JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

A Simple Synthesis of Alliin and *allo*-Alliin: X-ray Diffraction Analysis and Determination of Their Absolute Configurations

Wataru Hakamata,*[,†] Ryosuke Koyama,[†] Mizuki Tanida,[†] Tomomi Haga,[†] Takako Hirano,[†] Makoto Akao,[†] Hitomi Kumagai,[†] and Toshiyuki Nishio[†]

[†]Department of Chemistry and Life Science, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa-shi, Kanagawa 252-0880, Japan

Supporting Information

ABSTRACT: A simple method for the isolation of the bioactive compound alliin from garlic, as well as a method for the synthesis of diastereomerically pure alliin and *allo*-alliin on a preparative laboratory scale, was developed. The absolute configuration of the sulfur atom in alliin and *allo*-alliin was assigned on the basis of enzyme reactivity, optical rotatory dispersion, and circular dichroism analyses. A comparison of the results from these analyses, in combination with an X-ray diffraction study on a protected *allo*-alliin derivative, revealed *S* and *R* configurations of the sulfur atoms in alliin and *allo*-alliin, respectively. In addition, the same ^{1}H NMR spectrum was observed for synthetic and natural alliin. The absolute configuration of natural alliin was assigned for the first time on the basis of the NMR spectrum and X-ray coordinates.

KEYWORDS: *absolute configuration, alliin, allo-alliin, diastereoselective, garlic, synthesis, X-ray crystallography*

INTRODUCTION

The beneficial dietary properties of *Allium* species have been known for centuries and extensively exploited in food, spices, and herbal remedies.[1−5] Their major component, the sulfur-containing amino acid alliin, can be converted into other biologically active compounds such as allicin and ajoene via the enzyme alliinase.[6−10] It seems that alliin as a glycoside precursor of allicin appeared for the first time in the literature in 1909,[11] but in 1948, another amino acid precursor of allicin was isolated from *Allium sativum* and *Allium ursinum* and also called alliin.[12] Alliin exhibits attractive biological activities, for example, an antioxidant activity, an affinity to *N*-methyl-D-aspartate receptors, an inhibitory effect on platelet aggregation, an antimicrobial activity, a modification the membrane fluidity of tumor cells, and an effect on human tumor cell proliferation.[13−19] However, to gain a deeper understanding of these biological properties, a close examination of the chemistry of alliin and its diastereomer *allo*-alliin, the presence of which in garlic was first reported in 2005,[20] is required. Even though the structures of alliin, **1**, and *allo*-alliin, **2**, are shown in Figure 1, the absolute configurations of alliin and *allo*-alliin remain so far uncertain.

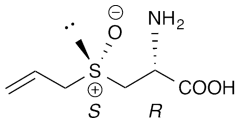
Since the late 19th century the active components of *Allium* species have attracted the attention of researchers, and many studies have been devoted to the understanding of their composition and biological activities.[21−24] In the 1940s, the presence of alliin in garlic and its role as a precursor in the alliinase-mediated formation of allicin was established.[12] In the same study, discrepancies between the optical rotation of natural and synthesized alliin were observed, which were attributed to a difference in the absolute configuration of cysteine derivatives **1**−**4** (Figure 1). Later, the same authors were the first to propose a relationship between the absolute configurations of **1**−**4** and the reactivity of alliinase.[24] However, the absolute configuration at the sulfur atom of the sulfoxide moiety could not be determined.

Other attempts to determine the absolute configuration of alliin unequivocally, for example, based on X-ray crystallographic analysis of structural analogues such as **5** or **6** (Figure 2),[25−27] or based on ORD and CD analyses of propyl derivatives of alliin such as **7** and **8**,[28−30] also failed to provide a definitive result. To the best of our best knowledge, no report of the absolute configuration of the sulfur atom in alliin has appeared in the literature since 1971. Accordingly, the absolute configuration of alliin has so far been deduced only on the basis of the reactivity of alliin toward alliinase and its ORD and CD curves compared to those of **5**. Hence, an unambiguous determination of the absolute configuration of the sulfur atom of alliin and *allo*-alliin for further chemical and biological studies remains to be carried out.

Moreover, the preparation of diastereomerically pure alliin and *allo*-alliin also remains challenging. Previous reported attempts to obtain diastereomerically pure alliin and *allo*-alliin by recrystallization failed to achieve a satisfactory diastereomeric separation of *allo*-alliin.[23,31,32] However, the diastereoselective synthesis of alliin via asymmetric oxidation of the sulfur atom using $[Ti(O\text{-}iPr)_4]$,[33] and the enzymatic syntheses of alliin via asymmetric oxidation of the sulfur atom using Fe(II)/α-ketoglutarate-dependent dioxygenase from *Bacillus thuringiensis* and dried cell powder of α-ketoglutarate-dependent dioxygenase-expressing *Escherichia coli* were reported.[34] Nevertheless, the diastereomeric excess of alliin did not exceed 83% in any of the cases and thus remains to be improved.

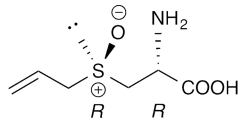
Received: August 21, 2015
Revised: December 3, 2015
Accepted: December 5, 2015
Published: December 5, 2015

DOI: 10.1021/acs.jafc.5b05501
J. Agric. Food Chem. 2015, 63, 10778−10784



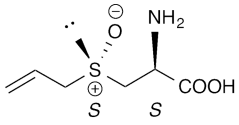
1: alliin, (*Rc,Ss*)-*S*-allyl-L-cysteine sulfoxide

Natural: major component



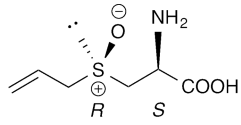
2: *allo*-alliin, (*Rc,Rs*)-*S*-allyl-L-cysteine sulfoxide

Natural: minor component



3: (*Sc,Ss*)-*S*-allyl-D-cysteine sulfoxide

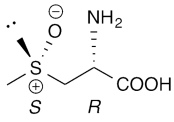
Synthetic



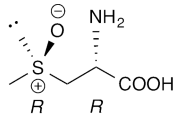
4: (*Sc,Rs*)-*S*-allyl-D-cysteine sulfoxide

Synthetic

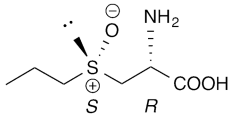
Figure 1. Structures of the *S*-allyl-cysteine sulfoxide stereoisomers.



