

Kinetics and mechanism of oxime formation from pyruvic acid

A. Malpica, M. Calzadilla and T. Cordova

Escuela de Quimica, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela 47102

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ABSTRACT: The following lines of evidence establish that oxime formation from pyruvic acid occurs with rate-determining carbinolamine dehydration under acidic and neutral conditions. First, saturation effects observed at pH 7 are strongly suggestive of carbinolamine accumulation, requiring that dehydration of the intermediate be the rate-determining step. Second, the reaction occurs exclusively with general acid catalysis in the entire range investigated. The pH–rate plot shows a break at pH near 2, which can be interpreted in terms of protolytic equilibrium of the substrate. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: Pyruvic acid; oxime; kinetics; mechanism

INTRODUCTION

Investigations on the kinetics and mechanism of imine formation from positively charged aldehydes have demonstrated that the acid-catalyzed dehydration of the carbinolamines is the rate-determining step in the entire range of pH \sim 0–7.^{1–3} The justification for this behavior was basically attributed to two factors: (i) very activated substrates to addition and (ii) the difficulty of the acid-catalyzed dehydration of the carbinolamines due to an unfavorable electrostatic situation. Depending on the pH, pyruvic acid exists as an acid, an anion or a mixture of both species, and there are different affinities to water between the acid and its anion. The relation of the equilibrium constants for hydration, defined as hydrate/ketone, is $K_{\text{acid}}/K_{\text{anion}} = 21$.⁴ This figure in some respects reflects the influence of the carboxyl group of pyruvic acid in comparison with the carboxylate group of pyruvate on the electrophilicity of the carbonyl carbon. Therefore, attack of the nucleophile on the acid must be easy in comparison with the same addition to anionic pyruvate, but the acid-catalyzed dehydration of the carbinolamine derived from the anion must be facilitated by the negative charge on this species. Based only on the foregoing consideration, in the pH range in which pyruvic acid exists as an anion, the rate-limiting step should be the addition of the nucleophile to form the intermediate, but the lack of enough protons in the medium and the nucleophilicity of the attacking amine

are other important factors to be considered. When the basicity of the amine is sufficiently increased, the dehydration of the carbinolamine intermediate is the rate-determining step, and the change to the rate-determining step of carbinolamine formation occurs at lower pH values.⁵

Oxime formation from pyruvate anion has been studied previously and values of the equilibrium constants for addition and rate constants for addition and dehydration of its carbinolamine have been reported.^{6,7} However, the study of this type of reaction over a range of pH values was initiated with semicarbazone formation from pyruvic acid.⁸ The results of this investigation revealed that the second-order rate constants show a complex dependence on the concentration of hydronium ions, a fact that was interpreted as the result of two factors: protolytic equilibrium of the substrate and changes in the rate-determining step from different additions of the nucleophile to the carbonyl carbon at low pH values to dehydration of the carbinolamine at higher pH values. In order to establish the influence that a stronger nucleophile exerts, in this work we studied oxime formation from pyruvic acid and its anion.

EXPERIMENTAL

Materials. Sodium pyruvate and hydroxylamine hydrochloride were obtained commercially. Solutions of these reagents were prepared just prior to use to minimize the possibility of decomposition. Buffer solutions from HCl–KCl, acetic acid and potassium phosphate–hydrogen phosphate were employed according to the pH investigated. Glass-distilled water was used throughout.

*Correspondence to: A. Malpica, Escuela de Quimica, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela 47102.

