Kinetics and mechanism of the formation and reactions of *S*-nitroso derivatives of some heterocyclic thiones

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Rate and equilibrium measurements have been obtained for the nitrosation (using nitrous acid in dilute acid aqueous solution) of the following thione–thiol nitrogen heterocyclic species and for the decomposition reactions of the formed *S*-NO^+ ions: 4-mercaptopyridine (**1**), 2-mercaptopyridine 1-oxide (**2**), 2-mercaptopyrimidine (**3**) and 2-mercapto-1*H*-imidazole (**4**). The nitrosation reactions were all rapid and rate constants were obtained by stopped-flow spectrophotometry. In all cases kinetic analysis showed that the halide ion-catalysed and the uncatalysed reactions occurred at or close to the diffusion-controlled limit and the reactive form of the substrate is its thione form. In some cases at high [nitrous acid] reaction occurred *via* N_2O_3 where the rate-limiting step is that of N_2O_3 formation. Similarly reaction of **1** and **3** in the presence of thiocyanate showed that reaction takes place *via* rate-limiting formation of ONSCN. All of the nitrosation reactions were somewhat reversible, with large equilibrium constants of 2.0×10^7, $\sim 4 \times 10^5$, 1.7×10^3 and 1.5×10^5 dm^6 mol^{-2} respectively for **1**, **2**, **3**, and **4**. All of the *S*-NO^+ ions decomposed rapidly in solution generating the disulfides and nitric oxide. This reaction was monitored only for the *S*-NO^+ derived from **1** and **2** because of stability problems. The deprotonated form of the *S*-nitroso product from **1** was identified from measurements at pH 7.4 and the pK_a value of the protonated form estimated as 4.5. It decomposed fairly rapidly to give the thione. We report some results where thiones show considerable catalytic activity in nitrosation reactions of *N*-methylaniline and ascorbic acid.

There continues to be a major interest in *S*-nitrosation, particularly of thiols, since the product *S*-nitrosothiols (or thionitrites) RSNO, are under active consideration as nitric oxide donors for therapeutic use, and they are also currently believed to be involved as carriers of NO *in vivo*.[1] Thiols generally react rapidly with all of the conventional electrophilic nitrosating species[2] mostly, but not exclusively, in aqueous acid solution. The simplest reagent is NO^+ (or $H_2NO_2^+$) generated from nitrous acid itself [eqn. (1)].[3]

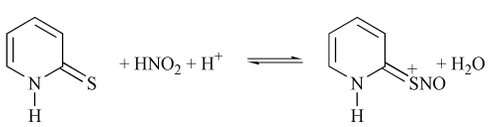
$$RSH + HNO_2 \rightleftharpoons RSNO + H_2O \qquad (1)$$

The reaction is very slightly reversible[4] with equilibrium constants in the range 10^5–10^6 mol dm^{-3}. Other reagents (XNO) can often be generated *in situ* by the addition of X^-, and produce pronounced catalytic effects in many cases. There is no complication arising from substrate protonation, which is usually the case with amine nitrosation. Thiocarbonyl compounds, notably thiourea derivatives, also undergo ready nitrosation. These reactions [eqn. (2)] generate *S*-nitroso

$$(NH_2)_2CS + HNO_2 + H^+ \rightleftharpoons (NH_2)_2CSNO^+ + H_2O \qquad (2)$$

cations, which have only a limited stability, but are sufficiently long-lived to allow rate and equilibrium constant determinations of the nitrosation reaction to be carried out. These reactions are also somewhat reversible, with the equilibrium constant for thiourea itself having a value of 5000 dm^6 mol^{-2} at 25 °C. Mechanistic work on the nitrosation of thiourea derivatives has been reported in a series of papers by Stedman and co-workers.[5,6]

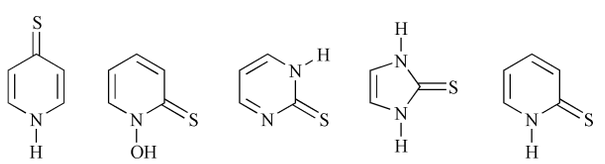
Recently we examined the kinetics and mechanism of the nitrosation of 2-mercaptopyridine (tautomer of pyridine-2(1*H*)-thione).[7] Reaction was rapid and occurred *via* the thione form of the reactant [eqn. (3)], generating the *S*-NO^+ cation. In



(3)

common with other *S*-nitrosation reactions the change was reversible, and the reactant was the dominant form at equilibrium with low [H^+] and low [HNO_2]. The equilibrium constant determined from spectral and kinetic measurements was found to be 1.5×10^5 dm^6 mol^{-2}. In acid solution the *S*-NO^+ cation decomposed to give di-2-pyridyl disulfide and NO, whereas quenching of the cation at pH 7.3 gave initially the deprotonated (aromatic) form *S*-NO, which decomposed fairly rapidly in parallel reactions leading to 2-mercaptopyridine (75%) and the disulfide (12%).

In this paper we have set out to examine an extended range of heterocyclic thiols to establish the generality of the chemistry in this area. We chose four substrates all of which were commercially available, (a) 4-mercaptopyridine, **1**, (b) 2-mercaptopyridine 1-oxide, **2**, (c) 2-mercaptopyrimidine, **3**, and (d) 2-mercapto-1*H*-imidazole, **4**, all shown here in their tautomeric thione forms. These substrates are also of some biological interest since they are good models for thiopurine and ergothioneine (a naturally occurring antioxidant). Compound **1** was chosen so that comparison could be made with the chemistry of 2-mercaptopyridine, **5**, and thus the influence of a ring-nitrogen atom *ortho* to the sulfur atom could be measured. Compound **2**



1 **2** **3** **4** **5**

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Table 1 Final absorbance values (A) at 325 nm in the nitrosation of **1** (2×10^{-5} mol dm^{-3}), (a) at pH 3.1 and (b) in $HClO_4$ (0.1 mol dm^{-3})

