Ajothiolanes: 3,4-Dimethylthiolane Natural Products from Garlic (*Allium sativum*)

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ABSTRACT: Stereoisomers of 5-(2-allylsulfinyl)-3,4-dimethylthiolane-2-ol, a family of 3,4-dimethylthiolanes of formula $C_9H_{16}O_2S_2$ we name ajothiolanes, were isolated from garlic (*Allium sativum*) macerates and characterized by a variety of analytical and spectroscopic techniques, including ultraperformance liquid chromatography (UPLC), direct analysis in real time-mass spectrometry (DART-MS), and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Ajothiolanes were found to be spectroscopically identical to a family of previously described compounds named garlicnins B_{1-4} ($C_9H_{16}O_2S_2$), whose structures we demonstrate have been misassigned. 2D $^{13}C-^{13}C$ NMR incredible natural abundance double quantum transfer experiments (INADEQUATE) were used to disprove the claim of nine contiguous carbons in these compounds, while X-ray absorption spectroscopy (XAS) along with computational modeling was used to disprove the claim that these compounds were thiolanesulfenic acids. On the basis of the similarity of their NMR spectra to those of the ajothiolanes, we propose that the structures of previously described, biologically active onionins A_{1-3} ($C_9H_{16}O_2S_2$), from extracts of onion (*Allium cepa*) and *Allium fistulosum*, and garlicnin A ($C_{12}H_{20}O_2S_4$), from garlic extracts, should also be reassigned, in each case as isomeric mixtures of 5-substituted-3,4-dimethylthiolane-2-ols. We conclude that 3,4-dimethylthiolanes may be a common motif in *Allium* chemistry. Finally, we show that another garlic extract component, garlicnin D ($C_7H_{12}O_2S_3$), claimed to have an unprecedented structure, is in fact a known compound from garlic with a structure different from that proposed, namely, 2(*E*)-3-(methylsulfinyl)-2-propenyl 2-propenyl disulfide.

KEYWORDS: *garlic, Allium sativum, onion, Allium cepa, Allium fistulosum, INADEQUATE NMR, X-ray absorption spectroscopy, thiolane-2-ol, DART-MS, UPLC, sulfenic acids, 3,4-dimethylthiolanes, ajothiolane, naturally occurring thiolanes*

INTRODUCTION

Garlic (*Allium sativum*) and other *Allium* preparations are noted for the structural diversity of the unusual organosulfur compounds they contain, and the contributions that these compounds make to the olfactory and gustatory qualities, and health benefits of these immensely popular seasonings and foods.[1] In 1992, one of us proposed the presence in onion extracts of families of $C_{12}H_{20}O_2S_4$ (*m*/*z* 324) 2,5-disubstituted 3,4-dimethylthiolanes (**5**; Figure 1) and homologues, detected by liquid chromatography–mass spectrometry (LC-MS).[2] Compounds **5** are presumably derived from thiolane-2-ol **4**, which in turn is formed by nucleophilic attack on 5,6-dimethyl-2-oxa-3,7-dithiabicyclo[2.2.1]heptane (**3**) isomers. Compound **3**, formed in several steps from 1-propenesulfenic acid (**2**), is in turn generated by alliinase cleavage of isoalliin (**1**), found in intact onion (*Allium cepa*) and garlic bulbs. The formation of proposed compound **5** is supported by model studies with thiophenol, which gave **6a** from **3**,[3] and, notably, by isolation of **4** ($C_9H_{16}O_2S_3$; *m*/*z* 252; named cepathiolane),[4−6] **6b**, and **6c**[7,8] from extracts of lachrymatory factor synthase (LFS)-suppressed onions. LFS-suppression prevents diversion of **2** to *Z*-propanethial *S*-oxide (**2a**; the onion lachrymatory factor (LF)), allowing **2** to self-condense to *S*-(1-propenyl) 1-propenethiosulfinate (**2b**). [3,3]-Sigmatropic rearrangement of **2b** gives **2c**, which then undergoes intramolecular 1,3-dipolar [2 + 3] cycloaddition giving **3**. Compounds **6b** and **6c**, also reported in extracts of LFS-suppressed onions, may originate from nucleophilic attack of H_2S and H_2S_2, respectively, on **3**. Compound **6d**, structurally related to **4**, **5**, and **6a–c** and recently reported to be present in extracts of ordinary onions, is thought to be formed from **1** by a more complex sequence of steps.[9]

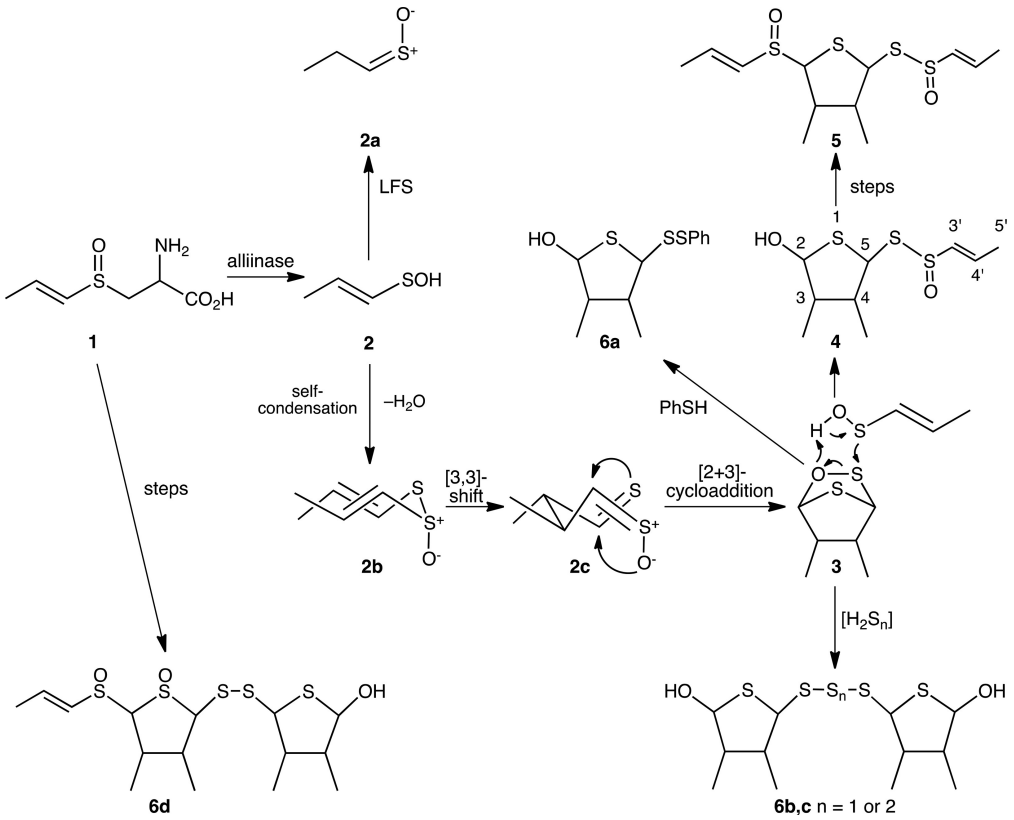


Figure 1. Proposed formation of *m/z* 324 compound **5** from isoalliin **1** via *m/z* 252 compound **4**; formation of thiophenol adduct **6a** and proposed formation of onion compounds **6b–6d**; stereochemistry omitted for **3–6** (LFS = lachrymatory factor synthase).

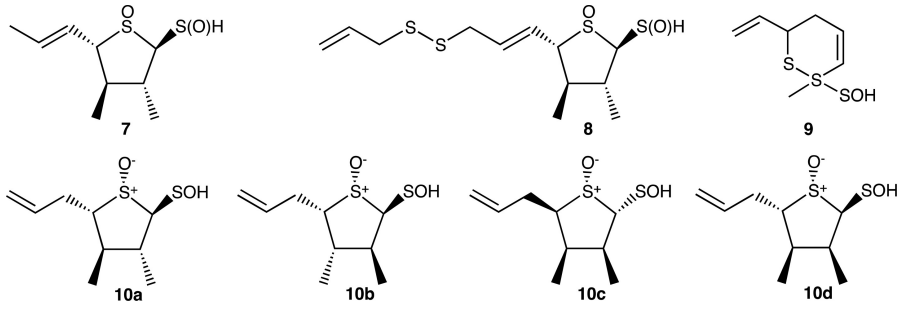


Figure 2. Structures proposed by Nohara et al. for compounds isolated from onion and garlic.

