Catalysis of *N*-Nitrosation and Diazotisation by Thiourea and Thiocyanate Ion

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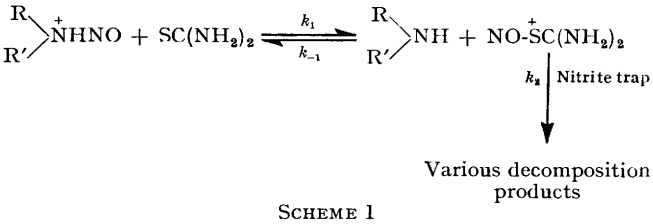
Thiourea is shown to be a very efficient catalyst in the nitrosation of morpholine in acid solution. Compared with other known catalysts, the order of efficiency is $SC(NH_2)_2 > SCN^- > Br^-$ in the ratio 4 200 : 240 : 1. The kinetic data are interpreted in terms of equilibrium formation of the $NO-\overset{+}{S}=$ ion from thiourea which acts directly as a nitrosating agent. The overall efficiency of thiourea as a catalyst arises from the large equilibrium constant for the formation of this ion rather than from the rate constant (k_2) for attack by the ion on morpholine. Values of k_2 for the series $SC(NH_2)_2$, SCN^-, and Br^- at 31° increase from 7×10^6 to 2.9×10^7 and 4.8×10^7 l mol^{-1} s^{-1}, respectively. Similarly the diazotisation of aniline proceeds *via* $NO-\overset{+}{S}=$ in the presence of added thiourea. Again the k_2 values were determined at 0° for the series $SC(NH_2)_2$, SCN^-, Br^- as 3.7×10^5, 1.1×10^7, and 1.6×10^9 l mol^{-1} s^{-1}. For both $SC(NH_2)_2$ and SCN^- reactions, the rate constant is well below that of the diffusion-controlled limit (*cf.* Br^-); the measured activation energies also support this conclusion. It is suggested that thiourea and possibly other sulphur-containing species act as excellent general catalysts for nitrosation and diazotisation reactions generally. Cysteine showed negligible catalytic activity whilst *S*-methylcysteine gave some indication of catalysis in the diazotisation of aniline, but the effect was small.

A FEATURE common to many nitrosation and diazotisation reactions is the ability of halide ions and thiocyanate ion to effect catalysis of these reactions in acid solution.[1] This is thought to be due to the equilibrium formation of the corresponding nitrosyl halides or nitrosyl thiocyanate (NOX generally) which are more efficient nitrosating agents than that derived from acidic nitrous acid alone, under the prevailing experimental conditions. The degree of catalysis is then dependent upon two factors: (a) the equilibrium constant for NOX formation, and (b) the rate constant for attack of the substrate by NOX. By and large, for those examples studied it appears that the magnitude of the equilibrium constant (K_X) is the more important term which governs the overall catalytic efficiency. Thus, values of K_X for nitrosyl chloride and nitrosyl bromide formation at 25° are 1.1×10^{-3} and 5.1×10^{-2} l^2 mol^{-2} respectively [2] and it is generally found that bromide ion catalysis is significantly more pronounced than chloride ion catalysis, even though the actual rate constants for NOX attack are larger for X = Cl than for X = Br for a number of substrates, *e.g.* aniline derivatives,[3] hydrazine,[4] and alkenes.[5] Simple arguments based on electronegativity differences between the halogens would predict that NOCl should be more reactive than NOBr. The particularly high efficiency of added thiocyanate ion [6] in nitrosation is readily attributable to the large value [7] (32 l^2 mol^{-2} at 20°) of K_X for nitrosyl thiocyanate formation.

