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Allium chemistry: Natural abundance of organoselenium compounds from garlic, onion and related plants and in human garlic breath

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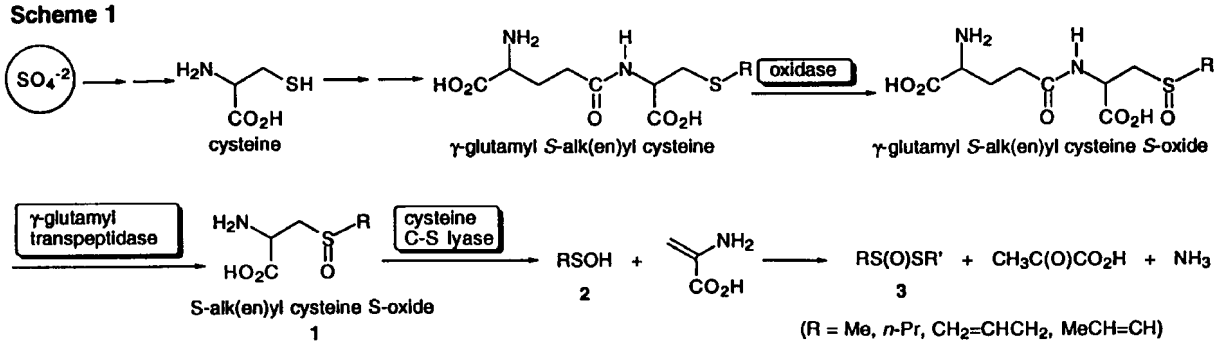
Abstract: By using the technique of gas chromatography with atomic emission detection (GC-AED), a variety of low molecular weight organoselenium and mixed organosulfur-organoselenium compounds have been identified in natural abundance in the headspace above chopped *Allium* and *Brassica* spp. and in human breath after ingestion of garlic. Selenoamino acid precursors to these organoselenium volatiles have been identified in these plants using the same technique.

INTRODUCTION

The characteristic flavors generated when garlic (*Allium sativum*), onion (*A. cepa*) and other genus *Allium* plants are cut or crushed are produced by the action of C-S lyase enzymes (alliinases) on *S*-alkyl cysteine *S*-oxides $RS(O)CH_2CH(NH_2)COOH$ (**1**), where R is variously Me, *n*-Pr, allyl, or 1-propenyl. The initially released sulfenic acid RSOH, **2**, condenses with itself or other sulfenic acids to form thiosulfinate ester, RS(O)SR' (**3**) and, when R = 1-propenyl, rearranges to the onion lachrymatory factor, propanethial *S*-oxide, EtCH=S=O (**4**). Other unusual cyclic and acyclic organosulfur compounds result from subsequent or parallel reactions of **2-4**. Compounds of type **1** are thought to originate from soil sulfate ion via cysteine through a series of steps culminating in oxidation of γ-glutamyl-*S*-alk(en)yl cysteines by oxidases to the corresponding *S*-oxides followed by cleavage of these to **1** by γ-glutamyl transpeptidases (Scheme 1)(1). Garlic is one of several vegetables containing elevated levels of selenium (Fig. 1)(2). The bioavailability of selenium in food products of vegetable origin is high (ca. 60% of total content), although little is known about the actual form in which selenium is found or about what flavor contributions selenium may make.

Scheme 1



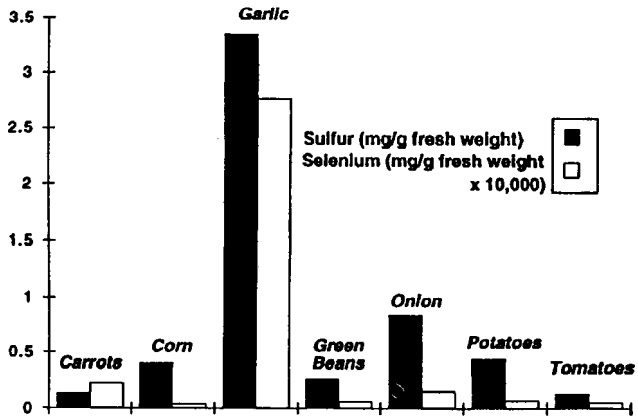
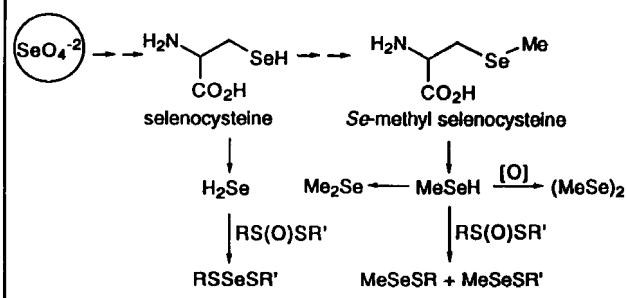


Fig. 1 Comparative sulfur and selenium content of common vegetables.

