Formation and Stability of Organic Zwitterions in Aqueous Solution: Enolates of the Amino Acid Glycine and Its Derivatives

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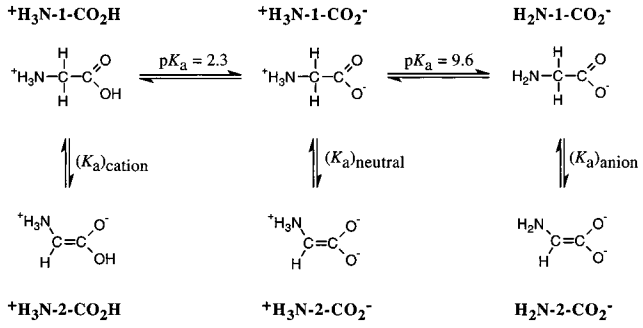
Abstract: Second-order rate constants for carbon deprotonation of glycine zwitterion, N-protonated glycine methyl ester, betaine methyl ester, and betaine by deuterioxide ion in D_2O have been determined by following deuterium exchange into these carbon acids in buffered solutions at 25 °C and $I = 1.0$ (KCl) by ^{1}H NMR spectroscopy. The data were used to calculate the following carbon acidities for glycine zwitterion and its derivatives in aqueous solution: $^+H_3NCH_2CO_2^-$, $pK_a = 28.9 \pm 0.5$; $^+H_3NCH_2CO_2Me$, $pK_a = 21.0 \pm 1.0$; $^+Me_3NCH_2CO_2Me$, $pK_a = 18.0 \pm 1.0$; $^+Me_3NCH_2CO_2^-$, $pK_a = 27.3 \pm 1.2$. The rate constants for deprotonation of glycine methyl ester by Brønsted base catalysts are correlated by $\beta = 0.92$. Two important differences between structure−reactivity relationships for deprotonation of neutral α-carbonyl carbon acids and cationic esters are attributed to the presence of the positively charged ammonium substituent at the latter carbon acids: (1) The smaller negative deviation of log k_{DO} from the Brønsted correlation for deprotonation of $^+H_3NCH_2CO_2Me$ than for deprotonation of ethyl acetate is attributed to stabilization of the transition state for enolization by electrostatic interactions between DO^- and the positively charged ammonium substituent. (2) The positive deviation of log k_{HO} for deprotonation of cationic esters from the rate−equilibrium correlation for deprotonation of neutral α-carbonyl carbon acids is attributed to both transition-state stabilization by these same electrostatic interactions and movement of negative charge at the product enolate away from oxygen and onto the α-carbon. This maximizes the stabilizing interaction of this negative charge with the positively charged ammonium substituent and leads to a reduction in the Marcus intrinsic barrier to proton transfer, as a result of the decreased resonance stabilization of the enolate. The implications of these results for enzymatic catalysis of racemization of amino acids is discussed.

Proteins show a high stereochemical integrity, because the α-protons of the repeating L-amino acid monomers are only weakly acidic. For example, the half-time for epimerization of isoleucine in bones is >100 000 years,[3] while the aspartyl residues in several mammalian proteins present in dentin, the ocular lens nucleus, and myelin have been shown to undergo racemization at a rate of ∼0.1%/year.[4] The known rate constants for these slow posttranslational modifications of proteins allow for the estimation of ages that are otherwise poorly documented.[5−7] Studies of the racemization of amino acids in water have been carried out under harsh conditions at temperatures of more than 100 °C, because these reactions were presumed to be too slow to study conveniently at room temperature.[8−13] On the other hand, significant racemization of amino acids is observed during peptide synthesis using many common methods under apparently mild reaction conditions.[14]

Scheme 1



The acidity of the α-proton of amino acids depends strongly upon the ionization state of these species (Scheme 1). For example, the deprotonation of glycine anion to give the enolate dianion $[(K_a)_{anion}]$ is much less favorable than deprotonation of the neutral zwitterion $[(K_a)_{neutral}]$. Deprotonation of glycine cation $[(K_a)_{cation}]$ should be even more favorable still. Although a decrease in pH results in protonation of glycine and hence an increased reactivity toward enolization, there is little change in

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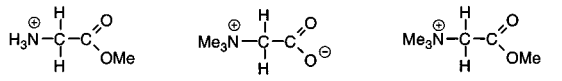
the overall rate of specific-base-catalyzed enolization because there is a compensating decrease in the concentration of hydroxide ion. However, amino acid derivatives where the charge at the substrate is held constant by "fixing" the ionization state, either by methylation of the carboxyl group of glycine to give glycine methyl ester (^+H_3N-1-CO_2Me) and/or by perm-



^+H_3N-1-CO_2Me **$^+Me_3N$-1-CO_2^-** **$^+Me_3N$-1-CO_2Me**

ethylation of the amino group to give betaine ($^+Me_3N$-1-CO_2^-) and betaine methyl ester ($^+Me_3N$-1-CO_2Me) are excellent models for the corresponding charged forms of glycine. We have developed methods to determine the pK_a of weak carbon acids in water,[15−18] and we were interested in their application to determination of the carbon acidity of the simple amino acid glycine ^+H_3N-1-CO_2^- and its derivatives ^+H_3N-1-CO_2Me, $^+Me_3N$-1-CO_2^-, and $^+Me_3N$-1-CO_2Me. This work was undertaken for the following reasons:

(1) There are no good estimates of the carbon acidity of amino acids in water under physiological conditions. These data are essential for characterization of the chemical reactivity of this important class of biological compounds.

(2) The origin of the rate acceleration for enzyme-catalyzed racemization of amino acids through highly unstable enolate intermediates is not well understood. Deprotonation of the amino acid is the first step of many such racemization reactions,[19−26] and we would like to know how the rate constant for this reaction might depend on the protonation state of the enzyme-bound amino acid.

(3) We are interested in understanding the effects of simple α-substituents on the rate and equilibrium constants for ionization of weak carbon acids in aqueous solution.[15−18,27] A comparison of the carbon acidity of glycine and its derivatives with data for simpler carbon acids such as ethyl acetate[16] and acetate ion[28] will provide insight into the effect of cationic substituents on carbanion stability and on the Marcus intrinsic barriers to proton transfer at carbon.

We have reported that deprotonation of the α-carbon of glycine methyl ester (^+H_3N-1-CO_2Me) by deuterioxide ion and Brønsted bases in D_2O can be *easily* followed at 25 °C and neutral pD; the data provide an estimate of pK_a = 21 for this carbon acid.[27] We report here the full details of our earlier communication and new data for the carbon acidity of glycine (^+H_3N-1-CO_2^-) and the derivatives betaine ($^+Me_3N$-1-CO_2^-) and betaine methyl ester ($^+Me_3N$-1-CO_2Me). These data give reliable values of the pK_a's for glycine and its derivatives which provide insight into the effects of cationic ammonium and trimethylammonium substituents on carbon acidity and Brønsted base catalysis of enolization. The relevance of these results to the mechanism for enzymatic catalysis of deprotonation of amino acids is discussed.

Experimental Section

Materials. Glycine methyl ester hydrochloride, betaine hydrochloride, methyl chloroacetate, 3-quinuclidinone hydrochloride, 3-chloroquinuclidine hydrochloride, quinuclidine hydrochloride, 3-quinuclidinol, cacodylic acid, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), 2,2,2-trifluoroethanol-d_3 (99.5% D), and potassium acetate were purchased from Aldrich. Glycine was purchased from Fluka, and methoxyacetic acid was purchased from Acros. Deuterium oxide (99.9% D) and deuterium chloride (35% w/w, 99.5% D) were from Cambridge Isotope Laboratories. KOD (40 wt %, 98+% D) was from Aldrich. All other organic and inorganic chemicals were reagent grade and were used without further purification.

The 3-substituted quinuclidines were purified by recrystallization as described previously.[16] The potassium salt of methoxyacetic acid was prepared by neutralization of an aqueous solution of the acid with 1 equiv of KOH. The water was removed under reduced pressure, and the salt was recrystallized from 10/1 ethanol/water. (Methoxycarbonylmethyl)trimethylammonium chloride (betaine methyl ester chloride) was synthesized by bubbling trimethylamine through a solution of methyl chloroacetate in toluene at −10 °C:[29] ^{1}H NMR (D_2O) δ ppm 4.36 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.34 (s, 9H, $N(CH_3)_3$).

Preparation of Solutions. As described previously,[15] the acidic protons of substrates and buffer components were generally exchanged for deuterium before preparation of solutions of these compounds in D_2O. Cacodylic acid, HFIP, 3-quinuclidinol, and CF_3CO_2OH were dissolved directly in D_2O (99.9% D), which resulted in <1 atom % increases in the protium content of this solvent.

Phosphate buffers were prepared by mixing stock solutions of K_2DPO_4 and KD_2PO_4 in D_2O (I = 1.0, KCl) to give the desired acid/base ratio. 3-Quinuclidinol and carboxylate buffers were prepared by dissolving their basic forms and KCl in D_2O followed by addition of DCl to give the desired acid/base ratio at I = 1.0 (KCl). Quinuclidine, 3-quinuclidinone, cacodylate, trifluoroethoxide, and glycine buffers were prepared by dissolving their acidic forms and KCl in D_2O followed by addition of KOD to give the desired acid/base ratio at I = 1.0 (KCl). Solutions of quinuclidine, 3-quinuclidinol, 3-chloroquinuclidine, and HFIP buffered by phosphate at pD 7.4−7.6 were prepared by dilution of solutions of their acidic forms (I = 1.0, KCl) with phosphate buffer (I = 1.0, KCl) and 1 M KCl to give a final phosphate buffer concentration of 80 mM.

Solution pH or pD was determined at 25 °C using an Orion model 720A pH meter equipped with a Radiometer GK2321C combination electrode. Values of pD were obtained by adding 0.40 to the observed reading of the pH meter.[30] The concentration of deuterioxide ion at any pD was calculated from eq 1, where $K_w = 10^{-14.87}$ is the ion product

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

of D_2O at 25 °C [31] and γ_{OL} = 0.79 is the apparent activity coefficient of lyoxide ion under our experimental conditions.[16]

The apparent pK_a's of the oxygen catalysts in D_2O at 25 °C and I = 1.0 (KCl), given by pK_{BD} = pD − log([B]/[BD$^+$]), were determined as the observed pD of gravimetrically prepared buffer solutions for which [B] = [BD$^+$] and the total buffer concentration was 0.05 M. Apparent pK_a's of pK_{BD} = 10.35 for glycine zwitterion and pK_{BD} = 8.48 for glycine methyl ester in D_2O at 25 °C and I = 1.0 (KCl) were

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determined by potentiometric titration of a 10 mM solution of the substrate with KOD.[32]

^{1}H NMR Spectroscopy. ^{1}H NMR spectra at 500 MHz were recorded in D_2O at 25 °C on a Varian Unity Inova 500 spectrometer. Relaxation times T_1 were determined using 10 mM solutions of substrate at I = 1.0 (KCl). Spectra were recorded with a sweep width of 2600 Hz, a 90° pulse angle, an acquisition time of 6 s, and zero-filling of the data to 64 K. The relaxation delay between pulses was 10−20-fold greater than the longest T_1 for the protons of interest. Chemical shifts are reported relative to HOD at 4.67 ppm. Baselines were subjected to a first-order drift correction before determination of integrated peak areas.

Kinetic Studies. All reactions were carried out in D_2O at 25 °C and a constant ionic strength of 1.0 maintained with potassium chloride. Reactions were initiated by mixing solutions of the substrate and buffer at the same pD and ionic strength (1.0, KCl) to give a final substrate concentration of 10−15 mM for glycine methyl ester, betaine methyl ester, and betaine. A concentration of 20 or 40 mM glycine was used for experiments in which glycine also served as the solution buffer.

