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Formation and Stability of Enolates of Acetamide and Acetate Anion: An Eigen Plot for Proton Transfer at α-Carbonyl Carbon

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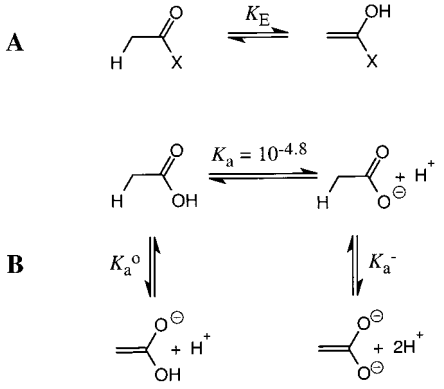
Abstract: Second-order rate constants were determined in D_2O for deprotonation of acetamide, *N,N*-dimethylacetamide, and acetate anion by deuterioxide ion and for deprotonation of acetamide by quinuclidine. The values of $k_B = 4.8 \times 10^{-8}$ M^{-1} s^{-1} for deprotonation of acetamide by quinuclidine ($pK_{BH} = 11.5$) and $k_{BH} = 2-5 \times 10^9$ M^{-1} s^{-1} for the encounter-limited reverse protonation of the enolate by protonated quinuclidine give $pK_a^C = 28.4$ for ionization of acetamide as a carbon acid. The limiting value of $k_{HOH} = 1 \times 10^{11}$ s^{-1} for protonation of the enolate of acetate anion by solvent water and $k_{HO} = 3.5 \times 10^{-9}$ M^{-1} s^{-1} for deprotonation of acetate anion by HO^- give $pK_a^C \approx 33.5$ for acetate anion. The change in the rate-limiting step from chemical proton transfer to solvent reorganization results in a downward break in the slope of the plot of log k_{HO} against carbon acid pK_a for deprotonation of a wide range of neutral α-carbonyl carbon acids by hydroxide ion, from −0.40 to −1.0. Good estimates are reported for the stabilization of the carbonyl group relative to the enol tautomer by electron donation from α-SEt, α-OMe, α-NH_2, and α-O^- substituents. The α-NH_2 and α-OMe groups show similar stabilizing interactions with the carbonyl group, while the interaction of α-O^- is only 3.4 kcal/mol more stabilizing than for α-OH. We propose that destabilization of the enolate intermediates of enzymatic reactions results in an increasing recruitment of metal ions by the enzyme to provide electrophilic catalysis of enolate formation.

Introduction

In recent years there has been good progress toward determining the kinetic and thermodynamic parameters for enolization of a variety of simple carbonyl compounds in water, including aldehydes,[1,2] ketones,[2,3] thiolesters,[4] oxygen esters,[5−7] carboxylic acids,[7] and more complex derivatives of these simple functional groups.[8] However, the kinetic and thermodynamic barrier to formation of the highly unstable enols of simple amides (Scheme 1A, X = NR_2) and carboxylate ions (X = O^-) in water is still a matter of speculation by chemists interested in defining the magnitude of the stabilizing interactions between α-NR_2 and α-O^- substituents and the carbonyl group, and by biochemists interested in defining the transition state stabilization by enzymes that catalyze deprotonation of α-carboxylate carbon.[9−12]

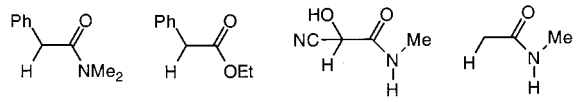
Scheme 1



Pearson and Dillon used the limited kinetic data of Bonhoeffer et al.[13] for the deuterioxide-ion-catalyzed exchange of the α-protons of acetamide for deuterium from D_2O to estimate a carbon acid pK_a of 25 for deprotonation of the α-methyl group of acetamide.[14] These authors also estimated a carbon acid pK_a of 25 for ethyl acetate, which is very similar to the pK_a of 25.6 for this carbon acid from modern work.[5] It is now known that amides are weaker carbon acids than oxygen esters, as a result of the greater π-donor ability of nitrogen as compared with oxygen, which stabilizes the amide as compared with the ester

(1) Chiang, Y.; Hojatti, M.; Keeffe, J. R.; Kresge, A. J.; Schepp, N. P.; Wirz, J. *J. Am. Chem. Soc.* **1987**, *109*, 4000−4009.
(2) Keeffe, J. R.; Kresge, A. J. In *The Chemistry of Enols*; Rappoport, Z., Ed.; John Wiley and Sons: Chichester, 1990; pp 399−480.
(3) Chiang, Y.; Kresge, A. J.; Tang, Y. S.; Wirz, J. *J. Am. Chem. Soc.* **1984**, *106*, 460−462.
(4) Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **1992**, *114*, 10297−10302.
(5) Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **1996**, *118*, 3129−3141.
(6) Rios, A.; Richard, J. P. *J. Am. Chem. Soc.* **1997**, *119*, 8375−8376.
(7) Rios, A.; Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **2000**, *122*, 9373−9385.
(8) Argyrou, A.; Washabaugh, M. W. *J. Am. Chem. Soc.* **1999**, *121*, 12054−12062. Tobin, J. B.; Frey, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 12253−12260. Bernasconi, C. F.; Moreira, J. A.; Huang, L. L.; Kittredge, K. W. *J. Am. Chem. Soc.* **1999**, *121*, 1674−1680.
(9) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 5928−5934. Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 11552−11568.
(10) Gerlt, J. A.; Gassman, P. G. *Biochemistry* **1993**, *32*, 11943−11952.

reactant,[15−19] and the greater electronegativity of oxygen as compared with nitrogen, which stabilizes an ester as compared with an amide enolate anion. There is good evidence from the



1 **2** **3** **4**

literature for pK_a > 25 for carbon ionization of acetamide in water. The pK_a of 26.6 for carbon ionization of phenyl dimethylacetamide (**1**) in DMSO is four units higher than the pK_a of 22.7 for phenyl ethyl acetate (**2**).[20] Kinetic data for the keto−enol tautomerization of the amide **3** were used to estimate p$K_E \approx 21.7$ for tautomerization of *N*-methyl acetamide (**4**) to give the corresponding enol.[21] This is larger than the estimated pK_E of 18.6 for ethyl acetate[5] and is consistent with greater stabilization of the keto tautomer by π-electron donation from an α-amino as compared with an α-alkoxy group. It is not known whether there is significant catalysis of deprotonation of acetamide in water by general (buffer) bases.

Less is known about the carbon acidity of acetate anion in water. A pK_a of 26.6 has been estimated for carbon ionization of acetic acid (K_a^o, Scheme 1B).[22a,b] A smaller carbon acidity is expected for acetate anion (K_a^-, Scheme 1B), as a result of strong destabilization of the conjugate base by the unfavorable electrostatic interaction between the neighboring anionic oxygens. The observed rate constant for exchange of the α-protons of acetate anion for deuterium from D_2O catalyzed by 1 M deuterioxide ion is about 1000-fold slower than that for the corresponding reaction of acetamide under the same conditions.[13] This shows that acetate anion is a substantially weaker carbon acid than acetamide.

We have developed methods to determine the pK_a's of a range of weak carbon acids in water.[4−7,23,24] We now report the determination of the pK_a's for carbon ionization of acetamide and acetate anion, together with estimates of the values of pK_E for keto−enol tautomerization of these compounds (Scheme 1A) and the lifetimes of their enolates in water. Our data for the carbon acidity of simple carboxylic acid derivatives provide a good measure of the relative stabilizing interactions of α-nitrogen, α-oxygen, and α-sulfur substituents with the carbonyl group, which are discussed within the context of recent computational and theoretical studies.[25−28]

The large intrinsic barrier to proton transfer at α-carbonyl carbon is generally sufficient to ensure that the rate of these reactions is limited by carbon−hydrogen bond cleavage. The present study of the carbon deprotonation of weakly acidic amides and carboxylate anions reveals a change in the rate-determining step for the deuterioxide-ion-catalyzed exchange of the α-protons for deuterium from D_2O, from proton transfer from the carbon acid to the reorganization of solvent that moves a molecule of D_2O into a "reactive" position to reprotonate the enolate to give the deuterated product. This change in the rate-determining step is similar to that observed by Eigen for "fast" proton transfer between the electronegative atoms of "normal" acids and bases.[29] In contrast to the fast proton transfer between normal acids and bases, where the change in the rate-determining step is observed to occur for nearly thermoneutral proton transfer, we find that the thermodynamic driving force to protonation of simple enolates by water must exceed 20 kcal/mol before there is a change in the rate-limiting step for proton transfer to solvent reorganization.

Experimental Section

Acetamide, *N*,*N*-dimethylacetamide, sodium acetate, quinuclidine hydrochloride, deuterium chloride (35 wt %, 99.5% D), and potassium deuterioxide (40 wt %, 98+% D) were purchased from Aldrich. Deuterium oxide (99.9% D) was purchased from Cambridge Isotope Laboratories.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra at 500 MHz were recorded at 25 °C on a Varian Unity Inova 500 NMR spectrometer. The relaxation times for the α-protons of acetamide, dimethylacetamide, and acetate anion were determined to be in the range $T_1 = 4-6$ s. In all cases the relaxation delay between pulses was at least 10-fold longer than the longest T_1 of the protons under examination. Chemical shifts were referenced to HOD at 4.67 ppm. The baseline of the NMR spectrum was subjected to a first-order drift correction before determination of integrated peak areas.

$$[DO^-] = \frac{10^{pD-pK_w}}{\gamma_{OL}} \quad (1)$$

Preparation of Solutions. The acidic protons of quinuclidine hydrochloride were exchanged for deuterium before use, and buffered solutions of quinuclidine in D_2O at $I = 1.0$ (KCl) were prepared as described previously.[4] The solution pD was determined at 25 °C using an Orion model 720A pH meter equipped with a Radiometer pHC4006-9 combination electrode. Values of pD were obtained by adding 0.40 to the reading of the pH meter.[30] The concentration of deuterioxide ion at any pD was calculated from eq 1, where $K_w = 10^{-14.87}$ M^2 is the ion product of D_2O at 25 °C,[31] and $\gamma_{OL} = 0.75$ is the apparent activity coefficient of lyoxide ion determined for the electrode under our experimental conditions ($I = 1.0$, 25 °C).

Deuterium Exchange Reactions. All reactions were carried out in D_2O at 25 °C and $I = 1.0$ (KCl).

