

Combinatorial Synthesis and Sensorial Properties of Mercapto Primary Alcohols and Analogues

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Combinatorial chemistry was used to extend the knowledge of beer thiols by synthesizing 13 mercapto primary alcohols and 6 other compounds (mercapto ketones and corresponding alcohols). Their respective mass spectra, retention indices on two columns, odor descriptors, and best estimated—gas chromatography—lowest amounts detected sniffing (BE-GC-LoADS) are reported. The descriptor “cheese”, commonly associated with mercapto aldehydes and thioesters, was rarely used for mercapto alcohols or mercapto ketones. Polyfunctional thiols containing at least six carbon atoms usually gave a delicate odor of rhubarb/carrot, whereas most of the mercapto primary alcohols of intermediate size were described as onion-like, plastic-like, or pungent. Sensorial analysis of beers spiked with copper(II) ions allowed confirmation of the participation of thiols in the overall odor of fresh beer. The finding of 4-mercapto-4-methyl-2-pentanone and 3-mercaptohexanol in an XAD-2 Amberlite resin beer extract indicates a requirement for a more efficient thiol extraction procedure allowing identification of relevant polyfunctional thiols for brewing scientists.

KEYWORDS: Combinatorial synthesis; polyfunctional thiols; flavor; aroma; beer; sulfur compounds; BE-GC-LoADS

INTRODUCTION

Dimethyl sulfide has long been known as a major odor compound of fresh beer. At concentrations approaching its threshold (~50 ppb), it imparts a desirable corn flavor to lagers (1). Sulfites excreted by yeast or arising through packaging and hop thioesters are other sulfur-containing molecules often perceived by beer consumers (2). Lermusieau et al. (3) recently suspected the occurrence of 2-methyl-3-furanthiol and *N*-(methyl)-mercaptoacetamide in fresh hopped beers. Sulfur compounds are also known to play a major role in aged beer off-flavors. Among them, dimethyl trisulfide and methional [3-(methylthio)propionaldehyde] confer very unpleasant notes such as onion and cooked potato to the beverage (4–7). In 1991, 3-mercapto-3-methylbutyl-formate (flavor dilution value < 1 in fresh beer and = 32 in stored beer), with its catty and ribes-like odor, was detected by Schieberle in pale lager beer (8). Isopentenyl mercaptan is another key compound for the brewer, being responsible for the well-known skunky lightstruck flavor, a defect found after exposure to radiation or light (9, 10). Recently, it has been shown that the occurrence of this thiol is already significant in fresh beers (6, 11). GC—olfactometry applied to beer extracts suggests that many other thiols could be relevant in beer. In most cases, unfortunately, no usual FID or MS peak can be recorded, so structure identification is very difficult. Without knowledge of Kovats indices on at least two GC

columns combined with sensorial characterization at the sniffing port, identification attempts are very problematic.

To expand the knowledge on beer thiols, 13 mercapto primary alcohols were here synthesized by combinatorial chemistry. This new methodology was recently applied successfully to obtain a large number of mercapto aldehydes (12), mercapto ketones, and mercapto secondary alcohols (13). Three new mercapto ketones and their corresponding mercapto alcohols have also been obtained in the present work. Although specific extraction procedures must still be optimized for beer thiols, an available thiol data bank was used here to assess the possible occurrence of polyfunctional thiols in fresh lager beers. Sensorial analyses were finally applied on samples “spiked” with copper(II) ions; the results demonstrate that thiols significantly contribute to the overall odor of a fresh beer.

MATERIALS AND METHODS

Chemicals. The reagents for the syntheses were of the highest purity commercially available and were not further purified before use. The solvents were anhydrous and stored over molecular sieves.

