Studies on Internal Nitroso Group Transfer. Nitrosation of Thiomorpholine

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The kinetics of nitrosation of thiomorpholine have been studied under different experimental conditions. At low nitrite concentration and high acidity the reaction rate is first order with respect to nitrite and thiomorpholine and independent of acidity. The results are interpreted through a mechanism in which the *S*-nitroso intermediate with a deprotonated amino group must acquire a boat conformation to facilitate the migration of the NO group from the sulphur to the nitrogen atom. The mechanism is supported by the low isotope effect observed when the reaction is studied in D_2O. In the pH range 2.3–3.6 and high nitrite concentration, the reaction rate is second order with respect to nitrite, the accepted mechanism being the nitrosation of deprotonated thiomorpholine by dinitrogen trioxide.

When a substrate susceptible to *N*-nitrosation (and with its nitrogen atom protonated) possesses a second nucleophilic position, the attack of the nitrosating agent can occur at this second centre, the final *N*-nitroso compound being obtained after an internal rearrangement of the nitroso group. This second centre can be an aromatic ring (as in nitrosation of aromatic amines at high acidities),[1–4] an oxygen atom (as in the hydroxylamine–nitrous acid reaction at high acidity),[5,6] a nitrogen atom (as in the hydrazine–nitrous acid reaction),[6,7] or a sulphur atom (as in nitrosation of thioproline).[8]

This behaviour is also found when the unprotonated nitrogen atom is less nucleophilic than other atoms in the molecule. Typical examples are the nitrosation of amides and ureas,[9–11] thioureas,[12] and tryptophan[13] which can be described as *O*-, *S*-, and *C*-nitrosation, respectively.

Being interested in the study of these internal nitroso-group transfers and having in mind our previous study on thioproline nitrosation,[8] we decided to investigate the thiomorpholine (TM)–nitrous acid reaction for the following reasons. First, the relative sulphur–nitrogen position in the ring is 1–3 for thioproline and 1–4 for TM. The corresponding transition states for internal NO migration would be expected to be a bicyclo[2.1.1] for thioproline and a bicyclo[2.2.1] for TM. From molecular models it seems that the second bicyclo transition state has a more favourable conformation. Second, the nitrosation of morpholine is well known[14,15] and could be used as a reference.

On the other hand, the existence of a sulphur atom at the heterocyclic ring modifies the carcinogenic and mutagenic properties of the corresponding secondary amines[16] and the knowledge of its *in vitro* formation (decomposition) could be useful for future *in vivo* work.

Experimental

The inorganic chemicals (Merck, *pro analisi* grade) were used without further purification. Thiomorpholine (Aldrich) was recrystallized as its perchlorate from propan-2-ol–diethyl ether and *N*-methylpiperidinium perchlorate from propan-2-ol. D_2O (99.8%) was supplied by the Spanish Junta de Energía Nuclear.

The kinetic measurements were carried out in a Kontron model Uvikon-820 spectrophotometer with a thermostatically controlled cell holder. The temperature (25.0 ± 0.1 °C) was kept constant with a Hetofrig thermostat. Acidity was measured using a Radiometer PHM-82 pH meter with a GK2401C combined electrode calibrated with standard buffer solutions (Merck) of pH 4 and 7.

The appearance of *N*-nitrosothiomorpholine was followed at λ 249 nm, its molar absorptivity being 3 520 $dm^3 mol^{-1} cm^{-1}$. The percentage of the reaction never exceeded 2% and a plot of absorbance *versus* time gave a straight line, its slope being the initial reaction rate.

Values of 8.48 and 8.82 for the pK_a of TM in H_2O and D_2O were obtained potentiometrically.

Results and Discussion

At $[H^+] = 0.1$ mol dm^{-3}, [nitrite] = 5.27×10^{-3}, and ionic strength 0.3 mol dm^{-3} ($NaClO_4$), the influence of the concentration of TM on the initial reaction rate was investigated. The obtained results (Figure 1) obey the rate equation (1):

$$r_0 = a[\text{total amine}]^2/(1 + b[\text{total amine}]) \quad (1)$$

where a and b are $(2.07 \pm 0.42) \times 10^{-4}$ $dm^3 mol^{-1} s^{-1}$ and $(3.25 \pm 0.65) \times 10^2$ $dm^3 mol^{-1}$, respectively.

