How Cysteine Reacts with Citral: An Unexpected Reaction of β,β-Disubstituted Acroleins with Cysteine Leading to Hexahydro-1,4-thiazepines[†]

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The reaction of β,β-disubstituted acroleins [3-methyl-2-butenal (**1**), 3-methyl-2-hexenal (**2**), and citral (**3**)] with cysteine gave 1:2 adducts of a novel structural type, namely hexahydro-1,4-thiazepines. To the best of our knowledge, the spontaneous formation of a seven-membered heterocycle from the addition of cysteine to α,β-unsaturated aldehydes is unprecedented. The adduct **6** obtained from citral, under acidic conditions, reacted further to give the new bicyclic compound **8**.

KEYWORDS: Cysteine conjugates; Michael addition; α,β-unsaturated aldehydes; hexahydro-1,4-thiazepine; 1,4-thiazepane; 3-methyl-2-butenal; 3-methyl-2-hexenal; citral

INTRODUCTION

In continuation of our work on cysteine conjugates, resulting from the addition of cysteine to α,β-unsaturated carbonyl compounds (*1*), we investigated the reaction of β,β-disubstituted acroleins with cysteine. We expected the formation of 1:2 conjugates of type A (**Figure 1**), in analogy to the known reaction of cysteine with other α,β-unsaturated aldehydes such as acrolein (*2*), (*E*)-2-butenal (crotonaldehyde) (*2*), (*E*)-2-hexenal (*1*, *3*), and (*E*)-2-methyl-2-butenal (tiglic aldehyde) (*1*). It is well-established that the first molecule of cysteine adds in a slow, reversible Michael-type reaction to give a mono-*S*-cysteinylated saturated aldehyde that cannot be isolated but reacts immediately in a very fast and reversible reaction with a second molecule of cysteine (even with an excess of aldehyde) to form 2-substituted thiazolidine-4-carboxylic acids (*2*).

Such thiazolidine 1:2 conjugates are interesting precursors in two ways: (i) due to the reversibility of their formation, they are rather unstable in aqueous solutions and tend to gradually release the parent aldehyde (*2*); (ii) β-lyase-catalyzed cleavage of these precursors leads to the formation of powerful flavor compounds such as 3-mercaptoalkanals (*3*).

It thus came as a surprise that the reaction of cysteine with β,β-disubstituted acroleins did not form thiazolidine derivatives (type A), but rather the seven-membered ring 1:2 conjugates of type B (**Figure 1**). To the best of our knowledge, this structural type for cysteine conjugates has not yet been reported.

The objectives of this study were (i) to establish the structures of these adducts, (ii) to establish the structure of the compound obtained from the primary citral adduct upon treatment with acid, and (iii) to assess the stability of the primary addition product of citral as a possible slow-release derivative for citral.

MATERIAL AND METHODS

Reagents. All reagents were purchased from Aldrich Chemicals, Fluka Switzerland, or Dr. Glaser (CH-4051 Basel, Switzerland). TLC plates were HP-TLC Merck cat. 1.05635.

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 500 spectrometer at 500.13 and 125.76 MHz, respectively, using D_2O solutions with TSP (sodium 3-(trimethylsilyl)-tetradeuteriopropionate) as internal standard. DCl in D_2O (7.6 N) was used to prepare acidic solutions. Two-dimensional gradient-enhanced COSY, HSQC, HMBC, and NOESY experiments were performed with standard Bruker software (XWINNMR 3.1). The signal assignments are based on proton–proton (COSY) and proton–carbon correlation experiments (HSQC, HMBC).

UPLC–MS Analyses. Ultraperformance liquid chromatography (UPLC) was performed on a Waters Acquity system coupled to a Thermo Finnigan LCQ mounted with an atmospheric pressure chemical ionization (APCI) source. Positive and negative ion mode spectra (scan range 80–800 Da) were recorded. Alternatively, electrospray ionization (ESI) was performed with the use of a spray voltage of 4.5 kV, capillary temperature at 200 °C, and N_2 gas at flow rate of 55 (Finnigan arbitrary units).

UPLC Column. UPLC separations were performed on an Acquity UPLC BEH C_{18}, 17 μm, 2.1 × 100 mm column (Cat. P/N 186002352). The analytes were eluted at 0.5 mL/min using an aqueous solution of 0.02% HCOOH (solvent A) and CH_3CN/MeOH 4/1 (v/v) containing HCOOH 0.02% (solvent B). The LC gradient was from 0% B to 95% B in 7 min.

HPLC Analyses. HPLC analyses were performed on an Agilent 1100 LC–MS system equipped with a B1312A binary pump and a G1314A UV detector.

