0.94 (m, 2 H); ^{13}C NMR (75 MHz, C$_6$D$_6$) δ 210.2, 172.0, 85.9, 84.6, 80.8, 68.7, 65.4, 51.2, 46.8, 34.2, 26.5; MS *m/z* (M$^+$) calcd 322.0140, obsd 322.0172.

C. 12b. Treatment of **12b** (105 mg, 0.36 mmol) in an analogous fashion followed by MPLC purification of the crude product (SiO$_2$, elution with 10% ether in petroleum ether) afforded **20** as a clear, yellow oil (35.5 mg, 36%): IR (CHCl$_3$, cm^{-1}) 2020, 1970, 1050; ^{1}H NMR (300 MHz, C$_6$D$_6$) δ 4.82 (dd, *J* = 8.4, 4.9 Hz, 1 H), 4.42 (dd, *J* = 8.5, 4.9 Hz, 1 H), 3.71 (AB q, *J* = 14.6, 7.4 Hz, 1 H), 3.55 (AB q, *J* = 13.8, 6.2 Hz, 1 H), 3.45 (AB q, *J* = 14.2, 7.8 Hz, 1 H), 1.70-1.15 (series of m, 4 H), 1.09 (d, *J* = 6.2 Hz, 3 H), 0.81 (m, 1 H), 0.68 (m, 1 H); ^{13}C NMR (300 MHz, C$_6$D$_6$) δ 85.0, 80.4, 80.1, 67.8, 65.8, 56.9, 34.6, 26.0, 19.1 (Fe(CO)$_3$ not seen); MS *m/z* (M$^+$) calcd 278.0241, obsd 278.0235.

D. 13b. Analogous processing of **13b** (143 mg, 0.48 mmol) gave **21** as a clear, yellow oil (73 mg, 54%) after MPLC purification: IR (CHCl$_3$, cm^{-1}) 2060, 1980, 1050; ^{1}H NMR (300 MHz, C$_6$D$_6$) δ 4.79 (m, 1 H), 4.37 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.69 (m, 1 H), 3.58-3.45 (m, 2 H), 1.86 (m, 1 H), 1.55-1.32 (m, 3 H), 1.04 (d, *J* = 6.2 Hz, 3 H), 0.77 (m, 2 H); ^{13}C NMR (75 MHz, C$_6$D$_6$) δ 86.6, 83.3, 81.8, 68.7, 63.5, 58.2, 34.3, 26.6, 19.9 (Fe(CO)$_3$ not seen); MS *m/z* (M$^+$) calcd 278.0241, obsd 278.0210.

Preparation of Dimethyl Acetals 14a and 14b and Hydrolytic Incorporation of Oxygen-18. A. 15a. A solution containing **9a** (2.78 g, 10 mmol), trimethyl orthoformate (1.2 g, 11.3 mmol), and *p*-toluenesulfonic acid (30 mg) in 50 mL of anhydrous methanol was stirred at room temperature for 5 h. Triethylamine (0.5 mL) was introduced, followed by water (25 mL). Following extraction with ether, the combined organic layers were washed with brine, dried, and evaporated to give **14a** as an orange oil (3.1 g, 96%), homogeneous by TLC.

This acetal (3.1 g, 9.6 mmol) dissolved in anhydrous THF (50 mL) was treated with H$_2^{18}$O (48% enriched, 209 μL, 11 mmol) and then with concentrated H$_2$SO$_4$ (2 drops). The reaction mixture was stirred at room temperature for 16 h, triethylamine (5 drops) was introduced, and the solvent was carefully removed under reduced pressure. The residual yellow solid was stirred in a 70:30 mixture of petroleum ether and ether, and the solution was decanted from the insoluble salts. Solvent evaporation gave **15a** as a yellow crystalline solid (2.52 g, 94%). ^{13}C NMR analysis showed the level of ^{18}O incorporation to be approximately 45%: ^{13}C=^{16}O, 195.40 ppm; ^{13}C=^{18}O, 195.37 ppm.

B. 15b. Treatment of **9b** (2.57 g, 10.9 mmol) in comparable fashion afforded the dimethyl acetal as an orange oil (2.96 g, 97%). Hydrolysis of this material in heavy water (48% ^{18}O) as described above resulted in the isolation of **15b** as an orange oil (1.99 g, 94%) into which 40% of the ^{18}O label had been incorporated: ^{13}C=^{16}O, 196.31 ppm; ^{13}C=^{18}O, 196.27 ppm.

Preparation of the Labeled Allylic Alcohols and 1,4-Diols. These reactions were performed in the previously described manner with essentially identical efficiencies. For **16a:** ^{13}C-^{16}O, 71.68 ppm; ^{13}O-^{18}O, 71.66 ppm. For **17a:** ^{13}C-^{16}O, 71.85 ppm; ^{13}O-^{18}O, 71.82 ppm. For **16b:** ^{13}C-^{16}O, 72.26 ppm; ^{13}O-^{18}O, 72.24 ppm. For **17b:** unresolved signals. For **18a:** unresolved signals. For **19a:** unresolved signals. For **18b:** ^{13}C-^{16}O, 73.54 ppm; ^{13}O-^{18}O, 73.52 ppm. For **19b:** ^{13}C-^{16}O, 73.95 ppm; ^{13}O-^{18}O, 73.92 ppm.

Acid-catalyzed cyclization of the diols was achieved as before. The relevant spectroscopic data for **22-25** are compiled in Table I.

