#-Glutamyl-S-alkylcysteines in Garlic and Other Allium spp.: Precursors of Age-Dependent trans-1-Propenyl Thiosulfinates

Larry D. Lawson, Zhen-yu J. Wang, and Bronwyn G. Hughes

J. Nat. Prod., **1991**, 54 (2), 436-444• DOI:
10.1021/np50074a014 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 2, pp. 436-444, Mar-Apr 1991

γ-GLUTAMYL-*S*-ALKYLCYSTEINES IN GARLIC AND OTHER *ALLIUM* SPP.: PRECURSORS OF AGE-DEPENDENT *TRANS*-1-PROPENYL THIOSULFINATES

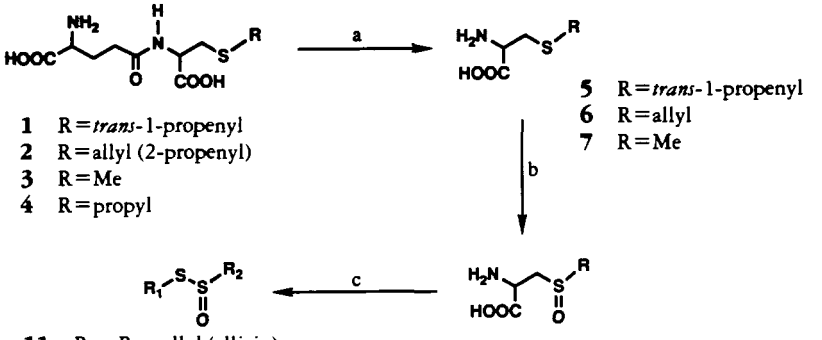
LARRY D. LAWSON,* ZHEN-YU J. WANG, and BRONWYN G. HUGHES

Murdock Healthcare, Springville, Utah 84663

ABSTRACT.—The γ-glutamyl-*S*-alkylcysteines of garlic (*Allium sativum*) were analyzed by reversed-phase hplc. Only two major compounds were found: γ-glutamyl-*S*-*trans*-1-propenylcysteine [**1**] and γ-glutamyl-*S*-allylcysteine [**2**]. Compounds **1** and **2** were the dominant uv_{220}-visible polar compounds found in homogenates of fresh-picked garlic cloves and were found to decrease markedly when fresh-picked garlic was stored, especially at 4°. Concomitant with the decrease in **1** and **2** was a 16-fold increase in *trans*-1-propenyl-allyl [**12**] and allyl-*trans*-1-propenyl [**13**] thiosulfinates in the homogenate of garlic stored for ten weeks at 4°. Evidence is given to show that **1** and **2** are the original sources of the *trans*-1-propenyl and allyl groups found in **12** and **13**. A comparison is given for the γ-glutamyl-*S*-alkylcysteine content of the bulbs and seeds of a number of *Allium* species.

About 45% of the organosulfur compounds of onion bulbs has been identified as γ-glutamyl-*S*-alkylcysteines and cysteine sulfoxides (1). Garlic, *Allium sativum* L. (Liliaceae), has also been reported to contain γ-glutamyl-*S*-alkylcysteines, namely γ-glutamyl-*S*-allylcysteine [**2**] and γ-glutamyl-*S*-propylcysteine [**4**] (2). γ-Glutamyl-*S*-alkylcysteines appear to provide a reserve for the alkylcysteine sulfoxides which are converted by alliinase to the antimicrobial dialkyl thiosulfinates (3).

We have recently reported the presence of *trans*-1-propenyl thiosulfinates in garlic clove homogenates (4). These compounds include *trans*-1-propenyl-allyl thiosulfinate [**12**] and allyl-*trans*-1-propenyl thiosulfinate [**13**] and much smaller amounts of *trans*-1-propenyl-methyl thiosulfinate [**14**] and methyl-*trans*-1-propenyl thiosulfinate [**15**] (the first-named residue being linked to the thio group and the second-named group being linked to the sulfinate group). They were present in only trace amounts in fresh-



1 R=*trans*-1-propenyl
2 R=allyl (2-propenyl)
3 R=Me
4 R=propyl

5 R=*trans*-1-propenyl
6 R=allyl
7 R=Me

8 R=*trans*-1-propenyl
9 R=allyl (alliin)
10 R=Me

11 R_1=R_2=allyl (allicin)
12 R_1=*trans*-1-propenyl, R_2=allyl
13 R_1=allyl, R_2=*trans*-1-propenyl
14 R_1=*trans*-1-propenyl, R_2=Me
15 R_1=Me, R_2=*trans*-1-propenyl
16 R_1=allyl, R_2=Me
17 R_1=Me, R_2=allyl

