Determination of Volatile Organic Selenium Compounds from the Maillard Reaction in a Selenomethionine−Glucose Model System

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In the past, biomedicine, toxicology, and other related disciplines have made some progress with regard to the detrimental and beneficial effects of selenium in biological systems. Therefore, it is of interest to determine the chemical structure and occurrence of organoselenium compounds generated in food systems via the Maillard reaction. The Maillard reaction involves the reaction of amino acids with reducing sugars and occurs in many food systems during processing under high-temperature conditions and is responsible for the generation of many volatiles. This research describes a model Maillard reaction system between the amino acid selenomethionine and a reducing sugar, glucose, to generate volatile organoselenium compounds. Using gas chromatography and gas chromatography−mass spectrometry, five selenium compounds were tentatively identified and semiquantitative values for concentration obtained.

Keywords: *Organoselenium; selenomethionine; Maillard reaction; gas chromatography (GC); gas chromatography/mass spectrometry (GC/MS)*

INTRODUCTION

The metal selenium, the 34th element on the periodic chart, has recently captured the interest of scientists in a gamut of research arenas. For example, the U.S. National Research Council has recommended a daily requirement of 60−120 μg per person. This number was directly extrapolated from numerical selenium levels that are safe and nutritionally adequate for laboratory animals (Food and Nutrition Board, 1976). An additionally recommended supplementation has been recommended for persons living in low-selenium areas (Food and Nutrition Board, 1977). Biomedical research has indicated that selenium may have certain antitumor and anticarcinogenic effects. A 1994 study indicated that diets with high amounts of Brazil nuts, which contain selenomethionine as a source of selenium, resulted in higher inhibition of tumor yield (Ip and Lisk, 1994). High levels of selenium in the diet are, however, toxic. Toxicologists have found that an excess of selenium in the diet of rats has effected biological changes such as a decrease in average kidney weight and an increase in average spleen weight (Palmer et al., 1982). Elemental selenium is found to be more toxic than methylated selenium, and the least toxic form of selenium is selenium containing amino acids (Lo and Sandi, 1980). In 1970, Morris and Levander determined the elemental selenium content of foods representative of the American diet (Morris and Levander, 1970). The selenium content of grain products ranged from 0.025 to 0.66 ppm, most likely because of the variation in selenium content of the soil in which they were grown. Meat samples ranged from 0.1 to 1.9 ppm. Most fruits and vegetables contained <0.01 ppm; notable exceptions were garlic, mushroom, and radish, which, respectively, contained 0.25, 0.13, and 0.04 ppm. In 1982, Palmer et al. showed that of 529 individual Brazil nuts (*Bertholletia excelsa*) sampled, 45% contained <10 ppm elemental selenium and 6% contained >100 ppm selenium (Palmer et al., 1982). In 1989, Secor and Lisk reported that the selenium content of Brazil nuts ranged from 0.20 to 253 ppm (Secor and Lisk, 1982). The intrinsically high concentration of selenium exists in Brazil nuts mainly in the organic form as the amino acid selenomethionine.

The Maillard reaction is the nonenzymatic browning reaction in foods that occurs between an amino acid and a reducing sugar in the presence of heat. The reaction begins with formation of a Schiff base (imine) between the carbonyl group of the sugar and the free amino group of an amino acid or protein. Through both Amadori and Heyns rearrangements, the Schiff bases rearrange to form a class of reactive intermediates called reductones and dehydroreductones. From here, these intermediates react in one of several mechanisms to form volatile compounds.

The research reported in this paper has resulted in the tentative identification of five organoselenium compounds from the Maillard reaction of selenomethionine and glucose in a model system. Two other selenium-containing organics were observed (see Table 2) but their structures were not obvious. Semiquantitative values were obtained for these compounds using the internal standard method.