A similar dependence of r_0 upon [amine] was found in the nitrosation of morpholine by nitroprusside ion,[17] however, it was demonstrated that this was not in fact a real rate equation and that a medium effect was involved. Other similar cases are described in the literature.[18] We tested this possibility in our system by using a different electrolyte to adjust the ionic strength, *N*-methylpiperidinium perchlorate being chosen for its similarity to thiomorpholinium perchlorate. We have corroborated that under the experimental conditions used, this tertiary amine does not react with nitrous acid. The reaction rate depends upon the electrolyte used, being *e.g.* 0.5 mol dm^{-3} 10% higher in $NaClO_4$. All the results presented here were obtained using *N*-methylpiperidinium perchlorate as the electrolyte. Figure 2 shows that the reaction rate is really first order in TM.

Table 1 shows some results obtained under different experimental conditions. They fit equation (2), where $k(H_2O)$ is $(5.70 \pm 0.18) \times 10^{-4}$ $dm^3 mol^{-1} s^{-1}$ ($\mu = 0.5$ mol dm^{-3}).

$$r_0 = k(H_2O)[\text{nitrite}][\text{total amine}] \quad (2)$$

This reaction rate equation is compatible with a mechanism in which the rate-limiting step is the attack of nitrosonium (or nitrous acidium) ion, formed by protonation of nitrous acid (equilibrium constant[19] $K_1 = 3 \times 10^{-7}$ $dm^3 mol^{-1}$), on free amine, with $k = k(H_2O)/(K_aK_1)$ as the kinetic constant. However, this gives a value of $k = 5.7 \times 10^{11}$ $dm^3 mol^{-1} s^{-1}$ which is

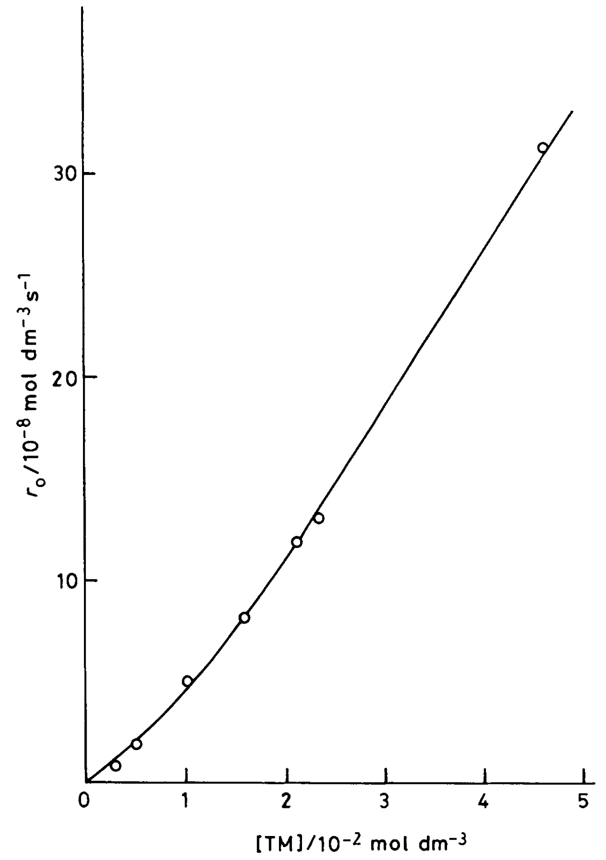


Figure 1. Dependence of initial rate of TM nitrosation on TM concentration at [nitrite] = 5.27×10^{-3} mol dm^{-3} and [H^+] = 0.100 mol dm^{-3} at 25 °C and μ = 0.3 mol dm^{-3} ($NaClO_4$).

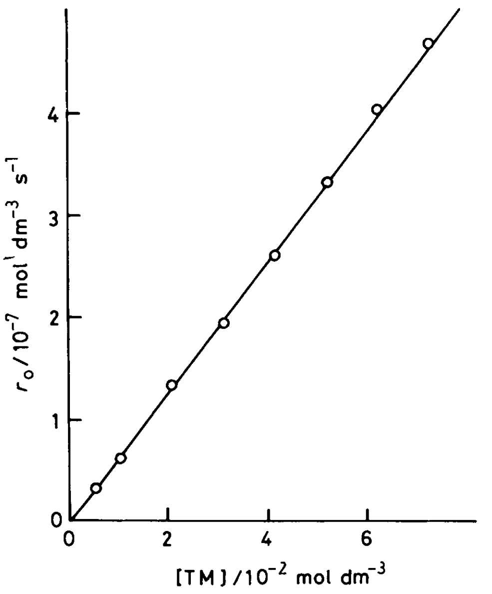
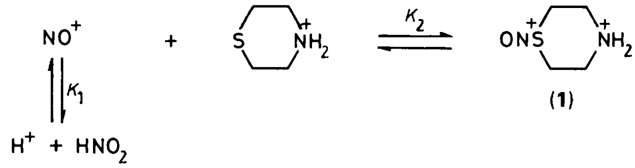