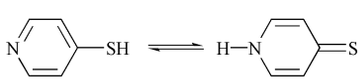
(a)		(b)	
$[HNO_2]_T$/mol dm^{-3}	A	$[HNO_2]_T$/mol dm^{-3}	A
0	0.445	8.00×10^{-6}	0.214
2.00×10^{-4}	0.191	1.60×10^{-5}	0.173
3.00×10^{-4}	0.154	2.40×10^{-5}	0.151
5.00×10^{-4}	0.119	3.20×10^{-5}	0.112
1.00×10^{-3}	0.078	4.00×10^{-5}	0.089
1.50×10^{-3}	0.066	8.00×10^{-5}	0.067
2.00×10^{-3}	0.060	1.20×10^{-4}	0.052
		2.00×10^{-4}	0.060

was selected to establish any effect due to the 1-oxygen atom, and **3** and **4**, to discover if a second ring-nitrogen atom plays a significant role. We will discuss the results together with those obtained earlier for **5**.[7]

Results

(a) Nitrosation of 4-mercaptopyridine 1

It is well-known that amino, hydroxy and thio derivatives of pyridines, pyrimidines and imidazoles can exhibit tautomerism *e.g.* as in eqn. (4). It has been established that, for the thio



(4)

derivatives, particularly in solution, the thione form is favoured to such an extent that the thiol form is not detectable by conventional spectral analysis. The quoted equilibrium constant for **1** [eqn. (4)] is 35000.[8] The thione form also predominates in the solid phase, whereas the thiol form dominates in the gas phase. In aqueous solution there is a major UV absorbance at 320 nm, which shifts to 280 nm on protonation in 2 M perchloric acid (literature pK_a 1.43[8]). Our measured absorption coefficients for the thione and its cation are in reasonably good agreement with the literature values.[8] Deprotonation of **1** also occurs at higher pH (pK_a 8.83[8]). A sample of **1** (5.2×10^{-4} mol dm^{-3}) in phosphate buffer pH 7.4 was reasonably stable, with a half-life of ~30 hours.

When **1** was added to sodium nitrite in dilute acid solution, a yellow colour developed rapidly which we take to be (by analogy with the reaction of the 2-isomer) the *S*-nitroso cation, **1**-NO$^+$. Examination of the UV–Visible spectrum showed a decrease in the absorbance at 320 nm and formation of an absorbance at 550 nm, characteristic of a *S*-nitroso species.[9] The absorbance at 320 nm was not completely removed when equimolar reactant concentrations were used, but did disappear when the nitrous acid was in ~5 fold excess, suggesting that this is an equilibrium reaction exactly as in eqn. (3) for the 2-isomer. We have attempted to obtain a value for the equilibrium constant K_N defined by eqn. (5), from absorbance measurements at

$$K_N = [\mathbf{1}\text{-NO}^+]/[\mathbf{1}]\,[HNO_2]\,[H^+] \quad (5)$$

325 nm. We kept the initial [**1**] constant at 2×10^{-5} mol dm^{-3} and varied the $[HNO_2]$ from 2–20 $\times 10^{-4}$ mol dm^{-3} (so that it is always effectively in constant excess) at a constant pH of 3.1 (citrate buffer). We added bromide ion (2×10^{-3} mol dm^{-3}) to catalyse the nitrosation, which then was effectively complete by the time that the first spectral measurement was taken. The absorbance at 325 nm decreased as shown in Table 1, as the total stoichiometric concentration of nitrous acid increased. At this pH the ionisation of nitrous acid is partial, so, in a quantitative analysis this must be taken into account (pK_a = 3.15[10]). We have derived an expression for $1/\Delta A$, where ΔA is the change

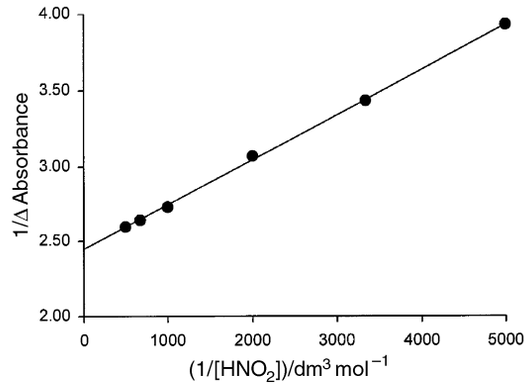


Fig. 1 Plot of 1/Δ Final Absorbance at 325 nm *vs.* $1/[HNO_2]_T$ in the nitrosation of **1** (2×10^{-5} mol dm^{-3}) at pH 3.1 in the presence of Br^- (2×10^{-3} mol dm^{-3}).

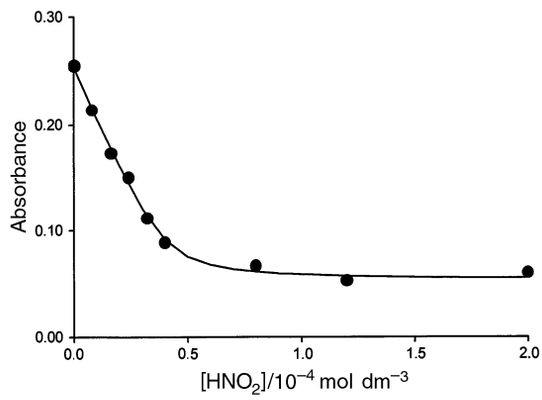


Fig. 2 The calculated fit and experimental points of the data in Table 1 (b).

in absorbance on addition of nitrous acid, *i.e.* 0.445–0.191 for the addition of 2.00×10^{-4} mol dm^{-3} HNO_2 *etc*. This is given as eqn. (6), and was derived by expressing the absorbance in

$$\frac{1}{\Delta A} = \frac{K_a + [H^+]}{(\varepsilon_1 - \varepsilon_{1\text{-NO}^+})K_N[H^+]^2[\mathbf{1}]_T[HNO_2]_T} + \frac{1}{(\varepsilon_1 - \varepsilon_{1\text{-NO}^+})[\mathbf{1}]_T} \quad (6)$$

