

## Kinetic and Equilibrium Studies of the Reactions of Some Thiolate Ions with Trinitro-aromatic Compounds: Intrinsic Reactivities

Michael R. Crampton\* and J. Andrew Stevens

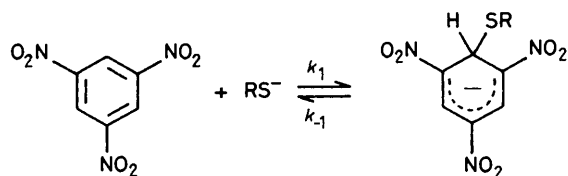
Department of Chemistry, Durham University, Durham, DH1 3LE

Kinetic and equilibrium data are reported for the formation of 1:1 and 1:2 adducts from 1,3,5-trinitrobenzene (TNB) and 2,4,6-trinitrotoluene with thiolate ions derived from mercaptoacetate, mercaptosuccinate, and glutathione. The results are used to determine intrinsic reactivities for the thiolate ions in adduct-forming reactions at the nitro-activated aromatic ring. The values for the three thiolate ions are all *ca.*  $5 \times 10^4$  and are considerably higher than comparable values, calculated from literature data, for the sulphite ion (300) and the hydroxide ion (10).

The results for the glutathione anion show that results reported previously for reaction with TNB refer to formation of the 1:2 adduct rather than 1:1 adduct formation.

The reactions of aromatic nitro-compounds with thiol-groups in biologically active compounds are of interest both as a means for the chemical modification of proteins<sup>1</sup> and as a primary step in the inhibition of leukocyte metabolism.<sup>2</sup> The excretion of halogenated aromatic nitro-compounds is likely to involve reaction with glutathione in the mammalian liver;<sup>3</sup> and it has been shown recently<sup>4</sup> that 1,3,5-trinitrobenzene (TNB) inhibits the action of glutathione *S*-transferase. The initial step in these reactions is thought to be  $\sigma$ -adduct formation<sup>5</sup> by thiolate attack at an aromatic ring.

Values of equilibrium constants have been reported for the formation of  $\sigma$ -adducts by reaction at unsubstituted positions of the TNB ring of ethanethiolate ions and substituted thiophenoxide ions (Scheme 1).<sup>6-9</sup> However there is only a single



Scheme 1.

report in the literature of rate measurements for this type of reaction. Thus Gan<sup>3</sup> found values of  $k_1$   $2.9 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  and  $k_{-1}$   $102 \text{ s}^{-1}$  for reaction with the glutathione anion in water.

We are currently interested in measuring intrinsic reactivities, in the Marcus sense, of nucleophiles for the trinitro-activated aromatic ring and in comparing values with those for proton transfer reactions.<sup>10</sup> Results have been reported<sup>11</sup> for some carbon and oxygen bases in methanol solvent. Here we present data for some sulphur bases, the anions derived from mercaptoacetic acid, mercaptosuccinic acid, and glutathione.

### Results and Discussion

Kinetic and equilibrium measurements were made for the reactions of thiolate ions with TNB and with 2,4,6-trinitrotoluene (TNT). For reasons of solubility measurements were usually made in a solvent consisting of water-dioxane (90:10, v/v). However the reaction of TNB with glutathione was studied in water-dioxane (99:1, v/v) to allow direct comparison with previous measurements.<sup>3</sup> The thiolate anions were generated from the parent acids by reaction with sodium hydroxide, sufficient base being added to ionise the carboxylic acid

functions as well as the thiol group. A slight excess, *ca.*  $0.005 \text{ mol dm}^{-3}$ , of base was used to ensure complete ionisation of the thiols. The equilibrium constant for direct reaction of TNB with hydroxide<sup>5</sup> in water has the value  $3 \text{ dm}^3 \text{ mol}^{-1}$  so that negligible interference from this reaction was observed. The ionic strength of the solutions was maintained at  $I = 0.25 \text{ mol dm}^{-3}$  with sodium chloride.