Scheme 2



In 1964, Finnish Nobel Laureate A. I. Virtanen, who did much of the pioneering research on onion sulfur biochemistry, reported on the basis of radioisotope studies that onion contained the selenoamino acids selenocystine ($(HOOCCH(NH_2)CH_2Se)_2$) and selenomethionine ($HOOCCH(NH_2)CH_2CH_2SeMe$)(3). Virtanen's novel discovery suggested that there might be a selenium-based flavor chemistry in *Allium* spp. parallel to that based on sulfur, e.g. originating from soil selenate (SeO_4^{-2}) or selenite (SeO_3^{-2})(Scheme 2). While typical levels of selenium in garlic are relatively low (0.28 μg/g of fresh weight), the odor threshhold for low molecular weight organoselenium compounds is lower than for the corresponding sulfur compounds so that trace amounts might still contribute. It is known that regions of California, which are major sources of domestic garlic, also contain higher than average levels of selenium in the soil. Furthermore, animal studies have shown that selenium-enriched garlic possesses cancer preventitive properties (4). For all of these reasons it was felt to be useful to obtain information on the nature and amounts of organoselenium compounds in garlic and related *Allium* and *Brassica* species, all well-known for their sulfur content, as well as information on how the body handles the selenium taken in from these plants. This review summarizes our discoveries in this area.

SELENIUM BIOCHEMISTRY

By way of background to our work, a brief review of selenium biochemistry is appropriate. Selenium (Se), an essential micronutrient whose absence causes skeletal and cardiac muscle dysfunction (5,6), is required for the proper function of the immune system and for cellular defense against oxidative damage, and thus may play a role in the prevention of cancer and premature aging (7). Selenocysteine ($HSeCH_2CH(NH_2)COOH$; Cys-SeH), whose incorporation into proteins is directed by a UGA codon, and has been called the 21st amino acid essential for ribosome-directed protein synthesis (8), is present at the active sites of glutathione peroxidase, 5'-deiodinase and selenoprotein P (9). In glutathione peroxidase, selenium (as Cys-SeH) removes an O atom from peroxides; in a catalytic cycle the resultant Cys-SeOH is then reduced back to Cys-SeH by glutathione. A unique biochemistry exists for Se, different from that seen with S: the pKa of free Cys-SeH is 5.2, compared with the >8 value for free cysteine; furthermore Se^- is more nucleophilic than S^- but at the same time bonds to Se are weaker than those to S. As a consequence of these properties, when involved in catalytic processes, Cys-SeH can be considered as a "super-active" cysteine. Garlic is claimed to contain selenoproteins (10) and a selenopolysaccharide (11), while broccoli is said to accumulate high levels of unknown forms of Se (12). Cabbage (*Brassica oleracea capitata*) grown with $H_2{}^{75}SeO_3$ contained various seleno-amino acids, -peptides, and -proteins (13).

ANALYTICAL METHODS

Analysis of flavorants and their precursors from *Allium* spp. plants presents a number of challenges: primary flavorants are formed on cutting or crushing the plants through action of the released enzymes on precursors. The flavorants themselves, which can exist in a variety of sometimes interconverting isomeric forms, are thermally and hydrolytically unstable, decomposing to give mixtures of secondary compounds, some of which are also thermally unstable. Selenium-containing flavorants/flavorant precursors, which may be important both from the standpoints of flavor as well as health benefits, have physical properties quite similar to those of far more abundant homologs containing sulfur. In particular, garlic and onion contain 0.28 and 0.015 μg Se per g fresh weight, respectively (14), compared to 3.3 and 0.84 mg S, e.g. 12,000 to 56,000 times higher levels of S than Se are present in these *Allium* spp.

