N-Nitroso Compounds. Part 3.[1] Hydrolysis of *N,N'*-Dimethyl-*N'*-(*p*-nitrophenyl)-*N*-nitrosourea in Aqueous Basic Solution. Effect of a Cationic Micelle on the Hydrolysis of Substituted *N*-Nitrosourea

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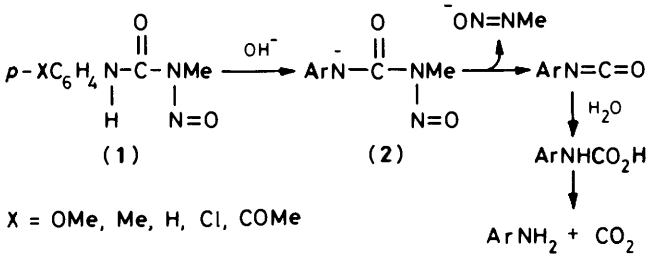
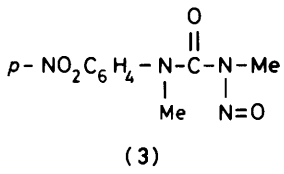
The hydrolysis of the title *N*-nitrosourea has been studied kinetically in various amine buffers (pH 8—11) at 36.8 °C. The reaction proceeds through hydroxide ion attack at the carbonyl carbon to form a tetrahedral intermediate, which collapses to an arylcarbamate ion, releasing an *N*-nitrosamino fragment. The effect of the buffer concentration on the hydrolysis rate is explained in terms of general base catalysis. Nucleophilic attack of unhindered amines at the carbonyl carbon also occurs under the hydrolytic conditions. With bulkier amines, however, the concurrent nucleophilic reaction is excluded because of steric hindrance around the carbonyl group. The micellar effect on the hydrolysis rate has also been investigated using cetyltrimethylammonium bromide (CTABr). The rate enhancement of the hydrolysis by the CTABr micelle is shown to be six-fold at pH 8.05. However, with an alternative *N*-nitrosourea bearing a labile hydrogen on N', a much larger micellar catalysis was observed for the elimination of the hydrogen in the presence of OH^-.

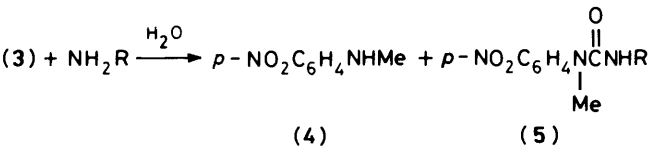
A number of *N*-nitroso compounds, such as *N*-nitroso-amines, -amides, or -ureas, are of current interest in connection with carcinogenesis or mutagenesis in living organisms.[2] However, only limited studies have been made on the chemical reactivity of *N*-nitrosourea. We have recently reported that the hydrolysis of *N*-methyl-*N'*-aryl-*N*-nitrosourea [ArNHCON(N=O)Me] (**1**) proceeds smoothly even under neutral conditions.[3] The reaction was found to occur by hydroxide ion initially abstracting the hydrogen from N' giving rise to anion (**2**) and an aryl isocyanate intermediate, as shown in Scheme 1.



Scheme 1.

Thus the presence of the labile hydrogen on N' is significant in the hydrolysis of (**1**). Indeed, we have also found that the structurally allied *N*-nitrosourea, where the H at the N'-position is replaced by a methyl group [ArNMeCON(N=O)Me], is markedly stable up to pH 7.6 at 36.8 °C.[3] In this paper we have extended our degradation studies of such neutrally stable compounds to a wider alkaline range. To the best of our knowledge, the only precedent for the hydrolysis of *N'*-blocked *N*-nitrosoureas is the work of Snyder and Stock concerning *N,N',N'*-trimethyl-*N*-nitrosourea in aqueous buffer solution.[4] We report here the hydrolysis of *N,N'*-dimethyl-*N'*-(*p*-nitrophenyl)-*N*-nitrosourea (**3**) in aqueous buffers of various amines. Kinetic results and a product study have been utilised to elucidate the hydrolytic mechanism. Furthermore, the micellar effect on the hydrolysis rates of (**1**) and (**3**) has been studied with cetyltrimethylammonium bromide (CTABr) as a cationic surfactant. The investigation of micellar catalysis in the hydrolysis of *N*-nitrosourea may be important in connection with the chemical reactivity in hydrophobic environments in biological systems.





Scheme 2.

