

Identification of New Sulfur-Containing Volatiles in Yellow Passion Fruits (*Passiflora edulis f. flavicarpa*)

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Capillary gas chromatographic analysis of aroma extracts obtained by simultaneous distillation-extraction from yellow passion fruits (*Passiflora edulis f. flavicarpa*) using a flame photometric detector revealed the presence of previously unreported sulfur-containing volatiles. 3-Mercaptohexanol and the acetates, butanoates, and hexanoates of 3-mercaptohexanol and 3-(methylthio)hexanol, respectively, were identified by comparison of chromatographic and mass spectral data with those of synthesized reference compounds. Approximate amounts of these trace constituents in freshly harvested fruits and in commercial passion fruit products were determined. The newly identified compounds, especially the acetates, exhibit powerful sensory properties.

INTRODUCTION

Due to their unique and delicate flavor, species of the genus *Passiflora* have been the subject of intensive research activities resulting in the characterization of a broad spectrum of volatile constituents (Whitfield and Last, 1986). One of the chemical classes standing out of the diverse mixture of components identified are sulfur-containing volatiles. 3-(Methylthio)hexanol and a mixture of *cis*- and *trans*-2-methyl-4-propyl-1,3-oxathiane and the corresponding oxide, 2-methyl-4-propyl-1,3-oxathiane 3-oxide (Winter et al., 1976; Winter, 1980), have been described as key ingredients for the aroma of the yellow passion fruit (*Passiflora edulis f. flavicarpa*). A strong influence of the configuration of these chiral volatiles on their sensory properties has been demonstrated (Heusinger and Mosandl, 1984; Pickenhagen and Brönnner-Schindler, 1984; Mosandl and Heusinger, 1985). The naturally occurring configuration of *cis*-2-methyl-4-propyl-1,3-oxathiane in the yellow passion fruit has been determined (Singer et al., 1986).

In the course of our own studies of passion fruit constituents [for references see Whitfield and Last (1986)] we noticed the presence of several sulfur-containing volatiles different from those previously reported. Considering the potential of sulfur compounds as flavor impact constituents (Demole et al., 1982), we decided to pursue the investigation of these unknown components. This paper reports the identification of seven new sulfur-containing volatiles and their (semi)quantification in yellow passion fruits and products thereof.

EXPERIMENTAL PROCEDURES

Materials. Freshly harvested yellow passion fruits were obtained by air freight from Brazil. They were analyzed within 24 h after arrival. Concentrates were supplied in frozen state by a commercial passion fruit juice producer.

Sample Preparation. Three hundred grams of fruit pulp was homogenized with 1 L of phosphate buffer solution (pH 6.8) for 30 s. The homogenate was subjected to simultaneous distillation-extraction for 2 h using pentane/ether (1:1) as solvent (Schultz et al., 1977). The aroma extract was dried over Na₂SO₄ and carefully concentrated to a volume of 0.3 mL by using a Vigreux column. The same procedure was carried out with 200 g of concentrate.

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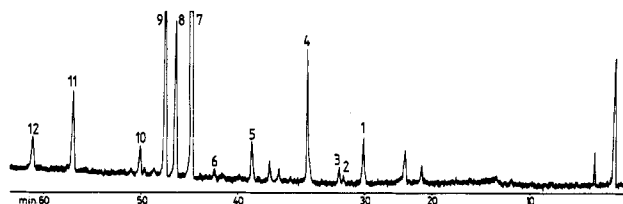


Figure 1. Capillary gas chromatogram (column 1) of an aroma extract (distillation-extraction) from yellow passion fruits using a flame photometric detector (for conditions, see Experimental Procedures). (1) *cis*-2-Methyl-4-propyl-1,3-oxathiane, 4-methyl-5-vinylthiazole; (2) *trans*-2-methyl-4-propyl-1,3-oxathiane; (3) ethyl 3-(methylthio)propanoate; (9) 3-(methylthio)hexanol. For other peaks see Table II.

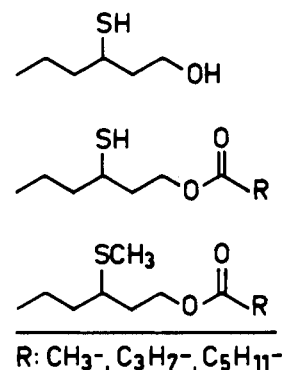


Figure 2. Compounds synthesized and identified as naturally occurring in yellow passion fruits.

