

## ON THE BEHAVIOUR OF L-DEHYDROASCORBIC ACID IN AQUEOUS SOLUTIONS

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Decomposition of L-dehydroascorbic acid in aqueous solutions under various conditions at 25°C has been studied. Cupric ions had no catalytic effect and the presence of oxygen did not affect the reaction rate either. The decomposition has been found to be catalysed by acids and bases, the catalytic effect of the hydroxyl ions being  $1.5 \cdot 10^7$  stronger than that of the hydroxonium ions. Up to approx. pH 5 the rate constant of the decomposition of L-dehydroascorbic acid obeyed the equation  $k = (0.53 + 10.8[\text{H}_3\text{O}^+]) \cdot 10^{-5} [\text{s}^{-1}]$ . The catalytic constants of various anions have also been measured and the coefficient in the Brønsted equation has been calculated as  $\beta = 0.28$ . In agreement with theory the effect of low concentrations of neutral salts (up to about 0.2M) was negligible. At pH 4 and an ionic strength of 0.54 the activation energy of the reaction was found to be 19.3 kcal/mol.

The stability of L-ascorbic acid and its oxidation product, L-dehydroascorbic acid (DAA), has been the subject of many studies. Ascorbic acid readily undergoes oxidation. Its kinetics in both model solutions and in natural systems have been investigated very thoroughly. Less attention has been given to DAA, the main reason being the unsatisfactory analytical methods of its quantitative determination. The reaction L-dehydroascorbic acid  $\rightarrow$  2,3-diketo-L-gulonic acid is of first order; its rate is enhanced by increasing pH of the medium and temperature. Terada and Omura<sup>1</sup> studied the kinetics of the decomposition of DAA in a buffered solution in a pH range from 2.0 to 7.2 and have found that the decomposition rate is independent of the buffer employed. The half-time of the reaction between pH 1.3 and 5.5 was longer than 1000 min at 20°C and 300 min at 37°C. At pH values below 2.4 and above 5.5 the stability of DAA is much weaker, the hydrolytic effect of the hydroxyl ions being  $10^7$  times stronger than that of the hydroxonium ions. Similar results have been reported by Nofre, Cier and Drevon<sup>2</sup> and by Penney and Zilva<sup>4</sup>. Omura and Terada<sup>4</sup> also investigated the effects of different salts on the decomposition of DAA. The strongest catalytic effect at pH 2.4 has been found with the ions  $\text{Fe}^{3+}$  and  $\text{MoO}_4^{2-}$ , at pH 4.5 with  $\text{Al}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{CNO}^-$ ,  $\text{MoO}_4^{2-}$  and  $\text{WO}_4^{2-}$ . The effect of  $\text{Cu}^{2+}$  ions was insignificant, though Strohecker and Schmidt<sup>5</sup> observed a weak catalysis by these ions. Dextrose and other saccharides, by contrast, inhibited the hydrolysis of DAA in proportion to their concentrations. Proteins and other nitrogen compounds<sup>2</sup> had no effect on the reaction rate. The decomposition of DAA proceeded at the same rate in the atmosphere of oxygen, nitrogen and carbon dioxide.

The present paper treats the kinetics of the decomposition of DAA more comprehensively; the study covers most factors controlling its rate. We have investigated the effects of oxygen, cupric, hydroxyl and hydroxonium ions, neutral salts, weak

acids and their anions. From the chemical point of view L-dehydroascorbic acid is  $\gamma$ -lactone of 2,3-diketo-L-gulonic acid, which readily undergoes hydrolysis by alkaline nucleophilic agents to alkali salts of the  $\gamma$ -hydroxy acid. Catalysis of hydrolytic reactions is generally acidobasic:

$$k = k_{\text{H}_2\text{O}}[\text{H}_2\text{O}] + k_{\text{H}_3\text{O}^+}[\text{H}_3\text{O}^+] + k_{\text{HA}}[\text{HA}] + k_{\text{A}^-}[\text{A}^-] + k_{\text{OH}^-}[\text{OH}^-]. \quad (1)$$

In dilute solutions the term  $k_{\text{H}_2\text{O}}[\text{H}_2\text{O}]$  can be set equal to  $k_0$ , i.e. the rate constants of the so-called spontaneous reaction, and  $k$  denotes the rate constant of decomposition<sup>7</sup> of DAA in respect to DAA.

