Decomposition Products of Allyl Isothiocyanate in Aqueous Solutions

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Solutions containing 1000 mg kg^{-1} of allyl isothiocyanate (AITC) in buffers of pH 4, 6, and 8 were stored at 80 °C and analyzed for the content of AITC, its isomerization product allyl thiocyanate (ATC), and 9 other decomposition products. The major reaction products in alkaline buffers were allylamine (AA), allyl dithiocarbamate (ADTC), diallylthiourea (DATU), and carbon disulfide (CDS). CDS and AA also arose as the major products in buffers of pH 6 and 4. Newly identified decomposition products of AITC were ADTC, diallylurea (DAU), and diallyl sulfide (DAS). Mechanisms explaining the decomposition of AITC are presented and discussed.

Keywords: *Allyl isothiocyanate; degradation products; allyl thiocyanate; allylamine; diallylthiourea; diallylurea; allyl dithiocarbamate; allyl allyldithiocarbamate; allyl mercaptan; diallyl sulfide; diallyl disulfide; carbon disulfide; HPLC; GLC*

INTRODUCTION

Isothiocyanates (ITCs), also referred to as mustard oils, have been found in a number of higher plants either in free form or as glucosinolates from which they are set free by enzymatic hydrolysis or by chemical conversion.

Allyl isothiocyanate (AITC) is one of the most widespread isothiocyanates. In plants, it is formed from the widely occurring glucosinolate sinigrin. AITC is the principal component of essential oils of numerous economically valuable vegetables, condiments, and oilseeds belonging to the Brassicaceae family, which are consumed via foliage (cabbage, Brussels sprouts), roots (horseradish), oilseeds (mustard), and meals derived from these plants. Some foods contain significant amounts of AITC. For example, 3−17 mg kg^{-1} of AITC have been found in shredded cabbage and coleslaw, respectively, and 1.3−1.8 g kg^{-1} volatile oil primarily consisting of AITC has been recovered from various horseradish roots (Delaquis and Mazza, 1995); requirements specified for mustard seed include AITC level >7 g kg^{-1} in *Brassica juncea* and >10 g kg^{-1} in *Brassica nigra* seeds (International Organisation for Standardization, 1987). The estimated world consumption for AITC (per year) via direct plant material and synthetic product is 455 000 and 79 000 kg, respectively (Clark, 1992).

AITC is an exceptionally pungent compound (showing organoleptic properties similar to those of other alkyl and aryl isothiocyanates), a strong lachrymator, and a skin vesicant. Nevertheless, this compound is used as a flavoring agent (e.g. in imitations of mayonnaise), as a flavor fortifier (for mustard and horseradish), and in so called "negative perfumery" as a repellent odor for cats and dogs and as a denaturant for ethanol etc. AITC appears on the FEMA/GRAS list but is not recommended for use in fragrances by the IFRA (Clark, 1992).

The research on ITCs and similar compounds such as thiocyanates, cyanates, and isocyanates has been mainly directed toward syntheses of pesticides and other agrochemicals, their degradation products, and metabolites. Similarly, AITC is a toxic compound showing a wide range of biological activities. Owing to the electrophilic character of the isothiocyanate functional group, the toxicity is assumed to be associated with reactions of AITC with biological thiols, amines, and alcohols *in vivo*. AITC (and its homologue methyl isothiocyanate) is used as a multipurpose soil fumigant for control of nematodes, soil fungi, soil insects, and weed seeds (Anonymous, 1992).

Use of AITC as a potent volatile antimicrobial compound (in preparations containing synthetic AITC, aqueous and/or alcoholic extracts of mustard seed) has been well-known for years (Drobnica et al., 1967). Its use is currently limited, but recent research suggests the compound may have useful applications in packaged foods such as meat and meat products (Delaquis and Mazza, 1995).

AITC has been shown to be goitrogenic, but its goitrogenic activity (compared to stronger goitrogens such as goitrin) is almost negligible (Duncan, 1991). It depresses thyroidal iodine concentration as well as thyroidal hormone biosynthesis (Langer and Greer, 1968).

