# Structure-Activity Relations of (2-Chloroethyl)nitrosoureas. 1. Deuterium Isotope Effects in the Hydrolysis of 1-(2-Chloroethyl)-1-nitrosoureas: Evidence for the Rate-Limiting Step

Neil Buckley*

*Brain Tumor Research Center of the Department of Neurological Surgery, School of Medicine, University of California, San Francisco, California 94143*

*Received July 7, 1986*

Large deuterium isotope effects for the hydrolysis of 1,3-bis(2-chloroethyl)-1-nitrosourea and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea in $H_2O$, $D_2O$ ($k^H/k^D$ = 6.04), and 1:1 $H_2O$:$D_2O$ ($k^{H:D}/k^D$ = 4.05) in cacodylate buffer (pH (pD) 7, 37.0 ± 0.1 °C) and the observation that $k^{H:D}$ and $k^D$ are the same and $k^H/k^D$ = 1.8 for hydrolysis of 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea, which undergoes an internal cyclization reaction in quantitative yield, suggest that the rate-limiting step is addition of water to the imino urea and not direct addition of water or hydroxyl to the carbonyl.

Despite three decades of often elegant experimental work on the complex organic chemistry of (2-chloroethyl)nitrosoureas (CENUs),[1] a clinically important class of anticancer drugs, many aspects of the hydrolysis reactions are still not understood. Of all mechanisms that have been proposed,[2-4] formation of the *gem*-diol tetrahedral intermediate **2** by direct addition of water to the urea carbonyl, first proposed by Snyder and Stock[3] for alkylnitrosoureas and later by Lown and Chauhan[4] for CENUs, is the most probable (Scheme I). The appeal of this mechanism is that antiperiplanar collapse of **2** nicely accounts for all products formed for CENUs.[4]

Nonetheless, the rate-limiting step of the reaction has not been well-defined. Rates determined by disappearance of parent CENU, loss of the nitroso group ($A^{230}$), and the appearance of products are the same for a given CENU.[5-7] For instance, $k_{obsd}$ for the disappearance of [$^{14}$C]cyclohexyl, [$^{14}$C]chloroethyl, and [$^{14}$C]carbonyl for hydrolysis of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in phosphate buffer, pH 7.4, 37 °C, calculated from data reported by Reed et al.,[5] are 0.012–0.013 min$^{-1}$. Negligible salt effects are observed in hydrolysis of alkyl nitrosoureas,[3] and high concentrations (1 M) of added salt affect the product distribution but not the rates of hydrolysis of CENUs.[5] Thus Snyder and Stock[3] suggested that, depending on conditions, the rate limiting step of hydrolysis of alkylnitrosoureas at alkaline pH may be either addition of water directly to **1** or collapse of **2** to products, and Lown and Chauhan[4] proposed that collapse of **2** is the rate-limiting step and that distribution of products, primarily 2-chloroethanol and acetaldehyde, is controlled in part by rotation about the $N_1$—N=O bond ($k_4$, Scheme II).

Scheme I is the most probable mechanism for hydrolysis of trialkylnitrosoureas.[8] The rate of hydrolysis of 1-(2-chloroethyl)-3,3-dimethyl-1-nitrosourea is much slower than the rate of hydrolysis for $N_3$-monoalkyl CENUs.[9] Hecht and Kozarich[10] found that phenoxide had a greater effect on the rate of hydrolysis of 1-methyl-1-nitrosourea than did thiophenoxide, which showed that basicity and not nucleophilicity of added anions is more important for a kinetic effect on the rate-determining step. Because 1-monoalkylnitrosoureas, 1,3-dialkylnitrosoureas, and CENUs have acidic protons on $N_3$ (p$K_a$ = 10–12[10]), hydrolysis could occur through the imino urea by the mechansim shown in Scheme II,[11] which unlike Scheme I would be subject to deuterium isotope solvent effects.

**Scheme I**

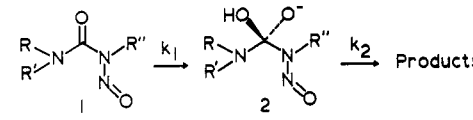



**Table I. $k_{obsd}$ for Hydrolysis of BCNU and ACNU in $H_2O$, $D_2O$, and 1:1 $H_2O$:$D_2O$[a]**

| | $10^3$ (min$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | $k^{H:H}{}_{obsd}$ | $k^{H:D}{}_{obsd}$ | $k^{D}{}_{obsd}$ | $k^{D}{}_{1}$ | $k^{H}{}_{3}/k^{D:H}{}_{3}$ | $k^{H}{}_{3}/k^{D}{}_{3}$ |
| BCNU | 5.5 | 1.35 | 0.86 | 44.0 | 4.07 | 6.05 |
| ACNU | 8.5 | 4.8 | 4.8 | – | 1.8 | 1.8 |

[a] Rates were determined spectrophotometrically ($A_{230}$) at pH (pD) 7.0, 37.0 ± 0.1 °C.