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Table 1. First-order rate constants for pyruvic acid oxime formation in aqueous solution at 30 °C and ionic strength 0.5

pH	[Amine] _{fb} (M) ^a	<i>k</i> _{obs} (min ⁻¹) ^b
0.25	8.39 × 10 ⁻⁸	0.79
0.49	2.20 × 10 ⁻⁸	0.13
0.80	8.73 × 10 ⁻⁷	2.40
1.06	8.15 × 10 ⁻⁸	0.21
1.54	2.54 × 10 ⁻⁷	0.35
1.95	2.30 × 10 ⁻⁶	2.17
2.30	2.03 × 10 ⁻⁶	1.09
2.74	3.93 × 10 ⁻⁶	1.26
3.06	6.89 × 10 ⁻⁶	1.19
3.56	2.44 × 10 ⁻⁵	1.57
3.93	6.69 × 10 ⁻⁵	1.85
4.60	2.43 × 10 ⁻⁴	1.60
5.04	8.01 × 10 ⁻⁴	1.66
5.44	4.42 × 10 ⁻⁴	0.41
5.50	2.05 × 10 ⁻³	1.32
6.12	3.54 × 10 ⁻³	0.64
6.61	1.08 × 10 ⁻²	0.56
7.10	1.50 × 10 ⁻¹	0.34

^a Concentration of hydroxylamine free base.^b All rate constants have been corrected to zero buffer concentration.

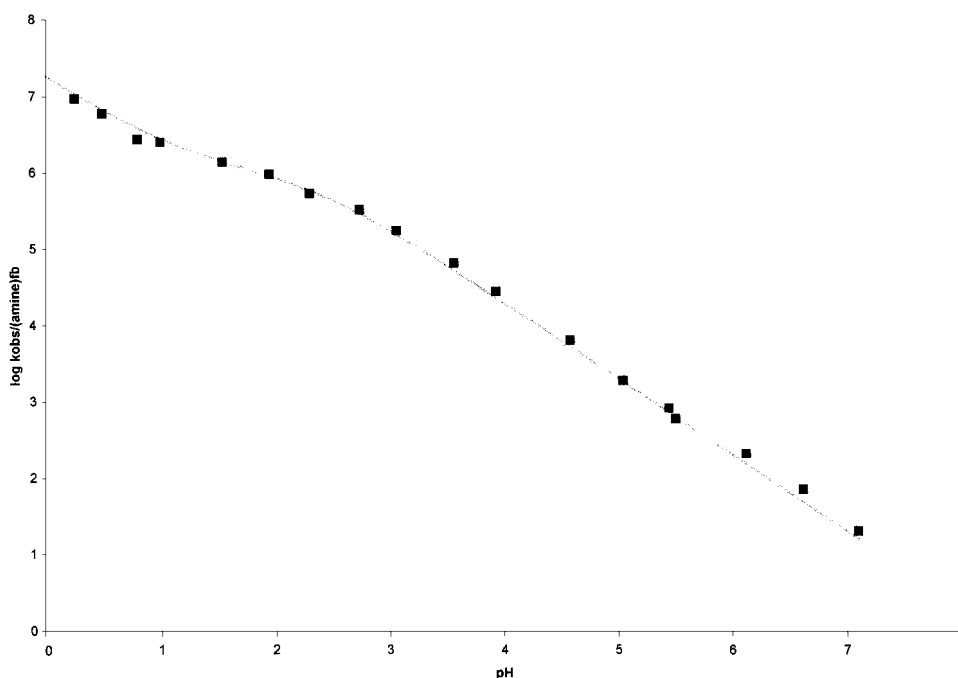
Kinetic measurements. Kinetic measurements were carried out spectrophotometrically with a Zeiss PMQ II spectrophotometer equipped with a jacketed cell holder through which water from a constant-temperature bath was circulated. The temperature was maintained at 30 °C throughout. The ionic strength was maintained at 0.5 throughout by the use of potassium chloride. The extent of the reaction was monitored by the increase in absorbance at 230 nm which accompanies oxime forma-

