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Biogenetic Studies in *Syringa vulgaris* L.: Bioconversion of $^{18}O(^{2}H)$-Labeled Precursors into Lilac Aldehydes and Lilac Alcohols

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Syringa vulgaris L. inflorescences, petals, and chloroplasts, isolated from lilac flower petals, were fed with aqueous solutions of ^{18}O-labeled linalool and [5,5-$^{2}H_2$]-deoxy-D-xylose (DOX). The chloroplasts of lilac flower petals were isolated after feeding experiments with labeled precursors. Volatiles from the chloroplasts were extracted by stir bar sorptive extraction (SBSE) and analyzed using enantioselective multidimensional gas chromatography−mass spectrometry (enantio-MDGC-MS). Feeding experiments with DOX indicate that the novel mevalonate-independent 1-deoxy-D-xylose 5-phosphate/2C-methyl-D-erythritol 4-phosphate (DOX/MEP) is the decisive pathway of lilac aldehyde and lilac alcohol, respectively. Bioconversion of [^{18}O]linalool into lilac aldehyde and lilac alcohol during in vivo feeding experiments was monitored, and the metabolic pathways are discussed.

KEYWORDS: ***Syringa vulgaris* L. biosynthesis; stir bar sorptive extraction (SBSE); enantioselective multidimensional gas chromatography−mass spectrometry (enantio-MDGC-MS); $^{18}O(^{2}H)$-labeled precursors**

INTRODUCTION

Lilac aldehydes and lilac alcohols have been described as characteristic monoterpenoids in *Syringa vulgaris* L. flowers with a desirable influence on the lilac flavor quality (*1*). Feeding experiments with aqueous solutions of deuterium-labeled monoterpenoids followed by enantioselective multidimensional gas chromatography-mas spectrometry (enantio-MDGC-MS) analysis have proved to be efficient methods for in vivo studies of biogenesis (**Chart 1**).

Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are the two basic precursors of monoterpenoids (*2*−*6*). Besides the classical mevalonate route, a mevalonate-independent pathway for the formation of IPP and DMAPP was recently discovered in bacteria, algae, and plants. The biosynthesis of several monoterpenes in plants via the 1-deoxy-D-xylose 5-phosphate/2C-methyl-D-erythritol 4-phosphate (DOX/MEP) pathway was investigated by Eisenreich et al. (*7*) for the first time.

Kreck et al. (*8*) investigated the biogenesis of lilac aldehyde and lilac alcohol in lilac using [5,5-$^{2}H_2$]mevalolactone (d_2-MVL) or [5,5-$^{2}H_2$]-1-deoxyxylulose (d_2-DOX) as indicative precursor molecules for the classic MVA and the novel DOX/MEP pathway. Although incorporation of d_2-MVL could not be detected, d_2-DOX was proved to be decisive for monoterpene biosynthesis in lilac inflorescences, leading to enantiopure (*S*)-linalool. (*S*)-Linalool must be enzymatically hydroxylated, leading to 8-hydroxylinalool, and subsequently oxidized to 8-oxolinalool. Cyclization of 8-oxolinalool exclusively leads to the four genuine (5′*S*)-configured diasteroisomers of lilac aldehyde, which are enzymatically reduced to the corresponding (5′*S*)-configured lilac alcohol diastereoisomers (*8, 9*) (**Scheme 1**).

This paper deals with feeding experiments with ^{18}O-labeled linalool, to clarify the cyclization mechanism of 8-oxolinalool to lilac aldehyde stereoisomers.

Furthermore, the location of de novo biosynthesis inside the cells of flower petals should be studied in more detail. For that reason the chloroplasts were isolated. Flower petals, which were either detached from inflorescences after feeding with suitable precursors or first detached from inflorescenses and subsequently incubated with solutions of suitable precursors, were investigated. Moreover, feeding experiments with isolated chloroplasts were carried out.

The volatiles of isolated chloroplasts were analyzed by stir bar sorptive extraction (SBSE) and enantio-MDGC-MS, allowing the simultaneous detection of genuine and labeled monoterpenoids.

