mole) of TCNEO in 50 ml. of 1,2-dibromoethane was heated to reflux, and a solution of 4.20 g. (0.02 mole) of 4-methoxystilbene in 50 ml. of 1,2-dibromoethane was added dropwise over a period of 2.5 hr. The heating was continued for another 15 min., and the rather dark reaction mixture was concentrated on a rotary evaporator to leave a dark semisolid residue that was taken up in boiling ethanol, treated with charcoal, and filtered. The product separated when the filtrate was cooled and was collected by filtration to give 6.6 g. (69%) of colorless needles, m.p. 203–204.5°. Subsequent recrystallization from ethanol appeared to cause some degradation and the analytical sample, m.p. 202.5–203°, was recrystallized from a mixture of benzene and hexane.

Anal. Calcd. for $C_{21}H_{14}N_4O_2$: C, 71.18; H, 3.98; N, 15.81. Found: C, 70.76; H, 4.12; N, 15.99.

Competition Experiment with cis- and trans-1,2-Dichloroethylene. *cis*-1,2-Dichloroethylene (b.p. 59°) and *trans*-1,2-dichloroethylene (b.p. 47.5–48°) were purified by distillation immediately before use. Into a 240-ml. Hastelloy-lined shaker tube there was placed 10.0 g. (0.069 mole) of TCNEO and 50 g. of a 50:50 mixture of the *cis* and *trans* olefins. The reaction vessel was heated at 130° and shaken for 16 hr. The pressure vessel was cooled, and the excess olefin was volatilized in a vacuum and collected in a cooled trap. Analysis of the composition of the recovered olefin by integration of the proton spectrum (*cis*, τ 3.57; *trans*, τ 3.65) gave 41% *trans* and 59% *cis*. The crude reaction product weighed 16.7 g. (100%) and was also analyzed by the proton spectrum determined in acetonitrile (*cis*, τ 4.23; *trans*, τ 4.44). The composition was 84% *trans* and 16% *cis*.

Acknowledgments. The author is indebted to Drs. D. C. Dittmer, C. G. Swain, R. E. Benson, and T. L. Cairns for helpful discussions during the course of this work.

Relative Nucleophilic Reactivities of Amino Groups and Mercaptide Ions in Addition Reactions with α,β-Unsaturated Compounds[1,2]

Mendel Friedman, J. F. Cavins, and J. S. Wall

Contribution from the Northern Regional Research Laboratory,[3] *Peoria, Illinois.* *Received March 17, 1965*

Factors were investigated which govern nucleophilic reactivities of functional groups in aminothiols, thiols, and other model compounds with α,β-unsaturated compounds such as acrylonitrile. Rates as a function of pH correlate with theoretical rate equations and indicate that mercaptide ions and nonprotonated amino groups participate in the rate-determining step. Possible reaction pathways of aminothiols are considered in terms of the two functional groups. A quantitative estimate of the influence of steric and polar parameters on rates of mercaptide ions was obtained from a Hammett–Taft-type, free-energy relation derived from observed linear Brønsted-type plots. The polar and steric reaction parameters of mercaptide ions are similar to those of amino groups. At comparable pK values and steric environments, sulfur anions are about 280 times more reactive than amino groups. This difference in reactivities is explained in terms of polarizabilities of nonbonded electrons on nitrogen and sulfur, charge distributions in ground and transition states, and solvation factors. A single, free-energy equation is developed that relates reactivities of mercaptide ions and amino groups to polar, steric, and nucleophilic parameters. Relative rates of these functional groups with several vinyl compounds remain essentially constant. The predictions of kinetic data that α,β-unsaturated compounds should react preferentially with SH groups in aminothiols attached to primary carbon atoms, but not with those attached to tertiary, were confirmed by synthetic applications.

Amino acids, peptides, and related natural products frequently contain two or more functional groups which may react concurrently with α,β-unsaturated compounds. The relative rates of reaction of these groups with vinyl derivatives determine the pathways of reactions and the nature of products. Kinetic studies of reaction rates of α,β-unsaturated compounds, such as acrylonitrile, with amino and sulfhydryl groups of amino acids, aminothiol acids, thiol acids, and related model compounds were made to determine the factors governing the relative reactivities of these groups and to establish the influence of one group upon the reactivity of the other.

Previously[2] it was demonstrated that the rates of reaction of amino groups in amino acids and peptides with acrylonitrile were a function of amino acid anion concentration which is determined by the pK values of the amino groups and the pH of the medium. The rates of reaction were also shown to obey a Hammett–Taft free-energy relationship of the type

$$\log \frac{k_A\text{-(any amino acid or peptide)}}{k_A\text{-(glycine)}} = \rho\sigma^A + Es \quad (1)$$

(1) Presented at the Division of Organic Chemistry, 148th National Meeting of the American Chemical Society, Chicago, Ill., Aug. 30–Sept. 4, 1964, Abstracts, p. 41S.

(2) Paper II of a series on reactions of amino acids, peptides, and proteins with α,β-unsaturated compounds. For paper I, see M. Friedman and J. S. Wall, *J. Am. Chem. Soc.*, 86, 3775 (1964).

(3) A laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

where k_{A^-} is the second-order anion rate constant, ρ is the slope of a plot of log k_{A^-} *vs.* pK of the amino groups, σ^A is the difference in pK values between any amino acid or peptide and glycine, and Es is the steric substituent constant.

In comparing nucleophilic reactivities of two different functional groups in addition reactions to conjugated double bonds, factors other than polar and steric environments of the nucleophiles must be considered as contributing to variations in rates. These factors are inherent to the nucleophiles and include charge, electronic structure, and size of the nucleophiles. The relative importance of these parameters and their quantitative values have been disputed.[4] The current experiments permit evaluation of the various parameters governing relative reactivities of mercaptide ions and amino groups in aliphatic compounds with vinyl derivatives. Reaction rates were studied as functions of pH, pK, and steric environments of functional groups. Proper evaluation of experimental pK values facilitated the development of linear free-energy relationships that encompass both amino and mercaptide reactivities. The necessity of including a factor which evaluates relative nucleophilicities of functional groups in similar steric and polar environments not only supports the validity of the concept of a difference in reactivity between nucleophiles but gives a quantitative measure of free-energy differences in the activation step.

Results and Discussion

Rates of Reaction of Sulfhydryl and Amino Groups. The rates of reaction of the sulfhydryl groups were followed by a microamperometric titration with $AgNO_3$ based on the procedures of Benesch, *et al.*,[5a] and Rosenberg, *et al.*,[5b] and those of the amino group by a modified Van Slyke[6] manometric amino nitrogen determination.

The reaction rates of the sulfhydryl group of homocysteine with acrylonitrile were examined as a function of concentration of both reactants. The results, summarized in Table I, show that the reaction is second order since the rate constants remain essentially invariant with concentration. Previously it was demonstrated that the reaction of the amino group in amino acids with acrylonitrile also follows second-order kinetics.[2]

Table I. Rates of Reaction of the Sulfhydryl Group in Homocysteine with Acrylonitrile at 30° and pH 8.1 (μ = 0.3)

Homocysteine, mole/l.	Acrylonitrile, mole/l.	k_2, l./mole/sec.
0.01	0.155	0.111
0.02	0.155	0.125
0.03	0.155	0.118
0.01	0.225	0.126
		Av. and std. dev. 0.120 ± 0.006

When a sufficient excess of the vinyl compound was employed, the graph of log C_t/C_0 *vs.* time, where C_t is the concentration at time t and C_0 the initial concentration, gave straight lines establishing that the reactions follow pseudo-first-order kinetics. The half-lives ($t_{1/2}$) were read directly from these graphs, and the pseudo-first-order rate constants (k_1) and second-order rate constants (k_2) were calculated by means of formulas $k_1 = 0.693/t_{1/2}$ and $k_2 = k_1/[\alpha,\beta$-unsaturated compound]. Several examples for the SH group are shown in Figure 1 and for the NH_3^+ group in Figure 2. To check the experimental accuracy of the results, the second-order rate constants were also determined directly from the integrated form of the second-order rate law by the procedure described by Frost and Pearson.[7] The ratio of vinyl compound to sulfhydryl group in these determinations was 2:1. These rate constants did not differ significantly from those obtained by pseudo-first-order kinetics. The direct determination of the second-order rate constants was also carried out in several cases in which the rates were too fast to be measured by the pseudo-first-order procedure.

