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Combinatorial Synthesis and Screening of Novel Odorants Such as Polyfunctional Thiols

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Abstract: Combinatorial chemistry was shown to be an efficient tool for the preparation of new aroma-impact compounds. In this case, polyfunctional thiols were synthesized quickly using halide reagents or Bunte salt intermediates. They were separated by gas chromatography and then characterized using low resolution EI and CI mass spectrometry. The individual sensorial properties of the thiol products (i.e. odor and perception threshold) were determined by GC-O (olfactometry) which uses the human nose as detector. The thiols were characterized based on their particular odors. 3-Methyl-2-buten-1-thiol, a relevant flavor naturally present in beer and coffee, emerged as the most powerful of the thiol library.

Keywords: Combinatorial synthesis, Bunte salts, polyfunctional thiols, flavor, aroma.

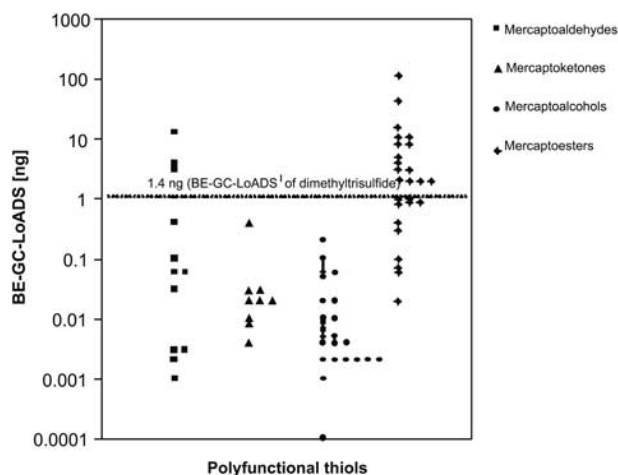
INTRODUCTION

Developed by Geysen [1], combinatorial chemistry is a recent methodology of which the major application has been to produce new drugs faster and less expensively. Taking advantage of this original approach, a few scientists have already used it to extend the knowledge on food flavors. In 1999, Khan *et al.* developed a “one-pot synthesis” of *S*-methyl thioesters by reacting methyl chlorothiolfomate with carboxylic acids [2]. They emphasized that substantial information on a relatively large number of compounds could be easily generated from only one library. Moreover, Berger *et al.* suggested that sensory analyses of a such synthetic mixture might be useful to find new marketable flavors or to identify unsuspected character-impact compounds [3].

Inspired by those studies, Vermeulen *et al.* created a combinatorial database on polyfunctional thiols (i.e. mercaptoaldehydes, mercaptoketones, mercaptoalcohols, and mercaptoesters) which might occur naturally in foods or beverages [4-7]. As shown in Fig. 1, in comparison with the powerful odorant dimethyltrisulfide (DMTS), these library compounds possessed very low odor perception threshold or BE-GC-LoADS (Best Estimated-Gas Chromatographic-Lowest Amount Detected by Sniffing) and exhibited different aromas. As summarized in Fig. 2, some scents were pleasant (e.g. 3-mercaptohexanol – rhubarb, lime, fruity odor) or suitable for using in food (e.g. 3-mercaptopentanal – broth, onion odor) while others were repulsive (e.g. 4-mercaptononyl-2-acetate – sweat, garlic odor).

Even though they impart very characteristic aromas or highly desirable notes to many foods and beverages [8], polyfunctional thiols are not so well-known and are rarely commercially available. Always present as traces and quite unstable when exposed to light, high temperatures or oxida-

tive conditions, it is very difficult to isolate or identify them using common extraction techniques or GC detectors. Considering their very low perception threshold, olfactometry is logically the most efficient detection system. That is why a database compiling the odors of many different thiols (eventually obtained by combinatorial synthesis) is very useful in flavor research.



