Substituent Effects on Amide Hydrogen Exchange Rates in Aqueous Solution[1,2]

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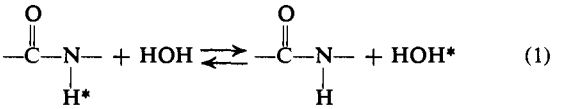
Received October 21, 1971

Abstract: Hydronium and hydroxide ion catalyzed amide hydrogen exchange rate constants have been measured for a series of acyl-substituted amides and several related compounds. Substituents which exert electron-withdrawing polar effects were found to *increase* the hydroxide ion rate constant (k_{OH}) by more than 10^3 ($\rho^I = +0.87$) and to *decrease* the hydronium ion (k_H) catalyzed rate constant, for a more limited series, by greater than tenfold. The relatively marked sensitivity of k_H values to polar effects favors an exchange mechanism which involves N-protonation rather than kinetically indistinguishable mechanisms which involve initial O-protonation of the amide. Substituent effects on amide hydrogen exchange rates are interpreted in terms of alterations in pK values for proton dissociation from neutral amides (pK_{N_2}) for the base-catalyzed pathway and from N-protonated amides (pK_{N_1}) for the acid-catalyzed path. From the rate constants (k_H and k_{OH}), the respective values of pK_{N_1} and pK_{N_2} for *N*-methylacetamide are computed at −7.8 and 17.7. The tautomerization constant for O–N protonation of cationic *N*-methylacetamide is 10.7 The k_H value for *N*-methylurea is about 10^4 greater than that for *N*-methylacetamide due to a much higher nitrogen basicity for *N*-methylurea. The kinetic isotope effect between CONH and COND is less than 15% for both hydronium and hydroxide ion catalyzed exchange pathways.

In order to more satisfactorily utilize amide hydrogen exchange (HX)[4,5] as a probe of static and dynamic aspects of polypeptide molecular structure, recent studies have been directed toward delineation of the factors which influence hydrogen exchange rates of simple amides[6-9] and small model peptides[10-13] (eq 1 where H* is protium, deuterium, or tritium). From

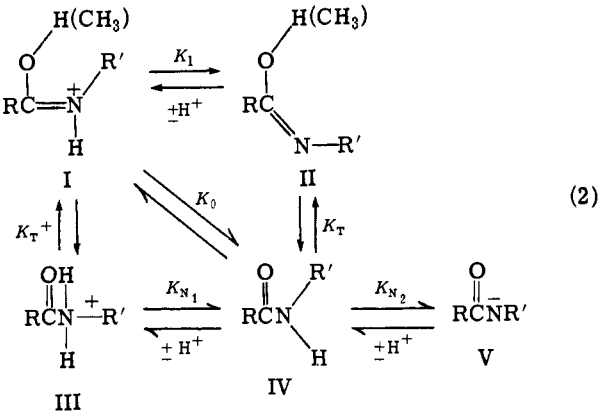


(1)

the rates of hydrogen–deuterium (H–D) exchange in dioxane–deuterium oxide for *N*-acetyl- and *N*-chloroacetylglycine derivatives measured by an ir technique and analysis of amide hydrogen exchange rates for several polypeptides, Leichtling and Klotz[9] concluded that in addition to inductive effects rather less well defined steric and/or local solvation effects may be operative.

We wish to present here results of a systematic study of polar effects of neighboring substituents on both the hydronium and hydroxide ion catalyzed amide hydrogen exchange rate constants of *N*-methylamide and peptide derivatives measured by nmr methods. These studies have shown marked substituent effects on both the specific acid and specific base catalyzed exchange rates which have been satisfactorily correlated solely with substituent constants for *inductive* effects.

From the formulation of the mechanisms of hydronium and hydroxide ion catalyzed hydrogen exchange as diffusion limited proton transfer reactions among oxygen and nitrogen atoms in aqueous solution,[14] the proton dissociation constants for the N-protonated (pK_{N_1}) and neutral (pK_{N_2}) amides are estimated (eq 2). While



(2)

the present work was in progress, Sheinblatt[13] reported the effect of ammonium and carboxylate ion and amide substituents on the hydroxide ion catalyzed amide hydrogen exchange (H–H) rates of model peptides in water[7] and related the variations in rates to differences in acidities of the peptide protons (*i.e.*, pK_{N_2}) in a manner analogous to that described herein. The pK_{N_1} value in conjunction with the macroscopic pK_M values for cationic *N*-methylacetamide proton dissociation permits an estimate of the values of K_0 and the related tautomerization constant K_{T^+}.

An extension of the present study to include the effects of vicinal peptide groups and amino acid side-chain

(1) This research was supported by the National Institutes of Health United States Public Health Service, Grants GM 13,777 (R. G. K.), FR 5415 (School of Medicine), and FR 15 (University of Pennsylvania Medical Computer Facility). Further details on the work in this paper are available in the Doctoral Dissertation of R. S. Molday, University of Pennsylvania, 1971.

(2) Abbreviations used are: DSS, dimethyl 2-silapentanesulfonate; DMSO, dimethyl sulfoxide; pK, the negative logarithm of the proton dissociation constant; TMS, tetramethylsilane.

(3) Predoctoral Fellow, National Institutes of Health, 1966–1971.

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(7) A. Berger, A. Lowenstein, and S. Meiboom, *ibid.*, **81**, 62 (1959).

(8) I. Klotz and B. Frank, *ibid.*, **87**, 2721 (1965).

(9) B. Leichtling and I. Klotz, *Biochemistry*, **5**, 4026 (1966).

(10) S. Nielsen, W. Bryan, and K. Mikkelsen, *Biochim. Biophys. Acta*, **42**, 550 (1960).

(11) M. Sheinblatt, *J. Amer. Chem. Soc.*, **87**, 572 (1965).

