

# **Allium Chemistry: HPLC Analysis of Thiosulfinates from Onion, Garlic, Wild Garlic (Ramsoms), Leek, Scallion, Shallot, Elephant (Great-Headed) Garlic, Chive, and Chinese Chive. Uniquely High Allyl to Methyl Ratios in Some Garlic Samples<sup>†</sup>**

Eric Block,\* Sriram Naganathan, David Putman, and Shu-Hai Zhao

Department of Chemistry, State University of New York at Albany, Albany, New York 12222

Room temperature vacuum distillates and extracts of onion (*Allium cepa*), garlic (*Allium sativum*), 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.) were analyzed by HPLC and <sup>1</sup>H NMR using authentic samples of suspected thiosulfinate components to evaluate the methods. Of the eight different thiosulfinates separated and identified in each plant extract, several are reported here for the first time. It is concluded that gas chromatography, as typically performed with high injector and column temperatures, presents an erroneous picture of the composition of room temperature extracts from *Allium* species and that HPLC provides a reliable qualitative and semiquantitative measure of what is actually present. A simple vacuum distillation procedure facilitating qualitative analysis of *Allium* volatiles is described. A number of significant trends are noted regarding the varying proportions of different thiosulfinates in each *Allium* species. In particular, some garlic varieties grown in cooler climates show a higher allyl to methyl ratio than garlic grown in warmer climates.

## INTRODUCTION

Since the identification in 1944 of the thiosulfinate allicin (4; CH<sub>2</sub>=CHCH<sub>2</sub>S(O)SCH<sub>2</sub>CH=CH<sub>2</sub>; see Table IV for numbering scheme and nomenclature) as the predominant olfactory and gustatory principle of freshly cut garlic (*Allium sativum*) (Cavallito and Bailey, 1944; Cavallito et al., 1944), considerable effort has been directed toward answering the question: "What compounds are primarily responsible for the characteristic flavor of freshly cut members of the genus *Allium*?" This question is of importance to the food and flavor industry and in chemotaxonomy (Bernhard, 1970; Mackenzie and Ferns, 1977; Saghir et al., 1964). The precise identification of thiosulfinates from *Allium* species takes on added significance in view of the recent report (Auger et al., 1990) that thiosulfinates are stable in the gas phase and can persist in the environment of *Allium* plants, acting as insect attractants or repellants. Finally, in connection with the widespread use of diverse *Allium* species in folk medicine (Augusti, 1990; Block, 1985, 1986, 1992), we note that a number of the unsaturated thiosulfinates seen in *Allium* species have been found to possess striking biological activity (Bayer et al., 1989a; Wagner et al., 1990; Sendl et al., 1992).

Because of their excellent resolution and mass identification capabilities, GC and GC-MS have figured prominently in the effort to characterize *Allium* volatiles. This has occurred despite the early cautionary note that many of the compounds from *Allium* species seen by GC may be "artifacts of analysis" (Saghir et al., 1964) and despite more recent work suggesting that better resolution may in fact be achieved using HPLC (see Table I)! Our

continuing interest in the organosulfur chemistry of the genus *Allium* (Bayer et al., 1989b; Block 1985, 1991a,b, 1992; Block and Bayer, 1990; Block et al., 1986, 1988, 1992a,b; Block and Zhao, 1990, 1992) has prompted us to undertake a comparative study of the utility of two methods of isolation, extraction and "room temperature steam distillation," and two chromatographic procedures, GC and HPLC, in the analysis of diverse *Allium* species. We have examined garlic, wild garlic (*Allium ursinum*), elephant (great-headed) garlic (*Allium ampeloprasum* L. var. *ampeloprasum* auct.), onion (*Allium cepa*), leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum* auct.), chive (*Allium schoenoprasum* L.), and Chinese chive (*Allium tuberosum* L.), using authentic samples (Naganathan, 1992) of the thiosulfinates thought to be present to evaluate analysis conditions and using LC-MS, GC-MS and UV, and <sup>1</sup>H NMR spectroscopy to confirm product identities. Table I lists prior chromatographic studies of extracts/distillates of these plants.

Our work, as described in this and the accompanying paper, leads us to the conclusion that *gas chromatography, as typically performed with elevated injector and column temperatures, presents an erroneous picture of the composition of both headspace volatiles and room temperature extracts from Allium species and that HPLC provides a reliable qualitative and quantitative measure of what is actually present.* Thus, analysis of *Allium* extracts or vacuum distillates by both normal (Si) and reverse phase (C<sub>18</sub>) HPLC, using diode array UV detection and LC-MS, accompanied by <sup>1</sup>H NMR analysis of these same samples, indicate that the predominant constituents are thiosulfinates. *We find no evidence from HPLC for the presence in these samples of significant quantities of the polysulfides and thiophenes claimed by prior GC-MS studies!* Using Si-HPLC, almost all of the thiosulfinates from *Allium* spp. can be separated and quantified using benzyl alcohol as internal standard. We describe a simple vacuum distillation procedure facilitating qualitative analysis of *Allium* volatiles and compare the

\* Author to whom correspondence should be addressed.

<sup>†</sup> This work was presented by Sriram Naganathan at the Division of Agricultural and Food Chemistry 2nd Graduate Candidate Symposium at the 203rd National Meeting of the American Chemical Society.

Table I. Survey of Papers Employing Chromatographic Methods in Analysis of Flavorants from Cut *Allium* Species<sup>a</sup>

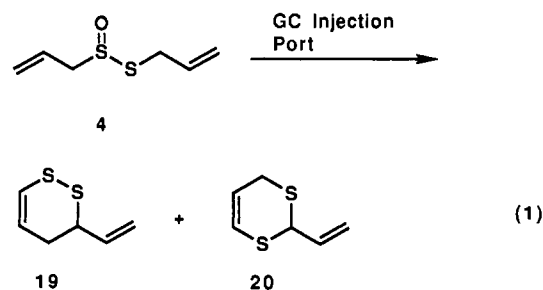
plant species	method <sup>b</sup>	RS(O)SR found? (n) <sup>c</sup>	ref <sup>d</sup>	plant species	method	RS(O)SR found? (n)	ref
garlic	E/PC	yes (6) <sup>e</sup>	a	onion	E/PC	yes (3)	a
	E,H/GC	no	b		VSD/GC	no	d
	H/GC	no	c		H/GC	no	c
	E/GC-MS	no	h		E,SD/GC-MS	no	i
	E/TLC <sup>f</sup>	yes (2)	j		E/TLC <sup>f</sup>	yes (2)	j
	E/GC-MS	no	n		D/GC-MS	no	o
	VSD/HPLC	yes (1)	g		D/GC-MS	no	e
	E/GC-MS	no	p		H/GC-MS	no	l
	D/GC-MS	no	q		H/GC-MS	no	s
	SD/GC-MS	no	r		SE/GC-MS	yes (1)	ee
	E/HPLC	yes (1)	v		E/HPLC	yes (3)	t
	E/HPLC	yes (7)	w		E/HPLC	yes (5)	w
	E/HPLC	yes (7)	x		E/HPLC	yes (1)	dd
	E/HPLC	yes (3)	y		elephant garlic	H/GC	no
SD/HPLC	no	z	E/HPLC	yes (7)		x	
wild garlic	E/SCF-MS	no	aa	chive	H/GC	no	c
	E/HPLC	yes (3)	u		H/GC-MS	no	f
scallion	E/PC	yes (3)	a	leek	H/GC	no	c
	H/GC	no	c		SD/GC-MS	no	k
	E/HPLC	yes (6)	w		E/HPLC	yes (6)	w
	D/GC-MS	no	bb		H/GC/TLC	yes (1)	cc
Chinese chive	D/GC-MS	no	gg	shallot	D/GC-MS	no	ff
	H/GC	no	c		SD/HPLC	no	m

<sup>a</sup> This survey does not include every one of the numerous GC studies of garlic and onion preparations. <sup>b</sup> Methods: E, extraction; SE, supercritical CO<sub>2</sub> extraction; D, distillation; GC, gas chromatography; H, headspace sampling; HPLC, high-pressure liquid chromatography; MS, mass spectrometry; PC, paper chromatography; SD, steam distillation; SCF, supercritical fluid chromatography; TLC, thin-layer chromatography; VD, vacuum distillation; VSD, vacuum steam distillation. <sup>c</sup> Number of different thiosulfates detected (*E/Z* isomers listed as *one* compound). <sup>d</sup> References: (a) Fujiwara et al., 1955; (b) Oaks et al., 1964; (c) Saghir et al., 1964; (d) Carson and Wong, 1961; (e) Brodnitz et al., 1969; (f) Wahroos and Virtanen, 1965; Leino, 1992; (g) Miething, 1985; (h) Brodnitz et al., 1971; (i) Boelens et al., 1971; Martín-Lagos et al., 1992; (j) Freeman and Whenhams, 1975; (k) Schreyen et al., 1976; (l) Mazza, 1980; (m) Wu and Wu, 1981; (n) Tokarska and Karwowska, 1983; (o) Talyzin et al., 1988; (p) Saito et al., 1989; Mazza et al., 1992; (q) Block et al., 1988; (r) Yu et al., 1989; (s) Kallio and Salorinne, 1990; (t) Bayer et al., 1989a; (u) Sendl and Wagner, 1991; (v) Jansen et al., 1987; Iberl et al., 1990a, 1990b; (w) Lawson and Hughes, 1990; (x) Lawson et al., 1991a; (y) Lawson et al., 1991b; Lawson and Hughes, 1992; (z) Lawson et al., 1990; (aa) Calvey et al., 1991; (bb) Kuo et al., 1990; Kuo and Ho, 1992; (cc) Auger et al., 1989; Auger and Thibout, 1981; (dd) Morimitsu et al., 1992; (ee) Sinha et al., 1992; (ff) Wu et al., 1982; Dembele and Dubois, 1973; (gg) Kameoka et al., 1984. <sup>e</sup> *n*-Propyl group claimed to be present. <sup>f</sup> GC analysis also conducted.

