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Further Polarographic Studies on the Condensation Products of Dehydro-L-ascorbic Acid with *o*-Phenylenediamine in Acetate Buffer, pH 3.6

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Three polarographic reduction waves, which appear when dehydro-L-ascorbic acid (DAA) is treated with *o*-phenylenediamine (OPD) in acetate buffer, pH 3.6, were reinvestigated by comparing with those of the synthesized products from DAA and OPD. A reaction scheme of DAA with OPD is presented and the characteristics of the reactions are discussed.

As previously known, dehydro-L-ascorbic acid (DAA), the oxidized form of L-ascorbic acid (AA), reacts with *o*-phenylenediamine (OPD) in acidic buffers to give three cathodic DC-waves ($E_{1/2}$: −0.240, −0.412, and −0.634 V *vs.* SCE, at pH 3.6), and this polarographic behavior differs appreciably from that of the other dehydro-reductones with OPD.[1] The third wave ($E_{1/2}$: −0.634 V), observed at an early stage of the measurement, is characteristic of DAA and can be used to detect both AA and DAA.[1,2] When the amount of OPD exceeds that of DAA, the ratio of the three wave heights changes with time, the sum of the three decreasing slightly.

With regard to the condensation products of DAA with OPD, many works have been reported.[3-12] Repeated discussions on the formulae of the products from 1 mole of DAA with 1 or 2 mol of OPD have not successfully assigned them to the three cathodic DC-waves.[1,13]

In the present work, each compound, synthesized according to the literature,[4,5,8,12] is studied polarographically at pH 3.6 and its polarographic behavior is compared with that of the three waves obtained when DAA is treated with OPD in the same buffer. In addition, the reaction mechanism of DAA with OPD is discussed, considering the bicyclic DAA structure recently suggested.[14-16]

Experimental

Chemicals. *o*-Phenylenediamine (reagent grade, Katayama Chemical Co., Osaka) was recrystallized before use. L-Ascorbic acid, acetone, methanol, and the chemicals used were of reagent grade. A pH 3.6 acetate buffer [0.2 M (mol dm^{-3})] was chosen. Norit "Extra" activated charcoal and O_2 gas of high purity were also employed.

Preparation of DAA. DAA-methanol solution was first prepared as before.[17] The evaporation of methanol under reduced pressure gave a viscous DAA-methanol adduct, which was directly diluted to a desired concentration of DAA with buffer solution of pH 3.6, for electrolyte solution. For solid preparation, DAA was prepared as previously described.[18]

Synthesis of the Condensation Products of DAA with OPD and Related Compounds. The condensation product of 1 mol DAA with 1 mol OPD (**A**)[5,12] was prepared according to Ogawa[5] or El Ashry *et al.*[12] and that of 1 mol DAA with 2 mol OPD (**B**)[4,5,8] according to Hasselquist.[4] The product **B** was refluxed in absolute methanol to give yellow crystals (**B′**).[4,8] A hydrolysis product from **B** in dilute HCl (**C**) was prepared according to Hasselquist.[4] The product **C** was also obtained from **B′**.

Apparatus. Polarographic measurements were carried out with a Yanagimoto Polarograph Type P-8, the characteristics of the capillary being m = 1.86 mg/s, t = 4.50 s^{-1} when the Hg level was 72.5 cm, and −0.6 V was applied. All the polarographic experiments were carried out at 25 ± 0.2 °C. ^{13}C-NMR Spectra were recorded on a JEOL FX-100 spectrometer.

*Preparation of Electrolyte Solutions of **B** and **B′**.* Neither **B** nor **B′** dissolved sufficiently in pH 3.6 acetate buffer, but both were freely soluble in *N*,*N*-dimethylformamide (DMF). For a stock solution, 0.1 M **B** or **B′** DMF solution was prepared. The solution was diluted 100 times with the acetate buffer before use, and the diluted solution was directly applied to the polarographic measurements.

Results and Discussion

Polarograms of the Condensation Products of DAA with OPD. The DAA concentration was kept constant at approximately 1×10^{-3} M, and the OPD concentration was changed. The polarograms shown in Fig. 1 were taken 1 h after mixing OPD with DAA in the buffer solution. As shown in Fig. 1(a), when the OPD concentration was below the DAA concentration, two reduction waves were observed, the half-wave potentials being −0.240 and −0.634 V (*vs.* SCE). The wave with more negative $E_{1/2}$ was much higher in

