JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Analysis of a Model Reaction System Containing Cysteine and (*E*)-2-Methyl-2-butenal, (*E*)-2-Hexenal, or Mesityl Oxide

CHRISTIAN STARKENMANN§

Firmenich S.A. 1, route des Jeunes, 1211 Geneva 8, Switzerland

Cysteine conjugates, resulting from the addition of cysteine to α,β-unsaturated carbonyl compounds, are important precursors of odorant sulfur compounds in food flavors. The aim of this work was to better understand this chemistry in the light of the unexpected double addition of cysteine to two unsaturated aldehydes. These reactions were studied as a function of pH. When *(E)*-2-methyl-2-butenal (tiglic aldehyde, **4**) was treated with cysteine in water at pH 8, the major product formed was the new compound (4*R*)-2-(2-{[(2*R*)-2-amino-2-carboxyethyl]thio}methylpropyl)-1,3-thiazolidine-4-carboxylic acid (**6**). Under acidic conditions (pH 1), we also observed a double addition, but the second cysteine was linked by a vinylic sulfide bond to form the previously unreported major product, (2*R*,2′*R*,*E*)-*S*,*S*′-(2,3-dimethyl-1-propene-1,3-diyl)bis-cysteine (**7**). When *(E)*-2-hexenal (**12**) was treated with cysteine under acidic conditions, the major product was the novel (4*R*,2″*R*)-2-[2′-(2″-amino-2″-carboxyethylthio)pentyl]-1,3-thiazolidine-4-carboxylic acid (**13**), and the formation of an vinylic sulfide compound analogous to **7** was not observed. Reduction of the acidic crude reaction mixture with $NaBH_4$ afforded **13** and the cysteine derivative (*R*)-*S*-[1-(2-hydroxyethyl)butyl]cysteine (**14**) in 14% yield. Treating (*E*)-2-hexenal with cysteine at pH 8 followed by $NaBH_4$ reduction yielded the new product (3*R*)-7-propylhexahydro-1,4-thiazepine-3-carboxylic acid (**15**). Addition of cysteine to mesityl oxide (**16**), at pH 8, followed by reduction with $NaBH_4$ furnished (*R*)-*S*-(3-hydroxy-1,1-dimethylbutyl)-cysteine (**3**) and the new compound (3*R*)-hexahydro-5,7,7-trimethyl-1,4-thiazepine-3-carboxylic acid (**18**).

KEYWORDS: Cysteine conjugate; (*E*)-2-methyl-2-butenal; (*E*)-2-hexenal; mesityl oxide; thiazepane-3-carboxylic acid; hexahydro-1,4-thiazepine; *S*-(1,2-dimethyl-3-oxopropyl)-L-cysteine; *S*-[1-(2-oxoethyl)butyl]-L-cysteine; 4-methyl-4-mercapto-2-pentanol; 3-mercaptohexanal

INTRODUCTION

In the course of our study of onion flavor precursors, we focused on new nonvolatile compounds that we suspected might be present in onion. It is known that aldehydes and cysteine are found in onion, and the importance of cysteine conjugates such as methyl, propyl, allyl, and 1-propenyl-L-cysteine sulfoxides has been well documented (*1*, *2*). Furthermore, it is well established that the reaction of cysteine with saturated aldehydes and ketones leads to the formation of thiazolidine-4-carboxylic acids (*3*, *4*). Methyl 2-methyl-thiazolidine-4-(*R*)-carboxylate has been recently identified in guava (*5*). Thiazolidine-4-carboxylic acids derived from cysteine and common food aldehydes have been studied as well as their decomposition into volatile products at typical roasting temperatures (*6*, *7*). In addition, cysteine has been reacted with furfural and the resulting cysteine−aldehyde conjugates were biotransformed into 2-furfurylthiol with use of Baker's yeast (*8*, *9*). When cysteine reacts with an aliphatic α,β-unsaturated aldehyde or ketone, the first reaction step is a Michael addition. This type of reaction was reported for pulegone (*10*, *11*), and also for a wide range of α,β-unsaturated aldehydes that were treated with cysteine and patented as molecules that improved the perceived freshness of foodstuff (*12*). This patent described only a general formula corresponding to the monoaddition of cysteine to unsaturated aldehydes without analytical data (*12*). A breakthrough concerning the role of cysteine conjugates in food flavors came in 1993 when they were identified as precursors of 4-mercapto-4-methylpentan-2-one, 4-mercapto-4-methylpentan-2-ol, and 3-mercaptohexan-1-ol, characteristic flavor molecules in Sauvignon Blanc wines and passion fruit (*13*−*15*). In the cited works the cysteine monoadducts were characterized by GC-MS after silylation. Few examples of the double addition of cysteine to α,β-unsaturated aldehydes have been reported. The double addition reactions of cysteine to acrolein, crotonaldehyde, cinnamaldehyde, and 4-hydroxy-(*E*)-2-pentenal have been well documented (*16*).

We were interested in studying the addition of cysteine to α,β-unsaturated aldehydes to form monoadducts such as **1** and **2** which could be precursors of 3-mercaptoalkanals. Little is known about mercaptoaldehydes in food; only 3-mercapto-3-methylpentanal and 3-mercaptohexanal have been found in onion (*17*, *18*) and beef liver (*19*), respectively. The syntheses

§ Contact information: phone +41 22 780 34 77; fax +41 22 780 33 34; e-mail christian.starkenmann@firmenich.com.

10.1021/jf0304268 CCC: $25.00
Published on Web 10/18/2003

and odor properties of these compounds have been recently discussed (*20*, *21*). We studied the addition of cysteine to tiglic aldehyde under acidic and basic conditions. The products were characterized before and after reduction with $NaBH_4$. The reactivity of tiglic aldehyde **4** was compared with that of (*E*)-2-hexenal (**12**), which has no substitution at the α position, and with that of the ketone mesityl oxide (**16**).

MATERIALS AND METHODS

Reagents. All reagents were purchased from Aldrich Chemicals or Fluka Switzerland. For flash chromatography, SiO_2−RP18 (Merck cat. 1.13900.0250) was used. TLC plates were HP-TLC Merck cat. 1.05635.

GC-FID Analysis. Fast GC was performed by using an HP 6850 gas chromatograph (Agilent Technologies) equipped with an HP-1 column (10 m × 0.1 mm i.d., microbore, 0.25 μm film thickness, Agilent cat. 127-1012-E), He flow ca. 1 mL/min; detector temperature 300 °C, injector temperature 250 °C, split ratio 1/50; and oven temperature program, 50 °C for 1 min then 60 °C per min to 300 °C.

GC-MS Analysis. GC-MS analyses were performed on HP 5890 or 6890 gas chromatographs (Agilent Technologies) equipped with SPB-1 columns (30 m × 25 mm i.d., 0.25 μm film thickness, Supelco). He carrier gas was used at a constant pressure of 82 kPa. The chromatograph was coupled to an MSD 5972 mass spectrometry detector (Agilent; electron energy, ca. 70 eV).

^{1}H- and ^{13}C NMR Spectra. NMR spectra were recorded on a Bruker AMX-360 spectrometer at 360 and 90 MHz. Chemical shifts in ppm are reported relative to tetramethylsilane ($(CH_3)_4Si$).

HPLC Analyses. HPLC analyses were performed on an Agilent 1100 HPLC equipped with a binary pump B1312A, with refractive index detection (G1362A). Separations were performed on a Phenomenex Aqua 5 μm C18 125A 150 mm × 2.0 mm i.d. column (cat. P/N 00F-4299-B0) or a Chromolith Merck RP18e (100 mm × 4.6 mm i.d.) (cat. 1.02129.0001). The analytes were eluted at 0.5 mL/min on the Phenomenex column and 1.5 mL/min on the Chromolith column, using an aqueous solution of 0.1% HCOOH (solvent A) and 0.1% HCOOH in CH_3CN (solvent B), 98/2 isocratic elution.

HPLC-MS Analyses. HPLC-MS analyses were performed with two types of equipment under different conditions. We used the Agilent 1100 LC-MS system equipped with a B1312A binary pump, a G1314A UV detector, and a G1946D mass spectrometer with an atmospheric-pressure chemical ionization (APCI) source. Positive and negative ion mode mass spectra (scan range 50−800 Da) were recorded simultaneously. HPLC separations were performed under the same conditions as described above. Alternatively Thermo Finnigan positive electrospray ionization (ESI) was performed with use of a spray voltage of 4.5 kV, capillary temperature at 200 °C, and N_2 gas at a flow rate of 55 (Finnigan arbitrary units) with an auxiliary gas flow rate set to ESI (Finnigan arbitrary units). In this instance the column used was an Xtera, 250 mm × 2 mm i.d., eluted with CH_3CN and water that contained 0.1% formic acid. The LC gradient started at 100% water for 5 min then increased from 0% CH_3CN to 50% CH_3CN in 30 min at a flow rate of 0.25 mL/min.

General Procedures. Derivatization with MSTFA was performed on dry products (∼20 mg) placed in a GC vial. Dry toluene (1.8 mL) was added followed by 0.1−0.15 mL of MSTFA. The mixture was then heated at 80 °C for 2 h and analyzed neat by GC-FID and GC-MS.

