Synthesis and Sensorial Properties of Mercaptoaldehydes

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Mercaptoalcohols and mercaptoketones have been described in the past decade as relevant character-impact compounds in various food products. On the other hand, very little is known of mercapto-aldehydes. Only 3-mercapto-2-methylpentanal and 3-mercaptohexanal have been found in onions and beef liver, respectively. The aim of this work was to synthesize 13 mercaptoaldehydes not commercially available by combinatorial chemistry. Chromatographic analyses of the mixture allowed the determination of their retention indices and mass spectroscopy fragments for further identification in foods. Gas chromatography−olfactometry demonstrated how some of them are characterized by unusual odor and very low perception threshold.

KEYWORDS: **Combinatorial chemistry; polyfunctional thiols; flavors; BE-GC-LOADS; sulfur compounds**

INTRODUCTION

Sulfur compounds such as thiazoles (*1*), thiophenes (*2*), polysulfides (*3*), and thioesters (*4*, *5*) have been investigated for years in many foods. Much less is reported on polyfunctional thiols, mainly because of their commercial unavailability and high reactivity. Thiols are very good nucleophiles. They readily add to double bonds, are liable to form thiyl radicals when exposed to light, and are thermally unstable. In addition, they are present in foods at such low levels that it is necessary to concentrate them prior to analysis. This increases the risk of losing them through oxidation and photochemical and/or thermal degradation, in addition to the usual physical loss of the most volatile compounds.

Careful extraction procedures and the use of a sulfur-selective flame photometric detector have recently led to the discovery of new aliphatic polyfunctional thiols present in trace amounts in food matrices such as wine (*6−9*), passion fruit (*10*, *11*), and olive oil (*12*, *13*). These new molecules impart very strong and characteristic aromas to foods, sometimes with highly desirable notes. Most of them are mercaptoketones, mercapto-alcohols, and mercaptoesters.

As for mercaptoaldehydes, only two have been described: 3-mercapto-2-methylpentanal in onions (*14*) and 3-mercapto-hexanal in beef liver (*15*). In low amounts (5 ppb), the former is characterized by a very pleasant meaty and roasty flavor profile, whereas at high concentration (1 ppm), its flavor is mainly described as sulfuric, pungent, and meaty. An odor threshold value of 0.95 ppb has been measured (*14*). On the other hand, 3-mercaptohexanal exhibits a complex fruity smell (*15*), very different from the "omelette" descriptor used by Badings et al. (*16*) in 1976 to define 3-mercaptobutanal.

In a recent paper (*17*), Vermeulen et al. showed that combinatorial chemistry is a promising technique for characterizing new polyfunctional thiols. They used a selective equimolar chemiluminescence detector to quantify mercaptoketones and mercapto secondary alcohols in a complex synthetic medium, without commercially pure standards. In the present study we have used a similar approach to describe the chromatographic behavior and sensorial properties of mercaptoaldehydes. We have also compared two synthetic pathways in order to sniff and analyze the less polluted sample. Additionally, Strecker degradation of homocysteine was tested as a third means of obtaining 3-mercaptopropanal.

EXPERIMENTAL PROCEDURES

Chemicals. The starting materials 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.

2,3-Butanedione 99%, DL-homocysteine 95%, 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), pentane 98%, diethyl ether 99.8%, 0.2-mm-thick silica plates, and 5,5′-dithiobis(2-nitro-benzoic acid) were provided by Aldrich Chemicals (Bornem, Belgium). 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 afforded by Janssen (Geel, Belgium). Hydrogen sulfide was obtained from Praxair (Antwerp, Belgium). 2,4-Dinitrophenylhydrazine was supplied by Merck (Overijse, Belgium).