SCHEME 1. Transformation of γ-glutamyl-*S*-alkyl-L-cysteines [**1–4**] via *S*-alkyl-L-cysteines [**5–7**] and *S*-alkyl-L-cysteine sulfoxides [**8–10**] to dialkyl thiosulfinates [**11–17**]. a, γ-glutamyl transpeptidase; b, H_2O_2; c, alliinase.

picked garlic but increased substantially upon storage of garlic at 4°. This finding may be particularly important because the *trans*-1-propenyl thiosulfinates have recently been shown to be responsible for the in vivo anti-asthmatic effects of onion homogenates (5). Concomitant with the rise in *trans*-1-propenyl thiosulfinates was a significant decline in a compound identified as a γ-glutamyl peptide. It was proposed that this γ-glutamyl peptide was the precursor of the *trans*-1-propenyl thiosulfinates. We now report the hplc separation, identification, and quantitation of this compound and other γ-glutamyl peptides found in homogenates of garlic and other *Allium* species.

RESULTS AND DISCUSSION

When an aqueous homogenate of fresh-picked garlic, or a properly prepared powder therefrom (4), was analyzed by C_{18} hplc only two main types of compounds were detected: the dialkyl thiosulfinates and two γ-glutamyl-*S*-alkylcysteines (Figure 1A). The dialkyl thiosulfinates of garlic have been previously identified and quantitated (4); however, the γ-glutamyl peptides were not initially identified because they had not been previously analyzed by hplc (6).

Comparative analysis of the contents of fresh-picked garlic and store-purchased garlic revealed two significant differences between these samples, namely that the homogenate of store-purchased garlic contained considerably larger amounts of *trans*-1-propenyl thiosulfinates and smaller amounts of the γ-glutamyl-*S*-alkylcysteines (Figure 1). The γ-glutamyl-*S*-alkylcysteines were initially characterized by their insolubility in organic solvents, a positive ninhydrin test, the dramatic effect of pH on their retention

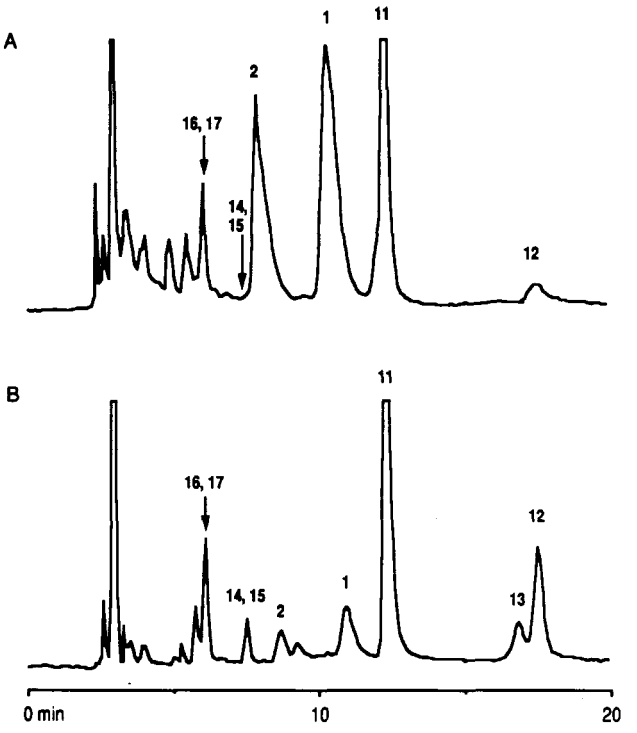


FIGURE 1. Hplc analysis of thiosulfinates and γ-glutamylcysteines in aqueous homogenates of (A) fresh-picked garlic cloves and (B) store-purchased garlic cloves. MeOH-H_2O-HCOOH (40:60:0.1), 1.0 ml/min, 220 nm. Compounds identified in Scheme 1.

times, and their disappearance upon treatment with γ-glutamyl transpeptidase. Because the γ-glutamyl-*S*-alkylcysteines produced rather broad peaks in a low ionic strength solvent system, significantly sharper peaks were obtained by using 50 mM KH_2PO_4 (pH 4.5)-MeOH (97.5:2.5) (Figure 2A). The earlier-eluting peptide was identified as γ-glutamyl-*S*-allylcysteine [**2**] as it was found to co-elute with synthetic **2** and to yield an identical uv spectrum. The later-eluting γ-glutamyl peptide was found to absorb much more strongly in the 240–254 nm region than γ-glutamyl-*S*-allylcysteine (Figure 3) and was identified by three means to be γ-glutamyl-*S*-*trans*-1-propenylcysteine [**1**]. First, both of the γ-glutamyl-*S*-alkylcysteines were found to be the parent compounds of *trans*-1-propenyl-allyl thiosulfinate [**12**] and allyl-*trans*-1-propenyl thiosulfinate [**13**]. Treatment of boiled (to inactivate alliinase after alliin depletion) homogenate of fresh-picked garlic (or a mixture of isolated **1** and **2**) with γ-glutamyl transpeptidase to give alkylcysteines (Figure 2B) followed by H_2O_2 oxidation to yield the alkylcysteine sulfoxides (Figure 2C) resulted in a large increase in the *trans*-

