Mechanism for the Solvolytic Decomposition of the Carcinogen *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine in Aqueous Solutions[1]

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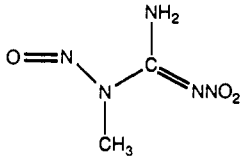
Abstract: A study of the kinetics and products of the decomposition of the carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in aqueous buffered solutions, 1 M ionic strength at 40 °C, has been carried out over the range pH 4–10. The pK_a of MNNG has been determined to be 7.73 ± 0.03. Rate constants k_{int} for the buffer-independent, solvolytic, decomposition reaction increase with increasing pH above pH 6. The slope of the plot of log k_{int} against pH is equal to 1 for data above pH ~8.5. Analogous experiments on the decomposition of the compound *N,N''*-dimethyl-*N'*-nitro-*N*-nitrosoguanidine (DMNNG) show the solvolytic decomposition reaction to be pH independent from pH 6 to 13, and the pK_a for this compound is found to be 5.72. DMNNG therefore is at least $10^{7.6}$ more stable toward hydroxide ion than MNNG. The currently accepted mechanism for solvolytic decomposition of MNNG is inconsistent with both the pH dependence of k_{int} for the reaction of MNNG and the extraordinarily low reactivity of DMNNG. The observed yield of nitrocyanamide anion in the base-catalyzed reaction of MNNG is 93%, and no nitrourea product is detected in these reactions. Reactions carried out in $H_2{}^{18}O$ with ^{16}O-containing MNNG indicate less than 1% exchange of the nitroso oxygen with solvent oxygen in MNNG reisolated after 50% of reaction. The above results rule out base-catalyzed decomposition by hydroxide ion attack at either the guanidino carbon or the nitroso nitrogen of MNNG. The most probable mechanism of this reaction involves the hydroxide ion catalyzed nitrile-forming elimination reaction of the anion of MNNG. The absence of buffer catalysis of the reaction and solvent deuterium isotope effects of k_{OD}/k_{OH} = 2.1 indicate that the leaving group expulsion step is rate limiting. The decomposition chemistry of the *N*-(*N*-nitroamidino)triazole (TRNG) has been similarly investigated. The plot of the log k_{int} against pH for TRNG has a slope of 1 above pH 6.5, the pK_a of TRNG has been determined to be 6.74 ± 0.02, and the yield of nitrocyanamide ion for the base-catalyzed decomposition of TRNG is 96%. These results are consistent with the mechanism described for the hydroxide ion catalyzed decomposition of MNNG, one that does not require a rearrangement reaction involving a nitroso group.

Introduction

The compound *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) is a powerful direct-acting carcinogen the biological activity of which is believed to be the result of the formation, in the course of its decomposition, of an electrophilic methyl group that reacts with DNA.[2,3] MNNG has demonstrated cancer



MNNG

chemotherapeutic potential.[4] It is routinely employed in studies of chemical carcinogenesis and is widely used in synthetic organic chemistry as a diazomethane generator that is activated upon treatment with aqueous base.[5] A detailed understanding of the aqueous chemistry is therefore of considerable interest.

The wide-ranging work of Lawley and Thatcher established the experimental basis for much of the current thinking about the aqueous reaction chemistry of MNNG.[6] They observed that the half-time of decomposition of MNNG decreased with increasing pH above pH 6 and that the only "UV-absorbing" product was nitrocyanamide ion. On this basis they proposed mechanism 1 (Scheme I), in analogy with what was then considered to be the mechanism of decomposition of *N*-methylnitrosourea. This mechanism persists in the modern review literature.[7]

We are currently investigating the aqueous chemistry of MNNG and related compounds in order to determine the elements of structure and reactivity that control their alkylating activities. We have obtained data on the solvolytic decomposition of MNNG with which mechanism 1 is inconsistent. The results rule out any mechanism proposed for the solvolytic decomposition of any structurally related *N*-nitroso compounds. It is concluded that the dominant mechanism of solvolytic decomposition at physiological pH is apparently a hydroxide ion catalyzed nitrile-forming elimination reaction of the anion of MNNG (see mechanism 2; Scheme II).

Experimental Section

Materials. Organic reagents were purified before use by recrystallization or distillation, and inorganic chemicals were ACS reagent grade or better. Water was glass distilled.

Methods. Kinetics. Decomposition reactions of MNNG and DMNNG at 40 °C were monitored, at 400 or 390 nm (MNNG) and 390 or 380 nm (DMNNG) using either a Milton Roy 1001 + or a Milton Roy 3000 spectrophotometer attached to thermostated water baths. Runs were carried out in 3 mL of reaction solution contained in a 1- × 1-cm cuvette stoppered with a septum. Runs were initiated, after 30 min of equilibration in the cell block, by injection of an aqueous solution (25% by volume in acetonitrile) of the nitrosoguanidine through the septum to a final concentration of between 0.000 25 and 0.005 M.

