# Quantification of Alk(en)yl-L-cysteine Sulfoxides and Related Amino Acids in Alliums by High-Performance Liquid Chromatography

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A liquid chromatographic technique was developed for the quantification of alk(en)yl-L-cysteine sulfoxides (ACSO) and selected amino acids (presumed to be involved in ACSO biosynthesis) in extracts of plant tissues. The method is based on fluorescent detection of 9-fluorenylmethyl chloroformate-derivatized ACSO adducts. On the basis of the sampling scheme used, conservative estimates of detection limits were 5 pmol of ACSO adduct, equivalent to ≤2.5 mg/100 g of fresh weight (g fw). Methyl-L-cysteine sulfoxide (MCSO) and 2-propenyl-L-cysteine sulfoxide (2-PECSO) were present in garlic (*Allium sativum*) cloves (16:84 w/w) with total ACSO levels of 300–500 mg/100 g fw. Some garlic cloves also contained lesser amounts of 1-propenyl-L-cysteine sulfoxide (1-PECSO). Pungent onion (*Allium cepa*) and leek (*Allium porrum* cv. Titan) bulbs contained MCSO and 1-PECSO (14:86 and 27:73 w/w, respectively) with total ACSO content of 100–200 mg/100 g fw. Cabbage (*Brassica oleracea*) leaves contained only MCSO at about 80 mg/100 g fw. None of the examined tissues contained the *n*-propyl-L-cysteine sulfoxide within the limits of detection, or ≤0.68–2.4% of the total ACSO in all samples. Of the amino acids quantified, glutamic acid (9–69 mg/100 g fw) was substantially more abundant than valine and glycine (up to 5 mg/100 g fw). Cysteine was also present in moderate amounts but was not quantified because of uncertainties in the extent of oxidation taking place during sample handling and analysis.

**Keywords:** *Alliums; brassicas; alk(en)ylcysteine sulfoxides; alliinase; non-protein amino acids; flavor precursors; pungency; liquid chromatography; fluorescence detection*

## INTRODUCTION

The distinctive flavor characteristics and purported therapeutic benefits of tissues of plants from the genus *Allium* are conferred by an array of organosulfur compounds generated from non-protein sulfur amino acid precursors, the *S*-alk(en)yl-L-cysteine sulfoxides (ACSO) (Lancaster and Boland, 1990). The profile and quantities of these ACSO are responsible for the nature and intensity of flavors derived from fresh and processed *Allium* tissues. For example, the methyl- (MCSO), 1-propenyl- (1-PECSO), and 2-propenyl- (allyl, 2-PECSO) cysteine sulfoxides are responsible for yielding character impact compounds of freshly comminuted cabbage leaves, onion bulbs, and garlic cloves, respectively (Saghir et al., 1964; Freeman and Whenham, 1976a,b). Thus, quantitative profiling of endogenous ACSO may allow for improved control of flavoring quality of these materials. Similarly, quantitation of the endogenous ACSO may benefit future efforts to use alliums for phytomedicinal preparations by allowing the standardization of raw materials (Ziegler and Sticher, 1989). From a product quality perspective, reactions of ACSO upon tissue disruption under certain conditions also have been implicated in the development of bitterness and discoloration in minced garlic and onion tissues (Shannon et al., 1967; Lukes, 1986).

Despite the awareness of the importance of ACSO, some confusion exists as to the relative levels of various ACSO in plant tissues. For example, *n*-propylcysteine sulfoxide (PCSO) has at times been reported to be a major ACSO component (Matikkala and Virtanen, 1959; Lancaster and Kelly, 1983; Lancaster et al., 1986; Lancaster and Boland, 1990), although others conclude that it is only present in trace quantities, if at all (Matikkala and Virtanen, 1967; Yagami et al., 1980; Ziegler and Sticher, 1989).

Various methods have been used to estimate ACSO content of alliums, although many of these are indirect methods. The determination of pyruvate, which is formed stoichiometrically upon action of *S*-alk(en)yl-L-cysteine sulfoxide lyase (C-S lyase; EC 4.4.1.4, alliin alkylsulfenate-lyase or alliinase) on ACSO, is an established method for estimating the pungency of alliums (Schwimmer and Guadagni, 1962). However, this method does not determine the profile or relative amounts of ACSO present. The measurement of thiosulfinates, formed by reactions succeeding C-S lyase action, has been proposed as an indicator of pungency (Carson and Wong, 1959; Nakata et al., 1970) but does not provide insight as to the relative proportions of the ACSO initially present. In addition, the relationship between thiosulfinate and original ACSO levels is not the same for minced onion and garlic (Thomas et al., 1992) and perhaps other ACSO-containing tissues. However, the recent development of comparatively mild liquid and gas–liquid chromatographic procedures for analyzing thiosulfinates represents a breakthrough in interpreting the chemical reactions that quickly follow enzymic action (Block et al., 1992a,b).

