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Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. cv. Scheurebe: demonstration of stereoselective reduction, *E*/*Z*-isomerization, oxidation and glycosylation

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Abstract

The metabolism of deuterium labeled geraniol in grape mesocarp of *Vitis vinifera* L. *cv.* Scheurebe was studied by in vivo-feeding experiments. Stereoselective reduction to (*S*)-citronellol, *E*/*Z*-isomerization to nerol, oxidation to neral/geranial and glycosylation of the corresponding monoterpene alcohols could be demonstrated. Time course studies including the determination of conversion rates revealed that the activity of these secondary transformations of monoterpenes is dependent on the ripening stage and can be distinguished from the development of the primary monoterpene synthase activities by the sharp increase at the end of the ripening period. The stereoselective biosynthesis of the potent odorant *cis*-(2*S*,4*R*)-rose oxide from labeled geraniol in grape berry mesocarp is demonstrated as well. Since (*S*)-citronellol is the precursor of *cis*-(2*S*,4*R*)-rose oxide it can be concluded that especially the last part of the ripening period is important for the generation of this potent odorant. This finding confirms the conclusion that a higher concentration of flavor compounds could be established in the berries by leaving the fruit on the vine for extended periods.

Keywords: *Vitis vinifera*; Monoterpenes; Rose oxide; Citronellol; Biosynthesis; Enantioselective multidimensional gas chromatography–mass spectrometry (enantio-MDGC–MS); Stir bar sorptive extraction (SBSE)

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1. Introduction

Monoterpenes contribute substantially to the characteristic floral aroma and flavor of the so called muscat and non-muscat aromatic grape varieties (*Vitis vinifera*). Well known representatives are Muscat of Alexandria and Gewürztraminer, respectively. Beside monoterpene alcohols (linalool, geraniol **6**, nerol **8**, citronellol **10** and α-terpineol) and monoterpene ethers (rose oxide **12**, nerol oxide) numerous other monoterpene compounds, such as polyhydroxylated derivatives, have been identified in grape must and wine (Rapp, 1992; Williams et al., 1980, 1985; Luan et al., 2004). In addition to the free odour-producing forms of monoterpenes, several glycosidically bound forms of monoterpenes have been described (Strauss et al., 1986).

Among the odiferous monoterpenes the cyclic ether rose oxide **12** (*cis*- and *trans*-4-methyl-2-(2′-methyl-1-propenyl)-tetrahydropyran) is a potent odorant in Scheurebe and Gewürztraminer wines, as could be shown by Guth (1997a,b) using gas chromatography/olfactometry. **12** has been previously detected in many fruits and essential oil producing plants (Naves et al., 1961; von Sydow and Karlsson, 1971; Kreis and Mosandl, 1994).

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Despite of their importance to wine flavor and aroma relatively little is known about the biochemistry of monoterpenoids in *V. vinifera*. The *de novo* biosynthesis of monoterpenes in grapes and leaves via the novel DOXP/MEP pathway has been demonstrated (Luan and Wüst, 2002) and recently a terpene synthase catalyzing the cyclization of geranyl diphosphate to (−)-α-terpineol has been cloned and functionally characterized for the first time in an angiosperm (Martin and Bohlmann, 2004). However, the mechanisms that underlie secondary transformations of monoterpenes like hydroxylation, reduction or glycosylation have not yet been characterized in vivo. Glycosylation activity in detached grape berries grown in vitro have been demonstrated (Bravdo et al., 1990) and cell suspension cultures of the variety Muscat de Frontignan are able to transform geraniol **6** into geranial **16** ((*E*)-3,7-dimethylocta-2,6-dien-1-al), neral **18** ((*Z*)-3,7-dimethylocta-2,6-dien-1-al), nerol **8** and citronellol **10** (Ambid et al., 1983). However, the cell suspension cultures used in these studies did not accumulate detectable amounts of monoterpenoids (as it is frequently observed in plant cell cultures of essential oil accumulating plants due to an increased activity of catabolic enzymes (Falk et al., 1990)) and therefore may not be comparable with the situation in vivo. First preliminary data on the in vivo metabolism of deuterium labeled linalool and geraniol have been previously published (Luan et al., 2003) and in this study we would like to present a more detailed investigation on the metabolism of geraniol, which includes time course studies, determination of conversion rates and subsequent enantioselective multidimensional GC/MS analysis of the metabolites giving information on the stereochemical aspects. The stereoselective biosynthesis of the potent odorant *cis*-(2*S*,4*R*)-rose oxide in grape berry mesocarp is demonstrated as well.

2. Results and discussion

Almost all monoterpens are derived from geranyl diphosphate. However, the metabolism of geraniol itself in intact grape berries in vivo has remained unknown.

In order to investigate the metabolism of geraniol **6** in grapes, in vivo-feeding experiments were performed with deuterium labeled d_6-geraniol **5**, which was prepared by a 4 step synthesis using geranyl acetate **1** as starting material. To avoid light induced oxygenation and *E*/*Z*-isomerization of geraniol **6** the grape berries were incubated with exclusion of light following the injection of labeled geraniol into the mesocarp of ripening berries at different stages of ripeness as characterized by their sugar content in degrees of Oechsle (°Oe). The isolation and fractionation of the target compounds was achieved by solid phase extraction (SPE) according to the method of Mateo et al. (1997).

