JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2007,** *55,* 4511−4517 **4511**

Volatile Organic Sulfur-Containing Constituents in *Poncirus trifoliata* (L.) Raf. (Rutaceae)

CHRISTIAN STARKENMANN,* YVAN NICLASS, AND SINA ESCHER

Firmenich SA, Corporate R&D Division, P.O. Box 239, CH-1211 Geneva 8, Switzerland

During our screening of plant materials to find new natural fragrance and flavor ingredients, we discovered two series of 3-sulfanylalkyl alkanoates in a peel extract of fruits of wild-growing *Poncirus trifoliata* (L.) Raf. (Rutaceae), a species closely related to *Citrus*. The two series belong to alkanoates of 3-methyl-3-sulfanylbutan-1-ol and 3-sulfanylhexan-1-ol, respectively, and thus are members of a family of natural molecules having in common a 1,3-positioned O,S moiety. The alkanoate residues comprise all even-numbered saturated fatty acids from C2 (acetate) to C18 (octadecanoate). Among the 20 sulfur-containing compounds identified, 14 are described for the first time as naturally occurring in a botanical species. Several cysteine-*S*-conjugates were synthesized as hypothetical precursors of the new volatile sulfur-containing constituents, where after *S*-(3-hydroxy-1,1-dimethylpropyl)-L-cysteine, *S*-[3-(acetyloxy)-1,1-dimethylpropyl]-L-cysteine, and *S*-[1-(2-hydroxyethyl)butyl]-L-cysteine were identified in the fruit peel. No cysteine-*S*-conjugates were detected in the fruit juice.

KEYWORDS: ***Poncirus trifoliata* (L.) Raf.; 3-methyl-3-sulfanylbutan-1-ol; 3-methyl-3-sulfanylhexan-1-ol; 3-methyl-3-sulfanylbutyl alkanoates; 3-sulfanylhexyl alkanoates; cysteine-*S*-conjugates; natural occurrence; fragrance and flavor chemical**

INTRODUCTION

Poncirus trifoliata (L.) Raf. (Rutaceae; previous classification *Citrus trifolata* L.) is native of China and closely related to *Citrus* species. It is a wild-growing, up to 2 m high shrub armed with big spines, and it survives temperatures as low as −20 °C. Many commercially important *Citrus* varieties are grown on *Poncirus* rootstocks, which provide resistance to the cold and raise the quality of the citrus fruit. The strong odor of *P. trifoliata* fruits reminds many of jasmine flowers and over ripe, rotten, sulfury mangos and is very different from the fresh odor of a lemon or an orange fruit. The essential oil of *P. trifoliata* peel is composed of monoterpene hydrocarbons such as β-pinene, myrcene, limonene, camphene, γ-terpinene, and *p*-cymene, as well as oxygenated compounds and sesquiterpenes. However, the published data (*1*, *2*) do not give enough information to understand the very peculiar odor tonalities. This prompted us to isolate and analyze the volatile sulfur-containing components of *P. trifoliata* fruits. In addition to the chemical and organoleptic analyses, this study discusses possible precursors of the new volatile sulfur-containing compounds identified.

EXPERIMENTAL PROCEDURES

General. Commercially available reagents and solvents of adequate quality were used without further purification. Reactions were performed in standard glassware. Yields were not optimized. Organic extracts were washed to neutrality with aqueous H_2SO_4 solution and/or $NaHCO_3$ and NaCl solutions, dried (Na_2SO_4), and evaporated.

* To whom correspondence should be addressed. Tel: +41 22 780 34 77. Fax: +41 22 780 33 34. E-mail: christian.starkenmann@firmenich.com.

Flash Chromatography (FC). Silica gel 60, 35−70 μm (sodium dodecyl sulfate), was used for normal-phase FC, and LiChroprep RP-18, 40−63 μm (Merck, catalog no. 1.1390.0250), was used for reversed-phase FC.

Gas Chromatography/Electron Impact-Mass Spectrometry (GC/EI-MS). An Agilent-GC-6890 system connected to an Agilent-MSD-5973 quadrupole mass spectrometer was operated at an electron energy of ca. 70 eV. Helium was the carrier gas at a constant flow rate of 0.7 mL/min. The spectra are listed as follows: fragment ions *m*/*z* (relative intensity). Separations were performed on fused-silica capillary columns, either coated with SPB-1 (apolar; 0.25 mm i.d. × 30 m; 0.25 μm d.f.; Supelco) or coated with Supelcowax10 (polar; SPWax; 0.25 mm i.d. × 30 m; 0.25 μm d.f.; Supelco). The standard oven program was the following: 50 °C for 5 min, then 50−240 °C at 5°/min, and hold at 240 °C. Retention indices (*I*) were calculated by linear extrapolation from the retention times (t_R; in min) of the analytes and the two closest alkanes eluting just before and just after the analyte. Injection modes were adapted accordingly to the quality of the sample; synthetic compounds were injected with a 50 to 1 split ratio, and natural extracts were injected in splitless mode.

Chiral GC/Atomic Emission Detector (AED). An Agilent-GC-6890 system was coupled to an AED (Jass, Germany). The chiral column was a fused-silica capillary column DiMePeBeta-1701 (0.25 mm i.d. × 10 m; 0.25 μm d.f.; Mega, from Brechbühler, Switzerland). The oven temperature was programmed from 50 to 200 °C at 1 °C/min. The enantiomeric ratios were measured at the S-specific emission detection wavelength 181 nm. The elution order t_R (*R*) < t_R (*S*) of the enantiomers (*R*)/(*S*)-**2** and (*R*)/(*S*)-**4** was confirmed by coinjections of authentic reference samples (*3*).

GC/MS−Olfaction (GC/MS-O). The GC part of a GC/EI-MS system as described above was equipped with an effluent splitter and a heated sniffing port. The apolar fused-silica capillary column was

10.1021/jf063453h CCC: $37.00
Published on Web 05/08/2007

coated with SPB-1 (0.53 mm i.d. × 30 m; 1.0 μm d.f.; Supelco). The polar column was a SPWax (0.53 mm i.d. × 30 m; 1.0 μm d.f.; Supelco).

HPLC/MS. An Agilent 1100 LC-MS system equipped with a B1312A binary pump was used. The separations were performed on a Zorbax SB-C18 column (rapid resolution HT; 2.1 mm i.d. × 50 mm; 1.8 μm particle size; Agilent, catalog no. 822700-902). The elution solvents were CH_3CN and water containing 0.02% formic acid. The gradient profile was started at 5% CH_3CN and held for 0.5 min and then increased to 100% CH_3CN in 5 min. The flow rate was 0.3 mL/min. The retention times (t_R) are expressed in minutes. The mass spectrometer was a Thermo Finnigan LCQ ion-trap spectrometer, with an electrospray ion source operated in positive mode (ESI^+). The spray voltage was 4.5 kV. The capillary temperature was heated at 250 °C. The sheath gas was nitrogen at a flow rate of 70 (Finnigan arbitrary units). The auxiliary gas was also nitrogen at a flow rate of 5 (Finnigan arbitrary units). Analyses were performed in selected ion monitoring (SIM) mode according to $[M + 1]^+$ of expected cysteine-*S*-conjugates. To achieve high sensitivity and selectivity, quantitative data were recorded as follows: The $[M + 1]^+$ ion of each compound was fragmented in the ion trap by applying 30% normalized collision energy. The most intense fragment was extracted, and the corresponding peak was integrated using Xcalibur software. Compound **26** was fragmented at 236, and ion 122 was extracted. Compound **21**: fragmentation at 208, extraction of ion 122. Compound **22**: fragmentation at 250, extraction of ion 233. Compound **24**: fragmentation at 222, extraction of ion 205.

