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Solvolysis of Organic Phosphates. V. Participation of the Neighboring Groups and Effects of Metal Ions in the Hydrolysis of 3-Hydroxy-2-pyridylmethyl Phosphate[1]

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The spontaneous hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate was investigated at 60, 70, and 80°C over the $-\log[H^+]$ range from 1 to 10 in aqueous media with an ionic strength of 0.10. The reactions followed apparent first-order kinetics with respect to the unreacted phosphate species, and the maximum rate was observed at $-\log[H^+]$ 7—8. Reactive species in hydrolysis were neutral zwitterion, monoanion, and dianion species, the reactivity increasing in this order. This trend in reactivity order has not been observed previously. In a moderately acidic region ($-\log[H^+]<2.0$), kinetic data were consistent with the acid catalysis acting on the zwitterion species. Activation parameters provide an evidence for the unimolecular nature of the transition state in spontaneous hydrolysis and suggest the A1 process for the acid catalyzed reaction. The anomalously high reactivity of the dianion species was attributed to participation of the 3-hydroxy group in the reaction process: intramolecular transfer of the hydroxyl proton to the ester oxygen in a pre-equilibrium stage, followed by the P–O bond cleavage in the subsequent transition state to give the products. Hydrolysis was also carried out in the presence of copper(II), nickel(II), zinc(II), aluminum(III), iron(III), and thorium(IV) ions at 60°C and $\mu=0.10$ in a relatively lower pH range. Introduction of the 3-hydroxyl group to 2-pyridylmethyl phosphate resulted in a change of catalytic status of various metal ions. Only iron(III) and thorium(IV) ions accelerated the rate to a significant extent. Validity of the two requirements originally proposed for the bivalent metal catalysis was extended to the polyvalent metal catalysis.

Intramolecular catalysis in the ester hydrolysis is much interesting from a biochemical viewpoint. In the enzymic reaction, the enzyme-substrate complex is formed prior to the rate-determining catalysis reaction as represented by

$$E + S \rightleftharpoons ES \rightarrow P \qquad (1)$$

Thus, the enzymic reaction follows first-order kinetics and the loss of entropy in the transition state is rather small. The geometry of the enzyme-substrate complex at the binding site is the major controlling factor for enzyme-specificity rather than the structural environment at the catalysis site. Such mechanistic criteria may also be applied in an analogous sense to the general organic intramolecular catalysis.[2] Recently, investigations of intramolecular catalysis in the ester hydrolyses, particularly in the hydrolyses of phosphates and sulfates, have been extensively carried out. Among them, hydrolyses of salicyl phosphate,[3] *S*-(2-carboxyphenyl)phosphorothioate,[4] and salicyl sulfate[5] are the cases of carboxyl group participation in the intramolecular general acid catalysis. Benkovic and Dunikoski have found the intramolecular acid catalysis by the imidazole-group participation in the hydrolysis of an imidazole substituted phenyl sulfate.[6] Meanwhile, we have actively investigated the

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[1] Contribution No. 231 from the Department of Organic Synthesis, Faculty of Engineering, Kyushu University. Preliminary communication: Y. Murakami, J. Sunamoto, and H. Ishizu, *Chem. Commun.*, **1970**, 1665.

[2] Y. Murakami and J. Sunamoto, *Kagaku no Ryoiki*, **25**, 800, 892, 989, and 1072 (1971).

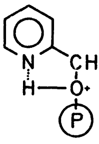
[3] M. L. Bender and J. M. Lawlor, *J. Amer. Chem. Soc.*, **85**, 3010 (1963).

[4] T. H. Fife and S. Milstein, *J. Org. Chem.*, **34**, 4007 (1969).

[5] S. J. Benkovic, *J. Amer. Chem. Soc.*, **88**, 5511 (1966).

[6] S. J. Benkovic and L. K. Dunikoski, Jr., *Biochem.*, **9**, 1390 (1970).

kinetics for the hydrolyses of aryl and alkyl phosphates containing a hetero-aromatic ring in their leaving alcohol groups. In this series of investigations, a unique intramolecular general acid catalysis has been confirmed in the hydrolyses of 2-pyridylmethyl,[7] 6-methyl-2-pyridylmethyl,[8] 2-pyridylethyl,[8] and 8-quinolyl phosphates.[9] We believe that the hydrolyses of the first three phosphates are the first examples of an intramolecular catalysis by the hetero-aromatic moiety in the hydrolyses of the open chain alkyl phosphates. The specific rate enhancement observed in the hydrolysis of these phosphates has been attributed to the participation of hetero-aromatic nitrogen. The intramolecular proton-transfer from pyridyl or quinolyl nitrogen to the ester oxygen seems to be facilitated in the pre-equilibrium step through a five- or six-membered chelate-ring formation, as illustrated by structures **1**, **2**, and **3**. In this work, we designed a competing state of affair for the intramolecular general acid catalysis and investigated the hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate which has an additional functional group, 3-hydroxy, to 2-pyridylmethyl phosphate in the proximity of a phosphate moiety. As a result, the present work clarifies which neighboring group, 1-pyridinium or 3-hydroxy group, provides more significant intramolecular catalysis in the hydrolysis.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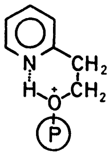
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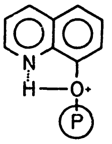
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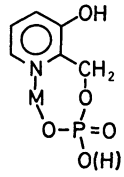
Ⓟ: phosphate moiety

We also studied the metal-ion catalysis in the hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate. Previously, we investigated the metal-ion catalysis in the hydrolyses of pyridylmethyl phosphates,[7] and 3-pyridyl and 8-quinolyl phosphates.[10] From these studies, we have proposed two requirements for metal ions to yield catalytic efficiency.[7,10] (1) For preliminary chelate formation, the metal ion should form an appropriate chelate of significant stability with an organic phosphate prior to the hydrolysis reaction. (2) For transitional chelate formation, the metal ion should demonstrate an appropriate affinity for the ester oxygen in the transition state, which would give rise to formation of a chelate ring involving the ester oxygen.

