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ARTICLE

# Straightforward Synthesis of Deuterated Precursors To Demonstrate the Biogenesis of Aromatic Thiols In Wine

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Straightforward synthesis of labeled *S*-3-(hexan-1-ol)-glutathione and *S*-4-(4-methylpentan-2-one)-glutathione has been developed through a conjugate addition optimization study. Sauvignon blanc fermentation experiments with the [$^2H_{10}$] *S*-4-(4-methylpentan-2-one)-glutathione used as a tracer released the corresponding deuterated thiol, thus proving the direct relationship with the 4-mercapto-4-methylpentan-2-one under enological conditions. The conversion yield of such transformation was estimated to be close to 0.3%, opening an avenue for additional study on varietal thiol biogenesis.

**KEYWORDS: Glutathione; precursor; grapes; wine**

## INTRODUCTION

More than a thousand aroma compounds have been identified so far in grapes and wine, but only few of them contribute to the wine aroma (*1*). During the past 10 years, some varietal thiols, such as 4-mercapto-4-methylpentan-2-one (**1**), 3-mercaptohexan-1-ol (**2**), and 3-mercaptohexylacetate (**3**), have been identified and mainly studied in wines of Sauvignon blanc (*2*, *3*), Macabeo (*4*), Traminer (*5*, *6*), Muscadet and Bacchus (*7*), Mueller-Thurgau and Kerner (*8*), Verdejo and Albarino (*9*), Gewurztraminer and Scheurebe (*10*), Petite Arvine (*11*), Cabernet Sauvignon, Cabernet franc, and Merlot (*12*, *13*). These thiols are responsible for the wines' fruity notes (**Figure 1**). Compounds **2** and **3** are reminiscent of grape fruit, passion fruit, and box tree, especially for **3**, whereas **1** exhibits broom, black currant bud, and box tree odors (*14*). These varietal thiols contribute positively to the fruity notes of young wines due to their very low perception thresholds: 0.8, 4.2, and 60 ng/L in hydroalcoholic solution for compounds **1**, **3**, and **2**, respectively (*14*).

These varietal thiols are released during alcoholic fermentation from nonvolatile precursors identified as glutathione (*15*, *16*) and cysteine conjugates (*17*−*19*) under the action of the yeast through its $\beta$-lyase enzymatic activity (*19*). The formal relationship between compounds **7** and **2** has been established in Sauvignon blanc (*20*), but this has never been investigated for **5**; neither have the enological conditions.

To better understand the biogenesis of fruity notes in wines, the quantification of their natural precursors in must would enable the determination of the aromatic potential of grapes. Accurate quantification of such aroma precursors at trace levels in grapes (*21*) is achieved by the stable isotope dilution assay (SIDA), which involves labeled analogues. Up to now, synthesis of 4-mercapto-4-methylpentan-2-one precursors, *S*-4-(4-methylpentan-2-one)-L-cysteine (**4**) and *S*-4-(4-methylpentan-2-one)-glutathione (**5**), has been reported as natural and deuterated compounds (*19*, *22*−*24*). Precursors of 3-mercaptohexan-1-ol, *S*-3-(hexan-1-ol)-cysteine (**6**) and *S*-3-(hexan-1-ol)-glutathione (**7**), were synthesized as a racemic mixture in natural and deuterated forms (*16*, *19*, *20*, *22*, *25*−*28*) and diastereomerically pure products (*22*, *29*).

Direct synthesis of **7** and **5** was already described in the literature (*22*); unfortunately, we did not succeed in reproducing the protocol. Therefore, we developed a new, shorter *S*-glutathione conjugate synthesis, which is easily reproducible by nonchemist specialists.

In this paper, we report a straightforward synthesis of **7** and compare it with the multistep synthetic strategies already described refs *20* and *22*. Then, we adapted the optimized synthesis to obtain the deuterated analogues, that is, **7**-$d_2$ and **5**-$d_{10}$, the latter being used as tracer to bring some insight into the biogenesis of aromatic thiols in wine.

## MATERIAL AND METHODS

**Chemicals.** Reduced glutathione, (*E*)-2-hexenal, hydrochloric acid, sodium dihydrogenphosphate, sodium monohydrogenphosphate, sodium borohydride, and [$^2H_{10}$]mesityl oxide were purchased from Sigma-Aldrich (St Quentin en Fallavier, France). Sodium sulfate was purchased from Merck (Darmstadt, Germany), and absolute ethanol and sodium hydroxide were from Carlo Erba (Val de Reuil, France). Pyridine was supplied from Fluka (St Quentin en Fallavier, France) and dichloromethane from Riedel de Haën (St Quentin en Fallavier, France). All of the chemicals required to elaborate the synthetic must were supplied from Sigma-Aldrich.