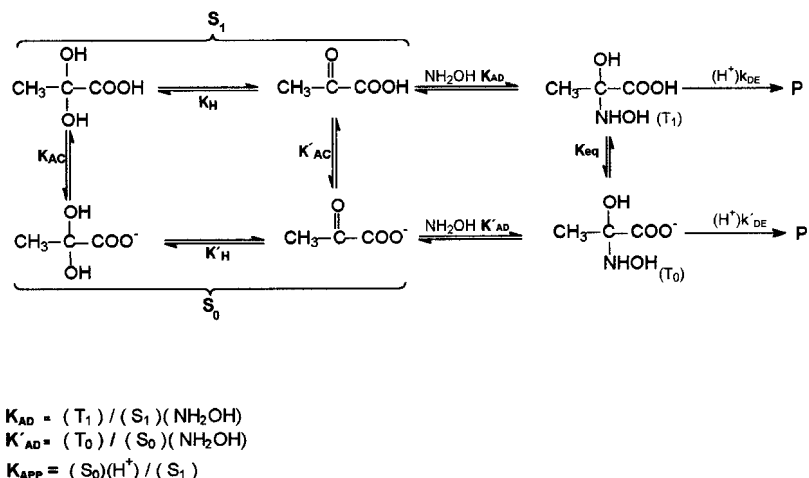
tion. First-order rate constants, *k*_{obs}, based on the total (hydrate + unhydrate) ketone, were obtained from plots of the difference between the absorbance at infinite time and at various times against time. Second-order rate constants, the relation between *k*_{obs} and the concentration of amine free base, were obtained from plots of first-order rate constants against hydroxylamine concentration and corrections for general acid catalysis by buffers were made by extrapolation to zero of plots of *k*_{obs}/[amine]_{fb} against buffer concentration.

Equilibrium constant measurements for carbinolamine formation were made spectrophotometrically at 30 °C and μ = 0.5 (KCl), at 240 nm, pH 9.11, in 0.125 M borate buffer and averages of 20 determinations were taken. The equilibrium constant was obtained from the negative intercept of a plot of 1/Δ*A*_{eq}^o vs 1/[amine]_{fb}. A value of 58.0 ± 0.2 l mol⁻¹ was obtained (literature values 57 l mol⁻¹ at 25 °C, pH 6.8 and μ = 0.3⁶ and 54.3 at 30 °C, pH 7.5 and μ = 1.3⁷). The apparent p*K*_a value of pyruvic acid was computer calculated from a non-linear least-squares procedure as the best fit to the experimental kinetic data, p*K*_a = 2.22 (literature value 2.5 at 25 °C and μ = 0⁹).

RESULTS AND DISCUSSION

First-order rate constants (*k*_{obs}) for pyruvic acid oxime formation were determined as a function of amine concentration over the pH range 0.25–7.00 at 30 °C in aqueous solution and ionic strength 0.5 (Table 1). In Fig.

**Figure 1.** Logarithms of second-order rate constants for pyruvic acid oxime formation plotted as a function of pH. □, Experimental points. The solid line was calculated based on the rate law in Eqn. (1) and the constants in Table 2



Scheme 1

1, logarithms of the second-order rate constants (obtained by extrapolation to zero of plots of $k_{obs}/[amine]_{fb}$ against buffer concentration), are plotted against pH. The pH-rate profile shows two breaks at pH values near 2 and near 1. In the pH ranges from 7 to ~ 2 and from ~ 1 to 0.25 the constants are linearly dependent on the concentration of the hydronium ion. This behavior is interpreted in terms of the mechanism outlined in Scheme 1, in which we propose that carbinolamine dehydration is considered to be the sole rate-limiting step. Carbinolamine dehydration occurs via $[T_0]k'_{DE}[H^+]$ and $[T_1]k_{DE}[H^+]$. The rate law for the mechanism of the reaction is

$$k_{obs}/[amine]_{fb} = \frac{([H^+]/[K_{app} + [H^+]]) [K_{app} K'_{AD} k'_{DE} + K_{AD} k_{DE} [H^+]]}{K_{AD} k_{DE} [H^+]} \quad (1)$$

At high pH values (pH higher than the apparent pK_a of the substrate), Eqn. (1) simplifies to

$$k_{obs}/[amine]_{fb} = K'_{AD} k'_{DE} [H^+] \quad (2)$$

Therefore, the antilogarithm of the intercept of the line on the ordinate (slope -1) obtained in the neutral zone of pH in Fig. 1 gives the value of $K'_{AD} k'_{DE}$. The value of K'_{AD} was obtained experimentally at pH 9.11, a condition in which the low proton concentration allows carbinolamine accumulation and therefore the equilibrium between S_0 and carbinolamine is well established, and with its values and the value of $K'_{AD} k'_{DE}$, k'_{DE} was estimated.

