Acetopyruvic Acid. Rate and Equilibrium Constants for Hydration and Enolization[1]

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Received September 14, 1971

Abstract: The behavior of acetopyruvic acid (2,4-dioxovaleric acid) in aqueous solution has been studied; equilibrium constants relating to enol, keto, and hydrate forms have been measured, as have the acid dissociation constants of the three forms: $pK_a = 2.79$, 1.30, and 3.21, respectively. The rates of hydration and dehydration have been measured between pH 2 and 7, and limits placed on the rate of enolization.

Acetopyruvic acid (2,4-dioxovaleric acid) has been known for some time as a very potent inhibitor of acetoacetate decarboxylase.[2,3] The inhibition has been shown to involve reaction of acetopyruvate with the enzyme to form a covalent product whose spectral properties suggest that it is an enamine.[3]

It was thus of considerable interest to study the reactions of a simple model compound with acetopyruvate, in the hope that comparisons of rates and equilibria would lead to better understanding of the chemistry of the enzyme. However, it rapidly became clear that acetopyruvate alone in aqueous solution is a complex mixture. The species which were found to be present are the diketo form of acetopyruvic acid, and its carboxylate anion, referred to as *keto*, the enol form of both acid and anion, *enol*, and the monohydrate, where a molecule of water has added to the α-keto group of the acid or anion, *hydrate*. The second pK_a of acetopyruvate was measured and found to be 7.6; thus, at pH values much above 6, the enolate will be present in significant concentration. The determination of equilibrium constants relating these species, and the rate constants for hydration and dehydration, will be reported in this paper; the reactions of acetopyruvate with a simple amine will be reported in the accompanying paper.[4]

Experimental Section

Materials and Methods. Acetylacetone was freshly distilled before use. Chloroacetic acid and cyanoacetic acid were recrystallized; for other buffers, reagent grade chemicals were used without further purification. Ionic strength was adjusted with KCl. All pH values were recorded using a Radiometer pH Meter 4. Hamilton microliter syringes were calibrated by repeated weighing of the amount of distilled water contained at a given setting; reproducibility was found to be 0.25%.

Acetopyruvic Acid (APY). A mixture of ethyl acetopyruvate[5] (100 g, 0.63 mol) and acetone (50 ml) was cooled to 0° (Dry Ice–acetone bath) and 253 ml of 5 *N* NaOH was added slowly. After stirring 4 hr at 0°, 212 ml of 6 *N* H_2SO_4 was added, and the mixture was filtered. The filtrate was extracted continuously with ether overnight and the residue after evaporation was sublimed three times *in vacuo*, giving 23.3 g (29% yield) of almost colorless product. APY was recrystallized from CCl_4: mp 98–100° (lit.[6] mp 98°). Lithium acetopyruvate (LiAPY) was prepared by careful neutralization of APY with lithium hydroxide, and was purified by precipitation from methanol with anhydrous ether.

Nmr Spectra of APY in Buffered Aqueous Solution. APY or LiAPY was dissolved in a suitable buffer (formate, HCl, or NaOH), containing *ca.* 0.02 *M tert*-butyl alcohol as internal nmr standard, to give solutions with ionic strength 0.6 *M* and total acetopyruvate 0.4 *M*. Spectra were recorded, with 50-Hz sweep width, on a Varian Associates HA-100 nmr spectrometer. The pH values of these solutions were recorded at the conclusion of the experiment. Spectra of APY at the higher pH values showed additional small peaks, which grew slowly with time, attributed to products of aldol condensation; these were not included in integrations. Peak areas were measured using a planimeter (Keuffel and Esser, No. 620005). Error limits were calculated from the thickness of the base line and the width of the peaks. For the spectra at pH 4.3 and 6.1, the two highest field peaks were not resolved, so it was necessary to run spectra at 60 MHz using a Varian A-60 spectrometer from which the ratio of high-field (hydrate) methylene to total methyl signal could be obtained. From this ratio, the areas of the unresolved higher field (enol and hydrate) methyl signals could be calculated.