**Analytical Procedures.** Electrospray ionization (ESI) mass spectra were recorded on a Micromass II quadrupole mass spectrometer fitted with an electrospray source coupled with a Waters HPLC. The injection

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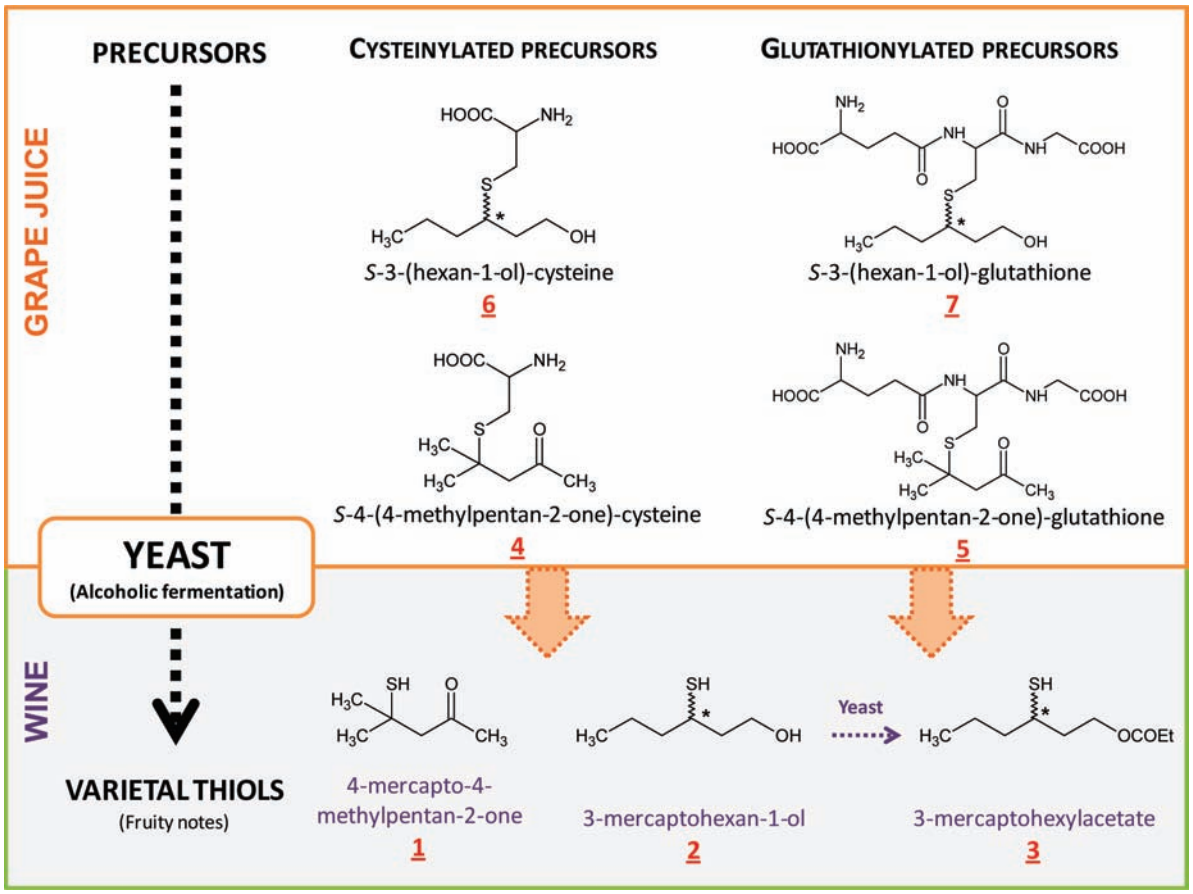


**Figure 1.** Release of varietal thiols from *S*-conjugate glutathione and cysteine precursors.

volume was equal to 2 $\mu$L, and the column temperature was maintained at 22 °C during the analysis. Analytes were first separated on a reverse phase ONYX 18 monolithic column (Phenomenex, 4.6 mm, 2.5 cm, 2 $\mu$m). The flow rate was maintained at 3 mL/min. A linear gradient was set from 0 to 100% of mobile phase B (acetonitrile with 0.1% of TFA) over a 2.5 min period and then 100% of B during 1 min before returning to the initial conditions (100% of mobile phase A, water with 0.1% of TFA). Detection was performed in positive ionization mode with the spray voltage and temperature source maintained at 3 kV and 120 °C, respectively.

Purifications were performed on preparative HPLC (Waters) using a reverse phase C18 (RP18, 19 × 100 mm, 5 $\mu$m). Detection was carried out by UV at 214 nm, and the flow rate was maintained at 50 mL/min.

