

Hydrogen Sulfide Reactivity with Thiols in the Presence of Copper(II) in Hydroalcoholic Solutions or Cognac Brandies: Formation of Symmetrical and Unsymmetrical Dialkyl Trisulfides

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In the presence of copper(II), hydrogen sulfide reacts with thiols (methanethiol and ethanethiol) to yield symmetrical or unsymmetrical trisulfides (dimethyl trisulfide, diethyl trisulfide, and ethyl methyl trisulfide). In alcoholic beverages, these compounds are known for their nauseous character, reminiscent of onion smell, and for their low detection levels (dimethyl trisulfide: 0.1 $\mu\text{g/L}$). A mechanism for trisulfide formation is proposed that involves a two-step redox reaction.

Keywords: Symmetrical and unsymmetrical disulfides; symmetrical and unsymmetrical trisulfides; copper catalysis

INTRODUCTION

In many cases, yeast cells are responsible for the appearance and the biotransformation of sulfur compounds. The presence of yeast strains causes the formation of hydrogen sulfide (H_2S), depending on the fermentation temperature and the medium pH value (Rankine, 1968; Eschenbruch, 1973; Vos and Gray, 1979; Wenzel and Dittrich, 1983; Monk, 1986; De Mora et al., 1986). H_2S formation is, however, also observed during malolactic fermentation. This latter phenomenon remains unexplained (Monk, 1986). The large quantities of the H_2S generated in young wines after fermentation may lead to nauseous sulfur-linked smells. Sulfur-based phytoprotecting substances are also known as sources of volatile sulfur compounds and, in particular, hydrogen sulfide (Eschenbruch, 1974; Wenzel et al., 1980; Schütz and Kunkee, 1979; Maujean et al., 1993; Rauhut and Kürbel, 1994).

The nucleophilic character of the sulfur atom should cause a high reactivity of H_2S with the major products of alcoholic fermentation (ethanol, carbon dioxide), with carbonyl compounds (aldehyde and ketone), or with other constituents present in wines and brandies. Because of this reactivity, H_2S may be the precursor of nauseous volatile products with lower detection levels (Maujean et al., 1993; Rauhut et al., 1993).

Hitherto, among the dialkyl trisulfides, only dimethyl trisulfide (DMTS) has been detected in cognac, whisky, wine, and beer samples (Peppard, 1978; Leppänen et al., 1979). The quantities of DMTS found in cognac and whisky samples by Leppänen et al. (1979) were, respectively, 1 and 5.4 $\mu\text{g/L}$. The perception level was found by these authors to be extremely low ($\approx 0.1 \mu\text{g/L}$), with a noxious onion-like smell in synthetic media [hydroalcoholic solution containing 10% ethanol (v/v) at pH 3.2] and in beer samples. Moreover, Ronkainen (1973) observed trisulfide formation during the distillation of

alcoholic beverages. But, the origin and formation mechanism of trisulfide are still unknown. S-Methylcysteine sulfoxide is the DMTS precursor in plants such as cabbage, turnip, or broccoli, and different mechanisms of trisulfide formation have been discussed.

In *Brassica* and *Allium* plants, the action of C-S lyases on S-methylcysteine sulfoxide has been proposed as a mechanism of trisulfide formation by Chin and Lindsay (1994). According to Boelens et al. (1971), these trisulfides might arise from the reaction of disulfides with elemental sulfur. Murayama (1970), however, proposed that the second step of the mechanism results from the reaction of the unstable methanesulfenic acid with H_2S , which leads to DMTS. It has also been reported that broccoli florets stored in the absence of oxygen produced higher amounts of DMTS (Hansen et al., 1992). Nevertheless, S-methylcysteine sulfoxide is not found in cognac brandies or cognacs.

In this study, we present results concerning the formation of symmetrical and unsymmetrical dialkyl trisulfides in hydroalcoholic media via the reactivity of H_2S with thiols (methanethiol and ethanethiol) in the presence of copper (II).

EXPERIMENTAL PROCEDURES

Chromatograms were obtained by a static headspace method with a gas chromatograph (Shimadzu GC 17A) coupled to a chemiluminescence detector (SCD 355 from Sievers). The separation of sulfur products was carried out on a 30 m $\times$ 0.32 mm poly(dimethylsiloxane) (4- μm film thickness) capillary column (SPB1-sulfur from Supelco); Nedjma and Maujean, 1995). The operating conditions were as follows. Column temperature program, 1 min step at 35 $^\circ\text{C}$ followed by a 10 $^\circ\text{C}/\text{min}$ temperature gradient to reach 55 $^\circ\text{C}$ and a 25 $^\circ\text{C}/\text{min}$ temperature gradient to 250 $^\circ\text{C}$; pressure program, 1 min step at 82 kPa followed by a 30 kPa/min pressure gradient to reach 320 kPa; injector temperature, 115 $^\circ\text{C}$. The carrier flow rate was 3 mL/min at the beginning of the analysis. Hydrogen and air input in the burner were, respectively, set at 100 and 40 mL/min. The oxygen pressure generator was maintained at 48 kPa (7 psig).

