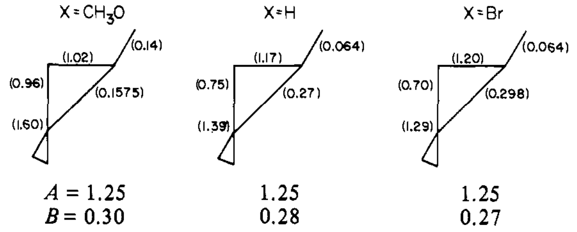
Scheme III



force constant for out-of-plane coordinates, Saunders[66] has used relation 11′ (with $g_\alpha = 1.0$) in calculations of KIE for elimination

$$F_\alpha = g_\alpha(n_i n_j)F^0{}_\alpha(T_d) + 0.235 \qquad (11')$$

reactions. In the present model, eq 11′, with g_α given by eq 12, was used for bending coordinates ϕ_{169} and ϕ_{627}, retaining the value of $F^\circ(T_d)$ from Table IV. For coordinates ϕ_{269} and ϕ_{127}, eq 11 was used, but the value of $F^\circ(T_d)$ was increased by 0.235 over that in Table IV in order to account for the fact that sp^2 bending force constants are larger than the corresponding sp^3 values. All other force constants were the same as in the previous model. Despite the rather large differences in several force constants, the resulting TS models (Scheme III) differ significantly from those of Scheme II only in that the C_α–O bond is slightly less ruptured in the present case. Otherwise, the conclusions are essentially unchanged from the previous model, suggesting that the results are not seriously affected by the form of the empirical relations used to generate the TS force field.

Acknowledgment. Financial support of this research by the Japan Ministry of Education (Grant No. 747018) and by the U.S. National Science Foundation (Grant No. CHE 76-09809) is gratefully acknowledged.

Supplementary Material Available: Activity data for the results obtained in this study (12 pages). Ordering information is given on any current masthead page.

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(66) Saunders, W. H., Jr. *Chem. Scr.* **1975**, *8*, 82.

Mechanism of Disproportionation of Ascorbate Radicals

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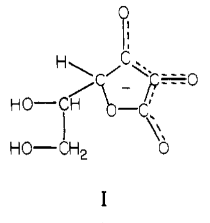
Abstract: Existing data on the kinetics of ascorbate radical decay, together with some new data on the effects of temperature, ionic strength, and presence of phosphate buffers, suggest a mechanism in which the ascorbate radical ion is in equilibrium with a dimer. This dimer reacts with hydrogen ion, or with other proton donors present including water and buffers (at rates depending upon their acid strengths), to form the disproportionation products ascorbate ion and dehydroascorbate acid.

The rate of spontaneous bimolecular decay of ascorbate radicals was first measured in 1960 in the course of a study of the kinetics of enzymatic oxidation of ascorbate.[1] In 1971, Bielski, Comstock, and Bowen[2] studied the reaction by pulse radiolysis, generating the radical by reaction of OH with ascorbate. They found that increasing the pH from 2 to 9 reduced the decay rate by a factor of 2000, and that at any given pH the rate increased with the concentration of added phosphate buffer.

The following year, a detailed study by Schöneshöfer[3] appeared, which included spectroscopic evidence for several different radicals formed by the action of OH on ascorbic acid. He showed that this complication could be avoided by the addition of halide or thiocyanate ions to the ascorbate solution; e.g., $OH + Br^- \rightarrow BrOH^- \xrightarrow{Br^-} OH^- + Br_2^-$, and Br_2^- reacts with ascorbate ion to form only the principal ascorbic acid radical I. Bielski et al.[2] had reported that the extinction coefficient of the radical changed with pH, but this was an error resulting from the formation of different types of radicals from oxidation of ascorbate by OH; Schöneshöfer[3] showed that when halide radicals were used as oxidants the extinction coefficient at 360 nm was essentially independent of pH. Fessenden, Schuler, and co-workers[4,5] studied the reaction by electron spin resonance (ESR) spectra and verified that only I forms in the reaction of Br_2^- with ascorbate.