^{1}H and ^{13}C NMR Spectra. The NMR spectra were recorded on a Bruker-Avance-500 spectrometer at 500.13 and 125.76 MHz, respectively. If not stated otherwise, the solvent was $CDCl_3$. δ Values are in ppm downfield from $(CH_3)_4Si$ (= 0 ppm). The cysteine-*S*-conjugates were measured in D_2O with sodium 3-(trimethylsilyl)tetradeuteriopropionate as an internal standard. The assignments by correlation spectroscopy, heteronuclear single quantum correlation, and heteronuclear multiple quantum coherence experiments were performed with standard Bruker software (XWINNMR 3.1).

Organic Extract of *P. trifoliata* (L.) Raf. Fruit Peel. In October 2005, 80 fruits of *P. trifoliata* (L.) Raf. were collected in the botanical garden of Geneva, Switzerland (registry CJBG 19801106). The outer peel layer, which corresponded mostly to the flavedo, was removed with a kitchen rasp, and the material (300 g) was macerated in CH_2Cl_2 (500 mL) at 4 °C for 16 h. The solvent extract was decanted, dried (Na_2SO_4), and concentrated to 67.9 g in a Vigreux apparatus at 40 °C. This extract was used for further analysis. The same operation was repeated with 40 fruits coming from a private garden in the Geneva area. The analytical results were comparable.

Isolation of the Fraction Containing Compounds with Free SH Groups ("Thiol" Fraction). To a 10 g aliquot of the organic extract was added octane-1-thiol (0.01 mg) as an internal standard, and the spiked extract was submitted to affinity chromatography on Affi-Gel 501 (*4*, *5*). The thiol fraction was carefully concentrated in a Vigreux apparatus to 0.1 mL and immediately submitted to GC/EI-MS analyses on SPB-1 and SPWax columns (injection volume, 1 μL; closed split). The natural thiol compounds of interest were confirmed by comparing their MS and retention indices (*I*) with those of authentic reference compounds on both polar and apolar columns. Quantitative data are only an approximation based on the comparison with the GC peak area of the internal standard.

Preparation of Extracts for the Analysis of Cys-*S*-Conjugates in the Fruit Juice. To a juice (160 g), which had been prepared from 20 fruits using a kitchen juicer, was added *S*-[1-(2-hydroxyethyl)-1-methylbutyl]-L-cysteine (**26**) (*6*) (1 mg) as an internal standard and lyophilized to give 21 g. This solid (1 g) was diluted in 0.02% aqueous formic acid (10 mL), centrifuged, and filtered on an Acrodisk (0.45 μm pore size). A 5 μL aliquot of the filtrate was injected to HPLC/ESI^+-MS. Only **26** was detected. The remaining 20 g was rediluted in water (100 mL) and filtered on SiO_2-RP18 (column 7 cm i.d. × 5 cm; conditioned with ethanol and then rinsed with 10 volumes of water). Elution was performed with five 200 mL portions (water, water/ethanol 9:1, 8:2, 7:3, and 1:1, respectively). Except for **26**, present in the fraction 7:3, no other Cys-*S*-conjugates were detected.

Preparation of Extracts for the Analysis of Cys-*S*-Conjugates in the Fruit Peel. The peel material, which was recovered from the above juice preparation (300 g), was homogenized in a kitchen aid in the presence of ice, and *S*-[1-(2-hydroxyethyl)-1-methylbutyl]-L-cysteine (**26**) (*6*) (1 mg) was added as an internal standard. The slurry was centrifuged (1 h, 4000 rpm/min, 10 °C). The solid was removed, and the liquid was lyophilized to give 20 g. This material was rediluted with water and filtered on SiO_2-RP18 as described above. The internal standard **26** was in the fraction (2.3 g) eluted with water/ethanol 7:3. Further purification of this fraction was performed on an ion exchange column (10 g, Dowex 50WX8, Aldrich, catalog no. 21751-4). The elution was performed with an aqueous 0.3−1.5 M ammonia gradient. The internal standard **26** as well as **21**, **22**, and **24** were detected in the fraction eluted with 1.2 M ammonia (20 mg) (HPLC/ESI^+-MS). Quantification of **21** and **24**, as well as the determination of the recovery factor of **26**, was measured in this fraction.

Quantification of the Cys-*S*-Conjugates. The calibration curve was established by replicate injections of 5 μL of standard solutions containing **21**, **22**, **24**, and **26**, each at 1, 5, 10, 20, and 50 μg/mL (HPLC/ESI^+-MS). The precursors were then quantified in the purified peel extract. The recovery of the internal standard **26** was only 0.033 ± 0.004 mg instead of 1 mg added to 300 g of peel.

General Procedure for the Synthesis of 3-Sulfanylalkyl Esters as Reference Compounds. The 3-methyl-3-sulfanylbutan-1-ol (**1**) (Firmenich, catalog no. 958055) or 3-sulfanylhexan-1-ol (**2**) (Alfa Aesus, catalog no. 821965) (10 mmol) was diluted in CH_2Cl_2 (10 mL). The desired acid chloride (10 mmol; diluted in 2 mL of CH_2Cl_2) was added dropwise. After stirring at 22 °C for 18 h, the reaction mixture was concentrated under partial vacuum to remove CH_2Cl_2. The crude product was submitted to FC on SiO_2 (250 g). The elution was done with cyclohexane/diethyl ether 95:5. The reaction yield after purification is given below for each preparation. The purified esters were analyzed by ^{1}H NMR, ^{13}C NMR, and GC/MS. The retention indices were calculated after injection on SPB-1 and SPWax GC columns.

*3-Methyl-3-sulfanylbutyl Acetate (**3**).* Yield: 1.45 g (89.5%). I_{SPB-1} 1080; I_{SPWax} 1566. ^{1}H NMR: 4.29 (t, J = 7.1 Hz, 2H), 1.98 (s, 3H), 1.90 (t, J = 7.1 Hz, 2H), 1.69 (br, SH), 1.42 (s, 6H). ^{13}C NMR: 180.0 (s), 62.0 (t), 44.4 (t), 42.9 (s), 33.1 (2q), 21.1(q). MS: 162 (2, M^+), 102 (40), 87 (12), 75 (15), 69 (100), 59 (9), 43 (60), 41 (35).

*3-Methyl-3-sulfanylbutyl Butanoate (**5**).* Yield: 1.65 g (86.8%). I_{SPB-1} 1266; I_{SPWax} 1725. ^{1}H NMR: 4.28 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.93 (t, J = 7.1 Hz, 2H), 1.70 (br, SH), 1.59−1.68 (m, 2H), 1.42 (s, 6H), 0.95 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.5 (s), 61.8 (t), 44.4 (t), 42.9 (s), 36.3 (t), 33.1 (2q), 18.4 (t), 13.7 (q). MS: 190 (2, M^+), 102 (40), 87 (12), 75 (10), 71 (48), 69 (100), 59 (9), 43 (35), 41 (35).

*3-Methyl-3-sulfanylbutyl Hexanoate (**7**).* Yield: 1.7 g (78.0%). I_{SPB-1} 1468; I_{SPWax} 1920. ^{1}H NMR: 4.28 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.94 (t, J = 7.1 Hz, 2H), 1.75 (br, SH), 1.58−1.67 (m, 2H), 1.42 (s, 6H), 1.24−1.35 (m, 4H), 0.90 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.8 (s), 61.8 (t), 44.4 (t), 42.9 (s), 34.4 (t), 33.1 (2q), 31.3 (t), 24.6 (t), 22.3 (t), 13.9 (q). MS: 218 (2, M^+), 102 (45), 99 (32), 87 (12), 71 (22), 69 (100), 59 (9), 55 (12), 43 (28), 41 (31).