MATERIALS AND METHODS

Synthesis of ^{18}O-Labeled (*R/S*)-Linalool. *Synthesis of [^{18}O]-6-Methyl-5-hepten-2-one (2).* The method of Croteau et al. (*10*) was applied. 6-Methyl-5-hepten-2-one **(1)** (39.7 mmol, 5 g) was treated with 6 g (40.5 mmol) of freshly distilled triethyl orthoformate in 6 mL of ethanol in the presence of 10 μL of concentrated sulfuric acid for 18 h at room temperature. The reaction was then heated at 60 °C for 4.5

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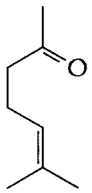
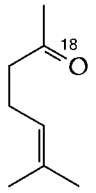
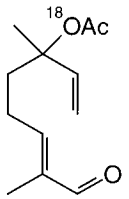
Chart 1. Starting Material (**1**) and Intermediates (**2**, **5**) Used in the Synthesis of Precursors **3** and **14** and Target Molecules (**6a**,**b**–**13a**,**b**) Investigated in Biogenetic Studies with *S. vulgaris* L.



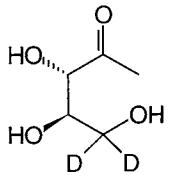
6-methyl-5-heptene-2-one (1)



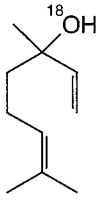
[^{18}O]-6-methyl-5-heptene-2-one (2)



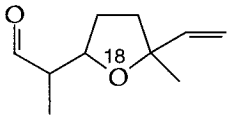
(R,S)-2,6-dimethyl-[6-^{18}O]-6-acetoxy-2,7-octadienal (5)



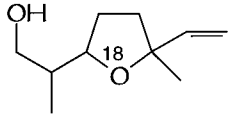
d_2-DOX (14)



(R,S)-[3-^{18}O]-linalool (3)



[1′-^{18}O]-lilac aldehyde (6a/b - 9a/b)



[1′-^{18}O]-lilac alcohol (10a/b - 13a/b)

h, quenched with 3 mL of ammonium hydroxide, and extracted with 60 mL of diethyl ether. After removal of the solvent, the residue was distilled under reduced pressure [bp (8 mm) 83−85 °C]; yield of the diethyl ketal = 6.5 g (82%). The ketal was hydrolyzed with 150 μL of $H_2[^{18}O]$ (99% ^{18}O atom) and 5 mg of *p*-toluensulfonic acid in 20 mL of methylene chloride for 0.5 h at room temperature. Solid anhydrous Na_2CO_3 was added to the reaction mixture to neutralize the acid, the solid was removed by filtration, using a hydrophob folded filter, and the solvent was removed under vacuum to yield 3.5 g (84%) of [^{18}O]-6-methyl-5-hepten-2-one (**2**): MS, *m*/*z* 129 (9, M^+), 109 (39), 108 (37), 93 (100), 67 (41), 55 (30).

Synthesis of ^{18}O-Labeled (R/S)-Linalool (3). Two grams (15.6 mmol) of the labeled ketone (**2**) was dissolved in 3 mL of THF at 0 °C, and 20 mL (20 mmol) of 1 M vinylmagnesium bromide was added dropwise. After 15 min at 0 °C, the solution was stirred for 1 h at room temperature. Excess vinylmagnesium bromide was destroyed by the addition of 5 mL of methanol followed by 5 mL of 50% saturated NH_4Cl. Extraction with 50 mL of diethyl ether, washing with 25 mL of saturated $KHCO_3$, and flash chromatography of the dried and concentrated extract (column i.d. = 20 mm; 20 g of silica gel 60, 220−440 mesh; diethyl ether/hexane, 1:9 v/v) yielded 1.8 g of [^{18}O]linalool (**3**) (74%): MS, *m*/*z* 155 (1, M^+), 121 (18), 93 (63), 91 (100), 79 (69), 67 (38).

Synthesis of ^{18}O-Labeled (*R*/*S*)-8-Oxolinalyl Acetate (5). *Synthesis of (R/S)-[3-^{18}O]-3-Acetoxy-3,7-dimethyl-1,6-octadiene (4).* According to the method of Vidari et al. (*11*) 2.14 mmol (218 mg) of Ac_2O and 0.053 mmol (6.5 mg) of DMAP were added to a solution of 1.60 mmol (250 mg) of **3** in 25.3 mmol (2 mL) of pyridine. The reaction mixture was stirred at 90 °C for 44 h and then quenched by adding 3 mL of methanol and 5 mL of aqueous $NaHSO_4$. The aqueous layer was extracted three times with 20 mL of CH_2Cl_2, and the combined organic layers were dried over sodium sulfate. Flash chromatography of the concentrated extract (column i.d. = 20 mm; 20 g of silica gel 60, 220−440 mesh; diethyl ether/hexane, 1:9 v/v) yielded 297 mg (94%) of **4**: MS, *m*/*z* 198 (1, M^+), 121 (10), 93 (50), 91 (100), 79 (50), 67 (20).