Figure 2. Dependence of initial rate of TM nitrosation on TM concentration at [nitrite] = 0.0108 mol dm^{-3} and [H^+] = 0.100 mol dm^{-3} at 25 °C and μ = 0.5 mol dm^{-3}. (*N*-Methylpiperidinium perchlorate).

one hundred times the maximum accepted value for a diffusion-controlled process.[19] Therefore, this reaction mechanism must be ruled out.

The reaction mechanism cannot be the same as that proposed for thioproline[8] in which the rate-limiting step is the loss of a proton from the intermediate formed by the protonated amine and the nitrosating agent, since it is not compatible with the experimental rate equation.

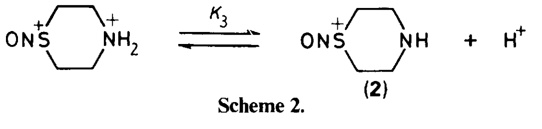
As has been proposed for other thiocompounds,[8,12,20] we accept that the initial step is the formation of the *S*-nitroso intermediate (**1**) in equilibrium with the reactants (Scheme 1).



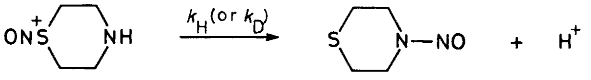
Scheme 1.

Although we have looked for direct spectrophotometric evidence for sulphur–nitroso species formation, we have not been able to find it. As we have pointed out, there is evidence in the literature for the formation of some $\overset{+}{S}$–N=O species. This difference may be due to a very low value for the formation equilibrium constant (K_2 in Scheme 1) as consequence of an unusually fast decomposition reaction because of the double positive charge on intermediate (**1**).

We can allow an acid–base equilibrium of this intermediate with its deprotonated form (Scheme 2). It is expected that the pK_a of this intermediate is lower than that corresponding to TM, although the effect of the NO group cannot be as dramatic as it is in *N*-nitrosoamines.[21] The reaction rate is compatible with a mechanism in which the rate-limiting step (*k* in Scheme 3)



Scheme 2.



Scheme 3.

is: (*a*) internal rearrangement of the NO group from sulphur to nitrogen in intermediate (**2**), followed by a fast hydrogen transfer from the amino group to the solvent; (*b*) simultaneous rearrangement and loss of a proton; and (*c*) later rearrangement of the nitroso group by the loss of a proton. To distinguish between these three possibilities we studied the reaction in D_2O since no primary isotope solvent effect should be observed in case (*a*), a large one in case (*c*), and a rather low effect in case (*b*).

Table 2 shows the results obtained in D_2O, the kinetic constant value being $k(D_2O) = (3.94 \pm 0.24) \times 10^{-4}$ dm^3 mol^{-1} s^{-1}. According to the proposed mechanism it follows the ratio

$$\frac{k(H_2O)}{k(D_2O)} = \frac{k_H(K_1K_2K_3)_{H_2O}}{k_D(K_1K_2K_3)_{D_2O}} \qquad (3)$$

where $k(H_2O)/k(D_2O)$ is 1.49 ± 0.09. The isotope effect for the formation of NO^+ has recently been revised[22] and the ratio $K_1(D_2O)/K_1(H_2O) = 2.55 \pm 0.25$ has been proposed. The equilibrium defined by K_2 should not exhibit isotope effects, *i.e.*, $K_2(H_2O)/K_2(D_2O) = 1$ and for the ratio $K_3(H_2O)/K_3(D_2O)$ we can accept that the corresponding isotope effect on the acidity constant of TM is 2.2 (see Experimental). This proposal is supported by the difference $\Delta pK_a = pK_a(D_2O) - pK_a(H_2O)$ which is almost constant for amines of different pK_a values and equal to 0.48.[23] From equation (3), we conclude that k_H/k_D =

Table 1. Initial rate values for the nitrosation of TM at different nitrite, acidity, and TM concentrations at 25 °C and $\mu = 0.5$ mol dm^{-3}.