HPLC Columns. HPLC separations were performed on a Phenomenex Aqua 5 μm C_{18} 125A 150 mm × 2.0 mm i.d. column (cat. P/N 00F-4299-B0). The analytes were eluted at 0.5 mL/min using an aqueous solution of 0.1% HCOOH (solvent A) and 0.1% HCOOH in CH_3CN (solvent B), 98/2 isocratic elution. Preparative HPLC was performed on a SP 250/10 Nucleosil 100–7 C_{18} column (Macherey-Nagel art. 715002), with isocratic elution with water/acetonitrile 9/1 (v/v) containing 0.2% of formic acid at a flow rate of 6 mL/min.

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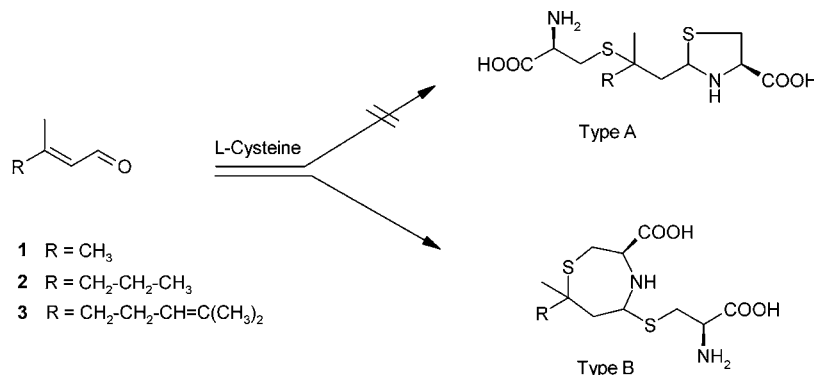*J. Agric. Food Chem.,* Vol. 53, No. 23, 2005   **9245**

Figure 1. Two types of cysteine conjugates from β,β-disubstituted acroleins.

Table 1. NMR Data of **4** (Diastereoisomers A and B)

position[a] C/H	chemical shift (multiplicity) δ ^{1}H, ppm	δ ^{13}C, ppm	coupling constant J(HH), Hz	correlation pattern HMBC	COSY
2A	3.58 (dd)	35.5 (t)	12.2, 7.7	C-3A, 15A	H-2A′, 3A
2A′	3.43 (dd)		12.2, 5.8	C-3A, 15A	H-2A, 3A
2B	3.51 (dd)	35.2 (t)	11.8, 7.7	C-3B, 15B	H-2B′, 3B
2B′	3.42 (dd)		11.8, 7.0	C-3B, 15B	H-2B, 3B
3A	4.68 (dd)	64.8 (d)	7.7, 5.8	C-2A, 5A, 15A	H-2A, 2A′
3B	4.55 (dd)	65.6 (d)	7.7, 7.0	C-2B, 5B, 15B	H-2B, 2B′
5A	4.968 (dd)	64.6 (d)[b]	9.3, 3.2	C-7	H-6A, 6A′
5B	4.965 (dd)	64.7 (d)[b]	8.6, 3.8	C-7	H-6B, 6B′
6A	2.47 (dd)	46.7 (t)	14.8, 3.2	C-5A, 7, 8A, 9A	H-5A, 6A′
6A′	2.27 (dd)		14.8, 9.3	C-5A, 7, 8A, 9A	H-5A, 6A
6B	2.51 (dd)	46.7 (t)	15.1, 3.8	C-5B, 7, 8B, 9B	H-5B, 6B′
6B′	2.30 (dd)		15.1, 8.6	C-5B, 7, 8B, 9B	H-5B, 6B
7 (A + B)		47.4/47.5 (s)			
8A	1.39 (s)	30.5 (q)		C-7, 9A	
8B	1.41 (s)	30.7 (q)		C-7, 9B	
9A	1.41 (s)	31.1 (q)		C-7, 8A	
9B	1.42 (s)	31.2 (q)		C-7, 8B	
15A		173.8 (s)			
15B		173.5 (s)			
1′ (A + B)		173.8/173.9 (s)			
2′ (A + B)	4.24 (m)	56.0 (d)			H-3′
3′ (A + B)	3.17 (m)	31.0 (t)			H-2′

[a] Numbering according to **Figure 2**. [b] Signals may be interchanged.

