Structure Elucidation, Enantioselective Analysis, and Biogenesis of Nerol Oxide in *Pelargonium* Species

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A novel enantioselective synthesis of nerol oxide (3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-pyran) was used for the determination of the absolute configuration at C-2. The order of elution of the enantiomers on octakis-(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin in OV 1701-vi as the chiral stationary phase in enantioselective GC was determined as (2*R*) before (2*S*). Enantioselective multidimensional GC/MS (enantio-MDGC/MS) was used for the determination of the enantiomeric ratios of nerol oxide in different geranium oils. As a result, in all investigated oils nerol oxide occurs as a racemate. The biogenesis of nerol oxide in *Pelargonium* species was investigated by feeding experiments using deuterium-labeled neryl glucoside as the precursor. The *Pelargonium* plants were able to convert the fed precursor into racemic nerol oxide, which has to be considered as a "natural racemate".

Keywords: *Pelargonium graveolens; Geraniaceae; stable isotope labeling; nerol oxide; biogenesis; geranium oil*

INTRODUCTION

Nerol oxide (3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-pyran, **9a**/**b**, see Figure 1) is a chiral monoterpenoid ether. It was isolated from Bulgarian rose oil in 1965 by E. sz. Kováts (Ohloff et al., 1980) and is nowadays a valuable base material in perfumery. In 1974, nerol oxide was detected in wine from several grape varieties where it contributes to the unique flavor (Schreier and Drawert, 1974). Since that time, nerol oxide has been detected in numerous plants as in *Pelargonium* species, which are used for the production of the so-called geranium oil. The olfactory properties of the enantiomers were investigated by an enantioselective synthesis (Ohloff et al., 1980). The first enantiomer separation of nerol oxide was performed by complexation GC (Schurig, 1987). In 1993, nerol oxide enantiomers were separated using a modified cyclodextrin derivative as the chiral stationary phase (Lindström et al., 1993). However, the elution order of nerol oxide enantiomers on modified cyclodextrin phases is still unknown. Moreover, biogenetic studies on the biogenesis of nerol oxide using labeled precursors have not been performed yet. Hence, this paper deals with a novel enantioselective synthesis of nerol oxide, the elucidation of the absolute configuration of its optical antipodes, the investigation of its biogenesis in *Pelargonium* plants using deuterium-labeled precursors, and its enantioselective analysis in different geranium oils.

EXPERIMENTAL PROCEDURES

Plant Material. Young plants of *Pelargonium graveolens* L'Héritier and an unknown *Pelargonium* hybrid were kindly provided by Gartenbau Stegmeier, Essingen, Germany.

Essential Oils. Commercially available samples of geranium oils were purchased from the German fragrance market. Authentic samples were self-prepared oils from *Pelargonium graveolens* and an unknown *Pelargonium* hybrid (see above). The oils were isolated using steam distillation (officinal apparatus DAB 9).

Reference Compounds. *cis*-(2*R*,4*S*)/*trans*-(2*S*,4*S*)-Rose oxides (4-methyl-2-(2-methyl-1-propenyl)-tetrahydropyran, enantioselective GC: ee = 65%, *cis*/*trans* = 75/25) were obtained from Dragoco, Holzminden, Germany. Nerol oxide (3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-pyran, **9a**/**b**) was obtained from Roth, Karlsruhe, Germany.

^{1}H NMR. The spectra were recorded on a Bruker AMX 500 or ARX 300, $CDCl_3$/TMS. NMR assignments were clarified by ^{1}H/^{1}H−COSY.

Enantio-MDGC-MS. Enantio-MDGC-MS (enantio-multidimensional gas chromatography−mass spectrometry) analysis of the SPME (solid-phase microextraction) headspace extracts, synthetic products, essential oils, and reference compounds was performed with a Siemens SICHROMAT 2, equipped with 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; transfer line 250 °C; open split interface 250 °C; helium sweeping flow 1 mL/min; ion trap manifold 230 °C; EI 70 eV. The reference compounds and synthetic products were analyzed in full scan mode (40−250 amu).

*Column Combination for the Separation of cis-/trans-Rose Oxide (**10**,**11**).* Precolumn conditions: Duran glass capillary (28 m × 0.23 mm) coated with a 0.23 μm film of PS-268; carrier gas hydrogen 125 kPa; split 25 mL/min; injector temperature 220 °C; detector FID 250 °C; oven temperature 110 °C (60 min isothermal), then 2 °C/min to 250 °C. Main column conditions: Duran glass capillary (30 m × 0.23 mm), coated with a 0.23 μm film of 50% heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS-268 polysiloxane derivative, column was self-prepared according to Dietrich et al. (1995); carrier gas hydrogen at 100 kPa; oven temperature 65 °C (20 min isothermal), then 1 °C/min to 150 °C; live-T-cutting 11.000−13.000 min.