*3-Methyl-3-sulfanylbutyl Octanoate (**9**).* Yield: 2.35 g (95.5%). I_{SPB-1} 1678; I_{SPWax} 2130. ^{1}H NMR: 4.28 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.94 (t, J = 7.1 Hz, 2H), 1.76 (br, SH), 1.57−1.66 (m, 2H), 1.43 (s, 6H), 1.22−1.36 (m, 8H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.8 (s), 61.8 (t), 44.4 (t), 42.9 (s), 34.4 (t), 33.1 (2q), 31.6 (t), 29.1 (t), 28.9 (t), 24.9 (t), 22.5 (t), 14.0 (q). MS: 246 (2, M^+), 127 (28), 102 (58), 87 (12), 71 (8), 69 (100), 57 (40), 55 (15), 43 (15), 41 (32).

*3-Methyl-3-sulfanylbutyl Decanoate (**11**).* Yield: 2.45 g (89.4%). I_{SPB-1} 1876; I_{SPWax} 2342. ^{1}H NMR: 4.28 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.94 (t, J = 7.1 Hz, 2H), 1.76 (br, SH), 1.57−1.66 (m, 2H), 1.43 (s, 6H), 1.22−1.36 (m, 12H), 0.88 (t, J = 7.1, 3H). ^{13}C NMR: 173.8 (s), 61.8 (t), 44.4 (t), 42.9 (s), 34.4 (t), 33.1 (2q), 31.9 (t), 29.4 (t), 29.3 (2t), 29.1 (t), 24.9 (t), 22.7 (t), 14.1 (q). MS: 274 (2, M^+), 155 (22), 102 (68), 87 (12), 71 (18), 69 (100), 68 (21), 57 (17), 55 (19), 43 (20), 41 (31).

*3-Methyl-3-sulfanylbutyl Dodecanoate (**13**).* Yield: 2.0 g (66.2%). I_{SPB-1} 2082; I_{SPWax} 2557. ^{1}H NMR: 4.28 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.94 (t, J = 7.1 Hz, 2H), 1.76 (br, SH), 1.57−1.66

(m, 2H), 1.43 (s, 6H), 1.22−1.36 (m, 16H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.8 (s), 61.8 (t), 44.4 (t), 42.9 (s), 34.4 (t), 33.1 (2q), 31.9 (t), 29.6−29.2 (6t), 25.0 (t), 22.7 (t), 14.1 (q). MS: 183 (18), 102 (82), 87 (12), 69 (100), 68 (30), 57 (22), 55 (19), 43 (22), 41 (31).

*3-Methyl-3-sulfanylbutyl Tetradecanoate (**15**).* Yield: 3.1 g (94.0%). I_{SPB-1} 2287; I_{SPWax} 2770. ^{1}H NMR: 4.28 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.94 (t, J = 7.1 Hz, 2H), 1.76 (br, SH), 1.57−1.66 (m, 2H), 1.43 (s, 6H), 1.22−1.36 (m, 20H), 0.89 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.8 (s), 61.8 (t), 44.4 (t), 42.9 (s), 34.4 (t), 33.1 (2q), 31.9 (t), 29.7−29.2 (8t), 25.0 (t), 22.7 (t), 14.1 (q). MS: 211 (18), 102 (92), 87 (15), 69 (100), 68 (30), 57 (22), 55 (20), 43 (22), 41 (34).

*3-Methyl-3-sulfanylbutyl Hexadecanoate (**17**).* Yield: 3.4 g (95.0%). I_{SPB-1} 2492; I_{SPWax} > 2800. ^{1}H NMR: 4.28 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.94 (t, J = 7.1 Hz, 2H), 1.76 (br, SH), 1.57−1.66 (m, 2H), 1.43 (s, 6H), 1.22−1.36 (m, 24H), 0.89 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.8 (s), 61.8 (t), 44.4 (t), 42.9 (s), 34.4 (t), 33.1 (2q), 31.9 (t), 29.7−29.2 (10t), 25.0 (t), 22.7 (t), 14.1 (q). MS: 340 (2, M^+ −18), 239 (18), 102 (100), 87 (15), 69 (95), 68 (24), 57 (22), 55 (20), 43 (22), 41 (29).

*3-Methyl-3-sulfanylbutyl Octadecanoate (**19**).* Yield: 3.8 g (98.4%). I_{SPB-1} 2696; I_{SPWax} >2800. ^{1}H NMR: 4.28 (t, J = 7.1 Hz, 2H), 2.29 (t, J = 7.1 Hz, 2H), 1.94 (t, J = 7.1 Hz, 2H), 1.76 (br, SH), 1.57−1.66 (m, 2H), 1.43 (s, 6H), 1.22−1.36 (m, 28H), 0.89 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.8 (s), 61.8 (t), 44.4 (t), 42.9 (s), 34.4 (t), 33.1 (2q), 31.9 (t), 29.7−29.2 (12t), 25.0 (t), 22.7 (t), 14.1 (q). MS: 368 (2, M^+ −18), 281 (8), 267 (12), 207 (18), 102 (100), 87 (15), 69 (78), 68 (12), 57 (18), 55 (17), 43 (18), 41 (25).

*3-Sulfanylhexyl Octanoate (**10**).* Yield: 2.4 g (93.8%). I_{SPB-1} 1799; I_{SPWax} 2271. ^{1}H NMR: 4.22−4.28 (m, 2H), 2.82−2.92 (m, 1H), 2.29 (t, J = 7.1 Hz, 2H), 1.97−2.06 (m, 1H), 1.24−1.79 (m, 16H), 0.93 (t, J = 7.1 Hz, 2H), 0.89 (t, J = 7.1 Hz, 2H). ^{13}C NMR: 173.7 (s), 62.0 (t), 41.2 (t), 37.9 (t), 37.3 (d), 34.3 (t), 31.7 (t), 29.1 (t), 28.9 (t), 25.0 (t), 22.6 (t), 20.1 (t), 14.1 (q), 13.7 (q). MS: 242 (2, M^+ −18), 145 (10), 127 (48), 116 (100), 101 (30), 88 (90), 87 (40), 83 (68), 73 (20), 57 (68), 55 (59), 43 (22), 41 (30).

*3-Sulfanylhexyl Decanoate (**12**).* Yield: 2.6 g (89.9%). I_{SPB-1} 2003; I_{SPWax} 2480. ^{1}H NMR: 4.22−4.28 (m, 2H), 2.82−2.92 (m, 1H), 2.29 (t, J = 7.1 Hz, 3H), 1.97−2.06 (m, 1H), 1.24−1.79 (m, 20H), 0.93 (t, J = 7.1 Hz, 3H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.7 (s), 62.0 (t), 41.2 (t), 37.9 (t), 37.3 (d), 34.3 (t), 31.9 (t), 29.1−29.4 (4t), 25.0 (t), 22.6 (t), 20.1 (t), 14.1 (q), 13.7 (q). MS: 288 (2, M^+), 173 (8), 155 (25), 116 (100), 101 (20), 88 (75), 87 (35), 83 (68), 71 (20), 57 (18), 55 (45), 43 (28), 41 (23).

