JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Enantioselective Analysis of Free and Glycosidically Bound Monoterpene Polyols in *Vitis vinifera* L. Cvs. Morio Muscat and Muscat Ottonel: Evidence for an Oxidative Monoterpene Metabolism in Grapes

FANG LUAN,† DANIELA HAMPEL,† ARMIN MOSANDL,† AND MATTHIAS WÜST*,§

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

The enantiomeric ratios of various free and glycosidically bound monoterpene polyols in musts of the aromatic grapes *Vitis vinifera* L. cvs. Morio Muscat and Muscat Ottonel were determined by means of enantioselective multidimensional gas chromatography−mass spectrometry. Reference compounds of defined stereochemistry were synthesized and permitted the unequivocal determination of the elution order of the target compounds on chiral columns with modified cyclodextrins as stationary phases. It could be shown for the first time that the linalool-derived polyol 3,7-dimethylocta-1,7-dien-3,6-diol occurs predominately as the (3*S*,6*S*)-configured stereoisomer, providing evidence that this compound is generated by an enzymatic process in grape berries. The involvement of a cytochrome P450 monooxygenase in the oxidative metabolism of monoterpenes in grapes is dicussed.

KEYWORDS: ***Vitis vinifera* L.; monoterpene polyols; oxidative metabolism; biosynthesis; enantioselective multidimensional gas chromatography−mass spectrometry (enantio-MDGC/MS)**

INTRODUCTION

Monoterpenes, which are genuine compounds in grape berries (*Vitis vinifera*), belong to the most important flavor compounds of several white wine varieties such as Muscat, Gewürztraminer, and Scheurebe and are responsible for their characteristic floral aroma (*1*). Besides linalool **1**, nerol, and geraniol, several highly odiferous cyclic ethers and lactones have been identified as key compounds that are generated by cyclization of oxygenation products from these monoterpene alcohols (see **Figure 1** for linalool oxygenation products). An example is the formation of wine lactone **13** from acid-catalyzed cyclization of the linalool derivative **12** (*2*) (see **Figure 1**).

Recently, it was shown by in vivo feeding experiments that the novel 1-deoxy-D-xylulose 5-phosphate/2*C*-methyl-D-erythritol-4-phosphate pathway is the dominant metabolic route for de novo monoterpene biosynthesis in grape berry exocarp and mesocarp (*3*). However, the oxygenation process of monoterpenes that leads to the formation of reactive higher oxidation state intermediates is poorly understood. The opinion, long held by viticulturists, that bunches of grapes exposed to sunlight during growth are more flavorful than fruit not exposed has resulted in the postulation of a photooxidation mechanism involving an ene-type addition of singlet oxygen to give hydroperoxides (*4*). However, vineyard experiments using the bunch-shading approach were rather inconclusive, because shading generally causes a delay of ripening and explains some differences in aroma levels insofar as their levels were shown to increase during ripening in previous studies (*5*). Whereas terpene biochemistry in fruit is largely unexplored, detailed studies have been carried out on essential oil producing plants such as mint (*Mentha piperita*) and sage (*Salvia officinalis*) that exhibit specialized oil-producing tissues (glandular trichomes) (*6*). Despite morphological and physiological differences, it seems reasonable to assume that the basic principles of monoterpene biochemistry in essential oil producing plants and fruits are closely related. It is important to note that cytochrome P450 monooxygenases have been shown to play a central role in monoterpene oxygenation in several plants (*7*). Detailed studies on the isolation, cloning, and structure−function relationships on P450 enzymes have been carried out and give evidence of a remarkable regio- and stereoselectivity of the oxygenation reaction catalyzed by these enzymes (*8*, *9*). It is the high degree of stereoselectivity that distinguishes an enzymatic oxygenation from a nonselective photooxygenation. Consequently, a stereoselective analysis of the oxgenation products should be a suitable probe for distinguishing between enzymatic and nonenzymatic reactions. However, such a stereoselective analysis of monoterpene oxygenation products in grapevine has not been performed. Despite the fact that numerous oxygenation products of linalool **1** have been detected in musts (see **Figure 1**), no information on the stereochemistry of the generated chiral

* Corresponding author (telephone +41-27-606-86-54; fax +41-27-606-85-15; e-mail matthias.wuest@hevs.ch).
† Johann Wolfgang Goethe-Universität.
§ University of Applied Sciences Valais.

