S-Nitrosation of Thiourea and Thiocyanate Ion. Nitrosyl Thiocyanate and the *S*-Nitroso-adduct of Thiourea as Nitrosating Agents

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Thiourea reacts with *N*-methyl-*N*-nitrosoaniline in aqueous acid solution to give *N*-methylaniline and a *S*-nitroso-intermediate, which decomposes to form the *CC'*-dithiodiformamidinium ion. The products are believed to be the same as those from the reaction of thiourea with nitrous acid. The nitrosoamine reaction is first order in both thiourea and the nitrosoamine, and is also acid-catalysed. Thiourea is shown to be almost as reactive towards the nitrosoamine in this reaction as is iodide ion. Added *N*-methylaniline reduces the rate of the reaction, showing that the formation of the *S*-nitroso-intermediate is reversible *i.e.* it is capable of acting directly as a nitrosating agent. Rate measurements with added *N*-methylaniline and various 'nitrite traps' [X = HN_3, $NH_2\overset{+}{N}H_3$, NH_2SO_3H, $\overset{+}{N}H_3OH$, and $CO(NH_2)_2$] have enabled the reactivity (relative to *N*-methylaniline) of the various X species towards $NO-\overset{+}{S}<$ to be established. The same order of reactivity of X is found as was recently observed for other more well known nitrosating agents NOCl, NOBr, and $H_2\overset{+}{N}O_2$, but the discrimination by $NO-\overset{+}{S}<$ is significantly greater than for these, implying that $NO-\overset{+}{S}<$ is a less reactive species. Rate constant ratios obtained similarly for nitrosyl thiocyanate are remarkably similar to those found for $NO-\overset{+}{S}<$ suggesting that they have much the same reactivity as nitrosating agents.

DENITROSATION of aromatic *N*-nitrosoamines has been shown [1] to involve rate-determining attack by a nucleophile at the nitroso-nitrogen atom of the protonated form of the nitrosoamine. This step, normally reversible, can be examined kinetically without complications due to the reversibility, if there is present a sufficient excess of a 'nitrite trap' such as sulphamic acid or hydrazoic acid, which effectively removes the free nitrosating agent as soon as it is formed. The denitrosation process is very susceptible to the reactivity of the nucleophile ($H_2O < Cl^- < Br^- < SCN^- < I^-$). This paper presents the results of a study of the effect of a neutral sulphur containing nucleophile, thiourea, in this system. For more conventional S_N2 substitutions at saturated carbon, it is known that thiourea is a powerful nucleophile, the Pearson [2] nucleophilicity parameter *n* having a value close to that of iodide ion. A preliminary account of some of our results has been published.[3] Early work by Werner [4] showed that nitrous acid reacted with thiourea at moderate acidities to give a coloured solution which faded rapidly. Salts of the *CC'*-dithiodiformamidinium ion were isolated from the reaction solutions. The structure of these salts has been established by *X*-ray crystallography [5] which shows quite clearly the presence of an S–S bond. Other oxidising agents, such as hydrogen peroxide, the halogens, and peracetic acid also yield these salts on reaction with thiourea,[5] but as far as we are aware, the reaction with a nitrosoamine has not been previously reported.

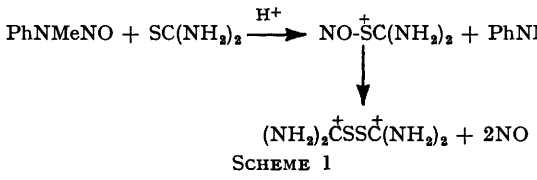
More recently Stedman and his co-workers have examined quantitatively the reactions of nitrous acid with thiourea [6] and alkylthioureas.[7] They conclude that *S*-nitrosation occurs to give the intermediate $(NH_2)_2C=\overset{+}{S}-N=O$ (which is the coloured species observed by Werner), which decomposes fairly rapidly to give the *CC'*-dithiodiformamidinium ion $(NH_2)_2\overset{+}{C}SS\overset{+}{C}(NH_2)_2$ and nitric oxide. Disulphides of this type have been isolated from the nitrosation of thiols,[8] and thioamides are believed [9] to undergo *S*-nitrosation with nitrous acid. Thiols are also attacked at the sulphur atom by alkyl nitrites to yield thionitrite esters.[10] If *S*-nitroso-intermediates are formed from nitrosoamines and thiourea, it is of interest to establish whether they can act directly as nitrosating agents (without the intermediacy of free nitrous acid), and to determine their reactivity relative to other well established nitrosating agents. This could be effected indirectly, by noting the variation of the observed rate constant as a function of added [secondary amine] (which is formed in the reaction), when reaction is carried out in the presence of a constant amount of a 'nitrite trap' X. This method has already been used to establish the relative reactivity of such species towards nitrosyl chloride, nitrosyl bromide, and the nitrous acidium ion.[11]

