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Solvolysis of Organic Phosphates. II. Pyridylmethyl Phosphates as Effected by the Presence of Polyvalent Metal Ions*1

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Influence of polyvalent metal ions on the hydrolysis of 2-, 3- and 4-pyridylmethyl phosphate was investigated at μ=0.10 in a lower pH range. The thorium ion accelerated the hydrolysis rate of all the phosphates to a remarkable extent as investigated at 40, 70 and 90°C. The primary participation of the phosphate moiety of each pyridylmethyl phosphate in the interaction with thorium(IV) was considered to be the main cause of this catalysis. Thus, the thorium catalysis was mechanistically distinguished from the copper catalysis which has been clarified previously. In addition, the vanadyl ion also demonstrated positive catalytic effect. On the other hand, iron(III) and aluminum(III) retarded the hydrolysis rate. This was ascribed to the reduced fraction of the active monoionic species as a result of metal-complex formation with the phosphates in the course of hydrolysis.

The effect of metal ions on the hydrolytic reactions of phosphate monoesters is an interesting aspect of metal-ion catalysis, and has been the subject of a number of research groups.[1] However, previous investigations have not provided any satisfactory explanation for the reaction mechanism involved in the catalysis.

In continuation of our previous work[2] on the hydrolysis of the three isomeric pyridylmethyl phosphates, *i.e.*, 2-pyridylmethyl phosphate, 3-pyridylmethyl phosphate and 4-pyridylmethyl phosphate, the catalytic effects of polyvalent metal ions on these hydrolytic reactions were investigated in the present work. The hydrolysis of phenyl phosphate was also studied in the presence of some metal ions in an attempt to obtain an additional information on the catalytic reaction mechanism.

Experimental

Source of the organic phosphates and apparatus used in the present work have been described previously.[2] The stock solutions of metal ions were prepared from their nitrate salts, with the exception of vanadyl ion which was obtained as sulfate. These solutions were standardized by means of chelatometric titration.

Kinetic Measurements. The initial concentration of a substrate in solution was adjusted to 2×10^{-3} M and the ionic strength was maintained at 0.10 with an inorganic salt such as potassium nitrate, potassium chloride or sodium perchlorate. The rate of hydrolysis was determined by measuring the amount of inorganic phosphate liberated in the course of reaction. The pH-value of a solution was adjusted to a desired value by adding standard acid or base (0.1 N perchloric acid or 0.1 N sodium hydroxide). The experimental solution was made up to 100 m*l* in total volume and placed in a thermostated cell. After a desired temperature was attained, an aliquot sample (usually 2.5 m*l*) was taken out at an appropriate time interval and placed in a test tube. The tube was quickly cooled in cold water, and a specified volume (usually 2 m*l*) of the sample was pipetted into a 25-m*l* volumetric flask which contained a cold mixture of 60 % perchloric acid (2 m*l*) and water (15 m*l*). The whole solution was then kept in a refrigerator for some time before the subsequent colorimetric analysis of inorganic phosphate.

Analysis of Inorganic Phosphate. The analytical method employed for the analysis of phosphoric acid was essentially that of Allen.[3] To a mixture which contained a 2.0-m*l* sample of the experimental solution, 2 m*l* of 60 % perchloric acid and 15 m*l* of water in a 25-m*l* volumetric flask, 2.0 m*l* of amidol reagent (a 100 m*l* aqueous solution contained 1.0 g of amidol and 20 g of sodium bisulfite) and 1.0 m*l* of 8.3% aqueous ammonium molybdate were added in succession. The mixture was diluted to 25 m*l* with water, and then placed in a constant temperature bath at 25°C for 15—25 min to develop the blue color. The absorbance was measured at 720 mμ with a Shimadzu-Bausch & Lomb Spectronic 20 colorimeter immediately after development of the molybdenum blue color. The amount of inorganic phosphate was

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[*1] Contribution No. 171 from the Department of Organic Synthesis, Faculty of Engineering, Kyushu University.

