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A Kinetic Analysis of the Iodination of Pyruvic Acid and Pyruvate Esters

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A KINETIC ANALYSIS OF THE IODINATION OF PYRUVIC ACID
AND PYRUVATE ESTERS

A Thesis
Presented to
the Graduate Faculty
Central Washington State College

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Mohamed M. Fahmi Hegazi

August, 1971

APPROVED FOR THE GRADUATE FACULTY

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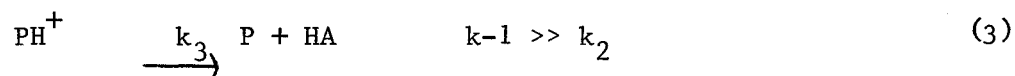
I. INTRODUCTION

Brønsted acids and bases are best characterized as proton donors and proton acceptors.¹ Reactions involving catalysis by Brønsted acids and bases generally proceed with the transfer of a proton between the catalyst and the substrate. Accordingly, the substrate must itself behave as an acid or base and the catalytic process is categorized as a typical acid-base or protolytic reaction.

It is possible to divide acid (and base) catalysed reactions into two categories² depending on whether the catalysis is due to all acids or bases present in solution, or specifically due to the conjugate acid or base of the solvent. Reactions of the first type are referred to as general acid (or base) catalysed reactions and those of the second type, specific acid (or base) catalysed reactions. If the rate of a reaction in an aqueous solution is proportional only to the hydronium ion concentration, it is said to be a specific acid catalysed (or specific hydronium ion catalysed) reaction: $v = (S) \{k_{\text{H}_3\text{O}^+} (\text{H}_3\text{O}^+)\}$. Whereas for a general acid catalysed process, the rate is dependent upon the concentration of undissociated molecular acids as well:

$v = (S) \{k_{\text{H}_3\text{O}^+} (\text{H}_3\text{O}^+) + k_{\text{HA}} (\text{HA})\}$. In the case of base promoted reactions, for specific base catalysis: $v = (S) \{k_{\text{OH}^-} (\text{OH}^-)\}$, and for general base catalysis: $v = (S) \{k_{\text{OH}^-} (\text{OH}^-) + k_{\text{B}} (\text{B})\}$.

The experimentally determined acid or base dependency of the rate of a reaction often indicates the extent of proton transfer which has taken place at the time the transition state associated with the rate determining step is reached. For example, consider a reaction which involves a rapid reversible transformation of the reactant into its conjugate acid which subsequently undergoes a first-order rate determining step forming an intermediate which is then rapidly transformed to the final product P.²



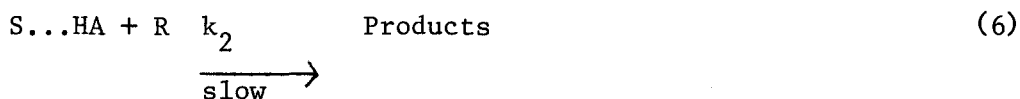
$$v = k_2 (SH^+) = k_2 K_{eq} \frac{(S)(HA)}{(A^-)} \quad k_3 \gg k_2$$

$$= \frac{k_2 K_{eq}}{K_a} (S) (H_3O^+) \quad (4)$$

(where K_{eq} is the equilibrium constant for reaction 1 and K_a is the ionization constant of the acid HA.)

The above equation shows that the reaction is proportional to the hydronium ion concentration i.e. the reaction represents an example of a specific acid catalysed process. It will be noted that the proton transfer is complete prior to the rate determining step for this process.

For some processes, however, the proton transfer may not be complete prior to the rate determining step as would be the case for a system involving the rapid equilibration of reactant and its hydrogen-bonded complex followed by the rate determining formation of products:



$$\text{for which: } v = k_2 (S \dots HA) (R) = k_2 K_{eq} (S) (HA) \quad (7)$$

This mechanism appears to be operative in the acid catalysed hydration of acetaldehyde where R is a water molecule. As indicated by eq.7, general acid catalysis is observed. Similar considerations apply to base catalysed reactions, where a typical specific base catalysed process could be represented by the mechanism:



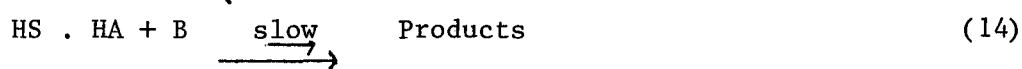
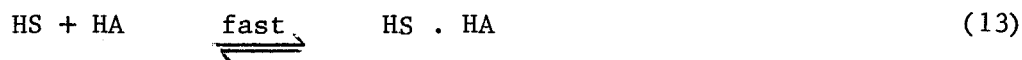
$$v = k_2 (S^-) (R) = k_2 K_{eq} (HS) (B)$$

and a general base catalysed process by:



$$v = k (HS) (B) \quad (12)$$

Finally, it is sometimes observed that the process under consideration is catalysed by both general acids and general bases. This does not imply a three body collision between substrate, acid and base in one step but may be the result of a prior equilibrium involving hydrogen bonding of the substrate with the acid followed by a rate determining proton transfer:



$$v = k (\text{HS} \cdot \text{HA}) (\text{B}) = k K_{\text{eq}} (\text{HS}) (\text{HA}) (\text{B}) \quad (15)$$

Experimentally, the pseudo first-order rate constants for reactions that are catalysed by both acids and bases are usually found to follow the equation:^{2, 5, 6.}

$$v = (S) \left\{ \sum k_i (A_i) + \sum k_j (B_j) \right\}, \quad (16)$$

$$\text{where } \frac{v}{(S)} = k_{\text{obsd}} = \sum k_i (A_i) + \sum k_j (B_j) \quad (17)$$

since the termolecular term is generally relatively small. The absence of any termolecular terms in the kinetic equation, however, cannot be given as a compelling evidence that the reaction represented by equations 13 and 14 does not occur. Many such processes are carried out in solvents possessing amphoteric properties such as water. Thus in general acid (or base) catalysis, water can act as a base (or an acid). The observed rate constant associated with reaction schemes represented by eq 13 and 14 in amphoteric solvents may be represented by:

$$k_{\text{obsd}} = k_o + \sum k_i (A_i) + \sum k_j (B_j) \quad (18)$$

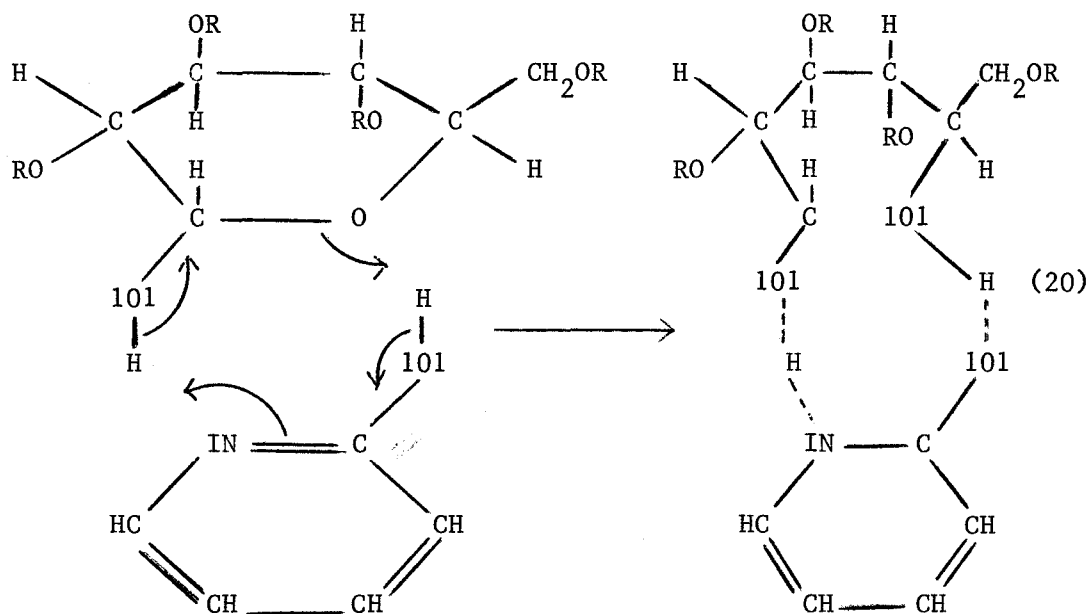
where k_o is called the spontaneous rate of the reaction and is best interpreted as that due to the catalysis by the solvent molecules.

If the solvent is water:

$$k_o = k_{H_2O} (H_2O)^x \quad (19)$$

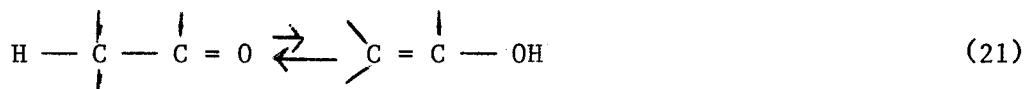
where the exponent, x , implies that the stiochiometry of the transition state with respect to water may be uncertain.

Prototropic isomerizations offer one of the most important classes of acid-base catalysed reactions. One such system which has been studied extensively is the mutarotation of glucose.² This reaction consists of the transformation of α -glucose into an equilibrium mixture of α - and β -glucose through the intermediacy of the open chain aldehydic form. Brønsted and Guggenheim⁷ found that the mutarotation of glucose is both general-acid and general-base catalysed. Lowry and Faulkner⁵ have demonstrated that in benzene solvent this reaction proceeds more rapidly in the presence of a mixture of pyridine and phenol than with either substance alone. Swain and Brown^{6, 2} have shown that 2-hydroxypyridine is 7000 times as an effective catalyst for a mutarotation reaction as a mixture of the same concentrations of pyridine and phenol. They attributed the effectiveness of 2-hydroxypyridine to the capacity of this compound to act both as a weak acid and as a weak base since the acidic and basic sites are held together rigidly in convenient position for simultaneous action on the hemiacetal linkage:

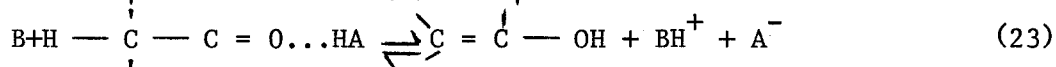
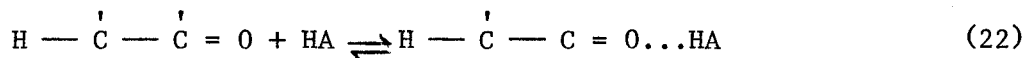


Such polyfunctionality is believed to be one of the factors responsible for the effectiveness of enzyme catalysts.

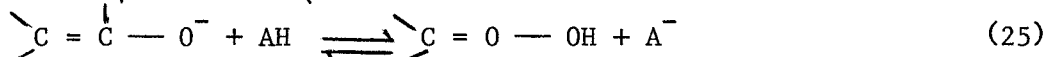
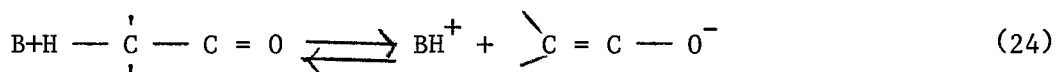
Another classic example involving the participations of general acid and general base catalysis is the enolization of ketones:



Either of two possible mechanisms may be operative for these reactions depending upon whether carbon-hydrogen bond breaking and oxygen-hydrogen bond making occur simultaneously:⁴



or in a stepwise fashion:



It has often been observed for general acid-base promoted reactions that the catalytic efficiency can be correlated with the acidic or basic strength of the catalyst. For example, for catalysis by a series of similar bases, the relationship can be quantitatively expressed by the following equation:²

$$\text{Log } k_B = \beta \log K_B + C \quad (26)$$

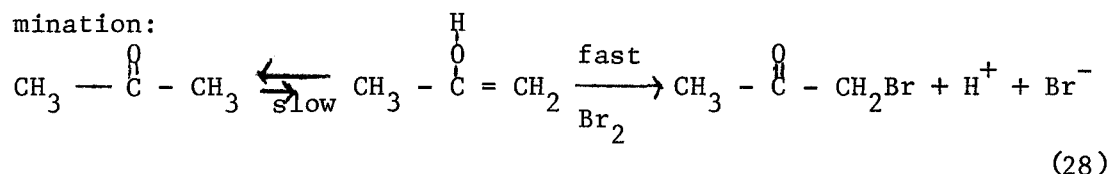
where k_B is the catalytic constant, K_B is a measure of a basic strength of the catalyst, β and C are constants associated with a given reaction, temperature and solvent. The analogous relationship exists for acid catalysis:

$$\text{Log } k_a = \alpha \log K_a + D \quad (27)$$

where k_a is the catalytic constant, K_a is the ionization constant of the acid, α and D are constants associated with a given reaction, temperature and the solvent. Equations 26 and 27 summarize the Brønsted catalysis law.

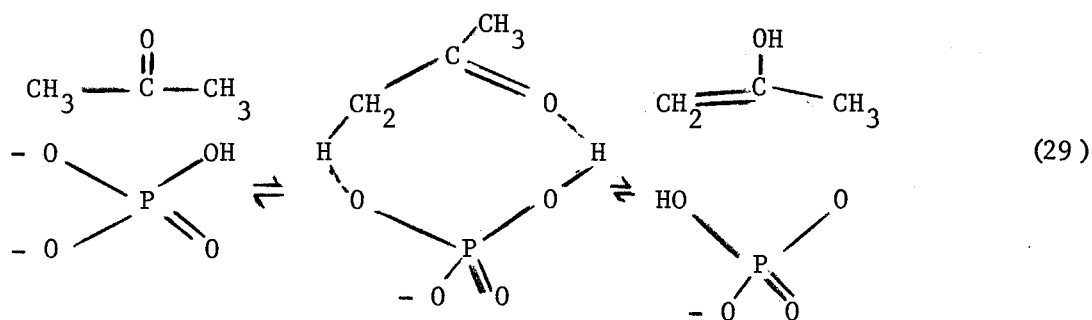
Since the equilibria associated with keto-enol isomerizations usually lie far to the left, indirect methods are needed to study the kinetics of such transformations. Most of the kinetic measurements reported in the literature have been carried out on the halogenation of acetone and other simple aliphatic ketones. Lapworth⁸, for example, has investigated the bromination of acetone in aqueous solution. He found that this process is a first-order reaction, the rate of which is proportional to the concentration of acetone, but independent of that of bromine. These results suggest that the

rate controlling step of the reaction is the transformation of acetone into its enol form, which subsequently undergoes rapid bromination:



Lapworth also found that the reaction was accelerated both by acids and bases such that the reaction rates are proportional to the concentration of all acids and the bases present in the buffering media.

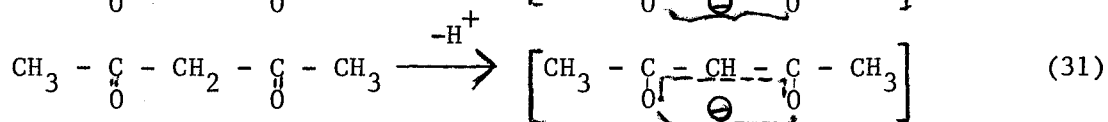
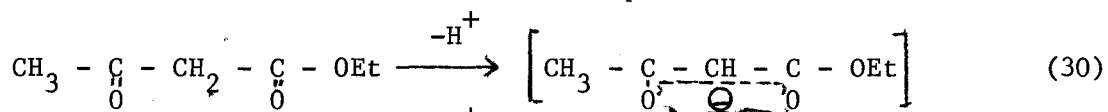
Larson and Meany⁹ have studied the iodination of acetone using phosphate buffers at 25.0°C. They observed that the catalysis of the enolization of acetone by monohydrogen phosphate is ten times faster than that due to diethylmalonate dianion. These results suggest that HPO_4^{2-} participates in the enolization by a mechanism which involves simultaneous general base and general acid catalysis.



Substantiating this theory is the finding by Meany and Lee¹⁰ that alkyl phosphate dianions, the basicities of which are comparable to HPO_4^{2-} , are much less efficient catalysts than the latter.

Significantly, it will be noted that alkyl phosphate dianions have no acidic proton.

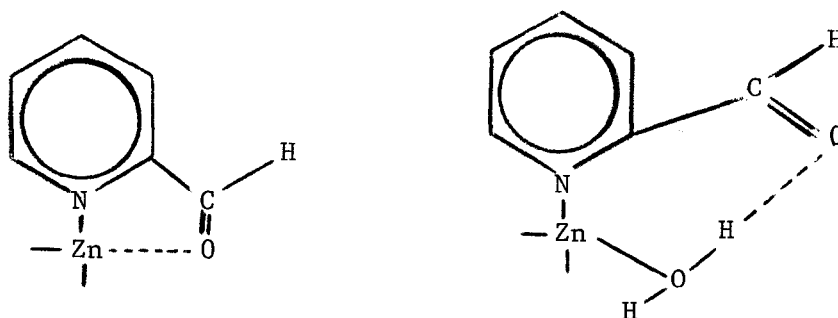
In carbonyl systems where one or more of the hydrogens of acetone has been replaced by an electron withdrawing substituent ($\text{>C} = \text{O}, -\text{COOEt}$) the rate of halogenation is greatly increased.^{4, 11} In these compounds, the ionization of C—H bond is facilitated both by the presence of adjacent electron attracting substituents ($\text{>C} = \text{O}, -\text{COOEt}$) and by the delocalization of the negative charge in the transition state leading to the formation of the enolate anion. Both of these factors enhance proton removal.



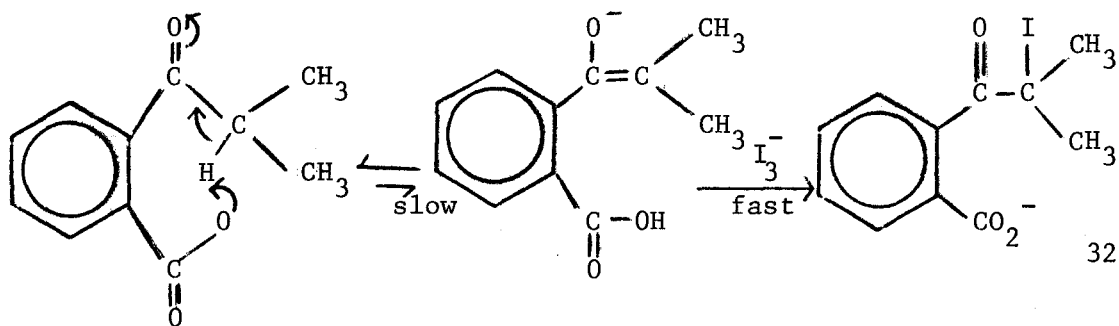
Acid catalysis of acetylacetone tautomerization (eq.31) has been investigated by Meany¹² using the metal ions, Ni^{2+} , Co^{2+} , Zn^{2+} , Co^{2+} , and Mn^{2+} . The powerful catalysis exhibited by these ions was attributed to the ability of acetylacetone to act as bidentate ligand.

Comparative studies of the hydration of 2- and 4-pyridine aldehyde have been carried out by Pocker and Meany¹³. The divalent zinc and cobalt ions are about 10^8 times more effective than water for 2-pyridine aldehyde hydration and practically inactive in the catalysis of the 4-pyridine aldehyde hydration. These results have been interpreted as arising from the chelation of 2-pyridine aldehyde to the divalent metal via the ring nitrogen and the carbonyl oxygen. This chelation would be expected to increase the reactivity of

carbonyl carbon towards H_2O or OH^- as suggested by either of the following structures:



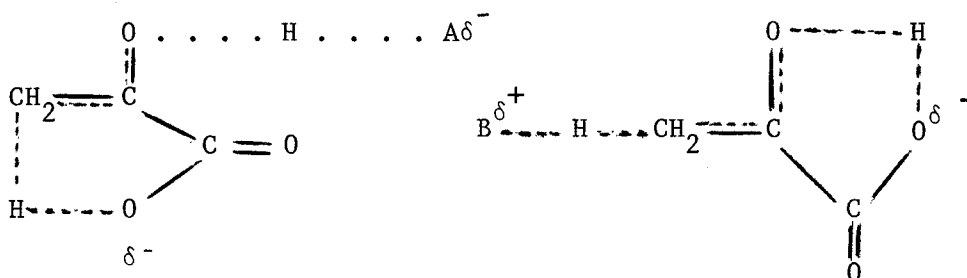
Another efficient mode of catalysis often arises with reactants which contain acidic and basic groups or both, suitably oriented with respect to the seat of the reaction, so that intramolecular catalysis may be operative. In this respect, Harper and Bender¹⁴ have investigated the iodination of *o*-isobutyrylbenzoate ion and have proposed that the enolization proceeds through intramolecular carboxylate ion catalysis.



Their calculations imply that the intramolecular catalysis by the *o*-carboxylate ion is just as efficient as that expected from a concentration of the corresponding intermolecular catalyst (benzoate ion) of ca. 50 M^{15} . They compare these results to those

obtained from the enolization of levulinic acid,¹⁶ in which it might be anticipated that the intramolecular catalytic effects would be smaller than in the stereochemically fixed-*o*-isobutyrylbenzoate. In the iodination of levulinic acid, the intramolecular catalysis by the carboxylate ion was found to be just as efficient as that from the corresponding intermolecular catalyst anticipated from only 1M levulinate ion.

