bicycloheptadienyl structure or the tropylium structure. Direct loss of CO_2H after loss of CO (H being lost from the bridge methylene group) would give the bicycloheptadienyl structure, or a rearrangement involving alkyl group migration after loss of CO would give the tropylium structure.

The adduct of 2,3-dimethylbutadiene with tetracyanoethylene gives important peaks at *m/e* 210 (M), 0.6% Σ_{12}, 82 (C_6H_{10}), 4.0% Σ_{12} (retro-Diels–Alder), and 28, 53.1% Σ_{12}. The peak at *m/e* 28 might be $H—C\equiv NH^+$, or possibly N_2^+ or $C_2H_4^+$.

[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY,[1] PEORIA, ILL.]

Application of a Hammett–Taft Relation to Kinetics of Alkylation of Amino Acid and Peptide Model Compounds with Acrylonitrile[2]

BY MENDEL FRIEDMAN AND JOSEPH S. WALL

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The reaction rates of amino acid and peptide model compounds with acrylonitrile were studied as a function of pH of the reaction medium, pK_2 of the amino group, temperature, and structure of the amino compounds. Logarithms of the second-order rate constants increased with amino acid anion concentration as the pH was increased. At any given pH the rates decreased with increasing pK_2's of the amino groups. Correction of the second-order rate constants to identical amino acid anion concentration gave a series of computed rate constants whose logarithms showed a direct linear dependence on the pK_2's of the amino groups in similar steric environments. A quantitative estimate of the influence of the steric and polar parameters on the rates was obtained from a Hammett–Taft-type free-energy relationship, which was derived from the observed linear variation of the second-order anion rate constants with the pK_2's of the amino groups for three distinct steric series of amino acids and peptides. The derived equation should be considered an extension of the Taft relationship to amino acids and peptides and should be useful for predicting reaction rates in nucleophilic displacements and additions of amino acids, peptides, and proteins. The activation parameters for the cyanoethylation of several compounds were determined and related to polar and structural variations in these compounds.

Hammett, Taft, and collaborators[3-5] demonstrated that the rates of reaction of individual members of a series of related reagents with certain compounds are a function of polar and steric parameters of the reagents. The present investigation of the effect of variables on reactivities of amino groups in a number of amino acids and peptides with α,β-unsaturated compounds, such as acrylonitrile, established that the observed rates are also determined by polar and steric substituent factors which are related to readily measured physical constants and evident structural characteristics of the amino compounds. These observations support a postulated reaction mechanism and allow predictions of reaction rates of new amino acids, peptides, and proteins with acrylate derivatives under various conditions of pH, temperature, and concentration. The results of this study should also be useful in the designing of experiments for selective chemical modification of free functional groups in proteins.[6]

This paper will present kinetic data obtained for the reaction of the amino group in several model compounds with acrylonitrile. The reaction rates were studied as a function of pH of the reaction medium, pK_2 of the amino group, temperature, and structure of the amino compounds.

The observed differences in the activation parameters were related to polar and structural factors.

Results and Discussion

Order of the Reaction.—The rates of reaction were followed by means of a ninhydrin colorimetric procedure, which measures the amount of primary amino compound in the reaction mixture and gives negligible color with alkylated amino compounds, such as N-cyanoethyl-β-alanine.[7] The fraction of starting material left unreacted is given by A_t/A_0, where A_t is the absorbance at 570 mμ at time t and A_0 is the initial absorbance (see Experimental).

When a sufficient excess of vinyl over amino compound was employed, the graph of log A_t/A_0 *vs.* time gave straight lines establishing that the reaction followed pseudo-first-order kinetics. Several examples are shown in Fig. 1. The half-lives ($t_{1/1}$) were read directly from the graph (Fig. 1) and the pseudo-first-order rate constants (k_1) and second-order rate constants (k_2) were calculated by means of the formulas: $k_1 = 0.693/t_{1/1}$ and $k_2 = k_1/\text{concentration of vinyl compound}$. Table I summarizes kinetic data for the reaction of β-alanine with acrylonitrile. The second-order rate constants (listed in the last column) are essentially invariant over a range of concentrations.

TABLE I

RATES OF REACTION OF β-ALANINE AT 30° IN BORATE BUFFER AT pH 8.4 (μ = 1.2)

β-Alanine, mole/l.	Acrylonitrile, mole/l.	$t_{1/2}$, min.	$k_1 \times 10^3$, sec.$^{-1}$	$k_2 \times 10^4$, l./mole/sec.
0.01	0.173	350	3.30	1.90
.01	.346	180	6.40	1.85
.01	.0865	725	1.59	1.84
.02	.300	225	5.13	1.71
			Av. and s.d.	1.82 ± 0.07

Effect of pH on Rates.—The rates of reaction of one representative amino acid from each steric series were determined as a function of pH on the alkaline side and the results are summarized in Table II.

In Fig. 2 the logarithms of the second-order rate con-

(1) This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(2) Presented at the 146th National Meeting of the American Chemical Society, Denver, Colo., Jan., 1964; Abstracts, p. 17C.

(3) L. P. Hammett, "Physical Organic Chemistry," McGraw–Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII.

(4) 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.

(5) H. H. Jaffé, *Chem. Rev.*, **53**, 214 (1953).

(6) L. Weil and T. S. Seibles, *Arch. Biochem. Biophys.*, **95**, 470 (1961), have recently used acrylonitrile as a selective blocking agent for sulfhydryl groups in proteins.

(7) (a) P. F. Butskus, *Izv. Vysshykh Uchebn. Zavedenii, Khim. i Khim. Teknol.*, **3**, 122 (1960); (b) P. F. Butskus, G. I. Denis, and A. I. Butskene, *ibid.*, **3**, 469 (1960).

