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Polarographic Studies on Ninhydrin in DMF and Dehydration from *gem*-Diol Structure

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The d.c.-polarogram of ninhydrin (I) in DMF containing 0.1 M TEAP shows three cathodic waves whose $E_{1/2}$ are −0.38, −0.65, and −1.23 V (*vs.* SCE), respectively. The first wave with a large maximum is attributed to one-electron reduction of 1,2,3-indantrione (II), the hydrated form of I, the second to one-electron reduction of I, and the third to one-electron reduction of the preceding electrochemical product from both I and II. CV Curves of I show three cathodic peaks, pc_1, pc_2, and pc_3, and two anodic peaks, pa_1, and pa_2, showing reversible character of pc_1 and pc_3 but not of pc_2. Different equilibria seem to take place between I and II in the bulk of the solution and at electrode surface. Hydration of II and dehydration of I in DMF were observed at 550 nm spectroscopically. A scheme is proposed for the electrolytic reduction of I in DMF.

The electrochemical behavior of carbonyl compounds is of interest as regards the electron transfer in biological systems. Aldehydes,[1,2] ketones,[3] α-keto acids,[4,5] di- and tricarbonyl compounds[6,7] and many other carbonyl compounds have been studied. However, ambiguities with origins mostly attributed to the hydration of carbonyl groups in aqueous systems remain and studies in nonaqueous systems are expected to clarify them.

Ninhydrin (I) is available as a stable monohydrated form of the corresponding tricarbonyl compound 1,2,3-indantrione (II). Its polarographic behavior in aqueous[8–12] and nonaqueous[13] solutions was reported. The effect of hydration and dehydration in nonaqueous solvents should be examined more in detail for the clarification of its electrochemical characteristics. We are interested in this study in relation to its partial structural similarity to dehydro-L-ascorbic acid[14,15] of biological importance.

Experimental

Chemicals. Ninhydrin (I) (analytical grade, Ishizu Pharmaceutical Co., Ltd., Osaka) was used without further purification. 1,2,3-Indantrione (II) was prepared as a purple powder by heating I under reduced pressure at 125—130 °C.[13]

Tetraethylammonium perchlorate (TEAP) (prepared for polarography, Nakarai Chemical Co., Ltd., Kyoto) was used after cautious drying under reduced pressure with appropriate heating.

Nonaqueous Solvent. *N*,*N*-Dimethylformamide (DMF) (analytical grade, Wako Pure Chemical Co., Ltd., Osaka) was dried as described by Mann.[16] For polarographic measurements, DMF containing 0.1 M (1 M=1 mol dm^{-3}) TEAP was deoxygenated by bubbling nitrogen gas dried with silica gel and phosphorus pentaoxide.

Apparatus. Polarographic measurements were carried out with a Yanagimoto Voltammetric Analyzer Type P-1000, characteristics of the capillary being $m=1.52$ mg/s, $t=4.00$ s^{-1} in DMF containing 0.1 M TEAP, when Hg level was 51.5 cm and −1.0 V was applied. Cyclic voltammetry was carried out with the Analyzer Type P-1000 with a function generator (NF Circuit Design Block Co., Ltd., Osaka). The working electrode was a hanging mercury electrode or a Pt electrode, a saturated calomel electrode (SCE), with a salt bridge[5] constructed of three gel layers, and an Ag/AgCl electrode being used as reference electrodes.

Results and Discussion

Polarographic Behavior of Ninhydrin (I). Polarograms of I were examined in dried and deoxygenated DMF, containing 0.1 M TEAP. A polarogram recorded immediately after direct dissolution of a necessary amount of crystalline I in the electrolyte solution (Fig. 1-a) showed an anomalous first wave with a large maximum and two other successive waves. The height of the first wave decreased with time, polarogram (a) changing into d *via* b and c. At the state of d, the first wave became stable, the shape of the wave not being anomalous. The half-wave potentials ($E_{1/2}$) of the three reduction waves were −0.38, −0.65, and −1.23V, respectively. At a very early period, the sum of the first and the second wave heights was a little higher than the height of the third wave, but decreased gradually with time and finally became equal to it. The slopes of logarithmic analysis for the three waves $dE/d\log[i_d/(i_d-i)]$ are 61, 38, and 85 mV, respectively.

