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Analytical Methods

Determination of volatile compounds formed in a glucose–selenomethionine model system by gas chromatography–atomic emission detector and gas chromatography–mass spectrometry

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ABSTRACT

In order to gain a better understanding of the formation of organoselenium compounds in food system, the Maillard reaction of selenomethionine and glucose was studied in a model system. The effects of heating time and pH on the volatile compounds formed in a glucose–selenomethionine reaction were also investigated. Nine organoselenium compounds were identified. Pyrazines and dimethyldiselenide are major volatile compounds generated from the glucose–selenomethionine model system. A high pH level favours the formation of pyrazine and dimethyldiselenide. In unbuffered systems, a pH change of three or more pH units may occur, and this may significantly affect the formation of Maillard reaction products.

1. Introduction

Selenium, discovered by Berzelius in 1817, is an element toxic in large quantities, but an essential trace metal for mammals, birds, and many bacteria. It is believed that selenium toxicity was first reported by Marco Polo when he described a disease called "hoof rot" in horses in Turkestan. Symptoms of this disease include loss of hooves and hair, liver damage and respiratory failure. The first evidence that selenium may be an anticarcinogenic element was presented by Clayton and Baumann (1949). They found hepatic tumour incidence induced by azo dye was decreased by a diet containing 5 ppm of selenium. This work was confirmed 28 years later (Griffin & Jacobs, 1977). In 1957, Schwarz and Foltz reported that selenium can effectively protect experimental rats against liver necrosis, and they also concluded "*selenium is an essential trace element*" (Schwarz & Foltz, 1957). This breakthrough research demonstrated that selenium is associated with antioxidant activity in biological systems. Selenium was first elucidated by Rotruck as an essential component of glutathione peroxidase, which is important in the protection of red blood cell membranes and other tissues from damage by peroxides. This enzyme is deficient in both animals and humans who have selenium-poor diets. Selenium supplementation of healthy subjects with low levels of selenium increases glutathione peroxidase activities (Rotruck et al., 1973). Now, it is clear that the toxicity and anticarcinogenic properties of selenium compounds are coming from the same mechanism, the generation of superoxide in the presence of reduced glutathione (Spallholz, Palace, & Reid, 2004).

Selenomethionine is the primary form of organoselenium compounds present in wheat, corn, rice and selenium-enriched yeast (Whanger, 2002). Some vegetables and nuts also contain high level of selenium, such as garlic, onion, and Brazil nuts (Inam & Somer, 1999; Palmer, Herr, & Nelson, 1982). The concentration of selenium in agricultural products is correlated with selenium concentration of soil in which they are grown. However, the selenium concentration of plant foods can be enriched by selenium-containing fertilisers and consumption of these selenium-enriched foods results in higher inhibition of tumour yield (Ip & Lisk, 1994; Ip, Lisk, & Thompson, 1996).

The Maillard reaction is one of the most complex and important flavour and colour generating reactions in food system. This

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complex degradative reaction is initiated by the interaction of a free amino group (amino acid) and a carbonyl group (reducing sugar). The Maillard reaction is known to be affected by pH, and temperature time combination, as well as water activity. Changes in each of these factors affect the profile of reaction products (Ames, 1990; Romero & Ho, 2007). Of all the factors affecting the reaction, temperature has the greatest influence. Many studies of the Maillard reaction have been carried out in model systems. Even in a simple system of a given amino acid and a reducing sugar, numerous volatile products are generated. Due to analytical limitation, often many minor compounds cannot be detected or identified. However, a high selectivity and sensitivity detector, such as the atomic emission detector (AED) coupled to a GC, could be useful in identification of a group of specific compounds, such as selenium-containing compounds.

Atomic emission spectroscopy (AES) is a method used to identify an atom by measuring the emission of photons of radiation released by the electrons of the atom after they are excited to a high energy level. Using GC in combination with AES, it is possible to detect elements in compounds after GC separation. In the interface, the eluent is atomised and excited by microwave-energised helium plasma that is coupled to a diode-array optical emission spectrometer.

