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Structure and Bioactivity of Thiosulfinates Resulting from Suppression of Lachrymatory Factor Synthase in Onion

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ABSTRACT: In normal onion (*Allium cepa*), *trans*-*S*-1-propenyl-L-cysteine sulfoxide is transformed via 1-propenesulfenic acid into propanethial *S*-oxide, a lachrymatory factor, through successive reactions catalyzed by alliinase and lachrymatory factor synthase (LFS). A recent report showed that suppression of the LFS activity caused a dramatic increase in thiosulfinates previously reported as "zwiebelane isomers". After purification by recycle high-performance liquid chromatography and subsequent analyses, we established the planar structure of the putative "zwiebelane isomers" as *S*-3,4-dimethyl-5-hydroxythiolane-2-yl 1-propenethiosulfinate, in which two of the three molecules of 1-propenesulfenic acid involved in the formation gave the thiolane backbone, and the third molecule gave the thiosulfinate structure. Of at least three stereoisomers observed, one in the (2′*R*,3′*R*,4′*R*,5′*R*)-configuration was collected as an isolated fraction, and the other isomers were collected as a combined fraction because spontaneous tautomerization prevented further purification. Both fractions showed inhibitory activities against cyclooxygenase-1 and α-glucosidase in vitro.

KEYWORDS: *Allium cepa*, alliinase, 1-propenyl cysteine sulfoxide, LFS, LF, 1-propenesulfenic acid, thiosulfinate, cepathiolane, zwiebelane, zwiebelane isomer, *S*-3,4-dimethyl-5-hydroxythiolane-2-yl 1-propenethiosulfinate, 5,6-dimethyl-2-oxa-3,7-dithiabicycloheptane, tearless onion, LFS suppressed onion, COX inhibition, α-glucosidase inhibition

INTRODUCTION

Allium plants, such as onion (*Allium cepa*) and garlic (*Allium sativum*), are among the most popular vegetables grown and consumed worldwide. They are known to have various health-promoting properties,[1,2] which have been ascribed in part to their rich content of organosulfur compounds. For example, thiosulfinates such as allicin in garlic have been shown to inhibit collagen-induced platelet aggregation.[3] Because aqueous extracts of fresh garlic cloves reportedly inhibited a pathway in the arachidonic acid (AA) cascade,[4] it is anticipated that the antiplatelet aggregation effect of thiosulfinates is due to inhibition of AA cascade. It was also reported that garlic showed 13 times more potent antiaggregatory effect than onion.[5] This may be explained by the fact that the amount of thiosulfinates generated in onion is much less than that in garlic. In garlic, 2-propenesulfenic acid generated from alliin by alliinase condenses nonenzymatically into a relatively stable thiosulfinate, allicin. In onion, however, most of 1-propenesulfenic acid generated from *trans*-*S*-1-propenyl-L-cysteine sulfoxide (PRENCSO) is rapidly converted into volatile lachrymatory factor (LF) before it condenses into thiosulfinates. Hence, in the absence of lachrymatory factor synthase (LFS), it can be expected that the 1-propenesulfenic acid would condense into di-1-propenyl thiosulfinate.[6] Although increase in thiosulfinate content in the PRENCSO−alliinase system devoid of LFS was demonstrated indirectly by the use of pinking reaction,[7,8] the presence of di-1-propenyl thiosulfinate has not been confirmed yet because this particular thiosulfinate is too reactive to exist at room temperature.[9]

Recently, Eady et al. reported that suppression of LFS expression in onion by RNAi caused a dramatic change in gas chromatography−mass spectrometry (GC-MS) profiles of downstream sulfur compounds in fresh macerates of onion, with marked increase in such compounds as di-1-propenyl disulfide and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene.[10] Interestingly, the GC-MS spectrum of a compound present among the compounds that increased most markedly by the suppression of LFS differed significantly from those of *cis*- or *trans*-zwiebelane reported by Block et al.[11−14] but agreed with that of the putative zwiebelane isomer first detected by Arnault et al.[15] in a trace amount during a GC-MS analysis of conventional onion juice. Because the compound was not isolated, its molecular structure remained unidentified.

The first objective of this work was to determine the molecular structure of the putative zwiebelane isomer observed in the LFS suppressed onion. The compound was prepared by use of a model reaction system to facilitate purification. The second objective was to study the bioactivities of the compound. Because the compound had a thiosulfinate moiety, the antiplatelet activity of the compound was assessed by measuring inhibition of cyclooxygenase-1 (COX-1) that catalyzes the formation of prostaglandin G2 from AA. Similarly, we investigated the α-glucosidase inhibiting activity of the compound, because of its thiolane backbone and sulfinate anion similar to that of kotalanol or salacinol, the active principles of antidiabetic herbal medicine, *Salacia*.[16,17]

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MATERIALS AND METHODS

Preparation of PRENCSO and Alliinase. PRENCSO and alliinase were prepared from heat-treated onion bulbs and from fresh garlic cloves, respectively, following the procedures previously described.[8] Briefly, PRENCSO was purified by cation-exchange resin and preparative medium-pressure liquid chromatography (MPLC), and purified alliinase was prepared by using hydroxyapatite and a concanavalin A−sepharose 4B column. The purified PRENCSO was dissolved in water at 20 mg/mL. The purified alliinase was dissolved in 500 mM sodium phosphate buffer (pH7.0) at 250 units/mL with 10% glycerol and 25 μM pyridoxal phosphate.

