Decomposition of Allyl Isothiocyanate in Aqueous Solution

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Allyl isothiocyanate was gradually decomposed in aqueous solution to produce a garlic like odor. The decomposition of this isothiocyanate was not based on hydrolysis of R-NCS as in the case of *p*-hydroxybenzyl isothiocyanate, but the addition reaction on --N=C=S . Four substances formed with the decomposition of the isothiocyanate were isolated, and their chemical constitutions were clarified. Allyl isothiocyanate was decomposed to allyl allyldithiocarbamate (III), which was degraded to diallyl tetra- and penta-sulfide (II), and this poly-sulfide was further degraded to paraffin like hydrocarbon (I) and sulfur. Moreover, N,N'-diallylthiourea (IV) was produced by the addition reaction of allylamine, formed from the isothiocyanate by action of water, to residual isothiocyanate.

It is well known that a mustard oil (alkyl isothiocyanate), in general, is unstable and apt to decompose, as shown in the cases of *p*-hydroxybenzyl isothiocyanate,^{1,2)} 3-indolylmethyl isothiocyanate^{3,4)} and 1-methoxy-3-indolylmethyl isothiocyanate⁵⁾ formed from sinalbin, glucobrassicin and neoglucobrassicin, respectively. The decomposition reaction of these isothiocyanates is mainly hydrolysis: $\text{R-NCS} \rightarrow \text{R-OH} + \text{SCN}^-$. Allyl isothiocyanate, in naturally, is formed from its precursor-glucoside sinigrin by action of myrosinase. Sinigrin is widely present in seed, root and leaf of many *Cruciferae*, namely in Wasabi (*Eutrema wasabi* Maxim), horse radish (*Cochlearia armoracia* L.), black mustard (*Brassica nigra* L.) and *etc.* This isothiocyanate formed by crushing of these plant tissues, however, is gradually decomposed by standing for a long

time at room temperature and produced a garlic like odor, and the rate of its decomposition is more slower as compared with *p*-hydroxybenzyl isothiocyanate. It seemed to be considered that the difference of decomposition rate in several isothiocyanate depends upon the kinds of their alkyl group and they give some influences for decomposition route of isothiocyanate. Water, also, may be performed an important role in their decomposition.

The present work was undertaken to identify the decomposition products of allyl isothiocyanate in aqueous solution, to make clear its decomposition mechanism, and to compare with that of *p*-hydroxybenzyl isothiocyanate.

The reaction mixtures of sinigrin-myrosinase and the aqueous solution of allyl isothiocyanate were prepared in sealed vessels and incubated at 37°C for several days. The amounts of residual isothiocyanate and formed SCN^- were determined in time course by the method of Z. Nagashima⁶⁾ and the method described in

1) S. Kawakishi and K. Muramatsu, *Agr. Biol. Chem.*, **30**, 688 (1966).

2) S. Kawakishi, M. Namiki, H. Watanabe and K. Muramatsu, *ibid.*, **31**, 823 (1967).

3) R. Gmelin, M. Saarivirta and A. I. Virtanen, *Suomen Kemistilehti*, **B33**, 172 (1967).

4) R. Gmelin und A. I. Virtanen, *ibid.*, **B34**, 15 (1961).

5) R. Gmelin und A. I. Virtanen, *Acta Chem. Scand.*, **16**, 1378 (1962).

6) Z. Nagashima and M. Uchiyama, *Nippon Nogei-kagaku Kaishi*, **31**, 416 (1957).

FIG. 1. Decomposition of Allyl Isothiocyanate in the Reaction Mixture of Sinigrin-Myrosinase.

○—○ allyl isothiocyanate

×—× SCN^-

The amounts of allyl isothiocyanate in zero time shows with the value determined after two hours in incubation at 37°C.

FIG. 2. Decomposition of Allyl Isothiocyanate in the Aqueous solution.

○—○ allyl isothiocyanate

△—△ elemental sulfur

×—× SCN^-

the previous paper,¹⁾ respectively. As shown in Figs. 1 and 2, allyl isothiocyanate was slowly decomposed and its residual amounts were reached to about ten and twenty five per cent of the initial state within ten days in the reaction mixture and the aqueous solution, respectively, and the formation of SCN^- from this isothiocyanate was negligible.

This suggested that hydrolysis of allyl isothiocyanate is not proceeded like the case of *p*-hydroxybenzyl derivative,^{1,2)} but water have a part in its decomposition. Since elemental sulfur was also formed during decomposition of the isothiocyanate, sulfur was determined by the titration method using sodium cyanide,⁷⁾ and the amounts of sulfur formed in aqueous solution were corresponded to about fourteen per cent of the decomposed isothiocyanate (Fig. 2). Changes in UV spectra of allyl isothiocyanate aqueous solution by the time course are shown in Fig. 3. This isothiocyanate

FIG. 3. Change in UV Spectra of Allyl isothiocyanate Aqueous Solution.

