

Organosulfur chemistry of garlic and onion: Recent results

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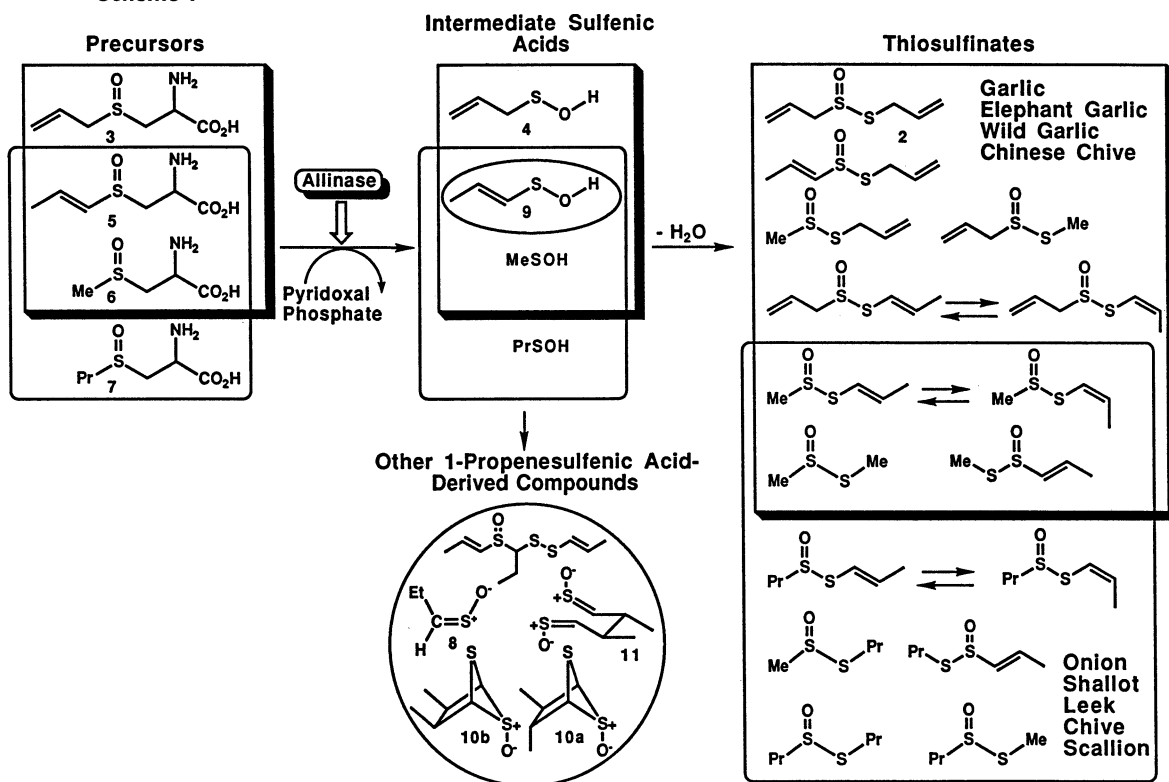
Abstract - Room temperature vacuum distillates and extracts of onion, garlic, and other genus *Allium* plants (wild garlic, leek, scallion, shallot, elephant (or great-headed) garlic, chive, Chinese chive) were analyzed by HPLC, "cryogenic" GC-MS and proton NMR spectroscopy, using authentic samples of suspected thiosulfinate components to evaluate the methods. Eight thiosulfates (RS(O)SR') and related organosulfur compounds (*cis*- and *trans*-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxides; (*Z,Z*)-*d,l*-2,3-dimethyl-1,4-butanedithial S,S'-dioxide) were separated and identified in each plant extract, several for the first time. Garlic grown in cooler climates was found to show enhanced allyl to methyl thiosulfinate levels in extracts.

