Acid Catalysis of the Denitrosation of *N*-Methyl-*N′*-cyclohexyl-*N*-nitrosothiourea

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Synopsis. The kinetics of denitrosation of *N*-methyl-*N′*-cyclohexyl-*N*-nitrosothiourea has been studied in the range of pH 3.45—3.73 using four organic acids; acetic acid, formic acid, chloroacetic acid, and cyanoacetic acid. Analyses of the kinetic data including the Brönsted plots have shown that the reaction is subject to a general acid catalysis.

It has been observed that many aliphatic nitroso compounds can act as nitrosating agents of naturally existing amines under conditions analogous to those found in the rat or human stomach (pH 1.7—3.6 with thiocyanate or other nucleophiles as catalyst).[1] Certain aromatic *N*-nitroso compounds have also long been known to be good transnitrosating agents.[2] In addition to above substances, *N*-methyl-*N′*-cyclohexyl-*N*-nitrosothiourea(**1**) was found to undergo denitrosation under nearly neutral conditions (pH 6.7). To investigate acid catalysis of the denitrosation of **1**, the buffer dilution study was performed in the range of pH 3.45—3.73.

Results and Discussion

Pseudo first-order rate constants were measured in a series of buffer solutions of four carboxylic acids at different buffer concentration. The results are summarized in Table 1. The plots of k_{obsd} *vs.* $[\text{buffer}]_T$ (total concentration of buffer) are shown in Fig. 1. Since the denitrosation of **1** is catalyzed by buffer and H_3O^+, the k_{obsd} is given by Eq. 1,

$$k_{obsd} = k_{H^+}[H_3O^+] + k_{HA}'[HA] \qquad (1)$$

where k_{HA}' and k_{H^+} are second-order rate constants for the buffer catalysis and the hydronium-ion catalysis, respectively. Therefore, the slope and the intercept in Fig. 1 correspond to k_{HA}' and $k_{H^+}[H_3O^+]$, respectively. The slopes and the intercepts were calculated by least squares computation. The k_{H^+} values were obtained from the intercepts. Since the true buffer catalysis is associated with the concentration of acid form of buffer, true second-order rate constants for the general acid catalysis (k_{HA}) are equal to $k_{HA}'/1-\alpha'$ (α'; dissociated fraction of acid). The k_{H^+} and k_{HA} determined are summarized in Table 2. From the data of Table 2, the average k_{H^+} value of **1** for all buffers determined at pH 3.45—3.73 was calculated to be 4.85 ± 0.93. The lowest k_{H^+} value for cyanoacetic acid buffer among those for other acids may be caused by somewhat hindered approach of hydronium ion to the amino nitrogen atom of **1** due to the interaction between hydronium ion and the resonance-stabilized anion partly formed by the abstraction of the α-hydrogen of the parent acid. This anion must be analogous to that formed from mandelonitrile by the attack of base. This average k_{H^+} value was 45 times greater than k_{HA} value for cyanoacetic acid and about 260 times greater than that for acetic acid, respectively. The former value was almost the same with that of acid catalysis in ether hydrolysis (*ca.* 40)[3] and the latter was about 1.7 times greater than that of acid catalysis in hydrolysis of 1,1-dimethoxy-2-phenylethene (*ca.* 160).[4]

Table 1. Denitrosation rate constants of *N*-methyl-*N′*-cyclohexyl-*N*-nitrosothiourea[a] at 36.9 °C

Buffer	pH	[Buffer]/M	$10^3 \times k/\text{s}^{-1}$ [b]
$NCCH_2COOH$	3.45	0.01	1.33±0.02
		0.02	1.46±0.01
		0.04	1.64±0.03
$ClCH_2COOH$	3.73	0.01	1.12±0.03
		0.02	1.27±0.02
		0.04	1.45±0.01
HCOOH	3.58	0.01	1.56±0.02
		0.02	1.76±0.02
		0.03	1.93±0.05
CH_3COOH	3.54	0.01	1.63±0.01
		0.02	1.94±0.03
		0.04	2.18±0.01

a) 8.28×10^{-5} M, μ=0.2(NaCl). b) The errors are deviation from the average of two runs.

