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Kinetic Dependence of the Aqueous Reaction of *N*-(Hydroxymethyl)benzamide Derivatives upon Addition of Electron-Withdrawing Groups

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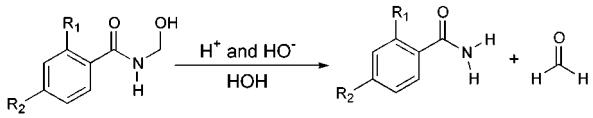
ABSTRACT



	R_1	R_2
1	H	H
2	H	Cl
3	Cl	Cl

The rate constants for the hydronium ion, hydroxide, and water-catalyzed breakdown of *N*-(hydroxymethyl)benzamide (1), 4-chloro-*N*-(hydroxymethyl)benzamide (2), and 2,4-dichloro-*N*-(hydroxymethyl)benzamide (3) in H_2O, at 25 °C, $I = 1.0$ (KCl), have been determined. The reactions of 1, 2, and 3 were found to be specific acid and specific base catalyzed with a first-order dependence on hydronium and hydroxide ions. At higher hydroxide concentrations, the reactions were found to be pH independent for each compound studied.

Carbinolamides have traditionally been used as sources of electrophilic carbon for aromatic substitution via acid-catalyzed breakdown producing amidinium ion intermediates.[1] More recently, carbinolamides have been found to be intermediates in the peptidylglycine α-amidating monooxygenase (EC 1.14.17.3) mediated transformation of glycine extended precursor peptides to the biologically active α-amidated peptide hormones and glyoxylic acid.[2] The carbinolamide functionality has also been proposed to play a critical role in the activity of bicyclomycin, a commercially used antibiotic.[3] Considering the important role this functionality plays in various biological venues, a thorough understanding of the mechanism of reaction and the factors affecting the carbinolamide reaction became an interesting problem. Herein, we describe our investigation of the acid and base-catalyzed reaction of *N*-(hydroxymethyl)benzamide (**1**), 4-chloro-*N*-(hydroxymethyl)benzamide (**2**), and 2,4-dichloro-*N*-(hydroxymethyl)benzamide (**3**) in H_2O, $I = 1.0$ (KCl), at 25 °C.

The carbinolamides studied were synthesized and purified using a known procedure.[1,4] The conversion of **1**, **2**, and **3** into their respective benzamides was followed by UV spectroscopy[5] and HPLC analyses.[6] No detectable catalysis of the reaction by buffers (≤0.05 M), used to maintain pH

(1) Zaugg, H. E.; Martin, W. B. *Org. React.* **1965**, *14*, 52−269.

(2) (a) Eipper, B. A.; Mains, R. E. *Annu. Rev. Physiol.* **1988**, *50*, 333−344. (b) Burgus, R.; Dunn, T. F.; Desiderio, D. M.; Ward, D. N.; Vale, W.; Guillemin, R.; Felix, A. M.; Gillessen, D.; Studer, R. O. *Endocrinology* **1970**, *86*, 573−582. (c) Rittel, W.; Maier, R.; Brugger, M.; Kamber, B.; Riniker, B.; Sieber, P. *Experientia* **1976**, *32*, 246−248. (d) Fournier, A.; Couture, R.; Magnan, J.; Gendreau, M.; Regoli, D.; St. Pierre, S. *Can. J. Biochem.* **1980**, *58*, 272−280. (e) Young, S. D.; Tamburini, P. P. *J. Am. Chem. Soc.* **1989**, *111*, 1933−34. (f) Francisco, W. A.; Merkler, D. J.; Blackburn, N. J.; Klinman, J. P. *Biochemistry* **1998**, *37*, 8244−8252. (g) Wilcox, B. J.; Ritenour-Rodgers, K. J.; Asser, A. S.; Baumgart, L. E.; Baumgart, M. A.; Boger, D. L.; DeBlassio, J. L.; deLong, M. A.; Glufke, U.; Henz, M. E.; King, L.; Merkler, K. A.; Patterson, J. E.; Robleski, J. J.; Vederas, J. C.; Merkler, D. J. *Biochemistry* **1999**, *38*, 3235−3245. (h) Kulathila, R.; Merkler, K. A.; Merkler, D. J. *Nat. Prod. Rep.* **1999**, *16*, 145−154. (i) Merkler, D. J.; Glufke, U.; Ritenour-Rodgers, K. J.; Baumgart, L. E.; DeBlassio, J. L.; Merkler, K. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1999**, *121*, 4904−4905.

