

## First Identification of *O,S*-Diethyl Thiocarbonate in Indian Cress Absolute and Odor Evaluation of Its Synthesized Homologues by GC-Sniffing

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Indian cress (*Tropaeolum majus* L.) absolute was studied by GC-olfactometry (VIDEO-Sniff method) in order to identify odor-active aroma compounds. Because of its fruity-sulfury odor note, a compound that has never been identified in plant extracts before stood out: *O,S*-diethyl thiocarbonate, present at 0.1% (percentage of the total GC/FID area) in the extract. GC×GC-TOFMS allowed for a clean mass spectrum to be obtained, and isolation by preparative GC followed by NMR studies allowed its identification. Here, we report on the first detection of *O,S*-diethyl thiocarbonate in Indian cress absolute by GC-olfactometry/VIDEO-Sniff and on its isolation and identification. The synthesis and odor evaluation of its homologues are presented.

**KEYWORDS:** Thiocarbonates; Indian cress; *Brassicales*; GC-olfactometry; sulfur; odor; VIDEO-Sniff; absolute; GC-sniffing

### INTRODUCTION

Plant extracts such as essential oils, concretes, and absolutes are an inexhaustible source of odorant molecules, and flavor and fragrance research continues their study in order to find new flavoring substances. Because of their frequent low perception thresholds and various olfactive notes, volatile organosulfur compounds (VOSCs) are of particular interest (1–3). A great number of such compounds have already been discovered in the past, but further studies of natural extracts benefit from the constant improvement in analytical techniques, and especially from the use of GC-olfactometry (GC-O).

GC-O consists in using the human nose as a detector, and is in most cases performed simultaneously with physical detection. This allows the detection and odor evaluation of volatile compounds separated by GC and thus makes possible the odor evaluation of single compounds of a complex mixture without previous chemical separation (4). In order to obtain information on the olfactory impact of the mixture's constituents, different GC-O methodologies have been developed: detection frequency methods (NIF, SNIF), time-intensity methods (OSME, FSCM, and posterior intensity), and dilution to threshold methods (AEDA, CHARM) as well as a hybrid method, the VIDEO-

Sniff, which combines detection frequency and intensity methods. The VIDEO-Sniff method allows for a complete analysis of the data to be done, including the number of panelists detecting an odor, the intensities, as well as the semantic description of the stimuli. Additionally, in order to facilitate visual interpretation, an aromagram displaying a color for each olfactory class is obtained (4–8).

Indian cress, whose botanical name is *Tropaeolum majus* L., belongs to the botanical order *Brassicales* and to the *Tropaeolaceae* family (9). The plant (flowers, leaves, and seeds) is edible and is also called *Nasturtium* as an allusion to its spicy flavor (in Latin: *nasum* = nose and *torquere* = to grimace) (10). Its absolute is obtained by preparation of the concrete by extraction of the plant material with a nonpolar solvent (hexane) followed by dissolution of the concrete in ethanol, freezing, filtration, and distillation of the alcohol (11).

Here, we report on the first identification of *O,S*-diethyl thiocarbonate in a plant extract and on the synthesis and odor evaluation of its homologues by GC-sniffing.

### MATERIALS AND METHODS

**Chemicals and Extracts.** *Botanical Source and Plant Material.* Commercial Indian cress absolute was provided by Robertet S.A., 06131 Grasse, France. It had been produced in Rustenburg, South Africa, by extraction of the fresh plant with *n*-hexane followed by treatment with ethanol.

*NMR.* NMR spectroscopy was carried out on a Bruker Avance AC200 (200 MHz) and a DRX500 (500 MHz) system (Bruker,

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Wissembourg, France). The solvent was  $\text{CDCl}_3$  with 0.03% TMS (Aldrich), chemical shift ( $\delta$ ) is given in parts per million (ppm), and coupling constants ( $J$ ) are given in Hz.

**Elemental Composition Analysis.** One synthesized compound (*O*-methyl *S*-propyl thiocarbonate) does not have a known CAS Registry Number (CAS RN) and was submitted to elemental composition analysis at the Service Central d'Analyse de the CNRS, Solaize, France.

