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Determination of *S*-Methyl-, *S*-Propyl-, and *S*-Propenyl-L-Cysteine Sulfoxides by Gas Chromatography−Mass Spectrometry after *tert*-Butyldimethylsilylation

KOUICHIRO TSUGE, MIEKO KATAOKA, AND YASUO SETO*

National Research Institute of Police Science, 6-3-1 Kashiwanoha, Kashiwa, Chiba 277-0882, Japan

A gas chromatographic−mass spectrometric method for the determination of *S*-methyl-L-cysteine sulfoxide (**1**), *S*-propyl-L-cysteine sulfoxide (**2**), and *S*-propenyl-L-cysteine sulfoxide (**3**), specific marker compounds in the genus *Allium*, is described. The target amino acids were converted to the *tert*-butyldimethylsilyl derivatives. The products were silylated on the amino and carboxyl groups and on an additional oxygen atom and were separated on a nonpolar capillary column. That incorporation of three *tert*-butyldimethylsilyl groups had occurred was verified by mass spectrometry, which gave an *m*/*z* 302 fragment as base peak (amino acid side chain eliminated ion) and *m*/*z* 436 (**1**), 464 (**2**), or 462 (**3**) as major peaks (*tert*-butyl function eliminated ion), by electron impact ionization. The detection limits for **1** and **2** under selected ion monitoring at *m*/*z* 436 (**1**) and *m*/*z* 464 (**2**), respectively, were determined to be 0.3 and 1.8 ng per injection. To clean up the analytes from the solvent extract of onion, as a representative food material, onion, the sample solution was subjected to combined solid phase extraction. The eluate from a Sep-Pak C_{18} cartridge was applied to a Bond Elut SCX cartridge (H^+ form), followed by washing with 0.1 M hydrochloric acid and elution with 0.5 M ammonia. From a simulated matrix solution containing 5% sucrose, **1** and **2** were extracted quantitatively, and the detection yield was ∼75%. The contents of **1**, **2**, and **3** in commercial onion were estimated to be 0.3, 3.1, and 3.0 mg, respectively, per gram of fresh weight.

KEYWORDS: ***S*-Alk(en)yl-L-cysteine sulfoxide; onion; GC-MS; solid phase extraction; *tert*-butyldimethylsilylation**

INTRODUCTION

Food materials contain characteristic components that are species-specific. To verify the existence of a specific type of food material in foodstuff, the identification of marker components that are specific to the materials is necessary. *S*-Alk(en)yl-L-cysteine sulfoxides are the specific components in the genus *Allium* (*1*, *2*). Volatile alkyl disulfides produced from *S*-alk(en)yl-L-cysteine sulfoxides by CS-lyase are the main source of the characteristic odor of *Allium* species. Quantification of *S*-alk(en)yl-L-cysteine sulfoxides is an important issue in plant physiology investigations. A variety of methods have been developed for the determination of *S*-alk(en)yl-L-cysteine sulfoxides. Headspace gas chromatography of thiosulfinates, formed by reactions after the CS-lyase action, has been proposed as an indicator of the extent of pungency (*3*). Thin layer chromatography combined with thin layer electrophoresis has been developed for the quantitation of *S*-alk(en)yl-L-cysteine sulfoxides (*4*−*6*). Subsequently, HPLC has been applied to the analysis of *S*-alk(en)yl-L-cysteine sulfoxides after derivatization with *o*-phthalaldehyde (*7*) or 9-fluorenylmethyl chloroformate (*8*). In general, for an analysis of such hydrophilic compounds, GC-MS after *tert*-butyldimethylsilylation (TBDMS) derivatization appears to be the method of choice, and a number of papers have indicated its utility in the analysis of amino acids (*9*−*11*). Our laboratory has applied a TBDMS GC-MS method for the analysis of nerve gas hydrolysis products (*12*−*14*). In this paper, we report on a method for the determination of *S*-methyl-L-cysteine sulfoxide (**1**), *S*-propyl-L-cysteine sulfoxide (**2**), and *S*-propenyl-L-cysteine sulfoxide (**3**), the main *S*-alk(en)yl-L-cysteine sulfoxide components in *Allium*, together with a pretreatment method using solid phase extraction for the cleanup of *S*-alk(en)yl-L-cysteine sulfoxides from the extract of onion, one of the most popular food materials belonging to the *Allium* genus.

MATERIALS AND METHODS

Reagents. *S*-Methyl-L-cysteine was purchased from Sigma (St. Louis, MO). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide was obtained from Pierce (Rockford, IL). l-Cysteine was purchased from Wako Pure Chemicals (Osaka, Japan). The Bond Elut SCX cartridge (regular type, 500 mg/3 mL) was obtained from Varian (Harbor City, CA). The

* Corresponding author [telephone (81)-471-35-8001; fax (81)-471-33-9159; e-mail seto@nrips.go.jp].

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Sep-Pak C_{18} Plus cartridge was obtained from Waters (Milford, MA). All other chemicals used were of analytical grade. All aqueous solutions were prepared with distilled, deionized water.

Onion Sample. An onion bulb cultivated in Ibaraki prefecture was commercially obtained from the local supermarket in Kashiwa, Chiba.

Spectroscopy and Melting Point Measurements. Melting points were measured using an MP-500V micro melting point apparatus (Yanako, Kyoto, Japan) and are uncorrected. ^{1}H NMR spectra were recorded on a JNM-ECP 600 spectrometer (600 MHz, JEOL, Tokyo, Japan). Electrospray ionization (ESI) time-of-flight (TOF) mass spectra were recorded on a Q-TOF2 (Micromass, Manchester, U.K.) instrument with direct sample infusion using a solvent of 0.1% formic acid in water. IR spectra were recorded on a JIR-WINSPEC 50 FT-IR spectrometer (JEOL, Tokyo, Japan) using a potassium bromide disk.

