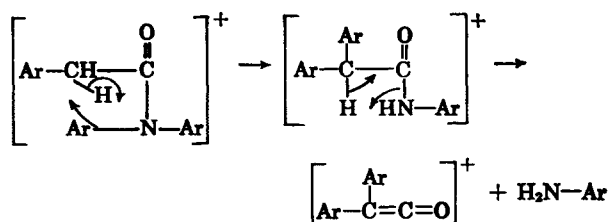


terated amide, then the deuterium isotope effect must have slowed down the aryl migration to a significant extent. Deuterium isotope effect in hydrogen rearrangement processes⁷ and in reducing the rate of reactions is well known.

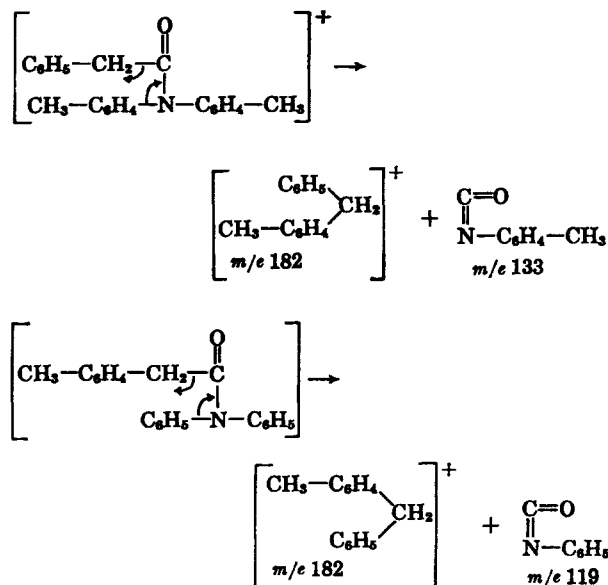
Significant peaks were observed at m/e 93 ($C_6H_5-NH_2$) and at m/e 107 ($CH_3-C_6H_4-NH_2$) in the mass spectra of *N,N*-diphenylphenylacetamide (Ia) and *N,N*-ditolylphenylacetamide (Ic), respectively. These fragments can only be formed by a double proton transfer from the methylene group to the nitrogen atom in two stages as shown below. Double proton transfers have been postulated before by other workers.⁸ We propose the following mechanism for aryl migration and double proton transfer.



Another line of evidence for aryl migration is the identification of significant peaks at m/e 182 and 181 in the mass spectra of *N,N*-ditolylphenylacetamide (Ic) and *N,N*-diphenyl-*p*-tolylacetamide (Ib). These fragments must have been formed by the migration of *p*-tolyl and phenyl groups, respectively, from the nitrogen to carbon. No peaks corresponding to the fragments formed by the migration of an aryl group from

(7) D. H. Williams, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 284 (1964).

(8) See C. Beard, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 269 (1964), and F. W. McLafferty and M. C. Hamming, *Chem. Ind. (London)*, 1366 (1958).



the carbon to nitrogen were observed in the mass spectra of the amides that were examined.

Experimental⁹

All the amides studied were prepared from the corresponding acid chlorides and amines in dry benzene solution. Their authenticity was established by comparison with the physical data cited in literature. The deuterated analogs of these amides were prepared by repeated equilibration of the amides in a 1:1 mixture of deuterated methanol and deuterated water with a trace of sodium methoxide. *N*-Deuterated diphenylacetanilide was prepared by first deuterating aniline and then treating it with diphenylacetyl chloride.

Acknowledgment.—This work was supported by a grant (MH-03930) from the U. S. Public Health Service.

(9) All mass spectra were obtained with a Consolidated Electrodynamic Corporation mass spectrometer Model No. 21-103C using an all-glass inlet system heated to temperatures well below the melting points of the samples. The ionizing energy was kept at 70 e.v. and the ionizing current at 50 μ a.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA 4, PENNSYLVANIA]

Mass Spectral Mechanisms. Homoallylic Participation in Fragmentation of Butadiene-Maleic Anhydride Adduct¹

BY STEPHEN J. WEININGER,² VU THI MAI, AND EDWARD R. THORNTON

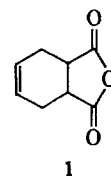
RECEIVED MARCH 14, 1964

The mechanistic path of the mass spectral fragmentation of 1,2,3,6-tetrahydrophthalic anhydride has been investigated by comparison with several model compounds, including the 3,3,6,6-tetradeuterated derivative. The fragmentation appears to derive some driving force from loss of CO from the molecular ion leading to a homoallylic carbonium ion.