(11) Gerlt, J. A.; Kozarich, J. W.; Kenyon, G. L.; Gassman, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9667−9669.
(12) Guthrie, J. P.; Kluger, R. *J. Am. Chem. Soc.* **1993**, *115*, 11569−11572.
(13) Bonhoeffer, K. F.; Geib, K. H.; Reitz, O. *J. Chem. Phys.* **1939**, *7*, 664−670.
(14) Pearson, R. G.; Dillon, R. L. *J. Am. Chem. Soc.* **1953**, *75*, 2439−2443.
(15) Bernardi, F.; Bottoni, A.; Venturini, A. *J. Am. Chem. Soc.* **1986**, *108*, 5395−5400.
(16) Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935−5943.
(17) Evanseck, J. D.; Houk, K. N.; Briggs, J. M.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1994**, *116*, 10630−10638.
(18) Wiberg, K. B.; Breneman, C. M. *J. Am. Chem. Soc.* **1992**, *114*, 831−840.
(19) Wiberg, K. B.; Rablen, P. R.; Rush, D. J.; Keith, T. A. *J. Am. Chem. Soc.* **1995**, *117*, 4261−4270.
(20) Bordwell, F. G.; Fried, H. E. *J. Org. Chem.* **1981**, *46*, 4327−4331.
(21) Bakulev, V. A.; Chiang, Y.; Kresge, A. J.; Meng, Q.; Morzherin, Y. Y.; Popik, V. V. *J. Am. Chem. Soc.* **2001**, *123*, 2681−2682.
(22) (a) Gao, J. *Theochem* **1996**, *370*, 203−208. (b) Guthrie, J. P. *Can. J. Chem.* **1993**, *71*, 2123−2128. (c) A pK_a of 31.6 for acetate anion can be calculated from the estimated values of pK_E for enolization of acetate anion to form the anionic enol and the pK_a for oxygen ionization of this enol to form the enolate dianion given in ref 22b.
(23) (a) Nagorski, R. W.; Mizerski, T.; Richard, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 4718−4719. (b) Richard, J. P.; Nagorski, R. W. *J. Am. Chem. Soc.* **1999**, *121*, 4763−4770. (c) Rios, A.; Crugeiras, J.; Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **2001**, *123*, 7949−7950.
(24) Richard, J. P.; Williams, G.; Gao, J. *J. Am. Chem. Soc.* **1999**, *121*, 715−726.
(25) Sklenak, S.; Apeloig, Y.; Rappoport, Z. *J. Am. Chem. Soc.* **1998**, *120*, 10359−10364.
(26) Rablen, P. R. *J. Am. Chem. Soc.* **2000**, *122*, 357−368.
(27) Siggel, M. R. F.; Streitwieser, A.; Thomas, T. D. *J. Am. Chem. Soc.* **1988**, *110*, 8022−8028.
(28) Hiberty, P. C.; Byrman, C. P. *J. Am. Chem. Soc.* **1995**, *117*, 9875−9880.
(29) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1−72.
(30) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188−190.
(31) Covington, A. K.; Robinson, R. A.; Bates, R. G. *J. Phys. Chem.* **1966**, *70*, 3820−3824.

Acetamide. The exchange for deuterium of the α-protons of acetamide in alkaline D_2O was initiated by the addition of solid acetamide to 10 mL of D_2O containing KOD ($I = 1.0$, KCl) to give a final substrate concentration of 30 mM. At timed intervals, 1.0 mL aliquots were withdrawn, and the KOD was neutralized by the addition of 2−3 M DCl. The samples were analyzed by ^{1}H NMR either immediately, or they were frozen for NMR analysis at a later time. Deuterium exchange reactions of acetamide in the presence of quinuclidine buffer were initiated by making a 10-fold dilution of a 1.1 M solution of acetamide in D_2O ($I = 1.0$, KCl) into D_2O containing 10% free base quinuclidine buffer (pD 11.2) to give a final substrate concentration of 0.11 M. Hydrolysis of acetamide in basic solutions produces acid so that the pD of the reaction mixture was closely monitored during the course of the reaction. A constant pD (±0.04) was maintained by the periodic addition of small amounts of 1.5 M KOD. At timed intervals, 0.75 mL aliquots were withdrawn and were analyzed directly by ^{1}H NMR.

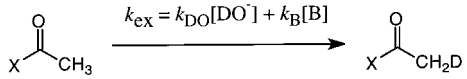
Dimethylacetamide. The exchange for deuterium of the α-protons of dimethylacetamide was initiated by the addition of 10−30 μL of dimethylacetamide to 10 mL of D_2O containing KOD ($I = 1.0$, KCl) to give a final substrate concentration of 10−30 mM. At timed intervals, 1.0 mL aliquots were withdrawn, and the KOD was neutralized by the addition of 2−3 M DCl. The samples were analyzed by ^{1}H NMR either immediately, or they were frozen for NMR analysis at a later time.

Acetate Anion. The exchange for deuterium of the α-protons of acetate anion was initiated by the addition of 75 μL of a 4 M solution of sodium acetate in D_2O to 10 mL of D_2O containing KOD ($I = 1.0$, KCl) to give a final substrate concentration of 30 mM. At timed intervals, 1.0 mL aliquots were withdrawn, and the KOD was neutralized by the addition of 2−3 M DCl. The samples were analyzed by ^{1}H NMR either immediately, or they were frozen for NMR analysis at a later time.

Results

The exchange for deuterium of the first α-proton of acetamide (Scheme 2, X = ND_2) in D_2O at 25 °C ($I = 1.0$, KCl) was followed by monitoring the disappearance of the α-CH_3 group of the substrate and the appearance of the α-CH_2D group of the product by ^{1}H NMR spectroscopy at 500 MHz.[4−7] Deuterium exchange leads to the disappearance of the singlet at 1.984 ppm due to the α-CH_3 group of reactant and the appearance of an upfield triplet at 1.970 ppm due to the α-CH_2D group of product, in which the remaining α-protons are coupled to the α-deuterium. The deuterium perturbation of the ^{1}H chemical shift (0.014 ppm upfield) and the H−D coupling constant (J_{HD} = 2.5 Hz) for $CH_2DC(O)ND_2$ are similar to those observed for $CH_2DC(O)SEt$,[4] $CH_2DC(O)OEt$,[5] and CH_2DCN.[24]

Scheme 2



A similar procedure was used to monitor the exchange for deuterium of the first α-proton of *N*,*N*-dimethylacetamide (Scheme 2, X = NMe_2) and acetate anion (Scheme 2, X = O^-) in D_2O at 25 °C ($I = 1.0$, KCl). The deuterium exchange reaction of dimethylacetamide leads to the disappearance of the singlet at 2.076 ppm due to the α-CH_3 group of reactant and the appearance of an upfield triplet at 2.062 ppm due to the α-CH_2D group of product (J_{HD} = 2.5 Hz). The deuterium exchange reaction of acetate anion leads to the disappearance of the singlet at 1.986 ppm due to the α-CH_3 group of reactant and the appearance of an upfield triplet at 1.974 ppm due to the α-CH_2D group of product (J_{HD} = 2.5 Hz). The chemical shifts for acetate refer to those obtained after acidification of the reaction mixture (see Experimental Section).

The hydrolysis of acetamide and dimethylacetamide to give acetate and ammonium ions is competitive with deuterium exchange into these compounds.[32] The hydrolysis reaction produces acid, but this did not interfere with studies of the reactions catalyzed by DO^- because the concentration of substrate (30 mM) was smaller than $[DO^-] \geq 0.10$ M, and the reactions were followed only during the initial stages. The higher substrate concentration of 0.11 M used in studies of the quinuclidine-catalyzed deuterium exchange reactions of acetamide resulted in the formation of significant amounts of acid, which was neutralized during the course of the reaction as described in the Experimental Section.

$$R = \frac{A_{CH_3}}{A_{CH_3} + \frac{A_{CH_2D}}{2}} \qquad (2)$$

The progress, *R*, of the deuterium exchange reactions of acetamide, dimethylacetamide, and acetate anion was determined from the integrated areas of the singlet due to the α-CH_3 group of CH_3COX (A_{CH_3}) and the triplet due to the α-CH_2D group of the monodeuterated product CH_2DCOX (A_{CH_2D}), according to eq 2.[24,33] The triplet due to the α-CH_2D group of CH_2DCOX was not completely resolved from the singlet due to the α-CH_3 group of CH_3COX. Therefore, the integrated area of the triplet (A_{CH_2D}) was calculated by multiplying the integrated area of only the most upfield of the three peaks by three. The integrated area of the singlet due to the α-CH_3 group of CH_3COX (A_{CH_3}) was then calculated as the difference between the total integrated area of all signals due to the α-CH_3 and α-CH_2D groups and that calculated for the triplet due to the α-CH_2D group of CH_2-DCOX.

Semilogarithmic plots (not shown) of reaction progress *R* against time were linear during exchange of 30% of the first α-proton of CH_3COND_2, 10% of the first α-proton of CH_3-$CONMe_2$, and 10% of the first α-proton of $CH_3CO_2^-$. The negative slope of these plots is equal to k_{obsd}, which is the first-order rate constant for exchange of a *single* α-proton of CH_3-COX.[24,33] The reaction of substrate to give monodeuterated product occurs three times faster than the exchange of a single proton of the α-CH_3 group, so that $k_{ex} = 3k_{obsd}$, where k_{ex} (s^{-1}) is the first-order rate constant for deprotonation of the substrate and formation of the monodeuterated product (Scheme 2).

Table S1 of the Supporting Information gives the dependence of $k_{ex} = k_{DO}[DO^-]$ for the deuterioxide-ion-catalyzed deuterium exchange reactions of acetamide, dimethylacetamide, and acetate anion on the concentration of deuterioxide ion in D_2O at 25 °C ($I = 1.0$, KCl). Figure 1 shows the pD-rate profiles for the DO^--catalyzed exchange reactions of acetamide (●), dimethylacetamide (■), and acetate anion (▲), where $k_{ex} = k_{DO}[DO^-]$. The solid lines show the least-squares fits of the data to eq 3, where

(32) A value of $k_{hyd} = 5 \times 10^{-5}$ M^{-1} s^{-1} for hydrolysis of acetamide catalyzed by hydroxide ion at 25 °C can be calculated from $k_{hyd} = 1.7 \times 10^{-4}$ M^{-1} s^{-1} at 45 °C [Meresaar, U.; Bratt, L. *Acta Chem. Scand. A* **1974**, *28*, 715−722] and the thermodynamic activation parameters for the reaction [Bolton, P. D.; Jackson, G. L. *Aust. J. Chem.* **1971**, *24*, 969−974]. By comparison, $k_{DO} = 1.9 \times 10^{-5}$ M^{-1} s^{-1} was determined in this work for the deuterium exchange reaction of acetamide catalyzed by deuterioxide ion (Table 1).

(33) Halkides, C. J.; Frey, P. A.; Tobin, J. B. *J. Am. Chem. Soc.* **1993**, *115*, 3332−3333.