INTRODUCTION AND BACKGROUND

Garlic (*Allium sativum*) and onion (*Allium cepa*) were among the earliest of cultivated foods and spices, easily identified by primitive food-seekers by their distinctive smell, now known to be associated with organosulfur compounds. The popularity of garlic and onions in folk medicine through the centuries for treatment of such varied disorders as dog bites, insect stings, earaches, burns and wounds, baldness, headaches, chest colds, respiratory ailments, asthma, pneumonia, diabetes, cardiovascular disorders, and rheumatism, among others, can be attributed to their pungent aroma, strong taste and, in the case of onion, its potent lachrymatory effect. These plants are members of the well known and widely appreciated genus *allium*, comprising more than 600 different species, other common members being leek, chive, shallot, and scallion. Common *allium* species were valued by early civilizations both as important dietary constituents and as medicinals for the treatment of many disorders, for example as recorded in the Bible, "...[the Jews who fled Egypt to wander the Sinai wilderness for forty years fondly remembered] the fish which we did eat in Egypt so freely, and the pumpkins and melons, and the leeks, onions and garlic," and as documented by discovery of dried garlic cloves and wooden models of onions among the relics found in the burial chambers of the pharaohs, and by references to medicinal uses of these plants in the writings of Aristotle, Hippocrates, Aristophanes, and Pliny the Elder (Ref. 1).

Both the popularity of *allium* plants as foodstuff as well as the reputation of garlic and onion as "cure-alls" stimulated scientific investigations, such as the early work by Pasteur in France (ca. 1858) into garlic's antibacterial activity and by Wertheim (1844) and later Semmler (1892) in Germany into the composition of distilled garlic and onion oils (mainly diallyl disulfide, $\text{CH}_2=\text{CH}-\text{CH}_2\text{SSCH}_2\text{CH}=\text{CH}_2$, 1, in the former case, and propenyl propyl disulfide in the latter case). Only with the advent of modern spectroscopic and chromatographic techniques has it become possible to determine the molecular basis for the odor, taste and biological activity of the fresh or processed plants. Key early discoveries on this subject were made by Cavallito (1944) and Wilkens (1962) in the United States, by Stoll and Seebeck in Switzerland (1948) and by Virtanen in Finland (1960) (Ref. 2,3). Cavallito discovered an unstable, odoriferous liquid substance in extracts of fresh garlic he termed allicin ($\text{CH}_2=\text{CHCH}_2\text{S(O)SCH}_2\text{CH}=\text{CH}_2 \equiv \text{AllS(O)SAll}$, 2-propene-1-sulfinothioic acid S-2-propenyl ester; 2) possessing antibacterial properties, while Stoll and Seebeck identified the immediate precursor of allicin as alliin, ($\text{CH}_2=\text{CHCH}_2\text{S(O)CH}_2\text{CH}(\text{NH}_2)\text{COOH}$, (+)-S-allyl-L-cysteine S-oxide; 3). Present evidence indicates that allicin 2 is formed by action of the C-S lyase enzyme alliinase (released by cutting or crushing garlic cloves) on the stable precursor alliin 3 by way of the intermediate 2-propenesulfenic acid (4, Scheme 1). Alliin 3 occurs abundantly in garlic, up to $0.76 \pm 0.40\%$ of fresh weight. From onion preparations Virtanen isolated three homologues of

Scheme 1



alliin, namely (+)-S-(prop-1-enyl)-L-cysteine S-oxide, **5** ($\text{CH}_3\text{CH}=\text{CHS}(\text{O})\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$), (+)-S-methyl-L-cysteine S-oxide, **6** ($\text{CH}_3\text{S}(\text{O})\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$) and (+)-S-propyl-L-cysteine S-oxide, **7** ($\text{CH}_3\text{CH}_2\text{CH}_2\text{S}(\text{O})\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$). Wilkens and Virtanen independently investigated the structure of the onion lachrymatory factor (LF, $\text{C}_3\text{H}_6\text{SO}$; **8**). 1-Propenesulfenic acid S-H tautomer ($\text{CH}_3\text{CH}=\text{CHS}(\text{O})\text{H}$) and propanethial S-oxide ($\text{CH}_3\text{CH}_2\text{CH}=\text{S}=\text{O}$) were suggested by Virtanen and Wilkens, respectively, as the structure of **8**. More recent work by the author has established that the LF **8** has the structure (*Z*)-propanethial S-oxide, and that it most likely originates from rearrangement of 1-propenesulfenic acid O-H tautomer **9** (ref. 4).