1) As reviews: H. Trapmann, *Arzneim.-Forsch.*, **9**, 341, 403 (1959); T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. II, W. A. Benjamin, Inc., New York, N. Y. (1966), Chapter 5; Y. Murakami, "Topics in Chelate Chemistry and Biochemistry," Special Publication No. 79 of *Kagaku no Ryoiki*, A. Nakahara, Ed., Nankodo, Tokyo (1967), pp. 153—188.

2) Y. Murakami and M. Takagi, *J. Am. Chem. Soc.*, **91**, 5130 (1969).

3) R. J. L. Allen, *Biochem. J. (London)*, **34**, 858 (1940).

determined by referring to a calibration curve established by the use of a standard KH_2PO_4 solution. Some of the metal ions were found to interfere with the phosphate analysis to some extent. The vanadyl ion has absorption in the 720 mμ range, and the observed absorbance had to be corrected for the presence of this metal ion. On the other hand, the ferric ion was found to decrease the absorbance of the molybdenum blue slightly through interaction with the molybdenum complex, while the thorium ion to increase it appreciably. Nevertheless, the interference of the ferric ion was of a negligible order under the present experimental conditions. The absorbance increase due to the presence of the thorium ion was corrected by establishing a relevant calibration curve. The presence of chelating agents employed in this work did not show any interference with the colorimetric determination of inorganic phosphate.

pH Measurements. Since the reaction temperature was so high (usually 90°C), the glass electrode could not demonstrate a sufficiently linear response to the pH values established by standard buffers[4] over the pH range studied. Thus, calibration of the electrode system was performed by dividing the whole pH range into three parts: pH 1.0—4.2, oxalate and phthalate buffers; pH 4.2—6.9, phthalate and phosphate buffers; pH above 6.9, phosphate and borate buffers. Since the glass electrode was not durable for prolonged use at elevated temperatures in a relatively high pH range (above 6), particularly in the presence of metal ions, the electrode membrane had to be reactivated rather often by treating with aqueous ammonium fluoride. However, careful operation made it possible to achieve the pH reproducibility within ± 0.01. The pH meter was calibrated before and after each kinetic run, and the pH variation was usually controlled within ± 0.03.

pH-Values obtained from the procedure described above need to be converted into hydrogen ion concentrations. Thermodynamic data for weak electrolytes, which make the conversion of pH into hydrogen ion concentration possible, are not available at present. Thus, a conventional method was employed in this work: the pH values of aqueous perchloric acid of known concentrations were measured at an ionic strength of 0.10 to determine the apparent activity coefficient. The apparent activity coefficient thus obtained was used over the whole pH range. The following relation holds at 80°C and $\mu = 0.10$.

$$-\log [H^+] = pH - 0.09$$

Results

Polyvalent Metal Catalysis. With a 1 : 1 molar ratio of substrate to metal ion, the hydrolysis reactions of pyridylmethyl phosphates were studied at 90°C and the ionic strength of 0.10. In general, metal ions of tervalence or higher are far more susceptible to hydrolysis than bivalent metal ions. Thus, the choice of conditions for the homogeneous catalytic reaction is often restricted. The reaction followed first-order kinetics with respect to unreacted substrate species in most cases. When deviation from the first-order rate law was extreme, the apparent rate constants were evaluated from the initial stage of hydrolysis. The results for aluminum-ion and the vanadyl-ion catalysis are listed in Table 1. The equimolar existence of the aluminum ion reduces the hydrolysis rates of pyridylmethyl phosphates into about half of the corresponding spontaneous rates at $-\log[H^+] \approx 2.5$, regardless of the type of the phosphate isomers. This retardation effect is pH-dependent and is not noticeable in a lower pH region as shown in Table 1. The presence of a ligand capable of coordinating to metal ion, nitrilotriacetic acid in this case, tends to restore the rate to the spontaneous one.

