Polarographic and Spectrophotometric Studies on Trimethylpyruvic Acid

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Recent studies on pH-dependence of the limiting current of α-keto acids[1] have revealed that the theory given by Brdicka et al.[2-4] for pyruvic acid and phenylglyoxylic acid, based on the kinetic character of the current due to the recombination of the dissociated acid anion with proton, does not fully explain the behavior of α-keto acids, including the above two acids. In order to interpret the decrease of the limiting current with decreasing pH in acid media, the roles of hydration of the keto form and also enolization in some cases have been suggested, compared with the spectrophotometric measurements[1]. However, the contributions of these factors to the limiting current are in most cases difficult to analyze separately, consequently the interpretation of the phenomenon has remained qualitative.

Although the polarographic and spectrophotometric behavior of trimethylpyruvic acid has already been briefly described with other α-keto acids[1], more detailed investigations on this acid seem to be interesting, since the acid has at least no possibility of enolization and would be therefore expected to give some quantitative knowledge concerning the relation between the polarographic behavior and the spectrophotometric.

Experimental

Materials.—Trimethylpyruvic acid was prepared according to Richard[5] by oxidizing pinacolone with alkaline potassium permanganate. B. p. 75°C at 10 mmHg. Freshly prepared trimethylpyruvic acid was dissolved into distilled water to be 5×10^{-3} M aqueous solution, which was used as stock solution for polarographic measurements.

Buffer Solutions.—McIlvain's buffer for pH 2.2 to 8.0 and Walpole's buffer for pH 0.65 to 5.20 were used. For pH below 1, hydrochloric acid or sulfuric acid solutions of various concentrations were used.

Apparatus and Procedure.—A Heyrovsky-Shikata type polarograph was used with a Yanagimoto Galvarecorder. The characteristics of the capillary used were $m=2.45$ mg./sec., $t=2.92$ sec. at the applied potential of -1.40 (vs. N.C.E.) in a buffer solution of pH 4.35, when the height of mercury reservoir was 60 cm. A normal calomel electrode was used as an outer reference electrode. The reference electrode was connected with the electrolytic cell by an agar bridge saturated with potassium nitrate, which was attached to the cell through sintered glass. The total cell resistance was any time less than 500 ohm. The dissolved oxygen in electrolytic solution was removed by bubbling nitrogen gas. The concentration of trimethylpyruvic acid in final electrolytic solutions was 5×10^{-4} M and the ionic strength was adjusted to be 0.5 by adding sodium chloride, except for the strong acid range. Polarograms were taken at 25 ± 0.05°C. No maximum suppressor was used throughout the experiments. For spectrophotometric measurements, Shimadzu Type QB-50 spectrophotometer with a 1.0 cm. quartz cell was employed. Temperature control was made within ±0.05°C by circulating water of constant temperatures through the jacket attached to the cell. pH Measurements for the electrolytic solutions and also for the determination of pK value of trimethylpyruvic acid were carried out with Horiba Model-P pH meter with a glass electrode.

In order to determine the hydration constant of

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[1] S. Ono, M. Takagi and T. Wasa, *Collection Czechoslov. Chem. Communs.*, **26**, 141 (1961).

[2] R. Brdicka and K. Wiesner, ibid., **12**, 39 (1947).

[3] R. Brdicka, ibid., **12**, 212 (1947).

[4] J. Koutecky and R. Brdicka, ibid., **12**, 337 (1947).

[5] A. Richard, *Ann. chim.* (Paris), [8], **21**, 360 (1910).

