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ALLIUM CHEMISTRY: IDENTIFICATION OF ORGANOSULFUR COMPOUNDS IN RAMP (*ALLIUM TRICOCCUM*) HOMOGENATES*

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

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Key Word Index—*Allium tricoccum* Ait.; Alliaceae (Liliaceae); ramp; supercritical fluid extraction; liquid chromatography-mass spectrometry; thiosulfinates; cepaenes

Abstract—Supercritical fluid (SF) extracts of homogenized ramp (*Allium tricoccum* Ait.) were separated and characterized with liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometric identification. The profiles of SF extracts of aqueous homogenates of ramp bulbs from three different seasons and growing regions revealed that the thiosulfinates were major components. In addition, some of the cepaenes (α-sulfinyldisulfides) found in extracts of onion juice, as well as allyl containing cepaenes (2-propenyl 1-(2-propenylsulfinyl)propyl disulfide), are present in the ramp extracts. The amount of allicin in ramp bulb homogenates ranged from approximately 10% to 50% of that found in extracts of aqueous garlic homogenates. The greater amount of the methyl 1-propenyl thiosulfinates in the ramp extracts relative to that found in the garlic extracts correlates with the flavor characteristics of ramp bulbs. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Ramp (*Allium tricoccum* Ait.; also known as wild leek; Figure 1) is found in moist, shady woods from New Brunswick to Minnesota and south to North Carolina. The Menomini Indians supposedly called an area on the shore of Lake Michigan where ramp grew abundantly "Shik'Ko" ("skunk place"), a name Anglicized to Chicago [1]. The Cherokee Indians consumed ramp leaves for colds and croup or as a spring tonic, while the warm juice of the leaves and bulbs was used for earaches [2]; the Iroquois Indians gave a decoction of ramp to children for worms [3]. Its edible bulbs are widely harvested and sold commercially [4]. Banquets featuring this plant are a spring tradition in southern Appalachia [1]. Ramp is a perennial which gives off a strong onion odor and has a taste similar to, but stronger than that of, garlic. These flavor characteristics suggest that it contains a mixture of many of the volatile organosulfur compounds found in onion and garlic.

While the volatile organosulfur compounds from European and Asian varieties of wild garlic (*A. ursinum* and *A. victorialis*) have been identified [5–7], and the latter two plants are considered to be closely related to the ramp (personal communication, Dr. Andrée Nault, 1996), very little is known of the amounts and types of organosulfur compounds present in ramp. In connection with a survey of the organosulfur chemistry of genus *Allium* plants [8], we have previously characterized the thiosulfinates contained in supercritical CO_2 (SC–CO_2) extracts of ramp homogenates and compared the profile to that of garlic (*A. sativum*) [9, 10]. These ramp SC–CO_2 extracts also appeared to contain cepaenes (α-sulfinyldisulfides), some of which had molecular masses and retention times suggesting the incorporation of the allyl (2-propenyl) group into the cepaene backbone. The present paper compares SC–CO_2 extracts of homogenates of ramp harvested in three different growing seasons and three different geographical areas for a total of five distinct plant samples. The thiosulfinates and cepaenes found in these extracts were more fully characterized by using reversed-phase liquid chromatography (LC)-mass spectrometry (MS) with atmospheric pressure chemical ionization (APCI) and

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* Dedicated with best wishes to Professor G. H. Neil Towers on the occasion of his 75th birthday.

