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# Degradation of 1,3-Diaryl-1-nitrosoureas in Aqueous Solutions and in Organic Solvents

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The degradation of 1,3-di(p,p'-disubstituted)aryl-1-nitrosoureas (I) in aqueous buffer solutions (at 0 °C over pH -0.5 to 5.4) and in several organic solvents (benzene, chloroform,*etc.* $) has been kinetically studied. In aqueous solution, the overall rates of degradation followed pseudo-first-order kinetics at constant pH. The decomposition reaction of 1,3-diphenyl-1-nitrosourea (Ia) was first-order in hydrogen ion over the pH range of -0.5 to 1.5 and denitrosated 1,3-diphenylurea (IIa) was formed predominantly. In the range of pH 1.5 to 5.4, the degradation of Ia was catalyzed by hydroxide ion and the main product was IIa, which was formed by the recombination of reactive products. In organic solvents, thermal degradation of Ia obeyed first-order kinetics and gave similar products to those yielded in the base-catalyzed degradation. The acid- and base-catalyzed and thermal degradations of Ia were about <math>10^2$  to  $10^3$  times more rapid than those of related 1,3-dialkyl-1-nitrosoureas. The reaction mechanisms are discussed.

**Keywords**—1,3-diaryl-1-nitrosourea; kinetics; acid catalysis; base catalysis; pyrolysis; denitrosation; degradation

It is well known that *N*-nitrosoureas [RN(NO)CONHR'] generally act on deoxyribonucleic acid (DNA) of cells as alkylating reagents and that the alkyl derivatives [ $R = CH_3$ ,  $C_2H_5$ ,  $C_3H_7$ ,  $C_4H_9$ ] are carcinogenic, while on the other hand, some of the 2-chloroethyl derivatives [ $R = ClCH_2CH_2$ ] are highly carcinostatic. The relationship between their chemical properties and biological activities (including carcinogenicity and antitumor activity), has been studied for more than ten years.<sup>1)</sup> A few years ago, we reported that some 1,3-diaryl-1-nitrosoureas (I)



are highly effective against rat asites hepatoma AH-13 cells.<sup>2)</sup> It was shown that they were fairly reactive as compared with the 1,3-dialkyl-1-nitrosoureas, and their chemical properties were also very different from those of the corresponding 1,3-dialkyl-1-nitrosoureas. Consequently, based on their chemical actions on DNA and protein model compounds, it was proposed that the manifestation of their antitumor activities is mainly due to the formation of active chemical species, a phenyldiazonium salt and a phenyl isocyanate, in tumor cells.<sup>3)</sup> However, the mode of degradation of 1,3-diaryl-1-nitrosoureas in aqueous solution, that may be closer to cellular conditions, is still not clear.

This paper describes kinetic studies on the degradation of 1,3-diaryl-1-nitrosoureas in

aqueous solutions and in organic solvents, in comparison with that of related 1,3-dialkyl-1nitrosoureas.

#### Experimental

Materials—All 1,3-diaryl-1-nitrosoureas [1,3-diphenyl-1-nitrosourea (Ia), 1,3-bis(4-tolyl)-1-nitrosourea (Ib), 1,3-bis(4-chlorophenyl)-1-nitrosourea (Ic)] used in this study were prepared by the method reported in our previous paper.<sup>4)</sup> Other organic chemicals used were purchased from Wako Pure Chemical Industries Co., Ltd. and Tokyo Kasei Industry Co., Ltd., "extra pure" grade. Organic solvents used were purchased from Wako Pure Chemical Industries Co., Ltd. and Ishizu Seiyaku Co., Ltd., "guaranteed" grade.

**Kinetics**—Rate constants for degradation were determined in the following manner. In aqueous solution: 1,3diaryl-1-nitrosoureas and internal standard were dissolved in acetone at 0 °C. The buffer solutions were cooled at 0 °C and mixed with the acetone solution. A volume of  $2\mu l$  of the reaction mixture was taken at timed intervals and immediately subjected to analysis by means of high-performance liquid chromatography (HPLC). In organic solvent: 1,3-diaryl-1-nitrosoureas and an internal standard were dissolved in organic solvent at the indicated temperature. The degradation of 1,3-diaryl-1-nitrosoureas was monitored by using HPLC as described below.