Bell and Fluendy¹⁶ have studied the iodination of a series of keto acids, $\text{CH}_3\text{CO}(\text{CH}_2)_n\text{COOH}$, where $n = 2, 3, 4,$ and 5 . In the case of pyruvic acid they attributed a large portion of the observed velocity to the intramolecular catalysis of the ionization or enolization by $-\text{CO}_2^-$ or CO_2H group in the same molecule. Fluendy¹⁷ using the results obtained from Monte Carlo calculations of the cyclization probabilities of aliphatic chains, has come to the conclusion that the enolization process of such keto acids proceeds predominantly through a cyclic transition state. Albery, Bell, and Powell¹⁸ have attributed the observed velocity of the iodination of pyruvic acid to intramolecular catalysis. They apparently view the transition states associated with enolization of pyruvate and the pyruvic acid in the following manner:



Recently, Meany,¹¹ however, has investigated the spontaneous rates of iodination of pyruvate esters at 25.0°C and preliminary studies show that these reactions proceed at comparable rates to the iodination of pyruvic acid. Thus the assumption that an intramolecular acid catalyzed enolization of pyruvic acid is difficult to justify.

A similar comparison has recently been reported by Pocker, Meany, and Zadorojny¹⁹ concerning the spontaneous hydration of pyruvate esters and pyruvic acid. These studies also indicate the absence of an intramolecular acid catalyzed path. Earlier, the intramolecular catalyzed path for the hydration of pyruvic acid was postulated by Eigen *et. al*²⁰ and Strehlow²¹ to account for the relatively large spontaneous rate of hydration of pyruvic acid as compared to other carbonyl compounds.

In the present work the relative rates of iodination of pyruvic acid and ethyl and methyl pyruvate have been compared at 25.0°C. These studies allow the determination of the respective free energies of activation for these processes. Measurements have also been carried out at various temperatures to determine

the enthalpies and entropies of activation for the spontaneous and base catalysed iodinations of these pyruvate systems. The present thesis also includes a comparison of the solvent deuterium isotope effects associated with the spontaneous rate of iodination of pyruvic acid and the alkyl pyruvates. Also, the catalytic rate coefficients associated with the basic components of various buffers have been determined for the iodination of methyl pyruvate. These values together with the spontaneous rate of iodination allow the determination of the Brønsted coefficient, β , associated with this process.

From these kinetic results, we hope to delineate the mechanism of catalysis for the enolization of pyruvate systems: by distinguishing between intra- and intermolecular catalysed pathways and by accessing the relative importance of carbon-hydrogen bond breaking, and oxygen-hydrogen bond making in the transition states of these enolization processes.

II. EXPERIMENTAL

A. Materials

Substrates

The pyruvic acid used in this work was obtained from Eastman Organic Chemical Company and the ethyl and methyl pyruvates from Aldrich Chemical Company. Since pyruvic acid tends to polymerize and the pyruvate esters are slowly hydrolysed, it is necessary to distill these substances prior to use. The distillation processes were carried out under reduced pressure. A small vacuum distillation apparatus was employed with a rotating receiver for collecting the middle fraction: bp (pyruvic acid) 59-61° (18 mm); bp (ethyl pyruvate) 53-54° (15 mm); bp (methyl pyruvate) 50-52° (15 mm). After distillation the substrates were packed in small, teflon-sealed glass vials in an atmosphere of nitrogen and were refrigerated until use. The concentration of the distilled pyruvic acid was checked by titration with a previously standardized sodium hydroxide solution¹⁸ and the pyruvic acid content was always found to be in the range 97-98%.

Solutions

The formate and acetate buffer components were obtained from commercially available sources either in analytical or reagent grade and were prepared in distilled water. The diethyl malonate buffer components were prepared by the hydrolysis of ethyl ester.⁴²

In all the buffer solutions used in this work the ratio of the general acid concentration (HA) to the conjugate base concentration (A^-) is equal to unity.

Deuterium oxide was a product of ICN Chemical Company and labeled as 99.8% pure.

Table I shows the concentration of each general acid and its conjugate base used in formate, acetate and diethylmalonate buffers and their respective pK_a values.

Table I

Concentration of Acidic and Basic Components in Formate, Acetate, Diethylmalonate Buffers and their Respective pK_a Values.

Buffer	Acid (<u>M</u>)	Base (<u>M</u>)	pK_a in H_2O	pK_a in D_2O
Formate	0.1	0.100	3.66	4.10
Acetate	0.1	0.100	4.65	
Diethylmalonate	0.025	0.025	7.10	

For diluting the buffers, various volumes of 0.1 M NaCl were added to the buffer solutions so that a constant ionic strength of 0.1 M was maintained. The dilution of formate buffer in both H_2O and D_2O for acetate and diethylmalonate buffers are represented in Table II. The table also includes the final concentrations of the respective conjugate bases used in the kinetic runs.

Table II

Dilution of Formate, Acetate and Diethylmalonate
Buffers by 0.1 M NaCl and the Final
Concentrations of the Respective
Conjugate Bases used in the
Kinetic Runs.

Buffer	Solution	ml. of Buffer	ml. of NaCl	(B ⁻)final (<u>M</u>)
Formate	1	10	40	0.019
	2	20	30	0.038
	3	30	20	0.058
	4	40	10	0.077
Acetate	1	0.5	9.5	0.005
	2	2.0	8.0	0.019
	3	4.0	6.0	0.039
	4	6.0	4.0	0.059
	5	8.0	2.0	0.078
DEM	1	0.5	9.0	0.0012
	2	2.0	8.0	0.0049
	3	2.4	7.6	0.0059
	4	4.0	6.0	0.0098

Pyruvic Acid - Pyruvate Solutions

These solutions were prepared by adding previously standardized 0.1 N NaCl containing 0.070 ml of freshly distilled pyruvic acid. The volumes added from these solutions and the pH values of the resulting pyruvic acid-pyruvate solutions are shown in Table III.

Table III

Volumes added from 0.1 N HCl and 0.1 N NaCl and pH
Values of the Pyruvic Acid-Pyruvate Solutions

ml of 0.1 N HCl	ml of 0.1 N NaCl	pH
20	80	1.75
30	70	1.60
40	60	1.49
50	50	1.38

Triiodide Solutions

This solution contained 2.1×10^{-3} M of iodine in 1.0 M potassium iodide and was used in the kinetic runs from 0 up to 30°C. Another iodine solution, 3.0×10^{-3} M iodine in 1.0 M potassium iodide was employed for the iodination from 30 up to 50°C. For the D₂O studies, the triiodide solutions were prepared in the same manner.

B. Analytical Instruments

Source of Kinetic Data

Kinetic measurements for both the hydration and enolization studies were obtained on a Beckman Kintrac VII recording spectrophotometer. Beckman and Unicam glass cells having a path length of 10 mm were used throughout the work. The cell basket in the instrument was attached to a Beckman thermocirculator

accessory for temperature control within the cells. For cooling the cell chamber to less than 12°C , a low temperature accessory was required. An external refrigeration unit was employed to cool a large bath (containing a mixture of 50% ethylene glycol - water) to the required temperature. The coolant from this bath was circulated through a coil in the thermocirculator unit which was connected to the cell compartment of the Kintrac by means of insulated polyethylene tubing. The cell chamber was flushed with nitrogen gas to prevent the condensation of water vapor on the mirrors of the monochromitor.

pH Measurements

The pH values of all solutions were determined using a Beckman Century SS pH meter, the reproducibility of which is ± 0.01 pH units. The instrument was standardized with standard buffer solution before taking the measurements. The electrodes used with this pH meter were Beckman glass and Calomel reference electrodes. In work utilizing D_2O as a solvent, the glass electrode correction formula, $\text{pD} = \text{pH} (\text{meter reading}) + 0.41$ was employed. The pK_a of pyruvic acid in D_2O was experimentally determined by using titrimetric methods. A solution of pyruvic in D_2O was titrated with a previously standardized solution of 0.100 M NaOD . The change in pD of the solution was followed during the entire course of titration. Figure 1 represents a plot of pD versus the ml. of NaOD added. From this plot, the pK_a of pyruvic acid in D_2O was found to be 2.95 at 25°C .

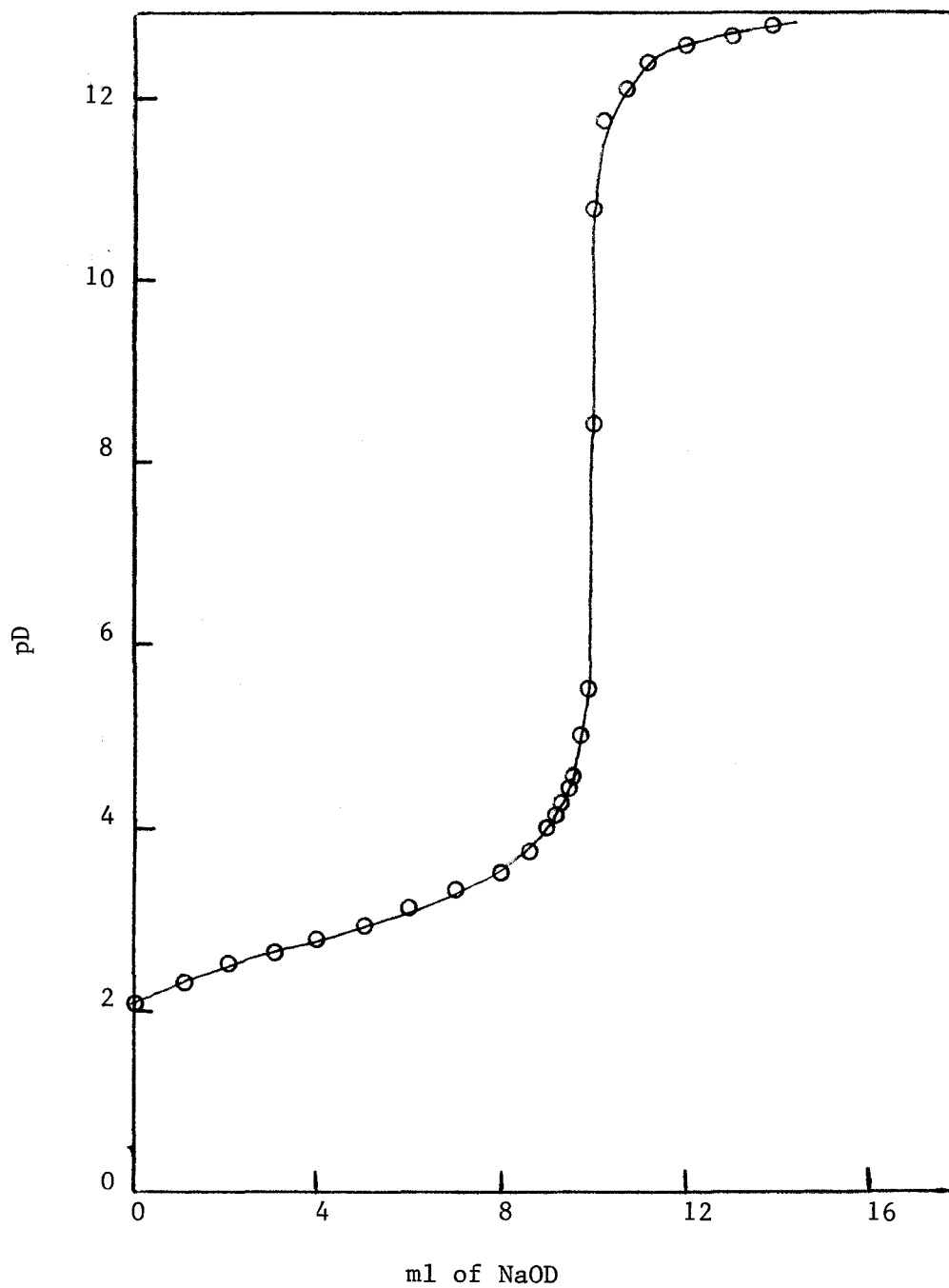


Figure 1 Determination of pK_a of pyruvic acid in D_2O

C. Kinetic Methods

Hydration Studies

The hydration of methyl pyruvate in 0.004 M formate buffer using D₂O as a solvent was followed spectrophotometrically by the diminution of methyl pyruvate signal at 330 nm. The method used in these studies is similar to that used by Pocker and Meany¹⁹ in the hydration studies of pyruvate systems. From these spectrophotometric readings, the relationship between $\log (A_t - A_\infty)$ and the time is represented in Figure 2. From the intercept of the straight line obtained, A_0 , the absorbancy at the kinetic zero, was calculated. The absorption at equilibrium, A_∞ , was determined spectrophotometrically at various temperatures. The ratio, R, of the hydrated to the unhydrated forms of methyl pyruvate at the temperature under consideration was deduced from the following equation,

$$R = \frac{A_0 - A_\infty}{A_0} \quad (33)$$

It was assumed that A_0 is constant over the range of temperatures studied. A plot of $\log R$ versus the reciprocal of absolute temperature is shown in Figure 3. From a comparison of these results with those obtained in the earlier work on the hydration of methyl pyruvate in H₂O, it was found that the extent of hydration of pyruvate esters is the same in both H₂O and D₂O at all temperatures studied.

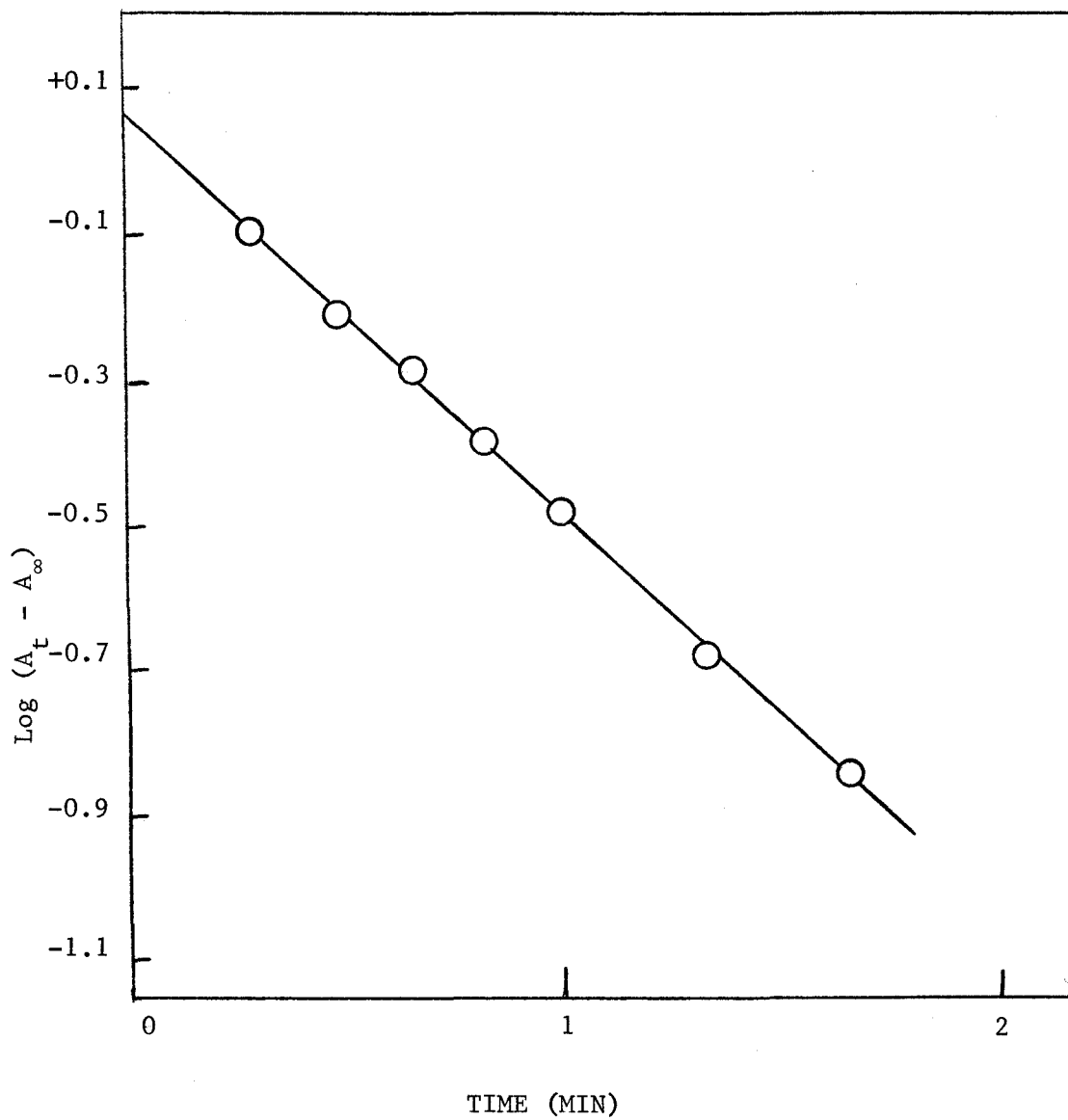


Figure 2 Typical methyl pyruvate hydration in D_2O , at 12°C , 0.004 M Formate buffer, $\text{pD} = 4.10$.

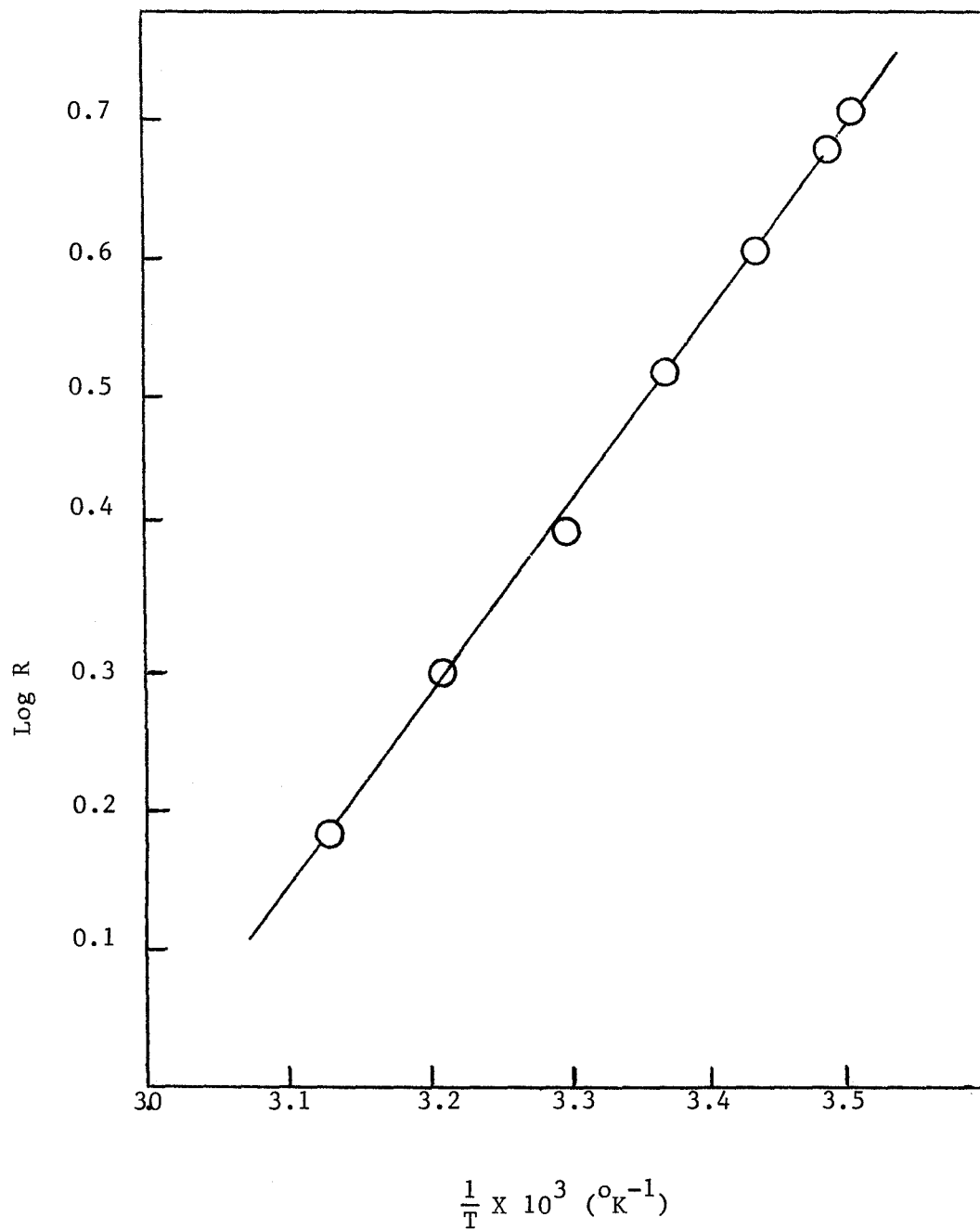


Figure 3 Log R plotted against reciprocal absolute temperatures for hydration of methyl pyruvate in D_2O .

The hydration studies of pyruvic acid in D_2O were carried out at $25^\circ C$. These studies were made in both H_2O and D_2O using equivalent concentrations of pyruvic acid and HCl . The latter was added to insure that the concentration of pyruvate anion would be negligibly small. Comparison of the spectrophotometric readings at 340 nm ¹⁹ led us to conclude that the extent to which pyruvic acid undergoes hydration in D_2O is the same as in H_2O .

