Kinetics and Mechanism for Pyruvic Acid Semicarbazone Formation[1]

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Received September 3, 1970

Second-order rate constants or pyruvic acid semicarbazone formation exhibit a complex dependence on pH: in the pH ranges 0–2, 3–4, and 6–7, these constants are linearly dependent on the concentration of the hydrated proton; in the pH ranges 2–3 and 4–6, breaks in the pH-rate profile occur and the second-order rate constants are relatively insensitive to the concentration of the hydrated proton. These results suggest that, below pH 2, attack of semicarbazide on pyruvic acid is the rate-determining step, between pH 3 and 6, attack of semicarbazide on pyruvate is the rate-determining step, and, above pH 6, decomposition of the carbinolamine intermediate is rate determining. Second-order rate constants for pyruvic acid methyl ester semicarbazone formation exhibit only the latter break in the pH-rate profile in accordance with these suggestions. The attack of semicarbazide on pyruvate is subject to general acid catalysis by carboxylic acids; this catalysis is characterized by a value of the Brønsted exponent α of 0.37.

The kinetics of semicarbazone formation have been intensively studied in recent years.[3,4] It seems safe to conclude that the principal features of the mechanism for these reactions are in hand. Specifically, attack of semicarbazide, subject to marked specific and general acid catalysis, is the rate-determining step under mildly acidic conditions and dehydration of the carbinolamine intermediate, subject to marked specific acid catalysis and to weak general acid catalysis, is rate determining under neutral and basic conditions.[3] Nevertheless, these conclusions are based on studies employing a limited number of structurally related substrates. Consequently, expansion of kinetic investigation of semicarbazone formation to include substrates of novel structure is likely to provide further insight into the mechanism and catalysis for these reactions. Results of one such investigation, employing pyruvic acid and methyl pyruvate as substrates, is reported herein.

Experimental Section

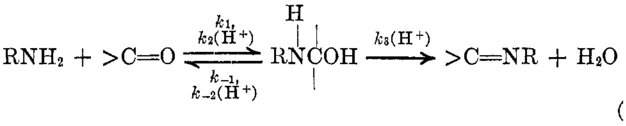
Materials.—Pyruvic acid and methyl pyruvate were obtained commercially and were redistilled prior to use. Semicarbazide hydrochloride was recrystallized from ethanol–water several times prior to use. Solutions containing semicarbazide as the free base were prepared just prior to use. Other reagents employed were of reagent grade and were used without further purification. Glass distilled water was employed throughout.

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 constantly circulated.[4a,b] Temperature was maintained at 30° throughout. The extent of reaction was monitored by the increase in optical density near 240 mμ which accompanies semicarbazide formation. First-order rate constants, based on the total (hydrated + unhydrated) ketone, were obtained in the usual way from semilogarithmic plots of the difference in optical density at infinite time and at various times against time. Second-order rate constants under conditions in which semicarbazide attack is rate determining were calculated by dividing first-order rate constants by the concentration of semicarbazide free base. Second-order rate constants for semicarbazone formation in the region of rate-determining carbinolamine dehydration were calculated from the expression, $k_2 = k_1/(\text{semicarbazide})_{\text{free base}}\ (f)$ where f is the fraction of total substrate present as the (hydrated + unhydrated) ketone. Ionic strength was maintained at 0.50 throughout with KCl. Values of pH were obtained with a Radiometer Model 22 pH meter equipped with a glass electrode.

Equilibrium constant measurements for carbinolamine formation were made spectrophotometrically at 30° as previously described.[4a] A value of 3.65 for the pK_a of the conjugate acid of semicarbazide was employed.

Results and Discussion

For nonionizable substrates, the pathway for semicarbazone formation may be formulated as



$$\mathrm{RNH_2} + {>}\mathrm{C{=}O} \underset{k_{-1},\ k_{-2}(\mathrm{H^+})}{\overset{k_1,\ k_2(\mathrm{H^+})}{\rightleftharpoons}} \mathrm{R\overset{H}{\underset{|}{N}}\overset{|}{\underset{|}{C}}OH} \xrightarrow{k_3(\mathrm{H^+})} {>}\mathrm{C{=}NR} + \mathrm{H_2O} \tag{1}$$

