Thiolytic Decomposition of the Carcinogen *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine. A Change in Rate-Limiting Step with Nucleophile Basicity Controls Alkylating Activity[1]

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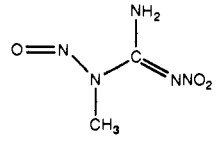
Abstract: The kinetics of the reaction of seven alkanethiolates with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine over the pH range 3–8.5 at 40 °C, ionic strength 1 M (KCl), are reported. Plots of k_{obs} against total thiol concentration are linear, and the slopes of these plots change as a function of pH. The changes in slope with pH are well-described by a rate law for decomposition of MNNG that is first-order in thiolate ion and first-order in neutral MNNG. Rate constants k_2' for the reaction of the thiolates are determined. There is no significant buffer catalysis of the reactions of any of the thiolates in the pH range studied. In the case of the reactions of propanethiolate and trifluoroethanethiolate, two products, methylnitroguanidine (MNG) and the thiol ((*RS*)-*N*-nitroformamidine) adducts **1**, were found to account quantitatively (98 ± 3%) for the nitroguanidine skeleton of the starting material. In the case of the other five thiolates, the percent yield of MNG was determined. The yields of MNG are independent of thiolate ion concentration or buffer concentration. The yield of MNG changes from 5% for the reaction of propanethiolate, the most basic thiolate, to 90% for the reaction of pentafluoropropanethiolate, the least basic thiolate. On the basis of the yields of MNG, which indicate the extent of reaction at the nitroso nitrogen for the different thiolates, specific second-order rate constants for the thiolate ion reaction at the nitroso nitrogen, k_{DN}, and for the thiolate ion reaction at the guanidino carbon, k_{DA}, are calculated from the total second-order rate constant, k_2'. The plot of log k_{DN} against pK_{aRSH} is linear with a slope $\beta_{nuc} = 0.54 \pm 0.02$. A similar plot for log k_{DA} shows a downward break with decreasing thiol pK_a. The plot is consistent with a reaction that involves an anionic intermediate, T^-, the formation of which is rate-limiting for basic thiolates and the decomposition of which is rate-limiting for weakly basic thiolates. Limiting values of β_{nuc} consistent with the data were determined to be $\beta_{nuc} = 0.70 \pm 0.12$ and 2.4 ± 0.2 for rate-limiting formation and breakdown reactions, respectively. The latter value is attributed to a late transition state for leaving group expulsion with a large imbalance in which C–N double bond formation lags behind leaving group expulsion. The results represent a good chemical model for the recently reported chemoprotective denitrosation reaction between glutathione and MNNG that is catalyzed by a glutathione *S*-transferase.

Introduction

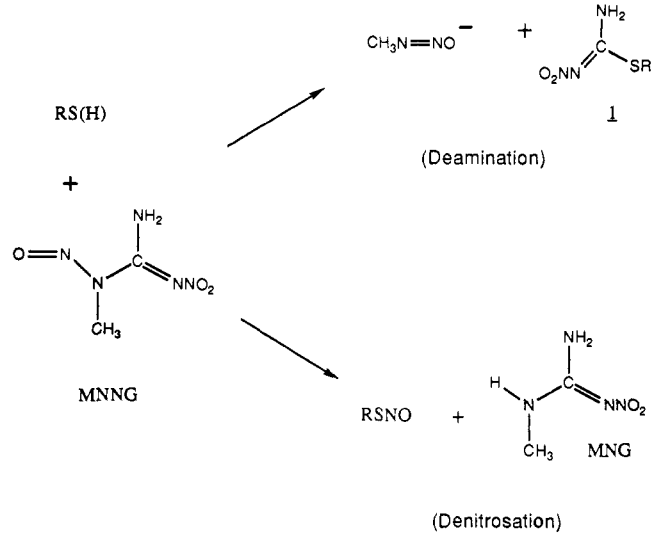
The compound *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) is a powerful direct acting carcinogen, the biological activity of which is believed to be the result of formation, in the course of its decomposition, of an electrophilic methyl group that reacts with DNA.[2,3] MNNG has demonstrated a cancer chemotherapeutic potential, as do a wide variety of structurally related compounds posessing the *N*-nitroso-*N*-alkyl functionality.[4] MNNG is also routinely used in the study of alkylating patterns of DNA.[5]



MNNG

The thiolytic decomposition chemistry of MNNG is of special interest because it was shown some time ago by Lawley and Thatcher that thiols strongly stimulate the decomposition of MNNG and further that there is, in certain cases, a correlation between higher cellular thiol concentration and more extensive alkylation of DNA in vitro.[6] It was found that thiols react both at the guanidino carbon and at the nitroso nitrogen of MNNG, and this observation has been repeated by others.[7] The partitioning of thiol reactivity between the two electrophilic sites in MNNG is particularly important because, as illustrated in Scheme I, reaction at the guanidino carbon (deamination) liberates the alkanediazoate that ultimately alkylates DNA, whereas reaction at the nitroso nitrogen (denitrosation) removes the nitroso group

Scheme I



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(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, Vol. 1*; Searle, C. E., Ed.; ACS Monograph Series 182; American Chemical Society: Washington, DC, 1984.