We have used GC with atomic plasma spectral emission detection (GC-AED) for element specific detection of natural abundance organoselenium compounds in both *Allium* and *Brassica* spp. plants, plant extracts and volatiles, and human exhaled breath following plant consumption. The technique

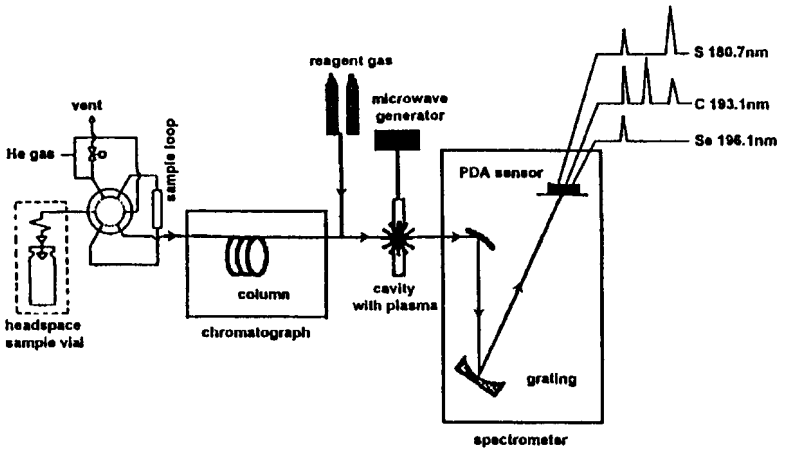


Figure 2. Schematic diagram of headspace-gas chromatograph-atomic emission detector (HS-GC-AED; Hewlett-Packard Co., Palo Alto, CA, USA). Sample is capped in the headspace sample vial which is placed in a heated bath to achieve thermal equilibration. The headspace gas is then driven through the sample 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 which 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).

of GC-AED, represented schematically in Fig. 2, has the important advantages of high sensitivity, elemental selectivity, and the possibility of simultaneous multi-element analysis (15). The AED response can flag compounds in the GC effluent which contain specific elements even though these compounds may be present in very small amounts or may coelute with other components. We have used the selenium emission line at 196 nm to detect organoselenium species while concurrently monitoring S and C by lines at 181 and 193 nm, respectively. These assignments were confirmed by GC-MS. The above techniques were used to analyze the headspace above homogenized garlic, elephant garlic, onion, Chinese chive and broccoli (16,17). As the headspace (HS) GC-MS procedure was not sufficiently sensitive and selective to directly detect natural levels of *Allium* and *Brassica* Se compounds, garlic, onion or broccoli grown in a Se-fertilized medium (Se-enriched plants), or garlic homogenates augmented by addition of selenoamino acids (see below) were utilized.

Headspace Analysis

In the case of the HS-GC-AED analysis of volatiles from natural garlic (Fig. 3), 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 MeS_nMe, MeS_nAll, and $AllS_nAll$ (n = 1-3, All = allyl), typical of garlic and garlic-like Alliums (18). The Se channel shows seven peaks: dimethyl selenide (MeSeMe), methanesulfenoselenoic acid methyl ester (MeSeSMe), dimethyl diselenide (MeSeSeMe), bis(methylthio)selenide ($(MeS)_2Se$), allyl methyl selenide (MeSeAll), 2-propenesulfenoselenoic acid methyl ester (MeSeSAll), and (allylthio)(methylthio)selenide (MeSSeSAll). Structures were established by mass spectrometry, in most cases through comparison with spectra of synthetic samples prepared from bis(*N*-benzotriazolyl)selenide and disulfide-diselenide interchange (19,20). Of the seven compounds, MeSeMe, MeSeSMe, and MeSeSeMe have been previously found in nature (21-24). The headspace above chopped broccoli, analyzed by GC-AED, showed MeSeMe, MeSeSMe, MeSeSeMe, $MeSeSC_3H_5$, $MeSSeSC_3H_5$, $C_3H_5SSeSC_3H_5$, together with six thus far unidentified peaks, in the Se channel and MeSSMe (major), MeSSSMe, $C_3H_5SSC_3H_5$ and $C_3H_5SSSC_3H_5$ in the S channel. The headspace above chopped onion showed the presence of methyl propyl selenide, MeSePr.