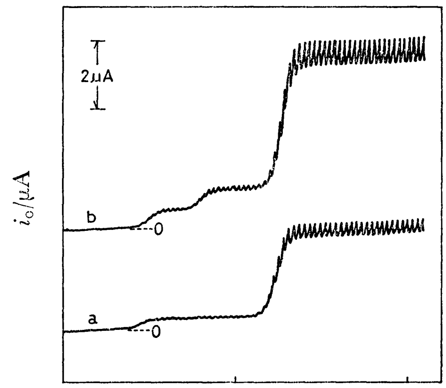


Fig. 1. Polarograms of the condensation products of DAA with OPD in acetate buffer pH 3.6.
Concn of DAA: *ca.* 1×10^{-3} M, concn of OPD: (a) 5×10^{-4} M, (b) 5×10^{-3} M, temp: 25 °C. Polarograms were taken 1 h after mixing OPD with DAA in the buffer solution.

wave height than the other. The relationships between the condensation time and the wave heights of the two reduction waves are shown in Fig. 2(a). The wave heights did not change after they reached equilibrium. When the OPD concentration was higher than the DAA concentration, another reduction wave appeared between the two waves (Fig. 1(b)). The half-wave potential was −0.412 V (*vs.* SCE). The three wave heights changed in a complex way with time as shown in Fig. 2(b). The third wave height decreased, while the first and second wave heights increased gradually. The total wave height of the three waves gradually decreased, though not large, as seen in Fig. 2(b).

Cyclic Voltammograms of the Condensation Products of DAA with OPD. Cyclic voltammograms (CV) were recorded under the same experimental conditions as for the DC-polarograms. When the OPD concentration was below that of DAA, two cathodic peaks (P_{c1} and P_{c3}) corresponding to the first and the third DC-waves, respectively, and one anodic peak (P_{a1}) corresponding to the P_{c1} product, were observed, as shown in Fig. 3(a). When the OPD concentration was higher than that of DAA, three cathodic peaks (P_{c1}, P_{c2}, and P_{c3}) corresponding to the first, the second and the third DC-waves, and two anodic peaks (P_{a1} and P_{a2}) corresponding to the P_{c1} and P_{c2} products, were observed as shown in Fig. 3(b). These results indicate that the first and the second waves are reversible but the third wave is irreversible.

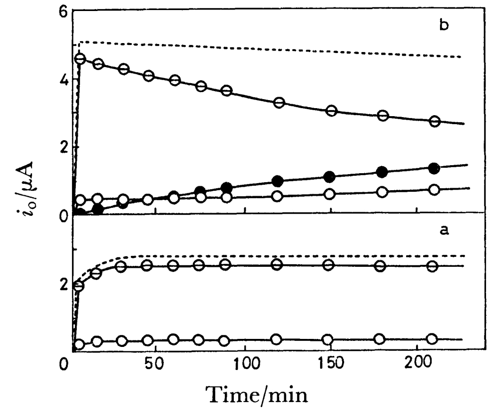


Fig. 2. Relationships between the condensation time and the wave height.
Concn of DAA: *ca.* 1×10^{-3} M, concn of OPD: (a) 5×10^{-4} M, (b) 5×10^{-3} M, temp: 25 °C, pH 3.6. ○: 1st wave, ●: 2nd wave, ⊖: 3rd wave, ---: total wave height.

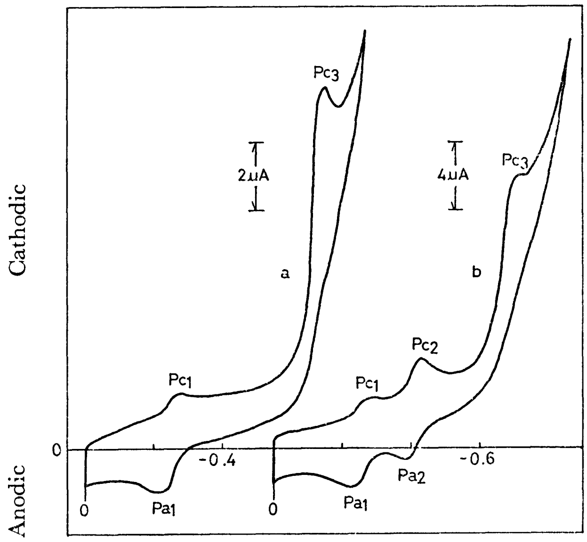


Fig. 3. CV curves of the condensation products of DAA with OPD in acetate buffer pH 3.6.
Concn of DAA: *ca.* 1×10^{-3} M, concn of OPD: (a) 5×10^{-4} M, (b) 5×10^{-3} M, temp: 25 °C, sweep rate: 50 mV/s, electrode: hanging mercury electrode (HME).

Polarograms of the Synthesized Products from DAA with OPD and Related Compounds. The polarogram of **A** (Fig. 4) was identical with that in Fig. 1(a), since the two reduction waves corresponded to the first and third waves. We attributed the polarogram shown in Fig. 1(a) to the equimolar condensation product of DAA with OPD.

