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Allium Chemistry: GC–MS Analysis of Thiosulfinates and Related Compounds from Onion, Leek, Scallion, Shallot, Chive, and Chinese Chive

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Extracts of 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.) were analyzed by 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. Authentic samples of suspected components were used to verify identities and quantitate amounts, employing benzyl alcohol as internal standard. Under these conditions, thiosulfinates MeS(O)SMe (**18**), PrS(O)SPr (**9**), MeSS(O)Pr (**13**), MeS(O)SPr (**14**), (*Z*)- and (*E*)-MeS(O)SCH=CHMe (**12**, **15**), (*E*)-MeSS(O)CH=CHMe (**10**), and (*Z*)- and (*E*)-PrS(O)SCH=CHMe (**6**, **8**) and 2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxides (**31**, **30**; *cis*- and *trans*-zwiebelanes) were all identified in onion, leek, scallion, shallot, and chive while MeS(O)SMe and (*E*,*Z*)-MeS(O)SCH=CHMe were identified in Chinese chive. On the other hand, we could not obtain satisfactory peaks for MeCH=CHS(O)SPr isomers or any thiosulfinates containing a 2-propenyl (allyl) group, which are best identified by HPLC. Sensory data are provided on compounds **30** and **31**.

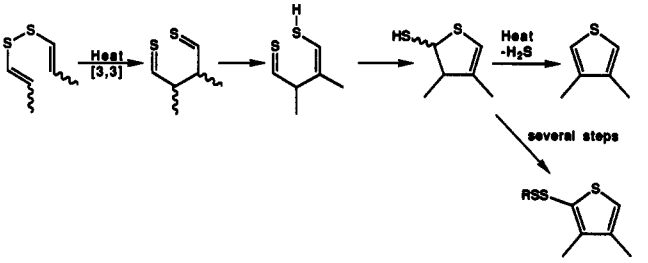
INTRODUCTION

The accompanying paper demonstrates the utility of HPLC in answering the question: "What compounds are primarily responsible for the characteristic flavor of freshly cut members of the genus *Allium* (Block et al., 1992)?" Our HPLC study of nine different *Allium* species shows that in each case the predominant flavor principles are thiosulfinates and propanethial *S*-oxide (in the case of onion), with only minimal amounts of polysulfides and other compounds. Several weaknesses exist with the HPLC method: (1) incomplete separation of some of the peaks; (2) retention time variation leading to possible misidentification of peaks; (3) compounds having minor UV activity may be overlooked; (4) present limitations of LC–MS in characterizing volatile compounds. In view of the fact that our conclusions on the composition of *Allium* flavorants are at variance with many earlier studies (see accompanying paper for prior citations) and that the HPLC method has its limitations, we sought to develop other "hyphenated" techniques to independently separate, characterize, and quantify these flavorants. We describe here the use of GC–MS for this purpose.

While GC–MS is of great value in *Allium* chemistry in the study of compounds of moderate thermal stability, such as those found in the distilled oils of garlic and onion (Block et al., 1988; Block and Zhao, 1990), thiosulfinates from *Allium* species are known to decompose on heating or attempted GC analysis, as illustrated by the formation of a pair of *m/e* 144 isomers from allicin (see eq 1 of accompanying paper) (Brodnitz et al., 1971). Furthermore, we have shown that bis(1-propenyl)disulfide, a common component of *Allium* distilled oils, rearranges at 85 °C to 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, which could then form 3,4-dimethylthiophene or 3,4-dimethyl-2-thienyl disulfides (Scheme I), reported to be present in *Allium* distillates (Sinha et al., 1992).

Many authors have drawn conclusions based on the pattern of products formed on GC analysis of *Allium* preparations using injection port temperatures as high as 280 °C (Martín-Lagos et al., 1992; Mazza et al., 1992; Sihna et al., 1992; Kallio and Salorinne, 1990; Kuo, 1990; Schreyen et al., 1976; Talyzin et al., 1988; Vernin et al., 1986; Wijaya et al., 1991; Yu et al., 1989) despite the earlier recognized risk that these products are "artifacts of analysis" (Bernhard, 1970). In 1974 we demonstrated that many thiosulfinates possessing up to eight carbons survive GC analysis if short packed columns (2 m × 3 mm i.d. 10% silicone rubber) and injection port and column temperatures below 100 °C are employed, that regioisomeric thiosulfinates such as MeS(O)SEt and MeSS(O)Et can be nicely separated, and that unique EI-GC–MS data can be obtained for each regioisomer (Block and O'Connor, 1974). More recently, conditions of the above type have been used in a study of the gas-phase stability of the leek component PrS(O)SPr (Auger et al., 1989). Since we have synthesized the various thiosulfinates shown by HPLC to be present in *Allium* extracts, we experimented with different GC columns and GC and GC–MS conditions to maximize resolution, peak shape, and sample detection; our results are described below. We find that injection port conditions and other GC and GC–MS variables can have a dramatic effect on peak characteristics for thermally labile compounds and that for some compounds excellent results are possible using on-column injection together with cryogenic injector/oven conditions.

Scheme I



RESULTS AND DISCUSSION

Initial GC–MS studies employed a 12-m × 0.2-mm capillary column and on-column injection. It was soon

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Table I. Thiosulfinates and Related Compounds from Extracts of *Allium* Species As Determined by GC–MS (Concentrations in Mole Percent of Total; Comparative HPLC Data[a] in Brackets)

compd no.	compd name[b]	white onion A, B[k]	yellow onion C[k]	red onion D[k]	shallot E[k]	scallion F[k]	leek[i,l] G[k]	chive[i] H[k]	Chinese chive[i] I[k]
5	*n*-PrSS(O)Propenyl-(*E*)[h]	(10) [10]	(12) [12]	(10) [10]	(14) [14]	(2) [2]	(5) [8]	(3) [3]	
6, 8	*n*-PrS(O)SPropenyl-(*Z*,*E*)	33 [17]	12 [10]	29 [14]	21 [22]	25 [17]	5 [15]	25 [16]	
9	*n*-PrS(O)SPr-*n*	8 [14]	9 [13]	6 [5]	26 [27]	35 [33]	25 [25]	57 [58]	
10	MeSS(O)Propenyl-(*E*)	14 [24]	24 [24]	19 [26]	8 [9]	3 [7]	21 [12]	1	
11	AllS(O)SMe								[9][g]
12, 15	MeS(O)SPropenyl-(*Z*,*E*)[j]	33 [33]	31 [25]	27 [33]	15 [15]	18 [22]	29 [27]	5 [5]	13 [5][g]
13	MeSS(O)Pr	1 [1]	1 [1]	4 [5]	6 [2.8]	8 [11]	7 [5]	4 [6]	
14	MeS(O)SPr[j]	1 [1]	1 [1]	4 [5]	3 [1.2]	8 [8]	6 [5]	7 [10]	
16	AllSS(O)Me								[13][g]
18	MeS(O)SMe	1	10 [14]	1 [3]	7 [9]	1 [1]	2 [3]	1 [2]	74 [72][g]
31, 30	zwiebelanes (*cis*/*trans*)[c]	20/8	14/6	17/7	17/12	5/3	7.5/3.3	2.3/1.6	
	total % MeS	19	32	21	22	18	30	9	93 [84][g]
	total % AllS								[10][g]
	total % 1-propenylS[e]	58	50	56	32	30	37	18	7 [6][g]
	total % *n*-PrS	23	18	23	46	52	33	73	
	total thiosulfinates[d]	0.2[m] [0.14]	0.35 [0.35]	0.19 [0.2]	0.20 [0.25]	0.1 [0.08]	0.19 [0.15]	0.26 [0.19]	1.7[f] [2.0][g]