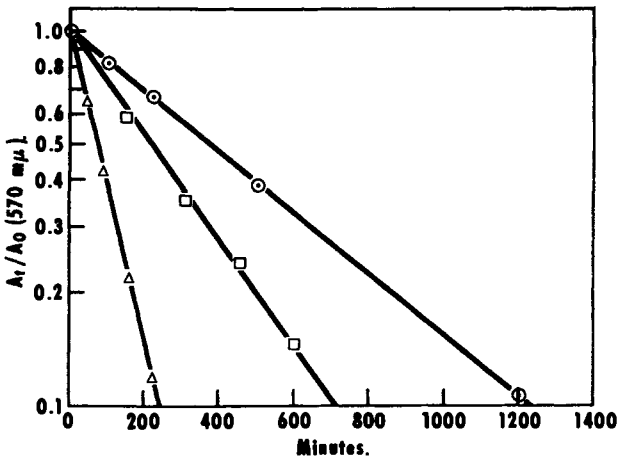


Fig. 1.—Plot of log A_t/A_0 *vs.* time for the reaction of amino acids (0.01 *M*) and acrylonitrile (0.173 *M*) in borate buffer at pH 8.4 and 30°: Δ, glycylglycine; □, DL-methionine; ⊙, DL-α-alanine.

stants are plotted as a function of pH. The variation of the logarithms of the rate constants with pH can be ascribed to the effect of pH on the concentration of the

TABLE II

RATES OF REACTION OF AMINO ACIDS WITH ACRYLONITRILE AS A FUNCTION OF pH IN BORATE BUFFERS (μ 1.2)

	β-Alanine		DL-α-Alanine		DL-α-Phenyl-α-alanine	
pH	$k_2 \times 10^4$	$k_{A^-} \times 10^4$	$k_2 \times 10^4$	$k_{A^-} \times 10^4$	$k_2 \times 10^4$	$k_{A^-} \times 10^4$
8.4	1.91	89.2	2.23	35.3	0.167	1.05
9.4	15.7	87.4	13.4	33.3	0.809	1.19
10.2	41.7	72.2	27.8	34.8		
10.9	62.5	72.0	36.0	37.3	1.11	1.12
11.5	73.0	81.5	37.0	37.2	1.19	1.19
Av. and s.d.		80.5 ± 8.1		35.6 ± 1.7		1.14 ± 0.04

various ionized species of the amino acids and their relative reactivities as nucleophiles. The rates increase rapidly as the pH approaches the pK of the amino group and, with further increase in pH, approach an asymptotic value. Expressions for the change in the reaction rate constant with pH in terms of the concentration of each of the amino acid species may be derived from the ionization constants of the amino acids.

The following equations represent the equilibria of amino acids in solution[8]

$$H_2A^+ \rightleftharpoons (H^+) + (HA);\ K_1 = (H^+)(HA)/(H_2A^+) \quad (1)$$

$$HA \rightleftharpoons HA^{\pm};\ K_D = (HA^{\pm})/(HA) \quad (2)$$

$$HA^{\pm} \rightleftharpoons (H^+) + (A^-);\ K_2 = (H^+)(A^-)/(HA^{\pm}) \quad (3)$$

where H_2A^+ is the amino acid cation; HA, the neutral form; $HA^{\pm}$, the zwitterion; and A^-, the anion.

The following terms are defined as

V_1 = velocity due to the reaction of acrylonitrile with HA

V_2 = velocity due to the reaction of acrylonitrile with A^-

k_2 = observed over-all second-order rate constant

k_{HA} = second-order rate constant associated with species HA

k_{A^-} = second-order anion rate constant associated with species A^-

(B) = concentration of acrylonitrile

(8) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1961, Chapter 4.

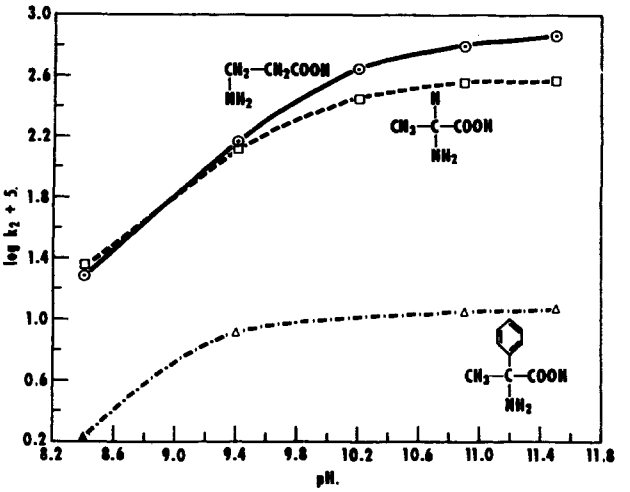


Fig. 2.—Rates of cyanoethylation of amino acids as a function of pH at 30°

Since $H_2A^+ = (H^+)^2(A^-)/K_1K_2^-$, HA = $(H^+)(A^-)/K_2K_D$, and $HA^{\pm} = (H^+)(A^-)/K_2$, the concentration of all unreacted amino acid species $(AA)_T$ equals

$$(AA)_T = (H_2A^+) + (HA) + (HA^{\pm}) + (A^-)$$

$$= [(H^+)^2/K_1K_2 + (H^+)/K_2K_D + (H^+)/K_2 + 1](A^-)$$

$$= \frac{[(H^+)^2K_D + K_1(1 + K_D)(H^+) + K_1K_2K_D]}{K_1K_2K_D}(A^-)$$

Solving for (A^-)

