of surface area. The supporting electrolyte was KBr 0.1 M. After completion of the electrolysis the products were identified by cyclic voltammetry or after extraction according to the following procedure: the solution was neutralized with an excess of NH_4Cl and then 100 mL of diethyl ether was added; the ammonia was allowed to evaporate through a condenser, the central well of which contained 2-propanol held at about -20 °C by occasional addition of pieces of solid CO_2.

6-Chloroquinoline with Benzhydrol Alcoholate. The ether extract was titrated by HPLC on a 25 cm RP 18 column eluted with a 75-25 methanol-water mixture and by gas chromatography on a 3 m 3% OV17 column.

2-Chloroquinoline with Isopropylate. The ether extract was separated into two portions. The first portion was analyzed by gas chromatography and NMR. 2-Propanone-1 (2-quinolinyl) was identified by its mass spectrum obtained by gas chromatography-mass spectroscopy: *m/e* 187 (11), 186 (28), 171 (13), 170 (32), 145 (5), 144 (36), 143 (100), 142 (27), 128 (8), 116 (17), 115 (26), 89 (23). The second portion was treated with 2,4-dinitrophenylhydrazine and the acetone derivative was identified by its melting point and NMR spectrum.

SNAr Reactions. 2-Methoxyquinoline was identified by comparison with an authentic sample[17]—UV spectrum, retention time in VPC (3% OV17), and HPLC (25 cm RP 18, 75-25 $MeOH/H_2O$). Its mass spectrum was identical with that previously published.[17]

2-Ethoxyquinoline was identified by comparison with an authentic sample—HPLC (25 cm Lichrosorb 65-35 $MeOH/H_2O$)—and by its mass spectrum: *m/e* 173 (57) (M), 158 (88) (M - 15), 145 (100) (M - 28), 129 (97) (M - 44), 128 (26) (M - 45), 117 (58), 116 (33), 102 (16) (129 (quinoline) - 27), 101 (12), 90 (42), 89 (66), 76 (11), 75 (19).

2-Isopropoxyquinoline was identified after evaporation of ammonia and extraction of the residue by ether: NMR spectrum ($CDCl_3$) (ppm by reference to Me_4Si) δ 1.38 (doublet, 6 H, CH_3), 5, 6 (septuplet, 1 H, O-C*H*<), 7.0-8.3 (multiplet, 6 H, aromatic protons); mass spectrum 187 (25) (M), 162 (38) (M - 15), 145 (100) (M - 42), 129 (45) (M - 58, quinoline), 117 (73), 102 (73) (quinoline - 27), 101 (15), 90 (66), 89 (73).

2-(Cyclohexyloxy)quinoline was identified through its mass spectrum obtained by gas chromatography-mass spectrometry coupling: 227 (47) (M), 146 (32), 145 (100) (M - C_6H_{11}), 129 (10) (M - 98, quinoline), 128 (10), 117 (22), 116 (9.6), 102 (2), 90 (13), 89 (15).

The other products of the S_NAr reactions were not formally identified but their voltammograms and their kinetics of formation were similar to other cases (see Table I).

Acknowledgment. This work was supported in part by the CNRS (Equipe de Recherche Associée No. 309 "Electrochimie Moléculaire").

Registry No. 2-Chloroquinoline, 612-62-4; 6-chloroquinoline, 612-57-7; methylate, 3315-60-4; ethylate, 16331-64-9; isopropylate, 15520-32-8; 3-methylbutylate, 35730-35-9; cyclohexanolate, 80754-03-6; 4-aminobutylate, 80754-04-7; 5-aminopentylate, 80754-05-8; diphenylcarbinolate, 80754-06-9; 1-chloronaphthalene, 90-13-1; chlorobenzene, 108-90-7; 2-ethoxyquinoline, 46185-83-5; 2-isopropoxyquinoline, 60814-31-5; 2-(cyclohexyloxy)quinoline, 80754-07-0; (2-quinolinyl)-2-propanone, 1531-30-2.

(17) Clugston, D. M.; Mc Lean, D. B. *Can. J. Chem.* **1966**, 44, 781.

Phosphonosulfates. Metal Ion Catalysis in the Hydrolysis of 2-Pyridyl- and 2-Pyridylmethylphosphonosulfate

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Abstract: The hydrolysis rates of phosphonosulfates having a neighboring 2-pyridyl group, 2-pyridyl- (**1**) and 2-pyridylmethylphosphonosulfate (**2**), have been compared with those of the corresponding phenyl- (**3**) and benzylphosphonosulfate (**4**) in a pH range of 1-9. All of the esters were found to undergo the acid-catalyzed reaction via the selective S-O bond cleavage. Under neutral pH conditions, the P-O bond was selectively cleaved in the solvolysis of all the esters regardless of whether Zn^{2+} or Mg^{2+} is present or not. In the absence of metal ion, an intramolecular catalysis by the 2-pyridyl group was observed for the hydrolysis of **2**, but not for that of **1**. The intramolecular catalysis was inhibited by the Zn^{2+} ion. Such an inhibition, however, appears to be largely compensated by the Zn^{2+} ion catalysis which leads to the formation of a pentavalent phosphorus intermediate upon metal chelation. Even more remarkable catalysis by the Zn^{2+} ion was observed for the hydrolysis of **1**.