Synthesis of (R/S)-2,6-Dimethyl-[6-^{18}O]-6-acetoxy-2,7-octadienal (5). The method of Wakayama et al. (*1*) was used. Compound **4** (1.5 mmol, 297 mg) and selenium dioxide (1.5 mmol, 166 mg) 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 60 mL of diethyl ether/petroleum ether 1:1 (v/v), and after removal of the solvent, the residue was purified by flash chromatography (column i.d. = 20 mm; 20 g of silica gel 60, 220−440 mesh; petroleum ether/diethyl ether, 3:2 v/v), affording 202 mg (64%) of compound **5**: MS, *m*/*z* 212 (1, M^+), 121 (21), 93 (38), 91 (100), 79 (77), 67 (31).

Synthesis of [1′-^{18}O]-Labeled (*R*/*S*)-Lilac Aldehyde. *Synthesis of (R/S)-2-[1′-^{18}O-(5′-Methyl-5′-vinyl)]tetrahydrofuran-2′-yl]propanal, [1′-^{18}O]Lilac Aldehyde (6a/b−9a/b).* The method of Kreck et al. (*8*) was applied. In dry methanol (30 mL) were dissolved catalytic amounts of sodium, and compound **5** (0.94 mmol, 200 mg) was added dropwise. After 48 h of stirring under an argon atmosphere at room temperature, 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 by using a Vigreux column, and the residue was purified by preparative thin-layer chromatography; conditions were as follows: PLC plates 20 × 20 cm silica gel 60 F_{254} 2 mm; mobile phase petroleum ether/diethyl ether 1:4 (v/v); 17 cm development distance; chamber saturation, affording 12 mg (7.5%) of compounds **6a/b**−**9a/b**: MS 170 (3, M^+), 155 (7), 127 (7), 113 (12), 93 (55), 91 (100), 79 (72).

