

Combinatorial Synthesis and Sensorial Properties of 21 Mercapto Esters

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The aim of this work was to determine the chromatographic and sensorial properties of 21 synthetic mercapto acetates, among which two—3-mercaptohexyl-acetate and 3-methyl-3-mercaptobutyl-acetate—have been previously detected in foods, especially passion fruit and wine. Combinatorial chemistry was used to synthesize them from α,β -unsaturated ketones and aldehydes in a very short time. GC-SCD, GC-MS, and GC-olfactometry proved to be very helpful techniques for analyzing them individually in the reaction mixture obtained. Compared to other polyfunctional thiols, mercapto esters show relatively high BE-GC-LoADS values (best estimate—gas chromatography—lowest amount detected by sniffing), with very diverse descriptors.

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

INTRODUCTION

Among other polyfunctional thiols, mercapto esters are known to be relevant in foods and beverages. 3-Mercapto-3-methylbutyl-formate, characterized by an exceptionally low odor threshold (2–5 ppt), has been found in coffee (1–3) and beer (4). According to Schieberle, this black currant odorant may appear in lager beers through aging (4). In 1983, Kolor et al. described ethyl-3-mercapto-propanoate as a fruity/skunky flavor in grape (5). In wine, 2-mercaptoethyl-acetate (roasted meat), 3-mercaptopropyl-acetate (roasted meat), and 3-mercaptohexyl-acetate (fruity) have been identified (6, 7). The *S* form of the latter, which is naturally dominant in passion fruit, is very repellent and sulfury, whereas its *R* enantiomer is rather pleasant and fruity (8). In passion fruit, this acetate is found together with the butanoate, pentanoate, and hexanoate analogues (9) as well as the 3-mercapto-3-methylbutyl-acetate (10).

Very recently we have shown that combinatorial syntheses combined with powerful analytical tools such as GC-MS, GC-SCD and GC-olfactometry make it possible quickly to obtain a large number of mercapto ketones, mercapto aldehydes, and mercapto alcohols (11, 12). This new methodology is applied here to characterize 21 mercapto esters.

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.

3-Buten-2-one (99%), 1-penten-3-one (97%), (*E*)-3-nonen-2-one (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%), acetyl chloride (99+%), piperidine (99%), anhydrous diethyl ether (99+%), and diethyl ether (99.8%) 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 afforded by Janssen (Geel, Belgium).

Synthesis. As previously published by Vermeulen et al. (13), a catalytic quantity of piperidine was added, under an inert atmosphere, to about four α,β -unsaturated aldehydes or ketones (25 mmol/L), which were characterized by different molecular weights. Next, thioacetic acid (a 50% excess) was added dropwise at a temperature in the range of 0–10 °C. 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 LiAlH₄ within 45 min. After the LiAlH₄ excess elimination, the aqueous phase was extracted twice with 20 mL of diethyl ether. The combined organic phases containing mercapto alcohols were washed with saturated sodium bicarbonate solution and dried over sodium sulfate, and the solvent was evaporated.

Five milliliters of the reaction mixture were then diluted with 5 mL of diethyl ether in a 25-mL flask under an inert atmosphere. Then 1 equiv of acetyl chloride was cautiously added, and the mixture was stirred magnetically at 0 °C (ice bath). After 45 min of stirring at room temperature, the new library obtained was stored at –80 °C prior to GC analysis.

Analytical Methods. *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

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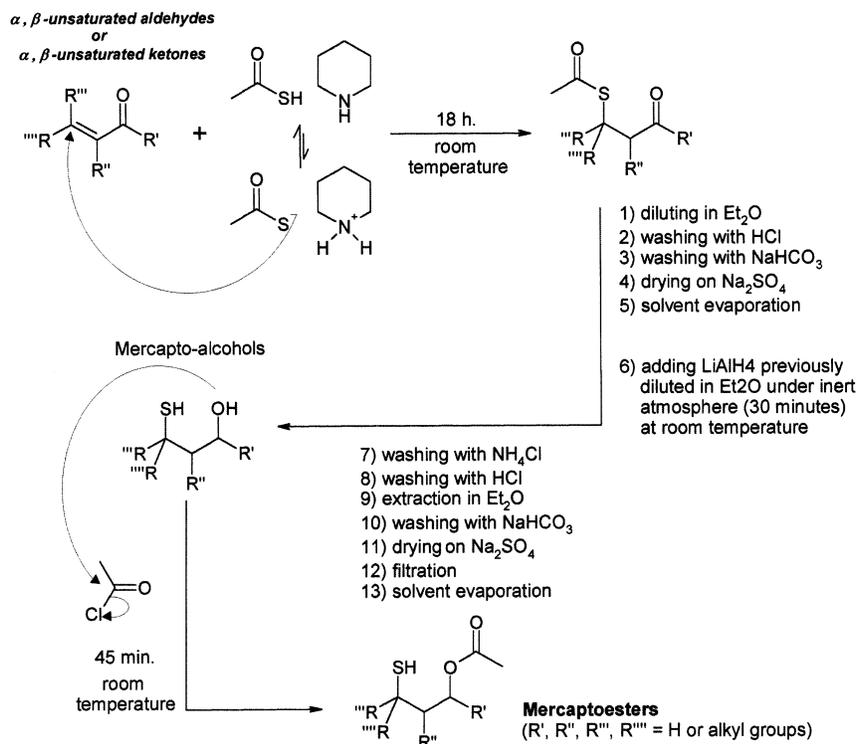