Phosphosulfates such as adenosine-5′-phosphosulfate (APS) and 3′-phosphoadenosine-5′-phosphosulfate (PAPS) are classified as active sulfates due to biological reasons and taken as the key intermediates for the biological sulfur metabolism.[2] The P-O-S linkage involved in such phosphosulfates undergoes S-O bond cleavage and transfers its sulfate moiety to numerous nucleophiles such as steroids and phenols under biological conditions. Reduction of the sulfate group in the active sulfates also takes place most plausibly via the S-O bond cleavage under certain enzymic conditions. However, information on the enzymic mechanisms of these reactions is very limited up to the present time. Various reactions of biochemical concern have often been enhanced by metal ions under nonenzymatic conditions. Thus, it is meaningful to examine metal-ion catalysis in the cleavage of the P-O-S linkage of the active sulfates and to clarify which bond, P-O or S-O, is selectively cleaved under such catalytic conditions. We have previously reported that the S-O bond of phosphosulfates was selectively cleaved in the acid-catalyzed hydrolysis and the catalysis was enhanced by the Mg^{2+} ion when the reaction was carried out in organic solvents containing low water contents.[3,4] However, the metal-ion catalysis was not detected without acids, partly due to low reactivity of a model substrate under specified conditions.

In this work, we selected another type of active sulfates, phosphonosulfates, and investigated the hydrolysis of 2-pyridyl- and 2-pyridylmethylphosphonosulfate (**1** and **2**) and of the related

(1) All correspondences should be addressed to this author at Osaka City University.

(2) (a) Lipman, F. *Science* **1958**, *128*, 575. (b) Peck, H. D., Jr. *Enzymes* **1974**, *10*, 651. (c) Roy, A. B.; Trudinger, P. A. "The Biochemistry of Inorganic Compounds of Sulfur"; Cambridge University Press: Cambridge, 1970. (d) Schiff, J. A.; Hodson, R. C. *Annu. Rev. Plant Physiol.* **1973**, *24*, 381. (e) Roy, A. B. *Enzymes* **1971**, *8*, 1.

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

(4) Tagaki, W.; Eiki, T. *Adv. Chem. Ser.* **1980**, *191*, 407.

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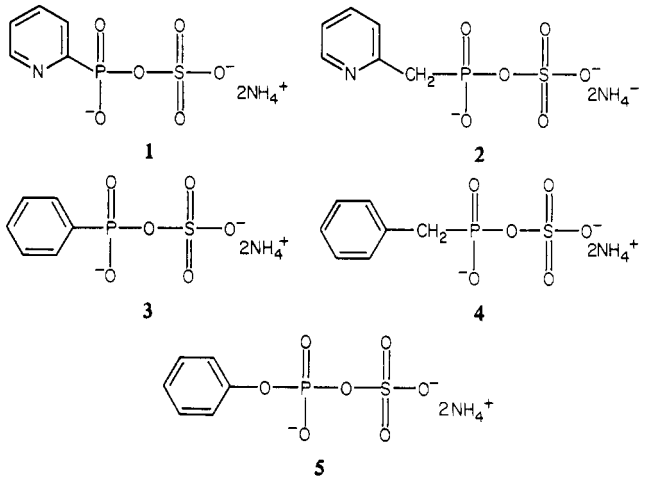
Table I. Physical and Analytical Data for Phosphonates and Phosphonosulfates

compound[e] (diammonium salt)	R_f[a]	λ_{max} (ϵ)[b]	$\delta(D_2O)$[c] for CH_2	elemental analysis,[d] % C	H	N
1	0.50	261 (4100)		21.59 (21.98)	4.48 (4.42)	15.60 (15.38)
2	0.54	262 (4400)	3.48 (d)	24.74 (25.08)	4.91 (4.89)	14.84 (14.63)
3	0.59	263.5 (280)		26.75 (26.47)	4.91 (4.81)	10.08 (10.09)
4	0.57	260 (220)	3.25 (d)	29.26 (29.37)	5.30 (5.28)	9.35 (9.78)
phosphonates:						
2-pyridyl-	0.27	262 (4300)				
2-pyridylmethyl-	0.37	264 (4860)	3.28 (d)			
phenyl-	0.37	258 (410)				
benzyl-	0.35	263 (260)	2.85 (d)			

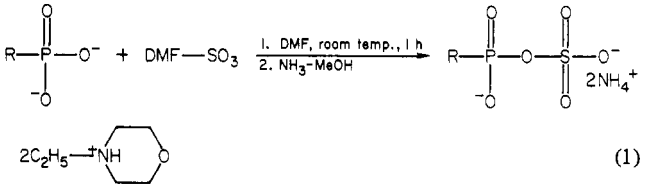
[a] Toyo Filter Paper No. 50, *n*-PrOH–NH_3 (28%)–H_2O. [b] In 0.1 N NaOH. [c] From sodium 2,2-dimethyl-2-silapentane-5-sulfonate. [d] Calculated values are given in parentheses. [e] 1, $C_5H_{12}N_3O_6PS$; 2, $C_6H_{14}N_3O_6PS$; 3, $C_6H_{13}N_2O_6PS$; 4, $C_7H_{15}N_2O_6PS$.

esters (**3**, **4**, and **5**) as well as their methanolysis reactions. The 2-pyridyl moiety was incorporated into the present phosphonosulfates with the expectation that a metal ion may undergo coordination with it together with the neighboring phosphonosulfate group.