**Chemicals.** All chemicals were purchased from Sigma-Aldrich (St. Quentin Fallavier, France), Merck (Fontenay-sous-Bois, France), and VWR Prolabo (Fontenay-sous-Bois, France). Methanol (Riedel-de-Haën), absolute ethanol (Merck), 1-propanol (Fluka), dichloromethane (Riedel-de-Haën), *S*-methyl chlorothioformate (Aldrich), *S*-ethyl chlorothioformate (Aldrich), *S*-propyl chlorothioformate (Aldrich),  $\text{CDCl}_3$  with 0.03% v/v of TMS (Aldrich), and magnesium sulfate (VWR). Solvents were distilled prior to use.

**Analytical Studies.** *Eight-Way Gas Chromatography–Olfactometry (8W-GC-O).* 8W-GC-O was carried out as described by Berdagué et al. on the given system, and the complete results will be presented in a future article (8).

**Headspace Solid Phase Microextraction-2D Gas Chromatography–Time-of-Flight-Mass Spectrometry (HS-SPME-2DGC-TOFMS).** A SPME 75  $\mu\text{m}$  fiber (Carboxen/PDMS) purchased from Supelco (Bellefonte, PA, USA) was used for the extraction of volatiles from the absolute headspace. The fiber was conditioned according to manufacturer's recommendations prior to analysis. 8.6 mg of absolute were placed in a sealed 20 mL SPME vial. After the headspace equilibrium procedure (30 min), the SPME needle was picked through the vial, and the fiber was exposed to the headspace for 2 min at 21 °C (2 cm; without agitation of the sample). After sampling, the fiber was thermally desorbed in the glass SPME liner of the GC injection port during 2 min at 280 °C. Split injection (split ratio 1:20) was performed with a SPME Combipal autosampler (CTC Analytics AG, Industriestrasse 20, 4222 Zwingen, Switzerland) on a 6890N chromatograph (Agilent, Massy, France) equipped with a time-of-flight mass spectrometer LECO Pegasus 4D (LECO Corporation, St. Joseph, MI, USA) and a cryogenic modulator (LECO Quad Jet Modulator). The two separate columns and the modulator are placed in the oven of the 6890N chromatograph. The first dimension chromatographic separation (column 1) was performed on a SPB-5 capillary column (5% diphenyl-, 95% dimethylpolysiloxane, length 30 m, internal diameter 0.32 mm, film thickness 1  $\mu\text{m}$ ; Supelco, St-Germain-en-Laye, France). The second dimension chromatographic separation (column 2) was performed on a DB-17 capillary column (50% dimethyl-, 50% diphenylpolysiloxane, length 2.50 m, internal diameter 0.178 mm, film thickness 0.30  $\mu\text{m}$ ; J&W, Folsom, USA). The column 1 oven was held at 40 °C for 5 min, then ramped at 3 °C/min to 230 °C and held for 10 min. The column 2 oven was constantly set 15 °C higher than the column 1 oven. Ultra high purity helium (Air Liquide U quality, 99.9995%; flow, 1 mL/min) was used as carrier flow. The transfer line was heated at 250 °C, and the ion source set point was 200 °C. The detector voltage was 1600 V. The modulator sequences were modulation period 7 s and hot pulse 0.8 s. Mass spectra (EI) were collected from  $m/z$  33 to 230 u at a scan rate of 200 spectra/s. Chromatograms were processed using the automated data processing software ChromTOF with a signal-to-noise ratio of 50 (meaning that for the software to recognize a signal as a peak, the S/N-ratio had to be at least 50). The NIST/EPA/NIH mass spectral library (NIST05) (NIST, Gaithersburg, MD, USA) was used for peak identification. Van den Dool retention indices were calculated according to the polar and the nonpolar columns.

**Preparative Gas Chromatography.** The unknown analyte was isolated on a Varian GC3300 chromatograph equipped with a TCD (Varian, Les Ulis, France) by using a stainless steel column (length 3 m  $\times$  internal diameter 0.64 cm packed with 25% polyethyleneglycol 20 M on Chromosorb W AW 60–80 mesh, homemade column by Robertet S.A., Grasse, France). Carrier gas, helium; pressure, 138 kPa; injector temperature, 220 °C; detector temperature, 260 °C; auxiliary temperature, 220 °C; temperature of the TCD filament, 300 °C; splitless injection. Temperature program of the GC oven: 110 to 230 °C at 5 °C/min. In order to protect the preparative GC system, a volatile extract of Indian cress absolute was prepared by hydrodistillation as

follows: 15 g of absolute were hydrodistilled during 4.5 h in order to give 238 mg of volatile extract (yield 1.6%).