*Column Combination for the Separation of Nerol Oxide (**9a**/**b**).* Precolumn conditions: Duran glass capillary (30 m × 0.23 mm), coated with a 0.23 μm film of SE-52; carrier gas hydrogen at 121 kPa; split 25 mL/min; injector temperature 220 °C; detector FID 250 °C; oven temperature 60 °C (5 min isothermal), then 5 °C/min to 250 °C. Main column conditions: Duran

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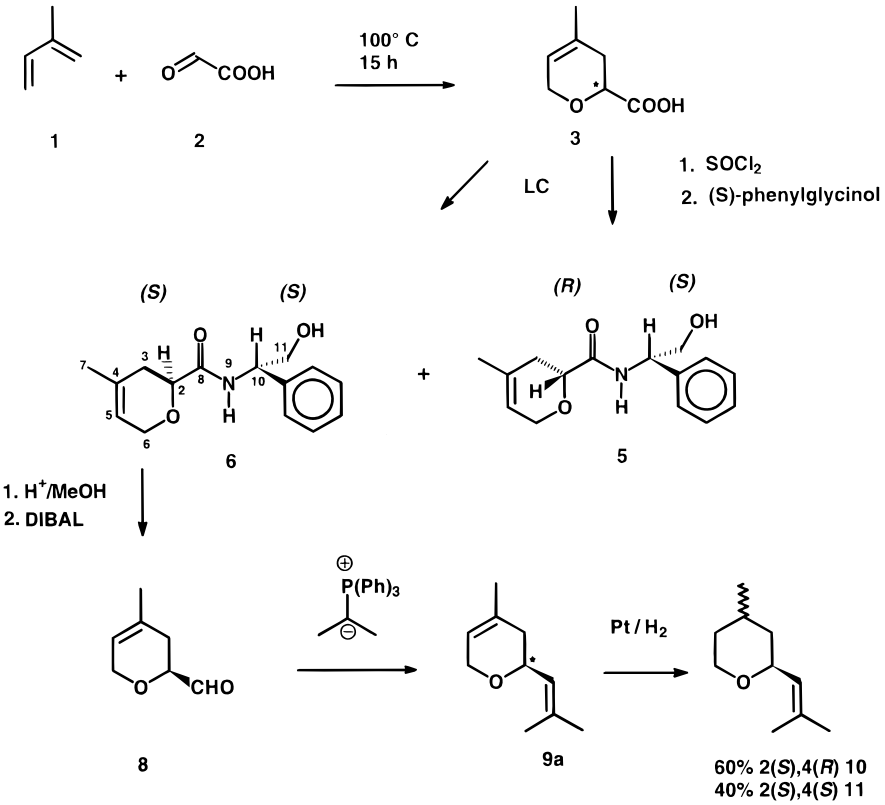
10.1021/jf981245m CCC: $18.00
Published on Web 07/03/1999

Scheme 1. Enantioselective Synthesis of (*S*)-Nerol Oxide 9a and *cis*- and *trans*-Rose Oxide 10, 11



glass capillary (30 m × 0.23 mm) coated with a 0.23 μm film of 50% octakis(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin in OV 1701-vi, column was self-prepared according to Schmarr (1992); carrier gas hydrogen at 100 kPa; oven temperature 60 °C (25 min isothermal), then 1.5 °C/min to 150 °C; live-T-cutting 23.500−24.500 min.

GC-MS. The GC-MS analysis of the synthetic products and the reference compounds was performed with a Fisons Instruments GC 8065 coupled to a Fisons Instruments MD 800 mass spectrometer, equipped with a HTFS capillary column (30 m × 0.25 mm; coated with SE-52; film thickness 0.5 μm; column was self-prepared according to Grob (1986)). GC conditions: carrier gas helium 70 kPa; split 30 mL/min; injector temperature 230 °C; oven temperature 40 °C (5 min isothermal), then 3 °C/min to 250 °C (30 min isothermal); ion source temperature 200 °C; mass range 40−250 amu; electron energy 70 eV. The molecular ions (M^+) and fragment ions are given as *m*/*z* with relative peak intensities in % of the most abundant peaks.

Administration of the Labeled Precursor. A solution of **16** with a concentration of 1.0 mg/mL was prepared by dissolving the same amount of **16** and Tween 20 in water. A 100 μL amount of this solution and two pieces of the leaf blade of *P. graveolens* (weight 100−200 mg) were incubated at room temperature for 24 h with exclusion of light in a sealed 2 mL vial. After incubation, the essential oil evaporating from the glandular trichomes was analyzed by headspace SPME.

SPME. A SPME fiber holder for manual use equipped with a fused silica fiber coated with poly(dimethylsiloxane) (film thickness 100 μm) was used (both SUPELCO, Munich, Germany). For headspace sampling, the septum of the vial containing the pieces of leaf blade was pierced and the fiber exposed for 3 min to the headspace. For thermal desorption, the SPME fiber remained in the injector for 3 min. Splitless injection mode was used, the split valve being opened after 2 min.