HPLC Analysis — Chromatography was performed with a system consisting of a Shimadzu model LC-6A pump equipped with a reverse phase column (Whatman Partisil 5-ODS-3,  $4.6 \times 150$  mm) and a Shimadzu model SPD-6AV UV-Vis spectrophotometric detector monitor. Peak areas of 1,3-diphenyl-1-nitrosourea were analyzed with a Shimadzu model C-R3A data processor. The mobile phase consisted of acetonitrile-water and the flow rate was 2 ml/min. The injection volume was 2  $\mu$ l, and an appropriate compound was used as an internal standard.

### Results

# Acid-Catalyzed Degradation

1,3-Diphenyl-1-nitrosourea (Ia) was decomposed at  $0 \,^{\circ}$ C in mixtures of acid solution (pH < 1.5) and acetone (50%, v/v). The rates of disappearance of Ia were measured by means of HPLC. As shown in Fig. 1 and Table I, the rate data adhere to a first-order rate law over the entire reaction process. The rate of disappearance of Ia depended upon the acid strength over



TABLE I. Degradation Rates<sup>a)</sup> of 1,3-Diphenyl-1-nitrosourea in Various pH Buffer<sup>b)</sup>

pН	$k_{obs}^{c)}(s^{-1})$	$\log k_{\rm obs}$	r <sup>d)</sup>
5.4 4.2 3.5 2.5 1.5 1.1 0.3 0.0 -0.2 (1.5 N HCl) -0.5	$3.53 \times 10^{-3} \\ 6.13 \times 10^{-4} \\ 1.73 \times 10^{-4} \\ 3.73 \times 10^{-5} \\ 5.75 \times 10^{-6} \\ 1.04 \times 10^{-5} \\ 5.25 \times 10^{-5} \\ 8.97 \times 10^{-5} \\ 3.13 \times 10^{-4} \\ 8.70 \times 10^{-4} \\ $	$\begin{array}{r} -2.45 \\ -3.21 \\ -3.76 \\ -4.42 \\ -5.24 \\ -4.98 \\ -4.27 \\ -4.05 \\ -3.50 \\ -3.06 \end{array}$	$\begin{array}{r} -0.972 \\ -0.990 \\ -0.997 \\ -0.987 \\ -0.984 \\ -0.995 \\ -0.999 \\ -0.999 \\ -0.999 \\ -0.992 \\ -0.995 \end{array}$
(3 N HCl)		2100	0.770

Fig. 1. Time Course of Disappearance of 1,3-Diphenyl-1-nitrosourea (Ia) and Formation of 1,3-Diphenylurea (IIa)

•, disappearance of 1,3-diphenyl-1-nitrosourea (Ia) at pH 0.3;  $\bigcirc$ , formation of 1,3-diphenylurea (IIa) at pH 0.3;  $\triangle$ , disappearance of 1,3-diphenyl-1-nitrosourea (Ia) at pH 4.2;  $\triangle$ , formation of 1,3-diphenylurea (IIa) at pH 4.2.

a) Determined by using HPLC. The mobile phase consisted of acetonitrile-water (5:5) and the flow rate was 2 ml/min. Naphthalene was used as an internal standard. 1,3-Diphenyl-1-nitrosourea (I) had an elution time of 3.0 min. b) Each run was carried out at  $0^{\circ}C \pm 0.2^{\circ}C$ . The reactions were initiated by mixing 1,3-diphenyl-1-nitrosourea (Ia) (1-2 mg) in acetone (2.5 ml) with Walpole's buffer (HCl-acetate buffer) (2.5 ml) or diluted HCl (2.5 ml). c) Rate constants were calculated by means of the least-squares method. d) Correlation coefficient between time and concentration of 1,3-diphenyl-1-nitrosourea (Ia).



