

The Reversible Hydration of Pyruvic Acid. I. Equilibrium Studies¹

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The position of equilibrium for the reversible hydration of pyruvic acid was determined using an nmr technique. Ratios of the hydrated to unhydrated forms in aqueous solutions were determined as a function both of temperature and pH by comparing the relative areas of signals derived from the hydrated and unhydrated forms. These appeared at 2.58 and 3.44 ppm, respectively, upfield from the nmr signal of water which was used both as the lock and internal reference. The temperature studies which were carried out at pH 0.35 allowed the evaluation of the thermodynamic parameters for the hydration of pyruvic acid from a plot of $\log K$ vs. $1/T$: $\Delta G^\circ = -0.24 \text{ kcal mol}^{-1}$, $\Delta H^\circ = -7.8 \text{ kcal mol}^{-1}$, and $\Delta S^\circ = -26.8 \text{ eu}$. The variation of the fraction of hydration, χ , with pH clearly showed that although the hydration of pyruvic acid is favorable ($\chi_{0.0^\circ} = 0.82$, $\chi_{25.0^\circ} = 0.61$) the corresponding hydration of pyruvate anion is slight ($\chi_{0.0^\circ} = 0.17$, $\chi_{25.0^\circ} = 0.06$). This dependency of the extent of hydration upon the relative concentration of pyruvic acid and pyruvate anion is quantitatively illustrated by plots of the fraction of hydration vs. pH at 0.0° and 25.0°. The resulting sigmoid curves showing maxima at low pH reveal points of inflection at pH 2.2 which correspond to the pK_a of pyruvic acid.

Introduction

Pyruvic acid occupies a central position in the metabolism of carbohydrates. It constitutes, moreover, a link between the metabolism of carbohydrates and that of proteins, for it arises from the products of deamination of a number of amino acids.^{3,4} Consequently, the fate of pyruvic acid when introduced into aqueous solutions has long been of physiological as well as chemical interest. Unfortunately, studies of the ultraviolet spectra of pyruvic acid in solutions have led to somewhat inconclusive interpretations.⁵ Recently, however, Becker⁶ has shown by nmr experiments that around pH 1 less than 2% of the total concentration of pyruvic acid exists in the enol form. Gold, *et al.*,^{7a} Griffiths and Socrates,^{7b} and Becker⁶ using the nmr technique, and Eigen, *et al.*,^{8a} and Strehlow^{8b} using relaxation methods have determined the relative concentrations of pyruvic acid and its hydrate at pH < 1; however, few or no data have been reported for this hydration at pH values which are generally employed in the study of enzymatic catalysis.

Recently, investigations in these laboratories have considered the spontaneous and the acid-catalyzed hydration of pyruvic acid as well as the metal-ion and the enzymatically catalyzed dehydration of 2,2-dihydroxypropionate anion.^{9,10} As a prelude toward these kinetic investigations we have sought to evaluate the equilibrium position for the hydration process as a function both of pH and of temperature using the nmr technique. Because of the reversibility of this process, such determinations are necessary for the quantitative analysis of our kinetic data. In much of our previous work^{11,12} we were able to evaluate the fractions of hydration of aliphatic aldehydes spectrophotometrically by a comparison of the absorbancy of the substrates at zero reaction to that observed at the kinetic infinity. However, be-

cause of the rapidity of the reaction presently under investigation, it is difficult to determine the extinction coefficient of the completely unhydrated form of pyruvic acid in aqueous media. The determination of the extinction coefficient in inert solvents may be of only qualitative use since it is often observed that carbonyl absorptions which have little tendency to hydrate are stronger in water than in hydrocarbon solvents.¹³ Thus, the nmr method has the advantage in that the relative areas of the signals associated with the hydrated and unhydrated forms of pyruvic acid (and its anion) may be accurately obtained through integration even at relatively high temperatures or values of pH at which the very rapid kinetics of the process preclude the determination of the equilibrium position through extrapolation to initial absorbancy.

In addition to the necessity of these data in connection with our kinetic studies, the present experiments

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also allow the evaluation of the standard free energies, ΔG° , heats, ΔH° , and entropies, ΔS° , of hydration and the pK_a of pyruvic acid.

