

Identification of Additional Pineapple Volatiles[†]

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The volatiles of pineapple essence were isolated by liquid-liquid extraction, fractionated on neutral alumina, and analyzed by high-resolution gas chromatography and coupled gas chromatography/mass spectrometry (GC/MS). Twenty-six constituents (1 tentative) were identified for the first time in pineapple including the following sulfur compounds: methyl 3-(methylthio)-(E)-2-propenoate, methyl 3-(methylthio)-(Z)-2-propenoate, ethyl 3-(methylthio)-(E)-2-propenoate, ethyl 3-(methylthio)-(Z)-2-propenoate, methyl 4-(methylthio)butanoate, ethyl 4-(methylthio)butanoate (tentative), and dimethyl trisulfide. Their odor contribution to pineapple aroma is assessed.

INTRODUCTION

Pineapple flavor has been extensively studied with over 140 volatiles identified (van Straten and Maarse, 1983; Berger et al., 1983, 1985; Ohta et al., 1987). Wu and co-workers (Wu et al., 1991) studied free and glycosidically bound pineapple constituents and identified 2-pentanol, 2-butoxyethanol, hexanoic acid, phenol, 4-hydroxybenzaldehyde, vanillin, and syringaldehyde as aglycons.

During our recent studies of pineapple volatiles (Takeoka et al., 1989) we noticed several unknown mass spectra that appeared to be sulfur-containing constituents. Since sulfur compounds often possess interesting sensory characteristics and low odor thresholds, this study focused on the identification of these constituents and the evaluation of their contribution to pineapple odor.

EXPERIMENTAL PROCEDURES

Isolation of Volatiles from Pineapple Essence. Freshly received Hawaiian pineapple essence (38 L; Nabisco 1987) was added to a carboy (40-L glass bottle) and continuously extracted for 48 h with pentane (6 L). After the first 24 h of extraction, the pentane was replaced by a new batch. The solvent was removed by distillation to give 38 g of extract. A portion of the extract (11.6 g) was distilled under reduced pressure (25 mmHg) using a Claisen distilling head with a 3-in. Vigreux column to remove residual pentane (3.56 g). The remaining extract was distilled under reduced pressure [40 °C (1 mmHg)] in the same apparatus. The resulting distillate (6.48 g) possessed a fruity pineapple odor while the pot residue (1.56 g) had a heavier pineapple odor.

Dry-Column Chromatography on Neutral Alumina. The pot residue (1.0 g) was fractionated on neutral aluminum oxide (Alumina Woelm TSC, Woelm Pharma, Eschwege, FRG), activity III, using pentane. Alumina (239 g) was packed into nylon tubing forming a 2.4 × 55 cm column. After development, the column was cut lengthways into 10 sections. The components were eluted from the alumina using freshly distilled diethyl ether. The eluates were concentrated prior to analysis by GC and GC/MS.

Vacuum Steam Distillation-Extraction. The sample was prepared according to the procedure described by Takeoka et al. (1988).

Gas Chromatography. A Hewlett-Packard (HP) 5890A gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with

a flame ionization detector (FID) was used. Separations were achieved on a 60 m × 0.32 mm i.d. DB-Wax column [$d_f = 0.25 \mu\text{m}$, bonded poly(ethylene glycol), J&W Scientific, Folsom, CA] using helium as the carrier gas ($\mu = 33.4 \text{ cm/s}$, 30 °C). The oven temperature was programmed as follows: 30 °C for 4 min, increased at 2 °C/min to 180 °C, and held for 25 min. A split ratio of 1:25 was employed. Injector and detector temperatures were 200 and 220 °C, respectively. A 60 m × 0.32 mm i.d. DB-1 column ($d_f = 0.25 \mu\text{m}$, bonded dimethyl polysiloxane, J&W Scientific) was used to analyze the sample prepared by vacuum steam distillation-extraction (SDE). Helium carrier gas was used ($\mu = 33.1 \text{ cm/s}$, 30 °C). A split ratio of 1:22 was employed. The injector and detector were maintained at 200 and 230 °C, respectively. A HP 5895 GC ChemStation was used for data processing.