Flash chromatography of crude products was performed with SiO_2−RP18 solid support, eluted with a water/ethanol gradient. The ratio between SiO_2−RP18/product, column size, and sample volume was adjusted according to Still et al. (*22*). The SiO_2−RP18 was suspended in EtOH and poured into the column. The column was washed with 4 bed volumes of water under 1 atm of pressure. A solution of the crude sample was added to the column. The gradient elution was executed by successive additions of 1 bed volume each of water, water/ethanol 9:1, water/ethanol 4:1, and finally 100% ethanol. Reactions were monitored by HP-TLC (water/*n*-butanol/ethyl acetate/acetic acid 1:1:2:1) and developed by using 0.3% ninhydrin in acetone. Fractions were pooled as appropriate and the solvent was removed by freeze-drying. Some reactions were conducted in D_2O to acquire ^{1}H and ^{13}C NMR spectra of the crude reaction products. NaOD was made from dry NaOH and D_2O (0.1 M aqueous NaOD).

Organoleptic evaluations of the crude mixture were done by diluting the solutions in plain water 1000 times. The crude reaction mixtures that contained **6**, **7**, and **13** were tasted at 10 ppm in plain mineral water.

*Preparation of (4R)-2-(2-{[(2R)-2-amino-2-carboxyethyl]thio}-methylpropyl)-1,3-thiazolidine-4-carboxylic Acid (**6**).* Tiglic aldehyde **4** (3 g, 0.36 mol) was added to cysteine **5a** (4.3 g, 0.36 mol) in water (80 mL) adjusted to pH 8 with a 10% aqueous solution of NaOH. After 1 h the mixture was concentrated (40 °C, 10 mbars) to give a pink viscous crude product (6 g). Analysis of the crude product was done by GC-FID on the corresponding MSTFA derivative. The chromatogram contained cysteine (at 3.3 min, ∼30% by peak area) and four closely eluting product peaks (at 4.88−4.92 min, ∼60% by peak area, approximate relative ratio 1:1:1:8). By GC-MS the fragmentation pattern of the major peak was *m*/*z* (rel intensity) 524 (5), 347 (14), 292 (25), 290 (20), 218 (100), 73 (40). LCMS ESI+ (Nucleosil column, 2 mm × 250 mm, CH_3CN, water, 0.1% formic acid, 5−100% CH_3CN for 30 min), 11 min M + 1 = 188 (mono adduct − H_2O), 13 min M + 1 = 309 (**6**, br). ^{1}H NMR (D_2O), two major diastereoisomers of **6** were designated **a** (46%) and **b** (42%). Two minor diastereoisomers comprised approximately 8% and 4% of the mixture by ^{1}H NMR.

^{1}H NMR δ 1.18 (d, *J* = 7 Hz, C**H**$_3$-11, isomer a and b), 1.38 (d, *J* = 7 Hz, 2C**H**$_3$-10, a and b), 2.1−2.25 (m, 2 C**H**-5, a and b), 3.0−3.5 (m, C**H**$_2$-3, C**H**-4, C**H**$_2$-7, a and b), 3.91−4.03 (m, C**H**-2, a and b), 4.28−4.32 (m, C**H**-8, a or b), 4.60−4.63 (m, C**H**-8, a or b), 4.75 (d, C**H**-6, b), 5.05 (d, *J* = 7 Hz, C**H**-6, a); ^{13}C NMR (D_2O) for diastereoisomers a and b, δ 14.9, 15.6 (q, C-11), 21.8, 22.9 (q, C-10), 32.8, 34.9, 35.7, 36.8 (t, C-3, C-9), 44.7, 45.0, 45.8, 49.1 (d, C-4, C-5), 56.2, 57.1 (d, C-2), 66.7, 68.6 (d, C-8), 71.7, 73.8 (d, C-6), 175.4, 176.5 (s, C-1, C-9).

The ^{1}H NMR experiment on a reaction mixture was carried out using the following conditions: **4** (42 mg, 0.5 mmol) was added to **5a** (60 mg, 0.5 mmol) and Na_2CO_3 (5 mg, 0.05 mmol) in D_2O (1 mL). The solution was stirred overnight. ^{1}H NMR and ^{13}C NMR data were the obtained.

*Preparation of (2R,2′R,E)-S,S′-(2,3-Dimethyl-1-propene-1,3-diyl)-bis-cysteine (**7**).* Compound **4** (16.8 g, 0.2 mol) was added to cysteine·HCl **5b** (15.7 g, 0.1 mol) in water (100 mL) and the mixture was stirred for 16 h. The water phase was concentrated (40 °C, 10 mbars) to give a pink viscous crude product (26 g). Flash chromatography of an aliquot (230 mg) was performed on reverse-phase silica gel (SiO_2−RP18, 16 g, column 25 mm diameter, elution system: water to water/ethanol 9:1). The first fraction (52 mg) was a mixture that contained **6** and the second fraction contained pure **7** (104 mg, yield 78%).

Analysis of the crude product was done by GC-FID analysis of the MSTFA derivatives and the chromatogram contained cysteine (at 3.3 min, ∼3% of peak area), an unidentified peak at 4.97 min (11% peak area), a peak at 5.00 min (12% peak area) that was tentatively assigned as an isomer of **7** + 4XMe_3Si, and a peak at 5.03 min (74% peak area) that was assigned as **7** + 4XMe_3Si. From GC-MS analyses, the fragmentation pattern for **7** + 4XMe_3Si was found to be *m*/*z* (rel intensity) 596 (5), 332 (25), 232 (40), 218 (100), 147 (24), 73 (70). This pattern was found to be identical for both the peaks at 5.00 and 5.03 min. LCMS ESI+: 3.8 min, M + 1 = 206 (**1**, minor peak, 20%), 6.0 min, M + 1 = 310 (**6**, major peak, 75%). ^{1}H NMR (0.1 M aqueous NaOD) of **7**: δ 1.31 (d, *J* = 7.0 Hz, C**H**$_3$-10), 1.73 (d, *J* = 1.0 Hz, C**H**$_3$-11), 2.59 (dd, *J* = 7.0, 13.2 Hz, C**H**$_2$-3, 1H), 2.71 (dd, *J* = 5.2, 13.2 Hz, C**H**$_2$-3, 1H), 3.36 (dd, *J* = 5.2, 7.0 Hz, C**H**-2), 3.46 (dd, *J* = 4.8 and 6.8 Hz, C**H**-8), 3.61 (q, *J* = 7.0 Hz, C**H**-4), 6.02 (s, br, C**H**-6). ^{13}C NMR δ 15.7 (q, C-10), 21.4 (q, C-11), 38.5 (t, C-3), 41.6 (t, C-7), 50.6 (d, C-4H), 58.2 (d, C-2), 58.6 (d, C-8), 123.9 (s), δ 139.2 (s), 183.5 (s), δ 183.9 (s).

*Reaction of N-Acetyl-cysteine **8** with **4** at pH 1.* Tiglic aldehyde **4** (0.5 g, 6.1 mmol) was added to *N*-acetyl cysteine **8** (1 g, 6.1 mmol) in HCl (0.1 M, 50 mL) and the mixture was stirred for 3 h at room temperature and then 2 h at 50 °C. The crude mixture was purified by flash chromatography to give 800 mg of **9** (yield 65%). GC-MS analysis of the MSTFA-derivatized mixture on an SPB-1 column (30 m, oven

program set to 130−300 °C at 7 deg/min) resulted in a chromatogram that contained minor peaks at 16.6−16.8 min *m/z* (% abundance) 260 (30), 217 (35), 202 (70), 190 (40), 189 (100), 187 (70), 159 (60), 131 (60) tentatively assigned as the silylenol ether of the Michael-addition monoadduct. Major peaks were observed at 25.27 *min* (32% peak area) and 25.33 min (28% peak area) and these had identical mass spectra: *m/z* (rel intensity) 536 (2), 302 (70), 286 (25), 260 (40), 202 (100), 73 (35) assigned as the silylated form of **9**.

LC-MS (APCI + mode) M + 1 observed: 325 minor peak assigned as *N*-acetylcystine; 409 minor peak, identity unknown; 248 tentatively monoadduct; 393 major double peak assigned as diastereomers of **9**. ^{1}H NMR of **9** (aqueous DCl at pH 4.7, purity of **9** was ∼90% of two isomers a and b, the remainder consisted of other diastereomers) δ 1.26 (d, J = 6.6, C**H**$_3$-10), 1.27 (d, J = 6.6, C**H**$_3$-10), 1.68 (s, 2 × C**H**$_3$-11), 2.05 (s, 2 × CH_3CO−), 2.65−2.92 (m, 2 × C**H**$_2$-3), 3.05−3.25 (m, 2 x C**H**$_2$-7), 3.55−3.65 (m, 2 × C**H**-4), 4.51−4.54 (m, 2 × C**H**-2), 4.58−4.62 (m, 2 × C**H**$_2$-8), 5.98 (s, 2 × C**H**-6). ^{13}C NMR (mixture of 2 diastereoisomers) δ 15.2, 15.6 (q, Me-11), 20.8, 21.2 (q, Me-10), 24.3, 24.5 (q, **Me**CO), 34.1, 34.3 (t, C-3), 36.9, 37.0 (t, C-7), 49.9, 50.7 (d, C-4), 55.3, 55.4 (d, C-8), 54.7, 54.9 (d, C-2), 122.9, 123.3 (d, C-6), 139.9, 140.2 (s, C-5), 175.8−176.5 (s).

*Reduction of the Crude Reaction Mixture of **5b** with **4** (Acidic Conditions).* Compound **4** (125 mg, 1.45 mmol) was added to **5b** (467 mg, 2.98 mmol) in water (5 mL) and the mixture was stirred for 2 h at pH 1. $NaBH_4$ (340 mg, 8.9 mmol) was added, and the pH was adjusted to pH 9 with a few drops of 10% aqueous NaOH. After 1 h, the crude reaction mixture was filtered on an RP18−SiO_2 column eluted with water/ethanol 95:5. After concentration, 148 mg were isolated as a mixture of **7**, **10**, and **11**.

