Electronic and Steric Effects of Alkyl Group on Denitrosation of 3-Alkyl-1-methyl-1-nitrosothioureas

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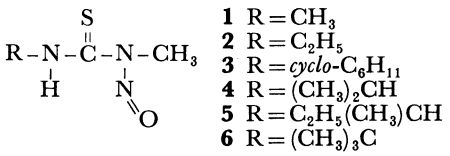
A series of 3-alkyl-1-methyl-1-nitrosothioureas with R=CH_3, C_2H_5, *cyclo*-C_6H_{11} (**3**), $(CH_3)_2CH$ (**4**), $C_2H_5(CH_3)CH$, and $(CH_3)_3C$ were synthesized and their rates of acid catalyzed (pH<6.5) denitrosation measured. According to rate measurements in CH_3COOD-D_2O, the kinetic isotope effect $k_H:k_D$ is 1.25 for **4**. Except **3**, a linear plot of log k_R/k_{Me} for the denitrosation of $RNHCSN(NO)CH_3$ *vs.* σ^* provides $\rho^*=-0.98$ ($r=-0.997$). The significant factor affecting the rate-determining step of the denitrosation of these *N*-nitrosothioureas at pH 4.6 is the electronic effect of the substituent at the N_3 position.

Carcinogenic nitrosamines are caused to result from reaction of a dietary or endogenous nitrite with ingested amines.[1] However, nitrite *in vivo* is not the only possible origin of nitroso compounds. Certain aromatic nitrosamines[2,3] and many aliphatic nitrosamines[4] act as nitrosating agents toward naturally occurring amines under acidic conditions. In aqueous acid solutions, nucleophiles usually attack the protonated form of nitrosamine in the rate-determining step,[5] but when strongly electron-withdrawing groups are present in nitrosamine, proton transfer to nitrosamine is believed to be rate-limiting. The latter situation arises in reactions of *N*-nitroso amides[6-9] and *N*-nitroso sulfonamide.[10] Besides the above *N*-nitroso compounds 3-cyclohexyl-1-methyl-1-nitrosothiourea was also found to be protonated in the rate-determining step in denitrosation.[11]

On the other hand, reactions of thiourea (or alkylthiourea) with nitrous acid[12] or *N*-methyl-*N*-nitrosoaniline[13] have been investigated, with a conclusion that *S*-nitrosation occurs to form the intermediate $(NH_2)_2C=\overset{+}{S}-N=O$, which is capable of acting directly as a nitrosating agent. Synthesis and decomposition of a series of *N*-nitrosothioureas have recently been studied with an anticipation that isothiocyanate released from them should be less reactive toward proteins than isocyanate, and the formation mechanism and conformation of *N*-nitrosothioureas and stereoelectronic control of their aqueous decomposition have been revealed by Lown and Chauhan,[14] but no effect of substituents at N_3 on the denitrosation has been examined. In relation to isothiocyanates it should be noted that some isothiocyanates have exhibited antitumor properties in their own right.[15] Therefore, it is interesting and necessary for understanding the mechanism of denitrosation to study the effect of substituents at the N_3 position of 3-alkyl-1-methyl-1-nitrosothioureas quantitatively and evaluate their trans-



1 R=CH_3
2 R=C_2H_5
3 R=*cyclo*-C_6H_{11}
4 R=$(CH_3)_2CH$
5 R=$C_2H_5(CH_3)CH$
6 R=$(CH_3)_3C$

Table 1. Decomposition product from 3-alkyl-1-methyl-1-nitrosothioureas under acidic conditions[a]

Nitroso-thiourea	3-Alkyl-1-methyl-thiourea/%	3-Alkyl-1-methyl-urea/%
1	80[b]	—
3	66	17[c]
4	38	11[c]
6	24	40
6(N_2)[d]	22.4	4.5[c]
6(N_2, $NaNO_2$)[e]	37	37[c]

a) Acetate buffer (pH 4.6), 0.05 M, and μ=0.2 (NaCl). b) Acetate buffer (pH 5.0), 0.05 M, and μ=0.2 (NaCl). c) From NMR analysis; not isolated yield. d) Under nitrogen atmosphere. e) With sodium nitrite under nitrogen atmosphere.

nitrosating abilities from a chemical viewpoint. In this research the denitrosation of a series of *N*-methyl-*N*-nitrosothioureas having various substituents at the N_3 position has been studied under acidic conditions.

