

# Allium Chemistry: Identification of Natural Abundance Organoselenium Volatiles from Garlic, Elephant Garlic, Onion, and Chinese Chive Using Headspace Gas Chromatography with Atomic Emission Detection

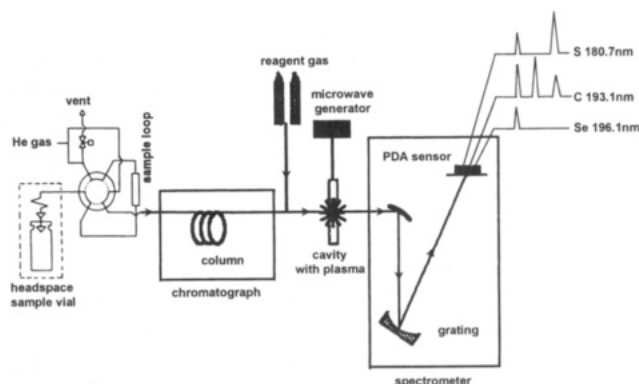
**Keywords:** Garlic; elephant garlic; onion; Chinese chive; *Allium sativum*; *Allium ampeloprasum*; *Allium cepa*; *Allium tuberosum*; headspace gas chromatography; gas chromatography–atomic emission detection; dimethyl selenide; methanesulfenoselenoic acid methyl ester; dimethyl diselenide; bis(methylthio)selenide; allyl methyl selenide; 2-propenesulfenoselenoic acid methyl ester; 1-propenesulfenoselenoic acid methyl ester; (allylthio)(methylthio)selenide; bis(allylthio)selenide; organoselenium compounds

## INTRODUCTION

Genus *Allium* plants, especially garlic (*Allium sativum*), contain more selenium than most vegetables (Morris, 1970). While onion (*Allium cepa*) contains selenocystine (1), selenomethionine (2), and possibly S-methylselenocysteine Se-oxide (3) (Spåre and Virtanen, 1964), the form in which Se is released when *Allium* spp. are cut is unknown, because volatile Se compounds would be obscured by approximately 12 000 times higher levels of similar organosulfur compounds (Block, 1992; Block et al., 1993). In view of the interest in Se biochemistry (Shamberger, 1983; Stadtman, 1990; Böck et al., 1991) and in *Allium* medicinal properties (Block, 1992, 1994; Block et al., 1993; Lawson, 1993), including using Se-enriched garlic to treat Se-deficiency diseases (Ip and Lisk, 1993) and inhibit tumorigenesis (Ip et al., 1992), we sought to identify volatile Se compounds from cut *Allium* spp. We have identified  $RS_nSeR'$  ( $n = 0-2$ , R, R' = methyl, allyl, 1-propenyl) and MeSeSeMe from *Allium* spp. by headspace analysis using the element-specific technique of gas chromatography with atomic emission detection (GC–AED) and mass spectrometry (GC–MS), confirming spectroscopic assignments of new compounds by synthesis.

When *Allium* spp. are cut, alliinase cleaves precursor S-alk(en)yl cysteine S-oxides [ $RS(O)CH_2CH(NH_2)CO_2H$ , R = methyl, allyl, 1-propenyl, or *n*-propyl] to reactive sulfenic acids RSOH which condense, giving thiosulfinate esters  $RS(O)SR'$ , the principal *Allium* flavorants (Block et al., 1992a,b), whose slow hydrolysis affords polysulfides,  $RS_nR'$ . The most volatile polysulfides can be detected by headspace gas chromatography (HS/GC), a technique that can increase detection sensitivity by 2–3 orders of magnitude over conventional sampling methods and simultaneously minimize chemical changes in volatile flavor and aroma components during analysis (Block and Calvey, 1994). We have used HS/GC–AED to detect and identify trace levels of volatile Se compounds from cut *Allium* spp. [garlic and onion contain 0.28 and 0.015  $\mu\text{g}$  of Se/g of fresh weight (Morris, 1970), respectively, compared to 3.3 and 0.84 mg of S].

