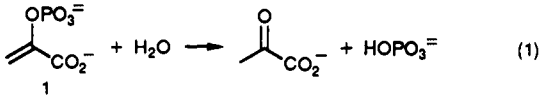
Keto-Enol Equilibria in the Pyruvic Acid System: Determination of the Keto-Enol Equilibrium Constants of Pyruvic Acid and Pyruvate Anion and the Acidity Constant of Pyruvate Enol in Aqueous Solution

Y. Chiang, A. J. Kresge,* and P. Pruszynski

Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario M5S 1A1, Canada. *Received September 5, 1991*

Abstract: Keto-enol equilibrium constants for the pyruvic acid system in aqueous solution at 25 °C were determined by Meyer halogen titration and also by another method that evaluates these constants as ratios of enolization to ketonization rate constants, $K_E = k^E/k^K$. Measurements by each method were made in both acidic and basic solution, and enol required for the ketonization rate measurements was supplied by hydrolysis of a silyl derivative and also by an equilibrated DMSO solution in which the enol content is greater than it is in water. The various methods gave nicely consistent results, which nevertheless differed between acidic and basic solutions, in accord with the different states of ionization of pyruvic acid in the two media; the values obtained were $pK_E = 3.21$ for pyruvic acid in the carboxylic acid form and $pK_E = 5.03$ for the pyruvate ion. The latter gives a free energy change for the ketonization of pyruvate enol that is 47% of the free energy liberated by the hydrolysis of the high-energy molecule, phosphoenolpyruvate; this shows that nearly half of the high energy content of this molecule resides in its masked enol function. An acidity constant for ionization of the enol hydroxyl group of pyruvate enol, $pK_a^E = 11.55$, was also determined, and this, when combined with pK_E for this species, gives $pK_a^K = 16.58$ as the acidity constant of the pyruvate ion ionizing as a carbon acid.

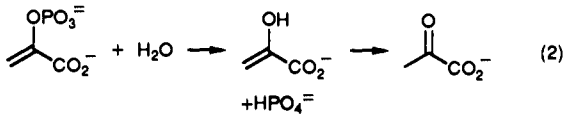
Thermodynamically unfavorable biological reactions are often driven by being coupled to favorable transformations of so-called high-energy compounds. One of the most energy-rich of these substances is phosphoenolpyruvate, the phosphate ester of the enol of pyruvate ion, **1**. Its hydrolysis, eq 1, is exoergic by $\Delta G° = -14.6$



(1)

kcal mol^{-1};[1] this is nearly twice the free energy liberated in the hydrolysis of ATP to ADP, $\Delta G° = -8.2$ kcal mol^{-1},[2] which itself is the principal energy-producing reaction in living systems. The unfavorable conversion of ADP to ATP is in fact driven by the transformation of phosphoenolpyruvate to pyruvate.

The hydrolysis of phosphoenolpyruvate may be divided formally into two parts: (1) hydrolysis of the phosphate ester to give pyruvate enol followed by (2) ketonization of the enol, eq 2. A

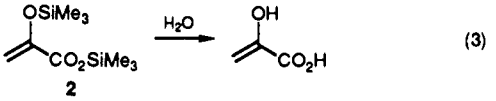


(2)

portion of the energy liberated in the overall reaction can then be attributed to ketonization, and some of the high energy content of phosphoenolpyruvate can be considered to reside in its masked enol function. In order to learn just how much this portion might be, we have determined keto-enol equilibrium constants for the pyruvic acid system. In the course of doing this, we also determined the acidity constant of the enol ionizing as an oxygen acid and the acidity constant of pyruvic acid ionizing as a carbon acid.

The classic way of determining keto-enol equilibrium constants is by halogen titration, a method invented by Meyer nearly a century ago.[3] This technique works well when enol contents are large, but it is not very satisfactory when they are small,[4] as is the case for the pyruvic acid system under some conditions. In the present work, therefore, in addition to using the Meyer technique, we employed another, kinetic method which evaluates keto-enol equilibrium constants as ratios of specific rates of enolization, k^E, to specific rates of ketonization, k^K: $K_E = k^E/k^K$.

This kinetic method requires generating the enol in greater than equilibrium amount in the medium of interest, in this case wholly aqueous solution. We accomplished this by hydrolysis of the bis-trimethylsilyl derivative of the enol, trimethylsilyl 2-(trimethylsiloxy)-2-propenoate[5] (**2**) (eq 3), and also by supplying the enol as an equilibrated keto-enol mixture in DMSO solution. The



(3)

latter method makes use of the fact that enols of simple carbonyl compounds are more stable in good hydrogen-bond-accepting solvents such as DMSO than they are in water, and enol contents are consequently greater;[6] addition of a small quantity of a keto-enol mixture equilibrated in such a solvent to a large amount of water then gives an essentially wholly aqueous solution whose enol content exceeds the equilibrium value for that medium.