Experimental Section

Pyruvic acid was purchased from Baker Analyzed Products and was twice distilled through a Vigreux column in the presence of nitrogen. The middle fraction (bp 68–69° (18 mm)) from the second distillation was used shortly after its purification in order to avoid the formation of appreciable quantities of polymer. The second distillation was carried out because small amounts of acetic acid were detected in the distillate from early fractions. Thus, the nmr spectrum of aqueous solutions of these early fractions showed the two expected signals arising from the methyl hydrogens associated with pyruvic acid (2.58 ppm) and its conjugate hydrate (3.44 ppm) as well as a third peak in between (2.76 ppm) the intensity of which increased upon addition of small quantities of acetic acid. The pyruvic acid solutions employed in this work were titrated to the desired acid or base strength using concentrated hydrochloric acid or sodium hydroxide such that the total concentration of hydrated and unhydrated pyruvic acid and its anion was 1.3 *M*.

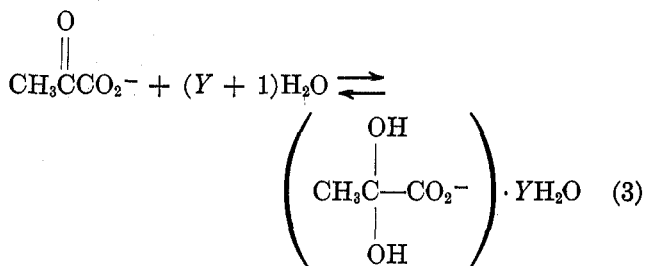
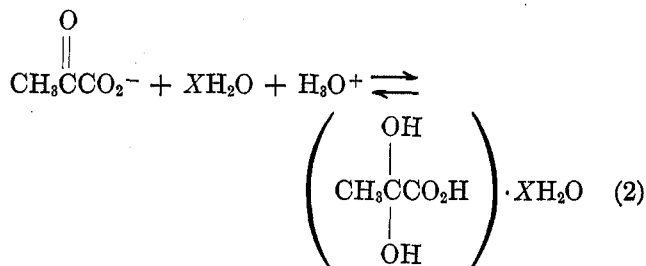
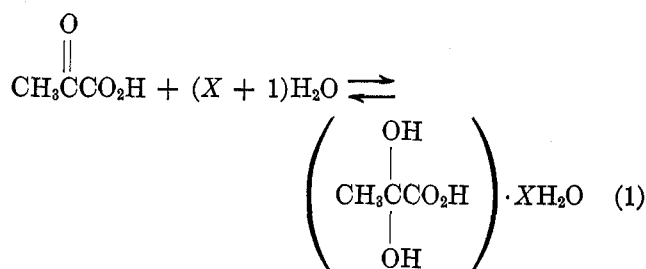
Measurements of pH were carried out on a Beckman 101900 research pH meter. The nuclear magnetic resonance spectra were determined on a Varian Model DA-60IL spectrometer using the frequency sweep mode. Since aqueous solutions were employed in these investigations, the nmr signal of water was utilized both as the lock and internal reference. An arbitrary ppm scale was used with the water signal assigned a value of zero because variations in concentration and temperature would be expected to alter its exact position. Temperatures were controlled to within $\pm 0.50^\circ$ by means of a variable-temperature accessory. Where confirmation of the results obtained by nmr was possible, a Gilford high-speed recording spectrophotometer was employed.

The relative amounts of the hydrated and unhydrated forms of pyruvic acid under the various conditions of temperature and pH investigated were deduced through integration of the spectra of pyruvic acid (or its anion) in equilibrium with its hydrate (or its anion). In general, these measurements were obtained shortly after the solutions were mixed although it was observed that no variation in spectra occurred when the runs were repeated a day later. When possible, the fraction of hydration, χ , of pyruvic acid was deduced spectrophotometrically. Kinetic runs, initiated by injecting neat pyruvic acid into the cuvettes, were carried out in which the initial absorbancy of pyruvic acid, A_0 , was determined by extrapolation to zero reaction and the final absorbancy, A_∞ , was that observed at equilibrium: $\chi = (A_0 - A_\infty)/A_0$. Typically, such a spectrophotometric determination at 340 m μ , pH 1.19 at 0.0° resulted in a fraction of hydration, $\chi = 0.80$. The frac-

tion of hydration obtained through nmr measurements at 0.0° gives the value $\chi = 0.81$ at the corresponding pH. However, such spectrophotometric determinations were generally less accurate since the reaction rate was often so rapid that it was impractical to deduce A_0 accurately from extrapolations of absorbancy to zero reaction.

