

## Quantification of Cysteine *S*-Conjugate of 3-Sulfanylhhexan-1-ol in Must and Wine of Petite Arvine Vine by Stable Isotope Dilution Analysis

JEAN-LUC LUISIER,<sup>†</sup> HERMANN BUETTNER,<sup>§</sup> SEBASTIAN VÖLKER,<sup>†,§</sup>  
THIERRY RAUSIS,<sup>†</sup> AND URBAN FREY<sup>\*,†</sup>

Institute of Life Technologies, University of Applied Sciences Western Switzerland Valais, Route de Rawyl 64, CH-1950 Sion 2, Switzerland, and Fachhochschule Muenster, Fachbereich Chemieingenieurwesen, Stegerwaldstrasse 39, D-48565 Steinfurt, Germany

Making use of a convenient synthetic approach to prepare the deuterated *S*-3-(hexan-1-ol)-cysteine by a Michael addition reaction, an analytical method was developed to measure the presence of the cysteine *S*-conjugate, precursor of 3-sulfanylhhexan-1-ol (3-mercaptohexan-1-ol), in must and wine from Petite Arvine vine. The method uses a stable isotope dilution assay with a suitable one-step sample preparation and HPLC-MS detection. The method has limits of detection and quantification of 3 and 10 µg/L, respectively. A correlation between the increase of the precursor concentration and the increase of the degree of rot has been established.

**KEYWORDS:** Cysteine *S*-conjugate; *S*-3-(hexan-1-ol)-cysteine; 3-sulfanylhhexan-1-ol; 3-mercaptohexan-1-ol; stable isotope dilution assay; deuterated analogue; Petite Arvine vine

### INTRODUCTION

Over the past few years, several studies have been published on the subject of identification and quantification of flavors in wines. Especially volatile thiols, which are highly odorous compounds, contribute to the aroma of white wines such as Sauvignon Blanc (1, 2) or rosé wines such as Cabernet Sauvignon and Merlot (3). A typical sulfur-containing aroma compound, 3-mercaptohexan-1-ol (2), provides the characteristic aroma of Petite Arvine wine (4, 5) as grapefruit and rhubarb flavors (6). The present work focuses on this part of aroma of Petite Arvine.

3-Mercaptohexan-1-ol (3-sulfanylhhexan-1-ol) (2) and other volatile thiols are almost absent from must, but are produced during alcoholic fermentation from cysteine *S*-conjugate (7) or its precursor glutathione *S* conjugates (8). This explains the amplification of aroma in Petite Arvine wine. The degradation of the precursor *S*-3-(hexan-1-ol)-cysteine (1) by yeasts (Figure 1) releases the volatile thiol 3-mercaptohexan-1-ol (2) by means of a carbon–sulfur bond cleavage catalyzed by  $\beta$ -lyase (EC 4.4.1.13) enzymes (9, 10).

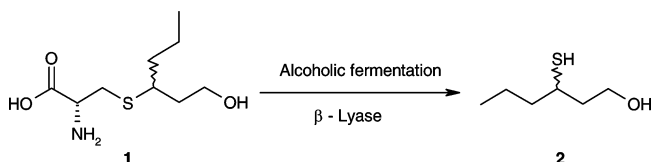
To investigate the biosynthesis of 3-mercaptohexan-1-ol (2) during the fermentation process of Petite Arvine wine, it is essential to quantify the cysteine *S* precursor. Classical thiol-specific analytical methods are based on gas chromatography–mass spectrometry (GC-MS), after selective extraction and derivati-

zation, either with an internal standard (7) or with the <sup>15</sup>N analogue used as internal standard (3). Starkenmann et al. used high-performance liquid chromatography–electrospray ionization positive mode mass spectrometry (HPLC-ESI+MS) with a standard curve of cysteine *S*-conjugate (11).

Another relevant analytical approach uses a stable isotope dilution method for the determination of low flavor concentrations (12, 13). Moreover, a stable deuterated analogue has been found to have physicochemical properties that are very similar to those of the compound of interest and is also well adapted to the quantitative determination of aroma in wines (14, 15). Consequently, isotope dilution analysis can be used for the determination of cysteine *S*-conjugate in must and wine.