3-Hydroxy-2-pyridylmethyl phosphate has three chelating sites. Thus, three kinds of structures for metal-chelate formation can be depicted as shown below by structures **4**, **5**, and **6**. The extent of catalysis due to these metal chelates is subject to the structural nature of the phosphate and the coordination affinity of a metal ion for the donor atoms. We qualitatively investigated the catalytic effects of various metal ions, *i.e.*, copper(II), nickel(II), zinc(II), aluminum(III), iron(III), and thorium(IV). Thus, the present work accumulates the kinetic and mechanistic data for confirmation of the above requirements established for the bivalent metal catalysis, and provides further mechanistic criterion for the polyvalent metal catalysis.[10]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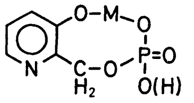
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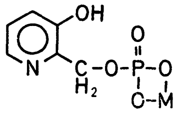
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Experimental

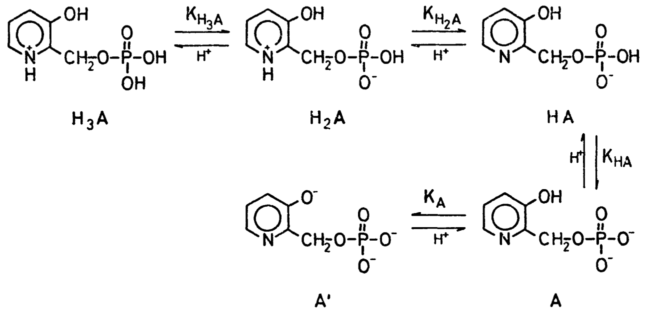
Materials. Preparation and purification of 3-hydroxy-2-pyridylmethyl phosphate have been described.[11] Other materials of analytical grade were commercially obtained and used without further purification. The stock solutions of metal ions were prepared from their nitrate salts. The solutions were standardized by means of the usual chelatometric titration.

Kinetic and Potentiometric Measurements. The apparatus and experimental procedures were essentially the same as those described previously.[9,10]

Determination of Acid Dissociation Constants and Specific Rate Constants. The overall rate constant k_0 is given by a summation of each product of a specific rate constant (k) and the corresponding mole fraction (X) over the all ionic species as represented by

$$k_0 = k_{A'}X_{A'} + k_A X_A + k_{HA}X_{HA} + k_{H_2A}X_{H_2A} + k_{H_3A}X_{H_3A} \tag{2}$$

To the present phosphate, the acid dissociation processes shown in Scheme 1 can be reasonably assigned.[11] The symbols suffixed to k's and X's in Eq. (2) (*i,e.*, A′, A, HA,



Scheme 1

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[7] Y. Murakami and M. Takagi, *J. Amer. Chem. Soc.*, **91**, 5130 (1969).

[8] Y. Murakami, J. Sunamoto, and N. Kanamoto, presented at the 24th Annual Meeting of the Chemical Society of Japan, Osaka, April, 1971.

[9] Y. Murakami and J. Sunamoto, This Bulletin, **44**, 1939 (1971).

[10] Y. Murakami and J. Sunamoto, *ibid.* **44**, 1827 (1971).

[11] Y. Murakami, J. Sunamoto, H. Sadamori, H. Kondo, and M. Takagi, *ibid.* **43**, 2518 (1970).

H_2A, and H_3A) stand for the ionic species shown in Scheme 1. A mole fraction of each ionic species was calculated by the aid of acid dissociation constants[11] which were determined by the pH-titration at low temperature (25°C) over the whole pH range (1—10.0). The results are illustrated by broken lines in Fig. 1. Thus, if all the acid dissociation constants at a given temperature are known, the specific rate constant for each ionic species can be easily evaluated by means of Eq. (2). However, the acid dissociation constants obtained by potentiometric measurements at higher temperatures (60—80°C) were suspected to involve a little uncertainty. These unavoidable errors might be caused by trace hydrolysis of the phosphate during the course of measurements. Thus, under the circumstances, the dynamic acid dissociation constants and the specific rate constants were simultaneously calculated from the kinetic data by the following procedures.

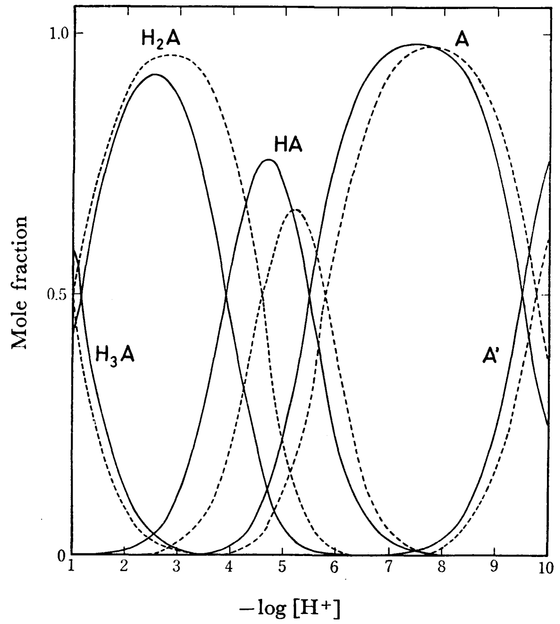

Fig. 1. Distribution of ionic species of 3-hydroxy-2-pyridylmethyl phosphate as a function of $-\log[H^+]$ at $\mu=0.10$, and at 25° (broken lines) and 60°C (solid lines). They were calculated using the pK_a values listed in Table 2.

1) Assumption is made that the trianionic species of the present phosphate (A′) is unreactive in the spontaneous hydrolysis over a pH-range 8.0—10.0 where only two species, trianionic and dianionic, are present. Then, the following relations are valid:

$$T_E = [A] + [A'] \tag{3}$$

$$k_{obs}T_E = k_A[A] \tag{4}$$

where T_E stands for the total concentration of the phosphate species and k_{obs} refers to the observed apparent first-order rate constant. The following equation is derived from Eqs. (3) and (4).

$$[H^+] = k_A\frac{[H^+]}{k_{obs}} - K_A \tag{5}$$

where K_A is the acid dissociation constant of the dianionic species. This equation indicates that a plot of $[H^+]$ *vs.* $[H^+]/k_{obs}$ should give a straight line. The slope of the line is equal to k_A and the intercept at $[H^+]/k_{obs}=0$ corresponds to K_A.

2) In a pH-range 6.0—7.5 where dianionic and monoanionic (HA) species exist exclusively, the following equations are applied to the system:

$$T_E = [HA] + [A] \tag{6}$$

$$k_{obs}T_E = k_{HA}[HA] + k_A[A] \tag{7}$$

where k_A value evaluated in Step 1 can be used. From Eqs. (6) and (7), the following relationship is given:

$$\frac{k_{obs}[H^+]}{k_A-k_{obs}} = k_{HA}\frac{[H^+]}{k_A-k_{obs}} + K_{HA} \tag{8}$$

The relationship between the left hand side and $[H^+]/(k_A-k_{obs})$ in the first term of the right hand side needs to be linear. Thus, k_{HA} and K_{HA} are to be obtained from the slope and the intercept at $[H^+]/(k_A-k_{obs})=0$, respectively.

