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MECHANISM OF ACTION OF 2-HALOETHYLNITROSOUREAS ON DEOXYRIBONUCLEIC ACID

NATURE OF THE INTERMEDIATES FROM NITROSOUREA DECOMPOSITION *

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Abstract—Direct current (DC) and differential pulse polarographic analyses were used to measure the rates of decomposition of a series of 2-haloethylnitrosoureas in aqueous solution. Measured by these methods, the rates of the first and rate-determining step which show a marked pH and solvent dependence agree with the overall rate of decomposition measured by gas evolution. In the 1,3-bis(haloethyl)-1-nitrosourea series, changing the nature of the halogen X has a small effect on the rate of decomposition. In the 3-cyclohexyl-1-(2-haloethyl)-1-nitrosourea series, changing X for OH or OCH_3 results in the rate of hydrolysis being reduced considerably. A free —NH_2 group in the nitrosourea structure as in CNU, MNU, ENU, CPNU, 4-CBNU and 5-CPNU accelerates considerably the rate of decomposition relative to the BCNU and CCNU series. Arrhenius parameters for the decomposition in aqueous pH 7.1 solution in the temperature range 28–47° were obtained for BFNU, BCNU and BBNU: log A, -20.1 ± 1.4, -21.6 ± 0.7 and -22.3 ± 1.6; E_a, 24.4 ± 2.0, 26.5 ± 1.0 and 27.2 m 2.3 kcal/mole. The corresponding values for BINU were estimated as log A, -23.3 ± 3.0; E_a, 28.0 ± 3.0 kcal/mole. Examination of the decomposition products of 1,3-bis(2-chloropropyl)-1-nitrosourea (BCNU-β-Me) and 1,3-bis[1-(chloromethyl)ethyl]-1-nitrosourea (BCNU-α-Me) favors decomposition pathway B via the diazohydroxide and cyclic chloronium ion for BCNU-β-Me and via the diazohydroxide and/or 2-chloro-1-methylethyl carbonium ion for BCNU-α-Me. While there is no evidence for the contribution of pathway A via a 2-imino-*N*-nitrosooxazolidinone for these compounds, consideration of product type and yields implicates a third decomposition pathway, via a 1,2,3-oxadiazoline intermediate. Additional evidence for an oxadiazoline intermediate is obtained by the isolation of 2-bromoethanol when BCNU is decomposed in the presence of a high concentration of sodium bromide.

2-Haloethylnitrosoureas including BCNU,†, BFNU, CCNU, chlorozotocin and streptozotocin are of clinical value in the treatment of a range of neoplasms including Hodgkin's disease, Burkitt's lymphoma, and cerebral neoplasms [1–6]. Nitrosoureas decompose readily in aqueous solution giving rise to a number of species including electrophiles and isocyanates which react with the biological macromolecules, DNA and proteins, respectively. The broad outlines of a molecular mechanism of action of the nitrosoureas is developing in terms of attack by cationic species on DNA leading to (i) alkylation of both bases and phosphates, (ii) depurination and strand scission, and (iii) inter- and intra-strand cross-linking and carbamoylation of amino groups by released isocyanates [1, 5, 6]. An analysis of these reactions permitted the rational synthetic design of new nitrosoureas which display promising antileukemic properties [7]. Thus, the present understanding of the mode of action of the 2-haloethylnitrosoureas suggests that the proposed molecular mechanism is essentially correct. However, inevitably in such a complex system, many details require clarification.

We report herein observations concerning the nature of the intermediates produced from nitrosourea decomposition.

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†Abbreviations: BBNU = 1,3-bis(2-bromoethyl)-nitrosourea; BCNU = 1,3-bis(2-chloroethyl)nitrosourea; BFNU = 1,3-bis(2-fluoroethyl)nitrosourea; BINU = 1,3-bis(2-iodethyl)nitrosourea; BCNU-α-Me = 1,3-bis[1-(chloromethyl)ethyl]-1-nitrosourea; BCNU-β-Me = 1,3-bis(2-chloropropyl)-1-nitrosourea; MNU = 1-methyl-1-nitrosourea; ENU = 1-ethyl-1-nitrosourea; CNU = 1-(2-chloroethyl)-1-nitrosourea; CPNU = 1-(3-chloropropyl)-1-nitrosourea; 4-CBNU = 1-(4-chlorobutyl)-1-nitrosourea; 5-CPNU = 1-(5-chloropentyl)-1-nitrosourea; and GANU = 1-(2-chloroethyl)-3-glucopyranosyl-1-nitrosourea.