thiosulfate composition of different *Allium* species as determined by HPLC. Our analytical data agree with limited HPLC thiosulfate analyses of extracts of several of the plants reported by other workers (see Table I). In this and the accompanying paper (Block et al., 1992a), describing the utility of GC-MS *under gentle conditions* in studying *Allium* volatiles, we have identified a significant number of compounds not previously reported in these plants. We also present the novel observation that certain garlic samples show a higher than normal alliin to methyl thiosulfate ratio and offer an explanation for this observation.

## RESULTS AND DISCUSSION

In 1974 we reported that (1) aliphatic thiosulfates (RS-(O)SR') related to alliin 4 possess both low stability and high reactivity associated with the weak S-S bond (bond energy 46 kcal mol<sup>-1</sup> or less) and have facile pathways available for decomposition; (2) most aliphatic thiosulfates, possessing up to eight carbons, survive high vacuum distillation below 50 °C as well as GC analysis, using a 1.8-m × 3-mm packed silicon rubber column and low injection port and column temperatures (Block and O'Connor, 1974a,b). Similar observations on GC conditions have been made in a more recent paper (Auger et al., 1989). 1-Propenyl propyl disulfide and other  $\alpha,\beta$ -unsaturated disulfides seen upon GC analysis of *Allium* extracts or distillates are known to be formed on heating *n*-PrS-(O)SPr-*n* (9) (Block and O'Connor, 1974; Brodnitz et al., 1971) and other thiosulfates (Jones and Helmy, 1969). The ready decomposition of alliin 4 on attempted GC analysis giving two C<sub>6</sub>H<sub>8</sub>S<sub>2</sub> isomers (Brodnitz et al., 1971), subsequently identified by us as thioacrolein dimers 19 and 20 (eq 1; Block et al., 1986), is also well-known. Since



1971, a number of papers have appeared using these thioacrolein dimers as GC markers for the presence of 4 in extracts of garlic and other plants and even as the basis for quantitative determination of 4 in the analysis of garlic-derived products (Saito et al., 1989). This analytical technique is flawed because other thiosulfates containing the *S*-2-propenyl group (e.g. RS(O)SCH<sub>2</sub>CH=CH<sub>2</sub>) could also give rise to dimers 19 and 20. Finally, many GC studies of *Allium* volatiles employ high column temperatures and/or injection port temperatures as high as 280 °C (see Table I), conditions which we find are especially conducive to thiosulfate decomposition. The accompanying paper reports our success in using wider bore capillary (Megabore) columns together with gentle injection port temperatures and minimal column temperatures in the analysis of C<sub>2</sub>-C<sub>6</sub> thiosulfates. However, even under the gentlest GC conditions alliin and other allylic thiosulfates fail to survive!

The preparation of fresh extracts of *Allium* species for HPLC analysis is described under Experimental Procedures. Because we sometimes experienced problems in these procedures with severe emulsion formation (e.g. elephant garlic) and found scale-up difficult due to the presence of plant pigments, waxy materials, and other

Table II. Comparative Thiosulfinate Concentrations in Garlic and Elephant Garlic Extracts and Room Temperature Distillate Extracts<sup>a</sup>

compd no.	compound	garlic (NY)				elephant garlic			
		extract		distillate		extract		distillate	
		$\mu\text{mol/g}^c$	mol %	$\mu\text{mol/g}^c$	mol %	$\mu\text{mol/g}^c$	mol %	$\mu\text{mol/g}^c$	mol %
1	AllSS(O)Propenyl-( <i>E</i> )	0.23	1.6	0.06	0.8	0.02	0.6	0.03	0.6
2,3	AllS(O)SPropenyl-( <i>Z,E</i> )	0.75	5.3	0.41	5.5	0.17	4.5	0.19	3.7
4	AllS(O)SAlI	13.0	89.0	6.7	89.0	1.4	37.0	1.9	38.0
10	MeSS(O)Propenyl-( <i>E</i> )	—	—	—	—	0.06	1.7	<i>b</i>	<i>b</i>
11	AllS(O)SMe	0.2	1.4	0.13	1.7	0.54	15.0	0.87	17.0
12,15	MeS(O)SPropenyl-( <i>Z,E</i> )	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	0.13	3.6	0.12	2.2
16	AllSS(O)Me	0.41	2.9	0.22	3.0	0.95	27.0	1.5	29.0
18	MeS(O)SMe	—	—	—	—	0.36	10.0	0.58	11.0
	total thiosulfinates	14.3		7.5		3.6		5.2	
	total % AllS		94.5		94.5		61.2		61.7
	total % MeS		2.1		2.3		33.6		35.0
	total % 1-propenylS		3.4		3.1		5.2		3.3

<sup>a</sup> Garlic from NY state farm; elephant garlic store purchased. <sup>b</sup> Trace amounts present. <sup>c</sup> Fresh weight.

plant components (e.g. onion), we experimented with distillation procedures (Carson and Wong, 1961; Cavallito and Bailey, 1944; Miething, 1985; Edwards et al., 1991). We subjected chopped *Allium* species to high vacuum at room temperature (using an oil bath to prevent the flask contents from freezing) and collected the aqueous condensate at  $-196^\circ\text{C}$ . We found that HPLC and NMR spectroscopic analysis of the  $\text{CH}_2\text{Cl}_2$  extract of the salt-saturated condensate gave good qualitative thiosulfinate composition profiles. Furthermore, in the case of onion distillates, sensory evaluation indicated that these distillates closely reproduce the true flavor of the freshly cut plant. Both allicin 4 and MeS(O)SMe (18), the least stable and most reactive of the *Allium* thiosulfinates under dry distillation conditions, can be easily purified by this "room temperature steam distillation" procedure. We believe this method succeeds because of the stabilizing effect of water, through hydrogen bonding, on the thiosulfinates (Block and O'Connor, 1974b; Lawson et al., 1991a). It is probable that thiosulfinates form azeotropes under our distillation conditions. Comparison of samples subjected to both the distillation and extraction procedures indicate that in some, but not all cases, more thiosulfinate is lost by distillation than by extraction (Table II shows 14.3 vs 7.5  $\mu\text{mol/g}$  total thiosulfinates by extraction compared to distillation for garlic; comparable values for elephant garlic are 3.6 vs 5.2  $\mu\text{mol/g}$ , indicating that distillation is more efficient in this case). There was excellent qualitative agreement in thiosulfinate profiles between the samples prepared by distillation and extraction (compare individual mole percent values in Table II). In onion samples, levels of non-thiosulfinate components (e.g. sulfines, cepaenes) were significantly reduced in the distillates compared to the extracts. A particular advantage of the distillation method is that the distillate contains the flavor components as an aqueous solution: there is no interference from extraneous plant material and there is total exclusion of organic solvents.

A comparison of Si- and  $\text{C}_{18}$ -HPLC separations established that the best resolution was obtained using Si-HPLC with 2-propanol/hexane gradients. Although poorer separation of regio- and stereoisomers was obtained with  $\text{C}_{18}$ -HPLC (1:1 methanol/water), the latter conditions were preferable for thermospray ionization LC-MS. Representative  $\text{C}_{18}$ - and Si-HPLC traces for the different *Allium* samples are shown in Figures 1 and 2. These analyses show the presence of up to eight different thiosulfinates (plus separable *E/Z* isomers) whose identity could be established by comparison of HPLC behavior with that of authentic samples (Naganathan, 1992), by  $^1\text{H}$  NMR

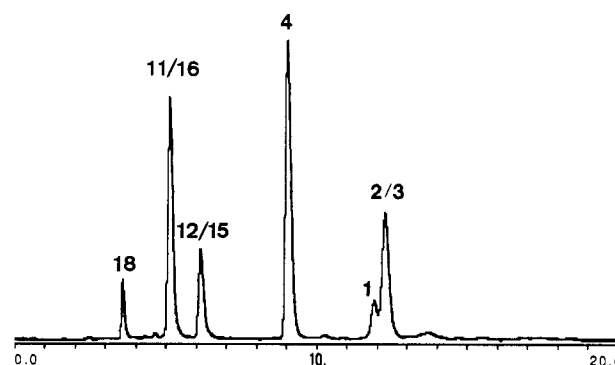


Figure 1.  $\text{C}_{18}$ -HPLC separation of thiosulfinates in garlic (*Allium sativum*; NY) extract: detection at 254 nm; injections on the right hand side at 0.0 min; see Table IV for peak identification and Experimental Procedures for details. The unnumbered peaks have not yet been identified.

analysis of the various methyl peaks (see Table III), by using a diode array UV detector, and by LC-MS methods. Data on thiosulfinate composition of the extracted plants are given in Table IV. Quantitation is based on calibration of the HPLC 254-nm UV detector with authentic samples whose molar UV extinction coefficients relative to an internal standard (benzyl alcohol) were independently measured. In most cases HPLC quantitation was substantiated by  $^1\text{H}$  NMR integration and GC-MS analysis (Block et al., 1992a). Each plant analysis was repeated several times with good qualitative agreement between runs; data are given in each case for a single representative run.