Synthesis. *Protocol a (H2S Addition).* Seventy-five milliliters of THF, 12 drops of piperidine, and α,β-unsaturated aldehydes (final individual concentrations = 25 mM) were mixed in a 100-mL three-neck flask. The flask was then immersed in an acetone/liquid N_2 bath at about −15 °C and the mixture stirred magnetically. Hydrogen sulfide was

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[1]
3-Mercaptopropanal

[5]
3-Mercapto-2-methylbutanal

[9]
3-Mercaptohexanal

[13]
3-Mercaptononanal

[2]
3-Mercaptobutanal

[6]
3-Mercaptopentanal

[10]
3-Mercaptoheptanal

[3]
3-Mercapto-3-methylbutanal

[7]
3-Mercapto-2-ethylpropanal

[11]
3-Mercapto-2-butylpropanal

[4]
3-Mercapto-2-methylpropanal

[8]
3-Mercapto-2-methylpentanal

[12]
3-Mercaptooctanal
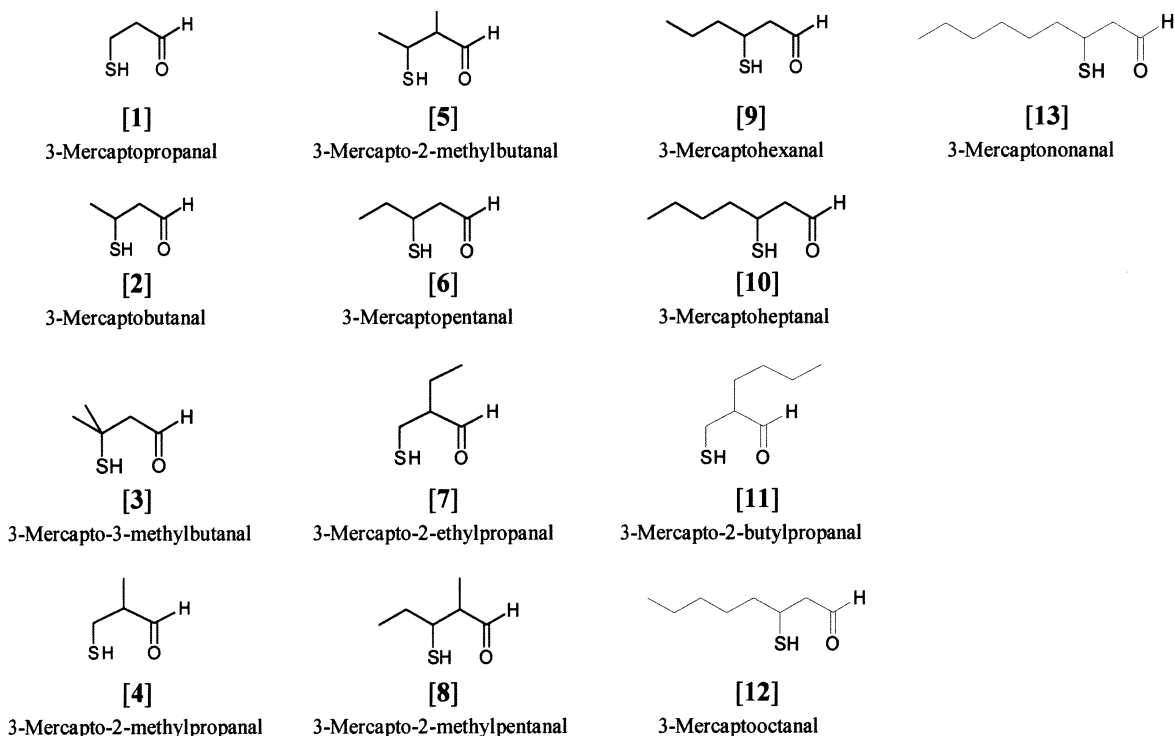
Figure 1. Structures of the 13 synthesized mercaptoaldehydes.
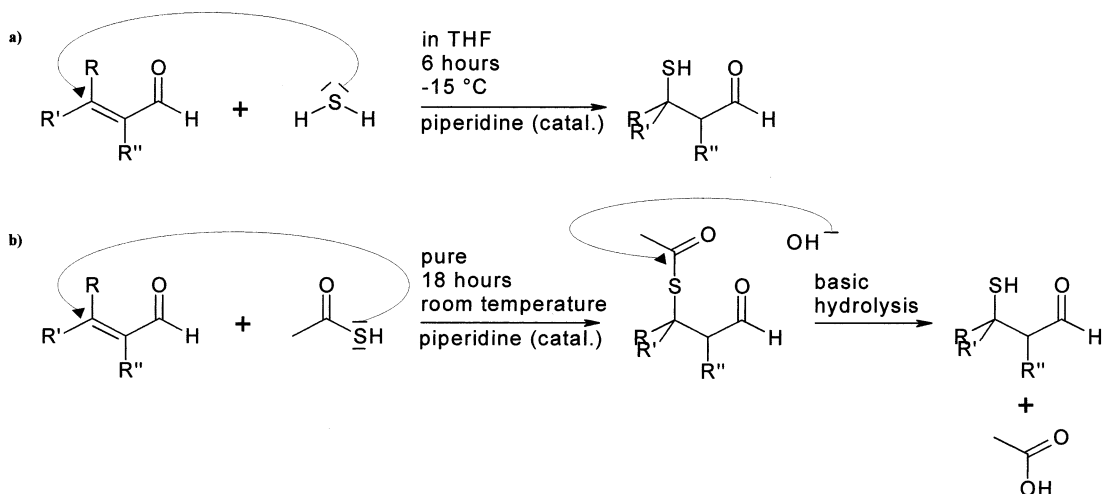
Figure 2. Two synthetic formation pathways of mercaptoaldehydes.
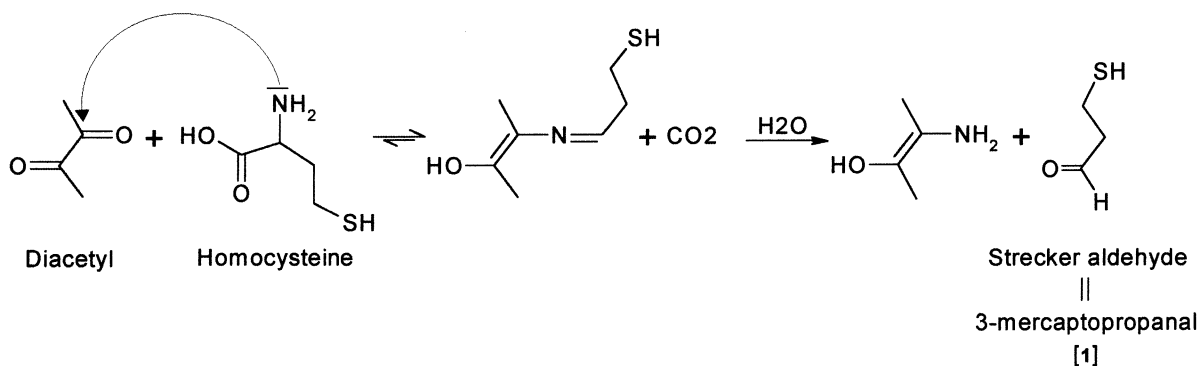
Figure 3. Strecker degradation of homocysteine.