HRMS (Q-TOF) were recorded on a JEOL JMS-SX-102A spectrometer.

NMR spectra were recorded on a Varian Unity Inova 500 equipped with a 3 mm reverse probe, and chemical shifts were referenced to trimethylsilylpropionic acid. NMR acquisitions were performed using VNMRJ software.

Analysis of **1** and **1**-$d_{10}$ was carried out using a Shimadzu GC-MS QP5050 coupled to a quadrupole mass spectrometer detector operating in selected ion monitoring (SIM) mode. Detection was performed in negative chemical ionization mode with methane using $m/z$ 160 and 170 as quantifier ions and $m/z$ 301 and 309 as qualifier ions for **1** and **1**-$d_{10}$, respectively. The compound **1**-$d_{10}$ was synthesized according to the method of Kotseridis et al. (*30*) with good yield and purity.

**Synthesis of *S*-3-(Hexan-1-ol)-glutathione (7).** (*E*)-2-Hexenal was added to a solution of glutathione (500 mg, 1.64 mmol) in a phosphate buffer ($NaH_2PO_4/Na_2HPO_4$, 1 M, pH 8, 10 mL) in three steps (3 × 0.33 equiv), with a 3 h interval between each addition. The reaction was stirred for 10 h (total duration of the reaction) at room temperature. Then, sodium borohydride (177 mg, 4.3 mmol) in water (4 mL) was added dropwise to the reaction mixture, which was left for 2 h at room temperature. The mixture was then concentrated under vacuum after the pH was adjusted to 3 with 2 M HCl. The resulting residue was dissolved in ethanol (10 mL), and a sodium hydroxide solution was added dropwise until a white precipitate was formed, which was filtered and purified on a reverse phase C18 to give the pure expected product (70 mg, 11%): mp = 154 °C; LC-MS, $[M + H]^+$ = 408.3 Da, rt = 0.84 min. HRMS (Q-TOF) calculated for $C_{16}H_{30}N_3O_7S$, 408.1804 Da; found, 408.1799 Da. $^1$H NMR (500 MHz, $D_2O$), $\delta$ 0.89 ($CH_3$, 3H, m, $J$ = 4.2 Hz), 1.42 ($CH_2$, 2H, m, $J$ = 7.3 Hz), 1.58 ($CH_2$, 2H, m, $J$ = 7.2 Hz), 1.71 and 1.85 ($CH_2$, 2H, m, $J$ = 7.4 Hz), 2.19 ($CH_2\beta$Glu, 2H, m, $J$ = 7.5 Hz), 2.56 ($CH_2\gamma$Glu, 2H, m, $J$ = 5.5 Hz), 2.84 (CH, 1H, m, $J$ = 7.2 Hz), 2.87 and 3.07 ($CH_2\beta$Cys, 2H, m, $J$ = 4.8 Hz), 3.71 ($CH_2$, 2H, t, $J$ = 6.7 Hz), 3.91 ($CH_\alpha$Glu, 1H, m, $J$ = 5.7 Hz), 4.00 ($CH_2\alpha$Gly, 2H, s), 4.56 ($CH_\alpha$Cys, 1H, m, $J$ = 2.7 Hz); $^{13}$C NMR (500 MHz, $D_2O$), $\delta$ 177.52, 175.97, 175.73, 175.71, 62.22, 56.39, 56.18, 45.35, 44.33, 39.49, 39.40, 34.09, 33.98, 28.87, 22.24, 16.10.

**Synthesis of [$^2H_2$]-*S*-3-(Hexan-1-ol)-glutathione (7-$d_2$).** [2,3-$^2H_2$]-(*Z*)-2-Hexenal (150 $\mu$L), synthesized according to the method of Roland et al. (*20*), was added to a solution of glutathione (1.17 g, 3.8 mmol) in phosphate buffer ($NaH_2PO_4/Na_2HPO_4$, 1 M, pH 8, 10 mL). [$^2H_2$]-*S*-3-(Hexan-1-ol)-glutathione (**7**-$d_2$) was synthesized as a natural analogue with good yield as previously described for **7**. LC-MS, $[M + H]^+$ = 410.2 Da, rt = 0.83 min. HRMS (Q-TOF) calculated for $C_{16}H_{29}N_3O_7SD_2$, 410.1930 Da; found, 410.1935 Da. Spectral data were entirely consistent with those of the unlabeled compound **7** with the only difference being the absence of signals corresponding to the labeled position in the $^1$H NMR spectrum.