3-Buten-2-one (99%), 1-penten-3-one (97%), (*E*)-3-nonen-2-on (95%), 3-penten-2-one (65%), 4-hexen-3-one (95%), 4-methyl-3-penten-2-one (98%), 5-methyl-3-hexen-2-one (75%), 2-propenal (90%), 2-ethyl-2-propenal (85%), 2-butyl-2-propenal (97%), (*E*)-2-octenal (94%), (*E*)-2-nonenal (97%), (*E*)-2-methyl-2-butenal (97%), (*E*)-2-hexenal (98%), 3-methyl-2-butenal (97%), 2-methyl-2-pentenal (98%), thioacetic acid (96%), piperidine (99%), tetrahydrofuran (99.9%) (THF), anhydrous diethyl ether (99+%), diethyl ether (99.8%), pentane (98%), 0.2-mm-

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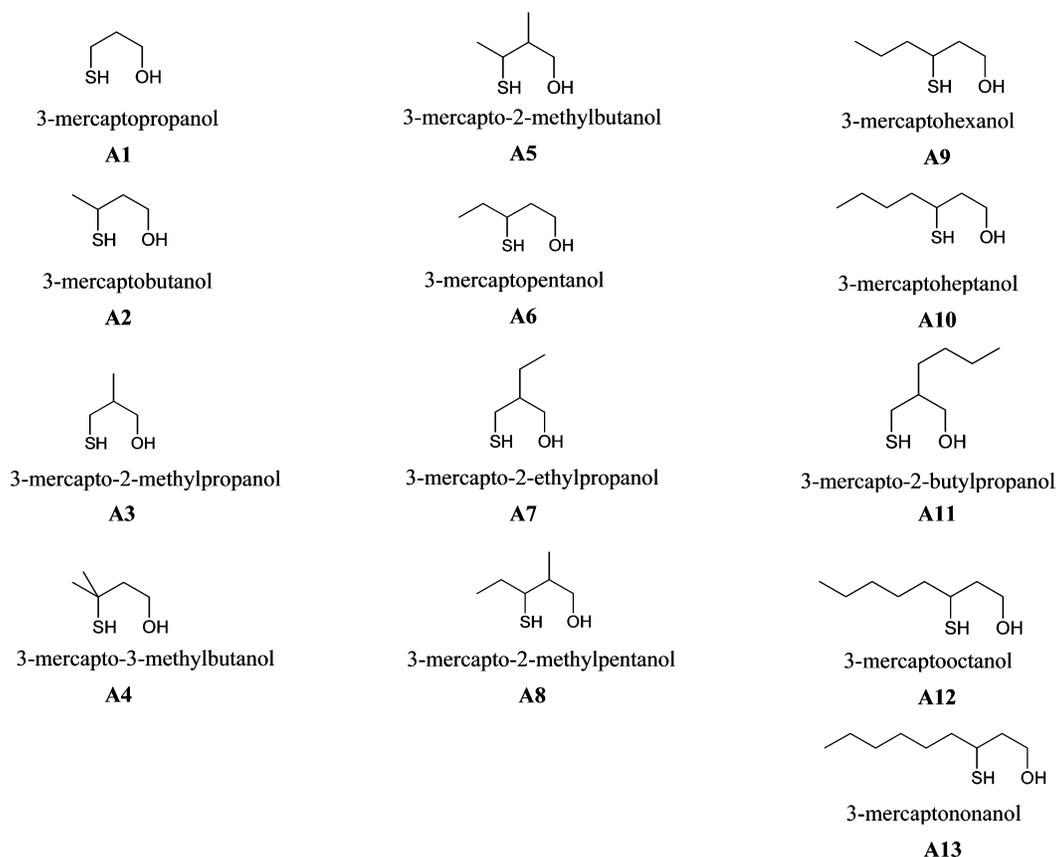


Figure 1. Structures of the synthesized mercapto primary alcohols.

