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Polarography of α-Keto Acids in Aqueous and Nonaqueous Solutions

Mitsuaki OHMORI* and Masanosuke TAKAGI

Laboratory of Biophysical Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai 591
**Osaka City Institute of Public Health and Environmental Sciences, Tennoji-ku, Osaka 543*

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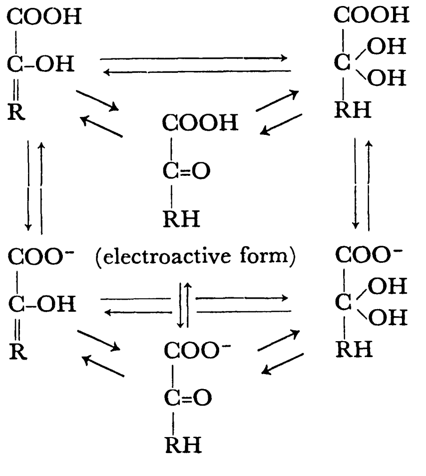
Phenylglyoxylic acid (I), phenylpyruvic acid (II), and pyruvic acid (III) were polarographically studied in buffer solutions and in DMF. Their reduction waves were reinvestigated in a wider pH range. Cyclic voltammetry showed that most electrode reactions of these acids in acidic pH are irreversible. pH-Dependence of $E_{1/2}$ and limiting current was studied. The observed decrease of the limiting currents of II and III in the strongly alkaline region was attributed to the dissociation of their corresponding enol forms. The *i-E* curves of these acids in DMF showed successive poorly-defined reduction waves, while those of the corresponding tetraethylammonium salts (TEA salts) were found to be much simpler in shape. A small oxidation wave was also observed with TEA salts of II and III, suggesting the presence of the enol form in DMF. The reduction products of I and its salt in DMF were studied by means of controlled potential electrolysis and NMR-spectroscopy. The electrode reaction mechanism of α-keto acids in nonaqueous solution is discussed, emphasizing the role of the free acid form as a proton-donor and the formation of a radical anion followed by a disproportionation reaction.

In polarographic investigations of biologically important α-keto acids, the first systematic study on the reduction of pyruvic acid was reported by Müller and Baumberger in 1939.[1] They found that the limiting currents of the two successive reduction waves change with pH, taking the form of an acid dissociation curve, the total current of the two waves being constant in the pH range studied. They attributed this phenomenon to the keto and enol forms of the acid in equilibrium in the buffer solutions.

It was pointed out by Brdička and Wiesner[2,3] that the two waves of pyruvic acid should be ascribed to the free acid and anion of the keto form and not to the enol form of the compound. The rate constants of the dissociation and recombination reactions of α-keto acids were determined by the polarographic method on the basis of the theory of kinetic current.[2-4] Because of this successful application of polarography, pyruvic acid has been considered to be representative of organic compounds whose rates of reaction in acid dissociation and recombination can thus be readily determined.

Later, participation of the hydrate form of α-keto acids in their polarographic behavior was suggested.[5-8] In 1961, Ono, Takagi, and Wasa[8] showed that the pH dependence of the limiting current of various α-keto acids could not be interpreted unless hydration and even enolization of the keto form when possible, in addition to the dissociation and recombination of the acid, were considered as factors in their equilibria in solution. The presented equilibria between an electroactive free keto form and various electroinactive ones should be considered in most cases even for the first reduction wave of α-keto acid.



