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SHORT COMMUNICATIONS

Allium Chemistry: Identification of Natural Abundance Organoselenium Compounds in Human Breath after Ingestion of Garlic Using Gas Chromatography with Atomic Emission Detection

Keywords: *Garlic; garlic breath; Allium sativum; gas chromatography–atomic emission detection; dimethyl selenide*

INTRODUCTION

Ingestion of garlic (*Allium sativum*) is well-known to cause bad breath (e.g., "eat no onions nor garlic, for we are to utter sweet breath"; Shakespeare, 1600). Garlic breath odor comes from the lungs and not from particles of garlic retained in the mouth (Blankenhorn and Richards, 1936; Block, 1992). Allyl methyl sulfide and disulfide (**1** and **2**, respectively), diallyl sulfide (**3**) and disulfide (**4**), 2-propenethiol (**5**), hydrogen sulfide, *p*-cymene, and (+)-limonene have all been identified as components of garlic breath through the use of GC–MS techniques (Laakso et al., 1989; Minami et al., 1989; Ruiz et al., 1994). We have previously established the presence of natural abundance organoselenium compounds in the headspace volatiles from cut or crushed garlic, elephant garlic (*Allium ampeloprasum*), onion (*Allium cepa*), and Chinese chive (*Allium tuberosum*) using the highly sensitive, element-specific technique of gas chromatography with atomic emission detection (GC–AED) (Cai et al., 1994a,b). We have described the capability of the GC–AED technique to perform simultaneous multielement analysis (Uden et al., 1986; Quimby and Sullivan, 1990; Sullivan and Quimby, 1990; Uden, 1992) and to flag compounds in GC effluents which contain specific elements such as selenium (Se), even though these compounds may be present in very small amounts or may coelute with other components. We now report the application of this technique to the analysis of human breath at various time periods following consumption of fresh garlic and report the presence of significant quantities of organoselenium and mixed organoselenium–sulfur compounds, which we have characterized for the first time in garlic breath, as well as a number of organosulfur compounds not previously identified.

EXPERIMENTAL PROCEDURES

Materials and Instruments. A Hewlett-Packard HP 5921A atomic emission detector (AED) interfaced with a HP 5890II cryogenic gas chromatograph was used, with a HP-1 30 m × 0.53 mm × 2.65 μm (film thickness) column; the thick film reduces peak tailing. The He carrier gas was kept at a constant pressure of 12.8 psi (12.7 mL/min at 60 °C) with an electric pressure control (EPC) system. The GC–AED employed an oxygen/hydrogen reagent gas mixture, with detection at 180.7 (S), 193.1 (C), and 196.1 nm (Se). Traps were prepared by placing 150 mg of 60–80 mesh Tenax (HP 8501–0008) in a GC liner and heating at 220 °C for 2 h. The Tedlar (poly(vinyl fluoride)) air sampling bags were purchased from Analabs Inc.

Human Breath Samples. Fresh garlic was purchased at a local supermarket. A 31-year-old female consumed 3 g of garlic (2–3 skinned cloves) after dinner. The garlic cloves were briefly chewed together with small pieces of white bread and swallowed, the garlic was washed down with ca. 50 mL of cold water, and the teeth were immediately brushed using cold water. The experiment was conducted in this manner in an effort to limit the garlic headspace gases and particles of crushed garlic remaining in the mouth. Breath samples were collected immediately after brushing the teeth and at four hourly intervals thereafter. A control breath sample was also collected prior to ingestion of garlic. Breath samples were collected by expiration into two 1.5 L Tedlar air sampling bags. The bag contents were then suctioned through the Tenax trap at a rate of 100 mL/min using a small vacuum pump. The trap was held at 0 °C during the suction to ensure absorption of volatile compounds. The trap was purged for 30 min with helium to drive off excess moisture before desorption. The trap was inserted into the GC injection port at 30 °C with the carrier flow turned off. Then the GC program was started and the helium carrier flow was turned on at 12.8 psi after 10 s. The injection port was then heated after 2 min at the rate of 30 °C/min to 180 °C. The GC oven was maintained at −60 °C for 3 min and was then increased to 200 °C at the rate of 10 °C/min. The cryogenic GC was used to refocus the light compounds generated by the thermal desorption. As a control,

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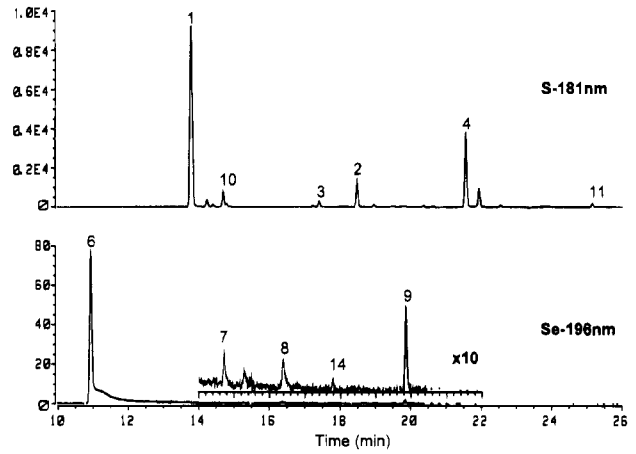

