N-Nitrosiminium Ions Are Ambident Electrophiles

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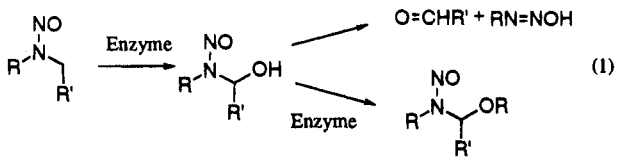
The carcinogenic and mutagenic activity of *N*-nitroso-*N*-dialkylamines is thought to be due in part to the fact that they are α-hydroxylated enzymatically whereupon they may decompose to precursors of DNA-alkylating diazonium ions and carbocations or they may be enzymatically or otherwise converted to acyl, sulfate, or phosphate esters or glucuronyl conjugates, as in eq 1.[1–3]



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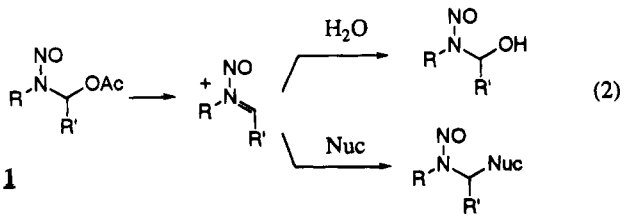
α-Acetoxy-*N*-nitrosodialkylamines **1** (eq 2) continue to play a central role in understanding nitrosamine carcinogenesis so that the solution chemistry of these compounds is of considerable importance.[1] There is



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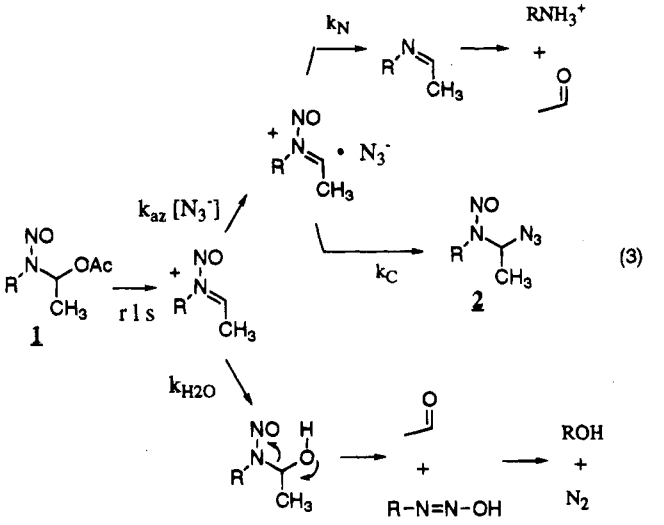
evidence to suggest that some α-acetoxy-*N*-nitrosodialkylamines decompose non-enzymatically through the intermediacy of *N*-nitrosiminium ions that can react with solvent or other nucleophiles to reform α-substituted *N*-nitrosodialkylamines of varying biological activity, eq 2.[1,2,4–6] We report here the first evidence that *N*-nitrosiminium ions are ambident electrophiles—certain nucleophiles denitrosate the *N*-nitrosiminium cation.[7] Denitrosation of this reactive intermediate represents a novel detoxification pathway because removal of the nitroso group destroys the alkylating activity of the *N*-nitrosamine precursor. Rate constants for the reactions of simple *N*-nitrosiminium cations with water, estimated by the azide ion "clock" method, are reported.

Analysis by ^{1}H-NMR of products formed by the decomposition of 1-(*N*-nitroso-*N*-ethylamino)ethyl acetate (**1a**, R = ethyl), 1-(*N*-nitroso-*N*-isopropylamino)ethyl acetate (**1b**, R = isopropyl), and 1-(*N*-nitroso-*N*-*tert*-butylamino)-ethyl acetate (**1c**, R= *tert*-butyl)[8] indicates predominant formation of ethanol (71%), 2-propanol (68%), and 2-methyl-2-propanol (72%), respectively, and no detectable yield (<3%) of ethylammonium, isopropylammonium, or *tert*-butylammonium ions, respectively. Decomposition in the presence of azide ion yields two new products in each case. First, the respective ammonium ions are formed in 3%, 24%, and 62% yields, respectively, at 0.2 M azide ion. The second product in each case, upon scale-up, isolation, and characterization, is revealed to be the azide adduct (**2a–c**), in eq 3).[10] The changes in the fractional yield of azide



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adduct as a function of azide ion concentration, as determined by HPLC, are as indicated in Figure 1. At 0.2 M azide ion the azide adduct accounts for 92%, 78%, and 34% of the starting material. In each of the three cases, analysis by spectrophotometry or HPLC indicates that there is less than a 8% increase in k_{obsd} for decomposition with up to 0.2 M azide ion. These data indicate that azide ion reacts after the rate-limiting step, presumably with the respective *N*-nitrosiminium cations to quantitatively yield, at 0.2 M azide ion, either the azide adduct, through attachment at carbon, or the ammonium ion, through denitrosation and subsequent hydrolysis of the imine—as in eq 3.