A slow downward drift in the solution pD was observed during the deuterium exchange reactions of glycine methyl ester and betaine methyl ester, due to formation of D_3O^+ as a product of the competing hydrolysis of the methyl ester.[16] The pD of these solutions was monitored closely and was maintained within 0.05 unit of the initial value by the addition of small aliquots of 2.9 M KOD.

The exchange for deuterium of the α-protons of glycine and of glycine derivatives was followed by monitoring the disappearance of the singlet due to the α-CH_2 group of the substrate and the appearance of the triplet due to the α-CHD group of the monodeuterated product by ^{1}H NMR spectroscopy at 500 MHz (see above). The latter signals exhibit an upfield shift of 0.014 (glycine and glycine methyl ester) or 0.016 ppm (betaine and betaine methyl ester) from the singlet[15−18,27] and coupling between the remaining α-proton and the α-deuterium (J_{HD} = 2.5 Hz). These deuterium perturbations of ^{1}H chemical shifts and H−D coupling constants are similar to those observed in previous work.[15−18,33a,34]

At timed intervals an aliquot was withdrawn from the reaction mixture and, in most cases, was prepared for ^{1}H NMR analysis by adjusting the pD to ≤6 with concentrated DCl, to quench the deuterium exchange reaction. Values of R, which is a measure of the progress of deuterium exchange,[17,18,33a] were calculated according to eq 2, where

$$R = A_{CH_2}/(A_{CH_2} + A_{CHD}) \quad (2)$$

A_{CH_2} and A_{CHD} are the integrated areas of the singlet due to the α-CH_2 group of the substrate and the triplet due to the α-CHD group of the monodeuterated product, respectively. At early reaction times, the upfield ^{13}C satellite of the signal due to the α-CH_2 group of the substrate was not completely resolved from the most downfield peak of the triplet due to the α-CHD group of the product.[16] Therefore, the total integrated area of the triplet due to the α-CHD group (A_{CHD}, eq 2) was obtained by multiplying the integrated area of the most upfield peak of this triplet by three. The area for the singlet due to the α-CH_2 group (A_{CH_2}) was then calculated as the difference between the total integrated area of all signals due to the α-protons and the calculated value of A_{CHD}.

The reactions of glycine methyl ester, glycine, betaine methyl ester, and betaine were followed during exchange for deuterium of 20−30%, 1−3%, up to 90%, and 30−90%, respectively, of the first proton of the α-CH_2 group of the substrate. Semilogarithmic plots of reaction progress, R, against time were linear with negative slopes equal to k_{obsd} (s^{-1}, eq 3), which is the rate constant for exchange of a *single* proton

$$\ln R = -k_{obsd}t \quad (3)$$

of the α-CH_2 group of the substrate.[33a] The values of k_{obsd} were reproducible to within 10%.

The ester hydrolysis reactions of glycine methyl ester were followed by ^{1}H NMR by monitoring the loss and appearance, respectively, of the singlets due to the CH_3 group of the methyl ester substrate and the methanol product. The progress of hydrolysis, f_{hyd}, was calculated using eq 4, where A_{MeOR} and A_{MeOH} are the integrated areas of the signals

$$f_{hyd} = A_{MeOR}/(A_{MeOR} + A_{MeOH}) \quad (4)$$

due to the CH_3 group of glycine methyl ester and methanol, respectively. There was significant overlap of the signals for the CH_3 protons of methanol and protons of the 3-substituted quinuclidines. In these cases, f_{hyd} was calculated using eq 5, where A_{std} is the integrated area for a

$$f_{hyd} = [A_{MeOR}/A_{std}]/[A_{MeOR}/A_{std}]_o \quad (5)$$

set of signals due to the 3-substituted quinuclidine, which acts as an internal standard, and $[A_{MeOR}/A_{std}]_o$ is the initial ratio of the integrated areas of the signals for the CH_3 protons of glycine methyl ester and the internal standard. Observed first-order rate constants for hydrolysis of glycine methyl ester, k_{hyd} (s^{-1}), were obtained as the negative slope of semilogarithmic plots of f_{hyd} against time, which were linear for at least three half-times for ester hydrolysis. The values of k_{hyd} determined in different experiments were reproducible to within 10%.

The deuterium enrichment of glycine obtained after ≥10 half-times for hydrolysis of glycine methyl ester in D_2O was determined by ^{1}H NMR analysis. The fraction of deuterated glycine, f_D, was calculated using eq 6, where A_{CH2} and A_{CHD} are the integrated areas of the singlet

$$f_D = 2A_{CHD}/(A_{CH2} + 2A_{CHD}) \quad (6)$$

and the upfield-shifted triplet due to the α-CH_2 group of unlabeled glycine and the α-CHD group of monodeuterated glycine, respectively. There was overlap of the signals for the α-CH_2 protons of glycine and those for 3-chloroquinuclidinium cation. Therefore, prior to NMR analysis, the latter component was removed from the reaction mixture by adjusting to pD ≈ 13 with concentrated KOD followed by rapid extraction of the tertiary amine into CCl_4. The remaining aqueous solution was then readjusted to pD ≈7 with concentrated DCl. A control study showed that there was no deuterium exchange into glycine during this extraction procedure.

Equation 6 was derived with the assumption that there is essentially no formation of dideuterated glycine labeled with an α-CD_2 group during the hydrolysis of glycine methyl ester in D_2O. The rate constants k_{hyd} (s^{-1}) for hydrolysis of glycine methyl ester are ∼10−20-fold larger than the rate constants k_{ex} (s^{-1}) for deuterium exchange to give monodeuterated glycine methyl ester (see Results), so that little ester will survive long enough for two exchange events to occur. The good agreement (±10%) between the rate constants for exchange determined directly by monitoring the incorporation of deuterium into glycine methyl ester, and indirectly from the values of k_{hyd} and f_D (see Results), shows that there would be no significant improvement using a more complicated treatment of these data that explicitly accounts for the formation of glycine containing an α-CD_2 group.

Results

Glycine Methyl Ester and Glycine. The exchange for deuterium of the first α-proton of glycine methyl ester and of glycine in D_2O at 25 °C and I = 1.0 (KCl) was followed by ^{1}H NMR spectroscopy at 500 MHz.[15,16] Representative partial ^{1}H NMR spectra of glycine methyl ester obtained during exchange of the first α-proton for deuterium in the presence of 80 mM phosphate buffer (pD 7.4) in D_2O at 25 °C and I = 1.0 (KCl) were shown in a preliminary report of this work.[27] Deuterium exchange results in decay of the singlet at 3.921 ppm due to the α-CH_2 group of the substrate and appearance of an upfield triplet at 3.907 ppm due to the α-CHD group of the monodeu-

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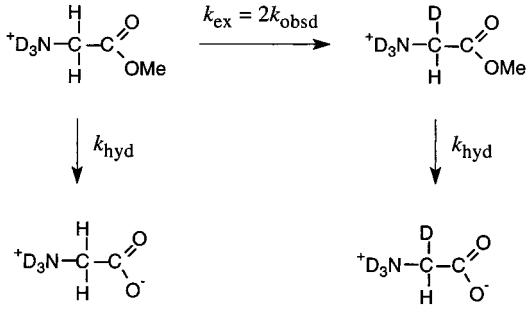
Scheme 2



teriated product, in which the remaining α-proton is coupled to the α-deuterium (J_{HD} = 2.5 Hz).

First-order rate constants for exchange for deuterium of the first α-proton of glycine methyl ester, k_{ex} (s^{-1}), were determined by two different methods.

(1) Method A. The deuterium exchange reactions of glycine methyl ester were followed for exchange of 20−30% of the first α-proton of the substrate, during which time there was relatively rapid loss of both the substrate and the monodeuterated product due to the competing ester hydrolysis reaction (Scheme 2). Values of *R*, a measure of reaction progress, were calculated from the integrated areas of the signals due to the α-CH_2 group of substrate and the α-CHD group of product according to eq 2,[17,18,33a] and rate constants k_{obsd} (s^{-1}) for exchange of a *single* α-proton were determined according to eq 3. However, the reaction of substrate to give monodeuterated product occurs at twice the rate of exchange of a single proton of the α-CH_2 group, so that $k_{ex} = 2k_{obsd}$, where k_{ex} (s^{-1}) is the first-order rate constant for exchange of the first proton of the α-CH_2 group of the substrate. Table S1 of the Supporting Information gives values of k_{ex} (s^{-1}) for the deuterium exchange reactions of glycine methyl ester in the presence of phosphate buffers (pD 7.0−8.1) and HFIP, 3-quinuclidinol, and quinuclidine at pD 7.4−7.5 (buffered by 80 mM phosphate) in D_2O at 25 °C and I = 1.0 (KCl).

(2) Method B. Rate constants for hydrolysis of glycine methyl ester, k_{hyd} (s^{-1}), were determined by following the disappearance of the CH_3 group of the substrate and the appearance of the CH_3 group of the methanol product by ^{1}H NMR spectroscopy, as described in the Experimental Section. After at least 10 half-times for ester hydrolysis, the fraction of the glycine product that contains deuterium, f_D, was determined by ^{1}H NMR analysis. The values of k_{hyd} and f_D were substituted into eq 7,

$$k_{ex} = k_{hyd} f_D/(1 - f_D) \qquad (7)$$

derived for Scheme 2, to give rate constants, k_{ex} (s^{-1}), for exchange of the first α-proton of glycine methyl ester. Table S2 of the Supporting Information gives values of k_{hyd} (s^{-1}), f_D, and the derived rate constants k_{ex} (s^{-1}) for the hydrolysis and deuterium exchange reactions of glycine methyl ester in the presence of phosphate buffers (pD 7.0−8.1) and HFIP, 3-chloroquinuclidine, and 3-quinuclidinol at pD 7.4−7.5 (buffered by 80 mM phosphate) in D_2O at 25 °C and I = 1.0 (KCl).

In many cases, deuterium exchange and hydrolysis were monitored concurrently in order to compare the rate constants for exchange obtained by methods A and B. The values of k_{ex} determined by these two methods agree to ±10% (Tables S1 and S2). For reactions in the presence of 3-chloroquinuclidinium cation, the values of k_{ex} could be obtained only by method B (Table S2).

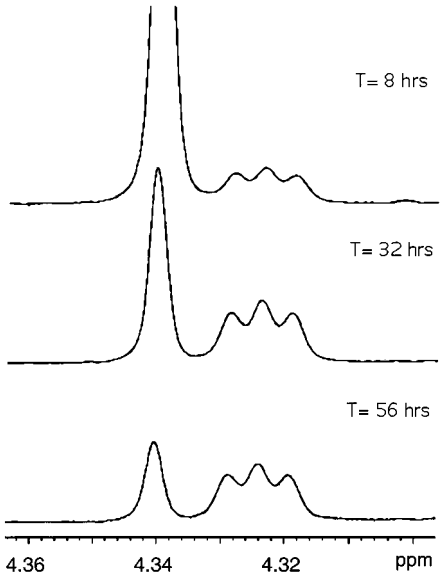


Figure 1. Representative partial ^{1}H NMR spectra at 500 MHz of betaine methyl ester obtained during exchange of the α-protons for deuterium in the presence of 50 mM phosphate buffer (pD 7.0) in D_2O at 25 °C and I = 1.0 (KCl). Deuterium exchange results in decay of the singlet at 4.340 ppm due to the α-CH_2 group of the substrate and appearance of an upfield triplet (J_{HD} = 2.5 Hz) at 4.324 ppm due to the α-CHD group of monodeuterated product.

