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## Stereoisomeric Distribution of 3-Mercaptohexan-1-ol and 3-Mercaptohexyl Acetate in Dry and Sweet White Wines Made from *Vitis vinifera* (Var. Sauvignon Blanc and Semillon)

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The enantiomeric distribution of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) in Vitis vinifera wines was determined by combining two techniques: specific purification of volatile thiols from the wines using p-hydroxymercuribenzoate and separation of the chiral molecules by gasphase chromatography on a cyclodextrin capillary column. The R and S enantiomer ratios of these two thiols in dry white Sauvignon blanc and Semillon wines are approximately 30:70 for A3MH and 50:50 for 3MH. However, in sweet white wines made from grapes affected by "noble rot" due to the development of Botrytis cinerea on ripe grapes, the proportion of the R and S forms of 3MH is in the vicinity of 30:70. During alcoholic fermentation, a change in the ratio of the two enantiomers of 3MH in dry white wines was observed. At the beginning of fermentation (around density 1.08), the S form represented over 60%; then, at lower density, as fermentation proceeded, the enatiomeric ratio approached 50:50. The ratio of the two 3MHA enantiomers remained constant throughout fermentation. On the contrary, the distribution of the two 3MH enantiomers changed very little during fermentation of the botrytized sweet wines. The perception thresholds for the R and S forms of 3MH in hydroalcoholic model solution are similar (50 and 60 ng/L). These two enantiomers have quite different aromas: The R form is fruitier, with a zesty aroma reminiscent of grapefruit, while the S form smells more of passion fruit. The perception thresholds of the R and S enantiomers of 3MHA are slightly different (9 and 2.5 ng/L). The less odoriferous R form is reminiscent of passion fruit, while the S form has a more herbaceous odor of boxwood.

KEYWORDS: Enantiomeric distribution; 3-mercaptohexan-1-ol; 3-mercaptohexyl acetate; Botrytis cinerea

### INTRODUCTION

3-Mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) were initially identified in passion fruit (1), and the enantiomer distribution of these volatile thiols was studied (2–4). These volatile thiols have also been identified in Sauvignon blanc wines (5, 6). The presence of these volatile thiols has also been reported in wines made from Alsacian grapes, such as Riesling and Gewürztraminer (7), as well as Petit and Gros Manseng (7), Cabernet Sauvignon, and Merlot (8, 9). More recently, these volatile thiols were identified in wines from the Canary Islands (10) and those made from Petite Arvine, a Swiss grape variety (11). There have been no previous publications concerning the enantiomers of 3MH and its acetate in wine.

This article reports on the distribution of enantiomers of these volatile thiols in dry and sweet Sauvignon blanc and Semillon wines and compares their perception thresholds in model hydroalcoholic solution.

#### MATERIALS AND METHODS

Wines and Must Analyzed. The Sauvignon blanc and Semillon must and dry and sweet white wines analyzed (vintages 2002–2005) came from two Bordeaux vineyards (Clos Floridène, Graves and Château Doisy-Daëne, Sauternes).

**Specific Extraction of Volatile Thiols from the Wines.** The volatile thiols were extracted from the wines with *p*-hydroxymercuribenzoate, using the method described by Tominaga et al. (*12*). A 500 mL sample of wine containing 2.5 nmol of 4-methoxy-2-methyl-2-mercaptobutane as an internal standard was extracted twice in a 2 L flask, using 100 mL of ethyl acetate for 5 min, with magnetic stirring each time. The organic phases collected were centrifuged for 5 min at 3800g to break the emulsion and were decanted in a separating funnel. The organic phase obtained was then extracted twice with 20 mL of *p*-hydroxymercuribenzoate solution (1 mM in a 0.2 M Tris solution), for 5 min each time. The two aqueous phases were combined and then loaded on a (1.5 cm  $\times$  3 cm) strong anion exchange resin column (Dowex 1,

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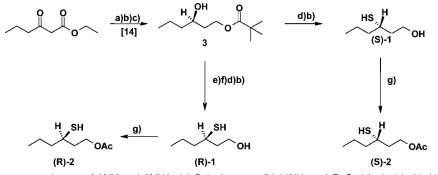


Figure 1. Synthesis of the two enantiomers of 3MH and 3MHA. (a) Baker's yeast; (b) LiAlH<sub>4</sub> and Et<sub>2</sub>O; (c) pivalyl chloride and pyridine; (d) Ph<sub>3</sub>P, diisopropyl azodicarboxylate, HCOOH, and THF; (f) NH<sub>3</sub> and MeOH; and (g) acetyl chloride, pyridine, and CH<sub>2</sub>Cl<sub>2</sub>.