RESULTS AND DISCUSSION

The same product, *CC'*-dithiodiformamidinium dichloride was obtained in high yield from the reaction of hydrogen peroxide with thiourea, and from *N*-methyl-*N*-nitrosoaniline and thiourea. If however the reaction of the nitrosoamine and thiourea was carried out in the

[1] I. D. Biggs and D. L. H. Williams, *J.C.S. Perkin II*, 1975, 107.
[2] R. G. Pearson, H. Sobel, and J. Songstad, *J. Amer. Chem. Soc.*, 1968, **90**, 319.
[3] D. L. H. Williams, *J.C.S. Chem. Comm.*, 1975, 375.
[4] E. A. Werner, *J. Chem. Soc.*, 1912, 2166, 2180.
[5] O. Foss, J. Johnsen, and O. Tvedten, *Acta Chem. Scand.*, 1958, **12**, 1782 and references therein.
[6] K. Al-Mallah, P. Collings, and G. Stedman, *J.C.S. Dalton*, 1974, 2469.
[7] P. Collings, K. Al-Mallah, and G. Stedman, *J.C.S. Perkin II*, 1975, 1734.
[8] U. Schultz and D. R. McCalla, *Canad. J. Chem.*, 1969, **47**, 2021.
[9] W. Walter and J. Voss in 'The Chemistry of Amides,' ed. J. Zabicky, Interscience, New York, 1970, p. 449.
[10] G. Kresze and J. Winkler, *Chem. Ber.*, 1963, **96**, 1203; B. Saville, *Analyst*, 1958, **83**, 670.
[11] D. L. H. Williams, *J.C.S. Perkin II*, 1975, 655.

presence of an excess of a ' nitrite trap ', in our case hydrazine, then no such product was obtained. Without such a trap the reaction mixture showed an initial yellow colour, characteristic of a NO–$\overset{+}{S}<$ intermediate. This colour faded quite rapidly and gave a solution which liberated iodine from potassium iodide. These disulphide salts are known to react with iodide in this way.[4] The yellow colour is also destroyed instantaneously by added sodium azide. The results suggest that, as for the reaction with nitrous acid,[6] thiourea reacts with the nitrosoamine to give initially the *S*-nitroso-adduct which decomposed (in the absence of a ' nitrite trap ') to give the disulphide (Scheme 1).



SCHEME 1

The kinetics of the reaction were studied at somewhat lower concentrations so that the rate of the disappearance of the absorption due to the nitrosoamine could be followed. The experiments were carried out under first-order conditions, with [thiourea] ≫ [nitrosoamine]. Each individual run showed a first-order dependence upon the [nitrosoamine] (see the typical run in the Experimental section), and a first-order dependence

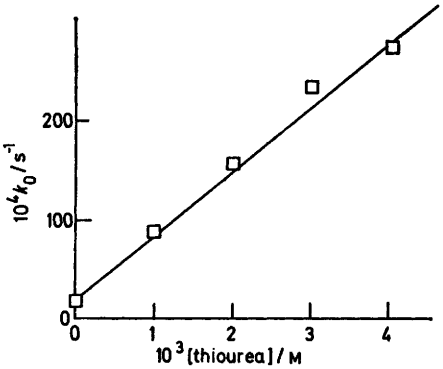


FIGURE 1 Variation of k_0 with [thiourea]

upon [thiourea] was found by varying the [thiourea] (see Figure 1). These experiments were done with 3.05M-hydrochloric acid (where there was a small contribution to the rate constant due to the reaction of chloride ion as the nucleophile), in the presence of an excess of sodium azide. The latter destroys the NO–$\overset{+}{S}<$ intermediate rapidly, so that there are no kinetic complications due to the possible reversibility of the reaction *i.e.* *N*-nitrosation of *N*-methylaniline (NMA) by NO–$\overset{+}{S}<$. In the event, the observed first-order rate constant k_0 (defined by -d[Nitrosoamine]/dt = k_0[Nitrosoamine]) was not altered very much when sodium azide was not present (see Table 1) so that the reversibility is not very marked, even in the absence of HN_3. There is no kinetic effect upon a five-fold increase of $[HN_3]$, so it is reasonable to assume that with $[HN_3] = 2.7 \times 10^{-4}$M the reaction is totally irreversible. The results show that thiourea is involved in the rate-determining step. A mechanism whereby a prior hydrolysis of the nitrosoamine to NMA and nitrous acid, followed by nitrosation of thiourea by the nitrous acid, is eliminated since the rate constant for hydrolysis is known [1] to be much smaller than those observed for the thiourea reaction (Figure 1). Further, it is likely that the nitrous acid would be preferentially destroyed by HN_3.