Enolization Studies

The triiodide ion, I_3^- , has a maximum absorption at 353 nm ($\epsilon = 24,500\text{ l. M}^{-1}\text{ cm}^{-1}$)¹⁸. The rates of iodination of the systems under consideration were determined by following the uptake of I_3^- ion at this wavelength. In these iodination runs, the concentration of pyruvic acid or the pyruvate esters, always greatly exceeded that of the triiodide ion so that pseudo zero-order kinetics were observed. The reaction velocities were calculated from the slopes of the linear traces by dividing by ϵ .¹¹

It is interesting to note that both the iodine and the triiodide ion are capable of halogenating enols.²² Thus, the calculated reaction velocities using the above equation are not exactly correct, since it takes into account only the disappearance of I_3^- ion and not both I_2 and I_3^- species. For this reason the above equation needs to be corrected by multiplying by $\frac{(I_2) + (I_3^-)}{(I_3^-)}$.

$$v_{\text{corr}} = \frac{\text{slope}}{\epsilon_{\text{I}_3^-}} \times \frac{(\text{I}_3^-) + (\text{I}_2)}{(\text{I}_3^-)} \quad (34)$$

This equation can be written in terms of the equilibrium constant K^{23} for the process $\text{I}_2 + \text{I}^- \rightleftharpoons \text{I}_3^-$ and the iodide ion concentration, in the following way:

$$v_{\text{corr}} = \frac{\text{slope}}{\epsilon_{\text{I}_3^-}} \times \frac{1 + K (\text{I}^-)}{K (\text{I}^-)} \quad (35)$$

Thus, the correction for the observed reaction velocity of the iodination can be made if K and (I^-) are known at the temperatures under consideration. Table IV summarizes the values of $\frac{1 + K (\text{I}^-)}{K (\text{I}^-)}$ at various temperatures.

Table IV

Variation of $\frac{1 + K (\text{I}^-)}{K (\text{I}^-)}$ with Temperature.^{a, b}

Temp (C ^o)	0.0	10	20	30	40	50
$\frac{1 + K (\text{I}^-)}{K (\text{I}^-)}$	1.02	1.03	1.04	1.05	1.06	1.08

a) $(\text{I}^-) = 0.032 \text{ M.l}^{-1}$

b) reference 23

In the determination of the activation parameters for the iodination processes, kinetic runs were carried out in pyruvic

acid-pyruvate solutions, as well as for alkyl pyruvate (using formate buffers in both H_2O and D_2O) at seven temperatures from 0 up to $50^\circ C$. Generally, the rates were determined in duplicate at each temperature to insure reproducibility. In addition, the iodination of methylpyruvate was carried out at $25.0^\circ C$ using acetate and diethylmalonate buffers.

For the iodination of the alkyl pyruvates, 3 ml of the solution containing the buffer and enough sodium chloride to adjust the ionic strength to 0.1 M were placed in a 3 cc cuvette. The solutions were thermostated for at least ten minutes in the cell compartment of the Kintrac to the desired temperatures. An accurately measured volume of alkyl pyruvate was introduced into the buffer solution by means of a calibrated Hamilton syringe. The kinetic runs were initiated by injection of 0.1 ml of the triiodide solution again using a microlitre syringe. Since these alkyl pyruvates are partially hydrated in aqueous solution, the solutions were allowed to pre-equilibrate for at least one minute before the iodination process was initiated.^{11, 25, 26} Earlier work^{26, 27} has shown that the hydrolysis of these alkyl pyruvates is exceedingly slow in these buffer solutions. Consequently the iodination was complete before any detectible quantity of pyruvic acid was generated.

In D_2O work, the alkyl pyruvate was injected last to prevent any deuterium exchange between the solvent and the substrate^{2, 3}

and the reaction with the triiodide ion was followed to completion. The reaction rates were calculated from the slope of the linear traces obtained, as was mentioned earlier.

It was observed that a slow decrease in the absorbancy of the triiodide ion was affected by the presence of formate buffer.¹¹ This decrease never exceeded 4-5% that which was caused by the iodination of alkyl pyruvate, and the observed rate constants were corrected accordingly.

The iodination of pyruvic acid in D_2O was carried out in a similar fashion except that they were run in DCl solutions. The ionic strength was adjusted to 0.1 M by adding appropriate quantities of sodium chloride. The hydration of pyruvic acid was allowed to reach the equilibrium before the iodination process was initiated.

III. RESULTS

Generally the conversion of ketones to their enol forms, is much slower than the halogenation of the enol. It has been shown previously that the rate of iodination of keto acids or keto esters is identical with the rate of enolization.^{18, 28} Therefore, the keto-enol transformations can be followed spectrophotometrically by monitoring the rate of disappearance of the halogenating agent, which is the triiodide ion in the present work.¹⁸

From the Lambert-Beer law:²⁹

$$A = \epsilon l c \quad (36)$$

where A = optical density

l = light pathlength in cm.

c = concentration of solute expressed as mole/liter

ϵ = extinction coefficient characteristic for the solute, solvent, and the temperature.

Thus for the triiodide ion using a path length of 10 mm

$$A = \epsilon_{\text{I}_3^-} (\text{I}_3^-), \quad \text{where } \epsilon_{\text{I}_3^-} = 24,500 \text{ l.mol}^{-1} \text{ cm}^{-1}$$

$$(\text{I}_3^-) = \frac{A}{\epsilon_{\text{I}_3^-}}$$

The rate of disappearance of the triiodide ion = $\frac{d(\text{I}_3^-)}{dt}$

$$\frac{d(I_3^-)}{dt} = \frac{(I_3^-)_o - (I_3^-)_t}{t} = \frac{A_o - A_t}{\epsilon_{I_3^-} t} \quad (37)$$

$$\frac{d(I_3^-)}{dt} = \frac{\text{slope}}{24,500 \text{ l.mol}^{-1} \text{ cm}^{-1}} \quad (38)$$

Accordingly, the rate of enolization is equal to the slope of the linear traces, obtained from the diminution of the triiodide peak at 353 nm, divided by the extinction coefficient of the triiodide ion. Since both iodine and the triiodide ions are capable of halogenating the enol,²² the rate (equation 38) was corrected throughout this work as was mentioned in the experimental section.

A. Iodination of Methyl- and Ethyl Pyruvates

The iodinations of methyl and ethyl pyruvates are general acid-general base catalysed processes. The overall rate constant for such a process in a buffered system may be written as a sum of the catalytic terms arising from all acidic and basic components present in the solution,³⁰

$$k_{\text{obsd}} = k_o + k_{L_3O^+} (L_3O^+) + k_{OL^-} (OL^-) + k_{LA} (LA) + k_B (B) \quad (39)$$

where k_o is the spontaneous rate constant and L is used to denote the isotopically different hydrogens.

The above equation does not contain a termolecular term containing both A^- and HA, indicating that the concerted action

of both A^- and LA is not a dominant pathway for the enolizations.

The pseudo-zero order rate constant for the iodination of the alkyl pyruvates in formate buffer ($pK_a = 3.66$) has been found to follow the equation.¹¹

$$k_{\text{obsd}} = k_o + k_{\text{HCO}_2^-} (\text{HCO}_2^-) \quad (40)$$

This equation indicates that the catalytic contribution arising from hydronium ions, hydroxide ion and formic acid are negligibly small under these conditions.

Since the rate constant for a reaction greatly depends on the total concentration of ions in solution,⁴ it is necessary to work under conditions where ionic strength is kept constant. Then the observed rate constants could be interpreted as due to the specific action of the buffer components and not due to the ionic strength of the solution.

By a proper choice of experiment, one may determine the value of the catalytic constant of formate, $k_{\text{HCO}_2^-}$, as well as the spontaneous rate of iodination, k_o . A series of runs were carried out varying simultaneously the concentration of the buffer components while maintaining a buffer ratio of unity and an ionic strength at 0.1 M. A plot of k_{obsd} versus formate ion concentration was found to give a straight line, the slope of which is equal to the catalytic rate constant for formate ion, $k_{\text{HCO}_2^-}$, and the intercept, the

spontaneous rate of iodination, k_0 . Such kinetic measurements were carried out for ethyl and methyl pyruvates over a range of formate buffer concentrations (0.019 - 0.097 mol. l^{-1}) at various temperatures. The observed rate constant for each run was calculated from the velocity of the reaction divided by the concentration of the unhydrated alkyl pyruvate.¹¹

$$k_{\text{obsd}} = \frac{v_{\text{obsd}}}{(\text{Alkyl pyruvate}) (1 - \chi)} \quad (41)$$

where χ is the fraction of hydration of an alkyl pyruvate at the temperature under consideration. The fraction of hydration of ethyl and methyl pyruvates at various temperatures, were obtained from the work of Pocker, Meany, and Zadorojny.¹⁹ It will be noted that the hydration equilibria of these alkyl pyruvates increases as the temperature decreases. These hydration equilibria show a second order dependence on the water concentration. Consequently, it is necessary to correct the ratio of the hydrated to the unhydrated form of the alkyl pyruvate to compensate for the higher concentrations of water used in the present work in comparison to those used by Pocker et. al.¹⁹ These corrections were made for ethyl and methyl pyruvates as follows:

$$K_{\text{eq}} = \frac{R}{(\text{H}_2\text{O})^2} = \frac{R^{\wedge}}{(\text{H}_2\text{O}_{\text{exp}})^2}$$

where $R' = \frac{\chi}{1-\chi}$ (χ : fraction of hydration under our experimental condition and R' is the corrected ratio)

$$\text{Thus } R' = \frac{R}{(55.1)^2} (\text{H}_2\text{O}_{\text{exp}})^2 \quad (42)$$

The corrected fraction of hydrations at various temperatures for ethyl- and methyl pyruvate are shown in Table V.

The formate catalysed rate constants of the iodination of methyl and ethyl pyruvates are represented in Tables VI and VII respectively.

The iodinations of methyl- and ethyl pyruvates were also followed in formate buffer (buffer ratio = 1) in D_2O over the concentration range (0.019 - 0.097 M. l^{-1}). The measurements were carried out at temperatures 2, 12, 25.1, 30, 34.9, 39.2 and 48.3. The pKa of the formate buffer is 4.1 and is essentially invariant over the temperature range studied. The fraction of hydration of methyl pyruvate in D_2O was briefly investigated and the results obtained, (Table VIII) are identical to those obtained by Pocker, Meany, and Zadorojny¹⁹ for the hydration of methyl pyruvate in H_2O . The formate catalyzed iodination of methyl and ethyl pyruvate in D_2O are represented in Table IX and X respectively.

TABLE V

The Corrected Fractions of Hydration of Ethyl
and Methyl Pyruvates at Various Temperatures

Methyl Pyruvate						
T			(H ₂ O)			
(°K)	R	a	Mol. l ⁻¹	R ^c	χ	1-χ
273	6.68		55.3	6.70	0.87	0.13
283	4.68		54.5	4.58	0.82	0.18
293	3.35		55.1	3.35	0.77	0.23
303	2.46		55.5	2.52	0.72	0.28
308	2.16		55.5	2.19	0.69	0.31
313	1.86		55.5	1.89	0.65	0.35
323	1.45		55.5	1.47	0.59	0.41
Ethyl Pyruvate						
273	5.52		55.3	5.54	0.85	0.15
283	3.80		54.5	3.73	0.79	0.21
293	2.78		55.1	2.78	0.74	0.27
303	2.04		55.5	2.07	0.67	0.33
308	1.83		55.5	1.86	0.65	0.35
313	1.55		55.5	1.57	0.61	0.39
323	1.20		55.5	1.22	0.55	0.45

a) Reference 19.

TABLE VI

The Formate Catalysed Rate Constants of Iodination
of Methyl Pyruvate at $\mu = 0.1 \text{ M}$.

-1.0 °C.

(HCO_2^-) M. l^{-1}	$v_{\text{obsd}} \times 10^9$ M. $\text{l}^{-1} \text{ sec}^{-1}$	$v_{\text{obsd}} \times 10^9$ M. $\text{l}^{-1} \text{ sec}^{-1}$	a	$k_{\text{obsd}} \times 10^7$ sec $^{-1}$	b
0.019	1.70	1.74		3.70	
0.039	5.02	5.14		10.94	
0.058	3.90	3.98		8.47	
0.076	3.40	4.47		9.51	
0.097	4.85	4.97		10.57	

10.0 °C.

(HCO_2^-) M. l^{-1}	$v_{\text{obsd}} \times 10^8$ M. $\text{l}^{-1} \text{ sec}^{-1}$	$C \times 10^8$ M. $\text{l}^{-1} \text{ sec}^{-1}$	$v_{\text{obsd}}^{\text{corr}} \times 10^8$ M. $\text{l}^{-1} \text{ sec}^{-1}$	c	$k_{\text{obsd}} \times 10^6$ sec $^{-1}$	b
0.019	4.76	0.054	4.70		1.47	
0.039	6.12	0.070	6.05		1.92	
0.058	7.48	0.086	7.39		2.32	
0.078	9.52	0.100	9.42		2.95	
0.097	12.93	0.119	12.8		4.02	

Table VI (Continued)

20.0 °C.

(HCO ₂ ⁻)	$v_{\text{obsd}} \times 10^7$	$C \times 10^7$	$v_{\text{obsd}} \times 10^7$	$k_{\text{obsd}} \times 10^6$ b
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹
water	0.17	-	0.17	1.06
0.019	0.49	0.006	0.48	3.00
0.039	0.94	0.011	0.92	5.75
0.058	1.29	0.016	1.28	7.95
0.078	1.63	0.021	1.61	10.04
0.097	1.91	0.025	1.88	11.71

30.0 °C.

(HCO ₂ ⁻)	$v_{\text{obsd}} \times 10^7$	$C \times 10^7$	$v_{\text{obsd}}^{\text{corr}} \times 10^7$ c	$k_{\text{obsd}} \times 10^5$ b
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹
0.019	0.49	0.024	0.47	0.967
0.039	0.64	0.051	0.58	1.20
0.058	1.12	0.073	1.05	2.16
0.076	1.63	0.094	1.54	3.16
0.091	1.50	0.116	1.38	2.85

Table VI (Continued)

35.0 °C.				
(HCO ₂ ⁻) M. l ⁻¹	$v_{\text{obsd}} \times 10^7$ M. l ⁻¹ sec ⁻¹	$C \times 10^7$ M. l ⁻¹ sec ⁻¹	$v_{\text{obsd}}^{\text{corr}} \times 10^7$ M. l ⁻¹ sec ⁻¹	$k_{\text{obsd}} \times 10^5$ sec ⁻¹
0.019	0.99	0.05	0.94	1.76
0.039	1.63	0.06	1.57	2.95
0.058	2.28	0.12	2.16	4.06
0.078	2.69	0.16	2.54	4.77
0.097	3.61	0.19	3.42	6.43
40.0 °C.				
0.019	1.6	0.06	1.54	2.58
0.038	2.5	0.12	2.33	3.91
0.058	3.5	0.17	3.34	5.61
0.077	4.9	0.21	4.68	7.86
0.097	6.0	0.27	5.72	9.59
50.0 °C.				
0.019	5.0	0.10	4.9	7.1
0.039	8.2	0.33	7.8	11.3
0.058	11.7	0.55	11.1	16.1
0.078	14.4	0.79	13.6	19.6
0.097	17.1	1.0	16.1	23.2

TABLE VII

The Formate Catalysed Rate Constants of the
Iodination of Ethyl Pyruvate at
Various Temperatures
and $\mu = 0.1 \text{ M}$.

-2.0 °C.

(HCO_2^-) M. l^{-1}	$v_{\text{obsd}} \times 10^9$ M. $\text{l}^{-1} \text{ sec}^{-1}$	$\hat{v}_{\text{obsd}} \times 10^9$ M. $\text{l}^{-1} \text{ sec}^{-1}$	a	$k_{\text{obsd}} \times 10^7$ sec $^{-1}$	b
0.019	0.77	0.79		1.92	
0.039	1.40	1.45		3.54	
0.058	1.94	1.99		4.85	
0.079	3.12	3.19		7.78	
0.097	3.54	3.62		8.83	

10.0 °C.

(HCO_2^-) M. l^{-1}	$v_{\text{obsd}} \times 10^7$ M. $\text{l}^{-1} \text{ sec}^{-1}$	$C \times 10^7$ M. $\text{l}^{-1} \text{ sec}^{-1}$	$v_{\text{obsd}}^{\text{corr}} \times 10^7$ M. $\text{l}^{-1} \text{ sec}^{-1}$	c	$k_{\text{obsd}} \times 10^6$ sec $^{-1}$	b
water	0.051	-	0.051		0.29	
0.019	0.150	0.005	0.144		0.81	
0.039	0.245	0.007	0.238		1.31	
0.058	0.374	0.009	0.366		2.05	
0.078	0.442	0.010	0.432		2.42	
0.097	0.544	0.012	0.532		3.00	

Table VII (Continued)

20.0 °C.				
(HCO ₂ ⁻)	$\nu_{\text{obsd}} \times 10^7$	$c \times 10^7$	$\nu_{\text{obsd}}^{\text{corr}} \times 10^7$	$k_{\text{obsd}} \times 10^6$
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹
water	0.17	-	0.17	1.13
0.019	0.35	0.007	0.35	2.31
0.038	0.68	0.011	0.67	4.44
0.058	0.92	0.016	0.90	5.99
0.097	1.43	0.025	1.40	9.36

30.0 °C.				
(HCO ₂ ⁻)	$\nu_{\text{obsd}} \times 10^7$	$c \times 10^7$	$\nu_{\text{obsd}}^{\text{corr}} \times 10^7$	$k_{\text{obsd}} \times 10^5$
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹
0.019	0.31	0.02	0.29	0.62
0.058	0.75	0.07	0.68	1.48
0.078	0.91	0.09	0.82	1.79
0.097	1.02	0.17	0.85	1.86

Table VII (Continued)

35.0 °C.				
(HCO ₂ ⁻)	$v_{\text{obsd}} \times 10^7$	$C \times 10^7$	$v_{\text{obsd}}^{\text{corr}} \times 10^7$	$k_{\text{obsd}} \times 10^5$
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹
0.019	0.88	0.05	0.83	1.71
0.039	1.29	0.06	1.23	2.52
0.058	1.84	0.12	1.72	3.52
0.078	2.38	0.16	2.23	4.56
0.097	2.59	0.19	2.40	4.91
40.0 °C.				
0.019	1.26	0.06	1.20	3.23
0.039	2.14	0.12	2.03	3.78
0.058	2.86	0.17	2.70	5.04
0.076	3.67	0.22	3.46	6.46
0.097	4.42	0.27	4.15	7.76
50.0 °C.				
water	1.05	-	1.05	1.74
0.019	3.67	0.10	3.57	5.83
0.039	6.26	0.33	5.93	9.77
0.058	8.44	0.56	7.88	13.0
0.078	10.75	0.79	9.96	16.4
0.097	14.42	1.02	13.40	22.1

TABLE VIII

The Fraction of Hydration of Methyl Pyruvate in D₂O
at Various Temperatures

Temperature (°C)	$R = \frac{\chi}{1-\chi}$	χ	$1-\chi$
12.0	4.90	0.83	0.17
25.0	3.06	0.75	0.25
30.0	2.60	0.72	0.28
34.9	2.21	0.69	0.31
39.1	1.90	0.65	0.35
48.1	1.43	0.59	0.41

TABLE IX

The Formate Catalyzed Iodination of
Methyl Pyruvate in D_2O
and $\mu = 0.1 \underline{M}$

2.0 °C.

(HCO_2^-) M. l^{-1}	$v_{obsd} \times 10^9$ M. $l^{-1} \text{ sec}^{-1}$	$v_{obsd} \times 10^9$ M. $l^{-1} \text{ sec}^{-1}$	a	$k_{obsd} \times 10^7$ sec $^{-1}$	b
0.019	1.36	1.39		2.96	
0.039	3.11	3.18		6.77	
0.058	4.37	4.47		9.51	
0.078	5.54	5.67		12.1	

12.5 °C.

(HCO_2^-) M. l^{-1}	$v_{obsd} \times 10^7$ M. $l^{-1} \text{ sec}^{-1}$	$v_{obsd} \times 10^7$ M. $l^{-1} \text{ sec}^{-1}$	a	$k_{obsd} \times 10^5$ sec $^{-1}$	b
0.000	0.109	0.112		0.033	
0.019	0.476	0.490		0.149	
0.039	0.680	0.701		0.214	
0.058	0.952	0.981		0.299	
0.078	1.19	1.23		0.374	
0.097	1.43	1.47		0.448	

Table IX (Continued)

25.0 °C.				
(HCO ₂ ⁻)	$v_{\text{obsd}} \times 10^7$	$c \times 10^7$	$v_{\text{obsd}}^{\text{corr}} \times 10^7$	$k_{\text{obsd}} \times 10^5$
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹
0.000	0.049	-	0.049	0.057
0.019	0.374	0.012	0.363	0.423
0.039	0.658	0.016	0.641	0.748
0.058	0.850	0.021	0.829	0.967
0.078	1.09	0.026	1.06	1.24
0.097	1.77	0.031	1.74	2.03
30.0 °C.				
0.000	0.095	-	0.095	0.20
0.019	0.408	0.012	0.396	0.83
0.039	0.612	0.027	0.586	1.2
0.058	0.952	0.040	0.912	1.9
0.078	1.36	0.054	1.31	2.7
0.097	1.70	0.068	1.63	3.4

Table IX (Continued)

34.9 °C.				
(HCO ₂ ⁻)	$v_{\text{obsd}} \times 10^7$	$C \times 10^7$	$v_{\text{obsd}}^{\text{corr}} \times 10^7$	$k_{\text{obsd}}^c \times 10^5$
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹
0.000	0.145	-	0.145	0.273
0.019	0.680	0.029	0.652	1.23
0.039	1.24	0.051	1.19	2.25
0.058	1.74	0.073	1.66	3.14
0.078	2.38	0.094	1.29	4.32
0.097	2.72	0.116	2.61	4.92
48.1 °C.				
0.000	0.537	-	0.537	0.784
0.019	2.72	0.135	2.59	3.77
0.039	4.42	0.238	4.18	6.11
0.058	6.26	0.340	5.92	8.64
0.078	10.2	0.445	9.76	14.2
0.097	12.0	0.544	11.4	16.7

TABLE X

The Formate Catalysed Iodination of
Ethyl Pyruvate at Various Temp-
eratures in D_2O
and $\mu = 0.1 \text{ M}$

2.0 °C.

