The Reactivity of Thiourea, Alkylthioureas, Cysteine, Glutathione, *S*-Methylcysteine, and Methionine towards *N*-Methyl-*N*-nitrosoaniline in Acid Solution

By **Geoffrey Hallett** and **D. Lyn H. Williams,*** Department of Chemistry, University Science Laboratories, South Road, Durham DH1 3LE

Thiourea and several alkylthioureas have very nearly the same rate constants for their reactions with *N*-methyl-*N*-nitrosoaniline in sulphuric acid containing hydrazine sulphate. The results are consistent with an initial *S*-nitrosation in which there is no significant delocalisation of the positive change to the amino-nitrogen atom in the transition state. For thiourea the detailed form of the acid catalysis is established, both H_0 and H_0''' acidity functions represent the initial protonation reasonably well. In the same reaction cysteine and glutathione have a small but measurable reactivity, which is comparable with that of chloride ion, whilst the effect of *S*-methylation (for *S*-methylcysteine and methionine) is to increase the rate constant for *S*-nitrosation by a factor of *ca.* 20, to about the same value as found for bromide ion. Alanine is totally unreactive under these conditions, a result which supports *S*- rather than *N*-nitrosation.

In aqueous acid solution the denitrosation of nitrosamines to yield the corresponding secondary amines can, in general, be effected by nucleophiles such as Cl^-, Br^-, I^-, and SCN^- as well as by the solvent.[1] The reactivity of the nucleophiles (towards two nitrosamines) correlated [2] surprisingly well with the Pearson nucleophilicity parameter [3] n. In a recent paper by one of us,[4] the direct reaction between *N*-methyl-*N*-nitrosoaniline and thiourea was described. All the kinetic and product studies were consistent with a reaction mechanism involving as the rate-determining stage, nucleophilic attack by thiourea (at the sulphur atom) of the protonated form of the nitrosamine, to give initially an unstable $\overset{+}{S}$–NO species. The reactivity of thiourea was quite similar to that of iodide ion for this nitrosamine and also for the reaction of *N*-nitrosodiphenylamine.[2] The direct nitrosation of thiourea and alkyl thioureas by nitrous acid itself has been kinetically studied by Stedman and his co-workers,[5,6] who also interpret their results in terms of an initial *S*-nitrosation. In this paper we report an extension of our earlier work with thiourea, where the detailed form of acid catalysis is examined, as well as establishing the reactivity towards nitrosamines in acid solution of *N*-alkylthiourea derivatives, cysteine, *S*-methylcysteine, methionine, and glutathione.

EXPERIMENTAL

N-Methyl-*N*-nitrosoaniline was prepared in the usual way [7] from *N*-methylaniline, and was distilled under reduced pressure before use. Thiourea and the *N*-alkyl thioureas were commercial samples which were recrystallised from ethanol. L-Cysteine, *S*-methyl-L-cysteine, L-alanine, L-methionine, and L-glutathione were all commercial samples of high purity which were used without further purification.

Kinetic measurements were all carried out in sulphuric acid solution at 31 °C in a Beckmann model 25 recording spectrophotometer. Most of the rate constants were determined by noting the disappearance of the nitrosamine absorption at fixed wavelength in the range 280—300 nm depending on the thiourea derivative used, whilst some of the slower reactions were followed by scanning an appropriate wavelength range at fixed time intervals. Good first-order behaviour was found to around 90% reaction for all nucleophiles except L-cysteine, where there was some deviation from first-order behaviour after 75% reaction. Duplicate runs agreed to better than ±5%; a typical run is given in Table 1.

