

Thermal Degradation of Allyl Isothiocyanate in Aqueous Solution

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Allyl isothiocyanate in an aqueous solution was heated and refluxed at 100 °C for 1 h. The reaction mixtures were then extracted with methylene chloride and analyzed using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The mixtures in aqueous phase were analyzed by high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS) equipped with an atmospheric-pressure chemical ionization (APCI) interface. The compounds identified in the methylene chloride extracts included diallyl sulfide, diallyl disulfide, diallyl trisulfide, diallyl tetrasulfide, allyl thiocyanate, 3*H*-1,2-dithiolene, 2-vinyl-4*H*-1,3-dithiin, 4*H*-1,2,3-trithiin, and 5-methyl-1,2,3,4-tetrathiane. *N,N*-Diallylthiourea, which was the major degradation product in the aqueous phase from the thermal reaction of allyl isothiocyanate, was identified by using LC–MS (APCI+), direct-probe EI-MS, and ¹H-NMR. The possible mechanism for the formation of these products was proposed.

Keywords: *Allyl isothiocyanate; thermal degradation; N,N-diallylthiourea; LC–MS; atmospheric-pressure chemical ionization*

INTRODUCTION

Allyl isothiocyanate (AITC), which is a major pungent flavor compound in mustard and Wasabi, is generated from its precursor, allyl glucosinolate, namely, sinigrin (Kawakishi and Namiki, 1969; Masuda et al., 1996). Sinigrin, which widely exists in mustard and other members of the *Brassica* species of the cruciferous vegetables such as cabbage and cauliflower, breaks down and releases AITC by the action of myrosinase (thioglucoside glucohydrolase) when the plant tissue is disrupted (Chin and Lindsay, 1993; Shahidi and Gabon, 1990; VanEtten et al., 1976; Zrybko and Rosen, 1997). The stability of sinigrin in the methanol/ammonia/water solution, which was used for the extraction of mustard seed, over a 24-h period at various temperatures was reported by Shahidi and Gabon (1990) and Shahidi (1993). Sinigrin was degraded by 16–21% at 45 °C but only degraded by 1–2% at 25 °C, and its decomposition products including allyl cyanide, thioglucose and its dimer, glucose, furfuryl alcohol, but-3-enynitrile, allyl isothiocyanate, and 3,4-epithiobutanenitrile were also reported (Shahidi, 1993; Shahidi and Gabon, 1990).

It has been shown that AITC is unstable and is gradually decomposed to other compounds having a garliclike odor in the presence of water at both room temperature and 37 °C (Kawakishi and Namiki, 1969). AITC is also reported to be sensitive to temperature and pH. The high temperature of 37 °C and alkaline conditions accelerate the decomposition of AITC (Ina et al., 1981).

Because no information exists about the stability and chemical reaction of AITC under normal cooking conditions, the purpose of this study was to investigate the thermal stability of AITC in boiling water and to identify thermal degradation products from AITC. This paper also reports the pH effect on the thermal stability of AITC.

EXPERIMENTAL PROCEDURES

Thermal Reaction of Allyl isothiocyanate (AITC). A sample of 1 g of allyl isothiocyanate (Aldrich Chemical Co., Milwaukee, WI) in 200 mL of distilled water was adjusted to pH 2.7, 7.0, or 9.0 with 0.1 N HCl or 0.1 N NaOH. The reaction solution was heated under reflux at 100 °C for 1 h. The reaction mixtures were then spiked with 0.7 mL of dodecane (10.9 mg/mL) as internal standard and extracted with 25 mL of methylene chloride. The methylene chloride phase was dried over anhydrous sodium sulfate and concentrated to 5 mL by a stream of nitrogen gas prior to gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analysis. The concentration of each individual compound was calculated by the equation of $A/IS \times R$; where *A* is the area count of the compound, *IS* is the area count of the internal standard, and *R* (=2.1) is the GC-FID response factor of dodecane to AITC.

The aqueous phase was concentrated to 20 mL by a vacuum rotary evaporator and analyzed using high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS). The compound of interest was collected by HPLC and identified by using direct-probe electron-impact MS and ¹H NMR.

GC and GC–MS Analysis. A Varian model 3400 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (DB-Wax, J&W 30 m × 0.25 mm i.d.) was used. The GC oven was temperature-programmed from 50 to 220 °C at a rate of 4 °C/min. The carrier-gas (He) flow rate was 2.0 mL/min. A split ratio of 25:1 was used. The temperatures of the injection port and detector were 240 and 250 °C, respectively. The GC–MS analysis was performed on an HP model 5890 coupled to an HP 5971 mass-selective detector. The same column and temperature program were used.