Synthesis of 3,6-Dihydro-4-methyl-2*H*-pyran-2-carboxylic Acid (3). Compound **3** was prepared according to Lubineau et al. (1994). The crude acid was purified by flash chromatography (eluent: pentane/diethyl ether/formic acid 70/25/5 (v/v/v)). ^{1}H NMR (δ): 1.75 (s, H-7, 3H), 2.27−2.36 (m, H-3, 2H), 4.22−4.36 (m, H-6, 2H), 5.44 (m, H-2, 1H), 9.35 (s, br, COO*H*, 1H).

Synthesis of 3,6-Dihydro-4-methyl-2*H*-pyran-2-carboxylic Acid Chloride (4). Compound **4** was prepared according to Becker et al. (1993). A 2.7 mmol amount of **3** yielded 1.7 mmol (63%) of **4** after distillation (Kugelrohr, 95 °C, 0.12 mbar). Because of its instability, compound **4** was used for the next step without GC/MS analysis.

Synthesis of (*R*)- and (*S*)-3,6-Dihydro-4-methyl-2*H*-pyran-2-carboxylic Acid (*S*)-Phenylglycinol Amide (5,6). The diastereomeric amides **5** and **6** were prepared according to Helmchen et al. (1979). A 1.7 mmol amount of **4** gave 0.7 mmol (44%) of (*R*)-**5** and 0.4 mmol (25%) of (*S*)-**6** after separation by flash chromatography (ethyl acetate/pentane 70/30 (v/v)). ^{1}H NMR (δ) of **5**: 1.74 (s, H-7, 3H), 2.19−2.36 (m, H-3, 2H), 2.98−3.00 (m, O*H*, 1H), 3.83−3.91 (m, H-11, 2H), 4.01 (dd, J = 3.9/10.9 Hz, H-2, 1H), 4.17−4.27 (m, H-6, 2H), 5.05−5.09 (m, H-10, 1H), 5.41 (s, br, H-5, 1H), 7.24−7.38 (m, phenyl-H, H-9, 6H). ^{1}H NMR (δ) of **6**: 1.72 (s, H-7, 3H), 2.10−2.33 (m, H-3, 2H), 2.71−2.74 (m, O*H*, 1H), 3.90−3.92 (m, H-11, 2H), 4.07 (dd, J = 3.9/10.9 Hz, H-2, 1H), 4.20−4.28 (m, H-6, 2H), 5.08−5.11 (m, H-10, 2H), 5.41 (s, br, H-5, 1H), 7.26−7.38 (m, phenyl-H, H-9, 6H).

Synthesis of (*S*)-3,6-Dihydro-4-methyl-2*H*-pyran-2-carboxylic Acid Methyl Ester (7). A 0.4 mmol amount of compound **6** was dissolved in 1 mL of THF and 25 mL of methanol, and 0.4 mmol of sulfuric acid was added. The mixture was refluxed for 18 h. The solvents were removed under reduced pressure, and the residue was suspended in water. It was extracted 3 times with portions of 30 mL of diethyl ether, and the combined extracts were dried over anhydrous sodium sulfate. After filtration over a layer of silica gel, the solvent was removed under reduced pressure to afford 0.24 mmol (56%) of **7**. GC-MS: 156 (M^+, 5), 141 (15), 138 (34), 128 (31), 97 (100), 69 (77), 41 (53).

Synthesis of (*S*)-3,6-Dihydro-4-methyl-2*H*-pyran-2-carbaldehyde (8). Compound **8** was prepared according to Zakharkin and Khorlina (1962) by reduction of **7** using DIBAL. The crude product was used for the next step without further

Table 1. Enantiomeric Ratio of Genuine and Labeled Nerol Oxide from Different Geranium Oils in Percent

		geranium oils				
		self-prepared		commercial samples		
compound		*Pelargonium graveolens*	*Pelargonium* unknown spp.	Réunion	China	Egypt
(+)-(*R*)-nerol oxide	**9b**	50.6	49.7	48.5	49.5	48.6
(−)-(*S*)-nerol oxide	**9a**	49.4	50.3	51.5	50.5	51.4
(+)-(*R*)-d_2-nerol oxide	**15b**	48.6	47.9			
(−)-(*S*)-d_2-nerol oxide	**15a**	51.4	52.1			

purification. GC-MS: 126 (M^+, 0.4), 97 (76), 80 (8), 69 (28), 53 (10), 41 (100).

Synthesis of (*S*)-3,6-Dihydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-pyran (9a). Compound **9a** was prepared according to Ogawa et al. (1978) using the Wittig reaction. A 0.01 mmol amount of **9a** was obtained after purification by flash chromatography (pentane/ether 90/7 (v/v). GC-MS: 152 (M^+, 4), 109 (5), 96 (9), 83 (62), 68 (80), 67 (100), 53 (32).