Consideration of rates for hydrolysis in $H_2O$ and $D_2O$ in Scheme II, then, predicts $k^H{}_1 > k^D{}_1$ and $k^H{}_3 > k^D{}_3$; in either Scheme I or II, deuterium should not affect rates after collapse of **6**. Note that $k_2$ and $k_{-2}$ would be subject to deuterium isotope effects, but, assuming that the effect is the same, $k^H{}_2/k^H{}_{-2}$ and $k^D{}_2/k^D{}_{-2}$ would be the same $K^H{}_2 = K^D{}_2$), and no deuterium isotope effect should be observed for this step. Both mechanisms are pH dependent.[12]

* Correspondence should be addressed to Neil Buckley, c/o The Editorial Office, Department of Neurological Surgery, 1360 Ninth Avenue, Suite 210, San Francisco, CA 94122.

(1) (a) Montgomery, J. A.; James, R.; McCaleb, G. S.; Kirk, M. C.; Johnston, T. P. *J. Med. Chem.* **1975**, *18*, 568-571. (b) Brundrett, R. B.; Colvin, M. *J. Org. Chem.* **1977**, *42*, 3538-3541. (c) Weinkam, R. J.; Liu, T. J.; Lin, H.-S. *Chem.-Biol. Interact.* **1980**, *31*, 167-177. (d) Brundrett, R. B. *J. Med. Chem.* **1980**, *23*, 1245-1247. (e) Lown, J. W.; Chauhan, S. M. S. *J. Org. Chem.* **1982**, *42*, 851-856. (f) Weinkam, R. J.; Deen, D. F. *Cancer Res.* **1982**, *42*, 1008-1014. (g) Weinkam, R. J.; Dolan, M. E. *J. Med. Chem.* **1983**, *26*, 1656-1659.

(2) The various mechanisms are reviewed in Weinkam and Lin (Weinkam, R. J.; Lin, H.-S. *Adv. Pharmacol. Chemother.* **1982**, *19*, 1-33) and ref 3 and 4.

(3) (a) Snyder, J. K.; Stock, L. M. *J. Org. Chem.* **1980**, *45*, 1990-1999. (b) Snyder, J. K.; Stock, L. M. *J. Org. Chem.* **1980**, *45*, 4494-4496.

(4) Lown, J. W.; Chauhan, S. M. S. *J. Org. Chem.* **1981**, *46*, 5309-5321.

(5) Reed, D. J.; May, H. E.; Boose, R. B.; Gregory, K. M.; Beilstein, M. A. *Cancer Res.* **1975**, *35*, 568-576.

(6) Garrett, E. R.; Goto, S.; Stubbins, J. F. *J. Pharm. Sci.* **1965**, *54*, 119-123.

(7) Loo, T. L.; Dion, R. L.; Dixon, R. L.; Rall, D. P. *J. Pharm. Sci.* **1966**, *55*, 492-497.

(8) Snyder and Stock[3a] have argued convincingly that addition of hydroxyl to the nitroso group cannot account for hydrolysis.

(9) Colvin, M.; Brundrett, R. B.; Cowens, W.; Jardine, I.; Ludlum, D. B. *Biochem. Pharmacol.* **1976**, *25*, 695-699.

(10) Hecht, S. M.; Kozarich, J. W. *Tetrahedron Lett.* **1972**, *50*, 5147-5150. Hecht, S. M.; Kozarich, J. W. *J. Org. Chem.* **1973**, *38*, 1821-1824.

(11) Garrett et al.[6] have proposed a very similar mechanism that involves addition of hydroxyl to the imino urea. This mechansim, discovered after experiments reported here were performed, would show little if any deuterium isotope effect.