HPLC and LC–MS Analysis. HPLC analysis was performed using a Varian 5000 liquid chromatograph equipped with a Varian 2050 detector. A TSK-GEL ODS-80TS reverse-phase column (250 mm × 4.6 mm i.d., TOSOH Co., Japan) was used. Elution was carried out at a flow rate of 1.0 mL/min using a solvent system of acetonitrile/water (40/60) in 10 min isocratically. The program was then linearly increased to 100% acetonitrile in 2 min and kept at 100% acetonitrile for another 15 min. The eluate was monitored at 254 nm. MS

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Table 1. Mass Spectral Data of Volatile Compounds from Thermal Degradation of Allyl Isothiocyanate

compound	RI ^a	MS spectral data <i>m/z</i> (relative intensity)	identification
1 diallyl sulfide	1118	116 ((M + 2) ⁺ , 1), 114 (M ⁺ , 27), 99 (31), 73 (57), 72 (34), 71 (28), 47 (21), 45 (100), 41 (47)	<i>b, c</i>
2 allyl isothiocyanate ^f	1353	101 ((M + 2) ⁺ , 3), 99 (M ⁺ , 74), 72 (33), 71 (9), 70 (6), 59 (5), 58 (5), 45 (12), 44 (8), 41 (100)	
3 allyl thiocyanate	1440	101 ((M + 2) ⁺ , 3), 99 (M ⁺ , 56), 98 (10), 72 (32), 71 (11), 58 (23), 46 (6), 45 (23), 41 (100)	<i>e</i>
4 diallyl disulfide	1468	148 ((M + 2) ⁺ , 1), 146 (M ⁺ , 10), 113 (12), 105 (12), 81 (19), 79 (11), 71 (12), 47 (12), 45 (45), 41 (100)	<i>b, c</i>
5 3 <i>H</i> -1,2-dithiolene	1513	106 ((M + 2) ⁺ , 5), 105 (13), 104 (M ⁺ , 56), 103 (100), 71 (14), 69 (12), 64 (10), 58 (9), 45 (33)	<i>d</i>
6 diallyl trisulfide	1787	180 ((M + 2) ⁺ , 1), 178 (M ⁺ , 7), 113 (96), 103 (9), 73 (100), 69 (4), 64 (13), 47 (15), 45 (64), 41 (80)	<i>c</i>
7 2-vinyl-4 <i>H</i> -1,3-dithiin	1833	146 ((M + 2) ⁺ , 7), 144 (M ⁺ , 57), 111 (45), 103 (15), 97 (20), 73 (13), 72 (86), 71 (100), 45 (45)	<i>b, c</i>
8 4 <i>H</i> -1,2,3-trithiin	1841	138 ((M + 2) ⁺ , 16), 136 (M ⁺ , 100), 103 (15), 72 (54), 71 (93), 69 (10), 64 (19), 45 (35)	<i>e</i>
9 5-methyl-1,2,3,4-tetrathiane	2005	172 ((M + 2) ⁺ , 18), 170 (M ⁺ , 100), 128 (43), 106 (30), 64 (34), 59 (10), 45 (13), 41 (18)	<i>c</i>
10 diallyl tetrasulfide		210 (M ⁺ , 12), 146 (55), 105 (37), 104 (16), 81 (24), 73 (78), 64 (52), 45 (45), 41 (100)	<i>c</i>

^a RI = retention index, CW-20M. ^b Mass spectral database of Wiley 138. ^c Mass spectral data from Block et al. (1988). ^d Mass spectral data from Yu et al. (1994). ^e Tentative identification according to mass spectral data of EI. ^f Starting material.

analysis was performed on a Micromass Platform II system (Micromass Co., MA) equipped with a Digital DECPc XL560 computer for data analysis. Positive-ion mass spectra were obtained using the heated nebulizer atmospheric-pressure chemical-ionization (APCI) interface. The ion-source temperature was set at 150 °C, and the sample cone voltage was 10 V.