### MATERIALS AND METHODS

**Reagents.** Commercial starting materials were purchased from Sigma-Aldrich or Acros Organics. Their purity was checked before use (melting range, refractive index, or <sup>1</sup>H NMR). When a known compound was prepared according to a literature procedure, pertinent references are given. *S*-3-(Hexan-1-ol)-cysteine was synthesized following the procedure developed recently (16), and the purity was checked by <sup>1</sup>H NMR.



**Figure 1.** Pathway of 3-mercaptohexan-1-ol (2) formation during alcoholic fermentation (9).

\* Corresponding author (telephone +41 27 606 86 57; fax +41 27 606 86 15; e-mail urban.frey@hevs.ch).

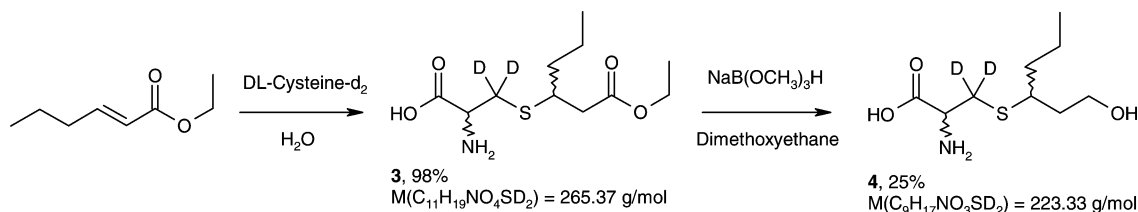
<sup>†</sup> University of Applied Sciences Western Switzerland Valais.

<sup>§</sup> Fachbereich Chemieingenieurwesen.

**Table 1.** Petite Arvine Samples and Cysteine S-Conjugate Concentrations in the Must

clone	rotten <sup>a</sup> (g)	rotten <sup>b</sup> (% Brix)	healthy <sup>a</sup> (g)	healthy <sup>b</sup> (% Brix)	rotten berries (%)	cysteine S-conjugate rotten <sup>c</sup> (μg/L)	cysteine S-conjugate healthy <sup>c</sup> (μg/L)
PA32	119	26.0	4557	16.0	2.5	40	31
PA87	145	25.0	3758	20.0	3.7	43	58
PA40	261	24.5	3833	19.0	6.4	30	51
PA826	468	21.5	4931	19.0	8.7	88	54
PA859	578	22.5	4951	19.5	10.5	75	55
PA836	493	20.5	3883	19.5	11.3	63	44
PA231	628	23.0	4440	18.0	12.4	80	48
PA208	409	21.5	2845	19.5	12.6	63	65
PA26	738	23.0	5083	18.0	12.7	25	57
PA806	476	24.0	3256	20.5	12.8	35	53
PA804	357	25.5	2106	20.0	14.5	53	48
PA35	625	22.5	2985	18.5	17.3	83	74
PA219	1261	23.0	5061	16.0	19.9	80	83
PA825	671	23.5	2262	20.0	22.9	55	43
PA104	1149	25.0	3513	18.5	24.6	25	73
PA10	848	22.5	2399	19.5	26.1	46	75
PA235	1868	25.5	4053	18.5	31.5	45	67
PA23	1765	23.5	2849	17.5	38.3	18	84
PA252	2222	25.0	3005	20.0	42.5	45	78
PA1003	2341	23.0	2609	19.5	47.3	34	85

<sup>a</sup> Weight of healthy, respectively rotten, berries in the collected samples. <sup>b</sup> % Brix measured in the must of healthy, respectively rotten, berries. <sup>c</sup> Concentrations of cysteine-S-conjugate of 3-mercaptohexan-1-ol obtained in the must of healthy, respectively rotten, berries.