3) For a pH-range 3.0—5.0, three phosphate species, neutral zwitterion (H_2A), monoanionic and dianionic forms, are to be taken into consideration. Then, the following equations can be given for this state of affair.

$$T_E = [H_2A] + [HA] + [A] \tag{9}$$

$$k_{obs}T_E = k_{H_2A}[H_2A] + k_{HA}[HA] + k_A[A] \tag{10}$$

where k_A and k_{HA} values evaluated in Steps 1 and 2, respectively, can be used. Combination of Eqs. (9) and (10) and the subsequent rearrangement give the following equations.

$$\frac{k_{obs}[H^+]^2}{(k_{HA}-k_{obs})[H^+] + (k_A-k_{obs})K_{HA}} = k_{H_2A}\frac{k[H^+]^2}{(k_{HA}-k_{obs})[H^+] + (k_A-k_{obs})K_{HA}} + K_{H_2A} \tag{11}$$

In a manner similar to those described in the preceding steps, k_{H_2A} and K_{H_2A} are evaluated from the plots.

4) Finally, by using all the values already obtained in the preceding steps and the kinetic data covering a pH-range 1.0—3.0 where cationic, neutral zwitterion, and monoanionic species are considered to be present, we have

$$T_E = [H_3A] + [H_2A] + [HA] \tag{12}$$

$$k_{obs}T_E = k_{H_3A}[H_3A] + k_{H_2A}[H_2A] + k_{HA}[HA] \tag{13}$$

$$\frac{k_{obs}[H^+]^2}{(k_{H_2A}-k_{obs})[H^+] + (k_{HA}-k_{obs})K_{H_2A}} = k_{H_3A}\frac{[H^+]^2}{(k_{H_2A}-k_{obs})[H^+] + (k_{HA}-k_{obs})K_{H_2A}} + K_{H_3A} \tag{14}$$

k_{H_3A} and K_{H_3A} would be evaluated from the linear plots based on Eq. (14). In general, however, the neutral species of the alkyl phosphates[12] and the monocationic species of pyridylalkyl phosphates[7,8] show much less reactivity in their spontaneous hydrolyses. Therefore, for the present phosphate the kinetic analysis due to Step 4 was discarded. Instead, an acid catalyzed hydrolysis of the neutral zwitterion species was confirmed to proceed in such a moderately acidic region more significantly. The overall rate constant k_0 is, thus, consistent with the equation

$$k_0T_E = k_{H^+}[H^+][H_2A] + k_{H_2A}[H_2A] + k_{HA}[HA] + k_A[A] \tag{15}$$

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[12] T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 2, W. A. Benjamin Inc., New York (1966), Chapt. 5.

TABLE 1. SPECIFIC RATE CONSTANTS FOR THE SPONTANEOUS HYDROLYSIS OF 3-HYDROXY-2-PYRIDYLMETHYL PHOSPHATE AT $\mu=0.10$ ($NaClO_4$ OR KNO_3)

Species	Temp. (°C)	$k_{H^+}\times 10^4$ $M^{-1}sec^{-1}$	$k\times 10^5$ sec^{-1}
Zwitterion	60	0.534	
(H_2A)	70	1.60	
	80	4.58	
Zwitterion	60		0.376
(H_2A)	70		1.55
	80		5.16
Monoanion	60		1.17
(HA)	70		4.75
	80		15.7
Dianion	60		2.52
(A)	70		9.23
	80		30.6

TABLE 2. ACID DISSOCIATION CONSTANTS OF 3-HYDROXY-2-PYRIDYLMETHYL PHOSPHATE AT $\mu=0.10$ ($NaClO_4$ OR KNO_3)

pK_a	25°C$^{a)}$	60°C$^{b)}$	70°C$^{b)}$	80°C$^{b)}$
pK_{H_3A}		1.16	1.08$^{c)}$	1.00
pK_{H_2A}	4.54	3.88	3.60	3.80
pK_{HA}	5.75	5.48	5.40	5.55
pK_A	9.67	9.48	9.40$^{d)}$	9.51

a) Obtained by means of potentiometric titration.
b) Calculated by the kinetic procedures described in the text.
c) Obtained by interpolation using values at 60° and 80°C.
d) Obtained by extrapolation using values at 25° and 60°C.

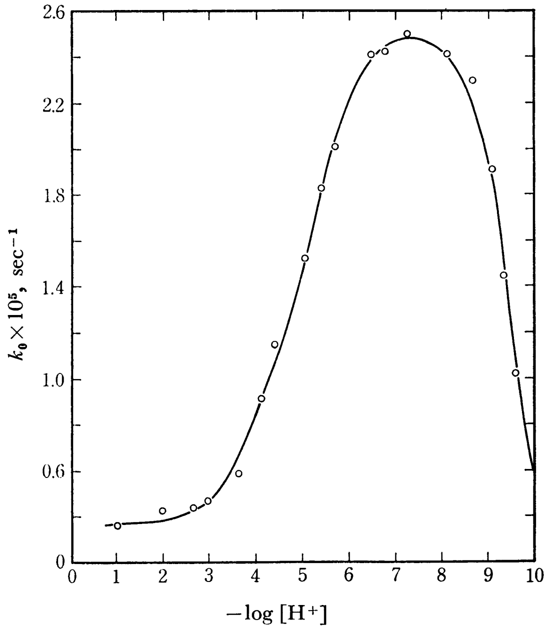

Fig. 2. Rate profile for the spontaneous hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate at 60°C and $\mu=0.10$. Solid line is a theoretical curve calculated from values listed in Tables 1 and 2 by using equation 15.

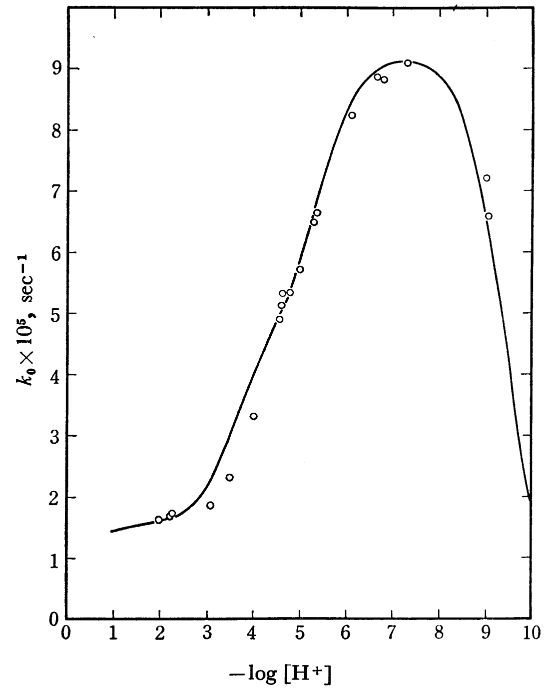

Fig. 3. Rate profile for the spontaneous hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate at 70°C and $\mu=0.10$. Solid line is a theoretical curve calculated from values listed in Tables 1 and 2 by using equation 15.