Atomic plasma spectral emission provides powerful element-specific detection for gas chromatography (Figure 1) (Uden, 1992). It has the important advantages of high sensitivity, elemental selectivity, and the possibility of simultaneous multielement analysis (Quimby and Sullivan, 1990; Sullivan and Quimby, 1990). The AED response can flag compounds in the GC effluent that contain specific elements, even though these compounds may be present in very small amounts or may coelute with other components. We use the selenium emission line at 196.1 nm to detect organoselenium species while concurrently monitoring S and C by lines at 180.7 and 193.1 nm, respectively. Empirical formula



**Figure 1.** Schematic diagram of headspace/gas chromatography–atomic emission detector (HS/GC–AED; Hewlett-Packard Co., Palo Alto, CA). Sample is capped in the headspace sample vial which is placed in a heated bath to achieve thermal equilibration. The headspace gas is then driven through the sample loop to the GC injection port. The GC eluent is introduced into a microwave-energized helium plasma that is coupled to a photodiode array (PDA) optical emission spectrometer. The plasma is sufficiently energetic to atomize all of the elements in a sample and to excite their characteristic atomic emission spectra. Up to four elements that have adjacent spectral emission wavelengths can be monitored simultaneously, illustrated here for sulfur (emission at 180.7 nm), carbon (emission at 193.1 nm), and selenium (emission at 196.1 nm).

calculation utilizing compound independent calibration (Uden et al., 1986) was employed for qualitative identification of the Se-containing peaks. These assignments were confirmed by HS/GC–MS. The above techniques were used to analyze the headspace above homogenized garlic, elephant (great-headed) garlic (*Allium ampeloprasum*), onion, and Chinese chive (*Allium tuberosum*). As the HS/GC–MS procedure was not sufficiently sensitive and selective to directly detect natural levels of *Allium* Se compounds, garlic grown in a Se-fertilized medium (Se-enriched garlic) (Ip et al., 1992) or garlic homogenates augmented by addition of selenoamino acids (see below) were utilized.

## EXPERIMENTAL PROCEDURES

**Materials and Instruments.** A HP 7694 headspace sampler and a HP 5921A GC–AED are used. Fresh elephant garlic (6 g) was homogenized with 13 mL of water, saturated with 5 g of  $Na_2SO_4$ , and capped in a 20 mL headspace vial. Each vial was heated with shaking at 90 °C for 45 min. The 1 mL sample loop and the transfer line were maintained at 90 °C. A 60 m  $\times$  0.32 mm  $\times$  0.25  $\mu\text{m}$  HP-INNOWax column was used. The GC oven temperature was set isothermal at 35 °C for 2 min and then increased at 5 °C/min to 180 °C. The helium carrier gas flow rate was 28  $\text{cm}^3/\text{s}$ . The GC–AED used  $O_2$  and  $H_2$  as reagent gases with detection at 180.7, 193.1, and 196.1 nm for S, C, and Se, respectively. As the mass

spectrometer (HP MSD) was not sufficiently sensitive and selective to directly detect natural levels of organoselenium compounds in unenriched *Allium* spp.. Se augmentation was achieved by addition of L-selenomethionine, L-selenoethionine, or L-selenocystine to the *Allium* homogenates at the level of 5 mg/g of plant with headspace sampling as above.

**Synthetic Standards: General.** Synthetic Se-S compounds, prepared according to the method of Ryan (1988) or Potapov et al. (1992), were malodorous, unstable liquids which were characterized immediately after preparation by GC-MS and  $^1\text{H}/^{13}\text{C}$  NMR spectroscopy and stored in solution under argon at  $-78^\circ\text{C}$  until used. GC-EI-mass spectra of the *Allium*-derived Se compounds were in excellent agreement with mass spectra obtained on synthetic samples. Molecular ions and major fragments match the natural ratio of stable Se and S isotopes; only peaks associated with the most abundant  $^{80}\text{Se}$  isotope are reported here.