(1) in zero time

(2) after two days

(3) after nine days

exhibits an absorption maximum near 240 $m\mu$ in aqueous solution, and this maximum did not much changes by its decomposition process, while a new absorption maximum at 260~270 $m\mu$ became to be observed. This was assumed to be caused by the decomposition products of the isothiocyanate. After the incubation of this isothiocyanate aqueous solution at 37°C

7) D. A. Skoog and J. K. Bartlett, *Anal. Chem.*, **27**, 369 (1955).

FIG. 4. Thin-layer Chromatogram of the Decomposition Products in Aqueous Solution.

Sample: ether extract of allyl isothiocyanate aqueous solution incubated at 37°C for a long time.

Solvent: *n*-hexane: acetone (9:1).

Coloring reagent: iodine-azide reagent and Dragendorff reagent (III and IV, positive).

for several days, the solution was extracted with ether, and the extract was chromatographed on silica gel plate as shown in Fig. 4, and many spots based on the decomposition products were found. From this chromatograms, it was deduced that four spots (I, II, III and IV) are due to main decomposition products of the isothiocyanate, and among them the containing of sulfur in II, III, IV and of nitrogen in III, IV could be detected by using iodine-azide and Dragendorff reagent, respectively. So, the isolation of these products from allyl isothiocyanate aqueous solution, in which about eighty per cent of this isothiocyanate were disappeared by incubation at 37°C for long time, was attempted. The aqueous solution was extracted with ether, and elemental sulfur as S_8 , mp 119°C, was crystallized from the concentrate of ether extracts, and the concentrate, after removal of sulfur, was chromatographed on silica gel column using *n*-hexane-ether. Each fraction, in which involved I, II, III and IV, respec-

tively, was purified by repeated column chromatography, and four substances given a single spot on thin-layer chromatograms were obtained.

The product I was colorless, odorless and oily liquid, and its IR spectrum was essentially identical with that of liquid paraffin. I was assumed to be a hydrocarbon like substance not containing nitrogen and sulfur.

The product II was pale yellow liquid and had strongly garlic like odor. Elementary analysis of II showed C 31.83, H 3.99, and S 62.82%, but these values were not corresponded with the calculated values for $C_6H_{10}S_4$ or $C_6H_{10}S_5$. Its molecular weight was 223.6 by use of vapor pressure osmometer and did not agreed with the values calculated from both formula. However, if II was a mixture of $C_6H_{10}S_4$ and $C_6H_{10}S_5$ in six to four ratio, its analytical values and molecular weight were well corresponded with the found. II exhibited a broad absorption band near 290 $m\mu$ (shoulder, $E_{1\%}^{1cm}$ 132). It is known that dialkyl disulfide shows a broad absorption band near 250 $m\mu$ (ϵ ca. 500) and in the case of polysulfide, the absorption band is displaced to longer wave lengths by the introduction of further sulfur atoms in the sulfur chain, and for example, diethyl tetrasulfide shows λ_{max} ca.

FIG. 5. NMR Spectrum of the Product II in $CDCl_3$.

290 $m\mu$ (ϵ 2400).⁸⁾ From these absorption properties, it was assumed that II is polysulfide or a mixture of polysulfide. IR spectrum showed the presence of allyl group (1635, 982 and 917 cm^{-1}) only and this was supported by NMR data (Fig. 5): δ 3.58 ppm (2H, Ha), 5.15 ppm (1H, Hb or Hc), 5.28 ppm (1H, Hc or Hb) and 5.80 ppm (1H, Hd). These data suggested that hydrogen atoms of II are based on allyl group only and II contains two allyl groups which are composed of symmetrically in its molecule. If II was estimated to diallyl polysulfide ($\text{S}=4\sim 5$), its mass spectrum (Fig. 6)

FIG. 6. Mass Spectrum of the Product II.

was illustrated reasonably: m/e 242 ($\text{CH}_2=\text{CHCH}_2-\text{S}_5-\text{CH}_2\text{CH}=\text{CH}_2$)⁺, 210 ($\text{CH}_2=\text{CHCH}_2-\text{S}_4-\text{CH}_2\text{CH}=\text{CH}_2$)⁺, 137 $\text{CH}_2=\text{CHCH}_2-\text{S}_3$ ⁺, 105 $\text{CH}_2=\text{CHCH}_2-\text{S}_2$ ⁺ and 73 $\text{CH}_2=\text{CHCH}_2-\text{S}$ ⁺. Then, II was deduced to be the mixture of diallyl tetra- and pentasulfide.