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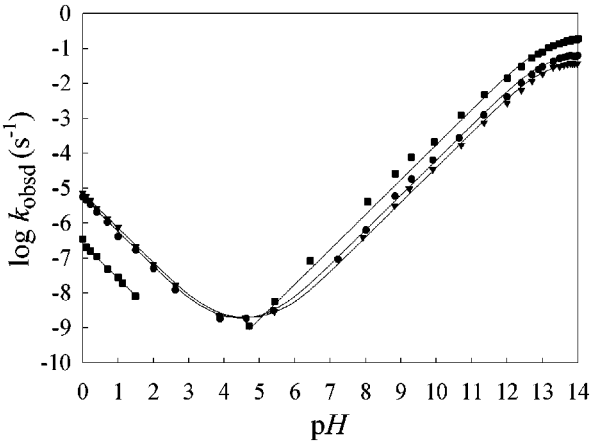


Figure 1. pH−rate profile for the aqueous reaction of *N*-(hydroxymethyl)benzamide (▼), 4-chloro-*N*-(hydroxymethyl)-benzamide (●), and 2,4-dichloro-*N*-(hydroxymethyl)benzamide (■) in H_2O, I = 1.0 (KCl), at 25 °C.

during the course of the reaction, was observed. No intermediates were detected by HPLC analysis, and the rate constants (k_{obsd}, s^{-1}) for the appearance of product and the disappearance of starting material (by HPLC) were found to be the same.

Shown in Figure 1 is the relationship between pH and the log k_{obsd} for the reaction of **1**, **2**, and **3** in H_2O, I = 1.0 (KCl), at 25 °C. Each carbinolamide studied has a domain that is first order in H_3O^+ and HO^-; however, the reaction becomes hydroxide independent at higher pH (more clearly observed in Figure 2). In addition, **1** and **2** have a pH-independent reaction occurring between pH 3 and 6.

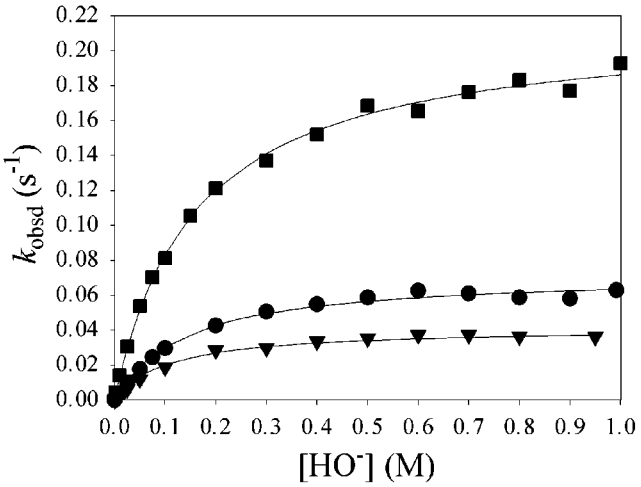
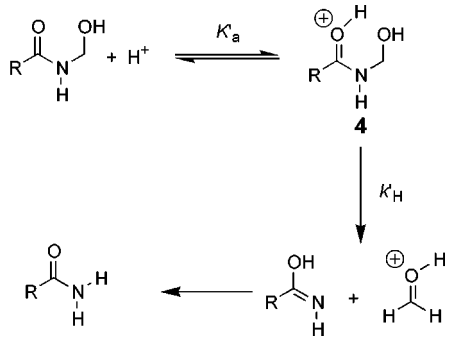


Figure 2. Effect of increasing hydroxide concentration (M) on the observed rates of reaction (k_{obsd}, s^{-1}) for *N*-(hydroxymethyl)-benzamide (▼), 4-chloro-*N*-(hydroxymethyl)-benzamide (●), and 2,4-dichloro-*N*-(hydroxymethyl)benzamide (■) in H_2O, I = 1.0 (KCl), at 25 °C.