The first-order rate constant for disappearance of the starting material was obtained by one of two methods. Analysis of the exponential decay of absorbance for between 3 and 5 half-lives of reaction was generally used for runs with halftimes of 1 h or less. Analyses were carried out by graphic means—a linear plot on semilogarithmic paper of $A_t - A_\infty$ against time yielded the half-time $t_{1/2}$ and the rate constant was obtained

(1) This research was funded by a grant from the North Carolina Board of Science and Technology, The Petroleum Research Fund, administered by the American Chemical Society, and the National Institutes of Health (Grant CA52881).

(2) The crystal structure of MNNG has recently been determined. It proves the nitrimino form in the solid state: Rice, S.; Cheng, M. Y.; Cramer, R. E.; Mandel, M.; Mower, H. F.; Seff, K. *J. Am. Chem. Soc.* **1984**, *106*, 239.

(3) Lawley, P. D. In *Chemical Carcinogens*; Searle, C. E., Ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984; Vol. 1.

(4) Skinner, W. A.; Gram, H. F.; Greene, M. O.; Greenberg, J.; Baker, B. R. *J. Med. Pharm. Chem.* **1960**, *2*, 299.

(5) For a review see: Black, T. H. *Aldrichimica Acta* **1983**, *16*, 3.

(6) Lawley, P. D.; Thatcher, C. J. *Biochem. J.* **1970**, *116*, 693.

(7) Hegarty, A. F. In *The Chemistry of Diazonium and Diazo Compounds*; Patai, S., Ed.; Wiley: New York, 1978; p 51. Regits, M. In *The Chemistry of the Diazonium and Diazo Groups*; Patai, S., Ed.; Wiley: New York, 1978; p 659. Regits, M.; Maas, G. *Diazo Compounds*; Academic Press: New York, 1986.

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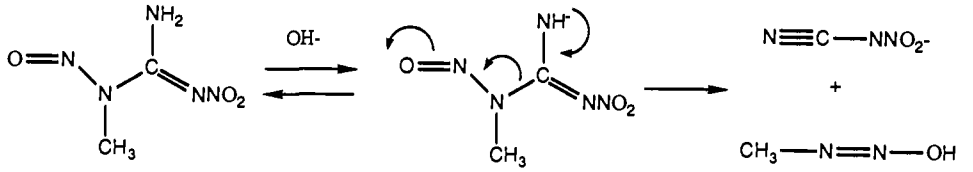
Scheme I



from the equation $k_{obs} = 0.693/t_{1/2}$—or with a commercially available fitting program. The two methods agreed within <5% using the same data set. The second method for determining k_{obs}, used in slower reactions, was that of initial rates. The required extinction coefficient for MNNG was determined under the particular experimental conditions from the change in absorbance observed on injection of MNNG into the reaction mix and from the gravimetrically determined concentration of MNNG. Both initial rate and first-order decay analysis on a single run gave agreement within 10% (five checks).

Experiments with the *N*-(*N*-nitroamidino)triazole (TRNG) were carried out similarly and monitored at 295 nm. TRNG stock solutions were made up in DMSO. Typical reaction concentrations of TRNG were 2×10^{-4} M, 1% by volume in DMSO.

The decomposition of DMNNG was also monitored either by high-performance liquid chromatography (HPLC) using a Waters C-18 column (mobile phase aqueous 0.01 M KH_2PO_4 buffer, 1 mL/min) with detection at 280 nm or by ^1H NMR in solutions containing D_2O.

Kinetics Controls. For MNNG the effect of chloride ion on reaction rate was found to be negligible. The value of k_{obs} varied randomly by less than 5% at KCl concentrations from 0 to 1 M (ionic strength 1 M with $NaClO_4$). For DMNNG the rate constants k_{obs} derived from the exponential decay monitored by HPLC to 57% and 91% completion agree to within 10% with the value determined from spectrophotometric determinations. For TRNG the decomposition kinetics at the standard concentration of 2×10^{-4} M, monitored at 295 nm, were compared with those observed at a 10-fold higher concentration, monitored at 330 nm. The higher concentration was typical of that used in the product analysis runs (below). The rate constants k_{obs} obtained in the two runs agreed to within 5%.

The value of pH was determined after the kinetic run using an Orion SA 720 meter with a Microelectrodes M13 combination probe. The meter was calibrated at two values of pH using either commercial standards or those perscribed by the *Merck Index*, 8th ed. The lyoxide ion concentration was calculated from the observed pH and the ion product of water. For experiments in D_2O, 0.4 was added to the observed pH and the ion product of D_2O was used to compute the lyoxide ion concentration.[8]

Products. Product analysis was carried out by HPLC using a Waters pump and UV detector set at 263 nm. Separations were effected at a flow rate of 1 mL/min, a mobile phase of either aqueous 0.01 M KH_2PO_4 with 0.2 M KCl or aqueous 1% acetic acid and 0.05 M KCl, and a Waters 30-cm amino column. Quantitation was performed by an Axxiom data system.