#### QUALITATIVE ANALYSIS

The data of Table IV are of value both qualitatively and semiquantitatively. The semiquantitative aspect of our data will be discussed below. Qualitatively, we conclude that, in addition to (*Z*)-propanethial *S*-oxide (7), the onion lachrymatory factor (LF), detected in the onion, leek, and shallot but probably formed in other *Allium* spp., the primary products from both the extraction and distillation procedures are thiosulfinates with only very minor quantities, if any, of di- and polysulfides, thiophenes, and thiosulfonates. The low levels of sulfides and thiosulfonates were established by HPLC as well as  $^1\text{H}$  NMR spectroscopy at 300 MHz and, as described in the accompanying paper, by GC-MS. For example, in those *Allium* spp. where methyl groups are abundant, distinctively shifted NMR peaks associated with polysulfides,  $\text{MeS}_n\text{R}$ , and thiosulfonates,  $\text{MeSO}_2\text{SR}$ , (Naga-

nathan, 1992) were far smaller than peaks associated with MeS(O)SR and MeSS(O)R. Thus, the response to the question posed earlier regarding the compounds primarily responsible for the flavor of freshly cut *Allium* species is "saturated and unsaturated thiosulfonates"! However because of their very low sensory threshold values (see below), minor amounts of disulfides, polysulfides, thiophenes, and thiosulfonates could still contribute to the flavor of the freshly cut plants.

While several of the thiosulfonates have been identified by HPLC and TLC methods in garlic and elephant garlic and in a few other *Allium* species [e.g. MeCH=CHSS(O)R, MeS(O)SMe, and PrS(O)SPr in onions; unresolved MeCH=CHSS(O)R/MeCH=CHS(O)SR isomers in onion, scallion, shallot, and leek; CH<sub>2</sub>=CHCH<sub>2</sub>SS(O)CH<sub>2</sub>-CH=CH<sub>2</sub>, CH<sub>2</sub>=CHCH<sub>2</sub>S(O)SMe/MeS(O)SCH<sub>2</sub>CH=CH<sub>2</sub>, and MeS(O)SMe in wild garlic (see Table I)], this and the accompanying paper represent the first complete identification of up to eight thiosulfonates in the each of the common *Allium* species, onion, shallot, scallion, leek, wild garlic, chive, and Chinese chive. In particular, we provide the first unequivocal evidence (based upon comparison with authentic standards) for thiosulfonates MeCH=CHS(O)SR (R = Me, Pr) in most of these species and for MeCH=CHSS(O)R in species not previously studied. Since thiosulfonates of type MeCH=CHSS(O)R have been found by us (Block, 1992) and by others (Bayer et al., 1989a, Lawson et al., 1991a) to undergo rapid *E-Z* interconversion, only the sums of the *E* and *Z* isomers for each R group are given in Tables II and IV even though in all cases the *E/Z* isomers can be separated and identified. Contrary to earlier reports (Fujiwara et al., 1955; Whitaker, 1976) but consistent with Lawson's observations (Lawson et al., 1991a), the *n*-propyl group is absent in garlic as well as in wild garlic, elephant garlic, and Chinese chive, the 1-propenyl group being present instead.

A number of interesting conclusions can be drawn from the data of Table IV:

(1) The major thiosulfonate from garlic is allicin 4, present to the extent of ca. 0.3% of the fresh weight. This value is in good agreement with that reported by others (Lawson et al., 1991a).

(2) The major unsymmetrical thiosulfonates from garlic, MeS(O)SCH<sub>2</sub>CH=CH<sub>2</sub> (16) and MeSS(O)CH<sub>2</sub>CH=CH<sub>2</sub> (11) are typically found in a 2:1 ratio. This interesting observation can be explained if it is recognized that in garlic, thiosulfonates containing only the 1- and 2-propenyl groups are formed 10 times more rapidly than thiosulfonates containing methyl groups (Lawson and Hughes, 1992). There would therefore be no 1- or 2-propenesulfenic acid (21 and 22, respectively) available to condense with the more slowly forming methanesulfenic acid (23). A mechanism involving reaction of 23 with allicin 4 (Scheme I) accommodates the facts that: (i) after 4 is maximally formed in 0.5 min it decreases until the mixed allyl methyl thiosulfonates 11/16 are maximally formed at 5 min (Lawson and Hughes, 1992); (ii) for each mole of 4 lost, 1.9 moles of mixed allyl methyl thiosulfonates is formed (Lawson and Hughes, 1992); (iii) the 16/11 ratio increases to ~2:1 after 1-5 min and 16 dominates; (iv) when powdered garlic (e.g. alliin (26)/alliinase) is added to excess aqueous *S*-methyl methanethiosulfinate (18), the major product is 11 (Scheme I).