*3-Sulfanylhexyl Dodecanoate (**14**).* Yield: 2.9 g (93.7%). I_{SPB-1} 2208; I_{SPWax} 2696. ^{1}H NMR: 4.22−4.28 (m, 2H), 2.82−2.92 (m, 1H), 2.29 (t, J = 7.1 Hz, 3H), 1.97−2.06 (m, 1H), 1.24−1.79 (m, 24H), 0.93 (t, J = 7.1 Hz, 3H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.7 (s), 62.0 (t), 41.2 (t), 37.9 (t), 37.3 (d), 34.3 (t), 31.9 (t), 29.1−29.6 (6t), 25.0 (t), 22.6 (t), 20.1 (t), 14.1 (q), 13.7 (q). MS: 201 (10), 183 (20), 116 (100), 101 (20), 88 (70), 87 (35), 83 (54), 71 (12), 57 (22), 55 (40), 43 (22), 41 (18).

*3-Sulfanylhexyl Tetradodecanoate (**16**).* Yield: 2.6 g (75.9%). I_{SPB-1} 2412; I_{SPWax} >2800. ^{1}H NMR: 4.22−4.28 (m, 2H), 2.82−2.92 (m, 1H), 2.29 (t, J = 7.1 Hz, 2H), 1.97−2.06 (m, 1H), 1.24−1.79 (m, 28H), 0.93 (t, J = 7.1 Hz, 3H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.7 (s), 62.0 (t), 41.2 (t), 37.9 (t), 37.3 (d), 34.3 (t), 31.7 (t), 29.1−29.4 (8t), 25.0 (t), 22.6 (t), 20.1 (t), 14.1 (q), 13.7 (q). MS: 229 (8), 211 (15), 116 (100), 101 (18), 88 (60), 87 (30), 83 (49), 71 (12), 57 (22), 55 (33), 43 (22), 41 (17).

*3-Sulfanylhexyl Hexadodecanoate (**18**).* Yield: 3.4 g (91.3%). I_{SPB-1} 2615; I_{SPWax} >2800. ^{1}H NMR: 4.22−4.28 (m, 2H), 2.82−2.92 (m, 1H), 2.29 (t, J = 7.1 Hz, 2H), 1.97−2.06 (m, 1H), 1.24−1.79 (m, 32H), 0.93 (t, J = 7.1 Hz, 3H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.7 (s), 62.0 (t), 41.2 (t), 37.9 (t), 37.3 (d), 34.3 (t), 31.9 (t), 29.1−29.7 (10t), 25.0 (t), 22.6 (t), 20.1 (t), 14.1 (q), 13.7 (q). MS: 372 (0.5, M^+), 258 (2), 239 (11), 116 (100), 101 (18), 88 (50), 87 (20), 83 (40), 71 (12), 57 (16), 55 (27), 43 (18), 41 (12).

*3-Sulfanylhexyl Octadodecanoate (**20**).* Yield: 3.5 g (87.5%). I_{SPB-1} 2816; I_{SPWax} >2800. ^{1}H NMR: 4.22−4.28 (m, 2H), 2.82−2.92 (m, 1H), 2.29 (t, J = 7.1 Hz, 2H), 1.97−2.06 (m, 1H), 1.24−1.79 (m, 36H), 0.93 (t, J = 7.1 Hz, 3H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR: 173.7 (s), 62.0 (t), 41.2 (t), 37.9 (t), 37.3 (d), 34.3 (t), 31.9 (t), 29.1−29.7 (12t), 25.0 (t), 22.6 (t), 20.1 (t), 14.1 (q), 13.7 (q). MS: 285 (6), 267 (10), 116 (100), 101 (15), 88 (45), 87 (20), 83 (38), 71 (12), 57 (16), 55 (22), 43 (18), 41 (11).

General Procedure for the Synthesis of Cys-*S*-Conjugates as Reference Compounds. *Preparation of S-(3-Hydroxy-1,1-dimethylpropyl)-L-cysteine (**21**).* *N*-Boc-cysteine (2 g, 9 mmol) was added to 3-methylbut-2-enal (1.21 g, 14.4 mmol) in CH_3CN (3 mL) in the presence of Cs_2CO_3 (1.5 g, 4.6 mmol). The mixture was stirred at 22 °C for 16 h. $NaBH_4$ (304 mg, 8 mmol) was added portion wise. After 1 h, the reaction was diluted with water, acidified to pH 1 with 2 M HCl, and extracted with ethyl acetate. The water phase was lyophilized and treated with 6 mL of concentrated HCl for 10 min. Water (20 mL) was added, and the mixture was loaded on a Dowex 50WX8 column (40 g, H^+ form). The resin was washed to neutrality with water (200 mL) and then eluted with an aqueous 0.3−2.1 M ammonia gradient. Compound **21** was obtained in 40% yield (750 mg). HPLC/ESI$^+$-MS: t_R 1.17 min; $[M + 1]^+$ 208. ^{1}H NMR (D_2O): 3.92 (dd, J = 4.5 and 8 Hz, 1H), 3.77 (t, J = 7 Hz, 2H), 3.14 (dd, J = 4.5 and 14 Hz, 1H), 3.02 (dd, J = 8 and 14 Hz, 1H), 1.85 (t, J = 8 Hz, 2H), 1.44 (s, 6H). ^{13}C NMR (D_2O): 175.6 (s), 61.4 (t), 57.1 (d), 47.8 (s), 45.9 (t), 31.8 (t), 31.0 (2q).

*Preparation of S-[3-(Acetyloxy)-1,1-dimethylpropyl]-L-cysteine (**22**).* Compound *N*-Boc-**21** (6.1 g, 20 mmol) was diluted in pyridine (20 mL) and treated with acetic anhydride (10 mL). After 4 h of stirring at room temperature, toluene was added (100 mL), and the solvent was distilled under vacuum. To the crude mixture, CH_2Cl_2 (20 mL) and trifluoroacetic acid (TFA, 40 mL) were added. After 1 h of stirring at 22 °C, toluene (100 mL) was added for the azeotropic distillation. The dried product was rediluted in water and submitted to FC on SiO_2-RP18 (130 g). The elution was performed by using 200 mL portions of water and then water/ethanol mixtures containing 10−50% ethanol. The solvent was distilled off. Compound **22** was obtained pure (730 mg, yield 15%). Note: During the *N*-Boc deprotection, the ester was partially cleaved. HPLC/ESI$^+$-MS: t_R 4.38 min; $[M + 1]^+$ 250. ^{1}H NMR (D_2O): 4.26 (t, J = 7 Hz, 2H), 3.92 (dd, J = 4 and 8 Hz, 1H), 3.13 (dd, J = 4 and 14 Hz, 1H), 3.03 (dd, J = 8 and 14 Hz, 1H), 2.08 (s, 3H), 1.94 (t, J = 8 Hz, 2H), 1.44 (s, 6H). ^{13}C NMR (D_2O): 177.5 (s), 175.4 (s), 65.4 (t), 57.1 (d), 47.8 (s), 42.1 (t), 31.2 (t), 31.0 (2q), 23.5 (q).

*Preparation of S-[3-(Butanoyloxy)-1,1-dimethylpropyl]-L-cysteine (**23**).* In the same way as described for **22**, compound **23** (1227 mg, 43%) was obtained. HPLC/ESI$^+$-MS: t_R 5.30 min; $[M + 1]^+$ 278. ^{1}H NMR (D_2O): 4.28 (t, J = 7 Hz, 2H), 4.01 (dd, J = 4 and 8 Hz, 1H), 3.17 (dd, J = 4 and 14 Hz, 1H), 3.06 (dd, J = 8 and 14 Hz, 1H), 2.37 (t, J = 7 Hz, 2H), 1.93−1.96 (m, 2H), 1.52−1.64 (m, 2H), 1.44 (s, 6H), 0.90 (t, J = 7 Hz, 3H). ^{13}C NMR (D_2O): 180.0 (s), 174.8 (s), 65.2 (t), 56.7 (d), 48.0 (s), 42.2 (t), 38.9 (t), 31.0 (t + 2q), 20.9 (t), 15.8 (q).