Effect of Ionic Strength on Rates. The Debye–Hückel theory predicts that the ionic strength of a medium should exert major effects on rates and equilibria when more than one ionic species react or are produced in a reaction.[7] Although the rate-determining step for the reaction of a mercaptide group with an α,β-unsaturated compound (see mechanism below) involves one charged species on each side of the equation, the ionization equilibrium occurring before the rate-determining step produces two charged species. Ionic strength might, therefore, influence the extent of ionization. To investigate such secondary salt effects,[8] rates of reaction of the sulfhydryl group in homocysteine with acrylonitrile were determined as a function of ionic strength at pH 8.1. The results, summarized in Table II, show that, although there appears to be a slight trend toward rate increase at higher ionic strength, the changes are within limits of experimental error. Ionic strength was maintained constant at 0.3 for all further rate studies.

Table II. Rates of Reaction of the Sulfhydryl Group in Homocysteine with Acrylonitrile at 30° and pH 8.1 as a Function of Ionic Strength

Ionic strength	k_2, l./mole/sec.
0.15	0.109
0.15	0.113
0.75	0.113
1.55	0.115
1.55	0.115
	Av. and std. dev. 0.113 ± 0.003

Effect of pH on Rates. The rates of reaction of SH groups in mercaptoacetic acid, cysteine, homocysteine, glutathione, penicillamine, and β-mercaptoisoleucine were determined as a function of pH (Figure 3 and Table III). Rates rapidly increase with pH, and when

(4) B. Miller, *J. Am. Chem. Soc.*, **84**, 403 (1962).
(5) (a) R. E. Benesch, H. A. Lardy, and R. Benesch, *J. Biol. Chem.* **216**, 663 (1955); (b) S. Rosenberg, J. C. Perrone, and P. L. Kirk, *Anal. Chem.*, **22**, 1186 (1950).
(6) (a) D. D. Van Slyke, *J. Biol. Chem.*, **83**, 425 (1929); (b) A. B. Kendrick and M. E. Hanke, *ibid.*, **132**, 739 (1940).
(7) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd. Ed., John Wiley and Sons, Inc., New York, N. Y., 1961, Chapter 2.
(8) K. B. Wiberg, "Physical Organic Chemistry," Part 3, John Wiley and Sons, Inc., New York, N. Y., 1964.

Table III. The pH Dependence of Contributions k_{2y} and k_{2z} of Nucleophilic Species $HAS^{\pm}$ and AS^{2-} to Calculated Second-Order Rate Constants for the Reaction of Mercaptide Groups with Acrylonitrile at 30°[a]

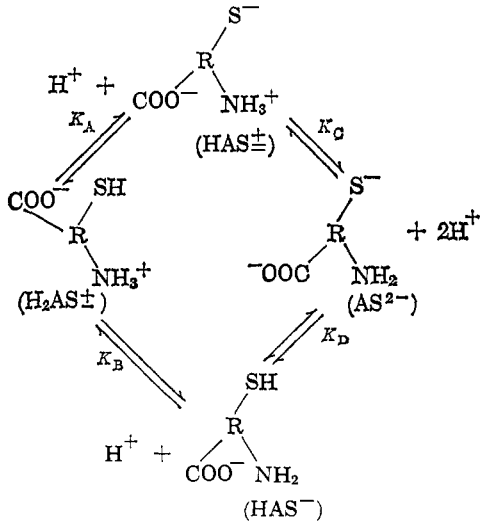
Compd.	pH	$k_{HAS^{\pm}}$, l./mole/sec.	$k_{AS^{2-}}$, l./mole/sec.	k_{2y}, l./mole/sec.	k_{2z}, l./mole/sec.	k_2', calcd. $(k_{2y} + k_{2z})$	k_2, obsd., l./mole/sec.
Penicillamine	6.50	0.0106		4.05×10^{-4}		4.05×10^{-4}	4.05×10^{-4}
	6.97	0.0104		1.11×10^{-3}		1.11×10^{-3}	1.11×10^{-3}
	7.40	0.0107		2.57×10^{-3}		2.57×10^{-3}	2.57×10^{-3}
	8.10	0.0106		6.46×10^{-3}	2.20×10^{-4}	6.68×10^{-3}	6.53×10^{-3}
	9.15			9.62×10^{-3}	3.68×10^{-3}	1.33×10^{-2}	1.31×10^{-2}
	10.00			8.01×10^{-3}	2.17×10^{-2}	2.97×10^{-2}	2.80×10^{-2}
	10.50		0.0920	5.29×10^{-3}	4.54×10^{-2}	5.07×10^{-2}	4.54×10^{-2}
	10.93		0.0845	2.86×10^{-3}	6.61×10^{-2}	6.90×10^{-2}	6.15×10^{-2}
	11.70		0.0917	6.07×10^{-3}	8.23×10^{-2}	8.29×10^{-2}	8.65×10^{-2}
	12.00		0.0878		8.51×10^{-2}	8.51×10^{-2}	8.51×10^{-2}
	12.45		0.0980		9.80×10^{-2}	9.80×10^{-2}	9.80×10^{-2}
β-Mercapto-isoleucine	6.80	0.00884		4.02×10^{-4}	0.008×10^{-4}	4.03×10^{-4}	4.20×10^{-4}
	8.10			4.21×10^{-3}	0.27×10^{-3}	4.38×10^{-3}	4.42×10^{-3}
	12.0		10.5×10^{-2}	3.34×10^{-4}	10.5×10^{-2}	10.5×10^{-2}	10.5×10^{-2}
Cysteine	5.97	0.443		2.93×10^{-3}		2.93×10^{-3}	2.93×10^{-3}
	6.92	0.478		2.66×10^{-2}		2.66×10^{-2}	2.66×10^{-2}
	7.48	0.359		6.31×10^{-2}		6.31×10^{-2}	6.31×10^{-2}
	8.10	0.456		2.03×10^{-1}	4.33×10^{-3}	2.07×10^{-1}	2.15×10^{-1}
	9.08			3.72×10^{-1}	7.55×10^{-2}	4.48×10^{-1}	4.65×10^{-1}
	9.95			3.16×10^{-1}	4.35×10^{-1}	7.49×10^{-1}	8.25×10^{-1}
	11.01		1.74	0.807×10^{-1}	1.44	1.52	1.44
	12.35		1.70		1.68	1.68	1.68
Glutathione	5.30	0.664		3.65×10^{-4}		3.65×10^{-4}	3.65×10^{-4}
	5.70	0.643		8.87×10^{-4}		8.87×10^{-4}	8.87×10^{-4}
	6.30	0.635		3.47×10^{-3}		3.47×10^{-3}	3.47×10^{-3}
	6.80	0.672		1.12×10^{-2}	4.03×10^{-5}	1.12×10^{-2}	1.15×10^{-2}
	8.12			1.73×10^{-1}	1.30×10^{-2}	1.86×10^{-1}	1.81×10^{-1}
	9.10			4.01×10^{-1}	2.88×10^{-1}	6.89×10^{-1}	6.98×10^{-1}
	9.50			3.32×10^{-1}	6.02×10^{-1}	9.34×10^{-1}	9.91×10^{-1}
	9.90			2.05×10^{-1}	9.36×10^{-1}	1.14	1.13
	10.50		1.41	6.87×10^{-2}	1.24	1.31	1.27
	11.90		1.37		1.38	1.38	1.35

[a] $k_{HAS^{\pm}} = k_2(1 + H^+/K_1)$; $k_{AS^{2-}} = k_2(1 + H^+/K_2)$; $k_{2y} = k_{HAS^{\pm}}K_1(H^+)/((H^+)^2 + K_1(H^+) + K_1K_2)$; $k_{2z} = k_{AS^{2-}}K_1K_2/((H^+)^2 + K_1(H^+) + K_1K_2)$.

pH exceeds the highest titration pK they begin approaching an asymptotic value.

The equilibria of the various ionized species of an

Scheme I



aminothiol carboxylate are illustrated in Scheme I.[9] The microscopic equilibrium constants shown in this scheme are related to the titration constants K_1 and K_2 as follows.

