reported complexes containing the related O=Tc—OR^{2+} core.[12,13]

Since the remaining complexes of type TcOLCl (L = $(sal)_2$en, $(sal)_2$phen) were isolated free of water, their structures were presumed to involve either a square-pyramidal coordination geometry with the oxo ligand occupying the axial site or a six-coordinate structure in which the axial ligands are oxo and chloro. Complexes showing related structures to each of these possibilities are known, but the former is considerably more common.[3] For the latter case, the Tc—Cl bond would be expected to be weak because of the trans influence of the oxo group. It is perhaps significant that [TcO(Ph-sal)$_2$Cl] (where Ph-sal = *N*-phenylsalicylideneaminato) has been shown to have an octahedral geometry, although in this case it is a phenolic oxygen that is trans to the Tc=O bond.[14] The distorted octahedral structure found for the two crystallographically independent [TcO($(sal)_2$en)Cl] molecules differs from the above in that the chloro ligand occupies the position trans to the oxo group. Consequently, the Tc—Cl bonds in the two independent molecules of [TcO($(sal)_2$en)Cl] (Table III) are significantly longer than in other technetium(V) complexes that show[10] lengths in the range 2.33–2.44 Å. The weak nature of the bonds to the atoms trans to the Tc=O units in $[Tc(H_2O)((acac)_2en)]^+$ and [TcO($(sal)_2$en)Cl] is reflected in the displacements of the Tc atoms from O_2N_2 coordination planes toward the axial oxo-oxygen atoms (by 0.37 Å in the $(acac)_2$en complex and by 0.28 and 0.25 Å in molecules 1 and 2, respectively, of the $(sal)_2$en complex).

Since many substituted derivatives of the parent Schiff base ligands **1–3** have been reported, the synthetic procedure adopted in the present work should be readily applicable to the production of a range of related Tc(V) complexes exhibiting a gradation of structural and electronic properties.[15] Such a variation in properties is expected to result in corresponding changes in the biological activity of the Tc complexes. The availability of such a series of complexes may thus prove useful for the systematic development of new Tc reagents for use in nuclear medicine. An investigation of this type is planned for the future.

Acknowledgment. Financial support for this work was provided by the National Institutes of Health, Grant No. HL-21276 (to E.D.). Diffractometer equipment and computing facilities were provided by the Science Research Council (U.K.) (to M.M. and P.A.T.).

Registry No. [TcO(H_2O)($(acac)_2$en)]Br, 87761-77-1; [TcO($(sal)_2$en)Cl], 87761-78-2; [TcO($(sal)_2$phen)Cl], 87761-79-3; [TcO(H_2O)($(acac)_2$en)]$Br_{0.25}Cl_{0.75}$, 87761-80-6; [TcO(H_2O)($(acac)_2$pn)]Br, 87761-81-7; [TcO(H_2O)($(BuOac)_2$en)]Br, 87761-82-8; [TcO(H_2O)($(Bzlac)_2$en)]Br, 87761-83-9; (n-Bu_4N)[$TcOCl_4$], 71341-65-6.

Supplementary Material Available: Tables giving full details of the determination and refinement of structures, anisotropic thermal parameters, least-squares planes and deviations, bond lengths and angles, H atom positional parameters, ^{1}H NMR data for all the complexes, and structure factors and Figure 3, showing the hydrogen bonding in [TcO(H_2O)($(acac)_2$en)]$Br_{0.25}Cl_{0.75}$ (41 pages). Ordering information is given on any current masthead page.

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(13) Related to this latter complex is [TcO($C_5H_9NO_2S$)($C_5H_{10}NO_2S$)] (where $C_5H_{11}NO_2S$ = D-penicillamine). An X-ray structural determination shows this complex to contain S_2N_2 equatorial donors with one axial site being filled by an oxo group while the other is occupied by a deprotonated carboxylic oxygen from one of the D-penicillamine ligands: Franklin, K. J.; Howard-Lock, H. E.; Lock, C. J. L. *Inorg. Chem.* **1982**, *21*, 1941.
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(15) Complexes of the type [TcO(H_2O)L] where L = *N*,*N*′-1,2-propylenebis(acetylacetone iminato), *N*,*N*′-ethylenebis(*tert*-butoxyacetoacetone iminato), and *N*,*N*′-ethylenebis(benzoylacetone iminato) were also isolated in the present study by using a procedure identical with that described in the Experimental Section. These products gave spectral and chromatographic properties similar to those of [TcO(H_2O)($(acac)_2$en)] and are undoubtedly similar in structure to this complex. However the microanalytical data for these products fell slightly outside the expected ranges in each case. The ^{1}H NMR data for all these complexes are included in Table 7S of the supplementary material.