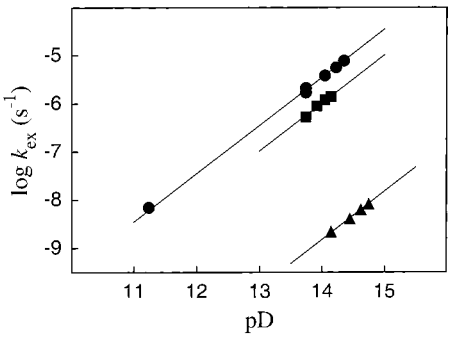


Figure 1. pD-rate profiles of k_{ex} (s^{-1}) for the buffer-independent exchange for deuterium of the first α-proton of acetamide (●), *N*,*N*-dimethylacetamide (■), and acetate anion (▲) in D_2O at 25 °C and $I = 1.0$ (KCl). The solid lines show the fits of the data to eq 3 (see text). The values of k_{DO} (M^{-1} s^{-1}) obtained by this fitting procedure are reported in Table 1.

$$\log k_{ex} = \log\left(\frac{k_{DO}K_w}{\gamma_{OL}}\right) + \mathrm{pD} \qquad (3)$$

k_{DO} (M^{-1} s^{-1}) is the second-order rate constant for the exchange catalyzed by deuterioxide ion, $K_w = 10^{-14.87}$ M^2 is the ion product of D_2O at 25 °C,[31] and $\gamma_{OL} = 0.75$ is the apparent activity coefficient of lyoxide ion determined for the electrode under our experimental conditions ($I = 1.0$, 25 °C). The values of k_{DO} (M^{-1} s^{-1}) obtained by this fitting procedure are reported in Table 1. The good fit to eq 3 of the data for deuterium exchange into acetamide in the presence of up to 0.4 M deuterioxide ion (pD 14.3, calculated using eq 1) shows that there is no significant conversion of acetamide to its nitrogen anion under these conditions.

Table S2 of the Supporting Information gives the dependence of $k_{ex} = k_{DO}[DO^-] + k_B[B]$ for the deuterium exchange reaction of acetamide on the concentration of 10% free base quinuclidine buffer in D_2O at pD = 11.2 and 25 °C ($I = 1.0$, KCl). An increase in the total concentration of quinuclidine buffer from

$$k_{rel} = \frac{k_{ex}}{k_{DO}[DO^-]} = 1 + \frac{k_B[B]}{k_{DO}[DO^-]} \qquad (4)$$

0.10 to 0.80 M results in an ca. 0.1 unit decrease in pD which complicates the determination of the second-order rate constant for exchange catalyzed by quinuclidine. Figure 2 (■) shows the plot of the normalized rate constant $k_{rel} = k_{ex}/k_{DO}[DO^-]$ for exchange for deuterium of the first α-proton of acetamide against [B]/[DO^-] (B = quinuclidine), according to eq 4. The solid line shows the fit of the experimental data to eq 4, with a slope $k_B/k_{DO} = 2.5 \times 10^{-3}$ that is equal to the ratio of the second-order rate constants for deuterium exchange catalyzed by quinuclidine and DO^-. This was combined with $k_{DO} = 1.9 \times 10^{-5}$ M^{-1} s^{-1} (Table 1) to give $k_B = 4.8 \times 10^{-8}$ M^{-1} s^{-1} for the quinuclidine-catalyzed deuterium exchange reaction of acetamide (Table 1).

Discussion

Acetate anion and amides of acetic acid are weak carbon acids that undergo slow deuterium exchange at 25 °C over periods of weeks to months. The value of $k_{DO} = 8.4 \times 10^{-9}$ M^{-1} s^{-1} for the DO^--catalyzed deuterium exchange reaction of acetate anion in D_2O at 25 °C (Table 1) is about the smallest rate constant that can be determined by our experimental methods. To observe deprotonation of carbon acids that are significantly weaker than acetate anion either these methods must be modified or the reaction temperature must be increased.

Only one rate constant, $k_{obsd} \approx 1 \times 10^{-3}$ min^{-1}, has been reported for exchange for deuterium of a single α-proton of acetamide in the presence of 1.0 M DO^- in D_2O at 25 °C.[13] This gives $k_{DO} \approx 5 \times 10^{-5}$ M^{-1} s^{-1} for DO^--catalyzed exchange of the first α-proton of acetamide, which is in fair agreement with $k_{DO} = 2.0 \times 10^{-5}$ M^{-1} s^{-1} determined in this work (Table 1). There are no earlier data for buffer catalysis of this exchange reaction. A temperature correction of the first-order rate constant determined for the deuterium exchange reaction of acetate anion in the presence of 1.0 M DO^- in D_2O at 100 °C gave $k_{obsd} \approx 1 \times 10^{-6}$ min^{-1} for this reaction at 25 °C.[13] This is ca. 1000-fold smaller than the value of k_{obsd} reported for the deuterium exchange reaction of acetamide under the same conditions.[13] By comparison, we observe a 2300-fold difference in the values of k_{DO} for the DO^--catalyzed exchange reactions of acetamide and acetate anion in D_2O at 25 °C and $I = 1.0$ (KCl) (Table 1).

Buffer-Catalyzed Deuterium Exchange into Acetamide. Figure 3 shows detailed mechanisms for the deuterium exchange reactions at α-carbonyl carbon catalyzed by deuterioxide ion (upper pathway) and general bases B (lower pathway).[5] General base catalysis of exchange is observed when the step for deprotonation of the substrate by DO^- (k_p, Figure 3) is rate-determining for the overall deuterioxide-catalyzed exchange reaction (k_{DO}). Brønsted (buffer) bases result in an increase in the rate constant for this proton-transfer step (k_1[B]) because lyoxide ion exhibits a low reactivity in proton transfer from carbon for its basicity,[34] a phenomenon known as the lyoxide ion anomaly.[35−37a] However, general base catalysis of the overall deuterium exchange reaction is not possible when the reprotonation of the enolate intermediate by solvent (k_{-p}) is so fast that the reorganization of solvent that places a molecule of DOD in a position to deliver a deuteron to the enolate ($k_{reorg} \approx 10^{11}$ s^{-1})[38−40] is rate-determining for the overall DO^--catalyzed exchange reaction ($k_{-p} > k_{reorg}$, Figure 3), because there is no mechanism by which Brønsted bases may lower this barrier to solvent reorganization.

Figure 2 shows the dependence of the increase in k_{ex}, given by the normalized rate constant $k_{rel} = k_{ex}/k_{DO}[DO^-]$ (eq 4), for the deuterium exchange reactions of ethyl acetate (●),[5] acetamide (■), and acetonitrile (▲)[24] on the ratio of the concentrations of the basic form of quinuclidine buffer (B = quinuclidine) and deuterioxide ion in D_2O at 25 °C. The relatively strong buffer catalysis observed for the deuterium exchange reaction of ethyl

(34) Jencks, W. P. *J. Am. Chem. Soc.* **1972**, *94*, 4731−4732.
(35) Kresge, A. J. *Chem. Soc. Rev.* **1973**, *2*, 475−503.
(36) Jencks, W. P.; Brant, S. R.; Gandler, J. R.; Fendrich, G.; Nakamura, C. *J. Am. Chem. Soc.* **1982**, *104*, 7045−7051.
(37) (a) Washabaugh, M. W.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 683−692. (b) There is a large increase in the secondary solvent deuterium isotope effect k_{DO}/k_{HO} for deprotonation of substituted thiazolium ions **5** by lyoxide ion, from $k_{DO}/k_{HO} = 1.3$ for nearly thermoneutral proton transfer to $k_{DO}/k_{HO} = 2.35$ for deprotonation of a carbon acid of $pK_a = 18.9$ for which the reaction barrier is 4 kcal/mol.[37a] The latter is close to the maximum secondary solvent deuterium isotope effect expected when proton transfer from the carbon acid is fast and reversible so that solvent reorganization is rate-determining for overall isotope exchange.[89] (c) Bednar, R. A.; Jencks, W. P. *J. Am. Chem. Soc.* **1985**, *107*, 7117−7126. (d) Lin, A. C.; Chiang, Y.; Dahlberg, D. B.; Kresge, A. J. *J. Am. Chem. Soc.* **1983**, *105*, 5380−5386.
(38) Giese, K.; Kaatze, U.; Pottel, R. *J. Phys. Chem.* **1970**, *74*, 3718−3725.
(39) Kaatze, U. *J. Chem. Eng. Data* **1989**, *34*, 371−374.
(40) Kaatze, U.; Pottel, R.; Schumacher, A. *J. Phys. Chem.* **1992**, *96*, 6, 6017−6020.

Table 1. Rate and Equilibrium Constants for the Ionization of Simple α-Carbonyl Carbon Acids in Water at 25 °C (I = 1.0, KCl)

carbon acid	base	k_{DO} or k_B[a] $M^{-1}s^{-1}$	k_{HO} or k_B[b] $M^{-1}s^{-1}$	k_{HOH} or k_{BH}[c]	pK_a^C[d]
CH_3COO^-	LO^-	8.4×10^{-9} [e]	3.5×10^{-9}[g]	$1 \times 10^{11}\ s^{-1}$[j]	≈ 33.5[m]
CH_3CONH_2	LO^-	1.9×10^{-5} [e]	9.5×10^{-6} [h]	$2.4 \times 10^{9}\ s^{-1}$ [k]	28.4[n]
		4.8×10^{-8} [f]	4.8×10^{-8} [i]	$3.5 \times 10^{9}\ M^{-1}\ s^{-1}$ [l]	
CH_3CONMe_2	LO^-	5.7×10^{-6} [e]	2.9×10^{-6} [h]	$7.3 \times 10^{9}\ s^{-1}$ [k]	29.4[o]

[a] Second-order rate constant for deprotonation of the carbon acid by deuterioxide ion (k_{DO}) or quinuclidine (k_B) in D_2O. [b] Second-order rate constant for deprotonation of the carbon acid by hydroxide ion (k_{HO}) or quinuclidine (k_B) in H_2O. [c] Rate constant for protonation of the enolate of the carbon acid by solvent water (k_{HOH}, s^{-1}) or the quinuclidinium cation (k_{BH}, $M^{-1}\ s^{-1}$). [d] pK_a for ionization of the carbon acid in water. [e] Obtained from the fit to eq 3 of the data in the pD-rate profiles in Figure 1. [f] Determined from $k_B/k_{DO} = 2.5 \times 10^{-3}$ (Figure 2) as the ratio of second-order rate constants for deprotonation by quinuclidine and deuterioxide ion and $k_{DO} = 1.9 \times 10^{-5}\ M^{-1}\ s^{-1}$. [g] Calculated from k_{DO} and a secondary solvent deuterium isotope effect of $k_{DO}/k_{HO} = 2.4$ for preequilibrium proton transfer from the carbon acid to LO^- followed by rate-determining solvent reorganization [ref 89]. [h] Calculated from k_{DO} and a secondary solvent deuterium isotope effect of $k_{DO}/k_{HO} = 2.0$ [ref 90]. [i] Calculated from the value of k_B for deprotonation of acetamide by quinuclidine in D_2O and a secondary solvent deuterium isotope effect of unity [ref 48]. [j] The limiting rate constant for protonation of the enolate with rate-determining reorganization of solvent, $(k_{HOH})_{lim} = k_{reorg} \approx 10^{11}\ s^{-1}$ [refs 24, 38−40], see text. [k] Calculated from the values of k_{HO} and pK_a^C for the carbon acid using eq 7. [l] The average of the range $k_{BH} = (2-5) \times 10^9\ M^{-1}\ s^{-1}$ for encounter-limited protonation of enolates by the quinuclidinium cation in water [ref 5]. [m] Calculated from the values of k_{HO} and k_{HOH} using eq 7 and assuming a stepwise mechanism for deuterium exchange (see text). [n] Calculated from the values of k_B and k_{BH} and $pK_{BH} = 11.5$ using eq 5. [o] Estimated by assuming that the effect of the NMe_2 for NH_2 substitution at acetamide is expressed equally on k_{HO} for formation and k_{HOH} for protonation of the amide enolate.