I

The ESR study[4] showed that I is in the anionic form over the pH range 1–12 and protonates only at still lower pH. The great change in the radical decay rate between pH 3 and 9 is therefore not due to a change in the nature of the radical and must result from some kind of acid catalysis. To understand the mechanism of the reaction and the phosphate effect, we have recalculated some of the older results[2] and have obtained some new pulse-radiolysis data.

Experimental Section

L-Ascorbic acid was "certified ACS" grade from Fisher Scientific Co. Hydrochloric and sulfuric acids were Aristar distilled and phosphoric acid was MCB reagent grade. Other chemicals were Baker analyzed. Water, after distillation, was passed through a Millipore ultrapurification system.

Pulse radiolysis was carried out with 2 MeV electron beams from a Van de Graaff generator. Pulses were 2–5 μs long; doses varied from 80 to 1700 rads. Radical concentrations were calculated from optical densities at 360 nm assuming an extinction coefficient of 3300 M^{-1} cm^{-1}.[6]

Solutions were 2×10^{-4} M in ascorbic acid and 0.02 M in KBr, and were saturated with N_2O at atmospheric pressure to convert solvated electrons to OH radicals. Runs with phosphate were always 0.0485 M in total phosphate, pH being adjusted by varying the relative amounts of H_3PO_4, NaH_2PO_4, and Na_2HPO_4. The highest pHs were adjusted with KOH.

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(1) Yamazaki, I.; Piette, L. H. *Biochim. Biophys. Acta* **1961**, *50*, 62.

(2) Bielski, B. H. J.; Comstock, D. A.; Bowen, R. A. *J. Am. Chem. Soc.* **1971**, *93*, 5624.

(3) Schöneshöfer, M. *Z. Naturforsch., B.* **1972**, *27B*, 649.

(4) Laroff, G. P.; Fessenden, R. W.; Schuler, R. H. *J. Am. Chem. Soc.* **1972**, *94*, 9062.

(5) Fessenden, R. W.; Verma, N. C. *Biophys. J.* **1978**, *24*, 93.

(6) Schuler, R. H. *Radiat. Res.* **1977**, *69*, 417.

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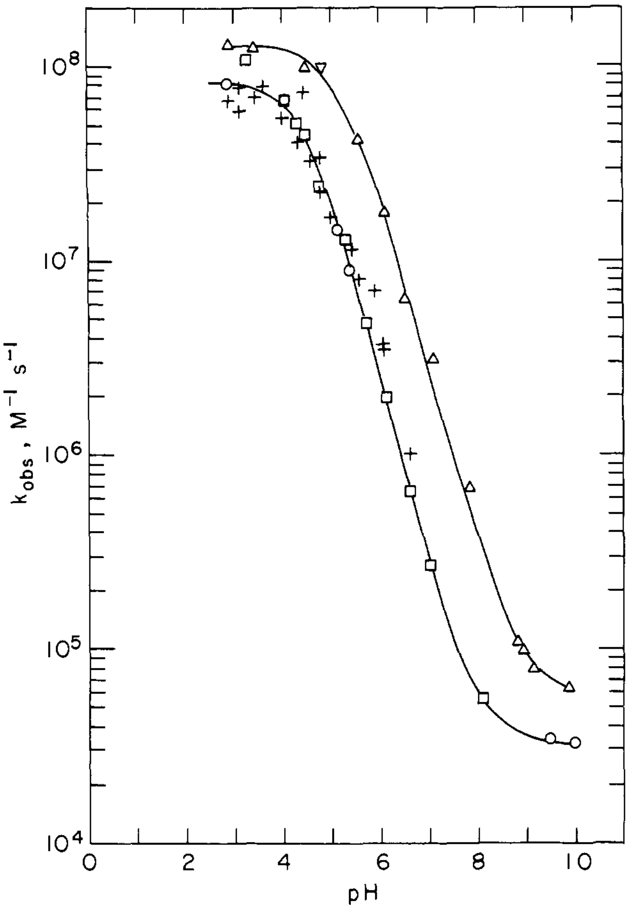