Syntheses. *(3R)-5-(S-L-Cysteinyl)-7,7-dimethylhexahydro-1,4-thiazepine-3-carboxylic Acid (4).* L-Cysteine (6.05 g, 50 mmol) was dissolved in water (60 mL) and THF (25 mL). 4-Methyl-3-butenal **1** (1.9 g, 23 mmol) was added dropwise within 15 min at 10 °C. After 2 h at room temperature, the white solid was filtered, washed with THF and water, and dried under vacuum to give **4** (5.5 g, yield 79%). The NMR solution was prepared by dissolving **4** (20 mg) in D$_2$O (0.8 mL) and DCl (0.02 mL). NMR data are given in **Table 1**.

UPLC−MS (APCI+): peak at 0.62 min with [M + H]$^+$ at *m/z* 308.9.

(3R)-5-(S-L-Cysteinyl)-7-methyl-7-propylhexahydro-1,4-thiazepine-3-carboxylic Acid (5). L-Cysteine (1.21 g, 10 mmol) was dissolved in aqueous KH$_2$PO$_4$ buffer (20 mL, 0.02 M, pH 7). 3-Methyl-2-hexenal **2** (E/Z mixture 7:3, 0.56 g, 5 mmol) was added dropwise during 10 min at 10 °C. After 2 h at room temperature, the white solid was filtered, washed with water, and dried under vacuum to give **5** (530 mg, yield 31%). The NMR solution was prepared by dissolving **5** (20 mg) in D$_2$O (0.8 mL) and DCl (0.02 mL). NMR data are given in **Table 2**. UPLC−MS (APCI+): peaks at 2.12 and 2.42 min, both with [M + H]$^+$ at *m/z* 336.8.

(3R)-5-(S-L-Cysteinyl)-7-methyl-7-(4-methyl-3-pentenyl)hexahydro-1,4-thiazepine-3-carboxylic Acid (6). L-Cysteine (3 g, 24.8 mmol) was dissolved in water (100 mL), pH 5.1, and a solution of geranial **3** (1.98 g, 13 mmol) in ethanol (50 mL) was added dropwise at 25 °C. A precipitate formed and the mixture was stirred for 2 h. The solid was filtered, washed with ethanol, and dried under high vacuum to give crude **6** (2.55 g, yield 72%). The NMR solution was prepared by

dissolving **6** (20 mg) in D$_2$O (0.8 mL) and DCl (0.02 mL). NMR data are given in **Table 3**. UPLC−MS (APCI+): two peaks at 2.75 and 3.20 min with [M + H]$^+$ at *m/z* 376.9 assigned to two diastereoisomers.

(4R)-2-[2-[S-(BOC-L-cysteinyl)]-2,6-dimethyl-5-heptenyl]-1,3-thiazolidine-4-carboxylic Acid (7). BOC-L-cysteine (2.21 g, 10 mmol) in acetonitrile (5 mL), cesium carbonate (1.63 g, 5 mmol), and geranial **3** (1.52 g, 10 mmol) were stirred for 24 h at room temperature. Excess geranial was removed under vacuum (0.1 mbar, 30 °C). The crude mixture was diluted in water (30 mL), L-cysteine (1.21 g, 10 mmol) was added (pH 7.3), and the mixture stirred for 2 h at room temperature. The solution was washed twice with diethyl ether. The aqueous phase was freeze-dried, rediluted in water, and subjected to chromatography on SiO$_2$ RP18 (40 × 63 μm, column diameter 4.5 cm, height of the solid phase 13 cm, elution with a gradient of water/ethanol by portions of 200 mL from 100% water to 50% water in gradient steps of 10%). The fraction that eluted with water/ethanol 4/1 contained compound **7** (1.59 g, yield 34%). The NMR solution was prepared with D$_2$O (**7** is not soluble in aqueous acid). NMR data are given in **Table 4**. MS (ESI−): [M − H]$^-$ at *m/z* 475. UPLC: 4.01 min.

(4R)-7-(1-Hydroxy-1-methylethyl)-1-methyl-2-thia-5-azabicyclo[4.3.1]-decane-4-carboxylic Acid (8). Compound **6** (5 g, 13 mmol) was dissolved at 25 °C in 0.2 M aqueous HCl (250 mL) using an ultrasonic bath. The clear solution was stirred for 36 h at room temperature and the new compound **8** isolated by preparative HPLC (retention time 12.4 min). The NMR solution was prepared by dissolving **8** (14 mg) in D$_2$O (0.8 mL) and DCl (0.02 mL). NMR data are given in **Table 5**. MS (APCI+): [M + H]$^+$ at *m/z* 274.