10.1021/jf030701q CCC: $27.50
Published on Web 03/09/2004

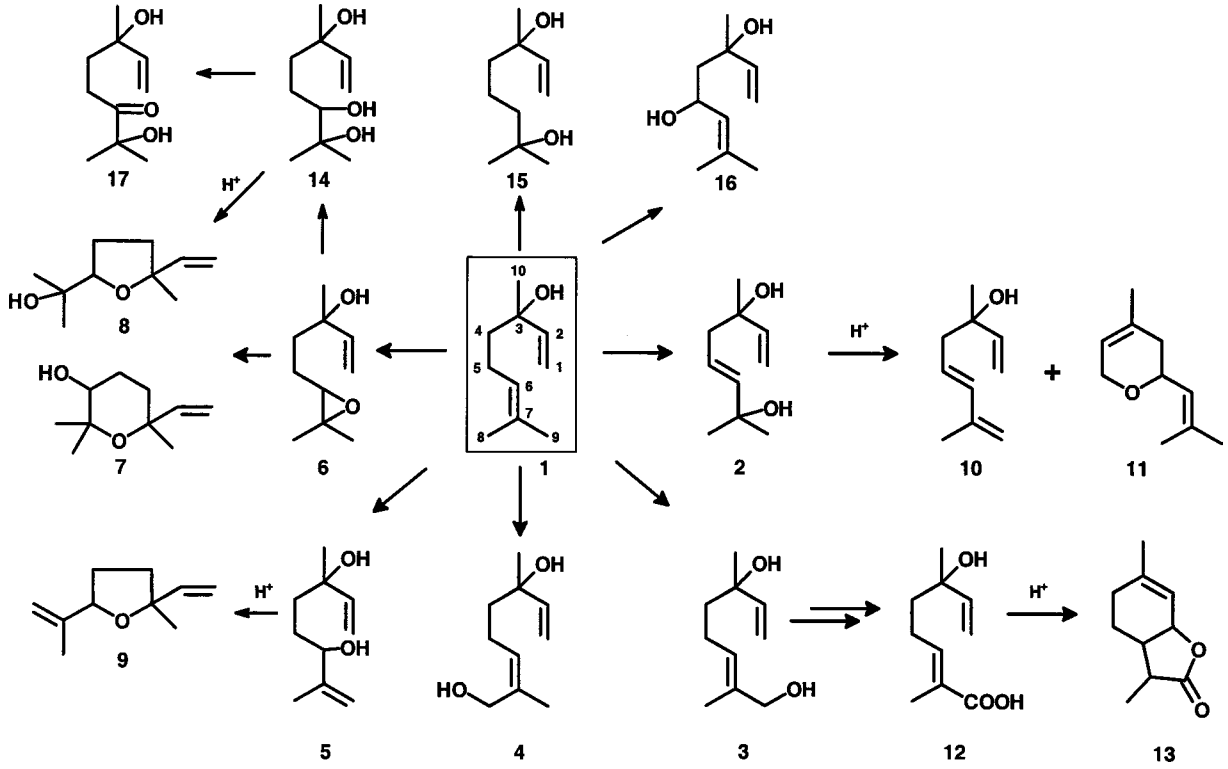


Figure 1. Oxygenation products of linalool.

centers in compounds **5**, **14**, and **16** is available in the literature. To fill this gap and to clarify the mechanism of monoterpene oxygenation in grapevine, we have determined the absolute configuration and enantiomeric ratios of several free and gylcosidically bound monoterpene polyols in musts of several aromatic white varieties. The results give evidence for the presence of a monoterpene oxygenase activity in grape berries.

EXPERIMENTAL PROCEDURES

Plant Material. Ripe grapes (degree Oechsle between 75 and 89) of *Vitis vinifera* cvs. Morio Muscat and Muscat Ottonel from vintages 2000 and 2001 were obtained from the Geisenheim Research Center, Department for Grapevine Breeding and Grafting, Geisenheim, Germany, and were stored at −20 °C until used.

Isolation of Free and Glycosidically Bound Monoterpenes. Seeds of ∼50 g of grape berries were removed, and the berries were ground with a mortar and pestle. During grinding the pH of the must was adjusted to 4 with phosphate buffer (pH 7). The mixture was completely homogenized 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 (*10*). 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 (*11*). The filtrate was passed through a 2-g C18-RP column (Supelco, Bellefonte, PA), previously activated with methanol. Free monoterpenes were eluted with methylene chloride 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/g of 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).

Synthesis of Reference Compounds. *Synthesis of (3R,6S)-3,7-Dimethylocta-1,7-dien-3,6-diol (**5**). (a) Synthesis of (3R)-3-Acetoxy-3,7-dimethylocta-1,6-diene.* The acetylation was carried out according to the method by Vidari (*12*) with some modifications: the crude product was purified by bulb-to-bulb distillation (90 °C, 0.1 mbar) to afford (3*R*)-3-acetoxy-3,7-dimethylocta-1,6-diene as a light yellow oil from (3*R*)-linalool **1**.