### EXPERIMENTAL

All polarographic measurements were performed with a Polarograph LP 60 with a recorder EZ II (Laboratorní přístroje, Prague). Kalousek's cell with a saturated calomel electrode was employed. The height of the level of mercury in the reservoir being 70 cm, the constants of the capillary used were  $m$  2.6 mg/s and  $t$  3 s. pH was measured with an apparatus Acidimeter AK (Laboratorní přístroje, Prague). The concentrations of DAA were determined polarographically after condensation with *o*-phenylenediamine according to Wasa, Tagaki and Ono<sup>6</sup>. To a 100-ml volumetric flask were pipetted 10 ml of  $2 \cdot 10^{-2}$ M-AgNO<sub>3</sub> and 10 ml of  $1 \cdot 10^{-2}$ M L-ascorbic acid and the contents were stirred. After the oxidation the mixture was made up to the volumetric mark with water. The precipitate of metallic silver was filtered off and the filtrate ( $1 \cdot 10^{-3}$ M-DAA) was used as a stock solution. To construct a calibration curve, 10-ml volumetric flasks with 1 ml of  $2 \cdot 10^{-2}$ M *o*-phenylenediamine were charged with 0, 0.4, 0.8, 1.2, 1.6 and 2.0 ml of the stock solution of DAA. The solution of *o*-phenylenediamine was freshly prepared every day and was tested whether it gave no reduction wave under the conditions of the experiment. The volumetric flasks were made up with the McIlvain buffer, pH 4.7. Each solution was left standing at room temperature for 50 min, then transferred into a polarographic cell. The oxygen was bubbled out with nitrogen and the polarographic curves were recorded.

In all the kinetic measurements, conducted in dilute HCl and buffered solutions, the initial concentration of DAA was  $2 \cdot 10^{-5}$ M. In these experiments 10-ml portions of  $2 \cdot 10^{-4}$ M-DAA were added to 100-ml volumetric flasks, the volume was made up with HCl or buffers and the flasks were kept at 25°C. From these solutions 2 ml were withdrawn and pipetted to 10-ml volumetric flasks along with 1 ml of  $2 \cdot 10^{-2}$ M *o*-phenylenediamine; the volume was made up with the McIlvain buffer, pH 4.7, to 10 ml. Further procedure was the same as that described above.

### RESULTS AND DISCUSSION

Employing the integrated form of the equation for the first order reactions the rate constants  $k$  for the individual experiments were calculated from the measured decrease in the concentration of DAA with time. The constant  $k_0$  was determined by measurements in pure water and in dilute solutions of HCl, where all terms of Eq. (1) excepting the first one can be disregarded, so that  $k = k_0$  (Fig. 1). The average value of  $k_0$  was  $0.53 \cdot 10^{-5} \text{ s}^{-1}$ . The constant  $k$  was also measured at higher concentrations of HCl at which full dissociation of this strong mineral acid could still be assumed,

so that its concentration equalled the concentration of  $\text{H}_3\text{O}^+$ . The dependence of  $k$  on the concentration of HCl, *i.e.*  $\text{H}_3\text{O}^+$ , is linear and can be described by the equation

$$k = k_0 + k_{\text{H}_3\text{O}^+}[\text{H}_3\text{O}^+], \quad (2)$$

where the value of  $k_0$  obtained by extrapolation to zero concentration of HCl agreed with that obtained by measurements in water and dilute solutions of HCl and was  $0.53 \cdot 10^{-5} \text{ s}^{-1}$ . The catalytic constant of the hydroxonium ion,  $k_{\text{H}_3\text{O}^+}$ , calculated as the slope of a straight line, was  $10.8 \cdot 10^{-5} \text{ l mol}^{-1} \text{ s}^{-1}$ . Eq. (2) describes the behaviour of DAA in acid media up to pH 4–5. At higher pH values the effect of  $\text{OH}^-$  ions becomes significant (Fig. 1). The white circles denote values calculated from Eq. (2). The black circles are the results of measurements in buffered media of different pH values (2 to 8) and different concentrations of the buffer constituents, the proportion of the acid and its salt being constant; the data were obtained by extrapolation to zero concentration.

