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Amidates as Leaving Groups: Structure/Reactivity Correlation of the Hydroxide-Dependent E1cB-like Breakdown of Carbinolamides in Aqueous Solution

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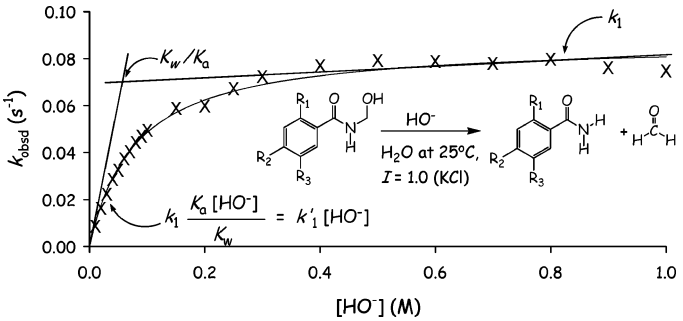
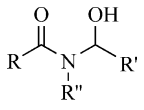
Received March 22, 2007



The kinetic study of the aqueous reaction, between pH 10 and 14, of eight *N*-(hydroxymethyl)benzamide derivatives in water at 25 °C, $I = 1.0$ M (KCl), has been performed. In all cases, the reaction proceeds via a specific-base-catalyzed deprotonation of the hydroxyl group followed by rate-limiting breakdown of the alkoxide to form aldehyde and amidate (E1cB-like). Such a mechanism was supported by the lack of general buffer catalysis and the first-order dependence of the rate of reaction at low hydroxide concentrations and the transition to zero-order dependence on hydroxide at high concentration. A ρ-value of 0.67 was found for the Hammett correlation between the maximum rate for the hydroxide independent breakdown of the deprotonated carbinolamide (k_1) and the substituent on the aromatic ring of the title compounds. Conversely, the substituents on the aromatic ring of the amide portion of the carbinolamide had only a small effect on the K_a of the hydroxyl group indicating that the amide group does not strongly transmit the electronic information of the substituents. These observations led to the conclusion that the major effect of electronic changes on the amide of carbinolamides is reflected in the nucleofugality of the amidate once the alkoxide is formed and not in the pK_a of the hydroxyl group of the carbinolamide.

Introduction

Carbinolamides (**1**) are formed by the reaction of an amide



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and an aldehyde/ketone with a accompanying proton shift.[1] While reports of carbinolamides appeared in the literature as early as the 1870s,[2,3] it has only been recently, with the discovery of their presence in molecules having interesting biological function, that the properties and reactions of carbinolamides have once again become of broader interest to the chemical community. For example; bicyclomycin,[4,5] talaroconvolutin,[6] azaspirene,[7] UCS1025A,[8] oteromycin,[9] and epolactaene[10] all contain the functionality. In addition, carbinolamides

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10.1021/jo070603u CCC: $37.00
Published on Web 07/13/2007

are intermediates both in peptide hormone formation[11−25] and in the catabolic pathway leading from purines to urea,[26] and they also have a role in the deleterious modification of DNA.[27] While the function of the carbinolamide in these molecules is in some cases unknown, with the diverse spectrum of biological venues in which carbinolamides have been implicated, it is surprising to realize how little is known about their inherent reactivity. In fact, carbinolamides exhibit a form of reactivity in water wherein the amide portion of their structure acts as a leaving group in what could be formally described as an elimination reaction (see Scheme 1).[28−34]

Typically, when the topic of amides is broached, it is their well-established stability with regard to acyl transfer[35,36] and their crucial role in the stability and structure of proteins[37] that are focused upon and not reactions involving their nucleofugality. However, this form of reactivity is utilized to some advantage in a number of biological systems.[11−26] One example involves the use of carbinolamides as intermediates in the generation of α-amidated peptide hormones.[11−25] (Approximately 50% of the known mammalian peptide hormones possess a C-terminal α-amide function that is critical for proper hormone function.)[13] The generation of peptide hormones is mediated by a bifunctional enzyme, peptidylglycine α-amidating monooxygenase (EC 1.14.17.3), which takes the glycine-extended peptide precursor and oxidizes the pro-*S* hydrogen[22] to create a carbinolamide. In a second active site, the catalytic breakdown of the carbinolamide yields the α-amidated peptide and glyoxalate.[38] The mechanism by which the breakdown of the carbinolamide intermediates occurs enzymatically is not understood but certain aspects of its catalysis have been elucidated. The one criterion for the activity of the PAL enzyme that all the investigators generally agree upon is the necessity of Zn^{2+}.[14,39−43] More recently, studies of the catalytic core of PAL have indicated that not only is Zn^{2+} required for enzymatic activity but also Fe^{3+}.[40] The exact role of the metal ions in the catalytic process has not been determined as arguments for a catalytic role and a structural role for enzyme activity have both been discussed.[14,39−43] While the exact function of metals in the catalytic cycle of PAL remains a matter of discussion, it is generally believed that the breakdown of the carbinolamide intermediate is catalyzed by acids and bases, with the possibility of a metal-bound hydroxide or water molecule.[42]

Such a mechanism does not correlate with what is currently known of the general reactivity of carbinolamides which, in the hydroxide region of the pH−rate profile, are known to react via a specific-base-catalyzed mechanism shown in Scheme

SCHEME 1. Accepted Mechanism for the Hydroxide-Dependent Breakdown of Carbinolamides

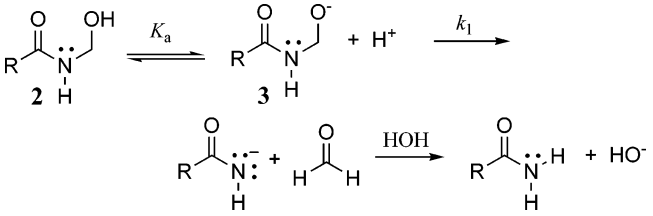

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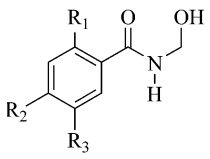
CHART 1. *N*-(Hydroxymethyl)benzamide Derivatives



