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ULTRAVIOLET SPECTRA OF L-ASCORBIC ACID AND CUPRIC ASCORBATE COMPLEX[1]

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Abstract—The UV absorption of ascorbic acid is assigned to $\pi \rightarrow \pi^*$ excitation. The shift of λ_{max} with the change of concentration of ascorbic acid in aqueous solutions is not attributed to the presence of keto form or the association of ascorbic acid at least under the conditions studied. The values of λ_{max} ($\log \varepsilon_{max}$) of free ascorbic acid (H_2A), ascorbate monoanion (HA^-) and dianion (A^{2-}) are 243·5 mμ (4·00), 265·5 mμ (4·17) and 265·5–267·5 mμ (too unstable to estimate), respectively. The solvent effect on the shift of λ_{max} is explained mainly as the dissociation of ascorbic acid. pK_a of ascorbic acid in EtOH and MeOH are estimated to be 6·0 and 5·1, respectively. Cu(II)-ascorbate complex in the presence of chelating agents in a phosphate buffer is identified by UV spectrophotometry.

IT HAS been reported that the UV spectrum of L-ascorbic acid varies not only in λ_{max} but also in ε_{max} with a change in the concentration, pH and solvent. But no rational interpretation for this variation of spectrum has yet been given.

The existence of a metal ascorbate complex composed of transition metal ion (e.g. Cu^{2+}) and ascorbate monoanion has been used as the basis for kinetic studies on autoxidation of ascorbic acid in the presence of metallic ions.

The present paper will give UV spectral evidence for the existence of three components, i.e., ascorbic acid molecule (H_2A), its monoanion (HA^-) and dianion (A^{2-}), in various ratios and also will give spectrophotometric identification of a metal-ascorbate complex.

RESULTS AND DISCUSSION

UV spectra of ascorbic acid in aqueous solution. The values of wave length (λ_{max}) and molar extinction coefficient (ε_{max}) of ascorbic acid are usually described together with its concentration, because these values vary with concentrations. Our experimental data shown in Table 1 uses E_{max} (absorbance) instead of ε_{max}.

Such a large shift in λ_{max} in Table 1 has sometimes been ascribed to the association[2] and/or the presence of a keto form[3] of ascorbic acid. But both these possibilities are excluded in view of the following facts.

If there were tautomerism of Eq. 1, conjugate system II is probably more stable than I.

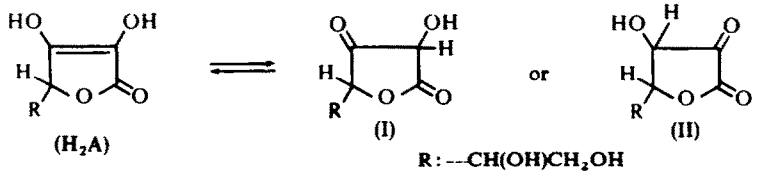

TABLE 1. UV SPECTRAL DATA OF λ_{max} AND E_{max} AT VARIOUS CONCENTRATIONS OF ASCORBIC ACID ($[H_2A]_T$) IN AQUEOUS SOLUTIONS.

$10^5[H_2A]_T$ (M)	λ_{max} (mμ)	E_{max}
1·2	263·5	0·136
3·0	263·5	0·326
6·0	263·0	0·616
12·0	257·0	1·19
30·0	251·0	2·80
60·0	247·0	5·75
90·0	246·0	8·51

But it is apparent that E_{max} increases with an increase of the total concentration of ascorbic acid ($[H_2A]_T$) and a plot of E_{max} *vs.* $[H_2A]_T$ gives an almost straight line, the slope of which gives the value of ε_{max} to be *ca.* 9600. The ε_{max} of *ca.* 10^4 suggests that the observable UV absorption curve of ascorbic acid is assigned to $\pi \rightarrow \pi^*$ excitation of C=C bond. The keto form (I or II) may have its λ_{max} for $n \rightarrow \pi^*$ in a longer wave length than the enol form. When the ratio of keto : enol increased according to Eq. 1 in a solution of lower concentration of ascorbic acid, E_{max} at such a low concentration would be smaller than that in Table 1 because the $n \rightarrow \pi^*$ absorbance is usually much smaller than the $\pi \rightarrow \pi^*$ absorbance. This means that the keto form[4] cannot be present, which is also supported by the failure of ascorbic acid to form a substituted hydrazone with 2,4-dinitrophenylhydrazine.[5] The keto form may have a strained ring, while the enol form is more stable by the conjugation of C=C and C=O groups.[6]

Jonaitis *et al.*[2] suggested that a marked shift of λ_{max} is caused by the association of ascorbic acid. But it is rather difficult to explain how association of ascorbic acid with the OH or C=O group can bring about a shift of $\pi \rightarrow \pi^*$ absorption. Even assuming that association not at direct C=C can affect the $\pi \rightarrow \pi^*$ absorption, it is abnormal that the association[7] brings about no "red shift" but "blue shift" as seen in Table 1, and causes no distinct change in ε_{max}.

