

Thermal Degradation of Sulforaphane in Aqueous Solution

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Sulforaphane, a cancer chemopreventive agent identified from broccoli, was degraded in an aqueous solution at 50 and 100 °C. The reaction mixtures were extracted with methylene chloride and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Dimethyl disulfide, S-methyl methylthiosulfinate, S-methyl methylthiosulfonate, methyl (methylthio)methyl disulfide, 1,2,4-trithiolane, 4-isothiocyanato-1-(methylthio)-1-butene, and 3-butenyl isothiocyanate were identified as volatile decomposition products. After methylene chloride extraction, the aqueous layer was dried and silica gel column chromatography was used to separate and purify the nonvolatile decomposition products. The major thermal degradation compound was determined by ^1H NMR, ^{13}C NMR, and FAB-MS as *N,N'*-di(4-methylsulfinyl)butyl thiourea. A possible mechanism for the formation of these products is proposed."

Keywords: *Sulforaphane; isothiocyanate; thermal decomposition; N,N'-di(methylsulfinyl)butyl thiourea*

INTRODUCTION

Epidemiological studies provide consistent evidence that individuals who consume high quantities of cruciferous vegetables experience a lower risk of developing several types of cancer (Zhang et al., 1992). Although the exact mechanism for this is not clear, the phytochemicals or secondary metabolites found in these vegetables cause much interest and are suggested to be responsible for this activity. Sulforaphane [(−)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane], one phytochemical produced by enzyme hydrolysis of glucoraphanin (a glucosinolate in broccoli, cabbage, etc.), has received the most attention for its cancer chemopreventive activity (Zhang et al., 1994). Sulforaphane is a potent inducer of phase II enzymes, including quinone reductase and glutathione *S*-transferase, which protect against carcinogens and other toxic electrophiles. Because sulforaphane is monofunctional, it does not influence the phase I enzymes (Zhang et al., 1994).

Due to the importance of this compound, various methods, including high-performance liquid chromatography (HPLC) (Zhang et al., 1992), gas chromatography (GC), GC/mass spectrometry (GC/MS) (Spencer and Daxenbichler, 1980; Chiang et al., 1998), and paired-ion chromatography (Fahey et al., 1997) have been used to analyze sulforaphane in *Brassica* vegetables. However, due to its instability, sulforaphane is difficult to analyze quantitatively. Recently, sulforaphane was found to decompose to other compounds due to the high-temperature conditions in the injection ports of GC or GC/MS equipment (Chiang et al., 1998). Although many studies about this compound have been published, no information is available on the stability and chemical reaction of sulforaphane under normal cooking conditions. The purpose of this study is to investigate the thermal stability of sulforaphane in aqueous solution

at different temperatures, to identify the thermal degradation products from sulforaphane, and to discuss the possible formation mechanism for these degradation products.

EXPERIMENTAL PROCEDURES

Material. TLC plates (250 μm thickness, 2–25 μm particle size), tridecane, and silica gel (130–270 mesh) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used for chromatography. All solvents used for chromatography were of analytical grade quality and purchased from Fisher Scientific (Springfield, NJ). Sulforaphane was purchased from LKT Laboratories, Inc. (St. Paul, MN). Its purity was checked by nuclear magnetic resonance (NMR) and thin-layer chromatography (TLC) methods, and it was stored below 0 °C.

NMR and Fast Atom Bombardment Mass Spectroscopy (FAB-MS). ^1H NMR and ^{13}C NMR spectra were obtained on a Varian Gemini-200 instrument (Varian Inc., Palo Alto, CA) at 200 and 50 MHz. $\text{CH}_3\text{OH}-d_4$ was used as a solvent, and chemical shifts were expressed in parts per million (δ). ^{13}C NMR multiplicity was determined by attached proton test (APT) experiment. FAB mass spectra were recorded on a Finnigan MAT-90 instrument (Finnigan Inc., San Jose, CA).

Thermal Decomposition of Sulforaphane. *Preparation of the Model System.* A sample of 30–60 mg of sulforaphane was added to 5–6 mL of distilled water. The mixture was heated in a 50 mL flask fitted with a condenser at different temperatures for 1 h in an oil bath. The reaction solution was then cooled to room temperature and extracted with 2 $\times$ 5 mL of methylene chloride. The methylene chloride phase was then dried using anhydrous sodium sulfate and concentrated to 0.2 mL by a stream of nitrogen gas after spiking with 4 μL of 1000 ppm tridecane as the internal standard. The concentrated sample was subjected to GC and GC/MS analysis directly.