RESULTS AND DISCUSSION

Reaction of β,β-Disubstituted α,β-Unsaturated Aldehydes with L-Cysteine. The first reaction of a β,β-disubstituted acroleine with L-cysteine was carried out with citral (**3**) (mixture of E/Z isomers 1:1) in order to evaluate the expected conjugate (**Figure 1**, type A) for its performance as a slow-release system for citral. It is known that such 1:2 adducts of conjugated unsaturated aldehydes and cysteine, due to their reversible formation, are quite unstable in aqueous solution and tend to release the starting aldehyde (*2*). As citral is an important ingredient in both flavors and fragrances, but suffers from low stability due to its sensitivity to oxidative and acid-catalyzed decomposition reactions (*4*, *5*), we hoped to find a way to "stabilize" citral in the form of its 1,3-thiazolidine conjugate with cysteine.

The idea of using cysteine conjugates to "stabilize" sensitive carbonyl compounds in flavor applications was patented more than 40 years ago (*6*), although citral was not among the examples given, and the chemical structure and the 1:2 stoichiometry of the conjugates were not known at that time.

The white precipitate from the reaction of citral with L-cysteine was analyzed by LC−MS in positive ion mode, and the abundant [M + H]$^+$ ion at *m/z* 377 confirmed the expected

Table 2. NMR Data of **5** (Diastereoisomers A and B)

position[a] C/H	chemical shift (multiplicity) δ 1H, ppm	δ 13C, ppm	coupling constant J(HH), Hz	correlation pattern HMBC	COSY	NOESY[b]
2A	3.62 (dd)	35.3 (t)	12.3, 7.7	C-3A, 15A	H-2A′, 3A	H-2A′, 3A
2A′	3.49 (dd)		12.3, 5.6	C-3A, 15A	H-2A, 3A	H-2A, 3A (5A)
2B	3.57 (dd)	34.6 (t)	12.0, 8.0	C-3B, 15B	H-2B′, 3B	H-2B′, 3B (5B)
2B′	3.45 (dd)		12.0, 8.0	C-3B, 15B	H-2B, 3B	H-2B (3B)
3A	4.84 (dd)	63.8 (d)	7.7, 5.6	C-2A, 5A, 15A	2A, 2A′	H-2A, 2A′
3B	4.70 (dd)	64.5 (d)	8.0, 8.0	C-2B, 5B, 15B	2B, 2B′	H-2B, 5B (2B′)
5A	4.98 (dd)	64.7 (d)	9.4, 2.9	C-7	6A, 6A′	H-6A, 8A, 9A (2A′, 6A′)
5B	5.00 (dd)	64.3 (d)	9.4, 3.2	C-7	6B, 6B′	H-3B, 6B, 8B, 9B (2B, 6B′)
6A	2.47 (dd)	45.3 (t)	14.8, 2.9	C-5A, 7	5A, 6A′	H-5A, 6A′ (8A, 9A)
6A′	2.30 (dd)		14.8, 9.4	C-5A, 7	5A, 6A	H-6A (5A, 8A, 9A)
6B	2.52 (dd)	45.0 (t)	15.1, 3.2	C-5B, 7	5B, 6B′	H-5B, 6B′ (8B, 9B)
6B′	2.29 (dd)		15.1, 9.4	C-5B, 7	5B, 6B	H-6B (5B, 8B, 9B)
7 (A + B)		51.1 (s)				
8A	1.34 (s)	27.0 (q)		C-6A, 7, 9A		H-5A, 9A (6A, 6A′, 10A, 2′, 3′)
8B	1.37 (s)	28.0 (q)		C-6B, 7, 9B		H-5B, 9B (6B, 6B′, 10B, 2′, 3′)
9A	1.60 (t)	45.0 (t)	8.0	C-7, 8A,	H-10	H-5A, 8A, 10, 11 (6A, 6A′, 3′)
9B	1.58 (t)	44.4 (t)	8.0	C-7, 8B	H-10	H-5B, 8B, 10, 11 (6B, 6B′, 3′)
10 (A + B)	1.41 (m)	19.8 (t)		C-11	H-9, 11	H-9, 11
11 (A + B)	0.91 (t)	16.4 (q)	7.0	C-9A, 9B, 10	H-10	H-9, 10
15A		172.7 (s)				
15B		172.4 (s)				
1′ (A + B)		173.1 (s)				
2′ (A + B)	4.36 (m)	55.5 (d)		C-1′, 3′	H-3′	H-3′
3′ (A + B)	3.16 (m)	30.3 (t)		C-1′, 2′	H-2′	H-2′

[a] Numbering according to **Figure 2**. [b] NOESY signals in parentheses are weak.

