Kinetic Study of Aqueous Decompositions of *N*-(2-Haloethyl)-*N'*-cyclohexyl-*N*-nitrosoureas. Effect of Haloethyl Group on the Preference in Decomposition Pathways

Kitaro YOSHIDA* and Kazuyuki YANO

Department of Chemistry, Saitama Medical School, 981 Kawakado, Moroyama, Iruma-gun, Saitama 350-04

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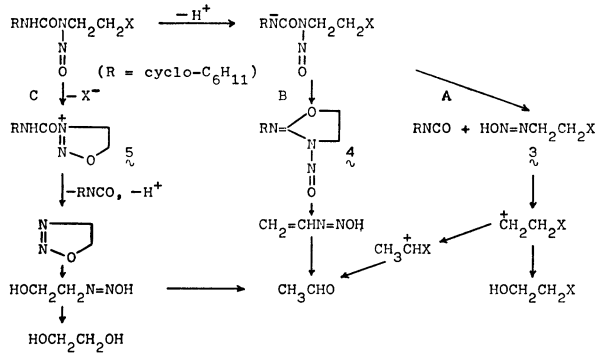
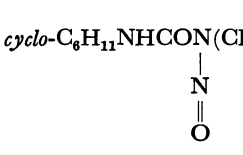
Synopsis. The aqueous decompositions of the title nitrosoureas under various pH conditions were studied kinetically to generalize their hydrolytic mechanisms. The relative decomposition rates of these analogs were in order of the leaving ability of the halogen moiety, which also affected on the preference in their reaction pathways.

Extensive studies[1] on the mechanism of action of antitumor agent *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU, **1-Cl**) under chemical hydrolytic conditions have established at least three competitive reaction pathways (A, B, and C, Scheme 1). Pathway A provides cyclohexyl isocyanate and 1-chloro-(2-hydroxyazo)-ethane **3** (X=Cl) with the latter intermediate becoming 2-chloroethyl cation which attacks certain nucleotides *in vivo*. Pathway B proceeds *via* an unstable nitrosooxazolidine **4** which breaks down to give acetaldehyde as a volatile product. Both processes are necessarily attended with proton elimination from N' as an initial step and are kinetically indistinguishable. The third pathway C, however, takes place by nucleophilic attack to C-2 by nitroso oxygen to liberate the chloride ion and affords oxadiazoline intermediate **5**. Although relative contributions of each competing pathways are varied depending on pH, solvent polarity, and nature of buffering medium, the propensity of **1-Cl** to provide a significant quantity of 2-chloroethyl cation through pathway A is a mainstay of its antitumor activity. Incidentally, little has been investigated for the chemical reactivities of other halogenated analogs, **1-F** and **1-Br**.[2] This note describes and compares a few kinetic results of aqueous decompositions of **1** in various buffer solutions.



Scheme 1.



	n	X
1-F:	2	F
1-Cl:	2	Cl
1-Br:	2	Br
2:	3	Br

Results and Discussion

The decomposition rates were measured by following the decrease of UV absorption of the substrates (230 nm). For each nitrosourea pseudo-first-order rate constant (k/s^{-1}) was observed and the kinetic data under various pH conditions are drawn in Fig. 1. As shown in Fig. 1, the rate of hydrolysis of **1-F** becomes larger with increasing hydroxide ion concentrations and is clearly dependent on pH over the whole range studied. A similar kinetic result was also observed for **1-Cl** within the range of pH 7—8. The decomposition rate of **1-Cl** is reduced at lower pH's, but the extent of the rate reduction becomes rather small. This trend is still more obvious in the case of bromine analog, **1-Br**. It is apparently shown that the reactivity of **1-Br** is higher than those of **1-F** and **1-Cl**. It decomposes at a rate 6 times faster than does **1-Cl** and is about 10 times more reactive than **1-F** at pH 7.1. Moreover, there is a broad pH range over which the decomposition rate is irrespective of acid or base.

Degradation study appears to be agreed with the above observations. When **1-Cl** was decomposed at pH 7.2, 2-chloroethanol was produced in 28% yield based on parent nitrosourea (Table 1), which must imply the occurence of elimination pathway A. Similarly, **1-Br** could afford the corresponding 2-haloethanol. Thus it would appear that the hydroxide ion catalyses are valid under the neutral conditions. However, as the pH is lowered, the contribution of the

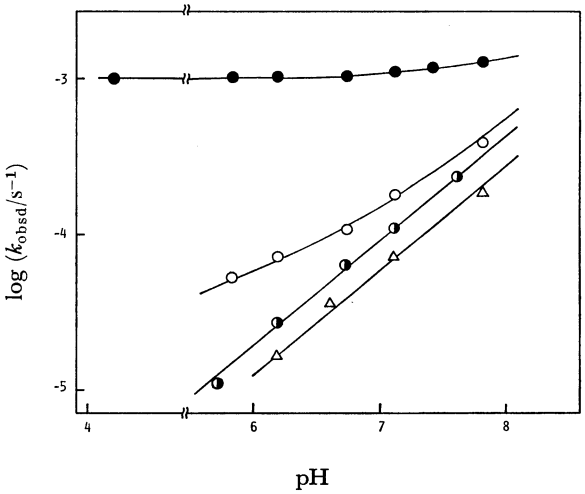


Fig. 1. Plots of $\log k_{obsd}$ *vs.* pH.
Nitrosourea, 1×10^{-4} M; 0.1 M buffer [acetate (pH—5), phosphate (5.8≤pH)]; μ=0.5 (NaCl); 36.9 °C.
○: **1-Cl**, ●: **1-Br**, ◑: **1-F**, △: **2**.