We took up the polarography of α-keto acids from our interest in electro-reduction of biologically-important conjugated carbonyl compounds such as dehydro-L-ascorbic acid.[9-11] We chose three α-keto acids: phenylglyoxylic acid (I), with no possibility of enolization; phenylpyruvic acid (II), which definitely includes enol forms in aqueous equilibria; pyruvic acid (III), in which enolization is possible but occurs in aqueous solution in a restricted concentration range (<2%).[12]

Experimental

Chemicals. Phenylglyoxylic acid (I) was prepared according to the method previously described.[13] For phenylpyruvic acid (II), since the instability of the free acid even as crystal is undesirable for quantitative experiments, α-acetoamidocinnamic acid was prepared and stored.[14] It was converted into α-hydroxycinnamic acid (the enol form of II) before each experiment, by hydrolysis in a dilute hydrochloric acid solution.[15] The specimen recrystallized from distilled water was dissolved in 0.1 M (mol dm^{-3}) HCl containing 10% ethanol. This was used as the stock solution for polarography in aqueous solutions. Pyruvic acid (III) (analytical grade, Wako Pure Chemicals Co., Ltd., Osaka) was used without further purification.

Tetraethylammonium hydroxide for neutralizing α-keto acids to the corresponding tetraethylammonium salts was of reagent grade (10% aqueous solution, Wako Pure Chemicals Co.)

Tetraethylammonium perchlorate (TEAP) was prepared according to Fujinaga *et al.*[16]

Nonaqueous Solvents. *N,N*-Dimethylformamide (DMF) and acetonitrile (AN) were purified and dried as prescribed by Mann.[17]

As buffer solutions, McIlvaine buffer and other buffers were employed. The ionic strength of the aqueous electrolytic solutions was not always controlled to a definite value. For the sake of confirmation, it was adjusted to 0.5 by adding the requisite amount of KCl in certain experiments.

Apparatus. A Yanagimoto Polarograph Type PA-202 combined with a Yanagimoto *iR*-drop compensator was used for most polarographic measurements. An aqueous saturated calomel electrode (SCE) was used as a reference electrode. The characteristics of the capillaries used were $m=2.40$ mg/s, $t=3.75$ s in pH 6.12 McIlvaine buffer when the Hg level was 60 cm and -1.0 V was applied, and $m=1.16$ mg/s, $t=3.77$ s in DMF at -1.5 V and 50 cm mercury head. All the polarographic experiments were carried out at (25 ± 0.1)°C.

For controlled potential electrolysis, a Yanagimoto Controlled Potential Electrolyzer was employed. NMR Spectra of the electro-reduction products were recorded on a JEOL MH-60 Spectrometer.

For cyclic voltammetry, a Yanagimoto Polarovision PA-20 was combined with a function generator of NF Circuit Design Block Co., Ltd., Osaka, Model FG-121A. The working electrode was a hanging mercury drop of appropriate size.

Experimental Procedures. Polarographic measurements in aqueous solutions were carried out by conventional methods. However, when the α-keto acids were not stable in the given pH, the buffer solution in an electrolytic cell was previously deoxygenated by bubbling nitrogen gas. To this oxygen-free buffer solution, the required volume of the stock solution was added and the polarogram was taken immediately.

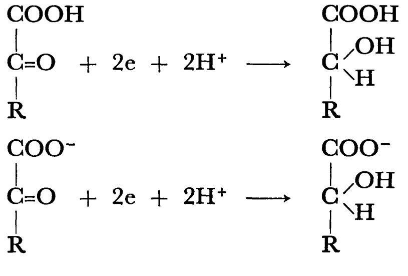
For nonaqueous solvents, a salt bridge to connect with the SCE was constructed of three gel layers, modifying the bridge devised by Takaoka.[18] The layers were 3% aqueous agar gel saturated with KCl, 3% agar gel containing 70% DMF, 30% H_2O, and 0.5 M TEAP, and 3% methylcellulose gel in DMF or in a DMF–AN mixture containing 0.5 M TEAP.

For the experiments with tetraethylammonium salts of α-keto acids, aqueous solutions of each α-keto acid were carefully neutralized to pH 7 by adding 10% aqueous solution of tetraethylammonium hydroxide. The neutralized solution was lyophyllized and stored in a refrigerator as a tetraethylammonium salt of the given α-keto acid.

Results and Discussion

Behavior of α-Keto Acids in Aqueous Buffer Solutions. In previous papers[8,19] the pH range was restricted to 0—8. Since additional data for enol forms of α-keto acids were expected in the alkaline range, this range was examined most carefully.