**Figure 2.** Reaction pathway to prepare the deuterated S-3-(hexan-1-ol)-cysteine (4).

**Samples.** The samples of Petite Arvine grapes were taken in 2002 at the experimental Valais State vineyard, where clones of Petite Arvine grapes are grown. Each sample originates from a different clone. For each clone, the harvest of three vine stocks was collected at maturity of the grape, and on the same day, the harvest was manually separated into healthy and rotten berries. The percent Brix (**Table 1**) was determined in each part. It should be noted that no distinction was made as to the type of rot. The samples were stored at  $-18^{\circ}\text{C}$  until they were squeezed to give the Petite Arvine must. For microvinification experiments, must of Petite Arvine of 2001 with a percent Brix of 23.8 and a total acidity expressed in tartaric acid of 7.4 g/L was used.

**Microvinification.** All of the must used for microvinification received an addition of sulfur dioxide of 50 mg/L of must. A solution of yeast was prepared by adding *Saccharomyces cerevisiae*, strain D96 (1.0 g), in must (5 mL) and water (5 mL). After inoculation of the 500 mL must with 3 mL of the yeast solution, alcoholic fermentation took place in 900 mL bottles at room temperature.

**<sup>1</sup>H NMR Spectra.** Nuclear magnetic resonance spectra of hydrogen nuclei (<sup>1</sup>H NMR) were recorded at 400 MHz, with a Bruker Avance 400 spectrometer. Chemical shifts  $\delta$  are noted relative to the signal of sodium 2,2-dimethyl-2-silapentane-5-sulfonate ( $\delta$  0.00 ppm), and coupling constants ( $J$ ) are noted in hertz. Coupling patterns are described by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

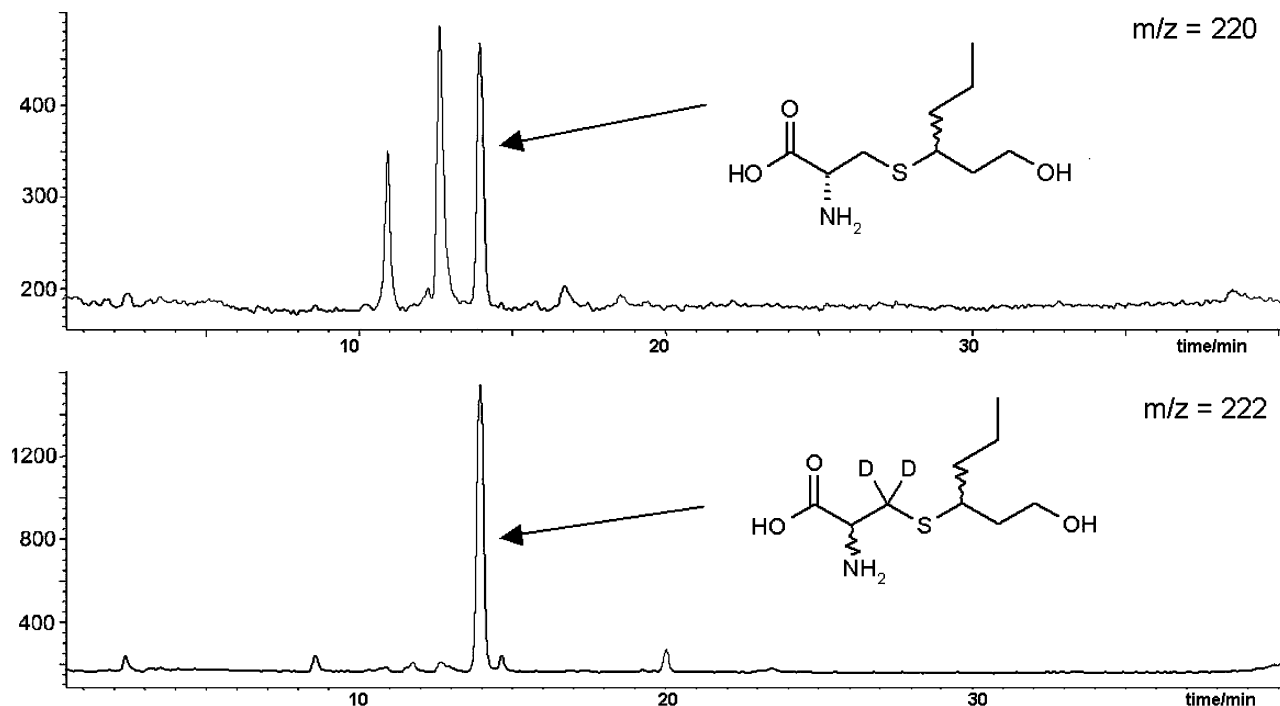
**HPLC-MS Analysis.** HPLC-MS analyses were performed using an Agilent 1100 MSD system equipped with a G1311A quaternary pump, a G1322A degasser, a G1314A UV detector, and a G1947-60101 mass spectrometer with an atmospheric pressure chemical ionization (APCI) source negative ion detection mode. Nebulizer pressure was 50 psi, dry gas temperature was  $300^{\circ}\text{C}$ , dry gas flow was 4.5 L/min, vaporizer temperature was  $375^{\circ}\text{C}$ , corona current was 10  $\mu\text{A}$ , and capillary voltage was 2000 V. Mass chromatograms, in the single ion monitoring mode (SIM), of the ions  $m/z$  220 ( $[\text{M} - \text{H}]^{-}$ , unlabeled precursor **1**) and  $m/z$  222 ( $[\text{M} - \text{H}]^{-}$ , labeled precursor) were recorded. For

