Bull. Chem. Soc. Jpn., 55, 1097—1101 (1982)

Phosphatosulfates. Metal-ion Effects on the Acid-catalyzed Hydrolysis of Phenyl Phosphatosulfate in an Acetonitrile–Water Mixed Solvent

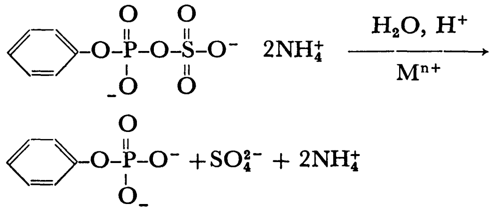
Toshio EIKI, Yoshio ASAI, and Waichiro TAGAKI*,†
Department of Chemistry, Faculty of Engineering, Gunma University, Kiryu, Gunma 376
†Department of Applied Chemistry, Faculty of Engineering, Osaka City University, 3-138, Sugimoto 3-chome, Sumiyoshi-ku, Osaka 558

(Received March 17, 1981)

The rates of the acid-catalyzed hydrolysis of phenyl phosphatosulfate (PPS) have been found to be highly dependent on the water content in the mixed solvent of acetonitrile–water. A remarkable rate acceleration was observed with a rate maximum near the 1 : 1 ratio of PPS and metal ion, and with a solvent with a low water content. Under such conditions, the site of the cleavage of the P–O–S linkage was observed to be predominantly the S–O bond.

Active sulfates[1] are the key starting intermediates in the sulfur metabolism in biological systems.[2,3] They contain the P–O–S phosphatosulfate linkage, and a number of biological transformations, such as the phosphate- or sulfate-group transfer to various nucleophilic acceptors or the reduction of sulfate groups, are all initiated by the cleavage of the P–O–S linkage. However, the mechanisms of the corresponding enzymatic catalyses have largely been left unclarified. The biomimetic chemistry of the phosphatosulfate linkage has also been relatively unexplored compared to that of the related P–O–P phosphate linkage.

An interesting feature of the chemistry of the P–O–S linkage is the role of metal ions—how they affect the rate and selectivity in the cleavage of the P–O *vs.* S–O bond. Previously we reported that the Mg^{2+} ion enhanced the rate of the acid-catalyzed hydrolysis of PPS, a simple model of active sulfates, in mixed solvents of acetonitrile–water when their water content was low.[4,5] The details will now be described in this paper, along with additional data for some other metal ions (Eq. 1). The substrate PPS was used in the form of diammonium salt.



(1)

diammonium salt of PPS

Results and Discussion

Kinetics. The reactions were generally carried out in an acetonitrile–water mixed solvent containing appropriate amounts of PPS (1.8—2.0×10^{-3} M), a metal ion (0—4×10^{-2} M) and buffer reagents of $HClO_4$ (0.3—3.0×10^{-3} M)-n-$Bu_4N^+ClO_4^-$ (2×10^{-2} M), at 25 °C (1 M=1 mol dm^{-3}). For some experiments with solvents of a low water content, the water was partially replaced by methanol (see Fig. 2b and Table 3). Under these conditions the hydrolysis of acetonitrile was not detected.

The rates were determined by measuring the formation of phenyl phosphate.[4—6] Most reaction mixtures were homogeneous during the reaction. Occasionally, ammonium sulfate was precipitated out at the end of reaction in the solvents with a low water content (3 M). Nevertheless, good pseudo-first-order rate constants were obtained up to more than 90% completion of the reaction.

Effect of Water Concentration. The rates of acid-catalyzed hydrolysis were found to be highly dependent on the water content of the medium, in both the absence and presence of a metal ion.