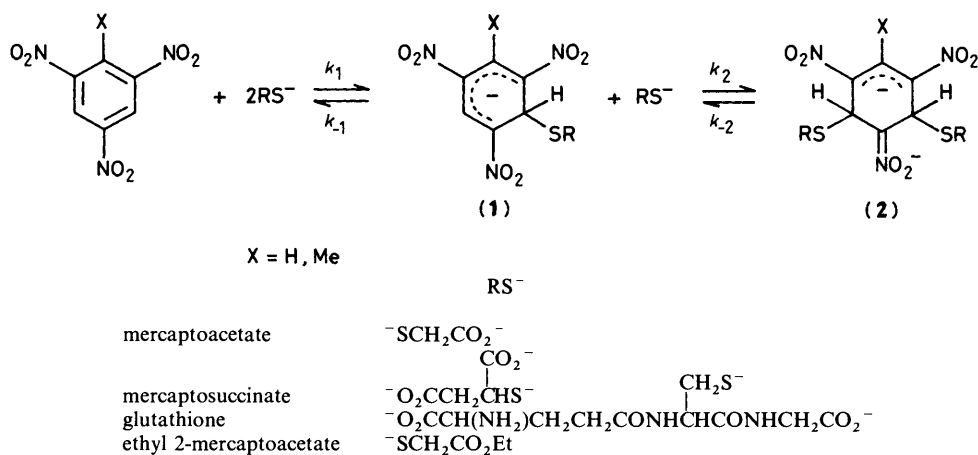
Our results indicate the reversible formation of 1:1 and 1:2 adducts as shown in Scheme 2. Slower irreversible reactions were also present but were not identified. All measurements were made with  $[\text{RS}^-] \gg [\text{TNB}]$  and  $[\text{TNT}]$  and it was found that formation of the 1:1 adducts, (1), was considerably faster than formation of the 1:2 adducts, (2). Under these conditions the rate expressions relevant to the two processes are given in equations (1) and (2).

$$k_{\text{fast}} = k_1[\text{RS}^-] + k_{-1} \quad (1)$$

$$k_{\text{slow}} = k_{-2} + \frac{k_2 K_1 [\text{RS}^-]^2}{1 + K_1 [\text{RS}^-]} \quad (2)$$

**Reactions with TNB.**—The visible spectra, measured on a recording spectrophotometer, of TNB in the presence of very low concentrations of thiolate ions show bands at 470 nm and 550 nm (shoulder). Measurements on related systems have shown<sup>5-8</sup> that these bands are attributable to the 1:1 adducts (1). With increasing concentration of thiolate ions a band at 500 nm, attributed to the 1:2 adducts (2), grows at the expense of these bands. Formation of the 1:1 adducts was in all cases too fast to measure on the stopped-flow timescale, reaction being complete within the mixing time of the apparatus. However temperature-jump measurements could be used to monitor this process. Equilibration of the 1:1 and 1:2 adducts fell conveniently in the timescale for stopped-flow evaluation.

Rate data for the reaction with mercaptoacetate ions are given in Table 1. A linear plot according to equation (1) gave values for  $k_1$   $1.4 \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  and  $k_{-1}$   $5\,500 \text{ s}^{-1}$ , leading to  $K_1 (=k_1/k_{-1})$   $250 \text{ dm}^3 \text{ mol}^{-1}$ . The best fit of the data for  $k_{\text{slow}}$  to equation (2) gave values for  $k_2$   $3\,300 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ,  $k_{-2}$   $8 \text{ s}^{-1}$  and  $K_1$   $220 \text{ dm}^3 \text{ mol}^{-1}$ . It was also possible to independently determine values for  $K_1$  and  $K_2$  using the absorbance values determined by stopped-flow spectrophotometry at completion of the fast and slow reactions respectively. The data, available as supplementary information, lead to values for  $K_1$   $240 \text{ dm}^3 \text{ mol}^{-1}$  and  $K_2$   $500 \text{ dm}^3 \text{ mol}^{-1}$  in good agreement with those obtained from the kinetic data.



Scheme 2.

**Table 1.** Rate data for the reaction of TNB with mercaptoacetate ions in water-dioxane (90:10, v/v) at 25 °C and with  $I = 0.25 \text{ mol dm}^{-3}$ .