Cepathiolanes **4** were isolated as mixtures of tautomerically interconvertable stereoisomers. Interestingly, the antiplatelet activity measured by the IC_{50} value for in vitro inhibition of cyclooxygenase-1 (COX-1) for **4** was 2 orders of magnitude smaller than the corresponding value for aspirin, a potent antiplatelet coagulation agent that inhibits COX-1.[6]

In a series of papers from 2010 to 2018, Nohara and coworkers reported the isolation and structural characterization of isomeric families of biologically active 3,4-dimethylthiolanesulfenic acids which they named "onionin A_{1-3}" ($C_9H_{16}O_2S_2$, *m/z* 220; **7**) from onion and *Allium fistulosum*,[10–14] and garlicnins A ($C_{12}H_{20}O_2S_4$, *m/z* 324; **8**), D ($C_7H_{12}OS_3$, *m/z* 208; **9**), and B_{1-4} ($C_9H_{16}O_2S_2$, *m/z* 220; **10a-d**), among others, from garlic (Figure 2).[15–19] The authors suggest that compounds **7–10** originate from alliinase-catalyzed reactions involving isoalliin **1** and its isomer alliin (**15**; see Figure 5 below) but propose subsequent steps that are at variance with the extensive body of knowledge on the biosynthetic pathways of *Allium* compounds[1,2] and with the structures of **4**, **6b,c**, and allium sulfoxide A_1 (**11**; Figure 3), recently isolated from *Allium fistulosum*.[20] For example, it is very difficult to imagine how nine carbons could be connected to form **7**, **8**, and **10** from any likely *Allium* precursor. Furthermore, sulfenic acids, which are highly reactive with solution lifetimes of less than 1 s[21] unless they are sterically encumbered, as in the case of compounds **12**,[22,23] have never been isolated from natural sources. For those sulfenic acids that have been studied experimentally or computationally, such as methanesulfenic acid (CH_3SOH)[24] and **12**,[22,23] the linear RS–O–H form is substantially favored over the branched structure, RS(O)H. Finally, structure **9** is inconsistent with bonding considerations for organosulfur compounds; tetravalent sulfur compounds have stringent bonding requirements for formation and are unknown in nature. For these and other reasons addressed below, structures **7–10** seem implausible.

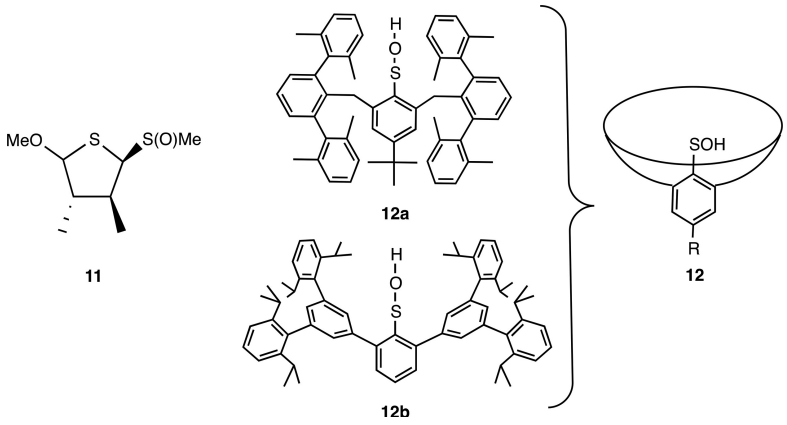


Figure 3. Left, allium sulfoxide A_1 (**11**), isolated from *Allium fistulosum*; center, bowl-shaped, sterically protected sulfenic acids **12a** (BmtSOH)[22] and **12b** (BpqSOH).[23]

We report here the isolation, spectroscopic, and computational studies of a family of 3,4-dimethylthiolanes from garlic we term "ajothiolanes," spectroscopically identical to Nohara's purported 3,4-dimethylthiolanesulfenic acids (**10a–d**; "garlicnin B_{1-4}") but whose structures we suggest are significantly different. We also propose an alternative structure for a compound we isolated from garlic which is spectroscopically identical to Nohara's compound **9** and which had been previously isolated from garlic extracts. Finally, we suggest that the structures proposed for compounds **7** and **8** are incorrect and propose alternative structures. Our studies illustrate the importance of using biosynthetic considerations in guiding structural assignments for natural products as well as the difficulties that can arise in assigning structures to new organosulfur compounds. These difficulties can be circumvented by the use of techniques such as ultraperformance liquid chromatography (UPLC)[25] and direct analysis in real time-mass spectrometry (DART-MS)[21,26] for analysis of complex mixtures, along with INADEQUATE NMR methods, sulfur-edge X-ray absorption spectroscopy (XAS), as well as computations to resolve ambiguities in structural assignments.

MATERIALS AND METHODS

General Methods. NMR spectra were recorded in $CDCl_3$ on a Bruker 400 Ultrashield (^{1}H, 400 MHz; ^{13}C, 100 MHz) or a Bruker 600 MHz Avance III NMR spectrometer (^{1}H, 600 MHz; ^{13}C, 125 MHz). Chemical shifts are in ppm downfield from tetramethylsilane, with residual $CHCl_3$ (7.27 ppm) as internal standard. Infrared spectra of neat compounds were recorded on a PerkinElmer UATR 2 FTIR. Mass spectra were obtained with a Hewlett-Packard 6890 GC and 5972A selective mass detector. A DART-AccuTOF mass spectrometer operating in positive or negative ion mode was employed with a polyethylene glycol spectrum as reference standard for exact mass measurements (PEG average molecular weight: 600). The atmospheric pressure interface was operated at the following potentials: orifice 1 = 15 V; orifice 2 = 5 V; and ring lens = 3 V. The RF ion guide voltage was set to 800 V to allow detection of ions greater than m/z 80. The DART ion source was operated with He at 200 °C and grid voltage = 530. The UPLC-MS/MS system consisted of an Eksigent Ultra LC100 System equipped with a XBridge C18 2.5 μm column (2.1 × 50 mm) 130 Å pore size operated at a flow rate of 0.5 mL/min. Water (mobile phase A) was acidified with 0.1% formic acid, and the water–acetonitrile gradient program was set as follows: time (min)/% acetonitrile (v/v), 0/5, 2/5, 6/80, 8/80, 8.02/5, 12/5. Detection was performed by a SCIEX TripleTOF mass spectrometer with various collision energies (standard collision energy = 15 V) after ionization either by electrospray or atmospheric pressure chemical ionization (declustering potential/energy of ionization = 40 V). Diethyl ether and THF were distilled from sodium-benzophenone ketyl. Ethyl acetate was purchased from Pharmaco-Aaper and used without further purification. Thiolane-2-ol was synthesized as described.[27] Solutions were dried with anhydrous $MgSO_4$. Analytical TLC was performed on precoated silica gel plates (Merck) with a 254 nm fluorescent indicator visualized under a UV lamp and by staining with $KMnO_4$ solution. Preparative TLC plates (Analtech) were 1 or 2 mm thick and were visualized under UV light. Because of the very low levels of the compounds found in the garlic extracts, exposure of solutions to plastic and rubber was rigorously avoided to prevent contamination of samples by plasticizers.