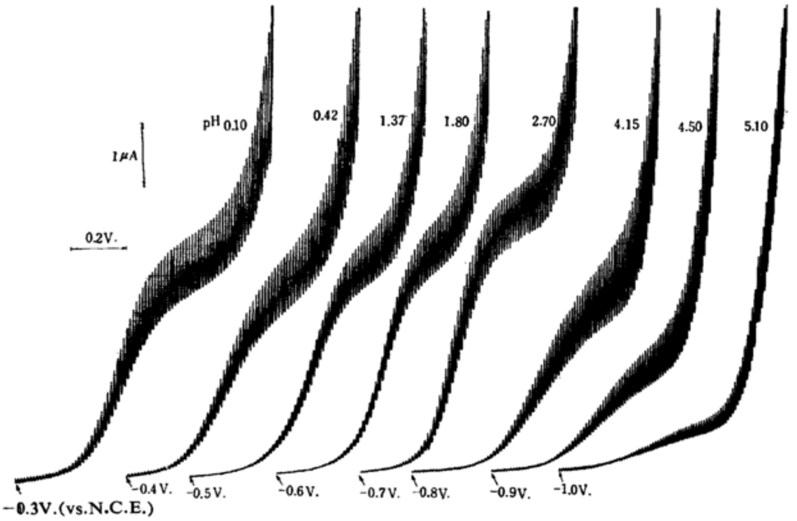
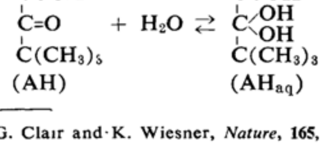


Fig. 1. Polarograms of trimethylpyruvic acid in various pH's, Concn.: 5×10^{-4} M, Temp.: 25°C, Ionic strength: 0.5.

trimethylpyruvic acid in undissociated state, the following kinetic measurments were carried out: about 0.3 ml. of 0.25 M trimethylpyruvic acid aqueous solution containing 1 N hydrochloric acid, which had been kept at 25°C, was rapidly mixed with about 2.7 ml. of McIlvain's buffer of pH 8, which had been taken in the spectrophotometric cell and also kept at 25°C. The change of the optical density at 310 mμ was followed with time.

Results and Discussion

Polarograms of trimethylpyruvic acid in various pH's are presented in Fig. 1. From Fig. 1 it can be seen that the limiting current of trimethylpyruvic acid has a minimum value in a rather acid pH as it has been known with other α-keto acids(1). The decrease of the current with increasing pH in pH above 3, coincides with the observation by Clair and Wiesner(6) and can be attributed to the decrease of the rate of recombination of the dissociated acid anion with proton. Since trimethylpyruvic acid has no possibility of enolization, the decrease of the current in acid pH has been accounted for by the hydration of carbonyl group in undissociated state(1). The hydration of carbonyl group is also considered from the absorption spectra as shown in Fig. 2. The fact that the optical density at 310 mμ due to C=O group, in 1.8 N hydrochloric acid, is smaller than those in alcohol or in acetate buffer of pH 5.09, can be interpreted by the formation of the hydrated form as



$$\begin{array}{c}COOH\\ |\\ C{=}O\\ |\\ C(CH_3)_3\\ (AH)\end{array} + H_2O \rightleftarrows \begin{array}{c}COOH\\ |\\ C\langle^{OH}_{OH}\\ |\\ C(CH_3)_3\\ (AH_{aq})\end{array}$$

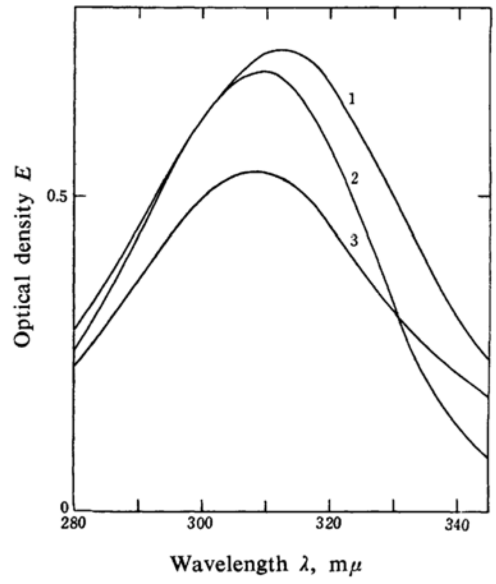


Fig. 2. Absorption spectra of trimethylpyruvic acid. Concn.: 0.025 M, Temp.: 25°C, 1 in alcohol, 2 in acetate buffer of pH 5.09, 3 in 1.8 N HCl aqueous solution.

