Polarographic Investigations of Vitamin C. II. On the Reduction Waves of Some Conjugated Tricarbonyl Compounds as Related Compounds of Dehydro-L-ascorbic Acid

By Sôzaburo ONO, Masanosuke TAKAGI and Tamotsu WASA

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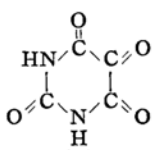
In the previous papers[1,2], it has been reported that dehydro-L-ascorbic acid (D.A.A.) shows a reduction wave which has the character as a kinetic current and the reduction product at the dropping mercury electrode is L-ascorbic acid (A.A.), and discussions were made on the inactive form of D.A.A. in aqueous solution and the hydration of carbonyl group was considered.

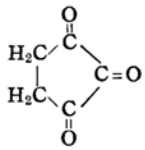
Since the possibility of the hydration in D.A.A. was proposed to be due to its conjugated three carbonyl groups, there must be seen also the same kind of kinetic current in other conjugated tricarbonyl compounds and it seems also interesting to study their behavior as kinetic current, in relation to their molecular configuration.

In the present investigations, following three conjugated tricarbonyl compounds were thus taken up.

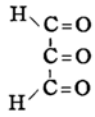


Alloxan
(I)

1) S. Ono, M. Takagi and T. Wasa, *J. Am. Chem. Soc.*, **75**, 4369 (1953).
2) S. Ono, M. Takagi and T. Wasa, This Bulletin, **31**, 356 (1958).



Dehyro-reductic acid
(II)



Mesoxalaldehyde
(III)

Of these three substances, the reduction wave of alloxan was already studied by Sartori and Liberti[3], but they do not seem to have paid any attention on its behavior as a kinetic current. Concerning other two substances, though their reduced forms i.e. reductic acid[4] and triose-reductone[5] have been polarographically studied, the reduction waves of dehydro-reductic acid and mesoxalaldehyde have not been hitherto believed to be available.

In the present paper, it will be therefore emphasized that each of the above three substances shows a reduction wave which has a similar behavior to that of D.A.A. and discussions will be developed on some correlations between the characteristics of the waves of these four substances including D.A.A. and their oxidation-reduction potentials.

Experimental

Materials.—L-Ascorbic acid was the same specimen as used in the previous works[1,2]. Triose-reductone was kindly given to us by Professor Hans von Euler of Stockholm University and later it was also prepared in our laboratory according to his method[6].

Reductic acid was prepared by Reichstein and Oppennauer's method[7].

Alloxan monohydrate was a product from Eastman Kodak Co.

Of the above four substances, alloxan could be directly used in aqueous solution for the purpose of the present experiments, while other three substances needed to be oxidized, in order to obtain their dehydro-forms. For that, various oxidizing reagents e.g. iodine, silver acetate and quinone were tried, since it would not be interesting if the polarograms should be unfavorably influenced by the oxidizing reagent used. The oxidation method finally used was as follows.

Each of the above substances, whose amount is weighed to produce 10 or 25 ml. of 0.1 mol./l. solution, is taken in a 10 or 25 ml. volumetric flask and dissolved with a small amount of the four times redistilled water (usually 2 or 5 ml.). Silver nitrate in aqueous solution, whose amount is so weighed as to be bimolar of the sample, is added into the same volumetric flask. After the oxidation has been completed, sodium acetate is added and then the whole solution is filled up with distilled water. The amount of the sodium acetate is taken according to the components of the acetate buffer so that the pH of the resulting solution is to be about 3.5. The solution is then filtered and the filtrate is immediately used as polarographic solution, in which the dehydro-form of 0.1 mol./l., sodium nitrate of 0.2 mol./l. and the components of the acetate buffer are included. In order to prepare the solutions of the dehydro-form whose concentrations are less than 0.1 mol./l., sodium nitrate and components of the acetate buffer are added into the preparation before filling up so that the resulting solutions contain the same concentrations of sodium nitrate and buffer components as above.

Alloxan was directly dissolved into the acetate buffer with sodium nitrate.

The final pH of the polarographic solutions was checked with a glass electrode. McIlvaine's buffer solutions were sometimes used instead of the distilled water to fill up the solutions of samples, and pH's were randomly changed in a limited range between 2.2 and 4.0.