The behavior of ions formed by electron bombardment promises to provide information about reactions of ions in the gas phase, for comparison with reactions of ions in solution. Also, structures which have not been identified in solution may easily be formed and studied under the high energy conditions of the mass spectrometer.

We have observed an unexpected fragmentation pattern in the mass spectrum of the Diels-Alder adduct, 1, of butadiene with maleic anhydride. This compound exhibits *no* molecular ion peak (*M*) and

exhibits intense peaks at $M - 28$ ($M - CO$) and $M -$



73 ($M - C_2O_3H$)³, using 76-v. electrons.

(3) Since it is confusing to list only masses, we include in parentheses probable assignments of the fragments. In many cases only one assignment is possible, but assignments are checked by isotopic natural abundance peak intensities wherever possible. In principle, all assignments could be made with certainty by using a double-focusing mass spectrometer.

(1) Supported in part by a PHS research grant, GM 10693, from the National Institutes of Health, Public Health Service.

(2) Socony-Mobil Fellow, 1962-1964.

TABLE I
 IMPORTANT IONS IN THE MASS SPECTRA OF CYCLIC ANHYDRIDES

Assignment	1		3		4		5		6		7	
	<i>m/e</i>	% Σ_{12}^a	<i>m/e</i>	% Σ_{12}	<i>m/e</i>	% Σ_{12}	<i>m/e</i>	% Σ_{12}	<i>m/e</i>	% Σ_{12}	<i>m/e</i>	% Σ_{12}
M	152	0.0	100	0.0	154	0.0	156	0.0	180	1.7	164	0.4
M - CO	124	5.8	72	0.0	126	.0	128	8.4	152	2.9	136	0.1
M - CO ₂	108	0.0	56	9.5	110	.0	112	0.0	136	0.0	120	1.0
M - C ₂ O ₂	80	10.0	28	52.9	82	14.0	84	13.3	108	2.6	92	3.7
M - C ₂ O ₂ H	79	25.1	27	8.8	81	1.3	83	11.6	107	16.3	91	7.8
M - C ₂ O ₂ D	82	16.9
Retro-Diels-Alder diene	54	0.9	58	0.8	82	0.2	66	35.8

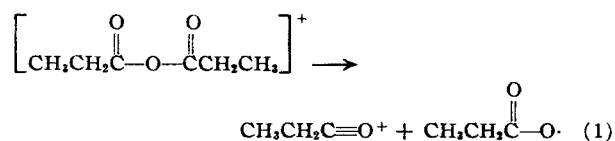
^a The designation $x\% \Sigma_{12}$ means that the peak comprises $x\%$ of all the ions having m/e 12 or more which are found in the mass spectrum of the compound, i.e., $x\%$ of the sum of the heights of all peaks with m/e 12 or higher.

Several other processes would be expected to be preferred. Retro-Diels-Alder reactions seem to occur easily in many cases,⁴ reflecting the favorability of forming new bonds to replace those broken in fragmentation. Usually the positive charge ends up in the π -system of the conjugated diene. A very small peak at m/e 54 (C₄H₆) but no peak at m/e 98 (C₄H₂O₃) occurs in the mass spectrum of 1. Even in the case of bicycloheptadiene, 2, which gives rise to the very

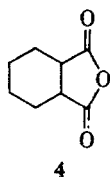
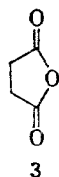


stable tropylium ion, C₇H₇⁺, the retro-Diels-Alder mechanism competes successfully⁵ to give nearly half as much C₅H₆⁺ as C₇H₇⁺. We have observed that the retro-Diels-Alder reaction is an important mode of fragmentation of the adduct of 2,3-dimethylbutadiene with tetracyanoethylene, one of the most powerful dienophiles known.