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

(5) See, for example: Wurdeman, R. L.; Church, K. M.; Gold, B. *J. Am. Chem. Soc.* **1989**, *111*, 6408.

(6) Lawley, P. D.; Thatcher, C. J. *Biochem. J.* **1970**, *116*, 693 and ref 7d.

(7) (a) Schulz, U.; McCalla, D. R. *Can. J. Chem.* **1969**, *47*, 2021. (b) Jensen, D. E.; Stelman, G. J. *Carcinogenesis* **1987**, *8*, 1791. (c) Jensen, D. E.; Gombar, C. T. *Banbury Rep.* **1982**, 379.

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and deactivates the alkylating activity of MNNG. Data from the literature on previous studies of the reactions of thiols with MNNG indicate a broad range between "negligible" and 68%[6-8] for the extent of denitrosation. The range of reaction conditions and span of percentages provide little evidence concerning what factors control the partitioning in the reaction of MNNG with thiols.

Understanding what factors of structure and reaction conditions control reactivity of these types of functionalities is presently of great practical significance. In part, tumor cell resistance to the cancer chemotherapeutic *N*-nitroso compound 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) is thought to be due to induction of a class of glutathione *S*-transferases that catalyzes the denitrosation, and thus inactivation, of BCNU by the biological thiol glutathione.[9] Another glutathione *S*-transferase has recently been isolated that affords chemoprotection against the alkylating activity of MNNG by selectively catalyzing the denitrosation by glutathione.[10]

In this report we summarize the results of a systematic investigation of the alkanethiolate–stimulated decomposition of MNNG in aqueous solutions. It is concluded that the deamination reaction occurs via an intermediate, T^-, analogous to that observed in the reactions of MNNG with some cyclic amines[11] and that there is a change in rate-limiting step with thiol anion basicity. This change in rate-limiting step, in parallel with a concurrent denitrosation reaction that does not undergo a similar change in rate-limiting step, is manifest in a change from predominant deamination for the reaction of strongly basic thiolate anions to predominant denitrosation for the reaction of less basic thiolate ions.

Experimental Section

Materials. Organic materials were recrystallized or distilled from a good commercial grade and, in the case of thiols, stored under nitrogen. Fluorinated thiols were prepared as previously reported and stored under argon at 0 °C.[12] Inorganic materials were ACS grade reagents and were used as purchased.

Methods. Kinetics. Most of the kinetic runs were carried out as previously described.[11] The reaction was initiated by an injection of MNNG-containing solution into a temperature-equilibrated cuvette containing the reaction solution with at least a 10-fold molar excess of thiol over MNNG. The decay of absorbance of MNNG at 400 nm was monitored spectrophotometrically. In the case of the reactions of pentafluoropropanethiol, extensive denitrosylation gave a product, presumably RSNO,[13] that absorbed in the same spectral region and resulted in a small overall change in absorbance. Some runs were therefore followed by HPLC, monitoring the disappearance of the MNNG peak at 275 nm after separation from the reaction mix by means of a C-18 column and a mobile phase containing 0.05 M each of KH_2PO_4 and K_2HPO_4 in 50% methanol/water. The reaction solution was maintained at 40 °C, checked by means of a flexible thermocouple, in a Waters Model 715 Autoinjector with an attached heater/cooler.

Thiol concentration and stability were checked by Ellman's assay using dithionitrobenzoic acid.[14] Stability was checked by using identical "dummy" reaction solutions containing no MNNG. The thiol concentration was assayed at time 0 in one solution and after 5–6 half-lives of the MNNG-containing reaction. With mercaptoacetic acid, mercaptoethanesulfonic acid, cysteamine, and methyl thioglycolate the thiols decomposed less than 5%, and for reaction tubes and thiol stock solutions, the concentration of thiol determined by weight was in good agreement with that determined by Ellman's assay. In the cases of propanethiol and the fluorinated thiols, oxidation was found to be extensive in the absence of additional precautions including purging of stock solutions with argon (propanethiol) or making up solutions in an argon atmosphere (trifluoroethanethiol). Thiol concentration changed over 5–6 half-lives due to oxidation generally by less than 10%, with a maximum of 13% in one run with trifluoroethanethiol. Good first-order kinetics were observed for more than 3 half-lives of reaction.

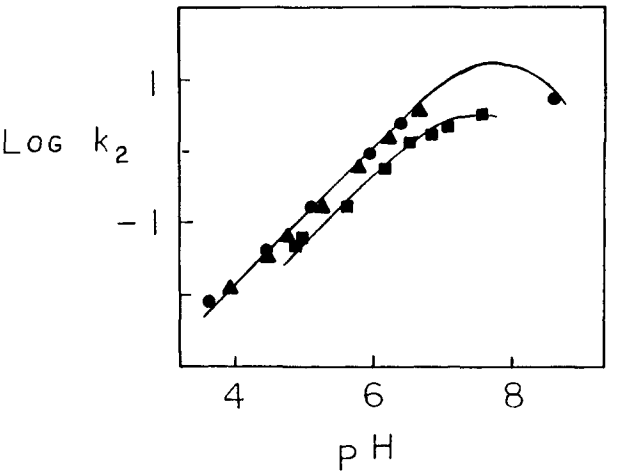

Figure 1. Plot of log k_2 against pH for the reaction of methyl thioglycolate (●), trifluoroethanethiol (■), and mercaptoethanesulfonic acid anion (▲) with MNNG at 40 °C, ionic strength 1 M (KCl). Solid lines are calculated for methyl thioglycolate and trifluoroethanethiol using the values of k_2' and pK_{aRSH} in Table I according to eq 1; line for mercaptoethanesulfonic acid anion omitted for clarity.

