S-Methylcysteine Sulfoxide in *Brassica* Vegetables and Formation of Methyl Methanethiosulfinate from Brussels Sprouts

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The naturally occurring sulfur compound S-methylcysteine sulfoxide (SMCSO) was determined for five common *Brassica* vegetables, broccoflower, broccoli, Brussels sprouts, cabbage, and cauliflower. This natural chemical has a role in the aroma and flavor of these vegetables and, perhaps more importantly, appears to be involved with the inhibition of experimental carcinogenesis. Brussels sprouts contained the highest level of SMCSO. This vegetable was then used to identify the cystine lyase-mediated enzymatic conversion products including methyl methanethiosulfinate (MMTSO). After it was demonstrated that MMTSO, dimethyl trisulfide, and pyruvate can be formed facilely in a simplified enzymatic model system, generation of MMTSO was confirmed in a water extract of macerated Brussels sprouts. Formation of MMTSO was shown to be strongly dependent on pH of the vegetable juice. After 24 h, only trace amounts of MMTSO were observed at the developed acidic pH of Brussels sprouts, while considerable amounts were formed at an adjusted basic pH. This is the first evidence that MMTSO is enzymatically derived from SMCSO under natural conditions.

INTRODUCTION

Consumption of Brassiceae vegetables such as cabbage, Brussels sprouts, broccoli, and cauliflower has been associated with a decreased incidence of human cancers (Graham et al., 1978). In addition, dietary incorporation of *Brassica* vegetables has been observed to inhibit experimental carcinogenesis in laboratory animals (Boyd et al., 1982; Wattenberg, 1983). Numerous investigations have focused on various organosulfur compounds found in these vegetables possessing these effects. For example, isothiocyanates and their metabolites as well as alkylic polysulfides have all been shown to have some carcinogenesis inhibition activity (Miller and Stoewsand, 1983; Wargovich and Goldberg, 1985). The biological activity of these individual components, though, has not been able to explain the full carcinogenesis inhibitory properties of these vegetables (Miller, 1982; Godlewski et al., 1985). One class of *Brassica* organosulfur compounds recently investigated (Marks, 1992) for their ability to inhibit carcinogenesis is the non-protein α amino acid (+)-S-methyl-L-cysteine sulfoxide (SMCSO) and its enzymatic byproduct, methyl methanethiosulfinate (MMTSO).

SMCSO was discovered to occur naturally in *Brassica* in 1956 by two independent research groups (Synge and Wood, 1956; Morris and Thompson, 1956) and is found throughout the *Brassica* genera with concentrations ranging between 1 and 2% on a dry weight basis (Mae et al., 1971; Whittle et al., 1976; Maw, 1982).

In 1963, Mazelis initially characterized an enzyme present in *Brassica* that is released upon disruption of plant tissue and catabolizes S-alkylcysteine sulfoxides, including SMCSO, into pyruvate, ammonia, and suspected alkyl thiosulfinates (Figure 1). This enzyme, subsequently termed cystine lyase (EC 4.4.1.8), was found to have a behavior similar to that of an enzyme present in garlic, alliinase (EC 4.4.1.4) (Stoll and Seebeck, 1951), except that it also has the ability to cleave L-cystine (Hamamoto and Mazelis, 1986).

Neither alkyl thiosulfinates (from *Brassica*) nor allyl thiosulfinates (from *Allium*, i.e., garlic or onion) have been isolated in intact plant tissue; however, evidence for their occurrence is strong (Block, 1985; Fenwick and Hanley, 1985). Recently, Kuo and Ho (1992) have identified two novel sulfur-containing aldehydes in Welsh onions and scallions that contain the thiosulfinate (S(O)S) moiety. In addition, it was hypothesized that alk(en)yl thiosulfinates eventually decompose into their corresponding mercaptans, sulfides, disulfides, trisulfides, and other miscellaneous polyorganosulfur compounds (Moore and O'Conner, 1966; Brodnitz et al., 1971; Block, 1985; Fenwick and Hanley, 1985). The enzymatic conversion of SMCSO to MMTSO and other polysulfides is illustrated in Figure 1. This study investigated the occurrence of SMCSO in *Brassica* and the formation of SMCSO-derived organosulfur compounds including MMTSO and dimethyl trisulfide (DMTS).