AITC showed mutagenicity on *Salmonella typhimurium* TA 100 and had the highest potency among other ITCs studied (Yamaguchi, 1980).

Reaction of AITC and other isothiocyanates with proteins of *Brassica* seed meals leads to partial transformation of proteins to the modified ones, which may be indigestible (Kawakishi and Kaneko, 1987).

Recent data indicate that AITC and ITCs as a group are one of a class of phytochemicals offering significant hope in preventing the development of cancer as potent anticarcinogens and may play a role in the future to construct "designer foods" used to control this disease (Caragay, 1992).

Surprisingly little is still known about the fate of ITCs in the environment, foods, or biological systems, including animals and humans. It was shown that AITC also arises by isomerization of allyl thiocyanate (ATC) in organic solvents (Smith and Emerson, 1960). Partial isomerization of both compounds to each other was also detectable in commercial preparations of AITC and ATC

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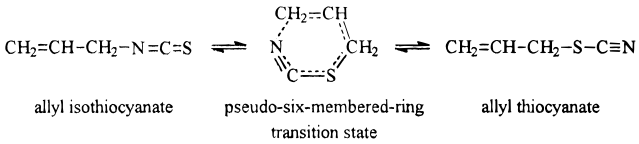
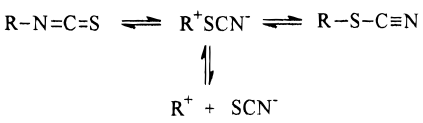
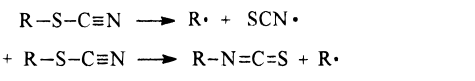
Scheme 1. Major Path of AITC and ATC Isomerization



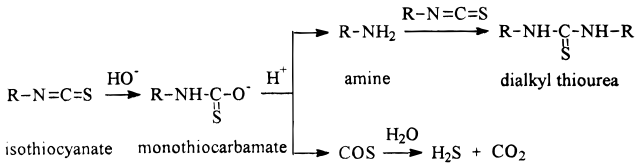
Scheme 2. Heterolytic Cleavage of ITCs



Scheme 3. Homolytic Cleavage of ITCs



Scheme 4. Decomposition of ITCs



and during gas chromatography at higher injector temperatures (Slater, 1992). Little of this isomerization has been shown to take place under physiological conditions.

The rearrangement of ITCs to thiocyanates (TCs) and *vice versa*, R−N=C=S ↔ R−S−C≡N, may generally occur by way of several mechanisms with sufficient *a priori* probability according to the nature of the organic residue R (Drobnica et al., 1977). The rearrangement passes through a cyclic intramolecular process involving an allylic shift, which is especially characteristic for AITC and ATC isomerization in organic solvents (Smith and Emerson, 1960) (Scheme 1). In addition to the intramolecular path, there are other general paths of ITC rearrangement. One such path is ionization and recombination of ions (Scheme 2). Finally, the analogous homolytic cleavage into alkyl and thiocyanogen radicals followed by their recombination may lead to the same product (Scheme 3).

The reaction pathway currently proposed for ITC decomposition in alkaline medium involves irreversible addition of hydroxyl ion to the carbon atom of the isothiocyanate group and formation of monothiocarbamates (Scheme 4). There exists an equilibrium of thiono and thiolo forms, which is almost entirely shifted to the side of thiolo structure (R−NH−CO−S$^-$) owing to the difficulty with which sulfur atoms form double bonds (Drobnica et al., 1977). In strongly alkaline solutions thiocarbamates are stable. In weakly alkaline, neutral, and acid medium, amine and carbonyl sulfide are formed; the latter compound further decomposes to hydrogen sulfide and carbon dioxide. At higher temperatures decomposition takes place even in an alkaline medium. The amine formed can further react with ITC, producing N,N′-disubstituted thiourea (Scheme 4).