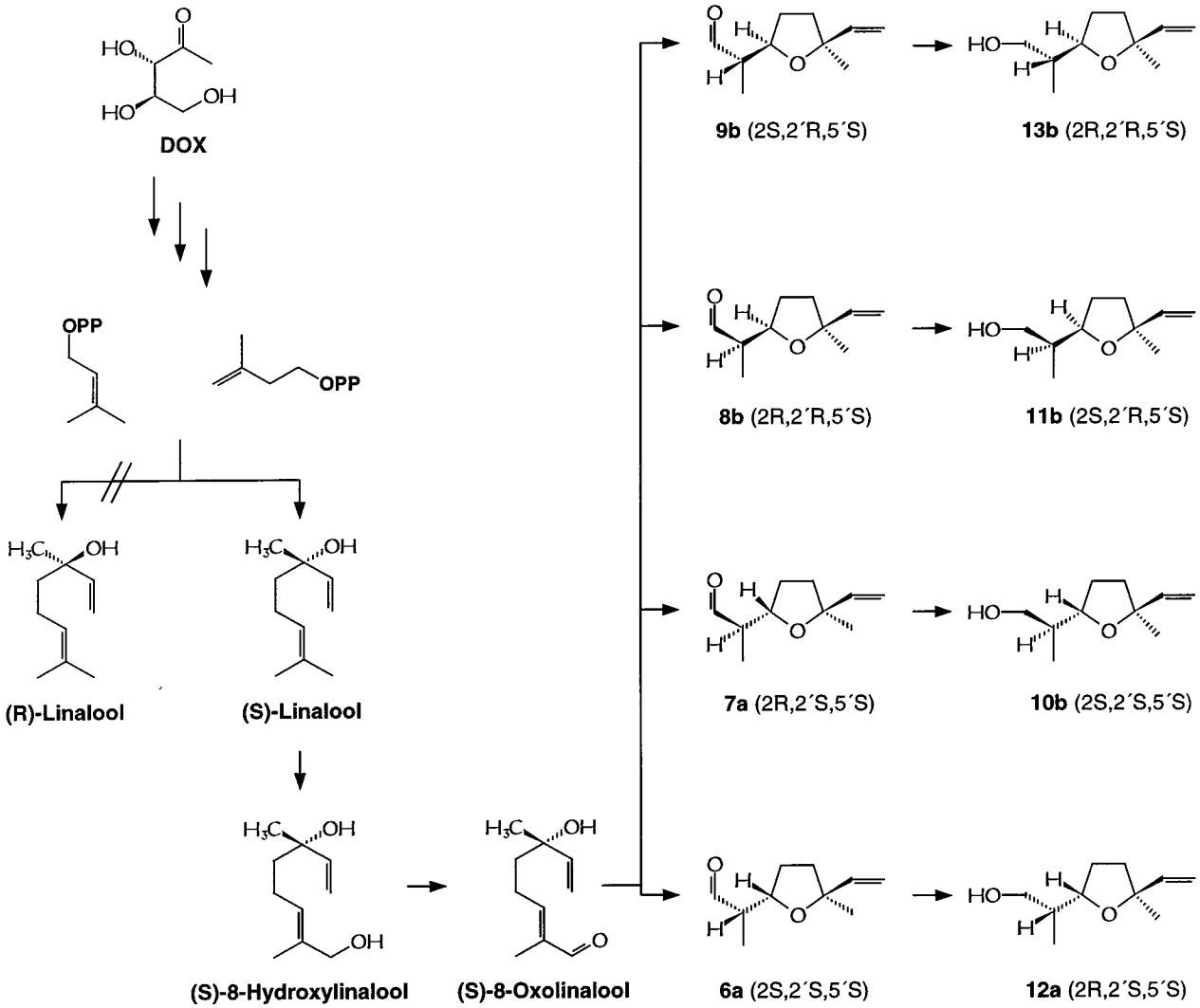
Synthesis of [1′-^{18}O]-Labeled (*R*/*S*)-Lilac Alcohol. *Synthesis of (R/S)-2-[1′-^{18}O-(5′-Methyl-5′vinyl)]tetrahydrofuran-2′-yl]propanol,[1′-^{18}O]-Lilac Alcohol (10a/b−13a/b).* The method of Kreck et al. (*8*) was applied. Compounds **6a/b**−**9a/b** (0.035 mmol, 6.0 mg) were dissolved in 10 mL of dry diethyl ether, and 3 mg (0.078 mmol) of lithium aluminum hydride was added in small portions. After 2 h of stirring at room temperature, 5 mL of water was added, and the reaction mixture was acidified with sulfuric acid and extracted with 60 mL 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 purified by preparative thin-layer chromatography; conditions were as follows: PLC plates 20 × 20 cm silica gel 60 F_{254} 2 mm; mobile phase petroleum ether/diethyl ether 1:1 (v/v); 17 cm development distance; chamber saturation, affording 0.02 mmol (4 mg) of **10a/b**−**13a/b**: MS 172 (2, M^+), 157 (4), 127 (9), 113 (11), 93 (100), 91 (98), 79 (42).

Synthesis of [5,5-$^2H_2$]-1-Deoxy-D-xylose (14). [5,5-$^2H_2$]-1-Deoxy-D-xylose was prepared according to the method of Jux and Boland (*12*).

Plant Material. While the lilac bushes were in blossom, cuttings from wild and domesticated bushes of *S. vulgaris* L. with different colored flowers were available. The inflorescences were 10−15 cm high, containing 5 cm long pedicles (~10 g of plant material).

Feeding Experiments. [^{18}O]-(*R*/*S*)-Linalool was dissolved in distilled water containing 0.1 mg/mL Tween 20 to get a solution of 0.1 mg/mL of the monoterpene. d_2-DOX was dissolved in distilled water to a concentration of 2 mg/mL. The inflorescences were cut off and put into the feeding solutions. In any case, blank experiments were carried out with distilled water containing 0.1 mg/mL Tween 20. During a

Scheme 1. Biogenetic Pathway of the Monoterpenoids Lilac Aldehyde and Lilac Alcohol in *S. vulgaris* L. and Absolute Configurations of the Genuine (5′*S*)-Configured Lilac Aldehyde and Lilac Alcohol Stereoisomers



period of 48 h, 3 μmol of the precursor was taken up by the plant. Furthermore, isolated lilac flower petals and isolated chloroplasts from lilac flower petals were incubated directly with a solution containing 0.1 mg/mL [^{18}O]-(*R/S*)-linalool and 0.1 mg/mL Tween 20 and with a solution containing 2 mg/mL d_2-DOX, respectively.