When a very small amount of water (below 0.25%) was added to the electrolyte solution after recording

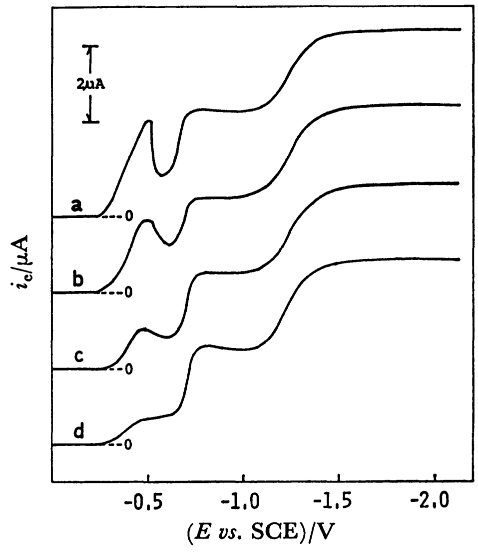


Fig. 1. Polarograms of ninhydrin.
Concn: 1×10^{-3} M, temp: 22 °C, a: Immediately after dissolving ninhydrin, b: 30 min, c: 60 min, d: 90 min.

(Fig. 1-a), a similar polarogram to d was obtained much more quickly than without addition of water.

The decrease in the first wave height seems to be related to the water content in the system. However, it is difficult to attribute the maximum observed in a to the water content. An equilibrium between the dehydrated form (II) and the hydrated form (I) in DMF should be considered, not only in the bulk of the solution but also at the electrode surface.

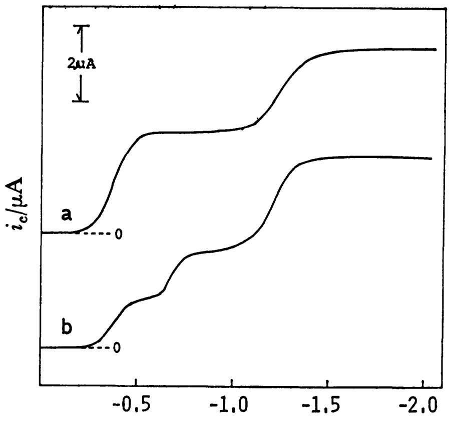
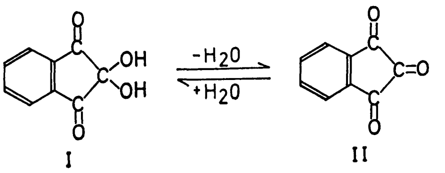


Fig. 2. Polarograms of 1,2,3-indantrione.
Concn: 1×10^{-3} M, temp: 19 °C, H_2O: a-0%, b-0.11%.

A polarogram of 1,2,3-indantrione (II), a dehydrated form of I, was also examined (Fig. 2-a). It showed two successive waves ($E_{1/2}=-0.38$ and -1.23 V). Kalinowski and Lasia reported that they observed two successive waves of II in absolute DMF containing 0.2 M $NaNO_3$ ($E_{1/2}=-0.39$ and -0.86 V vs. SCE),[13] each of which was ascribed by the authors to one-electron reduction.

When a small amount of water (0.11%) was added to the electrolyte solution after recording the polarogram (Fig. 2-a), a new wave ($E_{1/2}=-0.67$ V) appeared between the two waves at the expense of the first wave, *i.e.* in total, three waves were observed (Fig. 2-b). The first wave showed no maximum during the course of current decrease, the three waves corresponding to the three in Fig. 1-d, respectively. The newly appearing wave in Fig. 2-b may thus be attributed to I which is the hydration product of II in Eq. 1.



(1)

I II

It is suggested that the first wave in Fig. 1 is attributed to II, since it has the same $E_{1/2}$ value as that of the first wave in Fig. 2. Polarogram a in Fig. 3 was observed when the necessary amount of I was added in dried DMF containing TEAP which had been exposed to atmosphere in crystalline form, the exposure time not exceeding 1 h. Polarograms b and c showed a very small but more positive wave with time (Fig. 3).

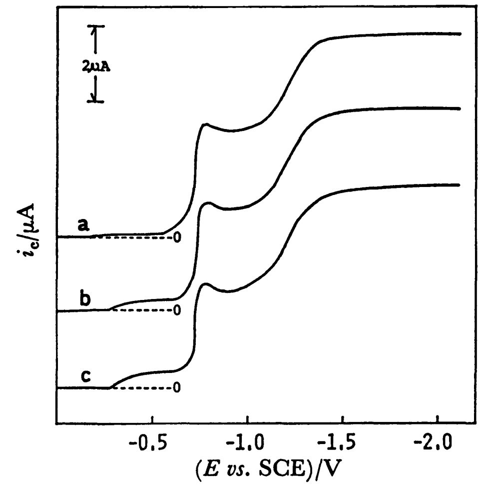


Fig. 3. Polarograms of ninhydrin taken with TEAP exposed to the atmosphere for about 1 h.
Concn: 1.1×10^{-3} M, temp: 22 °C, a: immediately after dissolving ninhydrin, b: 20 min, c: 60 min.