Change of Limiting Currents with pH. As shown in Figs. 1 and 2, each of the α-keto acids showed two reduction waves whose limiting currents change with pH.



$$\mathrm{R{-}C({=}O){-}COOH} + 2e + 2H^+ \longrightarrow \mathrm{R{-}CH(OH){-}COOH}$$

$$\mathrm{R{-}C({=}O){-}COO^-} + 2e + 2H^+ \longrightarrow \mathrm{R{-}CH(OH){-}COO^-}$$

We see that the first wave corresponds to the reduction of the free α-keto acid and the second to that of its anion form, involving the kinetic current due to the recombination of the anion form with hydrogen ion.[2–8)]

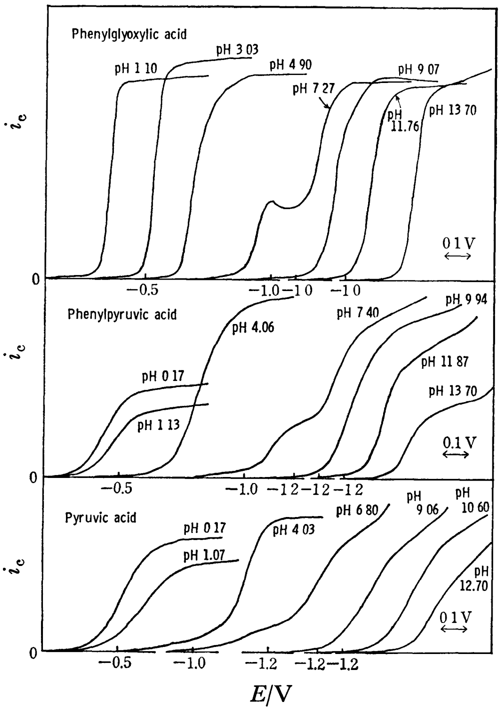


Fig. 1. Polarograms of α-keto acids in various pH.

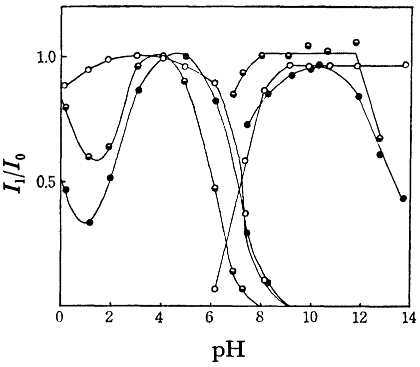
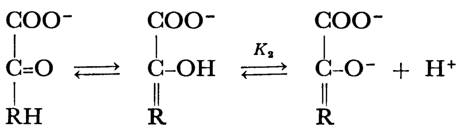


Fig. 2. pH Dependence of the reduction waves of α-keto acids.
Concn of keto acid 5×10^{-4}M, 25°C.
○: Phenylglyoxylic acid, ●: phenylpyruvic acid, ◒: pyruvic acid. I_0: the largest limiting current of each keto acid in acidic pH range was taken as I_0 for the corresponding acid.

In the reduction of I, with no possibility of enolization, the small decrease of the limiting current of the first wave with decreasing pH in the acidic range may be attributed to the effect of hydration of the free acid.[8,19)] The changes of the limiting current in the pH range above 4 can be interpreted by the kinetics of acid dissociation.[2–4)]

However, for II, in which enolization was observed by UV spectroscopy,[8)] a pronounced minimum in the limiting current-pH curve of the first wave near pH 1 was observed. A decrease of the limiting current of the second wave with pH could be seen in the pH range above 12. The decrease in the acidic range is due to the inactivation of the keto form by hydration and enolization, but that in the alkaline range may be explained by the inactivation of the anion by dissociation of the enol form and the increase of the enolate form with pH:



$$\begin{array}{c}COO^-\\ |\\ C{=}O\\ |\\ RH\end{array} \rightleftarrows \begin{array}{c}COO^-\\ |\\ C{-}OH\\ \|\\ R\end{array} \overset{K_2}{\rightleftarrows} \begin{array}{c}COO^-\\ |\\ C{-}O^-\\ \|\\ R\end{array} + H^+$$