N-Nitrosourea (**3**) was chosen as the substrate for the following reasons. (*a*) Our previous study showed that a series of *N*-nitrosoureas (**1**) have powerful mutagenic potency on *S. typhimurium* TA 1535, and also that while the mutagenic activity of (**3**) is of the same order of magnitude as that of (**1**), no significant activity was found for other *N'*-methylated homologues [*i.e.*, H instead of NO_2 on the phenyl ring of (**3**)].[5] Therefore, investigation of the decomposition of (**3**) in aqueous solution is of primary importance in understanding its chemical properties in physiological conditions. (*b*) Since both of the moieties, the *N*-nitroso group and the *N'*-*p*-nitrophenyl group of (**3**), are strongly electron-withdrawing, it is interesting to know which of the mechanisms would operate on the ureido linkage, C–N bond scission or N'–C scission. (*c*) Compound (**3**) decomposes gradually at a rate that allows the kinetic measurements to be made within the pH range 8—11. Also, *N*-methyl-*p*-nitroaniline (*p*-$NO_2C_6H_4NHMe$) liberated from (**3**) has a characteristic u.v. absorption at a longer wavelength ($\lambda_{max.}$ = 408 nm), which allows the use of conventional spectrophotometric methods with various buffer species.

Results

The hydrolysis of *N*-nitrosourea (**3**) (3.3×10^{-5}M) was carried out in various aliphatic amine buffers at 36.8 °C. The substrate decomposed at a moderate rate under basic conditions to afford

Table 1. Kinetic data in the hydrolysis of *N,N'*-dimethyl-*N'*-(*p*-nitrophenyl)-*N*-nitrosourea[a] at 36.8 °C

Buffer free base (M)[b]	pH[c]	10^3k_{obsd}/min^{-1}
Tris		
0.06	8.05	0.46
0.10	8.05	0.54
0.18	8.05	0.69
2-Amino-2-methyl-propane-1,3-diol		
0.025	8.79	1.41
0.05	8.79	1.67
0.10	8.79	2.06
Diethanolamine		
0.025	8.80	1.81
0.05	8.80	2.36
0.10	8.80	3.47
Ethanolamine		
0.025	9.36	6.93
0.05	9.36	9.04
0.10	9.36	13.33
Glycine		
0.025	9.46	7.37
0.05	9.46	9.12
0.106	9.46	14.44
β-Alanine		
0.025	9.89	24.57
0.05	9.89	33.80
0.10	9.89	56.80
Methylamine		
0.01	10.43	54.34
0.025	10.43	63.00
0.05	10.43	77.03
t-Butylamine		
0.025	10.48	33.35
0.05	10.48	34.74
0.10	10.48	38.11
n-Butylamine		
0.025	10.50	48.89
0.05	10.50	56.82
0.10	10.50	81.52
Triethylamine		
0.025	10.84	43.92
0.05	10.84	48.81
0.10	10.84	49.56
Piperidine		
0.025	11.09	69.30
0.05	11.09	85.56
0.10	11.09	100.43

[a] 3.32×10^{-5}M. [b] Ionic strength = 1.0 (NaCl). [c] Measured at 37 °C.

N-methyl-*p*-nitroaniline (**4**) and *N*-substituted-*N'*-methyl-*N'*-(*p*-nitrophenyl)urea (**5**) as the major products (Scheme 2). The reaction was followed by monitoring the increase with time of u.v. absorption of (**4**), at 408 nm, which showed good first-order kinetics up to three half-lives for all the runs.

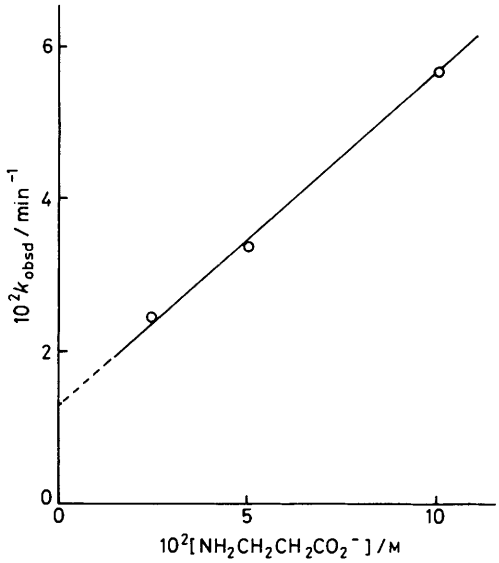


Figure 1. Effect of buffer concentration of the observed rate constant in the hydrolysis of (**3**) at pH 9.89 (36.8 °C)