Gas Chromatography/Mass Spectrometry. A Finnigan MAT 4500 GC/MS/INCOS system (quadrupole, Finnigan MAT, San Jose, CA) equipped with a 60 m × 0.32 mm i.d. DB-Wax column ($d_f = 0.25 \mu\text{m}$) was used. The oven temperature was programmed from 50 (5 min isothermal) to 220 °C at 4 °C/min. A 60 m × 0.32 mm i.d. DB-1 column was used for analyzing the sample prepared by vacuum SDE. Helium was used as the carrier gas for all three columns. The instrument was operated in the electron impact mode at 70 eV, taking scans from 33 to 350 m/z in a 1-s cycle. A second mass spectrometer, HP 5970B mass selective detector (MSD), was directly coupled (capillary direct interface) with a HP 5890A gas chromatograph. Separations were achieved on a 60 m × 0.25 mm i.d. DB-1 column ($d_f = 0.25 \mu\text{m}$). Helium was used as the carrier carrier gas with the head pressure maintained at 9.5 psi (65 kPa). Injector and transfer line temperatures were 190 and 210 °C, respectively. The oven temperature was programmed from 30 to 200 °C at 3 °C/min.

Reference Compounds. Methyl 3-(methylthio)-(E)-2-propenoate and methyl 3-(methylthio)-(Z)-2-propenoate were prepared (in a 1.5:1 ratio, respectively) by reaction of methyl propiolate (Aldrich Chemical Co., Milwaukee, WI), and methanethiol according to established procedures (Hasegawa, 1980). The isomers were separated and purified by preparative GC. Methyl 3-(methylthio)-(E)-2-propenoate had the following spectral properties: mass spectrum, m/z (relative intensity) 134 (3), 133 (5), 132 (M^+ , 69), 119 (3), 118 (3), 117 (56), 103 (6), 102 (7), 101 (100), 100 (35), 87 (4), 85 (26), 73 (57), 72 (22), 59 (13), 58 (42), 57 (19), 45 (63); $^1\text{H NMR}$ (200 MHz, CDCl_3) δ 2.34 (3 H, s, CH_3S), 3.74 (3 H, s, OCH_3), 5.66 (1 H, d, $=\text{CHCO}$, $J = 15 \text{ Hz}$, trans), 7.76 (1 H, d, $\text{SCH}=\text{}$, $J = 15 \text{ Hz}$, trans); Kovats index (DB-1) 1062. Methyl 3-(methylthio)-(Z)-2-propenoate had the following spectral properties: mass spectrum, m/z (relative intensity) 134 (3), 133 (5), 132 (M^+ , 76), 119 (3), 118 (4), 117 (61), 103 (6), 102 (9), 101 (100), 87 (6), 85 (27), 73 (63), 72 (27), 59 (14), 58 (48), 57 (15), 45 (69); $^1\text{H NMR}$ (200 MHz, CDCl_3) δ 2.40 (3 H, s, CH_3S), 3.75 (3 H, s, OCH_3), 5.86 (1 H, d, $=\text{CHCO}$, $J = 10 \text{ Hz}$, cis), 7.06 (1 H, d, $\text{SCH}=\text{}$, $J = 10 \text{ Hz}$, cis); Kovats index (DB-1) 1076. Ethyl 3-(methylthio)-(E)-2-propenoate and ethyl 3-(me-