Evidence for Intermediates and a Change in Rate-Limiting Step in the Aminolysis of the Carcinogen N-Methyl-N'-nitro-N-nitrosoguanidine by Cyclic Amines[1]

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Abstract: Rate constants and products are reported for the decomposition of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) stimulated by cyclic amines in aqueous solutions at 40 °C, ionic strength 1 M (KCl). Plots of k_{obs} against nucleophile are linear to a concentration of nucleophile up to 0.3 M. The slopes of the plots change as a function of pH. There is no evidence of significant buffer catalysis of the reactions in control experiments containing up to 0.5 M buffer. In the pH region from 4 to 8.5, the second-order rate constants, corrected for concentration of the acid form of MNNG and free base of the amine, increase with increasing pH and level off to a pH-independent reaction in the cases of imidazole, 3,5-dimethylpyrazole, and pyrazole. The downward break in the pH rate profiles and the absence of buffer catalysis require a change in the rate-limiting step involving two, presumably tetrahedral, intermediates that are in protonic equilibrium in the pH-dependent region. It is concluded that the rate-limiting step for the pH-independent reaction involves nucleophilic attack on MNNG while leaving group expulsion from an anionic intermediate, T$^-$, is rate-limiting for the pH-dependent region. This represents the first evidence for reaction intermediates in the nucleophile-stimulated decomposition of MNNG. The corrected second-order rate constants for the reactions of 1,2,3- and 1,2,4-triazoles are strictly pH-dependent over the same pH range. A comparison of the rate constants for the reaction of 1,2,4-triazole with those of pyrazole indicates that the reaction of triazoles involves a direct attack of triazole anion on MNNG with subsequent rate-limiting leaving group expulsion from T$^-$. These conclusions require that the N-nitrosomethylamine anion is a worse leaving group from the intermediate T$^-$ than the 1,2,4-triazole anion in spite of the fact that the triazole anion is estimated to be more than 3 orders of magnitude more basic. The unreactivity toward MNNG of certain amine nucleophiles, such as 4-(dimethylamino)pyridine, that are incapable of proton loss subsequent to nucleophilic attack further substantiates the conclusion that the N-nitrosomethylamine anion is an unexpectedly poor leaving group.
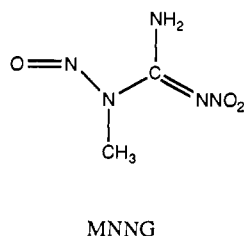
Introduction

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

MNNG

potential, as do a wide variety of structurally related compounds possessing the *N*-nitroso-*N*-alkyl functionality.[4] MNNG is also routinely used in the study of alkylating patterns of DNA.[5] An understanding of the detailed aqueous decomposition chemistry of MNNG is therefore of general importance.

We have recently determined that the solvolytic decomposition of MNNG at physiological pH involves the hydroxide ion-catalyzed elimination reaction of the anion of MNNG, yielding the nitrocyanamide ion and the alkanediazoate.[6] It was determined some time ago that nucleophiles other than hydroxide ion, for example, some amines,[7] thiol anions,[8] and (bi)phosphate ions,[8] stimulate the decomposition of MNNG. Little is known of the mechanisms of the nucleophile-stimulated reactions. In the case of amines, primary amines,[7a] dimethylamine,[7a] and some cyclic secondary amines[7b,c] react exclusively or predominantly at the guanidino carbon. Some larger acylcic secondary amines[7a] stimulate denitrosylation or are unreactive.[9]

The present investigation was initiated on the basis of results from our earlier investigation[6] which indicated that, in contrast to the unreactivity of *N*-methylimidazole, imidazole strongly stimulates the decomposition of MNNG under certain conditions of pH. We report here a detailed investigation of the reaction of some cyclic amines that provides the first evidence, in the form of a change in rate-limiting step, for intermediates in the reactions of MNNG. The results provide new insight into the nucleofugality of the *N*-nitrosomethylamine anion—which gives rise to the alkylating activity of MNNG and related compounds—and leads to the conclusion that the nitrosomethylamine anion is a surprisingly poor leaving group.

Experimental Section

Materials. Organic reagents were purified prior to use either by distillation or by recrystallization. Inorganic reagents were ACS grade or better.

Methods. Kinetics. Routine procedures and controls were the same as previously reported.[6]

Products. Product analysis was performed by high performance liquid chromatography with computer-assisted quantitation.[6] Separations were effected on a C-18 column (Column Resolution Inc., San Jose, CA) using an aqueous mobile phase (0.8 mL/min) containing 25% methanol and 0.05 M each of di- and monopotassium phosphate, monitored at 263 nm.

Synthesis. The synthesis of the reaction product of the 1,2,4-triazole reaction, 3 (TRNG), was reported previously.[6]