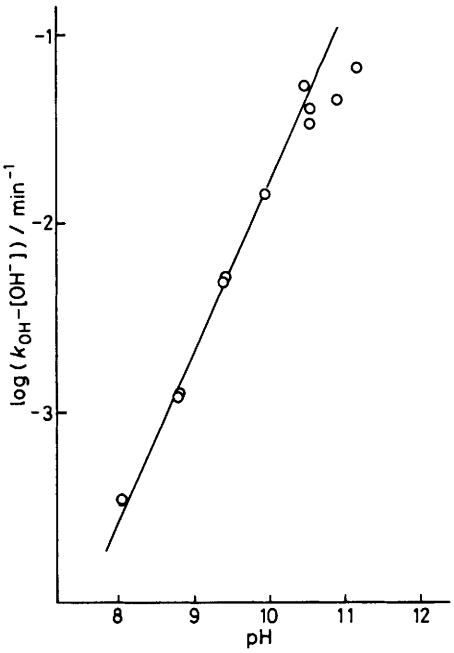


Figure 2. pH–rate profile for (**3**) at 36.8 °C

pH–Rate Profile.—The observed pseudo-first-order rate constants (k_{obsd}) obtained for various buffer concentrations at various pH values are summarized in Table 1. It can be seen from Table 1 that k_{obsd} increases with increase in the buffer concentration. Figure 1 shows a linear relationship between k_{obsd} and buffer concentration obtained at pH 9.89. Similar relationships have also been observed at other pH values. The k_{obsd} was extrapolated to zero buffer concentration and the intercept (k_{OH^-}[OH$^-$]) was obtained at various pH values. A plot of log(k_{OH^-}[OH$^-$]) *versus* pH gave a straight line of slope 0.90 as shown in Figure 2, which suggests that the alkaline hydrolysis of (**3**) is of first order with respect to the hydroxide ion.

Table 2. Catalytic coefficient (k_B) in the hydrolysis of *N*-nitrosourea (**3**) at 36.8 °C

Amine	pK_a[a]	10^2k_B/l mol^{-1} min^{-1}
Tris	7.75	0.192
2-Amino-2-methyl-propane-1,3-diol	8.52	0.87
Diethanolamine	8.59	2.21
Ethanolamine	9.15	8.53
Glycine	9.48	8.8
β-Alanine	9.91	42.9
n-Butylamine	10.35	65
Methylamine	10.36	56
t-Butylamine	10.36	6.4
Triethylamine	10.40	8
Piperidine	10.70	41

[a] Values at 37 °C cited from ref. 6.

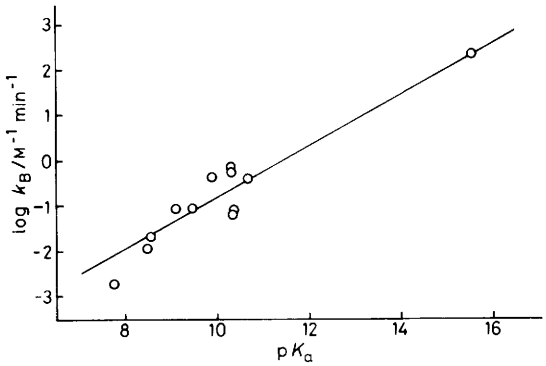


Figure 3. Plot of log k_B *versus* pK_a of base (36.8 °C)

Effect of Buffer Concentration.—The presence of a catalytic term due to the amine is also of significance. In order to compare the relative magnitudes of the catalysis, the slope (k_B) of k_{obsd} *versus* buffer concentration (*e.g.*, Figure 1) was calculated and summarized in Table 2. As shown in Table 2, k_B values increase with the increase in the pK_a of amines, and Brönsted plots, which include the catalytic term due to hydroxide ion (k_{OH^-}), gave a positive slope of 0.55 (Figure 3). Considering other reported β values for the base-catalysed hydrolysis of carboxylic acid esters or amides,[7] the β value of 0.55 obtained in our system suggests that the hydrolysis of (**3**) is subjected to the general base catalysis of amines.

Hydrolysis Products.—When (**3**) was decomposed in 0.1M-NaOH, (**4**) was produced stoicheiometrically. Other products, such as gaseous nitrogen or methanol arising from the *N*-methyl-*N*-nitrosamino fragment of (**3**), were not trapped or determined in this study. When the kinetics were studied in buffer solution, the u.v. spectrum at time infinity indicated the formation of *N*-substituted urea (**5**) as well as (**4**) in substantial yield. The yields of (**4**) and (**5**) in 0.2M-buffer solution under various pH conditions were determined spectrophotometrically and are summarized in Table 3. The hydrolysis with unhindered amines such as methylamine or n-butylamine afforded (**5**) as the major product. On the other hand, (**4**) was the sole product with t-butylamine or triethylamine, suggesting that the steric requirement of the amine has a marked effect on the product ratios.

