

Synthesis of the Individual Diastereomers of the Cysteine Conjugate of 3-Mercaptohexanol (3-MH)

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The individual diastereoisomers of the cysteine conjugate of 3-mercaptohexanol (**4**) were synthesized with high isomeric purity (>98%). On treatment with Apotryptophanase enzyme, the 3*R* diastereoisomer of **4** gave an 82% yield of the *R* enantiomer of **1**, with no trace of the 3*S* enantiomer present. Conversely, the 3*S* diastereoisomer of **4** gave the 3*S* enantiomer of **1** (43%) accompanied by a trace of the 3*R* form (*S/R* = 98.5:1.5), reflecting the diastereomeric purity of the cysteine conjugate. The same stereochemical outcome was observed when the individual diastereoisomers of **4** were added to fermentations with the *Saccharomyces cerevisiae* AWRI 1655 yeast strain, which gave **1** in 1% yield. A *d*₁₀-analogue of **1** was synthesized and used as an internal standard to determine, by gas chromatography–mass spectrometry (GC-MS), the amounts of **1** formed in these transformations.

KEYWORDS: Wine; aroma, flavor; 3-MH; cysteine conjugates; thiols

INTRODUCTION

In recent years, the contribution of thiol-containing compounds to the aroma and flavor of wines has been an important focus for research (1). Many such compounds are extremely potent, with aroma detection thresholds typically in the nanograms per liter range, and are characterized by distinctive aroma qualities. Three thiols (Figure 1) that have been found to be important to the aroma of wine are 3-mercaptohexanol (3-MH, **1**), the *O*-acetate of this compound (3-MHA, **2**), and 4-mercapto-4-methylpentan-2-one (4-MMP, **3**). **1** is characterized by a tropical fruit/passion fruit aroma and was first identified in yellow passion fruit (2–4). It has subsequently been identified in several wine varieties (5–7). **2** has also been described as having a tropical fruit aroma and was found to occur, in passion fruit, predominantly as the *S* enantiomer (8). **3** has an aroma reminiscent of box tree and black currant but, at higher concentrations, has been found to have a rather unpleasant cat-urine odor (9–11). The importance of thiols to the overall aroma of a wine is exemplified by the fact that **3** was found to be the most potent aroma compound (of 42 measured) in one study (9).

These thiols themselves are not present in grape juice in measurable concentration prior to fermentation. Rather, **1** and **3** are believed to accumulate as L-cysteine and glutathione

conjugates (12–15), with the amino acid moiety of the former being cleaved by the action of the yeast during fermentation (16–18). **2** is presumed to be formed from **1** during fermentation as a result of yeast alcohol acetyltransferase activity (19). More recently, Schneider et al. have shown that volatile thiols can be formed by the addition of hydrogen sulfide, produced by yeast under fermentation conditions, to olefinic precursors of **1** and **3** (20). However, under the conditions reported, this alternative mode of generation accounted for approximately 10% only of the total thiols generated.

One factor that must be taken into consideration with **1** and **2** (but not **3**) is the stereochemistry at the carbon to which the sulfur is attached, as, for both compounds, the two enantiomers have differing sensory properties (21). The cysteine conjugate of **1** has two diastereomeric forms, namely, 3*S*-**4** and 3*R*-**4**. Thus, the stereochemical outcome of the transformation of the conjugate **4** by the yeast may be dependent on the starting configuration of **4**. Wakabayashi et al. (18) prepared a 1:1 diastereomeric mixture of **4**, and when this mixture was subjected to enzymatic cleavage, using two different sources of β -lyase, the two enantiomers of **1** were found in similar proportions.

As an expansion of our earlier work concerning the liberation of **3** from its cysteine conjugate (16, 17), we have now synthesized the two cysteine conjugate forms of **4**, in diastereomerically pure fashion, to investigate the stereoselectivity of thiol formation more thoroughly and to obtain authentic samples for characterizing grape composition.

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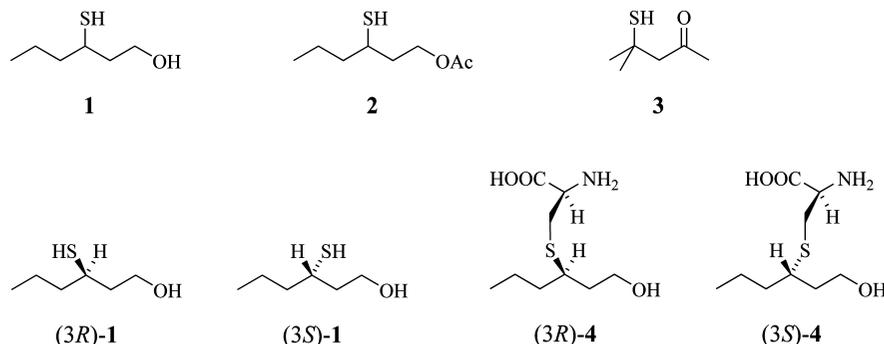


Figure 1. Structures of 3-MH (1), 3-MHA (2), 4-MMP (3), and the cysteinylated conjugates of 3-MH (4).