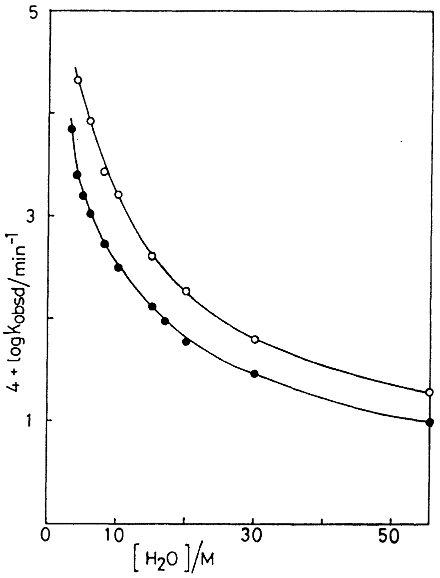


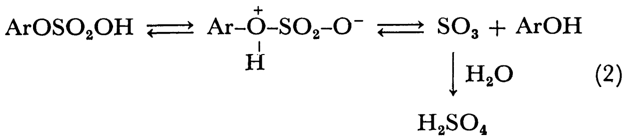
Fig. 1. Effect of water concentration on the rates of hydrolysis of PPS (1.8×10^{-3} M) in the presence of $HClO_4$(●; 2×10^{-3} M, ○ ; 4×10^{-3} M) and $(n\text{-}Bu)_4NClO_4$ (2×10^{-2} M) at 25 °C.

In the absence of a metal ion, Fig. 1 shows that the rates in pure water (55.5 M) are enhanced by some 10^3 times in an acetonitrile–water mixed solvent when the water content is reduced to 3 M. A similarly large rate enhancement has also been reported for other acid-catalyzed sulfate hydrolysis in solvents of low polarity (ether or moist dioxane).[7,8] A suggestion has been made that the hydrolysis of aryl hydrogensulfate based on an Al mechanism (Eq. 2) is due to a specific solvation of the zwitter ionic-transition state of sulfur trioxide by ether.[9] Since an Al mechanism to give sulfur trioxide has been suggested for the acid-catalyzed hydrolysis of PPS in pure water,[6,10,11] the specific solvation of sulfur

TABLE 1. Mg^{2+} ION EFFECT ON THE RATES OF HYDROLYSIS OF PPS AT 25 °C[a)]

$[Mg(ClO_4)_2]\times10^3$/M	k_{obsd}/min^{-1}, $[H_2O]$/M in acetonitrile					
	3	4	4.5	5	6	8
0	2.66×10^{-2}	1.78×10^{-2}	1.30×10^{-2}	1.14×10^{-2}	8.94×10^{-3}	8.28×10^{-3}
0.5	8.88×10^{-2}	—	—	4.57×10^{-2}	1.66×10^{-2}	7.98×10^{-3}
1.0	1.90×10^{-1}	8.82×10^{-2}	7.38×10^{-2}	—	—	—
1.8	2.67×10^{-1}	1.23×10^{-1}	7.74×10^{-2}	5.25×10^{-2}	3.18×10^{-2}	7.80×10^{-3}
2.2	1.44×10^{-1}	8.40×10^{-2}	—	—	—	—
2.5	1.20×10^{-1}	7.20×10^{-2}	3.84×10^{-2}	2.31×10^{-2}	1.38×10^{-2}	5.29×10^{-3}
5.0	2.53×10^{-2}	2.10×10^{-2}	1.27×10^{-2}	8.88×10^{-2}	5.41×10^{-3}	2.96×10^{-3}
10.0	1.06×10^{-2}	9.42×10^{-3}	7.20×10^{-3}	4.09×10^{-3}	4.06×10^{-3}	$2\ 23\times10^{-3}$
20.0	7.08×10^{-3}	5.10×10^{-3}	3.15×10^{-3}	2.86×10^{-3}	2.44×10^{-3}	1.53×10^{-3}

a) $[PPS]=1.8\times10^{-3}$ M, $[HClO_4]=1.0\times10^{-3}$ M, $[(n\text{-}Bu)_4NClO_4]=2.0\times10^{-2}$ M.



$$ArOSO_2OH \rightleftharpoons Ar\text{-}\underset{H}{\overset{+}{O}}\text{-}SO_2\text{-}O^- \rightleftharpoons SO_3 + ArOH \xrightarrow{H_2O} H_2SO_4 \qquad (2)$$

trioxide by acetonitrile may also be possible as an explanation in such a medium with a low water content. However, the effect of water seems to be too complex to allow a simple interpretation, as will be described below.