(b) Synthesis of (2E,6R)-6-Acetoxy-2,6-dimethylocta-2,7-dien-1-ol. The synthesis was carried out according to the method of Schwab (*13*).

(c) Synthesis of (2S,3S,6R)-6-Acetoxy-2,6-dimethylocta-2,3-epoxy-7-en-1-ol. The epoxide was prepared as previously described (*14*).

(d) Synthesis of (3R,6S)-3-Acetoxy-3,7-dimethylocta-1,7-dien-6-ol. The synthesis was carried out according to the method of Liu et al. (*14*).

*(e) Synthesis of (3R,6S)-3,7-Dimethylocta-1,7-dien-3,6-diol (**5**).* The diol was prepared as previously described (*15*).

The MS and ^{1}H NMR data of the synthesized compounds were in all cases identical with the data given in the references cited above.

Instrumental Methods. *Gas Chromatography/Mass Spectrometry (GC/MS).* The GC/MS analysis was performed on a Fisons Instruments GC 8000, coupled to a Fisons Instruments MD800 mass spectrometer, equipped with a fused silica column (30 m × 0,25 mm; coated with SE 52; film thickness = 0.25 μm). GC conditions were as follows: carrier gas, helium at 50 kPa; split, 20 mL/min; injector temperature, 230 °C; oven temperature, 40 °C (5 min isothermal) raised to 260 °C (20 min isothermal) at 5 °C/min with a 2 mL/min flow rate. Mass spectra were obtained at 70 eV with an ion source temperature of 200 °C and mass range of 40−250 amu.

Enantio-MDGC/MS System 1. The enantio-MDGC/MS analysis was performed with a Siemens SiChromat 2-8 coupled with a Finnigan MAT GCQ. The two capillary columns of the MDGC were coupled with a live-T-switching device. The precolumn was identical with the column of the GC/MS system described above; carrier gas, helium; split, 20 mL/min; injector temperature, 250 °C; detector FID, 250 °C; oven temperature, 60 °C (5 min isothermal) raised to 250 °C at 5 °C/min. The main column used was a 30 m × 0.25 mm i.d., 0.25 μm film thickness [heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin] (DiMe-β) in SE 52; carrier gas, helium; oven temperature, 60 °C (25 min isothermal) raised 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 sweeping flow of 1 mL/min; temperature of the transfer line, 250 °C; temperature of ion trap manifold, 170 °C; EI, 70 eV.

Enantio-MDGC/MS System 2. Most conditions of enantio-MDGC-MS-2 were identical with those of enantio-MDGC-MS-1. The differ-

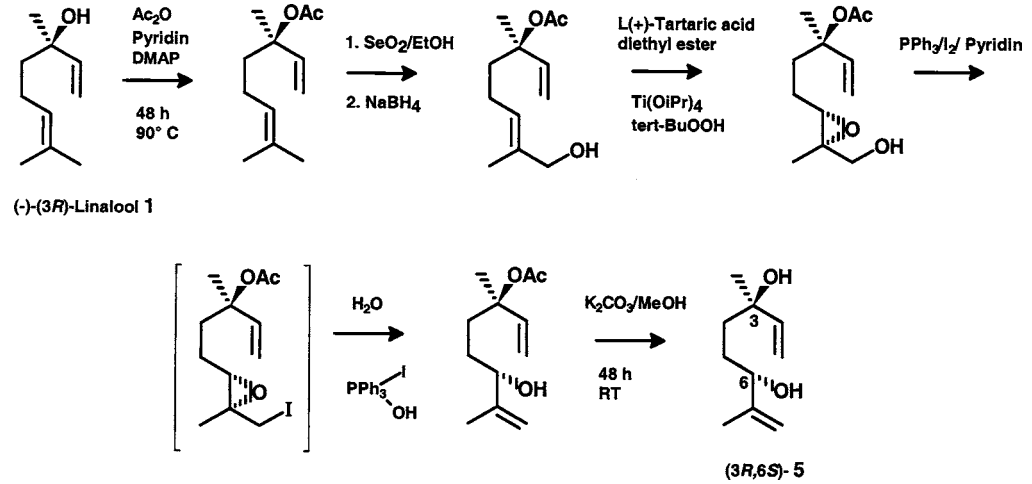
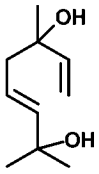
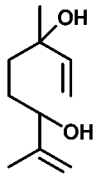
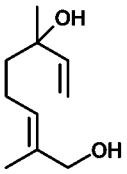
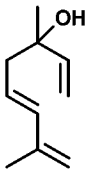
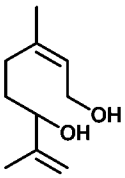
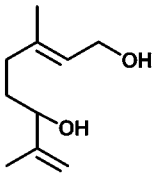


Figure 2. Stereoselective synthesis of (3*R*,6*S*)-3,7-dimethylocta-1,7-dien-3,6-diol (**5**).