The limiting value of $k_{obs}/[amine]_{fb}$ at low pH is $K_{AD} k_{DE} [H^+]$ and the antilogarithm of the intercept on the ordinate of the line obtained in the acidic zone in Fig. 1 gives the value of $K_{AD} k_{DE}$. The procedure to evaluate K'_{AD} cannot be used in this case because the high concentration of proton does not permit carbinolamine accumulation and for that reason K_{AD} cannot be measured; it was estimated assuming that the carbonyl

carbon in pyruvic acid has the same effect on the equilibrium constant for addition of hydroxylamine as it does on that for water; since the hydrate of pyruvic acid and the carbinolamine are closely related structurally, this assumption appears reasonable, and therefore $K_{AD} = K'_{AD} K_H / K'_H$. The relative extent of hydration of pyruvic acid and its anion is equal to 21,⁴ and with this figure and the value of K'_{AD} , K_{AD} was obtained.

Note that the values of K_{AD} and K'_{AD} provide measurements of equilibrium constants for the addition of hydroxylamine to pyruvic acid-hydrate and pyruvate anion-hydrate mixtures, respectively. In order to obtain the equilibrium constants for the addition of the nucleophile to the unhydrated substrates, it is necessary to correct K_{AD} and K'_{AD} for the extent of hydration:

$$K_{ADcor} = K_{AD}(1 + K_H) \quad \text{and} \quad K'_{ADcor} = K'_{AD}(1 + K'_H) \quad (3)$$

Table 2. Summary of rate and equilibrium constants for pyruvic acid oxime formation

Constant	Value
$K_{AD} k_{DE}$ ($l^2 \text{ mol}^{-2} \text{ min}^{-1}$)	1.78×10^7
$K'_{AD} k'_{DE}$ ($l^2 \text{ mol}^{-2} \text{ min}^{-1}$)	2×10^8
K'_{AD} ($l \text{ mol}^{-1}$)	58
K_{AD} ($l \text{ mol}^{-1}$)	1.22×10^3
k'_{DE} ($l \text{ mol}^{-1} \text{ min}^{-1}$)	3.45×10^6
k_{DE} ($l \text{ mol}^{-1} \text{ min}^{-1}$)	1.46×10^4
K'_{ADcor} ($l \text{ mol}^{-1}$)	63
K_{ADcor} ($l \text{ mol}^{-1}$)	3.5×10^3
K_{app} (M)	6.03×10^{-3}
k_{AD}^{0pp} (min^{-1}) ^a	33
k' ($l \text{ mol}^{-1} \text{ min}^{-1}$) ^b	3.78×10^6

^a Rate constant for addition of water to pyruvic acid at 25 °C.¹²

^b Rate constant for addition of hydroxylamine to pyruvate anion at 30 °C and ionic strength 1.3.⁷

Table 3. First-order rate constants for pyruvic acid oxime formation in aqueous solution at 30°C, pH 6.97 and ionic strength 0.5^a

[Amine] _{fb}	<i>k</i> _{obs} (min ⁻¹)
4.37 × 10 ⁻³	0.27
1.00 × 10 ⁻²	0.45
1.55 × 10 ⁻²	0.69
2.34 × 10 ⁻²	0.78
3.32 × 10 ⁻²	0.87
3.55 × 10 ⁻²	0.85
4.24 × 10 ⁻²	0.92
5.55 × 10 ⁻²	1.07
8.90 × 10 ⁻²	1.06

^a All reactions were carried out in 0.25 M phosphate buffer and the constants have not been corrected to zero buffer concentration.