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Kinetics of the Primary Interaction of Pentacyanonitrosylferrate(2–) (Nitroprusside) with Aliphatic Thiols

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The kinetics of formation and dissociation of adducts of pentacyanonitrosylferrate(2–) (nitroprusside) with a number of aliphatic thiols (RSH) have been measured by the temperature-jump/stopped-flow combination. The second-order rate constant for the formation of $Fe(CN)_5N(O)SR^{3-}$ is pH dependent, and this is rationalized by assuming that only the RS^- species is reactive (k_{RS^-}) with 2-mercaptoethanol, 1-pentanethiol, *N*-acetylcysteine, mercaptosuccinate, cysteine, 2-aminoethanethiol, and glutathione. With the three amino thiols, it is found that the forms containing the H_3N^+–S^- and the H_2N–S^- moieties are similar in reactivity. The dissociation of the adducts of nitroprusside with the simpler thiols is pH independent (from 6 to 12), but those of the amino thiols except glutathione are pH dependent and this is ascribed to NH_2 protonation weakening the N–S bond. The value of k_{RS^-} (3×10^3–4×10^4 M^{-1} s^{-1} at 25 °C) varies little with the variety of thiols studied, and the associated $\Delta H^{\ddagger}$ values are similar (~8 kcal mol^{-1}). There is a much larger variation in the adduct dissociation rate constants (from 12 to 3×10^3 s^{-1}) and attendant $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values.

The pentacyanonitrosylferrate(2–) (nitroprusside) ion undergoes addition reactions with a variety of bases (X^{n-}):[1,2]

$$Fe(CN)_5NO^{2-} + X^{n-} \rightleftarrows Fe(CN)_5NOX^{(n+2)-} \quad (1)$$

The base may attack either the oxygen or nitrogen atom of the coordinated nitrosyl. Adduct formation (eq 1) is usually heralded by a marked color increase, and this is followed by color fading as further reactions occur. These are usually redox in nature with $Fe(CN)_5OH_2^{3-}$ (or a polymeric derivative) as the iron product.[1,2] Kinetic data are available for reactions of nitroprusside with OH^-,[3,4] SO_3,[2-5] N_3^-,[6] NH_2OH,[6]

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$RCH{=}C(O^-)R$,[7] and RS^-,[8,9] including HS^-.[10] In certain cases[6] only indirect evidence for adduct formation as part of an overall reaction is obtained.

The red color developing when sodium nitroprusside is added to thiols in alkaline solution is the oldest method to be used for their detection.[1] This color fades fairly rapidly, and the overall reaction appears kinetically complex (with cysteine,[8,9] thioglycolate,[8] and 2-mercaptoethanol[8]). We have studied the kinetics of reaction 2 with a number of thiols by using a temperature-jump/stopped-flow combination. The rates are too rapid for accurate stopped-flow experiments, and the temperature jump must be made within 5–10 s of mixing reactants, before subsequent reactions can occur and disturb the equilibrium (2). This further decomposition is much more serious when thiols are in excess of nitroprusside, and so the reverse conditions were used in all kinetic experiments.

$$Fe(CN)_5NO^{2-} + RS^- (RSH) \rightleftarrows Fe(CN)_5N(O)SR^{3-} (+H^+) \quad k_f, k_d \quad (2)$$

Experimental Section

The chemicals used were commercial products, the purest available, and were used as received. The complex $[Co(en)_2cysSH]S_2O_6$[11] was a gift from Dr. Alan M. Sargeson. Sodium perchlorate solutions were standardized by passing an aliquot through an ion-exchange resin (Dowex 2-X8) in the acid form and titrating the released H^+ with standard base. All pH measurements were made with a Beckman D46 Expandomatic pH meter and an Orion Research pH electrode, which were standarized daily. Solutions of reactants were prepared fresh for each set of runs and stored out of light. Distilled ion-exchange water was used, and a N_2 atmosphere was maintained above the deaerated thiol solutions to reduce aerial oxidation. Kinetic experiments were carried out by using a Dionex D-130 stopped-flow apparatus and a Dionex D-150 temperature-jump combined with a Dionex D-130 stopped-flow apparatus. The magnitude of the temperature jump was determined by measuring with a spectrophotometer the absorbance change of a phenolphthalein–glycine buffer (0.1 M NaCl, pH 9.87) with temperature. The temperature change could then be correlated with the observed absorbance change for that system in the temperature-jump apparatus.[12] It was found that a 4-kV pulse resulted in a 10 ± 0.2 °C jump with our arrangement (100-μs heating pulse width). The solutions were therefore maintained in the syringes at 10 °C below the final temperature (usually 25 °C) for which relaxation data were required. Sodium nitroprusside (7–70 mM) at pH ~7 and I = 0.4 M and thiol (0.1 mM) at higher pH (phosphate buffer) and I = 0.4 M were separately equilibrated in the two syringes of the temperature-jump apparatus. They were then mixed, and within 5 s the temperature jump was imposed (the equilibrium (2) is established within 1 s). At least three jumps on fresh mixtures for each conditions were collected and averaged on an OLIS 3820 data-collecting module interfaced to the system. All relaxations were single first order, and fitting rate constants was accomplished by an OLIS computer routine developed by Dr. R. DeSa. The dissociation of the adduct was studied in the stopped-flow apparatus by adding freshly formed adduct (0.1 mM thiol; ~30 mM nitroprusside at high pH) in one syringe to buffer (phosphate at lower pH) in the other syringe. In all kinetic work, the wavelength of the observation was usually at 520 nm, near the peak for the nitroprusside adduct ($\epsilon \sim (5\text{–}10) \times 10^3$ M^{-1} cm^{-1}).[9,10] Observations at other nearby wavelengths gave similar results. All measurements were at I = 0.40 M, maintained with sodium perchlorate, and 25.0 ± 0.2 °C, unless otherwise indicated. The quoted rate constants are ±5–8%.