For III, decrease of the limiting current similar to that of II could be observed in both acidic and alkaline regions. The apparent pK_2 value of II and III is estimated to be *ca.* 13, provided that no kinetic current due to acid recombination is involved in such a high pH region. It is interesting that this pK_2 value is in good accordance with pK_3 value obtained by Bamann and Sethi for oxalacetic acid.[20)]

Even in strongly alkaline pH, no decrease of the limiting current such as those of II and III was observed in I, in which enolization is not possible.

However, it is not clear whether the enolization of the free acid form would participate in the decrease of the limiting current of III in acidic region.

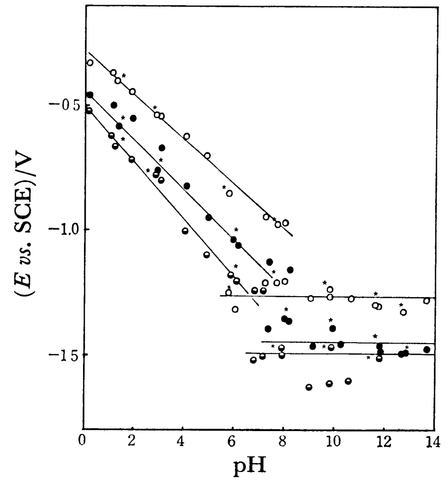
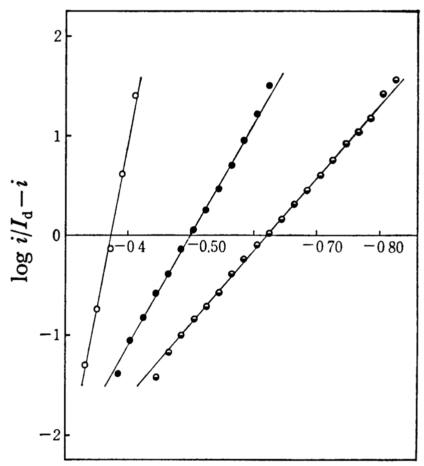
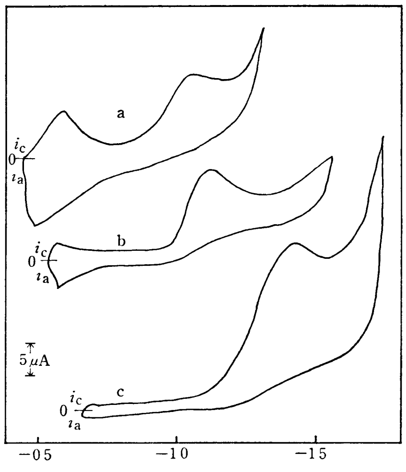


Fig. 3. Relation between the half-wave potentials of α-keto acids with pH.
The experimental conditions were the same as for Fig. 2.
* Ionic strength was adjusted to be 0.5.



(*E vs* SCE)/V

Fig. 4. Logarithmic analysis of the first wave of α-keto acids.
○: Phenylglyoxylic acid, ●: phenylpyruvic acid, ◒: pyruvic acid.

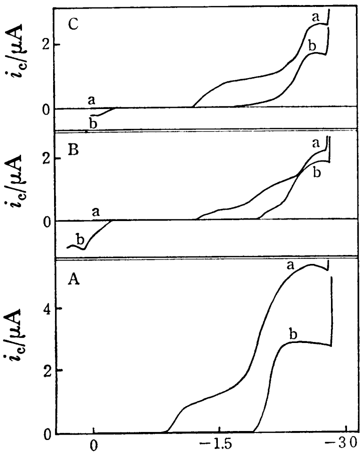


(*E vs.* SCE)/V

Fig. 5. Cyclic voltammetry of phenylglyoxylic acid in buffer solutions.
a: pH 1.13, b: pH 1.87, c: pH 4.95. Potential sweep: 2 V/s.