Others have attempted to use gas–liquid chromatography (GLC) measurements of the headspace of minced *Allium* tissues (Saghir et al., 1964; Brodnitz et al., 1971; Boelens et al., 1971; Kallio and Salorinne, 1990) or in distilled oils or solvent extracts prepared therefrom (Brodnitz et al., 1969; Boelens et al., 1971) as a means to estimate the original quantities of endogenous ACSO present. Volatile sulfides identified in the headspace are dominated by propenylated and *n*-propylated spe-

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cies, the latter especially in onion (Kallio and Salorinne, 1990; Kuo et al., 1990; Tokitomo and Kobayashi, 1992), but it is uncertain if the *n*-propyl fragments arose from endogenous PCSO or by transformation of 1- or 2-propenyl fragments to *n*-propyl fragments during sample handling and analysis. Some investigators have opined that the *n*-propylated sulfides are artifacts and arise from the transformation of 1-propenyl fragments from the temperatures encountered during GLC analysis (Boelens et al., 1971; Freeman and Whenham, 1975) or by endogenous reducing agents in macerated tissue (Freeman and Whenham, 1975, 1976a; Yagami et al., 1980). In view of these possibilities and the prior results of Matikkala and Virtanen (1959, 1967), Freeman and Whenham (1975) estimated the original mass proportions of PCSO, 1-PECSO, and MCSO in intact onion bulbs as 2:80:18. Further clouding the issue of the ACSO profile endogenous to onion bulbs are the findings that thiosulfinates produced in homogenized onion tissue are composed of nearly equimolar levels of 1-propenyl, *n*-propyl, and methyl fragments (Block et al., 1992a,b).

Attempts have been made to directly analyze ACSO contents in *Allium* and related plant species. Employing two-dimensional separation on silica gel thin-layer plates by electrophoresis and classical chromatography, Granroth (1968) qualitatively found only MCSO and 1-PECSO in onion bulb extracts. Using a similar approach with densitometry to analyze extracts of white onion, ACSO content was reported to be 4-fold greater than that estimated by pyruvate analysis, and the relative mass proportions of PCSO, 1-PECSO, and MCSO were found to be 66:14:20 (Lancaster and Kelly, 1983). This contrasts with the previous findings of Granroth (1968) and the ratio of trace:92:8 as determined by conventional amino acid analysis of onion extracts (Matikkala and Virtanen, 1967). However, Matikkala and Virtanen (1959) previously reported PCSO to be present in onion, on the basis of cochromatography with an authentic standard, at a mass ratio 1:4 PCSO/MCSO. These examples underscore the confusion that abounds in the literature regarding PCSO and relative ACSO levels in some plant tissues.

More recently, high-performance liquid chromatography (HPLC) methods have been applied to the analysis of ACSO in *Allium* species and ACSO-containing tissues of plants of other genera. Using sample derivatization with *o*-phthaldialdehyde (OPA) and 2-mercaptoethanol, Gustine (1985) detected MCSO in extracts of plants of the genus *Brassica*. Ziegler and Sticher (1988, 1989) used an OPA/2-methyl-2-propanethiol sample derivatization procedure and HPLC analysis to detect MCSO and quantify 2-PECSO in garlic. Subsequent analysis of thiosulfinates in garlic preparations confirms that 2-propenyl is the dominant constitutive alkyl fragment, with the methyl fragment being a minor component and the *n*-propyl fragment not detected (Block et al., 1992a,b; Lawson et al., 1991b). PCSO was reported to be absent in garlic (Ziegler and Sticher, 1988), whereas 1-PECSO is sometimes present (Lukes, 1986; Lawson et al., 1991b). MCSO has been verified to be present in five common *Brassica* plant tissues using OPA derivatization and HPLC (Marks et al., 1992).

An alternative method to preparing OPA derivatives for analysis of amino acids involves sample derivatization with 9-fluorenylmethyl chloroformate (FMOC) (Einarsson et al., 1983; Cunico et al., 1986). Preparation of FMOC−amino acid derivatives yields adducts with good stability and avoids the use of noxious thiol reagents, such as 2-mercaptoethanol and 2-methyl-2-propanethiol.

Our intent was to adapt an FMOC sample derivatization technique and HPLC method to quantitatively profile endogenous ACSO naturally occurring in tissues of selected plants of the genera *Allium* and *Brassica*. Materials chosen for study were garlic (*Allium sativum*) cloves, onion (*Allium cepa*) bulbs, leek (*Allium porrum*) bulbs, and cabbage (*Brassica oleracea*) leaves.