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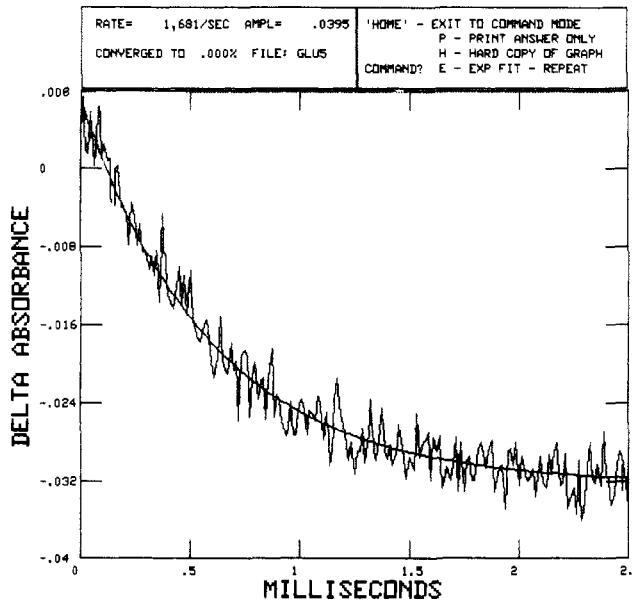


Figure 1. Computer-treated relaxation curve for 15–25 °C jump on a solution containing 0.1 mM glutathione and 67 mM nitroprusside at pH 9.8 and I = 0.4 M. The solutions were jumped within 5 s of mixing on the stopped-flow/temperature-jump apparatus.

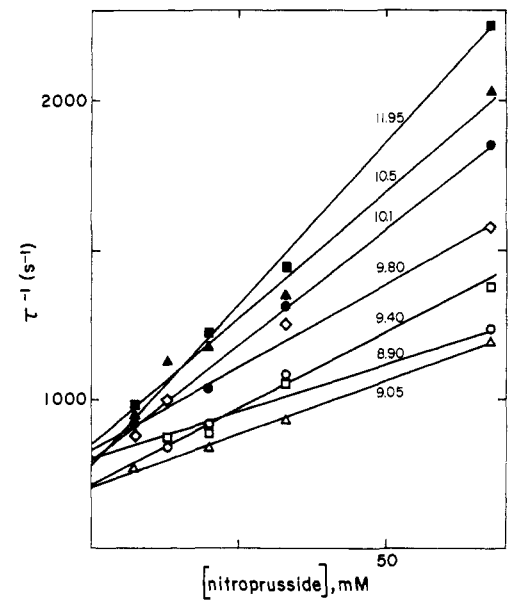


Figure 2. Plot of τ^{-1} vs. [nitroprusside] for relaxation of the glutathione/nitroprusside system at 25 °C, I = 0.4 M, and pH indicated on curve ([glutathione] = 0.1 mM).

Results

Temperature-jump relaxations of the system (2) were uniphasic for all thiols examined. A typical relaxation is shown in Figure 1. Since nitroprusside was used in excess over thiol, the relaxation time τ is given by (3) for a simple second-order

$$\tau^{-1} = k_f[Fe(CN)_5NO^{2-}] + k_d \quad (3)$$

forward and first-order reverse reaction. The plots of τ^{-1} vs. $[Fe(CN)_5NO^{2-}]$ for reaction of glutathione at a number of pHs are shown in Figure 2. The values of k_f and k_d from the slopes and intercepts of such figures for all the systems examined are shown in Table I. In the cases where $k_d < 3 \times 10^2$ s^{-1}, its value could be determined separately on the stopped-flow apparatus. Plunging the adduct formed at high pH (approximately 9–10) into buffers at low pH (4–6) effected it's dissociation, and the first-order rate constant k_d could thus be measured directly. The experimental value was independent