quantification the natural abundance of the  $\text{M} + 2$  peak of the unlabeled precursor, due to the <sup>34</sup>S and two <sup>13</sup>C isotopes, was taken into account. Separation was performed through a Nucleosil 100-5 C<sub>18</sub> HD column (5  $\mu\text{m}$ , 100 Å,  $250 \times 3.0$  mm). The gradient profile was started at 1% CH<sub>3</sub>CN (A), 1% aqueous solution of 1% formic acid (B), and 98% water (C) at a flow rate of 0.60 mL/min. A linear gradient was applied for 40 min, and the final composition was 40% A, 1% B, and 59% C.

**Synthesis of the Deuterated Cysteine S-Conjugate.** Deuterated S-3-(hexan-1-ol)-cysteine was synthesized (**Figure 2**) using the method described by Luisier et al. (16).

( $\pm$ )-2-Amino-3-[1-(2-ethoxy-2-oxoethyl)butyl]sulfanyl[3,3-<sup>2</sup>H<sub>2</sub>]propanoic Acid (**3**). At  $25^{\circ}\text{C}$  and under argon, the pH of a solution of [3,3-<sup>2</sup>H<sub>2</sub>]-DL-cysteine (0.20 g, 1.63 mmol) in water (3 mL) was brought up to 7–8 with a 10% aqueous solution of sodium hydroxide; this was followed by the addition of ethyl (2E)-2-hexenoate (**17**, **18**) (460 mg, 3.26 mmol). After 50 h under stirring, the mixture was acidified to pH 5 with a 15% solution of hydrochloric acid and washed with diethyl ether ( $2 \times 1.5$  mL). After evaporation, trituration of the residue in diethyl ether afforded a white powder **3**: yield = 0.43 g (99%); <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  0.83 (t, 3,  $J = 7.3$  Hz, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.19 (t, 3,  $J = 7.2$  Hz, OCH<sub>2</sub>CH<sub>3</sub>), 1.4 (m, 2, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.5 (m, 2, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.6 (m, 2, CH<sub>2</sub>CO), 3.1 (m, 1, SCH), 3.82 and 3.86 (s, 1, CHNH<sub>2</sub>), 4.12 (q, 2,  $J = 7.2$  Hz, OCH<sub>2</sub>CH<sub>3</sub>); APCI<sup>+</sup>  $[\text{M} + \text{H}]^{+}$  266.

( $\pm$ )-2-Amino-3-[1-(2-hydroxyethyl)butyl]sulfanyl[3,3-<sup>2</sup>H<sub>2</sub>]propanoic Acid (**4**). At  $50^{\circ}\text{C}$  and under argon, sodium trimethoxyborohydride (1.9 g, 15 mmol) was added to a solution of the propanoic acid derivative (**3**, 0.43 g, 1.8 mmol) in dimethoxyethane (25 mL). After 4 h under reflux, methanol (2 mL) was added, followed by water (2 mL). The solution was acidified to pH 3 with concentrated hydrochloric acid and evaporated. The crude product was purified using preparative HPLC with a C<sub>18</sub> column to afford a white powder **4**: yield = 0.09 g (25%); <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  0.78 (t, 3,  $J = 7.3$  Hz, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)



**Figure 3.** HPLC-MS(APCI) SIM mass chromatograms of a typical Petite Arvine must isotopic dilution assay with 100  $\mu\text{g/L}$  of added internal standard **4** with SPE sample concentration step.