Results and Discussion

Preparation of Phosphonosulfates. All of the phosphonosulfates (**1–4**) were prepared by reacting *N*-ethylmorpholinium salts of the corresponding phosphonates with the complex of *N*,*N*-dimethylformamide (DMF)–SO_3 in DMF according to our previous methods (eq 1).[5,6] The esters are acid labile but stable as the diammonium salts. Therefore, precaution was exercised so as



(1)

to maintain a sufficiently low temperature during the decomposition of excess SO_3 complex with ammonia–methanol. The physical and analytical data of the esters are summarized in Table I. In all cases, a starting material (phosphonate) and the corresponding product (phosphonosulfate) were separated satisfactorialy by paper chromatography; the latter was more mobile than the former with 1-propanol–NH_3 (28%)–water (6:3:1 v/v/v) as an eluant. The UV absorption spectra of a pair of phosphonates and phosphonosulfates, originating from π–π transitions of an aromatic ring, were almost identical with each other for all of the phosphonates and phosphonosulfates. The NMR signals for

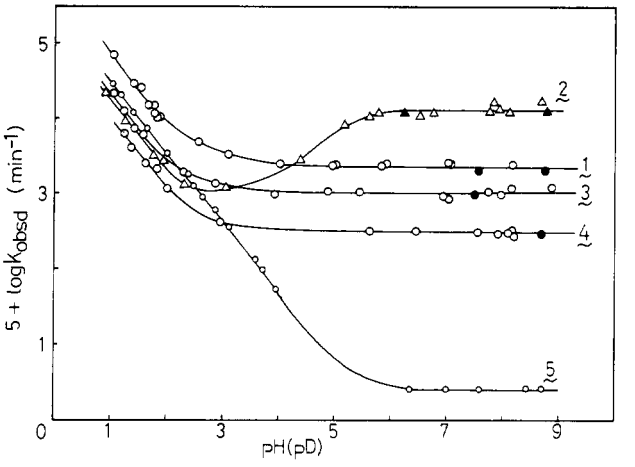


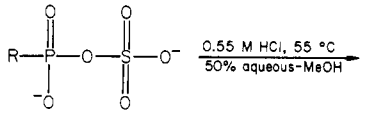
Figure 1. log k_{obsd}–pH profiles for the hydrolysis (formation of inorganic sulfate) of **1–5** at 55 °C, μ = 1.0 (KCl): [substrate] = 0.01 M; buffers (0.1 M) were HCl–KCl (pH 1–2), Cl_2CHCO_2H–Cl_2CHCO_2Na (pH 1.4–2.6), HCO_2H–HCO_2Na (pH 3–4), CH_3CO_2H–CH_3CO_2Na (pH 4–6), 2,6-lutidine–HCl (pH 6.5–7), N-ethylmorpholine–HCl (pH 7.8–8.9). Solid circles and triangles refer to the experimental points obtained in D_2O.

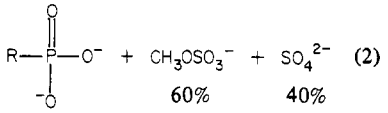
methylene protons of **2** and **4** appeared in a normal range.

Hydrolysis in the Absence of Metal Ion. The hydrolysis rates were determined by monitoring the release of inorganic sulfate colorimetrically upon treating aliquot reaction samples with barium chloranilate.[6-8] Good pseudo-first-order rate constants (k_{obsd}) were generally obtained up to more than 90% conversion of a phosphonosulfate. The results are shown in Figure 1,[9] which indicates that all of the esters undergo acid catalysis with slopes of −1 in the acidic pH region. The reactivities of the five esters do not differ greatly from each other. Such close resemblance in reactivity, which indicates that the substituent effect takes only a minor role, is similar to our previous results; substituents on the phenyl ring only had a small effect on the acid hydrolysis of phenylphosphosulfates (**5**) (Hammett ρ +0.22).[5] Nevertheless, the critical observation indicates that the reactivity follows the sequence **1** > **5** > **3** > **2** > **4** below pH 2. This order appears to reflect the leaving ability of phosphoryl moieties when the S–O cleavage occurs. In agreement with this view, methyl sulfate was obtained in a good yield from all five esters when the acid solvolysis was carried out in aqueous methanol (eq 2).[10] Thus the S–O cleavage is the predominant pathway of reaction under acidic conditions.

On the other hand, the solvolysis rates of the five esters were widely spread in the neutral pH region, spanning more than a

(5) Tagaki, W.; Eiki, T.; Tanaka, I. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 1139. This paper described the preparation of **5**.
(6) Eiki, T.; Tomuro, K.; Aoshima, S.; Suda, M.; Tagaki, W. *Nippon Kagaku Kaishi* **1980**, 454.
(7) Benkovic, S. J.; Hevey, R. C. *J. Am. Chem. Soc.* **1970**, *92*, 4971.
(8) Wainer, A.; Koch, L. *Anal. Biol. Chem.* **1962**, *3*, 457.
(9) The data for **2**, **4**, and **5** are taken from Figure 17 in ref 4.
(10) Methyl sulfate was confirmed to be stable under the conditions employed in this work. For the stability of this ester see: Calhoun, G. M.; Burwell, R. L., Jr. *J. Am. Chem. Soc.* **1955**, *77*, 6441. Kurz, J. L. *J. Phys. Chem.* **1962**, *66*, 2239.