Alliin **2** is a member of a class of unstable and reactive organosulfur compounds known as thiosulfonates. Alliin is an antimicrobial agent which affects RNA synthesis in microorganisms and lipid biosynthesis in mammals, yeast and higher plants, inhibiting acetyl-CoA synthetase in the latter (ref. 3). Thiosulfonates such as **2** can be synthesized by oxidation of the corresponding disulfide with peracids, e.g. **1** in the case of alliin. Since the above key initial discoveries, much effort has been expended developing procedures to detect and accurately quantitate alliin in fresh and processed garlic and to understand how alliin forms and decomposes. These studies have taken on added importance because of the considerable current interest in biological properties of organosulfur compounds from garlic, especially as related to cardiovascular disease, cancer and infectious diseases.

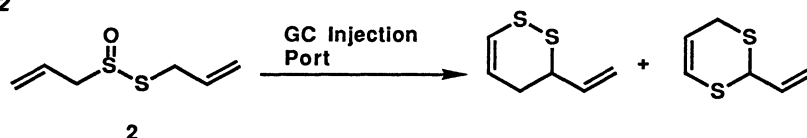
After the discovery of alliin **3** and related cysteine S-oxides $\text{RS}(\text{O})\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ **5-7** in allium species, it was recognized that upon cutting the plants, allinase-induced cleavage of these precursors could give thiosulfonates of type $\text{R}'\text{S}(\text{O})\text{SR}''$, where R' and R'' represent such groups as methyl, *n*-propyl, 1-propenyl and 2-propenyl. These thiosulfonates, and derived polysulfides, were assumed to be the principle source of flavor and aroma in allium species and were viewed as important taxonomic markers. Indirect methods were used to identify and quantify the allium thiosulfonates, e.g. paper chromatographic separation of cysteine derivatives, $\text{RSSCH}_2\text{CH}(\text{NH}_2)\text{COOH}$, of the thiosulfonates (Ref. 5), or gas chromatographic (GC) analysis of volatile disulfides, $\text{R}'\text{SSR}''$, thought to reflect to thiosulfonate composition (Ref. 6). Paper chromatography suggested the presence in garlic, onion and Chinese chive extracts of thiosulfonates such as $\text{MeS}(\text{O})\text{SMe}$, $\text{PrS}(\text{O})\text{SPr}$, $\text{AlIS}(\text{O})\text{SAlI}$, $\text{MeS}(\text{O})\text{SAlI}$, $\text{MeS}(\text{O})\text{SPr}$, $\text{AlIS}(\text{O})\text{SPr}$, although not all compounds were present in extracts of each plant.

RESULTS

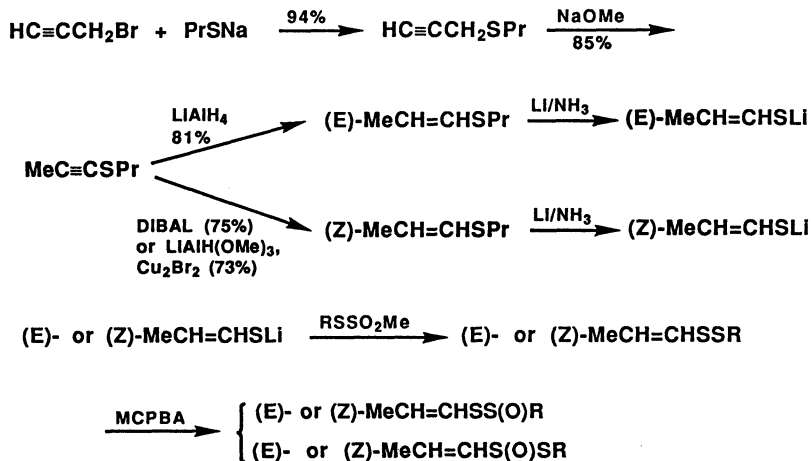
We have sought a direct answer to the question "What compounds are primarily responsible for the characteristic flavor of freshly cut members of the genus *Allium*?" This question is of considerable importance to the food and flavor industry, in chemotaxonomy, in dealing with chemical attraction/repulsion of potential allium insect predators (ref. 7), and in evaluating possible health benefits of these plants. While GC and GC-MS are of great value in the study of compounds of moderate thermal stability, such as those found in distilled oils of garlic and onion, thiosulfonates from allium species are known to decompose on attempted GC analysis (ref. 7), as illustrated by the formation of a pair of *m/e* 144 isomers from allicin **2** (ref. 8,9), as shown in Scheme 2. The thermal instability of thiosulfonates can be associated with the weak S-S bond (bond energy 46 kcal⁻¹ mol or less) and the facile pathways available for decomposition (ref. 10). In view of the complexity of thermal decomposition processes for aliphatic thiosulfonates, it seemed risky to draw conclusions on allium thiosulfonate composition from the pattern of decomposition products found on GC-MS analysis. We therefore undertook a study of the utility of HPLC, "cryogenic" GC-MS, and proton NMR methods to directly characterize thiosulfonates in allium extracts (ref. 11,12). At the same time we sought information on the stability of typical thiosulfonates to various conditions of isolation and chromatography. We also sought information on the chemistry of α,β -unsaturated thiosulfonates of type MeCH=CHS(O)SR, MeCH=CHSS(O)R and MeCH=CHS(O)SCH=CHMe. There are indications that such compounds, which have been little studied, show significant biological activity (ref. 13-15).