Oxygen Exchange. Experiments were carried out to investigate the possibility of exchange of the nitroso oxygen of MNNG with that of water enriched in $H_2{}^{18}O$. Reaction solutions were made up by weighing solid DABCO buffer base and KCl into a vial containing a stir bar. A volume of 1.00 M HCl in $H_2{}^{16}O$ was added to give a buffer ratio of 1/1. Volumes of $H_2{}^{16}O$ (distilled water) and $H_2{}^{18}O$ (97 atom %, Aldrich Chemical Co.) were added to give the final volume. Control experiments (natural abundance of ^{18}O) were also run. An injection of MNNG in acetonitrile initiated the reaction. The reaction was terminated after 1 half-time by addition of a volume of acetic acid and extracted with ethyl acetate that was subsequently passed over a column containing magnesium sulfate and evaporated under of a stream of dry nitrogen. The sample was then analyzed by desorptive chemical ionization mass spectroscopy (NH_3 gas).

Synthesis. DMNNG. A 5.4-g portion of nitrosonium tetrafluoroborate was added to a round-bottom flask maintained at −25 °C containing 3 g of *N'*,*N''*-dimethyl-*N*-nitroguanidine[10] and 4.5 g of di-*tert*-butyl-4-methylpyridine stirred in 150 mL of dry methylene chloride. After 15 min the flask was warmed to room temperature over the course of 1 h. The solvent was removed, leaving a yellow solid to which was added 25 mL of chloroform. Undissolved solid was removed by filtration and discarded, and the filtrate was evaporated to dryness. The remaining solid was chromatographed on a silica flash column using methylene chloride. The yellow solid from a concentrated fraction was recrystallized twice in ethanol/water to give the pure compound. ^1H NMR ($CDCl_3$): (s, 3 H) 3.25, (d, 3 H) 3.24, (br, 1 H) 9.6 ppm. The two methyl signals collapsed to one broad signal when the sample was irradiated with a decoupling pulse at 9.6 ppm. ^1H NMR (D_2O, 0.105 M KOD): (s, 3 H) 3.00, (s, 3 H) 3.10 ppm. ^1H NMR (DMSO-d_6, distilled from CaH): (br, 3 H) 2.96, (s, 3 H) 3.13, (br, 1 H) 9.90 ppm. The signal at 2.96 ppm collapsed to a clean singlet when the sample was irradiated with a decoupling pulse at 9.90 ppm. Anal. Calcd: C, 22.36; H, 4.37; N, 43.46. Found: C, 22.56; H, 4.32; N, 43.22. Exact mass: calcd, 161.05492; found, 161.0550.

TRNG. MNNG (0.015 mol) was added to 25 mL of an aqueous solution (20% ethanol), 0.38 M in acetic acid buffer (90% anion), containing 0.053 mol of triazole stirred at 50 °C. After 30 min the reaction was brought to 0 °C. A precipitate was filtered and washed with 25 mL of ice water, dissolved in 25 mL of water containing 0.015 mol of NaOH, and within 1 min neutralized with HCl. A solid was collected by filtration and recrystallized in ethanol/water. ^1H NMR (DMSO-d_6): (1 H, s) 9.24, (1 H, s) 8.45, (2 H, br) 9.99 ppm. Anal. Calcd: C, 23.08; H, 2.58; N, 53.84. Found: C, 23.27; H, 2.59; N, 53.81. The structure of TRNG (see Discussion), with attachment of the *N*-nitroamidino group at N-1 of triazole, is presumed based on the large difference (0.8 ppm) in chemical shift of the triazolo protons.

Results

Kinetics. The first-order rate constants for the decomposition of MNNG, DMNNG, and TRNG were measured at 40 °C, 1 M ionic strength (KCl), in a number of aqueous buffer systems from pH 3 to 10, 7 to ~13, and 4 to 9, respectively, by spectrophotometric methods. Several checks of the methodology and self-consistency were made (see Experimental Section). Values of k_{obs} for the reactions of MNNG, DMNNG, and TRNG are contained in Tables S1–S3, respectively (supplementary material).

Values for the rate constant k_{int} for the buffer-independent decomposition reactions were obtained by extrapolation of plots of k_{obs} against buffer to zero buffer concentration using the least-squares line. Typically the value of k_{obs} changed, linearly, by less than 30% with changes in buffer concentration that routinely spanned from 0.02 to 0.2 M (Figure S1a,b) (supplementary material). Triethanolamine was notable in that, under some conditions, it modestly stimulated the decomposition of all three substrates. The largest effect of this buffer was a 175% increase in k_{obs} for the reaction of MNNG at the highest buffer concentration (0.23 M). More typical modest increases, 40–80%, did not prevent extrapolation to zero buffer concentration to obtain a reasonably accurate value, ±10%, of k_{int}. Other buffers used include DABCO, *N*-methylimidazole, morpholinoethanesulfonate, carbonate, phosphate, acetate, and formate. Values of k_{int} for MNNG, DMNNG, and TRNG are contained in Tables S1–S3, respectively.

The validity of extrapolating to zero buffer concentration to obtain the rate constant k_{int} from typical experiments carried out at 0.02–0.2 M buffer is affirmed by several experiments carried out at millimolar buffer concentrations with MNNG. Three experiments show that the value of k_{obs} changes by less than 10% with a change from 0.003 or 0.007 to 0.03 M buffer.

Plots of log k_{int} versus pH are shown in Figure 1 for the decomposition of MNNG (circles) and DMNNG (squares) while that for the decomposition of TRNG is presented in Figure 2.