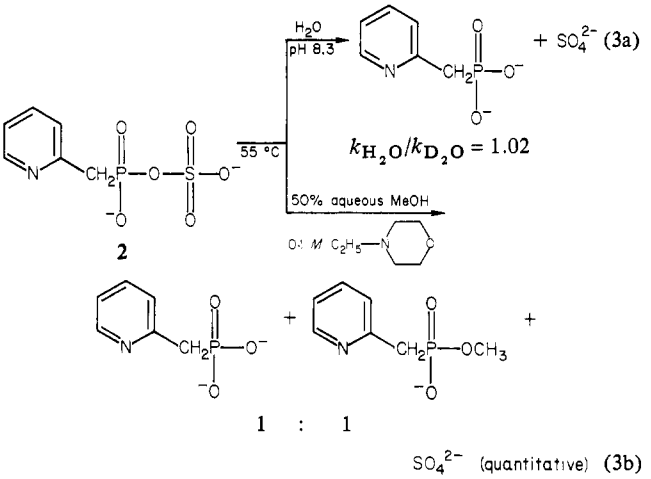




quantitative

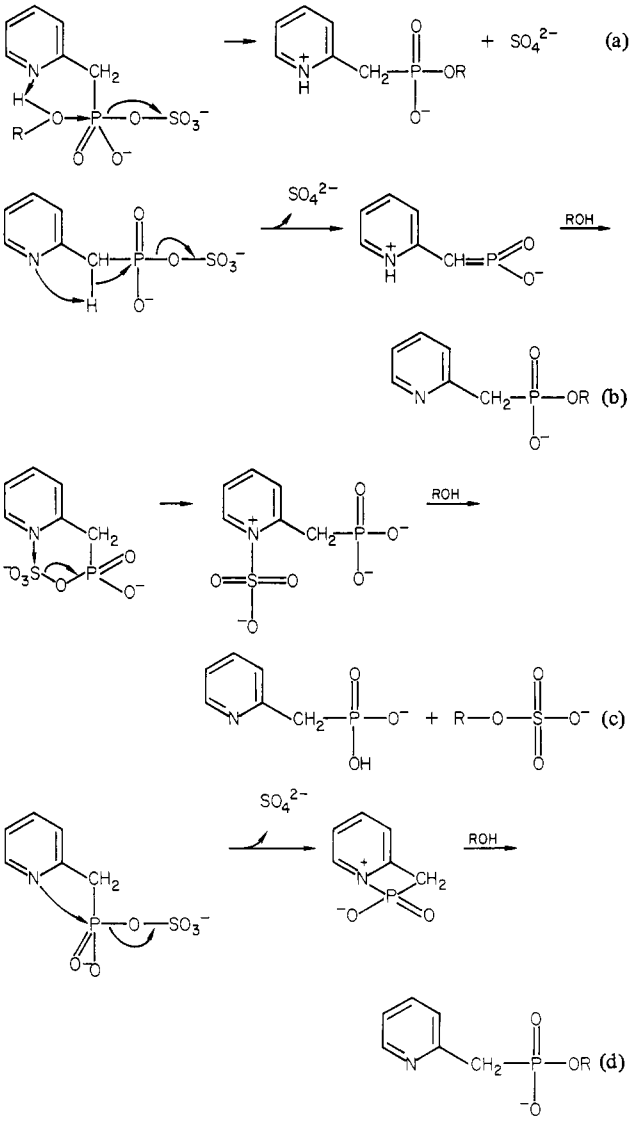
10^3-fold range. The reactivity order also underwent a change from **1** > **5** > **3** > **2** > **4** to **2** > **1** > **3** > **4** > **5**; the latter sequence contradicts the leaving ability of phosphoryl moieties and may not be accounted for by the S–O cleavage mechanism, regardless of whether the reaction proceeds unimolecularly or bimolecularly by nucleophilic attack on the sulfur atom. Alternatively, the reaction may occur through attack on the phosphorus atom which leads to the P–O cleavage as described later.

Another interesting feature observed in Figure 1 is a sigmoid pH–rate profile for 2-pyridylmethylphosphonosulfate (**2**) with an inflection at around pH 5, which appears to correspond to the acid dissociation of the pyridinium cation. This leads to several postulates illustrated in Scheme I that the neutral pyridyl group acts either as an intramolecular general base or as a nucleophilic catalyst. As for the general base catalysis, (a) the pyridyl nitrogen may assist the attack of either water or alcohol on the phosphorus atom, or (b) it may abstract methylene proton; both result in elimination of the sulfate moiety. The solvent isotope effect was found to be close to unity for both **2** and **4**, i.e., k_{H_2O}/k_{D_2O} = 1.02 and 1.09, respectively (eq 3a), excluding mechanism a and suggesting the nucleophilic catalysis. The incorporation of deuterium



into both phosphonosulfate **2** and the produced phosphonate was not detected under the hydrolysis conditions, although the methylene protons of the latter underwent complete exchange with the solvent deuterium (0.1 M NaOD) after 16 h at 55 °C. This fact also appears to rule out the possible participation of mechanism b. As for the nucleophilic catalysis, the pyridyl nitrogen may attack either (c) on the sulfur atom or (d) on the phosphorus. In the former pathway, the pyridinium sulfate intermediate would act as a sulfate donor toward various acceptors. If this were the case, it would provide an interesting model of sulfate-transfer enzymes. However, all of our attempts to trap the sulfate moiety with effective sulfate acceptors such as morpholine, phenyl phosphate, and methanol failed to prove the formation of such species in the course of hydrolysis. On the other hand, the major products in aqueous methanol were methyl pyridylmethylphosphonate and inorganic sulfate; indicating exclusively a P–O cleavage pathway (eq 3b). The solvent isotope effect again was found to be nearly unity in water–methanol (1:1 v/v) (k_{SOH}/k_{SOD} = 1.24). Consequently, the most plausible mechanism consistent with the pH–rate profile, the solvent isotope effect, and the product analysis must involve the nucleophilic attack of the pyridyl nitrogen on the phosphorus atom to form a four-membered cyclic intermediate (mechanism d in Scheme I), even though attempts to isolate or characterize the intermediate have been unsuccessful. The strain energy, which is unfavorable for the effective catalysis,[11] seems to be generated by the formation of such a four-membered ring and to provide the reason why a relatively small rate enhancement (k(**2**)/k(**4**) = 30–40 at the rate plateau region) is observed compared with other examples of intramolecular catalyses.[12]