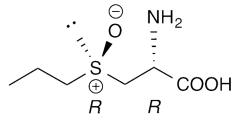
5: (+)-(*Rc,Ss*)-*S*-methyl-L-cysteine sulfoxide



6: (-)-(*Rc,Rs*)-*S*-methyl-L-cysteine sulfoxide



7: (*Rc,Ss*)-*S*-propyl-L-cysteine sulfoxide



8: (*Rc,Rs*)-*S*-propyl-L-cysteine sulfoxide

Figure 2. Structures of the *S*-methyl-L-cysteine sulfoxide and *S*-propyl-L-cysteine sulfoxide diastereoisomers.

In this study, we developed a simple method for the isolation of pure alliin from garlic and a synthetic method for the preparation of diastereomerically pure alliin and *allo*-alliin and also determined the absolute configuration of the sulfur atoms in alliin and *allo*-alliin based on NMR spectroscopy and X-ray diffraction analysis.

MATERIALS AND METHODS

Synthesis of Alliin Derivatives. All new compounds were characterized by ^{1}H NMR, ^{1}H−^{1}H COSY, HMQC, and ^{13}C NMR spectrometry, mass spectrometry, and elemental analysis. The NMR spectra were recorded on an ECA500 spectrometer (JEOL, Tokyo, Japan) (500 MHz for ^{1}H and 125 MHz for ^{13}C). Chemical shifts are expressed in parts per million relative to Me_4Si (0 ppm). Low-resolution mass spectra were obtained on a Quattro premier XE instrument under positive and negative ion ESI conditions (Waters, Milford, MA, USA). The single-crystal structure analysis was performed using X-ray diffraction on a D8 Venture diffractometer (Bruker AXS, Madison, WI, USA). Melting points were determined on an MP-21 capillary apparatus (Yamato Scientific, Tokyo, Japan) and are uncorrected. Optical rotation was measured on a P-1020 digital polarimeter (JASCO, Tokyo, Japan). Column chromatography was carried out using silica gel 60N (spherical neutral, particle size = 100−210 μm) (Kanto Chemical, Tokyo, Japan). The progress of all reactions was monitored by TLC on silica gel 60 F_{254} (0.25 mm) (Merck Millipore, Darmstadt, Germany).

Synthesis of *S*-Allyl-L-cysteine Methyl Ester, 9. L-Cysteine methyl ester hydrochloride (1.0 g, 5.83 mmol) (TCI Japan, Tokyo, Japan) was dissolved in EtOH/H_2O (2:1, v/v, 10 mL), before triethylamine (1.3 mL, 17.5 mmol) and allyl bromide (0.9 mL, 5.83 mmol) were added. After for 20 h of stirring, the solvent was removed under reduced pressure, and the obtained residue was purified by column chromatography on silica gel (MeOH/2-propanol, 1:2, v/v) to afford 0.73 g (71.5%) of *S*-allyl-L-cysteine methyl ester, **9**.

Synthesis and Separation of Alliin Methyl Ester, 10, and *allo*-Alliin Methyl Ester, 11. A solution of *S*-allyl-L-cysteine methyl ester, **9** (501.2 mg, 2.85 mmol), in MeCN (5 mL) was treated with a 30% w/w solution of H_2O_2 in water (450 μL). After 26 h of stirring at room temperature, 30 drops of a saturated aqueous solution of sodium thiosulfate were added. A hydrogen peroxide concentration in the mixture of <0.5 mg/mL was established by using a Quantofix peroxide 25 test stick (Sigma-Aldrich, St. Louis, MO, USA). Subsequently, all solvents were removed to afford a residue containing 294.4 mg of a mixture of alliin methyl ester, **9**, and *allo*-alliin methyl ester, **10**, which were separated by column chromatography on silica gel (MeOH/2-propanol, 1:2, v/v). After three purification cycles under these conditions, 107.9 mg (32.4%) of *allo*-alliin methyl ester, **10**, and 162.6 mg (21.5%) of alliin methyl ester, **9**, were obtained with a good purity (>99%).

TLC Analysis of the Diastereomer Mixture of Alliin Methyl Ester, 10, and *allo*-Alliin Methyl Ester, 11. The diastereomeric mixture was analyzed by TLC on silica gel using MeOH/2-propanol (1:2, v/v), followed by spray-staining with a 2% ninhydrin solution (Ninhydrin Spray II) (Wako Pure Chemical Industries, Tokyo, Japan). The diastereomeric mixture displayed two spots that represent *allo*-alliin methyl ester, **10** (R_f = 0.55), and alliin methyl ester, **11** (R_f = 0.47). The mixture of diastereomers was also analyzed by TLC on