Effects of Metal Ions. The metal-ion effect in water was examined by Benkovic and Hevey.[10)] They observed rates faster by 1.26—3.16 times in the hydrolysis of PPS by Mg^{2+}, Ca^{2+}, or Al^{3+} ions at pH 3.1 and 55 °C. They also observed that the rates were 1.2—2.05 times slower in the cases of Mn^{2+}, Co^{2+}, or Zn^{2+} ions. Thus, the effect of these metal ions is not large in pure water. Meanwhile, we have found remarkable metal-ion effects in the medium of acetonitrile–water mixed solvents.[4)]

As is shown in Fig. 2a and Table 1, the rate acceleration by the Mg^{2+} ion is not observable when the water content of the medium exceeds 8 M; rather, the addition of the Mg^{2+} ion tends to inhibit the acid catalysis (see the k_{obsd} values in the absence of a metal ion). On the other hand, a large rate acceleration takes place in a medium with a low water content, showing a maximum in each plot of k_{obsd} *vs.* $[Mg(ClO_4)_2]$ where the concentration of the Mg^{2+} ion is very close to the initial concentration of the substrate ($[PPS]=1.8\times10^{-3}$ M). For example, in the solvent of $H_2O=3$ M, the k_{obsd} at

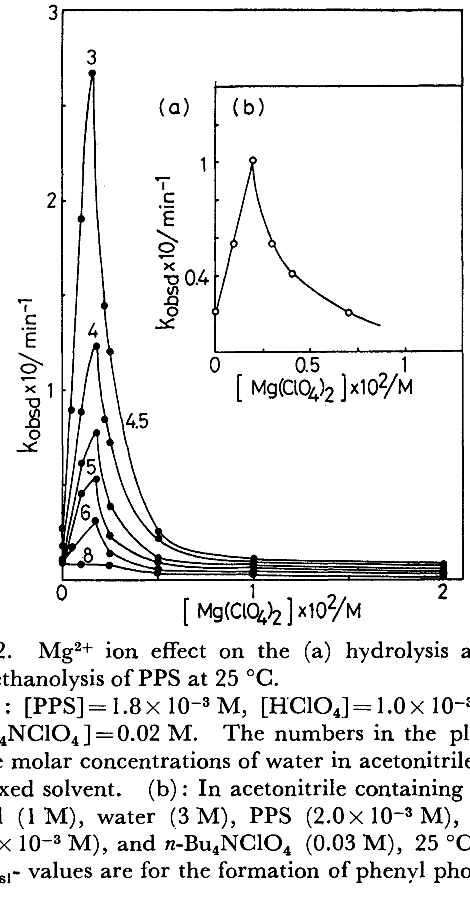


Fig. 2. Mg^{2+} ion effect on the (a) hydrolysis and (b) methanolysis of PPS at 25 °C.
(a): $[PPS]=1.8\times10^{-3}$ M, $[HClO_4]=1.0\times10^{-3}$ M [n-Bu_4NClO_4]=0.02 M. The numbers in the plots are the molar concentrations of water in acetonitrile–water mixed solvent. (b): In acetonitrile containing methanol (1 M), water (3 M), PPS (2.0×10^{-3} M), $HClO_4$ (1×10^{-3} M), and n-Bu_4NClO_4 (0.03 M), 25 °C. The k_{obsl}- values are for the formation of phenyl phosphate.