The ferric ion yielded a similar catalytic effect

TABLE 1. EFFECTS OF ALUMINUM AND VANADYL IONS ON THE HYDROLYSIS OF PYRIDYLMETHYL PHOSPHATES AT 90.0 ± 0.1°C AND $\mu = 0.10$ ($HClO_4$-$NaClO_4$)[a]

Phosphate	Metal ion	$-\log[H^+]$[b]	k, hr^{-1}	$(k/k_0) \times 100$[e]
2-Pyridylmethyl	Al^{3+}	1.06	0.055	96
		1.91	0.068	87
		2.46	0.046	57
		2.47	0.078[c]	98
	VO^{2+}	1.06	0.058	101
		1.93	0.081	103
		2.67	0.20[d]	250
3-Pyridylmethyl	Al^{3+}	2.47	0.019	58
4-Pyridylmethyl	Al^{3+}	2.47	0.019	56

a) $T_E = 2.00 \times 10^{-3}$ M, $T_M/T_E = 1$ where T_E and T_M stand for the total molar concentration of the substrate species and of the metal ion, respectively.

b) Variation within ± 0.01.

c) NTA present, $T_M/T_L = 1$ where T_L stands for the total molar concentration of the ligand.

d) Deviation from the first-order rate law is large.

e) k_0, spontaneous hydrolysis rate.

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[4] Four standard buffers, oxalate, phthalate, phosphate and borate buffers, were prepared in accordance with JIS (Z8802-1964).

as observed for the aluminum-ion catalysis under the same experimental condition. As an example, the presence of equimolar amounts of the ferric ion and 2-pyridylmethyl phosphate at 90°C and $\mu=0.10$ resulted in the reduction of hydrolysis rate to 65 % and 53 % of the corresponding spontaneous rates at $-\log[\mathrm{H}^+]=1.04$ and 1.89, respectively.

A survey on the catalytic effects of other tervalent metal ions, *i.e.*, indium, lanthanum and cerium, revealed that the tendency of precipitate-formation was remarkable. Thus, the metal-ion catalysis was investigated in an acidic region ($-\log[\mathrm{H}^+]=1-2$). The results indicate these metal ions were inactive in this low pH region. For example, in the presence of equimolar amounts of lanthanum(III) and 2-pyridylmethyl phosphate the hydrolysis rates of the phosphate at 90°C were 100 % and 99 % of the corresponding spontaneous ones at 1.06 and 1.91 in $-\log[\mathrm{H}^+]$, respectively. The first-order rate law holds in both cases. However, in the latter, the first-order kinetics was disturbed after some precipitates were produced during the course of hydrolysis.

Among other polyvalent metal ions, the uranyl ion revealed the distinct tendency to form precipitates with the substrates. Thus, no reasonable experiment was carried out. On the other hand, the vanadyl ion showed an appreciable catalytic effect in promoting the hydrolysis of 2-pyridylmethyl phosphate even in a relatively low pH range as shown in Table 1. It should be noted that the hydrolysis at $-\log[\mathrm{H}^+]=2.67$ did not follow first-order kinetics, and was accelerated with the progress of the reaction. Such an acceleration effect was observed only with the vanadyl ion, although the cause of this phenomenon is not obvious at present. Meanwhile, whenever a deviation from the first-order plots was observed in other metal-ion-effected hydrolysis of pyridylmethyl phosphates, the reaction rate was invariably decelerated and the rate plot appeared below the initial first-order rate curve.

Thorium(IV) Catalysis. The hydrolytic behavior of pyridylmethyl phosphates in the presence of the thorium ion at 90°C and $\mu=0.10$ is shown in Fig. 1. The reaction rates were so rapid that the hydrolysis proceeded to a considerable extent before thermal equilibrium was attained. It seems that the excessive enhancement of the catalytic reaction rate is reduced by the decrease of the concentration of catalyst species. However, the relative deficiency of the thorium ion ($T_\mathrm{M}/T_\mathrm{E}<1$) resulted in precipitate-formation even in a strongly acidic region (pH$\approx$1) at the beginning or during the course of reaction. This is most likely due to the complex formation between thorium(IV) and the substrate species.