Synthesis of *S*-Alkyl-L-cysteine Sulfoxides. **1** was prepared by the oxidation of *S*-methyl-L-cysteine with hydrogen peroxide, according to the method of Synge and Wood (*15*) with slight modifications. *S*-Methyl-L-cysteine (2.7 g) dissolved in 2.5 mL of water was mixed with 2.5 mL of 30% hydrogen peroxide solution and stirred at 25 °C for 1 h. **1** was crystallized by the addition of cold ethanol and recrystallized from water/ethanol: yield, 87%; mp, 166 °C [lit. 167−168 °C (*18*)]; IR, 1640 cm^{-1} (carboxylic acid), 2900 (amine), 1000 (sulfoxide); ESI-TOF-MS, *m*/*z* 152.0386 [(M + 1)$^+$], $C_4H_{10}NO_3S$; ^{1}H NMR (in D_2O) δ 2.69 (d, 3H, CH_3SO-), 3.10 (q) and 3.35 (q) (1H, $CCHSO-$), 3.27 (m, 1H, $CCHCS$), 4.14 (q) and 4.07 (m) (1H, NH_2CHC).

2 was prepared according to the method of Lancaster and Kathleen (*6*). *S*-Propyl-L-cysteine was prepared by using the method of Armstrong and Lewis (*16*) with slight modifications, as follows. l-Cysteine (11.2 g) dissolved in a mixture of 15 mL of 20 M sodium hydroxide and 200 mL of ethanol was mixed with 11.6 mL of 1-bromopropane, and the resulting solution was stirred at 25 °C for 3 min. The solution was adjusted to pH 5.25 by the addition of acetic acid and stirred for 30 min in an ice bath. The precipitate (*S*-propyl-L-cysteine) was collected by centrifugation (3.3 g), dissolved in 150 mL of water containing 0.5 mL of 5 M sodium hydroxide, mixed with 2.0 mL of 30% hydrogen peroxide, and stirred for 1 h at 25 °C. **2** was crystallized by the addition of cold ethanol and recrystallized from water/ethanol: yield, 44%; mp, 160−161 °C [lit. 163−164 °C (*17*)]; IR, 1600 cm^{-1} (carboxylic acid), 2900 (amine), 1030 (sulfoxide); ESI-TOF-MS, *m*/*z* 180.0701 [(M + H)$^+$], $C_4H_{10}NO_3S$; ^{1}H NMR (in D_2O), δ 0.93 (t, 3H, $CH_3CH_2CH_2SO-$), 1.65 (m, 2H, $CH_3CH_2CH_2SO-$), 2.84 (m, 2H, $CH_3CH_2CH_2SO-$), 3.07 (q) and 3.32 (q) (1H, $CCHSO-$), 3.27 (m, 1H, $CCHCS$), 4.10 (d of t, 1H, NH_2CHC).

Extraction of *S*-Alk(en)yl-L-cysteine Sulfoxides from Onion. The extraction of *S*-alk(en)yl-L-cysteine sulfoxides from onion was carried out according to the method of Bieleski and Turner (*4*) with slight modifications (*6*). Fresh onion bulbs (10 g) were peeled and cut into small pieces with a knife and then immersed in 100 mL of a methanol/chloroform/water (12:5:3, v/v) solution at −20 °C for 24 h. The liquid phase was removed, an additional 100 mL of the solvent was added to the residue material, and the extraction was repeated. The solvent phase was separated, and 100 mL of a 80% ethanol solution was added to the resulting solid materials. After 2 h, the supernatant was removed and used as the ethanol extract. The combined solvent fraction was supplemented with 90 mL of chloroform and 110 mL of water, and the mixture was vortexed. The aqueous upper phase was removed, combined with the ethanol extract, and concentrated to ∼5 mL under reduced pressure at 60 °C. The concentrate was diluted with 0.1 M hydrochloric acid (HCl) to be 25 mL.

Capillary Electrophoresis (CE). **1** and **2** were quantified electrophoretically using an HP ^{3D}CE CE system (Yokogawa Analytical Systems, Tokyo, Japan), using an HP Forensic Anion Analysis Kit (Yokogawa Analytical Systems; *18*). A 140 cm × 50 mm i.d. capillary fused silica column was used, and the electrophoresis buffer was an HP basic anion buffer. The voltage was set at 30 kV with a negative power supply. Detection was by indirect ultraviolet absorption at 350 nm (reference at 200 nm), and the column temperature was maintained at 30 °C. Samples were applied hydrodynamically at 50 mbar for 4 s.

High-Performance Liquid Chromatography (HPLC). *S*-Alk(en)yl-L-cysteine sulfoxides were quantified by HPLC after derivatization with *o*-phthalaldehyde according to the method of Ziegler and Sticke (*19*). A 20 μL aliquot of the onion extract was mixed with 180 μL of a 2.8 mg/mL *o*-phthalaldehyde solution in 50 mM sodium borate (pH 9.5) including 10% methanol and 0.2% *tert*-butylthiol (*20*). A 50 μL aliquot of the mixture was injected to the HPLC system, which consisted of an HP 1050 series liquid chromatograph (Yokogawa Analytical Systems). The HPLC column was a 25 cm × 4.6 mm i.d., 5 μm Inertsil ODS-2 (G-L Science, Tokyo, Japan), and the isocratic elution was done with a 32:68 mixture of 50 mM sodium phosphate (pH 7.15) and methanol. The flow rate was 1.0 mL/min and the column was maintained at room temperature. Absorbance at 339 nm was monitored. (−)-*S*-Methyl-L-cysteine sulfoxide, (+)-*S*-methyl-L-cysteine sulfoxide, (−)-*S*-propyl-L-cysteine sulfoxide, and (+)-*S*-propyl-L-cysteine sulfoxide were eluted at 8.8, 10.0, 15.1, and 17.7 min, respectively. The synthetic compounds of **1** gave two peaks eluting at 8.8 and 10.0 min, and those of **2** eluted at 15.1 and 17.7 min.

