Tetrahedron Letters 49 (2008) 6547–6549

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Rate of formation of *N*-(hydroxymethyl)benzamide derivatives in water as a function of pH and their equilibrium constants

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ARTICLE INFO

Article history:
Received 13 August 2008
Accepted 2 September 2008
Available online 6 September 2008

ABSTRACT

The third-order rate constants for the pH-dependent formation of the carbinolamides generated from the reaction of formaldehyde and benzamide, 4-chloro, 4-nitro, 4-methyl and 4-methoxybenzamide, are reported. The acid-catalyzed reaction was found to occur via rate-limiting proton transfer, whereas the hydroxide-dependent reaction occurred via a specific-base process. Coupling the rate constants for carbinolamide formation reported herein with the previously established rates for carbinolamide breakdown yielded equilibrium constants for the carbinolamides studied in water.

Carbinolamides have been found to have both positive[1,2] and negative[3] roles as intermediates in a number of biological venues. The functionality is a constituent in a number of molecules of medicinal importance;[4,5] however, its role in those molecules is poorly defined. Studies focused on the reactivity and mechanisms by which this functionality reacts in water have shown that these compounds are remarkably stable at biological pH, and exhibit a broad pH-dependent reactivity.[6,7] At the same time, carbinolamide formation and their equilibrium positions in aqueous solution have been almost completely ignored.[8] Such data may help to define the role of carbinolamides as intermediates in the reaction of exogenous and endogenous aldehydes with DNA which has been shown to lead to a modification that correlates to a higher incidence of certain types of cancers.[3] In addition, the equilibrium position for carbinolamides in solution will be important in clarifying their function in molecules such as bicyclomycin,[4] a commercially available antibiotic, and others.[5]

Reported here is the kinetic investigation of the rate of formation of *N*-(hydroxymethyl)benzamide compounds as a function of pH for the reaction of formaldehyde with benzamide (**1**), 4-chlorobenzamide (**2**), 4-nitrobenzamide (**3**), 4-methylbenzamide (**4**), and 4-methoxybenzamide (**5**) in water, I = 1.0 M (KCl) at 25 °C. The formation of each carbinolamide was followed by UV–Vis and/or HPLC using techniques detailed in the Supplementary data. Pseudo-first order kinetics were observed in all cases, and no intermediates or products other than the *N*-(hydroxymethyl)benzamide compounds were observed by HPLC.

Stock formaldehyde solutions were generated, and the $[CH_2O]^{tot}$ was determined using a formalized method.[9] The $[CH_2O]^{tot}$ in all of the kinetic solutions was 1.21 M. The large concentrations of formaldehyde were required due to the high degree of hydration of the carbonyl group (~98%),[10] and therefore, the relatively low $[CH_2O]$ in the reactive keto-form. Due to this large $[CH_2O]^{tot}$, the range in the pH that could be studied was diminished due to its hydrate buffering the solution (pK_a^{hyd} = 13.3).[10a]

The pH-rate profile (see graphical abstract) illustrates the relationship between the pH of the reaction and k_{obsd} for the reaction of **1**–**5** with formaldehyde to form the carbinolamide. Similar to that observed for the reaction of the carbinolamides,[7b] the pH-rate profile shows domains that are first-order in $[H_3O^+]$ and $[HO^-]$. A water-dependent reaction must also be postulated between pH values 3–6, so that a proper fit line can be generated.

The effect of changes in the $[HO^-]$ on the hydroxide-dependent reaction is shown in Figure 1. No evidence for the presence of buffer catalysis in the hydroxide-dependent reaction was found for the benzamides studied. For example, at pH 8.5, with

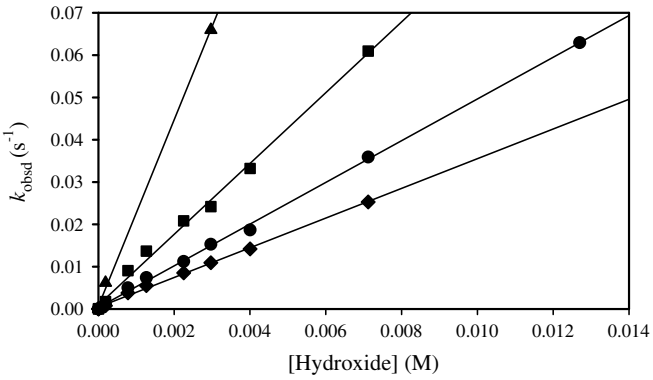

Figure 1. Effect of [hydroxide] (M) on the observed rate of carbinolamide formation (k_{obsd} (s^{-1})) for 4-methylbenzamide (♦), benzamide (●), 4-chlorobenzamide (■), and 4-nitrobenzamide (▲) in H_2O, I = 1.0 M (KCl), at 25 °C.