## MATERIALS AND METHODS

**Materials.** Chemicals and HPLC grade solvents were obtained from either Sigma Chemical Co. (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI). All water used was deionized and glass-distilled.

Garlic cloves, white onion and leek bulbs, and cabbage leaves were obtained from a local market and analyzed within 2 days of purchase. In addition, local garden grown leeks (cv. Titan) and "dehydrator" onions were also analyzed for ACSO content (the "dehydrator" bulbs were a gift from McCormick and Co., Inc., Hunt Valley, MD).

**Extraction of Alk(en)yl-L-cysteine Sulfoxides (ACSO) and Amino Acids.** Using the approach of Lancaster and Kelly (1983), the ACSO derivatives were extracted from the plant tissues as follows. After the outer skins or leaves were carefully removed, about 50 g of tissue was steeped for at least 24 h at −20 °C in 100 mL of methanol/chloroform/water (MCW; 12:5:3 v/v/v) which contained ethyl cysteine (1.0 mM) as an internal standard. The MCW extract was phase-separated by the addition of 1 volume of chloroform/water (2:3 v/v). The chloroform phase was discarded and the methanol/water phase reduced to about 50 mL under vacuum (127 mmHg) at 60 °C. The extract was then filtered through a 0.20-$\mu$m nylon filter (Gelman Science, Inc., Ann Arbor, MI) prior to analysis by HPLC.

Pyruvate contents [analyzed as in Thomas et al. (1992)] in steeped extracts of onion, leek, and cabbage tissues were similar to extracts prepared as homogenates of the tissue with the same solvent system, indicating negligible C-S lyase action on ACSO during the steeping period. For garlic, steeped extracts had pyruvate levels above background levels, indicating that up to 26% degradation of endogenous ACSO was noted. Even directly homogenizing garlic clove tissue in MCW allowed for limited degradation (5−10%) of endogenous ACSO components. It is likely that the greater solids content of garlic and the increased tortuosity to solvent penetration relative to that of the other tissues evaluated account for this observation with garlic. True background levels of pyruvate in garlic were determined after the tissue was frozen indirectly in a dry ice/ethanol mixture, lyophilized, boiled for 5 min in 4 volumes methanol, and finally aqueous extracted. Our calculations compensate for the loss of ACSO during the steeping (extraction) step routinely used. There was no selective loss of either ACSO during steeping, as direct homogenates and steeped extracts had the same relative proportions of ACSO reported in Table 1.

**Synthetic ACSO Standards.** *S-Methyl-L-cysteine Sulfoxide (MCSO).* Diastereomeric MCSO was prepared according to a modified method of Synge and Wood (1956). A mixture of 1.65 mL of 30% $H_2O_2$ and 23 mL of 0.6 M *S*-methyl-L-cysteine was kept at 25 °C with continuous stirring for 24 h. Two hundred milliliters of cold (4 °C) ethanol was added, and after 30 min at 4 °C, a white precipitate was recovered by filtration with a typical yield of 73%. The resultant (±) MCSO preparation decomposed at ca. 168 °C.

*S-2-Propenyl-L-cysteine Sulfoxide (2-PECSO).* Diastereomeric 2-PECSO was synthesized according to the procedure of Lancaster and Kelly (1983). L-Cysteine hydrochloride (4 g) was stirred into 75 mL of ethanol followed by the addition of 5.6 mL of 20 M NaOH. After 5 min, 4.4 mL of 2-propenyl bromide was added and the mixture was adjusted to pH 5.25 using glacial acetic acid. The mixture was slowly stirred for

**Table 1. Quantitation of ACSO and Related Amino Acids in Extracts of Selected Plant Tissues As Determined by HPLC**

| | mass tissue levels (±SD) for ACSO and amino acids (mg/100 g fw) | | | | | |
|---|---|---|---|---|---|---|
| tissue | MCSO | 2-PECSO | 1-PECSO | glutamic acid | glycine | valine |
| garlic cloves | 60.4 (29.4) | 305 (136) | (±)[a] | 34.1 (11.9) | ND[b] | 1.1 (0.9) |
| onion bulbs | | | | | | |
| white | 23.6 (7.6) | ND | 131 (18) | 9.8 (8.3) | tr[c] | 0.7 (0.4) |
| "dehydrator" | 14.7 (2.7) | ND | 97.8 (52.6) | 9.0 (1.2) | 2.3 (0.1) | tr |
| leek bulbs | 28.2 (12.5) | ND | 75.3 (16.2) | 12.1 (4.6) | 0.5 (0.4) | tr |
| cabbage leaves[d] | 79.7 | ND | ND | 68.9 | tr | 4.4 |

[a] (±) indicates component was detected only in some samples (see Results and Discussion). [b] ND indicates component was not detected. [c] tr indicates component was present at nonquantifiable levels (<0.1 mg/100 g fw) only in some samples. [d] Mean values presented for only two individual samples prepared and analyzed.