TABLE 2. EFFECTS OF ACID AND WATER CONCENTRATION ON THE RATES OF HYDROLYSIS OF PPS IN THE ABSENCE (k_o) AND THE PRESENCE OF Mg^{2+} ION (k_{max})[a)] IN MIXED SOLVENTS OF WATER–ACETONITRILE

$[HClO_4]\times10^3$/M	$-\log[HClO_4]$	$k_o(k_{max})$/min^{-1}, $[H_2O]$/M in acetonitrile		
		4.0	5.0	6.0
0.30	3.52	$3.83\times10^{-4}(1.92\times10^{-2})$	$2.63\times10^{-4}(1.06\times10^{-2})$	— (4.06×10^{-3})
0.50	3.30	$2.01\times10^{-4}(4.53\times10^{-2})$	$1.29\times10^{-3}(1.86\times10^{-2})$	$9.54\times10^{-4}(9.96\times10^{-3})$
0.75	3.12	$7.94\times10^{-3}(8.88\times10^{-2})$	$4.83\times10^{-3}(3.92\times10^{-2})$	$3.78\times10^{-3}(2.11\times10^{-2})$
1.00	3.00	$1.78\times10^{-2}(1.23\times10^{-1})$	$1.14\times10^{-2}(5.25\times10^{-2})$	$8.94\times10^{-3}(3.18\times10^{-2})$
1.50	2.82	$7.77\times10^{-2}(2.80\times10^{-1})$	$6.07\times10^{-2}(1.53\times10^{-1})$	$3.37\times10^{-2}(6.78\times10^{-2})$
2.00	2.69	$2.36\times10^{-1}(3.42\times10^{-1})$	$1.54\times10^{-1}(2.13\times10^{-1})$	$1.06\times10^{-1}(9.60\times10^{-2})$
3.00	2.52	$12.3\times10^{-1}(7.80\times10^{-1})$	$4.28\times10^{-1}(3.63\times10^{-1})$	$3.42\times10^{-1}(1.86\times10^{-1})$

a) $[PPS]=1.8\times10^{-3}$ M; $k_o([Mg(ClO_4)_2]=0$ M), k_{max} ($[Mg(ClO_4)_2]=1.8\times10^{-3}$ M) $[(n\text{-}Bu)_4NClO_4^-]=2.0\times10^{-2}$ M, 25 °C.

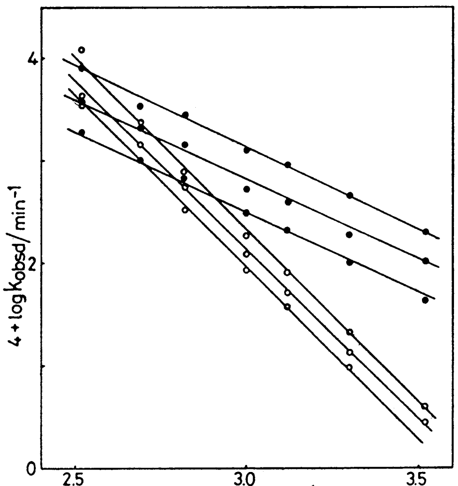


Fig. 3. Effect of acid and water concentration on the rates of hydrolysis of PPS in the absence (○) and presence of Mg^{2+} ion (●) in the mixed solvents of water–acetonitrile. Water concentrations are from the bottom 4,5, and 6 M, respectively (see Table 2).

the maximum ($k_{max}=2.67\times10^{-1}$ min^{-1}) is ten times larger than the k_{obsd} in the absence of the Mg^{2+} ion ($k_0=2.66\times10^{-2}$ min^{-1}). These k_{max} and k_0 values are dependent on both the water and acid concentrations as shown in Fig. 3 and Table 2.

As has previously been reported,[4,10,11] the acid hydrolysis of PPS in water gives a linear pH-log k_{obsd} plot with a slope of -1. In Fig. 3, the corresponding slopes of the plots of $-\log[HClO_4]$ *vs.* $\log k_0$ and $\log k_{max}$ are -3.2 and -1.6 respectively in the ranges of $[HClO_4]=0.3$—3.0×10^{-3} M and $[H_2O]=4$—6 M. Thus, the apparent number of protons involved in the rate-limiting step changes from one in pure water to more than three in the medium with a low water content and in the absence of a metal ion, while the number changes from -3.2 in the absence of the Mg^{2+} ion to -1.6 in its presence. Whatever the cause, these dependencies of k_{max} and k_0 on both the water and acid concentrations lead to the conclusion that the ratio of k_{max}/k_0 (*i.e.*, the metal-ion effect) increases linearly with a decrease in the water concentration (see Table 1) and exponentially with a decrease in the acid concentration (see Fig. 3 and Table 2), at least in the ranges of $[H_2O]=$ 3—8 M and $[HClO_4]=0.3$—3×10^{-3} M. Conversely, such metal-ion effects become unimportant at higher acid concentrations and in pure water.