Utilizing the difference in optical density at 310 mμ of the two states, the dissociated and undissociated, the apparent dissociation constant of trimethylpyruvic acid can be determined by measuring the change in optical density with pH as shown in Fig. 3. pH was changed by adding sodium acetate or hydrochloric acid and the concentration of trimethylpyruvic acid was kept constant. The pK_a value has been found to be 1.52 at 25°C at the ionic strength of 0.04, which is in agreement with the value of 1.47 cited in "International Critical Tables" but which

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6) E. G. Clair and K. Wiesner, *Nature*, 165, 202 (1950).

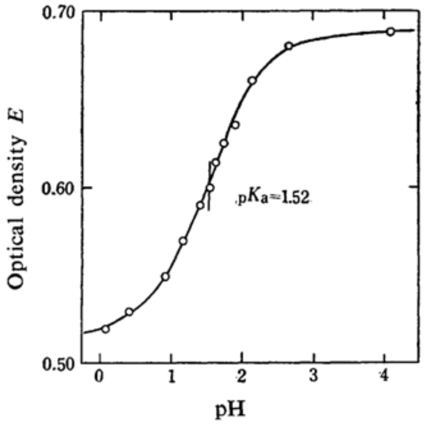


Fig. 3. Change of the optical density of trimethylpyruvic acid. At 310 mμ with pH. Concn.: 0.025 M, Temp.: 25°C. pH was changed by adding either HCl or sodium acetate.

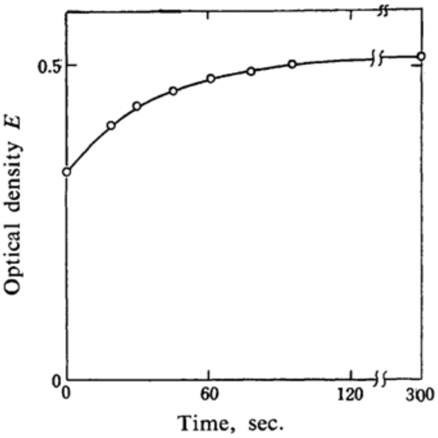


Fig. 4. Measurement of the rate of dehydration of hydrated trimethylpyruvate in pH about 5.
Change of the optical density at 310 mμ was followed with time. The optical density at zero time (E_0) was obtained by extrapolation of the log plot of Fig. 5.

considerably differs from the value of 2.3 used by Clair and Wiesner[6].

From the above change in optical density with pH and also from the observation that the optical density in undissociated state changed with temperature, while that in the dissociated remained constant, it has been assumed that trimethylpyruvic acid is appreciably hydrated in undissociated state, while its anion (A^-) is almost free from the hydration. This assumption may be allowed, referred to the same kind of property of phenylglyoxylic acid, for which the same assumption was deduced, comparing the ultraviolet absorption areas of the free acid anion with those of benzoic acid and cinnamic acid[1]. As in the present experiments, molecular extinction coefficients of AH and A^- can not be taken to be equal as in the case of phenylglyoxylic acid, the direct comparison of the optical densities between the dissociated and undissociated states has little significance for obtaining the quantitative knowledge on the hydration in undissociated state. A more direct attempt was therefore made kinetically, under the assumption that, when the undissociated acid, which exists in an equilibrium between AH and AH_{aq}, is mixed with a sufficiently larger volume of basic buffer solution, the dissociation of the acid occurs instantly to attain its equilibrium, while the dehydration reaction of the hydrated anion (A^-_{aq}) takes place so slowly that it can be followed photometrically as the change of the optical density at 310 mμ. An example of the experimental results is presented in Figs. 4 and 5. Since the dehydration is a first order reaction, the hydration constant of the undissociated acid can be obtained as