Preparation of Recombinant LFS. The LFS was expressed as a GST fusion protein in *Escherichia coli*, and the protein was purified by using glutathione−sepharose 4B affinity column followed by thrombin cleavage.[18] With 2.5 μg of the purified recombinant LFS (rLFS) and 5.0 units of alliinase, a maximum amount of LF was generated from 200 μg of PRENCSO.

GC-MS Analysis of Model Reaction Products. Model reaction mixtures comprising 10 μL of PRENCSO (200 μg) and 20 μL of the alliinase solution (250 units/mL) with or without the rLFS (2.5 μg) were incubated for 3 min at room temperature and extracted with 200 μL of diethyl ether containing 0.01 μL of benzyl alcohol as an internal standard. Immediately after extraction, the extracts were subjected to GC-MS analysis as described by Eady et al.[10] A 5975C inert XL MSD MS system (Agilent Technologies) equipped with a 7890A GC system (Agilent Technologies) was used for the analysis. GC separation was achieved using a 15 m × 0.32 mm i.d. DB-1 column with a thick coating (5 μm; Agilent Technologies). The carrier was 99.999% helium at 3.5 mL/min, and the column temperature program was 5 °C/min from 70 to 250 °C. Transfer line and detector temperature were held at 150 °C, and total ion chromatograms and mass spectra were analyzed with the electron-impact mode.

Liquid Chromatography−Mass Spectrometry (LC-MS) Analysis of Reaction Products from LFS (±) Models, LFS Suppressed Onion, and Normal Onion. *LFS (±) Models.* Model reaction mixtures comprising 40 μL of the PRENCSO (800 μg) and 80 μL of the alliinase (250 units/mL) with or without the rLFS (30 μg) were used to mimic normal and LFS minus onion juices, respectively. They were incubated for 30 s at room temperature, to which 270 μL of 2-propanol with 10 μg/mL of formononetin as an internal standard was added and vortexed for 30 s. The mixtures were then applied to the LC-MS as described below.

LFS Suppressed Onion and Normal Onion. Bulbs of an LFS suppressed onion and its nontransgenic control (a pungent dehydration mid-daylength variety) described in Eady et al.[10] were obtained by courtesy of Plant and Food Research, NZ, and individually frozen and pulverized into fine powders. The frozen powder (1.5 g each) was combined with 5 mL of H_2O in a 10 mL glass tube. After it was vortexed for 30 s, the tube was allowed to stand for 3 min at room temperature. Then, 5 mL of diethyl ether with a small amount of NaCl was added to the tube, and the tube was vortexed for 1 min. After centrifugation at 3500 rpm for 10 min, the organic phase was collected, and the precipitate was extracted with diethyl ether again. The combined organic phase was dried with Na_2SO_4, filtered, and evaporated to dryness. The residue was dissolved in methanol with 10 μg/mL formononetin as an internal standard. The methanol solution was applied to LC-MS.

LC-MS Conditions. ThermoFisher ODS Hypersil column (250 mm × 4.6 mm, 5 μm) on Agilent 1100 coupled to Finnigan LTQ-FT (Thermo Scientific). Water (LC-MS grade; solvent A) with a 10 mM concentration of ammonium formate and acetonitrile (LC-MS grade; solvent B) was used as the mobile phase. The gradient program was as follows: 18−90% B over 45 min; 90% B for 5 min; 90−18% B over 0.1 min; 18% B for 10 min. The flow rate was set to 0.5 mL/min, and the column oven temperature was set at 30 °C. A photodiode array detector was used in the range 200−650 nm. The ESI setting was as follows: spray voltage, 4.0 kV; and capillary temperature, 300 °C for positive mode. A full MS scan was performed in the m/z range 70−1000 at a resolution of 100000. The data were analyzed using Xcalibur software version 2.0.7 (Thermo Scientific).

Purification of Reaction Products from the LFS Minus Model. Two milliliters of the 20 mg/mL PRENCSO solution was added to 4 mL of the alliinase (250 units/mL), and after 30 s at room temperature, 3 mL of chloroform was added to the reaction mixture to quench the reaction and to extract the reaction products into the organic phase. After the extraction was repeated twice, the organic phase was combined and dried with Na_2SO_4. The chloroform was distilled off, and the residue was dissolved in a small amount of acetonitrile for fractionation by a recycle high-performance liquid chromatography (HPLC) (LC-9110NEXT; Japan Analytical Industry Co.). Two fractions containing the target compounds were obtained using two 250 mm × 20 mm i.d. ODS-3 columns (GL science) connected in series and acetonitrile/H_2O/trifluoroacetic acid = 39.4:59.6:1 at a flow rate of 5 mL/min as the mobile phase. The detection wavelength was set at ultraviolet (UV) 254 nm. By adding an excess amount of NaCl to the fractions, the organic phase was collected. The organic phase from each fraction was dried with Na_2SO_4 and evaporated to dryness to obtain 11.2 mg of fraction 1 (Fr1) and 2.5 mg of fraction 2 (Fr2).

Structural Analysis of the Purified Compounds. Structural analysis of the purified compounds was carried out by nuclear magnetic resonance (NMR) spectrometries, infrared (IR) spectroscopy, and elemental analysis. ^{1}H NMR and ^{13}C NMR spectra of the purified compound were obtained on JEOL ECA-500 at 500 and 125 MHz, respectively. The IR spectra were obtained using Perkin-Elmer SYSTEM2000 FT-IR spectrometer at neat condition. Perkin-Elmer 2400 CHNS analyzer was used for the elemental analysis.