(HCO_2^-) M. l^{-1}	$v_{\text{obsd}} \times 10^9$ M. $l^{-1} \text{ sec}^{-1}$	$v_{\text{obsd}} \times 10^9$ M. $l^{-1} \text{ sec}^{-1}$	a	$k_{\text{obsd}} \times 10^7$ sec $^{-1}$	b
0.019	1.26	1.28		3.12	
0.039	1.94	1.98		4.85	
0.058	3.21	3.28		8.00	
0.078	3.89	3.98		9.69	
0.097	6.08	6.30		16.97	

12.0 °C.

(HCO_2^-) M. l^{-1}	mlof EP	(EP) (1-X) M. l^{-1}	$v_{\text{obsd}} \times 10^7$ M. $l^{-1} \text{ sec}^{-1}$	a	$k_{\text{obsd}} \times 10^5$ sec $^{-1}$	b
0.000	0.025	0.0155	0.046		0.030	
0.019	0.030	0.0186	0.187		0.100	
0.039	0.050	0.0312	0.532		0.170	
0.058	0.050	0.0312	0.869		0.278	
0.078	0.050	0.0312	1.00		0.318	
0.097	0.050	0.0312	1.44		0.460	

Table X (Continued)

25.1 °C.				
(HCO ₂ ⁻)	$v_{\text{obsd}} \times 10^7$	$C \times 10^7$	$v_{\text{obsd}}^{\text{corr}} \times 10^7$	$k_{\text{obsd}}^c \times 10^5$
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹ b
0.000	0.036	-	0.036	0.042
0.019	0.289	0.011	0.277	0.329
0.039	0.510	0.016	0.494	0.680
0.058	0.748	0.021	0.727	.863
0.078	0.969	0.026	0.943	1.12
0.097	1.29	0.031	1.26	1.50
30.0 °C.				
0.000	0.075	-	0.075	0.164
0.019	0.272	0.012	0.260	0.569
0.039	0.544	0.027	0.518	1.13
0.058	0.782	0.040	0.742	1.63
0.078	1.050	0.054	1.00	2.19
0.097	1.260	0.068	1.19	2.61

Table X (Continued)

34.9 °C.				
(HCO ₂ ⁻)	$\nu_{\text{obsd}} \times 10^7$	$c \times 10^7$	$\nu_{\text{obsd}}^{\text{corr}} \times 10^7$	$k_{\text{obsd}} \times 10^5$
M. l ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	M. l ⁻¹ sec ⁻¹	sec ⁻¹
0.000	0.119	-	0.119	0.247
0.019	0.510	0.029	0.481	0.260
0.039	0.884	0.051	0.833	1.73
0.058	1.34	0.073	1.27	2.63
0.078	1.75	0.094	1.66	3.44
0.097	2.72	0.116	2.61	5.40
39.4 °C.				
0.000	0.172	-	0.172	0.322
0.019	0.850	0.041	0.809	1.51
0.039	1.63	0.067	1.57	2.92
0.058	2.38	0.093	2.29	4.27
0.078	3.06	0.119	2.94	5.49
0.097	3.74	0.145	3.60	6.71

Table X (Continued)

48.3 °C.

(HCO ₂ ⁻) M. l ⁻¹	$\nu_{\text{obsd}} \times 10^7$ M. l ⁻¹ sec ⁻¹	C X 10 ⁷ M. l ⁻¹ sec ⁻¹	$\nu_{\text{obsd}}^{\text{corr}} \times 10^7$ M. l ⁻¹ sec ⁻¹	c	$k_{\text{obsd}}^{\wedge} \times 10^5$ sec ⁻¹	b
0.000	0.456	-	0.456		0.746	
0.019	2.17	0.135	2.04		3.34	
0.039	4.29	0.238	4.05		6.62	
0.058	5.71	0.340	5.37		8.79	
0.078	8.30	0.445	7.85		12.9	
0.097	9.25	0.544	8.71		14.30	

$$a) \quad \nu_{\text{obsd}}^{\wedge} = \nu_{\text{obsd}} \times 1 + \frac{K (I^-)}{K (I^-)} \quad \text{see Table IV}$$

$$b) \quad k_{\text{obsd}}^{\wedge} = \frac{\nu_{\text{obsd}}^{\wedge}}{(AIK \text{ PYR}) (1 - \chi)} = \frac{\nu_{\text{obsd}}}{(AIK \text{ PYR}) (1 - \chi)} \times 1 + \frac{K (I^-)}{K (I^-)}$$

$$= k_{\text{obsd}} \times \frac{1 + K (I^-)}{K (I^-)}$$

$$c) \quad \nu_{\text{obsd}}^{\text{corr}} = \text{Rate of uptake of the triiodide ion by the buffer and alkyl pyruvate } (\nu_{\text{obsd}}) - \text{Rate of uptake of the triiodide ion by the buffer components only (C).}$$

The relationships between the rate constants and formate ion concentrations for the iodination of methyl and ethyl pyruvate in both H₂O and D₂O at various temperatures are represented in Figures 4-12.

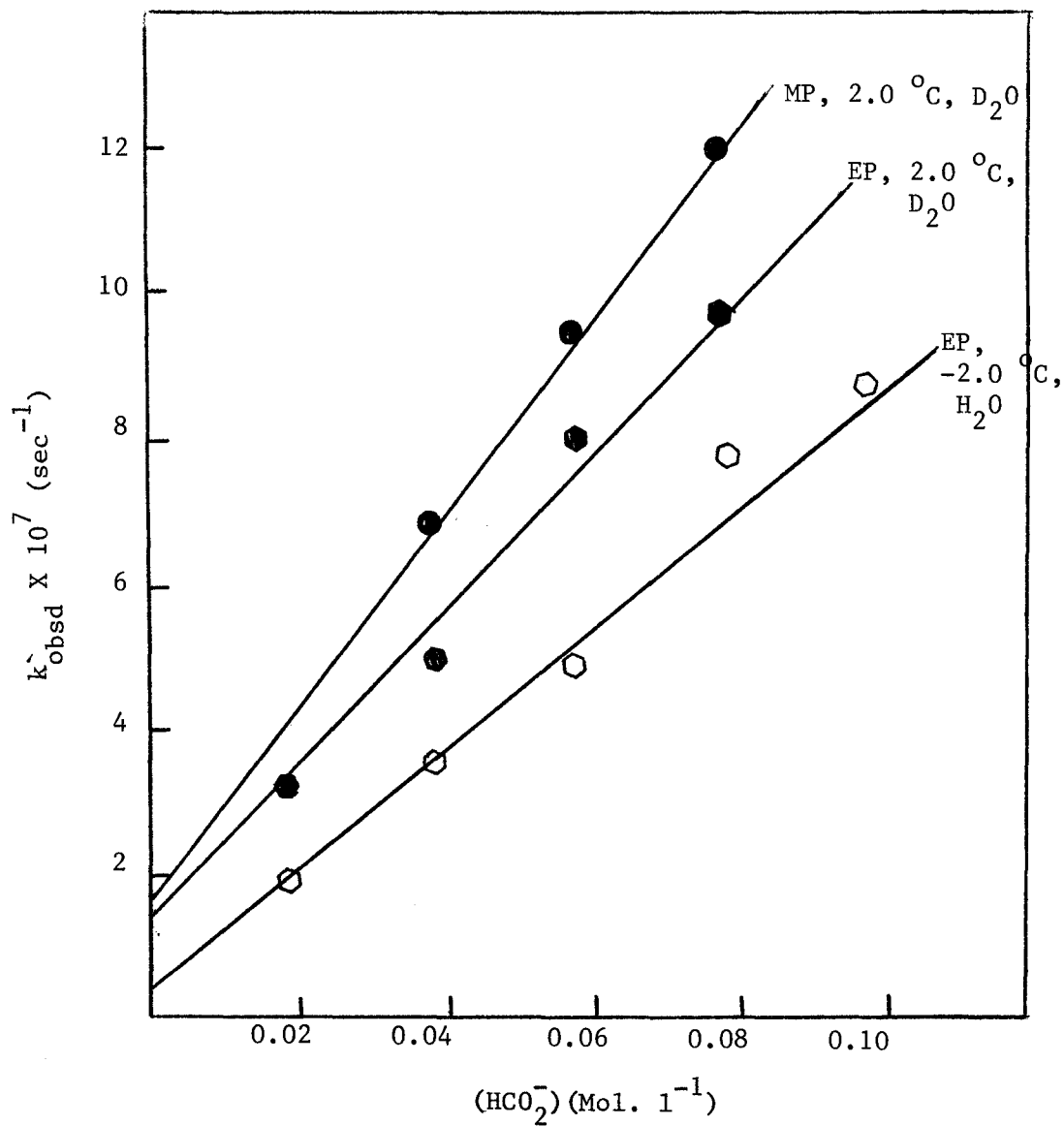


Figure 4: Catalysis of the iodination of ethyl pyruvate (EP) and methyl pyruvate (MP) in H_2O and D_2O at -1.0 and 2.0 °C. respectively and $\mu = 0.1 \text{ M}$.

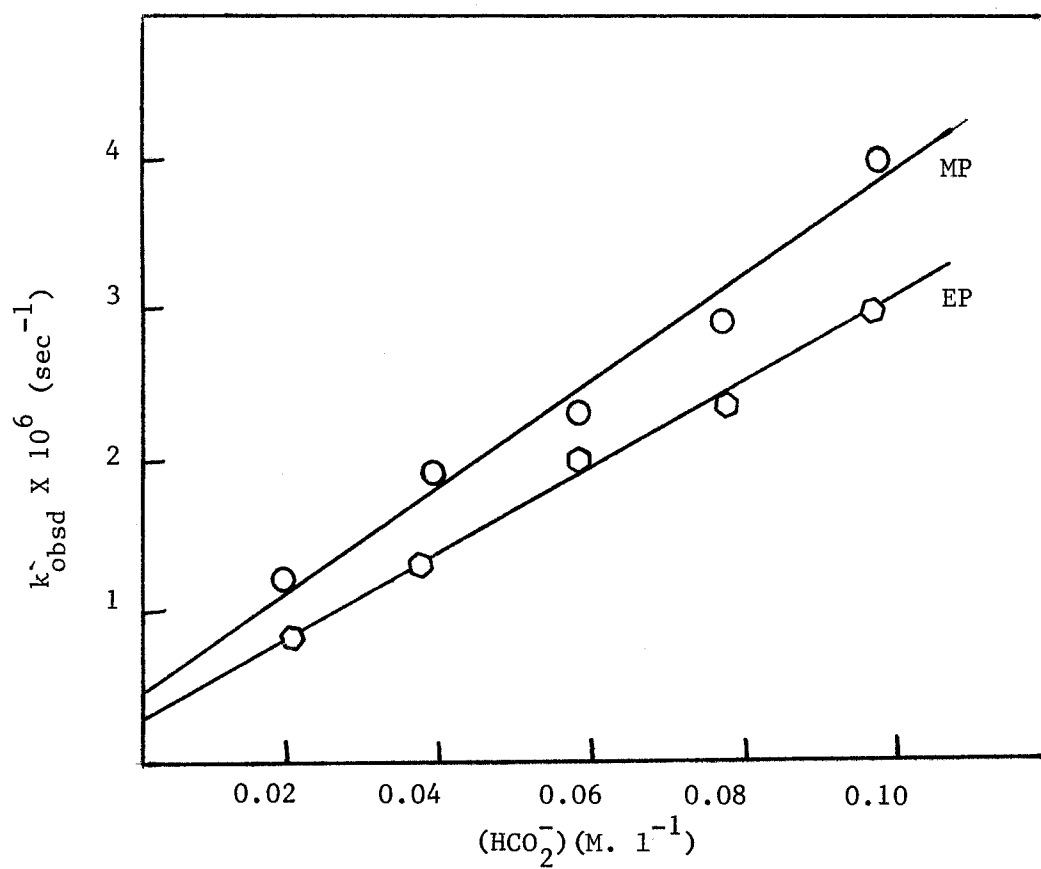


Figure 5: Catalysis of the iodination of ethyl and methyl pyruvates by formate buffer at 10°C and $\mu = 0.1 \text{ M}$.

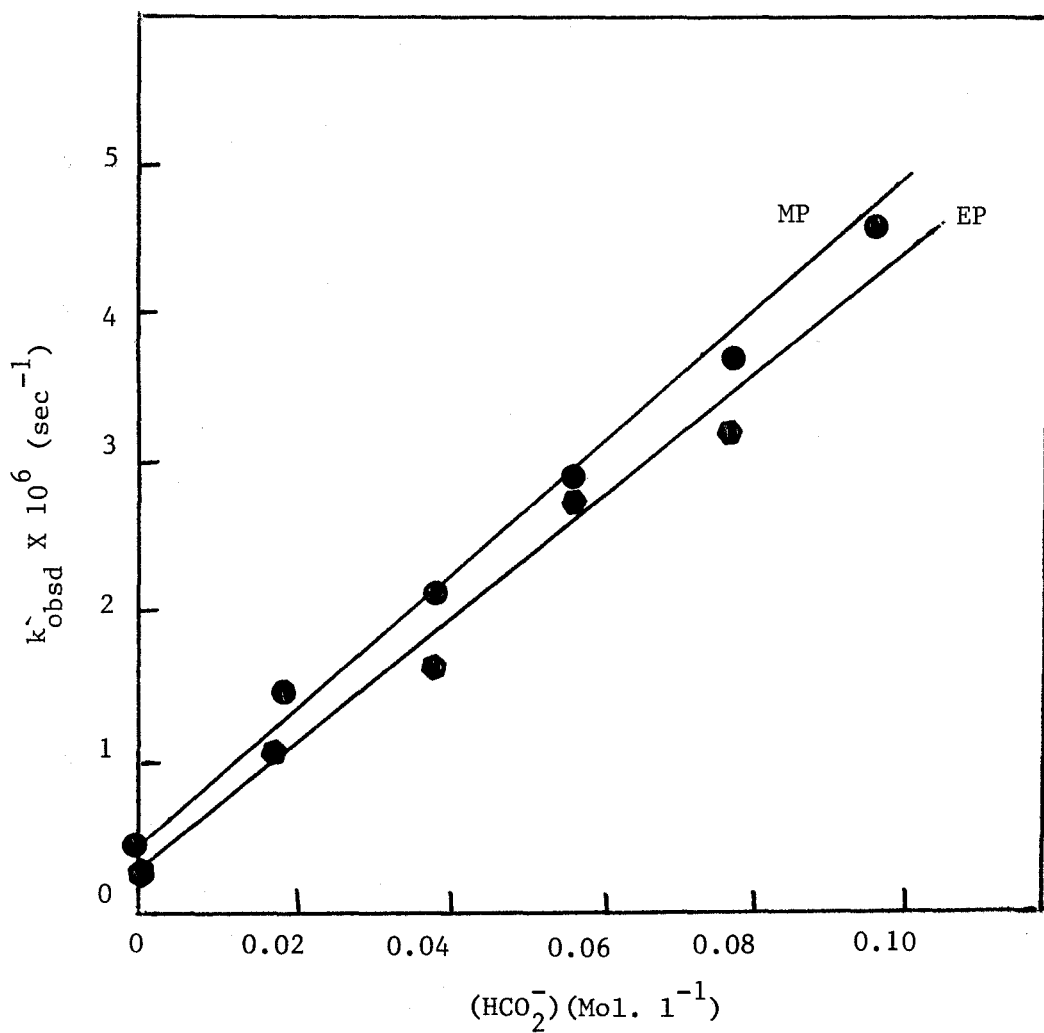


Figure 6: Catalysis of the iodination of ethyl and methyl pyruvates by formate buffer in D_2O , 12.0°C . and $\mu = 0.1 \text{ M}$.

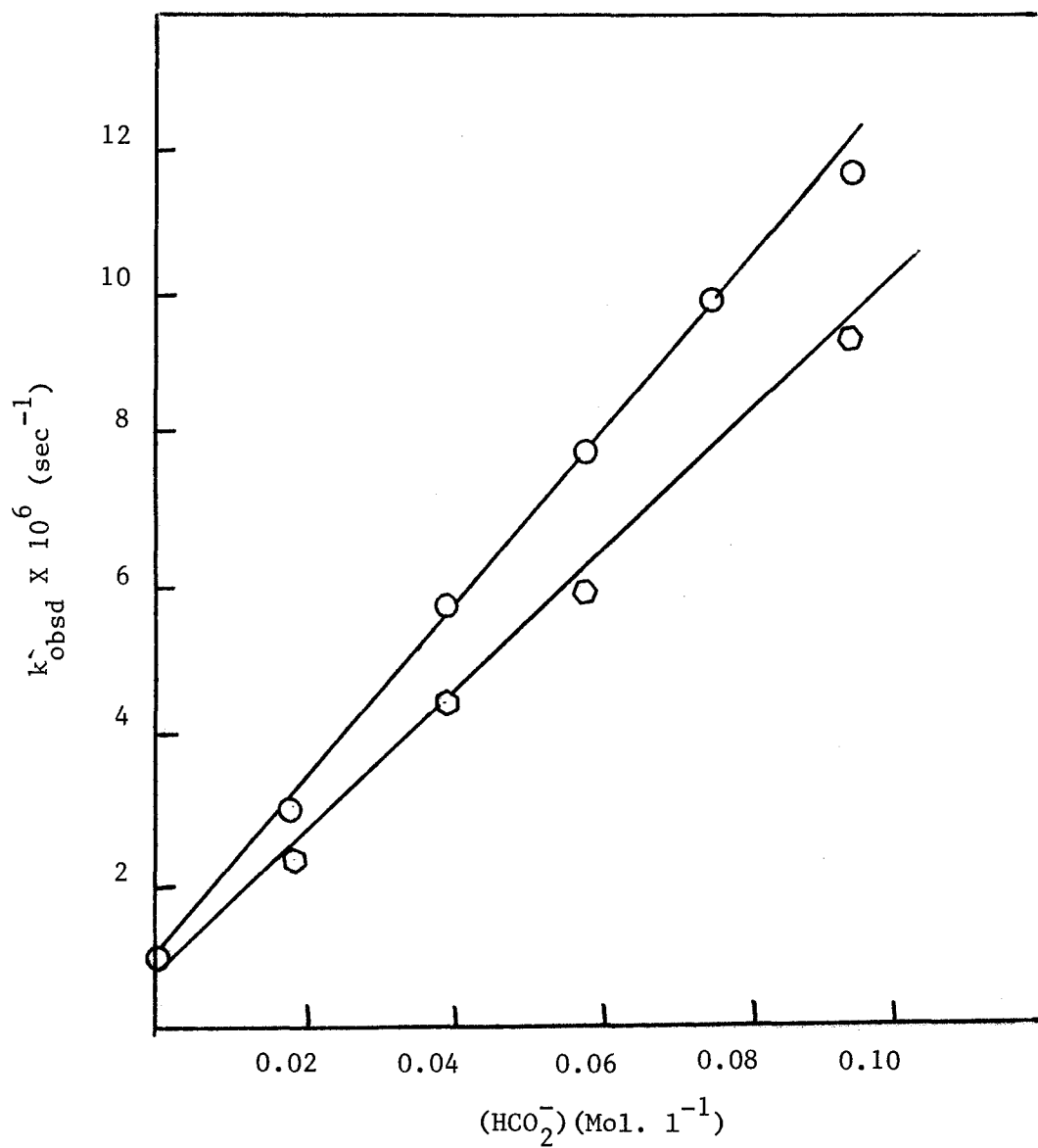


Figure 7: Catalysis of the iodination of ethyl and methyl pyruvates by formate buffers at 20 °C. and $\mu = 0.1 \text{ M}$.