Two methods were used for gas volume sampling. Hamilton Teflon luer-lock 5-mL gastight (1005 LTN) and solid-phase microextraction (SPME; from Supelco) with a fused silica fiber coated with a stationary phase [poly(dimethylsiloxane), 100 μm o.d.) to adsorb volatile and semivolatile sulfur compounds.

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The ^1H and ^{13}C NMR spectra were recorded in CDCl_3 with a Bruker AC 250 spectrometer.

Reagents and Chemicals. Normapur ethanol (analytical grade), L(+)-tartaric acid (TH2), and potassium hydrogenotartarate (THK) from Prolabo were used. Only distilled and deionized water (resistivity equal to $18.2 \text{ M}\Omega/\text{cm}$) were used. Ethanethiol and gaseous methanethiol were obtained from Aldrich. Sodium sulfide from Merck was dissolved in aqueous solution buffered at pH 3.2 (TH2/THK) to generate H_2S . The chloroform used for the preparation of the standards was distilled from calcium hydride (CaH_2). All of these compounds were at least 97% pure.

Preparation of Standards. *Solution A.* Hydroalcoholic solution contained 20% ethanol (v/v) was buffered at pH 3.2 (wine pH) by adding TH2 (3 g/L) and THK (3 g/L).

Trisulfides Formation. The solutions of H_2S , methanethiol, and ethanethiol were prepared according to the method of Nedjma et al. (1995).

A 250-mL flask was filled with 200 mL of the solution A containing copper chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $500 \mu\text{g/L}$). This solution was stored at 4°C overnight. Then, $500 \mu\text{L}$ of the solutions of $4 \times 10^{-2} \text{ g/L}$ of H_2S , methanethiol, or ethanethiol were added in different combinations to this medium. The flask containing the resulting mixture was then hermetically capped with silicone rubber septa (sleeve stoppers), and the mixture was allowed to warm to room temperature. The reaction was monitored by gas chromatography. The products of the reaction were identified by comparison with authentic standards that were synthesized according to Capozzi et al. (1989a,b).

Ethyl Methyl Disulfide (EMDS). A solution of 500 mg (3.25 mmol) of *S*-ethyl ethyl thiosulfonate and 902 mg of (methylthio)trimethylsilane (7.5 mmol) in 20 mL of chloroform was heated under reflux until there was total conversion of the initial compound (2 h). The solvent was eliminated by distillation. The residue was chromatographed by thin-layer chromatography (TLC; petroleum ether/AcOEt at 95/5) to provide 122 mg (35%) of EMDS: ^1H NMR (CDCl_3 , 250 MHz) δ 1.4 (3H, t, $J = 7 \text{ Hz}$), 2.4 (3H, s), 2.7 (2H, q, $J = 7 \text{ Hz}$); ^{13}C NMR 14.43 (CH_2), 22.1 (CH_3), 32.84 (CH_3); GC; $t_R = 6.28 \text{ min}$.

Dimethyl Trisulfide (DMTS). First, 1.36 g (7.64 mmol) of bis(trimethylsilyl) sulfide was added to a solution of 840 mg (7.64 mmol) of *S*-methyl methylthiosulfonate in 20 mL of chloroform. The reaction mixture was then heated and refluxed for 16 h. The solvent was removed by distillation (atmospheric pressure). The crude product was purified by TLC (petroleum ether/AcOEt at 95/5) to give 625 mg (65%) of pure DMTS: ^1H NMR (CDCl_3 , 250 MHz) δ 2.6 (6H, s); ^{13}C NMR δ 22.62 (2 CH_3); GC $t_R = 7.39 \text{ min}$.

Diethyl Trisulfide (DETS). The preceding procedure was also used for the preparation of DETS. First, 1 g (6.49 mmol) of *S*-ethyl ethylthiosulfonate and 1.18 g (6.49 mmol) of bis(trimethylsilyl)sulfide were converted. Flash chromatography with petroleum ether/AcOEt (95/5) gave 718 mg (72%) of pure DETS: ^1H NMR (CDCl_3 , 250 MHz) δ 1.35 (6H, t, $J = 7 \text{ Hz}$); 2.9 (4H, q, $J = 7 \text{ Hz}$), ^{13}C NMR δ 14.3 (2 CH_2), 32.6 (2 CH_3); GC $t_R = 8.62 \text{ min}$.