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	R_1	R_2	R_3
4a	H	H	H
4b	H	Cl	H
4c	Cl	Cl	H
4d	H	H	Cl
4e	Cl	H	H
4f	H	Cl	Cl
4g	H	NO_2	H
4h	H	H	NO_2

1.[28−33] However, very little information exists concerning the reactivity of molecules wherein an amide is acting as a leaving group and, therefore, the enzymatic requirements essential for catalysis are, by necessity, speculative. Previous studies investigating $(E1cB)_R$ reactions where an amidate acted as a leaving group were shown to undergo relatively slow rate determining departure of *N*-methylacetamidate in ethanol in the presence of ethoxide ($k_{obsd} = 4.6 \times 10^{-6}$ s^{-1} for $PhSO_2CH_2CH_2NHCOCH_3$ in EtOH/NaOEt at 25 °C).[44] To understand those catalytic components required to lower the energy barrier to reaction, we must first understand the fundamental reactivity of the functionality undergoing transformation. The study of the hydroxide-dependent breakdown of carbinolamides provides a unique opportunity to dissect the two steps of the reaction and gain information about the energetic requirements for an amidate to act as a leaving group. Presented here are the results of the hydroxide-dependent breakdown of a series of aromatic substituted *N*-(hydroxymethyl)benzamides (**4**) where we have obtained both the pK_a values for the hydroxyl group of the carbinolamide and the rate of the hydroxide-independent departure of the amide leaving group in this specific base/E1cB-like reaction.

Results

All kinetic experiments were performed in H_2O at 25 °C and $I = 1.0$ M (KCl) and were initiated by the injection of a concentrated stock solution of the carbinolamide of interest, dissolved in CH_3CN, into the aqueous reaction solution. Rate constants for the reaction of compounds **4a**−**h** at pH >10.5 were obtained from the change in absorbance as a function of time, obtained with a UV−vis spectrophotometer, and exhibited good first-order behavior with steady infinity absorbance values. In all cases, the reaction of the carbinolamide was very much faster than subsequent hydroxide-catalyzed hydrolysis of the amide, as evidenced by the constant infinity absorbance values. Also, the generation of formaldehyde as the reaction progressed did not complicate the observed rates (amide could potentially react with formaldehyde to reform the carbinolamide) as formaldehyde in aqueous solution is predominantly hydrated, $K_H = 2420$, where K_H = [hydrate]/[free aldehyde].[45] The only product observed by HPLC was parent amide and reactions quenched prior to the completion of the reaction contained only starting material and amide (formaldehyde could not be detected by the photodiode array detector on the HPLC).

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$$\ln\left\{\frac{A_{car}}{(A_{car} + (R_x)(A_{amide}))}\right\} = -k_{obsd}t \qquad (1)$$

At pH values below 10.5, the observed rate constant for some of the carbinolamides studied became too slow to be conveniently followed by UV−vis spectrophotometer and, thus, HPLC was used to concurrently follow the disappearance of **4** and appearance of amide product (**5**). The observed area of the peak for the amide needed to be corrected for differences in the molar extinction coefficients for the amides vs the carbinolamides (R_x, see Table 1S in the Supporting Information). The observed rate of reaction was then determined by comparing the area of the peak for the carbinolamide (A_{car}) to the area of the peak for the amide (A_{amide}) according to eq 1.

Figures 1 and 2 show the relationship between k_{obsd}, for the conversion of the carbinolamide into the amide/formaldehyde, and the [HO$^-$] in H_2O at 25 °C, $I = 1.0$ M (KCl). No evidence for catalysis by the buffers, used to maintain the pH between 10 and 12, was observed; however, the total buffer concentrations were kept quite low (0.1−0.05 M [$Buff_{tot}$]). To further explore this possibility, experiments with **4a** were performed at pH values of 10.00 (carbonate, total buffer [0.33M]) and 11.46 (phosphate, total buffer [0.150M]) yielding k_{obsd} values of 3.64×10^{-5} and 9.55×10^{-4} s^{-1}, respectively, which are kinetically similar to the reaction rates in the absence of buffer (see Figure 5S for a plot of [phosphate] vs k_{obsd} in the Supporting Information). From Figures 1 and 2, two distinct regions can be observed. The first region has a first-order dependence on [HO$^-$] that changes to a zero-order dependence at [HO$^-$] greater than ~0.3 M. Shown in Table 1 are the apparent second-order rate constants (k'_1) for the hydroxide-dependent reaction calculated by using k_1 and the K_a for the hydroxyl group determined as described below but k'_1 can also be determined from the linear portion of a plot of k_{obsd} vs [HO$^-$] (see Figure 1 and eq 2).

$$(k_{obsd})^{HO} = k_1\frac{K_a[HO^-]}{K_w} = k'_1[HO^-] \qquad (2)$$

Discussion

Previous studies involving the aqueous reaction of carbinolamides had focused on the linear correlation between the [HO$^-$] and the rate of reaction observed at lower pH values.[28−32] As a result, it is the apparent second-order rate constant (k'_1) from the correlation between hydroxide and k_{obsd} (see eq 2) that has been most frequently reported for the hydroxide-catalyzed reaction of carbinolamides under aqueous conditions.[28−30,32] However, other studies had shown that, at higher pH, the first-order dependence between k_{obsd} and [HO$^-$] does not hold.[31,33] The outcome was that while some information existed about the aqueous reaction of carbinolamides, the available second-order rate constants did not clarify how the structure of the carbinolamide affected the different steps of the hydroxide reaction. It is evident from Figures 1 and 2 that at lower concentrations of hydroxide a linear dependence between k_{obsd} and [HO$^-$] was observed. However, as the pH approaches 13, k_{obsd} was no longer linearly dependent on the hydroxide concentration and further increases in the amount of hydroxide in solution lead to no significant changes in k_{obsd}. By extending the hydroxide studies to higher [HO$^-$] it was possible to acquire information about both the hydroxide-dependent and hydroxide-independent reactions.