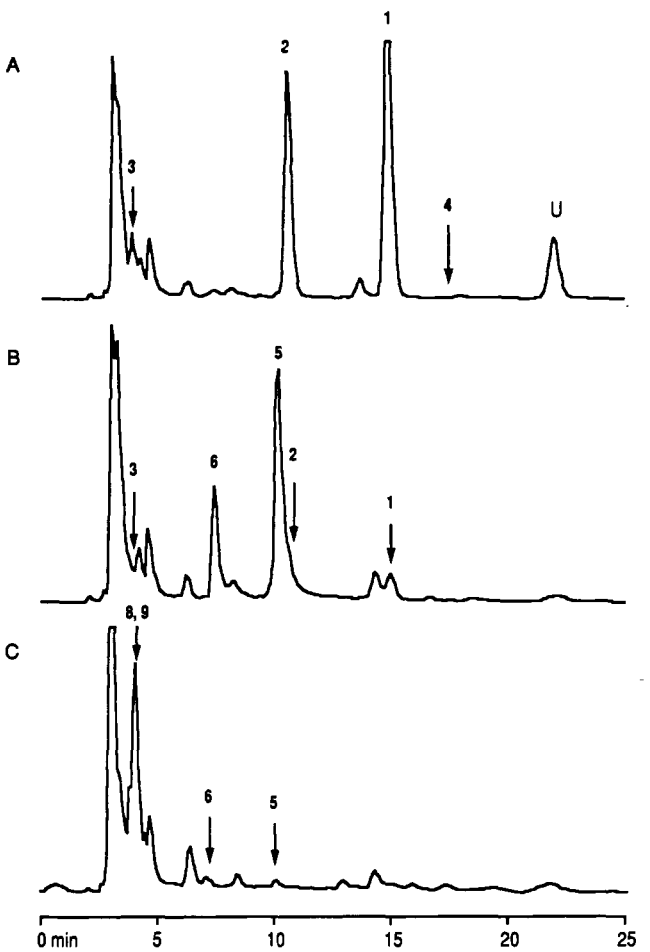


FIGURE 2. Conversion of the (A) γ-glutamyl-*S*-alkylcysteines of a boiled aqueous homogenate of fresh-picked garlic to (B) alkylcysteines by incubation with γ-glutamyl transpeptidase, and then to (C) alkylcysteine sulfoxides upon H_2O_2 oxidation. 0.05 M KH_2PO_4 (pH 4.5)-MeOH (97.5:2.5), 1.0 ml/min, 220 nm. Compounds identified in Scheme 1. U, unidentified γ-glutamyl peptide.

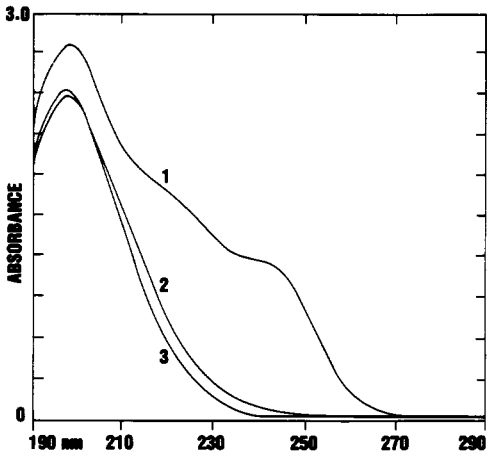


FIGURE 3. Uv spectra of γ-glutamyl-*S*-alkyl-L-cysteines **1–3** at 0.100 mg/ml in H_2O.

1-propenyl/allyl thiosulfinates (not shown) after treatment with the alliinase-containing powder of fresh-picked garlic. Omitting the oxidation step resulted in no increase in the *trans*-1-propenyl thiosulfinates, indicating that the original γ-glutamyl peptide was not oxidized and that it was indeed the source of the *trans*-1-propenyl group of the *trans*-1-propenyl thiosulfinates. The second proof of this structure came by showing that migration of the double bond of either isolated or synthetic γ-glutamyl-*S*-allylcysteine [**2**] from the 2 position to the 1 position, induced by potassium *t*-butoxide (7), resulted in converting the earlier-eluting γ-glutamyl peptide **2** to a compound with the same retention time and same uv spectrum as the later-eluting peptide **1** and a second peak corresponding to the cis isomer. Lastly, H_2O_2 treatment of **1** resulted in the formation of a compound with the same retention time and uv spectrum as the major γ-glutamyl peptide reported for onion, namely γ-glutamyl-*S*-*trans*-1-propenylcysteine sulfoxide [**1**].

