

Applications of Direct Analysis in Real Time Mass Spectrometry (DART-MS) in *Allium* Chemistry. 2-Propenesulfenic and 2-Propenesulfinic Acids, Diallyl Trisulfane S-Oxide, and Other Reactive Sulfur Compounds from Crushed Garlic and Other Alliums

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Through the use of direct analysis in real time mass spectrometry (DART-MS), 2-propenesulfenic acid, an intermediate long postulated as being formed when garlic (*Allium sativum*) is crushed, has been detected for the first time and determined by mass spectrometric methods to have a half-life of <1 s at room temperature. Two other key intermediates, 2-propenesulfinic acid and diallyl trisulfane S-oxide, have also been detected for the first time in volatiles from crushed garlic, along with allicin and related thiosulfinates, allyl alcohol, sulfur dioxide, propene, and pyruvate as coproducts. A commercial dietary supplement containing garlic powder, which was sampled after crushing, was found to contain alliin, methiin, and S-allylcysteine and produced allicin upon addition of water. DART-MS detection of 1-butenesulfenic acid from the ornamental *A. sicutum* is also reported. (Z)-Propanethial S-oxide (onion lachrymatory factor), absent in garlic, is found to be formed from crushed elephant garlic (*Allium ampeloprasum*), consistent with the classification of this plant as a closer relative of leek than of garlic. Mixtures of thiosulfinates, lachrymatory thial S-oxides, and related compounds are directly observed from crushed leek (*Allium porrum*) and Chinese chive (*Allium tuberosum*). Disulfanes and polysulfanes are detected only when the *Allium* samples are heated, consistent with earlier conclusions that these are not primary products from cut or crushed alliums.

KEYWORDS: DART mass spectrometry; garlic; elephant garlic; leek; 2-propenesulfenic acid; 2-propenesulfinic acid; propanethial S-oxide

INTRODUCTION

2-Propenesulfenic acid, $\text{CH}_2=\text{CHCH}_2\text{S}-\text{O}-\text{H}$ (**2**), postulated in 1951 to be formed by the action of the alliinase enzyme on the precursor alliin (**1**) when a clove of garlic (*Allium sativum*) is cut (**Figure 1**), has long eluded direct detection because of its very facile self-condensation to allicin (**3**) (1–4). Other elusive compounds, including 2-propenesulfinic acid (**9**) and diallyl trisulfane S-oxide (**7**), have been postulated as intermediates in the decomposition of **3** to diallyl trisulfane (**12**), among other products (2, 3). We describe here a novel approach that for the first time allows direct observation and determination of half-lives for species such as **2**, **9**, and **12**, using direct analysis in real time mass spectrometry (DART-MS). We also demonstrate the utility of DART-MS in the analysis, under exceptionally mild conditions, of volatiles formed upon cutting garlic, elephant garlic (*Allium ampeloprasum*), leek (*Allium porrum*), and Chinese chive (*Allium tuberosum*).

We recently used DART-MS to characterize reactive secondary metabolites formed upon crushing genus *Allium* plants, including

previously unknown lachrymatory thial S-oxides ($\text{RCH}=\text{SO}$) and irritant thiosulfinates ($\text{RS}(\text{O})\text{SR}$) from the ornamental *Allium sicutum* (Mediterranean bells) as well as related compounds from onion (*Allium cepa*) (5, 6). These studies employed positive ion DART (PI-DART-MS), to produce $[\text{M} + \text{H}]^+$ ions for analyte molecules, such as sulfinyl (S^+-O^-) compounds, that have high proton affinities due to strong bond polarity. Protonation occurs when analyte molecules collide with protonated water clusters $[(\text{H}_2\text{O})_n + \text{H}]^+$. The resultant $[\text{M} + \text{H}]^+$ ions are measured under high-resolution (HR) conditions. This work takes advantage of the ability of DART to directly analyze gases, liquids, and solids in open air, without prior treatment, simply by momentarily holding the sample in the DART gas stream, either manually or using an automatic sampling device (7–9). If used under negative ion conditions, for example, NI-DART-MS, $[\text{M} - \text{H}]^-$ ions are formed from analyte molecules containing acidic functional groups through proton abstraction by gaseous $[\text{O}_2]^{*-}$ (10). This latter technique has been used to demonstrate the presence of S_n^- species ($n = 2-4$) in drywall manufactured in China that emitted a sulfur odor (11). Because sulfenic and sulfinic acids play a central role in the formation of reactive secondary metabolites from garlic and other alliums, we have used NI-DART-MS to examine this aspect of *Allium* chemistry.

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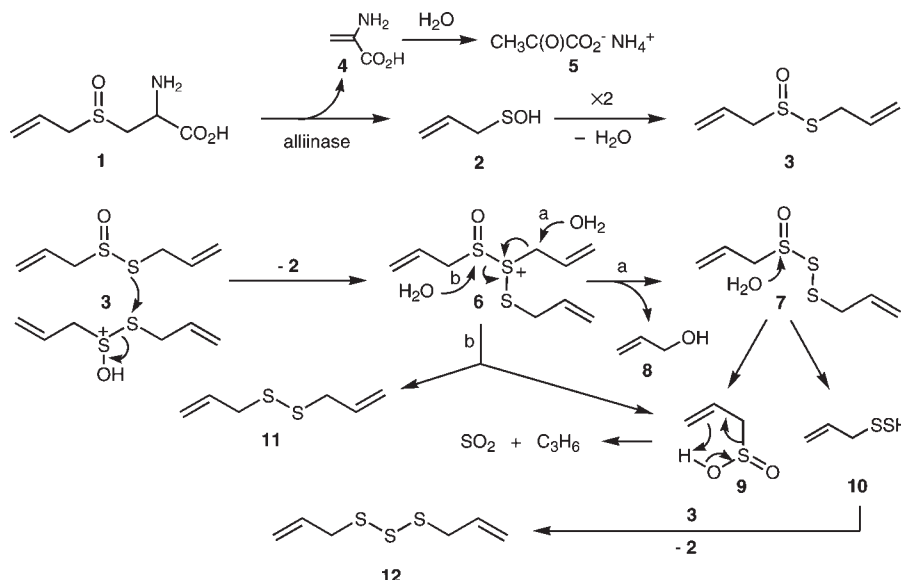


Figure 1. Reactions of alliin-derived compounds.