MATERIALS AND METHODS

NMR spectra were recorded as solutions in chloroform-*d* and were obtained on a Varian Gemini spectrometer operating at either 300 MHz (^1H) or 75.5 MHz (^{13}C). Specific rotations were recorded on a PolAAR 21 polarimeter. All reagents were purchased from Sigma-Aldrich. All solvents were of the highest commercial grade available. Diethyl ether and THF were distilled from sodium/benzophenone immediately prior to use. All organic solutions were dried over anhydrous sodium sulfate prior to filtration.

Methyl *N*-Butoxycarbonyl-L-cysteine (6). L-Cysteine methyl ester (5) (5.05 g, 29.4 mmol) in dichloromethane (30 mL) was stirred with di-*tert*-butyl dicarbonate (6.42 g, 29.4 mmol) and triethylamine (2.97 g, 29.4 mmol) at room temperature for 18 h. The solvent was removed and the residue chromatographed on silica (20:80 EtOAc/ CHCl_3) to give the *N*-protected compound (7.01 g, 100%), the spectral parameters of which were identical with those reported previously (25).

Methyl 2-Butoxycarbonylamino-3-[(3-hydroxy-1-propylpropyl)sulfanyl]propanoate (8). (*E*)-2-Hexenal (0.75 g, 7.6 mmol), triethylamine (1.55 g, 15.3 mmol), and the diprotected compound **6** (1.80 g, 7.6 mmol) in acetonitrile (35 mL) were stirred at room temperature for 3 days. Removal of the solvent and silica chromatography gave the desired aldehyde adduct **7** (2.17 g, 85%) as an inseparable mixture of diastereomers.

This adduct, **7** (1.69 g, 5.08 mmol), in methanol (10 mL) at 0 °C was treated with sodium borohydride (0.09 g, 2.38 mmol), which was added in two portions separated by 5 min. The reaction was stirred at room temperature for 3 h before being quenched with ammonium chloride solution. The solution was extracted with dichloromethane, dried, and concentrated to give the desired alcohol **8** (1.31 g, 77%).

Separation into the component diastereomers was achieved on silica using a ternary eluant comprising EtOAc (35%), CHCl_3 (52.5%), and hexane (12.5%). Obtained were pure samples of the first eluting, or "front" diastereomer (0.565 g), the second eluting "rear" diastereomer (0.38 g), and a fraction containing both (0.315 g).

(3R)-8 (front diastereomer): $[\alpha]_{\text{D}} -16.4$ (*c* 0.86, CHCl_3); ^1H NMR (CDCl_3) δ 5.43 (1H, br s, NH), 4.53 (1H, m, H_8), 3.90–3.64 (2H, m, H_1), 3.75 (3H, s, OMe), 3.04–2.76 (3H, m, $\text{H}_{3,7}$), 2.39 (1H, br s, OH), 1.91–1.82 (2H, m, H_2), 1.62–1.34 (4H, m, $\text{H}_{4,5}$), 1.44 (9H, s, tBu), 0.91 (3H, t, $J = 7.0$ Hz, H_6); ^{13}C NMR (CDCl_3) δ 171.9, 155.1, 80.6, 59.9, 53.3, 52.8, 42.6, 38.5, 37.3, 33.8, 28.5, 20.0, 14.2.

(3S)-8 (rear diastereomer): $[\alpha]_{\text{D}} +47.3$ (*c* 1.1, CHCl_3); ^1H NMR (CDCl_3) δ 5.40 (1H, br s, NH), 4.52 (1H, m, H_8), 3.88–3.64 (2H, m, H_1), 3.74 (3H, s, OMe), 3.02–2.84 (2H, m, H_7), 2.82–2.66 (1H, m, H_3), 2.32 (1H, br s, OH), 1.89–1.78 (2H, m, H_2), 1.67–1.35 (4H, m, $\text{H}_{4,5}$), 1.42 (9H, s, tBu), 0.89 (3H, t, $J = 7.0$ Hz, H_6); ^{13}C NMR (CDCl_3) δ 171.7, 155.5, 80.2, 60.1, 53.4, 52.5, 43.4, 37.9, 37.3, 32.9, 28.2, 19.9, 13.9.