For the polarograms of **B**, as shown in Fig. 5, two reduction waves were observed, whose wave heights changed with time. The two waves corresponded to the first and the second waves. The polarograms of

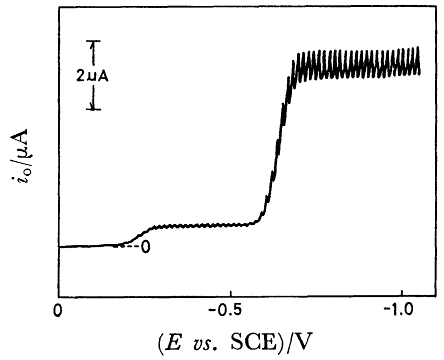


Fig. 4. Polarogram of **A**.
Concn of **A**: 2.64 mg/10 ml of buffer solution, temp: 25 °C, pH 3.6.

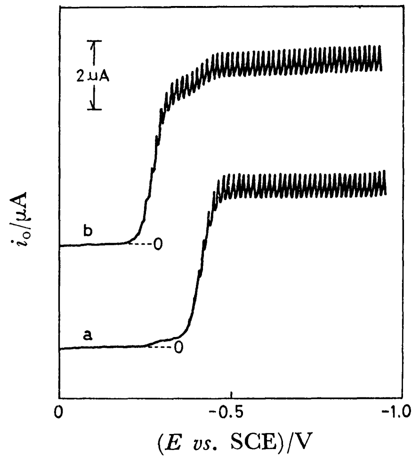


Fig. 5. Polarograms of **B**.
Concn of **B**: 3.54 mg/10 ml of buffer solution, temp: 25 °C, a: immediately after dissolving **B**, b: one day after.

B′ were similar to those of **B** shown in Fig. 5. It was also found by a separate experiment that **B**′ changed to **B** quantitatively when refluxed with a mixture of methanol and water (4:1). The electronic spectra showed that **B** is favored whenever water is added.

The polarogram of **C** is shown in Fig. 6. Only one reduction wave, corresponding to the first wave, was observed.

The polarograms of the further reaction products of **A** with OPD were recorded after the direct dissolution of excess OPD into the buffer solution containing **A**. Three reduction waves appeared, and the polarogram which was recorded 1 h after the addition of OPD agreed with that shown in Fig. 1(b). The time courses of the reaction of **A** with OPD observed by the change of heights of three waves are shown in Fig. 7. The polarographic behavior of the reaction products of **A** with OPD in the buffer was similar to that previously observed in Fig. 2(b). It is probable that, when DAA is treated with excess OPD, the condensation product, corresponding to **A**, is first produced and then it reacts further with another 1 mol of OPD.

By comparing this behavior with that of **B** or **B**′, and considering that the total wave height of the three waves began to decrease gradually as soon as the second wave appeared, we came to the conclusion that the appearance of the second wave and the increase in the first wave were caused by the formation of the condensation product of 1 mol DAA with 2 mol OPD as either **B** or **B**′.

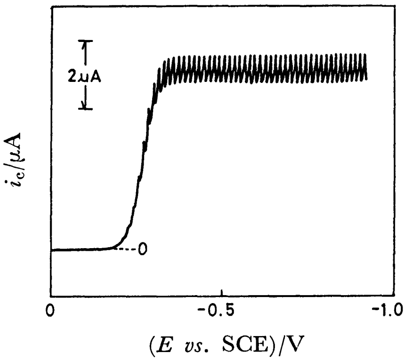


Fig. 6. Polarogram of **C**.
Concn of **C**: 2.46 mg/10 ml of buffer solution, temp: 25 °C, pH 3.6.

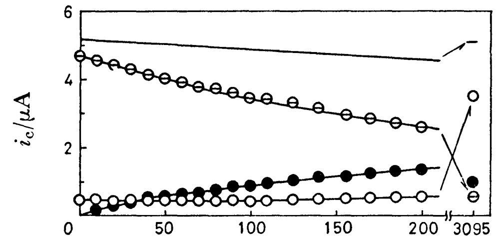
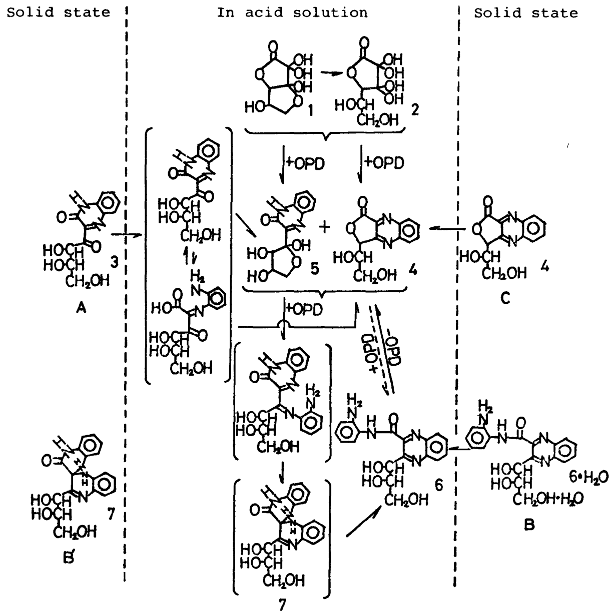


