Mechanisms of Formation of Volatile Sulfur Compounds following the Action of Cysteine Sulfoxide Lyases†

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Mechanisms for the formation of methanethiol, dimethyl disulfide, and dimethyl trisulfide in disrupted cabbage tissues were investigated. Dimethyl disulfide was produced in both air- and nitrogen-saturated disrupted cabbage tissues without significant differences ($p \leq 0.05$), which indicated that air oxidation of methanethiol is not the predominant mechanism for the formation of dimethyl disulfide. These results favored the mechanism in which the formation of dimethyl disulfide occurs from chemical disproportionation of methyl methanethiosulfinate. Methanethiol and dimethyl trisulfide were formed rapidly in model systems containing either methyl methanethiosulfinate or methyl methanethiosulfonate and hydrogen sulfide. This indicated that the reactions of the thiosulfinate and thiosulfonate compounds with hydrogen sulfide are prominent mechanisms for the formation of methanethiol and dimethyl trisulfide following the action of cysteine sulfoxide lyases. Methyl methanethiosulfinate and methyl methanethiosulfonates were found to possess characterizing sauerkraut aroma notes.

INTRODUCTION

Brassica (cabbage, Brussels sprouts, broccoli, and cauliflower) vegetables possess *S*-methyl-L-cysteine sulfoxide (1 in Scheme I) that occurs as a nonprotein amino acid (Maw, 1982). The characteristic flavors of these vegetables arise in part from the degradation of this amino acid following the action of cysteine sulfoxide lyases (C-S lyases). C-S lyases were first described in *Allium* plants (onion and garlic) by Stoll and Seebeck (1951) and later were demonstrated in *Brassica* vegetables by Mazelis (1963).

Dimethyl disulfide (4 in Scheme I) is a major volatile sulfur compound in the headspace of freshly disrupted tissues of cabbage (Bailey et al., 1961; Chin and Lindsay, 1993), broccoli (Forney et al., 1991; Hansen et al., 1992), garlic (Oaks et al., 1964) and onion (Boelens et al., 1971) and is considered a secondary product of the primary C-S lyases action on *S*-methyl-L-cysteine sulfoxide (Whitaker, 1976). The primary reaction product, presumably methanesulfenic acid (2 in Scheme I), has not been isolated because of its high reactivity (Penn et al., 1978). Stoll and Seebeck (1951) and Penn et al. (1978) proposed that the unstable sulfenic acid condenses and dehydrates to form the more stable methyl methanethiosulfinate (3 in Scheme I). The formation of dimethyl disulfide (4 in Scheme I) can thus be rationalized by the subsequent chemical disproportionation of the methyl methanethiosulfinate (3 in Scheme I; Ostermayer and Tarbell, 1960; Block and O'Connor, 1974). However, this scheme (Scheme I) does not account for the presence of methanethiol (CH_3SH) that is observed in the headspace of freshly disrupted tissues of cabbage (Chin and Lindsay, 1993), garlic (Oaks et al., 1964), and broccoli florets (Forney et al., 1991; Hansen et al., 1992).

In a survey of a large number of cabbage cultivars for the production of volatile sulfur compounds (Chin and Lindsay, 1993), methanethiol was observed to reach near maximum concentrations in the headspace of most cabbage samples soon after tissue disruption (<30 min at 30 °C), and then it slowly disappeared from the headspace. On the other hand, dimethyl disulfide was produced continuously in cabbage samples throughout the experimental period (100 min at 30 °C). Forney et al. (1991) also reported that methanethiol was one of the first compounds that appeared in the headspace of broccoli florets stored in sealed jars, whereas the level of dimethyl disulfide increased slowly in the headspace. Since methanethiol has been shown to readily undergo oxidation to dimethyl disulfide in the presence of air (Miller et al., 1973; Lindsay et al., 1986), an alternative mechanism can be proposed which involves atmospheric oxygen in the formation of dimethyl disulfide (Scheme II), and this mechanism also could accommodate the rapid formation of methanethiol and its gradual disappearance in the headspace of disrupted cabbage tissues.

Dimethyl trisulfide (8 in Scheme III) also occurs in the headspace of freshly disrupted tissues of cabbage (Bailey et al., 1961; Chin and Lindsay, 1993), broccoli (Hansen et al., 1992), and garlic (Oaks et al., 1964; Pino, 1992). Two mechanisms for the formation of trisulfides in *Brassica* and *Allium* plants following the action of C-S lyases have been proposed. Boelens et al. (1971) proposed that trisulfides might arise from the reaction of disulfides with elemental sulfur (Scheme III), whereas Maruyama (1970) speculated that the formation of dimethyl trisulfide might result from a reaction of the unstable methanesulfenic acid with hydrogen sulfide (Scheme IV). However, neither of these mechanisms has been tested for validity.

Marks et al. (1992) recently reported results of studies on the products of the C-S lyase system in *Brassica* vegetables, and they found methyl methanethiosulfinate (3 in Scheme I) in macerated Brussels sprouts after 24 h at pH 8.0. They also detected methyl methanethiosulfinate, methyl methanethiosulfonate (5 in Scheme I), and dimethyl trisulfide in a model system composed of *S*-methyl-L-cysteine sulfoxide and partially purified cabbage C-S lyase. However, they did not report the production of dimethyl disulfide from either the model system or the Brussels sprouts homogenates.

Some organosulfur compounds formed in disrupted tissues of *Brassica* vegetables possess anticarcinogenic properties (Miller and Stoewsand, 1983; Wattenberg, 1987;

† Research supported by the College of Agricultural and Life Sciences, University of Wisconsin—Madison.

0021-8561/94/1442-1529$04.50/0

Zhang et al., 1992; Bailey and Williams, 1993), and unpleasant volatile sulfur compounds diminish consumer acceptance of these foods. For example, Forney et al. (1991) reported that methanethiol production limits the use of modified-atmosphere storage technologies for extending the shelf life of broccoli. Information about the mechanisms of formation and regulation of production of undesirable volatile sulfur compounds in *Brassica* vegetables is needed. Therefore, the objective of this study was to further elucidate the mechanisms involved in the formation of methanethiol, dimethyl disulfide, and dimethyl trisulfide using disrupted cabbage tissues and selected model systems.

MATERIALS AND METHODS

Materials. Various cabbage (*Brassica oleracea* var. *capitata*) cultivars were provided by Dr. Paul H. Williams of the Department of Plant Pathology, University of Wisconsin—Madison. Cabbage heads were promptly cooled and stored at 4 °C after harvest and were used within 4 weeks. Quantification of each volatile sulfur compound produced by cabbage was carried out by preparing standard curves using authentic compounds (Chin and Lindsay, 1993). Methanethiol and dimethyl trisulfide were obtained from Eastman Fine Chemicals (Rochester, NY). Dimethyl disulfide was purchased from Aldrich Chemical Co. (Milwaukee, WI). Hydrogen sulfide (H_2S) was prepared by acidification of sodium sulfide (Whitten et al., 1988).