Solid Phase Extraction. A Sep-Pak C_{18} cartridge was activated by washing with methanol followed by water and equilibrated with a 0.1 M HCl solution. A 1 mL aliquot of the sample was applied to the C_{18} cartridge, followed by elution with 0.1 M HCl. The combined fraction of the cartridge pass-through and the 0.1 N HCl elution was applied to the Bond Elut SCX cartridge, which was activated by washing with ethanol and water and then equilibrated with 0.1 M HCl, and the SCX cartridge was washed with 4 mL of 0.1 M HCl followed by 6 mL of water. *S*-Alk(en)yl-L-cysteine sulfoxides were eluted with 4 mL of 500 mM ammonia water. The eluted fraction was dried by evaporation under reduced pressure, dissolved with 1 mL of distilled water, and filtered through a 0.45 mm cellulose acetate membrane.

***tert*-Butyldimethylsilylation (TBDMS) and Gas Chromatography—Mass Spectrometry (GC-MS)**. The aqueous sample was dried at 80 °C under a stream of nitrogen gas in a 1 mL volume glass vial (Nichiden Rika Garasu, Tokyo, Japan). Thirty microliters of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide and 20 μL of dried pyridine were added, and the vial was then closed with a Teflon screw cap, homogenized by sonication for 5 min, and incubated at 90 °C for 30 min. One milliliter of the mixture was applied to the GC-MS system, which consisted of an HP 6890 gas chromatograph interfaced with an HP 5973 quadrupole mass spectrometer (Yokogawa Analytical Systems). The stationary phase was a capillary 30 m × 0.25 mm i.d., 0.25 μm thickness, DB-5MS column (J&W Scientific, Folsom, CA). The carrier gas (helium) flow rate and split ratio were adjusted to 1.0 mL/min and 50:1, respectively. The injection port, transfer line, and ion source were maintained at 250, 280, and 250 °C, respectively. The oven temperature was controlled by a program [initial temperature, 100 °C (1 min hold), a ramp to 250 °C at 10 °C/min, then a ramp to 300 °C at 25 °C/min (3 min hold)]. Electron impact ionization (EI, ionization energy = 70 eV, ionization current = 60 μA) was used as the ionization mode for the general analysis. The acquisition mass range was *m*/*z* 50−550, and sampling was 0.8 scan/s. The acquisition was started 4 min after sample injection.

For GC-MS quantification, an internal standard (IS) calibration method was performed. A 10 μL aliquot of the solid phase extraction fraction was dried in a 1 mL volume glass vial. Thirty microliters of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide and 20 mL of a 1 μg/mL anthracene (IS) solution in dried pyridine were added and incubated at 90 °C for 30 min. One microliter of the mixture was applied to the GC-MS system under the above-mentioned analytical conditions except for the split ratio (20:1). Selected ion monitoring (SIM) was performed at *m*/*z* 302 and 436 for **1**, *m*/*z* 302 and 464 for **2**, *m*/*z* 302 and 462 for **3**, and *m*/*z* 178 for IS, respectively.

Chemical ionization (CI) was adopted using methane as the reagent gas, and the acquisition mode and mass range were positive and *m*/*z* 80−600, respectively.

High-resolution mass spectra were obtained using an HP 6890 gas chromatograph combined with an MSroute JMS-600W sector mass spectrometer (JEOL, Tokyo, Japan), under the same conditions as above.

RESULTS AND DISCUSSION

TBDMS Derivatives of Methyl-L-cysteine Sulfoxide and Propyl-L-cysteine Sulfoxide. As shown in **Figure 1**, under our