$10^3[\text{Mercaptoacetate}]$	$k_{\text{fast}}^a/\text{s}^{-1}$	$k_{\text{slow}}^b/\text{s}^{-1}$	$k_{\text{calc}}^c$
1.0	6 900	8	8
2.0	8 100	11	10
3.0	9 700	—	—
4.0	11 000	16	14
6.0	—	22	19
8.0	—	26	25
10	—	31	31
12	—	37	36

<sup>a</sup> Measured by T-jump spectrophotometry at 470 nm. <sup>b</sup> Measured by stopped-flow spectrophotometry at 500 nm as a colour-forming reaction. <sup>c</sup> Calculated from equation (2) using  $k_2 3 300 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ,  $k_{-2} 8 \text{ s}^{-1}$  and  $K_1 220 \text{ dm}^3 \text{ mol}^{-1}$ .

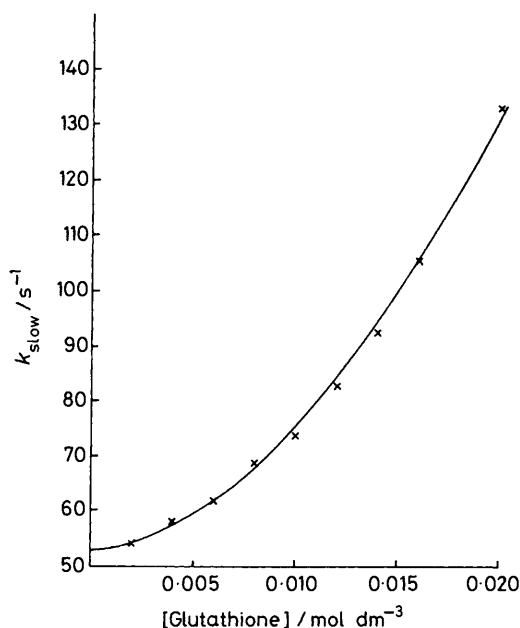
**Table 2.** Rate data for the reaction of TNB with mercaptosuccinate ions in water-dioxane (90:10, v/v) at 25 °C and with  $I = 0.25 \text{ mol dm}^{-3}$ .

$10^3[\text{Mercaptosuccinate}]$	$k_{\text{fast}}^a/\text{s}^{-1}$	$k_{\text{slow}}^b/\text{s}^{-1}$	$k_{\text{calc}}^c$
3.0	34 000	—	—
8.0	—	34	33
9.0	41 000	—	—
12	—	34	34
15	49 000	—	—
20	52 000	35	35
30	67 000	—	—
36	74 000	—	—
40	—	39	39
60	—	43	43
80	—	48	48

<sup>a</sup> Measured by T-jump spectrophotometry at 470 nm. <sup>b</sup> Measured by stopped-flow spectrophotometry at 500 nm. <sup>c</sup> Calculated from equation (2) with  $k_2 250 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ,  $k_{-2} 33 \text{ s}^{-1}$ , and  $K_1 33 \text{ dm}^3 \text{ mol}^{-1}$ .

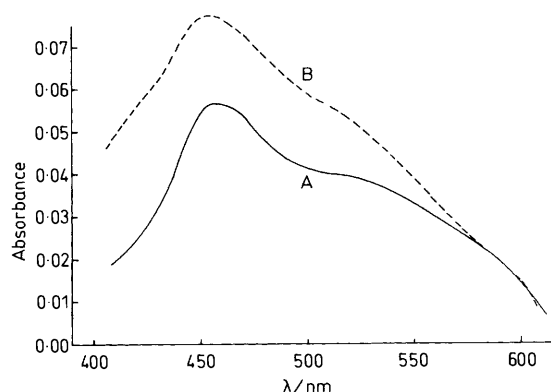
The rate data for reaction with mercaptosuccinate ions are given in Table 2 and lead to values for  $k_1 1.2 \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ,  $k_{-1} 3 \times 10^4 \text{ s}^{-1}$ ,  $k_2 250 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  and  $k_{-2} 33 \text{ s}^{-1}$ . The absorbance values lead to  $K_1 33 \text{ dm}^3 \text{ mol}^{-1}$  and  $K_2 9 \text{ dm}^3 \text{ mol}^{-1}$  and are available as supplementary data [sup. no. 56756 (5pp)]\*