Results and Discussion

The reversible hydration of pyruvic acid is somewhat more complex than other reversible hydration processes which have been investigated previously in these laboratories^{11,12} because of the acid properties of both pyruvic acid ($pK_a = 2.18$)⁶ and its hydrate ($pK_a = 3.6$).¹⁴ Thus, depending upon the pH of the reaction media, there are three possible equilibria which must be considered



At values of pH considerably lower than pH 2.18, the system is limited to equilibrium 1. As the pH is increased to around the pK_a of pyruvic acid, there exist comparable quantities of pyruvic acid and its conjugate base so that both equilibria 1 and 2 are operative. This would be expected to result in a decrease in the extent of hydration since the electron-withdrawing influence of the unprotonated carboxylate group is weak. As the pH of the media continues to increase, equilibrium 3 becomes operative with the formation of the 2,2-dihy-

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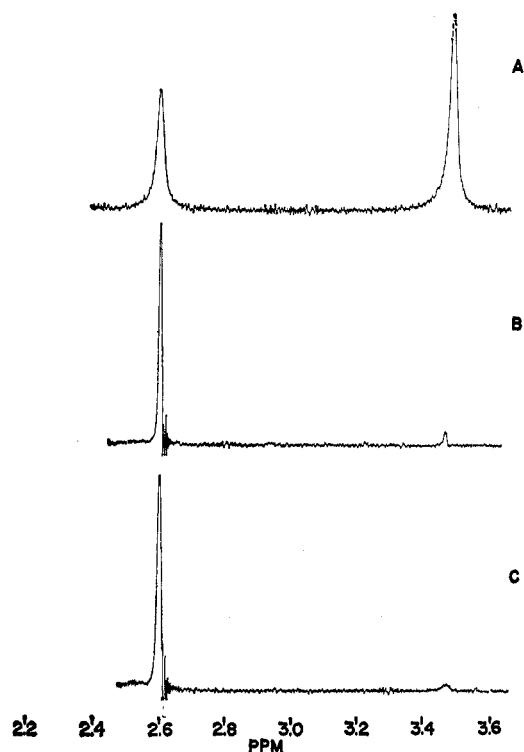


Figure 1. A, nmr spectrum of pyruvic acid in H_2O at 25.0° , pH 0.35; B, nmr spectrum of pyruvic acid in H_2O at 25.0° , pH 4.22; C, nmr spectrum of pyruvic acid in H_2O at 25.0° , pH 6.22.

droxypropionate anion. Again, the forward process is not favored for reasons stated above.

The nmr spectra of aqueous solutions of pyruvic acid at 25.0° are shown in Figure 1 at pH 0.35, 4.22, and 6.22. These spectra show the appearance of two peaks at 2.58 and 3.44 ppm which represent the signals arising from the methyl hydrogens associated with the hydrated and unhydrated forms, respectively. The relative positions of these peaks are consistent with this assignment since the shielding of the methyl hydrogens of the hydrate would be expected to be greater than that associated with the corresponding methyl hydrogens in the unhydrated form. It will be noted that at pH 0.35 a considerable quantity of the hydrate exists whereas at pH 4.22 and 6.22 the extent of hydration has markedly decreased following the deprotonation of pyruvic acid.

The variation in the fraction of hydration, χ , with pH was studied quantitatively as a function of pH in the range pH 0.35 to pH 6.22 at both 0.0° and 25.0° . When χ is plotted against pH, the data listed in Table I give rise to sigmoid curves having points of inflection at pH 2.2 as shown in Figure 2. These curves show maxima at low pH of $\chi^{\max}_{0.0^\circ} = 0.82$ and $\chi^{\max}_{25.0^\circ} = 0.61$ while at higher values of pH very low fractions of hydration are obtained: $\chi^{\min}_{0.0^\circ} = 0.17$ and $\chi^{\min}_{25.0^\circ} = 0.06$. These latter values appear to remain constant with further increases of pH. Because the fraction of hydration of pyruvic acid is so intimately related to the

Table I: Fraction of Hydration, χ , and Equilibrium Constant, $K = \chi/(1 - \chi)$, for the Hydration of Pyruvic Acid as a Function of pH at 25.0° and 0.0°

pH	25.0°		0.0°	
	K	χ	K	χ
0.35	1.55	0.608	4.42	0.816
1.13	1.39	0.583	4.42	0.816
1.50	1.06	0.514	3.27	0.764
1.95	0.711	0.416	1.64	0.622
2.53	0.294	0.227	0.593	0.372
2.92	0.163	0.140	0.303	0.233
3.47	0.0667	0.0624	0.213	0.176
4.29	0.0726	0.0677	0.207	0.172
4.51	0.0500	0.0476		
6.22	0.0572	0.0540		