(12) M. Sheinblatt, *ibid.*, **88**, 2123 (1966).

(13) M. Sheinblatt, *ibid.*, **92**, 2505 (1970).

(14) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964).

groups on hydrogen exchange rates of peptides is reported elsewhere.[15]

Experimental Section

Materials. The following compounds were obtained from commercial sources: *N*-methylacetamide (Eastman, vacuum redistilled), *N*-methylformamide (Aldrich, vacuum redistilled), *N*-methylurea (Aldrich), and *N*-acetylglycine and *N*-(acetyl)-glycine-*N'*-methylamide (Cyclo). The following $RCONHCH_3$ derivatives were synthesized[1] by aminolysis of the corresponding ethyl ester using 40% aqueous methylamine solution and recrystallized: R = $ClCH_2$, mp 45–46° (lit.[16] 46°); Cl_2CH, mp 73–75°; Cl_3C, mp 102–103° (lit.[17] 105°); F_3C, mp 48–50° (lit.[18] 50°); $H_3NCH_2^+$ (hydrochloride), mp 153–155°. *N*-(Trifluoroacetyl)glycine-*N'*-methylamide was prepared[1] from ethyl trifluoroacetate and glycine-*N'*-methyamide; dec >160°. The nmr chemical shifts, fine structures, and integrated areas were those expected for the compounds. Reagent grade chemicals and deionized water of specific resistance greater than 5×10^5 ohm cm were used without further purification. Deuterium oxide (Bio-Rad) of purity 99.8% was redistilled. DMSO was redistilled from calcium hydride[19] and stored over a molecular sieve (activated, Linde Type 4A, Matheson).

Instruments. Proton magnetic resonance measurements were made with a Jeolco Model JNM-C-60H, 60-MHz high-resolution nmr spectrometer with a probe temperature of 25 ± 1° and, in general, a tenfold recorder X-scale expansion. A Radiometer Model 4 pH meter calibrated with buffers at pH 1.00, 4.01, 7.43, and/or 10.0 was used with a combination glass–calomel electrode for pH measurements at the termination of reactions. A Digital Equipment Corporation PDP-6 digital computer was used in the analysis of nmr line shapes in conjunction with an Oscar Analog to Digital Converter (Benson-Lehner) to digitalize frequencies and intensities.[1]

Nmr Methods. Chemical shifts are reported in parts per million (δ) downfield from the internal references, DSS in aqueous solutions and TMS in DMSO.

(1) **Deuterium–Hydrogen (D–H) Exchange.** Samples of *N*-(acetyl)glycine-*N'*-methylamide with deuterium on the amide nitrogens were prepared by repeated dissolution in deuterium oxide and lyophilization. The D–H exchange was initiated by dissolving the amide-deuterated sample in 0.8 ml of 0.1 *M* aqueous KCl solution buffered at the desired pH with acetate or formate (0.02 *M*) maintained at 25°. The solution was quickly transferred into an nmr tube and inserted into the probe, and recordings were taken (initial recordings were taken within 1 min of initiation with the nmr spectrometer locked on an external control signal). During the exchange, the amplitude of the acetyl proton signal was used as an internal monitor of resolution. Rate measurements for amide D–H exchange were made by scanning the signals of the protons on the carbon adjacent to the nitrogen atom at various times. From the slopes of semilogarithmic plots of $A_t - A_\infty$ *vs.* time, where A_t and A_∞ are the amplitude values at the given and infinite times, respectively, observed pseudo-first-order rate constants were obtained.

(2) **Hydrogen (H–H) Exchange.** Amide hydrogen exchange rates at 25 ± 1° were determined from analysis of the nmr line shape of the *N*-methyl (or *N*-methylene) protons adjacent to the amide nitrogen with the spectrometer locked on an internal signal. No dependence of line shape on amide concentration (0.05–0.5 *M*, depending upon solubility) was detected. The solutions were adjusted to desired pH values with concentrated NaOH or HCl and pH was maintained with HCl, acetate, phosphate, or borate buffers. Except for acidic solutions of pH <1, the ionic strength was maintained at 0.1 *M* with KCl, and for *N*-methylformamide no effect on the rate of variation of ionic strength over the range 0.1–1.0 *M* was detected.

H–H Exchange Rate Constants from Line-Shape Analysis. The mean residence time, τ, of the amide proton as a function of pH was determined from (a) the peak-to-peak separation[6] or (b) the line width at half-maximum amplitude for the *doublet* to *singlet*[6] coalescence or, more reliably, (c) *total* line-shape analysis[20,21] (Table I). In the former methods, values from ten scans were averaged for each reaction mixture and the constant parameters for (i) the peak-to-peak separation and (ii) the effective line width at half-maximum amplitude for the doublet in the *absence* of exchange were obtained from the spectrum of an aqueous solution of the amide at pH values for which amide hydrogen exchange is too slow to affect the shape of the signal, $\tau > 1$ sec. In the analysis by *total* line shape, the value of τ was varied systematically to minimize the squared deviation between the normalized experimental and theoretical intensities at 48 frequencies.[1]

Table I. Amide Proton Mean Residence Times[a]

pH	τ, sec	
	Method 1[b]	Method 2[c]
7.29	0.262	0.329
7.44	0.190	0.241
7.60	0.150	0.150
7.72	0.120	0.117
7.87	0.084	0.084
7.96	0.061	0.059
8.11	0.044	0.044
8.24	0.031	0.032

[a] Obtained from coalescence of the *N*-methyl doublet of *N*-(acetyl)-glycine-*N'*-methylamide at 25°, ionic strength 0.1 *M*. [b] Peak–peak separation or width at half-maximum amplitude. [c] Total line-shape analysis.

