(3-methyl-1,3-butadienyl)benzene, 68036-69-1; methyl (*E,Z*)-2-methyl-5-phenyl-2,4-pentadienoate, 20414-96-4; methyl (*E,Z*)-5-cyclohexyl-2-methyl-2,4-pentadienoate, 75066-89-6; methyl (*E,Z*)-2-methyl-2,4-nonadienoate, 75066-90-9; methyl nonanoate, 1731-84-6; methyl (*E*)-2-nonenoate, 14952-06-8; methyl (*E,Z*)-2,4-nonadienoate, 39924-44-2; methyl (*Z,Z*)-2,4-nonadienoate, 75066-91-0; methyl (*Z,E*)-2,4-nonadienoate, 75066-92-1; methyl (*Z*)-2-nonenoate, 68872-72-0; (*E*)-1,1-dimethoxy-5-morpholino-3-hexene, 75066-93-2; (*E*)-1,1-dimethyoxy-5-piperidino-3-hexene, 75066-94-3; (*E*)-1,1-dimethoxy-5-(*N*-methylpiperidinium)-3-hexene I^-, 75066-95-4; (*E*)-2,5-dimethyl-6-piperidino-4-hexen-2-ol, 75066-96-5; (*E*)-2-methyl-1-piperidino-4-phenyl-2-butene, 74312-51-9; (*E*)-1,1-dimethoxy-5-methyl-5-piperidino-3-hexene, 75066-97-6; (*Z*)-6,6-dimethoxy-2-hexene, 75066-98-7; (*Z*)-1,1-dimethoxy-3-hexene, 55444-65-0; 1,1-dimethoxyhexane, 1599-47-9; 2,5-dimethyl-4-hexen-2-ol, 14908-27-1; 2,5-dimethyl-5-hexen-2-ol, 75066-99-8; (3-methyl-2-butenyl)benzene, 4489-84-3; (3-methyl-3-butenyl)benzene, 6683-51-8; (3-methylbutyl)benzene, 2049-94-7; 6,6-dimethoxy-2-methyl-2-hexene, 2006-05-5; 1,1-dimethoxy-5-methyl-3-hexene, 75067-00-4; ethynylcyclohexane, 931-48-6; ethynylbenzene, 536-74-3; 1-hexyne, 693-02-7; methyl (*E*)-3-bromo-2-methylpropenoate, 40053-01-8; methyl (*E*)-3-bromo-2-propenoate, 6213-87-2; methyl (*Z*)-3-bromo-2-propenoate, 6214-22-8; *p*-bromonitrobenzene, 586-78-7; (*Z*)-3-bromopropenoic acid, 1609-92-3; propiolic acid, 471-25-0; (*E*)-3-bromopropenoic acid, 6213-89-4; (*Z*)-1-bromo-1-propene, 590-13-6; acrolein dimethyl acetal, 6044-68-4; morpholine, 110-91-8; piperidine, 110-89-4; 2-bromo-1-propene, 557-93-7; 2-methyl-3-buten-2-ol, 115-18-4; (*E*)-2,5-dimethyl-3,5-hexadien-2-ol, 75082-96-1; 2-methyl-1-bromo-1-propene, 3017-69-4; 5-methyl-2,4-hexadienal acetal, 75067-01-5; 5-methyl-3,5-hexadiene acetal, 75067-02-6; 2-nitroacetophenone, 577-59-3; 4-nitroacetophenone, 100-19-6; 2-aminoacetophenone, 551-93-9; 4-aminoacetophenone, 99-92-3; palladium, 7440-05-3; triethylammonium formate, 585-29-5.

Supplementary Material Available: Table IV containing physical properties and NMR and mass spectral data for the compounds prepared in this study (7 pages). Ordering information is given on any current masthead page.

Alicyclic Nitrosamines and Nitrosamino Acids as Transnitrosating Agents

Sandra S. Singer,* George M. Singer, and Barbara B. Cole

Chemical Carcinogenesis Program, NCI Frederick Cancer Research Center, Frederick, Maryland 21701

Received June 24, 1980

Many alicyclic nitrosamines act as nitrosating agents under mild conditions (pH 1–3, in the presence of nucleophilic catalysts such as thiocyanate). All nitrosopiperazines, nitrosomorpholines, and nitrosamino acids tested were found to act as nitrosating agents, and certain nitrosopiperidines also showed this capability. Acyclic nitrosamines are far less reactive than functionally similar cyclic compounds.