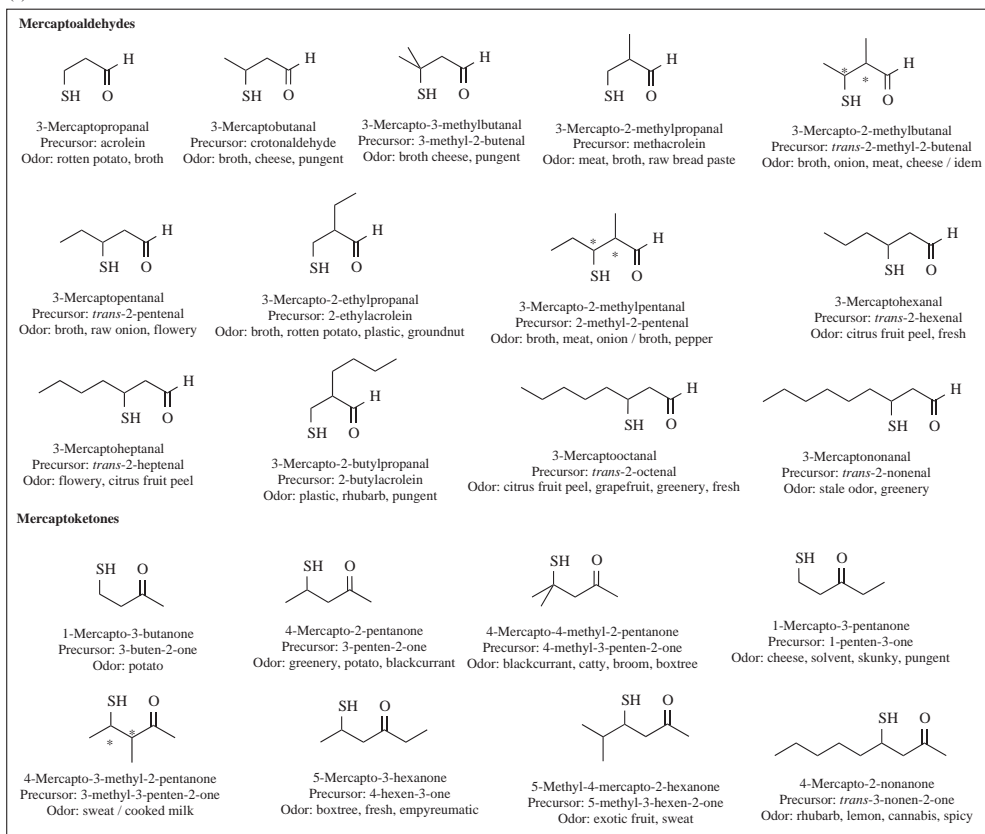
¹The BE-GC-LoADS or best estimated-gas chromatographic-lowest amount detected by sniffing is defined as the GC-O perception threshold of an odorant and is expressed in ng (see the Materials and Methods section for details).

Fig. (1). BE-GC-LoADS characterizing polyfunctional thiols in comparison with dimethyltrisulfide.

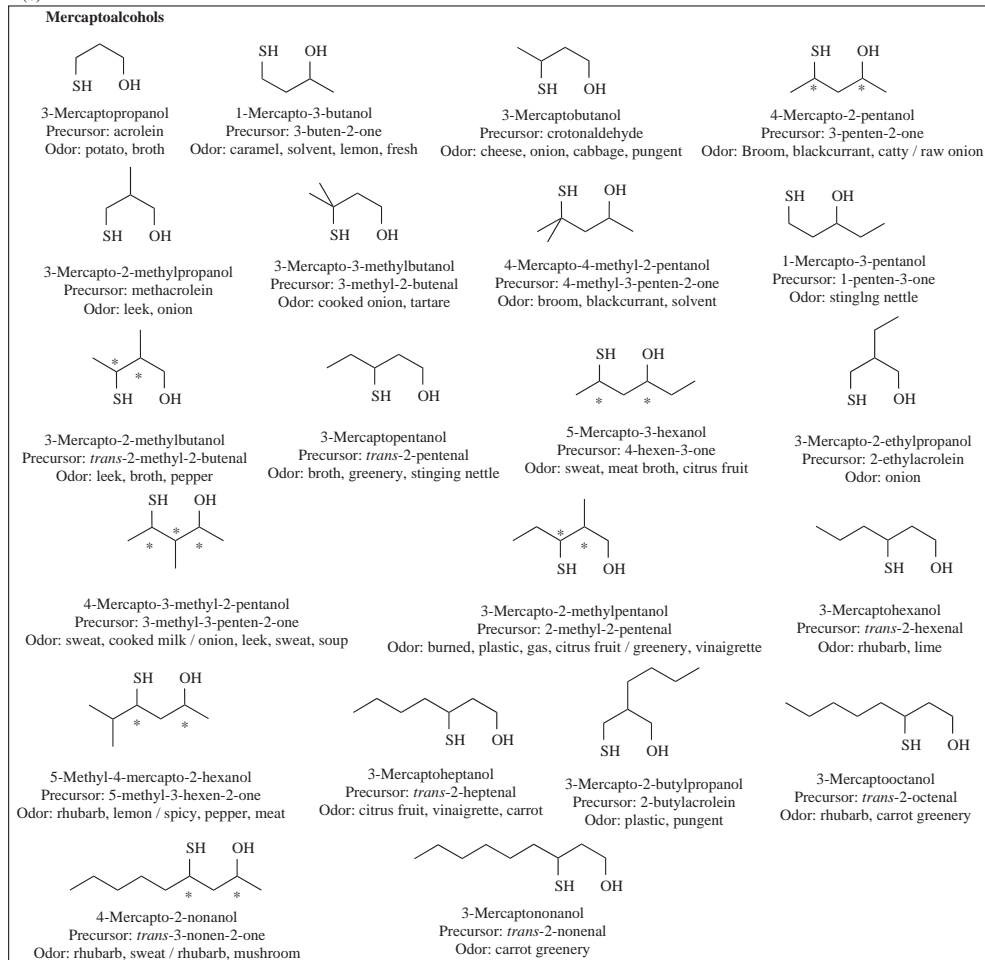
The aim of the present work was to find an additional reaction pathway to synthesize and characterize new polyfunctional thiols. So, bromides and chlorides with an extra chemical function were converted to their corresponding mercaptans *via* the Bunte salt reaction [9].