$$(A^-) = \frac{K_1K_2K_D(AA)_T}{K_1K_2K_D + K_1(1 + K_D)(H^+) + K_D(H^+)^2}$$

$$V_1 = k_{HA}(HA)(B);\quad V_2 = k_{A^-}(A^-)(B)$$

$$V_T = V_1 + V_2 = -[k_{HA}(H^+) + k_{A^-}K_2K_D](A^-)(B)/K_2K_D$$

Substituting for (A^-) and equating theoretical and experimental velocities

$$-\frac{[k_{HA}(H^+) + k_{A^-}K_2K_D]K_1(AA)_T(B)}{[K_1K_2K_D + (H^+)K_1(1 + K_D)(H^+)^2]} = k_2(B)(AA)_T$$

$$k_2 = \frac{k_{HA}(H^+)K_1}{[K_1K_2K_D + (H^+)K_1(1 + K_D) + K_D(H^+)^2]} + \frac{k_{A^-}K_1K_2K_D}{K_1K_2K_D + (H^+)K_1(1 + K_D) + K_D(H^+)^2} \quad (4)$$

Equation 4 relates the observed second-order rate constant to the second-order rate constants of the two nucleophiles in solution, the neutral amino acid HA and the amino acid anion A^-, the hydrogen ion concentration of the medium, and the three equilibrium constants of the amino acid.

Equation 4 may be simplified for the amino acids studied by making the following approximations: Since $K_D \sim 10^6$, $1 + K_D \cong K_D$, and since H^+ is small, $(H^+)^2$ may be neglected and $(H^+)/K_D \rightarrow 0$ and the first term drops out resulting in[9]

(9) It should be pointed out that the simplified form of the equation is not applicable to amino acids, such as *p*-aminobenzoic acid, whose K_D is small.[8] At low pH's eq. 4 is simplified to $k_{HA} = k_2K_D[K_1 + (H^+)]/K_1$ and at the isoelectric point $k_i = k_2\frac{(2K_2 + \sqrt{K_1K_2})}{K_2}$ for the amino acids studied.

$$k_{A^-} = k_2\left[1 + \frac{(H^+)}{K_2}\right] \qquad (5)$$

The second-order anion rate constants k_{A^-} shown in Table II were calculated by means of eq. 5 from the observed second-order rate constants, k_2, at the various pH's. The K_2's used for these calculations were taken from Table IV. The results show that the second-order anion rate constants are essentially invariant over the pH range studied demonstrating that eq. 5 agrees with the experimental observations.

It can also be shown that the slope of a plot of log k_2 against pH, $d(\log k_2)/d(pH) = (H^+)/[K_2 + (H^+)]$. The slopes of the curves in Fig. 2 are in accord with this equation.

Since eq. 5 has no k_{HA} term, these observations suggest that the amino acid anion is the species which participates with acrylonitrile in the rate-determining step. This is consistent with the following mechanism for the alkylation of the amino group as illustrated for the case of β-alanine and acrylonitrile

$$\underset{\text{I}}{NH_3^+CH_2CH_2COO^-} + OH^- \rightleftharpoons \underset{\text{II}}{NH_2CH_2CH_2COO^-} + H_2O$$

$$\underset{\text{II}}{^-OOCCH_2CH_2NH_2} + \underset{\text{III}}{CH_2{=}CHCN} \rightleftharpoons {}^-OOCCH_2CH_2\overset{+}{N}H_2CH_2\overset{-}{C}HCN \quad (\text{IV})$$

$$\underset{\text{IV}}{^-OOCCH_2CH_2\overset{+}{N}H_2CH_2\overset{-}{C}HCN} \rightleftharpoons \underset{\text{V}}{^-OOCCH_2CH_2NHCH_2CH_2CN}$$

A hydroxide ion removes a proton from zwitterion I to yield amino acid anion II, which adds in the rate-determining step to acrylonitrile to give intermediate IV, which is transformed to product V *via* a proton shift. Similar mechanisms have been suggested previously for the cyanoethylation reaction.[10]

It should be noted that V still contains an active hydrogen on the nitrogen and may add by a similar mechanism to another molecule of acrylonitrile but under more drastic conditions.[11] To confirm the large drop in the basicities of the amino group on cyanoethylation,[11] the monocyanoethyl derivatives of β-alanine and asparagine were synthesized and their pK_2 values, together with those of the starting materials, were determined at 30° (Table III).

TABLE III

pK_2'S OF AMINO ACIDS AND MONOCYANOETHYL DERIVATIVES AT 30°

Compound	pK_2
β-Alanine	10.06 ± 0.03
N-Cyanoethyl-β-alanine	7.85 ± .03
Asparagine	8.72 ± .03
α-N-Cyanoethylasparagine	6.46 ± .03

These data demonstrate that the primary amino group of the starting material is inherently a much stronger base as well as nucleophile than the secondary amino group of the monocyanoethyl derivative which is, in turn, a stronger base than the tertiary amino group of the dicyanoethyl derivative.[12] The larger steric factor in the monocyanoethylated derivative would cause an additional decrease in its reactivity as compared to the starting material. Thus, very little dicyanoethylation should take place until all the primary amino compound has been transformed to the monocyanoethyl derivative. This conclusion is supported by the experimental observation that in the presence of 1 mole of acrylonitrile only monocyanoethylated product is formed in high yield.[11] Moreover, the change of pK_2's of amino groups upon reaction with acrylonitrile indicates that the electrostatic characteristics of proteins will be significantly altered on cyanoethylation of the free amino groups.

Effect of Polar and Steric Factors on Rates.—The pK_2 of an amino acid is a constant that gives a measure not only of the concentration of the amino acid anion at any given pH, but also of the inherent basicity of the amino group. The dependence of reaction rates on differences in inherent basicities of the amino groups, as indicated by their pK_2's, can be deduced by comparing the rates of reaction of the several amino acids at the same amino acid anion concentrations.[13] The second-order anion rate constants (k_{A^-}) were calculated by means of eq. 5 for three series of experiments and the data are summarized in Table IV.