Isolation of Chloroplasts from Lilac Flower Petals. Chloroplasts were isolated as described by Gruissem et al. (*13*) from 150 g of lilac flower petals. The petals were divided into 50 g portions. The following procedures were carried out at 0−4 °C. Batches of lilac flower petals were homogenized in a blender in 150 mL of grinding solution [grinding solution (1× GM mix): 5× GM mix; 5 mM $Na_4P_2O_7$, 250 mM HEPES, 1.65 M sorbitol, 10 mM EDTA, 5 mM $MgCl_2$, 5 mM $MnCl_2$, adjusted to pH 6.8 with NaOH; diluted with water to 1× GM mix before use and with DTT added to a final concentration of 10 mM] for 5 s and filtered through four layers of Miracloth (Calbiochem).

The filtrate was centrifuged for 15 min in an SW-28 rotor at 5000*g*, and the resulting pellet was resuspended in ∼3 mL of the grinding solution. The resuspended chloroplast solution was loaded onto a Percoll step gradient. [Percoll step gradients: 20 mL of a 40% (v/v) Percoll solution was layered on 7.5 mL of 70% (v/v) Percoll solution. Percoll−bovine serum albumin−Ficoll solution (PCBF): 100 mL of Percoll, 3 g of polyethylene glycol (molecular weight 8000), 1 g of bovine serum albumin (BSA), 1 g of Ficoll. Percoll solution (40%, v/v): 20 mL of 5× GM mix, 6 mg of reduced glutathione, 1 mL of 1 M DTT, 40 mL of PCBF, H_2O to 100 mL. Percoll solution (70%, v/v): 20 mL of 5× GM mix, 6 mg of reduced glutathione, 1 mL of 1 M DTT, 70 mL of PCBF, H_2O to 100 mL.]

The solution was centrifuged at 8000*g* for 25 min at 4 °C in an SW-28 rotor. The chloroplasts that accumulate at the 40−70% interphase were fractionated and purified by a second Percoll step gradient centrifugation as described above. To remove the Percoll, the repurified chloroplasts were washed three times with grinding solution.

Isolation of Monoterpenoids. Monoterpenoids were extracted using the novel SBSE method. Theory and practice have been described recently (*14*). Stir bars (film thickness = 0.5 mm; length = 10 mm) were obtained from Gerstel (Mühlheim, Germany) under the trade name Twister. The lilac flower petals were isolated from the inflorescences. The chloroplasts from lilac flower petals were isolated as described above. The chloroplasts 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. The Twister was desorbed in the TDS system and subsequently analyzed by enantio-SBSE-MDGC-MS analysis of the volatile compounds.

Gas Chromatography—Mass Spectrometry (GC-MS). GC-MS analysis of the synthesized monoterpenoids was carried out with a Fisons Instrument GC 8065, coupled to a Fisons Instruments MD800 mass spectrometer, equipped with a self-prepared fused silica capillary coated with SE-52 (30 m × 0.25 mm i.d., film thickness = 0.23 μm).

Conditions were as follows: carrier gas, helium, 65 kPa; injection volume, 1 μL; 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.

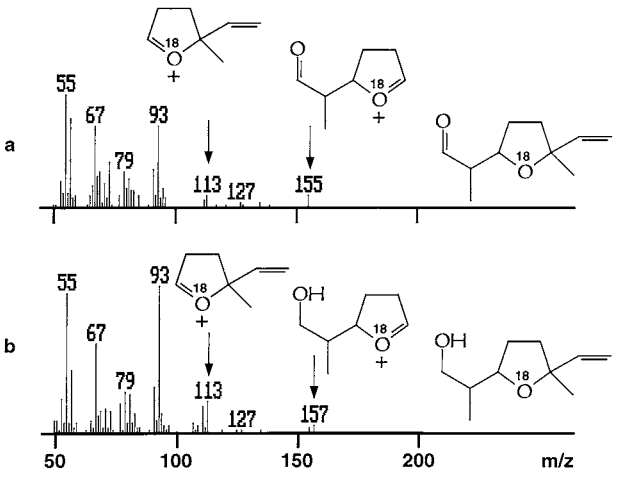


Figure 1. SBSE-enantio-MDGC-MS pattern of ^{18}O-labeled target molecules lilac aldehyde **(a)** and lilac alcohol **(b)**.

The molecular ion (M^+) and the fragmentation ions were given as *m*/*z* with relative peak intensities to the base peak (percent).

Enantio-SBSE-MDGC-MS. The enantio-SBSE-MDGC-MS consists of a Gerstel TDS thermal desorption system, mounted on a Siemens SiChromat 2, with two independent column oven programs and a live T-switching device, coupled to the transfer line of a Finnigan MAT ITD 800, using an open split interface. For thermal desorption the following conditions were applied: desorption temperature program, 10 °C at 60 °C/min to 250 °C, 2 min isothermal (6.0 min); flow mode TDS, splitless; transfer line temperature set at 250 °C. A Gerstel CIS-3 PTV injector was used for crygenic focusing of the released analytes.