Table 1. Determination of the Elution Order of Synthetic Polyol Stereoisomers on Two Chiral Stationary Phases

column	order of elution					
						
	2	**5**	**3**	**10**	**19**	**18**
DiMe-β	(3R) (I)[a] (3S) (II)	(3R,6S)+(3R,6R) (I + II) (3S,6S) (III) (3S,6R) (IV)	not resolved	(3R) (I) (3S) (II)	(3R) (I) (3S) (II)	(3R) (I) (3S) (II)
DiAc-β	not resolved	(3R,6S)+(3S,6S) (I +II) (3R,6R) (III) (3S,6R) (IV)	(6S) (I) (6R) (II)	(3R) (I) (3S) (II)	no baseline resolution	no baseline resolution

[a] Elution order is indicated by Roman numerals.

ences were as follows: for MDGC, the Siemens SiChromat 2 main column was coated with [heptakis-(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin] (DiAc-β) in SE 52, the carrier gas was H_2, and the oven temperature was 60 °C (30 min isothermal) raised to 200 °C at 2 °C/min; for MS, a Finnigan MAT ITD800 was used.

^{1}H NMR. The NMR spectra were obtained with a Bruker ARX 300, at 300 MHz, in $CDCl_3$ with TMS as the internal standard. The chemical shifts are given in δ (parts per million)

RESULTS AND DISCUSSION

To clarify the mechanism of the oxygenation reaction that leads to the formation of monoterpene polyols in grapes, we have focused our attention on polyols where the introduction of oxygen creates a new chiral center in the molecule. We have chosen three polyols that are derived from linalool **1**, geraniol, and nerol, which appear in musts in substantial amounts: 3,7-dimethylocta-1,7-dien-3,6-diol (**5**), (*E*)-3,7-dimethylocta-3,7-dien-1,6-diol (**18**), and (*Z*)-3,7-dimethylocta-3,7-dien-1,6-diol (**19**) (*16*). Racemic reference compounds were prepared using literature procedures (*17*, *18*), and compounds with defined absolute configurations at C6 were obtained via the rearrangement of enantiopure 2,3-epoxy alcohols, with the system Ph_3P, pyridine, I_2 and water (*14*) [see **Figure 2** for the synthesis of (3*R*,6*S*)-**5**; enantiopure **18** and **19** were prepared in an analogous manner using geranyl and neryl acetate, respectively, as starting materials]. Thus, the order of elution of the enantiomers of **5**, **18**, and **19** could be determined unequivocally on two chiral stationary phases by co-injection of enantiopure and racemic standards (see **Table 1**). As a result, heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodexrin (Dime-β) shows excellent selectivity as a chiral stationary phase and allows the simultaneous separation of almost all monoterpene polyols that were tested. However, the (3*R*)-configured diastereomeric polyols of **5** as well as the enantiomers of (*E*)-2,6-dimethylocta-2,7-dien-1,6-diol (**3**) could not be separated on heptakis(2,3-