DOI: 10.1021/acs.jafc.5b05501
J. Agric. Food Chem. 2015, 63, 10778−10784

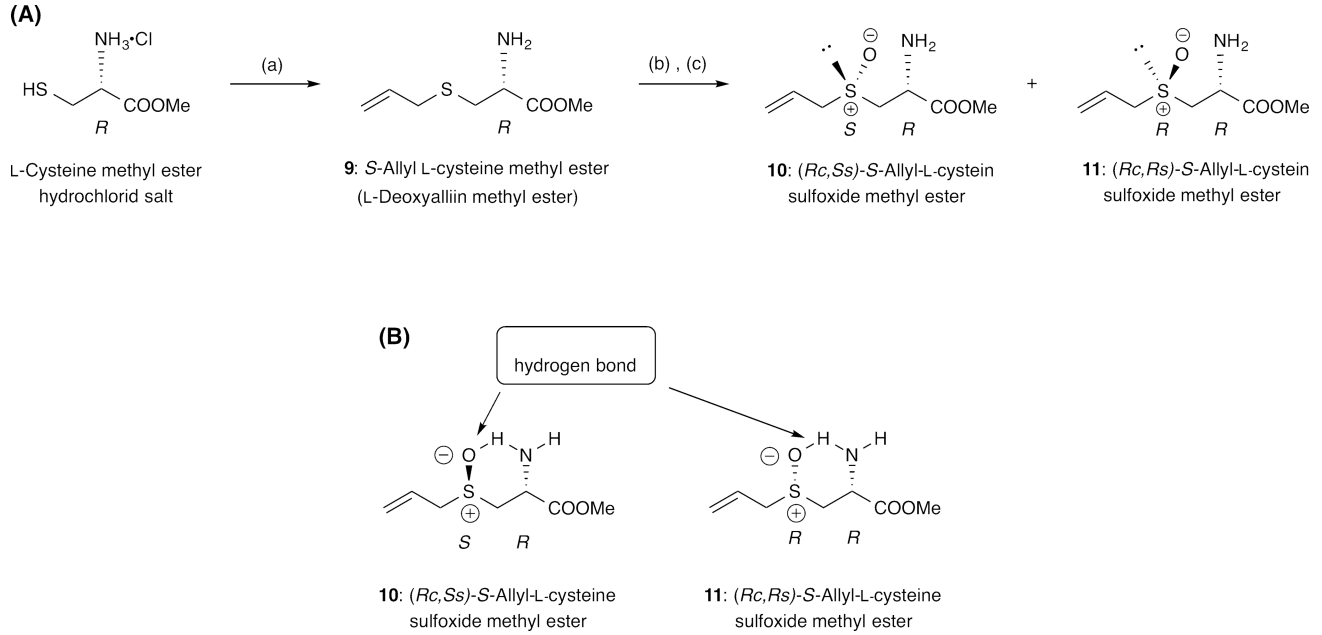


Figure 3. (A) Synthetic route to **10** and **11**: (a) allyl bromide, EtOH/H_2O (2:1, v/v), 71.5%; (b) 30% H_2O_2, MeCN, 54.0%. (B) Formation of intramolecular hydrogen bonds in **10** and **11**.

silica gel using two other solvent systems, (A) CH_2Cl_2/MeOH/25% aqueous NH_4OH (7:1:0.1) and (B) EtOH/2-propanol/H_2O (1:2:0.5), followed by spray-staining with a 2% ninhydrin solution. Both solvent systems exhibited two isomer spots (A, R_f = 0.53/0.47; B, R_f = 0.50/0.45).

Alliin Methyl Ester, 9: ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.05 (s, 2H, −N$\underline{H}_2$), 2.80 (t, 1H, J = 10.5 Hz, −C$\underline{H}_2$CH(NH$_2$)−), 2.91 (dd, 1H, J = 3.5 Hz, J = 13.0 Hz, −C$\underline{H}_2$CH(NH$_2$)−), 3.45 (dd, 1H, J = 8.0 Hz, J = 13.0 Hz, −C$\underline{H}_2$CH═CH$_2$), 3.62 (dd, 1H, J = 7.0 Hz, J = 13.0 Hz, −C$\underline{H}_2$CH═CH$_2$), 3.66 (s, 3H, −COOC$\underline{H}_3$), 3.67 (dd, 1H, J = 2.5 Hz, J = 10.5 Hz, −C$\underline{H}$(NH$_2$)−), 5.35 (dd, 1H, J = 2.0 Hz, J = 9.5 Hz, −CH═C$\underline{H}_2$), 5.37 (d, 1H, J = 1.0 Hz, −CH═C$\underline{H}_2$), 5.87 (dddd, 1H, J = 7.5 Hz, J = 10.0 Hz, J = 14.5 Hz, −C$\underline{H}$═CH$_2$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 49.15 (−$\underline{C}$H—(NH$_2$)−), 51.88 (−O—$\underline{C}$H$_3$), 55.07 (−$\underline{C}$H$_2$CH(NH$_2$)− and −$\underline{C}$H$_2$CH═CH$_2$), 122.63 (−CH═$\underline{C}$H$_2$), 127.38 (−$\underline{C}$H═CH$_2$), 174.85 (−$\underline{C}$OOCH$_3$); ESI-MS (positive) m/z 192 [M + H]$^+$. Anal. Calcd for $C_7H_{13}NO_3S$: C, 43.96; H, 6.85; N, 7.32. Found: C, 43.94; H, 6.61; N, 7.10. $[\alpha]_D^{23}$ −50.3° (c 1.0, MeOH).

***allo*-Alliin Methyl Ester, 10:** ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.10 (s, 2H, −N$\underline{H}_2$), 2.87 (dd, 1H, J = 7.0 Hz, J = 13.5 Hz, −C$\underline{H}_2$CH(NH$_2$)−), 3.06 (dd, 1H, J = 6.5 Hz, J = 13.5 Hz, −C$\underline{H}_2$CH(NH$_2$)−), 3.51 (dd, 1H, J = 8.0 Hz, J = 13.0 Hz, −C$\underline{H}_2$CH═CH$_2$), 3.64 (s, 3H, −COOC$\underline{H}_3$), 3.69 (dd, 1H, J = 7.5 Hz, J = 13.5 Hz, −C$\underline{H}_2$CH═CH$_2$), 3.82 (t, 1H, J = 7.0 Hz, −C$\underline{H}$(NH$_2$)−), 5.36 (dd, 1H, J = 2.5 Hz, J = 10.0 Hz, −CH═C$\underline{H}_2$), 5.39 (d, 1H, J = 1.0 Hz, −CH═C$\underline{H}_2$), 5.87 (dddd, 1H, J = 7.0 Hz, J = 10.0 Hz, J = 14.5 Hz, −C$\underline{H}$═CH$_2$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 49.30 (−$\underline{C}$H—(NH$_2$)−), 51.76 (−O—$\underline{C}$H$_3$), 54.19 (−$\underline{C}$H$_2$CH-(NH$_2$)−), 54.72 (−$\underline{C}$H$_2$CH═CH$_2$), 122.72 (−CH═$\underline{C}$H$_2$), 127.28 (−$\underline{C}$H═CH$_2$), 174.00 (−$\underline{C}$OOCH$_3$); ESI-MS (positive) m/z 214 [M + Na]$^+$. Anal. Calcd for $C_7H_{13}NO_3S$: C, 43.96; H, 6.85; N, 7.32. Found: C, 43.95; H, 6.80; N, 6.97. $[\alpha]_D^{23}$ +52.9° (c 1.0, MeOH).

