formation of 20(β) hydroxy compounds even when the reduction is done in the presence of mineral acids.

STATE COLLEGE, PENNA.   RECEIVED NOVEMBER 21, 1938

The Keto-Enol Tautomerism of Pyruvate Ion Studied Polarographically*

BY OTTO H. MÜLLER AND J. PERCY BAUMBERGER

Introduction

Pyruvate ion holds a key position in practically all schemes of carbohydrate metabolism in the intermediary steps of which the enol form is often postulated. The only evidence for this enol form has been spectroscopic[1] as the usual methods for its determination have failed in this particular case.

In this paper, we present data regarding the keto/enol + enolate ratio and polymerization of pyruvic acid and of pyruvate ion in relation to pH, and determine the apparent reduction potential of both forms of pyruvate ion at different pH. These determinations are made electrochemically, using the polarographic method.

Method

Materials.—The pyruvate used in these experiments was kindly given to one of us (J. P. B.) by Dr. H. Borsook of Pasadena in the form of solid lithium pyruvate of great purity. All other chemicals were c. p. products. The McIlvane series of buffers was used for pH 2.2–8.0 and Clark and Lubs HCl–KCl for pH 1.0–2.2. The pH was checked by means of the glass electrode. The temperature at which these experiments were carried out was 25 ± 0.1° maintained in a water thermostat.

General Description of Polarographic Method.—The method makes use of a dropping mercury electrode as half-cell connected to a calomel half-cell by an agar bridge saturated with potassium chloride. A battery, slide wire potentiometer, and galvanometer are also in the circuit so that the voltage applied to the system may be varied and the resulting current measured. The procedure depends upon the fact that many compounds may be reduced at the surface of mercury dropping slowly from a capillary when the voltage applied is sufficiently negative. The resulting current is a function of the concentration of reducible substance, while the potential at which the reduction occurs is characteristic of the compound reduced. This principle has been incorporated into a very convenient apparatus, the polarograph, in which the current and voltage are automatically graphically coördinated on photographic paper to give a current–voltage curve, called a polarogram. J. Heyrovský and his school[2] are responsible

for this development. On such a polarogram, the voltage is indicated by abscissas, and changes in current appear as waves.

Apparatus.—Our experiments were carried out with a Nejedlý polarograph Model VIII and a Nejedlý galvanometer with a sensitivity of 2.3 × 10⁻⁹ amp./mm./m. A special shunt permitted the modification of this sensitivity to any desired fraction, while critical damping was maintained.

The apparatus was so adjusted that the distance between two abscissas on the polarogram corresponded to 200 mv.

The dropping mercury electrode was made according to instructions given by Heyrovský[2]; its drop time in distilled water was six seconds when the mercury reservoir was 60 cm. above the capillary.

As oxygen is also reducible at the dropping mercury electrode, hydrogen gas was bubbled through all solutions before each experiment to remove the dissolved oxygen.

Instead of using a large layer of mercury at the bottom of the electrolysis vessel as non-polarizable anode, as is customary in polarographic work, we made use of a saturated calomel electrode with a large surface which was connected to the test solution by an agar bridge, saturated with potassium chloride. The potential of this separate electrode is constant[3] and its value is known, thus eliminating the troublesome measurement of the anode potential. All polarograms reproduced in this paper, therefore, show automatically the applied e. m. f. referred to the saturated calomel electrode.

For the plotting of graphs, liquid junction potentials have been neglected, while corrections for the iR drop[3] have been made in calculations of the half-wave potentials[4] which are all referred to the normal hydrogen electrode as zero. The accuracy of these values is ±20 mv., which seems sufficient for the present treatment. As we have shown[3] polarographic potentials may be read with an accuracy of ±1 mv. if proper precautions are taken.

Keto-Enol Tautomerism

This attempt to determine the concentration of keto and enol pyruvate ion was the direct outgrowth of the work on the coupled oxidation–reduction potential of pyruvate ⇌ lactate, carried out in this Laboratory.[5]

(*) Original manuscript received November 26, 1937.

(1) V. Henri and C. Fromageot, *Bull. soc. chim.*, [4] **37**, 845 (1925).