No attempt was made to correct the data for the estimated 4% inverse secondary deuterium isotope effect on k_{hyd} resulting from incorporation of a single α-deuterium into glycine methyl ester,[35] because this isotope effect is too small to be detected in these experiments. It was shown in earlier work that this small isotope effect does not affect the rate constants for deuterium exchange into ethyl acetate determined by monitoring the incorporation of deuterium into the substrate against the background of ester hydrolysis.[16]

Deuterium exchange into glycine zwitterion results in decay of the singlet due to the α-CH_2 group of the substrate and appearance of an upfield triplet (J_{HD} = 2.5 Hz) due to the α-CHD group of monodeuterated product. These *very* slow reactions ($k_{ex} \approx 10^{-9}$ s^{-1}) were followed during exchange of only ∼1−3% of the first α-proton, and rate constants were determined using eqs 2 and 3, as described above for glycine methyl ester. Table S3 of the Supporting Information gives values of k_{ex} (s^{-1}) for the deuterium exchange reactions of glycine (self-buffered, pD 9.9−10.9) in D_2O at 25 °C and I = 1.0 (KCl).

Betaine Methyl Ester and Betaine. Figure 1 shows representative partial ^{1}H NMR spectra of betaine methyl ester obtained during exchange of the α-protons for deuterium in the presence of 50 mM phosphate buffer (pD 7.0) in D_2O at 25 °C and I = 1.0 (KCl). Deuterium exchange results in decay of the singlet at 4.340 ppm due to the α-CH_2 group of the substrate and appearance of an upfield triplet (J_{HD} = 2.5 Hz) at 4.324 ppm due to the α-CHD group of monodeuterated product. The much slower deuterium exchange reactions of betaine result in decay of the singlet at 3.902 ppm due to the α-CH_2 group of substrate and appearance of an upfield triplet (J_{HD} = 2.5 Hz) at 3.886 ppm due to the α-CHD group of monodeuterated product.

(35) A secondary β-deuterium isotope effect on deuterioxide-catalyzed hydrolysis of monodeuterated ethyl acetate of $(k_{hyd})_H/(k_{hyd})_D$ = 0.96 was estimated[16] from $(k_{hyd})_{3H}/(k_{hyd})_{3D}$ = 0.90 for the isotope effect on the hydroxide-catalyzed hydrolysis of trideuterated ethyl acetate in water: Bender, M. L.; Feng, M. S. *J. Am. Chem. Soc.* **1960**, *82*, 6318−6321.

Table 1. Second-Order Rate Constants for Carbon Deprotonation of Glycine and Glycine Derivatives by Deuterioxide Ion and Brønsted Bases in D_2O[a]

base catalyst	pK_{BD}[b]	carbon acid	k_B[c] (M^{-1} s^{-1})
DO^-	16.6	$^+H_3NCH_2CO_2Me$	6.0 ± 0.2
		$^+H_3NCH_2CO_2^-$	$(8.9 \pm 0.3) \times 10^{-5}$
		$^+Me_3NCH_2CO_2Me$	570 ± 40
		$^+Me_3NCH_2CO_2^-$	$(6.6 \pm 0.1) \times 10^{-4}$
quinuclidine	12.1[d]	$^+H_3NCH_2CO_2Me$	$(1.4 \pm 0.3) \times 10^{-2}$
3-quinuclidinol	10.7[d]	$^+H_3NCH_2CO_2Me$	$(6.8 \pm 1.0) \times 10^{-4}$
		$^+Me_3NCH_2CO_2Me$	$(3.8 \pm 0.3) \times 10^{-2}$
3-chloroquinuclidine	9.7[d]	$^+H_3NCH_2CO_2Me$	$(7.0 \pm 1.0) \times 10^{-5}$
$(CF_3)_2CHO^-$	9.9	$^+H_3NCH_2CO_2Me$	$(6.0 \pm 0.6) \times 10^{-5}$
DPO_4^{2-}	7.0	$^+H_3NCH_2CO_2Me$	$(5.6 \pm 0.8) \times 10^{-7}$

[a] At 25 °C and $I = 1.0$ (KCl). [b] Apparent pK_a of the conjugate acid of the base catalyst in D_2O at 25 °C and $I = 1.0$ (KCl) given by $pK_{BD} = pD - \log([B]/[BD^+])$, determined as described in the Experimental Section, unless noted otherwise. [c] Second-order rate constant for deprotonation of the carbon acid by the base catalyst, determined from the effect of increasing concentrations of the catalyst on the rate constant k_{ex} (s^{-1}) for exchange of the first α-proton of the carbon acid in D_2O (see text). For deuterioxide ion as the base, $k_B = k_{DO}$. [d] Data from ref 16.

The deuterium exchange reactions of betaine methyl ester are ~6-fold faster than the competing ester hydrolysis and were generally followed for two or three half-times for exchange. The slower exchange reactions of this substrate in acetate buffers (pD 5.2) and methoxyacetate buffers (pD 3.2−4.0) were followed during exchange of about 50 and 5−15%, respectively, of the first α-proton. The exchange reactions of betaine were following during exchange of up to 90% of the first α-proton. Rate constants for exchange were determined using eqs 2 and 3, as described above for glycine methyl ester. Table S4 of the Supporting Information gives values of k_{ex} (s^{-1}) for the deuterium exchange reactions of betaine methyl ester in the presence of 3-quinuclidinone (pD 8.3), phosphate (pD 7.0), cacodylate (pD 6.5), acetate (pD 5.2), and methoxyacetate (pD 3.2−4.0) buffers and 3-quinuclidinol buffered by phosphate (pD 7.6) in D_2O at 25 °C and $I = 1.0$ (KCl). Table S5 of the Supporting Information gives values of k_{ex} (s^{-1}) for the deuterium exchange reactions of betaine in the presence of 3-quinuclidinol (pD 9.7−11.2), quinuclidine (pD 11.9), and trifluoroethoxide (pD 12.6) buffers in D_2O at 25 °C and $I = 1.0$ (KCl).

Catalysis by Lyoxide Ion and General Bases. (1) Glycine Methyl Ester. The first-order rate constants k_{ex} (s^{-1}) for the exchange for deuterium of the first α-proton of glycine methyl ester in the presence of 20 mM phosphate buffer (pD 7.0−8.1) in D_2O at 25 °C and $I = 1.0$ (KCl) (Tables S1 and S2) are essentially equal to the rate constants $(k_{ex})_o$ for exchange catalyzed by solvent species only (eq 8), because there is no

$$(k_{ex})_o/f_{N^+} = k_o = k_w + k_{DO}[DO^-] \quad (8)$$

$$\frac{k_{ex}}{f_{N^+}} = k_o + k_B[B] \quad (9)$$

significant general base catalysis of exchange by phosphate at this low buffer concentration ($k_o > k_B[B]$, eq 9). The plot (not shown) of $k_o = (k_{ex})_o/f_{N^+}$ (s^{-1}) against $[DO^-]$ (pD 7.0−8.1) according to eq 8, where f_{N^+}, the fraction of substrate in the reactive NR_3^+ cationic form ($pK_{BD} = 8.48$), changes by ~30%, is linear. The slope gives the second-order rate constant for exchange catalyzed by deuterioxide ion, $k_{DO} = 6.0$ M^{-1} s^{-1} (Table 1). Figure 2 (■) shows the pD−rate profile for the deuterium exchange reaction of glycine methyl ester. The solid line through the data was calculated from the value of k_{DO} using the logarithmic form of eq 8 with $k_w = 0$.

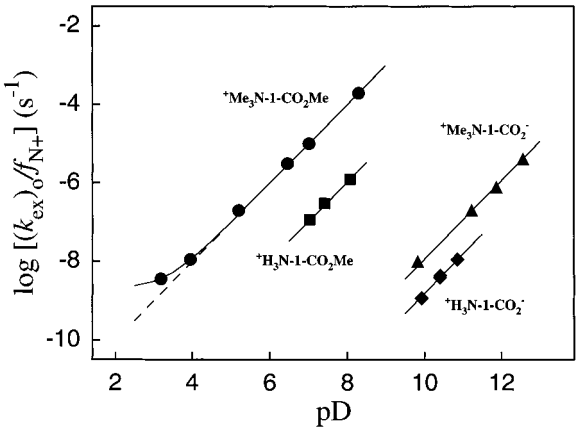


Figure 2. pD−rate profiles of $(k_{ex})_o/f_{N^+}$ (s^{-1}) for the buffer-independent exchange for deuterium of the first α-proton of glycine and glycine derivatives in D_2O at 25 °C and $I = 1.0$ (KCl). The solid lines through the data were calculated from k_{DO} (M^{-1} s^{-1}) using the logarithmic form of eq 8, with $k_w = 0$ or $k_w = 2.1 \times 10^{-9}$ s^{-1} for betaine methyl ester. (●) Data for betaine methyl ester ($f_{N+} = 1$). The dashed line was calculated from k_{DO} (M^{-1} s^{-1}) using the logarithmic form of eq 8 with $k_w = 0$. (■) Data for glycine methyl ester. Values of f_{N+} were calculated from the solution pD and $pK_{BD} = 8.48$ for $^+D_3NCH_2CO_2Me$ in D_2O (25 °C, $I = 1.0$, KCl). (▲) Data for betaine ($f_{N+} = 1$). (◆) Data for glycine zwitterion. Values of f_{N+} were calculated from the solution pD and $pK_{BD} = 10.35$ for $^+D_3NCH_2CO_2^-$ in D_2O (25 °C, $I = 1.0$, KCl).

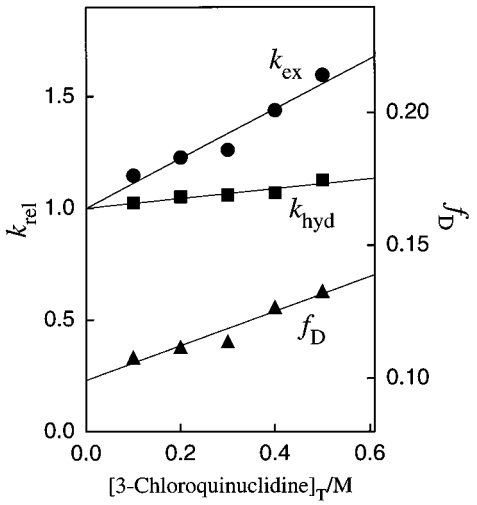


Figure 3. Dependence of the normalized rate constants k_{rel} for the deuterium exchange and hydrolysis reactions of glycine methyl ester, and the fraction of the glycine hydrolysis product that contains deuterium, on the total concentration of 3-chloroquinuclidine at pD 7.4 (buffered by 80 mM phosphate) in D_2O at 25 °C and $I = 1.0$ (KCl). (●) $k_{rel} = k_{ex}/(k_{ex})_o$ for exchange for deuterium of the first α-proton of glycine methyl ester. $(k_{ex})_o$ (s^{-1}) was determined as the intercept of a plot of k_{ex} against $[3\text{-chloroquinuclidine}]_T$. (■) $k_{rel} = k_{hyd}/(k_{hyd})_o$ for hydrolysis of glycine methyl ester. $(k_{hyd})_o$ (s^{-1}) was determined as the intercept of a plot of k_{hyd} against $[3\text{-chloroquinuclidine}]_T$. (▲) Fraction f_D of glycine hydrolysis product that contains deuterium.