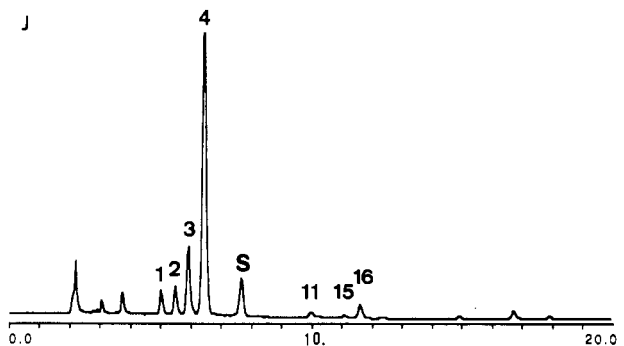
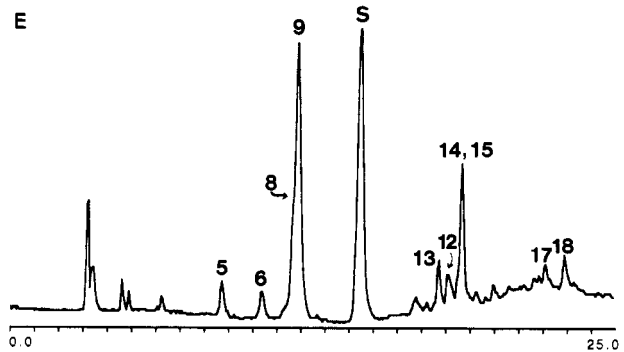
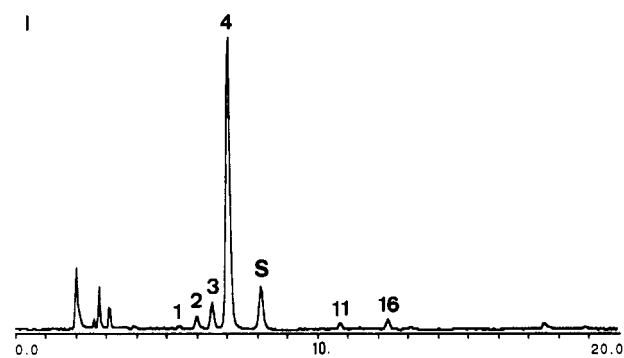
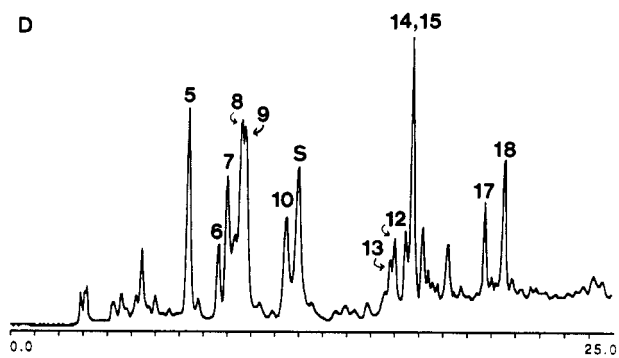
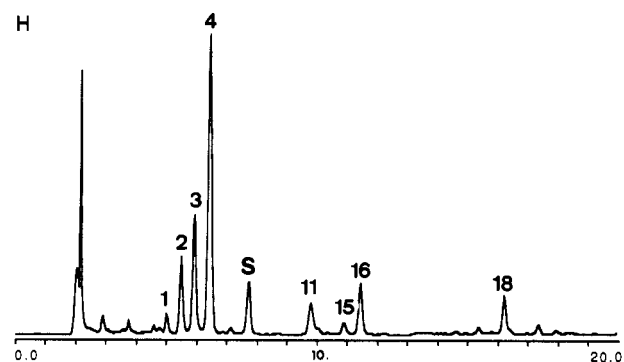
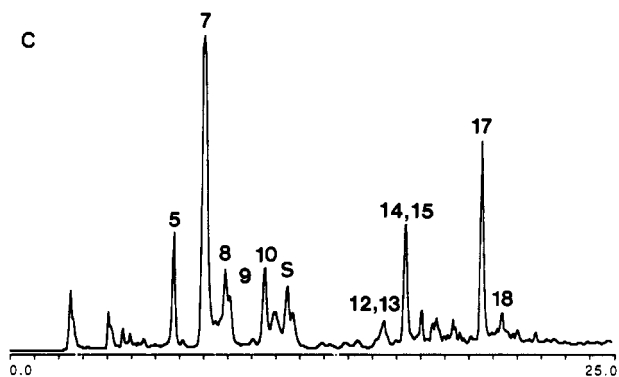
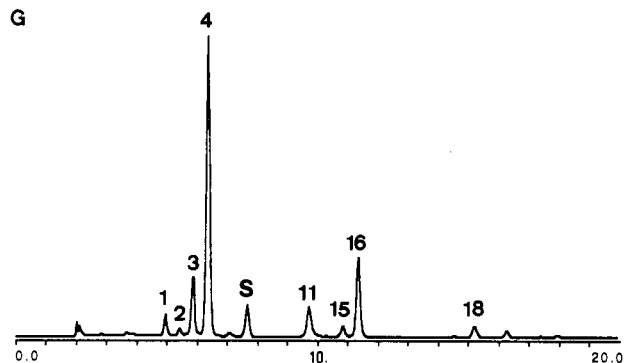
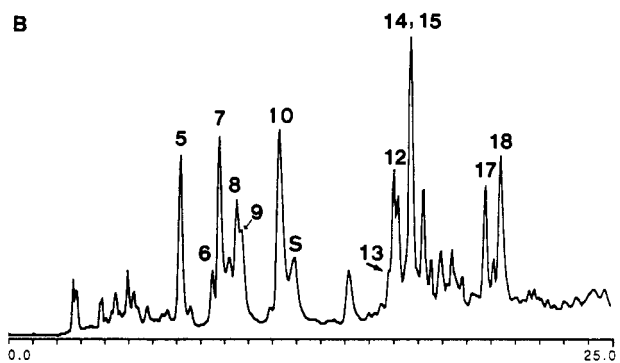
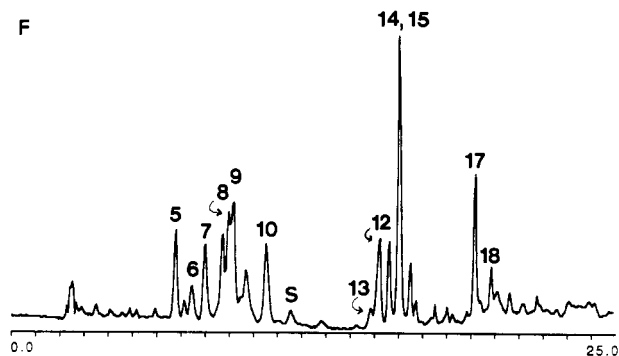
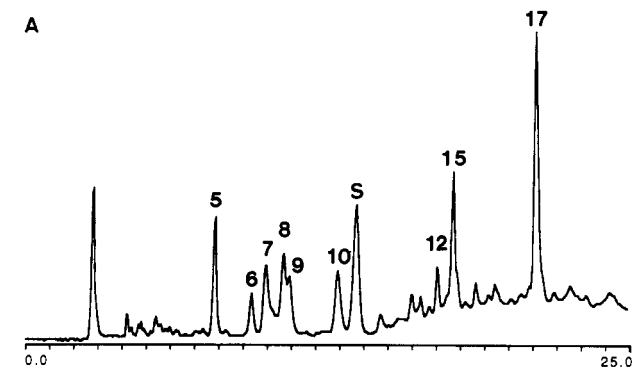
(3) Remarkably, the ratio of allicin 4 to the methyl/allyl thiosulfonates 11/16 is quite different comparing garlic grown locally (New York state) with that obtained in stores (typically from California or Mexico). Thus, some freshly

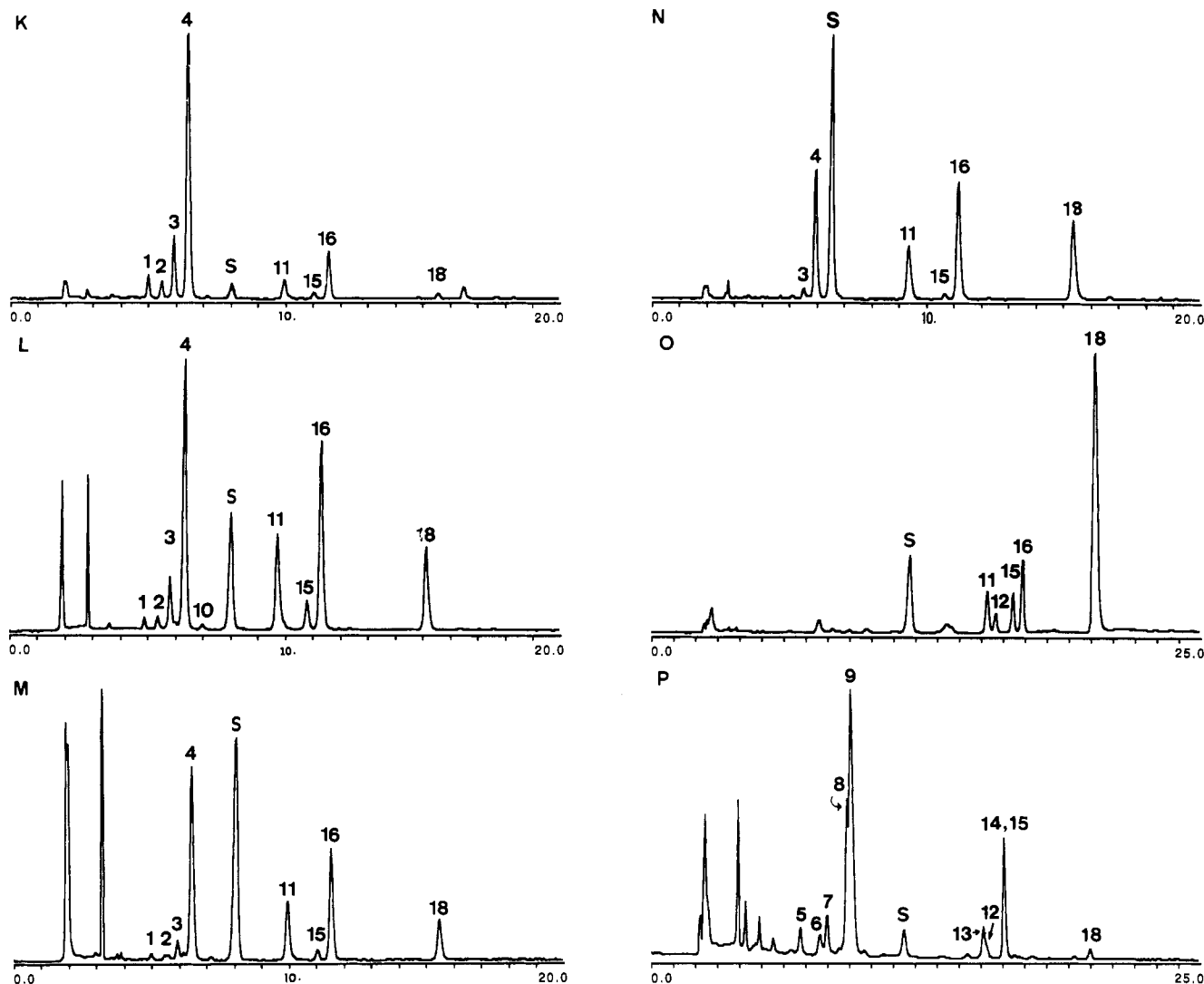
harvested New York state (NY)-grown garlic shows more than 95% of thiosulfonates present as allicin, an unprecedented observation. Both soft necked and hard necked ("top set") NY cultivars showed similar levels of allicin relative to other thiosulfonates. Analysis of the freshly harvested NY-grown garlic revealed abnormally low levels of (+)-*S*-methyl-L-cysteine sulfoxide (28, 0.08-0.25 mg/g of garlic compared to 1-1.6 mg/g in California garlic) with normal levels of other major cysteine derivatives and thiosulfonate precursors such as allyl cysteine sulfoxide (26), and methyl-, allyl-, and *trans*-1-propenyl- $\gamma$ -glutamyl cysteine (Lawson, Murdock Healthcare, unpublished analyses performed for the authors, 1992). A comparison of samples of Indian garlic obtained from a mountainous area (22-23 °C average temperature) and from an area with a climate averaging 10 °C warmer show a similar trend: the allyl/methyl ratio is larger for the garlic grown in a cooler climate. The Indian garlic samples are notable in that in both cases unusually high levels of methyl thiosulfonates are seen. Garlic treated with "mesoinositol hexaphosphate and silicic acid sol", which is claimed to destroy alliinase (Sakai, 1990), exhibits a deficiency of methyl thiosulfonates similar to that of NY-grown garlic. Finally, analysis of NY-grown elephant garlic shows that the methyl thiosulfonates/allicin ratio is smaller than the ratio observed in store-purchased (Mexican?) elephant garlic. We suggest that *Allium* spp., particularly garlic but also elephant garlic, grown in colder climates are subject to stress and that this stress manifests itself in reduced synthesis of (+)-*S*-methyl-L-cysteine sulfoxide (28), the immediate precursor of the methyl thiosulfonates. Chemical treatment of garlic can also result in diminished formation of methyl thiosulfonates, possibly by selective destruction of a methyl-specific alliinase (Lawson and Hughes, 1992).

