Development of a Method for Analyzing the Volatile Thiols Involved in the Characteristic Aroma of Wines Made from *Vitis vinifera* L. Cv. Sauvignon Blanc

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Volatile thiols were purified from a dichloromethane extract of Sauvignon blanc wine by the reversible combination of the thiols with *p*-hydroxymercuribenzoate, fixation of the resulting complex in an anion exchange column, and finally release of the thiols with cysteine. By analyzing the purified thiol extract from 500 mL of wine, using gas chromatography coupled with mass spectrometry, it was possible to measure the concentrations of five volatile compounds at once: 4-mercapto-4-methylpentan-2-one, 3-mercaptohexyl acetate, 3-mercaptohexan-1-ol, 4-mercapto-4-methylpentan-2-ol, and 3-mercapto-3-methylbutan-1-ol. Analysis of 10 typical Sauvignon Blanc wines (Bordeaux, Sancerre) showed that the first three compounds listed above are expected to be involved in varietal aroma, as they may be present at concentrations greatly in excess of their respective perception thresholds.

Keywords: *Volatile thiol; aroma; Sauvignon blanc wine; Vitis vinifera; p-hydroxymercuribenzoate*

INTRODUCTION

Sauvignon blanc is one of the most widely grown *Vitis vinifera* L. grape varieties in the world. Its wines have a characteristic aroma, usually described as green pepper, box tree, broom, grapefruit, passionfruit, and smoke. The compound responsible for the green pepper character, 2-methoxy-3-isobutylmethoxypyrazine (Augustyn et al., 1982; Allen et al., 1991), is more marked if the grapes are under-ripe. Two sulfur compounds reminiscent of box tree and broom have been identified in Sauvignon blanc wines: 4-mercapto-4-methylpentan-2-one (Darriet et al., 1995) and 3-mercaptohexyl acetate (Tominaga et al., 1996). Other compounds recently characterized in Sauvignon blanc wines, 4-mercapto-4-methylpentan-2-ol, 3-mercaptohexan-1-ol, and 3-mercapto-3-methylbutan-1-ol (Tominaga et al., 1998), have quite different aromas. The aromas of 4-mercapto-4-methylpentan-2-ol and 3-mercaptohexan-1-ol are reminiscent of citrus zest, grapefruit, and passionfruit, while that of 3-mercapto-3-methylbutan-1-ol is similar to cooked leeks. The fact that concentrations of 4-mercapto-4-methylpentan-2-one in some Sauvignon blanc wines (Bouchilloux et al., 1996) were much higher than the perception threshold suggests that they play a decisive role in the characteristic aroma of wines made from this grape variety. As the precise concentration of the other volatile thiols identified in Sauvignon blanc wines has not yet been determined, their contribution to the wines' aroma remains hypothetical.

This paper describes the quantitative analysis of five volatile thiols identified in Sauvignon blanc wines by gas chromatography coupled with mass spectrometry (GC/MS). This technique is based on an original method for the specific extraction of traces of a volatile thiol from a complex organic extract. The results of the analyses of various Sauvignon blanc wines have made it possible to define the role of these volatile thiols in the aroma of wines made from this grape variety.

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MATERIALS AND METHODS

Wines Analyzed. Analyses were carried out on French Sauvignon blanc wines from the Bordeaux (vintage from 1992 to 1996) and Sancerre (vintage 1996) Appellations.

Reference Compounds. 4-Mercapto-4-methylpentan-2-one (code OM469980), 3-mercaptohexyl acetate (code OM468972), and 3-mercaptohexan-1-ol (code OM 984640) were supplied by Interchim (Montluçon, France). 3-Mercapto-3-methylbutan-1-ol was kindly donated by Dr. Joulain from Société Robertet in Grasse (France). 4-Mercapto-4-methylpentan-2-ol was synthesized from 4-mercapto-4-methylpentan-2-one (Tominaga et al., 1998).