1.30 (m, 2,  $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 1.5 (m, 2,  $\text{CH}_2\text{CH}_2\text{CH}_3$ ), 1.61 (m, 1,  $\text{CH}_2\text{CH}_2\text{OH}$ ), 1.76 (m, 1,  $\text{CH}_2\text{CH}_2\text{OH}$ ), 2.8 (m, 1,  $\text{SCH}$ ), 3.6 (m, 2,  $\text{CH}_2\text{OH}$ ), 3.78 (s, 1,  $\text{CHNH}_2$ );  $\text{APCI}^+ [\text{M} + \text{H}]^+ 224$ .

**Sample Preparation.** At room temperature, the deuterated analogue **4** (100  $\mu\text{L}$  of a 20 mg/L aqueous solution) was added to the must or wine (20 mL). An aliquot (8 mL) of the samples to be analyzed, either must or wine, was passed through a SPE cartridge (solid phase extraction) (Supelclean Envi-18 SPE tube, 3 mL, 0.5 g packing), previously conditioned with methanol, followed by elution with methanol (2 mL). After evaporation, the concentrated extract (500  $\mu\text{L}$ ) was ready to be injected into the HPLC-MS system.

## RESULTS AND DISCUSSION

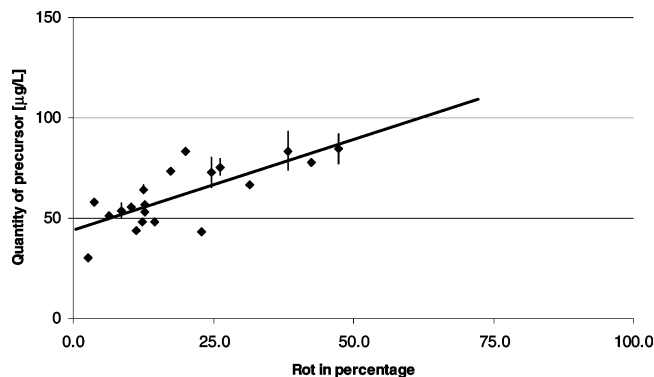
**Isotopic Dilution Method.** Figure 3 shows a typical chromatogram of a must of Petite Arvine after adsorption on a SPE cartridge followed by elution. Under the chromatographic conditions used, the diastereomers elute with the same retention time. The limit of detection (LOD), expressed as the concentration at which the signal-to-noise intensity ratio is 3, and the limit of quantification (LOQ), expressed as the concentration at which the signal-to-noise intensity ratio is 10, determined in must are 3 and 10  $\mu\text{g/L}$ , respectively. These results are similar to that of the published method (3). The major advantage of the method presented here is the simplified sample preparation step, which requires only one preconcentration step on a commercial SPE column and no derivatization prior to analysis. The recovery of the SPE preconcentration step was determined in a must sample with a concentration of **4** of 104  $\mu\text{g/L}$  to be 81%.

Integrated peak area ratios were plotted against concentration of **4**, in must samples, for a concentration range of 10.6–209.6  $\mu\text{g/L}$  and a **1** concentration of 20.7  $\mu\text{g/L}$ . The resultant curve was linear [area ratio =  $(0.0471 \times \text{concentration}) + 0.0566$ ;  $R^2 = 0.998$ ]. In addition, integrated peak area ratios were plotted against concentration of **1**, in must samples, for a concentration range of 20.7–175.5  $\mu\text{g/L}$  and a **4** concentration of 104.8  $\mu\text{g/L}$ . The resultant curve was linear [area ratio =  $(0.00908 \times \text{concentration}) + 0.02417$ ;  $R^2 = 0.998$ ]. A typical must sample