Synthesis of *N*-(*tert*-Butoxycarbonyl) Alliin Methyl Ester, 12. A solution of alliin methyl ester, **9** (348.0 mg, 1.8 mmol), in DMF (5 mL) was treated with di-*tert*-butyl dicarbonate (836.2 mg, 3.6 mmol). After 24 h of stirring, the solvent was removed in a centrifugal evaporator at room temperature for 30 min. The obtained residue was purified by column chromatography on silica gel (hexane/EtOAc, 1:3, v/v) to afford 169.5 mg (31.9%) of *N*-(*tert*-butoxycarbonyl) alliin methyl ester **12**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.45 (s, 9H, -C(C$\underline{H}_3$)$_3$), 3.18 (dd, 1H, J = 5.5 Hz, J = 13.5 Hz, −S(O)—C$\underline{H}_2$−), 3.35 (dd, 1H, J = 5.5 Hz, J = 13.5 Hz, −S(O)—C$\underline{H}_2$−), 3.49 (dd, 1H, J = 7.5 Hz, J = 13.0 Hz, −C$\underline{H}_2$CH═CH$_2$), 3.61 (dd, 1H, J = 7.5 Hz, J = 13.0 Hz, −C$\underline{H}_2$CH═CH$_2$), 3.80 (s, 3H, −COOC$\underline{H}_3$), 4.66 (dd, 1H, J = 7.5 Hz, J = 11.0 Hz, −C$\underline{H}$COOCH$_3$), 5.42 (dd, 1H, J = 1.0 Hz, J = 17.0 Hz, −CH═C$\underline{H}_2$), 5.47 (d, 1H, J = 10.0 Hz, −CH═C$\underline{H}_2$), 5.61 (d, 1H, J = 7.0 Hz, −CON$\underline{H}$−), 5.90 (dddd, 1H, J = 7.5 Hz, J = 10.5 Hz, J = 15.0 Hz, −C$\underline{H}$═CH$_2$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 28.25 (−O—$\underline{C}$—(CH$_3$)$_3$), 49.71 (−$\underline{C}$H(NH)−), 52.82 (−CH(NHBoc)—$\underline{C}$H$_2$−), 53.06 (−O—$\underline{C}$H$_3$), 56.12 (−$\underline{C}$H$_2$—CH═CH$_2$), 80.64 (−O—$\underline{C}$—(CH$_3$)$_3$), 124.08 (−CH═$\underline{C}$H$_2$), 125.29 (−$\underline{C}$H═CH$_2$), 155.15 (−$\underline{C}$ONH−), 170.51 (−$\underline{C}$OOCH$_3$); ESI-MS (positive) m/z 314 [M + Na]$^+$. Anal. Calcd for $C_{12}H_{21}NO_5S$: C, 49.47; H, 7.27; N, 4.81; S, 11.00. Found: C, 49.19; H, 7.16; N, 4.81; S, 10.69. $[\alpha]_D^{23}$ +57.1° (c 1.0, CHCl$_3$).

Synthesis of *N*-(*tert*-Butoxycarbonyl) *allo*-Alliin Methyl Ester, 13. A solution of alliin methyl ester, **10** (206.2 mg, 1.1 mmol), in DMF (5 mL) was treated with di-*tert*-butyl dicarbonate (495.4 mg, 2.2 mmol). After 24 h of stirring, the solvent was removed in a centrifugal evaporator at room temperature for 30 min. The obtained residue was purified by column chromatography on silica gel (hexane/EtOAc, 1:3, v/v) to afford 218.5 mg (70.0%) of *N*-(*tert*-butoxycarbonyl) *allo*-alliin methyl ester, **13**. Compound **13** was recrystallized from a hexane/EtOAc solution to afford a single crystal suitable for X-ray crystallography: ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.45 (s, 9H, −C(C$\underline{H}_3$)$_3$), 3.15 (dd, 1H, J = 4.0 Hz, J = 13.0 Hz, −S(O)—C$\underline{H}_2$−), 3.24 (dd, 1H, J = 8.0 Hz, J = 13.0 Hz, −S(O)—C$\underline{H}_2$−), 3.49 (dd, 1H, J = 7.5 Hz, J = 13.0 Hz, −C$\underline{H}_2$CH═CH$_2$), 3.59 (dd, 1H, J = 7.5 Hz, J = 13.0 Hz, −C$\underline{H}_2$CH═CH$_2$), 3.80 (s, 3H, −COOC$\underline{H}_3$), 4.72 (dd, 1H, J = 7.5 Hz, J = 11.0 Hz, −C$\underline{H}$COOCH$_3$), 5.42 (dd, 1H, J = 1.0 Hz, J = 17.0 Hz, −CH═C$\underline{H}_2$), 5.48 (d, 1H, J = 10.0 Hz, −CH═C$\underline{H}_2$), 5.73 (d, 1H, J = 7.0 Hz, −CON$\underline{H}$−), 5.89 (dddd, 1H, J = 7.5 Hz, J = 10.5 Hz, J = 15.0 Hz, −C$\underline{H}$═CH$_2$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 28.25 (−O—C—($\underline{C}$H$_3$)$_3$), 50.19 (−$\underline{C}$H(NH)−), 51.79 (−CH(NHBoc)—$\underline{C}$H$_2$−), 52.94 (−O—$\underline{C}$H$_3$), 56.61 (−$\underline{C}$H$_2$−CH═CH$_2$), 80.51 (−O—$\underline{C}$—(CH$_3$)$_3$), 124.28 (−CH═$\underline{C}$H$_2$), 125.12 (−$\underline{C}$H═CH$_2$), 155.31 (−$\underline{C}$ONH−), 170.86 (−$\underline{C}$OOCH$_3$); ESI-MS (positive) m/z 314 [M + Na]$^+$. Anal. Calcd for $C_{12}H_{21}NO_5S$: C, 49.47; H, 7.27; N, 4.81; S, 11.00. Found: C, 49.27; H, 7.19; N, 4.82; S, 10.72. $[\alpha]_D^{23}$ −101.1° (c 1.0, CHCl$_3$). mp, 104.0−104.8 °C.