MATERIALS AND METHODS

Materials and Reagents. *Brassica* vegetables including broccoflower, broccoli, Brussels sprouts, cabbage, and cauliflower were purchased from a local supermarket and rinsed. The outer leaves were removed when appropriate. SMCSO was synthesized via the oxidation of S-methylcysteine (Sigma Chemical Co., St. Louis, MO) with hydrogen peroxide (Synge and Wood, 1956) and recrystallized twice in ethanol. MMTSO synthesis followed the procedure of Moore and O'Connor (1966) by selective oxidation of dimethyl disulfide (DMDS) (Sigma) with 32% peracetic acid (Aldrich Chemical Co., Inc., Milwaukee, WI). All other chemical reagents and redistilled solvents were of analytical grade.

Purification and Enzymatic Analysis of Cystine Lyase in *Brassica*. Cystine lyase was partially purified from cabbage according to the methods of Hamamoto and Mazelis (1986) and Smith and Hall (1987). Enzymatic analysis of cystine lyase was accomplished by measuring pyruvate formation (Smith and Hall, 1987).

HPLC Analysis of SMCSO. SMCSO was analyzed as the o-phthaldialdehyde (OPA) derivative utilizing RP-HPLC (Gustine, 1985; Woolfson et al., 1987; Ziegler and Sticher, 1988, 1989). Isocratic separation was accomplished using 16% acetonitrile in 50 mM potassium phosphate buffer (pH 7.0). System hardware consisted of a Rainin Rabbit HP pump, a 20-μL sample loop, a

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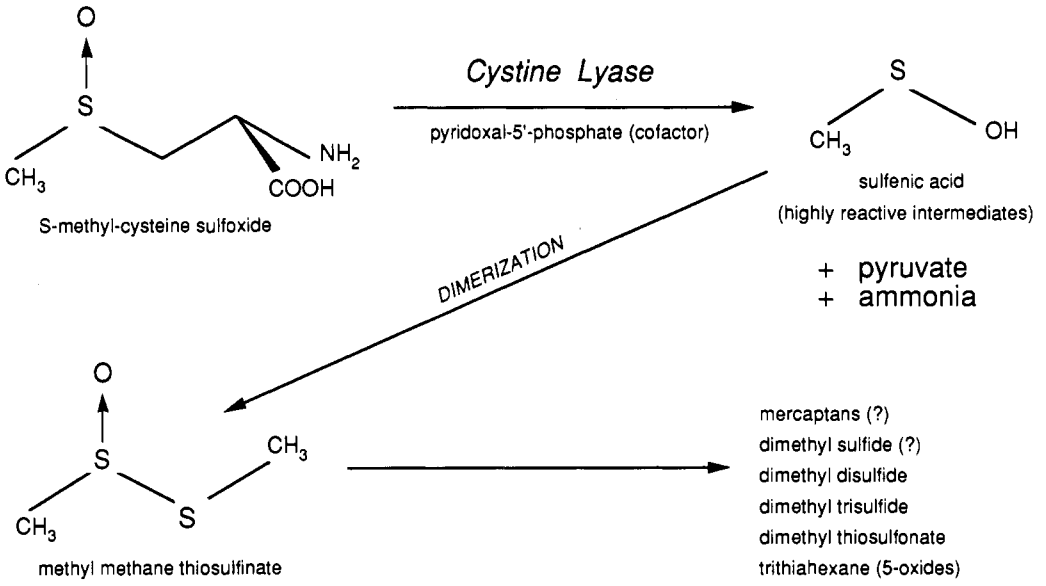

Figure 1. Enzymatic conversion of *S*-methylcysteine sulfoxide.

