The Kinetics of Alkaline Hydrolysis of N^1-Methylnicotinamide Cation

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Abstract: The kinetics of the base-catalyzed hydrolysis of N^1-methylnicotinamide cation were studied over a wide range of hydroxide ion concentrations and at several different temperatures. The apparent first-order rate constant which characterized the disappearance of substrate exhibited a complex dependence on hydroxide ion concentration. A reaction mechanism which incorporated specific base catalysis, superimposed general base catalysis by hydroxide ion, and ionization of an amide hydrogen is proposed to explain the observed behavior. The influence of temperature on the parameters of the derived rate law indicated the existence, in the reaction pathway, of a stabilized intermediate which required the addition of energy to form either reactants or products. The possibility of an internal electrostatic interaction which contributed to the stability of the intermediate is proposed.

A limited number of previous studies[3,4] have been concerned with the hydrolytic behavior of the N^1-methylnicotinamide cation (NMN). Burton and Kaplan[3] reported the appearance of a new absorption band with a peak absorbance at 282 mμ in the difference spectrum of NMN in alkaline *vs.* water solution. The authors attributed the new band to the formation of a charge-transfer complex between NMN and hydroxide ion, and its disappearance to hydrolysis of the amide in the complex. Martin and Hull[4] also studied this behavior and suggested that the new band was due to ionization of an amide hydrogen. They determined rate constants which characterized the disappearance of the new band and showed that the order with respect to hydroxide ion, for the disappearance, decreased toward zero as hydroxide ion activity was increased. On this basis they suggested that deprotonated NMN did not undergo hydrolysis but that the band disappeared as a consequence of hydrolysis of the NMN in equilibrium with deprotonated NMN.

The present investigation was designed to characterize the kinetics of the base-catalyzed hydrolysis of NMN in greater detail than had been done previously in order to gain an insight into the mechanism of the hydrolytic reaction. It was felt that such a study would be of interest in view of the fact that inferences have often been drawn about pyridine coenzyme activity from studies involving N^1-substituted nicotinamides.[3–7] As will be seen, the kinetics of alkaline hydrolysis of NMN were considerably more complex than had been realized and were very similar to those of "acyl-activated" amides such as 2,2,2-trifluoroacetanilide[8,9] and its N-methyl derivative.[8,10–13]

It is generally recognized that the mechanisms by which amides undergo alkaline hydrolysis involve the formation of a tetrahedral intermediate by nucleophilic attack of hydroxide ion upon the amide carbonyl. This was shown by Bender and Ginger[14] who demonstrated that the alkaline hydrolysis of benzamide which contained ^{18}O in the amide carbonyl proceeded concurrently with the exchange of ^{18}O between solvent and the substrate carbonyl. This proved that a tetrahedral intermediate existed for a period of time sufficient to allow proton exchange between the two oxygen atoms of the intermediate. The intermediate most often decomposed to form reactants rather than products, expelling the weaker base, hydroxide ion, rather than the stronger base, amido anion. Biechler and Taft[8] showed that the rate equations for the hydrolysis of acyl-activated acetanilides contained terms which were second-order in respect to hydroxide ion and suggested that hydrolysis proceeded through the conjugate base of the intermediate. Schowen and his coworkers[10–13] also investigated the kinetics of hydrolysis of an activated acetanilide and demonstrated the involvement of general base catalysis superimposed upon specific base catalysis not only for hydroxide ion but for general bases such as glycine. The consequences of ionization of an amide hydrogen of acyl-activated acetanilides have been considered by Biechler and Taft[8] and by Mader.[9] Biechler and Taft considered the anion formed by ionization of an amide hydrogen to be unreactive toward hydrolysis but Mader suggested that the deprotonated amide reacted with water to form the tetrahedral intermediate. The reaction of this amido anion with hydroxide ion to form products was also suggested by Mader.

Experimental Section

Materials. The chloride salts of N^1-methylnicotinamide, mp 241° (uncorrected), and N^1-methylnicotinic acid, mp 244–245° (uncorrected), were prepared by established methods.[15,16] Other chemicals used were of reagent grade.

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[3] R. M. Burton and N. O. Kaplan, *Arch. Biochem. Biophys.*, **101**, 139 (1961).

[4] R. B. Martin and J. G. Hull, *J. Biol. Chem.*, **239**, 1237 (1964).

[5] G. Cilento and D. L. Sanioto, *Arch. Biochem. Biophys.*, **110**, 133 (1965).

[6] S. Shifrin, *Biochim. Biophys. Acta*, **81**, 205 (1964).

[7] S. Shifrin, *Biochemistry*, **3**, 829 (1964).

[8] S. S. Biechler and R. W. Taft, Jr., *J. Amer. Chem. Soc.*, **79**, 4927 (1957).

[9] P. M. Mader, *ibid.*, 87, 3191 (1965).

[10] R. L. Schowen, H. Jayaraman, and L. Kershner, *ibid.*, **88**, 3373 (1966).

[11] R. L. Schowen and G. W. Zuorick, *ibid.*, 88, 1223 (1966).

[12] R. L. Schowen and G. W. Zuorick, *Tetrahedron Lett.*, 3839 (1965).

[13] R. L. Schowen, H. Jayaraman, and L. Kershner, *ibid.*, 497 (1966).

[14] M. L. Bender and R. D. Ginger, *J. Amer. Chem. Soc.*, 77, 58 (1955).

[15] J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.*, **150**, 395 (1943).

[16] W. Ciusa and G. Nebbia, *Gazz. Chim. Ital.*, **80**, 98 (1950).

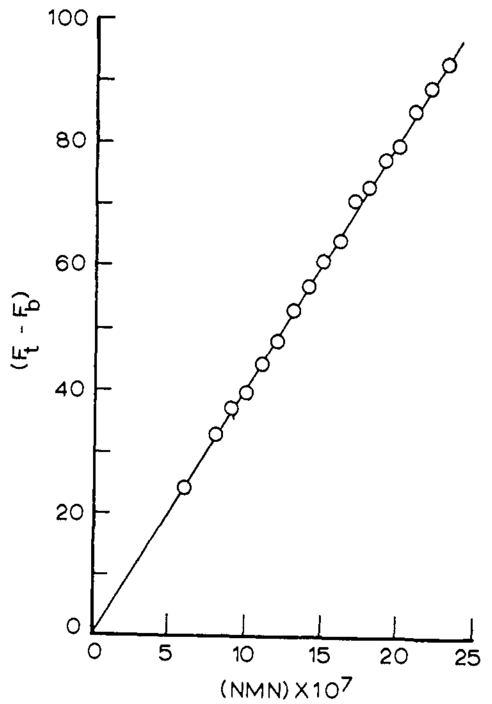

Figure 1. A plot illustrating the linearity of $(F_t - F_b)$ with concentration of NMN. See text.