Bioactivity Assays of Reaction Products Unique to LFS Minus Model. *COX-1 Inhibiting Activity.* The COX-1 inhibiting activity was evaluated by measuring prostaglandin H2 (PGH2) derived from AA by COX-1. To a tube containing 75 μL of 200 mM Tris-HCl buffer (pH 8.0), 75 μL of distilled water, 10 μL of heme (Cayman Chemical:760116) in 100 mM Tris-HCl buffer (pH 8.0) at 22.7-fold dilution, 10 μL of 2.6 units/μL COX-1 (Cayman Chemical:76011) and 10 μL of various concentrations (2.8, 11.2, 44.8, and 179.2 mM) of aspirin (Cayman Chemical:70260) in ethanol (positive control) or various concentrations (0.175, 0.7, 2.8, and 11.2 mM) of the test sample were added. After 5 min at 24 °C, 20 μL of 110 μM AA prepared by diluting 22 mM AA−ethanol solution (Cayman Chemical) with 0.5 mM KOH was added to the reaction solution and allowed to stand for an additional 1 min at 24 °C. The reaction was quenched by adding 800 μL of ethanol, and the solution was analyzed by ultraperformance liquid chromatography (UPLC)-MS/MS to determine the amount of PGH2. The COX-1 inhibition activity was calculated by the following equation: [COX-1 inhibition activity] (%) = {([PGH2 peak area of blank] − [PGH2 peak area of test sample])/[PGH2 peak area of blank]} × 100. A Waters Acquity UPLC with a BEH C18 column (100 mm × 2.1 mm, 1.7 μm) and a Waters Quatro Premier XE triple quadruple mass spectrometer were used for the quantitative analysis of PGH2. UPLC-MS/MS conditions were as follows: A 1 mM triethylamine in acetonitrile/1 mM triethylamine in H_2O gradient with a constant flow rate of 0.3 mL/min was used as the mobile phase (A, 1 mM triethylamine in acetonitrile; B, 1 mM triethylamine in H_2O). Gradient: 10−60% A over 10 min; 60−80% A over 0.5 min; 80% A for 5 min; 80−10% A over 0.01 min; and 10% A for 20 min. The sample tray temperature was set to 5 °C. Electrospray ionization−mass spectrometry (ESI-MS) conditions: negative ionization mode; source temperature, 120 °C; capillary voltage, 3 kV; cone voltage, 15 V; desolvation temperature, 350 °C; cone gas flow, 50 L/h; desolvation gas flow, 600 L/h; collision energy,