The reaction is clearly acid-catalysed (see Table 1) and

TABLE 1

Variation of k_0 with acidity

[Acid]/M	$10^4k_0/s^{-1}$	[Acid]/M	$10^4k_0/s^{-1}$
3.05 HCl	17 [a]	2.06 H_2SO_4	56
3.05 HCl	92 [b]	2.57 H_2SO_4	80
3.05 HCl	88	2.57 H_2SO_4	82 [c]
2.46 HCl	67	3.08 H_2SO_4	112
3.64 HCl	129	3.62 H_2SO_4	138
1.55 H_2SO_4	34	4.10 H_2SO_4	153

[PhNMeNO] = 1×10^{-4}M [thiourea] = 1×10^{-3}M, except *a* where [thiourea] = 0. $[HN_3] = 2.7 \times 10^{-4}$M except for *a* and *b* where $[HN_3] = 0$ and *c* where $[HN_3] = 13.5 \times 10^{-4}$M.

so it is reasonable to assume (as was found for reaction of other nucleophiles [1]) that the protonated form of the nitrosoamine reacts directly with thiourea in the rate-determining step (k_1). log k_0 is proportional to H_0 for both acids, except at the higher acidities in H_2SO_4, where a tailing off occurs, which is probably due to the significant degree of protonation of either the nitrosoamine or thiourea (or both) in that region. The rate constants for both acids are very similar at a given H_0 value, if account is taken of the chloride ion reaction in hydrochloric acid. From Figure 1 it is possible to evaluate k_1K (where K is the equilibrium constant for the initial protonation of the nitrosoamine) as 0.55, if a Hammett acidity dependence is assumed for the protonation. This value compares with 0.63 for I^-, 0.22 for SCN^-, 2.2×10^{-3} for Br^-, and 0.42×10^{-4} for Cl^-. There is a reasonably good correlation (particularly bearing in mind that we are effecting a nucleophilic substitution at nitrogen) between log k_1K and n the Pearson nucleophilicity parameter, as is shown in Figure 2. The slope of the line is 1.40, significantly larger than the slopes generally found for the conventional S_N2 substitution at carbon, and shows that the reactivity of the nucleophile (*i.e.* the bond-making process is going to the transition state) is particularly important for attack at the nitroso-nitrogen atom. A similar correlation (with a smaller slope) is found [12] for the reactions of the more reactive *N*-nitrosodiphenylamine with these nucleophiles. These results contrast with the very small spread of rate constants for the reaction of nucleophiles with the still more reactive nitrous acidium ion,[13] where it is thought that the

[12] J. T. Thompson and D. L. H. Williams, to be published.
[13] J. H. Ridd, *Quart. Rev.*, 1961, 432, and references quoted therein.

reaction rates approach that of the diffusion-controlled process.

The intermediate NO–$\overset{+}{S}<$ can in principle, itself act as a nitrosating agent, although it is in equilibrium with free

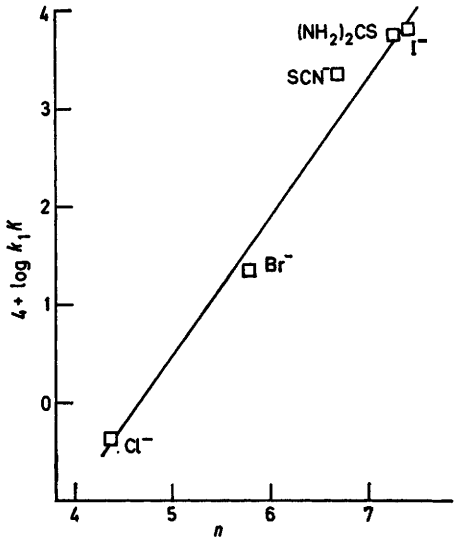


FIGURE 2 Plot of log k_1K *versus* n, the Pearson nucleophilicity parameter

nitrous acid,[6] which could complicate the issue. It is apparently not a very powerful reagent (compared with $H_2\overset{+}{N}O_2$ or NOCl) since the rate constant is not greatly changed by the addition of HN_3. We have examined this possibility in more detail by noting any variation of k_o with added NMA, which should increase the rate of *N*-nitrosation by NO–$\overset{+}{S}<$ if it is a significant process here Scheme 2 represents the reaction of the nitrosoamine with thiourea in the presence of a trap X (HN_3,