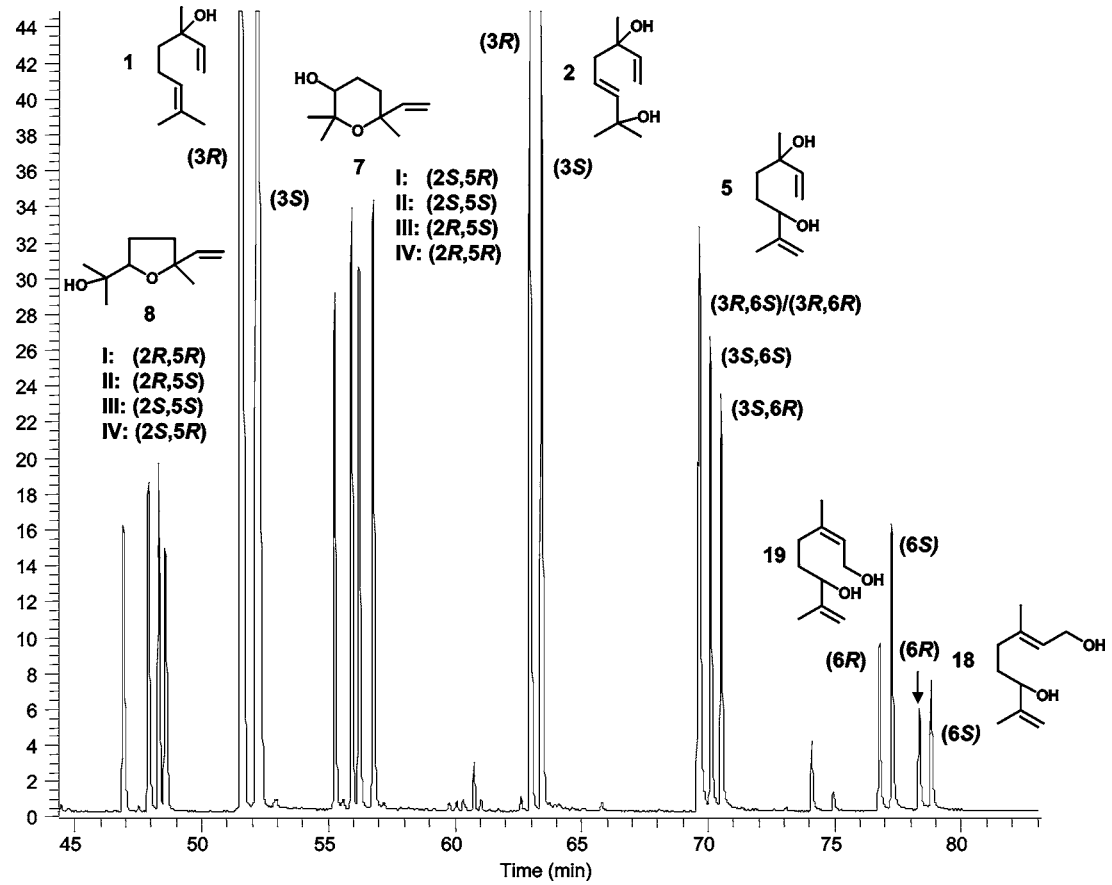


Figure 3. Chiral main column chromatogram (enantio-MDGC/MS-1) of racemic standard compounds **8**, **1**, **7**, **2**, **5**, **19**, and **18**. The elution order of linalool oxide stereoisomers is indicated by Roman numerals.

di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin but could be on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin. These two chiral stationary phases are therefore valuable tools for the enantioselective analysis of monoterpene polyols. The simultaneous enantioselective analysis of linalool **1** and the polyols **2**, **5**, **18**, and **19** was achieved by using an enantio-MDGC/MS system equipped with an achiral precolumn and a chiral main column (Dime-β) (enantio-MDGC/MS-1). The system has been described under Experimental Procedures. **Figure 3** shows a standard chromatogram of the chiral main column including the furanoid and pyranoid linalool oxides.

For the isolation and fractionation of free and glycosidically bound monoterpenes solid phase extraction was used and the glycosidically bound monoterpenes were enzymatically liberated (*11*). The fractions were analyzed using the enantio-MDGC/MS system described above (enantio-MDGC/MS-1). A typical main column chromatogram of free and glycosidically bound polyols is shown in **Figure 4**. The results for two different aromatic white wine varieties (Morio Muscat and Muscat Ottonel) are presented in **Table 2**. As expected (*19*, *20*), the enantiomeric purity of free linalool **1** was >90% in favor of the *S*-configured enantiomer in both varieties. The glycosidically bound linalool shows a somewhat lower purity (<90%), which might be attributed to a slight enantiodiscrimination during the enzymatic glycosidation process *in planta* or to a slight enantiodiscrimination during hydrolysis with the glucanase used to hydrolyze the isolated glycosides. These results are in good agreement with previous studies (*19*, *20*). In this context it is noteworthy that the enantiomeric ratio of free linalool in must decreases with time because of acid-catalyzed dehydration/hydration (*21*) and formation of racemic linalool from grape geraniol or geraniol glycosides. In general, all allylic alcohols are unstable in acidic solutions. It is therefore necessary to carry out the isolation of monoterpenes immediately after homogenization of the grape material.