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(a)



(b)



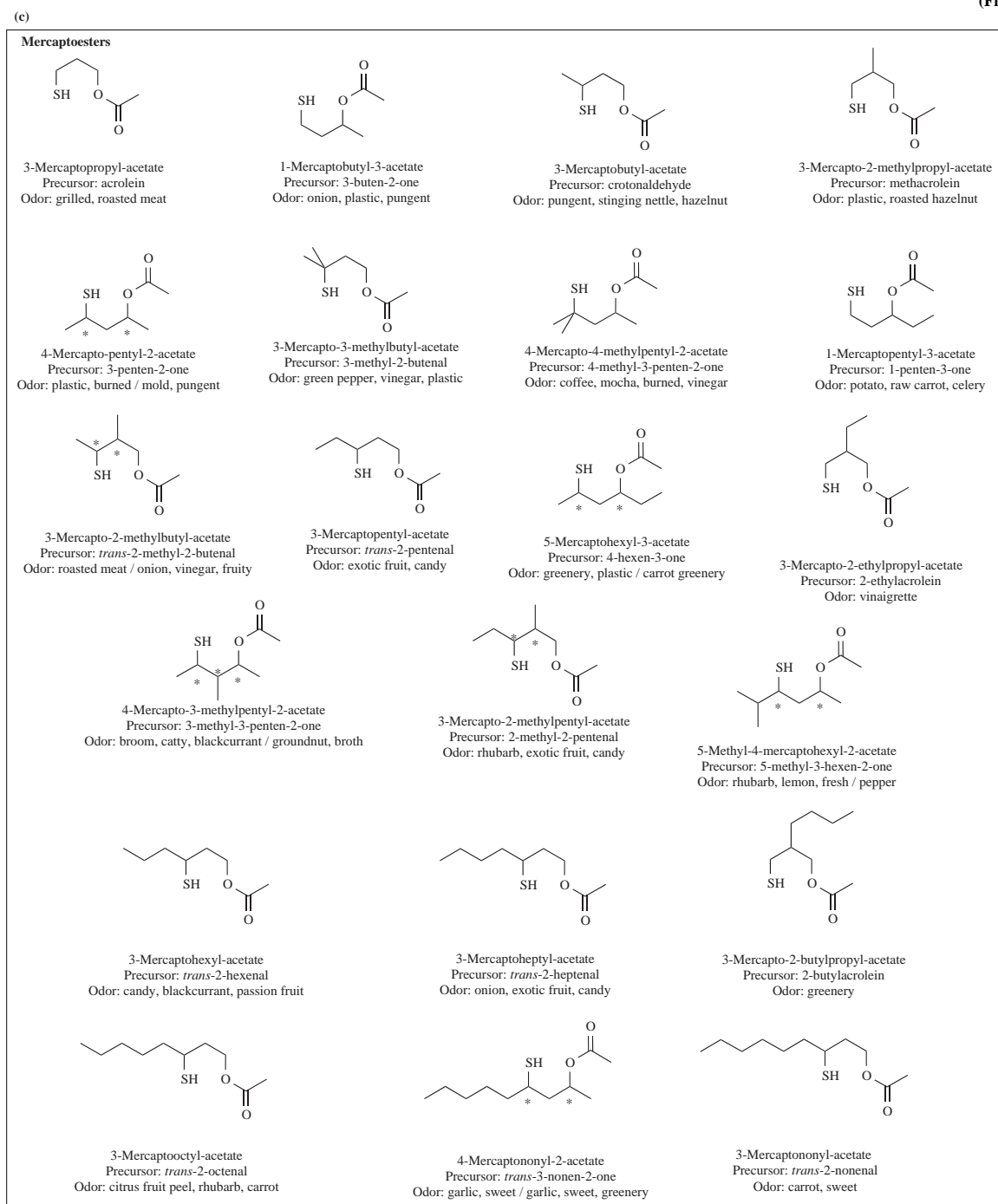


Fig. (2). Structures, chemical precursors and odor of some polyfunctional thiols obtained by combinatorial chemistry: (a) mercaptoaldehydes and mercaptoketones; (b) mercaptoalcohols; (c) mercaptoesters.

MATERIALS AND METHODS

Chemicals

Diethylether (99,8%, [60-29-7]) as well as halides were purchased from Aldrich: 1-bromo-2-butanone 90% [816-40-0], 1-bromo-3,3-dimethyl-2-butanone 97% [19686-73-8], 1-bromo-3,3-dimethyl-2-butanone 97% [5469-26-1], 1-chloro-2-methyl-2-propanol 97% [558-42-9], 1-chloro-2-propanol 70% [127-00-4], 1-chloro-3,3-dimethyl-2-butanone 95% [13547-70-1], 1-chloro-3-pentanone 85% [32830-97-0], 2-bromoethanol 95% [540-51-2], 2-chlorocyclohexanol 90%

