[Chem. Pharm. Bull. 19(2) 341—354 (1971)] UDC 547.475.2.04

Studies on L-Ascorbic Acid Derivatives. V.[1] Hydrolysis of L-Ascorbic Acid 3-Phosphate[2]

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(Received August 5, 1970)

The rate constants for the hydrolysis of L-ascorbic acid 3-phosphate were measured in aqueous solutions at 50—100° from pH 1 to 9.6 and also in strong acid solutions up to 4M hydrochloric acid. The first order kinetics with respect to the unreacted phosphate were obtained by determining the remaining phosphate. The pH rate profile (Fig. 3) for the hydrolysis of L-ascorbic acid 3-phosphate is represented by the rate equation. $K_{obsd} = k_a[H^+][H_3A]/C + k_N[H_3A]/C + k_1[H_2A^-]/C + k_2[HA^{2-}]/C + k_3[A_3^-]/C$. Where $[H_3A]/C$, $[H_2A^-]/C$, $[HA^{2-}]/C$ and $[A^{3-}]/C$ are molar fractions of the neutral species, monoanion, dianion and trianion, and k_a, k_N, k_1, k_2, and k_3 are their associated specific rate constants. From the observed hydrolysis rate and pK values, these specific rate constants were evaluated. The pK values of L-ascorbic acid 3-phosphate were determined to be $pK_1=0.01$, $pK_2=3.27$, and $pK_3=6.70$ at 28.5°.

In hydrochloric acid solutions (1—4M), the rate of hydrolysis is proportional to the hydrogen ion concentration. This result coupled with the moderately large solvent isotope effect and with the activation parameters suggests that the hydrolysis of the conjugate acid of L-ascorbic acid 3-phosphate is bimolecular and a water molecule is participated in the transition state.

Hydrolysis of the monoanion which is predominant at pH ~2 involves mainly P–O bond fission. Measurements of the solvolysis product composition indicated that the molar ratio, methyl phosphate/inorganic phosphate, was slightly smaller than the ratio, methanol/water, present in the reaction mixture. Decrease in the rate by the addition of an organic solvent, the large negative value of the activation entropy and the insignificant D_2O effect suggested the reaction mechanism as in Chart 2.

When the dianion was hydrolyzed in $H_2{}^{18}O$, ^{18}O was introduced mainly into inorganic phosphate. Different from those of common monoalkyl- or monoarylphosphates, the solvolysis in methanol–water mixture afforded almost exclusively inorganic phosphates. These data taken in conjunction with the relatively large solvent isotope effect and with the negatively large entropy value led to the postualation that the hydrolysis of the dianion underwent through a slow proton transfer to the carbonyl oxygen concerted with the nucleophilic attack by hydroxide ion which brought about the rapid scission of the P–O bond.

In the case of the trianion an extremely low hydrolysis rate was observed and possible mechanistic discussion was given to account for the stability.

As a result of considerable progress in the art of preparing formulations, various ways have been successfully devised for producing multi-vitamin products having acceptable stability. However, multicomponent liquid pharmaceuticals and cosmetics containing L-ascorbic acid exhibit a problem in respect to the periodical stability. Even in the case where loss in the ascorbic acid concentration is of no major concern, the gas evolution or discoloration often leads to a serious problem from the pharmaceutical point of view. With the aim of finding a solution in this connection, a variety of enol phosphates of L-ascorbic acid was prepared and the chemical properties[4] were examined. L-Ascorbic acid 3-phosphate, in particular, was

1) Part IV: H. Nomura and S. Morimoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 335 (1971).
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3) Location: *Higashiyodogawa-ku, Osaka.*
4) H. Nomura, T. Ishiguro, and S. Morimoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 381, 387 (1969); H. Nomura and K. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **14**, 1039 (1966).

distinguiched from L-ascorbic acid on enhanced stabilization against alkali treatment, oxidation and heating. The compound was ntiscorbutically active by oral or percutaneous administrations.[5] Furthermore, for some kind of pigmented dermatoses remedial effects have been shown by the percutaneous application.[6] In these cases L-ascorbic acid 3-phosphate has been ultimately dephosphorylated into the parent L-ascorbic acid by phosphatases in the living cells. This suggests that there is ample possibility for L-ascorbic acid 3-phosphate to be used as an excellent ingredient of the pharmaceuticals as well as cosmetics. In view of the current progress of kinetic studies on the hydrolysis of organic phosphates, it will be interesting to make a detailed study on the hydrolysis mechanism of L-ascorbic acid 3-phosphate which is regarded as a vinylogous acylphosphate possessing a unique leaving moiety. The experiments described in this paper were carried out for the purpose of obtaining definite information concerning the stability against hydrolysis, especially the main features of the pH dependency on the rate of hydrolysis.

Experimental

Reagent——All reagents used were of anlytical grade without further purification. Deuterium oxide and deuterochloric acid used were of 99.8 mole percent *D* purity and were purchased from Showa Denko Co. (Japan) and Ciba Products Ltd. (Switzerland), respectively. Water containing 8.6% of ^{18}O was from Miles Laboratories (U.S.A.). L-Ascorbic acid 3-phosphate[4] ($C_6H_6O_9P \cdot Ca3/2 \cdot 2H_2O$ $[\alpha]_D^{24} = +57.5$ ($c=1.0$ in H_2O)) and methylphosphate[7] used in the experiments were prepared by the method of the previous reports.

Instruments——For all pH measurements, Hitachi-Horiba pH-meter model M-4 was used. Determinations of the optical densities were made on Hitachi spectrophotometer model EPU-2A or Hitachi automatic UV spectrophotometer. Mass spectra were measured on Hitachi RMU-6D spectrometer.

Acid Ionization Constants and Mole Fraction——a) Spectrophotometric Method: The ionization constants of L-ascorbic acid 3-phosphate were determined essentially by the same technique as described in an earlier work.[8] The standard stock solution of L-ascorbic acid 3-phosphate was prepared by dissolving 156.7 mg of the magnesium salt in 50 ml of distilled water and stored at 20°. Using a pipett, accurately measured aliquots of the solution (1 ml) were transferred to 20 ml volumetric flasks and diluted to the mark with aqueous sulfuric acid of desired concentration varing from 0.5 to 40% (w/v). The measurement of the optical densities (210 to 270 mμ) was performed at a constant temperature (28.5°). Immediately after the sample preparation using the same solution of sulfuric acid as reference. The pK_1 value was obtained by plotting the absorbance at the acidity function of the sulfuric acid solution. The second and third ionization constants were determined as follows. Each L-ascorbic acid 3-phosphate solution (0.528×10^{-3}M) in 0.5M Britton-Robinson buffer[9] varing from pH 2.1 to 12.0 (17 different buffers) was prepared. The ionic strength of each solution was adjusted to $\mu=0.2$ with KCl. Buffer solutions of the same pH and ionic strengths were used as controls. The pK values were obtained by plotting the absorbance at 250 mμ against the pH value of the buffer solution.

b) Potentiometric Titration: The determination of pK_2 and pK_3 was performed as previously described.[4] The ionic strength was adjusted to $\mu=0.2$ by KCl.