Figure 3 shows the effect of increasing concentrations of 3-chloroquinuclidine at pD 7.4 (buffered by 80 mM phosphate) on the fraction, f_D, of the glycine obtained from hydrolysis of glycine methyl ester that contains deuterium and on the normalized rate constants $k_{hyd}/(k_{hyd})_o$ for ester hydrolysis and $k_{ex}/(k_{ex})_o$ for exchange for deuterium of the first α-proton of

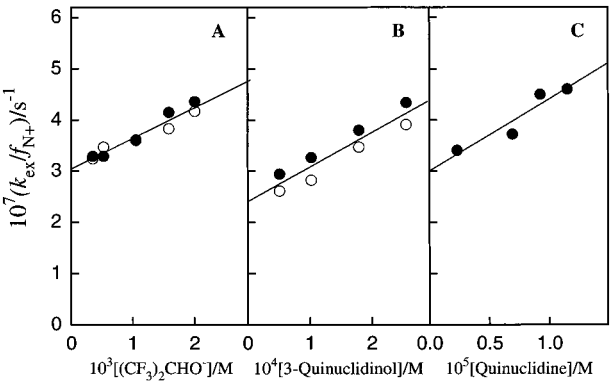


Figure 4. Dependence of k_{ex}/f_{N+} (s^{-1}) for exchange for deuterium of the first α-proton of glycine methyl ester on the concentration of Brønsted base catalysts at pD 7.4−7.5 (buffered by 80 mM phosphate) in D_2O at 25 °C and $I = 1.0$ (KCl). The slopes of these plots give the second-order rate constants k_B (M^{-1} s^{-1}) for general base catalysis of exchange (Table 1). (A) Data for HFIP anion ($pK_{BD} = 9.9$). (B) Data for 3-quinuclidinol ($pK_{BD} = 10.7$). (C) Data for quinuclidine ($pK_{BD} = 12.1$). (●) Values of k_{ex} (s^{-1}) determined by monitoring deuterium exchange into glycine methyl ester directly. (○) Values of k_{ex} (s^{-1}) determined from analysis of the deuterium enrichment of the glycine hydrolysis product, according to eq 7.

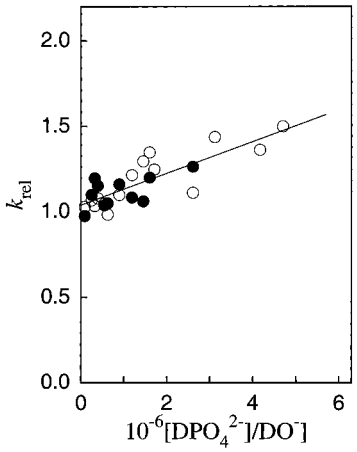


Figure 5. Dependence of the normalized rate constant $k_{rel} = k_{ex}/f_{N+}k_{DO}$-$[DO^-]$ for exchange for deuterium of the first α-proton of glycine methyl ester on ($[DPO_4^{2-}]/[DO^-]$) in phosphate buffers (pD 7.0−8.1) in D_2O at 25 °C and $I = 1.0$ (KCl). (●) Values of k_{ex} (s^{-1}) determined by monitoring deuterium exchange into glycine methyl ester directly. (○) Values of k_{ex} (s^{-1}) determined from analysis of the deuterium enrichment of the glycine hydrolysis product, according to eq 7. The solid line shows the fit of the data to eq 10; the slope gives $k_B/k_{DO} = 9.3 \times 10^{-8}$ as the ratio of second-order rate constants for exchange catalyzed by phosphate dianion and deuterioxide ion.

glycine methyl ester, in D_2O at 25 °C and $I = 1.0$ (KCl). Table 1 gives the second-order rate constant k_B (M^{-1} s^{-1}) for general base catalysis of exchange by 3-chloroquinuclidine that was determined as the slope of the plot (not shown) of k_{ex}/f_{N^+} (s^{-1}) against the concentration of the basic form of 3-chloroquinuclidine, according to eq 9.

Figure 4 shows the effect of increasing concentrations of the basic forms of HFIP, 3-quinuclidinol, and quinuclidine at pD 7.4−7.5 (buffered by 80 mM phosphate) on k_{ex}/f_{N^+} (s^{-1}) for exchange for deuterium of the first α-proton of glycine methyl ester in D_2O at 25 °C and $I = 1.0$ (KCl). The solid circles represent values of k_{ex} determined by monitoring deuterium incorporation into glycine methyl ester directly, and the open circles represent values of k_{ex} determined from analysis of the deuterium enrichment of the glycine hydrolysis product, according to eq 7. Table 1 gives the second-order rate constants k_B (M^{-1} s^{-1}) for general base catalysis of exchange by these bases, determined as the slopes of the plots in Figure 4 (eq 9).

An increase in the total concentration of phosphate buffer from 0.02 to 0.38 M ($[DPO_4^{2-}]/D_2PO_4^-] = 7/3$) results in a ∼0.1 unit increase in solution pD which complicates determination of the second-order rate constant for exchange catalyzed by phosphate dianion.

Figure 5 shows the plot of the normalized rate constant $k_{rel} = k_{ex}/f_{N+}k_{DO}[DO^-]$ for exchange for deuterium of the first α-proton of glycine methyl ester against $[DPO_4^{2-}]/[DO^-]$ according to eq 10 ($B = DPO_4^{2-}$). We attribute the scatter in

$$k_{rel} = \frac{k_{ex}}{f_{N^+}k_{DO}[DO^-]} = 1 + \frac{k_B[B]}{k_{DO}[DO^-]} \qquad (10)$$

these data to propagation of the errors in k_{ex} (±5%) and in $[DO^-]$ (±5%). The solid line shows the fit of the experimental data to eq 10; the slope gives $k_B/k_{DO} = 9.3 \times 10^{-8}$ as the ratio of second-order rate constants for exchange catalyzed by phosphate dianion and deuterioxide ion. This was combined with $k_{DO} = 6.0$ M^{-1} s^{-1} to give the second-order rate constant k_B (M^{-1} s^{-1}) for general base catalysis of exchange by phosphate dianion (Table 1).[36]

(2) Glycine. The exchange for deuterium of the first α-proton of glycine zwitterion was studied in D_2O at 25 °C and $I = 1.0$ (KCl) in solutions where glycine ($pK_{BD} = 10.35$) also served as the buffer to maintain constant pD (pD 9.9−10.9). An increase in the total concentration of glycine from 20 to 40 mM ($f_{N^+} = 0.5$) does not result in a significant increase in k_{ex} (s^{-1}), which shows that there is no general base catalysis of exchange at these low concentrations of glycine ($k_o > k_B[B]$, eq 9). The plot (not shown) of $k_o = (k_{ex})_o/f_{N^+}$ (s^{-1}) against $[DO^-]$ according to eq 8 is linear; the slope gives the second-order rate constant for exchange catalyzed by deuterioxide ion, $k_{DO} = 8.9 \times 10^{-5}$ M^{-1} s^{-1} (Table 1). Figure 2 (◆) shows the pD−rate profile for the deuterium exchange reaction of glycine. The solid line through the data was calculated from the value of k_{DO} using the logarithmic form of eq 8 with $k_w = 0$.

(3) Betaine Methyl Ester. Table S4 of the Supporting Information gives rate constants k_o (s^{-1}, eq 8, $f_{N^+} = 1$) for the buffer-independent solvent-catalyzed exchange for deuterium of the first α-proton of betaine methyl ester in D_2O at 25 °C and $I = 1.0$ (KCl) that were obtained as the *y*-intercepts of plots (not shown) of k_{ex} (s^{-1}) against the total buffer concentration (pD 3.2−8.3). The plot (not shown) of k_o (s^{-1}) against $[DO^-]$ according to eq 8 is linear; the slope gives the second-order rate constant for exchange catalyzed by deuterioxide ion, $k_{DO} = 570$ M^{-1} s^{-1} (Table 1). The fit of these data to the logarithmic form of eq 8, with $k_{DO} = 570$ M^{-1} s^{-1}, gives $k_w = (2.1 \pm 0.7) \times 10^{-9}$ s^{-1} as the pD-independent rate constant for exchange catalyzed by D_2O acting as a base. Figure 2 (●) shows the pD−rate profile for the deuterium exchange reaction of betaine methyl ester. The solid line through the data was calculated from the values of k_{DO} and k_w using the logarithmic form of eq 8.

Increasing the concentration of buffers at 25 °C and $I = 1.0$ (KCl) results in small 10−30% increases in k_{ex} (s^{-1}) for

(36) There is no significant general base catalysis of exchange by phosphate monoanion because it is ∼5 units less basic than phosphate dianion and the value of $\beta = 0.92$ (vide infra) for deprotonation of ^+H_3N-1-CO_2Me shows that there is a sharp falloff in k_B with decreasing catalyst basicity.

exchange of the first α-proton of betaine methyl ester, along with small (≤0.07 unit) changes in solution pD (Table S4). The plot (not shown) of $k_{rel} = k_{ex}/k_{DO}[DO^-]$ against [3-quinuclidinol]/$[DO^-]$ at pD 7.6 (buffered by 80 mM phosphate) according to eq 10 (B = 3-quinuclidinol, $f_{N^+} = 1$) is linear with slope $k_B/k_{DO} = 6.6 \times 10^{-5}$. This was combined with $k_{DO} = 570\ M^{-1}\ s^{-1}$ to give the second-order rate constant k_B ($M^{-1}\ s^{-1}$) for general base catalysis of exchange by 3-quinuclidinol (Table 1).[37]

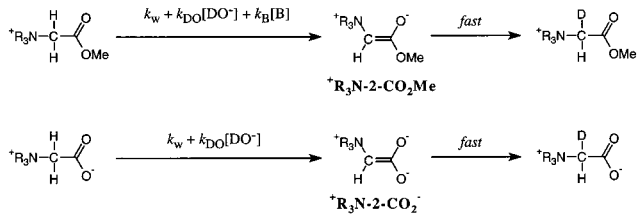
(4) Betaine. There is no significant change with changing buffer concentration in k_{ex} (s^{-1}) for exchange for deuterium of the first α-proton of betaine in D_2O at 25 °C and $I = 1.0$ (KCl) (Table S5), so that $k_{ex} = k_o$ (eq 9, $f_{N^+} = 1$). The plot (not shown) of k_o against $[DO^-]$ according to eq 8 is linear; the slope gives the second-order rate constant for exchange catalyzed by deuterioxide ion, $k_{DO} = 6.6 \times 10^{-4}\ M^{-1}\ s^{-1}$ (Table 1). Figure 2 (▲) shows the pD−rate profile for the deuterium exchange reaction of betaine. The solid line through the data was calculated from the value of k_{DO} using the logarithmic form of eq 8 with $k_w = 0$.