0022-3263/87/1952-0484$01.50/0 

**Scheme II**

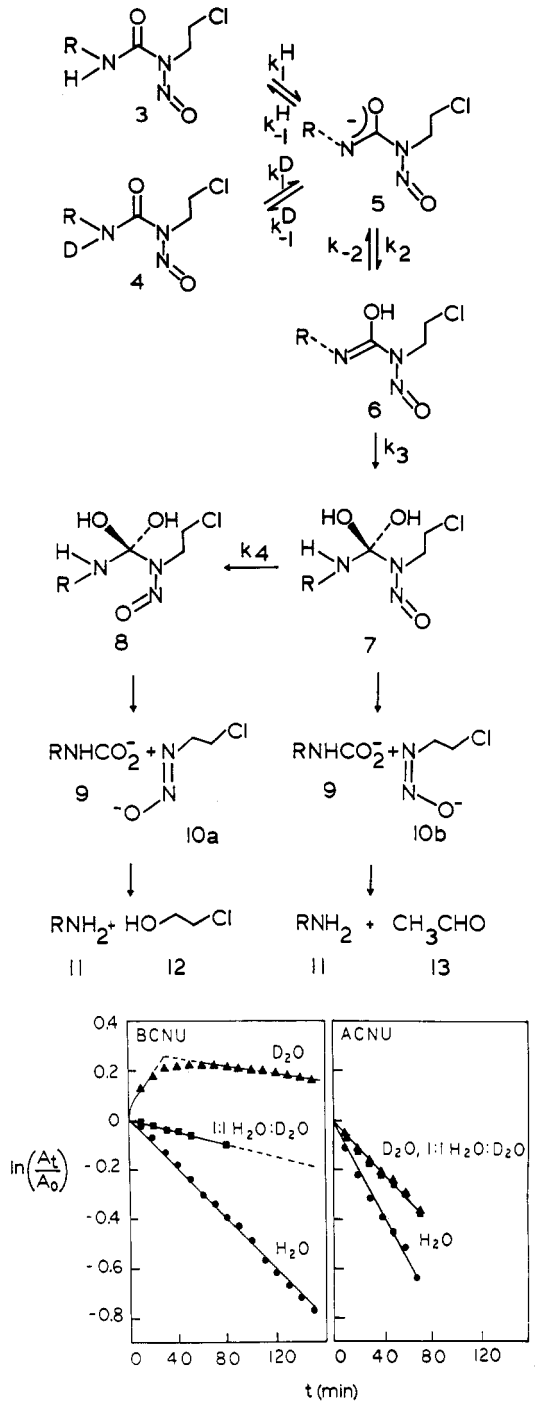






**Figure 1.** Rate profiles for BCNU (left) and ACNU (right) hydrolysis in sodium cacodylate buffer (pH[pD] = 7, 37 °C) made in H2O, 1:1 $H_2O$:$D_2O$, and 98% $D_2O$.

## Results and Discussion

Rate profiles for the hydrolysis of CCNU and 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) in sodium cacodylate buffer at pH (pD) 7, 37 °C, are distinctly different for buffer made in $H_2O$, $D_2O$, and 1:1 $H_2O$:$D_2O$.[11] Rate plots for BCNU are shown in Figure 1 (*left*) and $k_{obsd}$ are listed in Table I; CCNU (not shown) gave the same profile and essentially the same rate constants. Slopes of rate plots for BCNU in $H_2O$ and 1:1 $H_2O$:$D_2O$ decrease exponentially ($k^H_{obsd} > k^{H:D}_{obsd}$). The rate profile for hydrolysis of BCNU in 98% $D_2O$ buffer is strikingly different: the absorbance at $A_{230}$ increases exponentially initially, plateaus, and then decreases exponentially.[15] A plot of $k^H_3/k^D_3$ vs. the fraction of $D_2O$ is linear (Figure 2, assuming $k^H_3 = k^D_3$ for low fractions of $D_2O$); Wiberg[16] has noted that the linearity of such plots indicates a specific solvent isotope effect. The first-order $k^D_{obsd} = 0.044$ min$^{-1}$ for the initial increase is faster than the rate of hydrolysis and is consistent with the predicted effect of deuterium on the preequilibrum step and the observation that there is no initial increase in absorbance for $H_2O$ or mixed solvent hydrolysis ($k^H_1 > k^D_1$). Moreover, Lown and Chauhan[4] reported that H–D exchange at the $N_3$ proton of CENUs, measured by NMR, is faster than the rate of hydrolysis.

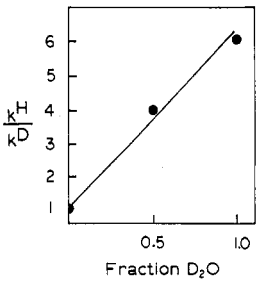
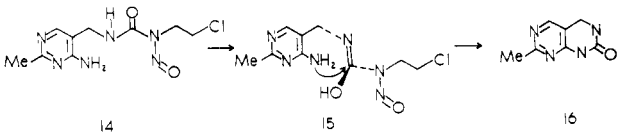



**Figure 2.** Plot of $k^H/k^D$ vs. fraction $D_2O$ for BCNU. Linearity shows a solvent isotope effect.[16]

By themselves, these results are not sufficient to differentiate between the effects of deuterium on $k_1$ and $k_3$, Scheme II. Upon hydrolysis 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU, **14**) cyclizes to give the urea **16** in essentially quantitative




yield.[17] $E_a$ and $\Delta S^{\ddagger}$ values calculated from published data for the hydrolysis of ACNU[17] and BCNU[12] in $H_2O$ are lower for ACNU. Thus for hydrolysis of ACNU in $H_2O$ and $D_2O$, only $k_1$ should be affected by deuterium. The rate profile for ACNU is shown in Figure 1 (*right*).[18] $k^H_{obsd}$ is faster than $k^D_{obsd}$ ($k^H/k^D = 1.8$), and the values of $k_{obsd}$ for ACNU hydrolysis in $D_2O$ and 1:1 $H_2O$:$D_2O$ are the same ($k_{obsd} = 4.8 \times 10^{-3}$ min$^{-1}$). Therefore, as predicted, $k^D_1$ and not $k^D_3$ is affected, and the large value of $k^H_3/k^D_3$ for hydrolysis of BCNU and CCNU must reflect the effect of deuterium in the activated complex and not in the preequilibrum step. Note that $(k^H/k^D)-(k^H/k^{H:D})$ for BCNU = 2 is essentially equivalent to the $k^H/k^{D,H:D} = 1.8$ for ACNU. While it may be inferred that the total value of $k^H/k^D$ for BCNU includes deuterium isotope effects that

(12) (a) Laskar, P. A.; Ayres, J. W. *J. Pharm. Sci.* **1977**, *66*, 1073–1076. (b) Chatterji, D. C.; Greene, R. F.; Gallelli, J. F. *J. Pharm. Sci.* **1978**, *67*, 1527–1532.