**Gas Chromatography–Mass Spectrometry.** GC-MS analysis was carried out using a 6890N chromatograph coupled to a 5973N mass spectrometer (Agilent, Massy, France). Samples were analyzed on fused-silica capillary columns HP-1 (polydimethylsiloxane, length 50 m  $\times$  internal diameter 0.20 mm  $\times$  film thickness 0.33  $\mu\text{m}$ ; Interchim, Montluçon, France) and HP-20 M (polyethyleneglycol, length 50 m  $\times$  internal diameter 0.20 mm  $\times$  film thickness 0.10  $\mu\text{m}$ ; Interchim, Montluçon, France). Carrier gas, helium; constant flow, 1 mL/min; injector temperature, 250 °C (nonpolar column) or 230 °C (polar column); split ratio, 1:100; temperature program, 60 to 250 °C at 2 °C/min then held isothermal (20 min) at 250 °C (nonpolar column) or 220 °C (polar column); ion source temperature, 155 °C; transfer line temperature, 250 °C (nonpolar column) or 230 °C (polar column); ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range 35–400 u. LRI were calculated with the help of a series of linear alkanes C6–C26 on nonpolar and polar columns.

**Gas Chromatography–Sniffing.** Odor evaluation by GC-sniffing was performed on a Shimadzu GC-2010 GC (Shimadzu, Champs-sur-Marne, France) equipped with an automatic injector type AOC-20i, a FID, and an ATAS olfactory port OP275 with a glass nasal cone (ATAS, Veldhoven, Netherlands). The thiocarbonates were analyzed on a capillary column DB-1 (polydimethylsiloxane, 50 m  $\times$  internal diameter 0.32 mm  $\times$  film thickness 0.52  $\mu\text{m}$ ; J&W, Folsom, USA). Carrier gas, nitrogen; constant pressure, 80 kPa; injector and temperature, 250 °C; split ratio, 1:100; injection volume, 1  $\mu\text{L}$ . 60% of the flow was directed to the FID, while 40% was directed into the heated sniffing port. Transfer line for nearly simultaneous detection: length inside oven; 0.89 m, length outside oven, 1.49 m; internal diameter, 0.25 mm; temperature, 250 °C. Capillary transfer line leading to the FID: length, 1.50 m; internal diameter, 0.25 mm. Temperature program: 60 °C, held during 10 min, then 10 °C/min up to 250 °C. Samples were prepared at 0.1% (0.5% for *O,S*-dimethyl thiocarbonate) in ether. Analysis was conducted by two nonsmokers trained according to J.N. Jaubert's Field of Odors and used to the organoleptic evaluation of natural extracts as well as to GC-olfactometry (12). Training was realized by first smelling standard compounds classified in odorant poles according to the Field of Odors in a static evaluation and then by realizing GC-O/AEDA on a standard mixture of 12 odorants in order to evaluate panelists' performance and to exclude specific anosmia.

**Determination of the Odor Threshold of *O,S*-diethyl Thiocarbonate by GC-Sniffing.** Threshold determination by GC-sniffing was done on the instrument described above according to Ferreira et al. (13). Solutions of the thiocarbonate were prepared in ethanol and analyzed by six panelists. Splitless injection, injection volume: 1  $\mu\text{L}$ . Temperature program of the GC oven: 60 to 220 °C at 10 °C/min. The last solution perceived by more than 50% of the panel determined the odor threshold, which is given in ng (mass perceived by the human nose in the sniffing cone).

**Synthesis.** *Synthesis of Thiocarbonates.* Anhydrous alcohol (MeOH, EtOH, or *n*-PrOH, 4 mmol) and pyridine (4 mmol) were placed in 3 mL of dichloromethane in a two-neck flask equipped with a cooler and a rubber septum. The mixture was stirred and cooled with a water bath. *S*-Alkyl chlorothioformate (*S*-methyl, *S*-ethyl or *S*-propyl, 4 mmol) was added via the septum with a syringe. After addition was completed, the mixture was heated to reflux during 15 min, and then cooled down to room temperature. Two milliliters of water was added, and stirring was continued during 5 min. The phases were separated, the organic phase dried over magnesium sulfate, and the residual solvent evaporated carefully under reduced pressure. Thiocarbonates were obtained in yields from 9% to 93% with GC purities between 90% and 96%.