TABLE 1. Constants for the Hydroxide-Independent Reaction of *N*-(Hydroxymethyl)benzamide and Derivatives and Their pK_a Values in Water at 25 °C, I = 1.0 M (KCl)

compd	temp (°C)	k'_1[a] ($M^{-1}s^{-1}$)	k_1 (s^{-1})[b,c]	pK_a[b,d]	k_{rel}[e]	k'_1 ($M^{-1}s^{-1}$, lit.)[f]
4a	25[g]	0.37	0.042 ± 0.003	13.05 ± 0.05	1	1.83
4b	25[g]	0.62	0.068 ± 0.005	13.04 ± 0.05	1.6	3.67
4c	25[g]	1.73	0.19 ± 0.01	13.04 ± 0.05	4.5	
4d	25	0.67	0.070 ± 0.005	13.02 ± 0.05	1.7	
4e	25	0.88	0.13 ± 0.01	13.14 ± 0.09	3.3	
4f	25	1.10	0.087 ± 0.005	12.90 ± 0.06	2.1	
4g	25	1.27	0.16 ± 0.01	13.14 ± 0.1	3.8	14.83
4h	25	1.30	0.13 ± 0.009	13.00 ± 0.05	3.1	12.33

[a] Apparent second-order rate constant for the hydroxide-dependent breakdown of the carbinolamides in water calculated from k_1 and K_a, using $k'_1 = (k_1K_a)/K_w$ and $K_w = 1 \times 10^{-14}$ M^2. [b] Errors obtained from nonlinear least-squares fits of k_{obsd} vs [HO^-] according to eq 3. [c] First-order rate constant for the pH-independent breakdown of the deprotonated carbinolamide. [d] Ionization constant for the hydroxyl group of the carbinolamide. [e] Calculated by dividing k_1 for **4a** into the k_1 value for each amide. [f] k'_1 values obtained from ref 32 and were determined in H_2O at 37 °C, I = 0.5. [g] Previously reported in ref 33.

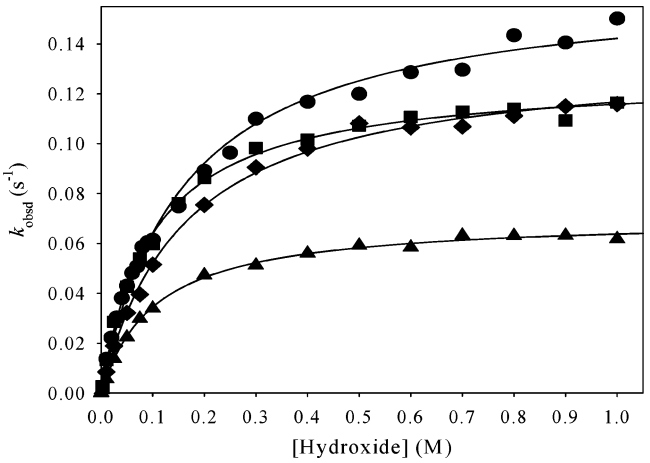


FIGURE 1. Plot of the effect of hydroxide concentration (M) on the observed rates of reaction (k_{obsd}, s^{-1}) for 4-nitro-*N*-(hydroxymethyl)-benzamide (●), 3-nitro-*N*-(hydroxymethyl)benzamide (■), 2-chloro-*N*-(hydroxymethyl)benzamide (◆), and 3-chloro-*N*-(hydroxymethyl)-benzamide (▲) in H_2O, I = 1.0 M (KCl), at 25 °C; lines through the data are best fit lines based on eq 3.

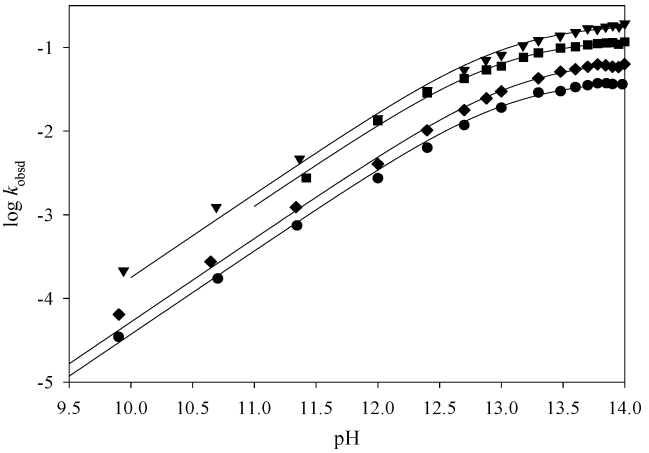


FIGURE 2. Partial pH−rate profile for the aqueous reaction of 2,4-dichloro-*N*-(hydroxymethyl)benzamide (▼), 3-nitro-*N*-(hydroxymethyl)-benzamide (■), 4-chloro-*N*-(hydroxymethyl)benzamide (◆), and *N*-(hydroxymethyl)benzamide (●) in H_2O, I = 1.0 M (KCl), at 25 °C.