(3R)-2-Amino-3-[(3-hydroxy-1-propylpropyl)sulfanyl]propanoic Acid (3R)-4. (3R)-**8** (0.41 g, 1.24 mmol) was treated with sodium hydroxide (47 mg, 0.95 equiv) in methanol (20 mL) at room temperature for 3 h and then allowed to stand in the refrigerator overnight. The solution was diluted with water and extracted with dichloromethane. The aqueous layer was acidified (to pH 2), saturated with solid sodium chloride, and thoroughly extracted with EtOAc. The EtOAc extracts were dried and concentrated to give the product (0.34 g, 84%): $[\alpha]_{\text{D}} -23.5$ (*c* 0.34, CHCl_3).

This compound (0.32 g, 1.0 mmol) was then stirred in dichloromethane containing TFA (3.42 g, 30.0 mmol) at room temperature for 2 h. The solvents were removed, and the residue was passed through a short ion-exchange resin column (Amberlite IRA-400), eluting with water, to give, after evaporation, the desired cysteinylated compound (3R)-**4** as its hydrochloride salt (0.18 g, 70%): $[\alpha]_{\text{D}} -14.1$ (*c* 0.32, H_2O); ^1H NMR (D_2O) δ 4.25 (1H, dd, $J = 6.5, 4.7$ Hz, H_8), 3.77–3.65 (2H, m, H_1), 3.23–3.02 (2H, m, H_7), 2.88 (1H, app quintet, $J \sim 6.2$ Hz, H_3), 1.96–1.30 (6H, m, $\text{H}_{2,4,5}$), 0.86 (3H, t, $J = 7.2$ Hz, H_6); ^{13}C NMR (D_2O) δ 170.7, 59.3, 53.0, 43.0, 36.6, 36.2, 29.8, 19.4, 13.3.

(3S)-2-Amino-3-[(3-hydroxy-1-propylpropyl)sulfanyl]propanoic Acid (3S)-4. (3S)-**8** (0.26 g, 0.77 mmol) was demethylated exactly as above using sodium hydroxide (29 mg, 0.95 equiv) to give the monoprotected intermediate (0.21 g, 83%): $[\alpha]_{\text{D}} +97.8$ (*c* 0.45, CHCl_3).

This product (0.20 g, 0.63 mmol) was then treated with TFA (2.14 g, 18.8 mmol) exactly as above to give (3S)-**3** as its hydrochloride salt (0.12 g, 73%): $[\alpha]_{\text{D}} +20.8$ (*c* 0.14, H_2O); ^1H NMR (D_2O) δ 4.29 (1H, app t, $J \sim 5.6$ Hz, H_8), 3.69 (2H, t, $J = 6.6$ Hz, H_1), 3.24–3.04 (2H, m, H_7), 2.86 (1H, app quintet, $3J \sim 6.4$ Hz, H_3), 1.94–1.29 (6H, m, $\text{H}_{2,4,5}$), 0.86 (3H, t, $J = 7.0$ Hz, H_6); ^{13}C NMR (D_2O) δ 170.6, 59.3, 52.8, 43.1, 36.5, 36.2, 29.6, 19.4, 13.2.

Ethyl *d*₈-Hexenoate (*d*₈-11). Commercially available *d*₁₀-butanol (9) (5.0 g, 59.5 mmol) was oxidized to the corresponding aldehyde **10** by the procedure outlined in Mancuso and Swern (21). To the final dichloromethane solution of **10** (used directly because of its high volatility) was added (carboxymethylene)triphenylphosphorane (23.0 g, 66 mmol), and the mixture was allowed to stir at room temperature for 7 days. The solvent was then removed, and hexane (200 mL) was added to the residue and allowed to stir for 30 min. The mixture was filtered and the filtrate concentrated and distilled (60–70 °C at 8 mmHg) to give *d*₈-**11** as a clear oil (4.8 g, 54%).

(±)-*d*₁₀-3-Mercaptohexanol (1). To labeled ester *d*₈-**11** (4.8 g, 32 mmol) in THF (25 mL) was added triethylamine (4.04 g, 40 mmol) and thioacetic acid (4.84 g, 64 mmol). After 3 days of stirring at room temperature, the mixture was diluted with dichloromethane (100 mL) and washed with 1 M HCl solution and brine. The solvent was removed and the residue, (±)-*d*₁₀-**12**, was diluted in dry ether (50 mL). This ether solution was added dropwise to another ether solution containing LiAlD₄ (1.74 g, 41.6 mmol) at 0 °C. After 2 h of stirring at room temperature, the reaction was cooled in ice and quenched by dropwise addition of a saturated aqueous solution of sodium sulfate. The aqueous layer was acidified with 1 M HCl (to pH 3) and extracted with ether. The combined ether extracts were washed with brine, dried, concentrated, and distilled (70–80 °C at 15 mmHg) to give the target (±)-*d*₁₀-**1** (1.27 g, 32%). The very high degree of isotopic substitution made characterization by NMR problematic. However, the compound showed appropriate GC retention time and mass spectral fragmentation pattern when compared with the unlabeled compound: *d*₁₀-**1** *m/z* (%) 144 (30), 143 (<1), 109 (32), 91 (31), 62 (100), 60 (78), 46 (47); *d*₀-**1** *m/z* (%) 134 (17), 100 (50), 82 (37), 57 (63), 55 (100), 41 (52).