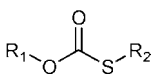
**Synthesized Thiocarbonates.** *O,S*-Dimethyl thiocarbonate,  $\text{C}_3\text{H}_6\text{O}_2\text{S}$  (106.01 u). CAS RN 38103-95-6. Yield n.d.

$^1\text{H}$  NMR (200 MHz):  $\delta$  2.35 (s, 3,  $\text{SCH}_3$ ), 3.83 (s, 3,  $\text{OCH}_3$ ).  $^{13}\text{C}$  NMR (200 MHz):  $\delta$  13.32 ( $\text{SCH}_3$ ), 54.09 ( $\text{OCH}_3$ ), 172.09 ( $\text{CO}$ ).

*O*-Methyl *S*-ethyl thiocarbonate,  $\text{C}_4\text{H}_8\text{O}_2\text{S}$  (120.02 u). CAS RN 38103-96-7. Yield 90%.

$^1\text{H}$  NMR (200 MHz):  $\delta$  1.32 (t, 3,  $J = 7.4$  Hz,  $\text{SCH}_2\text{CH}_3$ ), 2.87 (q, 2,  $J = 7.4$  Hz,  $\text{SCH}_2\text{CH}_3$ ), 3.81 (s,  $\text{OCH}_3$ ).  $^{13}\text{C}$  NMR (200 MHz):  $\delta$  14.95 ( $\text{SCH}_2\text{CH}_3$ ), 25.26 ( $\text{SCH}_2\text{CH}_3$ ), 53.81 ( $\text{OCH}_3$ ), 171.53 ( $\text{CO}$ ).

**Table 1.** Thiocarbonates Synthesized, Their LRIs, MS Data, and Odors

thiocarbonate 	LRI <sup>a</sup>	El-MS fragmentation <i>m/z</i> (%)	odor (GC-sniffing) <sup>b</sup>
		R <sub>1</sub> = methyl	
R <sub>2</sub> = methyl	767/1148	108 (4), 106 (85) [M <sup>+</sup> ·], 75 (43), 61 (18), 59 (61), 49 (5), 48 (5), 47 (100), 46 (30), 45 (42)	fruity, very faint <sup>c</sup>
R <sub>2</sub> = ethyl	825/1200	120 (77) [M <sup>+</sup> ·], 89 (14), 88 (15), 61 (93), 60 (13), 59 (100), 48 (22), 47 (12), 45 (18), 35 (18)	sulfury-fruity, pineapple
R <sub>2</sub> = propyl	924/1280	134 (49) [M <sup>+</sup> ·], 93 (37), 92 (41), 61 (77), 59 (100), 47 (27), 45 (19), 43 (47), 41 (56), 39 (20)	alliaceus-fruity, strong
		R <sub>1</sub> = ethyl	
R <sub>2</sub> = methyl	829/1200	120 (77) [M <sup>+</sup> ·], 75 (62), 61 (57), 50 (5), 49 (5), 48 (100), 47 (51), 46 (13), 45 (35), 43 (5)	sulfury, pineapple
R <sub>2</sub> = ethyl	906/1240	34 (89) [M <sup>+</sup> ·], 89 (29), 75 (56), 62 (100), 61 (40), 60 (10), 59 (8), 47 (47), 46 (20), 45 (20)	fruity, red fruit then sulfury
R <sub>2</sub> = propyl	1002/1325	148 (50) [M <sup>+</sup> ·], 76 (39), 75 (75), 62 (28), 47 (61), 45 (26), 43 (79), 42 (100), 41 (61), 39 (21)	red fruit, alliaceous
		R <sub>1</sub> = propyl	
R <sub>2</sub> = methyl	926/1273	134 (20) [M <sup>+</sup> ·], 75 (51), 61 (8), 49 (13), 48 (24), 47 (22), 45 (13), 43 (100), 41 (62), 39 (8)	sulfury, mains gas
R <sub>2</sub> = ethyl	1001/1317	148 (17) [M <sup>+</sup> ·], 89 (26), 75 (8), 63 (7), 62 (27), 61 (9), 47 (6), 43 (100), 41 (41), 39 (5)	sulfury
R <sub>2</sub> = propyl	1096/1399	162 (9) [M <sup>+</sup> ·], 103 (9), 89 (7), 76 (13), 61 (4), 47 (8), 43 (100), 42 (22), 41 (38), 39 (8)	sulfury, alliaceous

<sup>a</sup> Retention indices as determined on HP-1 and HP-20 M using the homologous series of *n*-alkanes. <sup>b</sup> Samples were prepared at 0.1% in ether. <sup>c</sup> Prepared at 0.5%.