The enantioselective analysis of the fractions was carried out with an enantio-MDGC–MS system equipped with an achiral pre column and a chiral main column. The elution order of the enantiomers of citronellal **14a/b**, citronellol **10a/b** and d_6-linalool **19a/b** was determined on the chiral stationary phase heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (diAc-β) by co-injection of enantiopure and racemic standards. The enantioselective analysis of the SBSE extracts were carried out with an enantio-SBSE-MDGC–MS system equipped with an achiral precolumn and a chiral main column. The elution order of the enantiomers of *cis*- and *trans*-rose oxide **12a–d** was determined on the chiral stationary phase heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (diMe-β) by co-injection of enantiopure and racemic standards.

All assays were performed at least three times. Control experiments with musts that had been boiled for 10 min showed no enzymatic activity. Fig. 1 shows a standard chromatogram using the chiral main column of the enantio-MDGC-MS system. The enantiomers of linalool (19), citronellol (10) and citronellal (14) are well separated with Di-Ac-β as the chiral stationary phase.

A typical main column chromatogram of free labeled and genuine geraniol **5/6**, nerol **7/8**, and citronellol **9/10** obtained from grape berries after administration of labeled geraniol is shown in Figs. 2(A)–(C). The conversion of d_6-geraniol **5** into d_6-nerol **7** and d_6-(*S*)-citronellol **9b** is clearly detectable as illustrated by Fig. 2(B). The labeled terpenes can be detected selectively on mass lane $m/z = 75$ because the base peaks of the corresponding unlabeled geraniol **6**, nerol **8** and citronellol **10** [$m/z = 69$] are shifted by 6 mass units as illustrated by Fig. 3. Additionally, inverse isotope effects (Matucha et al., 1991) were observed during the enantio-

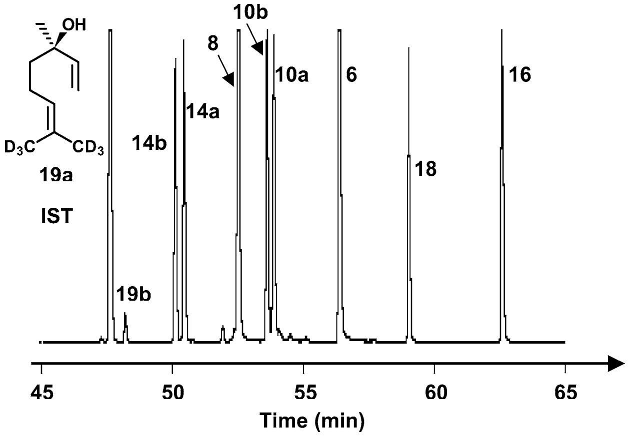

Fig. 1. Chromatogram of chiral and nonchiral reference compounds, using enantio-MDGC–MS (main column Di-Ac-β-CD): geraniol (**6**); nerol (**8**); geranial (**16**); neral (**18**); Chiral compounds are well differentiated into their enantiomers: (3*R*)-citronellol (**10a**); (3*S*)-citronellol (**10b**); (3*R*)-citronellal (**14a**); (3*S*)-citronellal (**14b**); d_6-(3*R*)-linalool (**19a**), used as internal standard; d_6-(3*S*)-linalool (**19b**).