then continuously and gently bubbled into the liquid phase for 6 h. After this period, thiols were detected by thin-layer chromatography (TLC). The solution was stored at −80 °C.

Protocol b (Thioacetic Acid Addition and Basic Hydrolysis). A catalytic quantity of piperidine (12 drops) was added to α,β-unsaturated aldehydes under an inert atmosphere. Next, thioacetic acid (∼15 g, representing a 50% excess) was added dropwise at a temperature between 0 and 10 °C. After addition, the reaction mixture was stirred for another 18 h at room temperature. The mixture was diluted with 100 mL of diethyl ether. The whole was washed first with 20 mL of

Table 1. GC-MS and Sensorial Properties of Mercaptoaldehydes

| | | | Kovats index | | | |
| | name, | eight most intense MS | CP-Sil 5 | | BE-GC- | odor at the |
no.	3- mercapto-	fragments [relative percentages]	CB	FFAP	LOADS (ng)	sniffing port
1	propanal	72 [100]; 90[a] [77]; 62 [74]; 57 [51]; 61 [45]; 47 [45]; 45 [28]; 56 [20]	756	1382	0.06	rotten patatoes, broth
2	butanal	41 [100]; 42 [78]; 86 [65]; 71[51]; 61 [46]; 43 [40]; 104[a] [30]; 70 [28]	803	1372	4	broth, cheese, pungent
3	3-methylbutanal	41 [100]; 56 [79]; 57 [34]; 55 [27]; 59 [26]; 43 [22]; 75 [21]; 84 [20]; 118[a] [15] present	842	1353	0.06	broth, cheese, pungent
4	2-methylpropanal	41[100]; 71 [87]; 42 [66]; 47[45]; 76 [27]; 86 [25]; 104[a] [21]; 45 [23]	826	1400	3	meat, broth, raw bread paste
5	2-methylbutanal	56 [100]; 61 [98]; 85 [92]; 55 [87]; 41 [74]; 100 [45]; 43 [40]; 57 [37]; 118[a] [10] present	892 −	1424[b] 1429[b]	0.002 −	broth, onion, meat, cheese[b] broth, onion, meat, cheese[b]
6	pentanal	41 [100]; 56 [95]; 100 [53]; 55 [48]; 45 [38]; 61 [42]; 43 [42]; 118[a] [35]	910	1476	1	broth, raw onion, flowery
7	2-ethylpropanal	85 [100]; 56 [98]; 55 [98]; 41 [93]; 47 [88]; 57 [61]; 43 [25]; 118[a] [22]	926	1481	13	broth, rotten patatoes, plastic, groundnut
8	2-methylpentanal	70 [100]; 41 [80]; 55 [49]; 75 [30]; 43 [30]; 99 [30]; 47 [25]; 69 [22]; 132[a] [8] present	991[b] 995[b]	1508[b] 1524[b]	0.003[b] 0.1[b]	broth, meat, onion[b] broth, pepper[b]
9	hexanal	55 [100]; 41 [75]; 70 [75]; 42 [669]; 61 [34]; 69 [34]; 81 [32]; 99 [29]; 132[a] [19] present	1004	1560	0.003	citrus fruit peel, fresh
10	heptanal	41 [100]; 69 [81]; 56 [78]; 55 [75]; 43 [45]; 42 [43]; 104 [39]; 61 [39]; 146[a] [9] present	1108	1659	0.03	flowery, citrus fruit peel
11	2-butylpropanal	55 [100]; 57 [98]; 56 [90]; 41 [88]; 43 [54]; 42 [46]; 113 [44]; 84 [39]; 146[a] [4] present	1133	1667	0.4	plastic, rhubarb, pungent
12	octanal	55 [100]; 41 [84]; 70 [70]; 56 [62]; 69 [44]; 57 [42]; 83 [42]; 67 [38]; 160[a] [4] present	1214	1764	0.001	citrus fruit peel, grapefruit, greenery, fresh
13	nonanal	55 [100]; 41 [83]; 70[83]; 43 [72]; 69 [58]; 83 [51]; 56 [46]; 42 [32]; 174[a] [4] present	1320	1873	no data	stale odor, greenery

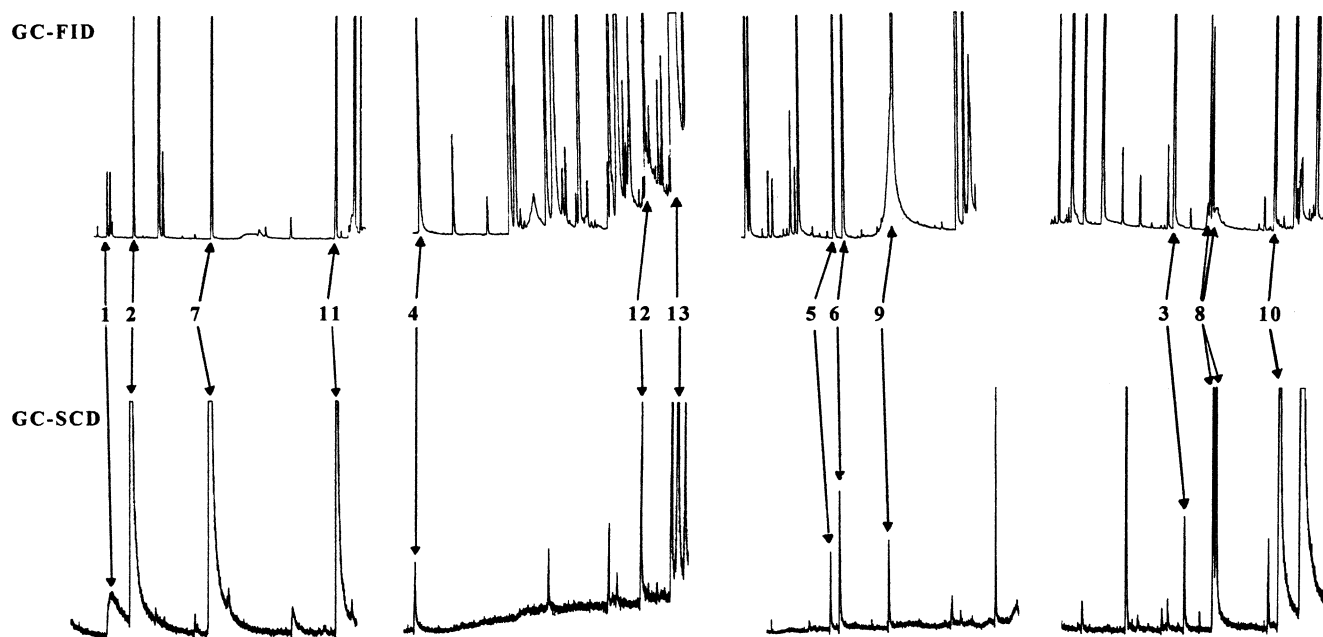[a] Molecular radical cation. [b] The two data correspond to diastereoisomers.