Fig. 7. Time courses of the reaction of **A** with OPD by the change of heights of the three waves.
Concn of **A**: 2.64 mg/10 ml of buffer solution, concn of OPD: 4×10^{-3} M, temp: 25 °C, pH 3.6. ○: 1st wave, ●: 2nd wave, ⊖: 3rd wave, ——: total wave height.

The decrease of the total wave height with time may be explained by a decrease of the diffusion constant of the depolarizer caused by the addition of one more OPD to **A**. However, the total wave height reaches its maximum value at the final equilibrated state, when the chemical species corresponding to **B** or **B**′ is scarce. That is, when DAA reacts with OPD, the formation of **B** or **B**′ is transitory, if the concentration of OPD is not much larger than that of the DAA; say maximum 3 times of DAA. When OPD is more than 10 times of DAA, the final state shows the presence of **C** and **B** or **B**′ only. The equilibrium between **C** and **B** (**B**′) is approximately 3:1, coinciding with the observation by Erlbach and Ohle for iso-DAA with OPD.[3]

Reaction Mechanism of DAA with OPD. Recently, it has been confirmed that the structure of DAA is predominantly a bicyclic form, shown as **1** at the top of Scheme 1, in solution, and the bicyclic form of DAA changes to the compound **2** with time.[14,16] We measured ^{13}C-NMR spectra of DAA in the mixture of D_2O and hydrochloric acid (about pH 3.0) to identify the structure of DAA in acidic solution. Six signals at 174.2, 92.0, 106.3, 73.6, 88.3, and 76.8 ppm were found. They were in accord with the chemical shifts in D_2O solution (C-1: 174.2, C-2: 92.0, C-3: 106.3, C-4: 73.5, C-5: 88.2, and C-6: 76.8 ppm), which were assigned to **1** by Hvoslef and Pedersen.[14] We thus conclude that DAA is predominantly in the form of structure **1** in acidic solution.

The structure of **A** has recently been correctly described as **3** by El Ashry *et al.*[11] The anomeric structure of **C** was first reported to be that of **4** by Erlbach and Ohle[3] in a reaction system of iso-DAA with OPD, and then **4** was reported by Ogawa,[5] Hasselquist,[4] and Dahn and Moll[8] in a reaction system of DAA with OPD.



Scheme 1. Reaction mechanism of DAA with OPD in acetate buffer pH 3.6.

As previously mentioned, DAA reacts equimolarly with OPD to exhibit two reduction waves. The first wave seems to be caused by **C**, which is formed by the reaction of **2** with OPD, whereas the characteristic wave, corresponding to the third, seems to be due to the reaction product of bicyclic DAA with OPD.

With regard to the structure of **B**, Dahn and Moll[8)] corrected the structure suggested by earlier workers[3,4)] and reported **6** for **B**, while **B′** has not been confirmed. Recently, we have studied the structures of **B** and **B′** on the basis of elemental analysis and spectroscopic data, and have concluded that **B** seemed to be a monohydrate of **6** and that **B′** might be **7**.[19)]

When OPD is in excess in the reaction with DAA the condensation product of 1 mol DAA with 2 mol OPD seems to be produced *via* **5**. Because **B′** could easily change to **B** in aqueous solution, **B′** is probably first produced *via* product **5** and seems to change immediately to **B**. This possibility is supported by the fact that the polarographic behavior of **B′** was identical with that of **B**. The product **B** was then hydrolyzed to **C**. When the polarogram of **C** was recorded with a large excess of OPD, the second wave was observed as well as the first. The reverse reaction from **C** to **B** seems to proceed when there is a large excess of OPD. This explains why the increment of the first wave was low in Fig. 2(b) and in Fig. 7.

Consequently, the reaction mechanism may be as shown in Scheme 1. Refering to the two-electron transfer found for each electrode reaction,[1)] and the cyclic voltammetric results of the three waves, it is reasonable to assume that the electrode reaction of the first and the second waves occurs reversibly with a two-electron transfer for the quinoxalines of **4** and **6**. In contrast, the electrode reaction of the third wave occurs irreversibly in a two-electron transfer which results in the quinoxalinone, **5**.

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