Figure 1. Synthetic pathway followed to obtain mercapto esters from α,β -unsaturated aldehydes or ketones.

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 $^{\circ}\text{C}$ was maintained for 4 min and then programmed to rise to 132 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$ followed by 132–250 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$. 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 $^{\circ}\text{C}$ 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 \times 0.32 mm i.d., WCOT silica polar FFAP CB capillary column (film thickness = 0.3 μm). Oven temperature, initially kept at 40 $^{\circ}\text{C}$ for 4 min, was programmed to rise to 132 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$ and, thereafter, from 132 to 250 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$, 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, which was equipped with a splitless injector maintained at 250 $^{\circ}\text{C}$ and opened after 0.5 min. Sulfur compounds were analyzed using a 50 m \times 0.32 mm i.d., WCOT apolar CP-Sil 5 CB capillary column (film thickness = 1.2 μm). An initial oven temperature of 40 $^{\circ}\text{C}$ was maintained for 4 min and then programmed to rise to 132 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$ followed by 132–250 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$. 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 the FID maintained at 250 $^{\circ}\text{C}$ and connected to a Shimadzu C-R3A integrator, whereas the other part was directed to a GC odor port at 250 $^{\circ}\text{C}$. 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 (14, 15). 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. (16), the best estimated GC lower amount detected by sniffing (BE-GC-LoADS) is defined as the geometric mean

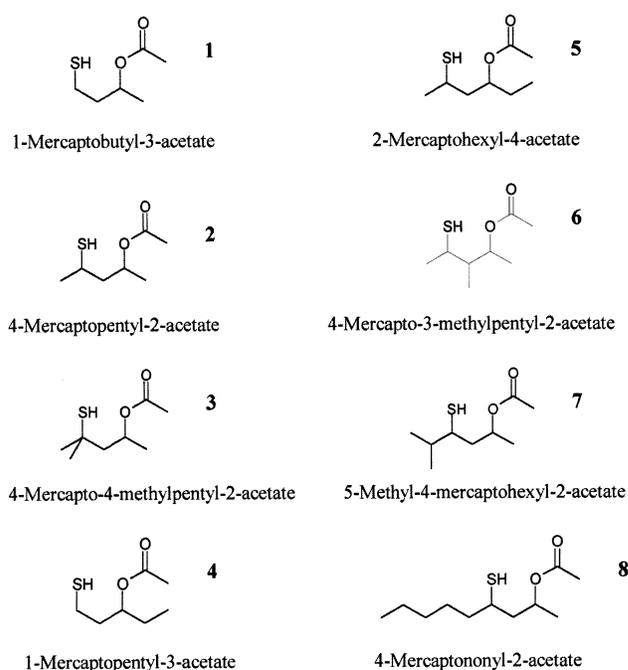


Figure 2. Structure of mercapto esters synthesized from α,β -unsaturated ketones.

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 1/50 000 dilution. Two judges working independently performed sensory analysis, and a verbal description of the odor was obtained at the same time.