Discussion

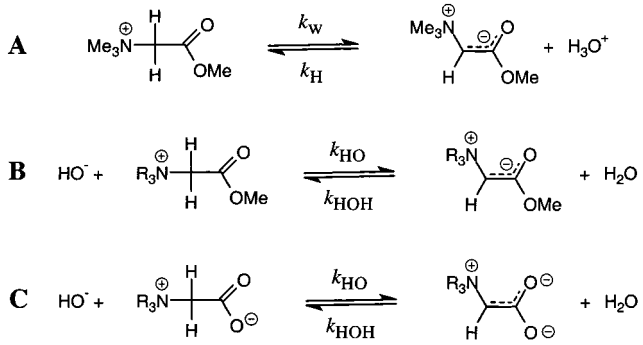
Reactive Species and Reaction Mechanisms. There have been several studies of the racemization of amino acids in water at elevated temperatures (>100 °C).[10−13,38,39] An observed rate constant for the racemization of alanine at pH 7.6 and 25 °C, $k_{rac} = 1.5 \times 10^{-12}\ s^{-1}$, can be calculated from the activation parameters for racemization obtained from rate constants at higher temperatures.[13] By comparison, a value of $k_o = 2 \times 10^{-11}\ s^{-1}$ ($t_{1/2}$ = 1100 years!) for conversion of glycine zwitterion to the enolate at pH 7.6 and 25 °C can be calculated from $k_{DO} = 8.9 \times 10^{-5}\ M^{-1}\ s^{-1}$ determined in this work (Table 1) and an estimated secondary solvent deuterium isotope effect of $k_{DO}/k_{HO} = 2$.[40]

We have shown previously that Brønsted catalysis of exchange for deuterium of the α-protons of ethyl acetate proceeds by direct deprotonation of the ester by general bases to give the free ester enolate.[16] This provides strong evidence that catalysis of the deuterium exchange reactions of glycine methyl ester by Brønsted catalysts of $pK_{BD} \geq 7$ (Figures 3−5) also occurs by direct deprotonation of ^+H_3N-1-CO_2Me by the basic form of these catalysts give the free enolate ^+H_3N-2-CO_2-Me (Scheme 3). The alternative mechanism, general acid catalysis with protonation of the carbonyl oxygen of the substrate, is forbidden by the libido rule of Jencks.[41] This is because there is no thermodynamic driving force for proton transfer from the acidic form of these catalysts ($pK_{BD} \geq 7$) to the oxygen of the product enolate, which has a pK_a of less than 7.[42] If there is no stabilization of the enolate by complete proton transfer from a general acid catalyst ($pK_{BD} \geq 7$) to form the enol ($pK_a < 7.0$), then there can be no stabilization of the transition state for enolization by concerted proton transfer from a general acid catalyst to the substrate carbonyl oxygen. We conclude that the rate constants for exchange of the first α-proton of ^+R_3N-1-CO_2Me and ^+R_3N-1-CO_2^- represent rate constants for deprotonation of these carbon acids to give the free enolates ^+R_3N-2-CO_2Me and ^+R_3N-2-CO_2^- (Scheme 3).

Scheme 3



Scheme 4



Rate and Equilibrium Constants. **(1) Betaine Methyl Ester.** The rate constant for deprotonation of $^+Me_3N$-1-CO_2-Me by solvent D_2O acting as a base, $k_w = 2 \times 10^{-9}\ s^{-1}$ (Results) is ~4-fold larger than $k_w = 5 \times 10^{-10}\ s^{-1}$ for deprotonation of acetone by H_2O,[43a] for which a near-limiting value of $k_H = 6.7 \times 10^9\ M^{-1}\ s^{-1}$ has been determined for the reverse protonation of the enolate by H_3O^+.[43b] A similar rate constant is expected for protonation of the enolate $^+Me_3N$-2-CO_2Me (k_H, Scheme 4A) for the following reasons: (1) The transition state for proton transfer from the strongly acidic H_3O^+ to these strongly basic enolates is "early",[44a] so that there are relatively small changes in k_H with changing enolate stability. (2) The similar values of k_w for deprotonation of acetone and $^+Me_3N$-1-CO_2Me to give the corresponding enolates are consistent with similar stabilities of these enolates relative to the neutral carbon acids.

(37) This value of k_B was calculated with the assumption that the observed small dependence of k_{ex} (s^{-1}) on [3-quinuclidinol] is due to general base catalysis of enolization rather than a specific salt or medium effect. We consider it to be tentative, because we have no data for specific salt and medium effects on the deprotonation of these glycine derivatives.

(38) Smith, G. G.; Sivakua, T. *J. Org. Chem.* **1983**, *48*, 627−634.

(39) Bada, J. L. *J. Am. Chem. Soc.* **1972**, *94*, 1371−1373.

(40) The secondary solvent deuterium isotope effects for lyoxide-catalyzed formation of the unstable enolates $^+Me_3N$-2-CO_2^- and ^+H_3N-2-CO_2^- are expected to be larger than $k_{DO}/k_{HO} = 1.46$ for deprotonation of acetone.[46] However, we have no evidence that they are close to the maximum value of $k_{DO}/k_{HO} = 2.4$ for formation of very unstable carbanions, for which lyoxide-catalyzed exchange is limited by solvent reorganization that exchanges the hydron of substrate with a labeled hydron from solvent: 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. Therefore, we use the average of the these values as the estimated secondary solvent deuterium isotope effect.

(41) Jencks, W. P. *J. Am. Chem. Soc.* **1972**, *94*, 4731−4732.

(42) Values of $pK_a = 7.3$ (Gao, J. *Theochem.* **1996**, *370*, 203−208) and $pK_a \approx 7$ (ref 16) have been estimated for the oxygen acidities of the enols of acetic acid and ethyl acetate, respectively. The NH_3^+ group should result in at least a 2-unit decrease in these pK_a's, because this substituent results in a 2 p*K* unit increase in the oxygen acidity of acetic acid.[83]

(43) (a) Bell, R. P. *The Proton in Chemistry*, 2nd ed.; Cornell University Press: Ithaca, NY, 1973; p 150. (b) Keeffe, J. R.; Kresge, A. J. In *The Chemistry of Enols*; Rappoport, Z., Ed.; John Wiley and Sons: Chichester, 1990; pp 399−480. (c) Carey, A. R. E.; Al-Quatami, S.; More O'Ferrall, R. A.; Murray, B. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1097−1098. (d) Chiang, Y.; Kresge, A. J.; Morimoto, H.; Williams, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 3981−3982.

(44) (a) For example, there is only a 5-fold difference in the values of k_H for protonation of the enolates of acetone ($k_H = 6.7 \times 10^9\ M^{-1}\ s^{-1}$) and acetaldehyde ($k_H = 1.3 \times 10^9\ M^{-1}\ s^{-1}$), whose statistically corrected pK_a's differ by 3 units (see Tables 13 and 14 of ref 43b). (b) The chosen near-limiting value for k_H is marginally smaller than that for protonation of the slightly more unstable enolate of acetone.

Table 2. Rate and Equilibrium Constants for Carbon Deprotonation of Ketones, Esters, and Amino Acid Derivatives in Water[a]

carbon acid	k_{HO}[b] (M^{-1} s^{-1})	k_{HOH}[c] (s^{-1})	pK_a[d]	k_B[e] (M^{-1} s^{-1})	k_B/k_{HO}[f]
4[g]	0.10	5×10^4	19.6	0.021	0.21
CH_3CO_2Et[h]	1.2×10^{-3}	5×10^8	25.6	1.3×10^{-6} [i]	1.1×10^{-3}
$^+H_3NCH_2CO_2Me$	4.1[j]	4×10^7 [k]	21.0 ± 1.0	6.8×10^{-4} [i]	1.7×10^{-4}
$^+Me_3NCH_2CO_2Me$	390[j]	4×10^6 [k]	18.0 ± 1.0	0.038[i]	9.7×10^{-5}
$^+H_3NCH_2CO_2^-$	4.5×10^{-5} [l]	4×10^{10} [k]	28.9 ± 0.5		
$^+Me_3NCH_2CO_2^-$	3.3×10^{-4} [l]	7×10^9 [k]	27.3 ± 1.2		

[a] At 25 °C and $I = 1.0$ (KCl). [b] Second-order rate constant for deprotonation of the carbon acid by hydroxide ion. [c] Rate constant for protonation of the enolate of the carbon acid by solvent water. [d] pK_a for ionization of the carbon acid in water. [e] Second-order rate constant for deprotonation of the carbon acid by 3-quinuclidinol. [f] Ratio of rate constants for deprotonation of the carbon acid by 3-quinuclidinol and hydroxide ion. [g] Data from ref 18. [h] Data from ref 16. [i] Data for reactions in D_2O. Solvent isotope effects of close to unity have been determined for general base-catalyzed deprotonation of other carbon acids (ref 16). [j] Calculated from k_{DO} for deprotonation by deuterioxide ion in D_2O (Table 1) with the assumption that the secondary solvent isotope effect is the same as that for deprotonation of acetone, $k_{DO}/k_{HO} = 1.46$.[46] [k] Calculated from the values of pK_a and k_{HO} using eq 12. [l] Calculated from k_{DO} for deprotonation by deuterioxide ion in D_2O (Table 1) and an estimated secondary solvent isotope effect of $k_{DO}/k_{HO} = 2.0$.[40]

A value of pK_a = 18.0 for the carbon acidity of $^+Me_3N$-1-CO_2Me (Table 2) can be calculated using eq 11 derived for

$$pK_a = \log(k_H/k_w) \qquad (11)$$

Scheme 4A, with $k_w = 5 \times 10^{-9}$ s^{-1} for deprotonation by H_2O, and an estimated value of $k_H = 5 \times 10^9$ M^{-1} s^{-1} for the reverse protonation of the enolate by H_3O^+.[44b] This assumes a solvent isotope effect of $(k_w)_H/(k_w)_D = 2.6$ for reactions in H_2O and D_2O.[45] The uncertainty in this pK_a is estimated to be less than 1 unit and is due largely to the uncertainty in the chosen value of k_H, because the values of k_H for protonation of simple enolates show only a small dependence on enolate structure and stability.[44a]

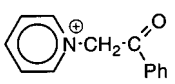
A value of $k_{HO} = 390$ M^{-1} s^{-1} for deprotonation of $^+Me_3N$-1-CO_2Me by hydroxide ion in H_2O (Table 2) can be calculated from the value of k_{DO} (Table 1), with the assumption that the secondary solvent deuterium isotope effect is the same as that for deprotonation of acetone, $k_{DO}/k_{HO} = 1.46$.[46] This value of k_{HO} and pK_a = 18.0 can be substituted into eq 12 derived for

$$pK_a = pK_w + \log(k_{HOH}/k_{HO}) \qquad (12)$$

Scheme 4B to give $k_{HOH} = 4 \times 10^6$ s^{-1} for the reverse protonation of the enolate $^+Me_3N$-2-CO_2Me by solvent water (Table 2).

(2) Betaine and Glycine Zwitterions. Rate constants k_{HO} (M^{-1} s^{-1}, Table 2) for deprotonation of $^+Me_3N$-1-CO_2^- and ^+H_3N-1-CO_2^- by hydroxide ion in H_2O were calculated from the values of k_{DO} (Table 1) and an estimated secondary solvent isotope effect of $k_{DO}/k_{HO} = 2$.[40] These rate constants are 4- and 30-fold smaller, respectively, than $k_{HO} = 1.2 \times 10^{-3}$ M^{-1} s^{-1} for deprotonation of ethyl acetate,[16] so that by this criterion the simple amino acid zwitterions $^+Me_3N$-1-CO_2^- and ^+H_3N-1-CO_2^- are *weaker* carbon acids than the simple oxygen ester ethyl acetate (pK_a = 25.6).[16]

Figure 6 shows extensive linear rate−equilibrium correlations of log k_{HO} (M^{-1} s^{-1}, ●) for the hydroxide ion-catalyzed formation and log k_{HOH} (s^{-1}, ○) for the reverse protonation by solvent water of the enolates of simple aldehydes, ketones, and esters with the pK_a of the parent carbon acid.[16] Data for deprotonation of acetate anion ($k_{HO} = 3.3 \times 10^{-9}$ M^{-1} s^{-1}, pK_a = 33.5)[28] have been included, to emphasize the requirement for a negative deviation from these linear correlations when the rate-determining step for the reverse protonation of the enolate by solvent water changes from the chemical step of proton transfer to the physical step of rotation or reorganization of a molecule of water into a "reactive position",[17] with a limiting rate constant of $k_{HOH} = k_{reorg} \approx 10^{11}$ s^{-1}.[47−49] Figure 6 also shows data for the positively charged carbon acids **3**



3

($k_{HO} = 1.8 \times 10^5$ M^{-1} s^{-1}, pK_a = 10.9)[43b,c] and $^+Me_3N$-1-CO_2Me (data from Table 2).

