

Hop Allylic Alcohols Are Precursors of Sulfur-Containing Odorants in Fresh Beer

J. Gros, S. Nizet and S. Collin
Unité de brasserie et des industries alimentaires
Université catholique de Louvain
Louvain-la-Neuve
Belgium

Keywords: onion, polyfunctional thiols, sulfur flavors, hop, allylic alcohols

Abstract

Hop is known to bring methanesulfenic acid to wort, the precursor of an onion-like odorant in beer: dimethyltrisulfide. Recently, 2-mercapto-3-methylbutanol was also suspected to be responsible of some onion off-flavors in beer. In model media, 2-mercapto-3-methylbutanol, 3-mercapto-3-methylbutanol and 3-methyl-2-buten-1-thiol were shown to be synthesized from 3-methyl-2-buten-1-ol and hydrogen sulfide (radical anti-Markovnikov addition, electrophilic Markovnikov addition and nucleophilic substitution, respectively). The present work shows that these mechanisms also occur in pilot-scale beer productions by the presence of hydrogen sulfide excreted by yeast. 1.8 µg/L of 2-mercapto-3-methylbutanol was detected after fermentation of a wort spiked with 10 mg/L of 3-methyl-2-buten-1-ol. A strong onion-off-flavor was also detected in the fresh beer. Sensorial analyses were performed on purified 2-mercapto-3-methylbutanol (radical addition favored by adding hydrogen peroxide and catalytic traces of FeS). Its typical freshly-cut onion flavor revealed very different from the “onion soup” descriptor currently used to describe dimethyltrisulfide.

INTRODUCTION

Isohumulone degradation is the main source of the well-known lightstruck off-flavor imparted by 3-methyl-2-buten-1-thiol (MBT). At higher concentration, this compound exhibits a repulsive “skunky” odor while at lower concentration, it may be appreciated by consumers (Gijs et al., 2002).

Hop is also known to bring methanesulfenic acid to wort, the precursor of the onion-like odorant dimethyltrisulfide (Callemien et al., 2006; Gijs et al., 2000; Lermusieau and Collin, 2003).

Recently, 2-mercapto-3-methylbutanol (2S3MBol) was shown to be another compound involved in beer onion off-flavors (Olsen et al., 1988; Vermeulen et al., 2005, 2006). 3-Methyl-2-buten-1-ol (3MBenol, well-known hop constituent) was suspected to be its precursor. In model media, hydrogen sulfide is able to induce 3 different kinds of chemical reactions involving this allylic alcohol: nucleophilic substitution, electrophilic addition (Markovnikov), and radical addition (anti-Markovnikov), leading to MBT, 3-mercapto-3-methylbutanol (3S3MBol) and 2S3MBol, respectively (Fig. 1). As yeast naturally excretes hydrogen sulfide, the same reaction could occur during brewing fermentations.

In the present work, 3MBenol was added to wort before fermentation. Specific thiol extraction with *p*HMB was used to isolate polyfunctional thiols in the resulting beer (Tominaga et al., 1998). 2S3MBol was also chemically synthesized and purified. Sensorial analysis was applied in order to distinguish its odor from the “onion soup” descriptor currently used to describe dimethyltrisulfide.

MATERIALS AND METHODS

Materials

Monohydrated *L*-cysteine hydrochloride, *p*-hydroxymercuribenzoic acid,

hydrogen chloride 37% and 3M Benol were provided by Aldrich Chemicals (Bornem, Belgium). Tris(hydroxymethyl)aminomethane and sodium acetate were supplied by USB (Cleveland, Ohio, USA) and UCB (Bruxelles, Belgium), respectively. Sodium hydroxide and anhydrous sodium sulfate 99% were supplied by Janssen (Geel, Belgium). Dichloromethane 99.9% from Romil (Cambridge, UK) was distilled before use. Dimethyltrisulfide was provided by Acros Organics (Geel, Belgium), absolute ethanol by VWR (Fontenay-sous-Bois, France) and hydrogen peroxide 30% (Perhydrol) by Merck (Darmstadt, Germany).