The Mg^{2+} ion is most frequently encountered as a cofactor in the reactions of phosphates including active sulfates in biological systems.[2,3,12] The above findings may offer important suggestions regarding the role of the Mg^{2+} ion in such a biological reaction. In order to obtain further knowledge, the effects of some other metal ions have also been examined, as will be described below.

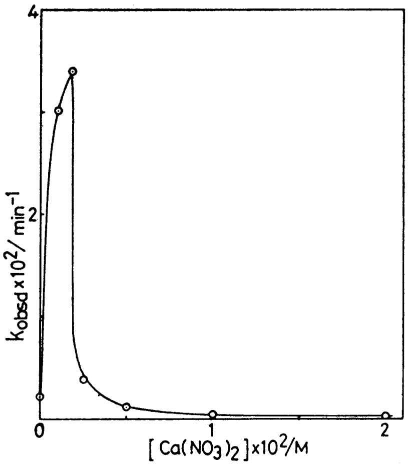


Fig. 4. Effect of Ca^{2+} ion on the rates of hydrolysis of PPS, 25 °C in 4 M water–acetonitrile.
$[PPS]=1.8\times10^{-3}$ M; $[HClO_4]=5.0\times10^{-4}$ M; $[(n\text{-}Bu)_4NClO_4]=0.02$ M.

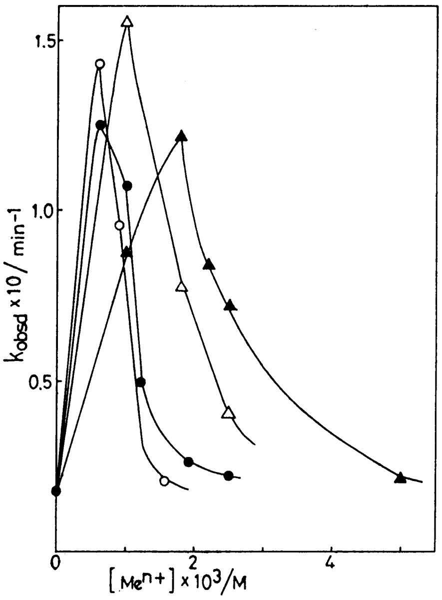


Fig. 5. Effect of metal ions on the rates of hydrolysis of PPS. 25 °C in 4 M water–acetonitrile.
$[PPS]=1.8\times10^{-3}$ M; $[HClO_4]=1.0\times10^{-3}$ M; $[(n\text{-}Bu)_4ClO_4]=0.02$ M; Me^{n+} are Zn^{2+} (△), Al^{3+} (●), Th^{4+} (○), and Mg^{2+} (▲).

As is shown in Figs. 4 and 5, the effects of Ca^{2+}, Zn^{2+}, Al^{3+}, and Th^{4+} on the rates were found to be similar to those of Mg^{2+} in Fig. 2. For example, the [PPS]/[metal ion]=1 ratio at the k_{max} is the same for both Ca^{2+} and Mg^{2+}, and they give comparable k_{max}/k_0 values of 16 and 23 respectively under the same conditions ($[H_2O]=4$ M, $[HClO_4]=5\times10^{-4}$ M). However, the details are different; the metal-ion concentrations which give the rate maximum with $[PPS]=1.8\times10^{-3}$ M are $[Th^{4+}]=0.6\times10^{-3}$ M, $[Al^{3+}]=0.6\times10^{-3}$ M, $[Zn^{2+}]=1.0\times10^{-3}$ M, $[Ca^{2+}]=1.8\times10^{-3}$ M, and $[Mg^{2+}]=1.8\times10^{-3}$ M; all the curves in Figs. 2, 4, and 5 are unusually sharp, but those of Th^{4+} and Al^{3+} are much sharper than for those of Ca^{2+} and Mg^{2+}. Apparently, complicated equilibria are taking place between PPS and a metal ion, which are both highly dependent on both the water and acid concentrations. The fact that [PPS]/[metal ion]=1—3 at the rate maximum suggests that the 1 : 1, 1 : 2, or 1 : 3 complex of a metal ion and PPS is catalytically active depending on the nature of metal ion, but that a further increase in the metal-ion coordination stabilizes the complex toward the hydrolysis.