We previous performed Maillard reaction of selenomethionine and glucose, and reported the tentative identification of five novel organoselenium compounds (Tsai, Hiserodt, Ho, Hartman, & Rosen, 1998). This paper reports the use of GC–AED and GC–MS to further characterise the products formed from a selenomethionine–glucose system under various pH conditions.

2. Materials and methods

2.1. Reagents

Selenomethionine was a gift from Sabinsa, Inc. (Piscataway, NJ).

Glucose and dodecane were purchased from Aldrich Chemical Co. (St. Louis, MO). Dichloromethane was purchased from J.T. Baker (Phillipsburg, NJ).

2.2. Sample preparation

Selenomethionine (0.15 g) and glucose (0.2 g) were dissolved in either 25 ml of water (unbuffered solution) or 25 ml 0.05 M sodium phosphate buffer solution, and the pH values of the solutions were adjusted to 3.0, 5.0, 7.0 and 9.0, using 85% phosphoric acid or 1 M NaOH solution. Each solution was sealed, individually, in a 100 ml glass bottle. The reaction temperature was 160 °C. The reaction times were 40, 60 and 80 min. After the reaction, the solution was adjusted to pH 7 and extracted with 50 ml of CH_2Cl_2. The organic phase was concentrated to 2 ml by a Kuderna-Danish concentrator and further to 1 ml under a nitrogen flow. Dodecane was used as an internal standard.

2.3. GC–FID analysis

The volatile compounds isolated from the thermal reaction systems were analysed by a Siemens SiChromat 2-8 gas chromatograph with an AS-20 auto-injection system (Cologne, Germany). The GC was equipped with an HP-5 fused silica capillary column (25 m × 0.32 mm i.d.; 0.52 μm film thickness; Agilent, Santa Clara, CA) and a flame ionisation detector. The injection volume was 1 μl for each sample with a split ratio of 50:1. The GC was run with an injector temperature of 275 °C and a detector temperature of 275 °C. The column temperature was programmed from 40 to 285 °C at a rate of 5 °C/min.

2.4. GC–MS analysis

GC–MS analysis was performed using an HP 5989A Mass Engine coupled with an HP 5890 II GC. Mass spectra were obtained by electron impact (EI) at 70 eV or ammonia chemical ionisation (NH_3-CI) and a mass scan from 40–450 amu. The ion source temperature was 230 °C, and the analyser temperature was 150 °C.

2.5. GC–AED analysis

An Agilent G2350A GC–AED (Agilent Technologies, Wilmington, DE) was used. Oxygen and hydrogen were used as reagent gases with detection at 179, 196, and 174 nm for carbon, selenium, and nitrogen. Helium carrier gas was used for all analyses. The cavity temperature was 250 °C. The transfer line temperature was 250 °C. The hydrogen pressure was 8.8 psi. The oxygen pressure was 24 psi, and the auxiliary gas pressure was 30.6 psi.

3. Results and discussion

Nine selenium-containing compounds are identified from this model system (Fig. 1) by GC–MS and GC–AED. The mass spectral data of these selenium-containing compounds identified are summarised in Table 1. Among them, compounds **IV** and **IX** were reported in a previous study (Tsai et al., 1998).

Compound **I** was identified as CH_3SeH. The monoisotopic molecular ion is *m/z* 96, which represents the $CH_3{}^{80}SeH^{\cdot+}$ radical ion. The major fragment ion is from the loss of methane.

Compound **II** was tentatively identified as $HSeCH_2CH_2CHO$. The base peak *m/z* 109 is $H^{80}SeCH_2CH_2^+$, which is generated by losing a $CHO^{\cdot}$ radical from the molecular ion. The ion at *m/z* 95 is due to $H^{80}SeCH_2^+$.

The ion at *m/z* 142 of compound **III** is from the molecular ion $C_2H_6O_2{}^{80}Se^{\cdot+}$. Losing a $CH_3^{\cdot}$ radical from the molecular ion results in the generation of $CH_3O_2{}^{80}Se^+$ at *m/z* 127.

