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Activation parameters for the reversible hydration of pyruvic acid

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ABSTRACT

Variable temperature stopped-flow investigations of the reversible hydration of pyruvic acid were carried out and reveal that for the spontaneous reaction $\Delta H^{\ddagger} = 36 \pm 7$ kJmol^{-1} and $\Delta S^{\ddagger} = -171 \pm 25$ Jmol^{-1}K^{-1} and for the hydronium ion-catalyzed reaction $\Delta H^{\ddagger} = 33 \pm 2$ kJmol^{-1} with $\Delta S^{\ddagger} = -121 \pm 7$ Jmol^{-1}K^{-1}. These values at 298 K correspond to a $\Delta G^{\ddagger} = 87 \pm 10$ kJmol^{-1} for the spontaneous reaction and a $\Delta G^{\ddagger} = 69 \pm 3$ kJmol^{-1} for the hydronium ion-catalyzed hydration. These kinetic results are interpreted in terms of the mechanisms of the spontaneous and hydronium ion-catalyzed hydration of pyruvic acid.

KEYWORDS: pyruvic acid, pyruvate, hydration, stopped-flow.

INTRODUCTION

Aqueous solutions of pyruvic acid and pyruvate ion exist as equilibrated mixtures consisting of keto, gem diol and enol species (Scheme 1) [1-5].

Numerous pyruvate-converting enzymes exist. For example, under anaerobic conditions pyruvate is reduced to lactate by the coenzyme NADH in a reaction catalyzed by lactate dehydrogenase (LDH:EC1.1.1.27) [6]. For the heart isoenzymes of LDH, “substrate inhibition” has been observed as indicated by deviations from normal Michaelis-Menten kinetics in which enzymatic velocities begin to drop off rapidly at higher substrate concentrations [7a]. Thus for this enzyme the question arises as to which structure of the pyruvic acid-pyruvate system serves as the “substrate” and which serves as the “substrate inhibitor”. For this enzymatic oxidation of NADH by pyruvate, the analysis supporting the conclusion that the keto form serves as substrate while the hydrated form serves as “substrate inhibitor” demands knowledge of the positions of equilibria and the rates of interconversion of the structures shown in Scheme 1 [7a, 7b].

Pyruvic acid has a much greater tendency to undergo nucleophilic acyl addition of water (K_{hyd} = 1.3 at 25.0 °C) than does pyruvate anion (K_{hyd} = 0.07 at 25.0 °C) because of the electron withdrawing effect of the carboxyl group [1-3]. At the same time, the reversible hydration of pyruvic acid is much more rapid than the corresponding reaction of pyruvate anion [2]. As the equilibrium constants for the enolization of both pyruvic acid and pyruvate anion are quite small, these reactions need not be considered in the present study [3-5].

The hydration of pyruvic acid is a pseudo-first order reaction which is specific acid catalyzed [2]. In dilute aqueous solutions of hydrochloric acid, rate constants for the reaction, k_{obs}, can be represented by the sum of catalytic terms for the spontaneous reaction, k_o, and the acid-catalyzed component, $k_{H_3O^+}$ (Eq. 1).

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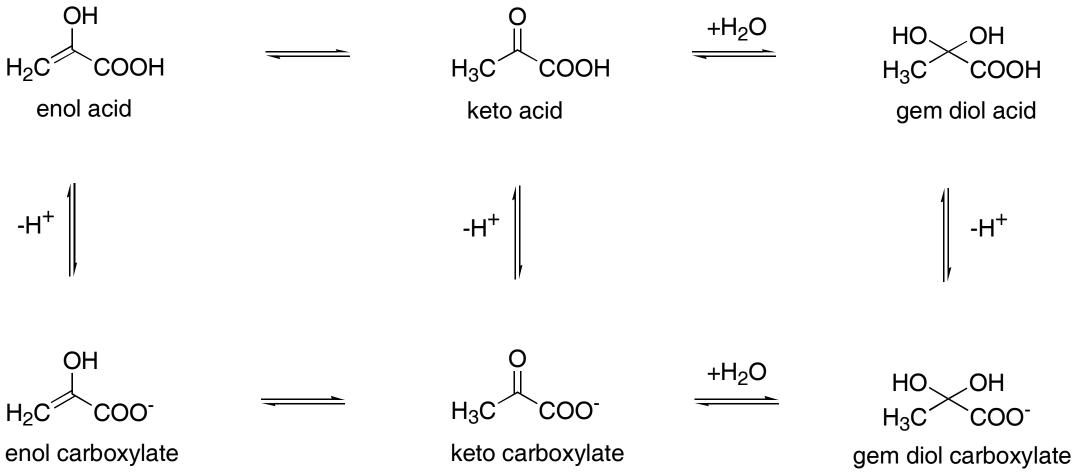
Dedicated to the memory of Professor Yeshayau Pocker, 1928-2010.