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thylthio)-(Z)-2-propenoate were prepared (in a 1.6:1 ratio, respectively) by reaction of ethyl propiolate (Aldrich) and methanethiol. The isomers were separated and purified by preparative GC. Ethyl 3-(methylthio)-(E)-2-propenoate had the following spectral properties: mass spectrum, m/z (relative intensity) 148 (2), 147 (3), 146 (M^+ , 45), 132 (2), 131 (25), 118 (6), 103 (37), 102 (6), 101 (100), 100 (36), 87 (9), 85 (10), 73 (45), 72 (17), 59 (5), 57 (11), 45 (36); 1H NMR (200 MHz, $CDCl_3$) δ 1.29 (3 H, t, CH_3CH_2), 2.34 (3 H, s, CH_3S), 4.20 (2 H, q, OCH_2), 5.66 (1 H, d, $=CHCO$, $J = 15$ Hz, trans), 7.75 (1 H, d, $SCH=$, $J = 15$ Hz, trans); Kovats index (DB-1) 1144. Ethyl 3-(methylthio)-(Z)-2-propenoate had the following spectral properties: mass spectrum, m/z (relative intensity) 148 (3), 147 (4), 146 (M^+ , 55), 132 (1), 131 (27), 118 (8), 103 (47), 102 (7), 101 (100), 100 (40), 87 (13), 85 (9), 73 (45), 72 (17), 59 (7), 58 (35), 57 (9), 45 (43); 1H NMR (200 MHz, $CDCl_3$) δ 1.29 (3 H, t, CH_3CH_2), 2.40 (3 H, s, CH_3S), 4.22 (2 H, q, OCH_2), 5.88 (1 H, d, $=CHCO$, $J = 10$ Hz, cis); Kovats index (DB-1) 1158. Ethyl 3-acetoxybutanoate was prepared by acetylation of ethyl 3-hydroxybutanoate with acetic anhydride. Ethyl 3-acetoxybutanoate had the following mass spectrum, m/z (relative intensity): 131 (27), 129 (15), 117 (9), 114 (21), 103 (5), 99 (7), 88 (13), 87 (10), 85 (18), 69 (67), 61 (10), 43 (100). Methyl (E)-4-hexenoate, prepared from the corresponding acid and alcohol, had the following mass spectrum, m/z (relative intensity): 128 (M^+ , 43), 113 (10), 97 (32), 96 (16), 81 (10), 74 (74), 71 (20), 69 (69), 68 (100), 59 (27), 55 (70), 53 (26), 43 (35), 41 (88); Kovats index (DB-1) 909. Methyl 5-hexenoate, prepared from the transesterification of ethyl 5-hexenoate, had the following mass spectrum, m/z (relative intensity): 128 (M^+ , 5), 113 (7), 97 (30), 96 (22), 87 (7), 74 (100), 71 (5), 69 (34), 68 (64), 67 (17), 59 (27), 55 (31), 54 (14), 53 (12), 43 (50), 42 (18), 41 (66); Kovats index (DB-1) 894. Ethyl 5-hexenoate had the following mass spectrum, m/z (relative intensity): 142 (M^+ , 9), 127 (1), 114 (9), 113 (5), 97 (58), 96 (34), 88 (62), 71 (20), 70 (45), 69 (81), 68 (96), 67 (27), 61 (27), 60 (70), 55 (52), 54 (20), 53 (21), 45 (14), 42 (27), 41 (100); Kovats index (DB-1) 965. Methyl 4-(methylthio)butanoate had the following mass spectrum, m/z (relative intensity): 150 (5), 149 (6), 148 (M^+ , 100), 119 (2), 118 (3), 117 (43), 116 (12), 101 (32), 89 (9), 87 (28), 75 (51), 74 (88), 69 (14), 61 (42), 59 (43), 43 (18), 41 (29); Kovats index (DB-1) 1098. Ethyl 4-(methylthio)butanoate had the following mass spectrum, m/z (relative intensity): 164 (5), 163 (8), 162 (M^+ , 100), 117 (86), 116 (19), 115 (24), 101 (22), 89 (27), 88 (41), 87 (32), 75 (60), 74 (62), 73 (47), 70 (27), 69 (29), 62 (21), 61 (98), 60 (51), 47 (28), 45 (51), 42 (31), 41 (58); Kovats index (DB-1) 1176.