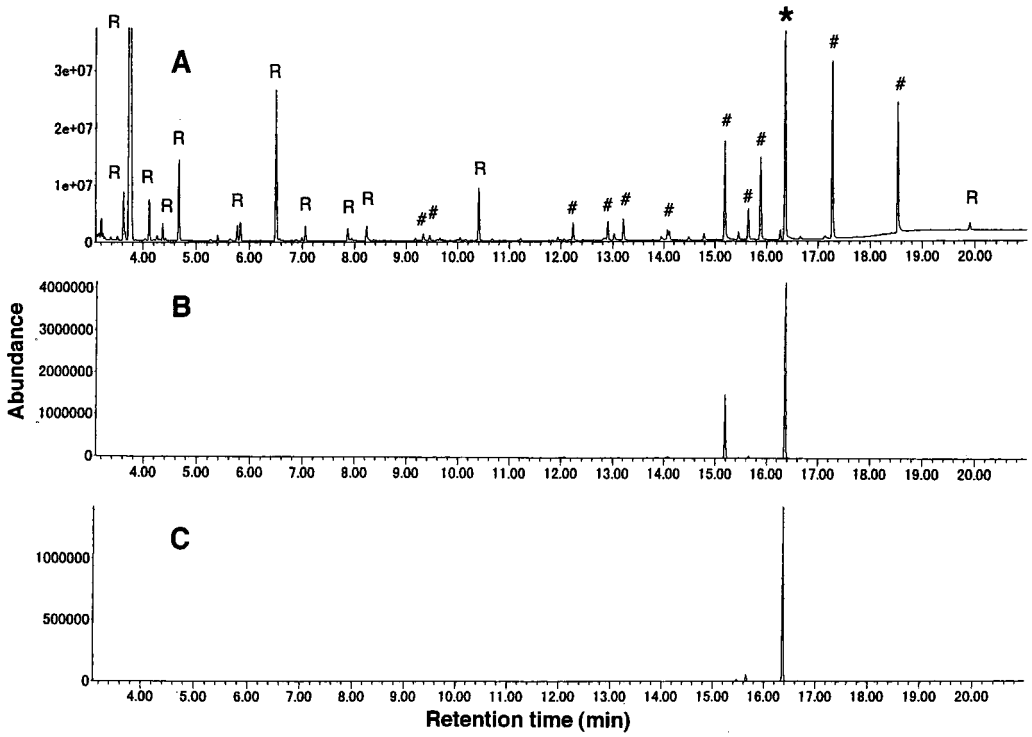

Figure 1. Gas chromatogram of the TBDMS derivative of *S*-methyl-L-cysteine sulfoxide (**1**): (A) total ion chromatogram; (B) extracted ion chromatogram at *m*/*z* 302; (C) extracted ion chromatogram at *m*/*z* 436. The TBDMS derivative of **1** (200 ng) was applied to GC-MS with electron impact ionization. The asterisk (∗) indicates a peak of tri(*tert*-butyldimethylsilyl) derivative of **1**, and number signs (#) are the other peaks derived from **1**. R indicates peaks derived from the derivatization reaction mixture.

GC conditions, the TBDMS derivative of **1** gave a major peak eluting at a retention time of 16.4 min. The EI mass spectrum of the peak (**Figure 2A**) gave an ion peak of *m*/*z* 302 as the base peak, and the characteristic ion peak of *m*/*z* 436, as well as a minor peak at *m*/*z* 478. The *m*/*z* 302 ion was assumed to be the ion corresponding to the molecule with the amino acid side chain eliminated, which is typical of TBDMS derivatives of many amino acids (*10*). The *m*/*z* 436 ion was assumed to be the des-*tert*-butyl form (M − 57), for which the molecular ion was the tri(*tert*-butyldimethylsilyl) derivative of **1**. The high-resolution mass spectrum was measured for the ion, giving the exact mass of *m*/*z* 436.2197. This value is consistent with the molecular composition $C_{18}H_{42}NO_3Si_3S$, the calculated mass of which is 436.2193. The methane CI mass spectrum of the TBDMS derivative of **1** (**Figure 2B**) gave ion peaks at *m*/*z* 522, 494, 478, and 436. They were assumed to be the C_2H_5 adduct ion (M + 29), the pseudomolecular ion (M + 1), the desmethyl molecular ion (M − 15), and the des-*tert*-butyl molecular ion (M − 57), which are commonly observed for TBDMS derivatives of amino acids under methane chemical ionization (*11*). Besides the peak of the tri(*tert*-butyldimethylsilyl) derivative of **1**, there were observed several peaks that were derived from **1** and not from the derivatization reagent (**Figure 1A**). They were not structurally elucidated. The heights of these peaks increased with the concentration of **1** in the derivatization reaction mixture. In the extracted ion chromatogram of *m*/*z* 302 under the EI conditions, no peak corresponding to a di-TBDMS derivative of *S*-methyl-L-cysteine sulfoxide was observed, which is commonly seen for the TBDMS derivatives of amino acids.

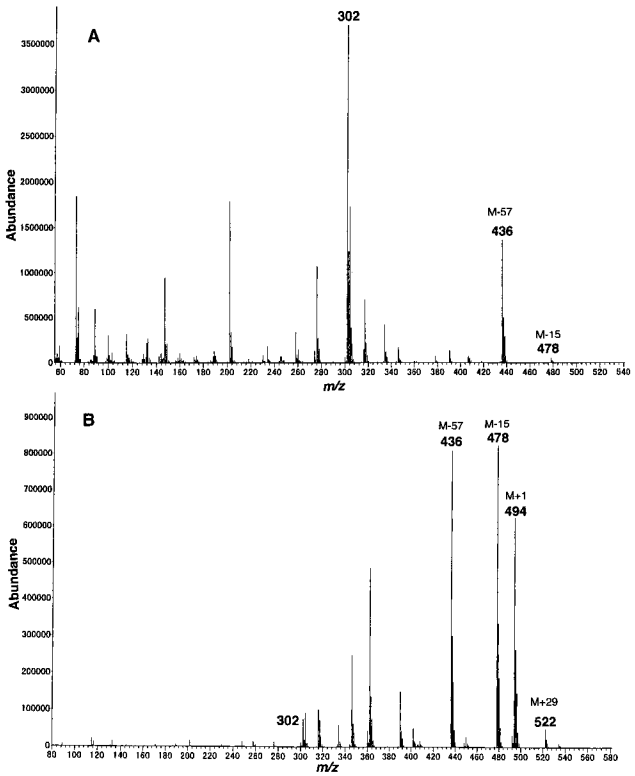

Figure 2. Mass spectra of the TBDMS derivative of *S*-methyl-L-cysteine sulfoxide (peak at 16.4 min in **Figure 1**): (A) mass spectrum under electron impact ionization; (B) mass spectrum under methane chemical ionization.