Micellar Effect.—The effect of CTABr micelle on the hydrolysis rate of (**3**) was examined at pH 8.05 and at 24.8 °C,

Table 3. Yields of *N*-methyl-*p*-nitroaniline (**4**) and *N*-substituted urea (**5**) in the hydrolysis of (**3**) in 0.2M-buffer at 36.8 °C

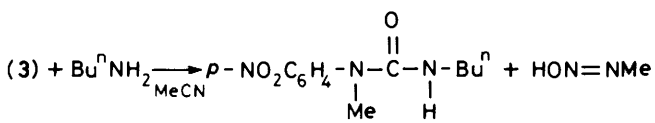
	Yield (%) ($\lambda_{max.}$/nm)	
Buffer amine	(**4**)	(**5**)
Tris	41	[a]
2-Amino-2-methyl-propane-1,3-diol	58	[a]
Diethanolamine	81	[a]
Ethanolamine	32	67 (322)
Glycine	35	63 (324)
β-Alanine	21	78 (326)
Methylamine	13	81 (329)
n-Butylamine	28	70 (329)
t-Butylamine	100	0
Triethylamine	100	0
Piperidine	94	0

[a] The yields were not determined. The u.v. spectrum at time infinity showed a small absorption near 308 nm, presumably due to the corresponding (**5**).

Table 4. Pseudo-first-order rate constants in the hydrolyses of (**1**) and (**3**) at 24.8 °C in the presence of CTABr (ionic strength = 0.05)

	10^3k_{obsd}/min^{-1}			
10^3[CTABr]/M	(**1**)-OMe[a]	(**1**)-H[a]	(**1**)-Cl[a]	(**3**)[b]
0	6.91	9.45	14.04	0.06
1	63.04	100.40	385.04	0.13
2	86.58	123.76	477.93	0.22
5	133.33	203.83	660.12	0.31
10	198.03	286.37	660.02	0.34
20	161.18	271.77	533.14	0.32

[a] [Substrate] = 9.9 × 10^{-5}M, 0.02M-NaH_2PO_4–Na_2HPO_4 buffer, pH 7.11. [b] [Substrate] = 3.3 × 10^{-5}M, 0.02M-Tris-HCl buffer, pH 8.05.

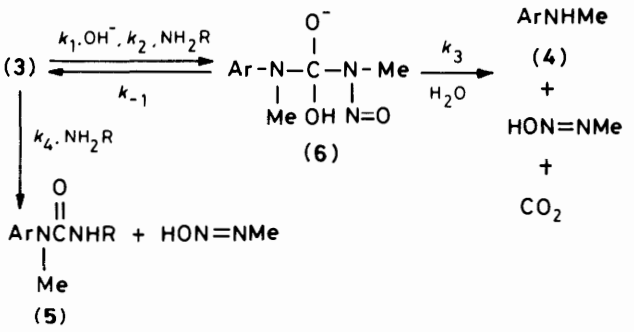


Scheme 3.

where the lowest limit of buffer concentration (0.02M-Tris) was employed. Our previous study indicated that the rate of hydrolysis of *N*-nitrosourea (**1**) increases linearly with increase in hydroxide ion concentration within the pH range 5.4—7.6.[3] A micellar effect on the hydrolysis of (**1**) was also investigated at pH 7.11. The observed rate constants of (**3**) and of three of the homologues of (**1**) at various surfactant concentrations are summarized in Table 4. For both substrates (**1**) and (**3**), the hydrolysis products were essentially the same to those under non-micellar conditions; only the rates were affected.

Discussion

Hydrolysis.—Prior to the discussion on the mechanism of hydrolysis, the reactivity of (**3**) with several aliphatic amines under anhydrous conditions will be discussed. The *N*-nitrosourea (**3**) was found to be stable for some days in an aprotic solvent at ambient temperature. When (**3**) was treated with an excess of molar n-butylamine in refluxing acetonitrile, the urea [(**5**), R = Bu^n] was formed in quantitative yield. Ethanolamine also reacted with (**3**) to afford the corresponding



Scheme 4.

urea. The reaction was found to occur by nucleophilic attack of the amine at the carbonyl group (Scheme 3), demonstrating that the *N*-methyl-*N*-nitrosamino moiety is a better leaving group than the *N'*-methyl-*N'*-*p*-nitrophenyl group; C–N scission is dominant over N'–C scission. However, when t-butylamine was used, the recovery of (**3**) was almost quantitative. These results imply that steric constraint exists on the ureido structure of (**3**). According to an inspection by a CPK molecular model, it was confirmed that the steric hindrance around the ureido carbon of (**3**) is huge, owing to the presence of the *N*-nitroso group and the *N'*-methyl substituent, which may constitute large restrictions to the amine nucleophile.