Odor Thresholds. These were determined on GC purified samples using methods previously described (Guadagni et al., 1966), with a panel of 16–20 judges.

RESULTS AND DISCUSSION

Volatiles from pineapple essence were isolated by continuous extraction with pentane. Following distillation of the extract to remove solvent and low-boiling volatiles, the pot residue was fractionated by dry-column chromatography on neutral alumina. Of the 10 fractions obtained, the majority of the components resided in fractions 1–3. These fractions were analyzed by capillary gas chromatography and gas chromatography/mass spectrometry (GC/MS). The analysis led to the identification of 26 (1 tentative) newly reported pineapple constituents (Table I).

Methyl 4-(methylthio)butanoate appears to be related to methionine, both possessing the same acid portion of the molecule. The corresponding ethyl ester, ethyl 4-(methylthio)butanoate, is strongly believed to be present, but its identification is tentative since a clean mass spectrum could not be obtained (ethyl 3-hydroxyhexanoate had the same retention index on the DB-Wax column employed). This compound has been used to enhance the pineapple character of fruit flavor added to tobacco (Pittet et al., 1975a). It has also been used as a flavoring in foods and in fragrance mixtures such as perfumes (Pittet et al., 1974, 1975a,b). The presence of esters of 3-(methylthio)pro-

Table I. Additional Volatiles Identified in Pineapple Essence

compound ^a	Kovats index (DB-Wax)	% area ^b of fraction ^c		
		1	2	3
methyl (E)-2-butenolate	1094	tr	0.03	nd
ethyl (E)-2-butenolate	1154	0.09	0.08	nd
3-methylbut-3-enyl acetate	1190	0.04	0.05	nd
methyl 5-hexenoate	1223	0.09	0.04	nd
methyl (E)-4-hexenoate	1237	<i>d</i>	0.02	nd
3-methyl-3-buten-1-ol	1239	<i>d</i>	0.04	nd
ethyl 5-hexenoate	1272	0.18	0.06	nd
3-methyl-2-buten-1-ol	1311	0.53	0.27	0.05
dimethyl trisulfide	1365	tr	nd	nd
trans-furan linalool oxide	1425	nd	nd	0.11
1-octen-3-ol	1443	nd	nd	0.03
heptanol	1446	nd	nd	0.08
cis-furan linalool oxide	1454	nd	nd	0.05
3-methylbutyl hexanoate	1456	0.04	nd	nd
ethyl 3-acetoxybutanoate	1569	nd	0.03	1.33
methyl benzoate	1594	0.02	nd	nd
methyl 4-(methylthio)butanoate	1617	nd	0.04	nd
nonanol	1651	nd	nd	0.16
(ethyl 4-(methylthio)butanoate) ^e	1652	nd	0.01	nd
methyl 3-(methylthio)-(E)-2-propenoate	1663	<i>d</i>	0.33	0.16
ethyl 3-(methylthio)-(E)-2-propenoate	1719	0.03	0.11	<i>d</i>
geranyl acetate	1743	0.03	0.01	nd
methyl 3-(methylthio)-(Z)-2-propenoate	1773	nd	0.03	0.08
ethyl 3-(methylthio)-(Z)-2-propenoate	1824	nd	0.13	0.16
geraniol	1835	0.05	<i>d</i>	nd
methyl cinnamate	2047	nd	0.01	nd
ethyl cinnamate	2102	0.08	0.19	nd

^a Mass spectrum and Kovats retention index consistent with those of an authentic reference sample. ^b Peak area percentage of total FID area excluding the solvent peaks (assuming all response factors of 1). ^c From dry column chromatography on neutral alumina. nd, not detected; tr, peak area less than 0.01%. ^d Peak area percentage could not be determined since peak was not completely resolved from neighboring constituent. ^e Tentative identification enclosed in parentheses.