LC-MS (APCI mode) 3.1−3.4 min, M + 1 = 208, minor component identified as **10**, 4.2−4.4 min, M + 1 =3 09, assigned to **7**, 5.0−5.2 min, M + 1 = 190, identified as **11**. GC-MS of the MSTFA derivatives, 3.7 min, 20% peak area silylated form of **11**, *m/z* (rel intensity) 333 (2), 290 (5), 216 (100), 160 (30), 147 (8), 115 (9), 73 (28); 4.97 min, 28% peak area, silylated form of **10**; *m/z* (rel intensity) 408 (5), 306 (20), 218 (100), 147 (10), 103 (12), 73 (45); 5.00 min, 50% peak area (*m/z* 596 for silylated **7**, for fragment ion data see above).

*Reduction of the Crude Reaction Mixture of **5a** with **4** (Neutral Conditions) To Yield Thiazepane **11**.* Tiglic aldehyde **4** (250 mg, 3 mmol) was added to **5a** (360 mg, 3 mmol) and Na_2CO_3 (31 mg, 0.3 mmol) in water (5 mL) and the mixture was stirred over the course of 2 h. $NaBH_4$ (338 mg, 9 mmol) was added and the reaction was stirred for 2 h more. Flash chromatography yielded a first fraction that contained cysteine and the double adduct **6** (140 mg) and a second fraction comprised of thiazepine **11 (**145 mg, yield 25%). Analysis of the second fraction by GC-FID of the MSTFA derivative gave peaks at 3.71−3.67 (80% peak area) determined to be a mixture of three silylated diastereoisomers of **11** which had identical MS data: *m/z* (rel intensity) 333 (5), 217 (25), 216 (100), 160 (35), 115 (10), 73 (30). LC-MS (APCI+ mode) 4.3 and 5.0 min, M + 1 = 190, diastereomers of **11**. ^{1}H NMR (0.1 M aqueous DCl) δ 1.08 (d, J = 7.1 Hz, Me-11), 1.21 (d, J = 7.1 Hz, Me-10), 2.38−2.42 (m, C**H**-6), 3.10−3.20 (m, C**H**$_2$-2 and -5, C**H**-7), 3.95−4.00 (m, C**H**-3). ^{13}C NMR δ 13.8 (q, C-11), 19.8 (q, C-10), 34.5 (t, C-2), 41.4 (d, C-6), 48.7 (d, C-7), 50.9 (t, C-5), 64.5 (d, C-3), 178.0 (s).

*Preparation of (4R,2″R)-2-[2′-(2″-Amino-2″-carboxyethylthio)-pentyl]-1,3-thiazolidine-4-carboxylic Acid **13**. (E)*-(2)-Hexenal (**12**, 0.3 mL, 2.6 mmol) was added to **5b** (800 mg, 5 mmol) in water (40 mL) and the mixture was stirred for 2 h at room temperature (pH 1). Diethyl ether was added to extract hydrophobic polymers. The aqueous solution was then concentrated under vacuum at 40 °C. Flash chromatography gave 640 mg of **13** (80% theoretical yield).

^{1}H NMR (aqueous DCl, pH 2, of the diastereomeric mixture) δ 0.92 (t, J = 7 Hz, C**H**$_3$-12), 1.37−1.48 (m, C**H**$_2$-11), 1.58−1.70 (m, C**H**$_2$-10), 2.10−2.50 (m, C**H**$_2$-5), 2.85−2.95 (m, C**H**-4), 3.15−3.05 (m, C**H**$_3$-3), 3.25−3.56 (m, C**H**$_2$-7), 4.30−4.25, 4.33−4.39, 4.48−4.52, 4.52−4.56, −4.40 (m, C**H**-8), 4.90−4.95, 5.00−5.05, 5.09−5.20 (m, C**H**-6). ^{13}C NMR δ 15.8 (s, C-12), 22.0, 22.11, 22.13, 22.17 (t, C-11), 33.3, 33.6 (t, C-3), 35.5, 35.6, 35.9, 36.8 (t, C-7), 39.1, 39.4, 39.9 (t, C-10), 39.9, 40.0, 40.1 (t, C-5), 45.6, 46.1, 46.7, 47.3 (d, C-4), 55.6−55.7 (d, C-2), 65.7, 66.7, 66.8, 67.8 (d, C-6), 66.4, 66.7, 67.8, 67.7 (d, C-8), 175.2−175.4 (s, C-1 and C-9).

GC-MS of the silylated crude reaction mixture on an SPB-1 column (30 m, oven temperature held at 130 °C for 5 min then increased to 320 °C at 7 °C/min and held for 8 min) contained peaks assigned to cysteine (6% peak area at 9 min, *m/z* 273), a dihydrothiazepine (8% peak area at 12.5 min, *m/z* (rel abundance) 348 (2), 246 (35), 218 (100), 148 (25), 100 (15), 73 (40)), and major product **13** at 24.8 min (66% peak area, 2 peaks at ratio 95/5, *m/z* (rel abundance) 538 (5), 306 (30), 274 (40), 218 (100), 156 (40), 73 (60).

*Preparation of (R)-S-[1-(2-Hydroxyethyl)butyl]cysteine (**14**).* (*E*)-2-Hexenal (**12**, 0.150 mL, 1.3 mmol) was added to **5b** (200 mg, 1.3 mmol) in water (20 mL) and the mixture was stirred over 2 h at room temperature (pH 1). Sodium borohydride (50 mg, 1.3 mmol) was added together with one drop of 30% NaOH (pH 10). After 1 h at room temperature, HCl was added to attain a pH of 7. The solution was concentrated under vacuum (10 mbars) at 40 °C. The crude product was filtered on 15 g of RP18 with a gradient starting from pure water to water/ethanol 9:1. The first fraction contained **13** (100 mg) and the second fraction consisted of **14** (40 mg, yield = 14%). ^{1}H NMR (aqueous DCl at pH 4.7, purity of **14** ∼90%) δ 0.9 (t, J = 7.0 Hz, C**H**$_3$), 1.40−1.50 (m, C**H**$_2$), 1.55−1.60 (m, C**H**$_2$), 1.72−1.95 (m, C**H**$_2$), 2.85−2.95 (m, C**H**-5), 3.0−3.15 (m, C**H**$_2$-3), 3.68−3.80 (m, C**H**$_2$-7), 3.87−3.95 (m, 1H C**H**-2). ^{13}C NMR (major diastereomer) δ 16.0 (q), 22.0 (t), 22.1 (t), 33.4 (t), 38.8 (t), 45.2 (d), 56.9 (d), 61.6 (t), 175.8 (s). LC-MS (APCI + mode): Phenomenex aqua 7.1 and 8.1 min <10% 1,4-adduct of aldehyde oxidized to carboxylic acid M + 1 = 238, 12.9 min >75% M + 1 = 222 alcohol **14**, 14.5 min <10% M + 1 = 202 cyclic form. GC-MS (silylation of the crude product) run on an SPB-1 column (30 m, oven temperature of 130−310 °C at 7 deg/min held for 12.5 min) 90% of the major diastereomer *m/z* (rel abundance): 437 (2), 320 (35), 219 (30), 218 (100), 73 (40).

*Preparation of (3R)-7-Propylhexahydro-1,4-thiazepine-3-carboxylic Acid (**15**).* Compound **12** (0.59 mL, 5 mmol) was added to **5a** (600 mg, 5 mmol) and Na_2CO_3 in water (30 mL) and the mixture was stirred over 2 h at room temperature (pH 8). HPLC analysis showed a poorly resolved mixture of products.

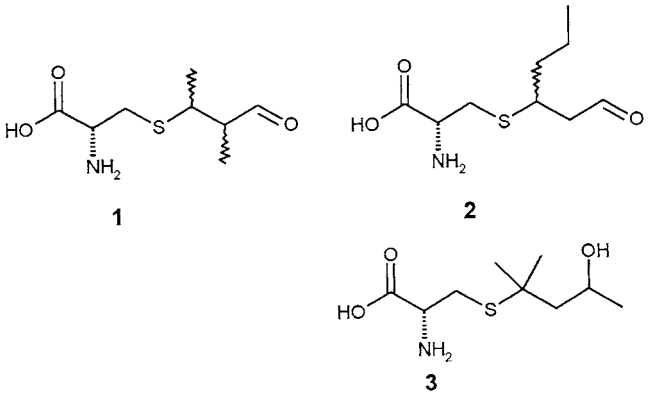
$NaBH_4$ (194 mg, 5 mmol) was added plus 0.1 mL of aqueous 30% NaOH and the mixture was stirred for 16 h. The water was removed under vacuum. Filtration on SiO_2−RP18 (16 g) afforded **15** (360 mg, yield = 36%).

GC-MS analysis of the silylated crude reaction mixture on a SPB-1 column (30 m, oven temperature set to 130 °C for 5 min then increased to 320 °C at 7 deg/min) 8 min cysteine (24% peak area), 12.1 min **15** diastereoisomer **a** (41% peak area) [MW = 347, *m/z* (rel intensity) 347 (3), 231 (20), 230 (100), 160 (10), 73 (25)], 12.7 min **15** distereoisomer **b** (29% peak area) [*m/z* (rel intensity) 230 (100), 160 (15), 134 (2), 129 (15), 73 (25)]. **15**: LCMS(APCI+) confirmed M + 1 = 204 of **15**.