As shown in **Figure 3**, the TBDMS derivative of **2** gave a peak eluting at a retention time of 16.8 min. The EI mass spectrum of the peak (**Figure 4A**) gave an ion at *m*/*z* 302 as the base peak and a characteristic ion of *m*/*z* 464, along with a minor ion of *m*/*z* 506. The *m*/*z* 464 ion was assumed to be the des-*tert*-butyl form (M − 57), the molecular ion of which was tri-*tert*-butyldimethylsilylated **2**. The high-resolution mass spectrum was measured for the ion, giving *m*/*z* 464.2509. This value is consistent with the molecular composition $C_{20}H_{46}NO_3Si_3S$,

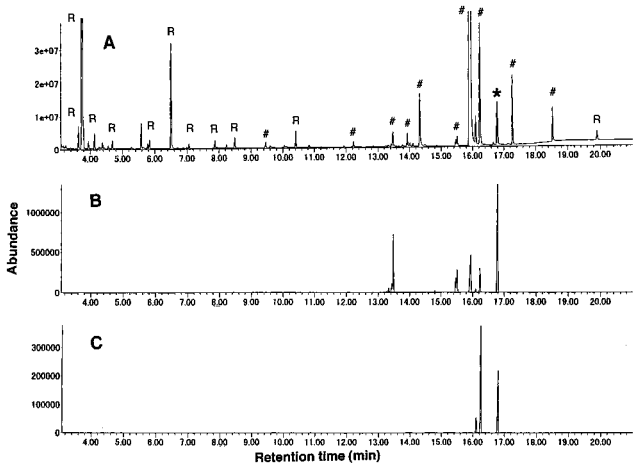

Figure 3. Gas chromatogram of the TBDMS derivative of *S*-propyl-L-cysteine sulfoxide (**2**): (A) total ion chromatogram; (B) extracted ion chromatogram at *m*/*z* 302; (C) extracted ion chromatogram at *m*/*z* 464. The TBDMS derivative of **2** (2 μg) was applied to GC-MS with electron impact ionization. The asterisk (∗) indicates a peak of tri(*tert*-butyldimethylsilyl) derivative of **2**, and number signs (#) are the other peaks derived from **2**. R indicates peaks derived from the derivatization reaction mixture.

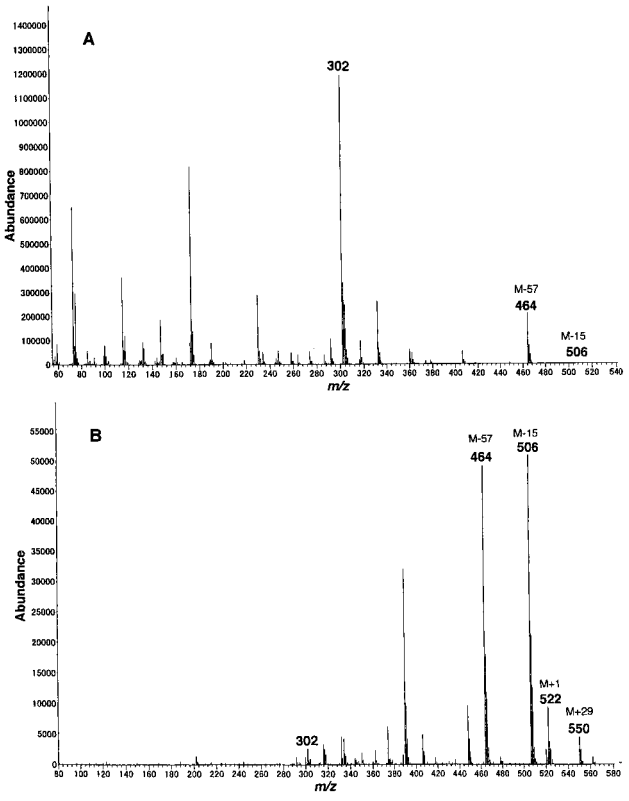

Figure 4. Mass spectra of the TBDMS derivative of *S*-propyl-L-cysteine sulfoxide (peak at 16.8 min in **Figure 3**): (A) mass spectrum under electron impact ionization; (B) mass spectrum under methane chemical ionization.

the calculated mass of which is 464.2506. The methane CI mass spectrum of TBDMS derivative of **2** (**Figure 4B**) gave *m*/*z* 550, 522, 506, and *m*/*z* 464, which were assumed to be the C_2H_5 adduct ion (M + 29), the pseudomolecular ion (M + H), the desmethyl molecular ion (M − 15), and the des-*tert*-butyl molecular ion (M − 57). Besides the peak of the tri(*tert*-butyldimethylsilyl) derivative of **2**, there were observed several peaks that were derived from **2** and not from the derivatization reagent (**Figure 3A**). They were not structurally elucidated. The heights of these peaks increased with the concentration of **2** in the derivatization reaction mixture. In the extracted ion chromatogram of *m*/*z* 302 under EI conditions, no peak corresponding to di-TBDMS-*S*-propyl-L-cysteine sulfoxide was observed.

Besides silylation at the amino and carboxyl functions as in the case of usual amino acids, one additional TBDMS group was introduced into **1** and **2**, probably at the sulfoxide, as shown by EI-MS, methane CI-MS, and high-resolution MS. Thus, we have experimentally confirmed that sulfoxide can be silylated. Dimethyl sulfoxide was derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide and identified by GC-MS (data not shown). In EI-MS, it gave fragment ions of *m*/*z* 105 (M − *tert*-butylmethyl − methyl), *m*/*z* 135 (M − *tert*-butyl), *m*/*z* 73 (trimethylsilyl), and *m*/*z* 177 (M − methyl). In the case of methane CI-MS, it gave fragment ions of *m*/*z* 135 (M − *tert*-butyl), *m*/*z* 177 (M − methyl), and *m*/*z* 191 (M − H). An active methyl hydrogen may be transferred to the sulfoxyl group, and the resultant thiol may be silylated with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide to form the silylated sulfoxide species. Then, the resulting silylated sulfoxide may rearrange probably via a $-S^+=CH$-type intermediate to form a silylated α-hydroxysulfide. This Pummerer-type rearrangement was also reported in the case of the TBDMS and other derivatives of thiodiglycol sulfoxide (*19*).