(9) (a) H. Lindley, *Biochem. J.*, 74, 577 (1960); (b) *ibid.*, 82, 418 (1962).

$$K_1 = K_A + K_B;\ K_1K_2 = K_AK_C = K_BK_D;\ 1/K_2 = 1/K_D + 1/K_C \quad (2)$$

The over-all reaction rate of an aminothiol involving nucleophilic species $HAS^{\pm}$ and AS^{2-} of the sulfhydryl group is described by eq. 3[9] wherein k_2 is the ob-

$$k_2 = \frac{k_{HAS^{\pm}}(H^+)K_A + k_{AS^{2-}}K_1K_2}{(H^+)^2 + K_1(H^+) + K_1K_2} \quad (3)$$

served, second-order rate constant, $k_{HAS^{\pm}}$ the second-order rate constant associated with nucleophilic species $HAS^{\pm}$, and $k_{AS^{2-}}$ with AS^{2-}. Note that the first part of the right-hand side of eq. 3 describes the contribution to the over-all rate due to $HAS^{\pm}$ (k_{2z}) and the second part due to AS^{2-} (k_{2y}). The derivation of this equation is similar to that given by Friedman and Wall[2] for rates of amino acids. On rearrangement and differentiation it can be shown that

$$\frac{d \log [(k_{AS^{2-}} - k_2)/k_2]}{d(pH)} = -1 + \frac{k_{HAS^{\pm}}K_A(H^+)}{k_{AS^{2-}}K_1K_2 + k_{HAS^{\pm}}K_A(H^+)} - \frac{k_{AS^{2-}}(H^+)}{k_{AS^{2-}}[K_1 + (H^+)] - k_{HAS^{\pm}}K_A} \quad (4)$$

Equation 4 predicts that a plot of log $(k_{AS^{2-}} - k_2)/k_2$ *vs.* pH should have a slope of -1 at the extremes of pH and an inflection point which depends upon the values for $k_{HAS^{\pm}}$, $k_{AS^{2-}}$, K_1, K_A, and K_2. Such a plot is shown schematically in Figure 4. The tangent

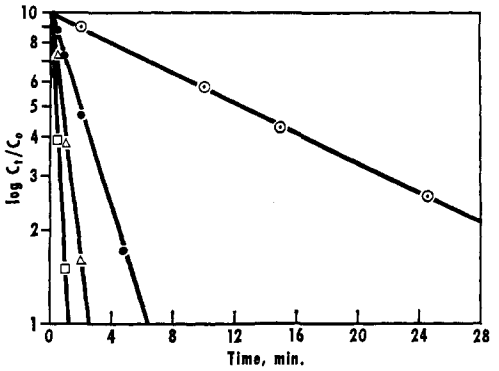


Figure 1. Plot of log C_t/C_0 *vs.* time for reaction of sulfhydryl (0.01 *M*) groups with acrylonitrile (0.155 *M*) at pH 8.1 and 30° (μ = 0.3): ⊙, penicillamine (β,β-dimethylcysteine); ●, mercaptoacetic acid; △, homocysteine; and □, cysteine ethyl ester.

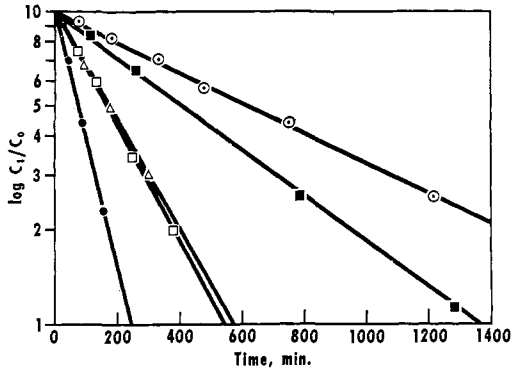


Figure 2. Plot of log C_t/C_0 *vs.* time for the reaction of amino groups (0.01 *M*) with acrylonitrile (0.15 *M*) at pH 8.1 and 30° (μ = 0.3): ⊙, cysteine ethyl ester; ■, glutathione; △, cysteine (free base); □, S-cyanoethylcysteine; and ●, penicillamine (β,β-dimethylcysteine).

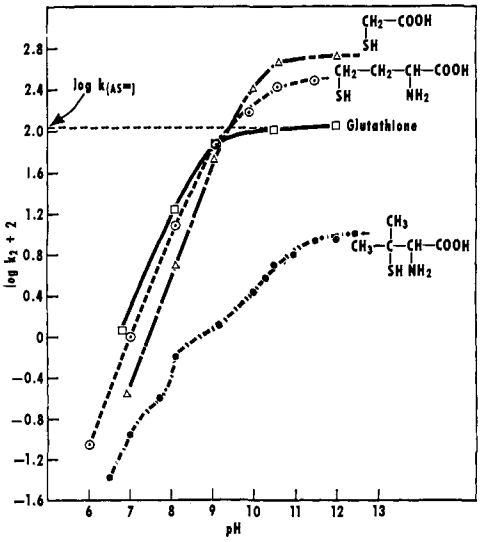


Figure 3. Logarithms of second-order rate constants as a function of pH for the reaction of mercaptide groups with acrylonitrile at 30° (μ = 0.3).

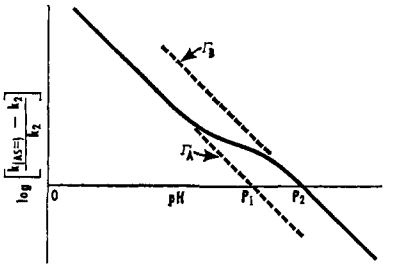


Figure 4. Schematic plot of log $(k_{AS^{2-}} - k_2)/k_2$ *vs.* pH.

in acid solution is designated τ_A and in basic solution τ_B. The equations for the two parallel tangents are 5 and 6. The points P_1 and P_2 in Figure 4 are the

$$[\log (k_{AS^{2-}} - k_2)/k_2]\tau_A = -\mathrm{pH} + \mathrm{p}K_A + \log k_{AS^{2-}}/k_{HAS^{\pm}} \quad (5)$$

$$[\log (k_{AS^{2-}} - k_2)/k_2]\tau_B = -\mathrm{pH} + \mathrm{p}K_2 + \log \frac{[k_{AS^{2-}}K_1 - k_{HAS^{\pm}}K_A]}{k_{AS^{2-}}K_1} \quad (6)$$

points of intersection of lines τ_A and τ_B on the pH axis. The distance between these points is given by eq. 7 and 8.

$$\Delta\mathrm{pH} = (\mathrm{pH})_{p_2} - (\mathrm{pH})_{p_1} = \mathrm{p}K_2 - \mathrm{p}K_A + \log\left[\frac{(k_{AS^{2-}}K_1 - k_{HAS^{\pm}}K_A)k_{HAS^{\pm}}}{k_{AS^{2-}}{}^2K_1}\right] \quad (7)$$

If $k_{AS^{2-}}K_1 \gg k_{HAS^{\pm}}$ and $K_A \cong K_1$ then eq. 7 becomes

$$\Delta\mathrm{pH} = \mathrm{p}K_2 - \mathrm{p}K_1 + \log k_{HAS^{\pm}}/k_{AS^{2-}} \quad (8)$$

Equation 7 gives a general relation between the distance of the inflection and the rate and equilibrium constants. The magnitude of this distance depends upon how severely the solid curves deviate from a straight line. Equation 8 is verified by the experimental observations that, as the plots of log $(k_{AS^{2-}} - k_2)/k_2$ *vs.* pH for mercaptoacetic acid, homocysteine, penicillamine, and cysteine in Figure 5 approach a straight line, the differences between pK_1 and pK_2 approach zero. The $k_{AS^{2-}}$ values for these compounds were directly determined at a pH about two units above the highest titration pK. At this high pH, $k_2 \rightarrow k_{AS^{2-}}$ (see glutathione plot in Figure 3).

Plots of log $(k_{AS^{2-}} - k_2)/k_2$ *vs.* pH in Figure 5 for mercaptoacetic acid data give a straight line with an intercept of 10.2, which is identical with the titration pK of the SH group for this compound. In contrast, similar plots for penicillamine give a curve with an inflection between pH 7.5 and 10.0. The extension of the linear portion below pH 10.0 intercepts the abscissa at pH 10.55, which is identical, within experimental error, with the second titration pK for this compound. The linear portions of the curve indicate the changes in concentration with pH of a single anion species that acts as a nucleophile in the designated pH regions. The inflections appear to be due to changes in the nature and reactivity of the nucleophiles. Thus, for penicillamine, the nucleophilic species $HAS^{\pm}$ predominates in the upper linear region of the curve and AS^{2-} in the lower. The curves for cysteine and homocysteine exhibit similar, but lower, inflections.