terms of the concentrations and absorption coefficients of **1**, and of **1**-NO$^+$, and by consideration of the various mass balance equations. In this equation K_a is the acid dissociation constant of nitrous acid, K_N the equilibrium constant for the nitrosation of **1**, $[\mathbf{1}]_T$ and $[HNO_2]_T$ the total stoichiometric concentrations of **1** and HNO_2 respectively and ε_1 and $\varepsilon_{1\text{-NO}^+}$ the absorption coefficients of **1** and **1**-NO$^+$. This analysis predicts a linear dependence of $1/\Delta A$ *vs.* $1/[HNO_2]_T$. This is indeed the case, as is shown in Fig. 1 for measurements at 325 nm. From the values of the slope and intercept, and other constants, we can obtain a value for K_N of $(1.95 \pm 0.6) \times 10^7$ dm^6 mol^{-2}. We repeated the absorbance measurements at different $[HNO_2]_T$ and at 0.1 mol dm^{-3} $HClO_4$, and these results are given also in Table 1. At this acidity it is not possible to use a large constant excess of HNO_2 and so the results were fitted directly using the Scientist computer package.[11] It is also necessary here to take into account the protonation equilibrium of **1**, but we can neglect the ionisation of HNO_2. The calculated dependence of the absorbance at 325 nm upon $[HNO_2]_T$ together with the experimental points are given in Fig. 2. The agreement is excellent, and values of K_N of $(2.0 \pm 0.4) \times 10^7$ dm^6 mol^{-2} and of the pK_a of the protonated form of **1** of 1.45 ± 0.02 were obtained. These agree very well with the value of K_N obtained at pH 3.1 and the literature pK_a value[8] of 1.43.

The kinetics of the nitrosation of **1** were carried out at 400 nm where there is a small increasing absorbance due to the product formation, with $[H^+]$ and $[\mathbf{1}] \gg [HNO_2]$. Reactions

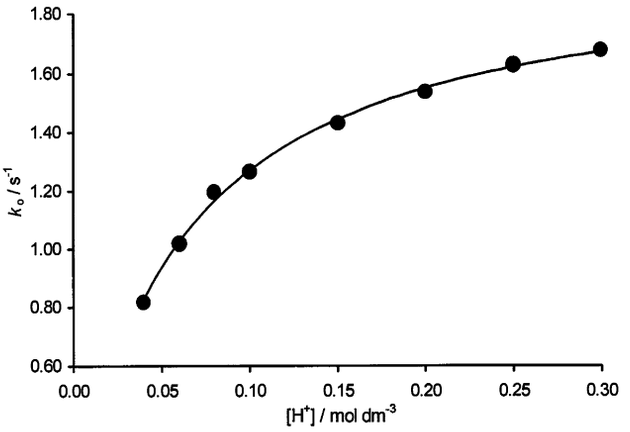


Fig. 3 The calculated fit and experimental points for the plot of the first-order rate constant k_o against $[H^+]$ in the nitrosation of **1** (6×10^{-3}) by HNO_2 (4×10^{-4} mol dm^{-3}).

were first-order and there was a first-order dependence on [**1**] as demonstrated by the linear relation between the observed first-order rate constant k_o and [**1**], with a small intercept at [**1**] = 0 (resulting from the slight reversibility of the process), which was too small to use reliably to calculate a value for K_N. The results yielded a value of 7500 ± 100 dm^6 mol^{-2} s^{-1} for the third-order rate constant k_N, defined in eqn. (7). Reactions were

$$\text{Rate} = k_N\,[\mathbf{1}]\,[HNO_2]\,[H^+] \qquad (7)$$

also studied by varying $[H^+]$ instead of [**1**]. The plot of k_o *vs.* $[H^+]$ was not linear (see Fig. 3), since the protonation of **1** now is important. Analysis of the results in terms of eqn. (8) when

$$k_o = k_N K_a [H^+]\,[\mathbf{1}]/(K_a + [H^+]) \qquad (8)$$

[**1**] ⩾ $[HNO_2]$ and where K_a is the acid dissociation constant of **1**-H$^+$, following the reaction by the decreasing thione absorbance at 325 nm, yields a value for k_N of 5900 ± 300 dm^6 mol^{-2} s^{-1}, in fair agreement with the value obtained earlier.

Measurements were also carried out with $[HNO_2]$ ⩾ [**1**]. Reactions were not now first-order, but tended towards zero-order particularly as the $[HNO_2]$ was increased. This suggests that reaction now occurs primarily *via* the N_2O_3 pathway in which the formation of N_2O_3 is the rate-limiting step, and which takes place in parallel with the minor pathway *via* NO^+ (or $H_2NO_2^+$) as per rate eqn. (9). This mixed order situation was

$$\text{Rate} = k_2\,[HNO_2]^2 + k_N\,[HNO_2]\,[\mathbf{1}]\,[H^+] \qquad (9)$$

analysed in terms of initial rates v_0 using a measured absorption coefficient for **1** at 325 nm of 5400 ± 60 dm^3 mol^{-1} cm^{-1}. A plot of $v_0/[HNO_2]$ *vs.* $[HNO_2]$ was linear with a very small intercept (too small to use in any calculations), and from the slope we get a value of 32 ± 2 dm^3 mol^{-1} s^{-1} for k_2, the second-order rate constant for N_2O_3 formation.

As expected these nitrosation reactions are strongly catalysed by halide ions. The results from the dependence of k_o upon [halide ion] (not shown) have been analysed in terms of rate-limiting attack by the corresponding nitrosyl halide. We obtained values for these second-order rate constants [k_{XNO} defined by eqn. (10)] of (3.2 ± 0.1) × 10^9 and (5.4 ± 0.1) × 10^9

$$\text{Rate} = k_{XNO}\,[XNO]\,[\mathbf{1}] \qquad (10)$$

dm^3 mol^{-1} s^{-1} respectively for the reactions of nitrosyl chloride and nitrosyl bromide, using the literature values for the equilibrium constants for their formation.[12] As expected there was also pronounced catalysis by added iodide ion, but the results were not analysed further since the equilibrium constant for

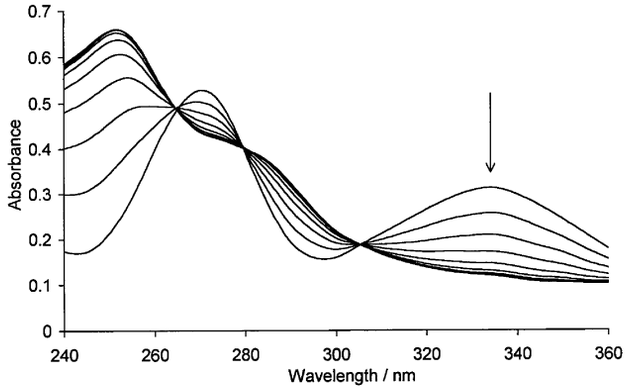


Fig. 4 Repeat spectra in the nitrosation of **2** (5×10^{-5} mol dm^{-3}) by HNO_2 (1×10^{-3} mol dm^{-3}) in $HClO_4$ (0.1 mol dm^{-3}) at 0.02, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60 and 1.80 s after mixing).