At $\tau > 1$ sec for the several amide solutions for which each component of the *N*-methyl doublet consists of partially resolved multiplets (due to long-range spin–spin interaction with the acyl (acetyl) protons) effective line widths[7] and transverse relaxation times, T_2', were measured from a Lorentzian line forming the envelope of the multiplet. The pseudo-first-order rate constants are related to the mean residence times by $k_{obsd} = 1/\tau$.

Results

Nmr Spectral Characteristics of Amides. Proton magnetic resonance spectra of substituted *N*-methylamides exhibit features similar to those of *N*-methylacetamide:[7] the *N*-methyl and *N*-methylene proton singlets at 2.7–2.9 and 3.8–4.2 ppm, respectively, in deuterium oxide appear as doublets (J = 4.8 and 5.8–6.1 Hz, respectively) under conditions of slow exchange in aqueous solutions as a result of spin–spin interactions with the adjacent amide proton.

At tenfold recorder X-scale expansion, each component of the *N*-methyl doublet of *N*-methylacetamide can be resolved into a quartet[7] and the *N*-methyl doublet components of *N*-methylchloroacetamide, glycine-*N'*-methylamide, and *N*-(acetyl)glycine-*N'*-methylamide can be resolved into triplets as a result of coupling between the acetyl or *N*-methylene protons and the *N*-methyl protons (J = 0.5 Hz). Long-range coupling could not be resolved for *N*-methyldichloroacetamide or *N*-methylurea. Upon expanded scale each component of the *N*-methyl doublet for *N*-methyltrifluoroacetamide can be resolved into a quartet as a result of fluorine–proton coupling (J = 0.7 Hz), as has been observed for *N*,*N*-dimethyltrifluoroacetamide (trans methyl group).[22]

In anhydrous DMSO, as the electron-withdrawing nature of the substituent increases, the broadened amide proton signals appear progressively downfield from TMS (δ 7.7–9.4 ppm). Additions of water up

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(19) B. M. Lynch, B. C. Macdonald, and J. G. K. Webb, *Tetrahedron*, 24, 3595 (1968).
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(21) A. Allerhand, H. S. Gutowsky, J. Jonas, and R. A. Meinzer, *J. Amer. Chem. Soc.*, 88, 3185 (1966).
(22) M. T. Rogers and J. C. Woodbrey, *J. Phys. Chem.*, 66, 540 (1962).

Table II. Second-Order Rate Constants for Hydronium and Hydroxide Ion Catalyzed Amide Proton Exchange at 25°, Ionic Strength 0.1 M[a]

Substituted N-methylamide	k_{OH}, M^{-1} sec^{-1}	k_H, M^{-1} sec^{-1}
N-Methylacetamide	$4.19 \pm 0.30 \times 10^6$	$3.60 \pm 0.15 \times 10^2$
N-Methylformamide	$3.39 \pm 0.30 \times 10^7$	$1.13 \pm 0.08 \times 10$
N-Methylchloroacetamide	$1.70 \pm 0.17 \times 10^8$	
N-Methyldichloroacetamide	$1.78 \pm 0.12 \times 10^9$	
N-Methyltrichloroacetamide	$1.65 \pm 0.14 \times 10^9$	
N-Methyltrifluoroacetamide	$4.07 \pm 0.25 \times 10^9$	
N-Methylurea	$9.11 \pm 0.45 \times 10^5$	$2.66 \pm 0.18 \times 10^6$
N-Acetylglycine[b]	$3.08 \pm 0.20 \times 10^6$ [b]	$2.95 \pm 0.25 \times 10^b$
N-(Acetyl)glycine-N'-methylamide[c]	$1.70 \pm 0.12 \times 10^7$	$4.57 \pm 0.32 \times 10$
N-(Acetyl)glycine-N'-methylamide[d]	$7.10 \pm 0.70 \times 10^7$	$2.00 \pm 0.20 \times 10$
Glycine-N'-methylamide	$4.90 \pm 0.40 \times 10^8$	
N-(Trifluoroacetyl)glycine-N'-methylamide[b]	$2.16 \pm 0.22 \times 10^{10}$	

[a] ± standard errors. [b] k_H is for $CH_3C(=O)NHCH_2C(=O)OH$; k_{OH} is for $CH_3C(=O)NHCH_2C(=O)O^-$ (pK = 3.60). [c] N-Methylamide hydrogen exchange rates. [d] N-Methylenamide hydrogen exchange rates.

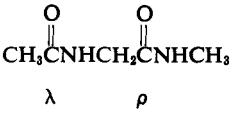
to 1% (v/v) had no effect on the position of the amide proton signal but caused large upfield shifts in the position of the signal of the ammonium ion protons of glycine-N'-methylamide hydrochloride.

Amide Hydrogen Exchange Rates. The exchange of amide hydrogens with water follows pseudo-first-order kinetics at constant pH with the observed rate constant independent of amide concentration.[7,13] The pH–rate profiles for amide hydrogen exchange (Figure 1) indicate catalysis by hydronium and hydroxide ions as described by eq 3 where k_H and k_{OH} are the specific

$$k_{obsd} = k_H a_H + k_{OH} a_{OH} = k_H a_H + k_{OH} K_w / a_H \quad (3)$$

acid and specific base catalyzed rate constants, respectively, K_w is the autoprotolysis constant for water, and a_H and a_{OH} are the activities of the solvated proton and hydroxide ion, respectively. There is no evidence for potassium phosphate buffer catalysis of amide proton exchange in aqueous solutions (pH 8, 0.001–0.1 M) with N-methylformamide, which is in accord with other reports.[9,15,23]