Purification of the Major Degradation Products in Aqueous Solution. The aqueous phase was evaporated to dryness by a rotary evaporator with the water bath at 45 °C. The residue was subjected to silica gel column (30 g) chromatography. The solvent system used was chloroform/methanol (6:1). The fractions containing the major degradation products were collected, and the purity of the isolated compound was monitored by TLC (the solvent used was chloroform/methanol 4:1). The solvents were removed under reduced pressure by a rotary evaporator at a temperature of 45 °C. Approximately 25 mg of colorless

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Table 1. Mass Spectral Data of Decomposition Products of Sulforaphane

compound	RI ^a	MS spectral data <i>m/z</i> (relative intensity)	identification
dimethyl disulfide	1355	96 [(M + 2) ⁺ , 10], 94 (M ⁺ , 100), 79 (64), 64 (16), 61 (24), 48 (24), 47 (42), 46 (54), 45 (78)	ref 1 ^a
S-methyl methylthiosulfinate	3272	112 [(M + 2) ⁺ , 1], 110 (M ⁺ , 16), 95 (11), 64 (64), 63 (26), 47 (64), 46 (19), 45 (100)	ref 1
S-methyl methylthiosulfonate	3535	128 [(M + 2) ⁺ , 2], 126 (M ⁺ , 40), 111 (4), 81 (66), 79 (52), 64 (40), 63 (64), 48 (26), 47 (100), 45 (98), 46 (46)	ref 1
methyl (methylthio)methyl disulfide	4230	142 [(M + 2) ⁺ ,1], 140 (M ⁺ , 8), 93 (4), 79 (8), 61 (100), 45 (50)	ref 1
1,2,4-trithiolane	4438	126 [(M + 2) ⁺ , 12], 124 (M ⁺ , 60), 78 (78), 60 (24), 59 (18), 46 (40), 45 (100), 44 (18)	ref 1
4-isothiocyanato-1-(methylthio)-1-butene	6452	161 [(M + 2) ⁺ , 1], 159 (M ⁺ , 12), 87 (80), 85 (20), 72 (74), 61 (20), 59 (16), 53 (20), 47 (30), 46 (20), 45 (100)	ref 1
3-butenyl isothiocyanate	3205	113 (M ⁺ , 47), 72 (100), 55 (23), 39 (48)	

^a RI, retention index on DB-1 column. ^b Reference 1, Wiley 138.

oil was obtained. The pure sample was subjected to NMR and MS analysis directly after the purifying procedure.

GC and GC/MS Analysis. A Varian Model 3400 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column [DB-1, 60 m × 0.32 mm (i.d.), 1.0 μm film thickness, J&W Scientific] was used. The oven temperature was programmed from 40 to 220 °C at an increase rate of 3 °C/min. The temperatures of the detector and injector were maintained at 280 and 210 °C, respectively. The flow rate of the helium carrier gas was 1 mL/min, and the split ratio was 10:1. GC/MS analysis was performed on an HP Model 5980 coupled with an HP 5971 mass selective detector. The capillary column and temperature program were the same as for the GC analysis. Mass spectra were obtained by electron ionization at 70 eV, and mass scan was from 33 to 300. Compound quantification was based on the GC/FID data. The concentration of the compound was calculated by using the following equation: $A \times 0.004/IS \times M$, where *A* is the area count of the compound, *IS* is the area count of the internal standard, and *M* is the mass of sulforaphane (mg). The identification of volatile compounds was based on the mass spectra obtained from the GC/MS.

RESULTS AND DISCUSSION

Volatile Compounds Generated from the Thermal Degradation of Sulforaphane. After thermal degradation of sulforaphane in aqueous solution, the resulting mixture was extracted with methylene chloride and analyzed by GC and GC/MS. Table 1 lists volatile compounds identified from the methylene chloride extracts. Their structures were tentatively determined by comparing their mass spectral data with reference (Table 1). Among them, 3-butenyl isothiocyanate has been reported to be a thermal degradation product of sulforaphane caused by the high temperature of the injection ports of GC and GC/MS (Chiang et al., 1998). Other compounds were reported for the first time as degradation products of sulforaphane.

The mechanisms for the formation of these volatile compounds from sulforaphane are proposed as shown in Figure 1. 4-Methylthio-4-hydroxybutyl isothiocyanate (compound **B**), which could be formed by the transaction of oxygen from sulfur to carbon via an epoxide (compound **A**), is an important intermediate for generating the methylthio radical and is the source of the methylthio group in those decomposition compounds. 4-Isothiocyanato-1-(methylthio)-1-butene can be produced by dehydration of 4-methylthio-4-hydroxybutyl isothiocyanate. The methylsulfinyl radical can be produced directly from sulforaphane. Different combinations of methylsulfinyl radical and methylthio radical will lead to different adducts.