Hydrolysis Experiments. Aliquots of solutions of sodium hydroxide of accurately known titer were placed in 100-ml volumetric flasks containing appropriate amounts of sodium acetate, or sodium perchlorate, or both. The flasks were brought to the desired temperature in an oil bath. An aliquot of a stock solution of NMN was added to yield a concentration in the reaction system of about 2.5×10^{-6} *M*. The total concentration of NMN was always negligibly small compared to the concentration of sodium hydroxide. Samples (3 ml) were withdrawn from the reaction solutions at various time intervals and placed in 50-ml volumetric flasks containing sufficient acid to neutralize the sodium hydroxide. The residual concentration of NMN in the samples was determined by a fluorometric assay. Spectral examination of reaction solutions showed that only NMN or N^1-methylnicotinic acid was present.

Assay. A fluorometric assay based on published procedures[17,18] was used to determine residual NMN. Samples (3 ml) were placed in 50-ml volumetric flasks and 5 ml of redistilled acetone containing 2×10^{-4} *M* manganous chloride was added to each flask. Sodium hydroxide (1.5 *M*, 2 ml) was added and the flasks were allowed to stand for 30 min. Hydrochloric acid (4 *N*, 2 ml) was then added and the flasks were allowed to stand for 30 min. Finally, each flask was made to volume with 2.5% monobasic potassium phosphate. Blanks containing no NMN were prepared in the same manner. The fluorescence of each solution was determined with a Turner Model 110 fluorometer using a 365-mμ excitation filter and a 436-mμ emission filter. The instrument was adjusted to zero with distilled water. The difference between the fluorescence of a sample (F_t) and that of the blank (F_b) for NMN solutions of known concentrations was found to be linear with concentration. This is illustrated in Figure 1. Addition of N^1-methylnicotinic acid to solutions of NMN before assay had no effect on the fluorescence readings. Since $(F_t - F_b)$ was linear with concentration, the logarithm of this term was plotted against time to determine the apparent first-order rate constants for the hydrolysis of NMN. Representative kinetic plots are shown in Figure 2.

Results and Discussion

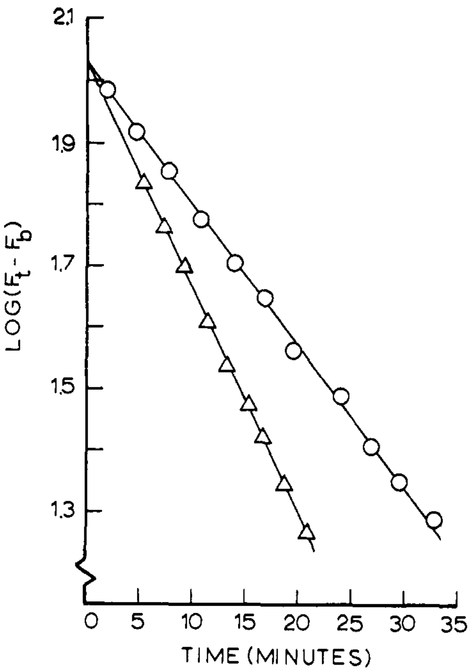

Figure 2. Example kinetic plots showing log $(F_t - F_b)$ plotted against time for the hydrolysis of NMN in 0.0991 *M* sodium hydroxide (△) and 0.0694 *M* sodium hydroxide (○). The solutions were adjusted to an ionic strength of 1.00 with sodium perchlorate and maintained at 40°. The initial concentration of NMN was 2.64×10^{-6} *M*.

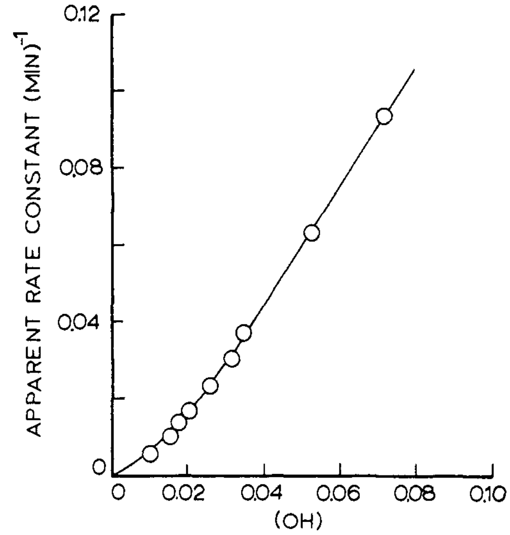

Figure 3. Apparent first-order rate constants at 40° for hydrolysis of NMN in dilute solutions of sodium hydroxide which were adjusted to an ionic strength of 1.00 with sodium acetate. The circles represent experimental points and the solid line was calculated using eq 14. Abscissa values refer to the normality of hydroxide ion.

Apparent first-order rate constants for the hydrolysis of NMN at 40° in alkaline solutions adjusted to an ionic strength of 1.00 with sodium acetate are presented in Table I and Figures 3 and 4. It is clear that the hydrolytic reaction exhibited a complex dependence on hydroxide ion concentration. The order of the reaction with respect to hydroxide ion changed continuously throughout the range of concentrations utilized, and varied from one at low concentrations, to greater than one at moderate concentrations, to less than one at high concentrations. This behavior can reasonably be explained by a mechanism which involves specific

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[17] J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.*, **167**, 157 (1947).

[18] V. M. Vivian, M. S. Reynolds, and J. M. Price, *Anal. Biochem.*, **10**, 274 (1965).

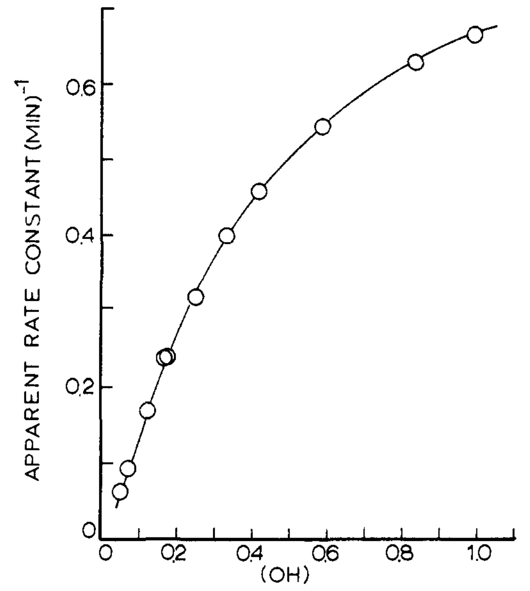

Figure 4. Apparent first-order rate constants at 40° for hydrolysis of NMN in alkaline solutions adjusted to an ionic strength of 1.00 with sodium acetate. The circles represent experimental points and the solid line was calculated using eq 14. Abscissa values refer to the normality of hydroxide ion.

base catalysis, superimposed general base catalysis by hydroxide ion, and ionization of an amide hydrogen.