TABLE 1

Rate data for the reaction of *N*-methyl-*N*-nitrosoaniline (1.52×10^{-4}M) with tetramethylthiourea (8.04×10^{-4}M) in sulphuric acid (4.24M) containing hydrazine sulphate (1.0×10^{-3}M)

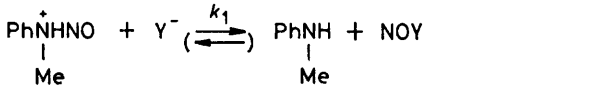
t/s	0	30	60	90	120	150	180
OD	0.648	0.583	0.523	0.471	0.427	0.387	0.349
$10^3k_0/s^{-1}$		4.19	4.30	4.32	4.28	4.29	4.36

t/s	210	240	270	300	∞
OD	0.317	0.288	0.264	0.243	0.098
$10^3k_0/s^{-1}$	4.39	4.43	4.44	4.44	

Mean value of $k_0 = (4.34 \pm 0.03) \times 10^{-3}$ s^{-1}.

RESULTS AND DISCUSSION

Reactions of Thiourea and the N-*Alkylthioureas.*—In order to eliminate complications due to reversibility we have examined the reactivities of nucleophiles towards nitrosamines (in acid solution) in the presence of an excess of a so-called nitrite trap X, such as sodium azide, hydrazine sulphate, sulphamic acid, *etc.* When [X] exceeds a certain lower limit denitrosation proceeds irreversibly and quantitatively (see Scheme 1). Under



SCHEME 1

these conditions the observed first-order rate constant k_0 (defined by $-d[\text{Nitrosamine}]/dt = k_0[\text{Nitrosamine}]$) is given by equation (1), if we assume some dependence upon the Hammett acidity function h_0, for the initial protonation (of equilibrium constant K), and if the concentration of the nucleophile $[Y^-]$ is effectively

$$k_0 = k_1 K h_0^a [Y^-] \qquad (1)$$

constant during any one kinetic experiment. Even though K is not known, it is constant for any one nitrosamine, so that relative reactivities of nucleophiles can readily be obtained quantitatively from plots of k_0 *vs.* [Y^-] at any one acidity. The exponent a is the experimentally determined order in h_0, which would be expected to be close to 1.

It was found that hydrazine sulphate behaved as the most satisfactory trap for the range of *N*-alkylthioureas studied and so was used throughout. Under these circumstances *N*-methylaniline is formed quantitatively. There was evidence of extensive side reactions involving some of the traps examined. In particular when using sulphamic acid for the reaction with tetramethylthiourea, a reproducible *S*-shaped curve was obtained for k_0 *vs.* [tetramethylthiourea]. The earlier kinetic results [4] were consistent with the decomposition of the $\overset{+}{\text{S}}$–NO intermediate by X with the regeneration of thiourea. Previous product analysis studies [4,5] had shown that in the absence of such a trap the intermediate yields dithioformamidinium ion $(NH_2)_2\overset{+}{C}SS\overset{+}{C}(NH_2)_2$ from thiourea itself. Variation of k_0 with [hydrazine sulphate] showed, for a number of different acidities, that the limiting condition prevailed so long as [hydrazine sulphate] $> 1 \times 10^{-3}$M. Under these circumstances good linear plots of k_0 *vs.* [Thiourea] were obtained at each acidity studied, showing clearly that the reaction is first-order in thiourea as predicted by equation (1). There is a small positive intercept in each case, which represents denitrosation brought about by the solvent water acting as a nucleophile. The slopes of these plots increase with acidity, as expected and are presented in Table 2. It is reasonable to assume that reaction occurs *via* the unprotonated form of thiourea. The pK_a value as measured by Janssen [8] is −1.19 (based on the H_0 scale); there is much evidence [9] that the protonation occurs at the sulphur atom. We can now correct the slope to give the reactivity of the unprotonated form of thiourea We have re-examined the acidity dependence by plotting log (corrected slope) *vs.* H_0, H_0''' (for tertiary amines) [10] and H_A (for amides).[11] All give reasonably good straight lines with slopes of 1.2, 0.9, and 1.6 respectively. It appears therefore that both H_0 and H_0''' are reasonably 'good' acidity functions to be used in connection here with the nitrosamine protonation in these sulphuric acid solutions. The kinetic results are all consistent with the outline mechanism given in Scheme 1.

TABLE 2

Variation of the slope of k_0 *vs.* [Thiourea] with acidity

$[H_2SO_4]$/M	Slope	Corrected slope †
0.78	0.66	0.72
1.57	2.24	2.87
2.36	6.31	11.3
3.14	10.3	29.9
3.92	15.7	84.8

† Corrected for protonation of thiourea.