(2) J. Heyrovský, "Polarographie," in W. Böttger, "Physikalische Methoden der analytischen Chemie," Akademische Verlagsgesellschaft, Leipzig, 1936, Vol. II, pp. 260–322.
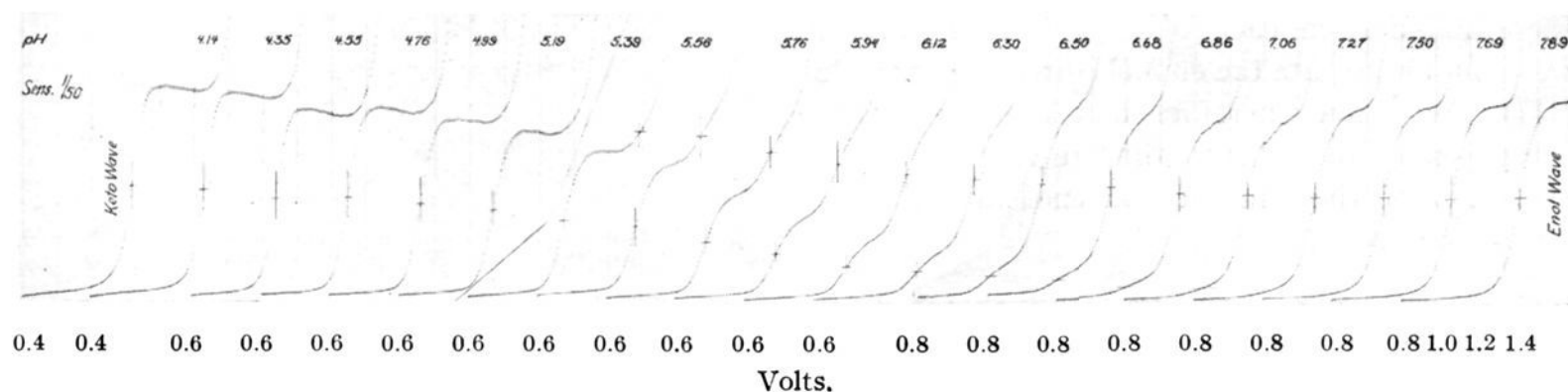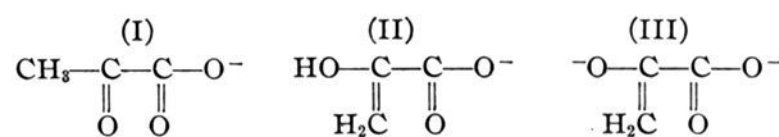
(3) O. H. Müller and J. P. Baumberger, *Trans. Electrochem. Soc.*, **71**, 181 (1937).

(4) J. Heyrovský and D. Ilković, *Coll. Czechoslov. Chem. Comm.*, **7**, 198 (1935).

(5) J. P. Baumberger, J. J. Jürgensen, and K. Bardwell, *J. Gen. Physiol.*, **16**, 961 (1933).

Fig. 1.—Polarograms of pyruvate reduction at different pH. The initial applied voltage for each curve is indicated; abscissas are 0.2 v. apart and progressively more negative. The applied voltage at the half-wave is indicated in each case

The reduction of pyruvic acid at the dropping mercury electrode seemed to present a satisfactory method for analytical determination, especially as several authors had mentioned such a reaction.[6,7] However, closer investigation showed that the polarogram of pyruvate really involves three waves and that the voltage at which each occurs is shifted by pH. This has not been recognized previously as no systematic study has been made. We therefore carried out a series of experiments in buffered solutions of different pH. Lithium pyruvate was used when it was found to give identical results with freshly distilled pyruvic acid.

We found it most convenient to use solutions of low concentration, because the iR correction would be small and there would be a minimum of polymerization. Figure 1 is a reproduction of three polarograms pieced together showing curves due to the reduction of 0.001 M pyruvate in buffers of different pH. We see that as the pH increases, the single wave at pH 4.14 becomes smaller and another wave appears. In the rest of this paper, these two waves are called keto and "enol" respectively, "enol" referring to the sum of enol and enolate pyruvate. It may be noticed that the sum total of the two waves (keto + "enol") is a constant, proportional to the concentration of pyruvate present. At pH 8 the keto wave has practically vanished and the "enol" wave is now as big as the original keto wave. The reduction of pyruvic acid is known to require two electrons per molecule[6]; from our curves we must conclude that this holds at any pH, i. e., for pyruvate ion also and that the reduction goes to completion in a single step.[8] The reason for the two different

(6) L. Schwaer, Coll. Czechoslov. Chem. Comm., **7**, 326 (1935).

(7) A. Winkel and G. Proske, Ber., **69**, 1917 (1936).

(8) In this fact we have a fundamental difference from the irreversible reductions of benzaldehyde [M. Tokuoka, Coll. Czechoslov. Chem. Comm., **7**, 392 (1935)] and benzophenone [H. Schweitzer and

waves must be that two distinctly different substances are reduced which are in equilibrium with each other. This equilibrium changes with pH. The most logical cause for such an equilibrium in the case of pyruvate ion is the keto–enol tautomerism which has been mentioned frequently in the literature but for which no definite and conclusive evidence has so far been presented. It is our opinion that the first wave which we obtain on the more acid side is due to the reduction of the keto form of pyruvic acid or pyruvate ion while the second wave is due to the "enol" form.