We described our use of the silyl derivative **2** for the purpose of generating the enol of pyruvic acid in a preliminary account of this work;[7] since then another report of the use of this substance for the same purpose has appeared.[8]

Experimental Section

Materials. Trimethylsilyl 2-(trimethylsiloxy)-2-propenoate was prepared as described[5] from pyruvic acid and trimethylsilyl chloride: ^{1}H NMR ($CDCl_3$) δ/ppm 5.70 (d, J = 1.1 Hz, 1 H), 4.75 (d, J = 1.1 Hz, 1 H), 0.16 (s, 9 H), 0.07 (s, 9 H). Pyruvic acid (Aldrich) was distilled before use. All other materials were the best available commercial grades and were used as received.

Kinetics, Ketonization. Rates of ketonization of the enols of pyruvic acid and pyruvate ion were determined spectrophotometrically by monitoring the decay in absorbance of the strong enol band at λ = 230-240 nm. Measurements were made at 25.0 ± 0.1 °C using a Cary Model

(1) Wood, H. G.; Davis, J. J.; Lochmüller, H. *J. Biol. Chem.* **1966**, *241*, 5692-5704.
(2) Zubay, G. *Biochemistry*, 2nd ed.; MacMillan: New York, 1988; p 409.
(3) Meyer, K. *Justus Liebigs Ann. Chem.* **1911**, *380*, 212-242.
(4) Kresge, A. J. *Acc. Chem. Res.* **1990**, *23*, 43-48. Keeffe, J. R.; Kresge, A. J.; Schepp, N. P. *J. Am. Chem. Soc.* **1990**, *112*, 4862-4868.
(5) Sekine, M.; Futatsugi, T.; Yamada, K.; Hata, T. *Tetrahedron Lett.* **1980**, *21*, 371-372.
(6) Ahlbrecht, H.; Funk, W.; Reiner, M. T. *Tetrahedron* **1976**, *32*, 479-482. Rochlin, E.; Rappoport, Z. *Abstracts*, 10th IUPAC Conference on Physical Organic Chemistry, Haifa, Israel, Aug 5-10, 1990; A-13.
(7) Kresge, A. J.; Plenary lecture, 10th IUPAC Conference on Physical Organic Chemistry, Haifa, Israel, August 5-10, 1990; *Pure Appl. Chem.* **1991**, *63*, 213-221.
(8) Peliska, J. A.; O'Leary, M. H. *J. Am. Chem. Soc.* **1991**, *113*, 1841-1842.

0002-7863/92/1514-3103$03.00/0

2200 spectrometer for the slower reactions and a Hi-Tech Scientific Model SF-S1 stopped-flow spectrometer for the faster ones.

Enol substrate solutions for the stopped-flow experiments were prepared from trimethylsilyl 2-(trimethylsiloxy)-2-propenoate by dissolving that substance in 10^{-4} M aqueous $HClO_4$ solution, in which the enol has a half-life of ca. 1 min, and then quickly loading the resulting solution into one of the driving syringes of the stopped-flow apparatus; several kinetic runs could then be made before the concentration of the enol substrate in this syringe dropped to a nonusable level. The silyl precursor was very sensitive to atmospheric moisture, but stock solutions in rigorously dried THF could be kept for several days under argon in Pierce Reacti-Vials equipped with Mininert Valves, through which samples could be withdrawn by microsyringe.

For the slower reactions monitored by conventional spectroscopy, enol was supplied either by hydrolysis of the silyl derivative or as a keto–enol equilibrium mixture in DMSO solution. In the latter case, enough pyruvic acid was added to DMSO to make a 0.5 M solution that was then allowed to reach equilibrium overnight. Reactions were initiated by adding 10 μL of this equilibrated stock solution to 1.2 mL of aqueous acid or base contained in a cuvette that had reached the temperature of the spectrometer cell compartment. Reactions using the silyl derivative were initiated in the same way, with THF solutions of that substance.

The kinetic data fit the first-order rate law well, and observed first-order rate constants were obtained by least-squares fitting to an exponential function.

Kinetics, Enolization. Rates of enolization of pyruvic acid and pyruvate ion were determined by halogen scavenging, using iodine as the scavenger in sodium hydroxide solutions and bromine as the scavenger in perchloric acid solutions. Measurements were made spectrophotometrically using a Cary Model 2200 spectrometer. The reactions in sodium hydroxide solutions were monitored by following the rise in absorbance at λ = 338 nm due to iodoform formation.[9] An excess of iodine over pyruvate ion was used (initial concentrations: $[I_2]_{stoich} = 7 \times 10^{-4}$ M and [pyruvate] = 1.2×10^{-4} M), and reactions were followed to completion. The rate data so obtained obeyed the first-order rate law well, and observed first-order rate constants were determined by least-squares fitting to an exponential function.