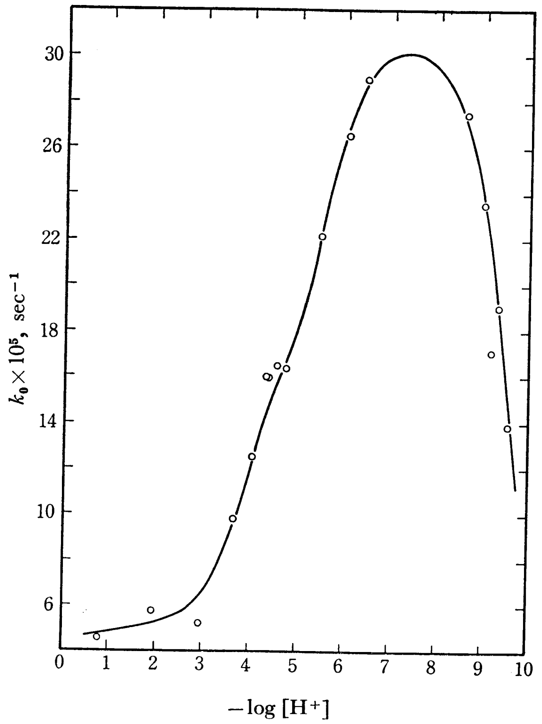

Fig. 4. Rate profile for the spontaneous hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate at 80°C and $\mu=0.10$. Solid line is a theoretical curve calculated from values listed in Tables 1 and 2 by using equation 15.

where

$$T_E = [H_3A] + [H_2A] + [HA] + [A] + [A'] \tag{16}$$

$$\left.\begin{aligned} [H_3A] &= \frac{1}{K_{H_3A}}[H^+][H_2A] \\ [H_2A] &= \frac{1}{K_{HA}}[H^+][HA] \\ [HA] &= \frac{1}{K_{HA}}[H^+][A] \\ [A] &= \frac{1}{K_A}[H^+][A'] \end{aligned}\right\} \tag{17}$$

Results

Spontaneous Hydrolysis. The specific rate constants evaluated by the preceding procedure and the acid dissociation constants are listed in Tables 1 and 2, respectively. Moreover, the agreement between the observed and the calculated rate constants at 60, 70, and 80°C is quite satisfactory as illustrated in Figs. 2, 3, and 4, respectively.

In our previous studies on the spotaneous hydrolyses of 2-, 3-, and 4-pyridylmethyl phosphates,[7] 6-methyl-2-pyridylmethyl,[8] 3-pyridyl, and 8-quinolyl phosphates,[9] the reactive species were always the neutral zwitterion and the monoanionic form, and rate-maxima in the pH-rate profiles were observed at $-\log[H^+]$ 2—3 and 5—6 for all the phosphates. In the present case, however, the maximum rate in the pH-rate profile for the spontaneous hydrolysis appeared at $-\log[H^+]$ 7—8 with a shoulder at 4.5. Consequently, the most reactive species was the dianionic form followed by the monoanion and then the neutral zwitterion in this order.

Activation Parameters. Each specific rate constant followed the Arrhenius law. The energy, enthalpy, and entropy of activation for each species are listed in Table 3. These values are quite similar in magnitude to those for the monoanions of organic phosphates previously reported,[13] and seem to indicate that all the reactive species of the present phosphate undergo hydrolysis through a unimolecular mechanism in which a pre-equilibrium proton-transfer process followed by P–O fission in the rate-determining step takes place.

Metal Ion Catalysis. In the presence of metal ions, the hydrolysis conditions were set at the upper limit pH and temperature in a range where no noticeable precipitates appeared and no partial hydrolysis of metal ions occurred. Consequently, a good homogeneous phase was maintained during the course of each run. All the reactions were carried out in the presence of equimolar amounts of a metal ion and the substrate at 60°C and $\mu=0.10$ (KNO_3). The hydrolysis reactions in the presence of copper(II), nickel(II), zinc(II), and aluminum(III) ions followed ap-

TABLE 3. ACTIVATION PARAMETERS FOR THE SPONTANEOUS HYDROLYSIS OF 3-HYDROXY-2-PYRIDYLMETHYL PHOSPHATE[a]

Species	E kcal mol^{-1}	$\Delta H^{\ddagger}$ kcal mol^{-1}	$\Delta S^{\ddagger}$ e. u.
Acid catalysis zwitterion (H_2A)	25.1[b]	24.4[b]	−5[b]
Zwitterion (H_2A)	30.6	29.9	+6
Monoanion (HA)	30.4	29.7	+8
Dianion (A)	29.1	28.5	+6

a) Errors, standard deviation, in individual rate constants are not greater than ±2%; those in E and $\Delta H^{\ddagger}$ are approximately ±0.1 kcal mol^{-1}; and those in $\Delta S^{\ddagger}$ are ±0.1 e.u.

b) Calculated from the second-order rate constants (M^{-1} sec^{-1}), k_{H^+}.

TABLE 4. METAL-ION-CATALYZED HYDROLYSIS OF 3-HYDROXY-2-PYRIDYLMETHYL PHOSPHATE AT 60°C AND $\mu=0.10$ (KNO_3)[a]

$-\text{Log}[H^+]$	M^{n+}	$k_c\times10^5$[b] sec^{-1}	$k_0\times10^5$[c] sec^{-1}	k_c/k_0
2.44	Cu^{2+}	0.388	0.403	0.95
3.53	Ni^{2+}	0.865	0.914	0.95
4.11	Ni^{2+}	0.574	0.616	0.93
3.57	Zn^{2+}	0.634	0.618	1.03
2.74	Al^{3+}	0.360	0.427	0.84
8.57	Al^{3+}	0.224	0.224	1.00
2.86	Fe^{3+}	0.635[d]	0.444	1.43
2.33	Th^{4+}	10.2[d]	0.396	*ca.* 26

a) $T_E=T_M=2.0\times10^{-3}$ M, where T_E and T_M stand for the total molar concentrations of substrate and metal ion, respectively.

b) k_c is the observed overall rate contant for the hydrolysis in the presence of metal ion.

c) k_0 is the overall rate constant for spontaneous hydrolysis.

d) Deviated from the first-order plot; estimated from the initial stage of reaction.