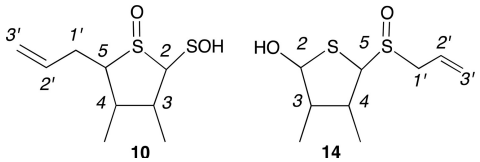
Stable sulfenic acid Bmt–SOH [Bmt = 1,3-*bis*[[2,6-*bis*(2,6-dimethylphenyl)phenyl]methyl]-5-*tert*-butylbenzene] was prepared as previously described.[22] All other compounds were purchased from Sigma-Aldrich Corporation and were of the highest quality available. For X-ray absorption spectroscopy (XAS) measurements, solutions were prepared in toluene at total sulfur concentrations of 100 mM and were placed in SPEX CertiPrep (Metuchen NJ, USA) X-cell 6.3 mm sample cups employing a 3 μm Etnom window (Chemplex Industries, Inc., Palm City, FL, USA). The concentrations of the solutions were chosen so that only minimal fluorescence self-absorption artifacts would be present in the X-ray absorption spectra.[28,29]

Preparation of Plant Extracts. Six kilograms of peeled garlic cloves (softneck garlic from California; purchased locally) were macerated in a food processor. Distilled acetone (6 L) was added to the macerate and the mixture was blended for 1 min and then stored in the dark for 30 h. The garlic pulp was separated from the liquid by filtration through a bed of Celite in a Büchner funnel, and the filtrate was concentrated on a rotary evaporator at 40 °C, affording a dark brown oil. The oil was saturated with NaCl, extracted with CH_2Cl_2 (3 × 200 mL), and the combined organic layers dried ($MgSO_4$) and concentrated in vacuo, yielding 17 g of sticky yellow–brown oil. The garlic concentrate was eluted from a silica gel column with five column volumes (CV) of CH_2Cl_2/MeOH (99:1) followed by five CV of pentane/acetone (4:1). The desired compounds eluted as a bright yellow band in the second CV with the second mobile phase (pentane/acetone). The eluate was concentrated in vacuo and eluted again from a silica gel column this time using 5 CV of pentane/EtOAc (2:1). Concentration and analysis of fractions by DART-MS showed the presence of a mixture of ajothiolane (H^+ adduct; m/z 221) along with allicin (H^+ adduct; m/z 163) and other garlic-derived compounds. The fraction enriched in ajothiolanes typically eluted as a yellow band after 2 CV of the second mobile phase (pentane/EtOAc 2:1). DART-MS and NMR indicate the presence of the target compounds, methylisoajoene, ajoenes, and higher mass compounds. Fractions enriched in ajothiolane are collected after 4–5 CV, combined, and concentrated, yielding 750 mg of a yellow liquid. The material (200 mg) was then loaded on a 1 mm thick preparative TLC plate and repeatedly eluted with pentane/EtOAc (2:1). The yellow, second-to-last band (R_f = 0.102), extracted

with CH_2Cl_2, contained 40 mg of ajothiolane **14** at a purity of about 85%. Methylisoajoene (**16**) eluted just after ajothiolane (R_f < 0.102).

5-(Allylsulfinyl)-3,4-dimethylthiolane-2-ol (14; ajothiolane). IR: ν_{max} 3331 (s, OH), 2961 (s, C−H), 2917 (s, C−H), 2849 (s, C−H), 1722 (w), 1461 (w), 1036 (s, S═O); HRMS (ESI) calcd for $[M + H]^+$ ($C_9H_{17}O_2S_2$) *m/z* 221.0670, found *m/z* 221.0655; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data for the major isomer of **14** are summarized in Table 3. The NMR data is in excellent agreement with that reported by Nohara for **10a**, as summarized in Table 1. Comparative chemical shift data of **4**, **6**, **11**, **14**, and thiolane-2-ol (**17**)[27] are given in Table 2. The 1D and 2D ^{1}H and ^{13}C NMR data for ajothiolane (**14**) are summarized in Table 3. Analysis of **14** by LC-MS/MS on an SCIEX TripleTOF (10 V) showed the protonated parent ion ($M + H^+$; $C_9H_{16}O_2S_2 + H^+$) with a mass of 221.0693 (calcd 221.0670). The parent ion fragmented giving ions with *m/z* = 203.0591 (calcd for $C_9H_{15}OS_2$, 203.0565, $[M - OH]^+$), 131.0551 (calcd for $C_6H_{10}OS$, 131.0531, $[M\text{-}C_3H_5SO]^+$), and 113.0443 (calcd for C_6H_9S, 113.0425, $[M\text{-}C_3H_5SO$ and $H_2O]^+$). More than four ajothiolane isomers (**14**) were present as indicated by pairs of methyl doublets at δ 1.00−1.40 ppm, e.g., δ 1.01 [2%], 1.07/1.28 [73%], 1.14/1.35 [20%], and 1.19/1.30 [5%] (Figure S1); LC-MS/MS showed six peaks corresponding in mass to ajothiolane (**14**) as its proton and sodium ion adducts. Stable sulfenic acid **12a** shows an OH band in the IR at 3460 cm^{-1} and is devoid of significant bands in the S═O region (1000−1100 cm^{-1}).[22]

Table 1. Comparative ^{1}H and ^{13}C NMR Data for 10 (Garlicnins B) and 14 (Ajothiolanes)[b]



carbon #	10a	14a[a]	10b	14b[a]
2	5.04 (84.0)	5.06 (84.0)	5.07 (90.2)	5.10 (90.1)
3	1.98 (54.9)	2.00 (54.9)	1.92 (53.5)	1.93 (53−54)
4	2.15 (42.8)	2.18 (42.8)	2.35 (50.5)	2.40 (50.8)
5	4.08 (74.9	4.10 (74.9)	4.06 (73.5)	4.05 (73.4)
3-Me	1.04 (13.9)	1.06 (13.9)	1.11 (15.7)	1.14 (15.6)
4-Me	1.23 (18.3)	1.28 (18.3)	1.35 (17.9)	1.35 (17.9)
1′	3.33, 3.59 (55.6)	3.34, 3.61 (55.6)	3.43, 3.59 (54.4)	3.46, 3.59 (53−54)
2′	5.68 (125.1)	5.74 (125.1)	5.68 (125.8)	overlap
3′	5.36, 5.38 (124.5)	5.37, 5.41 (124.6)	5.35, 5.37 (124.3)	overlap

[a]By ^{1}H NMR, ca. 73% of **14a** and 20% of **14b**. [b]The ^{1}H NMR spectra of **9c** and **9d** each show two methyl groups at δ 0.95/1.19 and 1.10/1.30 ppm, respectively; the ^{1}H NMR spectrum of isomers of **14** shows pairs of doublets (*J* = 6.7−6.9 Hz) at δ 1.01/1.19 and 1.09/1.30, corresponding to the respective doublet peaks seen for **9c** and **9d**, which have similar coupling constants.

(2*E*)-3-(Methylsulfinyl)-2-propenyl 2-propenyl disulfide (16; methylisoajoene). DART-MS indicated $C_7H_{12}OS_3$+H^+, *m/z* 209.0130, calcd *m/z* 209.0129. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) determined in both $CDCl_3$ and C_6D_6 is summarized in Table 4. The ^{1}H and ^{13}C NMR spectra in $CDCl_3$ are identical to those reported for **10** (garlicnin D); the ^{1}H NMR spectra are both missing one peak, which is seen with C_6D_6 as solvent. Compound **16** has been previously reported.[30]

Separation of Stereoisomers of Ajothiolane 14 by Preparative HPLC. An ajothiolane-rich sample (50 mg) is diluted in MeOH and injected on a preparative HPLC column (RP-C_{18} column, 2.5 × 40 cm; Dynamax 60A model 83-221-C (S/N 10634), Rainin Instrument Company, Inc.). A gradient of MeOH and H_2O + 0.05% formic acid is applied at 10 mL/min, the proportion of MeOH varying as follows (% MeOH (time(min)): 15 (0 min), 15 (10 min), 25 (30 min), 25 (55 min), 40 (65 min), 40 (70 min), 80 (75 min), and 80 (100 min). UV detection is not sensitive for ajothiolanes (limited absorption of UV light); therefore, fractions (15 mL) are collected, and the presence of **14** is assessed by DART-MS. The fractions containing **14** are salted with NaCl then extracted by distilled CH_2Cl_2. Organic layers are dried ($MgSO_4$), concentrated, and dissolved in $CDCl_3$ for NMR analysis. A total of 22 different methyl doublets were identified by NMR (11 stereoisomers) from these fractions. Unfortunately, grease and other pollutants from the HPLC system led to poor quality spectra. Two stereoisomers are consistently major in the mixture: ajothiolane **14a** (methyls at 1.08 and 1.29 ppm) and **14b** (methyls at 1.15 and 1.35 ppm). Stereoisomers of **14** were also separated by UPLC-MS using the above-described conditions.