MATERIALS AND METHODS

Isolation of Volatile Organic Selenium Compounds. Selenomethionine (0.1 g) and glucose (0.2) were dissolved in 25 mL of water and heated in a closed reaction vessel at 180 °C for 90 min. The reaction vessel was cooled to room temperature and opened, and the crude reaction mixture was extracted with three 30 mL portions of methylene chloride. The methylene chloride extracts were combined and concentrated to 10 mL using a Buchi rotary evaporator (Brinkmann Instruments, Westbury, NY) at 40 °C. The concentrated extract was quantitatively transferred to a graduated vial with a Teflon-lined cap and concentrated to dryness using a stream

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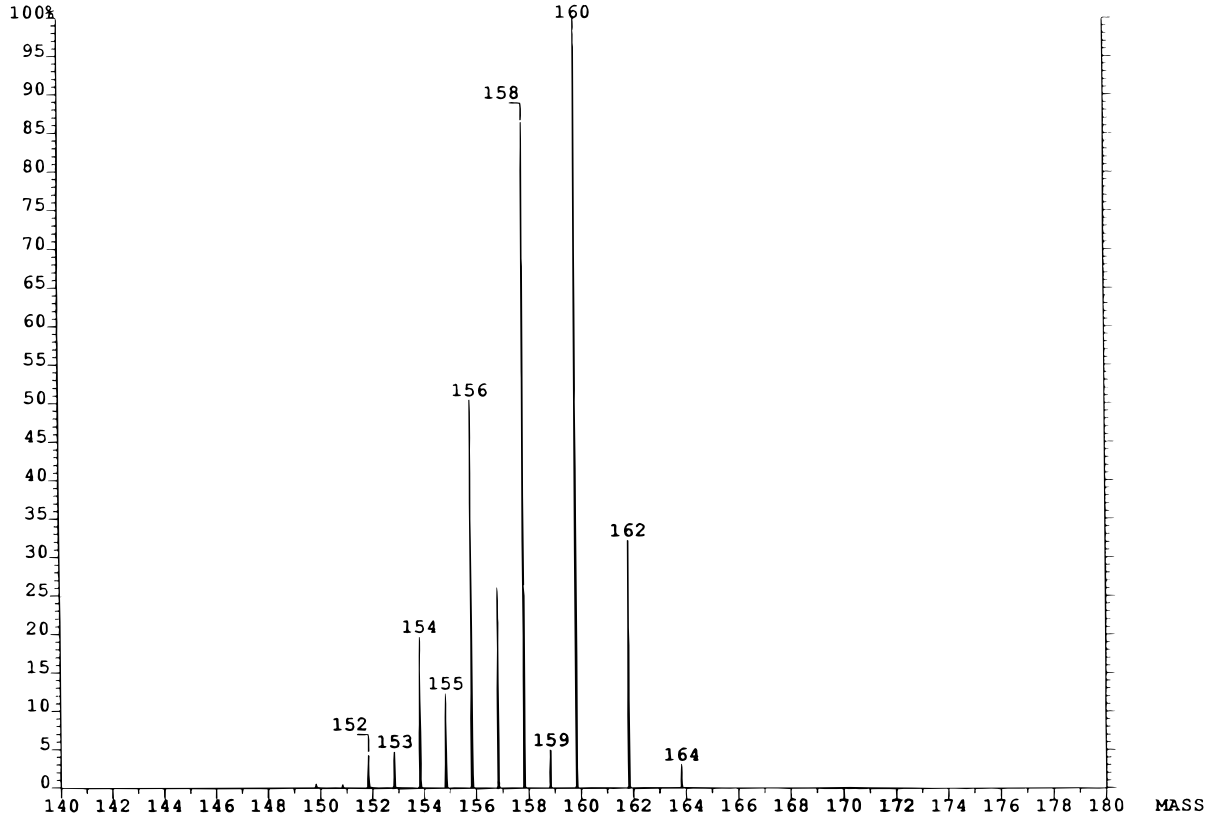
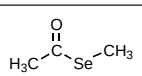
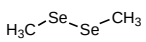
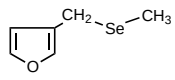
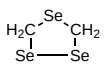


Figure 1. Computer-generated mass spectrum showing isotopes of Se_2.