Compounds **1** and **2** were the major polar sulfur compounds found in the homogenate of fresh-picked garlic (Figure 2A). Although **2** has been previously found in garlic (3), **1** has previously been reported only in chive seeds (3,6). γ-Glutamyl-*S*-propylcysteine [**4**] was not found, even though its presence in garlic has been previously reported (8). Because all previous work on garlic γ-glutamyl-*S*-alkylcysteines was performed before 1970 using paper chromatography or an amino acid analyzer, it is likely that γ-glutamyl-*S*-*trans*-1-propenylcysteine [**1**] was erroneously identified as γ-glutamyl-*S*-propylcysteine [**4**]. Further support for the absence of compound **4** was the fact that no propyl thiosulfinates are found in homogenates of fresh-picked or stored garlic (4). Much smaller amounts of the methyl compound **3** were found. An unidentified γ-glutamyl peptide (U) was found at a retention time of 22 min (Figure 2A). It disappeared upon teatment with γ-glutamyl transpeptidase but not when treated with H_2O_2 (not shown), indicating that it may be an oxidized compound or that it does not contain cysteine.

Initial observations showed that when garlic cloves were stored in the refrigerator they began to sprout sooner than when cloves were stored at room temperature. Analysis of the refrigerated cloves revealed an increase in the *trans*-1-propenyl/allyl thiosulfinates and a decrease in the γ-glutamyl-*S*-alkylcysteines. This phenomenon was studied in greater detail starting with freshly harvested garlic bulbs which were kept at room temperature or stored at 4°. The results of this experiment are shown in Figure 4.

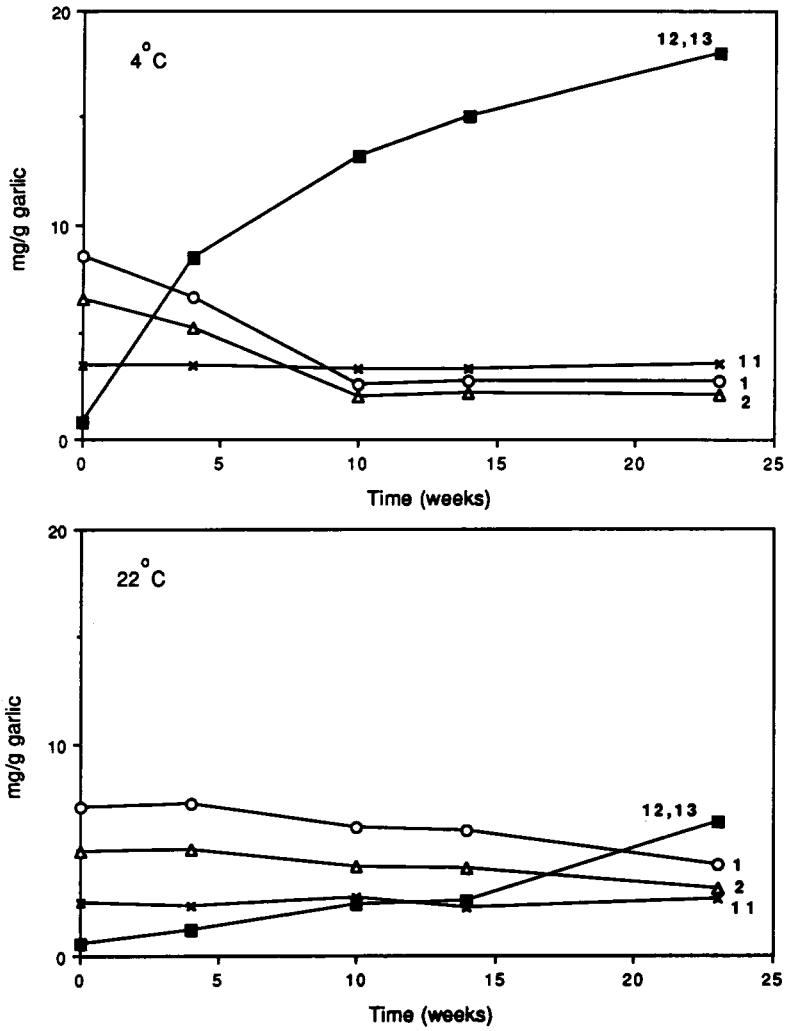


FIGURE 4. Effect of storage on the homogenate content of γ-glutamyl-*S*-*trans*-1-propenyl cysteine [**1**], γ-glutamyl-*S*-allylcysteine [**2**], *trans*-1-propenyl/allyl thiosulfinates (×10) [**12** and **13**], and allicin [**11**] of fresh-picked garlic cloves. The moisture content remained constant throughout the experiment.