Table 3. NMR Data of **6** (Stereoisomers A and B)

position[a] C/H	chemical shift (multiplicity) δ 1H, ppm	δ 13C, ppm	coupling constant J(HH), Hz	correlation pattern HMBC	COSY
2A	3.63 (dd)	35.3 (t)	12.2, 7.7	C-3A, 15A	H-2A′, 3A
2A′	3.50 (dd)		12.2, 5.5	C-3A, 15A	H-2A, 3A
2B	3.57 (dd)	34.4 (t)	11.9, 8.0	C-3B, 15B	H-2B′, 3B
2B′	3.46 (dd)		11.9, 8.0	C-3B, 15B	H-2B, 3B
3A	4.87 (dd)	63.6 (d)	7.7, 5.5	C-2A, 5A, 15A	H-2A, 2A′
3B	4.73 (dd)	64.3 (d)	8.0, 8.0	C-2B, 5B, 15B	H-2B, 2B′
5A	4.99 (dd)	64.6 (d)	9.7, 2.7	C-6A, 7, 3A	H-6A, 6A′
5B	5.01 (dd)	64.1 (d)	9.3, 3.3	C-6B, 7, 3B	H-6B, 6B′
6A	2.48 (dd)	45.2 (d)	15.0, 2.7	C-5A, 7, 8A, 9A	H-5A, 6A′
6A′	2.33 (dd)		15.0, 9.7	C-5A, 7, 8A, 9A	H-5A, 6A
6B	2.54 (dd)	45.0 (d)	15.1, 3.3	C-5B, 7, 8B, 9B	H-5B, 6B′
6B′	2.32 (dd)		15.1, 9.3	C-5B, 7, 8B, 9B	H-5B, 6B
7 (A + B)		51.0 (s)			
8A	1.37 (s)	26.9 (q)		C-6A, 7, 9A	
8B	1.40 (s)	27.8 (q)		C-6B, 7, 9B	
9A	1.60–1.66 (μ)	42.5 (t)		C-6A, 7,10	H-10
9B	1.60–1.66 (μ)	42.1 (t)		C-6B, 7,10	H-10
10 (A + B)	2.06–2.19 (μ)	25.3 (t)		C-9, 11, 12	H-11, 13
11 (A + B)	5.19 (m)	126.2 (d)		C-10, 13, 14	H-10, 13, 14
12 (A + B)		137.1 (s)			
13 (A + B)	1.64 (s)	19.9 (q)		C-11, 12, 14	H-10,11
14 (A + B)	1.69 (s)	27.8 (q)		C-11, 12, 13	H-11
15A		172.6 (s)			
15B		172.2 (s)			
1′ (A + B)		173.0 (s)			
2′ (A + B)	4.39 (m)	55.4 (d)		C-1′, 3′	H-3′
3′ (A + B)	3.09–3.24 (m)	30.3 (t)		C-1′, 2′	H-2′

[a] Numbering according to **Figure 2**.

Table 4. NMR Data of **7** (Mixture of Diastereoisomers)

position[a] C/H	chemical shift (multiplicity) δ 1H, ppm	δ 13C, ppm	coupling constant J(HH), Hz	correlation pattern HMBC	COSY
2	4.54 (m)	68.5 (d)		C-6, 7	H-6
4	3.56 (m)	69.3 (d)		C-5, 15	H-5
5	2.85 (m), 3.34 (m)	41.1 (t)		C-4, 15	H-4
6	2.16 (m), 2.33 (m)	47.9 (t)		C-2, 7, 8, 9	H-2
7		50.5 (s)			
8	1.33 (s)	27.8 (q)		C-6, 7, 9	
9	1.60 (m)	43.0 (t)		C-7, 8, 10	H-10
10	2.16 (m)	25.5 (t)		C-11,12	H-9, 11
11	5.23 (m)	126.8 (d)		C-10, 13, 14	H-10, 13, 14
12		136.4 (s)			
13	1.66 (s)	19.9 (q)		C-11, 12, 14	H-11
14	1.71 (s)	27.8 (q)		C-11, 12, 13	H-11
15		180.5 (s)			
1′		180.2 (s)			
2′	4.09 (m)	58.2 (d)			H-3′
3′	2.87 (m), 2.99 (m)	33.3 (t)		C-1′, 2′, 7	H-2′
(CH3)3C	1.45 (s)	30.6 (q)		(CH3)3C	
(CH3)3C		83.9 (s)			
NH-CO-		160.0 (s)			

[a] Numbering according to **Figure 2**.