The peak areas on the SIM chromatograms at *m*/*z* 302 (TBDMS derivatives of both **1** and **2**), *m*/*z* 436 (TBDMS derivative of **1**), *m*/*z* 464 (TBDMS derivative of **2**), and *m*/*z* 178 (IS) were calculated. Anthracene (IS) was eluted at 11.4 min. The calibration curves of the ratios of the **1** peak areas monitored at *m*/*z* 302 and 436 to those at *m*/*z* 178 (IS) were linear for GC injections ranging from 0.6 to 20 ng for **1** with correlation coefficients >0.999. The calibration curves of the ratios of the **2** peak areas monitored at *m*/*z* 302 and 464 to those at *m*/*z* 178 (IS) were linear for GC injections ranging from 2.5 to 20 ng for **2** with correlation coefficients >0.999. The within-day repeatability was determined from five successive injections of the standards (5 ng). The relative standard deviations were 6.0% (monitored at *m*/*z* 302) and 5.5% (*m*/*z* 436) for **1** and 4.9% (*m*/*z* 302) and 7.5% (*m*/*z* 464) for **2**, respectively. The detection limits (per injection, signal/noise = 3) were 0.063 ng (monitored at *m*/*z* 302) and 0.28 ng (*m*/*z* 436) for **1** and 0.43 ng (*m*/*z* 302) and 1.84 ng (*m*/*z* 464) for **2**, respectively. It is possible to detect **1** at the sub-nanogram level. The detection limit for **1** was several times lower than that for **2**.

The TBDMS derivatization mixture of synthesized **1** and **2** gave not only the tri(*tert*-butyldimethylsilyl) derivatives but also several compounds appearing as peaks on GC, which were derived from the analyte sulfoxides (**Figures 1A** and **3A**). The structures of the peaks could not be elucidated. The derivatization reaction of *S*-alkyl-L-cysteine sulfoxides with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide may provide many byproducts besides the expected tri(*tert*-butyldimethylsilyl) derivatives. Especially, a large peak eluting at 16.0 min was observed in the TBDMS reaction mixture of **2** (**Figure 3A**). However, the product level of the tri(*tert*-butyldimethylsilyl) derivatives was proportional to the analyte concentration. The detection sensitivity for **1** was several times higher than that for **2**. As derived from the comparison of the peak area on the total ion chromatograms (**Figures 1** and **3**), the total ion amount of **1** derivative per injection also was >10-fold higher than that of **2**. The yield of TBDMS derivative appears to be higher for **1** than for **2**, and this difference in derivative yields can be attributed to the steric hindrance to the silylation reaction at

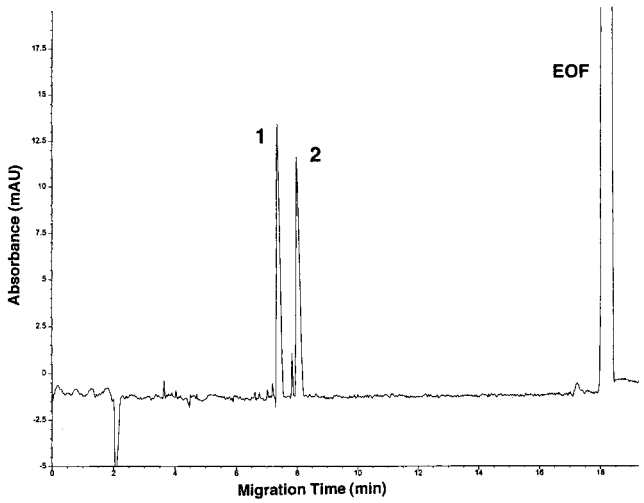

Figure 5. Electropherogram of *S*-methyl-L-cysteine sulfoxide (**1**) and *S*-propyl-L-cysteine sulfoxide (**2**) (each 1 mg/mL). Electroosmotic flow (EOF) appeared at 18 min.

the sulfoxide or stability of the produced tri(*tert*-butyldimethylsilyl) derivatives.

Efficiency of Solid Phase Extraction. The yield of TBDMS derivatization is influenced by sample matrix components. The solvent extract of onion consists of the complex matrix, which could interfere with the GC-MS analysis. The detection of the TBDMS peaks could be affected, and the yields of TBDMS derivatization would be suppressed. The majority of the components in the onion extract are probably carbohydrates. In this experiment, we adopted sucrose as a suspected interferent and developed a cleanup method using solid phase extraction, by which *S*-alk(en)yl-L-cysteine sulfoxides were isolated and sucrose was removed. A Sep-Pak C_{18} cartridge was used for the removal of hydrophobic interferents. Amino acids would not be retained by the cartridge and would be recovered in the eluted fraction. Bond Elut SCX (strong cation exchanger) was also used for the purification of *S*-alk(en)yl-L-cysteine sulfoxides. Noncationic compounds can be eluted, and the retained cationic compounds can then be eluted with ammonia. Ammonia was successfully removed by evaporation from the elution fraction. CE was used for the direct quantification of *S*-alkyl-L-cysteine sulfoxides in the solid phase extracts. **1** (migration time = 7.3 min) and **2** (8.0 min) could be separated with high resolution (**Figure 5**) and quantified in the presence of high levels of sucrose (15 min).

The solutions containing 5% sucrose and designated **1** and **2** were treated by solid phase extraction, and the starting and subsequent extracts were subject to CE and TBDMS GC-MS. As shown in **Table 1**, **1** and **2** derivatives were not detected by GC-MS in either the original sucrose solution or the Sep-Pak C_{18} pass-through fraction. Nearly all of the sucrose was removed by the combined solid phase extraction treatment. The recoveries of **1** and **2** in the final solid phase extract were ~90%. The GC-MS detection yields were ~75%.