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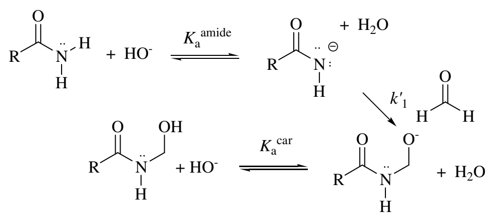
doi:10.1016/j.tetlet.2008.09.009



Scheme 1. Mechanism for hydroxide-dependent carbinolamide formation.

[pyrophosphate] (total buffer) of 0.15, 0.12, and 0.10, the k_{obsd} values for the reaction of **3** were $1.1 \times 10^{-4}\ s^{-1}$, $1.4 \times 10^{-4}\ s^{-1}$, and $1.4 \times 10^{-4}\ s^{-1}$, respectively. No evidence for buffer catalysis was found with the other buffers used to maintain pH in the hydroxide-dependent reaction, leading to the conclusion that proton transfer was not occurring in the rate-limiting step of the reaction.

Thus, hydroxide-dependent carbinolamide formation occurred via a specific-base catalyzed mechanism (see Scheme 1). The proposed mechanism involves deprotonation of the benzamide to generate a benzamidate intermediate. The benzamidate then undergoes rate-limiting nucleophilic attack on the keto-form of formaldehyde to generate the conjugate base of the carbinolamide, which was then protonated to form the product. Such a route is in accord with the currently accepted mechanism for the hydroxide-dependent breakdown of carbinolamides in aqueous solution.[6,7b,c]

It can be seen from Figure 1 that as electron-withdrawing groups are added to the aromatic ring of benzamide, the relative rate of carbinolamide formation increases. For the mechanism shown in Scheme 1, the addition of an electron-withdrawing group would result in an increase in the acidity of the amidic hydrogen. Available data indicate that 4-bromobenzamide and 4-nitrobenzamide have pK_a values of 17.13 and 15.85, respectively.[11] (The assumption that 4-chloro vs 4-bromo does not lead to significant differences in acidity is supported by both benzoic acid and phenol acidities.[12]) The increase in acidity of the amides, with the addition of electron-withdrawing groups, would lead to a larger [benzamidate] at a specific pH and to an increase in the rate of reaction.

The effect of the substituent group on the nucleophilicity of benzamidate was not as clear, as it is well-known that nucleophilicity and pK_a do not necessarily correlate in protic solvents.[13] Assuming that electron-withdrawing groups decrease the nucleophilicity of the benzamidate derivatives, this effect must be less pronounced than the substituents effect on the pK_a based upon changes in observed rates.

$$(k_{obsd})^{HO} = \frac{k'_1 K_a^{amide}[HO^-]}{K_w}\left[\mathrm{H_2C{=}O}\right] = k'_{HO}[HO^-] \qquad (1)$$

The rate expression for the hydroxide-dependent reaction is shown in Eq. 1, and this equation illustrates how the rate of the reaction was coupled to both the K_a^{amide} and the rate of benzamidate attack (k'_1) on the keto-form of the formaldehyde. The pseudo second-order rate constants (k'_{HO}) for the hydroxide-dependent reaction are shown in Table 1. As illustrated in Figure 1, the addition of electron-withdrawing groups leads to an increase in rate of the hydroxide-dependent reaction. The Hammett correlation of the pseudo second-order hydroxide reaction yields a ρ-value of 0.79 (see plot in Supplementary data).

Shown in Figure 2 is the relationship between $[H_3O^+]$ and k_{obsd} for the formation of the carbinolamide products. In contrary to the hydroxide-dependent data, the acid-catalyzed reaction does exhibit buffer catalysis (See Supplementary data). The y-intercepts of the plots of [buffer] versus k_{obsd} yielded the rates of the buffer independent reactions. Buffer catalysis implied that proton transfer occurs during the rate-limiting step of the acid-catalyzed reaction. Such a result was in agreement with current data concerning the acid-catalyzed breakdown of carbinolamides in water.[7a]

Two mechanisms could be postulated having rate determining proton transfer in the acid-catalyzed reaction. Scheme 2 shows a general acid-catalyzed mechanism where protonation of formaldehyde is concurrent with attack by the amide nitrogen on the carbonyl carbon of formaldehyde. This is followed by the loss of a proton to generate the carbinolamide product. Alternatively, a mechanism (see Scheme 3) could be postulated involving specific acid protonation of formaldehyde followed by general base catalyzed deprotonation of the amidic nitrogen. Both mechanisms lead to the same general equation shown in Eq. 2, where K_a^{HA} and K_a^{form}