Volatile Sulfur Compounds Formed in Disrupted Cabbage Tissues under Aerobic and Anaerobic Conditions. For the study on the anaerobic formation of volatile sulfur compounds, cabbage samples were homogenized in specially prepared blender jars to fully initiate C-S lyase activities under anaerobic conditions. Glass blender jars (1 qt each; Waring Products, New Hartford, CT) equipped with stainless steel blades were prepared for use as follows. A one-piece Bakelite-type lid was used for each blender jar. Three holes of about 4-mm diameter each had previously been drilled in each lid. Tubing (Tygon, Norton Performance Plastics, Akron, OH) was inserted into each of two holes for use as the purging inlet and outlet ports. Silicone sealant (Dow Corning Co., Midland, MI) was applied to the contact surfaces of tubings and the lid. The tubing sections were closed with clamps whenever gas purging was not applied. The third hole was closed using silicone sealant and a disk (10-mm diameter) of silicone material (Alltech Associates, Deerfield, IL) so that it could be used as a sampling port for gastight syringes. The silicone material did not absorb volatile sulfur compounds (<5%) during 1 h of contact.

Cabbage samples (140 g each) were cut as wedges from two randomly selected heads. Samples were then coarsely chopped, and each was transferred to a Waring blender jar that contained 570 mL of distilled water. The distilled water had previously been boiled and cooled to remove dissolved gases. The blender jars were then closed with lids prepared as described earlier and sealed with silicone sealant. After the silicone sealant was cured (about 1 h), cabbage samples in the blender jars were subjected to nitrogen purging at room temperature for 1 h. Samples were then blended at the highest speed for 30 s and held at 30 °C in a water bath (Cambridge Instruments, Buffalo, NY).

Immediately after cabbage samples were placed in the water bath (time zero) and 60 min after holding, headspace gases (4 mL) were withdrawn from each sample with a 5-mL gastight syringe (Hamilton Co., Reno, NV). All samples were exposed to the same holding conditions. The headspace samples were then injected into a Varian (Palo Alto, CA) 3700 gas chromatograph (GC) equipped with a flame photometric detector (FPD) and a Varian 4200 integrator. A glass column of 6 ft × 2 mm i.d., packed with 40/60 Carbopack B HT 100 (Supelco Co., Bellefonte, PA), was used to separate the volatile sulfur compounds. GC conditions were described in Chin and Lindsay (1993).

For the study of volatile sulfur compounds formed in disrupted cabbage tissues under aerobic conditions, corresponding samples from the same cabbage heads used in anaerobic studies were employed. These aerobic samples were subjected to the same treatments as the anaerobic samples except blender jars were purged with purified air for 1 h before blending. The GC analysis of volatile sulfur compounds was the same as described earlier.

Factors Influencing Methanethiol Formation in Disrupted Cabbage Tissues. *S*-Methyl-L-cysteine (Aldrich), L-cysteine (Aldrich), and glutathione (Aldrich) were each added separately to cabbage samples to determine the effects of these compounds on methanethiol production in disrupted cabbage tissues. Untreated cabbage samples were used as control samples. One-hundred micromolar *S*-methyl-L-cysteine, L-cysteine, or glutathione was added to cabbage samples before blending (cabbage/distilled water = 1/4) in Waring blenders at the highest speed for 30 s. Each cabbage homogenate was transferred to a series of 140-mL flasks (100 g/flask) closed with silicone septa that did not absorb volatile sulfur compounds (<5%) in 1 h. Methanethiol production at various holding times (30 °C) was analyzed by the headspace GC method described by Chin and Lindsay (1993).

The relationship between initial ascorbic acid concentrations and methanethiol production in disrupted cabbage tissues was also investigated. Ten cabbage heads, each from a different cultivar, were analyzed for ascorbic acid and ability to produce methanethiol after tissue disruption. Ascorbic acid was determined by the 2,6-dichloroindophenol AOAC Method 43.051 (Horwitz et al., 1975), and determinations were carried out concurrently with analyses for methanethiol.

Model Systems for Determination of Mechanisms for the Formation of Dimethyl Trisulfide. Mechanisms for the formation of dimethyl trisulfide were studied in model systems prepared by placing various concentrations and combinations of suspected precursors (see Table III) in 140-mL flasks described earlier, and each flask contained 100 mL of 50 mM pyrophosphate buffer (pH 6.3). Formation of dimethyl trisulfide at various holding times at 30 °C was determined by headspace GC (Chin and Lindsay, 1993).

Methyl methanethiosulfinate (3 in Scheme I) was synthesized according to the method of Moore and O'Connor (1966). It was purified by vacuum distillation at 2 mmHg, and a fraction boiling at 65–72 °C was collected. Gas chromatographic analysis (Block and O'Connor, 1974) on a packed column containing 3% OV-101 on 100/120 Chromosorb W-HP (Alltech Associates) revealed that about 96% purity was achieved.

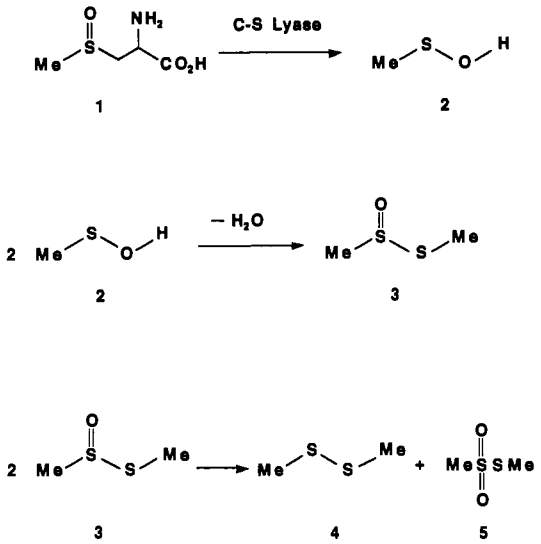
Methyl methanethiosulfonate (5 in Scheme I) was synthesized according to the method of Backer (1948). It was purified by vacuum distillation at 3 mmHg to collect a fraction boiling at 105 °C, and analysis by gas chromatography (Block and O'Connor, 1974) revealed about 97% purity was achieved for this compound.