Figure 1. Organosulfur (upper trace) and organoselenium compounds (lower trace) in human garlic breath 1 h after consumption of fresh garlic as determined by GC–AED: allyl methyl sulfide (**1**), allyl methyl disulfide (**2**), diallyl sulfide (**3**), diallyl disulfide (**4**), dimethyl selenide (**6**), allyl methyl selenide (**7**), methanesulfenoselenoic acid methyl ester (**8**, MeSeSMe), 2-propenesulfenoselenoic acid methyl ester (**9**, MeSeSAll), dimethyl disulfide (**10**), diallyl trisulfide (**11**), and dimethyl selenide (**14**). The small peak following peak 4 in the upper sulfur trace is an isomer of **4**, most likely allyl 1-propenyl disulfide. The sulfur vertical scale is in units 10^4 times larger (shown as "E4") than the corresponding selenium vertical scale.

garlic headspace was also collected using the Tedlar bags; the results were qualitatively similar to those using the headspace sampler.

Compound Identification. The GC–AED peaks were identified by GC retention time and GC–MS mass spectral fragmentation patterns of authentic samples (Cai et al., 1994a).

RESULTS AND DISCUSSION

As shown in Figure 1, the major organoselenium compound found in garlic breath is dimethyl selenide (**6**, Me_2Se), along with lesser amounts of allyl methyl selenide (**7**, MeSeAll), methanesulfenoselenoic acid methyl ester (**8**, MeSeSMe), 2-propenesulfenoselenoic acid methyl ester (**9**, MeSeSAll), and dimethyl selenide (**14**). The major organosulfur compounds identified include compounds **1**–**5** (2-propenethiol, **5**, not shown in Figure 1, is found only in breath samples taken within 1 h of garlic ingestion) as well as dimethyl disulfide (**10**, MeSSMe) and diallyl trisulfide (**11**, AllSSSAll), not previously identified in garlic breath. Garlic breath GC–AED showed enhanced amounts of **6** and **1** and diminished amounts of **11** and **13** compared to garlic headspace HS/GC–AED (Figure 2) (also see Deruaz et al., 1994). Hydrogen sulfide, which has previously been found in garlic breath (Ruiz et al., 1994), would not be detectable under our analytical conditions. Although we did not search for hydrogen selenide, it is unlikely to be present due to its facile *in vivo* methylation.

To determine the variation in concentrations of the various exhaled Se and S compounds with time, breath samples were collected prior to and shortly after ingestion of garlic, and at four hourly intervals thereafter. As seen in Figure 3, the levels of Me_2Se (**6**), AllSMe (**1**), and AllSSAll (**4**), as well as compounds **2**, **3**, **10**, and **11** (not shown), slowly decrease with time during the course of 4 h, while the level of AllSH (**5**) decreases rapidly (see Ruiz et al., 1994). It has been reported that garlic breath persists more than 18 h after ingestion of fresh garlic (Blankenhorn and Richards, 1936).

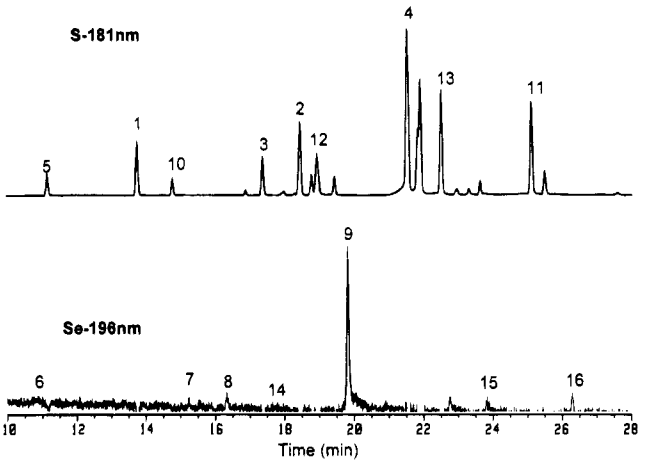

Figure 2. Headspace GC–AED analysis of fresh garlic ingested in this work. Compound identity is given in the Figure 1 caption with the exception of the following compounds which were not detected in garlic breath: dimethyl trisulfide (**12**), allyl methyl trisulfide (**13**), dimethyl diselenide (**14**), bis(methylthio)selenide (**15**), and (allylthio)(methylthio)selenide (**16**). In the upper sulfur trace, the large double peak between peaks 4 and 13 is presumably due to allyl (*E*)- and (*Z*)-1-propenyl disulfide. In garlic samples previously tested under slightly different GC conditions, the intensities of compounds **6**–**8** and **14**–**16** were stronger relative to that of **9**, and the other Se peaks were more easily seen, as in the case of elephant garlic (Cai et al., 1994). Bulb-to-bulb variation in garlic thiosulfinates has previously been noted (Block, 1992). Allicin, if present in the headspace volatiles, would not be detectable under our GC conditions.