Figure 1. Observed second-order rate constants for the decay of the principal ascorbic acid radical: ○, present work, no buffer; △, present work, 0.045 M phosphate; ▽, ref 1; □, ref 2 corrected, no buffer; +, ref 3, corrected for ionic strength when Br^-, Cl^-, or SCN^- was used. Lower curve for eq 4′ with $\mu = 10^{-3}$ M; upper curve, for eq 5.

Data in ref 2 on decay rates in the absence of phosphate were recalculated on the assumption that Schuler's extinction coefficient of 3300[6] holds at all pHs used; those taken in the presence of phosphate showed anomalous concentration effects and are not used in this paper. Below pH 2.8, some solvated electrons are converted to H atoms which react with ascorbic acid to form a different radical, which complicates the kinetics; only higher pHs are considered in this paper.

Runs without buffer were pH adjusted with small additions of KOH or HCl. In the pH range between 5 and 8, drifting of pH by as much as 0.1 unit sometimes occurred, which would lead to a 30% error in the measured rate constants. On each solution used, several runs were made at different doses; they usually showed a standard deviation of about ±5%.

Results

Figure 1 shows the second-order rate constants for the decay of the principal ascorbic acid radical obtained at room temperature in the absence of phosphate by recalculating data from ref 2, together with Schöneshöfer's data[3] obtained in Br^- and SCN^- solutions, and some new determinations by us. The new data and some of those of ref 3 were taken in the presence of halide to avoid problems resulting from formation of other radicals by reaction of OH with ascorbate. Although OH was used in ref 2, the other radicals are shorter lived than radical I and perturb the observed kinetics only early in the run. The effect of the other radicals on the decay kinetics led to a wrong evaluation of the extinction coefficient. From the change of optical density with time later in the runs which were accurately second order, correct results should be obtained by using the correct extinction coefficient, and the recalculated values indeed agree well with those of Schöneshöfer and with our new data.

Figure 1 also includes new data on solutions in 0.0485 M phosphate buffers. The data of ref 1, also obtained in phosphate buffer, agree well with ours.

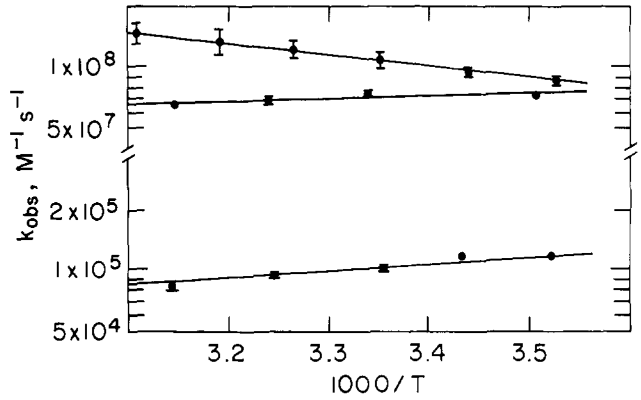

Figure 2. Temperature coefficient for rate constant of decay of ascorbate radicals at pH 2.95 (upper line), pH 4.0 (middle line), and pH 8.94 with phosphate buffer (lower line).