RESULTS AND DISCUSSION

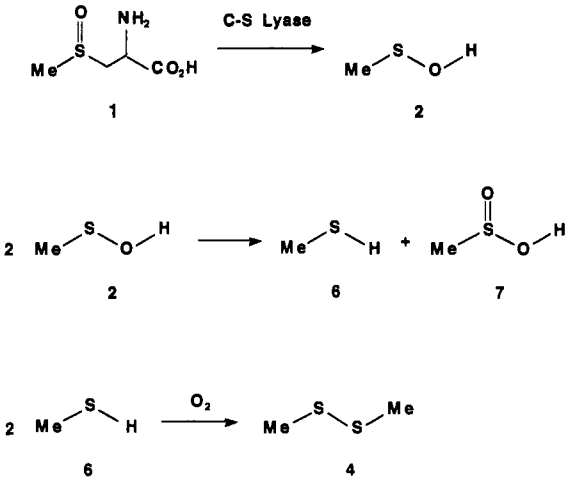
Importance of Air on the Formation of Volatile Sulfur Compounds in Cabbage Tissues. *Dimethyl Disulfide Formation.* The earlier proposed mechanism for the formation of dimethyl disulfide (Scheme I; Stoll and Seebeck, 1951; Ostermayer and Tarbell, 1960; Block and O'Connor, 1974; Penn et al., 1978) does not require oxygen, and the chemical reactions leading to dimethyl disulfide (4 in Scheme I) from methanesulfenic acid (2 in Scheme I) are dehydration and disproportionation. The alternative mechanism for the formation of dimethyl disulfide (Scheme II) requires oxygen for the oxidation of methanethiol (6 in Scheme II). To test the validity of both mechanisms, C-S lyase activity in cabbage was initiated and continued under both aerobic and anaerobic conditions. Headspace GC analyses of volatile sulfur compounds were conducted immediately after blending (time zero) as well as after 60 min of holding at 30 °C (Table I).

Dimethyl disulfide was not detected in either air- or nitrogen-saturated samples immediately after blending (time zero), but after 60 min at 30 °C, both treatments yielded dimethyl disulfide as a major volatile sulfur compound (Table I). The mean concentration of dimethyl disulfide in the anaerobic sample (0.38 ppm) was somewhat lower than that in the aerobic sample (0.52 ppm), but

Scheme I. Proposed Mechanism for the Formation of Dimethyl Disulfide Involving Condensation and Dehydration of Methanesulfenic Acid following the Action of C-S Lyase on *S*-Methyl-L-cysteine Sulfoxide (Stoll and Seebeck, 1951; Ostermayer and Tarbell, 1960; Block and O'Connor, 1974; Penn et al., 1978)



Scheme II. Proposed Mechanism for the Formation of Dimethyl Disulfide Involving Oxidation of Methanethiol following the Action of C-S Lyase on *S*-Methyl-L-cysteine Sulfoxide



statistical analysis (t-test, df = 1, one-sided) indicated that the samples were not significantly different ($p < 0.05$). These data appear to favor the mechanism (Scheme I) in which the formation of dimethyl disulfide occurs mainly from chemical disproportionation of methyl methanethiosulfinate (3 in Scheme I). However, the slightly lower concentration of methanethiol found in the air-saturated samples compared to the nitrogen-saturated samples (Table I) might indicate that some air oxidation of methanethiol to dimethyl disulfide had occurred.

Dimethyl Trisulfide Formation. Dimethyl trisulfide was not detected in the air-saturated samples (Table I), but the nitrogen-saturated samples yielded a mean concentration of 0.78 ppm of this compound after 60 min holding at 30 °C. Hansen et al. (1992) also reported that broccoli florets stored in the absence of oxygen produced higher amounts of dimethyl trisulfide than those stored in the presence of oxygen. The absence of dimethyl trisulfide in the air-saturated cabbage samples was probably not caused by the oxidation of dimethyl trisulfide to oxygen-bearing compounds analogous to sulfoxides or sulfones because dimethyl disulfide should also have been oxidized to oxygen-bearing compounds (Murray and Jindal, 1972) and removed from the headspace GC patterns. Thus, these results (Table I) provide evidence that the precursor(s) of dimethyl trisulfide in disrupted cabbage tissues is (are) labile under oxidative conditions.

Table I. Volatile Sulfur Compounds Formed in Disrupted Tissues of Cabbage under Aerobic and Anaerobic Conditions at 30 °C

		concn[a] (ppm)			
treatment	time (min)	hydrogen sulfide	methanethiol	dimethyl disulfide	dimethyl trisulfide
air	0	0.28 ± 0.23	0.01 ± 0.01	nd[b]	nd
	60	nd	0.20 ± 0.03	0.52 ± 0.19	nd
nitrogen	0	0.63 ± 0.35	0.02 ± 0.02	nd	nd
	60	0.51 ± 0.47	0.26 ● 0.08	0.38 ± 0.23	0.78 ● 0.13

[a] Values are means and standard deviations from two samples. [b] nd, not detected.

Table II. Effects of *S*-Methyl-L-cysteine, L-Cysteine, and Glutathione on the Formation of Methanethiol in Disrupted Tissues of Cabbage at 30 °C

treatment	ppb[a]		
(100 μM)	at 10 min	at 40 min	at 70 min
control	5.8 ± 8.2	35.5 ± 25.5	31.7 ± 23.4
S-MC[b]	5.8 ± 8.2	20.3 ± 14.5	35.7 ● 25.3
L-cysteine	10.2 ± 7.4	10.3 ● 14.6	29.3 ± 22.9
glutathione	13.0 ● 9.2	13.7 ± 9.7	16.7 ± 11.8

[a] Values are means and standard deviations from three samples. [b] S-MC, *S*-methyl-L-cysteine.

Hydrogen Sulfide Formation. Hydrogen sulfide was present in both air- and nitrogen-saturated cabbage samples immediately after blending (Table I). However, it was completely depleted in the air-saturated samples after 60 min of holding at 30 °C, whereas the nitrogen-saturated samples retained a relatively high level of this compound (Table I). Hydrogen sulfide has been reported to be unstable in the presence of oxygen (Budavari et al., 1989).

Methanethiol Formation in Disrupted Cabbage Tissues. *Role of S-Methyl-L-cysteine.* Arnold and Thompson (1962) showed that *S*-methyl-L-cysteine sulfoxide (1 in Scheme I) is formed from the oxidation of *S*-methyl-L-cysteine in tissues of Cruciferae, including whole leaves of broccoli and disks of turnip leaves. The action of C-S lyases on *S*-methyl-L-cysteine has been reported to yield methanethiol directly as a primary product (Nomura et al., 1963). Hall and Smith (1983) showed that *S*-methyl-L-cysteine is a substrate in vitro for a C-S lyase isolated from cabbage, and Hamamoto and Mazelis (1986) showed a similar in vitro activity for a C-S lyase isolated from broccoli. Thus, the level of free *S*-methyl-L-cysteine in cabbage could determine the concentration of methanethiol produced following tissue disruption and the action of C-S lyases. This hypothesis was tested by supplementing 100 μM (15 ppm) *S*-methyl-L-cysteine into cabbage samples before tissue disruption (Table II).

Methanethiol concentrations in the *S*-methyl-L-cysteine-supplemented samples of disrupted cabbage tissues were similar to those in control samples following holding for 10, 40, and 70 min at 30 °C (Table II). Statistical analysis (t-test, df = 2) indicated that the differences in methanethiol concentrations all were not significant at $p < 0.05$. Therefore, these data indicate that *S*-methyl-L-cysteine does not appear to contribute to the formation of methanethiol in freshly disrupted cabbage tissues.