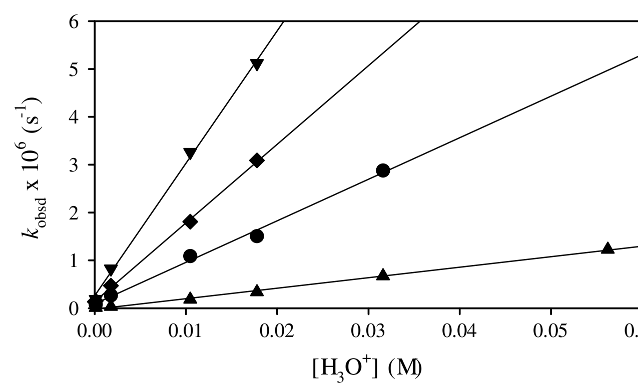
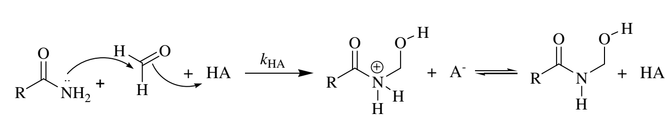


Figure 2. Effect of [hydronium ion] (M) on the rate of carbinolamide formation (k_{obsd} (s^{-1})) for 4-methoxybenzamide (▼), 4-methylbenzamide (◆), benzamide (●), and 4-nitrobenzamide (▲) in H_2O, I = 1.0 M (KCl), at 25 °C.



Scheme 2. General acid-catalyzed mechanism.

Table 1

Pseudo second-order and third-order rate constants for the acid-catalyzed and hydroxide-dependent formation of the *N*-(hydroxymethyl)benzamide derivatives in H_2O, with 1.21 M formaldehyde at 25 °C, I = 1.0 M (KCl) and the equilibrium constants for the *N*-(hydroxymethyl)benzamide derivatives under the same conditions

Amide	k'_H ($M^{-1}\ s^{-1}$)	k_H ($M^{-2}\ s^{-1}$)[a]	K_{eq}^{H} (M^{-1})	k'_{HO} ($M^{-1}\ s^{-1}$)	k_{HO} ($M^{-2}\ s^{-1}$)[b]	K_{eq}^{HO} (M^{-1})
1	$(8.7 \pm 0.2) \times 10^{-5}$	0.17	$(2.4 \pm 0.2) \times 10^{4}$	4.9 ± 0.1	9.9×10^{3}	$(2.7 \pm 0.2) \times 10^{4}$
2	$(8.3 \pm 0.2) \times 10^{-5}$	0.17	$(2.9 \pm 0.2) \times 10^{4}$	8.4 ± 0.2	1.7×10^{4}	$(2.7 \pm 0.2) \times 10^{4}$
3	$(2.2 \pm 0.1) \times 10^{-5}$	0.044	—[c]	22.0 ± 0.2	4.4×10^{4}	$(3.5 \pm 0.3) \times 10^{4}$
4	$(1.6 \pm 0.1) \times 10^{-4}$	0.32	—[c]	3.5 ± 0.1	7.0×10^{3}	$(4.5 \pm 0.2) \times 10^{4}$[d]
5	$(2.8 \pm 0.1) \times 10^{-4}$	0.56	$(3.7 \pm 0.2) \times 10^{4}$[e]	3.6 ± 0.1	7.2×10^{3}	$(4.6 \pm 0.2) \times 10^{4}$[d]

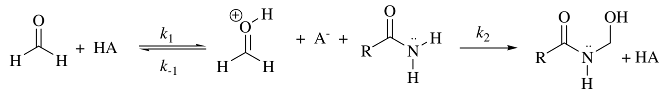
[a] Calculated using $k_H = ((k'_H(1 + K_H))/[CH_2O]^{tot})$ where $[CH_2O]^{tot}$ was 1.21 M.

[b] Calculated using $k_{HO} = ((k'_{HO}(1 + K_H))/[CH_2O]^{tot})$ where $[CH_2O]^{tot}$ was 1.21 M.

[c] No acid-catalyzed rates for carbinolamide breakdown were available.

[d] Apparent second-order rate constant for hydroxide-dependent carbinolamide breakdown obtained from Ref. [16].

[e] Second-order rate constant for acid-catalyzed carbinolamide breakdown obtained from Ref. [16].