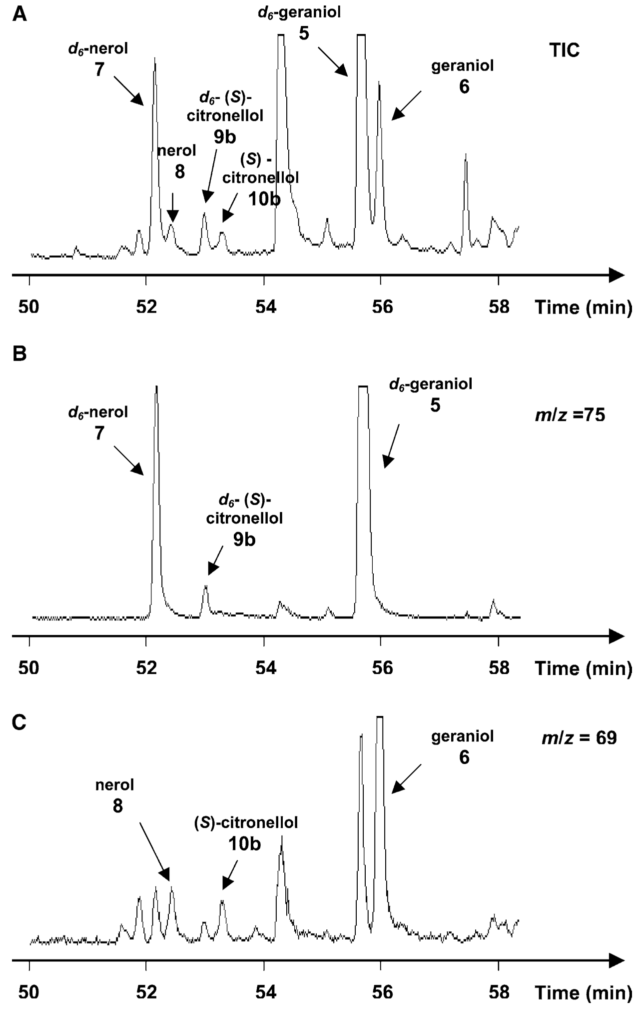

Fig. 2. Main column chromatogram (Di-Ac-β-CD) of labelled metabolites, generated from d_6-geraniol (conditions: see Fig. 1).

MDGC–MS analysis. The deuterium labeled monoterpene isotopomers show a shift of their peak maximum of approximately 20 s when compared with the unlabeled ones.

Generated free and glycosidically bound d_6-citronellol **9** shows an enantiomeric purity better than 95% in favor of the (*S*)-configured enantiomer during the whole ripening period (see below). Thus, the enzymatic reduction of the C2/C3 double bond of geraniol is a stereoselective process. Whether the corresponding reductase is able to reduce nerol as well, that is generated by an isomerase (see below) can not be answered at this stage because this requires the separation of the reductase and isomerase by a partial purification. However, a geraniol-nerol reductase has been isolated from rose petals (Dunphy and Allcock, 1972). The formation of (*S*)-citronellol via stereoselective reduction of geranial and concomitant reduction of the generated citronellal can be excluded because traces of citronellal could not be detected. Interestingly, the reduction of geraniol by *Saccharomyces cerevisiae* yields stereoselectively (*R*)-citronellol (Gramatica et al., 1982). It should be, therefore, possible to distinguish the origin of citronellol from wine berries and yeast fermentation in wines, respectively, by means of an enantioselective analysis.

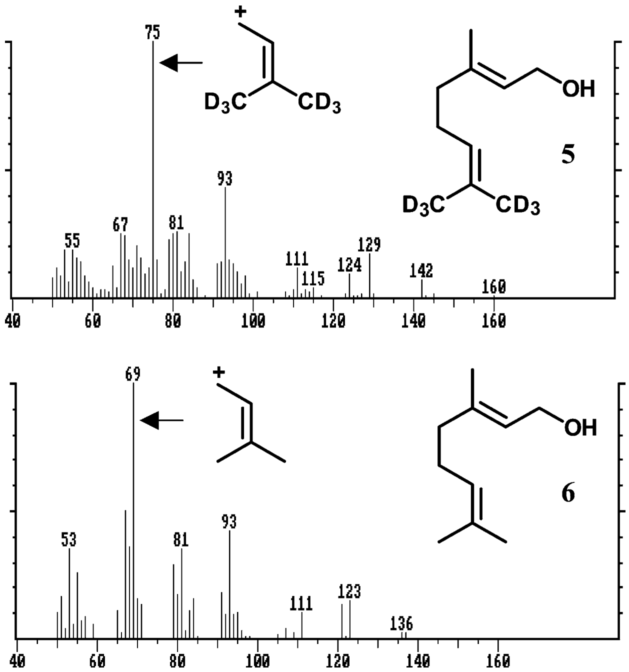

Fig. 3. Mass spectra of genuine and corresponding deuterium labelled geraniol.

Beside a stereoselective reduction, an *E*/*Z*-isomerization of labeled geraniol to nerol could be detected demonstrating the presence of an isomerase. In flower heads of *Rosa damascena* such an isomerase activity has been previously detected (Banthorpe and Poots, 1979) and isomerization of geraniol and geranyl phosphate to nerol and neryl phosphate, respectively, by cell-free enzyme systems from carrot leaves have been reported as well (Shine and Loomis, 1974). It is conceivable that in intact berries a non-enzymatic *E*/*Z* isomerization is triggered by natural light. If this is a process of significance in vivo still needs to be verified by experiments with berries illuminated in a diurnal fashion. Furthermore, in the present study the generation of geranial and neral in berry mesocarp could be detected, which requires the presence of an oxidase and/or a dehydrogenase. However, only trace amounts of neral and geranial were generated and were thus not quantified. A very low monoterpenol oxidase and dehydrogenase activity has been previously detected in cell-free extracts from *Pelargonium graveolens* leaves (Banthorpe et al., 1983).