Introduction

Secondary and tertiary amines react with nitrite under mildly acidic conditions to form *N*-nitroso compounds. Carcinogenic nitrosamines can be formed by the reaction of dietary or endogenous nitrite with ingested amines, and this has, in fact, been demonstrated in laboratory animals.[1,2] However, nitrosation in vivo is not the only possible source of nitroso compounds. We have shown that many aliphatic nitroso compounds are capable of acting as nitrosating agents of naturally occurring amines under conditions similar to those found in the rat or human stomach (pH 1.7–3.6 with thiocyanate or other nucleophiles as catalyst).[3] While it has long been known that certain aromatic *N*-nitroso compounds are excellent transnitrosating agents,[4,5] the generality of this reaction has not been realized previously. In this paper we report the behavior of several widely varying classes of alicyclic nitrosamines as nitrosating agents. Included are certain derivatives of natural products such as nitrosoproline and nitrosopipecolic acid previously thought biologically innocuous but now shown to have potentially hazardous roles in the formation of carcinogenic nitrosamines.

Results and Discussion

Many nitrosamines are capable of acting as nitrosating agents at pH's of 1–3 in the presence of a nucleophilic catalyst.[3,6] The reaction, or transnitrosation, may be represented by eq 1–4.

$$R_2NNO + H^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R_2\overset{+}{N}HNO \quad (1)$$

$$R_2\overset{+}{N}HNO + {}^-SCN \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R_2NH + NOSCN \quad (2)$$

$$NOSCN + R_2'NH \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} R_2'NNO + SCN^- \quad (3)$$

$$NOSCN + Z \rightarrow \text{unstable product} \rightarrow N_2, \text{ etc.} \quad (4)$$

$$Z = HSO_3NH_2,\ NaN_3,\ NH_2CONH_2, \text{ etc.}$$

The reactions shown in eq 1–3 are all reversible, and the extent of reaction observed will depend on the relative rates k_{-3} vs. k_{-2}. The reaction shown in eq 4 is a special case in which the nitroso recipient forms an unstable nitroso compound which rapidly decomposes to give nitrogen (N_2), thus rendering the sequence irreversible.[7,8] Such reactions are referred to as denitrosations, and they provide a convenient method for studying the reaction sequence shown in eq 1 and 2 without complications from reversibility.

We have previously shown[6,21] that transnitrosation by aliphatic nitrosamines occurs in acidic aqueous solutions with nucleophile catalysis and no direct and/or uncatalyzed reaction occurs. A steady-state concentration of NOSCN[21] can be monitored throughout the course of the reaction. In effect, a transnitrosation by an aliphatic nitrosamine may be considered as a nitrosation reaction in

(1) Lijinsky, W.; Taylor, H. W.; Snyder, C.; Nettesheim, P. *Nature (London)* **1973**, *244*, 176.
(2) Sander, J. *Arch. Hyg. Bakteriol.* **1967**, *151*, 22.
(3) Singer, S. S.; Lijinsky, W.; Singer, G. M. *Tetrahedron Lett.* **1977**, 1613.
(4) Baumgardner, C. L.; McCallum, K. A.; Freeman, J. P. *J. Am. Chem. Soc.* **1961**, *83*, 4417.
(5) Sieper, H. *Chem. Ber.* **1967**, *100*, 1646.
(6) Singer, S. S.; Lijinsky, W.; Singer, G. M. IARC Scientific Publications, 1978, No. 19, 175.

0022-3263/80/1945-4931$01.00/0

Table I. Reactivities of Nitrosamines

	denitrosation k, s^{-1}	relative rate of denitrosation	pK_a of relevant N[h]	% yield of nitroso-piperidine[b]	carcinogen +, − (ref)
N-nitroso-					
isonipecotic acid (10)	7.4×10^{-6}	1	10.60	3.1	− (14)
proline (8)	3.7×10^{-5}	6	10.640[f]	2.1	− (16)
morpholine	1.00×10^{-4}	14	8.33[f]	11	+ (18)
hydroxyproline (7)	1.48×10^{-4}	20	9.662[f]	1.5	− (16)
pipecolic acid (6)	1.80×10^{-4}	24	10.58	15	− (16)
cis-2,6-dimethylmorpholine	6.60×10^{-4}	89	-		+ (9)
trans-2,6-dimethylmorpholine	3.55×10^{-4}	47	-		+ (9)
3-methyl-2-phenylmorpholine	1.05×10^{-3}	140	-	3.3	− (11)
3,5-dimethylpiperazine (5)	2.45×10^{-4} [c]	256	6.70	none	+[e]
piperazine (1)	3.50×10^{-4} [c]	364	5.56		− (12, 15)
4-methylpiperazine (3)	8.20×10^{-4} [c] 1.7×10^{-3} [a]	852	4.86	none	− (14)
2,6-dicyanopiperidine	3.6×10^{-4}	49			
2,6-dimethylpiperidine	4.52×10^{-4}	61		60	− (13)
2,2,6,6-tetramethylpiperidine	3.10×10^{-2} [c,d]	4200	11.07		− (13)
2-ethylpiperidine	6×10^{-6}	0.8			
2-*sec*-butylpiperidine	5×10^{-6}	0.7			
piperidine	1.1×10^{-6}	0.1	11.23[f]		+ (13)
sarcosine (9)	3.50×10^{-5}	8	10.12[f]	4.1	+
O,*N*-dimethylhydroxylamine	2.26×10^{-3}	2368	4.72		− (14)
1,4-dinitroso-					
piperazine (2)	1.08×10^{-3} [a] 1.30×10^{-4} [c,g]	135	7.05		+ (10)
2,6-dimethylpiperazine (4)	1.78×10^{-3} [c,g]	2025		15	+ (10)