Imidazole Product 1. To 1 g of MNNG in ~10 mL of acetonitrile stirring in a 50-mL round-bottom flask was added 1 g of imidazole. A slow evolution of gas commenced after dissolution, and over the course
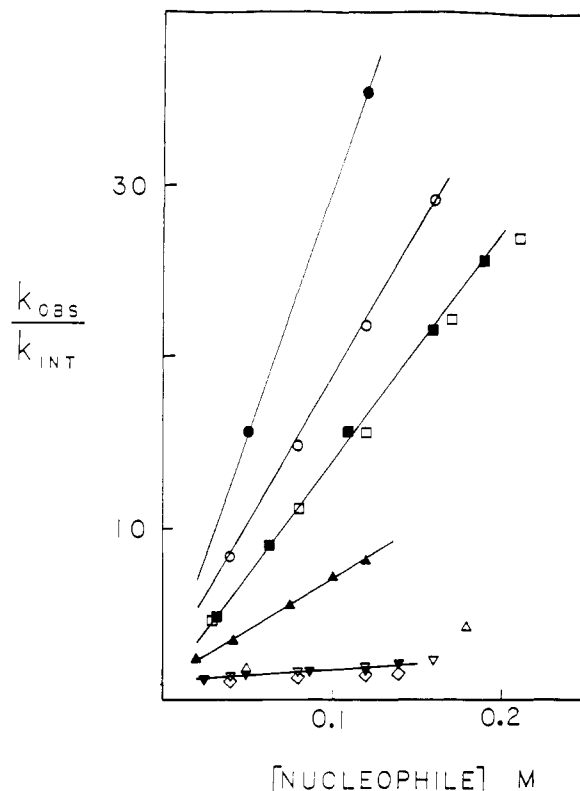
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Figure 1. Plot of k_{obs}/k_{int} against nucleophile concentration for the decomposition of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by imidazole, 3,5-dimethylpyrazole, and pyrazole at various values of pH, 40 °C, ionic strength 1 M (KCl). Imidazole: ▼, pH 8.48; ■, pH 7.85; ▲, pH 5.74. 3,5-Dimethylimidazole: ▽, pH 7.06; O, pH 5.80; ◇, pH 4.28. Pyrazole, △ pH 6.41; ●, pH 5.25; □, pH 4.06. Lines are drawn through points for the first, second, fifth, and eighth reactions above; others are omitted for clarity. Line for the reaction of pyrazole at pH 5.25 passes through points at 0.18 and 0.24 M nucleophile where the ordinate values are 52 and 71, respectively. In reactions where nucleophile was not buffering, biphosphate and acetic acid buffers were used at a concentration of 0.04 or 0.05 M (see Table S1).

of 18 h a white precipitate appeared. The solvent was removed by rotary evaporation, and the remaining solid was triturated with two 50-mL portions of ether, suspended for 30 min in 10 mL of H_2O, and then filtered and washed with H_2O. (Note! In subsequent syntheses of this material, in order to remove traces of unreacted carcinogen the solid isolated after removal of the solvent was treated for 5 min with 5 mL of water containing 0.3 g of NaOH, at which point the solid dissolved, followed by 0.7 mL of concentrated HCl, after which a precipitate reappeared. The solid was collected by filtration, washed with H_2O, and subsequently recrystallized in methanol.) ^{1}H NMR (DMSO-*d6*): (1 H, s) 7.12, (1 H, 2) 7.76, (1 H, s) 8.35, (2 H, br) 9.85 ppm. Anal. Calcd C, 30.97; H, 3.25; N, 45.15. Found for unrecrystallized material: C, 31.23; H, 3.24; H, 45.39.

Pyrazole Product 2. The product of the reaction of pyrazole with MNNG was synthesized by a procedure similar to that above for the imidazole product. An additional chromatography step (silica, ethyl acetate) preceded the final recrystallization in water. ^{1}H NMR (CDCl₃): (1 H, m) 6.52, (1 H, m) 7.82, (1 H, br) 8.1, (1 H, d) 8.36, (1 H, br) 9.15 ppm.

Results

First-order rate constants k_{obs} for the decomposition of MNNG in the presence of imidazole, 3,5-dimethylpyrazole, pyrazole, 1,2,3-triazole, or 1,2,4-triazole were determined in the pH region from 4 to 8. The first-order rate constants were found to increase linearly with amine concentration. For a given amine concentration, the increase in k_{obs} above the amine-independent rate constant, k_{int}, is dependent upon nucleophile type and pH. These observations are illustrated in Figure 1, in which the value of k_{obs}/k_{int} is plotted against nucleophile concentration for experiments at three different pH values for each of three of the amines studied. Inspection of the plot with attention to a particular

nucleophile indicates qualitatively that the effect of these nucleophiles is maximal at intermediate values of the pH range studied. Second-order rate constants, k_s, for the reactions of amines with MNNG were taken as the values of slopes of the plots of k_{obs} against total amine, typically based on four amine concentrations.

The products of the amine-stimulated decomposition were identified for the reaction of imidazole, pyrazole, and 1,2,4-triazole with MNNG as **1, 2,** and **3,** respectively (below). Identification

1, R = N⟍N—

NH₂ structure — C with NH_2, R, and $=NNO_2$ groups

2, R = N—N—

3, R = N—N—
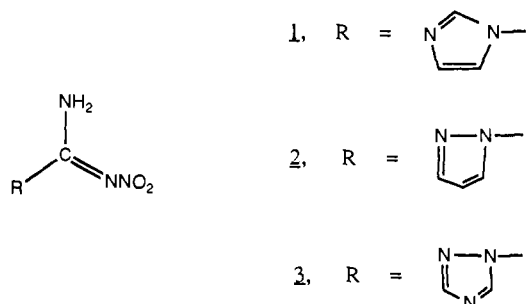
was based on coelution on high performance liquid chromatography with authentic standards. Quantitation after 7–10 half-lives of reaction indicates that the above products account for an average yield of 95% of the amine stimulated reaction (seven determinations).

The absence of a significant effect of buffer concentration upon the amine-stimulated decomposition of MNNG is evident in the absence of detectable upward curvature of plots of k_{obs} against amine concentration in nine of the experiments in which the amine itself was buffering (see, for example, Figure 1 solid squares, solid triangles, and diamonds). Additional experiments with acetate, pyridine, and N-methylimidazole buffers confirmed the absence of significant catalysis of the nucleophilic reaction of the imidazole, 1,2,4-triazole, and pyrazoles. The buffer concentration range tested varied from 0.05 to 0.5 M and from 0.05 to 0.21 M with an average change in k_{obs} of 10% and a maximum effect in one case of 18% (seven experiments).