For analyzing non-polar metabolites of geraniol in the ppb range an enantio-SBSE–MDGC–MS system (Kreck et al., 2002) was employed. Thus, the conversion of d_6-geraniol into *cis*- and *trans*-d_6-rose oxide **11a–c** in grape berry mesocarp could be detected (see Fig. 4). The mass spectrum of the labeled rose oxide is in good

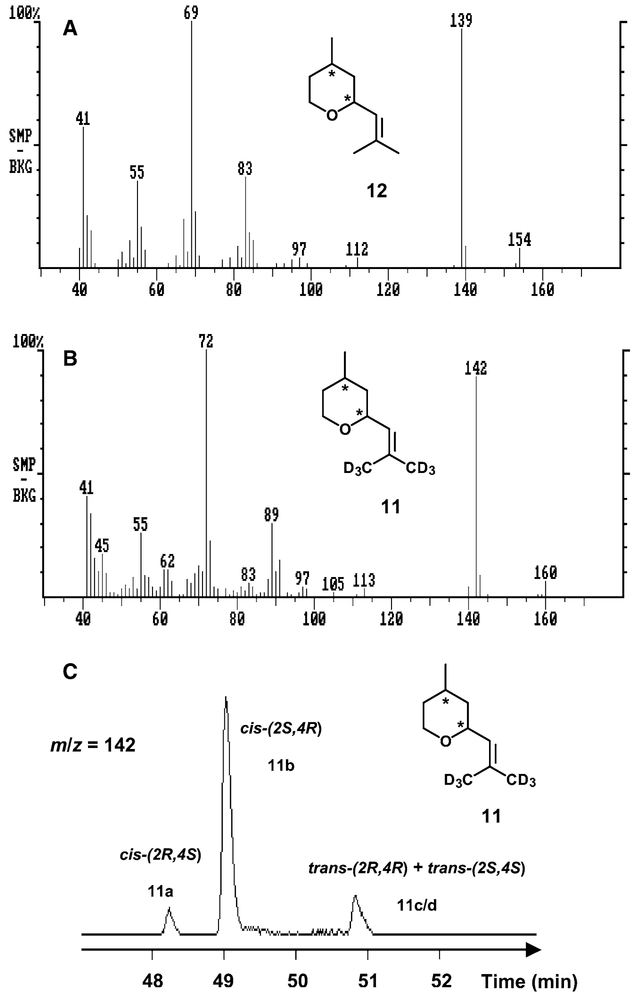

Fig. 4. Mass spectra of genuine (A) and deuterium labeled (B) rose oxide; Chiral main column chromatogram of labelled rose oxide stereoisomers, generated from d_6-(*S*)-citronellol (C).

agreement with that of a synthesized reference compound (Wüst et al., 1998a,b). The predominant stereoisomer generated is *cis*-d_6-(2*S*,4*R*)-rose oxide **11b**, which is in agreement with a previous study by Guth (1997a) showing the same predominant stereoisomer in Scheurebe and Gewürztraminer wines. However, the enantiomeric purity of labeled *cis*-rose oxide (ratio of (2*R*,4*S*)/(2*S*/4*R*) = 10/90) is somewhat higher than in Gewürztraminer wine (ratio of (2*R*,4*S*)/(2*S*/4*R*)= 30/70). The absolute configuration at C4 of *cis* d_6-(2*S*,4*R*)-rose oxide **11b** corresponds with the absolute configuration at C3 of the generated d_6-(*S*)-citronellol **9b** (see Fig. 5). It is, therefore, highly probable that rose oxide is generated from geraniol and/or nerol by a reaction sequence, that is shown in Fig. 5. After stereoselective reduction the generated (*S*)-citronellol is cyclized to *cis*- and *trans*-rose oxide. It is reasonable to assume that the cyclization of citronellol is accomplished by the same mechanism that has been demonstrated earlier in *Pelargonium* plants (Wüst et al., 1996, 1998a,b), when rose oxide biosynthesis has been investigated using deuterium-labelled (*R*)- and (*S*)-citronellol and their corresponding glucosides. It could be shown that citronellol (3,7-dimethyl-6-octen-1-ol) is hydroxylated by a rather unspecific enzyme to yield two labile isomeric diols of which (*E*)-3,7-dimethyloct-5-en-1,7-diol can easily cyclize in acidic media to yield *cis*- and *trans*-rose oxide. Because of its instability in acidic media free (*E*)-3,7-dimethyloct-5-en-1,7-diol has not been yet detected in musts or wines. However, 3,7-dimethyloct-7-en-1,6-diol, which is also generated by hydroxylation of citronellol, could be detected in Morio-Muscat grape must and wine together with *cis*- and *trans*-rose oxide (Rapp et al., 1984). It is noteworthy that in less acidic plant material like rose petals free and glucosylated (*E*)-3,7-dimethyloct-5-en-1,7-diol could be isolated and characterized (Watanabe et al., 1998). Due to the fact that the rose oxide concentration is constantly rising during wine aging (Guth, 1998), it seems reasonable to assume that glycosylated (*E*)-3,7-dimethyloct-5-en-1,7-diol is present in grape must as well. Hydrolysis of glycosylated flavor precursors during wine ageing with subsequent formation of odiferous compounds from the liberated reactive precursors is a well known process (Strauss et al., 1986). The metabolism of d_6-geraniol **5** in grape berries is summarized in Fig. 5. It is remarkable that the main stereoisomer of rose oxide that is generated in the berry is the one with the lowest odor threshold value (*cis*-(2*S*/4*R*)-rose oxide: 0.2 ppb as determined by Guth (1997a,b)). Even a small net conversion of the precursor citronellol has therefore a significant influence on the wine aroma.