By making the assumption that $K_1 \cong K_A$ for the investigated compounds, it can be shown[9] that at high pH values eq. 3 reduces to eq. 9, and at intermediate

$$\log (k_{AS^{2-}} - k_2)/k_2 = \mathrm{p}K_2 - \mathrm{pH} \quad (9)$$

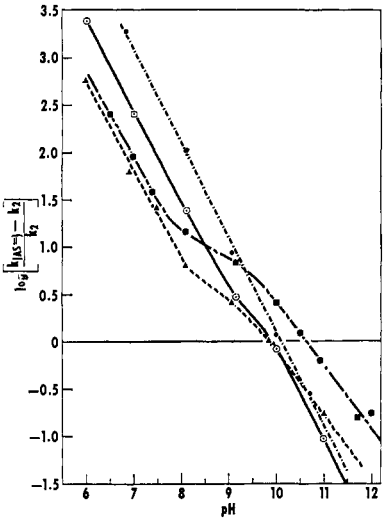


Figure 5. Dependence of log $(k_{AS^{2-}} - k_2)/k_2$ on pH for the reaction of sulfhydryl groups with acrylonitrile at pH 8.1 and 30° (μ = 0.3): ●, mercaptoacetic acid; ⊙, homocysteine; ■, penicillamine; and ▲, cysteine.

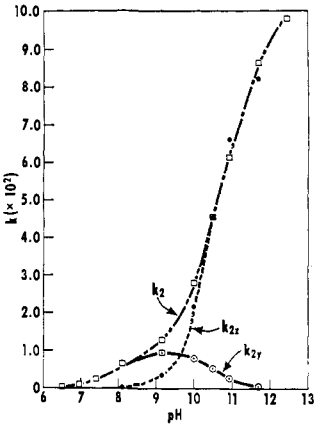


Figure 6. Trends in k_2, k_{2y} (fraction of k_2 due to $HAS^{\pm}$), and k_{2z} (fraction of k_2 due to AS^{2-}) with pH for the reaction of the SH group in penicillamine with acrylonitrile at 30° (μ = 0.3).

pH values below pK_1 eq. 3 simplifies to eq. 10.

$$\log (k_{HAS^{\pm}} - k_2)/k_2 = pK_1 - pH \qquad (10)$$

Equations 9 and 10 permit calculations of $k_{HAS^{\pm}}$ and $k_{AS^{2-}}$ given in Table III. In each case the value for $k_{AS^{2-}}$ exceeded that for $k_{HAS^{\pm}}$, demonstrating that the sulfur anion in AS^{2-} is a stronger nucleophilic species than $HAS^{\pm}$. The ratio $k_{AS^{2-}}/k_{HAS^{\pm}}$ ranges from 2 in glutathione to 9 in penicillamine. The low ratio exhibited by glutathione is probably due to the greater charge separation in this peptide as compared to the other compounds. These results imply that reaction rates at any pH are determined not only by the relative concentrations of the two nucleophilic species but also by their inherent strengths as nucleophiles.

The earlier assumptions that $K_1 \cong K_A$ and that the calculated k_{HAS^-} values at low pH and determined $k_{AS^{2-}}$ at high pH are close approximations for the constants associated with nucleophilic species $HAS^{\pm}$ and AS^{2-}, respectively, are verified by the fact that substitution of the values for these rate constants into eq. 3 yields calculated, second-order rate constants (k_2') which agree well with the observed values (k_2) for rates covering a wide pH range (Table III). The trend in the contributions of fractions k_{2y} and k_{2z} of the total rate (k_2) due to nucleophilic species $HAS^{\pm}$ and AS^{2-}, respectively, as a function of pH is illustrated in Figure 6 for penicillamine. Similar curves may be drawn for the other compounds.

The influence of pH on rates demonstrates that sulfur anions participate with acrylonitrile in the rate-determining step, which is consistent with the following mechanism for cyanoethylation of sulfhydryl groups

$$RSH + H_2O \rightleftharpoons RS^- + H_3O^+$$

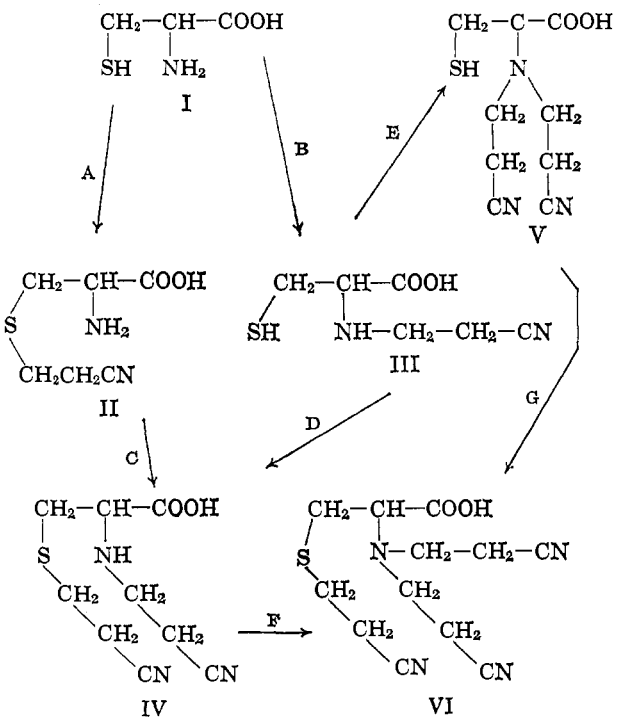
$$RS^- + CH_2{=}CH{-}CN \rightleftharpoons RS{-}CH_2{-}\bar{C}H{-}CN$$

$$RS{-}CH_2{-}\bar{C}H{-}CN + H_3O^+ \rightleftharpoons RS{-}CH_2{-}CH_2{-}CN + H_2O$$

where RS^- consists of species $HAS^{\pm}$ and AS^{2-} for an aminothiol acid. These two species react at different rates due to inductive effects brought about by change in charge caused by further ionization of the molecule. These conclusions are in agreement with theoretical discussions by Lindley[9] but, unlike his system, reaction rates with acrylonitrile could be studied at higher pH values, and determination of separate rate constants associated with each of the nucleophilic species was possible.

Reaction Pathways of Aminothiols with α,β-Unsaturated Compounds. The various possibilities for reaction of the two functional groups present in an aminothiol, exemplified by cysteine, with α,β-conjugated compounds, such as acrylonitrile, are illustrated in Scheme II. The over-all reaction to give VI

Scheme II



involves two parallel reactions, A and B, which yield II and III, followed by reactions C, D, and E to give IV and V which can react further *via* F and G. Analysis of the kinetics of this complex system would be difficult. However, based on the observed relative

Table IV. Rates of Reaction of Amino and Mercaptide Groups with Acrylonitrile at pH 8.1 and 30° (μ = 0.3)[a]

Compd.	$k_2(S^-)$, l./mole/sec.	pK_1[b]	$k_2(NH_2) \times 10^4$, l./mole/sec.	pK_2[b]	Ratio of observed rates (S^-/NH_2)
Mercaptoacetic acid	0.0390	10.20			
β-Mercaptopropionic acid	0.0270	10.05			
N-Acetylcysteine	0.0573	9.52			
DL-Homocysteine	0.120	8.70	4.20	10.46	285
Glutathione	0.173	8.56	3.68	9.57	470
Cysteine	0.134	8.15	4.40	10.37	304
Cysteine ethyl ester	0.183	6.53	1.42	9.05	1290
Penicillamine (β,β-dimethylcysteine)	0.00650	7.90	9.44	10.42	6.4
β-Mercaptoisoleucine (β,β-methylethylcysteine)	0.00442	8.10	5.60	10.6	7.9
N-Acetylpenicillamine	0.00104	9.90			
N-Acetyl-β-mercaptoisoleucine	0.00059	10.30			
β-Alanine			0.968	10.06	
S-Cyanoethylcysteine			4.03	8.60	
S-Carbethoxyethylcysteine			4.18	8.65	

[a] All pK values were determined at 30° [b] pK_1 equals pK of SH groups in thiol acids, and pK_2 equals pK of NH_3^+ groups in amino acids.

reactivities of the mercaptide ions and amino groups, it is possible to make a number of approximations that permit a simplified description of the system.