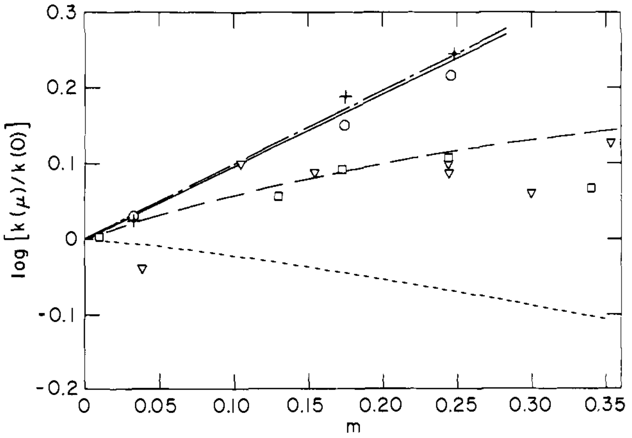

Figure 3. Effect of ionic strength on rate of decay of ascorbate radicals at various pH values. Predicted curves and experimental points: pH 2.95 (—, ○); pH 4.1 (– – –, □); pH 5.15 (- - - -, ▽); pH 9.8 (·‐·‐·, +).

The temperature coefficient of the decay of the ascorbate radical was determined for buffer-free solutions at pH 2.95 and 4.0 and for a solution with 0.0485 M phosphate buffer at pH 8.94. The results are shown in Figure 2.

The effect of ionic strength on the rate of a reaction between two ions is given approximately[7] by

$$\log\ [k(\mu)/k(0)] = z_A z_B \left(\frac{1.02\mu^{1/2}}{1 + \mu^{1/2}} \right) \equiv z_A z_B m \qquad (1)$$

where zA and z_B are the charges on reactants A and B, and μ is the total ionic strength, given by $^1/_2 \Sigma c_i z_i^2$ where the c_i's are the concentrations of all the ions in the solution. Radical decay rates were determined at four different pHs with ionic strength varied by additions of sodium perchlorate; the results are shown in Figure 3, where observed values of log $k(\mu)$ are plotted as a function of m.

Discussion

Though the radical decay constant k_{obsd} rises rapidly with increasing acidity from pH 8 to 4, its rate of change slows down at lower pHs and it appears to approach independence of the acid concentration at higher acidities. This behavior would result if two ascorbate radicals, reacting together, form a dimer which may react with H^+ to form ascorbic and dehydroascorbic acids, but which otherwise dissociates back to two radicals. At pH above 8, the rate again levels off at a finite value, showing that the dimer undergoes a similar but slower reaction with water, which is of course a much weaker proton donor than H^+. Phosphates or other buffers may similarly act as proton donors. In the following

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(7) Weston, R. E.; Schwarz, H. A. "Chemical Kinetics"; Prentice-Hall, Inc.: Englewood Cliffs, N. J., 1972; p 169.

reaction schemes, $A^{-}\cdot$ is the ascorbate radical I, HA^- is the ascorbate ion, A is the dehydroascorbic acid, A_2^{2-} is the dimer of I, P_0 is H_3PO_4, P_1 is $H_2PO_4^-$, and P_2 is HPO_4^{2-}. The observed overall reaction is[2]

$$2A^{-}\cdot + H^+ = HA^- + A$$

We postulate:

$$2A^{-}\cdot \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A_2^{2-}$$

$$A_2^{2-} + H^+ \xrightarrow{k_3} HA^- + A$$

$$A_2^{2-} + H_2O \xrightarrow{k_4} HA^- + A + OH^-$$

$$A_2^{2-} + P_0 \xrightarrow{k_5} HA^- + A + P_1$$

$$A_2^{2-} + P_1 \xrightarrow{k_6} HA^- + A + P_2$$

$$A_2^{2-} + P_2 \xrightarrow{k_7} HA^- + A + P_3$$

The observation of second-order kinetics implies that A_2^{2-} is at a steady state on the time scale of the reaction:

$$d(A_2^{2-})/dt = 0 = k_1(A^{-}\cdot)^2 - (A_2^{2-})[k_2 + k_3(H^+) + k_4 + k_5(P_0) + k_6(P_1) + k_7(P_2)] \quad (2)$$