Table 1. Isotopic Distribution Pattern for Selenium

isotope (nominal mass)	% abundance	isotope (nominal mass)	% abundance
82	9.19	77	7.58
80	49.82	76	9.02
78	23.52	74	0.87

Table 2. Summary of Data from the Maillard Reaction of Selenomethionine and Glucose

compd no.	assigned structure	monoisotopic mol wt[a]	approx concn[b] (ppm)	retention index
I		138	1.0	662.59
II		190	4.2	886.40
III	 (two isomers found)	176	2.1	968.33
IV			3.2	972.11
V		268	0.57	1134.20
VI	unknown	204 (1 Se)	0.30	N/A[c]
VI	unknown	232 (2 Se)	0.13	N/A

[a] Based on ^{80}Se isotope. [b] Based on 300 mg of starting material: 100 mg of selenomethionine and 200 mg of glucose. [c] N/A, not applicable.

of nitrogen at room temperature. The residue was taken up in 1.0 mL of nonadecane (internal standard) in methylene chloride (106 μg/mL of nonadecane). This solution was concentrated to 100 μL and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

GC. Results were obtained with a Varian 3400 gas chromatograph (Varian Associates, Sugar Land, TX) with a flame ionization detector using nonadecane as an internal standard. The separation was achieved using a 60 m × 0.32 mm (i.d.) DB-I capillary column with a 0.25 μm film thickness (J&W Scientific, Folsom, CA). The other GC instrument parameters were as follows: helium flow rate, 1 mL/min; injector temperature, 270 °C; detector temperature, 300 °C; initial column temperature, 40 °C with a 2 min hold time; final column temperature, 280 °C with a 30 min hold time; temperature program rate, 2 °C/min; injection volume, 1 μL; split ratio, 1:10.

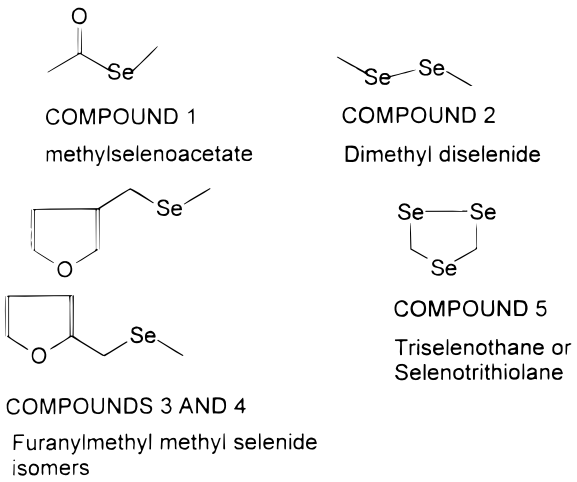


Figure 2. Hypothesized structures of volatile selenium compounds.

GC/MS. EI mass spectra of the organoselenium compounds were obtained using a Varian 3400 gas chromatograph (Varian Associates) directly interfaced with a Finnigan MAT 8230 double-focusing mass spectrometer. The GC separation conditions are discussed above. The mass spectrometer was operated in the EI mode (70 eV) scanning masses 35−650 amu at 1 s/decade. The other mass spectrometer conditions were as follows: ion source temperature, 260 °C; GC/MS interface line