Mole Fractions——The mole fractions of various ionic species of L-ascorbic acid 3-phosphate present in a given solution were evaluated from the measured pH and the ionization constants according to the procedure described by Chanley.[10] The calculation was carried out by using the pK_1 at 20° and the pK_2 and the pK_3 at 43°.

Buffer Solutions——The compositions of the buffers which were used in the present experiments are the same as those given by Stene[11] (Tables I and II). The pH values of the buffers were determined at room temperature 20° and corrected to the values at the reaction temperatures by the simple linear interpolation.

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Hydrolysis of L-Ascorbic Acid 3-Phosphate——We have determined the rate of hydrolysis of L-ascorbic acid 3-phosphate at several temperatures (50°, 100°, partly 80° and 90°).

At all pHs and temperatures, the same procedure was employed. Solutions (5 ml) of 0.07M L-ascorbic acid 3-phosphate were delivered with a pipett into volumetric flasks and diluted with appropriate buffers to make up to 100 ml respectively. The resulting solutions were initially 3.5 mM in the concentration of the substrate and the final ionic strengths were individually adjusted to $\mu=0.2$ with KCl unless otherwise indicated. Aliquots (10 ml) of each solution were sealed in glass ampoules and placed in a constant temperature bath which was regulated by thermostat with $\pm0.3°$ precision. At appropriate intervals, the ampoules were taken out and cooled in ice–water. Before analysis, the temperature of the solutions was adjusted to 20°. Since the present phosphate is too unstable in the presence of molybdate to permit the determination of inorganic phosphate, the L-ascorbic acid 3-phosphate remained was analyzed by the colorimetric method as described in the preceding paper.[1] The pH of the solutions was measured at the beginning and the end of each run. The pH drift was generally less than ±0.05 units; for runs with pH drift of more than 0.05 units, the average pH values were employed.

Isotope Effect of D_2O on the Hydrolysis Rate——The hydrolysis of L-ascorbic acid 3-phosphate was carried out in deuterium oxide. The experimental conditions were given in Table IV. For the determination of the pD values, the following equation[12] was used.

$$pD = \text{pH mether reading} + \frac{4.29\times10^2}{T} - 1.04 \qquad \text{Eq. 1}$$

Products of Solvolysis——A buffered methanol–water solution (25—67%) of L-ascorbic acid 3-phosphate ($C_6H_6O_9P\cdot Ca3/2\cdot 2H_2O$; 3.5×10^{-3}M) was allowed to be hydrolyzed in a sealed tube at 100°. The hydrolysate, *ca.* 8 μl, was spotted on a Whatman No. 1 filter paper (9×9 inches) and chromatographed (descending) for 18 hr with propanol–water–acetic acid–trichloroacetic acid (70:20:2.5:2.5). A mixture containing methyl phosphate, KH_2PO_4 and $Na_4P_2O_7$ was spotted alongside the hydrolysate. Determination of the products was carried out as previously described.[13]

Hydrolysis in $H_2{}^{18}O$——a) The Monoanion Hydrolysis: Calcium salt of L-ascorbic acid 3-phosphate ($C_6H_6O_9P\cdot Ca3/2\cdot 2H_2O$; 35 mg ($1\times10^{-4}$ mole)) and 0.1 ml of 2N hydrochloric acid (2×10^{-4} mole) were dissolved in 3 ml of water containing 8.67 atom % of ^{18}O. The solution was sealed in a glass ampoule and placed in a boiling water bath for 7 hours. The reaction mixture, after being neutralized, was chromatographed on Dowex-1-bicarbonate (1×20 cm) with 0.2M sodium bicarbonate. The fraction containing inorganic phosphate was collected and concentrated *in vacuo* to a small volume. Silver nitrate was added to the residue and the resulting precipitate of silver phosphate, after being collected by centrifugation and dried, was suspended in chloroform and allowed to react with ethyl iodide to yield triethylphosphate as pale yellow oil. NMR (60 Mc, $CDCl_3$): 1.33 (9H, triplet, $J=7.0$ cps, CH_3), 4.11 (6H, multiplet, $J_1=7.0$, $J_2=7.2$ cps, $-CH_2-$). Mass Spectrum m/e: 182 (M^+).

b) The dianion and the trianion of L-ascorbic acid 3-phosphate were respectively hydrolyzed under the condition given in Table VIII, followed by the working up as mentioned above. The results of mass spectrometric analysis are presented in Table VIII.

Result and Discussion

Ionization Constants

The first ionization constant of L-ascorbic acid 3-phosphate could not be determined at the temperature higher than 20° due to the rapid hydrolysis of the phosphate in a strong acid. Therefore the value at 20° was used for the calculation of the mole fraction of monoanionic species.

The second and the third ionization constants pK_2 and pK_3 at 28.5° and 43.5° obtained in the present study were given below.

Since the temperature dependence of an ionization constant has been found to be relatively small,[14] pK values at 43.5° may well be used, without any correction, for the calculation of the mole fraction of the various ionic species at 50°, where the kinetic measurements were performed. As shown in Fig. 3, a good agreement can be seen between the calculated (solid

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Temp. (°C)	pK_1	pK_2	pK_3
28.5	0.01[a]	3.27[a]	6.70[a]
43.5	—	3.30[b]	6.75[b]

a) Measured by the spectrophototometric method.
b) Measured by the potentiometric method.

line) and experimental values (open circle) of the rate constant *vs.* pH's, thus suggesting that no significant error would be involved in the estimation of the pK values. Based on the comparison of the ionization constants of L-ascorbic acid 3-phosphate with those of monoalkyl phosphoric acids,[15a] especially those of phosphoenol pyruvic acid,[15b] the pK_2 of L-ascorbic acid 3-phosphate is assigned to the ionization of the enol group at C_2 and therefore the pK_3 will be the second ionization of the phosphoryl group. The pK_1 value of L-ascorbic acid 3-phosphate was found to be extremely low as compared with those of simple monoalkyl phosphates.[15,16]

The greater acidity of the present phosphate may be primarily due to strong intramolecular hydrogen bonding of the hydroxyl hydrogen[16] either at C_2 or at C_5 to the negative center of the ionized phosphoryl group (IIIa, IIIa'). The strong ionization can be also ascribed to the increased dipolar character of L-ascorbic acid moiety[17] which may arise from the electron-attracting carbonyl group conjugated with double bond.

Rates of Hydrolysis

In each instance apparent first-order kinetics with respect to L-ascorbic acid 3-phosphate were obtained through the course of the hydrolysis.