The cyclic tertiary amines 4-(dimethylamino)pyridine, 4-aminopyridine, pyridine, 2-aminopyridine, 2-aminopyrimidine, 4-pyrimidone, and N-methylimidazole[6] failed to appreciably and consistently stimulate the decomposition of MNNG under conditions of pH and of nucleophile concentration comparable to those of the amines discussed above. In the case of the first five compounds, for seven experiments, increases in k_{obs} of less than 15% were observed, with increases in nucleophile concentration in most cases up to 0.3 M, with one exception being an increase of 20% at 0.3 M 2-aminopyridine. In the case of 4-aminopyrimidone, increases in k_{obs} of as much as 30% were observed at 0.3 M.

Discussion

The decomposition of the carcinogen MNNG is strongly stimulated by imidazole, pyrazoles, and triazoles between pH 4 and 8, and the effect is quantitatively accounted for by a nucleophilic reaction at the guanidino carbon. Such a reaction has been previously reported for primary and some secondary amines[7] and thiols.[8]

Imidazole and Pyrazoles. For these nucleophiles, the values of k_s, the slopes of plots of k_{obs} against total amine concentration, vary as a function pH. In part the pH dependence reflects the changing ionization state of both nucleophiles and the electrophile MNNG ($pK_a = 7.73$),[6] the active forms of which are presumably neutral. The specific second-order rate constant for reaction of neutral nucleophile with neutral MNNG, k_N, can be obtained from k_s according to eq 1, where K_{aM} and K_{aN} are the acidity constants for MNNG and the cationic acid forms of the nucleophiles, respectively.

$$k_N = k_s[(1 + (K_{aM}/[H^+]))/(K_{aN}/([H^+] + K_{aN}))] \quad (1)$$

It can be seen from inspection of Figure 2, the plot of log k_N against pH (solid points), that the value of k_N is itself in some
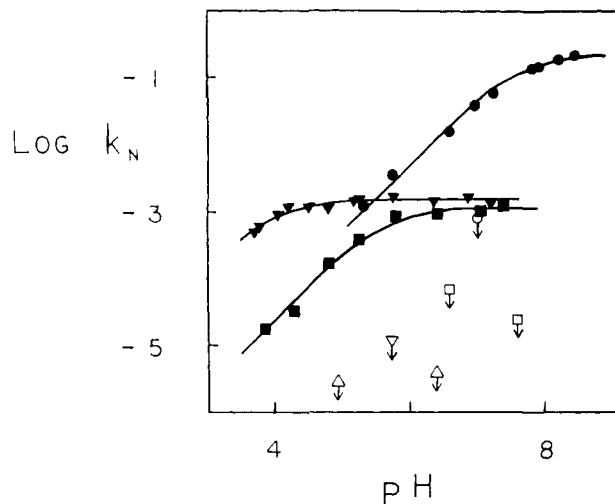
Figure 2. Plot of the log of k_N, the specific second-order rate constant for reaction of neutral MNNG and the free base form of the amine, against pH, 40 °C, ionic strength 1 M (KCl). Imidazole, ●; 3,5-dimethylpyrazole, ■; and pyrazole, ▼. All open symbols are upper limit values for 4-(dimethylamino)pyridine, O; 2-aminopyrimidine, ▽; N-methylimidazole, □; and pyridine, △.

cases pH-dependent, depending on the nucleophile and pH. The slope of the plot is equal to 1 in the cases of imidazole and 3,5-dimethylpyrazole at the lower end of the pH range studied, meaning that the rate constant is inversely proportional to hydrogen ion concentration, but the plots level off, becoming pH-independent, at higher values of pH. Over most of the range studied, the value of k_N for the reaction of pyrazole with MNNG is pH-independent, changing to a pH-dependent reaction at the lower extreme of pH studied.

The downward break in all three profiles in Figure 2 indicates that there is a change in rate-limiting step in the reactions of these nucleophiles with MNNG, and it rules out a single-step, concerted reaction. The pH-dependent change in rate-limiting step and the observed absence of significant buffer catalysis require that there are at least two intermediates in protonic equilibrium. The absence of buffer catalysis rules out the possibility of a pH-dependent mechanism with a single intermediate, because such a mechanism would require a rate-limiting formation or decomposition step involving proton transfer that would be subject to buffer catalysis.

The simplest mechanism consistent with the data is presented in eq 2. In the pH-dependent regions of Figure 2 the rate-limiting
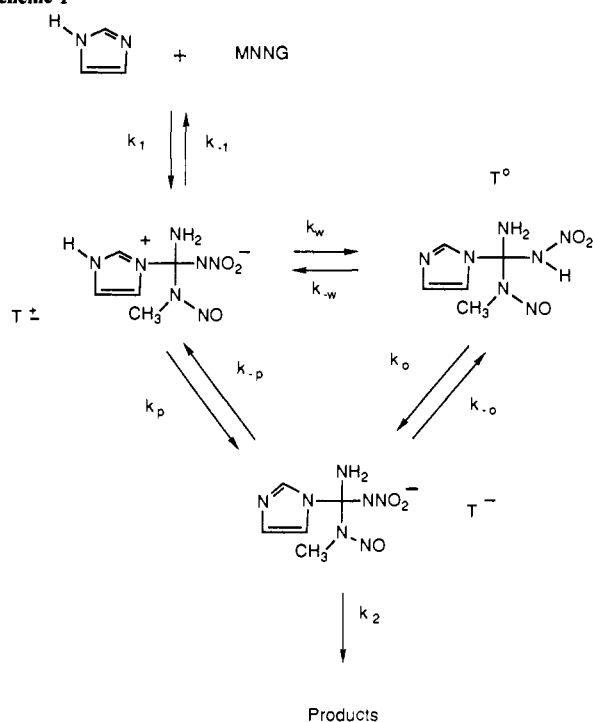
H-Nuc k_1 K_p