*O*-Methyl *S*-propyl thiocarbonate, C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>S (134.04 u). New compound. Yield 76%.

<sup>1</sup>H NMR (200 MHz): δ 1.00 (t, 3, *J* = 7.3 Hz, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.67 (sextuplet, 2, *J* = 7.3 Hz, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.85 (t, 2, *J* = 7.2 Hz, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3.81 (s, 3, OCH<sub>3</sub>). <sup>13</sup>C NMR (200 MHz): δ 13.10 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 23.08 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 32.86 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 53.85 (OCH<sub>3</sub>), 171.70 (CO). Elemental composition analysis: calculated % C 44.76, H 7.52, S 23.85; measured %, 44.49, H 7.41, S 23.46.

*O*-Ethyl *S*-methyl thiocarbonate, C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>S (120.02 u). CAS RN 14919-12-1. Yield 9%.

<sup>1</sup>H NMR (200 MHz): δ 1.29 (t, 3, *J* = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 2.32 (s, 3, SCH<sub>3</sub>) 4.27 (q, 2, *J* = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR (200 MHz): δ 13.37 (OCH<sub>2</sub>CH<sub>3</sub>), 14.28 (SCH<sub>3</sub>), 63.49 (OCH<sub>2</sub>CH<sub>3</sub>), 171.52 (CO).

*O,S*-Diethyl thiocarbonate, C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>S (134.04 u). CAS RN 3554-12-9. Yield 75%.

<sup>1</sup>H NMR (200 MHz): δ 1.31 (t, 3, *J* = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 1.32 (t, 3, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>), 2.87 (q, 2, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>), 4.28 (q, 2, *J* = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR (200 MHz): δ 14.18 (SCH<sub>2</sub>CH<sub>3</sub>), 14.94 (OCH<sub>2</sub>CH<sub>3</sub>), 25.13 (SCH<sub>2</sub>CH<sub>3</sub>), 63.14 (OCH<sub>2</sub>CH<sub>3</sub>), 170.19 (CO).

*O*-Ethyl *S*-propyl thiocarbonate, C<sub>6</sub>H<sub>12</sub>O<sub>2</sub>S (148.06 u). CAS RN 53120-91-5. Yield 80%.

<sup>1</sup>H NMR (200 MHz): δ 1.00 (t, 3, *J* = 7.3 Hz, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.30 (t, 3, *J* = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 1.67 (sextuplet, 2, *J* = 7.2 Hz, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.84 (t, 2, *J* = 7.2 Hz, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.27 (q, 2, OCH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR (200 MHz): δ 13.13 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 14.24 (OCH<sub>2</sub>CH<sub>3</sub>), 23.14 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 32.80 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 63.24 (OCH<sub>2</sub>CH<sub>3</sub>), 171.12 (CO).

*O*-Propyl *S*-methyl thiocarbonate, C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>S (134.04 u). CAS RN 135742-57-3. Yield 30%.

<sup>1</sup>H NMR (200 MHz): δ 0.95 (t, 3, *J* = 7.4 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.70 (sextuplet, 2, *J* = 7.1 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.34 (s, 3, SCH<sub>3</sub>), 4.20 (t, 2, *J* = 6.7 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR (200 MHz): δ 10.22 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 13.39 (SCH<sub>3</sub>), 22.05 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 69.01 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 171.64 (CO).

*O*-Propyl *S*-ethyl thiocarbonate, C<sub>6</sub>H<sub>12</sub>O<sub>2</sub>S (148.06 u). CAS RN 35363-44-1. Yield 90%.

<sup>1</sup>H NMR (200 MHz): δ 0.95 (t, 3, *J* = 7.4 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.32 (t, 3, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>), 1.69 (sextuplet, 2, *J* = 7.1 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.87 (q, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>), 4.18 (t, 2, *J* = 6.7 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR (200 MHz): δ 10.22 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 15.01 (SCH<sub>2</sub>CH<sub>3</sub>), 22.04 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 25.23 (SCH<sub>2</sub>CH<sub>3</sub>), 68.76 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 171.10 (CO).