Specific Base Catalyzed Amide Hydrogen Exchange. At pH values above 5, the contribution of specific base catalysis to the observed rate of amide hydrogen exchange is dominant (second term in eq 3). Substituents which exert progressively greater electron-withdrawing ability result in an *increase* in the base-catalyzed amide hydrogen exchange rate constants of greater than 10^3 when N-methylacetamide and N-methyltrifluoroacetamide are compared. Replacement of a proton of the N-methyl groups of N-methylacetamide with an adjacent amide group, $C(=O)NHCH_3$, as in N-(acetyl)glycine-N'-methylamide (λ peptide)



results in a 17-fold increase in the rate constant for specific base catalyzed proton exchange. The effect of a negatively charged carboxylate group in N-acetylglycine, however, decreases the exchange rate less than a factor of 1.4 relative to N-methylacetamide. A second-order hydroxide ion catalyzed rate constant of 3.1×10^6 M^{-1} sec^{-1}, determined for hydroxide ion catalyzed amide hydrogen exchange of N-acetylglycine at 25 ± 1°, is comparable with the value 2.7×10^6 M^{-1} sec^{-1} reported by Sheinblatt[13] at 23 ± 2°. The rate constants for hydrogen exchange of substituted amides at 25° are summarized in Table II.

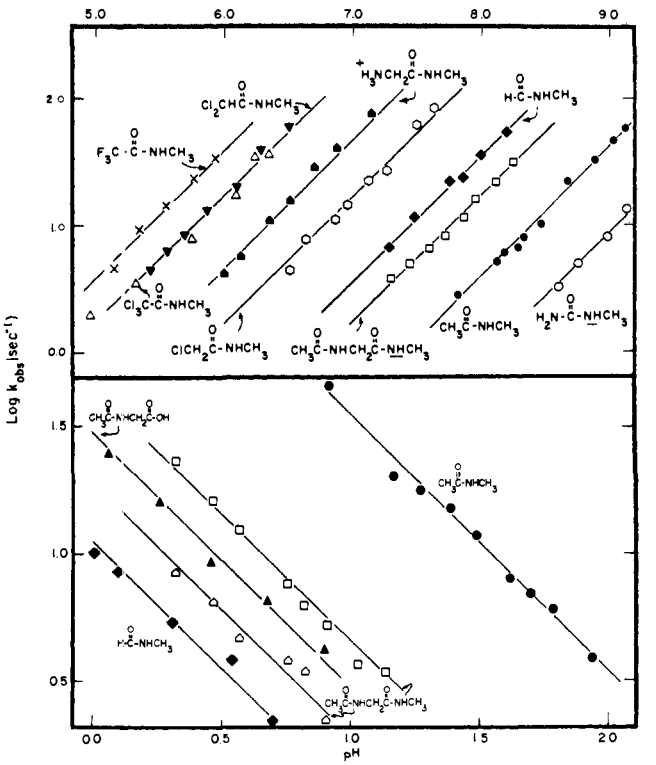


Figure 1. Dependence of the observed pseudo-first-order rate constants for amide proton exchange of substituted N-methylamides upon pH, at 25°, ionic strength 0.1 M.

Specific Acid Catalyzed Amide Hydrogen Exchange. There is a first-order dependence of the rate of amide hydrogen exchange on hydronium ion activity below pH 4 (first term in eq 3) for several amide derivatives: for N-methylacetamide at 25 ± 1° the second-order acid-catalyzed rate constant, k_H, is 360 M^{-1} sec^{-1} which may be compared to the value of 380 M^{-1} sec^{-1} at 23 ± 2° reported by Berger, *et al.*[7]

In general, electron-withdrawing substituents are associated with a *decrease* in the hydronium ion catalyzed rate constants and the values of k_H for amide protons adjacent to the N'-methyl and N-methylene groups of N-(acetyl)glycine-N'-methylamide are lower by about 10- and 20-fold, respectively, relative to N-

(23) S. W. Englander and A. Poulsen, *Biopolymers*, 7, 379 (1969).

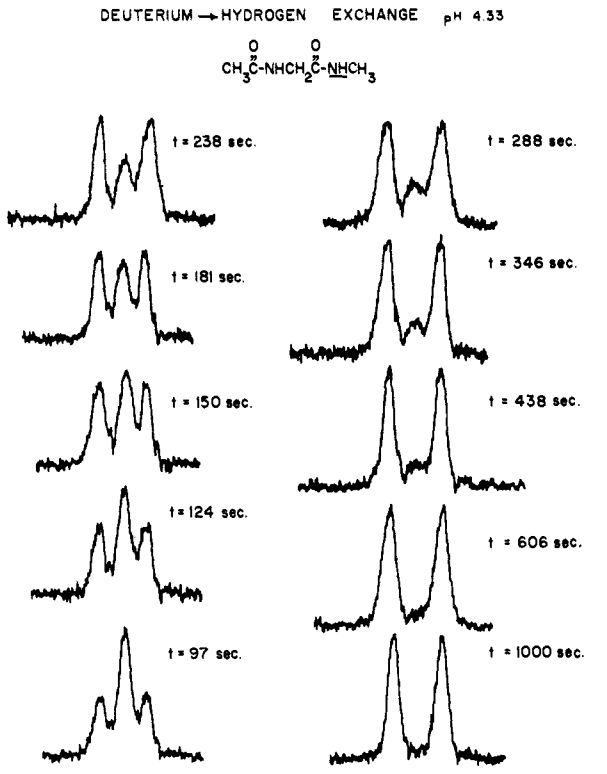


Figure 2. Time dependence of the *N*-methyl singlet to doublet transition associated with the exchange of deuterium with hydrogen for *N*-(acetyl)glycine-*N'*-methylamide at pH 4.33, 25°, ionic strength 0.1 *M*.