There have been two previous studies of the reaction of TNB with the thiolate ion of glutathione.<sup>3,4</sup> These have assumed that only a 1:1 interaction is involved with reported equilibrium

**Figure 1.** Variation with concentration of glutathione anion of values of  $k_{\text{slow}}$  for the 1:2 adduct forming reaction with TNB. Experimental points are marked (x); the full line is calculated from equation (2) with  $k_2 17 000 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ,  $k_{-2} 53 \text{ s}^{-1}$ , and  $K_1 15 \text{ dm}^3 \text{ mol}^{-1}$ .

constants of  $28 \text{ dm}^3 \text{ mol}^{-1}$ <sup>3</sup> and  $42 \text{ dm}^3 \text{ mol}^{-1}$ .<sup>4</sup> Our results show that even at very low concentrations of glutathione ( $10^{-3} \text{ mol dm}^{-3}$ ) both 1:1 and 1:2 adducts are present. The visible spectra show bands at 470 nm typical of the 1:1 adduct and at 500 nm typical of the 1:2 adduct.<sup>5-8</sup> Absorbance values, given as supplementary data, lead to values of  $K_1 15 \pm 5 \text{ dm}^3 \text{ mol}^{-1}$  and  $K_2 300 \pm 100 \text{ dm}^3 \text{ mol}^{-1}$ . Formation of the 1:1 adduct is too fast to measure on the stopped-flow timescale. Data for formation of the 1:2 adduct are shown in Figure 1 and these fit equation (2) well with  $k_2 17 000 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ,  $k_{-2} 53 \text{ s}^{-1}$  and  $K_1 15 \text{ dm}^3 \text{ mol}^{-1}$ . Combination of  $k_2$  and  $k_{-2}$  gives  $K_2 320 \text{ dm}^3 \text{ mol}^{-1}$ . These measurements refer to  $I = 0.25 \text{ mol dm}^{-3}$ . Gan has previously reported rate measurements at lower (uncontrolled) ionic strength. He used increases in absorbance at 500 nm to obtain values of  $k_{\text{obs}}$  in the range 100–130  $\text{s}^{-1}$ . Allowing for differences in ionic strength, which will be important for reactions of multi-charged species, these values are close to those observed by us for 1:2 adduct formation but were erroneously attributed to formation of the 1:1 adduct. In fact

\* For details of the Supplementary publications scheme see 'Instructions for Authors' (1989) in the January issue.



**Figure 2.** Visible spectra of TNT ( $6 \times 10^{-4}$  mol dm $^{-3}$ ) and mercaptoacetate ions ( $5 \times 10^{-3}$  mol dm $^{-3}$ ) measured by stopped-flow spectrophotometry; A, at completion of rapid reaction (corresponds to 1:1 adduct); and B, at completion of slow reaction (corresponds to a mixture of 1:1 and 1:2 adducts). Spectra have been normalised to 1 cm pathlength cells.

**Table 3.** Rate data for reaction of TNT with mercaptoacetate ions in water-dioxane (90:10, v/v) at 25 °C and with  $I = 0.25$  mol dm $^{-3}$ .

[Mercaptoacetate]	$k_{\text{fast}}^a/10^4 \text{ s}^{-1}$	$k_{\text{slow}}^b/\text{s}^{-1}$	$k_{\text{calc}}^c$
0.01		$28 \pm 1$	29
0.02		28	30
0.03		33	33
0.04	$5 \pm 1$		
0.05		40	42
0.07		53	56
0.08	$5 \pm 1$		
0.09		76	73
0.10		84	83

<sup>a</sup> Measured by T-jump at 460 nm. <sup>b</sup> Measured by stopped-flow as a colour forming reaction at 430 nm. <sup>c</sup> Calculated from equation (2) with  $k_2$  5 500 dm $^3$  mol $^{-1}$  s $^{-1}$ ,  $k_{-2}$  28 s $^{-1}$ , and  $K_1$  1.1 dm $^3$  mol $^{-1}$ .