Fig. 2. First-Order Plots of Disappearance of 1,3-Diphenyl-1-nitrosourea (Ia) at Several pH Values

 $\blacktriangle$ , acid-catalyzed reaction;  $\triangle$ , base-catalyzed reaction.

			pH 2.5				pH 0.3		
No.	R	σ	$k (s^{-1})^{b}$ (r) <sup>c)</sup>	log k	$\log k/k_0$	$k (s^{-1})^{b}$ (r) <sup>c)</sup>	log k	$\log k/k_0$	
Ia	н								
		0.00	$3.73 \times 10^{-5}$	-4.43	_	$5.25 \times 10^{-5}$	-5 -4.28		
		0.00	(-0.987)			(-0.999	)		
Ib	$CH_3$								
		-0.17	$2.33 \times 10^{-5}$	-4.63	-0.200	$8.12 \times 10^{-5}$	-5 -4.05	0.225	
		-0.12	(-0.987)			(-0.998	)		
Ic	Cl								
		0.23	$5.17 \times 10^{-4}$	- 3.29	1.14	$1.28 \times 10^{-5}$	-4 - 3.89	0.388	
		0.28	(-0.997)			(-0.998	)		

TABLE II. Substitution Effects of Degradation<sup>a)</sup> of 1,3-Diaryl-1-nitrosoureas (Ia-c)

a) Each run was carried out at  $0^{\circ}C \pm 0.2^{\circ}C$ . The reactions were initiated by mixing 1,3-diaryl-1nitrosourea (1-2 mg) in acetone (5 ml) with Walpole's buffer (HCl-acetate buffer, pH 0.3 or 2.5) (5 ml). b) Determined by using HPLC. The mobile phase consisted of acetonitrile-water (H, 5:5; CH<sub>3</sub>, 55:45; Cl, 6:4). 1,3-Diphenyl-1-nitrosourea (Ia), 1,3-di(4-tolyl)-1-nitrosourea (Ib) and 1,3-bis(4chlorophenyl)-1-nitrosourea (Ic) had elution times of 4.7, 3.0, and 4.8 min, respectively. Naphthalene or biphenyl was used as an internal standard. Rate constants were calculated by means of the least-squares method. c) Correlation coefficient between time and concentration of 1,3-diaryl-1-nitrosoureas.

### pH 1.5. The base-catalyzed reaction of Ia is discussed later.

The only detectable product was 1,3-diphenylurea (IIa), a denitrosated urea. The time courses of degradation of Ia and formation of IIa at 0 °C in pH 0.3 buffer solution were measured by means of HPLC. As shown in Fig. 2, the amount of IIa increased proportionally as the reaction proceeded. When 14% of Ia had disappeared, 12% of IIa had been formed. At the half-life time of Ia (220 min), the yield of IIa was about 50%.

The isolated yield of IIa at pH 0.3 and 0  $^{\circ}$ C was 91% at 1 d after the reaction had started. The structure was confirmed by means of melting point determination and infrared (IR), proton nuclear magnetic resonance (<sup>1</sup>H-NMR) and HPLC analyses.

The *p*-substitution effects on the rates were examined at pH 0.3 and the results are shown in Table II. The disappearance rates of Ia and Ib were  $5.25 \times 10^{-5}$  and  $12.8 \times 10^{-5}$  s<sup>-1</sup>, which are similar to each other. It can be concluded that substitution effects are small in the acid-

No.	Compounds	Temp. <sup><math>b</math></sup> (°C)	$k_{obs}^{c)}(s^{-1})$	r <sup>d)</sup>
Ia	NO N-CONH	25	$5.35 \times 10^{-3} e$	-0.972
		0	$3.13 \times 10^{-4} e$	-0.990
III	NO -CH <sub>2</sub> NHCON-CH <sub>3</sub>	25	$8.70 \times 10^{-5 g}$	- 0.999
IV	ON-N_NH	25	$1.50 \times 10^{-4}$ h)	- 0.998
v	ON-NO ON-NO	25	$9.70 \times 10^{-4f}$	- 0.999

 TABLE III.
 Comparison of the Stability of 1,3-Diaryl-1-nitrosourea (Ia) with Those of Related 1,3-Dialkyl-1-nitrosoureas in Acidic Solution<sup>a</sup> (1.5 N HCl)

a) The reaction was initiated by mixing an N-nitroso compound (1-2mg) in acetone (5ml) with Walpole's buffer (HCl-acetate buffer) (5ml). b)  $\pm 0.4$  °C. c) Rate constants were calculated by means of the least-squares method. d) Correlation coefficient between time and concentration of N-nitroso compounds. e) Determined by using HPLC. f) Determined spectrophotometrically. Degradation was monitored with a Shimadzu UV-240 spectrophotometer at 419 nm. g) Determined by using HPLC. The monitoring wavelength was 392 nm, and p-nitrotoluene was used as an internal standard. The mobile phase consisted of acetonitrile-water (5:5). Compound III had an elution time of 3.6 min. h) Determined spectrophotometrically at (392 nm).

catalyzed degradation of 1,3-diaryl-1-nitrosoureas.