Compound **IV** was identified as dimethyldiselenide. The ammonia chemical ionisation (CI) mass spectrum of compound **IV** is shown in Fig. 2, which matches the isotopic distribution pattern for a diselenium-containing compound generated by computer (Tsai et al., 1998). Compared to electron impact ionisation (EI), chemical ionisation (CI) causes less fragmentation and generates abundant protonated molecular ions. Compound **V** has the empirical formula C_4H_8OSe and was tentatively identified as $CH_3SeCH_2(C{=}O)CH_3$. The base peak at *m/z* 109 is $CH_3{}^{80}SeCH_2^+$, which is generated by losing the $C{=}OCH_3^{\cdot}$ radical. *m/z* 43 is due to $^+C{=}OCH_3$.

Compound **VI** has the same empirical formula, C_4H_8OSe, as compound **V**; however the structure was tentatively identified as

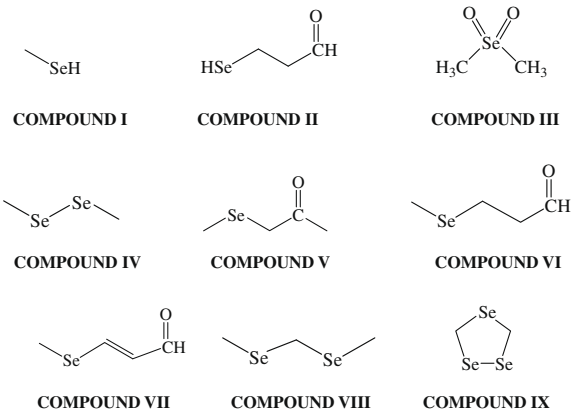

Fig. 1. The structures of organoselenium compounds identified.

Table 1
The mass spectra of selenium-containing compounds identified.

Compound no.	*m/z* (%)	Monoisotopic mol. wt.
I	98(24), 96(100), 95(21), 93(76), 91(44), 80(48), 78(29)	96
II	140(16), 138(97), 136(46), 134(23), 109(100), 107(54), 95(50), 93(88), 83(27), 74(23), 61(29), 57(38)	138
III	144(20), 142(100), 140(43), 138(15), 127(54), 125(24), 112(19), 93(29), 91(19), 78(21), 61(21), 45(30)	142
IV	192(31), 190(100), 188(90), 186(53), 175(64), 173(59), 171(36), 160(29), 109(23), 95(34), 93(63)	190
V	154(17), 152(92), 150(42), 148(19), 110(48), 109(100), 107(58), 95(27), 93(29), 91(28), 43(85)	152
VI	154(14), 152(82), 150(36), 124(9), 109(20), 107(19), 98(17), 96(100), 94(60), 80(17), 57(9), 41(9)	152
VII	152(19), 150(100), 148(48), 121(37), 119(20), 106(24), 95(52), 93(74), 91(35), 80(19), 55(89)	150
VIII	206(14), 204(39), 190(10), 172(12), 111(28), 109(100), 107(50), 95(31), 93(54), 83(16), 44(15)	204
IX	270(34), 268(100), 266(80), 264(45), 175(77), 173(68), 171(42), 160(53), 158(42), 156(25), 95(80), 93(89), 91(43), 80(22)	268

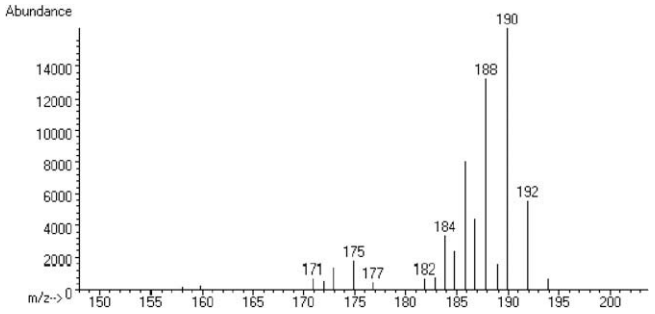

Fig. 2. NH_3-CI/MS spectrum of compound **IV**.