Values of k_1K can then readily be calculated at each acidity. For thiourea itself a value of 0.52 ± 0.03 is obtained. This process was repeated over the same range of acidities for each of a number of *N*-alkylthioureas. For all of the *N*-alkylthioureas there appears to be a rate dependence upon h_0 to the power 1.11—1.21 (averaged at 1.15), except for tetramethylthiourea where the value is a little lower at 0.99. In principle, for a single nitrosamine, we would expect the dependence upon the acidity to be independent of the nucleophile used. The variation that we find (which is not large) is taken to be the experimental error. Values of the order in h_0 and of k_1K' are given in Table 3 together with the original pK_a values of Janssen.[8] These were corrected by Stedman [6] using a different acidity function H_t of Tissier,[12] but we have retained the original values, since we are not here concerned with the absolute values of the pK_a, but have used the same acidity function (H_0) to calculate the amount of unprotonated thiourea as Janssen used in the determination of these pK_a values. The striking feature of the k_1K values in Table 3 is their similarity. All the values fall almost within the standard error. This reaction is particularly sensitive to the reactivity of the nucleophile as shown by the large range exhibited by the halide ions resulting in a slope of 1.41 in the Pearson plot,[2] so the almost constant value of k_1K throughout the series does mean that the reactivities are virtually identical. Stedman [8] found also that the *N*-alkylthioureas were all approximately equally reactive towards free nitrous acid, a result which was interpreted in terms of diffusion-controlled reactions. In that case, support was lent to this suggestion by the similarity between the observed rate constants and those for the diazotisation of aniline derivatives, where it has been established [13] that for a number of aniline derivatives the rate constants do approach the value expected for diffusion-controlled reactions. In our case, however, we believe that this is not the explanation for the constancy of the k_1K values, since if the k_1 values are close to the diffusion-controlled limit of *ca.* 1×10^{10} mol^{-1} s^{-1} then K must be of the order of 5×10^{-11}, which corresponds to a pK_a value for the nitrosamine of *ca.* −10. This has never been measured directly but it has an estimated [14] value of *ca.* −2. Further the Pearson plot of log k_1K *vs.* n shows no indication of levelling off at $n = 7.3$ for thiourea, and k_1K is 0.63 for I^- ($n = 7.4$). Another explanation for the constant values, which seems more likely in our case, is that in the transition state there is

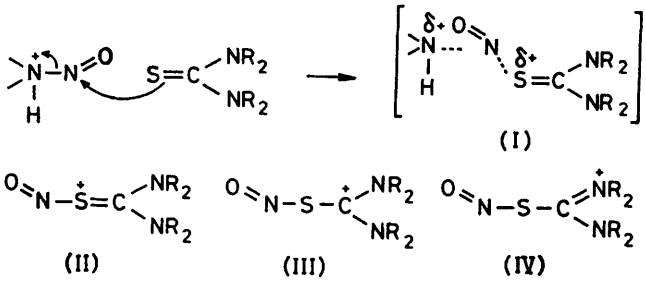
TABLE 3

Values of k_1K and the order in h_0 for the reaction of *N*-methyl-*N*-nitrosoaniline with thiourea and some *N*-alkylthioureas

	pK_a	Order in in h_0 †	k_1K
Thiourea	−1.19	1.21	0.52 ± 0.03
N-Methylthiourea	−1.12	1.15	0.57 ± 0.02
NN'-Dimethylthiourea	−1.32	1.11	0.58 ± 0.02
Trimethylthiourea	−1.53	1.11	0.49 ± 0.03
Tetramethylthiourea	−1.00	0.99	0.54 ± 0.03

† This is the exponent a in equation (1).

very little, if any, positive charge delocalised to the amino nitrogen atoms, *i.e.* contribution from structure (IV) to the transition state is negligible. Important contributions would then be expected towards the