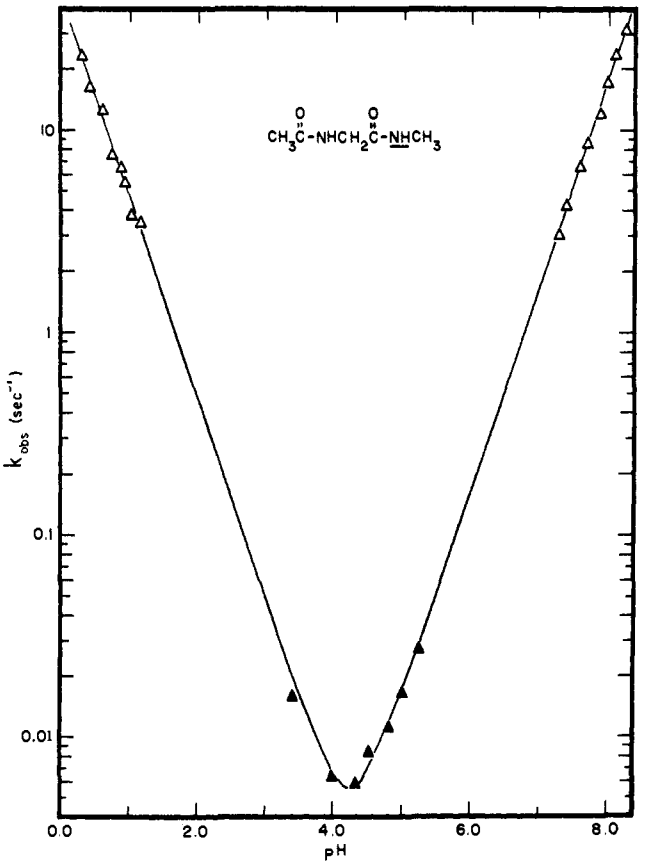


Figure 3. Dependence of the first-order rate constant for hydrogen or deuterium exchange upon pH for the amide adjacent to the *N'*-methyl group of *N*-(acetyl)glycine-*N'*-methylamide at 25°: hydrogen exchange rates (△) from coalescence of *N'*-methyl doublet by nmr line-shape analysis; deuterium–hydrogen exchange rates (▲) from time-dependent change in the *N'*-methyl signal. The *solid line* is calculated from eq 3 and the rate constants in Table II.

methylacetamide due to the polar effect exerted by the neighboring peptide groups. *N*-Methylamides with more strongly electron-withdrawing substituents such as fluoro, chloro, or ammonium ion groups exhibit no observable *N*-methyl line broadening even as low as pH ~0, indicating that these substituents have slowed the exchange rate so much that exchange is not detectable by the line-shape technique. Leichtling and Klotz[9] have shown a similar negative polar effect of electron-withdrawing substituents on the acid-catalyzed H–D exchange rate of amides in dioxane–deuterium oxide.

Unlike most *N*-methylamides, acid-catalyzed exchange of *N*-methylurea is detectable at pH 5–6 as a result of a value of k_H that is 10^4 higher than that for *N*-methylacetamide. The value of 2.5×10^4 M^{-1} sec^{-1} at 25° for the hydronium ion catalyzed amide hydrogen exchange rate constant for thiourea[24a] is 100-fold smaller than the values for k_H for *N*-methylurea[24c] and urea.[24f] The second-order hydronium ion catalyzed rate constants for amide hydrogen exchange at 25° are contained in Table II.

(24) (a) R. L. Vold and A. Correa, *J. Phys. Chem.*, 74, 2674 (1970). (b) The utilization of pK_{H^+} and pK_w values of 0 and 14 for the proton dissociations from H_3O^+ and HOH, respectively, allow for the 55.5 *M* concentration of water in the calculation of the pK_{N_1} and pK_{N_2} values. (c) This is probably due to the lesser N basicity related to the greater contribution of the valence bond structure $H_2N^+=C(S^-)NH_2$ to the analogous resonance hybrid of thiourea compared with urea as a consequence of the difficulty in forming double bonds between carbon and the large sulfur atom.[24d] These resonance differences are consistent with the decreased nucleophilicity of thiourea compared with urea toward formaldehyde.[24e] (d) W. P. Jencks, C. Moore, F. Perini, and J. Roberts, *Arch. Biochem. Biophys.*, 88, 193 (1960). (e) K. Dusek, *Collect. Czech. Chem. Commun.*, 25, 108 (1960); (f) R. L. Vold, E. S. Daniel, and S. O. Chan, *J. Amer. Chem. Soc.*, 92, 6771 (1970). (g) L. C. Martinelli, C. D. Blanton, and J. F. Whidby, *ibid.*, 93, 5111 (1970).

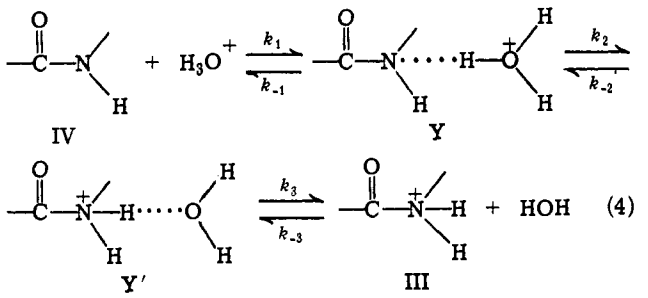
Amide Deuterium–Hydrogen (D–H) Exchange. Exchange of deuterium for hydrogen (eq 1, where H* is deuterium) can be measured from the time-dependent change in the nmr signal of the protons on the carbon adjacent to the amide nitrogen following dissolution of the deuterated amide into water. There is a decrease in amplitude of the central singlet peak and an increase in amplitude of the flanking doublet peaks for the *N*-methyl nmr signal of *N*-(acetyl)glycine-*N'*-methylamide (Figure 2) upon D–H exchange. The kinetics of the amplitude changes are pseudo first order for at least three half-times. The dependence of the observed pseudo-first-order rate constants of D–H exchange upon pH (Figure 3) shows no significant pH-independent region and confirms that the contribution of solvent catalysis to the rate expression for amide hydrogen exchange is negligible (eq 3) in aqueous solution, in agreement with previous studies.[4,7,9]

Comparison of hydrogen (H–H) and deuterium (D–H) exchange rates in water (Figure 3) for measurements in different regions of acidity shows that the second-order rate constants obtained for specific acid–specific base catalyzed D–H exchange are within 15% of the constants for H–H exchange.