Product Analysis. Reactions of all thiols with MNNG were assayed for the denitrosation product methylnitroguanidine (MNG). The product was identified by spiking the solution with authentic material and was quantitated after 10 half-lives of reaction of HPLC using the system described above with an aqueous mobile phase containing 0.1 M each of KH_2PO_4 and K_2HPO_4 and 0.2 M KCl, monitoring at 265 nm. Yields of MNG were checked at 20 half-lives and were within 5% of those determined at 10 half-lives in the case of all thiols studied, attesting to the stability of MNG under the reaction conditions.

The deamination products for the reaction of propanethiol and trifluoroethanethiol were quantitated after identification by spiking the solution with authentic materials independently synthesized. The analysis was carried out at after 4 half-lives of reaction in the case of the trifluoroethanethiol product. This was necessary because a comparison of deamination product yields at 10 and 20 half-lives showed that a 10% decrease occurred between the 10th and 20th half-life. Analysis was carried out after 10 half-lives in the case of the propanethiol reaction. The aqueous elution buffers contained 0.05 M each of KH_2PO_4 and K_2HPO_4 and were 50% methanol in the case of the trifluoroethanethiol reaction or 20% methanol in the case of the propanethiol reaction. Eluants were monitored at 280 and 285 nm, respectively.

Product Synthesis. Propanethiol Adduct. A solution of 2 g of MNNG in 20 mL of acetonitrile was added dropwise to 50 mL of a stirred solution of 2.0 M 1-propanethiol and 0.8 M phosphate buffer (60% dianion, 40% monoanion). A mild evolution of gas commenced immediately with the subsequent appearance of a pink color. After 1 h the solution was extracted with ether, the ether phase was dried with magnesium sulfate and filtered, and the ether was removed by rotary evaporation. White crystals formed in the remaining pink oil. The colorless solid was recrystallized twice in chloroform/hexane (9/1). ^1H NMR ($CDCl_3$): (3 H, t) 1.10, (2 H, m) 1.73, (2 H, t) 3.10 ppm. Anal. Found: C, 29.59; H, 5.41; N, 25.92. Calcd: C, 29.43; H, 5.57; N, 25.75.

Trifluoroethanethiol Product. The reaction was carried out as above with the following additional steps. After evaporation of the ether, the yellow slurry was washed with hexane, and the hexane was decanted off and discarded. After recrystallization in chloroform/hexane (9/1), contaminating MNG was removed by chromatography on silica gel with ethyl acetate/hexane eluant (30/70). The final product was recrystallized in chloroform/pentane. ^1H NMR ($CDCl_3$): (2 H, q) 3.9 ppm. Anal. Found: C, 17.81; H, 1.98; N, 20.63. Calcd: C, 17.73; H, 1.99; N, 20.69.

Results

First-order rate constants k_{obs} for the decomposition of MNNG in the presence of seven thiols were measured at 40 °C, ionic strength 1 M (KCl), in the pH range from 3 to 8.5. For a given thiol the typical pH range was 4–7. Plots of k_{obs} versus thiol concentration were linear and spanned a thiol concentration range of 0.005–0.1 M, most typically from 0.01 to 0.05 M, and contained

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(9) Smith, M. T.; Evans, C. G.; Doane-Setzer, P.; Castro, V. M.; Tahir, M. K.; Mannervik, R. L. *Cancer Res.* **1989**, *49*, 2621. For a review, see: Waxman, D. J. *Cancer Res.* **1990**, *50*, 6449.
(10) Jensen, D. E.; Mackay, R. L. *Cancer Res.* **1990**, *50*, 1440. Reference 7b.
(11) Wichems, D. N.; Nag, S.; Mills, J.; Fishbein, J. C. *J. Am. Chem. Soc.*, preceding paper in this issue.
(12) Gregory, M. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1967**, *89*, 2121.
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(14) Ellman, G. L. *Arch. Biochem. Biophys.* **1958**, *74*, 443.

