

Structural Determination and Odor Characterization of *N*-(2-Mercaptoethyl)-1,3-thiazolidine, a New Intense Popcorn-like-Smelling Odorant

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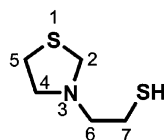
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The chemical structure of a novel, roasty, popcorn-like-smelling aroma compound formed from the reaction of fructose with cysteamine was studied by high-resolution mass spectrometry and nuclear magnetic resonance experiments. The structure of *N*-(2-mercaptoethyl)-1,3-thiazolidine exhibiting the extremely low odor threshold of 0.005 ng/L in air was finally confirmed by synthesis.

KEYWORDS: *N*-(2-Mercaptoethyl)-1,3-thiazolidine; odor threshold; cysteamine; fructose; Maillard reaction

INTRODUCTION

The most intense odorants in a thermally treated aqueous mixture of fructose and cysteamine have recently been evaluated (1), and it has been shown that *N*-(2-mercaptoethyl)-1,3-thiazolidine (1)



significantly contributes to the overall aroma of the Maillard model mixture. Compound 1 elicits an intense roasty, popcorn-like odor at the low odor threshold of 0.005 ng/L in air. Because the aroma compound to the best of our knowledge has not yet been reported among the reaction flavors or food aroma compounds, respectively, details on its identification are given in the present paper.

MATERIALS AND METHODS

All chemicals and reagents used in the syntheses were purchased from Aldrich (Steinheim, Germany).

Synthesis of *N*-(2-Mercaptoethyl)-1,3-thiazolidine (1). The synthetic route was based on methods described by Yasuhara and Shibamoto (2) and Barbaro et al. (3) for compounds with similar structural elements. In the first step, 1,3-thiazolidine was prepared in a two-step synthesis from cysteamine and formaldehyde (Figure 1), followed by the nucleophilic addition of the cyclic amine to the thioepoxide thiirane.

1,3-Thiazolidine. Cysteamine hydrochloride (25 mmol) and formaldehyde (35 mmol, 35% aqueous solution) were dissolved in an aqueous, freshly prepared, saturated sodium hydrogen carbonate solution (40 mL; pH 8.6). After 2 h of stirring at 20 °C under an argon atmosphere, the solution was extracted five times with dichloromethane

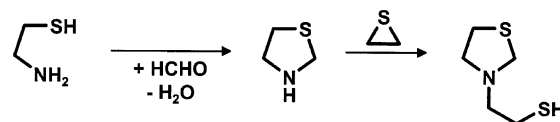


Figure 1. Synthetic route used in the preparation of *N*-(2-mercaptoethyl)-1,3-thiazolidine.

(150 mL total volume). The 1,3-thiazolidine formed (yield of the crude product = 95%) was freed from the solvent at 20 °C under a slight vacuum and was immediately used for the next step.

***N*-(2-Mercaptoethyl)-1,3-thiazolidine.** A mixture of 1,3-thiazolidine (~10 mmol; 890 mg) and thiirane (10 mmol; 600 mg; stabilized with 0.5% butylmercaptan to inhibit polymerization) was heated at 80 °C for 24 h without using a solvent. The mixture was then cooled to room temperature and, after addition of aqueous sodium hydroxide (50 mL; 1 mol/L), the unreacted 1,3-thiazolidine was removed by extraction with diethyl ether (3 × 100 mL). The aqueous phase was then adjusted to pH 8.0 by dropwise addition of concentrated HCl, and the target compound was extracted with diethyl ether (3 × 100 mL) in a yield of 1.20 g (80%).

¹H NMR signals in CDCl₃ (360 MHz; assignment of the carbon atoms refers to structure 1): δ 1.70 (t; 1H; SH), 2.54 (m; 2H; C6), 2.58 (m; 2H; C7), 2.85 (t; 2H; J = 6.2 Hz; C5), 3.03 (t; 2H; J = 6.2 Hz; C4), 4.03 (s; 2H; C2).

¹³C NMR signals in CDCl₃ (360 MHz; assignment of the carbon atoms refers to structure 1): δ 60.8 (C-2), 58.4 (C-4), 30.0 (C-5), 56.5 (C-6), 24.6 (C-7). ¹³C DEPT, performed as previously described (4), indicated five methylene groups.