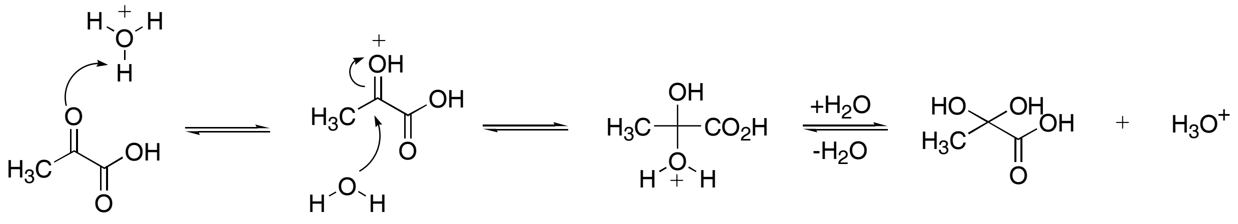
$$k_{obs} = k_o + k_{H_3O^+}[H_3O^+] \quad (1)$$

where it is assumed that $k_o = k_{H_2O}[H_2O]$. The mechanism proposed for the acid-catalyzed component is shown in Scheme 2 and that for the spontaneous reaction is illustrated in Scheme 3 [2]. It has been shown in earlier work that the transition states for both the acid-catalyzed and spontaneous paths probably involve one or more molecules of waters of solvation [1].

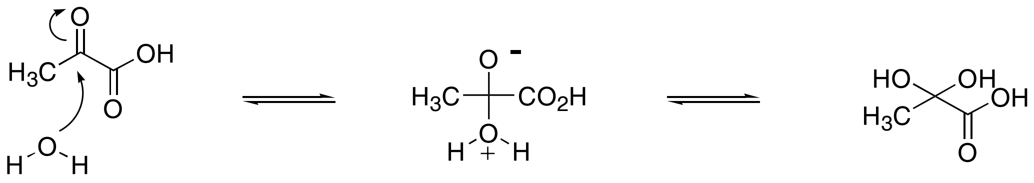
The reversible hydrations of pyruvic acid and pyruvate ion have been monitored by a number of methods including spectrophotometric, polaragraphic and NMR [1-8]. In this paper we describe the use of a stopped-flow spectrophotometric method to obtain the spontaneous and the hydronium ion-catalyzed rate constants for the conversion of pyruvic acid to its gem diol. The use of the stopped-flow spectrophotometric method was necessitated because rates of hydration of pyruvic acid as a function of hydronium concentration and as a function of temperature are far too rapid to be determined using conventional spectrophotometric methods.



Scheme 1. The keto, enol, and gem diol species of pyruvic acid and pyruvate anion.



Scheme 2. Mechanism for the acid-catalyzed hydration of pyruvic acid.



Scheme 3. Mechanism for the spontaneous hydration of pyruvic acid.

Shoemaker *et al*. have described methods for obtaining kinetic data for the acid-catalyzed hydration of pyruvic acid *via* NMR methods [8c]. A comparison must be made of the stopped-flow and NMR methods from operational points of view. First, stopped-flow directly measures reaction kinetics by graphically plotting reaction progress, while NMR bases its measurements of reaction rates on line broadening. Second, stopped-flow determines the order of the reaction under consideration, in this case of first-order kinetics, while that must be assumed utilizing NMR. Third, stopped-flow can utilize very dilute concentrations of pyruvic acid and HCl while NMR requires roughly equal volumes of water, pyruvic acid and aqueous HCl.

The kinetic parameters obtained in the present study allow direct comparisons to be made between the hydration of pyruvic acid to those obtained for the hydration of alkyl pyruvate esters and the enolization of alkyl pyruvate esters [1, 9, 10].

