Inhibition of Nitrosation by Steric Hindrance

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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 (Coultate 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 NH_3: $-\Delta G$ (kcal mol^{-1}) for $NH_4^+ + B \rightleftharpoons NH_3 + BH^+$].

a. R=H
b. R= t-Bu
c. R= CH_3

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, $NaNO_2$, $NaClO_4$ (for adjustment of ionic strength), and $HClO_4$ (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:

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

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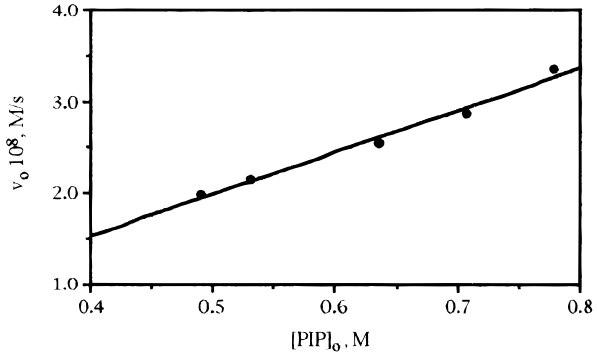

Figure 1. Influence of $[PIP]_0$ on the initial rate of PIP nitrosation: $[nitrite]_0 = 9.6 \times 10^{-3}$ M; $T = 25$ °C; pH = 3.0; $I = 2.0$ M.

Second order with respect to $[nitrite]_0$ shows that the nitrosating species is N_2O_3 (Mirvish, 1970, 1975). The unvarying order of magnitude 10^8 M^{-1} 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:

$$NO_2^- + H^+ \rightleftharpoons NHO_2 \qquad K_1$$

$$R_2NH + H^+ \rightleftharpoons R_2NH_2^+ \qquad K_2$$

$$HNO_2 + H^+ \rightleftharpoons H_2NO_2^+ \qquad K_3$$

$$H_2NO_2^+ + NO_2^- \rightleftharpoons N_2O_3 + H_2O \qquad K_4$$

$$R_2NH + N_2O_3 \rightarrow R_2NNO + HNO_2 \qquad k \text{ slow}$$

Since under the working conditions, $[amine]_0 = [R_2NH_2^+]$ and $[nitrite]_0 = [NO_2^-] + [HNO_2]$, the rate equation is readily achieved

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

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 $[2HNO_2 = N_2O_3 + H_2O] = K_3K_4/K_1 = 3.03 \times 10^{-3}$ 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 pK_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 $pK_a = 3.148$ at zero ionic strength at 25 °C), the agreement observed supports the proposed mechanism.

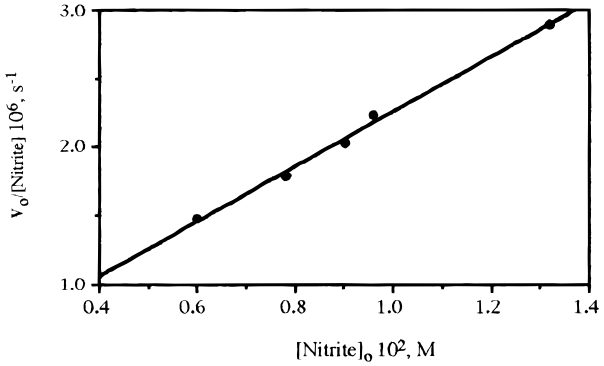

Figure 2. Influence of $[nitrite]_0$ on the initial rate of PIP nitrosation: $[PIP]_0 = 0.530$ M; $T = 25$ °C; pH = 3.0; $I = 2.0$ M.

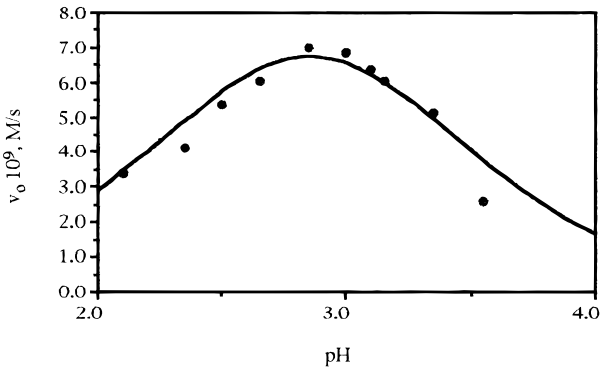

Figure 3. Influence of $[H^+]$ on the initial rate of PIP nitrosation: $[PIP]_0 = 0.332$ M; $[nitrite]_0 = 7.0 \times 10^{-3}$ M; $T = 25$ °C; $I = 2.0$ M.

Table 1. Values of *k* and pK_a of Nitrous Acid

substrate	$\alpha \times 10^6$ (M^{-1} s^{-1})	$\beta \times 10^3$ (M)	pK_a	$k \times 10^{-8}$ (M^{-1} 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 $\Delta H^{\#}$ for the Reaction of N_2O_3 with Various Substrates[a]

substrate	$\Delta H^{\#}$ (kcal mol^{-1})	ΔH_a (kcal mol^{-1})	$\Delta H^{\#}_{el}$ (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] $\Delta H^{\#}$, experimental enthalpies of activation; ΔH_a, enthalpies of protonation; $\Delta H^{\#}_{el}$, 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 $\Delta H^{\#}$ 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, $\Delta H_m = 1.6$ 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

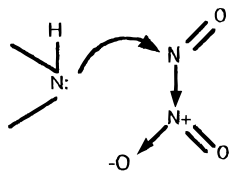

Figure 4. Interaction substrate-nitrosating agent.

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 ≈ 4-methylpiperidine > 2-methylpiperidine ≈ 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.

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