Table 1. The yields of 2-haloethanols from **1** in 0.1 M buffer at 36.9 °C

Nitrosourea	pH 4.30[a] Yield/%	pH 7.20[b] Yield/%
1-Cl	13.3	28.2
1-Br	0	21.5

a) Acetate. b) Phosphate.

catalysis tends to decrease. At pH 4.3, the amount of 2-chloroethanol from **1-Cl** reduced to 13% yield. Most obviously, none of the formation of 2-bromoethanol from **1-Br** was looked under the same conditions.

The results suggest that at higher pH values, the abstraction of the ureido proton is substantial, while as the pH is decreased, the nitroso oxygen assisted mechanism (pathway C) becomes increasingly more important. In the case of **1-F**, a poor leaving group attached to C-2 may resist to such an intramolecular process.[3] Further consistent evidence with the favored formation of the oxadiazoline intermediate from **1-Br** was also available. We synthesized the next higher homolog with three methylene units **2**, since it has already been reported that an introducing of the third methylene group into methyl-(2-tosyloxyethyl)-*N*-nitrosamine [$CH_3N(NO)CH_2CH_2OTs$] causes to prohibit its intramolecular process in such a way as to retard its decomposition rate.[4] As would be expected, **2** was quite modest under the present conditions. It is less reactive than **1-Br** by a factor of 15 at pH 7.1. Also, the kinetic feature of **2** is distinctly separated from that of **1-Br** and is closely parallel to **1-F**. The remarkable difference between two reactivities of **1-Br** and **2** must be due to a conformational restriction whereby the cyclic oxadiazoline from **2** with six membered ring is much less favorable than that from **1-Br** with five membered one. Probably, **2** decomposes by ordinary mechanisms (A or B).

The present study shows that an alteration of haloethyl moiety of the title nitrosoureas causes significant influences not only on their relative decomposition rates but on the preference in particular reaction pathways. Such the variations are related to the leaving properties of the C-2 substituent.

Experimental

Materials. *N*-(2-Haloethyl)-*N'*-cyclohexyl-*N*-nitrosoureas (**1**) were prepared by the method of Johnston.[2] *N*-(3-Bromopropyl)-*N'*-cyclohexyl-*N*-nitrosourea (**2**) was prepared from condensation of 3-bromopropylamine with cyclohexyl isocyanate, followed by nitrosation by sodium nitrite (63% overall yield): mp 42—44 °C; IR (KBr) 3280 (NH), 1690 (C=O), 1520 (C-NH), and 1470 cm^{-1} (N=O); ^1H NMR ($CDCl_3$) δ=0.91—2.26 (12H, m), 3.28 (2H, t, J=6 Hz, $CH_2CH_2C\underline{H}_2Br$), 3.94 (2H, t, J=6 Hz, $C\underline{H}_2CH_2CH_2Br$), 3.96 (1H, m, methine), and 6.81 (1H, m, NH). Found: C, 40.86; H, 6.16; N, 14.35; Br, 27.03%. Calcd for $C_{10}H_{18}N_3O_2Br$: C, 41.11; H, 6.21; N, 14.38; Br, 27.35%.

Kinetics were performed with a Shimadzu UV 200 spectrophotometer according to our previous method.[5] The pH of the buffer solution was measured with a Toa-denpa pH meter (HM-5A).

Product Analysis. Nitrosourea **1-Cl** (70 mg) in acetonitrile (3 ml) was added to 300 ml of 0.1 M (1 M=1 mol dm^{-3}) phosphate buffer solution (pH 7.2, μ=0.5). After the solution was stirred at 36.9 °C for 23 h, 2-bromoethanol (18 mg) was added as an internal standard. The cooled solution was saturated by sodium chloride, extracted with three portions of ether (450 ml), dried and evaporated. The GLPC analysis of the crude product showed the formation of 2-chloroethanol in 28% yield. The yield of 2-bromoethanol from **1-Br** was also determined by using 2-chloroethanol as an internal standard. The results are summarized in Table 1. Control experiments indicated that both of the 2-haloethanols did not hydrolyzed at pH 7.2. Owing to its insolubility in ether, attempts to trap ethylene glycol were unsuccessful.

This paper is dedicated to Professor Kazuo Nagamatsu in our laboratory on the 60th anniversary of his birth.

References

1) Recent article, for example; J. W. Lown and S. M. S. Chauhan, *J. Org. Chem.*, **46**, 5309 (1981).

2) The screening data indicated that **1-F** was effective against certain leukemic cells, while **1-Br** was inactive; T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *J. Med. Chem.*, **9**, 892 (1966).

3) The pH dependency of the decomposition rate of **1-F** was the same as that of *N*-ethyl-*N'*-cyclohexyl-*N*-nitrosourea, where the substitution on C-2 can be ruled out.

4) S. R. Koepke, R. Kupper, and C. J. Michejda, *J. Org. Chem.*, **44**, 2718 (1979).

5) K. Yoshida and K. Yano, *Bull. Chem. Soc. Jpn.*, **55**, 2200 (1982).