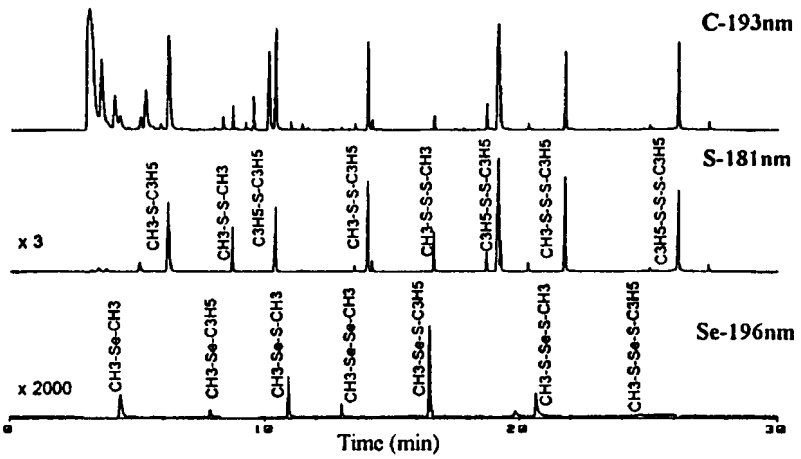


Figure 3. Organoselenium and organosulfur compounds in garlic determined by HS-GC-AED. The sulfur and selenium vertical scales are in units 3 and 2000 times larger, repectively, than the corresponding carbon vertical scale.

Selenoamino Acid Analysis

To determine the origin of the headspace selenium compounds, lyophilized normal garlic (0.02 ppm Se) or moderately selenium-enriched (68 ppm Se) garlic was derivatized with ethyl chloroformate to volatize the selenoamino acids (25-27). Subsequent GC-AED analysis showed a single peak in the Se channel, identified as selenocysteine by comparison with the retention time and mass spectral fragmentation of an authentic sample (Fig. 4). In garlic more heavily selenium-enriched (1355 ppm Se), *Se*-methyl selenocysteine was the major selenoamino acid found along with minor amounts of selenocysteine and traces of Se-methionine (Fig. 5, 6; Scheme 2); the S channel showed 2:1 allylcysteine and allyl-cysteine *S*-oxide along with minor amounts of methionine (28). In contrast to the situation with the selenoamino acids, there were only minor changes in the relative ratios of the sulfur amino acid as the level of selenium was varied from 0.02 to 1355 ppm. Analogous analysis of selenium-enriched onion (96 ppm Se) revealed the presence of equal amounts of *Se*-methyl selenocysteine and selenocysteine in the Se channel; analysis of selenium-enriched (150 ppm Se) broccoli

showed the presence of ca. 2:1 *Se*-methyl selenocysteine and selenocysteine along with much smaller amounts of Se-methionine in the Se channel and *S*-methyl cysteine and lower amounts of methionine in the S channel (the presence of *S*-methyl cysteine *S*-oxide has been previously reported (29)). The presence of *Se*-methyl selenocysteine as the major source of selenium in selenium-enriched *Allium* and *Brassica* spp. is significant since *Se*-methyl selenocysteine is known to exhibit cancer chemopreventitive activity (30). *Se*-Methyl L-selenocysteine is superior to *S*-methyl L-cysteine as a substrate for L-methionine γ-lyases and *S*-alkylcysteine α,β-lyases in bacteria, due to the superior leaving group ability of $MeSe^-$ compared to MeS^- (31,32). It is therefore probable that related enzymes in *Allium* and *Brassica* spp. cleave this selenoamino acid to MeSeH (or $MeSe^-$), which reacts with RSS(O)R' formed when the plant is cut to give MeSeSR and MeSeSR', or forms MeSeSeMe on oxidation. Analogous reaction of H_2Se, released from selenocysteine, could afford RSSeSR', while Se-methylation of *Se*-methyl L-selenocysteine followed by enzymatic cleavage would afford MeSeMe (see Scheme 2).