MATERIALS AND METHODS

All reagents used in this experiment were purchased from Millipore Sigma and used as received. Mixtures of 0.50 M hydrochloric acid and 0.50 M sodium chloride solutions were used to obtain appropriate dilutions of the acid.

Kinetic runs were conducted on a Shimadsu UV-2101PC UV spectrophotometer interfaced to a HiTech Scientific SFA-20 Rapid Kinetics Stopped-Flow Accessory. The mixing chamber was thermostated with a VWR-1160 constant temperature bath and temperatures were measured using a HiTech OPT-12T digital thermometer and determined to be within ±0.1 °C. An Orion Digital pH meter was used to determine pH values.

Prior to each run, the apparatus was flushed out at least twice with the reagent mixtures and kinetic runs were initiated by injecting equal volumes of 0.1 M sodium pyruvate and the appropriate concentration of hydrochloric acid and monitoring the decrease in absorbance at 340 nm. Kinetic runs were found to be first order with respect to pyruvic acid and each run was followed for at least 10 half-lives. Figure 1A illustrates a typical kinetic run while Figure 1B shows the corresponding plot of $\ln(A_t - A_\infty)$ *vs.* time. From these plots the observed rate constant for equilibration between pyruvic acid and its hydrate, k_{obs}, was taken as the negative slope and rate constants for the forward reaction, hydration of pyruvic acid to give the gem diol, k^f, were calculated using Eq. 2 [2].

$$k^f = k_{obs}K_{hyd}/(1 + K_{hyd}) \qquad (2)$$

RESULTS AND DISCUSSION

The pK_A of pyruvic acid is 2.18 so that at the dilute concentrations of hydrochloric acid utilized significant amounts of pyruvate anion exist [1]. Consequently, we took into consideration that the pyruvate anion will hydrate along with pyruvic acid thereby contributing to the diminution of absorbance. However, it has been shown that such contribution would be negligible since the hydration of pyruvate anion is much slower than that of pyruvic acid [9]. It was also considered that the existing pyruvate anion in the reaction solutions could serve as a general base and as such catalyze the hydration of pyruvic acid, giving rise to a greater apparent value of k_o. However, we were able to demonstrate that general base-catalysis by pyruvate anion was negligible by carrying out experiments in which the initial pyruvate anion concentration was varied.

Kinetic data for the hydration of pyruvic acid at various temperatures and hydronium concentrations are shown in Table l while Figure 2 depicts the variation of k^f as a function of $[H_3O^+]$ at 5 different temperatures taken from Table 1. Values for $\Delta H^‡$ and $\Delta S^‡$ were obtained using Eq. 3.

$$\ln(k/T) = \ln[(k_BT/h) + (\Delta S^‡/R) - (\Delta H^‡/RT)] \qquad (3)$$

where k is the rate constant under consideration, k_B is Boltzmann's constant, h is Plank's constant, R is the gas constant, and T the absolute temperature [11]. Figure 3 shows an Eyring plot for the second-order rate constant for water and Figure 4 depicts the corresponding plot for hydronium ion catalysis. From these graphs, the activation parameters for both modes of catalysis were evaluated and Table 2 lists the values of $\Delta H^‡$, $\Delta S^‡$ and $\Delta G^‡$ for the spontaneous and acid-catalyzed pathways in addition to the corresponding activation parameters for methyl and ethyl pyruvate.

The data in Table 2 shows that the values of $\Delta S^‡$ for both the spontaneous and acid-catalyzed modes of pyruvic acid hydration are highly negative.