Table 2. Second-order rate constants for general acid catalysis

Catalyst	p*Ka*[a]	Reaction pH	α'	$\text{Slope}((1-\alpha')k_{HA})/\text{M}^{-1}\,\text{s}^{-1}$	$k_{HA}/\text{M}^{-1}\,\text{s}^{-1}$
$NCCH_2COOH$	2.47	3.45	0.904	$(1.02\pm0.04)\times10^{-2}$	0.107 ±0.005
$ClCH_2COOH$	2.87	3.73	0.878	$(1.08\pm0.06)\times10^{-2}$	0.0888±0.0053
HCOOH	3.75	3.58	0.403	$(1.81\pm0.15)\times10^{-2}$	0.0304±0.0025
CH_3COOH	4.76	3.54	0.056	$(1.76\pm0.01)\times10^{-2}$	0.0186±0.0001
H_3O^+	−1.74				3.46±0.03[b]
					5.48±0.19[c]
					5.27±0.00[d]
					5.18±0.07[e]

a) Ref. 3. b—e) From the intercept of [Buffer] dependence: b) cyanoacetic acid; c) chloroacetic acid; d) formic acid; e) acetic acid.

The Brönsted plots of pK_a *vs.* log k_{HA} provided the linear relationship (r=0.998, slope($-\alpha$)=−0.38) presented by Eq. 2, as shown in Fig. 2.

$$\log k_{HA} = 0.006 - 0.38\,\text{p}K_a \qquad (2)$$

This indicates that the denitrosation of **1** is a general acid catalysis. The higher general acid catalysis by the acids possessing electron-withdrawing substituent at the α position suggests that the acid-catalyzed denitrosation of **1** would occur through rate-determining protonation (Scheme 1). The rate of denitrosation at 36.9 °C was measured in CH_3COOD/D_2O to evaluate the solvent deuterium isotope effect. The results at the same buffer concentration (0.05 M, $[HA(DA)]/[A^-]$=0.42, μ=0.2) also showed that denitrosation of **1** involves rate-determining protonation ($k(CH_3COOH/H_2O)/k(CH_3COOD/D_2O)$=1.36, $k(CH_3COOH/$

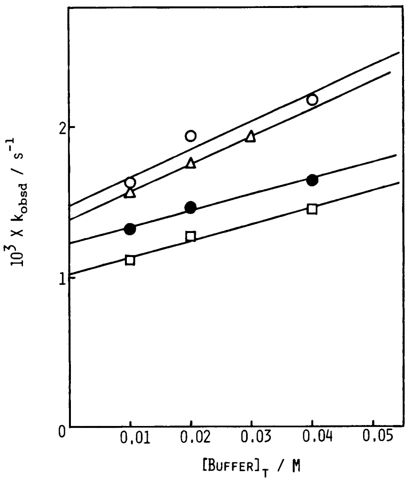


Fig. 1. Plots of k_{obsd} *vs.* buffer concentration (cyanoacetic acid (●), chloroacetic acid (□), formic acid (△), and acetic acid (○)) for **1**.

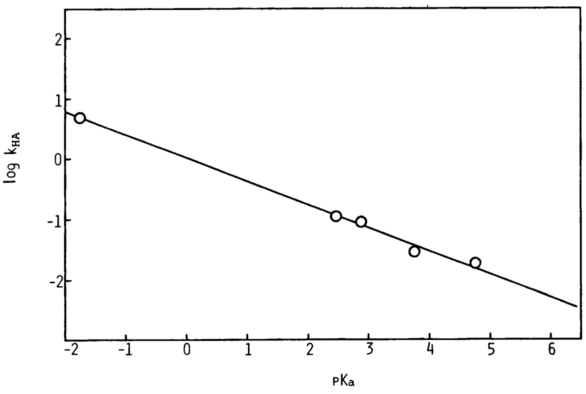
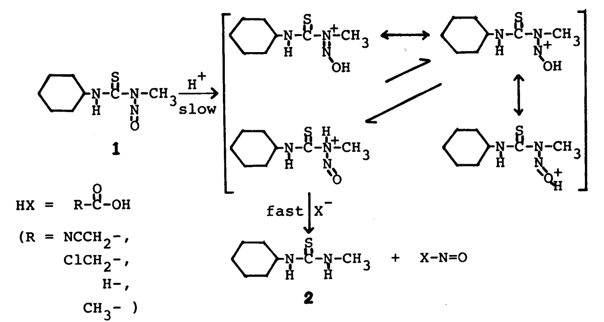


Fig. 2. The Brönsted plots for denitrosation of **1**.



Scheme 1.

H_2O)=$1.38 \times 10^{-3}\,s^{-1}$, $k(CH_3COOD/D_2O)=1.01 \times 10^{-3}\,s^{-1}$). The analogous general acid-catalyzed denitrosation has also been observed in decomposition of *N*-*n*-butyl-*N*-nitrosoacetamide[5] or *N*-nitroso-2-pyrrolidone.[6] The latter showed the Brönsted relationship with a slope(α) of 0.64. Since the rate of protonation of **1** is influenced by the reduced basicity of the amino nitrogen atom due to electron-withdrawing property of the nitroso-substituent, which may also be true for the protonation of *N*-nitroso-2-pyrrolidone, the difference in α between **1** (α=0.38) and *N*-nitroso-2-pyrrolidone (α=0.64) must be caused by the basicity difference of the amino nitrogen atom which is affected by the thiocarbonyl group in **1** or the carbonyl group in the latter. These results suggest that **1** would also be subject to general acid catalysis by carboxylic acid residue of an enzyme under acidic conditions.