The PTV was cooled to −150 °C using liquid nitrogen. The PTV was programmed from −150 °C at 12 °C/s to 250 °C, 1 min isothermal (1.5 min). Flow mode CAS was splitless (1 min). The liner was filled with Tenax TA (Alltech, Deerfield, IL). GC conditions were as follows: precolumn, self-prepared fused silica capillary (30 m × 0.23 mm i.d.), coated with a 0.23 μm film of SE-52; carrier gas, helium, 1.85 bar; detector, FID, 250 °C. Main column: self-prepared fused silica capillary (30 m × 0.23 mm i.d.), coated with a 0.23 μm film of 4‰ heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DIME-β-CD) (30%) in SE-52 (70%); carrier gas, helium, 1.45 bar; detector, ITD 800; transfer line temperature, 250 °C; open split interface, 250 °C; helium sweeping flow, 1 mL/min; ion trap manifold, 200 °C; EI, 70 eV; oven temperature program, precolumn, 60 °C (5 min isothermal), raised at 3 °C/min to 250 °C (30 min isothermal); main column temperature program, 60 °C (25 min isothermal), raised at 1.5 °C/min to 200 °C; cut times, lilac aldehyde, 26.5−28.0 min; lilac alcohol, 29.5−32.5 min.

RESULTS AND DISCUSSION

In previous studies Kreck et al. reported feeding experiments with aqueous solutions of ^{2}H-labeled precursors. This technique has proved to be highly efficient for in vivo studies of biogenesis. Consequently, this paper reports on extended in vivo studies of biogenesis using aqueous solutions of the precursor ^{18}O-labeled (*R*/*S*)-linalool in order to clarify the cyclization mechanism of 8-oxolinalool to lilac aldehyde stereoisomers. Furthermore, the location of the novel mevalonate-independent DOX/MEP pathway inside the plant cells of lilac flower petals was investigated for the first time.

Feeding experiments showed that [3-^{18}O]-(*R*/*S*)-linalool (**3**) is transferred to [1′-^{18}O]lilac aldehyde.

Figure 1 shows the SBSE-enantio-MDGC-MS pattern of lilac aldehydes and lilac alcohols obtained from chloroplasts from flower petals when [3-^{18}O]-(*R*/*S*)-linalool **(3)** was administered. A significant incorporation of [^{18}O]-(*R*/*S*)-linalool **(3)** into lilac aldehydes and lilac alcohols is detectable so that the fragment

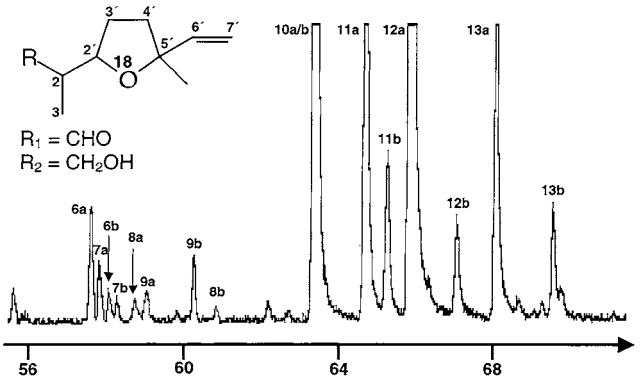


Figure 2. SBSE-enantio-MDGC-MS analysis of *S. vulgaris* L., fed with (*R*/*S*)-[3-^{18}O]-linalool (main column chromatogram; *m*/*z* 93): **6**–**9**, lilac aldehyde diastereomers; **10**–**13**, lilac alcohol diastereomers; **a/b**, enantiomers [**6a** (2*S*,2′*S*,5′*S*), **6b** (2*R*,2′*R*,5′*R*), **7a** (2*R*,2′*S*,5′*S*), **7b** (2*S*,2′*R*,5′*R*), **8a** (2*S*,2′*S*,5′*R*), **8b** (2*R*,2′*R*,5′*S*), **9a** (2*R*,2′*S*,5′*R*), **9b** (2*S*,2′*R*,5′*S*), **10a** (2*R*,2′*R*,5′*R*), **10b** (2*S*,2′*S*,5′*S*), **11a** (2*R*,2′*S*,5′*R*), **11b** (2*S*,2′*R*,5′*S*), **12a** (2*R*,2′*S*,5′*S*), **12b** (2*S*,2′*R*,5′*R*), **13a** (2*S*,2′*S*,5′*R*), **13b** (2*R*,2′*R*,5′*S*)].