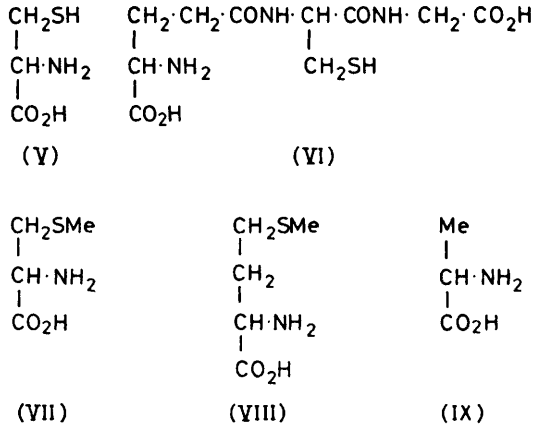
(I) (II) (III) (IV)

SCHEME 2

transition state (I) from structures (II) and perhaps (III) where the positive change is located on C and S respectively and where increasing methyl substitution on N would not be expected to stabilise the ion significantly.

More complex schemes are, of course, possible including protonation at the oxygen site of the nitroso-group, but reaction *via* the *N*-protonated species. At this stage however there is no conclusive evidence regarding this point and so it is not introduced into the reaction scheme.

Reactions of Cysteine, Glutathione, S-Methylcysteine, and Methionine.—In an attempt to discover whether other types of neutral sulphur sites have a significant reactivity towards nitrosamines, we have measured the rate constants for the reaction of *N*-methyl-*N*-nitrosoaniline in sulphuric acid with cysteine (V), glutathione (VI), *S*-methylcysteine (VII), and methionine (VIII). Again we have carried out the kinetic experiments in the presence of an excess of hydrazine sulphate, so that any



(V) (VI) (VII) (VIII) (IX)

direct nitrosation of these substances is irreversible and any *S*-nitroso-intermediate would be expected to react rapidly with hydrazine resulting in overall decomposition of the nitroso-species. This procedure also ensures that we do not have here a case of solvent-promoted denitrosation followed by nitrosation of cysteine *etc.* by the free nitrous acid thus formed. As before for thiourea and the *N*-alkylthioureas we have measured the first-order rate coefficient k_0 as a function of the concentration of the nucleophile (cysteine *etc.*) at constant acidity of 4.48M-H_2SO_4. The results are presented in Tables 4—7. It is clear that for both cysteine and glutathione (both of which contain the CH_2SH group) the reactivity towards the nitrosamine is very small. Nevertheless we believe

TABLE 4

Variation of k_0 with [cysteine]

10^3[Cysteine]/M	10^4k_0/s^{-1}
0	8.41
2.05	9.63
6.14	9.89
10.23	10.52

TABLE 5

Variation of k_0 with [glutathione]

10^3[Glutathione]/M	10^4k_0/s^{-1}
0	8.41
1.99	8.40
6.00	9.80
9.92	10.85

TABLE 6

Variation of k_0 with [*S*-methylcysteine]

10^3[*S*-Methylcysteine]/M	10^4k_0/s^{-1}
0	8.41
1.24	62.8
3.73	153.8
6.22	221.9

TABLE 7

Variation of k_0 with [methionine]

10^3[Methionine]	10^4k_0/s^{-1}
0	8.41
1.21	15.8
3.64	31.2
6.06	46.1

TABLE 8

Variation of k_0 with [alanine]

10^3[Alanine]/M	10^4k_0/s^{-1}
0	8.41
8.69	8.26
26.1	8.48
43.5	8.90

that the significant increases in k_0 with concentration for both Tables 4 and 5 do represent a direct reaction. The reactivity of *S*-methylcysteine and methionine (both of which contain the CH_2SCH_3 group) is much greater. If we assume that $k_0 = k_1Kh_0^{1.15}$ then it is easy to calculate k_1K values for the series. These are gathered together in Table 9 together with values obtained earlier from studies with the same nitrosamine [1] (Cl^-, Br^-, SCN^-, I^-). We examined the denitrosation also in the presence of alanine (IX) and found no detectable change in the rate constant with increasing [alanine]. The value of