Preparation of Samples for Analysis of 1. An aliquot (70 μL) of a solution containing racemic $^2\text{H}_{10}$ -**1** (14.32 $\mu\text{g}/\text{mL}$ in ethanol) was added, as an internal standard (IS) using a glass syringe (100 μL SGE), to a fermented sample (10 mL) containing salt (2 g, NaCl, BDH) and ethylenediaminetetraacetic acid (EDTA, approximately 20 mg) in a 20 mL SPME vial with a magnetic crimp cap (Gerstel). A similar sample

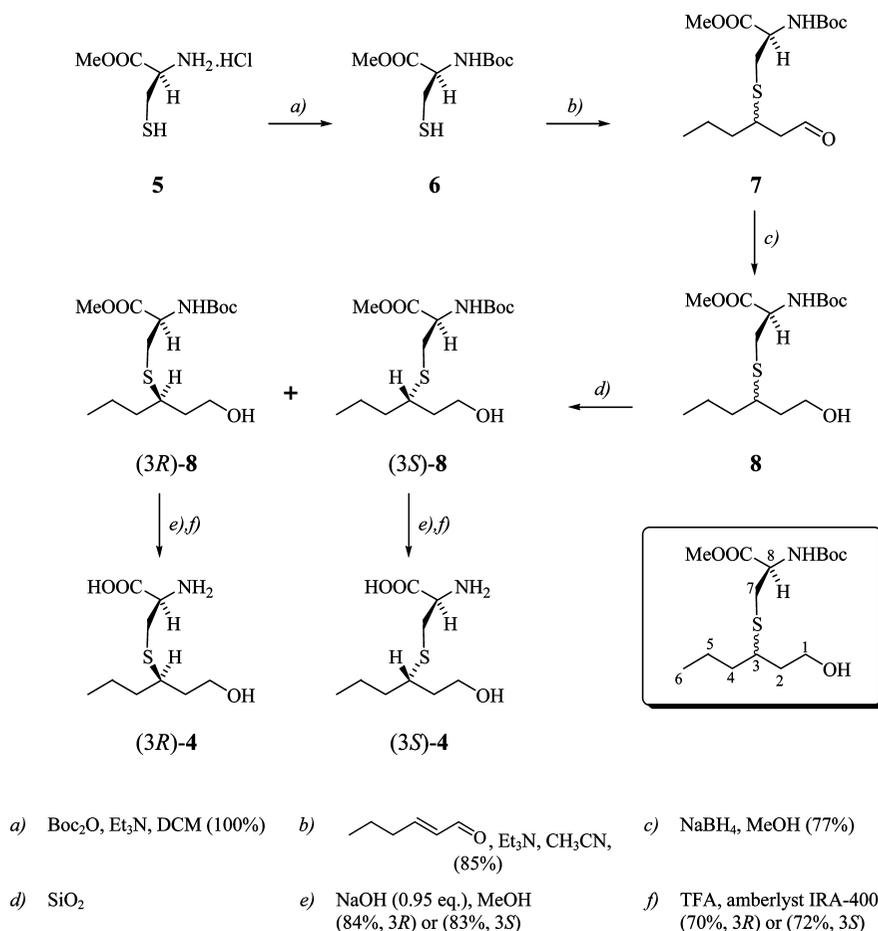


Figure 2. Syntheses of (3R)-4 and (3S)-4.

was prepared without added IS. For analysis of enzyme hydrolysates, samples were prepared in the same manner except that IS (7 μL) was added to the hydrolysate sample (1 mL) diluted in Milli-Q water (4 mL) containing salt (200 mg, NaCl, BDH) and EDTA (approximately 2 mg, added to suppress sample oxidation catalyzed by metal ions). Standard samples of aqueous ethanol (10%) spiked with $^2\text{H}_{10}$ -1 and with or without unlabeled 1 were prepared and analyzed at the same time to calibrate the method.