m/*z* 153 of unlabeled lilac aldehyde was shifted to *m*/*z* 155 and the fragment *m*/*z* 155 of unlabeled lilac alcohol was shifted to *m*/*z* 157. Furthermore, the fragment of the corresponding unlabeled lilac alcohol and lilac aldehyde (*m*/*z* 111) was shifted to the higher ratio (*m*/*z* 113) (see **Figure 1**). In view of the cyclization mechanism, there is no doubt the furanoid ring oxygen of the target molecules is delivered by the alcoholic function of the precursor linalool. In conjunction with earlier findings (*8*, *9*) some remarkable facts were detected, proving the enzyme catalyzed cyclization of the intermediate linalool derivative to yield lilac aldehyde and lilac alcohol stereoisomers: (*S*)-linalool, the fundamental precursor of lilac derivatives, is biosynthesized enantiospecifically, exclusively yielding (5′*S*)-configured genuine stereoisomers. In feeding experiments with ^{2}H(^{18}O)-labeled racemates of linalool and other precursors, enantiodiscrimination effects were significant (see **Figure 2**).

In feeding experiments with labeled (*R*)-linalool exclusively (5′*R*)-configured stereoisomers were detected, proving cyclization without any plant cell induced racemization effect on linalool. In conclusion, the cyclization mechanism is controlled by still unknown enzymes. Whereas the generation of (*S*)-linalool occurs as an enantiospecific reaction, the enantioselectivity of all other cyclization steps seems to be rather low.

Furthermore, in vivo feeding experiments using aqueous solutions of the precursor DOX **(14)** were administered to *S. vulgaris* L. to locate the DOX/MEP pathway inside the plant cells of lilac flower petals.

Three different methods of feeding experiments were carried out. In the first case lilac inflorescences were cut off and put into the feeding solution. After the incubation time, the flower petals were detached and the chloroplasts were isolated. In the second case the flower petals were detached from the inflorescences and then incubated directly with the feeding solution, and after that, the chloroplasts were isolated. Furthermore, the chloroplasts were isolated first and incubated directly with the feeding solution.

In the first two cases deuterium-labeled lilac aldehydes and lilac alcohols during the enantio-MDGC-MS analysis were observed.

Figure 3 shows the main column chromatogram of lilac aldehyde and lilac alcohol stereoisomers after feeding with DOX **(14)**. The incorporation of one or two units of labeled IPP/DMAPP that are generated from administered **14** leads to d_2-

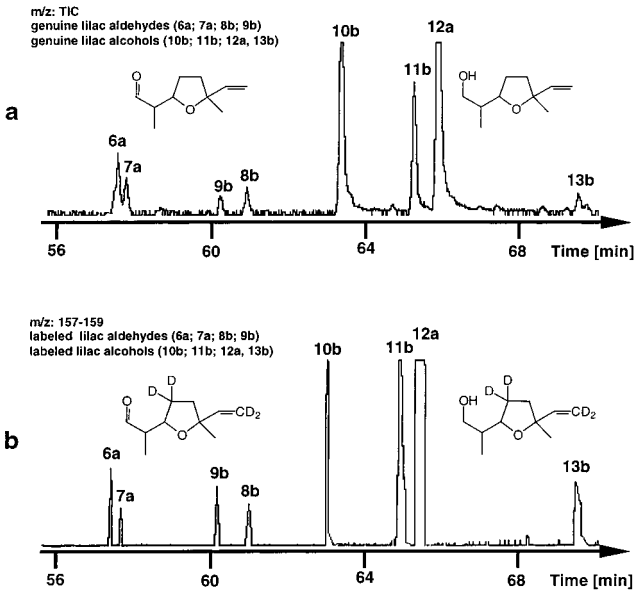


Figure 3. SBSE-enantio-MDGC-MS analysis of *S. vulgaris* L., fed with [5,5-$^2H_2$]-deoxy-D-xylose [DOX] (main column chromatogram): **(a)** *m/z* TIC [distribution of the genuine lilac aldehyde and lilac alcohol stereoisomers (area %) **6a/7a/9b/8b** (40/26/20/14); **10b/11b/12a/13b** (20/16/59/9)]; **(b)** *m/z* 157–159 [distribution of the d_4-labeled lilac aldehyde and d_4-lilac alcohol stereoisomers (area %) **6a/7a/9b/8b** (40/22/24/13); **10b/11b/12a/13b** (17/14/63/5)].