[a] HPLC data from accompanying paper (Block et al., 1992). [b] Numbers correspond to those used in accompanying paper. Chemical Abstracts Service names of compounds: **5**, (*E*)-1-propenesulfinothioic acid *S*-*n*-propyl ester; **6**, 1-propanesulfinothioic acid *S*-(*Z*)-1-propenyl ester; **8**, 1-propanesulfinothioic acid *S*-(*E*)-1-propenyl ester; **9**, 1-propanesulfinothioic acid *S*-1-propyl ester; **10**, (*E*)-1-propenesulfinothioic acid *S*-methyl ester; **11**, 2-propene-1-sulfinthioic acid *S*-methyl ester; **12**, methanesulfinothioic acid *S*-(*Z*)-1-propenyl ester; **13**, 1-propanesulfinothioic acid *S*-methyl ester; **14**, methanesulfinothioic acid *S*-1-propyl ester; **15**, methanesulfinothioic acid *S*-(*E*)-1-propenyl ester; **16**, methanesulfinothioic acid *S*-2-propenyl ester; **18**, methanesulfinothioic acid *S*-methyl ester; **31**, (1α,2β,3β,4α,5β)-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide; **30**, (±)-(1α,2α,3β,4α,5α)-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide. [c] Given as mol % based upon total thiosulfinate. [d] μmol/g of fresh weight. [e] Includes zwiebelanes calculated as MeCH=CHS(O)SCH=CHMe equivalent. [f] Allyl thiosulfinates not detected by GC–MS so omitted from calculations. [g] HPLC data includes allyl thiosulfinates, which cannot be detected by GC–MS. [h] Not detected by GC–MS; amounts in parentheses from NMR and/or HPLC. [i] Compound identity confirmed by ^{1}H NMR spectroscopy. [j] HPLC data corrected for MeS(O)SPr/MeS(O)Spropenyl-(*E*) peak overlap. [k] Letters refer to GC–MS traces in Figure 1. [l] GC–MS quantitative analysis in good agreement with GC quantitative analysis (with TCD). [m] Approximately 0.5 μmol/g of fresh weight of (*Z*)-propanethial *S*-oxide (LF 7) also present (see Experimental Procedures).

Table II. Electron Impact Mass Spectra of Sulfine, Thiosulfinates, and Zwiebelanes Found in *Allium* Species

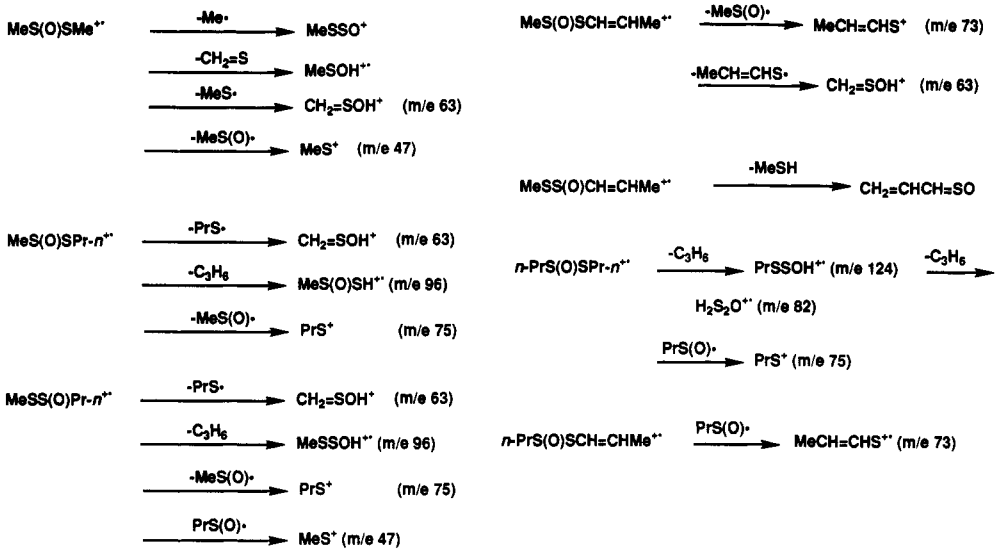
compd	formulas, M^+	mass spectral data (relative abundance in parentheses)[a]
7	EtCH=S$^+$O$^-$, C_3H_6SO, 90	92 (5), 91 (28), 90 (100), 86 (7), 84 (12), 75 (6), 74 (9), 73 (36), 72 (14), 71 (30), 69 (14), 63 (15), 62 (11), 61 (8), 60 (6), 59 (23), 58 (81), 57 (34)
18	MeS(O)SMe, $C_2H_6S_2O$, 110	112 (1), 111 (1), 110 (6), 95 (5), 79 (2), 66 (2), 65 (3), 64 (30), 63 (14), 62 (2), 49 (4), 48 (21), 47 (21), 46 (37), 45 (100)
12, 15	MeS(O)SCH=CHMe, $C_4H_8S_2O$, 136	136 (1), 74 (2), 73 (19), 72 (6), 71 (9), 69 (3), 64 (4), 63 (9), 59 (2), 58 (6), 57 (3), 48 (8), 47 (16), 46 (10), 45 (100)
10	MeSS(O)CH=CHMe, $C_4H_8S_2O$, 136	136 (0.5), 96 (2), 90 (2), 89 (3), 88 (18), 79 (2), 74 (3), 73 (11), 72 (5), 71 (9), 69 (3), 64 (4), 63 (5), 61 (4), 59 (4), 58 (6), 57 (4), 49 (3), 48 (10), 47 (27), 46 (18), 45 (100)
14	MeS(O)SPr-*n*, $C_4H_{10}S_2O$, 138	138 (1), 117 (1), 115 (1), 96 (4), 95 (2), 81 (5), 80 (4), 75 (23), 65 (5), 64 (12), 63 (29), 60 (2), 59 (8), 58 (10), 49 (5), 48 (21), 47 (56), 46 (46), 45 (100)
13	MeSS(O)Pr-*n*, $C_4H_{10}S_2O$, 138	138 (3), 119 (4), 117 (2), 116 (1), 115 (3), 96 (28), 95 (7), 92 (2), 91 (7), 88 (2), 81 (6), 80 (5), 65 (2), 63 (9), 62 (2), 49 (6), 48 (34), 47 (60), 46 (29), 45 (100)
6, 8	*n*-PrS(O)SCH=CHMe, $C_6H_{12}S_2O$, 164	164 (1), 122 (1), 103 (1), 92 (2), 87 (2), 75 (3), 74 (13), 73 (37), 72 (5), 71 (9), 63 (6), 59 (2), 58 (6), 57 (10), 48 (4), 47 (16), 46 (7), 45 (100)
9	*n*-PrS(O)SPr-*n*, $C_6H_{14}S_2O$, 166	166 (0.5), 124 (3), 106 (2), 92 (2), 82 (3), 76 (2), 75 (10), 74 (2), 73 (3), 64 (3), 63 (7), 59 (8), 58 (4), 49 (2), 48 (4), 47 (17), 46 (12), 45 (28), 44 (5), 43 (100)
31	*cis*-zwiebelane, $C_6H_{10}S_2O$, 162	162 (1), 130 (1), 116 (1), 115 (4), 114 (19), 113 (65), 111 (3), 105 (1), 103 (1), 101 (6), 100 (7), 99 (100), 98 (7), 97 (21), 85 (12), 84 (5), 79 (18), 77 (10), 74 (10), 73 (17), 72 (13), 71 (32), 69 (16), 67 (9), 66 (4), 65 (27), 64 (6), 63 (6), 61 (6), 60 (4), 59 (26), 58 (23), 57 (11), 55 (12), 53 (29), 51 (9)
30	*trans*-zwiebelane, $C_6H_{10}S_2O$, 162	162 (1), 130 (1), 116 (1), 115 (6), 114 (16), 113 (100), 112 (5), 111 (6), 101 (7), 100 (7), 99 (94), 98 (10), 97 (29), 85 (16), 84 (6), 80 (5), 79 (31), 77 (18), 74 (12), 73 (17), 72 (16), 71 (38), 69 (23), 67 (14), 66 (6), 65 (33), 64 (12), 63 (6), 61 (9), 60 (5), 59 (35), 58 (27), 57 (15), 55 (20), 53 (37), 51 (13)