Scheme 3. Specific acid followed by general base.

are the acidity constants for the acid catalyst and protonated formaldehyde, respectively. As a result, no definitive conclusions concerning the mechanism of the acid-catalyzed reaction can be made based upon available data. The pseudo second-order rate constants for the acid-catalyzed reaction (k'_H) are listed in Table 1. These rates show that as electron-withdrawing groups are added to the amide, the rate of the acid-catalyzed reaction slows. The Hammett plot of the pseudo second-order rate constants for the acid-catalyzed reaction leads to a ρ-value of -0.96 (see Supplementary data).

$$(k_{obsd})^H = \frac{k_2 K_a^{HA} [HA]}{K_a^{form}} [H_2C{=}O] = k_{HA} [HA] [H_2C{=}O] \quad (2)$$

As stated above, the $[CH_2O]^{total}$ in the reaction solutions was 1.21 M. The high $[CH_2O]$ relative to substrate was required to ensure sufficient quantity of formaldehyde in its reactive keto-form ($[CH_2O]^{keto}$). The $[CH_2O]^{keto}$ can be calculated using Eq. 3 where $K_H = 2420$ is the equilibrium constant for formaldehyde hydration (K_H = [hydrate]/[free aldehyde])[14] and $[CH_2O]^{total}$ was 1.21 M. Between pH 0 and 12, $[CH_2O]^{keto}$ was approximately 5×10^{-4} M, which was ~50 times the concentration of the benzamide derivatives in solution upon the initiation of the reaction. The third-order rate constants were then calculated by dividing the pseudo second-order rates by the $[CH_2O]^{keto}$. Table 1 lists the third-order rate constants for both the hydroxide-dependent and the acid-catalyzed reactions.

$$[CH_2O]^{keto} = (1 + K_H)/[CH_2O]^{tot} \quad (3)$$

Dividing the rates of formation of the *N*-(hydroxymethyl)benzamide derivatives by the rates of their breakdown, under similar conditions,[7b,c] yield the equilibrium constants shown in Table 1. Reasonable correlation between the K_{eq} calculated using the acid-catalyzed rates and those determined from the hydroxide-dependent rates was found. The results show that the presence of substituents on the aromatic ring of the amide does not lead to any significant trends in the equilibrium position for carbinolamide in aqueous solution.

These results were in agreement with previous studies investigating the equilibrium position of the reaction of amide derivatives with formaldehyde. Crowe and co-workers reported a similar equilibrium constant of $K_{eq} = \sim 5 \times 10^4$ M^{-1} for both acetamide's and benzamide's reaction with formaldehyde.[8a] Jencks and co-workers determined the equilibrium constants for the addition of a number of nucleophiles to formaldehyde and found $K_{eq} = 5.8 \times 10^4$ M^{-1} for urea.[15] The results reported here do not show any apparent 'amide' dependent trend in equilibrium position for the reaction of amides with formaldehyde to form carbinolamides, and are similar in magnitude to the previously reported values. This lack of sensitivity in K_{eq} to the nature of the amide in these experiments may be due to subtle effects being masked by error in the measurement of the rate constants.

Presented here is the first investigation of the rate constants for the formation of carbinolamides for a series of benzamides and formaldehyde as a function of pH. A general acid-catalyzed reaction operates in the acidic region of the pH-rate profile and a specific-base mechanism in the hydroxide-dependent region. The proposed reaction mechanisms were in accord with the currently accepted mechanisms for carbinolamide breakdown in aqueous solution.[6,7] Coupling these rates with previously reported rates of carbinolamide breakdown[7b,c] yielded K_{eq} values for carbinolamides in solution. The equilibrium constants clearly indicated that amide addition to formaldehyde was favored although the nature of the amide does not appear to have a significant effect on the energetics of the equilibrium. Although other aldehydes were not investigated, the electrophilicity of the aldehyde must play a role in the overall stability of the carbinolamide generated as seen in the hydration of the aldehydes.[14] While this must remain speculative, clearly the reaction of formaldehyde with benzamide derivatives generated stable adducts. Thus, the speculative role of the carbinolamide in bicyclomycin[4c] was supported by these results. In addition, the carbinolamide intermediates generated by the reaction of DNA with endogenous and exogenous aldehydes[3] would be expected to produce similarly stable products with lifetimes long enough that further modification could occur.

Acknowledgment

This work was supported by an award from the National Science Foundation under Grant No. CHE-0518130.

Supplementary data

Solution preparation for kinetic experiments, Table with wavelengths and correction factors used for various amides, Hammett plots for both the acid and hydroxide-dependent reactions, and a plot of buffer catalysis. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.09.009.

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