Specific Extraction of Volatile Thiols. A volume of 500 mL of wine containing 2.5 nmol of 4-methoxy-2-methyl-2-mercaptobutane (Rigaud et al., 1986) as an internal standard was brought to pH 7.0 with a sodium hydroxide solution (10 N) and extracted with two successive additions of 100 mL of dichloromethane (Pestipur, SDS; France; code 02922E) in a 2 L flask with magnetic stirring for 5 min each time. The combined organic phases were centrifuged for 5 min at 3800*g* to break the emulsion and separated in a separating funnel. The organic phase obtained was then extracted with two successive additions of 20 mL of a *p*-hydroxymercuribenzoate solution for 5 min each time (Sigma; code H0642) (1 mM in sodium hydroxide at 0.01 N). During this extraction, the aqueous phase had to be maintained at a pH >7 by adding a 10 N sodium hydroxide solution, if necessary. The two aqueous phases, from the extraction, were combined and brought to pH 7 by very slow addition of a 5% solution of hydrochloric acid to avoid precipitation of *p*-hydroxymercuribenzoate. They were then loaded into a strongly basic anion exchanger column (1.5 × 3 cm) (Dowex 1, Sigma; code 1X2-100) previously reactivated using 50 mL of hydrochloric acid at 0.1 M and then rinsed with ultrapure water (MilliQ,

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Table 1. Perception Thresholds and Olfactory Descriptions for the Odors of Volatile Thiols Identified in Sauvignon Blanc Wines

compound	perception threshold[a] (ng/L)	olfactory description
4-mercapto-4-methylpentan-2-one	0.8	box tree, broom
3-mercaptohexyl acetate	4	box tree, passion-fruit
3-mercaptohexan-1-ol	60	grapefruit
4-mercapto-4-methylpentan-2-ol	55	citrus zest
3-mercapto-3-methylbutan-1-ol	1500	cooked leeks

[a] In aqueous alcohol solution (12 v/v %).

Millipore). Percolation of the aqueous phase took 15 min. The column was then washed with 10 mL of potassium phosphate buffer (2 mM, pH 7.2) and then 50 mL of sodium acetate buffer (0.1 M, pH 6) to which 0.1 M sodium chloride had been added. The volatile thiols were released from the complex thiol−*p*-hydroxymercuribenzoate fixed on the column by percolating for 30 min using a cysteine solution (640 mg/60 mL) (Sigma; code C4820) adjusted to pH 7, previously purified of any volatile contaminants by repeated extractions with dichloromethane (5 mL × 3). The eluate containing the volatile thiols released from the column by cysteine was collected in a 100 mL flask and extracted using 4, 2.5, and 2.5 mL of dichloromethane (Atrasol, SDS, France; code 02932E) for 5 min each, with magnetic stirring. The organic phases were collected, dried on anhydrous sodium sulfate (Merck, Darmstadt, Germany), and then concentrated under nitrogen flow in a 10 mL graduated tube to ≈500 μL. The concentrate was then transferred to a 1 mL vial and concentrated to 25 μL.

Calibration. The white wine selected for calibration purpose is a Muscadet (vintage 1996). Further analysis of this wine presented slight amounts of 3-mercapto-3-methylbutan-1-ol and 3-mercaptohexan-1-ol but the three other volatile thiols were absent. The calibration of these compounds was therefore corrected by subtracting the blank ratios [area formed by a selected ion (see below) of these compounds contained naturally in this wine/that of internal standard].

Standard charts for the compounds to be analyzed were prepared by adding increasing quantities of the five volatile thiol reference compounds to the Muscadet wine (1−30 ng of 4-mercapto-4-methylpentan-2-one, 5−160 ng of 3-mercaptohexyl acetate, 12.5−400 ng of 4-mercapto-4-methylpentan-2-ol, 75−1200 ng of 3-mercaptohexan-1-ol, and 25−800 ng of 3-mercapto-3-methylbutan-1-ol in 500 mL, respectively, to make five or six points of different concentrations). For each concentration, the volatile thiols were extracted from the wine according to the method described above.

GC/MS. The GC/MS analysis conditions were identical to those described by Tominaga and Dubourdieu (1997) except for the initial isotherm (10 min). Two microliters of extract was injected. The five compounds were detected in SIM mode with the ions selected as follows: 4-mercapto-4-methylpentan-2-one, *m*/*z* 75; 4-mercapto-4-methylpentan-2-ol, *m*/*z* 134; 3-mercapto-3-methylbutan-1-ol, *m*/*z* 120; 3-mercaptohexyl acetate, *m*/*z* 116; 3-mercaptohexan-1-ol, *m*/*z* 134; 4-methoxy-2-methyl-2-mercaptobutane (internal standard), *m*/*z* 134.

Repeatability. Five analyses (extraction and measurement) were carried out on each wine to determine the variation coefficient for measuring each component.