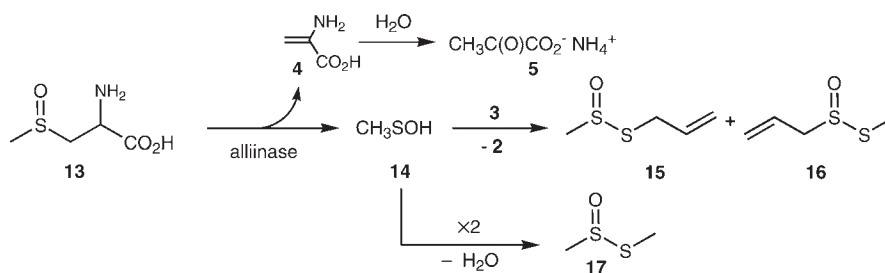


Figure 2. Reactions of methiin-derived compounds.

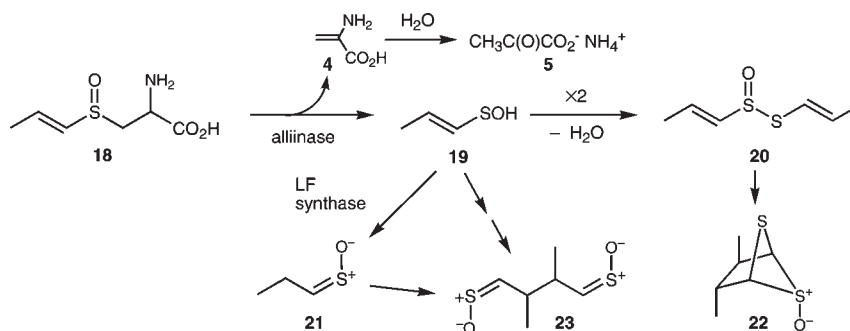


Figure 3. Reactions of isoalliin-derived compounds.

The chemistry that occurs when alliums are cut has been studied previously using techniques such as GC-MS, LC-MS, and NMR (2–4). Alliums store defense-related secondary metabolites as precursor *S*-alk(en)ylcysteine *S*-oxides, for example, alliin (1), which are enzymatically converted into unstable, chemically and biologically active agents in response to a challenge. When pest attack damages garlic, comingling of the normally separated alliinase and alliin occurs. The ensuing cascade of reactions releases a burst of defensive compounds including alliin (3) and aminoacrylic acid (4). The latter compound rapidly hydrolyzes to ammonium pyruvate (5). Alliin, an electrophilic defensive compound, is postulated to produce pain and inflammation in animal predators by activating TRPA1 and TRPV1, excitatory ion channels on primary sensory neurons of the pain pathway, to achieve deterrence (12–14). At room

temperature or with heating, alliin is converted by water into diallyl disulfane (11), trisulfane (12), and polysulfanes, principal components of the distilled oil of garlic (2).

The above processes are postulated to involve one or more intermediates, such as 2-propenesulfenic acid (2), thiosulfonium ion 6, diallyl trisulfane *S*-oxide (7), 2-propenesulfinic acid (9), and 2-propenepersulfide (10) (2, 4). None of these intermediates has been previously observed. Hydrolysis of 3 is thought to also afford allyl alcohol (8), sulfur dioxide, and propene. In addition to alliin, lesser amounts of cysteine *S*-oxides methiin (13; Figure 2) and isoalliin (18; Figure 3) also occur in intact garlic. Alliinase cleaves 13, giving methanesulfenic acid (14), and 18, giving 1-propenesulfenic acid (19). Sulfenic acids 14 and 19 can self-condense, forming the corresponding thiosulfates, 17 and 20, respectively, or react with alliin (3) to form isomeric thiosulfates such

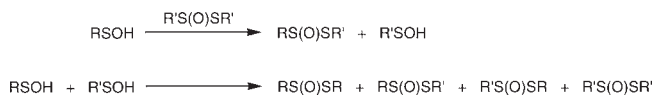


Figure 4. Formation of mixed thiosulfinates.

as **15** and **16** or mixed allyl 1-propenyl thiosulfinates (**Figure 4**) (2, 4). Bis-sulfine **23** ($\text{C}_6\text{H}_{10}\text{S}_2\text{O}_2$; (*Z,Z*)-*d,l*-2,3-dimethyl-1,4-butanedithial 1,4-dioxide) is commonly seen in alliums containing isoalliin (2, 4). In the case of onion, leek, and presumably elephant garlic, a second enzyme, lachrymatory factor synthase (LFS) (2, 15–17), absent in garlic, converts sulfenic acid **19** to (*Z*)-propanethial *S*-oxide (**21**), the onion lachrymatory factor (LF). Here, we illustrate the utility of DART-MS in characterizing under very mild conditions the organosulfur compounds formed when garlic, elephant garlic, leek, and Chinese chives are crushed.

MATERIALS AND METHODS

General Methods. DART operating conditions, as well as general analytical methods, are described elsewhere (5). For repetitive analyses an AutoDART-96 autosampler was employed.

Plant Samples and Chemical Standards. Garlic, elephant garlic, leek, Chinese chives, and a commercial dietary supplement containing garlic powder were purchased in local markets. *A. siculum* bulbs were obtained as described elsewhere (5). Standard compounds such as MeS(O)SMe and AllS(O)SAll were synthesized in Albany, NY, as described elsewhere (2).

Extraction of Elephant Garlic To Establish the Presence of Compounds **21 and **23**.** Two elephant garlic bulbs (200 g each) were peeled and homogenized with water in a food blender (200 mL). After 15 min, the homogenate was saturated with sodium chloride (40 g) and vigorously shaken with diethyl ether (2 × 200 mL). Each time the resultant emulsion was broken by centrifugation. The upper yellow ether layers were combined with an ether extract (100 mL) of the combined aqueous layers from centrifugation. The ether layers were dried (MgSO_4) and filtered through cotton. Ether was reduced to 20% of initial volume on a rotary evaporator, maintaining the flask at -15°C . The solution, kept at 0°C , was then carefully purged with Ar to remove remaining ether. The whole process was repeated with two more elephant garlic bulbs. Analysis of the concentrate by ^1H NMR (CDCl_3 ; TMS) showed minor peaks at δ 8.20 (t, $J = 7.8$ Hz) and 8.11 (d, $J = 9.6$ Hz) in a 1.4:1 ratio. Under these same conditions authentic **21** shows δ 8.17 (t, $J = 7.9$ Hz), whereas authentic **23** shows 8.09 (d, $J = 9.6$ Hz) (18). Most of the LF **21** originally present was presumably lost during the isolation procedure because the initial **21:23** ratio by PI-DART was 100:1.