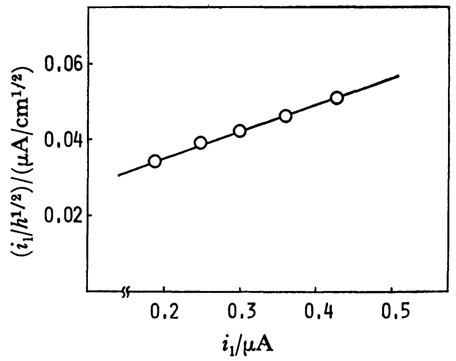


Fig. 4. Relation between i_l and $i_l/h^{1/2}$.
Concn: 1.1×10^{-3} M, h: 30—70 cm.

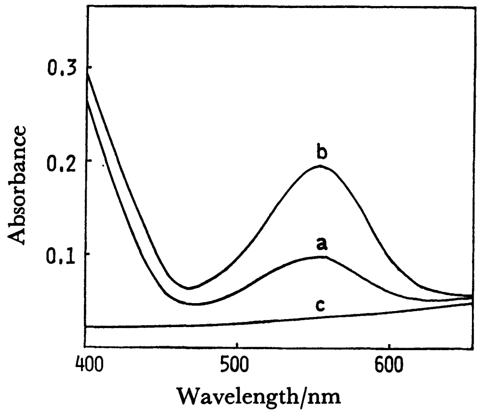


Fig. 5. Absorption spectra of ninhydrin and 1,2,3-indantrione in DMF.
a: Ninhydrin 1×10^{-2} M, b: 1,2,3-indantrione 1×10^{-2} M, c: DMF only.
Spectra were taken immediately after dissolving the specimen.

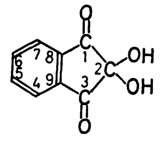
The results can be interpreted as follows. The chemically bound H_2O in I, *i.e.* in *gem*-diol structure, can be slowly removed even in an electrolyte solution dried insufficiently; equilibrium between I and II in

DMF can be attained with time.

However, as shown in Fig. 4, the plot of i_l of the first wave in Fig. 1-d against $i_l/h^{1/2}$ (i_l: limiting current, h: the level of the Hg reservoir) exhibited no kinetic character, suggesting a diffusion accompanied with adsorption of the electroactive chemical species for the corresponding electrode reaction.[17] It is difficult to demonstrate the direct participation of the dehydration reaction at the electrode.

On the other hand, the absorption spectra taken with I and II (Fig. 5), qualitatively supported an equilibrium between the two species in DMF. A relatively small absorption peak at 550 nm was obtained with I and a larger one with II. Prevention of the intrusion of atmospheric moisture into DMF medium was not satisfactory as compared with polarographic conditions, and the observed purple color due to the absorption at 550 nm faded with time for both I and II.

TABLE 1. ^{13}C-NMR CHEMICAL SHIFTS FOR NINHYDRIN IN DMF-d_7

Compound	Carbon	δ ^{13}C[ppm]
	1,3	197.6
	2	139.5
	8,9	137.7
	4,7	124.4
	5,6	88.4

(Reference: TMS)

The predominance of the hydrated form (I) in DMF was also observed by ^{13}C-NMR spectroscopy (Table 1), where no signal due to C-2 carbonyl group of II was detected, when each signal was assigned to the possible structure.

Let us discuss the first wave of reduction current with a large maximum in the polarogram (Fig. 1;. It is evidently due to the reduction of dehydrated form (II) at the electrode. If the current is due to species II in equilibrium, it would be difficult to interpret the presence of the maximum.

We might assume a different equilibrium between I and II, accompanied by adsorption at the electrode or a relatively fast dehydration from I to II, the equilibrium or rate of which may be largely dependent on the applied potential in a limited range. It is interesting to consider this characteristic phenomenon of ninhydrin in nonaqueous solution, in comparison with the observation made by Holleck and Lehman[10] that ninhydrin in aqueous buffer solutions also shows the reduction wave due to II, whose shape is very similar to the present one in DMF, though intensity and potential, *etc.* largely differ from each other.

The electrocapillary curve of the present system was also studied, an abnormal change of drop time being observed with applied potential corresponding to the maximum of curve a in Fig. 1. However, the drop time gave fairly scattered values for repeated measurements at the potential. This may suggest possible adsorption of I or II at the electrode.