In the first series of compounds listed (1–6), the amino group is attached to a primary carbon atom of an amino acid or peptide; in the second series (7–13), to a secondary carbon atom; and in the last series (14–18), to a tertiary carbon atom. The amino group has a similar steric environment within each series. Brønsted-type plots of the logarithms of the second-order anion rate constants (k_{A^-}) against the pK_2's of the amino groups at 30° gave straight lines for each series (Fig. 3). This linear relationship indicates that the calculated anion rate constants are a function of the basicities, and thus nucleophilicities,[14] of the amino groups.

An analysis of the rates for the three steric series of amino acids studied suggested that both polar and steric factors affect their reactivities. Treatment of the rate data obtained for the cyanoethylation reaction, in a manner analogous to that described by Taft[4,15] for other reactions, makes it possible to separate polar and

(10) (a) Y. Ogata, M. Okano, Y. Furaya, and I. Tabushi, *J. Am. Chem. Soc.*, **78**, 5426 (1956); (b) B. A. Feit and Z. Zilkha, *J. Org. Chem.*, **28**, 406 (1963).

(11) (a) L. L. McKinney, E. H. Uhing, E. A. Setzkorn, and J. C. Cowan, *J. Am. Chem. Soc.*, **72**, 2599 (1950); (b) *ibid.*, **73**, 1641 (1951).

(12) The observed large decrease in the basicity of the amino group on cyanoethylation contrasts with the effect on the pK_a of the amino group of propylamine upon further propylation. The pK_a's of the amino group in propylamine, dipropylamine, and tripropylamine are 10.67, 11.01, and 10.74, respectively. Thus, the introduction of an additional propyl group into propylamine causes an increase in the basicity of the amino group which is presumably due to an inductive effect resulting in an increased electron density on the nitrogen. The introduction of another propyl group into dipropylamine results in a slight decrease in the pK_a of the amino group, which is probably due to steric effects. It is possible that the bulky substituents on the amino group combined with an inductive electron-withdrawing effect of the cyano group favor the left-hand side of the following equilibrium to a greater extent than for the case of propylamines

$$\begin{matrix} CH_2{-}CH_2{-}COO^- \\ \diagdown \\ N{-}H \\ \diagup \\ CH_2{-}CH_2{-}C{\equiv}N \end{matrix} + H^+ \rightleftharpoons \begin{matrix} CH_2{-}CH_2{-}COO^- \\ \diagdown \\ N^+{-}H \\ \diagup \quad \diagdown \\ CH_2{-}CH_2{-}C{\equiv}N \quad H \end{matrix}$$

For a discussion of the effect of electron-withdrawing groups on the acidities of carboxylic acids see A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1962, Chapter 8.

(13) (a) P. D. Bartlett and R. H. Jones, *J. Am. Chem. Soc.*, **79**, 2153 (1957); (b) P. D. Bartlett and D. C. Dittmer, *ibid.*, **79**, 2159 (1957).

(14) J. O. Edwards and R. C. Pearson, *ibid.*, **84**, 16 (1962).

(15) W. A. Pavelich and R. W. Taft, Jr., *ibid.*, **79**, 4935 (1957).

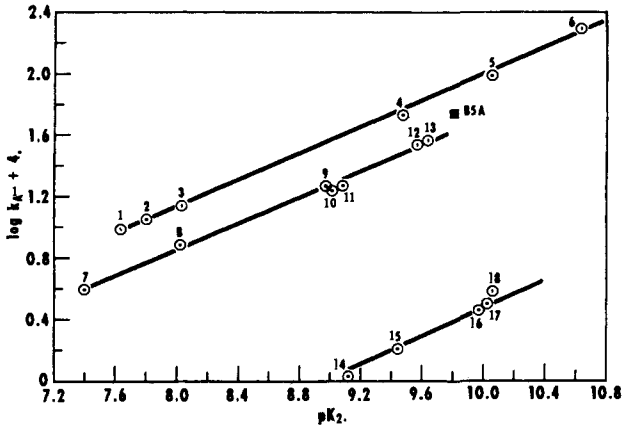


Fig. 3.—Variation of log k_{A^-} with the pK_2's of the amino groups at 30°. The numbers correspond to the compounds listed in Table IV; BSA is for bovine serum albumin.

steric factors which influence rates. From the data in Table IV and Fig. 3, it may be shown that the following relationship holds true for each steric series

$$\log k_{A^-} = \rho(\text{slope}) \times pK_2 + b(\text{intercept}) \quad (6)$$

The slopes of the three structurally different series shown in Fig. 3 reacting with acrylonitrile are nearly identical with an average value of 0.427 ± 0.01. Equation 6 is an extension of the Brønsted catalysis law and may be expressed as a Hammett–Taft-type free energy relationship (eq. 7) which relates the logarithm of the ratio of the second-order anion rate constants of any amino acid or peptide designated as II and glycine (I) to differences in steric and polar factors

$$\log \frac{k_A\text{-(II)}}{k_A\text{-(I)}} = \rho \times [pK_2(II) - pK_2(I)] + [b(II) - b(I)] \quad (7)$$
$$= \rho \times \sigma^A + Es$$

where ρ, the slope, is the parameter that measures the sensitivity of the logarithm of the ratio of the rate constants to polar effect; σ^A is the parameter that represents the polar effects of substituents and is simply the difference in the pK_2 values of the two amino acids; and Es, the difference in intercepts of the lines for the series containing the two amino acids, is the steric substituent constant that depends on the size and steric requirements of the substituents in the reaction. The σ^A value is analogous to the σ proposed by Hammett[3] for aromatic compounds, which he defined as the logarithm of the ratio of the equilibrium constant of a substituted benzoic acid to that of benzoic acid rather than the σ proposed by Taft[4] for the rates of normal alkaline and acid hydrolysis of aliphatic esters. The σ^A is written with the superscript A to denote its origin and applicability to aliphatic amino compounds.