Sampling Methods. An autosampler was used to precisely position a sample tube in the DART source region. Inverted melting point capillaries, which serve as sample tubes, were flamed before use to remove traces of lactic acid, found on skin, and other organic materials. With the inverted capillary positioned above the source area, the cleaned *Allium* plant sample was repeatedly punctured by the capillary, to disrupt the plant tissue (note: the term “crushed” is used below as a shorthand description of this capillary-puncture sampling procedure). The capillary was then automatically lowered into the proper source position. With practice, DART measurements began within 1–2 s of sampling. In the case of garlic, a peeled clove was used either at room temperature or prewarmed in a water bath to 40°C for maximum alliinase activity.

For NI-DART kinetic studies, data were continuously collected with 0.1 s scans while the m/z 89 region was monitored for the appearance of the 2-propenesulfenate anion at 89.0069, under high-resolution conditions to distinguish it from lactate at 89.0239. After 4 min, the capillary was automatically raised from the source region and the garlic was again sampled as described above. Repeated analysis was necessary to optimize the initial intensity of the m/z 89.0069 peak and maximize the instrument conditions to cleanly resolve the 89.0069 and 89.0239 peaks. When this was achieved, the intensity of the m/z 89.0069 peak, as it appeared and then disappeared within a few seconds, was plotted at 0.1 s increments as a fraction of total ion current. In this manner, the kinetic behavior of the m/z 89.0069 peak was compared to that of other NI peaks of interest, such as pyruvate, 2-propenesulfinate, sulfur dioxide, and background lactate.

Similar techniques were used under PI conditions to examine peaks that appeared at approximately the same time. For analyses done on a short time scale, the low gas velocity in the DART source should minimize evaporation effects (a concern for runs of longer duration).

In studies of the reproducibility of the PI-DART-MS analysis, garlic cloves were homogenized in a food processor. After 15 min, nine separate samples of the homogenate were taken up in melting point capillaries and analyzed as described above, integrating across each of the nine total ion chromatograms (TIC; peaks at m/z 163, 180, 325, 342 (all major), 137, 299 (both minor)). Good reproducibility was found, with a relative standard deviation (RSD) of 3.02%.

RESULTS AND DISCUSSION

Garlic. When a peeled garlic clove is punctured, as described above, and analyzed by PI-DART-TOF-MS at room temperature, the predominant products are $[\text{All}_2\text{S}_2\text{O} + \text{H}]^+$ (m/z 163), the adduct $[\text{All}_2\text{S}_2\text{O} + \text{NH}_4]^+$ (m/z 180), from the stoichiometric ammonia released on hydrolysis of aminoacrylic acid (4), and dimeric species $[(\text{All}_2\text{S}_2\text{O})_2 + \text{H}]^+$ (m/z 325) and $[(\text{All}_2\text{S}_2\text{O})_2 + \text{NH}_4]^+$ (m/z 342), with formula identification confirmed by HR-MS in all cases (see **Figure 7** and **Table 1**). In the DART gas flow, the TIC diminishes with time, and the m/z 325 and 342 peaks become less intense than the m/z 163 and 180 peaks. This is expected because the concentration of allicin diminishes as it evaporates and formation of the dimeric ions requires higher concentrations of allicin than the monomeric ions. The intensity of the dimeric peaks can be reduced by increasing the ionizing voltage. As described under Materials and Methods, good reproducibility was found by PI-DART-MS for the total ion chromatograms for nine separate samples of garlic cloves homogenized in a food processor and then taken up in capillaries (RSD = 3.02%).

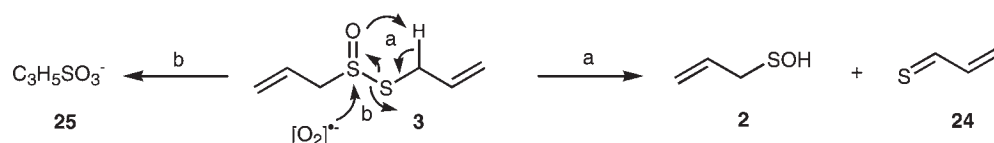
Of particular interest are various minor products, including diallyl trisulfane *S*-oxide (**7**; $[\text{C}_6\text{H}_{10}\text{S}_3\text{O} + \text{H}]^+$; m/z 195), allyl alcohol (**8**; $[\text{C}_3\text{H}_5\text{OH} + \text{H}]^+$; m/z 59), isomeric allyl methyl thiosulfinates **15** and **16** ($[\text{AllMeS}_2\text{O} + \text{H}]^+$; m/z 137), methyl methanethiosulfinate (**17**; $[\text{Me}_2\text{S}_2\text{O} + \text{H}]^+$; m/z 111), the mixed dimer of **15**, **16** + **3**, $[(\text{All}_2\text{S}_2\text{O})(\text{AllMeS}_2\text{O}) + \text{H}]^+$ (m/z 299), a bis-sulfine **23** ($[\text{O}=\text{S}=\text{CHCHMeCHMeCH}=\text{S}=\text{O} + \text{H}]^+$; m/z 179), and propene ($[\text{C}_3\text{H}_6 + \text{H}]^+$; m/z 43). The compositions of all peaks were confirmed by HR-MS. At room temperature there is no evidence in the PI-DART spectra from garlic (sampled as described above using a melting point capillary) for disulfanes or polysulfanes, which is consistent with these compounds being secondary products associated with decomposition of the thiosulfinates (19). When the DART measurements are made with a gas heater temperature of 250°C , additional significant peaks are seen including diallyl disulfane (observed m/z 147.0344, calculated m/z 147.0302 for $[\text{C}_6\text{H}_{10}\text{S}_2 + \text{H}]^+$), diallyl trisulfane (observed m/z 179.0054, calculated m/z 179.0023 for $[\text{C}_6\text{H}_{10}\text{S}_3 + \text{H}]^+$), and alliin (observed 178.0576, calculated m/z 178.0538 for $[\text{C}_6\text{H}_{11}\text{NO}_3\text{S} + \text{H}]^+$).

A limitation of the DART technique should be mentioned. From other work it is known that whereas the 2-propenyl (allyl) group is the dominant C_3H_5 fragment found in garlic, minor amounts of the isomeric 1-propenyl group also occur (2, 4, 20). This group cannot be distinguished from the allyl group by HR-MS, requiring additional MS-MS studies. Thus, both the m/z 163 and 137 species and related NH_4 adducts formed from garlic could contain both allyl and 1-propenyl fragments.