Table 2 Initial rates (v_0) in the nitrosation of **1** as a function of [added SCN^-]

$[SCN^-]$/mol dm^{-3}	v_0/mol dm^{-3} s^{-1}
1.00×10^{-4}	2.3×10^{-5}
2.00×10^{-4}	5.0×10^{-5}
4.00×10^{-4}	9.1×10^{-5}
8.00×10^{-4}	1.6×10^{-4}
1.00×10^{-3}	2.2×10^{-4}
2.00×10^{-3}	4.0×10^{-4}

nitrosyl iodide is not known, as NOI readily decomposes even in dilute solution. Nitrosation of **1** is also strongly catalysed by thiocyanate ion, see Table 2, where we report initial rates as a function of $[SCN^-]$. However individual kinetic experiments were now much closer to following zero-order rather than first-order behaviour. This suggests that nitrosyl thiocyanate formation is now partly if not wholly rate limiting. The data in Table 2 show that the contribution of the uncatalysed reaction is negligible. A more detailed analysis where the thione concentration is varied was carried out for the nitrosation of 2-mercapto-1*H*-imidazole **4**, which is presented later in this paper.

(b) Nitrosation of 2-mercaptopyridine 1-oxide (pyrithione) 2

The 2-thio 1-oxide derivative of pyridine is also believed to exist in aqueous solution (and also in the solid state) overwhelmingly in the thione form.[13] Its pK_a value has been determined as 4.67.[13] We find in acid solution absorption maxima at 271 and 333 nm with absorption coefficients 9710 and 4780 dm^3 mol^{-1} cm^{-1} respectively. As the pH is increased these maxima are replaced by two at 243 and 281 nm as the thione is deprotonated. Absorbance measurements at 243 nm as a function of pH gave the expected sigmoid curve yielding a pK_a value of 4.42 ± 0.04. On addition of sodium nitrite to an acidified solution of **2**, a more intense yellow colour developed which gradually faded over 10 minutes. The nitrosation reaction was too rapid to be followed by conventional spectrophotometry, and so measurements were made with the stopped-flow system. Fig. 4 shows the time-resolved spectra when **2** (5×10^{-5} mol dm^{-3}) reacts with excess nitrous acid (1×10^{-3} mol dm^{-3}) in perchloric acid (0.1 mol dm^{-3}). Reaction is effectively over in 1.8 s. Kinetic measurements were made at either 258, 290 or 329 nm and the results followed the first-order rate law well when [**2**] ⩾ $[HNO_2]$ at constant acidity (0.1 mol dm^{-3} $HClO_4$): there was also a first-order dependence on [**2**]. The rate constants, obtained by noting the increasing absorbance at 290 nm, are in Table 3. When the concentration limits were changed to $[HNO_2]$ ⩾ [**2**], we also obtained good first-order fit for the individual kinetic experiments (data in Table 3), but reactions were

Table 3 First-order rate constants (k_o) for the nitrosation of **2** by HNO_2 (5×10^{-5} mol dm^{-3}) and $HClO_4$ (0.100 mol dm^{-3})

[**2**]/mol dm^{-3}	k_0/s^{-1}
6.0×10^{-4}	0.463
6.0×10^{-4}	0.476
7.0×10^{-4}	0.530
8.0×10^{-4}	0.544
9.0×10^{-4}	0.626
1.0×10^{-3}	0.677

not now first-order in $[HNO_2]$. These results analysed satisfactorily in terms of eqn. (11), where we now have nitrosation

$$\text{Rate} = k_3[HNO_2]^2\,[\mathbf{2}] + k_N[HNO_2]\,[H^+]\,[\mathbf{2}] \qquad (11)$$

via the N_2O_3 and NO^+ (or $H_2NO_2^+$) pathways taking place concurrently. At low $[HNO_2]$ the second term is completely dominant. Analysis of the results in Table 3 yields values of $(5.1 \pm 0.4) \times 10^3$ dm^6 mol^{-2} s^{-1} for k_N and $(6.4 \pm 0.3) \times 10^8$ dm^3 mol^{-1} s^{-1} for $k_{N_2O_3}$ (the bimolecular rate constant for reaction of **2** with N_2O_3), using the most reliable value in the literature[14] for $K_{N_2O_3}$. Again, as for **1**, there was substantial halide ion catalysis and from plots of k_o against [halide] (data not shown), we obtained values of $(2.9 \pm 0.5) \times 10^9$ and $(3.2 \pm 0.5) \times 10^9$ dm^3 mol^{-1} s^{-1} respectively (k_{XNO}) for the reactions of ClNO and BrNO with **2**. Reversibility of the reaction was confirmed by the presence of a small intercept at $[H^+] = 0$ for plots of k_o against $[H^+]$ at constant [halide]. These intercept values gave estimated values of 25 ± 2 and 290 ± 60 dm^3 mol^{-1} s^{-1} respectively for the second-order rate constants for reaction of chloride and bromide ions with the **2**-NO^+ product (*i.e.* the reverse process) and hence approximate values for K_N of 2×10^5 and 6×10^5 dm^6 mol^{-2}. As in the case of **1**, reactions in the presence of thiocyanate ion when $[HNO_2] \gg [\mathbf{2}]$, tended towards zero-order behaviour when rate-limiting formation of nitrosyl thiocyanate occurs. The results gave a value of 2.1×10^4 dm^6 mol^{-2} s^{-1} for the third-order rate constant k_3' defined by eqn. (12).

$$\text{Rate} = k_3'[HNO_2]\,[H^+]\,[SCN^-] \qquad (12)$$

The activation energy E was determined for the chloride and bromide ion catalysed reactions. Allowance was made for the variation of K_{XNO} with temperature, and the activation energy for the rate-limiting step of attack by XNO extracted. We find values of 37 ± 2 and 15 ± 1 kJ mol^{-1} for the ClNO and BrNO reactions respectively.