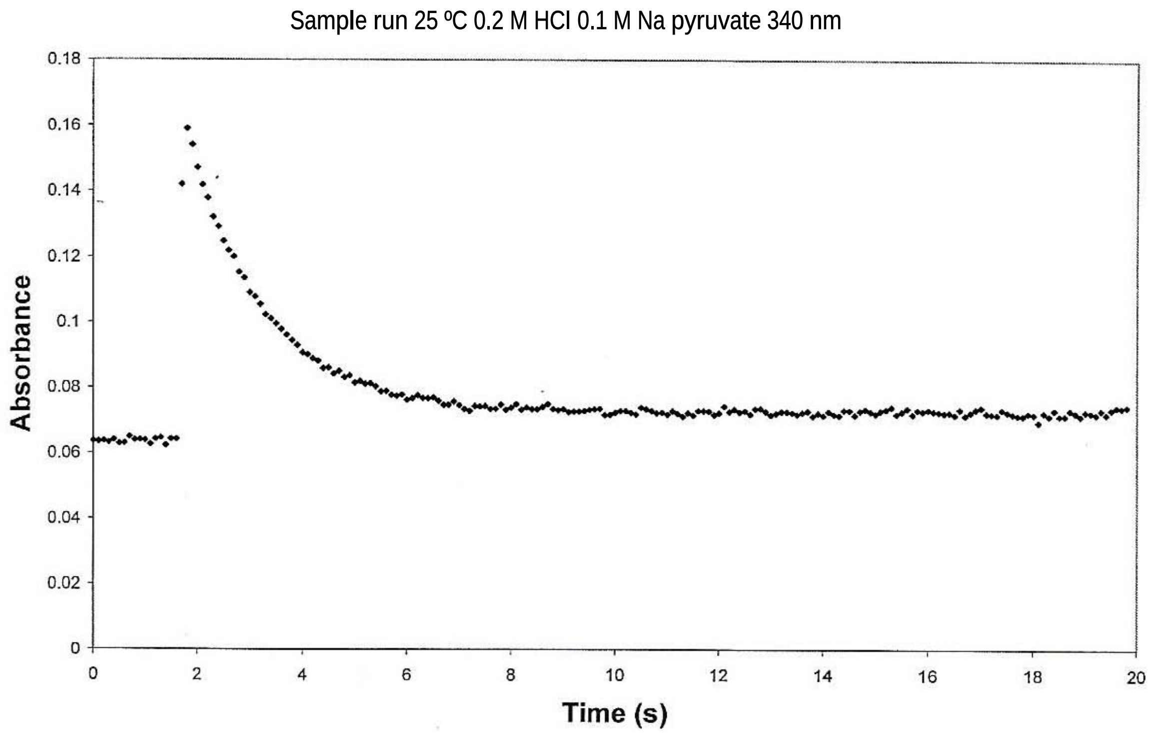


Figure 1A. A typical kinetic run for the acid-catalyzed hydration of pyruvic acid. Plot of absorbance at 340 nm *vs*. time (s) at 25 °C. Reaction conditions were as follows: $[HCl]_{initial}$ = 0.20 M and $[NaO_2CCOCH_3]_{initial}$ = 0.10 M.