Reference samples of the various thiosulfonates thought to be present in allium extracts were prepared by oxidation of the corresponding symmetrical or unsymmetrical disulfides R'SSR" or by condensation of sulfinyl chlorides R'S(O)Cl with thiols R'SH. Alkyl 1-propenyl or bis(1-propenyl) disulfides were synthesized by stereospecific *cis* or *trans* reduction of 1-propynyl propyl sulfide followed by lithium-ammonia cleavage of the S-propyl bond, trapping (E)- or (Z)-1-propenethiolate either with MeSSO₂Me or PrSSO₂Pr forming (E)- or (Z)-MeCH=CHSSR (R = Me or Pr), or oxidizing the thiolate to bis(1-propenyl) disulfide (Scheme 3). Using these methods, the following thiosulfonates were prepared: MeS(O)SMe, MeS(O)SPr, MeSS(O)Pr, PrS(O)SPr, MeS(O)SAll, MeS(O)SAll, AllS(O)SAll, (E/Z)-MeCH=CHSS(O)Me, (E/Z)-MeCH=CHSS(O)Pr, (E/Z)-MeCH=CHSS(O)All, (E)-MeCH=CHS(O)SMe, (E)-MeCH=CHS(O)SPr and (E)-MeCH=CHS(O)SAll. In the course of these studies it was discovered that (E)- or (Z)-isomers of MeCH=CHSS(O)R readily interconvert at room temperature (see Scheme 4 for proposed mechanism), that monooxidation of (Z,Z)- and (E,Z)-bis(1-propenyl) disulfide led to *trans*- and *cis*-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide (**10a,b**; *trans*- and *cis*-zwiebelene)(ref.16), respectively, and that bis-oxidation of (E,E)-bis(1-propenyl) disulfide led to (Z,Z)-*d,l*-2,3-dimethyl-1,4-butanedithial S,S'dioxide (**11**) (ref. 17). Compounds **10a,b** and **11** were shown to be present in onion extracts (ref. 16,17).

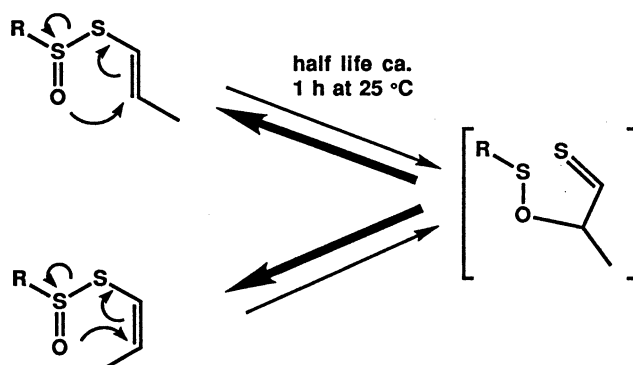
Scheme 2



Scheme 3



Scheme 4



Both normal phase (Si) and reverse phase (C18) HPLC methods were developed to separate isomeric thiosulfonates. The most satisfactory results were achieved with Si-HPLC with *i*-propanol/hexane gradients, as shown for an onion extract in Figure 1 (ref. 11). We also discovered that separation of most isomeric thiosulfonates could be achieved by GC and GC-MS using wide bore capillary columns (0.53 mm *i.d.*) with cryogenic (0 °C) on-column injection and initial column temperature conditions, slow column heating rates (2-5 °C/min), and GC-MS transfer line temperatures of 80-100 °C. A typical GC-MS separation of an onion extract is shown in Figure 2 (ref. 12).