*O,S*-Dipropyl thiocarbonate, C<sub>7</sub>H<sub>14</sub>O<sub>2</sub>S (162.07 u). CAS RN 136558-84-4. Yield 93%.

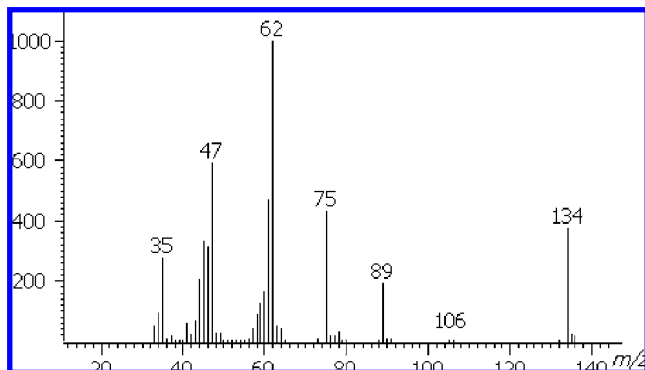
<sup>1</sup>H NMR (200 MHz): δ 0.96 (t, 3, *J* = 7.4 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.00 (t, 3, *J* = 7.4 Hz, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.68 (m, 6, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub> and OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.84 (t, 2, *J* = 7.3 Hz, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.17 (t, 2, *J* = 6.7 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR (200 MHz): δ 10.27 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 13.21 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 22.04 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 23.18 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 32.83 (SCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 68.84 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 171.32 (CO).

## RESULTS AND DISCUSSION

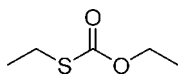
Indian cress absolute is a complex matrix that is dominated by the presence of a major nitrogen and sulfur containing compound: *N*-benzyl *O*-ethyl thiocarbamate (83.7% of the total GC/FID area) (*14*). Further major compounds are benzaldehyde (0.3%), *N*-(phenylmethylene)-*N*-benzylamine (0.7%), ethyl linolenate (0.9%), ethyl palmitate (1.0%), benzyl cyanide (1.1%), *N*-benzyl *O*-ethyl carbamate (3.8%), and benzyl isothiocyanate (3.9%), and numerous nitrogen and sulfur containing compounds are present in the absolute.

During the study of Indian cress absolute by GC-O on a multiport 8W-GC-O device (simultaneous analysis by eight sniffers) with the VIDEO-Sniff method, a fruity-sulfury odor with a LRI<sub>RTX-5</sub> = 931 stood out for several reasons: (a) the odor was perceived by 15 out of 16 panelists (94% frequency of detection), (b) the mean intensity over eight panelists of the odor was 4.3 on a scale from 1 (not intense) to 5 (very intense), (c) its fruity-sulfury odor was not common, (d) the corresponding mass spectrum showed sulfur isotopic peaks (M + 1)<sup>+</sup> and (M + 2)<sup>+</sup> and a molecular ion at 134 *m/z*, and (e) the compound had previously been classified as an unknown sulfur compound since it is present at only 0.1% in the extract and only GC-O revealed its olfactory impact.

During GC-O, the judges gave two olfactory signals for the unknown compound: the perception started by being described as fruity and changed rapidly into fruity-sulfury. This could have indicated a coelution of a sulfur compound with a compound having a fruity note, such as an ester. However, SPME-GC×GC-TOFMS analyses showed no major coelutions at



**Figure 1.** Time-of-flight mass spectrum of *O,S*-diethyl thiocarbonate, obtained by GC  $\times$  GC-TOFMS.



**Figure 2.** Molecular structure of *O,S*-diethyl thiocarbonate identified in Indian cress absolute.

$LRI_{RTX-5} = 931$  (1st dimension LRI) and also allowed for a clean mass spectrum to be obtained (**Figure 1**). The mass spectrum showed the presence of an ethoxy group ( $m/z = 45$ ) as well as sulfur isotopic patterns. Another explanation for the fruity-sulfury note could be the fact that diethyl disulfide, also present in the absolute with a  $LRI_{RTX-5} = 926$ , either tails chromatographically or tails in the glass nose cone, thus giving the sulfury odor. But this could be excluded because the judges smelled the fruity note at first, which then turned into fruity-sulfury. Plus, the fruity perception was well separated from the sulfury perception of diethyl disulfide.