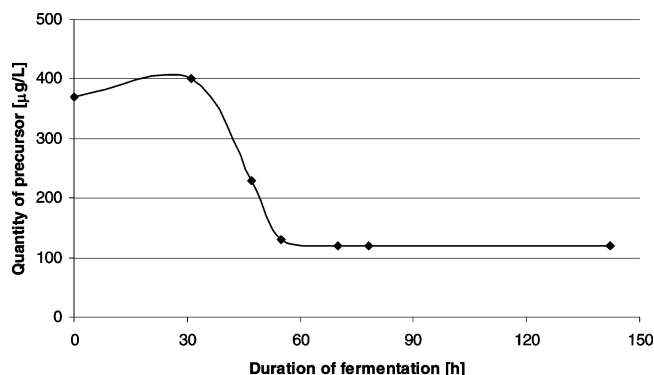
that contained **1** at a concentration of 21  $\mu\text{g/L}$  was analyzed ( $n = 5$ ) to check the coefficient of variance (CV) of the method. The CV was determined to be 3.4%. The linearity was also determined in wine, where integrated peak area ratios were plotted against concentration of **1** for a concentration range of 27.8–208.4  $\mu\text{g/L}$  and a **4** concentration of 104.8  $\mu\text{g/L}$ . The resultant curve was linear [area ratio =  $(0.00856 \times \text{concentration}) + 0.03876$ ;  $R^2 = 0.998$ ]. To check the response factor ratio of labeled **4** versus unlabeled **1**, cysteine *S*-precursor, an aqueous solution of (20.5 mg/L) and its deuterated analogue (20.3 mg/L) was used for serial dilutions with water in the concentration range of 0.203–2.03 mg/L. These solutions were extracted as described under Sample Preparation. The resultant curve was linear ( $R^2 = 0.9999$ ) with a response ratio of 1.0012.

**Concentration of Precursor in Petite Arvine Must.** The measurement of the concentration of the precursor of 3-mercaptohexan-1-ol (**2**) in the 20 samples of grape juice gave results between 30 and 90  $\mu\text{g/L}$  (Table 1), which were difficult to interpret. It is known that this concentration changes considerably and in an unpredictable way during maturation of the grape (19). We did find a good correlation between the percentage of rot in the harvest and the concentration in the healthy berries of the grapes. Figure 4 shows the correlation between the precursor concentrations in the healthy berries of the grape plotted against the percentage by weight of rotten berries in the sample. With 20 samples and a correlation coefficient ( $R$ ) of 0.745, the confidence level for this relationship is >99.9%. This means that the formation of the cysteine *S*-conjugate is strongly enhanced when the grape begins to rot. The same analysis was also carried out on the must obtained from the rotten berries of the grape. The result shows an inverse correlation between the concentration of precursor and the degree of rot. However, the confidence level for this correlation is only 91% ( $N = 20$ ;  $R = 0.319$ ).

A similar observation was made by the Dubourdieu group (20) when they found a low level of precursors in healthy grapes, a high level in “pourri plein”, in which the rotten berries are



**Figure 4.** Correlation between the concentration of S-3-(hexan-1-ol)-cysteine (1) in Petite Arvine must of healthy berries of the grape and percentage by weight of rotten berries. The error bars represent one standard deviation of replicates ( $n = 2$ ).



**Figure 5.** Transformation of S-3-(hexan-1-ol)-cysteine (1) during fermentation of the Petite Arvine must. The results are obtained from a single experiment.

always round, and a lower level in “pourri rôti”, in which the berries are partly evaporated. This could be interpreted by taking in account the well-known fact that in injured vegetable cells production of hexenal takes place (21), which will rapidly bind to glutathione (22), and that finally the glutathione adduct is very quickly degraded to S-3-(hexan-1-ol)-cysteine (1). The decrease of precursor concentration in the pourri rôti could be interpreted in terms of a transformation of the precursor to 3-mercaptohexan-1-ol (2), which can oxidize and thus disappear from berries.