This pathway generates the following steady-state rate equation.[3]

$$v = \frac{k_3[k_1 + k_2\ (\mathrm{H^+})]}{[k_{-1}/(\mathrm{H^+}) + k_{-2} + k_3]}(\mathrm{RNH_2})({>}\mathrm{C{=}O}) \tag{2}$$

Quite generally, under neutral or alkaline conditions the rate of dehydration of the carbinolamine is slower than that for its formation. Under these conditions, the rate law, in terms of first-order rate constants, becomes

$$k_{\text{obsd}} = k_3 k_{\text{eq}}(\mathrm{H^+})\frac{(\mathrm{RNH_2})}{1 + (\mathrm{RNH_2})K_{\text{eq}}} \tag{3}$$

in which K_{eq} is the equilibrium constant for carbinolamine formation; $K_{\text{eq}} = k_1/k_{-1}$.

Jencks has previously shown that the reaction of pyruvate with semicarbazide at pH 6.5 obeys a rate equation of this form and, therefore, that dehydration of the carbinolamine must be rate determining at this pH.[4a] In Table I, values of first-order rate constants for this reaction in the region of rate-determining dehydration are collected for several concentrations of semicarbazide. These data clearly show, as expected on the basis of eq 3, that values of $k_{\text{obsd}}/(\mathrm{H^+})$ approach a limiting value as the concentration of semicarbazide is increased. This limiting value is just k_3 which has a value of $2.4 \times 10^6\ M^{-1}\ \text{min}^{-1}$. These data confirm the observation of Jencks noted above and provide a rate constant in reasonable agreement with a value of $4.8 \times 10^6\ M^{-1}\ \text{min}^{-1}$ measured at 25° and

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(1) Supported by Grant AM 08232 from the National Institutes of Health and by the Escuela de Quimica, Universidad Central. Publication No. 1930 from the Department of Chemistry, Indiana University.

(2) Career Development Awardee of the National Institutes of Health; Research Fellow of the Alfred P. Sloan Foundation.

(3) Two comprehensive reviews are available: W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964); W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, Chapter 10.

(4) Leading references include (a) W. P. Jencks, *J. Amer. Chem. Soc.*, **81**, 475 (1959); (b) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 4319 (1962); (c) B. M. Anderson and W. P. Jencks, *ibid.*, **82**, 1773 (1960); (d) L. do Amaral, W. A. Sandstrom, and E. H. Cordes, *ibid.*, **88**, 2225 (1966); (e) R. K. Chaturvedi and E. H. Cordes, *ibid.*, **89**, 4631 (1967).

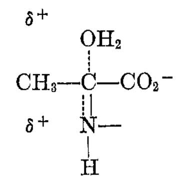
Table I

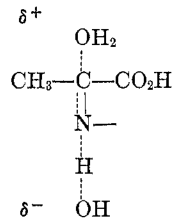
First-Order Rate Constants for Pyruvate Semicarbazone Formation in Aqueous Solution at 30° and Ionic Strength 0.50 under Conditions of Rate-Determining Carbinolamine Dehydration

Semicarbazide, M	pH	k_{obsd}, min^{-1}	$k_{obsd}/(H^+)$, M^{-1} min^{-1}
0.01	7.26	0.027	4.85×10^5
0.02	7.24	0.048	8.30×10^5
0.05	7.21	0.098	16.0×10^5
0.10	7.03	0.173	18.5×10^5
0.20	6.82	0.342	22.6×10^5
0.30	6.65	0.483	21.5×10^5

ionic strength 0.3 based on a single measurement at pH 6.5.[4a] Use of this value for k_3, the data of Table I, and eq 3 leads to a best value for K_{eq} near 30 M^{-1}. This value is somewhat larger than one of 10 M^{-1} measured at 25° and ionic strength 0.30. The latter value was measured spectrophotometrically rather than derived from kinetic data.[4a]

Note that there are several kinetically indistinguishable transition states for carbinolamine dehydration, two of which appear most reasonable: one based on addition of the amine to *pyruvate* coupled with specific acid catalysis of dehydration and one based on addition of amine to *pyruvic acid* coupled with both specific acid and base catalysis of dehydration.