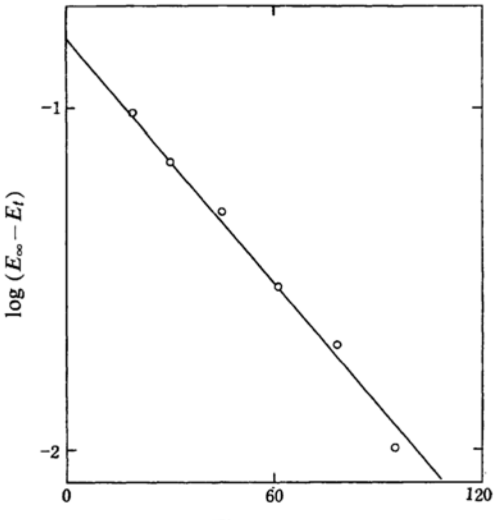


Fig. 5. Log plot of $(E_\infty - E_t)$ of Fig. 4 against time.

$$K_h = [AH_{aq}]/[AH] = (E_\infty - E_0)/E_0 \qquad (1)$$

where E_∞ and E_0 are the optical densities of the anion at the reaction times, $t=\infty$ and 0, respectively, the latter of which can be obtained from the extrapolation of the log plot in Fig. 5. The average hydration constant from three runs was 0.46 at 25°C. From the same log plot the average rate constant of dehydration in pH about 5 at 25°C has been obtained to be $2.8\times10^{-2}\,\text{sec}^{-1}$. The hydration constants of different temperatures were calculated from

TABLE I. TEMPERATURE DEPENDENCE OF THE OPTICAL DENSITY AND HYDRATION CONSTANT OF TRIMETHYLPYRUVIC ACID IN 1 N HYDROCHLORIC ACID AQUEOUS SOLUTIONS

Temp., °C	E at 310 mμ	$K_h=(E^*-E)/E$
13.0	0.467	0.814
19.5	0.530	0.598
25.0	0.580	0.460
31.5	0.630	0·344
37.0	0.670	0.264

Concn. of trimethylpyruvic acid: 0.0274 M.
$E^*=0.847$ is the value corresponding to the optical density when no hydration occurs, which was evaluated from E at 25°C with the corresponding K_h 0.460 as obtained above.

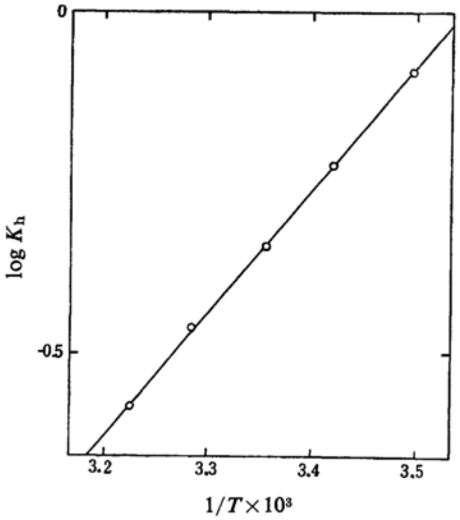
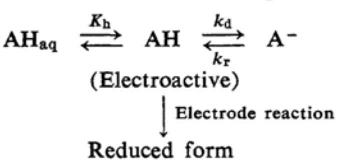


Fig. 6. Log plot of the hydration constant of trimethylpyruvic acid against reciprocal absolute temperature.

the corresponding optical densities versus the above obtained value at 25°C (Table I). The log plot of these constants against the reciprocal absolute temperatures gives a straight line, from whose slope the heat of hydration has been obtained to be −8.2 kcal. (Fig. 6).

The pH-dependence of the polarographic limiting current of trimethylpyruvic acid can be considered with the following scheme



$$\begin{tikzcd} AH_{aq} \arrow[r, shift left, "K_h"] & AH \arrow[l, shift left] \arrow[r, shift left, "k_d"] \arrow[d, "\text{Electrode reaction}"] & A^- \arrow[l, shift left, "k_r"] \\ & \text{Reduced form} & \end{tikzcd}$$

(Electroactive)

where k_d and k_r are the rate constants of dissociation and recombination of AH, respectively, and AH is only the electroactive form for the reduction at the dropping mercury electrode. A^-_{aq} and also the transformation between AH_{aq} and A^- via A^-_{aq} are neglected, since the equilibrium between A^- and A^-_{aq} has been known to be much shifted toward A^- from the above experimental results and consequently the concentration of A^-_{aq} in equilibrium is negligible in any pH.