EXPERIMENTAL

Materials

Chlorozotocin was a gift from Dr. Gerald Goldenberg, Manitoba Institute of Cell Biology, Winnipeg, Manitoba. 1-(β-D-Glucopyranosyl)-3-(2-chloroethyl)-1-nitrosourea (GANU) and 1,3-bis(2-fluoroethyl)-1-nitrosourea were obtained from Dr. Harry B. Wood Jr., Division of Cancer Treatment, National Cancer Institute, Washington, DC.

Authentic samples used in the gas chromatographic analysis were obtained as follows: 2-fluoroethanol, 2-

chloroethanol and 2-iodoethanol from the Aldrich Chemical Co., Milwaukee, WI; 2-bromoethanol from Matheson, Coleman & Bell, Norwood (Cincinnati), OH; and 2-chloro-1-propanol and 1-chloro-2-propanol from the Eastman Kodak Co., Rochester, NY. Streptozotocin was purchased from CalBiochem, San Diego, CA.

The remaining nitrosoureas used in this work were prepared by literature procedures [3, 7, 8] or are described below.

Melting points were determined on a Fisher Johns Apparatus and are uncorrected. The i.r. spectra were recorded on a Nicolet 7199 F.T. spectrometer and only the principal, sharply defined peaks are reported. The n.m.r. spectra were recorded on Varian A-60 and A-100 analytical spectrometers. The spectra were measured on approximately 10–15% (w/v) solutions in appropriate deuterated solvents with tetramethylsilane as reference. Line positions are reported in parts per million from the reference. Mass spectra were determined on an Associated Electrical Industries MS-9 double-focussing high-resolution mass spectrometer. The ionization energy, in general, was 70 eV. Peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15,000. Kieselgel DF-5 (Camag, Switzerland) and Eastman Kodak precoated sheets were used for thin-layer chromatography. Microanalyses were carried out by Mrs. D. Mahlow of this department.

1,3-Bis[1-(chloromethyl)ethyl]urea. 1-(Chloromethyl)ethyl isocyanate [9] was added to a solution of 1 ml triethylamine in 9 ml H_2O at 0°, and the mixture was stirred for 2 hr. The white solid was collected and purified by recrystallization from $CHCl_3$/pet. ether (275 mg, estimated 40 per cent yield), m.p. 117–119°.

Anal. Calc. for $C_7H_{14}Cl_2N_2O$ (mol. wt 212.0483): C, 39.45; H, 6.63; N, 13.15; Cl, 33.27. Found (212.0490, mass spectrum); C, 39.47; H, 6.54; N, 13.15; Cl, 33.23. P.m.r. ($CDCl_3$) δ 1.2 (d, 6H, CH_3); 3.5 (m, 4H, CH_2); 4.2 (m, 2H, CH); 4.6 (d, 2H, exchangeable). I.r. ν_{max} ($CHCl_3$) 3000 (N—H), 1705 (C=O) cm^{-1}.

1,3-Bis[1-(chloromethyl)ethyl]nitrosourea. To 100 mg of 1,3-bis[1-(chloromethyl) ethyl]urea in 2 ml of 98% HCOOH at 0° was added, during 2 hr, 200 mg $NaNO_2$. The mixture was stirred for an additional 2 hr at 0°. Five ml H_2O was then added cautiously and the resulting solution was extracted with ether. The ether extract was washed with H_2O, dried ($MgSO_4$), and the ether removed to yield a pale yellow oil which could be crystallized from pet. ether (60 mg, 55 per cent yield), m.p. 30–31°.

Anal. Calc. for $C_7H_{13}Cl_2N_3O_2$ (mol. wt 241.0385): C, 34.87; H, 5.42; N, 17.43; Cl, 29.40. Found (241.0389, mass spectrum): C, 34.72; H, 5.51; N, 17.22; Cl, 29.58. P.m.r. ($CDCl_3$) δ 1.3 (d, 3H, CH_3); 1.4 (d, 3H, CH_3); 3.5–4.0 (m, 4H, CH_2); 4.4 (m, 1H, CH); 5.1 (m, 1H, CH), 7.0 (s, 1H, exchangeable). I.r. ν_{max} ($CHCl_3$) 3300 (N—H); 1695 (C=O); 1505 (N=O) cm^{-1}.