Buffer Catalysis. Kinetic studies performed below pH 12 required the presence of buffers to maintain the pH of the reaction solutions (pH 10−10.6, carbonate; pH 11.4, phosphate). The total buffer concentrations were normally modest (0.10−0.05 M = [$Buff_{tot}$]) with kinetic experiments being performed at, at least, three different concentrations of buffer at each pH. No evidence for the presence of buffer catalysis was observed during the course of these studies (slopes of ~0 were found for plots of k_{obsd} vs [$Buff_{tot}$], plots not shown). However, it has recently been shown that the acid-catalyzed breakdown of *N*-(hydroxymethyl)benzamide (**4a**) occurs with buffer catalysis, indicating that the rate determining step of the acid-catalyzed reaction involves proton transfer.[34] Although there is no necessary connection between buffer catalysis for the acid-catalyzed reaction and its observation in the hydroxide-dependent reaction, prior studies had missed buffer catalysis in the acid region[29,31,33] and it was important to probe such a possibility for the hydroxide reaction at higher buffer concentrations.

The buffer studies were performed on **4a**, at two different pH values, utilizing a different buffer at each pH. For the studies performed at pH 10.0 in the presence of 0.33 M total carbonate (B^-/BH = 0.47), the k_{obsd}^{buffer} for the reaction of **4a** was found to be 3.64×10^{-5} s^{-1}, where the expected rate constant (based on the apparent second-order rate constant, see Table 1 and eq 2) was $k_{obsd}^{expected} = 3.7 \times 10^{-5}$ s^{-1} at the same pH. At pH 11.46 in the presence of 0.150 M total phosphate (B^-/BH = 0.14) $k_{obsd}^{buffer} = 9.55 \times 10^{-4}$ s^{-1} vs $k_{obsd}^{expected} = 1.07 \times 10^{-3}$ s^{-1} (see the plot of [$phosphate_{tot}$] vs k_{obsd} in the Supporting Information). The correlation between the k_{obsd}^{buffer} at high [buffer] and the $k_{obsd}^{expected}$ calculated from the apparent second-order rate for the hydroxide reaction does not support buffer catalysis in the hydroxide-dependent reaction.

Mechanism of the Hydroxide Reaction. The observed dependence of the rate of reaction on the hydroxide concentration (Figures 1 and 2) was indicative of a process in which an intermediate was being created prior to the rate determining step (E1cB-like or a specific base-catalyzed process). The first-order dependence of k_{obsd} on hydroxide, at lower hydroxide concentrations, can be explained by a shift in the equilibrium between **4** and **6** that occurs by neutralization of H^+, generated upon ionization of the hydroxyl group (see Scheme 2). At higher [HO^-], the pK_a of the hydroxyl group of the carbinolamide was surpassed, and further increases in hydroxide had no effect on the concentration of **6** and the rate of the reaction becomes hydroxide independent. The hydroxide-independent region when coupled with the lack of buffer catalysis provides strong evidence that it is alkoxide **6** that undergoes rate-limiting breakdown to form formaldehyde with the departure of the amidate.

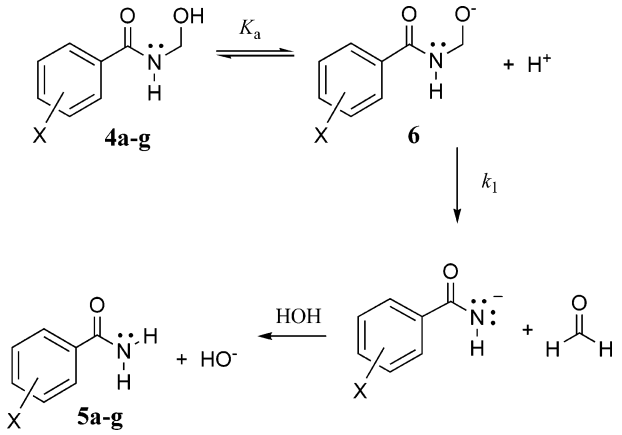
Another mechanistic possibility comes from the study of the aqueous reactions of *N*-acyloxymethyl amide derivatives (see Scheme 3).[46−50] Iley and Moreira[47] found that the onset of an observable hydroxide-catalyzed reaction for a series of structurally similar *N*-acyloxymethylbenzamides (**7**) was dependent upon whether the amidic nitrogen had an available proton. In those compounds where the amidic nitrogen was methylated, *N*-methyl-**7** primarily underwent hydroxide-dependent hydrolysis of the ester portion of the functionality with the onset of an observable hydroxide-dependent reaction at approximately pH 10, whereas those compounds having a hydrogen on the amidic nitrogen were found to undergo an observable hydroxide-dependent reaction at pH of approximately 6. On the basis of differences in the second-order rate constants between the *N*-methyl-**7** and **7**, and buffer catalysis studies, Iley and Moreira concluded that an elimination reaction was occurring wherein the proton on the nitrogen was being removed and an *N*-acyl imine was generated as an intermediate.[47]

A reaction such as that suggested in Scheme 3 could occur with the *N*-(hydroxymethyl)benzamide compounds discussed here. However, such a reaction would be largely invisible in our study as subsequent addition of water to the *N*-acyl imine would regenerate the carbinolamide. However, kinetic studies, involving pH values between 10 and 12, described here and in previously published work,[33] between pH 7 to 12, were performed in the presence of buffers used to maintain pH. In all cases, good pseudo-first-order kinetics were observed and, for those experiments performed by HPLC, no evidence for the generation of products involving attack of the *N*-acyl imine by the buffer was observed. Furthermore, KCl was used to maintain the ionic strength of the solution and once again no evidence for the production of products resulting from the attack of chloride on the carbon of the amidinium ion was observed by HPLC. While the absolute stability of the *N*-chloromethylbenzamide derivatives in water is not known, available data would suggest that they are very reactive and susceptible to nucleophilic attack.[46,51]

$$(k_{obsd})^{HO} = k_1 \frac{K_a[HO^-]}{K_w + K_a[HO^-]} \qquad (3)$$

Thus, available evidence supports a mechanism such as outlined in Scheme 2 where the rate expression for the reaction is as shown in eq 3. A variety of modifications of eq 3 can be performed depending upon the conditions of the reactions. For example, at low [HO$^-$] where K_w (dissociation constant for water) is much larger than the product of K_a and the [HO$^-$], eq 3 simplifies to eq 2. In eq 2 there is a first-order dependence between k_{obsd} and the [HO$^-$] and the constant k'_1 that consists of k_1, the rate for the hydroxide-independent breakdown of the conjugate base of the carbinolamide, K_a, the ionization constant for the hydroxyl group of the carbinolamide, and K_w. We have