Additionally, the quantification of the enzymatic transformation of d_6-geraniol **5** into free and bound d_6-nerol **7** and d_6-(*S*/*R*)-citronellol **9** was carried out with d_6-(*R*)-linalool **19a** as internal standard. This conversion rate was measured with ripening berries at different stages of maturity as characterized by their sugar content in °Oe degrees. However, the conversion rates presented in the following section should be regarded as semi-quantitative given the rather imprecise method of precursor application. Nevertheless, the data show that d_6-geraniol was quite efficiently metabolized in the mesocarp. The total conversion rate in % (free and bound metabolites) raised from 34% in immature berries (°Oe: 14.6) to 62% in ripe berries (°Oe: 65.6). The absolute conversion rate in nmol/g berry showed a sharp increase at the end of the ripening period for all three enzymatic activities (geraniol-nerol reductase, geraniol isomerase, and geraniol-nerol-citronellol glycosidase; see Fig. 6), especially those of reductase and glycosidase. These results show that the activity of secondary transformations of monoterpenes like reduction, isomerization and glycosylation is dependent on the ripening stage and can be distinguished from the development of the primary monoterpene synthase activity by the sharp increase at the end of the ripening

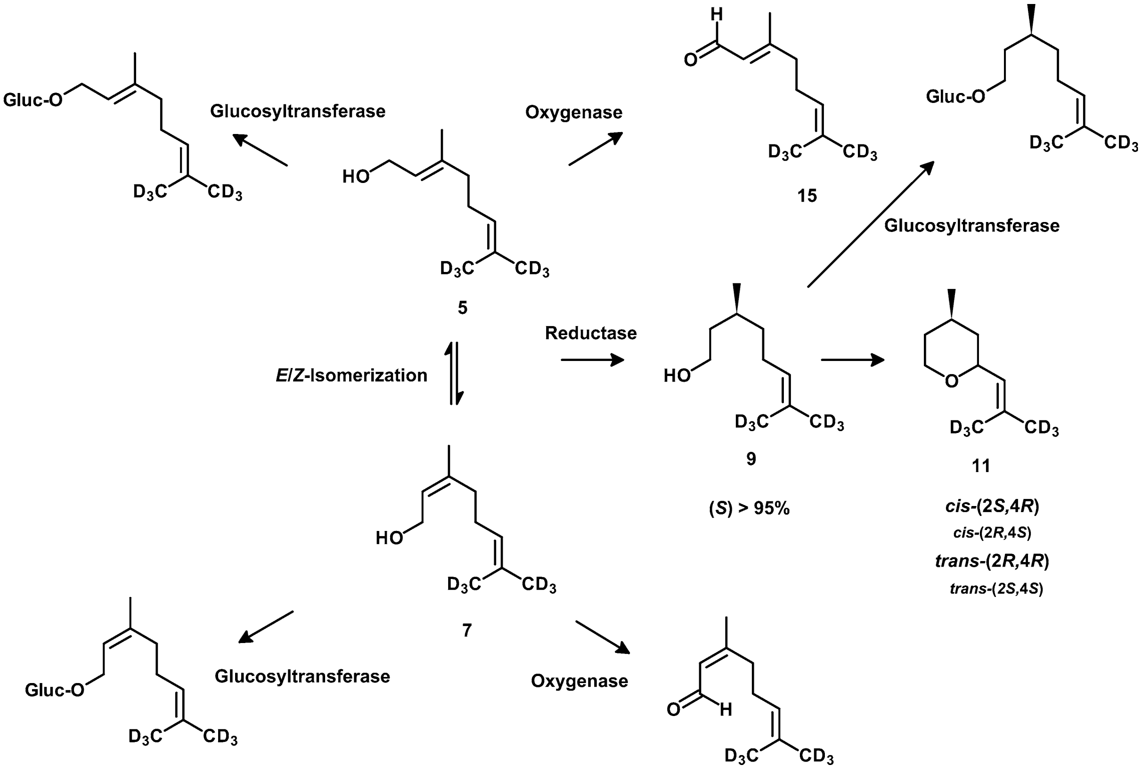

Fig. 5. Proposed metabolization scheme of d_6-geraniol.