For HPLC or GC analysis, peeled garlic cloves or onion pieces were homogenized in water (1:10 or 1:1 w/w, respectively) and, after 30 minutes, extracted with methylene chloride and immediately analyzed. A similar procedure was followed with other alliaceous plants (wild garlic (*Allium ursinum*), leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum* auct.), elephant (or great-headed) garlic (*Allium ampeloprasum* L. var. *ampeloprasum* auct.), chive (*Allium schoenoprasum* L.), and Chinese chive (*Allium tuberosum* L.). Because we sometimes experienced problems in the extraction procedure with severe emulsion formation and found scale-up difficult

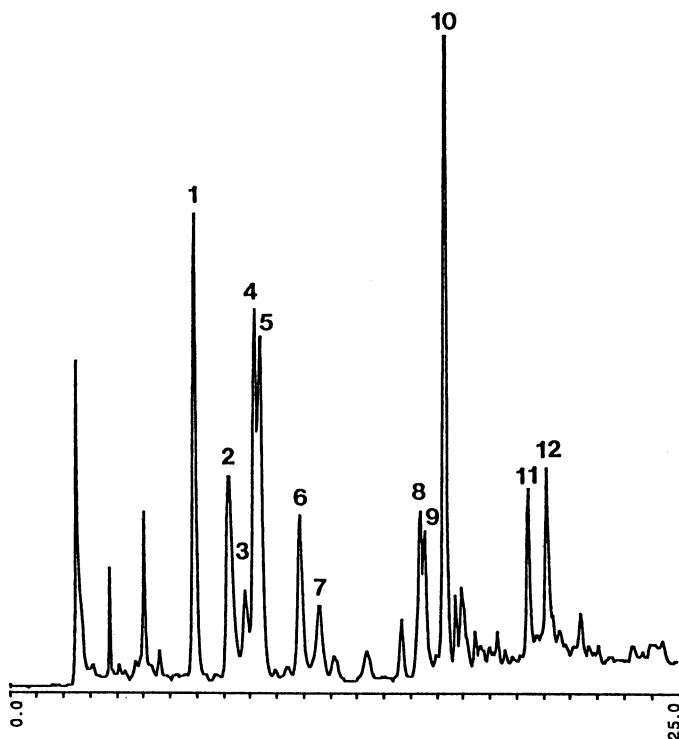


Figure 1 Si-HPLC of onion extract. Peak identification: 1, (E)-MeCH=CHS(O)SPr-*n*; 2, (Z)-MeCH=CHSS(O)Pr-*n*; 3 EtCHSO; 4, (E)-MeCH=CHSS(O)Pr-*n*; 5, *n*-PrS(O)SPr-*n*; 6, (E)-MeCH=CHS(O)SMe; 7, PhCH₂OH (internal standard); 8, MeSS(O)Pr-*n*; 9, (Z)-MeCH=CHSS(O)Me; 10, (E)-MeCH=CHSS(O)Me and MeS(O)SPr-*n* (overlap); 11, OSCH(CHMe)₂CHSO, 12, MeS(O)SMe.