After 10 weeks at 4°, the content of compounds **1** and **2** decreased by 70% each. Over the same period thiosulfinates **12** and **13** as well as **14** and **15** (not shown) increased 16-fold, while the allicin [**11**] content remained constant. At room temperature these effects were diminished to one-fourth of this extent. Virtanen (3) has reported that the γ-glutamyl peptides of onions disappear when onions begin to sprout and that there is a corresponding increase in onion γ-glutamyl transpeptidase (1,9) when onions sprout. Apparently an increase in γ-glutamyl transpeptidase also occurs as garlic cloves begin to germinate, a process which is accelerated by a cool temperature which mimics wintering. Unlike onion, however, the disappearance of the γ-glutamyl peptides of garlic was near maximal long before external sprouting was visible, although internal examination of the cloves revealed that sprouts were nearly ready to protrude.

Aging of garlic also resulted in the formation of additional unidentified compounds (X_1, X_2, X_3) at retention times 7.0 min, 12.3 min, and 25.9 min (Figure 5A), al-

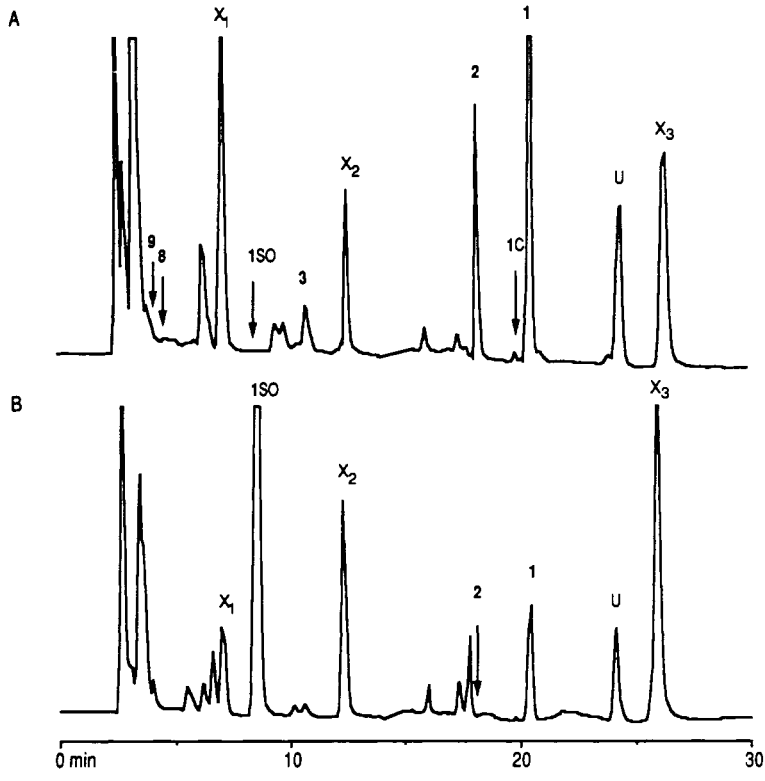


FIGURE 5. γ-Glutamylcysteines in aqueous homogenates of store-purchased garlic (A) and yellow onion (B). 0.05 M KH_2PO_4 (pH 2.5) 1.2 ml/min for 12 min, then 0.05 M KH_2PO_4 (pH 4.5)-MeOH (97.5:2.5), 2.0 ml/min, 220 nm. Compounds identified in Scheme 1. 1SO, sulfoxide of compound **1**. 1C, cis isomer of compound **1**. U, unidentified γ-glutamyl peptide. X_1, X_2, X_3, unidentified compounds.

though none of these peaks were decreased by γ-glutamyl transpeptidase treatment. The peak at 7.0 min was not found in the homogenates of boiled garlic cloves (not shown), indicating that it was probably formed enzymatically upon homogenization. These compounds were also found in store-purchased onion bulbs (Figure 5B), but not in onion seeds (not shown).