Table I. Apparent First-Order Rate Constants at 40° for Hydrolysis of NMN in Alkaline Solutions Adjusted to an Ionic Strength of 1.00 with Sodium Acetate

(OH)	Apparent rate constant, min^{-1} Obsd	Calcd[a]
0.0104	0.00582	0.00578
0.0155	0.0101	0.0107
0.0176	0.0137	0.0130
0.0207	0.0166	0.0166
0.0259	0.0232	0.0232
0.0320	0.0304	0.0316
0.0352	0.0372	0.0361
0.0528	0.0632	0.0630
0.0720	0.0935	0.0932
0.125	0.168	0.173
0.168	0.239	0.230
0.179	0.241	0.244
0.252	0.318	0.323
0.336	0.400	0.397
0.420	0.458	0.455
0.590	0.545	0.544
0.840	0.630	0.631
1.00	0.667	0.670

[a] Calculated values obtained with eq 14 and parameter values from Table VII.

A mechanism which seemed reasonable on the basis of previous studies of amide hydrolysis[8-13] is outlined by eq 1-7. It describes the formation of a tetrahedral intermediate (Y) by nucleophilic attack of hydroxide ion upon the amide carbonyl; the spontaneous decomposition of this intermediate to re-form reactants or to form products; the general base catalyzed decomposition of the intermediate by hydroxide ion or by the general base, acetate ion, to form products; the ionization of an amide hydrogen to form an amido anion (X); the reaction of this amido anion with hydroxide ion to form products; or the reaction of the amido anion with water to form intermediate Y.

$$\mathrm{NMN} + \mathrm{OH} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \mathrm{Y} \tag{1}$$

$$\mathrm{Y} \xrightarrow{k_1} \mathrm{N^1\text{-}methylnicotinic\ acid} \tag{2}$$

$$\mathrm{Y} + \mathrm{OH} \xrightarrow{k_2} \mathrm{N^1\text{-}methylnicotinate} \tag{3}$$

$$\mathrm{NMN} + \mathrm{OH} \overset{K}{\rightleftharpoons} \mathrm{X} \tag{4}$$

$$\mathrm{X} + \mathrm{H_2O} \xrightarrow{k_x} \mathrm{Y} \tag{5}$$

$$\mathrm{Y} + \mathrm{Ac}\cdot \xrightarrow{k_3} \mathrm{N^1\text{-}methylnicotinate} \tag{6}$$

$$\mathrm{X} + \mathrm{OH} \xrightarrow{k_4} \mathrm{N^1\text{-}methylnicotinate} \tag{7}$$

Here K represents the equilibrium constant for the ionization reaction shown in eq 4, and the various k's represent specific rate constants for the rate processes described.

The rate law for this mechanism was derived by assuming a steady-state condition for Y, by defining the acetate ion concentration as

$$(\mathrm{Ac}) = 1.00 - (\mathrm{OH}) \tag{8}$$

and by assuming that the concentration of Y was negligible compared to the total concentration of NMN. The total concentration of NMN, $(\mathrm{NMN})_t$, was taken to be the sum of the concentrations of free and deprotonated NMN. The resulting rate law was found to have the form

$$-\frac{d(\mathrm{NMN})_t}{dt} = k_{\mathrm{obsd}}(\mathrm{NMN})_t = \frac{A_1(\mathrm{OH}) + A_2(\mathrm{OH})^2 + A_3(\mathrm{OH})^3}{1.0 + B_1(\mathrm{OH}) + B_2(\mathrm{OH})^2}(\mathrm{NMN})_t \tag{9}$$

where A_1, A_2, A_3, B_1, and B_2 are composites of the various specific rate and equilibrium constants, and k_{obsd} is the experimentally determined apparent first-order rate constant for hydrolysis of NMN. Various modifications in this mechanism, obtained by excluding certain of the steps, resulted in six different forms of the rate law given in eq 9. Table II defines the parameters of eq 9 in terms of the specific constants for the processes in the various mechanisms considered.

It is obvious that the rate laws for several of the mechanisms (I and II) indicated in Table II cannot characterize the experimental data. The rate laws for the mechanisms III–VI were extensively tested to determine which of these most adequately described the observed hydrolytic behavior. The fitting of the data to these rate laws was accomplished on an IBM 7044 computer using a nonlinear regression program[19] provided with an appropriate weighting subroutine. The results of these tests are illustrated in Table III. The rate law for mechanism V yielded a significantly better description of the data than did those for mechanisms III and

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[19] The data were treated by a nonlinear regression program (Share Catalog No. 1428) which adjusted the parameters of eq 9 to minimize the error sum of squares

$$\sum\left(k_{\mathrm{obsd}} - \frac{A_1(\mathrm{OH}) + A_2(\mathrm{OH})^2 + A_3(\mathrm{OH})^3}{1.0 + B_1(\mathrm{OH}) + B_2(\mathrm{OH})^2}\right)^2$$

The data were weighted in inverse proportion to

$$\frac{A_1(\mathrm{OH}) + A_2(\mathrm{OH})^2 + A_3(\mathrm{OH})^3}{1 + B_1(\mathrm{OH}) + B_2(\mathrm{OH})^2}$$

Table II. The Parameters for Equation 9 in Terms of the Specific Rate and Equilibrium Constants for Various Possible Mechanisms