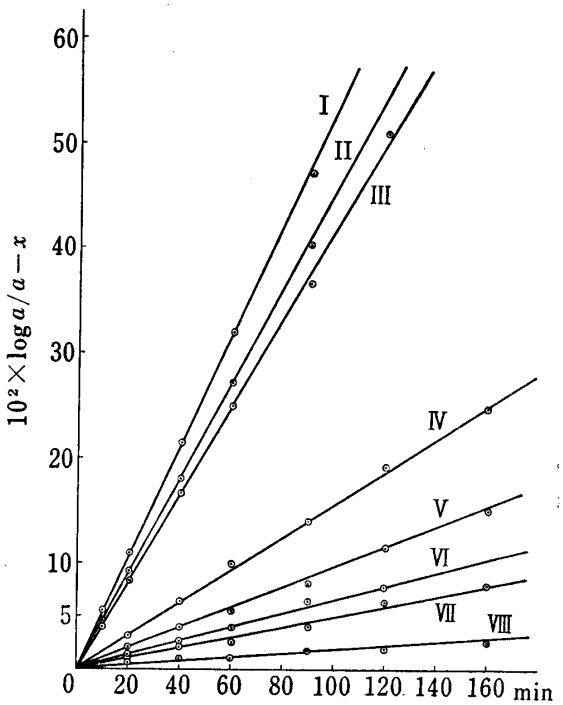
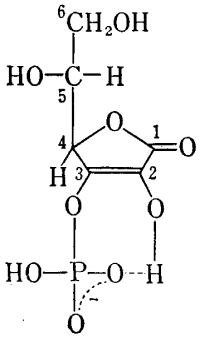
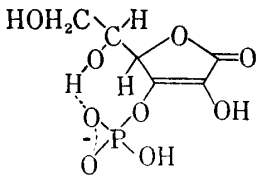


Fig. 1. Hydrolysis of L-Ascorbic Acid 3-Phosphate at 100° at Various pH Values:[a] I, 1.85; II, 2.85; III, 3.17; IV, 4.28; V, 4.96; VI, 5.73; VII, 6.73; VIII, 8.32

a) All these reaction gave satisfactory first-order kinetics and the values of rate constants k_{obsd} were calculated from slopes of plots of $\log a/a-x$ *vs.* time, where a is an absorbance of reaction mixture at zero time and $a-x$ is at time t.



IIIa



IIIa'

15) *a*) J.R. Van Wazer, "Phosphorus and its compound," Vol. 1, Interscience Publishers Inc., New York, 1958, p. 538; *b*) W. Kiessling, *Biochem. Z.*, **273**, 105 (1934).
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17) E.R.H. Jones and M.C. Whiting, *J. Chem. Soc.*, **1949**, 1423.

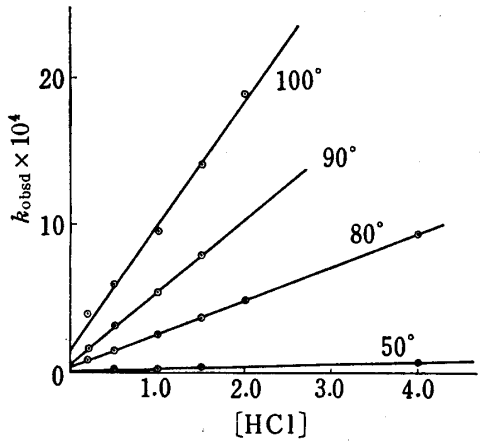

Fig. 2. Acid Catalyzed Hydrolysis of L-Ascorbic Acid 3-Phosphate at Various Temperature

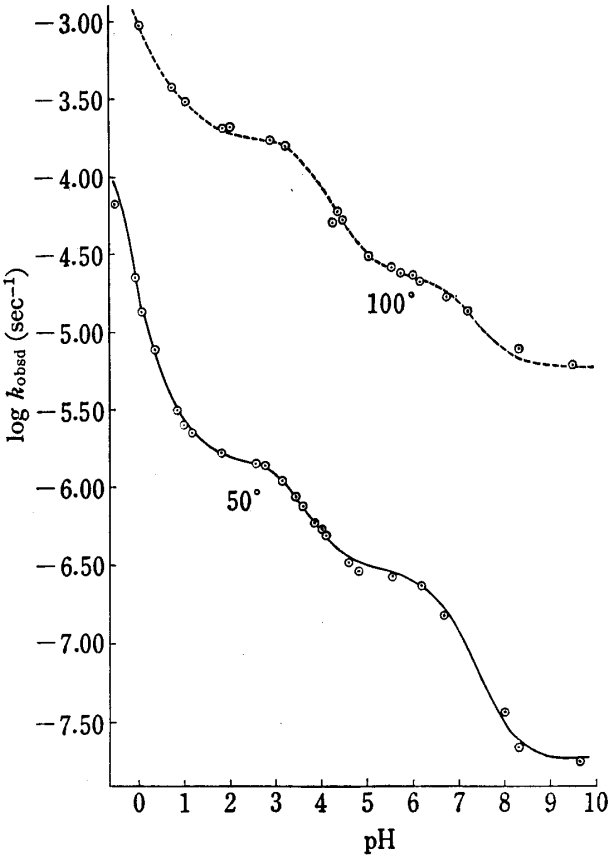

Fig. 3. Plot of log k_{obsd} against pH at 50° and 100°

Solid line, calculated: broken line, drawn through experimental points; ⊙, exprimental point plotted from data presented in Table I, II and III.

The pH rate profiles over the pH range 1—10 at 50 and 100° and at the ionic strength of μ=0.2M are illustrated in Fig. 3. In contrast to the most other phosphormonoesters[10,18-27] the hydrolysis of L-ascorbic acid 3-phosphate has no maximum rate at pH ~4. In low pH range the rate of hydrolysis decreases steadily with increasing pH value, and then, flattens around the pH ~2 where the monoanion species (III) is predominant. In the pH range higher than 4, the rate of hydrolysis decreases stepwise with plateaus at pH ~5.5 and ~7.5. This fact shows that the hydrolysis is not dependent on an apparent pH value but on the mole fraction of the respective ionic species. Chart 1 and, more concretely, equation 2 illustrate the reactions under consideration. Each of the ionic species is hydrolyzed with a respective specific rate constant.

$$k_{obsd} = k_a[H^+]\frac{[AH_3]}{C} + k_N\frac{[AH_3]}{C} + k_1\frac{[AH_2^-]}{C} + k_2\frac{[AH^{2-}]}{C} + k_3\frac{[A^{3-}]}{C} \qquad \text{Eq. 2}$$

where $[H_3A]/C$, $[H_2A^-]/C$, $[HA^{2-}]/C$ and $[A^{3-}]/C$ are molar fractions of the neutral species,

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21) *a*) L. Kugel and M. Halman, *J. Org. Chem.*, **32**, 642 (1967); A. Lapidot, D. Samuel, and M. Weiss-Broday, *J. Chem. Soc.*, **1964**, 637; *b*) C.A. Bunton, D. Kellerman, K.G. Oldham, and C.A. Vernon, *J. Chem. Soc.*, B, **1966**, 292.
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the monoanion, the dianion and the trianion, and k_a, k_N, k_1, k_2, and k_3 are their associated specific rate constants.