Figure 4. GC-FID and GC-SCD analyses of mercaptoaldehydes in the combinatorial synthetic media (three or four thiols by chromatogram). Numbers in bold correspond to mercaptoaldehydes (see **Figure 1**).

1 N HCl and then twice with 20 mL of saturated sodium bicarbonate solution. Next, 50 mL of 5 N NaOH was added for hydrolysis. The organic phase was separated and the aqueous phase extracted twice with 100-mL portions of diethyl ether. The combined organic phases were washed twice with 20 mL of saturated sodium bicarbonate solution and dried over sodium sulfate, and the solvent was evaporated. The final solution was stored at −80 °C.

Strecker Degradation of Homocysteine. In a 50-mL flask, 3 mL of 2,3-butanedione was mixed with 2 g of DL-homocysteine. After addition of three drops of 5 N NaOH and 2 mL of water, a thick pap was

obtained. The flask was then placed in a warm water bath (40 °C) for 36 h. Finally, 100 mL of distilled water was used to dilute this viscous mixture before filtration on glass filters. The filtrate was extracted with 2 × 20 mL of dichloromethane before concentration to 3 mL in a Snyder−Kuderna system.

Analytical Methods. *Thin-Layer Chromatography.* Pentane/diethyl ether (60:40) was used as chromatographic eluent with 0.2-mm silica plates. The colorless non-UV-absorbing chemicals were revealed with two different reagents: 5,5-dithiobis(2-nitrobenzoic acid) for thiols (*18*) and 2,4-dinitrophenylhydrazine for mercaptoaldehydes (*19*).

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a) 3-Mercaptobutanal [2]:
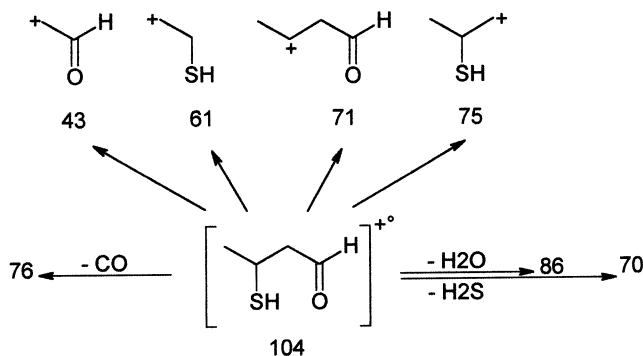
b) 3-Mercapto-2-butylpropanal [11]:
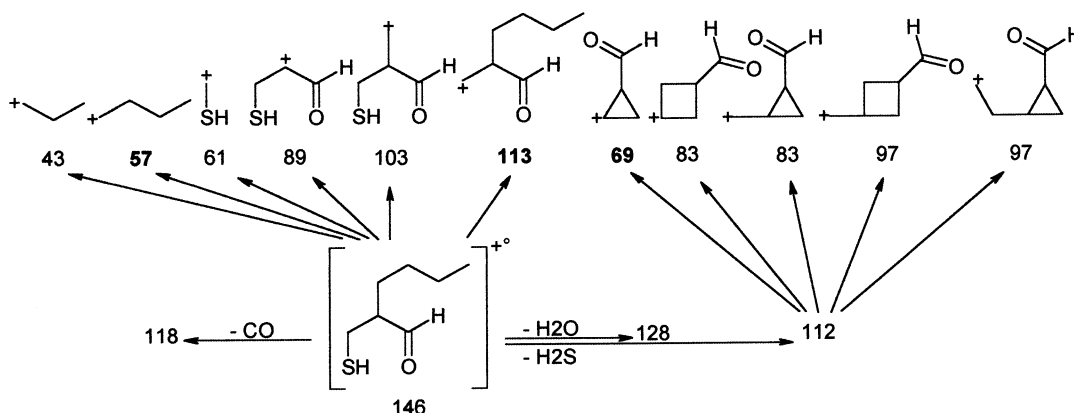
c) 3-Mercaptononanal [13]:

