

Inhibition of Nitrosation by Steric Hindrance

Samuel González-Mancebo, Emilio Calle, M. Pilar García-Santos, and Julio Casado*

Departamento de Química física, Facultad de Química, Universidad de Salamanca,
E-37008 Salamanca, Spain

The nitrosation of piperidine, 2-methylpiperidine, 4-methylpiperidine, 2,6-dimethylpiperidine, and 2-ethylpiperidine in acid medium was studied. Kinetic monitoring of the reactions was accomplished by spectrophotometric analysis of the nitrosamine formed at $\lambda = 249$ nm. In each case, the value of the rate constant of the determining step $R_2NH + N_2O_3 \rightarrow R_2NNO + HNO_2$ was calculated, observing the following sequence of reactivities: piperidine \approx 4-methylpiperidine $>$ 2-methylpiperidine \approx 2-ethylpiperidine $>$ 2,6-dimethylpiperidine. In the case of 2,6-dimethylpiperidine, no nitrosation was observed. The results are interpreted in terms of the steric hindrance of the alkyl groups to attack of the secondary amine group by dinitrogen trioxide. The results open the possibility of blocking the undesirable mechanisms of formation of *N*-nitroso compounds.

Keywords: Nitrosation; steric hindrance; piperidine and related compounds

INTRODUCTION

The mechanisms of formation of nitroso compounds have currently received increased attention owing to the carcinogenic, mutagenic, and teratogenic effects of many of them (Loeppky and Michejda, 1994; Preussmann and Steward, 1984; Mirvish, 1970, 1995).

Derivatives of piperidine and pyrrolidine, especially as alkaloids, are quite common in plants, and their *N*-nitroso derivatives would be expected to be formed by the nitrosation of such products (Lijinsky, 1992). The presence of nitrosopiperidine in pickling spice can be attributed to the nitrosation of piperine and other piperidine derivatives by the nitrite added to the spice mixture (Lijinsky et al., 1972). Exposure to the secondary amines dimethylamine, diethylamine, pyrrolidine, and piperidine is 7.4 mg/day (Tricker et al., 1994).

The endogenous formation of amines and volatile *N*-nitrosamines under certain clinical conditions is considerably higher than the exogenous exposure (Tricker et al., 1994). The anthelmintic agent piperazine, which undergoes rapid nitrosation (Bellander et al., 1984), is similar to the model compound chosen in this paper.

As a result, many attempts have been made, particularly in the field of food science, to block or inhibit the mechanisms of formation of these pathogenic species (Coultaite and Davies, 1994; Casado, 1982; Wilcox et al., 1991; Bao and Loeppky, 1991).

Day, in 1981, observed that the addition of flanking *tert*-butyl groups reduces the basicity of pyridine by a factor of 10 but that the same structural feature reduces the basicity of piperidine some 650 times. The dramatic reduction in the basicity of the secondary amine **b** seems only attributable to its conjugate acid (**b.H**⁺) having both acidic hydrogens sterically inaccessible to solvent H bonding. Gas-phase basicity measurements (pulsed ICR; Wolf et al., 1977) show that **b** is more basic than **a** by 8.5 kcal mol⁻¹ [relative to NH₃: $-\Delta G$ (kcal mol⁻¹) for NH₄⁺ + B \rightleftharpoons NH₃ + BH⁺].



- a. R=H
b. R= *t*-Bu
c. R= CH₃

In light of these findings and to investigate whether the introduction of alkyl groups into a potentially nitrosatable molecule is able to block its nitrosation, we conducted a kinetic study of the nitrosation of five cyclic secondary amines in acid medium: piperidine (PIP), 2-methylpiperidine (M2P), 4-methylpiperidine (M4P), 2,6-dimethylpiperidine (D26P), and 2-ethylpiperidine (E2P).

EXPERIMENTAL PROCEDURES

The substrates M2P, M4P, and PIP were from Merck and were 98% pure. D26P and E2P were from Aldrich and were 99% pure. *N*-Nitrosopiperidine was from Sigma. The other nitrosamines were prepared following procedures reported in the literature (Roberts and Ingold, 1973). The reagents, NaNO₂, NaClO₄ (for adjustment of ionic strength), and HClO₄ (for adjustment of acidity), were from Merck, *p.a.* Reactions were monitored kinetically by spectrophotometric analysis ($\lambda = 249$ nm) of the nitrosamines formed. We employed a Shimadzu UV-2101 double-beam apparatus with thermoelectric control to maintain temperature within ± 0.1 °C. The spectrophotometer was connected to a GRIDEC-386 computer for processing absorbance–time data. The thermostating chamber was equipped with six quartz cuvettes of 1.0 cm optical pathway, permitting several simultaneous kinetic measurements.