Mechanism	Eq	A_1	A_2	A_3	B_1	B_2
I	1, 2	$\frac{k_1k_f}{k_1 + k_r}$	Zero	Zero	Zero	Zero
IIIa	1, 2, 3	$\frac{k_1k_f}{k_1 + k_r}$	$\frac{k_2k_f}{k_1 + k_r}$	Zero	$\frac{k_2}{k_1 + k_r}$	Zero
IIIb	1, 2, 6	$\frac{k_f(k_1 + k_3)}{k_1 + k_r + k_3}$	$-\frac{k_3k_f}{k_1 + k_r + k_3}$	Zero	$-\frac{k_3}{k_1 + k_r + k_3}$	Zero
II	1, 3	Zero	$\frac{k_fk_2}{k_r}$	Zero	$\frac{k_2}{k_r}$	Zero
IV	1, 3, 4	Zero	$\frac{k_fk_2}{k_r}$	Zero	$\frac{k_2}{k_r} + K$	$\frac{k_2K}{k_r}$
Va	1–4	$\frac{k_1k_f}{k_1 + k_r}$	$\frac{k_2k_f}{k_1 + k_r}$	Zero	$\frac{k_2}{k_1 + k_r} + K$	$\frac{k_2K}{k_1 + k_r}$
Vb	1–4, 5	$\frac{k_1[k_f + k_xK(H_2O)]}{k_1 + k_r}$	$\frac{k_2[k_f + k_xK(H_2O)]}{k_1 + k_r}$	Zero	$\frac{k_2}{k_1 + k_r} + K$	$\frac{k_2K}{k_1 + k_r}$
Vc	1–4, 6	$\frac{k_f(k_1 + k_3)}{k_1 + k_r + k_3}$	$\frac{k_f(k_2 - k_3)}{k_1 + k_r + k_3}$	Zero	$\frac{k_2 - k_3}{k_1 + k_r + k_3} + K$	$\frac{(k_2 - k_3)K}{k_1 + k_r + k_3}$
Vd	1–6	$\frac{(k_1 + k_3)[k_f + k_xK(H_2O)]}{k_1 + k_r + k_3}$	$\frac{(k_2 - k_3)[k_f + k_xK(H_2O)]}{k_1 + k_r + k_3}$	Zero	$\frac{k_2 - k_3}{k_1 + k_r + k_3} + K$	$\frac{(k_2 - k_3)K}{k_1 + k_r + k_3}$
Ve	1, 2, 4, 6	$\frac{k_f(k_1 + k_3)}{k_1 + k_r + k_3}$	$-\frac{k_3k_f}{k_1 + k_r + k_3}$	Zero	$K - \frac{k_3}{k_1 + k_r + k_3}$	$-\frac{k_3K}{k_1 + k_r + k_3}$
VIa	1–4, 7	$\frac{k_1k_f}{k_1 + k_r}$	$\frac{k_fk_2}{k_1 + k_r} + k_4K$	$\frac{k_2k_4K}{k_1 + k_r}$	$\frac{k_2}{k_1 + k_r} + K$	$\frac{k_2K}{k_1 + k_r}$
VIb	1–4, 6, 7	$\frac{k_f(k_1 + k_3)}{k_1 + k_r + k_3}$	$\frac{k_f(k_2 - k_3)}{k_1 + k_r + k_3} + k_4K$	$\frac{(k_2 - k_3)k_4K}{k_1 + k_r + k_3}$	$\frac{k_2 - k_3}{k_1 + k_r + k_3} + K$	$\frac{(k_2 - k_3)K}{k_1 + k_r + k_3}$
VIc	1–7	$\frac{[k_f + k_xK(H_2O)](k_1 + k_3)}{k_1 + k_r + k_3}$	$\left\{\left(\frac{[k_f + k_xK(H_2O)](k_2 - k_3)}{k_1 + k_r + k_3}\right) + k_4K\right\}$	$\frac{(k_2 - k_3)k_4K}{k_1 + k_r + k_3}$	$\frac{k_2 - k_3}{k_1 + k_r + k_3} + K$	$\frac{(k_2 - k_3)K}{k_1 + k_r + k_3}$

Table III. The Value of the Parameters[a] of Equation 9 Obtained by Fitting the Data in Table I to the Rate Laws for the Various Mechanisms

	A_1	A_2	A_3	B_1	B_2	Sum of squares[b]
			Mechanism III			
Lower	−4678	6.33×10^5		-1.47×10^7		
Best	−3891	6.58×10^5		5.48×10^5		378.0×10^{-3}
Upper	−3067	7.10×10^5		6.83×10^5		
			Mechanism IV			
Lower		66.20		31.96	59.12	
Best		67.99		33.65	64.90	0.645×10^{-3}
Upper		69.77		35.37	70.55	
			Mechanism V			
Lower	0.1487	46.34		20.24	45.36	
Best	0.1885	47.50		21.31	48.90	0.169×10^{-3}
Upper	0.2260	48.70		22.43	52.41	
			Mechanism VI			
Lower	0.1323	47.48	−4.61	21.06	43.58	
Best	0.1787	48.82	−1.65	22.27	47.69	0.152×10^{-3}
Upper	0.2228	50.23	2.01	23.58	51.76	

[a] The parameters are given with their nonlinear confidence intervals at the 95% level. [b] The sum of the squares of the differences between observed and calculated apparent rate constants. Tests for significance at the 95% level using an F test for one added parameter showed that V is significantly better than III or IV but that VI is not significantly better than V.

IV. The characterization of the data by VI was not significantly better than that by V and, on the basis that the parameters A_3 and B_2 must have the same sign (see Table II), it is clear that the rate law for mechanism V best described the observed behavior. This indicates that eq 1–4, and possibly eq 5 and 6, are involved in the hydrolytic mechanism.[20]

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[20] It is clear that mechanism Ve of Table II, which involves eq 1,

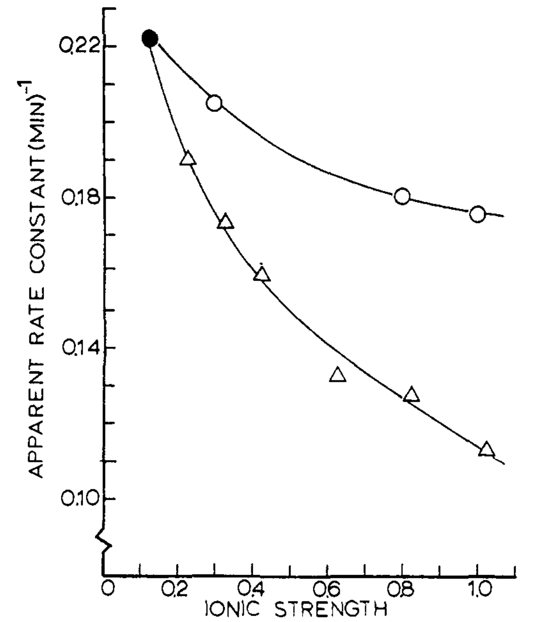

Figure 5. Apparent first-order rate constants for hydrolysis of NMN in 0.1223 M sodium hydroxide at 40° and at various ionic strengths adjusted with sodium acetate (○) or sodium perchlorate (△); ● represents no added salt.

It is possible, on the basis of other experiments, to show that the general base catalyzed decomposition of the intermediate by acetate ion, eq 6, is unlikely. Figure 5 illustrates the dependency of the apparent hydrolytic rate constant on salt concentration and demonstrates that the influence of sodium acetate was considerably different from that of sodium perchlorate. That the apparent rate constants were always larger when sodium acetate was employed might indicate that the expected decrease in the apparent rate constant due to an increase in ionic strength was offset, in part, by catalytic activity of the acetate ion. An alternative explanation is that acetate ion caused no catalysis, but that the observed behavior was due to a specific ionic association between perchlorate anion and NMN cation which resulted in a species which was much less reactive toward hydrolysis than the parent compound. A further insight into this behavior is provided by the results of studies which were conducted at constant hydroxide ion concentration and at an ionic strength adjusted to a value of 1.00 with different combinations of sodium acetate and sodium perchlorate. The results are shown in Figure 6. If it is assumed that sodium acetate functioned as both a salt and a catalyst, then it can be shown that

$$k_{\mathrm{obsd}} = \frac{k_1k_{\mathrm{f}}(\mathrm{OH}) + k_2k_{\mathrm{f}}(\mathrm{OH})^2 + k_3k_{\mathrm{f}}(\mathrm{OH})(\mathrm{Ac})}{[1.0 + K(\mathrm{OH})][k_1 + k_{\mathrm{r}} + k_3 + k_2(\mathrm{OH}) + k_3(\mathrm{Ac})]} \tag{10}$$

and that

$$\frac{\mathrm{d}k_{\mathrm{obsd}}}{\mathrm{d(Ac)}} = \frac{k_3k_{\mathrm{f}}(\mathrm{OH})(k_{\mathrm{r}} + k_3)}{[1.0 + K(\mathrm{OH})][k_1 + k_{\mathrm{r}} + k_3 + k_2(\mathrm{OH}) + k_3(\mathrm{Ac})]^2} \tag{11}$$