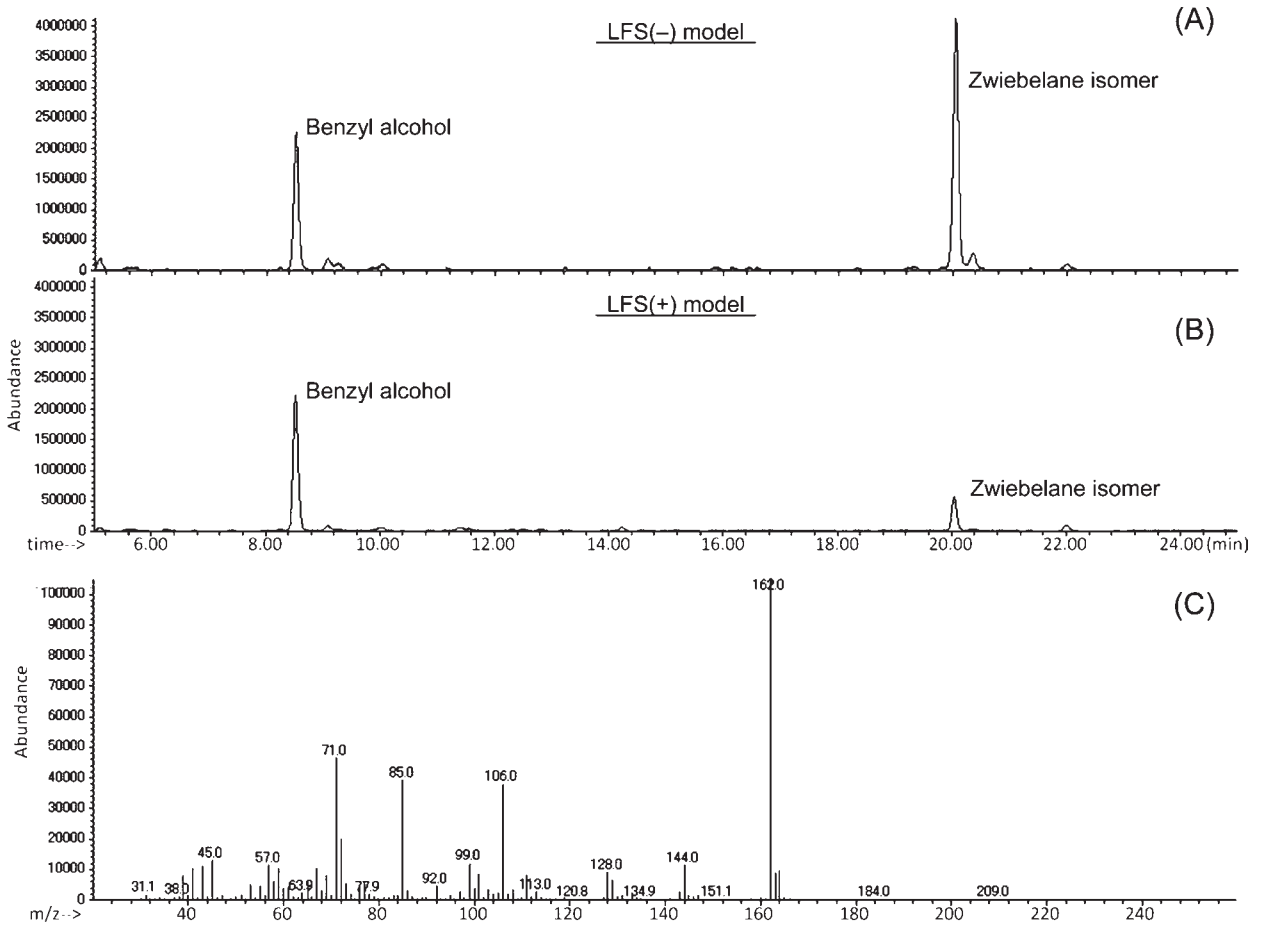

Figure 1. GC-MS total ion chromatogram and EI-MS spectrum of two model reaction extracts. The reaction time was 30 s. GC-MS total ion chromatogram of a model reaction without rLFS (A) and a model reaction with rLFS (B). MS spectrum at RT = 20 min in A (C). For GC-MS conditions, see the Materials and Methods.

16 eV; and MRM mode setting on parent ion $m/z = 351$ and on daughter ion $m/z = 271$.

α-Glucosidase Inhibiting Activity. α-Glucosidase from *Saccharomyces* sp. (Wako Chemical: 076-02841) was used for this experiment, and its enzyme activity was measured according to the manual supplied with the α-glucosidase in a reduced total reaction volume of 0.2 mL instead of 4 mL given in the manual. An increase in absorbance at 400 nm due to hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside (Wako Chemical: 325-34671) after incubation at 37 °C for 15 min was measured on a microplate spectrophotometer, with caffeic acid in ethanol as a positive control and the same amount of ethanol as a blank. In the 0.2 mL of reaction solution, the amount of the ethanolic solution of sample or the ethanol was 3.75 μL.

RESULTS AND DISCUSSION

Comparison of Constituents Between LFS Suppressed Onion, Normal Onion, and Corresponding Model Reaction Systems. GC-MS analysis of our model reaction products showed that, like the LFS suppressed onion, the LFS(−) model (PRENCSO and alliinase without rLFS) yielded the putative zwiebelane isomer in much larger amount than the LFS(+) model (PRENCSO, alliinase and rLFS). In the total ion chromatograms of the model reaction products (Figure 1A,B), the putative zwiebelane isomer was detected as a pair of large and small peaks both at around 20 min. The MS spectrum of these peaks (Figure 1C) agreed with that of the putative zwiebelane isomer: m/z (relative intensity) 162 (60), 106 (51), 85 (78), 71 (100), 57 (40), 45 (82), reported by Arnault,[15] or m/z 162 (100), 106 (40), 85 (41), 71 (51), 57 (13), 45 (13), reported by Eady.[10]

LC-MS analysis of the reaction products from the LFS(±) models, the LFS suppressed onion juice, and the normal onion juice (Figure 2) revealed that three peaks detected between retention time (RT) = 23 min and RT = 25 min were predominant in amounts and were specific to the LFS(−) model and the LFS suppressed onion juice. Essentially, the same MS spectra were obtained from these three peaks. Figure 2E shows a representative spectrum obtained from the peak at 25.18 min of Figure 2A. When a fraction containing these three peaks was collected and analyzed by GC-MS, a pair of large and small peaks both at around 20 min was obtained in the total ion chromatogram, and its MS spectrum was identical to that of the putative zwiebelane isomer (data not shown). These results revealed that the GC-MS peaks at around 20 min were the artifacts induced from at least three chemical species exhibiting the same MS spectrum as shown in Figure 2E under the milder LC-MS conditions. Artifacts formation during GC-MS analysis due to the high injection port temperature is not uncommon in *Allium* chemistry.[19] The peak at 27.29 min of Figure 2C was detected only in the LFS suppressed onion extract. MS analysis revealed that this peak was comprised of a mixture of two or more unidentified constituents.