UV spectra of ascorbic acid in an acetate buffer. It can be estimated that about one half of the total amount of ascorbic acid dissociates in water at the concentration of 6×10^{-5} M ascorbic acid in view of its K_1 of 8×10^{-5} at 25°. Hence, the effect of concentrations of ascorbic acid on the shift of λ_{max} seems to be due to variation of dissociation degree of ascorbic acid or concentration of ascorbate ion. In order to check this suggestion, the acetate buffer of pH 4·1 was employed as a solution for UV spectra measurement. The results are given in Table 2.

TABLE 2. λ_{max} and E_{max} IN ACETATE BUFFER OF pH 4·1.

$10^5[H_2A]_T$ (M)	λ_{max} (mμ)	E_{max}
3·0	254·0	0·257
6·0	254·0	0·540
15·0	254·0	1·285
30·0	254·0	2·70
60·0	254·0	5·625
90·0	253·5	7·89

As obvious in Table 2, λ_{max} is constant in the buffer and plot of E_{max} *vs.* $[H_2A]_T$ gives a straight line. ε_{max} is calculated to be 9200. This fact suggests that the shift of λ_{max} in aqueous solutions is based on equilibria of Eqs 2 or 3.

$$H_2A \overset{K_1}{\rightleftarrows} HA^- + H^+ \tag{2}$$

$$HA^- \overset{K_2}{\rightleftarrows} A^{2-} + H^+ \tag{3}$$

That is to say, the ratio of the concentration of ascorbate monoanion (HA^-) to that of free acid (H_2A) is maintained constant in a buffer solution in spite of wide variation of total concentration of ascorbic acid ($[H_2A]_T$).

UV spectra of ascorbic acid in an acidic or basic solution. According to the literature,[8] the shift of λ_{max} in acidic solutions is large—242–245 mμ. The shift on gradual addition of acid is listed in Table 3.

TABLE 3. λ_{max} AND E_{max} WITH ASCORBIC ACID OF $4{\cdot}5 \times 10^{-5}$M AT VARIOUS CONCENTRATIONS OF SULFURIC ACID IN AQUEOUS SOLUTIONS.

Concentration of H_2SO_4 aq. (N)	λ_{max} (mμ)	E_{max}
1×10^{-5}	261·5	0·443
2×10^{-5}	259·5	0·402
5×10^{-5}	254·0	0·375
1×10^{-4}	2490	0·390
2×10^{-4}	245·0	0·390
5×10^{-4}	244·5	0·420
1×10^{-3}	244·0	0·434
1×10^{-2}	243·5	0·443
1×10^{-1}	243·5	0·443
1×10^{-4}	243·5	0·604*

* Ascorbic acid 6×10^{-5} M

The value of ε_{max} seems to vary with the concentration of sulfuric acid, which might be related to the association or protonation of ascorbic acid. An isobestic point at 252·8 mμ, however, indicates that only two components must coexist in these pH ranges. Association is unlikely as discussed in previous sections. Protonation to OH or C=O is far less probable because of the very low equilibrium constant for conjugate acid of ordinary alcohol, aliphatic carboxylic acid, of which acidity is almost the same as OH in ascorbic acid, or carbonyl compounds.

The change of apparent E_{max} accompanied by the shift of λ_{max} caused by changing the ratio of two components is often seen when the absorption curve is rather broad. The present results may be a case, where E_{max} decreases when the λ_{max} shifts towards the wave length midway between those of two components. Assuming that the two components are H_2A and HA^-, ratio $[HA^-]/[H_2A]$ can be calculated to be *ca.* unity in 10^{-4} N H_2SO_4, in which λ_{max} is at 249 mμ and apparent E_{max} decreases more than 10% compared with those in solutions of much higher or much lower acidity.

Ratios of two or three components (i.e., H_2A, HA^- or A^{2-}) in a solution of a given pH can be calculated from Eq 4.

$$\begin{aligned} [HA^-]/[H_2A] &= K_1/[H^+] \\ [A^{2-}]/[H_2A] &= K_1K_2/[H^+]^2 \end{aligned} \tag{4}$$

where pK_1 and pK_2 are 4·1 and 11·8, respectively.