RESULTS AND DISCUSSION

Twenty-one mercapto acetates were synthesized by combinatorial chemistry, that is, by “one-pot syntheses” procedure

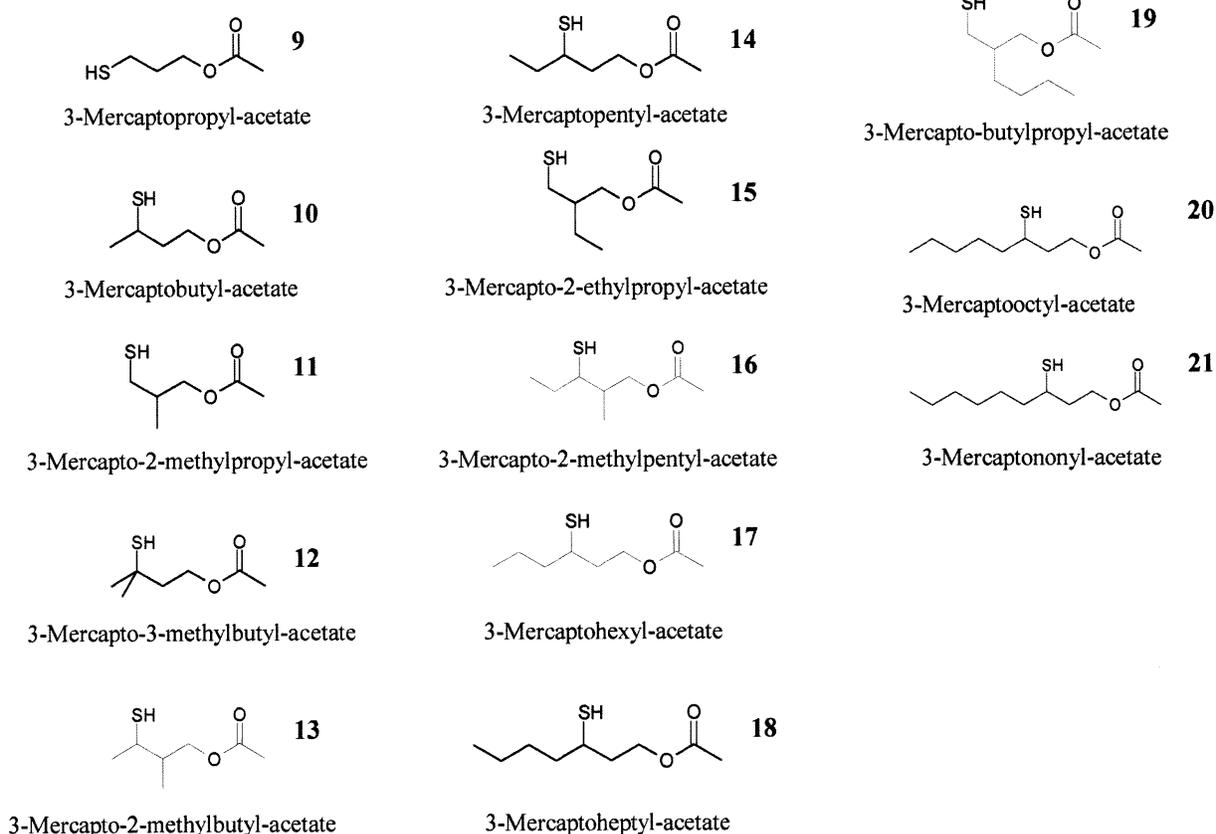


Figure 3. Structure of mercapto esters synthesized from α,β -unsaturated aldehydes.

Table 1. GC-MS and Sensorial Properties of Mercapto Esters Synthesized from α,β -Unsaturated Ketones

no.	name	MS fragments [relative percentage]	Kovats index		BE-GC-LoADS (ng)	odor at the sniffing port
			CP Sii5 CB	FFAP		
1	1-mercaptobutyl-3-acetate	43 [100]; 88 [51]; 55 [45]; 60 [22]; 47 [18] (148 ^a [2] present)	1025	1539	3	onion, plastic, pungent
2	4-mercaptopentyl-2-acetate	43 [100]; 69 [50]; 60 [40]; 102 [40]; 61 [30] (162 ^a absent)	1053 ^b 1063 ^b	1509 ^b 1531 ^b	2 ^b 0.8 ^b	plastic, burned ^b mold, pungent ^b
3	4-mercapto-4-methylpentyl-2-acetate	43 [100]; 83 [58]; 55 [33]; 41 [25]; 118 [25] (176 ^a [3] present)	1096	1518	8	coffee, mocha, burned, vinegar
4	1-mercaptopentyl-3-acetate	43 [100]; 102 [28]; 87 [28]; 55 [28]; 60 [24] (162 ^a [4] present)	1118	1611	4	potato, raw carrot, celery
5	2-mercaptohexyl-4-acetate	43 [100]; 61 [45]; 74 [41]; 116 [34]; 87 [24] (176 ^a absent)	1149 ^b 1157 ^b	1578 ^b 1596 ^b	3 ^b 0.4 ^b	greenery, plastic ^b carrot greenery ^b
6	4-mercapto-3-methylpentyl-2-acetate	43 [100]; 61 [38]; 83 [38]; 116 [38]; 55 [25] (176 ^a absent)	1166 ^b 1170 ^b	1613 ^b 1623 ^b	2 ^b 0.1 ^b	broom, catty, black currant ^b groundnut, broth ^b
7	5-methyl-4-mercaptohexyl-2-acetate	43 [100]; 87 [63]; 55 [47]; 130 [37]; 97 [33] (190 ^a absent)	1224 ^b 1238 ^b	1650 ^b 1675 ^b	8 ^b 5 ^b	rhubarb, lemon, fresh ^b pepper ^b
8	4-mercaptononyl-2-acetate	43 [100]; 55 [40]; 69 [36]; 87 [34]; 102 [32] (218 ^a absent)	1430 ^b 1438 ^b	1857 ^b 1871 ^b	44 ^b 115 ^b	garlic, sweet ^b garlic, sweet, greenery ^b