The values of k_{A^-} and pK_2 for glycine given in Table IV were taken as the standard state, and the σ^A and Es values for the other amino acids calculated according to eq. 7 are shown in Table V.

The data in Table V show that the observed rates of reaction decrease with increasing values of Es. The average Es value for the seven compounds in which the amino group is attached to a secondary carbon atom is −0.231 with a standard deviation of 0.038 and for the five compounds in which the amino group is attached to a tertiary carbon atom −1.43 with a standard deviation of 0.081. Minor changes in Es values are caused by variation of the chain length of the substituent attached to the carbon atom that bears the amino group. The introduction of an additional substituent into the same carbon atom causes a large decrease in Es values, the so-called "telescoping effect," which is due to the increased steric strain and steric interference of the bulky groups in the transition state.[4] On the other hand, the formation of a peptide causes a large decrease in the polar substituent constant while the steric factor is decreased only slightly as indicated by the constants for alanine and alanylalanine. Analogous effects are observed on comparison of the polar and steric substituent constants for norleucine and methionine and for α-alanine, phenylalanine, and β-methoxy-α-alanine. The observed changes in polar and steric parameters in proceeding from norleucine to methionine, from α-alanine to phenylalanine, and from α-alanine to β-methoxy-α-alanine are due to the replacement of a methylene group by a sulfur and of a hydrogen by a phenyl and methoxy group, respectively. Figure 3, together with the σ^A, ρ, and Es values listed in Table V, may be used as a basis for predicting relative reactivities for similarly substituted amino acids and peptides in nucleophilic additions and displacements. Examples of such reaction would be the nucleophilic additions of amino acids and peptides to α,β-unsaturated acetylenic compounds and nucleophilic displacement reactions with epoxides and ethylenimines.

An additional element which may cause an increase in steric strain in the activation process during the addi-

TABLE IV

RATES OF REACTION WITH ACRYLONITRILE AS A FUNCTION OF pK_2'S OF THE AMINO GROUPS IN BORATE BUFFER AT pH 8.4 AND 30° (μ = 1.2)

	Amino compound	$k_1 \times 10^5$, sec.$^{-1}$	$k_2 \times 10^4$, l./mole/sec.	pK_2[a]	$k_{A^-} \times 10^4$
1	Tetraglycine	14.4	8.32	7.63	9.81
2	Triglycine	15.4	8.90	7.79	11.0
3	Diglycine	16.5	9.54	8.04	13.7
4	Glycine	6.79	3.92	9.47	50.0
5	β-Alanine	3.30	1.91	10.06	89.2
6	ε-Aminocaproic acid	2.10	1.21	10.62	203 0
7	L-Methionyl-L-methionine	5.78	3.34	7.40	3.67
8	L-α-Alanyl-L-α-alanine	9.35	5.58	8.01	7.85
9	β-Methoxy-α-alanine	6.80	3.93	8.97	18.6
10	DL-Phenylalanine	6.07	3.51	9.00	17.6
11	DL-Methionine	5.25	3.04	9.08	17.6
12	DL-α-Alanine	3.40	2.23	9.57	35.3
13	DL-Norleucine	3.55	2.05	9.63	34.9
14	DL-α-Phenyl-α-alanine	0.289	0.167	9.12	1.05
15	DL-α-Methylmethionine	.222	.128	9.45	1.56
16	DL-α-Amino-α-methyl butyric acid	.123	.0715	9.98	2.79
17	α-Aminoisobutyric acid	.128	.0742	10.02	3.17
18	1-Aminocyclopentane-1-carboxylic acid	.167	.0966	10.06	4.51

[a] All pK_2's were determined at 30°.

TABLE V

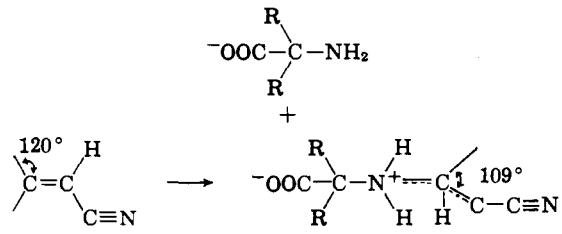
POLAR AND STERIC SUBSTITUENT CONSTANTS FOR THE RATES OF REACTION OF AMINO ACIDS AND PEPTIDES WITH ACRYLONITRILE IN BORATE BUFFER AT pH 8.4 AND 30° (μ = 1.2)

$$\log \frac{k_{A^-}[R(NH_2)COOH]}{k_{A^-}[CH_2(NH_2)COOH]} = \rho \times \sigma + Es$$

No.[a]	R	σ^A	Es	Rel. obsd. rates
4	$-CH_2-$	0	0	100
7	$-C(CH_2CH_2SCH_3)H-CO-NH-C(CH_2CH_2SCH_3)H-$	−2.07	−0.254	83.7
8	$-C(CH_3)H-CO-NH-C(CH_3)H-$	−1.46	− .182	140
9	$CH_3OCH_2-\dot{C}H-$	−0.50	− .219	100
10	$C_6H_5-CH_2-\dot{C}H-$	− .47	− .253	89.5
11	$CH_3-S-CH_2-CH_2-\dot{C}H-$	− .39	− .288	77.5
12	$CH_3-\dot{C}H-$	+ .10	− .200	56.9
13	$CH_3-CH_2-CH_2-\dot{C}H-$	+ .16	− .223	52.3
14	$(C_6H_5)(CH_3)\dot{C}-$	− .35	−1.53	4.26
15	$CH_3SCH_2CH_2-\dot{C}(CH_3)-$	− .02	−1.43	3.26
16	$(CH_3CH_2)(CH_3)\dot{C}-$	+ .51	−1.47	1.82
17	$(CH_3)_2\dot{C}-$	+ .55	−1.43	1.89
18		+ .59	−1.30	2.46

[a] Numbers refer to compounds listed in Table IV.

tion of an amino acid to an α,β-unsaturated compound is the change in hybridization of the sp^2-carbon atoms in the unsaturated compound to sp^3 in the transition state resulting in a decrease of bond angles from 120° in the starting material to around 109° in the activated complex, as



The steric effect on this angle compression will be a function of the size and bulkiness of the R groups in the amino compound. This effect may be of importance in proteins.