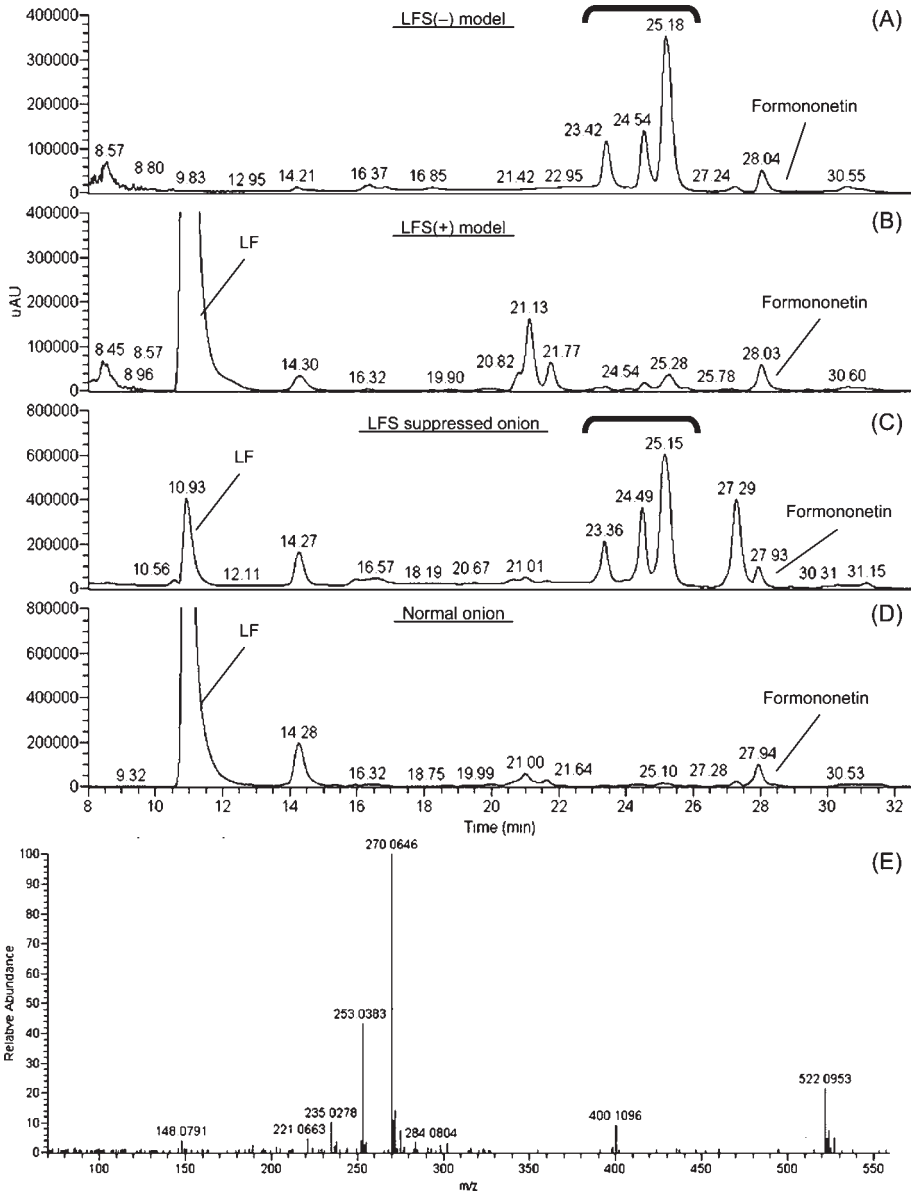

Figure 2. PDA chromatograms and ESI-MS spectrum of model reaction products and fresh onion juice extracts analyzed by LC-MS. PDA chromatogram of a model reaction without rLFS (A), a model reaction with rLFS (B), an LFS suppressed onion extract (C), and a normal onion extract (D). MS spectrum at RT = 25.18 min in A (E). For LC-MS conditions, see the Materials and Methods.

Isolation and Identification of the Putative Zwiebelane Isomers. The LFS(−) model reaction products were then prepared in a larger scale and extracted with chloroform. After two cycles of HPLC purification, two fractions, Fr1 (11.2 mg) and Fr2 (2.5 mg), were obtained from 40.0 mg of PRENCSO. The Fr1 corresponded to the peaks at RT = 23.42−24.54 min of the HPLC chromatogram, which suggested that it consisted of at least two isomers. Because isolation of these isomers was hindered by spontaneous tautomerization, further purification was not attempted, and these peaks were collected as a single fraction. The Fr2 corresponded to the single peak at RT = 25.18 min and was deemed isolated.

The structure of the isolated compound in fraction Fr2 was elucidated by spectroscopic and chemical methods. First, the molecular formula was determined as $C_9H_{16}O_2S_3$ by Fourier transform ion cyclotron resonance−mass spectrometry (FT-ICR-MS) (m/z found, 253.0383 $[M + H]^+$; calcd, 253.0385) and elemental analysis (found: C, 42.29%; H, 6.46%; O, 12.86%; S, 38.39%; calcd: C, 42.82%; H, 6.39%; O, 12.68%; S, 38.11%). The ^{1}H NMR and ^{13}C NMR data in $CDCl_3$ are shown in Table 1. The ^{13}C NMR spectrum in $CDCl_3$ showed nine carbons, and the ^{1}H NMR spectrum in $CDCl_3$ showed 15 protons. When the solvent was changed to DMSO-d_6, the ^{1}H NMR spectrum showed an exchangeable doublet signal at 6.20 ppm coupled with H5′ of Figure 3. In line with these observations, the presence of hydroxyl and thiosulfinate groups was suggested by strong IR absorption bands at 3342 and 1092 cm^{-1}, respectively.

The correlation signals obtained with heteronuclear multiple bond correlation (HMBC) and correlation spectroscopy (COSY) are shown in Figure 3. From the correlations of COSY, −C2′−C3′(−3′Me)−C4′(−4′Me)−C5′− and −C1−C2−C3 structures were elucidated. In addition, correlations were found