The basis of our opinion is the well established fact that only those acids and their ions are reducible at the dropping mercury electrode which have conjugated double bonds. The pyruvate ion (I) has two $C{=}O$ double bonds which are conjugated. The enol (II) and the enolate (III) forms of the pyruvate ion have one $C{=}C$ double bond and a $C{=}O$ group which are also conjugated. We may therefore expect them to be also reducible, although most likely at a different potential.

$$\underset{(I)}{CH_3{-}\underset{\underset{O}{\|}}{C}{-}\underset{\underset{O}{\|}}{C}{-}O^-} \qquad \underset{(II)}{HO{-}\underset{\underset{H_2C}{\|}}{C}{-}\underset{\underset{O}{\|}}{C}{-}O^-} \qquad \underset{(III)}{{}^-O{-}\underset{\underset{H_2C}{\|}}{C}{-}\underset{\underset{O}{\|}}{C}{-}O^-}$$

The need for conjugation of double bonds eliminates the possibility that the two waves may represent reductions of different hydrates of the pyruvate.

As associated and dissociated acids are indis-

E. Laqueur, Rec. trav. chim., **55**, 959 (1936)]. There with a change in pH another wave appears which, however, never becomes larger than one-half the original wave. This means that at a certain pH the reduction goes on in two steps, each requiring one electron per molecule, while at another pH the reduction goes to completion in one step, each molecule adding two electrons at once.

The reversible two-step oxidations and reductions[3] are another entirely different case of electro-reduction at the dropping mercury electrode.

tinguishable from each other polarographically, we cannot segregate the enol (II) from the enolate (III). We have therefore labelled our second wave "enol" to indicate that it represents the reduction of the sum of the enol and enolate forms of pyruvate ion.
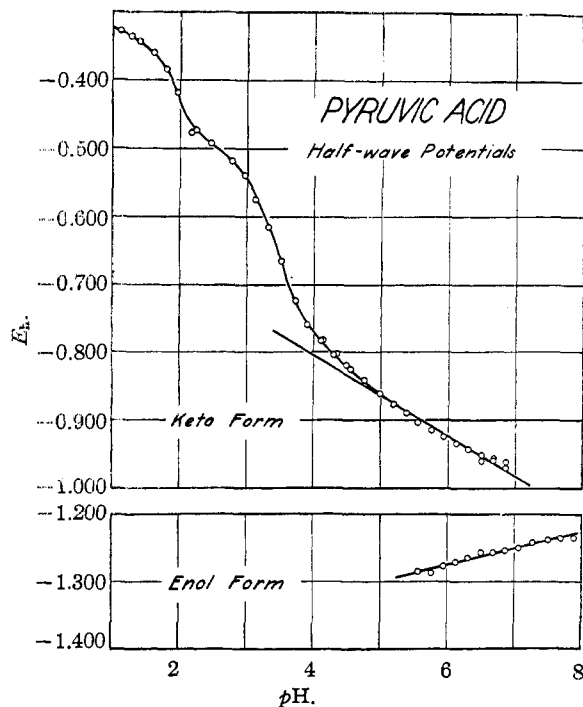

Fig. 2.—Apparent reduction potentials of pyruvic acid in its different forms at different pH's calculated from polarograms similar to and including those in Fig. 1 after making proper corrections.

In Fig. 2 we have plotted the changes of potential of keto and "enol" reductions against the changes in pH. We have only included the pH range from 1 to 8, as the "enol" wave loses its smooth "S" shape, which makes the potential measurements unreliable. The curves represent the values obtained when the half-wave method[4] of measuring potentials was used. For the case of reversible organic electro-reductions we have established the significant value of this half-wave potential.[3] Although we no doubt deal with a fairly irreversible process we have preferred this method of measurement to others because of its relationship to the "apparent reduction potential" (A.R.P.) of Conant[9] as will be shown later on.

Figure 3 shows a comparison of the heights of the keto and "enol" waves at different pH values; it may be seen that at pH 5.8 they are equal.
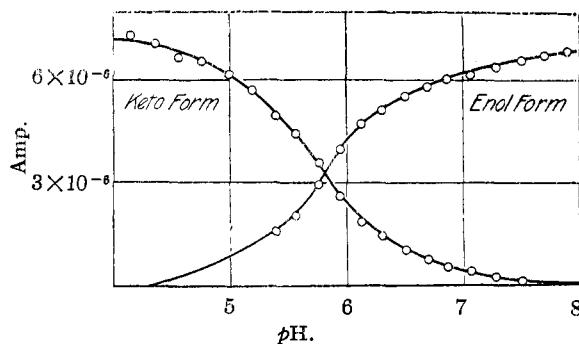
(9) J. B. Conant, *Chem. Rev.*, **3**, 1 (1926).