Kinetics of Hydration. Chloroacetate and cyanoacetate buffers were made up on the same day they were used; APY solutions were adjusted to pH 11 with 0.1 *M* NaOH. A Cary-15 was used, and optical density was followed at 285 nm. This is the absorption maximum of APY and is attributed to the enol, since neither keto nor hydrate forms should absorb at this wavelength. Thus, the formation of the hydrate is followed by watching the disappearance of the enol. Buffer (3.0 ml) was placed in a 1.0-cm cell and equilibrated for 15 min at 30°. Reaction was initiated by adding 0.2 ml of APY stock solution. Rate constants were evaluated from plots of $A - A_\infty$ *vs.* t on semilogarithmic paper. Because the change in absorbance is small, the absorbance is high (A_∞ is about 1), and the rate of the reactions is rapid, the rate constants are inaccurate. The uncertainty in the rate constants was estimated by assigning error bounds of 0.003 Å to the $A - A_\infty$ points, and drawing the extreme lines which would fit the data within these limits. An apparent equilibrium constant (which is really a complicated function of microscopic equilibrium constants) can be calculated as

$$K = \frac{A_0 - A_\infty}{A_\infty} = \frac{[\text{keto}]_\infty + [\text{hydrate}]_\infty}{[\text{enol}]_\infty}$$

Limits can be placed on K by considering the extreme values of $(A_0 - A_\infty)$ from the lines drawn to place limits on the rate constant.

Spectrophotometric Titration of Acetopyruvate. The first pK_a of APY is 2.58[6]; thus, the carboxylic acid group of APY is completely ionized at pH values higher than 5. The second pK_a of APY was evaluated from extinction coefficients at 295 nm; see Table I.

Rates of Enolization of Acetylacetone and APY. The buffers were acetic acid–sodium hydroxide, brought to ionic strength 0.1 with KCl. A stock solution of iodine, 0.0195 *M*, in KI, 0.151 *M*, was

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(1) Abstracted in part from the Ph.D. Thesis of J. P. G. (1968). Supported by Grant No. GM 04712 from the Institute of General Medical Sciences of the National Institutes of Health, and by a National Research Council of Canada Special Scholarship.

(2) (a) R. Davies, *Biochem. J.*, 37, 230 (1943); (b) R. Colman, Ph.D. Thesis, Radcliffe College, Cambridge, Mass., 1962.

(3) W. Tagaki, J. P. Guthrie, and F. H. Westheimer, *Biochemistry*, 7, 905 (1968).

(4) J. P. Guthrie, *J. Amer. Chem. Soc.*, **94**, 7024 (1972).

(5) C. S. Marvell and E. E. Dreger, "Organic Syntheses," Collect. Vol. I, Wiley, New York, N. Y., 1941, p 238.

(6) A. L. Lehninger and E. J. Witzemann, *J. Amer. Chem. Soc.*, **64**, 874 (1942).

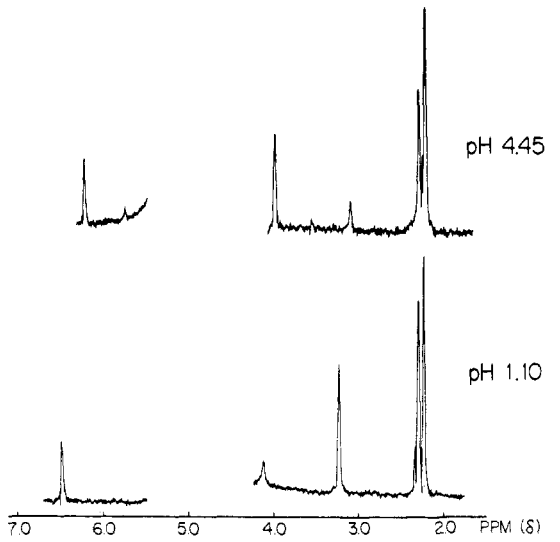


Figure 1. 60-MHz nmr spectrum of acetopyruvate in aqueous solution.