[a] All samples were also characterized by NH_3 CI-MS which showed $M + H^+$ *and* $M + NH_3^+$ ions in each case.

discovered that a narrow bore column rapidly lost resolution, presumably due to deposition of nonvolatiles, rendering it useless. Fortunately, we found that a 30-m × 0.53-mm i.d., *wide-bore* column did not suffer this fate and gave excellent resolution of most C_2–C_6 thiosulfinates. While a 15-m × 0.53-mm i.d. column was initially used for analysis of the chive sample, since it allows fast analysis of C_6 thiosulfinates without degradation, this column produces a high source pressure in the MS. However, by using the proper temperature program, C_6 thiosulfinate analysis can be conducted on the 30-m column. A comparative study employing a 50-m × 0.53-mm i.d. column gave decomposition of thiosulfinates under all temperature programs.

We find that by initially cooling the GC injector and oven to 0 °C and the GC–MS transfer line to 100 °C, resolution of C_2–C_6 thiosulfinates is maximized while degradation is minimized. This set of initial oven parameters, with a 5 °C/min oven/injector ramp, gives almost baseline resolution of C_4 thiosulfinate regioisomers MeS(O)SCH=CHMe/MeSS(O)CH=CHMe (geometric isomers could not be separated) and MeS(O)SPr/MeSS(O)Pr (see Table I for numbering scheme and nomenclature). Even under these conditions, while (*E*,*Z*)-PrS(O)-

Scheme II



SCH=CHMe gives a single, clean GC peak, *n*-PrS(O)-SPr-*n* shows significant decomposition. Using the same injector/oven initial conditions and a slower injector/oven ramp of 2 °C/min, with a cooler transfer line (80 °C) *n*-PrS-(O)SPr–*n* elutes as a sharp peak, with some loss of resolution of the C_4 thiosulfinates. However, even under these mild conditions we could not obtain satisfactory peaks for MeCH=CHS(O)SPr isomers or *any* of the thiosulfinates containing an allyl group, which are best identified by HPLC. In summary, using GC–MS methods, analysis is possible for *Allium* thiosulfinates MeS(O)-SCH=CHMe, MeSS(O)CH=CHMe, MeS(O)SPr, MeSS-(O)Pr, PrS(O)SCH=CHMe, and *n*-PrS(O)SPr-*n* as well as MeS(O)SMe, propanethial *S*-oxide, and 2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxides (zwiebelanes). Of the thiosulfinates, only MeS(O)SMe (Block and O'Connor, 1974) and *n*-PrS(O)SPr-*n* (Auger et al., 1989) have been previously studied by GC–MS methods; zwiebelanes have previously only been detected in onions (Bayer et al., 1989).

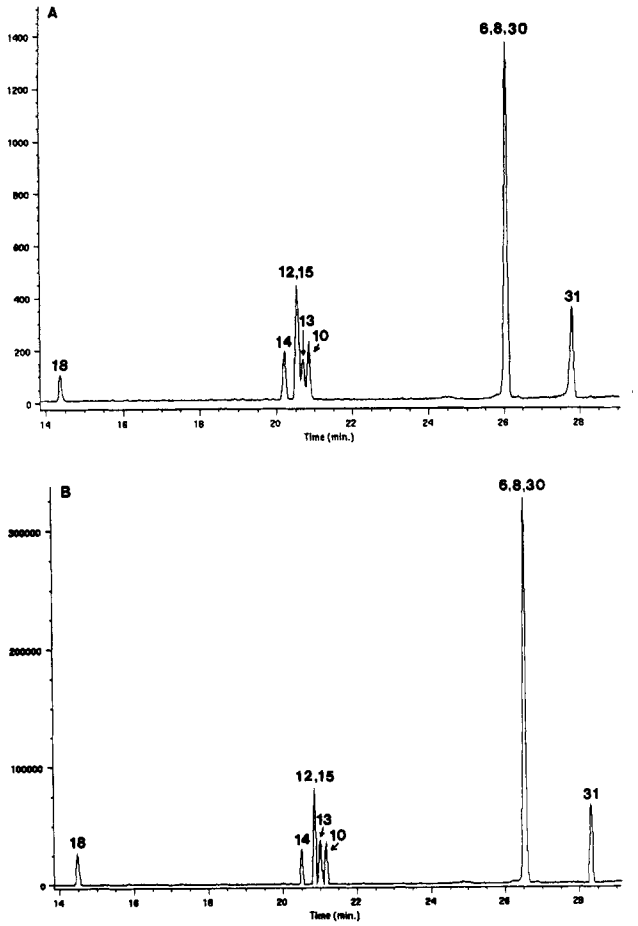
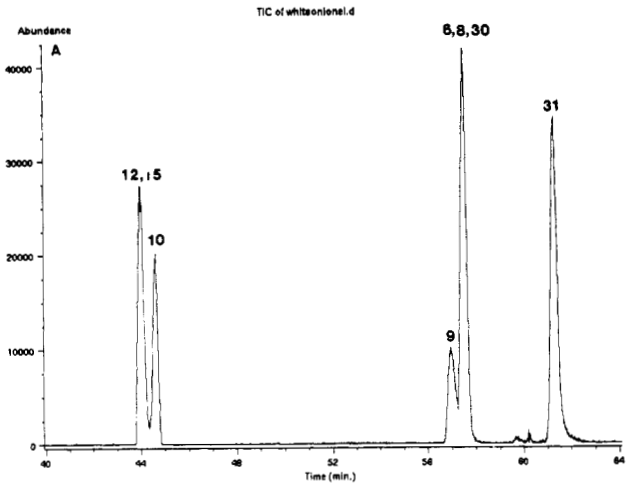