**Table 4.** Rate data for reaction of TNT with mercaptosuccinate ions in water-dioxane (90:10, v/v) at 25 °C.

[Mercaptosuccinate]	$k_{\text{slow}}^a/\text{s}^{-1}$	$k_{\text{calc}}^b$
0.1	$170 \pm 20$	170
0.2	180	180
0.3	210	210
0.4	260	240
0.5	330	280

<sup>a</sup> Measured by stopped-flow as a colour-forming process at 430 nm or as a fading reaction at 600 nm. <sup>b</sup> Calculated from equation (2) with  $k_2$  3 500 dm $^3$  mol $^{-1}$  s $^{-1}$ ,  $k_{-2}$  160 s $^{-1}$ , and  $K_1$  0.15 dm $^3$  mol $^{-1}$ .

measurement of the rate of 1:1 adduct formation requires the T-jump method. We measured values of  $k_{\text{fast}}$  ( $1.3 \pm 0.2$ )  $\times 10^5$  s $^{-1}$  and ( $1.5 \pm 0.2$ )  $\times 10^5$  s $^{-1}$  with concentrations of glutathione anion 0.01 mol dm $^{-3}$  and 0.012 mol dm $^{-3}$  respectively. Using the known value of 15 dm $^3$  mol $^{-1}$  for  $K_1$  we obtain values for  $k_1$  ( $1.8 \pm 0.6$ )  $\times 10^6$  dm $^3$  mol $^{-1}$  s $^{-1}$  and  $k_{-1}$  ( $1.2 \pm 0.2$ )  $\times 10^5$  s $^{-1}$ . The precision is relatively low because of the small conversion of TNB to its adduct in these solutions.

The rates of equilibration of TNB with both the 1:1 and 1:2 adducts with ethyl 2-mercaptoacetate were too fast for measurement with the stopped-flow method at 25 °C. Our results here are limited to values of  $K_1$  19 dm $^3$  mol $^{-1}$  and  $K_2$  20 dm $^3$  mol $^{-1}$  at  $I = 0.1$  mol dm $^{-3}$ . Data are in Table 10 (available as supplementary information).

**Reactions with TNT.**—U.v.-visible measurements indicated qualitative similarities with the corresponding reactions of thiolate ions with TNB. At sufficiently low thiolate concentrations species with  $\lambda_{\text{max}}$  460 nm, 550 (shoulder), characteristic of 1:1 adducts are formed, while at higher concentrations 1:2 adducts with  $\lambda_{\text{max}}$  430 nm were produced. Spectra obtained by stopped-flow spectrophotometry with mercaptoacetate ions are shown in Figure 2. The spectrum at completion of the fast reaction corresponds to the 1:1 adduct while that at completion of the slower reaction represents a mixture of 1:1 and 1:2 adducts. In the case of TNT isomeric attack involving adduct formation at the unsubstituted or the methyl-substituted ring-positions is possible. N.m.r. measurements were not possible here because of solubility problems. However the 1:1 adducts formed from TNT by attack of sulphite, cyanide, hydride, acetate, and dialkyl hydrogen phosphite ions all involve<sup>5</sup> reaction at an unsubstituted position so we formulate the 1:1 adduct with thiolate ions as (1; X = Me). Similarly the 1:2 adduct<sup>12</sup> with sulphite,  $\lambda_{\text{max}}$  420 nm, has been shown to involve attack at two unsubstituted ring-positions so that we expect the 1:2 thiolate adduct to have structure (2; X = Me). In accord with the high affinity of thiolate ions for carbon relative to their affinity for protons<sup>6</sup> we found no evidence for formation of trinitrobenzyl anions ( $\lambda_{\text{max}}$  370, 514, 620 nm) by abstraction of a side chain proton. Interference in the reactions by hydroxide ion was minimised by maintaining [free OH $^-$ ]  $\leq 0.005$  mol dm $^{-3}$ .