**Synthesis of [$^2H_{10}$]-*S*-4-(4-Methylpentan-2-one)-glutathione (5-$d_{10}$).** Pyridine (500 $\mu$L, 6.2 mmol) and [$^2H_{10}$]mesityl oxide (450 $\mu$L, 3.6 mmol) were added sequentially to a solution of glutathione (1.11 g, 3.6 mmol) in water (8 mL). Two additional portions of [$^2H_{10}$]mesityl oxide (2 × 150 $\mu$L) were added, the first after 8 h and the second after 20 h. The reaction mixture was stirred for 36 h at room temperature under nitrogen atmosphere and washed with dichloromethane (3 × 20 mL), and the aqueous layer was concentrated to dryness under vacuum. The resulting yellow oil was dissolved in absolute ethanol (40 mL) to obtain the desired product as a white precipitate, which was removed by filtration (1.23 g, 84%). mp = 135 °C; LC-MS, $[M + H]^+$ = 416.3 Da, rt = 0.74 min. HRMS (Q-TOF) calculated for $C_{16}H_{19}N_3O_7SD_{10}$, 416.2276 Da; found, 416.2270 Da. $^1$H NMR (500 MHz, $D_2O$), $\delta$ 2.81 (CDH, 1H, m), 2.16 ($CH_2\beta$Glu, 2H, m,

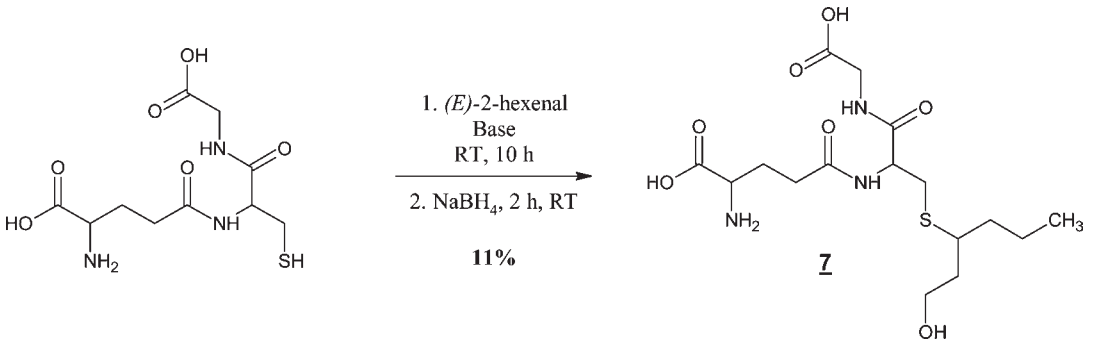


**Figure 2.** Direct synthesis of *S*-3-(hexan-1-ol)-glutathione (**7**).

$J$ = 7.5 Hz), 2.53 ($CH_2\gamma$Glu, 2H, m, $J$ = 5.5 Hz), 2.94 and 3.12 ($CH_2\beta$Cys, 2H, m, $J$ = 4.8 Hz), 3.77 ($CH_\alpha$Glu, 1H, m, $J$ = 5.7 Hz), 3.80 ($CH_2\alpha$Gly, 2H, s), 4.59 ($CH_\alpha$Cys, 1H, m, $J$ = 2.7 Hz); $^{13}$C NMR (500 MHz, $D_2O$), $\delta$ 216.62, 178.26, 177.70, 176.82, 174.87, 57.00, 56.43, 56.43, 56.04, 34.57, 34.24, 32.26, 30.07, 29.09.

**Fermentations.** Fermentations were conducted at laboratory scale (1.5 L) under synthetic and natural conditions (Sauvignon blanc must from Touraine, sugar = 205 g/L, total acidity = 4.11 g/L, pH 3.31, assimilable nitrogen = 47 mg/L). The synthetic media simulating standard grape juice was composed of (per liter) (*31*) glucose (200 g), citric acid (6 g); DL-malic acid (6 ng); mineral salts ($KH_2PO_4$, 750 mg; $K_2SO_4$, 500 mg; $MgSO_4 \cdot 7H_2O$, 250 mg; $CaCl_2 \cdot 2H_2O$, 155 mg; NaCl, 200 mg; $MnSO_4 \cdot H_2O$, 4 mg; $ZnSO_4$, 4 mg; $CuSO_4 \cdot 5H_2O$, 1 mg; KI, 1 mg; $CoCl_2 \cdot 6H_2O$, 0.4 mg; $H_3BO_3$, 1 mg; $NaMoO_4 \cdot 2H_2O$, 1 mg); vitamins (myoinositol, 20 mg; nicotinic acid, 2 mg; calcium pantothenate, 1.5 mg; thiamin−HCl, 0.25 mg; pyridoxine−HCl, 0.25 mg; biotin, 0.003 mg); anaerobic growth factors (ergosterol, 15 mg; sodium oleate, 5 mg; Tween 80, 0.5 mL); nitrogen source, 300 mg of nitrogen as ammoniacal nitrogen (18.6% $NH_4Cl$); and amino acids (L-proline, 20.5%; L-glutamine, 16.9%; L-arginine, 12.5%; L-tryptophan, 6%; L-alanine, 4.9%; L-glutamic acid, 4%; L-serine, 2.6%; L-threonine, 2.6%; L-leucine, 1.6%; L-aspartic acid, 1.5%; L-valine, 1.5%; L-phenylalanine, 1.3%; L-isoleucine, 1.1%; L-histidine, 1.1%; L-methionine, 1.1%; L-tyrosine, 0.6%; L-glycine, 0.6%; L-lysine, 0.6%; and L-cysteine, 0.4%).