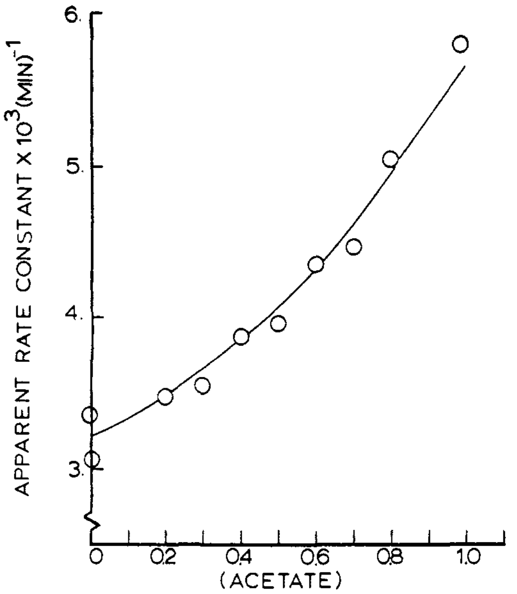

Figure 6. Apparent first-order rate constants for hydrolysis of NMN at 40° in 0.0102 M sodium hydroxide solutions adjusted to an ionic strength of 1.00 with varying concentrations of sodium acetate and sodium perchlorate. The circles represent experimental points and the solid line was calculated using eq 13. Abscissa values refer to the normality of acetate ion.

It is apparent from this relationship that if acetate ion functions as a catalyst, then the slope of the curve in Figure 6 should decrease with increasing acetate concentration. That the slope of the curve increased suggests that acetate catalysis superimposed upon specific base catalysis was not important in the over-all reaction scheme. This evidence supports the premise that a specific association occurred between NMN and perchlorate ion. Additional support for this premise is provided by the report[21] on the influence of sodium acetate and sodium perchlorate on the complexometric behavior of NMN.

The extent of complex formation at 40°, as determined spectrally, between NMN and the electron donor, 8-chlorotheophyllinate anion, was shown to decrease on the addition of either sodium acetate or sodium perchlorate to the system.[21] However, the extent of complex formation was always found to be smaller when sodium perchlorate was the salt present. This effect was quantitatively related to ion pairing between NMN and perchlorate ion. An ion-pair formation constant of 0.86 M^{-1} (ionic strength of 1.0) was reported.

To test further the possibility that sodium perchlorate influenced the hydrolytic reaction differently from sodium acetate due to the formation of an ion pair which was significantly more resistant to hydrolysis than was NMN, apparent first-order rate constants were determined at 40° for the alkaline hydrolysis of NMN in systems adjusted to an ionic strength of 1.00 with sodium perchlorate. The results are presented in Table IV. Here the hydrolytic mechanism was assumed to involve eq 1–4, and

$$\mathrm{NMN} + \mathrm{ClO_4} \overset{G}{\rightleftarrows} \mathrm{NMN\text{-}ClO_4} \tag{12}$$

where NMN-ClO$_4$ is an ion pair assumed to be re-

2, 4, and 6, cannot describe the data since parameters A_2 and B_2 of that mechanism must be negative.

[21] D. Brooke and D. E. Guttman, *J. Pharm. Sci.*, in press.

Table IV. Apparent First-Order Rate Constants at 40° for Hydrolysis of NMN in Alkaline Solutions Adjusted to an Ionic Strength of 1.00 with Sodium Perchlorate

(OH)	Apparent rate constant, min^{-1} Obsd	Calcd[a]
0.0104	0.00336	0.00328
0.0155	0.00600	0.00610
0.0198	0.00850	0.00892
0.0207	0.00950	0.00955
0.0259	0.0136	0.0135
0.0397	0.0237	0.0253
0.0496	0.0345	0.0348
0.0694	0.0532	0.0548
0.0792	0.0639	0.0651
0.0991	0.0854	0.0862
0.109	0.102	0.0967
0.123	0.117	0.111
0.139	0.129	0.128
0.197	0.187	0.187
0.271	0.262	0.255
0.345	0.329	0.317
0.436	0.398	0.385
0.582	0.474	0.478
0.727	0.548	0.555
1.00	0.667	0.670

[a] Calculated values are based on eq 13. See text.

sistant to hydrolysis and G is the ion-pair formation constant. The apparent rate constant can be described by

$$k_{\mathrm{obsd}} = \frac{[k_1k_f/(k_1 + k_r)](\mathrm{OH}) + [k_2k_f/(k_1 + k_r)](\mathrm{OH})^2}{\{1.0 + [k_2/(k_1 + k_r)](\mathrm{OH})\}[1.0 + K(\mathrm{OH}) + G(\mathrm{C10_4})]} \tag{13}$$

Since the only difference between the hydrolytic reactions in systems containing sodium acetate and those containing sodium perchlorate was assumed to be the ion-pair formation, the composites $k_1k_f/(k_1 + k_r)$, $k_2k_f/(k_1 + k_r)$, and $k_2/(k_1 + k_r)$, and K can be calculated from the parameters for the rate law for mechanism V in Table III. Employing these considerations, the value for G was found to be 0.79 M^{-1}. The close agreement between the ion-pair formation constants found by the two different methods support the assumptions made. The evidence is, however, indirect and the possibility that acetate functioned as a general base and contributed to a small extent to the over-all rate cannot be ruled out.

It has been shown that the rate equation for the alkaline hydrolysis of NMN, in systems maintained at an ionic strength of 1.00 with sodium acetate, which is consistent with all of the experimental data, is

$$-\frac{d(\mathrm{NMN})_t}{dt} = \frac{k_f(\mathrm{OH})\{[k_1/(k_1 + k_r)] + [k_2/(k_1 + k_r)](\mathrm{OH})\}}{\{1.0 + [k_2/(k_1 + k_r)](\mathrm{OH})\}[1.0 + K(\mathrm{OH})]}(\mathrm{NMN})_t \tag{14}$$

The dependencies of k_f and composites $k_1/(k_1 + k_r)$ and $k_2/(k_1 + k_r)$ on temperature provide an additional insight into the over-all mechanism of alkaline hydrolysis. Apparent first-order rate constants for the alkaline hydrolysis in systems adjusted to an ionic strength of 1.00 with sodium acetate at 30 and 50° are reported in Tables V and VI. The values for K, k_f, and the composites $k_1/(k_1 + k_r)$ and $k_2/(k_1 + k_r)$, at the different temperatures, are presented with their nonlinear confidence intervals in Table VII. The activation parameters for k_f, and the calculated ratios k_r/k_1, k_2/k_1, and k_r/k_2, are presented in Table VIII.

