

differences might be due to specific conformational preferences with respect to rotation about the $C_\alpha-C_\beta$ bond, a consideration which does not enter for the ethyl compounds. When factors like this are evaluated with confidence then studies of $^{13}\text{C}-^{13}\text{C}$ coupling constants such as this will become a useful addition to other nmr studies of peptide conformation and function.

Acknowledgments. The authors would like to thank Dr. George Peterson for helpful discussions and Mr. Sergiu Marcus for excellent technical assistance. This work was supported in part by National Institutes of Health Grant AM 02493.

John A. Sogn, Lyman C. Craig,* William A. Gibbons

The Rockefeller University
New York, New York 10021

Received February 4, 1974

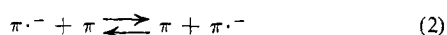
Equilibrium Studies by Electron Spin Resonance. VII. The Use of Time-Averaged Electron Transfer Kinetics to Determine Free Ion-Ion Pair Equilibrium Constants

Sir:

Although esr spectroscopy has proven itself to be the most promising tool for the investigation of the nature of ion pairing in solution, there still exists only one esr technique for the determination of the thermodynamic equilibrium constant for the dissociation of ion pair into free ion (eq 1).¹ To utilize this technique



one must be able to observe the esr spectra of the ion pair and free ion simultaneously.² However, it has been previously pointed out that the conditions for simultaneous observation of the free ion and ion pair are met only rarely.³ This means that for the vast majority of free ion-ion pair equilibria there is no experimental method for the determination of the equilibrium constant. Here we wish to report a time averaging technique based upon the electron transfer rates from anion radical to neutral molecule (eq 2).



Since these rates are readily obtainable,⁴ the technique described here should be applicable for the determination of ion pair dissociation equilibrium constants for a variety of anion radical ion pairs.

It has been previously observed that anion radicals in hexamethylphosphoramide (HMPA) are virtually fully dissociated,⁵ but the addition of alkali metal salts to these anion radical solutions in HMPA results in the formation of the ion pair,⁶ which in some cases can be observed simultaneously with the free ion.⁷ Chang

and Johnson⁸ have demonstrated that the rate of electron exchange (eq 2) for naphthalene systems is slower for the ion pair than it is for the free ion. In agreement with these results we have found that the addition of NaClO_3 to solutions of the free anion radical of *p*-dinitrobenzene in HMPA formed by reduction with sodium⁹ resulted in a smooth decrease in the rate of electron transfer with increasing concentration of sodium cation.¹⁰

The rate constant for the electron transfer between the free *p*-dinitrobenzene anion radical and neutral molecule was determined by esr line broadening under slow exchange conditions utilizing the low field esr line (Figure 1).

Since the observed rate constant for the electron exchange reaction (k_{obsd}) decreases with addition of Na^+ , this value must be a weighted average between the rate constant for the free ion k_{ex}^0 and that for the ion pair (k_{ex}'). This relationship is given by eq 3 providing that

$$k_{\text{obsd}} = \{(\alpha)k_{\text{ex}}^0 + (\beta)k_{\text{ex}}'\} / \{(\alpha) + (\beta)\} \quad (3)$$

the two-jump model (ion pair-free ion) adequately represents the system.

The thermodynamic equilibrium constant for the ion pair dissociation is given by $K_{\text{eq}} = (\alpha)(\text{Na}^+)/(\beta)$, where (Na^+) represents the concentration of added salt since the concentration of added salt is larger by more than two orders of magnitude than the anion radical concentration. Combining this equation with eq 3, we obtain the expression

$$1/(k_{\text{obsd}} - k_{\text{ex}}^0) = K_{\text{eq}}/(\text{Na}^+)(k_{\text{ex}}' - k_{\text{ex}}^0) + 1/(k_{\text{ex}}' - k_{\text{ex}}^0) \quad (4)$$

K_{eq} was obtained from the slope of a plot of $1/(k_{\text{obsd}} - k_{\text{ex}}^0)$ vs. $1/(\text{Na}^+)$ as shown in Figure 2. k_{ex}' was obtained from the intercept, $1/(k_{\text{ex}}' - k_{\text{ex}}^0)$. Typical data for a single experiment are shown in Table I.