[a] Reaction of nitrosamine (0.05 M) with ammonium sulfamate (0.05 M) in 1 N HClO + 0.05 M $^-$SCN catalyst. [b] Reaction of nitrosamine (0.05 M) with piperidinium perchlorate (0.05 M) at pH 1.7, with 0.50 M $^-$SCN catalyst, 18 h. [c] Rates determined in 3 N HCl, 0.05 M nitrosamine, 0.05 M $NH_4SO_3NH_2$. [d] Rate determined in 0.5 M HCl and adjusted accordingly. [e] Unpublished results. [f] Literature values (Weast, R. C. "CRC Handbook of Chemistry and Physics", 55th ed.; CRC Press: Cleveland, OH, 1975; p D126). [g] 1-Nitroso only. [h] For example, 7.05 is the pK_a of the 4-imino in 1-nitrosopiperazine and, hence, is the relevant pK_a for loss of one nitroso in dinitrosopiperazine.

Table II. Rates of Denitrosation of Acyclic Nitrosamines

N-nitroso-	k, s^{-1}
O,*N*-dimethylhydroxylamine	2.3×10^{-3}
sarcosine	3.5×10^{-5}
bis(β,β,β-trifluoroethyl)amine	no reaction
diisopropylamine	no reaction
diethanolamine	no reaction
iminodiacetonitrile	7.1×10^{-6}

which a very small amount of nitrite is added very slowly over the course of the reaction. In a transnitrosation reaction it is convenient to study the formation of the product nitrosamine, while the disappearance of the donor is often difficult to follow with accuracy at the early stages of the reaction because changes in its concentration are small. We have used "denitrosation" reactions with ammonium sulfamate as the recipient to evaluate the relative ease of denitrosation of a number of alicyclic and a few acyclic nitrosamines. Results for the alicyclic nitrosamines are given in Table I. Denitrosation data is given both in the form of first-order rate constants and relative rates, relative to *N*-nitroso-isonipecotic acid.

Because of the wide range of denitrosation rates of the compounds we have studied, it was necessary to use two sets of reaction conditions. In a standard denitrosation experiment, a solution 0.05 M in nitrosamine was prepared in 1 N $HClO_4$ containing 0.05 M NaSCN and was maintained in a thermostatted bath at 50 °C. Ammonium sulfamate (0.05 M) was added to initiate the reaction. Aliquots were taken at timed intervals, quenched by 50-fold dilution with water and analyzed by high-pressure LC to monitor the disappearance of nitrosamine with time. First-order rate constants for nitrosamine dissappearance were determined by a plot of ln (a/x) vs. t, where a is the initial concentration of nitrosamine and x is the concentration at time t.

Results are summarized in Table I. Rate constants for the nitrosamines studied fell in the range 1×10^{-3} to 1×10^{-6} s^{-1}. Under these conditions several compounds gave rate constants of 1×10^{-3} s^{-1}, which was the fastest measurable. We then sought conditions under which reaction would be slower, so that the rate constants for these rapid reactions could be more accurately determined. In 3 N HCl, all of these faster reacting compounds could be denitrosated with rate constants that were somewhat slower than those obtained in 1 N $HClO_4$ with 0.05 M NaSCN as catalyst since chloride ion is a far less effective nucleophile than thiocyanate. *N*-Nitroso-2,2,6,6-tetramethylpiperidine reacted so rapidly that it was necessary to use 0.5 M HCl as the denitrosation medium in order to get a reaction rate that was slow enough to measure. The relative rate constant shown in Table I for this compound was then calculated, assuming the reaction is first order in H^+ and Cl^-, as has been shown for related reactions.[3,7]

All alicyclic compounds that were found to undergo denitrosation in 1 N $HClO_4$/0.05 M $^-$SCN were also studied in transnitrosation reactions with one or more of the following amines: *N*-methylpiperazine, morpholine,[3,6] or piperidine. Reactions were routinely carried out at pH 1.7 in $HClO_4$ with 0.5 M NaSCN. Concentration of the ni-