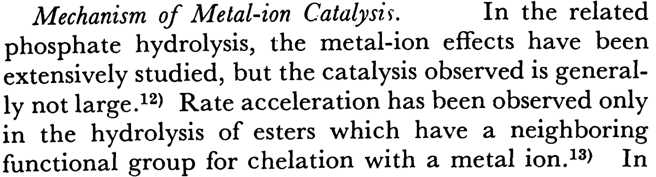
Selective S–O Bond Cleavage. It has already been shown by an ^{18}O-tracer study that the S–O bond of PPS was selectively cleaved in the acid-catalyzed hydrolysis in water.[4,10] Similarly, methyl hydrogensulfate was obtained in a reaction in aqueous methanol.[10] However, the metal-ion effect was found to be small in water, as has been mentioned above, and so it was not examined in the latter aqueous methanol.

TABLE 3. Mg^{2+} ION EFFECT ON THE RATE OF HYDROLYSIS OF PPS IN MIXED SOLVENTS OF METHANOL (1.0 M)–WATER (3.0 M)–ACETONITRILE AT 25 °C[a]

$[Mg(ClO_4)_2]$/M	k_{obsd}/min^{-1}
0	2.06×10^{-2}
1.0×10^{-3}	5.69×10^{-2}
2.0×10^{-3}	1.04×10^{-1}
3.0×10^{-3}	5.72×10^{-2}
4.0×10^{-3}	4.15×10^{-2}
7.0×10^{-3}	2.00×10^{-2}

a) $[PPS]=2.0\times10^{-3}$ M; $[HClO_4]=1.0\times10^{-3}$ M; $[(n\text{-}Bu)_4NClO_4]=3.0\times10^{-2}$ M.

As is shown in Table 3 and Fig. 2b, a partial substitution of 4 M water by methanol in acetonitrile did not change the essential features of the Mg^{2+}-ion effect; a rate maximum was also observed at [PPS] : [metal ion] = 1 : 1. From the reaction mixture at the rate maximum, methyl hydrogensulfate was then detected in a 60% yield by NMR analysis. The alternative formation of methyl phenyl phosphate by the P–O bond cleavage was not detected in spite of the fact that it is stable under the present reaction conditions (Eq. 3a) and should be easily detected by means of the NMR doublet of the CH_3 signal. Thus, the methanolysis of

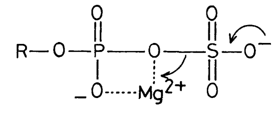


$$C_6H_5O\text{-}\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}\text{-}O\text{-}\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}\text{-}OH \begin{cases} \xrightarrow{H_2O} C_6H_5O\text{-}PO_3H_2 + H_2SO_4 & (3a) \\ \xrightarrow{CH_3OH} C_6H_5O\text{-}PO_3H_2 + CH_3O\text{-}SO_3H & (3b) \end{cases}$$

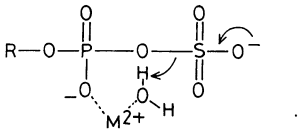
PPS occurs by means of the predominant S–O bond cleavage (Eq. 3b). Here, it should be noted that this S–O cleavage is the subject of Mg^{2+}-ion catalysis.