Table I. The Observed Rate Constant for the Electron Exchange Reaction with Added NaClO_3

$(\text{Na}^+), M$	$k_{\text{obsd}}, M^{-1} \text{sec}^{-1}$	$(\text{Na}^+), M$	$k_{\text{obsd}}, M^{-1} \text{sec}^{-1}$
0	1.90×10^8	0.309	1.631×10^8
0.164	1.75×10^8	0.321	1.615×10^8
0.184	1.732×10^8		

It should be noted here that the intercept of the plots of line width vs. the concentration of neutral molecule (Figure 1) vary with the concentration of added salt as shown in Figure 3. Further, this variation is dependent upon the concentration of the anion radical. For this reason k_{obsd} must be determined for each salt concentration using the same anion radical solution, and k_{ex}^0 and k_{ex}' will vary slightly from experiment to experiment. However, the data from all experiments using different anion radical concentrations fit on the same line in Figure 2; thus $k_{\text{ex}}' - k_{\text{ex}}^0$ is a constant for each experiment.

(8) R. Chang and C. S. Johnson, Jr., *J. Amer. Chem. Soc.*, **88**, 2338 (1966).

(9) The sodium reduction in HMPA and the purification of the HMPA were carried out exactly as previously described.⁷

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(2) (a) R. D. Allendoerfer and R. J. Papez, *J. Phys. Chem.*, **76**, 1012 (1972); (b) G. R. Stevenson, L. Echegoyen, and L. R. Lizardi, *ibid.*, **76**, 2058 (1972).

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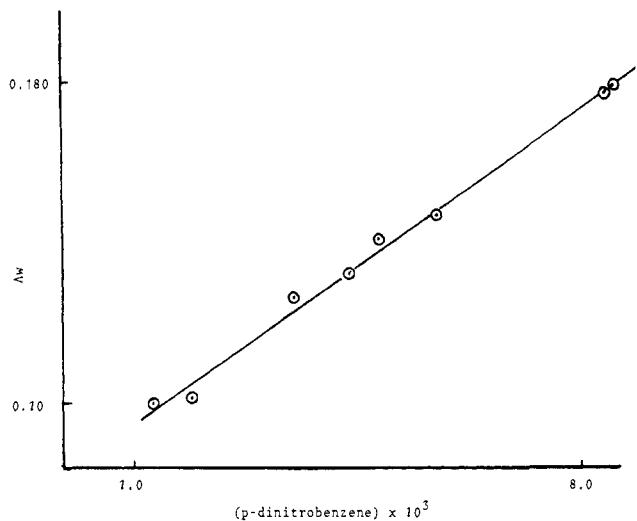


Figure 1. Plot of the line width (Δw) vs. the concentration of *p*-dinitrobenzene in HMPA at 23° without added salt. k_{ex}^0 is calculated for the slope of this plot.

The experiments were carried out by generating a large amount of (ca. 150 ml) of the anion radical solution and passing this solution under high vacuum into 10-ml tubes containing weighed portions of neutral *p*-dinitrobenzene and methodically dried salt.¹¹ Each 10-ml tube was connected with an esr tube, and after all of the salt and neutral molecule had dissolved the sample was submitted to esr analysis. The exact anion radical concentrations were not determined but all were between 10^{-5} and 10^{-3} M. While the experimental rate constants vary for specific salt concentrations from one anion radical solution to another, all of the kinetic data fit quantitatively to eq 4. The equilibrium constant for the ion pair dissociation (eq 1) was found to be 0.74 ± 0.07 at 23°.

Deranleau¹² has recently pointed out that equilibrium constants for weak complexes are most reliable when they are based upon data that extend as much as possible into the region where the saturation factor (*s*) is between 0.2 and 0.8. For the kinetic experiment described here

$$(s) = (\beta) / \{(\alpha) + (\beta)\} = (k_{obsd} - k_{ex}^0) / (k_{ex}' - k_{ex}^0) \quad (5)$$

Calculated from eq 5 our saturation factor (*s*) varied from 0.15 to 0.33.

The fact that a single rate constant (k_{obsd}) is observed means that the mean time between ion association-dissociation events is short compared to the mean time between electron transfer events. If the reverse were true, simultaneous observation of the ion pair and free ion would be observed experimentally. That is, a superposition of lines of different line widths would be observed, as was found by Chang and Johnson.⁸ It is interesting to note here that the line broadening for the addition of salt without increasing the neutral molecule concentration is most likely due to a rapid exchange of the cation between the ion pair and the solvent. This effect has been previously observed.¹³

(11) The NaClO_3 was kept in a vacuum oven for 48 hr at 75° before use.