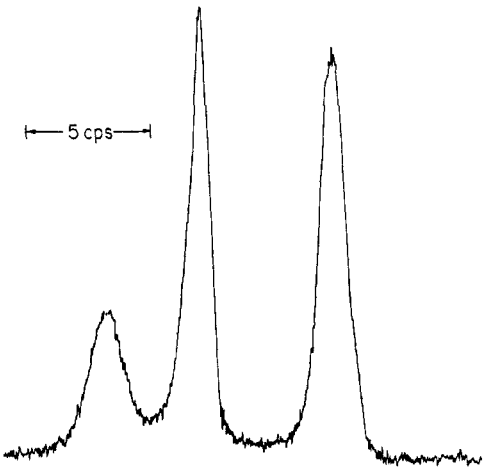


Figure 2. 100-MHz nmr spectrum of the signals from the methyl groups of acetopyruvate at pH 1.62.

Table I. Spectrophotometric Titration of APY

pH	ϵ_{app},[a] M^{-1} cm^{-1}	Log x[b]	pK_{a_2}	
5.76	4,080[c]			
6.71	6,100	0.802	7.51	av 7.70 ± 0.16[e]
7.69	12,100	−0.072	7.76	
9.01	18,000	−1.188	7.82	
9.88	18,800	−2.171	7.71	
10.85	18,900[d]			

[a] At 295 nm. [b] $x = \{(\epsilon_{A^{2-}} - \epsilon_{app})/(\epsilon_{app} - \epsilon_{HA^-})\}$. [c] Used as ϵ_{HA^-}. [d] Used as $\epsilon_{A^{2-}}$. [e] Lit.[6] 8.5. It is not clear how the value of 8.5 for pK_{a_2} in ref 6 was arrived at. Furthermore, these workers state that, at pH 7.4, 1.27 equiv of NaOH had reacted during a potentiometric titration; this corresponds to $pK_{a_2} = 7.8$.

prepared. Stock solutions were prepared of the diketones in 0.00174 *M* acetate buffer and brought to ionic strength 0.1 with KCl. Buffer (3.0 ml) in a 1.0-cm uv cell was equilibrated for 15 min at 30°; 100 μl of iodine solution and then 50 or 100 μl of diketone solution were added. The disappearance of triiodide absorption at 351 nm was recorded.

Calculations. The equilibrium constants for the keto–enol–hydrate system were evaluated using a rigorous nonlinear least-squares procedure based on that described by Wentworth,[7,8] but suitably modified, since in this case two equations (1 and 3, defined below) were fitted simultaneously. Estimated standard deviations for the parameters and for functions of the parameters were calculated from the covariance matrix.[7,8]

Results

The nmr spectrum of acetopyruvate in $CDCl_3$ shows only signals attributable to the enol form:[9] a singlet for the methyl at δ 2.30 and one for the vinyl proton at δ 6.50. In aqueous solution, however, there are *three* singlets at *ca.* δ 2.3 where the methyl group is expected to absorb, two singlets at *ca.* δ 4.1 and 3.2, attributable to methylenes, and a singlet at *ca.* δ 6.5 attributable to an olefinic proton. Furthermore, the positions and relative intensities of these peaks are pH dependent. It is quite apparent from Figure 1 that of the two methylene signals, the one at higher field predominates at low pH, while the one at lower field predominates at pH values above 3; the relative intensity of the signal from the olefinic proton is much less sensitive to pH. The two methylene-containing species are identified as the keto tautomer and the hydrate resulting from addition of water to the α-carbonyl group of acetopyruvate. It is known that pyruvic acid is hydrated to a considerable extent in water, and that the anion forms a hydrate to a much smaller extent.[10,11] The methylene signal at higher field (which is larger at low pH) may be assigned to the hydrate, and the one at lower field to the keto form.

Since the methylene of the keto form absorbs quite close to the massive solvent water signal, simple integration of the spectrum could not be used to determine the composition of the mixture. D_2O cannot be used as solvent because exchange rapidly washes out the methylene protons.