Table 1. ^{1}H NMR and ^{13}C NMR Data[a] of the Compound from Fr2

C no.	^{13}C ($CDCl_3$)	^{1}H ($CDCl_3$)	^{1}H (DMSO-d_6)
1	129.19	6.52 (dq, 1, J = 14.9, 1.7)	6.82 (dq, 1, J = 14.7, 1.7)[b]
2	137.72	6.65 (dq, 1, J = 14.9, 6.9)	6.41 (dq, 1, J = 14.7, 7.3)[b]
3	18.02	2.00 (dd, 3, J = 6.9, 1.7)	1.91 (dd, 3, J = 7.3, 1.7)
2′	59.93	4.87 (d, 1, J = 4.0)	4.97 (d, 1, J = 4.9)
3′	48.95	2.10−2.19 (ddq, 1, J = 12.3, 6.4, 4.0)	2.08−2.15 (m, 1)
3′-Me	14.60	1.07 (d, 3, J = 6.4)	0.98 (d, 3, J = 6.4)
4′	48.83	1.99−2.06 (ddq, 1, J = 12.3, 7.4, 6.8)	1.69−1.75 (m, 1)
4′-Me	14.67	1.15 (d, 3, J = 6.8)	1.01 (d, 3, J = 6.9)
5′	90.87	5.20 (d, 1, J = 7.4)	5.06 (dd, 1, J = 7.5, 7.5)
5′−OH			6.20 (d, 1, J = 7.5)

[a] Chemical shifts δ are given in ppm and coupling constants J in Hz. [b] The chemical shifts for H1 and H2 in DMSO-d_6 are inverted from their normal positioning in $CDCl_3$.

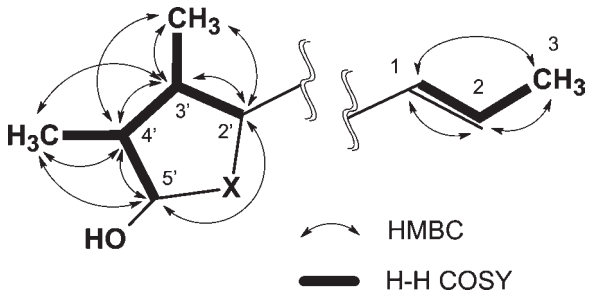

Figure 3. Key ^{1}H−^{1}H COSY and HMBC interactions of the compound from Fr2. X, the heteroatom.

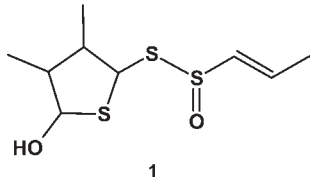

Figure 4. Planar structure of *S*-3,4,-dimethyl-5-hydroxythiolane-2-yl 1-propenethiosulfinate (compound **1**) having a thiosulfinate bond and a thiolane backbone.

between H2′ and C5′ and between H5′ and C2′ in HMBC. These HMBC correlations indicated that the partial structure −C2′−C3′(−3′Me)−C4′(−4′Me)−C5′− forms a five-membered ring via one heteroatom (indicated by X in Figure 3). Taking into consideration the molecular formula, $C_9H_{16}O_2S_3$, and the presence of a hydroxyl group (−OH) and a thiosulfinate group [−S(O)−S−] in the molecule, we concluded that the heteroatom was sulfur and that the compound had a thiolane backbone. Furthermore, it was confirmed that the C2′ of the thiolane backbone and C1 of the partial structure −C1−C2−C3 were connected together via a thiosulfinate structure, because no HMBC correlations were found between the thiolane backbone and the partial structure −C1−C2−C3. Note that the chemical shifts for H1 and H2 in DMSO-d_6 are inverted from their normal positioning in $CDCl_3$ (Table 1). Such inversion due to the strong shielding effect of the solvent on the vinylic β-site was also observed in ^{1}H NMR spectra of PRENCSO (data not shown) and has been reported for vinyl sulfoxides, such as glucoraphenin (4-methylsulfinyl-3-butenyl glucosinolate) and desulfoglucoraphenin,[20] and vinyl ketones, such as chalcone and (*E*)-1-phenyl-2-buten-1-one.[21] The chemical shift determined for C1 suggested the position of the oxidized sulfur atom to be right next to C1. Thus, the planar structure was established as *S*-3,4-dimethyl-5-hydroxythiolane-2-yl 1-propenethiosulfinate (compound **1**) shown in Figure 4. The structure of the compound **1** had been predicted by Block[12] as a precursor of an unidentified compound, which showed m/z = 325−326 ($C_{12}H_{20}S_4O_2$/$C_{12}H_{22}S_4O_2$) in the LC-MS analysis of normal onion juice. However, the details of compound **1** were not given. More recently, one of the stereoisomer of compound **1** in (2′*R*,3′*S*,4′*S*,5′*R*) configuration was isolated from a normal onion bulb and named "cepathiolane" (**1b**).[22] Some other stereoisomers were predicted but were not detected.

From the results of this study along with the knowledge from the previous studies,[12,22,23] we propose the formation of compound **1** in onion juice as shown in Figure 5: In the absence of LFS, two molecules of unstable 1-propenesulfenic acid derived from the reaction of PRENCSO and alliinase go through self-condensation and form a transient 5,6-dimethyl-2-oxa-3,7-dithiabicycloheptane (compound **2**) via (*E*,*E*)-di-1-propenyl thiosulfinate. Immediately after this, another molecule of 1-propenesulfenic acid reacts with compound **2** via nucleophilic attack to form compound **1**.[12] These series of self-reactions are considered to occur instantaneously. In normal onion, most of 1-propenesulfenic acid is rapidly converted into LF before it condenses into thiosulfinates. In LFS suppressed onion, on the other hand, three molecules of the sulfenic acid are converted into compound **1** as shown in Figure 5.