Two experimental sets were performed as follows:

*Set 1*. Two fermenters using synthetic must, one for the control and one spiked with **5**-$d_{10}$ at a concentration equal to 4.5 nmol/L, were inoculated with VIN13 as yeast strain at 10 g/hL. Fermentations were maintained at 20 °C.

*Set 2*. The same protocol as for set 1 was followed using a Sauvignon blanc must from the Touraine region.

Fermentation progress was followed by fermenter weighing (total time approximately 12 days): the amount of $CO_2$ released was determined by automatic measurement of fermenter weight loss every 20 min (*32*). The resulting synthetic and Sauvignon blanc wines were clarified (3000 rpm, 15 min, 4 °C), and 100 mL of each sample was aliquoted. Wine samples were then analyzed according to the Rodriguez-Bencomo et al. method (*33*).

**Estimation of Conversion Yields from 5-$d_{10}$ to 1-$d_{10}$ Using a Standard Addition Method.** Sauvignon blanc wines obtained from experiment set 2 underwent four standard additions of **1**-$d_{10}$ (2, 4, 6, and 8 ng/L) to estimate the amount of **1**-$d_{10}$ naturally released by the yeast. The absolute value of the $x$-intercept for the relationship $\text{area}_{\mathbf{1}\text{-}d10} = f(\text{conc}_{\mathbf{1}\text{-}d10})$ corresponds to the amount of **1**-$d_{10}$ released by the yeast.

## RESULTS AND DISCUSSION

This study intended to provide small amounts of deuterated analogues of glutathione conjugates occurring in grapes. Falck et al. reported one method for deuterated analogue synthesis, which was based on acid labile protecting groups of *N*-$^t$Boc L-glutathione dimethyl and di-*tert*-butyl esters (*34*). According to our experience, it was very difficult to obtain *S*-(hexan-1-ol)-glutathione (**7**). Even after some improvements, the synthesis required lengthy (seven steps) and tedious efforts to obtain the desired product with a very moderate yield (*20*). Indeed, the three first steps represented a total yield of below 14%. Moreover, the esterification of glutathione conjugate led to two byproducts. The first byproduct came from the oxidation of free thiol and the second from the cleavage of the thioester bond by dimethylamine. Moreover, the final addition of glutathione conjugate on (*E*)-2-hexenal did not reach completion, and after a reduction step with sodium borohydride, a mixture of the desired product with glutathione was obtained. Recent investigations reported the direct synthesis of **7** and **7**-$d_9$ using a conjugate addition on (*E*)-2-hexenal with pyridine giving pure products in satisfactory yields equal to 34 and 44%, respectively (*22*). Unfortunately, we did not succeed in reproducing the Grant-Preece synthesis. Therefore, we developed a new strategy for the synthesis of the compound **7** in racemic form as well as for the synthesis of **5**-$d_{10}$. Interestingly, this method can also be used as a powerful tracer in fermentation experiments that involve **5**-$d_{10}$.

The free thiol of glutathione could be a good candidate for a nucleophilic addition on (*E*)-2-hexenal. Many references in the literature describe the addition of glutathione on unsaturated esters such as dimethyl fumarate (*35*), polyethylene glycol (*36*), conjugate ketones (*37*, *38*), and quinones (*39*, *40*). The basic idea was to use this chemistry to our benefit and design a more efficient and straightforward method for the synthesis of **7** (with a reduced number of steps and a satisfactory yield.

**Direct Synthesis of *S*-3-(Hexan-1-ol)-glutathione (7).** Conjugate addition of glutathione on (*E*)-2-hexenal led to **7** after a reduction step with sodium borohydride (**Figure 2**). The conjugate addition was the only limiting step (incomplete, generation of byproduct), whereas the reduction facilitated the production of the expected compound **7** in good yield and purity as already reported (*20*, *22*). To improve the global reaction yield and the purity of the desired product, investigations were necessary to optimize the conjugate addition.