Figure 1. (A) GC analysis (with TCD) of standard mixture of MeS(O)SMe (18), MeS(O)SCH=CHMe (12, 15), MeSS(O)-CH=CHMe (10), MeSS(O)Pr (13), MeS(O)SPr (14), PrS(O)-SCH=CHMe (6, 8), and *cis*- and *trans*-zwiebelanes (31, 30). (B) GC–MS TIC of same standard mixture. In both cases a 30-m × 0.53-mm wide-bore capillary column was used: program—heat from 0 °C at rate of 5 °C/min to 200 °C; GC–MS transfer line 100 °C; helium head pressure 5 psi.

We have examined the effect of more severe GC conditions on pure samples of thiosulfinates as well as bis(1-propenyl) disulfide. The results are striking. For example, while (*E*,*Z*)-MeS(O)SCH=CHMe gives a single peak under our optimum temperature conditions, under more severe conditions (injector at 270 °C, GC–MS transfer line at 250 °C) it gives a very complex series of peaks. Decomposition of the thiosulfinates, like sulfoxides (Clement, 1990; Fedorak and Andersson, 1992), can occur in the injection port (homogeneously under gas-phase conditions, heterogeneously on heated surfaces), in the GC column and in the GC–MS transfer line. Similar results are obtained with bis(1-propenyl) disulfide; at 250–280 °C injector temperatures we detect formation of significant quantities of 3,4-dimethyl-2-mercapto-2,3-dihydrothiophene and 3,4-dimethylthiophene, not found in analyses conducted with 120 °C injection port temperatures. *Given the complexity of the decomposition process, we are of the opinion that in the case of especially thermally unstable flavor components, such as alkyl thiosulfinates, little if any useful information can be obtained by GC–MS analysis of plant extracts under conditions employing high injection port/transfer line temperatures.