The initial rate method was used. In no case was 2% of the product formed reached.

RESULTS AND DISCUSSION

First order with respect to the initial substrate concentration, [amine]₀, was observed (Figure 1). Plotting of the v_0 /[nitrite]₀ values against those of [nitrite]₀ furnished straight lines with the intercept not significantly different from 0 (Figure 2); this points to second order with respect to the initial nitrite concentration, [nitrite]₀. Studying the variation in the rate of nitrosation with pH, we obtained the results shown in eq 1 and Figure 3.

$$v_0 = \alpha[H^+]/(\beta + [H^+])^2 \quad (1)$$

The results obtained afford the following kinetic equation:

$$v_0 = \alpha[\text{amine}]_0[\text{nitrite}]_0^2[H^+]/(\beta + [H^+])^2 \quad (2)$$

* Author to whom correspondence should be addressed.

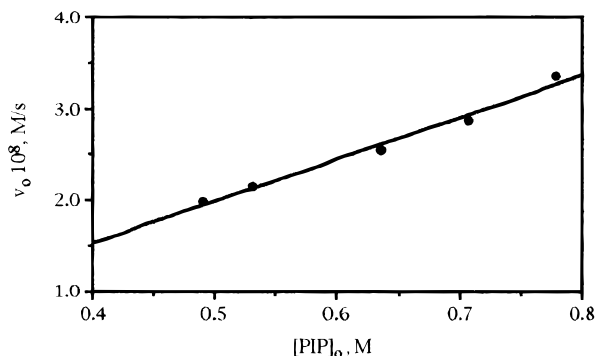
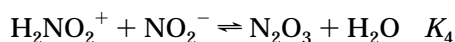
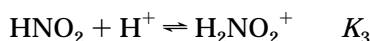
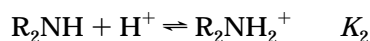
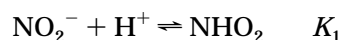


Figure 1. Influence of $[PIP]_0$ on the initial rate of PIP nitrosation: $[\text{nitrite}]_0 = 9.6 \times 10^{-3} \text{ M}$; $T = 25 \text{ }^\circ\text{C}$; $\text{pH} = 3.0$; $I = 2.0 \text{ M}$.

Second order with respect to $[\text{nitrite}]_0$ shows that the nitrosating species is N_2O_3 (Mirvish, 1970, 1975). The unvarying order of magnitude $10^8 \text{ M}^{-1} \text{ s}^{-1}$ found for the true rate coefficient for the *N*-nitrosation step when one is working with many secondary amines under conditions in which the effective nitrosating agent is thought to be dinitrogen trioxide indicates that the attack of the N_2O_3 upon free amines must be diffusion-controlled (Casado et al., 1983, 1994). This permits us to propose the following mechanism of reaction:



Since under the working conditions, $[\text{amine}]_0 = [\text{R}_2\text{NH}_2^+]$ and $[\text{nitrite}]_0 = [\text{NO}_2^-] + [\text{HNO}_2]$, the rate equation is readily achieved

$$v_0 = (kK_3K_4/K_1K_2)[\text{amine}]_0[\text{nitrite}]_0^2[\text{H}^+]/(1/K_1 + [\text{H}^+])^2 \quad (3)$$

in agreement with the experimental data.

Comparison of the experimental (eq 2) and theoretical (eq 3) rate equations affords the values of α and β and hence that of k , the attack constant of N_2O_3 on each substrate, since

$$\alpha = kK_3K_4/K_1K_2 = kK_m/K_2 \quad (4)$$

$$\beta = 1/K_1 \quad (5)$$

where $K_m [2\text{HNO}_2 = \text{N}_2\text{O}_3 + \text{H}_2\text{O}] = K_3K_4/K_1 = 3.03 \times 10^{-3} \text{ M}^{-1}$ (Markovits et al., 1981).