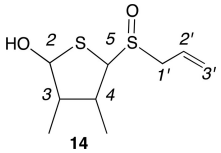
Analysis of Sulfur Chemical Form Using Sulfur K-Edge X-ray Absorption Spectroscopy (XAS). Sulfur K-edge X-ray absorption spectra were recorded at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamlines 6−2 and 4−3. Both were equipped with helium flight paths having 6.3 μm-thick polypropylene windows to minimize X-ray attenuation, and with Si(111) double crystal monochromators. Spectra were measured by monitoring the total X-ray fluorescence using a Stern-Heald-Lytle detector (the EXAFS company, Pioche NV, USA).[31] The incident X-ray energy was calibrated with reference to the spectrum of a solid anhydrous sodium thiosulfate ($Na_2S_2O_3$) standard using the literature value of 2469.2 eV as the energy of maximum intensity of the lowest energy K-edge absorption peak.[32] The spectrum of sodium thiosulfate was also used to optimize the energy resolution, by adjusting apertures in the different beamlines. Analysis of X-ray absorption spectroscopic data used the EXAFSPAK program suite.[33] Spectra were normalized to the edge-jump to give a per-unit-sulfur absorption spectrum, using the spline method, which employs a rigid spline above the absorption edge to estimate the edge jump.

Density Functional Theory (DFT) Calculations. DFT geometry optimizations were carried out using DMol^3 and Biovia Materials Studio Version 2017 R2[34,35] using the Perdew−Burke−Ernzerhof functional for both the potential during the self-consistent field procedure and the energy. DMol^3 double numerical basis sets included

Table 2. Comparative ^{1}H (and ^{13}C) NMR Data for 14a (Ajothiolane), 4 (Cepathiolane), 6 (3,4-Dimethyl-2-hydroxy-5-(phenyldithio)thiolane),[4] 11 (Allium Sulfoxide A1),[20] and Thiolane-2-ol (26a)[27]

carbon	14a	4	6	11	26a
2	5.06 (84.0)	5.20 (90.9)	5.29	5.00 (94.2)	5.53−5.59 (81.8)
3	2.00 (54.9)	1.99−2.06 (48.8)	2.46	2.27 (45.7)	(32.8)
4	2.18 (42.8)	2.10−2.19 (49.0)	2.08	2.65 (44.6)	(27.8)
5	4.10 (74.9)	4.87 (59.9)	4.61	3.87 (72.9)	(40.7)
3-Me	1.06 (13.9)	1.15 (14.7)	1.25	1.01 (12.9)	
4-Me	1.28 (18.3)	1.07 (14.6)	1.13	1.40 (14.3)	
1′	3.34, 3.61 (55.6)	6.52 (129.2)		$CH_3S(O)$ 2.40 (36.0)	
2′	5.74 (125.1)	6.65 (137.7)			
3′	5.37, 5.41 (124.6)	2.00 (18.0)			

Table 3. Summary of 1D and 2D NMR Data for the Major Stereoisomer of Ajothiolane (14)



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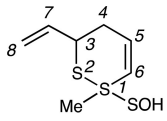
	δ ^{13}C (ppm)	δ ^{1}H (ppm)	multiciplicity (J in Hz), integration	HMBC (^{1}H–^{13}C correlation)	INADEQUATE (^{13}C–^{13}C corr.)	COSY (^{1}H–^{1}H corr.)
–OH		4.34	d, J = 11.0, 1H			
2	84.0	5.05	dd, J = 8.2, 1.0, 1H	5.05 to 42.8 and 74.9	84.0 to 54.9	5.05 to 2.00
3	54.9	2.00	ddd, J = 10.0, 7.0, 3.7, 1H	2.00 to 13.9, 18.3, 42.8 and 84.0	54.9 to 13.9, 42.8 and 84.0	2.00 to 1.06
CH_3-3	13.9	1.06	d, J = 6.7, 3H	1.06 to 42.78, 55 and 84.0		1.07 to 2.01
4	42.8	2.18	dd, J = 13.9, 9.4, 1H	2.18 to 18.3, 54.9 and 74.9	42.8 to 18.3	2.19 to 1.28
CH_3-4	18.3	1.27	d, J = 6.7, 3H	1.27 to 42.8, 54.9 and 74.9		1.27 to 2.17
5	74.9	4.09	d, J = 5.8, 1H	4.09 to 18.3, 42.8 and 84.0	74.9 to 42.8	4.11 to 2.18
1′	55.6	3.61 3.35	dd, J = 12.5, 6.5, 1H dd, J = 12.6, 8.5, 1H	3.61 to 74.9 and 125.1 3.35 to 74.9 and 125.1		3.61 to 3.35
2′	125.1	5.72	m, 1H	5.74 to 55.6		5.71 to 5.37
3′	124.6	5.41 5.37	s, 1H d, J = 6.7, 1H	5.37, 5.41 to 55.6 and 125.1		5.37 to 5.71 5.40 to 5.70

Table 4. Comparison of the Spectral Data for Methylisoajoene (25) in $CDCl_3$ and C_6D_6, 9 in $CDCl_3$, and Yoshida's Isolated Sample[30] of 25 in C_6D_6



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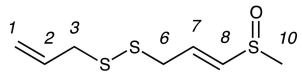


25

carbon #	**25**, ^{1}H, $CDCl_3$	**25**, ^{1}H, C_6D_6	**25**, ^{13}C, $CDCl_3$	**25**, ^{13}C, C_6D_6	**9**, ^{1}H, $CDCl_3$	**9**, ^{13}C, $CDCl_3$	**25**, ^{1}H, C_6D_6, Yoshida[30]	**25**, ^{13}C, C_6D_6, Yoshida[30]
1, CH_2=	5.21 (dd, J = 17.0, 1.2, 1H) 5.16 (d, J = 9.9, 1H)	5.03 (d, J = 16.9, 1H) 4.95 (d, J = 10.4, 1H)	118	118.9	5.16 (d, J = 12, 1H; Ha-8)	119.1 (C-8)	4.77 (dd, J = 17, 0.9, 1H)	118.7
2, =CH	5.82 (ddt, J = 17.2, 9.9, 7.3, 1H)	5.60 (dt, J = 17.1, 7.3, 1H)	132	133.1	5.79 (1H, m, H-7)	133.2 (C-7)	5.3 (m, 2H)	133.5
3 CH_2	3.33 (d, J = 7.3, 2H)	3.00 (d, J = 7.4, 2H)	42	42.9	3.30 (1H, d, J = 7.5; H-3)	42.5 (C-3)	2.74 (d, J = 7.4, 2H)	42.5
6 CH_2	3.45 (d, J = 6.2, 2H)	2.86 (d, J = 7.6, 2H)	39	38.8	3.43 (d, J = 9.8, 2H; H_2-4)	39.4 (C-4)	2.65 (d, J = 1.3, 2H)	38.3
7 CH=	6.44–6.46 (m, 1H)	6.38 (dt, J = 14.9, 7.5, 1H)	134	131.4	6.42 (2H, s, H-5, H-6)	133.6 (C-5)	6.1 (dt, J = 14.6, 7.6, 1H)	132.2
8 =CH	6.44–6.46 (m, 1H)	5.74 (d, J = 14.7, 1H)	137	137.9		137.3 (C-6)	5.6 (d, J = 14.6, 1H)	138.4
9 CH_3	2.65 (s, 3H)	1.87 (s, 3H)	41	39.8	2.62 (3H, s, S-1-CH_3)	41.3 (S-1-CH_3)	1.7 (s, 3H)	40.4

polarization functions for all atoms with all-electron core treatments. Solvation effects, when present, were modeled using the conductor-like screening model (COSMO)[36] in DMol^3 with a dielectric value representing toluene (ε = 2.38). Convergence during geometry optimizations were taken to be achieved when changes in energies were less than 0.05 kJ·mol^{-1}, the maximum force was less than 10 kJ/mol Å, and the maximum atomic displacement was less than 0.05 Å. While the presence of local and unexplored energy minima cannot always be rigorously excluded in any geometry optimization process, the tolerances for the energies determined by DFT geometry optimization are therefore assumed to be 0.05 kJ/mol. DFT simulations of near-edge spectra were calculated using the StoBe-deMon code[37] employing the so-called half-core-hole approximation for the core-hole, incorporating relaxation of selected excited states at the absorption edge, employing the coordinates from DMol^3 geometry optimizations.