+   ⇌   I—H   ⇌   I⁻ + H⁺   (2)

    k_{-1}

MNNG   ↓ k_2

Products
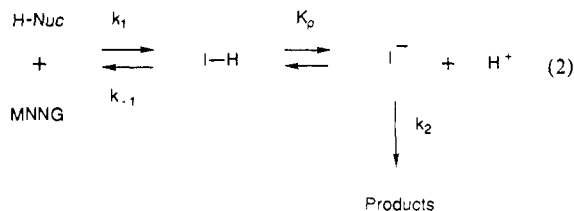
step is k_2, while k_1 is rate-limiting in the pH-independent regions of Figure 2. The rate law for k_N for such a mechanism is given in eq 3, and good agreement to the points in Figure 2 is obtained

$$k_N = k_1/[([H^+]/(K_pk_2/k_{-1})) + 1] \quad (3)$$

(solid lines in Figure 2) using values for the constants summarized in Table I.

On the basis of the observed products and in analogy to the chemistry observed in the substitution reactions at the carbonyl groups[10a] and nitro-activated olefins,[10b-d] the required intermediates are presumably tetrahedral. Intermediates consistent with the terms in eq 3 are illustrated in Scheme I for the nucleophile imidazole with T± and T⁻ analogous to I—H and I⁻, respectively, of eq 2. The rate constant k_2 is the only one consistent with being rate-limiting in the pH-dependent region. The possibility that the rate constants k_p or k_o are rate-limiting can be ruled out

Scheme I

Figure 3. Plot of the log of k_N, the specific second-order rate constant for reaction of neutral MNNG and the free base form of 1,2,3- and 1,2,4-triazoles against pH, 40 °C, ionic strength 1 M (KCl). Squares for 1,2,3-triazole and circles for 1,2,4-triazole.

Table I. Microscopic Rate and Equilibrium Constants for the Decomposition of N-Methyl-N'-nitro-N-nitrosoguanidine by Cyclic Amines at 40 °C, Ionic Strength 1 M (KCl)[a]

amine	pK_a^b (M)	k_1^c (M^{-1} s^{-1})	$K_p k_2/k_{-1}^{c,d}$ (M)	k_T (M^{-1} s^{-1})
imidazole	6.97	0.23	2.0 × 10^{-8}	
3,5-dimethylpyrazole	4.17	0.0012	1.9 × 10^{-6}	
pyrazole	2.65	0.0015	1.0 × 10^{-4}	
1,2,3-triazole	8.79			0.69[e]
1,2,4-triazole	9.59			2.95

[a] Standard errors are ±10% or better unless noted. [b] Measured potentiometrically under the experimental conditions. [c] Constant as expressed in eq 2 and Scheme I. [d] ±15%. [e] ±20%.

because, in all but one case, the proton-transfer steps are expected to be pH-independent, due to the low pK_a values of the intermediates.[11]

The rate constant k_1 represents the rate-limiting pH-independent reaction. The only other possibility, k_w, can be ruled out due to the absence of buffer catalysis that is expected if k_w is rate-limiting. Conservative values of 10^8 s^{-1}[21] and 10^9 M^{-1} s^{-1}[17,22]

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(11) The k_o step cannot represent the rate-limiting step in the pH-independent reaction. This is because the pK_a values of the intermediates T^0 are so low that the water-catalyzed proton abstraction from T^0 is dominant in the relevant region of pH. The pK_a of T^0 (Scheme I) is calculated to be less than 2.9 on the basis of the value of $\rho_1 = -4.27$ for ionization of substituted methyl nitramides calculated from the data of Serov et al.,[12] the pK_a of methylnitramide at 40 °C of 6.09,[12] and the sum of σ_1 values estimated for the substituents attached to the central carbon of T^0. The value of $\sigma_1 = 0.12$ for NH$_2$ is reported,[13] but upper limits for σ_1 for imidazole and N-nitrosomethylamine were taken as $\sigma_1 > 0.27$ and $\sigma_1 > 0.36$, respectively, on the basis of the value of $\sigma_1 = 0.27$ for imidazole (2-yl)[14] and $\sigma_1 = 0.36$ for N(CH$_3$)COCH$_3$,[13,15] respectively. The rate constant for water-catalyzed dissociation of T^0 can then be calculated to be >6.3 × 10^6 s^{-1}, more than 10^3 larger than the first-order rate constant for proton transfer to hydroxide ion of 1000 s^{-1} at pH 6, on the basis of rate constants for thermodynamically favorable proton transfers of 5 × 10^9 M^{-1} s^{-1}[17] and the ion product of water of 10$^{13.29}$ at 40 °C, 1 M ionic strength (KCl).[18] Thus, hydroxide ion-catalyzed proton abstraction from T^0 is expected to be insignificant relative to the water-catalyzed reaction at pH values less than 6. The same analysis applies to the reactions of the other nucleophiles because of the lower pH at which the reactions are pH-dependent and the lower pK_a values of the intermediates. The pK_a values will be less than the value calculated for the imidazole adduct due to the greater electron-withdrawing effect expected for the pyrazoles compared to imidazole. A similar analysis for the intermediates T$^\pm$ in the reactions of the pyrazole and 3,5-dimethylpyrazole indicates that k_p cannot represent a pH-dependent rate-limiting step for these reactions. The pK_a values in these cases are expected to be somewhat less than those of the protonated heterocycles themselves,[19] indicating pK_a values of about 3 or less. In the case of T$^\pm$ for the reaction of imidazole, the pK_a is uncertain enough that a hydroxide ion-catalyzed reaction might be dominant—consistent with the observed pH dependence—but such a reaction would also exhibit buffer catalysis, and the fact that such catalysis is thus inconsistent with k_p being the rate-limiting step in the pH-dependent reaction of imidazole.