At 100 MHz, the signals from the methyl groups of the three species were resolved at pH <4; see Figure 2. Of the three signals the one at highest field is relatively large in acid solution and becomes much smaller as the pH is raised. The one at lowest field is very small in acid solution and becomes relatively large as the pH is raised. These changes parallel those observed for the peaks assigned to the methylene protons. Therefore, the highest field methyl signal is assigned to the hydrate form; the lowest field peak is assigned to the keto form, and the peak at intermediate position is assigned to the enol form. As the pH is raised the separation between the signals from the enol and hydrate forms decreases, until for pH >4 they are no longer well resolved. However, the area of the composite peak can be factored by integrating the signal from the hydrate methylene and the signals from the methyl groups of all three forms.

It is possible to obtain relative amounts of the three forms as a function of pH by integration of nmr spectra of acetopyruvate, as shown in Table II, and, from these data, the following apparent equilibrium constants: K_1^{app} = [keto]/[enol]; K_2^{app} = [hydrate]/[keto]; K_3^{app}

(7) W. E. Wentworth, *J. Chem. Educ.*, 42, 96, 162 (1965).
(8) W. E. Deming, "Statistical Adjustment of Data," Dover Publications, New York, N. Y., 1964.
(9) In principle several forms of the enol are possible, with the enolic proton on the α or γ oxygen, and with cis or trans geometry about the double bond; under the conditions of the experiments reported here these are indistinguishable.
(10) M. Becker and H. Strehlow, *Ber. Bunsenges. Phys. Chem.*, 64, 813 (1960).
(11) M. Becker, *ibid.*, 68, 669 (1964).

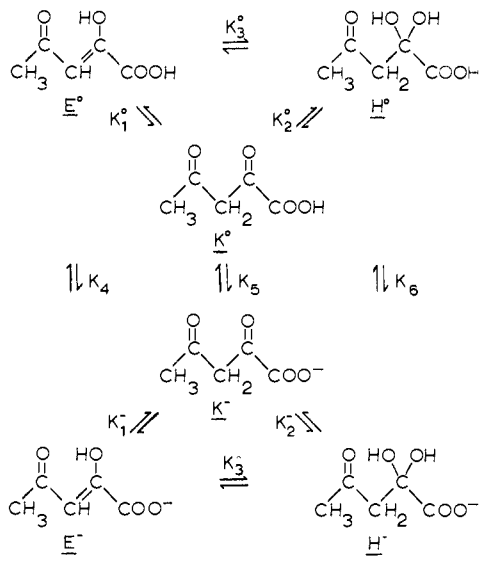
Table II. Areas of Peaks in 100-MHz Spectra of APY and Apparent Equilibrium Constants Relating Keto, Enol, and Hydrate Forms[a]

pH	Area of peaks, as measured by planimeter—— K	E	H	K_1^{app}	K_2^{app}	K_3^{app}
0.43	3.9 ± 0.8	15.5 ± 0.8	20.4 ± 1.4	0.252 ± 0.053	5.23 ± 1.10	1.32 ± 0.11
0.90	5.8 ± 1.0	18.2 ± 0.8	22.7 ± 1.3	0.319 ± 0.061	3.91 ± 0.71	1.25 ± 0.087
1.62	12.4 ± 1.2	23.2 ± 1.0	24.5 ± 1.0	0.534 ± 0.057	1.98 ± 0.21	1.06 ± 0.063
2.96	24.0 ± 1.8	26.6 ± 1.5	10.8 ± 1.4	0.866 ± 0.080	0.450 ± 0.068	0.390 ± 0.055
4.33	46.2 ± 2.4	47.3 ± 3.1[b]	6.0 ± 2.0[b]	0.975 ± 0.083	0.129 ± 0.044	0.126 ± 0.044
6.17	27.4 ± 2.8	33.0 ± 3.0[b]	3.8 ± 1.2[b]	0.830 ± 0.108	0.139 ± 0.046	0.115 ± 0.037

[a] Aqueous solution 28°, μ = 0.6 *M*. [b] E + H peaks were not resolved at 100 MHz; the area of E + H was factored using the ratio of hydrate to total APY measured in separate experiments using 60-MHz nmr spectra; for pH 4.3, E + H = 53.3 ± 2.4, hydrate/total = 0.060 ± 0.020; for pH 6.2, E + H = 36.8 ± 2.8, hydrate/total = 0.059 ± 0.17.