^{1}H NMR (aqueous DCl, pH 2, major diastereoisomer of **15**) δ 0.88 (t, J = 7.0 Hz, Me), 1.37−1.60 (m, 2 × C**H**$_2$), 1.90−2.05 (m, C**H**$_2$-6, 1H), 2.35−2.45 (m, C**H**$_2$-6, 1H), 2.90−3.01 (m, C**H**-7), 3.15−3.58 (m, C**H**$_2$-5 and C**H**$_2$-2), 4.45−4.55 (m, C**H**-3). ^{13}C NMR δ 15.8 (q), 22.55 (t, C-Me), 32.2 (t, C-2), 38.2 (t, C-6), 39.4 (t, CH_2-CH), 46.3 (t, C-5), 49.2 (d, C-7), 63.1 (d, C-3), 173.1 (s).

*Preparation of (2R)-S-(1,1-Dimethyl-3-oxobutyl-cysteine) (**17**).* Mesityl oxide (**16**, 6.27 g, 64 mmol) was added to **5a** (7.7 g, 64 mmol) and Na_2CO_3 (1.5 g) in water (100 mL) at room temperature (pH 8). After 2 h excess **16** was removed by extraction with Et_2O. The water phase was concentrated under vacuum at 40 °C. Ethanol (100 mL) was added and the mixture was heated at reflux for 15 min. The hot solution was filtered and concentrated under vacuum to afford 10 g of solid, which was a 2/1 mixture of cysteine and **17** by ^{1}H NMR. LC-MS (Xtera column, 2 mm × 250 mm eluted with 10% CH_3CN, 90% water 0.1% formic acid) 2.1 min M + 1 = 220 plus small fragment *m/z* (**18**) 202. GC-MS of the silylated form (MW = 363, not observed, **17** + 2XMe_3Si) was acquired on a SPB-1 column (30 m, oven temperature program from 130 to 300 °C at 10 deg/min) 6.5 min: *m/z* (relative abundance) 273 (50), 202 (25), 156 (100), 129 (40), 100 (60), 73 (55). ^{1}H NMR (0.1 M aqueous DCl) δ 1.42 (s, 2 × Me), 2.25 (s, MeCO), 2.81 (d, J = 7 Hz, C**H**$_2$a-5), 2.90 (d, J = 7 Hz, C**H**$_2$b-5), 3.02

Scheme 1. Target Molecules and a Flavor Precursor[a]



[a] **1** and **2** are the target molecules, and **3** is a known example of a flavor precursor (*14*–*23*).

(dd, J = 4.3−13.5 Hz, C**H**$_2$a-3), 3.20 (dd, J = 7.2−13.5 Hz, C**H**$_2$b-3), 3.97 (dd, J = 4.3−7.2 Hz, C**H**-2). ^{13}C NMR δ 30.9 (q, 2 × Me), 31.4 (t, C-3), 34.7 (q, C-7), 47.1 (s, C-4), 56.4 (t, C-5), 56.9 (d, C-2), 175.2 (s, C-1), 216.4 (s, C-6).

*Preparation of (R)-S-(3-Hydroxy-1,1-dimethylbutyl)cysteine (**3**) and (3R)-Hexahydro-5,7,7-trimethyl-1,4-thiazepine-3-carboxylic Acid (**18**).* Compound **16** (0.120 mL, 1 mmol) was added to **5a** (120 mg, 1 mmol) in water (20 mL). The pH was adjusted to 8 by the addition of Na_2CO_3 (30 mg) and the mixture was stirred for 16 h at room temperature. Sodium borohydride (80 mg, 2 mmol) was added together with one drop of 30% aqueous NaOH (pH 10). After 1 h at room temperature, HCl was added until the solution was at pH 7. The solution was concentrated under vacuum at 40 °C and ethanol was added to the residue. Filtration was followed by removal of ethanol under vacuum (40 °C, 10 mbars). The crude product (0.31 g) was filtered on 15 g of RP18 with a gradient starting from pure water to water/ethanol 9/1. The first fraction was mainly the alcohol **3** (60 mg, yield 27%) and the second fraction contained **18** (130 mg, yield 61%).

*Analysis of **3***: ^{1}H NMR (aqueous DCl, pH 4.7, purity ∼90%) δ 1.22 (d, J = 7.0 Hz, Me-7), 1.38 (s, 2 × Me), 1.7−1.8 (m, C**H**$_2$-5), 2.95−3.18 (m, C**H**$_2$-3), 3.90−4.0 (m, C**H**-2), 4.10−4.25 (m, C**H**-6). ^{13}C NMR δ 27 (q, C-7), 31.1 (q, 2×), 31.2 (t, C-3), 48.5 (s, C-4), 52.6 (t, C-5), 57.1 (d, C-2), 68.2 (d, C-6), 175.5 (s, C-1).

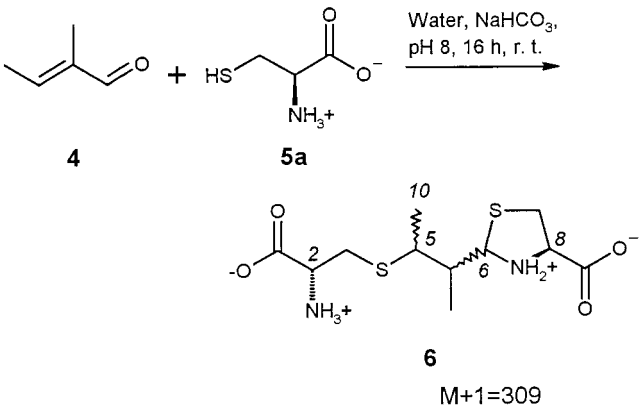
*Analysis of **18***: ^{1}H NMR (aqueous DCl, pH 2, purity >95%, one diasteomer) δ 1.32 (s, Me), 1.39 (s, Me), 1.42 (d, J = 6.4 Hz, **Me**-CH-5), 1.98 (d, J = 15.7 Hz, C**H**a-6), 2.22 (dd, J = 9.8, 15.7 Hz, C**H**b-6), 3.23 (dd, J = 5.4, 16.7 Hz, C**H**a-2), 3.41 (dd, J = 4.4, 16.7 Hz, C**H**b-2), 3.75−3.80 (m, C**H**-5), 4.35 (t, J = 4.9 Hz, C**H**-3). ^{13}C NMR δ 23.8 (q, **Me**-C-5), 29.3 (t, C-2), 29.6 (q, **Me**-C-7), 33.4 (q, **Me**-C-7), 45.5 (s, **C**-7), 52.8 (t, **C**-6), 53.6 (d, **C**-5), 63.7 (d, **C**-3), 173.6 (s, COOH).

LC-MS: M + 1 = 222 first peak **3**, M + 1 = 204 s peak **18**; GC-MS of the MSTFA derivative of the crude product run on an SPB-1 column (30 m, oven temperature set to 130−300 °C at 7 deg/min) 8.8 min, 50% peak area, cysteine, 12.9 min, 16% peak area, product **18**: *m*/*z* (rel abundance) 347 (10), 172 (30), 157 (50), 117 (100), 89 (30), 73 (60), 14 min, 34% peak area, alcohol **3**; *m*/*z* (rel abundance) 437 (2), 320 (20), 218 (100), 117 (25), 73 (30).

RESULTS AND DISCUSSION

Addition of Cysteine 5a to 2-Methyl-2-butenal (Tiglic Aldehyde, 4). When cysteine was treated with tiglic aldehyde and (*E*)-2-hexenal to prepare monoadducts **1** and **2** (**Scheme 1**), which we speculated to be flavor precursors (*14*, *15*, *23*) of 3-mercaptoalkanals, we obtained complex mixtures. We then attempted to prepare the known cysteine monoadduct **3**, identified in Sauvignon white wine, by the published method of cysteine addition to mesityl oxide followed by reduction (*14*). However, we found the major product of this reaction was the novel thiazepane **18**. These observations led us to study more carefully the Michael addition of cysteine to tiglic aldehyde **4**, (*E*)-2-hexenal (**12**), and mesityl oxide (**16**).

Scheme 2. Reaction of Cysteine **5a** with Tiglic Aldehyde **4** at pH 8

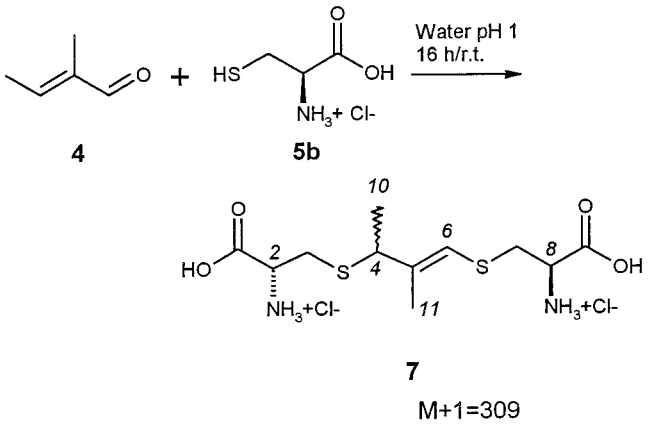


Addition of 1 equiv of cysteine **5a** to 1 equiv of tiglic aldehyde **4** in slightly basic aqueous solution (pH 8) was affected in D_2O and analysis by ^{1}H NMR spectroscopy showed a complex mixture containing as the major components a new compound **6** and tiglic aldehyde **4** (**Scheme 2**).