The total amount of ascorbic acid ($[H_2A]_T$) is expressed as:

$$[H_2A]_T = [H_2A] + [HA^-] + [A^{2-}] \quad (5)$$

The percentage of free H_2A in 10^{-1} N H_2SO_4 is thus calculated to be 99·91 % and that of HA^- is only 0·09 %. These figures mean that H_2A have λ_{max} at 243·5 mμ, which may be abbreviated as $\lambda_{max(H_2A)}$. $\varepsilon_{max(H_2A)}$ is calculated to be $1{\cdot}00 \times 10^4$, using data of E_{max} with $4{\cdot}5 \times 10^{-5}$ M of $[H_2A]_T$ in Table 3, where $\varepsilon_{max(H_2A)}$ denotes ε_{max} of H_2A species. The value of $\varepsilon_{max(H_2A)}$ $1{\cdot}00 \times 10^4$ is reconfirmed by our previous results[9] in 10^{-1} N and 4×10^{-1} N acids (HCl and H_3PO_4).

In order to obtain spectral data on HA^- and A^{2-}, UV absorptions were measured in solutions of higher pH. Some results are shown in Table 4 with ratios of three components calculated by Eq 4.

TABLE 4. UV DATA OF $6{\cdot}0 \times 10^{-5}$M ASCORBIC ACID AND THE CALCULATED RATIO OF THREE COMPONENTS; FREE ASCORBIC ACID (H_2A), ASCORBATE MONOANION (HA^-) AND DIANION (A^{2-})

Solution	pH	λ_{max} (mμ)	E_{max}	Ratio in % $[H_2A]$: $[HA^-]$: $[A^{2-}]$		
$KH_2PO_4 - K_2HPO_4$	5·2	265·0	0·855	7·31	92·69	0
$H_3BO_3 - KCl - KOH$	7·8	265·5	0·893	0·02	99·97	0·01
$H_3BO_3 - KCl - KOH$	10·0	265·5	0·863	0	98·43	1·57
KOH	12·0	265·5–267·5	—	0	13·79	86·21

UV data of HA^- are easily obtained from the results in the solution of pH 7·8 to be $\lambda_{max(HA^-)}$ of 265·5 mμ and $\varepsilon_{max(HA^-)}$ of $1{\cdot}47 \times 10^4$.

On the other hand, ascorbic acid is very unstable in alkaline solutions even in the presence of a small amount of EDTA which is effective for stabilizing ascorbic acid in solutions of pH below 7·8. $\lambda_{max(A^{2-})}$ was *ca.* 265·5–267·5 mμ, but $\varepsilon_{max(A^{2-})}$ could not be estimated.

UV spectra of ascorbic acid in alcohols. UV spectral data of ascorbic acid in alcoholic solutions are also listed in Table 5.

The results in Table 5 together with the results in Table 1 seem to confirm the ordinary bathochromic solvent effect on λ_{max} of $\pi \rightarrow \pi^*$. But apparent E_{max} in various concentrations of aqueous ethanol solutions is similar to that in Table 3.

TABLE 5. λ_{max} AND E_{max} FOR $6{\cdot}0 \times 10^{-5}$M ASCORBIC ACID IN ALCOHOLIC SOLUTIONS.

Solvent	λ_{max} (mμ)	E_{max}
23 % EtOH aq.*	259·0	0·569
48 % EtOH aq.	253·0	0·508
73 % EtOH aq.	249·0	0·522
95 % EtOH aq.	247·0	0·523
EtOH	244·5	0·590
MeOH	247·0	0·513

* % in volume/volume

Thus, apparent ε_{max} in EtOH or MeOH is calculated to be 9800 or 8600 and these figures are comparable to 9300 or 8700 of ε_{max} in water at the λ_{max} same as that in EtOH (244·5 mμ) or MeOH (247·0 mμ). This suggests that solvent effect on UV absorption is mainly caused by the dissociation degree of ascorbic acid. These facts supposedly give a way of estimation of pK_a of ascorbic acid at dilute concentration in these organic solvents compared with the data in acidic aqueous solutions.

The ratios of three components, $[H_2A]:[HA^-]:[A^{2-}]$ are calculated using data in Table 3 to be 86·2:13·8:0 and 65·2:34·8:0 at λ_{max} of 244·5 mμ (in 5×10^{-4} N H_2SO_4) and 247 mμ (in $1·5 \times 10^{-4}$ N H_2SO_4). Since $[H_2A]_T$ is known to be $4·5 \times 10^{-5}$ M where association are negligible, pK_a of ascorbic acid in EtOH and MeOH are calculated to be 6·0 and 5·1, respectively.