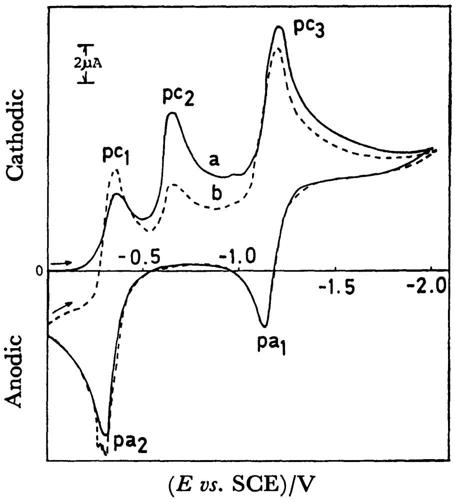


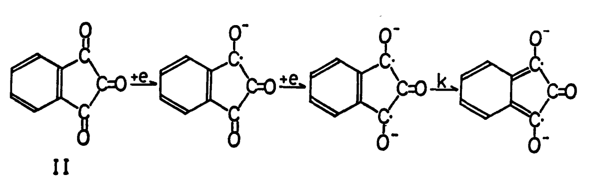
Fig. 6. Cyclic voltammograms of ninhydrin in DMF. Concn: 2.1×10^{-3} M, temp: 26 °C, sweep: 50 mV/s, electrode: HME, a: single sweep, b: multiple sweep.

Cyclic Voltammetry of Ninhydrin. Cyclic voltammetry of I was studied with use of the hanging mercury electrode (HME). The cyclic voltammogram (CV) is shown in Fig. 6, curve (a) being recorded by a single sweep and curve (b) by a multiple sweep. In both cases three cathodic peaks (pc_1, pc_2, and pc_3), corresponding to the polarographic three waves, respectively, and two anodic peaks (pa_1 and pa_2) corresponding to pc_3 and pc_1, respectively, were observed. Peak potential differences, $|E_{pc1}-E_{pa2}|$ and $|E_{pc3}-E_{pa1}|$, were found to be 60 mV for both cases, suggesting one-electron reversible redox system.[18]

In b, the peak current of pc_2 was smaller, while that of pc_1 became larger as compared with those in a. Irregularity of pa_2 in b was observed, suggesting an abrupt change in surface conditions.

A similar CV was observed with use of the Pt electrode, $|E_{pc3}-E_{pa1}|$ being approximately 70 mV. These cyclic voltammograms are similar to those of II observed by Kalinowski and Lasia[13] except for peak height of pc_2.

Mechanism of the Electrode Reaction. From the results of d.c.-polarography, CV and the proposed mechanism[13] of DME reaction of II, the electrode reaction mechanism of I in DMF can be discussed. Kalinowski and Lasia simplified the problem of ninhydrin (I) only in its dehydrated form (II), as proposed in Eq. 2.[13] However, the equilibrium between I and II in the bulk of the solution and also at the electrode surface should be taken into consideration. The reversibility of the electrode reaction for three steps of reduction is more clearly visualized by CV than by d.c.-polarography. The first and third waves in d.c.-polarography can be described each as reversible one-electron transfer on the basis of logarithmic analysis

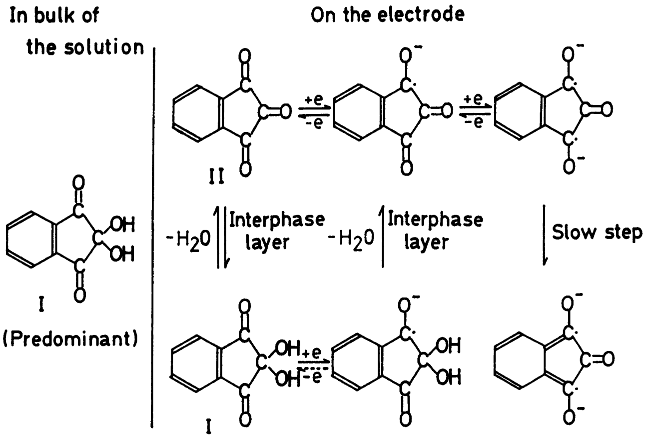


II

(2)

and the peak potential differences $|E_{pc_1}-E_{pa_2}|$ and $|E_{pc_3}-E_{pa_1}|$. The second wave is typically irreversible, since peak pc_2 does not exhibit its corresponding anodic peak.

The following scheme is proposed for the principal process of electrolytic reduction of ninhydrin in DMF.



Scheme 1.

The hydrated form can be reduced at the electrode as the second wave, but the reduction product easily liberates H_2O and is converted into the dehydrated form identical with the direct reduction product from II. In nonaqueous solvents the dehydration is understood as an irreversible process.

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