panoic acid and 4-(methylthio)butanoic acid can be explained by the Stickland reaction (Stickland, 1934). In this reaction involving methionine and another amino acid, 3-(methylthio)propanoic acid and 4-(methylthio)butanoic acid can theoretically be formed. Methyl and ethyl 3-(methylthio)propanoate are major constituents found in pineapple. Some other interesting sulfur-containing esters include methyl 3-(methylthio)-(E)-2-propenoate, methyl 3-(methylthio)-(Z)-2-propenoate, ethyl 3-(methylthio)-(E)-2-propenoate, and ethyl 3-(methylthio)-(Z)-2-propenoate. These esters are similar to the major esters found in pineapple, methyl and ethyl 3-(methylthio)propanoate. To our knowledge this is the first time these compounds have been reported in food, although they have been patented as useful perfume compounds (Hasegawa, 1980). The mass spectra of these esters are shown in Figures 1–4. The esters of 3-(methylthio)-2-propenoic acid with petasol have been found in *Petasites officinalis* Moench (Stoll et al., 1956). Additionally, the esters of 3-(methylthio)-(Z)-2-propenoic acid with petasol and isopetasol have been found in the roots and flowers of *Petasites hybridus* (L.) G.M. and SCH (Steinegger et al., 1979). Planchonelline, a 3-(methylthio)-(E)-2-propenoic acid ester of laburnine [(+)-trachelanthamidine] was found as a major alkaloid of the leaves of *Planchonella thyrsoides* C.T. White and *P. anteridifera* (White & Francis) H.J. Lam (Hart and Lambertson, 1966). The origin of these esters is unclear. However, many *Streptomyces* and some fungi are known to produce 3-(methylthio)-(E)-2-propenoic acid from me-

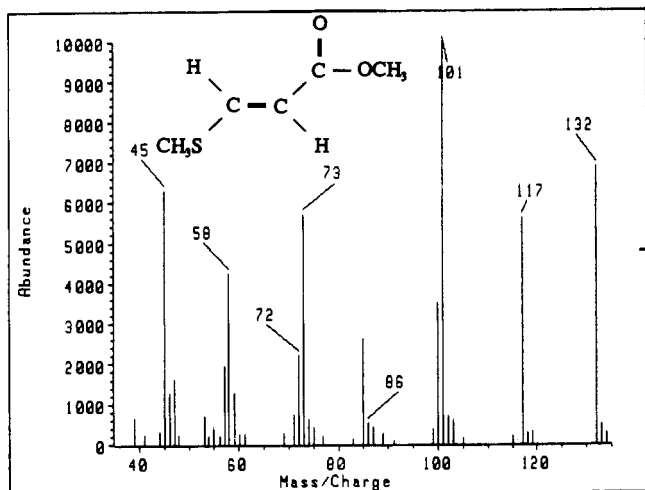


Figure 1. Mass spectrum of methyl 3-(methylthio)-(*E*)-2-propenoate.

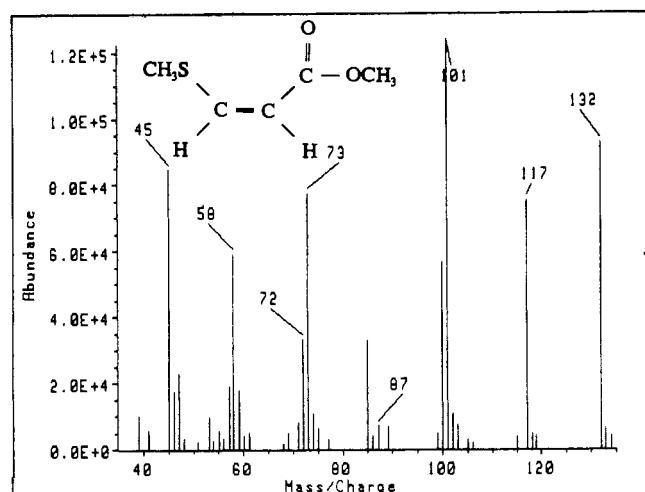


Figure 2. Mass spectrum of methyl 3-(methylthio)-(*Z*)-2-propenoate.