5-μm (22 cm × 4.6 mm i.d.) C_{18} column (Brownlee Labs) enclosed in a column heater at 30 °C, and a Gilson Holochrome UV–vis variable-wavelength detector. Detection of OPA–SMCSO was monitored at 337 nm. Limit of detection (S/N = 20) was 10 ng injected. Data analysis was performed utilizing the Rainin Dynamax HPLC Method Manager, which controlled both the operational aspects of the HPLC system and the acquisition, analysis, and editing of generated data. Synthesized SMCSO was found to be approximately an equimolar mixture of the ± racemates, of which one enantiomer had identical retention time to an "acidic" amino acid, presumably (+)-SMCSO, isolated from cabbage according to the procedures of Synge and Wood (1956), Morris and Thompson (1956), and Mae et al. (1971). Quantitation of the racemates was unable to be determined since analytical standards were unavailable.

GC–MS Analysis of SMCSO-Derived Compounds. SMCSO-derived organosulfur compounds were identified by GC–MS. DMTS (Eastman Kodak Co., Rochester, NY), synthesized MMTSO (≥95% purity), and methyl methanethiosulfonate (MMTSOO) (Sigma) were used as standards for GC analysis.

A Hewlett-Packard Model 5890A capillary gas chromatograph equipped with a mass selective detector (MSD) Model 5970B was used for the detection and mass spectral determination of DMTS, MMTSO, and MMTSOO. A Restek Rtx-5 (5% phenyl; 95% methylsilicone, Restek Corp., Bellefonte, PA) bonded-phase fused silica capillary column (30 m × 0.25 mm i.d., d_f = 0.2 μm) was coupled directly to the MSD capillary interface.

Operating conditions of the GC were as follows: injection volume, 1.0 μL; injector temperature, 150 °C; transfer line, 150 °C; He flow rate, 1 mL/min; oven temperature, 40 °C (held for 1 min) programmed to 200 °C at 5 °C/min. Mass spectra were obtained via electron impact ionization (EI) over the range 30–150 amu at 3.57 scans/s. The ion source temperature was 240 °C, and the electron impact energy was 70 eV.

Occurrence of SMCSO in Five *Brassica* Vegetables. SMCSO was initially isolated in the edible portion of five *Brassica* vegetables following the procedures of Morris and Thompson (1956), Synge and Wood (1956), and Mae et al. (1971) by using both strong and weak cation exchangers. Enzymatic action of these aqueous macerated *Brassica* extracts was inhibited through the addition of 95% ethanol. The entire isolation procedure has been outlined by Marks (1992).

Formation of MMTSO in an Enzymatic Model System. MMTSO formation over time was investigated using an enzymatic model system. SMCSO and partially purified cystine lyase was incubated at 35 °C with occasional shaking in a buffered Tris-HCl (pH 8.5) solution according to the procedure of Smith and Hall (1987). Samples were analyzed for SMCSO, pyruvate, and SMCSO-derived compounds over a 24-h time period. Aliquots of 250 μL were used for the analysis of SMCSO and pyruvate.

Table I. Concentration of SMCSO in Various *Brassica*[a]

vegetable	SMCSO, mg/100 g of fresh wt	vegetable	SMCSO, mg/100 g of fresh wt
broccoflower	24.9 ± 5.8[c]	cabbage	18.5 ± 4.7[c,d]
broccoli	19.1 ± 5.5[c,d]	cauliflower	14.3 ± 3.8[d]
Brussels sprouts	68.0 ± 22.8[b]		

[a] Values are means ± SE (n = 5). Means with different letters indicate significant differences as determined by ANOVA ($p < 0.05$) and Duncan's multiple-range test ($p < 0.05$).