RESULTS AND DISCUSSION

In beer, the concentration of transition metals are generally in the range 0.01–1.6 mg/L (Binns et al., 1978; Hough et al., 1982). The presence of copper and H_2S leads to copper sulfide (CuS) precipitation (Thorne et al., 1970). Thus, the nauseous hydrosulfurous smells are diminished in beer. In the case of cognac brandies and cognacs, the copper concentration is also the most important one (Table 1). The concentration of these metals are even higher and exceed the concentration of sulfur products, in particular thiols (methanethiol and ethanethiol) that are in the $\mu\text{g/L}$ range. Our results obtained with hydroalcoholic solutions and cognac brandies show that after 24 h at room temperature, the reaction of H_2S in the presence of copper(II) with

Table 1. Concentration of Transition Metals in Cognac Brandy and Cognac

beverage	concentration (mg/L)			
	copper	iron	manganese	zinc
cognac brandy	0.5–3	< 0.1	0.03–0.06	0.01–0.22
cognac	0.3–5	0.01–0.65	0.01–0.45	0.01–1.00

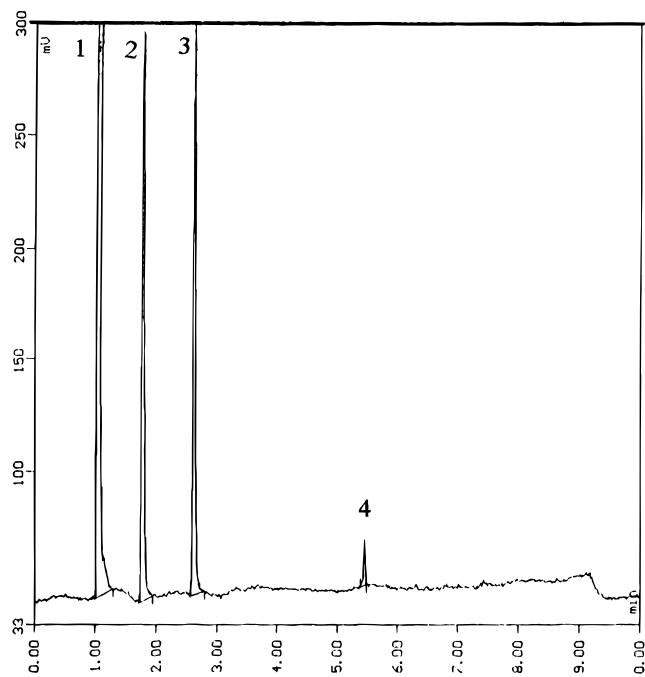
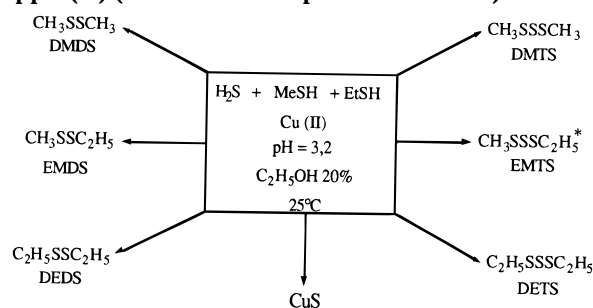


Figure 1. Chromatogram obtained after 24 h of reaction at room temperature in the absence of copper(II). (1) H_2S ; (2) MeSH ; (3) EtSH ; (4) DMDS.

Scheme 1. Sulfur Products Obtained from the Reaction of H_2S with Thiols in the Presence of Copper(II) (* Indicates Proposed Structure)



methanethiol leads to DMDS and DMTS. Reaction with ethanethiol under the same conditions leads to DEDS and DETS.

If methanethiol and ethanethiol were treated under these conditions, symmetrical and unsymmetrical disulfides and trisulfides were formed, whereas no reaction was observed when copper was absent (Figure 1). Among these products, we have identified two symmetrical disulfides (DMDS and DEDS), two symmetrical trisulfides (DMTS and DETS), and one unsymmetrical disulfide [ethyl methyl disulfide (EMDS)]. The chromatographic behavior of the remaining unidentified compound indicates it is ethyl methyl trisulfide (EMTS; Scheme 1).