Isolation of 1 from the Fructose/Cysteamine Mixture. Fructose (100 mmol) and cysteamine (33 mmol) were reacted in phosphate buffer (1 L; 0.1 mol/L; pH 7.0) as previously described (1). The mixture was extracted with diethyl ether, and after concentration of the extract to 1 mL, the target compound was isolated by preparative gas chromatography as follows: 50 μL of the extract was repeatedly injected onto a stainless steel column (3 m × 2.4 mm i.d.) packed with silicone SE-54 (10% w/w) on Chromosorb W AW DMCS (<100 mesh). Helium was used as the carrier gas (20 mL/min). The effluent in the retention index region from 1300 to 1400 (based on *n*-alkanes) was condensed into a self-constructed U-shaped glass trap (20 cm × 0.4 cm) via a

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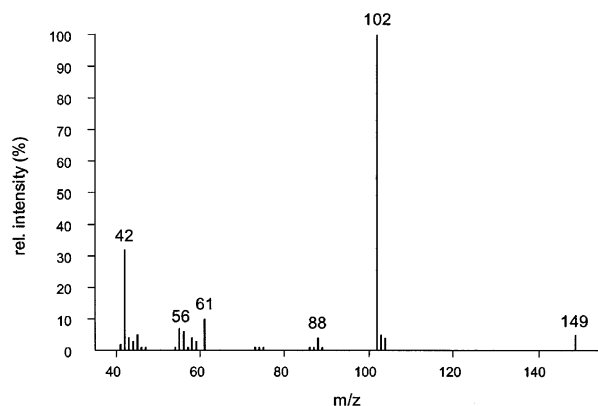


Figure 2. Mass spectrum (MS/EI) of compound 1.

Table 1. Key Ions and Sum Formulas Obtained by High-Resolution Mass Spectrometry of 1

ion (<i>m/z</i>)	molecular formula	possible fragment
149 ^a	C ₅ H ₁₁ NS ₂ ⁺	(M) ⁺
102	C ₄ H ₈ NS ⁺	(M ⁺ - CH ₂ S) ⁺
88	C ₃ H ₆ NS ⁺	(M ⁺ - C ₂ H ₅ S) ⁺
61	C ₂ H ₅ S ⁺	(M ⁺ - C ₃ H ₆ NS) ⁺
56	C ₃ H ₆ N ⁺	(<i>m/z</i> 102 - HCHS) ⁺
42	C ₂ H ₄ N ⁺	(<i>m/z</i> 88 - HCHS) ⁺

^a The theoretical mass was calculated as 149.0333, found 149.0337.

13:1 effluent splitter. During condensation the traps were maintained at -50 °C. After about four runs, the material was flushed with diethyl ether (0.5 mL) into a small vessel. To avoid condensation of water, the traps were connected to CaCl₂ tubes between the single GC runs. It should be stressed that this method of preparation leads to considerable losses of 1 due to its thermal degradation in the hot injector; a cold on-column injection would probably result in higher yields.

High-Resolution Gas Chromatography—Mass Spectrometry (HRGC-MS). The material isolated by preparative gas chromatography was chromatographed on a fused silica column (30 m × 0.32 mm DB-5; 0.5 μm; Fisons Instruments, Mainz, Germany). Mass spectral analysis was done as previously reported (5).

Nuclear Magnetic Resonance (NMR) Spectrometry. NMR spectra were recorded using an AM 360 spectrometer (Bruker, Karlsruhe, Germany) in CDCl₃ at 298 K and at 360 MHz in a Wilmad 535 PP tube. The program WIN NMR (version 4.0) was used for spectra calculation based on either TMS or CHCl₃ as the internal standard. ¹³C DEPT spectra were measured as reported previously (4).

RESULTS AND DISCUSSION

Characterization of 1. In Figure 2 is displayed the mass spectrum (MS/EI) of 1 isolated by preparative GC from ~40 model reactions. Because the molecular mass of *m/z* 149 could clearly be established by MS/CI (data not shown), the presence of one nitrogen atom was suggested by the uneven molecular mass. Furthermore, because the isotopic distribution in the molecular ion (data not shown) suggested the presence of two sulfur atoms, the very intense base fragment at *m/z* 102 hints at either a morpholine ring or a methyl-substituted thiazolidine ring, which was in good agreement with the isotopic pattern in this fragment.