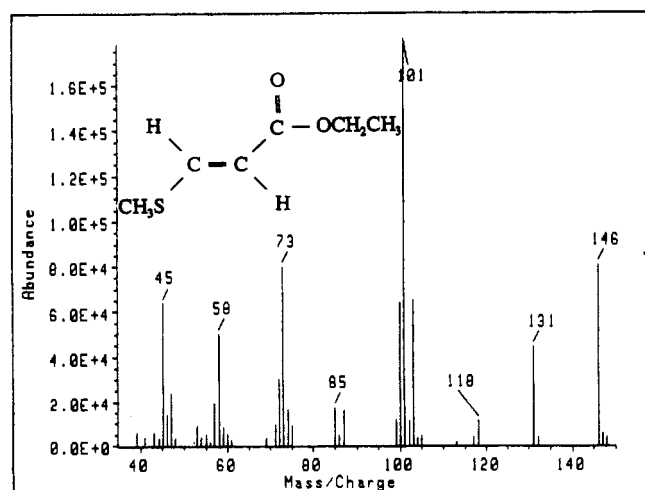


Figure 3. Mass spectrum of ethyl 3-(methylthio)-(*E*)-2-propenoate.

thionine (Arima et al., 1970). The potent odorant dimethyl trisulfide is reported for the first time in pineapple, although dimethyl disulfide was previously identified by Näf-Müller and Wilhalm (1971). Dimethyl trisulfide is considered to be an important component of cooked cabbage, broccoli, and cauliflower (Buttery et al., 1976).

The presence of methyl and ethyl cinnamate is not surprising since cinnamic acid has been identified by Ohta

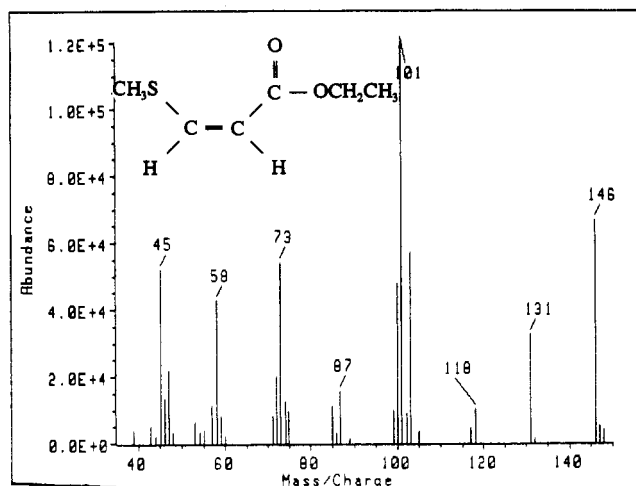


Figure 4. Mass spectrum of ethyl 3-(methylthio)-(*Z*)-2-propenoate.

Table II. Odor Thresholds of Some of the Newly Identified Pineapple Constituents

compound	odor threshold in water, ppb
ethyl <i>S</i> -(+)-2-methylbutanoate	0.006
dimethyl trisulfide	0.01
1-octen-3-ol	1.3
geranyl acetate	9
ethyl 4-methylthiobutanoate	19
methyl 3-(methylthio)-(<i>Z</i>)-2-propenoate	25
nonanol	50
methyl 3-(methylthio)-(<i>E</i>)-2-propenoate	95
methyl 4-(methylthio)butanoate	133
methyl (<i>E</i>)-4-hexenoate	147
methyl 5-hexenoate	194
ethyl 3-(methylthio)-(<i>E</i>)-2-propenoate	246

et al. (1987). It has been recently reported that this acid occurs as a glycosidically bound constituent in pineapple (Wu et al., 1991) and raspberry (Pabst et al., 1991). Prior to its identification in Chinese quince peel oil, ethyl 5-hexenoate had not been reported in any essential oil (Mihara et al., 1987). The presence of the previously tentatively identified constituent, methyl 5-hexenoate, has been confirmed (Takeoka et al., 1989).