GC-MS Analysis. Samples were analyzed with a Hewlett-Packard (HP) 6890N gas chromatograph fitted with a Gerstel MPS2 autosampler and coupled to a HP 5973N mass spectrometer. The autosampler was fitted with an automated 65 μm carbowax/divinylbenzene (CW/DVB) SPME fiber (Supelco). The gas chromatograph was fitted with an approximately 30 m \times 0.25 mm i.d. AlphaDex 120 (Supelco) fused silica capillary column having 0.25 μm film thickness. The carrier gas was helium (BOC gases, ultrahigh purity), and the flow rate was 1.1 mL/min. The oven temperature started at 50 $^\circ\text{C}$, was held at this temperature for 1 min, then increased to 140 at 2 $^\circ\text{C}/\text{min}$, then to 260 at 40 $^\circ\text{C}/\text{min}$, and held at this temperature for 10 min. The injector was held at 220 $^\circ\text{C}$ and the transfer line at 260 $^\circ\text{C}$. The sample was extracted at 40 $^\circ\text{C}$ for 30 min and desorbed in the inlet for 15 min. The splitter, at 45:1, was opened after 36 s. Fast injection was done in pulsed splitless mode with an inlet pressure of 25.0 psi maintained until splitting. The injection sleeve (Supelco, 0.75 mm i.d.) was borosilicate glass. Positive ion electron impact spectra at 70 eV were recorded in the range m/z 35–350 for scan runs. For quantification of 1, mass spectra were recorded in the selective ion monitoring (SIM) mode. The ions monitored in SIM runs were m/z 60, 62, 92, 109, and 144 for [$^2\text{H}_{10}$]-1 and 55, 82, 88, 100, and 134 for 1, respectively. Selected fragment ions were monitored for 30 ms each. The underlined ion for each compound was the ion typically used for quantitation, having the best signal-to-noise and the least interference from other wine components. The other ions were used as qualifiers. The limit of detection for 1 was 5 $\mu\text{g}/\text{L}$, and the coefficient of determination (r^2) was 0.994.

Enzyme Assay. The assay mixture was prepared by adding (3S)-4 (10 μL , 1 mg/mL) or (3R)-4 (12.8 μL , 0.78 mg/mL), pyridoxal 5'-phosphate (20 μL , 1 mM), and assay buffer (1.88 or 1.852 mL, 20 mM phosphate buffer, pH 7.0; 1 mM EDTA) to total 2 mL. The reaction was initiated by adding 1 mg of Apotryptophanase (Sigma-Aldrich, St. Louis, MO), and the reaction mixture was incubated at 37 $^\circ\text{C}$ for 1 h. The reaction was stopped by placing it on ice, and the mixture was then stored at -20 $^\circ\text{C}$ prior to analysis. Assays were conducted in duplicate.

Yeast Ferment. *Saccharomyces cerevisiae* strain AWRI 1655 was grown in 5 mL of YPD overnight at 30 $^\circ\text{C}$. A total of 1.5 mL of culture was inoculated into two separate 250 mL SCD liquid media [6.7 g/L yeast nitrogen base (Difco, Detroit, MI); 6% glucose] spiked with 2.5 mL (3S)-4 (1.0 mg/mL) or 3.2 mL (3S)-4 (0.78 mg/mL), and contained in a fermentation flask with an airlock and sidearm septum for sampling. Duplicate ferments were conducted at 27 $^\circ\text{C}$ for 3 days, after which samples were centrifuged to remove yeast and the supernatant was stored at -20 $^\circ\text{C}$ prior to analysis.

RESULTS AND DISCUSSION

Tominaga et al. (12) recently reported a preparation of a cysteinylated conjugate of 1. They treated hexenal with L-cysteine and reduced the crude product with sodium borohydride. However, there was no direct assessment of the composition of the final product obtained other than by trimethylsilylation and GC-MS analysis. Subsequently, and independently of each other, both Starkenmann (26) and Wakabayashi et al. (18) conducted this same reaction and found, in both cases, that the major product of the first step was in fact a substituted 1,3-thiazolidine-4-carboxylic acid, with the desired 1,4-cysteine adduct being only a minor component in the product mixture. Under these circumstances, the observation of 1 in the enzymatic reactions conducted by Tominaga et al. (12), using their