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

(Figure 1), 8 from α,β -unsaturated ketones (Figure 2) and 13 from α,β -unsaturated aldehydes (Figure 3). Although three successive chemical reactions were required for each of them, "one week/one person" was enough to obtain the complete library.

GC-MS enabled us to tentatively identify the mercaptans individually (Tables 1 and 2). Contrarily to our results with mercapto ketones, mercapto aldehydes, and mercapto alcohols, molecular ions were found for only four compounds (1, 3, 4, and 9). In all cases the major peaks were 43 for $[\text{H}_3\text{CCO}]^+$ and the radical cation resulting from the loss of a neutral acetic acid ($\text{M}^+ - 60$). Simultaneous loss of H_2S was also possible in most cases (Figure 4).

Except for 1, 3, and 4, all mercapto esters issued from unsaturated ketones yielded two peaks as expected on both nonchiral columns, owing to diastereoisomers (Table 1).

The presence of a sulfur atom was checked by GC-SCD. With the help of a simple pentanethiol calibration curve, the same equimolar sulfur detector was also used to quantify all of the mercapto esters present in our library.

The mercapto esters emerged as strong odorants at the sniffing port (Tables 1 and 2). Some reminded us of typical sulfur notes such as onion (1 and 13), garlic (8), potato (4), meat (9), and black currant (6 and 17), whereas others (usually with at least eight carbon atoms) were pleasant with coffee-mocha (3), carrot (21), rhubarb (7 and 20) odors. Only a few mercapto alkyl-

Table 2. GC-MS and Sensorial Properties of Mercapto Esters Synthesized from α,β -Unsaturated Aldehydes

no.	name	MS fragments [relative percentage]	Kovats index		BE-GC-LoADS (ng)	odor at the sniffing port
			CP SII5 CB	FFAP		
9	3-mercaptopropyl-acetate	43 [100]; 74 [79]; 41 [21]; 47 [14]; 61 [14] (134 ^a [3] present)	992	1565	0.9	grilled, roasted meat
10	3-mercaptobutyl-acetate	43 [100]; 88 [58]; 55 [46]; 59 [25]; 61 [21] (148 ^a absent)	1035	1559	0.9	pungent, stinging nettle, hazelnut
11	3-mercapto-2-methylpropyl-acetate	43 [100]; 88 [58]; 55 [40]; 47 [17]; 41 [17] (148 ^a absent)	1051	1583	11	plastic, roasted hazelnut
12	3-mercapto-3-methylbutyl-acetate	69 [100]; 43 [86]; 41 [46]; 102 [39]; 75 [19] (162 ^a absent)	1082	1568	0.3	green pepper, vinegar, plastic
13	3-mercapto-2-methylbutyl-acetate	43 [100]; 61 [47]; 69 [40]; 60 [40]; 102 [40] (162 ^a absent)	1119 ^b 1122 ^b	1612 ^b 1619 ^b	2 ^b 2 ^b	roasted meat ^b onion, vinegar, fruity ^b
14	3-mercaptopentyl-acetate	43 [100]; 73 [60]; 102 [57]; 69 [45]; 41 [37] (162 ^a absent)	1144	1649	1	exotic fruit, candy
15	3-mercapto-2-ethylpropyl-acetate	43 [100]; 102 [38]; 60 [38]; 69 [35]; 87 [25] (162 ^a absent)	1154	1665	11	vinagrette
16	3-mercapto-2-methylpentyl-acetate	43 [100]; 74 [51]; 41 [34]; 116 [34]; 83 [34] (176 ^a absent)	1207	1687 ^b 1692 ^b	0.07	rhubarb, exotic fruit, candy
17	3-mercaptohexyl-acetate	43 [100]; 55 [50]; 88 [47]; 116 [40]; 63 [40] (176 ^a absent)	1227	1727	0.02	candy, black currant, passion fruit
18	3-mercaptoheptyl-acetate	43 [100]; 69 [46]; 88 [46]; 55 [29]; 41 [27] (190 ^a absent)	1324	1823	0.06	onion, exotic fruit, candy
19	3-mercapto-2-butylpropyl-acetate	43 [100]; 55 [40]; 41 [30]; 60 [30]; 130 [25] (190 ^a absent)	1331	1835	1	greenery
20	3-mercaptooctyl-acetate	43 [100]; 88 [76]; 55 [40]; 41 [32]; 69 [32] (204 ^a absent)	1425	1930	0.9	citrus fruit peel, rhubarb, carrot
21	3-mercaptononyl-acetate	43 [100]; 88 [80]; 55 [40]; 41 [36]; 69 [30] (218 ^a absent)	1526	>2000	15	carrot, sweet