Enolization in perchloric acid solutions was very much slower than in sodium hydroxide solutions, and reactions could not be followed to completion in a convenient length of time; zero-order initial-rate methods were therefore used instead. Measurements were made in the presence of bromide ion, and consumption of the bromine scavenger was monitored by following changes in the absorbance of tribromide ion at λ = 310 nm, ε = 7535 cm^{-1} M^{-1}.[10] Initial concentrations in the reaction mixtures were $[Br_2]_{stoich} = 2 \times 10^{-4}$ M, $[Br^-]_{stoich} = 0.1$ M, and [pyruvic acid] = 0.02 M, and reactions were followed for about 10 min, which corresponded to ca. 0.1–0.2% enolization. Traces of absorbance vs time were accurately linear, and gradients, $\Delta A/\Delta t$, were obtained by linear least-squares analysis. The gradients were then converted into observed first-order rate constants according to eq 4, in which K_{assoc} (= 17 M^{-1})[11] is the equilibrium constant for the $Br_2 + Br^- = Br_3^-$ association reaction.

$$k_{obsd} = (\Delta A/\Delta t)(1 + K_{assoc}^{-1}[Br^-]^{-1})/\epsilon[\text{pyruvic acid}] \quad (4)$$

Keto–Enol Equilibria, Meyer Method. Keto–enol equilibrium constants were determined from the initial bursts of iodine scavenger consumption which occurred prior to slower zero-order decreases in scavenger concentration when pyruvic acid was combined with iodine in dilute perchloric acid or cacodylic acid buffer solution. Measurements were made spectroscopically with a Cary Model 2200 spectrometer; the reaction mixtures contained iodide ion, and the absorption maximum of I_3^- at λ = 351 nm, ε = 2.6×10^4 cm^{-1} M^{-1},[12] was used to monitor concentrations. Initial concentrations in the reaction mixtures were $[I_2]_{stoich} = 3 \times 10^{-5}$ M, $[I^-]_{stoich} = 7 \times 10^{-3}$ M, and [pyruvic acid] = $(1.7–3.4) \times 10^{-2}$ M for the perchloric acid solutions and $[I_2]_{stoich} = 1 \times 10^{-4}$ M, $[I^-]_{stoich} = 7 \times 10^{-3}$ M, and [pyruvate ion] = $(0.7–1.1) \times 10^{-2}$ M for the cacodylic acid buffers (buffer concentration 0.02 M, buffer ratio = 1.0); in all cases, the ionic strength was maintained at 0.10 M. Enol concentrations at equilibrium were calculated using eq 5, in which ΔA is the change in absorbance that occurred during the burst and K_{assoc} (= 670 M^{-1})[12] is the equilibrium constant for the $I_2 + I^- = I_3^-$ association reaction.

$$[\text{enol}] = \Delta A(1 + K_{assoc}^{-1}[I^-]^{-1})/\epsilon \quad (5)$$

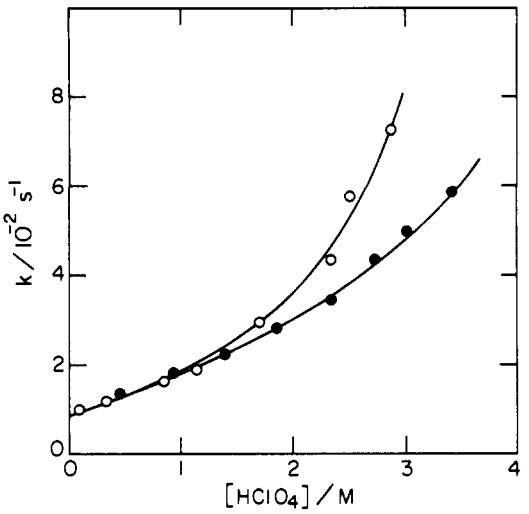


Figure 1. Rate data for the ketonization of pyruvic acid enol in aqueous perchloric acid solutions at 25 °C: enol supplied as equilibrated DMSO solution (○); enol supplied by hydrolysis of trimethylsilyl 2-(trimethylsiloxy)-2-propenoate (●). Values at each acid concentration are averages of 2–7 separate determinations.

Hydration Equilibrium. The extent of hydration of the keto group of pyruvic acid in aqueous solution was determined spectrophotometrically by measuring the absorbance of solutions of the acid at the absorption maximum of the keto form λ = 320 nm; the hydrate does not absorb light at this wavelength. Measurements were made in wholly aqueous solutions containing added perchloric acid to suppress the ionization of pyruvic acid; the stoichiometric concentration of the latter was 0.0108 M. Hydration equilibrium constants, K_h, were calculated using the relationship of eq 6, in which A is the absorbance of the solution being measured and A_0 is the absorbance of the unhydrated acid at the same stoichiometric concentration. The latter was determined by measuring

$$K_h = (A_0 - A)/A \quad (6)$$

the absorbance of solutions of sodium pyruvate and correcting the values obtained for the extent of hydration (6.6%; vide infra) of the keto group of the pyruvate ion. This method assumes that the extinction coefficients at λ = 320 nm of the keto groups of pyruvic acid and the pyruvate ion are the same; the validity of this assumption is supported by the fact that the results obtained here agree well with those obtained by a UV absorption method for which this assumption did not have to be made.[13]

Results

Ketonization, Acid Solutions. Rates of ketonization of pyruvic acid enol were measured in concentrated aqueous perchloric acid solutions using enol supplied as an equilibrated keto–enol DMSO solution as well as by hydrolysis of trimethylsilyl 2-(trimethylsiloxy)-2-propenoate. The data are summarized in Tables S1 and S2 of the supplementary material[14] and are displayed in Figure 1.