Procedure and Apparatus.—The polarograph was the same one as used in the previous work[1,2]. The maximum galvanometer sensitivity was 1.72×10^{-9} A. The characteristics of the capillary used were $m=0.908$ mg./sec. and $t=4.21$ sec./drop in the buffer solution (pH 3.4) when the height of the mercury reservoir was 60 cm. and the applied potential was -0.4 V. (vs. N.C.E.) at 25°C. For the temperature control, the same cell fitted with a jacket as used in the previous work, was employed. Polarograms were taken from 10°C to 50°C at 5°C intervals. The accuracy of the temperature control was ±0.05°C. As the reference electrode, a normal calomel electrode was used.

Controlled Potential Electrolysis.—The controlled potential electrolysis of alloxan was carried out by means of the same apparatus as described in the previous paper[2].

Results and Discussion

The polarograms of alloxan, dehydro-reductic acid and mesoxalaldehyde are presented in Figs. 1, 2 and 3, respectively. As immediately seen from the above figures, the temperature coefficient of the wave height of each substance is so great as in the case of D.A.A. The wave height was also found to be independent of the height of the mercury reservoir. Therefore it may be said that all the three substances show the character as kinetic currents which are similar to that of D.A.A.

The relationship between the wave height of each substance and the temperature

3) G. Sartori and A. Liberti, *Ricerca sci.*, **16**, 313 (1946).
4) F. Santavy and B. Bitter, *Coll. Czech. Chem. Communs.*, **15**, 112 (1950).
5) R. Brdicka and P. Zuman, ibid., **15**, 766 (1950).
6) H. v. Euler and H. Hasselquist, "Reduktone", Stuttgart, Enke (1950).
7) T. Reichstein and R. Oppennauer, *Helv. Chim. Acta*, **16**, 988 (1933).

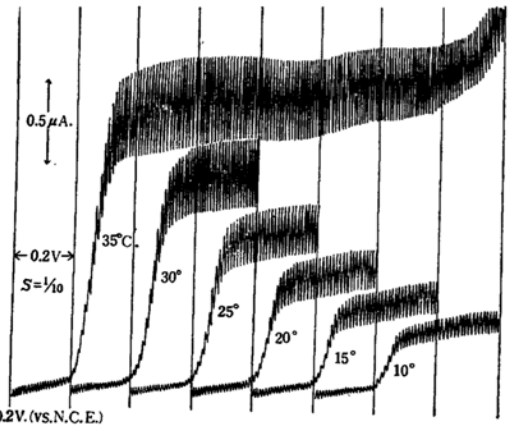


Fig. 1. Temperature dependence of the wave height of alloxan. Concn. of Alloxan = 5×10^{-3} mol./l., pH 3.6, Hg 60 cm.

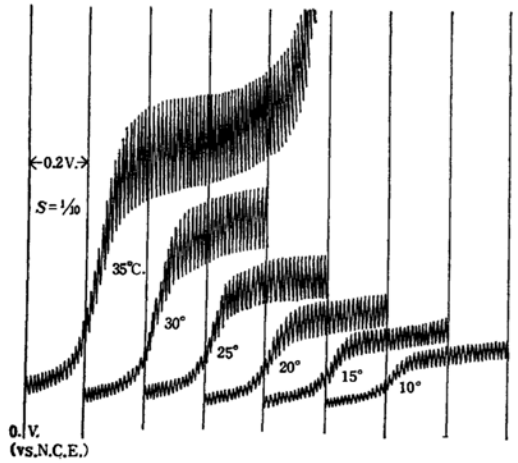


Fig. 2. Temperature dependence of the wave height of Dehydro-reductic acid. Concn. of dehydro-reductic acid = 0.05 mol./l., pH 2.8, Hg 60 cm.

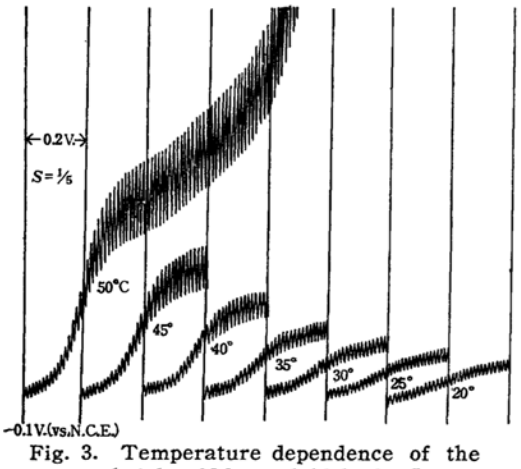


Fig. 3. Temperature dependence of the wave height of Mesoxalaldehyde. Concn. of mesoxalaldehyde = 0.1 mol./l., pH ≒3.0, Hg 60 cm.