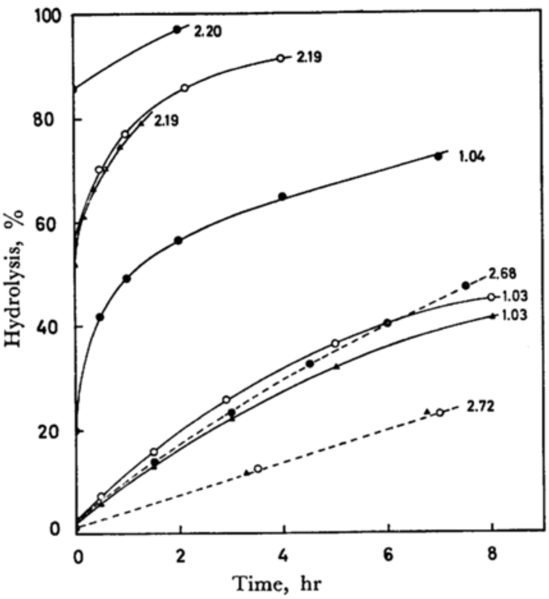


Fig. 1. Hydrolysis of pyridylmethyl phosphates in the presence of thorium(IV) ion: 90.0±0.1°C, $\mu=0.10$ ($\mathrm{HClO_4}$-$\mathrm{NaClO_4}$), $T_\mathrm{E}=2.00\times10^{-3}$ M, $T_\mathrm{M}/T_\mathrm{E}=1$; ●, 2-isomer ; ○, 3-isomer; ▲, 4-isomer; the broken lines indicates the spontaneous hydrolysis at their maximum rate; number refers to $-\log[\mathrm{H}^+]$ value. First sampling was made 15—19 min after the solution was introduced into the reaction vessel (time=0).

In order to study the catalytic effects in more detail, the hydrolysis of 2- and 3-pyridylmethyl phosphate was also investigated at lower temperatures, and the results are shown in Figs. 2, 3 and 4. As the pH value of the system was raised, an increasing tendency of precipitate-formation was observed. However, when the amount of precipitates was small, they disappeared gradually as hydrolysis proceeded to a certain extent. Thus, precipitates formed at the initial stage seem to be a thorium complex of the substrate. As the rate plots in Figs. 2 and 4 indicate, the hydrolysis curve tends to level off after a certain period of time. As shown in Figs. 2 and 3, the thorium-ion catalyzed hydrolyses of 2- and 3-pyridylmethyl phosphate reach their maximum rate at around pH 2.7 and slow down as the pH of the system departs from this optimum value. This fact suggests that essentially the same catalytic process is operative for these substrates, although the rate-promoting effect of the thorium ion for the hydrolysis of the 2-isomer was found to be considerably greater than that for the hydrolysis of the 3-isomer. The catalytic reaction rates at 40°C ($T_\mathrm{M}/T_\mathrm{L}=1$, Fig. 4) are now compared with the corresponding spontaneous rates, which were calculated by the aid of activation energies evaluated previously, in terms of the required reaction time to carry out 30 % hydrolysis. The $-\log[\mathrm{H}^+]$ value of 2.7 was selected for this comparison, since the maximum rates were observed both in the presence and in the absence of thorium: 2-isomer, 2300 hr (spontaneous), 0.8 hr (catalytic); 3-isomer, 7000 hr (spontaneous), 8.5 hr (catalytic).