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(15) The σ_1 value for the N-nitrosomethylamino group is likely to be larger than that of an N-methylacetamido group ($\sigma_1 = 0.28$)[13] on the basis of the significantly weaker basicity of dimethylnitrosamine compared to the corresponding carboxamide that indicates significantly more delocalization into the nitroso group when compared with the carbonyl group.[16]
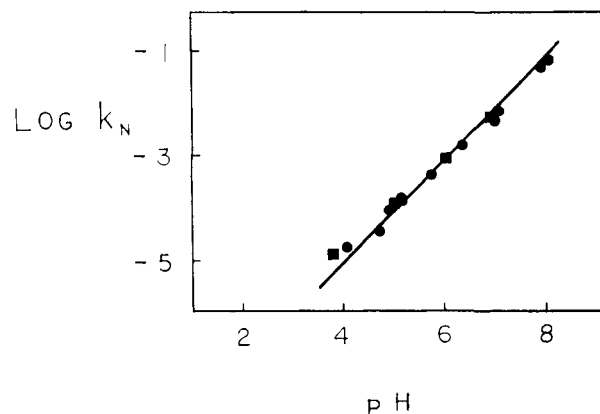
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(19) This conclusion is made on the basis of what is known about the acidity of similar intermediates formed in ester aminolysis[10a] and in the reactions of amines with nitro-activated olefins.[10b-d] In the aminolysis of phenyl acetate, the pK_a of the tetrahedral intermediate T$^\pm$ is not significantly different from that of the ammonium ion due to the counterbalancing acid-strengthening effect of the phenoxy group ($\sigma_1 = 0.38$)[13] and the acid-weakening electrostatic effect of the oxyanion. In the present case the acid strengthening effects of the amino and nitrosamino group are at least as large as the phenoxy group.[20] In contrast, the counterbalancing electrostatic interaction must be much weaker in the T$^\pm$ of Scheme 1 due to the fact that the negative charge is delocalized in the nitro group while the positive charge is delocalized in the heterocycle. The pK_a values of the intermediates T$^\pm$ formed from the reaction of piperidine or morpholine with nitrostilbene in 50% DMSO/50% water are 1.3 and 1.5 units less, respectively, than those of the protonated amines.[10b] The groups attached to the central carbon in the intermediates T$^\pm$ are, in sum, likely to be more electron-withdrawing in the reactions with MNNG compared to nitrostilbene. This is concluded on the basis of a comparison of the relative electron-withdrawing abilities of the different functionalities that comprise T$^\pm$ in the reaction of MNNG compared to those that comprise T$^\pm$ in the nitrostilbene reaction. The largest difference is in the electron-withdrawing ability of the N-nitrosoamino group (MNNG reaction, $\sigma_1 > 0.28$)[15] compared to that of hydrogen (nitrostilbene reaction, $\sigma_1 = 0$). The other functionalities are likely to be roughly equivalent. The acidity of the intermediate T$^\pm$ in the nitrostilbene reaction may be relatively diminished due to intramolecular hydrogen bonding based on the lower pK_a values observed for the T$^\pm$ adducts formed in the reactions of the same amines with nitrostyrene.[10c] In the case of the reactions of piperidine or morpholine with β-methoxy-α-nitrostilbene in 50% DMSO/50% water, the pK_a values of the intermediates T$^\pm$ are 3.3 and 5.4 units less, respectively, than those of the protonated amines.[10d]

(20) The limit $\sigma_1 > 0.28$ for the nitrosomethylamino group[15] combined additively with the electron-withdrawing effect of the amino group ($\sigma_1 = 0.1$)[13] suggests an acidifying effect at least equal to that of the phenoxy group ($\sigma_1 = 0.38$).[13]

(21) Grunwald, E.; Chang, K. C.; Skipper, P. L.; Anderson, V. K. *J. Phys. Chem.* **1976**, *80*, 1425. In the present case a substantially smaller value is expected given the extremely unfavorable geometry and entropy of this process.

for the uncatalyzed and buffer-catalyzed k_w reactions, respectively, predict 2–3-fold increases in the amine-stimulated decomposition reactions when buffer concentration is varied up to 0.5 M. In contrast, the increases in buffer concentration failed to increase the rate constants for aminolysis by more than 20% (see Results).
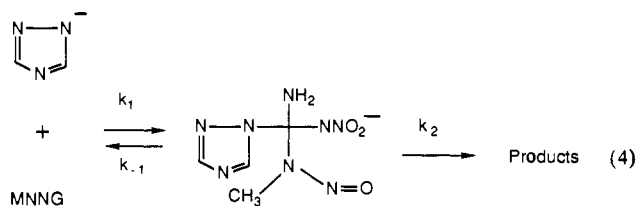
Triazoles. The second-order rate constants k_s were corrected, as above, for the neutral forms of MNNG and 1,2,3- and 1,2,4-triazoles to obtain values of k_N for these nucleophiles. The pH-rate profiles for these reactions are presented in Figure 3, and the slope of 1 of the solid line indicates that the rate constants are inversely proportional to hydrogen ion concentration. This is in contrast to the behavior that might have been expected on the basis of the behavior of the rate constants for pyrazole, Figure 2, which is of similar or slightly greater basicity (conjugate acid pK_a = 1.1, 2.40, and 2.65 for 1,2,3-triazole, 1,2,4-triazole, and pyrazole, respectively) and for which the values of k_N are pH-independent over most of the same range of pH.