StoBe-deMon calculations employed the nonlocal exchange function of Perdew and Wang[38] and the Perdew correlation fuctional.[39,40] The (6311/311/1) basis set was used for C and (311/1) for H. In order to localize the core hole to the sulfur atom of interest, the atom of interest (the absorber) employed the IGLO III basis set,[41] and in those species with more than one sulfur, an effective core potential combined with the (311/211/1) basis set was employed for the spectator sulfur atom. Interpolation of the exchange-correlation potential employed auxiliary the basis sets (5,4;5,4) for S, (5,2;5,2) for C, and (3,1;3,1) for H. A pseudopotential (4:6,4) was applied to the spectator sulfur atom. Calculations were carried out in this way for each sulfur atom in multisulfur species. Convolution with pseudo-Voigt line shape functions was conducted as previously described,[42] with a shift of calculated transition energies of +5.2 eV to provide alignment with experiment.

RESULTS AND DISCUSSION

Isolation, NMR Studies, and Mechanistic Considerations. With DART-MS,[21,26] we reexamined research done by one of us more than 25 years ago, which suggested the presence of families of heterocyclic organosulfur compounds of unknown structure in *Allium* extracts, wondering if processes analogous to those shown in Figure 1 involving attack on **3** by other nucleophiles could occur in garlic extracts to afford analogues of **4**. Indeed, analysis of garlic preparations by DART-MS indicated

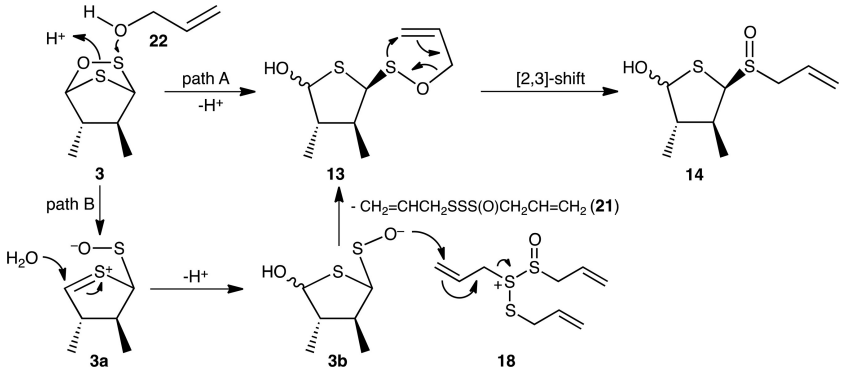


Figure 4. Proposed reaction sequence for the formation of **14** from **3** in garlic extracts.

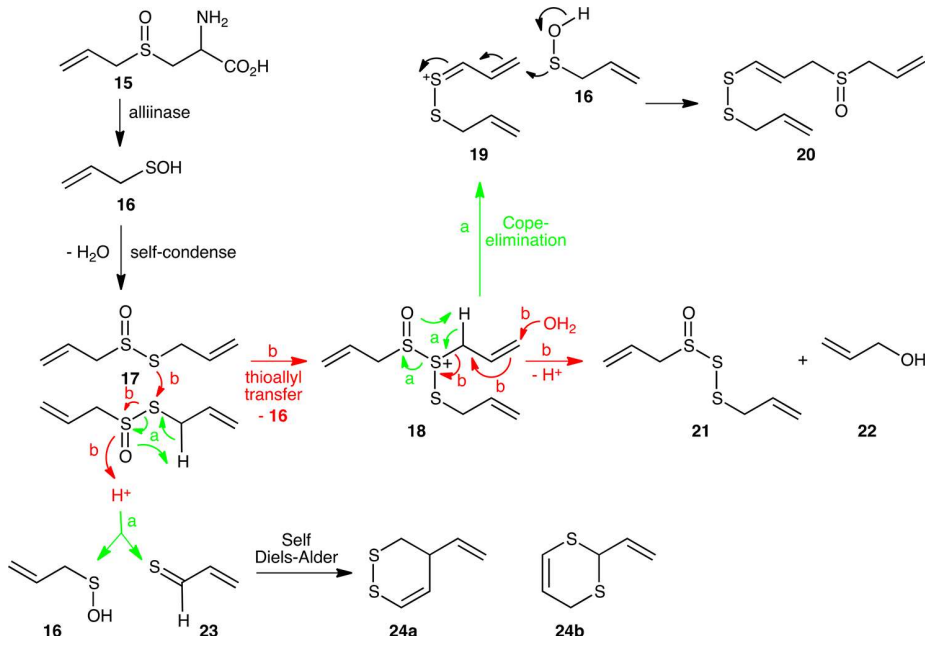


Figure 5. Major reactions occurring upon maceration of garlic and alliinase induced release of 2-propenesulfenic acid (**16**) from alliin (**15**): conversion of allicin (**17**) to ajoene (**20**) via intermediates **18** and **19**, to trisulfide *S*-oxide **21** and allyl alcohol (**22**), and to vinyl dithiins **24a** and **24b** via thioacrolein (**23**).

the presence of compounds of mass $C_9H_{16}O_2S_2$ ($C_9H_{16}O_2S_2$+H^+ *m/z* 221.070), also identified as its NH_4^+ adduct (*m/z* 238.094). We speculated that the *m/z* 221 peak was due to a condensation product between **3** and allyl alcohol (**22**, Figure 4) since *m/z* 221 differs from the mass of **3** ($C_6H_{10}S_2O$+H^+, *m/z* 163) by the addition of C_3H_6O (*m/z* 58), and allyl alcohol is known to be present in garlic extracts (Figure 5).[21] Nucleophilic attack by allyl alcohol on isomer **3** could afford allyl sulfenate **13**, which would undergo a 2,3-sigmatropic rearrangement[43] to **14** (path A). Alternatively (path B), **3** could ring-open to intermediate **3a** followed by attack by water and O-allylation of the sulfenate anion by sulfonium ion **18**, giving **13** and **21** (path B). S-Allylation of the sulfenate anion by **18** could also directly give **14**. Figure 5 illustrates the likely sequence of steps from alliin (**15**) via 2-propenesulfenic acid (**16**), allicin (**17**), and sulfonium ions **18** and **19** to ajoene (**20**), diallyl trisulfide *S*-oxide (**21**), and allyl alcohol (**22**) based on DART-MS studies of crushed garlic.[21]

We became aware of a series of papers by Nohara et al. describing biologically active sulfur-heterocycles of unprecedented structures isolated from *Allium* extracts and characterized primarily by spectroscopic methods. Among the compounds they isolated from garlic extracts are a series of compounds **10a–d** of mass 220 termed garlicnins B_{1-4} having the same formula, $C_9H_{16}O_2S_2$, as our compound **14**. Given the differences in proposed structure, we sought to isolate isomers of **14**, which we term "ajothiolane" (ajo is Spanish for garlic), to compare spectroscopic properties with those of Nohara's garlicnin B_{1-4}. Our procedure for the isolation of ajothiolane is summarized in Figure 6. Thus, from 6 kg of peeled garlic, macerated in acetone and then extracted with CH_2Cl_2 followed by column chromatography, fractions were obtained which were analyzed by DART-MS, showing the presence of known garlic-derived compounds vinyldithiins (**24a,b**), allicin (**17**), ajoene (**20**), and allyl methyl thiosulfinates ($MeS(O)SCH_2CH{=}CH_2$ and $MeSS(O)CH_2CH{=}CH_2$), along with new compounds showing MH^+ ions at *m/z* 221 (ajothiolane/garlicnin B_{1-4}) and at *m/z* 209 (Nohara's garlicnin D). Sequential column and preparative thick layer chromatography (TLC) gave pure fractions of the *m/z* 221 and 209 compounds. As can be seen