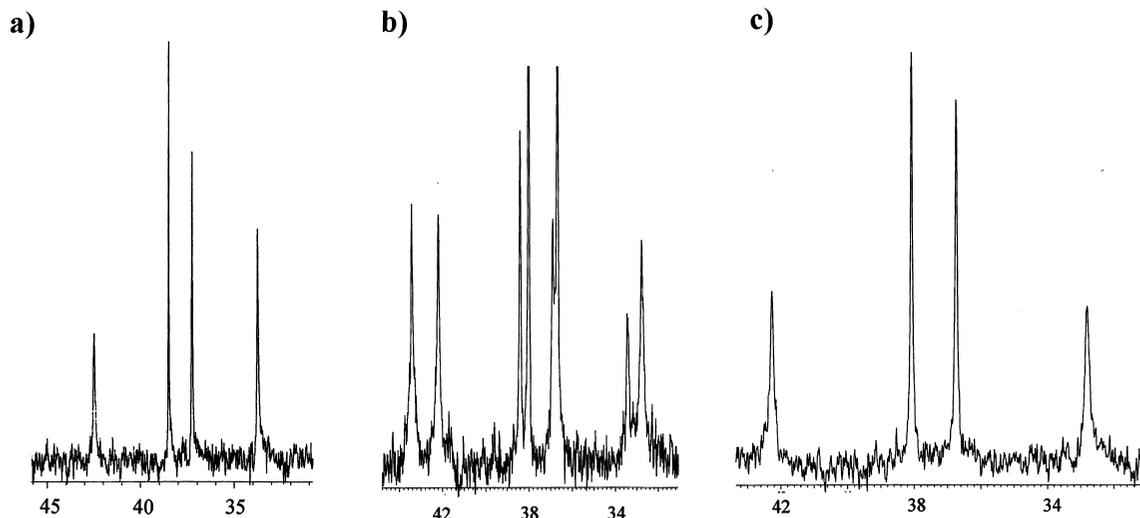


Figure 3. Portions of ^{13}C NMR spectra indicating diastereomeric purity of (a) front diastereomer of **8** (from left to right, the peaks correspond to C_3 , C_4 , C_2 , and C_7); (b) the product of treating the front diastereomer of **8** with 2.5 equiv of NaOH; and (c) the product of treating the front diastereomer of **8** with 0.95 equiv of NaOH.

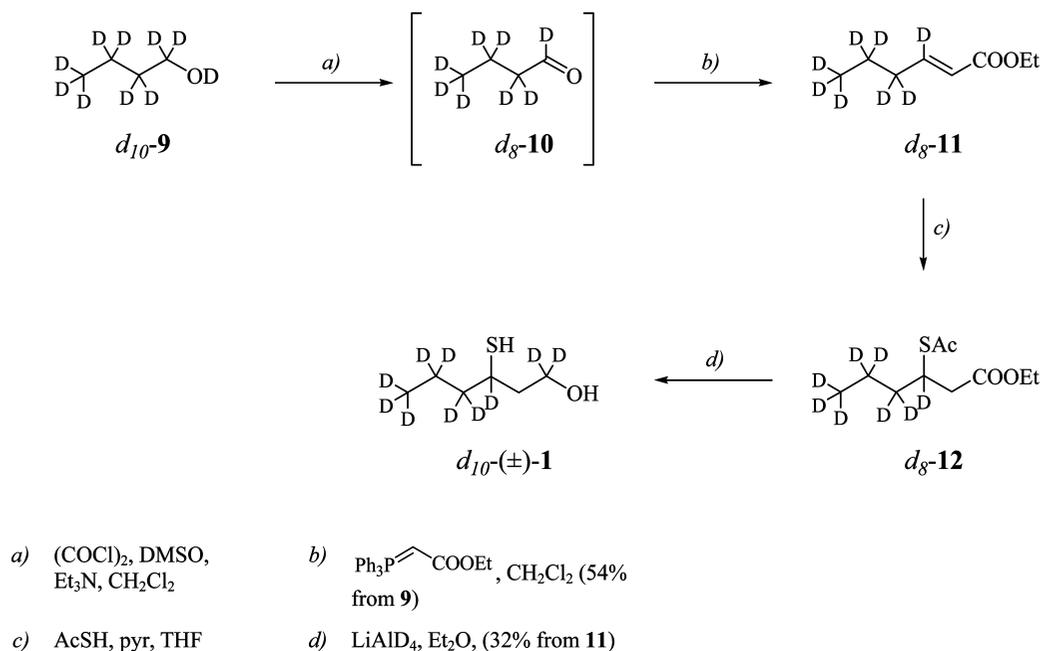


Figure 4. Synthesis of racemic $d_{10}\text{-1}$.

substrate, cannot be assigned unambiguously to the action of the microbiological agent on the cysteinylated analogue. The problem of cyclization was overcome by the use of N-Boc-protected L-cysteine, which produced, after chromatography, a diastereomeric mixture of ~90% purity (26). More recently, Luisier et al. described a synthesis of a diastereomeric mixture of the cysteinylated analogues by addition of cysteine to ethyl hex-2-enoate followed by reduction of the ester (27).

For our synthesis of the precursors **4** (Figure 2), we chose to begin with commercially available L-methylcysteine hydrochloride (**5**), which we felt would give rise to products that would be soluble in organic solvents and would therefore be amenable to a standard purification regimen using normal phase silica gel. In our hands, the final products proved to be of higher purity (>95% by NMR) than those prepared earlier using more water soluble reagents. L-Methylcysteine hydrochloride (**5**) was converted into its N-Boc analogue **6** as described elsewhere (25). Addition of **6** in a conjugate fashion to (*E*)-2-hexenal provided an inseparable diastereomeric mixture of the cysteinylated

aldehydes **7**. The mixture was reduced with sodium borohydride to give a mixture of the cysteinylated alcohols **8**, which were able to be separated on silica gel. The two diastereomers were originally designated “front”-**8** and “rear”-**8**, in reference to the order in which they eluted off the column: front-**8** and rear-**8** were subsequently shown to be (3*R*)-**8** and (3*S*)-**8**, respectively, after the enzyme and ferment experiments.