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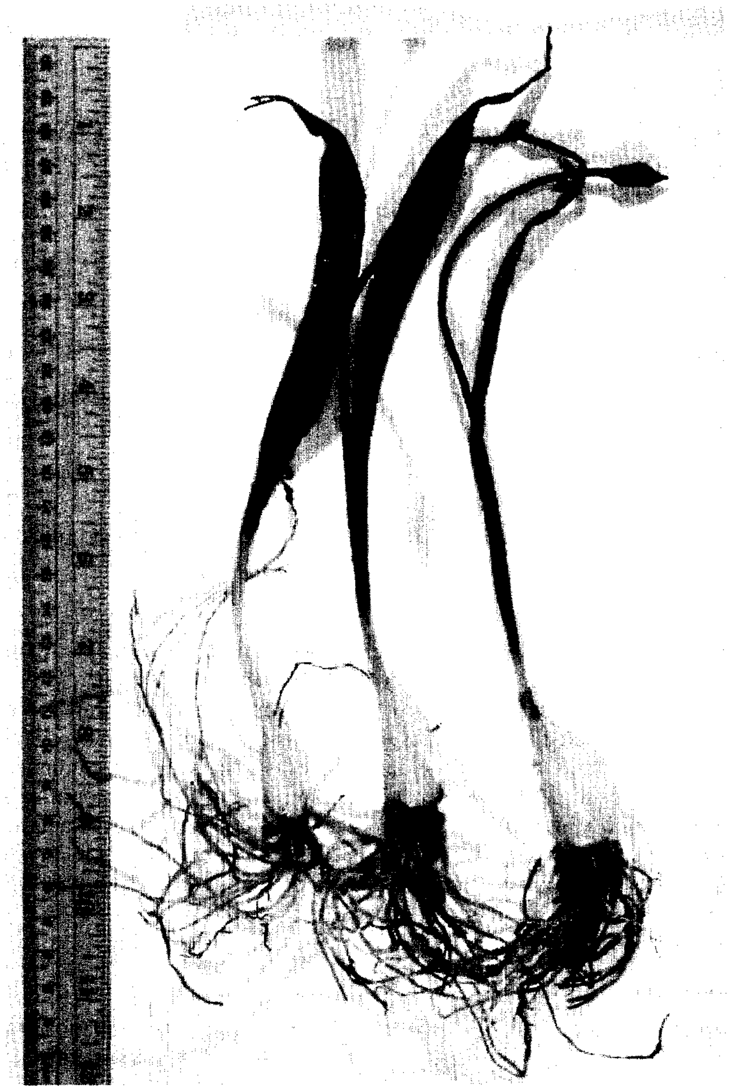

Fig. 1. Ramp (*Allium tricoccum*) plant collected in Greensboro County, WV.

LC tandem MS (LC-MS-MS) detection methods. Chromatographic retention behavior and mass spectra of the naturally occurring cepaenes are compared with synthetic standards. Prior to this report some of these compounds (2-propenyl containing cepaenes) were found in homogenates prepared by combining onion and garlic juices [11]. Cepaenes are important both in terms of their flavor and biological activity, *e.g.* as antithrombotic agents [12–16]. This is the first compilation of the volatile organosulfur compounds, including the 2-propenyl containing cepaenes, found in homogenates prepared from ramp.

RESULTS AND DISCUSSION

LC-APCI-MS methods provide an effective alternative to gas chromatography-MS for analysis of thermally labile organosulfur compounds found in extracts of *Allium* spp. The LC-APCI-MS total ion chromatograms (TIC) of SF extracts of ramp harvested in 1996 near the Potomac River, McLean, VA (P96) and Greensboro County, WV (WV96) are shown in Figure 2, traces A and B, respectively. The major components in the ramp extracts are a mixture of thiosulfinates, **1–7** and **9–11** (see Table 1 for compound identification), which are also found in SF extracts of aqueous homogenates of garlic. Several other thiosulfinates, **8** and **12–13**, are found in minor amounts and are more easily observed with a selected ion trace of m/z 165 [9]. The data suggest that 2-propene-1-sulfinothioic acid *S*-*n*-propyl ester (**14**), 1-propanesulfinothioic acid *S*-methyl ester (**15**) and methanesulfinothioic acid *S*-*n*-propyl ester (**16**) are present although **14** is not chromatographically resolved from **9–11**, and **15/16** are not resolved from **4–6**. Their presence is suggested because the relative abundance of the $[M+H+2]^+$ ions is greater than it