Aromatic Index. The aromatic potential of each compound was assessed by calculating the aromatic index (*I*) using the equation $I = c/s$ (Boidron et al., 1988), where *c* is the concentration found in the wines and *s* is the olfactory perception threshold in an aqueous alcohol solution (12 v/v %) as determined in previous experiments (Darriet et al., 1995; Tominaga et al., 1996, 1998) (Table 1).

RESULTS

Specific Aspects of the Method for Extracting Volatile Thiols. The volatile compounds were first extracted from 500 mL of wine using dichloromethane as described under Materials and Methods. The volatile thiols in this organic extract were then extracted using an aqueous solution of *p*-hydroxymercuribenzoate. The thiol−*p*-hydroxymercuribenzoate complex formed was then fixed on an anion exchange column that had been rinsed with a sodium acetate buffer to eliminate any contaminant compounds. The thiols held in the column via *p*-hydroxymercuribenzoate were released from *p*-hydroxymercuribenzoate by eluting the column with a cysteine solution, and they were finally extracted with dichloromethane and analyzed using GC/MS. In these conditions, the recovery of internal standard was 75−80%. Figure 1 shows the GC/MS analysis results of volatile thiols in a Sauvignon blanc wine (Bordeaux, 1996). The five compounds to be measured were then easily detectable in SIM mode. They could also be detected in SCAN mode (results not presented).

Standard Curves and Repeatability. The area of each peak corresponding to the selected ion was calculated, and the ratio of the area Hs/His (area formed by a selected ion of each compound/that of internal standard) was expressed graphically for the aforementioned concentration standards. The correlation between the two parameters was linear for all of the compounds analyzed. Their regression equations were as follows: 4-mercapto-4-methylpentan-2-one, $y = 0.01x + 0.001$ ($r = 1.000$); 3-mercaptohexyl acetate, $y = 0.006x + 0.012$ ($r = 0.996$); 3-mercaptohexan-1-ol, $y = 0.008x - 0.051$ ($r = 0.998$); 4-mercapto-4-methylpentan-2-ol, $y = 0.002x - 0.001$ ($r = 1.000$); 3-mercapto-3-methylbutan-1-ol, $y = 0.002x + 0.001$ ($r = 0.999$). Figure 2 shows, as an example, the standard curve for 4-mercapto-4-methylpentan-2-one, which had the lowest range of concentrations.

Repeatability of the measuring system was assessed over a series of five extractions. Table 2 shows the variation coefficients obtained for each of the five compounds. These were in the vicinity of 3% for 4-mercapto-4-methylpentan-2-ol, 3-mercapto-3-methylbutan-1-ol, and 3-mercaptohexan-1-ol, whereas they were under 10% for the other two compounds.

Concentrations and Aromatic Potential of the Volatile Thiols in Sauvignon Blanc Wines. The volatile thiols in four Sauvignon blanc wines (Bordeaux, 1996) were analyzed according to the above method (Table 3). The same analyses were also carried out on Sancerre wines from the same vintage (Table 4) as well as various vintages of the same Bordeaux wine (Table 5). The 4-mercapto-4-methylpentan-2-one content of these various wines, which all had characteristic Sauvignon blanc noses, ranged from a few nanograms per liter to ≈40 ng/L. These values, confirming those found by Bouchilloux et al. (1996), were well above the perception threshold for this component (0.8 ng/L). Consequently, the aromatic index of 4-mercapto-4-methylpentan-2-one was >1 in all cases and could be as high as 50 in certain wines. This component had an undeniable impact on the aroma of the Sauvignon blanc wines.

The 3-mercaptohexyl acetate content of the Sauvignon blanc wines analyzed ranged from zero to several hundred nanograms per liter. As the perception threshold is 4 ng/L, its impact on the aroma was therefore quite variable, although it could be considerable in certain wines for which the aromatic index could be as high as 190. 3-Mercaptohexan-1-ol was always present