The absorbances at completion of the rapid 1:1 adduct forming reaction with mercaptoacetate ions were measured at 450 nm using stopped-flow spectrophotometry. We assumed a value for  $\epsilon$  of  $1.7 \times 10^4$  dm $^3$  mol $^{-1}$  cm $^{-1}$  typical of 1:1 adducts and calculated a value for  $K_1$  of 1.1 dm $^3$  mol $^{-1}$ . Rate data are in Table 3. Relatively low conversion to 1:1 adduct was achieved so that the values of  $k_{\text{fast}}$  approximate to  $k_{-1}$ , giving a value for  $k_1$  ( $=K_1k_{-1}$ ) of  $5.5 \times 10^4$  dm $^3$  mol $^{-1}$  s $^{-1}$ . The values of  $k_{\text{slow}}$  give a good fit with equation (2) with  $k_2$  5 500 dm $^3$  mol $^{-1}$  s $^{-1}$ ,  $k_{-2}$  28 s $^{-1}$ , and  $K_1$  1.1 dm $^3$  mol $^{-1}$ .

Equilibrium constants for reaction of TNT with mercaptosuccinate were low, necessitating the use of high concentrations of thiolate ions and it was not possible to keep the ionic strength constant at 0.25 mol dm $^{-3}$ . The initial absorbances at 470 nm relating to 1:1 adduct formation yielded a value for  $K_1$  of 0.15 dm $^3$  mol $^{-1}$ ; and a value for  $k_{-1}$  of  $6 \times 10^4$  s $^{-1}$  was obtained by T-jump methods. Combination of these values gives  $k_1$   $7.5 \times 10^3$  dm $^3$  mol $^{-1}$  s $^{-1}$ . Rate data for the formation of the 1:2 adduct in Table 4 accord with equation (2) with  $k_2$  3 500 dm $^3$  mol $^{-1}$  s $^{-1}$  and  $k_{-2}$  160 s $^{-1}$ .

Measurements with glutathione were restricted to 1:1 adduct formation. Initial absorbances gave a value for  $K_1$  of 0.12 dm $^3$  mol $^{-1}$ . T-Jump measurements gave  $k_{-1}$   $4 \times 10^4$  s $^{-1}$  leading to  $k_1$  ( $=K_1k_{-1}$ ) 5 000 dm $^3$  mol $^{-1}$  s $^{-1}$ .

**Comparisons.**—Data are summarised in Table 5 and are compared with  $\text{p}K_{\text{a}}$  values for the thiols in water. Values of  $K_1$  for 1:1 adduct formation with TNB decrease in the order mercaptoacetate > mercaptosuccinate > ethyl 2-mercaptoacetate > glutathione and show (in terms of log  $K_1$  values) a much smaller spread than the  $\text{p}K_{\text{a}}$  values. The correlation between the two quantities is poor although the higher basicity of mercaptoacetate relative to its ethyl derivative is reflected in the  $K_1$  values. However mercaptosuccinate shows lower affinity for TNB than expected from its  $\text{p}K_{\text{a}}$  value and the chain branching at the carbon atom  $\alpha$  to the thiolate ion may be responsible. This chain branching will result<sup>5</sup> in unfavourable steric interactions in the adduct (1) and it is probable that electrostatic repulsion will be present involving the two carboxylate groups and the negative charge delocalised in the nitro groups in the adduct. These factors are also likely to be

**Table 5.** Comparison of kinetic and equilibrium data for reactions at 25 °C with  $I = 0.25 \text{ mol dm}^{-3}$ , medium water-dioxane.

Thiol	Substrate	$k_1/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	$k_{-1}/\text{s}^{-1}$	$K_1/\text{dm}^3 \text{ mol}^{-1}$	$k_2/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	$k_{-2}/\text{s}^{-1}$	$K_2/\text{dm}^3 \text{ mol}^{-1}$	$pK_a^a$	Medium composition (v/v)
Ethyl 2-mercaptoacetate	TNB			19			20	7.9 <sup>b</sup>	90:10
Mercaptoacetate	TNB	$1.4 \times 10^6$	5 500	250	3 300	8	400	10.7 <sup>c</sup>	90:10
Mercaptosuccinate	TNB	$1.2 \times 10^6$	$3 \times 10^4$	33	250	33	8	11.1 <sup>d</sup>	90:10
Glutathione	TNB	$1.8 \times 10^6$	$1.2 \times 10^5$	15	$1.7 \times 10^4$	53	320	8.7 <sup>e</sup>	99:1
Mercaptoacetate	TNT	$5.5 \times 10^4$	$5 \times 10^4$	1.1	5 500	28	200	10.7 <sup>c</sup>	90:10
Mercaptosuccinate	TNT	7 500	$6 \times 10^4$	0.15	3 500	160	22	11.1 <sup>d</sup>	90:10
Glutathione	TNT	5 000	$4 \times 10^4$	0.12				8.7 <sup>e</sup>	99:1