* Authentic samples of the corresponding disulfides and thiosulfonates were also analyzed by GC–MS. Evidence for the presence of these compounds in *Allium* extracts was then sought by GC–MS. *In no case was there any evidence for the presence of disulfides or thiosulfonates in fresh Allium extracts, indicating that in cases where they are reported they may arise from thermal degradation.* In particular, based on the response factors determined for representative disulfides and thiosulfonates, we can confidently exclude the presence of these compounds at levels greater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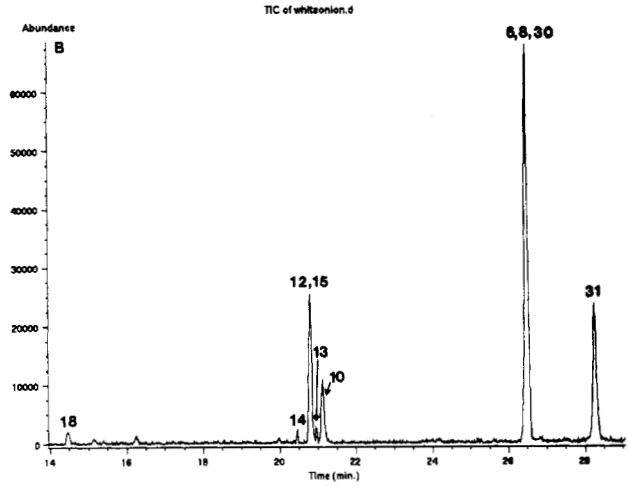
A
6,8,30
31
12,15
10
9
Time (min.)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Abundance
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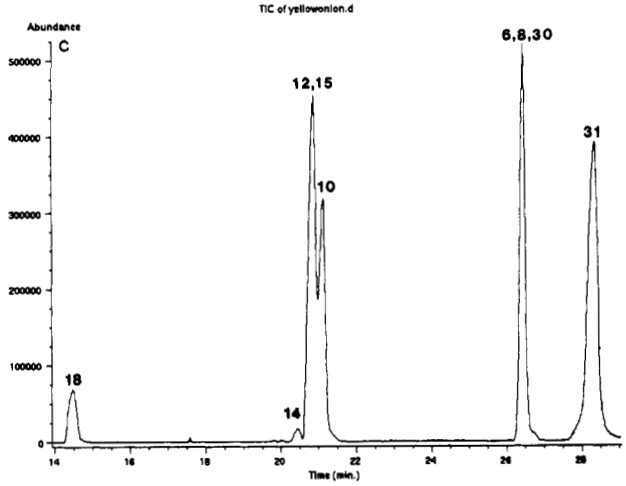
12,15
31
13
10
18
14
Time (min.)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Abundance
C
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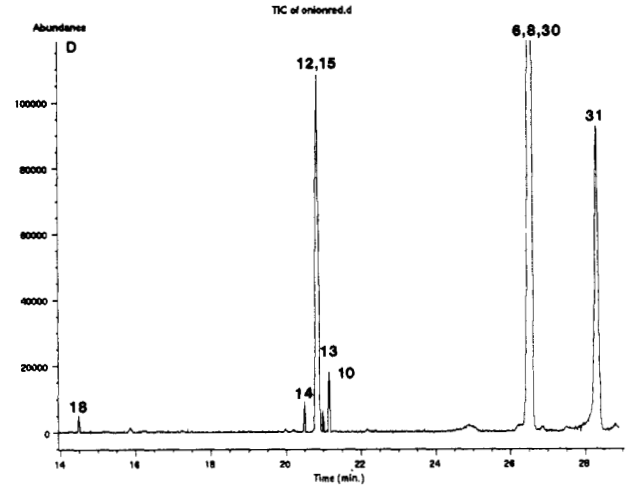
12,15
31
10
18
14
Time (min.)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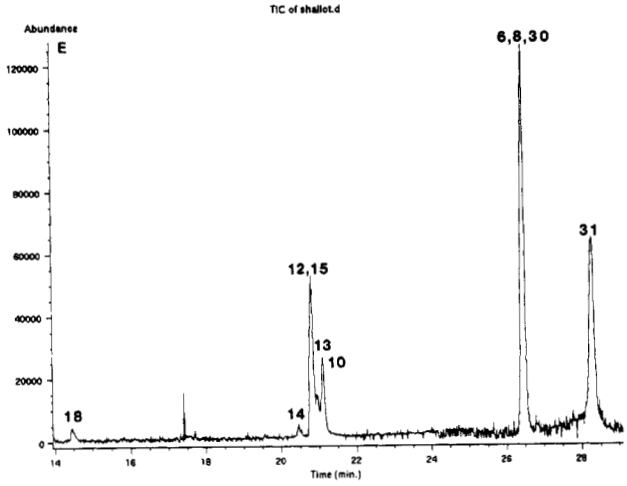
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Abundance
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12,15
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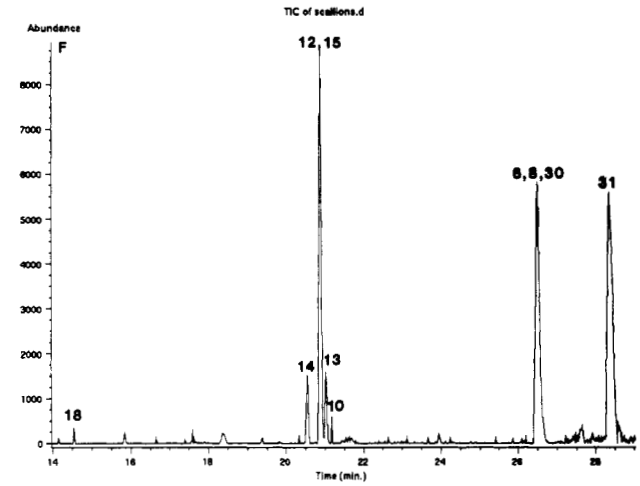
14
Time (min.)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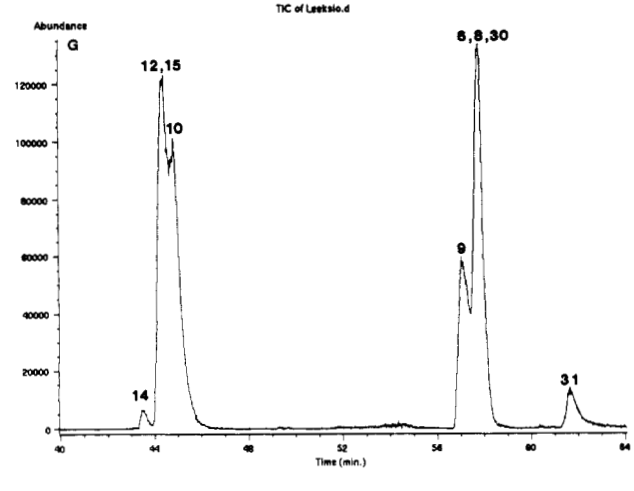
Time (min.)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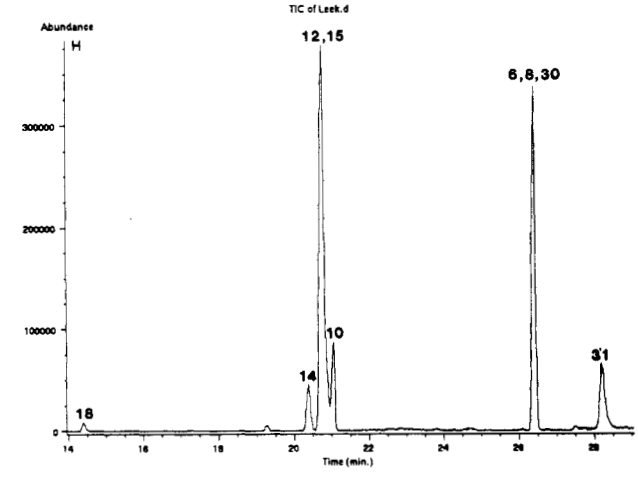
31
Time (min.)