The values of parameters α and β were calculated using an optimization algorithm (Casado et al., 1978, 1979).

With the values of β it is possible to calculate that of K_1 and hence of K_2 , the $\text{p}K_a$ of the nitrous acid. Table 1 shows the values obtained when each of the amines was studied; when one compares these with the values reported by Tummavuori and Lumme in 1968 (thermodynamic value $\text{p}K_a = 3.148$ at zero ionic strength at $25 \text{ }^\circ\text{C}$), the agreement observed supports the proposed mechanism.

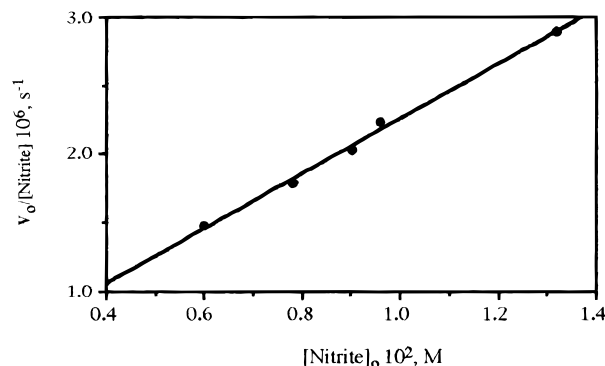


Figure 2. Influence of $[\text{nitrite}]_0$ on the initial rate of PIP nitrosation: $[PIP]_0 = 0.530 \text{ M}$; $T = 25 \text{ }^\circ\text{C}$; $\text{pH} = 3.0$; $I = 2.0 \text{ M}$.

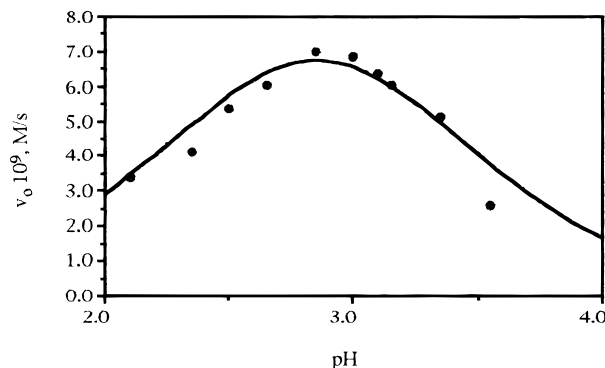


Figure 3. Influence of $[\text{H}^+]$ on the initial rate of PIP nitrosation: $[PIP]_0 = 0.332 \text{ M}$; $[\text{nitrite}]_0 = 7.0 \times 10^{-3} \text{ M}$; $T = 25 \text{ }^\circ\text{C}$; $I = 2.0 \text{ M}$.

Table 1. Values of k and $\text{p}K_a$ of Nitrous Acid

substrate	$\alpha \times 10^6$ ($\text{M}^{-1} \text{ s}^{-1}$)	$\beta \times 10^3$ (M)	$\text{p}K_a$	$k \times 10^{-8}$ ($\text{M}^{-1} \text{ s}^{-1}$)
PIP	2.3 ± 0.2	1.41 ± 0.10	2.85	1.3 ± 0.2
M2P	1.5 ± 0.3	1.22 ± 0.10	2.91	0.7 ± 0.3
M4P	2.5 ± 0.2	1.37 ± 0.10	2.85	1.0 ± 0.2
D26P				no reaction
E2P	1.6 ± 0.3	1.55 ± 0.20	2.83	0.8 ± 0.3

Table 2. Values of ΔH^\ddagger for the Reaction of N_2O_3 with Various Substrates^a

substrate	ΔH^\ddagger (kcal mol ⁻¹)	ΔH_a (kcal mol ⁻¹)	ΔH^\ddagger_{el} (kcal mol ⁻¹)
PIP	17.5 ± 0.5	13.2 ± 0.1	2.7 ± 0.5
M2P	20.8 ± 0.7	14.0 ± 0.1	5.2 ± 0.7

^a ΔH^\ddagger , experimental enthalpies of activation; ΔH_a , enthalpies of protonation; ΔH^\ddagger_{el} , enthalpies of activation of slow step.