The structure of **6** was determined from spectra acquired on an isolated sample consisting of two major diastereomers. The molecular ion of **6**, at *m*/*z* 308, was consistent with the molecular formula $C_{11}H_{20}N_2O_4S_2$. From the molecular formula it was evident that **6** contained three sites of unsaturation. Two sites of unsaturation were accounted for by carbonyl carbons which resonated in the ^{13}C NMR spectrum at δ 175.4 and 176.5 ppm and were attributed to the acid moieties of two cysteine equivalents. No other carbon signals were present downfield of δ 80 ppm, indicating that the remaining unsaturation was not a double bond and so must be due to the presence of a cyclic moiety. Three coupled spin systems were observed in the COSY spectrum. Multiplets, arising from the two diastereoisomers, at δ 3.93 and 3.99 ppm (H-2) were coupled respectively to two multiplets at 3.05 and 3.28 ppm (H-3), and this system was attributed to an unmodified cysteine moiety. Multiplets at δ 3.05 and 3.19 ppm (H-4) were coupled to methyl doublets at 1.40 and 1.41 ppm (Me-10) and multiplets at 2.10 and 2.25 ppm (H-5). The H-5 methine protons were further coupled to methyl doublets at 1.05 and 1.18 ppm (Me-11) and to doublets at 4.80 and 5.01 ppm (H-6). HSQC data indicated that the proton resonances at δ 3.05 and 3.19 ppm (H-4) were attached to carbons at 45.1 and 49.0 ppm, suggesting that these carbons were single bonded to heteroatoms while the doublets at 4.80 and 5.01 ppm (H-6) were attached to carbons of resonance 71.7 and 73.8 ppm consistent with each carbon single bonded to two heteroatoms. This spin system was attributed to the reacted tiglic aldehyde, attached to one cysteine moiety via a sulfide bond at C-4 and attached to the other cysteine moiety, cyclized to form a thiazolidine ring, at C-6. The third spin system observed in the COSY spectrum consisted of a multiplet at δ 3.22−3.52 ppm (H-7) coupled to multiplets at 4.54 and 4.18 ppm (H-8) and was attributed to the second, cyclized cysteine moiety. This type of double reaction has been described with crotonaldehyde as the substrate (*16*) and according to this publication, the rate-determining step was the addition of sulfhydryl anions of cysteine to the C−C double bond. The reaction was highly pH dependent and proceeded well at an optimum pH between 8 and 9. The formation of the thiazolidine was very fast and reversible.

In our study, the monoadduct (M + 1 = 206) **1** was not detected by LC-MS, but a compound M + 1 = 188, which we

Scheme 3. Reaction of Cysteine HCl **5b** with Tiglic Aldehyde **4** under Acidic Conditions



believe resulted from the formation of an intramolecular Schiff base from the corresponding monoadduct, was observed. It was not possible to confirm the structure of the M + 1 = 188 compound by ^{1}H NMR or by ^{13}C NMR analysis of the reaction mixture nor was the compound sufficiently stable to permit isolation. However, the cyclic structure of this monoadduct was inferred from the isolation of the corresponding thiazepane **11** from a similar reaction mixture after reduction with $NaBH_4$ (see below).

The reaction was repeated with 1 equiv of tiglic aldehyde in D_2O, in the presence of 1 equiv of cysteine·HCl **5b**, for 16 h at room temperature, as shown in **Scheme 3**. Acidic conditions were chosen to decrease the nucleophilicity of the cysteine nitrogen and in hopes of avoiding the formation of the double adduct resulting from formation of the thiazolidine moiety. The ^{1}H NMR spectrum of the crude reaction mixture showed a lot of tiglic aldehyde remaining, small doublets at δ 4.80 and 5.01 ppm indicated the presence of compound **6**, but the major product existed as two isomers possessing singlets in the ^{1}H NMR spectrum at 6.02 and 6.04 ppm, typical of a vinylic proton. The structures of these two isomers were inferred from NMR data, including the ^{13}C NMR spectrum that contained two quartets at δ 15.8 and 16.31 ppm (C −11), two doublets at 123.3 and 123.4 ppm (C-6), and two singlets at 141.4 and 141.5 ppm (C-5). HPLC traces of this reaction mixture were different from those obtained with the pH 8 reaction mixture. By LC-MS two new peaks with molecular ions of M + 1 = 309 were observed, indicating that double addition of cysteine to tiglic aldehyde had occurred but yielding products that differed from **6**. Both peaks had molecular weights of 308, consistent with the molecular formula $C_{11}H_{20}N_2O_4S_2$, which indicated that **7** contained two cysteine moieties and three sites of unsaturation. These isomers were tentatively assigned the structure **7**.

Workup of the reaction mixture resulted in isolation of **7** in 78% yield. The ^{1}H NMR analysis of isolated **7** (**Figure 1**) was performed in a 0.1 M NaOD solution, because it was not possible to solubilize purified **7** in aqueous DCl, but fortunately a signal for a vinylic proton, seen when the reaction was performed in D_2O and followed by ^{1}H NMR, was still present. Whereas the crude reaction mixture appeared to contain two isomers of **7**, upon purification, only one isomer remained. The absence of one of the isomers might be due to unintended separation during flash chromatography or instability of that isomer during the workup or in the basic NMR solution.

Two sites of unsaturation in **7** were accounted for by carbonyl carbons which resonated at δ 183.9 and 183.5 ppm in the ^{13}C NMR spectrum and were attributed to the acid moieties of two

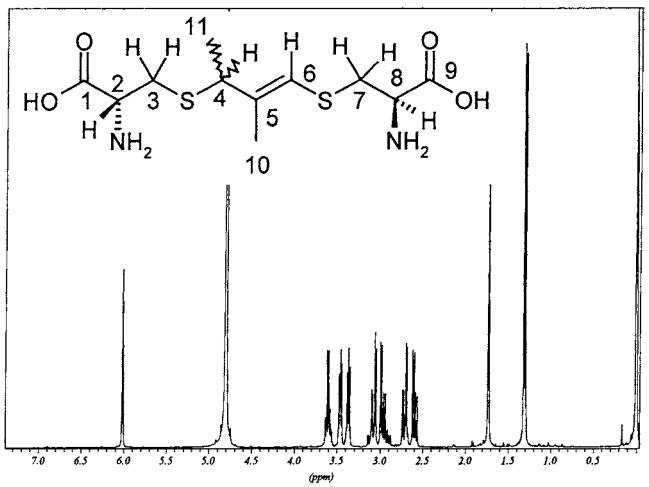


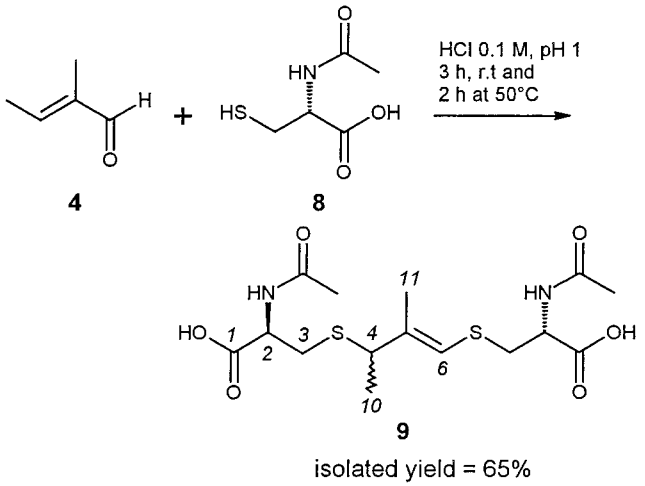
Figure 1. ^{1}H NMR (D_2O) spectrum of vinyl sulfide **7**.

cysteine moieties. Carbon resonances at δ 139.2 (singlet, C-5) and 123.9 (doublet, C-6) indicated that **7** contained a C=C double bond that bore three substituents and one proton. The presence of the double bond accounted for the last remaining site of unsaturation and eliminated the possibility that **7** contained a cyclic thiazolidine moiety. Four coupled spin systems were observed in the COSY spectrum. Two that were attributed to the unmodified cysteine moieties exhibited correlations at δ 2.59 and 2.71 ppm (H-3) that were coupled to one another and to a doublet of doublets at δ 3.36 ppm (H-2) while a multiplet at δ 3.07 ppm (H-7) was coupled to a doublet of doublets at δ 3.46 ppm (H-8). The third spin system consisted of a broad singlet at δ 6.02 ppm (H-6) coupled to a doublet at 1.73 ppm with a coupling contant of J = 1 Hz (Me-11), indicating the presence of a vinylic proton on the same double bond as a methyl group. HSQC correlations indicated that this vinylic proton was attached to an sp^2 carbon at δ 123.9 ppm (C-6). The fourth spin system contained a broad quadruplet at δ 3.61 (H-4) coupled to a doublet at 1.31 ppm (Me-10). By HSQC analysis the proton resonating at 3.61 ppm was attached to a carbon with a chemical shift at δ 50.6 ppm (C-4), consistent with a C−S bond. The ^{13}C NMR chemical shift of 15.8 ppm assigned to Me-11 gives an indication of the stereochemistry of the double bond. There are many examples of alkenyl methyl groups on trisubstituted olefins trans to a vinylic proton that have ^{13}C NMR chemical shifts of approximately 15 ppm while those that are cis to the proton have chemical shifts around 27 ppm (*24*). On the basis of this information, the double bond configuration of **7** was assigned to be *E* (CH_3-11 syn to S atom). The NMR solution was injected on LC-MS and while the crude reaction mixture contained two peaks of M + 1 = 309 only one peak was present in the NMR solution, consistent with the presence of a single isomer. The initial NMR experiments conducted with the reaction mixture indicated the formation of isomers with the same double bond configuration (*E*). The absence of multiple diastereomers indicated that epimerization at the α-position of the cysteine carbonyl was, at most, minor.