64.6 ppm was slightly shifted upfield with respect to our published values (66.4−68.6 ppm) for C-2 of 1,3-thiazolidine cysteine conjugates (*1*). In fact, these measured chemical shifts were in better agreement with C-3 of several hexahydro-1,4-thiazepines, where we had measured values between 63.1 and 64.5 ppm (*1*) under similar conditions. Because this single difference was too small to allow for a safe distinction between conjugates of types A and B, we synthesized the BOC-derivative **7** (**Figure 2**) by Michael-type monoaddition of BOC-L-cysteine to citral followed by the conversion of the aldehyde group into a 1,3-thiazolidine by the addition of L-cysteine in a second step.

This allowed the NMR data of structurally closely related compounds **6** (**Table 3**) and **7** (**Table 4**) to be compared directly. Indeed, the 1,3-thiazolidine-4-carboxylic acid derivative **7** gave

empirical formula $C_{16}H_{28}N_2O_4S_2$ for the double addition conjugate. APCI-MS in negative ion mode gave an abundant $[M − H]^−$ ion at *m/z* 375, thus further confirming our result.

According to known precedent (*1−3*), we expected the 1,3-thiazolidine structure (**Figure 1**, type A) for this compound.

The 1H and 13C NMR data (**Table 3**) were also in support of the anticipated structure, except for one signal that did not fit well: the pair of 13C doublets (for two diastereoisomers) at 64.1/

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Table 5. NMR Data of **8**

position[a] C/H	chemical shift (multiplicity)		coupling constant	correlation pattern	
	δ ^{1}H, ppm	δ ^{13}C, ppm	J(HH), Hz	HMBC	COSY
2	2.98 (dd)	30.7 (t)	14.0, 7.2	C-3, 7, 15	H-2′, 3
2′	3.08 (dd)		14.0, 4.0	C-3, 7, 15	H-2, 3
3	3.92 (dd)	57.2 (d)	7.2, 4.0	C-2, 15	H-2, 2′
5	4.15 (ddd)	72.0 (d)	10.0, 10.0, 4.0		H-6ax, 6eq, 11
6ax	1.50 (m, overlap)	48.1 (t)			
6eq	2.03 (ddd)		14.0, 4.0, 2.7		H-5, 6ax, 9eq
7		51.1 (s)			
8	1.39 (s)	33.0 (q)		C-6, 7, 9	
9ax	1.49 (m, overlap)	39.5 (t)			
9eq	1.79 (m)				H-6eq, 9ax, 10 eq, 10 ax
10ax	1.47 (m, overlap)	25.8 (t)			
10eq	1.68 (m)				H-9ax, 9eq, 10ax, 11
11	1.48 (m, overlap)	55.3 (d)			
12		78.2 (s)			
13	1.23 (s)	30.7 (q)		C-11, 12, 14	
14	1.29 (s)	26.2 (q)		C-11, 12, 13	
15		175.5 (s)			

a Numbering according to **Figure 5**.
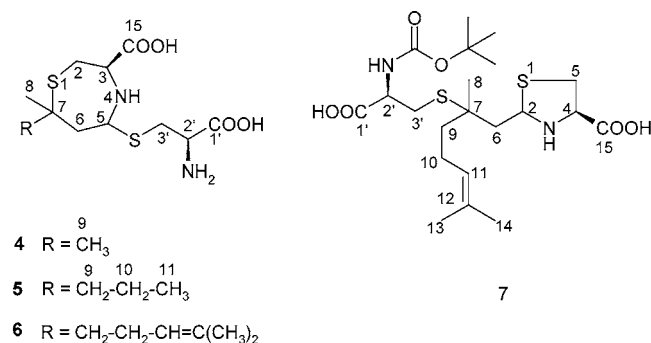
Figure 2. Structures and their numbering for NMR data (**Tables 1–5**).

rise to pairs of doublets (due to diastereoisomers) at 68.52/68.47 (C-2) and 69.33/69.28 ppm (C-4). The corresponding protons for **7** resonated at 4.54 (H-2) and 3.56 ppm (H-4).

In contrast, the corresponding signals for the seven-membered ring compound **6** were found at 64.6/64.1 (C-5) and 63.6/64.3 ppm (C-3) and at 4.99/5.01 (H-5) and 4.87/4.73 ppm (H-3). With these significant and characteristic differences for type A and type B conjugates established, we feel confident of our structural assignments. That Michael-type monoadducts between cysteine and α,β-unsaturated aldehydes under certain conditions lead to seven-membered heterocycles has been shown previously (*1*). A possible explanation for the exclusive formation of type B conjugates with β,β-disubstituted acroleins might be that the Michael-type addition of cysteine to the fully substituted β-carbon is very slow and that it is in fact the amino group of cysteine reacting with the aldehyde to give an imine, which then would undergo ring closure to form the type B conjugates (**Figure 3**). Such a "7-endo-trig" cyclization would be favored by the Baldwin rules (*7*) over the "5-endo-trig" cyclization involving the imine carbon, although Baldwin has shown (*8*) that second-row elements such as sulfur have less geometrical constraint for an endocyclic ring closure.