(12) D. A. Deranleau, *J. Amer. Chem. Soc.*, **91**, 4044 (1969).

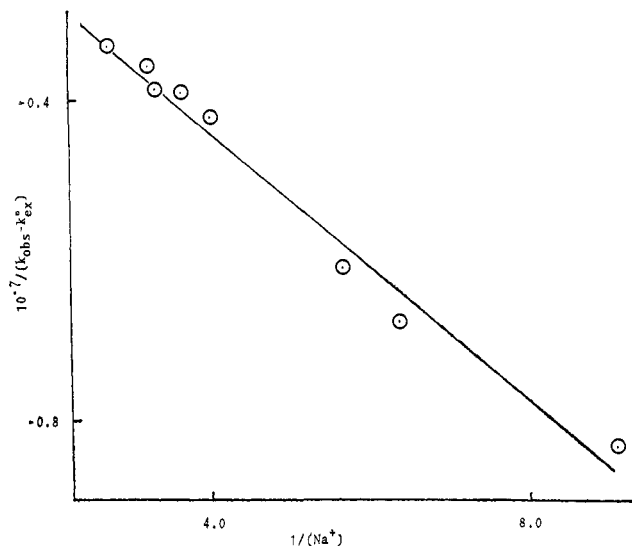


Figure 2. Plot of $10^{-7}/(k_{obsd} - k_{ex}^0)$ vs. one over the concentration of added salt.

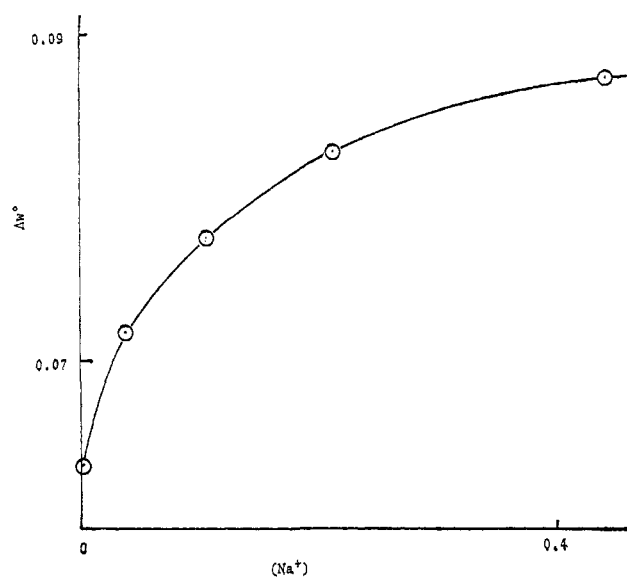


Figure 3. Plot of line width (Δw^0) vs. the concentration of added salt for systems not containing neutral molecule.

The use of rate constants in the time averaging equation (eq 4) is not unprecedented. Several workers have previously used kinetic data to obtain association constants between substrate and enzyme;¹⁴ however, this is the first report to our knowledge of the use of time-averaged kinetics for the determination of ion pair dissociation constants. Time-averaging experiments have previously been carried out, utilizing alkali metal splitting constants, for the determination of equilibrium constants controlling the equilibria between ion pairs and solvent separated ion pairs,¹⁵ but some drawbacks to this technique have recently been pointed out by Szwarc and coworkers.¹⁶

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Acknowledgment. The authors are grateful to Research Corporation for the support of this work.

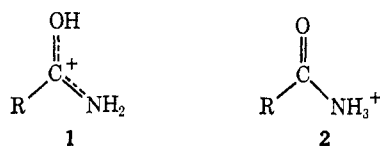
Gerald R. Stevenson,* Rosario Concepción
Department of Chemistry, University of Puerto Rico
Rio Piedras, Puerto Rico 00931
Received March 7, 1974

Position of Protonation and Mechanism of Hydrolysis of Simple Amides

Sir:

The nmr spectra of simple amides dissolved in very acidic solvents such as 100% H_2SO_4 ¹ or FSO_3H ^{2,3} provide strong evidence that the principal conjugate acids of these substances in these media are the oxygen-protonated species, **1**. In less acidic solvents, rapid exchange of the added proton prevents a similar positive structural assignment; nevertheless, it has been widely assumed that protonation occurs chiefly on oxygen under these conditions as well.⁴

Quite recently, however, this assumption was questioned, and an argument was advanced favoring predominant formation of the nitrogen-protonated species, **2**, in dilute or moderately concentrated aqueous acids.^{3,5}



This proposal has aroused considerable interest and has generated a number of counter arguments.⁶ None of these rebuttals, however, is free of unproven assumptions, and the issue therefore still remains unsettled. We wish here to describe new experimental work which provides an especially clear answer to the question of the position of protonation of amides in dilute and moderately concentrated aqueous acids and has an important bearing on the mechanism of acid-catalyzed amide hydrolysis as well.