Recently, Stedman and his co-workers [8,9] have measured the rate constant for the forward and reverse steps for the reactions of nitrous acid with thiourea, alkylthioureas, and cysteine. The reactions are very rapid, forming initially the unstable nitrososulphonium ion $NO-\overset{+}{S}=$. The derived equilibrium constant for thiourea is 5000 l^2 mol^{-2} at 25° and the reaction is virtually quantitative for cysteine. Similar *S*-nitrosations of this kind have been effected using nitrosamines in acid solution as the nitrosating agents,[10] where it has been shown that a direct nitrosation reaction (cross- or trans-nitrosation) occurs rather than an initial hydrolysis followed by a nitrous acid reaction. As expected the nitrosamine reaction is much slower than the nitrous acid reaction. There is some evidence from earlier work [11] that these nitrososulphonium ions can themselves act as nitrosating agents. By noting the variation of the observed rate constants for thiourea nitrosation by a nitrosamine with added secondary amine (RR'NH), at constant acidity and $[SC(NH_2)_2]$, it was possible [11] to obtain the rate constant ratio $k_{-1} : k_2$ shown in Scheme 1.



SCHEME 1

A range of conventional nitrite traps was used including hydrazine, hydrazoic acid, and sulphamic acid. Step k_{-1} of course represents the direct nitrosation of RR'NH by the nitrososulphonium ion. The range of selectivity shown by $NO-\overset{+}{S}C(NH_2)_2$ (for a given secondary amine) for these nitrite traps was very similar to that shown by NOSCN, implying that these reagents are of comparable reactivity, whereas both showed a significantly larger selectivity range than either NOCl or NOBr, again suggesting that the nitrosyl halides are the more reactive nitrosating agents.

Any reaction *via* $NO-\overset{+}{S}C(NH_2)_2$ can of course be studied directly by the observation of any catalysis of nitrosation by thiourea. This does not seem to have been reported in the literature; consequently we have examined the nitrosation of morpholine (as a typical secondary amine), and the diazotisation of aniline (a typical primary aromatic amine) both in the presence of thiourea, together with the corresponding reactions in

the presence of thiocyanate ion and bromide ion for comparison purposes. Reaction rate constants for the morpholine reactions were determined by conventional spectrophotometric methods whereas the aniline reactions were sufficiently rapid to require a stopped-flow procedure. The results are presented in this paper and are discussed in terms of the reactivity of the various nitrosating agents involved.

EXPERIMENTAL

Morpholine and aniline were both redistilled and centre fractions collected at constant b.p.s. Cysteine and *S*-methylcysteine were commercial samples of the highest purity grade available, as were all other materials used.

The kinetic measurements of the morpholine reactions were conducted spectrophotometrically, the reactions being carried out in the cell of a Beckman model 25 recording spectrophotometer at 31° in aqueous solution. The absorbance was monitored continuously at 342 nm, measuring the formation of the product *N*-nitrosomorpholine. Reactions were all performed under first-order conditions with the morpholine in a constant excess over the nitrous acid concentration. Good first-order behaviour was generally found for at least 80% of the reaction. A typical run is quoted in Table 1. The kinetic measurements for diazotisation were carried out using a Canterbury stopped-flow spectrophotometer either at 0 or 30°, under first-order conditions with [aniline] $\gg$ [HNO_2]. The bromide ion- and thiocyanate ion- catalysed reactions were followed by noting the increasing absorbance at 325 nm due to the diazonium ion. A different procedure was adopted for the thiourea catalysed reactions since the rate of formation of the nitrososulphonium ion was comparable with that of its reaction. So a solution of the ion was first prepared by mixing an acidic solution of thiourea and one of sodium nitrite and immediately (*i.e.* within a few seconds) the mixture was added to a solution of aniline within the stopped-flow system. The reaction was then followed by noting the disappearance of the 420 nm absorption due to the yellow nitrososulphonium ion. Good first-order plots were obtained for each individual run, and generally the mean value of at least five separate runs was taken. The reproducibility of the measurement was better than ±5%.

TABLE 1

A typical run for the reaction of nitrous acid (9×10^{-3}M) with morpholine (0.154M) in sulphuric acid (0.113M) containing thiourea (2.54×10^{-3}M)

t/s	0	12	24	36	48	60	72	∞
Absorbance	0.173	0.226	0.269	0.303	0.332	0.356	0.374	0.458
10^2k_0/s^{-1}		1.72	1.70	1.69	1.70	1.71	1.69	