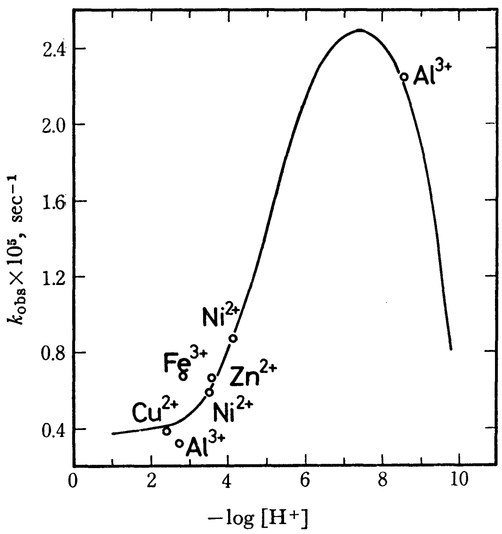

Fig. 5. Effects of metal ions on the hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate at 60°C and $\mu=0.10$ (KNO_3). $T_E=T_M=2.0\times10^{-3}$ M. Solid line represents the rate profile for spontaneous hydrolysis under the same conditions.

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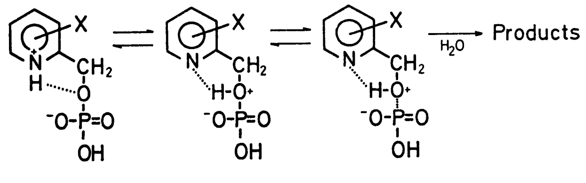
[13] J. A. Maynard and J. M. Swan, *Aust. J. Chem.*, **16**, 596 (1963).

parent first-order kinetics with respect to the total concentration of the unreacted phosphate in homogeneous phase at pH's and metal concentrations studied. However, all the metal ions showed no significant catalytic effect. On the other hand, the presence of iron(III) and thorium(IV) ions resulted in a significant acceleration of rate, although the reactions did not follow the simple first-order kinetics and the hydrolysis rates were retarded as reactions proceeded further. During the course of reactions, no formation of insoluble materials was detected and a good homogeneous system was maintained. In particular, the thorium ion showed a profound rate enhancement. The catalytic efficiency of these metal ions is listed in Table 4. The catalytic status of the present metal ions with the exception of thorium(IV) is illustrated in Fig. 5 in reference to the spontaneous reaction.

Discussion

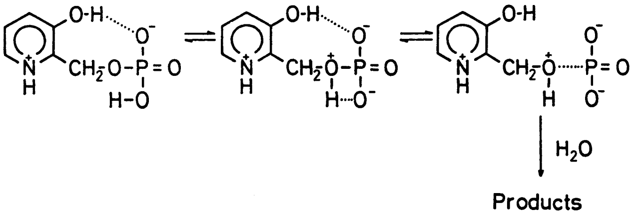
Hydrolysis of Neutral Zwitterion Species. In the hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate, the neutral zwitterion form (H_2A) was the least reactive of the three reactive species, H_2A, HA, and A. This behavior differs noticeably from other heteroaromatic phosphates studied previously.[7-9] Furthermore, for the hydrolyses of the zwitterion species of 2-pyridylalkyl phosphates and 8-quinolyl phosphate, a unique intramolecular general acid catalysis was observed upon participation of 1-pyridinium or 1-quinolinium proton which was transferred to the ester oxygen in the pre-equilibrium stage of reaction. The mechanistic aspect is shown in Scheme 2.



X= H, 6-CH$_3$

Scheme 2

Thus, for the zwitterion form of the present phosphate, the following two aspects of mechanistic criteria may be considered: (1) An intramolecular general acid catalysis played by the 1-pyridinium proton occurs and the 3-hydroxyl group provides only an electronic effect on the reaction rate as an electron-withdrawing group,[14] in a manner similar to the cases of 2-pyridylalkyl phosphates (Scheme 2). (2) An intramolecular hydrogen bonding of the 3-hydroxyl group to the terminal phosphate oxygen is formed and the phosphate proton is transferred intramolecularly to the ester oxygen, as seen in the usual hydrolyses of alkyl phosphate monoanions. Therefore, if the latter holds, the positive pyridinium group acts only as an electron-withdrawing group as illustrated in Scheme 3. If the driving force for the intramolecular proton transfer is due to a preliminary intramolecular hydrogen bonding, Scheme 3 may be more favorable for interpretation of the results than Scheme 2. Since an O–H···O bond is tighter in general than an N–H···O bond in hydrogen bonding strength, the phosphate moiety is to be fixed in space far away from the 1-pyridinium proton due to the intramolecular O–H···O bonding as shown in Scheme 3. Consequently, an intramolecular acid catalysis by the pyridinium group would not be expected, even though the present phosphate is one of 2-pyridylmethyl phosphates. Meanwhile, the absolute hydrolysis rate of the present phosphate is 2.3 times larger than that of 2-pyridylmethyl phosphate in zwitterion form at 70°C. Thus, the 3-hydroxyl group must participate in the reaction process so that the hydrolysis rate is enhanced due to its electronic effect and/or additional intramolecular interaction. As a result, there still remains some uncertainty in elucidating the hydrolysis mechanism. Further confirmation of the present mechanism waits studies of the linear free energy relationship on the hydrolysis of pyridylalkyl phosphates.



Scheme 3

Linear free energy relationships (LFER) for the hydrolyses of phosphates and sulfates have been extended by Bunton,[15] Fendler,[16] and Kirby.[17] However, all the esters they studied were aryl esters. So far, the acidity of a leaving group (pK_{OH}) has been usually adopted as the structural parameter for LFER study of ester hydrolyses, where P–O or S–O bond cleavage is the rate-determining step. The pK_{OH} value of an alcohol is, however, generally very large (13—16)[18], even those of the alcohols having an aromatic moiety,[19] compared with those of the phenols (~10).[20] It is, therefore, difficult to determine the correct and reliable acid dissociation constants of alcohols, particularly at higher temperatures, by the usual techniques. Thus, it is quite important to establish some reliable structural parameters other than acid dissociation constants of the leaving alcohols for the LFER study of the hydrolyses of alkyl phosphates.

Activation parameters E, $\Delta H^{\neq}$, and $\Delta S^{\neq}$ for the

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[14] J. M. Vundennbelt, C. Henrich, and S. G. Vandenberg, *Anal. Chem.*, **26**, 726 (1954).

[15] C. A. Bunton, E. J. Fendler, E. Humeres, and K. U. Yang, *J. Org. Chem.*, **32**, 2806 (1967).

[16] E. J. Fendler and J. H. Fendler, *ibid.*, **33**, 3852 (1968).

[17] A. J. Kirby and A. G. Varvoglis, *J. Amer. Chem. Soc.*, **89**, 415 (1967).

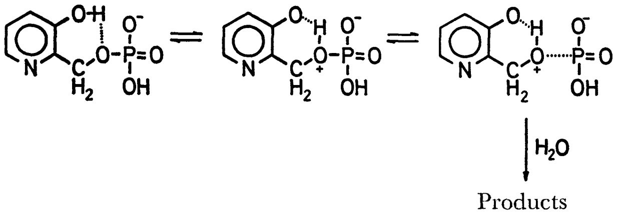
[18] P. Ballinger and F. A. Long, *ibid.*, **82**, 795 (1960).