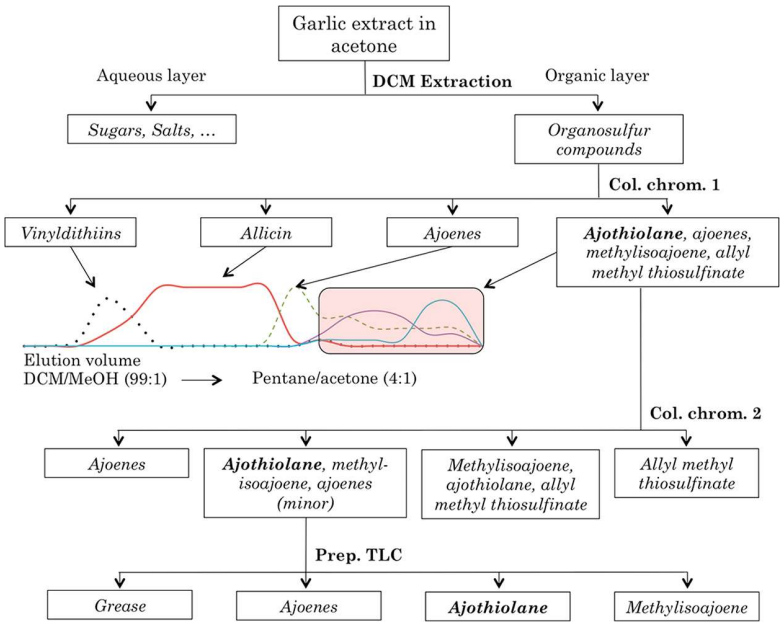


Figure 6. Procedure for the separation and isolation of garlic extract components.

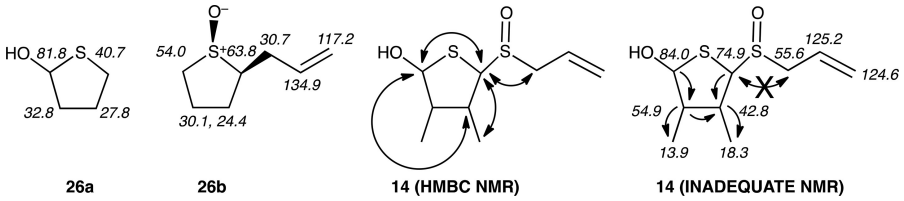


Figure 7. ^{13}C NMR chemical shifts for model compounds thiolane-2-ol (**26a**) and *cis*-2-allylthiolane 1-oxide (**26b**);[44] long-range HMBC $^{1}H-^{13}C$ NMR correlations (HMBC) for **14**; and sp^3-sp^3 INADEQUATE $^{13}C-^{13}C$ NMR correlations for **14**.

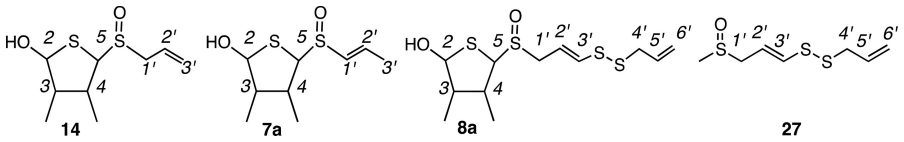
from Table 1, there was excellent agreement between our ^{1}H and ^{13}C NMR data for compounds **14a,b** and Nohara's compounds **10a,b**; similar agreement is found for the NMR data for the m/z 209 compound **25** we term "methylisoajoene" isolated by us and Nohara's compounds **9**, as shown in Table 4. A literature search revealed that **25** had been previously isolated from garlic extracts and fully characterized by Yoshida et al.,[30] whose NMR data is included in Table 4.

A challenge we faced in the above procedure was to recover very minor, polar components of the total extract, resolving them from a variety of other coeluting compounds of similar polarity. While the low preparative TLC R_f value (0.1) of the target compounds required repeating the elution multiple times until sufficient purity was realized, the advantage of preparative TLC over column chromatography was the ability to recover weakly eluting compounds. Because we were isolating very minor components, it was essential to distill all solvents to remove traces of plasticizers; furthermore, contact of all extracts with rubber and plastics of any kind had to be strictly avoided.

A second challenge we faced was resolving the differing interpretations of the 1D and 2D NMR data for **10** and **14**. Our chemical shift data are fully consistent with comparative data shown in Table 2 and Figure 7 for **14a**, **4**, **6**, **11**, and thiolane-2-ol (**26a**) for ^{1}H and ^{13}C values for S−**CH**−OH C-2 of thiolane containing an OH-group as well as for S−**CH**−S(O) or S−**CH**−S. Furthermore, the ^{13}C NMR data for proposed structure **10** is inconsistent with published NMR data for *cis*-2-allylthiolane 1-oxide (**26b**).[44] The different interpretations of COSY and HMBC 2D NMR data are likely due to failure to consider the effect of long-range (^{4}J) couplings which occur across heteroatoms,[5,6,9] particularly when there is a W-effect, which can erroneously suggest direct bonding when atoms are in fact separated by a heteroatom (see Figure 7). To resolve any possible ambiguity, we employed an INADEQUATE (Incredible Natural Abundance DoublE QUAntum Transfer Experiment) $^{13}C-^{13}C$ correlation experiment. Given the low natural abundance of ^{13}C, it was necessary to adjust conditions to enhance sensitivity, employing 24 h of accumulation with 45 mg of sample, as described in the Materials and Methods section. The results, shown in Table 3, Figure 7 and Supporting Information Figures S6A,B confirm the absence of $^{13}C-^{13}C$ correlation between C5 and C1′ as well as between C2 and C5, favoring structure **14a** and making structure **10a** implausible.

Our proposed structure for **14** is a 5-allylsulfinylthiolane-2-ol rather than an isomeric 5-allylthiylthiolane-2-ol 1-oxide. The latter structure is precluded not only based on spectroscopic and biosynthetic considerations but also because α-hydroxysulfoxides are known to be kinetically unstable, rapidly decomposing to carbonyl compounds and sulfenic acids, which then undergo further transformations.[45] Finally, based on the close similarity of NMR data for onionin A_1 (**7**) and garlicnin A (**8**) with that for ajothiolane **14** (and the side chain of **8** with the NMR data for the known[46] garlic extract constituent (1*E*)-3-(methylsulfinyl)-1-propenyl 2-propenyl disulfide (**27**) (see Table 5), termed garlicnin L-2 by Nohara[47]) we propose revised structures **7a** and **8a** for **7** and **8**, respectively, as shown in

Table 5. Comparison of NMR Data for Ajothiolane (14), Proposed Revised Structure 7a for Previously Reported Onionin A_1 (7), Proposed Revised Structure 8a for Previously Reported Garlicnin A (8), and ((1*E*)-3-(Methylsulfinyl)-1-propenyl 2-propenyl Disulfide (27)[a]



	14 δ ^{13}C (ppm)	**14** δ ^{1}H (ppm)	**7a** δ ^{13}C (ppm)	**7a** δ ^{1}H (ppm)	**8a** δ ^{13}C (ppm)	**8a** δ ^{1}H (ppm)	**27** δ ^{13}C (ppm)	**27** δ ^{1}H (ppm)
−OH		4.34, d, *J* = 11.0, 1H		4.31, d, *J* = 10.9, 1H		4.32, d, *J* = 11.3, 1H	[37.4] (CH_3)	[2.60] (CH_3)
2	84.0	5.05, dd, *J* = 8.2, 1.0, 1H	83.5	4.99, dd, *J* = 6.9, 1.7, 3H	84.0	5.00, d, *J* = 11.4		
3	54.9	2.00, ddd, *J* = 10.0, 7.0, 3.7, 1H	55.0	1.97, m, 1H	54.9	1.97		
CH_3-3	13.9	1.06, d, *J* = 6.7, 3H	13.9	1.05, d, *J* = 6.3, 3H	13.8	1.01, d, *J* = 6.8		
4	42.8	2.18, dd, *J* = 13.9, 9.4, 1H	42.9	2.16, m, 1H	43.3	2.12		
CH_3-4	18.3	1.27, d,, *J* = 6.7, 3H	18.1	1.28, d, *J* = 6.9, 3H	18.4	1.25, d, *J* = 6.8, 3H		
5	74.9	4.09, d, *J* = 5.8, 1H	79.2	4.01, d, *J* = 5.8, 1H	78.2	3.91, d, *J* = 5.7, 1H		
1′	55.6	3.61, dd, *J* = 12.5, 6.5, 1H 3.34, dd, *J* = 12.6, 8.5, 1H	131.7	6.03, dd, *J* = 13.8, 1.7, 1H	53.0	3.28−3.45	56.5	3.50, d, *J* = 7
2′	125.1	5.74, m, 1H	139.6	6.47, dq, *J* = 6.9, 13.8, 1H	116.8	5.85	134.9	5.45−6.28
3′	124.6	5.41, s, 1H 5.37, d, *J* = 6.7, 1H	18.3	1.90, dd, *J* = 6.9, 1.7, 3H	119.4	6.31, d, *J* = 14.9	116.7	6.37, d, *J* = 15
4′					41.4	3.28−3.45	41.4	3.36
5′					132.6	5.85	132.5	5.45−6.28
6′					123.9	5.39, 5.40	119.2	4.94−5.30