The results show that the reaction of DAA is catalysed both by the hydroxonium and by the hydroxyl ions, the effect of the latter being much stronger. The catalytic effect of  $\text{OH}^-$  ions is so strong that the constant  $k_{\text{OH}^-}$  cannot be exactly determined by measurements in selected concentrations of an alkali hydroxide, as was the case in determining  $k_{\text{H}_3\text{O}^+}$ . Consequently, the values of  $k_{\text{OH}^-}$  could be estimated from measurements in buffers of pH 7 and 8, employing Eq. (2). These values were 1780 and 1290, respectively, *i.e.* about  $1.5 \cdot 10^7$  times greater than the values of  $k_{\text{H}_3\text{O}^+}$ . Terada and Omura<sup>1</sup> found the ratio  $k_{\text{OH}^-}/k_{\text{H}_3\text{O}^+}$  to be  $10^7$ . In view of the considerable inaccuracy in the determination of  $k_{\text{OH}^-}$  this agreement can be considered satisfactory. The great catalytic effect of  $\text{OH}^-$  ions and the impossibility of the determination of  $k_{\text{OH}^-}$  make narrower the pH range where the constant  $k$  is not affected by a change of pH. This fact is an unfavourable circumstance in determining the catalytic constants of weak acids (HA) and their anions ( $\text{A}^-$ ).

The effect of low concentrations of neutral salts on the hydrolytic rate of DAA was investigated in solutions which were 0.02, 0.05, 0.10, 0.20 and 0.40M in KCl and

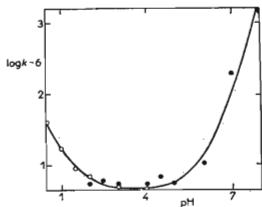


FIG. 1  
Constant  $k$  as a Function of pH at 25°C

$9 \cdot 10^{-3} \text{M}$  in HCl. Up to the molarity of 0.20 the effect of KCl on the constant  $k$  was not analytically perceptible. The average value of  $k$  in the presence of KCl was  $0.65 \cdot 10^{-5} \text{ s}^{-1}$ . In the absence of neutral salts, *i.e.* in the pure  $9 \cdot 10^{-3} \text{M-HCl}$ , the value of  $k$  was  $0.61 \cdot 10^{-5} \text{ s}^{-1}$ . Apart from the error of the method the difference may be due to the following reasons: 1. The presence of KCl somewhat suppresses the dissociation of HCl, which otherwise would be practically complete. The solution then behaves as a weak buffer of a little higher pH than would correspond to the acid itself and the value of  $k$  is consequently higher than that corresponding to the equation  $k = k_{\text{H}_3\text{O}^+}[\text{H}_3\text{O}^+]$ . 2. The primary salt effect, which raises the value of  $k$  by as much as 10%. In our case the increase due to this effect may be approx. 5%.

The decomposition of DAA is generally an acidobase-catalysed reaction. Comparison of the constants  $k_{\text{OH}^-}$  and  $k_{\text{H}_3\text{O}^+}$  indicates that even in the catalysis by Brönsted acids the anions of weak acids will have a stronger catalytic effect than undissociated molecules of weak acids. We have investigated the catalytic effects of weak acids and their anions. The solutions of DAA in buffers from these acids and their salts covered a pH range where  $k \approx k_0$ . Consequently, catalysis by  $\text{H}_3\text{O}^+$  and  $\text{OH}^-$ , and the primary salt effect could be disregarded (the concentration of salts did not exceed 0.2M, dissociation of weak acids was not considered). Under the given assumptions the increase of  $k$  above the value of  $k_0$  is caused mainly by anions behaving as bases and, to some extent, by undissociated weak acids, operative as acids in Brönsted's conception<sup>8</sup>. With each anion and the corresponding acid a series of measurements was carried out at different concentrations of the anions, various concentrations of the acid being employed with each concentration of the anions. In accordance with Brönsted's theory, anions have proved a stronger catalysts than undissociated acids, their catalytic effect being proportional to their concentration.

In a number of experiments with acids with high dissociation constants (acetic acid, phenylacetic acid, formic acid) this assumption was not fulfilled. The effect of  $\text{OH}^-$  ions was not negligible in these cases and distorted the results of the measurements. With these acids the catalytic constants are difficult to estimate. The same is true of acids with low dissociation constants in the region of low pH values (salicylic

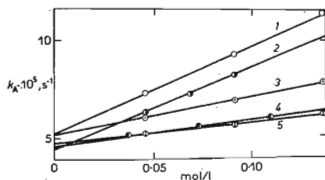


FIG. 2

The Catalytic Constants of Anions,  $k_{\text{A}^-}$ , in Relation to their Concentrations at 25°C