Relation between Half-wave Potentials of α-Keto Acids and pH. We see from Fig. 3 that each first wave of I, II, and III shows a linear relation of the $E_{1/2}$ with pH, the slopes being 84, 100, and 112 mV/pH, respectively, whereas the half-wave potentials of the second wave are almost unchanged with pH.

Reversibility of the Electrode Reaction. Since the presence of acid dissociation, enolization and hydration equilibria for three acids in the wide pH range makes analysis of the electrode reaction difficult, an acidic pH range was chosen in which the effects of acid recombination kinetics and dehydration kinetics, at least, can be minimized (pH near 1.1). In pH range below 1.1, increase of the limiting current with decreasing pH observed for II and III may be explained by the acid-catalyzed dehydration reaction of the hydrated free acid form.[8)] The conventional logarithmic analysis



(*E vs.* SCE)/V

Fig. 6. Polarograms of α-keto acids and their tetraethylammonium salts in DMF.
A (a) Phenylglyoxylic acid (b) TEA salt
B (a) Phenylpyruvic acid (b) TEA salt
C (a) Pyruvic acid (b) TEA salt
Concn of the depolarizer around 5×10^{-4}M.

of the current-potential curve was applied. Each acid exhibited a linear relation with a slope of 31, 89, and 140 mV for I, II, and III, respectively (Fig. 4), suggesting the largest reversibility of the electrode reaction for I and the smallest for III. However, the results of cyclic voltammetry indicate no ideally reversible character of the electrode reaction, even for I, as seen in Fig. 5.

Behavior of α-Keto Acid in Nonaqueous Solvents. Polarograms of the Free α-Keto Acids: Polarograms of each α-keto acid examined, given in Fig. 6, show two or three poorly-defined reduction waves in DMF containing 0.1 M TEAP (curve a). Almost the same of results were obtained in AN. Of the three α-keto acids, the total wave height of I greatly exceeds that of II and III. The phenomena may be partly due to the presence of intramolecular proton donors, probably those of carboxylic groups. In order to eliminate the proton-donating effect of the carboxylic group, the acids were neutralized with tetraethylammonium hydroxide having the same cation as that of the supporting electrolyte. The results obtained with these tetraethylammonium salts of α-keto acids show much simpler reduction waves (curve b, Fig. 6). The limiting currents indicate a certain intermedial number of transferred electrons between 1 and 2 as compared with the 2-electron transferred current in aqueous solution, considering effects of viscosity on the limiting current in nonaqueous solvents.[21]

A small oxidation wave was observed for II and III, when the polarogram was taken with tetraethylammonium salt. The wave, which is absent in I, may be attributed to the oxidation of the enol form, but no further discussion is possible on this.

In order to examine the mechanism of electrode reactions of α-keto acids in nonaqueous solvents, electro-reduction products were studied with free acid and tetraethylammonium salt by applying controlled potential electrolysis. In preliminary experiments carried out with the three keto acids, only I gave reproducible results. This might be due to the instability of the reduction products. The experiment carried out with I is given in the following.

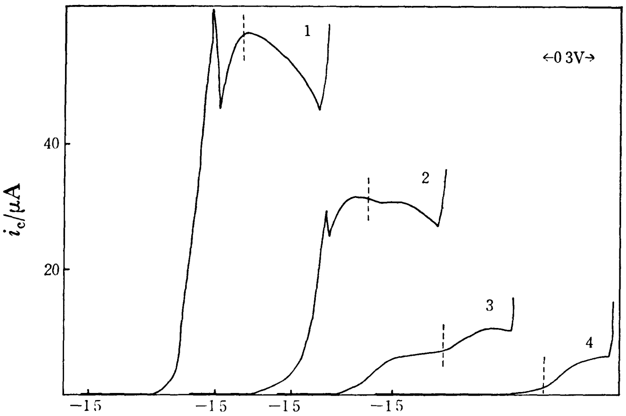


Fig. 7. Change of polarograms of phenylglyoxylic acid TEA salt by the controlled potential electrolysis in DMF.