Activation Parameters.—The rates of alkylation of several representative compounds from each steric series by acrylonitrile were examined at three different temperatures and the results are shown in Table VI.

The rate studies for the compounds listed in Table VI were carried out at pH's two or more units above the pK_2's. At these high pH's the amino acids are essentially completely in the ionized form. Under these conditions eq. 5 reduces to $k_2 = k_{A^-}$ and the activation parameters calculated[16,17] from the directly determined anion rate constants do not involve terms for the thermodynamics of deprotonation but include only activation terms for the rate-determining step.

(16) J. F. Bunnett in "Technique of Organic Chemistry, Vol. VIII, Part 1, 2nd Ed., A. Weissberger, Ed., Interscience Publishers, Inc., New York, N. Y., 1961, p. 376.

(17) For a discussion of the accuracy of the experimental procedures and calculations used to obtain the activation parameters, see L. L. Schalger and F. A. Long in "Advances in Physical Organic Chemistry," Vol. 1, V. Gold, Ed., Academic Press, Inc., New York, N. Y., 1963, p. 7.

TABLE VI

TEMPERATURE DEPENDENCE OF THE RATES OF CYANOETHYLATION AT pH 12.4[a] (μ = 1.2) AND ACTIVATION PARAMETERS[b] AT 30°

Amino compound	k_{A^-} × 10^4, l./mole/sec. 10°	20°	30°	ΔH^*, kcal./mole	ΔF^* kcal./mole	ΔS^*, e.u.
Glycylglycine	2.72	6.65	14.5	13.6	22.7	−26.5
Glycine	13.3	26.7	53.4	11.8	20.9	−30.0
β-Alanine	16.7	37.1	84.0	13.4	20.6	−23.8
L-α-Alanyl-L-α-alanine	1.67	4.17	8.78	13.8	22.0	−26.9
DL-Methionine	3.93	9.54	20.3	14.1	21.5	−24.1
DL-α-Alanine	6.70	15.9	35.6	14.1	21.1	−23.2
DL-α-Phenyl-α-alanine	0.171	0.417	1.11	15.2	23.2	−26.4
DL-α-Methyl-methionine	.223	0.578	1.51	15.6	23.0	−24.4
α-Aminoisobutyric acid	.495	1.23	3.32	15.6	22.6	−23.0

[a] The rates for glycylglycine and alanylalanine were determined at pH 11.0. [b] The accuracy in the activation parameters should be of the same order of magnitude as given for the rate constants in Table I.

The data in Table VI establish that with increase in structural and steric complexity of the amino compounds the enthalpy of activation of the cyanoethylation reaction increases to a greater extent than the free energy change. The activation entropy thus becomes more positive as molecular size and α-carbon substitution increases but not in a systematic manner definitely relating to the steric series. This trend in the entropies would appear to contrast with the rule of Price and Hammett[4,18] which states that a decrease in the entropy of activation should occur in a reaction series with increasing complexity.

In Table VII the differences between the activation parameters of the several amino compounds and glycine are compared and related to the steric and polar com-

(18) J. D. Gettler and L. P. Hammett, *J. Am. Chem. Soc.*, **65**, 1824 (1943).

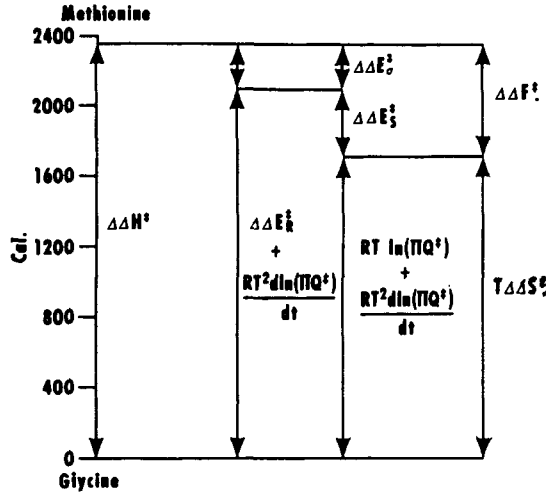


Fig. 4.—Differences in activation parameters between glycine and methionine.

ponents of the free energy of activation relative to glycine. From analysis of Fig. 3 the following relationships[4] are evident

$$\Delta\Delta F^* = -RT \ln k/k_0 = \Delta\Delta E_\sigma^* + \Delta\Delta E_s^* = \Delta\Delta H^* - T\Delta\Delta S^*$$

$$\Delta\Delta E_\sigma^* = -2.3RT(\sigma\rho);\ \Delta\Delta E_s^* = -2.3RT(E_s)$$

where $\Delta\Delta E_\sigma^*$ is the polar activation energy and $\Delta\Delta E_s^*$ the total steric energy of activation. These values relative to glycine are summarized also in Fig. 4 for methionine.