The solvent deuterium isotope effect on the hydroxide ion catalyzed decomposition of the anion of MNNG was determined to be $k_{OD}/k_{OH} = 2.1 \pm 0.1$ based on runs at 50% and 20% DABCO cation buffers. The final values of k_{OD} and k_{OH} were obtained by dividing the observed value of k_{int} by the calculated lyoxide ion concentration.

(8) The ion product for H_2O was taken as $K_w = 10^{-13.29}$ M^2 as calculated by Harned and Hamer.[9] That for D_2O was calculated to be $10^{-14.16}$ M^2 by assuming the difference between H_2O and D_2O to be the same as at 25 °C for the pure solvents.

(9) Harned, H. S.; Hamer, W. J. *J. Am. Chem. Soc.* **1933**, *33*, 2194.

(10) Hardy-Klein, M. L. *J. Chem. Soc.* **1957**, 71.

Table I. Experiment To Determine Extent of Oxygen Exchange from Solvent Water into Unreacted MNNG

buffer base, pH	total buffer concn	% ^{18}O[a] in H_2O	signal area for mass[b]		signal intens for mass 167/ sum signal intens for mass 165 + 167
			mass 165	mass 167	
DABCO[c]	0.040	0.20	197562	4273	2.12
8.88	0.040	47	52145	1106	2.08
DABCO[c]	0.20	0.20	3507840	73464	2.05
8.88	0.20	49	2660270	66539	2.44

[a] The first listing of each pair of runs at the same buffer ratio represents a control experiment with "unenriched" H_2O. The ^{18}O isotopic content in these experiments is assumed to be "natural abundance" as described by: CRC Handbook of Chemistry and Physics, 56th ed.; Weast, R. C., CRC Press: Boca Raton, FL, 1975; p B-254. The second listing is a calculated percent based on the volumes of dilute aqueous solutions (unenriched) and 97% ^{18}O enriched H_2O used to make to reaction solution. [b] Masses are for the M + 18 protonated ammonia adducts of the ^{16}O- and putative ^{18}O-containing MNNG that are generated in the desorptive chemical ionization mass spectrometry using ammonia gas. [c] DABCO = diazabicyclo-[2.2.2]octane.

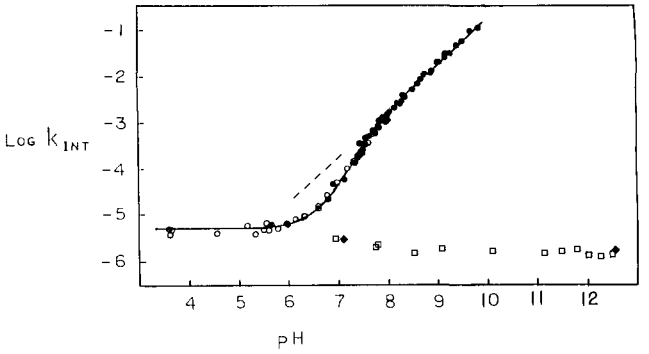


Figure 1. Plot of the logarithm of the buffer-independent first-order rate constant for decomposition k_{int} against pH for MNNG (circles) and DMNNG (squares and diamonds) at 40 °C, 1 M ionic strength, 1% by volume in acetonitrile in H_2O except as noted. Rate constants were obtained from experiments employing initial rate measurements (open symbols) and exponential decay measurements (filled symbols). The solid line is calculated using the rate equation and constants in the text (see Discussion). The dashed line is of slope 1 extended from the data above pH 8.5 (circles). Rate constants denoted by diamonds were measured in D_2O.

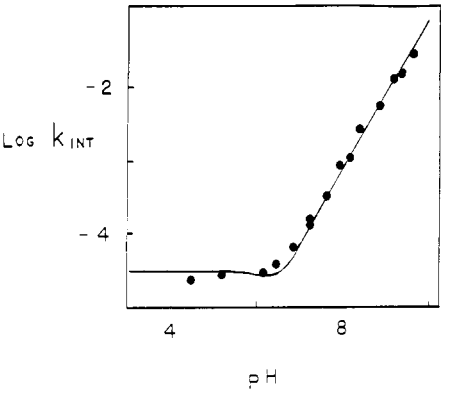


Figure 2. Plot of the logarithm of the buffer-independent first-order rate constant for decomposition k_{int} against pH for TRNG at 40 °C and 1 M ionic strength (KCl), 1% by volume DMSO. The solid line is as calculated using the rate equation and constants given in the text (see Discussion).

pK_a Values. The pK_a of MNNG was determined from the change in the measured extinction coefficient at 400 nm as a function of pH. It was not possible to accurately measure the extinction coefficient at the high-pH end point because of the rate of decomposition. At the highest pH for which an extinction coefficient was measured, less than 5% of the MNNG had decomposed by the time the initial absorbance of MNNG was recorded. Despite some scatter, the large amount of data (40 points) allows a good fit to a simple titration curve with a pK_a of 7.73 ± 0.03. This value is in reasonable agreement with the value of 7.57 ± 0.02 calculated from measurements of pH of 10% and 20% ionized solutions of 0.05 M MNNG containing 2% by volume of acetonitrile. The pK_a values for DMNNG (5.71 ± 0.02) and TRNG (6.74 ± 0.02) were similarly obtained spectrophotometrically. Titration curves for MNNG, DMNNG, and TRNG are included in Figures S2 (MNNG and DMNNG) and S4 (TRNG).