The mechanism in **Figure 1** proposed for hydrolysis of allicin is consistent with earlier studies by Kice on diaryl thiosulfinates (21) and with the various compounds found in this and earlier work. It is particularly satisfying to find small peaks corresponding to $[\text{C}_6\text{H}_{10}\text{S}_3\text{O} + \text{H}]^+$ and $[\text{C}_6\text{H}_{10}\text{S}_3\text{O} + \text{NH}_4]^+$, even though the precise structural formulas cannot be determined from the MS

Table 1. Garlic (*Allium sativum*) PI-DART and NI-DART Peak Measurements

species	neutral parent	measured	calcd	diff	abundance
positive ion					
[C ₃ H ₆ + H] ⁺	C ₃ H ₆	43.0543	43.0548	-0.0005	0.1
[C ₃ H ₆ + NH ₄] ⁺	C ₃ H ₆	60.0797	60.0813	-0.0016	0.2
[C ₃ H ₅ OH + H] ⁺	8	59.0498	59.0497	0.0001	9.4
[C ₂ H ₆ S ₂ O + H] ⁺	17	110.9940	110.9938	0.0002	1.0
[C ₂ H ₆ S ₂ O + NH ₄] ⁺	17	128.0212	128.0204	0.0008	0.1
[C ₄ H ₈ S ₂ O + H] ⁺	14, 15	137.0100	137.0095	0.0005	13.3
[C ₄ H ₈ S ₂ O + NH ₄] ⁺	14, 15	154.0362	154.0360	0.0002	0.8
[C ₆ H ₁₀ S ₂ O + H] ⁺	3	163.0230	163.0251	-0.0021	100
[C ₆ H ₁₀ S ₂ O + NH ₄] ⁺	3	180.0495	180.0517	-0.0022	7.6
[C ₆ H ₁₀ S ₂ O ₂ + H] ⁺	23	179.0197	179.0201	-0.0004	0.2
[C ₆ H ₁₀ S ₂ O ₂ + NH ₄] ⁺	23	196.0450	196.0466	-0.0016	0.1
[C ₆ H ₁₀ S ₃ O + H] ⁺	7	195.0018	194.9972	0.0046	0.3
[C ₆ H ₁₀ S ₃ O + NH ₄] ⁺	7	212.0205	212.0238	-0.0033	0.1
[C ₁₀ H ₁₈ S ₄ O ₂ + H] ⁺	3 + 14, 15	299.0239	299.0268	-0.0029	3.1
[(C ₆ H ₁₀ S ₂ O) ₂ + H] ⁺	3	325.0366	325.0425	-0.0059	15.8
negative ion					
[SO ₄] ⁻		95.9538	95.9517	0.0021	39.4
[HSO ₄] ⁻		96.9617	96.9596	0.0021	16.5
[C ₃ H ₃ O ₃] ⁻	5	87.0099	87.0082	0.0017	3.2
[C ₃ H ₅ SO] ⁻	2	89.0081	89.0061	0.0020	22.6
[C ₃ H ₅ SO ₂] ⁻	9	105.0050	105.0010	0.0040	100
[SO ₅] ⁻		111.9488	111.9467	0.0021	26.0
[C ₃ H ₅ SO ₃] ⁻	25	120.9968	120.9959	0.0009	93.1

**Figure 5.** (a) Formation of 2-propenesulfenic acid from alliin; (b) reaction of alliin with superoxide radical anion.

data alone. Because theoretical calculations (22) and synthetic studies (23–27) indicate that compounds of type RS(O)SSR are favored over isomeric compounds RSS(O)SR, we suggest that the structure of the C₆H₁₀S₃O species is **7**. PI-DART analysis of elephant garlic, discussed in detail below, also shows the presence of peaks corresponding to **7** (see **Figure 8** and **Table 3**).

When garlic is sampled at 24 °C, the summed ratio of the *m/z* 137 peaks associated with [(C₃H₅)MeS₂O + H]⁺ to those associated with [All₂S₂O + H]⁺ and isomeric *m/z* 163 protonated thiosulfonates is 5:100, although this ratio can be as low as 1:100 if data are collected immediately after garlic is crushed. This ratio increases to 6.8:100 when garlic is sampled at 40 °C. The 24 °C data compare well with published data on ratios of methiin, alliin, and isoalliin in garlic (28, 29). Indeed, PI-DART analysis (150 °C source gas temperature) of a commercial garlic supplement prepared from powdered, dehydrated garlic showed alliin (**1**) to be the dominant product (100%; calcd for C₆H₁₁NO₃S + H, 178.0538; found 178.0562), along with methiin (**13**; 11.4%; calcd for C₄H₉NO₃S + H, 152.0381; found 152.0444), and *S*-allylcysteine (27.8%, calcd for C₆H₁₁NO₂S + H, 162.0589; found 162.0609). It is possible that some of the *S*-allylcysteine detected arises from deoxygenation of alliin (**1**) upon heating in the DART source. Exposure of the powdered garlic to water results in the immediate formation of alliin (**3**) and *m/z* 137 protonated thiosulfonates as in the case of fresh garlic.

The increase in the *m/z* 137 to 163 ratio in crushed fresh garlic with temperature and with time is consistent with expectations that, whereas methiin (**13**) is cleaved more slowly than alliin (**1**) (30), a greater percentage of **13** reacts during the sampling time at 40 °C compared to 24 °C. Thus, the time to 50% of maximum yield for **15** and **16** is reported to decrease from 0.6 to 0.2 min as

the temperature increases from 23 to 37 °C, whereas formation of alliin (**3**) is almost instantaneous at both temperatures (31).