It appears that the anhydride group determines the course of fragmentation of 1, but other anhydrides behave quite differently. Propionic anhydride molecular ion decomposes thus



the acylium ion being the major peak in the mass spectrum.⁶ Phthalic anhydride produces important peaks at M - 44 (M - CO₂) and M - 72 (M - C₂O₃) and no M - 28 (M - CO),⁷ as does succinic anhydride, 3. Our observations on 3 led us to conclude that both the anhydride and the cyclohex-4-enyl moieties are



(4) K. Biemann, "Mass Spectrometry, Organic Chemical Applications," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 102-107; see, however, H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1964, p. 155, and C. Djerassi, J. M. Wilson, H. Budzikiewicz, and J. W. Chamberlin, *J. Am. Chem. Soc.*, **84**, 4544 (1962).

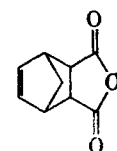
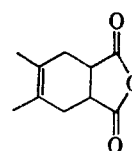
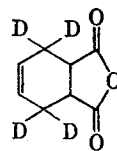
(5) S. Meyerson, J. D. McCollum, and P. N. Rylander, *ibid.*, **83**, 1401 (1961).

(6) American Petroleum Institute, Research Project 44, "Catalog of Mass Spectral Data," No. 1152.

(7) *API, ibid.*, No. 85.

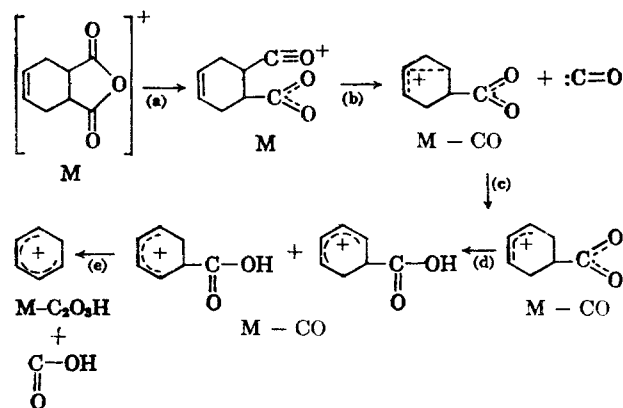
necessary to allow the unexpected loss of CO from 1. Confirmation of this conclusion was provided by showing that the saturated analog of 1, *cis*-4, behaves similarly to 3, producing a peak at M - 72 (M - C₂O₃) which is *ca.* 11 times more intense than the peak at M - 73 (M - C₂O₃H), and no peak at M - 28 (M - CO).

We therefore studied three other Diels-Alder adducts containing the anhydride group along with the double bond



The intensities of the peaks most pertinent to our discussion are in Table I. Some discussion of other peaks is in the Experimental section.

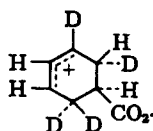
We believe that these results lead to, and are consistent with, a reasonable picture of the mechanism of decomposition of 1 and its derivatives as shown here.



Step a involves cleavage of the anhydride group, analogous to eq. 1, but in this cyclic case does not cleave the molecule. Loss of the stable CO molecule occurs in step b, leading to a cation stabilized by homoallylic overlap, which provides a driving force for loss of CO instead of loss of CO₂ as in the case of 4.⁸ It would be attractive to assume that steps b and c were concerted, but the experiment with the tetradeuterio derivative 5 would then be difficult to explain, since both C₂O₃H and C₂O₃D are lost, and loss of C₂O₃D is favored by about 1.5:1. Stereospecific migration of deuterium

(8) Initial, rather than competitive, loss of CO is supported by our observation that with 1 the M - 28 (M - CO) peak is last to disappear at low ionizing voltages.

trans to the $\text{—C}\equiv\text{O}^+$ group simultaneously breaking away as CO would lead to *cis*-H on one side of the —CO_2 group and *cis*-D on the other