Experimental

N-*Methyl*-N′-*cyclohexyl*-N-*nitrosothiourea (1).* *N*-Methyl-*N*′-cyclohexylthiourea (**2**) (0.6 g, 3.48 mmol), which was obtained from the reaction of cyclohexylamine and methyl isothiocyanate in acetone at room temperature (18 h), was treated with sodium nitrite (0.36 g, 5.23 mmol) in acetic acid (5 ml)–acetic anhydride (20 ml) at 0 °C (1 h). The reaction mixture was poured in ice-water and extracted with ether (80 ml) three times. The ethereal solution was washed with 5% Na_2CO_3 solution (4 times), 5% $NaHCO_3$ solution (3 times), saturated NaCl solution, dried ($MgSO_4$), and evaporated to give an orange liquid, which upon trituration with pentane gave yellow crystals of **1** (0.39 g, 56%). **1**: Mp 44.5—45.5 °C (lit,[7] mp 35—36 °C); IR (KBr) 3310 (NH), 2930, 2860, 1515, 1495, 1407, 1175, 1000, 965, 910, and 790 cm^{-1}; NMR (CCl_4) δ 8.36 (1H, br, NH), 4.34 (1H, m, methine), 3.43 (3H, s, $-CH_3$), 2.40—1.00 (10H, m, $-CH_2-$). Found: C, 47.63; H, 7.46; N, 21.20; S, 15.90%. Calcd for $C_8H_{15}ON_3S$: C, 47.73; H, 7.51; N, 20.88; S, 15.93%. **2**: Mp 163—164 °C (lit,[7] mp 165—166 °C).

Product Analysis. A solution of *N*-methyl-*N*′-cyclohexyl-*N*-nitrosothiourea(**1**)(150 mg, 0.745 mmol in 5 ml of methanol was added to 150 ml of Tris buffer solution (pH 6.70, 0.05 M, μ=0.2 with NaCl) and stirred for 5 h at 37 °C. After cooling, the contents were saturated with NaCl and extracted with ether (80 ml × 3) and the combined extracts were washed with saturated NaCl solution, dried ($MgSO_4$), and evaporated to give a solid and yellow liquid. The product was separated with pentane to **2** (white solid, 53 mg, 41%) insoluble in pentane and cyclohexyl isothiocyanate (**3**, yellow liquid, 45 mg, 43%) soluble in pentane. **3**: IR(neat) 2950, 2870, 2110(N=C=S), 1450, 1360, and 1307 cm^{-1}; NMR (CCl_4) δ 3.85—3.45 (1H, m, methine), 2.25—1.20 (10H, m, $-CH_2-$).

Kinetic Measurements. The kinetic measurements of the denitrosation of **1** were carried out at 36.9 ± 0.3 °C in a thermostated UV cell. The appropriate buffer solution (3 ml) was transferred into each cell with a pipet, and after standing for thermal equilibration, the reaction was initiated by adding 20 μl of the substrate solution (1 mg/0.4 ml-MeOH) with the aid of a microsyringe. The progress of the reaction was monitored spectrophotometrically by following the disappearance of the absorption maximum at 270 nm. The reaction followed pseudo first-order kinetics; infinity readings were obtained after 8—10 half lives.

References

1) S. S. Singer, W. Lijinsky, and G. M. Singer, *Tetrahedron Lett.*, **1977**, 1613; S. S. Singer, *J. Org. Chem.*, **43**, 4612 (1978); S. S. Singer, G. M. Singer, and B. B. Cole, *ibid.*, **45**, 4931 (1980).

2) C. L. Baumgardner, K. A. MaCallum, and J. P. Freeman, *J. Am. Chem. Soc.*, **83**, 4417 (1961); H. Sieper, *Chem. Ber.*, **100**, 1646 (1967).

3) A. J. Kresge and Y. Chiang, *J. Am. Chem. Soc.*, **95**, 803 (1973).

4) T. Okuyama, S. Kawano, and T. Fueno, *J. Org. Chem.*, **46**, 4372 (1981).

5) C. N. Berry and B. C. Challis, *J. Chem. Soc., Perkin Trans. 2*, **1974**, 1638.

6) B. C. Challis and S. P. Jones, *J. Chem. Soc., Perkin Trans. 2*, **1975**, 153.

7) J. W. Lown and S. M. S. Chauhan, *J. Org. Chem.*, **48**, 507 (1983).