A number of stereoisomers are possible for compound **1**, and our LC-MS analysis suggested the presence of at least three isomers. Two of the isomers (2′*R*,3′*R*,4′*R*,5′*R*)-**1** (**1a**) and (2′*R*,3′*S*,4′*S*,5′*R*)-**1** (**1b**) were probably generated from (1*R*,4*R*,5*R*,6*R*)-**2** (**2a**) and (1*R*,4*R*,5*S*,6*S*)-**2** (**2b**), respectively, through reaction with a third sulfenic acid. The remaining isomers were thought to be generated from 1-propenesulfenic acid and **2** in configurations other than those of **2a** and **2b**, although the occurrence of such configuration change was yet to be confirmed.

It was reported that when di-1-propenyl disulfide was synthetically oxidized, the resulting (*E*,*E*)-di-1-propenyl thiosulfinate formed **2a** and **2b** in a 4:1 ratio via intramolecular rearrangement, while (*Z*,*Z*)-di-1-propenyl thiosulfinate formed zwiebelanes.[23,24] Although zwiebelanes have been often detected in onion juice, detection of **2a** or **2b** has not been reported. This absence of detection may be explained by the fact that, in contrast to the synthetic oxidation of disulfides where sulfenic acid involvement is unlikely, a supply of sulfenic acid should be plentiful in onion juice for the rapid transformation of compound **2a** and **2b** into various stereoisomers of compound **1**, which, up to the present study, have been dubbed collectively as "zwiebelane isomers" based on the agreement of the molecular ion of m/z = 162 in their GC-MS spectra with that of zwiebelanes.[11−14] A plausible explanation for the detection of zwiebelane isomers by the earlier investigators is that, under the high temperature condition of GC-MS, compound **1** would revert back to compound **2** as shown by the dashed arrow in Figure 5.

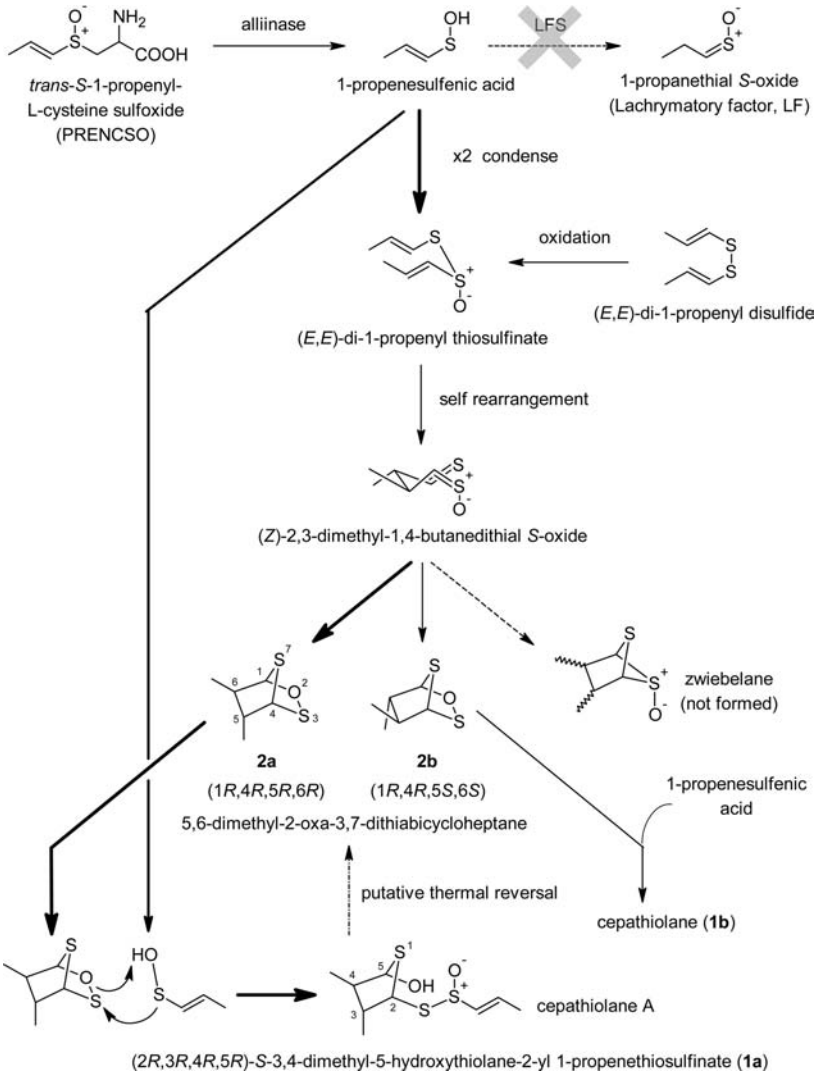

Figure 5. Proposed scheme for the formation of compound **1** (cepathiolanes).