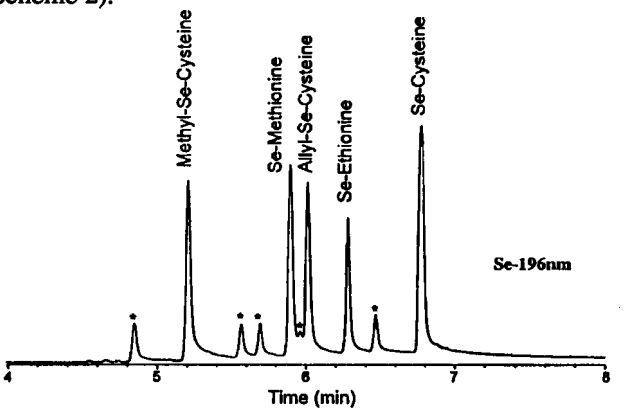


Figure 4. GC-AED analysis (Se channel, monitored at 196.1 nm) of $ClCO_2Et$ derivatized selenocysteine ($HSeCH_2CH(NH_2)COOH$; Cys-SeH), *Se*-methyl selenocysteine ($MeSeCH_2CH(NH_2)COOH$; Cys-Se-Me), seleno-D,L-methionine ($MeSeCH_2CH_2CH(NH_2)COOH$; Se-Met), seleno-D,L-ethionine ($EtSeCH_2CH_2CH(NH_2)COOH$; Se-Eth), and *Se*-allyl selenocysteine (CH_2=CH-$CH_2SeCH_2CH(NH_2)COOH$; Cys-Se-All). Peaks labelled with an asterisk are byproducts associated with the derivatization procedure.

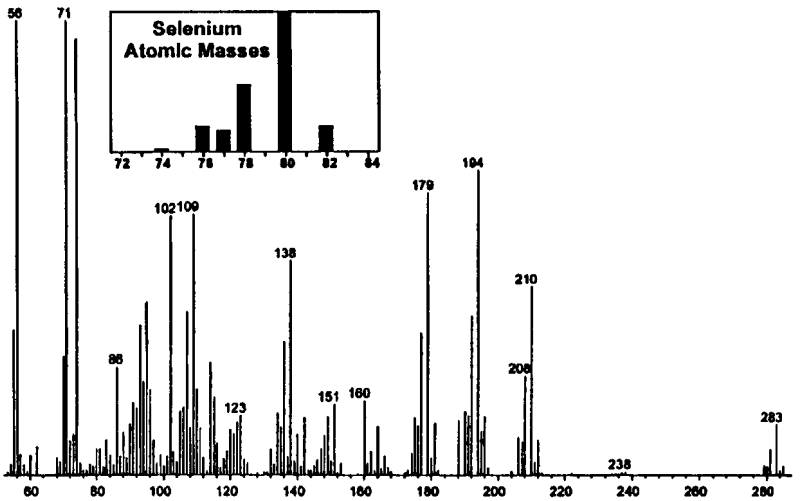


Figure 5. Mass spectrum of $ClCO_2Et$ derivatized *Se*-methyl selenocysteine (Cys-Se-Me), $MeSeCH_2CH(NHCO_2Et)CO_2Et$ (M^+ 283), showing ions of m/e ≥ 53. Major fragments are seen at m/e 210 ($M^+ - CO_2Et$), 194 ($M^+ - NHCO_2Et, H^+$), 179 ($M^+ - NHCO_2Et, Me, H^+$), 138 ($MeSeCH_2C{\equiv}NH^+$), 109 ($MeSeCH_2^+$), and 74 ($HCO_2Et^{\cdot+}$). The selenium stable natural abundance isotopic ratios are shown in the inset.

Analysis of the headspace above selenium-enriched garlic by HS-GC-AED showed a very similar profile of compounds whether or not synthetic Se-methyl selenocysteine was added, although all of the peak abundances are enhanced by the addition of the synthetic selenoamino acid. On the other hand, addition of synthetic *Se*-allyl selenocysteine to selenium-enriched garlic followed by HS-GC-AED showed a profile quite different from that of selenium-enriched garlic, with the major peaks being diallyl selenide (major peak; not seen in normal garlic; small peak in selenium-enriched garlic) and AllSSeSAll (or isomer; small peak in selenium-enriched garlic). We conclude that *Se*-allyl selenocysteine is not present in our lyophilized garlic samples. Further work is necessary to establish