Figure 5. Hypothetical mass spectrometric degradation pathways of three mercaptoaldehydes.
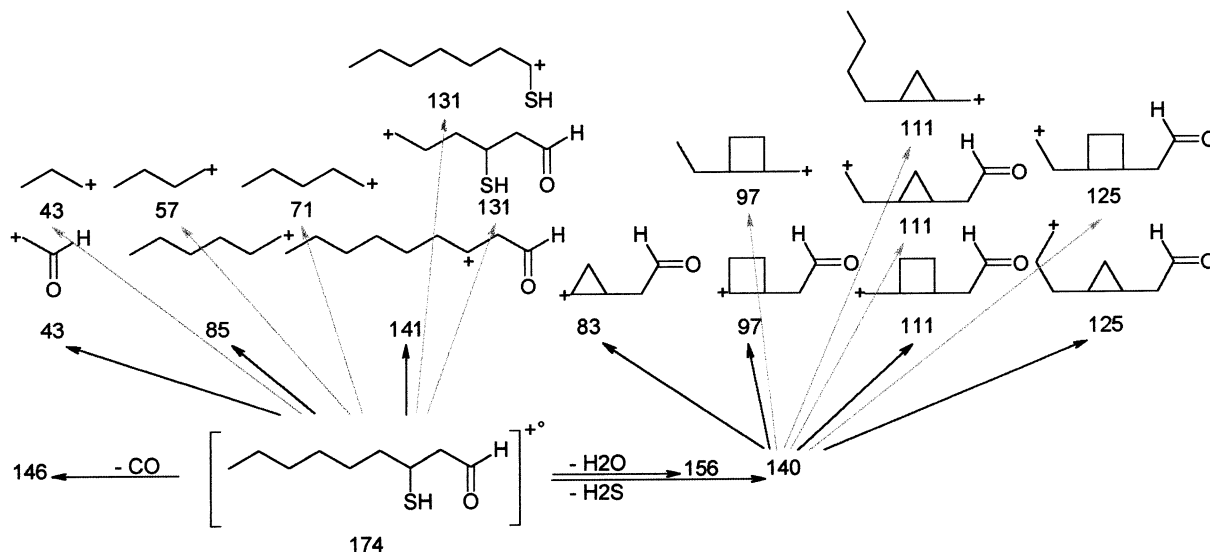
Gas Chromatography Coupled with Sulfur Chemiluminescence Detection (GC-SCD). GC was performed using a Chrompack CP9001 chromatograph equipped with a splitless injector maintained at 250 °C and opened after 0.5 min. Analysis of sulfur compounds was performed using a 50 m × 0.32 mm i.d., wall-coated open tubular (WCOT) apolar CP-SIL 5 CB capillary column (film thickness = 1.2 μm) 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 from 40 to 132 °C at 2 °C/min followed by a rise from 132 to 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 polar FFAP CB capillary column (film thickness = 0.3 μm). Oven temperature, initially kept at 40 °C for 4 min, was programmed to rise from 40 to 132 °C at 2 °C/min and

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thereafter from 132 to 250 °C at 10 °C/min, remaining at the maximum temperature for 15 min. Spectral recording was automatic throughout elution using Xcalibur software. The compounds were identified on the basis of their fragmentation patterns.