$$PhNMeNO + H_3\overset{+}{O} \underset{}{\overset{K}{\rightleftharpoons}} Ph\overset{+}{N}HMeNO + H_2O$$

$$Ph\overset{+}{N}HMeNO + SC(NH_2)_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PhNHMe + NO\text{-}\overset{+}{S}C(NH_2)_2$$

$$NO\text{-}\overset{+}{S}C(NH_2)_2 + X \xrightarrow{k_3} SC(NH_2)_2 + \text{various products}$$

SCHEME 2

NH_2SO_3H, *etc.*). The general expression for k_o is given by equation (1). The ratio of rate constants k_{-1}/k_3 can readily be obtained from a plot of $1/k_o$ *versus* [NMA] at constant acidity, [thiourea], and [X], from equation (2). This ratio represents the relative reactivities of NMA and X towards NO–$\overset{+}{S}<$. Such a plot is shown in Figure 3 for

$$k_o = \frac{k_1Kh_o[SC(NH_2)_2]k_3[X]}{k_{-1}[NMA] + k_3[X]} \quad (1)$$

$$\frac{1}{k_o} = \frac{k_{-1}[NMA]}{k_1Kh_o[SC(NH_2)_2]k_3[X]} + \frac{1}{k_1Kh_o[SC(NH_2)_2]} \quad (2)$$

reaction in H_2SO_4 using sulphamic acid as X. Values of k_{-1}/k_3 (which do not take into account any protonation equilibria of NMA or X) have been obtained for the five X species in Table 2. The ratios have also been determined for nitrosyl thiocyanate NOSCN, by using SCN^- instead of thiourea as the nucleophile, so that comparison between the reactivities of these two nitrosating agents can be made. Figure 4 shows a plot of equation (2) for the thiocyanate ion–nitrosoamine reaction at constant [HN_3]. The data for nitrosyl bromide (from a previous paper [11]) are also included in Table 2 for

TABLE 2
k_{-1}/k_3 Values

X	NOBr	$NO\overset{+}{S}C(NH_2)_2$	NOSCN
HN_3	3.2×10^{-2}	5.0×10^{-2}	4.5×10^{-2}
$NH_2\overset{+}{N}H_3$	4.8×10^{-2}	1.9×10^{-1}	1.8×10^{-1}
NH_2SO_3H	1.8	4.3	4.0
$\overset{+}{N}H_3OH$	30	85	88
$CO(NH_2)_2$	1 170		2 670

comparison. The X compounds are arranged in decreasing order of efficiency as traps for nitrosating agents *i.e.* HN_3 is the most, and urea the least reactive of those studied, with a range of *ca.* 10^4 covering the sequence

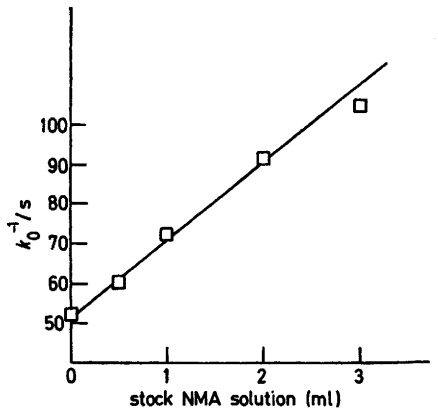


FIGURE 3 $1/k_o$ *versus* [NMA] for reaction of thiourea with the nitrosoamine in the presence of sulphamic acid