Results and Discussion

Decomposition of **3** and **4** in acetate buffer (pH 4.6) produced predominantly 3-cyclohexyl-1-methylthiourea (**3a**) (66%) and 3-isopropyl-1-methylthiourea (**4a**) (38%), respectively, and that of **1** in acetate buffer (pH 5.0) produced 1,3-dimethylthiourea (**1a**) (80%). These results suggest that in the decomposition of **1**, **3**, or **4** under these acidic conditions the protonated *N*-nitrosothioureas are led markedly to the unimolecular excision of the NO^+ group or the denitrosation by the attack of nucleophile. Decomposition of **6** in acetate buffer (pH 4.6) produced a mixture of 3-*t*-butyl-1-methylthiourea (**6a**) and 3-*t*-butyl-1-methylurea (**6b**) in a ratio of **6a**:**6b**=37:63, which implies formation of protonated *N*-nitrosothiourea and *S*-nitrosoisothiourea, followed by unimolecular denitrosation or nucleophilic attack leading to thiourea or urea derivative (Scheme 1). The results are summarized in Table 1. The formation of these products is different from that of products (urea, nitrosourea, and propyl isothiocyanate) from 1,3-dipropyl-1-nitrosothiourea under acidic conditions (acetone:1 M HCl=5:1)(1 M=1 mol dm^{-3}) studied by Lown and Chauhan.[14]

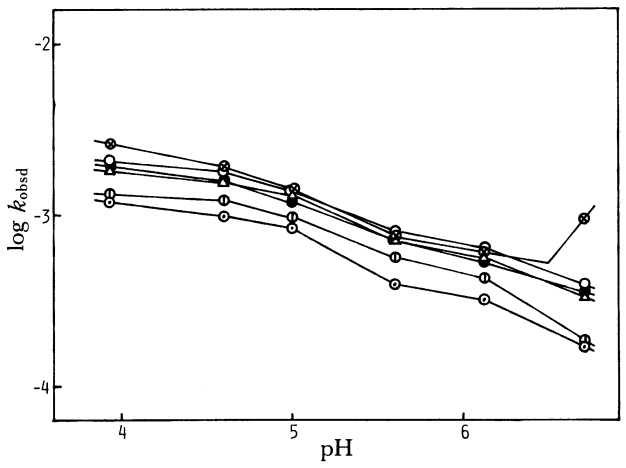


Fig. 1. pH-Rate profiles for decomposition of **1** — **6** at 36.9°C, pH 3.90—5.60 (acetate buffer), pH 6.12 (imidazole buffer), and pH 6.70 (Tris buffer), 0.05 M, μ=0.2 (NaCl), **1** (⊙), **2** (Φ), **3** (○), **4** (△), **5** (●), and **6** (⊗).

Table 2. Test for catalysis by nucleophile in the denitrosation of **4** at pH 4.6[a]

Added Nucleophile	$10^3k_0/s^{-1}$[b]
0	1.37±0.01
0.176 M NaCl	1.56±0.03
0.176 M NaBr	1.71±0.02
1.05 M NaBr	1.61±0.01
0.176 M NaSCN	1.63±0.03
1.05 M NaSCN	1.56±0.01
0.176 M NaI	1.70±0.02
0.176 M NaN_3	1.72±0.04

a) [**4**]=8.28×10^{-5} M in acetate buffer at 36.9°C. b) The errors are given by deviation from average of two runs.

pH-log k_{obsd} plots (pH 3.9—6.7) for the decomposition of compounds **1—6** are shown in Fig. 1. As shown in Fig. 1, decomposition rate constants for **1—6** decrease with increase in pH (pH<6.5), which indicates that all of them are subject to acid catalysis[11] and that the reactivity follows the sequence **6>3>5>4>2>1** at pH 4.6. The pH-rate profile for the decomposition of **6** shows an inverted-bell curve around pH 6.5, exhibiting the highest reactivity in this series above pH 6.5. The rate constant for the denitrosation of **4** at pH 4.6 is almost unaffected by the presence of nucleophile Br^- or SCN^-, as shown by the data in Table 2. This feature contrasts with the denitrosation of nitrosamine previously studied[5] but resembles the pattern of denitrosation for *N*-nitroso amide[9] and *N*-nitroso sulfonamide.[10]