Mechanism of Metal-ion Catalysis. In the related phosphate hydrolysis, the metal-ion effects have been extensively studied, but the catalysis observed is generally not large.[12] Rate acceleration has been observed only in the hydrolysis of esters which have a neighboring functional group for chelation with a metal ion.[13] In other well-designed models for ATP hydrolysis, neighboring polyamine ligands showed essentially no effect on the metal-ion catalysis.[12] An exception was the observation that the hydrolysis or phosphate group-transfer reaction of ATP was accelerated in the presence of the Mg^{2+} ion when the reactions were conducted in dimethyl sulfoxide with a low water content.[14]

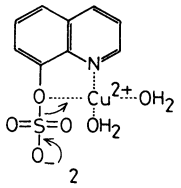
For an efficient catalysis to occur, a metal ion and phosphate (or sulfate) must form a complex. This process would, however, not be favored in water because of the favored hydration of the metal ion and the ester, whereas in an enzymatic reaction the medium at the active site is not necessarily water, but rather would be hydrophobic or dipolar aprotic in favor of metal ion-substrate binding. This idea may be supported by the findings shown above in Figs. 2, 4, and 5. However, even if this is true, the question still remains of how such complexation can activate the selective S–O bond cleavage. One may consider the present metal-ion effect to be electrophilic catalysis, as is illustrated in **1a**.



1a



1b



2

This type of catalysis was proposed in the Cu^{2+}-ion catalyzed hydrolysis of 8-quinolyl sulfate, as is shown in **2**.[13f] However, the present metal-ion effect is highly acid-dependent; under neutral conditions, no such metal-ion-assisted S–O cleavage was detected with divalent metal ions.[15] Therefore, the results may be tentatively accounted for by a mechanism of metal-ion-assisted proton-transfer catalysis, as is illustrated in **1b**, where the leaving group to capture the proton is the phosphoryl group. This type of catalysis is essentially the same as that considered in the hydrolysis of aryl hydrogensulfates, where a neighboring carboxyl or imidazolium group acts as the proton-transfer catalyst to the leaving phenoxyl group.[16,17] In **Ib**, the proton transfer seems to be smooth when the metal ion coordinates only to the phosphate group, leaving the sulfate group free. Thus, any further coordination of the metal ion to the sulfate group would stabilize the complex and inhibit the reaction.

Experimental

Materials. The diammonium phenyl phosphatosulfate (PPS) was prepared by the previously reported method.[6] The tetrabutylammonium perchlorate was prepared by the literature method.[18] Deionized water was treated with $KMnO_4$ and distilled twice before use. The metal ions were used in the forms of perchlorate (Mg^{2+}) and nitrate (Zn^{2+}, Ca^{2+}, Al^{2+}, Th^{4+}) of an analytical grade.

Product Analyses. The hydrolysis products were found to be solely phenyl phosphate and inorganic sulfate, regardless of the presence or absence of metal ions. No phenol or other possible organic products were detected. In the reactions shown in Fig. 2b, methyl hydrogensulfate, phenyl phosphate, and inorganic sulfate were the sole products, and methyl phenyl phosphate was not detected.

Phenyl phosphate was analyzed by UV absorption and paper chromatography.[6] Inorganic sulfate was analyzed colorimetrically by the barium chloranilate method.[19] Methyl hydrogensulfate was analyzed by NMR in the following way: the evaporation of acetonitrile gave $(n\text{-Bu})_4\text{NClO}_4$ precipitates, which were then removed by filtration. The filtrate was adjusted to pH 11 with aqueous sodium hydroxide to give insoluble magnesium hydroxide, which was subsequently removed by centrifugation. The supernatant was then neutralized with hydrochloric acid, and the solution was lyophilized. The residue was dissolved in D_2O and analyzed by NMR for phenyl and methyl (3H, δ 3.81, singlet) protons. The yield of methyl hydrogensulfate thus analyzed was 60% for the reaction mixture at the rate maximum shown in Fig. 2b.