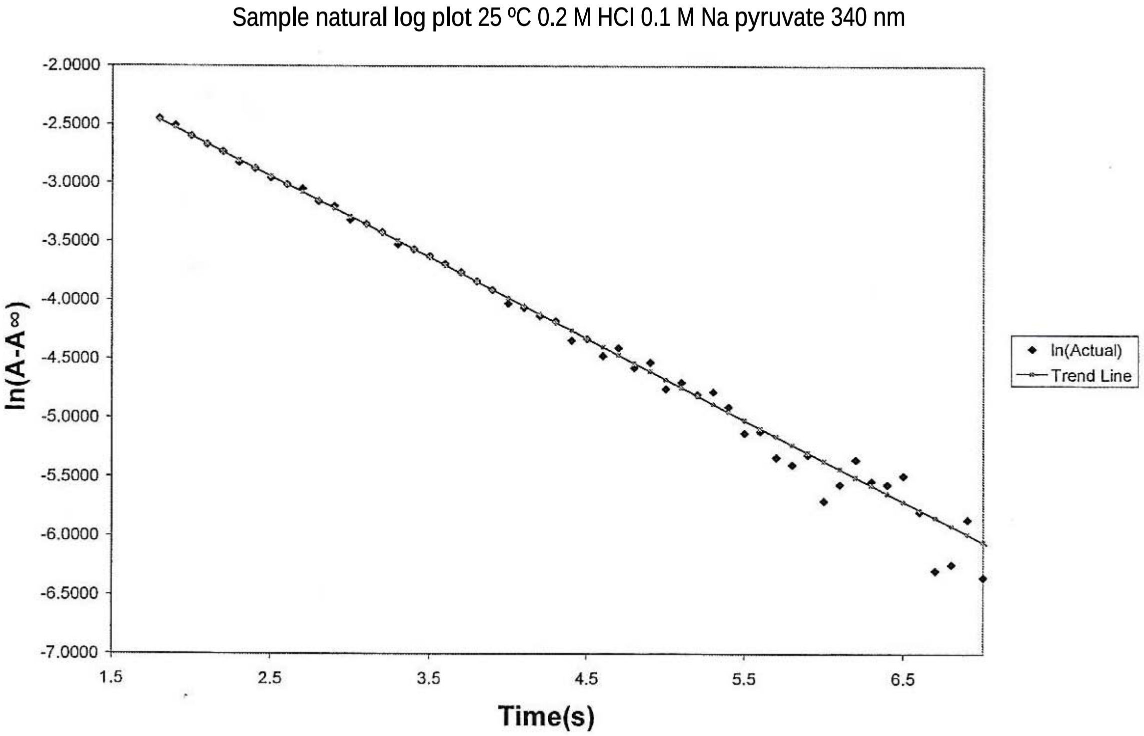


Figure 1B. Plot of $\ln(A_t-A_\infty)$ *vs*. time for the data shown in Figure 1A.

Table 1. Hydration rate constants at various pyruvic acid (HPyr), hydronium ion concentrations, and temperature.

Temp. (°C)	**[NaPyr] (M) Initial**	**[HCl] (M) Initial**	**$[H_3O^+]$[a] (M) Final**	**[HPyr] (M) Final**	**$K_{hyd}/(1+K_{hyd})$[b]**	**k_{obs}[c] (s^{-1})**	**k^f[d] (s^{-1})**
5.0	0.0989	0.1908	0.0547	0.0902	0.776	0.186	0.144
5.0	0.0989	0.2862	0.0989	0.0937	0.776	0.294	0.228
5.0	0.0989	0.3816	0.1451	0.0952	0.776	0.384	0.298
5.0	0.0989	0.4770	0.1919	0.0960	0.776	0.477	0.370
10.0	0.0989	0.1908	0.0547	0.0902	0.719	0.262	0.188
10.0	0.0989	0.2862	0.0989	0.0937	0.719	0.370	0.266
10.0	0.0989	0.3816	0.1451	0.0952	0.719	0.508	0.365
10.0	0.0989	0.4770	0.1919	0.0960	0.719	0.655	0.471
15.0	0.0989	0.1908	0.0547	0.0902	0.678	0.363	0.246
15.0	0.0989	0.2862	0.0989	0.0937	0.678	0.518	0.351
15.0	0.0989	0.3816	0.1451	0.0952	0.678	0.704	0.477
15.0	0.0989	0.4770	0.1919	0.0960	0.678	0.895	0.606
20.0	0.0989	0.1908	0.0547	0.0902	0.637	0.502	0.320
20.0	0.0989	0.2862	0.0989	0.0937	0.637	0.728	0.463
20.0	0.0989	0.3816	0.1451	0.0952	0.637	0.960	0.612
20.0	0.0989	0.4770	0.1919	0.0960	0.637	1.243	0.791
25.0	0.0989	0.1908	0.0547	0.0902	0.565	0.691	0.391
25.0	0.0989	0.2862	0.0989	0.0937	0.565	1.004	0.568
25.0	0.0989	0.3816	0.1451	0.0952	0.565	1.300	0.735
25.0	0.0989	0.4770	0.1919	0.0960	0.565	1.685	0.952

[a] Measurements of pH were used in calculating actual $[H_3O^+]$ assuming an activity coefficient, f, of 0.789. The value of the activity coefficient was determined from the expression: $\log f = -[0.49z^2\mu^{1/2}]/(1 + 1.5\mu^{1/2})$ where μ and z stand for the ionic strength and charge, respectively. [b] K_{hyd} is the equilibrium constant for the hydration of pyruvic acid taken from reference 2. [c] k_{obs} is the negative slope of a plot of $\ln(A_t\text{-}A_\infty)$ *vs*. time averaged over four kinetic runs. [d] $k^f = k_{obs}K_{hyd}/(1 + K_{hyd})$.

Highly negative entropies of activation for the hydration would be expected from the requirement of an ordered transition state involving one or more waters of solvation. It is known that the stoichiometry for the hydration of pyruvic acid actually involves multiple molecules of water in which one water molecule covalently bonds to form the gem diol while additional water molecules hydrogen bond to the gem diol [1]. Thus, it is easily conceivable that at the transition state for the spontaneous hydration some stabilization (and structural organization) would occur through more than one water of solvation. Since the entropy of activation for the acid-catalyzed pathway is less negative than the spontaneous reaction, the involvement of additional waters of hydration in the transition state may not be as important.