**Concentration Changes of Cysteine S-Conjugate during Fermentation.** It is known that <10% of the cysteine S-conjugate is transformed into the volatile 3-mercaptohexanol (3) and about 60% is probably degraded during the process (23). We have found that after the alcoholic fermentation by yeasts, 30% of the precursor remains untransformed (Figure 5). The concentration of the latter was stabilized after 50–60 h. It should be noted that in this must, collected in 2001, the initial concentration of precursor was about 4–5 times higher than for the samples used above, collected in 2002. The annual variation is one of the subjects in a further study.

In an second experiment, in which must was doped with the precursor to an initial concentration of 1680 µg/L, the transformation of cysteine S-conjugate in the must stopped after 60 h and the remaining concentration was stable at 1200 µg/L, so approximately 70% was not converted in the yeast medium. In a similar experiment by Fretz (24) with a cysteine marked with  $^{35}\text{S}$ , about half of the radioactive precursor remained nontransformed in the water phase. This can be interpreted as a specific

cleavage of one of the racemic forms by the yeast, as was found by Wakabayashi for tryptophanase and *E. limosum* (9). As the synthesized cysteine S precursor, according to  $^{13}\text{C}$  NMR data (16), was a 45/55% diastereoisomeric mixture arising from the reaction of L-cysteine with ethyl hexenoate, we can postulate that one of the diastereoisomers was transformed preferentially by yeasts, whereas the second one remained almost unchanged during the fermentation. In this case one should observe in the wine an enantiomeric distribution of 3-mercaptohexanol in favor of one of the enantiomers. In fact, Tominaga et al. observed that, in Semillon and Sauvignon wines made from botrytized grapes with a higher precursor concentration, the enantiomeric distribution of 3-mercaptohexanol was 30:70 in favor of the S form and that, in dry white wines with a low precursor concentration, a uniform enantiomeric distribution of 50:50 was obtained (25). Another possible explanation for the fact that the total amount of precursor is not converted is an inhibition of the enzymatic activity (9).

## ACKNOWLEDGMENT

We are thankful for technical support by Michel Pont, Claudia Barbara Fretz, Fabrice Micau, and Pascal Jacquemettaz.

## LITERATURE CITED

- (1) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J. N.; Dubourdieu, D. Identification of a powerful aromatic of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385–392.
- (2) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon wines. *Flavour Fragrance J.* **1998**, *13*, 159–162.
- (3) Murat, M.-L.; Tominaga, T.; Dubourdieu, D. Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. *J. Agric. Food Chem.* **2001**, *49*, 5412–5417.
- (4) Fretz, C. B.; Luisier, J.-L.; Tominaga, T.; Amado, R. 3-Mercaptohexanol: an aroma impact compound of petite arvine wine. *Am. J. Enol. Vitic.* **2005**, *56*, 407–410.
- (5) Fretz, C. B.; Känel, S.; Luisier, J.-L. Analysis of volatile components of Petite Arvine wine. *Eur. Food Res. Technol.* **2005**, 504–510.
- (6) Fretz, C. B.; Luisier, J.-L.; Amado, R. Sensory characterisation of Petite Arvine wines. *Mitt. Lebensm. Hyg.* **2006**, *96*, 189–198.
- (7) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D. A new type of flavor precursor in *Vitis vinifera* L. cv. Sauvignon Blanc S-cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215–5219.
- (8) Peyrot des Gachons, C.; Tominaga, T.; Dubourdieu, D. Sulfur aroma precursor present in S-glutathione conjugate form: identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Agric. Food Chem.* **2002**, *50*, 4076–4079.
- (9) Wakabayashi, H.; Wakabayashi, M.; Eisenreich, W.; Engel, K.-H. Stereochemical course of the generation of 3-mercaptohexanol and 3-mercaptohexanol by  $\beta$ -lyase-catalyzed cleavage of cysteine conjugates. *J. Agric. Food Chem.* **2004**, *52*, 110–116.
- (10) Swiegers, J. H.; Pretorius, I. S. Modulation of volatile sulfur compounds by wine yeast. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 954–960.
- (11) Starkenmann, C.; Niclass, Y.; Escher, S. Volatile organic sulfur-containing constituents in *Poncirus trifoliata* (L.) Raf. (Rutaceae). *J. Agric. Food Chem.* **2007**, *55*, 4511–4517.
- (12) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of  $\beta$ -damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757–759.
- (13) Pfner, P.; Matsui, T.; Grosch, W.; Guth, H.; Hofmann, T.; Schieberle, P. Development of a stable isotope dilution assay for the quantification of 5-methyl-(E)-2-hepten-4-one: application to