**Bis(allylthio)selenide (13).** *N*-Chlorobenzotriazole (1.5 g, 20 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (5 mL) was cooled under argon to  $-20^\circ\text{C}$ , and a precipitate appeared. A solution of diallyl selenide (0.8 g, 5 mmol) (Amosova et al., 1991) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added dropwise by syringe. The resulting homogeneous solution was warmed to room temperature and stirred for 12 h; a white precipitate of **12** appeared. A solution of 2-propenethiol (0.74 g, 10 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added, giving a yellow solution, which was subjected to rapid flash chromatography (silica gel;  $\text{CHCl}_3$ ). The eluate was concentrated in vacuo to give **13** as an unstable yellow oil (0.35 g, 39% yield):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.85 (m, 2H), 5.17–5.19 (m, 4H), 3.59 (d,  $J = 6.9$  Hz, 4H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  133.9 ( $=\text{CH}_2$ ), 118.4 ( $=\text{CH}-$ ), 41.1 ( $\text{CH}_2$ ) [diallyl disulfide:  $^1\text{H}$  NMR  $\delta$  5.8–5.9 (m, 2H), 5.1–5.2 (m, 4H), 3.31 (d,  $J = 7.5$ );  $^{13}\text{C}$  NMR  $\delta$  133.4, 118.4, 42.2]; EI-GC-MS  $m/z$  226 ( $\text{M}^+$ , 3%), 194 (1%), 153 (7%), 113 (78%), 73 (80%), 41 (100%).

**Bis(methylthio)selenide (7).** In a similar manner, **7** was prepared via reaction of methanethiol (0.48 g, 10 mmol) with **12** (5 mmol). The crude yellow title compound, **7**, was unstable and was used as a  $\text{CDCl}_3$  solution without concentrating:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.72 (s, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  22.5 [MeSSMe shows  $^1\text{H}$  and  $^{13}\text{C}$  NMR  $\delta$  2.38 and 22.0; MeSeSeMe shows  $^1\text{H}$  and  $^{13}\text{C}$  NMR  $\delta$  2.55 and 10.4; MeSSeMe shows  $^1\text{H}$  NMR  $\delta$  2.42 and 2.57 (Potapov et al., 1992)]; EI-GC-MS  $m/z$  174 ( $\text{M}^+$ , 57%), 159 (8%), 127 (46%), 112 (30%), 94 (9%), 80 (4%), 61 (3%), 45 ( $\text{CH}_3^+$ , 61%).

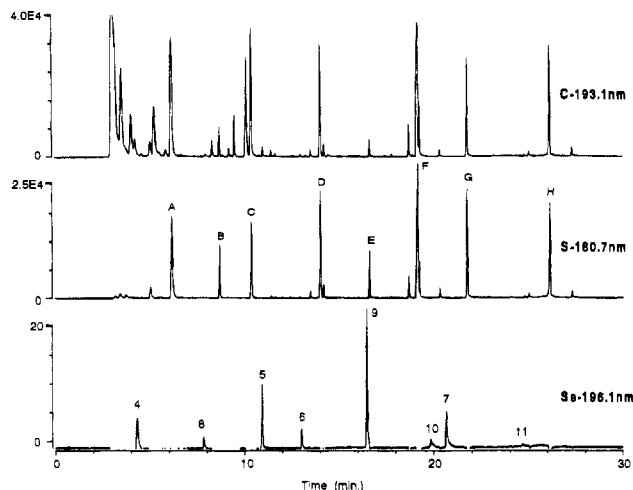
**(Allylthio)(methylthio)selenide (11).** In a similar manner, **11** was prepared via reaction of methanethiol (0.24 g, 5 mmol) and 2-propenethiol (0.38 g, 5 mmol) with **12** (5 mmol). Compound **11** was identified by EI-GC-MS:  $m/z$  200 ( $\text{M}^+$ , 7%), 159 (8%), 127 (10%), 112 (12%), 87 (100%), 73 (52%), 45 (66%), 41 (55%).

**2-Propenesulfenoseleonic Acid Methyl Ester (9).** A solution of bromine (0.16 g, 1 mmol) in  $\text{CHCl}_3$  (10 mL) was added to a solution of **6** (0.188 g, 1 mmol) in  $\text{CHCl}_3$  (10 mL), giving a dark red solution. A solution of diallyl disulfide (0.146 g, 1 mmol) in  $\text{CHCl}_3$  (10 mL) was added dropwise, whereupon the solution turned yellow. GC-MS analysis indicated a ca. 1:1:1 mixture of **6**:**9**: $\text{All}_2\text{S}_2$ ; for **9**, EI-GC-MS shows  $m/z$  168 ( $\text{M}^+$ , 68%), 127 (12%), 112 (11%), 93 (14%), 73 (62%).