Similar kinetic investigations have also been carried out on the spontaneous hydrations of the methyl and ethyl esters of pyruvic acid (Table 2) [9]. Again, the values of entropies of activation

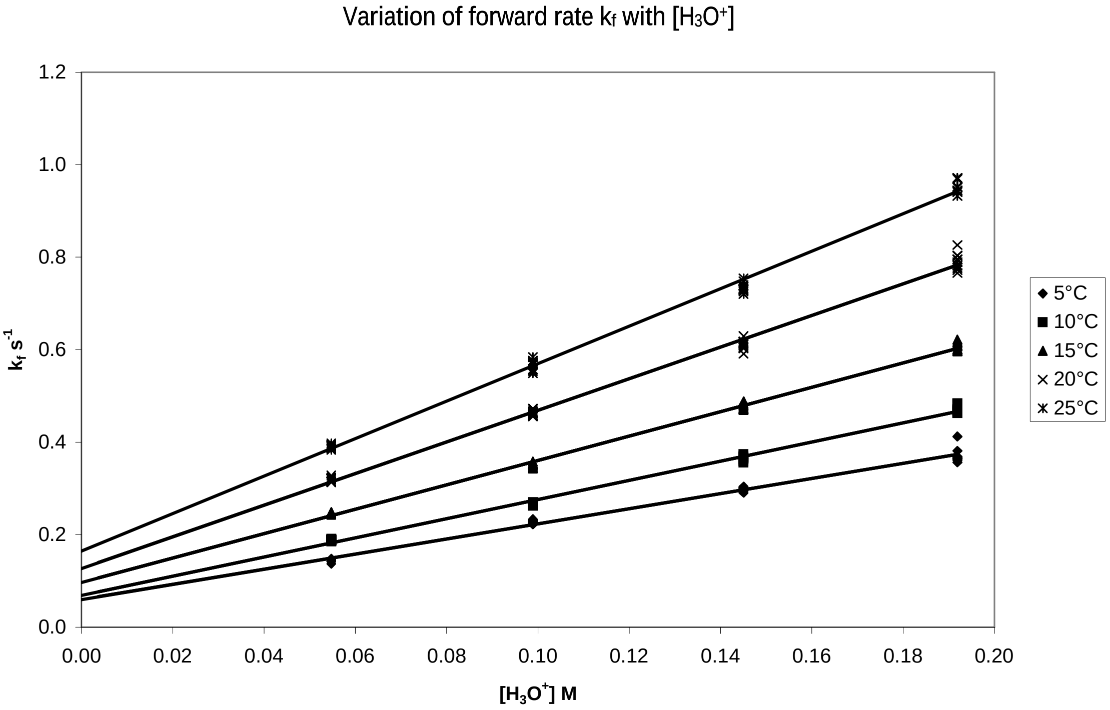


Figure 2. Plot of k^f *vs*. $[H_3O^+]$ for the acid-catalyzed hydration of pyruvic acid at 5.0 °C (◆), 10.0 °C (■), 15.0 °C (▲), 20.0 °C (×) and 25.0 °C (*).