<sup>a</sup> Values in water. <sup>b</sup> Ref. 13. <sup>c</sup> Ref. 14. <sup>d</sup> Ref. 15. <sup>e</sup> Ref. 16.

**Table 6.** Intrinsic rate constants for reaction at an unsubstituted position of trinitro-activated aromatic rings.

Nucleophile	$k_0$	Medium
Glutathione	$6 \times 10^4$	Water-dioxane (99:1, v/v)
Mercaptoacetate	$5.6 \times 10^4$	Water-dioxane (90:10, v/v)
Mercaptosuccinate	$5 \times 10^4$	Water-dioxane (90:10, v/v)
Sulphite	300	Water
Hydroxide	10	Water

present in the 1:2 adduct with mercaptosuccinate which has the lowest value for  $K_2$  of the thiols studied.

Values of  $K_1$  for reaction with TNT are lower than those for reaction with TNB by factors between 125 (glutathione) and 227 (mercaptoacetate). The polar effect of the methyl group in TNT will be an important factor in reducing adduct stability. However the disruption in planarity of the nitro-groups in TNT by the methyl group will reduce their ability to delocalise negative charge and will also be important.<sup>17</sup> In the 1:2 adducts the rings are necessarily non-planar so that steric effects are less important and the values of  $K_2$  for reactions with TNT are similar to those for the corresponding reactions of TNB.

Comparisons of nucleophilic reactivity are usefully discussed<sup>10,11</sup> in terms of intrinsic rate constants,  $k_0$ , which may be obtained by extrapolation of  $\log k$  versus  $\log K$  plots to  $K = 1$  ( $\Delta G^\circ = 0$ ). Using the data for 1:1 adduct formation for reaction of each thiolate ion with TNB and TNT we obtained the values of  $k_0$  shown in Table 6. These refer to reaction at an unsubstituted position of the nitro-activated ring. (The plots of  $\log k_1$  and of  $\log k_{-1}$  versus  $\log K_1$  are given as Figure 3 in the supplementary data.) From literature values we also obtained intrinsic rate constants for reaction of the sulphite ion<sup>12</sup> and the hydroxide ion<sup>18</sup> at unsubstituted ring-positions of trinitro-activated aromatics (Figures 4 and 5 in the supplementary data).

The results in Table 6 show that the intrinsic reactivities of the three thiolate ions studied are closely similar and are considerably higher than that of the sulphite ion, which in turn is higher than that of the hydroxide ion. Factors thought to be important in determining intrinsic reactivity are the electronic-structural reorganisation accompanying reaction and the solvent reorganisation during reaction.<sup>10</sup> The more reorganisation required the lower the intrinsic rate. In the three thiolate ions for which we have information, all of which contain carboxylic groups, there is unlikely to be extensive delocalisation of the negative charge away from the sulphur atom, so that electronic re-organisation will be minimal. There is also evidence<sup>19</sup> that thiolate ions are poorly solvated, relative to alkoxide ions, by hydrogen-bonding solvents. Thus there will be little need for desolvation of the sulphur centre during reaction. However in the sulphite ion the negative charges will largely reside on oxygen atoms so that adduct formation, which involves reaction through the sulphur atom,<sup>5,6</sup> will require

electronic reorganisation with associated changes in solvation. The hydroxide ion will be very strongly solvated in water and its low intrinsic reactivity probably reflects the need for desolvation during bond formation at the aromatic ring.