TIC of Leek.d
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Time (min.)

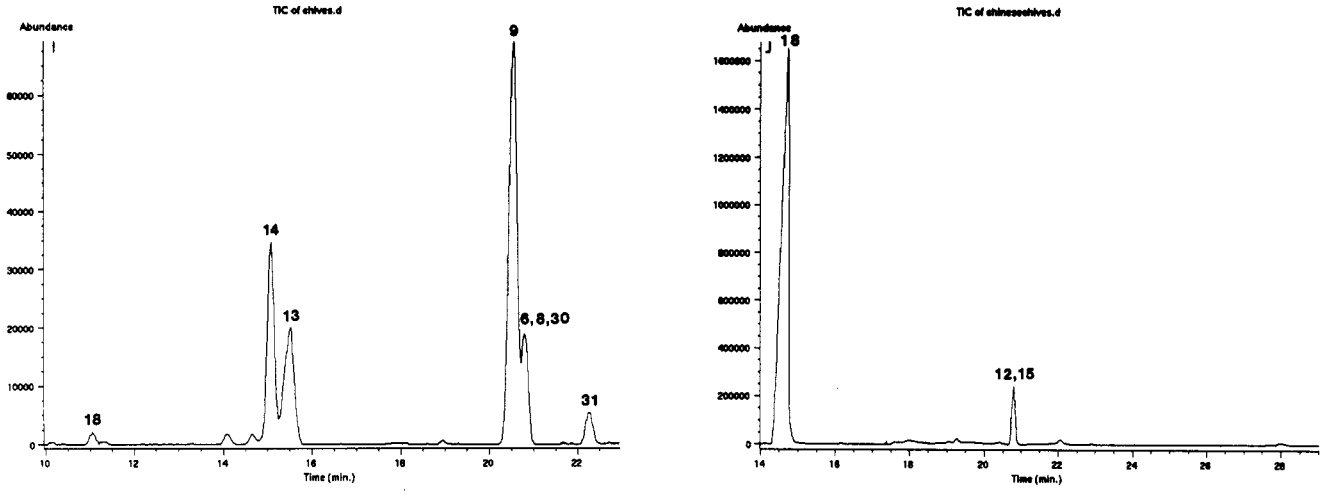

Figure 2. GC–MS separation of thiosulfinates (see Table I for compound identification) in *Allium* species, shown as total ion chromatograms: (A) white onion (*A. cepa*), condition 1; (B) same sample, condition 2; (C) yellow onion, condition 2; (D) red onion, condition 2; (E) shallot, condition 2; (F) scallion, condition 2; (G) leek, condition 1; (H) leek, condition 2; (I) chive, condition 3; (J) Chinese chive, condition 2. Conditions: (1) 30-m × 0.53-mm wide-bore capillary column, program—hold at 0 °C for 5 min, increase to 200 °C at 2 °C/min, GC–MS transfer line 80 °C, helium head pressure 10 psi; (2) 30 m × 0.53 mm wide-bore capillary column, program—heat from 0 °C at rate of 5 °C/min to 200 °C, GC–MS transfer line 100 °C, helium head pressure 5 psi; (3) 15-m × 0.54-mm wide-bore capillary column, program—heat from 0 °C at rate of 5 °C/min 200 °C, GC–MS transfer line 100 °C, helium head pressure 5 psi.

than 1% of the typical total concentrations of thiosulfinates (e.g., at levels ≥1 nmol/g of wet weight).

Mass spectra of the non-allylic C_2, C_4, and C_6 thiosulfinates and zwiebelanes and (*Z*)-propanethial *S*-oxide [the onion lachyrmatory factor (LF), 7] found in common *Allium* species are given in Table II, while suggested fragmentation pathways are presented in Scheme II. It can be seen that regioisomeric pairs MeS(O)SPr and MeSS(O)Pr are distinguished by the *m/e* 75 peak (PrS^+) in the mass spectrum of MeS(O)SPr, which is absent in the spectrum of MeSS(O)Pr, and the predominant *m/e* 96 (MeSSOH) peak present in the mass spectrum of MeSS(O)Pr but much weaker in that of MeS(O)SPr. Similarly, MeS(O)SCH=CHMe and MeSS(O)CH=CHMe can be distinguished by an *m/e* 88 peak (CH_2=CHCH=SO) in the latter and the predominant *m/e* 73 peak (MeCH=CHS^+) in the former. By calibration of authentic thiosulfinate samples with benzyl alcohol we were able to quantify amounts of thiosulfinates present in various *Allium* samples. The calibration could be done through the total ion chromatogram or with ions specific to benzyl alcohol and the thiosulfinate in question. The latter technique allows overlapped peaks to be deconvoluted on the basis of single ions and quantified on the basis of the calibration to a specific ion of benzyl alcohol. For example, the calibration of MeSS(O)CH=CHMe under EI conditions was accomplished by comparing the TIC area of the benzyl alcohol peak with the TIC area of the thiosulfinate peak or by comparing the area of the characteristic benzyl alcohol *m/e* 107 peak with the area of the characteristic MeSS(O)CH=CHMe *m/e* 88 peak. These ratios allowed us to calculate response factors for the different thiosulfinates, which were used to calculate absolute amounts of thiosulfinates. A linear relationship exists between the concentration of thiosulfinates and their MS signal over a 10-fold range in concentration encompassing the range typically found in onion samples. The amounts of thiosulfinates determined by GC–MS were compared to amounts calculated by ^{1}H NMR (C_4 thiosulfinates) and HPLC. The results are generally in good agreement, reinforcing the complementarity of the techniques.

The relative abundances of ions in a given compound may vary under different mass spectral acquisition conditions. Therefore, we used the same acquisition conditions (tune file, spectral range, and NH_3 gas flow for CI) in recording GC–MS results, allowing meaningful integration of single or total ion areas. Two types of comparisons were made of GC vs GC–MS analysis of *Allium* thiosulfinates: (1) A leek extract was analyzed using a GC equipped with thermal conductivity detector (TCD) and an HP-5 cross-linked 5% methyl phenyl silicone column (30 m × 0.53 mm i.d.). By using a packed column inlet system and a *slow* temperature program (40 °C for 5 min, then 1 °C/min, injector temperature 115 °C, detector temperature 200 °C, column head pressure 20 psi), all C_4 thiosulfinates, *n*-PrS(O)SPr-*n*, and (*E,Z*)-MeCH=CHSS(O)Pr-*n* gave sharp peaks without detectable decomposition. *We find that a slow temperature program and mild initial oven/injector conditions are crucial to minimize decomposition of thiosulfinates in GC analysis.* We were able to quantify the peaks that were well resolved under these conditions such as (*E,Z*)-MeCH=CHSS(O)Pr-*n*, *cis*-zwiebelane, and *n*-PrS(O)SPr-*n* by calibration of synthetic authentic samples with benzyl alcohol. The results are in good agreement with those obtained by GC–MS analysis (see Table I). (2) A standard synthetic mixture of MeS(O)SMe, MeS(O)SCH=CHMe, MeSS(O)CH=CHMe, MeS(O)SPr, MeSS(O)Pr, PrS(O)SCH=CHMe, and *cis*- and *trans*-zwiebelanes was analyzed using a GC containing two 30-m wide-bore columns, one of which was connected to a TCD and the other to the mass spectrometer. Each analysis was performed using a 5 °C/min oven/injector ramp from 0 °C (cryogenic conditions). Parts A and B of Figure 1 show the GC and TIC traces, respectively. Considering the differences in detectors, the traces are remarkably similar!

In the accompanying paper (Block et al., 1992) we observe that, contrary to a recent paper (Sinha et al., 1992), there is no HPLC evidence for the presence of allicin 4 or other thiosulfinates containing the 2-propenyl group in the extracts of any of the onion varieties analyzed. In our GC–MS analysis of extracts of onion, we note the presence of a variety of other thiosulfinates but none of the polysulfides, thiophenes, thiosulfonates, or other compounds claimed by Sinha et al. to be present in their

supercritical CO_2 extract of onion. We suspect that these compounds may be artifacts of their analytical procedure, which employs a high injection port temperature (280 °C).

PLANT ANALYSIS

Fresh extracts of the different *Allium* samples were prepared by homogenizing washed and trimmed plants with an equal weight of water as described elsewhere (Block et al., 1992). The homogenate was allowed to stand at room temperature for 30 min and then was filtered through cheesecloth. The filtrate was saturated with salt, allowed to stand for an additional 5 min, and then extracted with an equal volume of CH_2Cl_2. The CH_2Cl_2 extract was dried and concentrated in vacuo at room temperature. Without delay, the ^1H NMR spectrum was recorded and the GC–MS and HPLC analyses performed (if a delay was necessary, the CH_2Cl_2 extract was stored at −78 °C). Typical GC–MS TIC traces are given in Figure 2, while quantitative data are recorded in Table I.