The ratio of rates (S^-/NH_2) for the alkylation by acrylonitrile in compounds in which the SH groups are attached to primary carbon atoms range from 300 to 1300 (Table IV). For these compounds the major pathway must involve an almost complete substitution of the SH group before the amino group reacts to any significant extent. This conclusion is confirmed by the similarity of the rate constants for the reaction of the amino group in cysteine and S-cyanoethylcysteine, the product of pathway A. Because of the greater reactivity of the S^- group, reaction pathway A–C predominates and pathway B–D need not be considered in an approximate solution. When rate ratios of consecutive reactions, such as A and C in Scheme II, exceed 300, conversion of I to II and of II to IV may be treated as separate reactions.[8] The kinetics of reaction A were analyzed above and those of B in a previous communication[2] where it was also shown that rates *via* F may be neglected under present reaction conditions.

The SH groups in penicillamine and β-mercaptoisoleucine are attached to tertiary carbon atoms. Their mercaptide ions are only around seven times more reactive than the amino groups (Table IV). For these compounds, an analysis of the kinetics has to include pathway B–D. Such an analysis would require complete product yield determinations at intervals during the reaction.

Relative Nucleophilic Reactivities of Amino Groups and Mercaptide Ions. In Figure 7 are plotted log $k_{HAS^\pm}$ against pK_1 and log $k_{AS^{2-}}$ against pK_2 values for two steric series of thiols and aminothiols. In the first series (upper plot), the SH groups are attached to primary and in the second series (middle plot), they are attached to tertiary carbon atoms. For the first steric series the best straight line was drawn through the points designating log $k_{HAS^\pm}$ and pK_1 for the aminothiols and log $k_{AS^{2-}}$ and pK_{SH} for mercaptoacetic and mercaptopropionic acids. Two points for log $k_{AS^{2-}}$ *vs.* pK_2 for the aminothiols deviate significantly from this straight line. This deviation is probably due to the fact that pK_2 is not a true measure of the basicity of nucleophilic species AS^{2-} (which is more closely given by pK_D). The points designated as X on the graph when read on the pK scale give the predicted pK_D values associated with AS^{2-} For the second steric series the points fall on a straight line (Figure 7, middle plot).

Treatment of the rate data in a manner similar to that described by Taft[10] and adapted[2] for other reactions makes it possible to separate polar and steric factors which influence rates of cyanoethylation of the mercaptide ions. From Figure 7, it may be shown that

$$\log k_{S^-} = \rho(\text{slope}) \times pK_{SH} + b(\text{intercept}) \quad (11)$$

where log k_{S^-} refers to anion rate constants associated with $^-S—R—COO^-$, $HAS^\pm$, and AS^{2-}. Equation 11 is an extension of the Brønsted catalysis law and may be expressed as a Hammett–Taft-type free-energy relationship (eq. 12) which relates the logarithm of the ratio of second-order anion rate constants of any thiol or aminothiol acid to that of mercaptoacetic acid, the simplest thiol acid

$$\log \frac{k_{S^-}\left(R\begin{smallmatrix}COO^-\\S^-\end{smallmatrix}\right)}{k_{S^-}\left(CH_2\begin{smallmatrix}COO^-\\S^-\end{smallmatrix}\right)} = \rho \times \sigma^{S^-} + Es \quad (12)$$

where ρ, the slope, is the parameter that measures the sensitivity of the logarithm of the ratio of rate constants to polar effects, σ^{S^-} is the parameter that represents the polar effect of substituents and is the difference between the pK_{SH} values in HSRCOOH and $HSCH_2$-COOH (Table IV), and *Es*, the difference in intercepts of the parallel lines with the ordinate (Figure 7, upper and middle lines) of the two steric series containing the sulfhydryl group, is the steric substituent

(10) (a) R. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, Chapter 13; (b) W. A. Pavelich and R. W. Taft, Jr., *J. Am. Chem. Soc.*, 79, 4935 (1957).

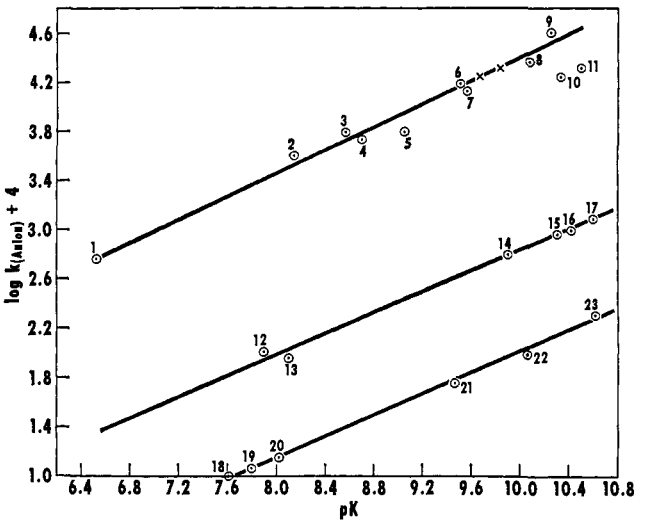


Figure 7. Plot of log second-order anion rate constants *vs.* pK for the reaction of mercaptide groups (1–17) and amino groups (18–23) with acrylonitrile at 30° (see text). Data for amino groups are from ref. 3. Upper plot, mercaptide groups attached to primary carbon atoms: 1, $^{-}SCH_2CH(NH_3^+)COOC_2H_5$; 2, $^{-}SCH_2CH(NH_3^+)COO^-$; 3, $^{-}$S-glutathione-NH_3^+; 4, $^{-}SCH_2CH_2CH(NH_3^+)COO^-$; 5, $^{-}SCH_2CH(NH_2)COOC_2H_5$; 6, $^{-}SCH_2CH(NHCOCH_3)COO^-$; 7, $^{-}$S-glutathione-NH_2; 8, $^{-}SCH_2CH_2COO^-$; 9, $^{-}SCH_2COO^-$; 10, $^{-}SCH_2CH(NH_2)COO^-$; 11, $^{-}SCH_2CH_2CH(NH_2)COO^-$. Middle plot, mercaptide groups attached to tertiary carbon atoms: 12, $CH_3CCH_3(S^-)CH(NH_3^+)COO^-$; 13, $CH_3CH_2CCH_3(S^-)CH(NH_3^+)COO^-$; 14, $CH_3CCH_3(S^-)CH(NHCOCH_3)COO^-$; 15, $CH_3CH_2CCH_3(S^-)CH(NHCOCH_3)COO^-$; 16, $CH_3CCH_3(S^-)CH(NH_2)COO^-$; 17, $CH_3CH_2CCH_3(S^-)CH(NH_2)COO^-$. Lower plot, amino groups attached to primary carbon atoms: 18, tetraglycine; 19, triglycine; 20, diglycine; 21, glycine; 22, β-alanine; 23, ϵ-aminocaproic acid.

constant that depends on the size and steric requirements of the substituents in the reaction.

In Table V are shown the σ^{S^-} and Es values calculated according to eq. 12, using the average ρ-value of 0.450 and the indicated $k_{AS^{2-}}$ values for a number of sterically hindered thiol and aminothiol acids.

Table V. Polar and Steric Substituent Constants for Rates of Reaction of Mercaptide Ions $R(S^-)COO^-$ with Acrylonitrile at 30° (μ = 0.3[a])

R	σ^{S^-}	Es	$k_{AS^{2-}}$
$-CH_2-$	0	0	4.26
$-C(CH_3)(NH_2)-CH_2CH_2-$	+0.22	−1.73	9.88×10^{-2}
$-C(CH_3)(NH_2)-CH_3-$	+0.40	−1.74	10.5×10^{-2}
$-C(CH_3)(NHCOCH_3)-CH_2CH_3-$	−0.30	−1.66	6.65×10^{-2}
$-C(CH_3)(NHCOCH_3)-CH_3-$	−0.10	−1.61	9.45×10^{-2}

[a] The $k_{AS^{2-}}$ was determined directly for each compound at pH 12.4.