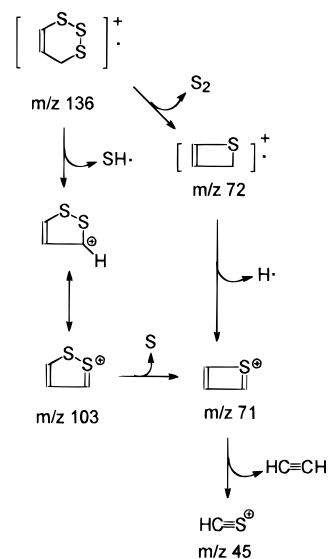
Direct Probe EI-MS Analysis. Direct probe EI-MS was conducted on a Finnigan MAT 8230 high-resolution mass spectrometer. The temperature of the probe was increased from 35 to 350 °C at 5 °C/s.

¹H-NMR Analysis. The ¹H-NMR (360 MHz) spectrum was obtained in pyridine-*d*₅ solution on a modified Nicolet NT 360 instrument. Chemical shifts are reported as parts per million relative to tetramethylsilane (TMS), and coupling constants are in hertz.

Synthesis of *N,N*-Diallylthiourea. AITC (0.1 mol) was diluted with 30 mL of toluene, and allylamine (0.1 mol) was added dropwise. The reaction solution was then stirred at 60 °C for 2 h. The reaction mixture was concentrated and kept in a -20 °C freezer for overnight. The resulting solid was washed with hexane three times. The purity of this product was 99% based on HPLC analysis. This method was modified from the method of Pascual and Rindlisbacher (1994).

RESULTS AND DISCUSSION

Volatile Compounds Generated from the Thermal Degradation of AITC. After thermal reaction in boiling water for 1 h, the odor of AITC was totally changed from a pungent, mustardlike flavor note to a strong garliclike note. The degraded solution of AITC was extracted and then analyzed by GC and GC-MS. Table 1 lists the volatile compounds identified from the thermal degradation of AITC. These compounds were tentatively identified by comparing their mass spectral data with references or by interpreting the CI mass spectra (compounds **3** and **8**). The mass spectral data of compound **3** is very similar to that of AITC (compound **2**). This compound was tentatively identified as allyl thiocyanate, an isomer of AITC. Allyl thiocyanate can also be thermally generated from AITC when pure AITC was introduced into the GC. The thermal isomerization of AITC to allyl thiocyanate is due to the high temperature of the injection port based on our study. According to the mass spectral data of compound **8** (Table 1), the fragment intensity of (M + 2)⁺ is 16% of the molecular ion. This suggests that compound **8** contains three

**Figure 1.** Proposed mass fragmentation for the compound **8**.

atoms of sulfur. This compound was proposed as 4*H*-1,2,3-trithiin based on the mass fragmentation as shown in Figure 1.

It has been reported that AITC can gradually be decomposed to allyl dithiocarbamate (compound **A** in Figure 2), which is then degraded to diallyl polysulfides when AITC is kept in an aqueous solution at 37 °C for several days (Kawakishi and Namiki, 1969). However, compound **A** was not detected when AITC was heated at 100 °C for 1 h according to our study. It may be possible that compound **A** is thermally labile and easily decomposed under such a vigorous heating condition or that generation of this compound is not favorable in this cooking condition.

The mechanisms for the formation of these volatile compounds from AITC are proposed as shown in Figure 2. Allyl mercaptan (compound **B**), which could be a decomposition product from compounds **3** or **A** through a hydrolysis process or reduction reaction (Kawakishi and Namiki, 1969), is a key intermediate for generating aliphatic sulfides and cyclic sulfides. The dimerization of allyl mercaptan leads to the formation of diallyl disulfide (compound **4**) as proposed by Kawakishi and

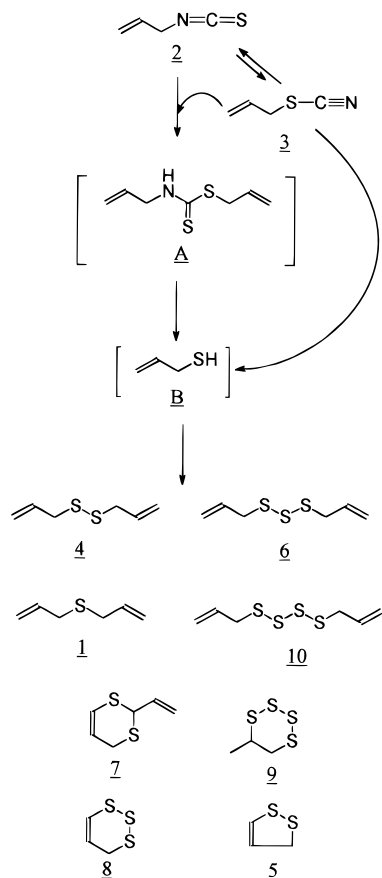


Figure 2. Possible pathways for the formation of volatile compounds generated from thermally degraded allyl isothiocyanate.