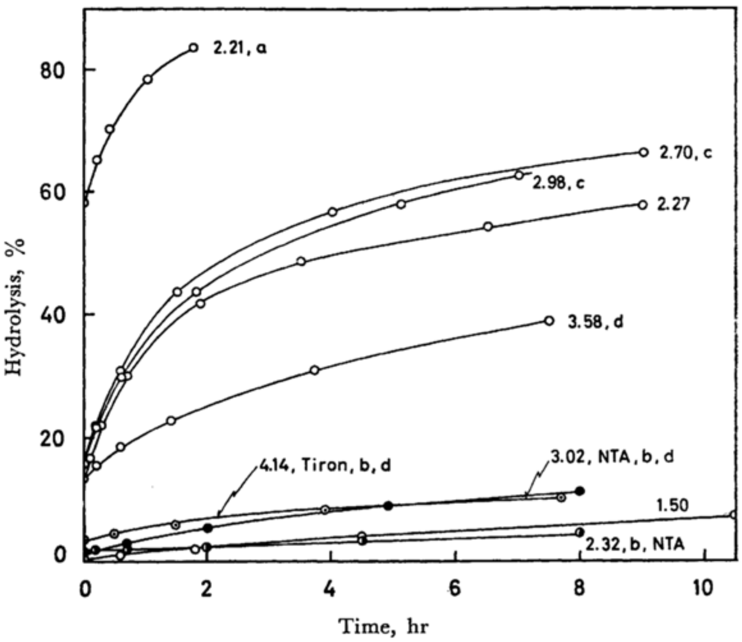


Fig. 2. Hydrolysis of 2-pyridylmethyl phosphate in the presence of thorium(IV) ion and its chelates: 40.0 ± 0.1°C, $\mu=0.10$ ($HClO_4$-$NaClO_4$), $T_E=2.00\times10^{-3}$ M, $T_M/T_E=1$; *a*, 70°C; *b*, $T_M/T_L=1$; number refers to $-\log[H^+]$ value. In some cases, precipitation was observed at the initial stage (*c*) or throughout the reaction (*d*). First sampling was made 15 min after the solution was introduced into the reaction vessel (time=0).

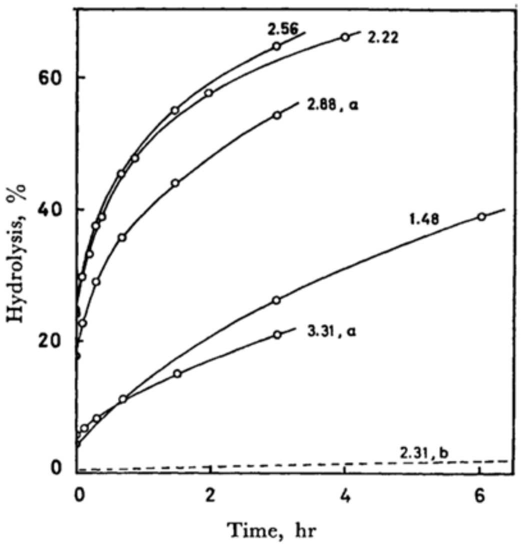


Fig. 3. Hydrolysis of 3-pyridylmethyl phosphate in the presence of thorium(IV) ion: 70.0 ± 0.1°C, $\mu=0.10$ ($HClO_4$-$NaClO_4$), $T_E=2.00\times10^{-3}$ M, $T_M/T_E=1$; a, precipitates exist; b, spontaneous hydrolysis ; number refers to $-\log[H^+]$ value. First sampling was made 15 min after the solution was introduced into the reaction vessel except the run at 1.48 and the spontaneous one at 2.31 in $-\log[H^+]$ value, where 23 min was taken instead (time = 0).

Thus, the catalytic effect of the thorium ion is striking, and the observableh ydrolysis is entirelydue to the catalytic process in the low temperature range.

The catalytic effect of the thorium ion in these hydrolysis reactions diminished when a certain kind of chelating agent was present (Fig. 2). This is probably due to the so-called masking effect of these ligands on the thorium ion, so that the affinity of the metal ion toward the substrate species was considerably reduced. The thorium-ion catalysis did not follow simple kinetic laws after all.

Hydrolysis of Phenyl Phosphate. The hydrolysis of phenyl phosphate was studied at 80°C and the ionic strength of 0.10 in aqueous media in the presence of metal ions as listed in Table 2. It should be noted that all the bivalent metal ions listed in this table tend to retard the hydrolysis rate. This is even more marked with the vanadyl and the aluminum ion, and particularly with the ferric ion. In the ferric-ion catalysis, the reaction followed first-order kinetics fairly well up to 20 % hydrolysis of the substrate initially present, but deviated from this significantly thereafter.

The thorium ion produced some insoluble precipitates of unknown character with phenyl phosphate over the wide pH range even in the presence of some chelating agents, *e. g.*, NTA or Tiron. Thus, the hydrolysis reaction in the presence of the thorium ion was inadequate for study in a homo-