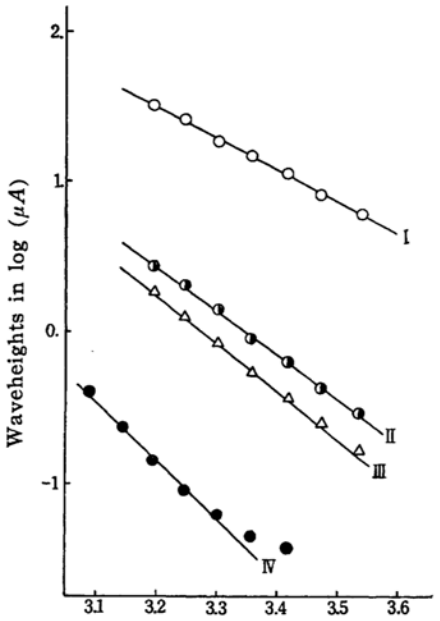


Fig. 4. Plot of logarithm of the reduction wave height against reciprocal absolute temperature.
I Alloxan
II Dehydro-reductic acid
III Dehydroascorbic acid
IV Mesoxalaldehyde
Concn. of each substance = 0.1 mol./l., pH 3.5, Hg 60 cm.

is shown in Fig. 4, where the logarithm of the wave height is plotted against the reciprocal absolute temperature. Since the wave height of each substance, including D.A.A., was found to be roughly proportional to its concentration at a given temperature, the wave heights of the substances of 0.1 mol./l. were in some cases recalculated from the experimental values obtained with more dilute concentrations. Although it was not strictly examined whether the relationship presented in Fig. 4 is valid for a wider pH range, no appreciable change in the wave height has been observed, in a range between pH 2.2 and 4.0.

Since all the substances are not stable enough in aqueous solution, the experiments with changing pH were not made in the outer range other than above mentioned. The half-wave potentials of these substances in pH 3.5 are listed in Table I.

It seems to be difficult to find any correlation between the wave height and the half-wave potential from Fig. 4 and Table I, while it may be of interest to consider

TABLE I

$\pi_{1/2}$ in pH 3.5	Alloxan	Dehydro-reductic acid	Dehydroascorbic acid	Mesoxalaldehyde
V(vs. N.C.E.)	−0.05	−0.27	−0.42	−0.35

TABLE II

$\pi_0-\pi_{1/2}$ of the reduced form	Dialuric[8] acid, Alloxan	<	Reductic acid,[9] Dehydro-reductic acid	<	Ascorbic acid,[10] Dehydro-ascorbic acid	< Triose-reductone,[11] Mesoxalaldehyde
Height of the reduction wave	Alloxan	>	Dehydro-reductic acid	>	Dehydro-ascorbic acid	> Mesoxalaldehyde
Temp. coeff. of the reduction wave	Alloxan	<	Dehydro-reductic acid	<	Dehydro-ascorbic acid	< Mesoxalaldehyde

the present results in connection with the standard oxidation-reduction potential π_0 of the corresponding system and the half-wave potential $\pi_{1/2}$ of the anodic wave of the reduced form.

As Brdicka and Zuman pointed out[5], each of the reduced forms of the above substances (i.e. dialuric acid, reductic acid, triose-reductone and D.A.A.), shows a considerable shift of the $\pi_{1/2}$ of the anodic wave from its corresponding π_0 to a more positive potential. Therefore, they described this fact as a characteristic of endiol group. In their paper, however, no further considerations on these potential shifts were made.

In Table II, these four substances are arranged in the order of magnitude of the above stated potential shift, the reduction wave height and its temperature coefficient, respectively. It may be said, of these four oxidation-reduction systems, that the smaller the height of the reduction wave, the larger both the shift of $\pi_{1/2}$ of the anodic wave from the corresponding π_0 and the temperature coefficient of the reduction wave height.

In the previous paper[2], discussions were made on the problem of the reversibility of the A.A. and D.A.A. system, on the basis of the experimental results, referring to the theoretical studies made by Koutecky[12]. Considerable disagreements between the experimental values and Koutecky's theoretical ones were found when the electrode reaction of the system was assumed to be reversible. Then, as a cause of these disagreements, the irreversible electrode reaction was assumed by the present authors. However, the correlation between the height of the reduction wave and $\pi_0-\pi_{1/2}$ of the anodic wave, presented in Table II, may suggest that although the electrode reaction itself is not reversible, the inactivation of the oxidized form in aqueous solution must be one of the most important factors which may cause both the potential shift and the kinetic current. The inactivation may be due to the hydration at the central carbonyl group among the conjugated three, as it was in the case of D.A.A.