To our knowledge, the formation of a vinylic sulfide derived from the 1,4-addition of cysteine to an α,β-unsaturated aldehyde followed by the reaction of a second cysteine on the aldehyde function has not been reported. The only known vinylic sulfide originating from cysteine is *S*-2-propenylcysteine reportedly found in *Alliums* species (*1*, *2*).

Addition of *N*-Acetyl-cysteine 8 to (*E*)-2-Methyl-2-butenal (Tiglic Aldehyde 4). Using acidic conditions to avoid the double

Scheme 4. Addition of *N*-Acetyl-cysteine **8** to *(E)*-2-Methyl-2-butenal (tiglic aldehyde **4**)



reaction of cysteine with tiglic aldehyde **4** failed. In an effort to prevent the double reaction of cysteine and to obtain a β-mercaptocarbonyl derivative, *N*-acetyl-cysteine **8** was chosen as the candidate for the next experiments. Natural γ-glutamyl-cysteine present in onion (*1*) may have a similar reactivity with α,β-unsaturated aldehydes as that of *N*-acetyl-cysteine **8**.

Under acidic conditions we observed the formation of two isomers of the vinylic sulfide **9** (**Scheme 4**). The structure was elucidated by ^{1}H NMR, ^{13}C NMR, and 2-D NMR experiments on the one purified isomer, isolated in 65% yield. In the ^{13}C NMR spectrum two cysteine carbonyls were present at δ 176.4 ppm in addition to two carbonyls attributed to the acetyl groups at 176.1 and 175.8 ppm. Signals at δ 140.2 and 139.9 ppm (C-5) and at 123.3 and 122.9 ppm (C-6) show the presence of a C=C double bond. The methyl groups attached to the double bond appeared at δ 15.6 and 15.2 consistent with an *E* configuration of the trisubstitued double bond. Correlations in the COSY spectrum resembled those observed for **7**. The use of *N*-acetyl-cysteine did not avoid a double addition to tiglic aldehyde. The presence of two diastereomers resulted from the nonstereoselective addition of cysteine at carbon C-4 of tiglic aldehyde **4**.

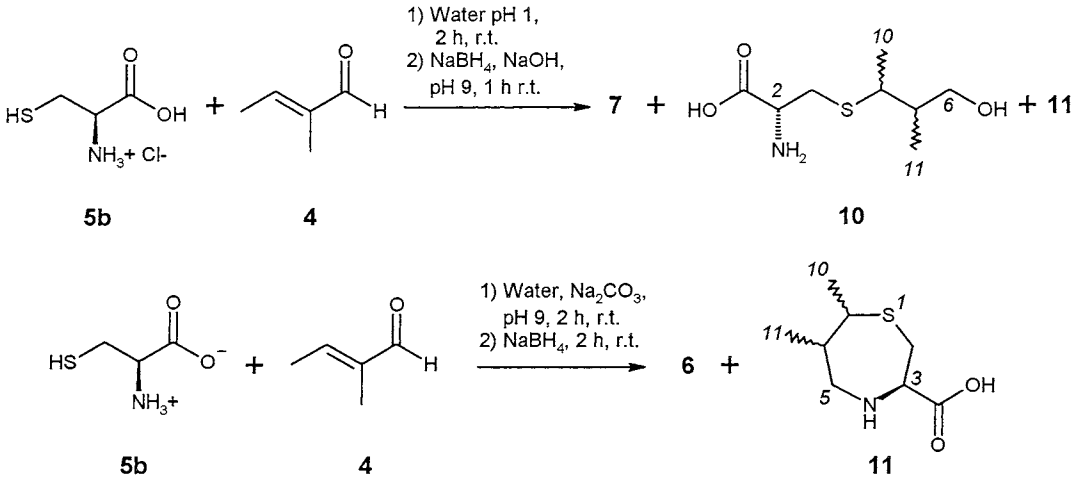
With use of basic conditions (pH 8), a complex mixture was formed, composed mainly of bis-*N*-acetyl-cysteine disulfide but no readily identifiable aldehyde adducts.

Reduction of Cysteine Adducts. LC-MS analysis suggested that free aldehyde **1** (*m/z* 205) may be formed in the course of the reaction of tiglic aldehyde **4** with cysteine **5a** and **5b**. The apparent instability of this molecule made isolation and characterization difficult. Consequently we reduced the crude reaction mixture of **4** with **5a** or **5b** using $NaBH_4$ to trap **1** as the corresponding alcohol. $NaBH_4$ was added directly to the reaction mixtures and the pH adjusted to about nine by addition of aqueous NaOH. This change of pH did not seem to have a major effect on the composition of the two reaction mixtures since differences in the reduced mixtures were evident (**Scheme 5**).

Products **6** and **7**, formed in the first steps of the acidic and basic reactions, respectively, were not reduced by $NaBH_4$ and they were detected by LC-MS or isolated as side products after flash chromatography. Compound **7**, alcohol **10**, and thiazepane **11** were detected by LC-MS and GC-MS in the mixture prepared under acidic conditions. The LC-MS analysis showed a product with an M + 1 ion of *m/z* 208, which is consistent with the molecular formula for **10**, $C_8H_{17}NO_3S$. By GC-MS of the MSTFA-derivatized reaction mixture, a peak was detected at 4.97 min, with an *m/z* 408 ion, corresponding to the loss of a methyl radical from triply silylated **10**. The molecular ion of silylated **10** (*m/z* 423) was not observed. Silylated thiazepane **11** also was detected and gave an expected molecular ion, *m/z* 333, with a GC retention time of 3.7 min. LC-MS analysis showed a peak yielding an ion of *m/z* 189, which is consistent with the molecular formula of **11**, $C_8H_{15}NO_2S$. The thiazepane **11** was also present in the basic reaction mixture (**Scheme 5**). The formation of the thiazepane is consistent with the presence of the corresponding intramolecular cyclic Schiff base in the unreduced reaction mixtures.

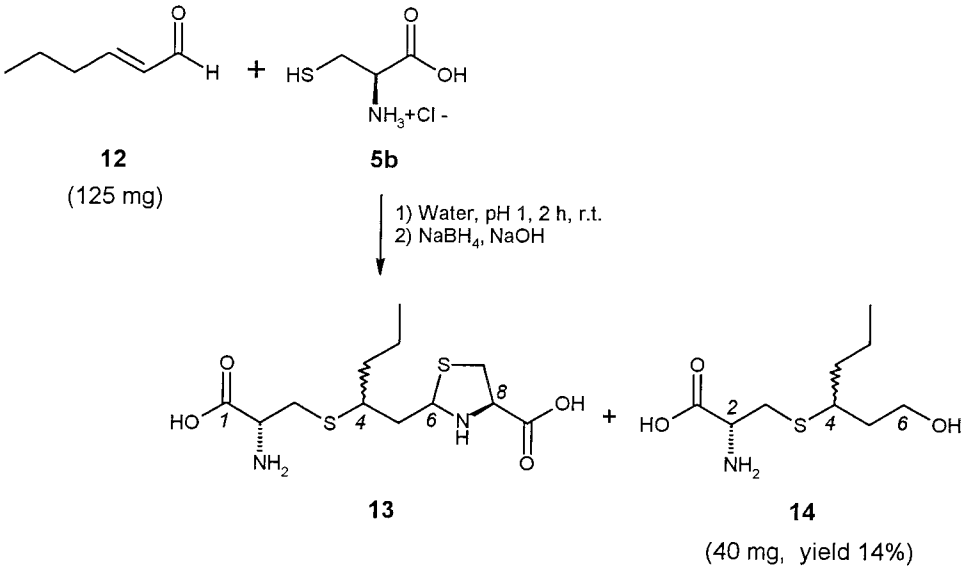
Thiazepane **11** was isolated as a mixture of three diastereomers from the basic reaction mixture. NMR data for the major diastereomer allowed elucidation of the structure. The ^{13}C NMR spectrum was consistent with structure **11** showing two triplets at δ 34.5 (C-3) and 50.9 ppm (C-5), three doublets at 64.5, 48.7, 41.4 ppm (C-3, 7, 6, respectively), and one carbonyl signal at δ 178.0 ppm. No alkene carbon signals were evident. Two coupled spin systems were observed in the COSY spectrum corresponding to the reacted cysteine and tiglic aldehyde moieties. The cysteine residue yielded two coupled multiplets at 3.95−4.00 and 3.10−3.20 ppm corresponding to H-3 and H-2, respectively. HSQC data showed that the multiplet at δ 3.95−4.00 (H-3) was attached to a methine carbon resonating

Scheme 5. Reduction of Tiglic Aldehyde Adducts under Basic or Acidic Conditions (non-ionic forms displayed)[a]

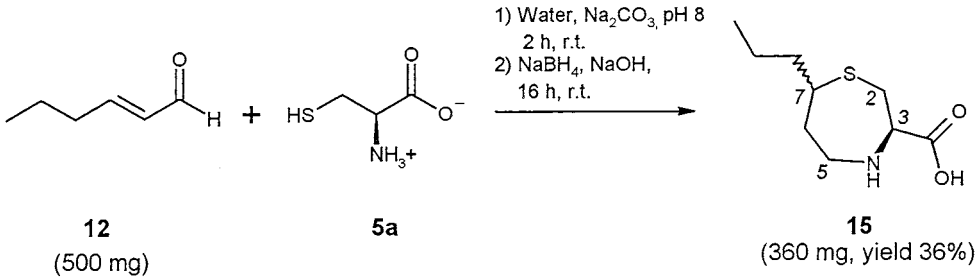


[a] **11** was numbered according to UPAC, but to avoid confusion, both methyls were still numbered 10 and 11 to be consistent with its precursor.