*Preparation of S-[1-(2-Hydroxyethyl)butyl]-L-cysteine (**24**).* Compound **24** was synthesized in the same way as was described for **22** but purified as a side product during of the *N*-Boc deprotection to prepare **25**. HPLC/ESI$^+$-MS: t_R 5.27 min; $[M + 1]^+$ 222. ^{1}H NMR (D_2O): 3.98−4.02 (m, 1H), 3.67−3.77 (m, 2H), 3.00−3.18 (m, 2H), 2.82−2.93 (m, 1H), 1.82−1.91 (m, 1H), 1.70−1.78 (m, 1H), 1.56−1.61 (m, 2H), 1.38−1.46 (m, 2H), 0.90 (t, J = 7 Hz, 3H). ^{13}C NMR (D_2O): 175.1 (s), 62.1 (t), 56.7 (d), 45.5 (d), 39.4 (t), 39.0 (t), 33.1 (t), 22.1 (t), 16.1 (q).

*Preparation of S-{1-[2-(Acetyloxy)ethyl]butyl}-L-cysteine (**25**).* Compound **25** was prepared in the same way as was described for **22** (900 mg, yield 16%). HPLC/ESI$^+$-MS: t_R 6.74 and 6.77 min (two diastereoisomers); $[M + 1]^+$ 264. ^{1}H NMR (D_2O): 4.22−4.28 (m, 2H), 3.98−4.02 (m, 1H), 3.00−3.18 (m, 2H), 2.88−2.93 (m, 1H), 2.10 (s, 3H), 1.97−2.02 (m, 1H), 1.80−1.90 (m, 1H), 1.56−1.61 (m, 2H), 1.39−1.46 (m, 2H), 0.90 (t, J = 7 Hz, 3H). ^{13}C NMR (D_2O): 177.6 (s), 175.4 (s), 65.9 (t), 56.9 (d), 45.5 (d), 39.2 (t), 35.3 (t), 33.4 (t), 23.4 (q), 22.1 (t), 16.0 (q).

RESULTS

Identification of Volatile Organic Sulfur-Containing Compounds in *P. trifoliata* (L.) Raf. The fruits were collected on

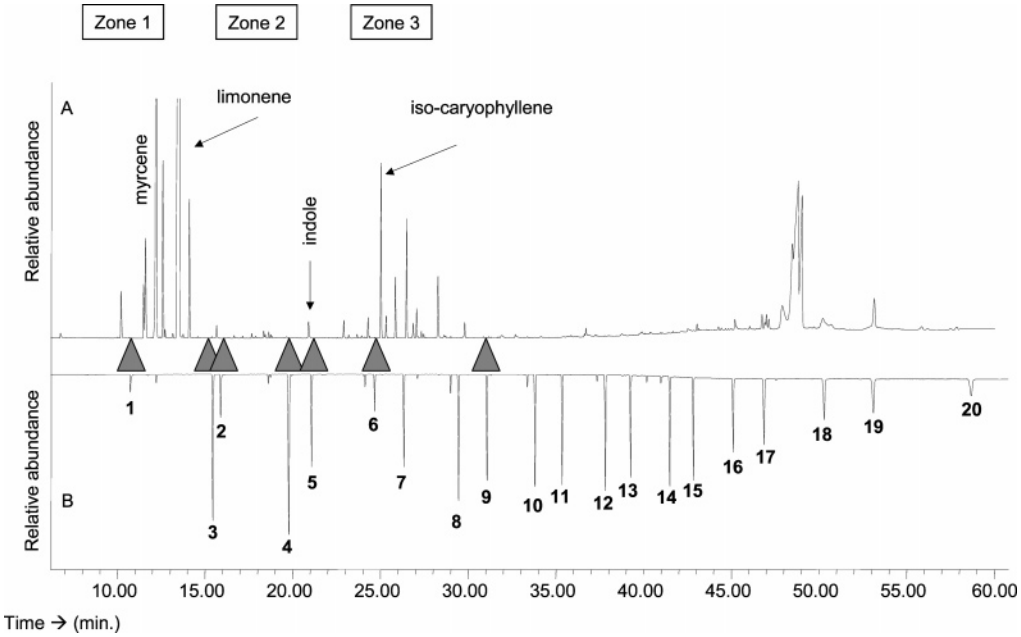


Figure 1. (**A**) GC-TIC trace on apolar column of the crude extract. (**B**) Inverted GC-TIC trace of an equimolar mixture of synthetic thiols from **1** to **20**. Marks represent retention times where sulfury odors were smelled.

two different local shrubs. The crude peel extract was submitted to GC/MS-O and GC/MS analyses. The odor profile was very rich. It started with green notes produced by hexanal and (*E*)-hex-2-en-1-ol, cheesy tonalities from butanoic acid, and fruity notes from esters such as 3-methylbutyl and 2-methylbutyl acetate, respectively. Between the calculated retention indices I_{SPB-1} 900 and 1060 (**Figure 1**, zone 1), typical odors deriving from terpenes such as α-pinene, sabinene, *β*-pinene, myrcene, 1,5-*p*-menthadiene, and limonene were observed and confirmed by GC/MS and by retention indices on both polar and apolar columns. After this zone and before a group of sequiterpenes (**Figure 1**, zone 3), many very well-known odors corresponding to linalool, citronellal, decanal, citronellol, indole, neryl acetate, and geranyl acetate were perceived. Indole was detected at I_{SPB-1} 1260 and I_{SPWax} 2452. It is responsible for the jasmine type smell of this *Citrus*-related species. We were mainly interested in the strong tropical fruit and sulfury odors of the fruit that were detected within these three zones at seven different retention times (**Figure 1**). Unfortunately, the concentrations were too low to record the mass spectra of these odorant compounds suspected to contain a sulfur atom. For this reason, the crude extract was submitted to affinity chromatography on Affi-Gel 501 (*4*, *5*), an efficient method to enrich compounds with free SH groups. The thiol extract was analyzed by GC/MS on apolar and polar columns. The retention indices *I* were measured, and the mass spectra were compared with those of authentic samples. The first thiol smelled sweaty, cheesy, roasted pork skin, and had I_{SPB-1} 942 and I_{SPWax} 1663 with a typical MS fragmentation pattern for **1**. The next compound smelled like mustard, rubbery, and tomato leafs. It had I_{SPB-1} 1080 and I_{SPWax} 1716 and a mass spectrum corresponding to **3**. We reinjected the thiol extract in SIM mode monitoring the ions at *m*/*z* 69 and 102 to screen for higher ester homologues. This experiment allowed us to discover a whole series of 3-methyl-3-sulfanylbutyl alkanoates from acetate **3** to octadecanoate **19** (**Scheme 1**).