Scheme 1



The mechanism for the acid-catalyzed breakdown of the carbinolamides studies here (shown in Scheme 1) involves a preequilibrium protonation of the carbonyl oxygen (**4**), followed by rate-limiting cleavage of the carbon−nitrogen bond to form protonated formaldehyde and the tautomeric form of the product amide. This proposed mechanism is supported by the observed first-order dependence on [H_3O^+] and the lack of buffer catalysis ([buffer] ≤ 0.05 M).

In principle, protonation in the acid-catalyzed reaction could occur on either the carbonyl oxygen or the nitrogen. It is generally accepted in acid-catalyzed amide hydrolysis studies that the thermodynamically favored position of protonation is the carbonyl oxygen (pK_a of O-protonated amide 0 to −3;[7] of N-protonated amide −7 to −8[8]). The addition of a methylene unit bearing a hydroxyl group should

(3) (a) Miyoshi, T.; Miyari, N.; Aoki, H.; Kohsaka, M.; Sakai, H.; Imanaka, H. *J. Antibiot.* **1972**, *25*, 569−575. (b) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Takana, H.; Take, T.; Uchiyama, T.; Ochiai, H.; Abe, K.; Koizumi, K.; Asao, K.; Matuski, K.; Hoshino, T. *J. Antibiot.* **1972**, *25*, 610−612. (c) Williams, R. M.; Durham, C. A. *Chem. Rev.* **1988**, *88*, 511−540. (d) Park, H.-g.; Vela, M.; Kohn, H. *J. Am. Chem. Soc.* **1994**, *116*, 471−478. (e) Vela, M.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 5223−5231. (f) Williams, R. M.; Tomizawa, K.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1987**, *109*, 4028−4035. (g) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1988**, *110*, 4089−4090. (h) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1990**, *112*, 3114−3121. (i) Santillan, A.; Zhang, X. D.; Widger, R.; Kohn, H. *J. Org. Chem.* **1998**, *63*, 1290−1298. (j) Santillan, A.; Park, H.; Zhang, X. D.; Lee, O. S.; Widger, W. R.; Kohn, H. *J. Org. Chem.* **1996**, *61*, 7756−7763. (k) Park, H.; Zhang, X. D.; Widger, W. R.; Kohn, H. *J. Org. Chem.* **1996**, *61*, 7750−7755. (l) Park, H.; Zhang, Z. M.; Zhang, X. D.; Widger, W. R.; Kohn, H. *J. Org. Chem.* **1996**, *61*, 7764−7776. (m) Tanaka, H. *Antibiotics (N.Y.)* **1979**, *5*, 18−25. (n) Sorel, R. H. A.; Roseboom, H. *Int. J. Pharm.* **1979**, *3*, 93−99. (o) Cho, H. J.; Park, H. G.; Zhang, X. D.; Riba, I.; Gaskell, S. J.; Widger, W. R.; Kohn, H. *J. Org. Chem.* **1997**, *62*, 5432−5440.

(4) Nair, B. R.; Francis, J. D. *J. Chromatogr.* **1980**, *195*, 158−161.

(5) The reactions of **1**, **2**, and **3** were monitored spectrophometrically by following the decrease in absorbance at 227, 238, and 230 nm, respectively, for the disappearance of starting material.

(6) Slower reactions were followed by HPLC until the reaction was complete or for the first 4% reaction. The disappearance of **1**, **2**, and **3** and the appearance of the respective amide products were monitored at 238 nm with a correction factor used to adjust peak areas for differences in their molar extinction coefficients.

(7) (a) Arnett, E. M. *Quantitative Comparisons of Weak Organic Bases*; Cohen, S. G., Streitwieser, A., Jr., Taft, R. W., Eds.; Interscience Publishers: New York, 1963; Vol. 1, pp 223−403. (b) Caplow, M.; Jencks, W. P. *Biochemistry* **1962**, *1*, 883−893. (c) Guthrie, J. P. *J. Am. Chem. Soc.* **1974**, *96*, 3608−3615. (d) Modro, T. A.; Yates, K.; Beaufays, F. *Can. J. Chem.* **1977**, *55*, 3050−3057. (e) Yates, K.; Riordan, J. C. *Can. J. Chem.* **1965**, *43*, 2328−2335. (f) Yates, K.; Stevens, J. B. *Can. J. Chem.* **1965**, *43*, 529−537.