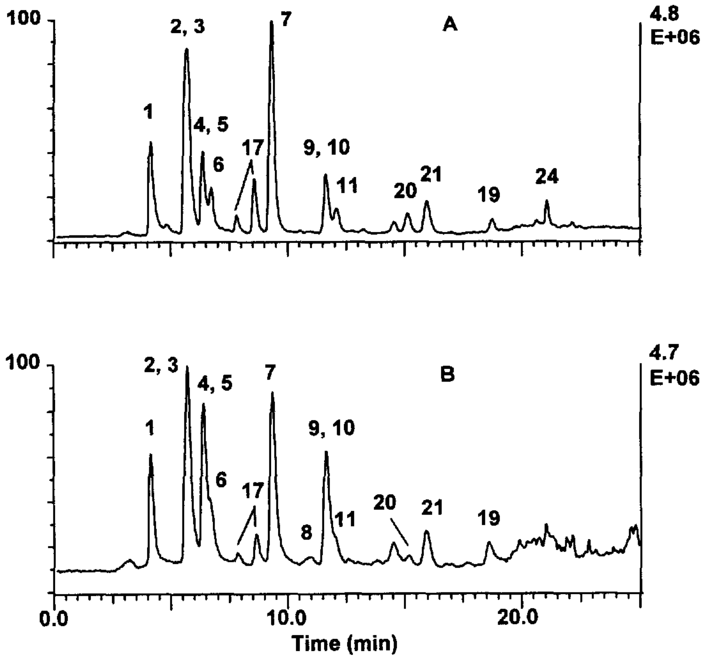

Fig. 2. Total ion chromatogram of supercritical fluid (SF) extracts of (A) fresh ramp, P96, and (B) fresh ramp, WV96. See Tables 1 and 2 for peak identification.

should be for the isotope ions associated with the molecular ions $[M+H]^+$.

The percent peak areas corresponding to the various thiosulfinates from ramp harvested by the Potomac River in the spring of 1995 (P95), 1996 (P96) and 1997 (P97) and two additional geographic locations are reported in Table 1. These data are provided as a means of comparison of 'amounts' of the compounds within the ramp extracts analyzed. Estimates of approximate amounts of allicin (7) present in the ramp extracts can be made by comparing the ramp data to data obtained from the analysis of garlic extracts used as method controls. Based on the average allicin content we have previously reported in aqueous homogenates of store bought garlic (13 μmol/g wet weight, $n=7$) [17], homogenates from ramp bulbs harvested by the Potomac River contain approximately 5–7 μmol/g wet weight, from ramp bulbs harvested in West Virginia contain approximately 3 μmol/g, and from those harvested in Quebec, Canada contain <1 μmol/g. The water content of fresh ramp bulbs and garlic cloves are 77–81% and 62–68%, respectively (personal communication, P. Whanger, 1997). Other differences in the thiosulfinate content are readily observed in the ramp extracts. These differences were anticipated because variations in the amounts of the thiosulfinates found in homogenates of fresh garlic bulbs from the same crop, different growing seasons, different geographical areas, and different storage conditions have been reported [18]. Of most interest is the variation in thiosulfinate content between the ramp extracts from bulbs harvested during different years near the Potomac river. The P97 extract appears to be more garlic like than the P95 and P96 extracts with a higher percentage of thiosulfinates **2**/**3** and **7** present. The Canadian ramp extract (C97) contains predominately thiosulfinates **7** and **9**–**11**, while the West Virginia ramp extract (WV96) contains similar amounts of thiosulfinates **1**, **2**/**3**, **4**–**6**, **7** and **9**–**11**. The greater amount of the methyl 1-propenyl thiosulfinates (**4**–**6**) in P95, P96, and WV96 as compared to garlic (~7%, [9]) correlates well with the flavor characteristics perceived when consuming ramp. The aroma of ramp is described as more cabbage-like than garlic (personal communication, P. Whanger, 1997). The flavor threshold for compounds **4**–**6** is 0.01 ppm with flavor descriptors including sulfur, cabbage and onion as compared to allicin (7) at 0.1 ppm with flavor descriptors of raw garlic, sulfur, rubbery, and green [19]. The ramp extracts of P97 and C97 on the other hand have an odor more closely associated with garlic extracts.