= [hydrate]/[enol]. The values so calculated are shown in Table II. Using the following relationships (with symbols defined by Scheme I), K_1^0 = [K⁰]/[E⁰],

Scheme I



K_2^0 = [H⁰]/[K⁰], K_3^0 = [H⁰]/[E⁰], K_4 = [E⁻]([H⁺]/[E⁰]), K_5 = [K⁻]([H⁺]/[K⁰]), and K_6 = [H⁻]([H⁺]/[H⁰]), one obtains

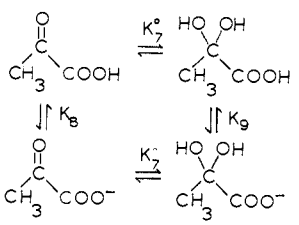
$$K_1^{app} = K_1^0([H^+] + K_5)/([H^+] + K_4) \quad (1)$$

$$K_2^{app} = K_2^0([H^+] + K_6)/([H^+] + K_5) \quad (2)$$

$$K_3^{app} = K_3^0([H^+] + K_6)/([H^+] + K_4) \quad (3)$$

The microscopic equilibrium constants were then obtained by fitting eq 1 and 3 to the experimental data by the method of weighted nonlinear least squares. This procedure led to the following results: pK_4 = 2.79 ± 0.14, pK_5 = 1.30 ± 0.17, pK_6 = 3.21 ± 0.18, K_1^0 = 0.299 ± 0.040, K_2^0 = 4.40 ± 0.65, and K_3^0 = 1.32 ± 0.070. These values may be compared with Becker's[10,11] results on pyruvate (with constants defined by Scheme II): K_7^0 = 1.85, pK_8 = 2.07, pK_9 = 3.60. Thus, as

Scheme II



would be expected, replacing one of the methyl hydrogens in pyruvate by CH_3CO has increased the equilibrium constants for hydration, and made both the keto and hydrate forms more acidic.

Rate of Enolization. Since the keto, enol, and hydrate forms of acetopyruvate gave distinct and sharp peaks in the nmr spectrum, the rates of interconversion must be less than about 5 sec⁻¹, since for $k \geq \Delta\nu$ broadening or merging of the peaks would be observed.

The rate of enolization was measured at pH 5, using the iodination reaction; the reaction of the enol with iodine was presumed to be very fast.

$$\text{hydrate} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \text{keto} \xrightarrow{k_{23}} \text{enol} \xrightarrow[\text{fast}]{+I_2} \text{product} \quad (4)$$

Because acetopyruvate is about 50% enolized at pH 5 these experiments were done with iodine at higher concentration than acetopyruvate; thus, one is looking at a small decrease in a large absorbance—an inherently inaccurate procedure. Curved first-order plots were obtained in these experiments, suggesting an integrated rate equation with *two* exponential terms. Such an equation can be derived for this system; however, the inherent imprecision of the data made it unrealistic to attempt this detailed a kinetic analysis; instead the best lines fitting first-order plots of the data from 10 sec onward within experimental error (taken as 0.003 absorbance unit in $A - A_\infty$) were drawn. The rate constants measured in the iodination experiments are found in Table III. The "rate constants" for the

Table III. Enolization of Acetopyruvate and Acetylacetone[a]

pH	Buffer concn, *M*	Substrate	k_{obsd}
4.97	0.0408	Acetopyruvate[b]	0.18[d]
4.96	0.00816	Acetopyruvate[b]	0.066[d]
4.95	0.00408	Acetopyruvate[b]	0.11[d]
4.97	0.00816	Acetylacetone[c]	0.0427
4.98	0.00408	Acetylacetone[c]	0.0303

[a] Temperature = 30.0 ± 0.1°; μ = 0.1 *M* (KCl). [b] 1.92×10^{-5} *M*. [c] 1.33×10^{-5} *M*. [d] Curved first-order plots were obtained; see text.