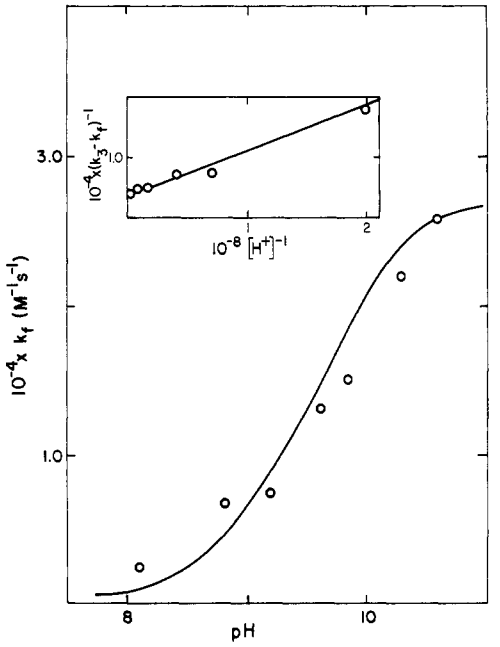


Figure 3. Plots of functions 7 and 9, inset, for the formation of the nitroprusside–mercaptoethanol adduct at 25 °C and $I = 0.4$ M. Values of $k_3 = 2.8 \times 10^4$ M^{-1} s^{-1}, $k_2 = 0$, and $K_1 = 3 \times 10^{-10}$ M were used in calculating the solid lines.

of the concentration of adduct used and, except for mercaptosuccinate, was independent of the pH at the start and at the completion of the experiment. Excellent agreement with the value of k_d from the relaxation experiments was noted (Table I).

For the group of thiols containing no other functional group that would ionize in the pH ~7–10 region (we shall term these monofunctional), the equilibria are simply represented by (4)–(6), where R may contain a CO_2^- group or groups. The

$$RSH \rightleftarrows RS^- + H^+ \quad K_1 \tag{4}$$

$$Fe(CN)_5NO^{2-} + RSH \rightleftarrows Fe(CN)_5N(O)SR^{3-} + H^+ \quad k_2, k_{-2}, K_2 \tag{5}$$

$$Fe(CN)_5NO^{2-} + RS^- \rightleftarrows Fe(CN)_5N(O)SR^{3-} \quad k_3, k_{-3}, K_3 \tag{6}$$

expressions (7)–(9) represent the expected kinetic behavior.[13]

$$k_f = \frac{k_2[H^+] + k_3K_1}{[H^+] + K_1} \tag{7}$$

$$k_d = k_{-3} + k_{-2}[H^+] \tag{8}$$

$$(k_3 - k_f)^{-1} = (k_3 - k_2)^{-1} + K_1[H^+]^{-1}(k_3 - k_2)^{-1} \tag{9}$$

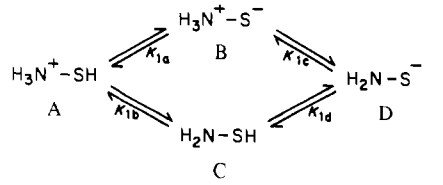
The experimental data were best fitted to function 9 with use of the values of k_3 and K_1 shown in Table II. The value of k_3 was equated to that of k_f at the highest pH. The value of k_2 was assumed to be zero.[14] The reasonable agreement between experimental and calculated values for k_f for mercaptoethanol is illustrated in Figure 3 and for the other thiols in Table I. It was possible to determine activation parameters associated with (6) by examining the effect of temperature on the value of k_f (k_3) at high pH. All the rate constants and associated temperature parameters are collected in Table II. Also included are literature values for K_1.[15-17]