Four substances formed by AITC decomposition in aqueous medium at 37 °C were identified, being diallylthiourea, allyl allyldithiocarbamate, diallyl tetrasulfide, and diallyl pentasulfide. Mechanisms of some of these product formations have been presented (Kawakishi and Namiki, 1969). AITC also decomposed to a small extent to thiocyanate (rhodanide) ions. About 5% of AITC was transformed to these ions after storage for 10 days of AITC solution (4 mmol dm^{-3}) at 37 °C. Other reaction products formed were a paraffin-like hydrocarbon and elemental sulfur, which accounted for ∼50% of AITC decomposition products after 10 days of storage of AITC solution.

Physiological properties of AITC decomposition products are almost unknown. Some compounds of similar structure (allylthiourea) showed mutagenicity, though it was of moderate degree. No significant mutagenicity was shown by allyl alcohol, AA, allylurea, and allyl cyanide (Yamaguchi, 1980). Various *N*-(*N*-substituted thiocarbamoyl)cysteines obtained from Cys and various ITCs and some substituted thioureas possessed antimicrobial activity (Kojima and Kado, 1976). 1-Carboxyalkyl-3-substituted-2-thioureas derived from amino acids [RNHCSNH(CH$_2$)$_n$COOH] showed fungicidal properties (Dash and Mahapatra, 1973).

Despite years of study, the decomposition products of AITC and the corresponding mechanisms still are not completely understood as well as factors influencing their formation, stability, and secondary reactions. The research described here was intended to determine AITC stability in aqueous solutions at different pH values and at different temperatures. This work was part of a study dealing with reactions of AITC with amino acids. It was intended to identify and quantify the AITC decomposition products that may have arisen either in solutions of AITC alone or in solutions containing AITC and amino acids. Such a knowledege is a prerequisite in understanding the mechanisms of action of AITC and its toxic and/or protective effects.

MATERIALS AND METHODS

Materials. Allyl isothiocyanate (AITC), diallylurea (DAU), diallylthiourea (DATU), allyl mercaptan (AM), and diallyl sulfide (DAS) were products of Aldrich Chemie (Germany); allylamine (AA) and diallyl disulfide (DADS) were obtained from Fluka Chemika (Switzerland), and carbon disulfide (CDS) was produced by Ferak (Germany). Allyl thiocyanate (ATC) was synthesized according to the method of Slater (1992), and allyl dithiocarbamate sodium salt (ADTC) and allyl allyldithiocarbamate (AADTC) were prepared according to the procedure described by Kawakishi and Namiki (1969). The purity of the synthesized chemicals, verified by HPLC method, was >95%. Buffers of pH 4, 6, and 8 were solutions of 0.2 M disodium hydrogen phosphate and 0.1 M citric acid. Trifluoroacetic anhydride (TFAA) in dichloromethane (1:3, v/v) was used as the acylation reagent.

Model Solutions. Aqueous solutions (a series of 20 mL vials provided with a silicone rubber cap) containing 1 g/L of AITC (10.1 mM/L) in buffers of pH 4, 6, and 8 were held at 20, 40, and 80 °C for 80 min in a water bath. In intervals of 10 min the vials were cooled, and aliquots of 2 mL were taken and analyzed using either GLC (AA, DAU, and DATU as their trifluoroacetates) or HPLC (all other compounds). Two parallel solutions were analyzed in all cases, and the average values were calculated.

GLC Analysis. A Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (FID) and a fused silica capillary column with HP-5 (30 m × 0.25 mm i.d., Hewlett-Packard) was used. The operating conditions were as follows: injector, 180 °C; detector, 250 °C; HP-5 column program, from 40 to 230 °C (at a rate of 4 °C/min); HP-INNOWax column program, from 80 to 250 °C (5 °C/min); nitrogen carrier gas flow rate, 2 mL/min.

Aliquots of the reaction mixtures (2 mL) were extracted with two 2 mL portions of dichloromethane, the combined extracts were evaporated to dryness, and the residue was acylated with 0.3 mL of TFAA in dichloromethane (1:3, v/v) for 30 min at room temperature. An aliquot of 1 μL was analyzed by GLC.