Replacing citral by geranial (the pure (*E*) isomer of **3**), as expected, did not change the diastereoisomeric ratio of the cysteine 1:2 adducts, as was evident from the identical NMR spectra of both samples. Unfortunately, we were unable to remove the BOC protecting group of **7** to see if the deprotected 1,3-thiazolidine derivative would spontaneously isomerize into the 1,4-thiazepine derivative **6**: exposure of **7** to typical acidic

conditions (trifluoroacetic acid) for the deprotection step resulted in intractable decomposition of **7**.

To check if β,β-disubstituted acroleins other than citral also give cysteine conjugates of type B, we submitted 4-methyl-3-butenal **1** and 3-methyl-2-hexenal **2** (*E/Z*-mixture 7:3) to the same reaction with L-cysteine. In both cases, the conjugates precipitated from the reaction mixture, and by inspection of their NMR spectra, it was immediately evident that analogous 1,4-thiazepines had been obtained: **4** (from **1**, **Table 3**) and **5** (from **2**, **Table 4**). In fact, all chemical shifts and coupling constants of the ring atoms were close to the corresponding signals of the citral conjugate **6**.

Whereas **5** and **6**, due to the two newly formed asymmetric C-atoms (C-5 and C-7), give rise to four diastereoisomers (assuming that the (*R*)-chirality of the two cysteine moieties is preserved during the reaction), compound **4** has only one additional stereogenic center (C-5) and, therefore, can give only two diastereoisomers (ratio ca. 1:1), thus simplifying the interpretation of the NMR spectra. Clearly, the slight but characteristic differences observed especially in the ^{1}H NMR spectra (**Table 1**) of the two diastereoisomers of **4** (**4A** and **4B**) (**Figure 4**) show that these differences are due to the relative configuration (trans or cis) of the C-3 carboxy group and the C-5 S-cysteinyl moiety. The assignment of the trans configuration to **4A** is based on the close analogy (chemical shifts and coupling constants) of its ^{1}H signals with those of **5A**. For compound **5**, NOESY experiments (**Table 2**) show a strong NOE between H-3 and H-5 for **5B**, which is absent in **5A**, thus establishing the cis configuration for **5B**. It is noteworthy that the four methyl groups of the mixture **4A** and **4B** have very similar ^{1}H and ^{13}C chemical shifts, which makes their precise assignment difficult.

The NOESY experiment of **5** (for both isomers A and B) revealed strong interactions between H-5 and both H-8 and H-9 (**Table 2**), indicating that both isomers, **5A** and **5B**, are themselves mixtures of diastereoisomers due to C-7. By analogy, the same must also be true for **6A** and **6B**. However, the ^{1}H and ^{13}C NMR spectra of **5** and **6** do not indicate such complex mixtures: a few signals are just slightly broadened, so small are apparently the spectral differences for the C-7 epimers. This observation is in line with the above-mentioned fact that the methyl groups of **4A** and **4B** (where C-7 epimers do not exist) are barely different in both their ^{1}H and ^{13}C NMR spectra.
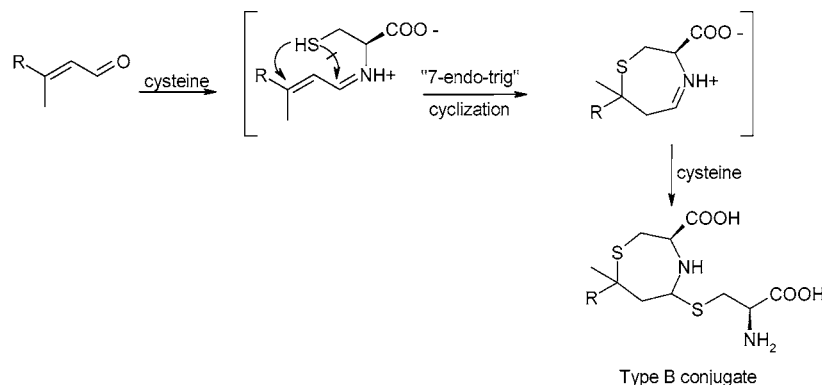
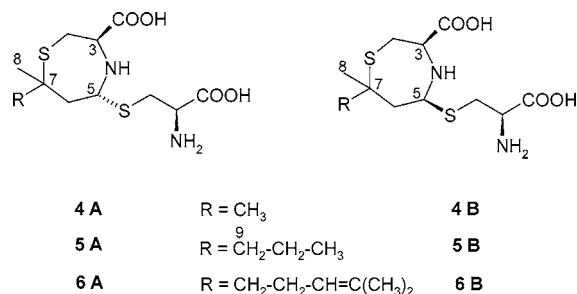**Figure 3.** Hypothetical pathway for the formation of type B conjugates.