SCHEME 2. Specific Base-Catalyzed Breakdown of *N*-(Hydroxymethyl)benzamide Derivatives



reported the apparent second-order rate constants, determined in this study, in Table 1 along with those previously reported and found good agreement between the relative magnitudes of our results observed at 25 °C, I = 1.0 M (KCl), and those performed at 37 °C, I = 0.5 M. It is clear from the data in Table 1 that the addition of electron-withdrawing groups to the aromatic ring of the amide portion of the carbinolamide results in an overall increase in the velocity of the reaction. This was clearly illustrated by **4a** (4-H), **4b** (4-Cl), and **4f** (4-NO_2) where the relative rates for the apparent second-order rate constant were 1, 1.7, and 5.4, respectively. The Hammett correlation for the apparent second-order rate constants (k'_1) vs σ was linear and has a ρ-value of 0.86 (Figure 2S in the Supporting Information) whereas Bundgaard and co-workers found a ρ-value of 1.11 in their studies at 37 °C.[32]

The k'_1 values provide an interesting profile of the substituent effects of the rates of the hydroxide-dependent reaction but also illustrate their limitation in providing information to increase our mechanistic understanding of the reaction. To understand the true effect of the substituents on the hydroxide-dependent reaction, the effects of the substituents on the individual rates and equilibrium constants must be dissected. Such a determination requires that full dependence of k_{obsd} on [HO$^-$] be obtained such as that shown in Figures 1 and 2. Having established both the first-order and the zero-order dependence of k_{obsd} on the [HO$^-$], the experimental data can be fit to eq 3 by using a nonlinear least-squares method to determine values for k_1 and K_a for **4a**−**h**. The values of k_1 and K_a so determined for the compounds studied are listed in Table 1.

***K*$_a$ of the Hydroxyl Group.** The K_a values for the compounds studied were determined from the nonlinear least-squares fit of eq 3 to the hydroxide dependence of k_{obsd} (see example in Figure 1). The K_a values determined are listed in Table 1, and it is immediately obvious that there is no significant effect on the K_a of the hydroxyl group upon substitution of the aromatic ring of the amide portion of the carbinolamide. From the data in Table 1, all of the carbinolamides reported here have pK_a values of ∼13. These pK_a values correlate reasonably well with the reported pK_a values for the hydrates of formaldehyde and acetaldehyde which are 13.29 and 13.48, respectively.[52] The greater acidity found for the hydroxyl group of the carbinolamides can be explained by the benzamide group being better able to inductively stabilize the negative charge being generated

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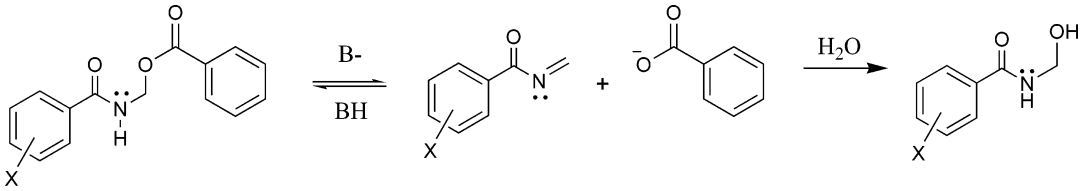
SCHEME 3. Mechanism of Base-Catalyzed Aqueous Reaction of *N*-Acyloxymethylbenzamides



7

on the oxygen vs the hydroxyl group in the hydrate of formaldehyde.

The lack of any apparent variation in the acidity of the hydroxyl group is also clear in the Hammett plot shown in Figure 3S (Supporting Information), which illustrates a poor correlation between $\log(K_a^X/K_a^H)$ and σ that has a $\rho = 0.003$. This result further supports the observational conclusion discussed above; however, the data in Figure 3S (Supporting Information) show two of the points deviating significantly from the remainder of the data and an argument could be put forth as to whether these points should be included in the plot. If the data points for **4f** and **4g** are not included in the Hammett plot (see Figure 4S of the Supporting Information), there is a good linear correlation that has a $\rho = 0.07$. If this was compared to the well-known effect of addition of methylene units between a carboxylic acid group and an aromatic ring where $\rho = 1.0$, 0.56, and 0.24 have been determined for benzoic acid,[52] 2-phenyl acetic acid,[53] and 3-phenylpropanoic acid derivatives,[53] respectively, the results presented here are somewhat surprising. On the basis of the carboxylic acid results, a larger effect on the K_a of the hydroxyl group was expected due to the similar number of atoms between the ionizing group and the aromatic system as compared to 3-phenylpropanoic acid. We conclude that the inductive effects of substituents on the aromatic ring must not be transmitted as effectively by the amide σ bonds as compared to the σ bonds of an alkane system or the amide marginalizes/dominates the electronic effects of substituents on the aromatic ring with the net effect being a hydroxyl group whose pK_a is relatively insensitive to the structure of the molecule.