X-ray Diffraction Study of *N*-(*tert*-Butoxycarbonyl) *allo*-Alliin Methyl Ester, 13. Colorless pieces of **13** were mounted on a quartz fiber. Cell dimensions and intensities were measured using a D8

DOI: 10.1021/acs.jafc.5b05501
J. Agric. Food Chem. 2015, 63, 10778−10784

Venture diffractometer using Cu Kα radiation (λ = 1.5418 Å). The structure was resolved with X-Presso and APEX2. Crystallographic data in CIF format are in the Supporting Information.[35]

Synthesis of Alliin, 1. Sodium methoxide (28% methanolic solution, 50 μL) was added to a solution of alliin methyl ester, **9** (99.6 mg, 0.52 mmol) in MeOH/H_2O (4:1, v/v, 10 mL) cooled to −20 °C. After 2 h of stirring at −20 °C, dry Dowex 50W-X8, 200−400 mesh (H^+ form, 100 mg), was added, before the resin was removed by filtration and the solvent was evaporated. The obtained residue was purified by column chromatography on silica gel (12:3:1:0.5:0.5, CH_2Cl_2/EtOH/MeOH/H_2O/CF_3COOH) to afford 26.1 mg (28.2%) of alliin, **1**.

Synthesis of *allo*-Alliin, 2. Sodium methoxide (28% methanolic solution, 50 μL) was added to a solution of *allo*-alliin methyl ester, **10** (103.8 mg, 0.59 mmol) in MeOH/H_2O (4:1, v/v, 10 mL) cooled to −20 °C. After stirring at −20 °C for 2 h, dry Dowex 50W-X8, 200−400 mesh (H^+ form, 100 mg), was added, before the resin was removed by filtration and the solvent evaporated. The obtained residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOH/MeOH/H_2O/CF_3COOH, 12:3:1:0.5:0.5) to afford 15.2 mg (16.4%) of *allo*-alliin, **2**.

Preparation of Garlic Lyophilization Powder. Peeled fresh garlic was heated using a 500 W microwave oven (30 min/3 g). The thus obtained garlic was homogenized with the corresponding weight of water, before the homogenate was filtered using four layers of gauze. The obtained solution was centrifuged at 15000*g* for 20 min at 4 °C. The resulting supernatant was lyophilized.

Extraction and Purification of Alliin from Garlic Lyophilization Powder. Garlic lyophilization powder (1.0 g) was ground in a mortar and placed in an ice bath before CH_2Cl_2/EtOH/MeOH/H_2O (12:3:1:0.5, v/v, 10 mL) was added according to a modified reported method.[36] After 30 min of extraction, the precipitate was removed by filtration and the solvent was evaporated to afford 30.9 mg of a crude residue, which was purified by column chromatography on silica gel (CH_2Cl_2/EtOH/MeOH/H_2O/CF_3COOH, 12:3:1:0.5:0.5, v/v) to afford 14.7 mg of alliin. On the basis of a comparison of NMR measurements, this method provided diastereomerically pure alliin.

RESULTS AND DISCUSSION

Synthesis of Diastereomerically Pure Alliin Derivatives. To determine the absolute configuration of the sulfur atom in alliin, **1**, and *allo*-alliin, **2**, the availability of diastereomerically pure samples of **1** and **2**, suitable for single-crystal X-ray diffraction analysis, is essential. As protected amino acid derivatives generally crystallize easily, we focused on the synthesis of diastereomerically pure protected derivatives of **1** and **2**. Herein, we report a strategy for the preparation of sulfoxide derivatives **10** and **11** that were obtained from a diastereoselective oxidation with H_2O_2, which pre-empts the need for further HPLC purification. An L-cysteine methyl ester hydrochloride was chosen as starting material for the syntheses of compounds **10** and **11**. Treatment of this starting material with triethylamine and allyl bromide in EtOH/H_2O yielded *S*-allyl compound **9**. The oxidation of the sulfur atom in **9** using a 30% H_2O_2 solution in MeCN furnished a diastereomeric mixture of sulfoxides **10** and **11**. After separation by column chromatography on silica gel (MeOH/2-propanol, 1:2), **10** and **11** were obtained in pure form (Figure 3A). The easy separation of these diastereomers by conventional column chromatography on silica gel is probably facilitated by the formation of an intramolecular hydrogen bond between the hydrogen atoms of the amino groups and the oxygen atoms of the sulfoxide moieties, which may induce a significant polarity difference between conformers **10** and **11** (Figure 3B). Thus, simple access to **10** and **11** is provided, which is especially important for biological assays.

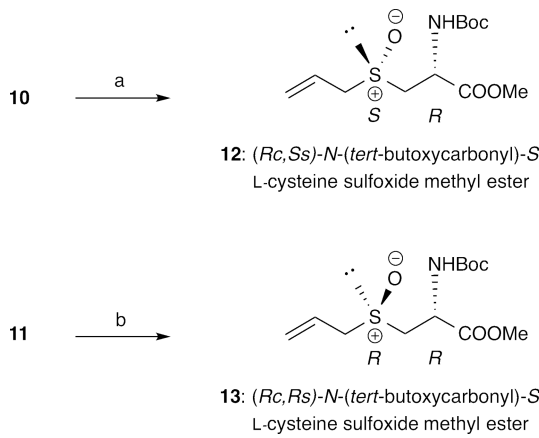


Figure 4. Synthesis of *N*-Boc derivatives **12** and **13**: (a) Boc_2O, DMF, 31.9%; (b) Boc_2O, DMF, 70.0%.