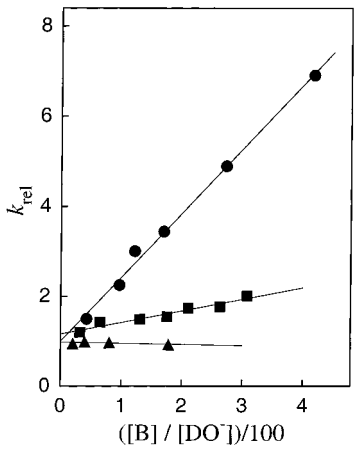


Figure 2. Dependence of the normalized rate constants $k_{rel} = k_{ex}/k_{DO}[DO^-]$ for exchange for deuterium of the first α-proton of carbon acids on the ratio of the concentrations of quinuclidine (B) and deuterioxide ion. Key: (●) Deuterium exchange into ethyl acetate at pD 12.3, 12.1, and 11.1 (data from ref 5). (■) Deuterium exchange into acetamide at pD 11.2 (this work). (▲) Deuterium exchange into acetonitrile at pD 12.3 (data from ref 24). The solid lines show the fit of the experimental data to eq 4, with slopes k_B/k_{DO} that are equal to the ratio of the second-order rate constants for deuterium exchange catalyzed by quinuclidine and deuterioxide ion.

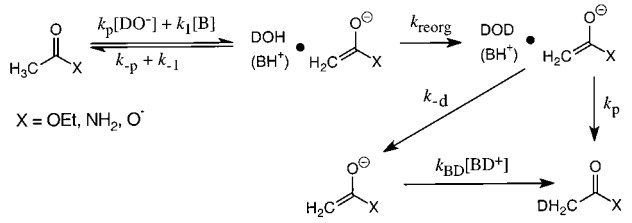


Figure 3. Mechanisms for exchange for deuterium of the first α-proton of acetic acid derivatives catalyzed by deuterioxide ion and general bases in D_2O. Solvent reorganization ($k_{reorg} \approx 10^{11}\ s^{-1}$)[38−40] is faster than diffusional separation of the ion pair to free ions ($k_{-d} \approx 1.6 \times 10^{10}\ s^{-1}$).[91]

acetate requires that proton transfer be rate-determining for the corresponding DO^--catalyzed exchange reaction ($k_{-p} < k_{reorg}$, Figure 3). However, the lack of detectable buffer catalysis of deuterium exchange into acetonitrile provides evidence for a change to rate-determining solvent reorganization for the overall exchange reaction catalyzed by DO^- ($k_{-p} > k_{reorg}$, Figure 3).[24,41]

The observation of weak, but significant, buffer catalysis of the deuterium exchange reaction of acetamide in this work is consistent with a stepwise mechanism for deuterium exchange catalyzed by DO^-, through an amide enolate intermediate for which $k_{-p} < k_{reorg} \approx 10^{11}\ s^{-1}$ for protonation of the enolate by solvent.

The rate-determining step for the buffer-catalyzed deuterium exchange reaction of acetamide is either deprotonation of the carbon acid (k_1) or diffusional separation of the ion pair intermediate to the free ions (k_{-d}), depending upon the relative values of k_{-1} and k_{-d} for partitioning of the ion pair intermediate (Figure 3).[5] The Brønsted exponent $\beta = 1.09 \pm 0.05$ determined for general base catalysis of the deuterium exchange reaction of ethyl acetate by 3-substituted quinuclidines shows that there is complete proton transfer from this carbon acid to general base catalysts in the rate-limiting transition state for exchange.[5] It was concluded that these thermodynamically uphill proton transfers are limited by the diffusional separation of the reversibly formed ammonium cation-enolate ion pair intermediate ($k_{-1} \gg k_{-d}$, Figure 3).[5]

It was not possible to determine a Brønsted coefficient β for the deuterium exchange reaction of acetamide because this reaction is too slow to monitor at pD < 11[42] and the weak general base catalysis of deuterium exchange is most easily detectable when the pH of the solution is below the pK_a of the general base catalyst.[43] However, our data require that the enolate of acetamide be more unstable relative to the parent carbon acid than is the enolate of ethyl acetate.[44] Therefore, the same inequality $k_{-1} \gg k_{-d}$ (Figure 3) and rate-determining step (k_{-d}) will be observed for the general-base-catalyzed deuterium exchange reactions of ethyl acetate and acetamide,

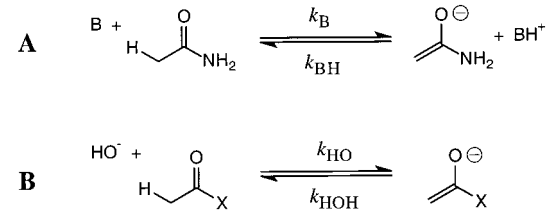
(41) Fishbein, J. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1988**, *110*, 5087−5095.

(42) For example, at pD 10 and 25 °C the calculated halftime for exchange for deuterium of the first α-proton of acetamide in the absence of buffer catalysts is about 65 years!

(43) Jensen, J. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 1476−1488. It is shown in this reference that general acid catalysis of hydrolysis of acetals is most easily detected against the background of a competing specific-acid-catalyzed reaction when pH ≫ pK_a for the acid catalyst. It can be shown by analogy that general base catalysis is most easily detected against the background of a competing specific-base-catalyzed reaction when pH ≪ pK_a for the base catalyst.

(44) The second-order rate constant $k_B = 2.4 \times 10^{-5}\ M^{-1}\ s^{-1}$ for formation of the enolate of ethyl acetate by quinuclidine-catalyzed deprotonation of the parent carbon acid[5] is 500-fold larger than $k_B = 4.8 \times 10^{-8}\ M^{-1}\ s^{-1}$ (this work) for the corresponding reaction of acetamide. This requires that the enolate of acetamide be at least 3.7 kcal/mol more unstable relative to the parent carbon acid than the enolate of ethyl acetate.

Scheme 3



because the rate constant k_{-1} for collapse of the amide enolate ion pair must be at least as large as that for the more stable ester enolate. If diffusional separation of the ion pair complex between the amide enolate and the quinuclidinium cation is rate-determining for deprotonation of acetamide by quinuclidine, then microscopic reversibility requires that encounter-controlled formation of this ion pair be rate-determining for the reverse protonation of this enolate. In other words, protonation of the enolate of acetamide by the quinuclidinium cation (protonated quinuclidine) is an encounter-controlled reaction.

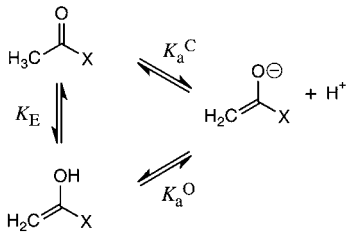
Carbon Acid Acidity and Enol Content of Acetamide. Scheme 3A shows that pK_a^C for carbon ionization of acetamide can be obtained from the rate constants for its deprotonation by a buffer base to give the free enolate (k_B) and for protonation of the free enolate by the conjugate acid of this base (k_{BH}), according to eq 5. Protonation of the enolate of acetamide by the quinuclidinium cation is an encounter-controlled reaction ($k_{BH} = k_{enc}$). Evidence was presented in earlier work that the rate constants for encounter-limited protonation of the enolate of ethyl acetate by substituted tertiary ammonium ions may be smaller than that for a diffusion-controlled reaction ($k_d = 5 \times 10^9$ M^{-1} s^{-1}),[45−47] and a range of $k_{BH} = k_{enc} = (2-5) \times 10^9$ M^{-1} s^{-1} was estimated for k_{BH}.[5] Therefore, the values of $k_{BH} = 3.5 \times 10^9$ M^{-1} s^{-1} and $k_B = 4.8 \times 10^{-8}$ M^{-1} s^{-1} (Table 1) for proton transfer between acetamide and quinuclidine ($pK_{BH} = 11.5$) were substituted into eq 5 to give $pK_a^C = 28.4$ for ionization of acetamide as a carbon acid in water. This treatment neglects the secondary solvent deuterium isotope effect on k_B arising from its determination here in D_2O rather than H_2O. However, there is good evidence that this solvent deuterium isotope effect is close to unity.[48] The uncertainty in $pK_a^C = 28.4$ arises mainly from the uncertainty in the value of $k_{BH} = (2-5) \times 10^9$ M^{-1} s^{-1} for encounter-controlled protonation of the enolate and is estimated to be at most ±0.5 units.[5]

$$pK_a^C = pK_{BH} + \log\left(\frac{k_{BH}}{k_B}\right) \quad (5)$$

$$pK_a^O = \rho_I \sigma_I^X + C \quad (6)$$

$$pK_a^C = pK_w + \log\left(\frac{k_{HOH}}{k_{HO}}\right) \quad (7)$$

Scheme 4



The thermodynamic cycle in Scheme 4 shows that if the acidity constants of acetamide (K_a^C, X = NH_2) and its enol (K_a^O) are known, then the equilibrium constant for keto−enol tautomerization can be calculated from the relationship $pK_E = pK_a^C - pK_a^O$. There is no experimental value of pK_a^O for the enol of acetamide. However, there is a reasonable correlation (eq 6) of pK_a^O for oxygen ionization of several simple enols $H_2C{=}C(OH)X$ with the inductive substituent constant σ_I of Ehrenson and Taft for the substituent X:[49,50] $\rho_I = -12.8$, $C = 10.7$, $r = 0.96$ (eq 6).[51] The value of $\sigma_I = 0.12$ for NH_2[50] then gives $pK_a^O = 9.2$ for oxygen ionization of the enol of acetamide. This can be combined with $pK_a^C = 28.4$ (see above) to give $pK_E = 28.4 - 9.2 = 19.2$ for keto−enol tautomerization of acetamide. By comparison, $pK_E = 21.7$ has been estimated for the closely related *N*-methyl acetamide starting from the experimental value of $pK_E = 12.1$ for tautomerization of **3**, with corrections for the estimated effects of β-cyano (−9.2 units) and β-hydroxy (−0.4 units) groups on pK_E for the parent amide.[21]

Lifetime of the Enolate of Acetamide in Water. Equation 7 derived for Scheme 3B shows that the rate constant for deprotonation of acetamide by hydroxide ion, $k_{HO} = 9.5 \times 10^{-6}$ M^{-1} s^{-1} (Table 1), can be combined with $pK_a^C = 28.4$ and $K_w = 10^{-14}$ M^2 to give $k_{HOH} = 2.4 \times 10^9$ s^{-1} for the reverse protonation of the enolate of acetamide by solvent water. This corresponds to an enolate lifetime in water ($1/k_{HOH}$) of 4×10^{-10} s.