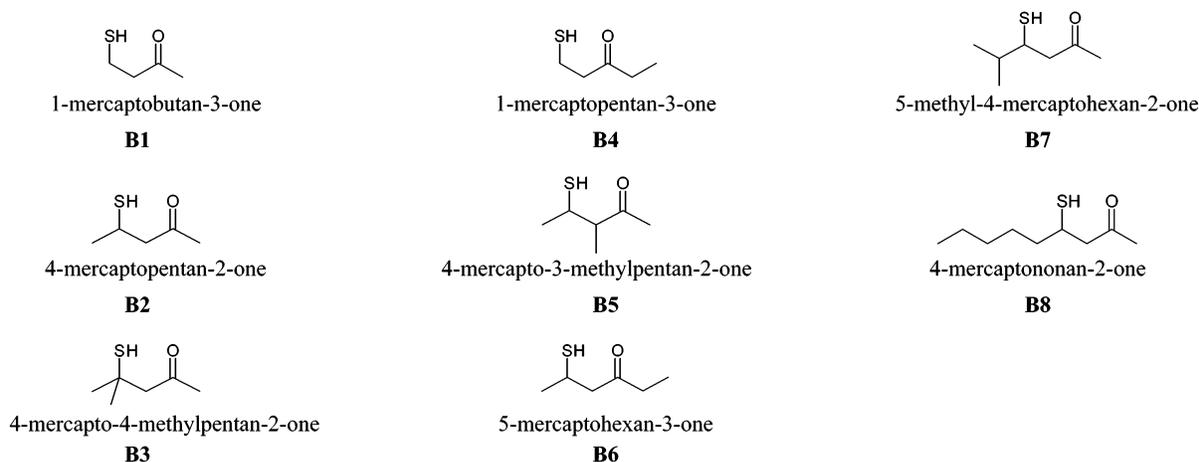


Figure 2. Structures of the synthesized mercapto ketones.

thick silica plates, and 5,5'-dithiobis(2-nitrobenzoic acid) were provided by Aldrich Chemicals (Bornem, Belgium). 3-Methyl-3-penten-2-one (95%), 2-butenal (99.5%), (*E*)-2-pentenal (98%), and (*E*)-2-heptenal (98%) were supplied by Fluka (Bornem, Belgium). 2-Methyl-2-propenal (97%) was provided by Janssen (Geel, Belgium). Hydrogen sulfide was obtained from Praxair (Antwerp, Belgium). 2,4-Dinitrophenylhydrazine and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were supplied by Merck (Overijse, Belgium). The Amberlite XAD-2 resins were purchased from Supelco Inc. (Bellefonte, PA).

Combinatorial Synthesis of Mercapto Primary or Secondary Alcohols. A catalytic quantity of piperidine (12 drops) was added to α,β -unsaturated aldehydes or ketones (25 mmol/L) under an inert atmosphere. Next, thioacetic acid (15 g, representing a 50% excess) was added dropwise at a temperature in the range of 0–10 °C (ice bath). After this addition, the reaction mixture was stirred for 18 h at room temperature. The whole was diluted with 100 mL of diethyl ether and then washed with 20 mL of 1 N HCl and twice with 20 mL of a

saturated sodium bicarbonate solution. After drying over sodium sulfate, the solvent was evaporated.

The crude product was reduced by LiAlH_4 (3.6 g of LiAlH_4 in dry diethyl ether) within 45 min. After the addition of 40 mL of NH_4Cl (10% aqueous solution) and 40 mL of 2 N HCl, the aqueous phase was extracted twice with 20 mL of diethyl ether. The combined organic phases were washed with saturated sodium bicarbonate solution and dried over sodium sulfate, and the solvent was evaporated before storage at -80 °C prior to analysis.

Analytical Methods. *Thin Layer Chromatography.* Pentane/diethyl ether (60:40) was used as chromatographic eluent with 0.2 mm silica plates to follow the course of the syntheses. The colorless non-UV-absorbing thiols were revealed with 5,5'-dithiobis(2-nitrobenzoic acid) (14), whereas 2,4-dinitrophenyl-hydrazine was used for identifying mercapto ketones (15).

Gas Chromatography Coupled with Sulfur Chemiluminescence Detection (GC-SCD). GC was performed using a Chrompack CP9001