Scheme I. Possible Mechanism of Catalysis by the Neighboring Pyridyl Group



The solvolysis of 2-pyridylphosphonosulfate (**1**) did not show any evidence for such neighboring participation by the pyridyl nitrogen and the pH–rate profile for **1** was generally similar to those for **3** and **4**. An intramolecular nucleophilic catalysis was observed in the hydrolysis of 4-nitrophenyl 8-quinolyl phosphate,[13] while such catalysis was not detected in the hydrolysis of 2-pyridylphosphonate.[14] The former ester involves a five-membered cyclic intermediate, but the latter would form a three-membered one if there is any.

(11) A four-membered ring intermediate is considered in the Wittig reaction; for example, see: Cadogan, J. G., Ed. "Organophosphorus Reagents in Organic Synthesis"; Academic Press: New York, 1979. For a related four-center process at phosphorus, see also: Harrowfield, J. M.; Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1980**, *102*, 7733.

(12) For example, see: Kirby, A. J.; Fersht, A. *Prog. Bioorg. Chem.* **1971**, *1*, 1.

(13) Loran, J. S.; Williams, A. *J. Chem. Soc., Prekin Trans. 2* **1977**, 64.

(14) Loran, J. S.; Naylor, R. A.; Williams, A. *J. Chem. Soc., Prekin Trans. 2* **1977**, 418.

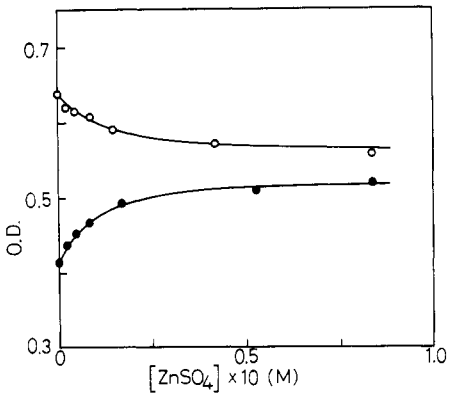


Figure 2. Plots of optical density of the **1** and **2** systems vs. Zn^{2+} ion concentration: (●) [**1**] = 1×10^{-4} M (260 nm); (○) [**2**] = 1×10^{-4} M (264 nm); μ = 1.0 (KCl); pH 5.40; 25 °C.

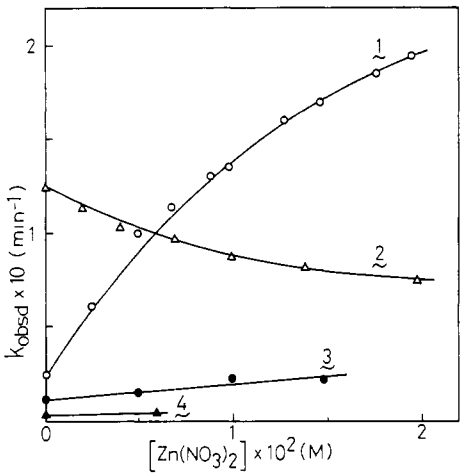


Figure 3. Zn^{2+} ion effects on the hydrolysis rates; see Table II for experimental conditions.

In conclusion, the intramolecular nucleophilic catalysis by a heterocyclic nitrogen atom seems to proceed through formation of a cyclic intermediate, a four-membered ring being a minimum allowable ring size.

Metal Ion Catalysis. Coordination of **1** and **2** with the Zn^{2+} ion was detected by the change of absorption intensity originating from electronic transitions within the pyridine ring (Figure 2). A 1:1 complex, in which the Zn^{2+} ion is bridged between the pyridyl nitrogen and the phosphoryl oxyanion, is likely involved in each case. Meanwhile, no detectable spectral change was observed in the presence of the Mg^{2+} ion, consistent with a very weak coordination tendency of the Mg^{2+} ion toward pyridine bases in general.

The effects of Zn^{2+} and Mg^{2+} ions on the hydrolysis rates under neutral pH conditions are shown in Figures 3 and 4, respectively. The Zn^{2+} ion inhibits the hydrolysis of **2** which is the most reactive species in the absence of metal ions, while it enhances the hydrolysis of **1**. The apparently different metal ion effects between the hydrolysis of **1** and **2** seem to be accounted for by assuming the same reaction scheme involving the preequilibrium formation of a 1:1 complex (C) of substrate (S) and metal ion (M) on the basis of eq 4. Equation 5 is formulated in accordance with eq 4,[15] where $[M]_T$ and $[S]_T$ are the initial stoichiometric concentrations of metal ion and substrate, respectively, k_0 and k_m are the rate constants for the hydrolyses of metal-free and metal-coordinated substrate, respectively, and K stands for the formation constant for a 1:1 complex (C). The calculated curves in Figure 3 for the hydrolysis of **1** and **2** are obtained by eq 5, using the evaluated K and k_m values given in Table II.