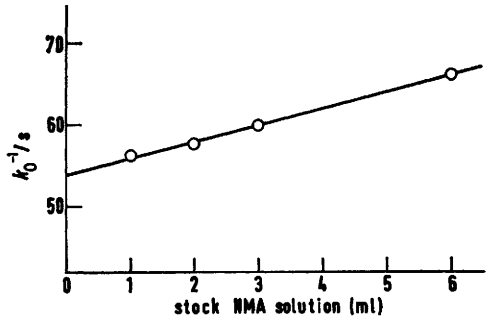


FIGURE 4 $1/k_o$ *versus* [NMA] for reaction of SCN^- with the nitrosoamine in the presence of hydrazoic acid

for NOBr. It is clear that for all the X species the same trend of reactivity is shown for both NOSCN and $NO\overset{+}{S}C(NH_2)_2$ as for NOBr (and also for NOCl and $H_2\overset{+}{N}O_2$, not shown here [11]). The striking feature of Table 2 is the remarkable similarity between the rate constant ratios for NOSCN and $NO\overset{+}{S}C(NH_2)_2$. This indicates

that they both show virtually the same selectivity among the various X species studied, which in turn suggests that they show an almost identical reactivity in these nitrosation reactions. The rate constant ratios for both of these species are 2—3 fold greater than for the corresponding NOBr reactions, suggesting again, by the selectivity–reactivity principle, that NOBr is the more reactive reagent. It proved impossible to get a value of k_{-1}/k_3 for urea in the thiourea reaction, since in each individual kinetic run there was a marked departure from first-order behaviour, with the apparent first-order rate constant decreasing as reaction proceeded. This did not occur in the corresponding thiocyanate reaction. The rate reduction was greater the higher the NMA concentration, suggesting that for some reason there could be a build-up, beyond the stationary-state limit, of [NO–$\overset{+}{S}C(NH_2)_2$] in this case. This would arise, if, for example urea is a particularly inefficient reagent for the removal of the nitrosating agent; in this case possibly some steric effect operates with the larger NO–$\overset{+}{S}C(NH_2)_2$ which is not present for NOSCN and NOBr.

Catalysis by added halide and thiocyanate ions is of course well known in nitrosation processes,[13] and is interpreted in terms of the formation and reaction of the nitrosyl halide and nitrosyl thiocyanate species NOY. The greater catalytic effect of thiocyanate ion than of either chloride or bromide ion is due to the relative values of the equilibrium constants for the formation of NOY from nitrous acid and the anions Y^-. This effect is so large that it is the main factor in deciding the extent of catalysis, rather than the actual reactivities of the NOY species. When this is taken into account it is possible to compare the reactivities of such species. Nitrosyl chloride is *ca.* six times more reactive than nitrosyl bromide in electrophilic addition to alkenes.[14] The factor is much greater for the nitrosation of the hydrazinium ion.[15] This order is to be expected from the inductive effects of the halogens. So far as NOSCN is concerned, it has been found to be significantly less reactive than either nitrosyl chloride or nitrosyl bromide in the direct nitrosation of hydroxylamine and *O*-methylhydroxylamine,[16] and also in the nitrosation of the hydrazinium ion [15] by nitrous acid in the presence of added anions. The results support our data for the relative reactivities of NOBr and NOSCN based on selectivity arguments.

Reaction of thiourea with *N*-methyl-*N*-nitrosoaniline in the absence of any X species proceeded normally to the NO–$\overset{+}{S}<$ intermediate (which subsequently was converted to the disulphide salt). It was found however, that over a longer period of time the Fischer–Hepp rearrangement product (*p*-nitroso-*N*-methylaniline) of the nitrosoamine was formed in yields >60% (based on the nitrosoamine used up) together with amounts of a yellow product, which often accompanies the rearrangement reaction, but which has not yet been isolated and identified because of its instability. The rate of formation of the rearrangement product was much the same as for the 'normal' rearrangement process at this acidity,[17] except for the relatively long induction period after the formation of the NO–$\overset{+}{S}<$ intermediate. This result demands that the nitrosoamine is regenerated in the reaction mixture, probably when all the thiourea has been converted to the disulphide salt. This is confirmed by an examination of the spectrum of the reaction solutions at various times. It is not to be expected that the nitric oxide released in the formation of the disulphide salt would recombine with the *N*-methylaniline to give the nitrosoamine under these conditions. Al-Mallah [18] has similarly observed the total regeneration of nitrous acid on the reaction of nitrous acid with thiourea, once the thiourea has been fully reacted. The reaction sequence in Scheme 3 was suggested. We have tested this suggestion in our system, by carrying out the reaction of thiourea

$$\begin{aligned} 2NO + O_2 &= 2NO_2 \\ NO_2 + NO &= N_2O_3 \\ N_2O_3 + H_2O &= 2HNO_2 \end{aligned}$$