Because the diols **2** and **5** are directly derived from linalool **1** by oxygenation, the (3*R*)-configured stereoisomers of **2** and **5** are clearly dominating. Free **2** and glycosidically bound diol **2** show enantiomeric purities of >98%. Concerning diol **5**, the (3*R*)-configured stereoisomers are barely detectable. In both varieties the diastereomeric purity of free diol **5** is >90%, whereas the diastereomeric purity of glycosidically bound diol **5** is considerably lower. Again, this might be attributed to a discrimination during gylcosidation or to a slight enantiodiscrimination during hydrolysis with the glucanase used to hydrolyze the isolated glycosides. However, the clear dominance of the (3*S*,6*S*)-configured stereoisomers of free and glycosidically bound **5** shows that the introduction of oxygen at C6 of linalool is a stereoselective process. A photooxygenation mechanism can therefore be excluded, because the photooxygenation of racemic or enantiopure linalool yields a 1:1 mixture of the (6*R*)- and (6*S*)-configured stereoisomers (data not shown). The stereoselective generation of the predominant (3*S*,6*S*)-**5** gave clear evidence for an enzymatic oxygenation of linalool. Because the oxygenation at C6 is accompanied by an allylic rearrangement to yield a C7−C8 double bond, the generation of an intermediate radical can be assumed. The appearance of such an intermediate radical is a known feature of cytochrome P450 catalyzed oxygenations and is generated by abstraction of a hydrogen radical (*22*). The hydrogen radical is accepted by the reactive iron-oxo species of the activated cytochrome P450 enzyme to form an iron-bound hydroxy radical. If the generated radical of the substrate molecule is in conjugation with a double bond, allylic rearrangement can occur before the iron-bound

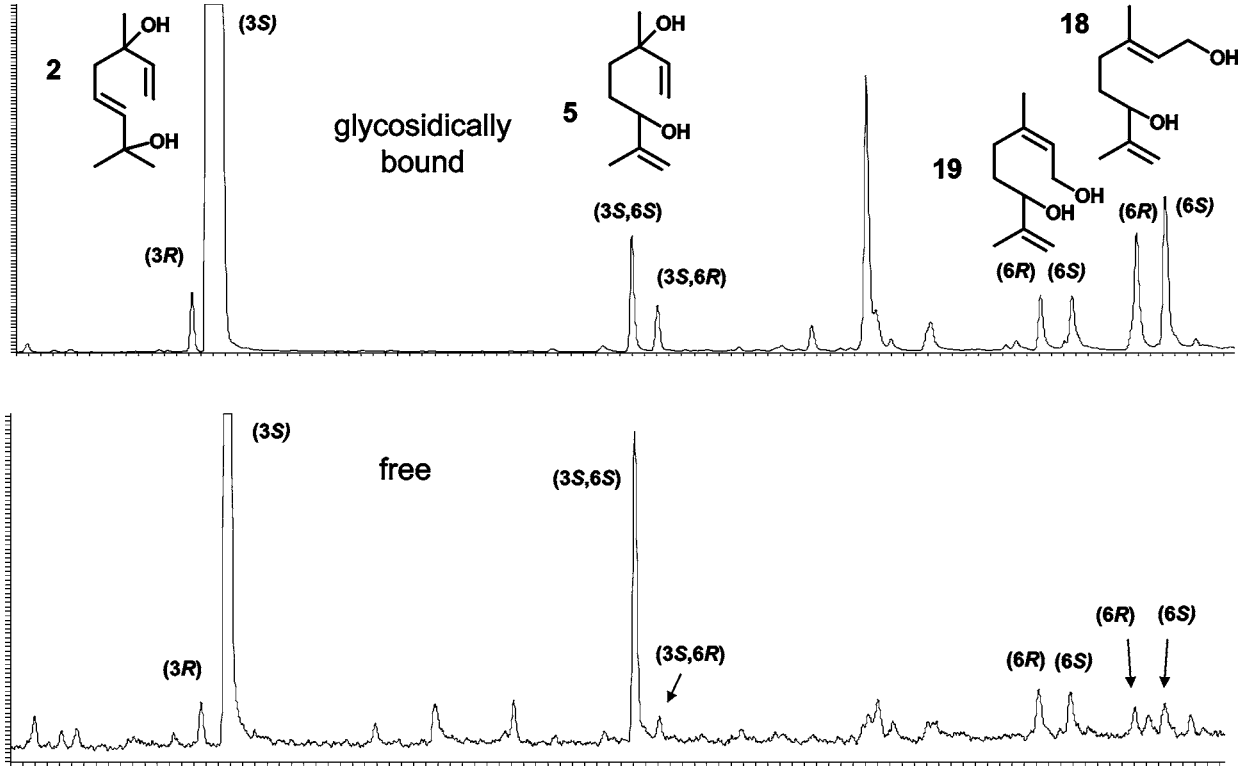
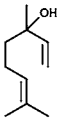
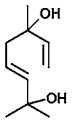
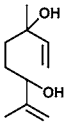
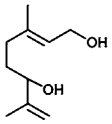
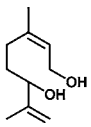


Figure 4. Chiral main column chromatogram (enantio-MDGC-MS-1) of free and glycosidically bound polyols **2**, **5**, **18**, and **19**.