Synthesis of (2*S*,4*R*)-4-Methyl-2-(2-methyl-1-propenyl)-tetrahydropyran (10) and (2*S*,4*S*)-4-Methyl-2-(2-methyl-1-propenyl)tetrahydropyran (11). Compounds **10** and **11** were prepared by partial reduction of **9a** in a micromole scale according to Ohloff et al. (1964). MS data are in agreement with the data given by Snowden et al. (1987).

Synthesis of (*Z*)-Methyl-3,7-dimethyl-2,6-octadienoate (13). Compound **13** was prepared according to Corey et al. (1968). A 3.3 mmol amount of (*Z*)-3,7-dimethyl-2,6-octadienal yielded 2.5 mmol (75%) of crude product. The product was used for the next step without further purification. GC-MS: 182 (M^+, 3), 123 (17), 114 (25), 83 (29), 69 (79), 41 (100).

Synthesis of (*Z*)-3,7-Dimethyl-2,6-(1,1-$^2H_2$)octadien-1-ol (14). Compound **14** was prepared according to Becker et al. (1993) using lithium aluminum deuteride (>99% atom D; Fluka, Deisenhofen, Germany). A 2.6 mmol amount of **13** yielded 0.3 mmol (11%) of **14** after purification by column chromatography (silica gel, pentane/diethyl ether 2/5 (v/v)). Purity >95% (GC). Deuterium content: d_2 > 97% (^{1}H NMR). GC-MS: 156 (M^+, 0), 138 (15), 123 (21), 122 (6), 95 (80), 69 (69), 41 (100). ^{1}H NMR (δ): 1.62 (s, H-8 or H-10, 3H), 1.71 (s, H-8 or H-10, 3H), 1.77 (s, H-9, 3H), 2.09−2.12 (m, H-4, H-5, 4H), 5.10−5.15 (m, H-6, 1H), 5.46 (s, H-2, 1H).

Synthesis of 3,6-Dihydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-(6,6-$^2H_2$)pyran (15a/b). Compound **15a/b** was prepared according to Taneja et al. (1978) in a micromole scale with some modifications. A 25 mg amount of **14** and a catalytic amount of *N*-iodosuccinimide (NIS) were taken up in 1 mL of CCl_4 in a GC vial. The vial was sealed and heated at 90 °C for 25 min. The dark violet solution was shaken several times with an aqueous solution of $Na_2S_2O_3$ until the iodine was completely removed. The reaction mixture was subjected to preparative TLC (Polygram SIL G/UV_{254}, Machery-Nagel, Düren, Germany; eluent pentane/ether 9/1 (v/v)). The nerol oxide fraction was removed from the TLC plate and extracted with ether. GC-MS: 154 (M^+, 5), 111 (5), 96 (10), 83 (83), 70 (97), 69 (100), 55 (36).

Synthesis of (*Z*)-3,7-Dimethyl-2,6-(1,1-$^2H_2$)octadien-1-yl-β-D-glucopyranoside (16). Compound **16** was prepared according to Paulsen et al. (1985). A 0.7 mmol amount of **14** yielded 0.13 mmol (19%) of **16** after purification by column chromatography (silica gel, ethyl acetate/pentane/methanol 5/5/1 (v/v/v)). ^{1}H NMR (δ): 1.61 (s, H-8 or H-10, 3H), 1.70 (s, H-8 or H-10, 3H), 1.77 (s, H-9, 3H), 2.0−2.2 (m, H-4, H-5, 4H), 3.3−3.9 (m, 11H), 4.36 (d, J = 7.6 Hz, H-1', 1H), 5.0−5.2 (m, H-6, 1H), 5.37 (s, H-2, 1H).

RESULTS AND DISCUSSION

The first enantioselective synthesis of both nerol oxide enantiomers was performed by Ohloff et al. (1980) using (*R*)-linalool as the chiral building block. However, the elucidation of the absolute configuration of the optical antipodes remained unclear. Therefore, a novel enantioselective synthesis of nerol oxide was elaborated (see Scheme 1). To determine the absolute configuration of the stereocenter at C-2, enantiopure nerol oxide was hydrogenated to afford a mixture of enantiopure *cis*- and *trans*-rose oxide (**10**,**11**).

