Combinatorial Synthesis and Screening of Novel Odorants Such as Poly-functional Thiols

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\textbf{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.

\textbf{Keywords:} Combinatorial synthesis, Bunte salts, polyfunctional thiols, flavor, aroma.

\section*{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 \textit{et al.} developed a “one-pot synthesis” of \textit{S}-methyl thioesters by reacting methyl chlorothioformate 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 \textit{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 \textit{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 oxidative 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.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{BE-GC-LoADS.png}
\caption{BE-GC-LoADS characterizing polyfunctional thiols in comparison with dimethyltrisulfide.}
\end{figure}

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Mercaptoketones

**Mercaptoaldehydes**

**Mercaptoalcohols**

**Mercaptoketones**

**Mercaptocarboxylates**
MATERIALS AND METHODS

Chemicals

Diethylether (99.8%, [60-29-7]) as well as halides were purchased from Aldrich: 1-bromo-2-butane 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-butanol 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% [23144-52-7], chloroacetaldehyde 50% [107-20-0], chloroacetone 95% [78-95-5], R-3-bromo-

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

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An equimolar quantity of Na$_2$S$_2$O$_3$ (6 mmol) was dissolved of each) were diluted in 5 mL of ethanol in a 25 mL flask. Four halides with different molecular weights (1.5 mmol) were hydrolyzed by H$_2$SO$_4$ to give the corresponding thiol. Expressed in ng, it corresponds to the geometric mean between the lowest mass of compound measured 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 C$_0$) was diluted to obtain a range of solutions with decreasing concentrations ([1/50×C$_0$], [1/100×C$_0$], [1/200×C$_0$], [1/500×C$_0$], [1/1000×C$_0$], [1/2000×C$_0$], [1/5000×C$_0$], 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 × 0.25 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 (CH$_4$ – positive mode) were performed with a TQ 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 Na$_2$S$_2$O$_3$, were hydrolyzed by H$_2$SO$_4$ to give the corresponding thiol.

**Determination of BE-GC-LoADS**

As suggested previously [3, 11], BE-GC-LoADS was used to assess the odor potency of each thiol. 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 Na$_2$S$_2$O$_3$, were hydrolyzed by H$_2$SO$_4$ to give the corresponding thiol.
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-mercaptoctylhexanol (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 $[M-34-18]$.\(^{1}\)

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 odors 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).

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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

<table>
<thead>
<tr>
<th>Ri CP-Sil 5 CB</th>
<th>RI FFAP</th>
<th>Odor CP-Sil 5 CB</th>
<th>BE-GC-LOADS [ng] CP-Sil 5 CB</th>
<th>5 MOST ABUNDANT MS-EI IONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>658</td>
<td>nd</td>
<td>Sulfur, garbage</td>
<td>0.006</td>
<td>47, 76*, 59, 45, 48</td>
</tr>
</tbody>
</table>

**MERCAPTOALDEHYDE**

- Mercaptoacetaldehyde **5**
  - 658
  - Odor CP-Sil 5 CB: Sulfur, garlic
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 0.006
  - 5 MOST ABUNDANT MS-EI IONS: 47, 76*, 59, 45, 48

**MERCAPTOKETONE**

- Mercaptoaceton **5**
  - 739
  - Odor CP-Sil 5 CB: Pungent, plastic, sulfur, meat
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 1.9
  - 5 MOST ABUNDANT MS-EI IONS: 43, 90*, 47, 45, 42

- 3-Mercapto-2-butanol **5**
  - 768
  - Odor CP-Sil 5 CB: Broth, blackcurrant
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 0.6
  - 5 MOST ABUNDANT MS-EI IONS: 43, 61, 104*, 60, 59

- 1-Mercapto-2-butanol **5**
  - 836
  - Odor CP-Sil 5 CB: Broth, fresh
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 0.4
  - 5 MOST ABUNDANT MS-EI IONS: 57, 47, 104*, 45, 76

- 5-Mercapto-2-pentanone **5**
  - 947
  - Odor CP-Sil 5 CB: Solvent, sulfur
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 0.002
  - 5 MOST ABUNDANT MS-EI IONS: 55, 57, 61, 85, 118*

**MERCAPTOALCOHOL**

- 2-Mercaptoethanol **4,5,6**
  - 717
  - Odor CP-Sil 5 CB: Sewage, H2S, onion, garbage, sulfur
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 24
  - 5 MOST ABUNDANT MS-EI IONS: 78*, 47, 60, 48, 45

- 1-Mercapto-2-propanol **4,5,7**
  - 742
  - Odor CP-Sil 5 CB: Garlic, onion
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 4.6
  - 5 MOST ABUNDANT MS-EI IONS: 45, 48, 59, 74, 49 (92* present)

**MERCAPTOPOLYMER**

- 3-Methyl-2-buten-1-thiol **4,5,6,7**
  - 808
  - Odor CP-Sil 5 CB: Aged beer, hop, skunky, pungent
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 0.00005
  - 5 MOST ABUNDANT MS-EI IONS: 41, 69, 102*, 68, 53

**UNSATURATED THIOL**

- 2-Mercaptohexanol **4,5**
  - 1085
  - Odor CP-Sil 5 CB: Broth, mushroom, meat
  - BE-GC-LOADS [ng] CP-Sil 5 CB: 1.5
  - 5 MOST ABUNDANT MS-EI IONS: 81, 80, 114, 41, 132*
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

REFERENCES