This observation was further illustrated by the insensitivity of the pK_a to substitution on the 2-position of the aromatic ring. It is recognized that the pK_a values of compounds with substituents on the 2-position of the aromatic ring do not follow the same correlation as those with substituents on the 3- and 4-positions.[54] For example, the pK_a values for 4-chloro-, 3-chloro-, and 2-chlorobenzoic acid are 3.99, 3.83, and 2.94, respectively, where substitution on the 2-position leads to a larger effect due, presumably, to inductive, electrostatic, and steric effects.[55] In the case of the carbinolamides studied here, such effects do not appear to be in operation as the pK_a values found for the hydroxyl groups are all very similar given experimental error.

Rate-Limiting Step (k_1). On the basis of the data presented here, the departure of the benzamidate to generate formaldehyde (Scheme 2) was the rate determining step of this process. This conclusion was supported by the observed hydroxide dependence and the lack of buffer catalysis in this same region. The rate determining step (k_1) involves the formation of the carbon oxygen double bond as the carbon−nitrogen bond is cleaved to form formaldehyde and release the amidate. This type of reactivity, i.e., an amide acting as a leaving group in what is essentially an elimination reaction, has received only cursory attention[44] and the results presented here represent the first structurally analogous series of compounds where a broad enough range in the pH rate studies has been performed so that the values of k_1 could be determined. As stated earlier, the mechanism for the reaction is shown in Scheme 2 and the correlation between k_{obsd} and the [HO$^-$] can be fit to eq 3 separating the effect of aromatic substituents on the K_a of the hydroxyl group and on k_1. The values determined for k_1 from the nonlinear least-squares fit of eq 3 to the k_{obsd} values vs [HO$^-$] are listed in Table 1.

From the data in Table 1, as the electronic demand of the substituents on the aromatic ring increases, the nucleofugality of the amide increases, as evidenced by the increase in the magnitude of k_1 with the addition of stronger electron withdrawing groups. This result is illustrated in Figure 3, which shows a linear correlation between the σ-value and $\log(k_{1X}/k_{1H})$. The ρ-value from Figure 3 is 0.67 whereas a previous study involving a similar series of compounds[32] found a much greater sensitivity to substitution on the aromatic ring ($\rho = 1.11$). However, the previous study had the combined effects of both the K_a and k_1 in their plot and the experiments were performed at $I = 0.5$, suggesting that the substituent could play a larger role in stabilizing the developing charge in the transition state of these reactions due to differences in the ionizing power of the solvent. The positive value of ρ is further evidence that there is buildup of negative charge on the amide leaving group in the transition state of the rate determining step that is stabilized by the addition of electron-withdrawing groups.

Although not discussed in the buffer catalysis section, which focused on general base catalysis, the possibility exists that the benzamidate leaving group may require assistance during heterolytic cleavage of the C−N bond. Catalysis during the departure of the benzamidate group could occur via a specific acid mechanism or a general acid mechanism. A specific acid-catalyzed mechanism is unlikely due to the basic conditions of the reaction, the protonation state of the hydroxyl group of the carbinolamide, and the pK_a values of the protonated forms of amides (pK_a of *O*-protonated amide 0 to −3;[56−61] and *N*-protonated amide −7 to −8).[62,63] The most logical form for this

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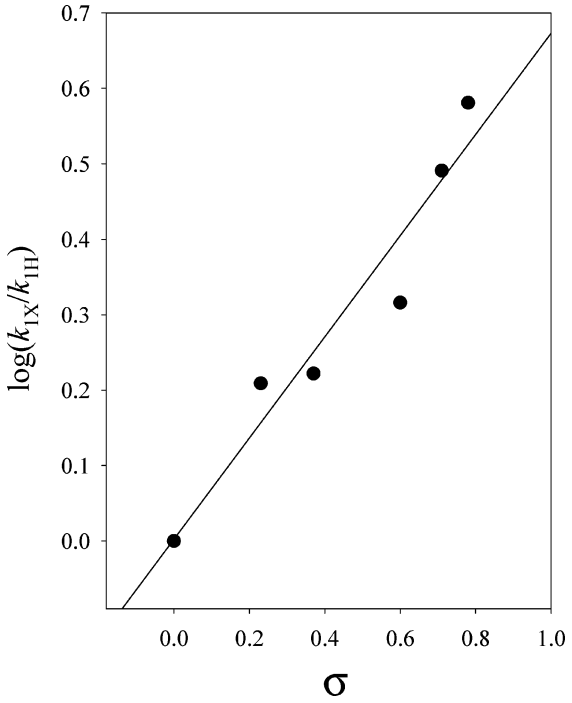
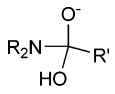


FIGURE 3. Hammett plot of $\log(k_{1X}/k_{1H})$ for the hydroxide-independent reaction of a series of *N*-(hydroxymethyl)benzamide derivatives in H_2O at 25 °C, $I = 1.0$ M (KCl), vs σ.

assistance in the departure of the benzamidate to take would be protonation of amidate as C−N bond cleavage was occurring. However, no evidence for any buffer catalysis was observed during the buffer catalysis studies performed at pH values of 10 and 11.4. This provides circumstantial support for a mechanism wherein the benzamidate is acting as the leaving group in this reaction.