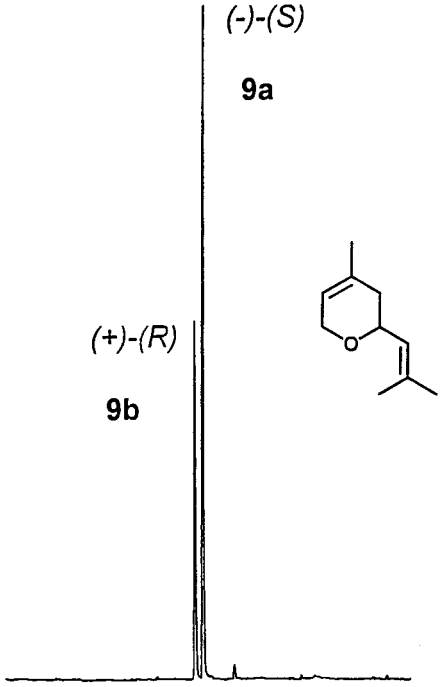
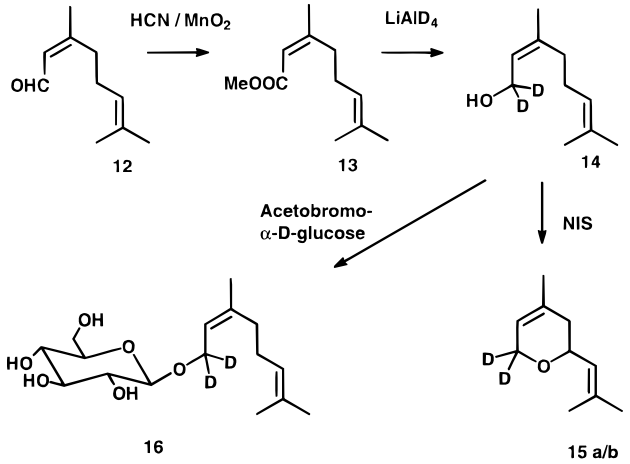


Figure 1. Separation of nerol oxide enantiomers on octakis-(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin in OV 1701-vi.

The absolute configurations at C-2 and C-4 of **10** and **11** were determined by enantioselective GC analysis using heptakis-(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin as the chiral stationary phase. All four stereoisomers of rose oxide can be separated on this phase with known order of elution (Wüst et al., 1998a). Hence, the absolute configuration of the stereocenter at C-2 of nerol oxide can be deduced. The elution order of the nerol oxide enantiomers on an octakis(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin phase was determined as (*R*)-**9b** before (*S*)-**9a** by injection of (*S*)-enriched nerol oxide (see Figure 1).

The same cyclodextrin phase column was used in a MDGC/MS system for the determination of the enantiomeric ratios of nerol oxide in different geranium oils (see Table 1). In all investigated oils, nerol oxide occurs as a racemate. This result is in agreement with the result of Kaiser (1984) who measured the optical rotation power of nerol oxide isolated from Reunion geranium oil and with the result of Werkhoff et al., who determined the enantiomeric ratio of nerol oxide in a geranium oil of undefined origin by enantioselective GC. Racemic nerol oxide was also found in rose oil (Ohloff

Scheme 2. Synthesis of Labeled Neryl Glucoside 16 and Reference Compounds 15a/b



et al., 1980; Kováts, 1987; Lindström et al., 1993), labdanum oil, osmanthus oil, and lavender oil (Werkhoff et al.).

These findings led to the hypothesis that nerol oxide is not formed directly in the plant but from a precursor which is preformed to a large extent. Nerol is discussed as a potential precursor, and singlet oxygen might act as an active agent for the introduction of a second allylic hydroxy group (Ohloff et al., 1980). To verify this hypothesis, deuterium-labeled nerol oxide **14** was prepared (see Scheme 2).

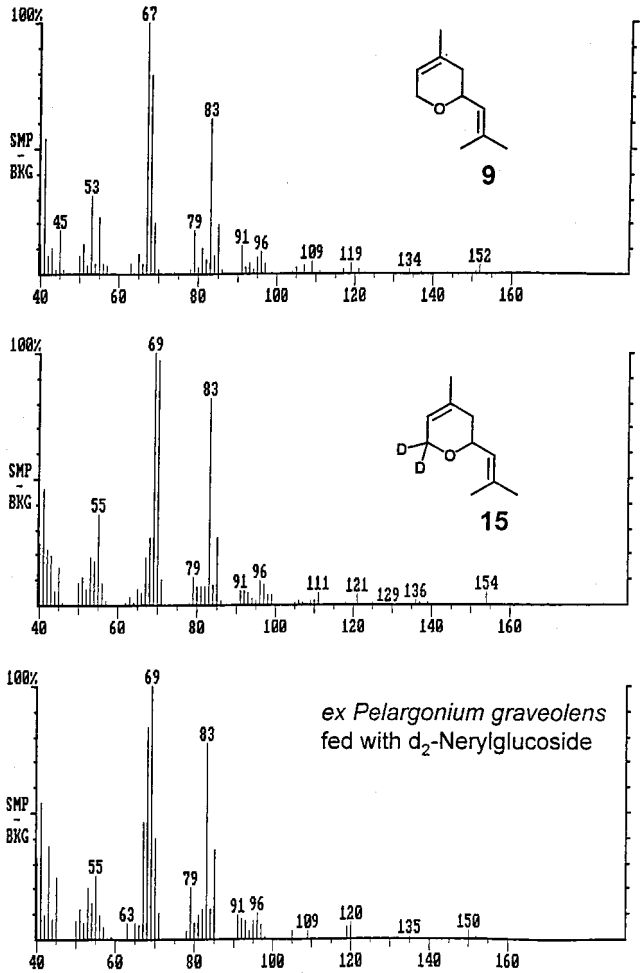


Figure 2. Mass spectra of unlabeled (**9**) and labeled (reference compound **15**) nerol oxide in comparison with nerol oxide obtained from *P. graveolens* fed with **16**.