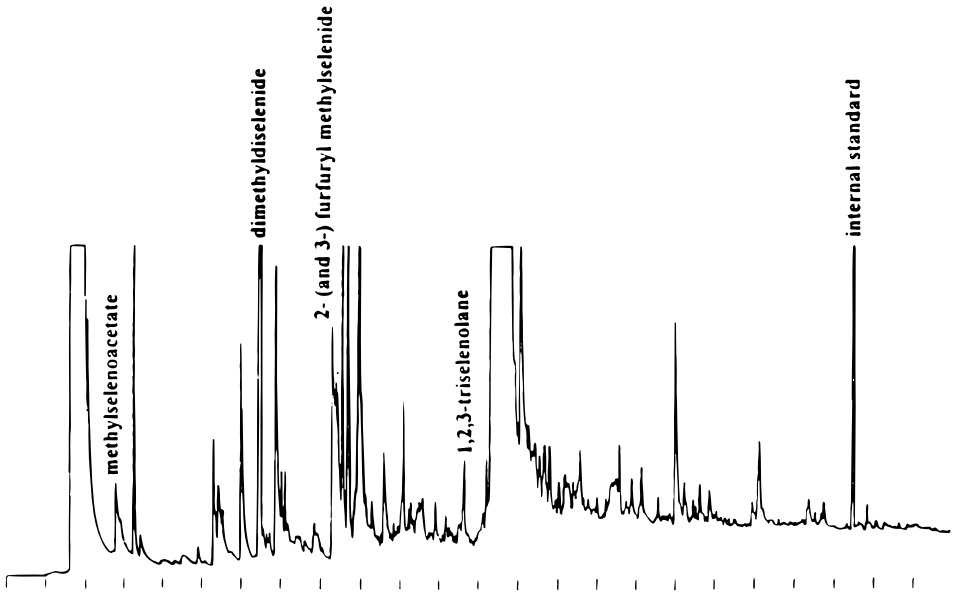


Figure 3. Gas chromatogram of crude Maillard reaction mix. Each tic on the *X*-axis is 5 min.

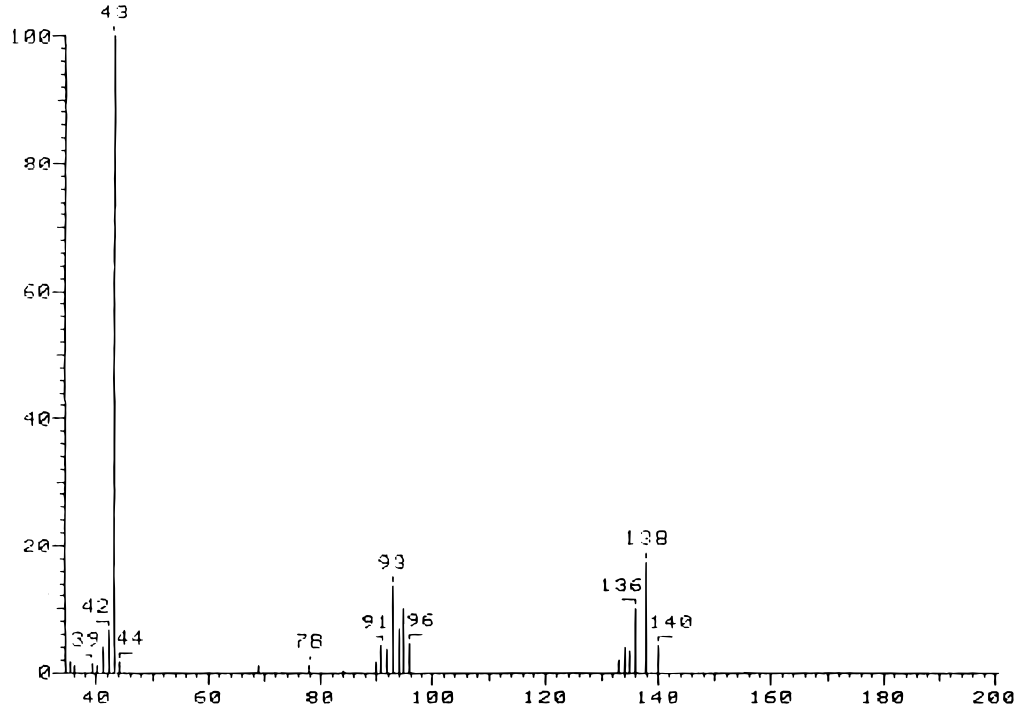


Figure 4. Electron ionization mass spectrum of compound **I**.

temperature, 260 °C; MS inlet temperature, 270 °C; emission current, 1 mA; interscan time, 0.8 s; resolution, 1000.

Chemicals and Reagents. Seleno-DL-methionine was purchased from Sigma Chemical Co. (St. Louis, MO). Nonadecane (99%), D-glucose (dextrose), (ACS reagent grade), and a standard of dimethyl diselenide were purchased from Aldrich Chemical Co. (Milwaukee, WI). The water and methylene chloride were of HPLC grade and purchased from Fisher Scientific Co. (Springfield, NJ).