Table V. Apparent First-Order Rate Constants at 30° for Hydrolysis of NMN in Alkaline Solutions Adjusted to an Ionic Strength of 1.00 with Sodium Acetate

(OH)	Apparent rate constant, min^{-1} Obsd	Calcd[a]
0.0174	0.00710	0.00712
0.0290	0.0147	0.0146
0.0435	0.0253	0.0252
0.0652	0.0416	0.0418
0.0894	0.0605	0.0599
0.119	0.0808	0.0806
0.164	0.106	0.108
0.208	0.128	0.132
0.317	0.180	0.177
0.380	0.203	0.197
0.507	0.227	0.228
0.570	0.241	0.241
0.633	0.260	0.252
0.760	0.271	0.270
1.00	0.288	0.294
1.00	0.290	0.294

[a] Calculated values obtained with eq 14 and parameter values from Table VII.

Table VI. Apparent First-Order Rate Constants at 50° for Hydrolysis of NMN in Alkaline Solutions Adjusted to an Ionic Strength of 1.00 with Sodium Acetate

(OH)	Apparent rate constant, min^{-1} Obsd	Calcd[a]
0.0348	0.0594	0.0598
0.0532	0.113	0.110
0.0709	0.163	0.163
0.0886	0.214	0.215
0.0985	0.237	0.245
0.0985	0.246	0.245
0.173	0.458	0.452
0.197	0.518	0.511
0.271	0.698	0.676
0.296	0.679	0.724
0.345	0.840	0.813
0.370	0.845	0.854

[a] Calculated values obtained with eq 14 and parameter values in Table VII.

Table VII. Values for the Constants k_f, K, $k_1/(k_1 + k_r)$, and $k_2/(k_1 + k_r)$ of Equation 14 at Three Different Temperatures

Temp, °C	Constant	Value[a] Best	Lower	Upper
30.0	k_f, M^{-1} min^{-1}	1.287	1.266	1.309
	$k_1/(k_1 + k_r)$	0.123	0.101	0.144
	$k_2/(k_1 + k_r)$, M^{-1}	18.345	17.323	19.552
	K, M^{-1}	3.176	3.070	3.283
40.0	k_f, M^{-1} min^{-1}	2.532	2.474	2.590
	$k_1/(k_1 + k_r)$	0.0732	0.0575	0.0881
	$k_2/(k_1 + k_r)$, M^{-1}	18.88	17.83	20.04
	K, M^{-1}	2.604	2.437	2.770
50.0	k_f, M^{-1} min^{-1}	4.802	4.646	4.958
	$k_1/(k_1 + k_r)$	0.0476	0.0020	0.0901
	$k_2/(k_1 + k_r)$, M^{-1}	15.73	15.23	17.44
	K, M^{-1}	2.130	1.910	2.379

[a] Constants are given with their nonlinear confidence intervals at the 95% level.

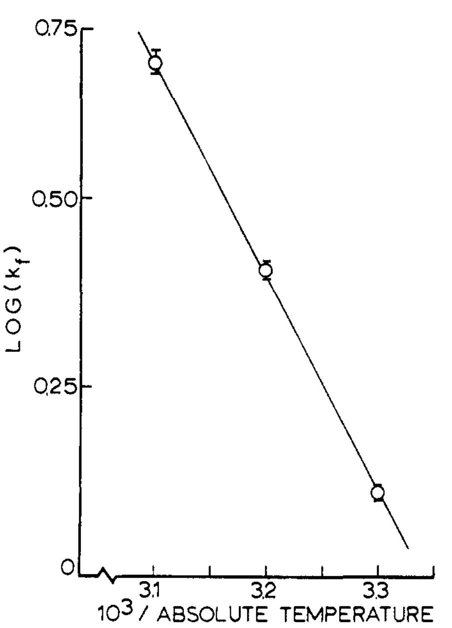

Figure 7. An Arrhenius plot for k_f. The nonlinear confidence interval at the 95% level is indicated.

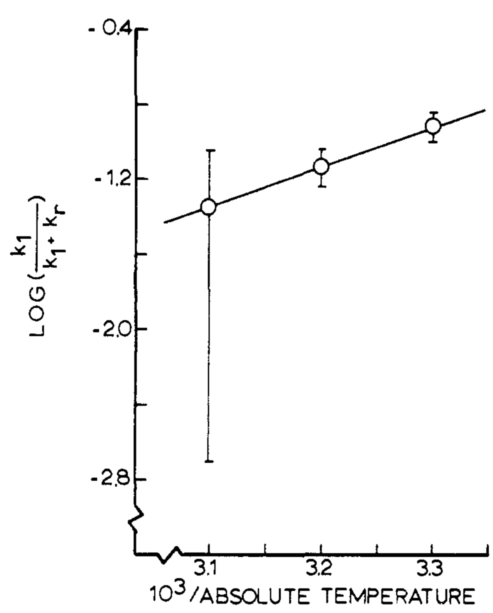

Figure 8. An Arrhenius plot for the composite $k_1/(k_1 + k_r)$. The nonlinear confidence interval at the 95% level for the composite is indicated.

An Arrhenius plot for k_f is given in Figure 7. The linearity of this plot suggests, in an indirect manner, that the tetrahedral intermediate was not formed by the reaction of water with deprotonated NMN. If the tetrahedral intermediate were formed by both the nucleophilic attack of hydroxide ion on NMN and the reaction of water with deprotonated NMN, the experimentally determined k_f would actually be the sum of two terms, k_f and $k_xK(H_2O)$ (see Table II, mechanism Vb). The equilibrium constant, K, exhibited a marked decrease with increasing temperature. Therefore, the term $k_xK(H_2O)$ might be expected to remain relatively constant over a range of temperatures and would result in a nonlinear Arrhenius plot. This evidence is equivocal, however, and a water reaction with deprotonated NMN cannot be completely ruled out.

Table VIII. Activation Parameters[a] for k_f, and the Calculated Ratios k_r/k_1,[b] k_r/k_2, and k_2/k_1[b]

Activation parameter	k_f	k_r/k_1	k_r/k_2	k_2/k_1
ΔH^*, kcal/mol	13.6	9.7	1.9	7.2
$\Delta G_{303^\circ}{}^*$, kcal/mol	17.2	−1.2	1.8	−3.0
$\Delta S_{303^\circ}{}^*$, eu	−11.5	38.0	2.2	35.0

[a] Standard states taken as 1.0 M in aqueous solution for solutes and pure liquid for water. [b] Because of the great uncertainty in $k_1/(k_1 + k_r)$, the ratios k_r/k_1 and k_2/k_1 and their activation parameters are quite uncertain. The activation parameters for k_f and k_f/k_2 can be estimated with much more surety.