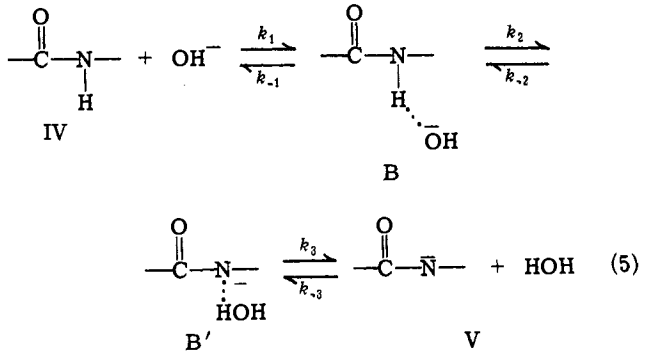
Discussion

The mechanisms of the specific acid and base catalyzed proton exchange of amides, as originally for-

mulated by Berger, *et al.*,[7] proceed through the less stable[25] N-protonated amide tautomer (III in eq 2) as described by eq 4 or through the amide anion V as



described by eq 5, respectively. These formulations



incorporate the generalized treatment of diffusion-limited proton transfers[14] in which, following the formation of a hydrogen-bridged complex (Y or B) by diffusion-limited encounter steps (k_1) and rapid equilibration of Y $\rightleftharpoons$ Y′ or B $\rightleftharpoons$ B′, the N-protonated amide and water (eq 4) or amide anion and water (eq 5) separate in diffusion-limited processes, respectively.

The observed rates of proton transfer for the schemes of eq 4 and 5 are given by eq 6 from Eigen[14] which

$$k_{\text{exchange}} = k_1/\{1 + 10^{-(\text{p}K_{\text{acceptor}} - \text{p}K_{\text{donor}})}\} \qquad (6)$$

applies to the present data. With eq 6 the pK_{N_1} values for the conjugate acid of the *acceptor* amides can be calculated from the rate constants for specific acid catalyzed exchange and the pK_{H^+} value of zero for the solvated proton *donor*.[24b] Similarly, with eq 6 the pK_{N_2} values for the amide *donors* can be calculated from the rate constants for specific base catalyzed exchange and the pK_w value of 14 for the conjugate acid of the acceptor hydroxide ion.[24b]

Specific Base Catalyzed Amide Hydrogen Exchange. The relative effect of substituents on the specific base catalyzed amide proton exchange rate constants can be correlated with the pK values of corresponding carboxylic acids from which they are derived (Figure 4) or alternatively with inductive substituent constant (σ_I) values from Charton[26] (not shown). With the exception of *N*-(trifluoroacetyl)glycine-*N*′-methylamide the observed rates of hydroxide ion catalyzed amide hydrogen exchange rates are less than the diffusion-controlled limit of about 10^{10} M^{-1} sec^{-1} which indicates that the pK_{N_2} values of these amides (eq 2) are greater than that of water ($pK_w = 14$).[24b] The slope of $+0.87 \pm 0.14$ (Figure 4, p$K > 2$) indicates that the substituent

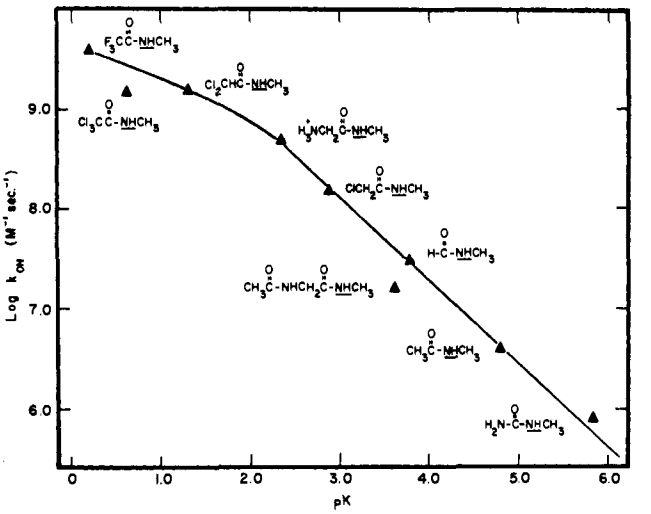


Figure 4. Dependence of the second-order rate constant for hydroxide ion catalyzed amide proton exchange of substituted amides upon the pK value of the corresponding substituted carboxylic acid at 25°, ionic strength 0.1 M (see ref 33). The pK value of carbamic acid is from ref 28. The slope for the data for p$K > 2$ is 0.87 ± 0.14 by least-squares methods.