1-(4-Chlorobutyl)-1-nitrosourea. Potassium cyanate (310 mg, 4.0 m-moles) was added to 600 mg (4.0 m-moles) of 4-chlorobutylamine hydrochloride in 5 ml water, and the mixture was stirred overnight. After chilling, the resulting precipitate was collected and air dried (520 mg, 85 per cent yield). This crude product, although slightly contaminated with the starting amine hydrochloride, was found suitable for nitrosation. Two hundred mg of crude 4-chlorobutylurea in 1 ml of 98% formic acid at 0° was treated with 150 mg sodium nitrite added in portions over 20 min. After an additional 30 min of stirring at 0°, 5 ml water was added cautiously. The pale yellow solid was collected, dried and recrystallized from ether/pet. ether (140 mg, 59 per cent yield), m.p. 64–65°.

Anal. Calc. for $C_5H_{10}ClN_3O_2$ (mol. wt 179.0461): C, 33.43; H, 5.62; N, 23.40; Cl, 19.74. Found (179.0460, mass spectrum): C, 33.50; H, 5.64; N, 23.70: Cl, 19.96. P.m.r. ($CDCl_3$) δ 2.6 (m, 4H, CH_2); 3.5 (t, 2H, CH_2); 3.8 (t, 2H, CH_2); 5.8 (s, 1H, exchangeable); 6.8 (s, 1H, exchangeable). I.r. ν_{max} ($CHCl_3$) 3300, 3220 (N—H), 1730 (C=O), 1480 cm^{-1} (N=O).

1-(5-Chloropentyl)-1-nitrosourea. This compound was prepared by the same method as 1-(4-chlorobutyl)-1-nitrosourea. The nitrosation of a crude 250-mg sample of 1-(5-chloropentyl)urea gave the nitrosourea as a pale yellow solid (225 mg, 64 per cent yield), m.p. 65–66°.

Anal. Calc. for $C_6H_{12}ClN_3O_2$ (mol. wt 193.0614): C, 37.21; H, 6.26; N, 21.70; Cl, 18.31. Found (193.0616, mass spectrum): C, 37.15; H, 6.23; N, 21.86; Cl, 18.38. P.m.r. ($CDCl_3$) δ 1.3–1.9 (m, 6H, CH_2); 3.5 (t, 2H, CH_2); 3.8 (t, 2H, CH_2); 5.9 (s, 1H, exchangeable); 6.8 (s, 1H, exchangeable). I.r. ν_{max} ($CHCl_3$) 3400, 3240 (N—H), 1770 (C=O), 1480 cm^{-1} (N=O).

N-nitroso-2-oxazolidinone. This compound was prepared according to the method of Newman and Kutner [10]; 380 mg (68 per cent yield), m.p. 48–50° (lit. [10] 50–53°). P.m.r. ($CDCl_3$) δ 3.5 (t, 2H, CH_2), 4.1 (t, 2H, CH_2).

Methods

Polarographic determination of decomposition rates for nitrosoureas. The Princeton Applied Research (PAR) model 174A polarograph and 9300-9301 polarographic cell were used in a three-electrode configuration which included an aqueous saturated calomel reference electrode (SCE), to which all potentials in this paper are relative, a platinum counter electrode, and a dropping mercury electrode (DME) with a controlled 2 s drop time. The temperature in the cell was maintained at 37.5 ± 0.2° by circulation of thermostatted water unless otherwise indicated [11]. The resulting curves were recorded on a Houston 200 X-Y recorder. The sample solutions were buffered at pH 7.1 with 0.01 M potassium phosphate buffer in 0.01 M KCl supporting electrolyte. The pH values of the sample solutions were measured with an Accumet model 520 pH meter before each run.

For compounds which showed extremely low solubility in aqueous solution, a 5% acetonitrile aqueous solution was used; in some cases differential pulse polarography of the aqueous solution was sufficiently sensitive and this was used whenever possible. All solutions were deaerated with purified nitrogen for 10 min before a run and blanketed with it during the run. The Arrhenius parameters for the 1,3-bis(2-halo-

ethylnitrosoureas were determined from the rate data at different temperatures.

Determination of nitrosourea decomposition products. The decompositions were carried out at pH 7.2 and 37° for 24 hr, as described previously [7], for the 1,3-bis(2-haloethyl)-1-nitrosoureas. Gas chromatographic analyses were performed on a Hewlett–Packard model 5830 A temperature programmable research chromatograph equipped with a flame ionization detector. Samples were injected onto a 6 m, 6.4 mm-o.d. column of Carbowax on Chromosorb W. The column was heated at 50° for acetaldehyde, acetone and propionaldehyde measurements and at 150° for 2-chloroethanol, 2-chloro-1-propanol and 1-chloro-2-propanol measurements. Identification was done using retention times of authentic samples: acetaldehyde, 2.8 min; acetone, 4.4 min; propionaldehyde, 4.0 min; 2-chloroethanol, 10.1 min; 2-chloro-1-propanol, 7.4 min; and 1-chloro-2-propanol, 6.2 min.