Role of Sulfhydryl Reducing Agents. Since in cabbage the action of C-S lyases on *S*-methyl-L-cysteine sulfoxide

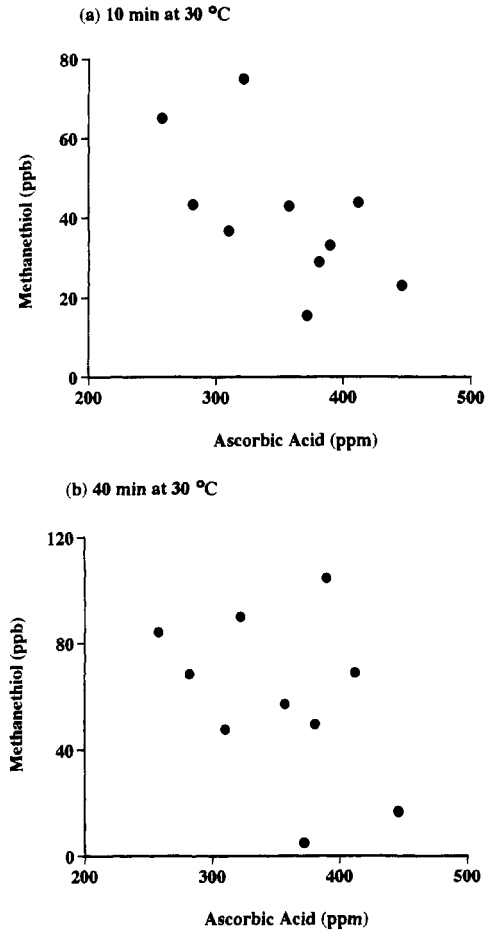


Figure 1. Relationship of initial ascorbic acid concentrations to methanethiol production in 10 cabbage cultivars (a) 10 min after blending and holding at 30 °C and (b) 40 min after blending and holding at 30 °C.

after tissue disruption leads to the formation of dimethyl disulfide (Scheme I), methanethiol might be formed by the reduction of dimethyl disulfide in the presence of reducing agents. Therefore, 100 μM L-cysteine or glutathione (reduced form) was added to cabbage samples before blending to determine whether this mechanism was operative. Both treatments gave somewhat lower methanethiol concentrations at 40 and 70 min (30 °C) compared to control samples (Table II), but none of the differences were significant at $p < 0.05$. Thus, these results indicate that the reduction of dimethyl disulfide by L-cysteine and glutathione does not occur in freshly disrupted cabbage tissues.

Role of Ascorbic Acid. The relationship between initial ascorbic acid concentrations and methanethiol production in disrupted cabbage tissues was investigated by determining ascorbic acid concentrations in cabbage samples and correlating the ascorbic acid contents with methanethiol concentrations (Figure 1). Data in Figure 1, parts a and b, show the relationships following holding for 10 and 40 min at 30 °C, respectively. Pearson correlation coefficients of −0.617 and −0.388 were obtained for 10 and 40 min, respectively. Only a weakly statistically significant negative correlation ($p < 0.10$) was found between ascorbic acid and methanethiol after 10 min. In this case, lower concentrations of methanethiol were produced in the presence of higher initial concentrations of ascorbic acid. Foyer and Halliwell (1976) demonstrated that glutathione can nonenzymatically reduce dehydroascorbic acid and then suggested that sulfhydryl-containing compounds might be involved in ascorbic acid retention mechanisms. Thus, the negative correlation (Figure 1a) between methanethiol concentrations at 10 min and the initial ascorbic acid contents might have resulted from the oxidation of methanethiol and regeneration of ascorbic acid from dehydroascorbic acid. On the other hand, other researchers (Kanner et al., 1977; Mahoney and Graf, 1986) have demonstrated that ascorbic acid in the presence of transition metals may act as a prooxidant. Such a system might also have been responsible for the diminished concentrations of methanethiol observed at 10 min (30 °C) with higher levels of initial ascorbic acid.

Role of Methyl Methanethiosulfinate, Methyl Methanethiosulfonate, and Hydrogen Sulfide. In detailed studies on the mechanisms of formation of dimethyl trisulfide, we found that when 1 ppm of hydrogen sulfide was added to a buffered solution (pH 6.3) of either 1 ppm of methyl methanethiosulfinate (3 in Scheme I) or 1 ppm of methyl methanethiosulfonate (5 in Scheme I), trace amounts of methanethiol were detected in the headspace of the solution immediately after the reactants were combined. After 20 min at 30 °C, 12 and 15 ppb of methanethiol were found in the samples of methyl methanethiosulfinate and methyl methanethiosulfonate, respectively. These concentrations were similar to those detected in the headspace of freshly disrupted cabbage tissues which had been held at 30 °C for 10 min (Chin and Lindsay, 1993). Because hydrogen sulfide has been identified as a volatile sulfur component in freshly disrupted cabbage tissues (Bailey et al., 1961; Chin and Lindsay, 1993) and broccoli florets (Forney et al., 1991), we therefore have concluded that the reaction of hydrogen sulfide with methyl methanethiosulfinate or methyl methanethiosulfonate is a major pathway leading to the formation of methanethiol in freshly disrupted cabbage tissues (Scheme V). Because methanesulfenic acid is unavailable for use in model systems owing to its instability, the contribution of Scheme II to the formation of methanethiol in cabbage cannot be determined. Thus, that pathway might also be a significant contributor of methanethiol to freshly disrupted cabbage tissues.

Furthermore, the lower methanethiol production observed when either L-cysteine or glutathione was added to cabbage samples (Table II) was probably caused by diminished concentrations of methyl methanethiosulfinate and methyl methanethiosulfonate that resulted from the presence of cysteine or glutathione. Indeed, Small et al. (1947, 1949) and Ostermayer and Tarbell (1960) have shown that cysteine reacts with the thiosulfinate and thiosulfonate compounds to form mixed disulfides.