effects on the base-catalyzed exchange rates for this series of amides are similar to the effects of substituents on the pK of the corresponding carboxylic acids. Sheinblatt[13] has reported hydroxide ion catalyzed amide hydrogen exchange rate constants for a series of glycine derivatives, $H_3N^+(CH_2)_nC(=O)NHCH_2COO^-$, in which n was varied from 1 to 4 in order to vary the distance and interaction between the ammonium ion and the amide group. The slope for the linear free-energy correlation of the log k_{OH} values and substituent effects (as expressed by the parent carboxylic acid pK values) for the glycine derivatives was $+1.0$ and not significantly different from the value of $+0.87$ in the present work (Figure 4). The rate constants for hydroxide ion catalyzed amide hydrogen exchange become less sensitive to substituent effects for extremely electron-withdrawing groups as indicated by the downward deviations from a slope of $+0.87$ that occur for compounds with pK values for the parent carboxylic acid of less than 2 (Figure 4). This result is consistent with an approach to the condition ($pK_{acceptor} - pK_{donor} > 0$ (eq 6) and the diffusion-limited rate constant of *ca.* 10^{10} M^{-1} sec^{-1}.[14] That substituents do *not* reach a limiting ability to lower amide pK_{N_2} values is suggested by the continued progression of N–H chemical shifts in DMSO to lower field with increases in the electron-withdrawal effect of the substituent (Figure 5) even for the trifluoro and trichloro groups: such shifts appear to be related to the ability of substituents to progressively lower the electron density at and increase the acidity of the amide proton.[19] The value of 2.2×10^{10} M^{-1} sec^{-1} measured for the *N*-methylenamide proton exchange rate constant of *N*-(trifluoroacetyl)glycine-*N*′-methylamide, which contains two electron-withdrawing groups, is in the range for diffusion-limited proton transfer reactions[14,27] and comparable to the value of 1×10^{10} M^{-1} sec^{-1} reported by Sheinblatt[13] for the *N*-methylenamide group of glycylglycinamide.

(25) G. Fraenkal and C. Franconi, *J. Amer. Chem. Soc.*, **82**, 4478 (1960).
(26) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).
(27) P. Debye, *Trans. Electrochem. Soc.*, **82**, 265 (1942).

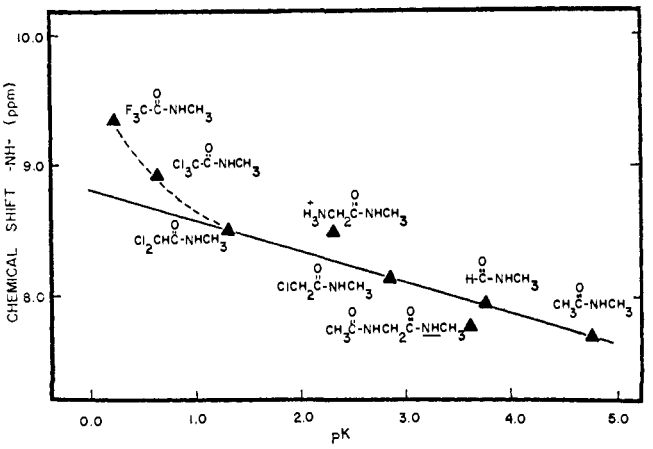
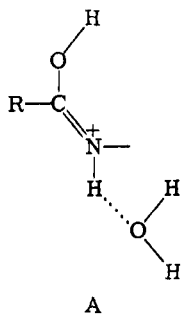


Figure 5. Dependence of the amide hydrogen chemical shift for substituted *N*-methylamides in DMSO (TMS internal reference) upon the p*K* value of the corresponding carboxylic acid at 25°.

The decrease in the hydroxide ion catalyzed amide hydrogen exchange rate constant of *N*-methylurea relative to *N*-methylacetamide can be rationalized by the contribution of the formal valence bond structure, $H_2^+N{=}C(O^-)NHCH_3$, to the resonance hydrid which introduces a type of resonance that would be expected to decrease the acidity of the proton at the *N*-methyl site. In spite of the somewhat different nature of the ureido group compared to the amide group, the log k_{OH}–p*K* correlation for amides satisfactorily encompasses *N*-methylurea and carbamic acid[28] (Figure 4).

Specific Acid Catalyzed Amide Hydrogen Exchange. The slope is about −1.3 for the correlation of log k_H values with p*K* values of the parent carboxylic acid for *N*-methylacetamide, *N*-methylformamide, and the ρ peptide of *N*-(acetyl)glycine-*N'*-methylamide (Table II). The effect of electron-withdrawing substituents is to decrease the hydronium ion catalyzed amide hydrogen exchange rate constant and is consistent with a decrease in basicity of the amide nitrogen atom[24g] (pK_{N_1} in eq 2). There is, however, a kinetically indistinguishable mechanism for hydronium ion catalyzed amide hydrogen exchange which involves an initial protonation of the amide oxygen followed by removal of the nitrogen-bound proton from the O-protonated tautomer of the conjugate acid of the amide (I in eq 2) by water. Such a mechanism (A) may be expected



A

to be relatively insensitive to substituents, since the *decrease* in basicity of the carbonyl oxygen, K_O, may be expected to be balanced by an *increase* in acidity of the nitrogen-bound proton of the O-protonated amide, K_I (eq 2), for electron-withdrawing substituents. Thus, the observed substantial substituent effects upon k_H values, which are similar in magnitude but opposite in direction to the substituent effects upon k_{OH} values for which the mechanism of exchange appears unambiguous, provide evidence to support the mechanism of eq 4 rather than mechanisms which involve the more stable O-protonated amide intermediates.[25]

The value of the hydronium ion catalyzed amide hydrogen exchange rate constant for *N*-methylurea is 10^4 larger than that found for *N*-methylacetamide and may be explained in part by the contributions of the valence bond forms, $H_2N^+{=}C(O^-)NHCH_3$ and $H_2^+N{=}C(O^-)N^+H_2{-}CH_3$, to the resonance hybrids for neutral and, more importantly, cationic *N*-methylurea, respectively. The net effect of such resonance contributions is to provide relatively greater stabilization of N-protonated urea and Y′ with reference to the neutral species and Y (eq 4) in the case of ureas than in the case of amides, *i.e.* to make *N*-methylureas more basic than *N*-methylamides.[24g] This effect facilitates both N-protonation and hydronium ion catalyzed exchange in ureas relative to *N*-methylamides.