There is a good correlation between the effect of increasing concentrations of buffer catalysts on the observed rate constant for exchange of deuterium from D_2O into carbon acids and the lifetime of the carbanion intermediate of this reaction (Figure 2). Strong general base catalysis is observed for deuterium exchange into ethyl acetate (Figure 2, ●), a reaction that proceeds through a relatively long-lived ester enolate ($k_{HOH} = 5 \times 10^8$ s^{-1}) that freely diffuses through water.[5] By contrast, there is no detectable general base catalysis of stepwise deuterium exchange into acetonitrile (Figure 2, ▲), which proceeds through the short-lived cyanomethyl carbanion, and for which there is good evidence that solvent reorganization is at least partly rate-determining for DO^--catalyzed exchange ($k_{-p} > k_{reorg}$, Figure 3). Therefore, the microscopic reverse protonation of this carbanion by solvent is also limited by solvent reorganization, so that $k_{HOH} = (k_{HOH})_{lim} = k_{reorg} \approx 10^{11}$ $s^{-1} > k_{-d}$.[5,24,41] In this case, general base catalysis of exchange is not possible because there is no mechanism by which buffer bases

(45) McClelland, R. A.; Kanagasabapathy, V. M.; Steenken, S. *J. Am. Chem. Soc.* **1988**, *110*, 6913−6914.
(46) McClelland, R. A.; Kanagasabapathy, V. M.; Banait, N. S.; Steenken, S. *J. Am. Chem. Soc.* **1991**, *113*, 1009−1014.
(47) McClelland, R. A.; Cozens, F. L.; Steenken, S.; Amyes, T. L.; Richard, J. P. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1717−1722.
(48) This assumption does not lead to a large error in the calculated value of pK_a^C for acetamide in H_2O because a solvent isotope effect of $k_B(H_2O)/k_B(D_2O) = 1.1$ has been determined for deprotonation of L-glyceraldehyde 3-phosphate dianion by 3-quinuclidinone [Richard, J. P. *J. Am. Chem. Soc.* **1984**, *106*, 4926−4936].
(49) Ehrenson, S.; Brownlee, R. T. C.; Taft, R. W. *Prog. Phys. Org. Chem.* **1973**, *10*, 1−80.
(50) The values of σ_I are from Table 3.7 of: Hine, J. *Structural Effects on Equilibria in Organic Chemistry*; Wiley: New York, 1975.
(51) The following values of pK_a^O and $\sigma_I X$ [ref 50] were used for this correlation (eq 6). X = OMe: $\sigma_I = 0.27$, $pK_a^O = 7.1$ [Table 2, footnote j]. X = OH: $\sigma_I = 0.25$, $pK_a^O = 7.3$ [ref 22a]. X = Ph: $\sigma_I = 0.10$, $pK_a^O = 10.4$ [Jefferson, E. A.; Keeffe, J. R.; Kresge, A. J. *J. Chem. Soc., Perkin Trans. 2* **1995**, 2041−2046]. X = H: $\sigma_I = 0.00$, $pK_a^O = 10.5$ [ref 2]. X = Me: $\sigma_I = -0.04$, $pK_a^O = 10.9$ [ref 2].

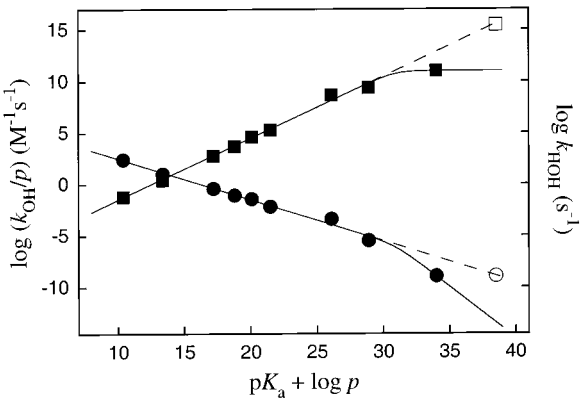


Figure 4. Correlations of rate constants for deprotonation of α-carbonyl carbon acids by hydroxide ion, k_{HO} (M^{-1} s^{-1}), and for the reverse protonation of the enolates by solvent water, k_{HOH} (s^{-1}), with the pK_a of the carbon acid. The values of k_{HO} and pK_a were statistically corrected for the number of acidic protons p at the carbon acid. These plots show data from earlier work[2,4,5] and new data from this work for acetamide and acetate anion (Table 1). (●) Correlation of log(k_{HO}/p) for deprotonation of neutral aldehydes, ketones, esters, and acetamide by hydroxide ion. Excluding the point for acetate anion (pK_a = 33.5), the data are correlated by log(k_{HO}/p) = 6.52 − 0.40(pK_a + log p). (■) Correlation of log k_{HOH} for the reverse protonation of the enolates of neutral α-carbonyl acids by solvent water. Excluding the point for the enolate of acetate anion, the data are correlated by log k_{HOH} = 0.60(pK_a + log p) − 7.48. The solid lines were calculated with the assumption that protonation of highly unstable enolates by solvent water is limited by the rotation of a molecule of water into a "reactive position" with $k_{HOH} = (k_{HOH})_{lim} = k_{reorg} \approx 10^{11}$ s^{-1}, using eqs 8 and 9 derived for Scheme 5 (see text). The dashed lines and open symbols show the extrapolations of the linear portions of these correlations to the "impossible" values of pK_a = 38.0 and $k_{HOH} = 4 \times 10^{15}$ s^{-1} for acetate anion (see text).

can lower the barrier to the physical transport step (k_{reorg}) that limits the solvent-catalyzed exchange reaction.[5,24,41] The lifetime of the enolate of acetamide in water (4×10^{-10} s) is close to that of the cyanomethyl carbanion (10^{-11} s),[24,41,52] and only weak general base catalysis by quinuclidine of the deuterium exchange reaction of acetamide (Figure 2, ■) is observed.

Carbon Acid Acidity of *N*,*N*-Dimethylacetamide. The pK_a for carbon deprotonation of *N*,*N*-dimethylacetamide must be at least 0.5 units higher than that for acetamide, because the second-order rate constant $k_{DO} = 5.7 \times 10^{-6}$ M^{-1} s^{-1} for DO^--catalyzed exchange for deuterium of the first α-proton is 3-fold smaller than that for acetamide (Table 1). A value of pK_a^C = 29.4 for *N*,*N*-dimethylacetamide can be estimated by assuming that the effect of *N*,*N*-dimethylation of the parent acetamide on carbon acidity is expressed equally in k_{HO} for formation (3-fold decrease) and in k_{HOH} for the reverse protonation (3-fold increase) of the enolate of *N*,*N*-dimethylacetamide.[53]

Carbon Acid Acidity of Acetate Anion. A pK_a of 38.0 for carbon deprotonation of acetate anion can be estimated from $k_{HO} = 3.5 \times 10^{-9}$ M^{-1} s^{-1} for deprotonation of this carbon acid by hydroxide ion (Table 1) and an extrapolation of the excellent linear correlation between the statistically corrected values of log k_{HO} and the carbon acid pK_a for a series of neutral methyl and benzylic monocarbonyl carbon acids (Figure 4, ○).[5,7,54a] However, this extrapolation is inappropriate because a value of pK_a^C = 38.0 for acetate anion would require the impossibly large rate constant $k_{HOH} = 4 \times 10^{15}$ s^{-1} (Figure 4, □) for the reverse protonation of the enolate dianion by solvent water (eq 7).[54b] The maximum limiting rate constant for protonation of a carbanion by solvent water is given by the rate constant for the solvent reorganization that places a molecule of solvent in a "reactive position" to protonate the carbanion, $k_{HOH} = (k_{HOH})_{lim} = k_{reorg} \approx 10^{11}$ s^{-1}.[24,38−40] This limiting value of $k_{HOH} = 10^{11}$ s^{-1} for protonation of the enolate of acetate anion by solvent water then gives pK_a^C = 33.5 (eq 7) for the carbon acidity of acetate anion.[22c] The unfavorable electrostatic interaction in the transition state for proton transfer between hydroxide ion and this negatively charged carbon acid is expected to result in a decrease in k_{HOH} below the extrapolated value of $k_{HOH} = 4 \times 10^{15}$ s^{-1} (see above). However, the following observations provide good evidence that electrostatic interactions do not result in a decrease in k_{HOH} to below $k_{HOH} = (k_{HOH})_{lim} = 10^{11}$ s^{-1} for protonation of the enolate of acetate anion by solvent water.

(1) The addition of an α-NH_3^+ substituent to the enolate of ethyl acetate results in a 12-fold decrease in reactivity from $k_{HOH} = 5 \times 10^8$ s^{-1} to $k_{HOH} = 4 \times 10^7$ s^{-1} for the enolate of N-protonated glycine methyl ester.[7] The value of $k_{HOH} = 4 \times 10^{10}$ s^{-1} for protonation of the enolate of glycine zwitterion ($^+H_3NCH_2CO_2^-$)[7] is only (2−3)-fold smaller than the limiting rate constant $(k_{HOH})_{lim} = 10^{11}$ s^{-1} so that removal of the α-NH_3^+ substituent is expected to result in an increase in reactivity to $k_{HOH} = (k_{HOH})_{lim} = 10^{11}$ s^{-1} for protonation of the enolate of acetate anion.

(2) The addition of an α-NH_3^+ substituent to ethyl acetate results in a large 4.6 unit increase in carbon acidity from pK_a^C = 25.6 for ethyl acetate to pK_a^C = 21.0 for N-protonated glycine methyl ester.[6,7] There should be a similar large effect of the addition of an α-NH_3^+ substituent to acetate anion to give glycine zwitterion (pK_a^C = 28.9)[7] which is consistent with the value of pK_a^C = 33.5 for acetate anion calculated from the observed value of $k_{HO} = 3.5 \times 10^{-9}$ M^{-1} s^{-1} and the limiting value of $k_{HOH} = 10^{11}$ s^{-1}.