Decomposition of BCNU in saturated NaBr. The decomposition was carried out at pH 7.2, 37°, in a saturated sodium bromide solution. One milliliter of a 40 mM BCNU solution was allowed to decompose in a sealed tube for 24 hr. Gas chromatographic (g.c.) analysis was done as above. Identification was done using retention times of authentic samples and by g.c. mass spectral analysis. Two new products were identified: (i) 1-bromo-2-chloroethane, retention time 4.5 min. Mass spectral data: *m/e* (relative intensity) [142 (5.3), 144 (6.9); M^+, $BrCH_2CH_2Cl$], [107 (3.1), 109 (2.3); M^+—Cl, $BrCH_2CH_2^+$], [63 (100), 65 (33); M^+—Br, $+CH_2CH_2Cl$], and (ii) 2-bromoethanol, retention time 13.5 min. Mass spectral data: *m/e* (relative intensity) [124 (4.7), 126 (4.8); M^+, $BrCH_2CH_2OH$], [45 (74); M^+—Br, $+CH_2CH_2OH$], [31 (100); M^+—CH_2Br, + CH_2OH].

A control experiment was run using 2-chloroethanol in place of the nitrosourea. Incubation of the mixture, followed by g.c. analysis, indicated that less than 2 per cent of the 2-chloroethanol could be converted to 2-bromoethanol under these conditions.

RESULTS AND DISCUSSION

Determination of rates of decomposition of nitrosoureas

While Wheeler *et al.* [1, 12] and Panasci *et al.* [13] have used u.v. absorbance of the nitroso function to monitor the decomposition of nitrosoureas in 5% ethanol/H_2O buffered to pH 7.4, other methods have been less direct. Loo and Dion [14] developed a colorimetric procedure based on the release of nitrous acid and Montgomery *et al.* [15] measured the rates of nitrogen and carbon dioxide evolution during decomposition. In the present work, polarographic analysis employing the electrochemically active nitroso group proved to be a convenient and sensitive method for determining the rate of the first step of decomposition of the nitrosoureas directly.

All of the nitrosoureas studied showed two polarographic waves (Table 1), [16, 17]. These waves in neutral solution correspond to the reversible reduction of the *N*-nitroso group to the hydroxyamino group [18] followed by reduction to the amino group, both processes requiring two electrons. The two waves are often but not always of equal height. In all cases these waves decreased with time following the aqueous decomposition of the nitrosoureas. In no cases were any waves from reducible decomposition products observed (see below). This implies that the decomposition products are transient and/or electrochemically inactive, in accord with the suggested primary decomposition by

Table 1. Polarographic behavior of nitrosoureas

	R—N(NO)CONHR′ at pH 7.1, 37.5°					
Entry	R	R′	$E_{1/2}$, 1	$E_{1/2}$, 2	$k(\times 10^{-3})$ min^{-1}	$T_{1/2}$
1	CH_3—	—H	−0.884	−1.041	14.8	7 ± 2
2	CH_3CH_2—	—H	−0.955	−1.155	43.3	16 ± 1
3	$ClCH_2CH_2$—	—H	−0.752	−1.010	88	8 ± 4
4	$Cl(CH_2)_2CH_2$—	—H	−0.785	−1.025	116	6 ± 2
5	$Cl(CH_2)_3CH_2$—	—H	−0.982		128	5 ± 0.5
6	$Cl(CH_2)_4CH_2$—	—H	−0.980		110	5 ± 0.5
7	FCH_2CH_2—	—CH_2CH_2F	−0.890	−1.117	8.9	78 ± 2
8	$ClCH_2CH_2$—	—CH_2CH_2Cl	−0.777	−1.110	8.8	79 ± 5
9	$BrCH_2CH_2$—	—CH_2CH_2Br	−0.705	−1.095	16.9	52 ± 3
10	ICH_2CH_2—	—CH_2CH_2I*	−0.775	−1.035	9.6	58 ± 3
11	cyclo-C_6H_{11}—	—CH_2CH_2F	−0.724	−1.050	9.5	73 ± 2
12	cyclo-C_6H_{11}—	—CH_2CH_2Cl*	−0.853	−1.168	10.0	69 ± 1
13	cyclo-C_6H_{11}—	—CH_2CH_2Br*	−0.845	−1.075	36.5	19 ± 1
14	cyclo-C_6H_{11}—	—CH_2CH_2OH	−0.771	−1.034	3.7	186 ± 6
15	cyclo-C_6H_{11}—	—$CH_2CH_2OCH_3$	−0.823	−1.125	0.5	1445 ± 30
16	$ClCH(CH_3)CH_2$—	—$CH_2CH(CH_3)Cl$	−0.835	−1.135	9.3	74
17	$ClCH_2CH(CH_3)$—	—$CH(CH_3)CH_2Cl$	−0.865	−1.015	32.1	22
18	Chlorozotocin		−0.770	−1.112	17.8	39 ± 1
19	Streptozotocin		−0.960	−1.140	16.9	41 ± 1
20	GANU		−0.755	−1.035	70.7	10 ± 1
21	*N*-nitrosooxazolidinone		−0.705	−1.145	16.9	41 ± 2
22	$(CH_3)_2NCON(NO)CH_2CH_2Cl$		−0.870	−1.115	0.25	> 2800