The results presented here also illustrate one of the fundamental difficulties experienced in the hydrolysis of amides. It is generally accepted that the hydroxide-dependent hydrolysis of amides involve a T_{O^-} intermediate (**8**) that has a number of



T_{O^-}, **8**

possible fates which could involve breakdown, hydroxide-catalyzed breakdown, or general-base-catalyzed breakdown.[64] It is also thought that in "normal" amides the breakdown of T_{O^-} is the rate-limiting step but available oxygen exchange data indicate that the nitrogen must be protonated or being protonated in the transition state for T_{O^-} to breakdown to form the carboxylate and amine products. However, there have been reports of an amide hydrolysis mechanism that was second order in hydroxide and other detailed series of ^{18}O incorporation experiments coupled with kinetic studies have clearly shown there is a change in the mechanism of amide hydrolysis that is connected to the pK_a of the amine leaving group.[36] Two amines that have been shown to depart as the anion in amide hydrolysis studies are 3,3,4,4-tetrafluoropyrrolidine[65] and pyrrole[65] whose pK_a values for their protonated states are 4.05[66] and −3.8,[67] respectively. Data concerning the acidity of the amine hydrogen to generate the anions of the parent compounds above is even sketchier; however, a pK_a of 17.3 has been determined for the proton attached to the nitrogen of pyrrole.[68] It is the expulsion of the amine anion in T_{O^-} that clearly resembles the reaction of **6** with the expulsion of the amidate for the hydroxide-dependent breakdown.

Limited data concerning the acidity of hydrogen attached to nitrogen in amides exist but available data suggest that the acidity can vary from benzamide having a pK_a > 19.0 to 4-nitrobenzamide that has a pK_a of 15.85.[69,70] These pK_a values bracket the pK_a quoted for pyrrole (pK_a = 17.3) and, thus, these benzamidates should be capable of acting as anionic leaving groups in carbinolamide breakdown. The foundation of this conclusion lies in the structural similarity of T_{O^-} and **6** where the breakdown involves the generation of a carbonyl from the negatively charged oxygen. All available data point to a mechanism of breakdown for carbinolamides that involves a benzamidate group acting as a leaving group in a manner outlined in Scheme 2.

Conclusions

Presented here are the results of the study of the aqueous reactions, at pH values between ~10 and 14, investigating the effects of substituents on the aromatic ring of the amide portion of a series of *N*-(hydroxymethyl)benzamide compounds. To fully understand the hydroxide-dependent mechanism of breakdown of these compounds and the effects of substitution, both k_1 and K_a for the reaction must be investigated. These studies show that electron-withdrawing substituents lead to the following general affects on the reactivity of the carbinolamides studied here: (a) As the strength of the electron-withdrawing group increases, the pK_a of the hydroxyl group decreases but the decrease in the pK_a values is much smaller than expected given the relative proximity of the substituents on the aromatic ring (ρ = 0.07). (b) Electron-withdrawing groups increase the nucleofugality of the benzamidate leaving group in the rate-limiting step of the reaction (ρ = 0.67). These studies clearly show that amides are capable of acting as leaving groups in elimination reaction supporting $(E1cB)_R$ studies performed by Stirling.[44] (c) The addition of ortho substituents (**4c**, **4e**) led to no changes in the mechanism of reaction of the compounds and no significant changes in the pK_a values of the hydroxyl group of the carbinolamide. All the evidence presented here supports a mechanism of reaction under basic conditions where this is a specific-base-catalyzed deprotonation of the hydroxyl group followed by rate-limiting breakdown of the alkoxide thus created (E1cB-like).

Implications to the Enzyme-Catalyzed Reaction. These results imply that the rate enhancement achieved by PAL is not acid/base catalysis involving ionization of the hydroxyl group of the carbinolamide, as the alkoxide appears to have

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significant lifetime in solution. This observation is due to the nature of the leaving group that is being expelled during the reaction. While the molecular machinery for removal of the hydroxyl proton may exist within the active site, it is clear that the departure of the amidate is the step that is thermodynamically most demanding and will require catalysis for rate enhancement to be realized. Implicit in these statements is the assumption that the hydroxide-dependent route is the mechanism that is being utilized by the enzyme and, to date, the exact mechanism utilized by PAL is unknown.

Experimental Section

Materials and Methods. Potassium dihydrogenphosphate, potassium phosphate, potassium hydrogenphosphate, potassium chloride, potassium hydroxide, potassium carbonate, and HCl were obtained from commercial sources. All materials used in these studies were reagent grade and were used without further purification. All column chromatography was performed with silica gel (70−230 mesh). The water used in the kinetic and HPLC studies was house DI-water that was then passed through a water purification system. Standard potassium hydroxide solutions were obtained by dilution of concentrated solutions with the hydroxide concentration obtained by titration to the phenolphthalein end point with standard HCl solutions.

All melting points are uncorrected and were obtained with a melting point apparatus. The ^{1}H NMR spectra reported here and in the Supporting Information were obtained in $CDCl_3$ (7.27 ppm) with a 400 MHz instrument unless otherwise stated.

Kinetics. The majority of the kinetic studies performed between pH 10 and 14 utilized a spectrophotometer with the temperature of the sample transport tray maintained at 25 °C and controlled by computer. A standard kinetic experiment was performed by injecting a concentrated stock solution of the carbinolamide dissolved in CH_3-CN into a thermally equilibrated (allowed to sit in the sample changer at least 10 min prior to injection) reaction solution contained in a cuvette, yielding a final substrate concentration of 5×10^{-5} to 1×10^{-4} M and a solution that was ∼0.05−0.1% acetonitrile. The change in absorbance was followed as a function of time and k_{obsd} was obtained with use of software supplied with the instrument (see Table 1S in the Supporting Information for the wavelengths where reactions were followed for each compound studied). The pH of the reaction solution was obtained subsequent to completion of the reaction by using a standardized combination glass electrode attached to a pH meter. The hydroxide concentration was calculated by using eq 4 with an apparent activity coefficient for hydroxide of $\gamma^{HO^-} = 0.79$,[71] determined from the measured pH of known [HO$^-$] in water at $I = 1.0$ M (KCl) at 25 °C.

$$[HO^-] = \frac{10^{(pH - pK_w)}}{\gamma^{HO-}} \quad (4)$$

As a result of the sluggish nature of the rates of reaction below pH 10.5 for some of the carbinolamides, these studies were performed utilizing an HPLC. Kinetic experiments at these pH values were initiated in the same manner as described above and the sample vials containing the reaction solutions were stored in a water bath maintained at 25 °C or in the temperature-controlled autosampler (see below). At timed intervals the progress of the reaction was determined by comparing peak sizes for the starting material vs those of the product determined by HPLC.