The average ρ-value (0.450) is similar to that obtained for amino groups[2] (0.427), demonstrating that rates for the two functional groups do not differ in their sensitivities to basicities as measured by pK. Similarity in ρ-values for amino and mercaptide groups is also indicated by the approximately parallel slopes of the curves in Figure 7 for amino (lower line) and mercaptide groups. The average Es value in the steric series in which the sulfhydryls are attached to tertiary carbon atoms (1.68 ± 0.064) is also of the same order of magnitude as the corresponding Es value[2] (1.43 ± 0.08) for amino groups attached to similarly substituted carbon atoms.

Equation 12 may be used to calculate predicted rates for similarly substituted thiol derivatives.

The previously demonstrated large difference in rate ratios S^-/NH_2 in cysteine and analogous compounds as compared to penicillamine and to β-mercaptoisoleucine is the result of the greater steric factor associated with the latter compounds and of changed basicities of the functional groups due to inductive effects from alkyl substituents near the reactive sites.

Since Es and ρ-values are similar for both functional groups, the following general equation is proposed which separates contributions of polar, steric, and nucleophilic parameters to relative reaction rates of amino and mercaptide anions in the several steric series of compounds studied.

$$\log \frac{k_S\text{-}\left(\mathrm{R}\begin{smallmatrix}\diagup\mathrm{COO^-}\\ \diagdown\mathrm{SH}\end{smallmatrix}\right)}{k_A\text{-}\left(\mathrm{CH_2}\begin{smallmatrix}\diagup\mathrm{COO^-}\\ \diagdown\mathrm{NH_2}\end{smallmatrix}\right)} = \rho\sigma^A + Es + N \quad (13)$$

where σ^A is the difference in pK values between any amino and sulfhydryl group and that for the amino group in glycine. Equation 13 is analogous to eq. 1 except for the additional nucleophilicity factor N. This factor gives a measure of the greater nucleophilic strength of mercaptide as compared to amino groups for any two compounds when σ^A and Es are zero.[11] The value of N is defined as zero for the amino group, and for the S^- group, N is equivalent to the difference in intercepts on the ordinate between top and bottom lines on Figure 7. This difference is 2.44. A striking example is the comparison of $HS-CH_2CH_2COOH$ and $NH_2-CH_2CH_2COOH$, which gives a direct measure of the nucleophilicity factor N. The SH and NH_2 groups of these compounds have identical pK values, and, therefore, identical σ-values and the compounds are structurally quite similar (Es = 0), and yet, β-mercaptopropionic acid reacts 280 times faster with acrylonitrile than does β-alanine (Table IV), which corresponds to an N-value of 2.45. The difference in ΔF^* for the two functional groups equals $2.303RTN$.

Similarly, by knowing Es and σ^A values for any amino or thiol acid it is possible to calculate its predicted reaction rate with acrylonitrile.

It should be emphasized that in order to apply eq. 13 to new nucleophiles it is first necessary to establish that both Es and ρ-values associated with the new

(11) For a discussion of other free-energy relations which include nucleophilicity factors, see P. R. Wells, *Chem. Rev.*, 63, 171 (1963), and references cited there.

nucleophilic species are approximately equal to the corresponding values for the amino groups.

A number of attempts have been made to explain the factors governing nucleophilic reactivities.[4,12-15] The relative reactivities of amino and mercaptide groups having comparable basicities and equal steric environments may be explained in terms of polarizabilities of nonbonded electrons on nitrogen and sulfur, relative stabilization of charges in the respective transition states, and solvation effects. Factors proposed to explain relative nucleophilicities have been applied mainly to the observed kinetics of nucleophilic displacements, whereas the present investigation is concerned with nucleophilic additions. However, the analogy between nucleophilic displacements and additions is quite close. In the nucleophilic addition to a conjugated system the leaving group may be visualized to be the pair of electrons which is polarized away from the terminal double bond as the nucleophile approaches the reactive site.

Model studies of transition states show that both the amino and mercaptide groups have to approach the double bond of acrylonitrile almost at right angles to the plane of the molecule and that the amino group has to assume a more restricted orientation than the sulfur anion in the course of formation of transition states. The mechanism of formation of the two transition states differs in several features which energetically favor the transition state of the mercaptide anion over that for the amino group. As the pair of electrons on nitrogen approaches the double bond, it forces an energetically unfavorable redistribution of electrons since the double bond is being broken. The antibonding empty orbitals of the double bond, which are in the process of assuming tetrahedral geometry, can overlap with the nitrogen orbitals. The sulfur anion has two lone pairs of electrons left after bonding is initiated, and the sulfur atom has empty 3d-orbitals. If the energetics are favorable, the 3d-orbitals may stabilize the high-energy electrons of the double bond polarized to the center carbon atom of acrylonitrile during the formation of the transition state.

The transition states for the reaction of mercaptide and amino groups differ by another feature. In going from the negatively charged ground state of the sulfur anion to the transition state a dissipation of charge takes place, whereas in the amino group charge separation occurs, as indicated below. This difference in the charge distribution favors the transition state of the sulfur anion.

$$^-OOC-R-S\cdots CH_2\cdots \overset{\delta^-}{C}H-C\equiv N \quad \text{and}$$

$$^-OOC-R-\overset{\delta^+}{N}H_2\cdots CH_2\cdots \overset{\delta^-}{C}H-C\equiv N$$

Since the two transition states being compared differ in charge distributions, they should also differ in the degree of solvation. As solvation energies increase with charge-to-size ratios of nucleophiles,[16] polar solvents should desolvate the sulfur anion with greater ease than the amino group. Such considerations imply that relative reactivities of the two functional groups should be a function of the polarity of the solvent.

To determine whether relative nucleophilic reactivities of mercaptide ions and amino groups are of the same order of magnitude with α,β-unsaturated compounds other than acrylonitrile, k_2 values for the reaction of the S^- group in mercaptopropionic acid and the NH_2 group of glycine were compared with three vinyl compounds (Table VI).

Table VI. Second-Order Rate Constants for the Reaction of the S^- Group in $^-S-CH_2CH_2COO^-$ and the NH_2 Group in $NH_2-CH_2COO^-$ at pH 8.1 and 30°

Vinyl compd.	$k_2(S^-)$ × 10^2, l./mole/sec.	$k_2(NH_2)$ × 10^5, l./mole/sec.	Ratio of rates (S^-/NH_2)
Acrylonitrile	2.70	20.4	132
Methyl acrylate	11.0	76.0	145
Acrylamide	0.46	2.60	178

From the observed rate ratios it may be concluded that the relative nucleophilicities of the two functional groups remain fairly constant for the three vinyl compounds listed in Table VI.

Synthetic Application of Kinetic Results. On the basis of the observed relative reactivities of the amino and mercaptide groups with acrylonitrile (Table IV), it would be predicted that the sulfhydryl groups attached to primary carbon atoms in aminothiols should add preferentially to acrylates. This prediction was confirmed by the synthesis of several S-alkyl derivatives in high yield from equimolar concentrations of cysteine and the corresponding acrylate.

$$\underset{\substack{SH\\ NH_2}}{CH_2-CH-COOH} + CH_2=CH-R \longrightarrow \underset{\substack{S-CH_2-CH_2-R\\ NH_2}}{CH_2-CH-COOH}$$

VII, R = CN
VIIa, R = $CONH_2$
VIIb, R = CO_2CH_3
VIIc, R = $CO_2CH_2CH_3$
VIId, R = $CO_2CH_2CH_2CH_2CH_3$

The structures of compounds VII–VIId were confirmed by elemental analyses, infrared, and quantitative n.m.r. data taken in trifluoroacetic acid. The chemical shifts assigned to the various kinds of hydrogens in these compounds are given under Experimental.

In contrast, attempts to synthesize S-cyanoethylpenicillamine from equimolar concentrations of penicillamine and acrylonitrile resulted in a mixture of products. This result is not surprising since in the kinetic studies it was shown that the mercaptide group in penicillamine is only around six times more reactive than the amino group.