(13) This buffer was used to allow comparison of results for experiments that measured H–D exchange reactions of DNA, spermine-bound DNA, and CENU-alkylated DNA that have been studied in our laboratory.[14] Moreover, because there is sufficient evidence to suggest that phosphate buffers affect hydrolysis of CENUs, the standard system was not used for these experiments. Results obtained in phophate buffered systems will be reported elsewhere.

(14) Basu, H. S.; Shafer, R. M.; Marton, L. J., submitted for publication.

(15) The UV spectrum for CCNU at $t = 30$ min, within the plateau region, is different in heavy than in light water, suggesting the formation of the intermediate **6**.

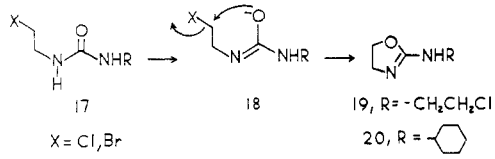
(16) Wiberg, K. B. *Chem. Rev.* **1955**, *55*, 713–743, and references cited therein.

(17) Nishigaka, T.; Nakamura, K-I.; Tanaka, M. *J. Pharmacobio-Dyn.* **1985**, *8*, 409–416.

(18) The ratio $k^H_{ACNU}/k^H_{BCNU} = 1.6$ in cacodylate is consistent with $k^H_{ACNU}/k^H_{BCNU} = 2$ for hydrolysis in 0.1 M phosphate buffer, pH 7.4, 37 °C.[2,17]

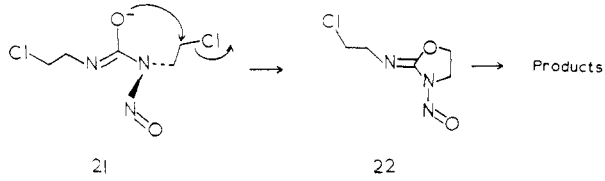
occur during steps that preceed the rate-limiting step, it is possible that the large $k^D/k^D$ is the result of a primary isotope effect.

Cyclized hydrolysis products for bis(2-haloethyl)ureas and CENUs are different, but are consistent with Scheme II. Kreling and McKay[19] found that **17** cyclizes to **20** in 93.5% yield in 1 h in 70–80 °C water, a much slower reaction than hydrolysis of CENUs. Their observation that **19** was the exclusive product for **17**, X = Br, suggests that **18** is sufficiently stable that preferential displacement of $Br^-$ takes place before addition of water, which in turn suggests that the electron-withdrawing nitroso group greatly affects the rate and product distribution of the hydrolysis of CENUs. Weinkam and Lin[20] isolated the oxozolines **19** and **20** from hydrolysis reactions of BCNU




and CCNU (12 and 3–5% of isolated products, respectively). Using isotope dilution methods they showed that **19** and **20** were products of the unnitrosated ureas that were presumably formed by addition of 2-chloroethylamine to the nitrosated imino urea with subsequent loss of 2-chloroethanediazohydroxide.[21]

Cyclization of CENUs takes place by reaction with the group on $N_1$, however. Conformations in which the alkyl group on $N_1$ is syn to the carbonyl are preferred in the crystal structure of *trans*-4-Me-CCNU,[22] and, as found in NMR studies, in aprotic and protic solvents for alkylnitrosoureas[23] and CENUs.[4] (Significantly, no products consistent with cyclization reactions between the anion of $N_3$ and the 2-chloroethyl group anti to the carbonyl have been isolated.) Lown and Chauhan[24] have prepared the 2-(alkylimino)-3-nitrosooxazolidine **22**, which is a minor