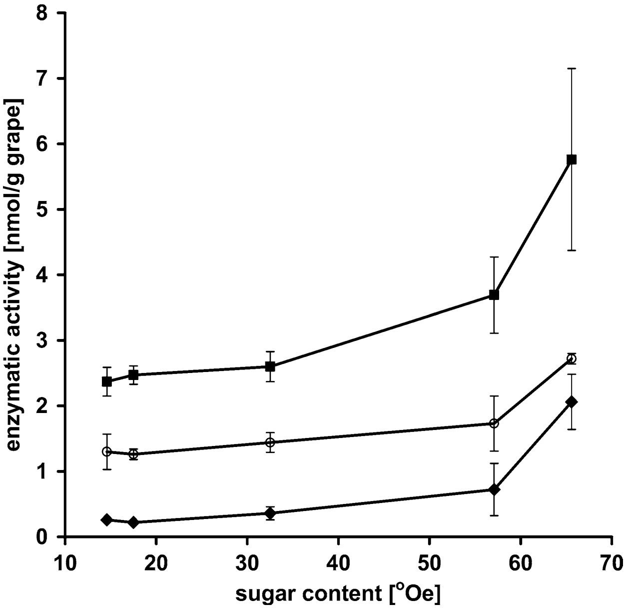

Fig. 6. Relationship between °Oe and enzymatic activity in nmol/g grape ($n = 3$ or 4): ◆, reductase activity (conversion rate of d_6-geraniol into free and bound d_6-citronellol); ○, isomerase activity (conversion rate of d_6-geraniol into free and bound d_6-nerol); ■, glycosidase activity (conversion rate of d_6-geraniol into labeled bound geraniol, nerol, and citronellol).

period. For example monoterpene synthase activity is characterized by a constant increase after the beginning of ripening, reaching its maximum at full ripeness as it has been demonstrated for linalool synthase (lis) in Muscat de Frontignan berries (Ebang-Oke et al., 2003).

3. Conclusions

The stereoselective reduction of labeled geraniol **5** with subsequent cyclization of generated d_6-(*S*)-citronellol **9b** to the potent odorant *cis*-(2*S*, 4*R*)-rose oxide **11b** has been demonstrated in Scheurebe grape berry mesocarp by in vivo feeding experiments. Additionally, glycosylation, *E*/*Z*-isomerization and oxidation were observed. The determination of conversion rates showed a sharp increase of the geraniol reductase activity towards the end of the ripening period. Since citronellol is the precursor of rose oxide it can be concluded that especially the last part of the ripening period is important for the generation of this potent odorant. This finding confirms the conclusion that a higher concentration of flavor compounds could be established in the berries by leaving the fruit on the vine for extended periods (Wilson et al., 1984).

The identification of grape mesocarp as a site of monoterpene secondary transformations is an essential result of this study and it sets the foundation for more definitive studies like the isolation, partial purification and characterization of the corresponding enzymes which is currently in progress.

4. Experimental

4.1. Plant material

Ripening grape berries (degree Oechsle between 14.6 and 65.6 as determined using an Abbé-refractometer) of *V. vinifera* L. cultivars Scheurebe were obtained from the Research Center Geisenheim, Department for Grapevine Breeding and Grafting (Geisenheim, Germany) during the vintage 2003.

4.2. In vivo feeding experiments

d_6-Geraniol **5** (10 mg/ml in DMSO), was injected into the mesocarp of ripening grape berries (5 μl/g grape) using a microliter syringe. A large part of the substrate solution was squirted out of the berry mesocarp after injection due to the pressure inside the berries and was removed by wiping off. The exact amount of labeled geraniol that rested in the berry was determined using d_6-(*R*)-linalool as internal standard as described below. The berries were incubated for 3 d in the dark at room temperature and then stored at −20 °C until used. Blank experiments were carried out with grape must and substrate: 50 g of grape berries were homogenised and the must was boiled for 10 min. After the must had cooled down d_6-geraniol **5** was added (0.5 μl/g must). The mixture was then incubated for 3 d in the dark at room temperature and then stored at −20 °C until used.

4.3. Isolation of free and glycosidically bound monoterpenes by SPE

Seeds of about 50 g grape berries were removed and the berries were ground with a mortar and pestle. During grinding the pH-value of the must was adjusted to 4 with phosphate buffer (pH 7). The mixture was completely homogenised with an Ultra Turrax blender for 2 min. After centrifugation at 3000*g* for 5 min, the supernatant was treated with Carrez I and II (Matissek et al., 1992). After filtration, the filtrate was used for the isolation of free and bound monoterpenes.

For the isolation and fractionation of the target compounds solid phase extraction was used according to the method of Mateo et al. (1997). The filtrate was passed through a 2-g C18-RP column (SUPELCO, Bellefonte, USA), previously activated with methanol. Free monoterpenes were eluted with dichloro methane and glycosides with methanol. The methanol was removed under reduced pressure, and the residue was taken up in citrate–HCl buffer (pH 4.0). The resulting solution was incubated with 2 mg of Glucanex® per gram tissue (Novo Nordisk, Dittingen, Switzerland) for 24 h at room temperature to release the glycosidically bound monoterpenes. The liberated monoterpenes were extracted with methyl *tert*-butyl ether (MTBE).

4.4. Isolation of rose oxide by SBSE

Rose oxide was extracted using the novel stir bar sorptive extraction method (SBSE; Baltussen et al. (1999)) instead of SPE because of its low concentration. Following the in vivo feeding of labeled geraniol grape berries were homogenized as described above and the juice was decanted. Rose oxide was extracted for 30 min by SBSE with stir bars obtained from Gerstel (Mühlheim Germany) under the trade name Twister® .

4.5. Determination of conversion rate and quantification of metabolites

The conversion rate of d_6-geraniol and the quantification of the generated metabolites was determined using [8,9-$^2H_6$]-(3*R*)-linalool **19a** as internal standard.