The steric hindrance around the carbonyl group of (**3**) is also present in the hydrolysis in aqueous buffer. As shown in Table 3, unhindered primary amines such as methylamine, β-alanine, or n-butylamine could react directly with (**3**) to afford the corresponding urea (**5**) in good yield. In these instances, the other product was aniline (**4**). The conversion of (**5**) into (**4**) during the reaction is excluded as a possible mechanism, since the authentic ureas (**5**) did not decompose at 36.8 °C up to pH 13. On the other hand, the hydrolysis with OH^- occurred preferentially in sterically hindered amine buffers (*e.g.*, t-butylamine) to afford only (**4**). These observations together with the foregoing kinetic results are in accord with the reaction in Scheme 4. The hydrolysis is initiated by OH^- attack at the carbonyl carbon to form the tetrahedral intermediate (**6**). The electron-withdrawing nature of the *N*-nitroso group of (**6**) makes the C–N bond scission easier, which rapidly leads to (**4**), methyldiazohydroxide, and CO_2.* A concurrent pathway is an S_N2 process with amine to expel methyldiazohydroxide.†

The kinetic equations for Scheme 4 are given in equations (1)—(3). Assuming a steady-state concentration of (**6**), $k_3 \gg k_{-1}$, and $d[(4)]/dt : d[(5)]/dt = k_1 + k_2 : k_4$, the rate of formation of (**4**) can be expressed by equation (4). When k_4 is ignored (*e.g.*, t-butylamine), the rate constant is given by equation (5).

$$\frac{d[(4)]}{dt} = k_3[(6)] \quad (1)$$

$$\frac{d[(5)]}{dt} = k_4[(3)][NH_2R] \quad (2)$$

$$\frac{d[(6)]}{dt} = k_1[(3)][OH^-] + k_2[(3)][H_2O][NH_2R] - k_{-1}[(6)] - k_3[(6)] \quad (3)$$

$$\frac{d[(4)]}{dt} = \frac{(k_1 + k_2)(k_1[OH^-] + k_2[H_2O][NH_2R] + k_4[NH_2R])}{k_1 + k_2 + k_4} \times [(3)] \quad (4)$$

$$k_{obsd} = k_1[OH^-] + k_2[H_2O][NH_2R] \quad (5)$$

The present study has thus shown that *N*-nitrosourea (**3**) readily decomposes not only in the presence of OH^-, but also through nucleophilic attack of several amines even at ambient temperature. The facile reaction with an amine nucleophile is attributed to the high leaving tendencies of the *N*-methyl-*N*-nitrosamino moiety of (**3**).[8] In both pathways methyldiazohydroxide is formed, which is assumed to be the active methylating species for cellular macromolecules under physiological conditions.[9]

It is worthy of note that (**3**) undergoes nucleophilic displacement by the amine to provide transaminated urea (**5**). Similar reactions of other *N*-nitroso compounds have also been reported for *N*-nitrosacetamides with imidazole in aqueous solution,[10] and for *N*-nitrosalkylamides or *N*-nitrosophenacylamides with aliphatic primary amines in organic solvents.[11] From a biochemical standpoint, the reaction of (**3**) with an amine nucleophile is of interest in that carbamoylation of some cellular amines[12] might possibly occur by *N*-nitrosourea (**3**). The relevance of such implications to the mutagenesis of (**3**)[5] may be of importance. Further investigation of (**3**) with other nucleophiles, such as phenols or thiols, is needed.