(8) (a) Williams, A. *J. Am. Chem. Soc.* **1976**, *98*, 5645−5651. (b) Fersht, A. R. *J. Am. Chem. Soc.* **1971**, *93*, 3504−3515.

Table 1. Dissociation and Rate Constants for the Hydronium Ion, Hydroxide, and Water-Catalyzed Breakdown of **1**, **2**, and **3** in H_2O ($T = 25$ °C, $I = 1.0$ (KCl))

	k_H[a] ($M^{-1}s^{-1}$)	k_{HOH} (s^{-1})	k_1[b] (s^{-1})	k_{rel}[c]	pK_a[b]
1	$(7.3 \pm 0.2) \times 10^{-6}$	$(1.7 \pm 0.1) \times 10^{-9}$	0.042 ± 0.003	1	13.05 ± 0.05
2	$(5.7 \pm 0.2) \times 10^{-6}$	$(1.6 \pm 0.1) \times 10^{-9}$	0.068 ± 0.005	1.6	13.04 ± 0.05
3	$(2.6 \pm 0.13) \times 10^{-7}$		0.19 ± 0.01	4.5	13.04 ± 0.05

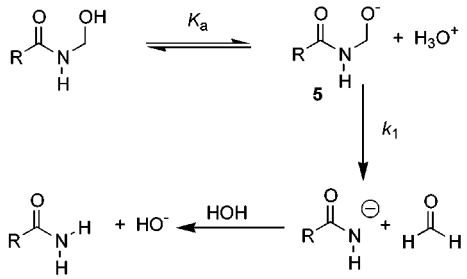
[a] Errors obtained from linear least-squares analysis of the plot of k_{obsd} vs $[H_3O^+]$. [b] Errors obtained from nonlinear least-squares fits of k_{obsd} vs $[HO^-]$ according to eq 1. [c]Calculated by dividing k_1 for **1** into the k_1 value for each amide studied.

lower the pK_a of the lone pair electrons on the nitrogen further, thereby increasing the thermodynamic preference for protonation on the carbonyl oxygen and leading to the mechanism proposed in Scheme 1.

The proposed mechanism (Scheme 1) is further supported by Perrin's studies of the acid-catalyzed proton exchange on nitrogen for amides,[9] wherein it was shown that proton exchange occurred by O-protonation when electron-withdrawing groups were attached to the carbonyl carbon or the nitrogen. In the case of the carbinolamides studied here, two of the compounds (**2**, **3**) have electron-withdrawing groups attached to the amide portion of the molecule and all have the hydroxy group attached to the methylene unit on the nitrogen, as was discussed above. The mechanism in Scheme 1 was proposed on the basis of these arguments; however the results presented here cannot distinguish between the two mechanisms (O-protonation vs N-protonation) and further study of the acid-catalyzed reaction is necessary.

Figure 2 shows the relationship between k_{obsd} and the $[HO^-]$ for the conversion of **1**, **2**, and **3** into their respective benzamides and formaldehyde in H_2O, $I = 1.0$ (KCl), at 25 °C. At lower and intermediate $[HO^-]$, there is a first-order dependence between the k_{obsd} and the concentration of hydroxide (Figures 1 and 2). At higher hydroxide concentrations, k_{obsd} becomes independent of $[HO^-]$. Coupling these observations with the absence of buffer catalysis yields a mechanism wherein specific base-catalyzed deprotonation of the hydroxyl group is followed by rate-determining breakdown of the anionic form of the carbinolamide (**5**, Scheme 2). As the carbinolamide becomes fully ionized (**5**), at high

Scheme 2



$[HO^-]$, the overall reaction becomes hydroxide independent. These results are in agreement with other studies involving the hydroxide-catalyzed reaction of carbinolamides.[10]

Using the mechanism outlined in Scheme 2, the observed rate for the hydroxide reaction ($(k_{obsd})^{HO}$) can be defined as in eq 1. The solid line in Figure 2 shows the nonlinear least-squares fit[11] of the our results for the hydroxide-catalyzed reactions of **1**, **2**, and **3** to eq 1, where K_a is the dissociation constant of the hydroxyl groups of **1**, **2,** and **3** and k_1 is the first-order rate constant for the breakdown of the deprotonated carbinolamide (**5**, Scheme 2).