[a]Isomers of compound **6b**, **6c**,[7,8] and **6d**[9] (Figure 1) show C2 ^{13}C/^{1}H shifts at δ 83.4−86.6/5.14−5.18, 83.6−86.6/5.17−5.19, and 83.0/5.15−5.17, respectively; the ^{13}C/^{1}H shifts of a reported isomer of **7a**[7,8] are similar to those described in Table 5 for **7a**.

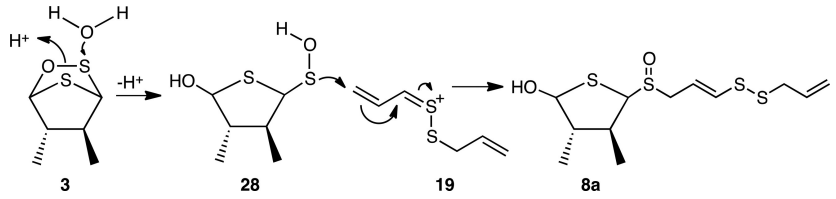


Figure 8. Proposed formation of **8a** in garlic extracts from the hydrolysis of **3**, where **8a** is the revised structure for garlicnin A (**8**).

Table 5; structures of onionins A_{2-3} should be similarly revised. With limited characterization, **7a** is described in two patents as being present in onion extracts.[7,8] Compound **8a** could be formed as shown in Figure 8 by hydrolysis of **3** followed by reaction of **28** with intermediate **19** derived from **18**, as proposed in Figure 5 for the formation of ajoene (**20**).[46]

In Figures 1, 4, and 8, nucleophilic attack on the oxygen-adjacent sulfur in compound **3** is proposed as a key mechanistic step. To examine this possibility, density functional theory (DFT; B3LYP/6-3111G**) electronic structure calculations were carried out on the parent compound, 2,7-dithia-3-oxabicyclo[2.2.1]heptane. They showed that the oxygen-adjacent sulfur has a net charge of 0.44, which is more positive than the bridging sulfur with a net charge of 0.16 (see Supporting Information Table S2). Since the oxygen-adjacent sulfur is more electron-depleted than the nonoxygen-adjacent sulfur atom, the difference in electrophilicity would be expected to favor nucleophilic attack on the oxygen-adjacent sulfur atom.

UPLC-MS/MS Separation and Mass Spectral Fragmentation Structural Evidence. ^{1}H NMR analysis of ajothiolane fractions indicated the presence of 22 different doublets, suggesting the presence of as many as 11 stereoisomers (with five chiral centers, 16 pairs of enantiomers are predicted). Use of a reverse phase column on both a UPLC-QTOF and UPLC-Orbitrap under MS/MS conditions afforded chromatograms showing at least seven stereoisomers of differing retention times with *m*/*z* 221.06 and 234.04, corresponding to $[C_9H_{16}O_2S_2+H]^+$ and $[C_9H_{16}O_2S_2+Na]^+$, respectively (see Supporting Information Figure S3). At low collision energy on a SCIEX TripleTOF MS/MS high resolution instrument, major fragmentation processes of the protonated molecular ion $C_9H_{17}O_2S_2$ involved loss of water giving *m*/*z* 203 ($C_9H_{15}OS_2$) and loss of C_3H_5SO giving *m*/*z* 131 ($C_6H_{10}OS$). At higher collision energy, the major fragment corresponded to a loss of both C_3H_5SO and water giving *m*/*z* 113 (C_6H_9S; protonated 3,4-dimethylthiophene). While these fragmentation processes are reasonable for structure **14**, they are more difficult to explain for structure **10**.

Computational Results: Ground State Density Functional Calculations. DFT calculations were carried out for sets of 16 stereoisomers of 32 possible (considering enantiomers) alternative sulfoxide and sulfenic acid structures of ajothiolane, and the energetic differences are summarized in Table S1. The structures of four selected stereoisomers are shown in Figure 9.

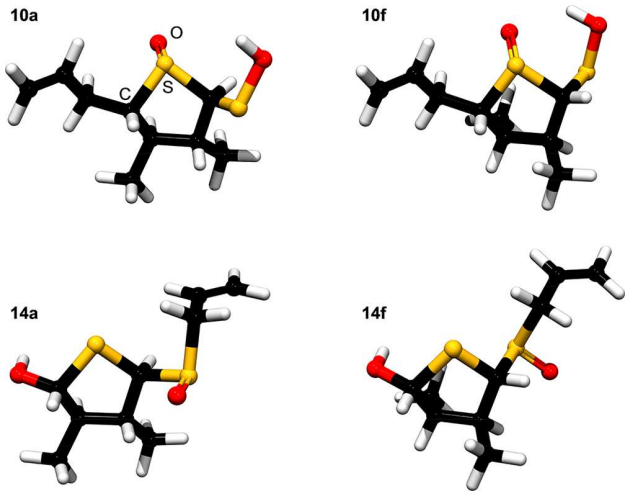


Figure 9. DFT energy minimized geometry optimized structures for four different $C_9H_{16}O_2S_2$ isomers (**10a**, **10f**, **14a**, and **14f**) from Table S1. The structures shown were calculated using a toluene solvation model.

As a check upon our calculations, the energies of a few selected enantiomers of both **10** and **14** were computed, and as expected, these were found to be identical within the energy tolerances specified by the geometry optimization refinement convergence criteria of 0.03 kJ·mol^{-1} (not illustrated). Considering matching pairs of stereoisomers for **10** and **14** in Table S1, the energies for the former are consistently higher in energy, on average by 44 kJ·mol^{-1}. For example, the computed energies for **10a** and **14a** differ by 39 kJ·mol^{-1}, with the smallest difference being between **10p** and **14p** at 10.5 kJ·mol^{-1}. For some stereoisomers of **10**, such as **10f**, a hydrogen bond between the sulfenic acid proton (−SOH) and the oxygen of the ring sulfoxide can form, and we estimate that this bond lowers the energies of these stereoisomers by ∼10−16 kJ·mol^{-1}. We also computed the energies of selected sulfinyl tautomers (−S(O)H)[48] of the sulfenic acids **10**, and these were found to be consistently higher in energy by as much as 90 kJ·mol^{-1}. Because an additional chiral center is introduced in these tautomers (the sulfinyl sulfur), the number of possible stereoisomers is doubled, and we elected not to compute all 32 of these (of 64 possible, considering enantiomers), but instead computed selected structures only. For the sulfinyl tautomers of **10** in which a hydrogen bond can form between the sulfinyl−SH and the oxygen of ring sulfoxide, such as the sulfinyl tautomer of **10f**, we estimate a stabilization of ∼10 kJ·mol^{-1} is provided by the formation of this bond. We also examined selected isomers of **14** in which the sulfoxide is present on the ring sulfur, and these were found to be higher in energy, for example, by 22 kJ·mol^{-1} for **14a**. However, this isomer is expected to be kinetically unstable as is known for other α-hydroxysulfoxides.[45]

From the calculations, we predicted that the most stable isomer is **14g** (rel E = 0.00), with the two methyl groups cis, followed by isomers **14b** (rel E = 1.87) and **14a** (rel E = 2.55). While **14g** may be the most stable isomer, the precursor bicyclic sultene **3** with cis methyl groups would be derived from the less abundant *E*,*Z*-isomer of **2b** (Figure 1).[3] The *cis*-dimethyl isomer of **3** would also be expected to be less stable than isomers of **3** with *trans*-methyl groups. The minor isolated isomer **14b** is predicted to be slightly more stable than the major isolated isomer **14a** with a computed energy difference between **14a** and **14b** of 0.68 kJ·mol^{-1}. On the basis of the nuclear Overhauser effect spectroscopy (NOESY) spectra of **10a** and **10b** reported by Nohara,[16] we propose the full structures **14a** and **14b** for **10a** and **10b**, respectively (Figure 10).