RESULTS AND DISCUSSION

(a) *Nitrosation of Morpholine.*—Since all kinetic experiments showed a good first-order dependence upon [HNO_2] we can exclude nitrosation *via* N_2O_3 as a mechanistic pathway under these experimental conditions. Virtually quantitative formation of *N*-nitrosomorpholine was noted in all the experiments. As expected the reaction was first-order with respect to the morpholine concentration as shown by the data presented in Figure 1. The first-order rate constant k_0 is defined by $d[HNO_2]/dt = k_0[HNO_2]$. The catalytic effect of added bromide ion, thiocyanate ion, and thiourea was investigated. The results of the variation of k_0 with [catalyst] are presented in Table 2. Clearly there is catalysis in all cases and mere inspection of the data reveals that the

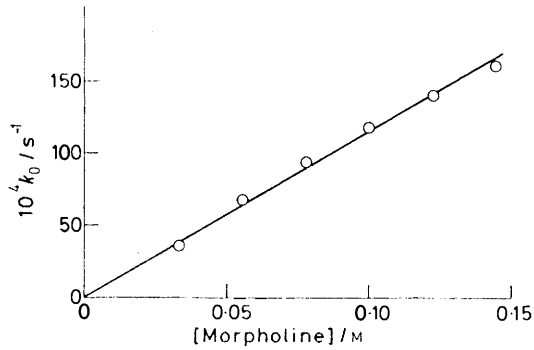


FIGURE 1 Variation of k_0 with [Morpholine] in the nitrosation of morpholine in the presence of SCN^-

extent of catalysis increases along the series $Br^- < SCN^- < SC(NH_2)_2$; in fact the overall catalytic efficiency for the series is, more quantitatively, 1 : 240 : 4200 as given by the slopes of the plots of k_0 *versus* [nucleophile] in Figure 2. It is generally believed that nitrosation of a secondary amine proceeds by direct attack of NOX at the free base form of the amine, as outlined in Scheme 2. K_X is the equilibrium constant for the formation of NOX from HNO_2, H^+, and X^-, and K_A is the acid dissociation constant of the protonated secondary amine $\overset{+}{\text{N}}H_2$. If we define the [Total nitrite] = [HNO_2] + [NOX] then the general rate equation is given by equation (1), where $[A]_T$ is the total amine concentration for an amine where

TABLE 2

Variation of k_0 with [Br^-], [SCN^-], and [$SC(NH_2)_2$] for the reaction of morpholine with nitrous acid (9×10^{-3}M) in sulphuric acid (0.113M) at 31°

$[Br^-]$/M	10^4k_0/s^{-1}	$10^3[SC(NH_2)_2]$/M	10^4k_0/s^{-1}
0.171	3.63	1.27	69.3
0.285	4.84	2.54	170
0.570	9.38	3.81	261
0.855	12.9	5.08	334
1.14	17.6	6.35	421
[Morpholine] = 0.111M		[Morpholine] = 0.154M	
$10^3[SCN^-]$/M	10^4k_0/s^{-1}	$10^3[SCN^-]$/M	10^4k_0/s^{-1}
7.22	29.0	50.9	186
10.8	43.4	64.4	224
14.4	55.8	70.8	252
18.1	70.3	77.3	274
21.8	83.0	96.6	342
32.2	123	129	440
36.4	138	161	552
[Morpholine] = 0.111M			

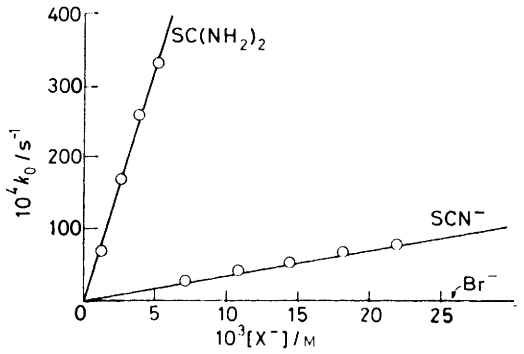


FIGURE 2 Comparison of the catalytic efficiencies of Br^-, SCN^-, and $SC(NH_2)_2$ in the nitrosation of morpholine