Namiki (1969). It has been shown that heating pure diallyl disulfide leads to significant amounts of diallyl sulfide, diallyl trisulfide, and diallyl tetrasulfide (Block et al., 1988). Diallyl sulfide (compound 1) and diallyl trisulfide (compound 6) can be generated from diallyl disulfide by a disproportionation or an allyldithio-radical addition reaction (Block et al., 1988). Diallyl tetrasulfide probably occurs from the interaction of two allyldithio radicals. 2-Vinyl-4*H*-1,3-dithiin (compound 7) is a thermal degradation product from garlic oil and can be generated from two molecules of thioacrolein ($\text{CH}_2=\text{CH}-\text{CH}=\text{S}$) (Block et al., 1988). It is possible that thioacrolein results from the oxidation of allyl mercaptan. Compounds 9 and 5 were reported to be the thermal degradation products from alliin and deoxyalliin, respectively, the flavor precursors of garlic (Yu et al., 1994). Yu et al. (1994) proposed that compound 9 could be from the interaction of one molecule of allyl mercaptan and three molecules of hydrogen sulfide, while compound 5 could be from interaction of one molecule of allyl mercaptan and one molecule of hydrogen sulfide followed by an oxidation reaction. Compound 8 has not been reported to be the thermal degradation product of garlic oil. However, its analogous compound, 1,2,3-trithiane, has been found in the thermally degraded alliin aqueous solution and its formation mechanism is proposed as the interaction of one molecule of allyl mercaptan and two molecules of hydrogen sulfide (Yu et al., 1994). 1,2,3-Trithiane can probably be further oxidized to generate compound 8.

pH Effect on the Generation of Volatile Compounds from AITC. The pH effect on the generation of volatile compounds from AITC is shown in Table 2.

Table 2. pH Effect on the Generation of Volatile Compounds from Thermal Degradation of Allyl Isothiocyanate (AITC)

compound	amount (mg/g of AITC)		
	pH 2.7	pH 7.0	pH 9.0
1 diallyl sulfide	13.09	22.16	26.40
2 allyl isothiocyanate	379.06	290.27	270.46
3 allyl thiocyanate	9.21	13.84	12.32
4 diallyl disulfide	8.09	11.47	12.71
5 3 <i>H</i> -1,2-dithiolene	0.13	0.17	0.19
6 diallyl trisulfide	10.31	13.63	14.11
7 2-vinyl-4 <i>H</i> -1,3-dithiin	tr ^a	tr	tr
8 4 <i>H</i> -1,2,3-trithiin	tr	tr	tr
9 5-methyl-1,2,3,4-tetrathiane	0.01	0.02	0.03
10 diallyl tetrasulfide	tr	tr	tr

^a tr = trace.

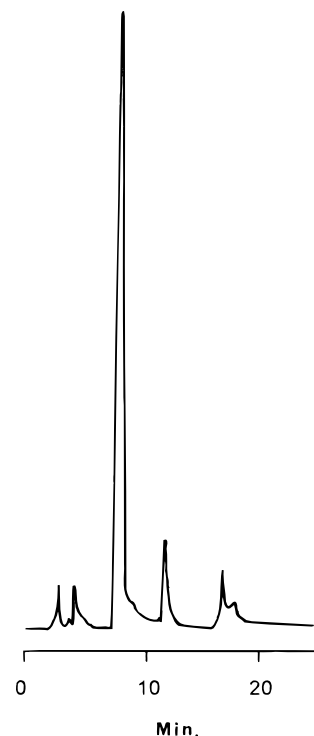


Figure 3. HPLC chromatograph of the nonvolatile products generated from thermally degraded allyl isothiocyanate: column, TSK-GEL ODS-80TS reverse-phase column (250 mm × 4.6 mm i.d.); flow rate, 1.0 mL/min; solvent system, acetonitrile/water (40/60) for 10 min, 40%–100% acetonitrile in 2 min, 100% acetonitrile for 15 min; detector wavelength, 254 nm.