Application of high-resolution mass spectrometry revealed a sum formula of C₅H₁₁NS₂ for the molecular ion (*m/z* 149; Table 1). From the ratio of carbon to hydrogen atoms one double bond or ring equivalent can be calculated. Because a ring structure was very probable due to the intense *m/z* 102 ion, no additional double bond was expected. Furthermore, the elimination of 47 from the molecular mass (*m/z* 149 - 47 = *m/z* 102) suggested

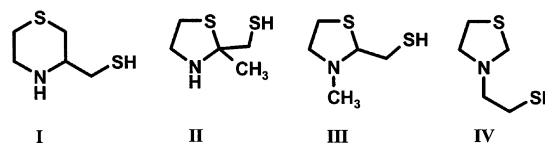


Figure 3. Possible structures suggested for 1 as derived from the mass spectral data.

Table 2. Odor Thresholds of Roasty, Popcorn-like Smelling Odorants Detected in Foods or Maillard Reaction Flavors, Respectively

odorant	odor threshold (ng/L in air)
<i>N</i> -(2-mercaptoethyl)-1,3-thiazolidine	0.005
2-acetyl-1-pyrroline	0.02 ^a
2-propionyl-1-pyrroline	0.02 ^a
2-acetyl-2-thiazolidine	0.06 ^a
5-acetyl-2,3-dihydro-1,4-thiazine	0.06 ^a
2-acetyl-2-thiazoline	0.05 ^a
2-propionyl-2-thiazoline	0.02
2-propionyl-2,3-dihydro-1,4-thiazine	0.1 ^a
2-acetylpyrazine	0.4 ^a
2-acetylthiazole	4 ^a

^a Data are taken from ref 8.

the loss of either a mercapto methylene or a thio methyl group. On the basis of these data, four structures were proposed for compound 1 (Figure 3). However, the elimination of a C₂H₅S radical from the molecular ion, yielding *m/z* 88 (Table 1), seems to be possible preferably from structure IV.

Following this suggestion and starting from formaldehyde and cysteamine, *N*-(2-mercaptoethyl)-1,3-thiazolidine was synthesized by a reaction of 1,3-thiazolidine with ethylene sulfide (thiirane) according to the scheme given in Figure 1. The mass spectra (MS/EI and MS/CI) obtained for the synthetic compound completely agreed with the spectra obtained for compound 1 isolated from the reaction of cysteamine with fructose.

The ¹H NMR measurement gave six signals, among which in particular the singlet of two H atoms at δ 4.03 and the two triplets of two H atoms at δ 2.85 and 3.03 with identical coupling constants confirmed the unsubstituted positions at carbon atoms 2, 4, and 5 in the thiazolidine ring. The results of the ¹³C NMR experiments, in particular, of the DEPT experiments indicating five methylene groups finally proved the structure of the *N*-substituted 1,3-thiazolidine.

Analytical and Sensory Attributes of 1. A determination of the retention indices of 1 on two stationary GC phases gave an RI of 1325 on DB-5 and an RI of 2035 on FFAP. The latter index is very close to that of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (RI = 2030), which is commonly formed in significant amounts in Maillard-type reactions and might be why 1 has not yet been reported in the literature. The retention indices of 1 were identical with the data obtained for *N*-(2-mercaptoethyl)-1,3-thiazolidine.

A determination of the odor threshold of *N*-(2-mercaptoethyl)-1,3-thiazolidine and of 1 in air using a GC-O method recently described (6) gave a value of 0.005 ng/L in air. Compared to several other compounds known in the literature to elicit roasty, popcorn-like aromas (Table 2), 1 proved to be by far the most potent aroma compound in this group. However, compared to the other compounds given in Table 2, 1 does not contain the same structural element of a cyclic amine with an adjacent α-oxo group, which is believed to cause roasty, popcorn-like aromas (7). Obviously, different receptors may exist in the human olfactory system causing similar odor qualities.

It should be noted that, due to the partially amphoteric character of **1**, with a protonated nitrogen and a sulfur anion, it can hardly be isolated from solvent extracts by treatment with either acids or bases and will, therefore, mostly be lost during isolation from foods. Furthermore, presumably due to the formation of an "inner salt", significant losses occur if chromatographic procedures are applied for enrichment and/or purification. We also observed a cleavage into 1,3-thiazolidine and thiirane at elevated temperatures in aqueous media. Therefore, application of **1** onto a GC column via a hot injector also may result in high losses.

In conclusion, this behavior might be another reason **1** has not been described previously in Maillard reaction systems containing cysteamine or cysteine.

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