Table 2. Enantiomeric Ratio of Free and Glycosidically Bound Polyols in *V. vinifera* Cv. Morio Muscat and Muscat Ottonel

enantiomeric ratio		Morio Muskat		Muscat Ottonel	
		free	bound	free	bound
	3S/3R-**1**	94.0/6.0	89.0/11.0	99.8/0.2	87.3/12.7
	σ	4.0	4.0	0.5	3.9
	3S/3R-**2**	99.7/0.3	98.9/1.1	99.4/0.6	99.2/0.8
	σ	0.5	0.6	1.3	0.8
	3S,6S/6R-**5**	98.8/1.2	70.6/29.4	90.8/9.2	54.6/45.4
	σ	1.5	6.6	4.9	13.4
	6S/6R-**18**	n.d.	63.5/36.5	n.d.	54.6/45.4
	σ		10.5		13.4
	6S/6R-**19**	n.d.	44.0/56.0	n.d.	n.d.
	σ		5.3		

hydroxy radical is captured by the substrate. This mechanism is called the oxygen rebound mechanism, and the allylic rearrangement has been called allylic scrambling. These mechanisms have been already described for several allylic hydroxylations of unsaturated monoterpenes (*8*, *23*). **Figure 5** illustrates the putative cytochrome P450 catalyzed hydroxylation of (*S*)-linalool. After hydrogen radical abstraction from one of the terminal methyl groups (C8 or C9), allylic rearrangement occurs. The oxygen rebound can take place from the *Si* or the *Re* site of the plane that is defined by C6, C7, C8, and C9 of the substrate molecule in the active site of the enzyme. Because the (6*S*)-configured diol **5** is predominately generated, the oxygen rebound takes place preferentially from the *Si* site.

It is remarkable that the geraniol- and nerol-derived glycosidically bound diols **18** and **19** are present as almost racemic mixtures. Free **18** and **19** were barely detectable, and their peak areas could not be properly integrated. At this stage the low stereoselectivity of the oxygenation reaction of geraniol and

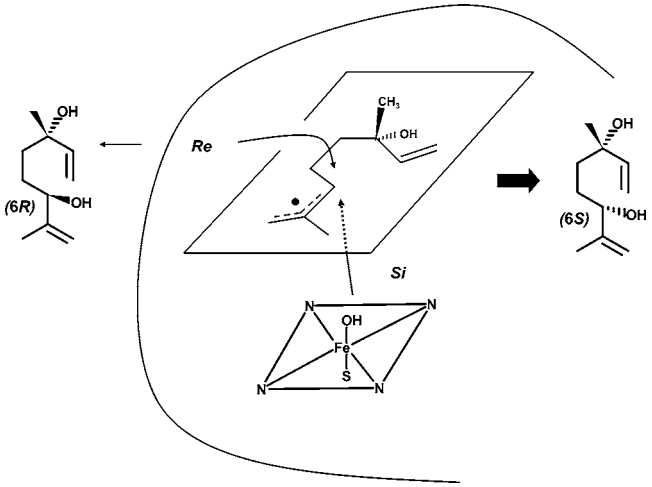


Figure 5. Putative cytochrome P450 catalyzed hydroxylation of (*S*)-linalool. After hydrogen radical abstraction from one of the terminal methyl groups (C8 or C9), allylic rearrangement occurs. The oxygen rebound can take place from the *Si* or *Re* site of the plane that is defined by C6, C7, C8, and C9 of the substrate molecule in the active site of the enzyme. Because the (6*S*)-configured diol **5** is predominately generated, the oxygen rebound takes place preferentially from the *Si* site.

nerol cannot be explained in detail. Possible explanations are a low streoselectivity of the enzyme toward nerol and geraniol and/or a competing nonspecific oxygenation that is induced by free radicals or light.

To investigate the oxygenation reaction in more detail, in vivo feeding studies with deuterium-labeled linalool and exclusion of light are necessary. The first preliminary results that were obtained by using this approach confirm the above postulated enzymatic oxygenation of monoterpenes in grape berries and will be published elsewhere.

ACKNOWLEDGMENT

We gratefully thank Günter Maier, Novo Nordisk, for supplying Glucanex and Ernst Rühl, Department for Grapevine Breeding and Grafting, Geisenheim, for making grape wines available.

LITERATURE CITED

(1) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022−3026.

(2) Bonnländer, B.; Baderschneider, B.; Messerer, M.; Winterhalter, P. Isolation of two novel terpenoid glucose esters from Riesling wine. *J. Agric. Food Chem.* **1998**, *46*, 1474−1478.

(3) Luan, F.; Wüst, M. Differential Incorporation of 1-Deoxy-D-xylulose into (3*S*)-Linalool and Geraniol in Grape Berry Exocarp and Mesocarp. *Phytochemistry* **2002**, *60*, 451−459.