Degradation rates of 1,3-diaryl-1-nitrosoureas under the same conditions were compared with those of the related 1,3-dialkyl-1-nitrosoureido derivatives. As shown in Table III, at 25 °C in 1.5 N HCl, the observed rate constant of Ia was  $5.35 \times 10^{-3} \text{ s}^{-1}$ . On the other hand, those of 1-nitroso-1-methyl-3-benzylurea (III) and a cyclic *N*-nitrosourea, 1-nitroso-2imidazolidinone (IV), were  $8.70 \times 10^{-5}$  and  $15.0 \times 10^{-5} \text{ s}^{-1}$ . Thus, under acid-catalyzed conditions, Ia degrades  $10^2$  fold more rapid than the related 1,3-dialkyl-1-nitrosoureido derivatives. 3-Nitroso-2-oxazolidinone (V), related to a cyclic *N*-nitrosourea IV, degraded with a rate constant of  $9.70 \times 10^{-4} \text{ s}^{-1}$ .

## **Base-Catalyzed Degradation**

The base-catalyzed reaction of 1,3-diphenyl-1-nitrosourea (Ia) could be studied by means of HPLC over a wide pH range. However, the reaction of Ia is quite rapid at pH 5.4 and, therefore, the pseudo-first degradation rates of 1,3-diaryl-1-nitrosoureas were measured in the range from pH 2 to 5. As shown in Fig. 1, the rate data for each of these reactions obeyed pseudo first-order kinetics over the entire reaction process. The product distribution for the reaction of Ia was studied in the buffer solution (pH 4.2) by using HPLC. The time courses of degradation of Ia and formation of IIa are shown in Fig. 2. When 32% of Ia had disappeared, less than 2% of IIa had been formed. Compound Ia had a half-life time of 19 min, and at this time the yield of IIa was only about 5%. Thus, the contribution of denitrosation is not important in the base-catalyzed pH region.

The degradation products of Ia were determined by using HPLC. Though the final set of products was complicated, the main product was IIa (42%) at one day after the reaction had started. The intermediates in the base catalysis seem to be mainly a diazonium ion and an isocyanate, and the urea should be produced by coupling of the isocyanate. To test this hypothesis, we tried to find recombined products of two 1,3-diaryl-1-nitrosoureas bearing



Chart 1. Formation of Different Ureas from Two Kinds of 1,3-Diaryl-1nitrosoureas (Ia, b)

different substituents in a reaction mixture: when mixture of Ia and Ib (1:1) was decomposed in pH 4.2 buffer-acetone solution at 0 °C (Chart 1), the formation of a recombined urea (1-(4tolyl)-3-phenylurea, VI) was indeed observed, accompanied with IIa and IIb. The ratio of 1,3di(4-tolyl)urea (IIb), 1-(4-tolyl)-3-phenylurea (VI) and 1,3-diphenylurea (IIa) was about 1:1.8:1.

The substitution effects on the rates of base-catalyzed degradation were examined at pH 2.5 and the results are shown in Table II. The degradation rates were in order of the substituent effect. Plots of  $\log k_{obs}$  against  $\sigma$  and  $\sigma^0$  gave positive slopes ( $\rho = 3.45$ , r = 0.915 and  $\rho = 3.41$ , r = 0.97, respectively) indicating that decreasing electron density at the nitrogen atom results in increasing degradation rate of 1,3-diaryl-1-nitrosoureas. This result is different from that at pH 0.3, and means that the reaction mechanism at pH 2.5 was different from that at pH 0.3.

The stability of 1,3-diphenyl-1-nitrosourea (Ia) was compared with those of the related 1,3-dialkyl-1-nitrosoureido derivatives at pH 5.4. At 0 °C, the rate of disappearance of Ia was  $3.53 \times 10^{-3} \, \text{s}^{-1}$  and that of 3-benzyl-1-methyl-1-nitrosourea (III) at 50 °C was  $8.70 \times 10^{-5} \, \text{s}^{-1}$ . The degradation reaction of Ia is more than  $10^3$  fold more rapid than those of 1,3-dialkyl-1-nitrosoureido derivatives.