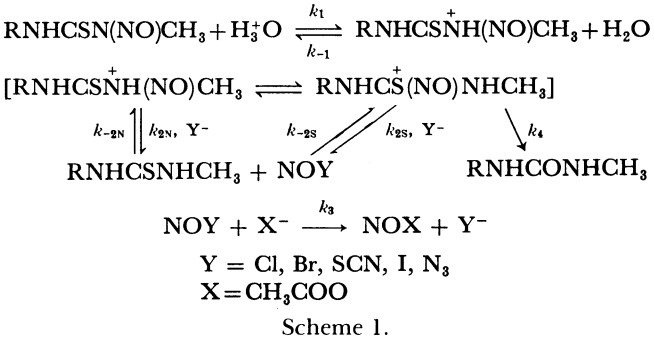
To evaluate the solvent deuterium isotope effect, the rate of denitrosation at 36.9°C was measured in CH_3COOD-D_2O. Results obtained at the same buffer ratio ([HA(DA)]/[A^-]=1.04, 0.05 M, μ=0.2) revealed that the denitrosation of **4** is slower in CH_3COOD-D_2O than in CH_3COOH-H_2O by a factor of 1.25 (Table 3). This feature argues against fast pre-equilibrium formation of the protonated form of *N*-nitrosothiourea, and rather suggests that the proton transfer is rate-limiting. The above results resemble those of *N*-nitroso amide (k_H/k_D=1.9 or 1.3)[6,8] and nitroso sulfonamide (k_H/k_D=1.5)[10] rather than those of nitrosamine (k_H/k_D=*ca.* 0.5—0.6).[16,17]

Table 3. Solvent deuterium isotope effect[a] in the denitrosation of **4**

$10^3k_H/s^{-1}$	1.56±0.03[b]
$10^3k_D/s^{-1}$	1.24±0.01
k_H/k_D	1.25

a) [HA(DA)]/[A^-]=1.04 (A=CH_3COO), [**4**]=8.28×10^{-5} M, and μ=0.2 (NaCl) at 36.9°C. b) The errors are given by deviation from average of two runs.

Scheme 1 is a probable outline mechanism for the denitrosation presented as capable of explaining the observations. The initial *N*-protonation of *N*-nitroso-



Scheme 1.

thiourea is written as a one-stage process for simplicity and *O*-protonation is not considered. The rate constant k_2 is composed of k_{2N} and k_{2S}. The step k_{-2}(*i.e.*, $k_{-2N}+k_{-2S}$) is negligible in the presence of excess X^-. The general expression of rate constant derived from Scheme 1

$$k_0 = k_1k_2[H_3^+O][Y^-]/(k_{-1}+k_2[Y^-]) \quad (1)$$

is simplified to two limiting forms

$$k_0 = k_1k_2[H_3^+O][Y^-]/k_{-1}, \quad (2)$$

$$k_0 = k_1[H_3^+O], \quad (3)$$

which correspond to different reaction patterns based on the inequalities $k_{-1} \gg k_2[Y^-]$ and $k_{-1} \ll k_2[Y^-]$, respectively. The catalysis by Y^- is in effect caused to disappear only if $k_{-1} \ll k_2[Y^-]$, with a result that Eq. 1 reduces to $k_0=k_1[H_3^+O]$, which is the form observed in this work.

In order to know electronic and steric effects of substituents at N_3 on denitrosation, Taft's Eq.[18]

$$\log k_R/k_{Me} = \rho^*\sigma^* + \delta Es \quad (4)$$

was applied to the data at pH 4.6 in Fig. 1. The ρ^* and δ values computed by using the least squares

method are −0.70 and −0.048, respectively. A plot of log $k_R/k_{Me}-\rho^*\sigma^*$ against *Es* gave an inferior correlation coefficient (r=−0.529) (Fig. 2). This indicates that the steric effect of substituent R is small for the transition state of protonation of these compounds **1—6**. Therefore, to know the electronic effect of substituents at the N_3 position on the protonation, log k_R/k_{Me} values from the data at pH 4.6 in Fig. 1 are plotted against σ^* into Fig. 3. The points for R=CH_3, C_2H_5, $(CH_3)_2CH$, $C_2H_5(CH_3)CH$, and $(CH_3)_3C$ provide a linear relationship (slope ρ^*=−0.98, r=−0.997) as given by