Sigma, code 1X2-100). The column was then rinsed with 50 mL of sodium acetate buffer (0.1 M, pH 6). The volatile thiols were separated from the thiol-*p*-hydroxymercuribenzoate complex fixed on the column by percolating with a cysteine solution (640 mg/60 mL) adjusted to pH 7, purified of any volatile contaminants by extraction three times with 5 mL of dichloromethane. The eluate containing the volatile thiols released was extracted with 4, 2.5, and 2.5 mL of dichloromethane in turn for 5 min each, with magnetic stirring. The organic phases were collected in a pill container, dried on anhydrous sodium sulfate, and then concentrated to approximately 500  $\mu$ L under a nitrogen flux (75 mL/min) in a 10 mL graduated tube. The concentrate was then transferred to a 1 mL vial and concentrated to 25  $\mu$ L. The purified extract was injected into a gas chromatography/mass spectrometry (GC/MS) to assay the 3MH and 3MHA and to quantify the two enantiomers of these volatile thiols (see below).

**Specific Extraction of Volatile Thiols from Fermenting Must.** The methods described above were also applied to fermenting must, previously stabilized using sulfur dioxide (50 mg/L) and centrifuged at 3800*g* for 10 min. The volatile thiols from the prepared must were analyzed immediately.

Assay of 3MH and 3MHA. The volatile thiols purified by the method described above were analyzed using GC/MS in selected ion monitoring (SIM) mode to assay the 3MH and 3MHA in wine or fermenting must by selecting the m/z = 134 and m/z = 116 ions, respectively (13).

Separation of Two Enantiomers of 3MH and 3MHA. The purified extracts of the volatile thiols obtained from wine or fermenting must were injected into a Lipodex C [50 m × 0.25 mm; chiral column, heptakis-(2,3,6-tri-O-pentyl)- $\alpha$ -cyclodextrines; Interchim] to separate the two enantiomers of 3MH and 3MHA. The chromatography conditions were identical to those described by Tominaga et al. (*13*) except that the oven temperature was programmed from 70 to 120 °C at a rate of 1.5 °C/min and from 120 to 200 °C at a rate of 8 °C/min, with initial and final isothermal hold times of 100 and 5 min, respectively. Helium was used as the carrier gas (1 mL/min). 3MH and 3MHA were detected in both SCAN and SIM mode by selecting the *m*/*z* = 55, 100, and 134 ions for 3MH and *m*/*z* = 43, 55, and 116 ions for 3MHA.

**Extracting Racemic Mixtures of 3MH and 3MHA Added to Must and Wines Made from Healthy and Botrytized Grapes.** To check that there was no matrix effect on the extraction of the enantiomers of 3MH and 3MHA, the racemic mixtures of these two volatile thiols were added to must and wine from which every trace of thiols had been removed and were then extracted by the method described above. The purified extracts were injected into a chiral column, and the enantiomer ratios of these volatile thiols were measured. All of the thiols were eliminated from the must and wine by extracting 500 mL with 100 mL of ethyl acetate twice for 5 min before the racemic mixture of 3MH and 3MHA was added.

Synthesis of the Two Enantiomers of 3MH and 3MHA. The two enantiomers of 3MH and 3MHA were synthesized from ethyl 3-oxohexanoate, as previously described (14) (Figure 1). The pivalic ester 3 was subjected to a Mitsunobu reaction with thioacetic acid, with expected inversion of the configuration. The deprotection of the

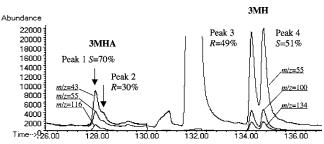


Figure 2. Separating the two enantiomers of 3MHA and 3MH in a dry Sauvignon blanc wine on a Lipodex C chiral column.