It may be seen that, whereas the rate data obtained by these two methods are in agreement at low acidities, systematic differences appear at higher acid concentrations. These differences are in the direction of slower rates for enol generated by the hydrolysis of the silyl derivative, and this suggests that production of enol by this method is not sufficiently more rapid than ketonization to give reliable ketonization rate constants. Difficulties such as this can often be remedied by adding fluoride ion to the reaction solution, for fluoride ion strongly accelerates the hydrolysis of silyl derivatives;[15] that, however, is not possible in strongly acidic solutions where fluoride ion would be converted to hydrofluoric acid (pK_a = 3.17).[16] Hydrolysis of the silyl derivative is therefore

(9) Tapuhi, E.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 5758–5765.
(10) Keeffe, J. R. Unpublished work.
(11) Keeffe, J. R.; Kresge, A. J.; Toullec, J. *Can. J. Chem.* **1986**, *64*, 1224–1227.
(12) Harper, E. T.; Bender, M. L. *J. Am. Chem. Soc.* **1965**, *87*, 5625–5632.
(13) Knoche, W.; Lopez-Quintela, M. A.; Weiften, J. *Ber. Bunsen-Ges. Phys. Chem.* **1985**, *89*, 1047–1050.
(14) Supplementary material; see the paragraph at the end of this paper regarding availability.
(15) Colvin, E. W. *Silicon in Organic Synthesis*; Butterworths: Toronto, 1981; pp 218–221.
(16) Kresge, A. J.; Chiang, Y. *J. Phys. Chem.* **1973**, *77*, 822–825.

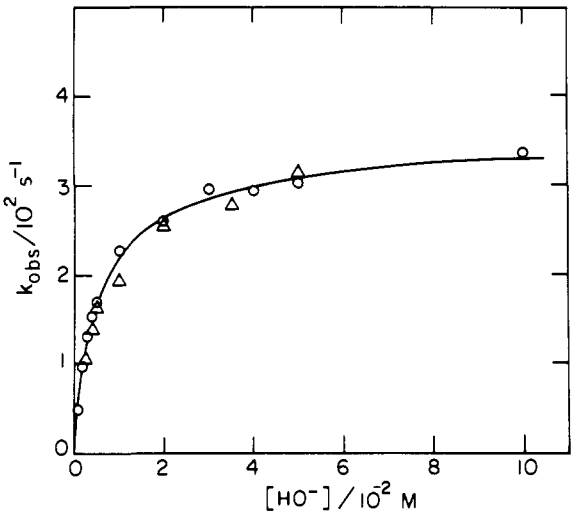


Figure 2. Rate data for the ketonization of pyruvic acid enol in aqueous sodium hydroxide solutions at 25 °C in the presence (△) and in the absence (○) of fluoride ion. Values at each base concentration are averages of 3–9 separate determinations.

not a useful way of generating pyruvic acid enol in strongly acidic solution for the purpose of measuring its rate of ketonization.

Figure 1 shows that the rate constants measured here in the more concentrated acids increase with acidity somewhat more rapidly than in direct proportion to acid concentration. Such behavior is not uncommon, and the situation is generally handled by using an acidity function to correlate the data. The X_0 function[17] appears to be the best scale currently available for this purpose.[18] The data were therefore fitted to eq 7,[19] the standard

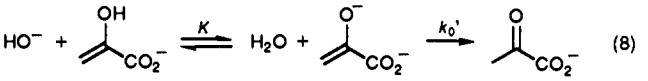
$$\log \{(k_{obsd} - k_{uc})/C_{H^+}\} = \log k_{H^+} + mX_0 \qquad (7)$$

expression generally used to correlate k_{obsd} with X_0[20] modified by the inclusion of an additional term, k_{uc}, to allow for the "uncatalyzed" component of the ketonization reaction.[21] The results obtained using enol supplied in DMSO solution gave a good linear relationship, which provided the rate constants $k_{H^+}{}^K = (3.34 \pm 0.42) \times 10^{-3}$ M^{-1} s^{-1} for ketonization catalyzed by the hydrogen ion and $k_{uc}{}^K = (1.06 \pm 0.07) \times 10^{-2}$ s^{-1} for the uncatalyzed reaction. The data obtained using enol generated by hydrolysis of trimethylsilyl 2-(trimethylsiloxy)-2-propenoate also gave a good linear correlation, but the value of $k_{H^+}{}^K$ which this produced was greater, by a factor of 1.9, than that based upon the DMSO data. This greater value of k_{H^+}, unlike that provided by the DMSO data, led to a keto–enol equilibrium constant for the pyruvic acid system in disagreement with the result produced by the Meyer method described below. This reinforces the conclusion reached above that hydrolysis of the silyl derivative is an unreliable method of generating pyruvic acid enol in concentrated acid solutions.

Ketonization, Basic Solutions. Rates of ketonization of pyruvic acid enol were also determined in sodium hydroxide solutions, using only hydrolysis of trimethylsilyl 2-(trimethylsiloxy)-2-propenoate as the source of enol. Fluoride ion can exist in these media, and rate measurements were therefore made in the presence as well as in the absence of this ion. The concentration of sodium hydroxide was varied from 0.001 to 0.1 M, and the ionic strength was kept constant at 0.10 M; in one series of experiments this was done by adding $NaClO_4$ and in another by adding NaF. The data are summarized in Table S3 of the supplementary material[14] and are displayed in Figure 2.

