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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

Fang Luan [a], Armin Mosandl [a], Andreas Münch [a], Matthias Wüst [b,*]

[a] *Institut für Lebensmittelchemie, Johann Wolfgang Goethe-Universität, Marie-Curie-Strasse 9, D-60439 Frankfurt (Main), Germany*
[b] *University of Applied Sciences Valais, Department of Life Technologies, Route du Rawyl 47, CH-1950 Sion 2, Switzerland*

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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)

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).

* Corresponding author. Tel.: +41 27 606 86 54; fax: +41 27 606 85 15.
E-mail address: matthias.wuest@hevs.ch (M. Wüst).

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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-

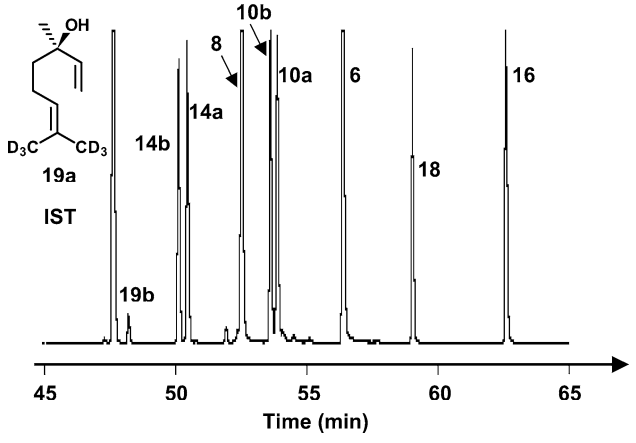

Fig. 1. Chromatogram of chiral and nonchiral reference compounds, using enantio-MDGC–MS (main column Di-Ac-β-CD): geraniol (**6**); nerol (**8**); geranial (**16**); neral (**18**); Chiral compounds are well differentiated into their enantiomers: (3*R*)-citronellol (**10a**); (3*S*)-citronellol (**10b**);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