Conclusions

This study clearly demonstrates that the reactivity of the mercaptide group as a nucleophile in an aminothiol acid is influenced by the presence or absence of a charge on the neighboring amino group. The results

(12) C. G. Swain and C. B. Scott, *J. Am. Chem. Soc.*, **75**, 141 (1953).
(13) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 1778 (1960).
(14) J. O. Edwards and R. G. Pearson, *ibid.*, **84**, 16 (1962).
(15) B. Miller, *Proc. Chem. Soc.*, 303 (1962).
(16) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, p. 260. See also footnote 38 in ref. 4.

also show that to make a valid comparison of nucleophilicity as a function of basicity it is essential to compare rate constants associated with individual reacting nucleophilic species and not observed rates. For each nucleophile studied, there appears to be an inherent reactivity which may be further varied by inductive and steric effects. The difference in rates between the S^- and NH_2 groups at equal pK values and steric environments is a constant.

The factor that relates the basicity of compounds containing the same nucleophilic functional group to their reactivity with α,β-unsaturated compounds is ρ, which is probably a function of the electrostatic interactions between the nucleophilic and electrophilic centers in the transition state. Since the ρ-value was similar for S^- and NH_2 groups, functional group basicity as a reaction parameter must be independent of the nature of the two nucleophiles. The *Es* values, which represent steric effects in the reaction and which are possibly related to entropy factors, are also of the same order of magnitude for the two functional groups. This finding indicates that similar structural changes influence reactivities of amino and mercaptide groups alike in terms of activation energies. If eq. 13 could be shown to apply to other nucleophiles, then it would be of value for the prediction of reaction rates of these nucleophiles with α,β-unsaturated compounds.

Moreover, since the results of the present investigation show that steric and inductive effects influence rates of reaction of the SH relative to that of NH_2 groups to such a large extent, caution should be exercised in the use of acrylonitrile and analogous compounds as supposedly specific blocking agents for sulfhydryl groups in aminothiols and proteins.[17]

Experimental

Melting points were taken on a Fisher-Johns melting point apparatus and are not corrected. Infrared spectra were measured with a Perkin-Elmer[18] Model 21 spectrophotometer on KBr micropellets. N.m.r. spectra were determined in trifluoroacetic acid with tetramethylsilane as the internal reference as described by Bovey and Tiers[19] on a Varian spectrophotometer Model A-60 with integrator at 60 Mc.

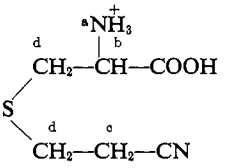
The source of materials is as follows: L-cysteine, reduced glutathione, and DL-penicillamine were obtained from Mann Research Laboratories; L-cysteine ethyl ester hydrochloride, N-acetyl-L-cysteine, N-acetyl-DL-penicillamine, and DL-homocysteine were obtained from Nutritional Biochemicals; DL-N-acetyl-β-mercaptoisoleucine was obtained from Aldrich; mercaptoacetic and mercaptopropionic acids were obtained from Eastman; acrylonitrile and acrylamide were obtained from Matheson; and methyl acrylate was obtained from Rohm and Haas. All thiol derivatives were titrated with $AgNO_3$ to ascertain their purity.

β-Mercaptoisoleucine. The compound was prepared in a nearly quantitative yield by the hydrolysis of N-acetyl-β-mercaptoisoleucine in acid solution.[20] The sulfhydryl group consumed the theoretical amount of $AgNO_3$.

S-Cyanoethylcysteine (VII). Weil and Seibles[17] report the isolation of S-cyanoethylcysteine on reaction of cysteine with 2 moles of acrylonitrile at pH 8. Attempts to prepare this compound by their procedure indicated that under the reaction conditions used the α-amino group became partially cyanoethylated. A modified procedure in which a stoichiometric amount (1 mole) of acrylonitrile was used gave the desired compound.

A solution of 10 g. (0.056 mole) of cysteine hydrochloride monohydrate dissolved in 15 ml. of water was brought to pH 8.1 with a dilute ammonia solution. Total volume was adjusted to 60 ml. and the cysteine solution was placed into a 100-ml., round-bottom flask equipped with a stirrer and nitrogen inlet. To this solution was added 3.6 ml. (0.057 mole) of freshly redistilled acrylonitrile with stirring under 1 atm. of nitrogen over a period of 10 min. Stirring was continued for 4 more hr. The solution was then evaporated to dryness by means of an aspirator (bath temperature 30–33°) and the residue was recrystallized once from 75% ethanol and a second time from 80% ethanol at 5° as fluffy white crystals, yielding 7.6 g. (80%).

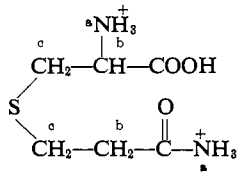
The infrared spectrum showed a nitrile peak at 2240 cm.$^{-1}$ and the following assignments were made to the n.m.r. spectrum.



protons	chemical shift (τ-units)
a	2.30
b	5.30
c	6.47
d	7.00

Anal. Calcd. for $C_6H_{10}N_2O_2S$ (174): C, 41.33; H, 5.85; N, 16.13; S, 18.50. Found: C, 41.44; H, 5.89; N, 15.99; S, 18.82.

Cysteine-S-propionamide (VIIa). A solution of 1.74 g. (0.01 mole) of cysteine hydrochloride monohydrate in 20 ml. of water was adjusted to pH 8.0 with ammonia water. To the cysteine solution was added 0.78 g. (0.011 mole) of acrylamide (Matheson) and the reaction mixture was stirred under 1 atm. of nitrogen at room temperature for a period of 48 hr. A precipitate settled out during the reaction time. It was filtered off and recrystallized from 75% ethanol–water as white needles. Additional fractions were obtained from the mother liquor by evaporation and recrystallization of the residue for a total yield of 82%, m.p. 213–214° with bubbling. The following assignments were made to the n.m.r. spectrum.



protons	chemical shift (τ-units)
a	2.23
b	5.30
c	7.10

(17) L. Weil and T. S. Seibles, *Arch. Biochem. Biophys.*, **95**, 470 (1961).

(18) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

(19) F. A. Bovey and G. V. D. Tiers, *J. Am. Chem. Soc.*, **81**, 2870 (1959).

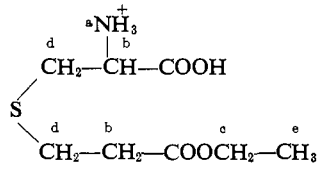
(20) H. M. Crooks in "The Chemistry of Penicillin," H. Clark, Ed., Princeton University Press, Princeton, N. J., 1949, Chapter 16.

Anal. Calcd. for $C_6H_{12}N_2O_3S$ (192): C, 37.50; H, 6.25; N, 14.58; S, 16.66. Found: C, 37.56; H, 6.31; N, 14.62; S, 16.61.

S-Carbomethoxyethylcysteine (*VIIb*). A solution of 8.7 g. (0.05 mole) of cysteine hydrochloride monohydrate in 15 ml. of water was brought to pH 8.1 with ammonia water. Final volume was 30 ml. To the cysteine solution was added 4.73 ml. (0.056 mole) of methyl acrylate dropwise over a period of 7 hr. with stirring and under nitrogen. Stirring was continued for another 26 hr. and the reaction mixture was worked up as described above for acrylonitrile. The compound was recrystallized from aqueous methanol and the yield was nearly quantitative, m.p. 176–178°. The infrared spectrum showed a peak at 1743 cm.$^{-1}$, which was assigned to the carbomethoxy group.

Anal. Calcd. for $C_7H_{13}NO_4S$ (207): C, 40.58; H, 6.28; N, 6.76; S, 15.45. Found: C, 40.22; H, 6.27; N, 6.88; S, 15.52.

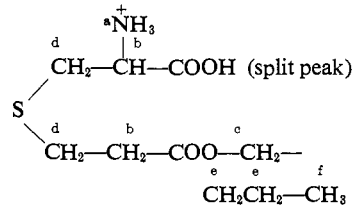
S-Carbethoxyethylcysteine (*VIIc*). The reaction was carried out in the same manner as described for acrylonitrile. The ethyl acrylate was added dropwise over a period of 75 min. and stirring was continued for 53 more hr. The cloudy reaction mixture was filtered and evaporated to dryness. The residue was taken up in hot 80% ethanol, filtered, and left standing at 5°. Long, fluffy, white needles crystallized out. Further crops were obtained from the mother liquor at −5° and the reaction appears to go quantitative, m.p. 183–184°. The infrared spectrum had a strong peak at 1745 cm.$^{-1}$, which is assigned to the carbethoxy group, and the following assignments were made to the n.m.r. spectrum.



protons	chemical shift (τ-units)
a	2.28
b	5.60
c	6.53
d	7.05
e	8.62

Anal. Calcd. for $C_8H_{15}NO_4S$ (221): C, 43.44; H, 6.78; N, 6.33; S, 14.48. Found: C, 43.48; H, 6.89; N, 6.08; S, 14.91.