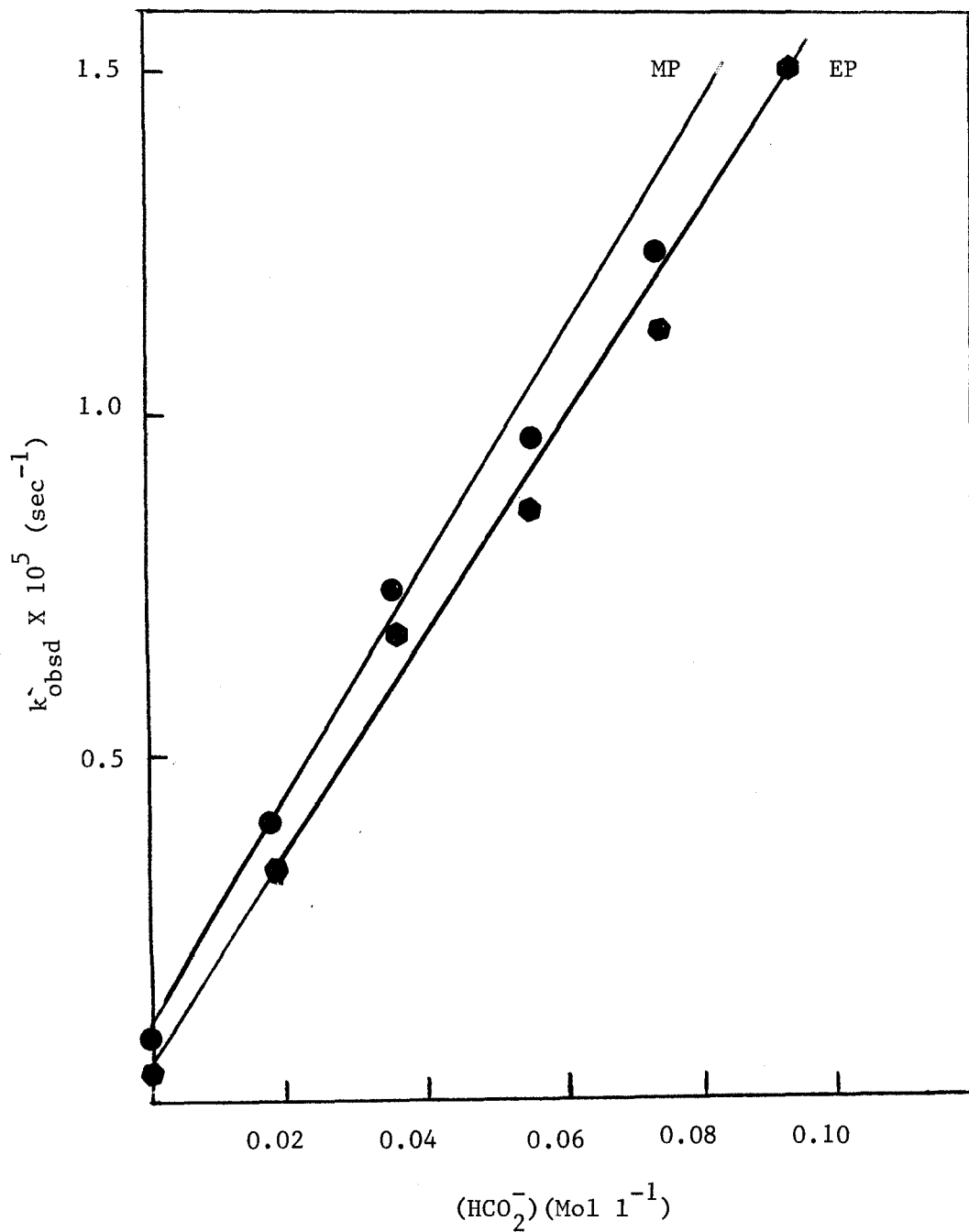


Figure 8: Catalysis of the iodination of methyl and ethyl pyruvates by formate buffer in D_2O , at 25°C . and $\mu = 0.1 \text{ M}$.

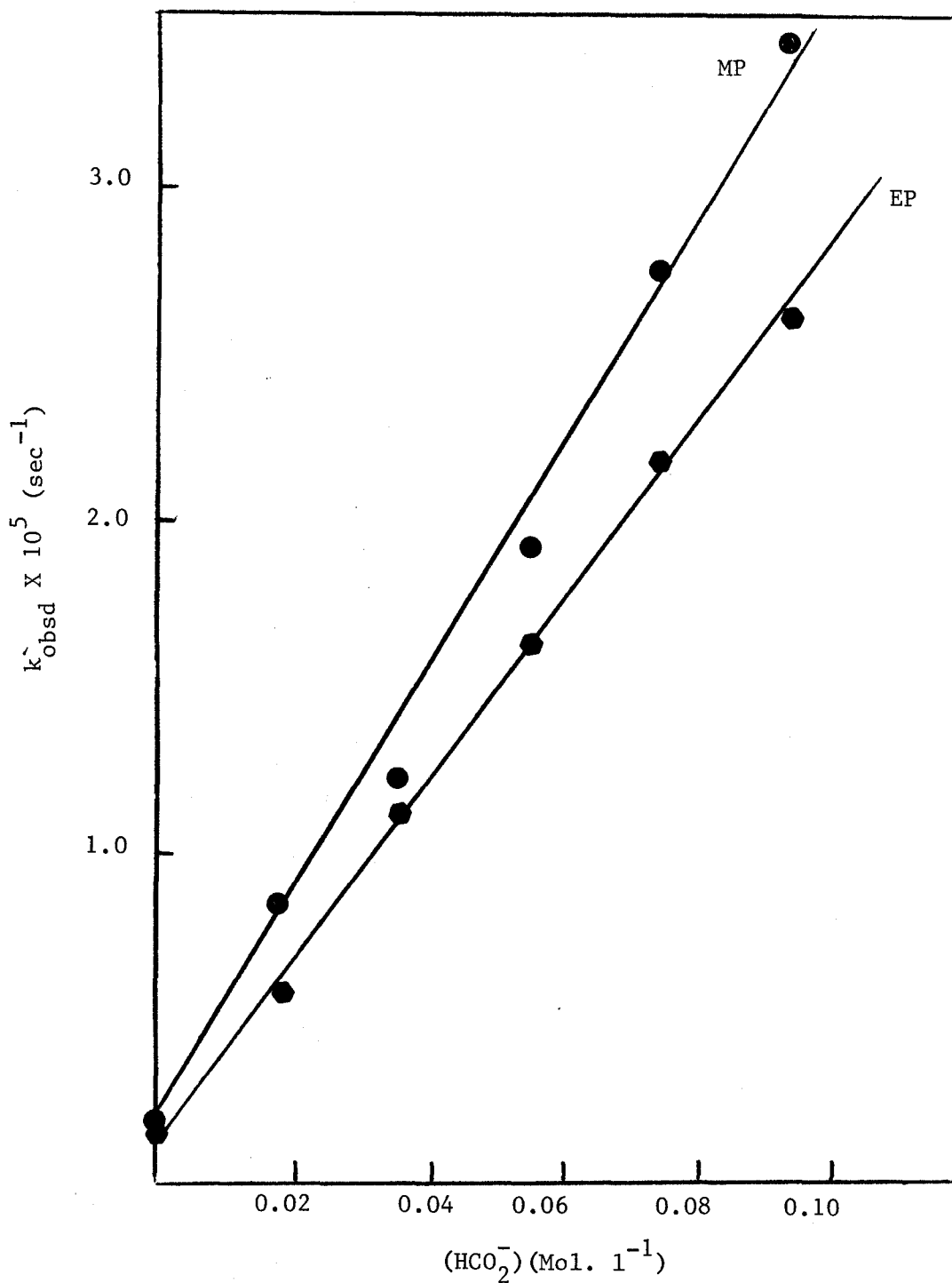


Figure 9: Catalysis of the iodination of ethyl and methyl pyruvate by formate buffer in D_2O , at 30°C . and $\mu = 0.1 \text{ M}$.

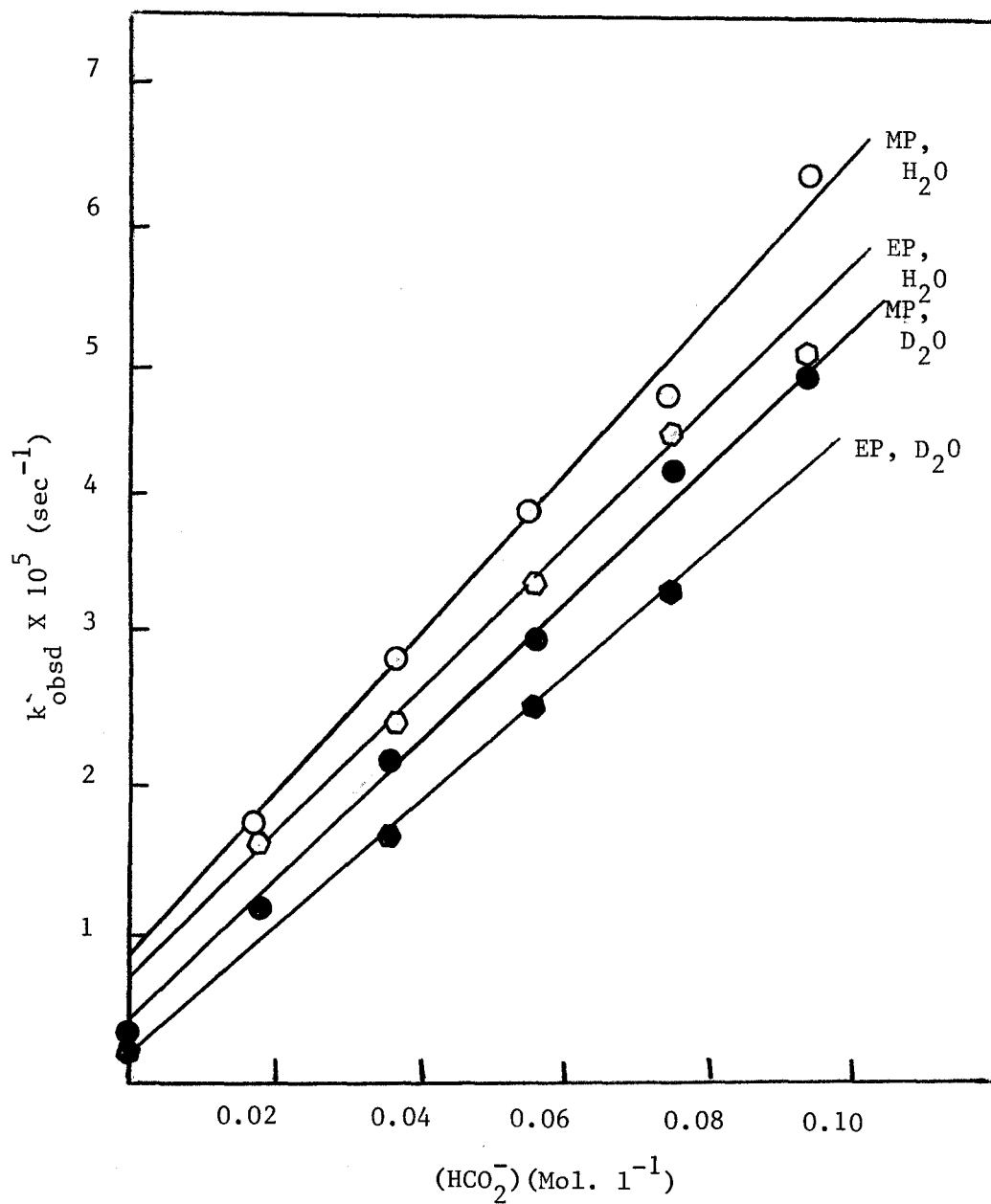


Figure 10: Catalysis of the iodination of ethyl and methyl pyruvates in formate buffer in both H_2O and D_2O , at 35°C . and $\mu = 0.1 \text{ M}$.

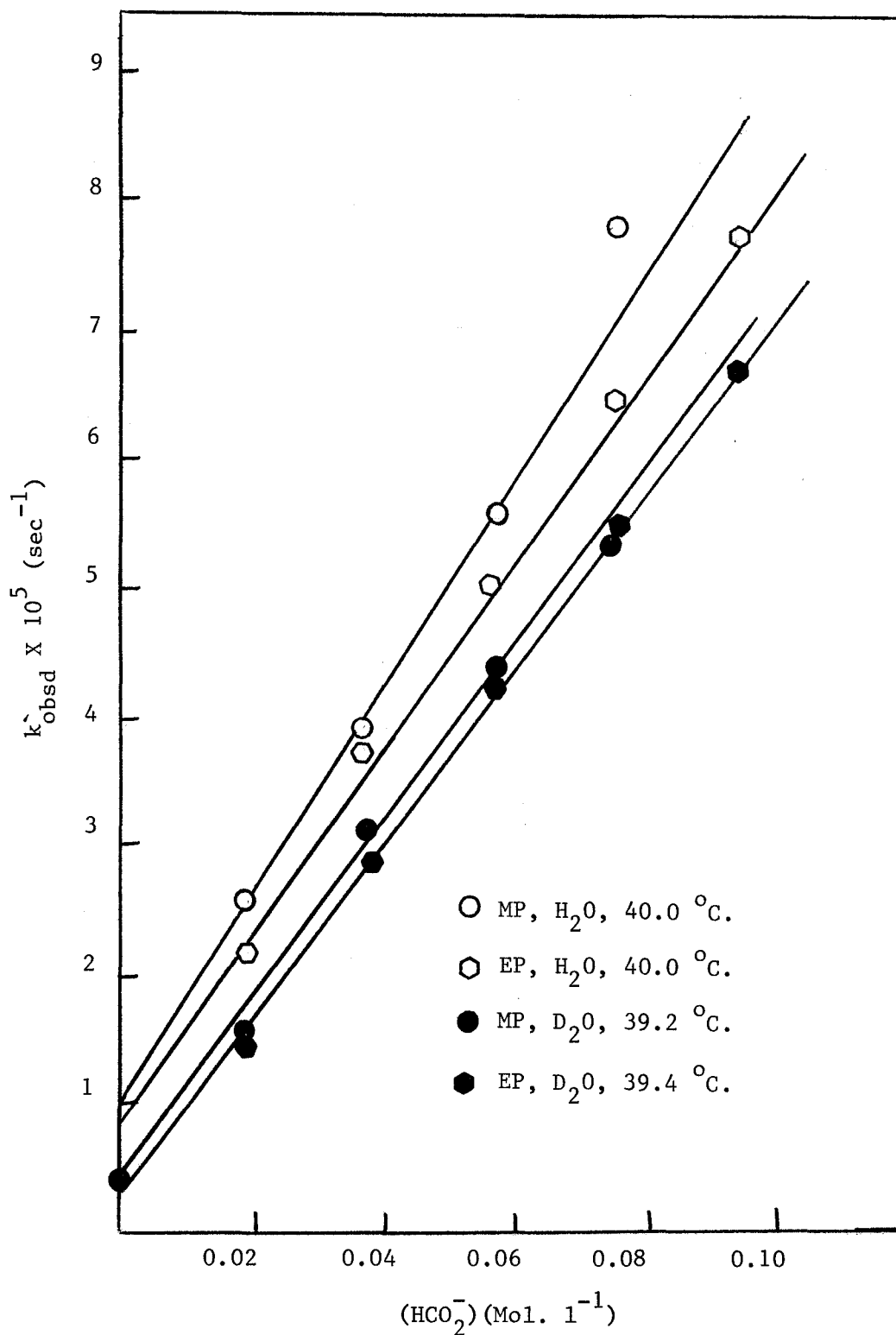


Figure 11: Catalysis of the iodination of ethyl and methyl pyruvate by formate buffer in both H₂O and D₂O at 40 and 39.2 °C. respectively, $\mu = 0.1 \text{ M}$.

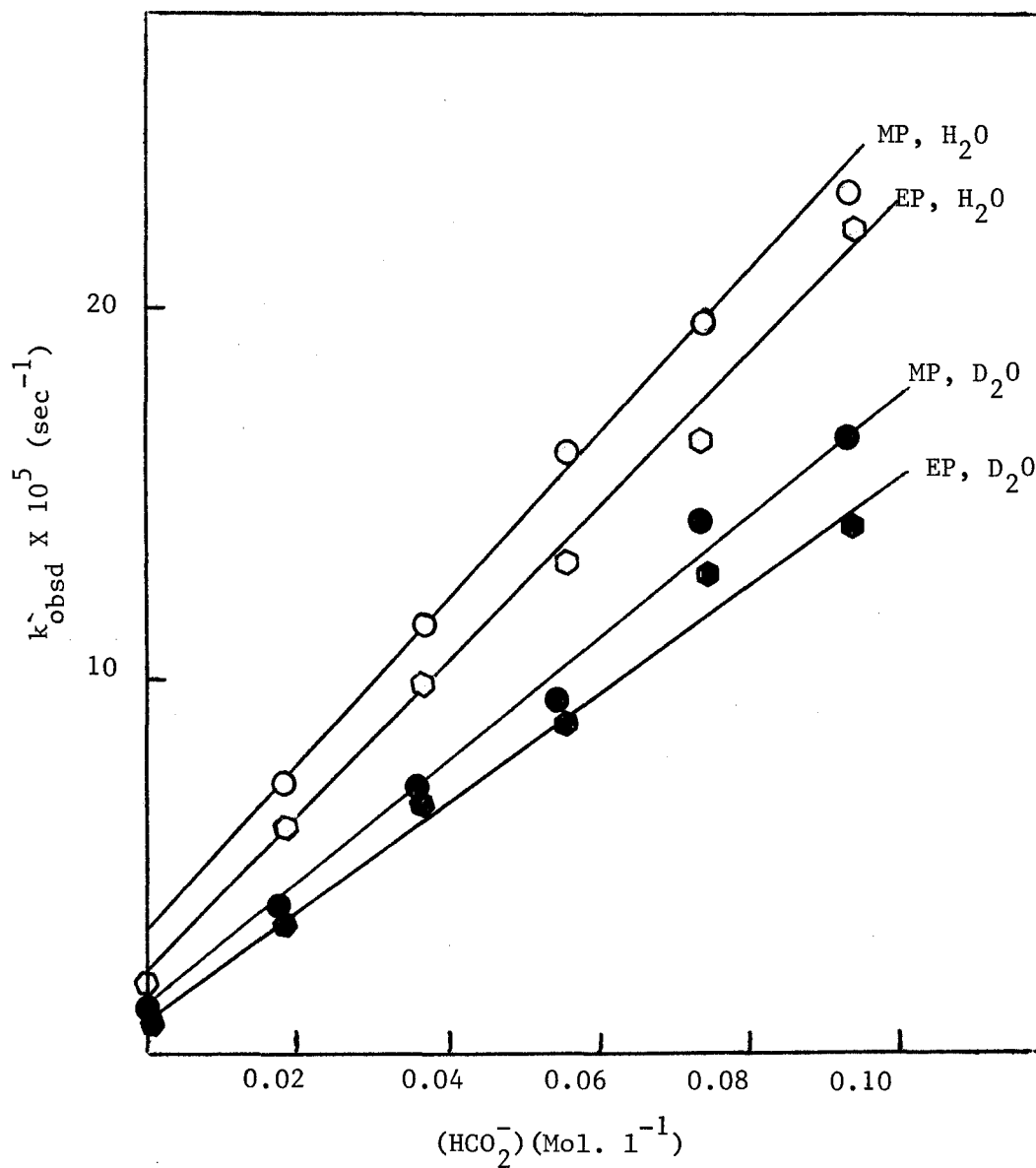
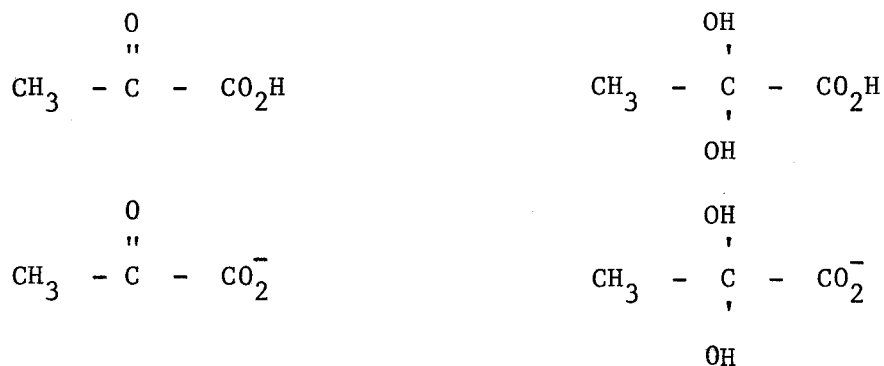


Figure 12: Catalysis of the iodination of methyl and ethyl pyruvates by formate buffer in both H₂O and D₂O, at 50 and 48 °C. respectively and $\mu = 0.1 \text{ M}$.

B. Iodination of Pyruvic Acid

Kinetic studies of the iodination of pyruvic acid in the region of pH around its pK_a is more complicated than the iodination of pyruvate esters. At the pK_a of pyruvic acid, the acid and its conjugate base (the pyruvate anion) exist in equilibrium. Therefore, two iodination processes must be taken into account. Furthermore, in an aqueous solution, both pyruvic acid and the pyruvate ion exist in equilibrium with their respective hydrated forms. Thus the aqueous solution containing formally pyruvic acid consists of four species.³¹



The iodination of pyruvic acid is a general acid-general base catalyzed reaction. The overall rate of iodination can be attributed to the catalytic contribution of all acidic and basic components present in the reaction solution. This can be expressed in the following manner:¹¹

$$k_{\text{obsd}} = k_0 + (\text{LP})\{ k_i (\text{LA})_i + k_j (\text{B})_j \} + (\text{P}^-)\{ k_i^- (\text{LA})_i + k_j^- (\text{B})_j \} \quad (43)$$

where k and k^- refer to the rate constants associated with pyruvic acid and pyruvate ion, respectively. The components, k_0 , is the solvent catalyzed rate constant, and L denotes the isotopically different hydrogens.