Table I. Formation and Dissociation Rate Constants at 25 °C and $I = 0.4$ M

pH	$10^{-4}k_f$,[a] M^{-1} s^{-1}	$10^{-2}k_d$, s^{-1}	pH (temp, °C)	$10^{-4}k_f$,[a] M^{-1} s^{-1}	$10^{-2}k_d$, s^{-1}
			N-Acetylcysteine		
12.0	2.7 (2.7)	2.2	5.76		1.8[b]
9.60	1.7 (1.8)	2.4	4.25		1.7[b]
9.20	1.0 (1.2)	2.1	12.0 (34)	3.1 (3.2)	3.4 (4.1)
8.70	0.89 (0.6)	2.2	12.0 (39)	3.8 (4.0)	6.0 (6.3)
8.20	0.41 (0.2)	2.3	12.0 (44)	5.6 (5.2)	9.8 (9.9)
7.9	0.12 (0.1)	1.9			
			Mercaptoethanol		
12.0	2.7 (2.8)	1.5	8.10	0.25 (0.10)	2.0
10.60	2.6 (2.6)	1.3	5.76		1.0[b]
10.30	2.2 (2.4)	1.5	4.25		1.1[b]
9.85	1.5 (1.9)	1.7	12.0	2.7 (2.4)	1.5 (1.5)
9.60	1.3 (1.5)	1.5	12.0 (19)	2.5 (1.8)	0.6 (0.8)
9.20	0.76 (0.90)	1.4	12.0 (34)	3.7 (3.6)	2.7 (3.8)
8.80	0.67 (0.44)	1.5			
			Mercaptosuccinate		
10.95	0.45 (0.45)	1.0	6.10		2.8[c]
10.10	0.32 (0.36)	1.1	5.75		4.3[c]
9.81	0.23 (0.27)	1.0	12.0 (20)	0.52 (0.52)	0.8 (0.76)
8.95	0.073 (0.05)	1.0	12.0 (29)	0.69 (0.79)	1.2 (1.3)
9.26		0.94[c]	12.0 (39)	1.3 (1.3)	2.4 (2.2)
			1-Pentanethiol		
12.0	3.0	~0	5.76		0.12[b]
8.45	0.12	~0			
			Cysteine		
11.32	2.6 (2.5)	1.4 (1.5)	10.0	2.2 (2.2)	3.4 (3.7)
10.71	2.5 (2.4)	1.7 (1.9)	9.75	2.1 (2.1)	7.3 (5.2)
10.53	2.3 (2.4)	1.5 (2.1)	9.20	1.9 (1.9)	8.2 (11)
10.30	2.2 (2.2)	2.1 (2.6)	8.75	1.9 (1.6)	18.1 (17)
10.15	2.2 (2.2)	2.4 (3.1)			
			2-Aminoethanethiol		
11.50	2.6 (2.6)	1.6 (1.8)	10.3	2.4 (2.5)	3.0 (3.2)
11.0	2.6 (2.5)	1.8 (2.0)	9.4	2.4 (2.2)	9.1 (9.6)
10.8	2.4 (2.5)	1.9 (2.2)	8.25	2.35	15.4 (2.0)
10.6	2.4 (2.5)	2.2 (2.5)			
			Glutathione		
11.95	2.1 (1.8)	7.6	9.4	0.98 (1.0)	7.3
10.50	1.8 (1.7)	8.3	9.05	0.74 (0.74)	7.0
10.10	1.6 (1.5)	7.8	8.90	0.66 (0.62)	8.2
9.8	1.2 (1.4)	8.1			
			Penicillamine		
11.2	0.26	5.1			

[a] Values in parentheses calculated on the basis of (7), $k_2 = 0$. [b] Plunges from pH 10.2 or 9.1. [c] Plunges from pH 11.0.

For three amino thiols that were examined, the microscopic ionization scheme (10) must be considered, where H_3N^+–SH



(10)

represents the diprotonated form of the amino thiol and

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Table II. Activation Parameters for Reaction of Monofunctional Thiols with Nitroprusside at 25 °C, I = 0.4 M

reactant	pK_1[a]	$10^{-4}k_3$, M^{-1} s^{-1}	$\Delta H_3^{\ddagger}$, kcal mol^{-1}	$\Delta S_3^{\ddagger}$, eu	$10^{-2}k_{-3}$,[b] s^{-1}	$\Delta H_{-3}^{\ddagger}$, kcal mol^{-1}	$\Delta S_{-3}^{\ddagger}$, eu	$10^{-2}K_3$,[c] M^{-1}	ΔH_3,[c] kcal mol^{-1}	ΔS_3,[c] eu
$HO(CH_2)_2S^-$	9.5 (9.6)[d]	2.8	7.5	−13	1.3 (1.1)	17.5	10	2.2	−10.0	−24
$CH_3(CH_2)_4S^-$		3.0			~0 (0.12)			2.5		
$CH_3CONHCH(CO_2^-)CH_2S^-$	9.3 (9.5)[e]	2.7	8.4	−11	1.9 (1.8)	15.3	3	1.4	−6.9	−14
$^-O_2CCH_2CH(CO_2^-)S^-$	9.7 (10.1)[f]	0.5	8.1	−14	1.0 (0.9)	9.8	−17	0.46	−1.7	+3

[a] First value from k_f/pH profile; literature value in parentheses. [b] First value from intercept of plot of (3); value in parentheses from pH plunge. [c] From k_3/k_{-3}, $\Delta H_3^{\ddagger} - \Delta H_{-3}^{\ddagger}$ and $\Delta S_3^{\ddagger} - \Delta S_{-3}^{\ddagger}$. [d] Reference 15, I = 0.02 M. [e] Reference 16, I = 0.3 M. [f] Reference 17, I = 0.5 M.