Products. The yields of nitrocyanamide ion from the decomposition of MNNG and TRNG were quantitated after 10 half-lives of reaction by high-performance liquid chromatography. For MNNG, above pH 7.1 the yield averages 93% with a standard deviation of ±3% (23 runs). Within this subset there is a probable, but not entirely consistent, slight decrease in the yield of nitrocyanamide anion with increasing buffer concentration. For TRNG, above pH 7.8 the yield of nitrocyanamide is 96% ± 3% (7 runs). The data are summarized in Tables S4 (MNNG) and S5 (TRNG). No product analysis was carried out for the reaction of DMNNG.

In no instance was there a positive identification of nitrourea in any of the product analysis experiments with MNNG. The limits of detection indicate that it could account for less than 2% of the products in any run. A control containing no MNNG but 0.0010 M nitrourea (pH 7.42, 0.18 M *N*-methylimidazole buffer) yielded no measurable nitrocyanamide while the yield of nitrocyanamide from MNNG under the same reaction time and conditions was measured as 92%.

Oxygen Exchange. Experiments were carried out to detect incorporation of ^{18}O from $H_2{}^{18}O$ into unreacted MNNG. The relative amounts of the protonated ammonia adducts of ^{16}O-containing (M^+ = 165) and ^{18}O-containing (M^+ = 167) MNNG were quantitated after 1 half-time of decomposition. Two experiments were run at the same pH using different buffer concentrations. For each buffer concentration a control containing H_2O of natural isotopic abundance was run. The data for these experiments are summarized in Table I.

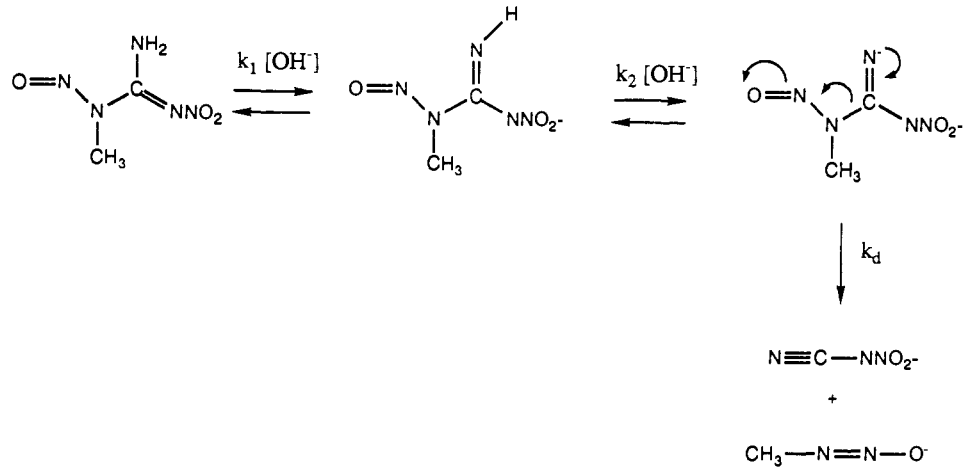
The results of these experiments indicate that there is less than 1% of exchange, to the equilibrium value of 47% or 49%, of oxygen from water into MNNG. As can be seen from the last column of Table I, the percent of the total signal from the two masses that was of mass 167 is nearly the same whether the reaction was run in ^{18}O-enriched or nonenriched H_2O.[11] In the experiment at 0.20 M buffer there was a slight increase of 0.4% in the percent of the total signal that was of M^+ = 167. In runs containing ^{18}O, the signal at M^+ = 169 was less than 1% that of the signal at 165, indicating no significant oxygen exchange involving the nitro group of MNNG.

Discussion

Analysis of Mechanism. The more quantitative observations reported here confirm those of Lawley and Thatcher but, with one additional piece of data, allow mechanism 1 to be ruled out as the dominant mechanism above pH 6. Mechanism 1 requires that a plot of the log k_{int}, the first-order rate constant for de-

(11) The observed percent in the control runs (Table I) is in excess of the 0.6% that would be expected based on the natural abundance of ^{18}O and the fact that MNNG contains three oxygens. Co-isolation of a small amount of an unknown that generates a signal at 167 is indicated by the fact that the background from a mass spectrum of solvent used to dissolve the samples for application to the solid probe gave no signal at 167.

Scheme II



composition of MNNG, against pH should have a slope of 1 below the pK_a of MNNG and should level off to a slope of zero above the pK_a. This is inconsistent with the experimental data reported here. The pK_a of MNNG is 7.73. Inspection of the pH–rate profile (Figure 1) for the decomposition of MNNG shows that above pH 6.5 the plot is curved, with tangent slopes sometimes greater than 1, until about pH 8, above which pH the plot is linear with a slope of 1.