(4) The concentrations of the minor unsymmetrical thiosulfonates, which possess 1-propenyl groups, vary with the age and storage conditions of the garlic following picking, as previously noted (Lawson et al., 1991a,b; Mütsch-Eckner, 1991). Thus, the allyl/methyl/1-propenyl ratio for store-purchased garlic, refrigerated store-purchased garlic, NY-grown garlic, refrigerated NY-grown garlic is 80:16:4, 78:11:11, 94:2:3, 90:3:8, respectively, demonstrating the increase in the 1-propenyl levels upon refrigeration for both the store-purchased and NY-grown garlic.

(5) In the *Allium* species examined, the allyl/methyl ratio ranged from 94:2 (NY garlic) to 80:16 (store bought garlic) to 74:24 (Indian garlic grown at 32 °C) to 62:35 (elephant garlic) to 50:49 (wild garlic) to 11:86 (Chinese chive). Allyl groups are absent in onion, scallion, shallot, leek, and chive.

(6) While all of the plants contained 1-propenyl groups, it was the dominant group in thiosulfonates from onion (largest amounts), scallion, leek, chive, and shallot. In the case of garlic, of the thiosulfonates containing 1-propenyl groups, the MeCH=CHSS(O) regioisomers predominate over the MeCH=CHS(O)S regioisomers by factors ranging from 2:1 to 20:1 (see Table IV and discussion below on variation of thiosulfonate concentrations with time) and (*E*)-RS(O)SCH=CHMe isomers predominate over *Z* isomers by a factor of at least 2.5:1, correcting the earlier, different conclusions (Lawson et al., 1991a) where authentic samples of the thiosulfonates in question were not available for LC calibration. The most abundant thiosulfonates (25-53% of total thiosulfonates) in the white, yellow, and red onion, shallot, and the scallion are (*E,Z*)-MeCH=CHSS(O)Me (12/15) and





**Figure 2.** Si-HPLC separation of thiosulfates in *Allium* species: A, white onion (*A. cepa*) extract; B, yellow onion extract; C, red onion extract; D, shallot (*A. ascalonicum* auct.) extract; E, scallion (*A. fistulosum* L.) extract; F, leek (*A. porrum* L.) extract; G, garlic (*A. sativum*; Mexican) extract; H, garlic (Mexican; refrigerated at 5 °C for 2 months) extract; I, garlic (NY) extract; J, garlic (NY, refrigerated at 5 °C for 2 months) extract; K, garlic (deodorized (Sakai, 1990)) extract; L, elephant garlic (*A. ampeloprasum* L. var. *ampeloprasum* auct.) distillate; M, elephant garlic (NY) distillate; N, wild garlic freeze-dried powder extract; O, Chinese chive (*A. tuberosum* L.) extract; P, chive (*A. schoenoprasum* L.) extract. Detection at 254 nm; injections on the right-hand side at 0.0 min; S identifies the peak for benzyl alcohol, the internal standard; see Table IV for identification of other peaks and Experimental Procedure for details. The unnumbered peaks have not yet been identified.

**Table III.** *Allium* Species Thiosulfinate Methyl <sup>1</sup>H NMR Chemical Shifts<sup>a</sup>

compd no.	R-1	R-2	C-3	C-2	C-1	S(O)	S	C-1'	C-2'	C-3'
11	allyl	methyl						2.64		
16	methyl	allyl			2.99					
18	methyl	methyl			2.96			2.67		
14	methyl	propyl			3.00					1.01
15	methyl	( <i>E</i> )-1-propenyl			2.98					1.93
12	methyl	( <i>Z</i> )-1-propenyl			3.04					1.86
13	propyl	methyl	1.06					2.63		
10	( <i>E</i> )-1-propenyl	methyl	1.99					2.60		
	( <i>Z</i> )-1-propenyl	methyl	1.96					2.69		

<sup>a</sup> Compounds of the type MeSO<sub>2</sub>SR typically show methyl singlets at 3.20–3.30 ppm while disulfides MeSSR show methyl singlets at 2.30–2.40 ppm.

(*E*)-MeCH=CHS(O)SMe (10); isomer (*Z*)-MeCH=CHS(O)SMe is not detected. The most abundant thiosulfinate in Chinese chive is MeS(O)SMe (18; 72% of total thiosulfates) while in chive it is *n*-PrS(O)SP*n* (9; 58% of total thiosulfates).

Of the thiosulfates found in onion, 37–47% contain the 1-propenyl group. This number gives a somewhat misleading indication of the actual amount of 1-propenyl

group present in onion since the majority of the propenyl group generated as 1-propenesulfenic acid (21) winds up as the lachrymatory factor (LF, 7). Thus, in one experiment using diethyl ether as solvent to extract yellow onions, 0.50 μmol/g of LF was isolated compared to 0.21 μmol/g total of thiosulfinate! Small quantities of several other compounds known to be derived from 21 were also positively identified in some of the extracts by spectro-

Table IV. Thiosulfinates and Sulfoxides from Extracts of *Allium* Species As Determined by SI-HPLC (Concentrations in Mole Percent of Total)

compd no.	compound <sup>c</sup> (response factor) <sup>b</sup>	white onion, A <sup>c,d</sup>	yellow onion, B <sup>c,d</sup>	red onion, C <sup>c,d</sup>	shallot, D <sup>c,d</sup>	scallion, E <sup>c,d</sup>	leek, F <sup>c,d</sup>	garlic store, G <sup>c,d</sup>	garlic store, H <sup>c,d</sup>	garlic R <sup>d</sup> , NY, I <sup>e</sup>	garlic R <sup>d</sup> , NY, J <sup>e,d</sup>	garlic <sup>d</sup> (Indian 1) <sup>h</sup>	garlic <sup>d</sup> (Indian 2) <sup>h</sup>	garlic <sup>d</sup> deod, K <sup>c</sup>	elephant garlic, L <sup>c</sup>	elephant garlic, NY, M <sup>c</sup>	wild garlic, N <sup>c,i</sup>	Chinese chive, O <sup>c,d</sup>	chive, P <sup>c,d</sup>
1	AlISS(O)Propenyl-(E)(18)							2.1	1.6	1.6	3.1	1	1	2.5	0.6	0.6			
2,3	AlIS(O)SPropenyl-(Z,E)(21)							5.9	18	5.3	13	3	3	8.3	3.7	2.6	0.9		
4	AlIS(O)SAlI (11)							62	59	89	79	64	53	69	37	43	28		
5	n-PrSS(O)Propenyl-(E)(18)	9	12	9	14	2	8												2.5
6,8	n-PrS(O)SPropenyl-(Z,E)(21)	16	10	12	22	17	15												16
7	EtCH=S=O (25)	++	++	++	++	++	++												58
9	n-PrS(O)SP-n (11)	13	13	4	26	33	24												*
10	MeSS(O)Propenyl-(E)(18)	22	23	23	9	7	12	8.1	7.5	1.4	1.6	9	11	*	*	*			
11	AlIS(O)SMe (11)							8.1	7.5	1.4	1.6	9	11	5.4	17	16	16	9	1 (5.5)
12,15	MeS(O)SPropenyl-(Z,E)(21)	31	25 (24)	29	16 (15)	22	31 (26)	1.2	1.9	*	*	1	1	1	2.2	1.5	0.7	5	5.9
13	MeSS(O)Pr (10)	1	1	4	2.8	11	5												15 (10)
14	MeS(O)SPr (14)	1	(1)	4	(1.2)	8	(5)												
16	AlISS(O)Me (11)							18	11	2.9	3.9	20	27	13	29	28	34	13	
17	OSCH(CHMe) <sub>2</sub> CHSO (48)	6	2	11	1.8	3	3												
18	MeS(O)SMe (14)	28	38	33	23	25	27	2.2	0.9			2	4	1.2	11	8.6	20	72	1.8
	total % MeS							16	11	2	3	18	24	11	35	31	49	86	13
	total % AlIS							80	78	94	89	80	74	83	62	67	50	11	
	total % 1-propenylS	45	37	47	32	24	33	4	11	3	8	2	2	6	3	2	1	3	10 (12)
	total % n-PrS	27	25	19	46	51	40												77 (75)
	total thiosulfinates <sup>e</sup>	0.14	0.35	0.20	0.25	0.08	0.15	25.6	20.7	14.3	22.1	15.5	36.5	24	5.2	2.7	21 <sup>i</sup>	2.0	0.19