The direct reversible formation of T^- from triazole anion and MNNG accounts for the pH dependence of the triazole reaction. This reaction is favored in the case of triazoles because they are appreciably more acidic (pK_a = 8.79 and 9.58 for 1,2,3-triazole and 1,2,4-triazole, respectively) than pyrazole or imidazole (pK_as $\approx$ 14).[23] The reaction pathway may be deduced by inspection of Figures 2 and 3 which show that the second-order rate constant for reaction of pyrazole and 1,2,4-triazole are equal at pH $\approx$ 6.3. If T^- formation from 1,2,4-triazole anion were irreversible, the fastest route to T^- at pH < 6.3 would become the pH-independent formation of $T^\pm$, with rate constants of magnitude comparable to that observed for the reaction of pyrazole, which is of comparable basicity. The fact that such a reaction is not observed is due to the much weaker basicity of the triazole anion compared to the pyrazole anion, so that the former is expelled more readily from T^- than the nitrosomethylamine anion, while the latter is not. The direct formation of T^- from triazole anion is required by the larger rate constants for reaction of triazole compared to pyrazole at pH > 6.3. If the intermediacy of $T^\pm$ were required, its pH-independent formation, with rate constants comparable to those observed for pyrazole, would become rate-limiting.

Rate constants k_T for the reactions of the triazole anions with MNNG, derived from the data in Figure 3 and the pK_a values of the neutral triazoles, are presented in Table I.

The Leaving Group. The N-nitrosomethylamine anion, its more stable resonance form being the alkanediazoate, utimately decomposes to generate the alkylating species that is responsible for the carcinogenic activity of MNNG. It and a number of related N-nitrosoalkylamine anions are leaving groups common to a large variety of N-alkyl-N-nitroso compounds that are potent carcinogenic and/or cancer chemotherapeutic agents. In spite of their importance, little of a quantitative nature is known of the chemistry of these species.[24] The present work allows some semiquantitative assessment of the N-nitrosomethylamine anion as a leaving group.[25]

The conclusion above that T^- decomposition is rate-limiting in the reaction of triazole anions with MNNG means that the nitrosomethylamine anion is a worse leaving group from T^- than the substantially more basic triazole anion ($k_{-1} > k_2$, eq 4). The nitrosomethylamine anion is a poorer leaving group in spite of the deduction below that it is a weaker base than the triazole anion by more than 3 pK_a units. Due to its instability, the pK_a of N-nitrosomethylamine is unknown. It is likely to be somewhat more acidic than methylnitramide, the measured pK_a for which

(22) The conclusion that the proton transfer in such cases is thermodynamically favorable is likely to be correct on the basis of what is known of the acidity of similar intermediates encountered in ester aminolysis[10a] and in the reactions of amines with nitro-activated olefins.[10b–d,19]

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(24) Moss, R. A. *Acc. Chem. Res.* **1974**, *7*, 421 and references therein. Hovinen, J.; Fishbein, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 366. Hovinen, J.; Finneman, J. l.; Satapathy, S. N.; Ho, J.; Fishbein, J. C. *J. Am. Chem. Soc.*, in press.

(25) As described in ref 24 (Moss), the isomeric E and Z forms have different stabilities and physical properties, and whether the leaving group departs in one or the other or both forms is unknown.

(4)

is 6.15 (25 °C, μ = 0.04),[12] on the basis of the slightly larger value of σ^- for the nitroso group (σ^- = 1.46) than for the nitro group (σ^- = 1.27).[13] The pK_a for triazole is 9.58 (Table I).

The poor leaving ability of the N-nitrosomethylamine anion is manifest in the unreactivity of relatively good nucleophiles that are incapable of forming the intermediate T^- via proton loss. Figure 2 contains upper limit values of k_N (open symbols) determined for nucleophiles including pyridine (triangles), N-methylimidazole (squares), and 4-(dimethylamino)pyridine (circles). The inactivity of these nucleophiles is due to the fact that partitioning of the intermediate $T^\pm$ lies far in favor of the starting materials, $k_{-1} \gg k_x$, eq 5. Lower limits for k_{-1}/k_x (eq

(5)
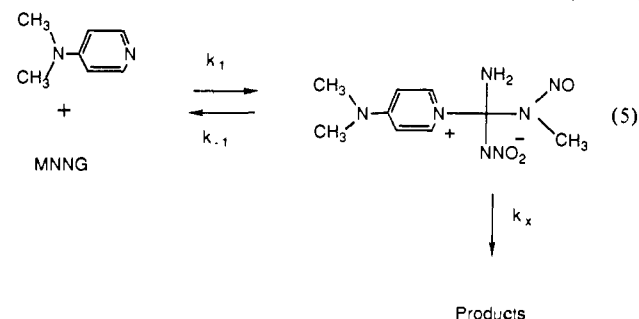
5) of 4700, 1 × 10⁴, and 6 × 10⁴ for 4-(dimethylamino)pyridine, N-methylimidazole, and pyridine, respectively, can be calculated from the upper limits for k_N, calculated from the observed upper limits of k_s (see Results), lower limits for k_1, and the steady-state expression for the mechanism of eq 5. The k_1 limits were estimated as follows. The rate constant k_1 (eq 5) for the reaction of N-methylimidazole is expected to be about the same as k_1 for imidazole, the experimentally measured rate constant for the pH-independent reaction of imidazole (Figure 2). Lower limits for the value of k_1 for the other nucleophiles were determined from a plot of log k_1 against conjugate acid pK_a for imidazole and pyrazole by interpolation, for pyridine (pK_a = 5.1), and by slight extrapolation for 4-(dimethylamino)pyridine (pK_a = 9.4). The latter two values represent lower limits because pyridine is known to be a stronger nucleophile than imidazole in acyl group transfer when the rate-limiting step is nucleophilic attack.[26]

Related Systems. Investigations of nucleophilic substitution reactions at N-nitrosoamides lend qualitative support to the characterization of the N-nitrosomethylamine anion as a surprisingly poor leaving group. The rate law for the alkaline hydrolysis of N-nitroso-2-imidazolidone (C, below) contains both

C   D

a term that is second-order in hydroxide ion and one that is both first-order in hydroxide ion and first-order in buffer base.[27] The observation requires that hydroxide ion expulsion from the anionic intermediate is rapid relative to the departure of the N-nitrosoalkylamine anion leaving group, assuming that a hydroxide ion-

(26) Palling, D. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 4869 and references therein. Page, M. l.; Jencks, W. P. *J. Am. Chem. Soc.* **1972**, *94*, 3263. Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436.