Arrhenius plots for the composites $k_1/(k_1 + k_r)$ and $k_2/(k_1 + k_r)$ are presented in Figures 8 and 9. The indication is that both composites decreased as the temperature was increased. This behavior would only be possible if the energy of activation for the return of the tetrahedral intermediate to reactants was greater than the energy of activation for the formation of products from the intermediate. It would appear that the reaction scheme for the alkaline hydrolysis of NMN is significantly more complex than was suggested by eq 1–4. A more complete mechanistic conception of the hydrolytic reaction is presented in Figure 10. Here it is suggested that the reactants proceed through activated complex Y_{fr} to form an intermediate Y which is stabilized by the electron-withdrawing property of the pyridinium cation and by an internal charge interaction. In forming reactants *via* activated complex Y_{fr} or N^1-methylnicotinic acid *via* activated complex Y_1 the internal charge interaction must be disrupted, a process which would proceed with concomitant solvation of charge and which would be entropically unfavorable. However, in proceeding to N^1-methylnicotinate *via* activated complex Y_2 it would appear that this interaction need not be greatly altered, a factor which may favor this process on an entropic basis.

A plot of pK_a against the reciprocal of absolute temperature is presented in Figure 11. The pK_a at 25° was estimated to be 13.4, which is quite close to the value of 13.2 determined by Martin and Hull[4] from their kinetic data. The good agreement, in spite of the fact that the rate law used by Martin and Hull to determine pK_a did not incorporate a superimposed general base catalytic step, can be rationalized by considering the rate law derived in eq 14 in a slightly different form. It can be shown that

$$k_{\text{obsd}} = \frac{\{[k_fk_1/(k_1 + k_r)]/[k_2/(k_1 + k_r)]\}(\text{OH}) + k_f(\text{OH})^2}{[1.0 + K(\text{OH})]/[k_2/(k_1 + k_r)] + (\text{OH}) + K(\text{OH})^2} \tag{15}$$

Since $k_2/(k_1 + k_r)$ was found to be much larger than 1.0, K, or $k_fk_1/(k_1 + k_r)$, the expression may be reduced to

$$k_{\text{obsd}} = \frac{k_f(\text{OH})}{1.0 + K(\text{OH})} \tag{16}$$

which is of the same form as the expression used by Martin and Hull to determine K, and therefore pK_a.

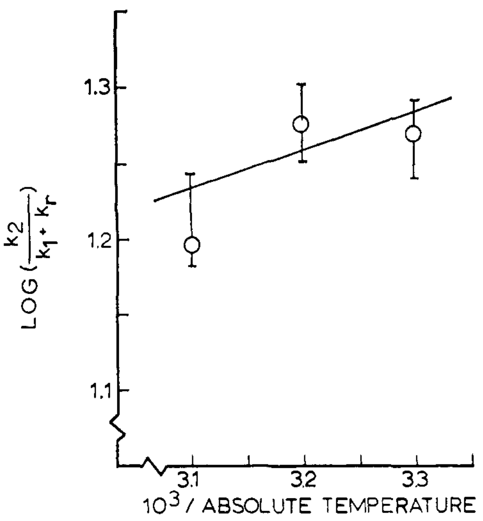

Figure 9. An Arrhenius plot for the composite $k_2/(k_1 + k_r)$. The nonlinear confidence interval at the 95% level for the composite is indicated.

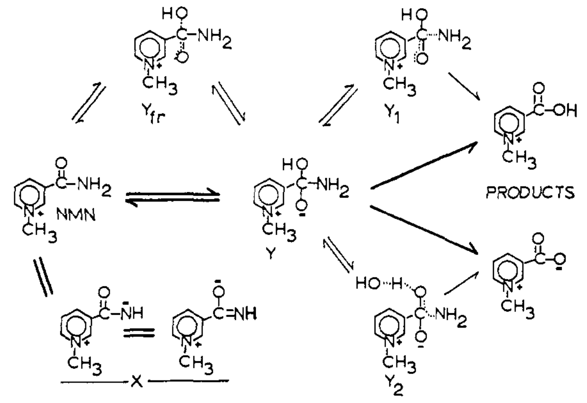

Figure 10. A proposed mechanism for the alkaline hydrolysis of NMN. See text.

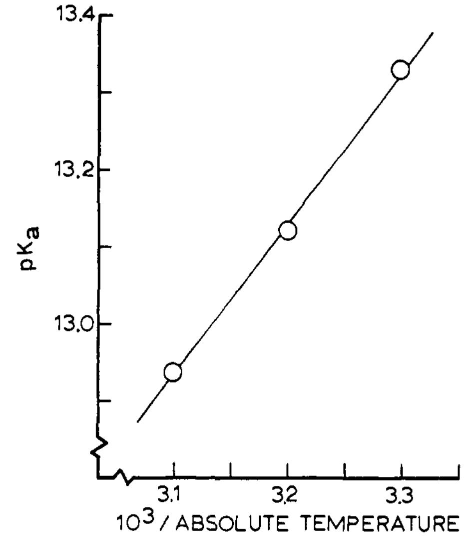

Figure 11. A plot illustrating the influence of temperature on pK_a for NMN.

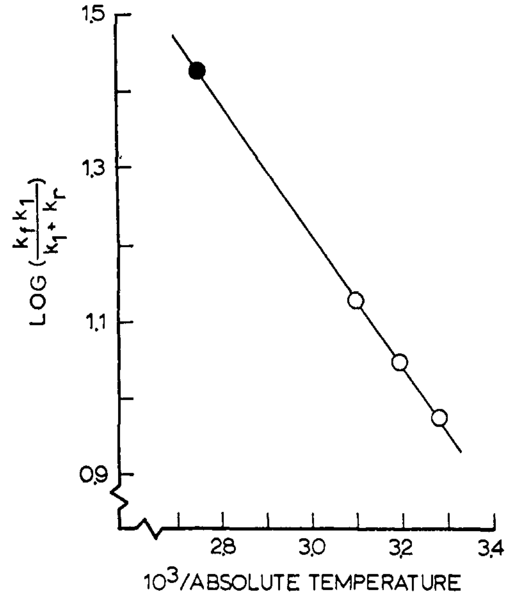

Figure 12. An Arrhenius plot for the term $k_f k_1/(k_1 + k_r)$. The interpolation at 90° is indicated by ●.

Because of the electron-withdrawing capability of the pyridinium nitrogen of NMN and the possibility of additional stabilization of its tetrahedral intermediate by an internal electrostatic interaction, the kinetics of alkaline hydrolysis of NMN would be expected to vary from those of nicotinamide in several ways. Finholt and Higuchi[22] studied the base-catalyzed hydrolysis of nicotinamide at 90° employing hydroxide ion concentrations as high as 0.2 *M*, and no evidence of general base catalysis superimposed upon specific base catalysis was apparent. The involvement of such a process in the kinetics of hydrolysis of NMN as contrasted with those of nicotinamide is conceivably due to the greater acidity of the groups about the carbonyl carbon of the tetrahedral intermediate of NMN. This is consistent with the conclusion of Schowen and Zuorick,[11] who worked with 2,2,2-trifluoro-N-methylacetanilide. The specific base catalytic rate constant, k_{OH}, for the hydrolysis of nicotinamide at 90° was reported to be 14.6 M^{-1} hr^{-1}.[21] The analogous constant, $k_f k_1/(k_1 + k_r)$, for NMN was estimated from Figure 12 to be 26.6 M^{-1} hr^{-1} at 90°. It is suggested that both acyl activation and internal stabilization of the intermediate would increase the forward rate constant, k_f, and decrease the return rate constant, k_r, making the specific base catalytic constant larger in the case of NMN than in the case of nicotinamide.