Concn of the depolarizer *ca.* 1×10^{-2}M. Applied potential −2.4 V *vs.* SCE, 1: Before electrolysis, 2: 1 h, 3: 3 h, 4: 5 h.

Controlled potential electrolysis was carried out with the salt of I exhibiting a relatively simple current-potential curve. When started with a relatively high concentration of the depolarizer, the polarogram of the initial stage showed a pronounced maximum which had not been seen with dilute solutions of the same specimen (Fig. 7). The electrolysis was carried out at −2.4 V, using a mercury pool as the working electrode. After completion of the electrolysis at the applied potential, a small reduction wave appeared at a more negative potential, for which we have no explanation.

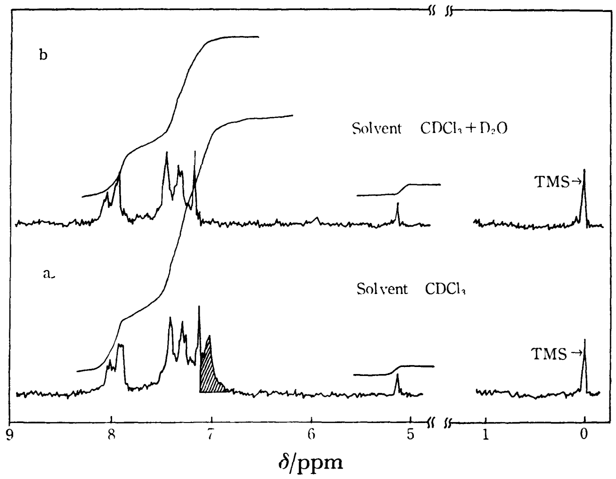


Fig. 8. NMR Spectra of the reduction products of phenylglyoxylic acid TEA salt in DMF at 60 MHz in $CDCl_3$.

The electrolyte solution after electrolysis was evaporated under reduced pressure, and the residue was acidified by addition of excess 0.5 M H_2SO_4 solution. The acid solution was then extracted with ethyl ether. The residue from the ether extract after evaporation was used for NMR measurement. The results are given in Fig. 8. In comparison with the spectrum of DL-mandelic acid,[22] a small signal at 5.13 ppm (a) can be attributed to the proton bound directly to the α-carbon of mandelic acid, while the proton of the alcoholic OH at the α-position seems to exhibit a signal near 7 ppm. It is not, however, a well-defined singlet, but a somewhat broad signal indicating the overlap with that of the carboxylic proton. The addition of D_2O to the electro-reduction product (Fig. 8-b) causes the disappearance of the hatched portion of Fig. 8-a. The change in the integrated absorption intensity caused by the addition of D_2O seems to correspond roughly to two protons, *i.e.*, carboxylic and hydroxyl, if we assume that the unchanged part is the total contribution of 5 phenyl protons. Consequently, the small signal at 5.13 ppm may indicate the formation of mandelic acid after the electrolysis, the yield of which may be 1/4 mol per 1 mol of the starting I. The remainder of the electrolytic product can therefore be attributed to the formation of the dimer.

The results suggest that the electrolytic product of I under these conditions is a mixture of DL-mandelic acid