The fact that the pH–rate profile for the decomposition of MNNG has slope 1 above the pK_a of 7.73 for MNNG requires a term in the rate law that is first order in hydroxide ion and first order in the anion of MNNG—or equally, a term that is second order in hydroxide ion and first order in the neutral form of MNNG. This mode of decomposition is dominant below the pK_a of MNNG as well, as evidenced by the curve for which tangents have slopes greater than 1 between pH 8 and 7. At pH 6 and below, a pH-independent reaction becomes dominant.

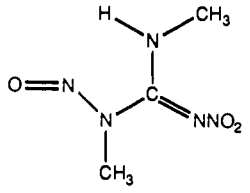
A simple two-term rate law that, when written in terms of the neutral form of MNNG, contains a pH-independent term and one that is second order in hydroxide ion as in eq 1 gives a good

$$V/[\text{MNNG}]_{\text{tot}} = k_{\text{int}} = k_1/(1 + K_a/[\text{H}^+]) + k_2[\text{OH}^-]^2/(1 + K_a/[\text{H}^+]) \quad (1)$$

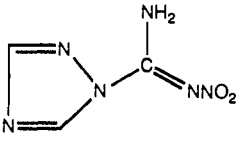
fit to the data (solid curve in Figure 2) using the values of k_1 = 5.2 (±0.2) × 10^{-6} s^{-1} and k_2 = 1.98 (±0.05) × 10^8 M^{-2} s^{-1} and the experimentally determined value for K_a = 1.86 × 10^{-8} M.[12]

Mechanism 2 (Scheme II) is consistent with the term in the rate law for the base-catalyzed decomposition of MNNG and is not inconsistent with any other experimental results obtained in the present work or previously.

The requirement of mechanism 2 that two protons are removed from the N″ nitrogen (NH_2 in the neutral form) is consistent with the extraordinary stability toward base of DMNNG (below)



DMNNG



TRNG

compared to MNNG. DMNNG has a single labile proton, the pK_a of which is measured to be 5.71. The pH-rate profile for the decomposition of DMNNG (Figure 1, squares) establishes that, in contrast to MNNG, there is no observed term in the rate law that is second order in hydroxide ion. Such a term, if it exists, is at least 7.6 orders of magnitude smaller than that for MNNG.[13] This effect of a methyl group is too large to be accounted for by electronic factors.[14]

A number of alternatives to mechanism 2 can be ruled out. The mechanism claimed to operate in the generation of diazomethane from MNNG in aqueous 5 M KOH gives nitrourea as a product, and this is consistent with the 93% yield of nitrocyanamide ion observed in the present study.[5] Further, the work of Boopsingh and Briody establishes that nitrourea decomposes, to products other than nitrocyanamide, with a pH-independent first-order rate constant of 2.4 × 10^{-4} s^{-1} that is kinetically inconsistent with its being an intermediate in the formation of nitrocyanamide ion from MNNG above pH ~8.[15,16]

The possibility that decomposition occurs via a hydroxide ion attack on the nitroso group can be ruled out because of the absence of ^{18}O exchange from $H_2{}^{18}O$ into unreacted starting material. There is substantial evidence in support of this type of mechanism in the ethoxide ion catalyzed decomposition of *N*-(2,2-diphenylcyclopropyl)-*N*-nitrosourea in ethanol.[17] An analogous mechanism in the case of MNNG must involve reversible hydroxide ion attack. This is based on the rate law that requires at least one subsequent slow step in which an additional molecule of hydroxide ion is consumed. Thus, ^{18}O exchange is predicted to be rapid relative to overall reaction by this mechanism. In contrast, we failed to detect more that 0.4% incorporation ^{18}O from ~50% enriched ^{18}O-containing water after 1 half-time of decomposition in DABCO buffers.

Any mechanism involving a rearrangement reaction is considered unlikely. Such a mechanism might be suggested based on the known chemistry of *N*-nitro- and *N*-nitrosoamides.[18-21]

(12) These values are obtained using a commercially available nonlinear least-squares fitting program. The values of [H^+] and [OH^-] were calculated from the observed values of pH and the ion product of water, 1 M KCl and 40 °C, equal to 5.12 × 10^{-14} M^2.[9]

(13) The lower limit for the effect of methyl substitution on this term in the rate law is obtained by extrapolating a line of slope 1 from the rate constant at the highest pH for experiments with DMNNG decomposition back to the pH value of the largest rate constant measured for MNNG.

(14) Substitution of a cyanoethyl group for the methyl group in MNNG increases the rate constant k_2 by less than a factor of 10: Nag, S.; Fishbein, J. C. Unpublished results.

(15) Boopsingh, B.; Briody, J. M. *J. Chem. Soc.* **1973**, 1487.