Adsorption Chromatography. The aroma concentrate was placed on a water-cooled column (200 × 9 mm i.d.) filled with 5 g of silica gel (activity II-III, Merck 7734). Fractionation of the compounds was achieved with the following series of solvents: 40 mL of (I) pentane, (II) pentane/CH₂Cl₂ (9:1), (III) pentane/CH₂Cl₂ (2:1), (IV) pentane/CH₂Cl₂ (1:2), (V) pentane/ether (9:1), and (VI) ether. The fractions were concentrated to volumes of 0.3 mL and subjected to GC and GC/MS investigations.

Capillary Gas Chromatography. Column 1 was a Carbowax 20M glass capillary column (25 m × 0.25 mm i.d.; WGA, Düsseldorf, Germany) installed in a Carlo Erba gas chromatograph Fractovap Series 4100, equipped with a sulfur-selective flame photometric detector (SSD, control Model 250) and split injector (1:20). The injector temperature was 220 °C and the detector temperature 230 °C. The carrier gas was helium at 2 mL/min. The temperature program was 70–180 °C at 2 °C/min.

Column 2 was a DB-Wax fused silica column (60 m × 0.32 mm

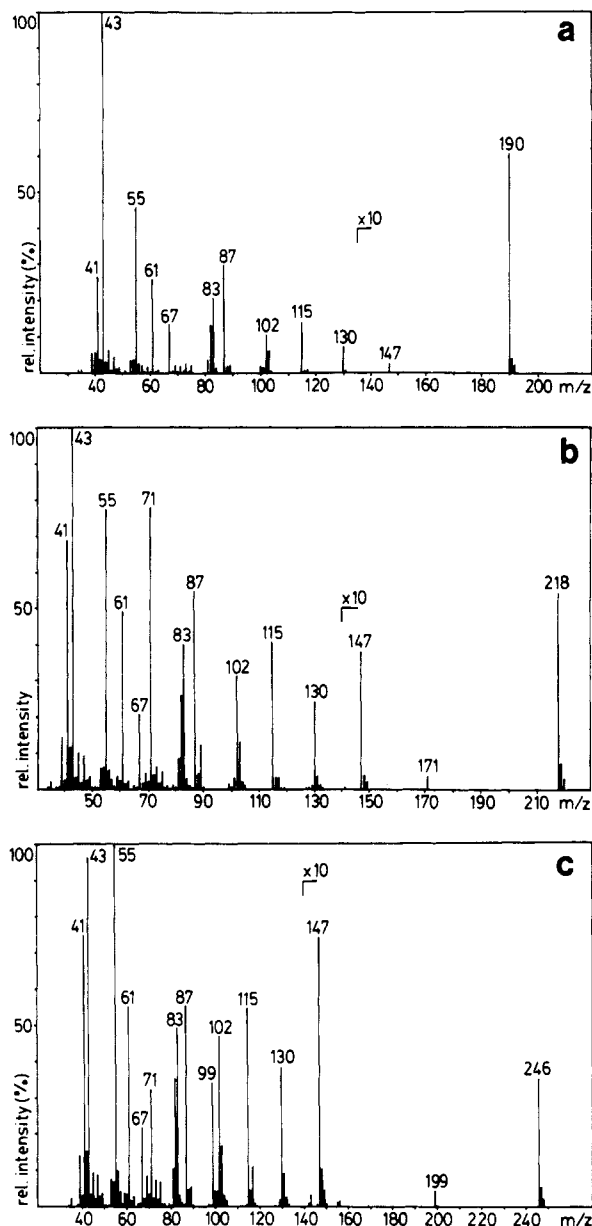


Figure 3. Mass spectra of (a) 3-(methylthio)hexyl acetate; (b) 3-(methylthio)hexyl butanoate; (c) 3-(methylthio)hexyl hexanoate.

i.d.; J&W Scientific Inc.) installed in a Carlo Erba gas chromatograph Fractovap Series 2050 with FID and split injector (1:25). The injector temperature was 200 °C and the detector temperature 220 °C. The carrier gas was helium at 2 mL/min. The temperature program was 70–230 °C at 2 °C/min.