It may be seen that there is no difference between rate constants determined in the presence of fluoride ion and those determined in its absence. This indicates that the generation of pyruvic acid enol from the silyl derivative is sufficiently fast in these media, even in the absence of fluoride ion, not to interfere with the kinetics of the ketonization reaction; unlike the situation in concentrated acids, therefore, hydrolysis of the silyl derivative is a useful way of producing the enol in sodium hydroxide solutions.

Figure 2 shows that ketonization of pyruvic acid enol is catalyzed by hydroxide ion at low basicities, but the catalysis becomes saturated at higher base concentrations. Such behavior is typical of enol ketonization reactions;[22] it is produced by a reaction scheme that involves rapid equilibrium formation of the much more reactive enolate ion followed by rate-determining ketonization of enolate through proton transfer to its β-carbon atom from a water molecule, eq 8. At low basicities the rate of this process is directly



(8)

proportional to hydroxide ion concentration, but, as the basicity increases, the position of equilibrium in the first step shifts from enol to enolate, and the advantage of converting a less reactive to a more reactive species is lost; hydroxide ion catalysis therefore disappears, and the rate of reaction assumes a constant limiting value.

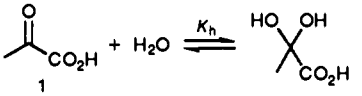
The rate law that applies to this reaction scheme is given as eq 9. Least-squares fitting of the rate data to this expression

$$k_{obsd} = \frac{k_0'K[HO^-]}{K[HO^-] + 1} \qquad (9)$$

produced values of both the limiting rate constant, k_0', and the equilibrium constant for the first step, K. Since the latter is a function of the acidity constant of the enol, $K_a{}^E$, and the autoprotolysis constant of water, K_w ($K = K_a{}^E/K_w$), this treatment also leads to the acid dissociation constant of pyruvic acid enol. The values obtained are $k_0' = 341 \pm 18$ s^{-1}, $K_a{}^E = (2.82 \pm 0.17) \times 10^{-12}$ M, and $pK_a{}^E = 11.55 \pm 0.03$;[23] these results may also be expressed as a hydroxide ion catalytic coefficient for the ketonization reaction, $k_{HO^-}{}^K = k_0'K_a{}^E/K_w = (6.08 \pm 0.18) \times 10^4$ M^{-1} s^{-1}.

Enolization, Acid Solutions. Rates of enolization of pyruvic acid were determined in concentrated perchloric acid solutions over the concentration range $[HClO_4] = 0.4$–2.7 M. The data, summarized in Table S4 of the supplementary material,[14] were fitted to eq 7; this gave the rate constants $k_{H^+} = (6.82 \pm 0.53) \times 10^{-7}$ M^{-1} s^{-1} and $k_{uc} = (1.68 \pm 0.04) \times 10^{-7}$ s^{-1}.

These rate constants refer to a mixture of pyruvic acid and its ketone group hydrate, which is formed to an appreciable extent in aqueous solution by a rapidly established equilibrium reaction, eq 10. Conversion of these rate constants into specific rates of



1 (10)

enolization, referenced to the reactive unhydrated form only, requires knowledge of the hydration equilibrium constant, K_h, i.e., $k^E = k(1 + K_h)$.

A number of determinations of K_h have been reported, but the results differ too widely to be of use for the present purpose; this equilibrium constant was therefore redetermined here. Replicate measurements were made in solutions to which perchloric acid had been added in order to keep pyruvic acid in its un-ionized form. Three perchloric acid concentrations were used: $[HClO_4] = 0.07$, 0.2, and 0.4 M; the data are summarized in Table S5 of the supplementary material.[14] No systematic variation in K_h with

(17) Cox, R. A.; Yates, K. *Can. J. Chem.* **1981**, *59*, 2116–2124.
(18) Kresge, A. J.; Chen, H. J.; Capen, G. L.; Powell, M. F. *Can. J. Chem.* **1983**, *61*, 249–256.
(19) Chiang, Y.; Kresge, A. J.; Krogh, E. T. *J. Am. Chem. Soc.* **1988**, *110*, 2600–2607.
(20) Cox, R. A. *Acc. Chem. Res.* **1987**, *20*, 27–31.
(21) Chiang, Y.; Kresge, A. J.; Santaballa, J. A.; Wirz, J. *J. Am. Chem. Soc.* **1988**, *110*, 5506–5510.
(22) Keeffe, J. R.; Kresge, A. J. In *The Chemistry of Enols*; Rappoport, Z., Ed.; Wiley: New York, 1990; Chapter 7.
(23) This acidity constant is a concentration quotient, appropriate to the ionic strength of the measurements, $\mu = 0.10$ M.

perchloric acid concentration could be discerned, and the best value was therefore taken to be a simple average of the 12 separate determinations: $K_h = 2.31 \pm 0.02$. This result agrees well with $K_h = 2.26$ reported recently[13] and also the earlier values K_h = 2.38,[24] 2.4,[25] and 2.38,[26] but it is significantly different from K_h = 0.68,[27] 1.55,[28] and 1.86.[29]

Use of the presently determined value, $K_h = 2.31$, gives the specific rate of enolization catalyzed by the hydrogen ion referenced to the unhydrated form of pyruvic acid, $k_{H^+}^E = (2.26 \pm 0.18) \times 10^{-6}$ M^{-1} s^{-1}, and the rate constant for the corresponding uncatalyzed reaction, $k_{uc}^E = (5.55 \pm 0.12) \times 10^{-6}$ s^{-1}.