Table I. Rates of Hydrolysis of L-Ascorbic Acid 3-Phosphate in Hydrochloric Acid Solutions[a]

HCl (M)	$10^4 \times k_{obsd}$ (sec^{-1})			
	100°	90°	80°	50°
0.2	3.84	1.55	0.798	—
0.5	5.87	3.10	1.30	0.078
1.0	9.48	5.40	2.47	0.138
				0.138[c]
1.5	14.1	7.96	3.66	0.232
2.0	19.0	—	4.94	0.321
4.0	—	—	9.55[b]	0.703[b]
				0.755[b]

a) Ionic strength of the solutions were adjusted to 2.5 with KCl unless otherwise indicated.
b) ionic strength, μ=4.0
c) ionic strength, μ=1.0
d) ionic strength, μ=5.0

Table II. Rates of Hydrolysis of L-Ascorbic Acid 3-Phosphate at 50° as a Function of pH

pH[a]	Buffer solution (M)[b]	$10^6 \times k_{obsd}$ (sec^{-1})
0	HCl (1.0)[c]	13.4
0	HCl (1.0)[d]	13.8
0	HCl (1.0)[e]	13.8
0.80	HCl (0.155)	3.12
0.86	HCl (0.132)[f]	2.57
1.09	HCl	2.25
1.75	HCl	1.68
2.52	KHP (0.02)–HCl	1.39
2,70	HCl	1.42
2.73	HCl[g]	1.28
3.09	KHP[h] (0.02)–HCl	1.05
3.40	KHP (0.02)–HCl	0.862
3.55	HCl	0.762
3.83	KHP (0.02)–NaOH	0.587
3.99	KHP (0.02)–NaOH	0.530
4.05	KHP (0.05)–NaOH	0.506
4.58	KHP (0.02)–NaOH	0.342
4.76	KHP (0.05)–NaOH	0.296
5.54	KHP (0.02)–NaOH	0.267
6.20	AcONa[i] (0.03)–HCl	0.231
6.35	KHP (0.02)–NaOH[g]	0.175
6.41	KHP (0.02)–NaOH	0.128
8.00	KHP (0.02)–NaOH	0.082
8.33	$NaHCO_3$ (0.05)–NaOH	0.052
9.60	H_3BO_3 (0.02)–NaOH	0.060

a) Corrected to a reaction temperature (50°).
b) Ionic strength (μ) of the buffer solutions were adjusted to 0.2 with KCl otherwise indicated.
c) μ=3.5 *d*) μ=2.5 *e*) μ=1.0 *f*) μ=0.7 *g*) μ=0.8
h) KHP=potassium hydrogen phthalate *i*) AcONa=sodium acetate

Table III. Rates of Hydrolysis of L-Ascorbic Acid 3-Phosphate at 100°

pH[a]	Buffer solution (M)[b]	$10^5 \times k_{obsd}$ (sec^{-1})
0	HCl (1.0)	94.8
0.301	HCl (0.5)	58.7
0.699	HCl (0.2)	38.4
1.03	HCl (0.1)	30.1
1.75	HCl (0.022)	20.6
1.75	HCl (0.022)	19.4[c]
1.85	HCl (0.02)	20.2
2.85	KHP (0.02)–HCl	17.3
3.17	KHP (0.02)–HCl	15.9
4.28	KHP (0.02)–HCl	5.97
4.33	KHP (0.02)–HCl	4.56
4.41	KHP (0.02)–NaOH	5.38
4.96	KHP (0.02)–NaOH	3.08
4.96	KHP (0.02)–NaOH	2.56[c]
5.54	KHP (0.02)–NaOH	2.61
5.73	KHP (0.02)–NaOH	2.46
6.07	KHP (0.02)–NaOH	2.42
6.11	KHP (0.02)–NaOH	2.30
6.73	KH_2PO_4 (0.011)–NaOH	1.68
7.18	KH_2PO_4 (0.011)–NaOH	1.50
7.23	KH_2PO_4 (0.011)–NaOH	1.39
8.32	H_3BO_3 (0.014)–NaOH	0.93
9.60	H_3BO_3 (0.02)–NaOH	0.71

a) Corrected to a reaction temperature (100°).
b) Ionic strength (μ) of the buffer solutions were adjusted to 0.2 with KCl otherwise specified.
c) μ=1.0

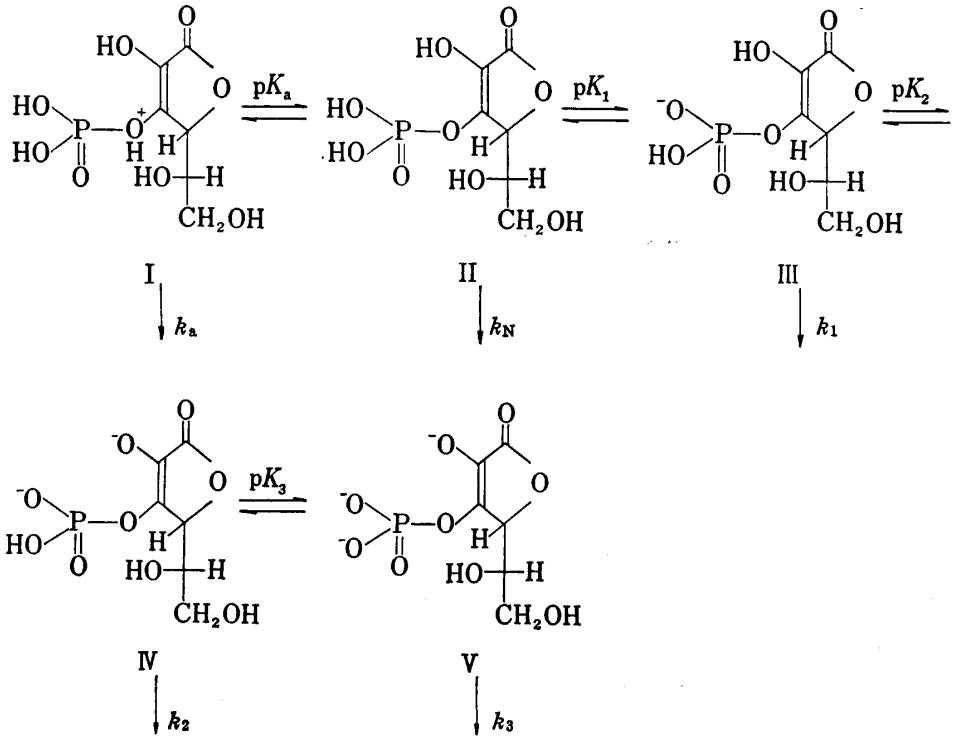

Chart 1

Acid Hydrolysis

In an aqueous hydrochloric acid solution, the hydrolysis rate of L-ascorbic acid 3-phosphate increases linearly in proportion with the stoichiometric acidity of the solution (Fig. 2). From the slope, the rate constant for the acid-catalyzed reaction, $k_a=1.3\times10^{-5}\cdot1$ mole^{-1} sec^{-1} (at 50° and at $\mu=2.5$) was obtained. A curve given by plotting log k_{obsd} against log [H$^+$] is reasonably linear from the range [H$^+$]=1 to 4.0 with a slope of 1. These results can be accounted for by the suggestion that the hydrolysis of the conjugate acid of L-ascorbic acid 3-phosphate proceeds by the participation of water molecule.[28] Similarly, plots of log k_{obsd} +Ho *vs.* log H$^+$+Ho gave a straight line with a slope of 0.86 ±0.1 (ϕ). According to the Bunnetts empirical criteria,[29] this value is in the range characteristic of hydrolysis involving