(27) Castro, A.; Leis, J. R.; Pena, M. E.; Tato, J. V. *J. Chem. Soc., Perkin Trans. 2* **1986**, 117.

catalyzed intramolecular rearrangement mechanism involving attack on the nitroso group can be ruled out.

The reaction of imidazole with N-nitroso-2-pyrrolidone (D) is kinetically second-order in imidazole concentration at low concentrations but first-order in imidazole at high concentrations of imidazole.[28] The change in order in imidazole requires a change in rate-limiting step and rules out a reaction with a single intermediate, the formation of which is buffer-catalyzed. The imidazole buffer-independent reaction implicates T$^\pm$ as the first-formed intermediate in the reaction (see Discussion, Imidazole and Pyrazoles, and Scheme I). The fact that a subsequent step is rate-limiting at low imidazole concentrations requires that T$^\pm$ revert to starting materials faster than the N-nitrosoalkylamine anion leaving group can be expelled. The poorer leaving ability of the N-nitrosoalkylamine anion compared to imidazole is qualitatively in accord with the similar conclusion from the present work.

The claim that the reactions of imidazole, N-methylimidazole, and pyridine with N-methyl-N-nitroamides involve rate-limiting nucleophilic attack[29] contrasts substantially with conclusions in the present work, assuming rough equivalence of the N-nitrosomethylamine and N-nitromethylamine anions. The data for the reaction of N-nitro-N-methylacetamide do not rule out the possibility of a stepwise reaction with rate-limiting decomposition of an intermediate such as T$^\pm$, leaving aside the possibility of a concerted reaction. The value of β_{nuc} = 0.71, based on the rate constants for pyridine, N-methylimidazole, imidazole, morpholine, and piperidine,[29] is not dissimilar from the values of ~0.8 obtained for reactions of phenylacetates with primary and secondary amines, for which decomposition of the intermediates T$^\pm$ is in fact rate-limiting.[30] The conclusion that the rate-limiting step for nitroamines involves nucleophilic attack is based in part on a plot, of approximate slope of 1, of log k for reaction of nucleophiles with (4-nitrophenyl)acetate against log k for reactions of N-nitro-N-methylacetamide. However, the interpretation of this plot is obscured by the fact that the plot contains a spectrum of nucleophiles for which the attack step (hydroxide and 4-chlorophenolate ions), the decomposition step (acetate ion, pyridine, morpholine), or a mixture (piperidine and imidazole) are rate-limiting in the reaction with (4-nitrophenyl)acetate.[30] Indeed, the larger rate constant for imidazole compared to pyridine in the reaction with N-nitro-N-methylacetamide is more consistent with a mechanism involving rate-limiting decomposition of T$^\pm$.[26]

The comparable reactivity of imidazole, pyridine, and N-methylimidazole with N-methyl-N-nitroamides also contrasts with the present reaction. The observation indicates that expulsion of the N-nitromethylamine anion leaving group from T$^\pm$ must be faster than proton loss and subsequent leaving group expulsion in the case of the reaction of imidazole. An explanation may lie in the fact that the highly localized charge on the oxyanion in T$^\pm$ formed from the reaction of N-nitroacetamide, compared to the delocalized negative charge on the nitro group of T$^\pm$ involved in the reactions of MNNG, will both increase the basicity and suppress the nucleofugality, relative to the T$^\pm$ from MNNG, of the ammonium ions due to a stronger electrostatic interaction. Such interactions have been calculated to retard the leaving ability of an alkylamine by as much as a factor of 10^3.[31] Both factors work in the direction of making the decomposition of T$^\pm$, rather than T$^-$, the rate-limiting step, as it is in the uncatalyzed aminolysis of phenylacetate and (4-nitrophenyl)acetate by most primary and secondary amines.[30]

Biological Significance. Recently, considerable evidence has been summarized which implies that the decomposition of some N-nitroso alkylating agents may occur by direct interaction with DNA, thereby explaining the site and sequence selectivity observed in the alkylation reactions of certain nitrosoureas.[32] Specific proposals formulated to rationalize the observations have been criticized, but the origins of site and sequence selectivity remain uncertain.[5,33] The results summarized in the present work illustrate some important considerations in thinking about site selectivity of molecules like MNNG that could alkylate DNA subsequent to direct nucleophilic attack by the DNA itself. This work illustrates that factors that govern which of the several nucleophilic sites on DNA in fact reacts with molecules like MNNG include not only the inherent nucleophilicity of the site, as measured by proton basicity or reactivity with S$_N$2 reagents such as alkyl halides, but also the partitioning of intermediates encountered on the reaction pathway.

For example, the unmeasurable nucleophilic activity toward MNNG of relatively good to strong nucleophiles, such as N-methylimidazole, pyridine, 4-aminopyridine, and 4-(dimethylamino)pyridine that cannot lose a proton subsequent to nucleophilic attack, compared to the strong activity of nucleophiles of equal or substantially lesser basicity, such as imidazole and pyrazoles, suggests that the most basic sites in DNA—N^7 of guanine and adenine—may not be the most active nucleophiles in reactions with MNNG.

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