# Thermal Degradation in Organic Solvents

Thermal degradation of Ia in organic solvents was studied by means of HPLC. The products of thermal decomposition have already been reported and they were derivatives of the reactive intermediates, such as aryldiazonium salts and arylisocyanates.<sup>3)</sup> The degradation of 1,3-diphenyl-1-nitrosourea (Ia) obeyed first-order kinetics in several organic solvents (Table IV). In benzene and chloroform at 20–40 °C, the rate constants of Ia were  $3.08 \times 10^{-4}$ – $3.10 \times 10^{-5} \text{ s}^{-1}$ . Compared with the degradation in aqueous solutions, the thermal degradation rates of Ia in nonpolar solvents were very slow.

In acetone and dimethylsulfoxide (DMSO), the degradation rates of Ia were  $4.58 \times 10^{-6} \text{ s}^{-1}$  (at 5 °C) and  $1.26 \times 10^{-4} \text{ s}^{-1}$  (at 10 °C), respectively. In aprotic polar solvents, the degradation rates of Ia were almost the same as those in nonpolar solvents.

In methanol, Ia was very unstable and its degradation rate was  $2.00 \times 10^{-3} \text{ s}^{-1}$  at 0 °C. It gave IIa and methyl *N*-phenylcarbamate [C<sub>6</sub>H<sub>5</sub>NHCOOCH<sub>3</sub>]. Those results suggest that methanol may play a role as a scavenger of nitrosonium ion and phenyl isocyanate.<sup>3)</sup>

In chloroform, the rates of disappearance of Ib and Ic bearing methyl and chloro groups on the phenyl ring at the *para* position, were  $1.95 \times 10^{-4}$  s<sup>-1</sup> (at 40 °C) and  $9.85 \times 10^{-5}$  s<sup>-1</sup> (at 30 °C), respectively (Table IV). Considering the difference of the temperature examined, these data suggest that there is little substituent effect on the degradation rates of 1,3-diaryl-1-

	0					
Compd. No.	Substituent	Temp. <sup>b)</sup> (°C)	Solvent <sup>c)</sup>	$k_{obs} (s^{-1})$	r <sup>d)</sup>	
Ia	Н	40	Benzene	$3.08 \times 10^{-4}$	-0.997	
		20	Benzene	$3.10 \times 10^{-5}$	-0.984	
		30	Chloroform <sup>e)</sup>	$1.32 \times 10^{-4}$	-0.989	
		5	Acetone	$4.58 \times 10^{-6}$	-0.949	
		10	DMSO	$1.26 \times 10^{-4}$	- 0.968	
		0	Methanol	$2.00 \times 10^{-3}$	-0.997	
Ib	CH3	40	Chloroform <sup>e)</sup>	$1.95 \times 10^{-4}$	-0.983	
Ic	Cl	30	Chloroform <sup>e)</sup>	$9.85 \times 10^{-4}$	-0.988	

TABLE IV. Degradation<sup>a</sup>) Rates of 1,3-Diaryl-1-nitrosoureas in Organic Solvents

a) The reactions were initiated by dissolving a 1,3-diaryl-1-ureido derivative (1-2 mg) in an organic solvent (5 ml) at the indicated temperature. In the case of degradation in DMSO, Ia was dissolved in acetome (5 ml, at 10 °C). The reaction was initiated by mixing the solution and DMSO (10 ml). Determined by using HPLC. In the case of degradation in benzene, the monitoring wavelength was 392 nm and *p*-nitrotoluene was used as an internal standard. The mobile phase consisted of acetonitrile-water (Ia, 5:5; 15; 55; 145; Ic, 6:4). Ia, Ib and Ic had elution times of 3.0, 4.7, and 4.8 min, respectively. Naphthalene or biphenyl was used as an internal standard. Rate constants were calculated by means of the least-squares method. b)  $\pm 0.2$  °C. c) All solvents used were of reagent grade. d) Correlation coefficient between time and concentration of 1,3-diaryl-1-nitrosoureas. e) The chloroform used contained about 0.5% methanol as a stabilizer.