The linear rate−equilibrium correlation between log k_{HO} and pK_a for deprotonation of neutral α-carbonyl carbon acids (Figure

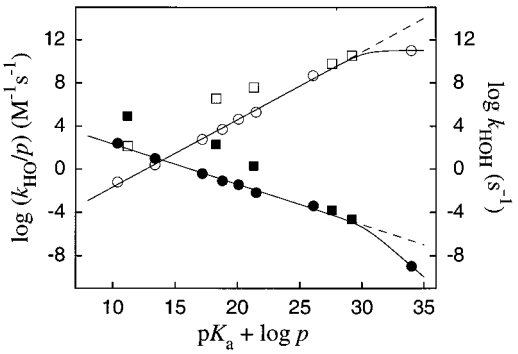


Figure 6. Rate−equilibrium correlations for deprotonation of α-carbonyl carbon acids by hydroxide ion, k_{HO} (M^{-1} s^{-1}), and the reverse protonation of the enolates by solvent water, k_{HOH} (s^{-1}), with the pK_a of the carbon acid. The values of k_{HO} and pK_a were statistically corrected for the number of acidic protons p at the carbon acid. (●) Correlation of log(k_{HO}/p) for deprotonation of neutral aldehydes, ketones and esters by hydroxide ion. The data were taken from earlier work,[16] with additional data for deprotonation of acetate anion (pK_a = 33.5).[28] Excluding the point for acetate anion, the data are correlated by log(k_{HO}/p) = 6.063−0.373(pK_a + log p). (○) Correlation of log k_{HOH} for the reverse protonation of the enolates of neutral α-carbonyl acids by solvent water. The data were taken from earlier work,[16] with additional data for the enolate of acetate anion ($k_{HOH} = 10^{11}$ s^{-1}).[28] Excluding the point for the enolate of acetate anion, the data are correlated by log k_{HOH} = 0.625(pK_a + log p) − 7.918. (■) Data for deprotonation of amino acid derivatives (Table 2) and the cationic ketone **3**. (□) Data for protonation of the enolates of amino acid derivatives (Table 2) and the enolate of **3**. The solid lines were calculated with the assumption that the protonation of highly unstable enolates is limited by the rotation of a molecule of water into a "reactive position" with $k_{HOH} = k_{reorg} \approx 10^{11}$ s^{-1}.[17]

(45) See footnote 48 in: Argyrou, A.; Washabaugh, M. W. *J. Am. Chem. Soc.* **1999**, *121*, 12054−12062.

(46) Pocker, Y. *Chem. Ind.* **1959**, 1383−1384.

(47) Giese, K.; Kaatze, U.; Pottel, R. *J. Phys. Chem.* **1970**, *74*, 3718−3725.

(48) Kaatze, U. *J. Chem. Eng. Data* **1989**, *34*, 371−374.

(49) Kaatze, U.; Pottel, R.; Schumacher, A. *J. Phys. Chem.* **1992**, *96*, 6017−6020.

Table 3. Substituent Effects on the Carbon Acidity of Acetate Anion in Water[a]

	carbon acid					
	$CH_3CO_2^-$	$^+H_3NCH_2CO_2^-$	$^+Me_3NCH_2CO_2^-$	CH_3CO_2 Et	$^+H_3NCH_2CO_2Me$	$^+Me_3NCH_2CO_2Me$
$pK_a + \log p$[b]	34.0[c]	29.2	27.6	26.1	21.3	18.3
$\Delta\Delta G_o$ H → NR_3^+ [d]		6.5	8.7		6.5	10.6
$\Delta\Delta G_o$ CO_2^- → CO_2R[e]				10.7	10.7	12.6

[a] Data from Table 2, unless noted otherwise. [b] pK_a for ionization of the carbon acid in water at 25 °C and $I = 1.0$ (KCl) with a statistical correction for the number of acidic protons p. [c] Data from ref 28. [d] Effect of NR_3^+ substituent on the Gibbs free energy change for ionization of the carbon acid in water at 298 K. [e] Effect of alkylation of the carboxylate group on the Gibbs free energy change for ionization of the carbon acid in water at 298 K.

6, ●) can be used to set lower limits of p$K_a \geq 26.1$ and p$K_a \geq 28.4$ for the formally neutral zwitterions $^+Me_3N$-1-CO_2^- and ^+H_3N-1-CO_2^-, respectively. These pK_as were calculated with the assumption that the values of log k_{HO} for these carbon acids (Table 2) lie on the linear correlation line shown in Figure 6.[50] They represent lower limits because the values of log k_{HO} for deprotonation of cationic ketones (e.g., **3**)[33,51] and the cationic ester $^+Me_3N$-1-CO_2Me exhibit positive deviations from this correlation (Figure 6). A lower limit of p$K_a \geq 26.2$ for $^+Me_3N$-1-CO_2^- can be calculated using eq 12 (Scheme 4C) with $k_{HOH} \geq 5 \times 10^8$ s^{-1} for protonation of $^+Me_3N$-2-CO_2^- by solvent water, where $k_{HOH} = 5 \times 10^8$ s^{-1} is the rate constant for protonation of the more stable enolate of ethyl acetate.[16] Upper limits of p$K_a \leq 28.5$ for $^+Me_3N$-1-CO_2^- and p$K_a \leq 29.3$ for ^+H_3N-1-CO_2^- can be calculated using eq 12 (Scheme 4C) with the limiting value[47−49] of $k_{HOH} = k_{reorg} \approx 10^{11}$ s^{-1} for protonation of the enolate with rate-limiting rotation of a molecule of water into a "reactive position".[17] The ranges of these limits are relatively small; the averages of the upper and lower limits give carbon acidities of p$K_a = 27.3 \pm 1.2$ for $^+Me_3N$-1-CO_2^- and 28.9 ± 0.5 for ^+H_3N-1-CO_2^- (Table 2). Rate constants k_{HOH} (s^{-1}) for the reverse protonation of the enolates $^+Me_3N$-2-CO_2^- and ^+H_3N-2-CO_2^- by solvent water (Scheme 4C) were calculated from these pK_as and k_{HO} using eq 12 (Table 2).

The value of p$K_a = 28.9 \pm 0.5$ for glycine zwitterion is substantially larger than earlier literature estimates of pK_a = 16−17 for several L-amino acids.[10] The latter values were obtained using an equation similar to eq 11, with the observed first-order rate constant $k_{rac} \approx k_w$ for racemization of the amino acid at pH 7.6 and 139 °C, and a rate constant of $\sim 6 \times 10^{10}$ s^{-1} for the reverse protonation of the amino acid enolate.[10] However, this calculation severely underestimates the true pK_a's because it assumes that the amino acid is deprotonated by solvent water at pH 7.6, when in fact the dominant pathway is deprotonation by hydroxide ion (eq 12).[52] If deprotonation of amino acids by hydroxide ion at pH 7.6 is *much faster* than deprotonation by solvent water, then $k_w \ll k_{rac}$, and the assumption that water is the reactive base will lead to an overestimate of the acidity of the amino and pK_a's that are much smaller than the true values.

(3) Glycine Methyl Ester. An upper limit of p$K_a \leq 22.1$ for the carbon acidity of ^+H_3N-1-CO_2Me can be calculated using eq 12 (Scheme 4B) with $k_{HOH} \leq 5 \times 10^8$ s^{-1} for the reverse protonation of the enolate by solvent water, where $k_{HOH} = 5 \times 10^8$ s^{-1} for protonation of the less stable enolate of ethyl acetate.[16] A value of $k_{HOH} = 5 \times 10^8$ s^{-1} for protonation of ^+H_3N-2-CO_2Me would apply in the limiting case where the effect of an α-NH_3^+ substituent on the acidity of ethyl acetate is expressed wholly in the rate constant k_{HO} for deprotonation. A lower limit of p$K_a \geq 20.0$ for ^+H_3N-1-CO_2Me can be calculated using $k_{HOH} \geq 4 \times 10^6$ s^{-1}, where $k_{HOH} = 4 \times 10^6$ s^{-1} for protonation of the more stable enolate $^+Me_3N$-2-CO_2-Me. The consensus value is p$K_a = 21.0 \pm 1$ for ^+H_3N-1-CO_2-Me (Table 2). The same value is obtained by making the assumption that 80% of the effect of an α-NH_3^+ substituent on the equilibrium stability of the enolate of ethyl acetate is expressed in k_{HO} and that 20% of the effect is expressed in k_{HOH}.[27] The rate constant $k_{HOH} = 4 \times 10^7$ s^{-1} for the reverse protonation of the enolate ^+H_3N-2-CO_2Me by solvent water was calculated from the values of pK_a and k_{HO} using eq 12 (Table 2).

Chart 1

	pK_a		pK_a		pK_a + log p
CH_3CO_2H	4.76	CH_3OH	15.5	CH_2CO_2R	26.1
$^+Me_3NCH_2CO_2H$	1.83	$^+Me_3NCH_2OH$	9.3	$^+Me_3NCHCO_2R$	18.3
ΔpK_a	**2.9**		**6.2**		**7.8**

Effects of α-NR_3^+ Substituents on Carbon Acidity. The effects of charged α-NR_3^+ (R = Me, H) substituents on the carbon acidity of simple carboxylic acid derivatives in aqueous solution determined in this work are summarized in Table 3. By comparison, substitution of carbon for the pyridinium nitrogen of **3** results in an estimated 5.3 unit increase in the pK_a from 10.9 for **3** to 16.2 for benzyl methyl ketone.[43b,c,93] The 2−3 unit larger acidifying effect of an α-NMe_3^+ than of an α-NH_3^+ substituent on carbon acidity (Table 3) corresponds to a normal difference in the polar effects of these substituents for which values of $\sigma_I = 0.92$ and 0.60, respectively, have been reported.[53] This larger polar effect of α-NMe_3^+ than of α-NH_3^+ on carbon acidity reflects the following: (1) the formation of hydrogen bonds between the α-NH_3^+ group and solvent, which has the effect of moving positive charge away from nitrogen and onto solvent, thereby increasing the "effective" separation between the interacting cationic and anionic centers at the enolate; (2) a greater stabilization of negative charge by intramolecular electrostatic interactions with α-NMe_3^+ than with α-NH_3^+, as a result of the tendency of the more "greasy" α-NMe_3^+ with three methyl groups to reduce the *effective* dielectric constant of the local medium through which these interactions occur.[54,55]

The data in Chart 1 show that the acidifying effect of an NMe_3^+ substituent increases as this charged substituent is moved

(50) The linear least-squares correlation lines shown in Figure 6 are given by: (●) log(k_{HO}/p) = 6.063−0.373(pK_a + log p); (○) log k_{HOH} = 0.625-(pK_a + log p) − 7.918.

(51) Bernasconi, C. F.; Moreira, J. A.; Huang, L. L.; Kittredge, K. W. *J. Am. Chem. Soc.* **1999**, *121*, 1674−1680.

(52) For example, deprotonation of $^+Me_3N$-1-CO_2Me by D_2O becomes kinetically significant only at pD <4 where $[DO^-] \approx 10^{-11}$ M (Figure 2).

(53) Hine, J. In *Structural Effects on Equilibria in Organic Chemistry*; Wiley: New York, 1975; p 98.

(54) Kirkwood, J. G.; Westheimer, F. H. *J. Chem. Phys.* **1938**, *6*, 506−512.