4.6. Instrumentation

4.6.1. Gas chromatography–mass spectrometry

The gas chromatography–mass spectrometry (GC–MS) analysis of the synthesised compounds was carried out with a Fisons Instruments GC 8065, coupled to a Fisons Instruments MD800 mass spectrometer. Chromatography was performed with a fused silica column (30 m × 0.25 mm i.d., 0.23 μm) coated with SE 52 and programmed from 60 (5 min isothermal) to 260 °C (20 min isothermal) at 5 °C/min with a 2 ml/min He flow rate and 20 ml/min split. Mass spectra were obtained at 70 eV with an ion source temperature of 200 °C.

4.6.2. Enantio-MDGC–MS system

The MDGC analysis was performed with a Siemens SiChromat 2 coupled to a Finnigan MAT ITD 800. The two capillary columns of the MDGC were coupled with a live-T-switching device. The pre column was the same as the column of GC–MS described above, carrier gas, H_2; split, 20 ml/min; injector temperature, 250 °C; detector, FID; oven temperature, 60 °C (5 min isothermal) to 250 °C at 5 °C/min. The chiral main column was a 30 m × 0.25 mm i.d., 0.25 μm film thickness DiAc-β (heptakis-(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin); carrier gas H_2; oven temperature, 60 °C (30 min isothermal) to 200 °C at 2 °C/min. The main column was coupled to the transfer line of the mass spectrometer, using an open split interface with a helium sweeping flow of 1 ml/min; temperature of ion trap manifold, 200 °C, temperature of transfer line: 250 °C; EI, 70 eV.

4.6.3. Enantio-SBSE–MDGC–MS system

The system has been previously described by Kreck et al. (2002).

4.6.4. ^1H NMR

The NMR spectra were obtained with a Bruker ARX 300, 300 MHz, in $CDCl_3$ with TMS as the internal standard. The chemical shifts are given in δ (ppm).

4.7. Synthesis

4.7.1. Synthesis of the precursor [8,8,8-$^2H_3$]-3-methyl-7-[$^2H_3$]methylocta-2E,6-dien-1-ol (d_6-geraniol) 5

4.7.1.1. Synthesis of 1-acetoxy-6 7-epoxy-3 7-dimethyl-octa-2-ene **2** *(Manning, 1979).* A solution containing 15.0 mmol (2.94 g) of 1-acetoxy-3,7-dimethylocta-2,6-diene **1** in 50 ml of methylene chloride was cooled to 0 °C. To this solution a suspension containing 20.2 mmol (4.37 g) of 78,7% *m*-chlorperoxybenzoic (MCPBA) in 25 ml of methylene chloride was added over 10 min. The mixture was stirred at 5 °C for 30 min and then treated with small portion of suspension of MCPBA until the starting material disappeared (TLC-control). The mixture was filtered and the filtrate was washed sequentially with aqueous $NaHCO_3$, water and brine and then dried (Na_2SO_4). The solution was concentrated to give an oil which gave 13.9 mmol (2.95 g, 92.8%) of epoxide **2**.

MS *m*/*e* (rel. int.) 152 (3), 137 (3), 109 (13), 94 (9), 85 (43), 81 (46), 79 (43), 71 (40), 59 (40), 43 (100), 41 (39).

^{1}H NMR: δ (ppm) 1.26 (*s*, 3H, 8-H, $(CH_3)_2CO$); 1.30 (*s*, 3H, 9-H, $(CH_3)_2CO$); 1.72 (*s*, 3H, 10-H, $CH_3C{=}$); 1.51–1.60 (*m*, 2H, 4-H, methylene); 2.05 (*s*, 3H, 12-H, OAc); 2.10–2.23 (*m*, 2H, 5-H, methylene); 2.68–2.72 (*t*, 1-H, 6H, HCO), 4.57–4.60 (*d*, 2H, 1-H, CH_2OAc); 5.35-5.41 (*t*, 1H, 2-H, $CH_2{=}$).

4.7.1.2. Synthesis of 6-acetoxy-4-methyl-4-hexenal **3** *(Manning, 1979).* To the solution of 1.70 g (8.0 mmol) of 1-acetoxy-6,7-epoxy-3,7-dimethylocta-2-ene **2** in 30 ml of anhydrous diethyl ether a solution containing 2.0 g (8.8 mmol) of periodic acid in 30 ml THF was added slowly with vigorous stirring. The resulting mixture was stirred for 90 min at room temperature. The mixture was quenched with ice water. The organic layer was separated and washed with aqueous $NaHCO_3$, water, and brine. The organic layer was dried and concentrated under reduced pressure to yield 1.0 g (5.9 mmol, 73.6%) of title compound **3**. This oil was used for the next step without purification.

MS *m*/*e* (rel. int.) 126 (23), 110 (34), 84 (96), 81 (49), 79 (26), 67 (44), 55 (30), 43 (100), 41 (35).