The product III was yellow liquid odoring like garlic and this contained of nitrogen and sulfur in its molecule from the color reaction using Dragendorff and iodine-azide reagents on silica gel plate. IR spectrum in chloroform solution suggested the presence of thioamide group (3400, 1500 and 1372 cm^{-1}) and allyl group (3090, 1640, 985 and 925 cm^{-1}). Molecular weight of III was determined as 173 by

mass spectroscopy. The presence of thioamide group was also supported by UV absorption maximum: $\lambda_{\text{max}}^{\text{EtOH}}$ 254 $m\mu$ ($\log \epsilon$ 4.01) and 275 $m\mu$ ($\log \epsilon$ 3.93) and the reaction with iodine-azide solution. Assuming the constitution of III to allyl allyldithiocarbamate from these results, the fragment peaks on its mass spectrum (Fig. 7) are possible to assign: m/e 99 ($\text{CH}_2=\text{CHCH}_2-\dot{\text{N}}^+=\text{C}=\text{S}$), 74 ($\text{CH}_2=\text{CHCH}_2-\dot{\text{S}}\text{H}$), 72 ($\text{CH}_2=\text{N}^+=\text{C}=\text{S}$) and 41 ($\text{CH}_2=\text{CHCH}_2^+$). And the mechanism of these fragmentation was quite reasonably illustrated as Fig. 8. The degradation of $\text{CH}_2=\text{CHCH}_2-\dot{\text{N}}^+=\text{C}=\text{S}$ to $\text{CH}_2=\text{N}^+=\text{C}=\text{S}$ was supported by the presence of meta stable ion 52.3. Then, this dithiocarbamate was synthesized from allylamine to identify of III. Synthesized and isolated samples were

FIG. 7. Mass Spectrum of the Product III.

FIG. 8. Fragmentation Scheme of the Product III on its Mass Spectrometry.

8) H. P. Koch, *J. Chem., Soc.*, **1949**, 394.

FIG. 9. UV Spectrum of the Product III.
— isolated, ---- synthesized

FIG. 10. UV Spectrum of the Product IV.
— isolated, ---- synthesized

quite identical in IR, UV (Fig. 9) spectra and chromatographic behaviors.

The product IV was colorless plates, mp 49°C and given $C_7H_{12}N_2S$ as its molecular formula. From IR, UV spectra (Fig. 10) and color reaction, it was assumed to be N, N'-diallylthiourea and identified by comparison with synthesized one.

As mentioned above, the UV spectrum of allyl isothiocyanate in aqueous solution was

changed according to its decomposition. This changes are well illustrated with allyl allyldithiocarbamate (III) and N, N'-diallylthiourea (IV) formed from allyl isothiocyanate. The absorption near 240 and 270 mμ in spectrum (3) of Fig. 3 are corresponded to λ_{max} 243 mμ of IV and λ_{max} 275 mμ of III, respectively.

Allyl isothiocyanate was decomposed slower than *p*-hydroxybenzyl isothiocyanate, because the hydrolysis of allyl isothiocyanate did not proceed as follows: $R-N=C=S \rightarrow R-OH + NCS^-$. So, the decomposition of this isothiocyanate was based on the addition of water or other decomposition products to isothiocyanate part. Actually, it may be considered that the products II, III and IV are formed by addition reaction on its isothiocyanate part.

Formation of dithiocarbamate from isothiocyanate occurred also on this isothiocyanate, as in the case of *p*-hydroxybenzyl isothiocyanate.²¹ Therefore, it may be possible to consider that this is one of the general decomposition reaction of isothiocyanate. Allyl allyldithiocarbamate is probably formed by addition of allyl-S- compound, which is also secondary decomposition product of this isothiocyanate, to allyl isothiocyanate without any enzyme action.

Next, on diallyl polysulfide, it seemed that the degradation of the dithiocarbamate by water affords allyl mercaptane or allyl disulfide which polymerizes to polysulfide spontaneously. This was supported by the fact that diallyl polysulfide is formed gradually in water saturated ether solution of the dithiocarbamate. It is interesting that polysulfide was not formed from *p*-hydroxybenzyl isothiocyanate but disulfide was formed, and the reverse was the case of allyl derivative. Diallyl polysulfide was found in garlic oil already,⁹¹ but its properties and formation mechanism are unknown. Furthermore, the reaction of allyl isothiocyanate to diallyl polysulfide was also remained unknown.