$$\log k_R/k_{Me} = \rho^*\sigma^* = -0.98\sigma^*. \quad (5)$$

This indicates that the denitrosation is influenced mainly by the electronic effect of substituents at the N_3 position. The more the substituent at N_3 releases electrons and increases the electron density at the N_1 position, the more the protonation on N_1 is facilitated.[19] The above result supports that the protonation on the N_1 nitrogen atom is the rate-determining step in the denitrosation of **1—6**. The positive deviation for R=*cyclo*-C_6H_{11}, which becomes pronounced at higher temperatures (36.9°C) at pH 5.0 (Table 4), may be due to inversion of the cyclohexyl group which permits the favorable conformation for the protonation on the N_1 position. This electronic effect (ρ^*=−0.98) of substituent R on the denitrosation is much smaller than that on alkaline hydrolyses of alkyl nitrite (ρ^*=2.67).[20]

In the denitrosation process of **6** (Scheme 1), the increase in electron density at the N_1 nitrogen atom by the electron-releasing effect of the *t*-butyl group may facilitate the protonation on the N_1 nitrogen atom to form a protonated intermediate. For **6**, the transition state to form a protonated *N*-nitroso or *S*-nitroso intermediate must be stabilized, by the electron-releasing effect of the *t*-butyl group, more than for **1**, **3**,

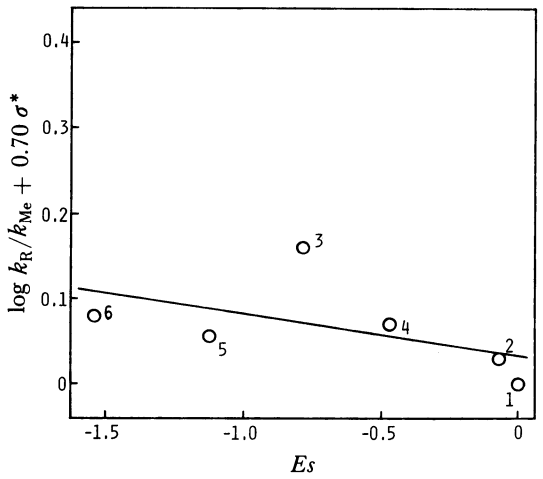


Fig. 2. Plot of log k_R/k_{Me}+0.70σ^* for the denitrosation of the $RNHCSN(NO)CH_3$ in acetate buffer (pH 4.6) against Taft's Es values, δ=−0.048 (r=−0.529).

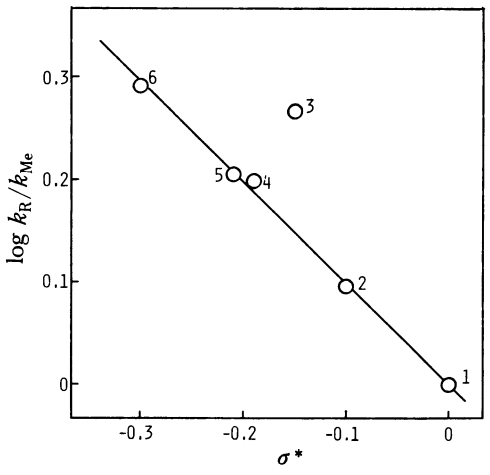


Fig. 3. Plot of log k_R/k_{Me} *vs.* σ^* for the denitrosation of the $RNHCSN(NO)CH_3$ series (pH 4.6). ρ^*=−0.98 (r=−0.997) for the R=CH_3, C_2H_5, $(CH_3)_2CH$, $C_2H_5(CH_3)CH$, and $(CH_3)_3C$.

Table 4. Kinetic data for denitrosation of 3-alkyl-1-methyl-1-nitrosothioureas

Nitrosothiourea[a]	Temp/°C	$10^4k_0/s^{-1}$[b]	ΔH^*/kJ mol^{-1}	ΔS^*/J mol^{-1}K^{-1}
1	17.0	1.05±0.02		
	25.0	2.50±0.03		
	36.9	8.32±0.02	75.3	−61.5
3	17.0	1.84±0.02		
	25.0	4.01±0.04		
	36.9	14.21±0.04	76.6	−52.3
4	17.0	1.80±0.02		
	25.0	3.97±0.03		
	36.9	12.98±0.02	72.8	−65.7
5	17.0	1.82±0.01		
	25.0	4.03±0.01		
	36.9	11.85±0.74	67.3	−83.7
6	17.0	2.26±0.01		
	25.0	5.03±0.02		
	36.9	14.27±0.18	65.7	−87.4

a) 8.28×10^{-5} M, acetate buffer (pH 5.0), 0.05 M, and μ=0.2 (NaCl). b) The errors are given by deviation from average of two runs.