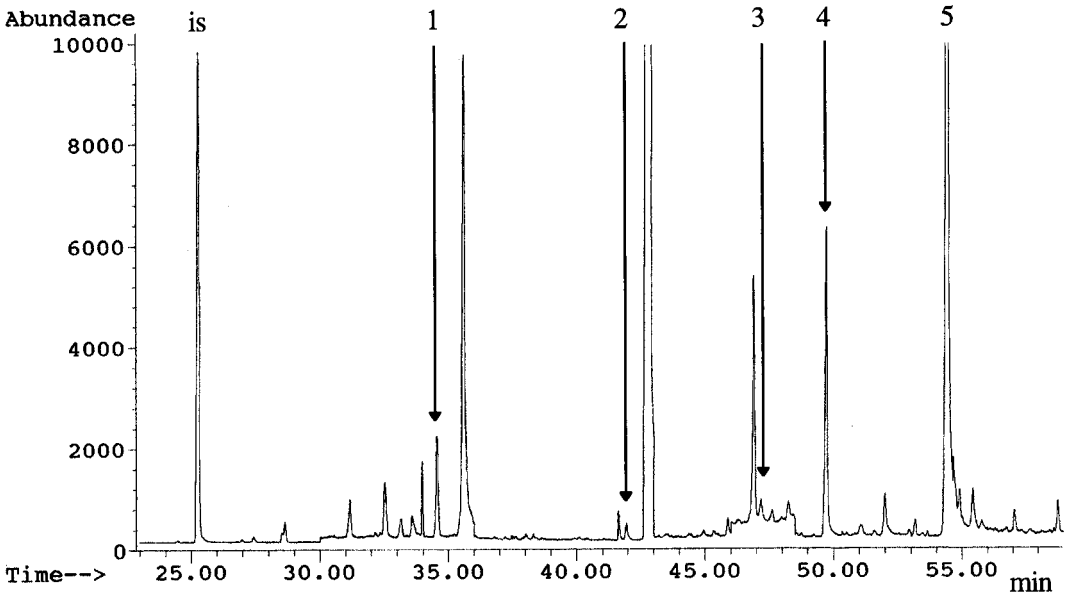

Figure 1. Analysis by coupled GC/MS of the volatile thiols extracted from a Sauvignon blanc wine (Bordeaux, 1996): 1, 4-mercapto-4-methylpentan-2-one; 2, 4-mercapto-4-methylpentan-2-ol; 3, 3-mercapto-3-methylbutan-1-ol; 4, 3-mercaptohexyl acetate; 5, 3-mercaptohexan-1-ol; i.s., internal standard.

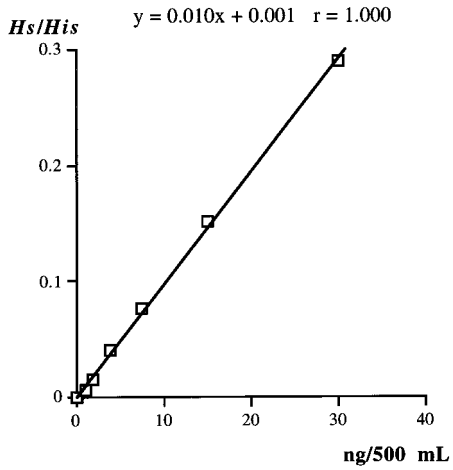

Figure 2. Standard curve for the analysis of 4-mercapto-4-methylpentan-2-one concentrations. Hs/His: Ratio of the area of the 4-mercapto-4-methylpentan-2-one peak to that of the internal standard (i.s., 4-methoxy-2-methyl-2-mercaptobutane).

in Sauvignon blanc wines at concentrations of several hundred nanograms per liter and, in some wines, the content could be measured in micrograms per liter. This component, with its characteristic odor of grapefruit and a perception threshold of 60 ng/L, was also a major feature of the bouquet. Those wines with the highest 3-mercaptohexanol content also had higher concentrations of the acetate.

The role of 4-mercapto-4-methylpentan-2-ol in wine aroma was less marked than that of the other three volatile thiols mentioned, and it was generally present in concentrations below the perception threshold (55 ng/L), although this value was reached in a few of the wines.

Finally, the 3-mercapto-3-methylbutan-1-ol content of the wines analyzed was always considerably lower than the perception threshold (1500 ng/L), confirming that, as was supposed when this component was initially identified, its contribution to the varietal aroma of Sauvignon blanc wines was nonexistent.

DISCUSSION AND CONCLUSION

Sulfur compounds, especially volatile thiols, may play a major role in the aroma of many fresh plants, vegetables, fruit, or processed products (roasted coffee, wine, etc.) (Shankaranarayana et al., 1982). However, the fact that these substances are present in these complex natural products in trace amounts makes it difficult to identify them and measure their concentrations. The analytical methods previously available involved tedious, complicated purification phases that were also rather nonspecific.