Values in parentheses are corrected for peak overlap using data from NMR or GC-MS analysis. \* Trace amounts detected. ++ Variable but significant amounts detected. <sup>a</sup> Chemical Abstracts names of compounds: 1, (E)-1-propenesulfinothioic acid S-2-propenyl ester; 2, 2-propene-1-sulfinothioic acid S-(Z)-1-propenyl ester; 3, 2-propene-1-sulfinothioic acid S-(E)-1-propenyl ester; 4, 2-propene-1-sulfinothioic acid S-2-propenyl ester; 5, (E)-1-propenesulfinothioic acid S-n-propyl ester; 6, 1-propenesulfinothioic acid S-(Z)-1-propenyl ester; 7, (Z)-propanethial S-oxide; 8, 1-propenesulfinothioic acid S-(E)-1-propenyl ester; 9, 1-propenesulfinothioic acid S-1-propyl ester; 10, (E)-1-propenesulfinothioic acid S-methyl ester; 11, 2-propene-1-sulfinothioic acid S-methyl ester; 12, methanesulfinothioic acid S-(Z)-1-propenyl ester; 13, 1-propenesulfinothioic acid S-methyl ester; 14, methanesulfinothioic acid S-propyl ester; 15, methanesulfinothioic acid S-(E)-1-propenyl ester; 16, methanesulfinothioic acid S-2-propenyl ester; 17, (Z,Z)-d,l-2,3-dimethyl-1,4-butanedithial S,S'-dioxide; 18, methanesulfinothioic acid S-methyl ester. <sup>b</sup> Molar absorption with HPLC: 254-nm UV detector relative to benzyl alcohol calibrated by NMR peak integration. <sup>c</sup> Amounts given in  $\mu\text{mol/g}$  wet (fresh) weight. Since garlic and elephant garlic contain ca. 60% water (Lawson, 1991a), approximate dry weight values for these plants are obtained by multiplying fresh weight values by 2.5. Comparative HPLC data (Lawson 1990; 1991a): white onion, yellow onion, shallot, scallion, leek, garlic, and elephant garlic, respectively, 0.40, 0.50, 1.89, 1.65, 1.77, 34, and 11  $\mu\text{mol/g}$  fresh weight (first five values refer to 1-propenyl thiosulfinate amounts only). <sup>d</sup> Abbreviations used: D, distilled and then distillate extracted; R, refrigerated for 2 months at 5 °C prior to analysis; NY, plant obtained from farms in upstate New York (average growing temperature 19–22 °C); deod, garlic treated to "deodorize" (Sakai, 1990); Indian 1, grown in mountainous area of India with average 25 °C average growing temperature; Indian 2, grown in plains area of India, with 30–32 °C average growing temperature; store, typical store-purchased garlic (generally of California origin with average 25 °C average growing temperature; Indian 2, grown in plains area of India, with 30–32 °C average growing temperature (average high 33 °C, average low 17 °C)); % MeS, % AlIS, % 1-PropenylS, % PrS, percent of total thiosulfinate containing methyl, allyl, 1-propenyl, or n-propyl group, respectively. <sup>e</sup> Letters refer to HPLC traces shown in Figure 2. / Composition confirmed by GC-MS (accompanying paper). <sup>f</sup> Composition confirmed by <sup>1</sup>H NMR at 300 MHz. <sup>h</sup> These data supported by similar data from Lawson (Murdock Healthcare, unpublished analyses performed for the authors, 1992). <sup>i</sup> Amounts of thiosulfinate are based on the weight of freeze-dried powder. Comparative HPLC data: 30% 4, 47% 11 + 16, 23% 18; total thiosulfinate concentration 107  $\mu\text{mol/g}$  dry weight (Sendi and Wagner, 1991).





**Table V. Variation of Concentrations of Thiosulfinates in an Extracted Garlic Homogenate<sup>a</sup> with Elapsed Time As Determined by Si-HPLC (Molar Amounts Relative to Alliin)**

compd no.	compound <sup>c</sup>	elapsed time of analysis following homogenization			
		1 min	30 min	6 h	26 h
1	AllSS(O)Propenyl-( <i>E</i> )	7.7	4.2	1.1	
2,3	AllS(O)SPropenyl-( <i>Z,E</i> )	16	14	12	9
4	AllS(O)SAll <sup>b</sup>	100	100	100	100
10	MeSS(O)Propenyl-( <i>E</i> )	1.2			
11	AllS(O)SMe	6.9	7.2	7.2	6.8
12,15	MeS(O)SPropenyl-( <i>Z,E</i> )	1.6	1.6	1.3	0.7
16	AllSS(O)Me	15	16	16	16
18	MeS(O)SMe	1.1	1.1	0.4	
	total % MeS	9	9	9	5
	total % AllS	82	84	86	90
	total % 1-propenylS	9	7	5	4
	total thiosulfinates (μmol/g)	40.4	52.7	31.8	35.7

<sup>a</sup> Store-purchased garlic refrigerated for 26 weeks to enhance 1-propenyl thiosulfinates. <sup>b</sup> Normalized to 100. <sup>c</sup> All amounts in table confirmed by <sup>1</sup>H NMR spectroscopy.

(4, thiosulfonates, di- and polysulfides, and thiophene derivatives) may in fact be artifacts of their analytical procedure.

#### LC-MS AND DIODE ARRAY UV ANALYSIS

Exploratory LC-MS analysis of extracts of store-purchased garlic under reversed-phase conditions (Figure 1) was performed under particle beam EI and CI as well as thermospray conditions. LC-MS analysis of garlic extracts has not been previously reported. Unseparated MeS(O)SCH<sub>2</sub>CH=CH<sub>2</sub>/MeSS(O)CH<sub>2</sub>CH=CH<sub>2</sub> (11/16) was identified under EI conditions by a base peak at *m/e* 73 (CH<sub>2</sub>=CHCH<sub>2</sub>S) along with an *m/e* 136 parent and *m/e* 95 (CH<sub>3</sub>SSO<sup>+</sup>) and 63 (CH<sub>3</sub>SO<sup>+</sup>) fragments, and under NH<sub>3</sub> CI and NH<sub>4</sub>OAc thermospray conditions by M + H<sup>+</sup> ions at *m/e* 137 and M + NH<sub>4</sub><sup>+</sup> ions at *m/e* 154. Alliin (4) was identified under EI conditions by a base peak at *m/e* 73 (CH<sub>2</sub>=CHCH<sub>2</sub>S) along with an *m/e* 162 parent and under NH<sub>3</sub> CI and thermospray conditions by M + H<sup>+</sup> ions at *m/e* 163 and M + NH<sub>4</sub><sup>+</sup> ions at *m/e* 180. Isomeric (*E,Z*)-MeCH=CHSS(O)CH<sub>2</sub>CH=CH<sub>2</sub> (2/3) was identified under NH<sub>3</sub> CI and thermospray conditions by M + H<sup>+</sup> ions at *m/e* 163 and M + NH<sub>4</sub><sup>+</sup> ions at *m/e* 180.

A comparison was made for us by E. Calvey and by L. Lawson of Si-HPLC diode array UV data on the individual components of extracts of garlic with the corresponding Si-HPLC/diode array UV data for our synthetic samples of compounds 1-3, 10, 12, and 15. Excellent agreement was obtained in both cases between data for our samples and the individual garlic extract components (see Experimental Procedures for UV data).

#### QUANTITATIVE ANALYSIS OF ALLIUM THIOSULFINATES AND ITS LIMITATIONS

We sought to determine the factors that effect the reliability and quantitative reproducibility of *Allium* thiosulfinate analysis by HPLC methods. Since thiosulfinates are known to be relatively unstable, the effect of varying the elapsed time between homogenization and HPLC analysis from 1 min to 26 h was determined (Table V). Garlic cloves were homogenized as above. The homogenate was divided into four portions which were maintained at room temperature and extracted and analyzed as above so that the elapsed time before injection into the HPLC was 1 min, 30 min, 6 h, and 26 h. Parallel studies were performed using <sup>1</sup>H NMR spectroscopy at 300 MHz. There was good agreement between the NMR

and HPLC data. Thiosulfinate concentrations were normalized relative to alliin. While the total thiosulfinate concentration remained roughly constant, thiosulfinates of type MeCH=CHS(O)SR diminished rapidly and were nearly gone after 6 h, regioisomeric thiosulfinates MeCH=CHSS(O)R were reduced to ca. 50% of their initial value after 26 h, and the small initial concentration of MeS(O)SMe was significantly reduced after 6 h. Apart from the significant reduction in concentrations of MeCH=CHS(O)SR thiosulfinates and a 12% reduction in concentration of MeCH=CHSS(O)R isomers, there was good agreement between the 1- and 30-min samples.