[1561-86-0], 3-bromo-1-propanol 97% [627-18-9], 3-bromo-2,2-dimethyl-1-propanol 96% [40894-00-6], 3-chloro-1-propanol 98% [627-30-5], 3-chloro-2,2-dimethyl-1-propanol 99% [13401-56-4], 3-chloro-2-butanone 97% [4091-39-8], 4-bromo-2-methyl-2-butene 90% [870-63-3], 5-chloro-2-pentanone 85% [5891-21-4], 6-bromo-1-hexanol 97% [4286-55-9], 6-chloro-1-hexanol 96% [2009-83-8], 6-chloro-2-hexanone 97% [10226-30-9], 7-bromo-1-heptanol 95% [10160-24-4], 8-bromo-1-octanol 95% [50816-19-8], 8-chloro-1-octanol 98% [23144-52-7], chloroacetaldehyde 50% [107-20-0], chloroacetone 95% [78-95-5], *R*-3-bromo-

2-methyl-1-propanol 97% [93381-28-3], *S*-2-chloro-1-propanol 97% [19210-21-0], *S*-3-bromo-2-methyl-1-propanol 97% [98244-48-5], H₂SO₄ 65% [8014-95-7] and ethanol 99,8% [64-17-5] were from Merck. Na₂S₂O₃ [7772-98-7] and Na₂SO₄ [7757-82-6] were respectively supplied by UCB and Janssen Chemica.

Thiol Synthesis

Four halides with different molecular weights (1.5 mmol of each) were diluted in 5 mL of ethanol in a 25 mL flask. An equimolar quantity of Na₂S₂O₃ (6 mmol) was dissolved in 5 mL and added to the ethanol solution. The whole mixture was heated at reflux for 18 h. Hydrolysis (120 °C, 7 h) was carried out by adding 6 mL of 10% aqueous sulfuric acid after which thiols were recovered by extraction with 2 × 5 mL of diethylether. The extract was finally dried over anhydrous Na₂SO₄ and stored at -80 °C before GC analysis.

Analytical Methods

Gas chromatography hyphenated to a sniffing port (GC-O) or to a flame ionization detector (GC-FID):

Gas chromatography was performed using a Chrompack CP9001 gas chromatograph which 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 × 1.2 μm, wall-coated open tubular (WCOT fused silica) apolar CP-Sil 5 CB capillary column (Chrompack, The Netherlands). Maintained at 40 °C for 4 min, the initial oven temperature was then programmed to rise from 40 to 132 °C at 2 °C/min and from 132 to 250 °C at 10 °C/min. The final temperature was held for 15 min. The eluent was sent to a GC-sniffing port (250 °C) and it was diluted with a large volume of humidified air (20 mL/min) to improve the transport of the effluent out of the funnel [10]. Sensorial analyses (odor detection and description) were performed by two judges working independently. Alternatively, FID was used instead of olfactometry.

Determination of Retention Index (RI)

Retention indexes (RI) were estimated by connecting the column to a FID maintained at 250 °C according to the following formula:

$$RI_i = (100 \times C_n) + \left[\frac{t_i - t_n}{t_{n+1} - t_n} \times 100 \right]$$

Where RI_i = retention index of unknown_i

C_n = number of C atoms of the alkane eluting just before unknown_i

t_i = retention time of unknown_i

t_n = retention time of alkane eluting just before unknown_i

t_{n+1} = retention time of alkane eluting just after unknown_i

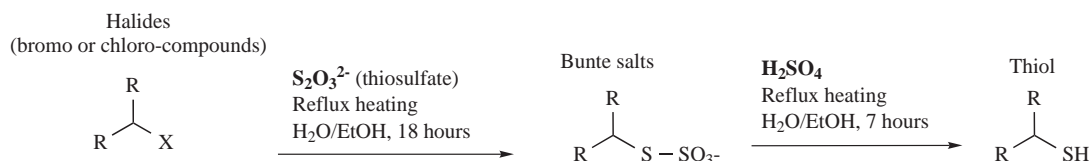


Fig. (3). Polyfunctional thiol synthesis pathway starting from halides and hydrolyzing Bunte salt intermediates.

Determination of BE-GC-LoADS

As suggested previously [3, 11], BE-GC-LoADS was used to assess the odor potency of each thiol. Expressed in ng, it corresponds to the geometric mean between the lowest mass of compound perceived at the outlet of the GC-sniffing port and the highest undetected amount injected onto the column. It is determined by injecting a series of diluted solutions. Therefore, the initial synthetic mixture (concentration C₀) was diluted to obtain a range of solutions with decreasing concentrations ([1/50×C₀], [1/100×C₀], [1/200×C₀], [1/500×C₀], [1/1000×C₀], [1/2000×C₀], [1/5000×C₀], etc.) which were successively analyzed by GC-O (at each polyfunctional thiol retention time) until no odor could be perceived.

Electron Ionization Gas Chromatography-Mass Spectrometry (GC-MS/EI)

Positive ion mass spectra (*m/z* 40 to 380) were recorded using electron ionization 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 same column as for GC-O or a 25 m × 0.32 mm × 0.3 μm WCOT fused silica polar CP-Wax 58 (FFAP) CB capillary column (Chrompack). The oven temperature program was the same as that described for GC-O. Spectral recording was automatic throughout the elution using the ThermoFinnigan Xcalibur software. The compounds were identified on the basis of their masses and fragmentation patterns.