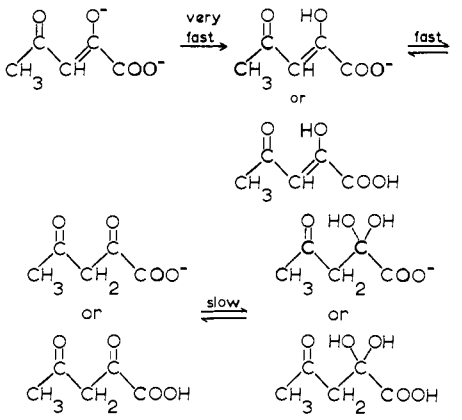
enolization of acetopyruvate evaluated in this way are obviously quite inaccurate and indeed are little better than lower limits. However, as we shall see enolization is at least fivefold faster than hydration.

The iodination of acetylacetone was measured as a check on the method and gave simple first-order kinetic behavior. Extrapolation of the observed rates (see Table III) to zero buffer gave a "water" rate of 0.018 sec⁻¹ at 30°; Pearson and Dillon[12] report a water rate of 0.017 sec⁻¹ at 25°.

(12) R. G. Pearson and R. L. Dillon, *J. Amer. Chem. Soc.*, 75, 2439 (1953).

Rate of Approach to Hydration Equilibrium. The procedure used to measure the rate of hydration was based upon conversion of acetopyruvate to its dianion, and then quenching a solution of the dianion into a buffer solution at a pH at which the monoanion or neutral acid is the major species. The second pK_a of acetopyruvate was measured spectrophotometrically (see Experimental Section) and found to be 7.70 ± 0.16. Thus, at pH 11 acetopyruvate is essentially completely converted to the dianion. The course of events which occurs during such a quenching process is shown in Scheme III. The uv absorption of the enol is being

Scheme III



monitored. Justification of the assumption that keto–enol equilibrium is established before measurements begin is deferred to the Discussion.

Rate constants for hydration of APY, extrapolated to zero buffer concentration, are given in Table IV.

Table IV. Rate Constants for Hydration and Dehydration of Acetopyruvate[a,b]

pH	k_0,[g] sec^{-1}	k_{-2},[h] sec^{-1}	k_2,[i] sec^{-1}
1.9[c]	0.098 ± 0.026	0.061 ± 0.016	0.11 ± 0.04
2.9[d]	0.066 ± 0.012	0.056 ± 0.010	0.022 ± 0.008
4.0[e]	0.028 ± 0.006	0.026 ± 0.005	0.004 ± 0.002
5.0[e]	0.012 ± 0.002	0.011 ± 0.002	0.0015 ± 0.0007
6.1[f]	0.013 ± 0.002	0.012 ± 0.002	0.0016 ± 0.0008
7.0[f]	0.020 ± 0.005	0.019 ± 0.005	0.003 ± 0.001

[a] Extrapolated to zero buffer concentration. [b] In H_2O, 25°, μ = 0.3 (KCl). [c] Cyanoacetate buffer. [d] Chloroacetate buffer. [e] Acetate buffer. [f] Phosphate buffer. [g] Apparent rate constant for approach to equilibrium. [h] Rate constnt for dehydration, calculated from eq 6. [i] Rate constant for hydration, calculated from eq 7.

Buffer catalysis was present, but the catalytic constants were only slightly larger than experimental error.

Figure 3 shows a graph of the rate constants for approach to hydration equilibrium as a function of pH.

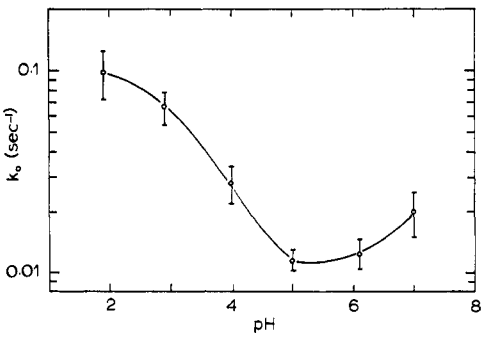


Figure 3. Rate constants, extrapolated to zero buffer concentration, for approach to hydration equilibrium, in H_2O at 30°, μ = 0.3 M(KCl).