In a similar fashion, the extract of a white onion was analyzed by NMR with elapsed times of 30 min and 24 h prior to analysis. The results paralleled those seen with the garlic, namely thiosulfinates of type MeCH=CHS(O)SR were gone after 24 h but otherwise the 30-min and 24-h extracts were quite similar. These results explain the failure of previous workers to identify thiosulfinates of type MeCH=CHS(O)SR in onion extracts (Bayer et al., 1989a). It is concluded that representative data is best obtained within 30 min of homogenization although most of the components survive in the homogenate for 24 h or more. Others have reported that aqueous solutions of alliin and other thiosulfinates undergo only ca. 9% decomposition after standing at room temperature overnight (Lawson and Hughes, 1992). It has been our experience, and that of others, that solutions of alliin and other thiosulfinates are much less stable in organic solvents at room temperature. Organic solutions are best analyzed immediately or stored in a freezer at temperatures below -20 °C. We have previously demonstrated the excellent hydrogen-bonding ability of thiosulfinates (Block and O'Connor, 1974b) and suggest that this property is responsible for the relative stability of thiosulfinates in water.

A variety of other factors can affect the reproducibility of *Allium* thiosulfinate analysis: (1) We have already noted that the "room temperature steam distillation procedure", while qualitatively very similar to direct extraction, typically provides half of the amounts of thiosulfinates obtained by extraction. (2) The relative percentages for different alkyl groups can vary with the part of the plant; e.g., in Chinese chive the methyl/allyl ratio varies from 71:29 to 36:64, analyzing leaf, roots, and rhizome (Mackenzie and Ferns, 1977), while in wild garlic this same ratio varies from 50:50 to 34:66, analyzing leaf and bulbs, respectively (Sendl and Wagner, 1991). (3) Thiosulfinate variation also occurs with the plant variety (as already seen in the case of yellow, white, or red onion), developmental stage (Lancaster and Kelly, 1983), and growing and storage conditions (as already discussed). Of course, our HPLC technique is of value in determining precisely these differences!

Sensory analysis of the "room temperature steam distillate" of onion showed it to have an odor and taste quite similar to that of fresh onions. Unsaturated thiosulfinates such as (*E*)-MeCH=CHSS(O)Me and (*E*)-MeCH=CHS(O)SMe both have threshold levels of 0.01 ppm; the former has a fresh raw onion or shallot flavor while the latter has a cabbage or radish note. Thiosulfinate (*E,Z*)-MeCH=CHSS(O)Pr has a green fresh raw onion odor and a threshold level of 0.01 ppm while MeCH=CHSS(O)CH<sub>2</sub>CH=CH<sub>2</sub> has a fresh garlic odor and a threshold level of 0.05 ppm. For comparison, disulfides such as *n*-PrSSPr-*n* and MeSSCH=CHMe are reported to have detection thresholds of ca. 0.002-0.006 ppm (Boelens et al., 1971); in the present work related

thiosulfates have been found to possess detection thresholds as low as 0.001 ppb.

## CONCLUSIONS

In our study of room temperature extracts or distillate extracts of nine different *Allium* species, we find good agreement between HPLC,  $^1\text{H}$  NMR, and GC/GC-MS analyses (Block et al., 1992b), using authentic samples to optimize analysis conditions and as calibrants for quantification and using a well-defined procedure for obtaining extracts. Analysis of thiosulfate mixtures by Si-HPLC is particularly useful because most isomers can be separated and even the most unstable compounds (such as those from garlic) survive analysis. While our exploratory studies of *Allium* thiosulfate profiles establish the broad utility of the analytical methods, and in our opinion are better representative for taxonomic purposes of the *Allium* species than earlier profiles based on secondary products (Bernhard, 1970; Freeman and Whenham, 1975; Saghir et al., 1964), both quantitative and qualitative differences have been found in samples of several species purchased at local markets during the course of 6 months. Therefore to best define the thiosulfate profiles characteristic of each species, a more extensive study should be conducted of the changes in thiosulfate ratios, for well-identified cultivars, with growing conditions and other controlled variables (e.g. fertilization, irrigation, humidity, plant varieties, storage, transport, etc.).

## EXPERIMENTAL PROCEDURES

**Materials.** All *Allium* varieties except wild garlic (ramson) were obtained from local stores. The wild garlic was provided in the form of a freeze-dried powder by Anna Sendl and H. Wagner. NY-grown garlic was obtained directly from the growers—Rose Valley Farms, Rose, NY; Hillside Organic Farms, Troy, NY; and Ron Bennett Farm, Victor, NY. Indian garlic was obtained on location. Solvent grade dichloromethane from Ashland Chemical Co. was freshly distilled prior to use. With samples for LC-MS analysis, great care was taken to avoid contamination from ubiquitous phthalate plasticizers. Benzyl alcohol was obtained from Eastman Kodak and used without further purification. Anhydrous sodium sulfate (Fisher Certified) was used as drying agent. Solvents used for HPLC were obtained from J. T. Baker (HPLC grade) and used as such. Authentic samples of each of the compounds 1–18 were prepared by us using methods described elsewhere (Naganathan, 1992; Putman, 1992).  $^1\text{H}$  NMR data for the methyl groups of compounds 10–16 and 18 are given in Table III and partial diode array UV data for compounds 1–3, 5, 6, 8, 10, 12, and 15 are given below; more extensive  $^1\text{H}$  and  $^{13}\text{C}$  NMR, mass, and IR spectra and diode array UV data for these and related compounds are given elsewhere (Naganathan, 1992). HPLC analysis of cysteine derivatives in NY state garlic was performed for us by L. Lawson.

**Equipment.** Si- and  $\text{C}_{18}$ -HPLC analyses were performed on Rainin Microsorb columns (silica gel or  $\text{C}_{18}$  silica gel, 5  $\mu\text{m}$ , 250 mm  $\times$  4.6 mm with a 15 mm  $\times$  4.6 mm guard) using a Rainin Solvent Deliver System. Data collection, processing, and gradient control were achieved using the Dynamax Method Manager programs on a Macintosh computer. Two different solvent gradient programs were used for Si-HPLC. The first program, used in those runs shown in Figure 2 with a 20-min time scale, was at a constant flow rate of 1.8 mL/min with an initial composition of 2% 2-propanol/98% hexanes for 6 min, a linear increase to 10% 2-propanol during 10 min, and a hold at 10% for 9 min before returning to the initial composition over 5 min. The second program, used in those runs with a 25-min time scale, was at a constant flow of 1.6 mL/min with an initial composition of 2% 2-propanol/98% hexanes for 10 min, a linear increase to 20% 2-propanol during 20 min, and a hold at 20% for 5 min before returning to the initial composition over 10 min. For  $\text{C}_{18}$ -HPLC, the solvent was 50% MeOH–50% water. LC-MS analysis employed a Hewlett-Packard 5989 MS Engine together with a

Rainin or Hewlett-Packard HPLC. LC-MS analysis was also performed for us by the Extrel Corp. The MS Engine was equipped with both particle beam and thermospray detectors. The latter detector required addition of  $\text{NH}_4\text{OAc}$  to the HPLC solvent. NMR spectra were obtained on a Varian XL 300 or Varian Gemini 300 operating at 300 MHz for proton.

**Homogenate Preparation.** Fresh extracts of garlic were prepared by homogenizing peeled garlic cloves in 10 mL of water/g of garlic for 1 min using a tissue homogenizer to ensure cell disruption, filtering through cheesecloth or nylon mesh, saturating the filtrate with sodium chloride, extracting twice with equal volumes of methylene chloride, drying, and rapidly concentrating at room temperature. In most cases the concentrates were then analyzed by normal (Si) or reversed-phase ( $\text{C}_{18}$ ) HPLC within 30 min of the time of homogenization. A similar procedure was applied to other *Allium* species, reducing the water/plant weight ratio to 2:1 or even 1:1 in the case of plants with higher water content. Plant material was homogenized with a Black and Decker "Handy-Chopper", a Waring blender, or a Brinkmann tissue homogenizer. If a garlic extract is prepared using a high-speed tissue homogenizer instead of a blender, the resulting clear solution affords after extraction twice the amount of each thiosulfate compared to the extract prepared with a blender, indicating that thiosulfate levels can depend on the homogenization procedure employed. For small-scale analyses of onion and shallot, ca. 20 g of the peeled and trimmed bulbs were placed in the chopper with an equal weight of water and blended. In the case of garlic/elephant garlic, trimmed, peeled cloves were used with 10 times their weight of water. For chive and Chinese chive the entire greens were used, for scallion the entire plant except the roots was used, while for leek the bottom two-thirds of the plant was used except for roots; in all of these cases twice as much water as plant (by weight) was used. The homogenate was then transferred to a beaker and homogenized for 1 min with the tissue homogenizer. For large-scale analyses, ca. 150 g of the peeled and trimmed plant was homogenized in a Waring blender for 1 min with an equal amount of water. In either case, the homogenate was allowed to stand for 30 min prior to extraction or distillation. In a few cases garlic or onion homogenates were allowed to stand at room temperature for time periods varying from 1 to 24 h prior to extraction and analysis to determine the effect of standing time on thiosulfate composition (data given in Table V).