Fig. 3.—Relative concentration of pyruvate in the keto and "enol" form at different pH's as indicated by the electro-reduction current of each form.

This value, of course, would be valid only if the rate of enolization, respectively, ketone formation, is infinitely slow compared to the rate of reduction. We may assume that both keto and "enol" forms diffuse to the electrode at about the same rate.[10] If keto is reduced first and the cathode surface depleted of keto molecules, no doubt some of the remaining "enol" molecules will change into keto and also be reduced. The following experiments show that the rate of ketone formation is too great to be measured by our procedure.

Solutions of lithium pyruvate in a fairly alkaline medium were shown polarographically to be practically free from the keto form. Then a constant potential of 1.2 v. was applied at which keto is reduced in acid solution while "enol" is not; the corresponding current was of course very small. When oxygen-free acid was added to the solution in a stream of hydrogen, the current reached the full value which it would have if only keto were present, after a time interval of only one-half minute. This was the minimum time necessary to ensure complete mixing of the solution by bubbling hydrogen gas through it. Even when the solution was kept in an ice-bath, the ketone formation had taken place before the mixing was considered adequate.

It would seem, therefore, that the wave for keto may be somewhat larger because of conversion of "enol" to keto locally at the surface of the electrode. However, this effect must be slight judging by the fact that the keto wave practically vanishes at pH 7.5.

The ratio of keto/"enol" becomes unity at pH 5.8 and decreases with increase in pH and *vice versa;* e. g., it is approximately 10 at pH 4.8 and 0.1 at pH 6.8. This relation of the equilibrium of the two forms of pyruvate to pH brings up the question as to whether or not the enol pyruvate ion (II) is dissociated to give an enolate ion (III). The polarographic method cannot be used to answer this question as it does not distinguish between associated and dissociated acids. A preliminary titration of pyruvic acid seemed

(10) The rate of diffusion of the reducible substance is one of the important factors governing the height of polarographic waves [D. Ilkovič, *Coll. Czechoslov. Chem. Comm.*, **6**, 498 (1934)].
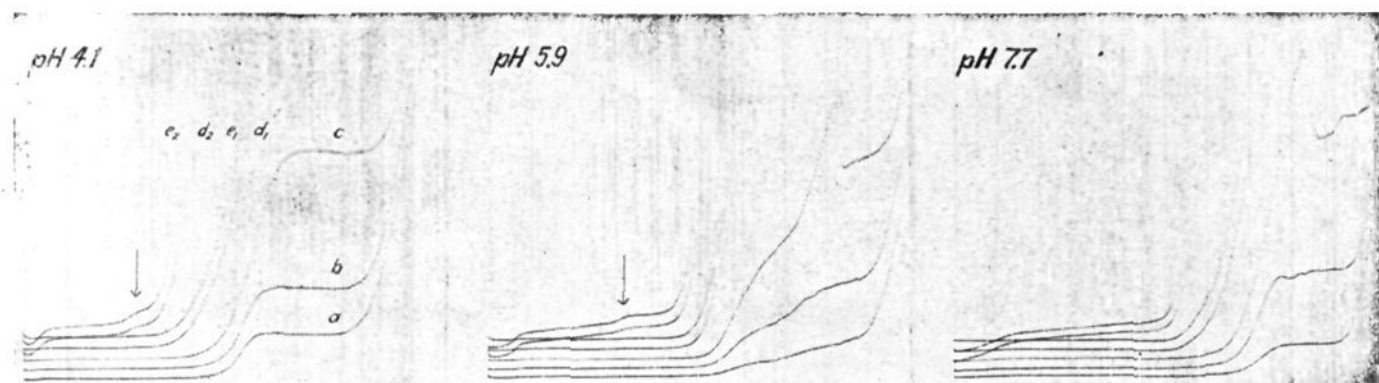
Fig. 4.—The polymerization of pyruvate as affected by pH and concentration. Three families of curves, all starting at zero applied voltage, with members at similar approximate concentrations and galvanometer sensitivities identically arranged as follows: (a) 0.001 M; (b) 0.002 M; (c) 0.005 M; (d$_1$) 0.1 M; (e$_1$) 0.2 M; all at 1/500 sensitivity. (d$_2$) 0.1 M; (e$_2$) 0.2 M; each at 1/50 sensitivity.

to indicate a second dissociation constant around pH 5.8 but speculations as to the steps in the enolization (either enolization and subsequent dissociation, or dissociation from the methyl groups with induced enolization) have to be postponed until more accurate measurements are made.