Discussion

Rate of Enolization. Comparison of the rate constants at pH 5 for enolization (*ca.* 0.1 sec^{-1}) and for approach to hydration equilibrium (0.012 sec^{-1}) shows that enolization is fast relative to hydration. That this is true for all of the hydration experiments is strongly implied by the simple first-order kinetic behavior which was observed. The most likely explanation for the complex kinetic behavior of APY in the iodination experiments is that dehydration becomes partly rate limiting by the final stages of the reaction. The hydrate is initially 5% of the total APY, but is 10% of the total remaining after the enol is consumed, and 20% of the total after 1 half-life for enolization has passed (*i.e.*, 5 or 6 sec after initiation of the reaction). Thus, under the conditions of the enolization experiments, one expects complex kinetics for APY. Acetylacetone, with only keto and enol forms present at appreciable concentrations, shows simple kinetics.

Hydration Equilibrium. From the kinetic experiments one can obtain an apparent equilibrium constant, related to the equilibrium constant for hydration of the α keto group. For acetopyruvate, formation of enol from the enolate and establishment of tautomeric equilibrium between keto and enol are complete by the time measurements begin. Thus, at $t = 0$

$$\text{enol} \overset{K_1^{app}}{\rightleftharpoons} \text{keto}$$

and at $t = \infty$

$$\text{enol} \overset{K_1^{app}}{\rightleftharpoons} \text{keto} \overset{K_2^{app}}{\rightleftharpoons} \text{hydrate}$$

Since the absorbance of the enol form was measured, $A_0 = \epsilon_{enol}[enol]_0 = \epsilon_{enol}[APY]_{total}/(1 + K_1^{app})$ and $A_\infty = \epsilon_{enol}[enol]_\infty = \epsilon_{enol}[APY]_{total}/(1 + K_1^{app}(1 + K_2^{app}))$. We may define an apparent equilibrium constant $K = (A_0 - A_\infty)/A_\infty = K_3^{app}/(1 + K_1^{app})$. This equation permits a comparison of the approach to equilibrium experiments with the nmr experiments discussed earlier.

Figure 4 shows the agreement between the experimental values of K from the uv experiments and a line calculated using the parameters determined from the nmr data.

Rate of Hydration. Since keto–enol equilibration is fast relative to hydration, the approach to hydration equilibrium experiments may be described by

$$\text{enol} \overset{K_1^{app}}{\rightleftharpoons} \text{keto} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{hydrate} \qquad (5)$$

The observed rate constant, k_{obsd}, obtained from a first-order plot of the absorbance data, may be expressed as

$$k_{obsd} = k_{-2}(1 + K_3^{app}/(1 + K_1^{app})) \qquad (6)$$

Also

$$k_2/k_{-2} = K_2^{app} \qquad (7)$$

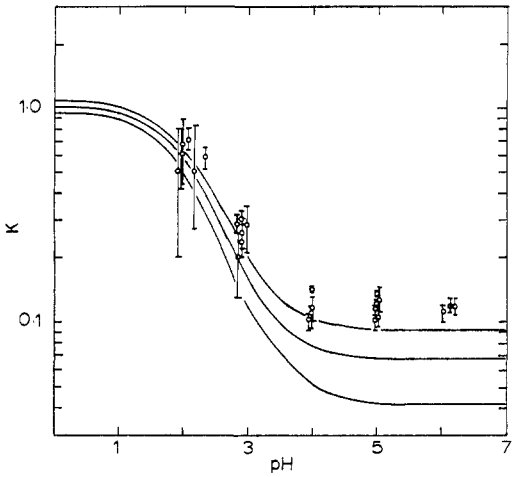


Figure 4. Apparent equilibrium constant for the hydration equilibrium experiments in H_2O at 30°, μ = 0.3 M (KCl). Points are derived from approach to equilibrium experiments; the central line is based on the equilibrium constants from the nmr experiments; the two lines on either side show the magnitude of the estimated standard deviations in K based on the standard deviations of the equilibrium constants from the nmr experiments.