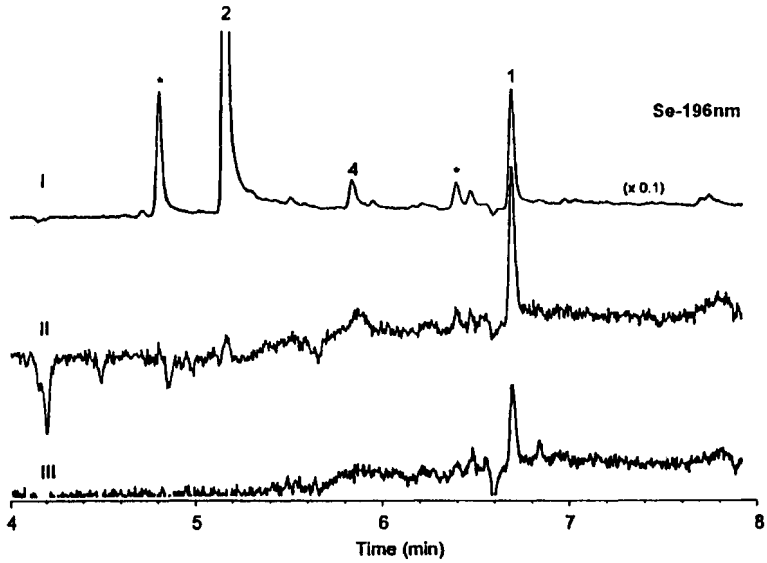


Figure 6. GC-AED analysis (Se channel, monitored at 196.1 nm) of $ClCO_2Et$ derivatized amino acids from lyophilized Se-enriched (I: 1355 ppm Se; II: 68 ppm Se) and unenriched (III: 0.02 ppm Se) garlic. Compound identification: $ClCO_2Et$ derivatized selenocysteine (1, $HSeCH_2CH(NH_2)$-COOH; Cys-SeH), *Se*-methyl selenocysteine (2, $MeSeCH_2CH(NH_2)COOH$; Cys-Se-Me), and seleno-D,L-methionine (4, $MeSeCH_2CH_2CH(NH_2)COOH$; Se-Met). Peaks labelled with an asterisk are byproducts associated with the derivatization procedure.

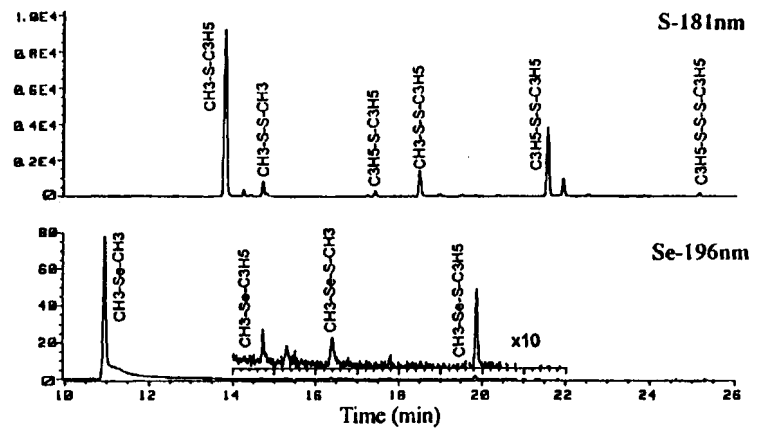


Figure 7. Organosulfur (upper trace) and organoselenium compounds (lower trace) in human garlic breath one hour after consumption of fresh garlic as determined by GC-AED: S, allyl methyl sulfide, dimethyl disulfide, diallyl sulfide, allyl methyl disulfide, diallyl disulfide, diallyl trisulfide; Se, dimethyl selenide, allyl methyl selenide, methanesulfenoselenoic acid methyl ester (MeSeSMe), 2-propenesulfenoselenoic acid methyl ester (MeSeSAll). The sulfur vertical scale is in units 10^4 times larger (shown as "E4") than the corresponding selenium vertical scale.

whether or not *Se*-allyl selenocysteine is synthesized to any extent in garlic cloves. We suggest that synthetic *Se*-allyl selenocysteine is cleaved in garlic homogenates probably forming CH_2=$CHCH_2$-SeH, which is oxidized to thermally unstable AllSeSeAll, which in turn loses selenium affording AllSeAll. We further suggest that when garlic is presented with high levels of inorganic selenium fertilizer, the excess selenocysteine formed is *Se*-methylated to give the major selenoamino acid, *Se*-methyl selenocysteine.