Polymerization

Our next study concerned the wave at -0.2 v. from the normal calomel zero which Schwaer[6] had reported as due to the undissociated, molecular form of pyruvic acid. As we had not seen it in our dilute solutions, we increased the concentration of lithium pyruvate. Figure 4 is a reproduction of 3 polarograms which are representative of these studies. All are carried out analogously, the starting solutions (curves 4a) were from the experiments which are presented in Fig. 1. To these solutions increasing amounts of lithium pyruvate were added until in the last curve on each polarogram the concentration of lithium pyruvate was about 0.2 M. The first group of curves in Fig. 4 is at pH 4.1 and represents typical keto-waves. The second group is at pH 5.9 and shows both keto and "enol" reductions. It should be noticed that while the keto reduction even at the higher concentration shows no maximum,[2] the "enol" reduction is marked by a very pronounced maximum. This can be seen again in the third group, which represents typical "enol" reductions, in a pH 7.7 buffer.[11]

In the first and second groups of curves (Fig. 4) we furthermore notice a little wave indicated by an arrow which is absent in the third group and only appears with higher sensitivities of the gal-

vanometer in the more concentrated solution. This wave changes to more positive potential and becomes greater with decreasing pH. In 1 N solution of hydrochloric acid, it is quite pronounced and is almost at -0.200 v. We therefore feel that this is the same wave which Schwaer reported as due to the associated, molecular form of pyruvic acid. However, we believe it to be caused by a polymerization product of pyruvic acid, because we observe that this wave grows with age; e. g., the two waves of pH 4.1 and 5.9 increased from 3.5 to 11 mm. and from 1.5 to 4 mm., respectively, in twenty days, while pH 7.7 never shows this wave. As is well known, pyruvic acid easily polymerizes upon standing; in most work, therefore, only freshly vacuum distilled pyruvic acid has been used.

Figure 5 represents polarograms of 0.01 M solutions of lithium pyruvate which have been left standing for a week after their preparation; the galvanometer sensitivity and the height of the waves are indicated in the figure. The highest wave is obtained at pH 8.0 where the pyruvate is all in the unpolymerized "enol" form. Other relative concentrations of keto, "enol," or polymer are calculated by referring the heights of their waves to this standard, assuming that the height of polarographic waves is a linear function of concentration.[12] Thus from a comparison of the diminution in heights of the keto wave and the correspondingly appearing polymer wave we can calculate n for the polymer on the assumption

(11) The significance of this maximum which only occurs in the "enol" reduction is still uncertain. We believe it indicates a difference in the nature of the electro-reduction of the two tautomeric forms.

(12) While the strict proportionality of height of wave to concentration has been proven in polarographic work for many systems, there is in general no reason to believe that this is true in all cases, especially if the conditions are changed. From our experience with pyruvic acid in buffers of different pH and at varying concentrations (see e. g., Figs. 1 and 4) we find, however, that the concentration/height-of-wave relationship is sufficiently constant to give significance to the deductions from our experiments which we make in this paper.
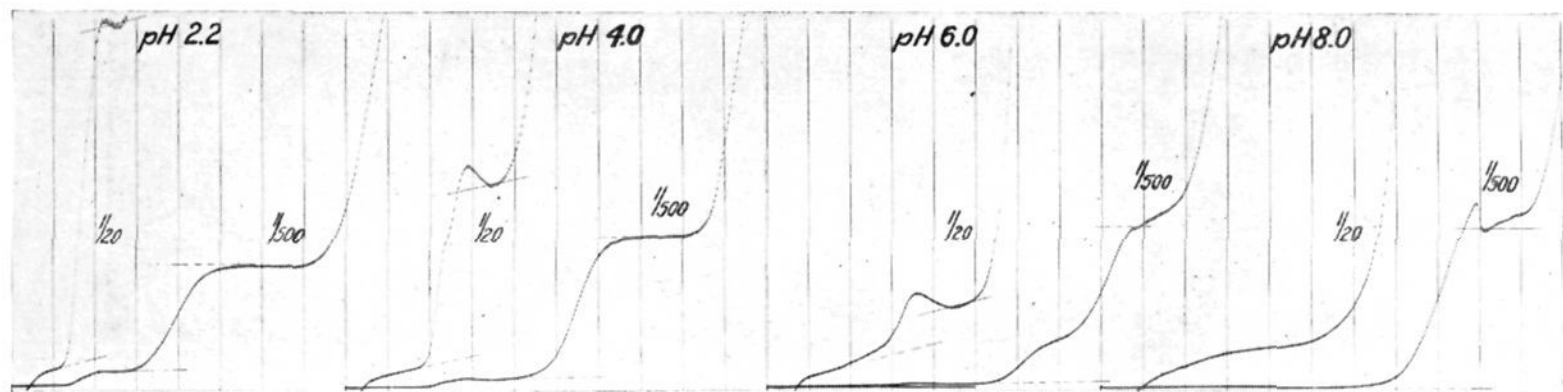
Fig. 5.—The relative concentrations of polymerized and unpolymerized pyruvate at different pH's are shown as heights of polarographic waves. The conditions for each curve at a specified pH are identical except that the galvanometer sensitivity is different.