From the above equation, it is apparent that the measurements of the spontaneous rate of iodination of pyruvic acid must be carried out under conditions where the second and third terms are negligibly small, that is, under conditions where the uptake of triiodide ion is mainly due to pyruvic acid. By carrying out the measurements in the pH range 1.4-1.8, the concentration of pyruvate ion is comparatively small so that its action as a catalyst or as a competitive reactant is negligible. Earlier work²⁸ has shown that the iodination of pyruvic acid is insensitive to acid catalysis. Therefore, the catalysis of the iodination by the hydronium ion or hydroxide ion is experimentally undetectable. By using a low concentration of pyruvic acid, the catalytic contribution by either pyruvic acid itself or by its hydrated form is negligibly small compared with the iodination of pyruvic acid.¹¹

The iodination of pyruvic acid was carried out in solutions of low pH, low pyruvic acid concentrations (97×10^{-4} mole per liter) and at an ionic strength of 0.1 M. These measurements were carried out over a wide range of temperatures and Table XIV summarizes the results obtained.

The spontaneous rate constant, k_o , associated with the iodination of pyruvic acid was obtained by dividing the observed velocity by the concentration of the unhydrated form of pyruvic acid¹¹

$$k_o = \frac{v_{\text{obsd}}}{(\text{HP}) (1 - \chi)} \quad (44)$$

where χ is the fraction of hydration at the temperature under consideration. As in the case of alkyl pyruvates, account was taken of the fact that the fraction of hydration of pyruvic acid varies with temperature. The extent of hydration of pyruvic acid at various temperatures was investigated by Pocker, Meany and Zadorojny.¹⁹ As these workers pointed out, these hydration equilibria show a third order dependence on the water concentration. Accordingly, the ratio of the hydrated to the unhydrated form of pyruvic acid (where the water concentration is 49 M. l^{-1}) needs to be corrected to correspond to our experimental conditions (where the water concentration is ca. 55.5 M. l^{-1}). These corrections were made as follows:

$$K_{\text{eq}} = \frac{R}{(\text{H}_2\text{O})^3} = \frac{R}{(49)^3} = \frac{R'}{(55.5)^3}$$

where K_{eq} refers to the equilibrium constant for the hydration and $R' = \frac{\chi}{1 - \chi}$ (χ is the fraction of hydration under the experimental condition and R' is the corrected ratio). Thus,

$$R^{\wedge} = \frac{R (55.5)^3}{(49)^3} = R \times 1.4 \quad (45)$$

Table XI shows the fraction of hydration of pyruvic acid at various temperatures and water concentrations of 55.5 Mole l^{-1} .

TABLE XI

Fraction of Hydration for Pyruvic Acid at Various Temperatures at a Water Concentration of 55.5 M. l^{-1}

T ($^{\circ}K$)	$\frac{1}{T} \times 10^3$ ($^{\circ}K^{-1}$)	$R_{(49.0)}$ ^a	$R_{55.5}$ ^b	χ	$1 - \chi$
273	3.66	4.42	6.19	0.86	0.14
281	3.56	2.95	4.13	0.81	0.19
288	3.47	2.17	3.03	0.75	0.25
298	3.36	1.45	2.03	0.67	0.33
302	3.30	1.06	1.48	0.60	0.40
306	3.26	1.05	1.45	0.58	0.42
313	3.19	0.73	1.02	0.51	0.49
323	3.09	0.48	0.67	0.40	0.60

a) Reference 19

b) $R_{55.5} = R_{49.0} \times 1.4$

The concentration of undissociated pyruvic acid was calculated in consideration of the pH and ionic strength of the reaction solution using the relationships:

$$\text{pH} = -\text{Log } a_{\text{H}^+} = -\text{Log } (\text{H}^+) (\gamma_{\text{H}^+}) \quad (46)$$

$$K_a = \frac{(\text{H}^+) \gamma_{\text{H}^+} (\text{P}^-) \gamma_{\text{P}^-}}{(\text{HP})} \quad (47)$$

The ionic activity coefficient γ_{\pm} was calculated using the relation³²

$$-\log \gamma_{\pm} = \frac{A \sqrt{\mu}}{1 + \sqrt{\mu}}$$

where μ is the ionic strength and A has a value dependent on the dielectric constant, density and temperature of the solvent. Since the activity coefficient shows a dependence on temperatures, it was necessary to calculate the activity coefficient at various temperatures. The square of the activity coefficients as calculated are shown in Table XII. It is clear from the table that the value of γ_{\pm}^2 does not differ significantly throughout the range 0 - 50 °C. Consequently, the following relationship was employed at the various temperatures from 0 up to 50 °C.,

$$-\log \gamma_{\pm} = \frac{0.5 \sqrt{\mu}}{1 + \sqrt{\mu}} \quad (48)$$

TABLE XII

Calculation of the Ionic Activity Coefficients
in Water at Various Temperatures
and $\mu = 0.1 \text{ M}$

Temperature (°C.)	A	a	-Log \pm	γ_{\pm}^2
0.0	0.4918		0.118	0.58
8.0	0.4989		0.119	0.57
15	0.5028		0.121	0.57
25	0.5115		0.122	0.57
29	0.5161		0.124	0.57
34	0.5211		0.125	0.56
40	0.5262		0.126	0.56
50	0.5373		0.129	0.55

a) Reference 32

Since the thermodynamic dissociation constant, K_a , of pyruvic acid varies with the temperature, it was necessary to determine these values at the temperatures at which the iodination runs were carried out. Values of K_a were deduced by using the Clausius-Clapyron equation²⁹

$$\log \frac{K_2}{K_1} = \frac{\Delta H (T_2 - T_1)}{2.303 R T_1 T_2} \quad (49)$$

where K_1 is the dissociation constant of pyruvic acid at 25 °C., $K_1 = 3.24 \times 10^{-3} \text{ M}$.³³ and K_2 the dissociation constant at any temperature T_2 . The enthalpy of ionization of pyruvic acid, ΔH , has the value 2.90 Kcal/mole.³⁴ The values of K_a for pyruvic acid as calculated from the above equation are shown in Table XIII.

TABLE XIII

Ionization Constants of Pyruvic Acid
at Various Temperatures

Temp. (°C)	0.00	8.0	15.0	25.0	29.4	33.7	40.0	50.0
$K_a \times 10^3$ (M)	2.09	2.42	2.75	3.24	3.55	3.74	4.12	4.76

TABLE XIV

The Spontaneous Rate of Iodination of Pyruvic
Acid at Various Temperatures and $\mu = 0.1 \text{ M}$.

0.0 °C.						
pH	$\frac{(\text{P}^-)}{(\text{HP})}$	$(\text{HP}) \times 10^4$ M. 1^{-1}	$(\text{HP})(1-\chi) 10^4$ M. 1^{-1}	$v_{\text{obsd}} \times 10^9$ M. 1^{-1} sec^{-1}	a	$k_o \times 10^6$ sec^{-1}
1.75	0.154	81	11.3	0.192		0.17
1.38	0.065	88	12.3	0.156		0.13
8.0 °C.						
1.75	0.181	80	15.2	0.364		0.239
1.60	0.131	83	15.8	0.672		0.425
1.49	0.099	86	16.3	0.224		0.137
1.38	0.077	87	16.5	0.364		0.220
15.0 °C.						
1.75	0.206	78	19.5	0.975		0.50
1.60	0.149	82	20.5	1.43		0.69
1.49	0.113	84	21.0	1.08		0.52
1.38	0.087	86	21.5	1.05		0.49
25.0 °C.						
1.75	0.243	76	25.0	3.84		1.54
1.60	0.175	80	26.4	5.07		1.92
1.49	0.134	83	27.4	3.84		1.40
1.38	0.103	85	28.0	3.95		1.40

Table XIV (Continued)

29.7 °C.					
pH	$\frac{(P^-)}{(HP)}$	$(HP) \times 10^4$ M. 1^{-1}	$(HP)(1-\chi) 10^4$ M. 1^{-1}	$u_{\text{obsd}} \times 10^9$ M. 1^{-1} sec $^{-1}$	$k_o \times 10^6$ sec $^{-1}$
1.75	0.267	74	29.6	6.21	2.10
1.60	0.192	79	31.6	8.93	2.83
1.49	0.147	82	32.8	6.38	1.94
1.38	0.113	84	33.6	7.36	2.19
33.7 °C.					
1.75	0.285	73	29.2	9.58	3.28
1.60	0.205	78	31.2	13.6	3.35
1.49	0.157	81	32.4	10.9	3.36
1.38	0.121	84	33.6	11.8	3.51
40.0 °C.					
1.75	0.316	71	34.8	22.6	6.50
1.60	0.226	77	37.7	28.9	7.68
1.49	0.173	80	38.2	22.6	5.77
1.38	0.134	83	39.2	27.8	6.78
50.1 °C.					
1.75	0.370	69	41.4	58.7	14.2
1.60	0.266	74	44.4	82.3	18.5
1.38	0.157	81.2	48.7	67.9	14.0

Table XIV on the previous page

$$a) \quad v_{\text{obsd}}^{\sim} = v_{\text{obsd}} \times \frac{1 + K(I^-)}{K(I^-)} \quad \text{see Table IV}$$

Studies of the iodination of pyruvic acid in D_2O were carried out under similar conditions as in H_2O . The runs were followed at $25^\circ C$. and at an ionic strength of $0.1 \underline{M}$. The pK_a at $25^\circ C$ for pyruvic acid in D_2O was determined experimentally, $pK_a = 2.96$ (Figure 1). The concentration of pyruvic acid and pyruvate ion were calculated in the same manner as described for H_2O . The activity coefficient at $25^\circ C$. was determined using the relationship^{4, 35}

$$- \log \gamma_{\pm} = 1.83 \times 10^6 \sqrt{\frac{\mu}{D^3 T^3 \rho}} \quad (50)$$

where D is the dielectric constant of D_2O , T the absolute temperature, μ the ionic strength and ρ is the density of D_2O at $25.0^\circ C$.³⁶

The results described in the experimental section on the hydration of pyruvic acid in H_2O and D_2O showed that the fraction of hydration in D_2O is the same as in H_2O . Table XV summarizes the results of the iodination of pyruvic acid in D_2O , at $25^\circ C$. and $0.1 \underline{M}$ ionic strength.

TABLE XV

The Spontaneous Rate of Iodination of
 Pyruvic Acid in D_2O ,
 at 25.0 °C.
 and $\mu = 0.1 \underline{M}$.

pD	$\frac{(P^-)}{(HP)}$	$(HP) \times 10^4$ M. l^{-1}	$10^4 (HP) (1-\chi)$ M. l^{-1}	$v_{\text{obsd}} \times 10^9$ M. $l^{-1} \text{ sec}^{-1}$	$k_o \times 10^6$ sec^{-1}
1.14	0.022	97	32	2.55	0.79
1.23	0.026	97	32	2.34	0.73
1.36	0.027	97	32	2.27	0.71

C. Determination of the Activation Parameters

The activation parameters for the spontaneous and base-catalyzed iodination of pyruvate esters in both H_2O and D_2O , as well as for the spontaneous iodination of pyruvic acid were determined from the respective rate coefficients associated with these processes as a function of temperature. Thus, Arrhenius plots were constructed by plotting the logarithms of the spontaneous and base catalyzed rate coefficients versus the reciprocal of the corresponding absolute temperatures. These plots are shown in Figures 13, 14, 15, and 16. It is important to note that in constructing such plots, the points were not given the same weight. For example, at higher temperatures uncertainties arise since the uptake of the triiodide ion by the formate buffers and impurities becomes more significant than that at lower temperatures. In addition, the Arrhenius frequency factor A varies over a wide range of temperatures.

$$k = A e^{-E_a/RT} \quad (51)$$

Since the purpose of this work is to determine the activation parameters at $25^\circ C.$, the points determined in the temperature range $10 - 35^\circ C.$ were weighed most heavily. The energy of activation associated with each process was calculated from the relation:

$$E_a = -2.303 R(\text{slope}) \quad (52)$$

From the activation energy E_a , the enthalpy of activation was determined:

$$\Delta H^* = E_a - RT \quad (53)$$

and the free energy of activation was derived from:

$$\begin{aligned} \Delta G^* &= -RT \ln k^* \\ &= -RT \ln \frac{k_r h}{kT} \end{aligned} \quad (54)$$

The entropy of activation ΔS^* was deduced using the relationship²⁹

$$\Delta S^* = \frac{\Delta H^* - \Delta G^*}{T} \quad (55)$$

The activation parameters associated with the spontaneous and base-catalyzed iodination of pyruvate systems are shown in Table XIX. Tables XVI - XVIII summarize the dependency of k_o and $k_{\text{HCO}_2^-}$ upon the absolute temperatures for the iodination of pyruvate systems in both H_2O and D_2O .

TABLE XVI

The Dependency of k_o and $k_{\text{HCO}_2^-}$ upon the Absolute
Temperature in H_2O as a Solvent

Methyl Pyruvate

T (°K)	$\frac{1}{T} \times 10^3$ (°K ⁻¹)	$k_{\text{HCO}_2^-} \times 10^5$ M. 1 ⁻¹ sec ⁻¹	$-\text{Log } k_{\text{HCO}_2^-}$	$k_o \times 10^7$ (sec ⁻¹)	$-\text{Log } k_o$
272	3.68	0.856	5.068	2.30	6.638
283	3.53	3.14	4.503	6.50	6.187
293	3.41	11.4	3.943	11.5	5.939
298 ^a	3.36	19.4	3.712	19.0	5.720
303	3.30	26.7	3.570	53.0	5.275
308	3.25	56.2	3.250	80.0	5.097
313	3.19	83.5	3.078	100.0	5.000
323	3.09	211	2.675	325.0	4.488

a) Reference 11

Table XVI (Continued)

Ethyl Pyruvate

T (°K)	$\frac{1}{T} \times 10^3$ (°K ⁻¹)	$k_{\text{HCO}_2^-} \times 10^5$ M. 1 ⁻¹ sec ⁻¹	-Log $k_{\text{HCO}_2^-}$	$k_o \times 10^6$ (sec ⁻¹)	-Log k_o
283	3.53	2.78	4.550	0.30	6.523
293	3.41	9.10	4.040	0.77	6.114
298 ^a	3.36	16.8	3.775	1.7	5.769
303	3.30	21.4	3.669	4.0	5.398
308	3.25	47.4	3.320	7.0	5.155
313	3.19	72.4	3.140	7,8	5.108
323	3.09	197	2.704	20.5	4.688

a) Reference 11

TABLE XVII

The Dependency of k_o of the Iodination of Pyruvic Acid upon Absolute Temperature.

T (°K)	$\frac{1}{T} \times 10^3$ (°K ⁻¹)	k_o (sec ⁻¹)	-Log k_o
273	3.66	1.3×10^{-7}	6.886
281	3.56	2.2×10^{-7}	6.657
288	3.47	4.9×10^{-7}	6.309
298	3.36	1.4×10^{-6}	5.851
302	3.30	2.1×10^{-6}	5.678
306	3.26	3.4×10^{-6}	5.474
313	3.20	6.8×10^{-6}	5.168
323	2.09	1.4×10^{-5}	4.855

TABLE XVIII

The Dependency of k_o and $k_{\text{HCO}_2^-}$ upon the
Absolute Temperature in D_2O .

Methyl Pyruvate

T (°K)	$\frac{1}{T} \times 10^{-3}$ (°K ⁻¹)	$k_{\text{HCO}_2^-} \times 10^5$ M. l ⁻¹ sec ⁻¹	$-\text{Log } k_{\text{HCO}_2^-}$ (sec ⁻¹)	$k_o \times 10^7$ (sec ⁻¹)	$-\text{Log } k_o$
275	3.64	1.34	4.87	1.6	6.79
285	3.51	4.26	4.37	3.0	6.52
298	3.36	15.5	3.81	9.0	6.04
303	3.30	32.7	3.49	20	5.70
308	3.25	50.5	3.29	40	5.40
312	3.20	73.0	3.14	50	5.30
321	3.11	164	2.78	100	5.00

Ethyl Pyruvate

275	3.64	1.20	4.919	1.4	6.854
285	3.51	4.12	4.385	2.0	6.699
298	3.36	14.7	3.833	7.5	6.125
303	3.30	26.3	3.579	10	6.000
308	3.25	40.5	3.392	25	5.602
312	3.20	68.4	3.165	32	5.495
322	3.10	149	2.825	80	5.097

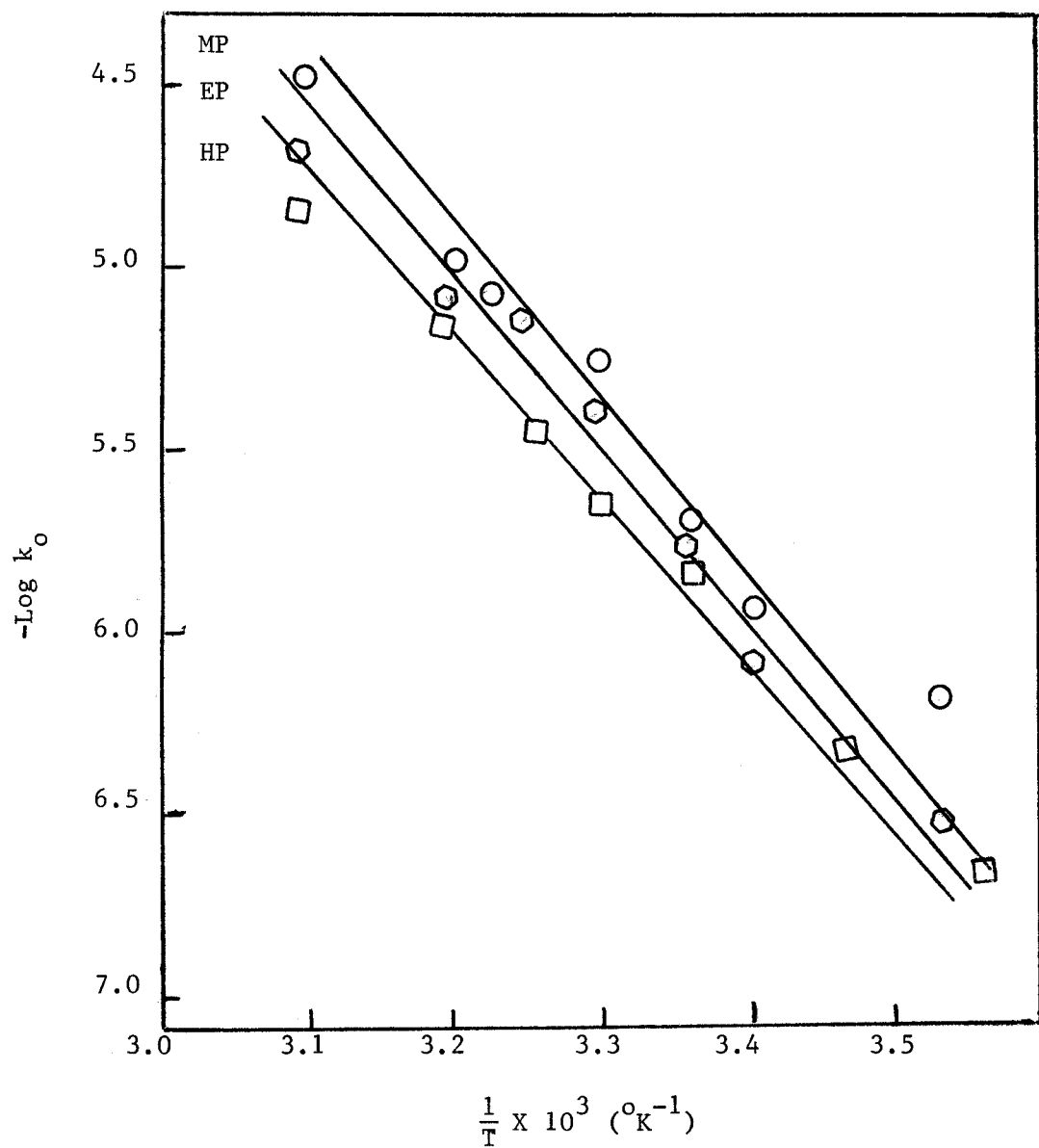


Figure 13: Arrhenius plots for the spontaneous iodination of methyl and ethyl pyruvates and pyruvic acid.