The values of K'_H and K_H are 0.087 l mol⁻¹ and 1.86 l mol⁻¹, respectively.⁴ The corrected equilibrium constants provide direct measurements of equilibrium constants for the addition of hydroxylamine to the unhydrated ketones, and therefore they represent the real affinity of pyruvic acid and its anion to the nucleophile.

A summary of rate and equilibrium constants is provided in Table 2. The solid line in the Fig. 1 was calculated based on the rate law in Eqn. (1) and the constants in Table 2. The agreement of the theory with experimental data is satisfactory.

The assumption that the velocity of the reaction is always determined by the dehydration of the carbinol-

amines of the species of the substrate is based in the following considerations:

1. In the pH range from 7 to ~2, all the experimental points fit on a line, which is commonly considered diagnostic of no transition from one type of rate-determining step to another,¹⁰ and a plot of the first-order rate constants obtained at pH 6.97 against the amine concentration (Fig. 2, Table 3) shows that the constants increase linearly with increasing amine concentration. At sufficiently high concentrations of the nucleophile, they show a tendency to become independent of this variable. This behavior has been observed previously¹¹ and strongly suggests that dehydration of the carbinolamine formed from the addition of hydroxylamine is the rate-limiting step.
2. In the pH range from ~1 to 0.25, owing to the pK_a value of pyruvic acid, the substrate largely exists as pyruvic acid. There are at least three kinetically indistinguishable possibilities regarding the limiting-step of the reaction: (a) the acid-catalyzed conversion of the hydrated ketone to the unhydrated species, (b) the acid-catalyzed attack of hydroxylamine to pyruvic acid and (c) the acid-catalyzed dehydration of carbinolamine derived from pyruvic acid. The first possibility is excluded by the fact that experimental first-order rate constants are linearly dependent on hydroxylamine concentration, tending to reach a limit at pH 0.5; with hydroxylamine total concentration in the range between 0.03 and 1.25 M they did not show a deviation from linearity. The rate law for conversion of the hydrated ketone to the unhydrated pyruvic acid

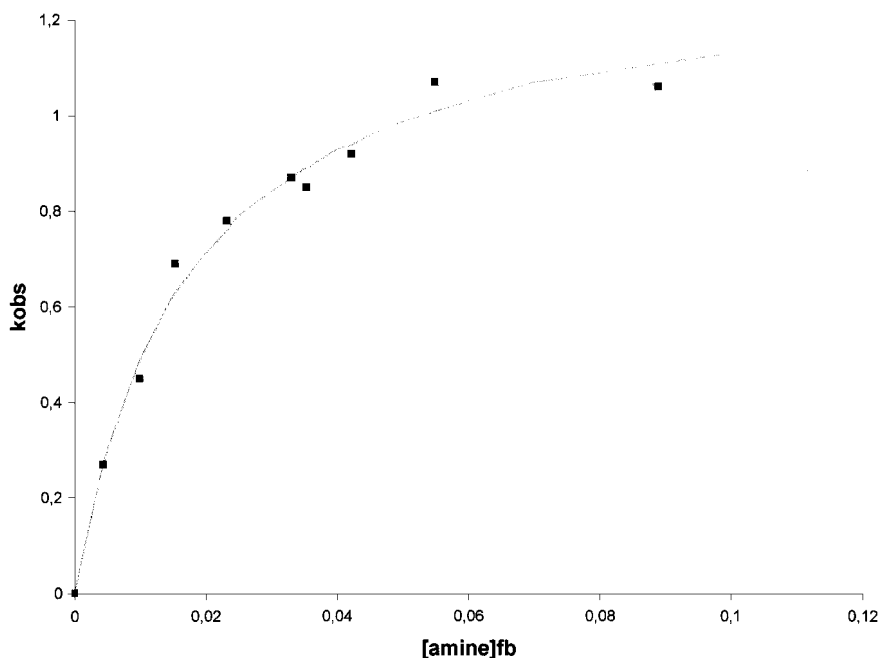


Figure 2. First-order rate constants for oxime formation from pyruvic acid plotted as a function of hydroxylamine free base concentration. The rate constants were measured at 30°C, ionic strength 0.5 and pH 6.97 (Table 3)