Because acidic compounds tend to form [M - H]⁻ ions under NI-DART conditions, it was significant to find that when garlic was sampled as above by NI-DART-TOF-MS, major peaks were seen, corresponding to [AllSO]⁻, [AllSO₂]⁻, [AllSO₃]⁻, [CH₃SO₂]⁻, [C₃H₃O₃]⁻ (pyruvate), and SO₂, and identified by their exact masses (see **Figure 9** and **Table 1**). The ratio of [C₃H₅SO₂]⁻ to [CH₃SO₂]⁻ was 9:1, which is similar to the expected alliin to methiin ratio (29), particularly if it is recognized that 2-propenesulfenic acid is unstable with regard to loss of SO₂ (32), whereas methanesulfenic acid is not. Authentic samples of AllS(O)SAll and MeS(O)SMe both showed peaks corresponding to RSO₂⁻ and RSO₃⁻, but neither showed RSO⁻. This observation is relevant because it is well-known that thermal decomposition of alliin and other thiosulfonates can give sulfenic acids (**Figure 5**) (2). The RSO₃⁻ may be associated with attack of O₂^{•-} on the sulfinyl sulfur of the thiosulfonates, as previously described for solution studies, and is likely to have the formula RS(O)OO⁻ rather than being a sulfonate anion (33). The RSO₂⁻ may be associated with hydrolysis of the thiosulfonates by processes involving direct attack of water on the sulfinyl sulfur, by attack at the sulfinyl sulfur of intermediates such as **6**, or, less likely, by attack of O₂^{•-} on the sulfonyl sulfur of thiosulfonates. In the latter instance, RSO₂⁻ would likely have the formula RS-O-O⁻; this structure would be unlikely to result in loss of SO₂ when R = allyl. It is also possible that some of the RSO₂⁻ arises through air oxidation of RSO⁻, as has been reported for 2-pyridinesulfonate (34).

The intensity of the 2-propenesulfenate peak at *m/z* 89 rapidly decreases relative to that of the 2-propenesulfinate and RSO₃⁻ ions (**Figure 10**), with a half-life of <1 s, eventually disappearing

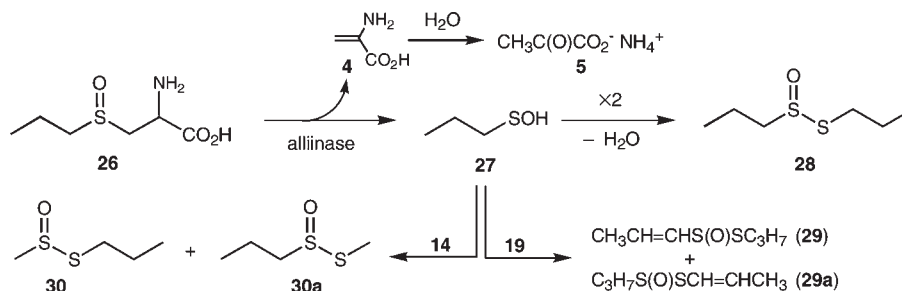


Figure 6. Reactions of propiin-derived compounds.

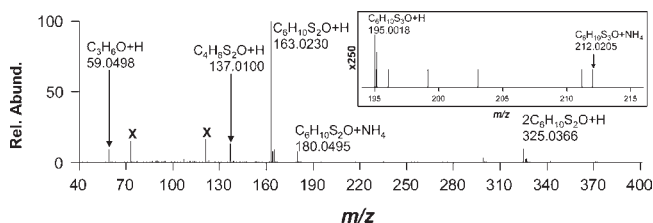
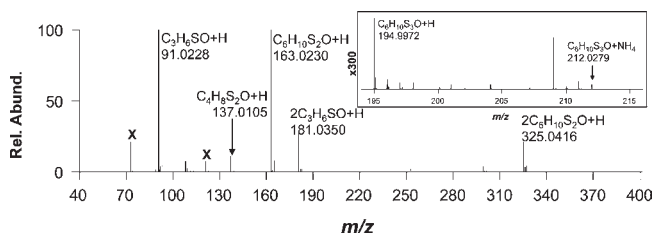
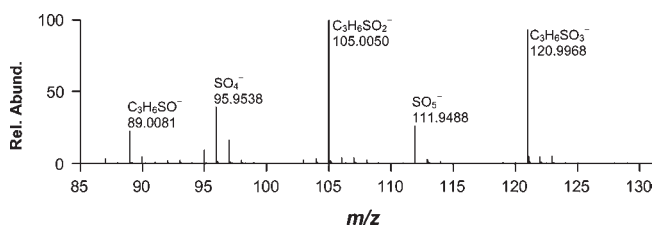
Figure 7. PI-DART mass spectrum from crushed garlic (*A. sativum*).Figure 8. PI-DART mass spectrum from crushed elephant garlic (*A. ampeloprasum*).

Figure 9. NI-DART mass spectrum from crushed garlic.

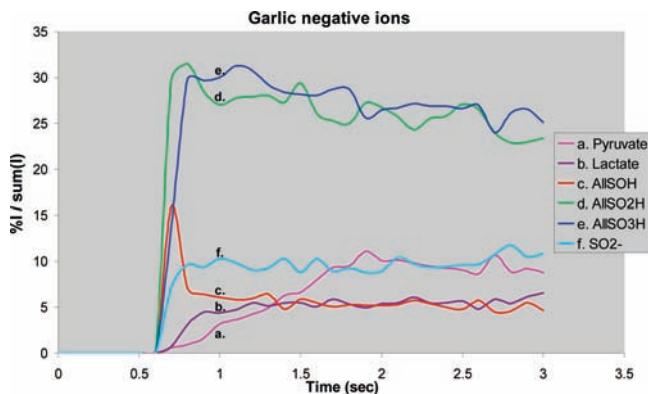


Figure 10. NI-DART of crushed garlic kinetic plot.

completely. At the same time the m/z 87 signal for pyruvate rapidly increases. The very short lifetime for 2-propenesulfenic acid is similar to our gas-phase results for methanesulfenic acid (35) and is consistent with the very rapid formation of alliin from alliin

determined previously using dehydrated garlic powder (31). In the PI-DART spectra of onion and leek, (*Z*)-propanethial *S*-oxide, which has the same molecular formula as 2-propenesulfenic acid, has a significantly longer gas-phase lifetime.

Because isoalliin comprises about 10% of the cysteine sulfenic acid content of garlic (2), without additional studies we cannot exclude the possibility that 1-propenesulfenic acid contributes to the observed short-lived m/z 89.0081 signal (and 1-propenesulfinate to the m/z 105.0050 signal). Garlic lacks the LF synthase enzyme, so 1-propenesulfenic acid should not rearrange to the LF and should show reactivity similar to that of 2-propenesulfenic acid; a signal for the protonated LF at m/z 91 is absent from the garlic PI-DART trace. Given the above limitation, we believe that we have determined the upper limit to the lifetime of 2-propenesulfenic acid from crushed garlic.