There is evidence that a change from a stepwise to a concerted reaction mechanism may be *enforced* when the putative intermediate of the stepwise reaction becomes too unstable to exist for the time of a bond vibration (10^{-13} s).[55−57] Our data do not distinguish between stepwise and concerted mechanisms for the DO^--catalyzed deuterium exchange reaction of acetate anion so that the pK_a for deprotonation of acetate anion that can be estimated from the kinetic data for deuterium exchange is a *lower* limit. If the formation of the highly unstable enolate dianion is avoided in a concerted exchange reaction, then the observed rate constant for deuterium exchange would be larger than that for the putative stepwise exchange reaction, so that the pK_a of 33.5 would underestimate the true pK_a for carbon deprotonation of acetate anion. The deuterium exchange reaction of acetate anion lies in the "borderline" region where it is difficult to determine whether a change from a stepwise to a

(52) Fishbein, J. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1988**, *110*, 5075−5086.
(53) The statistically corrected plots of log k_{HO} and log k_{HOH} against the carbon acid pK_a for a broad series of neutral α-carbonyl carbon acids shown in Figure 4 have slopes of −0.40 and +0.60, respectively (see ref 54). These correlations show that the substituent effects on enolate stability are expressed about equally in log k_{HO} and log k_{HOH}.
(54) (a) Calculated from $k_{HO} = 3.5 \times 10^{-9}$ M^{-1} s^{-1} (Table 1) and the correlation line for k_{HO} in Figure 4 given by log(k_{HO}/p) = 6.52 − 0.40(pK_a + log p). (b) Calculated from the hypothetical pK_a of 38.0 and the correlation line for k_{HOH} in Figure 4 given by log k_{HOH} = 0.60(pK_a + log p) − 7.48.
(55) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1383−1396.
(56) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161−169.
(57) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345−375.

concerted reaction mechanism has occurred, because there is only a small difference in the reaction barriers and transition state structures for competing stepwise and concerted mechanisms.[55−57] Therefore, even if the deuterium exchange reaction of acetate anion *does* proceed by a concerted mechanism: (a) the advantage of this concerted mechanism over the competing stepwise mechanism will be small; (b) the value of k_{HO} reported in Table 1 will lie close to the second-order rate constant for deprotonation of acetate anion by hydroxide ion to give the putative enolate dianion; and (c) the value of $pK_a^C = 33.5$ for acetate anion calculated by assuming a stepwise mechanism for deuterium exchange will lie close to the true pK_a for this carbon acid.

Structure−Reactivity Correlations. Brønsted-type correlations of rate constants for proton transfer with thermodynamic driving force are sharply curved for reactions of electronegative proton donor and acceptor atoms such as oxygen and nitrogen.[29] The rate-determining step for these reactions is the diffusion-controlled formation of an encounter complex between reactants (Brønsted coefficient = 0.0) for thermodynamically favorable proton transfer or the separation of the product complex (Brønsted coefficient = 1.0) for unfavorable proton transfer. For nearly thermoneutral reactions there is a narrow range of thermodynamic driving force for which the proton transfer step is partly rate-determining, and where the slope of the correlation changes sharply from 0.0 to 1.0. These distinctive "Eigen" correlations are observed when the intrinsic barrier to proton transfer is small and the intrinsic rate constant for thermoneutral proton transfer is close to the diffusion-controlled limit.[29,58]

By comparison, the intrinsic rate constant for thermoneutral proton transfer between α-carbonyl carbon and the hydroxide ion is small,[59] and Brønsted correlations of rate constants for proton transfer from carbon are usually linear with slopes of less than unity, even when the thermodynamic barrier to proton transfer is very large.[35,58] For example, the statistically corrected Brønsted-type correlation of log k_{HO} for deprotonation of neutral α-carbonyl carbon acids by hydroxide ion in Figure 4 (●) remains linear with a slope of −0.40 as the pK_a of the carbon acid is increased from 10 (thermodynamically favorable reaction) to 28 (highly unfavorable reaction).[60]

Scheme 5

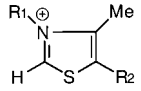
$$HO^- + {}^*H{-}C \underset{k_{-p}}{\overset{k_p}{\rightleftharpoons}} HO{}^*H \bullet {}^-C \underset{k_{reorg}}{\overset{k_{reorg}}{\rightleftharpoons}} HOH \bullet {}^-C$$

The data in Figure 4 provide rare examples of linear rate-equilibrium correlations for proton transfer at carbon which break down as the rate constant for protonation of the corresponding enolate approaches the limiting value of $k_{HOH} = (k_{HOH})_{lim} = k_{reorg} \approx 10^{11}$ s^{-1} for solvent reorganization. This corresponds to a change in the rate-determining step for deprotonation at carbon to give the enolate from proton transfer (k_p, Scheme 5 and Figure 3) to reorganization of solvent (k_{reorg}). A short extrapolation of the linear correlation for k_{HOH} in Figure 4 (■) shows that the limiting rate constant $k_{HOH} = 10^{11}$ s^{-1} is attained for protonation of the enolate of a hypothetical α-carbonyl carbon acid with a statistically corrected pK_a of 31, while weaker carbon acids such as acetate anion ($pK_a^C \approx 33.5$, Table 1) lie to the right of this break. The solid lines in Figure 4 show the fits of the data to eqs 8 and 9 derived for Scheme 5, where $k_p = 10^{\{-0.40(pK_a + \log p) + 6.52\}}$ and $k_{-p} = 10^{\{0.60(pK_a + \log p) - 7.48\}}$ are the rate constants for the proton-transfer steps calculated from the linear correlations observed up to $pK_a^C = 28.4$ for acetamide, and $k_{reorg} = 10^{11}$ s^{-1}.

$$(k_{HO}/p) = \frac{k_p k_{reorg}}{k_{-p} + k_{reorg}} \quad (8)$$

$$k_{HOH} = \frac{k_{-p} k_{reorg}}{k_{-p} + k_{reorg}} \quad (9)$$

The downward break in the Brønsted-type correlation for proton transfer between simple enolates and water will be centered on $pK_a = 15.7$ for water ($\Delta pK_a = 0$) when the intrinsic barrier for proton transfer is small.[29] By contrast, the downward



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break in the correlation at $k_{HOH} = 10^{11}$ s^{-1} in Figure 4 occurs at $pK_a = 31$ ($\Delta pK_a = 15$) and corresponds to a favorable thermodynamic driving force of 21 kcal/mol. This very large driving force is required to overcome the large intrinsic barrier of ca. 10 kcal/mol and reduce the observed barrier to protonation of the enolate to that for reorganization of solvent.[61]

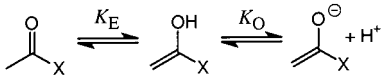
Three related changes in the rate-determining step for proton transfer to lyoxide ion have been observed for the isotope exchange reactions of carbon acids. (1) The deuterium exchange reaction of succinonitrile ($pK_a^C = 26.6$) catalyzed by deuterioxide ion is partly limited by solvent reorganization.[24] Therefore, the change in the rate-determining step for deprotonation of simple α-cyano carbon acids occurs at $pK_a < 26$ rather than at $pK_a \approx 31$ observed here for simple α-carbonyl carbon acids (Figure 4). This reflects the smaller intrinsic barrier for proton transfer from α-cyano as compared with α-carbonyl carbon acids.[62] (2) The small thermodynamic barrier to proton transfer of 4 kcal/mol at the point where solvent reorganization and proton transfer are equally rate-determining for the lyoxide-ion-catalyzed isotope exchange reactions of the C2-proton of thiazolium ions **5** is consistent with a small intrinsic barrier for carbon deprotonation to give a localized carbanion.[37b] (3) Nearly normal Eigen-type plots are observed for reversible deprotonation of HCN by carboxylate ions and amines, which shows that HCN is an almost "normal" acid.[37c] Similarly, the large Brønsted parameter of $\beta = 1.12$ observed for thermodynamically unfavorable deprotonation of chloroform by amine bases provides evidence that the rate-determining step for the isotope

(58) Kresge, A. J. *Acc. Chem. Res.* **1975**, *8*, 354−360.
(59) The correlation for k_{HO} in Figure 4 [ref 54a] gives $(k_{HO})_I = 2$ M^{-1} s^{-1} for thermoneutral deprotonation of a hypothetical monoprotic carbon acid which has a pK_a of 15.7 by hydroxide ion (pK_a 15.7).
(60) The Marcus equation predicts that these correlations should show detectable curvature, and the explanation for the failure of the Marcus treatment was discussed in our earlier work.[5]
(61) An intrinsic barrier of $\Lambda = 10$ kcal/mol can be calculated using eq 10 (derived at 298 K) with $k_{obsd} = k_{HOH} = 10^{11}$ s^{-1} and $K = 10^{(31-15.7)}$ [Guthrie, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 7249−7255].

$$\log k_{obsd} = \frac{1}{1.36}\left\{17.44 - \Lambda\left(1 - \frac{1.36 \log K}{4\Lambda}\right)^2\right\} \quad (10)$$

(62) Bernasconi, C. F. *Tetrahedron* **1985**, *41*, 3219−3234.

Table 2. Effects of α-Carbonyl Substituents X on the Equilibrium Constants for Carbon Ionization and Keto−Enol Tautomerization of Acetaldehyde



$K_C = K_E K_O$

X	pK_a^C [a] (−log K_C)	pK_E [b] (−log K_E)	pK_a^O [c] (−log K_O)	Δ log K_C	Δ log K_E	Δ log K_O
H[d]	16.7	6.2	10.5	0	0	0
Me[d]	19.3	8.3	10.9	−2.6	−2.1 (−1.6)[e]	−0.4
SEt	21.0[f]	13.2[g]	7.8[h]	−4.3	−7.0	2.7
OMe	25.6[i]	18.5[g]	7.1[j]	−8.9	−12.3 (−12.4)[e]	3.4
OH[k]	26.6	19.3	7.3	−9.9	−13.1 (−12.1)[e]	3.2
NH_2[l]	28.4[m]	19.2[g]	9.2[h]	−11.7	−13.0 (−11.4)[e]	1.3
O^- [l]	33.5[m]	21.8[n]	11.7[o]	−16.8	−15.6	−1.2

[a] Equilibrium constant for ionization of the carbon acid. [b] Equilibrium constant for keto−enol tautomerization. [c] Equilibrium constant for oxygen ionization of the enol. [d] Data from ref 2. [e] Data from B3LYP/6-31G**//B3LYP/6-31G** calculations taken from ref 25. [f] Data from ref 4. [g] Calculated from data in this table using the relationship $pK_E = pK_a^C - pK_a^O$ (Scheme 4). [h] Calculated from the correlation given by eq 6 with values of $\rho_I = -12.8$ and $C = 10.7$ [ref 51], and values of σ_I^X from ref 50. [i] Data for X = OEt from ref 5. [j] Calculated from $pK_a^O = 6.55$ for the enol of methyl mandelate [Chiang, Y.; Kresge, A. J.; Schepp, N. P.; Xie, R. Q. *J. Org. Chem.* **2000**, *65*, 1175−1180] with corrections for the presence of the β-phenyl (+1.04 units) and β-hydroxy (−0.46 units) groups [refs 5 and 88]. [k] Data from ref 22a. [l] Data from this work. [m] Data from Table 1. [n] Calculated from a thermodynamic cycle, see text. [o] Calculated using the relationship $pK_a^O = pK_a^C - pK_E$ (Scheme 4).