that the undeterminable percentage of prepared pyruvate is fully represented in these polymer waves. We then get

pH	2.2	4.0	6.0	8.0
n	4.0	2.9	2.9	No polymer

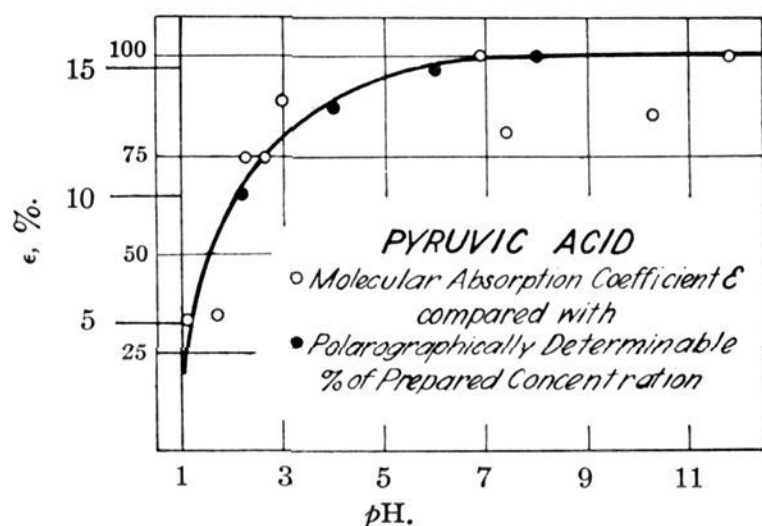A polymer of pyruvic acid with $n = 3$ was found by Massol[13] using cryoscopic methods.

Fig. 6.—The molecular absorption coefficient of pyruvic acid (associated and dissociated) at different pH's plotted from Henri and Fromageot's data[1] is shown by open circles. For comparison the percentage of total pyruvic acid which is determinable as keto and "enol" pyruvate is shown as solid circular dots.

Henri and Fromageot[1] presented spectrophotometric evidence for the enolization of pyruvate ion in alkali. We made a similar study[14] on buffer solutions of 0.001, 0.005 and 0.01 M lithium pyruvate. Only the 0.01 M pyruvate shows very marked differences in light absorption with pH and allows the calculation of values which agree with their findings. It appears, however, from Grossmann's[15] work that the wave length of light chosen to study the C=C concentration,

namely 3300 Å., is really the absorption maximum for C=O and that < 2300 Å. would have been proper for "enol" absorption. Therefore "enol" was probably not measured by Henri and Fromageot.

Our observation on polymerization and the spectrophotometric work of Henri and Fromageot[1] show an interesting analogy. This is illustrated in Fig. 6 where the molecular absorption coefficient, ϵ, and the polarographically determinable percentage of prepared concentration of pyruvate vary similarly with pH. It seems reasonable to conclude that the change in light absorption measured was the result of polymerization of pyruvate rather than enolization.

The supposition of the above polymer might also explain the later work of Fromageot and co-workers[16] on substituted pyruvic acids. Bromine titrations in which ferric chloride was used as indicator showed an increase of the reacting compound toward more acid solutions: e. g., 0% at pH 5.0 and 0.5% at pH 1. Fromageot therefore altered his previous viewpoint[1] and concluded that in the acid region the enol form predominated, while the increase in absorption of the ultraviolet toward the alkaline range should be attributed to a compound of the structure

$$R_2C{=}C{-}C\diagup^{OH}_{\diagdown OH}$$
$$\diagdown_{O}\diagup$$

If ferric chloride and bromine are specific reagents for a double bond, as is assumed for the "enol" titrations, then the last formula must be wrong, because it, too, has a double bond, and therefore should react. Second, as "enols" are generally known to form more toward the alkaline side than toward the acid side; it is very un-

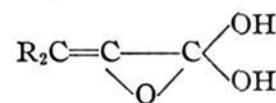(13) M. G. Massol, *Bull. soc. chim.*, [3] **33**, 335 (1905).