Various matrix components would be expected to interfere with the TBDMS derivatization reaction. In our previous work on the TBDMS GC-MS analysis of methylphosphonic acids, a severe suppression of TBDMS derivatization was observed in the presence of divalent cations and sugars (*13*). Neutral sugars in particular may inhibit the TBDMS derivatization by serving as competitors in the silylation reaction. The carbohydrate concentration in onion extract may be high, and it is reasonable that *S*-alk(en)yl-L-cysteine sulfoxides in the onion extract might not have been detected by TBDMS GC-MS. The cleanup of *S*-alk(en)yl-L-cysteine sulfoxides, using the combination of reversed phase and cation exchange cartridges, resulted in an almost quantitative recovery from the simulated matrix (high level of sucrose) solution. The reason for the GC-MS detection yields of ~75% (**Table 1**) may be that the remaining sucrose in the final solid phase extract, even though low, might interfere with the TBDMS derivatization of **1** and **2** to some extent.

Table 1. Recoveries of *S*-Alkyl-L-cysteine Sulfoxides in Solid Phase Extraction from Sucrose Matrix[a]

	S-methyl-L-cysteine sulfoxide		*S*-propyl-L-cysteine sulfoxide	
fraction	CE	GC-MS	CE	GC-MS
starting	100	0	100	0
Sep-Pak C_{18}	99.5	0	100	0
Bond Elut SCX	90.5 ± 3.3	74.5 ± 4.7	99.0	74.4 ± 5.3

[a] *S*-Methyl-L-cysteine sulfoxide (**1**) and *S*-propyl-L-cysteine sulfoxide (**2**) were dissolved in 5% sucrose in 0.1 M HCl solution, and 1 mL of the mixture (starting fraction) was applied to the Sep-Pak C_{18} Plus cartridge. The cartridge was eluted with 0.1 M HCl. The combined fraction of the cartridge pass-through and the 0.1 N HCl elution (Sep-Pak C_{18} fraction) was applied to the Bond Elut SCX cartridge, washed with 4 mL of 0.1 M HCl followed by 6 mL of water. **1** and **2** were eluted with 4 mL of 500 mM ammonia water. The eluted fraction was dried by evaporation under reduced pressure (Bond Elut SCX fraction). Each fraction was examined by CE and GC-MS after TBDMS derivatization.

Determination of Alk(en)yl-L-cysteine Sulfoxides in Onion. The method developed was applied for the quantification of alk(en)yl-L-cysteine sulfoxides in commercially available onion. The extract (5%) of onion slices (10 g) was treated with the combined solid phase extraction of Sep-Pak C_{18} and Bond Elut SCX, and the solid phase extract was subjected to TBDMS derivatization and GC-MS analysis. Without any pretreatment, alk(en)yl-L-cysteine sulfoxides were not detected from the onion extract by TBDMS GC-MS analysis. As shown in **Figure 6C**, the TBDMS derivative of **1** was detected in the solid phase extract of the onion extract, which was eluted at 16.4 min, precisely the same as the authentic compound. The mass spectrum was identical to that of the authentic compound (data not shown). The TBDMS derivative of **2** was also detected (**Figure 6D**), which showed the same retention time (16.8 min) and mass spectrum as the authentic compound. **3** was assumed to be present in the onion extract, the peak for which appeared at the expected elution position (16.8 min) slightly earlier than the derivative of **2** (**Figure 7**). The structure of **3** could be inferred from its EI mass spectrum, which gave ions at *m*/*z* 504 (M − 15), 462 (M − 57), 360 (M − 159), and 302 (M − propenyl) (**Figure 8**). Although the issue of whether the *S*-alkenyl function of the detected **3** peak was 1-propenyl or 2-propenyl could not be ascertained mass spectrometrically, it is said that onion contains only the 1-propenyl compound (*2*).

Synthetic *S*-alk(en)yl-L-cysteine sulfoxides give (±)-*S*-diastereomers. These isomers can be separated on a reversed phase HPLC column after *o*-phthalaldehyde derivatization (*7*, *20*, *21*). However, the capillary DB-5 column failed to separate the TBDMS derivatives of the diastereomers clearly (**Figures 1** and **3**). The resolution of the TBDMS derivatives of **2** and **3** was also poor, and the two peaks almost coeluted (**Figure 6**), in contrast to the good resolution of *o*-phthalaldehyde derivatives of **2** and **3** on a reversed phase HPLC column (*20*). However, using mass spectrometric analysis, **2** and **3** could be separately identified and quantified by GC-MS.

The concentrations of *S*-alk(en)yl-L-cysteine sulfoxides in the onion sample were determined by measuring the peak areas of

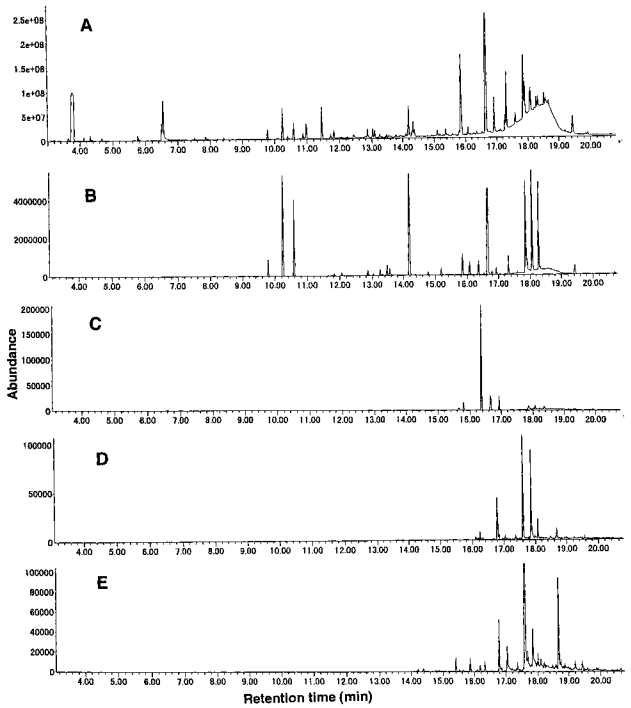

Figure 6. Gas chromatogram of the TBDMS reaction product of the solid phase extract of onion extract: (A) total ion chromatogram; (B) extracted ion chromatogram at *m*/*z* 302; (C) extracted ion chromatogram at *m*/*z* 436; (D) extracted ion chromatogram at *m*/*z* 464; (E) extracted ion chromatogram at *m*/*z* 462.