Figure 4. Mg^{2+} ion effects on the hydrolysis rates of **1** and **2**; see Table II for experimental conditions.

Table II. Kinetic Parameters for Metal Ion Catalyzed Hydrolysis[a]

substrate	pH	metal ion (nitrate)	k_m (k_0), min^{-1}		K, M^{-1}	$k_mK/(1 + K[S]_T)$ or k_M,[d] M^{-1} min^{-1}
1	5.56	Zn^{2+} [b]	2.86×10^{-1}	(2.38×10^{-2})	145	16.9
	5.50	Mg^{2+} [c]				0.789
2	5.77	Zn^{2+} [b]	0.634×10^{-1}	(1.24×10^{-1})	390	
	5.61	Mg^{2+} [c]		(1.09×10^{-1})		0.121
3	5.46	Zn^{2+} [c]		(1.04×10^{-2})		0.831
4	5.62	Zn^{2+} [c]		(3.15×10^{-3})		0.064

[a] Based on the data shown in Figures 3 and 4; in 0.1 M buffer solution of 2-(*N*-morpholino)ethanesulfonic acid–NaOH; [substrate] = 0.01 M; μ = 1.0 (KNO_3); 55 °C. [b] Rates were followed by high-pressure liquid chromatography. [c] Rates were followed by the barium chloranilate method in the presence of 8-hydroxyquinoline. [d] Metal-ion dependent second-order rate constant ($(k_{obsd} - k_0)/[M]$).

$$M^{2} + S \underset{}{\overset{K}{\rightleftharpoons}} C \xrightarrow{k_m} \text{products} \qquad (4)$$

$$k_{obsd} = k_0 + \frac{(k_m - k_0)K[M]_T}{1 + K[M]_T + K[S]_T\left(1 - \frac{k_{obsd} - k_0}{k_m - k_0}\right)} \qquad (5)$$

The complexation of **2** with the Zn^{2+} ion inhibits the intramolecular nucleophilic catalysis by the pyridyl group (mechanism d in Scheme I), i.e., $k_0 > k_m$ (Table II). The inhibition suggests that the coordination of Zn^{2+}, as bridged between the pyridyl nitrogen and the phosphoryl oxyanion, takes place. It is interesting to note that in spite of such an inhibition effect the k_m value for **2** is larger than the k_0 value for **1**. Thus, the complexation appears to activate the P–O–S linkage toward the attack of foreign nucleophiles (H_2O or OH^-) on **2**. As for the hydrolysis of **1**, the positive metal-ion catalysis may be expected on account of the absence of catalysis by the pyridyl group in the absence of metal ion. As shown in Table II, the k_m value for **1** is 4.5 times larger than that for **2**. A similar reactivity correlation was observed under acid-catalyzed conditions.

The formation constant for the **1**–Zn^{2+} (1:1) complex (K = 129 M^{-1}),[16] evaluated from the spectral data given in Figure 2, is close to that given in Table II (145 M^{-1}). The formation constants for both **1** and **2** complexes are comparable to those for 2-pyridylmethyl phosphate[17] and other related compounds.[18,19] It is now

(15) Benkovic, S. J.; Dunikoski, L. *J. Am. Chem. Soc.* **1971**, *93*, 1526. Equation 5 is essentially the same as that reported in this paper except for the inclusion of k_0.

(16) Based on the plots of $1/(OD_x - OD_0) = 1/(OD_\infty - OD_0)(1 + 1/K[M]_T)$; $[S]_T \ll [M]_T$.

(17) Murakami, Y.; Takagi, M. *J. Phys. Chem.* **1963**, *67*, 582.

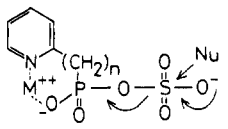


Figure 5. Possible mechanism for the selective S–O cleavage in an electrophilic catalysis by a metal ion (n = 0 and 1).

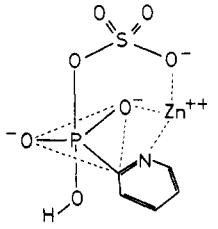
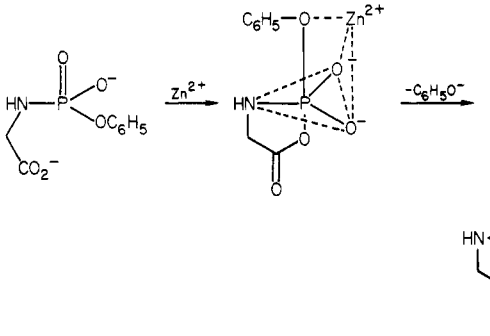


Figure 6. Proposed structure of the intermediate or transition state for the Zn^{2+} catalyzed hydrolysis of 2-pyridylphosphonosulfate (**1**).

of interest to compare the rate constants of **1** and **3** in order to demonstrate the metal-ion effects. In the absence of metal ion, the ratio $k_0(\mathbf{1})/k_0(\mathbf{3})$ is only 2 at pH 5–8. The initial slope ($k_mK/(1 + K[S]_T)$) of the curve for **1** in Figure 3 should correspond to the metal-ion-catalyzed second-order rate constant at a low metal-ion concentration and can be compared with the corresponding slope (k_M) of the line for **3** in Figure 3. The comparison gives a ratio of 16.9/0.831 = 20.3 for the Zn^{2+} ion catalysis at pH 5.5. Similarly, the ratio of 16.9/0.789 = 21.4 for the comparison of the Zn^{2+} ion with the Mg^{2+} ion catalysis indicates that the former is much more effective than the latter in the hydrolysis of **1**. Questions which arise next are how such metal ions catalyze the reaction and which is the site of bond cleavage, P–O or S–O bond.