(c) Nitrosation of 2-mercaptopyrimidine 3

The protonated form of **3** (pK_a 1.35[15]) has an absorbance maximum at 285 nm (ε 28900 ± 300 dm^3 mol^{-1} cm^{-1}). Addition of nitrous acid decreased this absorbance progressively, but a large excess was required to produce substantial changes (see Fig. 5). The absorbances due to the excess nitrous acid centred around 360 nm and also below 270 nm obscure any absorbance band(s) due to the nitrosated product **3**-NO^+. This demonstrates that the nitrosation reaction is reversible and that the equilibrium constant is much smaller than it is for the corresponding reactions of **1** and **2**. Final absorbance values for reactions with increasing $[HNO_2]$ are given in Table 4, and treatment with the Scientist package as in **(a)** yielded a value of 1700 ± 200 dm^6 mol^{-2} for K_N. Rate measurements were only possible for **3** using a large excess of $[HNO_2]$ in order to drive the reaction from left to right, when the active nitrosating species is N_2O_3, so it was not possible to obtain a value of k_N. As expected there was pronounced catalysis by both chloride and bromide ions, but no attempt was made to extract any individual rate constants because of the significant reversibility contribution.

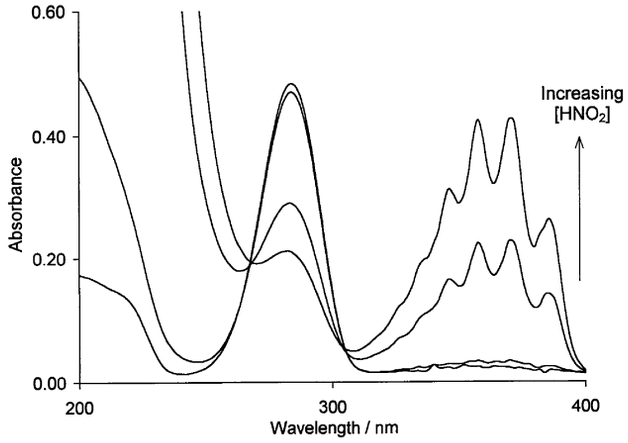


Fig. 5 Final spectra of the product of nitrosation of **3** (2×10^{-5} mol dm^{-3}) in $HClO_4$ (0.1 mol dm^{-3}) at $[HNO_2]$ 2×10^{-5}, 2×10^{-4}, 4×10^{-3} and 8×10^{-3} mol dm^{-3}.

Table 4 Final absorbance at 285 nm (A) in the nitrosation of **3** (2×10^{-5} mol dm^{-3}) in $HClO_4$ (0.10 mol dm^{-3}) as a function of $[HNO_2]_T$

$[HNO_2]_T$/mol dm^{-3}	A
0	0.475
2.00×10^{-5}	0.448
2.00×10^{-4}	0.434
4.00×10^{-4}	0.427
8.00×10^{-4}	0.399
1.60×10^{-3}	0.345
2.00×10^{-3}	0.334
4.00×10^{-3}	0.276
8.00×10^{-3}	0.198

Table 5 Final absorbance measurement at 285 nm (A) for the nitrosation of **4** (4×10^{-5} mol dm^{-3}), (a) at constant $[H^+]$ (0.10 mol dm^{-3}) and (b) at constant $[HNO_2]_T$ (4×10^{-4} mol dm^{-3})

(a)		(b)	
$[HNO_2]_T$/mol dm^{-3}	A	$[H^+]$/mol dm^{-3}	A
1.60×10^{-5}	0.052	0.0125	0.098
4.80×10^{-5}	0.099	0.0250	0.158
8.00×10^{-5}	0.147	0.0375	0.200
9.60×10^{-5}	0.150	0.050	0.232
1.20×10^{-4}	0.165	0.075	0.279
1.60×10^{-4}	0.190	0.100	0.311
2.40×10^{-4}	0.210	0.150	0.354
3.20×10^{-4}	0.220	0.200	0.383
4.00×10^{-4}	0.229	0.250	0.403
5.00×10^{-4}	0.232		
1.00×10^{-3}	0.250		

(d) Nitrosation of 2-mercapto-1*H*-imidazole 4

Again this compound exists primarily in the thione form in solution. The pK_a value for the protonated form is −1.6 and for deprotonation of the neutral form 11.6.[16] The thione has a major absorbance maximum at 252 nm and we have determined the absorption coefficient at this wavelength as 14500 ± 150 dm^3 mol^{-1} cm^{-1}. There was no significant decomposition in acid solution over 14 hours. Preliminary nitrosation experiments were consistent with a rapid nitrosation reaction generating a species which absorbs at around 280 nm (the *S*-nitroso cation), followed by a slower decomposition of the *S*-nitroso ion. Absorbance measurements at 280 nm, varying separately $[HNO_2]$ and $[H^+]$ at constant [**4**] are given in Table 5. These, together with the absorption coefficient data were fitted with Scientist to a model as before yielding values of $(1.70 \pm 0.16) \times 10^5$ and $(1.30 \pm 0.15) \times 10^5$ dm^6 mol^{-2} for K_N, from the

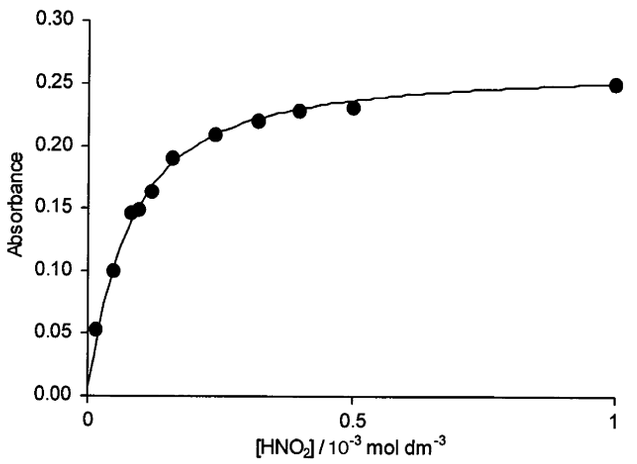


Fig. 6 Calculated curve and experimental points of the final absorbance at 280 nm from the data in Table 5 (a) for the nitrosation of **4**.