Effect of CTABr Micelle on the Hydrolysis Rates of (**1**) *and* (**3**).—The effect of cationic micelles on the hydroxide ion-catalysed reaction has been widely investigated.[13] Amongst the investigations carried out, preference for micellar catalysis to give the elimination reaction has been pointed out by Lapinte and Viout in the reaction of 1-bromo-2-phenylpropane in aqueous alkaline solution, where the rate of *E*2 reaction was enhanced by CTABr micelle by inhibiting the competitive S_N1 process.[14] A crucial example can be seen in the work of Tagaki *et al.* on the alkaline hydrolysis of α-substituted *p*-nitrophenyl acetates.[15] They found that the mechanism of hydrolysis changes over from an ordinary hydroxide ion attack at the carbonyl carbon under non-micellar conditions to an *E*1cB process forming an α-carbanion with large rate acceleration under CTABr micellar conditions. This is relevant to our study. For instance, as shown in the present work, the hydrolysis of *N*-nitrosourea (**3**) is initiated by hydroxide ion attack at the ureido carbon. However, we found previously that another series of compounds (**1**) bearing a labile hydrogen on N' undergo hydrolysis *via* an *E*1cB process forming anion (**2**) as the intermediate.[3] Thus, both hydrolyses are distinguished mechanistically according to active reaction sites. These findings prompted us to compare micellar catalysis on (**1**) and (**3**). There has been no report so far on the micellar-catalysed reaction of compounds that possess a ureido linkage in their molecules.

For substrates (**1**) and (**3**), the micellar effect was examined using CTABr at 24.8 °C and with a lower ionic strength of 0.05. The pseudo-first-order rate constants (Table 4) are plotted against the surfactant concentration, as shown in Figure 4. The Figure indicates that increase in the surfactant concentration (up to 10^{-2}M) results in an increase in k_{obsd} for all the substrates examined. The maximum rate (k_ψ, [CTABr] = 10^{-2}M) of (**3**) was six-fold larger than the non-micellar rate (k_o). This rate

* The decarboxylation step of *N*-methyl-*N*-arylcarbamate ion, which is omitted in Scheme 4, is not kinetically important, as the reported rate constant of decarboxylation of *N*-(*p*-nitrophenyl)carbamate ($p\text{-}NO_2C_6H_4NHCO_2^- \xrightarrow{H_2O} p\text{-}NO_2C_6H_4NH_2 + CO_2$, $k = 0.502$ min^{-1}, pH 8.14; S. L. Johnson and D. L. Morrison, *J. Am. Chem. Soc.*, 1972, **94**, 1323) is much too large when compared with that of the hydrolysis of (**3**).

† A referee suggested, for the S_N2 process, the presence of catalysis by amine as a general base. However, we cannot conclude from the present data whether such catalysis may affect k_4.

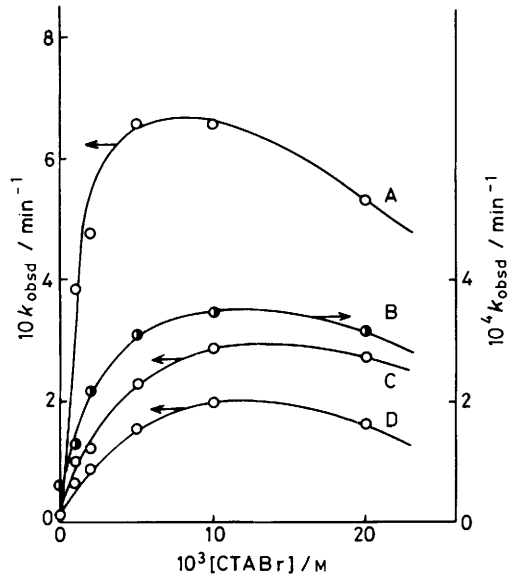


Figure 4. Effect of CTABr on the hydrolysis rates of (**1**) [A (**1**)-Cl, C (**1**)-H, D (**1**)-OMe] (○, 0.02M-phosphate buffer, pH 7.11) and (**3**) (B) (●, 0.02M-Tris buffer, pH 8.05); ionic strength = 0.05 (NaCl); 24.8 °C

ratio of (**3**) appears to be comparable to those of other reported catalyses by a simple cationic surfactant (CTABr) in the nucleophilic reaction of OH^- with the ester carbonyl group.[13] However, the micellar effect on (**1**) is more pronounced. For example, (**1**)-Cl showed a rate enhancement of $k_\psi/k_0 = 47$. It has been suggested that, in the hydrolysis of α-substituted *p*-nitrophenyl acetates, a carbanion with a charge system that is more delocalized is more stabilized on the positive electrostatic field of the cationic micellar surface.[15] This may also be the case for the micellar reaction of *N*-nitrosourea. Namely, as for the *E*1cB process of (**1**), the negative charge on the nitrogen (N′) of the intermediate (**2**) is possibly delocalized through the resonance effect of the aryl group directly attached to N′, whereas such delocalization of the negative charge is not anticipated for the tetrahedral intermediate (**6**). Consequently, the stabilization by the CTABr micelle is more pronounced for (**2**). This seems to be further substantiated by the much larger micellar catalysis of (**1**)-Cl, which has an electron-withdrawing substituent on the phenyl ring.