9) F. W. Semmler, *Arch. Pharm.*, **230**, 434 (1892).

FIG. 11. Proposed Degradation Scheme of Allyl Isothiocyanate in Aqueous Solution.

On the formation of liquid paraffin like substance (I) and free sulfur, they may be formed by degradation of II, considering that II purified through silica gel column gradually redecomposed to I and sulfur. Therefore, it was assumed to be a polymer of allyl group for I. It was shown by old workers¹⁰⁾ that allyl isothiocyanate in aqueous solution gradually decomposes to allyl cyanide and sulfur. In our experiments using gas chromatography, however, allylcyanide was undetectable in allyl isothiocyanate aqueous solution kept for a long time. This was suggested that elemental sulfur is not directly produced from allyl isothiocyanate.

Finally, the formation of N,N'-diallylthiourea is presumed as follows; water addition to the isothiocyanate gives to allylthiocarbamic acid which affords allylamine by hydrolysis spontaneously, and the nucleophilic attack of allylamine to residual isothiocyanate produces N,N'-diallylthiourea. Relationship between each product on allyl isothiocyanate decomposition is proposed as shown in Fig. 11.

EXPERIMENTAL

UV absorption spectra were measured with Hitachi-

Perkin Elmer 139 and Hitachi EPS-2, IR spectra with Hitachi EPI-2 in chloroform solution and KBr disks, mass spectra with Hitachi mass spectrometer RMU-6D, NMR with JMN-4H-100 and molecular weight with Hewlett-Packard Vapor Pressure Osmometer.

Disappearance of allyl isothiocyanate in the reaction mixture of sinigrin-myrosinase and the isothiocyanate aqueous solution. The reaction mixture were prepared in sealed flask to prevent the volatilization of formed isothiocyanate, and its composition was as follows:

0.1M acetate buffer solution (pH 5.2)	100 ml
substrate solution (containing sinigrin	
0.51 × 10 ⁻³ mole)	 50 ml
enzyme solution (containing myrosinase	
preparation 80 mg)	 50 ml
total	200 ml

This solution was incubated at 37°C, and residual allyl isothiocyanate was determined in time course by the method of Z. Nagashima.⁶⁾ Sinigrin and myrosinase were prepared from black mustard and white mustard seed, respectively.¹¹⁾ Allyl isothiocyanate aqueous solution was used of freshly distilled commercial source in 200 ml of water. Formed SCN⁻ was determined by the colorimetric method described in the previous paper^{1,2)} and the determination of elemental sulfur was followed with the titration method using sodium cyanide by D. A. Skoog *et al.*⁷⁾

10) *Beilstein Handbuch der Organischen Chemie*, 4, 214 (1922).

11) Z. Nagashima and M. Uchiyama, *Nippon Nogei-kagaku Kaishi*, 33, 478 (1959).

Isolation of decomposition products. Ten grams of freshly distilled allyl isothiocyanate was dissolved in 5 liters of water and stood for about 1 month at 37°C. This solution was extracted with ether, and the ether extract was dried on anhydrous sodium sulfate. When the ether solution was concentrated, free sulfur was crystallized (yield 0.34 g), mp 119°C. After filtration of sulfur, the concentrate (5.53 g) was chromatographed over silica gel (80 g) column, and the elution was performed with 200 ml of *n*-hexane, 200 ml of *n*-hexane-ether (9:1 v/v), 200 ml of *n*-hexane-ether (1:1 v/v) and then 300 ml of ether, and 50 ml aliquots of fractions were collected. All fractions were submitted to thin-layer chromatography and the results are summarized in Table I.

TABLE I. SILICA GEL CHROMATOGRAPHY OF THE ETHER EXTRACT

Fractions	Solvents	Yield (g)	Products
2~4	<i>n</i> -hexane	0.43	I, sulfur (0.05 g)*
5~8	<i>n</i> -hexane-ether (9:1)	0.93	I (trace) and II
12~14	ether	0.34	III and IV (trace)
15~17	ether	1.05	IV

* It was crystallized from the fractions by standing for several hours at room temperature.

From fractions 2~4, 0.30 g of I and 0.05 g of sulfur were obtained. Fractions 5~8 were combined, concentrated and rechromatographed on silica gel using *n*-hexane to give II, 0.51 g, as a pure liquid. Fractions 12~14 were also chromatographed repeatedly on silica gel to give a yellow liquid of III, 0.05 g. Since fractions 15~17 contained of IV only, these fractions were combined, concentrated and kept in refrigerator for several hours to give plates of IV, mp 49°C (from 20% ethanol), yield 0.60 g.