Column 3 was a DB-210 fused silica column (60 m × 0.32 mm i.d.; df = 0.25 μm; J&W Scientific) installed in a Carlo Erba gas chromatograph Series 2900 equipped with FID and split injector (1:20). The injector temperature was 230 °C and the detector temperature 250 °C. The carrier gas was hydrogen at 2.5 mL/min. The temperature program was 70–230 °C at 2 °C/min.

Gas Chromatography/Mass Spectrometry. Columns 2 and 3, respectively, were coupled to a double-focusing mass spectrometer CH 5-DF (Varian, Bremen, Germany) equipped with a MS-Data System DP 10 (AMD, Intetra, Harpstedt, Germany) installed in a SAM 68K (KWS, Karlsruhe, Germany). The ionization voltage was 70 eV and the ion source temperature 180 °C. The mass range (m/z) was 30–400 and the speed 1 scan/s.

Quantitative Analysis. The quantification was based on hexanethiol, which was added as external standard (15 μg) to the concentrated aroma extracts obtained by distillation–extraction. Peak areas were compared using a computing integrator (Spectra Physics, SP 4100).

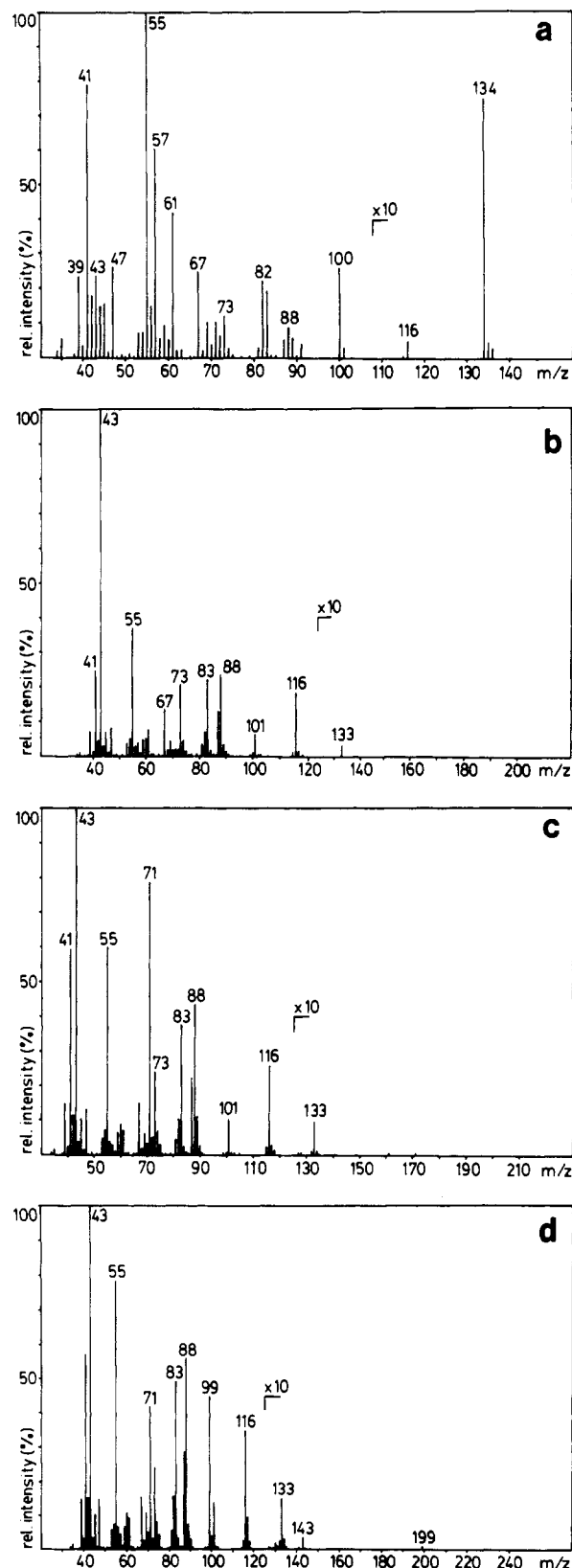


Figure 4. Mass spectra of (a) 3-mercaptohexanol; (b) 3-mercaptohexyl acetate; (c) 3-mercaptohexyl butanoate; (d) 3-mercaptohexyl hexanoate.