Table IV. Isotope Effects of D_2O on the Hydrolysis of L-Ascorbic Acid 3-Phophate

Temp. (°C)	Medium		$k^{H_2O}_{obsd}\times10^4$ sec^{-1}	Medium		$k^{D_2O}_{obsd}\times10^4$ sec^{-1}	k_{D_2O}/k_{H_2O}
	pH	Buffer		pD[12]	Buffer		
50	—	4N HCl	0.703	—	4N DCl	1.10	1.56
80	—	1N HCl	2.47	—	1N DCl	3.63	1.47
100	1.75	HCl–H_2O	2.06[a]	1.75	DCl–D_2O	2.37	1.15
100	5.22	KHP[b]–H_2O	0.289[a]	5.22	KHP[b]–D_2O	0.518	1.79

a) The rate constant was obtained by interpolation of the plots of log k_{obsd} *vs.* pH in Fig. 3.
b) The concentration of KHP used was 0.05M.

water as a proton transfer agent. The replacement of H_2O with D_2O resulted in an increase in the rate of hydrolysis. The value k_{D_2O}/k_{H_2O} is consistent with the occurrence of a partial rate limiting protonation step and corresponds to those for the A-2 hydrolyses of *p*-nitro- and 2,4-dinitrophenyl phosphate.[25,30] The small rate increase given by the addition of potassium chloride in 4N hydrochloric acid will be due to the salt effect presumably on the neutral species rather than on the conjugate species. From the hydrolysis rates determined at several temperatures in 1.5N[31] and 4N hydrochloric acid, the entropies of activation were calculated using the Eyring's equation (Eq. 3).

$$k=\left(e\frac{KT}{h}\right)\exp\left(\frac{\Delta S^{\ddagger}}{R}\right)\exp\left(\frac{-E_{expt}}{RT}\right) \qquad \text{Eq. 3}$$

The large negative entropy of activation (Table V) is compatible with the assumption that the hydrolysis of the conjugate acid undergoes by a bimolecular mechanism.[32]

Supposing that the observed rate constant in the pH range 0—2 can be represented as the sum of those due to the hydrolysis of the neutral (II) and of the monoanionic species (III), the equation 2 may be written as follows:

$$k_{obsd}=k_N\frac{[H_3A]}{C}+k_1\frac{[H_2A^-]}{C}=k_N\left(1-\frac{[H_2A^-]}{C}\right)+k_1\frac{[H_2A^-]}{C}$$

$$=(k_1-k_N)\frac{[H_2A^-]}{C}+k_N \qquad \text{Eq. 4}$$

28) *a*) L. Zucker and L.P. Hammett, *J. Am. Chem. Soc.*, **61**, 2791 (1939); *b*) M.A. Paul and F.A. Long, *Chem. Rev.*, **57**, 1 (1957); *idem*, *ibid.*, **57**, 935 (1957).
29) J.F. Bunnett and F.P. Olson, *Canad. J. Chem.*, **44**, 1899, 1917 (1966); J.F. Bunnett, *J. Am. Chem. Soc.*, **83**, 4956 (1961); D.R. Phillips and T.H. Fife, *ibid.*, **90**, 6808 (1968).
30) C.A. Bunton and S.J. Farber, *J. Org. Chem.*, **34**, 3396 (1969).
31) In the solution above 1.5N hydrochloric acid, the rate due to the conjugate species accounts almost predominantly for the overall reaction rate.
32) L.L. Schaleger and F.A. Long, *Advan. Phys. Org. Chem.*, **1**, 1 (1963).

TABLE V. The Activation Parameters of the Hydrolysis Reactions of L-Ascorbic Acid 3-Phosphate

Medium	Temp. (°C)	k_{obsd} sec^{-1}	$E_{expt.}$ kcal/mole	$\Delta S^{\ddagger}$ *e.u.* at 50°
4N HCl[a]	80	9.55×10^{-4}	20.3	−17.1
	50	6.75×10^{-5}		
1.5N HCl[a]	100	1.41×10^{-3}	20.4	−18.7
	90	7.96×10^{-4}		
	80	3.7×10^{-4}		
	50	2.30×10^{-5}		
pH 1.75[b]	100	2.06×10^{-4}	22.7	−17.0
	90	6.33×10^{-5}		
	50	1.68×10^{-6}		
pH 5.54[c]	100	2.61×10^{-5}	21.8	−23.2
	90	9.72×10^{-6}		
	80	4.90×10^{-6}		
	50	2.67×10^{-7}		
pH 9.60[d]	100	7.1×10^{-6}	21.9	−25.7
	90	2.86×10^{-6}		
	80	1.22×10^{-6}		
	50	6.0×10^{-8}		

a) Rates were measured at 4N (μ=4.0) and 1.5N HCl (μ=2.5 with KCl) where the acid catalyzed hydrolyses are almost predominant.
b) At this pH range (μ=0.2 with KCl), the predominant species is the monoanion.
c) At this pH range (0.05M KHP buffer, μ=0.2 with KCl) the dianion is the predominant species.
d) At this pH range (0.02M borate buffer, μ=0.2 with KCl) the trianion is predominant.

where $C=[H_3A]+[H_2A^-]$. The specific rate constant of each species can be estimated graphically from the linear plot of k_{obsd} against the mole fraction[33] of the monoanion, *i.e*, the intercept represents k_N and the slope k_1—k_N. Actually, in the pH range above 0.8, k_{obsd} was linearly related to the mole fraction with a slope of $k_1-k_N=-1.01\times10^{-5}$ sec^{-1}, whereas in the more strongly acidic solution, a positive deviation from the equation 4 was observed. The deviation is due to the contribution of an acid catalyzed hydrolysis. Similar situation has been noticed in the hydrolysis of benzyl phosphate,[34] glucose-1-phosphate[35] and *p*-nitrophenyl phosphate.[25] As a consequence the rate constants of the neutral species and the monoanion were obtained, $k_N=1.15\times10^{-5}$ sec^{-1} and $k_1=1.44\times10^{-6}$ sec^{-1}. The results indicate that in contrast to a series of simple monoalkylphosphate,[18-20,25,27] the neutral species of L-ascorbic acid 3-phosphate undergoes hydrolysis much more readily than the monoanion. Similar anomalies have been reported in the case of hydrolysis of *tert*-butyl phosphate,[21a] neopentyl phosphate,[21b] benzyl phosphate[34] and glucose-1-phosphate.[35] The pH rate profile calculated from equation 4 using the values of k_N and k_1 showed a good agreement with k_{obsd} in the range above pH 0.8—2.5. Moreover if contribution of the conjugate species are taken into a account and these specific rate constants, k_a, k_N and k_1, are substituted into equation 2 (where $[HA^{2-}]$ and $[A^{3-}]$ are negligible), the range of the exact agreement is extended from pH 2.5 to below pH zero.