(7) Buglass, A. J.; Challis, B. C.; Osborn, M. R. IARC Scientific Publications, 1974, No. 9, 94.
(8) Williams, D. L. H. *Food Cosmet. Toxicol.* **1978**, *16*, 365.
(9) Lijinsky, W.; Taylor, H. W. *Cancer Res.* **1975**, *35*, 2123.
(10) Lijinsky, W.; Taylor, H. W. *Cancer Res.* **1975**, *35*, 1270.
(11) Lijinsky, W.; Taylor, H. W. *Cancer Lett.* **1976**, *1*, 359.
(12) Garcia, H.; Keefer, L.; Lijinsky, W.; Wenyon, C. E. M. *Z. Krebsforsch.* **1970**, *74*, 179.
(13) Lijinsky, W.; Taylor, H. W. *Int. J. Cancer* **1975**, *16*, 318.
(14) Lijinsky, W.; Taylor, H. W. *Z. Krebsforsch.* **1977**, *89*, 31.
(15) Love, L. A.; Lijinsky, W.; Keefer, L. K.; Garcia, H. *Z. Krebsforsch.* **1977**, *89*, 69.
(16) Garcia, H.; Lijinsky, W. *Z. Krebsforsch.* **1973**, *79*, 141.

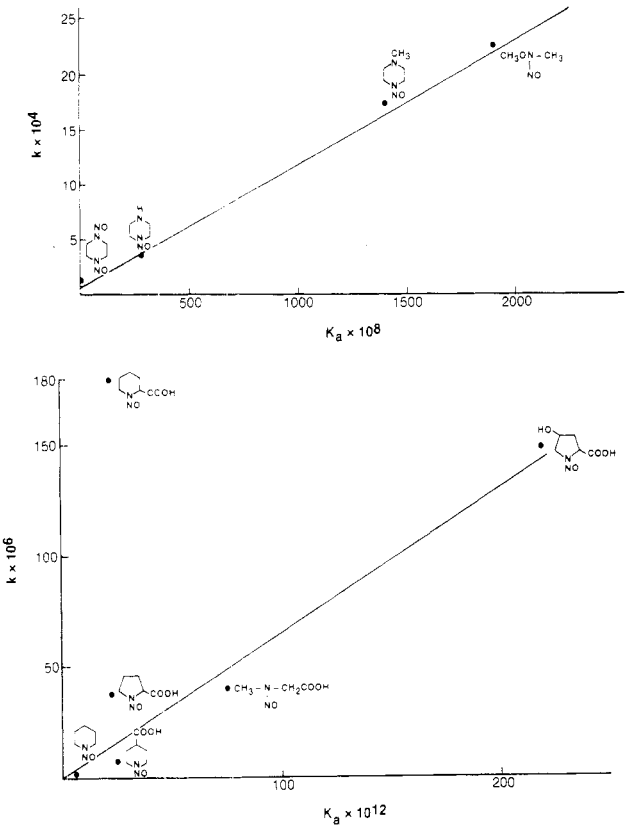
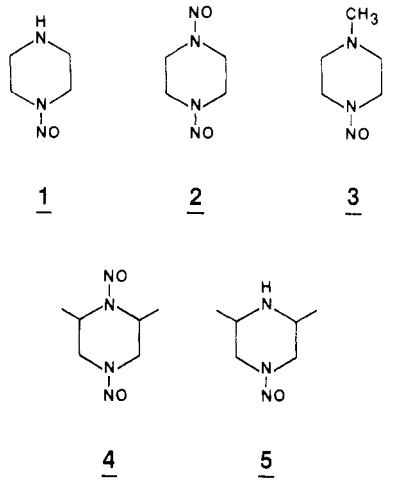


Figure 1. Rate constant for denitrosation vs. K_a (ionization constant of the conjugate acid) for (A, top) a series of rapidly denitrosated compounds and (B, bottom) a series of nitrosamino acids.

trosamine was 0.05 M. When piperidine was the recipient amine, the reaction was essentially irreversible but relatively slow. When either *N*-methylpiperazine or morpholine was the recipient, the reverse reaction became important. These reactions eventually reach an equilibrium, the position of which is governed by all factors which affect the denitrosation (see eq 2) of the nitrosamines involved. These factors include steric and inductive effects of α-substituents and the relative basicities of the parent amines. Weakly basic amines are nitrosated more readily[17] than those that are strong bases (Table I) and, conversely, their nitroso derivatives are more readily denitrosated than those of strong bases. In the absence of steric effects, a linear correlation can be obtained from a plot of the rate constant for denitrosation vs. the K_a of the parent amine (ionization constant of the conjugate acid; see Figure 1).

The nitrosamines that can act as nitrosating agents fall into four groups loosely related to the basicity of the parent amine: the piperazines, the morpholines, the alkyl piperidines, and the nitrosoaminocarboxylic acids.