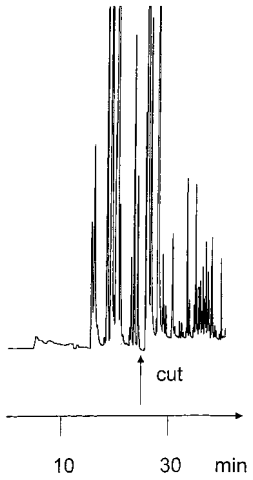


Figure 3. Precolumn chromatogram obtained from *P. graveolens* by headspace SPME.

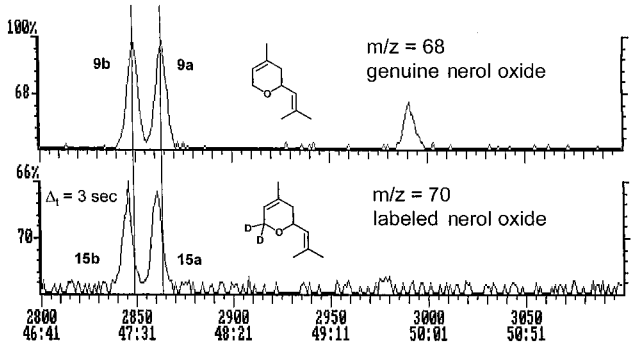
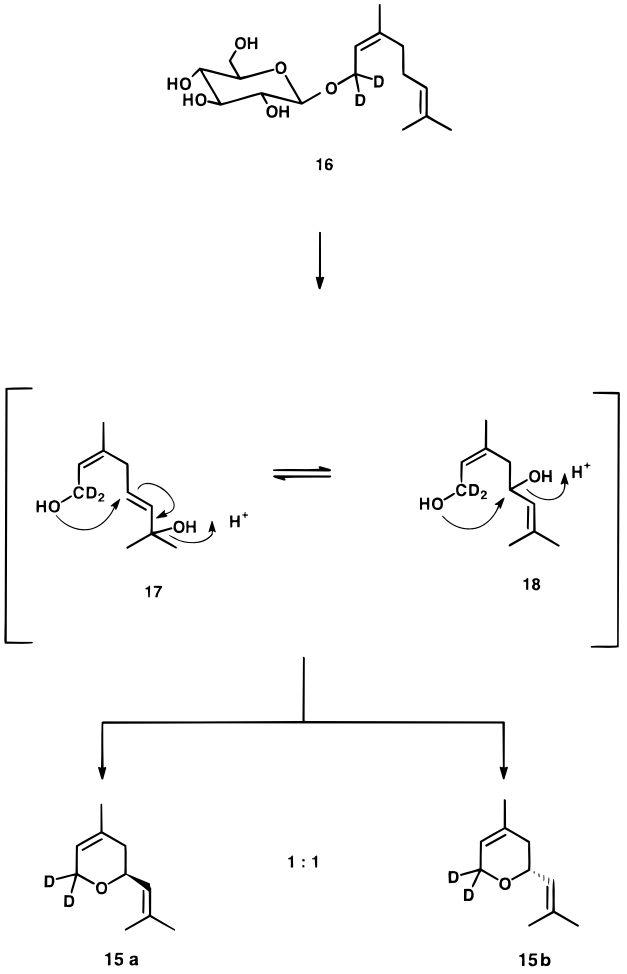


Figure 4. Main column chromatogram obtained from *P. graveolens* fed with **16**.

Since Croteau et al. (1987) could show that monoterpene glucosides have a transport function in monoterpene catabolism, the labeled nerol **14** was glucosidated and fed to the plants by incubating leaf disks with a solution of glucoside **16** in septum-sealed 2 mL vials for 24 h in the dark. These conditions were chosen in order to exclude a photooxygenation of labeled neryl glucoside **16** by singlet oxygen. After the incubation, the headspace of the septum-sealed vial was analyzed using solid-phase microextraction (SPME). The SPME extracts were analyzed by enantio-MDGC-MS. Figure 3 shows a precolumn chromatogram obtained from *P. graveolens* which was fed with **16**. Nerol oxide was transferred to the chiral main cloumn and detected by MS. Figure 4 shows the main column chromatogram.