Preferential removal of D would then seem impossible because the *cis*-H and -D would be the only atoms close enough to be removed by —CO_2 , and there would be a 1:1 statistical chance of removal of H or D, with loss of H further favored by a normal kinetic isotope effect. Separating steps b and c provides a ready explanation: the homoallylic ion formed in step b could lose its stereochemistry so that D-migration could occur either *cis* or *trans* to the —CO_2 group. The resulting 3:1 probability of D being *cis* would then explain the experimental 1.5:1 factor favoring loss of $\text{C}_2\text{O}_3\text{D}$ if a reasonable isotope effect of *ca.* 2 favoring H were superimposed. An allylic cation is formed in step c, followed by hydrogen atom migration in step d to give a yet more stable butadienyl radical-cation type structure. Loss of $\cdot\text{CO}_2\text{H}$ as in step e would then give a stable cyclic homolog of a pentadienyl cation ("protonated benzene").⁹ The possibility that CO_2 and H \cdot are lost separately without occurrence of step d seems unlikely because of less favorable energetics than for loss of $\cdot\text{CO}_2\text{H}$ and the fact that if CO_2 were lost, then loss of H_2 molecule should be favorable, producing a benzene cation, and only a small peak corresponding to benzene is observed. The existence of a molecular ion peak with 6, and the increased intensity of $M - 28$ ($M - \text{CO}$) relative to $M - 73$ ($M - \text{C}_2\text{O}_3\text{H}$), are reasonably explained by the cation-stabilizing influence of the methyl groups. The nonconcerted nature of steps b and c is supported by the presence of an $M - 28$ ($M - \text{CO}$) peak in the mass spectrum of 7, which could undergo step b but should not undergo concerted loss of CO and H migration because the product would be a bridgehead cation.

Whether excited electronic states are important in mass spectral fragmentations is not known.¹⁰ Since explanations based on consideration of only electronic ground states are so successful,^{4,10} we have considered only ground state cations in our mechanistic scheme.

The homoallylic participation which we believe is a big factor in determining the fragmentation of 1 and related structures appears to be a rather substantial effect. For reactions in solution the strength of homoallylic participation should be weakened by solvation of the carbonium ion. However, it seems possible that homoallylic participation could be strong in solution under certain conditions; in particular, steric hindrance to solvation of a carbonium ion (as in bicyclic structures).

Experimental

All melting points have been corrected.

***cis*-1,2,3,6-Tetrahydrophthalic Anhydride.**—A benzene solution of maleic anhydride was placed in a flask having a gas inlet side-arm and Dry Ice condenser. Butadiene was admitted through

(9) Loss of $\cdot\text{CO}_2\text{H}$ from acids or $\cdot\text{CO}_2\text{R}$ from esters is known to be a favorable process if a stabilized cation can be formed as the other fragment; for example, α -amino acids and esters show this tendency (K. Biemann and J. A. McCloskey, *J. Am. Chem. Soc.*, **84**, 3192 (1962)).

(10) F. W. McLafferty, Ed., "Mass Spectrometry of Organic Ions," Academic Press, Inc., New York, N. Y., 1963.

the side-arm from a gas cylinder until a sudden precipitate of product appeared. The solid was removed by filtration, washed, dried, and then recrystallized from ligroin (75–95°), m.p. 101.5–102.3° (lit.¹¹ m.p. 103–104°).

***cis*-3,3,3,6-Tetradeuterio-1,2,3,6-tetrahydrophthalic Anhydride.** Butadiene-1,1,4,4- d_4 was prepared by repeated exchange of commercial 2,5-dihydrothiophene 1,1-dioxide with D_2O -dioxane containing potassium carbonate,¹² followed by pyrolysis of the dioxide. The purified butadiene- d_4 was distilled into a tube containing benzene and maleic anhydride, and the tube was sealed and allowed to stand 1 week. The product was recrystallized from ligroin; m.p. 100.4–101.0°. The n.m.r. spectrum of this compound had a barely visible peak at 2.46 δ where the undeuterated compound had absorption corresponding to four protons. Electronic integration indicated the compound was 96.6% d_4 .

***cis*-4,5-Dimethyl-1,2,3,6-tetrahydrophthalic Anhydride.**—Equimolar quantities of 2,3-dimethylbutadiene and maleic anhydride were allowed to react in benzene. The white precipitate was recrystallized from 1:1 benzene-petroleum ether; m.p. 78–79° (lit.¹³ m.p. 78–79°).

***endo*-Bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic Anhydride.**—Equimolar quantities of freshly distilled cyclopentadiene and maleic anhydride were allowed to react in benzene. The product had m.p. 165.5–167.3° dec. (lit.¹⁴ 161–162°).

***cis*-1,2-Dimethyl-4,4,5,5-tetracyanocyclohexene.**—Tetracyanoethylene was allowed to react with 2,3-dimethylbutadiene in tetrahydrofuran. The product on recrystallization from 1:1 benzene-petroleum ether gave a white crystalline solid, m.p. 140–141° (lit.¹⁵ 136–137°).