TABLE VII

DIFFERENCES BETWEEN ACTIVATION PARAMETERS FOR CYANOETHYLATION OF VARIOUS AMINO COMPOUNDS AND GLYCINE AT 30°

Amino compound	$\Delta\Delta F^*$, cal.	$\Delta\Delta H^*$, cal.	$T\Delta\Delta S^*$, cal.	$\Delta\Delta E_\sigma^*$, cal.	$\Delta\Delta E_s^*$, cal.	$\Delta\Delta E_R^* + RT^2 \frac{d \ln (\pi Q^*)}{dt}$
Glycylglycine	782	1850	1070	846	−81	1000
Glycine	0	0	0	0	0	0
β-Alanine	−272	1600	1870	−346	74	1950
L-α-Alanyl-L-α-alanine	1090	2020	925	856	129	1160
DL-Methionine	583	2350	1770	230	355	2120
DL-α-Alanine	245	2300	2050	−59	304	2360
DL-α-Phenyl-α-alanine	2310	3400	1080	207	2100	3190
DL-α-Methyl-methionine	2100	3800	1590	12	2090	3790
α-Aminoisobutyric acid	1670	3800	2130	−325	2000	4120

For the general case the potential energy terms of activation are

$$\Delta\Delta H^* = \Delta\Delta E_\sigma^* + \Delta\Delta E_R^* + \Delta\Delta E_\psi^* + RT^2 \frac{d \ln (\pi Q^*)}{dt}$$

in which $\Delta\Delta E_R^*$ is a steric repulsion or strain effect, $\Delta\Delta E_\psi^*$ a resonance effect, and the last term is a kinetic energy factor. For the amino compounds studied the resonance term may be neglected. Also the $RT^2[d \ln (\pi Q^*)]/dt$ term has generally been regarded as negligible when plots of log k_2 *vs.* $1/T$ are apparently linear, as is the case when values obtained from Table VI are treated in this manner. However, when logs of the ratios of rates relative to glycine are plotted against $1/T$, certain of the curves were distinctly nonlinear. Small deviations from linearity are apparently accentuated by this technique. While the kinetic energy term in the enthalpy differences is small, it may be significant.

The enthalpy difference $\Delta\Delta H^*$ illustrated in Fig. 4 consists of

$$\Delta\Delta H^* = \Delta\Delta E_\sigma^* + \Delta\Delta E_s^* + T\Delta\Delta S^*$$

Since the changes in $\Delta\Delta E_\sigma^*$ and $\Delta\Delta E_s^*$ are ascribed to measurable changes in polar and steric effects, respectively, additional changes in $\Delta\Delta H^*$ must result from variations in $T\Delta\Delta S^*$ which consists of terms

$$T\Delta\Delta S^* = RT \ln (\pi Q^*) + RT^2 [d \ln (\pi Q^*)]/dt$$

in which πQ^* is composed of paritition functions involving temperature-dependent kinetic energies of motion. The entropy or total kinetic energy differences relative to glycine vary in a manner that can be related to the molecular structure and possible translational and bending motions of the amino acids. Thus, $T\Delta\Delta S^*$ for β-alanine is appreciably greater than zero, although the compound is in the same steric series as glycine. The additional methylene group in β-alanine undoubtedly accounts for the increase in the entropy difference. In contrast, the peptide derivatives show a lower value of $T\Delta\Delta S^*$ than other members of the steric series to which they belong. This may be due to the partial double bond nature of the peptide link restricting rotation around the –CO–N– bond. The plot of log k/k_0 for glycylglycine and alanylalanine relative to glycine showed the greatest deviation from linearity. This may be due to the kinetic energy term differences. The $T\Delta\Delta S^*$ value for α-phenyl-α-alanine is also lower than the other members of the same steric series despite the higher molecular weight of this molecule. The benzene ring apparently adds rigidity and steric hindrance to molecular motion.

In summary, the increase in $T\Delta\Delta S^*$ values with molecular complexity observed in the present study seems to be the result of an increased kinetic energy of motion of the molecule which is independent of steric hindrance in the cyanoethylation reaction. These observations agree with the data of Fitzpatric and Gettler[19] obtained on studies of oxime formation with various ketones. Their results and the present data emphasize that kinetic energy effects implicitly contained in entropy terms must be considered in evaluating the effect of substituent changes in a reaction series upon entropy. It is noteworthy that the trend in the activation parameters for ionization of amino groups expressed by eq. 3 for the series glycine, α-alanine, and α-aminoisobutyric acid is in the same direction[20] as observed in the present study for the kinetics of their cyanoethylation.

Application to Proteins.—To determine whether the kinetic data described could be applied to the prediction of reaction rates of the free amino groups

(19) F. W. Fitzpatric and J. D. Gettler, *J. Am. Chem. Soc.*, **78**, 530 (1956).
(20) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Academic Press, Inc., New York, N. Y., 1958, Chapter 8.

in proteins, the rate of alkylation of the protein bovine serum albumin (BSA) was measured at pH 9.4 and 30°. Since there are 57 ϵ-amino groups and only 1 α-amino group per BSA molecule, the second-order anion rate constant was calculated by using the intrinsic pK_a value, 9.8 ± 0.1, for the ϵ-amino groups of the lysine side chains given by Tanford and co-workers.[21] The observed rate constant (k_A-) is 5.1×10^{-3} l./mole/sec. and is designated in Fig. 3 as BSA. The predicted value (7.9×10^{-3}) for this constant lies directly above the BSA point on the top line in Fig. 3. The proximity of predicted and determined rate constants indicates that the Hammett–Taft-type relationship, derived for amino acids and peptides, may be used to predict rates for complex molecules. The difference between the predicted and determined rates for the protein is probably due to the uncertainty in the intrinsic pK_a of the ϵ-amino groups of the lysine side chains and to steric and configurational factors.

Future publications will be concerned with the effect of variation in structure of the vinyl compounds on rates and with the relative reactivities of other functional groups present in proteins.