Quantification of the Synthesized Thiols

Different amounts of pentanethiol were injected in GC-MS/EI to quantify each synthesized polyfunctional thiol using the following calibration trend line:

$$\text{Peak area}_{\text{pentanethiol}} = \text{Response coefficient}_{\text{pentanethiol}} \times \text{Concentration}_{\text{pentanethiol}}$$

Approximate but suitable for sensorial measurements, these concentrations were logically expressed in pentanethiol equivalents and used to evaluate BE-GC-LoADS.

Chemical Ionization GC-MS (GC-MS/CI)

GC-MS/CI analyses (CH₄ – positive mode) were performed with a TSQ 7000 Finnigan MAT mass spectrometer connected to a HP 5890 gas chromatograph equipped with a 30 m × 0.32 mm × 0.25 μm Econo-Cap EC-5 capillary column (Alltech, U.S.A.). The data were recorded automatically using Xcalibur software.

RESULTS AND DISCUSSION

In this study, twenty polyfunctional thiols were obtained using the combinatorial “one-pot synthesis” concept. As shown in Fig. 3, Bunte salt intermediates, which were created from bromo- or chloro-compounds and Na₂S₂O₃, were hydrolyzed by H₂SO₄ to give the corresponding thiol.

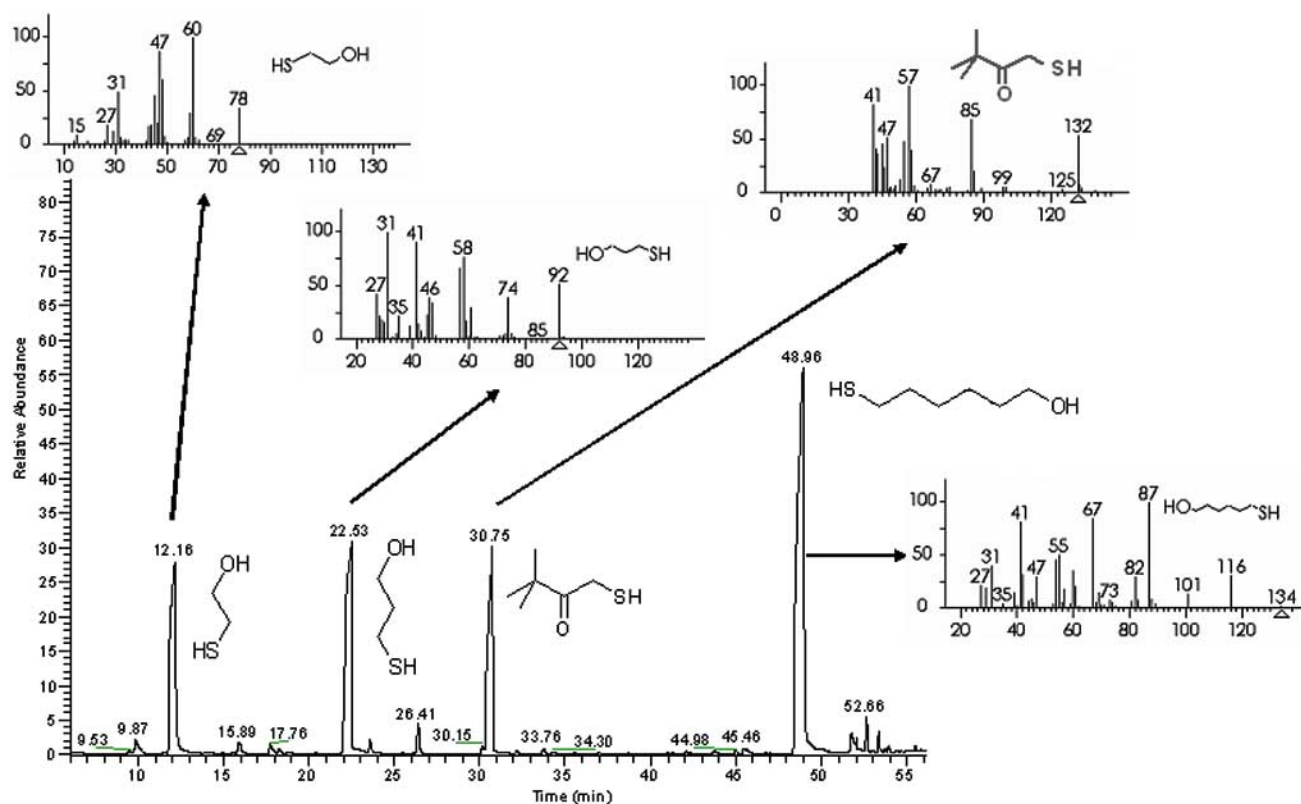


Fig. (4). GC-MS/EI analysis of the thiol derivatives of 2-bromoethanol, 3-bromopropanol, 1-bromopinacolone, and 6-bromohexanol.

Considering the high specificity of the reaction (Fig. 4) and the limited number of starting reagents, low resolution GC-MS/EI analysis was sufficient to identify each thiol. To support the chemical assignments shown in Table 1, all the molecular masses were confirmed by GC-MS/CI (see Fig. 5), mass spectra were compared to standards (NIST database) when possible, and duplicate samples were synthesized in some cases from different precursors or even through different reaction pathways. Moreover, analogs of our previous libraries were used as references to determine a range of retention indices where the new compounds might be detected or to predict potential MS fragmentation profiles.