Some properties and chemical structure of each products

Product I. This was odorless and colorless liquid hydrocarbon, in which was not contained of nitrogen and sulfur.

Product II. This was a pale yellow liquid and had a garlic like odor. The color reactions of iodine-azide and phloroglucinol-hydrochloric acid were positive. *Anal.* Found: C, 31.83; H, 3.99; S, 62.82% (cf. Calcd. for $C_6H_{10}S_4$: C, 34.29; H, 4.80; S, 60.91% and for $C_6H_{10}S_5$: C, 29.80; H, 4.16; S, 66.20%). Its molecular weight was determined by vapor pressure

osmometer method 223.6. Its molecular formula was not agreed with $C_6H_{10}S_4$ and $C_6H_{10}S_5$. UV absorption maximum: λ_{max}^{EtOH} 290 m μ ($E_{1\%}^{1cm}$ 132, shoulder). IR spectrum in chloroform solution: 1639, 982, 917 cm^{-1} ($-CH_2CH=CH_2$). NMR spectrum in $CDCl_3$ (Fig. 5): δ 3.58 (2H), 5.15 (1H), 5.28 (1H), 5.80 (1H) ppm ($\begin{smallmatrix} H \\ | \\ -CH_2-C=CH \\ | \\ H \end{smallmatrix}$). Mass spectrum (Fig. 6): m/e 242, 210, 137, 105, 73.

Product III. A yellow liquid, garlic like odor. The color reactions with iodine-azide and Dragendorff reagent were positive. Molecular weight, 173 (mass spectrometry). UV absorption maximum, λ_{max}^{EtOH} 254 m μ ($\log \epsilon$ 4.01), 275 m μ ($\log \epsilon$ 3.93) (Fig. 9). IR spectrum in chloroform solution: 3400, 1500, 1372 cm^{-1} ($-N-\overset{S}{\underset{H}{C}}-$) and 3090, 1640, 985, 925 cm^{-1} ($-CH_2-CH=CH_2$). Mass spectrum (Fig. 7): m/e 173 (M^+), 99, 74, 72, 41 and meta stable ion 52.3

$$\left(m^* = \frac{m_2}{m_1} = \frac{72^2}{99}\right)$$

Product IV. Plates from 20% ethanol, mp 49°C. Iodine-azide and Dragendorff reactions were both positive. λ_{max}^{EtOH} 243 m μ ($\log \epsilon$ 4.12) (Fig. 10). IR spectrum in KBr disk: 1649, 983, 925 cm^{-1} ($-CH_2CH=CH_2$) and 1550, 1220, 1075 cm^{-1} ($-N-\overset{S}{\underset{H}{C}}-$). *Anal.* Found:

C, 53.81; H, 7.73; N, 17.70; S, 20.43. Calcd. for $C_7H_{12}N_2S$: C, 53.83; H, 7.74; N, 17.94; S, 20.49%. The melting point of IV was undepressed by admixture with authentic N,N' -diallylthiourea which was synthesized from allylamine and allyl isothiocyanate. The isolated and authentic samples were identical in respects to UV (synthesized one: λ_{max}^{EtOH} 243 m μ , $\log \epsilon$ 4.13) (Fig. 10), IR spectra and thin-layer chromatographic behaviors.

Synthesis of allyl allyldithiocarbamate. To a mixture of allylamine (5.7 g) in ether and sodium hydroxide (4 g) in water, an ether solution of carbon disulfide (11.6 g) was added dropwise with stirring, and the white precipitate of Na allyldithiocarbamate was formed. The precipitates were collected, washed with ether, and then dried under reduced pressure. To a stirred suspension of Na allyldithiocarbamate (3 g) in ether, allyl bromide (2.3 g) in ether was added. The additional stirring was continued further for three hours at room temperature. The reaction mixture

was filtrated, and the filtrate was concentrated to give a yellow oil 0.55 g. This oil was chromatographed on silica gel using *n*-hexane-ether, and the fractions contained of allyl allyldithiocarbamate were collected, concentrated, and the repeated chromatography of this crude oil afforded pure oil, which gave a single spot of allyl allyldithiocarbamate on silica gel plate. Yield 0.44 g. *Anal.* Found: C, 48.05; H, 6.14; N, 8.15; S, 35.78. Calcd. for $C_7H_{11}NS_2$: C, 48.55; H, 6.40; N, 8.09; S, 36.96%. The UV absorption spectrum (Fig. 9) showed $\lambda_{\text{max}}^{\text{EtOH}}$ 254 m μ (log ϵ

4.03) and 275 m μ (log ϵ 3.99). IR spectrum was identical with the isolated one.

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