Extraction of Polyfunctional Thiols by pHMB

Beer (500 ml) and bidistilled dichloromethane (200 ml) were stirred for 30 minutes. After decantation (± 15 minutes), the lower phase and the interfacial emulsion were centrifuged for 20 minutes at 10000 rpm. The organic phase was then extracted by 2 \times 20 ml of a pHMB solution (360 mg of pHMB, 24.6 g of Tris in 1 L of Millipore water) for 5 and 10 minutes, respectively. The combined aqueous phases were loaded into a strongly basic anion exchanger column (Dowex 1WX2-100 resin from Aldrich Chemicals), washed before hand by NaOH 2 M, HCl 2 M, and in between rinsed by ultrapure water. Then 50 ml of sodium acetate buffer (0.1 M, pH 6) was poured on the resin to remove impurities. Next volatile thiols were released by percolating a purified cysteine solution (640 mg of hydrochloride *L*-cysteine monohydrated in 60 ml of Millipore water – this solution was washed with 2 \times 5 ml of distilled CH₂Cl₂ before use). The eluate containing the volatile thiols was collected and extracted by 4 and then 3 ml of distilled CH₂Cl₂ using magnetic stirring (5 minutes). The organic phases were pooled, dried on anhydrous Na₂SO₄, and finally concentrated under nitrogen to 70 μ l to be stored at -80°C before GC analysis. Note that 4-methoxy-4-methyl-2-butanethiol 99.5% (Oxford Chemicals) – added at the first extraction step – and thiazole 99% (Aldrich Chemicals) – added before the final concentration step – were used respectively as internal and external standards (IST and EST).

Gas Chromatography Hyphenated to a Sniffing Port (GC-O) or to a Flame Ionization Detector (GC-FID)

This was performed using a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250°C and opened after 0.5 min. Sulfur compounds were analyzed on a 50 m \times 0.32 mm i.d., wall-coated open tubular (WCOT) apolar CP-Sil 5 CB capillary column (film thickness: 1.2 μ m). The oven temperature, initially set and maintained 4 min at 36°C, was then programmed to rise from 36 to 85°C at 20°C/min and then from 85 to 141°C at 1°C/min and finally from 141°C to 250°C at 3°C/min. The final temperature was held for 30 min. The eluent was sent to a GC-sniffing port (250°C) and diluted with a large volume of humidified air (20 ml/min) to improve the transport of the effluent out of the funnel. Odor detection and description were performed by three judges working independently. Retention indices (RI) were estimated by connecting the column to a FID maintained at 250°C.

Gas Chromatography Hyphenated to a Mass Spectrometer (GC-MS)

Identification was confirmed by mass spectroscopy. Mass spectra ($m/z = 40$ to 380) were recorded at 70 eV on a ThermoFinnigan Trace MS mass spectrometer connected to a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector and a 50 m \times 0.32 mm i.d., wall-coated open tubular (WCOT) apolar CP-Sil 5 CB MS capillary column (film thickness: 1.2 μ m). The oven temperature program was the same as that described for GC-O. Spectral recording was automatic throughout elution; Xcalibur software was used.

Gas Chromatography Hyphenated to a Pulsed Flame Photometric Detector (GC-PFPD)

Quantification was obtained by using the PFPD detector. The injections were

carried out in the splitless mode at 250°C, the split being turned on after 0.5 minute. At the detector, the following parameters were selected : 250°C, 580 V, 18 ms for the gate width, 6 ms for the gate delay, 400 mV as trigger level and 3.45 Hz as pulse frequency. The oven temperature, initially set and maintained 4 min at 40°C, was then programmed to rise from 40 to 132°C at 2°C/min and then from 132 to 250°C at 3°C/min. The final temperature was held for 30 min.