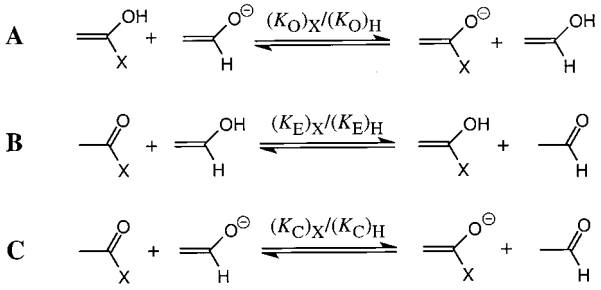
exchange reaction is diffusional separation of a contact ion pair intermediate (see Figure 3).[37d]

The data in Figure 4 do not show a good fit to either the simple Marcus equation for proton transfer or to an expanded equation that includes a work term.[5] Both treatments predict that the correlation between log k_{HO} and pK_a should be detectably curved because of a Hammond-type shift to a later transition state with increasing thermodynamic barrier to proton transfer, and hence should exhibit a slope > |−0.50| for unfavorable proton transfer.[35,63] Rather, this correlation is linear with a slope of −0.40 even for highly unfavorable proton transfer (up to 21 kcal/mol). The explanation for the failure of simple Marcus treatments of these data was discussed in earlier work.[5] The curvature shown in Figure 4 reflects the change in slope from −0.40 to −1.0 that occurs with the change in the rate-determining step for deprotonation of carbon acids by hydroxide ion, from proton transfer (k_p, Scheme 5) to solvent reorganization (k_{reorg}). Such "Eigen" curvature would have been more difficult to detect against the background of secondary Marcus-type curvature resulting from a simple Hammond effect on the structure of the transition state for proton transfer.

Substituent Effects on Enolization. Table 2 summarizes the effects of changing substituents X at simple carbon acids CH_3-COX on carbon acidity (log K_C) and on the equilibrium constant for keto−enol tautomerization (log K_E), relative to acetaldehyde (X = H). Most of these data are from experimental studies, except for acetic acid where the data from QM/MM calculations are used.[22a] In a related case these calculations have been found to show good agreement with the experimental studies.[24]

The effect of α-OR, α-SR, and α-NR_2 substituents on log K_C for carbon acid ionization, log K_O for ionization of the enol, and log K_E for keto−enol tautomerization (Scheme 6 and Table 2) depends on the following: (1) The polar effect of these electron-withdrawing groups which favor carbon and oxygen ionization by stabilizing negative charge at the enolate ion. (2) The π-donor effect of interactions between the lone pair(s) of electrons at these substituents and the electron deficient π-orbital at the adjacent carbonyl carbon.[15−19,64] This is more stabilizing

Scheme 6



than the corresponding interaction between these nonbonding electrons and the π-orbital of the adjacent carbon at the enol and would be expected to increase the barrier to both carbon ionization and keto−enol tautomerization. The data in Table 2 provide support for the following generalizations about polar and π-donor substituent effects on log K_C, log K_O, and log K_E (Scheme 6).

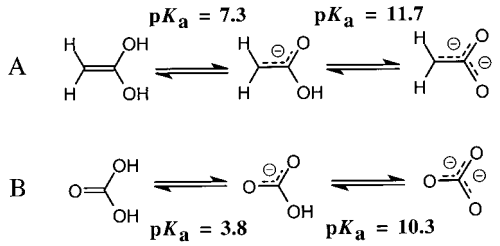
(1) The effects of α-OR, α-SR, and α-NR_2 groups on log K_O (Δlog K_O, Scheme 6A) are due mainly to polar stabilization of the enolate anion. For example, the 3.4 and 3.2 unit effects, respectively, of α-OMe and α-OH groups on log K_O (Table 2) are not much larger than the 2.1 and 2.4 unit effects of α-OEt and α-OH groups on the pK_a for ethanol,[65] where π-donor interactions are negligible.

(2) The π-donor effects of α-OR, α-SR, and α-NR_2 groups on log K_E (Δlog K_E, Scheme 6B) are much larger than any polar effect on this equilibrium constant. The values of log K_E in Table 2 change by up to 15 units, while log K_O changes by no more than 3.4 units due to the polar interaction of these substituents with the anionic oxygen at the enolate. The polar substituent effect on log K_E is substantially smaller than 3.4 units, because there is little difference in the polar interactions of these substituents with the formally neutral keto and enol groups.

(3) Both the π-donor and polar effects of α-OR, α-SR, and α-NR_2 groups contribute to the observed changes in log K_C (Δlog K_C, Scheme 6C). However, the π-donor effects, which result in up to a 15 unit change in log K_E, are much larger than the polar effects.

(63) Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891−899.
(64) Wiberg, K. B.; Rablen, P. R. *J. Am. Chem. Soc.* **1995**, *117*, 2201−2209.
(65) Sørensen, P. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1987**, *109*, 4675−4690.

Scheme 7



It can be shown from the appropriate thermodynamic cycle that the difference in the pK_a's for oxygen ionization of the enol of acetic acid (pK_a^O = 7.3, Table 2) and of acetic acid itself (pK_a = 4.8) is equal to the difference in pK_E for acetic acid (pK_E = 19.3, Table 2) and pK_E for acetate anion, so that pK_E = 21.8 for keto−enol tautomerization of acetate anion (Table 2). A value of pK_a^O = 11.7 for oxygen ionization of the enolate of acetic acid (which is also the enol of acetate anion) to give the enolate dianion can then be calculated from pK_E = 21.8 and pK_a^C = 33.5 using the relationship pK_a^O = pK_a^C − pK_E (Scheme 4). The 4.4 unit difference in the first pK_a of 7.3 and the second pK_a of 11.7 for ionization of the enol of acetic acid (Scheme 7A) is smaller than the 6.5 unit difference in the first pK_a of 3.8 and the second pK_a of 10.3 for the related ionization of carbonic acid (Scheme 7B).[66] The significance of the 6.5 − 4.4 = 2.1 unit difference in these two differences is unclear.[67]

Polar substituent effects on organic reactions such as the oxygen ionization of enols have been extensively documented.[68] By contrast, experimental data which provide a measure of the stabilizing interaction of the carbonyl group with α-OR, α-SR, and α-NR_2 groups are scarce, although in recent years there has been an explosion of computational work to model and rationalize these interactions.[15−19,25−28,64] The data in Table 2 support the following conclusions about the interactions of π-donor atoms with the carbonyl group.

(1) The 7.0 unit effect of the α-SEt group on log K_E for tautomerization of acetaldehyde is more than half as large as the 12.3 unit effect of the α-OMe group. Therefore, the interaction of the acetyl group with an α-SEt group is stabilizing, but significantly less so than the corresponding interaction with an α-OMe group. There is evidence that an α-SR group provides greater stabilization of carbocations than does the corresponding α-OR group,[69] but the interpretations of experimental[69] and computational[70] studies to compare these interactions are anything but straightforward. Ab initio calculations show that α-SR is a better π-electron donor than α-OR when the energy of the π-acceptor LUMO is low (e.g., at XCH_2^+), but that this order is reversed as the energy of the acceptor LUMO increases [e.g., at XC═O(R)], and are consistent with the observed differential stabilization of carbocations and the carbonyl group by α-SR and α-OR groups.[70b] These experiments and calculations are consistent with the notion that: (a) The relative interactions of α-OR and α-SR groups are governed by the greater polarizability of sulfur as compared with oxygen when there is a large transfer of positive charge from a strongly electrophilic π-acceptor site such as CH_2^+, which results in a greater stabilizing interaction of α-SR with these sites. (b) The relative interactions of α-OR and α-SR groups are governed by the better π-overlap of the matched 2p orbitals of carbon and oxygen as compared with the mismatched 2p and 3p orbitals of carbon and sulfur, respectively, when there is a relatively small transfer of positive charge from a weakly electrophilic π-acceptor site such as C═O(R), which results in a greater stabilizing interaction of α-OR with these sites.

(2) The values of pK_E for methyl acetate (18.5), acetic acid (19.3), and acetamide (19.2) are similar (Table 2). Nitrogen substituents are better π-donors than are oxygen substituents,[15−19] and this would be expected to favor greater ground-state stabilization and a more unfavorable equilibrium constant for tautomerization of amides than of esters. However, similar equilibrium constants for tautomerization of methyl acetate and acetamide are also predicted by recent calculations (Table 2, values in parentheses).[25] Both the calculations and the experimental data are consistent with the conclusion that these tautomeric equilibrium constants cannot be rationalized by a simple consideration of the π-donor effects of α-NR_2 and α-OR groups. It has been proposed that the second nonbonding electron pair at oxygen can provide additional ground-state stabilization of an ester by interaction with the σ^*_{C-O} antibonding orbital of the carbonyl group, so that the sum of the separate stabilizing interactions between the carbonyl group and the two lone pairs of electrons at oxygen is similar to the π-donor interaction of the single lone pair of electrons at nitrogen.[25]

(3) There is only a 2.5 unit difference in pK_E for tautomerization of acetic acid (19.3) and acetate anion (21.8). Therefore the ground-state stabilization of the carbonyl group by interaction with an α-O^- group is not much larger than its stabilization by interaction with an α-OH group. This is consistent with the results of qualitative analyses of substituent effects on oxygen acidity,[27,71] and with recent high level ab initio calculations,[26] which show that the resonance stabilization of acetate anion is not much greater than for acetic acid. There is the same 2.5 unit difference in the pK_a's for the enol of acetic acid (7.3) and for oxygen deprotonation of acetic acid (4.8). Here, this difference shows that an α-acetyl group provides a 3.4 kcal/mol greater stabilization of negative charge at oxygen than does an α-vinyl alcohol group. Part of this 3.4 kcal/mol effect is due to the greater stabilization of negative charge by the polar effect of the electron-withdrawing acetyl group,[72] so that the resonance stabilization of the negative charge is smaller than 3.4 kcal/mol. This falls within the range of other estimates of the resonance stabilization of charge at acetate anion.[71] The 2.5 log

(66) Jencks, W. P.; Regenstein, J. In *Handbook of Biochemistry and Molecular Biology, Physical and Chemical Data*, 3rd ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, OH, 1976; Vol. 1, pp 305−351.

(67) The value of k_{DO} determined for the deuterium exchange reaction of acetate anion may be larger than the second-order rate constant for stepwise deprotonation of acetate anion by DO^- that was used to calculate the value for pK_a^C (eq 7). However, as discussed in the text, we predict that this should not introduce a large error into the value of pK_a^C or of pK_a^O calculated from pK_a^C.

(68) Hine, J. *Structural Effects on Equilibria in Organic Chemistry*; Wiley: New York, 1975; Chapter 2.

(69) Jagannadham, V.; Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **1993**, *115*, 8465−8466. (b) References to other theoretical and gas-phase studies are summarized in ref 70a.