Under the assumption that the rate of dehydration from AH_{aq} is so slow that it causes no kinetic current, while the recombination of A^- with proton causes the kinetic current, the equation for the limiting current of trimethylpyruvic acid as a function of hydrogen ion concentration can be derived as follows.

Writing the apparent dissociation constant K_a in the form

$$K_a=[A^-][H^+]/([AH]+[AH_{aq}]) \qquad (2)$$

the dissociation constant for free acid K_0 can be expressed as

$$K_0=k_d/k_r=[A^-][H^+]/[AH]=K_a(K_h+1) \qquad (3)$$

As the analytical concentration of the acid [a] is

$$[a]=[AH]+[AH_{aq}]+[A^-] \qquad (4)$$

to a first approximation, the limiting current can be written as being proportional to the concentration of AH at the electrode surface, which is supplied by diffusion from AH in bulk or by the recombination reaction of A^- with proton, involving the kinetic current, for which Koutecky's equation[4] can be valid. The limiting current is then prexessed as

$$i_l=i_d\frac{[AH]}{[a]}+i_d\frac{[A^-]}{[a]}\times\frac{0.87[H^+](k_rt/K_0)^{1/2}}{1+0.87[H^+](k_rt/K_0)^{1/2}} \qquad (5)$$

where i_d is the diffusion current corresponding to [a] and t is the drop time.

Substituting relations 1 to 4 into Eq. 5 and rearranging the resulting equation, the following equation for the limiting current as a function of $[H^+]$ can be obtained.

$$i_l=\frac{i_d[H^+]}{K_a+[H^+]}\left(\frac{1}{K_h+1}+\frac{0.87K_a[k_rt/K_a(K_h+1)]^{1/2}}{1+0.87[H^+][k_rt/K_a(K_h+1)]^{1/2}}\right) \qquad (6)$$

The use of this equation may be allowed for the limited case where the diffusion current due to AH in bulk is not appreciable as far as the recombination reaction of A^- with proton causes the kinetic current and when the recombination reaction is no longer the rate-determining, the limiting current is the diffusion current due to AH corresponding to the concentration $[A^-]+[AH]$.

In Fig. 7 the pH-dependence of the limiting current of trimethylpyruvic acid is presented. The theoretical curve was drawn according to

Eq. 6, substituting, as K_a, K_h and t, the above experimentally obtained values into the equation and choosing other values i.e. k_r and i_d so as to fit the curve to the experimental points. The agreement of the theoretical curve with the experimental results is good except for the strong acid range, where the deviation of the experimental results from the theoretical may be qualitatively interpreted by the acid catalyzed dehydration of AH_{aq}, whose contribution is not considered in the equation. The experimental conditions as ionic strength, viscosity etc. in this range, can not be the same because of increasing acid concentration and moreover the measurements of the limiting current may have considerable error because of the deformation of the wave; consequently, no further analysis was attempted.

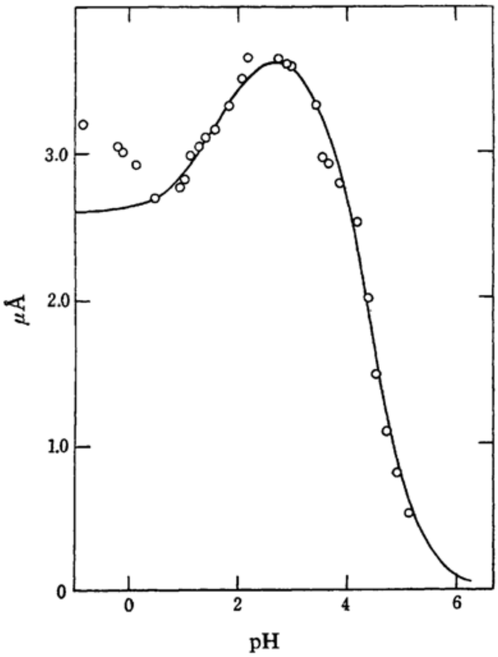


Fig. 7. pH-Dependence of the limiting current of trimethylpyruvic acid. Concn.: 5×10^{-4} M, Temp.: 25°C, ionic strength: 0.5. Solid line represents the theoretical curve according to Eq. 6. ○ experimental points.