TABLE V. Comparison of the Stability of 1,3-Diaryl-1-nitrosourea with Related N-Nitroso Compounds in Chloroform<sup>a)</sup>

No.	Compounds	Temp. <sup><math>b</math></sup> (°C)	$k_{obs}^{c)} (s^{-1})$	<i>r</i> <sup><i>d</i>)</sup>
Ia		30	$1.32 \times 10^{-4} e^{-4}$	-0.989
III	NO CH <sub>2</sub> NHCON-CH <sub>3</sub>	25	g)	
IV	ON-NO	25	$8.77 \times 10^{-5f}$	-0.999
	0			

a) The reactions were initiated by dissolving an N-nitroso compound (1-2 mg) in an organic solvent (5 ml) at the indicated temperature. b)  $\pm 0.4$  °C. c) Rate constants were calculated by means of the least-squares method. d) Correlation coefficient between time and concentration of N-nitroso compounds. e) Determined by using HPLC. f) Determined spectrophotometrically. Degradation was monitored with a Shimadzu model UV-240 spectrophotometer (cell length = 10 mm) at 419 nm. g) Determined by using HPLC. The monitoring wavelength was 392 nm and p-nitrotoluene was used as an internal standard. The mobile phase consisted of acetonitric-water (5:5). 1-Nitroso-1-methyl-3-benzylurea had an elution time of 3.6 min. The degradation rate was to slow to measure.

## nitrosoureas.

Degradation rates of III and IV were measured in chloroform for comparison with those of 1,3-diaryl-1-nitrosoureas (Table V). The observed degradation rate of Ia was  $1.32 \times 10^{-4} \text{ s}^{-1}$  at 30 °C. The degradation rate of III under the same conditions was too slow to be determined ( $< 10^{-8} \text{ s}^{-1}$ ). The observed degradation rate of IV was  $3.68 \times 10^{-5} \text{ s}^{-1}$ .

Thus, 1,3-diaryl-1-nitrosoureas are very unstable compounds in organic solvents as compared with these related 1,3-dialkyl-1-nitrosoureido compounds.

### Discussion

1,3-Diphenyl-1-nitrosourea (Ia) was decomposed at  $0^{\circ}$ C in the mixtures of acid solution



Fig. 3. The Relationship between the Logarithms of the Observed First-Order Rate Constants for 1,3-Diphenyl-1-nitrosourea (Ia) and the pH Values

and acetone (50%, v/v). In any pH, the degradation followed first-order kinetics. The relationship between the logarithms of the observed first-order rate constants ( $k_{obs}$ ) and the pH values is shown in Fig. 3. The data indicate that the rate of disappearance of Ia is greatly dependent on the pH value of the reaction medium. From the plots, the minimum rate constant among the observed first-order rate constants is  $5.25 \times 10^{-6} \,\mathrm{s}^{-1}$  at pH 1.5. In the region of pH 1.5—5.4, the pH profile for the degradation of Ia was linear with a slope of about 0.703, which means that the contribution of specific base catalysis is predominant in this region. On the other hand, in the region of pH < -1.5, the profile was linear with a slope about -1.05, which means that the contribution of specific acid catalysis is predominant. The following rate expression is derived, where [H<sup>+</sup>] and [OH<sup>-</sup>] are hydrogen ion and hydroxide ion concentrations, respectively,  $k_0$  is the zero-order rate constant of pyrolysis of Ia, and,  $k_1$  and  $k_2$  are the first-order rate constants for specific acid and base catalysis, respectively. From Fig. 3, the following parameters are calculated: n' = -1.05, n'' = 0.703,  $k_1 = 1.6 \times 10^{-4} \,\mathrm{s}^{-1}$ , and  $k_2 = 3.91 \times 10^{-7} \,\mathrm{s}^{-1}$ . In the region of pH 1.5—5.4, the reaction medium was a mixture of acetone and water, causing the reaction order to be fractional.