One of the properties of *p*-hydroxymercuribenzoate is that it combines in a reversible reaction with compounds containing a thiol function (Jocelyn, 1987). The volatile thiols in an organic extract may be extracted by combining them with an aqueous solution of *p*-hydroxymercuribenzoate. They are then released by adding excess quantities of another thiol, nonextractable by organic solvents, such as cysteine. Finally, the released thiols are concentrated from the aqueous phase by extraction using organic solvents. This extraction technique was used in previous experiments to identify 4-mercapto-4-methylpentan-2-one extracted from Sauvignon blanc wines (Darriet et al., 1995). It is, however, still difficult to extract and, especially, to measure the concentration of certain volatile thiols by using this method due to the extraction of compounds other than thiols in the aqueous phase of *p*-hydroxymercuribenzoate (Tominaga et al., 1996).

The original aspect of the method described in this paper is that it eliminates these impurities, as the substances extracted with *p*-hydroxymercuribenzoate were purified by percolation through a strongly basic anion exchanger column (Dowex 1X2-100). Any *p*-hydroxymercuribenzoate, either free or combined with the thiols, was fixed on the resin. Once the column had been washed, the volatile thiols left in it were released by elution with cysteine solution.

This method was preferable to the use of *p*-hydroxymercuribenzoate immobilized on Sepharose 4B to combine the volatile thiols in the organic extract, as the agarose gel could be denatured by the organic solvents.

Table 2. Repeatability of the Analysis of Volatile Thiols in Sauvignon Blanc Wine (Bordeaux, 1996)

sample	4-mercapto-4-methyl-pentan-2-one (ng/L)	3-mercaptohexyl acetate (ng/L)	3-mercaptohexan-1-ol (ng/L)	4-mercapto-4-methyl-pentan-2-ol (ng/L)	3-mercapto-3-methyl-butan-1-ol (ng/L)
1	23.4	30.9	943	28.1	85.4
2	26.8	32.3	1013	27.6	87.2
3	22.6	34.8	1038	26.0	90.3
4	19.4	36.5	1021	26.3	92.7
5	23.2	36.8	1015	24.0	83.3
av ($n = 5$)	23.1	34.3	1006	26.4	87.8
SD	2.62	2.59	36.5	1.02	3.78
CV (%) (5%)	9.99	6.61	3.10	3.40	3.77

Table 3. Analysis of Volatile Thiols (Nanograms per Liter) in Four Sauvignon Blanc Wines from Bordeaux, 1996 Vintage

	sample			
compound	1	2	3	4
4-mercapto-4-methylpentan-2-one	5 (6)[a]	4 (5)	10 (13)	4 (5)
3-mercaptohexyl acetate	724 (181)	451 (113)	451 (113)	275 (69)
3-mercaptohexan-1-ol	8402 (140)	12822 (214)	7465 (123)	3736 (63)
4-mercapto-4-methylpentan-2-ol	18 (0.3)	20 (0.4)	22 (0.4)	20 (0.4)
3-mercapto-3-methylbutan-1-ol	78 (0.05)	86 (0.06)	97 (0.07)	82 (0.06)

[a] Numbers in parenthese indicate the aromatic index of each component.

Table 4. Analysis of Volatile Thiols (Nanograms per Liter) in Three Sauvignon Blanc Wines from Sancerre, 1996 Vintage

	sample		
compound	1	2	3
4-mercapto-4-methylpentan-2-one	22 (28)[a]	24 (30)	4 (5)
3-mercaptohexyl acetate	254 (64)	212 (53)	777 (194)
3-mercaptohexan-1-ol	1291 (22)	733 (12)	3415 (57)
4-mercapto-4-methylpentan-2-ol	11 (0.2)	20 (0.4)	1 (0.02)
3-mercapto-3-methylbutan-1-ol	123 (0.08)	134 (0.09)	34 (0.02)

[a] Numbers in parentheses indicate the aromatic index of each component.

Table 5. Analysis of the Volatile Thiols (Nanograms per Liter) in Several Vintages of a Single Bordeaux Wine

	vintage			
compound	1992	1993	1994	1995
4-mercapto-4-methylpentan-2-one	7 (9)[a]	40 (50)	10 (13)	44 (55)
3-mercaptohexyl acetate	0 (0)	0 (0)	0.4 (0.08)	2.8 (0.7)
3-mercaptohexan-1-ol	871 (15)	1178 (20)	600 (10)	1686 (28)
4-mercapto-4-methylpentan-2-ol	46 (0.8)	111 (2)	25 (0.5)	33 (0.6)
3-mercapto-3-methylbutan-1-ol	128 (0.08)	89 (0.06)	97 (0.06)	104 (0.07)

[a] Numbers in parentheses indicate the aromatic index of each component.