As for the effort to interpret the above disagreements from different standpoint, though Zuman suggested the possibility of the opening of the lactone ring in the case of D.A.A.[13], such may not be taken into consideration, since the above three substances other than D. A. A. have different configurations and still show quite similar behavior of the kinetic current to that of D.A.A. Despite of that, there remains another kind of possibility, that the reducible form which causes the kinetic current is not the completely dehydrated one as shown in I, II and III, but the one in which hydration is still maintained at either one of the two carbonyl groups except the central.

Let us now consider again the reversibility of the above four oxidation-reduction systems. It is obviously seen that the $\pi_{1/2}$ of the reduction waves of D.A.A., dehydro-reductic acid and mesoxalaldehyde do not coincide with those of the corresponding oxidation waves, but in contrast with them, alloxan was expected to give the coincidence of the potentials in this respect, since the $\pi_{1/2}$ of alloxan is considerably more positive than those of the other three; also Sartori and Liberti[3] reported the reversible character of the dialuric acid and alloxan system,

8) G. M. Richardson and R. K. Cannan, *Biochem. J.*, **23**, 68 (1929).
9) N. Mayer, *J. chim. phys.*, **34**, 107 (1937).
10) G. Ball, *J. Biol. Chem.*, **118**, 219 (1937).
11) R. Wurmser, N. Mayer and O. Crepy, *J. chim. phys.*, **33**, 101 (1936).
12) J. Koutecký, *Coll. Czech. Chem. Communs.*, **20**, 116 (1955).
13) P. Zuman, A private communication to the authors. See also M. Brezina and P. Zuman, "Die Polarographie in der Medizin, Biochemie und Pharmazie" Akademische Verlagsgesellschaft, Leipzig, (1956), p. 423.

though they do not seem to have paid any attention on the characteristics of alloxan as a kinetic current.

As seen in Fig. 5, a practically perfect coincidence of the $\pi_{1/2}$ of the reduction wave with that of the oxidation wave, which is due to dialuric acid formed by

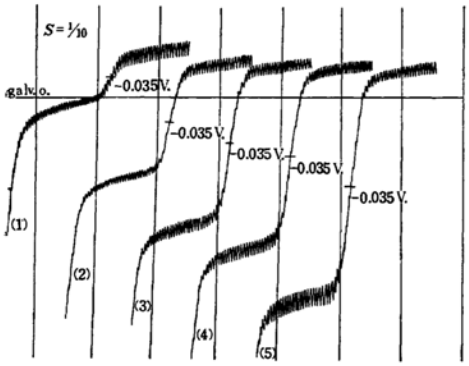


Fig. 5. Controlled potential electrolysis of alloxan at −0.2 V. (vs. N.C.E.). Polarograms were taken at 45 min. intervals. Conc. of alloxan $=1\times10^{-3}$ mol./l., pH 3.09, temp. 35°C, Hg 60 cm.

the electrolysis, has been observed. It may be readily understood that the height of the oxidation wave becomes larger than that of the initial reduction wave as the electrolysis proceeds, since the former is a diffusion current, while the latter a kinetic one.

From these observations, it may be said that the theory derived by Koutecky is at least applicable to the case of alloxan.

In order to solve the question why the electrode reaction is reversible only with the dialuric acid and alloxan system and irreversible with the other three systems, the investigations will be further developed.

Summary

Alloxan, dehydro-reductic acid and mesoxalaldehyde show reduction waves which are similar to that of dehydro-L-ascorbic acid. The half-wave potentials of alloxan, dehydro-reductic acid, dehydro-L-ascorbic acid and mesoxalaldehyde in pH 3.5 are −0.05, −0.27, −0.42 and −0.35 V. (vs. N.C.E.), respectively.

Of these four substances, the lower the height of the reduction wave, the greater both the shift of the half-wave potential of the anodic wave of the corresponding reduced form from the standard oxidation-reduction potential of the system and the temperature coefficient of the height of the reduction wave.

Inactive forms of these substances at the electrode reaction may be the hydrated ones as proposed in the case of dehydro-L-ascorbic acid.

Alloxan has been proved to be polarographically reversible by the controlled potential electrolysis and the half-wave potential of the reduction wave coincides with that of the oxidation wave of dialuric acid, while no such observations have been made with the other three.

The authors would like to express their sincere thanks to Professor Hans von Euler and his coworkers for their kindness that they have given them a specimen of pure triose-reductone.

Laboratory of Biophysical Chemistry
College of Agriculture, University
of Osaka Prefecture, Sakai