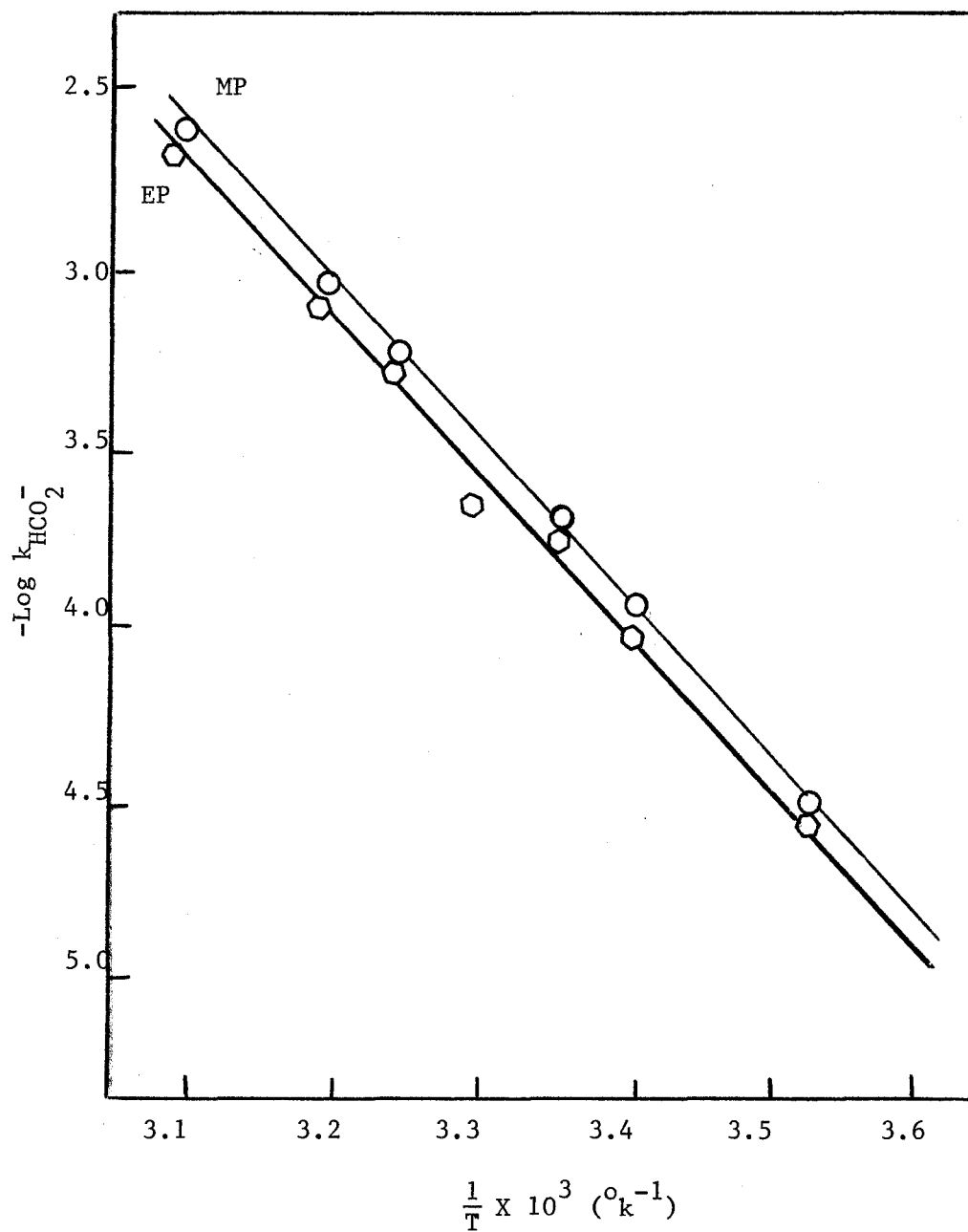


Figure 14: Arrhenius plots for the formate catalysed iodination of methyl and ethyl pyruvates.

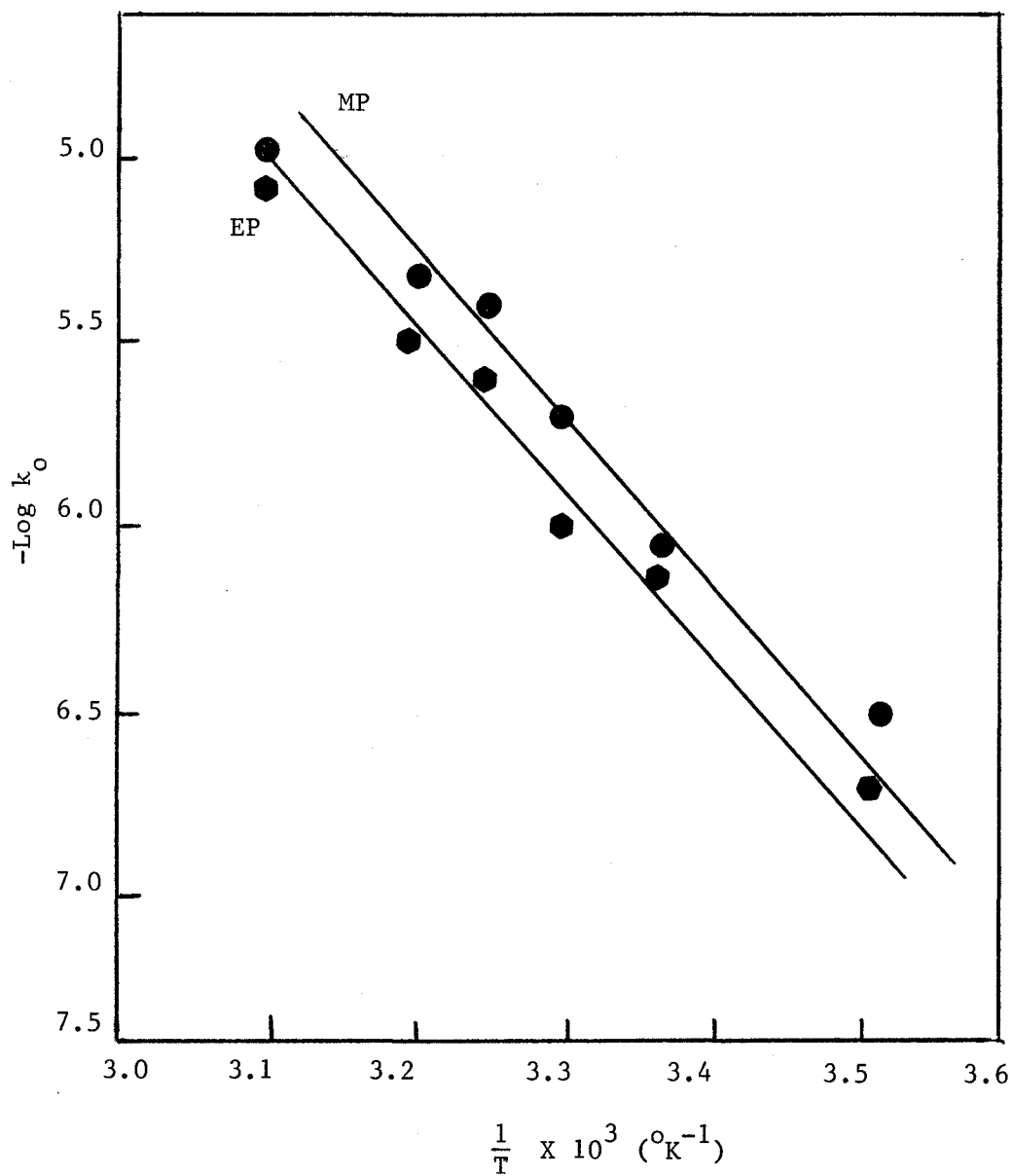


Figure 15: Arrhenius plots for the spontaneous iodination of methyl and ethyl pyruvates in D_2O .

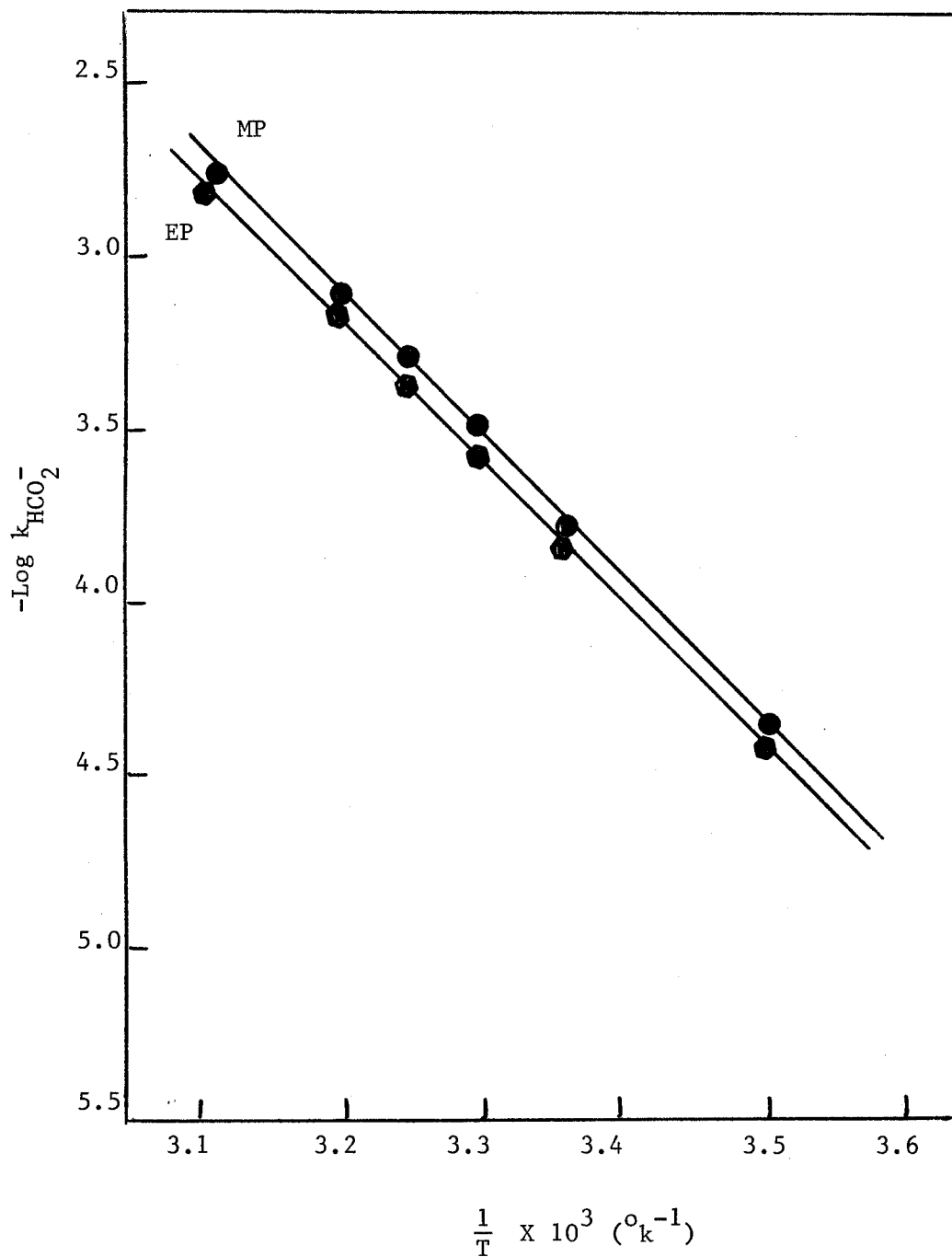


Figure 16: Arrhenius plots for the formate catalysed iodination of methyl and ethyl pyruvates in D_2O .

TABLE XIX

Activation Parameters for the Iodination in
Both D₂O and H₂O

Spontaneous Iodination

	D ₂ O		H ₂ O		
	MP	EP	MP	EP	HP
E _a (Kcal/mole)	20.3	20.3	20.7	21.6	20.3
Δ H* (Kcal/mole)	19.7	19.7	20.1	21.0	19.7
Δ G* (Kcal/mole)	25.6	25.8	25.2	25.4	25.4
Δ S* (Cal/deg/mole)	-20	-21	-17	-15	-19

Formate Catalyzed Iodination

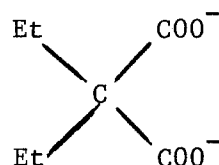
E _a (Kcal/mole)	18.3	17.9	19.6	19.9	-
Δ H* (Kcal/mole)	17.7	17.4	19.0	19.3	-
Δ G* (Kcal/mole)	22.6	22.7	22.6	22.7	-
Δ S* (Cal/deg/mole)	-16	-18	-12	-11	-

D. Determination of the Brønsted Coefficient (β) for the Iodination of Methyl Pyruvate

In order to construct a Brønsted plot for the base catalyzed iodination of methyl pyruvate at 25 °C., it was necessary to determine the catalytic rate constants for a number of bases which catalyse the iodination process. Values for the spontaneous (water-catalyzed) and Formate-Catalyzed rate coefficient were obtained from the work of Meany¹¹ on the iodination of methyl pyruvates in formate buffers at 25 °C.

Trials were also made in the present work in malonate buffer for the iodination of methyl pyruvate at 25 °C. It was observed that the uptake of the triiodide ion takes place very fast by the buffer solution to the extent that it was difficult to monitor the runs accurately.

Another series of runs were carried out in diethyl malonate buffer (Buffer ratio of unity) where there exists no removable protons on the α carbon



and consequently the enolization process is impossible. In addition, a series of runs were carried out in acetate buffer (buffer ratio = 1), at 0.1 M ionic strength at 25.0 °C. The observed rate constants

of the iodination of methyl pyruvate at 25.0 °C. by acetate and diethyl malonate buffers are shown in Table XX. Plots of k_{obsd} versus acetate and diethyl malonate dianion concentration were constructed and are represented in Figure 17.

TABLE XX

Observed Rate Constants of the Iodination of Methyl Pyruvate at 25.0 °C. and $\mu = 0.1 \text{ M}$.

Acetate Buffer				
(B ⁻) (Mol. l ⁻¹)	S _{obsd} X 10 ³ (cm. ⁻¹ sec ⁻¹)	S _{corr} X 10 ³ (cm. ⁻¹ sec ⁻¹)	a v _{obsd} X 10 ⁷ (M. l ⁻¹ sec ⁻¹)	k _{obsd} X 10 ⁵ (sec ⁻¹)
0.005	0.364	0.378	0.154	0.32
0.019	0.900	0.936	0.382	0.80
0.039	1.59	1.66	0.678	1.4
0.059	2.28	2.37	0.967	2.0
0.079	2.68	2.79	1.14	2.4
0.098	5.28	5.49	2.24	4.7
Diethyl Malonate Buffer				
0.0012	0.460	0.480	0.196	0.410
0.0049	1.76	1.83	0.747	1.56
0.0059	2.58	2.69	1.10	2.28
0.0098	3.60	3.75	1.53	3.19

a) $S_{\text{corr}} = \text{observed slope } (S_{\text{obsd}}) \times \frac{1 + K(\text{I}^-)}{K(\text{I}^-)}$ (see Table IV)

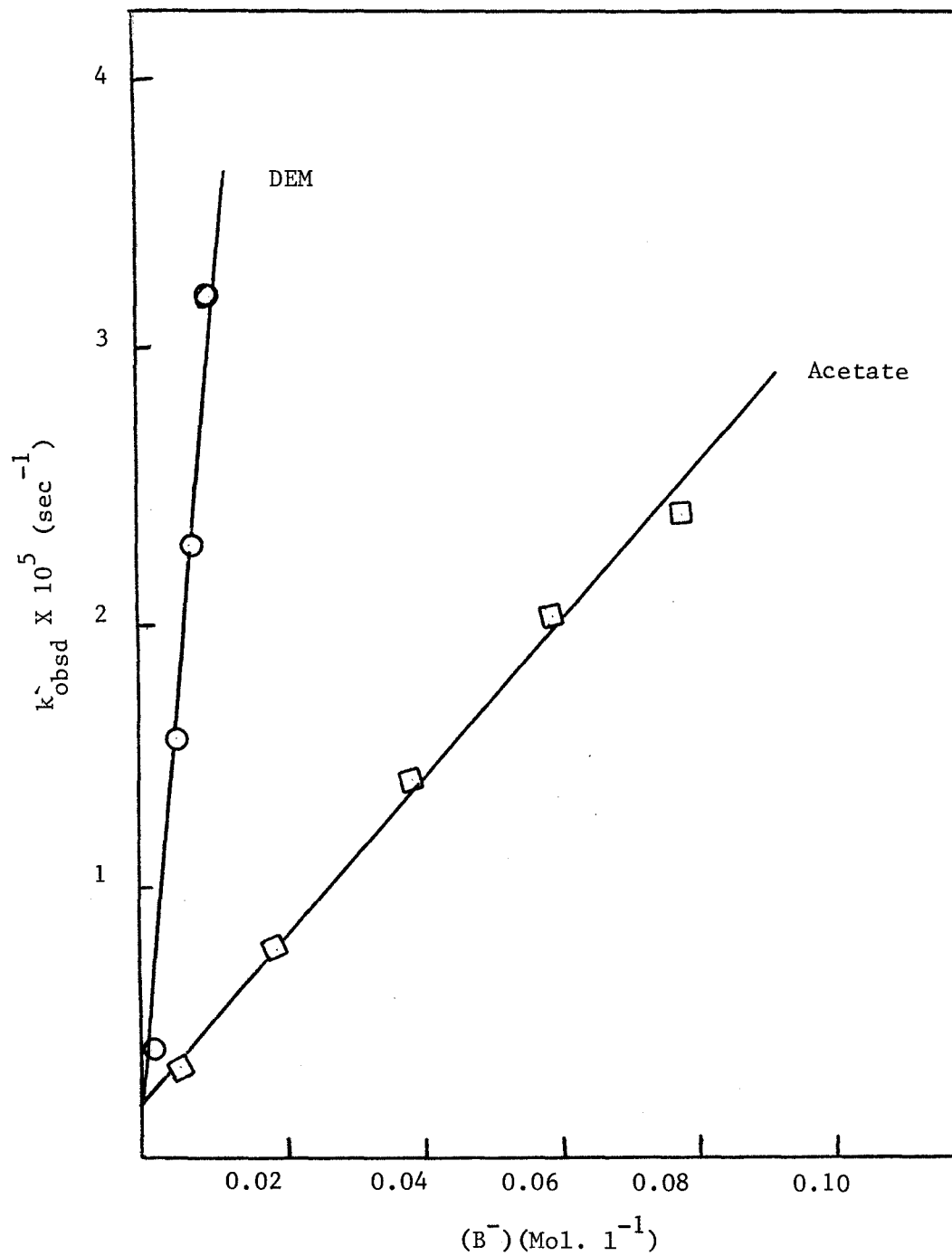


Figure 17: Catalysis of the iodination of methyl pyruvate by acetate and diethyl malonate dianion ions at 25 °C. and $\mu = 0.1 \text{ M}$.

The straight lines represented in Figure 17 can be represented by the following equation:

$$k_{\text{obsd}} = k + k_B^- (B^-) \quad (56)$$

where k represents the catalytic contribution arising from the solvent, hydroxide ion. The slopes of these lines give the catalytic coefficients associated with the acetate anion and diethyl malonate dianion. Table XXI summarizes the measurements taken in formate, acetate and diethylmalonate buffers.

TABLE XXI

The Slopes and Intercepts Associated with the Iodination of Methyl Pyruvate using Formate, Acetate and Diethyl Malonate Buffers at 25 °C. and an Ionic Strength of 0.1 M.

Buffer	pKa	Slope ($l.M^{-1} \text{ sec}^{-1}$)	Intercept (sec^{-1})
Formate ^{a, b}	3.65	19.4×10^{-5}	1.9×10^{-6}
Acetate	4.65	28.1×10^{-5}	2.0×10^{-6}
Diethyl Malonate	7.10	32.3×10^{-4}	2.0×10^{-6}

a) Reference 11

b) The value of this intercept was found equal to the spontaneous rate of iodination.¹¹

From the above table, it is apparent that in formate, diethyl malonate and acetate buffers the values of the intercepts are

almost the same, and represent the spontaneous rate of iodination of methyl pyruvate at 25 °C. These results, however, were surprising, since it was anticipated that the catalytic contribution arising from the hydroxide ion would become significant at the higher values of pH studied. Table XXII shows the catalytic rate constants of general base catalyzed iodination of methyl pyruvate at 25.0 °C. Brønsted plots were constructed by plotting the logarithm of the catalytic rate coefficients associated with water, formate, acetate and diethyl malonate dianion ions versus their respective values of pKa. This plot is represented in Figure 18 in which the slope of the straight line is equal to the Brønsted coefficient, ($\beta = 0.49$).

TABLE XXII

Catalytic Rate Constant of General Base Catalyzed
Iodination of Methyl Pyruvate at 25.0 °C. at
an Ionic Strength of 0.1 M.