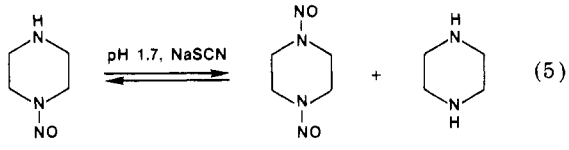
Piperazines. All of the nitrosopiperazines are good transnitrosating agents. The parent amines are weakly basic, and the N–NO bond is weak relative to that in nitroso derivatives of strong bases such as piperidine. We have studied the following piperazine derivatives: mononitrosopiperazine (**1**), dinitrosopiperazine (**2**), 4-methyl-1-nitrosopiperazine (**3**), 2,6-dimethyldinitrosopiperazine (**4**), and 3,5-dimethyl-1-nitrosopiperazine (**5**). Of these, the most rapidly denitrosated is **4**, in which the nitroso flanked by the methyls is removed about 10 times faster than the other nitroso group. The order of denitrosation



1 **2** **3**

4 **5**

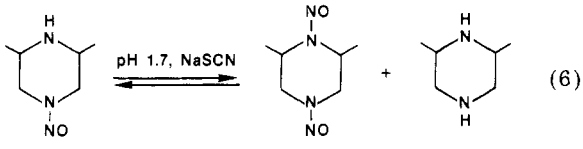
of the piperazines is **4** ≫ **3** > **1** > **5** > **2**, where the rates for **2** and **4** refer to removal of one nitroso group only. The great reactivity of **4** is undoubtedly due to the influence of the two α-methyl groups. The methyl groups are axial (as shown by the 7-Hz coupling to the geminal methine proton) and 1,3-diaxial interactions deform the ring sufficiently to weaken π overlap in the N–N bond. Harris et al.[19] recently reported that the barrier to rotation is 17 kJ mol^{-1} less for the 1-nitroso group in **4** than for the 4-nitroso. They attribute this lowered barrier for internal rotation to destabilization of the ground state of the 1-nitroso group of **4** by the 1,3-diaxial methyl interactions (ca. 11 kJ mol^{-1}) and the relief of this interaction possible in the transition state. Denitrosation should also occur more readily from a transition state of this type in which π overlap is not complete. The presence of the methyl groups greatly reduces the mononitroso–dinitroso disproportionation reaction in this case, in contrast to that which we have observed for **1**[3] (eq 5). Mononitrosopiperazine (**1**) dispro-



(5)

1 54% **2** 23% 23%

portionates to give an equilibrium mixture of 54% **1** and 23% **2** after 1 h at pH 1.5 with $^-$SCN catalyst. In contrast, 3,5-dimethylmononitrosopiperazine (**5**) gives 6.5% **4** immediately, and then **5** slowly disappears with no additional increase in **4** over 18 h (eq 6). Apparently, the presence



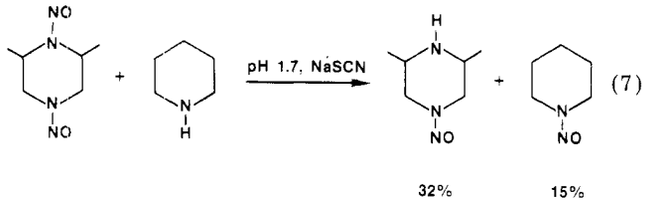
(6)

5 **4**, 6.5%

of the two α-methyls is sufficient to inhibit renitrosation greatly under these conditions. The pK_a for the 4-imino nitrogen in **5** is 6.70, not greatly different from that observed for the 4-imino nitrogen in **1** (7.05). The difference in basicity cannot account for the difference in reactivity. Furthermore, 2,6-dimethyldinitrosopiperazine (**4**) does

(17) Mirvish, S. S. IARC Scientific Publications, 1974, No. 9, 67.
(18) Druckrey, H.; Preussman, R.; Ivankovic, S.; Schmähl, D. *Z. Krebsforsch.* **1976**, *69*, 103.
(19) Harris, R. K.; Pryce-Jones, T.; Swinborne, F. J. *J. Chem. Soc., Perkin Trans 2* **1980**, 476.

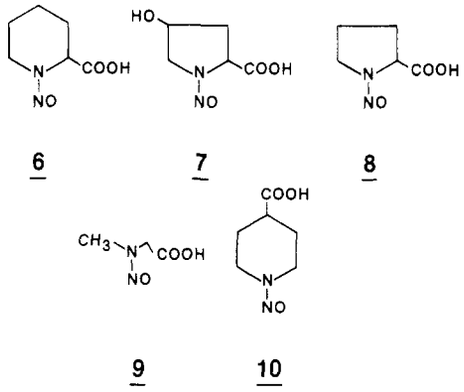
nitrosate piperidine in contrast to all the other nitrosopiperazines studied (eq 7; vide supra).