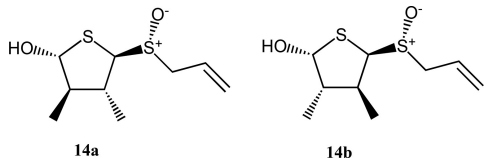


Figure 10. Proposed structures for **14a** and **14b**.

X-ray Absorption Spectroscopy (XAS) Studies. Figure 11 shows the sulfur K-edge X-ray absorption near-edge

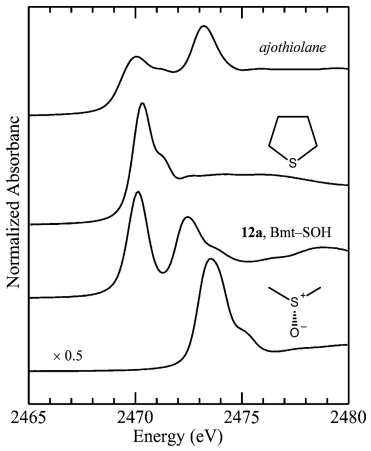


Figure 11. Comparison of the sulfur K near-edge X-ray absorption spectra of ajothiolane, a cyclic organic sulfide (thiolane), a sulfenic acid (**12**a, BmtSOH),[22] and dimethyl sulfoxide. The vertical scale of the dimethyl sulfoxide spectrum has been adjusted by a factor of 2. All compounds were solutions in toluene at ∼100 mM total sulfur concentration.

spectrum of ajothiolane compared with several standard compounds representing the various sulfur functional groups in **10** and **14**, including the stable sulfenic acid, 4-*tert*-butyl-2,6-bis[(2,2″,6,6′′-tetramethyl-*m*-terphenyl-2′-yl)methyl]-benzenesulfenic acid (**12a**, BmtSOH). We have previously used sulfur K-edge XAS to study onions and shiitake mushrooms,[49,50] but there are no previous reports of XAS studies of sulfenic acids. This is not surprising, given the short lifetimes of naturally formed sulfenic acids. With a stable sulfenic acid **12a** available, the experimental spectrum is compared with two simulated spectra corresponding to sulfoxide (e.g., **14**) and sulfenic acid (e.g., **10**) models, created by adding the spectra corresponding to the relevant sulfur functional groups in Figure 10. The ajothiolane spectrum is substantially broader than those of the standard compounds (Figure 12), which may be due to the presence of stereoisomers. Despite this, both ajothiolane and sulfoxide model spectra exhibit a distinct feature at 2471.2 eV (marked in Figure 12) that is not present in the sulfenic acid model. Figure 12 also compares the StoBe deMon DFT computed spectra for **10a** and **14a**. Similar to the simulation based on standard spectra, the computed sulfenic acid model spectra lack the feature at 2471.2 eV, which is present in the sulfoxide model and the experimental spectra. Taken together,

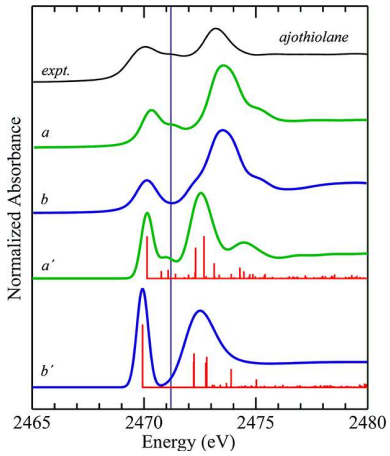


Figure 12. Comparison of the experimental sulfur K-edge near-edge spectra of ajothiolane (*expt.*) with different simulated spectra. Traces *a* (green line) and *b* (blue line) compare the simulated sulfoxide (green line) and sulfenic acid (blue line) model, respectively, comprising 50/50% DMSO and thiolane (*a*), and 50/50% BmtSOH (**12a**) and DMSO (*b*). Traces *a′* and *b′* compare the DFT (StoBe/DeMon) half-core-hole computed spectra for the sulfoxide **14a** and sulfenic acid **10a**, respectively. The curves convoluted with a line shape function are shown as green and blue lines (*a′* and *b′*), whereas the individual computed transitions are shown as the red stick spectra. Note the "bump" at ∼2471.2 eV, present in the experimental spectrum of ajothiolane, indicated by the gray vertical line, that is present in the sulfoxide models a and a' but not in the simulated and computed sulfenic acid models b and b'.

these results indicate that the most probable structure for ajothiolane is sulfoxide **14**.

In conclusion, it is clear from the above that compounds **9** and **10** have been misidentified by Nohara et al. and that our suggested ajothiolane structures are more in line with known structures of other *Allium* organosulfur compounds. On the basis of our work, we suggest that compounds **7** and **8** have also been misidentified and suggest alternative structures analogous to that proposed for **14**. Given the reported biological activity of **7**−**10**, revising the structures is a prelude to synthesis. We conclude that 3,4-dimethylthiolanes may be a common motif in *Allium* chemistry, representing a rich area for future study, and extending the growing list of thiolane-containing natural products such as biotin,[51] *Nuphar* alkaloids,[52,53] *Salacia* thiosugar thiolanium sulfonium salts,[54] *Breynia* spiroketal glycosides,[55] *Streptomyces*-derived tetronothiodin and albomycin,[56,57] and lanostane sulfides in crude oils,[58,59] in addition to the *Allium*-derived thiolanes described above.[4−19]

Because the intermediates leading to the formation of **14** are achiral, it is surprising that Nohara reports optical activity for garlicnin B_{1-4}. If this optical activity is not due to traces of adventitious chiral impurities, then it is possible that *Allium* enzymes may influence the several steps leading to the formation of 3,4-dimethylthiolanes as well as related compounds found in *Allium* extracts. This possibility remains to be explored.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b03638.

Separation methods and selected 1D and 2D NMR spectra for **14**, **16**, and **17**; computed energies for stereoisomers of **10** and **14**; DFT calculations for 2,7-dithia-3-oxabicyclo[2.2.1]heptane (PDF)

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Author Contributions

E.B., B.D., and B.B. conceived the natural products studies, and B.D. and B.B. performed separations and analyses. R.S. performed the UPLC chromatographic separations and analyses. G.N.G., E.Y.S., J.J.H.C., I.J.P., and E.M.R. conceived and performed the XAS studies and/or associated computations and modeling, K.G. provided the stable sulfenic acid samples and participated in their XAS analysis. E.B. wrote the paper.

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Notes

The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH.

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While we name ajothiolane (**14**) 5-(allylsulfinyl)-3,4-dimethylthiolane-2-ol, the *Chemical Abstracts* name for **14** would be tetrahydro-3,4-dimethyl-5-[(2-propen-1-ylsulfinyl]thiophene-2-ol; similarly, thiolane-2-ol (**26a**) and *cis*-2-allylthiolane 1-oxide (**26b**) are named by *Chemical Abstracts* as tetrahydrothiophene-2-ol and *cis*-tetrahydro-2-(2-propenyl)thiophene 1-oxide, respectively.

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DEDICATION

This article is dedicated with warmest wishes to Professor E. J. Corey in honor of his 90th birthday.

ABBREVIATIONS USED

COSY, correlation spectroscopy; DART-MS, direct analysis in real time mass spectrometry; DFT, density functional theory; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum correlation; INADEQUATE, incredible natural abundance double quantum transfer experiment; IR, infrared; LFS, lachrymatory factor synthase; SSRL, Stanford Synchrotron Radiation Laboratory; UPLC, ultra-performance liquid chromatography; XAS, X-ray absorption spectroscopy.

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