(K_1^{app}, K_2^{app}, and K_3^{app} are defined by eq 1, 2, and 3.) Rate constants calculated from eq 6 and 7 are given in Table IV; for this purpose K_2^{app} and $K_3^{app}/(1 + K_1^{app})$ were calculated using the parameters derived from the nmr experiments.

The kinetics of hydration and dehydration of pyruvic acid have been studied in other laboratories. Eigen, *et al.*,[13] reported, for the dehydration of 2,2-dihydroxypropionic acid (pyruvic acid hydrate) at 25°, an uncatalyzed path with rate constant 0.22 sec^{-1} and an acid-catalyzed path with rate constant 1.25 M^{-1} sec^{-1}. Pocker and Meany[14] reported a rate constant of 0.00092 sec^{-1} for the uncatalyzed dehydration of 2,2-dihydroxypropionate ion at 0°. Allowing a factor of about 10 for the 30° temperature difference between these experiments and the present work, this corresponds to a rate of dehydration of about 0.01 sec^{-1}.

These values may be compared with the rates of dehydration of the "hydrate" of acetopyruvic acid. The rate constant for the anion, taken as the value of k_{-2} at pH 5 and 6, is 0.011 sec^{-1}; although the low pH results from this study are of low precision, it appears that by pH 2, where the hydrate (pK_a = 3.2) is mainly present in the acid form, the rate constant for hydration (k_{-2}) has leveled off at about 0.06 sec^{-1}.[15] Thus, the rate constants for acetopyruvate are quite similar to those for pyruvate.

This study was undertaken as a foundation for studies of the reactions of acetopyruvate with amines, since it was found that these reactions gave complicated kinetic behavior. These studies are reported in the accompanying paper.[4] The results reported above define the equilibria established for acetopyruvate in dilute aqueous solution in the pH range 0–7, and the rate constants for processes taking more than 10 sec to come to equilibrium.

Acknowledgment. The author gratefully acknowledges critical discussions, advice, and encouragement provided by Professor F. H. Westheimer, who originally suggested this problem. I would like to thank Professor K. L. Williamson, Mt. Holyoke College, who kindly ran the 100-MHz nmr spectra.

(13) M. Eigen, K. Kustin, and H. Strehlow, *Z. Phys. Chem.*, 31, 140 (1962).

(14) Y. Pocker and J. E. Meany, *J. Phys. Chem.*, 74, 1486 (1970).

(15) The k_{obsd} values for hydration of 0.2 and 0.4 sec^{-1} obtained from the last stages of the reactions at pH 1 or 0, respectively, indicate that there *is* an acid-catalyzed reaction.

Acetopyruvate. Enamine Formation with Aminoacetonitrile. Models for Acetoacetate Decarboxylase[1]

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Received September 14, 1971

Abstract: The reaction of aminoacetonitrile with acetopyruvic acid (2,4-dioxovaleric acid) leads to the production of two isomeric products, which have been identified as the enamines formed by reaction at the α or γ keto function. The two enamines form at closely similar rates and the equilibrium constants are similar, so that the kinetics of the reaction are complicated. Analysis has led to rate constants for the two enamine forming reactions which may be compared with the rate constant for the reaction of acetopyruvate with acetoacetate decarboxylase, for which it is a powerful inhibitor.

Acetoacetate decarboxylase reacts rapidly and reversibly to form a stable compound with acetopyruvate.[2] This compound, which is believed to be an enamine on the basis of its uv spectrum, is apparently formed by reaction of the acetopyruvate with the ϵ amino group of a lysine residue in the active site.[2c] It

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(1) Abstracted in part from the Ph.D. Thesis of J. P. G. (1968). Supported by Grant No. GM 04712 from the Institute of General Medical Sciences of the National Institutes of Health, and by a National Research Council of Canada Special Scholarship.

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