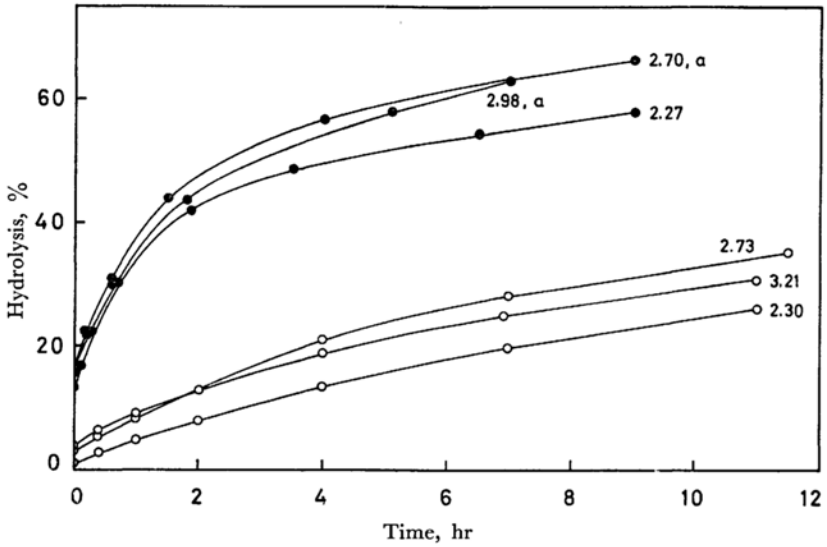


Fig. 4. Hydrolysis of pyridylmethyl phosphates in the presence of thorium(IV) ion: 40.0± 0.1°C, $\mu=0.10$ ($HClO_4$ - $NaClO_4$), $T_E=2.00\times10^{-3}$ M, $T_M/T_E=1$; ●, 2-isomer ; ○, 3-isomer; *a*, precipitates were noticed at the initial stage; number refers to $-\log[H^+]$ value. First sampling was made 15 min after the solution was introduced into the reaction vessel. (time=0).

TABLE 2. EFFECTS OF METAL IONS ON THE HYDROLYSIS OF PHENYL PHOSPHATE AT 80.0±0.1°C AND $\mu=0.10$[a, b)]

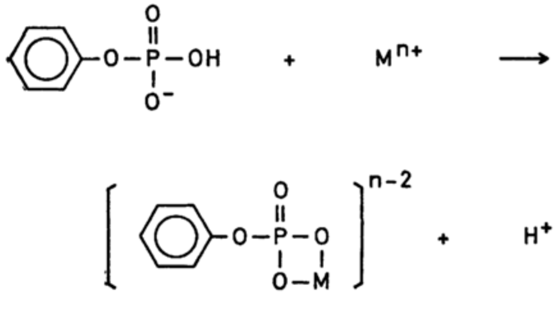
Metal ion	$-\log[H^+]$	k, hr^{-1}	k_0, hr^{-1}	$(k/k_0)\times100$	Ref.
Fe^{3+}	1.01±0.01	0.0233	0.0488	47.7	This work
Al^{3+}	1.02±0.01	0.0449	0.0495	90.7	This work
VO^{2+}	2.67±0.02	0.0990	0.1062	93.2	This work
Cu^{2+}	3.62±0.01	0.1069	0.1088	98.3	2
Zn^{2+}	4.05±0.02	0.1076	0.1083	99.4	2
Co^{2+}	4.55±0.02	0.1052	0.1060	99.2	2
Ni^{2+}	4.56±0.03	0.1050	0.1059	99.2	2
Mg^{2+}	4.61±0.03	0.1036	0.1055	98.2	2

a) $T_E=2.00\times10^{-3}$ M, $T_M/T_E=1$.
b) Supporting electrolyte: below $-\log[H^+]=2.67$, $HClO_4$ - $NaClO_4$; above $-\log[H^+]=2.67$, KNO_3.

geneous system.

Discussion

The ferric and the aluminum ion gave retardation effect to the hydrolysis of phenyl phosphate. These metal ions may form the corresponding phenyl phosphate complexes due to the following reaction.



Judging from the stability constants of metal complexes formed with inorganic phosphate (PO_4^{3-}),⁵⁾ tervalent metal ions would give much more stable metal complexes of simple organic phosphate, such as phenyl phosphate, than bivalent metal ions. Thus, the complex-formation may proceed to a certain extent even in an acidic region (pH≈1), where the metal complex is supposed to be inert, and the concentration of the active monoionic species is reduced as a result. Since iron(III) and aluminum(III) do not show significant affinity toward *N*-donor atom in general,⁵⁾ these metal ions would interact with pyridylmethyl phosphates at the phosphate group even in the case

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

of the 2-isomer. Thus, the same explanation can be given for the retarded hydrolysis rate of pyridylmethyl phosphates, regardless of metal species.