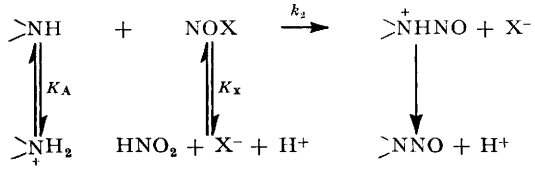
the protonation is substantial (as in the case of morpholine and aniline). Under first-order conditions with

$$\text{Rate} = \frac{k_2[\text{Total nitrite}]K_A[A]_T}{\left(1 + \frac{1}{K_x[H^+][X^-]}\right)[H^+]} \quad (1)$$

$[A]_T \gg [\text{Total nitrite}]$ the expression for k_0 is given then by equation (2). If K_x is very small (as it is for

$$k_0 = \frac{k_2K_A[A]_T}{\left(1 + \frac{1}{K_x[H^+][X^-]}\right)[H^+]} \quad (2)$$

$X^- = Br^-$, 5.1×10^{-2} l^2 mol^{-2} at 25°) then at reasonably low $[Br^-]$ and $[H^+]$ the limiting condition $1/K_x[H^+][X^-] \gg 1$ applies and k_0 reduces to equation (3) which



SCHEME 2

predicts a first-order dependence upon $[X^-]$ and independence of the acidity. The results in Table 2 for Br^- do indeed give a linear plot of k_0 *versus* $[X^-]$ and

$$k_0 = k_2K_A[A]_TK_x[X^-] \quad (3)$$

Table 3 shows the constancy of k_0 within the experimental error over the range 0.1—0.4M-H_2SO_4. The effect of protonation of the amine is thus offset by the acid concentration term in the equilibrium constant for NOBr formation. Similarly for SCN^-, although K_x is now greater, at low $[SCN^-]$ the limiting form (3) applies but at high $[SCN^-]$ the plot of k_0 *versus* $[SCN^-]$ does

TABLE 3

Values of k_0 at different acidities for the reaction of morpholine (0.170M) with nitrous acid (8.6×10^{-3}M) containing bromide ion (0.490M)

$[H_2SO_4]$/M	$10^4k_0/s^{-1}$
0.113	11.2
0.170	11.2
0.227	11.2
0.340	11.1
0.420	11.7

TABLE 4

Variation of k_0 with $[H_2SO_4]$ for the reaction of morpholine (0.170M) with nitrous acid (9×10^{-3}M) containing thiourea (2.59×10^{-3}M)

$[H_2SO_4]$/M	$10^4k_0/s^{-1}$
0.113	184
0.147	96.5
0.170	74.6
0.227	23.7

curve off as the limiting form becomes less applicable. Similarly for thiourea where K_x is now much larger the limiting form does not apply ($K_x[H^+][X^-]$ being now typically *ca.* 3) and k_0 *versus* [free thiourea] is significantly curved.

A further consequence in the thiourea case is that k_0 is not independent of $[H^+]$ if the general form for k_0 [equation (2)] applied. Table 4 shows in fact the variation of k_0 with $[H_2SO_4]$ at constant $[SC(NH_2)_2]$. The rate constants here, particularly at the higher acidities, were not very reliable since there was evidence of the decomposition of the nitrososulphonium intermediate.

The results can all be interpreted in terms of Scheme 2 and it is clear that for the three catalysts, nitrosation occurs *via* NOBr, NOSCN, and $NO\overset{+}{S}C(NH_2)_2$. This represents the first positive direct identification of $NO\overset{+}{S}C(NH_2)_2$ as a nitrosating agent in its own right, although the kinetic analysis referred to earlier [11] suggested this possibility.

One can calculate k_2 for each of these reactions either from equation (3) or (2). For $X^- = Br^-$ a value of 5.2×10^7 l mol^{-1} s^{-1} was obtained for reaction at 31°. A repeated set of experiments at a different morpholine concentration gave a value of 4.5×10^7 l mol^{-1} s^{-1}. Similarly for the NOSCN reaction two separate determinations of k_2 gave a mean value of 2.8×10^7 l mol^{-1} s^{-1}. The raw experimental data for one set are in Table 2. A value of k_2 for this reaction was also obtained from the variation of k_0 with $[\text{Morpholine}]_{\text{Total}}$ at constant $[SCN^-]$ as shown in Figure 1. This yielded a value of 2.9×10^7 l mol^{-1} s^{-1} in excellent agreement with the earlier results. For the $SC(NH_2)_2$-catalysed reactions, even though k_0 *versus* [Total $SC(NH_2)_2$] appears to be linear a corrected plot of k_0 *versus* [Free $SC(NH_2)_2$] is in fact quite curved. This is to be expected for now K_x is large making the limiting condition ($1/K_x[H^+][X^-] \gg 1$) not applicable in this case. It is possible, however to evaluate k_2 values for each $SC(NH_2)_2$ concentration from the more general equation (2); the average value found was 7×10^6 l mol^{-1} s^{-1}.