Others.—Commercial hexahydrophthalic anhydride, m.p. 35–36° (lit.¹⁶ m.p. 32°), was purified by vacuum sublimation. Commercial succinic anhydride was recrystallized from ether; m.p. 119–120° (lit.¹⁷ m.p. 119–120°).

Mass Spectra.—Mass spectra were taken with a CEC Model 21–130 mass spectrometer (cycloidal focusing). Normally the bombarding electrons were nominally 76 v., at a current of 20 $\mu\text{a.}$, and with an inlet temperature between 100 and 125°.

Further Discussion.—Although some of the compounds do undergo a retro-Diels-Alder type fragmentation of the molecular ion (M) as indicated in Table I by the formation of diene⁺, it is minor except in the case of the cyclopentadiene-maleic anhydride adduct 7. In all cases except 7 there is no peak at m/e 98 corresponding to the positive ion of maleic anhydride; in the spectrum of 7 m/e 98 has an intensity 0.1% Σ_{12} .

The butadiene-maleic anhydride adduct 1 has no major peaks other than those in Table I and peaks at m/e 27, 6.9% Σ_{12} , and m/e 39, 7.2% Σ_{12} . These peaks are probably the quite common¹⁸ C_2H_3^+ and C_2H_5^+ , respectively. At lower voltages the $M - 73$ ($M - \text{C}_2\text{O}_3\text{H}$) peak was the next to last to disappear, and the $M - 28$ ($M - \text{CO}$) was the last to disappear.

Succinic anhydride (3) exhibits no particularly interesting peaks other than those in Table I.

Hexahydrophthalic anhydride (4) exhibits several peaks of significant intensity, at m/e 82, 81, 67, 54, 41, 39, 28, and 27. The mass spectrum¹⁹ of cyclohexene has major peaks at just these m/e values and with nearly the same relative intensities. We therefore conclude that the anhydride loses C_2O_3 , forming the cyclohexene molecular ion $\text{C}_6\text{H}_{10}^+$, and that the smaller fragments are largely formed from $\text{C}_6\text{H}_{10}^+$ by a retro-Diels-Alder and other processes.

The tetradeuterio compound 5 exhibits a peak at m/e 29, 6.9% Σ_{12} , which is probably C_2HD_2^+ , corresponding to C_2H_3^+ in the spectrum of 1.

Compound 6, the adduct of 2,3-dimethylbutadiene with maleic anhydride, exhibits no peaks worthy of comment other than those in Table I.

The adduct of cyclopentadiene with maleic anhydride, 7, exhibits a peak at m/e 91 (C_7H_7), 7.8% Σ_{12} , which may have the

(11) A. C. Cope and E. C. Herrick, *Org. Syn.*, **30**, 93 (1950).

(12) A. C. Cope, G. A. Berchtold, and D. L. Ross, *J. Am. Chem. Soc.*, **83**, 3859 (1961).

(13) W. J. Bailey, J. Rosenberg, and L. J. Young, *ibid.*, **76**, 2251 (1954).

(14) H. Kwart and L. Kaplan, *ibid.*, **75**, 3356 (1953).

(15) W. J. Middleton, R. E. Heckert, and E. L. Little, *ibid.*, **80**, 2783 (1958).

(16) O. Baeyer, *Ann.*, **258**, 145 (1890).

(17) R. L. Shriner and H. C. Struck, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 560.

(18) F. W. McLafferty, "Mass Spectral Correlations," Advances in Chemistry Series No. 40, American Chemical Society, Washington, D. C., 1963.

(19) Reference 6, No. 209.

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 $\text{H}-\text{C}\equiv\text{NH}^+$, 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 Acrylonitrile²

BY MENDEL FRIEDMAN AND JOSEPH S. WALL

RECEIVED JANUARY 17, 1964

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 collaborators³⁻⁵ 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

(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.

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 $m\mu$ 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 ($t_{1/2}$) 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: $k_1 = 0.693/t_{1/2}$, 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 ($\mu = 1.2$)

β -Alanine, mole/l.	Acrylonitrile, mole/l.	$t_{1/2}$, min.	$k_1 \times 10^4$, 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 \pm 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-

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