These rate constants may be combined with their counterparts for the ketonization of pyruvic acid enol to give keto–enol equilibrium constants for this system according to the relationship $K_E = k^E/k^K$. The result obtained using hydrogen ion catalytic coefficients is $K_E = (6.76 \pm 1.00) \times 10^{-4}$, $pK_E = 3.17 \pm 0.07$, and that using the uncatalyzed rate constants is $K_E = (5.24 \pm 0.35) \times 10^{-4}$, $pK_E = 3.28 \pm 0.03$.

Enolization, Basic Solutions. Rates of enolization were also determined in dilute sodium hydroxide solution. Measurements were made over the concentration range [NaOH] = 0.02–0.09 M at a constant ionic strength, 0.10 M; the data so obtained are summarized in Table S6 of the supplementary material.[14] Observed first-order rate constants proved to be accurately proportional to hydroxide ion concentration, and linear least-squares analysis gave the bimolecular rate constant, $k_{HO^-} = (4.64 \pm 0.08) \times 10^{-1}$ M^{-1} s^{-1}.

Pyruvic acid is completely ionized to the pyruvate ion in the solutions in which these measurements are made, and the keto group of this ion is much less extensively hydrated than is that of the acid. The factor correcting rate constants for this hydration reaction, $(1 + K_h)$, is therefore less sensitive to variations in K_h, and literature values of K_h are consequently adequate in this case. Three values have been reported, K_h = 0.054,[25b,26] 0.057,[28] and 0.087,[29] and the average of these, $K_h = 0.063$, was used here. This gave the hydroxide ion catalytic coefficient for enolization of pyruvate ion, $k_{HO^-}^E = (4.94 \pm 0.08) \times 10^{-1}$ M^{-1} s^{-1}. Combination of this with the hydroxide ion catalytic coefficient for ketonization provides the keto–enol equilibrium constant $K_E = (8.12 \pm 0.27) \times 10^{-6}$, $pK_E = 5.09 \pm 0.01$.

Meyer Method. Keto–enol equilibrium constants were also determined by the Meyer halogen titration method, using iodine as the titrant. Measurements were made in perchloric acid solutions at three concentrations, $[HClO_4]$ = 0.056, 0.065, and 0.074 M, and in a cacodylic acid buffer solution at $[H^+] = 8.2 \times 10^{-7}$ M, $pC_{H^+} = 6.1$;[30] in all cases the ionic strength was held constant at 0.10 M. The data are summarized in Tables S7 and S8 of the supplementary material.[14]

The values of K_E obtained in perchloric acid solution showed no systematic variation with acid concentration. Twelve separate determinations gave an average result which, when corrected for hydration, provided the equilibrium constant $pK_E = 3.17 \pm 0.02$. Eleven separate determinations were made in the cacodylic acid buffer; the average result corrected for hydration gave $pK_E = 4.97 \pm 0.10$.

Discussion

The various values of the keto–enol equilibrium constant for the pyruvic acid system determined here are collected in Table I. It may be seen that the results fall into two groups, separated by about 2 orders of magnitude: those determined in acidic solution and those determined at $pC_{H^+} = 6.1$ and in basic solution. There is good agreement within each group. It is significant also that the Meyer method gives better results, as judged by the error limits in pK_E, in acidic solutions where the enol content is greater than in basic solutions where it is considerably lower; this reflects the difficulties inherent in halogen titration of substances present at very low concentrations.

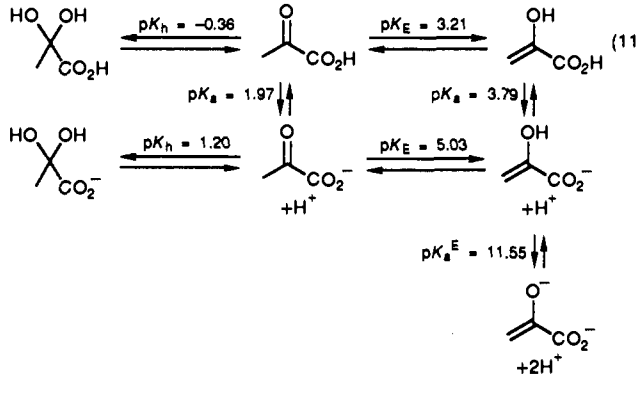
Table I. Keto–Enol Equilibrium Constants for the Pyruvic Acid System in Aqueous Solution at 25 °C

method	pK_E[a]
$k_{H^+}^E/k_{H^+}^K$	3.17 ± 0.07
k_{uc}^E/k_{uc}^K	3.28 ± 0.03
Meyer, pC_{H^+} = 1.1–1.2	3.17 ± 0.02
	av 3.21 ± 0.04
$k_{HO^-}^E/k_{HO^-}^K$	5.09 ± 0.01
Meyer, pC_{H^+} = 6.1	4.97 ± 0.10
	av 5.03 ± 0.06

[a] Error limits are standard deviations.