The content of γ-glutamyl-*S*-alkylcysteines and γ-glutamyl-*S*-*trans*-1-propenylcysteine sulfoxide in several *Allium* spp. is given in Table 1. Smaller amounts of **1** were found in store-purchased garlic than in fresh-picked garlic, which reflects both the length of time since harvesting and the probable cool temperatures at night at farms and warehouses. Bulbs of a variety of onion types, shallot, and leek contained much smaller amounts of **1** than garlic, and no detectable levels of the allyl **2** or methyl **3** compounds. Among onions, bulbs of boiling onion contained larger amounts of **1** than other onion types, on a fresh wt basis, due mostly to its lower moisture content. Compound **1** has not been previously reported in onion bulbs, although the methyl compound **3** has been reported to be 0.16–0.19 mg/g (1) (onion type not specified). Based on retention times, we initially thought there was a small amount (0.03 mg/g) of **3** in onion bulbs; however a diode-array uv-scan of the suspected peak did not match. When the initial hplc pH was changed from 2.5 to 3.0, the interfering peak separated from **3**, revealing its absence in onion bulbs. By far the dominant γ-glutamyl-*S*-alkylcysteine found in onion and shallot bulbs was the sulfoxide of **1**. Its content depended on the type of onion, was

TABLE 1. Content of γ-Glutamyl-*S*-alkylcysteines and γ-Glutamyl-*S*-*trans*-1-propenyl-cysteine Sulfoxide in *Allium* spp.

	Compound				
	trans-1-propenyl **1**	allyl **2**	methyl **3**	propyl **4**	*trans*-1-propenyl sulfoxide
Bulbs	(mg/g fresh weight)[a]				
Garlic[b] (*Allium sativum* L. cv. Calif. Early)	8.0 ± 1.4	6.9 ± 1.5	0.5 ± 0.2	n[c]	n
Garlic[b] (*A. sativum* L. cv. Calif. Late)	6.9 ± 1.4	5.5 ± 1.1	1.2 ± 0.5	n	n
Garlic[d] (*A. sativum* L.)	4.1 ± 0.6	4.0 ± 1.6	1.3 ± 0.7	n	n
Yellow onion[d] (*Allium cepa* L.)	0.08 ± 0.03	n	n	n	0.53 ± 0.24
Red onion[d] (*A. cepa* L.)	0.07 ± 0.03	n	n	n	0.48 ± 0.35
White onion[d] (*A. cepa* L.)	0.07 ± 0.02	n	n	n	0.98 ± 0.34
Boiling onion[d] (*A. cepa* L.)	0.27 ± 0.10	n	n	n	4.0 ± 1.9
Shallot[d] (*A. cepa* L. *aggregatum* group)	0.18 ± 0.11	n	n	n	0.85 ± 0.15
Green onion[d] (*A. cepa* L.)	0.04 ± 0.06	n	n	n	n
Leek[d] (*Allium ampeloprasum* L. *porrum* group)	0.02 ± 0.03	n	n	n	n
Seeds					
Yellow onion (*A. cepa* L.)	18.5	0.6	0.5	0.8	0.5
Red onion (*A. cepa* L.)	19.8	0.2	1.1	0.3	1.1
White onion (*A. cepa* L.)	22.7	0.4	0.4	0.6	0.5
Chive (*Allium schoenoprasum* L.)	30.5	0.2	0.8	0.7	0.5
Garlic chive (Chinese chive) (*Allium tuberosum* Rottl. ex K. Spreng)	13.0	15.3	16.9	n	0.3

[a]Percent dry wt in bulbs: garlic (38), yellow onion (6.6), red onion (8.0), white onion (11.7), boiling onion (20.3), green onion (11.4), shallot (18.2), leek (19.9). All seeds contained 93% dry wt.

[b]Fresh-picked. Mean ± SD of 4 bulbs.

[c]Not detected. Limit of detection is approximately 0.01 mg/g fresh wt.

[d]Store-purchased. Mean ± SD of 3–4 stores.

not detectable in green onions, and was especially high in boiling onions (small white onions of 3–3.5 cm diameter), even when the moisture content was taken into account. The content of the sulfoxide of **1** in onion bulbs was previously reported to be 2.0 mg/g (1). It was not found in garlic or leek bulbs and was present in only very small amounts (dry wt basis) in the seeds tested, even in onion seeds. The simplicity of the results of analyses of the seeds was striking (Figure 6). Hplc analysis of aqueous homogenates of onion and chive seeds revealed almost exclusively compound **1**. The seeds of garlic chive (Chinese chive) were unique in that they also contained large amounts of the allyl **2** and methyl **3** compounds. γ-Glutamyl-*S*-propylcysteine [**4**] was not found in any of the bulbs but was a minor constituent of most of the seeds tested. The cis isomer of **1** was found to occur at 1–2 wt percent of **1** in both garlic bulbs and all seeds tested. The cis isomer was found to increase at the expense of **1** when extracts were heated.