Azide ion is known to be an efficient trap of some unstable carbocations, reacting with a diffusion-limited

(1) Lawley, P. D. In *Chemical Carcinogens*; Searle, C. D., Ed.; ACS Monograph 182; American Chemical Society: Washington, D.C., 1984. Lijinsky, W. *Chemistry and Biology of N-nitroso Compounds*; Cambridge University Press: Cambridge, 1992.

(2) Braun, H.; Wiessler, M. *Angew. Chem., Int. Ed. Engl.* **1980** *19*, 400 and references within. Wiessler M.; Rossnagel, G.; Rugewitz-Blackholm, B. In *N-Nitroso Compounds: Occurence, Biological Effects and Relevence to Human Cancer*; O'Neill, I. K., von Borstel, R. C., Miller, R. C., Long, J.; Bartsch, H., Eds.; IARC Sci. Pub., **1984**; Vol. 52, p 465. Frank, N.; Wiessler, M. *Carcinogen* **1986** *7*, 365.

(3) Mochizuki, M.; Anjo, T.; Sekiguchi, N.; Ikarashi, A.; Suzuki, A.; Wakabayashi Y.; Okada, M. *Chem. Pharm. Bull.* **1986** *34*, 3956.

(4) Wiessler, M. In *N-Nitrosamines*; Anselme, J.-P., Ed.; American Chemical Society: Washington, D.C., 1979; p 57.

(5) Frei, E.; Frank, N.; Wiessler, M. *J. Canc. Res. Clin. Oncol.* **1990**, *116*, 569.

(6) Revis, C.; Rajamäki, M.; Fishbein, J. C. Unpublished results.

(7) It was previously reported that the major product of the Ce(IV) oxidation of cyclohexylethanolamine is cyclohexylamine but whether the denitrosation involved loss of NO from a radical or NO^+ from an *N*-nitrosiminium ion was not ascertained. Loeppky, R. N; Li, E. *Chem. Res. Toxicol.* **1988** *1*, 334.

(8) **1a**: ^{1}H-NMR ($CDCl_3$) δ 7.14 (1H, q); 3.59 (2H, m); 2.08 (3H, s); 1.78 (3H, d); 1.09 (3H, t). Anal. Calcd: C, 44.99; H, 7.55; N, 17.49. Obsd: C, 44.84; H, 7.49; N, 17.38. **1b**: ^{1}H-NMR ($CDCl_3$) δ (*E*) 7.14 (1h, q); 4.34 (1H, m); 2.06 (3H, s); 1.59/1.55 (6H, d); 1.41 (3H, d); (*Z*) 6.85 (1H, q); 4.79 (1H, m); 2.10 (3H, s); 1.88 (3H, d); 1.21/1.13 (6H, d). (*E*)/(*Z*) = 1/4. Anal. Calcd: C, 48.26; H, 8.10; N, 16.08. Obsd: C, 48.08; H, 8.17; N, 16.00. **1c**: ^{1}H-NMR ($CDCl_3$) δ (*E*) 6.80 (1H, q); 2.12 (3H, s); 1.91 (3H, d); 1.39 (9H, s); (*Z*) 6.54 (1H, q); 2.09 (3H, s); 1.61 (3H, d); 1.60 (9H, s). Anal. Calcd: C, 51.05; H, 8.57; N, 14.88. Obsd: C, 51.09; H, 8.52; N, 14.80.

(9) Reactions carried out at room temperature for 10 half-lives with an initial substrate concentration of 6×10^{-3} M.