First attempts with pyridine, triethylamine, or 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) as bases raised problems during the purification procedure. Consequently, only mineral bases were selected for further conjugate addition. To have a complete conjugate addition, an excess of (*E*)-2-hexenal was added in the reaction according to several procedures (1.2, 2.4, 3.0, 4.0, or 10.0 equiv). During several efforts with various amounts of (*E*)-2-hexenal a side-product was constantly formed (LC-MS $[M + H]^+$ = 1590.8 Da), which could not be eliminated but only minimized by the triple addition of the electrophile (3 × 0.3 equiv). Its removal from the reaction mixture was achieved by a purification procedure on a reverse phase C18 to give the expected product **7** as a pure white solid. To simultaneously generate the thiolate ion on glutathione, promote the conjugate addition, and prevent the oxidation of free thiol, the pH had to be adjusted carefully. The stability of each product was studied in a range of pH (pH 8.0−9.5). We observed that the first byproduct was formed (LC-MS $[M + H]^+$ = 1590.8 Da) at pH >8.5. When the pH

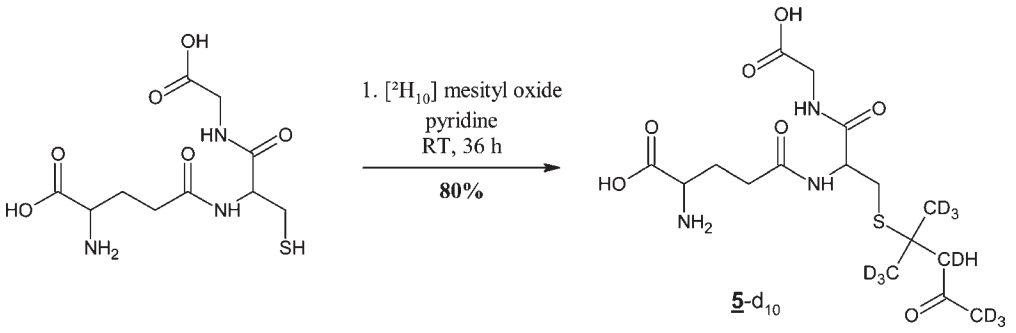


**Figure 3.** Direct synthesis of [$^2H_{10}$]-*S*-4-(4-methylpentan-2-one)-glutathione (**5**-$d_{10}$).

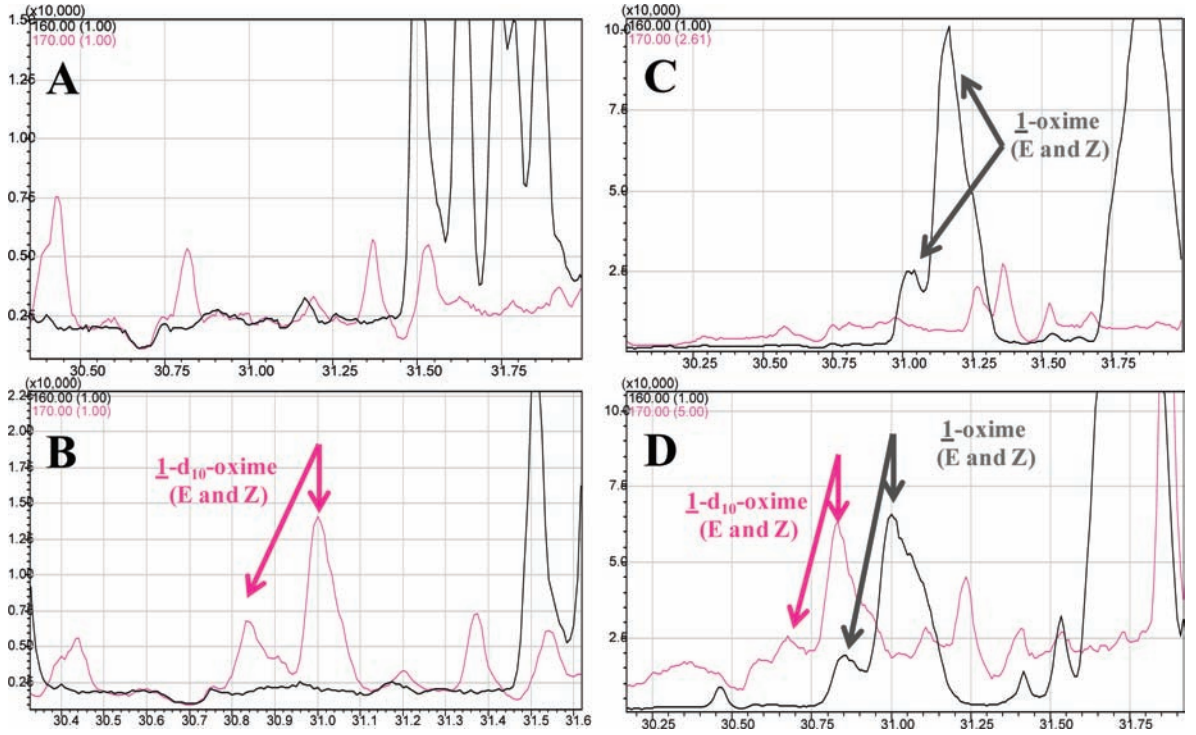


**Figure 4.** Identification of **1**-$d_{10}$ released in synthetic (**A**,**B**) and Sauvignon blanc (**C**,**D**) wines after alcoholic fermentation. **B** and **A** correspond to synthetic must with and without spiking of **5**-$d_{10}$ at 4.5 nmol/L, respectively. **D** and **C** correspond to Sauvignon blanc must with and without spiking of **5**-$d_{10}$ at 4.5 nmol/L. (The analysis of **1**-$d_{10}$ according to the Rodriguez-Bencomo et al. method (*33*) requires a methoximation of the ketone function involving two position isomers. The label "**1**-oxime (E and Z)" or "**1**-$d_{10}$-oxime (E and Z)" represents each produced isomer.)

was >9.0, an additional byproduct was observed (LC-MS $[M + H]^+$ = 794 Da). The best results were obtained at a pH close to 8.0. Furthermore, the conjugate addition was studied at 25 and 50 °C, and at the latter temperature, additional byproducts were formed, which presented problems in subsequent purification steps. In parallel, we studied the optimum reaction time (3 days, 2 days, 1 day, 10 h) and observed that the impurities increased along with the duration of the reaction.