Using the values of α and β we calculated that of k , the attack constant of the dinitrogen trioxide on each substrate (step five of the mechanism). The results are shown in Table 1.

The order of magnitude found for k lies within the range of values accepted for reactions that occur at the encounter-controlled limit (Ridd, 1978). [The definition of α means that knowledge of ΔH^\ddagger is not immediate; it is necessary to know the values of the protonation enthalpies, ΔH_a , of the amines and of that associated with the K_m equilibrium. The former have been determined by Berthon et al. in 1977 and the latter, $\Delta H_m = 1.6 \text{ kcal mol}^{-1}$, has been calculated by our team (Mira, 1989).]

As may be seen, alkylation of piperidine leads to a considerable increase in the activation enthalpy of the nitrosation reaction by N_2O_3 (Table 2), causing a reduction in the reaction rate (Table 1). In the case of double

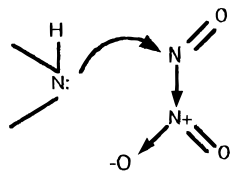


Figure 4. Interaction substrate-nitrosating agent.

methylation (2,6-dimethylpiperidine) this means that, under the working conditions, no nitrosation reaction occurs.

This pronounced decrease in the reactivity of the piperidine molecule against N_2O_3 , as a result of its alkylation, could be understood in terms of steric hindrance.

In the case of the reactions studied here, the approximation and interaction of the amine would take place with the N_2O_3 molecule (Figure 4). The steric hindrance would now arise from the size and configuration of the nitrosating agent rather than from the substituent R of the nitrosatable substrate.

Along this line of enquiry, the reactivity of nitrosatable substrates (expressed in terms of k) should decrease in the following order: piperidine \approx 4-methylpiperidine $>$ 2-methylpiperidine \approx 2-ethylpiperidine $>$ 2,6-dimethylpiperidine.

Comparison of the results obtained from study of the nitrosation of 2-methylpiperidine and 2-ethylpiperidine reveals almost identical reactivities, supporting the notion that the steric hindrance would mainly be due to the size of the dinitrogen trioxide.

We conclude that the addition of flanking alkyl groups to piperidine reduces or even prevents the nitrosations.

ACKNOWLEDGMENT

We thank the reviewers for their kind comments.