Kinetics. The rate of the reaction was followed spectrophotometrically by quenching the reaction mixture with aqueous sodium hydroxide according to the previously reported method, except for the centrifugation of the quenched solutions to remove magnesium hydroxide.[4,11] Good pseudo-first-order rate constants were obtained up to a 90% completion of the reaction in the range of $[\text{HClO}_4]=0.3-3.0\times10^{-3}$ M, both in the presence and in the absence of $\text{Mg(ClO}_4)_2$ (see Fig. 3), although significant deviation from the first-order kinetics due to autocatalytic rate acceleration was observed below $[\text{HClO}_4]=0.3\times10^{-3}$ M. The pseudo-first-order rate constants were computed as usual by using $k_{\text{obsd}}=\frac{1}{t}\ln\frac{\text{OD}_\infty-\text{OD}_0}{\text{OD}_\infty-\text{OD}_t}$. The rate of reaction shown in Fig. 2b was determined by the same method.

References

1) Adenosine 5′-phosphosulfate (APS) and 3′-phosphoadenosine 5′-phosphosulfate (PAPS).

2) F. Lipmann, *Science*, **128**, 575 (1958).

3) a) H. D. Peck, Jr., *Enzymes*, **10**, 651 (1974); b) A. B. Roy and P. A. Trudinger, "The Biochemistry of Inorganic Compounds of Sulphur," Cambridge Univ. Press, Cambridge, (1970); c) J. A. Schiff and R. C. Hodson, "The Metabolism of Sulfate," *Ann. Rev. Plant Physiol.*, **24**, 381 (1973); d) A. B. Roy, *Enzymes*, **8**, 1 (1971).

4) W. Tagaki, Y. Asai, and T. Eiki, *J. Am. Chem. Soc.*, **95**, 3037 (1973).

5) W. Tagaki and T. Eiki, *Adv. Chem. Ser.*, **191**, 407 (1980).

6) W. Tagaki, T. Eiki, and I. Tanaka, *Bull. Chem. Soc. Jpn.*, **44**, 1139 (1971).

7) S. Burstein and S. Lieberman, *J. Am. Chem. Soc.*, **80**, 5235 (1958).

8) B. D. Batts, *J. Chem. Soc.*, **1966**, 551.

9) A. Williams and K. T. Douglas, *Chem. Rev.*, **75**, 627 (1975).

10) S. J. Benkovic and R. C. Hevey, *J. Am. Chem. Soc.*, **92**, 4971 (1970).

11) T. Eiki, K. Tomuro, S. Aoshima, M. Suda, and W. Tagaki, *Nippon Kagaku Kaishi*, **1980**, 454.

12) B. S. Cooperman, *Biochemistry*, **8**, 5055 (1969).

13) a) R. Hofstetter, Y. Murakami, G. Mont, and A. E. Martell, *J. Am. Chem. Soc.*, **84**, 3041 (1962); b) Y. Murakami, J. Sunamoto, and H. Sadamori, *Chem. Commun.*, **1969**, 983; c) S. J. Benkovic and L. K. Dunikowski, Jr., *J. Am. Chem. Soc.*, **93**, 1526 (1971); d) C. M. Hsu and B. S. Cooperman, *ibid.*, **98**, 5652 (1976); e) R. W. Hay and J. A. G. Edmonds, *Chem. Commun.*, **1967**, 969; f) R. W. Hay, C. R. Clark, and J. A. G. Edmonds, *J. Chem. Soc., Dalton Trans.*, **1974**, 9.

14) N. Nelon and E. Racker, *Biochemistry.*, **12**, 563 (1973).

15) Under neutral or basic conditions, divalent metal ions (Mg^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, *etc.*) were found to catalyze nucleophilic reactions on phosphorus to give a P–O cleavage.[5]

16) S. J. Benkovic, *J. Am. Chem. Soc.*, **88**, 5511 (1966).

17) S. J. Benkovic and L. K. Dunikowski, *Biochemistry*, **9**, 1390 (1970).

18) a) H. Sadek and R. M. Fuoss, *J. Am. Chem. Soc.*, **72**, 301 (1950); b) R. L. Buckson and S. G. Smith, *J. Phys. Chem.*, **68**, 1875 (1964).

19) A. Wainer and L. Kock, *Anal. Biol. Chem.*, **3**, 457 (1962).