Consequently, the optimal conditions for the conjugate addition of glutathione on (*E*)-2-hexenal were room temperature, slow addition of (*E*)-2-hexenal in three portions, pH 8 (phosphate buffer), and stirring for 10 h. Then, the same conditions, including an additional reduction step with sodium borohydride, were applied to the formation of **7**-$d_2$ with (*E*)-2,3-[$^2H_2$]-2-hexenal as starting material (*20*).

**Direct Synthesis of [$^2H_{10}$]-*S*-4-(4-Methylpentan-2-one)-glutathione (5-$d_{10}$).** Following the same pathway, a different deuterated glutathione conjugate was synthesized: [$^2H_{10}$]-*S*-4-(4-methylpentan-2-one)-glutathione (**5**-$d_{10}$) (see **Figure 3**). The natural compound was first identified in Sauvignon blanc grape juice by Fedrizzi et al. (*15*), whereas the labeled analogue was synthesized for the first time by Grant-Preece et al. (*22*). Our synthesis strategy was slightly modified to reduce the reaction time to 36 h instead of 48 h. Under our conditions no byproduct was observed. The reaction time of conjugate addition between glutathione and [$^2H_{10}$]mesityl oxide was longer (36 h) than for **7** synthesis, but no byproduct was observed. The direct synthesis of **5**-$d_{10}$ was inexpensive and effective (yield close to 80%). This established protocol was optimized, and it can be easily carried out by nonspecialists.

**Study of the Relationship between *S*-4-(4-Methylpentan-2-one)-glutathione (5) and 4-Mercapto-4-methylpentan-2-one (1).** Glutathione conjugates (**7** and **5**) were recently identified in different grape varieties such as as Sauvignon blanc (*15*, *16*), Melon B., Riesling, Gewürztraminer (*21*), Pinot Grigio, and Chardonnay (*41*) and brought new insight into the biogenesis of varietal thiols in wine.

Compound **7** was first reported as a pro-precursor of 3-mercaptohexan-1-ol (**2**) (*42*), and then its direct relationship with **2** was demonstrated in Sauvignon blanc with a conversion yield