$CH_3{}^{80}SeCH_2CH_2CHO$. Losing the $CH_2CH_2CHO^{\cdot}$ radical results in the ion cluster at *m/z* 98, 96, and 94. The base peak *m/z* 96 is due to the $CH_3{}^{80}Se^+$ ion.

Compound **VII** has the empirical formula C_4H_6OSe. The ion at *m/z* 55 is due to $CH{=}CHCHO^+$ and *m/z* 121 is generated by losing CHO^+ from the ion at *m/z* 150, which is molecular ion. Thus, compound **VII** was identified as $CH_3SeCH{=}CHCHO$.

Compound **VIII** has the empirical formula $C_3H_8Se_2$ and the structure was tentatively assigned as $CH_3SeCH_2SeCH_3$. The base peak at *m/z* 109 is due to $CH_3{}^{80}SeCH_2^+$, which is generated by losing the $CH_3{}^{80}Se^{\cdot}$ radical from molecular ion *m/z* 204.

Compound **IX** was assigned as 1,2,4-selenotrithiolane, the only triselenium compound identified.

$CH_3{}^{80}Se^+$ ion fragment can be generated from several compounds identified in this study. The isotopic distribution for $CH_3{}^{80}Se^+$ is *m/z* 93, 95, 97 etc., and the ion at *m/z* 95 should be the most intense one. However, the unusual highly intense *m/z* 93 was observed in every compound except compound **VI**. This phenomenon was also observed in a previous study. Theoretically, *m/z* 93 is mainly due to $CH_3{}^{78}Se^+$; however, here we suspect that $^{80}Se{=}CH^+$ could also make a significant contribution to *m/z* 93.

The yields of pyrazines, compounds **IV** and **VI** at different times and pHs are listed in Tables 2. Unlike pyrazines and compound **IV**, the formation of compound **VI** is favoured at low pH, which has the maximum yield at pH 3 and 80 min, and the yields decreased with increasing pH. It was observed that the pH change of an unbuffered solution is significant after heating but not in buffered systems (Table 3). In unbuffered model systems a pH change of 3 or more units is not unusual during heating, and this can affect both the rate and the pathway of the formation of volatile and coloured products. Thus, it is very important to maintain a constant pH during heating when model systems are used to study the influence of pH on the Maillard reaction.

The GC–AED profiles of volatile compounds generated in a pH 9 solution is shown in Fig. 3. The N channel of pH 9 shows seven compounds: pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine, and tetramethylpyrazine. The Se channel shows that the major selenium-containing compound is compound **IV**. The possible formation mechanisms of compounds **I**, **IV** and **VI** are proposed in Fig. 4.

We had identified five volatile selenium compounds in our previous study and another six volatile selenium compounds were identified in this study. Dimethyldiselenide (compound **IV**) is the major volatile selenium compound generated in both studies. To our knowledge, none of these compounds has been found in uncooked food. Selenium can exist in a number of different forms. Their toxicities and biological functions are quite different. For both inorganic and organic selenium compounds, the generation of the metabolite, methylselenol (CH_3SeH, compound **I**) is critical for their anticarcinogenic properties (Ip, Thompson, Zhu, & Ganther, 2000). The catalytic nature of the selenide anion (RSe^-) will generate superoxide ($O_2^{\cdot-}$) subsequently, which may cause

Table 3
pH values in model system.

Time (min)	Unbuffered systems				Buffered systems			
0	3.01	5.00	6.99	9.01	3.00	4.98	7.01	9.00
40	3.02	4.82	5.11	5.95	3.03	5.01	6.98	8.96
60	3.01	4.94	5.56	5.84	3.01	4.97	7.00	8.93
80	2.99	4.93	5.77	6.38	3.02	4.96	6.97	8.95

Table 2
Yields of major volatile compounds (mg/g of glucose) generated in model system (buffered solutions).