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

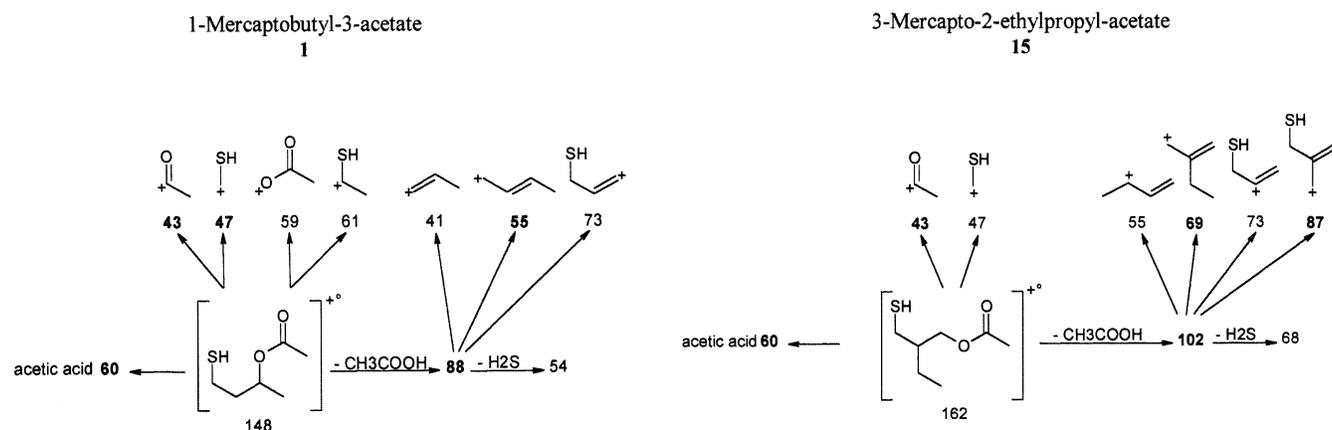


Figure 4. Hypothetical mass spectrometric degradation pathways of mercapto esters.

acetates revealed upon exhalation the odor of the corresponding alcohol (20 and 21). Among the mercapto esters here analyzed at the sniffing port, two have been previously described by usual sensorial analyses. The descriptors issued from both techniques were similar: grilled, roasted meat for 3-mercaptopropyl-acetate and black currant, tropical fruit for 3-mercaptohexyl-acetate (7–9, 17, 18). As for the latter, noteworthy is its similarity with the descriptors given by Werkhoff et al. for 3-mercaptohexyl-butanolate, -pentanoate, and -hexanoate.

Similarly to most sulfur compounds (e.g., 1.4 ng for dimethyl trisulfide), mercapto alkyl-acetates are characterized by low BE-GC-LoADS (from 0.02 to 11 ng, except 8 with 44 and 115 ng). However, for the 21 compounds, these threshold values are significantly higher than those previously determined for most mercapto alcohols [from 0.0001 to 0.06 ng (13)] or mercapto aldehydes [from 0.001 to 13 ng (12)]. Slight threshold differences can also be noticed between some diastereoisomers (2 and 5–8). The lowest value is here obtained with 3-mer-

captohexyl-acetate, for which Tominaga et al. published a 4 ppt odor threshold in a 12% ethanolic solution (19).

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