The esterifying methyl group in each of the two isomers of **8** was removed by treatment with sodium hydroxide in aqueous methanol. However, the amount of base used proved to be crucial; when treated with 2.5 equiv of hydroxide, following a procedure from the literature (28), there was observed almost total epimerization of the amino acid stereocenter, as indicated in Figure 3b. This problem was overcome by the use of just less than one full equivalent in base, as shown in Figure 3c. Following removal of the N-Boc group, (3*R*)-**4** and (3*S*)-**4** (from the front-**8** and rear-**8** isomers, respectively) were isolated. NMR spectral analysis indicated that the sample of (3*S*)-**4** contained

1–2% of the (3*R*)-4 diastereomer. No (3*S*)-4 was detected in the sample of the (3*R*)-4 isomer.

To determine the absolute stereochemistry of the two isomers of **4**, solutions of each in a model medium were fermented to dryness using a yeast strain developed in our laboratory that yields relatively high proportions of **1** (**22**). The ferments were then analyzed using an α -cyclodextrin chiral GC column and deuterium-labeled **1** as the internal standard. Previously, Kotseridis and Baumes described a preparation of ²H₂-**1** that they used for their quantification method (**23**). However, we chose to use a *d*₁₀ analogue, which has the advantage of giving complete baseline separation from the unlabeled analyte on both chiral and achiral chromatography. The synthesis of *d*₁₀-**1** is outlined in **Figure 4**. *d*₁₀-Butanol (**9**) was oxidized under Swern conditions (**24**) to produce *d*₈-butanal (**10**), which, because of its extreme volatility, was not isolated but rather obtained as a solution in dichloromethane. This solution was then treated with the stabilized phosphorane (carbethoxymethylene)triphenylphosphorane to produce the *d*₈-hexenoate ester **11**. Addition of thiolacetic acid proceeded as described earlier (**16**) to produce *d*₈-**12**, which was reduced with lithium aluminum deuteride to give the required *d*₁₀-**1**.

Chromatography of racemic **1** on the α -cyclodextrin column gave good separation of the enantiomers (*R* = 1.3). The first eluting isomer was the 3*R* form and the second isomer, the 3*S* form, of 3-MH, as shown by comparison with enantiomerically pure samples synthesized according to published methods (**21**). On treatment with Apotryptophanase enzyme, the front isomer of **4** gave exclusively the 3*R* isomer of **1** (82% yield) with no trace of the 3*S* enantiomer present. Conversely, the rear isomer of **4** gave the 3*S* enantiomer of **1** (43%) accompanied by a trace of the 3*R* form (*S/R* = 98.5:1.5), reflecting the diastereomeric purity of the starting conjugate. The same stereochemical outcome was observed when the two isomers of **4** were added separately to fermentations conducted by the *S. cerevisiae* AWRI 1655 yeast strain, which gave 1.0 and 1.1% yields of the 3*R* or 3*S* isomers of **1**, respectively. No **1** was observed in control samples (no fermentation). Accordingly, the front isomer of **4** was assigned the 3*R* stereochemistry and the rear isomer the 3*S* stereochemistry.

These results show that, under these experimental conditions and with this particular yeast strain, there is little difference in the efficiency of **1** release from the two isomeric forms of the cysteine conjugates **4**. The availability of the isomerically pure samples of **4** described here will enable further studies with different yeast strains and at various concentrations. They will also allow the development of analytical methods for determining the concentration of each isomer in grape samples.

