Kinetics and Mechanism of Decompositions of *N*-Methyl-*N'*-aryl-*N*-nitrosoureas and Related Compounds in Aqueous Buffer Solution

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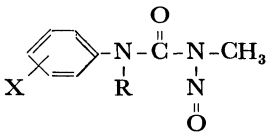
The decomposition reactions of *N*-methyl-*N'*-aryl-*N*-nitrosoureas (**1**) and of *N,N'*-dimethyl-*N'*-aryl-*N*-nitrosoureas (**2**) were studied kinetically in phosphate buffer solution. All nitrosoureas **1** were easily hydrolyzed even under neutral conditions and the rates of hydrolysis were found to be proportional to the hydroxide ion concentration over pH range 5.4—7.6. General base catalysis by buffer components was negligible. The reaction was subjected to the elimination mechanism in which an abstraction of the ureido proton was involved as an initial step. On the other hand, nitrosoureas **2** had no significant reactivity under the same conditions.

N-alkyl-*N*-nitrosoureas are unstable and are readily hydrolyzed to produce alkanediazohydroxides, which are thought to be active alkylating agents toward genetic material in biological systems.[1] The simplest member of this class of compounds is *N*-methyl-*N*-nitrosourea (MNU). This is known to act as a powerful carcinogen and mutagen without need of enzymatic hydroxylation and its chemical and biological aspects have been well documented.[2]

In earlier work, we synthesized two families of substituted MNU, namely, *N*-methyl-*N'*-aryl-*N*-nitrosoureas (**1a**—**1c**, **1e**, **1g**) and *N,N'*-dimethyl-*N'*-aryl-*N*-nitrosoureas (**2**), and their mutagenic activities on *S. Typhimurium* TA 1535 were investigated.[3] All *N'*-monosubstituted derivatives (**1**) exhibited the higher mutagenisity than that of MNU, whereas no respectable activity was found for *N',N'*-disubstituted ones (**2**). The study showed that the presence of the proton on *N'* is significant to the mutagenic activity and also that this activity is influenced by the electronic effect of the para substituent X on the phenyl group. The structural and electrostatic natures of these nitrosoureas are thus important factors that govern their biological efficiency. However, the mode of decomposition as well as the intrinsic stability under physiological conditions have been left unclear. Therefore, we undertook a systematic degradation study of these nitrosoureas in neutral aqueous solution and elucidated their hydrolytic mechanism.



	X	R
1a:	*p*-OCH_3	H
1b:	*p*-CH_3	H
1c:	H	H
1d:	*m*-OCH_3	H
1e:	*p*-Cl	H
1f:	*m*-Cl	H
1g:	*p*-$COCH_3$	H
2a:	H	CH_3
2b:	*p*-NO_2	CH_3

Results and Discussion

The decomposition reactions of **1** and **2** were conducted in 0.1 M (1 M=1 mol dm^{-3}) phosphate buffer solution at constant ionic strength. The reaction was followed by monitoring the decrease of UV absorption of the substrate. The observed pseudo-first order rate constants (k) at pH 6.95 are summarized in Table 1.

Table 1 indicates the following features: (a) All *N'*-monosubstituted derivatives (**1**) that contain the labile proton on *N'* were rapidly decomposed even under neutral conditions ($t_{1/2}$=7—19 min at 36.8 °C). On the contrary, *N',N'*-disubstituted derivatives (**2**) in which the proton was replaced by methyl group were markedly stable up to pH 7.6 (see Experimental).

Table 1. The kinetic data of **1** and **2** at pH 6.95[a]

Nitrosourea[b]	Temp/°C	$10^4 \times k/s^{-1}$ [c]	ΔH^*/kcal mol^{-1}	ΔS^*/eu
1a	25.2	1.01±0.01		
	36.8	6.15±0.10	27.3±0.1	14.9±0.4
1b	25.2	1.05±0.02		
	36.8	6.76±0.04	29.5±0.3	22.0±0.7
1c	25.2	1.34±0.01		
	36.8	8.17±0.02	28.0±0.3	17.6±1.0
1d	25.2	1.51±0.07		
	36.8	9.55±0.08	28.6±0.2	19.8±0.5
1e	36.8	12.30±0.10		
1f	25.2	2.31±0.06		
	36.8	14.80±0.08	28.7±0.9	21.2±2.9
1g	25.2	2.63±0.11		
2a	36.8	Unreacted		
2b	36.8	Unreacted		

a) 0.1 M phosphate buffer, μ=0.222. b) (1.2—1.6)×10^{-4} M. c) Average of two runs.