## Experimental

1,3,5-Trinitrobenzene was a recrystallised commercial specimen. 2,4,6-Trinitrotoluene was recrystallised from material supplied by Ministry of Defence, Waltham Abbey. The thiols were the purest grade commercially available. The distilled water was boiled to remove carbon dioxide and subsequently protected from the atmosphere.

U.v.-visible spectra were measured with a Beckman Lambda 3 instrument. A Hi-Tech SF3L stopped-flow spectrophotometer was used to measure rate coefficients. This instrument was also used to determine the spectral shapes of species present after short reaction times. This involved measuring absorbances at individual wavelengths and building up spectra point-by-point. Temperature-jump measurements were made with an instrument supplied by Hartley Measurements Ltd. consisting of a delay line energy-storage system with a square heating pulse. T-Jumps of 5 °C were obtained in a cell with a heated volume of 0.5 cm<sup>3</sup>.

All kinetic measurements were made under first-order conditions with the concentration of nucleophile in large excess of that of the nitro-compound. Reported rate coefficients are the mean of at least five separate determinations and are precise to  $\pm 5\%$  stopped-flow or  $\pm 10\%$  T-jump. For stopped-flow work a solution of the nitro-compound was contained in one syringe and a solution of the thiolate ion, generated from thiol and sodium hydroxide, in the second syringe. However for reactions involving glutathione, which is known to oxidise in alkaline solution, one syringe contained nitro-compound and glutathione and the other the sodium hydroxide solution.

## Acknowledgements

We thank the SERC for a research studentship (J. A. S.).

## References

- R. B. Freedman and G. K. Raddon, *Biochem. J.*, 1968, **108**, 383.
- M. J. Strauss, A. DeFusco, and F. Terrier, *Tetrahedron Lett.*, 1981, **22**, 1945.
- L.-H. Gan, *Aust. J. Chem.*, 1977, **30**, 1475.
- A. G. Clark and M. Sinclair, *Biochem. Pharmacol.*, 1988, **37**, 259.
- For reviews see: E. Buncl, M. R. Crampton, M. J. Strauss, and F. Terrier, 'Electron deficient aromatic- and heteroaromatic-base interactions,' Elsevier, Amsterdam 1984; M. R. Crampton, *Adv. Phys. Org. Chem.*, 1969, **7**, 211; F. Terrier, *Chem. Rev.*, 1982, **82**, 77.
- M. R. Crampton, *J. Chem. Soc. B*, 1968, 1208.
- M. R. Crampton and M. A. El-Ghariani, *J. Chem. Soc. B*, 1971, 1043.

- 8 M. R. Crampton, *J. Chem. Soc. B*, 1971, 2112.
- 9 J. W. Larsen, K. Amin, S. Ewing, and L. L. Magid, *J. Org. Chem.*, 1972, **37**, 3857.
- 10 C. F. Bernasconi, *Pure Appl. Chem.*, 1982, **54**, 2335.
- 11 J. P. L. Cox, M. R. Crampton, and P. Wight, *J. Chem. Soc., Perkin Trans. 2*, 1988, 25.
- 12 D. N. Brooke and M. R. Crampton, *J. Chem. Soc., Perkin Trans. 2*, 1980, 1850.
- 13 M. M. Kreevoy, E. T. Harper, R. E. Duvall, A. S. Wilgus, and L. T. Ditsch, *J. Am. Chem. Soc.*, 1960, **82**, 4899.
- 14 R. J. Irving, L. Nelander, and I. Wadso, *Acta Chem. Scand.*, 1964, **18**, 769.
- 15 O. Makitie and A. Ilvonen, *Acta Chem. Scand.*, 1972, **26**, 847.
- 16 F. A. Isherwood, *Symp. Biochem. Soc.*, 1957, **17**, 3.
- 17 D. N. Brooke and M. R. Crampton, *J. Chem. Res.*, 1980 (S) 340; (M) 4401.
- 18 B. Gibson and M. R. Crampton, *J. Chem. Soc., Perkin Trans. 2*, 1979, 648.
- 19 M. R. Crampton 'Chemistry of the Thiol Group,' ed. S. Patai, Wiley, London, 1974, 379.

*Received 16th November 1988; Paper 8/04569K*