Table I. Summary of Product Yields, Specific Second-Order Rate Constants, and Partial Second-Order Rate Constants for Reactions of Thiolate Ions with *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine, 40 °C, Ionic Strength 1 M (KCl)

thiol	pK_a[a]	%DN[b] (trials)	%DA[c] (trials)	k'_2,[d] M^{-1} s^{-1}	k_{DN},[e] M^{-1} s^{-1}	k_{DA},[f] M^{-1} s^{-1}
1-propanethiol	10.08	5.9 (2)	97 (2)	4400	260	4140
mercaptoacetic acid	9.87	4.6 (8)		3800	175	364
mercaptoethanesulfonic acid	8.91	4.7 (16)		930	44	886
cysteamine	8.17	12 (8)		132	15.6	116.4
methyl thioglycolate	7.74	23 (16)		69	15.7	53.3
trifluoroethanethiol	7.24	75 (12)	20 (7)	9.1	6.8	2.3
pentafluoropropanethiol	7.0[g]	89 (3)		4.9	4.4	0.54

[a] Measured pK_a (unless noted). [b] Percent denitrosation based on measured yield of methylnitroguanidine. [c] Percent deamination based on measured yield of (*RS*)-*N*-nitroformamidine adduct. [d] Total specific second-order rate constant for reaction of thiolate ion with MNNG. [e] Calculated partial rate constant for denitrosation (see text). [f] Calculated partial rate constant for deamination (see text). [g] Calculated from the measured pK_a of trifluoroethanethiol based on ΔpK_a between trifluoroethanethiol and pentafluoropropanethiol reported in ref 12.

five thiol concentrations. In accord with earlier workers,[7,8] it was found that all of the thiols studied strongly stimulate the decomposition of MNNG; values of k_{obs} at the thiol concentrations in this study were typically tens to thousands of times larger than the solvolytic rate constants measured in the same pH range.[15] The slopes of plots of k_{obs} against thiol concentration yielded the second-order rate constants, k_2, the values of which were found to be pH-dependent, as observed by others.[8] The pH dependence is illustrated in Figure 1 for three of the thiols studied.

No significant catalysis by buffers of the reaction of thiols with MNNG was detected. Ten experiments with buffers including formate, acetate, and phosphate dianion, ranging in concentration from 0.05 to 0.35 M (five concentrations per experiment), gave an average increase in k_{obs} of 9% for reactions of mercaptoacetic acid (one experiment, pH 4.45), 2-mercaptoethanethiol (three experiments, pH 2.35, 5.33, and 6.14), cysteamine (two experiments, pH 4.49 and 5.75), methyl thiolglycolate (three experiments, pH 2.24, 4.42, and 6.14), and trifluoroethanethiol (one experiment, pH 5.70). The largest increase, 20%, was with methyl thioglycolate and phosphate buffer (0.05–0.34 M); phosphate buffer has been reported to itself stimulate the decomposition of MNNG.[6]

The thiol-stimulated denitrosation of MNNG was quantitated for all seven thiols under various conditions of pH and buffer and thiol concentration. There was no marked change in the yields with any of these variables that could not be attributed to random error. Except for propanethiol and pentafluoropropanethiol, product yields were measured at two different values of pH, at two different buffer concentrations that varied typically from 0.05 to 0.3 M at each pH, and at two thiol concentrations that varied typically from 0.01 to 0.05 M at each particular buffer concentration. The mean yields of MNG and number of experiments are summarized for each thiol in Table I under the column %DN.

The thiol-stimulated deamination of MNNG was quantitated for the reaction of propanethiol and trifluoroethanethiol. In case of the latter thiol, quantitation was determined at pH 6.34 and 6.77 by varying buffer and thiol concentration in the same ranges as described for the denitrosylation reactions, above. The yield of deamination adduct was not measurably dependent upon any of the three variables. The mean yields of deamination products are included in Table I under the column %DA.

Discussion

The observed changes in values of k_2, the second-order rate constant for the reaction of total thiol with MNNG, as a function of pH (Figure 1) are consistent with a simple one-term rate law involving the reaction of the thiol anion with the neutral form of MNNG, as previously concluded.[8] The specific second-order rate constant, k_2', for the reaction of the thiol anions with neutral MNNG is related to k_2 by eq 1 in which the second and third

$$k_2' = k_2(1 + ([H^+]/K_{aRSH}))(1 + (K_{aMH}/[H^+])) \quad (1)$$

terms in the product normalize for the ionization of the thiol

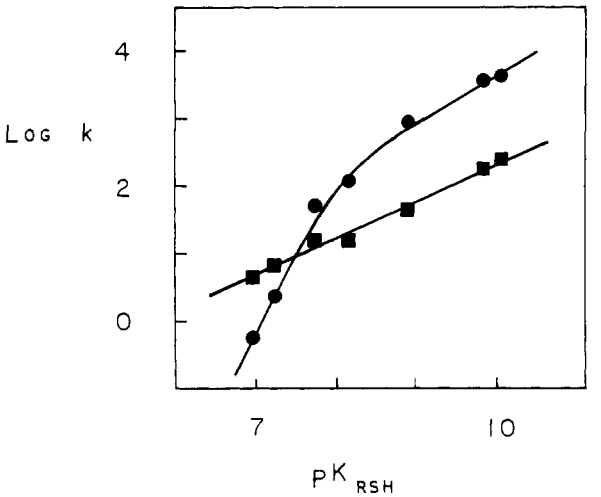


Figure 2. Plot of log k against thiol pK_a for the deamination (k_{DA}, ●) and denitrosation (k_{DN}, ■) reactions of thiol anions with MNNG at 40 °C, 1 M ionic strength (KCl). Solid line for k_{DN} is the line of least squares, line for k_{DA} calculated according to eqs 4–6.