For the observed rate constant of radical disappearance, k_{obsd}, we have in the absence of phosphate

$$-d(A^{-}\cdot)/dt = 2k_{obsd}(A^{-}\cdot)^2 = 2d(A)/dt = 2(A_2^{2-})[k_3(H^+) + k_4] \quad (3)$$

Combining (2) and (3), we find for k_{obsd}

$$k_{obsd} = k_1\Big/\left[1 + \frac{k_2}{k_3(H^+) + k_4}\right] \quad (4)$$

From (4) we see that as (H^+) becomes large, $k_{obsd} \rightarrow k_1$. On the alkaline side, since k_{obsd} becomes thousands of times smaller than k_1, unity in the denominator of (4) becomes negligible, and as (H^+) also becomes negligible $k_{obsd} \rightarrow k_1k_4/k_2$.

The ionic strength effect on k_{obsd} is complicated. From (1), we expect $\log [k_1(\mu)/k_1(0)] = +m$, $\log [k_3(\mu)/k_3(0)] = -2m$, and little effect of ionic strength on k_2 or k_4. In the presence of phosphate, k_5 is not affected but $\log [k_6(\mu)/k_6(0)] = +2m$ and $\log [k_7(\mu)/k_7(0)] = +4m$. The hydrogen ion concentration is related to pH by $\log [H^+] = -pH + m/2$ because the logarithm of the activity coefficient is $-m/2$. Equation 4 may now be written

$$k_{obsd} = \frac{10^{\log k_1 + m}}{1 + [k_4/k_2 + 10^{\log[(k_3/k_2) - pH - 1.5m]}]^{-1}} \quad (4')$$

Since it was apparent that at the lower pHs k_4 is negligible compared to $k_3(H^+)$, a plot of $1/k_{obsd}$ vs. $1/(H^+)$, using the low-pH, low-ionic-strength data, gave preliminary values of k_1 and k_3/k_2. From this k_1 and the apparent high-pH asymptote in k_{obsd}, a preliminary value of k_4/k_2 was obtained. Small adjustments in these constants were then made to improve the fit to eq 4′. The data used were the buffer-free data of ref 2, assumed to be at $\mu = 10^{-3}$, and the new data of the present work, including one run at pH 4 with $\mu = 10^{-4}$, while the others were with KBr added to $\mu = 10^{-2}$. In Figure 1 the experimental points are plotted, along with a curve calculated for $\mu = 10^{-3}$. The constants were $k_1 = 10^{7.89} = 7.8 \times 10^7$, $k_3/k_2 = 10^{4.56} = 3.6 \times 10^4$, and $k_4/k_2 = 4.0 \times 10^{-4}$.

The data of Schöneshöfer from Figure 8 of ref 3 were adjusted to the extinction coefficient of 3300 $M^{-1}\ cm^{-1}$ from his assumed value of 3700. Note also that we plot k, while he plots $2k$. The concentrations of added ions Cl^-, Br^-, and SCN^- are not stated, except for two presumably typical runs where 0.1 M salt was used; we assumed all these runs were at $\mu = 0.1$ and corrected these values to $\mu = 10^{-3}$ to fit our plot, while the runs without added salt were plotted without any adjustment for ionic strength. Schöneshöfer's results are seen to agree excellently with ours, except for his data with iodide, which appear aberrant and are omitted here.

Our new data on ionic strength effects at four pHs, with μ adjusted by additions of KBr and/or $NaClO_4$, are shown in Figure 3, together with the predictions of eq 4′. At the lowest and highest pHs, k_{obsd} is nearly proportional to k_1, and we predict a slope near unity for the curve of $\log [k(\mu)/k(0)]$ vs. m. The results agree closely with this prediction. At pH 4.1, k_1 and k_3 are working in opposite directions, and we predict that with increasing m, k_{obsd} will increase slowly. At pH 5.5, the rate is expected to decline with increasing ionic strength, but this is true only at $\mu = 10^{-3}$; at higher μ, the rates were higher. It seems that k_3 obeys eq 1 only at very low ionic strengths.