thioacetate obtained produced the (*S*)-(+)-1 (+1.0°, c = 1, CHCl<sub>3</sub>) enantiomer with a purity of 97% (GC-flame ionization detection) and an enantiomer excess of 96%, measured on a BetaDex column (30 m, 0.25 mm, 0.25  $\mu$ m film, 80°, 2 min, 80–200° at 2°/min; He, 10 psi). The same pivalic ester **3** was subjected to two Mitsunobu reactions: the first with formic acid to invert the alcohol configuration and then the second to introduce the thioacetate, resulting in an overall retention of the configuration to produce the (*R*)-(-)-1 (-1.0°, c = 1, CHCl<sub>3</sub>) enantiomer with a purity of 98% and an enantiomer excess of 94%. The operating methods for these reactions are given in ref *14*. The (*S*)-(+)-**2** (+11.3°, c = 1, CHCl<sub>3</sub>) and (*R*)-(-)-**2** (-11.0°, c = 1, CHCl<sub>3</sub>) acetates were obtained from the two alcohols in the usual way (*g*), with the corresponding enantiomer purity.

**Olfactory Perception Threshold of the Two Enantiomers of 3MH and 3MHA.** As described by Boidron et al. (15), this was assessed by a jury of 30 trained tasters in triangular tests, where increasing quantities of the compound were added to a hydroalcoholic model solution. The perception threshold corresponded to the minimum concentration perceived by 50% of the tasters.

#### RESULTS

Separation of the Two Enantiomers of 3MH and 3MHA. 3MH and 3MHA were specifically isolated from wine and purified using the above method and then injected into a chiral column to separate the two enantiomers of these volatile thiols. Most of the other compounds that make it difficult to detect these volatile thiols, such as fatty acids and higher alcohols, migrate faster and come out of the column sooner than the compounds studied. Both enantiomers of these volatile thiols are easily detected in SIM mode (Figure 2). Similarly, detection of these volatile thiols in SCAN mode showed that there were no contaminant ions due to impurities (results not shown).

The distribution of the 3MHA enantiomers in wine was tentatively determined. Although the separation was marginal, it was, however, still possible to determine the ratio of the two forms by comparing the height of the peaks, as we checked by analyzing known quantities of enantiomer added to a must (results not shown). On the other hand, Weber et al. achieved

 Table 1. Distribution of the Two Enantiomers of 3MH and 3MHA in

 Dry White Wines Made from Different Grape Varieties in Different

 Vintages<sup>a</sup>

vintages	varieties	3MH ( <i>R</i> : <i>S</i> )	3MHA ( <i>R</i> : <i>S</i> )
2002 dry wines	Sauvignon 1	44:56	28:72
	Sauvignon 2	45:55	ND
	Sauvignon 3	42:58	ND
	Semillon 1	41:59	28:72
	Semillon 2	42:58	ND
	Semillon 3	44:56	ND
2004 dry wines	Sauvignon 1	45:55	30:70
	Sauvignon 2	44:56	32:68
	Semillon 1	49:52	28:72
2005 dry wines	Sauvignon 1	51:49	ND
	Sauvignon 2	57:43	ND
	Sauvignon 3	55:45	27:73
	Semillon 1	52:48	ND
	Semillon 2	51:49	ND
	Semillon 3	52:48	ND

<sup>a</sup> ND, not detected.

 Table 2. Distribution of the Two Enantiomers of 3MH in Sweet Wines

 Made from Different Grape Varieties in Different Vintages

vintages	varieties	3MH ( <i>R</i> : <i>S</i> )
2002 sweet wines	Semillon 1 Semillon 2	29:71 32:68
2004 sweet wines	Semillon 1 Semillon 2	24:76 34:66
2005 sweet wines	Sauvignon Semillon 1 Semillon 2	34:66 33:67 32:68

a good separation of the compounds by means of multidimensional gas chromatography (2). The two enantiomers of 3MH were clearly separated under our chromatography conditions.

The peaks corresponding to the R and S forms were distinguished by injecting the corresponding pure reference thiols obtained by synthesis. Peaks 1 and 2, eluted with retention times of 127.89 and 128.18 min, corresponded to the S and R forms of 3MHA, respectively. Peaks 3 and 4, eluted with retention times of 134.18 and 134.67 min, corresponded to the R and S forms of 3MH, respectively.

**Distribution of the Two Enantiomers of 3MH and 3MHA in Wine.** In dry Sauvignon and Semillon wines made from healthy grape must, totally unaffected by *Botrytis cinerea*, the *R* and *S* enantiomers of 3MH are relatively uniformly distributed (approximately 50:50), with slight variations from one vintage to another: for example, there were slightly higher quantities of the *R* form of 3MH in wines from the 2005 vintage (**Table 1**). However, in sweet Semillon wines made from botrytized grapes, the ratio of the two enantiomers of 3MH (30:70) was very different from that in dry wines, irrespective of the vintage (**Table 2**).