^{1}H NMR: δ (ppm) 1.72 (*s*, 3H, 7-H, $CH_3C{=}$); 2.05 (*s*, 3H, 9-H, OAc); 2.35–2.40 (*t*, 2H, 2-H, methylene); 2.55–2.60 (*t*, 2H, 3-H, methylene); 4.56–4.58 (*d*, 2H, 6-H, CH_2COAc); 5.32–5.38 (*t*, 1H, 5-H, $CH_2{=}$).

4.7.1.3. Synthesis of [1,1,1,3,3,3-$^2H_6$]-[2-$^2H_1$]isopropyl triphenylphosphonium bromide (Pyun et al., 1993). A mixture of 5.54 g (21.1 mmol) of triphenylphosphine and 2.5 g (19.2 mmol) of 2-bromo-[1,1,1,2,3,3,3,3-$^2H_7$]-propane was heated at 150 °C for 40 h in a special thick wall tube with a sealed scew cap. Recrystallization of the crude product with diethyl ether/ethanol (3:2 v/v) yielded 6.53 g (86.7%) of the title compound; mp of the resulting crystals: 239–240 °C (lit. 238–239 °C for the unlabeled compound (Fagerlund and Idler, 1957)).

4.7.1.4. Synthesis of [8,8,8-$^2H_3$]-1-acetoxy-3-methyl-7-[$^2H_3$]methylocta-2E,6-diene **4** *(Pyun et al., 1993).* A solution of 1.6 M *n*-butyllithium in hexane (3.7 ml, 5.88 mmol) was added dropwise to a suspension of 2.31 g (5.88 mmol) [1,1,1,3,3,3-$^2H_6$]-[2-$^2H_1$]-isopropyl triphenylphosphoniumbromide in THF in ice-bath under N_2-atmosphere. After 1 h at room temperature 1.0 g (5.88 mmol) of 6-acetoxy-4-methyl-5-hexenal **3** was added to the solution at 0 °C. After 3 h stirring at room temperature, the solution was quenched by adding 1.2 ml of MeOH. Solvent removed under reduced pressure gave the crude product. The residue was extracted three times with hexane by refluxing. The combined hexane layer was concentrated and the residue was purified by flash chromatography (pentane/diethylether 3:1 v/v) to give 531 mg (44.6%) of the title compound **4**.

MS *m*/*e* (rel. int.) 160 (1, $M^+ - OAc$), 142 (12), 124 (12), 93 (39), 80 (20), 75 (100), 67 (28), 43 (68).

^{1}H NMR: δ (ppm) 1.70 (*s*, 3 H, 10-H, $CH_3C{=}$); 2.06 (*s*, 3H, 12-H, OAc); 2.05–2.13 (*m*, 4H, 4-H, 5-H, methylene); 4.57–4.58 (*d*, 2H, 1-H, CH_2COAc); 5.06–5.10 (*t*, 1H, 6-H, $CH_2{=}C(CD_3)_2$); 5.32–5.37 (*t*, 1H, 2-H, $CH{=}$).

4.7.1.5. Synthesis of [8,8,8-$^2H_3$]-3-methyl-7-[$^2H_3$]methylocta-2E,6-dien-1-ol (d_6-geraniol) **5** *(Streitwieser et al., 1994).* [8,8,8-$^2H_3$]-1-Acetoxy-3-methyl-7-[$^2H_3$]methylocta-2,6-diene **4** (531 mg, 2.62 mmol) were dissolved in 15 ml of dry methanol. To the solution 400 mg of anhydrous K_2CO_3 was added. The reaction mixture was stirred for 4 h at room temperature. After addition of 25 ml diethyl ether, the mixture was washed with water and brine. After drying the organic layer was removed under reduced pressure. The residue was purified by flash chromatography (pentane/diethylether 3:1 v/v) to give 310 mg (73.8%) of d_6-geraniol **5**.

MS *m*/*e* (rel. int.) 160 (1, M^+), 142 (7), 124 (9), 93 (46), 80 (18), 75 (100), 71 (11), 67 (11), 44 (48).

^{1}H NMR: δ (ppm) 1.68 (*s*, 3H, 10-H, $CH_3C{=}$); 1.99–2.17 (*m*, 4H, 4-H, 5-H, methylene); 3.21 (*s*, 1H, OH); 4.09–4.16 (*d*, 2H, 1-H, CH_2OH); 5.07–5.11 (*t*, 1H, 6-H, $CH_2{=}C(CD_3)_2$); 5.39–5.44 (*t*, 1H, 2-H, $CH{=}$).

4.7.2. Synthesis of the internal standard [8,8,8-$^2H_3$]-(3R)-methyl-7-[$^2H_3$]methylocta-1,6-dien-3-ol (d_6-(3R)-linalool) 19a

The internal standard d_6-(3*R*)-linalool **19a** was prepared as described for the synthesis of d_6-geraniol **5.**

(3*R*)-Linalyl acetate ((3*R*)-3-acetoxy-3,7-dimethylocta-1,6-diene), synthesised from (3*R*)-linalool according to the method of Vidari et al. (1999), was used as starting material.

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