Table 1 summarizes the five most abundant ions in the GC-MS/EI mass spectra of each polyfunctional thiol. Except for 6-mercaptohexanol (**17**, Fig. 6), 7-mercaptoheptanol (**18**) and 8-mercaptooctanol (**19**) which are prone to eliminate water during fragmentation, the molecular ion was always clearly present. The loss of hydrogen sulfide was also frequently observed. Depending on structure, a few mercaptoalcohols (e.g. 3-mercapto-2-methylpropanol (**13**), 3-mercapto-2,2-dimethylpropanol (**15**), 2-mercaptocyclohexanol (**16**), 6-mercaptohexanol (**17**, Fig. 6), 7-mercaptoheptanol (**18**) and 8-mercaptooctanol (**19**)) underwent two simultaneous rearrangements as indicated by the presence of a fragment ion of $[M-34-18]^+$.

Reviewing Table 1, it appears that most of the synthesized mercaptans exhibit a broth-like odor. However, the sensorial descriptors used to qualify polyfunctional thiols with 2 or 3 carbon atoms were “unpleasant, sulfur, onion,

and rot”, whilst long and linear molecules were often characterized by fresh, fruity, or green/flowery scents.

Because stereochemistry affects smell, it is not surprising that (*R*)-3-mercapto-2-methylpropanol (**13**) was perceived as slightly more pleasant and fruity than its (*S*)-enantiomeric form. On the other hand, the diastereoisomers of 2-mercaptocyclohexanol (**16**) yielding two peaks on the CP-Sil5 CB GC column had the same odor (i.e. broth, mushroom, meat).

All the synthesized mercaptans emerged as strong odorants at the sniffing port. Effectively, their BE-GC-LoADS or olfactometric detection threshold were usually lower than 1.4 ng, a value associated with dimethyltrisulfide which is considered as a very powerful aroma. Interestingly, 3-methyl-2-buten-1-thiol (**20**) with its typical odor of aged beer showed the lowest BE-GC-LoADS determined until now (i.e. 0.00005 ng). As expected, slight threshold differences could be noticed between enantiomers (e.g. 3-(*R/S*)-mercapto-2-methylpropanol (**13**)) or diastereoisomers (e.g. 2-mercaptocyclohexanol (**16**)).

In conclusion, we would like to emphasize that these original results were very easily obtained by taking advantage of two complementary and routine techniques: combinatorial chemistry syntheses and the gas chromatography (identification and characterization by GC-MS and GC-O analyses).

ACKNOWLEDGEMENT

The authors wish to thank the FNRS (Fonds National de la Recherche Scientifique) for financial support.

Table 1. Survey of the Retention Indexes on Apolar and Polar GC Capillary Columns, the Odor, the BE-GC-LoADS, and the 5 Most Abundant Ions in the GC-MS/EI Mass Spectra Associated with Each Synthesized Polyfunctional Thiol

Ri _{CP-Sil 5 CB} ¹	RI _{FFAP} ²	Odor _{CP-Sil 5 CB}	BE-GC-LOADS [ng] _{CP-Sil 5 CB} ³	5 MOST ABUNDANT MS-EI IONS	
MERCAPTOALDEHYDE					
Mercaptoacetaldehyde ^{4,5}				47, 76*, 59, 45, 48	1
658	nd	Sulfur, garbage	0,006		
MERCAPTOKETONE					
Mercaptoacetone ^{4,5}				43, 90*, 47, 45, 42	2
739	1359	Pungent, plastic, sulfur, meat	1,9		
3-Mercapto-2-butanone ^{4,5}				43, 61, 104*, 60, 59	3
768	1295	Broth, blackcurrant	0,6		
1-Mercapto-2-butanone ^{4,5}				57, 47, 104*, 45, 76	4
836	1431	Broth, fresh	0,4		
5-Mercapto-2-pentanone ^{4,5}				46, 41, 118*, 74, 103	5
910	nd	Solvent, sulfur	0,002		
1-Mercapto-3-pentanone ^{4,5,7}				55, 57, 61, 85, 118*	6
947	1517	Cheese, skunky, pungent, greenery	0,009		
1-Mercaptopinacolone ^{4,5,7}				57, 41, 85, 132*, 47	7
954	1435	Sulfur, skunky, pungent, fruity	0,2		
6-Mercapto-2-hexanone ^{4,5}				43, 71, 132*, 55, 81	8
1014	nd	Geranium, greenery	0,03		
MERCAPTOALCOHOL					
2-Mercaptoethanol ^{4,5,6}				78*, 47, 60, 48, 45	9
717	1501	Sewage, H ₂ S, onion, garbage, sulfur	24		
1-Mercapto-2-propanol ^{4,5,7}				45, 48, 59, 74, 49 (92* present)	10
742	1425	Garlic, onion	4,6		
2-Mercaptopropanol ^{4,5,6}				61, 60, 92*, 59, 74	11
769	1485	2-R form: broth, rotten potato	2-R form: 1		
3-Mercaptopropanol ^{4,5,7}				57, 58, 41, 92*, 47	12
840	1665	Broth, potato, sulfur	0,3		
3-Mercapto-2-methylpropanol ^{4,5,7}				72, 41, 57, 55, 47 (106* present)	13
920	1692	3-S form: <i>Allium</i> , broth, soup 3-R form: lemon, raw onion, soup	3-S form: 0,6 3-R form: 3,8		
1-Mercapto-2-methyl-2-propanol ^{4,5}				106*, 41, 64, 78, 45	14
954	1459	Broth, meat	0,5		
3-Mercapto-2,2-dimethylpropanol ^{4,5,7}				55, 43, 86, 73, 41 (120* present)	15
962	1680	Broth, coffee, sulfur	0,004		
2-Mercapto-cyclohexanol ^{4,5,6,8}				81, 80, 114, 41, 132*	16
1085 1097	1765	Broth, mushroom, meat Broth, mushroom, meat	1,5 0,5		
6-Mercaptohexanol ^{4,5,6,7}				87, 67, 55, 60, 41 (134* absent)	17
1176	2084	Mushroom, gas, violet	1,9		
7-Mercaptoheptanol ^{4,5}				87, 101, 55, 67, 68 (148* absent)	18
1285	2083	Carrot, fresh, greenery	1,2		
8-Mercaptooctanol ^{4,5,7}				87, 101, 67, 41, 55 (162* absent)	19
1393	2150	Fruity, greenery	3,3		
UNSATURATED THIOL					
3-Methyl-2-buten-1-thiol ^{4,5,6,7}				41, 69, 102*, 68, 53	20
808	1112	Aged beer, hop, skunky, pungent	0,00005		