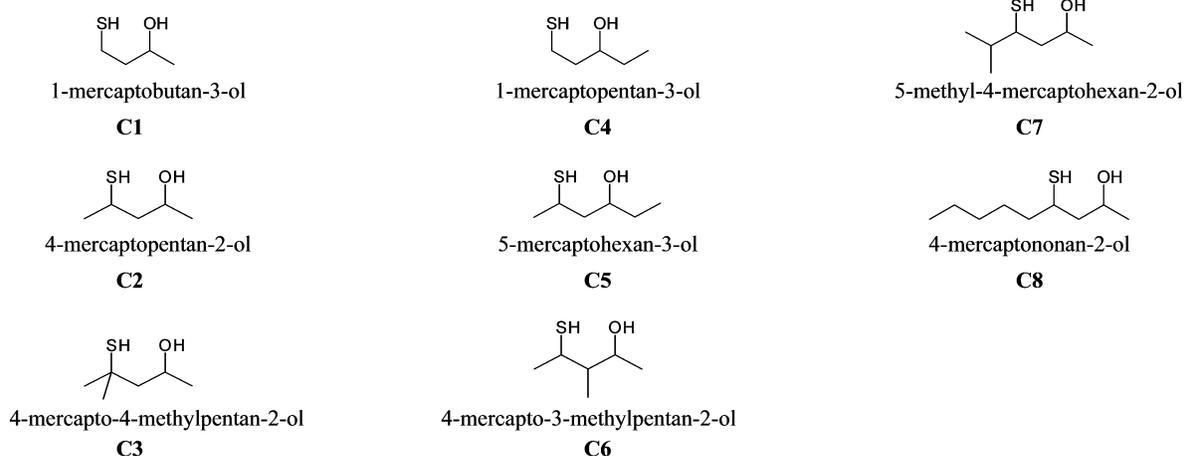


Figure 3. Structures of the synthesized mercapto secondary alcohols.

chromatograph equipped with a splitless injector maintained at 250 °C and opened after 0.5 min. Analysis of sulfur compounds was performed using a 50 m × 0.32 mm i.d., wall-coated 1.2 μm film thickness open tubular (WCOT fused silica) apolar CP-Sil 5 CB capillary column (Middelburg, The Netherlands) connected to a sulfur chemiluminescence detector (Sievers, model 355 SCD) and a Shimadzu CR3A integrator. An initial oven temperature of 40 °C was maintained for 4 min and then programmed to rise to 132 °C at 2 °C/min followed by 132–250 °C at 10 °C/min. The final temperature was then held for 45 min. Helium carrier gas was used at a flow of 32.0 cm/s (flow rate = 1.0 mL/min). Air and hydrogen flows were maintained at 40 and 100 mL/min, respectively, in the 800 °C combustion chamber. The air flow rate in the ozone generator was 6 psi, and a vacuum of 150–275 Torr was applied to the entire system.

Gas Chromatography Coupled with Electronic Impact Mass Spectrometry (GC-MS). Mass spectra (m/z 40–380) 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 either the previously described column or a 25 m × 0.32 mm i.d., WCOT 0.3 μm film thickness fused silica polar FFAP CB capillary column (Middelburg, The Netherlands). Oven temperature, initially kept at 40 °C for 4 min, was programmed to rise to 132 °C at 2 °C/min and, thereafter, from 132 to 250 °C at 10 °C/min, remaining at the maximum temperature for 15 min. Spectrometric recording was automatic throughout elution using Xcalibur software. The compounds were identified on the basis of their fragmentation patterns and library matching of spectra.

Gas Chromatography Coupled with Dual Flame Ionization and Olfactometric Detection (GC-FID-O). This was performed using a Chrompack CP9001 gas chromatograph that was equipped with a splitless injector maintained at 250 °C and opened after 0.5 min. Sulfur compounds were analyzed using a 50 m × 0.32 mm i.d., WCOT fused silica 1.2 μm film thickness apolar CP-Sil 5 CB capillary column (Middelburg, The Netherlands). An initial oven temperature of 40 °C was maintained for 4 min and then programmed to rise to 132 °C at 2 °C/min followed by 132–250 °C at 10 °C/min. The final temperature was held for 15 min. A fused silica T-junction was used at the end of the capillary column. Fifty percent of the eluent was sent to an FID maintained at 250 °C and connected to a Shimadzu C-R3A integrator, whereas the other part was directed to a GC odor port at 250 °C. In the latter case, the eluent was diluted with a large volume of air (20 mL/min) previously humidified in an aqueous copper(II) sulfate solution to improve the transport of the effluent out of the funnel (16). The volume brought by the GC is too low to really prevent odor assessors' mucous membranes from drying. To be sure that no oxidation occurred in the sniffing port, all compounds were also smelled one time without air. As described by Berger et al. (17), the best estimated-GC-lowest amount detected by sniffing (BE-GC-LoADS) is defined as the geometric mean between the lowest mass of compound perceived at the outlet of the GC odor port and the highest undetected amount injected onto the column. Experiments were performed by injecting 2