* Equals 5% CH_3CN.

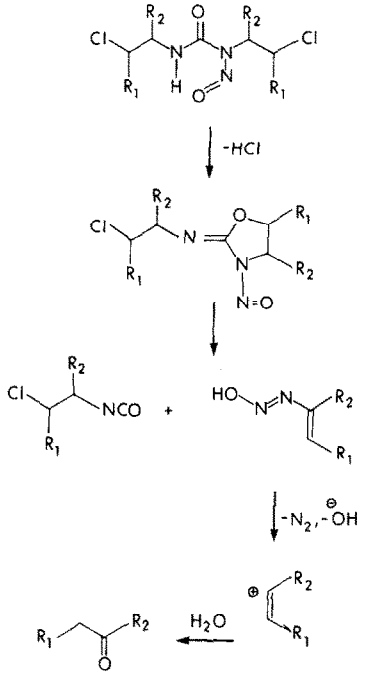


Fig. 1. Aqueous decomposition of 2-haloethylnitrosoureas via pathway A. (a) $R_1 = R_2 = H$, BCNU; (b) $R_1 = CH_3$, $R_2 = H$, BCNU-β-Me [1,3-bis(2-chloropropyl)nitrosourea]; and (c) $R_1 = H$, $R_2 = CH_3$, BCNU-α-Me [1,3-bis[1-(chloromethyl)ethyl]nitrosourea].

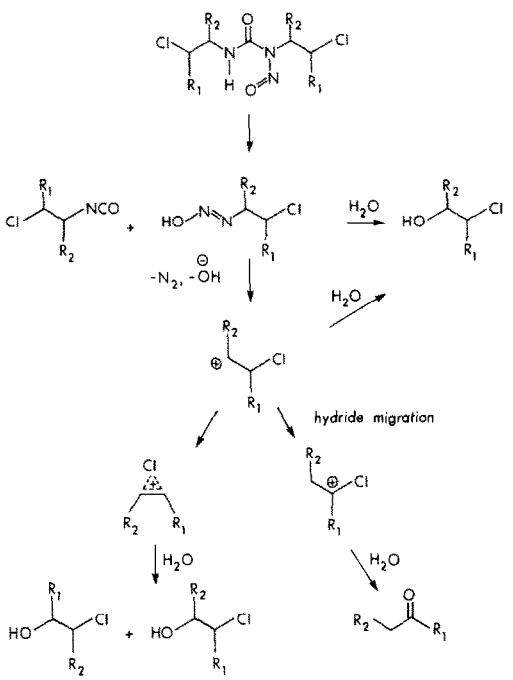


Fig. 2. Aqueous decomposition of 2-haloethylnitrosoureas via pathway B. (a) $R_1 = R_2 = H$, BCNU; (b) $R_1 = CH_3$, $R_2 = H$, BCNU-β-Me; and (c) $R_1 = H$, $R_2 = CH_3$, BCNU-α-Me.

pathways A (Fig. 1) and B (Fig. 2). Plots of the logarithm of the diffusion-limited current against time were always linear for both waves, permitting determination of the rate constants for the decomposition at pH 7.1 (Table 1). Values of the rate constants were determined separately for each wave. In nearly all cases these two calculated rate constants were identical within experimental error, and the reported values are the means of at least two measurements; the error cited is the authors' estimate and varies with the number of kinetic runs, definition of the polarographic waves, and linearity of the logarithmic plots. The reported rate constant values are calculated from half-life values measured over at least one half-life except for very slow decompositions. It is evident from Table 2 that the decomposition rates for nitrosoureas are dependent upon the solvent system used. Therefore, the rates measured in 5% acetonitrile are not strictly comparable with those measured in purely aqueous solution.