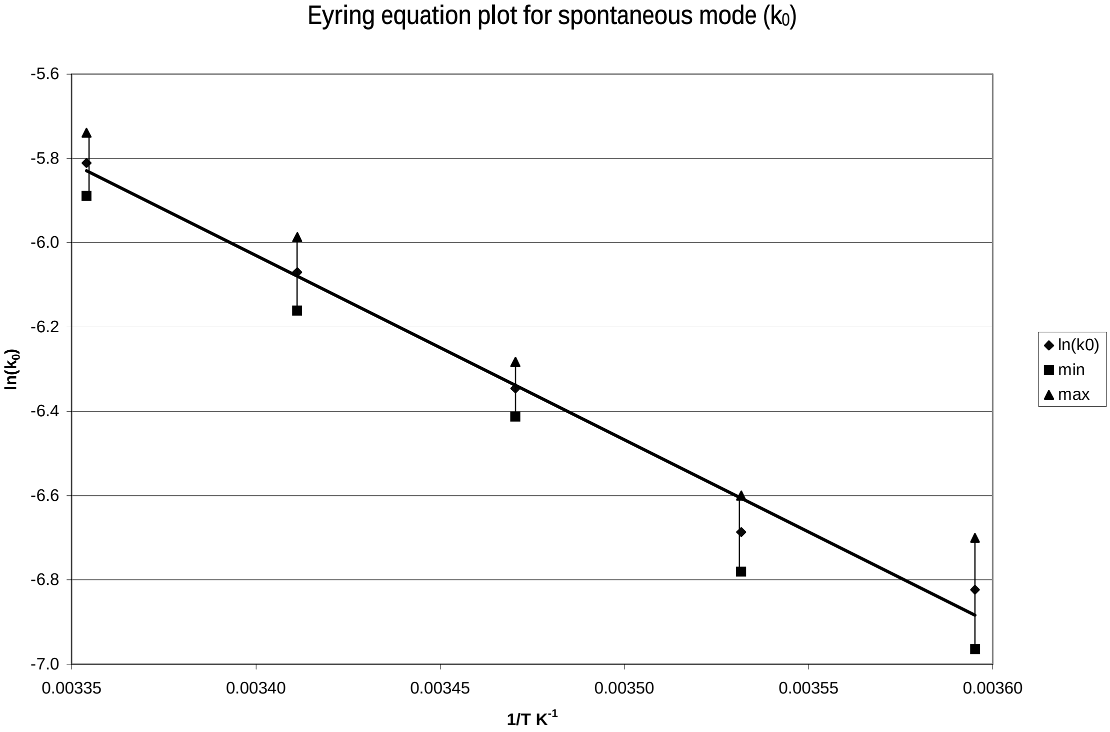


Figure 3. Plot of $\ln k^f_{H_2O}$ *vs*. 1/T for the spontaneous hydration of pyruvic acid.

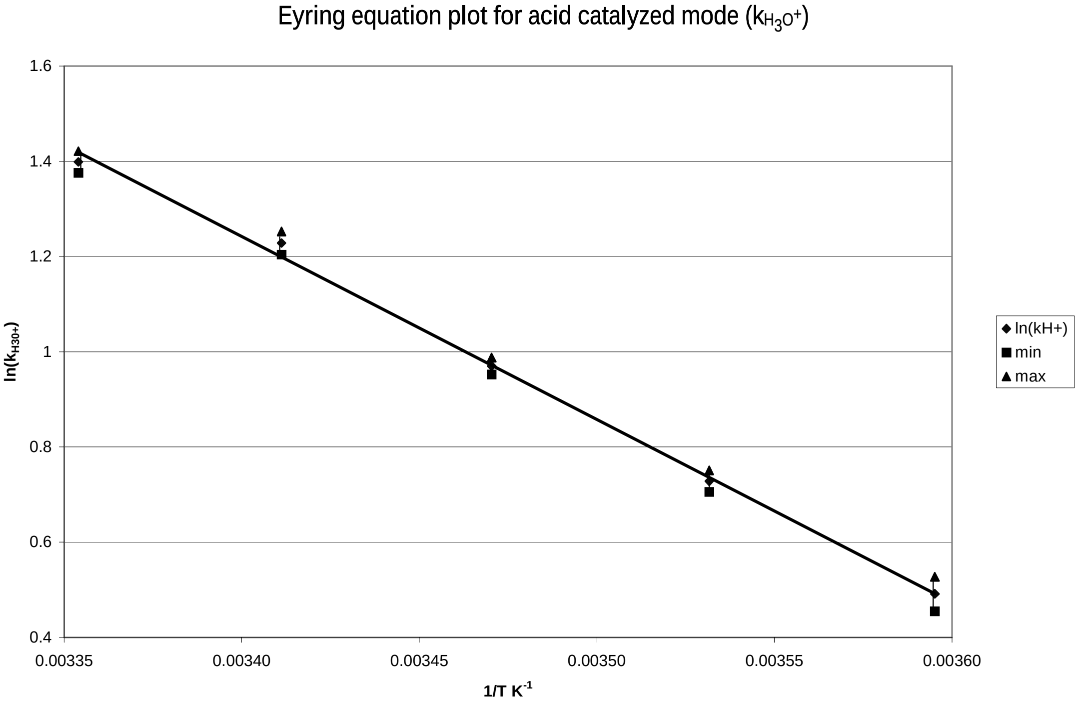


Figure 4. Plot of $\ln k^{f}_{H_3O^+}$ *vs.* 1/T for the acid-catalyzed hydration of pyruvic acid.