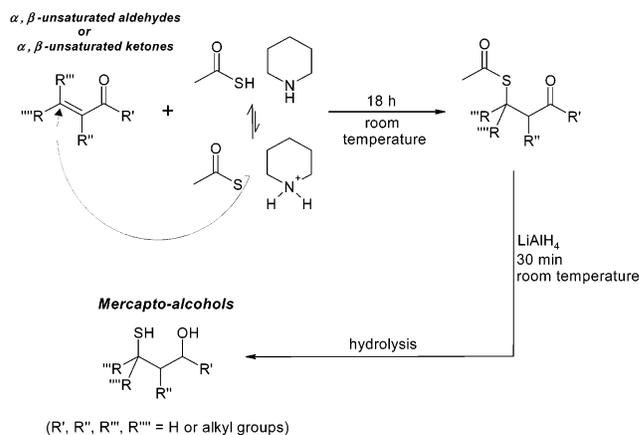


Figure 4. Synthetic formation pathway of mercapto alcohols.

μL of each solution at the following dilutions: 1/50, 1/100, 1/200, 1/500, 1/1000, 1/2000, 1/5000, and so on up to the 1/50 000 dilution. Two judges working completely independently of each other performed sensory analyses. During the course of all sensory analyses the verbal odor descriptions of the judges were recorded.

Sensorial Analysis of Beers Spiked with Copper. Five coded beer samples, two of which containing copper(II) (50 ppm), were presented to 10 assessors. Assessors were asked to smell each sample under a green light and to select the two samples that were different from the other three. Note that the copper was added just before the test to avoid thiol oxidation.

RESULTS AND DISCUSSION

As discussed previously for mercapto aldehydes (12), mercapto ketones, and mercapto secondary alcohols (13), combinatorial chemistry turned out to be an ingenious and rapid way to obtain new polyfunctional thiols. In this way, 13 mercapto primary alcohols (Figure 1), 3 additional mercapto ketones (Figure 2), and the mercapto secondary alcohols corresponding to these ketones (Figure 3) were synthesized. The mercapto ketones were obtained by bubbling H_2S through a mixture of α, β -unsaturated ketones as previously detailed by Vermeulen et al. (13). However, to improve reaction yields and avoid highly polluted samples, a new “one-pot” procedure for obtaining mercapto alcohols, using thioacetic acid instead of hydrogen sulfide, was tested (Figure 4). This resulted in cleaner chromatograms and easier GC analyses.

The composition of our libraries was confirmed by GC-MS (Tables 1–3). Each thiol proved to be identifiable through its molecular ion (M^+). All compounds lost a neutral H_2S molecule