¹H NMR Spectroscopy. ¹H NMR spectra were recorded on a Bruker WH 270 spectrometer at 270 MHz in CDCl₃ solutions using tetramethylsilane as internal standard.

Reference Compounds. 3-Mercaptohexanol and 3-(methylthio)hexanol were synthesized by addition of H₂S and CH₃SH, respectively, to (*E*)-2-hexenal and subsequent reduction with NaBH₄ as described by Winter et al. (1976). The esterification

Table I. Chromatographic and Mass Spectral Data of the Diesters Obtained from 3-Mercaptohexanol by Reaction with Acid Chlorides

compound	KI DB-Wax	MW	fragmentation <i>m/z</i> (rel intensity, %)
3-(acetylthio)hexyl acetate	1947	218	43 (100), 115 (17), 55 (16), 116 (9), 83 (9), 41 (8), 88 (7), 158 (6), 73 (6), 87 (5)
3-(butanoylthio)hexyl butanoate	2214	274	71 (100), 43 (69), 115 (16), 41 (15), 55 (9), 83 (7), 186 (5), 116 (5), 72 (5), 153 (2)
3-(hexanoylthio)hexyl hexanoate	2608	330	99 (100), 43 (91), 71 (55), 115 (30), 55 (23), 41 (22), 116 (11), 83 (10), 100 (7), 117 (5)

Table II. Chromatographic Data and Approximate Distributions of Sulfur-Containing Passion Fruit Volatiles

compound	peak ^a	LSC	KI		fruits	approximate concentration, ppb					
			DB-Wax	DB-210		concentrates					
						a	b	c	d	e	f
3-mercaptohexanol	7	VI	1795	1352	38	195	61	109	47	22	1
3-mercaptohexyl acetate	5	III	1675	1570	2	1	2	2	0.4	0.5	0.1
3-mercaptohexyl butanoate	8	III	1821	1734	2	2	7	2	1	1	0.5
3-mercaptohexyl hexanoate	11	III	2025	1940	1	1	4	1	2	1	0.5
3-(methylthio)hexanol	9	VI	1843	1457	71	16	19	3	21	1	1
3-(methylthio)hexyl acetate	6	IV	1749	1670	0.1	0.1	0.3	- ^b	-	-	-
3-(methylthio)hexyl butanoate	10	IV	1892	1833	0.3	0.2	1	-	-	-	-
3-(methylthio)hexyl hexanoate	12	IV	2095	2040	0.5	0.3	1	-	-	-	-

^a The peak numbers correspond to the chromatogram in Figure 1. ^b Not detected.

of the formed alcohols was carried out by reaction with the corresponding acid chlorides in CCl₄ for 30 min at room temperature according to the procedure reported by Heusinger and Mosandl (1984). Mass spectra of these esters are shown in Figures 3 and 4. ¹H NMR data (270 MHz, CDCl₃): for 3-mercaptohexyl acetate δ 4.25 (2 H, t, *J* = 6.7 Hz, H₂COCO), 2.88 (1 H, m, HCS), 2.05 (3 H, s, H₃CCO), 2 (1 H, m), 1.6 (5 H, m), 1.38 (1 H, d, *J* = 7.3 Hz, CSH), 0.92 (3 H, t, *J* = 6.9 Hz, H₃CCH₂); for 3-(methylthio)hexyl acetate δ 4.23 (2 H, t, *J* = 6.7 Hz, CH₂OCO), 2.6 (1 H, m, HCS), 2.05 (3 H, s, H₃CCO), 2.02 (3 H, s, H₃CS), 1.9 (2 H, m), 1.5 (4 H, m), 0.92 (3 H, t, *J* = 7.2 Hz, H₃CCH₂). *cis*- and *trans*-2-methyl-4-propyl-1,3-oxathiane were synthesized by condensation of 3-mercaptohexanol with acetaldehyde as described by Winter et al. (1976).