Table III. Ionization and Rate Constants for Reaction of Amino Thiol Species with Nitroprusside at 25 °C, I = 0.4 M

reactant	pK_{1a}	pK_{1b}	pK_{1c}	pK_{1d}	I, M	$10^{-4}k_4$, M^{-1} s^{-1}	$10^{-3}k_{-4}$, s^{-1}	K_4, M^{-1}	$10^{-4}k_5$, M^{-1} s^{-1}	$10^{-2}k_{-5}$, s^{-1}	K_5, M^{-1}	pK_6	pK_{1c}[a]
$(H^+)H_2NCH(CO_2^-)CH_2S^-$	8.65	8.75	10.05	9.95	0.15[b]	4.8	2.6	17	2.6	1.4	200	9.0	10.1
$(H^+)H_2N(CH_2)_2S^-$	8.35		10.81		0.15[c]	2.5	2.2	12	2.6	1.7	171	9.2	10.4
γ-Glu-Cys-Gly (glutathione)	8.93	9.13	9.28	9.08	0.2–0.5[d]	1.4	0.8	18	1.8	7.6	24	9.1[e]	9.3
$H_2NCH(CO_2^-)C(CH_3)_2S^-$									0.26	5.1	5		

[a] Calculated from $K_{1c} = (K_4K_6)K_5^{-1}$. [b] Reference 20. [c] Reference 21. [d] Reference 19. [e] pK_6 assumed to equal pK_{1b}.

K_{1a}–K_{1d} are the appropriate ionization constants[18] (again with CO_2^- groups ignored). It is assumed that only the S^--containing forms B and D are reactive:

$$Fe(CN)_5NO^{2-} + H_3N^+-S^- \rightleftarrows Fe(CN)_5N(O)S-NH_3^{2-} \quad k_4, k_{-4}, K_4 \quad (11)$$

$$Fe(CN)_5NO^{2-} + H_2N-S^- \rightleftarrows Fe(CN)_5N(O)S-NH_2^{3-} \quad k_5, k_{-5}, K_5 \quad (12)$$

The second-order rate of formation of the $Fe(CN)_5NO^{2-}$ adduct from the mixture of species A, B, C, and D considered to be in labile equilibria is therefore given by (13). It is not

$$(\text{rate})[Fe(CN)_5NO^{2-}]^{-1} = k_f([A] + [B] + [C] + [D]) = k_4[B] + k_5[D] \quad (13)$$

difficult to deduce (14).

$$k_f[H^+]^2 + (K_{1a} + K_{1b})[H^+] + K_{1a}K_{1c} = K_{1a}k_4[H^+] + K_{1a}K_{1c}k_5 \quad (14)$$

A plot of the product on the left side of (14) against $[H^+]$ for cysteine, 2-aminoethanethiol, and glutathione was nicely linear (not shown). From the slopes and intercepts and with use of the most appropriate ionization constant,[19-21] values of k_4 and k_5 could be deduced (Table III). The agreement between experimental values and the calculated curve according to (14) is demonstrated in Figure 4 for cysteine and shown for all thiolamines in Table I. Since there is evidence[18,22] that the acid strength of SH is much greater than NH_3^+ in 2-aminoethanethiol, the macroscopic ionization constants were used for K_{1a} and K_{1c} in the calculation of k_4 and k_5. Poor relaxation effects with the penicillamine–nitroprusside systems restricted examination to the highest pH where the amino acid is predominantly in the D form. Only values of k_5 and k_{-5} were therefore obtained.

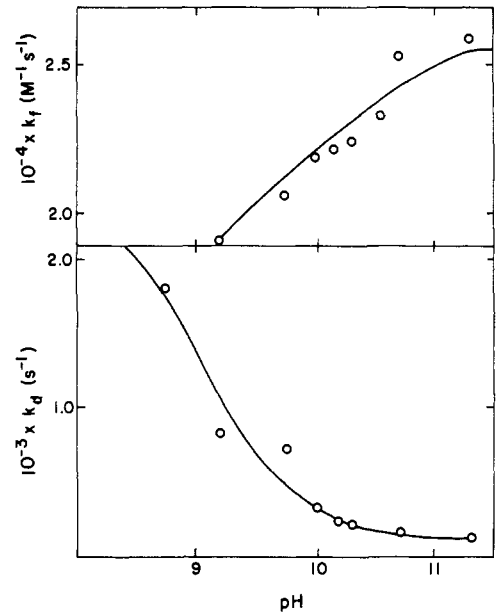

Figure 4. Formation (upper curve) and dissociation (lower curve) of the nitroprusside–cysteine complex. Solid lines are drawn according to (14) and (18) with use of the constants given in Table III.