As discussed above, it is likely that the Zn^{2+} ion bridges between the pyridyl nitrogen and the phosphoryl oxyanion in both **1** and **2**. If such were the case, the sulfate group would be left free from the metal chelation and would be attacked by a nucleophile or decomposed unimolecularly through the selective S–O bond cleavage as illustrated in Figure 5, where Zn^{2+} acts as an electrophilic catalyst. Similar electrophilic catalysis by metal ions was suggested for several reactions, such as hydrolyses of 8-quinolyl sulfate,[20] some monophosphates containing a neighboring imidazolyl[15] or pyridyl group,[19,21] or ATP.[22] However, experimental facts again indicate that the selective P–O bond cleavage takes place as in the absence of metal ion (eq 3b).

In methanol–water (1:1 v/v) containing Zn^{2+} and *N*-ethylmorpholine, both **1** and **2** gave methyl 2-pyridyl- and methyl 2-pyridylmethylphosphonate, respectively, in 50% yield. Thus, it seems to be clear that an intramolecular complexation of the 2-pyridyl nitrogen and the phosphoryl oxyanion simultaneously with a metal ion (Zn^{2+} or Mg^{2+}) can activate the phosphoryl phosphorus atom toward the attack of a nucleophile (OH^- or H_2O) which leads to the selective cleavage of the P–O bond. A similar mechanism has been suggested by Wiliiams and his co-workers for the metal-ion catalyzed hydrolysis of *O*-(4-nitrophenyl)-2-pyridylphosphonate.[14] They estimated the rate enhancement by Ni^{2+} to be greater than 930-fold over the spontaneous reaction. However, they limited the metal ion examined to a low concentration range and the kinetic data lack in comparison with the behavior of a 2-pyridylmethyl analogue. Other closely related examples are the metal-ion-catalyzed hydrolysis of phosphate diesters in which a neighboring hydroxyl or carboxyl group participates in the catalysis.[23] Benkovic proposed a mechanism in which a metal ion assists the formation of a pentavalent intermediate by neutralization of the developing negative charge on the phosphorus oxyanion brought about by the attack of a neighboring nucleophile (eq 6). In the present case, however, the neighboring 2-pyridyl nitrogen in **1** cannot take a role as a nucleophile due to steric reasons, while its nucleophilic reactivity in **2** is prohibited by complex formation with a metal ion.



(6)

In conclusion, we propose a mechanism for the present metal-ion catalysis such as illustrated in Figure 6. An important feature in this mechanism is the participation of the 2-pyridyl group in the catalysis which acts as an efficient metal binder for the favorable formation of the pentavalent phosphorus intermediate. The role of metal ion is most plausibly to faciliate the nucleophilic attack of hydroxide ion through its charge neutralization effect at the phosphoryl oxyanion and to stabilize the pentavalent phosphorus intermediate, acting as a template component. This mechanism seems to be essentially the same as that proposed by Williams et al.[14] but differs somewhat from that given by Benkovic (eq 6).[23]

The present results strongly suggest for enzymic reactions involving active sulfates that the P–O bond is also cleaved to afford a phosphoryl moiety if an enzyme requires a divalent metal ion as a cofactor, but sulfate transfer reactions are not favored under metal-ion-catalyzed conditions. It should be noted that in all of the model reactions so far examined,[4] the S–O bond cleavage was observed only under acidic conditions.[24]

Experimental Section

Materials and Methods. All melting points were uncorrected. UV and visible spectra were recorded on a Shimadzu UV-200 spectrophotometer and NMR spectra with a Varian A-60 spectrometer. pH was measured with a Hitachi-Horiba F-7DE pH meter. Water used for kinetics was purified by treating deionized water with $KMnO_4$ followed by double distillation. Buffer and other reagents for analysis were obtained from commercial sources as extra pure reagents.

Phosphonic Acids (Table I). 2-Pyridyl-,[25] 2-pyridylmethyl-,[26] phenyl-,[27] and benzylphosphonic acid[28] were all prepared according to the literature methods.

Phosphonosulfates (Table I) were prepared by the following general procedure.

2-Pyridylphosphonic (1 g; 6.28 mmol) was dissolved in a mixture of *N*-ethylmorpholine (5.07 g; 44 mmol) and anhydrous DMF (10 mL). To this mixture was added dropwise with stirring a DMF solution (11.2 mL) of SO_3 (1.4 M) and the stirring was continued for 1 h at room temperature. A methanol solution of dry ammonia (10.7 mL; NH_3 44 mmol) was added dropwise to the reaction mixture with stirring on an ice–salt bath. Anhydrous ether (160 mL) was further added to give an oily precipitate, which was separated by decantation and washed with a small amount of ethanol to give a solid. This solid was purified by reprecipitation from methanol–ether to give the diammonium salt of 2-pyridylphosphonosulfate (**1**) (740 mg; 43% yield). The yields for other esters were also around 40%.