^{1,2}RI_{CP-Sil 5 CB} or RI_{FFAP} are respectively the retention index on the apolar CP-Sil 5 CB or polar FFAP capillary columns (see the "analytical methods" section for more details); ³BE-GC-LoADS or Best Estimated-Gas Chromatographic-Lowest Amount Detected by Sniffing is defined as the GC-O perception threshold of an odorant and is given in pentanethiol equivalent (see the "analytical methods" section for more details); ⁴identified by EI mass spectrometry; ⁵identified by CI mass spectrometry; ⁶identified by comparison to NIST database MS spectra; ⁷synthesized from two different precursors (using the Bunte salt reaction or another pathway) and identified by comparison of retention index and MS data in both cases; ⁸separately and specifically synthesized from *R*- or *S*-reagents (the Bunte salt reaction corresponds to a SN₂ inducing an inversion of the initial configuration in the final product), the enantiomers were then individually analyzed by GC; ⁹characterized by different chemical and physical properties, the diastereoisomers were resolved by the GC CP-Sil 5 CB capillary column; *molecular ion.

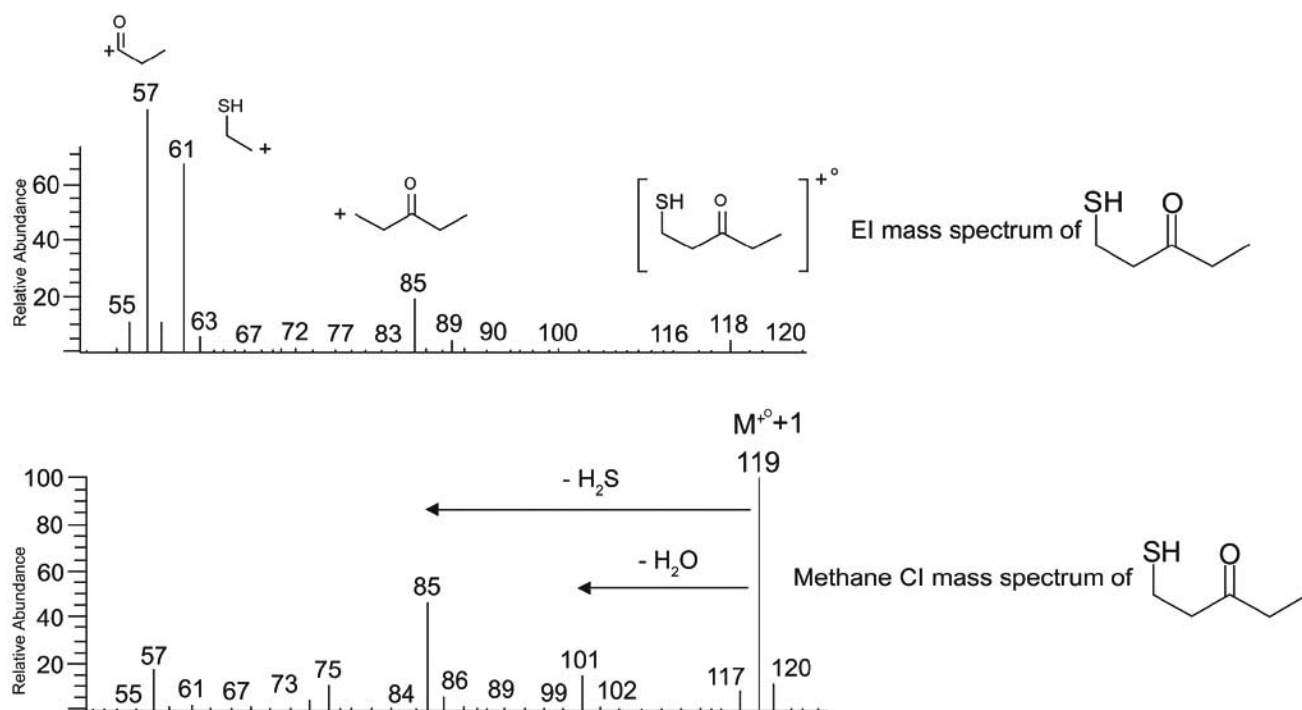


Fig. (5). GC-MS/EI mass spectrum of 1-mercapto-3-pentanone and confirmation of its mass and preliminary identification using GC-MS/CI.

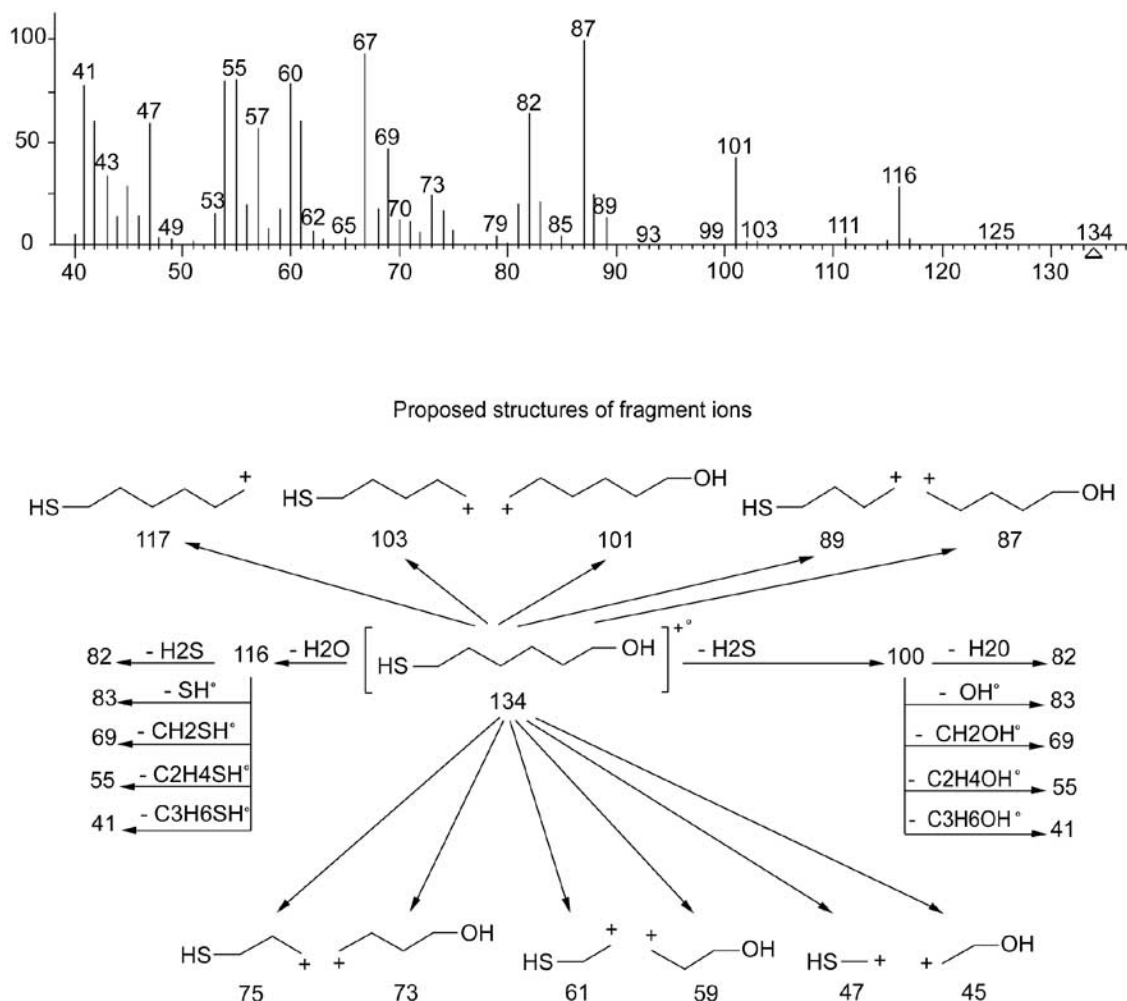


Fig. (6). Interpretation of 6-mercaptohexanol mass spectrometric results and presentation of its potential EI fragmentations or rearrangements.

ABBREVIATIONS

BE-GC-LoADS	=	Best estimated-gas chromatographic-lowest amount detected by sniffing
CI	=	Chemical ionization
DMTS	=	Dimethyltrisulfide
EI	=	Electron ionization
FID	=	Flame ionization detection
GC	=	Gas chromatography
M	=	Molecular mass
MS	=	Mass spectrometry
O	=	Olfactometry
RI	=	Retention index

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