Scheme 6. Cysteine HCl **5b** Addition to *(E)*-2-Hexenal (**12**) in Water at pH 1



Scheme 7. Reduction of the Crude Reaction Mixture Resulting from the Addition of Cysteine **5a** to *(E)*-2-Hexenal (**12**) at pH 8



at 64.5 ppm (C-3). The COSY spectrum contained correlations that were assigned to the reacted tiglic aldehyde residue and these consisted of a doublet at δ 1.08 ppm (Me-11) that was coupled to a multiplet at δ 2.40 ppm (H-6). The H-6 resonance was further coupled to a multiplet at 3.10−3.20 ppm (H-7) that was coupled to a doublet at δ 1.20 ppm (Me-10). The H-7 multiplet was further coupled to a multiplet at δ 3.35 ppm that was assigned to H-4. An alternative thiazepane structure resulting from Michael addition of the cysteine amine group followed by thiol addition and dehydration at the aldehyde carbonyl is not likely since reduction of the resulting thioalkene function by $NaBH_4$ would not be expected.

1,4-Addition of Cysteine to *(E)*-2-Hexenal (12). To compare our results with the published reports (*14*, *15*) of related flavor precursors in Sauvignon Blanc wine, the addition of cysteine to *(E)*-2-hexenal (**12**) was performed under both acidic and basic conditions, followed by reduction with $NaBH_4$ (**Schemes 6** and **7**).

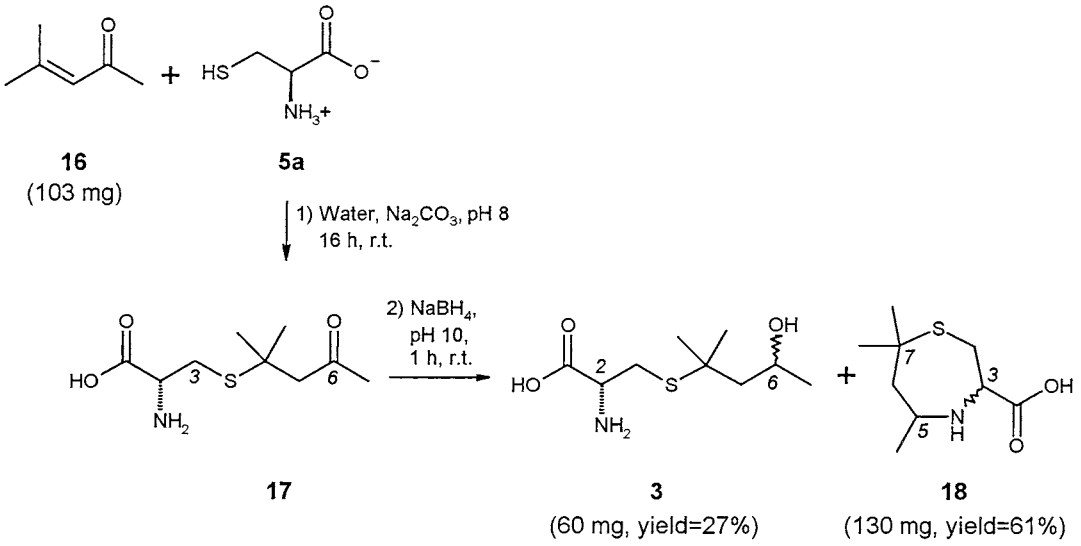
Under acidic conditions, LC-MS of the crude reaction mixture before reduction showed a mixture of compounds possessing molecular ions of *m*/*z* 201 (monoadduct formation followed by dehydration), *m*/*z* 219 (monoadduct, presumably **2**), and *m*/*z* 323 (a diastereoisomeric mixture of double adduct **13**). After flash chromatography the structure of product **13** was elucidated from spectra acquired of a mixture of two major diastereoisomers (90% of the mixture) and two minor diastereoisomers (10% of the mixture). The molecular ion of **13**, at *m*/*z* 322, was consistent with the molecular formula $C_{12}H_{22}N_2O_4S_2$. It was confirmed by GC-MS of the silylated form that gave a molecular ion of *m*/*z* 538 corresponding to addition of three Me_3Si moieties. The COSY spectrum showed the corresponding correlations as observed for **6**, including multiplets at δ 4.9 to 5.1 ppm attributable to the thiazolidine methine proton (H-6) located between the N and S atoms. The HSQC spectrum showed that these proton resonances at δ 4.9 to 5.1 ppm (H-6) were coupled to methine carbons at δ 65.8 and 66.4 ppm (C-6).

Major differences between acidic and basic conditions were observed after reduction with $NaBH_4$. Thus, the double adduct **13** (yield 80%) and the alcohol **14** (14% yield) were isolated by flash chromatography when the reaction was performed under acidic conditions. However, under basic conditions, only thiazepane **15** was isolated (36% yield) as a mixture of two diastereoisomers and the remainder of the material was a mixture of **13** and cysteine.

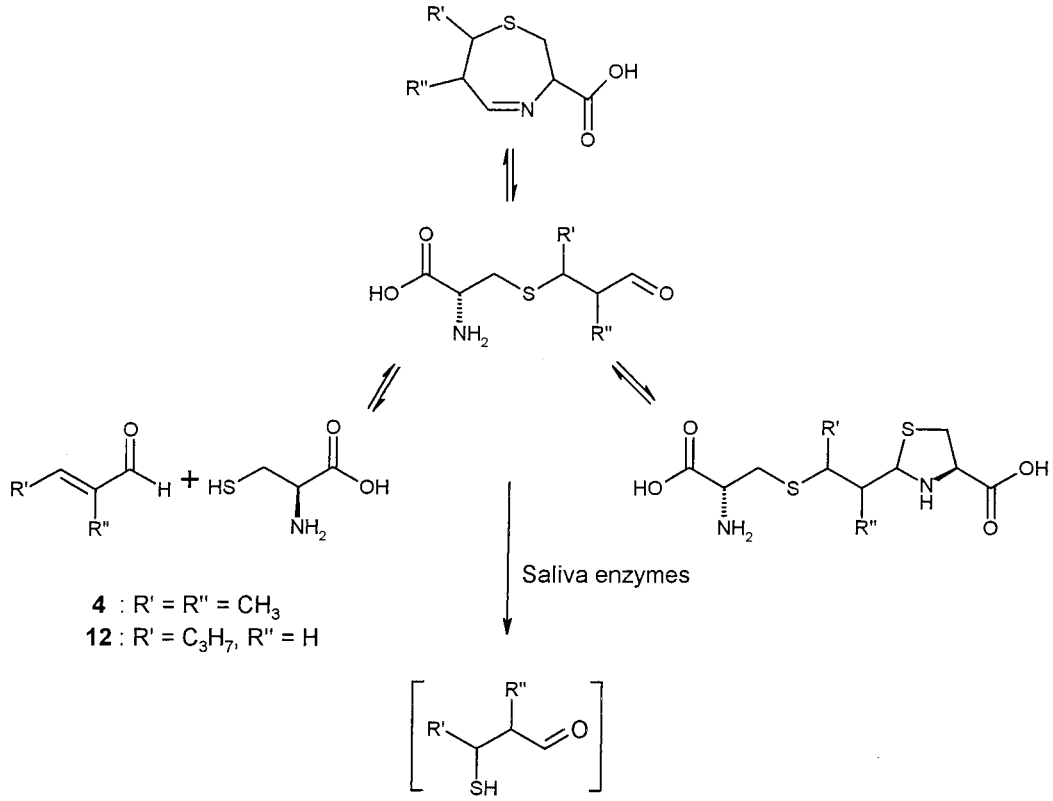
Mass spectral data for **14** and **15** were consistent with the assigned structures. By LC-MS, M + 1 ions of *m*/*z* 222 and *m*/*z* 204 were recorded for **14** and **15**, respectively. GC-MS analysis of the trimethylsilylated derivatives further confirmed the molecular compositions. Trimethylsilylated **14** produced a weak *m*/*z* 437 peak corresponding to the molecular ion (three trimethylsilyl groups added) and major fragment ions at *m*/*z* 320, 218 (base peak), and 73. These data are consistent with those previously published (*14*, *15*). Silylated thiazepane **15** also produced a weak molecular ion, *m*/*z* 347, and a base peak at *m*/*z* 230 resulting from loss of a Me_3SiCO_2 radical.

The major differences observed in the ^{13}C NMR spectra of **14** and **15** were a resonance at δ 57 ppm assigned to (C-2) of **14** and a resonance at δ 63 ppm assigned to C-3 of **15**. A resonance at δ 62 ppm in the spectrum of **14** was assinged to C-6 while the spectrum of **15** contained a signal at δ 46 ppm that was assigned to C-5. In contrast to the acidic tiglic aldehyde

Scheme 8. 1,4-Addition of Cysteine **5a** to Mesityl Oxide (**16**) at pH 8



Scheme 9. Proposed Generation Pathway of Odorant 3-Mercapto-alkanals in the Mouth



reactions, 2-hexenal did not produce alkenyl sulfide products corresponding to **7** or **9**. This suggests that lack of substitution in the α-position relative to the carbonyl group of (*E*)-2 hexenal disfavors the formation of such compounds with the olefin function of which is less stabilized than in **7** or **9**. At pH 8 it appears the major reaction between cysteine and 2-hexenal is formation of a monoadduct that exists as the cyclized Schiff base, reduction of which yielded thiazepane **15**.