Of all other metal ions, the thorium ion promoted the hydrolysis of the present phosphates to the greatest extent. Nevertheless, no simple kinetic law can be applied to this system. Since the catalytic effect does not show large isomer-dependency, the primary catalytic process must involve interaction between thorium(IV) and the phosphate moiety of the substrates. Large stability constants obtained for the thorium-phosphate complexes may provide a sound basis for this discussion.[6] In addition, the hydrolysis rate reached its maximum at $-\log[\mathrm{H}^+]\approx 2.7$, where the corresponding spontaneous hydrolysis also attained the maximum rate. This suggests that the substrate undergoes interaction with thorium(IV) in its zwitterion form. In spite of the primary participation of the phosphate moiety in the thorium-ion catalysis, the ring-nitrogen may be involved in the interaction with the metal ion in the hydrolysis of 2-pyridylmethyl phosphate since the highest acceleration effect on the rate was obtained with the 2-phosphate.

Bamann, Trapmann and their coworkers have studied in recent years the hydrolytic behavior of some phosphate monoesters in the presence of quadrivalent metal ions.[7-13] Quadrivalent titanium, zirconium, hafnium and thorium were found to show catalytic activity in an acidic region and the effect of thorium was the greatest. According to their work, solvolysis was accelerated several hundred times in most cases due to the presence of thorium ion, and the reaction rate was observed to be very rapid at the initial stage followed by leveling-off after a while. In spite of the fact that their experimental conditions are not directly comparable with ours, the catalytic pathway, which would elucidate the present thorium-ion catalysis, may also be applied to their system.

Meanwhile, in the hydrolysis of α-D-glucose-1-phosphate[14] the thorium ion retarded the hydrolysis rate in an acidic region below $-\log[\mathrm{H}^+]\approx 2$ and the positive catalytic effect was observed above $-\log[\mathrm{H}^+]=2.7$. Thus. the thorium-ion catalysis depends upon the structure of an alcoholic residue of a selected phosphate and also on the corresponding spontaneous reaction mechanism.

In our previous work,[2] the copper ion was found to promote the reaction rate of 2-pyridylmethyl phosphate to a significant extent in a $-\log[\mathrm{H}^+]$ range above 3.3, while the same metal ion did not show any meaningful catalysis for other pyridylmethyl phosphates. The particular copper complex formed with the 2-isomer in the transition state, where the chelate ring involves both pyridyl nitrogen and ester oxygen as donor atoms, was postulated in order to elucidate the enhanced catalytic effect. On the other hand, the thorium complexes of the present substrates were not well characterized under present experimental conditions. Thus, presentation of an acceptable catalytic mechanism is not possible at present. The thorium-ion promoted hydrolysis seems to be much different from the corresponding copper-ion-effected reaction in respest of their catalytic mechanisms.

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[6] E. L. Zebroski, H. W. Alter and F. K. Heumann, *J. Am. Chem. Soc.*, **73**, 5646 (1951).

[7] E. Bamann, H. Trapmann, J. Riehl, A. Gerl and B. Oechsner, *Arch. Pharm.*, **296**, 174 (1963).

[8] E. Bamann, A. Gerl, B. O. Oechsner, J. Riehl and H. Trapmann, *Naturwissenschaften*, **47**, 106 (1960).

[9] E. Bamann, H. Trapmann and H. J. Krauss, *Arch. Pharm.*, **295**, 330 (1962).

[10] E. Bamann, H. Trapmann and B. Oechsner, *ibid.*, **295**, 663 (1962).

[11] H. Trapmann and M. Devani, *ibid.*, **298**, 253 (1965).

[12] H. Trapmann and M. Devani, *ibid.*, **298**, 497 (1965).

[13] H. Trapmann and M. Devani, *Hoppe-Seyler's Z. Physiol. Chem.*, **340**, 81 (1965).

[14] Y. Murakami and M. Tanaka, This Bulletin, **39**, 122 (1966).