24 h at 25 °C. The suspension was cooled at 4 °C for 1 h, and a white precipitate (2-propenylcysteine) was collected by filtration. The 2-propenyl-L-cysteine was then oxidized to the sulfoxide by treating with $H_2O_2$ and recovered from ethanol as described for MCSO. The white, powdery (±) 2-PECSO preparation decomposed at ca. 166 °C.

*S-n-Propyl-L-cysteine Sulfoxide (PCSO) and S-1-Propenyl-L-cysteine Sulfoxide (1-PECSO).* Synthesized diastereomeric PCSO and 1-PECSO isolated from onion bulb tissue were gifts from McCormick and Co., Inc. (Hunt Valley, MD). The white, powdery (±) PCSO preparation decomposed at ca. 163 °C. Insufficient amounts of 1-PECSO precluded analysis of melting or decomposition point.

**Infrared Analysis of Standard ACSO.** Infrared analysis on ACSO was done using a Nicolet 5DX FT-IR spectrometer (Nicolet Instrument Corp., Madison, WI). A mixture of standard ACSO and KBr (1:2 w/w) was ground with a mortar and pestle, and pellets were prepared from this mixture by applying 10 tons of pressure for 2 min under vacuum. Infrared spectra of all ACSO samples featured $\gamma_{max}^{KBr}$ of 635, 2970 (amino acid), and 1020 $cm^{-1}$ (sulfoxide).

**Sample Derivatization and Preparation.** Sample derivatization was similar to the method of Cunico et al. (1986). A 0.2-mL sample (either standard solution or tissue extract diluted 100−1000-fold) in 0.1 M sodium bicarbonate (pH 8.0) was added to 0.2 mL of 4 mM FMOC in acetone. After incubation at 20−23 °C for 3 min, 0.4 mL of pentane was added and the resultant mixture shaken gently by hand for about 1 min to extract and remove potential chromatographic interference of the alcoholic reaction product of FMOC. The upper pentane phase was discarded. After two similar pentane extractions, 10 μL of washed sample was analyzed by HPLC.

**Liquid Chromatography.** HPLC analysis was done with a Perkin-Elmer 3b liquid chromatograph (Perkin-Elmer, Norwalk, CT) equipped with a 5-μm LiChrosorb RP-18 Hibar column (150 mm × 4.6 mm), using the procedure of Einarsson et al. (1983) as a starting point. The column was submerged in a water bath maintained at 42 °C. Detection of FMOC derivatives was achieved with a Hitachi Model F1000 fluorescence spectrophotometer (Hitachi, Ltd., Tokyo, Japan) set at an excitation wavelength of 264 nm and an emission wavelength of 340 nm. Peak areas were determined with a Spectra-Physics (San Jose, CA) integrator (Model SP4270).

Isocratic elution was achieved with buffered (pH 4.4) acetic acid/methanol/acetonitrile (55:42.5:2.5 v/v/v) at a flow rate of 1.0 mL/min. The acetic acid was prepared by adding 3 mL of glacial acetic acid and 1 mL of triethylamine to 1 L of distilled water (Einarsson et al., 1983), and the pH was adjusted to 4.4 with 9 N NaOH. Identification of ACSO and selected amino acids was done by matching retention times of components in sample extracts with those of authentic standards and/or spiking sample extracts with standards and obtaining coelution of peaks.

Quantification of ACSO and related amino acids was done relative to external and internal standards. At least three individual sample extracts were analyzed for each *Allium* species, and the mean values are reported. Calibration curves for both sulfoxides and amino acids were generated using solutions prepared from the synthesized or reference ACSO and commercially available amino acid standards. Standard curves were established by triplicate analyses where $r^2$ values for each of the curves was ≥0.986. The variation in analysis for each ACSO component (Table 1) is attributable primarily to sample-to-sample variation, since the coefficient of variation of chromatographic analysis was always less than 10% and in most cases less than 5%. These correlations and coefficients of variation were similar for the HPLC method of ACSO analysis reported by Ziegler and Sticher (1989).