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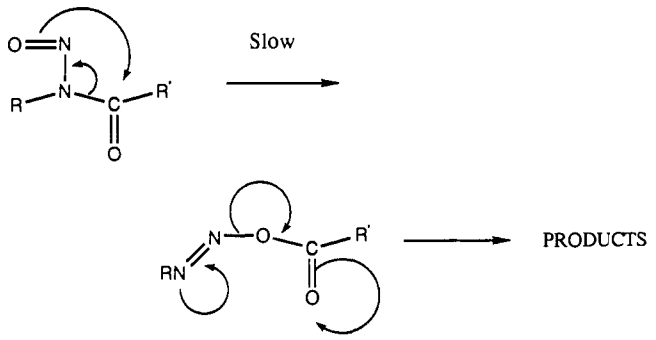
Such compounds undergo rate-limiting unimolecular rearrangements to yield ester and alkene products: To be consistent with



the observed rate law for decomposition of MNNG, a rearrangement mechanism must involve a rearrangement step that is fast and reversible relative to the subsequent consumption of 2 equiv of hydroxide ion. In contrast, the reactions of *N*-nitro- and *N*-nitrosoamides indicate that formation of the diazo ester intermediate is irreversible.[18-22] Further, the diazo ester intermediates (above) are known to decompose with nitrogen—oxygen bond cleavage to give N_2 whereas the product from MNNG, nitrocyanamide ion, requires an unprecedented oxygen—carbon bond cleavage.

Mechanism 2 (Scheme II) predicts that the reaction should be general for any nitroguanidine with a weakly basic anionic nitrogen leaving group—in contrast to a mechanism involving rearrangement that would only be operative for nitroguanidines containing nitroso or nitro functionalities in the leaving groups. This prediction was tested by the synthesis and study of *N*-(*N*-nitroamidino)triazole (TRNG, above), which has a weakly basic triazole anion leaving group, the conjugate acid pK_a of which is around 10.[23]

The prediction above is affirmed by the decomposition chemistry of TRNG. The pH–rate profile (Figure 2) for its decomposition shows a first-order dependence on hydroxide ion above the measured pK_a for TRNG of 6.74. The solid curve in Figure 2 is calculated from eq 1, with the values $k_1 = 3.1 \times 10^{-5}$ s^{-1}, $k_2 = 6.2 \times 10^8$ M^{-2} s^{-1}, and the experimentally determined value of $K_a = 1.82 \times 10^{-7}$ M, the values of other parameters being the same as for MNNG. The yield of nitrocyanamide anion in the hydroxide ion catalyzed reaction averages 96%.

The E1cb mechanism of hydroxide ion catalyzed nitrile-forming elimination reactions of imido esters represents a reasonable precedent for mechanism 2.[24] Similarities are also observed in the hydroxide ion catalyzed decomposition of nitramide ion, $HN_2O_2^-$, though in the case of nitramide ion proton abstraction is concerted with leaving group departure as evidenced by the intermediate value of Bronsted β equal to 0.5 for the observed general-base-catalyzed reaction.[25] A concerted reaction for the decomposition of MNNG is ruled out in the next section.

Nature of the Rate-Limiting Step. The absence of buffer catalysis of decomposition of MNNG by moderately weak buffer bases such as DABCO and *N*-methylimidazole requires that k_d (mechanism 2, Scheme II) is the rate-limiting step because it rules out both a concerted mechanism in which proton transfer and product formation are concurrent and rate-limiting proton transfer (k_2 in mechanism 2).

The step k_2 can be ruled out as rate limiting because it can be shown that if k_2 is rate limiting, catalysis by moderately weak bases will be observed even if the pK_a of MNNG anion is equal to or greater than that of water. The driving force for catalysis is the stabilizing interaction of the hydrogen bond between the conjugate acid of the catalyst and the dianion of MNNG. Catalysis of this type has been referred to as a form of stepwise preassociation mechanism, and the general-acid-catalyzed decomposition of carbamates is an example involving hydrogen bonding with an intermediate that is more acidic than the hydronium ion.[26,27]

In a rate-limiting reaction of the anion of MNNG with a moderately weak buffer base, the expectation of catalysis arises from the fact that the transition state for the k_2 step will be quite late and will be stabilized by hydrogen bonding to nearly the same extent as the product encounter complex. The amount of stabilization expected can be calculated based on the hydrogen-bonding interaction observed between ammonium and phenolate ions.[28] In the present case, a rate increase of at least 110%, in the presence of 0.3 M Dabco buffer (50% cation), and 360%, in the presence of 0.15 M *N*-methylimidazole (50% cation), is expected due to hydrogen-bonding stabilization if k_2 is rate limiting and the pK_a of MNNG anion is equal to or greater than that of H_2O.[29] Lower values for the pK_a of MNNG anion predict larger amounts of catalysis.[30] The observed amounts of catalysis in the actual experiments, identical to the conditions described above, give rate increases of less than 10% in both cases (Figure S1a,b).

The inverse solvent deuterium isotope effect of $k_{OD}/k_{OH} = 2.1$ on the lyoxide ion catalyzed decomposition reaction is consistent with the conclusion that proton transfer is not the rate-limiting step in this reaction. The isotope effect measurements were made in a region of pH in which the lowest energy form of MNNG is the anion, and thus the observed isotope effect arises from isotope

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(30) Larger values predict larger amounts of catalysis by hydrogen bonding; smaller values predict smaller amounts of catalysis by this mechanism but will result in compensating increases in catalysis by a simple trapping mechanism because of a resulting difference in rate-limiting step between the hydroxide ion catalyzed and buffer catalyzed reactions.