Pilot-Scale Beer Productions

Experimental beers (P1 and Reference) were produced from lager beer industrial wort (kind gift from Inbev, Leuven, Belgium). Strain INBR 291 (INBR collection of the Université catholique de Louvain, Louvain-la-Neuve, Belgium) was pitched at 15×10^6 cells/ml in the diluted wort (12° Plato). For P1, 10 mg/L of 3MBenol was added before fermentation. Fermentation occurred in cylindrical tubes at 12°C for seven days, and the fermented liquor was left at 4°C for another week. Yeast was separated from beer by centrifugation. Beer was saturated in CO₂ for 12 hours and bottled in 250 ml flasks.

Chemical Synthesis of 2S3MBol

Water containing 3% hydrogen peroxide (instead of 5% ethanol as used by Vermeulen et al., 2006) was adjusted to pH 4.2 by a 0.2 M acetate buffer, saturated with a continuous flow of hydrogen sulfide and spiked with catalytic traces of FeS (Fig. 2). Then, 100 mg/L of 3MBenol were added. After 3 hours, 5 ml of the reaction mixture were filtered and extracted with dichloromethane (3×1 ml). An external standard was added (nonane 50 µl, final concentration = 5 mg/L) to the organic phase before concentration under nitrogen to 70 µl.

Chromatographic Purification of 2S3MBol

Purification was performed on the CP-SIL 5 CB column connected to a T-junction splitting the effluent between a cold trap and a throw gap (ratio 1:1). The cold trap consisted of an uncoated glass capillary tube (Fused Silica Guard Column 0.35 m \times 0,53 mm i.d.; Agilent USA), wrapped in a copper sleeve, as previously described by Gallois (1987). The first end was cooled with ice while the other one was maintained in liquid nitrogen. In order to discard 3S3MBol (RI = 944), 2S3MBol (RI = 958) was only trapped in between 37.0 and 38.15 min (electronic switch). The tube was further disconnected and 2S3MBol desorbed with 3×10 µl of absolute ethanol.

Sensorial Analysis

Thirteen panelists individually described dimethyltrisulfide and 2S3MBol put on perfumers strips.

RESULTS AND DISCUSSION

In model media, hydrogen sulfide is able to produce 2S3MBol from 3MBenol (Vermeulen et al., 2006). In order to assess if yeast could induce the same reaction, pilot-scale beer productions were conducted on worts spiked (P1) or not (Reference) with 10 mg/L of 3MBenol. Worts were fermented with a lager yeast. The thiols were selectively extracted from the resulting beers with pHMB, identified by GC-MS and GC-O, and quantified by GC-PFPD.

While only 0.2 µg/L of 2S3MBol was detected in the Reference, up to 1.8 µg/L characterized P1 (spiked wort), described by the panelists as strongly pungent (onion off-flavors). The sensorial descriptor was linked to the presence of 2S3MBol since no similar odor was detected in the spiked wort containing its precursor. 3MBenol exhibits pleasant almond/herb odors.

Very interestingly, the spiked-wort also lead to a beer which was much richer in 3S3MBol (up to 7.0 µg/L against 1.6 µg/L in the Reference). As in the model medium, the electrophilic addition (Markovnikov) was favored compared to the radical pathway (anti-Markovnikov).

In order to help the brewers to better distinguish onion off-flavors issued from either dimethyltrisulfide or 2S3MBol, sensorial analyses were applied on both chemicals. Unfortunately, the latter was not commercially available. The olfactometric purity required for such experiments was reached by chromatographic purification. The chemical reaction was first optimized in order to increase the relative proportion of radical/electrophilic additions. As expected, hydrogen peroxide revealed able to increase hydroxyl radicals in presence of traces of FeS, leading to lower amounts of 3S3MBol (Fig. 2). The resulting mixture was then purified by GC-cold trapping (from RI = 948 to RI = 962). After 26 chromatographic injections of 2 μ l, 2S3MBol was eluted from the cold trap by 150 μ l of ethanol and concentrated to 30 μ l. GC-O applied on the alcoholic extract confirmed its olfactometric purity (no odor at RI of 3S3MBol = 944).