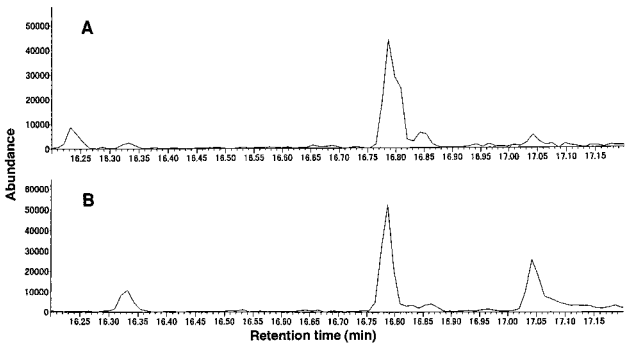

Figure 7. Extracted ion chromatograms at *m*/*z* 464 (A) and *m*/*z* 462 (B) of the TBMDS reaction product of the solid phase extract of an onion extract.

their TBDMS derivatives on SIM chromatograms at *m*/*z* 436 (**1**) and *m*/*z* 464 (**2**) and assuming that the recoveries in the solid phase extraction were 74.4 and 74.0%, respectively (**Table 1**). Although authentic compound **3** was not synthesized in this study, the concentration of **3** was estimated, under the supposition that the detectability of the M − 57 ion (SIM at *m*/*z* 462) and the solid phase extraction recovery are the same as those of **2**. The concentrations of **1**, **2**, and **3** in the onion sample were 0.3, 3.1, and 3.0 mg/g of fresh weight of onion.

The concentrations of **1**, **2**, and **3** were also determined by HPLC. From the onion extract, (−)-*S*-methyl-L-cysteine sulfoxide and (−)-*S*-propyl-L-cysteine sulfoxide were detected. In addition, the peak eluting at 14.0 min was observed, which was assumed to be (−)-*S*-propenyl-L-cysteine sulfoxide. We assumed that the *o*-phthalaldehyde derivatization efficiency and molar absorbency of (−)-*S*-propenyl-L-cysteine sulfoxide were the same as those of **2**. The concentrations of **1**, **2**, and **3** in the onion sample were 0.3, 2.7, and 1.8 mg/g of fresh weight of

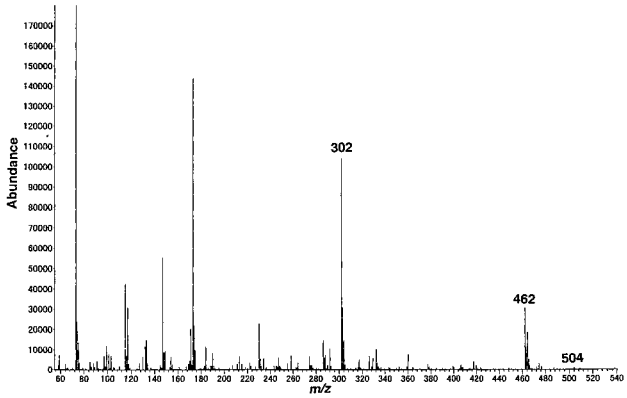

Figure 8. EI mass spectrum of the former part of the peak at 16.8 min shown in **Figure 6E**.

onion. The concentration of *S*-methyl-L-cysteine sulfoxide measured by GC-MS was almost equal to that by HPLC, and also the value of *S*-propyl-L-cysteine sulfoxide measured by GC-MS was similar to that by HPLC. However, the value of *S*-propenyl-L-cysteine sulfoxide measured by GC-MS was ∼70% higher than that by HPLC. The discrepancy may be ascribed to the situation that the detectability of **3** in TBDMS GC-MS and HPLC analyses was not examined.

Onion contains various types of *S*-alk(en)yl-L-cysteine sulfoxides. Thomas et al. (*8*) reported that the contents of **1** and *S*-1-propenyl-L-cysteine sulfoxide in white bulb onion were 23.6 and 131 mg/g, respectively, and that *S*-2-propenyl-L-cysteine sulfoxide and **2** were not detected. Lancaster et al. (*6*) reported that the contents of **1**, **2**, and *S*-1-propenyl-L-cysteine sulfoxide were 0.9, 2.9, and 0.6 mg/g, respectively. The levels of *S*-alk(en)yl-L-cysteine sulfoxides were variable during the growth of onion (*22*). Therefore, our estimated levels of *S*-alk(en)yl-L-cysteine sulfoxides in commercial onion appear to be reasonable.

The established GC-MS method is not complicated or time-consuming. It provides for the structural confirmation and detection of *S*-alk(en)yl-L-cysteine sulfoxides and its sensitive quantification, the detection limit (nanogram order) of which is compatible with that of the HPLC method (*8*). GC separation efficiency is superior to HPLC, providing detection of *S*-alk(en)yl-L-cysteine sulfoxides without interference by sample matrix components. MS offers specific detection, compared to ultraviolet−visible absorbance detection in HPLC and CE. Using the combined solid phase extraction cleanup pretreatment, this method has the potential for use in the areas of food and forensic sciences.

ABBREVIATIONS USED

CE, capillary electrophoresis; ESI, electrospray ionization; IS, internal standard; SIM, selected ion monitoring; TBDMS, *tert*-butyldimethylsilyl(ation); TOF, time-of-flight.

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