Experimental

M.p.s are uncorrected. I.r. spectra were recorded on a Shimadzu IR-400 spectrophotometer and u.v. spectra were carried out on a Shimadzu UV-200 spectrophotometer. N.m.r. spectra were taken by a Hitachi R-24 instrument (60 MHz) and chemical shifts are presented by δ values using Me_4Si as an internal standard.

Materials.—All the reagents used were of commercial grade. The preparation of *N*-nitrosourea (**3**) has been described previously.[3] Acetonitrile was distilled once over $CaCl_2$ and then with P_2O_5. The stock solution of (**3**) was made from the anhydrous acetonitrile, to an accurate concentration of 1×10^{-2}M. The buffer solutions were prepared from doubly distilled water with appropriate amines and the ionic strengths were adjusted with NaCl. The pH of the buffer solution was measured at 37 °C.

Kinetic Measurements.—An aliquot of the stock solution of (**3**) (10 μl) was dissolved in an appropriate buffer solution (3 ml), thermostatted in a u.v. cell (36.8 ± 0.3 °C). The initial concentration of (**3**) was 3.32×10^{-5}M. As the reaction proceeded, the absorption of the substrate (296 nm) gradually decreased and the absorption of (**4**) appeared at 408 nm. The increase of the latter absorption (A_t) was monitored with time. The infinity reading (A_∞) was recorded after at least eight half-lives had elapsed. The pseudo-first-order rate constants presented in Table 1 are those calculated from the plots of log-$[(A_\infty - A_0)/(A_\infty - A_t)]$ *versus* time. The errors in duplicate runs for the rapid reactions (pH ≧ 10.43) were within the range 0.07—4.0%. For the micellar reactions, the kinetic measurements were made in the same way as described above, except that an ionic strength of 0.05 was employed and the temperature was lowered to 24.8 °C.

Preparation of Authentic Samples of N-*Substituted*-N′-*methyl*-N′-(p-*nitrophenyl*)*urea* (**5**): N-*n*-*Butyl Derivative* (R = Bu^n).—According to the literature method,[11] direct transamination of *N*-nitrosourea (**3**) was carried out in an aprotic solvent. Namely, (**3**) (0.238 g, 0.001 mol) was allowed to react with Bu^nNH_2 (0.73 g, 0.01 mol) in anhydrous MeCN (10 ml) for 30 min at 70—80 °C. After cooling, the solvent and the excess of amine were evaporated under reduced pressure to leave an oil (0.251 g, 100%), which crystallized on standing at room temperature. Recrystallization from ether gave white needles of the desired urea, m.p. 78.5—80.0 °C (Found: C, 57.4; H, 6.9; N, 16.85. $C_{12}H_{17}N_3O_3$ requires C, 57.4; H, 6.8; N, 16.7%); $\nu_{max.}(CHCl_3)$ 3 460 (NH), 2 960, and 1 660 (C=O) cm^{-1}; $\lambda_{max.}(H_2O)$ 329 nm, $\varepsilon_{max.}$ 7 500 l mol^{-1} cm^{-1}; δ($CDCl_3$) 0.68—1.62 (7 H, m, $CH_2CH_2CH_3$), 3.08—3.44 (2 H, m, NCH_2), 3.36 (3 H, s, N′CH_3), 4.63 (1 H, m, NH), 7.35 and 8.15 (4 H, A_2B_2, *J* 11 Hz, C_6H_4).

N-2-*Hydroxyethyl Derivative* (R = CH_2CH_2OH). Substrate (**3**) was treated with ethanolamine (10 molar excess) as described above. M.p. 115.0—116.0 °C (from ether) (Found: C, 50.3; H, 5.4; N, 17.5. $C_{10}H_{13}N_3O_4$ requires C, 50.2; H, 5.5; N, 17.6%); $\nu_{max.}(CHCl_3)$ 3 460, 3 400 (OH), 2 950, and 1 650 cm^{-1}; $\lambda_{max.}(H_2O)$ 322 nm, $\varepsilon_{max.}$ 7 600 l mol^{-1} cm^{-1}; δ($CDCl_3$) 2.16 (1 H, s, OH), 3.14—3.82 (4 H, m, CH_2CH_2), 3.30 (3 H, s, N′CH_3), 5.14 (1 H, m, NH), 7.36 and 8.17 (4 H, A_2B_2, *J* 11 Hz, C_6H_4).