The ratio of the two enantiomers of 3MHA was only determined in dry wines as this compound is never present in sweet, botrytized wines. The distribution of the two enantiomers (R:S) of 3MHA in dry wines was approximately 30:70 for both grape varieties studied (**Table 1**).

**Confirming That One of the Enantiomers Was Not Extracted Preferentially.** As wine containing sugars, for example, may be considered a chiral medium and thiols are released from mercury complexes by L-cysteine, a chiral compound, it was necessary to check that the specific method

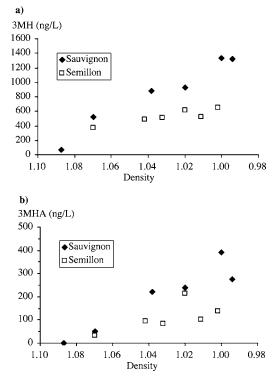


Figure 3. Changes in (a) 3MH and (b) 3MHA contents during alcoholic fermentation of must used to make dry wines.

used to extract the thiols did not isolate more of one enantiomer than the other. Racemic mixtures of 3MH and 3MHA were added to a dry white Sauvignon blanc wine that had previously been extracted with ethyl acetate to eliminate the thiols. Extracting the added thiols from this model wine, using the method described above, produced an enantiomer ratio identical to that of the two thiols added. Similarly, the racemic mixture of 3MH added to must or wine made from botrytized grapes was recovered without any change in the ratio of the two enantiomers (results not shown). This confirmed that the method was not selective toward certain enantiomers and confirmed the ratios found in the wine samples. Moreover, racemization during the purification was not evident (result not shown).

**Changes in the Distribution of 3MH and 3MHA Enantiomers during Alcoholic Fermentation.** 3MH and 3MHA concentrations increase gradually during alcoholic fermentation (**Figure 3**). By the end of fermentation, the 3MH and 3MHA contents of the two dry white wines analyzed (2002 Sauvignon blanc and Semillon) had reached approximately 1300 and 650 ng/L and 280 and 140 ng/L, respectively.

The distribution of the 3MH and 3MHA enantiomers was determined in the same wines during alcoholic fermentation. At the beginning of fermentation (around density 1.08), the *S* form represented over 60% (**Figure 4a**), and then, at lower density, as fermentation proceeded, the enatiomeric ratio approached 50:50, the ratio found in the dry white wines analyzed (**Table 1**). On the contrary, the ratio of the two 3MHA enantiomers remained constant throughout fermentation, with the *R* and *S* forms at a ratio of approximately 30:70 (**Figure 4b**). Botrytized sweet wines contain more 3MH than dry white wines made from healthy grapes (**Figure 5a**). The distribution of the two 3MH enantiomers changed very little during fermentation of the sweet wines (**Figure 5b**).

It was also confirmed that changes in the distribution of the two 3MH enantiomers in dry wines was not due to an analysis artifact caused by coelution of byproducts of alcoholic fermen-

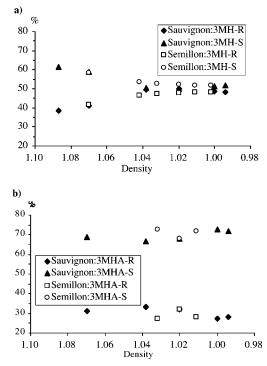


Figure 4. Changes in the distribution of the two enantiomers of (a) 3MH and (b) 3MHA during alcoholic fermentation of must used to make dry wines.

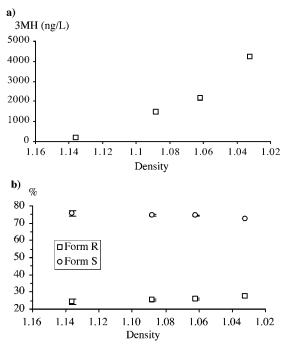
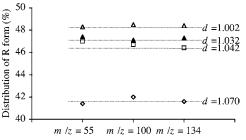


Figure 5. Release (a) and enantiomer distribution (b) of 3MH during alcoholic fermentation of must used to make a sweet wine.

tation with the enantiomers. Indeed, the stereoisomer ratio was calculated from the three ions selected as representative of the 3MH spectrum (m/z = 55, 100, and 134). These ratios remained stable at the various stages in alcoholic fermentation (d = 1.070-1.002), irrespective of the ions chosen (**Figure 6**). It was, therefore, impossible to overestimate the *R* form due to coelution.