Table 6 Initial rates (v_0) of the SCN^- catalysed nitrosation of **4** with HNO_2 (8×10^{-4} mol dm^{-3})

[**4**]/mol dm^{-3}	v_0/mol dm^{-3} s^{-1}
2.00×10^{-5}	3.2×10^{-4}
4.00×10^{-5}	3.3×10^{-4}
6.00×10^{-5}	3.4×10^{-4}
8.00×10^{-5}	3.5×10^{-4}
1.00×10^{-4}	3.6×10^{-4}
2.00×10^{-4}	4.1×10^{-4}
4.00×10^{-4}	5.1×10^{-4}
6.00×10^{-4}	6.1×10^{-4}
8.00×10^{-4}	7.4×10^{-4}
1.00×10^{-3}	8.1×10^{-4}
1.50×10^{-3}	1.1×10^{-3}
2.00×10^{-3}	1.3×10^{-3}

variation of absorbance with $[HNO_2]$ and $[H^+]$ respectively. There were excellent fits between the calculated curve and the experimental points. The results for one of the analyses are shown in Fig. 6. Kinetic data (not shown) gave values of 7730 ± 20, 8400 ± 200 and 8500 ± 100 dm^6 mol^{-2} s^{-1} for k_N from measurements when both [**4**] and $[H^+] \gg [HNO_2]$ whilst varying [**4**] or $[H^+]$. From the former we were also able to obtain an estimate for the reverse rate constant k_{-N} from the small intercept at [**4**] = 0, and hence an approximate value for K_N of 1.8×10^5 dm^6 mol^{-2} which is in good agreement with the values obtained from absorbance measurements. With $[HNO_2] \gg$ [**4**] individual reactions were almost zero-order, consistent again with a rate-limiting N_2O_3 process and we obtain a value for k_2 the second-order rate constant for N_2O_3 formation [eqn. (9)] of 46 ± 2 dm^3 mol^{-1} s^{-1}. We also obtained average values for k_{XNO} for reactions of nitrosyl chloride and nitrosyl bromide with **4** of $(2.7 \pm 0.2) \times 10^9$ and $(4.1 \pm 0.4) \times 10^9$ dm^3 mol^{-1} s^{-1} when reactions were carried out in the presence of added chloride and bromide ions. As in the case of **1** and **2**, addition of thiocyanate ion produced almost zero-order kinetics. The results (in Table 6) were analysed in terms of rate eqn. (13), where there

$$\text{Rate} = k_3 [HNO_2] [H^+] [SCN^-] + k_N [HNO_2] [H^+] [\mathbf{4}] \quad (13)$$

are two pathways (i), rate-limiting ONSCN formation, concurrent with (ii), a rate-limiting reaction of NO^+ ($H_2NO_2^+$) with **4**. Values of k_3 (the rate constant for ONSCN formation), were obtained from the plots of the initial rates (v_o) against $[SCN^-]$ (from the slope, 19800 ± 900 dm^3 mol^{-1} s^{-1}) and v_0 against [**4**] (from the intercept, 18800 ± 550 dm^3 mol^{-1} s^{-1}). Similarly a value of k_N, of 6250 ± 300 dm^6 mol^{-2} s^{-1}, was obtained from the slope of the plot of v_0 against [**4**], in fair agreement with the values obtained for the uncatalysed reactions earlier.

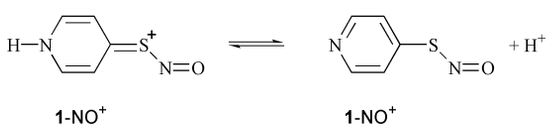
(e) Decomposition of the SNO^+ species

The *S*-nitroso ions from all five thiones decomposed in solution on standing to give the disulfide species and nitric oxide [eqn. (14)]. In some cases *e.g.* those derived from **1**-NO^+ and **2**-NO^+,

$$2 \text{=}S^+\text{–NO} \longrightarrow \text{–S–S–} + 2NO + 2H^+ \quad (14)$$

the disulfide is in the dication form, whereas the disulfide from **4**-NO^+ exists in the reaction solutions as the neutral species. The disulfides were identified by comparison of the UV spectra with authentic samples and nitric oxide was detected by the NO electrode system in all cases, and was quantified as ~100% for the decomposition of **1**-NO^+ in the presence of excess **1** in 0.1 mol dm^{-3} $HClO_4$. Only the nitroso cation from **1** was sufficiently stable to allow its use in further studies. We were able to show that it decomposed to the disulfide at pH 3.1 with a half life of ~16 minutes.

When a solution of **1**-NO^+ (prepared at pH 1 using a ten-fold excess of nitrous acid), was quenched in a buffer pH 7.4, the initial spectrum changed, the major absorbance at 280 nm being replaced by one at 255 nm. Lowering the pH to 1 regenerated the 280 nm absorbance. This suggests an acid–base equilibrium as in eqn. (15). A similar change was observed for the



1-NO^+ **1**-NO^+ (15)

reactions of the 2-isomer.[7] From initial spectral measurements at different pH values we estimated the pK_a here to be ~4.5. The deprotonated material however was not stable but reverted to the thione with a half-life of about 6 minutes, possibly *via* the protonated form and the reversal of eqn. (3) when nitrous acid at this pH would be deprotonated and so be unable to effect the re-nitrosation of the thione.

(f) *S*-NO^+ cations as nitrosating species

We carried out some experiments to examine the potential of *S*-NO^+ cations as electrophilic nitrosating species. **1**-NO^+ was generated at pH 1 from equimolar amounts of **1** and nitrous acid, and was used to react with an equimolar amount of ascorbic acid (H_2A) at pH 7.4 in the presence of EDTA to suppress any copper-ion mediated decomposition.[17] There was a very rapid decrease of absorbance at ~260 nm (due to ascorbic acid) to about half of its value (consistent with the expected stoichiometry), which was concurrent with the appearance of the absorbance at 325 nm due to the thione **1**. Kinetic measurements were carried out with $[H_2A] \gg$ [**1**-NO^+]. We found good first-order plots and there was a first-order dependence on $[H_2A]$, leading to a value of 4500 ± 300 dm^3 mol^{-1} s^{-1} for the overall second-order rate constant. The formation of nitric oxide was approximately quantitative as measured by the NO-electrode system.