As can be seen on mass lane $m/z = 68$ and 70, genuine and labeled nerol oxide occur as racemates. On mass lane $m/z = 70$, the labeled nerol oxide enantiomers are mainly detected, as can be seen by the position of the peak maxima on this mass lane: Because of the so-called inverse isotope effect (Matucha, 1991), the deuterium-labeled nerol oxide isotopomers show a shift of their peak maxima of approximately 3 s relative to the unlabeled one. The presence of labeled nerol oxide is evident from the mass spectra measured at the peak maximum on mass lane $m/z = 70$ (see Figure 2). The base peak at $m/z = 69$, which is shifted by two mass units compared to unlabeled nerol oxide, is clearly detectable. The fact that the cyclization of labeled nerol also proceeds in the dark excludes a photooxygenation

Scheme 3. Proposed Biosynthetic Pathway for the Conversion of Labeled Neryl Glucoside 16 into Nerol Oxide 9a/b in *Pelargonium* Species



mechanism which was previously advanced. It shows that the plant is able to convert the fed nerol into nerol oxide. Since a photooxygenation mechanism can be excluded, it is highly probable that nerol is enzymatically oxydized in an allylic position to give one of the diols in Scheme 3 (**17** or **18**), which are equivalent by allylic rearrangement. **17**/**18** can easily cyclize to nerol oxide by acid catalysis (Ohloff et al., 1964).

In petals of *Rosa damascena*, the diol **17** was recently isolated and spectroscopically characterized (Knapp et al., 1998), which supports the above-mentioned suggestions concerning the cyclization mechanism.

CONCLUSIONS

Since nerol oxide appears in all geranium oils as a racemate, it seems that the formation proceeds by acid-catalyzed cyclization of **17**/**18** without any enzymatic catalysis. It is highly probable that the formation of **17**/**18** is catalyzed by an enzymatic allylic oxidation of the corresponding monoterpenol nerol in analogy to the formation of rose oxide (Wüst et al., 1996, 1998a,b). A photooxygenation mechanism is rather unlikely. Nerol oxide has to be considered as a natural racemate.

ACKNOWLEDGMENT

We thank D. Stegmeier, Gartenbau Stegmeier, Essingen, Germany, for providing authentic plant material, F. Dettmar for technical assistance, and Dipl.-Ing. S. Bihler and Dr. H. Hanssum for recording the ^{1}H-NMR spectra.

LITERATURE CITED

Becker, H. G. O.; et al. *Organikum*, 19th ed.; Barth Verlagsgesellschaft mbH: Leipzig, 1993; pp 440−441, 511−12.

Corey, E. J.; Gilman, N. W.; Ganem, B. E. New Methods for the Oxidation of Aldehydes to Carboxylic Acids and Esters. *J. Am. Chem. Soc.* **1968**, *90*, 5616−5617.

Croteau, R. Biosynthesis and Catabolism of Monoterpenes. *Chem. Rev.* **1987**, *87*, 929−954.

Dietrich, A.; Maas, B.; Mosandl, A. Diluted modified cyclodextrins as chiral stationary phases—Influence of the polysiloxane solvent: Heptakis(2,3-di-O-acetyl-6-O-*tert*-butyldimethylsilyl)-*β*-cyclodextrin. *J. High Resolut. Chromatogr.* **1995**, *18*, 152−156.

Grob, K. *Making and Manipulating Capillary Columns for Gas Chromatography*; Dr. Alfred Hüthig Verlag: Heidelberg, Germany, 1986.

Helmchen, G.; Nill, G.; Flockerzi, D.; Yousef, M. S. Gezielte präparative Trennung enantiomerer Carbonsäuren und Lactone via Flüssigkeitschromatographie und nachbargruppenunterstützte Hydrolyze diastereomerer Amide (Separation of enantiomeric carboxylic acids and lactones by liquid chromatography and hydrolysis of diastereomeric amides). *Angew. Chem.* **1979**, *91*, 65−66.

Kaiser, R. 134. (5*R**,9*S**)- and (5*R**,9*R**)-2,2,9-Trimethyl-1,6-dioxaspiro[4.4]non-3-ene and their dihydro derivatives as new constituents of geranium oil. *Helv. Chim. Acta* **1984**, *67*, 1198−1203.

Knapp, H.; Straubinger, M.; Fornari, S.; Oka, N.; Watanabe, N.; Winterhalter, P. (*S*)-3,7-Dimethyl-5-octene-1,7-diol and related oxygenated monoterpenoids from petals of *Rosa damascena* Mill. *J. Agric. Food Chem.* **1998**, *46*, 1966−1970.

Kováts, E. sz. Composition of essential oils. Part 7. Bulgarian oil of Rose (*Rosa damascena* Mill). *J. Chromatogr.* **1987**, *406*, 185−222.

Lindström, M.; Jaquier, A. A gas chromatographic study of the enantiomeric composition of volatile compounds in Bulgarian rose oil (Rosa damascena Mill). Part 1. *Riv. Ital. EPPOS* **1993**, 130−139.