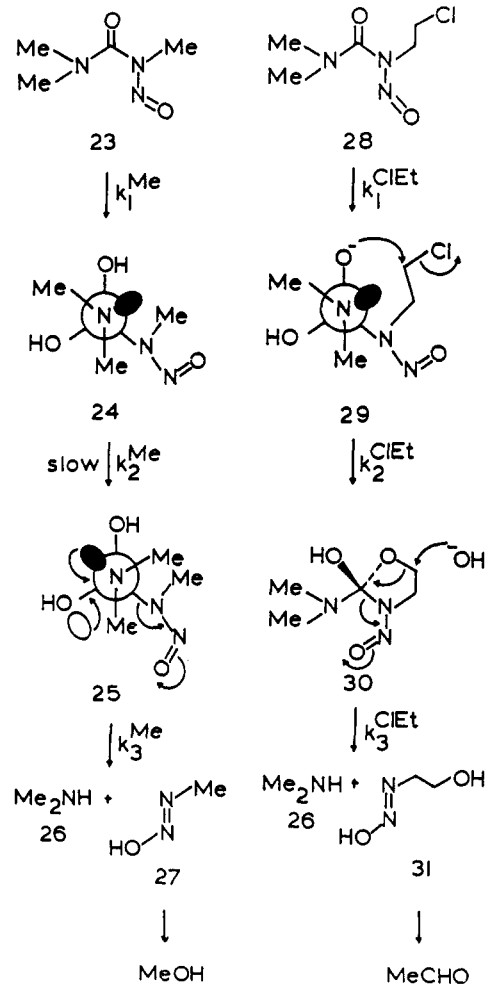

postulated intermediate in the hydrolysis of CENUs, and found that it decomposed to products found for CENUs. In contrast to reactions of unnitrosated ureas, **22** probably arises from cyclization of the 2-chloroethyl group on $H_1$ of the imino urea **21** and not from cyclization of the tetrahedral intermediate followed by a dehydration step. It should be noted that a plot of ln $k_{obsd}$ vs. pH for hydrolysis of 1-(2-bromoethyl)-CCNU is flat and that similar plots for 1-(2-fluoro)-, 1-(2-chloro)-, and 1-(3-bromo-*n*-propyl)-CCNU increase exponentially with essentially the same slope above pH 7.[25] The lack of pH dependence and the faster rate for hydrolysis of the 2-bromoethyl compound implies that the leaving group influences the rate-limiting step. However, because the only products identified were 2-haloethanols, it is not possible to suggest a mechanism for hydrolysis of the 2-bromoethyl compound. Nonetheless, from the pH profile it seems clear that neither Scheme I nor Scheme II is involved in the rate-limiting step.

**Table II. Values of $k_{obsd}$ and $k^{ClEt}/k^{Me}$ for Hydrolysis of Homologues of Alkylnitrosoureas and CENUs**

| $RN_3R'CON_1R''NO$ | $k_{obsd}$, $min^{-1}$ | $k^{ClEt}/k^{Me}$ |
|---|---|---|
| R = R′ = R″ = Me | $9.5 \times 10^{-6}$[a] | 280 |
| R = R′ = Me; R″ = $CH_2CH_2Cl$ | $2.7 \times 10^{-3}$[b] | |
| R = R″ = Me; R′ = H | $2.4 \times 10^{-4}$[a] | 58 |
| R = R″ = $CH_2CH_2Cl$; R′ = H | $14 \times 10^{-3}$[c] | |
| R = R′ = H; R″ = Me | 0.14[d] | 1 |
| R = R′ = H; R″ = $CH_2CH_2Cl$ | 0.14[e] | |

[a] Calculated from Snyder and Stock's[3] values for hydrolysis at 25 °C, pH 7.5, by assuming that the rate at 37 °C is approximately 2-fold greater; even a large error that could result from this crude assumption would not affect the qualitative interpretation. [b] Calculated from ref 9. [c] From ref 1(f). [d] From ref 6. [e] From ref 2.

**Scheme III**




Examination of the values for $k^{ClEt}/k^{Me}$ for hydrolysis of an homologous series of $N_1$-methylnitrosoureas and CENUs (Table II) shows the pronounced influence of the 2-chloroethyl group. 1,3,3,-Trimethyl-1-nitrosourea (**23**) and 1-(2-chloroethyl)-3,3-dimethyl-1-nitrosourea (**28**) must react via Scheme I. Addition of hydroxide to the less hindered face of the urea would give the tetrahedral intermediates **24** and **29**. Neither **24** nor **29**, which are the most stable rotomers shown as Newman projections in Scheme III, has the appropriate orientation for anitperiplanar collapse to products. Rotation about the $N_3$–C bond

(19) Kreling, M.-E.; McKay, A. F. *Can. J. Chem.* **1959**, *37*, 504–505.
(20) Weinkam, R. J.; Lin, H-S. *J. Med. Chem.* **1979**, *22*, 1193–1198.
(21) This exchange reaction may account for the high yield of unsymmetric ureas from the aqueous reaction of 1-methyl-1-nitrosourea and amines [Boivin, J. L.; Boivin, P. A. *Can. J. Chem.* **1951**, *29*, 478–481].
(22) Smith, H. W.; Camerman, A.; Camerman, N. *J. Med. Chem.* **1978**, *21*, 468–471.
(23) Snyder, J. K.; Stock, L. M. *J. Org. Chem.* **1980**, *45*, 886–891.
(24) Lown, J. W.; Chauhan, S. M. S. *J. Med. Chem.* **1981**, *24*, 270–279.
(25) Yoshida, K.; Yano, K. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 1557–1558.

to give the form than may collapse leads to the highly unfavorable conformation shown by **25**; rotation of **29** produces a similar conformation. In the absence of other factors, both tetrahedral intermediates should collapse to product at the same rate. It is surprising, then, that $k_{obsd}$ for **28** is 280-fold higher than $k_{obsd}$ for **23**. **29** but not **24** may cyclize and break down to the azohydroxide **31**, a process not possible for **24**. Therefore, $k^{Me}{}_3 \ll k^{ClEt}{}_2$ or $k^{ClEt}{}_3$ and the high value of $k_{obsd}$ for **28** may be the result of the cyclization of **29** and the collapse of **30**. Products are consistent with this interpretation. **23** yields dimethylamine and methanol,[10] the latter of which must arise from attack of hydroxide on the *anti*-diazohydroxide **27**.[26] **28** yields dimethylamine, acetaldehyde,[9] and ethylene glycol,[9,1d] presumably through **31**.[27] Lown and Chauhan[4] estimated that the rate of rotation about the $N_1$—N=O bond for the tetrahedral intermediate ($k_4$, Scheme II) is on the order of the rate of hydrolysis of CENUs (ca. 0.010–0.014 $min^{-1}$). Therefore, $k_{rot}$ would be faster than either $k_2$ or $k_3$ for either trialkylnitrousourea. Lown and Chauhan[4] have suggested that breakdown of the tetrahedral intermediate to produce the azohydroxide that leads to 2-chloroethanol requires a proton on $N_3$, which is not possible for these compounds.