Olfactory Perception Thresholds of the Two 3MH and 3MHA Enantiomers. A panel of 30 trained tasters carried out triangular tests where increasing quantities of the 3MH and



**Figure 6.** Changes in the stereoisomer ratio calculated according to three selected ions (m/z = 55, 100, and 134) during alcoholic fermentation (d = 1.070-1.002).

 Table 3. Perception Threshold and Olfactory Descriptors of the Two

 Enantiomers of 3MH and 3MHA

compounds	enantiomeric forms	threshold (ng/L)	olfactory descriptors
3MH	R	50	grapefruits citrus peels
	S	60	passion fruit
	racemic mixture	60 <sup>a</sup>	grapefruits passion fruit
АЗМН	R	9	passion fruit
	S	2.5	boxwood
	racemic mixture	4 <sup>b</sup>	boxwood passion fruit

<sup>a</sup> Ref 6. <sup>b</sup> Ref 5.

3MHA enantiomers were added to a hydroalcoholic model solution "similar to wine". Seventy percent of the tasters distinguished between model dilute alcohol solutions containing 2000 ng/L of the *R* and *S* enantiomers of 3MH. The perception thresholds measured are presented in **Table 3**, together with the olfactory descriptors of the *R* and *S* forms of these compounds.

#### DISCUSSION

This was the first time the distribution of the two enantiomers of 3MH and 3MHA in white *Vitis vinifera* wines had been analyzed, which was achieved by a combination of specific purification of volatile thiols and separation of chiral molecules on a cyclodextrin capillary column.

In dry white wines made from healthy grapes, 3MH is almost racemic, whereas 3MHA is predominantly in *S* form (70%). The yeast strain used (three strains of *Saccharomyces cerevisiae* and one strain of *Saccharomyces bayanus*) for fermentation had no impact on the enantiomer distribution of these volatile thiols (results not shown).

The 3MHA content of young dry white wines is known to be correlated with that of 3MH (16). It is also well-known that 3MH is formed from a cysteinylated precursor, metabolized by yeast during alcoholic fermentation (17). The racemic distribution of 3MH in dry wines may be due to the existence of a racemic mixture of the precursor of 3MH in must.

The cysteinylated precursor of 3MHA has not yet been identified. 3MHA is generally considered to be formed by esterification of 3MH by yeast during alcoholic fermentation. The esterase or lipase involved probably acetylates 3MH with a certain enantioselectivity.

However, in wines made from botrytized grapes (*B. cinerea*), the enantiomer distribution of 3MH is 30:70 in favor of the *S* 

form, indicating that these overripe grapes probably contain higher concentrations of its precursor.

Furthermore, the change in the distribution of the two 3MH enantiomers during alcoholic fermentation in dry wines indicates that the endogenous yeast enzyme responsible for releasing 3MH exhibits a certain diastereoselectivity in 3MH release. In fact, Wakabayashi et al. (18) reported on the chiral selectivity of 3MH release by certain  $\beta$ -lyases. The yeast enzyme responsible for cleaving the carbon–sulfur bond of an S conjugate cysteine may show a certain attraction toward the *S* form at the beginning of alcoholic fermentation.

The perception thresholds of the *R* and *S* forms of 3MH are very similar. However, these two enantiomers have different aromas that were significantly distinguished in the triangular tasting test. The R form's aroma is reminiscent of grapefruit, while the S form smells like passion fruit. The perception threshold of the S form of 3MHA is approximately four times lower than that of the R form. Furthermore, the S enantiomer is three times more abundant in wine than the R form. These two enantiomers have different aromas: the S form is more herbaceous, with a boxwood smell similar to that of 4-methyl-4-mercaptopentanone (13), while the S form is fruitier, reminiscent of passion fruit. These differences in aroma are in agreement with the findings of Weber et al. (4). In any case, these experiments demonstrated that the enantiomer distribution of thiols such as 3MH and 3MHA must be taken into account in assessing the olfactory impact of these compounds in wine.

To enhance our understanding of the impact of winemaking parameters on the enantiomer distribution of the thiols in wine, it will be necessary to study the chirality of the cysteinylated precursors in must made from healthy grapes and those affected by *B. cinerea*.

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