Our work represents the first observation of 2-propenesulfenic acid (9) in the gas phase and the first direct observation of 2-propenesulfenic acid from alliin, confirming our earlier mechanistic proposal for hydrolysis of alliin. Compound 9 is quite unstable with a low barrier to retro-ene desulfination to propene ($\Delta H^\ddagger = 11 \text{ kcal mol}^{-1}$; Figure 1) and a half-life of 21 min at 24 °C (32). It is likely that this decomposition accounts in part for the high abundance of sulfur dioxide seen in the DART spectra. Whereas the peak associated with propene is extremely small, this is expected in view of its high volatility and weak proton affinity.

Significantly, our work also represents the first case of direct observation of a sulfenic acid formed by the action of an alliinase enzyme on an *S*-alk(en)yl cysteine sulfoxide. Sulfenic acid formation from the cleavage of cysteine sulfoxides was first proposed by Stoll and Seebeck in 1951 (1) and has been widely accepted despite the fact that it has never been directly observed because of the short lifetime of these compounds. Virtanen reported in 1962 that he found no peak of m/z 90 corresponding to 2-propenesulfenic acid (2) in mass spectrometric studies of crushed garlic, in contrast to his success using these methods in detecting the m/z 90 onion lachrymatory factor (36, 37). Furthermore, sulfenic acids were not detected when sulfur K-edge absorption spectroscopy (XAS) was employed to directly image alliums crushed and rapidly chilled in liquid nitrogen (38). Sulfenic acids are thought to play a key role in the defensive strategy of diverse plants against microbial infection and insect predation through self-condensation or rearrangement to irritating (13) and antimicrobial compounds such as alliin or 2-propanethial *S*-oxide. 2-Propenesulfenic acid (2) is thought to play a major role in the antioxidant activity of garlic and itself is a powerful antioxidant (39, 40). Cysteine sulfenic acid, of interest as an important intermediate in the oxidation of albumin thiol and other proteins, has recently been identified by tandem mass spectrometry (41, 42).

***A. siculum* (Mediterranean Bells).** The PI-DART data for this plant appear elsewhere (5). The NI-DART spectra of bulbs and leaves of *A. siculum* (Figure 11 and Table 2) give results similar to those seen with garlic, showing anionic peaks corresponding to

$\text{CH}_3\text{CH}_2\text{CH}=\text{CHSO}^-$ ($\text{C}_4\text{H}_8\text{SO}$, m/z 103; 2.8%), $\text{CH}_3\text{CH}_2\text{CH}=\text{CHSO}_2^-$ ($\text{C}_4\text{H}_8\text{SO}_2$, m/z 119), and $\text{CH}_3\text{CH}_2\text{CH}=\text{CHSO}_3^-$ ($\text{C}_4\text{H}_8\text{SO}_3$, m/z 135), with the m/z 103 peak being short-lived and only 10–30% of the intensity of the m/z 119 and 135 peaks. Smaller peaks corresponding to $\text{C}_4\text{H}_9\text{SO}_2^-$ and $\text{C}_4\text{H}_9\text{SO}_3^-$ (but not $\text{C}_4\text{H}_9\text{SO}^-$) were also seen. All peaks were identified by their exact masses.

Elephant Garlic. Elephant garlic, also called “great-headed garlic” (*A. ampeloprasum* var. *ampeloprasum*), is not a true garlic but rather a type of leek (43). It is readily available in the produce section of markets in the United States and has a flavor more similar to that of garlic, albeit milder, than leek. The distilled oil of this plant has antimicrobial activity (44, 45). We previously reported, on the basis of HPLC analysis, that the thiosulfonates from crushed elephant garlic show an allyl/methyl/1-propenyl ratio of ca. 65:33:2 (20), which can be compared to the alliin/methiin/isoalliin cysteine precursor ratios of 60:17:20 (29).

The PI-DART spectrum for elephant garlic (Figure 8 and Table 3) reveals the presence of significant concentrations of propanethial *S*-oxide (21), the onion LF, in addition to alliin, methyl/allyl thiosulfinate (15/16) and/or methyl/1-propenyl thiosulfinate, and the bis-sulfine (23) along with traces of diallyl trisulfane *S*-oxide (7). The presence of both 21 and 23 was confirmed by ^1H NMR analysis of an ether extract of the crushed plant, which showed a characteristic triplet for 21 at 8.20 ppm and a doublet for 23 at 8.11 ppm, as previously observed with onions (18). Most of the LF 21 originally present in the elephant

garlic was presumably lost during the isolation procedure because the 21/23 ratio by PI-DART was 100:1, whereas the NMR ratio after isolation was 1.4:1. The occurrence of the onion LF in elephant garlic has not been previously reported and makes this plant unique in having both alliin as well as the LF; it is thus more than just “a milder version of garlic”. Our observations are identical to those made by us a year earlier using PI-DART on a separate sample of elephant garlic, indicating excellent reproducibility of our results. Upon crushing of elephant garlic a mild lachrymatory effect is readily detected. These results suggest the occurrence of both the alliinase and LFS enzymes in this plant, which is not surprising because leeks should have both enzymes. In contrast to the results from the onion PI-DART, which shows that the LF constitutes about 98% of the abundance of all peaks, the ratio of the LF to alliin in elephant garlic is 0.6:1. The LF is considerably more volatile than alliin, so the amount of the LF in elephant garlic may be exaggerated: on the basis of just the areas of the DART peaks, the ratio of the alliin/methiin/isoalliin cysteine precursors is calculated to be ca. 61:4:34.

Leek. In contrast to garlic and onion, where alliin and isoalliin are the respective dominant precursors, in leek, propiin (26, Figure 6), methiin, and isoalliin are all present in comparable amounts. Because both alliinase and the LF synthase are also present, a complex mixture of volatiles is expected when leek is crushed. Although that is indeed the case for PI-DART, as shown in Table 4 and the simplified summed Figure 12 (in the Supporting Information) showing only the neutral parents, it is encouraging that all of the peaks seen can be readily assigned. Thus, the data in Table 4 indicate that the thiosulfonates contain 34% methyl, 16% 1-propenyl, and 50% *n*-propyl groups. If the LF is included as a source of 1-propenyl groups, then the ratio of groups changes to 23% methyl, 42% 1-propenyl, and 34% *n*-propyl groups. These numbers have not been corrected to reflect the variable sensitivity of the DART to different compounds, the effect of differences in volatilities, or the effect of differences of rates of alliinase-catalyzed formation, all of which could affect peak intensity at the time of sampling.