Human Garlic Breath Analysis

Finally, analysis of human garlic breath by GC-AED (Fig. 7; the subject consumed, with brief chewing, 3 g of fresh garlic with small pieces of white bread, followed by 50 mL of cold water) revealed in the Se channel the presence of dimethyl selenide (MeSeMe) as the major component along with one tenth to one fortieth the amount of $MeSeC_3H_5$, CH_3SeSMe and $MeSeSC_3H_5$; the S channel showed the major components to be AllSH, CH_3SAll and AllSSAll with lesser amounts of MeSSMe, $CH_3SSC_3H_5$, an isomer of AllSSAll (presumably MeCH=CHSSAll), $C_3H_5SC_3H_5$ and $C_3H_5SSSC_3H_5$ (33). The composition of the Se and S compounds in garlic breath was examined as a function of time over the course of four hours. After four hours the levels of MeSeMe, AllSSAll, AllSAll and MeSSMe are reduced by 75% from the initial levels of 0.45 ng/L (MeSeMe), 45 ng/L (AllSSAll), 6.5 ng/mL (AllSAll), and 1.8 ng/L (MeSSMe)(Fig. 8). The AllSH could only be detected in breath immediately after ingestion of garlic. In view of the reported very low threshhold detection level for low molecular weight organoselenium compounds (34), it is likely that compounds such as MeSeMe contribute to the overall odor associated with garlic breath. It has been previously reported that dimethyl selenide, which has a garlic-like odor, is found in the breath air of animals fed inorganic selenium compounds (35) and humans who have accidentally ingested selenium compounds (36).

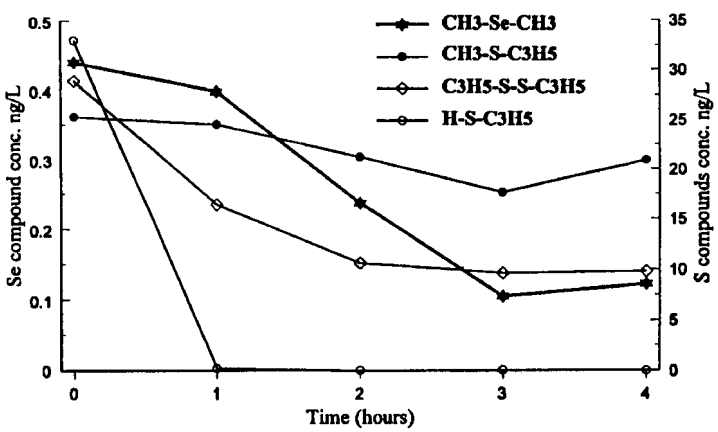


Figure 8.
Variation in the concentrations of allyl methyl sulfide, diallyl disulfide, 2-propenethiol and dimethyl selenide in human garlic breath with time.

CONCLUSION

In summary, our work constitutes the first identification of the organoselenium compounds formed on cutting *Allium* spp. plants, on the precursor compounds in the intact plants, and on the selenium-containing metabolites formed on ingestion of garlic. Since the derivatized amino acid GC analysis can only be used for volatile compounds, it is likely that a number of interesting and important natural, heavier selenoamino acids and selenopeptides in *Allium* spp. have been missed. The search for these compounds can best be done using element-specific LC-AED (or LC-ICP) methods. Work in this area is currently in progress.