TABLE 9

Values of k_1K for a number of nucleophilic species

Reactant	k_1K	Reactant	k_1K
Alanine	*ca.* 0	Bromide ion	2.2×10^{-3}
Chloride ion	4×10^{-5}	Methionine	2.6×10^{-3}
Cysteine	7×10^{-5}	Thiocyanate ion	0.22
Glutathione	1.3×10^{-4}	Thiourea	0.52
S-Methylcysteine	1.4×10^{-3}	Iodide ion	0.63

$k_0 = 8.4 \times 10^{-4}$ s^{-1} represents denitrosation by the solvent. It appears that nitrosation at the NH_2 (or NH_3^+) group is thus eliminated as a possible reaction site, so that we can deduce that for cysteine, glutathione, *S*-methylcysteine, and methionine, reaction does occur at the sulphur atom. So far as relative reactivities are concerned, it is clear that the reactivity of the CH_2SH group (in cysteine and glutathione) is very low and comparable with that of chloride ion, whereas the introduction of a *S*-methyl substituent (in *S*-methylcysteine and methionine) increases the reactivity towards nitrosation considerably, as expected, so that both of these reactants are about as reactive as bromide ion towards electrophilic nitrosation.

These kinetic results provide quantitative evidence that a direct reaction between thiols and nitrosamines (probably in their pronated forms) can occur, albeit rather slowly. Reactivity is significantly increased by *S*-methyl substitution so that generally sulphides R-S-R'' can be expected to undergo *S*-nitrosation by nitrosamines much more readily. Reactions of this type have been reported,[15] but have not been examined mechanistically. We would not expect to find the same products since under our kinetic conditions any $\overset{+}{S}$–NO intermediate would be expected to react rapidly and irreversibly with hydrazine, regenerating the reactant nucleophile. All the kinetic results are consistent with this scheme.

We thank the North of England Cancer Research Campaign for a research studentship (to G. H.) and the Royal Society for an equipment grant.

[9/822 *Received*, 25*th May*, 1979]

REFERENCES

[1] I. D. Biggs and D. L. H. Williams, *J.C.S. Perkin II*, 1975, 107.
[2] J. T. Thompson and D. L. H. Williams, *J.C.S. Perkin II*, 1977, 1932.
[3] R. G. Pearson, H. Sobel, and J. Songstad, *J. Amer. Chem. Soc.*, 1968, **90**, 319.
[4] D. L. H. Williams, *J.C.S. Perkin II*, 1977, 128.
[5] K. Al-Mallah, P. Collings, and G. Stedman, *J.C.S. Dalton*, 1974, 2469.
[6] P. Collings, K. Al-Mallah, and G. Stedman, *J.C.S. Perkin II*, 1975, 1734.
[7] A. I. Vogel, 'Textbook of Practical Organic Chemistry,' Longmans, London, 1954, p. 547.
[8] M. J. Janssen, *Rec. Trav. chim.*, 1962, **81**, 650.
[9] M. J. Janssen, *Spectrochim. Acta*, 1961, **17**, 475; W. Kutzelnigg and R. Mecke, *ibid.*, p. 530; R. J. Gillespie and T. Birchall, *Canad. J. Chem.*, 1963, **41**, 2643.
[10] E. M. Arnett and G. W. Mach, *J. Amer. Chem. Soc.*, 1964, **86**, 2671.
[11] K. Yates, J. B. Stevens, and A. R. Katritzky, *Canad. J. Chem.*, 1964, **42**, 1957.
[12] C. Tissier and M. Tissier, *Bull. Soc. chim. France*, 1972, 2109.
[13] J. H. Ridd, *Quart. Rev.*, 1961, **15**, 418; M. R. Crampton, J. T. Thompson, and D. L. H. Williams, *J.C.S. Perkin II*, 1979, 18.
[14] I. D. Biggs and D. L. H. Williams, *J.C.S. Perkin II*, 1976, 691.
[15] U. Shulz and D. R. McCalla, *Canad. J. Chem.*, 1969, **47**, 2021; R. Schoental and D. J. Rive, *Biochem. J.*, 1965, **97**, 466.