Table 2. Activation parameters for the spontaneous and acid-catalyzed pathways of the hydration of pyruvic acid and various pyruvate esters.

Compound	**$\Delta H^{\ddagger}$ ($kJmol^{-1}$)**	**$\Delta S^{\ddagger}$ ($Jmol^{-1}K^{-1}$)**	**$\Delta G^{\ddagger}$ ($kJmol^{-1}$)[a]**
Pyruvic acid[b]	36 ± 7	-171 ± 25	87 ± 10
Pyruvic acid[c]	33 ± 2	-121 ± 7	69 ± 3
Methyl pyruvate[b,d]	31.5	-192	88.7
Ethyl pyruvate[b,d]	31.9	-193	89.4

[a] At 298 K. [b] Taken from second-order rate constants associated with spontaneous reactions. [c] Acid-catalyzed rate. [d] Taken from reference 9. Values of entropies of activation from reference 9 for the pyruvate esters have been corrected to correspond to rate constants in s^{-1} divided by the concentration of water to be compared to the data for pyruvic acid in the present work.

for these esters are highly negative suggesting a demand for a greater degree of orientation during the development of the transition state for the rate determining step for these reactions. Interestingly, it will be noted that these entropies of activation are slightly more negative than that for pyruvic acid. Although these values are close to being within experimental error, a small decrease in the entropy of activation of the alkyl pyruvate esters might be expected as the developing transition state involves more crowding due to the proximity of the carbonyl group (site of addition of water) and the alkyl ester groups.

The relatively rapid spontaneous hydration of pyruvic acid led Strehlow in an earlier report to propose an intramolecular-catalyzed path for the spontaneous reaction in which the nearby carboxyl group of pyruvic acid served as an effective acid catalyst [12]. The similarity of the activation parameters for the spontaneous modes of hydration for pyruvic acid, methyl pyruvate and ethyl pyruvate (Table 2), however, indicates the absence of such an intramolecular acid-catalyzed mode since the potentially catalytic carboxyl proton has been replaced by an alkyl group on the esters. Thus, the large spontaneous reaction rates for the hydrations of pyruvic acid and the pyruvate esters relative to these reactions of other carbonyl compounds are simply due to the electron withdrawing inductive effect of the adjacent carboxyl group [1, 9].

It is also of interest to compare kinetic parameters for the spontaneous hydrations and enolizations of pyruvic acid and its alkyl pyruvate esters. For the enolizations, spontaneous rates and $\Delta S^{\ddagger}$ values for pyruvic acid (-105 $Jmol^{-1}K^{-1}$), methyl pyruvate (-100 $Jmol^{-1}K^{-1}$), and ethyl pyruvate (-100 $Jmol^{-1}K^{-1}$) are almost identical [10]. Again since the kinetic parameters for the enolizations are essentially identical for pyruvic acid and the alkyl pyruvate esters, an intramolecular acid-catalyzed path in the case of pyruvic acid is not a possibility. Thus the more rapid rates of enolization of these compounds than those of other carbonyls is due to the electron withdrawing inductive effect of the ester group facilitating the removal of a β-hydrogen by a water molecule [10].

It will also be noted that the $\Delta S^{\ddagger}$ values associated with the spontaneous enolizations of pyruvic acid and alkyl pyruvates are not as negative as those associated with the corresponding hydration reactions. This would imply the requirement of a less highly ordered transition state in the rate determining step for the spontaneous enolizations than for the corresponding hydrations. Although Alagona *et al*. have questioned the number of water molecules involved in the enolization of pyruvic acid, the less negative entropies of activation suggest that in the enolizations only a single water molecule need be involved in the rate determining step to remove the substrate's β-hydrogens, whereas for hydration multiple water molecules are involved in the formation of a solvated tetrahedral structure [13].

CONCLUSION

The stopped-flow spectrophotometric method used in the present work allowed determination of rapid rates of hydration of pyruvic acid to be made as a function of hydronium ion concentration and temperature. The data show that the hydration of pyruvic acid and alkyl pyruvate esters proceed through parallel mechanisms and, therefore, that an intramolecular acid-catalyzed path for pyruvic acid is nonexistent. Thus it is established that the rather rapid rate of hydration, in comparison to the corresponding reaction of other carbonyl compounds, is due to the electron withdrawing inductive effect stabilizing the transition state formed in the rate determining step of this hydration reaction.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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