Mechanisms for the Formation of Dimethyl Trisulfide. *Role of Dimethyl Disulfide and Elemental Sulfur.* Chin and Lindsay (1993) observed that dimethyl disulfide and dimethyl trisulfide shared similar production patterns in disrupted cabbage tissues and suggested that these two compounds might have a common precursor. Earlier, however, Boelens et al. (1971) had proposed that disulfides might serve as a precursor for trisulfides (Scheme III), but presented no experimental evidence to support the hypothesis. Therefore, an experiment was carried out (entry 1, Table III) to test the validity of Scheme III for cabbage by placing 1 ppm of dimethyl disulfide and 1 ppm of elemental sulfur in a series of 100-mL (pH 6.3) 50 mM pyrophosphate buffers and holding these solutions at 30 °C. The sulfur powder (sublimed) either was directly dispersed in the aqueous buffer solutions or was dissolved in olive oil first and then was added to the buffer solutions. In both cases, headspace GC analyses at various holding times showed no formation of dimethyl trisulfide even

Scheme III. Proposed Mechanism for the Formation of Dimethyl Trisulfide Involving Elemental Sulfur and Dimethyl Disulfide following the Action of C-S Lyase on *S*-Methyl-L-cysteine Sulfoxide (Boelens et al., 1971)

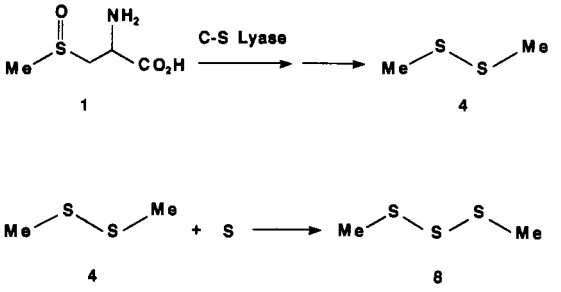


Table III. Formation of Dimethyl Trisulfide in Model Solutions for the C-S Lyase-Catalyzed Degradation of *S*-Methyl-L-cysteine Sulfoxide in Cabbage

	reaction mixture		ppm[a]					
entry	precursor	concn (ppm)	at 0 min	at 20 min	at 60 min	at 240 min	at 600 min	at 1200 min
1	DMDS[b]	1	nd[c]	nd	nd	nd	nd	nd
	sulfur	1						
2	CH_3SH	1	nd	nd	nd	nd	0.13	0.15
	H_2O_2	10						
	H_2S	1						
3	MTSI[d]	1	0.05	0.24	–[e]	–	–	–
	H_2S	1						
4	MTSO[f]	1	0.66	0.62	–	–	–	–
	H_2S	1						

[a] Values are averages of duplicate determinations. [b] DMDS, dimethyl disulfide. [c] nd, not detected. [d] MTSI, methyl methanethiosulfinate. [e] –, not determined. [f] MTSO, methyl methanethiosulfonate.

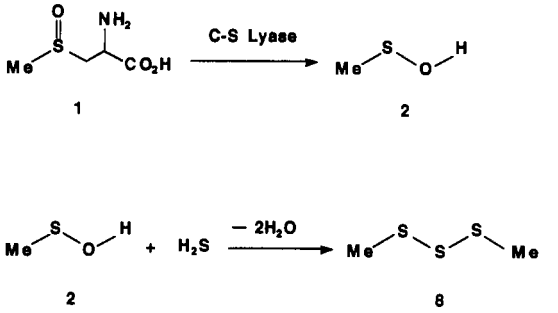
after 20 h (1200 min) of holding at 30 °C (entry 1, Table III). Therefore, Scheme III can be ruled out as a major pathway leading to dimethyl trisulfide production in freshly disrupted cabbage tissues.

However, the pathway (Scheme III) may have significance in processed cruciferous foods. Westlake et al. (1950) and Carson and Wong (1959) have reported the synthesis of alk(en)yl trisulfides by reacting disulfides with elemental sulfur in the presence of amines at elevated temperatures. Ammonia results as a product of the action of C-S lyases on *S*-alk(en)yl-L-cysteine sulfoxides (Stoll and Seebeck, 1951; Mazelis, 1963) and it also results from acid- or base-catalyzed hydrolysis of these amino acids at elevated temperatures (Ostermayer and Tarbell, 1960). Therefore, the reaction of dimethyl disulfide with elemental sulfur in the presence of amines at elevated temperatures could contribute appreciably to the production of dimethyl trisulfide in cooked *Brassica* vegetables.

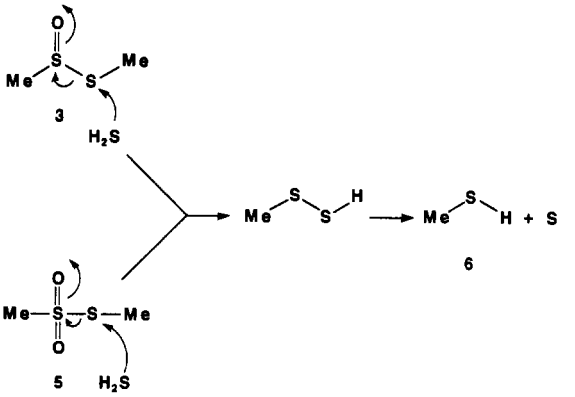
Role of Methanesulfenic Acid and Hydrogen Sulfide. The mechanism (Scheme IV) proposed by Maruyama (1970) is difficult to prove because the reaction leading to the formation of dimethyl trisulfide involves elusive methanesulfenic acid (2 in Scheme IV). Except for a few unusual sulfenic acids (Pal et al., 1969; Chou et al., 1974), the simple alkanesulfenic acids have not been isolated in pure forms. Furthermore, although simple alkanesulfenic acids can be generated by thermolysis or pyrolysis of appropriate sulfoxides (Shelton and Davis, 1973; Penn et al., 1978), such high-temperature generation of sulfenic acids is not appropriate for the study of C-S lyase-catalyzed generation of sulfenic acids under mild conditions.

However, several researchers (Barton et al., 1973; Gilbert et al., 1975; Armstrong and Buchanan, 1978) have

Scheme IV. Proposed Mechanism for the Formation of Dimethyl Trisulfide Involving Methanesulfenic Acid and Hydrogen Sulfide following the Action of C-S Lyase on *S*-Methyl-L-cysteine Sulfoxide (Maruyama, 1970)



Scheme V. Most Probable Mechanism for the Formation of Methanethiol from the Reactions of Methyl Methanethiosulfinate and Methyl Methanethiosulfonate with Hydrogen Sulfide following the Action of C-S Lyase on *S*-Methyl-L-cysteine Sulfoxide



indicated that sulfenic acids can be formed as transient intermediates from the reactions of thiols with hydrogen peroxide at room temperatures. Therefore, we employed a mixture of methanethiol (1 ppm) and hydrogen peroxide (10 ppm) as the source for methanesulfenic acid (CH_3SOH) and added to this mixture 1 ppm of hydrogen sulfide (entry 2, Table III) to test the mechanism (Scheme IV) proposed by Maruyama (1970). Methanesulfenic acid, if produced from methanethiol and hydrogen peroxide, would be trapped by hydrogen sulfide to produce dimethyl trisulfide according to Scheme IV.

Headspace GC analysis showed that up to 0.15 ppm (15% yield) of dimethyl trisulfide was slowly produced in this reaction mixture (entry 2, Table III). However, the time required for dimethyl trisulfide to appear in the headspace of this mixture was about 8 h. Chin and Lindsay (1993) showed that dimethyl trisulfide was one of the major volatile sulfur compounds in the headspace of freshly disrupted cabbage tissues after only 100 min holding at 30 °C. Similarly, Marks et al. (1992) reported rapid production of dimethyl trisulfide in a model system composed of *S*-methyl-L-cysteine sulfoxide and partially purified cabbage C-S lyase. Thus, the results of Table III (entry 2) do not strongly support the mechanism for the formation of dimethyl trisulfide (Scheme IV) proposed by Maruyama (1970).