Table 1. GC-MS and Sensorial Properties of Mercapto Primary Alcohols

code	name	MS fragments, <i>m/z</i> [relative percentage]	Kovats index		BE-GC-LoADS (ng)	odor at the sniffing port (CP-Sil5 CB)
			CP-Sil5 CB	FFAP		
A1	3-mercaptoopropanol	57 [100]; 41 [94]; 58 [82]; 45 [59]; 47 [59] (92 ^a [53] present)	852	1665	0.2	potato, broth
A2	3-mercaptobutanol	57 [100]; 55 [86]; 41 [86]; 72 [74]; 43 [74] (106 ^a [21] present)	898	1665	0.1	cheese, onion, cabbage, pungent
A3	3-mercapto-2-methylpropanol	41 [100]; 47 [67]; 57 [62]; 55 [49]; 72 [42] (106 ^a [20] present)	920	1698	0.07	leek, onion
A4	3-mercapto-3-methylbutanol	41 [100]; 69 [73]; 71 [35]; 86 [24]; 43 [24] (120 ^a [3] present)	944	1671	0.2	cooked onion, tartare, chervil
A5	3-mercapto-2-methylbutanol	41 [100]; 60 [93]; 45 [89]; 61 [86]; 71 [61] (120 ^a [14] present)	987	1738	0.01	leek, broth, pepper
A6	3-mercaptopentanol	41 [100]; 57 [70]; 55 [47]; 61 [43]; 69 [43] (120 ^a [3] present)	1002	1765	0.001	broth, greenery, stinging nettle
A7	3-mercapto-2-ethylpropanol	57 [100]; 41 [94]; 47 [79]; 55 [79]; 45 [68] (120 ^a [12] present)	1020	1790	0.01	onion
A8	3-mercapto-2-methylpentanol	41 [100]; 74 [47]; 55 [39]; 71 [37]; 45 [32] (134 ^a [8] present)	1082 ^b 1084 ^b	1822 ^b 1828 ^b	0.007 ^b 0.004 ^b	burned plastic, gas, citrus fruit ^b greenery, vinaigrette ^b
A9	3-mercaptohexanol	55 [100]; 41 [72]; 57 [64]; 61 [46]; 67 [30] (134 ^a [8] present)	1095	1853	0.004	rhubarb, lime
A10	3-mercaptoheptanol	55 [100]; 41 [86]; 57 [50]; 61 [46]; 81 [34] (148 ^a [11] present)	1198	1962	0.006	citrus fruit, vinaigrette, carrot
A11	3-mercapto-2-butylpropanol	43 [100]; 74 [35]; 45 [17]; 47 [11]; 57 [9] (148 ^a absent)	1218	1985	0.005	plastic, pungent
A12	3-mercaptooctanol	41 [100]; 55 [96]; 57 [65]; 69 [38]; 43 [35] (162 ^a [6] present)	1302	>2000	0.004	rhubarb, carrot greenery
A13	3-mercaptononanol	41 [100]; 55 [88]; 57 [53]; 43 [50]; 69 [44] (176 ^a [9] present)	1424	>2000	0.08	carrot greenery

^a Molecular ion. ^b The data correspond to diastereoisomers.

Table 2. GC-MS and Sensorial Properties of Mercapto Ketones

code	name	MS fragments, <i>m/z</i> [relative percentage]	Kovats index		BE-GC-LoADS (ng)	odor at the sniffing port (CP-Sil5CB)
			CP-Sil5 CB	FFAP		
B1	1-mercaptobutan-3-one	43 [100]; 71 [33]; 61 [18]; 55 [9]; 104 ^a [9]	844	1445	0.01	potato
B2 ^c	4-mercaptopentan-2-one	43 [100]; 85 [29]; 41 [26]; 118 ^a [26]; 61 [19]	884	1422	0.03	greenery, potato, black currant
B3 ^c	4-mercapto-4-methylpentan-2-one	43 [100]; 132 ^a [26]; 75 [21]; 55 [19]; 99 [12]	915	1382	0.004	black currant, catty, broom, ...
B4	1-mercaptopentan-3-one	57 [100]; 61 [64]; 85 [32]; 118 ^a [28]; 47 [20]	947	1517	0.009	cheese, solvent, skunky, pungent
B5 ^c	4-mercapto-3-methylpentan-2-one	43 [100]; 99 [69]; 55 [50]; 61 [19]; 132 ^a [6]	959 ^b 967 ^b	1469 ^b 1477 ^b	0.02 ^b 0.4 ^b	sweat ^b cooked milk ^b
B6 ^c	5-mercaptohexan-3-one	57 [100]; 61 [48]; 75 [44]; 132 ^a [30]; 99 [28]	984	1487	0.02	box tree, fresh, empyreumatic
B7 ^c	5-methyl-4-mercaptohexan-2-one	43 [100]; 112 [22]; 113 [19]; 55 [17]; 146 ^a [6]	1069	1585	0.03	exotic fruit, sweet
B8	4-mercaptononan-2-one	43 [100]; 55 [22]; 41 [16]; 141 [13]; 71 [13] (174 ^a absent)	1292	1802	0.02	rhubarb, lemon, cannabis, spicy

^a Molecular ion. ^b The data correspond to diastereoisomers. ^c As published by Vermeulen et al. (13).

leading to the ($M^{+} - 34$) radical cation. As could be anticipated, many cations stabilized by heteroatoms were easily detectable in the spectra. The loss of a water molecule in all mercapto alcohols also occurred.