The rates of the decompositions measured electrochemically are not entirely in agreement with previous studies [12, 13]. However, the half-lives reported by Wheeler [1, 12] referred to a 5% ethanol/H_2O system buffered to pH 7.4, with, in some cases, the compounds added in an acetone solution. Therefore, from the solvent dependence noted in Table 2, it is not unexpected that there is an apparent discrepancy in the decomposition rates reported herein and by Wheeler.

It is apparent from Table 3 that, as anticipated, the rate of decomposition of BCNU increases progressively with pH in the range 4.4 to 8.0. A free-NH_2 group in the nitrosourea structure accelerates the rate of decomposition relative to the disubstituted analogues (Table 1). Lack of an N—H proton (no. 22, Table 1) severely inhibits decomposition, in agreement with previous results [8]. The observations in this study suggest that loss of the N—H proton is the first step in the decomposition, in accord with the results of Hecht and Kozarich [19, 20] reported for MNU.

Table 2. Solvent effects on the decomposition rates of nitrosoureas

RN(NO)CONHR′ R	R′	Solvent system, pH 7.1	$T_{1/2}$ (min)
$BrCH_2CH_2$—	—CH_2CH_2Br	H_2O	52
$BrCH_2CH_2$—	—CH_2CH_2Br	5% CH_3CH/H_2O	36
$ClCH_2CH_2$—	—CH_2CH_2Cl	H_2O	79
$ClCH_2CH_2$—	—CH_2CH_2Cl	5% CH_3CN/H_2O	52
$ClCH_2CH_2$—	—CH_2CH_2Cl	5% Acetone/H_2O	72
$ClCH_2CH_2$—	—CH_2CH_2Cl	5% Ethanol/H_2O	69

Table 3. pH Effects on the decomposition rate of BCNU

Temperature (°)	pH	$T_{1/2}$ (min)
22	4.4	3890 ± 90
22	7.0	734 ± 70
22	8.0	481 ± 15

It is evident from Table 1 that compounds which differ only in the halogen substituent can display quite different half-lives. In the series of compounds which contain a cyclohexyl group and a 2-substituted ethylnitrosourea (no. 11–15, Table 1) the half-lives vary considerably. For the substituents —Br, —Cl, —F, —OH, —OCH_3, the respective half-lives are 19, 69, 73, 186 and 1445 min. As the leaving ability of the substituent decreases, the half-life increases. This suggests that proton abstraction followed by loss of the substituent is a significant decomposition pathway for some nitrosoureas.

Electrochemical study of N-*nitrosooxazolidinone*

Since a 2-imino-*N*-nitrosooxazolidinone intermediate, first suggested by Montgomery *et al.* [15], is in agreement with the previous two observations, the electrochemistry of the structurally similar *N*-nitrosooxazolidinone was investigated (no. 21, Table 1). Its rate of hydrolysis, under the same conditions, is considerably faster than that observed for the 2-fluoroethyl- and 2-chloroethyl nitrosoureas but comparable with the 2-bromoethyl and 2-iodoethyl derivatives. Its half-wave potentials are so close to those observed for the 2-haloethyl nitrosoureas that it cannot be distinguished from them in dilute solutions. Therefore, whether a 2-imino-*N*-nitrosooxazolidinone is an intermediate in the decomposition of some nitrosoureas cannot be determined from the electrochemical data presented.

Table 4. Temperature dependence of nitrosourea hydrolysis reaction

Compound	Temperature (°)	Half-life (min)	log k (k, sec^{-1})
BFNU	28	220	−2.436
BFNU	37	76	−2.898
BFNU	41	38	−3.202
BFNU	47	20	−3.468
BCNU	28	288	−2.318
BCNU	37	84	−2.852
BCNU	41	49	−3.085
BCNU	47	20	−3.468
BBNU	28	161	−2.571
BBNU	37	52	−3.063
BBNU	41	24	−3.403
BBNU	47	11	−3.729
BBNU*	28	103	−2.765
BBNU*	37	36	−2.317
BBNU*	41	27	−3.353
BBNU*	47	11	−3.745
BINU*	28	182	−2.517
BINU*	37	58	−3.012
BINU*	41	39	−3.189
BINU*	47	16	−3.566

* In 5% CH_3CN (v/v); otherwise aqueous; pH 7.1.