Similar to previous reports on onion (1,3), a major portion of the organosulfur compounds of garlic was contained in the γ-glutamylcysteines. The content of dialkyl thiosulfinates in garlic homogenates has been previously determined to be about 6.0 mg/g (4). Free L-cysteine and L-methionine are present at only trace levels in garlic (11). Assuming that the γ-glutamylcysteines listed in Table 1 constitute most of the remaining organosulfur compounds, the γ-glutamylcysteines would constitute about 70

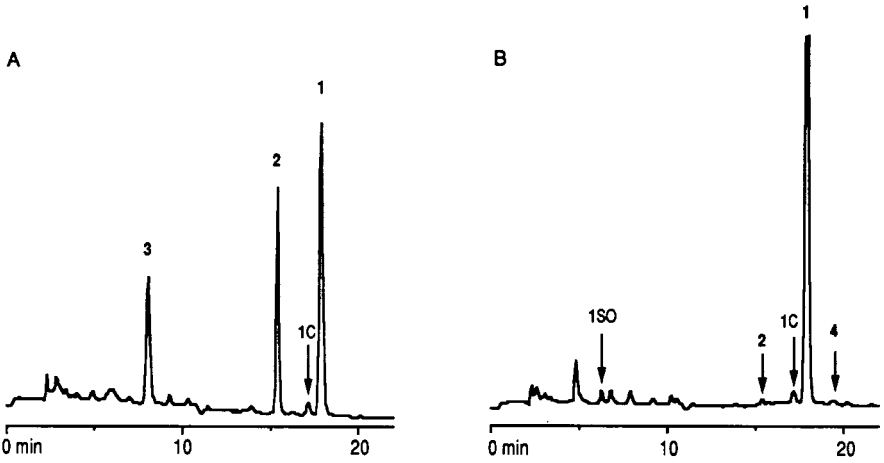


FIGURE 6. γ-Glutamylcysteines in aqueous homogenates of seeds of garlic chive (A) and red onion (B). 0.05 M KH_2PO_4 (pH 3.0) 1.2 ml/min for 9 min, then 0.05 M KH_2PO_4 (pH 4.5)-MeOH (97.5:2.5), 2.0 ml/min, 220 nm. Compounds identified in Scheme 1. **1C**, cis isomer of **1**. **1SO**, sulfoxide of **1**.

wt percent or 40 mol percent of the total organic bound sulfur in the homogenate of fresh-picked garlic and about 60 wt percent or 30 mol percent in the homogenate of store-purchased garlic.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hplc analyses were performed on a Beckman System Gold Model 126 chromatograph employing a Model 168 diode-array detector, a Model 507 autosampler cooled to 4°, and a Supelco (Bellefonte, PA) C18 column (5 μm, 250 mm × 4.6 mm). The uv spectra for Figure 3 were obtained with a Beckman DU-7 spectrophotometer.

PLANT MATERIAL.—Fresh-picked garlic was obtained from a farm in Yerington, Nevada. All other bulbs were purchased at local grocery stores. Voucher specimens are maintained in our laboratory. Chive and garlic chive seeds were obtained from Park Seed Co. (Greenwood, SC) and all onion seeds from Mellinger's Horticulture Merchants (N. Lima, OH). Homogenates were prepared in deionized H_2O (10 ml/g garlic clove or milled seed and 2 ml/g onion bulb) using a Virtis 45 Micro Ultrashear homogenizer (Gardiner, NY). Extraction appeared to be complete as homogenization with up to four times as much H_2O did not result in a greater yield. Extraction with H_3PO_4 at pH 2.5 also did not improve the yield. The homogenates were washed with 1 volume of CH_2Cl_2 to remove thiosulfinates. It was important to keep the homogenates cool after preparation to prevent bacterial hydrolysis to alkylcysteines.

γ-GLUTAMYL-*S*-ALLYL-L-CYSTEINE [**2**].—L-Cysteine hydrochloride (1.58 g) was dissolved in EtOH and converted to the sodium salt with 0.69 g of sodium. To the same solution was added 1.2 g of allyl bromide and enough H_2O to effect solution. The reaction was complete in 45 min, and the solvent was removed with a rotary evaporator. The residue was dissolved in EtOH and filtered, and ethanolic HCl was added to pH 8. The precipitate, *S*-allylcysteine hydrochloride, was recrystallized from EtOH-H_2O and had a melting point of 223° (dec) [lit. (10) 220°, dec]. A γ-carboxy-activated glutamic acid was prepared by treating 0.3 g of *N*-*t*-Boc-L-glutamic acid α-*t*-butyl ester with 0.12 g of *N*-hydroxysuccinimide and 0.21 g of 1,3-dicyclohexylcarbodiimide. γ-Glutamyl-*S*-allylcysteine was then prepared by reacting 40 mg of *S*-allylcysteine hydrochloride and 100 mg of activated blocked glutamate in the presence of 0.02 g of $NaHCO_3$ in MeCN, followed by solvent removal and deprotection with trifluoroacetic acid. The final product was used without further purification.