By employing LC-MS and LC-MS-MS (Table 2) we have been able to identify some of the cepaenes present in SF extracts of ramp homogenates. The LC-MS chromatographic profiles of the different ramp extracts were generally similar except for the peak area ratios (Table 1) to the P97 extract which was used in the MS-MS studies. The 'symmetrical' cepaenes, methyl 1-(methylsulfinyl)propyl disulfide isomers (**17**; $[M+H]^+$, *m*/*z* 185) and 2-propenyl 1-(2-propenylsulfinyl) propyl disulfide (**24**; $[M+H]^+$, *m*/*z* 237), were identified in the ramp extracts and verified with synthetic standards. This contrasts with the yellow onion extracts where **17** and (*E*)-1-propenyl 1-(1-propenylsulfinyl)propyl disulfide (**23**) were identified [9].

Table 1. Percent Peak Areas[*] Corresponding to the Various Thiosulfinates and Cepaenes found in Ramp Extracts[†]

Cmpd No.	**Compound**[‡]	**P95**	**P96**	**P97**[§]	**WV96**	**C97**
1	MeS(O)SMe	11	5	9	17	0.3
2/3	MeS(O)SAll/MeSS(O)All	21	17	34	20	7
4/5/6	MeS(O)S1-propenyl-(*E*,*Z*)/MeSS(O)1-propenyl-(*E*)	13	19	6	22	2
7	AllS(O)SAll	28	23	40	17	59
8	*n*-PrS(O)SAll	0.9	0.4	0.1	1	1
9/10/11	AllS(O)S1-propenyl-(*E*,*Z*)/AllSS(O)1-propenyl-(*E*)	11	14	8	14	29
12	*n*-PrS(O)S1-propenyl-(*E*)	0.2	0.1	0.02	1	0.5
13	*n*-PrSS(O)1-propenyl-(*E*)	[‖]	0.08	[‖]	[‖]	0.4
17	MeS(O)CHEtSSMe[¶]	2.0	8.5	0.8	3.8	0.1
	(*m*/*z* 211, unknown)	1.9	0.1	0.2	0.7	0.0
19	MeS(O)CHEtSS1-propenyl	0.5	1.3	0.1	0.5	0.0
20	AllS(O)CHEtSSMe	1.1	3.3	0.1	0.6	0.0
21	MeS(O)CHEtSSAll	3.9	6.2	0.8	1.8	0.3
	(*m*/*z* 237, unknown)	1.4	0.4	0.1	0.3	0.2
24	AllS(O)CHEtSSAll	3.9	1.7	0.8	0.6	0.5
	Total % Thiosulfinates	85.1	78.6	97.1	92.0	99.2
	Total % Cepaenes	14.7	21.5	2.9	8.3	1.1

[*] The values of the percent peak areas are the ratio of the area from the integration of the selected ion traces of the molecular ions ($[M+H]^+$) relative to the combined areas of both the thiosulfinates and cepaenes. Data is based on a single extraction unless otherwise noted.

[†] The ramp extracts are identified as follows: P95, P96, P97-harvested along the Potomac River, McLean, Virginia in 1995, 1996, and 1997, respectively; WV96-harvested in Greensboro County, West Virginia in 1996; and C97-harvested in Quebec, Canada in 1997.