- hazelnut oils and hazelnuts. *J. Agric. Food Chem.* **1999**, *47*, 2044–2047.
- (14) Schneider, R.; Kotseridis, Y.; Ray, J.-L.; Augier, C.; Baumes, R. Quantitative determination of sulfur-containing wine odorants at sub parts per billion levels. 2. Development and application of a stable isotope dilution assay. *J. Agric. Food Chem.* **2003**, *51*, 3242–3248.
- (15) Katherine, R.; Hebditch, R.; Nicolau, L.; Brimble, M. A. Synthesis of isotopically labelled thiol volatiles and cysteine conjugates for quantification of Sauvignon Blanc wine. *J. Labelled Compd. Radiopharm.* **2007**, *50*, 237–243.
- (16) Luisier, J.-L.; Veyrand, J.; Piantini, U. Characterisation and simple synthesis of S-[3-hydroxy-1-propylpropyl]-L-cysteine. *Chimia* **2007**, *61*, 533–535.
- (17) Tanaka, K.; Yamagishi, N.; Tanikaga, R.; Kaji, A. New method for synthesis of  $\alpha,\beta$ -unsaturated carboxylic esters from carbonyl compounds using monoanions of dithiocarbonates, and dianions of ethyl mercaptoacetate and ethyl 2-mercaptopropionate. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 3619–3625.
- (18) Yamada, K.; Miyaura, N.; Itoh, M.; Suzuki, A. Addition reaction of copper(I) methyltrialkylborates with ethyl propiolate. Stereospecific synthesis of (*E*)- $\alpha,\beta$ -unsaturated acid esters. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3431–3432.
- (19) Peyrot des Gachons, C.; Tominaga, T.; Dubourdieu, D. Measuring the aromatic potential of *Vitis vinifera* L. Cv. Sauvignon Blanc grapes by assaying S-cysteine conjugates, precursors of the volatile thiols responsible of their varietal aroma. *J. Agric. Food Chem.* **2000**, *48*, 3387–3391.
- (20) Thibon, C. Recherches sur les précurseurs cystéinylés de l'arôme variétal des vins de Sauvignon blanc. Ph.D. Thesis 1361, Université Victor Segalen Bordeaux II, 2006; pp 87–90.
- (21) Hatanaka, A.; Sekiya, J.; Kajiwar, T. Linolenic acid and its 13-hydroperoxide inhibit hexanal formation from linoleic acid in plant tissues. *J. Agric. Food Chem.* **1983**, *31*, 176–178.
- (22) Davoine, C.; Falletti, O.; Douki, T.; Iacazio, G.; Ennar, N.; Montillet, J.-L.; Triantaphylidès, C. Adducts of oxylipin electrophiles to glutathione reflect a 13 specificity of the downstream lipoxygenase pathway in the tobacco hypersensitive response. *Plant Physiol.* **2006**, *140*, 1484–1493.
- (23) Peyrot des Gachons, C. Recherches sur le potentiel aromatique des raisins de *Vitis vinifera* L. cv. Sauvignon blanc. Ph.D. Thesis 799, Université Victor Segalen Bordeaux II, 2000.
- (24) Fretz, C. B. Aroma active sulphur compounds and their precursors in Petite Arvine wine. Ph.D. Thesis 15932, Swiss Federal Institute of Technology Zürich, 2005.
- (25) Tominaga, T.; Niclass, Y.; Frerot, E.; Dubourdieu, D. J. Stereoisomeric distribution of 3-mercaptohexan-1-ol and 3-mercaptohexylacetate in dry and sweet white wines made from *Vitis vinifera* (var. Sauvignon Blanc and Semillon). *J. Agric. Food Chem.* **2006**, *54*, 7251–7255.

---

Received for review October 8, 2007. Revised manuscript received February 29, 2008. Accepted March 5, 2008. We acknowledge financial support by the HES-SO/Valais.

JF072963O