(14) We are very much indebted to Dr. B. L. Crawford, Jr., of the Chemistry Department of this University for helping us carry out this spectrophotometric study.

(15) P. Grossmann, *Z. physik. Chem.*, **109**, 305 (1924).

(16) C. Fromageot and S. Perraud, *Biochem. Z.*, **223**, 213 (1930); C. Fromageot, M. Pelletier and P. Ehrenstein, *Bull. soc. chim.*, [4] **51**, 1283 (1932).

likely that Fromageot titrated "enols" in his experiments. Third, the bromine reaction and that of ferric chloride is known to fail in a number of cases where the existence of "enols" is fairly well established. Hantzsch[17] and Dieckmann[18] came to the conclusion that the formation of colored ferric compounds between "enols" and ferric chloride may be expected only if inner complex salts or "conjugated enol-salts" with a secondary valence linkage between metal and carbonyl oxygen are possible, as may well be the case in polymerized pyruvate.

The Apparent Reduction Potential of Keto and "Enol" Pyruvate and of Pyruvic Acid.—Conant[9] describes an important characteristic of irreversibly reducible compounds which he calls the "apparent reduction potential" (A.R.P.). This is determined by observing the rate at which reversible oxidation–reduction systems (B) are oxidized by the irreversible system (A). He gives evidence that if the rate of oxidation of B is related to the E'_0 of B and the A.R.P. of A, there must be a *reversible* step in the reduction of A, followed by an *irreversible* step
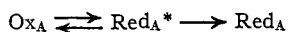
$$Ox_A \rightleftharpoons Red_A^* \longrightarrow Red_A$$

Thus considering the reversible stage in the reduction of A, the relation may be expressed

$$E'_{0A} - E'_{0B} = \frac{0.059}{n} \ln \frac{[Ox_A][Red_B]}{[Red_A^*][Ox_B]}$$

Therefore it follows that A.R.P. $= E'_{0A}$.

In the polarographic method a similar situation exists at the half-wave potential[3] which therefore should correspond to A.R.P. For the case of the reduction of pyruvic acid (P) to an electromotively active form of lactic acid (L*) and then to the stable form of lactic acid (L),[19] we have

$$P \rightleftharpoons L^* \longrightarrow L$$
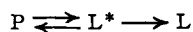
and

$$E'_{0 P \rightleftharpoons L^*} - E'_{0 Hg_2^{++} \rightleftharpoons Hg^0} = \frac{0.059}{n} \ln \frac{[P][Hg^0]}{[L^*][Hg_2^{++}]}$$

We have shown[3] that, by means of the polarograph, potentials of *reversible* oxidation–reduction systems can be measured, even if only oxidant or reductant alone is present in solution. The half-wave potential of a polarographic curve corresponds with the E'_0 of the particular system.

If the irreversible step in the above reactions is sufficiently slow, compared to the electron transfer itself in the reversible process, the product of the *reversible* reduction (L*) would remain at the electrode surface long enough to impress upon it a potential governed by the ratio of its concentration to that of the remaining, still unreduced, oxidant. As the dropping mercury electrode is unique in presenting a constantly renewed, fresh surface, we are able to pick out the reversible steps in such irreversible reactions and treat them separately.

Of the compounds studied by Conant only three have been investigated polarographically. We have estimated their half-wave potentials from the published data and find good agreement with Conant's determination of the A.R.P.[9] as shown below.[20] In all these cases the solvent was hydrochloric acid and the potential is expressed as E_h.

	Maleic acid	1,3 Dinitro-benzene	Nitro-benzene
A. R. P. Conant,[9] v.	−0.25	+0.16	+0.06
Polarographic half-wave, v.	−0.28[21]	+0.16[22]	+0.07[23]

It would appear that the polarographic method has the advantage (in cases where it can be used) that the reversible step $P \rightleftharpoons L^*$ is measured rather than the irreversible $L^* \longrightarrow L$ step, which Conant's method employs, i. e., we measure E_0' of $P \rightleftharpoons L^*$ directly whereas he obtains it indirectly. It also has the advantage that the A.R.P. of more than one irreversible compound can be determined in the same solution; this would be very difficult by any other method we know of.

Figure 2 shows the A.R.P. of keto-pyruvic acid at different pH. The general slope of this curve might be expected to be 60 mv./pH as in general for E'_0 of organic compounds, and to be modified by dissociation of the carboxyl groups of pyruvic acid and lactic acid. The curves show marked changes in slope at pH's close to the pKa of pyruvic acid (2.45) and lactic acid (3.85).

In our experiments with reversible oxidation–reduction systems we were able to oxidize the reductant at the dropping mercury electrode.[3] The half-wave potentials thus obtained from

(17) A. Hantzsch, *Ann. Chem.*, **392**, 286 (1912).