[‡] *Chemical Abstracts* names for compounds: **1**, methanesulfinothioic acid *S*-methyl ester; **2**, methanesulfinothioic acid *S*-2-propenyl ester; **3**, 2-propene-1-sulfinothioic acid *S*-methyl ester; **4**, methanesulfinothioic acid *S*-(*E*)-1-propenyl ester; **5**, methanesulfinothioic acid *S*-(*Z*)-1-propenyl ester; **6**, (*E*)-1-propenesulfinothioic acid *S*-methyl ester; **7**, 2-propene-1-sulfinothioic acid *S*-2-propenyl ester (allicin); **8**, 1-propanesulfinothioic acid *S*-2-propenyl ester; **9**, 2-propene-1-sulfinothioic acid *S*-(*E*)-1-propenyl ester; **10**, 2-propene-1-sulfinothioic acid *S*-(*Z*)-1-propenyl ester; **11**, (*E*)-1-propenesulfinothioic acid *S*-2-propenyl ester; **12**, 1-propanesulfinothioic acid *S*-(*E*)-1-propenyl ester; **13**, (*E*)-1-propenesulfinothioic acid *S*-*n*-propyl ester. See Table 2 for names of compounds **17**, **19**–**21**, and **24**.

[§] Extracts from two different bulbs afforded similar peak area ratios.

[‖] The response for this compound was observed in the selected ion trace (*m*/*z* 165) but not integrated.

[¶] Combined percent areas for both *m*/*z* 185 isomers.

The 'mixed' cepaenes with a molecular ion of *m*/*z* 211 are more difficult to distinguish. The two cepaenes, methyl (*E*)-1-(1-propenylsulfinyl)propyl disulfide (**18**) and 1-(methylsulfinyl)propyl (*E*,*Z*)-1-propenyl disulfide (**19**), with a molecular ion of *m*/*z* 211 have previously been identified in onion extracts [9]. These components, **18** and **19**, in the onion extracts elute between 16 and 19 min. The mixed cepaenes with a molecular ion of *m*/*z* 211 in the ramp extracts elute between 13 and 19 min. The *m*/*z* 211 component in the ramp extract eluting at ~18.5 min has been identified as **19**. The major *m*/*z* 211 component (retention time (t_r) ~16 min) in the ramp extracts has a similar retention time compared to component **18** but has a significantly different MS-MS spectrum. Based on the presence of ions at *m*/*z* 147 and 105 and the lack of ions at *m*/*z* 121 and 79 in the MS-MS spectrum, we propose that this component is an isomer of 1-(methylsulfinyl)propyl 2-propenyl disulfide (**21**). The latter two ions are present in the spectrum of the synthetic isomer methyl 1-(2-propenylsulfinyl)propyl disulfide (**20**). Two other m/z 211 components, eluting between 14 and 15.5 min, in the ramp extract afforded MS-MS spectra. These components elute before **18** and **19** in the onion extracts suggesting the presence of the 2-propenyl functionality in their structure. The second peak (t_r ~15 min) has a retention time and MS-MS spectrum similar to the synthetic isomer **20**. The first peak (t_r ~14.5 min) has a base peak at *m*/*z* 73 with additional ions at *m*/*z* 41, 147, and 211 all greater than 20% of the base peak. This contrasts with compound **21** which has a base peak at *m*/*z* 41.

Late eluting constituents in extracts of ramp homogenates which ionize poorly under APCI conditions but are observed with UV detection have previously been identified as dithiins, alkyl sulfides and polysulfides [10]. In addition, the LC-MS data suggest that compounds related to ajoene (**28**, AllS(O)-$CH_2CH{=}CHSSAll$, (*E*,*Z*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide) are present in ramp extracts. Ajoene is a major organosulfur component in oil-macerated garlic products [20, 21] and is structurally related to the cepaenes. The ajoene-homolog, where methyl functionalities are substituted for the allyl