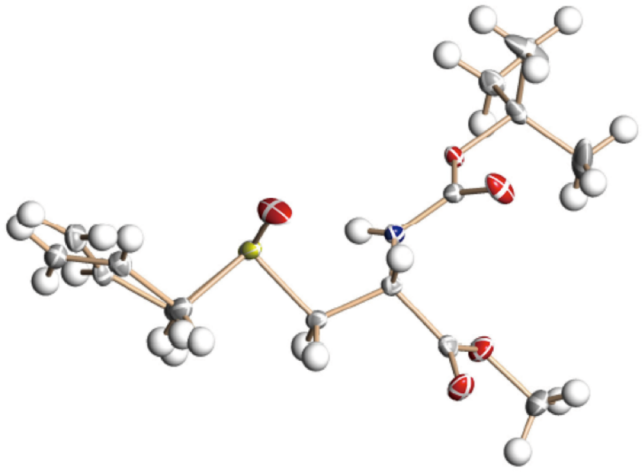


Figure 5. Molecular structure of **13** (thermal ellipsoids set at 50% probability; red, oxygen; yellow, sulfur; blue, nitrogen).

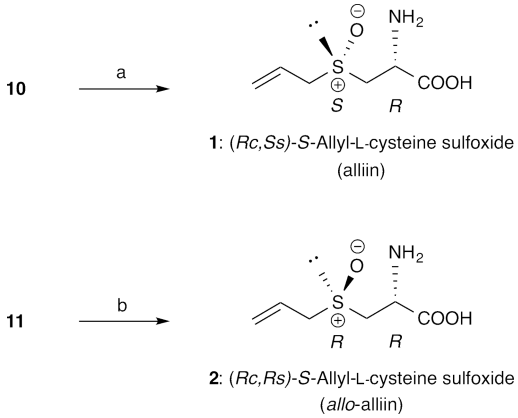


Figure 6. Syntheses of **1** and **2**: (a) sodium methoxide, MeOH, 28.2%; (b) sodium methoxide, MeOH, 16.4%.

Single-Crystal X-ray Structure of Protected *allo*-Alliin, 11. To determine the absolute configurations of the sulfur atoms in **10** and **11**, we decided to prepare the corresponding protected derivatives to obtain crystals suitable for X-ray diffraction studies. Accordingly, the amino groups of **10** and **11** were protected using Boc_2O to afford their *N*-Boc derivatives,

DOI: 10.1021/acs.jafc.5b05501
J. Agric. Food Chem. 2015, 63, 10778−10784

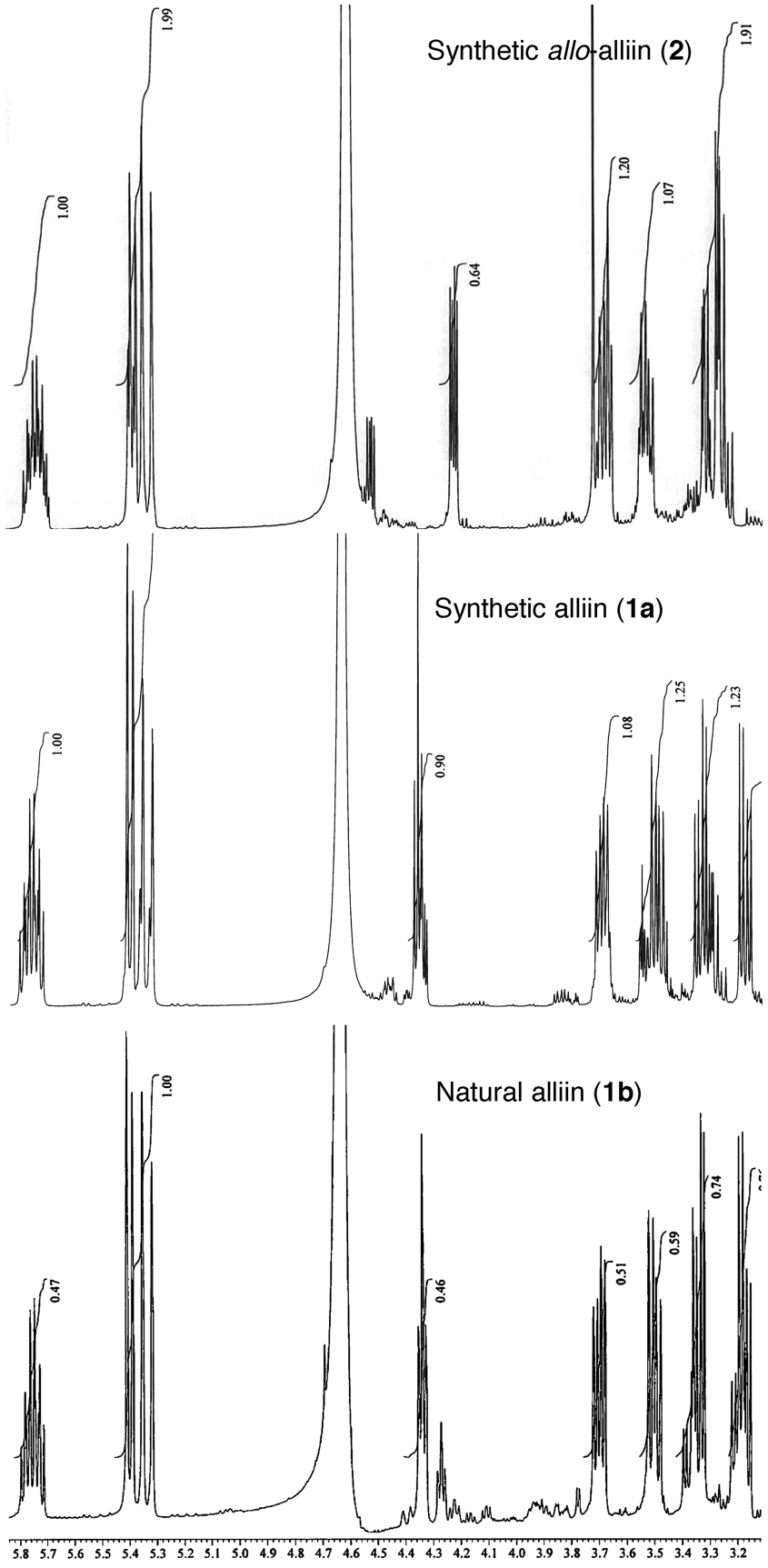


Figure 7. Comparison of the amino acid region in the ^{1}H NMR spectra of synthetic *allo*-alliin (**2**), synthetic alliin (**1a**), and natural alliin (**1b**).