Buffer	pKa	k_B^- ($1.M^{-1} \text{ sec}^{-1}$)	-Log k_B^-
Water	-1.74	3.42×10^{-8}	7.46
Formate	3.65	19.4×10^{-5}	3.71
Acetate	4.65	28.1×10^{-5}	3.55
Diethyl Malonate	7.10	32.3×10^{-4}	2.49

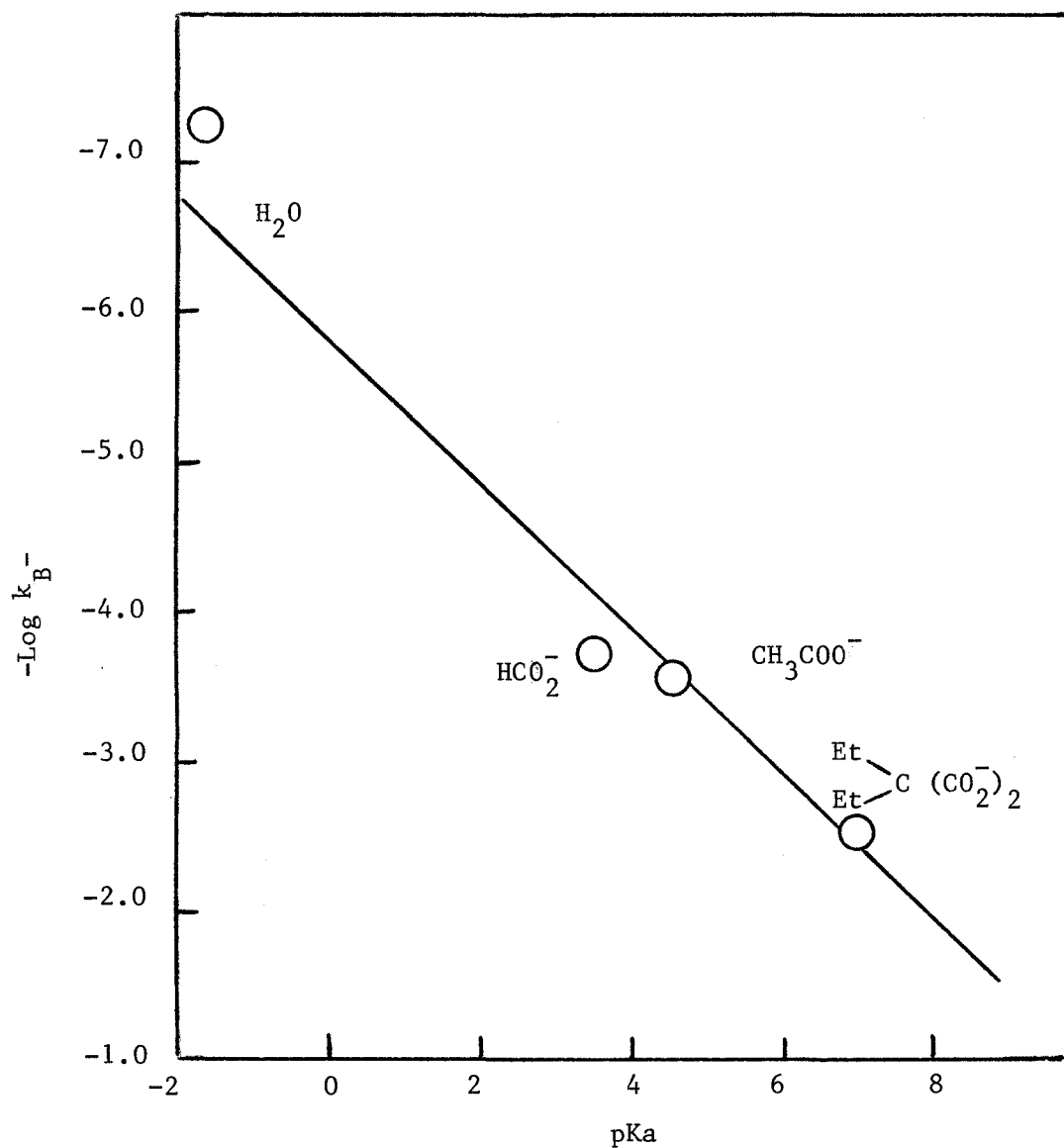
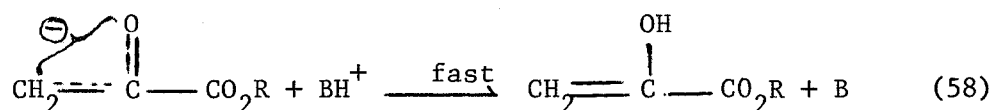
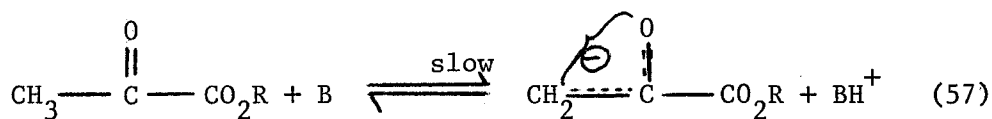


Figure 18: Brønsted plots for the base catalyzed iodination of methyl pyruvate at 25 °C.

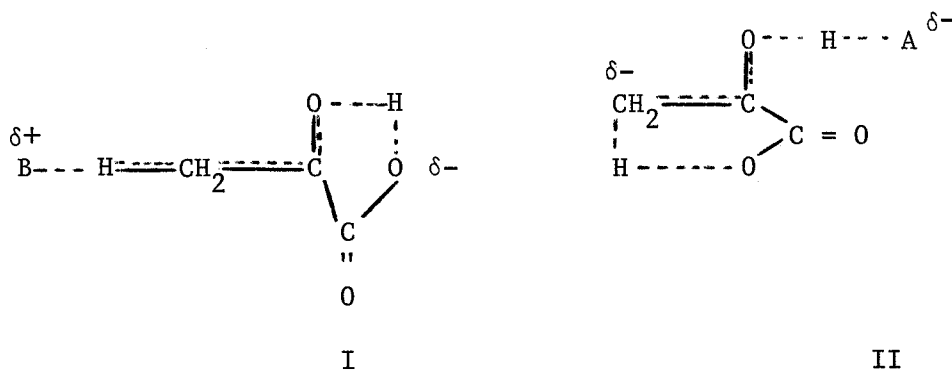
IV. DISCUSSION

The capacity of water and buffered systems to catalyze the iodination of pyruvic acid and pyruvate esters substantiates the earlier finding pertaining to the general-base catalyzed nature of this reaction.^{5, 11, 28} The mechanism associated with the general base catalyzed process may be expressed as follows:



where R = H, CH₃ or C₂H₅

Bell¹⁸ has proposed a mechanism for the catalysis of the iodination of pyruvic acid and pyruvate anion involving a cyclic transition state arising from the intramolecular catalysis by the carbonyl group (for pyruvic acid) or carboxylate group (for pyruvate ion):



One of the purposes of the present work was to distinguish between intra and intermolecular catalyzed pathways for this enolization process. Comparative iodination studies were carried out on pyruvic acid, ethyl and methyl pyruvates. It will be noted that the pyruvate esters cannot develop into transition states such as I and II due to the absence of acidic protons on the ethoxy or methoxy groups of these esters. Consequently, intramolecular catalysis in the alkyl pyruvate is not possible. The rates of iodination of the pyruvate systems were determined as a function of temperature and at an ionic strength of 0.1 M.

A. Brønsted Coefficient

The Brønsted relationship between $\log k_B$ and pK_a of the bases of Table XXII (in the results section) yield $\beta = 0.49$. A relationship has been suggested between β and the acidity of the enolizing proton for such proton transfer reaction.³⁷ For example, for reactions involving the abstraction of weakly acidic protons, one would expect that the transition state would develop late in the reaction progress and, consequently, one would conclude that the transition state would already resemble the product (s) formed. For such processes, it is found that the experimental value of β is close to unity. This is exemplified by the enolization of acetone where the value of β is ca.0.88.³⁸ On the other hand, abstraction of a proton from a species having a highly acidic proton, should have a transition state similar to the reactant

and a value of β close to zero is generally observed. Thus, the values of β for the abstraction of protons from bromoacetylacetone and methyl pyruvate are 0.42 and 0.49 respectively. Table XXIII shows a comparison of the Brønsted coefficients β , for the base catalyzed halogenation of methyl pyruvate and some other ketones. From this table, it is apparent that as the acidity of the enolizing proton increases the value of β decreases and the enolization of the substrate becomes more sensitive to base catalysis.

TABLE XXIII
Brønsted Coefficients for Base-Catalyzed
Halogenation^a

Substrate	β
$\text{CH}_3 \text{ CO CH}_3$	0.88
$\text{CH}_3 \text{ CO CH}_2\text{Cl}$	0.82
$\text{CH}_3 \text{ COCH}_2 \text{ CO}_2 \text{ C}_2\text{H}_5$	0.59
$\text{CH}_3 \text{ CO CO}_2 \text{ CH}_3$	0.49
$\text{CH}_3 \text{ CO CH}_2 \text{ CO CH}_3$	0.48
$\text{CH}_3 \text{ CO CH Br COCH}_3$	0.42

a) Reference 37

The observed insensitivity of iodination of pyruvic acid and the alkyl pyruvates towards acid catalysis is not surprising since it is generally observed that the sensitivity of reactions towards acid catalysis is inversely related to substrate acidity.³⁹

Thus, the results from the present work suggest that in the enolizations of pyruvic acid and of alkyl pyruvates, carbon-hydrogen bond breaking (already greatly assisted by the near proximity of the carboxyl or carboalkoxyl group) is considerably more important than oxygen to hydrogen bond making.

B. Comparison of the Spontaneous Rates of Iodination

The spontaneous rate constants for the iodination of alkyl pyruvates were determined from measurements in formate buffer. It was found earlier that the catalysis of the iodination of alkyl pyruvate in this buffer could be represented as follows:¹¹

$$k_{\text{obsd}} = k_o + k_{\text{HCO}_2^-} (\text{HCO}_2^-) \quad (40)$$

Thus, a plot of k_{obsd} versus formate ion concentration gives a straight line having a slope equal to the catalytic coefficient for formate anion, $k_{\text{HCO}_2^-}$, and an intercept equivalent to the spontaneous rate of iodination, k_o . The spontaneous rates of iodination of pyruvic acid were determined from measurements on solutions of low substrate concentration ($\approx 0.01 \text{ M. l}^{-1}$) in a pH range (1.4 - 1.8) well below the pK_a of pyruvic acid (2.49).³² Under these conditions, the catalytic contribution by pyruvate ion is negligibly small and the observed rate constant for the iodination is simply equal to the spontaneous rate constant for this process,¹¹ $k_{\text{obsd}} = k_o$.

The iodinations of pyruvic acid and alkyl pyruvates were carried out over a wide range of temperatures. A comparison of the spontaneous rate constants of the iodination of pyruvic acid and alkyl pyruvate at 10, 25, and 35 °C. are shown in Table XXIV.

TABLE XXIV

Comparison of the Spontaneous Rates of Iodination

Temperature (°C)	$k_o \times 10^6 \text{ (sec}^{-1}\text{)}$		
	MP	EP	HP
10	1.25	1.23	1.23
25 ^a	1.34	1.31	1.30
35	1.41	1.38	1.36

a) see Reference 11

The similar magnitudes of the spontaneous rates of iodination of pyruvic acid and alkyl pyruvates may indicate a similarity of mechanism of enolization for all three substrates and thus the intramolecular catalyzed path for the iodination of pyruvic acid is relatively unimportant. Thus, it would appear that for all three processes, the carbon-hydrogen bond rupture which takes place in the rate determining step is already aided to such an extent by the strong electron withdrawing effect of the adjacent carboxyl or carboalkoxy group, that neither inter nor intramolecular acid catalysis is required.

C. Activation Parameters

Studies of the iodination processes as a function of temperature allow the determination of the activation parameters. The activation parameters for the spontaneous iodinations of pyruvic acid and alkyl pyruvates at 298 °C are represented in Table XXV.

TABLE XXV

Activation Parameters for the Spontaneous Iodination of Pyruvic Acid, Methyl Pyruvate and Ethyl Pyruvate

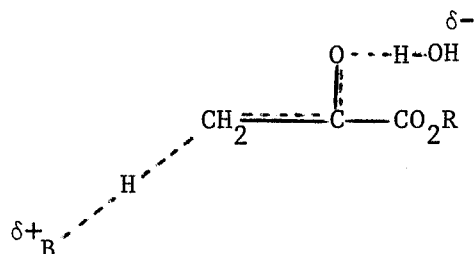
Substrate	E_a (Kcal/mole)	ΔH^* (Kcal/mole)	ΔG^* (Kcal/mole)	ΔS^* (e.u)
Pyruvic Acid	20.3	19.7	25.4	-19
Methyl Pyruvate	20.6	20.0	25.2	-17
Ethyl Pyruvate	21.6	21.0	25.4	-15

As implied earlier and from Table XXV, the free energies of activation are almost identical for all three substrates, therefore, indicating again, that the mechanisms by which the enolization processes take place are very similar. Consequently, the involvement of a cyclic transition state in the enolization of pyruvic acid as proposed by earlier workers is most doubtful since no such cyclic transition state is possible for the corresponding enolizations of the pyruvate esters.

First of all, it will be noted that for the enolizations of the three substrates under consideration, the enthalpies of activation are indeed very similar. The fact that the value for ethyl pyruvate is slightly higher may be due to steric crowding which arises from the interaction of the ethyl group with the solvation shell in the transition state. Since the rate determining step of these enolization processes involve the formation of a more polar transition state from neutral molecules (ethyl pyruvate and water), the degree of solvation of the transition state, and hence steric crowding, would be expected to exceed that in the ground state. In any event, the similarity of the enthalpies of activation associated with the processes under consideration strongly suggests that, in the case of pyruvic acid, if intramolecular hydrogen bonding does indeed exist, it must have just as much of a stabilizing influence in the ground state as it does in the transition state. Hence, the earlier proposal by Bell *et. al.*¹⁸ suggesting intramolecular acid catalysis is without foundation.

The variation of the enthalpies of activation throughout this series of compounds is compensated for by a slight variation of the corresponding entropies of activation, i.e. the free energies of activation for all three processes are essentially the same. As expected from reactions in which charge separation begins in the transition state and, also for reactions in which the total number of particles decreases in progressing from the ground state

to transition state, the entropies of activation for the enolizations studied in the present work are all negative. This indicates that in each case, there exists a higher degree of restriction motion associated with the transition state than in the ground state.



It is usually observed that reactions involving the formation of a cyclic transition state are accompanied with a relatively large negative entropy of activation, principally because of the generation of rotational restrictions not present in the ground state. It will be noted that the difference between the entropy of activation for pyruvic acid (ca. -19 Cal/mole/degree) and methyl pyruvate (ca. -17 Cal/mole/degree) enolization is small indicating the absence of a cyclic transition state in the former process. The entropy of activation for the enolization of ethyl pyruvate (ca. -15 Cal/mole/degree) continues a trend which suggests that the variation in ΔS^* may be a function of size of the group attached to the carboxylate group. It is not, however, a simple matter to accurately interpret the small differences in the entropies of activation observed in the pyruvic acid, methyl

pyruvate and ethyl pyruvate systems since these parameters are reflective not only of changes occurring with the substrate as the transition state is formed, but also include changes in the randomness of the solvent molecules as new species (which in this case require a higher degree of solvation) are produced. In this connection, it is perhaps noteworthy that earlier workers^{19,40} indicate that the degree of solvation of pyruvic acid hydration is considerably greater than that associated with the pyruvate esters hydration. It is, therefore, possible that the change of solvation occurring in the rate determining step of the enolization of pyruvic acid has a greater effect in the destruction of solvent randomness than in the case of methyl pyruvate. Consistent with this possible explanation is the fact that for ethyl pyruvate, the value of the entropy of activation is less negative than that for methyl pyruvate, implying that as the alkyl group on the ester increases in size, alteration of the solvent cage is further minimized during the development of the transition state, perhaps because the larger alkyl group partially blocks out the solvent.

D. Deuterium Solvent Isotope Effects

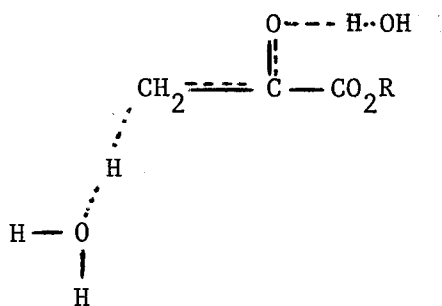
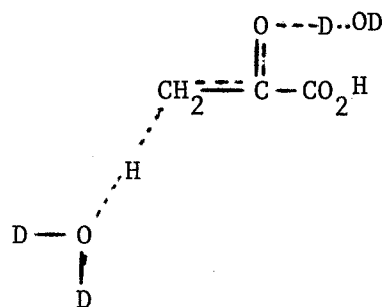
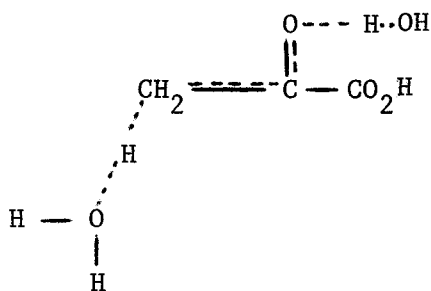
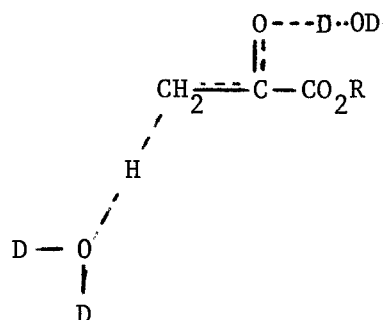
The deuterium solvent isotope effects for the spontaneous iodination of alkyl pyruvates and pyruvic acid are shown in Table XXVI.

TABLE XXVI

The Solvent Deuterium Isotope Effects for the Spontaneous Rate of Iodination

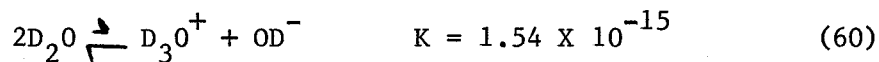
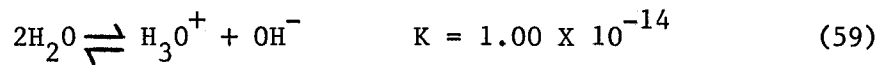
Substrate	$k_{\text{H}_2\text{O}}$ (sec^{-1})	$k_{\text{D}_2\text{O}}$ (sec^{-1})	$\frac{k_{\text{H}_2\text{O}}}{k_{\text{D}_2\text{O}}}$
Methyl Pyruvate	1.9×10^{-6}	1.0×10^{-6}	1.9
Ethyl Pyruvate	1.7×10^{-6}	0.81×10^{-6}	2.1
Pyruvic Acid	1.4×10^{-6}	0.73×10^{-6}	1.9

The data indicates that the ratio, $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$, is basically the same for these substrates, (ca. 2), again indicating the similarity of mechanism for the iodination of alkyl pyruvates and pyruvic acid. The transition states for the enolization of the alkyl pyruvates in both H_2O and D_2O can be represented as follows:

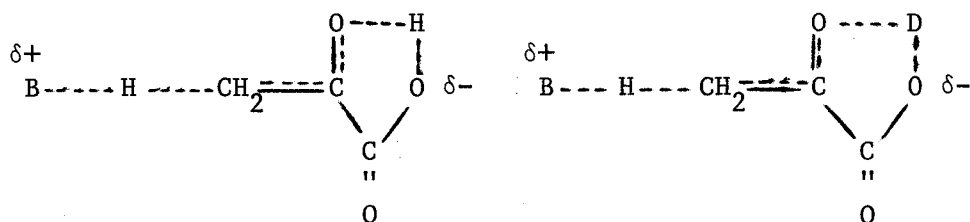
Catalysis by H₂OCatalysis by D₂O

It will be noted that carbon-hydrogen bond breaking in the transition state is more complete than oxygen-hydrogen bond making at the time the transition state is reached.

The slower the rates of iodination of these pyruvate systems in deuterium oxide as compared to water probably arise because of the stronger basicity of H₂O as shown from the values of the ionization constants of water and deuterium oxide:³⁶



On the other hand, if the proposal by Bell *et. al.* that the enolization of pyruvic acid proceeded through a cyclic transition state were correct:



One would expect a larger deuterium isotope effect for pyruvic acid since it is known that such intramolecular hydrogen bonding, when it exists, is more important in H_2O than in D_2O . Thus, the experimentally determined value of solvent deuterium isotope effect offers an additional piece of evidence that the intramolecular catalyzed path in the iodination of pyruvic acid is unimportant in the transition state.

It is of interest to compare the activation parameters for the spontaneous iodination of alkyl pyruvates with those obtained in the mutarotation of glucose⁴² in both H_2O and D_2O (Table XXVII). From this table it will be noted that the activation enthalpies obtained in D_2O are smaller than those determined in H_2O . Furthermore, the entropies of activation in D_2O are more negative than in H_2O . These results could be interpreted on the basis that the transition state is reached later in deuterium oxide than in water resulting in a more intimate involvement of solvent molecules in

D_2O than in H_2O and consequently, the entropies of activation for D_2O catalysis are more negative than those in H_2O catalysis.

TABLE XXVII

Activation Parameters for the Spontaneous Iodination of Alkyl Pyruvate and for the Spontaneous Mutarotation of Glucose in Both H_2O and D_2O

	Mutarotation ^a of Glucose		Iodination of Methyl Pyruvate	
	H_2O	D_2O	H_2O	D_2O
ΔH^* (Kcal/mole)	17.2	16.4	20.1	19.7
ΔS^* (Cal/mole/degree)	-25.0	-30.0	-17.0	-20.0
ΔG^* (Kcal/mole)	24.7	25.3	25.2	25.6

a) Reference 42

In summary, the values of Brønsted coefficient β , free energies of activation and solvent deuterium isotope effects give a strong indication that the mechanisms of enolizations of pyruvate esters and of pyruvic acid are the same and consequently, the cyclic transition state which was proposed by earlier workers¹⁸ is unimportant in the enolization of pyruvic acid.

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