To confirm the hypothesis, the 3-methyl-3-sulfanylbutyl esters **3**, **5**, **7**, **9**, **11**, **13**, **15**, **17**, and **19** were prepared. Each compound

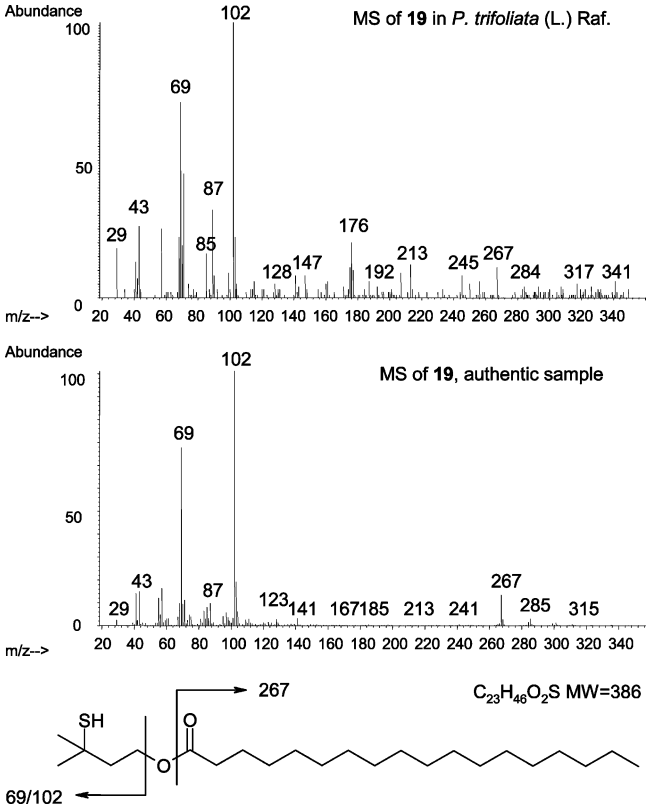


Figure 2. Comparison of mass spectra from natural **19** with that of synthetic **19**.

was fully characterized by ^{1}H and ^{13}C NMR, MS (**Figure 2**), as well as by I_{SPB-1} and I_{SPWax}.

We were still missing some organoleptically important molecules that had strong tropical fruity, sulfury types of odors. The compounds **4** and **6** were easily identified by GC/MS of the Affi-Gel extract. The analysis was repeated in the SIM mode monitoring the ions at *m*/*z* 116, 88, and 83. The ion *m*/*z* 116 results of the MacLafferty rearrangement involved the carbonyl

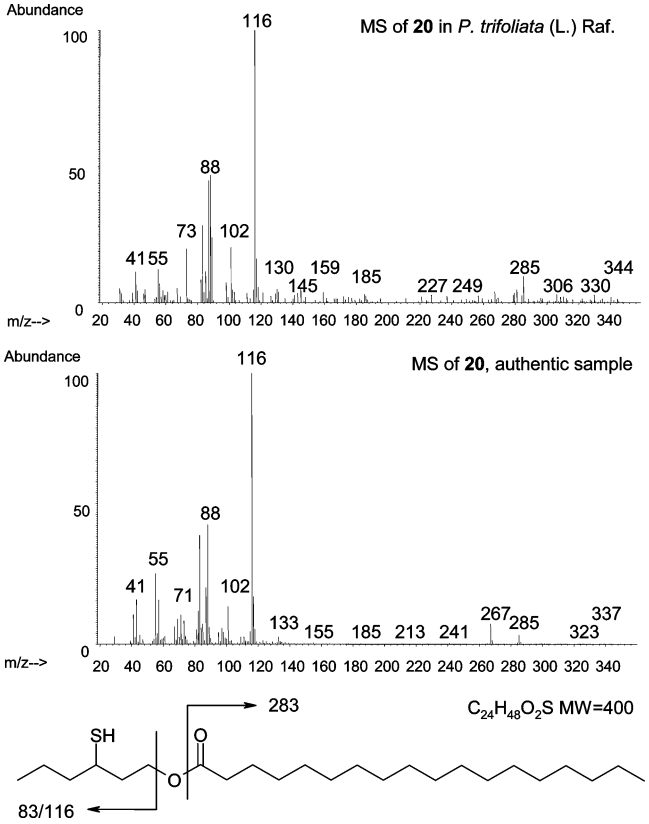


Figure 3. Comparison of mass spectra from natural **20** with that of synthetic **20**.

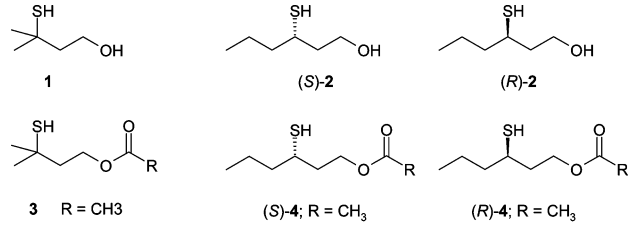
group of the ester moiety. The ion at *m*/*z* 88, an even-numbered mass, suggests a rearrangement rather than of a fragmentation. Furthermore, the *m*/*z* 116 fragment loses SH to give the ion at *m*/*z* 83. This selective MS analysis allowed us to bring out the background of the GC trace the following 3-mercaptohexyl alkanoates: **4**, **6**, **8**, **10**, **12**, **14**, **16**, **18**, and **20** (**Figure 3**).

On the basis of comparison with the GC-TIC peak area of octane-1-thiol (internal standard), the concentrations in the peel were estimated to be in the 50 ppb range for **1**−**4**, in the 40 ppb range for **6**, and in the 10 ppb range for **5**−**7**. The higher homologues were in the baseline of the TIC.

Preparation and Identification of Cysteine-*S*-Conjugates. There is growing evidence that compounds with such 1,3-positioned O,S moieties are formed in nature via an enzyme-mediated addition of an S-nucleophile, for example, glutathione or a metabolite thereof, to α,*β*-unsaturated carbonyl compounds. The volatile thiols are released from such *S*-conjugates upon the action of *β*-lyases (*6*−*11*). To screen *P. trifoliata* for such potential precursors, several cysteine-*S*-conjugates were prepared. *N*-Boc-cysteine was coupled to 3-methylbut-2-enal. The crude product was directly reduced with $NaBH_4$. The resulting *N*-Boc-Cys-*S*-alkanol was deprotected in TFA and purified by reversed phase FC. Compound **21** was then esterified with acetic anhydride to give **22** or with butanoic anhydride to give **23**. The same procedure was repeated with (*E*)-hex-2-enal to prepare **24** and **25** (**Scheme 2**).

The fruit juice was collected, and *S*-[1-(2-hydroxyethyl)-1-methylbutyl]-L-cysteine (**26**) was added as an internal standard (*6*). The lyophilized juice was analyzed by HPLC/ESI^+-MS. Except for the internal standard **26**, no cysteine-*S*-conjugates were detected. After the lyophilizate was desalted, **26** remained again the only conjugate detected. In a further experiment, the

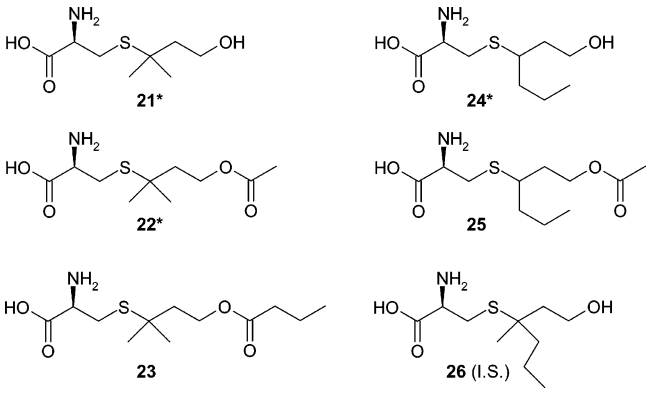
Scheme 1. Compounds with a 1,3-Positioned O,S Moiety Identified in *P. trifoliata* (L.) Raf.