(10) Isolated by extraction of scaled-up reactions with CH_2Cl_2 and purification by preparative chromatography on silica. **2a**: ^{1}H-NMR ($CDCl_3$) δ (*Z*) δ 6.24 (1H, q); 3.60 (2H, q); 1.67 (3H, d); 1.15 (3H, t); (*E*) δ 6.35 (1H, q); 4.19 (2H, m); 1.53 (3H, t); 1.38 (3H, d). Anal. Calcd: C, 33.85; H, 6.33; N, 48.66. Obsd: C, 33.56; H, 6.34; N, 48.92. **2b**: ^{1}H-NMR ($CDCl_3$) δ (*E* = *Z*) 6.38, (q, 1H); 5.63 (1H, q); 4.70 (1H, m); 4.35 (1H, m); 1.86 (3H, d); 1.65–1.55 (6H, overlap d's); 1.35 (3H, d); 1.28 (6H, overlap d's). Anal. Calcd: C, 38.36; H, 6.97; N, 44.38. Obsd: C, 38.20; H, 7.05; N, 44.55. **2c**: ^{1}H-NMR ($CDCl_3$) δ (*Z*) 4.82 (1H, q); 1.70 (3H, d) 1.56 (9H, s). (*E*) 5.40, (1H, q); 1.98 (3H, d); 1.42 (9H, s).

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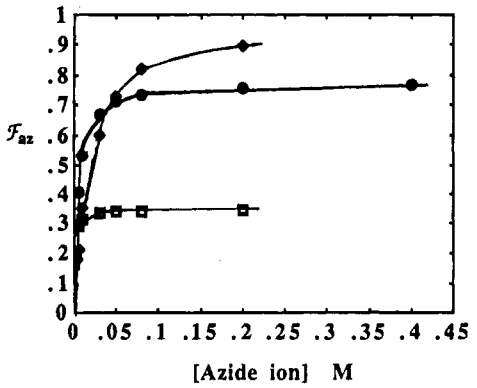


Figure 1. Plot of the yields of azide adducts **2a–c**, formed in the decomposition reactions of **1a–c** as a function of azide ion concentration in aqueous solutions, pH = 6.3, 0.3 M cacodylic acid buffer, ionic strength 1 M ($NaClO_4$), 25 °C: **1a** (◆); **1b** (●); **1c** (□).

rate constant of ~5 × 10^9 M^{-1} s^{-1} when the ratio of rate constants for reaction of azide ion compared to solvent, k_{az}/k_{solv}, is on the order of 10^3 M^{-1}.[11,12] Under such conditions the diffusion-limited trapping by azide ion can be used as a clock to estimate the rate constants for reactions of the cations with water and, thus, their lifetimes in solution.[11]

The fractional yield of azide adduct can be expressed as described in eq 4 (derived from eq 3). The slopes

$$\mathcal{F}_{az} = \frac{k_{az}[N_3^-]\left[\frac{k_C}{k_C + k_N}\right]}{k_{az}[N_3^-] + k_{H_2O}} \qquad (4)$$

divided by the intercepts of the least squares lines for the double reciprocal plot of $1/[N_3^-]$ against the inverse of the fraction of azide adducts, $1/\mathcal{F}_{az}$, give values for the ratios k_{H_2O}/k_{az}. The values of the inverse ratios are k_{az}/k_{H_2O} = 55 (± 1) M^{-1}, 218 (± 2) M^{-1}, and 1700 (±100) M^{-1} and $k_C/(k_N + k_C)$ = 1.00 ± 0.02, 0.78 ± 0.02, and 0.34 ± 0.02 for the cations, from **1a**, **1b**, and **1c**, respectively. These values of ratios generate the solid lines in Figure 1. Rate constants for the capture of these cations by water are k_{H_2O} = 9.1 × 10^7 s^{-1}, 2.3 × 10^7 s^{-1}, and 3.0 × 10^6 s^{-1}, respectively, assuming a value of k_{az} = 5 × 10^9 M^{-1} s^{-1}.[13]

More stable *N*-nitrosiminium cations are also ambident electrophiles. The rate constant for decay of (*N*-nitroso-*N*-methylamino)(4-methoxyphenyl)methyl azide (**4a**) is strongly inhibited by millimolar concentrations of added azide ion to a limiting rate constant that is ~4% of that in the absence of azide as indicated in Figure 2.[14] These data are consistent with reaction via a free *N*-nitrosiminium cation that is trapped by azide ion either at carbon to regenerate the starting materials or at the nitroso nitrogen to give products, as indicated in eq 5. Analysis by ^{1}H-NMR of the products of decay of (*N*-nitroso-*N*-methylamino)(4-methoxyphenyl)methyl azide (**4a**) in 25%/75% CD_3CN/D_2O in the presence of 0.1 M NaN_3 indicates a 66% yield of methylammonium ion and a 34% yield of imine ($k_N[N_3^-]$, eq 5). These data reflect the fact that at high azide ion concentration the exclusive pathway for decomposition of (*N*-nitroso-*N*-methylamino)-