Table 2. The pseudo-first order rate constants ($10^4 \times k/s^{-1}$) of **1** under various pH conditions (36.8 °C)

Nitrosourea[a]	pH[b]					
	5.40	5.82	6.29	6.72	7.20	7.60
1a	0.259	0.641	1.75	4.65	12.0	29.4
1b	0.263		1.83	4.91		30.4
1c	0.343	0.895	2.46	5.78	17.0	38.1
1d	0.410		2.85	7.13		47.0
1e	0.469	1.08	3.25	8.37		58.4
1f	0.583		3.73	10.2		73.0
1g	0.690			12.1	34.0	

a) $(1.2—1.6) \times 10^{-4}$ M. b) 0.1 M phosphate buffer, $\mu=0.5$.

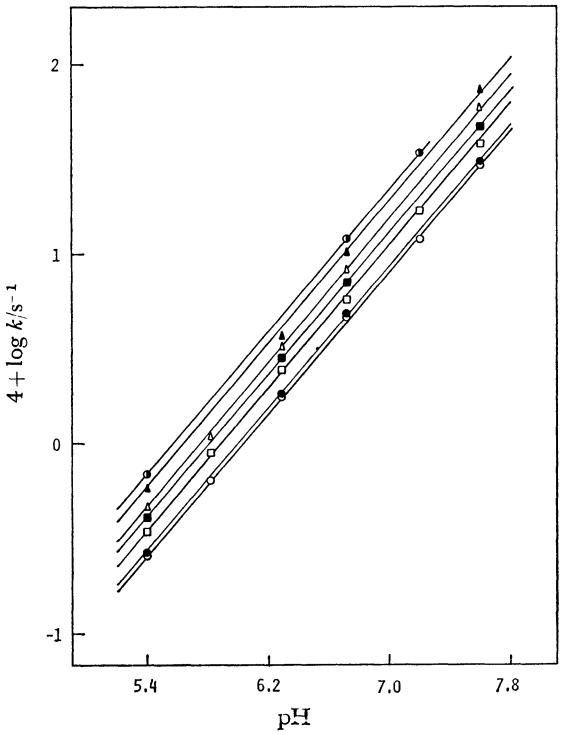


Fig. 1. pH-rate profiles for **1** at 36.8 °C (see Table 2).
○: **1a**, ●: **1b**, □: **1c**: ■: **1d**, △: **1e**, ▲: **1f**, ◑: **1g**.

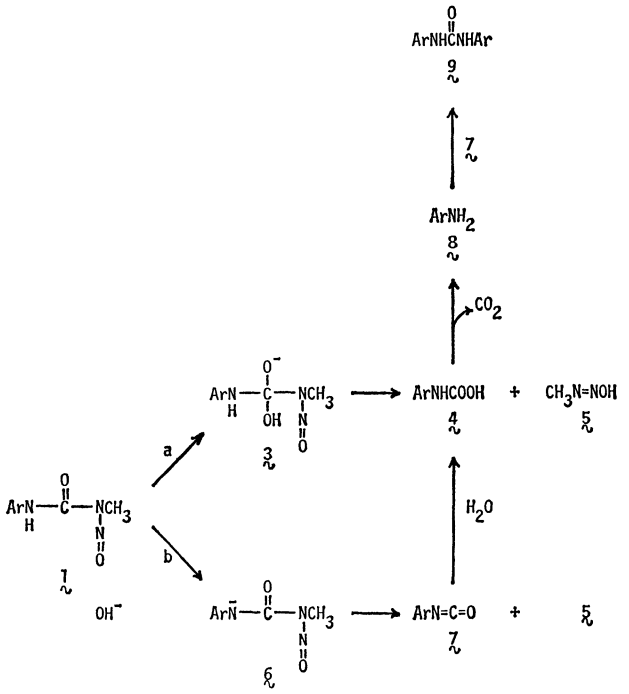
This blocking effect by *N'*-methyl substituent under chemical hydrolytic conditions appears to be correlated with our previous finding in the mutagenic study.[3] (b) The decomposition rates within the series of **1** were in order of the substituent effect of X. Plot of log k against σ gave a positive slope ($\rho=0.65$, $r=0.991$,[4] 36.8 °C) which indicates that decreasing in the electron density at nitrogen atom (N') results in increasing the decomposition rate of **1**.