Among the newly synthesized thiols, compounds **A8** and **C8** each expectedly yielded two peaks on both nonchiral columns, owing to the presence of two asymmetric carbon atoms. On the other hand, compound **A5** surprisingly appeared at only one Kovats index. The presence of a sulfur atom in each molecule was checked by GC-SCD. By means of a simple pentanethiol calibration curve, our equimolar sulfur detector enabled us to quantify accurately all of the thiols in our library.

Attention is directed to the very large variety of descriptors used to describe the 13 mercapto primary alcohols (**Table 1**). Among them, surprisingly, only one compound (**A2**) was reminiscent of cheese odors, a descriptor very common for sulfur

compounds such as mercapto aldehydes (**12**) or thioesters (**17**). On the other hand, pleasant descriptors such as carrot, rhubarb, and citrus fruit or more aggressive/pungent ones such as onion were more frequently proposed by the panelists. Most of these descriptors were also found for some mercapto ketones or mercapto secondary alcohols (**Table 3**). First attempts to establish odor activity relationships among mercapto alcohols and ketones are summarized in **Figure 5**. Most of the structures containing at least six carbon atoms emerged as more delicate, such as rhubarb and carrot, whereas many mercapto primary alcohols with intermediate size and branching were described as onion, plastic, or pungent. Also interesting is the structural analogy between methional [3-(methylthio)propionaldehyde] and compounds **A1**, **B1**, and **B2**, all described as potato-like.

In terms of threshold, the BE-GC-LoADS values were in many cases lower (from 0.0001 to 0.06 ng) for the mercapto

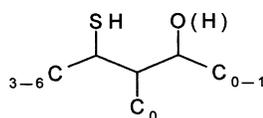
Table 3. GC-MS and Sensorial Properties of Mercapto Secondary Alcohols

code	name	MS fragments, <i>m/z</i> [relative percentage]	Kovats index		BE-GC-LoADS (ng)	odor at the sniffing port (CP-Sil5 CB)
			CP-Sil5 CB	FFAP		
C1	1-mercaptobutan-3-ol	45 [100]; 43 [39]; 47 [36]; 55 [29]; 72 [25] (106 ^a [7] present)	881	1622	0.005	caramel, solvent, lemon, fresh
C2 ^c	4-mercaptopentan-2-ol	45 [100]; 86 [68]; 71 [63]; 61 [53]; 69 [37] (120 ^a [11] present)	914 ^b	1587 ^b	0.002 ^b	broom, black currant, catty ^b
C3 ^c	4-mercapto-4-methylpentan-2-ol	57 [100]; 85 [97]; 45 [68]; 100 [58]; 41 [42] (134 ^a [16] present)	926 ^b	1614 ^b	0.02 ^b	raw onion ^b
C4	1-mercaptopentan-3-ol	57 [100]; 85 [97]; 45 [68]; 100 [58]; 41 [42] (134 ^a [16] present)	952	1547	0.009	broom, black currant, solvent, ...
C5 ^c	5-mercaptohexan-3-ol	47 [100]; 57 [99]; 59 [94]; 45 [79]; 41 [71] (120 ^a [4] present)	981	1698	0.05	stinging nettle
C6 ^c	4-mercapto-3-methylpentan-2-ol	61 [100]; 59 [96]; 71 [46]; 100 [43]; 116 [32] (134 ^a [11] present)	1012 ^b	1658 ^b	0.02 ^b	sweat, meat broth, citrus fruit ^b
C6 ^c	4-mercapto-3-methylpentan-2-ol	56 [100]; 45 [96]; 55 [71]; 61 [67]; 100 [58] (134 ^a [13] present)	1023 ^b	1682 ^b	0.06 ^b	sweat, cooked milk ^b
C6 ^c	4-mercapto-3-methylpentan-2-ol		1022 ^b	1671 ^b	0.06 ^b	sweat, cooked milk ^b
C6 ^c	4-mercapto-3-methylpentan-2-ol		1037 ^b	1677 ^b	0.0001 ^b	onion, leek, sweat, soup ^b
C6 ^c	4-mercapto-3-methylpentan-2-ol			1687 ^b		
C6 ^c	4-mercapto-3-methylpentan-2-ol			1739 ^b		
C7 ^c	5-methyl-4-mercaptohexan-2-ol	71 [100]; 45 [90]; 55 [76]; 61 [67]; 114 [45] (148 ^a [14] present)	1097 ^b	1747 ^b	0.002 ^b	rhubarb, lemon ^b
C7 ^c	5-methyl-4-mercaptohexan-2-ol		1107 ^b	1762 ^b	0.002 ^b	spicy, peppery, meaty ^b
C8	4-mercaptononan-2-ol	45 [100]; 71 [55]; 41 [55]; 43 [50]; 55 [50] (176 ^a [5] present)	1315 ^b	1971 ^b	0.002 ^b	rhubarb, sweat ^b
C8	4-mercaptononan-2-ol		1323 ^b	1985 ^b	0.008 ^b	rhubarb, mushroom ^b