Furthermore, it had a much lower capacity (Sigma; code H3133; 0.8 μmol of *p*-hydroxymercuribenzoate/mL of packed gel) for fixing volatile thiols than the Dowex resin, regarding *p*-hydroxymercuribenzoate combined with thiols. Finally, certain impurities may be fixed in a nonspecific way on the Sepharose matrix and then be eluted with the thiols. These phenomena were kept to a minimum by using the basic anion resin (Dowex 1X2-100).

This method also made it possible to analyze the following five volatile thiol compounds in 500 mL of wine: 4-mercapto-4-methylpentan-2-one, 3-mercaptohexyl acetate, 3-mercaptohexan-1-ol, 4-mercapto-4-methylpentan-2-ol, and 3-mercapto-3-methylbutan-1-ol.

Analysis of 10 typical Sauvignon blanc wines (Bordeaux, Sancerre) showed that the first three compounds listed above are expected to be involved in their varietal aroma, as concentrations were, in some cases, much higher than their respective perception thresholds. It was already known that the highly aromatic 4-mercapto-4-methylpentan-2-one (Darriet et al., 1995), also identified in box tree and broom (Tominaga and Dubourdieu, 1997), gave to Sauvignon blanc wines an aroma reminiscent of these plants. These experiments demonstrate that 3-mercaptohexan-1-ol and its acetate, already identified in passionfruit (Engel and Tressl, 1991), are also expected to contribute to the aroma of wines made from this grape variety, adding an increasing fruity note.

The specific method for extracting volatile thiols developed in this research may also be applied to analysis of these compounds in other plant extracts or derivatives (fruit, drinks, etc.).

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LITERATURE CITED

Allen, M. S.; Lacey, M. J.; Harris, R. L. N.; Brown, W. V. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* **1991**, *42*, 109−112.

Augustyn, O. P. H.; Rapp, A.; van Wyck, C. J. Some volatile aroma components of *Vitis vinifera* L. cv. Sauvignon blanc. *S. Afr. J. Enol. Viticult.* **1982**, *3*, 53−60.

Boidron, J. N.; Chatonnet, P.; Pons, M. Influence du bois sur certaines sur certaines substances odorantes des vins. *Connais. Vigne Vin* **1988**, *22*, 275−294.

Bouchilloux, P.; Darriet, P.; Dubourdieu, D. Mise au point d′une méthode de dosage de la 4-mercapto-4-méthylpentan-2-one dans les vins de Sauvignon. *J. Int. Sci. Vigne Vin* **1996**, *30*, 23−29.

Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J. N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-Mercapto-4-methylpentan-2-one. *Flavour Fragrance J*. **1995**, *10*, 385−392.

Engel, K. H.; Tressl, R. Identification of New Sulfur-Containing Volatiles in Yellow Passion Fruits (*Passiflora edulis f. flavicarpa*). *J. Agric. Food Chem*. **1991**, *39*, 2249−2252.

Jocelyn, P. C. In *Methods in Enzymology*; Jakoby, W. B., Griffith, O. W., Eds.; Academic Press: London, U.K., 1987; Vol. 143, p 44.

Rigaud, J.; Etievant, P.; Henry, R.; Latrasse, A. Le méthoxy-4-méthyl-2-butanethiol-2, un constituant majeur de l'arôme du bourgeon de cassis. *Sci. Aliments* **1986**, *6*, 213−220.

Shankaranarayana, M. L.; Raghavan, B.; Abraham, K. O.; Natarajan, C. P. Sulphur compounds in flavours. In *Food Flavors*; Morton, I. D., Macleod, A. J., Eds.; Elsevier Science Publishers: Amstedam, 1982; Part A, pp 169−281.

Tominaga, T.; Dubourdieu, D. Identification of 4-Mercapto-4-methylpentan-2-one from the Box Tree (*Buxus sempervirens* L.) and Broom (*Sarothamnus scoparius* (L.) Koch.). *Flavour Fragrance J*. **1997**, *12* (6), 373−376.

Tominaga, T.; Darriet, P.; Dubourdieu, D. Identification de l'acétate de 3-mercaptohexanol, composé à forte odeur de buis, intervenant dans l'arôme des vins de Sauvignon. *Vitis* **1996**, *35* (4), 207−210.

Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon. *Flavour Fragrance J*. **1998**, *13*, in press.

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