Morpholines. The nitrosomorpholines are transnitrosating agents, but not of the same order of magnitude as the piperazines. The most rapidly reacting donor in the series is nitrosophenmetrazine (3-methyl-2-phenylnitrosomorpholine). All the nitrosomorpholines, however, are capable of nitrosating piperidine, although the equilibrium does favor the nitrosomorpholine. We recently reported[20] the separation of *cis*- and *trans*-2,6-dimethylnitrosomorpholine as pure isomers. These compounds provide a unique opportunity to study the effect of ring conformation on nitrosation and denitrosation. In a denitrosation experiment, the cis isomer denitrosates twice as fast as the trans isomer.

Piperidines. Nitrosopiperidine is a very stable compound and will not act as a nitroso donor. The pK_a of piperidine is 11.23, and since piperidine is slow to nitrosate in mild acid compared to morpholine (pK_a = 8.33) or *N*-methylpiperazine (pK_a = 7.36), the nitroso derivative is not subject to denitrosation at the pH of the solutions employed in our transnitrosation. Various nitrosopiperidine derivatives, however, are transnitrosating agents. The effects of substitution α to the nitroso are profound. 2,6-Dimethyl-1-nitrosopiperidine and 2,2,6,6-tetramethyl-1-nitrosopiperidine are both transnitrosating agents and give high yields of nitrosopiperidine when piperidine is the recipient amine. As is the case with 2,6-dimethyldinitrosopiperazine, renitrosation of the donor is hindered and, thus, the transnitrosation reaction proceeds. With 2,6-dimethylnitrosopiperidine as donor, a 60% yield of nitrosopiperidine can be obtained in 18 h. These results complement those of Jones, Lijinsky, and Singer[22] in that the ratio of the rates of nitrosation of piperidine, 2,6-dimethylpiperidine, and 2,2,6,6-tetramethylpiperidine is 100:10:1.

Amino Acids. There are several naturally occurring amino acids which contain secondary amino groups and, hence, can form *N*-nitroso derivatives. We have studied five such compounds and found that denitrosation is accelerated when a carboxyl is α to an *N*-nitroso group. The compounds studied included, in order of decreasing reactivity, *N*-nitrosopipecolic acid (**6**), *N*-nitroso-4-hydroxyproline (**7**), *N*-nitrosoproline (**8**), *N*-nitrososarcosine (**9**), and *N*-nitrosoisonipecotic acid (**10**). A good linear correlation is obtained (Figure 1B) when K_a for the amino nitrogen is plotted against the rate of denitrosation for these five compounds, except for *N*-nitrosopipecolic acid (**6**) which is a striking outlier. It denitrosates faster than any other α-amino acid studied, reacting 5 times faster than *N*-nitrosoproline (**8**) and *N*-nitrososarcosine (**9**). There is a difference in the carboxyl group in **6** being axial,[23] while the carboxyl groups in **7** and **8** are pseudoequatorial.

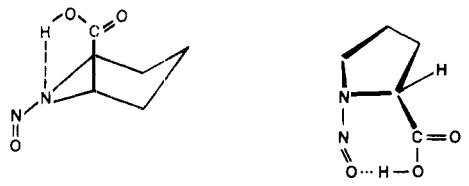


A comparison of the rate constants for denitrosation of *N*-nitrosopipecolic acid (**6**) ($k = 1.80 \times 10^{-4}$ s^{-1}) with that of *N*-nitroso-2-ethylpiperidine[24] ($k = 6 \times 10^{-6}$ s^{-1}) and *N*-nitroso-2-*sec*-butylpiperidine ($k = 5 \times 10^{-6}$ s^{-1}) permits us to evaluate the effect of the axial[23] α-carboxyl group on denitrosation vs. the steric effect of one α-axial alkyl substituent. The *N*-nitroso-2-alkylpiperidines react 5 to 6 times faster than piperidine itself, but **6** reacts over 160 times faster than nitrosopiperidine. Thus, the rate-enhancing effect of the carboxyl group in **6** cannot be attributed exclusively to a steric or an electronic effect, or even to a combination of both. It seems more likely that the rate enhancement is due to a neighboring-group effect that is geometrically favored in **6** as opposed to any of the other acids. Examination of Dreiding models indicates that intramolecular protonation of the amine nitrogen can occur in **6**, but not in **7** or **8** (Figure 2). This may be a contributing factor in the loss of the nitroso group.

Nitrosoamino acids are known to be formed in the environment, in foods, and therefore could be found in the human stomach.[1,2] While only *N*-nitrososarcosine has been identified as a carcinogen among those studied, compounds **6–9** are all effective transnitrosating agents and will nitrosate piperidine to give rise to *N*-nitrosopiperidine, a potent carcinogen and a very stable compound. The yields of *N*-nitrosopiperidine obtained in 18 h of reaction at pH 1.7 are shown in Table I. A pH profile has been established for the reaction of *N*-nitrosopipecolic acid and *N*-nitroso-2,6-dimethylpiperidine with piperidine and is shown graphically in Figure 3. The yield of *N*-nitrosopiperidine drops off rapidly with increasing pH. It is well-documented that the optimum pH for piperidine nitrosation is above 3.[25,26] The fact that the maximum yield of *N*-nitrosopiperidine in transnitrosation from an alicyclic nitrosamine is at such a low pH must be due to the importance of the protonation step in the denitrosation reaction (i.e., the nitrosation of piperidine is not rate determining).