N-*Carboxymethyl derivative* (R = CH_2CO_2H). To a solution of glycine (9.0 g, 0.12 mol) in 0.2M-NaOH (300 ml) with NaCl (6.1 g) was added a solution of (**3**) (1.0 g, 0.0043 mol, 15 ml of MeCN). The resulting suspension was stirred at 37 °C for 16 h. The mixture was extracted with $CHCl_3$ (100 ml × 3) to remove the resultant (**4**) (*ca.* 0.2 g). The aqueous layer was acidified by hydrochloric acid to pH 3, condensed (40 ml), and then extracted with $CHCl_3$ (50 ml × 3). The organic layer was washed with saturated brine, dried ($MgSO_4$), and evaporated to leave an oil (0.315 g, 30%) which crystallized at room temperature. M.p. 140.0—142.0 °C (from acetone–hexane) (Found: C, 47.5; H, 4.35; N, 16.6. $C_{10}H_{11}N_3O_5$ requires C, 47.4; H, 4.4; N, 16.6%); $\nu_{max.}$(KBr) 3 410, 3 000, 1 740, and 1 590 cm^{-1}; $\lambda_{max.}(H_2O)$ 324 nm, $\varepsilon_{max.}$ 7 800 l mol^{-1} cm^{-1}; δ[$^2H_6$]acetone) 2.75 (3 H, s, N′Me), 3.28 (2 H, d, NCH_2), 5.74 (1 H, m, NH), 6.92 and 7.52 (4 H, A_2B_2, *J* 11 Hz, C_6H_4).

N-2-*Carboxyethyl derivative* (R = $CH_2CH_2CO_2H$). Substrate (**3**) was hydrolysed in sodium hydroxide solution in the presence of excess of molar β-alanine (37 °C, 2 h) according to the procedure for R = CH_2CO_2H. M.p. 141.0—142.0 °C (from acetone–hexane) (Found: C, 49.4; H, 4.9; N, 15.6. $C_{11}H_{13}N_3O_5$ requires C, 49.4; H, 4.9; N, 15.7%); $\nu_{max.}$(KBr) 3 390, 2 900, 1 710, and 1 580 cm^{-1}; $\lambda_{max.}(H_2O)$ 326 nm, $\varepsilon_{max.}$ 7 500 l mol^{-1} cm^{-1}; δ([$^2H_6$]acetone) 1.94 (2 H, t, CH_2CO_2H), 2.60—3.10 (2 H, q, NCH_2), 2.72 (3 H, s, N′CH_3), 5.58 (1 H, m, NH), 6.93 and 7.53 (4 H, A_2B_2, *J* 11 Hz, C_6H_4).

N-*Methyl derivative* (R = Me). To a solution of *N*-methyl-*p*-nitroaniline (2 g, 0.013 mol) in toluene (100 ml) was added, dropwise, methyl isocyanate (20 ml, 0.339 mol) at room

temperature. The mixture was stirred at 80 °C for 3 days. After cooling, evaporation of the solvent left light yellow crystals (2.7 g, 98%). M.p. 128.0—130.0 °C (from ether–acetone) (Found: C, 51.2; H, 5.3; N, 20.0. $C_9H_{11}N_3O_3$ requires C, 51.7; H, 5.3; N, 20.1%); $\nu_{max.}(CHCl_3)$ 3 480, 3 010, and 1 660 cm^{-1}; $\lambda_{max.}(H_2O)$ 329 nm, $\varepsilon_{max.}$ 6 800 l mol^{-1} cm^{-1}; δ ([$^2H_6$]acetone) 2.08 (3 H, d, NCH_3), 2.67 (3 H, s, $N'CH_3$), 5.39 (1 H, m, NH), 6.88 and 7.48 (4 H, A_2B_2, *J* 11 Hz, C_6H_4).

Product Analysis.—Because of the limited solubility of (**3**), quantitation of the hydrolysis products was achieved from the kinetic runs. Namely, after the reaction was complete (more than eight half-lives), the u.v. spectrum of the solution displayed two characteristic bands, one was the urea (**5**) (308—329 nm) and the other was *N*-methyl-*p*-nitroaniline (**4**) (408 nm). Identification of these products was performed by t.l.c. comparison (silica gel, ether–$CHCl_3$ or ether–hexane) of the R_F values with those of their authentic samples, and also performed independently by preparative hydrolysis of (**3**) in buffer solutions. The yields of (**4**) and (**5**) were determined from the intensity of each absorption maxima and are summarized in Table 3.

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