Table II. Formation of SMCSO-Derived Compounds over Time in an Enzymatic Model System

time, h	pyruvate, mM	MMTSO, mM	DMTS, μM	MMTSOO, μM
0	0	0	0	0
0.25	0.70	~0.02[b]	0.44	nd[c]
0.5	1.38	~0.02[b]	0.33	nd
1.0	2.40	0.04	0.90	nd
2.0	4.47	0.08	1.27	nd
4.0	8.03	0.13	3.95	nd
24.0	28.40	1.72	3.16	9.55

[a] Reaction conditions given under Materials and Methods. [b] Below level of quantitation; determined by extrapolation. [c] Not detected.

Aliquots of 2.0 mL were diluted with 20 mL of water, separated with 20 mL of methylene chloride, dried over sodium sulfate, and analyzed for SMCSO-derived compounds via GC–MS.

Formation of MMTSO in Brussels Sprouts. Loss of SMCSO and formation of MMTSO were monitored in a Brussels sprouts water extract; 25 g of Brussels sprouts was macerated with 100 mL water and divided. One portion was treated with 5.0 mL of 600 mM Tris-HCl (pH 8.5). The other portion was left untreated. After both samples were incubated at 35 °C for 24 h, analyses of pH, SMCSO, and MMTSO were performed.

Statistical Analysis. Data were analyzed using a one-way analysis of variance (ANOVA) and the Duncan's multiple range test, using $p < 0.05$ as the level of significance (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The concentration of SMCSO in the edible portion of the five *Brassica* vegetables investigated is shown in Table I. Although these levels are slightly lower than those previously reported for *Brassica* (Mae et al., 1971; Bradshaw and Borzucki, 1982; Benevenga et al., 1983), factors such as plant maturity, time period required for transportation of vegetables, and water content may all have an effect on these measured SMCSO concentrations. A

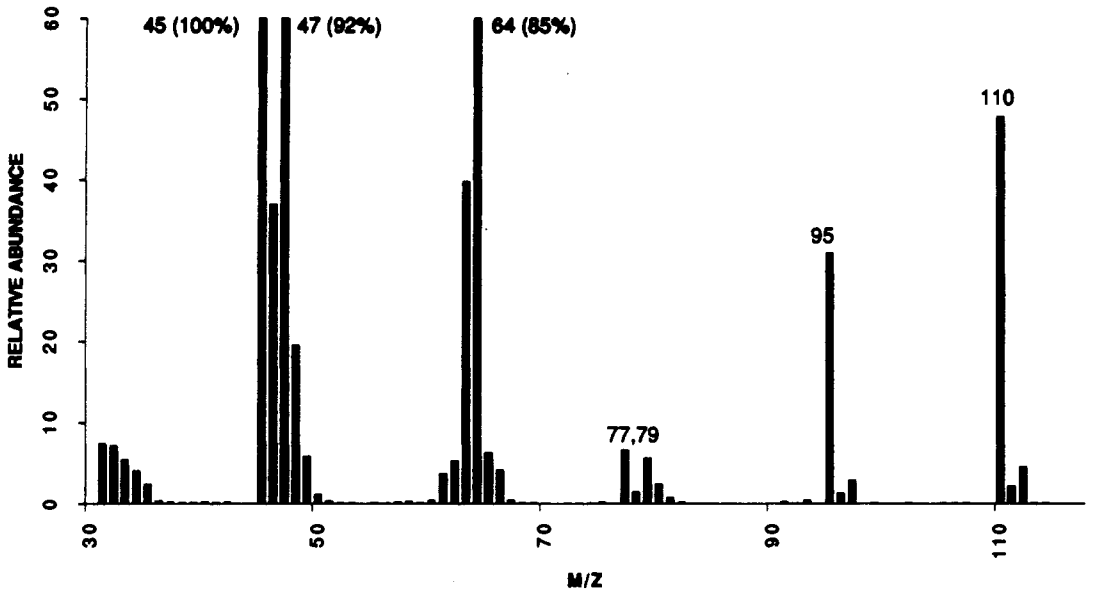

Figure 2. Mass spectrum of methyl methanethiosulfinate.