Aliphatic Acyclic Nitrosamines. Rates of denitrosation for representative nitrosamines derived from acyclic amines have been studied. Results are given in Table II. The geometrical constraints that occur in a ring system are not present in the acyclic systems, and there is no steric effect on denitrosation. Inductive effects, of course, are still apparent. *N*-Nitrososarcosine denitrosates at the same rate as *N*-nitrosoproline. *N*-Nitroso-*O*,*N*-dimethylhydroxylamine denitrosates extremely rapidly, as would be expected, as the pK_a of the parent amine is 4.72.

(20) Singer, S. S.; Singer, G. M. *J. Liq. Chromatogr.* **1979**, *2*, 1219.
(21) Singer, S. S. *J. Org. Chem.* **1978**, *43*, 4612.
(22) Jones, A. R.; Lijinsky, W.; Singer, G. M. *Cancer Res.* **1974**, *34*, 1079.
(23) Lijinsky, W.; Keefer, L.; Loo, J. *Tetrahedron* **1970**, *26*, 5137.
(24) Singer, G. M.; Wilbur, D. J., presented in part at the 178th ACS National Meeting, Washington, DC, Sept 10–14, 1979; ORGN 237.
(25) Sander, J.; Schweinsberg, F.; Menz, H. P. *Hoppe-Seyler's Z. Physiol. Chem.* **1968**, *349*, 1691.
(26) Yamada, T.; Yamamoto, M.; Tanimura, A. *Shokuhin Eiseigaku Zasshi* **1974**, *15*, 201.



6 8

Figure 2. Intramolecular protonation at N-1 is possible in **6** but not in **8**. In the latter, protonation of the oxygen would be likely. The observation that **6** denitrosates 4 times faster than **8** may be taken as evidence for the importance of protonation of the amine nitrogen in denitrosation.

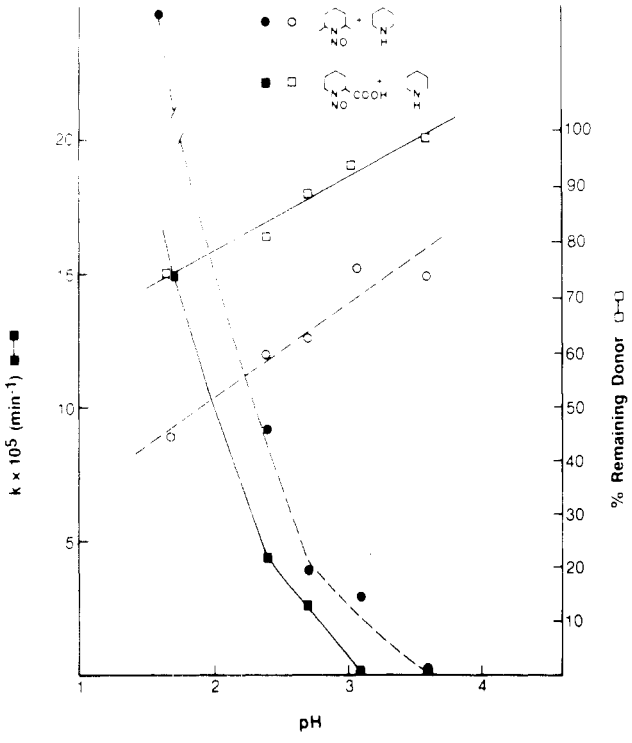


Figure 3. pH profile for the thiocyanate-catalyzed nitrosation of piperidine by two alicyclic nitrosamines.

Conclusions

Our studies of transnitrosation by aliphatic nitrosamines clearly show that several factors determine the strength of the N–N bond in a nitrosamine and hence the ability of any given compound to transnitrosate. The nitroso derivatives of weak bases such as the piperazines transnitrosate most rapidly. The presence of bulky substituents or COOH or CN substituents α to the nitroso group has considerable influence on rates of denitrosation. Perturbation of the π system of the *N*-nitroso group by α substituents is most pronounced in six-membered-ring systems, leading to marked acceleration of denitrosation rates in comparison with similarly substituted acyclic compounds.