Thus we have the bimolecular rate constants for the *N*-nitrosation of morpholine by each of the nitrosating agents, the values of k_2 decreasing in the order NOBr > NOSCN > $NO\overset{+}{S}C(NH_2)_2$. The overall range is, however, quite small, which in itself suggests that the reaction rates are approaching the diffusion-controlled limit. However, the actual values of k_2 are approximately 100 times smaller than the calculated values for such reactions in water at 31°. We have measured the activation energy

for the *N*-nitrosation step for the reaction of $NO\overset{+}{S}C(NH_2)_2$, and find a value of *ca.* 40 kJ mol^{-1}. For experimental reasons [principally the side reaction of the decomposition of $NO\overset{+}{S}C(NH_2)_2$] this value is subject to a large error, probably ± 8 kJ mol^{-1}, but nevertheless the value is significantly higher than the range 6—21 kJ mol^{-1} found [12] for other diffusion-controlled reactions. Similarly Fen and Tannenbaum [13] have found the activation energy for the nitrosation of morpholine in the presence of thiocyanate ion to be 40 kJ mol^{-1}.

The relatively small range of k_2 values is perhaps unexpected but Stedman and his co-workers [14] have found that a similar situation exists in the nitrosation of hydroxylamine, where NOBr and NOCl have approximately the same reactivity, yet the k_2 values are again, *ca.* 100 times less than those expected for the diffusion-controlled processes. It must be stressed that a number of approximations are made in the evaluation of the calculated and experimental k_2 values so that exact agreement is not to be expected. However, our order of reactivity is in agreement with earlier observation that NOBr is more reactive than NOSCN, in the nitrosation of hydroxylamine [14] and also the hydrazinium ion.[4] It is not possible to compare our findings regarding $NO\overset{+}{S}C(NH_2)_2$ with any other work since this is, as far as we are aware, the first report of direct thiourea catalysis in nitrosation. Our earlier work [11] concerning reversible denitrosation suggested (by selectivity arguments) that NOSCN and $NO\overset{+}{S}C(NH_2)_2$ are of comparable reactivity.

It is perhaps surprising at first sight to note that the free base form of morpholine (pK_a 8.38 [15] at 30°) is less reactive towards nitrosation than is aniline [3] (pK_a 4.51 [16] at 30°). There are, however, a number of examples where the basicity and nucleophilicity trends are not parallel, *e.g.* for imidazole and aniline, when the Pearson *n* value [17] is used as the measure of nucleophilicity.

(b) *Diazotisation of Aniline.*—We have measured the rate constants for diazotisation of aniline in the presence of thiourea, thiocyanate ion, and also bromide ion for comparison purposes. All reactions were very rapid and were measured in a stopped-flow spectrophotometer noting the appearance of the diazonium ion in the case of bromide and thiocyanate reactions. For thiourea the nitrososulphonium intermediate was generated first before the addition of aniline, since the rate of formation of the nitrososulphonium intermediate was sufficiently close to the rate of diazotisation to make the kinetic analysis difficult. These reactions were followed by noting the disappearance of the yellow nitrososulphonium intermediate at 420 nm. The results of the variation of k_0 with both [Br$^-$] and [SCN$^-$] are given in Table 5 and of k_0 with [Aniline]$_{Total}$ for the thiourea experiments in Table 6. The variation of k_0 with thiourea was examined but, as expected, did not produce much of a variation in k_0 because of the considerable conversion of HNO_2 to the nitrososulphonium intermediate under the experimental conditions prevailing.