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LITERATURE CITED

- Mestres, M.; Busto, O.; Guasch, J. Analysis of organic sulfur compounds in wine aroma. *J. Chromatogr., A* **2000**, *881*, 569–581.
- 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.
- Weber, B.; Maas, B.; Marz, A.; Olk, J.; Mosandl, A. Enantiomeric distribution of the chiral sulfur-containing alcohols in yellow and purple passion fruits. *Z. Lebensm. Unters. Forsch.* **1994**, *199*, 48–50.
- Werkhoff, P.; Guntert, M.; Krammer, G.; Sommer, H.; Kaulen, J. Vacuum headspace method in aroma research: flavor chemistry of yellow passion fruits. *J. Agric. Food Chem.* **1998**, *46*, 1076–1093.
- 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*, 159–162.
- Bouchilloux, P.; Darriet, P.; Henry, R.; Lavigne-Cruège, V.; Dubourdieu, D. Identification of volatile and powerful thiols in Bordeaux wines. *J. Agric. Food Chem.* **1998**, *46*, 3095–3099.
- Kotseridis, Y.; Baumes, R. Identification of impact odorants in Bordeaux reg grape juice, in the commercial yeast used for its fermentation and in the produced wine. *J. Agric. Food Chem.* **2000**, *48*, 400–406.
- Weber, B.; Maas, B.; Mosandl, A. Stereoisomeric flavor compounds. 72. Stereoisomeric distribution of some chiral sulfur-containing trace components of yellow passion fruits. *J. Agric. Food Chem.* **1995**, *43*, 2438–2441.
- Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022–3026.
- Pearce, T. J. P.; Peacock, J. M.; Aylward, F.; Haisman, D. R. Catty odors in food: reactions between hydrogen sulfide and unsaturated ketones. *Chem. Ind.* **1967**, *37*, 1562–1563.
- Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.; Dubourdieu, D. Identification of a powerful aromatic compound of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385–392.
- 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.
- Tominaga, T.; Dubourdieu, D. Identification of cysteinylated aroma precursors of certain volatile thiols in passion fruit juice. *J. Agric. Food Chem.* **2000**, *48*, 2874–2876.
- 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 for their varietal aroma. *J. Agric. Food Chem.* **2000**, *48*, 3387–3391.
- 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.
- Howell, K. S.; Swiegers, J. H.; Elsey, G. M.; Siebert, T. E.; Bartowsky, E. J.; Fleet, G. H.; Pretorius, I. S.; de Barros Lopes, M. Variation in 4-mercapto-4-methyl-pentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. *FEMS Microbiol. Lett.* **2004**, *240*, 125–129.
- Howell, K. S.; Klein, M.; Swiegers, J. H.; Hayasaka, Y.; Elsey, G. M.; Fleet, G. H.; Høj, P. B.; Pretorius, I. S.; de Barros Lopes, M. Genetic determinants of volatile thiol release by *Saccharomyces cerevisiae* during wine fermentation. *Appl. Env. Microbiol.* **2005**, *71*, 5420–5426.
- Wakabayashi, H.; Wakabayashi, M.; Eisenreich, W.; Engel, K.-H. Stereochemical course of the generation of 3-mercaptohexanal and 3-mercaptohexanol by β -lyase-catalyzed cleavage of cysteine conjugates. *J. Agric. Food Chem.* **2004**, *52*, 110–116.
- Swiegers, J. H.; Willmott, R.; Hill-Ling, A.; Capone, D. L.; Pardon, K. H.; Elsey, G. M.; Howell, K. S.; de Barros Lopes, M. A.; Sefton, M. A.; Lilly, M.; Pretorius, I. S. Modulation of volatile thiol and ester aromas in wine by modified wine yeast. In *Flavor Science Recent Advances and Trends*; Developments in Food Science 43; Bredie, W. L. P., Petersen, M. A., Eds.; Elsevier: Amsterdam, The Netherlands, 2006; pp 113–116.
- Schneider, R.; Charrier, F.; Razungles, A.; Baumes, R. Evidence for an alternative biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one in wines. *Anal. Chim. Acta* **2006**, *563*, 58–64.

- (21) Tominaga, T.; Niclass, Y.; Frérot, E.; Dubourdieu, D. 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). *J. Agric. Food Chem.* **2006**, *54*, 7251–7255.
- (22) Swiegers, J. H.; Capone, D. L.; Elsey, G. M.; Sefton, M. A.; Bramley, B. R.; Francis, I. L.; Pretorius, I. S. Engineering volatile thiol release in *Saccharomyces cerevisiae* for improved wine aroma. *Yeast* **2007**, *24*, 561–574.
- (23) Kotseridis, Y.; Baumes, R. Quantitative determination of sulfur-containing wine odorants at sub-ppb levels. 1. Synthesis of the deuterated analogues. *J. Agric. Food Chem.* **2000**, *48*, 5819–5823.
- (24) Mancuso, A. J.; Swern, D. Activated dimethyl sulfoxide: useful reagents for synthesis. *Synthesis* **1981**, 165–185.
- (25) West, C. W.; Estiarte, M. A.; Rich, D. H. New methods for side-chain protection of cysteine. *Org. Lett.* **2001**, *3*, 1205–1208.
- (26) Starkenmann, C. Analysis of a model reaction system containing cysteine and (*E*)-2-methyl-2-butenal, (*E*)-2-hexenal, or mesityl oxide. *J. Agric. Food Chem.* **2003**, *51*, 7146–7155.
- (27) Luisier, J.-L.; Veyrand, J.; Piantini, U. Characterisation and simple synthesis of *S*-[3-hydroxy-1-propylpropyl]-L-cysteine. *Chimia* **2007**, *61*, 533–535
- (28) Threadgill, M. D.; Gledhill, A. P. Synthesis of peptides containing *S*-(*N*-alkylcarbamoyl) cysteine residues, metabolites of *N*-alkylformamides in rodents and in humans. *J. Org. Chem.* **1989**, *54*, 2940–2949.

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