It is clearly shown that the decomposition rate of every nitrosourea **1** depends on the hydroxide ion concentration (Table 2). The pH-rate profile exhibited positive slopes of exactly 0.94±0.02 over pH range 5.4—7.6 (Fig. 1). This indicates that the reaction can be described in a first order with respect to the hydroxide ion term.[5] Further, changing the buffer concentration at constant pH gave little effect on the observed rate (Table 3). Therefore, general base catalysis by hydrogenphosphate dianion is negligible in comparison with the specific base catalysis.[5]

Table 3. The effect of buffer concentration on the decomposition rate of **1c** at pH 6.72 (36.8 °C)[a]

$[HPO_4^{2-}]$/M	$10^4 \times k/s^{-1}$
0.003	5.25
0.015	5.45
0.031	5.60
0.061	5.78
0.122	6.79

a) **1c** $=1.64 \times 10^{-4}$ M, $\mu=0.5$.



On the basis of these kinetic results, two possible mechanisms could be proposed to the aqueous decomposition of **1** as shown in the above scheme. One involves hydroxide ion attack on the carbonyl carbon of **1** to form tetrahedral intermediate **3** (or *via* direct S_N2 displacement), followed by the decomposition of **3** to give arylcarbamic acid **4** and methanediazohydroxide **5** ($B_{AC}2$ type mechanism, path a). The other involves an initial abstraction of the ureido proton by hydroxide ion to produce anion **6** which

releases aryl isocyanate **7** and **5** (E1cB type mechanism, path b). The aryl isocyanate is very reactive in water.[6] Accordingly, **4** may be formed again with this reaction mechanism. Thus, both types of mechanism can represent the same set of products.

The only different feature that distinguishes these two mechanisms is the presence of aryl isocyanate intermediate **7** along the reaction path b. Although **7** is not isolable from aqueous solution, this can be effectively trapped by aniline **8**[6a,7] which is formed from **4**. Degradation of **1c** in 0.1 M phosphate buffer under several pH conditions yielded *N,N'*-diphenylurea **9** as a major product. Undoubtedly, this is a striking evidence of the formation of the aryl isocyanate in the course of the reaction, indicating the occurrence of path b.

Generally, Arrhenius parameters are used to distinguish E1cB from $B_{AC}2$, where the former should show a considerably more positive entropy of activation than the latter.[8] The entropies of activation of **1** were 15—22 e.u. (see Table 1). There was no discernible difference in the kinetic behavior of each compound. Such a value is too large to be assigned to $B_{AC}2$ mechanism.[8b,8c] Consequently, the E1cB pathway is favored. Further, the effect of substituent X on the decomposition rate is relatively small ($\rho=0.65$), showing a close similarity to that reported for the ester hydrolysis involving an isocyanate intermediate ($XC_6H_4NHCOOC_6H_5$, $\rho=0.64$).[9]

Thus, the experimental results listed above are consistent with an interpretation that nitrosoureas **1** first react by the abstraction of the α proton to produce anion **6** and that the nucleophilic attack to the carbonyl group is excluded as possible mechanism. The presence of the isocyanate intermediate during the reaction of **1** may be of interest from the viewpoint of their carbamoylating abilities toward many nucleophiles in cellular environment.[10]

Snyder and Stock reported the aqueous decomposition of *N,N'*-dimethyl-*N*-nitrosourea (DMNU) in basic solution.[2d] This aliphatic nitrosourea represented evidence that the key reaction occurred with carbonyl group as a reaction site. In our neutral conditions, DMNU showed much lower reactivity ($k=6.40\times10^{-6}$ s^{-1}, pH 6.95, $\mu=0.222$, 36.8 °C) than those of **1**. The great increase of the decomposition rate from the aliphatic compound to the aromatic ones ($k_{1c}/k_{DMNU}=128$) can be interpreted in terms of the inductive effect of the aryl group of **1**.[11] The electron-withdrawing effect of the aryl substituent might more stabilize the negative charge on nitrogen (*N'*) and this facilitates the elimination process of **1**.