The dependence on pH of k_d, the first-order rate constant for dissociation of the nitroprusside adduct of cysteine (Figure 4) and 2-aminoethanethiol (Table I), can be rationalized as

$$Fe(CN)_5N(O)S-NH_3^{2-} \rightleftarrows Fe(CN)_5N(O)S-NH_2^{3-} + H^+ \quad K_6 \quad (15)$$

$$Fe(CN)_5N(O)S-NH_3^{2-} \rightarrow Fe(CN)_5NO^{2-} + S^-–{}^+NH_3 \quad k_{-4} \quad (16)$$

$$Fe(CN)_5N(O)S-NH_2^{3-} \rightarrow Fe(CN)_5NO^{2-} + S^-–NH_2 \quad k_{-5} \quad (17)$$

for which[13]

$$k_d = \frac{k_{-4}[H^+] + k_{-5}K_6}{[H^+] + K_6} \quad (18)$$

The theoretical curve for (18) with values of k_{-4}, k_{-5}, and K_6 given in Table III is in reasonable agreement with experimental

(18) A critical analysis of the ionization behavior of the amino thiols examined in this study is given in: Friedman, M. "The Chemistry and Biochemistry of the Sulfhydryl Group in Amino acids, Peptides and Proteins"; Pergamon Press: New York, 1973; Chapter 1.
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(21) Li, N. C.; Manning, R. A. *J. Am. Chem. Soc.* **1955**, *77*, 5225.
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Table IV. Activation Parameters for Nitroprusside Adduct Formation with X^{n-}

X^{n-}	k(25 °C), M^{-1} s^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, eu	ref
OH^-	0.55	12.6	−18	3
	0.22			4
SH^-	1.7×10^2	7.2	−24	10
SO_3^{2-}	4.5×10^2	5.8	−27	5
$CH_3(CO^-)=CH_2$	4.6×10^5 [a]			7
$HO(CH_2)_2S^-$	2.6×10^4	7.5	−13	this work
$OAcNHC(CO_2^-)HCH_2S^-$	3.4×10^4	8.4	−11	this work
$^-O_2CCH_2C(CO_2^-)HS^-$	4.8×10^3	8.1	−14	this work

[a] Estimated from the observed rate constant and concentration of enolate ion in equilibrium with propanone.

values for the cysteine adduct (Figure 4). The corresponding values for 2-aminoethanethiol are shown in Table III, and the agreement with experimental values indicated in Table I. There is no dependence of k_d on pH from 8.9 to 12.0 for the glutathione adduct.

Discussion

Single first-order relaxations were obtained for all systems studied, and the variation of relaxation times with reactant concentrations indicated that reaction 2 was second order in the forward direction and first order in the reverse direction. All kinetic data can be rationalized in terms of little or no reactivity toward nitroprusside of species containing the SH group. The second-order rate constant (k_f) vs. pH profile thus resembles the titration curve for the thiol (Figure 3), and the pK_1 values obtained from the kinetic experiments (expression 9) are in good agreement with literature titration values[15-17] in slightly different conditions (Table II). It follows that the often observed[8,9] decreasing value for the adduct formation constant with decreasing pH resides in variations of k_f but constant k_d values (Table I).

The formation rate constants for reaction of the RS^- forms of mercaptoethanol, 1-pentanethiol, and *N*-acetylcysteine (k_3, in Table II) and the zwitterionic B and anionic form D of cysteine, 2-aminoethanethiol, and glutathione (k_4 and k_5 in Table III) are remarkably similar, ranging from only 1.4×10^4 to 4.9×10^4 M^{-1} s^{-1}. The similarity in adduct formation rate constants for the B and D forms of the amino thiols may result from compensating factors. The zwitterion form might be expected to contain the less nucleophile sulfide ion but to react more rapidly with the negative nitroprusside ion from electrostatic considerations. Small decelerating effects on the formation rate constant are observed on placing an additional negative charge on the thiol (in mercaptosuccinate) and by introducing steric hindrance adjacent to the C–S^- bond (in penicillamine). The closeness of the formation rate constants discourages attempts to apply LFER to the data. The rate constants, enthalpies, and entropies of activation associated with the formation reactions are compared with those for interaction of nitroprusside with other nucleophiles in Table IV. All have negative entropies of activation associated with reactions between like charges. The energies of activation for the thiols examined are similar and close to that reported for the slower reacting SH^- ion.[10] They are lower than that for weaker nucleophilic OH^- ion.[3] The low $\Delta H^{\ddagger}$ for reaction of SO_3^{2-} has been ascribed to its attacking the oxygen of the NO group.[5] All other nucleophiles in Table IV are believed to react with the N part of NO.