(4) Williams, P. J.; Strauss, C. R.; Wilson, B.; Dimitriadis, E. Origins of some volatile monoterpenes and nor-isoprenoids in grapes and wines—biosynthetic and biogenetic considerations. In *Topics in Flavour Research*; Berger et al., Eds.; Eichhorn Verlag: Marzling-Hangenham, Germany, 1985; pp 336−352.

(5) Bureau, S.; Baumes, R. L.; Razungles, A. J. Effects of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera* L. cv. Syrah. *J. Agric. Food Chem.* **2000**, *48*, 1290−1297.

(6) Wise, M. L.; Croteau, R. Monoterpene biosynthesis. In *Comprehensive Natural Products Chemistry*; Barton et al., Eds.; Elsevier: New York, 1999; Vol. 2, pp 97−153.

(7) Schuler, M. Plant cytochrome P450 monooxygenases. *CRC Crit. Rev Plant Sci.* **1996**, *15*, 235.

(8) Wüst, M.; Little, D. B.; Schalk, M.; Croteau, R. Hydroxylation of limonene enantiomers and analogs by recombinant (−)-limonene 3- and 6-hydroxylases from mint (*Mentha*) species: Evidence for catalysis within sterically constrained active sites. *Arch. Biochem. Biophys.* **2001**, *387*, 125−136.

(9) Wüst, M.; Croteau, R. Hydroxylation of specifically deuterated limonene enantiomers by cytochrome P450 limonene-6-hydroxylase reveals mechanism of multiple product formation. *Biochemistry* **2002**, *41*, 1820−1827.

(10) Matissek, R.; Schnepel, F.; Steiner, G. *Lebensmittelanalytik*; Springer-Verlag: Berlin, Germany, 1992; p 128.

(11) Mateo, J. J.; Gentilini, N.; Huerta, T.; Jiménez, M.; Di Stefano, R. Fractionation of glycoside precursors of aroma in grapes and wine. *J. Chromatogr. A* **1997**, *778*, 219−224.

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

(13) Schwab, W. *Untersuchung über nichtflüchtige Metabolite pflanzlicher Aromastoffe*; Julius-Mayimilians-Universität: Würzburg, 1989; pp 183−184.

(14) Liu, Z.; Lan, J.; Li, Y.; Xing, Y.; Cen, W. Total partial synthesis of (3S,6S)-(+)-3,7-dimethyl-6-hydroxy-3-acetoxyocta-1,7-diene and (3S,6S)-(−)-3,7-dimethylocta-1,7-diene-3,6-diol from geraniol. *J. Chem. Res. (S)* **1999**, 324−325.

(15) Schneider, O. *Ananlysis of Chiral Organic Molecules*; de Gruyter Verlag: Berlin, Germany, 1995; p 182.

(16) Mateo, J. J.; Jiménez, M. Monoterpenes in grape juice and wines. *J. Chromatogr. A* **2000**, *881*, 557−567.

(17) Williams, P. J.; Strauss, C. R.; Wilson, B. New linalool derivatives in Muscat of Alexandria grapes and wines. *Phytochemistry* **1980**, *19*, 1137−1139.

(18) Strauss, C. R.; Wilson, B.; Williams, P. J. Novel monoterpene diols and diol glycosides in *Vitis vinifera* grapes. *J. Agric. Food Chem.* **1988**, *36*, 569−573.

(19) Doglia, G. The enantiomers of linalool in Asti Muscat. *Riv. Vitic. Enol.* **1993**, 23−36.

(20) Garcia Moruno, E. The chirality of α-terpineol in aromatic wines. Detection of chiral or recemic linalool addition in wines. *Sci. Aliments* **1999**, *19*, 207−214.

(21) Askari, C.; Hener, U.; Schmarr, H.-G.; Rapp, A.; Mosandl, A. Stereodifferentiation of some chiral monoterpenes using multidimensional gas chromatography. *Fresenius' J. Anal. Chem.* **1991**, *340*, 768−772.

(22) Groves, J. T.; Han, Y.-Z. Models and mechanism of cytochrome p450 action. In *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 2nd ed.; Ortriz de Montellano, P., Ed.; Plenum Press: New York, 1995; pp 3−48.

(23) Groves, J. T.; Subramanian, D. V. Hydroxylation by cytochrome p-450 and metalloporphyrin models. Evidence for allylic rearrangement. *J. Am. Chem. Soc.* **1984**, *106*, 2177−2181.

Received for review October 12, 2003. Revised manuscript received January 9, 2004. Accepted January 11, 2004. Financial support by the Deutsche Forschungsgemeinschaft (DFG), the Fonds der Chemischen Industrie, and Graduiertenförderungsstelle of Johann Wolfgang Goethe-Universität is gratefully acknowledged

JF030701Q