In Figure 1, logarithms of second-order rate constants for pyruvate semicarbazone formation at 30° are plotted against pH over the pH range 0–8. Where necessary, these rate constants have been extrapolated to 0 buffer concentration. Above pH 7, for which carbinolamine dehydration is rate determining, these rate constants are, as developed above, first order in the concentration of the hydrated proton. The third-order rate constant, which is equal to $K_{eq}k_3$ (eq 1), is 7.2×10^7 M^{-2} min^{-1}.

Under slightly more acidic conditions, the second-order rate constants generate a break in the pH-rate profile diagnostic of a transition to rate-determining attack of semicarbazide on pyruvate.[3] Plots of the second-order rate constants in the pH range 3.5–5.5 against the concentration of the hydrated proton yield values of k_1 and k_2 (eq 1) of 80 M^{-1} min^{-1} and 2×10^6 M^{-2} min^{-1}, respectively.

The transition state for the acid-catalyzed reaction between pH 2 and 4 is again kinetically ambiguous; it may be regarded as acid-catalyzed attack of amine on pyruvate (as noted above) or as uncatalyzed attack of amine on pyruvic acid. These transition states differ only in the site of protonation of the pyruvate moiety. If the latter possibility is correct, the second-order rate constant for addition of semicarbazide to pyruvic acid, the product of the experimental third-order rate constant and the dissociation constant for pyruvic acid, is 6.3×10^3 M^{-1} min^{-1}. This value is considerably greater than the rate constant for addition of semicarbazide to methyl pyruvate (see below). This result suggests that the former alternative is the correct one. However, the possibility of intramolecular general acid catalysis for the addition of semicarbazide to pyruvic acid, which may be important in the hydration of this substrate,[5,6] provides a possible explanation for this finding. Thus, although the data strongly suggest acid-catalyzed addition of semicarbazide to pyruvate, the alternative cannot be firmly excluded.

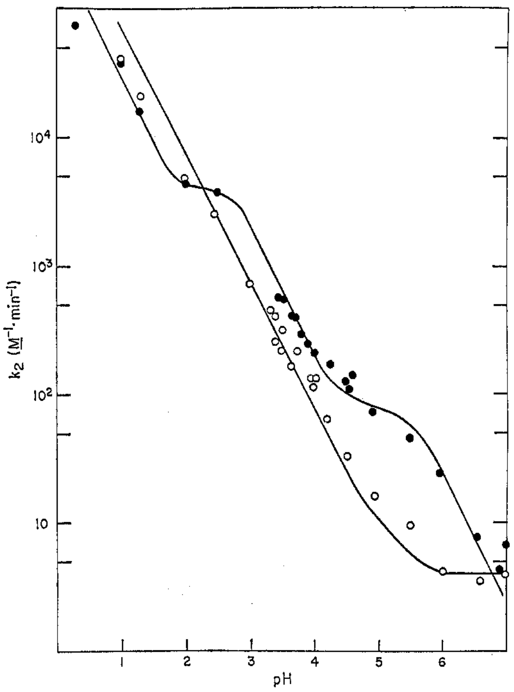


Figure 1.—Logarithm of second-order rate constants for semicarbazone formation from pyruvic acid (closed circles) and methyl pyruvate (open circles) measured at 30° and 0.50 ionic strength as a function of pH.

Under still more acidic conditions, a second break in the pH-rate profile is observed which occurs near the pK_a of pyruvic acid, 2.50.[7] This behavior reflects different reactivities of pyruvic acid and pyruvate anion toward nucleophilic attack by semicarbazide. The calculated third-order rate constant for acid-catalyzed attack of semicarbazide on pyruvic acid, k_2 of eq 1, is 3×10^5 M^{-2} min^{-1}. Thus pyruvate appears about sevenfold more reactive than pyruvic acid toward acid-catalyzed addition of semicarbazide. At first glance, this is a surprising conclusion since the carboxyl group of pyruvic acid is certainly more electron withdrawing than the carboxylate group of pyruvate. While this factor ought to increase the rate of attack of nucleophilic reagents at the carbonyl carbon, there are three other factors that need consideration as well. First, the effects of polar substituents on the rate of nucleophile attack and the extent of substrate protonation are opposite. Thus, the relatively electron-donating carboxylate group will favor substrate protonation compared to the carboxyl group. Second, aqueous solutions of pyruvic acid are converted more