Different modifications of the conditions were tested to increase the formation of trisulfides from H_2S , thiols, and copper. The optimal conditions were obtained with 5 equivalents of copper(II), 1 equivalent of thiols, and 5 equivalents of H_2S . Under these conditions and after

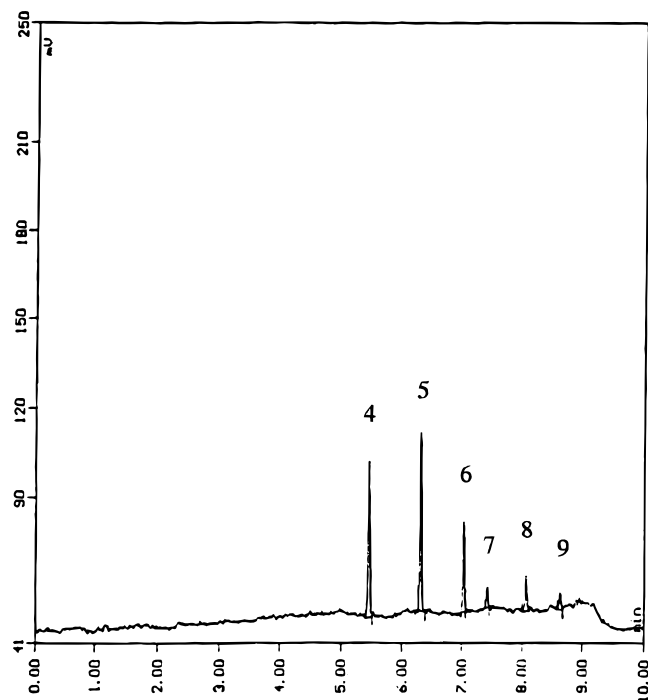


Figure 2. Gas chromatogram of disulfides and trisulfides obtained from the reaction of H₂S with thiols in the presence of copper chloride by the gas sample needle method. (4) DMDS; (5) EMDS; (6) DEDS; (7) DMTS; (8) EMTS; (9) DETS.

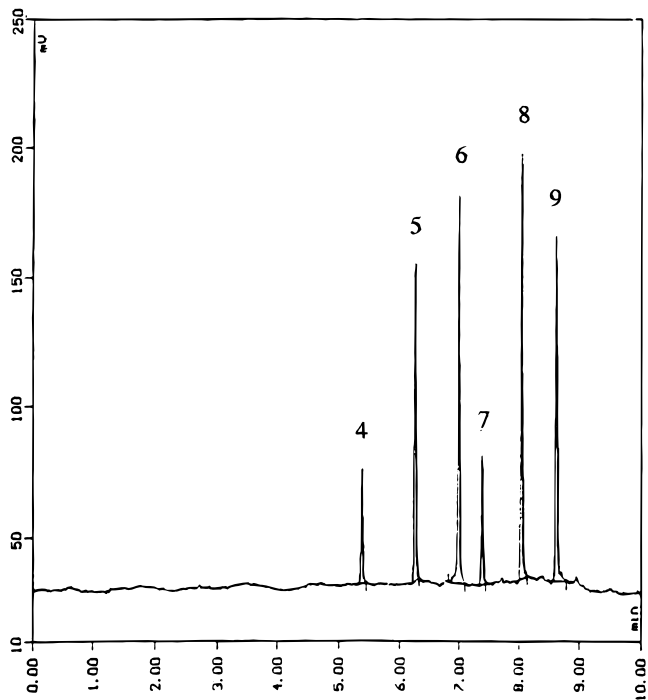


Figure 3. Gas chromatogram of disulfides and trisulfides obtained from the reaction of H₂S with thiols in the presence of copper chloride by the SPME method. (4) DMDS; (5) EMDS; (6) DEDS; (7) DMTS; (8) EMTS; (9) DETS.

24 h at room temperature, no H₂S and thiols were observed (Figure 2). These optimized conditions could be used in synthetic organic chemistry for the preparation of trisulfides.

Two chromatograms obtained by two different sampling techniques (gas needle and SPME) are displayed in Figures 2 and 3. With SPME, the responses of disulfides and trisulfides increased. However, this technique is not suitable for the follow-up of reactions