The difference between the two sets of results is a consequence of the fact that pyruvic acid exists in the acid solutions in its un-ionized carboxylic acid form but is converted to pyruvate ion at $pC_{H^+} = 6.1$ and in sodium hydroxide solutions. One set of results then gives the enol content of un-ionized pyruvic acid whereas the other gives that of the pyruvate ion. The difference observed is consistent with the substituent effects of the carboxylic acid and carboxylate groups and their influence on the stability of the keto isomer. Because the carboxylic acid group is electron-withdrawing, it will destabilize the keto isomer through an unfavorable interaction with its positively charged keto group carbonyl carbon atom; this unfavorable interaction is reduced when the carboxylic acid group is converted to a carboxylate ion, and that makes the keto isomer more stable, increasing the energy gap between the keto and enol forms and reducing the enol content.

The best values (simple averages) of pK_E for pyruvic acid and pyruvate ion, together with other relevant information, are summarized in the scheme of eq 11. The presently determined enol content of pyruvate ion, $pK_E = 5.03$, agrees well with an estimate, $pK_E = 5.1$, made previously[32] from rates of ketonization of the enol generated enzymatically in acetic acid buffer solutions[32] and rates of enolization measured by halogen scavenging in similar solutions.[33] The present result is also consistent with $pK_E = 5.4$ determined by Meyer titration.[34] Another comparison of the present results with literature data is afforded by the acidity constant of the carboxyl group in pyruvic acid enol, which may be calculated using the thermodynamic cycle in the center of the scheme of eq 11 plus the acidity constant of pyruvic acid, pK_a = 1.97;[35] the result obtained, $pK_a = 3.79$, agrees well with pK_a =

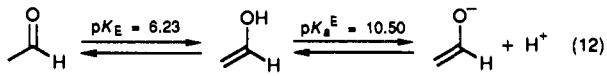


(11)

(24) Strehlow, H. Z. *Elektrochem.* **1962**, *66*, 392–396.
(25) (a) Gold, V.; Socrates, G.; Crampton, M. R. *J. Chem. Soc.* **1964**, 5888–5889. (b) Becker, M. *Ber. Bunsen-Ges. Phys. Chem.* **1964**, *68*, 669–676.
(26) Greenzaid, P.; Luz, Z.; Samuel, D. *J. Am. Chem. Soc.* **1967**, *89*, 749–756.
(27) Griffiths, V. S.; Socrates, G. *Trans. Faraday Soc.* **1967**, *63*, 673–677.
(28) Pocker, Y.; Meany, J. E.; Nist, B. J.; Zadorojny, C. *J. Phys. Chem.* **1969**, *73*, 2872–2882.
(29) Cooper, A. J. L.; Redfield, A. G. *J. Biol. Chem.* **1975**, *250*, 527–532.
(30) Calculated value based upon the acidity constant for cacodylic acid provided in ref 31.
(31) Kilpatrick, M. L. *J. Am. Chem. Soc.* **1949**, *71*, 2607–2610.
(32) Miller, B. A.; Leussing, D. L. *J. Am. Chem. Soc.* **1985**, *107*, 7146–7153.
(33) Schellenberger, A.; Hübner, G. *Chem. Ber.* **1965**, *98*, 1938–1948.
(34) Burgner, J. W.; Ray, W. J., Jr. *Biochemistry* **1974**, *13*, 4229–4237.
(35) This is the gross value, $pK_a = 2.49$,[36] corrected for hydration using $K_h = 2.31$.
(36) Pedersen, K. J. *Acta Chem. Scand.* **1952**, *6*, 243–256.

3.72 predicted by a correlation of acidity constants for α-substituted acrylic acids.[37]

The keto–enol equilibrium constant for pyruvic acid determined here, $pK_E = 3.21$, is 3 orders of magnitude greater than that for enolization of the parent unsubstituted carbonyl compound, acetaldehyde (eq 12), for which $pK_E = 6.23$.[38] This difference must



(12)

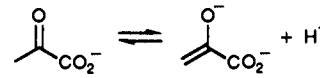
be due in large part to destabilization of the keto isomer of pyruvic acid by the adjacent carboxylic acid group, as pointed out above. In the case of pyruvate ion with $pK_E = 5.03$, the difference drops considerably to 1 order of magnitude, but it still leaves pyruvate ion with a greater enol content than acetaldehyde. This could be the result of a residual keto isomer destabilizing effect, but stabilization of the enol by carboxylate, for which a sizeable carbon–carbon double-bond-stabilizing effect ($D = 3.9$ kcal mol^{-1}) has been reported,[39] is likely to be a contributing factor.