The remaining AITC (compound 2) was less than 38% after thermal reaction for 1 h. The amount of allyl thiocyanate (compound 3) was calculated after deducing the thermally isomerized allyl thiocyanate (about 10% from AITC) caused by the high temperature at the GC injection port. The AITC was more stable when the reaction was conducted in an acidic solution than in neutral or alkaline solution. This result is consistent with the results of Ina et al. (1981). The amounts of volatile compounds increased when the pH increased (except compound 3). Table 2 shows that the amount of compound 3 generated at pH 7.0 is more than pH 2.7 and 9.0, suggesting the isomerization of AITC is more favorable in neutral conditions than in acidic and basic conditions.

Nonvolatile Compounds from Thermal Degradation of AITC. After thermal reaction, the nonvolatile compounds in the aqueous phase were analyzed by HPLC. There is one major peak (retention time = 7.6

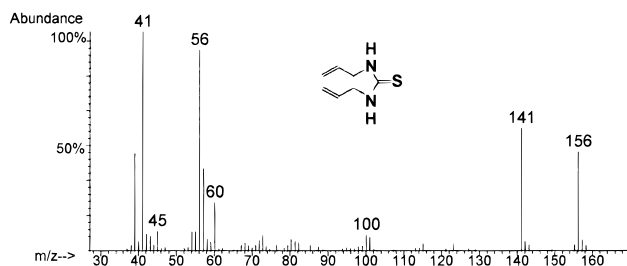


Figure 4. Direct-probe EI-MS of a major nonvolatile compound from the thermal degradation of allyl isothiocyanate.

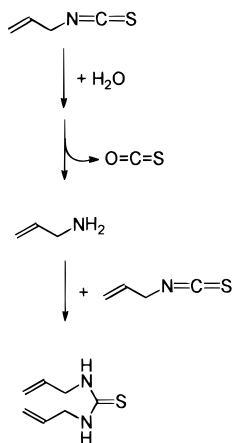


Figure 5. Possible pathways for the formation of *N,N'*-diallylthiourea from allyl isothiocyanate (Kawakishi and Namiki, 1969).

min) present in the HPLC chromatograph as shown in Figure 3. The molecular weight of this compound was determined as 156 using LC-MS (APCI+). The compound of interest was collected by HPLC and further identified by ^1H NMR and direct-probe EI-MS. The UV and ^1H -NMR data of this compound are as follows. UV max (in methanol): 211 and 243 nm. ^1H NMR (pyridine- d_5 vs TMS): 4.48 (2H, br), 5.08 (1H, dd, $J = 10.3, 1.3$ Hz), 5.27 (1H, dd, $J = 17.2, 1.4$ Hz), 6.05 (1H, m), 8.46 (1H, br). The mass spectrum of this compound is present in Figure 4. The strong fragment of m/z 41 suggests the presence of the allyl group in this structure. The fragment of m/z 56 could be $\text{CH}_2=\text{CH}-\text{CH}=\text{NH}_2^+$, and the fragment of m/z 141 is obtained after the double bond on the allyl group is shifted to the adjacent carbon followed by a loss of a methyl group. Combining the mass-spectral and ^1H -NMR data, this compound was proposed as *N,N'*-diallylthiourea. This structure was also confirmed by the mass-spectral and ^1H -NMR data of the synthesized compound.

N,N'-diallylthiourea was reported to be one of the major degradation products from AITC when AITC was incubated at 37 °C for a few days (Kawakishi and Namiki, 1969). However, there was insufficient data in the report of Kawakishi and Namiki (1969) to support this identification. Our study confirmed that *N,N'*-diallylthiourea was the major nonvolatile compound in the thermal degradation of AITC.

The mechanisms for the formation of *N,N'*-diallylthiourea were proposed by Kawakishi and Namiki (1969) as shown in Figure 5. AITC is hydrolyzed to allylamine, which then reacts with AITC to generate *N,N'*-diallylthiourea. This pathway was further confirmed by the reaction of allylamine and AITC for the synthesis of *N,N'*-diallylthiourea.

Conclusion. Our study demonstrate that AITC, a pungent mustardlike flavor compound, is a very unstable compound. It can be decomposed to aliphatic and cyclic sulfur-containing compounds with a garliclike flavor note under cooking condition. AITC can also be decomposed to nonvolatile compound *N,N'*-diallylthiourea. The disubstituted alkylthioureas have been reported to possess the activity against insect pests and spider mites (Pascual and Rindlisbacher, 1994). The biological activity of *N,N'*-diallylthiourea needs to be addressed. The generation of *N,N'*-diallylthiourea from foods containing AITC under cooking or storage conditions also need to be further studied.

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