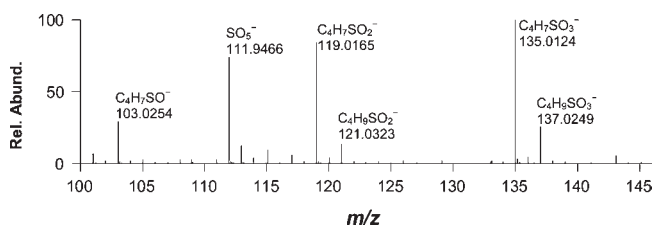


Figure 11. NI-DART mass spectrum from crushed *A. siculum*.

Table 2. *Allium siculum* (Mediterranean Bells) NI-DART Peak Measurements

species	neutral parent	measured	calcd	diff	abundance
$[\text{C}_4\text{H}_7\text{SO}]^-$	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHSOH}$	103.0254	103.0218	0.0036	29
$[\text{SO}_5]^-$		111.9466	111.9497	-0.0001	73.9
$[\text{C}_4\text{H}_7\text{SO}_2]^-$	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHSO}_2\text{H}$	119.0165	119.0167	-0.0002	84.3
$[\text{C}_4\text{H}_9\text{SO}_2]^-$	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2\text{H}$	121.0330	121.0323	0.0007	13.4
$[\text{C}_4\text{H}_7\text{SO}_3]^-$	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHS}(\text{O})\text{O}_2\text{H}$	135.0124	135.0116	0.0008	100
$[\text{C}_4\text{H}_9\text{SO}_3]^-$	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{S}(\text{O})\text{O}_2\text{H}$	137.0249	137.0272	-0.0023	25.3

Table 3. Elephant Garlic (*Allium ampeloprasum*) PI-DART Peak Measurements

species	neutral parent	measured	calcd	diff	abundance
$[\text{C}_3\text{H}_6\text{SO} + \text{H}]^+$	21 (LF)	91.0228	91.0218	0.0010	100
$[\text{C}_3\text{H}_6\text{SO} + \text{NH}_4]^+$	21	108.0483	108.0483	0.0000	7.9
$[\text{C}_2\text{H}_6\text{S}_2\text{O} + \text{H}]^+$	17	110.9933	110.9938	-0.0005	0.6
$[\text{C}_4\text{H}_8\text{S}_2\text{O} + \text{H}]^+$	14, 15	137.0105	137.0095	0.0010	11.5
$[\text{C}_6\text{H}_{10}\text{S}_2 + \text{H}]^+$	11	147.0301	147.0302	-0.0001	0.5
$[\text{C}_4\text{H}_8\text{S}_2\text{O} + \text{NH}_4]^+$	14, 15	154.0355	154.0360	-0.0005	0.8
$[\text{C}_6\text{H}_{10}\text{S}_2\text{O} + \text{H}]^+$	3	163.0255	163.0251	0.0004	85.5
$[\text{C}_6\text{H}_{10}\text{S}_2\text{O} + \text{NH}_4]^+$	3	180.0526	180.0517	0.0009	4.2
$[(\text{C}_3\text{H}_6\text{SO})_2 + \text{H}]^+$	21	181.0350	181.0357	-0.0007	27.3
$[\text{C}_6\text{H}_{10}\text{S}_2\text{O}_2 + \text{H}]^+$	23	179.0193	179.0201	-0.0008	0.3
$[\text{C}_6\text{H}_{10}\text{S}_3\text{O} + \text{H}]^+$	7	194.9951	194.9972	-0.0021	0.3
$[\text{C}_6\text{H}_{10}\text{S}_3\text{O} + \text{NH}_4]^+$	7	212.0279	212.0238	0.0041	0.1
$[\text{C}_{10}\text{H}_{18}\text{S}_4\text{O}_2 + \text{H}]^+$	3 + 14, 15	299.0262	299.0268	-0.0006	4.2
$[(\text{C}_6\text{H}_{10}\text{S}_2\text{O})_2 + \text{H}]^+$	3	325.0416	325.0425	-0.0009	21.6
$[(\text{C}_6\text{H}_{10}\text{S}_2\text{O})_2 + \text{NH}_4]^+$	3	342.0701	342.0690	0.0011	0.3

Table 4. Leek (*Allium porrum*) PI-DART Measurements

species	neutral parent	measured	calcd	diff	abundance (summed)
[C ₃ H ₆ SO + H] ⁺	21 (LF)	91.0223	91.0218	0.0005	100
[C ₃ H ₆ SO + NH ₄] ⁺	21	108.0494	108.0483	0.0011	
[C ₂ H ₆ S ₂ O + H] ⁺	17 (MeS(O)SMe)	110.9943	110.9938	0.0005	15.3
[C ₂ H ₆ S ₂ O + NH ₄] ⁺	17	128.0192	128.0204	-0.0012	
[C ₄ H ₈ S ₂ O + H] ⁺	MeS(O)SCH=CHMe MeSS(O)CH=CHMe	137.0111	137.0095	0.0016	14.6
[C ₄ H ₈ S ₂ O + NH ₄] ⁺	MeS(O)SCH=CHMe MeSS(O)CH=CHMe	154.0361	154.0360	0.0001	
[C ₄ H ₁₀ S ₂ O + H] ⁺	MeSS(O)Pr MeS(O)SPr	139.0256	139.0251	0.0005	30.0
[C ₄ H ₁₀ S ₂ O + NH ₄] ⁺	MeSS(O)Pr MeS(O)SPr	156.0531	156.0517	0.0014	
[C ₆ H ₁₀ S ₂ O + H] ⁺	22 (zwibelanes)	163.0266	163.0251	0.0015	2.5
[C ₆ H ₁₀ S ₂ O + NH ₄] ⁺	22	180.0539	180.0517	0.0014	
[C ₆ H ₁₂ S ₂ O + H] ⁺	PrS(O)SCH=CHMe PrSS(O)CH=CHMe	165.0415	165.0408	0.0007	17.2
[C ₆ H ₁₂ S ₂ O + NH ₄] ⁺	PrS(O)SCH=CHMe PrSS(O)CH=CHMe	182.0681	182.0673	0.0008	
[C ₆ H ₁₄ S ₂ O + H] ⁺	28 (PrS(O)SPr)	167.0550	167.0564	-0.0014	32.1
[C ₆ H ₁₄ S ₂ O + NH ₄] ⁺	28	184.0849	184.0830	0.0019	