Compounds	40 min				60 min				80 min			
	pH 3	pH 5	pH 7	pH 9	pH 3	pH 5	pH 7	pH 9	pH 3	pH 5	pH 7	pH 9
Pyrazine	ND	ND	0.021	0.041	ND	ND	0.029	0.024	ND	0.014	0.252	0.260
Methylpyrazine	ND	ND	0.022	0.014	ND	ND	0.033	0.164	ND	0.009	0.202	0.169
Compound **IV**	0.061	0.029	0.743	0.938	0.046	0.041	0.545	1.162	0.058	0.134	2.099	1.490
2,6-Dimethylpyrazine	ND	ND	0.039	0.014	ND	ND	0.067	0.144	ND	0.018	0.239	0.191
Ethylpyrazine	ND	ND	0.019	0.005	ND	0.002	0.008	0.027	ND	0.008	0.034	0.027
Compound **VI**	0.027	0.021	0.022	0.009	0.057	0.044	0.016	0.010	0.542	0.098	0.077	0.007
Trimethylpyrazine	ND	ND	0.006	0.003	ND	ND	0.011	0.043	ND	0.008	0.098	0.052

ND: not detected.

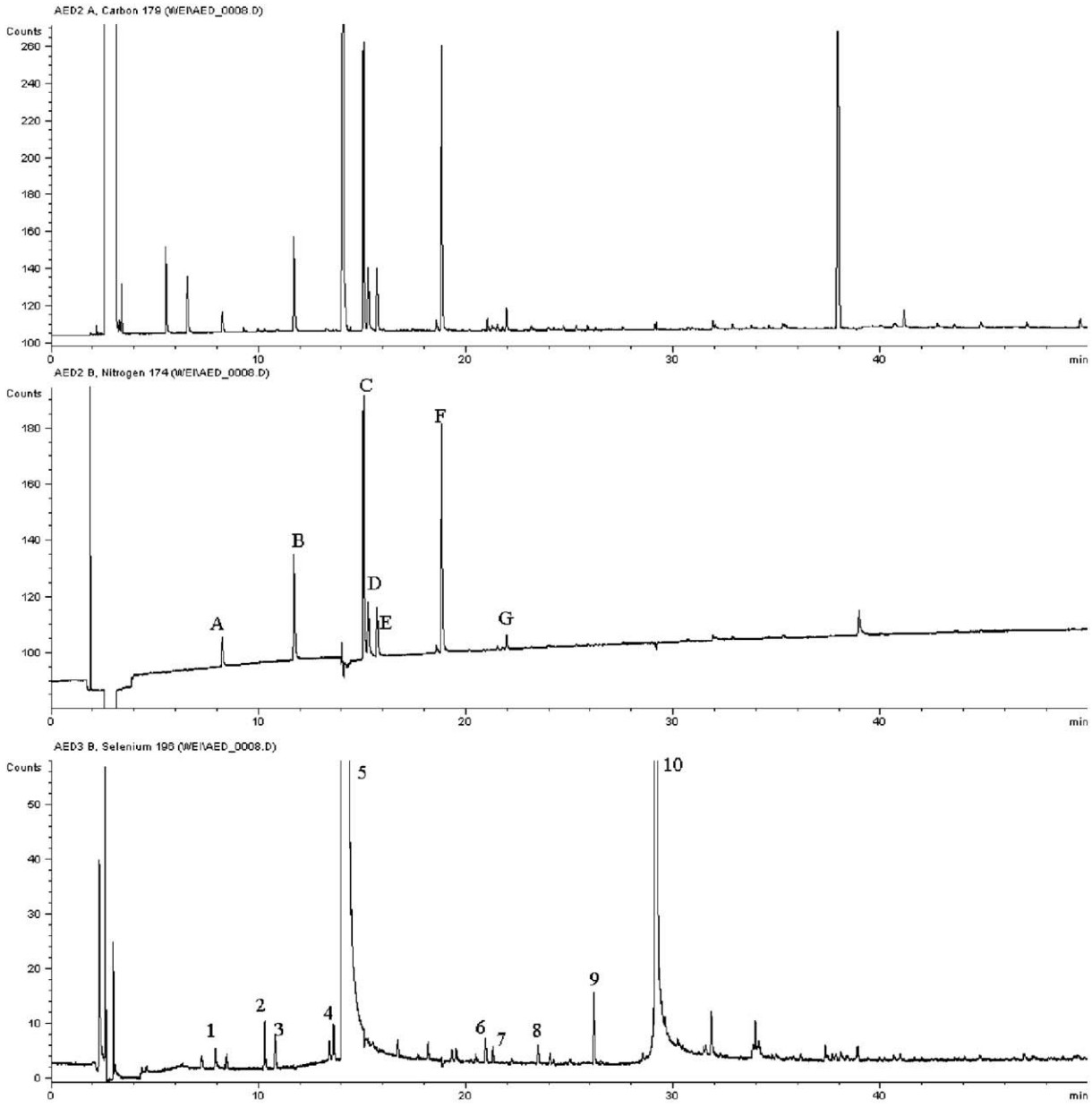

Fig. 3. GC–AED profile of volatile compounds formed from glucose–selenomethionine model system (pH 9): top, C channel; middle, N channel; bottom, Se channel; nitrogen-containing compounds identified: A = pyrazine; B = methylpyrazine; C = 2,5-dimethylpyrazine; D = 2,6-dimethylpyrazine; E = 2,3-dimethylpyrazine; F = trimethylpyrazine; G = tetramethylpyrazine. Selenium-containing compounds identified: 1 = compound **I**; 2 = compound **II**; 3 = compound **III**; 4 = unknown; 5 = compound **IV**; 6 = Compound **V**; 7 = compound **VI**; 8 = compound **VII**; 9 = compound **VIII**; 10 = compound **IX**.

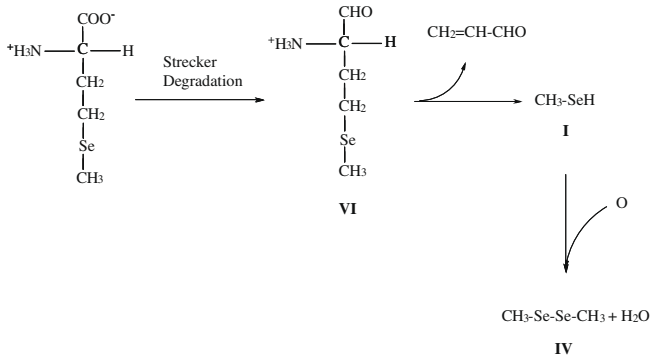

Fig. 4. The proposed formation mechanisms of compounds **I**, **IV**, and **VI**.

apoptosis in cancer cells (Spallholz, Shriver, & Reid, 2001). Selenomethionine is not able to generate superoxide. Among these selenium-containing compounds identified in this study, dimethyldiselenide (compound **IV)** was reported as being able to generate superoxide *in vitro* (Spallholz et al., 2004). It implies that toxicities and anticarcinogenic properties of selenium-containing food may be modified by cooking. Of course, a study of the toxicities and biological functions of other selenium-containing compound generated is needed.

4. Conclusion

Nine organoselenium compounds generated from the Maillard reaction model system of selenomethionine and glucose were

identified. Some of these organoselenium compounds are more active than selenomethionine in the generation of superoxide. Dimethyldiselenide (compound **IV**), which can generate superoxide more effectively than selenomethionine does, is the major volatile organoselenium generated in a glucose–selenomethionine model system; it was also the major organoselenium compound generated in our previous study. The pH value was also shown to have a significant effect on the formation of these organoselenium compounds.

Selenium is effective against cancer cells, and the most convenient way to deliver selenium into body is *via* food, and selenomethionine is the major seleno compound in many foods. Heating is a widely used means of food preparation and it may also be an easiest way to enhance the anti-cancer properties of selenium-containing foods. This study is expected to provide information for the evaluation of nutrition and toxicity values of selenium-rich food products.

We have also demonstrated that the atomic emission detector is a powerful tool for gas chromatography due to its high sensitivity, elemental selectivity, and the ability for multielement analysis. The chemical structures of some selenium-containing compounds detected by AED cannot be established by GC–MS because a lack of sensitivity and interference.

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