Experimental

Source of Material.—The α-amino-α-methylbutyric acid was a gift of Dr. F. H. Stodola of the Northern Laboratory, and the other amino acids and peptides were the best commercial grades available. Crystallized bovine plasma albumin was obtained from Pentex[22]; acrylonitrile from Matheson. Melting points were taken on a Fischer–Johns melting point apparatus and are not corrected. Infrared spectra were measured in KBr pellets on a Perkin–Elmer Model 21 spectrophotometer.

N-Cyanoethyl-β-alanine.—The procedure was adapted from McKinney, *et al.*[11] To 4.5 g. of β-alanine (Mann, 0.050 mole) dissolved in 50 ml. of a 1 *N* NaOH solution was added dropwise over a period of 20 min. 3.5 ml. of freshly redistilled acrylonitrile (0.052 mole) under an atmosphere of nitrogen. The reaction mixture was stirred for 32 hr. at room temperature and then heated at 50° for 1 hr. The cooled solution was neutralized with HCl and evaporated to dryness. The oily residue was recrystallized from ethanol–water–acetone and then twice from ethanol–water at −10° as fluffy long needles; yield 5.2 g. (73.4%), m.p. 135–137°, lit.[7a] m.p. 137°.

α-N-Cyanoethylasparagine.—To 4.0 g. (0.027 mole) of DL-asparagine (Eastman No. 5694) dissolved in 73 ml. of water containing an equivalent amount of NaOH was added 2 ml. (0.030 mole) of freshly redistilled acylonitrile dropwise under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 90 min. and then was maintained at 57° for 18 hr. The cooled solution was neutralized with acetic acid to pH 4 and evaporated to dryness. The residue recrystallized from water–acetone as fluffy white leaflets; final yield, 73%, m.p. 187–188° dec. The infrared spectrum[23] exhibits the characteristic frequencies: (a) NH_2- stretching frequency of primary amide group and secondary amino group at 3350 and 3160 cm.$^{-1}$; (b) C≡N peak at 2240 cm.$^{-1}$; (c) C=O stretching frequency of the primary amide at 1650 cm.$^{-1}$; (d) double peak due to primary amide group betwen 1375 and 1410 cm.$^{-1}$. The ninhydrin and Van Slyke amino nitrogen tests were negative.

Anal. Calcd. for $C_7H_{11}N_3O_3$: C, 45.4; H, 5.95; N, 22.7. Found: C, 45.7; H, 5.99; N, 22.7.

Kinetic Measurements.—A tightly stoppered flask of amino compound in the appropriate buffer containing the vinyl compound was placed in a constant temperature bath together with a blank solution that contained everything except the amino component. The concentration of all amino compounds used was 0.01 *M*, and of acrylonitrile 0.173 *M*. The concentration 0.01 *M* in reaction sites, of BSA, was based on a molecular weight of 66,000 and 95 reactive sites.[24] Any variations from these concentrations are shown in Table I. Periodically, 1-ml. aliquots were removed from the reaction mixture and blank solutions and diluted to 50 ml. with water which was equilibrated at the appropriate temperature to give solutions A and B, respectively. A solution of the amino compound of the same dilution was also prepared, solution C. The ninhydrin color reaction, according to the procedure of Stein and Moore,[25] was carried out in triplicate with three 1-ml. aliquots of solutions A, B, and C, and water, solution W. The tubes were heated for 15 min. at 100°. The absorbances were measured with a Beckman B spectrophotometer at 570 mμ. The fraction of amino component remaining unreacted was calculated from the formula

$$\frac{A_t}{A_0} = \frac{A_A - A_B}{A_C - A_W} = \text{fraction of primary amino group left unreacted in soln. A}$$

where A_A, A_B, A_C, and A_W are the absorbances of the solution A, B, C, and W, and where A_t and A_0 are the corrected absorbances at time t and zero, respectively.

A modified ninhydrin color reaction was used with the crystallized BSA,[26] except that the reaction was carried out for 30 min. and absorbances were read at 580 mμ. The accuracy of results is estimated to be ±3%.

Each kinetic experiment was left running for about 4 half-lives, and the pH's of the reaction mixture were measured with a Radiometer pH meter, Model TTT1C, at the beginning and at the end of the reaction. No significant change was observed.

Determination of pK_2's of the Amino Groups.—The automatic titrations were carried out by means of TTT1C Titrator with Titrigraph (Radiometer-Copenhagen) standardized at 30° with two NBS pH standards at pH 4.01 and 6.85.[27] Deionized distilled water was used for all determinations and a 0.758 *N* carbonate-free KOH solution was used for all titrations. A 0.005 *M* solution (10 ml.) of the amino acid in 0.15 *M* KCl solution, which was brought to a pH of around 2 with HCl, was placed into 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 by means of a magnetic stirrer. The pK_2 values were determined graphically, and the accuracy is estimated to be ±0.03 pK_2 unit.

Acknowledgment.—It is a pleasure to acknowledge the assistance of Mr. A. C. Beckwith with the mathematical derivations.

(21) C. Tanford, S. A. Swanson, and W. S. Shore, *J. Am. Chem. Soc.*, **77**, 6414 (1955).

(22) The mention of trade names or products does not constitute endorsement by the Department of Agriculture over those not named.

(23) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, p. 205.

(24) L. E. McClure, L. Schieler, and M. S. Dunn, *J. Am. Chem. Soc.*, **75**, 1980 (1953).

(25) W. H. Stein and S. Moore, *J. Biol. Chem.*, **211**, 907 (1954).

(26) L. H. Krull, A. C. Beckwith, J. S. Wall, and R. J. Dimler, Abstracts, 140th National Meeting of the American Chemical Society, Chicago, Ill., Sept., 1961, p. 32C.

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