[19] M. Tissier and C. Tissier, *Bull. Soc. Chim. Fr.*, **1967**, 3155.

[20] G. Kortum, W. Vogel, and K. Andrussow, "Dissociation Constants of Organic Acids in Aqueous Solution," Butterworths and Co., Ltd., London (1961).

hydrolysis of the zwitterion species are quite similar in their magnitude to those for other pyridylalkyl phosphates,[7,8] 3-pyridyl and 8-quinolyl phosphates.[9] Thus, it may be reasonable to assume that the nature of the transition state is essentially the same as that discussed previously, where a unimolecular P–O bond cleavage to give an alcohol and HPO_3^- occurs, irrespective of the source of the transferable proton in the pre-equilibrium stage.

Hydrolysis of Monoanionic Species. Contrary to what would be expected from the previous studies,[7-9] the monoanionic species was more reactive than the neutral zwitterion. The anomalous behavior of the monoanion must be interpreted from two aspects: (1) a simple electron-withdrawing effect of the 3-group (similar to Scheme 3), and (2) an intramolecular general acid catalysis played by the 3-hydroxyl group (Scheme 4).



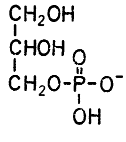
Scheme 4

If Scheme 3 is valid, the electronic effect of the 3-hydroxyl group for this species would be approximately in a similar magnitude to that expected for the zwitterion species. When the substituent effect due to the 3-hydroxyl group on the reaction rate is represented in terms of the ratio of reaction rates against 2-pyridylmethyl phosphate $k_{(3\text{-H-2-PMP})}/k_{(2\text{-PMP})}$, this value is about 2.3 for the hydrolysis of the zwitterion species. On the other hand, the rate ratio is 1.25×10^2 for the hydrolysis of the monoanionic species. This profound rate enhancement should be attributed to the additional proximity effect due to introduction of a hydroxyl group at the 3-position of 2-pyridylmethyl phosphate. Consequently, this neighboring group may be responsible for the intramolecular proton transfer in the transition state as seen in Scheme 4. The driving force for the proton transfer may be due to the preliminary intramolecular hydrogen bonding, primarily to the terminal phosphate oxygen, in which the hydroxyl group is brought about in the neighborhood of the ester oxygen. The hydrogen bonding is then switched to the ester oxygen in the pre-equilibrium stage prior to the transition state, where the stable six-membered ring is formed.

Activation parameters for the hydrolysis of the monoanion are consistent with a unimolecular mechanism accompanied with P–O bond cleavage.

Hydrolysis of Dianionic Species. Generally, the dianionic species of an organic phosphate is unreactive toward spontaneous hydrolysis, except for those of phosphate esters which have a strong electron-withdrawing group in a leaving alcohol moiety, *e.g.*, nitrophenyl and polynitrophenyl phosphates.[15,16] For the present phosphate, the dianionic species was the most reactive and the trianionic species was stable in alkaline media. This presents a unique case in which the dianionic species is more reactive than the zwitterion and monoanionic forms in the hydrolysis of alkyl phosphates. This interesting fact requires some characteristic explanation for the hydrolysis mechanism in reference to hydrolyses of the other ionic species of the present phosphate and of ordinary alkyl phosphates.

The profound rate enhancement must be ascribed to the participation of the 3-hydroxyl group situated in the proximity of the phosphate moiety. No work has been reported on the intramolecular participation of a hydroxyl group in the hydrolysis of alkyl phosphates or sulfates, which consequently gave out to some significant rate enhancement. Bender and Lawlor have shown that 8-hydroxy-1-naphthyl phosphate dianion hydrolyzed about 10-fold faster than the dianion of the 8-methoxyl ester.[3] They considered that this rate enhancement was due to the intramolecular proton transfer from the 8-hydroxyl group to the ester oxygen, although the extent of their investigation was rather limited. Glycerol-1-phosphate **7** was stable in alkaline media and had a rate maximum at around pH 4—5 in its hydrolysis in a manner similar to those observed for usual monoalkyl phosphates. Glycerol-1-methyl phosphate **8** was readily hydrolyzed in alkaline media to give a mixture of glycerol-1- and -2-phosphates, no methyl phosphate being produced.[12] Kugel and Halmann have reported the hydrolysis of 1,2-propanediol-1-dihydrogen phosphate,[21] where the phosphate was also hydrolyzed with a maximum rate at pH ~4. In this pH region, the mole fraction of the monoanion reaches its maximum. As a whole, these studies indicate that an alcoholic hydroxyl group at the β-position and/or the γ-position to the phosphate group of alkyl phosphates does not bring about any significant rate enhancement in hydrolysis.


$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2O\text{-}\overset{O}{\overset{\|}{P}}\text{-}O^- \\ \qquad\ \ |\\ \qquad\ OH \end{array}$$

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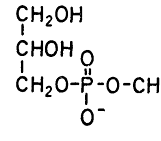
$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2O\text{-}\overset{O}{\overset{\|}{P}}\text{-}O\text{-}CH_3 \\ \qquad\ \ |\\ \qquad\ O^- \end{array}$$

8

The profound rate enhancement due to the participation of the 3-hydroxyl group may be interpreted from two different aspects: (1) nucleophilic attack of the negatively charged phenoxide group at the phosphorus atom, and (2) intramolecular transfer of the hydroxyl proton to the ester oxygen. Scheme 5 demonostrates the nucleophilic attack of the phenoxide ion at the phosphorus atom forming a cyclic transition state (or a cyclic intermediate), which is followed by the intramolecular transfer of the phosphate group to the phenolic oxygen. A similar state of affair was confirmed in the hydrolysis of glycerol hydrogen

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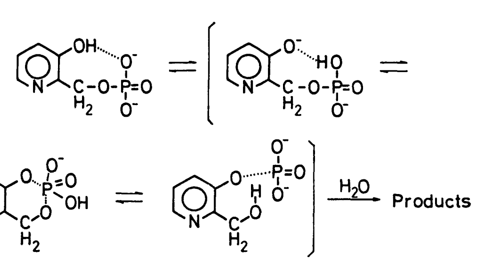
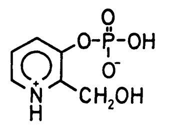
[21] L. Kugel and M. Halmann, *J. Amer. Chem. Soc.*, **88**, 3566 (1966).