Table 5. Chinese Chive (*Allium tuberosum*) PI-DART Measurements

species	neutral parent	measured	calcd	diff	abundance
[C ₃ H ₆ SO + H] ⁺	21 (LF)	91.0297	91.0218	0.0079	3.4
[C ₃ H ₆ SO + NH ₄] ⁺	21 (LF)	108.0550	108.0483	0.0067	2.5
[C ₂ H ₆ S ₂ O + H] ⁺	17 (MeS(O)SMe)	110.9919	110.9938	-0.0019	97.6
[C ₂ H ₆ S ₂ O + NH ₄] ⁺	17 (MeS(O)SMe)	128.0203	128.0204	-0.0001	37.7
[(C ₂ H ₆ S ₂ O) ₂ + H] ⁺	17 (MeS(O)SMe)	220.9777	220.9798	-0.0021	16.3
[(C ₂ H ₆ S ₂ O) ₂ + NH ₄] ⁺	17 (MeS(O)SMe)	238.0080	238.0064	0.0016	2.0
[C ₄ H ₈ S ₂ O + H] ⁺	15/16	137.0081	137.0095	-0.0014	100.0
[C ₄ H ₈ S ₂ O + NH ₄] ⁺	15/16	154.0352	154.0360	-0.0008	36.9
[(C ₄ H ₈ S ₂ O) ₂ + H] ⁺	15/16	273.0095	273.0112	-0.0017	33.0
[(C ₄ H ₈ S ₂ O) ₂ + NH ₄] ⁺	15/16	290.0363	290.0377	-0.0014	3.8
[C ₆ H ₁₀ S ₂ O + H] ⁺	3 (allicin)	163.0236	163.0251	-0.0015	26.8
[C ₆ H ₁₀ S ₂ O + NH ₄] ⁺	3 (allicin)	180.0520	180.0517	0.0003	8.6
[(C ₆ H ₁₀ S ₂ O) ₂ + H] ⁺	3 (allicin)	325.0403	325.0424	-0.0021	2.2
[(C ₂ H ₆ S ₂ O)(C ₄ H ₈ S ₂ O) + H] ⁺	15/16 + 17	246.9964	246.9955	0.0009	34.2
[(C ₂ H ₆ S ₂ O)(C ₄ H ₈ S ₂ O) + NH ₄] ⁺	15/16 + 17	264.0294	264.0220	0.0074	3.8
[(C ₆ H ₁₀ S ₂ O)(C ₄ H ₈ S ₂ O) + H] ⁺	15/16 + 3	299.0264	299.0268	-0.0004	13.1
[(C ₆ H ₁₀ S ₂ O)(C ₄ H ₈ S ₂ O) + NH ₄] ⁺	15/16 + 3	316.0504	316.0534	-0.0030	1.6

Despite these limitations, the ratios of the different compounds found are in reasonable agreement with earlier results from HPLC and GC-MS studies involving extraction and chromatography (20, 46). Earlier HPLC analysis of a fresh leek extract indicated the thiosulfinate composition to be 27% methyl, 33% 1-propenyl, and 40% *n*-propyl groups; allyl groups were absent. Similar ratios were previously determined by GC-MS analysis of leek extracts. The expected ratios of the precursors methiin, isoalliin, and propiin in uncut leek based on thiosulfinate and LF formation are similar to recent findings of Doran and co-workers (47). However, in contrast to the latter work, we find no evidence for thiosulfates containing ethyl groups, despite specifically searching for them. Furthermore, in our studies disulfanes and polysulfanes were absent, which is consistent with earlier work indicating that such compounds are secondary decomposition products of thiosulfates (2). The NI-DART results for leek were not especially informative, showing very strong signals for SO₂, HSO₄, and other inorganic forms of sulfur along with minor peaks for RSO₂H. The virtues of the PI-DART technique as applied to the analysis of leek volatiles are that only a few minutes are required, the fragile and highly volatile LF is seen in good abundance, and sulfane and polysulfane decomposition products are absent.

Chinese Chive. On the basis of the PI-DART data (Figure 13 in the Supporting Information and **Table 5**), the ratio of alk(en)yl groups in Chinese chive is 65% methyl and 35% allyl. Traces of 1-propenyl groups are present as indicated by detection of propanethial *S*-oxide (LF); *n*-propyl groups were absent. The amount of 1-propenyl group is underestimated because it is based on only the amount of LF. The ratios of the different compounds found are in reasonable agreement with earlier results from HPLC and GC-MS studies involving extraction and chromatography (20, 46). Earlier HPLC analysis of a fresh Chinese chive extract indicated the thiosulfinate composition to be 86% methyl, 11% allyl, and 3% 1-propenyl; *n*-propyl groups were absent. Similar ratios were previously determined by GC-MS analysis of Chinese chive extracts.

More than 50 years after 2-propenesulfenic acid (**2**) was first proposed as an intermediate in the formation of allicin from alliin (*I*), direct observation of this elusive species has now been achieved and an upper limit to its lifetime determined using DART-MS under negative ion conditions. 2-Propenesulfenic acid, another important short-lived intermediate in garlic chemistry, has also been directly observed for the first time. In our work, DART-MS is used in a motion picture camera-like manner to document the rapid chemical processes occurring in the

fractions of a second following the crushing of a garlic clove with a capillary tube. To obtain more precise kinetic information, it should be possible to fabricate a miniature garlic press that can crush garlic *within the DART source region* while simultaneously scanning under negative ion conditions. Such work is in progress.

The DART-MS technique has also been used to directly observe for the first time a trisulfane *S*-oxide (**7**) in nature. We have previously suggested that **7** is a key intermediate in the formation from allicin of diallyl trisulfane, the predominant polysulfane in the distilled oil of garlic (**48**), so it is satisfying to be able to confirm the presence of **7**, albeit at trace levels. In addition, DART-MS analysis reveals that when elephant garlic is crushed, substantial amounts of the LF are found, which had not been previously recognized. Our work underscores the utility of DART-MS as a powerful tool in natural products and food chemistry and sheds further light on the remarkable natural products chemistry of genus *Allium* plants.

ABBREVIATIONS USED

DART-MS, direct analysis in real time mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; GC-MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; HR-MS, high-resolution mass spectrometry; LF, lachrymatory factor; LFS, lachrymatory factor synthase; PI, positive ionization; TOF-MS, time-of-flight mass spectrometer; TRPA1, transient receptor potential ion-channel protein, member A1; TRPV1, transient receptor potential ion-channel protein, member V1; TIC, total ion current.

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Supporting Information Available: Figures 12 and 13: PI-DART traces of crushed leek and Chinese chive, respectively. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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