RESULTS AND DISCUSSION

Volatile constituents were isolated from yellow passion fruits by means of simultaneous distillation-extraction. Subsequent capillary gas chromatographic analysis was carried out by employing not only a conventional flame ionization detector (FID) but also a sulfur-selective flame photometric detector (FPD). This detection mode (Figure 1) revealed the presence of sulfur-containing volatiles other than the previously described 3-(methylthio)hexanol (peak 9), the isomeric 2-methyl-4-propyl-1,3-oxathianes (peaks 1 and 2), 4-methyl-5-vinylthiazole (peak 1), and ethyl 3-(methylthio)propanoate (peak 3).

Synthesis of Reference Compounds. Our search for potential candidates for the unknown sulfur compounds was guided by two aspects: (i) 3-Mercaptohexanol has been used as building block for the synthesis of the stereoisomeric 2-methyl-4-propyl-1,3-oxathianes (Winter et al., 1976). This alcohol might play a similar role in the biogenesis of the oxathianes, and its presence in passion fruits seemed plausible. (ii) Esters are the most abundant class of passion fruit volatiles. Short-chain even-numbered acids are major moieties in these esters (Winter and Klöti, 1972; Murray et al., 1972; Engel and Tressl, 1983). These characteristic features of the passion fruit aroma led us to synthesize the compounds shown in Figure 2 and to check for their natural occurrence in the passion fruit extract.

The reference samples were synthesized according to established procedures (Winter et al., 1976; Heusinger and Mosandl, 1984). Their mass spectra are presented in

Figures 3 and 4. The structures of 3-(methylthio)hexyl acetate and 3-mercaptohexyl acetate were further confirmed by means of ¹H NMR spectroscopy.

In accordance with the results reported by Heusinger and Mosandl (1984) the reaction of 3-mercaptohexanol with acid chlorides in CCl₄ at room temperature yielded the corresponding 3-mercaptohexyl esters as the main products. The additional esterification of the thiol group is only a minor reaction; chromatographic and mass spectral data of these byproducts are listed in Table I.

Identification. The retention times of the synthesized reference compounds matched those of the unknown constituents detected by means of gas chromatography using the FPD mode (Figure 1). Their final gas chromatographic/mass spectrometric identification in yellow passion fruits was achieved by applying the following procedure: (a) pre-separation of the aroma extract by liquid-solid chromatography on silica gel to reduce overlapping of the sulfur-containing trace constituents by major components (the distribution of the sulfur compounds in the fractions obtained is listed in Table II); (b) determination of the KI values of the synthesized reference compounds on two different stationary phases (Table II); (c) mass spectrometric screening of the LSC fractions at these retention indices.

To the authors' knowledge 3-mercaptohexanol and the acetates, butanoates, and hexanoates of 3-(methylthio)hexanol and 3-mercaptohexanol, respectively, have been described for the first time as naturally occurring volatiles. As can be seen in Figure 1, there still remain unidentified sulfur-containing passion fruit constituents. The retention times of peaks 3 and 4 in Figure 1 matched those of synthesized 3-mercaptohexanol and 3-(methylthio)hexanol, recently identified in tomato paste (Buttery et al., 1990). However, capillary gas chromatographic/mass spectrometric confirmation could not be achieved.

(Semi)quantification. The approximate amounts of the new sulfur compounds in yellow passion fruits (Table II) were determined by means of comparison of their peak areas in the FPD chromatogram with hexanethiol, which was added to the extract as external standard. This quantification procedure did not involve a determination of calibration factors considering differences in the ex-

traction efficiencies and the gas chromatographic responses. Therefore, it has to be pointed out that the values presented in Table II are only meant to give a rough idea of the concentrations and the relative distribution of the new sulfur compounds.

The procedure was also applied to passion fruit products. It is interesting to note the variations in the distribution of the sulfur-containing volatiles in concentrates, commercially employed as intermediates in the production of passion fruit beverages (Table II).