^a Molecular ion. ^b The data correspond to diastereoisomers. ^c As published by Vermeulen et al. (13).

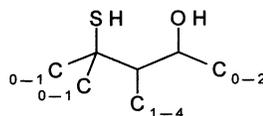
Odor descriptors : rhubarb, carrot

(A9, A12, A13, B8, C7, C8)



Odor descriptors : onion, sweat, pungent, leek

(A3, A4, A5, A7, A11, C5, C6)



Odor descriptor : potato

(A1, B1, B2, methional)

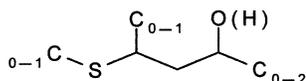


Figure 5. Minimal moieties found in mercapto alcohols and ketones for some descriptors.

secondary alcohols. Very low values also characterized many mercapto primary alcohols (from 0.001 to 0.2 ng). This was especially the case for four linear analogues: 3-mercaptopentanol (1 pg), 3-mercaptohexanol (4 pg), 3-mercaptoheptanol (6 pg), and 3-mercaptooctanol (4 pg).

Although specific extraction procedures are yet to be optimized for beer thiols, the thiol data bank developed in this study was used for the first time to search for potential polyfunctional thiols in fresh lager beers. An ethyl ether XAD-2 Amberlite extract was obtained from a fresh industrial Belgian beer as previously described by Lermusieau et al. (3). As recently shown by Gijs et al. (6), the most intense odor at the sniffing port proved to be isopentenyl mercaptan (flavor dilution = 729 in fresh beer and = 243 in aged beer). Among the 29 mercapto ketones and mercapto alcohols described here, only two were detected in the beer extract, with relatively low dilution factors (flavor dilution = 3 and 9, respectively). One was identified as

4-mercapto-4-methyl-pentan-2-one (B3) and the other as 3-mercaptohexanol (A9). Neither yielded a peak at the FID or MS detector, yet they could be fairly reliably identified on the basis of their Kovats indices on the CP-Sil 5 and FFAP columns and above all by their very typical odor descriptions [black currant, catty (B3); and rhubarb, lime (A9), respectively]. The presence of the mercapto ketone was formerly suspected by Tressl et al. in an aged-beer extract (18). Taking into account the unusual oxidizability of such compounds and their trace occurrence, one can logically suspect that both thiols as well as other analogues might appear to be more relevant in beer if an adequate extraction procedure was used. To confirm that thiols significantly contribute to the overall flavor of a fresh beer (19), sensorial analyses were applied on beers spiked with copper(II) (50 ppm). A "two-out-of-five" assay on sample with unmodified pH (4.4) indicated that copper is able to modify the sensorial profile of the beer by trapping thiols into odorless chemical complexes ($\alpha < 0.1\%$).

As the *p*-hydroxymercuribenzoic acid selective extraction appears to be very efficient to characterize thiols in Sauvignon wines (20), we are currently consulting our thiol data bank to identify new potent odorants in analogous types of beer extract.

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Received for review December 20, 2002. Revised manuscript received March 28, 2003. Accepted March 28, 2003. We thank the Fonds National de la Recherche Scientifique (FNRS) for financial support.

JF0212340