There is no obvious relation between the ability of a given compound to act as a transnitrosating agent and that compound's carcinogenic activity (or lack of it). It is possible that for specific compounds transnitrosation may be a biologically important reaction, but the data allow no generalizations. It should be noted that many compounds that are noncarcinogenic in animal tests act as transnitrosating agents and consequently might form carcinogenic nitrosamines by reaction with amines found in the environment (e.g., morpholine). The conditions normally found in the human stomach (pH 1.5–3, presence of nucleophiles such as chloride or thiocyanate ions) are conducive to transnitrosation. This suggests that even nitrosamines that are noncarcinogenic in animal tests should not be regarded as inocuous, since many could act as in vivo nitrosating agents.

Experimental Section

Organic chemicals were obtained from Aldrich or Eastman and were generally not purified further. Inorganic chemicals were Fisher ACS reagent grade. Solvents were Eastman ACS reagent or (for high-pressure LC) Burdick and Jackson, "Distilled in Glass".

IR spectra were recorded on a Perkin-Elmer Model 297 spectrometer. UV spectra were obtained on a Beckman Acta IV spectrometer. High-pressure liquid chromatography was carried out on a Waters liquid chromatograph equipped with a Model 440 absorbance detector.

The *N*-nitroso compounds used were synthesized by standard methods as described elsewhere.[9-14,17,18,21]

Kinetics. The rate constant for denitrosation was determined for each compound in the following manner: the nitrosamine (0.25 mequiv) was dissolved in 5 mL of 1 N $HClO_4$ that was 0.05 M in NaSCN and had been warmed to 50 °C. Reaction was initiated by addition of $NH_4SO_3NH_2$ (0.25 mequiv). Aliquots were taken at timed intervals, and the reaction was quenched by 1/50 dilution with H_2O. When solubility problems were encountered, the denitrosation solution used was 2 N $HClO_4$ (that was 0.10 M in NaSCN) mixed with an equal volume of dioxane. (The use of the mixture containing dioxane had no effect on reaction rates.)

Several nitrosamines gave rate constants of 1×10^{-3} s^{-1} in 1 N $HClO_4$/0.05 M NaSCN. Denitrosations of these compounds were run in the following manner: the nitrosamine (0.25 mequiv) was dissolved in 3 N HCl (5 mL), $NH_4SO_3NH_2$ (0.25 mequiv) was added to initiate the reaction, and aliquots were taken and diluted 1/50 with H_2O.

Kinetics were followed by monitoring the disappearance of the nitrosamine by following the UV absorbance at 245 mM or by high-pressure LC on a Waters μBondapak C_{18} or a LICROSORB C_{18} column, using water/methanol as the eluant at a percentage suitable to elute the compound in 3–5 min. When the compound under test was an acid or base, water was replaced with pH 5.6 acetate buffer (10 mM).

Transnitrosation to Amines. The nitrosamine (0.25 mequiv), piperidinium perchlorate (2.5 mequiv), and NaSCN (2.5 mequiv) were dissolved in 0.1 N $HClO_4$ (5 mL) which had been warmed to 50 °C. Aliquots were taken at timed intervals and analyzed by high-pressure LC. The percent yields of nitrosopiperidine shown in Table I were measured after 18 h of reaction at 50 °C.

pH Profile. Solutions of *N*-nitroso-2,6-dimethylpiperidine (0.05 M) or *N*-nitrosopipecolic acid (0.05 M), NaSCN (0.5 M), and piperidinium formate (0.5 M) in formate buffer of appropriate pH were maintained at 50 °C for 18 h. Aliquots were taken and the samples analyzed by high-pressure LC as described previously (vide supra).

Acknowledgment. We thank Dr. W. Lijinsky for providing some of the nitroso compounds used. This work was supported by Contract No. NO1-CO-75380 with the National Cancer Institute, NIH, Bethesda, MD 20205.

Registry No. **1**, 5632-47-3; **2**, 140-79-4; **3**, 16339-07-4; **4**, 55380-34-2; **5**, 67774-31-6; **6**, 4515-18-8; **7**, 30310-80-6; **8**, 7519-36-0; **9**, 13256-22-9; **10**, 6238-69-3; *N*-nitrosomorpholine, 59-89-2; *N*-nitroso-*cis*-2,6-dimethylmorpholine, 69091-16-3; *N*-nitroso-*trans*-2,6-dimethylmorpholine, 69091-15-2; *N*-nitroso-3-methyl-2-phenylmorpholine, 34993-08-3; *N*-nitroso-2,6-dicyanopiperidine, 22905-25-5; *N*-nitroso-2,6-dimethylpiperidine, 17721-95-8; *N*-nitroso-2,2,6,6-tetramethylpiperidine, 6130-93-4; *N*-nitroso-2-ethylpiperidine, 14300-04-0; *N*-nitroso-2-*sec*-butylpiperidine, 75101-89-2; *N*-nitrosopiperidine, 100-75-4; *N*-nitroso-*O*,*N*-dimethylhydroxylamine, 16339-12-1.