(18) W. Dieckmann, *Ber.*, **50**, 1375 (1917).

(19) In this paper lactic acid is stated to be the end-product of the reduction of pyruvic acid at the dropping mercury electrode. This assumption is made for convenience but also has support in the fact that lactic acid is formed when pyruvic acid is reduced in acid solution by zinc or by electrochemical means.

(20) M. Shikata [*Mem. College Agr., Kyoto Imp. Univ.*, No. 4, 7 (1927)] recognized an analogy between his polarographically determined electrode reduction potential and Conant's A.R.P.

(21) E. Vopička, *Coll. Czechoslov. Chem. Comm.*, **8**, 349 (1936).

(22) M. Shikata and N. Hozaki, *Mem. College Agr., Kyoto Imp. Univ.*, No. 17, 1 (1931).

(23) M. Shikata, *Trans. Faraday Soc.*, **21**, 42 (1925).

curves due to the anodic oxidation of reductant were identical with those due to the cathodic reduction of the oxidant of the same system. This fact serves as a very convenient criterion to determine the reversibility of an oxidation–reduction system. For instance we were unable to oxidize lactic acid in a solution of nitric acid at the dropping mercury electrode, using the most positive potential available at this electrode, viz., $E_h = +0.65$ v. This supports the view that the reversible step $P \rightleftarrows L^*$ is followed by an irreversible process $L^* \longrightarrow L$.

Using Conant's method, Barmore[24] found that lactic acid could be oxidized in acid solution by potassium permanganate which corresponded to an apparent oxidation potential (A. O. P.) of $E_h = +1.4$ v. However, when he tried to determine the A. R. P. of pyruvic acid by this method he failed, because the most negative reagent he could use, i. e., titanium trichloride ($E_h = -0.8$ at pH 7.2) was not negative enough to bring about a reduction. For such systems, therefore, the polarographic method and Conant's method supplement each other.

(24) M. A. Barmore, "Electrometric Studies on Pyruvic Acid, Lactic Acid, and Glyceric Aldehyde" (unpublished thesis), Stanford University Library, 1929.

This work was aided by a grant from the Rockefeller Foundation.

Summary

1. The application of the polarographic method to the study of keto–enol tautomerism, polymerization and apparent reduction potential is described.
2. Evidence is presented in support of the following facts: (a) The ratio of keto/enol + enolate forms of pyruvate ion becomes unity at pH 5.8 and decreases with increase in pH and vice versa. (b) Pyruvic acid polymerizes even in fairly dilute solutions and this polymerization increases with increase in acidity and with time. (c) The "polarographic half-wave potential" is equivalent to the "apparent reduction potential" of Conant and represents the E'_0 of the reversible step in an irreversible reaction. (d) The apparent reduction potentials of the various forms of pyruvic acid vary with pH.
3. An interpretation of the conflicting data in the literature concerning the keto–enol tautomerism of pyruvate ion is given on the basis of some new facts presented in this paper.

# The Synthesis from β-Naphthohydroquinone of a Tautomer of 4-Benzyl-1,2-naphthoquinone

BY LOUIS F. FIESER AND MARY FIESER

In the course of an investigation with Bradsher[1] it was observed in preliminary experiments that 4-benzyl-1,2-naphthoquinone (I) can be converted by treatment with concentrated sulfuric acid or with alkali into a yellow substance which, as shown in the present work, is a monomer isomeric with the starting material. In undertaking

I   II
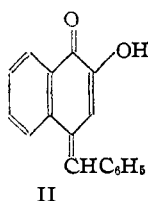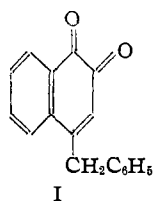
a further study of the problem, the possibility was considered that the yellow substance has the

structure of the acidic tautomer, II, even though there is no analogy in quinone chemistry for the independent existence of such a compound. It was thought that if this were the structure the substance might be obtainable more readily than through the rather inaccessible quinone I by application of a reaction employed successfully by Fieser and Hartwell[2] for the preparation of a similar compound, 2-hydroxy-1,4-naphthofuchsone-1 (III). This substance, or its tautomer, was ob-

III

(1) Fieser and Bradsher, THIS JOURNAL, 61, 417 (1939).
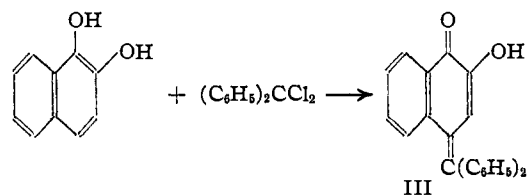
(2) Fieser and Hartwell, ibid., 57, 1484 (1935).