(70) (a) Apeloig, Y.; Karni, M. *J. Chem. Soc., Perkin Trans. 2* **1988**, 625−636. (b) Bernardi, F.; Mangini, A.; Epiotis, N. D.; Larson, J. R.; Shaik, S. *J. Am. Chem. Soc.* **1977**, *99*, 7465−7470. (c) Clark, T.; Schleyer, P. v. R. *Tetrahedron Lett.* **1979**, *48*, 4641−4644.

(71) Hine has estimated that there is an ca. 7 kcal/mol resonance stabilization of negative charge at acetate anion [ref 68, pp 164−165], and Siggel et al. have estimated this interaction to be 2−5 kcal/mol [ref 27].

(72) The inductive substituent constant σ_I for the acetyl group is 0.28 [ref 50], and σ_I = (0.05 + 0.10) = 0.15 can be estimated for the α-vinyl alcohol substituent, where 0.05 and 0.10 are the values of σ_I for a vinyl group and a CH_2OH group, respectively [ref 50].

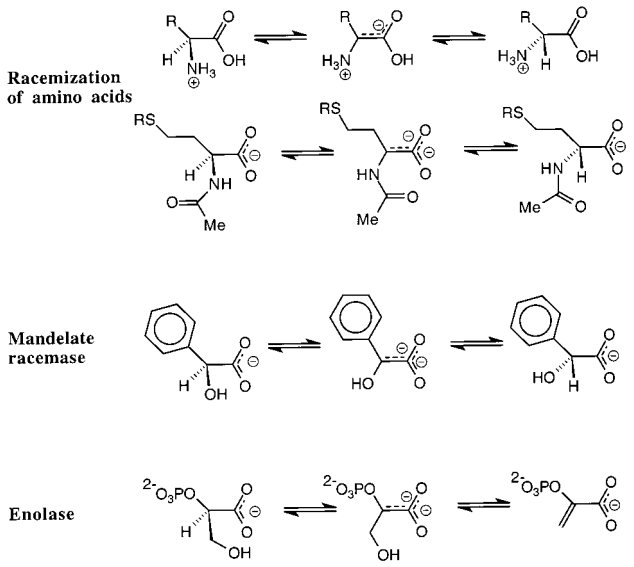


Figure 5. Enzyme-catalyzed reactions for which the first step is carbon deprotonation of a carboxylic acid or a carboxylate anion.

unit difference in the interaction of α-O^- as compared with α-OR with the carbonyl group reflects the advantage associated with spreading unit negative charge across two oxygen atoms at the delocalized oxygen anion. It is likely that this advantage is strongly attenuated by the strong stabilization of oxyanions by aqueous solvation, so that the stabilization from "resonance" delocalization of negative charge will be much larger for formation of "naked" anions in the gas phase.

Enzymatic Catalysis. The problem of catalysis of the deprotonation of α-carboxylate carbon is particularly difficult, and it is interesting to note two relationships between the catalytic strategies for enzymes that catalyze these reactions and the substrate structure.

(1) A variety of enzyme-catalyzed racemization reactions of α-amino acids proceed by abstraction of the α-amino proton to give an amino acid enolate, followed by protonation of this enolate to give the epimeric or racemic amino acid (Figure 5).[73−78] Simple amino acid racemases have no requirement for a metal ion cofactor, while the enzyme that catalyzes racemization of *N*-acyl methionine is a metalloenzyme member of the enolase superfamily.[79−82] We have proposed that there is effective stabilization of the zwitterionic enolate intermediate of α-amino acid racemases by intramolecular electrostatic interactions between the protonated α-amino group and the enolate oxygen (Figure 5) and that this interaction is enhanced by transfer of the enolate from water to an enzyme active site of low dielectric constant.[7] There can be no such intramolecular electrostatic stabilization of the enolate intermediate of the enzyme that catalyzes racemization of *N*-acyl methionine, because the weakly basic *N*-acyl group is not protonated. Rather, the enolate dianion intermediate of this enzymatic reaction is stabilized by interaction with an enzyme-bound metal dication.[82]

(2) Mandelate racemase[83−86] and enolase[87] catalyze proton transfer from the α-carboxylate carbon of mandelate and 2-phosphoglycerate, respectively (Figure 5). Each is a metalloenzyme and a member of the enolase superfamily.[79,80] However, while there is only one metal ion binding site for mandelate racemase,[80] there are two metal ion sites for enolase.[87] The enolate dianion intermediate of the reaction catalyzed by mandelate racemase is stabilized by interaction with the α-phenyl group,[88] while that for the reaction catalyzed by enolase is strongly destabilized by electrostatic interactions with the phosphate dianion. We suggest that the latter enzyme has recruited a second metal cation in order to "neutralize" these strongly destabilizing intramolecular interactions with the phosphate dianion.

It has been suggested that some carbanion intermediates of enzyme-catalyzed reactions are too unstable to exist for the time of a bond vibration (ca. 10^{-13} s) at enzyme active sites,[92] and that their formation is therefore avoided by a concerted reaction that is "enforced" by the insignificant lifetime of the putative intermediate of the stepwise reaction.[56,57] The lifetime of the enolate of acetate anion in water is less than $1/k_{HOH} = 10^{-11}$ s (Figure 4), and it is highly likely that the lifetime of this enolate in the presence of buffer acids that are more acidic than water, such as those found at enzyme active sites, is shorter than the vibrational limit of 10^{-13} s. Therefore, the formation of such carbanions in enzyme active sites might be avoided by concerted reaction mechanisms. At the same time there is good evidence that the elimination reaction catalyzed by enolase proceeds through an enzyme-bound enolate tetraanion intermediate (Figure 5),[93] which should be even more unstable toward protonation than the enolate of the acetate anion. The observation of this stepwise enzymatic reaction mechanism suggests that there are substantial chemical barriers to both protonation of, and elimination of HO^- from, the enzyme-bound carbanion,

(73) Cardinale, G. J.; Abeles, R. H. *Biochemistry* **1968**, *7*, 3970−3978.
(74) Rudnick, G.; Abeles, R. H. *Biochemistry* **1975**, *14*, 4515−4522.
(75) Gallo, K. A.; Tanner, M. E.; Knowles, J. R. *Biochemistry* **1993**, *32*, 3991−3997.
(76) Glavas, S.; Tanner, M. E. *Biochemistry* **1999**, *38*, 4106−4113.
(77) Albery, W. J.; Knowles, J. R. *Biochemistry* **1986**, *25*, 2572−2577.
(78) Koo, C. W.; Blanchard, J. S. *Biochemistry* **1999**, *38*, 4416−4422.
(79) Babbitt, P. C.; Gerlt, J. A. *J. Biol. Chem.* **1997**, *272*, 30591−30594.
(80) Babbitt, P. C.; Hasson, M. S.; Wedekind, J. E.; Palmer, D. R. J.; Barrett, W. C.; Reed, G. H.; Rayment, I.; Ringe, D.; Kenyon, G. L.; Gerlt, J. A. *Biochemistry* **1996**, *35*, 16489−16501.
(81) Palmer, D. R. J.; Garrett, J. B.; Sharma, V.; Meganathan, R.; Babbitt, P. C.; Gerlt, J. A. *Biochemistry* **1999**, *38*, 4252−4258.
(82) Thompson, T. B.; Garrett, J. B.; Taylor, E. A.; Meganathan, R.; Gerlt, J. A.; Rayment, I. *Biochemistry* **2000**, *39*, 10662−10676.
(83) Landro, J. A.; Kallarakal, A. T.; Ransom, S. C.; Gerlt, J. A.; Kozarich, J. W.; Neidhart, D. J.; Kenyon, G. L. *Biochemistry* **1991**, *30*, 9274−9281.
(84) Neidhart, D. J.; Howell, P. L.; Petsko, G. A.; Powers, V. M.; Li, R.; Kenyon, G. L.; Gerlt, J. A. *Biochemistry* **1991**, *30*, 9264−9273.
(85) Powers, V. M.; Koo, C. W.; Kenyon, G. L.; Gerlt, J. A.; Kozarich, J. W. *Biochemistry* **1991**, *30*, 9255−9263.
(86) Schafer, S. L.; Barrett, W. C.; Kallarakal, A. T.; Mitra, B.; Kozarich, J. W.; Gerlt, J. A.; Clifton, J. G.; Petsko, G. A.; Kenyon, G. L. *Biochemistry* **1996**, *35*, 5662−5669.
(87) Larsen, T. M.; Wedekind, J. E.; Rayment, I.; Reed, G. H. *Biochemistry* **1996**, *35*, 4349−4358.
(88) Chiang, Y.; Kresge, A. J.; Popik, V. V.; Schepp, N. P. *J. Am. Chem. Soc.* **1997**, *119*, 10203−10212.
(89) Gold, V.; Grist, S. *J. Chem. Soc., Perkin Trans. 2* **1972**, 89−95. Kresge, A. J.; O'Ferrall, R. A. M.; Powell, M. F. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: New York, 1987; Vol. 7.
(90) The secondary solvent deuterium isotope effect for deprotonation of simple amides by lyoxide ion is expected to be larger than $k_{DO}/k_{HO} = 1.46$ for deprotonation of acetone [Pocker, Y. *Chem. Ind.* **1959**, 1383−1384]. However, we have no evidence that it is close to the maximum value of $k_{DO}/k_{HO} = 2.4$ for deprotonation to form very unstable carbanions, for which isotope exchange is limited by solvent reorganization that exchanges the hydron of substrate with a labeled hydron from solvent.[89] Therefore, we use an intermediate value of $k_{DO}/k_{HO} = 2.0$ as the estimated secondary solvent deuterium isotope effect for deprotonation of acetamide and dimethylacetamide.
(91) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1373−1383.
(92) Thibblin, A.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 4963−4973.
(93) Stubbe, J.; Abeles, R. H. *Biochemistry* **1980**, *19*, 5505−5512. Poyner, R. R.; Laughlin, L. T.; Sowa, G. A.; Reed, G. H. *Biochemistry* **1996**, *35*, 1692−1699. Anderson, V. E.; Weiss, P. M.; Cleland, W. W. *Biochemistry* **1984**, *23*, 2779−2786. Anderson, S. R.; Anderson, V. E.; Knowles, J. R. *Biochemistry* **1994**, *33*, 10545−10555.

so that its lifetime is substantially longer than that for the free carbanion in water. This provides evidence that enolase effects an increase in both the *thermodynamic* and the *kinetic* stability of the bound carbanion; the effect of the enzyme on the kinetic stability of this intermediate reflects the requirement for the loss of stabilizing interactions with the enzyme at the transition state for enzymatic protonation of the carbanion.

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Supporting Information Available: Table S1: Rate constants k_{ex} (s^{-1}) for deuterium exchange into acetamide, *N*,*N*-dimethylacetamide, and acetate anion catalyzed by deuterioxide ion. Table S2: Rate constants k_{ex} (s^{-1}) for deuterium exchange into acetamide determined in the presence of quinuclidine buffer (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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