A linalool oxide has been previously reported by Ohta et al. (1987). They did not specify which isomer was present, but judging from its retention index it was probably the (*Z*)-linalool oxide, furanoid.

Identification of Constituents in Fresh Pineapple Fruit. Having identified these sulfur-containing constituents in pineapple essence, we checked for their presence in fresh fruit. In a sample prepared by vacuum SDE, methyl 3-(methylthio)-(*E*)-2-propenoate (not fully resolved from an adjacent peak), methyl 3-(methylthio)-(*Z*)-2-propenoate, methyl 4-(methylthio)butanoate, and ethyl 3-(methylthio)-(*E*)-2-propenoate were identified. The concentration of each constituent was in the <1–6 ppb range. Dimethyl trisulfide was not detected. Ethyl 4-(methylthio)butanoate was also not found; however, if present it would have coeluted with a large methyl 3-acetoxyhexanoate peak on the DB-1 column employed. The odor thresholds of various pineapple constituents are shown in Table II. Since the concentrations of the newly identified sulfur compounds are less than their odor thresholds, they probably have little, if any contribution to the overall aroma.

Reassessment of the Contribution of Ethyl 2-Methylbutanoate to Pineapple Aroma. Using a permethylated β -cyclodextrin capillary column, we recently

found that pineapple essence contains ethyl 2-methylbutanoate in exclusively the *S*-(+) configuration (Takeoka et al., 1990). The *S*-(+) enantiomer is a potent odorant with an odor threshold of 6 ppt. This value is considerably lower (0.006 vs 0.3 ppb) than the one we used previously to calculate the odor unit value in pineapple (Takeoka et al., 1989). The recalculated odor unit value of ethyl 2-methylbutanoate is 11 000, which makes it the second largest odor contributor (after furaneol) to pineapple aroma.

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Registry No. Methyl (*E*)-4-hexenoate, 14017-81-3; dimethyl trisulfide, 3658-80-8; 1-octen-3-ol, 3391-86-4; methyl 4-(methylthio)butanoate, 53053-51-3; nonanol, 28473-21-4; ethyl 4-(methylthio)butanoate, 22014-48-8; methyl 3-(methylthio)-(*E*)-2-propenoate, 15904-85-5; ethyl 3-(methylthio)-(*E*)-2-propenoate, 136115-65-6; geranyl acetate, 105-87-3; methyl 3-(methylthio)-(*Z*)-2-propenoate, 15904-84-4; methyl (*E*)-2-butenate, 623-43-8; ethyl (*E*)-2-butenate, 623-70-1; 3-methylbut-3-enyl acetate, 5205-07-2; methyl 5-hexenoate, 2396-80-7; 3-methyl-3-buten-1-ol, 763-32-6; ethyl 5-hexenoate, 54653-25-7; 3-methyl-2-buten-1-ol, 556-82-1; *trans*-furan linalool oxide, 34995-77-2; heptanol, 53535-33-4; *cis*-furan linalool oxide, 5989-33-3; 3-methylbutyl hexanoate, 2198-61-0; ethyl 3-acetoxybutanoate, 27846-49-7; methyl benzoate, 93-58-3; ethyl 3-(methylthio)-(*Z*)-2-propenoate, 136115-66-7; geraniol, 106-24-1; methyl cinnamate, 103-26-4; ethyl cinnamate, 103-36-6; ethyl *S*-(+)-2-methylbutanoate, 10307-61-6; methanethiol, 74-93-1; methyl propionate, 922-67-8; ethyl propionate, 623-47-2; ethyl 3-hydroxybutanoate, 5405-41-4; (*E*)-4-hexenoic acid, 1577-20-4.