has been established to be¹²

$$k = k^0 + k^H[H^+] \quad (4)$$

where k^0 and k^H represent the uncatalyzed and acid-catalyzed loss of water respectively, k^0 is reported to have a value of 33 min^{-1} and k^H $378 \text{ l mol}^{-1} \text{ min}^{-1}$, and therefore at pH 0.5 the limiting value of k should be 153 min^{-1} ; instead of this, the experimental value of k_{obs} at pH 0.5 and $[\text{amine}]_{\text{total}} = 1.25$ was 1.38 min^{-1} .

With respect to a rate-determining step of acid-catalyzed attack of the nucleophile on pyruvic acid, the following consideration appear to exclude this possibility. Although this behavior has been suggested to explain semicarbazone formation from pyruvic acid in the pH range 0–2,⁸ perhaps this reaction is not a good model owing to the use of a less efficient nucleophile. The rates of carbonyl additions depend on the factors which facilitate this process by increasing the charge density and nucleophilicity of the attacking reagent, and therefore there is a tendency for catalysis to occur at the points at which it is most needed; since hydroxylamine is a better nucleophile than semicarbazide, the necessity for assistance is minor. This argument is enforced by the fact that acid catalysis of the attack of hydroxylamine on pyruvate anion has not been detected in the pH range 6.5–7.5.⁷

The preceding arguments appear to support acid-catalyzed dehydration as the rate-limiting step over the entire range of pH investigated. The breaks observed in Fig. 1 occur near the $\text{p}K_a$ of pyruvic acid, which reflects different reactivities of the carbinolamines derived from pyruvic acid and its anion toward the acid-catalyzed dehydration.

Note that there are kinetically indistinguishable transition states for the dehydration of the carbinolamine derived from pyruvate anion, which appears reasonable: one based on a specific acid catalysis of dehydration and the other on an uncatalyzed dehydration of the neutral carbinolamine (presumably with intramolecular general acid catalysis) with transition states that differ only in the site of protonation of the pyruvate moiety. A plot of the logarithm of the catalytic constants of hydronium ion, acetic acid and dihydrogenphosphate monoanion, which are 3.45×10^6 , 58 and $0.79 \text{ l mol}^{-1} \text{ min}^{-1}$, respectively,⁷ against $\text{p}K_a$ values is linear ($r = 0.99$) with a Brönsted coefficient $\alpha = 0.72$, a value that corresponds to a considerable amount of change in the charge of the proton-donating or proton-accepting atom in the transi-

tion state. Although the data are fairly limited, there is a definite trend that suggests that the former alternative is the correct one.

Perhaps the most notable result here is the difference between k'_{DE} and k_{DE} ($k'_{\text{DE}}/k_{\text{DE}} \approx 240$). In semicarbazone formation from a series of substituted benzaldehydes,¹⁴ the dehydration process shows a negative value of ρ , indicating that this step is aided by electron donation to the reaction center; however, the highest ratio between the dehydration rate constants of the series (*p*-methoxy/*p*-nitro) is near 54, and in addition the nature of the nucleophile employed appears not to have great influence in this process, as indicated by the finding that the dehydration rate constants of carbinolamines formed from pyruvate anion and hydroxylamine, semicarbazide⁸ and *N*-methylhydroxylamine¹³ are 3.45×10^6 , 2.4×10^6 and $4.1 \times 10^6 \text{ l mol}^{-1} \text{ min}^{-1}$, respectively. All these facts strongly suggest that the presence of a negative charge near the reaction center of the carbinolamines formed by pyruvate anion exerts a great positive influence on the velocity of dehydration.

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