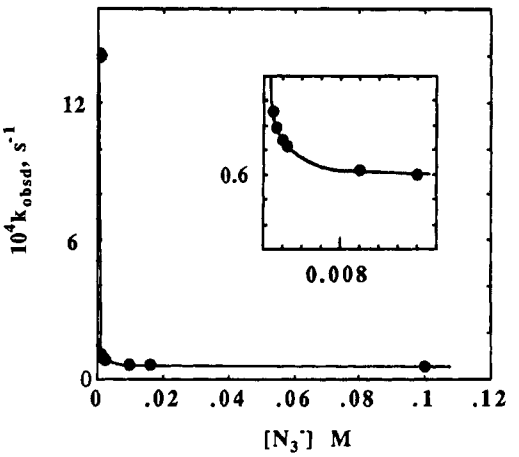
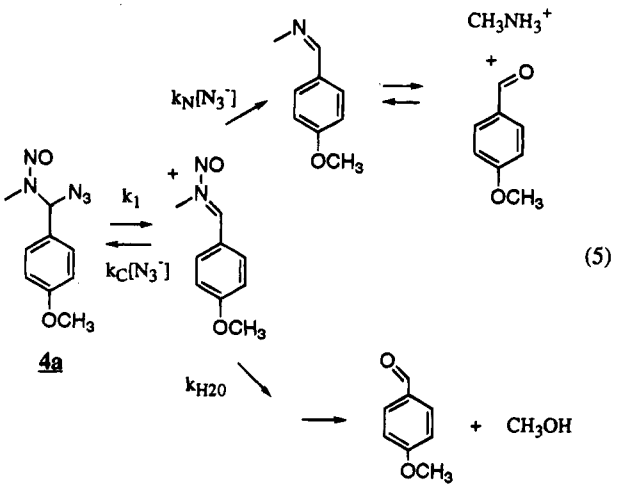


Figure 2. Plot of k_{obsd} against azide ion concentration in the decay of (*N*-nitroso-*N*-methylamino)(4-methoxyphenyl)methyl azide (**4a**) ([*S*] = 8 × 10^{-5} M) in aqueous solutions ionic strength 1 M ($NaClO_4$), 25 °C, pH 8.9 ± 0.1. Inset is detail of points at low azide ion concentration, axis labels are same as in main figure. The rate constant at $[N_3^-]$ = 0 is the intercept of a plot of k_{obsd} against $[HClO_4]$ for reactions carried out between 0.0016 and 0.01 M $HClO_4$. Rate constants not plotted at 0.5 and 1.0 M azide ion were 6.0 × 10^{-5} s^{-1} and 5.9 × 10^{-5} s^{-1}, respectively.



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(4-methoxyphenyl)methyl azide (**4a**) is via denitrosation ($k_N[N_3^-]$, eq 5).

The appropriate expression k_{obsd} for the decay of **4a** (eq 5) in the presence of azide is given in eq 6, and a

$$k_{obsd} = \frac{k_1(k_N[N_3^-] + k_{H_2O})}{(k_C + k_N)[N_3^-] + k_{H_2O}} \qquad (6)$$

nonlinear least-squares fitting of the data (solid line in Figure 2) gives values of k_C/k_{H_2O} = (2.2 ± 0.1) × 10^4 M^{-1}, k_N/k_{H_2O} = 940 (±60) M^{-1} and k_1 = (1.40 ± 0.04) × 10^{-3} s^{-1}.[15] Neglecting the reaction at nitrogen, the value of k_C/k_{H_2O} is larger by a factor of 9 than the value directly measured for the (*N*-nitroso-*N*-methylamino)(phenyl)-methaniminium cation.[13]

Finally, the denitrosation reaction reported here is not azide ion-specific. Decomposition in D_2O of 1-(*N*-nitroso-*N*-isopropylamino)ethyl acetate (**2**) in the presence of 0.2 M thiosalicylate, pD 8.5, ionic strength 1 M ($NaClO_4$) gives a 72% yield of isopropylammonium ion.

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(11) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 4689.
(12) McClelland, R. A.; Kanagasabapathy, V. M.; Banait, N. S.; Steenken, S. *J. Am. Chem. Soc.* **1991**, *113*, 1009.
(13) k_{az} = 2.0 × 10^9 M^{-1} s^{-1} and k_{az}/k_{H_2O} = 2600 M^{-1} have been directly measured for an *N*-nitrosiminium ion. Vigroux, A.; Kresge, A. J.; Fishbein, J. C. *J. Am. Chem. Soc.*, in press.
(14) Unbuffered azide solutions, 25 °C.
(15) Fit was carried out using proportional weighting.