The well-known *N*-nitrosation reaction of *N*-methylaniline (NMA) to give the nitrosamine (NNMA) was examined spectrophotometrically in the presence and absence of **2**. Even at low [**2**] the extent of catalysis is spectacular. The results are shown in Fig. 7, where the pseudo first-order rate constants are plotted against the concentration of added **2**. Initially the plot is linear, curving off at higher [**2**] as expected when essentially all of the nitrous acid has been converted to **2**-NO^+. Analysis of the data in terms of eqns. (16), (17) and (18) gave the predicted

$$\mathbf{2} + HNO_2 + H^+ \rightleftharpoons \mathbf{2}\text{-}NO^+ + H_2O \quad (16)$$

$$\text{NMA-}H^+ \rightleftharpoons \text{NMA} + H^+ \quad (17)$$

$$\mathbf{2}\text{-}NO^+ + \text{NMA} \rightleftharpoons \text{NNMA} + H^+ + \mathbf{2} \quad (18)$$

Table 7 Summary of equilibrium and rate constants for thione nitrosation

	Thione				
	1	**2**	**3**	**4**	**5**
K_N/dm^6 mol^{-2}	2.0×10^7	~4×10^5	1.7×10^3	1.5×10^5	~1×10^5
k_N/dm^6 mol^{-2} s^{-1}	6700	5900	—	8200	8200
k_{ClNO}/dm^3 mol^{-1} s^{-1}	3.2×10^9	2.9×10^9	—	2.7×10^9	3.5×10^9
k_{BrNO}/dm^3 mol^{-1} s^{-1}	5.4×10^9	3.2×10^9	—	4.1×10^9	3.7×10^9
k_2'/dm^6 mol^{-2} s^{-1}	—	1.0×10^4	—	—	2.0×10^4 1.9×10^4

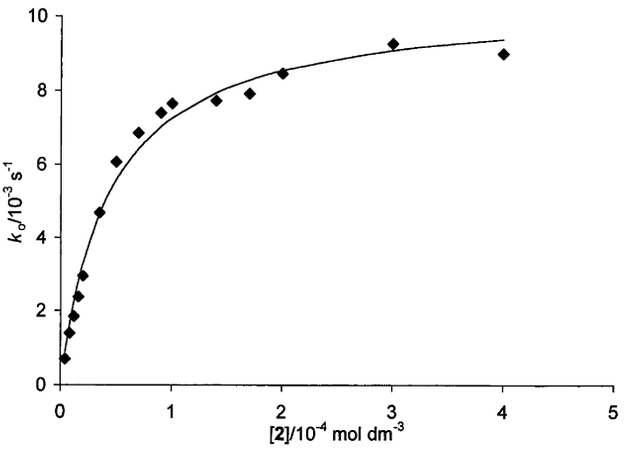


Fig. 7 Values of the observed first-order rate constant k_o for the nitrosation of *N*-methylaniline (4.43×10^{-3} mol dm^{-3}) by HNO_2 (2×10^{-5} mol dm^{-3}) in $HClO_4$ (0.1 mol dm^{-3}) as a function of added **2**.

linear correlation between $1/k_o$ and 1/[**2**] *i.e.* the double reciprocal plot, leading to values for K_N of $(1.5 \pm 0.3) \times 10^5$ dm^6 mol^{-2} and for k_2 the rate constant for reaction of **2**-NO$^+$ with NMA of $(2.0 \pm 0.1) \times 10^5$ dm^3 mol^{-1} s^{-1}.

Discussion

It is evident that all of the thiones studied are very reactive towards electrophilic nitrosation, initially generating the corresponding *S*-nitroso cations *S*-NO$^+$, which then decompose fairly rapidly in mildly acidic solution to give the corresponding disulfide and nitric oxide. The *S*-nitrosation is a somewhat reversible process but with very large equilibrium constants (K_N in eqn. (3)), in common with other *S*-nitrosation reactions of thiols[4] and thioureas.[5,6] Values of K_N are given in Table 7 together with the rate constant values obtained in this work, including for comparison purposes the data for the thione form of 2-mercaptopyridine.[7] In the case of nitrosation of simple thiols (such as cysteine), the low concentration of thiol remaining at equilibrium is important in that it provides the reducing agent to generate Cu^+ (from Cu^{2+}) which is the reagent for nitric oxide production. The value of K_N for the nitrosation of **1** was obtained at two different acidities by two different procedures and produced values of $(1.95 \pm 0.6) \times 10^7$ and $(2.0 \pm 0.4) \times 10^7$ dm^6 mol^{-2}, in excellent agreement. For the pyrimidine (**3**) reaction, the equilibrium constant however is some 10^2 smaller than the others, which is perhaps a reflection of the adjacent basic ring nitrogen atom, which will be substantially protonated in the acidic nitrosation media. Unfortunately we have no rate data to support this suggestion. The presence of the *N*-oxo group has no measurable effect on the value of K_N (see **3** and **5**) and the value for **1** is the largest equilibrium constant of this type that we have encountered—significantly bigger than for the isomeric **5**, presumably due to the greater distance from the ring-nitrogen atom.