For nitrosoureas that react via Scheme II, however, the value of $k^{ClEt}/k^{Me}$ is lower, which suggests that for this pathway cyclization of the $N_1$-(2-chloroethyl) group has a lesser influence on $k_{obsd}$. Addition of water to the imino urea should be much faster than addition of water to the carbonyl, and $k_3$, Scheme II, is probably rate-limiting for both alkylnitrosoureas and CENUs. The ratio of rates for the homologues with two protons on $N_3$ is unity.

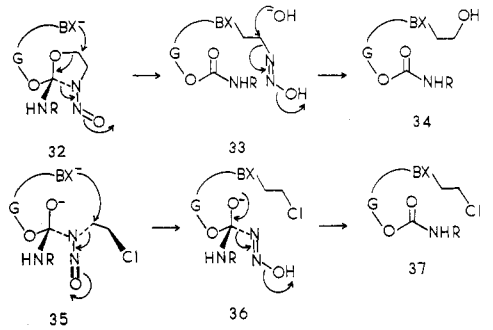
This analysis may explain the significant differences between the deuterium isotope effects reported here and the inverse isotope effects for the hydrolysis of mono-, di-, and trialkylnitrosoureas at pH 9.72, 25 °C, in either phosphate or borate buffers, reported by Snyder and Stock.[3a] The rate of collapse of the tetrahedral intermediate may be slower than the rate of addition of water to the imino urea for reactions of alkylnitrosoureas via Scheme II. At high buffer concentrations and pH, collapse of the tetrahedral intermediate is rate-limiting.[3a] This implies that for CENUs, the values of $k_3$ and the rates of collapse to product are on the same order. Alternatively, the preequilibrum step for 1,3-dialkylnitrosoureas may be swamped by $^-$OH at pH 9.72 ($k^H/k^D$ = 0.52–0.64). The half-time for hydrolysis of BCNU at pH 7.4 in phosphate buffer is 48 min,[7] but increases dramatically to 5 min at pH 8[7,11a], which would affect $k_1$ but not $k_3$, Scheme II. The value of $k^H/k^D$ = 0.96 for 1-methyl-1-nitrosourea[3a] and the finding that $k^{Me}/k^{ClEt}$ is unity for this compound and 1-(2-chloroethyl)-1-nitrosourea (Table II), both of which have two protons on $N_3$, probably reflect a statistical effect for proton abstraction ($k_1$, Scheme II) at pH 6.8.

It is important to note that an isocyanate is not formed in Scheme II.[28] With few exceptions,[3a] only amines derived from groups on $N_3$ of CENUs have been isolated. Based on the mechanism in Scheme II, we[30] have proposed a mechanism for the addition reaction of CENUs to the $O^6$-position of guanine, a DNA alkylation site that is probably important for tumor cell kill.[2] With interactive computer techniques, we modeled cross-linking reactions of $O^6$-guanine carbamate products derived from the 2-substituted ethyl group on $N_3$ of BCNU and have suggested that these adducts may be cytotoxic in conforma-

(26) Lown et al. [Lown, J. W.; Chauhan, S. M. S.; Koganty, R. R.; Sapse, A.-M. *J. Am. Chem. Soc.* **1984**, *106*, 6401–6408] have recently shown that diazohydroxides formed in solution from hydrolysis of CENUs have ratios of the *E*:*Z* (anti:syn) forms of 80:20, and that the rate of conversion between forms is very slow. These ratios may reflect rotation about the $N_1$—N=O bond in the imido urea or tetrahedral intermediate; rotation in the parent might be restricted by delocalization throughout the urea moiety.

(27) Diazohydroxide **31** is equivalent to the intermediate expected in the hemipinacol rearrangement for nitrous acid deamination of β-hydroxyamines to yield carbonyl compounds [see, e. g.: Saavedra, J. E. *J. Org. Chem.* **1981**, 46, 2610–2614 for deamination of a series of β-hydroxyamines. Acetaldehyde formed in the deamination of 2-chloroethylamine [Colvin, M.; Cowens, J. W.; Brundrett, R. B.; Kramer, B. S.; Ludlum, D. B. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 515–520] probably arises from 1,2,3-oxadiazoline that reacts with water to give **31**].