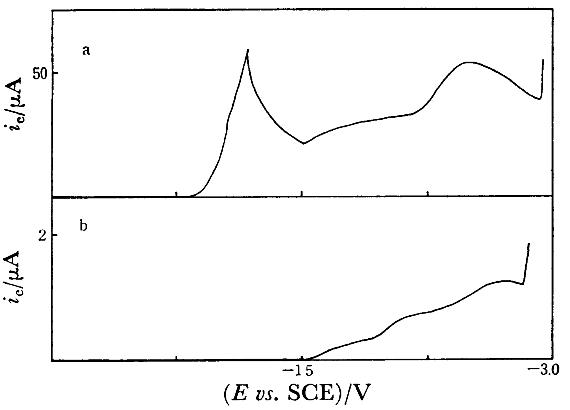


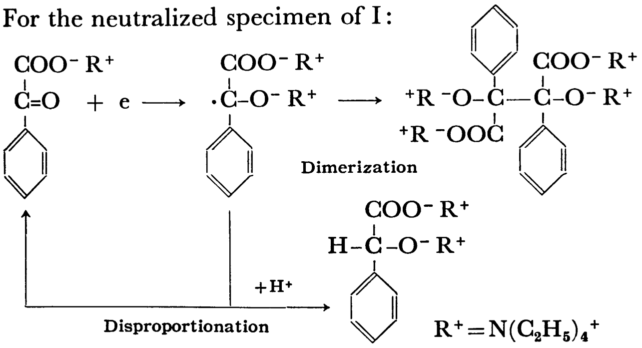
Fig. 9. Change of polarograms of phenylglyoxylic acid by controlled potential electrolysis in DMF.
Applied potential −1.2 V *vs.* SCE, a: before electrolysis (Concn of the depolarizer *ca.* 1.7×10^{-2}M), b: after the completion of the electrolysis (Concn of the depolarizer 6.6×10^{-4}M).

and the dimer due to the binding of two radicals from I

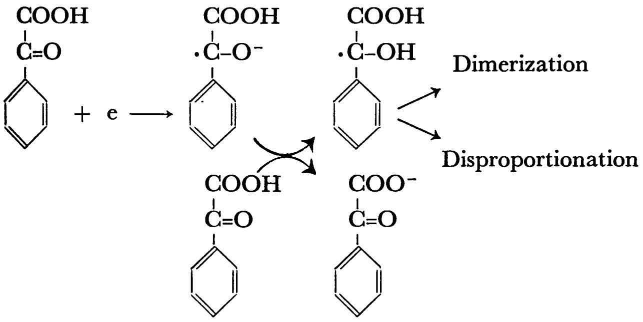
Electrolytic reduction was applied to the free acid form of I. The behavior of I in free acid form in relatively high concentrations differs a great deal from that in dilute concentrations (Fig. 9-a). −1.2 V was chosen for the applied potential. The polarogram after electrolysis still shows poorly-defined reduction waves (Fig. 9-b). The electro-reduction product was extracted in the same manner as in the treatment of the neutralized I. The products were confirmed to be a mixture of DL-mandelic acid and the dimer but in a different ratio from that in the above case, more mandelic acid formation being detected with the free acid form than with the neutralized specimen of I.

Proposed Scheme of the Electrode Reaction. From the results, the following schemes are proposed for the principal process of the electrolytic reduction of I in DMF.

For the neutralized specimen of I:



For the free acid:



For the salt of I, one-electron reduction can produce the radical, most of which may be converted into the dimer. The remainder then exhibits a disproportionation reaction yielding the salts of I and mandelic acid, as is sometimes seen in the polarography of carbonyl groups in nonaqueous solvents,[23] provided that protons can be supplied from either DMF or TEAP.

The possibility of the involvement of two-electron reduction to form dianions is not completely excluded. However, since no well-defined stepwise reduction waves were observed, it is preferable to represent the simplified schemes using disproportionation only in the production of mandelic acid.

Electrolysis of the free acid would be more complicated than that of the salt. The effect of proton-donors should be considered. The radical anion formed in the first step of one-electron reduction might react with an unreacted molecule, and the protonated radical might perform the same kind of dimerization and disproportionation as in the case of the salt. The anion produced by the reaction of a radical with an unreacted free acid molecule would be reduced at a more negative potential than in the case of the original free acid.

Besides the electro-reduction of the carbonyl group, the discharge of protons liberated from carboxylic groups might be considered in the polarograms of the free acid. Since various chemical species having carboxylic groups can be produced as a result of the first electrode reaction, these acids with slightly different p*K* values may cause successive and poorly-defined, more negative waves.[24]

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