and **4** so as to result in a larger increase of solvation, which may in turn lead to a larger decrease (21.7—35.1 $J\,mol^{-1}\,K^{-1}$) in the activation entropy of **6** than those of the others (**1**, **3**, and **4**) (Table 4). In nitrosation of thiourea (EtNHCSNHEt) by an equimolar amount of $NaNO_2$ and 0.5 M HCl in acetone at −10 °C, the *S*-nitroso intermediate leads to urea *via* elimination of HSNO and addition of water to the carbodiimide thus formed.[14] But the decomposition of **6** in this acetate buffer solution (pH 4.6) at 37 °C in the absence of oxygen (under nitrogen atmosphere) produced **6b** in one fifth amount of **6a**. This distribution of products suggests that the existence of oxygen greatly influences the production of **6b**.[21] Williams[13] has reported that the reaction of thiourea with *N*-methyl-*N*-nitrosoaniline in acid solution in the absence of oxygen forms no Fischer-Hepp rearrangement products but merely the denitrosation product *N*-methylaniline, which suggests that the nitrous acid can be regenerated using oxygen dissolved in the solution according to

$$2NO + O_2 = 2NO_2$$
$$NO_2 + NO = N_2O_3$$
$$N_2O_3 + H_2O = 2HNO_2$$

Scheme 2.

In fact, the decomposition of **6** in acetate buffer (pH 4.6) containing sodium nitrite under nitrogen atmosphere produced a mixture of **6a** and **6b** in a ratio of **6a**:**6b**=1:1 (Table 1). Therefore, the protonated intermediate of **6** is attacked mainly by the nitrous acid formed from oxygen to produce **6b** with no production of carbodiimide followed by addition of water.

These results lead to a conclusion that the electronic effect of substituents at the N_3 position has a predominant effect on the protonation on the N_1 nitrogen atom. It is probable that the denitrosation in this system (**1**—**6**) is not greatly affected by the steric factor of substituent R lying remote from the nitroso group. In this series, acid catalysis is a prerequisite for the denitrosation process different from hydrolysis of alkyl nitrite under neutral conditions[22] and the rate constants of denitrosation of these *N*-nitrosothioureas are in good correlation to σ^* values with one exception, which indicates that the values may be useful as an index of transnitrosating abilities of *N*-nitrosothioureas.

Experimental

Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-400 spectrophotometer and ^1H NMR spectra were recorded with a Hitachi R-24 instrument. Microanalyses were performed in the Microanalytical Laboratory of the Institute of Physical and Chemical Research, Wako-shi.

Materials. Ethylamine, *t*-butylamine, and methyl isothiocyanate were obtained from Wako Pure Chemical Co., and cyclohexylamine, isopropylamine, and *s*-butylamine from Tokyo Kasei Co.

General Method for the Preparation of N-Nitrosothioureas. 3-Alkyl-1-methylthiourea were synthesized through reaction of the corresponding alkylamines and methyl isothiocyanate. A dilute solution of HCl (0.07 M, 20 ml) was added dropwise for 20 min to a suspension of 3-alkyl-1-methylthiourea (2.0—6.0 mmol) and sodium nitrite (2.0—6.0 mmol) in CH_2Cl_2 (20 ml) with stirring and cooling (0 °C) under a nitrogen atmosphere, and the mixture was stirred with cooling (0 °C) for additional 40 min by following the method of Lown and Chauhan.[14] After the reaction was over, the organic layer was taken out, washed with water, dried ($MgSO_4$), and subjected to evaporation to afford crude *N*-nitrosothioureas **1**—**6**, which were purified except **2** by either crystallization from pentane or by chromatography over silica gel.

1,3-Dimethyl-1-nitrosothiourea (1): mp 46—47 °C (lit,[14] mp 46 °C).