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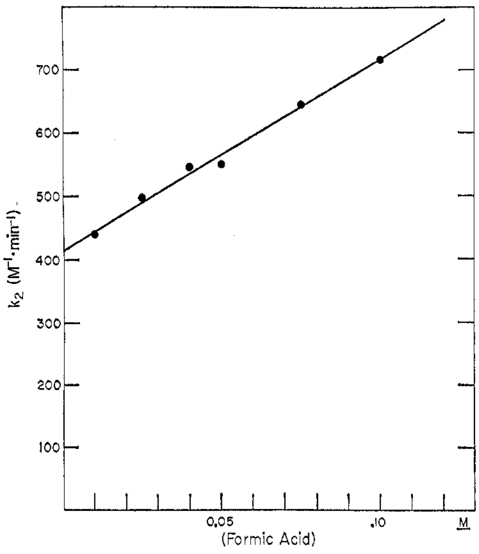

Figure 2.—Second-order rate constants for attack of semicarbazide on the pyruvate anion at 30° and pH 3.70 as a function of the concentration of formic acid.

fully to the unreactive hydrated form (*ca.* 65%) than are solutions of pyruvate (*ca.* 59%).[8,9] Finally, intramolecular general base catalysis by the pyruvate carboxylate group may contribute to its enhanced reactivity compared to pyruvic acid and methyl pyruvate (see below). The combination of these factors evidently outweighs the inherently greater electrophilicity of pyruvic acid compared to pyruvate. Of course, should the reaction from pH 2 to 4 reflect uncatalyzed attack of amine on pyruvic acid, these considerations become unnecessary.

These conclusions are strengthened by studies of the kinetics of pyruvic acid methyl ester semicarbazone formation. Second-order rate constants for this reaction are plotted against pH in Figure 1 for comparison with those for the corresponding reaction of pyruvate. Note that not all of the rate constants in the pH region greater than 3 have been extrapolated to 0 buffer concentration and may, therefore, be slighly too large. Nevertheless, the second-order rate constants and pH are best correlated by a single straight line with a slope of unity which breaks above pH 5 indicating a transition in rate-determining step. Note that the reactivity of pyruvate methyl ester toward attack by semicarbazide is nearly equal to that for pyruvic acid as expected on the basis of the similarity of the carboxyl and carbmethoxy groups. What is surprising is that the pyruvate anion appears to be about 20 times as reactive as pyruvate methyl ester toward water-catalyzed attack of semicarbazide (Figure 1). Differences in substrate hydration and basicity and intramolecular carboxylate catalysis may account for this difference.

The attack of semicarbazide on the pyruvate anion (or the kinetic equivalent) is subject to general acid catalysis by carboxylic acids. In Figure 2, second-order rate constants for this reaction at pH 3.70 are plotted as a function of the concentration of formic acid. Third-order rate constants evaluated from plots of this type for four carboxylic acids are collected in Table II. These values are well correlated by a single straight line in a Brønsted plot with a slope of 0.37. This value, while not large, is somewhat greater than those previously observed for general acid catalysis of semicarbazone formation.[4] This is a surprising finding since the pyruvate anion is the most reactive substrate for which general acid catalysis of semicarbazide attack has yet been studied and the transition state might have been expected to be reached progressively earlier along the reaction coordinate as the substrate becomes more reactive.[10–12] This suggests that values of Brønsted exponents should decrease with increasing substrate reactivity. It is, of course, possible that the degree of proton transfer in the transition state, as measured by the Brønsted exponent, is not representative of progress along the reaction coordinate. Finally, it is possible that the observed catalysis reflects general base catalysis of the attack of semicarbazide on pyruvic acid which, then, would have a value of $\beta = 0.63$.

Table II

Catalytic Constants for Several Acids for the Attack of Semicarbazide on Pyruvate at 30°

Catalyst	pH	Concn range, M	pK_a	$k_3 \times 10^{-3}$, M^{-2} min^{-1}
Cyanoacetic acid	2.49	0.02–0.15	2.45	13.4
Chloroacetic acid	3.00	0.008–0.06	2.90	7.2
Formic acid	3.70	0.01–0.10	3.75	3.0
Acetic acid	4.00	0.015–0.24	4.76	1.4

Registry No.—Pyruvic acid semicarbazone, 2704-30-5.

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