Table III. Generation of MMTSO in Brussels Sprouts

time, h	pH	SMCSO, μM	MMTSO, μM	DMTS, μM	allyl NCS[a]
0	6.3	1345	nd[b]	nd	nd
24	4.4	650	tr[c]	tr	+++[d]
24	8.0	40	~360	~2	nd

[a] Allyl isothiocyanate; identification based on mass spectrum. [b] Not detected. [c] Trace amount (not quantitated). [d] Large amount present; unable to quantitate since standard was not available at time of analysis.

significantly greater level of SMCSO was found in Brussels sprouts as compared to the other vegetables. This may, in part, reflect the comparably lower water content of Brussels sprouts, although there is only a 6.5% difference in water content between Brussels sprouts and cabbage or cauliflower (USDA, 1984). Interestingly, broccoflower (a broccoli–cauliflower cross) contained substantial amounts of SMCSO.

Results from the enzymatic model system, shown in Table II, indicate that pyruvate, MMTSO, and DMTS are being formed over time. Spike recovery of a three compound mixture (DMTS, MMTSO, MMTSOO) was >95% in the solvent extraction procedure. The mass spectrum of MMTSO is illustrated in Figure 2. m/z 95 corresponds to loss of CH_3, while m/z 64 may indicate loss of $S–CH_2$ via a hydrogen rearrangement (McLafferty, 1980).

It is suggested from Table II that formation of MMTSOO is slightly retarded compared to formation of other SMCSO-derived polysulfides. Apparently, other SMCSO-derived compounds are being formed during the enzymatic hydrolysis of SMCSO as illustrated by the disparity of pyruvate concentration compared to the total concentration of analyzed organosulfides. In a subsequent study, the formation of pyruvate was found to occur slightly more slowly than the loss of SMCSO, consistent with enzymatic kinetics.

Formation of SMCSO-derived organosulfur compounds in Brussels sprouts over time is shown in Table III. Spike recovery in the plant matrix was ≤20% for MMTSO and DMTS. MMTSOO was not recoverable via this isolation scheme. This would likely be due to a higher water solubility of MMTSOO compared to that of the other compounds in the spike mixture. After 24 h, the natural pH of the Brussels sprouts decreased from 6.3 to 4.4. The addition of Tris-HCl buffer increased juice pH to 8.0. Trace levels of MMTSO along with considerable amounts of isothiocyanates were observed to occur naturally in the pH 4.4 extract. Basifying the juice dramatically increased the formation of MMTSO. Not only did SMCSO concentration decrease by 52% after 24 h but the adjustment of pH to 8.0 increased loss of SMCSO by an additional 94%. In fact, there was very little SMCSO left (40 μM) after basic pH adjustment of the juice. The pH optimum of cystine lyase is reported to be between 8.0 and 8.5 (Hamamoto and Mazelis, 1986). Extrapolation of observed MMTSO and other organosulfur compounds to naturally occurring concentrations in Brussels sprouts could not be made due to the poor spike recovery when the more complex plant matrix was used.

Results of these experiments indicate that SMCSO is catabolized to form MMTSO in Brussels sprouts via the cystine lyase-mediated enzymatic reaction and that the formation of MMTSO and other organosulfur compounds are time- and pH-dependent. Conditions that are pH-dependent are also known to affect the formation of glucosinolate-derived compounds (Astwood et al., 1949; MacLeod, 1976; Daxenbichler et al., 1977). Both naturally occurring glucosinolate-derived and SMCSO-derived compounds are present in *Brassica* vegetables. These organosulfur compounds appear to have major roles in the inhibition of carcinogenesis (Wattenberg, 1977; Boyd et al., 1982; Marks, 1992) as well as in the vegetable flavor and aroma (MacLeod and MacLeod, 1970; Buttery et al., 1976; VanEtten et al., 1976).

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Registry No. SMCSO, 6853-87-8; MMTSO, 13882-12-7; MMTSOO, 2949-92-0; DMTS, 3658-80-8; allyl NCS, 57-06-7; cystine lyase, 73298-99-4; pyruvate, 127-17-3.