Table 1. Products Arising from AITC at pH 4[a]

	product (in mM/L)											
time (min)	AITC	ATC	DAU	DATU	ADTC	AADTC	AA	CDS	AM	DAS	DADS	total
0	10.1	1.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.6
10	8.7	1.8	nd	nd	nd	nd	0.1	0.1	nd	nd	nd	10.7
20	6.1	3.4	nd	nd	nd	nd	0.1	1.0	nd	0.2	nd	10.7
30	4.2	3.7	nd	nd	nd	nd	0.4	1.4	nd	0.2	nd	9.9
40	4.1	4.0	nd	nd	nd	nd	0.8	1.5	nd	0.4	nd	10.8
50	3.7	4.2	nd	nd	nd	nd	1.1	2.3	nd	0.6	tr	11.9
60	3.1	4.0	nd	nd	nd	nd	1.5	2.4	tr	0.6	tr	11.5
70	2.4	4.0	nd	nd	nd	0.1	2.1	2.5	tr	0.7	tr	11.7
80	2.0	4.0	nd	nd	nd	0.1	2.4	2.6	tr	0.7	tr	11.8

[a] tr, traces (0.01−0.04 mmol dm^{-3}); nd, not detected.

Table 2. Products Arising from AITC at pH 6[a]

	product (in mM/L)											
time (min)	AITC	ATC	DAU	DATU	ADTC	AADTC	AA	CDS	AM	DAS	DADS	total
0	10.1	1.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.0
10	5.1	3.4	nd	nd	nd	tr	0.3	0.2	nd	0.2	tr	9.2
20	3.1	3.6	nd	nd	nd	tr	0.8	0.3	tr	0.3	tr	8.1
30	1.3	3.1	nd	nd	nd	0.1	1.7	0.7	tr	0.4	tr	7.4
40	0.7	2.7	nd	nd	nd	0.2	2.5	0.9	tr	0.4	tr	7.2
50	0.3	2.4	nd	tr	nd	0.2	2.9	1.5	0.1	0.3	tr	7.6
60	0.2	1.7	nd	tr	nd	0.2	3.1	1.7	0.1	0.2	tr	7.3
70	0.1	1.2	nd	tr	nd	0.2	2.7	1.7	0.2	0.2	tr	6.4
80	0.1	1.0	nd	tr	nd	0.2	1.4	2.0	0.3	0.2	tr	5.2

[a] tr, traces (0.01−0.04 mmol dm^{-3}); nd, not detected.

Table 3. Products Arising from AITC at pH 8[a]

	product (in mM/L)											
time (min)	AITC	ATC	DAU	DATU	ADTC	AADTC	AA	CDS	AM	DAS	DADS	total
0	10.1	2.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.2
10	8.0	2.4	nd	tr	1.9	nd	0.5	nd	nd	nd	tr	12.8
20	5.4	3.1	tr	0.3	2.6	0.1	1.2	tr	nd	nd	tr	12.7
30	2.9	3.5	0.1	0.6	2.9	0.2	2.1	0.1	tr	0	tr	11.4
40	1.7	3.3	0.1	0.7	3.1	0.2	2.9	0.3	tr	0	tr	12.1
50	0.7	2.6	0.2	0.9	3.1	0.2	3.6	0.3	tr	0.1	tr	11.8
60	0.2	2.2	0.1	0.6	3.1	0.1	4.2	0.5	tr	0.1	tr	11.1
70	0.1	1.4	0.1	0.9	3.1	0.8	4.6	0.6	tr	0.2	tr	11.8
80	0.1	0.3	0.2	1.1	3.1	0.6	5.0	1.1	tr	0.3	0.1	11.7

[a] tr, traces (0.01−0.04 mmol dm^{-3}); nd, not detected.

Retention time data were as follows: AA, 4.0 min; DAU, 16.0 min; and DATU, 22.3 min.