On the other hand, Barton et al. (1973) have found that the reaction rates for the oxidation of low molecular weight sulfhydryl compounds by hydrogen peroxide increased as the pH was elevated and proposed a mechanism for the formation of sulfenic acids which involved a nucleophilic attack of the thiolate ion (RS^-) on the oxygen of the peroxide. Armstrong and Buchanan (1978) have noted

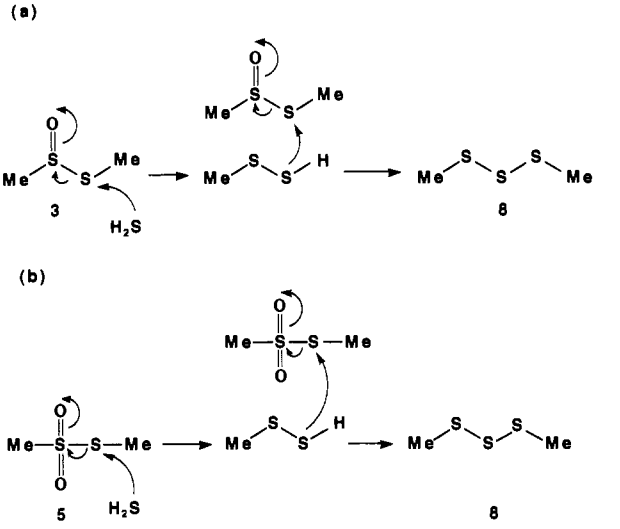
that the effective second-order rate constants for the oxidation of sulfhydryl compounds by hydrogen peroxide at neutral pH were about 0.1–1 M^{-1} s^{-1}, and thus the rates of oxidation were relatively low. The pH of the model solutions employed in the present study was adjusted to 6.3, which is similar to that of the cabbage homogenate. Thus, the slow appearance of dimethyl trisulfide in the model solutions (entry 2, Table III) was probably caused by the slow formation of methanesulfenic acid at near neutrality, which would be the rate-limiting step, from the reaction of methanethiol with hydrogen peroxide. Therefore, participation of methanesulfenic acid in the formation of dimethyl trisulfide (Scheme IV) in disrupted cabbage tissues cannot be completely ruled out.

Role of Methyl Methanethiosulfinate, Methyl Methanethiosulfonate, and Hydrogen Sulfide. Brodnitz et al. (1971) reported that a sample of synthetic allyl thiosulfinate (allicin) held at 20 °C for 20 h decomposed to a variety of volatile sulfur compounds including diallyl trisulfide. We carried out a similar experiment by dissolving 1.5 ppm of methyl methanethiosulfinate (3 in Scheme I) in a pH 6.3 buffer solution and found that methyl methanethiosulfinate was completely depleted after 20 h at 30 °C. Dimethyl sulfide (23%), dimethyl disulfide (44%), and dimethyl trisulfide (9%) were the major decomposition products of methyl methanethiosulfinate in the buffer solution. This result suggested that spontaneous decomposition of methyl methanethiosulfinate, albeit slow at mild temperatures, might contribute to the production of dimethyl trisulfide in disrupted cabbage tissues. On the other hand, because dimethyl trisulfide develops quite rapidly in freshly disrupted cabbage tissues (Chin and Lindsay, 1993) and C-S lyase model systems (Marks et al., 1992), alternate pathways are probably responsible for the production of dimethyl trisulfide in these cases.

When 1 ppm each of methyl methanethiosulfinate (3 in Scheme I) and hydrogen sulfide was mixed into a pH 6.3 buffer solution, dimethyl trisulfide was produced immediately after mixing (entry 3, Table III), and after 20 min at 30 °C, a 24% yield was obtained. Furthermore, when 1 ppm of hydrogen sulfide was added to a buffered solution of 1 ppm of methyl methanethiosulfonate (5 in Scheme I), even more dimethyl trisulfide was rapidly produced (entry 4, Table III). On the basis of these findings we have proposed mechanisms shown in Scheme VI for the formation of dimethyl trisulfide involving methyl methanethiosulfinate and methyl methanethiosulfonate. The slower reaction of methyl methanethiosulfinate with hydrogen sulfide compared to methyl methanethiosulfonate was probably the result of the presence of two competing sites that are subject to attack by nucleophiles (Kice and Liu, 1979). These are the sulfinyl sulfur (>S=O) and the sulfenyl sulfur (–S–) sites in methyl methanethiosulfinate. Nucleophilic attack occurs only at the sulfenyl sulfur for the thiosulfonate compounds (Kice et al., 1974), and only nucleophilic attack at the sulfenyl sulfur can lead to the formation of dimethyl trisulfide (Scheme VI). Kice et al. (1974) and Kice and Liu (1979) also have reported that the reactivity of various nucleophiles toward thiosulfonates is higher than that for thiosulfinates. Furthermore, Wijers et al. (1969) reported that RSO_2^- (from thiosulfonates) is a good leaving group, and this property has been utilized for the synthesis of a variety of mixed disulfides.

In studies by Marks et al. (1992), dimethyl trisulfide was reported to be produced from the action of C-S lyase upon *S*-methyl-L-cysteine sulfoxide, and dimethyl disulfide

Scheme VI. Proposed Mechanisms for the Formation of Dimethyl Trisulfide from the Reactions of (a) Methyl Methanethiosulfinate with Hydrogen Sulfide and (b) Methyl Methanethiosulfonate with Hydrogen Sulfide following the Action of C-S Lyase on *S*-Methyl-L-cysteine Sulfoxide



which has been widely found in other studies (Bailey et al., 1961; Oaks et al., 1964; Boelens et al., 1971; Forney et al., 1991; Hansen et al., 1992; Chin and Lindsay, 1993) was not reported. Marks et al. (1992) did not comment on the final steps of the mechanism of formation of dimethyl trisulfide, and they did not comment about the absence of dimethyl disulfide in the C-S lyase model systems. Hydrogen sulfide did not appear to have been present in their model systems, and therefore, another mechanism would be required to explain the results for the model enzymic system.

However, on the basis of our findings that hydrogen sulfide readily reacts with methyl methanethiosulfinate and methyl methanethiosulfonate, we have concluded that this mechanism (Scheme VI) is a major pathway leading to the formation of dimethyl trisulfide in freshly disrupted *Brassica* vegetables.