**Qualitative Analysis of ACSOs by Thin-Layer Chromatography (TLC).** Using a modified method of Lancaster and Kelly (1983), a 0.25-mm-thick silica gel plate with a preabsorbent area (Whatman LK5; 20 × 20 cm) was prewashed in solvent 1 (methyl ethyl ketone/pyridine/water/glacial acetic acid, 80:15:15:2 v/v/v/v) and dried in a fume hood for approximately 1 h. Extracts and standards were applied to the preabsorbent area and allowed to dry for 1 h, and chromatography was achieved with solvent 1. After a 24-h drying period, a second separation was done in the same direction with 1-propanol/water/propyl acetate/glacial acetic acid/pyridine (120:60:20:4:1 v/v/v/v/v). The plate was allowed to dry in a fume hood for about 30 min, and the bands were visualized by spraying with 0.5% ninhydrin in *tert*-butyl alcohol/ethylene glycol monomethyl ether/pyridine/glacial acetic acid (4:3:2:1 v/v/v/v; Underwood and Rockland, 1954) and the $R_f$ values calculated. It was observed that the ACSO bands turned orange-brown upon complete development as expected (Wagner et al., 1984).

## RESULTS AND DISCUSSION

**Development of the Chromatographic Method.** Our initial attempts to chromatographically resolve and quantify ACSO endogenous to alliums and related plant tissues employed sample derivatization using OPA, as described by Ziegler and Sticher (1989). However, we noted that peak areas for the standard OPA−PCSO derivative fluctuated erratically when samples were injected over time after derivatization. It is suspected that degradation of the OPA−PCSO derivative may be responsible for this observation (L. M. Norris, McCormick Co., personal communication, 1992), as OPA adducts of some amino acids are known to be unstable (Allison et al., 1984). Consequently, we turned to using the FMOC derivatization procedure for the balance of this work. No problems similar to that observed for the OPA−PCSO derivative were encountered for the standard ACSO and amino acids derivatized with FMOC.

Subsequent effort focused on developing a simple and rapid HPLC method capable of resolving and quantifying the endogenous ACSO and related amino acids. Mobile phase composition, isocratic or gradient elution, column temperature, and column length were the variables most closely evaluated. Complete resolution of the target components was obtained with a short (150 mm) reversed-phase column maintained at 42 °C and isocratic elution at a flow rate of 1.0 mL/min (Figure 1a,b). The original method of Einarsson et al. (1983) required gradient elution for resolving FMOC−amino acid adducts. Separation of the (−) and (+) isomers of