HPLC Analysis. All analytes in model solutions were analyzed simultaneously by RP-HPLC/UV using the apparatus consisting of programmable isocratic pump and fluid metering pump in binary arrangement, autosampler, UV detector with variable wavelength operating at 205 nm, and vacuum membrane degasser (Thermo Separation Products, Inc., U.S.A.). The pump system was completed by gradient dynamic mixer. A Nova Pak C_{18}, 250 × 4.6 mm, 4 μm column, equipped with a Guard-Pak Nova Pak C_{18} precolumn (Waters, U.S.A.) was used. Operating conditions were as follows: gradient elution by acetonitrile (A)/water (B) mixtures at room temperature and a flow rate of 1 mL/min (A:B = 0:100 for 2 min, 2−15 min a gradient of A:B = 65:35, 15−20 min A:B = 65:35, 20−30 min a gradient of A:B = 0:100 v/v). Aliquots of the reaction mixtures (20 μL) were analyzed.

Retention time data were as follows: ADTC, 2.5 min; ATC, 13.0 min; AM, 14.9 min; AITC, 16.8 min; AADTC, 17.5 min; CDS, 18.0 min; DAS, 19.0 min; DADS, 21.3 min.

Identification of Products. Identification of ATC, DAU, DATU, AA, AM, DAS, and DADS was performed by GC/MS using a Hewlett-Packard G1800A apparatus equipped with a HP-5 or HP-INNOWax column operating under the conditions described above (helium carrier gas flow rate was 2 mL/min). Mass spectra were obtained by EI ionization at 70 eV.

RESULTS AND DISCUSSION

The results presented in Tables 1−3 show the complexity of AITC decomposition reactions, which are significantly controlled by the pH of the reaction medium. Therefore, neutral, acid, and alkaline solutions were studied and compared. Ten different reaction products were identified and quantified in solutions arising at 80 °C. The same products (occurring in lower amounts) were also detected in solutions stored at 40 and 20 °C. The same products also arise in ATC solutions stored at the same conditions (Velíšek and Pecháček, 1997).

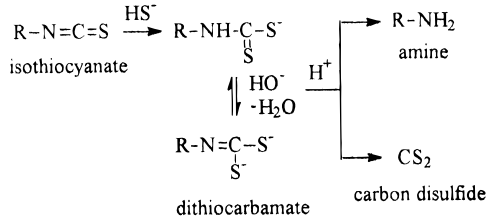
The total amount of the analyzed compounds remained practically constant in solutions of pH 4 and 8 but decreased to about 50% in solutions of pH 6, probably due to the formation of elemental sulfur. Concentrations of elemental sulfur and of thiocyanate ions were determined as the decomposition products of AITC by Kawakishi and Namiki (1969).

Newly identified AITC decomposition products were DAU, ADTC, and DAS. Kawakishi and Namiki (1969) identified DATU and AADTC as the decomposition products of AITC in water at 37 °C and proposed the formation of AA, AM, and DADS as potential reaction intermediates.

The rates of AITC decomposition in solutions of pH 6 and 8 are practically comparable. The slowest decomposition of AITC is found in solutions of pH 4.

On the basis of the facts presented in Schemes 1−4 it is possible to propose almost the complete reaction

Scheme 5. Formation and Decomposition of Dithiocarbamates



pathway for the decomposition of AITC in acid, neutral, and weakly alkaline medium.

Commercial preparations of natural or synthetic AITC always contain ATC and *vice versa*. Nevertheless, ATC further arises by isomerization of AITC in aqueous media and decomposes, possibly by both homolytic and heterolytic cleavages, in neutral and alkaline solutions. The rate of isomerization is relatively independent of solvent polarity, salt concentration, and other effects (Ben-Efraim, 1977).

As can be seen from Tables 1−3, the major product of AITC decomposition is AA. The compound arises in highest amounts in alkaline medium and reacts with AITC under the formation of DATU and possibly also enters some other reactions. DATU is partly desulfurized to DAU in alkaline medium.