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REFERENCES

1. E. Block, *Angew. Chem., Int. Ed. Engl.* **31**, 1135 (1992).
2. J.P. Diaz-Alarcón, M. Navarro-Alarcón, H. López-García de la Serrana, and M.C. López-Matínez, *J. Agric. Food Chem.* **42**, 2848-2851 (1994).
3. C.-G. Spåre and A.I. Virtanen, *Acta Chem. Scand.* **18**, 280-282 (1964).
4. C. Ip, D.J. Lisk, and G.S. Stoewsand *Nutr. Cancer* **17**, 279-286 (1992).
5. V.R. Young, *N. Engl. J. Med.* **304**, 1228-1230 (1981).
6. S.K. Sathe, A.C. Mason, R. Rodibaugh, C.M. Weaver, *J. Agric. Food Chem.* **40**, 2084-2091 (1992).
7. M.J. Axley, A. Böck, T.C. Stadtman, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8450-8454 (1991).
8. D. Söll, *Nature (London)* **331**, 662-663 (1988).
9. R.F. Burk, K.E. Hill, *J. Nutr.* **124**, 1891-1897 (1994).
10. W. Wang, J. Tang, A. Peng, A. *Shengwu Huaxue Zazhi* **5**, 229-234 (1989) [*Chem. Abstr.* **111**, 95847d (1989)].
11. M. Yang, K. Wang, L. Gao, Y. Han, J. Lu, T. Zou, *J. Chin. Pharm. Sci.* **1**, 28-32 (1992) [*Chem. Abstr.* **118**, 77092u (1993)].
12. G.S. Bañuelos, D. Dyer, R. Ahmad, S. Ismail, R.N. Raut, J.C. Dagar, *J. Soil and Water Cons.* **48**, 530-534 (1993).
13. J.W. Hamilton, *J. Agric. Food Chem.* **23**, 1150 (1975).
14. V.C. Morris, *J. Nutr.* **100**, 1385-1386 (1970).
15. P.C. Uden in *Element-Specific Chromatographic Detection by Atomic Emission Spectroscopy*, ACS Symp. Ser. 479, 1-24 (ACS, Washington, DC, 1992).
16. X.-J. Cai, P.C. Uden, J.J. Sullivan, B.D. Quimby, and E. Block, *Analyt. Proc.*, **31**, 325-327 (1994).
17. X.-J. Cai, P.C. Uden, E. Block, X. Zhang, B.D. Quimby, and J.J. Sullivan, *J. Agric. Food Chem.* **42**, 2081-2084 (1994).
18. D. Deruaz, F. Soussan-Marchal, I. Joseph, M. Desage, A. Bannier, J.L. Brazier, *J. Chromatogr. A.* **677**, 345-354 (1994).
19. M.D. Ryan and D. Harpp, *Tetrahedron Lett.* **33**, 2129-2132 (1992).
20. V.A. Potapov, S.V. Amosova, P.A. Petrov, L.S. Romanenko, V.V. Keiko, *Sulfur Lett.* **15**, 121-126 (1992).
21. K. Soda, H. Tanaka, N. Esaki, In *The Chemistry of Organic Selenium and Tellurium Compounds* (ed. S. Patai), Vol. 2, Ch. 7, (John Wiley & Sons, Chichester, 1987).
22. C.S. Evans, C.J. Asher, and C.M. Johnson, *Aust. J. Bio. Sci.* **21**, 13-20 (1968).
23. T.G. Chasteen, G.M. Silver, J.W. Birks, R. Fall, *Chromatographia* **30**, 181-185 (1990).
24. T.G. Chasteen, *Appl. Organomet. Chem.* **7**, 335-342 (1993).
25. P. Husek, *J. Chromatogr.* **552**, 289-299 (1991).
26. J. Wang, Z.-H. Huang, D.A. Gage, and J.T. Watson, *J. Chromatogr. A* **663**, 71-78 (1994).
27. J. Janák, H.A.H. Billiet, J. Frank, K.C.A.M. Luyben, and P. Husek, *J. Chromatogr. A* **677**, 192-196 (1994).
28. X.-J. Cai, E. Block, P.C. Uden, X. Zhang, B.D. Quimby, and J.J. Sullivan, *J. Agric. Food Chem.*, **43**, 1754-1757 (1995).
29. H.S. Marks, J.A. Hilson, H.C. Leichtweis, G.S. Stoewsand, *J. Agric. Food Chem.* **40**, 2098-2102 (1992).
30. S. Vadhanavikit, C. Ip, and H.E. Ganther, *Xenobiotica*, **23**, 731-745 (1993).
31. H. Takada, N. Esaki, H. Tanaka, and K. Soda, *Agric. Biol. Chem.* **52**, 2897-2901 (1988).
32. H. Kamitani, N. Esaki, H. Tanaka, and K. Soda, *Agric. Biol. Chem.* **54**, 2069-2076 (1990).
33. X.-J. Cai, E. Block, P.C. Uden, B.D. Quimby, and J.J. Sullivan, *J. Agric. Food Chem.* **43**, 1751-1753 (1995).
34. J.H. Ruth, *Am. Ind. Hyg. Assoc. J.* **47**, A-142-151 (1986).
35. N. Oyamada, M. Kikuchi, and M. Ishizaki, *Anal. Sci.* **3**, 373-376 (1987).
36. R.F. Buchan, *J. Amer. Med. Assoc.* **227**, 559-560 (1974).