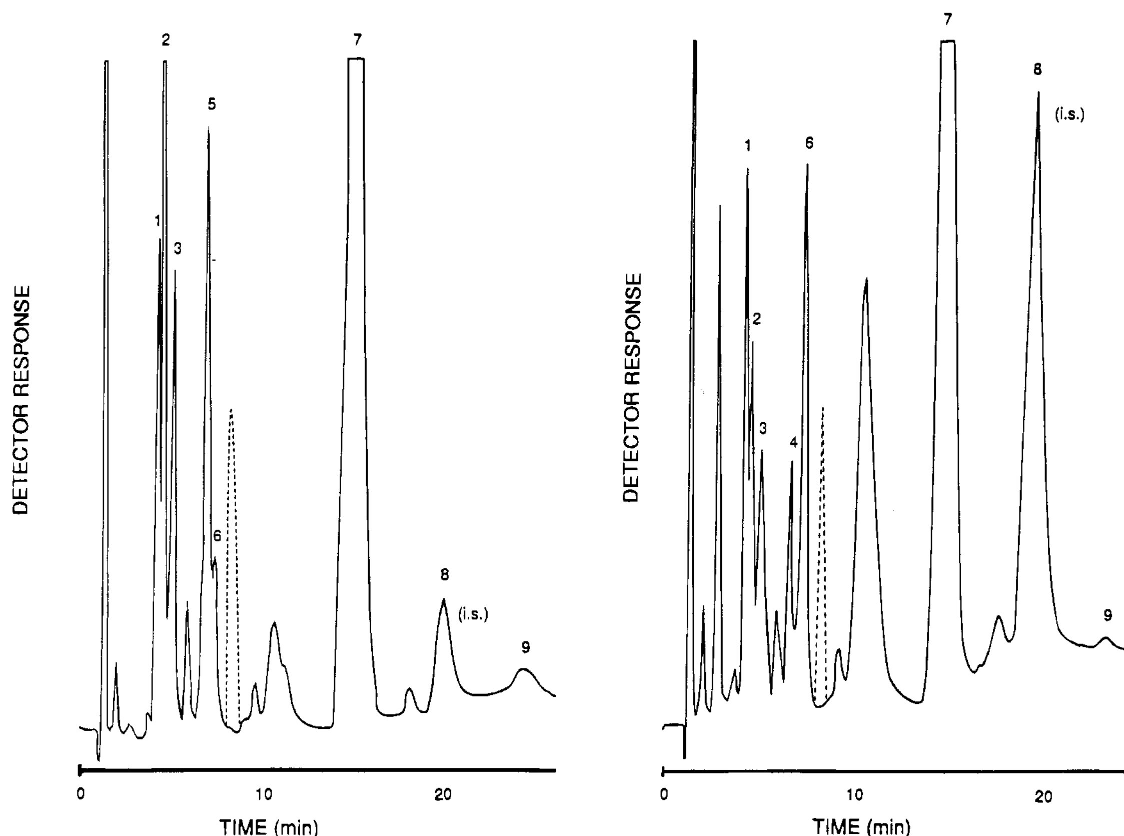


**Figure 1.** Typical HPLC chromatograms for garlic (a, left) and onion (b, right) tissue extracts. Peak identities of FMOC derivatives are as follows: 1, MCSO; 2, cysteine; 3, glutamic acid; 4, glycine; 5, 2-PECSO; 6, 1-PECSO; 7, FMOC alcohol derivative; 8, ethyl cysteine (internal standard); 9, valine. Broken line indicates elution pattern of PCSO derivative.

the synthetic ACSO was not judged necessary since only the (+) isomer is found in nature (Carson, 1987).

**ACSO Analysis of Selected Tissues.** A typical HPLC chromatogram of an extract of garlic is shown in Figure 1a. We found 2-PECSO to be the most dominant ACSO in garlic, with MCSO present at considerably lesser amounts (Table 1). On a molar basis, the total ACSO levels in garlic cloves are about 21 $\mu$mol/g fw. Surprisingly, there are few previous studies with which to compare results, since most focus has been placed on onion tissue component analysis. The level of 2-PECSO we found in fresh garlic is within the broad range 90–1150 mg/100 g fw and consistent with the average 580 mg/100 g fw reported for five samples by Ziegler and Sticher (1989). Ziegler and Sticher (1989) confirmed the presence of MCSO in fresh garlic but did not report endogenous quantities. We found the relative proportions of 2-PECSO and MCSO to be about 84:16, nearly identical to the relative proportions of the 2-propenyl and methyl fragments found in the headspace of freshly comminuted garlic as analyzed by GLC (82:16; Freeman and Whenham, 1975).

We did not detect any compounds in garlic extracts with the same retention time as that of synthetic PCSO. This is in agreement with the findings of Ziegler and Sticher (1989), who reported PCSO to be absent from garlic (*viz.*, below the detection limits of their HPLC method). Recently developed HPLC procedures for determining thiosulfinates in garlic homogenates also noted the relative absence of *n*-propylated species (Lawson et al., 1991b; Sendl and Wagner, 1991), presumably attributable to the absence of endogenous PCSO, or a lack of a reducing system capable of forming *n*-propyl derivatives from 1(2)-propenyl adducts, which appears to be present in onion tissues (Freeman and Whenham, 1975, 1976: Yagami et al., 1980). Since we could easily resolve synthetic PCSO by our method, we conclude that PCSO is a minor ACSO component and present at levels of $\leq$0.7% of the total ACSO pool of 365 mg/100 g fw in garlic, on the basis of the limits of detection and how the samples were handled. Ziegler and Sticher (1989) also reported the absence of PCSO in garlic, but from the information provided, it is difficult to estimate their limit of detection.

In some garlic extracts, we noted the presence of minor amounts of 1-PECSO. Elution of 1-PECSO as a shoulder of the peak representing 2-PECSO precluded the quantification of the former in the presence of copious quantities of the latter. Although 1-PECSO is generally not considered a major ACSO component in garlic, it has been reported to be present by direct analysis (Granroth, 1968; Lukes, 1986). In addition, analysis of thiosulfinates in homogenates of garlic cloves revealed the presence of 1-propenyl-containing species, presumed to have been derived from 1-PECSO (Lawson et al., 1991a,b; Block et al., 1992a,b).

A typical HPLC chromatogram of an extract from onion bulb tissue is shown in Figure 1b. The primary ACSO was 1-PECSO, and MCSO also was present in substantial quantities in white and "dehydrator" onion bulbs (Table 1). The proportion of 1-PECSO was 85–87% of the total ACSO for both onion types. No 2-PECSO or PCSO was detected, restricting the levels of these components to be $\leq$1.6–2.2% of the total ACSO pool of 112–154 mg/100 g fw in onion. Our values for 1-PECSO and MCSO are somewhat different from the 385 and 35 mg/100 g fw, respectively, reported by Matikkala and Virtanen (1967), and the 60 and 90 mg/100 g fw, respectively, reported by Lancaster and Kelly (1983). However, the proportion of 1-PECSO (92%) of

the total ACSO reported by Matikkala and Virtanen (1967) is similar to that reported here for pungent onion bulbs. Discrepancies in these values may be founded in varietal differences and method of analysis. In addition, it is difficult to speculate on how the reported presence of PCSO as the dominant ACSO in onion (Lancaster and Kelly, 1983) impacts on any comparison between this previous study and the present one (where PCSO was not detected). In any case, when calculated on a molar basis, our values for total ACSO content in white and "dehydrator" onion bulbs are about 9.9 and 7.1 μmol/g fw, respectively. These values agree well with, and are in the upper range (as expected for pungent onions) of, the 5–10 μmol of pyruvate/g fw for macerated onion bulbs of varying pungency (Thomas et al., 1992).