that is, (*Rc*,*Ss*)-*N*-(*tert*-butoxycarbonyl)-*S*-allyl-L-cysteine sulfoxide methyl ester, **12**, and (*Rc*,*Rs*)-*N*-(*tert*-butoxycarbonyl)-*S*-allyl-L-cysteine sulfoxide methyl ester, **13**, respectively (Figure 4). After recrystallization from hexane/EtOAc, colorless needles

DOI: 10.1021/acs.jafc.5b05501
J. Agric. Food Chem. 2015, 63, 10778−10784

of **13** were obtained. On the basis of a single-crystal X-ray diffraction analysis, the absolute configuration of the sulfur atom and the α-carbon atom of **13** were determined as *R* and *R*, respectively; the molecular structure of **13**, which has the empirical formula $C_{12}H_{21}NO_5S$, is shown in Figure 5. It can thus be concluded that the absolute configurations of the sulfur and α-carbon atoms of **11** are also *R* and *R*, respectively. Consequently, the absolute configuration of the sulfur and α-carbon atoms in **10** and **12** should be *S* and *R*, respectively.

Absolute Configuration of Natural Alliin. Having determined the absolute configurations of **10** and **11**, we subsequently focused on the elucidation of the absolute configuration of natural alliin obtained from garlic. Diastereomerically pure alliin and *allo*-alliin were synthesized from **10** and **11**, respectively, and deprotection of **10** and **11** was achieved with sodium methoxide in methanol (Figure 6). Subsequently, garlic-derived alliin was extracted from the garlic lyophilization powder and purified by column chromatography on silica gel. An NMR analysis of synthetic alliin from **10**, synthetic *allo*-alliin from **11**, and garlic-derived alliin, was carried out to determine the absolute configuration of natural alliin by comparison. As shown in Figure 7, the ^{1}H NMR spectrum of garlic-derived alliin is identical to that of synthetic alliin. From this result, it can be deduced that the absolute configurations of the sulfur and α-carbon atom of garlic-derived alliin are *S* and *R*, respectively. This is consistent with the configuration of alliin reported by Barnsley in 1968,[28] which was based on enzyme reactivity, as well as ORD and CD curves.

Although the absolute configuration of garlic-derived alliin has been the subject of research for many years, the focus of these studies was mainly on its reactivity toward alliinase and its ORD spectra. However, to better understand its biological activity, a thorough investigation of the absolute configuration was required. Therefore, we carefully determined the absolute configuration of alliin as (*Rc*,*Ss*)-*S*-allyl-L-cysteine sulfoxide and that of *allo*-alliin as (*Rc*,*Rs*)-*S*-allyl-L-cysteine sulfoxide in this study.

Moreover, a simple method for the preparation of diastereomerically pure alliin and *allo*-alliin on a laboratory scale is provided herein; we believe that this may be useful for the evaluation of the biological activity of alliin and *allo*-alliin. In addition, an easy and practical method for the isolation of diastereomerically pure alliin from garlic is described.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b05501.

Crystallographic data of compound **13** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*(W.H.) Phone: +81-466-84-3960. Fax: +81-466-84-3960. E-mail: hakamata.wataru@nihon-u.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We express our sincere gratitude to the General Research Institute of the College of Bioresource Sciences of Nihon University for NMR and MS measurements. We also gratefully acknowledge A. Sato of A-Rabbit-Science Japan Co., Ltd., for carrying out the elemental analyses, as well as Bruker AXS Japan Co., Ltd., for carrying out the X-ray crystallographic studies.

■ REFERENCES

(1) Block, E. *Garlic and Other Alliums: The Lore and The Science*; Royal Society of Chemistry: Cambridge, UK, 2010.

(2) Koch, H. P., Lawson, L. D., Eds. *Garlic: The Science and Therapeutic Applications of Allium sativum L. and Related Species*; Williams and Wilkins: Baltimore, MD, USA, 1996.

(3) Edwards, S. E.; Williamson, I. C. R. E. M.; Heinrich, M. Garlic. In *Phytopharmacy: An Evidence-Based Guide to Herbal Medicinal Products*, 1st ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2015; pp158−160.

(4) Block, E. Fifty years of smelling sulfur. *J. Sulfur Chem.* **2013**, *34*, 158−207.

(5) Block, E. The chemistry of garlic and onions. *Sci. Am.* **1985**, *252*, 114−119.

(6) Block, E. Biological activity of allium compounds: recent results. *Acta Hortic.* **2005**, *688*, 41−58.

(7) Trio, P. Z.; You, S.; He, X.; He, J.; Sakao, K.; Hou, D. X. Chemopreventive functions and molecular mechanisms of garlic organosulfur compounds. *Food Funct.* **2014**, *5*, 833−844.

(8) Borlinghaus, J.; Albrecht, F.; Gruhlke, M. C.; Nwachukwu, I. D.; Slusarenko, A. J. Allicin: chemistry and biological properties. *Molecules* **2014**, *19*, 12591−12618.

(9) Bayan, L.; Koulivand, P. H.; Gorji, A. Garlic: a review of potential therapeutic effects. *Avicenna J. Phytomed.* **2014**, *4*, 1−14.

(10) Block, E. The organosulfur chemistry of the genus *Allium* − implications for organic sulfur chemistry. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1135−1178.

(11) Rundqvist, C. Farmakokemisk underökning af Bulbus Allii. *Farm. Aikak.* **1909**, *18*, 323−333.

(12) Stoll, A.; Seebeck, E. Über alliin, die genuine Muttersubstanz des Knoblauchöls. 1. Mitteilung über Allium-Substanzen. *Helv. Chim. Acta* **1948**, *31*, 189−201.