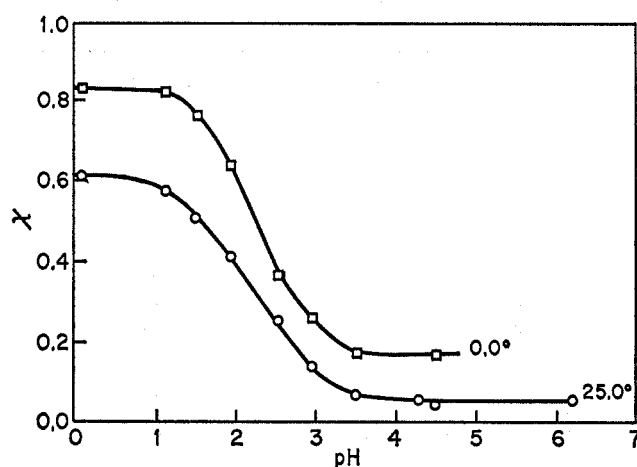


Figure 2. Fraction of hydration, χ , plotted against pH for the hydration of pyruvic acid: \square , data at 0.0° ; \circ , data at 25.0° .

relative concentrations of the acid and its conjugate base, these curves resemble the titration of a weak acid. The points of inflection represent the maximum variation of the fraction of hydration with pH which in turn is caused by the rapidly changing ratio of pyruvic acid to its anion. Thus the value of pH 2.2 at the point of inflection is in complete accord with the pK_a of pyruvic acid as obtained polarographically by Strehlow^{8b} ($pK_a = 2.18$). The close agreement between the values of pK_a as obtained by the two methods is further substantiation of the quantitative nature of the data resulting from the nmr measurements.

The equilibrium constant for the hydration of pyruvic acid, $K = [\text{hydrate}]/[\text{pyruvic acid}]$, was determined as a function of temperature. These studies were conducted using solutions of pH 0.35 so that almost 99% of the total concentration of pyruvic acid existed in its protonated form. Table II summarizes the data which were obtained using a total concentration of pyruvic acid of 1.3 M over a temperature range between 0.0° and 50.0° . These data allowed the evaluation of the thermodynamic parameters for the hydration. From the linear plot of $\log K$ against $1/T$ we deduced the standard

Table II: Dependency of $K = [\text{hydrate}]/[\text{pyruvic acid}]$ upon Absolute Temperature. Total Pyruvic Acid Concentration = 1.3 M

T , °K	$1/T \times 10^3$, °K ⁻¹	$[\text{hydrate}]/$ $[\text{pyruvic acid}]$	Log $[\text{hydrate}]/$ $[\text{pyruvic acid}]$
273	3.66	4.42	0.645
278	3.60	3.47	0.540
284	3.52	2.56	0.408
289	3.46	2.10	0.322
294	3.40	1.75	0.243
297	3.37	1.50	0.176
299	3.34	1.30	0.113
304	3.29	1.06	0.025
314	3.18	0.70	-0.155
324	3.09	0.47	-0.328

Table III: Thermodynamic Parameters for the Hydration of Carbonyl Compounds at 298.2°K

	$-\Delta H^\circ$, kcal mol ⁻¹	$-\Delta G^\circ$, kcal mol ⁻¹	ΔS° , eu
Formaldehyde ^a	14.6	5.4	-30.8
Chloral ^b	12.7	3.68	-30.2
Pyruvic acid	7.8	0.24	-26.8
s-Dichloroacetone	5.7	1.27	-14.8
as-Dichloroacetone	5.5	0.62	-16.4
Acetaldehyde ^c	5.1	0.23	-16.4
Diacetyl ^b	4.5	0.72	-12.7
4-Pyridinecarboxaldehyde ^d	4.2	0.08	-13.9
2-Pyridinecarboxaldehyde ^d	3.8	-0.35	-13.8
Monochloroacetone ^b	2.0	-0.28	-7.7

^a R. Bieber and G. Trumpler, *Helv. Chim. Acta*, **30**, 1860 (1947). ^b R. P. Bell and A. O. McDougall, *Trans. Faraday Soc.*, **56**, 12 (1960). ^c R. P. Bell and J. C. Clunie, *Trans. Faraday Soc.*, **48**, 440 (1952). ^d Y. Pocker, J. E. Meany, and B. J. Nist, *J. Phys. Chem.*, **71**, 4509 (1967).

free energy ΔG° , heat ΔH° , and entropy of hydration ΔS° , as -0.24 kcal mol⁻¹, -7.8 kcal mol⁻¹, and -26.8 eu, respectively. Becker⁶ has estimated quantities of similar magnitude for this process using a total pyruvic concentration of 2 M although his determinations were made at only two temperatures. The thermodynamic parameters associated with the hydration of pyruvic acid are compared with the similar quantities for the hydration of other carbonyl compounds in Table III.