$$(k_{obsd})^{HO} = k_1 \frac{K_a[HO^-]}{K_w + K_a[HO^-]} \qquad (1)$$

The lines in Figure 1 were generated using eq 2, where k_H is the rate constant for the acid-catalyzed reaction,[12] k_{HOH} is the rate for the water-catalyzed reaction, and the hydroxide relationship has been explained for eq 1.

$$\log k_{obsd} = \log\left(k_H[H^+] + k_{HOH} + k_1 \frac{K_a[HO^-]}{K_w + K_a[HO^-]}\right) \qquad (2)$$

Given in Table 1 are the rate constants for the hydroxide-catalyzed reactions of **1**, **2**, and **3** in H_2O, $I = 1.0$ (KCl), at 25 °C. It is clear, from the data in Table 1, that the addition of electron-withdrawing groups to the aromatic ring has very little effect on the pK_a of the hydroxyl group but there is an increase in the rate-determining breakdown of **5** (k_1) (see k_{rel} in Table 1). The increases in k_1 are attributable to the decreased basicity of the amide anion which is a result of the addition of the electron-withdrawing groups. These observations support the mechanism shown in Scheme 2 where the addition of electron-withdrawing groups should inductively stabilize the developing negative charge on the nitrogen in the transition state and lead to an increase in k_1.

(9) (a) Perrin, C. L.; Arrhenius, G. M. L. *J. Am. Chem. Soc.* **1982**, *104*, 6693−6696. (b) Perrin, C. L.; Lollo, C. P. *J. Am. Chem. Soc.* **1984**, *106* 2754−2757. (c) Perrin C. L. *Acc. Chem. Res.* **1989**, *22*, 268−275. (d) Perrin, C. L.; Chen, J.-H.; Ohta, B. K. *J. Am. Chem. Soc.* **1999**, *121*, 2448−2455.

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(11) The values of k_1 and K_a were determined by fitting the data in Figure 2 to eq 1 using the nonlinear least-squares curve fitting program associated with SigmaPlot 2000 from SPSS Science.

(12) Obtained from linear plots of k_{obsd} vs $[H_3O^+]$.

Also included in Table 1 are the second-order rate constants for the acid-catalyzed reaction of the compounds studied here. The effect of the electron-withdrawing groups on k_{obsd} is the opposite of what is observed for k_1 of the hydroxide-catalyzed reaction. That is, the overall rate of the reaction decreases with the addition of electron-withdrawing substituents. The exact nature of the effect of the substituents is difficult to quantify as k_H (eq 2) consists of both a rate constant for the breakdown of the protonated form of the carbinolamide (**4**, k'_H) and the dissociation constant for the protonated form of the carbinolamide (K'_a) (see Scheme 1 and eq 3).

$$(k_{obsd})^H = \frac{k'_H[H^+]}{K'_a} = k_H[H^+] \qquad (3)$$

In principle, the effect of the electron-withdrawing groups on k'_H should be smaller than those observed for the hydroxide reaction (k_1) because, in the case of the hydroxide reaction, it is necessary to stabilize the developing negative on the amide leaving group, whereas in the acid-catalyzed reaction the leaving group is neutral. The role that the substituents play in affecting the pK'_a would then be expected to dominate, leading to an overall decrease in the concentration of **4** and, thus, a decrease in the second-order rate constant of the hydronium ion catalyzed reaction with the addition of the electron-withdrawing substituents to the aromatic ring (see Table 1).

The results presented here represent the first complete pH−rate study for carbinolamides derived from formaldehyde and benzamide derivatives. As with previous studies,[10] we found that, at intermediate pH, the reaction was specific base catalyzed. In addition, we determined the rate constants for the hydroxide-independent reactions, where the hydroxyl group of the carbinolamide is fully deprotonated, and found that the addition of electron-withdrawing groups to the amide increases the nucleofugality of the amidic group, as evidenced by the increases in k_1 (k_{rel} in Table 1). The hydronium ion catalyzed reactions were also investigated, and it was determined that this reaction occurred by a preequilibrium protonation of the carbinolamide followed by rate-limiting breakdown to the amide and protonated aldehyde. The addition of electron-withdrawing groups to the amide portion of the carbinolamide slowed the acid-catalyzed reaction presumably through changes in the K'_a of the O-protonated carbinolamide (**4**).

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