RESULTS AND DISCUSSION

Qualitative identification of organoselenium compounds was based on the diagnostic isotopic distribution pattern for selenium (see Table 1 for the isotopic distribution of a monoselenide). Isotopic abundances for diselenium and triselenium compounds were computer generated and used to identify compounds (Figure 1 shows the pattern for a diselenium-containing compound). The structures of five organoselenium compounds were postulated (Table 2 and Figure 2). Also, two minor unknowns were observed, but their structures were not obvious. The chromatogram obtained from the Maillard reaction of selenomethionine and glucose is shown in Figure 3. Many peaks were observed in this chromatogram resulting from caramelization of the sugar, and the selenium compounds were minor compounds in the trace, which had to be extracted by utilization of mass chromatography.

Compound **I** (Figure 4) was tentatively identified as methyl selenoacetate. It was identified as an organoselenium compound on the basis of isotopic distribution surrounding the monoisotopic molecular ion at *m*/*z* 138, this ion resulting from the empirical formula

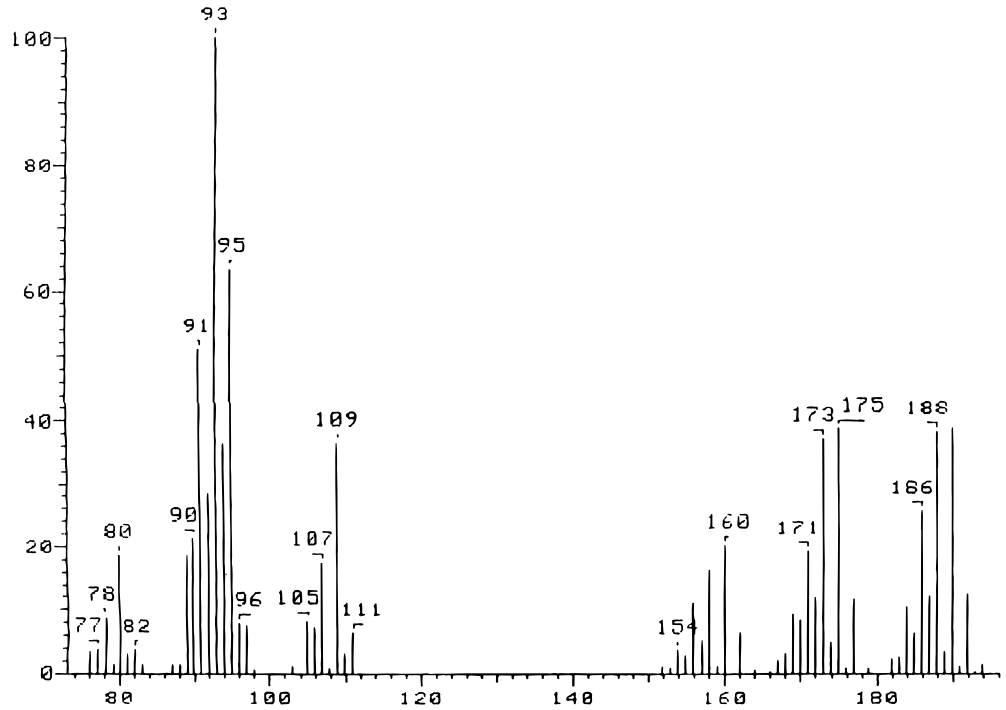


Figure 5. Electron ionization mass spectrum of compound **II** (dimethyl diselenide).