Lubineau, A.; Augé, J.; Lubin, N. Aqueous hetero Diels−Alder reactions: The carbonyl case. *Tetrahedron* **1994**, *50*, 10265−10265.

Matucha, M.; Jockisch, W.; Verner, P.; Anders, G. Isotope effect in gas−liquid chromatography of labelled compounds. *J. Chromatogr.* **1991**, *588*, 251−258.

Ogawa, T.; Takasaka, N.; Matsui, M. Synthesis of (−)-*cis*-rose oxide from D-glucose. *Carbohydr. Res.* **1978**, *60*, C4−C6.

Ohloff, G.; Schulte-Elte, K.-H.; Willhalm, B. Zur Darstellung von Tetrahydropyran und Tetrahydrofuranderivaten aus 1,7-bzw. 1,6-Allyldiolen durch Dehydratation in der Allylstellung (Preparation of tetrahydropyran and tetrahydrofuran derivatives by dehydratation in allylic position using 1,7- and 1,6 allylic diols). *Helv. Chim. Acta* **1964**, *47*, 602−626.

Ohloff, G.; Giersch, W.; Schulte-Elte, K.-H.; Enggist, P.; Demole, E. Synthesis of (*R*)- and (*S*)-4-Methyl-6-2′-methylprop-1′-enyl-5,6-dihydro-2*H*-pyran (Nerol oxide) and natural occurrence of its racemate. *Helv. Chim. Acta* **1980**, *63*, 1582−1588.

Paulsen, H.; Lê-Nguyên, B.; Sinnwell, V.; Heemann, V.; Seehofer, F. Synthese von Glycosiden von Mono-, Sesqui- und Diterpenalkoholen (Preparation of glycosides of mono-, sesqui- and diterpene alcohols). *Liebigs Ann. Chem.* **1985**, 1513−1536.

Schmarr, G. Beiträge zur On-line LC-GC Kopplung und modifizierte Cyclodextrine als chirale stationäre Phasen in der Kapillar-GC (On-line LC-GC coupling and modified cyclodextrin derivatives in c-GC). Ph.D. Thesis, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany, 1992.

Schreier, P.; Drawert, F. Gaschromatographisch-massenspektrometrische Untersuchung flüchtiger Inhaltsstoffe des Weines (Investigations on volatiles in wine using GC-MS). *Z. Lebensm.-Unters.-Forsch.* **1974**, *154*, 273−278.

Schurig, V. Enantiomer Separation by Complexation Gas Chromatography-Applications in Chiral Analysis of Pheromones and Flavours. In *Bioflavour '87: Analysis, Biochemistry, Biotechnology*; Proceedings of the International Conference, Würzburg, Federal Republic of Germany, September 29−30, 1987; Schreier, P., Ed.; de Gruyter: Berlin, Germany, 1987; pp 35−54.

Snowden, R. L.; Linder, S. M.; Muller, B. L.; Schulte-Elte, K. H. Beta-Cleavage of bis (homoallylic) potassium alkoxides. Preparation of 3-hydroxypropyl and 4-hydroxybutyl propenyl ketones from gamma- and delta-lactones. Synthesis of (±)-rose oxide. *Helv. Chim. Acta* **1987**, *70*, 1879−1885.

Taneja, S. C.; Dhar, K. L.; Atal, C. K. *N*-Iodosuccinimide for the synthesis of rose oxide. *J. Org. Chem.* **1978**, *43*, 997−998.

Werkhoff, P.; Brennecke, S.; Bretschneider, W.; Schreiber, K. Enantioselektive GC-Analyse chiraler Riech- und Aromastoffe. Teil 3 (Enantioselective analysis of chiral flavor compounds. Part 3). *Contact 65*, 4−7.

Wüst, M.; Beck, T.; Dietrich, A.; Mosandl, A. On the biogenesis of Rose oxide in *Pelargonium graveolens* L'Héritier and *Pelargonium radens* H.E. Moore. *Enantiomer* **1996**, *1*, 167−176.

Wüst, M.; Rexroth, A.; Beck, T.; Mosandl, A. Mechanistic aspects of the biogenesis of Rose oxide in *Pelargonium graveolens* L'Héritier. *Chirality* **1998a**, *9*, 229−238.

Wüst, M.; Beck, T.; Mosandl, A. Biogenesis of geranium oil compounds: On the origin of oxygen in *cis*-/*trans*-rose oxide. *J. Agric. Food Chem.* **1998b**, *46*, 3225−3229.

Zakharkin, L. I.; Khorlina, I. M. Reduction of esters of carboxylic acids into aldehydes with diisobutylaluminium hydride. *Tetrahedron Lett.* **1962**, 619−620.

Received for review November 12, 1998. Revised manuscript received May 7, 1999. Accepted May 10, 1999. The financial support by the Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged.

JF981245M