LITERATURE CITED

- Bao, Y. T.; Loeppky, R. N. Blocking nitrosamine formation with polymers. *Chem. Res. Toxicol.* **1991**, *4*, 382.
- Bellander, B. T. D.; Hagmar, L.; Österdahl, B. G. Nitrosation of piperazine in man, in *N-Nitroso Compound: Occurrence, Biological Effects and Relevance to Human Cancer*; IARC Scientific Publication 57; IARC: Lyon, France, 1984; pp 171–78.
- Berthon, G.; Blais, M. J.; Enea, O. The influence of solvent upon linear enthalpy-entropy relationships. Proton ionization of substituted piperidines in water-ethanol mixtures. *J. Phys. Chem.* **1977**, *81*, 1991.
- Casado, J., Ed. Proceedings of the Bilateral Hispano-British Seminar on Mechanisms of Formation of Carcinogenic Nitroso Compounds. *Acta Cient. Compost.* **1982**, *19*.
- Casado, J. Nitrosation reactions. Invited lecture. In *Fast Reactions in Solution*, Discussion Group, Annual Meeting, University of Burgos; Royal Society of Chemistry: London, 1994.
- Casado, J.; Castro, A.; López-Quintela, M. A.; Cachaza, J. M. Kinetic studies on the formation of nitrosamines. I. Formation of dimethylnitrosamine in aqueous solution of perchloric acid. *Z. Krebsforsch.* **1978**, *91*, 279.
- Casado, J.; Castro, A.; López-Quintela, M. A.; Rodríguez Prieto, M. F. Structure-reactivity correlations in nitrosation reactions of secondary amines by alkyl nitrites in basic media. *Z. Phys. Chem. Neue Folge* **1979**, *118*, 43.
- Casado, J.; Castro, A.; Leis, J. R.; López-Quintela, A.; Mosquera, M. Kinetic studies on the formation of *N*-nitroso compounds. VI. The reactivity of N_2O_3 as a nitrosating agent. *Monatsh. Chem.* **1983**, *114*, 639.
- Coulter, T.; Davies, J. *Food, The Definitive Guide*; Royal Society of Chemistry: London, 1994.
- Day, J. C. Severe steric hindrance: hydrogen bonding and the consequences of its inhibition. Equilibrium hydrogen isotope effects. *J. Am. Chem. Soc.* **1981**, *103*, 7355.
- Lijinsky, W. *Chemistry and Biology of N-nitroso Compounds*; Cambridge Monographs on Cancer Research; Cambridge University Press: Cambridge, U.K., 1992.
- Lijinsky, W.; Conrad, E.; Van de Bogart, R. Formation of carcinogenic nitrosamines by interaction of drugs with nitrite. In *N-Nitroso Compounds, Analysis and Formation*; IARC Scientific Publication 3; IARC: Lyon, France, 1972; pp 130–33.
- Loeppky, R. N.; Michejda, C. J., Eds. *Nitrosamines and Related N-Nitroso Compounds, Chemistry and Biochemistry*; ACS Symposium Series 553; American Chemical Society: Washington, DC, 1994.
- Markovits, G. Y.; Schwartz, S. E.; Newman, L. Hydrolysis equilibrium of dinitrogen trioxide in dilute acid solution. *Inorg. Chem.* **1981**, *20*, 445.
- Mira, J. S. Thesis of Licenciatura (Sp), University of Salamanca, 1989.
- Mirvish, S. S. Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. *J. Natl. Cancer Inst.* **1970**, *44*, 633.
- Mirvish, S. S. Formation of *N*-nitroso compounds: chemistry, kinetics, and *in vivo* occurrence. *Toxicol. Appl. Pharmacol.* **1975**, *31*, 325.
- Mirvish, S. S. Role of *N*-nitroso compounds (NOC) and *N*-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to *NOC*. *Cancer Lett.* **1995**, *93*, 17.
- Preussmann, R.; Steward, B. W. *N*-Nitroso carcinogens. In *Chemical Carcinogens*; Searle, C., Ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984, pp 643–828.
- Ridd, J. H. Diffusion control and preassociation in nitrosation, nitration and halogenation. *Adv. Phys. Org. Chem.* **1978**, *16*, 1.
- Roberts, J. R.; Ingold, K. U. Kinetic applications of electron paramagnetic resonance spectroscopy. X. Reactions of some alkylamino radicals in solution. *J. Am. Chem. Soc.* **1973**, *95*, 3228.
- Tricker, A. R.; Pfundstein, B.; Preussmann, R. Nitrosatable secondary amines. Exogenous and endogenous exposure and nitrosation *in vivo*. In *Nitrosamines and Related N-Nitroso Compounds, Chemistry and Biochemistry*; Loeppky, R. N., Michejda, C. J., Eds.; ACS Symposium Series 553; American Chemical Society: Washington, DC, 1994; pp 93–99.
- Tummavuori, J.; Lumme, P. Protolysis of nitrous acid in aqueous sodium nitrate and sodium nitrite solutions at different temperatures. *Acta Chem. Scand.* **1968**, *22*, 2003.
- Wilcox, A. L.; Bao, Y. T.; Loeppky, R. N. Pyrroles as effective agents for blocking amine nitrosation. *Chem. Res. Toxicol.* **1991**, *4*, 373.
- Wolf, J. F.; Staley, R. H.; Koppel, I.; Taagepera, M.; McIver, R. T., Jr.; Beauchamp, J. L.; Taft, R. W. Gas phase basicities and relative proton affinities of compounds between water and ammonia from pulsed ion cyclotron resonance thermal equilibria measurements. *J. Am. Chem. Soc.* **1977**, *99*, 5417.

Received for review May 30, 1996. Revised manuscript received November 6, 1996. Accepted November 11, 1996.® We thank the Commission of the European Community for supporting the research reported in this paper as part of a project on the nitrosable molecules present in foodstuffs (Contract 93CVVF1-610-0). We also thank the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) for its support of an investigation on precursor mechanisms of carcinogenesis by *N*-nitroso compounds (Project ALI389/90), the Spanish Ministerio de Educación y Ciencia, and the University of Salamanca for financial assistance to S.G.-M. and M.P.G.-S., respectively.

JF960371S

® Abstract published in *Advance ACS Abstracts*, January 1, 1997.