Some chemical shifts of ^{1}H NMR and ^{13}C NMR, especially the ^{1}H−^{1}H coupling constants at $J_{2'\text{-}3'}$ = 4.0 Hz and $J_{4'\text{-}5'}$ = 7.4 Hz, of compound **1** we obtained as Fr2 differed markedly from those of **1b** (cepathiolane) but agreed with those calculated from the dihedral angles for **1a** reported by Yoshida et al.[22] Relative configurations of C2′, C3′, and C4′ of compound **1** were confirmed by NOE correlations: H2′ and 3′Me, 3′Me and H4′, and H3′ and 4′Me. Hence, we concluded that the configuration of compound **1** (in Fr2) was (2′*R*,3′*R*,4′*R*,5′*R*) and call it "cepathiolane A" (**1a**). The remainder of the analytical data of **1a** are as follows: colorless oil; $[\alpha]_D^{23}$ −7 ($CHCl_3$). UV λ_{max} 260 nm (in 58% CH_3CN). IR (neat, ν_{max}) 3342 (b), 2964 (s), 2930 (m), 2873 (m), 1722 (w), 1627 (w), 1453 (m), 1377 (w), 1092 (s), 1043 (vs), 946 (s), 887 (w) cm^{-1}. FT-ICR-MS calculated for $C_9H_{15}OS_3$, 235.0280 $[M - H_2O + H]^+$; found, 235.0278; calculated for $C_9H_{20}NO_2S_3$, 270.0651 $[M + NH_4]^+$; found, 270.0646; calculated for $C_{18}H_{36}NO_4S_6$, 522.0963 $[2M + NH_4]^+$; found, 522.0953.

Combining Fr1 and Fr2, the total molar yield of cepathiolanes was calculated to be 24.2% [13.7 mg (54.3 μmol) of cepathiolanes from the 40.0 mg (224 μmol) of PRENCSO]. Because the formation of cepathiolanes requires three molecules of sulfenic acid or PRENCSO, this yield constitutes 72.7% of the theoretical yield of 33.3%. Thus, in the absence of LFS, cepathiolanes should be the predominant breakdown products of PRENCSO. Comparison of the peaks in Figure 2A,C suggests that cepathiolane A (RT = 25 min) is the major cepathiolane generated. This is consistent with the 4:1 yield ratio reported for **2a** and **2b**, the most likely precursors of cepathiolane A (**1a**) and cepathiolane (**1b**), respectively.[23]

Bioactivity of the Cepathiolanes. Because the cepathiolanes bear a thiosulfinate moiety, the antiplatelet activity was tested by evaluating the inhibition of COX-1 in vitro. COX-1 is a bifunctional enzyme with cyclooxygenase activity, which catalyzes the conversion of AA to prostaglandin G2 (PGG2), and peroxidase activity, which catalyzes the conversion of PGG2 to PGH2. As shown in Table 2, the IC_{50} values of the compounds were nearly 2 orders of magnitude smaller than that of aspirin, known and used as a potent antiplatelet coagulation agent that inhibits COX-1. When COX-1 was added to AA in the presence of an excess amount of cepathiolanes, the amount of AA did not decrease (data not shown). This result suggested that, as with aspirin, cepathiolanes inhibited the conversion of AA to PGG2 by the cyclooxygenase activity of COX-1.[25] On the other hand,

Table 2. IC_{50} Values[a] of Cepathiolane A (1a, Fr2), Crude Cepathiolanes (Fr1), and Aspirin for Inhibition of COX-1

substrate	IC_{50} (mM)[a]
cepathiolane A (**1a**, Fr2)	0.052 ± 0.008
crude cepathiolanes (Fr1)	0.042
aspirin	3.3 ± 0.3

[a] Each value represents the average ± SD of three determinations except for Fr1.

Table 3. IC_{50} Values[a] of Cepathiolane A (1a, Fr2), Crude Cepathiolanes (Fr1), and Caffeic Acid for Inhibition of α-Glucosidase

substrate	IC_{50} (mM)[a]
cepathiolane A (**1a**, Fr2)	0.71 ± 0.09
crude cepathiolanes (Fr1)	1.5 ± 0.12
caffeic acid	14 ± 3.3

[a] Each value represents the average ± SD of three determinations.

methyl allyl trisulfide (MATS), a component commonly present in steam-distilled garlic oil, was reported to inhibit the peroxidase activity,[26] because PGG2 was detected when COX-1 was added to AA in the presence of MATS. Thus, the inhibition mechanism of the cepathiolanes is likely to be different from that of MATS.

The α-glucosidase inhibitory activities of cepathiolanes (Fr1 and Fr2) were compared with that of caffeic acid, which had been reported to have moderate α-glucosidase inhibitory activity.[27] The IC_{50} values are shown in Table 3. The results showed that Fr2 (cepathiolane A, **1a**) had 15 times stronger inhibitory activity than caffeic acid. It was suggested that the side chain with sulfate anion of salacinol was essential for the reported α-glucosidase inhibitory activity.[28] Thus, the inhibitory activity of cepathiolanes may be due to the sulfinate anion in the structure.

In conclusion, the potential inhibitory activities of cepathiolanes against COX-1 and α-glucosidase would give more healthful properties to the LFS suppressed onion.

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ABBREVIATIONS USED

AA, arachidonic acid; COSY, correlation spectroscopy; COX-1, cyclooxygenase-1; ESI-MS, electrospray ionization−mass spectrometry; FT-ICR-MS, Fourier transform ion cyclotron resonance−mass spectrometry; GC-MS, gas chromatography−mass spectrometry; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum correlation; HPLC, high-performance liquid chromatography; IR, infrared; LC-MS, liquid chromatography−mass spectrometry; LF, lachrymatory factor; LFS, lachrymatory factor synthase; MATS, methyl allyl trisulfide; NMR, nuclear magnetic resonance; PDA, photo diode array; PGG2, prostaglandin G2; PGH2, prostaglandin H2; PRENCSO, *trans*-(+)-1-propenyl-L-cysteine sulfoxide; rLFS, recombinant LFS; RT, retention time; UPLC, ultraperformance liquid chromatography; UV, ultraviolet

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