SCHEME 3

with *N*-methyl-*N*-nitrosoaniline in acid solution in the absence of oxygen in two ways, (a) by keeping nitrogen continuously bubbling through the reaction solution and examining samples spectrophotometrically from time to time and (b) with the reaction solution (previously deoxygenated) enclosed in a tightly fitting stoppered cell in the spectrophotometer. In both cases, no rearrangement product was formed, but merely the denitrosation product *N*-methylaniline. This confirms the suggestion that the nitrous acid can be regenerated (in theory quantitatively) using oxygen dissolved in the solution. In effect we are then studying the nitrosoamine-catalysed oxidation of thiourea. When all the thiourea has been consumed, the regenerated nitrosoamine undergoes the only other reaction available to it under the conditions *i.e.* the acid-catalysed intramolecular rearrangement, together with the solvent-promoted denitrosation (reversible in the absence of a nitrous acid trap.)

The direct *S*-nitrosation of thiourea by a nitrosoamine established in this paper may well have a bearing on the carcinogenic nature of nitrosoamines.[19] Sulphur-containing compounds present in animals (*e.g.* cysteine) could readily be converted to the corresponding disulphides by the action of nitrosoamines. The reaction between some thiols and *N*-alkyl-*N*-nitrosourethanes has been reported,[20] but the mechanism of the reaction was not established. Cysteine reacts [8] with *N*-methyl-*N*-nitrosotoluene-*p*-sulphonamide (MNTS) to give cystine and the sulphonamide, but it is likely, in view of the very

[14] J. R. Park and D. L. H. Williams, *J.C.S. Perkin II*, 1972, 2158.
[15] G. Stedman, personal communication.
[16] T. D. B. Morgan, G. Stedman, and M. N. Hughes, *J. Chem. Soc.* (*B*), 1968, 344.
[17] T. D. B. Morgan, D. L. H. Williams, and J. A. Wilson, *J.C.S. Perkin II*, 1973, 473.
[18] K. Al-Mallah, Ph.D. Thesis, University of Wales, 1974.
[19] P. N. Magee and J. M. Barnes, *Adv. Cancer Research*, 1967, **10**, 163.
[20] R. Schoental and D. J. Rive, *Biochem. J.*, 1965, **97**, 466.

rapid hydrolysis of MNTS in dilute acids,[21] that nitrosation is effected by the nitrous acid so released rather than by MNTS itself. Preliminary work in these laboratories has shown that cysteine has a small, but significant reactivity as a nucleophile (towards *N*-methyl-*N*-nitrosoaniline) which is comparable with that of chloride ion. Further work is in progress.

EXPERIMENTAL

CC'-Dithiodiformamidinium dichloride was prepared by the method of Preisler and Berger,[22] from hydrogen peroxide and thiourea. The preparation was repeated using *N*-methyl-*N*-nitrosoaniline instead of hydrogen peroxide. In both cases, white crystals of the salt were obtained in high yield; these were filtered, washed with ethanol and ether and dried. The i.r. spectra of the two products were virtually identical. No salt precipitated when the preparation was repeated in the nitrosoamine case, when there was present an excess of hydrazine hydrochloride.

[21] D. R. McCalla, A. Reuvers, and R. Kitai, *Canad. J. Biochem.*, 1968, **46**, 807; D. L. H. Williams, *J.C.S. Perkin II*, 1976, 1838.

The kinetic measurements were carried out at 31° in aqueous solution in the thermostatted cell of a recording u.v.–visible spectrophotometer. Usually the decreasing absorption at 280 nm due to the reactant was recorded as a function of time. Good first-order plots were obtained and the rate constants were reproducible within ±4%. A typical run is shown for the reaction of the nitrosoamine (1×10^{-4}M) with thiourea (2×10^{-3}M), sulphamic acid (9.6×10^{-3}M) and *N*-methylaniline (2.4×10^{-3}M) in sulphuric acid (3.45M).

t (30 s units)	0	2	3	4	5
OD	0.889	0.598	0.509	0.440	0.383
$10^4k_o/s^{-1}$		96.8	95.2	95.0	97.0
t (30 s units)	6	7	8	9	∞
OD	0.347	0.316	0.292	0.276	0.229
$10^4k_o/s^{-1}$	95.6	96.5	97.9	97.8	

We thank Dr. G. Stedman for helpful discussion, and the Royal Society for an equipment grant.

[6/793 *Received*, 23*rd April*, 1976]

[22] P. W. Preisler and L. Berger, *J. Amer. Chem. Soc.*, 1947, **69** 322.