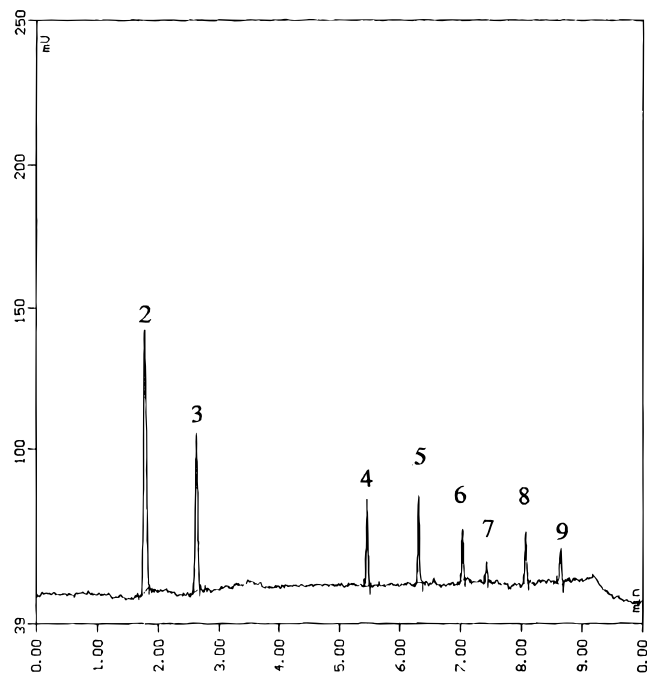
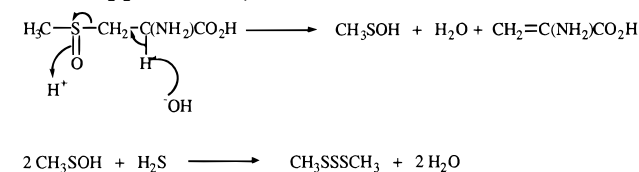


Figure 4. Gas chromatogram of disulfides and trisulfides obtained from the reaction of H₂S with thiols in the presence of copper sulfate by the gas sample method. (2) MeSH; (3) EtSH; (4) DMDS; (5) EMDS; (6) DEDS; (7) DMTS; (8) EMTS; (9) DETS.

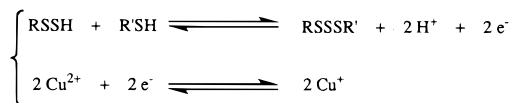
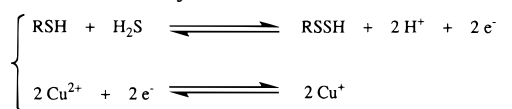
Scheme 2. Trisulfide Formation Step from S-Methylcysteine Sulfoxide β -Elimination (Murayama, 1970; Peppard, 1978)



because of the low adsorption of thiols and H₂S. In this reaction, copper appears to be an absolute requirement for formation of disulfides and trisulfides. We also showed that when H₂S is increased from 1 to 5 equivalents, with methanethiol and ethanethiol (at 1 equivalent each and in the presence of 5 equivalents of copper(II), trisulfide formation becomes the major reaction. The reaction yield is lower when copper chloride (CuCl₂·2H₂O) is replaced by copper sulfate (CuSO₄) at the same concentration (Figure 4). Indeed, an incomplete conversion was observed.

Murayama (1970) proposed a mechanism for DMTS formation in beer from S-methylcysteine sulfoxide β -elimination during the cooking of several brassicaceous vegetables leading to methanesulfenic acid, an unstable intermediate product. This intermediate reacts with H₂S giving DMTS (Scheme 2). It has also been observed that the presence of sulfur dioxide in the reaction medium diminishes the S-methylcysteine sulfoxide concentration (DMTS-precursor; Peppard, 1978). Murayama's mechanism cannot be envisaged in cognac brandies or cognacs because of the absence of S-methylcysteine sulfoxide. Moreover, we observed trisulfide formation in hydroalcoholic solutions without this compound. The mechanism presented in Scheme 3 appears to be the most reasonable one. This mechanism is based on the two coupled redox reactions, where copper is the oxidant. However, the hydroalkyl disulfide intermediate has not been found by chromatographic analysis, per-

Scheme 3. Proposed Mechanism for the Formation of Symmetrical or Unsymmetrical Trisulfides



R = Me ou Et

R' = Me ou Et

haps because of its low stationary concentration. A radical mechanism has to be considered. Further experimental study is needed to verify the validity of each step of the proposed mechanism.

CONCLUSION

The results reported here give new insight into the origin of symmetrical and unsymmetrical trisulfides and their mechanism of formation in cognac brandies. Indeed, for the first time, it has been shown that trisulfide formation was possible from the reaction of H_2S with thiols in the presence of copper(II). Concerning preparative organic chemistry, these observations may lead to a new method of synthesis of trisulfides. Further studies in this field are in progress in our laboratory.

ABBREVIATIONS USED

DMDS, dimethyl disulfide; EMDS, ethyl methyl disulfide; DEDS, diethyl disulfide; DMTS, dimethyl trisulfide; EMTS, ethyl methyl trisulfide; DETS, diethyl trisulfide; GC, gas chromatography; SCD, sulfur chemiluminescence detector.

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