In a consecutive reaction HS^- anions formed by decomposition of carbonyl sulfide (all thiol compounds react in dissociated form) react with AITC to yield allyl dithiocarbamate, which is responsible for the formation of other products (Scheme 5). Both S atoms are equivalent in strongly alkaline media (Drobnica et al., 1977). Alkyl dithiocarbamates are labile compounds, and their acid decomposition in mild acid medium and also in neutral and weakly alkaline medium leads to AA and CDS. The latter compound is the major decomposition product of AITC in pH 4. When the decomposition temperatures are low, formation of the side products via the corresponding monothiocarbamate (COS, H_2S and CO_2) at the expense of CS_2 is unavoidable (Schwack and Nyanzi, 1994).

In acid and weakly alkaline medium, oxidation of *N*-alkyl dithiocarbamates by air may further lead to *N*,*N*′-dialkyl thiuramdisulfides, which in alkaline medium decompose to ITCs and elemental sulfur or yield amine and carbon disulfide or a mixture of carbon disulfide, carbonyl sulfide, hydrogen disulfide, and carbon dioxide (Drobnica et al., 1977). The same reaction probably occurs in AITC solutions.

The heterolytic cleavage of ATC (Scheme 2) leads to thiocyanate ions. Another reaction by which the thiocyanate ions may arise is the alkaline degradation of AITC. In this case allyl alcohol would also arise as the second reaction product. However, allyl alcohol was not identified in our experiments. The homolytic cleavage of ATC is more probable. In the case of the homolytic cleavage (Scheme 3), the presence of free allyl radicals (CH_2═CH−CH_2•) would be expected to lead to side products resulting from olefinic polymerization. Such a product, a hydrocarbon-like substance, was really identified as a product of AITC decomposition in aqueous solutions at 37 °C (Kawakishi and Namiki, 1969). Furthermore, an unsaturated hydrocarbon-like substance was detected in a commercial AITC preparation by GC/MS (Slater, 1992).

Organic TCs react with alkali in aqueous solutions and yield the corresponding thiols or thiolate anions, RS^- or cyanides (Ben-Efraim, 1977). Kawakishi and Namiki (1969) did not detect cyanide ions. The former reaction is then probably the main source of CH_2═CH−CH_2^- anions and AM, respectively. The highest amounts of AM arise in solutions of pH 6. Oxidation of AM by air (even at low temperatures) then affords DADS. The formation of the allyl thiolate is believed to be the initial step of the reaction (Ohno and Oae, 1977). The second step is the formation of a thiyl radical by electron transfer between the thiolate anion and oxygen. Hence, the reaction is very much promoted under alkaline conditions (Scheme 6). DADS may also arise by reaction of ATC with AM in alkaline medium (R−SCN + R-SH → R-S−S−R + HCN; Field, 1977), but this reaction is also very improbable.

Scheme 6. Autoxidation of Thiols

$$RSH + HO^- \rightleftharpoons RS^- + H_2O$$
$$RS^- + O_2 \rightleftharpoons RS\bullet + O_2^-\bullet$$
$$2\,RS\bullet \longrightarrow RSSR$$
$$RSH + O_2^-\bullet \longrightarrow RS\bullet + HO_2^-$$
$$RSH + HO_2^- \longrightarrow RS\bullet + HO^- + HO\bullet$$
$$RSH + HO\bullet \longrightarrow RS\bullet + H_2O$$

Scheme 7. Dismutation of Disulfides

$$RSSR \longrightarrow RSS\bullet + R\bullet$$
$$RSSR + R\bullet \longrightarrow RSR + RS\bullet$$
$$RSSR + RS\bullet \longrightarrow RSSSR + R\bullet$$

Homolytic scission of the −S−S− bond of disulfides (R−S−S−R → 2R−S•) occurs in photolysis and certain other reactions (Field, 1977). However, the most probable is the homolytic cleavage of the C−S bond in DADS, which also predominates during the thermal degradation of DADS (Block et al., 1988), and additional routes (dismutation) leading to DAS and diallyl polysulfides can be proposed (Scheme 7). Similar reactions leading to DAS and diallyl polysulfides are also proposed in aqueous garlic preparations (Block, 1992).

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