Our present observation provides the fact that, as in the cases of other reported alkylnitrosoureas,[1d,2d,5] the aqueous decomposition rate of **1** thoroughly depends on the hydroxide ion concentration around neutral pH. Owing to the absence of a labile proton on *N'*, disubstituted derivatives **2** are inert under neutral conditions. This stability of **2** in chemical process appears to limit their mutagenic potency.

Experimental

All melting points were uncorrected. IR spectra were recorded on a Shimadzu IR 400 spectrometer and UV spectra with a Shimadzu UV 200 spectrometer. NMR spectra were taken in acetone-d_6 solutions using a Hitachi R-24 (60 MHz) instrument and chemical shifts are given in δ with TMS as an internal standard. Microanalyses were performed in the microanalytical laboratory of the Institute of Physical and Chemical Research, Wako-shi, Saitama. pH was measured with a Toa-denpa HM-5A pH meter. Doubly distilled water was used for kinetics and buffer reagents were of commercial special grade.

N-Methyl-*N'*-arylureas were synthesized by the condensation reactions[12] of *p*- or *m*-substituted anilines with methyl isocyanate in quantitative yield. *N,N'*-Dimethyl-*N'*-arylureas were prepared from corresponding *N*-methylanilines. The ureas were purified by recrystallization from acetone and were used for subsequent nitrosation reactions. A typical procedure of the nitrosation was as follows.

N-*Methyl*-N'-*(*p-*methoxyphenyl)*-N-*nitrosourea (**1a**)*: To a stirred solution of *N*-methyl-*N'*-(*p*-methoxyphenyl)urea (900 mg, 5 mmol) in acetic acid (10 ml) and acetic anhydride (50 ml) was added sodium nitrite (518 mg, 7.5 mmol) in small portions at 0 °C. The resulting solution was allowed to stand in a refrigerator overnight. The mixture was poured onto ice and extracted with ether three times. The etheral solution was washed with cold 5% sodium hydrogencarbonate solution, dried ($MgSO_4$), and evaporated to give crude product, which was recrystallized from ether–hexane to yield **1a** (355 mg, 34%): mp 97.0—98.0 °C (decomp). IR (KBr): 3350 (NH), 1705 (C=O), 1470 (N=O) cm^{-1}. NMR: 3.21 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 6.95 and 7.66 (2d, A_2B_2, $J=9$ Hz, 4H, C_6H_4), 9.59 (broad, 1H, NH). Found: C, 51.35; H, 5.27; N, 20.27%. Calcd for $C_9H_{11}O_3N_3$: C, 51.67; H, 5.30; N, 20.09%.

1b (44%): mp 102—103 °C (decomp). IR (KBr): 3310, 1720, 1460 cm^{-1}. NMR: 2.34 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 7.16 and 7.64 (2d, A_2B_2, $J=9$ Hz, 4H, C_6H_4), 9.67 (broad, 1H, NH). Found: C, 55.86; H, 5.74; N, 21.81%. Calcd for $C_9H_{11}O_2N_3$: C, 55.95; H, 5.74; N, 21.75%.

1c (52%): mp 83—84 °C (decomp) (lit,[13] 83—85 °C). IR (KBr): 3310, 1645, 1490 cm^{-1}. NMR: 3.19 (s, 3H, CH_3), 7.03—7.88 (m, 5H, C_6H_5), 9.69 (broad, 1H, NH). Found: C, 53.44; H, 5.07; N, 23.42%. Calcd for $C_8H_9O_2N_3$: C, 53.63; H, 5.06; N, 23.45%.

1d (55%): mp 98.0—98.5 °C (decomp). IR (KBr): 3330, 1730, 1460 cm^{-1}. NMR: 3.17 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.56—7.74 (m, 4H, C_6H_4), 9.60 (broad, 1H, NH). Found: C, 51.52; H, 5.26; N, 20.22%. Calcd for $C_9H_{11}O_3N_3$: C, 51.67; H, 5.30; N, 20.09%.