5*	$R = CH_2\text{-}CH_2\text{-}CH_3$	**6**	$R = CH_2\text{-}CH_2\text{-}CH_3$
7*	$R = CH_2\text{-}(CH_2)_3\text{-}CH_3$	**8**	$R = CH_2\text{-}(CH_2)_3\text{-}CH_3$
9*	$R = CH_2\text{-}(CH_2)_5\text{-}CH_3$	**10***	$R = CH_2\text{-}(CH_2)_5\text{-}CH_3$
11*	$R = CH_2\text{-}(CH_2)_7\text{-}CH_3$	**12***	$R = CH_2\text{-}(CH_2)_7\text{-}CH_3$
13*	$R = CH_2\text{-}(CH_2)_9\text{-}CH_3$	**14***	$R = CH_2\text{-}(CH_2)_9\text{-}CH_3$
15*	$R = CH_2\text{-}(CH_2)_{11}\text{-}CH_3$	**16***	$R = CH_2\text{-}(CH_2)_{11}\text{-}CH_3$
17*	$R = CH_2\text{-}(CH_2)_{13}\text{-}CH_3$	**18***	$R = CH_2\text{-}(CH_2)_{13}\text{-}CH_3$
19*	$R = CH_2\text{-}(CH_2)_{15}\text{-}CH_3$	**20***	$R = CH_2\text{-}(CH_2)_{15}\text{-}CH_3$

* Identified for the first time in a plant source.

Scheme 2. Chemical Structures of Synthesized Precursors



* Identified in *P. trifoliata* (L.) Raf.; IS, internal standard.

internal standard **26** (3.3 ppm) was added to the peel material that remained from the juice preparation. After homogenization, centrifugation, and filtration on reversed phase, the spiked peel sample was analyzed by HPLC/ESI^+-MS under instrumental conditions that had been optimized with the authentic samples. The Cys-*S*-conjugates **21**, **22**, and **24** were detected at the corresponding retention times. The natural occurrence in *P. trifoliata* peel has thus been proven. However, neither acetyloxy derivative **23** nor butanoyloxy derivative **25** was detected. The detection limits of the Cys-*S*-conjugates were 5 ng of **21** and 10-fold lower for the internal standard **26** due to the stronger ionization of this latter compound. The low recovery factor of 1/30 for **26** can be explained by interactions like adsorptions on the highly sticky and viscous peel matrix or by some enzymatic residual activities degrading the Cys-*S*-conjugates. Therefore, we are able to give only an indication of the amounts of **21** and **24** present in the peel: 0.045 ± 0.004 and 0.011 ± 0.04 mg/kg, respectively, without applying recovery factors. The presence of **22** was confirmed, but the amount was too low to give quantitative data.

DISCUSSION

The presence of 18 sulfanylalkyl alkanoates in *P. trifoliata*, a species closely related to *Citrus*, is quite remarkable, as if nature had made use of combinatorial synthesis using the two parent sulfanyl alcohols **1** and **2** or their respective precursors as substrates for esterification.

Only a few volatile sulfur-containing constituents have been reported to occur in *Citrus* species. Undoubtedly, the most famous among these compounds is *p*-menth-1-ene-8-thiol, a powerful trace component of grapefruit juice (*Citrus paradisi* Macfayden) (*12*−*14*), also identified in yuzu (*Citrus junos* Sieb.) (*15*) and in late Valencia orange juice (*16*). The catty-smelling 4-methyl-4-sulfanylpentan-2-one has been detected in grapefruit juice (*13*, *14*) and in yuzu peel oil (*17*). The roles of 4-methyl-4-sulfanylpentan-2-ol and methional were described in cold-pressed grapefruit oil (*18*). Finally, *S*,*S*′-ethylidene dithioacetate has been identified in the juice of blood oranges (*19*).

Among the 20 constituents with a free SH group identified in *P. trifoliata*, only **1**−**4**, **6**, and **8** have been reported in natural systems previously (for a recent review, see ref *20*). The 3-sulfanylhexan-1-ol (**2**) and derivatives thereof are quite common in nature [e.g., passion fruit (*21*, *22*), *Vitis vinifera* L. var. Sauvignon blanc wine (*23*), clary sage (*24*), *Ruta chalepensis* L. (*17*), and *Persicaria odorata* (*5*)]. The 3-methyl-3-sulfanylbutan-1-ol (**1**) was reported to be present in Sauvignon blanc wine (*23*) and in passion fruit (*8*). Acetate **3** was found in coffee (*25*), in wine (*26*), and in passion fruit (*8*). The esters **5**, **7**, and **9**−**20** are new natural compounds.

The 3-sulfanylhexan-1-ol (**2**) and its esters have an asymmetric carbon. Analysis of the Affi-Gel extract by chiral GC/AED allowed determination of the ratio (*R*)-**2**/(*S*)-**2** to be 2:3 whereas the ratio (*R*)-**4**/(*S*)-**4** was 3:7. *P. trifoliata* L. is thus a further natural organism in which such 3-sulfanylalkyl derivatives are formed preferentially with the (*S*)-configuration [passion fruit (*22*, *27*, *28*), clary sage (*24*), *R. chalepensis* L. (*17*), and human axillary sweat (*29*)].

The conjugates **21** and **24** have previously been found in nature. Both **21** and **24** act as flavor precursors in the must of *V. vinifera* L. cv. Sauvignon blanc (*7*). Compound **21** is known as "felinine", a constituent of cat urine (*30*, *31*), while **24** has also been reported in the juice of passion fruit (*8*). The presence of conjugate **23** has never been reported.

Organoleptic Evaluations. The new natural constituents have interesting organoleptic properties and may find use in a wide range of flavor applications. Compounds **3**, **5**, **7**, and **9** were tasted at 1 ppm in plain water. The flavor profile of **3** had tomato leaf, grapefruit, and mango tonalities. Compound **5** was less fruity but was mushroom, cheesy, and quince. Compound **7** was described as over ripe fruit, mango, and black pepper. Compound **9** had descriptors like watercress, fruity, and fatty. Compound **9** will find application in various fat flavors, in red fruits, in tropical notes, and in smoke flavors. The higher homologues were tasted at 10 ppm. Compound **11** was animalic, smoky, and beefy. It fits into many flavors such as tobacco, fruit, and meat. Compound **13** lost the tropical fruit character and turned to alliaceous, ripe, and heavy. Compound **13** is readily seen in cognac flavors and savory preparations. Compound **15** had over ripe fruit, leafy, chive, bucchu, and slightly alliaceous characters with a very long-lasting sensation in the mouth. These higher homologues induced strong retronasal odor sensations. Surprisingly, **17** and **19** still had an odor when tasted at 10 ppm. The 3-sulfanylhexyl derivatives **4**, **6**, **8**, **10**, **12**, **14**, **16**, **18**, and **20** were also tasted in water. All compounds have tropical fruit odors, alliaceous tonalities, and induce long-lasting retronasal odor sensations. The strength decreases proportionally with the increase of the alkanoate chain length. Compounds **16** and **18** were still well-detected at 10 ppm.

The present chemical analysis has not been exhaustive with respect to volatile organic sulfur-containing constituents. The Affi-Gel 501 methodology is a highly efficient tool for the extraction of thiols (*4*, *5*) but disulfides (*R*-*S*-*S*-*R*) or sulfides (*R*-*S*-*R*) will not be extracted. This may explain why no structure could be associated to some typical descriptors for sulfur compounds like skunk odor (I_{SPB-1} 810), rubbery (I_{SPB-1} 854), and cooked asparagus (I_{SPB-1} 891).