In our phosphate-buffered runs, the total phosphate concentration was kept constant at 0.0485 M, so that the ionic strength increased with increasing pH. For each pH used, the concentrations $[P_0]$, $[P_1]$, $[P_2]$, and $[P_3]$ were calculated by using the pK values[8] for phosphoric acid: 2.12, 7.21, and 12.66.

The ionic strength was calculated for each solution. Our reaction mechanism leads to eq 5, which was found to fit the data

$$k_{obsd} = [10^{7.89} + m]/\{1 + [4.0 \times 10^{-4} + 10^{4.56 - pH - 3/2m} + (k_5/k_2)[P_0] + (k_6/k_2)[P_1]10^{2m} + (k_7/k_2)[P_2]10^{4m}]^{-1}\} \quad (5)$$

well with $k_5/k_2 = 2.5 \times 10^4$, $k_6/k_2 = 7.9 \times 10^{-2}$, and $k_7/k_2 < 2 \times 10^{-6}$. The term in P_2 was found to be negligible compared to the term in P_1, even when, at the highest pH, 99.6% of the phosphate was in the P_2 form. The phosphate effect on k_{obsd} at the lowest and highest pHs results almost entirely from the ionic strength effect; the larger effects at intermediate pHs are due to reactions 5 and 6. The curve in Figure 1 is drawn through the points calculated for 0.0485 M total phosphate by eq 5 and is seen to fit our experimental points well.

From the temperature coefficient data of Figure 2, estimates of some activation energies can be made. Defining E_{obsd} as $-R\ d \ln k_{obsd}/d(1/T)$, we find at pH 2.95, $E_{obsd} = 2.45 \pm 0.75$ kcal/mol; at pH 4.0, $E_{obsd} = -0.6 \pm 0.5$ kcal/mol; and at pH 8.9, $E_{obsd} = -1.7 \pm 0.6$ kcal/mol. These values came from least-squares calculations but the probable errors shown came from subjective judgments. At the lower two pHs, k_4/k_2 is negligible, and differentiation of eq 4 with respect to $1/T$ gives

$$E_{obsd} = E_1 + [1 + (k_3(H^+)/k_2)]^{-1}(E_3 - E_2) \quad (6)$$

and at pH 2.95 and 4.0

$$E_1 + 0.025(E_3 - E_2) = 2.45 \pm 0.75$$

$$E_1 + 0.216(E3 - E_2) = -0.6 \pm 0.5$$

Hence $E_1 = 2.85 \pm 0.9$; $E_3 - E_2 = -16 \pm 5$ kcal/mol. At pH 8.9 k_3, k_5, and k_6 are all negligible and we have $E_{obsd} = E_1 + E_4 - E_2 = -1.7 \pm 0.6$; $E_4 - E_2 = -4.5 \pm 1.1$; and $E_4 - E_3 = 11.5 \pm 5.5$ kcal.

The reaction of A_2^{2-} with H^+ is presumably a simple exothermic protonation and E_3 should be small and k_3 perhaps of the order of $10^{10}\ M^{-1}\ s^{-1}$ at room temperature. Then k_2 should be of the order of $10^5\ s^{-1}$, suggesting an activation energy of 11–13 kcal, in agreement with our rough estimate of $E_2 - E_3$. The equilibrium constant $[A_2^{2-}]/[A^-]^2 = k_1/k_2 \simeq 10^3\ M^{-1}$. Thus if the radical concentration were 10^{-5} M, which is about the highest attained in our pulse radiolysis work, the radical would not be more than about 2% dimerized.

Acknowledgment. This research was carried out at Brookhaven National Laboratory under contract with U.S. Department of Energy and supported in part by its Office of Basic Energy Sciences and in part by NIH Grant No. 2 RO1 GM23656-04.

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(8) "Handbook of Chemistry and Physics"; CRC Press: Cleveland, Ohio, 56th ed.