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effects on all steps involved in product formation from the anion—a process that is first order in lyoxide ion. The observed isotope effect is close to that expected (k_{OD}/k_{OH} = 2.30) for a reaction involving complete consumption of a lyoxide ion in steps up to and including the rate-limiting step.[33] Rate-limiting proton transfer would result in a normal isotope effect that would offset the inverse effect.[34]

The near-maximal value of the solvent deuterium isotope effect suggests that there is substantial progress in the transition state (k_d, mechanism 2, Scheme II) toward the highly charge-delocalized products. This is because an early transition state that resembles the dianion of MNNG would be expected to be strongly hydrogen bonded to solvent and the fractionation factors for these hydrogen bonds would offset those lost in the consumption of the lyoxide ion.[33,34] The fact that the isotope effect caused by loss of lyoxide ion is nearly uncompensated suggests that the transition state is charge diffuse and productlike because the fractionation factors for weakly basic species, like the products diazotate anion and nitrocyanamide ion are near unity.[35]

Acceptance of mechanism 2 allows an upper limit value of 20.6 for the pK_a of the monoanion of MNNG to be calculated. This is based on the measured first pK_a of MNNG of 7.73, the rate law for mechanism 2 written in terms of constants for dissociation of MNNG, $MNNG^-$, and leaving group expulsion (k_d),[36] a value of k_{int} calculated according to eq 1 at a pH greater than 7 (the pH above which mechanism 2 accounts for more than 90% of the reaction), and an upper limit for k_d of 10^{10} s^{-1}.[37]

Related Systems. The requirement for a the loss of two protons for the elimination mechanism deduced here is without precedent in the aqueous reaction chemistry of *N*-nitrosoguanidines or any structurally related system. A similar mechanism involving the removal of a single proton followed by elimination was suggested for both the oxyanion and sodium hydride catalyzed decomposition reactions of *N*-nitrosomethyl urea in nonaqueous media.[38]

The present work is the second report of a decomposition reaction of an *N*-nitrosamide-type system that is kinetically second order in hydroxide ion. The first such report was the work of Peña and co-workers for the decomposition of *N*-nitroso-2-imidazolidone, but the reaction chemistry appears to be fundamentally different.[39]

The aqueous decomposition chemistry of MNNG is notable by comparison with that of structurally related compounds in that where there is definitive evidence, these other compounds undergo hydroxide ion catalyzed decomposition by nucleophilic attack at the electron-deficient carbon. A substitution mechanism is likely in the case of *N*-nitroso-*N*-methylurea,[40] benzyl-*N*-nitroamides,[41] and *N*-nitroso-2-imidazolidone.[39] In other cases, *N*-methyl-*N*-nitroacetamide[41] and *N*-nitroso-2-pyrrolidone,[42] the absence of an elimination pathway is less certain. The reaction of MNNG and the precedent of the elimination mechanism for the base-catalyzed decomposition of certain thiol esters indicate that the possibility of this reaction pathway should not be ignored.[43]

A Subsidiary Issue. The difference in pK_a between MNNG (7.73) and DMNNG (5.71) is larger than can be accounted for on the basis of inductive or hyperconjugative effects expected in a methyl for hydrogen substitution. The observed difference of 2 units may be an underestimate, by 0.3 unit, if a statistical correction for two protons on MNNG is warranted. A similar substitution lowers the pK_a of nitramide (NH_2NO_2) by 0.3 unit.[25] Exchange of a hydrogen for a methyl group may enforce a fundamentally different stereochemical configuration in DMNNG compared to MNNG. The difference could result in significantly different pK_a values due to intrinsic stereochemical effects[44,45] or a difference in intramolecular hydrogen bonding.[48]

Alternate explanations can be discounted. First, it is unlikely that the difference in pK_a is result of the two compounds being of different tautomeric forms. The nitrimino form is indicated for both based on the crystal structure of MNNG[2] and the ^1H NMR decoupling data for DMNNG in the polar aprotic solvent DMSO (see Experimental Section). Second, consideration of steric strain caused by the methyl group would predict a larger pK_a for DMNNG, opposite the observation, because strain will be maximal in the highly delocalized, planar, conjugate base.

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Registry No. D_2, 7782-39-0; D_2O, 7789-20-0; DMNNG, 138090-34-3; TRNG, 138090-35-4; *N*-methyl-*N'*-nitro-*N''*-nitrosoguanidine, 70-25-7; nitrocyanamide anion, 62722-85-4; *N',N''*-dimethyl-*N*-nitroguanidine, 101250-97-9; triazole, 288-88-0.

Supplementary Material Available: Figures S1–S3 and tables of rate constants for decomposition of MNNG (S1), yields of nitrocyanamide from decomposition of MNNG (S2), rate constants for decomposition of DMNNG (S3), rate constants for decomposition of TRNG (S4), and yields of nitrocyanamide from decomposition of TRNG (24 pages). Ordering information is given on any current masthead page.

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(37) The limit for k_d is set by the absence of observable buffer catalysis. If k_d were greater than 10^{10} s^{-1}, the dianion would decompose faster than a buffer base could diffuse away. This would enforce measurable buffer catalysis by a hydrogen-bonding preassociation mechanism,[26] as discussed above,[29] which is not observed.

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