$$k_{\rm obs} = k_0 + k_1 [{\rm H}^+]^{n'} + k_2 [{\rm OH}^-]^{n'}$$

Compared with related 1,3-dialkyl-1-nitrosoureido derivatives (Table III), the pH profiles of 1,3-diaryl-1-nitrosoureas are very different. Degradation of 1,3-diaryl-1-nitrosoureas was about  $10^2$  fold more rapid under acid catalysis and about  $10^3$  fold more rapid under base catalysis,<sup>6)</sup> as compared with 1,3-dialkyl-1-nitrosoureido derivatives. The most stable pH regions of 1,3-dialkyl-1-nitrosoureas are quite different from those of 1,3-diaryl-1-nitrosoureas. The disappearance of 1-methyl-1-nitrosourea (MNU) is independent of the acid strength from pH 2 to 4 and inversely proportional to the acid strength at higher pH values. In contrast, the rate of disappearance of 1,1,3-trimethyl-3-nitrosourea (TMNU) is proportional to the acid strength at lower than pH 7, and inversely proportional to the acid strength at higher than pH 7. The minimum observed rate constants of MNU and TMNU were  $7.08 \times 10^{-7} s^{-1}$  at pH 2–4 and  $4.03 \times 10^{-8} s^{-1}$  at pH 7, respectively.<sup>6</sup>

On the other hand, the rate of disappearance of Ia is entirely dependent on pH, and the minimum observed rate constant of Ia was  $5.25 \times 10^{-6} \text{ s}^{-1}$  at pH 1.5. This means that, even at such a low pH, base-catalyzed degradation can occur. These properties of Ia should be due to the specific conformation of 1,3-diaryl-1-nitrosoureas and the remarkable electron deficiency at the nitrogen atoms of the ureido group. The rate constants of 1-alkyl-3-aryl-1-nitrosoureas

and 1-alkyl-3-aryl-3-nitrosoureas were in the range of  $1.01-2.63 \times 10^{-4} s^{-1}$  at 25 °C at pH 6.95<sup>7</sup>) and 0.33-2.6 × 10<sup>-5</sup> s<sup>-1</sup> at 33 °C in chloroform.<sup>8</sup>) These reports show that the stability of *N*-nitrosoalkylarylureas is almost the same as that of 1,3-dialkyl-1-nitrosoureas, and suggest that the benzene ring attached to the ureido nitrogen atom does not affect the stability. On the other hand, in 1,3-diaryl-1-nitrosoureas in which both nitrogen atoms of the ureido group are substituted by benzene rings, they may restrict free rotation around the amide bond of the ureido group and force the *N*-nitrosoureas to adopt a rotamer form which is very easily attacked by hydroxide ion. This rotamer also readily undergoes thermal degradation. The electron-withdrawing effect of the benzene ring may also make 1,3-diaryl-1-nitrosoureas unstable. In the entire pH region in which acid-catalyzed degradation of TMNU occurs, 1,3-diaryl-1-nitrosoureas should have much greater positive charge than that of TMNU. These two effects make 1,3-diaryl-1-nitrosoureas more unstable than related 1,3-dialkyl-1-nitrosoureas in the base-catalyzed pH region and in organic solvents.

The pH profile and final products of acid-catalyzed degradation of 1,3-diaryl-1nitrosoureas are not similar to those of 1,3-alkyl-1-nitrosoureas. In the acid-catalyzed pH region, Ia gave the denitrosated urea quantitatively. This is presumably because the diarylureas formed are insensitive to acids and nitrous acid, and, therefore, the hydrolysis of IIa was negligible. This fact helps us to analyze the reaction clearly. On the other hand, the final degradation product of MNU was methanol, and those of TMNU were methanol and N,N'-dimethylamine.<sup>5,6)</sup> This means that the denitrosated ureas formed from the degradation of these 1,3-dialkyl-1-nitrosoureas are unstable. It appears that the degradation products of 1,3-diaryl-1-nitrosoureas, and the reaction mechanism for degradation of 1,3-diaryl-1nitrosoureas may be the same as that of 1,3-dialkyl-1-nitrosoureas in this pH region.



Chart 2. A Possible Mechanism of Acid-Catalyzed Degradation

Two possible reaction mechanisms could be proposed for the acid-catalyzed denitrosation of I, as shown in Chart 2. One involves proton attack on the nitrogen atom on which the nitroso group is attached, followed by degradation of this intermediate to give the denitrosated urea II (path b). Path b seems to apply to denitrosation of *N*-nitrosoamines.<sup>5</sup>) The other involves initial protonation on the carbonyl oxygen atom, to produce an enolic type urea and nitrosonium ion (path a). These two types of mechanism can account for the denitrosation of 1,3-diaryl-1-nitrosoureas. The mechanism of acid-catalyzed denitrosation of 1,3-diaryl-1-nitrosoureas can not be settled at the present time, but may involve an ionic key step. If the reaction site is near a benzene ring (path b), substituents should affect the rate of degradation. Since substituent effects on the degradation rate are relatively small, path a is