Sensorial analysis was applied by thirteen assessors. All of them characterized it by a freshly-cut onion odor, very different from the “onion soup-cooked vegetable” descriptors currently used to describe dimethyltrisulfide.

ACKNOWLEDGEMENTS

The authors wish to thank the FNRS (Fonds National de la Recherche Scientifique) for financial support. J. Gros thanks the INBEV-Baillet Latour Funds.

Literature Cited

- Callemien, D., Dasnoy, S. and Collin, S. 2006. Identification of a stale-beer-like odorant in extracts of naturally aged beer. *J. Agric. Food Chem.* 54:1409-1413.
- Gallois, A. 1987. Collection of gas chromatographic fractions for NMR analysis. *J. High Resol. Chrom. & Chrom Com.* 10:213-214.
- Gijs, L., Chevance, F., Jerkovic, V. and Collin, S. 2002. How low pH can intensify β -damascenone and dimethyltrisulfide production through beer aging. *J. Agric. Food Chem.* 50:5612-5616.
- Gijs, L., Perpète, P., Timmermans, A. and Collin, S. 2000. 3-Methylthiopropionaldehyde as precursor of dimethyl trisulfide in aged beers. *J. Agric. Food Chem.* 48:6196-6199.
- Lermusieau, G. and Collin, S. 2003. Volatile sulfur compounds in hops and residual concentrations in beer - A review. *J. Am. Soc. Brew. Chem.* 61:109-113.
- Olsen, A. 1988. Onion-like off-flavour in beer: isolation and identification of the culprits. *Carlsberg Res. Commun.* 53:1-9.
- Tominaga, T., Murat, M.-L. and Dubourdieu, D. 1998. Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* 46:1044-1048.
- Vermeulen, C., Gijs, L. and Collin, S. 2005. Sensorial contribution and formation pathways of thiols in foods: a review. *Food Rev. Int.* 21:69-137.
- Vermeulen, C., Lejeune, I., Tran, T.T.H. and Collin, S. 2006. Occurrence of polyfunctional thiols in fresh lager beers. *J. Agric. Food Chem.* 54:5061-5068.

