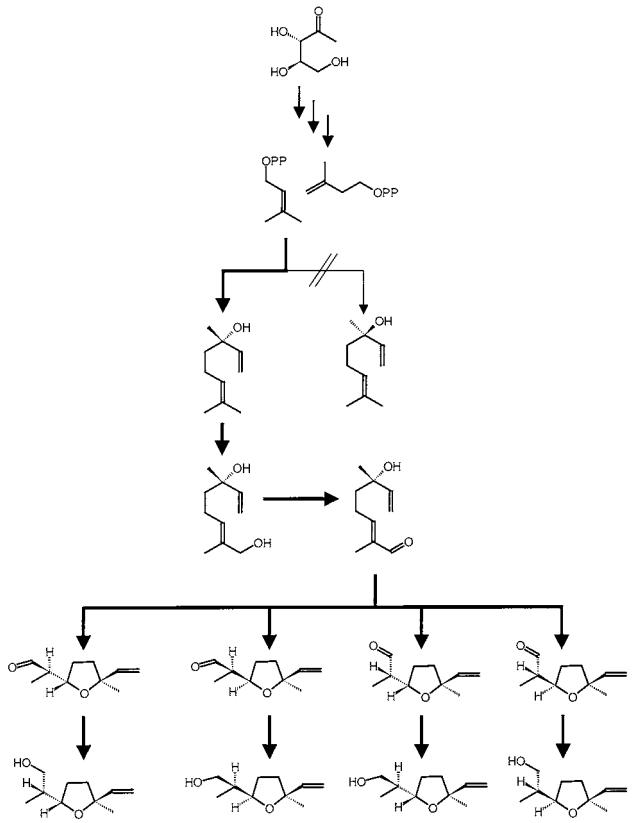

Figure 9. Biogenetic pathway of the monoterpenes lilac aldehyde and lilac alcohol in *S. vulgaris* L.

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