Determination of Arrhenius parameters for nitrosourea decompositions

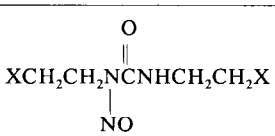
From rate data at different temperatures (separate study, Table 4), the Arrhenius parameters were derived for BFNU, BCNU and BBNU in aqueous pH 7.1 solutions as follows: log A, −20.1 ± 1.4, −21.6 ± 0.7 and −22.3 ± 1.6, E_a, 24.4 ± 2.0, 26.5 ± 1.0 and 27.2 ± 2.3 kcal/mole. Despite all efforts, no results could be obtained for BINU in aqueous solution, so BBNU and BINU were both examined in 5% acetonitrile with the following results: log A, −18.9 ± 1.6 and −19.9 ± 1.0; E_a, 24.0 ± 1.5 and 24.8 ± 1.5 kcal/mole. On the basis of these results we estimate the values for log A and E_a for BINU in aqueous solution as: log A, −23.3 ± 3.0; E_a, 28.0 ± 3.0 kcal/mole. The plots of the logarithm of the diffusion current against time from which the rate data were derived were, in all cases, linear over at least one half-life. The Arrhenius plots were also linear in all five cases and the error limits given are the standard deviations. The values of E_a obtained in this study are within the experimental error of those obtained for similar compounds under similar but not identical conditions in the spectrometric study of Garrett and Goto [21].

Consideration of alternative decomposition pathways for nitrosoureas

The suggested competing decomposition pathways for 2-haloethyl nitrosoureas are shown in detail in Figs. 1 and 2. The major products resulting from the decomposition of the four 1,3-bis(2-haloethyl)-1-nitrosoureas are listed in Table 5. Although the rate of decomposition for BFNU is comparable with that of BCNU, and the rate for BBNU comparable with that of BINU, the product ratios are quite different. The significant increase in acetaldehyde production for BBNU and BINU could result from a greater contribution by pathway A (Fig. 1) or pathway B (Fig. 2). Both the ability of the halogen to act as a leaving group and facilitate 2-imino-*N*-nitrosooxazolidine formation, as well as its polarizability which will stabilize the halocarbonium ion, formed after hydride migration, increase in the series fluorine, chlorine, bromine and iodine.

Steric effects as they relate to the decomposition of 2-chloroethylnitrosoureas were then investigated by preparing two appropriately substituted derivatives, 1,3-bis(2-chloropropyl)nitrosourea (BCNU-β-Me) and 1,3 - bis[1 - (chloromethyl) - ethyl]nitrosourea (BCNU-α-Me). Acetaldehyde results from the decom-

Table 5. Decomposition of 1,3-bis(2-haloethyl)-1-nitrosoureas



X	% CH_3CHO	% XCH_2CH_2OH
F	18	80
Cl	25	61
Br	39	14
I	66	0

Table 6. Decomposition of methyl substituted nitrosoureas

RN(NO)CONHR′ R	R′	Products (%)
$ClCH_2CH(CH_3)$—	—$CH(CH_3)CH_2Cl$	1-Chloro-2-propanol (30) 2-Chloro-1-propanol (0) Acetone (0) Propionaldehyde (38)
$ClCH(CH_3)CH_2$—	—$CH_2CH(CH_3)Cl$	1-Chloro-2-propanol (21) 2-Chloro-1-propanol (21) Acetone (21) Propionaldehyde (0)

position of BCNU via pathway A (Fig. 1) or pathway B (Fig. 2) after hydride migration.

While BCNU-α-Me is expected to produce only propionaldehyde via pathway A, it should produce both propionaldehyde and two isomeric chloropropanols by pathway B. By contrast, BCNU-β-Me should result in only acetone via pathway A and in acetone plus two isomeric chloropropanols via pathway B. In each case, the ratios of the two types of products should then give information on the relative contributions of the alternative pathways.

Decomposition of these two compounds in aqueous solution at 37° buffered to pH 7.2 followed by g.c. analysis resulted in the identification of the products listed in Table 6. The isolation of the isomeric chloropropanols in substantial yields in both cases is evidence for the participation of pathway B and does not favor pathway A.