(pK_{aRSH}) and MNNG (pK_{aMH} = 7.73),[15] respectively. A good fit to the data, for example the solid lines in Figure 1, is obtained for eq 1 using the values for k_2' and pK_{aRSH} in Table I. The values of k_2' increase with increasing thiol anion basicity.

At the extremes of the range of thiol anion basicity, the product yield data in Table I indicate that the reactions of thiol anions with MNNG can be quantitatively accounted for by parallel reactions at the nitroso nitrogen (denitrosation) and the guanidino carbon (deamination). The yield for the denitrosation reaction is found under the column %DN (Table I), while the yield for the deamination reaction is found under the column %DA (Table I). The reactions of propanethiol and pentafluoropropane thiol anions give a mean total yield (%DA + %DN) of 98 ± 3%. The product yields in Table I indicate that for strongly basic thiol anions deamination predominates, whereas for weakly basic thiol anions denitrosation predominates.

The values of the rate constants for the denitrosation and deamination reactions, k_{DN} and k_{DA}, respectively, can be obtained from the value of k_2' and the data for the percentage yield of MNG (%DN, Table I) that was determined for each of the thiols studied, according to eqs 2 and 3. The values of k_{DN} and k_{DA} are summarized in Table I.

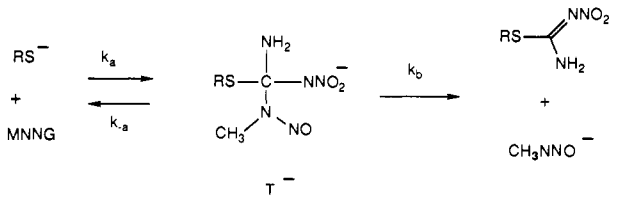
$$k_2' = k_{DN} + k_{DA} \quad (2)$$

$$\%DN = k_{DN}/(k_{DN} + k_{DA}) \quad (3)$$

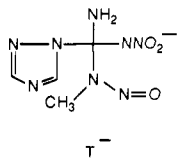
Deamination. The Bronsted-type plot of log k_{DA} against thiol pK_a is presented in Figure 2 (circles) and shows that, while there is a fairly regular decrease in log k_{DA} with decreasing pK_a from pK_a 10 to 8, there is a sharp decrease in log k_{DA} with thiol pK_a below this region. The two points for the low pK_a thiol anions deviate from a line passing through the other thiol anions by between 1 and 2 orders of magnitude—well outside experimental error.

(15) Galtress, C. L.; Morrow, P. R.; Nag, S.; Smalley, T. L.; Tschantz, M. F.; Vaughn, J. S.; Wichems, D. N.; Ziglar, S. K.; Fishbein, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 1406.

Scheme II



The downward break is consistent with a change in rate-limiting step with thiol anion pK_a, as explained below. We have previously presented evidence, in the form of a nonlinear dependence of reaction rate constant upon pH, that requires presumably tetrahedral intermediates in the reactions of certain cyclic amines with MNNG.[11] It was concluded that the reactions of triazole anions, a reasonable analogy for the reactions of thiol anions, occur with rate-limiting decomposition of the intermediate T^-, below. The



data for the deamination reaction of thiol anions (Figure 2) are consistent with the intermediacy of T^- in Scheme II, and a change in rate-limiting step with thiol anion basicity such that nucleophilic attack (k_a, Scheme II) is rate-limiting for the strongly basic thiol anions, whereas breakdown of T^- (k_b, Scheme II) is rate-limiting for the weakly basic thiol anions. The data give a good fit (solid curve through circles in Figure 2) to such a two-step mechanism using eq 4, and values of k_a and K_ak_b for the rate-limiting attack

$$k_{DA} = k_a(k_b/(k_{-a} + k_b)) \quad (4)$$

and decomposition reactions, respectively, as defined by eq 5 and 6. The dependencies of the two reactions upon thiol pK_a, β_{nuc}

$$\log k_a = (0.70\ (\pm 0.12))pK_{aRSH} - 3.33 \quad (5)$$

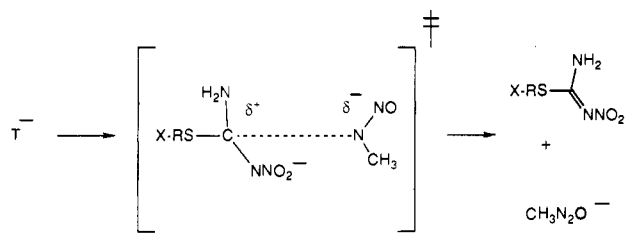
$$\log K_ak_b = (2.4\ (\pm 0.2))pK_{aRSH} - 17.0 \quad (6)$$

(= dlog k_{DA}/dpK_{aRSH}), are equal to the slope terms in eqs 5 and 6, respectively.