γ-GLUTAMYL-*S*-METHYL-L-CYSTEINE [**3**] AND γ-GLUTAMYL-*S*-PROPYL-L-CYSTEINE [**4**].—These compounds were prepared by incubation of *S*-methylglutathione or *S*-propylglutathione (Sigma, St. Louis, MO) with carboxypeptidase A (Sigma) in 0.05 M KH_2PO_4 buffer, pH 7.3, for 4 h at room temperature.

ISOLATION AND STABILITY OF **1–3**.—Compounds **1–3** were also obtained using a preparative C_{18} hplc column (250 mm × 21.4 mm) eluted with MeOH-H_2O-HCOOH (50:50:0.1) at 8 ml/min and de-

tected at 235 nm to separate the γ-glutamylcystyeines of homogenates of onion seeds (for **1**) or garlic chive seeds (for **1–3**). Prior to preparative hplc, the homogenates were freed from carbohydrates and protein on a Dowex-50w (H^+) column (Sigma) washed with H_2O, and γ-glutamylcysteines were eluted with 3N NH_4OH. The NH_4OH was removed by rotary evaporation and the compounds redissolved in H_2O. After collection of the preparative hplc fractions, the solvent was removed by rotary evaporation at 50° to one-half volume followed by lyophilization. The purity of compounds **1–3** was 95% or greater as determined by analytical hplc (Figures 2 and 5). In dry form, **1–3** were stable; however, in aqueous solutions at 0.1–4.0 mg/ml, compound **1** was unstable, decaying at a rate of 5% per week when frozen at −15°, although **2** and **3** were stable under the same conditions. It is recommended that solutions of **1** be stored at −70°.

γ-GLUTAMYL TRANSPEPTIDASE REACTION.—To 1 ml of a garlic homogenate (10 ml H_2O/g) was added 1 ml of tris buffer, pH 8, and 2 units of bovine kidney γ-glutamyl transpeptidase (Sigma), followed by incubation at 37° for 2 or more hours.

H_2O_2 OXIDATION.—Garlic homogenate (1 ml) was incubated with 0.05 ml of 1 M H_2O_2 overnight at 37°.

QUANTITATION.—The γ-glutamyl-*S*-alkylcysteines were quantitated according to the extinction coefficients at 0.10 mg/ml at 220 nm determined with a Beckman DU-7 spectrophotometer. The following extinction coefficients (ml/mg) were found in H_2O or 50 mM KH_2PO_4 (pH 4.5)-MeOH (97.5:2.5): **1** (17.1), **2** (8.1), **3** (5.4), **4** (6.0). The extinction coefficient for the sulfoxide of **1** was determined by hplc diode-array detector quantitation after H_2O_2 oxidation of a known amount of **1** and was found to be 6.5. The value for the cis isomer of **1** was estimated from normalized scans taken by the hplc diode-array detector to be 26. As is typical for peptides, the extinction coefficients increased with dilution; however, the hplc detector response was highly linear over the range of 0.01 and 0.20 mg/ml of injected peptide. The ratio of extinction coefficients found with the DU-7 was nearly identical to ratios found for peak areas obtained from the hplc diode-array detector.

ACKNOWLEDGMENTS

The γ-glutamyl-*S*-allylcysteine [**2**] was synthesized by Dr. Steven G. Wood.

LITERATURE CITED

1. E.J. Matikkala and A.I. Virtanen, *Acta Chem. Scand.*, **21**, 2891 (1967).
2. A.I. Virtanen, *Qual. Plant. Mater. Veg.*, **18**, 8 (1969).
3. A.I. Virtanen, *Phytochemistry*, **4**, 207 (1965).
4. L.D. Lawson, S.G. Wood, and B.G. Hughes, *Planta Med.*, (in press).
5. T. Bayer, W. Breu, O. Seligmann, Y. Wray, and H. Wagner, *Phytochemistry*, **28**, 2373 (1989).
6. G.R. Fenwick and A.B. Hanley, *CRC Crit. Rev. Food Sci. Nutr.*, **22**, 273 (1985).
7. J.F. Carson and L.E. Boggs, *J. Org. Chem.*, **31**, 2862 (1966).
8. A.I. Virtanen, M. Hatanaka, and M. Berlin, *Suom. Kemistil. B*, **35**, 245 (1962).
9. S. Schwimmer and S.J. Austin, *J. Food Sci.*, **36**, 807 (1971).
10. A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **31**, 189 (1948).
11. S.J. Ziegler and O. Sticher, *Planta Med.*, **55**, 372 (1989).

Received 18 July 1990