1e (56%): mp 110—111 °C (decomp). IR (KBr): 3340, 1715, 1490 cm^{-1}. NMR: 3.16 (s, 3H, CH_3), 7.30 and 7.76 (2d, A_2B_2, $J=9$ Hz, 4H, C_6H_4), 9.85 (broad, 1H, NH). Found: C, 44.88; H, 3.81; N, 19.69%. Calcd for $C_8H_8O_2N_3Cl$: C, 44.98; H, 3.77; N, 19.67%.

1f (47%): mp 87—88 °C (decomp). IR (KBr): 3300, 1730, 1470 cm^{-1}. NMR: 3.20 (s, 3H, CH_3), 7.00—7.95 (m, 4H, C_6H_4), 9.87 (broad, 1H, NH). Found: C, 44.76; H, 3.73; N, 19.88%. Calcd for $C_8H_8O_2N_3Cl$: C, 44.98; H, 3.77; N, 19.67%.

1g (55%): mp 119—121 °C (decomp). IR (KBr): 3360, 1705, 1470 cm^{-1}. NMR: 2.57 (s, 3H, $COCH_3$), 3.22 (s, 3H, CH_3), 7.99 (s, 4H, C_6H_4), 10.06 (broad, 1H, NH). Found: C, 54.29; H, 5.05; N, 19.12%. Calcd for $C_{10}H_{11}O_3N_3$: C, 54.30; H, 5.01; N, 19.00%.

2a (59%): oil. IR (film): 1690, 1480 cm^{-1}. NMR: 2.40

(s, 3H, N'CH_3), 2.88 (s, 3H, CH_3), 6.70 (s, 5H, C_6H_5). Found: C, 55.56; H, 5.72; N, 21.65%. Calcd for $C_9H_{11}O_2N_3$: C, 55.95; H, 5.74; N, 21.75%.

2b (54%): mp 78—80 °C. IR (KBr): 1690, 1470 cm^{-1}. NMR: 2.48 (s, 3H, N'CH_3), 2.98 (s, 3H, CH_3), 6.95 and 7.60 (2d, A_2B_2, J=9 Hz, 4H, C_6H_4). Found: C, 45.32; H, 4.23; N, 23.59%. Calcd for $C_9H_{10}O_4N_4$: C, 45.38; H, 4.23; N, 23.52%.

Kinetics. The ionic strength of the buffer solution was adjusted with sodium chloride.[14] Thirty microliters of a stock solution of nitrosourea **1** (*ca.* 1.4×10^{-2} M in anhydrous methanol) was added by a microsyringe to 3 ml of the appropriate buffer solution in a thermostatted (±0.3 °C) UV cell. The final methanol concentration was 1% by volume. The disappearence of the characteristic absorption of the substrate (**1a**, 278; **1b**, 270; **1c**, 264; **1d**, 278; **1e**, 268; **1f**, 262; **1g**, 219 nm) was followed at intervals. The infinity reading was obtained after at least eight half lives had elapsed. For all the substrates, the kinetic plots showed good first order behavior up to 90% of the overall reaction. The stabilities of *N'*,*N'*-disubstituted derivatives (**2**) were checked at pH 7.60 and at 36.8 °C. No spectral changes were observed for 52 h and 110 min for **2a** and **2b**, respectively.

Decomposition Products. Nitrosourea **1c** (50 mg) in 5 ml of ether was added to 300 ml of 0.1 M phosphate buffer solution (pH 7.20). The resulting suspension was allowed to stir for 140 min at 37 °C. The mixture was extracted with ether and evaporated to yield essentially pure *N*,*N'*-diphenylurea **9** [17 mg (57%), mp 238—239 °C, λ_{max} (H_2O) 252 nm]. Similar result was also obtained at pH 5.82 (37 °C, 43 h). An authentic sample of *N*,*N'*-diphenylurea (carbanilide) was prepared by the reaction of aniline with phenyl isocyanate [mp 238—240 °C,[15] λ_{max} (H_2O) 252 nm].

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