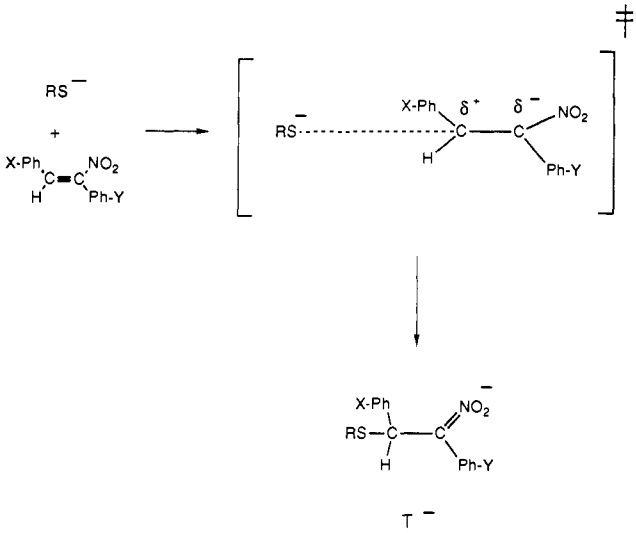
An alternative explanation for the change in dependence of log k_{DA} upon thiol pK_a (Figure 2, circles) is unlikely. A downward break in a plot of log k against pK_a of the nucleophile may be consistent with a Hammond effect on a single-step reaction in which the amount of bond formation to the nucleophile, measured by β_{nuc}, decreases with increasing nucleophile reactivity. Such effects are known but typically involve fairly small changes in structure–reactivity parameters, a few tenths of a unit, over larger changes of reactivity.[16] In the present case the value of β_{nuc} changes from 2.4 to 0.7 over a 2–3 pK_a unit change in thiol anion basicity.

The value of $\beta_{nuc} = 0.7$ for rate-limiting attack is notably large. For thiolate ions, a variety of reactions involving rate-limiting attack, or S_N2 displacement, give values of β_{nuc} in the range 0.25 ± 0.25.[17,18] A value of $\beta_{nuc} \approx 0.5$ has been recently reported for

Scheme III

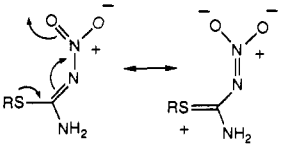


Scheme IV



thiol anion attack on α-thioethers of nitrostilbene.[18] The value of $\beta_{nuc} = 0.7$ observed in the present work indicates that S–C bond formation has proceeded nearly to completion in the reaction with MNNG, because the value of β_{eq}^-, the dependence on thiol pK_a of the logarithm of the equilibrium constant for formation of T^-, is likely to be between 0.8 and 1 on the basis of what is observed in similar reactions. For example, the rate-limiting diffusion-controlled trapping of T^- formed from thiol anion attack on acetaldehyde exhibits $\beta_{nuc} = 1$ that is due to the equilibrium formation of T^-.[17c] Similarly, the value of β_{nuc} is 0.80 and 0.84 for the equilibrium formation of T^- in the reactions of thiolate ions with α-methoxynitrostilbene and nitrostilbene, respectively.[17a,18]

The observed value of $\beta_{nuc} = 2.4$ for the rate-limiting decomposition of T^- is likely to be too large to be accounted for soley by a product-like transition state because such an explanation requires an unreasonable amount of effective charge on the sulfur in the product. The value of β_{nuc} is a reflection of the change in charge on the nucleophile that is experienced by the substituents in going from the ground state to the transition state. For the present reaction the total change in charge on the sulfur atom in going from the ground state to a very product-like transition state could exceed 1 due to the resonance interaction in the product, illustrated below, that places a positive charge on sulfur.



However, to suggest that the observed value of $\beta_{nuc} = 2.4$ is due solely to a product-like transition state requires that the sulfur atom in the product have an effective charge of 1.4. This is unreasonable on the basis of what is known about the effective charge on an atom attached to an acyl-type or alkyl-type system which ranges between 0.2 and 0.8 for systems including N, O, or S atoms.[19] An effective charge on sulfur of 0.7 can be cal-

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culated for α-thioethers of nitrostilbenes.[18] However, the effective charge on sulfur tends more typically to be at the small end of the range cited above because of the poorer overlap of the 3p orbitals of S with the 2p-π system.[17b]

Imbalance in a transition state that is product-like with respect to the amount of leaving group expulsion can account for the surprisingly large value of $\beta_{nuc} = 2.4$. Such a transition state is depicted in Scheme III, in which bond fission to the leaving group is essentially complete but there is an imbalance such that there is little in the way of double-bond character in the C–N bond. If the electrons that become the second bonding pair in the penultimate C–N double bond are largely localized on the imino nitrogen or in the nitro group, the substituents on the thiol will experience a greater positive charge, due to the electron-deficient carbon, than in the final product.