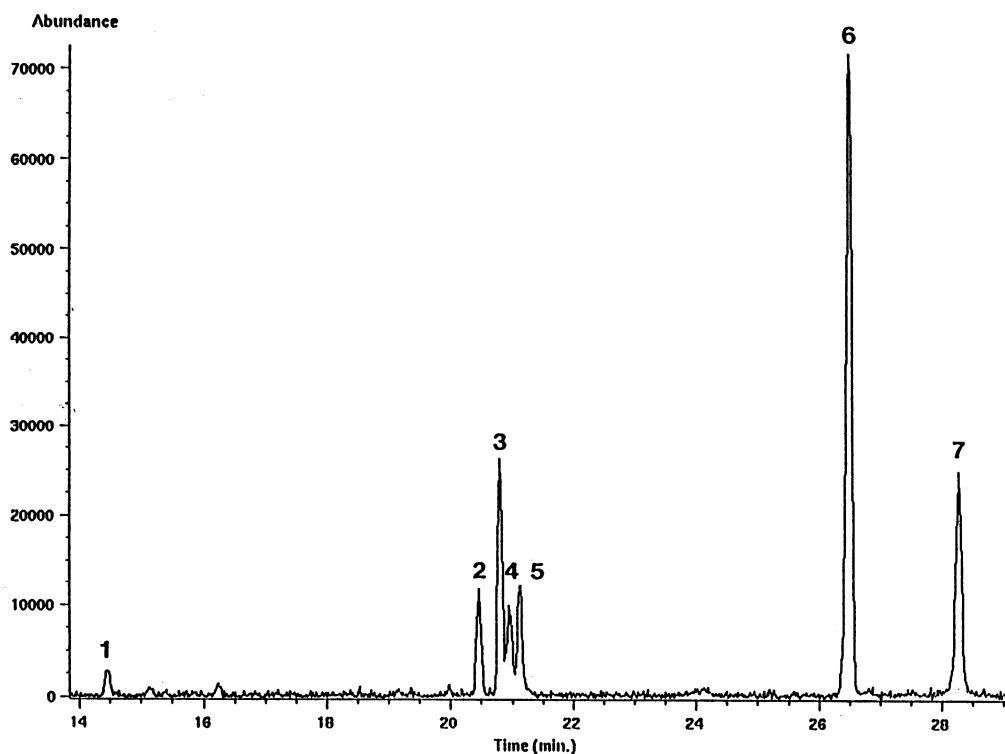


Figure 2 GC-MS total ion chromatogram of same onion extract using 30 m x 0.53 mm methyl silicone gum capillary column, injector and column programmed from 0 °C. Peak identification: 1, MeS(O)SMe; 2, MeS(O)SPr-*n*; 3, (E,Z)-MeCH=CHSS(O)Me; 4, MeSS(O)Pr-*n*; 5, (E)-MeCH=CHS(O)SMe; 6, (E,Z)-MeCH=CHSS(O)Pr-*n*, *trans*-zwiebelane; 7, *cis*-zwiebelane.

due to the presence of plant pigments, waxy material and other plant components, we experimented with distillation procedures (ref. 18). We subjected chopped allium species to high vacuum at room temperature (using an oil bath to prevent the flask contents from freezing), collected the aqueous condensate at liquid nitrogen temperatures, and then extracted this distillate with methylene chloride. We found excellent qualitative agreement in thiosulfinate profiles between the samples prepared by distillation and extraction. Furthermore, in the case of the onion distillates, sensory evaluation indicated that these distillates closely reproduce the true flavor of the freshly cut plant. We believe that this method succeeds because of the stabilizing effect of water, through hydrogen bonding, on the thiosulfates, which presumably undergo azeotropic distillation with water.

We sought to determine the factors that effect the reliability and quantitative reproducibility of allium thiosulfinate analysis by HPLC and "cryogenic" GC-MS methods. Since thiosulfates are known to be relatively unstable, the effect of varying the elapsed time between homogenization and analysis from one minute to 26 hours was determined for aqueous garlic and onion homogenates maintained at room temperature. In parallel studies using HPLC, "cryogenic" GC-MS, and proton NMR spectroscopy, it was found that while the total thiosulfinate concentration remained roughly constant during this period, thiosulfates of type MeCH=CHS(O)SR diminished rapidly and were nearly gone after 6 h, regioisomeric thiosulfates MeCH=CHSS(O)R were reduced to ca. 50% of their initial value after 26 h, and the initial concentration of MeS(O)SMe was significantly reduced after 6 h. These results explain the failure of previous workers to identify MeCH=CHS(O)SR-type thiosulfates in onion extracts (ref. 13). Apart from significant reduction in concentrations of MeCH=CHS(O)SR and a 12% reduction in concentration of MeCH=CHSS(O)R, there was good agreement between the 1 minute and 30 minute samples, supporting our choice of 30 minutes for the analysis protocol. While the "cryogenic" GC-MS methods fail for the garlic thiosulfates (LC-MS can be used here), there was otherwise good agreement between the three analytical methods. Other factors affecting our analysis include possible variation of thiosulfinate content from one part of the plant to another (e.g. leaf, root, rhizome), as others have already noted (ref. 19), with plant variety, developmental stage, growing and storage conditions (fertilization, irrigation, humidity, transport). Of course, our analytical methods are of value in determining precisely these differences!