Temperature Influence on the Generation of Volatile Compounds from Sulforaphane. Two different temperatures have been used in the experiment. Temperature was found to have a great effect on the concentrations of the volatile compounds generated. The

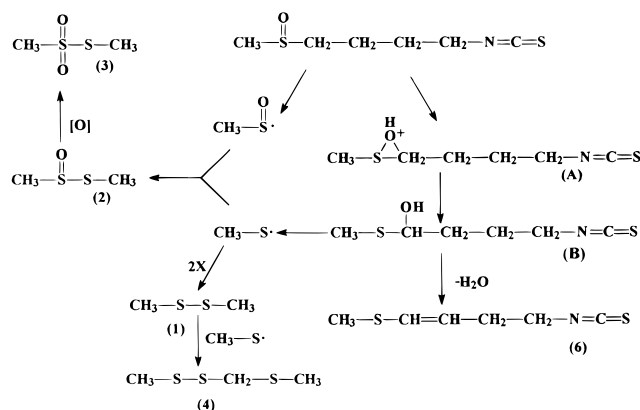


Figure 1. Possible pathways for the formation of volatile compounds generated from thermal degradation of sulforaphane [1, dimethyl disulfide; 2, S-methyl methylthiosulfinate; 3, S-methyl methylthiosulfonate; 4, methyl (methylthio)-methyl disulfide; 6, 4-isothiocyanato-1-(methylthio)-1-butene].

Table 2. Quantitation of Thermal Degradation Products from Sulforaphane at Two Different Temperatures

compound	50 °C (ppm) ^a	100 °C (ppm)
dimethyl disulfide	2.1	309.0
S-methyl methylthiosulfinate	17.2	184.8
S-methyl methylthiosulfonate	26.6	174.0
methyl (methylthio)methyl disulfide	4.9	38.7
1,2,4-trithiolane	6.5	23.0
4-isothiocyanato-1-(methylthio)-1-butene	13.2	134.9

^a Micrograms per gram of sulforaphane

concentrations of volatile decomposition products at 100 °C were much higher than those at 50 °C, suggesting that higher temperature accelerates the rate of degradation. The differences of the concentrations at the two temperatures are shown in Table 2.

Nonvolatile Compound from Thermal Degradation. After thermal reaction, the aqueous phase was subjected to silica gel column chromatography. Only one main compound was obtained as a colorless oil. It exhibited prominent quasi-molecular ion peaks in the positive FAB mass spectrum at *m/z* 313 [M + 1]⁺ and 335 [M + Na]⁺ about 2 times the molecular weight of sulforaphane. In the ¹H NMR (CD₃OD), it showed signals at δ 1.78 (8H, m, -CH₂CH₂-), 2.65 (6H, s, CH₃), 2.87 (4H, m-CH₂SO), and 3.53 (4H, m, CH₂N), whereas in the ¹³C NMR (CD₃OD), it showed signals at δ 24.0 (CH₂), 32.3 (CH₂), 41.2 (-CH₂N-), 47.2 (CH₃S-), and 57.4 (-SCH₂-). These NMR data were similar to those of sulforaphane (Kore et al., 1993; Zhang et al., 1992), suggesting this compound is a dimer of sulforaphane. Sulforaphane is an isothiocyanate compound. Recently one of these compounds, allyl isothiocyanate, was reported to convert to diallylthiourea in aqueous solution (Chen and Ho, 1998). This compound was, there-

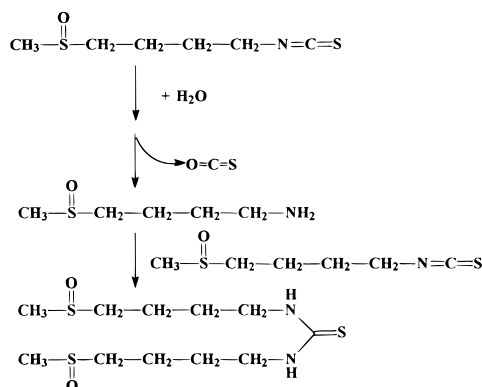


Figure 2. Possible pathways for the formation of *N,N'*-di(methylsulfinyl)butyl thiourea from sulforaphane.

fore, tentatively determined as (methylsulfinyl)butyl thiourea. After reexamination of the NMR and FAB-MS, it is uncertain whether the carbon signal for the thiourea group was observed in the ^{13}C NMR spectrum. To clarify this, diallylthiourea was synthesized (Chen and Ho, 1998). We did not observe the carbon signal for thiourea group in its ^{13}C NMR spectrum either. It is possible that the low sensitivity of the NMR instrument does not allow us to observe this weak signal. The major degradation product was elucidated as *N,N'*-di(methylsulfinyl)butyl thiourea (Figure 2).

The proposed mechanism for the formation of this compound is shown in Figure 2. A similar mechanism has been proposed by Kawakishi and Namiki (1969) and Chen and Ho (1998) for the formation of diallylthiourea from allyl isothiocyanate. Sulforaphane is first hydrolyzed to an amine, and the resulting amine reacts with sulforaphane to generate this thiourea compound.

Conclusion. Our study demonstrates that sulforaphane, a cancer chemopreventive agent, is an unstable compound. It will degrade under cooking conditions, and the major degradation product is a thiourea compound. The bioactivity of this thiourea

compound needs to be addressed, and the generation of the thiourea compound from foods containing isothiocyanate compounds needs to be studied further.

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Received for review January 29, 1999. Revised manuscript received June 25, 1999. Accepted July 7, 1999.

JF990082E