It has been shown that the kinetics of the alkaline hydrolysis of NMN are considerably more complex than had been previously realized.[3,4] The kinetic picture is, however, similar to those observed with activated acetanilides.[8-13] The possible existence in the reaction pathway of an internally stabilized intermediate makes ester and amide derivatives of pyridinium cations unusually attractive candidates for further kinetic studies.

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(22) P. Finholt and T. Higuchi, *J. Pharm. Sci.*, **51**, 655 (1962).

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Solvolysis of Heteroannularly Substituted Methylferrocenylcarbinyl Acetates

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Abstract: The nature of the transmission of substituent effects from one cyclopentadienyl ring of ferrocene to a reaction center on the other ring was investigated by means of solvolysis studies on heteroannularly substituted methylferrocenylcarbinyl acetates. The evidence indicates that inductive or field effects predominate, and that resonance interactions play at most a minor role. A linear relationship exists between substituent effects in the solvolysis reaction and the reversible quarter-wave potentials of the appropriately substituted ferrocenes.

Solvolyses of metallocenylcarbinyl acetates have demonstrated that an α-metallocenyl substituent imparts remarkable stability to a carbonium ion center.[2] The stereochemistry of these solvolyses shows that departure of the leaving acetate ion is strongly favored in the direction away from the central metal atom.[3] These and other results[1a] have been cited as evidence for the occurrence of some electron release from the central metal atom to the positive carbon, though this interpretation has recently been the subject of controversy in the literature.[4] In this paper we report a study of the effects of several simple substituents in the heteroannular position of the ferrocenylmethylcarbinyl acetate skeleton upon the solvolysis rate and show that inductive and field effects predominate in the transmission of these effects.

Experimental Section

Substituted Acetylferrocenes. The preparation and properties of 1′-bromo-, 1′-chloro-, and 1′-cyanoacetylferrocenes have previously been reported.[5]

1′-Carbomethoxyacetylferrocene. Nesmeyanov and Reutov reported the acetylation of carbomethoxyferrocene[6] in 58% yield.[7] An attempt to follow their procedure led to a 30% yield of the desired product, along with recovery of 62% of the starting material. Since the conditions specified appeared to be rather mild, the reaction was repeated under more vigorous conditions.

Acetyl chloride (3.67 g, 47 mmol) was added to a stirred suspension of powdered aluminum chloride (11.5 g, 86 mmol) in methylene chloride (70 ml) under nitrogen. Over a period of 10 min, carbomethoxyferrocene (8.0 g, 32.8 mmol) in methylene chloride (80 ml) was added. The solution warmed slightly. The reaction was stirred at room temperature for 3.25 hr and poured over ice. Sufficient acid was added to allow separation of the layers, and the product was extracted into methylene chloride. The combined organic phase was washed three times with sodium bicarbonate solution and once with water. After drying over magnesium sulfate, the solvent was removed to yield a dark red solid, mp 99–100.5°. One recrystallization from heptane produced 8.59 g of dark red crystals (91.5%), mp 101–101.7° (lit.[7,8] mp 92.5–94.5°, 102–103°).

Carbinols. The 1′-bromo-, 1′-chloro-, 1′-carbomethoxy-, and 1′-cyanoacetylferrocenes were reduced to the corresponding carbinols with sodium borohydride in methanol.

The general procedure consisted in adding sodium borohydride powder over a period of 2–5 min to a methanolic solution of the ketone stirred with a magnetic stirrer. A gentle stream of nitrogen was directed onto the surface of the solution during the reaction. The reductions were generally complete in about 0.5 hr. The solutions changed from the red-orange color of the ketones to the straw-yellow color of the alcohols. After approximately 1 hr, excess sodium borohydride was decomposed with acetone. The reaction mixture was poured into approximately 200 ml of water and ammonium chloride was added until the solution tested weakly basic. The alcohol was extracted with dichloromethane. Ether formation may occur if the solution of the alcohol is dried over anhydrous magnesium sulfate or chromatographed on acid-washed alumina. For this reason, the dichloromethane solutions were worked up by adding them to an equivalent volume of benzene in a solvent stripping apparatus, and then distilling solvent under reduced pressure. The last traces of solvent were removed either at room temperature or else on a warm water bath maintained at a temperature no greater than 50°. The crude alcohol was used directly to prepare the acetate or else it was further purified by chromatography on neutral or basic alumina that had been deactivated by the addition of water (roughly 5% by weight). The crude alcohol was used directly to prepare the acetate only when its infrared spectrum indicated that none of the starting ketone remained unreduced. Liquid methylferrocenylcarbinols in general give poor elemental analyses whereas the liquid acetates give satisfactory analyses. For this reason all the carbinols were not themselves analyzed, but rather only the corresponding acetates were analyzed.

Acetates. The carbinols were converted into the corresponding acetates with acetic anhydride in pyridine according to the pro-

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(1) (a) Ph.D. Thesis, California Institute of Technology, Pasadena, Calif., 1963. (b) Ph.D. Thesis, California Institute of Technology, Pasadena, Calif., 1961.

(2) (a) J. H. Richards and E. A. Hill, *J. Amer. Chem. Soc.*, **81**, 3484 (1959); (b) E. A. Hill and J. H. Richards, *ibid.*, **83**, 3840 (1961).

(3) (a) E. A. Hill and J. H. Richards, *ibid.*, **83**, 4216 (1961); (b) D. S. Trifan and R. Backsai, *Tetrahedron Lett.*, **No. 13**, 1 (1960).

(4) (a) J. C. Ware and T. G. Traylor, *ibid.*, 1295 (1965); (b) M. Cais, J. J. Dannenberg, A. Eisenstadt, M. I. Levenberg, and J. H. Richards, *ibid.*, 1695 (1966); (c) T. T. Tidwell and T. G. Traylor, *J. Amer. Chem. Soc.*, **88**, 3442 (1966); (d) T. G. Traylor and J. C. Ware, *ibid.*, **89**, 2304 (1967); (e) J. J. Dannenberg and J. H. Richards, *Tetrahedron Lett.*, 4747 (1965).

(5) D. W. Hall and J. H. Richards, *J. Org. Chem.*, 28, 1549 (1963).

(6) R. A. Benkeser, D. Goggin, and G. Schroll, *J. Amer. Chem. Soc.*, **76**, 4025 (1954).

(7) A. N. Nesmeyanov and O. A. Reutov, *Dokl. Akad. Nauk SSSR*, **115**, 518 (1957); *Chem. Abstr.*, **52**, 5393 (1958).

(8) M. Rosenblum, Ph.D. Thesis, Harvard University, 1953.