Sensory Aspects. The flavor intensities of the passion fruit concentrates correlated well with the detected differences in the patterns of sulfur volatiles; e.g., concentrate a possessed a strong, typical passion fruit aroma, whereas concentrate f was perceived as flat and stale. The sensory properties of 3-(methylthio)hexanol and 3-mercaptohexanol including the influence of their configurations on the odor qualities have been described in detail (Winter et al., 1976; Heusinger and Mosandl, 1984). The newly identified esters exhibited odor profiles typical for sulfur compounds: unpleasant at higher concentrations and very fruity and typical for tropical fruits in appropriate dilutions. The mercapto esters turned out to be more powerful than the corresponding methylthio compounds; the aroma intensities decreased from acetates to hexanoates. 3-(Methylthio)hexyl acetate exhibits aroma notes strongly reminiscent of grapefruit; 3-mercaptohexyl acetate is extremely fruity, suggestive of passion fruit, with a Riesling type note. Detailed investigations of the sensory properties and determinations of odor thresholds are currently in progress.

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LITERATURE CITED

- Buttery, R. G.; Teranishi, R.; Flath, R. A.; Ling, L. C. Identification of Additional Tomato Paste Volatiles. *J. Agric. Food Chem.* **1990**, *38*, 792-795.
- Demole, E.; Enggist, P.; Ohloff, G. 1-p-Menthene-8-thiol: A Powerful Impact Constituent of Grapefruit Juice (*Citrus paradisi* MACFAYDEN). *Helv. Chim. Acta* **1982**, *65*, 1785-1794.
- Engel, K.-H.; Tressl, R. Differentiation of Yellow and Purple Passion Fruits by Investigation of their Ester Composition. *Chem. Mikrobiol. Technol. Lebensm.* **1983**, *8*, 33-39.
- Heusinger, G.; Mosandl, A. *Tetrahedron Lett.* **1984**, *25*, 507-510.
- Mosandl, A.; Heusinger, G. 1,3-Oxathianes, Chiral Fruit Flavour Compounds. *Justus Liebigs Ann. Chem.* **1985**, 1185-1191.
- Murray, K. E.; Shipton, J.; Whitfield, F. B. The Chemistry of Food Flavour. 1. Volatile Constituents of Passionfruit, *Passiflora edulis*. *Aust. J. Chem.* **1972**, *25*, 1921-1933.
- Pickenhagen, W.; Brönnner-Schindler, H. Enantioselective Synthesis of (+)- and (-)-cis-2-Methyl-4-propyl-1,3-oxathiane and their Olfactory Properties. *Helv. Chim. Acta* **1984**, *67*, 947-952.
- Schultz, T. H.; Flath, R. A.; Mon, T. R.; Eggling, S. B.; Teranishi, R. Isolation of Volatile Components from a Model System. *J. Agric. Food Chem.* **1977**, *25*, 446-449.
- Singer, G.; Heusinger, G.; Fröhlich, O.; Schreier, P.; Mosandl, A. Chirality Evaluation of 2-Methyl-4-propyl-1,3-oxathiane from the Yellow Passion Fruit. *J. Agric. Food Chem.* **1986**, *34*, 1029-1033.
- Whitfield, F. B.; Last, J. H. The flavour of the passion fruit—a review. In *Progress in Essential Oil Research*; Brunke, E. J., Ed.; de Gruyter: Berlin, 1986; pp 3-48.
- Winter, M. U.S. Patent 4,220,561, Sept 2, 1980; *Chem. Abstr.* **1981**, *94*, 84141y.
- Winter, M.; Klöti, R. *Helv. Chim. Acta* **1972**, *55*, 1916-1921.
- Winter, M.; Furrer, A.; Willhalm, B.; Thommen, W. Identification and Synthesis of two New Organic Sulfur Compounds from the Yellow Passionfruit (*Passiflora edulis f. flavicarpa*). *Helv. Chim. Acta* **1976**, *59*, 1613-1620.

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Registry No. 3-Mercaptohexanol, 51755-83-0; 3-(methylthio)hexanol, 51755-66-9; 3-mercaptohexyl acetate, 136954-20-6; 3-mercaptohexyl butyrate, 136954-21-7; 3-mercaptohexyl hexanoate, 136954-22-8; 3-(methylthio)hexyl acetate, 51755-85-2; 3-(methylthio)hexyl butyrate, 136954-23-9; 3-(methylthio)hexyl hexanoate, 136954-24-0; 3-(acetylthio)hexyl acetate, 136954-25-1; 3-(butanoylthio)hexyl butyrate, 136954-26-2; 3-(hexanoylthio)hexyl hexanoate, 136954-27-3.