A typical HPLC experiment began by placing the samples in an autosampler, with the temperature maintained at 25 °C. The remainder of the HPLC system used for these studies consisted of two pumps, and a photodiode array detector, all remotely controlled by a computer. The column was a C-18 reverse phase column with guard column containing the same material. At timed intervals, a 100 μL volume of the reaction solution was injected into the HPLC system and the progress of the reaction was followed by observing the relative changes in the area of the carbinolamide peak (A_{car}) vs the area for the amide product (A_{amide}). The production of formaldehyde could not be detected by the photodiode array detector. The pH values of the reaction solutions were determined, prior to the injection of starting material and following the completion of the observed portion of the reaction, as described for the spectrophotometric experiments. No significant changes in pH (>0.05) were ever observed.

The observed rates of the reactions utilizing the HPLC were calculated with eq 1. The areas observed for the amide product were statistically corrected for differences in the molar absorptivities between the carbinolamide and the amide product. The correction factor (R_x) was determined by following the reaction of the carbinolamide by HPLC for 3−5 half-lives and determining the differences in the total area change for the carbinolamide vs the amide. For all compounds studied, the magnitude of the change in the area of the amide was less than the observed loss of area for the starting material in the same time period. The correction factor was determined independently at, at least, two other pH values and good agreement as to the magnitude of the correction factor was found in all cases. Table 1S (Supporting Information) lists the solvent systems and the wavelengths at which the individual compounds were studied by HPLC, as well as the correction factor used in the calculation of the observed rates.

Synthesis. Synthesis and characterization data for compounds **4a**, **4b**, **4d**, **4e**, **4g**, and **4h** are located in the Supporting Information.

2,4-Dichloro-*N*-(hydroxymethyl)benzamide (4c). 2,4-Dichlorobenzamide (**5c**) (mp 192−193 °C; lit.[72] mp 193−194 °C) was prepared from 2,4-dichlorobenzoic acid by using a general procedure outlined in Vogel[73] utilizing PCl_5 to synthesize the acid chloride and treating this with NH_4OH to yield the amide, which was then recrystallized from $CHCl_3$. The carbinolamide was prepared by using a published method,[74] and was purified by recrystallization from methanol to give a 25% yield. The product was a white crystalline powder that had a mp of 116−118 °C. ^{1}H NMR 400 MHz ($CDCl_3$) δ 7.71 (d, 1H), 7.46 (d, 1H), 7.35 (dd, 1H), 7.23−7.31 (br s, 1H, OH), 4.96 (s, 2H), 3.34 (t, 1H). ^{13}C NMR 100 MHz ($CDCl_3$) δ 166.9, 137.8, 132.3, 131.9, 131.8, 130.5, 127.9, 65.6.

General Synthetic Procedure. The remainder of the carbinolamides were synthesized by using the following general method. The substituted benzamide (2−4 g) was dissolved in ∼40 mL of methanol. To this solution was added 0.5 g of potassium carbonate and ∼10 g of paraformaldehyde. The mixture was then allowed to stir at room temperature for 24 h. After this time period, the progress of the reaction was determined by HPLC with use of an appropriate solvent system (see Table 1S, Supporting Information). Further additions of paraformaldehyde were performed if the reaction had not proceeded to a satisfactory degree (95−100%). Once the reaction was complete, the volume of the mixture was reduced to approximately half its original volume with a rotary evaporator, and the resulting solution was cooled to 4 °C. Upon cooling, a solid material that largely consisted of paraformaldehyde with a small amount of product precipitates out of solution. This solid was removed by vacuum filtration with the filtrate containing the desired product. Prior to final purification, the filtrate was further reduced in volume using a rotary evaporator and this solution was

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added directly to the chromatography column. The column used, in all cases, was composed of silica gel with methanol used as the eluant.

3,4-Dichloro-*N*-(hydroxymethyl)benzamide (4f). 4f was synthesized with use of 3,4-dichlorobenzamide (**5f**) (recrystallized from ethanol/water, mp 138−139 °C; lit.[75] mp 138−140 °C) from 3,4-dichlorobenzoic acid, which was produced in the same manner as described for **5c**. Mp 166−167 °C. ^{1}H NMR 400 MHz (DMSO) δ 9.30 (br t, 1H), 8.10 (d, 1H), 7.85 (dd, 1H), 7.78 (dd, 1H), 5.75 (t, 1H), 4.70 (t, 2H). ^{13}C NMR 100 MHz (DMSO) δ 164.0, 134.6, 134.2, 131.3, 130.7, 129.2, 127.5, 63.0.

Acknowledgment. This research was supported by an award from Research Corporation and by the National Science Foundation under Grant No. CHE-0518130.

Supporting Information Available: Synthesis and characterization data for compounds **4a**, **4b**, **4d**, **4e**, **4g**, and **4h**, a table with wavelengths and correction factors used to adjust peak areas in HPLC chromatograms for differences in molar extinction coefficients between starting material and products, plots for k_{obsd} vs [HO$^-$] for those compounds not shown in print, the Hammett Correlation for the apparent second-order rate constants (k'_1) for carbinolamide reaction and the Hammett Correlation for the K_a values of the hydroxyl group of the carbinolamide, and a plot of total phosphate concentration vs k_{obsd} for **4a** and the ^{1}H and ^{13}C NMR spectra for compounds **4c** and **4f**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO070603U