[nitrite]/mol dm^{-3}	[H$^+$]/mol dm^{-3}	[TM]/mol dm^{-3}	r_0/10^{-7}mol dm^{-3} s^{-1}
0.0108	0.119	0.0210	1.16
0.0108	0.181	0.0210	1.22
0.0108	0.243	0.0210	1.21
0.0108	0.305	0.0210	1.21
0.0108	0.367	0.0210	1.20
0.0108	0.429	0.0210	1.27
0.0108	0.460	0.0210	1.27
0.0108	0.491	0.0210	1.25
0.001 03	0.0930	0.009 75	0.0592
0.002 05	0.0930	0.009 75	0.0990
0.003 08	0.0930	0.009 75	0.176
0.004 10	0.0930	0.009 75	0.230
0.005 13	0.0930	0.009 75	0.292
0.006 15	0.0930	0.009 75	0.357

Table 2. Initial rate values for the nitrosation of TM at different nitrite, acidity, and TM concentrations at 25 °C and $\mu = 0.5$ mol dm^{-3} in D_2O.

[nitrite]/mol dm^{-3}	[H$^+$]/mol dm^{-3}	[TM]/mol dm^{-3}	r_0/10^{-7}mol dm^{-3} s^{-1}
0.005 61	0.180	0.0296	4.42
0.005 61	0.180	0.0412	9.08
0.005 61	0.180	0.0825	20.9
0.002 80	0.183	0.0206	2.25
0.001 69	0.169	0.0206	12.6
0.005 61	0.211	0.0206	4.76
0.005 61	0.149	0.0206	4.38
0.005 61	0.242	0.0206	4.68

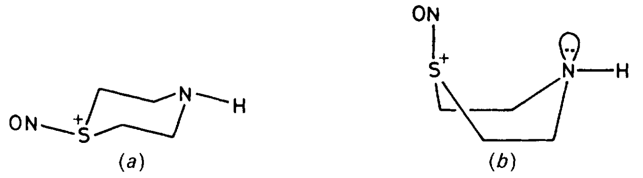


Figure 3. Chair and boat conformations of *S*-nitroso-deprotonated intermediate (**2**) (see text).

1.67 ± 0.11. We can understand this rather low value for a primary solvent isotope effect in terms of hypothesis (*b*) in which the loss of the proton is only a partial rate-determining step in the reaction. The conformation thermodynamically more favourable for the intermediate (**2**) must be one with the nitroso group and the hydrogen atom of the amino group in equatorial positions [see Figure 3(*a*)]. The internal transfer of the nitroso group from sulphur to nitrogen atoms requires a boat conformation [see Figure 3(*b*)]. For cyclohexane[24] the energy difference between both conformations is 30 kJ mol^{-1} and the activation energy is 46 kJ mol^{-1}. Since these figures correspond to the strain in a six-membered ring, similar values for the energy difference between conformations (**3a**) and (**3b**) are to be expected. This activation energy is large enough to partially control the reaction rate and therefore the loss of the proton is a rather faster later step. This is not the case for linear (non-rigid) molecules or ring molecules in which no special conformation is required for NO transfer (*e.g.* in thioproline) as can be seen from molecular models.

Furthermore, the experimental isotope effect does not support the notion that attack of NO^+ on the free amine is the rate-limiting step. A simple calculation shows that if we accept that this step should be diffusion controlled (which is the case for amines)[19] the experimental $k(H_2O)/k(D_2O)$ ratio should be 0.70 instead of 1.49.

There are no activation energy values in the literature to corroborate the previous ideas but for future discussions we have measured the activation energy for the total process; this value is 86 kJ mol^{-1}.