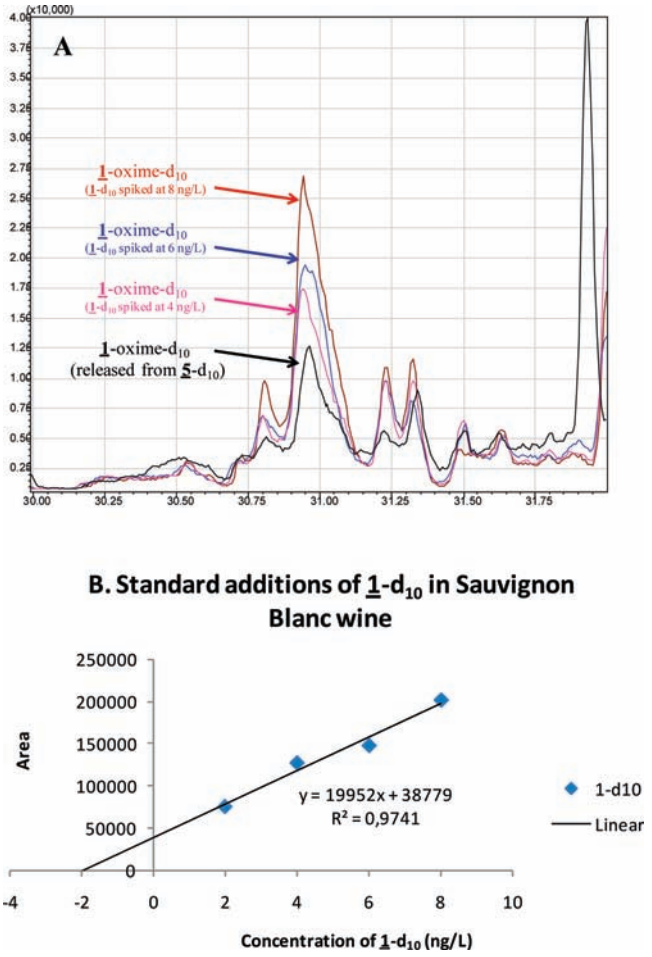


**Figure 5.** (**A**) Standard additions of **1**-$d_{10}$ in Sauvignon blanc wine and (**B**) calculation of conversion yields. (Standard additions of **1**-$d_{10}$ were performed in completely fermented Sauvignon blanc samples.)

close to 4.5% (*20*). Additional investigations showed that a modified yeast strain (overexpression of *Escherichia coli* gene *tna A*) compared to the conventional VIN13 was more efficacious to generate **2** from **7** under model conditions (*22*).

Compound **5** was first identified in Sauvignon blanc must (*15*), but its relationship with the corresponding thiol was never investigated. For the first time, we established the direct connection between the two compounds under enological conditions using synthetic and natural musts (Sauvignon blanc in the latter case). For this purpose, a synthetic must and a Sauvignon blanc must were spiked with **5**-$d_{10}$ at 4.5 nmol/L and were compared with their corresponding control experiments. The monitoring of **1**-$d_{10}$ release in wine was performed to study the direct relationship with the corresponding glutathionylated precursor.

Wines from synthetic and Sauvignon blanc musts were extracted and analyzed according to a well-optimized and published method (*33*). The formal identification of **1**-$d_{10}$ in both synthetic and Sauvignon blanc wines (same retention time and ion ratios as reference compound **1**-$d_{10}$) proved the direct relationship between compound **5** and the corresponding varietal thiol under enological conditions (**Figure 4**).

Standard additions of **1**-$d_{10}$ were performed in completely fermented Sauvignon blanc samples to estimate the conversion yield of **5**-$d_{10}$ into the corresponding varietal thiol (**Figure 5A**). The initial amount of **1**-$d_{10}$ released by the yeast during the alcoholic fermentation corresponded to the absolute value of the $x$-intercept for the following relationship: $\text{area}_{\mathbf{1}\text{-}d10} = f(\text{conc}_{\mathbf{1}\text{-}d10})$ with $\text{area}_{\mathbf{1}\text{-}d10}$, which was measured on chromatograms and $\text{conc}_{\mathbf{1}\text{-}d10}$, the concentration of **1**-$d_{10}$ spiked in completely fermented samples (**Figure 5B**). Under our conditions, the conversion yield of **5**-$d_{10}$ into the corresponding thiol was close to 0.3%. This value was similar to those reported by Subileau et al. (*43*) for the conversion yield of the cysteinylated precursor into **1** under equivalent conditions. The use of deuterated precursor probably induced some kinetic isotope effects during the enzymatic conversion of **5**-$d_{10}$ into **1**-$d_{10}$. However, compound **5** occurs in grapes at a mean level of about 1 μg/L, whereas **4** is present at 4 μg/L (*21*). Thus, the conversion of **5** into thiol by yeast could explain about 20% of the total **1** generated in Sauvignon blanc wines. Compound **5** constitutes the second major precursor of **1** in grapes.

## ABBREVIATIONS USED

ESI, electrospray ionization; NMR, nuclear magnetic resonance; HRMS, high-resolution mass spectrometry; GC-MS, gas chromatography−mass spectrometry; SIM, selected ion monitoring; TFA, trifluoroacetic acid; SIDA, stable isotope dilution assay; rt, retention time; mp, melting point.

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