Table 2. APCI-MS-MS Spectral Data of Cepaenes

Cmpd No.	Compound[*]	MS-MS spectral data of $[M+H]^+$ ions (relative abundance)[†]
17	MeS(O)CHEtSSMe[‡]	185(30); 121(70); 111(10); 79(98); 73(100); 64(16); 45(22)
18	1-PropenylS(O)CHEtSSMe[‡,§]	211(12); 121(65); 79(98); 73(100); 64(12); 45(24); 41(10)
19	MeS(O)CHEtSS1-propenyl[‡]	211(35); 147(75); 105(100); 73(70); 61(34); 45(24); 41(25)
20	AllS(O)CHEtSSMe[‖]	211(46); 136(14); 121(48); 91(18); 79(73); 73(100); 64(18); 45(32); 41(34); 39(15)
21	MeS(O)CHEtSSAll[¶]	211(38); 147(72); 113(20); 105(20); 91(15); 89(15); 73(76); 64(18); 45(68); 41(100); 39(14)
22	MeS(O)CHEtSSPr-*n*	213(48); 149(70); 107(20); 89(100); 73(100); 64(10); 45(26); 43(67); 41(31); 27(17)
23	1-PropenylS(O)CHEtSS1-propenyl[**]	237(40); 163(12); 147(74); 105(100); 90(22); 73(40); 61(26); 45(58); 41(56); 39(28); 29(15)
24	AllS(O)CHEtSSAll	237(30); 147(46); 105(14); 91(16); 73(100); 45(30); 41(36); 39(12)
25	1-PropenylS(O)CHEtSSPr-*n*[†]	239(6); 149(48); 107(20); 73(100); 45(16); 43(40); 41(10)
26	*n*-PrS(O)CHEtSS1-propenyl[†]	239(4); 147(100); 105(76); 73(30); 61(20); 45(12); 41(20)
27	*n*-PrS(O)CHEtSSPr-*n*[††]	241(26); 149(50); 107(28); 73(100); 45(26); 43(54); 41(35); 27(18)

[*] *Chemical Abstracts* names for the compounds: **17**, methyl 1-(methylsulfinyl)propyl disulfide; **18**, methyl (*E*)-1-(1-propenylsulfinyl)propyl disulfide; **19** 1-(methylsulfinyl)propyl (*E*,*Z*)-1-propenyl disulfide; **20**, methyl 1-(2-propenylsulfinyl)propyl disulfide; **21**, 1-(methylsulfinyl) propyl 2-propenyl disulfide; **22**, 1-(methylsulfinyl)propyl propyl disulfide; **23**, (*E*)-1-propenyl 1-(1-propenylsulfinyl)propyl disulfide; **24**, 2-propenyl 1-(2-propenylsulfinyl) propyl disulfide **25**, (*E*)-1-(1-propenylsulfinyl)propyl propyl disulfide; **26**, (*E*)-1-propenyl 1-(propylsulfinyl)propyl disulfide; **27**, propyl 1-(propylsulfinyl)propyl disulfide.

[†] Recorded only ions above 10% base peak.

[‡] Previously reported data [9]; added to table to complete data set; previous MS-MS conditions: −54 eV, scan range 40–240 daltons. [§] Review of previously reported spectrum indicates that *m*/*z* 137 and 90 were present at < 10% base peak.

[‖] Two peaks observed; data presented for major component; minor component (~40% of major component) afforded similar fragmentation with a base peak of *m*/*z* 73; other ions present had slightly different ratios.

[¶] Product ion spectrum of mixed cepaene found in extract P97.

[**] This spectrum differs slightly from the previously reported spectrum in reference [9]; current MS-MS conditions: −100 eV; scan range 15–240 daltons; *m*/*z* 163 and 90 previously observed at < 10% base peak. Spectrum from identified peak in onion extract used as chromatographic control.

[††] Two peaks observed; data presented for major component; minor component (~20% of major component) afforded similar fragmentation with a base peak of *m*/*z* 73.

groups, $MeS(O)CH_2CH{=}CHSSMe$, (**29**), (*E*,*Z*)-2,3,7-trithiaoct-4-ene 7-oxide) may be present in these SF extracts of ramp homogenates as evidenced by the presence of an apparent molecular ion of *m*/*z* 183. Sendl *et al.* [22–24] previously isolated ajoene-homologs including **29** from chloroform and chloroform/acetone extracts of wild garlic.