Figures

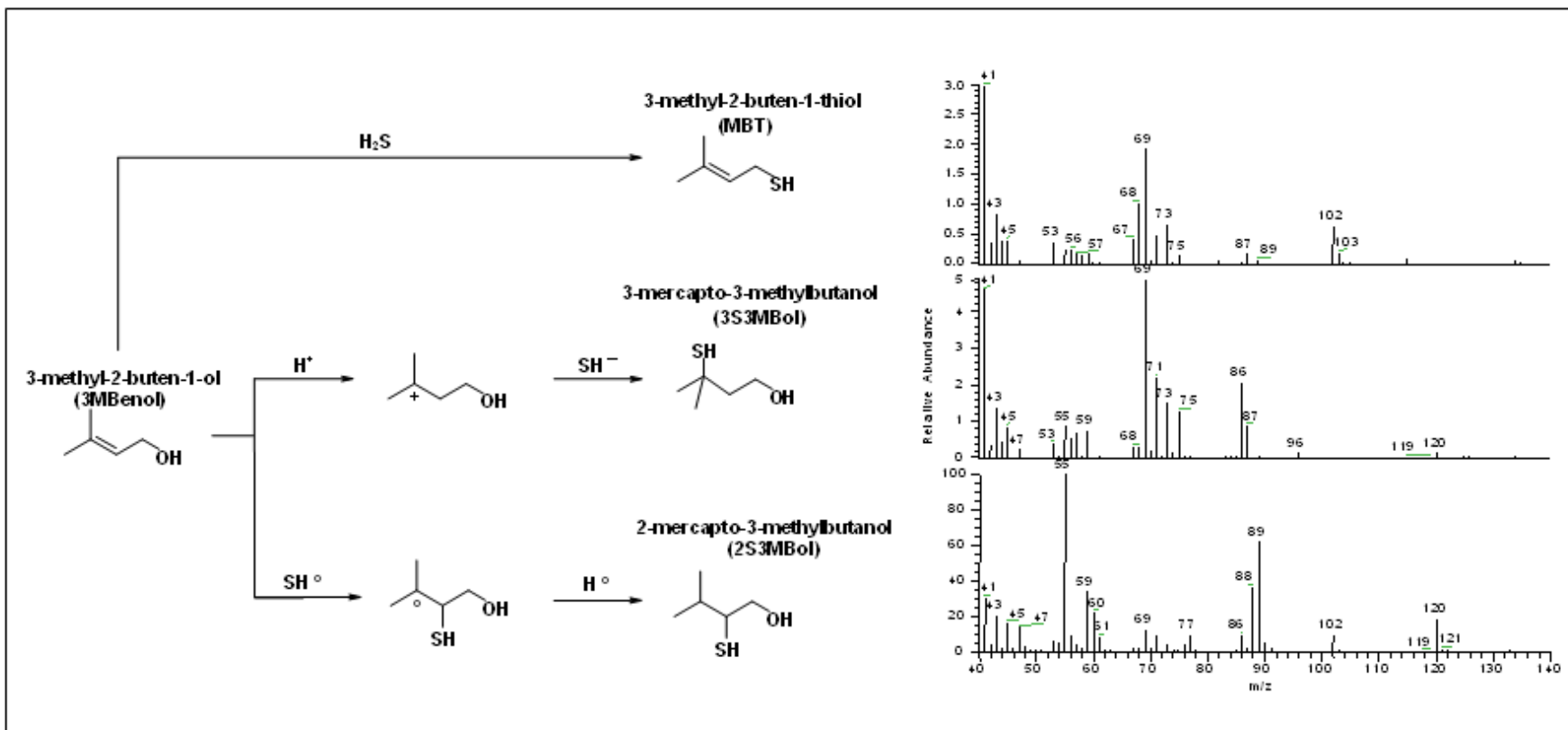
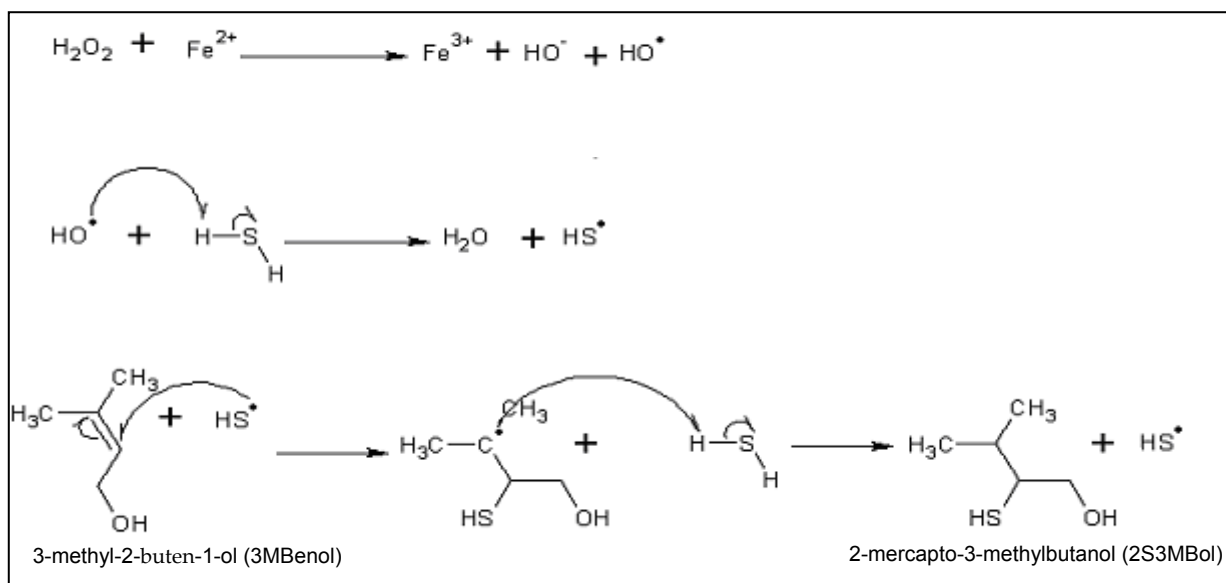
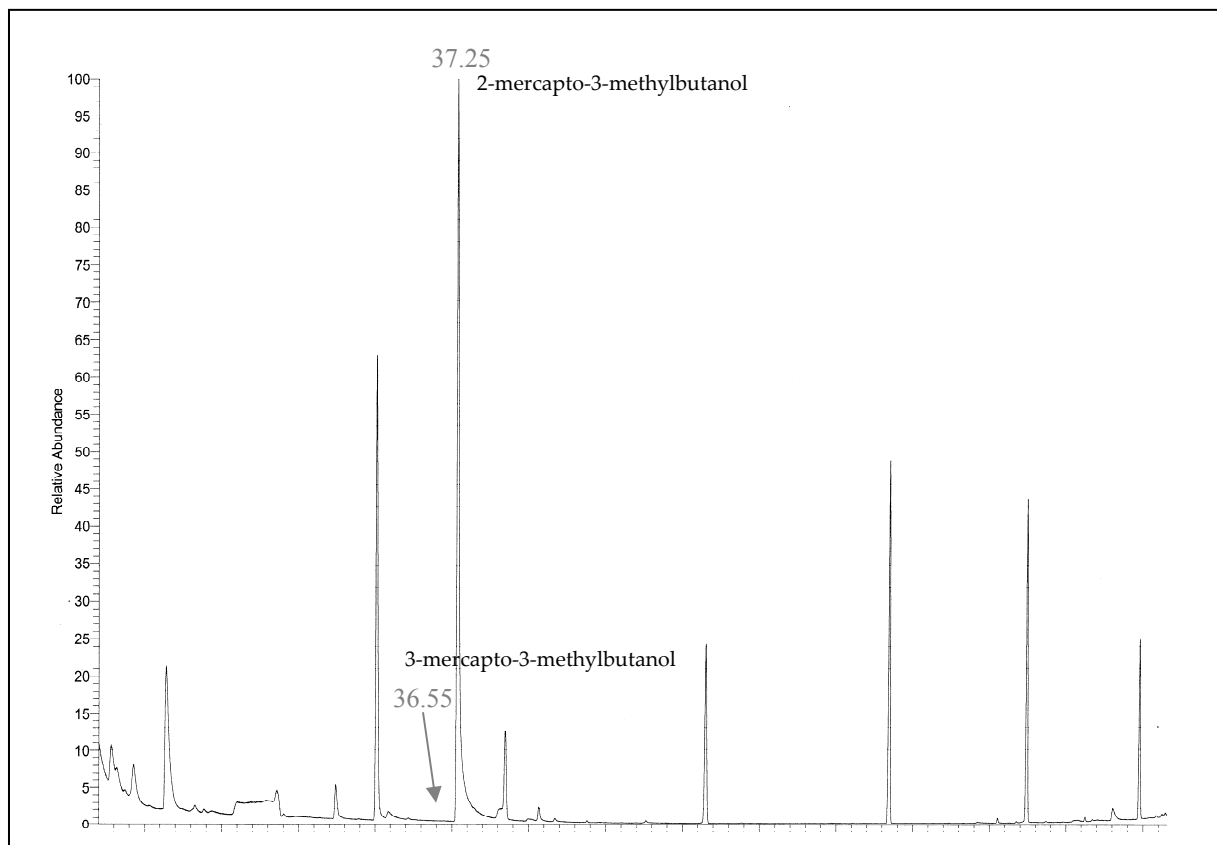


Fig. 1. Hypothetical formation pathways of 3-methyl-2-buten-1-thiol, 3-mercapto-3-methylbutanol, and 2-mercapto-3-methylbutanol in beer.



(a)



(b)

Fig. 2. (a) Chemical reaction leading to 2-mercapto-3-methylbutanol and (b) chromatogram of the resulting product.