UV spectra as an evidence for Cu(II)*-ascorbate complex.* Autoxidation of ascorbic acid is accelerated by metallic ions; kinetic data suggest that one electron transfer from ascorbate ion to metallic ion occurs after the formation of an intermediary complex. Since the complex may be very unstable, the presence of a complex has been mentioned only in a few communications.

Spectrophotometric identification has long been expected for a proof of the presence of a metallic ascorbate complex. Stability constants of UO_2-ascorbate[10] and VO_2-ascorbate[11] complexes were obtained by means of potentiometric determination, since UO^{2+} and VO_2^{2+} are very weak catalysts for the autoxidation.

Martell *et al.*[12] measured the change of pH in 25 sec, and suggested formation of a Cu(II)-ascorbate complex. But they reported the same procedure for ferric ion was unsuccessful owing to the very fast oxidation by ferric ion.[12] Although their method is unknown in detail, the change of pH of the solution seems to be caused by an oxidation-reduction between ascorbic acid and cupric ion and a pH change was not observed owing to the absence of a redox reaction in the case of the ferric ion which is a less active catalyst than the cupric ion. It is difficult to tell whether the rapid change in pH on mixing solutions of cupric salt and ascorbic acid is caused by the complex formation or by the completion of a redox reaction. Thus, it was hoped to obtain spectral evidence in the case of the cupric ion which is the best catalyst for the autoxidation of ascorbic acid.

Grinstead[13] tried to show a spectrophotometric detection of Fe(III)-ascorbate complex in the oxidation of ascorbic acid with Fe(III)-EDTA complex, but there was no significant change in UV spectra.

In the present approach for the confirmation of a cupric ion complex, the high oxidation potential of cupric ion was lowered by adding a chelating agent. A buffer solution was used to suppress any shift of absorption spectra caused by decreasing the concentration of ascorbic acid or changing pH of an aqueous solution as shown in the previous sections.

The shift of λ_{max} was observed neither when an ascorbic acid solution was mixed with Cu(II)-EDTA solution in an acetate buffer of pH 4·1, nor when cupric acetylacetonate or imidodiacetate of small stability constant (K_s) was substituted for EDTA.

The effect of anions on the rate of autoxidation of ascorbic acid results from the difference in coordination ability of anions to metallic ions.[14] Phosphate, sulfate or nitrate ions do not affect the reaction,[15] but the reaction in an acetate buffer is often changed and some interaction between acetate ion and metallic ions is suggested.[16] Therefore, a phosphate buffer of pH 5·2 was used in the following experiments.

When 2.67×10^{-5} M ascorbic acid was mixed with a Cu(II)-EDTA solution, λ_{max} shifted to 260·5 mμ from 265·5 mμ, where ascorbic acid itself in the buffer has λ_{max}. This shift of 5 mμ may suggest the formation of a Cu(II)-ascorbate complex according to the following equilibria.

$$HA^- + [Cu(II)\text{-}EDTA] \rightleftarrows [HA\text{-}Cu(II)\text{-}EDTA]^- \quad (6)$$

The fact that λ_{max} of free ion (HA^-) is of higher wave length then that of H_2A (non-ion) suggests the probability of the shift of λ_{max} to a shorter wave length than that of HA^-; but the wave length is longer than that of H_2A by the formation of the complex. A change of absorption in the visible region could not be estimated with the low concentration of Cu(II)-EDTA employed.

EXPERIMENTAL

Materials. Ascorbic acid was of 99·5% pure with decomposition point of 190·9° [lit.[17] 190–192° (dec)] and optical rotation of $[\alpha]_D^{25}$ +20·94° (lit.[17] 20·5–21·5°). Acetate and phosphate buffers were prepared by Michaelis' and borate buffer by Clark–Lubs' methods. Ion-exchanged water was used. EDTA and iminodiacetic acid were of commercial extra pure grade. Ethanol, methanol and acetylacetone were purified by ordinary methods before use. $CuSO_4 \cdot 5H_2O$ of commercial guaranteed reagent grade was used.

UV spectrometry. A Shimadzu automatic recording spectrophotometer and a Hitachi 124 spectrophotometer were used for spectral measurements in quartz cells of 10 mm wide. Spacers of 8 mm were employed at the ascorbic acid concentration over 2×10^{-4} M.

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