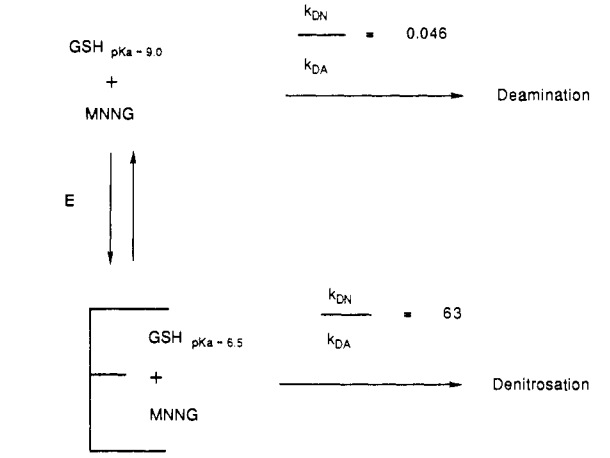
An analogous imbalance has been observed in the reaction of thiol anions with nitrostilbene, illustrated in Scheme IV.[17a] The value of β_{nuc} of 0.19 for this reaction indicates that there is little C–S bond formation as detected by substituents on the thiolate—the value of β_{eq} for T^- formation is 0.84. However, the value of $\rho = 0.96$ for substituents Y, compared to the equilibrium value of $\rho_{eq} = 1.10$ for T^- formation, indicates that there is substantial progress in the scission of the C–C double bond and charge buildup on the α-nitro carbon (Scheme IV). There must be a considerable corresponding positive charge on the adjacent carbon, as indicated in Scheme IV. When viewed in the reverse direction, that of T^- breakdown (Scheme IV), this imbalance is directly analogous to that proposed in the present case (Scheme III). The substituents X on the thiol in the reaction involving MNNG (Scheme III) are in a position analogous to the substituents X in the reaction of thiolate ions with nitrostilbene (Scheme IV). In both reactions, substituents X would be expected to experience a larger positive charge in the transition state than exists in reactants or products. A similar imbalance also exists in the transition states for the reactions of cyclic secondary amines with nitrostilbenes[20] and in the imine-forming elimination reactions of benzaldehyde carbinolamine ethers.[21]

Finally, the break in the plot of log k_{DA} against pK_{aRSH} (Figure 2) indicates that the *N*-nitrosamine anion is a surprisingly poor leaving group, as was concluded previously for the reactions of certain cyclic amines with MNNG.[11] The intersection of the lines of limiting slope for rate-determining attack and rate-determining breakdown indicate that the intermediate T^- partitions equally to products and reactants for a thiolate ion of conjugate acid $pK_a = 8.0$. The partitioning of T^- is equal in spite of the fact that the *N*-nitrosomethylamine anion is likely to be substantially less basic (conjugate acid $pK_a < 6.2$).[11,22]

Denitrosation. The values of log k_{DN}, the second-order rate constant for denitrosation by the thiolate anions, are plotted against the conjugate acid pK_a of the thiolates in Figure 2 (squares). The points give a good fit to a straight line (solid line, Figure 2) of slope $\beta_{nuc} = 0.54 \pm 0.02$. This compares with a value of $\beta_{nuc} = 0.58$ and ≈0.6 for the nitroso group transfer to secondary amines by phenethyl nitrite and *N*-methyl-*N*-nitrosotoluenesulfonamide, respectively.[23,24]

Our data do not distinguish among several possible mechanisms for the reaction. Others favor a concerted displacement for the reactions of amines. This conclusion rests largely on the basis of poor correlations between rate constants and amine basicity, where a structurally heterogeneous group of amines are compared, and "better" correlations between rate constants for a limited number of the nucleophiles and their vertical ionization potentials.[23,24] The thiol-mediated reduction of nitrosobenzenes has been shown to occur with the intermediacy of a semimercaptal.[25]

Scheme V



Biological Importance. The early work of Lawley and Thatcher[6] has led to the generalization that the DNA alkylating activity of MNNG is generally thiol-mediated. Other correlative evidence of this type has been obtained more recently.[26]

In contrast, results from other in vitro systems suggest a thiol-dependent activity that is chemoprotective against the alkylating action of MNNG. In these systems a depletion of soluble thiol by pretreatment of cells with diethyl malonate results in an increase in the DNA alkylating activity of MNNG.[7b,10,27] A key to how thiols may protect against alkylation has been identified by the recent finding that a purified glutathione *S*-transferase of the μ-class catalyzes a glutathione-dependent denitrosation reaction of MNNG.[10] Glutathione is the most abundant soluble thiol in mammalian systems, with concentrations of the reduced form varying from 1 to 10 mM. The isolated glutathione *S*-transferase stimulates >80% denitrosation of MNNG in the reaction with glutathione.[10]

The experimental results presented in this report provide a possible explanation for the fact that free glutathione stimulates decomposition of MNNG with predominant (~90%) deamination[10] whereas the glutathione *S*-transferase-mediated reaction stimulates decomposition of MNNG with predominant (>80%) denitrosation. The pK_a of glutathione is ~9.0 in solution, and our data predict that the reaction of such a thiol should occur with ~95% deamination, in good agreement with the measured values. The pK_a of the thiol of glutathione bound to the active site of a μ-class glutathione *S*-transferase has recently been reported to be 6.5 ± 0.2.[28] The reason for the perturbed pK_a is unknown. Our data predict that a thiol of this pK_a will react with MNNG to give a ~98% yield of denitrosation, in agreement with the >80% lower limit obtained in the enzymatic reaction. The predicted differential partitioning is illustrated in Scheme V, where E is the enzyme glutathione *S*-transferase.