The high negative entropy of hydration as observed from the present investigation may be indicative of a highly structured form of the hydrate of pyruvic acid. This may include the bridging of one or more molecules of water through hydrogen bonding. Indeed, a third-power dependence of the ratio of 2,2-dihydroxypropionic to pyruvic acid on the water concentration has previously been postulated^{6,7a} which implies that the stoichiometry of the hydrate involves two additional molecules of water. Data from the present work would

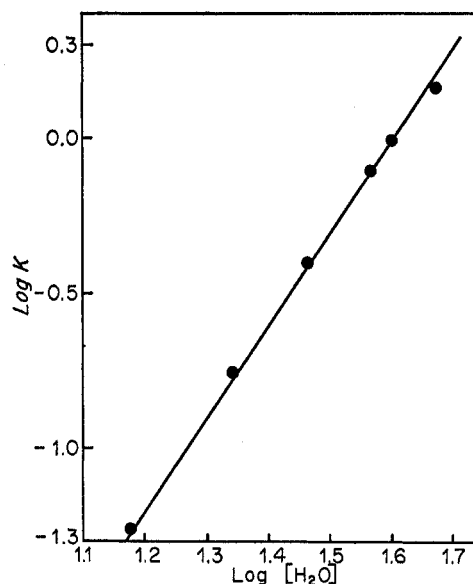


Figure 3. Log K plotted against log $[\text{H}_2\text{O}]$; slope of straight line 3.0. Points are deduced from our own data at 26.0° (see Table IV, ref d).

Table IV: Third-Order Dependence of the Equilibrium Ratio, $K = [\text{hydrate}]/[\text{pyruvic acid}]$, on Water Concentration^a at 26°

[pyruvic acid], mol l. ⁻¹	$[\text{hydrate}]/$ $[\text{pyruvic acid}]$	$K_{\text{eq}} =$ $K/[\text{H}_2\text{O}]^3$	Ref
0.00	2.310	1.54×10^{-5}	a, b
0.00	1.940	1.30×10^{-5}	c
1.30	1.360	1.30×10^{-5}	a, d
1.35	1.600	1.52×10^{-5}	a, d
1.65	1.480	1.59×10^{-5}	a, d
1.81	1.420	1.61×10^{-5}	a, d
3.18	1.000	1.61×10^{-5}	a, d
4.17	0.770	1.57×10^{-5}	a, d
4.20	0.610	1.35×10^{-5}	e
6.25	0.400	1.63×10^{-5}	a, d
8.34	0.177	1.61×10^{-5}	a, d
11.14	0.054	1.59×10^{-5}	a, d

^a The concentration of "free" water was calculated using the assumption that each dissociated proton in these solutions "binds" four water molecules to form H_3O_4^+ . In addition, account was taken of the fact that the equilibrium ratio, K , varies with the third power of the water concentration; i.e., the formal concentration of "free" water used in calculating K_{eq} was deduced from the equation $[\text{H}_2\text{O}] = [\text{H}_2\text{O}]_{\text{total}} - 4[\text{H}^+] - 3[\text{hydrate}]$. ^b From data reported in ref 9; $[\text{H}_3\text{O}_4^+] = 0.6$ M. ^c From data reported in ref 7a. ^d Present work; hydrochloric acid was added to bring all solutions to a constant hydronium ion concentration, $[\text{H}_3\text{O}_4^+] = 0.5$ M. ^e From data reported in ref 7b.

seem to confirm these findings. Table IV compares our values of

$$K_{\text{eq}} = \frac{[\text{hydrate}]}{[\text{pyruvic acid}][\text{H}_2\text{O}]^3} = K \frac{1}{[\text{H}_2\text{O}]^3}$$

to those obtained from other workers, and Figure 3 shows that by plotting our own data in the form of log K vs. log $[\text{H}_2\text{O}]$ a straight line of slope 3 is obtained.