Scheme 5

phosphates,[12] where a cyclic intermediate was formed and subsequently an intramolecular rearrangement of the phosphate group took place. We previously tried to isolate the cyclic phosphate **10** which would be derived from the present phosphate, and 2-hydroxymethyl-3-pyridyl phosphate under various conditions. However, the work failed to give these products, and gave 3-hydroxy-2-pyridylmethyl phosphate exclusively.[22] This suggests that the present phosphate, rather than



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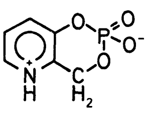

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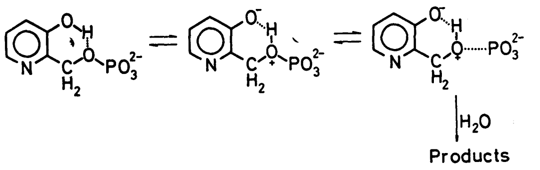
being the precursor of the cyclic phosphate **10** or of the intramolecularly rearranged phosphate **9** should be an intermediate in their hydrolytic decomposition. The dianionic species of 2-hydroxymethyl-3-pyridyl phosphate would not be as reactive as that of 3-hydroxy-2-pyridylmethyl phosphate toward hydrolysis, unless the 2-hydroxymethyl group would participate favorably in the reaction.

Thus, the alternative concept is adopted as the plausible mechanism for elucidation of the enhanced hydrolysis rate. As shown in Scheme 6, the hydroxyl



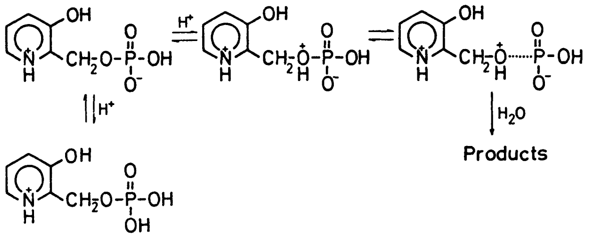
Scheme 6

proton is completely transferred to the ester oxygen in the pre-equilibrium stage, and the P–O bond is then weakened in the subsequent transition state to give the products. This mechanistic aspect is quite analogous to that employed for the hydrolysis of the monoanion as the most plausible mechanism. The six-membered ring formation would stabilize the pre-equilibrium stage in these hydrolytic pathways. For the dianionic species, however, an increase of negative charge on the phosphate moiety will make the proton transfer much more favorable. The small positive entropy of activation again suggests the unimolecular mechanism for the dianion hydrolysis.

Hydrolysis in Moderately Acidic Region. In the moderately acidic region, $-\log[H^+]$ 1.0—2.0, the hydrolysis rates of 8-quinolyl and 3-pyridyl phosphates increased in proportion to the stoichiometric concentration of acid,[9] while pyridylmethyl phosphates underwent no detectable acid catalyzed hydrolysis in a comparable acidic region.[7,8] We attributed the favorable acid catalysis for the former two aryl phosphates to the electron-withdrawing effect caused by the positively charged pyridinium or quinolinium group and also to resonance stabilization given to the generated alcohol of phenolic nature. For the present phosphate, the hydrolysis in a moderately acidic region was not subject to a marked acid catalysis along with the change of hydrogen ion concentration. Under these circumstances, two reaction mechanisms of kinetic equivalence are to be considered: (1) unimolecular spontaneous reaction of the monocationic species, and (2) acid catalyzed reaction of the zwitterion form.

If one assumes that the monocationic species is reactive in the hydrolysis of the present phosphate, the specific rate constants are $k_{H_3A}=0.368\times10^{-5}$, 1.33×10^{-5}, and $4.58\times10^{-5}\,\mathrm{sec}^{-1}$ at 60, 70, and 80°C, where $pK_{H_3A}=1.16$ (60°C), 1.08 (70°C), and 1.00 (80°C), respectively. From these kinetic data, the activation parameters may be estimated: $E=29.5\,\mathrm{kcal\,mol}^{-1}$, $\Delta H^{\neq}=28.8\,\mathrm{kcal\,mol}^{-1}$, and $\Delta S^{\neq}=+3$ e.u. The specific rate constants and the activation parameters cited here are not necessarily unfavorable to this mechanism. However, we can not show which one of the four protons may be transferred to the ester oxygen at the pre-equilibrium stage in this case.

Under consideration of the results previously obtained on the reactivity of the monocationic species,[7] this species should be much less reactive and the neutral zwitterion is subject to an acid catalysis in this moderately acidic region. The kinetic data and the corresponding activation parameters were evaluated from this aspect as listed in Tables 1 and 3. The entropy of activation in Table 3 suggests that the acid catalysis for the present phosphate proceeds through A1 process as shown in Scheme 7.



Scheme 7

Effects of Metal Ions. We proposed previously that the two successive interactions were required to yield the metal ion catalysis.[10] Thus, the hydrolysis reaction of 3-hydroxy-2-pyridylmethyl phosphate was investigated in the presence of various metal ions in order to examine the validity and applicability of the proposed mechanisms.

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[22] Y. Murakami, J. Sunamoto, K. Morooka, and H. Kondo, presented at the Local Meeting of the Chemical Society of Japan, Fukuoka, July, 1968.

3-Hydroxy-2-pyridylmethyl phosphate is related in structure to 2-pyridylmethyl phosphate. Therefore, it may be expected that the copper(II) ion would have a significant catalytic efficiency for the hydrolysis of the present phosphate. Contrary to what would be expected, the copper ion as well as other bivalent metal ions showed no detectable catalysis. When the reaction mixture prepared at pH above 4 in the presence of copper(II) was poured into a reaction vessel, a blue insoluble material appeared about ten minutes after the addition and simultaneously the pH value of the mixture decreased sharply. Apparently, a certain copper complex was produced with liberation of protons. The precipitates were insoluble in almost all of the polar and nonpolar organic solvents as well as in water. In order to examine the nature of the complex, we tried to prepare a complex of 3-hydroxy-2-pyridylmethanol with copper(II): equimolar amounts of the alcohol and copper nitrate were dissolved in water, pH was adjusted to 4—6 using 0.1 N-sodium hydroxide, and the resulting blue precipitates were isolated. The IR spectrum of this copper complex was compared with that of the complex produced during the course of hydrolysis. They differ from each other and the latter is more related to the spectrum of the substrate ester as shown in Fig. 6. As can be seen from Fig. 6-c, absorption bands assigned to the stretching vibration of P–O–C and P=O bonds (900—1200 cm^{-1}) are still observed in the spectrum of the complex formed in the hydrolysis. As a result, the complex is not the one with the hydrolysis product, but that with the unreacted substrate. With the knowledge obtained from the copper catalysis on the hydrolyses of 2-pyridylmethyl[7] and 8-quinolyl[10] phosphates, we can eliminate possibility of the formation of complexes **4** and **6**. If the copper chelate is primarily in structure **4**, the rate enhancement due to the copper ion must be necessarily obtained for the present hydrolysis. Thus, we may assign either **5** or **11** to the blue complex obtained during the reaction on the basis of the affinity of the copper ion for nitrogen and/or oxygen donor atoms. There also remains possibility that the complex would be of polymeric nature. Although we are not ready to give a persuasible geometry for the complex, precipitation of the substrate-copper complex may also cause a catalytic inertness of the copper ion. The 3-hydroxy group controlled the geometry of the complex formed in the preliminary equilibrium stage, which resulted in null catalytic efficiency of copper(II) in this work.