Furthermore, under the following experimental conditions, pH 2.3–3.6, [nitrite] 0.014–0.0245 mol dm^{-3}, and [total amine] 2.3 × 10^{-4} mol dm^{-3}, we have carried out several series of experiments from which the reaction kinetics are assumed to be described by equation (4).

$$r_0 = \frac{k[\text{nitrite}]^2[\text{total amine}][\text{H}^+]}{(k_a + [\text{H}^+])^2} \qquad (4)$$

Figure 4 shows a plot of [H$^+$] *versus* $([\text{H}^+]/(r_0))^{\frac{1}{2}}$, as required by equation (4). From the intercept:slope ratio a value of 3.17 ± 0.04 for the pK_a of nitrous acid was deduced. This value is comparable to previous published figures.[25] This same reaction rate equation has been found for other secondary amines,[26,27] and the proposed mechanism implies, as a rate-determining step, the attack of dinitrogen trioxide on free amine. The value for the kinetic constant is (6.0 ± 0.3) × 10^7 dm^3 mol^{-1} s^{-1} which is comparable to the value for morpholine[14] (see references 26 and 27 for a more detailed calculation of the kinetic constant). These results indicate that the nitrosation of TM by N_2O_3 is a normal nitrosation of a secondary amine and no further studies were carried out.

A final question concerns the fact that NO^+ (or $NO_2H_2^+$) can distinguish between sulphur and nitrogen atoms; this is not the case with the neutral nitrosating agents N_2O_3 (this paper) and NOBr.[8] No theoretical calculations have been performed for the species studied here but we can refer to the results published by Jørgensen and co-workers for 2-(methylthio)ethylamine[28] and different nitrosating agents.[29] Jørgensen concludes that the nitrosyl cation prefers to attack sulphur by an orbital-controlled reaction, rather than nitrogen by a charge-controlled reaction. For this substrate, the first ($E = -8.78$ eV) and second ($E = -10.53$ eV) HOMOs are

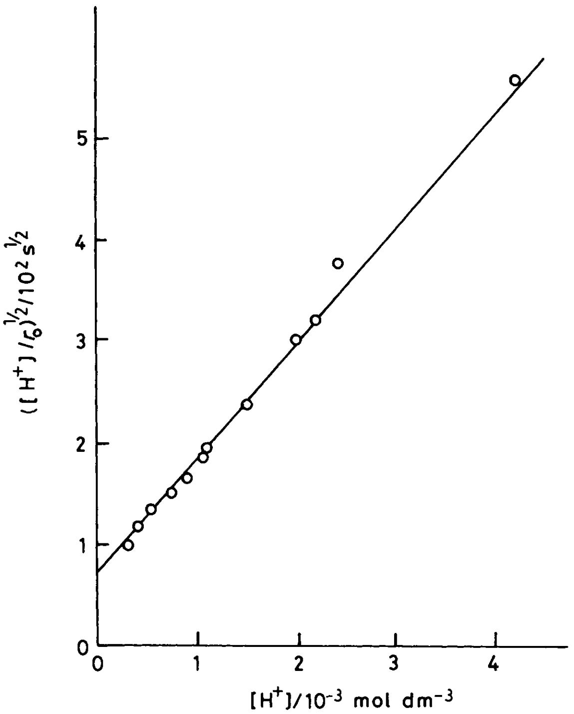


Figure 4. Linearization of the acidity dependence of the initial rate for the nitrosation of TM according to equation (4) at [nitrite] = 0.0245 mol dm^{-3} and [TM] = 2.30×10^{-4} mol dm^{-3}, at 25 °C and $\mu = 0.1$ mol dm^{-3}.

related to the sulphur and nitrogen atoms, respectively. Therefore, the energy difference between both HOMOs is 1.75 eV which is comparable to the value of 0.88 eV corresponding to the difference between the LUMO energy of NO^+ ($= -8.31$ eV) and the average energy of both HOMOs ($= -9.66$ eV). It means that NO^+ can distinguish between those orbitals and the reaction will take place with that orbital which originates a more favourable decrease in frontier orbital energy,[30,31] *i.e.*, the first HOMO.[28] LUMO energies[29] for NOCl and NCSNO, which are two common uncharged nitrosating agents, are 3.78 and 4.80 eV, respectively. Now, the average difference $E_{HOMO} - E_{LUMO}$ is 13.44 for NOCl and 14.46 eV for NCSNO. Consequently, two effects are to be expected: first, a neutral nitrosating agent will not distinguish between the first and second HOMO of the nitrosatable substrate; second, the substrate-neutral nitrosating agent reaction will be a charge-controlled reaction.[30] Therefore, the behaviour of neutral and cationic nitrosating agents are very different. Although different values for HOMO and LUMO energies will be involved for species studied in this paper, it is to be expected that the conclusions will be the same as those presented here.

Acknowledgements

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