1 Phenyl acetate, 2 benzoate, 3 formate, 4 mandelate, 5 salicylate.

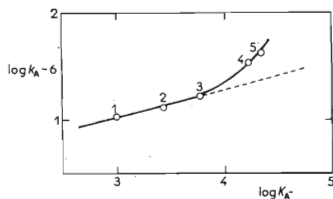


FIG. 3

$\log k_{\text{A}^-}$  as a Function of  $\log K_{\text{A}^-}$  at 25°C

acid, mandelic acid). Phenylacetic acid, benzoic acid and salicylic acid are very little soluble in water. The concentrations of these acids were consequently too low, so that their catalytic constants,  $k_{HA}$ , could not be determined; pH of these solutions could not be lowered to the region where  $k \approx k_0$ . The catalytic constants of anions of weak acids are plotted vs their concentrations in Fig. 2. Table I and Fig. 3 give the catalytic constants of anions in relation to the strength of an acid or base.

TABLE I  
Determined Catalytic Constants of Conjugated Acids and Bases

Conjugated acid	Dissociation constant <sup>9</sup> $K_{HA} \cdot 10^5$ at 25°C	$k_{HA} \cdot 10^5$ $s^{-1}$	Conjugated base	$k_{A^-} \cdot 10^5$ $s^{-1}$
Phenylacetic	4.875	—	phenyl acetate	44.0
Benzoic	6.295	—	benzoate	36.0
Formic	17.72	0.45	formate	17.3
Mandelic	38.9	—	mandelate	13.3
Salicylic	107	—	salicylate	10.7

TABLE II  
Comparison of the Determined Rate Constant,  $k$ , with the Reported Ones

pH	2.0	3.2	4.7	7.2
$k \cdot 10^{+5}$ at 20°C <sup>1</sup>	0.52	—	0.58	1.27
25°C	0.64 <sup>a</sup>	0.54 <sup>a</sup>	0.76 <sup>b</sup>	25.1 <sup>b</sup>
37°C <sup>2</sup>	—	0.83	—	33.3

<sup>a</sup> Calculated from Eq. 2; <sup>b</sup> interpolation of the data in Fig. 1.

TABLE III  
Activation Energy of the Decomposition of DAA at Different pH Values

pH	2.4	3.0	4.0	4.5	4.5	5.9	6.0	6.1	6.4	7.0
$E$ , kcal/mol	14.2	14.8	19.3	15.1	16.3	23.9	23.4	26.1	20.0	23.65
Ref.	1	10	this work	1	11	11	11	1	12	10

The relation plotted in Fig. 3 should be a straight line described by the equation

$$\log k_{A^-} = \log G_{A^-} + \beta \log K_{A^-}, \quad 0 < \beta < 1. \quad (3)$$

The first three points correspond to conjugated bases of salicylate (1) mandelate (2) and formate (3). Since the dissociation constants of the acids corresponding to these bases are rather low the assumption  $k = k_0$  is best fulfilled just in the case of these three points. The catalytic effect of  $\text{OH}^-$  ions can here be neglected and owing to the low value of  $k_{\text{H}_3\text{O}^+}$  catalysis by hydroxonium ions will not be significant either (after correction for catalysis by  $\text{H}_3\text{O}^+$  ions the values of  $k_{A^-}$  are practically the same). This, however, is not the case with benzoate and phenyl acetate (points 4 and 5), where the dissociation constants of the corresponding acids are relatively high, so that the effect of  $\text{OH}^-$  ions becomes significant. This explains why the measured values of  $k_{A^-}$  of these anions are higher than the actual ones, but cannot be corrected because of the impossibility of the determination of  $k_{\text{OH}^-}$ . Therefore, the straight line corresponding to equation (3) was drawn through the first three points only. The coefficient  $\beta$  than results as 0.28.

Terada and Omura<sup>1</sup> and Nofre, Cier and Drevon<sup>2</sup> obtained several values of the rate constant  $k$  at different pH values and temperatures, which are compared with our results in Table II. The agreement is satisfactory. Only the value of  $k$  for pH 7.2 and 20°C seems too low as against those we obtained at 25°C and 27°C; the discrepancy is evidently due to the considerable effect of  $\text{OH}^-$  ions at pH 7.2.

The activation energy of the studied reaction at different pH values has also been determined by a number of authors (Table III). The value determined by ourselves at pH 4.0 and an ionic strength of 0.54 is 19.3 kcal/mol. It is higher than the reported ones, which is probably due to the considerable ionic strength of the solution.

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