Aroma Properties of Methyl Methanethiosulfinate and Methyl Methanethiosulfonate. Following the synthesis of methyl methanethiosulfinate and methyl methanethiosulfonate, an aroma highly suggestive of sauerkraut was apparent in the laboratory. Aroma evaluations of dilutions of the two compounds (Stone and Sidel, 1985) revealed an estimated detection threshold of 550 ppb for methyl methanethiosulfinate and 5 ppm for methyl methanethiosulfonate. Descriptive sensory analysis (Stone and Sidel, 1985) using a sourness scale and a similarity to sauerkraut aroma scale revealed that the aroma of methyl methanethiosulfonate was more sour and typical of sauerkraut than methyl methanethiosulfinate, but the latter compound also possessed the same type of characterizing sauerkraut aroma notes. These observations suggest that although methanethiol, dimethyl disulfide, and dimethyl trisulfide contribute to sauerkraut flavor and aromas (Lee et al., 1974; Dahlson, 1986), some of the characteristic aroma of sauerkraut is provided by the thiosulfinate and thiosulfonate compounds. The concentrations of these compounds in sauerkraut should reflect the extent of C-S lyase activity (Scheme I) that occurs as a result of decompartmentalization of the enzyme and substrate during the cutting of cabbage and the early stages of fermentation.

Conclusions. Schemes I, V, and VI depict that methanethiol, dimethyl disulfide, and dimethyl trisulfide

all are derived from the common precursor methyl methanethiosulfinate. Since methyl methanethiosulfinate is a product of the C-S lyase-catalyzed degradation of *S*-methyl-L-cysteine sulfoxide, it follows that selection of plant cultivars that are low in *S*-methyl-L-cysteine sulfoxide or C-S lyase activity should afford a means to provide *Brassica* vegetables with less malodorous volatile sulfur compounds, which should enhance consumer acceptance of these vegetable foods.

Additionally, certain chemical modifications of the thiosulfinate compound should also aid in lowering concentrations of malodorous volatile sulfur compounds. In this regard, Fujiwara et al. (1954b, 1955) have shown that thiaminthiol can react with allicin (allyl thiosulfinate) to form allithiamine through nucleophilic substitution, and allithiamine retains the vitamin B_1 activity (Fujiwara et al., 1954a). Therefore, novel means for controlling objectionable volatile sulfur compounds in *Brassia* vegetable foods should result from an understanding of their mechanisms of formation, and additional research should be directed toward this goal.

ACKNOWLEDGMENT

We thank Dr. Paul H. Williams for cabbage samples.

LITERATURE CITED

Armstrong, D. A.; Buchanan, J. D. Reactions of superoxide anion radical, hydrogen peroxide, and other oxidants with sulfhydryl enzymes. *Photochem. Photobiol.* **1978**, *28*, 743–755.

Arnold, W. N.; Thompson, J. F. The formation of (+) *S*-methyl-L-cysteine sulfoxide from *S*-methyl-L-cysteine in crucifers. *Biochim. Biophys. Acta* **1962**, *57*, 604–606.

Backer, H. J. Properties of the sulfonyl group. III. Oxidation of tetramethyl tetrathioorthocarbonate. *Recl. Trav. Chim. Pays-Bas* **1948**, *67*, 894–906.

Bailey, G. S.; Williams, D. E. Potential mechanisms for food-related carcinogens and anticarcinogens. *Food Technol.* **1993**, *47* (2), 105–118.

Bailey, S. D.; Bazinet, M. L.; Driscoll, J. L.; McCarthy, A. I. The volatile sulfur components of cabbage. *J. Food Sci.* **1961**, *26*, 163–170.

Barton, J. P.; Packer, J. E.; Sims, R. J. Kinetics of the reaction of hydrogen peroxide with cysteine and cysteamine. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1547–1549.

Block, E.; O'Connor, J. The Chemistry of alkyl thiosulfinate esters. VII. Preparation and spectral studies. *J. Am. Chem. Soc.* **1974**, *96*, 3929–3944.

Boelens, M.; de Valois, P. J.; Wobben, H. J.; van der Gen, A. Volatile flavor compounds from onion. *J. Agric. Food Chem.* **1971**, *19*, 984–991.

Brodnitz, M. H.; Pascale, J. V.; Van Derslice, L. Flavor components of garlic extract. *J. Agric. Food Chem.* **1971**, *19*, 273–275.

Budavari, S., O'Neil, M. J., Smith, A., Heckelman, P. E., Eds. *The Merck Index*, 11th ed.; Merck: Rahway, NJ, 1989; p 4732.

Carson, J. F.; Wong, F. F. Separation of aliphatic disulfides and trisulfides by gas-liquid partition chromatography. *J. Org. Chem.* **1959**, *24*, 175–179.

Chin, H.-W.; Lindsay, R. C. Volatile sulfur compounds formed in disrupted tissues of different cabbage cultivars. *J. Food Sci.* **1993**, *58*, 835–839.

Chou, T. S.; Burgtorf, J. R.; Ellis, A. L.; Lammert, S. R.; Kukolja, S. P. Azetidinone sulfenic acid. Isolation of crystalline sulfenic acids from penicillin sulfoxides and a study of their reactivities. *J. Am. Chem. Soc.* **1974**, *96*, 1609–1610.

Dahlson, A. Increased utilization of sauerkraut and cabbage through technological development. M.S. Thesis, University of Wisconsin—Madison, Dec 1986.

Forney, C. F.; Matteis, J. P.; Austin, R. K. Volatile compounds produced by broccoli under anaerobic conditions. *J. Agric. Food Chem.* **1991**, *39*, 2257–2259.

Foyer, C. H.; Halliwell, B. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* **1976**, *33*, 21–25.

Fujiwara, M.; Nanjo, H.; Arai, T.; Suzuoki-Ziro. "Allithiamine", a newly found derivative of vitamin B_1. II. The effect of allithiamine on living organism. *J. Biochem. (Tokyo)* **1954a**, *41*, 273–285.

Fujiwara, M.; Watanabe, H.; Matsui, K. "Allithiamine", a newly found derivative of vitamin B_1. I. Discovery of allithiamine. *J. Biochem. (Tokyo)* **1954b**, *41*, 29–39.

Fujiwara, M.; Yoshimura, M.; Tsuno, S. "Allithiamine", a newly found derivative of vitamin B_1. III. On the allicin homoglogues in the plants of the *Allium* species. *J. Biochem. (Tokyo)* **1955**, *42*, 591–601.

Gilbert, B. A.; Laue, H. A. H.; Norman, R. O. C.; Sealy, R. C. Electron spin resonance studies. XLVI. Oxidation of thiols and disulfides in aqueous solution. Formation of thiyl, sulfinyl, and sulfonyl radicals, disulfide radical anions, and carbon radicals. *J. Chem. Soc., Perkin Trans. 2* **1975**, 892–900.

Hall, D.; Smith, I. K. Partial purification and characterization of cystine lyase from cabbage (*Brassica oleracea* var *capitata*). *Plant Physiol.* **1983**, *72*, 654–658.

Hamamoto, A.; Mazelis, M. The C-S lyases of higher plants. Isolation and properties of homogeneous cystine lyase from broccoli (*Brassica oleracea* var *botrytis*) buds. *Plant Physiol.* **1986**, *80*, 702–706.