and d_4-lilac aldehyde and lilac alcohol with characteristic distribution of the stereoisomers. In **Figure 3** d_4-isotopomers of lilac aldehyde and lilac alcohol are depicted. The fragmentation patterns of d_4-labeled lilac aldehyde and d_4-labeled lilac alcohol are in accordance with the findings of Kreck et al. (*8*). In the third case only the unlabeled structures of lilac aldehydes and lilac alcohols were detectable. These results demonstrate that lilac aldehydes and lilac alcohols are accumulated in the chloroplasts of lilac flower petal cells, and it is highly probable that the enzymes, which are involved in monoterpene metabolism based on the DOXP/MEP route, are located in the chloroplasts. The failure in monoterpenoid biosynthesis using isolated chloroplasts may be due to deactivation of the enzymes, which are involved in monoterpene metabolism during isolation. Final knowledge will be achieved by further investigations.

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LITERATURE CITED

(1) Wakayama, S.; Namba, S.; Hosoi, K.; Ohno, M. The Synthesis and the Absolute Configurations of Lilac Alcohols, New Naturally Occurring Odours Ingredients of Lilac Flower. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3183−3187.

(2) Watanabe, N.; Watanabe, S.; Nakajima, R.; Moon, J.; Shimokihira, K.; Inagaki, J.; Etoh, H.; Asai, T.; Sakata, K.; Ina, K. Formation of flower fragrance compounds from their precursors by enzymatic action during flowering opening. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 1101−1106.

(3) Lichtenthaler, H. K.; Rohmer, M.; Schwender, J. Two independent biochemical pathways for isopentenyl diphosphate (IPP) and isoprenoid biosynthesis in higher plants. *J. Physiol. Plant.* **1997**, *101*, 643−652.

(4) Lichtenthaler, H. K. The 1-deoxy-D-xylose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1999**, *50*, 47−65.

(5) Lichtenthaler, H. K. Non-mevalonate isoprenoid biosynthesis: enzymes, genes and inhibitors. *Biochem. Soc. Trans*. **2000**, *28*, 785−789.

(6) Luan, F.; Wüst, M. Differential incorporation of 1-deoxy-D-xylose into (3*S*)-linalool and geraniol in grape berry exocarp and mesocarp. *Phytochemistry* **2002**, *60*, 451−459.

(7) Eisenreich, W.; Sagner, S.; Zenk, M. H.; Bacher, A. Monoterpenoid essential oils are not of mevalonoid origin. *Tetrahedron Lett*. **1997**, *38*, 3889−3892.

(8) Kreck, M.; Püschel, S.; Wüst, M.; Mosandl, A. Biogenetic Studies in *Syringa vulgaris* L.: Synthesis of Deuterium-Labeled Precursors into Lilac Aldehydes and Lilac Alcohols. *J. Agric. Food Chem.* **2003**, *51*, 463−469.

(9) Kreck, M. Natürliche Duft- und Aromastoffe − Authentizitätsbewertung und Biogenesestudien mit neuen online-Methoden. Thesis, J. W. Goethe University, Frankfurt a. M., Germany, 2003.

(10) Croteau, R. B.; Shaskus, J. J.; Renstom, B.; Felton, M. N. Mechanism of the Pyrophosphate Migration in the Enzymatic Cyclization of Geranyl and Linalyl Pyrophosphates to (+)- and (−)-Bornyl Pyrophosphates. *Biochemistry* **1985**, *24*, 7077−7085.

(11) Vidari, G.; Di Rosa, A.; Zanoni, G.; Bicchi, C. Enantioselective synthesis of each stereoisomer of the pyranoid linalool oxides: the linalool route. *Tetrahedron: Asymmetry* **1999**, *10*, 3547−3557.

(12) Jux, A.; Boland, W. Improved protocol towards isotopically labelled 1-deoxy-D-xylose. *Tetrahedron Lett*. **1999**, *40*, 6913−6914.

(13) Gruissem, W.; Greenberg, B. M.; Zurawski, G.; Hallick, R. B. Chloroplast Gene Expression and Promoter Identification in Chloroplast Extracts. *Methods Enzymol*. **1986**, *118*, 253−270.

(14) Baltussen, E.; Sandra, P.; David, F.; Cramers, C. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *J. Microcolumn Sep*. **1999**, 737−747.

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