ACKNOWLEDGMENT

We thank Francine Willcocks for suggesting the analysis of *P. trifoliata* (L.) Raf. and Dr. Didier Roguet, curator of the botanical garden of Geneva, Switzerland, for botanical certifications.

LITERATURE CITED

(1) Scora, R.; England, A. B.; Bitters, W. P. Essential oils of *Poncirus trifoliata* and its selections in relation to classification. *Phytochemistry* **1966**, *5*, 1139−1146.

(2) Nobile, L.; Pozzo-Balbi, T. Composition of the essential oil from *Citrus trifoliata* fruit rinds I. *Ann. Chim.* **1968**, *58*, 968−981.

(3) Tominaga, T.; Niclass, Y.; Frérot, E.; Dubourdieu, D. Stereoisomeric distribution of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in dry and sweet white wines made from *Vitis vinifera* (var. Sauvignon Blanc and Semillon). *J. Agric. Food Chem.* **2006**, *54*, 7251−7255.

(4) Full, G.; Schreier, P. Kovalente chromatographie: Ein wertvolles hilfsmittel fuer die aromastoffanalytik von thiolen im spurenbereich. *Lebensmittelchemie* **1994**, *48*, 1−4.

(5) Starkenmann, C.; Luca, L.; Niclass, Y.; Praz, E.; Roguet, D. Comparison of volatile constituents of *Persicaria odorata* (Lour.) Soják, (*Polygonum odoratum* Lour.) and *Persicaria hydropiper* L. Spach (*Polygonum hydropiper* L.). *J. Agric. Food Chem.* **2006**, *54*, 3067−3071.

(6) Starkenmann, C.; Niclass, Y.; Troccaz, M.; Clark, A. J. Identification of the precursor of (*S*)-3-methyl-3-sulfanylhexan-1-ol, the sulfury malodour of human axilla sweat. *Chem. Biodiversity* **2005**, *2*, 705−716.

(7) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon Blanc: *S*-cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215−5219.

(8) Tominaga, T.; Dubourdieu, D. Identification of cysteinylated aroma precursors of certain volatile thiols in passion fruit juice. *J. Agric. Food Chem.* **2000**, *48*, 2874−2876.

(9) Peyrot des Gachons, C.; Tominaga, T.; Dubourdieu, D. Sulfur aroma precursor present in *S*-glutathione conjugate form: Identification of *S*-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Agric. Food Chem.* **2002**, *50*, 4076−4079.

(10) Wakabayashi, H.; Wakabayashi, M.; Eisenreich W.; Engel, K.-H. Stereoselectivity of the β-lyase-catalyzed cleavage of *S*-cysteine conjugates of pulegone. *Eur. Food Res. Technol.* **2002**, *215*, 287−292.

(11) Cooper, A. Mechanism of cysteine *S*-conjugate β-lyases. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1998**, *72*, 199−238.

(12) Demole, E.; Enggist, P.; Ohloff, G. 1-*p*-Menthene-8-thiol: A powerful flavor impact constituent of grapefruit juice (*Citrus paradisi* Macfayden). *Helv. Chim. Acta* **1982**, *65*, 1785−1794.

(13) Buettner, A.; Schieberle, P. Characterization of the most odor-active volatiles in fresh, hand-squeezed juice of grapefruit (*Citrus paradisi* Macfayden). *J. Agric. Food Chem.* **1999**, *47*, 5189−5193.

(14) Buettner, A.; Schieberle, P. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfayden) by quantitation and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 1358−1363.

(15) Yukawa, C.; Osaki, K.; Iwabuchi, H. Volatile components of yuzu (*Citrus junos* Sieb. ex Tanaka). *Jpn. J. Food Chem.* **1994**, *1*, 46−49.

(16) Hinterholzer, A.; Schieberle, P. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragrance J.* **1998**, *13*, 49−55.

(17) Escher, S.; Niclass, Y.; van de Waal, M.; Starkenmann, C. Combinatorial synthesis by nature: Volatile organic sulfur-containing constituents in *Ruta chalepensis* L. *Chem. Biodiversity* **2006**, *3*, 943−957.
(18) Lin, J.; Rousseff, R. L. Characterization of aroma-impact compounds in cold-pressed grapefruit oil using time-intensity GC−olfactometry and GC−MS. *Flavour Fragrance J.* **2001**, *16*, 457−463.
(19) Naef, R.; Velluz, A.; Meyer, A. P. J. Volatile constituents of blood and blond orange juices: A comparison. *J. Essent. Oil Res.* **1996**, *8*, 587−595.
(20) Vermeulen, C.; Gijs, L.; Collin, S. Sensorial contribution and formation pathway of thiols in foods: A review. *Food Rev. Int.* **2005**, *21*, 69−137.
(21) Engel, K. H.; Tressl, R. Identification of new sulfur-containing volatiles in yellow passionfruit (*Passiflora edulis* f. *flavicarpa*). *J. Agric. Food Chem.* **1991**, *39*, 2249−2252.
(22) Werkhoff, P.; Güntert, M.; Krammer, G.; Sommer, H.; Kaulen, J. Vacuum headspace method in aroma research: Flavor chemistry of yellow passion fruits. *J. Agric. Food Chem.* **1998**, *46*, 1076−1093.
(23) Tominaga, T.; Furrer, A.; Robert, H.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Fragrance J* **1998**, *13*, 159−162.
(24) van de Waal, M.; Niclass, Y.; Snowden, R.; Bernardinelli, G.; Escher, S. 1-Methoxyhexane-3-thiol, a powerful odorant of clary sage (*Salvia sclarea* L.). *Helv. Chim. Acta* **2002**, *85*, 1246−1260.
(25) Kumazawa, K.; Masuda, H. Identification of odor-active 3-mercapto-3-methylbutyl acetate in volatile fraction of roasted coffee brew isolated by steam distillation under reduced pressure. *J. Agric. Food Chem.* **2003**, *51*, 3079−3082.
(26) Bouchilloux, P.; Darriet, P.; Henry, R.; Lavigne-Cruege, V.; Dubourdieu, D. Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *J. Agric. Food Chem.* **1998**, *46*, 3095−3099.
(27) Weber, B.; Dietrich, A.; Maas, B.; Marx, A.; Olk, J.; Mosandl, A. Stereoisomeric flavour compounds. 66. Enantiomeric distribution of the chiral sulphur-containing alcohols in yellow and purple passion fruits. *Z. Lebensm.-Unters. Forsch.* **1994**, *199*, 48−50.
(28) Weber, B.; Maas, B.; Mosandl, A. Stereoisomeric flavor compounds. 72. Stereoisomeric distribution of some chiral sulfur-containing trace components of yellow passion fruits. *J. Agric. Food Chem.* **1995**, *43*, 2438−2441.
(29) Troccaz, M.; Starkenmann, C.; Niclass, Y.; van de Waal, M.; Clark, A. J. 3-Methyl-3-sulfanylhexan-1-ol as a major descriptor for the human axilla-sweat odour profile. *Chem. Biodiversity* **2004**, *1*, 1022−1035.
(30) Westall, R. G. The amino acids and other ampholytes of urine. The isolation of a new sulphur-containing amino acid from cat urine. *Biochem. J.* **1953**, *55*, 244−248.
(31) Mattina, M. J. I.; Pignatello, J. J.; Swihart, R. K. Identification of volatile components of bobcat (*Lynx rufus*) urine. *J. Chem. Ecol.* **1991**, *17*, 451−462.

Received for review November 29, 2006. Revised manuscript received March 26, 2007. Accepted March 30, 2007.

JF063453H