**Extractions.** The homogenate was filtered through cheesecloth, and the filtrate was saturated with salt and extracted with an equal volume of dichloromethane. It was usually necessary to use centrifugation followed by filtration in order to clear the resulting emulsion. The dichloromethane extract was concentrated on a rotary evaporator at room temperature. The residue was dissolved in 1 mL of dichloromethane, dried, and filtered through a 0.2- $\mu\text{m}$  Nylon 66 filter for immediate HPLC analysis. Thiosulfates are rather unstable in dichloromethane. If immediate analysis is not possible, the dichloromethane solutions should be stored below  $-20\text{ }^\circ\text{C}$  and preferably at  $-78\text{ }^\circ\text{C}$ . To determine the effect of varying the elapsed time between homogenization and HPLC analysis, 11 g of garlic was homogenized. The homogenate was divided into four portions which were maintained at  $20\text{ }^\circ\text{C}$  and analyzed by Si-HPLC; the elapsed time before injection into the HPLC was 1 min, 30 min, 6 h, and 26 h. The results are summarized in Table V. Extraction of the LF from yellow onions with ether is described in the accompanying paper (Block et al., 1992a).

**Distillations.** The homogenate was transferred into a round-bottom flask containing a magnetic stir-bar and vacuum distilled using an apparatus consisting of a 1-L flask immersed in an oil bath fitted with an elongated Claisen-type distillation head attached to a 90° glass connector terminating in a 3-neck 500-mL round-bottom flask half immersed in liquid nitrogen. This flask was connected via a pair of liquid nitrogen chilled vacuum traps to a vacuum pump. Initially, the vacuum was applied carefully and intermittently so as to prevent uncontrollable foaming, typically during 1 h. When a high vacuum (0.01–0.005 mm) was eventually achieved and the water started to distill at a rapid rate, the homogenate in the pot cooled rapidly and usually froze, causing frost to form on the outside of the pot. The oil bath temperature also dropped considerably even though heat

was still being applied. Sometimes, when large volumes of distillate were collected, water would fill the collection vessel and freeze in the inlet neck, preventing further distillation. This was accompanied by the clearing of the frost on the flask and a rise in the pot temperature to 40 °C even though there was still water to be distilled. Under these conditions, the distillation was continued after replacing the filled collection vessel with a fresh one. Completion of the distillation was indicated by the disappearance of the frost on the pot and an increase in the temperature of the oil bath to ca. 40 °C. The distillate was allowed to thaw at room temperature, then saturated with salt, and extracted twice with equal volumes of dichloromethane. The extracts were combined and concentrated on a rotary evaporator at room temperature. The residue was dissolved in 1 mL of dichloromethane, dried, and filtered through a 0.2- $\mu$ m Nylon 66 filter for HPLC analyses.

**Reaction of Garlic Homogenate with Methanesulfinothioic Acid *S*-Methyl Ester (18).** Garlic powder (0.30 g) was added to a vigorously stirred solution of 18 (0.30 g) in water (3 mL) at room temperature. After 20 min, CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added and the aqueous layer was saturated with salt. The organic layer was separated, concentrated, dried, and filtered. The filtrate, analyzed by Si-HPLC, showed 2-propene-1-sulfinothioic acid *S*-methyl ester (11, CH<sub>2</sub>=CHCH<sub>2</sub>S(O)SMe) and excess 18 as the only thiosulfinate peaks.

**LC-MS Analysis.** LC-MS analysis of garlic extracts under reverse-phase conditions (Figure 1) performed for us by both the Hewlett-Packard Co. and the Extrel Corp. under particle beam EI and CI as well as thermospray conditions showed unseparated MeS(O)SCH<sub>2</sub>CH=CH<sub>2</sub>/MeSS(O)CH<sub>2</sub>CH=CH<sub>2</sub> (16/11) [*m/e* 136 (4), 95 (5), 87 (7), 73 (100), 63 (6) by EI and 137 (M + H<sup>+</sup>), 154 (M + NH<sub>4</sub><sup>+</sup>)] by NH<sub>3</sub>-CI or thermospray; allicin (4) [*m/e* 162 (17), 145 (5), 114 (17), 113 (26), 90 (28), 73 (100)] by EI and NH<sub>3</sub>-CI or thermospray [163 (M + H<sup>+</sup>), 180 (M + NH<sub>4</sub><sup>+</sup>)] and MeCH=CHSS(O)CH<sub>2</sub>CH=CH<sub>2</sub> (2/3) [*m/e* 163 (M + H<sup>+</sup>), 180 (M + NH<sub>4</sub><sup>+</sup>)] by NH<sub>3</sub>-CI or thermospray.

**Diode Array UV Analysis.** Synthetic samples of compounds 1-3, 5, 6, 8, 10, 12, and 15 provided by us to E. Calvey were injected into a Si-HPLC column connected to a Hewlett-Packard Model 1040A diode array UV detector and the UV spectra were recorded in each case for the major HPLC peak. These data are given here as  $\lambda_{\max}$  (log  $\epsilon$ ); full UV spectra appear elsewhere (Naganathan, 1992): 1, (*E*)-1-propenesulfinothioic acid *S*-2-propenyl ester (AllSS(O)Propenyl-*E*), 200 (4.33), 268 (3.74); 2, 2-propene-1-sulfinothioic acid *S*-(*Z*)-1-propenyl ester (AllS(O)SPropenyl-*Z*), 216 (4.23); 3, 2-propene-1-sulfinothioic acid *S*-(*E*)-1-propenyl ester (AllS(O)SPropenyl-*E*), 222 (4.19); 5, (*E*)-1-propenesulfinothioic acid *S*-*n*-propyl ester (*n*-PrSS(O)Propenyl-*E*), 206 (4.11), 268 (3.74); 6, 1-propanesulfinothioic acid *S*-(*Z*)-1-propenyl ester (*n*-PrS(O)SPropenyl-*Z*), 216 (4.23), 264 (3.67); 8, 1-propanesulfinothioic acid *S*-(*E*)-1-propenyl ester (*n*-PrS(O)SPropenyl-*E*), 222 (4.13), 254 (3.68); 10, (*E*)-1-propenesulfinothioic acid *S*-methyl ester (MeSS(O)Propenyl-*E*), 208 (4.10), 266 (3.67); 12, methanesulfinothioic acid *S*-(*Z*)-1-propenyl ester, (MeS(O)SPropenyl-*Z*), 216 (4.17), 266 (3.68); 15, methanesulfinothioic acid *S*-(*E*)-1-propenyl ester; MeS(O)SPropenyl-*E*); 220 (4.12), 264 (3.69). There was excellent agreement between spectra obtained on diode array HPLC analysis of garlic extracts containing compounds 1-3, 10, 12, and 15 and the spectra of our authentic samples.

**Sensory Analysis of *Allium* Components.** Several pure components of *Allium* extracts/distillates available from synthetic studies were evaluated by "expert flavorists" who described odor and taste. The "room temperature steam distillate" of onions, consisting primarily of  $\alpha,\beta$ -unsaturated thiosulfinate, had an odor and taste quite similar to that of fresh onions. Of the pair (*E*)-MeCH=CHSS(O)Me/(*E*)-MeCH=CHS(O)SMe (13/10), the former had a pronounced fresh raw onion or shallot flavor while the latter had more of a cabbage or radish note; the detection threshold for both was 0.01 ppm. Of the pair (*E,Z*)-MeCH=CHSS(O)Pr-*n*/MeCH=CHSS(O)CH<sub>2</sub>CH=CH<sub>2</sub> (6,8/2,3) the former had a green, raw, fresh onion odor while the latter at 1-10 ppm had a very fresh garlic odor; the detection threshold for the former was 0.1 ppm and for the latter was 0.05 ppm.

## ABBREVIATIONS USED

C<sub>18</sub>-HPLC, high-pressure liquid chromatography with octadecyl bonded phase; Si-HPLC, high-pressure liquid chromatography with silica gel; HPLC, high-pressure liquid chromatography; LC-MS, coupled high-pressure liquid chromatography-mass spectrometry; GC-MS, coupled gas chromatography-mass spectrometry; CI, chemical ionization; EI, electron ionization; LF, onion lachrymatory factor, (*Z*)-propanethial *S*-oxide; MS, mass spectrometry; NMR, nuclear magnetic resonance; UV, ultraviolet.

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**Registry No.** (E)-1, 134595-70-3; (Z)-1, 143038-12-4; (Z)-2, 136516-39-7; (E)-3, 134568-42-6; 4, 539-86-6; (E)-5, 143063-27-8; (Z)-5, 143038-13-5; (Z)-6, 119052-97-0; (E)-7, 74635-27-1; (Z)-7, 70565-74-1; (E)-8, 119052-98-1; 9, 1948-52-3; (E)-10, 134568-43-7; (Z)-10, 136516-41-1; 11, 3736-98-9; (Z)-12, 119053-00-8; 13, 125895-81-0; 14, 119151-97-2; (E)-15, 119052-99-2; 16, 104228-49-1; (R\*,R\*)-(Z,Z)-(±)-17, 127793-93-5; 18, 13882-12-7.