(55) Kirkwood, J. G.; Westheimer, F. H. *J. Chem. Phys.* **1938**, *6*, 512−517.

progressively closer to the site of ionization.[56] The 2.9-unit effect of the NMe_3^+ group on the pK_a of acetic acid is the minimum effect of this substituent on deprotonation of a simple ester to give a *hypothetical* enolate in which the negative charge is completely localized at the enolate oxygen. The addition of an α-NMe_3^+ group to ethyl acetate to give $^+Me_3N$-1-CO_2Me results in an observed 7.8-unit increase in carbon acidity, which is larger than the 6.2-unit effect of an NMe_3^+ group on the acidity of methanol. Therefore, at least some of the negative charge at the enolate $^+Me_3N$-2-CO_2Me resides on the α-carbon; this ester enolate behaves as an anion with an "effective" center of charge that is slightly less than one atom removed from the cationic substituent.

The fraction of negative charge localized at the α-carbon of the ester enolate, f_C, is given by the solution of eq 13, where *a*

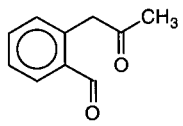
$$\Delta pK_a = 7.8 = 2.9(1 - f_C) + 6.2af_c \qquad (13)$$

is the attenuation factor that gives the falloff in charge−charge interactions as the separation between the NMe_3^+ substituent and the negative charge is increased by one atom. Equation 13 was derived with these assumptions: (1) The observed 7.8 unit effect of the α-NMe_3^+ substituent on the pK_a of a simple oxygen ester represents the sum of the effects due to its interaction with the negative charge at the enolate carbon (f_C) and at the enolate oxygen ($1 - f_C$). (2) The interaction of the NMe_3^+ group with a unit negative charge at the enolate oxygen results in a 2.9-unit decrease in pK_a, which increases to 6.2 units when the charge separation is decreased by one atom and 6.2*a* units when it is decreased by two atoms (Chart 1). Values of $f_C = 0.22$ and 0.31 were calculated from eq 13 using attenuation factors of $a = 4.0$ and $a = 3.0$, respectively.[57] These fractions are smaller than $f_C = 0.4$ for the enolate of acetaldehyde in a vacuum determined by ab initio calculations.[58] However, transfer of an enolate from the gas phase to water may result in movement of negative charge from carbon to oxygen and a smaller value of f_C, due to the greater stabilization of negative charge at strongly electronegative oxygen compared with carbon by hydrogen bonding to solvent.

Brønsted Base Catalysis. The Brønsted coefficient $\beta = 1.09$ for deprotonation of ethyl acetate by 3-substituted quinuclidines[16] shows that there is complete proton transfer to these bases in the rate-determining step. It was concluded that proton transfer from this simple ester is limited by the diffusional separation of the complex between the enolate and the conjugate acid of the Brønsted base to give the free enolate. By contrast, the following observations show that deprotonation of ^+H_3N-1-CO_2Me by general bases is limited by the chemical step of proton transfer from the carbon acid to the Brønsted base: (1) The value of $\beta = 0.92$ for deprotonation of ^+H_3N-1-CO_2Me is significantly smaller than $\beta = 1.0$ that is required for reactions in which proton transfer to the base catalyst is essentially complete in the transition state.[27,59] (2) The addition of an α-NH_3^+ group to ethyl acetate to give ^+H_3N-1-CO_2Me results in a 3400-fold increase in k_{HO} for deprotonation by hydroxide ion but only a 520-fold increase in k_B for deprotonation by 3-quinuclidinol (Table 2). This requires that the α-NH_3^+ group results in a corresponding 6-fold larger decrease in k_{BH} than in k_{HOH} for the reverse protonation of the enolate, because the overall substituent effect on the ratios of rate constants for enolate formation and reaction, e.g., $\Delta\log(k_{HOH}/k_{HO}) = \Delta pK_a$, must be the same for all bases. We conclude that the α-NH_3^+ substituent leads to a substantial reduction in k_{BH} for protonation of the enolate ^+H_3N-2-CO_2Me by protonated 3-quinuclidinol below the diffusion-limited rate constant for protonation of the enolate of ethyl acetate.[16]

The following results for catalysis by hydroxide ion and Brønsted bases of the deprotonation of weak neutral and cationic α-carbonyl carbon acids to give negatively charged enolates and neutral organic zwitterions, respectively, show that there is a systematic dependence of the relative reactivity of anionic and neutral base catalysts on the charge at the reacting carbon acid,[60−63] and they define the magnitude of these effects.

(1) There is a large difference between $\beta = 0.92$ for deprotonation of ^+H_3N-1-CO_2Me ($pK_a = 21.0$)[27,59] and $\beta = 0.55$ for deprotonation of the methyl group of the acetone-like ketone **4** ($pK_a = 19.6$)[18] by the same set of 3-substituted quinuclidines.[64]



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This is a surprising result because similar transition states with respect to proton transfer from the carbon acid are expected for these reactions of similar thermodynamic driving force. The very different Brønsted parameters for the two reactions show that a larger effective positive charge is "seen" by the substituent at the Brønsted base catalyst at the transition state for deprotonation of the cationic carbon acid.[65,66] Deprotonation of the neutral ketone **4** results in formation of a negatively charged enolate which interacts favorably with the developing positive charge at the base catalyst in the transition state. However, deprotonation of the cationic ^+H_3N-1-CO_2Me results in formation of a formally neutral enolate, so that the Brønsted coefficient may provide a more realistic measure of the progress of proton transfer in the transition state.

(2) Figure 7 shows the effect of increasing concentrations of 3-quinuclidinol on the normalized observed rate constants for the deuterium exchange reactions of ethyl acetate,[16] ^+H_3N-1-CO_2Me and $^+Me_3N$-1-CO_2Me. The different slopes of these correlations represent differences in the relative magnitude of the rate constants for deprotonation of these carbon acids by neutral general bases and lyoxide ion, which are summarized in Table 2. The rate constant ratio k_B/k_{HO} for deprotonation of the neutral carbon acid ethyl acetate by 3-quinuclidinol and

(56) Data for CH_3CO_2H, $^+Me_3NCH_2CO_2H$, and CH_3OH were taken from ref 83. The pK_a for $^+Me_3NCH_2OH$ was taken from: Hine, J.; Kokesh, F. C. *J. Am. Chem. Soc.* **1970**, *92*, 4383−4388.

(57) A simple electrostatic treatment of the interactions between a carbanion and polar substituents predicts an attenuation factor of 4.0 for the insertion of a methylene group between interacting charges and dipoles at adjacent atoms (ref 53, p 96). Smaller attenuation factors of 2−3 are observed in cases where the initial separation of interacting charges is larger (ref 53, pp 161−162).

(58) Wiberg, K. B.; Castejon, H. *J. Org. Chem.* **1995**, *60*, 6322−6334.

(59) The second-order rate constants k_B (M^{-1} s^{-1}, Table 1) for deprotonation of ^+H_3N-1-CO_2Me in D_2O define the Brønsted correlation $\log k_B = 0.92pK_{BD} - 13.09$. The Brønsted plot was published[27] in the preliminary communication of this work.

(60) Thibblin, A. *J. Am. Chem. Soc.* **1984**, *106*, 183−186.

(61) Chwang, W. K.; Eliason, R.; Kresge, A. J. *J. Am. Chem. Soc.* **1977**, *99*, 805−808.

(62) Richard, J. P. *J. Am. Chem. Soc.* **1984**, *106*, 4926−4936.

(63) Halvarsson, T.; Bergman, N. A. *J. Org. Chem.* **1991**, *56*, 251−254.

(64) The data for deprotonation of **4** in H_2O [18] and ^+H_3N-1-CO_2Me in D_2O (Table 1) by quinuclidine, 3-quinuclidinol, and 3-chloroquinuclidine give Brønsted coefficients of $\beta = 0.48$ and $\beta = 0.96$, respectively.

(65) Hupe, D. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 451−464.

(66) Williams, A. *Adv. Phys. Org. Chem.* **1992**, *27*, 1−55.

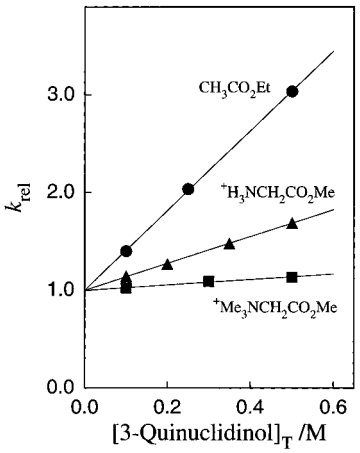
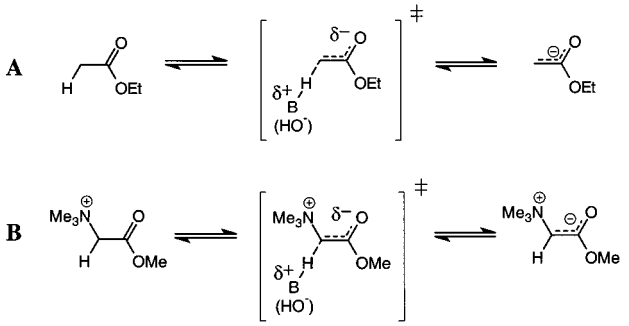


Figure 7. Dependence of the normalized rate constant $k_{rel} = k_{ex}/(k_{ex})_o$ for exchange for deuterium of the first α-proton of carbon acids on the total concentration of 3-quinuclidinol in D_2O at 25 °C and $I = 1.0$ (KCl). Values of $(k_{ex})_o$ (s^{-1}) were determined as the intercepts of plots of k_{ex} against [3-quinuclidinol]$_T$. (●) Deuterium exchange into ethyl acetate at pD 10.7.[16] (▲) Deuterium exchange into glycine methyl ester at pD 7.4 (buffered by 80 mM phosphate). (■) Deuterium exchange into betaine methyl ester at pD 7.6 (buffered by 80 mM phosphate).

Scheme 5



hydroxide ion is 11-fold larger than that for deprotonation of the cationic carbon acid $^+Me_3N$-1-CO_2Me by the same bases (Table 2). This corresponds to a 1.4 kcal/mol larger differential stabilization of the transition state for deprotonation of the cationic acid by the negatively charged hydroxide ion compared with neutral 3-quinuclidinol (Scheme 5B) than for deprotonation of a similar neutral carbon acid (Scheme 5A).