S-Carbobutoxyethylcysteine (*VIId*). A solution of 4.176 g. (0.024 mole) of cysteine hydrochloride monohydrate was prepared in 15 ml. of water with cooling in an ice bath. The cysteine solution was brought to pH 8.1 with ammonia water. Total volume was 30 ml. To the cysteine solution was added 4.0 ml. (0.0288 mole) of butyl acrylate dropwise over a period of 15 min. with stirring and under 1 atm. of nitrogen. A fluffy white precipitate started settling out of the solution. Stirring was continued for 24 hr. while the amount of the precipitate increased with time. The precipitate was filtered off and recrystallized from 90% aqueous ethanol as fluffy, silky needles. Further crops were obtained by working up the filtrate and the reaction was considered quantitative, m.p. 194–195°. The infrared spectrum showed a strong peak at 1740 cm.$^{-1}$, which was assigned to the ester group, and the following assignments were made to the n.m.r. spectrum.



protons	chemical shift (τ-units)
a	2.27
b	5.70
c	6.57
d	7.12
e	8.40
f	8.97

Anal. Calcd. for $C_{10}H_{19}NO_4S$ (249): C, 48.19; H, 7.63; N, 5.62; S, 12.85. Found: C, 48.60; H, 7.66; N, 5.53; S, 12.84.

Kinetic Measurements. Rate studies for the reaction of the sulfhydryl group were carried out with 0.01 *M* solutions in the appropriate buffer at 30°. Deionized distilled water was used for all determinations. The sample was weighed into the flask and the buffer, which was previously equilibrated at 30°, was added to a volume about 2 ml. less than needed to fill the flask. The material was dissolved with stirring, acrylonitrile was added in the desired concentration, and the flask was made up to volume, shaken vigorously, and placed in a 30° bath. A blank solution that contained everything except the acrylonitrile was run together with the reaction mixture. The disappearance of the sulfhydryl groups was followed by amperometric titration of the remaining sulfhydryl groups with $AgNO_3$ as described by Benesch, *et al.*,[5a] and Rosenberg, *et al.*[5b] Periodically, 1-ml. aliquots were removed and diluted along with 2 ml. of Tris buffer to 50 ml. with water. Five milliliters of this diluted solution, which contained 1 μmole of sulfhydryl at zero time, was titrated with 0.001 *N* $AgNO_3$. The data were plotted as milliliters of $AgNO_3$ *vs.* scale reading, and extrapolation of the straight-line portion to zero scale reading gave μmoles of $AgNO_3$ necessary for the titration of the free sulfhydryl group in the sample. Titration of the blank solution in the same manner gave the zero time value.

Disappearance of amino groups was followed by the Van Slyke procedure as modified by Kendrick and Hanke.[6b] Periodically, 3-ml. aliquots were removed and analyzed for the concentration of the remaining primary amino nitrogen. The accuracy is estimated to be ±5%.

Determination of the pK Values of the Sulfhydryl and Amino Groups. Automatic titrations were carried out by means of a TTTIC titrator with Titrigraph (Radiometer–Copenhagen) standardized with three standards from the National Bureau of Standards[21] at pH 4.01, 6.85, and 9.14. Deionized, distilled water was used for all determinations, and a 1 *N* carbonate-free KOH solution standardized with potassium acid phthalate was used for all titrations. Ten milliliters of a 0.005 *M* solution of the compound to be titrated in 0.3 *M* KCl solution, which was brought to a pH of around 2 with HCl, was placed in a 20-ml., condenser-type vessel. The temperature in the titration vessel was maintained at 30° by means of a circulating water bath. All titrations were carried out in duplicate under nitrogen while the solution was being stirred

(21) R. G. Bates, "Electrometric pH Determinations," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 74.

magnetically. The pK values were determined graphically, and the accuracy is estimated to be ± 0.05 pK unit.

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Stereochemistry of Acetolysis of Alkyl Sulfonates[1]

A. Streitwieser, Jr., T. D. Walsh,[2a] and J. R. Wolfe, Jr.[2b]

Contribution from the Department of Chemistry, University of California, Berkeley, California. *Received April 29, 1965*

Acetolysis o 2-octyl p-toluenesulfonate at 75° for varying lengths of time gives predominantly inverted 2-octyl acetate with varying amounts of racemization. Racemization is shown to come from reaction of 2-octyl acetate with the p-toluenesulfonic acid also formed, from addition of acetic acid to octene formed, and from concurrent racemization of the starting tosylate. The solvolytic displacement reaction itself proceeds with essentially complete inversion of configuration. Similarly, acetolysis of benzyl-α-d tosylate gives about 20% apparent racemization in the product; this apparent racemization probably also results from concurrent racemization of the starting tosylate.

Despite the continued widespread interest in solvolytic displacement reactions, comparatively few studies have been reported of the stereochemistry of simple alkyl systems[3] and even these have often lacked necessary control experiments. If we consider solvolysis in acetic acid of simple systems (not involving neighboring group participation or nonclassical carbonium ions) we find the following stereochemical outcomes: α-phenylethyl chloride, 15% net inversion[5]; α-phenylethyl tosylate, 12% net inversion[6]; α-phenylneopentyl tosylate, 10% net inversion[7]; 1-butyl *p*-nitrobenzenesulfonate, 85% net inversion[8]; and 2-butyl and 2-octyl tosylates, varying degrees of racemization.[9,10] In addition, there are examples of complete inversion in systems whose stereochemistry is followed by *cis–trans* isomerism, *e.g.*, 4-*t*-butylcyclohexyl tosylate[11] and 2-indanyl-1-*d* tosylate.[12]

Since it is known that many acetates racemize by treatment with strong acids in acetic acid,[10] equivalent alkali acetate is often used during acetolysis to neutralize the sulfonic acid liberated; however, in preliminary studies of the stereochemistry of acetolysis of 2-octyl tosylate we found indications of more highly racemized product when lithium acetate was present.[13] Accordingly, we carried out a more extensive study.

Results and Discussion

2-Octyl *p*-toluenesulfonate (2-OcOTs) was prepared in the usual way from optically active 2-octanol (2-OcOH) and tosyl chloride (TsCl) in pyridine. The oily product cannot be purified readily, and this product did contain some chloride, but alcohol was shown to be absent. Three separate preparations gave consistent results in the ratio of rotation of 2-OcOTs to that of 2-OcOH, 0.98 ± 0.01, a value considerably higher than the 0.84 reported by Phillips.[14] The relative rotations of 2-octyl acetate (2-OcOAc) and ethyl ether (2-OcOEt) to that of 2-OcOH, 0.71 ± 0.01 and 1.81 ± 0.01, respectively, were determined by tetraethylammonium acetate displacement and ethanolysis, respectively, of the 2-OcOTs; these results agree with those reported earlier.[15]

Solvolysis of 2-OcOTs was carried out in acetic acid under various conditions, as reported in Table I. The product acetate was distilled material which was examined for purity by g.c. The observed optical activity was corrected for the presence of nonester impurities (*e.g.*, solvent) which were considered to be racemic. The tabulated per cent net inversion is *not* corrected for the presence of several per cent of isomeric octyl acetates, mostly 3- with some 4-, whose total quantities are listed as "per cent rearrangement."

In run 73, the octyl acetate obtained shows 6% apparent racemization. This product contains 1.5% rearranged acetates, but these account for only a portion

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(2) (a) Woodrow Wilson Fellow, 1958–1960; United States Rubber Company Fellow, 1960–1961; (b) Monsanto Chemical Co. Fellow, 1956–1957.

(3) See summaries in A. Streitwieser, Jr., "Solvolytic Displacement Reactions," McGraw-Hill Book Co., Inc., New York, N. Y., 1963; C. A. Bunton, "Nucleophilic Substitution at a Saturated Carbon Atom," Elsevier Publishing Company, New York, N. Y., 1963, Chapter 3; E. R. Thornton, "Solvolysis Mechanisms," Ronald Press Co., New York, N. Y., 1964. See also ref. 4.

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