(28) The mechanisms by which the 2-chloroethanediazohydroxides **10a,b** decompose to give products are discussed elsewhere [Buckley, N., manuscript in preparation]. Addition of water to either side of the syn or anti forms of the imidourea and rotation about the C–$N_3$ bond directly yield all tetrahedral intermediates proposed by Lown and Chauhan.[4] Steric and stereochemical factors account for the breakdown of the tetrahedral intermediates and the products formed from subsequent intermediates. It should be noted, however, that the primary and chlorovinyl carbocations and chloronium ions often proposed[1a,1c,2,5] for these reactions are probably not involved. Brundrett and Colvin[1b] have pointed out that $S_N2$ and $E_2$ mechanisms account for products, including those that form via 1,2-hydride and 1,2-methyl shifts,[1b818] better than $S_N1$ mechanisms. Because hydroxyethylated products predominate in DNA,[29] the reverse of products found in hydrolysis reactions, the question of intermediacy has particular importance for reactions of CENUs with DNA. It is tempting to speculate that breakdown of tetrahedral intermediates formed by addition of $O^6$-guanine to **6** on the surface of DNA might be suppressed by steric factors, which allows cyclization to take place as shown in Scheme III. Nucleophiles on bases adjacent to the site of addition would be fixed relative to the adduct; if displacement reactions took place on a cyclized product (**32–34**, where $BX^-$ is an adjacent




base nucleophile on the same strand, for instance $O^6$- or $N_7$-guanine[29]), hydroxyethyl products (**34**) would form. [This sequence may have important implications for DNA cross-linking reactions (Buckley, N., unpublished results).] Chloroethylated bases could form by the sequence **35–37**, in which **36** is analogous to an intermediate postulated for the conversion of amides to carboxylic acids upon treatment with nitrous acid. This mechanism is more satisfying that the nonspecific, delocalized primary carbocationic mechanism cited recently to explain reactions of CENUs with DNA.[29] **Note added in proof**: Recently we measured the rate constants for cross-linking of calf thymus DNA pulse-treated in vitro with saturating doses of nitrogen mustard (HN2), BCNU, and (2-chloroethyl)(methylsulfonyl)methane sulfonate (2-ClEtSoSo) [Buckley, N.; Brent, T. P., submitted for publication]. The standard model for DNA cross-linking reactions[2] predicts that BCNU and 2-ClEtSoSo should form the same initial adduct and should have the same kinetics and mechanism of cross-linking. Cross-linking of DNA treated with HN2 and BCNU is a fast first-order process that probably reflects a rate-limiting rearrangement of initial adducts before the DNA cross-linking reaction occurs, while that for 2-ClEtSoSo is a slower, second-order process; the kinetics of the latter reaction are consistent with the putative 2-chloroethyl adduct at $O^6$-guanine. Cross-linking for BCNU is ca. 10-fold faster than for 2-ClEtSoSo. The rate constant for cross-linking of L-1210 cells treated with HN2 is the same as the rate constant for rearrangement of parent mustard in buffer. The rate of cross-linking in L-1210 and 9L cells treated with BCNU, CCNU, or CNU is essentially equivalent to the rates for three DNAs in vitro ($k_{obsd}$ = 2.7–7.1 × $10^{-3}$ $min^{-1}$) and is at most 2-fold faster than the rate of hydrolysis for **28**. We believe that lethal cross-linking reactions on the surface of cellular DNA occur by a process analogous to the sequence **32** → **34** (see above), in which cyclization to **32** is the rate-limiting step. Lethal cross-links form by reaction of BX-$(CH_2)_2N_2OH$ in **33** with a base nucleophile on the opposite strand. These results suggest that the standard model for CENU cross-linking reactions is not correct.

(29) Hartley, J. A.; Gibson, N. W.; Kohn, K. W.; Mattes, W. B. *Cancer Res.* **1986**, *46*, 1943–1947.

(30) Buckley, N.; Feuerstein, B. G.; Linfoot, P. A.; Deen, D. F.; Marton, L. J., submitted for publication. Linfoot, P. A.; Tofilon, P. J.; Buckley, N.; Marton, L. J.; Deen, D. F., submitted for publication.

tionally relaxed DNA.[30] The heuristic mechanism nicely explains results for CENU-treated DNA reported by others, including Babson and Reed's results for CENU deactivation of glutathione reductase,[31] Brent's results[32a] for alkylation of DNA and deactivation of an intracellular repair enzyme, and the sequential labeling of guanine-$N_7$ positions in DNA reported by Hartley et al.[29] In vitro experiments with polynucleotides [32b] and computer graphic studies[33] of the implications of the mechanism in Scheme II for CENU product distributions and the range of products formed in DNA that may affect tumor cell kill are being explored and will be reported elsewhere.

(31) Babson, J. R.; Reed, D. J. *Biochem. Biophys. Res. Commun.* **1978**, *84*, 754-756.
(32) (a) Brent, T. P. *Cancer Res.* 1986, *46*, 2320-2323. (b) Brent, T. P.; Buckley, N., unpublished results.
(33) Feuerstein, B. G.; Pattabiraman, N.; Marton, L. J., *Proc. Nat. Acad. Sci. U.S.A* 1986, 83, 5948-5952. Buckley, N.; Feuerstein, B. G.; Marton, L. J., manuscript in preparation.

## Experimental Section

CENUs were dissolved in absolute ethanol to give concentrations of approximately 2 mg/mL. Ten or 20 μL of stock ethanol solution were added to microcuvettes containing 350 μL of 1 mM sodium cacodylate, 50 mM NaCl buffer (pH (pD) = 7) that had been preequilibrated in the electrically heated (37 ± 0.1 °C) block of a Gilford Model 2600 spectrometer. Five minutes after addition and mixing, 0 time readings were taken and reactions were followed for 1-2 half-lives. Rate constants were calculated as the slopes of plots of ln $(A_t/A_0)$ vs. time, which assumes that $A_\infty = 0$.[6]

**Acknowledgment.** This work was supported in part by NIH program Project Grant CA-13525 and the Phi Beta Psi Sorority. I thank Professors John W. Larsen and John K. Snyder for helpful discussions and Burt G. Feuerstein, M.D., Ph.D., and Professors Leon M. Stock and Frank A. Carey for helpful comments on the manuscript.