Table I. Thiosulfinates and Related Compounds from Extracts of *Allium* Species by GC-MS and HPLC (Bracketed) as Mol % of Total

Compound	Garlic Store	Garlic Store (R)	Garlic NY	Elephant Garlic	Wild Garlic	Yellow Onion	Red Onion	Shallot	Scallion	Leek	Chive	Chinese Chive
AIIS(O)SAlI	[62]	[59]	[89]	[37]	[28]	---	---	---	---	---	---	---
AIIS(O)SCH=CHMe-(Z,E)	[5.9]	[18]	[5.3]	[3.7]	[0.9]	---	---	---	---	---	---	---
AIIS(O)CH=CHMe-(E)	[2.1]	[1.6]	[1.6]	[0.6]	---	---	---	---	---	---	---	---
<i>n</i> -PrSS(O)Propenyl-(E)	---	---	---	---	---	(12)[12]	(10) [10]	(14) [14]	(2) [2]	(5) [8]	(3) [3]	---
<i>n</i> -PrS(O)SPropenyl-(Z,E)	---	---	---	---	---	12 [10]	29 [14]	21 [22]	25 [17]	5 [15]	24 [16]	---
<i>n</i> -PrS(O)SPPr- <i>n</i>	---	---	---	---	---	9 [13]	6 [5]	26 [27]	35 [33]	25 [25]	57 [58]	---
MeSS(O)Propenyl-(E)	tr	[0.6]	---	tr	---	24 [24]	19 [26]	8 [9]	3 [7]	21 [12]	1	---
AIIS(O)SMe	[8.1]	[7.5]	[1.4]	[17]	[16]	---	---	---	---	---	---	[9]
MeS(O)SPropenyl-(Z,E)	[1.2]	[1.9]	tr	[2.2]	[0.7]	31 [25]	27 [33]	15 [15]	18 [22]	29 [27]	5 [5]	13 [5]
MeSS(O)Pr	---	---	---	---	---	1 [1]	4 [5]	6 [2.8]	8 [11]	7 [5]	4 [6]	---
MeS(O)SPr	---	---	---	---	---	1 [1]	4 [5]	3 [1.2]	8 [8]	6 [5]	7 [10]	---
AIIS(O)Me	[18]	[11]	[2.9]	[29]	[34]	---	---	---	---	---	---	[13]
MeS(O)SMe	[2.2]	[0.9]	---	[11]	[20]	10 [14]	1 [3]	7 [9]	1 [1]	2 [3]	1 [2]	74 [72]
Zwiebelanes (cis/trans) ^a	---	---	---	---	---	14/6	17/7	17/12	5/3	7.5/3.3	2.3/1.6	---
OSCH(CHMe) ₂ CHSO ^a	---	---	---	---	---	[2]	[11]	[1.8]	---	[3]	---	---
Total % MeS-	16	11	2	35	49	32	21	22	18	30	9	93 ^d [84]
Total % AIIS-	80	78	94	62	50	---	---	---	---	---	---	[10]
Total % 1-PropenylS- ^c	4	11	3	3	1	50	56	32	30	37	18	7 ^d [6]
Total % <i>n</i> -PrS-	---	---	---	---	---	18	23	46	52	33	73	---
Total Thiosulfinates ^b	25.6	20.7	14.3	5.2	21	0.35[.4]	0.19[.2]	0.2[.25]	0.1[.08]	0.19[.2]	0.26[.2]	1.7 ^d [2]

^aMol % based upon total thiosulfinate. ^bμmol/g wet weight. ^cFor onions, shallot, scallion, leek and chive, based on GC-MS and include zwiebelanes, but not OSCH(CHMe)₂CHSO, calcd. as MeCH=CHS(O)SCH=CHMe equiv. ^dGC-MS values exclude allyl methyl thiosulfinates, not detectable by this technique.