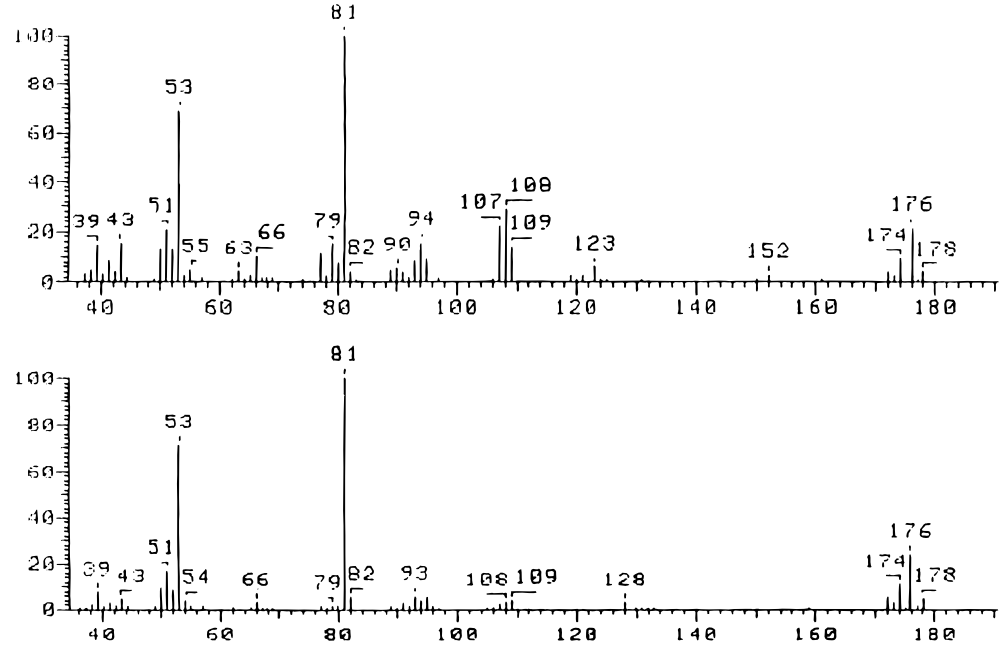


Figure 6. Electron ionization mass spectrum of compound **III** (top) and **IV** (bottom).

$C_3H_6O^{80}Se^{\bullet+}$. Important fragmentation results from the loss of the $CH_3CO^{\bullet}$ radical from the molecular ion, resulting in the even-electron ion fragments observed surrounding *m*/*z* 95. *m*/*z* 95 is due to $CH_3{}^{80}Se^{+}$. The isotopic distribution for the ions at masses 93, 95, 97, etc., is unusual because the ion resulting from $CH_3{}^{78}Se^{+}$ at *m*/*z* 93 is more intense than can be predicted from the theoretical isotopic ratios. However, this anomaly is also seen in the mass spectrum for compound **II**, which also produces this unusual ion cluster. This is not an artifact or interference and is seen in the spectrum of commerically obtained dimethyl diselenide. The mass spectrum for compound **I** is shown in Figure 4. The explanation for the unusual ion ratios observed at masses 91, 93, 95, etc., for these compounds is not readily apparent.

Compound **II** (dimethyl diselenide) shows the distinctive isotopic cluster for a diselenium compound with the monoisotopic molecular ion, at *m*/*z* 190, due to $C_2H_6{}^{80}Se^{80}Se^{\bullet+}$. Also present in the molecular ion cluster are the ions $C_2H_6{}^{78}Se^{80}Se^{\bullet+}$, $C_2H_6{}^{78}Se^{78}Se^{\bullet+}$, etc. Loss of a methyl radical results in the ion cluster at *m*/*z* 173, 175, etc., 175 being due to $CH_3{}^{80}Se^{80}Se^{+}$. This cluster shows the same isotopic distribution pattern as observed in the molecular ion (two Se present). A second methyl loss results in a cluter of ions surrounding *m*/*z* 160, which is due to $^{80}Se^{80}Se^{+}$. The ion at *m*/*z* 95 is due to $CH_3{}^{80}Se^{+}$. This mass spectrum is identical to the mass