The rate constants of dissociation and recombination of trimethylpyruvic acid obtained from the above treatment are $k_r=1.4\times10^7$ l. mol^{-1} sec^{-1} and $k_d=6.0\times10^5$ sec^{-1}, respectively. Although considerably larger values were obtained than those by Clair and Wiesner, k_r is still small, compared with those for other acids. Since k_r should not be very different of usual acids, for which $10^{10}\sim10^{11}$ l. mol^{-1} sec^{-1} have been reported by different authors with different methods⁽7-9⁾ of determination, indicating the diffusion velocity of hydronium ion, this relatively small value of k_r of trimethylpyruvic acid may partly attributed to the steric factor of the methyl groups against the recombination reaction. This kind of steric factor may be excluded in the case of pyruvic acid, for which larger values of k_r than that of trimethylpyruvic acid have been known⁽10⁾.

As for pyruvic acid, Becker and Strehlow⁽11⁾ have recently reported the revised values of k_r and k_d by using their equation, involving the contribution of hydration and the hydration constant estimated on the basis of their spectrophotometric measurements. However, as it was pointed out in the previous paper⁽1⁾, pyruvic acid is not free from the possibility of enolization. The spectrophotometric behavior of pyruvic acid differs from that of trimethylpyruvic acid, especially in alcoholic solution; the optical density of pyruvic acid in alcohol at the wave length of the maximum absorption is considerably lower than that of pyruvate in buffer solution (pH 6), while the reverse tendency is seen with trimethylpyruvic acid as in Fig. 2. Also the fact that pK_a value of pyruvic acid (2.50) is larger than that of trimethylpyruvic acid, is difficult to understand only with the difference in the hydration and the steric hindrance, when considered with the reverse order of pK values between acetic acid and trimethylacetic acid, though such an interpretation was attempted in the previous paper. As far as the question whether the enolization participates in the polarographic and spectrophotometric behavior of pyruvic acid, remains unsolved, the present results on trimethylpyruvic acid may be said to be more significant for the quantitative approach in the studies of this kind.

Summary

Polarographic and spectrophotometric behavior of trimethylpyruvic acid has been investigated.

The limiting current of trimethylpyruvic acid shows a minimum value in pH about 0.5. The absorption spectra show the absorption maximum at about 310 mμ in aqueous solutions, indicating the decrease of the optical density in strongly acid pH. These facts have been accounted for by the hydration of carbonyl group of undissociated acid.

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7) M. Eigen, *Z. physik. Chem. N. F.*, **1**, 176 (1954).

8) M. Eigen and J. Schoen, ibid., **3**, 126 (1955).

9) A. Weller, ibid., **3**, 238 (1955).

10) R. Brdicka, *Collection Czechoslov. Chem. Communs.*, **19**, 41 (1954).

11) M. Becker and H. Strehlow, *Z. Elektrochem.*, **64**, 129 (1960).

pK_a for apparent dissociation constant of trimethylpyruvic acid has been spectrophotometrically obtained to be 1.52 at 25°C.

Hydration constant of the undissociated acid ($K_h = [AH_{aq}]/[AH]$) has been kinetically obtained to be 0.46 at 25°C. The rate constant of dehydration of hydrated trimethylpyruvate in buffer solution of pH about 5 was 2.8×10^{-2} sec^{-1} at 25°C. From the temperature dependence of the hydration constant, the heat of hydration of unhydrated acid has been found to be -8.2 kcal.

A quantitative interpretation of the pH-dependence of the limiting current has been attempted by deriving the equation, involving the hydration and dissociation factors. The agreement of the theoretical curve with the experimental is good, except for the pH range below 0.5, where acid catalyzed dehydration of hydrated acid should be taken into consideration.

Rate constants of the dissociation and recombination of trimethylpyruvic acid have been found to be $k_d = 6.0 \times 10^5$ sec^{-1} and $k_r = 1.4 \times 10^7$ l. mol^{-1} sec^{-1}, respectively.

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