Gas Chromatography Coupled with Dual Flame Ionization and Olfactometric Detections (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 apolar CP-Sil 5 CB capillary column (film thickness = 1.2 μm). An initial oven temperature of 40 °C was maintained for 4 min and then programmed to rise from 40 to 132 °C at 2 °C/min followed by a rise from 132 to 250 °C at 10 °C/min. The final temperature was held for 15 min. A 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 Shimidazu C-R3A integrator, while 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 (*20, 21*). 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. (*4*), the best estimated GC−lower 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 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 1/50000 dilution. Two judges working independently performed sensory analysis, and a verbal description of the odor was obtained at the same time.
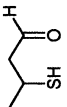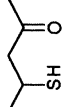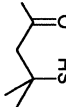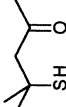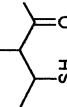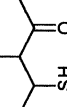
RESULTS AND DISCUSSION

As combinatorial syntheses have proved to be a good way to obtain Kovats indices, mass spectra, and sensorial properties (BE-GC-LOADS) for mercaptoketones and mercapto secondary alcohols, we used here a similar approach to characterize mercaptoaldehydes. Thirteen α,β-unsaturated aldehydes (from three to nine carbon atoms) were converted to the corresponding thiols (**Figure 1**).

To improve reaction yields and minimize cross-reactions, two alternative synthesis procedures were compared. The first was equivalent to the protocol used before for mercaptoketones (*17*), that is, continuous bubbling of hydrogen sulfide through the α,β-unsaturated aldehyde/piperidine/THF mixture (**Figure 2a**). In the second, mixing of pure thioacetic acid with α,β-unsaturated aldehydes led to thioesters, subsequently subjected to basic hydrolysis to thiols and acetic acid (**Figure 2b**). The second procedure resulted in cleaner chromatograms, thus facilitating mass spectroscopy and the olfactory analyses. In the case of 3-mercaptopropanal (**1**), Strecker degradation of homocysteine (**Figure 3**) emerged as a third successful way to obtain GC peaks with retention indices of 756 and 1382 on the CP-Sil 5 CB and FFAP columns, respectively (**Table 1**). It can be emphasized that this latter protocol applied on cysteine did not allow us to produce 2-mercaptoethanol (very rapid hydrogen sulfide loss).

The presence of a sulfur atom in all of our thiols was confirmed by a response at the SCD detector (**Figure 4**), whereas the structure was checked by GC-MS. In all cases (**Table 1**), the molecular ion (M$^{•+}$) was easily recognized on the mass spectrum. Moreover, all molecules were able to lose H$_2$S (M$^{•+}$ − 34) after electron impact bombardment. **Figure 5** shows the hypothetical degradation pathways of three selected compounds, indicating how easily neutral molecules such as H$_2$O (M$^{•+}$ − 18) and CO (M$^{•+}$ − 28) are also lost in subsequent degradations.

Table 2. Retention Indexes, Odors, and BE-GC-LOADS of Mercaptoaldehydes and Related Mercaptomethyl Ketones

Mercaptoaldehydes	Kovats index (CP-Sil 5 CB)	BE-GC-LOADS [ng]	Odor at the sniffing port	Mercaptoketones	Kovats index (CP-Sil 5 CB)	BE-GC-LOADS [ng]	Odor at the sniffing port
[2]	803	4	Broth, cheese, pungent		884	0,069	Greenery, potato, blackcurrant
[3]	842	0.06	Broth, cheese, pungent		915	0.004	Blackcurrant, catty, broom, vinaigrette, citrus fruit
[5]	892	0.002	Broth, onion, meat, cheese		959 [a] 967 [a]	0,098 [a] 0,098 [a]	Sweat [a] Cooked milk [a]

[a] The two data correspond to diastereoisomers.

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As expected, most of the synthesized mercaptoaldehydes were found to possess characteristic cheesy, onion-like flavors. It is noteworthy that the three 3-mercapto-2-methylaldehydes (**4**, **5**, and **8**) are described by panelists as having a meaty odor at the sniffing port. The compounds 3-mercapto-2-methylbutanal (**5**) and 3-mercapto-2-methylpentanal (**8**) were detected as diastereoisomers, at least on the FFAP column, with similar descriptors. 3-Mercaptohexanal (**9**), 3-mercaptoheptanal (**10**), and 3-mercaptooctanal (**12**) were the most interesting thioaldehydes, with fresh citrus fruit or flower descriptors.

Finally, we used the BE-GC-LOADS strategy to assess the olfactive power of the mercaptoaldehydes at the GC-sniffing port (**Table 1**). The mercaptoaldehydes often proved to be 10–1000 times more potent than dimethyl trisulfide (BE-GC-LOADS, 1.4 ng). Their odor profiles and activities were very different from those of related mercaptomethyl ketones (**Table 2**).

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