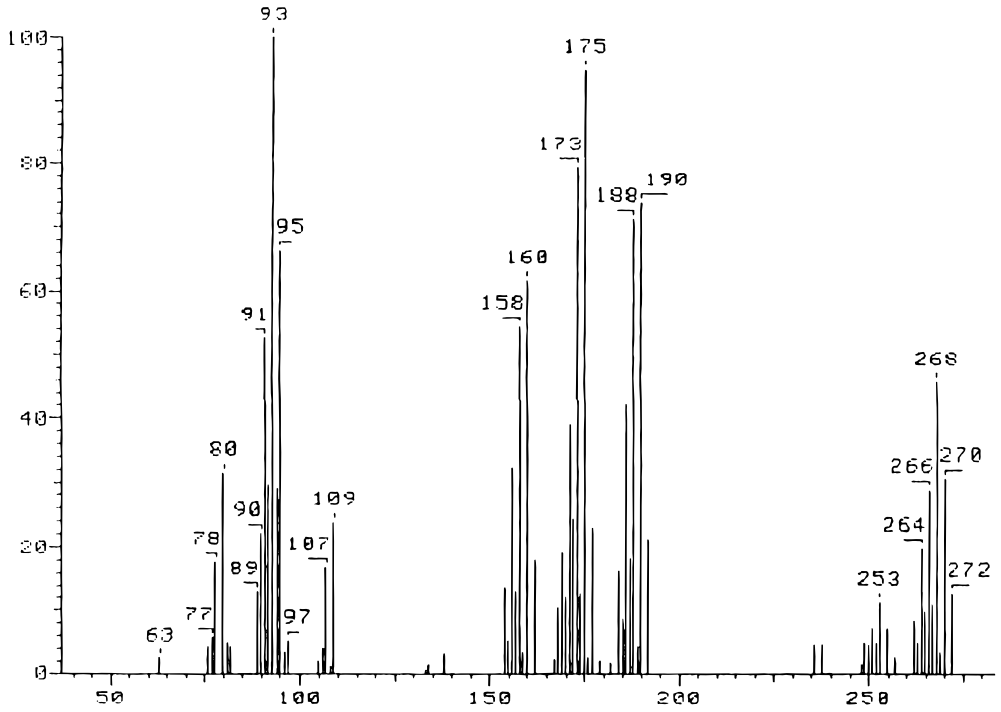


Figure 7. Electron ionization mass spectrum of compound **V**.

spectrum obtained from a commercial standard from Aldrich, and the retention index was identical within experimental parameters. The mass spectrum for compound **II** is shown in Figure 5.

The mass spectra for compounds having postulated structures of 2- and 3-methylfuranyl methyl selenide (compounds **III** and **IV**) show the distinctive isotopic cluster for a monoselenium compound as seen in the mass spectrum for Compound **I** (methyl selenoacetate). The ion at *m*/*z* 81 (base peak with no selenium isotopes) is due to β-cleavage to the ring and is the methylfuranyl ion. The ion at *m*/*z* 53 identifies this compound as a methylfuran and most probably results from the loss of carbon monoxide from the ion at *m*/*z* 81 (Figure 6). There were two chromatographically separated isomers present as observed by mass chromatography.

Compound **V** (postulated to be 1,2,4-selenotrithiolane) was identified as a triselenium compound on the basis of the characteristic isotopic distribution pattern about the monoisotopic molecular ion at *m*/*z* 268, 268 being due to $C_2H_4{}^{80}Se^{80}Se^{80}Se^{\bullet+}$. Important fragmentation results from loss of ^{80}Se to form $C_2H_4{}^{80}Se^{80}Se^{\bullet+}$ and the related cluster of isotopic peaks at *m*/*z* 186, 188, etc. This fragment has the characteristic pattern for a diselenium ion.

Other diselenium ion cluster occur around *m*/*z* 173 and 158 due to $CH_2{}^{80}Se^{80}Se^{+}$ and $^{80}Se^{80}Se^{+}$, respectively. The ion at *m*/*z* 95 is due to $CH_3{}^{80}Se^{+}$. The mass spectrum for compound **V** is shown in Figure 7.

CONCLUSION

The stuctures of five organoseleno compounds resulting from a model system involving the Maillard reaction of selenomethionine with glucose have been postulated. It is possible these volatile organoselenium compounds might be present at low levels in roasted Brazil nuts, which are known to contain the amino acid selenomethionine. The research described in this paper provides the foundation for screening roasted Brazil nuts for levels of these volatile compounds and for toxicologists to determine if there is a potential heath consideration.

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