3-Isopropyl-1-methyl-1-nitrosothiourea (4) (56%): An oil, IR (neat) 3340 (NH), 2990, 1505, 1405 (N=O), 1175 (C=S), and 1020 cm^{-1}; NMR ($CDCl_3$) δ=1.36 (6H, d, J=6.0 Hz, $C(CH_3)_2$), 3.46 (3H, s, N-CH_3), 4.67 (1H, q, J=6.4 Hz, $C\underline{H}Me_2$), and 8.26 (1H, br s, NH). Found: C, 37.32; H, 6.89; N, 26.12; S, 19.94. Calcd fo $C_5H_{11}N_3OS$: C, 37.25; H, 6.88; N, 26.06; S, 19.89.

3-s-Butyl-1-methyl-1-nitrosothiourea (5) (51%): An oil; IR (neat) 3330 (NH), 2980, 1505, 1405 (N=O), 1170 (C=S), and 1010 cm^{-1}; NMR ($CDCl_3$) δ=0.98 (3H, t, J=6.8 Hz, $CH_2C\underline{H}_3$), 1.30 (3H, d, J=6.0 Hz, $CHC\underline{H}_3$), 1.68 (2H, q, $C\underline{H}_2CH_3$), 3.48 (3H, s, N-CH_3), 4.85—4.25 (1H, m, $C\underline{H}(CH_3)C_2H_5$), and 8.40 (1H, br s, NH). Found: C, 41.10; H. 7.43; N, 24.16; S, 18.32. Calcd for $C_6H_{13}N_3OS$: C, 41.12; H, 7.48; N 23.98; S, 18.30.

3-t-Buthyl-1-methyl-1-nitrosothiourea (6) (69%): An oil; IR (neat) 3375 (NH), 2980, 1520, 1500, 1420 (N=O), 1200, 1160, and 935 cm^{-1}; NMR ($CDCl_3$) δ=1.59 (9H, s, $C(CH_3)_3$), 3.43 (3H, s, N-CH_3), 8.55 (1H, br s, NH). Found: C, 41.07; H, 7.46; N, 23.92; S, 18.39. Calcd for $C_6H_{13}N_3OS$: C, 41.12; H, 7.48; N, 23.98; S, 18.30.

Kinetic Measurements. The kinetic method was the same as described previously.[11] Reactions were monitored spectrophotometrically by following the disappearance of the absorption maximum at 266—270 nm with a Shimadzu UV 200 spectrophotometer. The reaction followed pseudo first-order kinetics; infinity readings were obtained after 8—10 half lives. The value of the pH of buffer was determined with a Toa Denpa HM-5A pH meter. A mixture of 3-ethyl-1-methyl-1-nitrosothiourea (**2**) and 3-methyl-1-ethyl-1-nitrosothiourea (**2′**) was obtained from nitrosation of 3-ethyl-1-methylthiourea and its separation by column chromatography (silica gel) with 30% ether-hexane as eluent failed of success after successive two trials. Therefore the rate constant of **2** was obtained from kinetic measurements on both the first fraction (**2**:**2′**=68:32) and the last fraction (**2**:**2′**=23:77) separated by column chromatography. Estimated values in acetate buffer (pH 4.6, 36.9 °C) are as follows: $k=1.22\times10^{-3}\,s^{-1}$ for **2** and $k=2.08\times10^{-3}\,s^{-1}$ for **2′**; NMR ($CDCl_3$) for **2** δ=1.37 (3H, t, J=7.2 Hz, $CH_2C\underline{H}_3$), 3.46 (3H, s, N-CH_3), 3.56—4.02 (2H, m, $C\underline{H}_2CH_3$), and 8.58 (1H, br s, NH) and for **2′** δ=1.04 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 3.26 (3H, d, J=5.0 Hz, N-CH_3), 4.24 (2H, q, J=7.2 Hz, $C\underline{H}_2CH_3$), and 8.52 (1H, br s, NH). The first fraction (**2**:**2′**=68:32): an oil. Found; C, 33.08; H, 6.21; N, 27.88; S, 21.44. Calcd

for $C_4H_9N_3OS$: C, 32.64; H, 6.16; N, 28.55; S, 21.78.