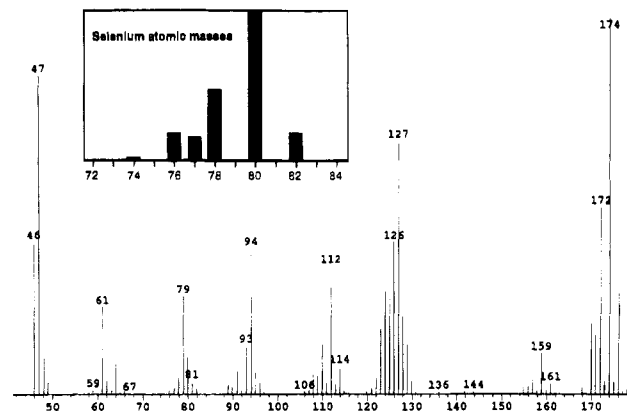
**(*E,Z*)-1-Propenesulfenoseleonic Acid Methyl Ester (10).** In a similar manner 1 mmol piece of **6** and bis(1-propenyl) disulfide [76% (*E,E*)-isomer] (Block and Zhao, 1992; Nagathan, 1992; Thiruvazhi, 1993) was reacted with  $\text{Br}_2$ , giving a yellow solution which showed a 3:1 ratio of (*E*)-**10**:(*Z*)-**10** with the **10**:bis(1-propenyl) disulfide ratio of ca. 1:1; for both isomers of **10**, EI-GC-MS shows  $m/z$  168 ( $\text{M}^+$ , 83%), 153 (11%), 128 (10%), 112 (7%), 93 (19%), 73 (100%), 45 (98%).

## RESULTS AND DISCUSSION

In the case of elephant garlic (Figure 2) there are essentially no Se-containing peaks observed in the C or S channels because at those signal levels they are so small as to be lost within the background signals. The S channel shows  $\text{MeS}_n\text{Me}$ ,  $\text{MeS}_n\text{All}$ , and  $\text{AllS}_n\text{All}$  ( $n = 1-3$ , All = allyl), typical of garlic and garlic-like alliums.



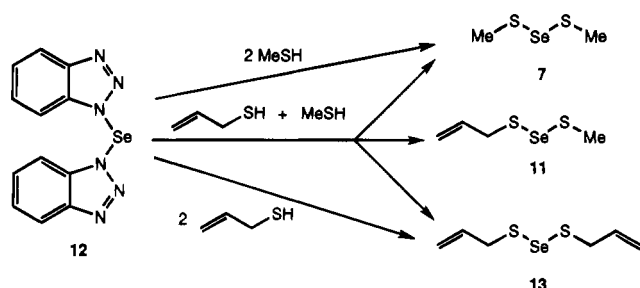
**Figure 2.** Organoselenium compounds in elephant garlic determined by HS/GC-AED-MSD. Organosulfur compound identification: **A**, MeSAll; **B**, MeSSMe; **C**, AllSAll; **D**, MeSSAll; **E**, MeSSSMe; **F**, AllSSAll; **G**, MeSSSAll; **H**, AllSSSAll. On the basis of other studies, the small peaks associated with larger peaks **D** and **F** are most likely isomers of  $\text{MeSSCH}=\text{CHMe}$  and  $(\text{MeCH}=\text{CHS})_2$ , respectively.



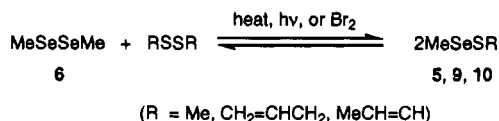
**Figure 3.** Mass spectrum of bis(methylthio)selenide,  $(\text{MeS})_2\text{Se}$  (**7**), showing ions of  $m/e \geq 46$ . The selenium stable natural abundance isotopic ratios are shown in the inset.