(13) Zhang, Z.; Lei, M.; Liu, R.; Gao, Y.; Xu, M.; Zhang, M. Evaluation of alliin, saccharide contents and antioxidant activities of black garlic during thermal processing. *J. Food Biochem.* **2015**, *39*, 39−47.

(14) Ieong Tou, W.; Chang, S. S.; Wu, D.; Lai, T. W.; Wang, Y. T.; Hsu, C. Y.; Chen, C. Y. Molecular level activation insights from a NR2A/NR2B agonist. *J. Biomol. Struct. Dyn.* **2014**, *32*, 683−693.

(15) Akao, M.; Shibuya, T.; Shimada, S.; Sakurai, H.; Kumagai, H. *In vivo* production of bioactive compounds from *S*-allyl-L-cysteine sulfoxide, garlic odor precursor, that inhibit platelet aggregation. *J. Clin. Biochem. Nutr.* Supple. **2008**, *43*, 1−3.

(16) Tsuchiya, H.; Nagayama, M. Garlic allyl derivatives interact with membrane lipids to modify the membrane fluidity. *J. Biomed. Sci. (London, U. K.)* **2008**, *15*, 653−660.

(17) Choi, M. K.; Chae, K. Y.; Lee, J. Y.; Kyung, K. H. Antimicrobial activity of chemical substances derived from *S*-alk(en)yl-L-cysteine sulfoxide (alliin) in garlic, *Allium sativum* L. *Food Sci. Biotechnol.* **2007**, *16*, 1−7.

(18) Siegers, C. P.; Steffen, B.; Röbke, A.; Pentz, R. The effects of garlic preparations against human tumor cell proliferation. *Phytomedicine* **1999**, *6*, 7−11.

(19) Liakopoulou-Kyriakides, M. Relation between the structure of alliin analogues and their inhibitory effect on platelet aggregation. *Phytochemistry* **1985**, *24*, 1593−1594.

(20) Yamazaki, Y.; Tokunaga, T.; Okuno, T. Quantitative determination of eleven flavor precursors (*S*-alk(en)yl cysteine derivatives) in garlic with an HPLC method. *Nippon Shokuhin Kagaku Kogaku Kaishi* **2005**, *52*, 160−166 (in Japanese)..

(21) Cavallito, C. J.; Bailey, J. H. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *J. Am. Chem. Soc.* **1944**, *66*, 1950−1951.

DOI: 10.1021/acs.jafc.5b05501
J. Agric. Food Chem. 2015, 63, 10778−10784

(22) Cavallito, C. J.; Buck, J. S.; Suter, C. M. Allicin, the antibacterial principle of *Allium sativum*, II. Determination of the chemical structure. *J. Am. Chem. Soc.* **1944**, *66*, 1952−1954.
(23) Small, L. D.; Bailey, J. H.; Cavallito, C. J. Alkyl thiosulfinates. *J. Am. Chem. Soc.* **1947**, *69*, 1710−1713.
(24) Stoll, A.; Seebeck, E. Die synthese des natürlichen Alliins und seiner drei optisch aktiven Isomeren. 5. Mitteilung über Allium-Substanzen. *Helv. Chim. Acta* **1951**, *34*, 481−487.
(25) Synge, R. L. M.; Wood, J. C. (+)-(*S*-Methyl-L-cysteine *S*-oxide) in cabbage. *Biochem. J.* **1956**, *64*, 252−259.
(26) Hine, R.; Rogers, D. The crystal and molecular structure of (+)-(*S*-methyl-L-cysteine *S*-oxide); a standard of absolute configuration for asymmetric sulphur. *Chem. Ind. (Chichester, U. K.)* **1956**, 1428−1430.
(27) Hine, R. The crystal structure and molecular configuration of (+)-*S*-methyl-L-cysteine sulfoxide. *Acta Crystallogr.* **1962**, *15*, 635−641.
(28) Barnsley, E. A. Correlation of the configuration of some sulfoxides with (+)-*S*-methyl-L-cysteine *S*-oxide. *Tetrahedron* **1968**, *24*, 3747−3752.
(29) Henson, P. D.; Mislow, K. Optical rotatory dispersion and circular dichroism of diastereomeric *S*-allyl-L-cysteine *S*-oxides. *J. Chem. Soc. D* **1969**, 413−414.
(30) Fowden, L.; Scopes, P. M.; Thomas, R. N. Optical rotatory dispersion and circular dichroism. Part LXX. The circular dichroism of some less common amino-acids. *J. Chem. Soc. C* **1971**, 833−840.
(31) Iberl, B.; Winkler, G.; Müller, B.; Knobloch, K. Quantitative determination of allicin and alliin from garlic by HPLC. *Planta Med.* **1990**, *56*, 320−326.
(32) Freeman, F.; Huang, B.-G.; Lin, R. I.-S. Garlic chemistry. Nitric oxide oxidation of *S*-2-propenylcysteine and (+)-*S*-2-propenyl-L-cysteine sulfoxide. *J. Org. Chem.* **1994**, *59*, 3227−3229.
(33) Koch, I.; Keusgen, M. Diastereoselective synthesis of alliin by an asymmetric sulfur oxidation. *Pharmazie* **1998**, *53*, 668−671.
(34) Hibi, M.; Kawashima, T.; Yajima, H.; Smirnov, S. V.; Kodera, T.; Sugiyama, M.; Shimizu, S.; Yokozeki, K.; Ogawa, J. Enzymatic synthesis of chiral amino acid sulfoxides by Fe(II)/α-ketoglutarate-dependent dioxygenase. *Tetrahedron: Asymmetry* **2013**, *24*, 990−994.
(35) The crystallographic data have been deposited with the Cambridge Crystallographic Data Centre with deposition Number CCDC 1440071. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, United Kingdom; e-mail:deposit@ccdc.cam.ac.uk.
(36) Lancaster, J. E.; Kelly, K. E. Quantitative analysis of the *S*-alk(en)yl-L-cysteine sulfoxides in onion (*Allium cepa* L.). *J. Sci. Food Agric.* **1983**, *34*, 1229−1235.

DOI: 10.1021/acs.jafc.5b05501
J. Agric. Food Chem. 2015, 63, 10778−10784