Product Analyses. A solution of **1** (15 mg, 0.144 mmol) in 3 ml of MeOH was added to 100 ml of acetate buffer solution (pH 5.0, 0.05 M, μ=0.2 with NaCl) and stirred for 2 h and 50 min at 37 °C. After cooling, the contents were neutralized with 0.1 M NaOH and saturated with NaCl and extracted with chloroform (40 ml×3) and the combined extracts were washed with saturated NaCl solution, dried ($MgSO_4$), and evaporated to give 1,3-dimethylthiourea (9 mg, 80%): mp 62—63 °C; NMR ($CDCl_3$) δ=2.99 (6H, d, J=4.8 Hz, CH_3) and 6.30 (2H, br s, NH). **3** (50 mg, 0.248 mmol) in acetate buffer solution (pH 4.6) for 1 h at 37 °C was allowed to produce 3-cyclohexyl-1-methylthiourea (**3a**) and 3-cyclohexyl-1-methylurea (**3b**) (white solids, 34.7 mg, 83%, **3a**:**3b**=80:20 from NMR spectrum) by following a similar procedure as described above. **3a**: mp 163—164 °C; NMR ($CDCl_3$) δ=1.00—2.20 (10H, m, CH_2), 2.94 (3H, d, J=5.2 Hz, N-CH_3), 3.55—4.00 (1H, m, CH-N), 5.66 (1H, br s, NH), and 5.90 (1H, br s, NH). **4** (16 mg, 0.099 mmol) in acetate buffer solution (pH 4.6) for 1 h at 37 °C produced mainly 3-isopropyl-1-methylthiourea (**4a**) together with 3-isopropyl-1-methylurea (**4b**) (9.3 mg, 50%, **4a**:**4b**=78:22 from NMR spectrum). **4a**: NMR ($CDCl_3$) δ=1.21 (6H, d, J=6.6 Hz, $C(CH_3)_2$), 2.96 (3H, d, J=4.8 Hz, N-CH_3), 4.24 (1H, o, 7.0 Hz, $C\underline{H}(CH_3)_2$), 5.90 (1H, br s, NH), and 6.26 (1H, br s, NH). **6** (100 mg, 0.571 mmol) in acetate buffer solution (pH 4.6) for 1 h at 37 °C through a similar course as described above produced a mixture of white solids (**6x** and 3-*t*-butyl-1-methylurea (**6b**), 49 mg, 64%, **6x**:**6b**=37:63 from NMR spectrum). **6b** was isolated by recrystallization from hexane-acetone; **6x** was not isolated from the mixture but its IR and NMR spectra were in accord with those of **6a**. **6b**: mp 134—135 °C; IR (KBr) 3390 (NH), 3330 (NH), 1625 (C=O), 1560, 1360, 1275, and 1215 cm^{-1}; NMR ($CDCl_3$) δ=1.30 (9H, s, $C(CH_3)_3$), 2.65 (3H, d, J=4.8 Hz, N-CH_3), 5.01 (1H, br s, NH), and 5.21 (1H, br s, NH); UV λ_{max}^{MeOH} 245 (ε 170) and 206 nm (1940). Found: C, 55.29; H, 10.95; N, 21.38. Calcd for $C_6H_{14}N_2O$: C, 55.35; H, 10.84; N, 21.52. **6x**=**6a**: NMR ($CDCl_3$) δ=1.42 (9H, s, $C(CH_3)_3$), 3.02 (3H, d, J=4.8 Hz, N-CH_3), 6.14 (2H, br s, NH); UV λ_{max}^{MeOH} 245 (ε 11600) and 212 nm (9300). **6** (51 mg, 0.291 mmol) in acetate buffer solution (pH 4.6) for 1 h at 37 °C under nitrogen atmosphere produced a mixture of white solids (**6a** and **6b**, 11 mg, 27%, **6a**:**6b**=83:17 from NMR spectrum). **6** (51 mg, 0.291 mmol) in acetate buffer solution (pH 4.6) containing sodium nitrite (20 mg, 0.291 mmol) for 1 h at 37 °C under nitrogen atmosphere produced a mixture of white solids (**6a** and **6b**, 30 mg, 74%, **6a**:**6b**=50:50 from NMR spectrum).

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19) ^{15}N Chemical shifts (δ) of various *N*-nitrosothioureas including **1** and **3** have been investigated by Lown and Chauhan[14] (**1**: N_3H (δ=98.5), $>N_1$ (273.7); **3**: N_3H (δ=141.9), $>N_1$ (273.6)) and the progressive effect of the increase in bulk of the N_3 substituent in the *N*-nitrosothioureas on the preferred conformations has also been examined by them, with a suggestion that the NH proton becomes less accessible for interacting with the solvent.[14] These results support that the protonation on the N_1 position is much more facilitated in the *N*-nitrosothiourea which has a strongly electron-releasing and bulkier substituent at the N_3.
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