(3) Lyoxide ion often exhibits a low reactivity relative to Brønsted bases for deprotonation of carbon acids and this is known as the "lyoxide ion anomaly".[67−69] This anomaly results in a 1400-fold negative deviation of k_{DO} from the Brønsted correlation for deprotonation of ethyl acetate by 3-substituted quinuclidines,[16] where the interaction between DO^- and the developing negative charge at the enolate is destabilizing (Scheme 5A). However, the enolate of ^+H_3N-1-CO_2Me is formally neutral overall and there is 50-fold *smaller* deviation of k_{DO} from the corresponding correlation for deprotonation of this cationic carbon acid, for which the destabilizing interaction is offset by a stabilizing interaction between DO^- and the positively charged ammonium group of the substrate (Scheme 5B).[27,59] The residual 27-fold negative deviation of k_{DO} from the Brønsted plot for deprotonation of ^+H_3N-1-CO_2Me is probably not due to an electrostatic effect because the deprotonation of ^+H_3N-1-CO_2Me by neutral and anionic general bases obey a single Brønsted correlation.[27,59] It likely constitutes a lyoxide ion anomaly for deprotonation of ^+H_3N-1-CO_2Me, and it may reflect the strong solvation of lyoxide ion and the requirement for a nearly complete loss of this stabilizing solvation in the transition state.[68,69] This is because any requirement for a large fractional loss of a reactant-stabilizing interaction, such as solvation, will result in an increase in the intrinsic barrier over that for reactions where the stabilizing interaction is absent.[70−74]

(4) The positive deviation of log k_{HO} for deprotonation of ^+H_3N-1-CO_2Me ($pK_a = 21.0$) from the linear rate−equilibrium correlation shown in Figure 6 corresponds to a 160-fold larger rate constant for deprotonation of the cationic ^+H_3N-1-CO_2Me by hydroxide ion than for deprotonation of a neutral carbon acid of the same pK_a. By comparison, the rate constant ratio for deprotonation of the neutral ketone **4** ($pK_a = 19.6$) by 3-quinuclidinol and hydroxide ion, $k_B/k_{HO} = 0.21$, is 1200-fold larger than $k_B/k_{HO} = 1.7 \times 10^{-4}$ for deprotonation of the cationic ester ^+H_3N-1-CO_2Me (Table 2). Similarly, there is a large difference in the rate constant ratios for deprotonation of the cationic $^+Me_3N$-1-CO_2Me ($pK_a = 18.0$) and the neutral acetone ($pK_a = 19.3$)[43b] by hydroxide ion, $k_{BME}/k_{acetone} = 1800$,[43d] and by water, $k_{BME}/k_{acetone} = 10$.[43a] These results suggest that rate constants for deprotonation of ^+H_3N-1-CO_2Me by neutral bases such as water and 3-quinuclidinol will exhibit smaller deviations from a rate−equilibrium correlation for deprotonation of related neutral carbon acids than that observed for deprotonation of ^+H_3N-1-CO_2Me by the anionic base hydroxide ion (Figure 6).

Intrinsic Reaction Barriers. The values of log k_{HO} (M^{-1} s^{-1}) for deprotonation of the cationic esters $^+Me_3N$-1-CO_2Me and ^+H_3N-1-CO_2Me by hydroxide ion exhibit large positive deviations from the linear rate−equilibrium correlation for deprotonation of neutral aldehydes, ketones, and esters (Figure 6). A similar positive deviation for deprotonation of cationic ketones has been noted in earlier work.[33b,51] This observation that reactions of similar thermodynamic driving force proceed at very different rates requires that there be a smaller Marcus intrinsic barrier Λ for the faster deprotonation of cationic carbon acids by hydroxide ion than for the deprotonation of neutral α-carbonyl carbon acids.

The rate constants for deprotonation of the very weakly acidic zwitterions ^+H_3N-1-CO_2^- and $^+Me_3N$-1-CO_2^- by hydroxide ion exhibit small (<3-fold) deviations from the correlation in Figure 6. Here, the manifestation of any positive deviation is restricted by the rate constant for the reverse protonation of the enolate by solvent water (Table 2),[17] which is approaching the limiting value of $k_{HOH} = k_{reorg} \approx 10^{11}$ s^{-1}.[47−49] Furthermore, it is not clear whether these formally neutral zwitterions should behave as neutral α-carbonyl carbon acids and obey the correlation in Figure 6 or whether they should behave as cationic carbon acids and exhibit positive deviations from this correlation. These uncertainties are reflected in the uncertainties in the pK_a's for these carbon acids given in Table 2.

There are at least two effects of cationic substituents that may act to lower the Marcus intrinsic barrier Λ for proton transfer reactions of resonance-stabilized α-carbonyl carbanions.

(1) Electrostatic interactions between hydroxide ion and a cationic substituent at the carbon acid may stabilize the transition

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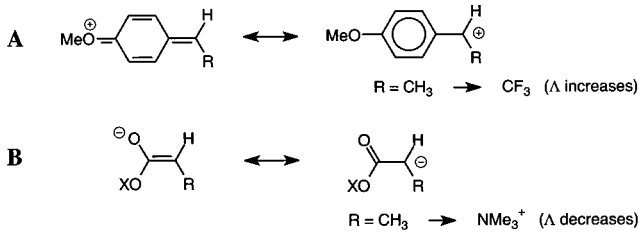
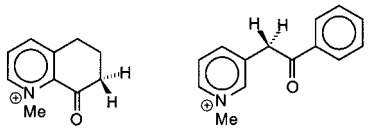
Scheme 6



state for enolization without affecting the thermodynamic driving force for deprotonation of the carbon acid (Scheme 5). The evidence for the importance of these electrostatic interactions was discussed in the preceding section.

(2) There is good evidence that the addition of electron-withdrawing α-substituents to ring-substituted benzylic carbocations results in a movement of positive charge onto the aromatic ring and an increase in the resonance stabilization of the carbocation (Scheme 6A).[75] This reduces the destabilizing charge−dipole interaction with the electron-withdrawing α-substituent and results in an increase in the intrinsic barrier for addition of nucleophiles to the carbocation.[76−79] Similarly, the addition of electron-withdrawing cationic substituents to an ester or ketone enolate might induce movement of negative charge away from oxygen and onto the α-carbon, because this will result in a larger stabilizing electrostatic interaction between unlike charges (Scheme 6B). Such localization of charge at the α-carbon would result in a decrease in the resonance stabilization of the carbanion and a reduction in the intrinsic barrier for proton transfer.

The cationic ketone **6** (pK_a = 11.0, k_{HO} = 5300 M^{-1} s^{-1})[43b,c] is only slightly more acidic than **5** (pK_a = 11.9, k_{HO} = 11 M^{-1} s^{-1}),[33b] but it undergoes a 500-fold faster deprotonation by hydroxide ion. Consequently, the value of log k_{HO} for deprotonation of **5** falls close to the correlation line shown in Figure 6 whereas log k_{HO} for **6** lies 1.6 units above this line.[50] These data show that the cationic substituents at **5** and **6** lead to a similar reduction in pK_a over that for a simple ketone[43b] but that the cationic substituent at **6** results in a significantly smaller intrinsic barrier to proton transfer to hydroxide ion. This suggests that placement of a cationic group close to a formal carbanionic carbon (as at the enolate of **6**) favors localization of negative charge at this carbon and results in a reduction in the Marcus intrinsic barrier to proton transfer.



5 **6**

Enzymatic Catalysis of Deprotonation of Amino Acids. A variety of enzyme-catalyzed racemization reactions proceed by abstraction of the α-proton of an amino acid to give an amino acid enolate, followed by protonation of this enolate to give the epimeric or racemic amino acid.[19,22−26,80,81] The results of our work suggest two related roles for an enzyme catalyst in optimizing the inherently strong electrostatic stabilization of these enolates from interactions between the closely spaced positive ammonium group and the negative charge of the enolate oxygen.

(1) The most abundant form of amino acids at physiological pH is the zwitterion and there is a requirement for activation of these species toward enolization by protonation of the carboxylate group to give the cationic form. We propose that amino acid racemases bind the more abundant zwitterion, $^+H_3NCH(R)CO_2^-$,[82] and activate the bound zwitterion toward enolization either by preequilibrium proton transfer to form enzyme-bound $^+H_3NCH(R)CO_2H$ or by formal general acid catalysis at the carbonyl group.[85−88] Brønsted acid catalysis of enolization will be favored if binding of the substrate induces a downward shift in the pK_a of the catalytic acidic residue at the enzyme to a value below the pH of the solution.[89]

(2) A nonpolar active site will favor enzymatic catalysis of amino acid racemization by providing a medium of low effective dielectric constant that maximizes stabilization of the zwitterionic enolate intermediate $^+H_3NC^-(R)CO_2H$ by intramolecular electrostatic interactions. There is an enormous dependence on the medium of the NMe_3^+ substituent effect on the oxygen acidity of carboxylic acids. The addition of a NMe_3^+ group to acetate ion results in only a small decrease in the basicity of the carboxylate group in water, $\Delta G_w = -4$ kcal/mol (ΔpK_a = 2.9, Chart 1); but it results in a very large decrease in gas-phase basicity, from 348.5 kcal/mol for $CH_3CO_2^-$ to 239.3 kcal/mol for $^+Me_3NCH_2CO_2^-$, $\Delta G_g = -109$ kcal/mol (Scheme 7).[90,91] The large difference in the substituent effects in the gas phase and water reflects the much larger free energy of solvation of an ammonium cation and a carboxylate anion when these groups are placed at separate molecules, ΔG_{solv}^R, than when they are placed in the same molecule to give a formally neutral zwitterion, ΔG_{solv}^P (Scheme 7).

We suggest that there is a similar influence of the medium on the effect of an α-NR_3^+ group on the ionization of carboxylic acids at carbon, so that the transfer of amino acids $^+H_3NCH(R)CO_2H$ from water to a nonpolar environment in an enzyme active site will stabilize the zwitterionic enolate $^+H_3NC^-(R)CO_2H$

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(82) For example, the pK_a of the carboxylic acid group of proline cation is 2.0.[83] Therefore, at pH 8, the fraction of proline present in the reactive cationic form is only 10^{-6} and the limiting rate constant for reaction of proline would be $k_{cat}/K_m \le (10^{-6})(10^9\ M^{-1}\ s^{-1}) = 10^3\ M^{-1}\ s^{-1}$, where $10^9\ M^{-1}\ s^{-1}$ is an estimated rate constant for a diffusion-limited enzymatic reaction.[84] However, a much larger rate constant of $k_{cat}/K_m = 7 \times 10^5\ M^{-1}\ s^{-1}$ is observed for racemization of D- and L-proline by proline isomerase, which shows that the substrate for this enzyme is proline zwitterion.[80a]
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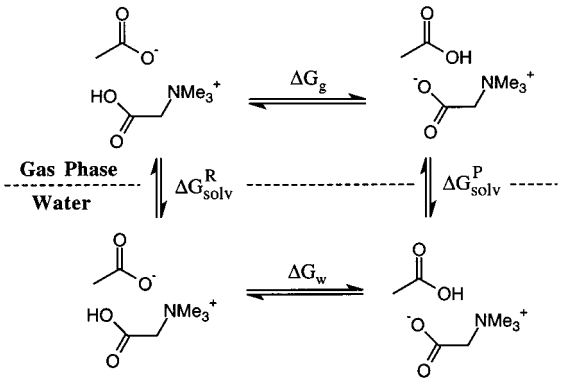
Scheme 7



relative to the cationic substrate. In this case, the intrinsic binding energy of the substrate is utilized to stabilize the ground-state complex of the enzyme and the amino acid cation $^{+}H_3NCH(R)CO_2H$ at an active site of low dielectric constant.[92] This binding energy would then be expressed at the zwitterionic enolate intermediate $^{+}H_3NC^{-}(R)CO_2H$, where the destabilization resulting from placement of the cationic substrate at an active site of low dielectric constant is replaced by stabilizing intramolecular electrostatic interactions. A similar "medium" effect of transfer from water to the active site of pyruvate decarboxylase of the carbanionic zwitterion generated by deprotonation of hydroxybenzylthiamine diphosphate at the benzylic carbon has been proposed to account for the more than 9-unit lower pK_a of the enzyme-bound than of the free carbon acid.[94]

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Supporting Information Available: Table S1: First-order rate constants for exchange of the first α-proton of glycine methyl ester in D_2O determined by monitoring incorporation of deuterium into glycine methyl ester by ^1H NMR. Table S2: First-order rate constants for exchange of the first α-proton of glycine methyl ester in D_2O determined from the rate constants for ester hydrolysis and the deuterium enrichment of the glycine hydrolysis product. Table S3: First-order rate constants for exchange of the first α-proton of glycine in D_2O. Table S4: First-order rate constants for exchange of the first α-proton of betaine methyl ester in D_2O. Table S5: First-order rate constants for exchange of the first α-proton of betaine in D_2O (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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