# Reactivity–Selectivity Relationships in Reactions of Ambident Nucleophiles with the Superelectrophiles 4,6-Dinitrobenzofuroxan and 4,6-Dinitro-2-(2′,4′,6′-trinitrophenyl)benzotriazole 1-Oxide[1]

Erwin Buncel* and Richard A. Renfrow

*Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6*

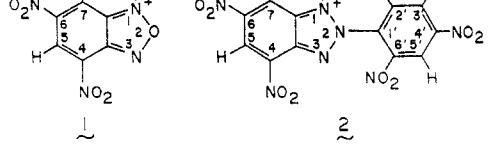
Michael J. Strauss

*Department of Chemistry, University of Vermont, Burlington, Vermont 05405*

*Received May 23, 1986*

The reactions of 4,6-dinitrobenzofuroxan (1) and 4,6-dinitro-2-(2′,4′,6′-trinitrophenyl)benzotriazole 1-oxide (2) with aryl oxides and arylamines have been investigated. Phenoxide ion reacted with 1 to give a carbon-bonded adduct by reaction at C-7, but in contrast with the 1,3,5-trinitrobenzene (TNB) system, aniline reacted to give both nitrogen- and carbon-bonded adducts as observable species, depending on the reaction conditions. Reaction of 2 with phenoxide ion gave products arising solely from nucleophilic attack at C-1′ of the picryl moiety. Reaction of 2 with aniline also yielded a nitrogen-bonded adduct when carried out in the presence of 1 equiv of triethylamine as catalyst. Inhibiting the reactivity of aniline through ortho or *N*-methyl substitution resulted in the formation of C-7 carbon-bonded adducts of 2. These reactions generally involve kinetically preferred but reversible formation of a σ complex that is bonded via the heteroatom, followed by conversion to a carbon-bonded product of thermodynamic control. The extent to which the kinetically and thermodynamically preferred products can be observed is rationalized according to reactivity–selectivity arguments. The formation of carbon-bonded aniline complexes with 1, but not with TNB, is a result of the greater reactivity of the former (a superelectrophile). The observed regiochemistry in the reaction of nucleophiles with 2 depends on the stability of the adduct at the benzotriazole moiety (C-7) and the selectivity of the nucleophile for reaction at the benzotriazole (C-7) vs. the picryl moiety (C-1′). Decreased selectivity will result from an increase in thermodynamic driving force for the addition reaction (more negative $\Delta G_0$) and/or, for exergonic reactions, a decrease in intrinsic barrier (i.e., decreased $\Delta G_0^{\ddagger}$). Decreased selectivity resulting from decreased intrinsic barriers will favor rapid formation of the products of thermodynamic control. Previous results with related systems are also rationalized on the basis of stability–selectivity relationships.

The recent discovery of the highly electrophilic nature of certain heteroaromatic substrates such as 4,6-dinitrobenzofuroxan (DNBF, 1) and 4,6-dinitro-2-(2′,4′,6′-trinitrophenyl)benzotriazole 1-oxide (PiDNBT, 2) has given new impetus to the study of the anionic σ complexes formed between nucleophiles and electron-deficient species.[4] Of particular interest recently has been the study of nucleophiles which are potentially ambidentate.[2,5-13] It



1 2

(1) Ambident Nucleophilic Reactivity in σ-Complex Formation. 6. Part 5: reference 2. Reactivity–Selectivity Correlations. 6. Parts 4 and 5: reference 3.

(2) Buncel, E.; Murarka, S. K.; Norris, A. R. *Can. J. Chem.* **1984**, *62*, 534.
(3) (a) Buncel, E.; Wilson, H.; Chuaqui, C. *J. Am. Chem. Soc.* **1982**, *104*, 4896. (b) Buncel, E.; Wilson, H. *J. Chem. Educ.*, in press.
(4) Buncel, E.; Crampton, M. R.; Strauss, M. J.; Terrier, F. *Electron Deficient Aromatic- and Heteroaromatic-Base Interactions. The Chemistry of Anionic Sigma Complexes*; Elsevier: Amsterdam, 1984.
(5) Strauss, M. J.; Renfrow, R. A.; Buncel, E. *J. Am. Chem. Soc.* **1983**, *105*, 2473.
(6) Buncel, E.; Renfrow, R. A.; Strauss, M. J. *Can. J. Chem.* **1983**, *61*, 1690.
(7) Terrier, F.; Debleds, F.; Halle, J. C.; Simonnin, M. P. *Tetrahedron Lett.* **1982**, *23*, 4079.
(8) Terrier, F.; Halle, J. C.; Simonnin, M. P.; Pouet, M. J. *J. Org. Chem.* **1984**, *49*, 4363.

0022-3263/87/1952-0488$01.50/0 