4 A	R = CH_3	**4 B**
5 A	R = CH_2-CH_2-CH_3	**5 B**
6 A	R = CH_2-CH_2-CH=C(CH_3)$_2$	**6 B**

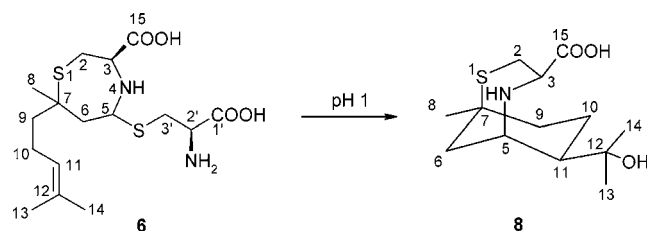**Figure 4.** Diastereoisomeric forms A and B of cysteine conjugates **4**–**6**.

Figure 5. Acid-catalyzed transformation of the citral conjugate **6** into the bicyclic alcohol **8**. (Numbering of the figure is used in the NMR tables.)

Cysteine Conjugate 6 as a Slow-Release System for Citral. With the structure of **6** established, we investigated the behavior of this compound as a precursor for the slow release of citral **3**. In neutral and slightly basic solution (concentration 0.2% of **6** in acetonitrile/water 1:1, pH 8.0, room temperature), the compound gradually released citral (yield ca. 14% after 1 day by HPLC), but sulfury notes were also perceptible. Also, the performance (long-lasting effect) as a slow release system in perfumery applications did not show a significant advantage over using free citral. Although a slight slow release effect was observed, the idea of using **6** in fabric softener applications was abandoned.

When **6** was dissolved in aqueous acid (DCl, D_2O) for NMR measurements, after 1 day at room temperature, we observed the formation of a new product from the change of the spectra. Judging from the MS (APCI+), the $[M + H]^+$ peak at m/z 274 indicated that one moiety of cysteine had been lost from **6** and the elements of water added. The C=C double bond was not present anymore. Preparation on a larger scale and purification of this new compound allowed its structure to be determined as the bicyclic alcohol **8** (**Figure 5**).

The structure of **8** (except for the *relative* configuration of C-3) follows unambiguously from the NMR data (**Table 5**),

which seem to indicate the presence of a single diastereoisomer (only one set of signals). The equatorial position of C-12 follows from the large coupling constant J^3(H-5,H-11) of 10 Hz. Assuming that the (*R*)-configuration at C-3 has been preserved during the reaction, there are two possible stereoisomers for **8**: 3*R*,5*S*,7*S*,11*R* (drawn) and 3*R*,5*R*,7*R*,11*S*, which may not be distinguished by our NMR data.

LITERATURE CITED

(1) 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.

(2) Esterbauer, H.; Ertl, A.; Scholz, N. The reaction of cysteine with α, β-unsaturated aldehydes. *Tetrahedron* **1976**, *32*, 285−289.

(3) 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.

(4) Ueno, T.; Masuda, H.; Ho, C.-T. Formation mechanism of *p*-methylacetophenone from citral via a *tert*-alkoxy radical intermediate. *J. Agric. Food Chem.* **2004**, *52*, 5677−5684.

(5) Freeburg, E. J.; Mistry, B. S.; Reineccius, G. A.; Scire, J. Stability of citral-containing and citral-less lemon oils in flavor emulsions and beverages. *Perfumer Flavorist* **1994**, *19*, 23−32.

(6) Schogt, J. C. M.; Haverkamp Begemann, P.; Jong, K.; Rademaker-Koster, J.; Wel, H.; Unilever. Verfahren zur Herstellung von Aromatisierungsmitteln mit gespeicherter Aromawirkung. German Pat. Application DE 1517130, 1964; *Chem. Abstr.* **1966**, *64*, 4860d−h.

(7) Baldwin, J. E. Rules for ring closure. *J. Chem. Soc., Chem. Commun.* **1976**, 734−736.

(8) Baldwin, J. E.; Cutting, J.; Dupont, W.; Kruse, L.; Silberman, L.; Thomas, R. C. 5-Endo-trigonal reactions: A disfavoured ring closure. *J. Chem. Soc. Chem. Commun.* **1976**, 736−738.