The extent to which data from a solution reaction can be extrapolated to behavior at an enzyme active site is uncertain. For this type of enzyme, the extrapolation may be less inaccurate than for most due to the general enzymatic function, which is to inactivate, by reaction with glutathione, an array of electrophiles of varying structure. The GST enzymes exhibit broad substrate specificity and compared to other enzymes are only modestly stereo- and regiospecific, with some exceptions.[29] Thus it is reasonable that both the nitroso group and the guanidino carbon

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of MNNG may be accessible to the thiol at the active site. Given this, the chemoprotective activity of the glutathione *S*-transferase-mediated reaction of glutathione may be due to a denitrosation reaction that is predominant because the thiolate of GSH, when it reacts at carbon, generates an intermediate T^- that mainly reverts to starting materials due to the low conjugate acid pK_a of the thiolate at the active site.

Supplementary Material Available: Tables S1-S4 of data from reactions of thiols with *N*-methyl-*N'*-nitro-*N*-nitroguanidine, 40 °C, ionic strength 1 M (KCl) (14 pages). Ordering information is given on any current masthead page.

Coordination Isomerism in Pentamethylcyclopentadienyl-Substituted Iminophosphanes: From Classical Structures to a π-Complexed Iminophosphenium Ion

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Abstract: Novel iminophosphanes of the type $Me_5C_5P{=}NR$ (R = $C_6H_2tBu_3$ (**5a**), Si-*i*-Pr_3 (**5b**), $SiMe_3$ (**5c**)) are prepared via either thermolytic or base-promoted elimination reactions and characterized by analytical and spectroscopic methods. The X-ray crystal structures of **5a,b** establish the presence of coordination isomerism of the cyclopentadienyl ring, which at the same time strongly alters the characteristics of the P-N multiple bond. For **5a**, η^1-attachment of the Me_5C_5 moiety to the phosphorus and a localized phosphorus-carbon σ-bond are found, and the P-N double bond compares to that of alkylated iminophosphanes (r_{PN} = 155.1 (8) pm). **5b** exhibits η^2-coordination of the cyclopentadienyl ring leading to increased P-N triple-bond character, as evidenced by shortening of the P-N distance (153.3 (3) pm) and the remarkable opening of the nitrogen valence angle (153.3 (2)°). The structure is discussed as an intramolecular π-complex between a formal cyclopentadienyl anion and an iminophosphenium cation. The ^1H and ^{13}C NMR spectra show that rapid elementotropic rearrangements around the five-membered ring take place both in solution and in the solid state. The unique temperature dependence of δ ^{31}P in solution together with the considerable differences in δ ^{31}P between the solution and solid states are interpreted in a model assuming a "haptotropic" mechanism for the fluxionality involving dynamic η^1/η^2-coordination isomerizations in solution. As expected, nucleophilic displacement of the Me_5C_5 moiety takes place in the reaction of **5b,c** with $LiC_6H_2tBu_3$, yielding the iminophosphanes $tBu_3H_2C_6P{=}NR$ (R = Si-*i*-Pr_3 (**8b**), $SiMe_3$ (**8c**)) as products.

Introduction

Pentamethylcyclopentadienyl-substituted (Me_5C_5) phosphorus-p(π) multiple-bond systems are of both high synthetic and theoretical interest due to their unique stereoelectronic properties. While the Me_5C_5 group provides sufficient steric bulk for the stabilization of the low coordination number,[1-3] its liability toward nucleophilic substitution[3,4] at the same time permits a synthetically valuable functionalization of the double bond. In addition, the capacity for multihapto π-bonding[5] leads to a versatility in structure and bonding. Thus, the cyclopentadienyls in the neutral systems $Me_5C_5P{=}ER_n$ (ER_n = $C(SiMe_3)_2$,[1] PC_5Me_5[3]) are η^1-(σ)-bonded in the solid state, while "nonclassical" $\eta^2(\pi)$-coordination prevails in the phosphenium cation $[Me_5C_5PN(H)tBu]^+$.[6] In solution, all known systems are fluxional due to fast elementotropic rearrangements.[6,7]

Although an Me_5C_5-substituted iminophosphane has been prepared,[2] no structure determinations of such compounds, which should constitute a "missing link" between η^1-$Me_5C_5P{=}PR$ and $[\eta^2$-$Me_5C_5PNHR]^+$, have been performed yet. Here, we report the preparation and first structural characterization of novel Me_5C_5-substituted iminophosphanes. It is found that a substitution-induced coordination isomerism occurs between $\eta^1(\sigma)$- and $\eta^2(\pi)$-bonding of the Me_5C_5 ligand, which has most interesting implications on the structure and bonding of the phosphorus-nitrogen multiple bond. The investigation of the dynamic aspects of the molecular structure was attempted by NMR spectroscopic studies of **5a** both in the solid state and in solution, and evidence is presented that the dynamic process may follow different mechanisms in both cases.

Experimental Section

All manipulations were carried out under a dry argon atmosphere using standard Schlenk techniques. Solvents were dried by refluxing over appropriate reagents and distilled before use. Me_5C_5Li,[8] (2,4,6-tri-*tert*-butylanilino)dichlorophosphane (**2a**),[9] lithium bis(trimethylsilyl)-amide (**4**),[10] [bis(trimethylsilyl)amino]dichlorophosphane (**6**),[11] and (2,4,6-tri-*tert*-butylphenyl)lithium[12] were prepared according to literature

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