The Se channel shows eight peaks: dimethyl selenide (**4**, MeSeMe), methanesulfenoseleonic acid methyl ester (**5**, MeSeSMe), dimethyl diselenide (**6**, MeSeSeMe), bis(methylthio)selenide [**7**,  $(\text{MeS})_2\text{Se}$ ], allyl methyl selenide (**8**, MeSeAll), 2-propenesulfenoseleonic acid methyl ester (**9**, MeSeSAll), 1-propenesulfenoseleonic acid methyl ester (**10**, MeSeSCH=CHMe), and (allylthio)-(methylthio)selenide (**11**, MeSSeSAll). Structures were established by mass spectrometry (Figure 3), in most cases through comparison with spectra of synthetic samples prepared from bis(*N*-benzotriazolyl) selenide (**12**, Scheme 1) (Ryan and Harpp, 1992; Ryan, 1988) and disulfide-diselenide interchange (Scheme 2) (Potapov et al., 1992). Compounds **4-6** have been previously found in nature (Soda et al., 1987; Evans et al., 1968; Chasteen et al., 1990; Chasteen, 1993); the mass spectrum of **5** agrees with that published (Chasteen, 1993). Distilled onion oil, analyzed by direct injection, as well as the headspace above chopped onion showed **7**; in the case of onion oil, three other longer retention time Se compounds (possibly PrSSeSMe) were also seen, while in the case of Se-enriched onions (96 ppm of Se) **4**, **5**, and **10** were also found. The headspace above chopped Chinese chive contained **4-7** and **9**. No sulfenyl [ $-\text{S}(\text{O})-$ ,  $-\text{S}(\text{O})\text{S}-$ , or  $\text{S}(\text{O})\text{Se}-$ ] compounds (Block

## Scheme 1



## Scheme 2



et al., 1992a,b) were detected by HS/GC methods, due to their water solubility and diminished volatility.

Efforts to increase Se levels in the volatiles to facilitate GC-MS identification elucidated possible sources of Se in 4-11. Addition of selenocystine (1) to onion or garlic homogenates (5 mg/g of plant) enhanced formation of 7 and in the case of garlic, 11 and bis(allylthio)selenide [13, (AllS)<sub>2</sub>Se], suggesting that 1 can serve as an H<sub>2</sub>Se equivalent for RSX group transfer. Similarly, addition of selenomethionine (2) to onion and garlic homogenates enhances formation of 5 in both cases and, in the case of garlic, 8 and 9; addition of selenoethionine (14) to onion and garlic homogenates enhances formation of 6 in both cases and affords ethanesulfenoseleonic acid methyl ester (15, EtSSeMe) and 2-propenesulfenoseleonic acid ethyl ester (16, EtSeSCH<sub>2</sub>CH=CH<sub>2</sub>) in the case of garlic. From 2 and 14, formation of RSeSR' via RSe transfer as well as transalkylation via R-Se cleavage is seen. Despite the fact that in garlic allyl-group-containing flavorant precursors are more abundant than the analogous methyl compounds, of the compounds of type RSeSR only compounds MeSeSMe and MeSeSAl are seen [however, MeSeAll (8) is found], suggesting a preference for attachment of methyl over allyl to Se in *Allium* spp. The 6:5 ratios, much higher than the overall 1:10<sup>4</sup> Se/S abundance ratio, may reflect the greater acidity of MeSeH compared to MeSH (Shrift, 1973), favoring in the *Allium* homogenates MeSeH → MeSe<sup>-</sup> over MeSH → MeS<sup>-</sup>. We assume that formation of 6 involves direct oxidative coupling of MeSe<sup>-</sup>, known to be very facile [note also the presence in onion of 1 (Spåre and Virtanen, 1964) rather than 2,7-diamino-4-thia-5-selenooctanedioic acid (HO<sub>2</sub>CCH(NH<sub>2</sub>)CH<sub>2</sub>SeSCH<sub>2</sub>CH(NH<sub>2</sub>)-CO<sub>2</sub>H)] (Shrift, 1973).

With the occurrence of selenium compounds 4-11 in *Allium* headspace volatiles, and the strong, garlicky odor of the synthetic compounds, it is probable that many of these compounds are exhaled in trace amounts in "garlic breath" (Ruiz et al., 1994) and should be detectable using the GC-AED technique. Efforts are underway to answer the myriad questions raised by our initial studies of *Allium* Se chemistry.

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