The similar values for the rate constants for dissociation of the nitroprusside adducts of mercaptoethanol, *N*-acetylcysteine, and mercaptosuccinate are however a result of widely varying $\Delta H_{-3}^{\ddagger}$ and $\Delta S_{-3}^{\ddagger}$ values. There are (compensating) decreasing values of $\Delta H_{-3}^{\ddagger}$ and $\Delta S_{-3}^{\ddagger}$ as the overall negative charge on the adduct increases from −3 to −5. The dissociative behavior therefore dominates the differences in ΔH_3 and ΔS_3 for the overall reaction of these three thiols. This is also indicated with 1-pentanethiol whose high stability with nitroprusside ion is solely as a result of a very small k_{-3} (=12 s^{-1}). Replacing an NH_2 by an NH_3^+ group near the decomposing N(O)SR bond accelerates the decomposition markedly (10–20-fold) as shown by the values of k_{-4} for cysteine and 2-aminoethanethiol. With glutathione, where the NH_2 group is probably some distance away in the tripeptide, protonation has little effect on the dissociation rate constant for the adduct.

For cysteine, the value of K_6 is quite close to that of K_{1b}. This indicates that the $Fe(CN)_5NO^{2-}$ moiety and H^+ have similar effects, when attached to the sulfur, on the ionization tendency of the NH_3^+ group. This similarity is also shown in the pK's for the processes (19) and (20), being respectively

$$CH_3S(CH_2)_2NH_3^+ \rightleftarrows CH_3S(CH_2)_2NH_2 + H^+ \quad (19)$$

$$Fe(CN)_5N(O)S(CH_2)_2NH_3^{3-} \rightleftarrows Fe(CN)_5N(O)S(CH_2)_2NH_2^+ + H^+ \quad (20)$$

9.5 and 9.2 (pK_6). The independence of k_d on pH for the glutathione adduct (Table I) probably results from protonation of the distant NH_2 group having little effect on N(O)–S cleavage. This therefore precluded a determination of pK_6. However, it is known that replacement of the sulfhydryl proton by the methylmercury cation in glutathione has a negligible effect on the acidities of the other functional groups.[19] It is reasonable then to equate pK_6 with pK_{1b} for glutathione, as with cysteine. It is easily shown that $K_{1c} = K_4K_6/K_5$, and we can therefore check our data and, in part, our mechanistic interpretation. Values of K_{1c} computed from our kinetic results are in satisfying agreement with literature values (Table III).

Finally, a number of thiols and proteins containing SH groups were found to give no color with nitroprusside. These included 4-nitrothiocresol, β-D-thioglucose, and $Co(en)_2(cysSH\text{-}N,O)^{2+}$ (in which the thiol portion of cysteine is not coordinated to the cobalt(III)[11]). Presumably in these, electron density has been withdrawn sufficiently from the sulfur atom to reduce its nucleophilicity. This is supported by transient color formation when *p*-methoxythiophenol was added to nitroprusside, although insolubility in aqueous solution precluded further studies. The proteins bovine serum albumin, human carbonic anhydrase, and hemerythrin all contain cysteine groups and give positive tests with such thiol reagents as 2-(chloromercuri)-4-nitrophenol and Ellman's reagent. Nevertheless these proteins did not give coloration with nitroprusside even at higher pH. This somewhat puzzling and disappointing result may reside in inaccessibility and/or a negatively charged environment for the sulfur site toward the negative octahedral complex, although this seems unlikely. We intend to probe this point further and to examine other thiols and dithiols, as a first step to the elucidation of the complex mechanism for the overall redox reaction.[8,9]

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Registry No. $Fe(CN)_5N(O)SR^{3-}$ (SR = 2-mercaptoethanolato), 87985-64-6; $Fe(CN)_5N(O)SR^{3-}$ (SR = 1-pentanethiolato), 87985-65-7; $Fe(CN)_5N(O)SR^{3-}$ (SR = *N*-acetylcysteinato), 87985-66-8; $Fe(CN)_5N(O)SR^{3-}$ (SR = mercaptosuccinato), 87985-67-9; $Fe(CN)_5N(O)SR^{3-}$ (SR = cysteinato), 87985-68-0; $Fe(CN)_5N(O)SR^{3-}$ (SR = 2-aminoethanethiolato), 87985-69-1; $Fe(CN)_5N(O)SR^{3-}$ (SR = glutathionato), 87999-36-8; $Fe(CN)_5N(O)^{2-}$, 15078-28-1; 2-mercaptoethanol, 60-24-2; 1-pentanethiol, 110-66-7; *N*-acetylcysteine, 616-91-1; mercaptosuccinic acid, 70-49-5; cysteine, 52-90-4; 2-aminoethanethiol, 60-23-1; glutathione, 70-18-8.