TABLE 5

k_0 as a function of [Br$^-$] and of [SCN$^-$] for the diazotisation of aniline at 0°

10^2[Br$^-$]/M	k_0/s^{-1}	10^3[SCN$^-$]/M	k_0/s^{-1}
3.45	0.226	4.08	0.466
4.32	0.274	8.15	0.851
5.18	0.320	12.3	1.21
6.04	0.368	16.3	1.56
7.77	0.450	20.4	1.85
9.49	0.590	[Aniline]$_{Total}$ = 2.57×10^{-2}M	
10.11	0.672		

[Aniline]$_{Total}$ = 1.89×10^{-2}M

TABLE 6

k_0 as a function of [Aniline]$_{Total}$ for the diazotisation of aniline in the presence of thiourea (1.08×10^{-2}M) at 0°

10^2[Aniline]$_{Total}$/M	k_0/s^{-1}
1.09	0.136
1.56	0.216
1.95	0.270
2.34	0.330
3.12	0.484

From the linear plots of k_0 *versus* [Br$^-$] and [SCN$^-$], values of k_2 at 0° were found to be 1.6×10^9 and 1.1×10^7 l mol^{-1} s^{-1}, respectively, *i.e.* NOSCN is significantly less reactive than is NOBr, where the reaction rate constant approaches that expected for a diffusion-controlled reaction. Rate measurements were also carried out at 30° for both of these anion-catalysed reactions, giving 2.7×10^9 and 9.1×10^7 l mol^{-1} s^{-1}, respectively, for the k_2 values. The activation energies for both reactions are 12 (NOBr) and 48 (NOSCN) kJ mol^{-1}. The value of 12 kJ mol^{-1} falls within the range expected [12] for diffusion-controlled reactions.

For the thiourea-catalysed reaction at 0°, k_0 *versus* [Aniline]$_{Total}$ is linear and from the slope k_2 can easily be calculated as 3.7×10^5 l mol^{-1} s^{-1}. In this case K_x[H$^+$][SC(NH$_2$)$_2$] is *ca.* 30 so that $(1 + 1/K_x[H^+][SC(NH_2)_2]) \simeq 1$ and k_0 reduces to k_2K_A[Aniline]$_{Total}$/[H$^+$]. Clearly $NO\overset{+}{S}C(NH_2)_2$ is not as reactive towards aniline as is NOSCN (or NOBr) although, because of the large equilibrium constant for its formation from nitrous acid and thiourea, the overall effect is that $SC(NH_2)_2$ makes a good catalyst for diazotisation of aniline, better than Br$^-$ but not, in this case, as good as in SCN$^-$. The effect here is not so marked as for the nitrosation of morpholine where the actual rate-constant (k_2) values are much closer together (possibly due to some steric reasons) so that the overall catalytic efficiency is there governed almost totally by the magnitude of the K_x values.

Stedman and his co-workers [9] have also reported that the nitrosation of cysteine proceeds rapidly and virtually quantitatively to give initially the nitrososulphonium ion. We have examined the possibility of the intervention of this ion and its *S*-methyl derivative in nitrosation by looking for a dependence of the rate constant for the reaction upon the concentration of added cysteine and also *S*-methylcysteine. For the morpholine nitrosation cysteine proved to be a totally ineffective catalyst

and whilst there was evidence of catalysis by *S*-methylcysteine, the effect was not large and the decomposition of the nitrososulphonium ion interfered with the rate measurements. Some results were, however, obtained (for the *S*-methylcysteine case) for the aniline diazotisation. The observed rate constant (after correction for the decomposition) for the reaction of aniline (total concentration 5.54×10^{-2}M) with nitrous acid (1.92×10^{-3}M) containing *S*-methylcysteine (5.14×10^{-2}M) and H^+ (0.294M) was 98.4×10^{-4} s^{-1}, leading to a k_2 value of 1.6×10^3 l mol^{-1} s^{-1}. Another experiment with different reactant concentrations gave 2.0×10^3 l mol^{-1} s^{-1}. These results show that even though the equilibrium constant for the formation of the $\overset{+}{>}$S-NO ion from *S*-methylcysteine is very large, the ion itself is not a very efficient nitrosating agent so that it is likely that thiols and sulphides generally are poor overall catalysts for nitrosation and diazotisation reactions.

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