Monoanion and Dianion

In the pH range 2.5—5.5, only the monoanion and the dianion must be considered. The variation of the rate constant given by equation 2 where $[H_3A]$ and $[A^{3-}]$ are negligible may be represented as

33) The mole fractions of the neutral species and the monoanion, $[H_3A]/C$, $[H_2A]/C$, present at any given pH were estimated by using the ionization constant, k_1 determined at 28.5°. The numerical calculation was referred to the foot note, 8*a*.
34) J. Kumamoto and F.H. Westheimer, *J. Am. Chem. Soc.*, **77**, 2515 (1955).
35) C.A. Buton, D.R. Llewelly, K.G. Oldhen, and C.A. Vernon, *J. Chem. Soc.*, **1958**, 3588.

$$k_{obsd} = k_1 \frac{[H_2A^-]}{C} + k_2 \frac{[HA^{2-}]}{C} = k_1 + (k_2 - k_1)\frac{[HA_2^-]}{C} \qquad \text{Eq. 5}$$

where $C=[H_2A^-]+[HA^{2-}]$. By the similar procedure described in the preceeding section, the specific rate constants were determined as follows.

$k_1=1.67\times10^{-6}$ sec^{-1} at 50°

$k_2=2.65\times10^{-7}$ sec^{-1} at 50°

The hydrolysis of the monoanion proceeds 6.3 times faster than that of the dianion which in turn undergoes about 6 times more rapidly than that of the trianion as shown later. From these specific rate constants and the pK values a theoretical curve of the pH-rate profile can be constructed (50°) as shown in Fig. 3 and fits well with an individual experimental point.

The hydrolysis rates of monoanion of alkyl or aryl phosphates have been known to be predicted with the equation 6.[36,37]

$$\log k = 0.91 - 0.27\ pK_a \text{ in min}^{-1} \text{ at } 100° \qquad \text{Eq. 6}$$

However, a negative deviation from the line has been noted when a leaving alcohol from the phosphoric monoester is more acidic than phenol, as in the case of 2,4-dinitrophenyl phosphate (pK_a=4.07).[36] Since the pK_a of L-ascorbic acid is 4.25, this type of deviation of the rate is expected on the hydrolysis of L-ascorbic acid 3-phosphate. Actually, the rate is much smaller than the predicted value, the deviation from the above equation being by a factor of about 48 in rate, or 6.3 in pK_a and the magnitude being much larger than of 2,4-dinitrophenyl phosphate.[36] This unusually low reactivity of the monoanion of L-ascorbic acid 3-phosphate is probably attributed to electronic effect caused by the conjugation of *d*-orbitals in the phosphorus atom with the electron withdrawing group *via* the ester oxygen. This effect may reduce the polarity of the ester P–O bond and decrease either the dissociation into methaphosphoric acid or the electrostatic interaction with an approaching nucleophile. The vicinal substituents may also decrease reactivity of the present monoanion.[37]

The studies on the hydrolysis of L-ascorbic acid 3-phosphate monoanion provide several mechanistic criteria as follows:

1) The inorganic phosphate formed by the hydrolysis of the monoanion in an ^{18}O-enriched aqueous solution was chromatographically isolated and analyzed for its ^{18}O-content by mass spectrometry. The results given in Table VIII show that the hydrolysis proceeds mainly by P–O bond fission which is in accord with the foregoing analyses for monoanions of phosphoric acid monoesters.[27]

2) The monoanionic species of the present phosphate showed characteristically a small activation energy as well as a more negative entropy of acitvation than those found for common monophosphate monoanions[27] (Table V).

3) Addtion of an organic solvent generally destabilizes a strongly polarized transition state and retards A-2 hydrolysis.[28b,38] As described in Table VI the decreased solvolytic rate of the monoanion was observed.

4) The deuterium solvent isotope effect, k_{D_2O}/k_{H_2O}=1.15, found for hydrolysis of the monoanion is in accord with the A-2 mechanism in which proton transfers between oxygen atoms are assumed to be important in the rate limitting step but in which the values of k_{D_2O}/k_{H_2O} are close to unity or large.[30,39]

36) A.J. Kirby and A.G. Varvoglis, *J. Am. Chem. Soc.*, **89**, 415 (1967).
37) C.A. Bunton, E.J. Fendler, E. Humeres, and K. Yang, *J. Org. Chem.*, **32**, 2806 (1967).
38) C.K. Inogold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N.Y., 1953, p. 345; C.A. Bunton and S.G. Perry, *J. Chem. Soc.*, **1960**, 3070.
39) R.H. De Wolfe, K.M. Ivanetich, and N.F. Perry, *J. Org. Chem.*, **34**, 848 (1969); C.A. Bunton and R.H. De Wolfe, *ibid.*, **30**, 1371 (1965).

Table VI. Solvolysis of L-Ascorbic Acid 3-Phosphate in Methanol-, Ethanol-, and Other Organic Solvents Water Mixtures

Solvent	k_{obsd} (sec^{-1})[a]	
	Monoanion	Dianion
H_2O	2.06×10^{-4} (100°)	2.89×10^{-5} (100°) 2.80×10^{-7} (50°)
50% CH_3OH–H_2O	5.85×10^{-5} (100°)	1.11×10^{-5} (100°)
50% EtOH–H_2O	6.58×10^{-5} (100°)	9.26×10^{-6} (100°)
50% dioxane–H_2O	9.20×10^{-5} (100°)	—
50% acetonitril–H_2O	—	2.47×10^{-7} (50°)

a) To a 100 ml volumetric flask, L-ascorbic acid 3-phosphate ($C_6H_9O_9P\cdot Ca3/2\cdot 2H_2O$, 3×10^{-3} mole) and calculated amount of 0.1N HCl (or dry HCl in methanol) were transferred. The resulting mixture was diluted with water–organic solvent mixture given in the table and made up to the mark. The ionic strength is adjusted to 0.1 with KCl and an appropriate buffer agent. A kinetic procedure was performed by the method already mentioned.

5) Experiments on the solvolysis of the monoanion in mixed aqueous methanolic solvents showed that relative to that for the dianion the molar ratio, methyl phosphate/inorganic phosphate, formed was close comparatively to the molar ratio, methanol/water present in the solvent mixture (Table VII). This fact leads to the suggestion that the reactive intermediate, methaphosphoric acid, is liberated in the hydrolysis.[27,36]

Table VII. Percents of Methylphosphate, *ortho*-Phosphate and Condensed Inorganic Phosphates Formed in the Solvolysis in Methanol–Water Mixtures at 100°

Solvent composition		Solvolysis products of L-ascorbic acid 3-phosphate[a]								
		Monoanion			Dianion			Trianion		
MeOH–H_2O v/v%	Mole% of MeOH in the solvent mixture	Mole % of			Mole % of			Mole % of		
		CH_3-H_2PO_4	H_3PO_4	$H_4P_2O_7$	CH_3-H_2PO_4	H_3PO_4	$H_4P_2O_7$	CH_3-H_2PO_4	H_3PO_4	$H_4P_2O_7$
25:75	12.5	19.6[b]	73.2	7.1	0.3[c]	37.6	62.0	—	—	—
50:50	29.0	22.5[b]	69.3	8.3	4.3[c]	55.6	40.1	10.4[d]	89.6	0
66.5:33.5	47.0	26.7[b]	49.4	24.2	23.4[c]	25.0	51.0	—	—	—

a) The methanol–water solution of L-ascorbic acid 3-phosphate was allowed to hydrolyze at 100° for *b*) 3 hr, *c*) 10 hr, *d*) 15 hr.