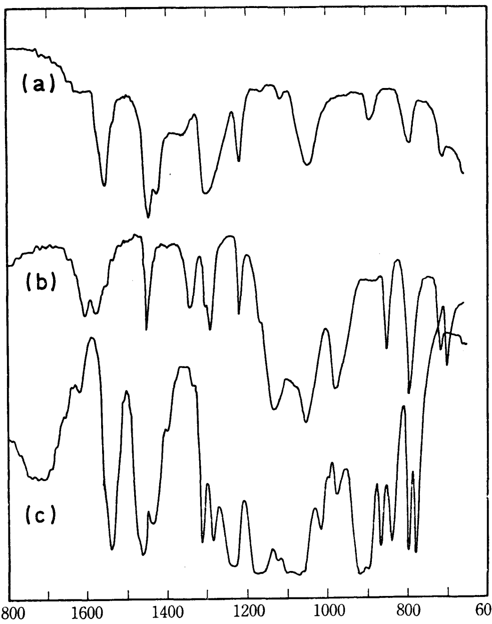
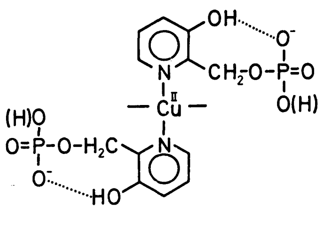


Fig. 6. Infrared spectra (KBr disk method): (a), the copper(II) complex of 3-hydroxy-2-pyridylmethanol; (b), the complex precipitated during the hydrolysis reaction of 3-hydroxy-2-pyridylmethyl phosphate in the presence of copper(II) (see text); (c), 3-hydroxy-2-pyridylmethyl phosphate.



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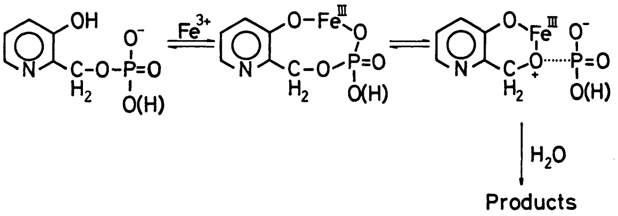
For 2-pyridylmethyl[7] and 8-quinolyl[23] phosphoates, the iron(III) ion demonstrated a negative catalytic effect in both hydrolyses. On the other hand, the metal ion showed a large positive catalytic efficiency in the hydrolysis of salicyl phosphate.[24] We ascribed these opposite effects to the difference in affinity of the ferric ion for nitrogen and oxygen donor atoms.[25,26] The iron(III) ion usually demonstrates a larger affinity for an oxygen donor atom rather than for a nitrogen atom. The donor atom existing in the leaving alcohol group of the former two phosphates is nitrogen, while that of the latter phosphate is carboxyl oxygen. 3-Hydroxy-2-pyridylmethyl phosphate has donor atoms of both kinds, the 1-pyridinium nitrogen and the 3-hydroxyl oxygen. Thus, it is of quite interest to examine which donor atom is more effective for catalysis, in reference to the catalytic inertness of the copper ion which demonstrates a larger affinity for a nitrogen donor atom. We see from Fig. 5, that the iron(III) ion shows a positive catalytic effect although the reaction did not follow the strict first-order kinetics. At this stage, validity of the two requirements for the ef-



Scheme 8

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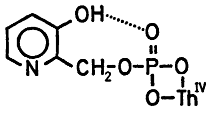
[23] Y. Murakami, J. Sunamoto, and H. Sadamori, *Chem. Commun.*, **1969**, 983.

[24] R. Hofstetter, Y. Murakami, G. Mont, and A. E. Martell, *J. Amer. Chem. Soc.*, **84**, 3041 (1962).

fective metal ion catalysis, originally proposed for the bivalent metal ions, is extended to the trivalent metal ion catalysis and a reaction mechanism is put forward in Scheme 8. We take it for granted that this scheme is the most plausible mechanism for the iron catalyzed hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate.

The thorium(IV) ion demonstrated a profound catalytic effect in the hydrolyses of organic phosphates, irrespective of the nature of a leaving alcohol moiety.[10,25] We could, therefore, expect a significant catalysis also for the hydrolysis of the present phosphate. The reaction rate was progressively depressed and consequently deviated from the first-order plot. Nevertheless, a significant effect was observed by all means. For the thorium catalysis observed in the hydrolyses of 3-pyridyl and 8-quinolyl phosphates, the extent of catalytic efficiency in terms of k_c/k_0 was about 10^3 at $-\log[H^+] \sim 2.0$.[10] For the present phosphate, the hydrolysis rate was accelerated only by 30-fold. Although we are not ready to interpret this large difference in catalytic efficiency at present, there is no doubt as to the participation of the 3-hydroxyl group in the present thorium ion catalysis. Judging from the fact that this metal ion does not show a large substrate specificity in hydrolysis relative to other metal ions and possesses the large stability constants with inorganic phosphate,[27] we proposed previously that the catalytic site may be located at the phosphate moiety. The preliminary chelate formation may occur at the terminal phosphate oxygen atoms as illustrated by structure **6**. If this is the case, the relatively strong intramolecular hydrogen bonding between the 3-hydroxyl proton and the phosphate oxygen may interfere with the preliminary chelate formation, shown by structure **12**. Consequently the extent of the catalytic efficiency would be reduced. In addition, the steric hindrance and the inductive effect due to the presence of the 3-hydroxyl group may also affect this chelate formation. In conclusion, through our surveying studies on the polyvalent metal ion catalysis for the hydrolysis of 3-hydroxy-2-pyridylmethyl phosphate, we may advance a concept that the introduction of the 3-hydroxyl group to the 2-pyridylmethyl moiety influences to a great extent the metal ion catalysis and spontaneous hydrolysis due to its electronic and environmental nature.



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[25] Y. Murakami and M. Takagi, This Bulletin, **42**, 3478 (1969).

[26] Y. Murakami, *Nippon Kagaku Zasshi*, **91**, 185 (1970).

[27] L. G. Sillén and A. E. Martell, "Stability Constants of Metal-Ion Complexes," Special Publication No. 17, The Chemical Society, London (1964).