Hansen, M.; Buttery, R. G.; Stern, D. J.; Cantwell, M. I.; Ling, L. C. Broccoli storage under low-oxygen atmosphere: identification of higher boiling volatiles. *J. Agric. Food Chem.* **1992**, *40*, 850–852.

Horwitz, W., Senzel, A., Reynolds, H., Park, D. L., Eds. *Official Methods of Analysis*, 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1975; pp 829–830.

Kanner, J.; Mendel, H.; Budowski, P. Prooxidant and antioxidant effects of ascorbic acid and metal salts in a β-carotene-linoleate model system. *J. Food Sci.* **1977**, *42*, 60–64.

Kice, J. L.; Liu, C. A. Reactivity of nucleophiles toward and the site of nucleophilic attack on phenyl benzenethiolsulfinate. *J. Org. Chem.* **1979**, *44*, 1918–1923.

Kice, J. L.; Rogers, T. E.; Warheit, A. C. The relative nucleophilicity of common nucleophiles toward sulfenyl sulfur. Comparison of the relative reactivity of different nucleophiles toward sulfenyl vs. sulfonyl sulfur. *J. Am. Chem. Soc.* **1974**, *96*, 8020–8026.

Lee, C. Y.; Acree, T. E.; Butts, R. M.; Stamer, J. R. Flavor constituents of fermented cabbage. *Proc. Int. Congr. Food Sci. Technol., 4th* **1974**, *1*, 175–178.

Lindsay, R. C.; Josephson, D. B.; Olafsdottir, G. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In *Seafood Quality Determination*; Kramer, D. E., Liston, J., Eds.; Elsevier Science Publishers: Amsterdam, 1986.

Mahoney, J. R.; Graf, E. Role of alpha-tocopherol, ascorbic acid, citric acid, and EDTA as oxidants in model systems. *J. Food Sci.* **1986**, *51*, 1293–1296.

Marks, H. S.; Hilson, J. A.; Leichtweis, H. C.; Stoewsand, G. S. *S*-Methylcysteine sulfoxide in *Brassica* vegetables and formation of methyl methanethiosulfinate from Brussels sprouts. *J. Agric. Food Chem.* **1992**, 2098–2101.

Maruyama, F. T. Identification of dimethyl trisulfide as a major aroma component of cooked brassicaceous vegetables. *J. Food Sci.* **1970**, *35*, 540–543.

Maw, G. A. Biochemistry of *S*-methyl-L-cysteine and its principle derivatives. *Sulfur Rep.* **1982**, *2*, 1–32.

Mazelis, M. Demonstration and characterization of cysteine sulfoxide lyase in the *Cruciferae*. *Phytochemistry* **1963**, *2*, 15–22.

Miller, A.; Scanlan, R. A.; Lee, J. S.; Libbey, L. M.; Morgan, M. E. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas perolens*. *Appl. Microbiol.* **1973**, *25*, 257–261.

Miller, K. W.; Stoewsand, G. S. Dietary Brussels sprouts and glucosinolate influence on rat hepatic polysubstrate monoxygenases. *J. Plant Foods* **1983**, 67–74.

Moore, T. L.; O'Connor D. E. The reaction of methanesulfenyl chloride with alkoxide and alcohols. Preparation of aliphatic sulfenate and sulfinate esters. *J. Org. Chem.* **1966**, *31*, 3587–3592.

Murray, R. W.; Jindal, S. L. Photosensitized oxidation of dialkyl disulfides. *J. Org. Chem.* **1972**, *37*, 3516–3520.

Nomura, J.; Nishizuka, Y.; Hayaishi, O. S-alkylcysteinase: enzymatic cleavage of *S*-methyl-L-cysteine and its sulfoxide. *J. Biol. Chem.* **1963**, *238*, 1441–1446.

Oaks, D. M.; Hartmann, H.; Dimick, K. P. Analysis of sulfur compounds with electron capture/hydrogen flame dual channel gas chromatography. *Anal. Chem.* **1964**, *36*, 1560–1565.

Ostermayer, F.; Tarbell, D. S. Products of acidic hydrolysis of *S*-methyl-L-cysteine sulfoxide; the isolation of methyl methanethiolsulfonate, and mechanism of the hydrolysis. *J. Am. Chem. Soc.* **1960**, *82*, 3752–3755.

Pal, B. C.; Uziel, M.; Doherty, D. G.; Cohn, W. E. Isolation and characterization of a pyrimidine sulfenic acid via scission of the sulfur-sulfur bond in the methyl analog of bis(4-thiouridine) disulfide. *J. Am. Chem. Soc.* **1969**, *91*, 3634–3638.

Penn, R. E.; Block, E.; Revelle, L. K. Methanesulfenic acid. *J. Am. Chem. Soc.* **1978**, *100*, 3622–3623.

Pino, J. A. Headspace sampling methods for the volatile components of garlic (*Allium sativum*). *J. Sci. Food Agric.* **1992**, *59*, 131–133.

Shelton, J. R.; Davis, K. E. Decomposition of sulfoxides. II. Formation of sulfenic acids. *Int. J. Sulfur Chem.* **1973**, *3*, 205–216.

Small, L. D.; Bailey, J. H.; Cavallito, C. J. Allyl thiosulfinates. *J. Am. Chem. Soc.* **1947**, *69*, 1710–1713.

Small, L. D.; Bailey, J. H.; Cavallito, C. J. Comparison of some properties of thiolsulfonates and thiosulfinates. *J. Am. Chem. Soc.* **1949**, *71*, 3565–3566.

Stoll, A.; Seebeck, E. Chemical investigations on alliin, the specific principle of garlic. *Adv. Enzymol.* **1951**, *11*, 377–400.

Stone, H.; Sidel, J. *Sensory Evaluation Practices*; Academic Press: New York, 1985.

Wattenberg, L. W. Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine or benzo[*a*]pyrene on pulmonary and forestomach neoplasia in A/J mice. *Carcinogenesis* **1987**, *8*, 1971–1973.

Westlake, H. E., Laquer, H. L.; Smyth, C. P. The dipole moments and the interconvertibility of diethyl disulfide and trisulfide. *J. Am. Chem. Soc.* **1950**, *72*, 436–438.

Whitaker, J. R. Development of flavor, odor, and pungency in onion and garlic. *Adv. Food Res.* **1976**, *22*, 73–133.

Whitten, K. W., Gailey, K. D., Bishop, C. B., Bishop, M. B., Eds. *Experiments in General Chemistry*, 1st ed.; Saunders College Publishing: Fort Worth, TX, 1988.

Wijers, H. E.; Boelens, H.; van der Gen, A. Synthesis and some properties of 1-alkenyl alkyl disulfides and di(1-alkenyl) disulfides. *Recl. Trav. Chim. Pays-Bas* **1969**, *88*, 519–529.

Zhang, Y.; Talalay, P.; Cho, C.-G.; Posner, G. H. A major inducer of anticarcinogenic protective enzymes from broccoli: Isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399–2403.

Received for review September 16, 1993. Accepted September 27, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, November 15, 1993.