The base etiolated tissue of leek contained MCSO and 1-PECSO at a mass ratio of 27:73 and at total levels similar to those found in pungent onion bulbs (Table 1). As with onion tissue, no 2-PECSO or PCSO was detected, limiting the levels of either of these components to ≤2.4% of the total ACSO pool (104 mg/100 g fw or about 6.0 μmol/g fw). Since onions are generally perceived to be more pungent and lachrymatory than leeks, it is tempting to speculate that the property of pungency may be somewhat dependent on the proportion of 1-PECSO as well as the total amounts of 1-PECSO. The onion and leek samples used in our studies were particularly pungent. This may also explain why garlic tissue had only 3–4 times the levels of ACSO of the onion and leek tissues, rather than the 5–13 greater ACSO content as expected (Saghir et al., 1964; Thomas et al., 1992).

Cabbage leaves were found to contain only MCSO, at levels (about 5.2 μmol/g fw) greater than found in the alliums evaluated (Table 1). Other ACSO components are estimated to be at levels ≤1.6% (mass basis) of the total ACSO pool. Gustine (1985) determined MCSO as the only ACSO in *Brassica* tissue extracts but did not report quantitative results. Marks et al. (1992) quantified MCSO in extracts of five *Brassica* species, including cabbage, at levels of 14–68 mg/100 g fw.

**Analysis of Relevant Amino Acids in Selected Tissues.** In addition to ACSOs, we were also able to estimate endogenous levels of selected amino acids in tissue extracts by this HPLC method. The amino acids chosen for evaluation have been reported as intermediates in the pathway of ACSO biosynthesis. Granroth (1970) suggested the biosynthesis of 1-PECSO from valine and cysteine. More recently, Lancaster and Shaw (1989) proposed that glutathione (formed from cysteine, glutamic acid, and glycine) is the key metabolite for the biosynthesis of all ACSOs. We did not attempt to estimate cysteine contents in the extracts because of uncertainties of the stability of cysteine during sample handling and processing. For all tissues evaluated, glutamic acid was relatively abundant, whereas glycine and valine were present in lesser, often trace, amounts (Table 1). These results generally agree with previous amino acid analysis of onion bulb tissue (Matikkala and Virtanen, 1967).

**Qualitative TLC Analysis of Extracts.** As a comparative technique, we analyzed synthesized standards and tissue extracts using TLC (Figure 2). For MCSO, 2-PECSO and 1-PECSO, and PCSO standards, the average $R_f$ values were 0.34, 0.54–0.55, and 0.58, respectively. Although minor contaminates were sometimes present, synthetic or reference ACSO preparations usually appeared as single spots and the order of separation is in agreement with previous studies (Lukes 1971; Lancaster and Kelly, 1983). The bands for the sulfoxide standards turned a dull orange-brown after development with ninhydrin reagent, as expected (Wagner et al., 1984). The banding pattern of each tissue extract was consistent with the results obtained by HPLC. However, the presence of many unidentified bands, especially in the zones to where the ACSO components migrate, makes it difficult to use TLC as the primary means to identify, even qualitatively, the profile of ACSO present.