To evaluate the stability with time of the various *Allium* thiosulfinates in the freshly prepared homogenates, the following experiment was performed. A white onion homogenate was prepared as described above except that the time it was allowed to stand at room temperature prior to extraction was varied from 15–30 min to 8–24 h (Figure 3). The only significant change was the gradual disappearance of MeSS(O)CH=CHMe. Thus, we conclude that our standard analysis procedure provides an accurate profile of the initially formed thiosulfinates in each plant specimen. For reasons described elsewhere (Block et al., 1992), considerable plant-to-plant variation in thiosulfinate composition is possible.

When onions are subjected to the CH_2Cl_2 extraction/concentration procedure, there is virtually complete loss of the volatile lachrymatory factor, **7** (Block et al., 1992). If a commercial juicer is used to rapidly express the juice of an onion and this juice is extracted with cold ether, which is carefully concentrated allowing some ether to remain, a major peak for the LF (*m/e* 90) is seen by GC–MS (Figure 4). Quantitative analysis indicates that the LF exceeds the amount of total thiosulfinates plus zwiebelanes by a factor of at least 2.5:1 (see footnote *m*, Table I).

Some trends can be summarized. In extracts of onions, leeks, and scallions, thiosulfinates containing the 1-propenyl group predominate. Also found in these extracts are significant amounts of zwiebelanes, which are thought to arise from the rearrangement of 1-propenyl 1-propenethiosulfinate. The chive extract gave thiosulfinates containing mostly *n*-propyl groups. Extracts of Chinese chive yielded thiosulfinates all containing methyl groups. These thiosulfinates, and related compounds, give the *Allium* species their characteristic flavor, with differences in flavor arising from the variation in abundance of these compounds. Sensory analysis of synthetic samples of *cis*- and *trans*-zwiebelanes (**31** and **30**, respectively) was performed by "expert flavorists" who described odor and taste. The *trans*-zwiebelane had a green or raw onion and a sweet sulfur taste with a detection threshold of 0.1 ppm. The *cis*-zwiebelane had a sweet or brown sautee taste with liver and hydrogen sulfide notes and a detection threshold of 0.5 ppm.

CONCLUSION

The mass spectrum of each GC peak, obtained by GC–MS under both EI and CI (NH_3) conditions, is in excellent agreement with data from the authentic sample introduced into the mass spectrometer by both our GC conditions and particle beam LC–MS methods. By using internal standards and test mixtures of synthetic thiosulfinates, quantitative GC–MS thiosulfinate data may be obtained for comparison with HPLC data. The HPLC and GC–MS techniques are complementary because compounds that overlap under Si-HPLC conditions (polarity separation) can often be separated under GC conditions (volatility separation), and vice versa, and compounds that are weakly UV absorbing may give strong MS signals. Thus,

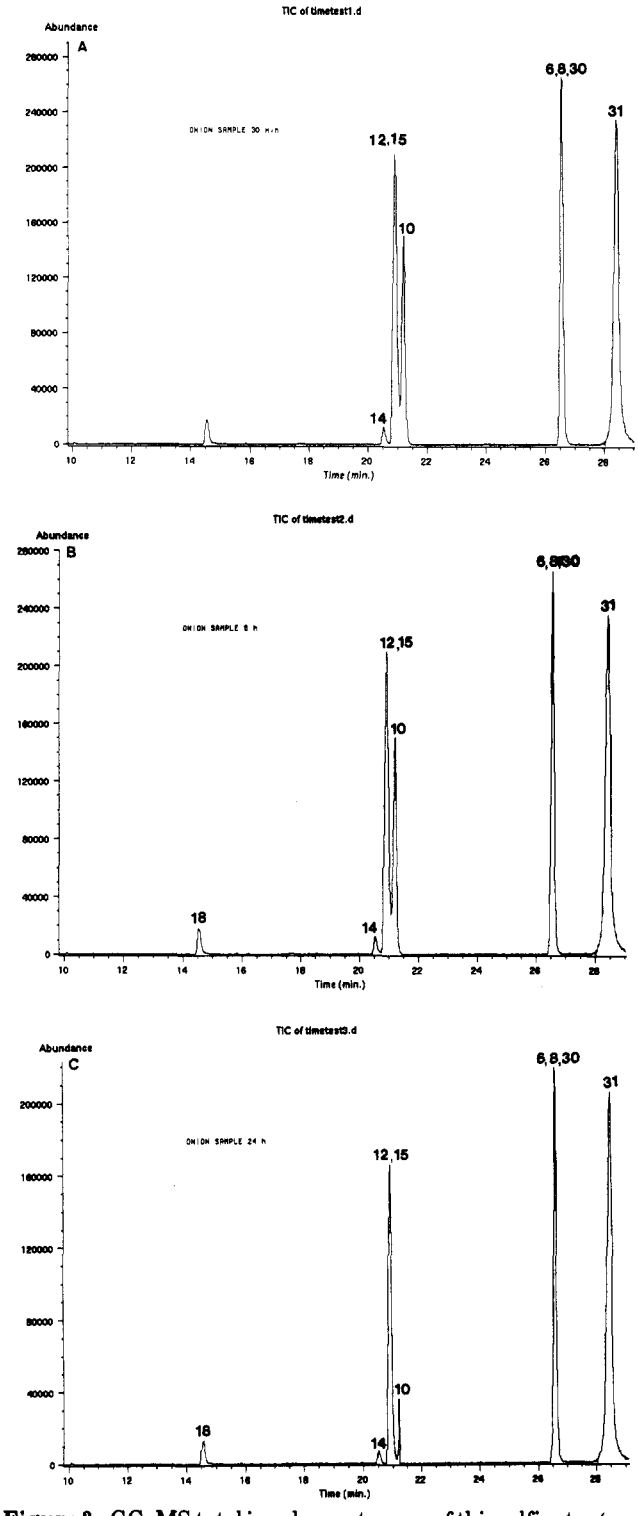

Figure 3. GC–MS total ion chromatogram of thiosulfinates (see Table I for compound identification) in white onion (*A. cepa*) homogenate after (A) standing for 15–30 min at room temperature, (B) standing for 8 h at room temperature, and (C) standing for 24 h at room temperature (conditions 2 of Figure 2 caption used).