Proton Dissociation and Tautomerization Constants for Neutral and Protonated Amides. From the hydroxide ion catalyzed amide hydrogen exchange rate constant for *N*-methylacetamide (Table II), eq 6 and the value for k_1 of 2×10^{10} M^{-1} sec^{-1}, the pK_{N_2} value for *N*-methylacetamide is estimated at 17.7. The electron-withdrawal effects of neighboring peptide groups decrease the pK_{N_2} value of a peptide proton in a polypeptide molecule such as poly-D,L-alanine approximately 2 units relative to *N*-methylacetamide.[23] If the diffusion-controlled rate constant for hydronium ion encounter with an amide is similar to that of hydroxide then k_1 in eq 6 is 2×10^{10} M^{-1} sec^{-1} and pK_{N_1} values for N-protonated conjugate acid tautomers (eq 2) of *N*-methylacetamide and *N*-methylureas are estimated from eq 6 at −7.8 and −3.9, respectively. From the value of pK_{N_1} the macroscopic p*K* value of −0.46 for the conjugate acids of *N*-methylacetamide,[29] where $K_M = [\text{amide}][H^+]/\{[C(OH){=}N^+HR] + [C(=O)N^+H_2R]\}$ and the relation $1/K_M = 1/K_O + 1/K_{N_1}$, the value of the tautomerization constant, $K_{T^-} = [C(OH){=}N^+HR]/[C(=O)N^+H_2R] = K_{N_1}/K_O$, is about 10^7 in favor of O-protonation (eq 2). From pK_M and pK_I values of −0.46 and 7.5, respectively (the latter for the conjugate acid of O,*N*-dimethylacetimidate[30]), the value of the tautomerization constant, $K_T = [C(OH){=}NR]/[C(=O)NHR] = K_I/K_O$, is computed at 10^{-8} (eq 2 and ref 31). The values for pK_{N_1} and K_T reported by Fersht[31] based upon a different approach are in good agreement with our estimates. The values of K_{T^+} and K_T reflect the differences in basicity on O and N atoms and the differences in resonance stabilization within the prototropic tautomers.

Isotope Effects. The absence of a significant kinetic deuterium isotope effect on the hydronium and hydroxide ion catalyzed amide exchange rate constants (*i.e.*, H–H exchange compared with D–H exchange,

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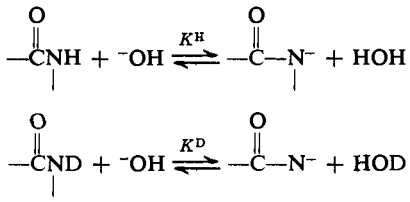
Figure 3) is consistent with the mechanisms of eq 4 and 5.

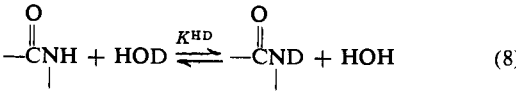
In the mechanism for specific acid catalysis, proton transfer to the amide nitrogen by hydronium ion does not contribute to the rate determining step for exchange which involves the diffusion-limited separation and approach of water and N-protonated amide (eq 4). Secondary kinetic isotope effects, if present, are expected to be small.[32]

For specific base catalysis, a competition for the protium or deuterium exists between the amide nitrogen and the hydroxide ion oxygen (k_{-2}/k_2 in eq 5). If little or no isotope effect is present in the diffusion rate constant, k_1, the isotope effect should be a reflection of the equilibrium constants for amide deprotonation (eq 7). Thus, the ratio of equilibrium constants is



(7)

equal to the equilibrium expression for the binding of deuterium *vs.* protium (eq 8), where $K^{HD} = K^H/K^D$.



(8)

In this regard, an equilibrium isotope effect of 21% has been measured for tritium–hydrogen exchange and of 13% for tritium–deuterium exchange with poly-D,L-alanine by Englander and Poulsen.[23]

An accurate estimate of the isotope effects in the present work is not possible but the results indicate that such effects are within the experimental uncertainty of about 15%.

Comparison of amide hydrogen exchange in H_2O with hydrogen exchange in D_2O[8,10] for *N*-methylacetamide indicates that amide hydrogen exchange is faster in D_2O by approximately a factor of two for both specific acid and specific base catalysis. This inverse kinetic isotope effect is related in part to the difference in basicity of H_2O (K_w = 14.0 at 25°) and D_2O (K_w' = 14.8 at 25°).[33]

Acknowledgment. We wish to extend our appreciation to Dr. S. W. Englander for his active interest, support, and numerous valuable discussions pertinent to this work. We wish also to thank Dr. R. O. Viale and Mr. C. Choi for helpful discussions and assistance.

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Conformational Properties of Some Ortho-Substituted 1,1-Diphenylethanes

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Abstract: We report here the results of an investigation of the conformational properties of some 1,1-diphenylethanes. Nmr spectra provide a generally applicable method for studying the conformational preference of these compounds through the detection of the ring current shielding effects. In our approach, semiempirical conformational energy calculations have been used to build contour maps of relative conformational energy as a function of the two internal rotation angles of these molecules. Conformations of minimum energy, as detected from the contour maps, have been assumed as the most stable. The theoretical ring current effects corresponding to these conformations have been calculated and the predicted shieldings on the ortho nuclear positions and α-hydrogen atoms have been found in agreement with those experimentally observed. Barriers to internal rotation, as detected from the energy contour maps, have been compared with the experimental barriers but only a semiquantitative agreement has been found.

In previous studies,[1] we have investigated (mainly by nmr) the conformational properties of bridged aromatic compounds of the type Ar–X–Ar (X = CH_2, O, S, SO_2, CO). We report here a similar study on some 1,1-diphenylethanes (compounds I–III). Nmr spectra provide a generally applicable method to detect the conformational preference in compounds of the type Ar–X–Ar.

In fact, due to the proximity of the two aromatic rings, the shielding of the ring current[2] of the adjacent nucleus on the ortho positions of the other ring is a function of the molecular conformation. The presence of a methyl group at the bridge position in 1,1-diphenylethanes (X = $CHCH_3$; DPE) poses steric restraints to

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