These criteria are considered to be characteristic of reactions that the rate limiting step is the slow proton transfer to the substrate followed by fragmentation to metaphosphoric acid and to L-ascorbic acid (route a in Chart 2). However, it has been shown that metaphosphate reacts with methanol producing methyl phosphate much more readily than expected from the molar ratio, methanol/water in methanol–water solution, since methanol is a more effective nucleophile than water.[36,40] Therefore the product distribution studies appear to indicate a possibility that part of the reaction involves slow protonation concerned with simultaneous attack of nucleophile upon the monoanionic species (route b). The proposed pathway are shown in Chart 2.

As for the hydrolysis of the dianion, we can provide the following several mechanistic criteria:

40) J.D. Chanley and E. Feageson, *J. Am. Chem. Soc.*, **85**, 1181 (1963); C.A. Bunton, E.J. Fendler, and J.H. Fendler, *ibid.*, **89**, 1221 (1967).

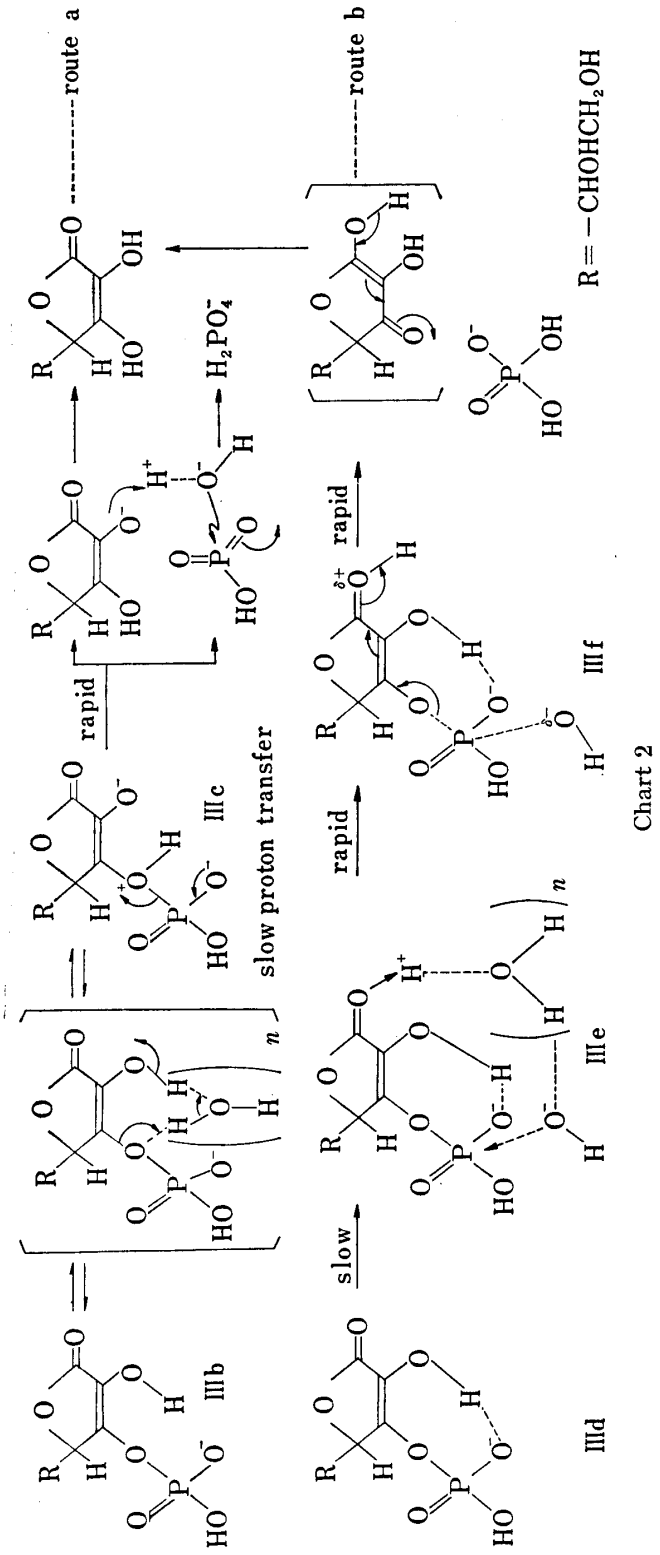

Chart 2

1) The isotope experiments using $H_2{}^{18}O$ showed that the hydrolysis of the dianion proceeded mainly by way of P–O bond fission (Table VIII).

2) The hydrolysis rate of the dianion is slow in that the reaction possesses low apparent activation and large negative entropy of activation. The Arrhenius parameters suggest that the transition state is more hydrated than the initial state[32] (Table V).

3) The solvolysis in methanol–water mixture at 100° showed that methyl phosphate formation was considerably small relative to the molar fraction of methanol in the reaction mixture. The result is contrasted to that for the monoanion and incompatible with a mechanism which requires the decomposition to the monomeric metaphosphate ion.

4) The value of the deuterium isotope effect was greater than unity, $k_{D_2O}/k_{H_2O}=1.79$. Generally, nucleophilic attack by water in the rate limiting step is expected to show a solvent isotope effect of k_{D_2O}/k_{H_2O} close to 0.5.[41] While, hydrolysis catalyzed by the protonation of the leaving group may be expected to show an opposite effect $k_{D_2O}/k_{H_2O}>1$, since the transfer of a proton to a basic oxygen should always have a larger equilibrium constant in D_2O than in H_2O.[41] The value of k_{D_2O}/k_{H_2O} in the present case approximates to those found for the hydrolyses which proceed by slow proton transfer as the rate limiting step as exemplified by *p*-nitrophenyl and 2,4-dinitrophenylphosphate (k_{D_2O}/k_{H_2O}= 1.4—1.5).[25,30]

5) As shown in Table VI the hydrolysis rate of the dianion decreased by addition of organic solvents.[38]

6) During the solvolysis of the dianion in methanol–water the formations of *ortho-*, *pyro-* and a small amount of trimetaphosphate were observed (Table VII). Such condensed inorganic phosphate formation which could scarcely be observed in the case of the monoanion

41) K.B. Wiberg, *Chem. Rev.*, **55**, 718 (1955); C.A. Bunton and V.T. Shiner, *J. Am. Chem. Soc.*, **83**, 3214 (1961); F.H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).