This previously unknown compound could not be identified using MS and retention indices on polar and nonpolar columns alone using the databases available to the authors (commercial libraries Wiley, MassFinder 2.1 Library, NIST98, and NIST05 as well as laboratory mass spectra libraries built from pure substances). Since it was present at only 0.1% in the absolute ( $LRI_{HP-1} = 905$ ), isolation by preparative GC seemed to be the most efficient way. In order to avoid damage to the preparative GC system by repeatedly injecting the absolute (solvent extract, and thus presence of nonvolatile compounds), a volatile extract was prepared by hydrodistillation (yield: 238 mg). The target compound was present at 2.6% of the total GC/FID area in the obtained volatile extract. After preparative GC, 3 mg of analyte was obtained and analyzed by NMR.  $^1H$  NMR confirmed the ethoxy group but also indicated another  $CH_3-CH_2-X-$  motif, which, according to the chemical shift in  $^1H$  NMR and mass fragments (MS:  $m/z = 61$ ), seemed to contain sulfur:  $CH_3-CH_2-S-$ . No further signals were visible in  $^1H$  NMR, but  $^{13}C$  NMR showed a carbonyl group at 171 ppm (corresponding to the loss of a mass fragment  $m/z = 28$  by the molecular ion, resulting in a fragment of  $m/z = 106$ ). According to this information, we identified the analyte as *O,S*-diethyl thiocarbonate (**Figure 2**). Olfactory evaluation by GC-sniffing of the isolated compound confirmed the fruity-sulfury odor perceived during 8W-GC-O.

The identification of two thiocarbonates (*O,S*-dimethyl and *O*-ethyl *S*-methyl thiocarbonate) has previously been reported in pineapple extracts by Akioka et al. and Tamura et al., and *O,S*-dimethyl thiocarbonate has also been identified in wine, but *O,S*-diethyl thiocarbonate has never been found in plants or plant extracts before (15–17). Concerning its formation in Indian cress absolute, it could take place during the processing of the concrete in order to obtain the absolute (treatment of the

concrete with ethanol). It might result from the action of ethanol on the corresponding acid (*S*-ethyl carbonothioate), but this is yet to be elucidated. The acid as well as thiocarbonates in general have never been described in plants or plant extracts before, and the origin of these compounds will need further study. However, the natural occurrence of *O,S*-diethyl thiocarbonate cannot be excluded either.

The target compound was synthesized by reaction of *S*-ethyl chlorothioformate and ethanol with satisfying yields (75%), and GC purity ( $\sim 96\%$ ) and its spectral and olfactory analyses as well as comparison of its linear retention index ( $LRI_{HP-1} = 906$ ) with the one of the target compound in the absolute ( $LRI_{HP-1} = 905$ ) confirmed the identification. This compound and its homologues have never been identified in plants or plant extracts before. They are mostly reported in the literature as byproducts, or their synthesis is described (18–20). However, since *O,S*-diethyl thiocarbonate presents an interesting odor, we decided to synthesize eight homologues for odor evaluation (**Table 1**). The odor notes are fruity-sulfury, but with perceivable differences. The sulfury notes go from alliaceous to mains gas, whereas the fruity note varies between red fruit and pineapple. The odor threshold of *O,S*-diethyl thiocarbonate was further determined by GC-O to be 0.2 ng.

Indian cress absolute has already led to the identification of odorant thiocarbonates in the past (14), and the identification of a thiocarbonate presented here proves that natural matrices still are an important source of new compounds. GC-olfactometry highly contributes to the discovery of new odorant compounds since it permits rapid identification of odorant zones in a chromatogram, combined with the evaluation of the odorant impact and interest of the analytes. Hence, unnecessary isolation of odorless compounds is avoided, and research focused on odorant analytes. The complete study of Indian cress by GC-olfactometry/VIDEO-Sniff will be given in a future article (Breme et al., manuscript in preparation).

**Supporting Information Available:**  $^1H$  NMR of *O,S*-diethyl thiocarbonate and  $^{13}C$  NMR of *O,S*-diethyl thiocarbonate. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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