1,4-Addition of Cysteine to Mesityl Oxide (4-Methyl-3-penten-2-one, 16). The addition of cysteine to an α,*β*-unsaturated carbonyl functionality was repeated with **16**, a ketone, as the substrate. It has been reported that under acidic conditions, at pH 1, the Michael addition was slow and incomplete (*16*). As shown in **Scheme 8**, we treated mesityl oxide with cysteine at pH 1 under the same conditions used with tiglic aldehyde. After reduction of the crude mixture with $NaBH_4$, the alcohol **3** was obtained (12% yield). The remainder of the reaction mixture was composed of unreacted starting materials. However, at pH 8, the monoaddition product **17** was isolated (yield 22%) as a mixture with cysteine (2/1 cysteine/**17** by NMR). After reduction of the crude, pH 8 reaction mixture with $NaBH_4$, the major compound isolated was once again a thiazepane, **18** in 61% yield, in addition to the targeted alcohol **3** in 27% yield.

LC-MS analysis of **17** produced an M + 1 ion of *m*/*z* 220. The mass difference between the reduced monoaddition products **3** and **18** was observed by LC-MS. Compound **3** possessed an M + 1 ion of *m*/*z* 222 consistent with the molecular formula

$C_9H_{19}NO_3S$, while compound **18** had an M + 1 ion of *m*/*z* 204 consistent with the molecular formula $C_9H_{17}NO_2S$.

Although previously described in the literature, the only spectral data published for **17** and **3** are mass spectra of the trimethylsilylated derivatives (*14*). In addition to these spectra, we were able to obtain NMR data that were consistent with the proposed structures. Thiazepane **18**, like **11** and **15**, produced spectral data consistent with the seven-member ring structure. We could not find any references in the literature concerning thiazepanes **11**, **15**, and **18** so this is the first time that their formation is described.

It is interesting to mention that the diastereomeric mixture of **13** was odorless. However, when tasted at 10 ppm in mineral water, a strong persistent retronasal odor developed after a few seconds of tasting. This odor was probably due to the formation of 3-mercaptohexanal in the mouth (*21*−*25*) (**Scheme 9**). Compounds **6** and **7** also developed a strong and persistent retronasal onion-like flavor when tasted at 10 ppm in mineral water. These observations suggest that these double cysteine adducts stick to the mouth mucosa and saliva enzymes generate the mercaptoaldehydes (*20*, *21*, and unpublished results) from the mono-cysteine, Michael-addition adducts (**Scheme 9**). The reduction of S−C bonds by saliva enzymes has not yet been documented.

CONCLUSIONS

When cysteine was added to the α-substituted, α,*β*-unsaturated aldehyde, tiglic aldehyde, **4** under slightly basic conditions, a double addition of cysteine was observed and product **6** was formed. Under acidic conditions a double addition also occurred; however, the second cysteine molecule added to form a vinylic sulfide bond and product **7** was formed. Reduction of the crude mixture prepared under acidic conditions gave the alcohol **10**, presumptive evidence of the existence of the monoadduct **1**. Under basic conditions, after reduction with $NaBH_4$, thiazepane **11** was formed. Similar results were obtained with *(E)*-2-hexenal (**12**) but in this case the corresponding vinylic sulfide, double addition product was not observed, probably because it was not stabilized by an α-substituent. In the case of mesityl oxide (**16**), the carbonyl group was less reactive and basic conditions gave the monoadduct **17** in better yield than under acidic conditions. After reduction of the crude pH 8 mixture with $NaBH_4$ two products were isolated: the corresponding alcohol **3** and thiazepane **18**.

A better understanding of the biogeneration of volatile sulfur compounds from these types of products is a challenge for the future.

ABBREVIATIONS USED

MSTFA, *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide; $NaBH_4$, sodium borohydride; LC-MS, high-performance liquid chromatography−mass spectrometry; HP-TLC SiO_2−RP18, high-performance silica gel thin-layer chromatography with a hydrophobic C18 coating; COSY, *co*rrelation *s*pectroscop*y*; HMQC, *h*eteronuclear *m*ultiple *q*uantum *c*orrelation spectroscopy; HMBC, *h*eteronuclear *m*ultiple *b*ond *c*orrelation spectroscopy.

ACKNOWLEDGMENT

I thank Eric Frérot and Olivier Haefliger for the LC-MS measurements and scientific advise, Anthony Buchs for the practical work in the lab, François Benzi for tasting assistance, Robert Brauchli and Walter Thommen for NMR measurements and discussion, and Jana Pika, Gary Womack, and Roger Snowden for discussion and writing assistance.

LITERATURE CITED

(1) Lancaster, J. E.; Boland, M. J. Flavor Biochemistry. *CRC Onion Allied Crops* **1990**, *3*, 33−68.
(2) Block, E. The organosulfur chemistry of the genus *Allium*. Implications for the organic chemistry of sulfur. *Angw. Chem., Int. Ed. Engl.* **1992**, *31*, 1135−1178.
(3) Lieberman, S.; Brazeau, P.; Hariton, L. B. Spiro (steroid) Thiazolidines. *J. Am. Chem. Soc.* **1948**, *70*, 3094.
(4) Scholka, I. R.; Spoerri, P. E. Thiazolidine Chemistry. The preparation of 2-substituted thiazolidine-4-carboxylic acids. *J. Org. Chem.* **1957**, *22*, 943−946.
(5) Fernandez, X.; Duñach, E.; Fellous, R.; Lizzani-Cuvelier, L.; Loiseau, M.; Dompe, V.; Cozzolino, F.; George, G.; Rochard, S.; Schippa, C. Identification of thiazolidines in guava: stereochemical studies. *Flavour Fragrance J.* **2002**, *17*, 432−439.
(6) Rizzi, J. P.; Steimle, A. R.; Patton, D. R. Formation of dialkylthiophenes in Maillard reactions involving cysteine. *Dev. Food Sci.* **1992**, *29*, 731−741.
(7) De Roos, K. B. Meat Flavor generation from cysteine and sugars. *ACS Symp. Ser.* **1992**, *490*, 203−216 (Chapter 16).
(8) Tuong, H. B. Procédé de préparation de thiols. EP 0770686 A1, 1995.
(9) Tuong, H. B.; Matthey-Doret, W.; Fay, L. B.; Bel Rhlid, R. Generation of thiols by biotransformation of cysteine−aldehyde conjugates with Baker's Yeast. *J. Agric. Food Chem.* **2003**, *51*, 3629−3635.
(10) Kerkenaar, A.; Schmedding, D. J. M.; Berg, J. Method for preparing p-mentha-8-thiol-3-one. US Patent 5,182,194, 1993.
(11) Wakabayashi, H.; Wakabayashi, M.; Eisenreich, W.; Engel, K.-H. Stereoselectivity of the B-lyase-catalyzed cleavage of S-cysteine conjugates of pulegone. *Eur. Food Res. Technol.* **2002**, *215*, 287−292.
(12) Buckholz, L. Flavor freshness enhancers. Patent WO 01/35768 A1, 2001.
(13) Darriet, P.; Tominaga, T.; Demole, E.; Dubourdieux, D. Mise en évidence dans le raisin de Vitis vinifera (var. Sauvignon) d'un précurseur de la 4-mercapto-méthylpentan-2-one. *C. R. Acad. Sci. Paris, Bio. Pathol. Végétale* **1993**, *316*, 1332−1335.
(14) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D. A new type of flavor precursors in Vitis vinifera L. cv. Sauvignon Blanc, S-cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215−5219.
(15) Tominaga, T.; Dubourdieu, D. Identification of cysteinylated arome precursors of certain volatile thiols in passion fruits. *J. Agric. Food Chem.* **2000**, *48*, 2874−2876.
(16) Esterbrauer, E.; Ertl, A.; Scholz, N. The reaction of cysteine with α;*β*-unsaturated aldehydes. *Tetrahedron* **1976**, *32*, 285−289.
(17) Widder, S.; Sabater Lüntzel, C.; Dittner, T.; Pickenhagen, W. 3-Mercapto-2-methylpentan-1-ol, a new Powerful Aroma Compound. *J. Agric. Food Chem.* **2000**, *48*, 418−423.
(18) Widder, S.; Dittner, T.; Sabater Lüntzel, C.; Pickenhagen, W.; Vollhardt, J. 3-mercapto-2-alkyl-alkan-1-ole und ihre Anwendung als Duft- und Aromastoffe. EP 0924198 A1, 1999.
(19) Werkhoff, P.; Brüning, J.; Günter, M.; Kaulen, J.; Krammer, G.; Sommer, H. Potent, mercapto/methylthio-substituted aldehydes and ketones in cooked beef liver. *J. Food Sci.* **1996**, *18*, 19−27.
(20) Winter, M.; Furrer, A.; Willhalm, B.; Tommen, W. Identification and synthesis of two new organic sulfur compounds from the yellow passion fruit (*Passiflora edulis f. flavicarpa*). *Helv. Chim. Acta* **1976**, *59*, 1613−1620.
(21) Vermeulen, C.; Collin, S. Synthesis and sensorial properties of mercaptoaldehydes, *J. Agri. Food Chem.* **2002**, *50*, 5654−5659.

(22) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.
(23) 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.
(24) Wehrli, F. W.; Nishida, T. The use of Carbon-13 Nuclear Magnetic Resonance Spectroscopy in Natural Products Chemistry. *Prog. Chem. Org. Nat. Prod.* **1979**, *36*, 24−25.
(25) Badings, H. T.; Maarse, H.; Kleipool, R. J. C.; Tas, A. C.; Neeter, R.; ten Noever de Brauw, M. C. Formation of odorous compounds from hydrogen sulfide and 2-butenal. *Z. Lebensm. Unters. Forsch* **1976**, *161*, 53−59.

Received for review June 11, 2003. Revised manuscript received August 29, 2003. Accepted August 31, 2003.

JF0304268