Chart 3. A Possible Mechanism of Base-Catalyzed Degradation

more likely. These results do not correspond with the results obtained in the study of three types of 1,3-dialkyl-1-nitrosoureas reported by Snyder *et al.*<sup>6)</sup>

Two mechanisms for base-catalyzed degradation could also be proposed, as shown in Chart 3. One involves initial abstraction of the ureido hydrogen atom by hydroxide ion to produce a phenyldiazohydroxide (VII) and an isocyanate (VIII) (path c). The other involves attack of a hydroxide ion on the carbonyl carbon of I to give a tetrahedral intermediate which produces phenyldiazohydroxide (VII) and *N*-phenylcarbamic acid (IX) (path d). The following evidence suggests that path c is more likely. 1. As mentioned above, considerable amounts of 1,3-diarylureas (II) were formed from 1,3-diaryl-1-nitrosoureas. These products can be easily derived from aryl isocyanates (VIII) in aqueous solution. Path c accounts for this product (II), but path d can not. 2. These reaction rates are affected by substitution on the benzene ring, which means that the reaction site is relatively near the phenyl ring and/or a considerable negative charge is placed on the benzene ring in the transition state. 3. As shown



Chart 4. A Possible Mechanism of Thermal Decomposition in Organic Solvents

in Fig. 1, there is a time lag between the decrease of Ia and increase of the combination urea (IIa). This mechanism accounts for the slow formation of the urea. This conclusion is contrary to results obtained in a study on 1,3-dialkyl-1-nitrosoureas<sup>6</sup> but consistent with results obtained in the studies of 1-methyl-1-nitroso-3-arylureas.<sup>7,9</sup>

On the basis of thermal degradation product analysis,<sup>3)</sup> two possible mechanisms were presented (Chart 4). One involves intramolecular rearrangement through a 4-membered ring to afford a phenyldiazo carbamate (XI), which decomposes to give a phenyldiazonium ion (VII) and a phenyl isocyanate (VIII) (path e). The other involves internal prototropy through a 6-membered ring followed by intramolecular rearrangement to give a phenyl isocyanate (VIII) and a diazonium ion (VII) (path f). Since the kinetic results show no substitution effects, the reaction site may be located far from the phenyl ring and/or a phenyldiazohydroxide ion is not involved in the rate-determining step.<sup>10)</sup> The base-catalyzed degradation of I involves intermediates similar to those in the pyrolysis in organic solvents, but the rate of this reaction depends strongly on the substituents on the benzene rings. If the reaction proceeded *via* path f, substituents should influence the rate constants, because the acidity of the NH proton is controlled by substituents on the phenyl rings.<sup>11)</sup>

### Conclusion

In aqueous solutions, in the pH range of -0.5 to 1.5, the degradation of Ia was firstorder in hydrogen ion and yielded denitrosated IIa as the main product. Our kinetic data show that a slow protonation at the carbonyl oxygen atom is the key step in the reaction. In the range of pH 1.5—5.4, the degradation of Ia was catalyzed by hydroxide ion, but the products were II, formed by recombination of the reactive intermediate. The analysis of reaction products and our kinetic data support the conclusion that the reaction occurs *via* initial proton abstraction.

In organic solvents, I was more stable than in aqueous solutions, and the first-order rate constant for Ia was greater than those of 1,3-dialkyl-1-nitrosoureas. Thermal degradation of I could proceed *via* a 4-membered ring intermediate to give phenyldiazo *N*-phenylcarbamate (XI) as a reactive intermediate.

Compared with related 1,3-dialkyl-1-nitrosoureas, degradation of I is several hundred fold more rapid under all the conditions we examined. This can be interpreted in terms of a special conformation of I and the electron-withdrawing effect of the benzene rings.

This work has shown that 1,3-diaryl-1-nitrosoureas (I) undergo base-catalyzed degradation, even at low pH, much more readily than 1,3-dialkylureido derivatives and generate active chemical species. Accordingly, similar degradation reactions may proceed in AH-13 tumor cells and antitumor-active chemical species may be formed effectively. This might result in high activity for killing the tumor cells.

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