The percentage of propionaldehyde produced from the decomposition of BCNU-α-Me is much greater (38 per cent) than the percentage of acetone produced in the decomposition of BCNU-β-Me (21 per cent). This result is in accord with that portion of pathway B (Fig. 2) involving hydride migration to form the intermediate chlorocarbonium ion followed by hydrolysis, as suggested by Brundrett *et al.* [22], for BCNU decomposition to be the major pathway to carbonyl-containing decomposition products. Thus, the decomposition of BCNU-α-Me proceeds through the more stable initially formed 2-chloro-1-methylethyl cation,* whereas the decomposition of BCNU-β-Me proceeds via the less stable 2-chloro-2-methylethyl cation. The decomposition of BCNU-α-Me and BCNU-β-Me also produced the chloropropanols listed in Table 6 which, as pointed out above, is evidence for overall participation of pathway B. The identification of both 1-chloro-2-propanol and 2-chloro-1-propanol after decomposition of BCNU-β-Me implicates the cyclic chloronium ion (Fig. 2) as an intermediate.

The calculations of Hehre and Hiberty [23] (although it should be noted that they neglect solvation effects) indicate that the rearrangement of the initially produced 2-chloro-2-methylethyl carbonium ion to the methyl-substituted cyclic chloronium (Fig. 2) is an exothermic process in accord with this finding. In contrast, the absence of 2-chloro-1-propanol after decomposition of BCNU-α-Me argues against the cyclic chloronium ion in this case.

The pertinent calculations of Hehre and Hiberty [23] suggest that in this case rearrangement of the initially produced 2-chloro-1-methylethyl cation to the methyl-substituted cyclic chloronium ion is an endothermic process. Therefore, the only chloropropanol produced in the decomposition of BCNU-α-Me is that which results from hydrolysis of the initially produced 2-chloro-1-methylethyl carbonium ion (Fig. 2). Further information concerning the contribution of this decomposition pathway is anticipated from specific deuteration studies.

The isolated products from these decompositions suggest the involvement of a third decomposition pathway. A 1,2,3-oxadiazoline intermediate, pathway C (Fig. 3), is in accord with the reported data. Initial proton loss followed by isocyanate formation with concomitant loss of the halogen produces a transient oxadiazoline (Fig. 3). Proton loss from such an intermediate could result in the carbonyl containing products. Montgomery [24] has suggested recently that such an intermediate could also result by cyclization of a 2-chloroethyl diazohydroxide (it would, of course, require a *syn*-geometry). While this may be a valid mechanistic pathway, the rapid decomposition of *syn*-diazotates

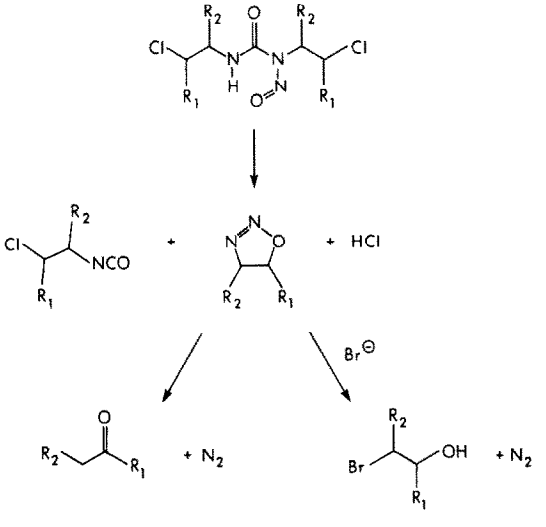


Fig. 3. Aqueous decomposition of 2-haloethylnitrosoureas via pathway C.

* For reasons of clarity, we refer to the alkyl-substituted systems as derivatives of the parent ethyl cations, rather than in the usual manner based on derivatives of the longest carbon chain [23].

[25] to produce carbonium ions and similar species would be expected to compete favorably with such a cyclization.

Further evidence for the intermediacy of a 1,2,3-oxadiazoline was obtained by an additional experiment. It appeared that such an intermediate would be susceptible to nucleophilic attack at the carbon bearing the nitrogen producing hydroxyethylated nucleophiles and liberating nitrogen (Fig. 3). Therefore, the aqueous decomposition of BCNU was carried out in a saturated sodium bromide solution. Gas chromatographic mass spectral analysis of the reaction solution indicated two new products: 1-bromo-2-chloroethane (previously observed by Montgomery [26] in a similar experiment) and 2-bromoethanol (which accounted for 6 per cent of the volatile products). A control experiment indicated that bromide substitution for chloride in the 2-chloroethanol formed did not occur significantly during the 24-hr incubation.

A 1,2,3-oxadiazoline intermediate appears to be a competing pathway to the acetaldehyde produced in the decomposition of BCNU. It also accounts for the hydroxyethylated nucleosides reported by Kramer *et al.* [27, 28] and may contribute significantly to DNA degradation, since hydroxyethyl alkylating agents have been reported [29] to result in extensive strand scission.

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