Pyruvate enol, with $pK_a^E = 11.55$, is a weaker acid than the parent enol, vinyl alcohol (eq 12), for which $pK_a^E = 10.50$.[38] This is undoubtedly due to the presence of the negative charge in pyruvate ion, which will oppose formation of a second negative charge in the enolate ion. The structural relationship between


3 4

the substituent and the ionizing hydroxyl group in these two enols is similar to that in the pair formic acid (**3**) and malonate ion (**4**), and it is interesting that the difference in pK_a for the enols, $\Delta K_a = 1.05$, is greater than that between formic acid ($pK_a = 3.75$)[40] and malonate ion ($pK_a = 4.27$),[41] $\Delta pK_a = 0.52$; this is probably because the second negative charge is more delocalized in the malonate ion than it is in pyruvate enol and the unfavorable electrostatic interaction is consequently reduced.

The keto–enol equilibrium constant for pyruvate ion may be combined with the acidity constant of its enol to provide an acidity constant for pyruvate ionizing as a carbon acid, eq 13. The result,



(13)

pK_a^K ($= pK_E + pK_a^E$) $= 16.58 \pm 0.07$,[23] is not very different from that for the parent acetaldehyde system, $pK_a^K = 16.73$; this follows from the fact that the difference in keto–enol equilibrium constants for the two systems, $\Delta pK_E = 1.20$, is very nearly completely offset by the difference in their enol acidity constants, $\Delta pK_a^E = -1.05$.

Pyruvic acid will be converted to pyruvate ion at physiological pH, and it is the keto–enol equilibrium constant for this ion, $pK_E = 5.03$, that is relevant to biological systems. This value gives $\Delta G° = -6.9$ kcal mol^{-1} as the free energy change for the ketonization reaction, which is 47% of the free energy liberated by the hydrolysis of phosphoenolpyruvate (eq 1). This shows that nearly half of the energy content of this high-energy substance resides in its masked enol function.

Acknowledgment. We are grateful to the Natural Sciences and Engineering Research Council of Canada, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the U. S. National Institutes of Health for financial support of this work.

Supplementary Material Available: Tables S1–S8 containing rate and equilibrium data (8 pages). Ordering information is given on any current masthead page.

(37) Charton, M. *J. Org. Chem.* **1965**, *30*, 557–560.
(38) Chiang, Y.; Hojatti, J.; Keeffe, J. R.; Kresge, A. J.; Schepp, N. P.; Wirz, J. *J. Am. Chem. Soc.* **1987**, *109*, 4000–4009.
(39) Hine, J.; Skoglund, M. J. *J. Org. Chem.* **1982**, *47*, 4766–4770.
(40) Harned, H. S.; Embree, N. D. *J. Am. Chem. Soc.* **1934**, *56*, 1042–1044.
(41) Pinching, G. D.; Bates, R. G. *J. Res. Natl. Bur. Stand. (U.S.)* **1948**, *40*, 405–416.

Reduction of Daunomycin in Dimethyl Sulfoxide. Long-Lived Semiquinones and Quinone Methide and Formation of an Enolate at the 14-Position via the Quinone Methide

Giorgio Gaudiano,[†,‡] Massimo Frigerio,[†] Pierfrancesco Bravo,[†] and Tad H. Koch*[,‡]

Contribution from the CNR—Centro di Studio per le Sostanze Organiche Naturali, Dipartimento di Chimica, Politecnico, I-20133 Milano, Italy, and Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215. *Received September 18, 1991*

Abstract: Anaerobic reduction of daunomycin (**1**, daunorubicin) in 5%/95% H_2O/DMSO (DMSO = dimethyl sulfoxide) or in DMSO with sodium dithionite or bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer), respectively, yields 7-deoxydaunomycinone (**7**) and a mixture of the diastereomers of bi(7-deoxydaunomycinon-7-yl) (**8**). A precursor to both **7** and **8** is 7-deoxydaunomycinone quinone methide (**4**) formed from glycosidic cleavage of daunomycin hydroquinone (**3**). The hydroquinone **3** is estabished as a precursor to the quinone methide **4** from relative rates. In 5%/95% H_2O/DMSO or DMSO, daunomycin semiquinone (**2**) and quinone methide (**4**) have much longer lifetimes than in 100% protic solvents such as H_2O or methanol. The quinone methide reacts to form the side chain enolate most likely by intramolecular proton transfer from the methyl group at the 14-position to the 7-position.

Introduction

Anthracycline antitumor drugs as represented by daunomycin (**1**, daunorubicin) show complex redox chemistry which is biologically accessible[1,2] and fall in the category of bioreductively activated compounds.[3] The anaerobic reactivity of the transients from reduction of daunomycin in one-electron steps, the semiquinone (**2**), hydroquinone (**3**), and quinone methide (**4**), is medium dependent. In protic solvents such as water and methanol

[†] Politecnico di Milano.
[‡] University of Colorado.

(1) Abdella, B. R. J.; Fisher, J. *Environ. Health Perspect.* **1985**, *64*, 3.
(2) Powis, G. *Pharmacol. Ther.* **1987**, *35*, 57.
(3) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249.

0002-7863/92/1514-3107$03.00/0