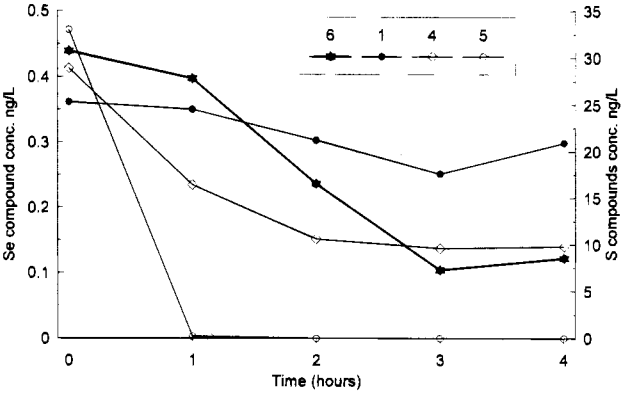

Figure 3. Variation in the concentrations of allyl methyl sulfide (**1**), diallyl disulfide (**4**), 2-propenethiol (**5**), and dimethyl selenide (**6**) in human garlic breath with time.

If we select the 1 h breath sample as typical, the 0.4: 24.5:13.5 ng/L **6**:**1**:**4** ratio is interesting for several reasons: (1) If it is assumed that the 0.4 ng of **6** represents 90% of exhaled organic Se and that the 38 ng of **1** + **4** represents 75% of total exhaled organic S, then the calculated total S:total Se ratio (assuming elemental Se is 73% of the weight of volatile organic Se as **6**, and S is 50% of the average weight of **1** + **4**) is ca. 80:1, far smaller than the 1.2×10^4:1 ratio of the total amounts of S and Se found in fresh garlic. (2) Further calculations show that, assuming 300 L/h expired air, then in the first hour 0.04 μg of MeSeMe = 0.029 μg of elemental Se/g of consumed garlic is exhaled, representing ca. 10% of the typical amount of elemental Se (0.28 μg/g) in fresh garlic (Morris, 1970). Similarly, in the first hour the equivalent of 2.5 μg of elemental S/g of consumed garlic is exhaled, representing less than 0.1% of the amount of elemental S (3.3 mg/g) present in fresh garlic. (3) The reported (Ruth, 1986) odor thresholds for **4**, diethyl sulfide, and diethyl selenide are 0.5, 0.9,

and 0.3 ng/L, respectively. From these values it is clear that, at 14–25 ng/L, compounds **1** and **4** should be easily detected by smell, while, at 0.4 ng/L, **6** should just barely be detectable (assuming the threshold value for **6** is similar to that of diethyl selenide). The 150-fold difference in the ratio of exhaled S:Se (80:1) compared to the S:Se value in the intact plant (1.2×10^4:1) may be a reflection of the larger fraction of Allium S compounds being metabolized compared to the Se compounds, and hence not exhaled. Alternatively, the rate of excretion of Se as **6** may be much faster than the rate of excretion of sulfides and disulfides.

In the accompanying paper (Cai et al., 1995), we identify the selenoamino acids found in normal as well as Se-enriched garlic. We suggest that these compounds are the source of the exhaled Se compounds in garlic breath. It is likely that some of the S compounds in garlic breath originate from hydrolysis of the initially formed thiosulfinates (garlic flavorants; RS(O)SR′), such as allicin (CH_2=$CHCH_2S(O)SCH_2CH$=CH_2), in the digestive tract, affording odorous compounds which are then absorbed in the blood and exchanged with exhaled gases in the lungs. A similar mechanism might exist for the Se compounds, although **8** and **9** might also be formed via MeSeH enzymatically directly released (Takada et al., 1988; Kamitani et al., 1990) from the precursor selenoamino acids (Cai et al., 1995). Dimethyl selenide (**6**) could result from *in vivo* methylation of H_2Se released from selenocysteine and of MeSeH released from *Se*-methylselenocysteine (**17**), as is the case with **17** administered to rats (Vadhanavikit et al., 1993). It is known that **6** has a garlicky odor and, by analogy to Et_2Se (Ruth, 1986), should have a very low odor threshold. Thus, even when present at levels of 0.4 ng/L, **6** still may contribute to garlic breath odor. The breath air of animals which have been given inorganic Se as selenite or selenate contains **6**. When significant amounts of **6** are present in human breath, it suggests exposure to Se compounds, e.g., in rectifiers, etc. (Buchan, 1974).

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