Table I summarizes our findings on the composition of extracts of common allium species. Based on this data, together with information from the literature, some general observations can be made:

1) Gas chromatography and GC-MS, as typically performed with elevated injector, column and GC-MS transfer line temperatures (ref. 20; also see references cited in ref. 11), presents an erroneous picture of the composition of both headspace volatiles and room temperature extracts from allium species. There is no evidence from either our HPLC or cryogenic GC-MS analyses of allium extracts for the presence of significant quantities of the polysulfides and thiophenes claimed by prior GC-MS studies, even at concentrations two orders or magnitude lower than that of the thiosulfonates! The answer to the question posed earlier ("What compounds are primarily responsible for the characteristic flavor of freshly cut members of the genus *Allium*?") is "Saturated and unsaturated thiosulfonates and related sulfinyl compounds!"

2) For thiosulfonates $RS(O)SCH=CHMe$, both (E)- and (Z)-isomers are seen, reflecting the facile room temperature isomerization. For thiosulfonates $RSS(O)CH=CHMe$, only the (E)-isomer is found. These results are consistent with a [2,3]-sigmatropic rearrangement process that is possible for thiosulfonates of type $RS(O)SCH=CHMe$ but not those of type $RSS(O)CH=CHMe$.

3) The different *Allium* spp. can be categorized by the types of alkyl groups present in the thiosulfonates. This type of chemotaxonomic information has been used to establish relationships between different members of the *Allium* genus. Only the Chinese chive showed a predominance of methyl groups; the methyl group is present in all of the *Allium* species examined. In garlic, elephant garlic, and wild garlic, allyl is the major alkyl group; minor quantities of allyl thiosulfonates occur in Chinese chive. Contrary to a recent report (ref. 20), we find no evidence for allyl thiosulfonates in onion extracts. The *n*-propyl group is the major alkyl group in chive, scallion, shallot, and leek, is present in onion, and is absent in garlic (contrary to earlier work (ref. 5) but in agreement with a recent report (ref. 19)), elephant garlic, wild garlic and Chinese chive. While all of the plants contain 1-propenyl groups, it is the dominant group only in onion. The total percentage of the 1-propenyl group incorporated in onion thiosulfonates, zwiebelanes and bis-sulfine (e.g. 59% for white onions) is somewhat misleading since most of the 1-propenyl group generated as 1-propenesulfenic acid winds up as the LF, which is not included in the calculation because it is lost during analysis. Scheme 1 summarizes our observations.

4) Store-purchased garlic (grown in warm climates such as Mexico or California) shows a typical allyl:methyl:1-propenyl ratio of 80:16:4. The major thiosulfonate from garlic is allicin, present to the extent of ca. 0.3% of fresh weight, a value in good agreement with data of others (ref. 19). Following refrigeration for two months this same garlic shows an allyl:methyl:1-propenyl ratio of 78:11:11. New York grown garlic (average growing temperature 19-22 °C) shows an allyl:methyl:1-propenyl ratio of 95:2:3 while refrigerated New York grown garlic shows an allyl:methyl:1-propenyl ratio of 89:3:8. Garlic grown in a plains region of India (average growing temperature 30-32 °C) shows an allyl:methyl:1-propenyl ratio of 74:24:2 while garlic grown in a mountainous region of India (average growing temperature 22-23 °C) shows an allyl:methyl:1-propenyl ratio of 80:18:2. California or Mexico-grown elephant garlic shows an allyl:methyl:1-propenyl ratio of 61:34:5 while New York grown elephant garlic (average growing temperature 19-22 °C) shows an allyl:methyl:1-propenyl ratio of 67:31:2.

Based on the above data it seems that garlic or elephant garlic grown in a cooler climate (e.g. upstate New York or mountainous regions of India) has a higher allyl:methyl ratio than plants grown in warmer climates. Other studies reveal that this same trend is seen in the $CH_2=CHCH_2S(O)CH_2CH(NH_2)COOH$ to $CH_3S(O)CH_2CH(NH_2)COOH$ ratio, the former predominating in plants grown in cooler climates. Refrigeration of garlic appears to increase the relative amount of 1-propenyl thiosulfonates, as previously found by Lawson (ref. 19).

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