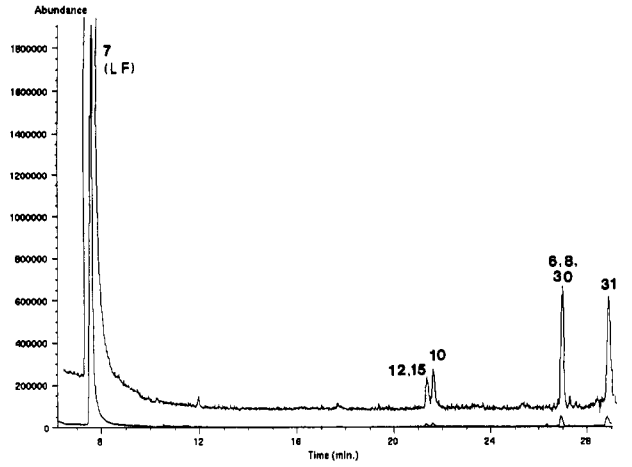

Figure 4. GC–MS total ion chromatogram of ether extract of juice of white onion showing (*Z*)-propanethial *S*-oxide (**7**) along with thiosulfinates **6**, **8**, **10**, **12**, and **15** and zwiebelanes **30** and **31**, with a superimposed 10× attenuated trace (30-m × 0.53-mm wide-bore capillary column; program—heat from 0 °C at rate of 5 °C/min to 200 °C; GC–MS transfer line 100 °C; helium head pressure 2 psi).

with Si-HPLC, MeS(O)SMe is the *last* thiosulfinate to elute and propanethial *S*-oxide, the predominant onion component, overlaps with the C_6 thiosulfinates; with GC, propanethial *S*-oxide elutes well in advance of MeS(O)SMe, the *first* thiosulfinate to elute. Where peak overlap does occur, computer processing is possible, allowing deconvolution of peaks based on a selected ion(s). This technique allows even multiply overlapped peaks to be accurately quantified. Because cryogenic injection conditions are employed, it is possible to detect highly volatile components, like propanethial *S*-oxide, which may be important both from an olfactory standpoint and for attraction/repulsion of insect predators.

In summary, our results underscore the importance of using authentic samples in establishing valid conditions for their analysis. We suggest that serious problems of artifact formation exist with much of the prior GC and GC–MS work on *Allium* flavorants because of the use of excessively hot (for thiosulfinates!) GC injection ports, GC–MS transfer lines, or column conditions. We show that by using a GC equipped with a cryogenic injector/column system and 15–30-m wide-bore (0.53 mm) capillary columns, quantitative information on thiosulfinate and zwiebelane composition in a variety of *Allium* species can now be obtained in the course of a few minutes.

EXPERIMENTAL PROCEDURES

Materials. All *Allium* varieties were obtained from local grocery stores. Solvent grade CH_2Cl_2 was purchased from Ashland Chemical Co. and distilled from CaH_2 in an all-glass apparatus just prior to use; diethyl ether was freshly distilled from sodium benzophenone ketyl. Benzyl alcohol from Eastman Kodak was used without further purification. Anhydrous magnesium sulfate (Fisher Certified) was used as a drying agent. The synthesis and characterization of the various *Allium* thiosulfinates, thiosulfonates, and disulfides appear elsewhere (Naganathan, 1992).

Equipment. Mass spectra were collected using a Hewlett-Packard 5898 mass spectrometer ("MS Engine") equipped with a GC, LC (particle beam and thermospray), and direct insertion interfaces, EI MS at 70 eV and CI MS with NH_3. A dual-column Hewlett-Packard 5890 II GC with programmable on-column injector and cryogenic cooling (CO_2) was used for GC separations; one column of this GC was connected to a TCD, while the other column was connected to the MS Engine. A Rainin solvent delivery system was used for LC separations (see accompanying paper for LC conditions) and *flow injections* for mass spectral analysis. Data processing was achieved using an HP/Apollo 400 series computer employing standard Hewlett-Packard HP UX Chemstation software. GC separations were accomplished using a 15-m × 0.54-mm i.d. J&W Scientific DB-1 ("Durabond") column, a 30-m × 0.53-mm i.d. HP-1 (cross-linked methyl silicone gum), column or a 30-m × 0.53-mm i.d. HP-5 (cross-linked 5% phenyl methyl silicone gum) column, using 99.999% helium as a carrier gas. The temperature profiles employed were as follows: 0–200 °C at 5 °C/min, injector under oven tracking control, transfer line at 100 °C, and a column head pressure of 5 psi (2 psi for analysis of LF **7**); or 0 °C hold for 5 min to 200 °C at 2 °C/min, injector under oven tracking control, transfer line at 80 °C, and a column head pressure of 10 psi. The MS source and quadrupole magnet temperatures were maintained at 200 and 100 °C, respectively. NMR spectra were obtained using either a Varian XL 300 or Gemini 300 spectrometer operating at 300 MHz.

Homogenate Preparation and Extraction. Plant material was homogenized with a Black and Decker Handy Chopper or Waring blender. In all cases *Allium* samples were washed and trimmed and then placed into the homogenizer with an equal weight of distilled water. The material was homogenized for approximately 1 min and then allowed to stand for 30 min at room temperature (approximately 18 °C). The homogenate was filtered through cheesecloth, and the filtrate was saturated with sodium chloride. The filtrate was allowed to stand for an additional 5 min and then extracted with an equal volume of CH_2Cl_2; the resultant emulsion was separated by centifugation and filtration. The CH_2Cl_2 extract was dried over magnesium sulfate and concentrated at aspirator vacuum at room temperature and then at 0.005 mmHg for 10 min to remove residual CH_2Cl_2.

Lachrymatory Factor Determination. A Hamilton Beach Model 395W juice extractor was employed, affording 87 g of juice from 165 g of sliced white onion. The juice was saturated with sodium chloride, allowed to sit for 5 min, vacuum filtered through Celite, and then extracted twice with equal volumes of freshly distilled ether. The ether extracts were combined and concentrated at 20 mmHg at room temperature to a volume of 1 mL. Analysis by GC–MS using benzyl alcohol as internal standard indicated 0.4 μmol of total thiosulfinates–zwiebelanes and 0.94 μmol of total LF per gram of juice (0.21 μmol of total thiosulfinates–zwiebelanes and 0.50 μmol of total LF per gram of fresh weight).

Control Experiments. A white onion homogenate was prepared as described above except that the time it was allowed to stand at room temperature prior to extraction was varied from 30 min to 8–24 h (Figure 3A–C). The only significant change was the gradual disappearance of MeSS(O)CH=CHMe (**10**). Synthetic sample of isomers of bis(1-propenyl) disulfide of good purity were analyzed by GC–MS using a 30-m × 0.53-mm poly(methylsilicone) capillary column under conditions where the on-column GC injector temperature was varied from 120 to 280 °C. At lower temperatures the major products were the *E*,*E*, *E*,*Z*, and *Z*,*Z* isomers of the disulfide, while at temperatures above 250 °C significant quantities of compounds identified by GC–MS as *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene and 3,4-dimethylthiophene were found to be present.

ABBREVIATIONS USED

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; TIC, total ion chromatograph; MS, mass spectrometry; NMR, nuclear magnetic resonance; TCD, thermal conductivity detector; UV, ultraviolet; LF, onion lachrymatory factor.

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