In summary, we have shown that aqueous homogenates of ramp contain allicin (**7**) as a major component along with other thiosulfinates, RS(O)SR′, and cepaenes, RS(O)CHEtSSR′ (R, R′ = methyl, 1-propenyl, or 2-propenyl). Based on the known chemistry of other genus *Allium* plants [8], it is likely that bulbs of intact ramp contain *S*-alk(en)ylcysteine *S*-oxides ($RS(O)CH_2CH(NH_2)COOH$) and related γ-glutamyl peptides. Our work identifies for the first time the organosulfur components of crushed ramp and establishes a chemical link between *A. tricoccum*, *A. ursinum* and *A. victorialis*. Thus, comparison of our data for *A. tricoccum* with published HPLC analyses for *A. ursinum* [5, 24] reveal very similar patterns for thiosulfinates **1**, **2**/**3**, and **7** with levels of **7** less than half of that found in garlic homogenates and elevated levels of methyl thiosulfinates present in both; we also have noted similarities in occurrence of ajoenes **28** and **29** in homogenates from both plants. While only GC-MS data for volatiles from *A. victorialis* has been reported [6, 7], the relative abundance of Me, All, 1-propenyl and *n*-propyl polysulfides is consistent with our results for *A. tricoccum*. Precise comparison would require preparation and analysis of homogenates of all three plants under identical conditions (*e.g.* SF-CO_2/LC-APCI-MS).

EXPERIMENTAL

Plant materials

Ramp plants were collected along the banks of the Potomac River near Washington, DC, in the Spring of 1995, 1996 and 1997; from Greensboro County, West Virginia in the Spring of 1996; and from Quebec, Canada in Spring of 1997. Ramp bulbs were immediately frozen at −80° or stored in the refrigerator until extracted. Voucher specimen are available in the FDA's Center for Food Safety and Applied Nutrition botanicals collection. Syntheses of the cepaene standards are described elsewhere [25].

Supercritical fluid extraction.

Ramp bulbs were extracted using a PrepMaster with an AccuTrap. Bulbs (2–5 g) were homogenized in 10 ml/g of H_2O at ambient temperature by using a Tissumizer. The solutions were allowed to stand at room temperature for 5–10 min to ensure complete enzymatic conversion to the thiosulfinates and related compounds. Hydromatrix (a mixture of diatomaceous earth and crystalline silica) was mixed with 15 ml of ramp homogenate (representing ≈ 1.5 g) and placed in a 50 ml extraction vessel. The mixtures were extracted at 35° and 240 atm at a flow rate of 2 ml/min for *ca.* 30 min, resulting in 60 g of liquid CO_2 used. The effluent was trapped on glass beads at 0–1° and desorbed with 1 ml of MeOH.

LC-MS and LC-MS-MS analysis.

LC-MS data were collected by using a Finnigan TSQ 7000 equipped with an APCI interface, and a Hewlett Packard 1050 liquid chromatograph. Chromatographic separation was achieved with a YMC J'sphere ODS-M80 column (250 × 2.0 mm I.D., S-4 μm, 80A). A linear gradient employing H_2O-MeCN as the mobile phase was pumped at 200 μl/min. The initial mobile phase composition of 70%A (95:5 H_2O-MeCN), 30%B (5:95 H_2O-MeCN) was held constant for 10 min. The final mobile phase composition of 40%A:60%B was reached at 25 min, followed by an isocratic period of 15 min. Ionization conditions were: vaporizer, 350 or 400°; heated capillary, 150–200°; corona discharge needle, 5.0 μA. Nitrogen was employed, at a sheath gas pressure of 70 psi and auxiliary gas was on at 50 mm (65 mm flow tube, maximum flow 12 L/min), to assist in nebulization. Ions were decomposed by using argon in the collision cell at 0.8 mTorr and an optimized collision energy of -12 to -15 eV for the thiosulfinates and -55 or -100 eV for the cepaenes. Day to day instrument conditions were set based on the mass spectrum of allicin (**7**), such that the spectrum was the same every day.

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