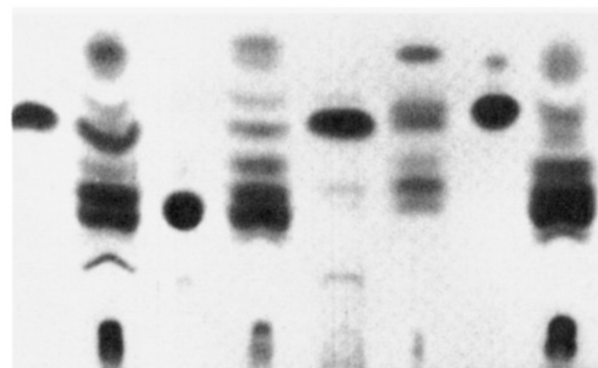


**Figure 2.** Thin-layer chromatography of synthetic or reference ACSO preparations and plant tissue extracts.

**Relationship between ACSO and Thiosulfinate Profiles.** Our estimation of total ACSO content for garlic is about 21 μmol/g fw (from Table 1). Thiosulfinate levels in seven samples of garlic range from 14 to 36 (mean of 23) μmol/g fw (Block et al., 1992a). On the basis of the molar ratio of alk(en)yl groups of 2:1 for thiosulfinate/ACSO, twice as much thiosulfinate has been observed in garlic aqueous extracts than would be expected from the ACSO levels reported in Table 1. The additional ACSO precursors required to account for the level of thiosulfinates formed in garlic may come from those coupled as γ-glutamyl peptides, which would not be detected by our procedure. The 1- and 2-PECSO γ-glutamyl peptide derivatives have been identified in garlic (Mütsch-Eckner et al., 1992), and while we are not aware of reports on the quantities of these peptides in garlic, the 1-PECSO γ-glutamyl peptide has been found (Matikkala and Virtanen, 1967) in onion at levels approaching 2 mg/g fw (equivalent to 11 μmol/g fw).

Our analysis of total ACSO levels in onion range from 6.4 to 8.8 μmol/g fw (Table 1), whereas a range of thiosulfinate levels of 0.14–0.35 (mean of 0.23) μmol/g fw was found in three onion extracts (Block et al., 1992a). Thus, only 5–7% of the alk(en)yl fragments nascent to onion ACSO can be accounted for in thiosulfinate formation. The reduced degree of conversion of ACSO in onion to thiosulfinates can be attributed to the 1-PECSO species being dominant (Table 1). Following C-S lyase (alliinase) action, the sulfenic acid derivative of 1-PECSO is more likely to yield the propanethial *S*-oxide (lachrymatory factor), which may be partially lost as a volatile, or cyclic structures (zwiebelanes) and α-sulfinyl disulfides (cepaenes), rather than a thiosulfinate adduct (Bayer et al., 1989a,b; Block, 1992). In addition, the mixed 1-propenyl/methyl and dimethyl thiosulfinate species have limited stability in aqueous extracts of fresh-cut onion (Block et al., 1992a). In contrast, mixed methyl/allyl and diallyl thiosulfinates are stable in garlic.

On average, extracts of freshly comminuted garlic

contain about 100-fold (and on a paired comparison basis, range 41–260-fold) molar excess of thiosulfinate relative to similar preparations of onion (Block et al., 1992a). On the basis of a rapid colorimetric assay, fresh aqueous extracts of garlic were reported to have 1000-fold greater levels of thiosulfinate than those of onion (Thomas et al., 1992). The added order of magnitude difference in thiosulfinate levels produced by onion and garlic between these studies lies in the colorimetric response of the di-2-propenyl thiosulfinate species (allicin) being about 7-fold greater than the dimethyl thiosulfinate species (Nakata et al., 1970). The dominant ACSO in garlic is 2-PECSO (Table 1), and the allyl fragment accounts for 74–94% of alk(en)yl groups in thiosulfinates in aqueous extracts of garlic (Block et al., 1992a). In contrast, the methyl group constitutes about 33% of the alk(en)yl groups in onion thiosulfinates (Block et al., 1992a).

One final point of discussion is that *n*-propyl groups account for 37–47% of the alk(en)yl fragments in thiosulfinates identified in onion but are not detected as such in garlic (Block et al., 1992a). Although our inability to detect PCSO in garlic appears consistent with the studies by Ziegler and Sticher (1988) and Block et al. (1992a), a dilemma can be averted if one considers any or all of the following. By our calculation, only 0.2 μmol/g fw of endogenous PCSO can account for all of the *n*-propyl groups found in onion thiosulfinates (Block et al., 1992a), provided all of the PCSO yields thiosulfinate. Our limit of detection of PCSO is equivalent to about 0.14 μmol/g fw in onion. If actual PCSO levels are near our detection limit, then low levels of endogenous PCSO come close to accounting for the *n*-propyl groups in thiosulfinates. Furthermore, we cannot account for any PCSO that exists in onion as γ-glutamyl peptides, and this pool is substantial in onion (Matikkala and Virtanen, 1967) and could provide a reservoir of PCSO. Finally, in view of the demonstrable reducing power in onion aqueous extracts (Freeman and Whenham, 1975, 1976a; Yagami et al., 1980), a limited conversion of 1-PECSO or any of its monomeric adducts to PCSO (or other *n*-propyl adducts) may account for some of the *n*-propyl fragments arising in the thiosulfinates.

## ABBREVIATIONS USED

ACSO, alk(en)yl-L-cysteine sulfoxide; PCSO, *n*-propylcysteine sulfoxide; 1-PECSO, 1-propenylcysteine sulfoxide; 2-PECSO, 2-propenylcysteine sulfoxide; MCSO, methylcysteine sulfoxide; OPA, *o*-phthaldialdehyde; FMOC, 9-fluorenylmethyl chloroformate; MCW, methanol/chloroform/water; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

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