Our study is based upon the well-known ring-size effect of five- and six-membered cyclic structures commonly called I strain: five-membered rings resist a change in hybridization of one of the ring atoms from sp^2 to sp^3 , whereas six-membered rings favor such a process.⁷ We applied this effect to the present problem

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(2) R. J. Gillespie and T. Birchall, *Can. J. Chem.*, **41**, 148 (1963); T. Birchall and R. J. Gillespie, *ibid.*, **41**, 2642 (1963).

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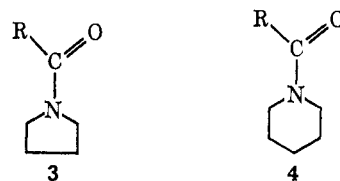
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by examining the behavior of amides in which the nitrogen atom is incorporated in five- and six-membered rings, **3** and **4**.⁸ Since the amide group is planar, or



very nearly so,¹⁰ the nitrogen atom in unprotonated amides is sp^2 hybridized. Protonation on nitrogen makes this atom tetravalent and changes its hybridization to sp^3 ; *N*-acetylpyrrolidines, **3**, should therefore be weaker nitrogen bases than *N*-acetylpiperidines, **4**. Protonation on oxygen, on the other hand, simply reinforces the conjugation which gives the nitrogen atom in the free bases sp^2 hybridization, and O protonation should therefore show no ring-size effect. These phenomena may be seen, for example, in aniline derivatives with planar nitrogen such as *N*-(*p*-nitrophenyl)pyrrolidine ($\text{p}K_a = -1.1$)¹¹ and *N*-(*p*-nitrophenyl)piperidine ($\text{p}K_a = +1.8$);¹¹ here the five-membered cyclic amine is nearly three orders of magnitude less basic than its six-membered homolog. On the other hand, the basic strengths of the parent amines, pyrrolidine ($\text{p}K_a = 11.31$)^{12a} and piperidine ($\text{p}K_a = 11.12$),^{12b} are nearly identical, for here the nitrogen atom is already pyramidal in the free amine and no change occurs upon protonation.

We examined the effect of ring size upon basicity in two series of substrates, the acetamides, **3** and **4** $\text{R} = \text{CH}_3$, and the *p*-nitrobenzamides, **3** and **4** $\text{R} = \text{p-NO}_2\text{-C}_6\text{H}_4$. Basic strengths were measured in dilute to moderately concentrated aqueous HClO_4 by standard indicator methods, making use of the decrease in uv absorption which these amides show upon protonation. Ratios of concentrations of protonated to unprotonated amide, $C_{\text{BH}^+}/C_{\text{B}}$, were determined at a number of points over the range $C_{\text{BH}^+}/C_{\text{B}} \approx 0.1-10$; these values were then extrapolated down to the point where acidity functions cease to deviate from C_{H^+} , *i.e.*, down

Table I. Basicities and Rates of Hydrolysis of Cyclic Amides

Substrate	$\text{p}K_a^a$	$10^6k,^b \text{ sec}^{-1}$
<i>N</i> -Acetylpyrrolidine	0.08 ± 0.02	8.5 ± 0.9
<i>N</i> -Acetylpiperidine	0.04 ± 0.02	43.9 ± 0.2
<i>N</i> -(<i>p</i> -nitrobenzoyl)pyrrolidine	-1.97 ± 0.03	
<i>N</i> -(<i>p</i> -nitrobenzoyl)piperidine	-2.20 ± 0.03	

^a Measured in aqueous HClO_4 at 25°. ^b Measured in 20 wt % aqueous HClO_4 at 81.9°.

(8) The protonation of cyclic amides in which the carbonyl group as well as the nitrogen atom is in the ring, *i.e.*, lactams, had been studied before.⁹ In these substances, however, two ring atoms are initially sp^2 ; simple I-strain concepts therefore do not apply, and comparison with the behavior of polymethylenimines, as was made in that work, is not completely valid.

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