The third-order rate constants k_N [eqn. (7)] are all reasonably constant for the four thiones for which values were obtained, within the range 6000–8000 dm^6 mol^{-2} s^{-1}, which appears to be the upper limit for a wide range of neutral reactants including thiourea, alkyl thioureas, some aniline derivatives, some thiols, nitronic acids, *etc.*[18,19] It has been argued that this limit arises when the reaction of NO$^+$ (or $H_2NO_2^+$) occurs at the encounter-controlled limit,[20] which means that the equilibrium constant for NO$^+$ formation is ~1×10^{-6} dm^3 mol^{-1}. The reactions of ClNO and BrNO with **1**, **2**, **4** and **5** similarly all appear to react at or close to the encounter limit, since the derived second-order rate constants are all very near to the calculated encounter limit of ~7×10^9 dm^3 mol^{-1} s^{-1}. This conclusion is supported by the finding of low values of the activation energies (15 and 37 kJ mol^{-1}) for the reactions of ClNO and BrNO with **2**, as expected for an encounter-controlled process, The corresponding rate constant for N_2O_3 attack at **2** is a power of 10 less than the encounter limit, reinforcing the belief[20] that it is a less powerful nitrosating species than are ClNO and BrNO. Strangely for **2** the rate of its reaction with N_2O_3 is rate limiting whereas for both **1** and **5**, under our reaction conditions at high [HNO_2], the rate-limiting step is now the formation of N_2O_3. This must be due to the electron attracting effect of –OH in **2**, which reduces reactivity—a factor which is not evident in the reactions of NO$^+$, ClNO or BrNO, since these reagents react at the encounter limit with all the reagents studied. We obtain, from two different thiones, values of 32 and 46 dm^3 mol^{-1} s^{-1} for k_2 [eqn. (9)], the rate constant for N_2O_3 formation. The literature values[21,22] vary somewhat within the range 4–30 dm^3 mol^{-1} s^{-1} for reactions at 25 °C, derived from a spectrum of reactants. The range is somewhat larger than one would expect to see. Possibly the discrepancies arise from different ionic strength conditions and also, more probably, from the possiblity that in some cases reactions were not fully zero-order in [substrate], *i.e.* when a small undetectable component of a first-order process was also present, arising from the mechanism of rate-limiting attack of NO$^+$ (or $H_2NO_2^+$).

For the nitrosation of both **2** and **4** in the presence of thiocyanate ion, the results show that we are measuring the rate-limiting formation of nitrosyl thiocyanate, with an overall third-order rate constant (k_2') of ~2×10^4 dm^6 mol^{-2} s^{-1}. This has only been observed previously at 25 °C in the nitrosation of hydrazoic acid[23] and thioglycolic acid,[3] and also at 0 °C for other reactants. Our values of k_2' are in acceptable agreement with the earlier values of 1.1×10^4 and 1.2×10^4 dm^6 mol^{-2} s^{-1}.

The (even slight) reversibility of these reactions allows the calculation of some of the parameters for the reverse reaction. It is perhaps worth noting that in the halide ion catalysed denitrosation of the **2**-NO$^+$ cation [eqn. (19)], bromide ion is

$$X^- + \mathbf{2}\text{-}NO^+ \rightleftharpoons \mathbf{2} + XNO \qquad (19)$$

shown to be more reactive than chloride ion as expected from their nucleophilicities in water, and as has been demonstrated experimentally for example in the halide ion catalysed denitrosation of nitrosamines,[24] and in many other reaction types.

It would appear that these thione nitrosation reactions are quite general for these heterocyclic systems. The chemistry is very similar, as expected, to that established for the nitrosation of the open-chain forms as in the reactions of thiourea and its *N*-alkylated derivatives.[5,6] One difference lies in the fact that for

both **1** and **5**, the deprotonated forms of the *S*-NO^+ cations have been identified, whereas this has not been the case for the thioureas. It would be of some interest to compare our present results with those for 3-mercaptopyridine, which cannot exist in the thione form.

All of the *S*-NO^+ species decompose fairly rapidly in mildly acidic solution to give the corresponding disulfides and nitric oxide. We have characterised the former by comparison of UV spectra with known samples and the latter by quantitative analysis using the NO-electrode system. We have not attempted in this work to look at the detailed mechanism of the decomposition of the *S*-NO^+ cations: it is likely that they follow the same pathways as do the *S*-nitroso cations derived from thiourea.[25]

As expected the *S*-NO^+ species show significant reactivity as independent nitrosating species. We have shown this for the reactions of **1**-NO^+ in reaction with ascorbic acid, of **2**-NO^+ with *N*-methylaniline and earlier[7] of **5**-NO^+ with *N*-methylaniline and a thiol. Interestingly the actual rate constants for reactions of *S*-NO^+ ions are not particularly large. For example we find that the bimolecular rate constant for reaction of **2**-NO^+ with the free base form of *N*-methylaniline is 2.0×10^5 dm^3 mol^{-1} s^{-1}, whereas both ClNO and BrNO react at or near to the encounter limit. This is fully in line with other results where the sequence of reactivity has been established[26] as ClNO > BrNO > ONSCN > $(NH_2)_2CSNO^+$ for a number of substrates. This must be due in part at least to the strength of the S–N bond in these cations. The spectacular catalysis of thiourea and now these thiones in nitrosation reactions arises from the very large equilibrium constants for their reaction with nitrous acid in generation of the active nitrosating species XNO—*i.e.* it is a concentration rather than a kinetic effect. On the other hand as expected these *S*-NO^+ cations are shown to be much more reactive as electrophilic nitrosating agents than are the non-charged *S*-nitrosothiols RSNO, since **1** reacts with ascorbic acid with a rate constant of 4500 ± 300 dm^3 mol^{-1} s^{-1}, whereas for RSNO species in the same reaction,[17] typical values of the rate constant (which varies with structure) at the same pH (7.4) are 1.5×10^{-2} and 2.5×10^{-1} dm^3 mol^{-1} s^{-1} respectively for *S*-nitrosoglutathione and *S*-nitrosocysteine. This is a large effect, even though the structures are not comparable, and reflect the better leaving group ability of thione compared with the thiolate anion.

Experimental

All materials were of the highest purity grade available and were used as such. The *N*-oxide **2** was generated from its sodium salt hydrate. Aqueous stock solutions of the thiones were freshly prepared for each set of experiments and were protected from light exposure with aluminium foil. Spectra were obtained on conventional spectrophotometers interfaced with PCs or in some cases using a stopped-flow spectrophotometer with a diode array attachment. Most of the kinetic experiments were carried out using an Applied Photophysics SX-17MV BioSequential stopped-flow ASVD spectrophotometer and rate constants obtained from the software package supplied. Each trace was the average of at least three runs and at least three of these averages were obtained for each reaction. Rate constants were usually reproducible within ±3%. Errors quoted were obtained by linear regression in Microsoft Excel and the Statistics option in Scientist. Both linear and non-linear least squares fitting procedures were employed.

Nitric oxide analyses were achieved with a commercial World Precision ISO-NO mark II specific electrode which was calibrated using NO generated in solution from either ascorbic acid–sodium nitrite or potassium iodide–sulfuric acid–sodium nitrite solutions. All measurements were made after purging with nitrogen for at least 15 minutes, so that oxidation of nitric oxide was not a significant reaction.

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