Table VIII. % of Entering Oxygen at H_3PO_4 produced by the Hydrolysis at 100°

Species	Substrate[a] mg (mole)	Solution (ml)	a (H_2O, atom % excess ^{18}O)	Hydrolysis reaction time (hr)	b (H_3PO_4, atom % excess ^{18}O)	100 b/a
Monoanion	35 (1×10^{-4})	1/15N HCl (3.1)	8.3	7	6.2	74.5
Dianion	35 (1×10^{-4})	1/30N HCl (3.1)	8.3	27	6.7	81
Trianion	35 (1×10^{-4})	1/30N NaOH (3.1)	7.1	27	5.1	72

a) calcium salt of L-ascorbic acid 3-phosphate ($C_6H_6O_9P\cdot Ca3/2\cdot 2H_2O$)

may be accounted for by the reaction mechanism which proceeds by the attack of orthophosphate anion to the electrophilic center of the dianion (IVc in Chart 3).

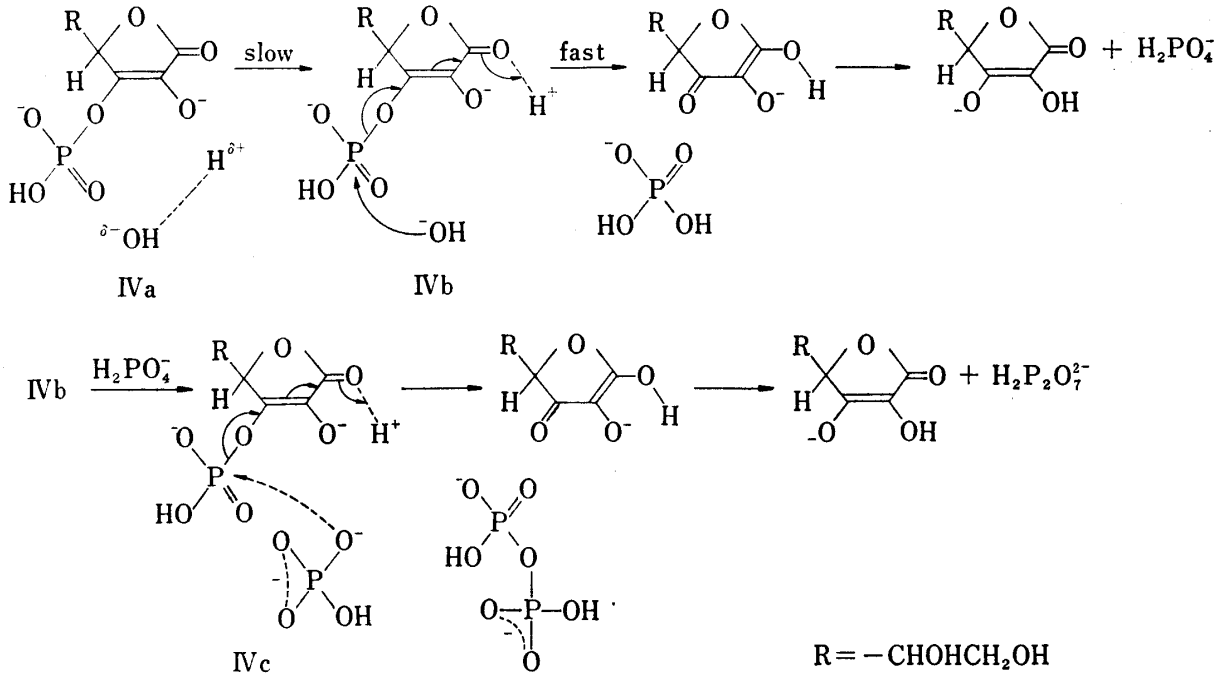

Chart 3

Based on these observations, it is tempted to propose the mechanism for the dianion hydrolysis as in Chart 3. The rate determining step may involve the protonation to the carbonyl oxygen concerted with the simultaneous nucleophilic attack by hydroxide ion (IVb). It should be noted, that the mechanism is quite different from those for the hydrolyses of a series of simple monoalkylphosphate monoanions.[27] The difference may be attributed to the unique structure of L-ascorbic acid 3-phosphate which can be regarded as a vinylogous acylphosphate or as an activated enolphosphate.

Recently, Clark, *et al.* have classified a numerous activated phosphate into P-XYZ systems in which the element Z must be capable of accommodating the electrons of the P-X bond.[42] According to the concept, L-ascorbic acid 3-phosphate is regarded as a sort of P-XYZ (X=oxygen, Y=vinylogous carbonyl carbon, Z=carbonyl oxygen) and therefore becomes a phosphoryl transfer agent under the condition leading to electron withdrawal from the carbon–carbon double bond, *e.g.*, by protonation to Z. In other words, electrophilic

42) V.M. Clark and D.W. Hutchinson, *Progress in Org. Chem.*, 7, 75 (1968); V.M. Clark, D.W. Huchinson, A.J. Kirby, and S.G. Warren, *Angew. Chem. Intern. Ed. Engl.*, 3, 678 (1964).

attack by proton on Z must reduce p_π–d_π bonding between phosphorus and X (oxygen). This should assist both attack of a nucleophile on phosphorus and breaking of the existing P–O bond. The present mechanism is considered as in Chart 3.

Trianion

In the pH range above 5.5 only the di- and trianion must be considered.

$$k_{obsd}=k_2[HA^{2-}]/C+k_3[A^{3-}]/C$$
$$=k_2+(k_3-k_2)[A^{3-}]/C \qquad \text{Eq. 6}$$

where $C=[HA^{2-}]+[A^{3-}]$.

The specific rate constants $k_2=2.80\times10^{-7}$ sec^{-1} and $k_3=4.5\times10^{-8}$ sec^{-1} (at 50°) were obtained from the equation. The values for k_2 agrees well with that obtained in the preceeding section. The extreme stability of a number of simple alkyl phosphates in alkaline medium has been reported by numerous investigators.[18,25,27] The trianion of L-ascorbic acid 3-phosphate no way alters the above generalization in spite of the presence of a neighboring hydroxyl group.[43]

Decrease in the hydrolysis rate of the trianion is accounted for by a negative value in the activation entropy (Table V). Presumably the large negative charge surrounding the phosphorus atom in the trianion may restrict the attack of a nucleophile. Based on the mechanistic criteria which are very similar to those of the dianion, *i.e.*, the activation parameters, P–O bond cleavage and insignificant formation of methyl phosphate on the methanol–water solvolysis, the hydrolysis of the trianion may be interpreted by the mechanism analogous to that assumed for the dianion.

Acknowledgement The authors express their gratitude to Dr. S. Tatsuoka, General Manager of the division, and Drs. J. Manaka and T. Masuda for their kind encouragement; H. N. wishes to thank Drs. M. Nishikawa, Y. Asahi, and H. Mima for very helpful discussions of our results.

43) C.A. Bunton and H. Chaimovich, *J. Am. Chem. Soc.*, **88**, 4082 (1966).