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Table III. Methanolysis Products in 50% Aqueous Methanol in the Presence of 2,6-Lutidine (55 °C)

substrate	methyl arylphosphonate (product) $\delta(CH_3)^a$	$\delta(CH_2)^a$	R_f^b	yield,[c] %
1	3.55 (d, 3 H)		0.62	49
2	3.35 (d, 3 H)	3.52 (d, 2 H)	0.64	50
3	3.10 (d, 3 H)	3.60 (d, 2 H)	0.67	39
4	3.53 (d, 3 H)		0.72	42

[a] ^1H NMR signal in D_2O; from sodium 2,2-dimethyl-2-silapentane-5-sulfonate. [b] Toyo Filter Paper No. 50; *n*-PrOH–NH_3 (28%)–H_2O (6:3:1). [c] Based on NMR analysis.

Kinetic Measurements. All of the rates were determined under pseudo-first-order conditions.

(a) In the Absence of Metal Ion. The reaction mixture (substrate = 0.01 M, and μ = 1.0 (KCl)) was transferred into ampules and kept in a constant temperature bath. The hydrolysis rate was then determined by monitoring the amounts of inorganic sulfate released at appropriate time intervals according to the barium chloranilate method previously reported.[7-9]

(b) In the Presence of Metal Ion. 2-(*N*-Morpholine)ethanesulfonic acid–NaOH (0.1 M) was used as the buffer. In the presence of $Mg(NO_3)_2$, the kinetic method was exactly the same as described above. In the presence of $Zn(NO_3)_2$, two methods were employed. (1) The barium chloranilate method was applied to the cases of phenyl- (**3**) and benzylphosphonosulfate (**4**), for which a sample solution (5 mL) for analysis was composed of the reaction mixture (0.5 mL), acetate buffer (1 mL, 2.5 M, pH 4), an ethanol solution of 8-hydroxyquinoline (2.5 mL, 6×10^{-3} M), and water. This composition is the same as that employed for usual analysis except for the presence of 8-hydroxyquinoline. (2) In the cases of 2-pyridyl- (**1**) and 2-pyridylmethylphosphonosulfate (**2**), the rates were followed by means of high-pressure liquid chromatography [HPLC Yanako L-2000; column, Yanako PEL-AX (o.d. 2 mm × 500 mm); eluant, 5×10^{-2} M KH_2PO_4; sample size, 4 μL]; a phosphonate produced during the reaction was well separated from the corresponding phosphonosulfate. Method 1 was also applied to the latter cases.

Product Analysis. (a) Hydrolysis. Amounts of the liberated inorganic sulfate were determined colorimetrically by using barium chloranilate as mentioned above. Phosphonates were identified by paper chromatography and their yields were evaluated on the basis of UV absorption intensity (Table I). In the cases of **1** and **2** the phosphonates could be accurately determined by high-pressure liquid chromatography as mentioned above.

(b) Methanolysis in the Absence of Metal Ion. (1) Under Acidic Conditions in 50% Aqueous Methanol (55 °C). The reaction mixtures were neutralized to pH 7 and concentrated to dryness. Dried solid residues were analyzed by NMR in D_2O for methyl sulfate: $\delta(CH_3)$ (D_2O) 3.73 (3 H, singlet).

(2) Under Neutral pH Conditions in 50% Aqueous Methanol (55 °C). 2-Pyridylmethylphosphonosulfate (**2**) (150 mg) was dissolved in 50% aqueous methanol containing 2,6-lutidine (1 M) to prepare its 0.1 M solution. The reaction mixture was kept for 24 h at 55 °C and concentrated to dryness. The solid residue was dissolved in 5 mL of water, to which was added a few drops of aqueous ammonia (28%). This aqueous solution was again evaporated to dryness, and the residue was dried on P_2O_5 and analyzed by NMR in D_2O for methyl 2-pyridylmethylphosphonate. This ester was also analyzed by paper and high-pressure liquid chromatography. The results including those for other substrates are shown in Table III.

(c) Methanolysis in the Presence of $Zn(NO_3)_2$ in 50% Aqueous Methanol (55 °C). The reaction gave essentially the same results as described above for the case without metal ion as regards product distribution. In all the cases including that of Cu^{2+} ion, the formation of methyl sulfate was not detected.

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Registry No. 1, 80642-94-0; **2**, 80642-95-1; **3**, 80642-96-2; **4**, 80642-97-3; **5**, 32599-82-9; 2-pyridylphosphonate diammonium salt, 80642-98-4; 2-pyridylmethylphosphonate diammonium salt, 80642-99-5; phenylphosphonate diammonium salt, 63119-09-5; benzylphosphonate diammonium salt, 80643-00-1; methyl 2-pyridylphosphonate, 80643-01-2; methyl 2-pyridylmethylphosphonate, 80643-02-3; methyl phenylphosphonate, 10088-45-6; methyl benzylphosphonate, 63581-66-8; Zn, 7440-66-6; Mg, 7439-95-4.

Studies of Hydrogen-Bonded 5′-Guanosine Monophosphate Self-Associates Using Low-Frequency Raman Scattering

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Abstract: The temperature and concentration dependence of the low-frequency (20–400 cm^{-1}) Raman spectra of the disodium and dipotassium salt in the gel state have been studied by calculation of $R(\bar{\nu})$. The spectra showed two bands below 150 cm^{-1} with maxima at ca. 115 and ca. 80 cm^{-1}, respectively. The former is assigned to hydrogen-bonded self-associates of 5′-GMP, whereas the latter is ascribed to non-hydrogen-bonded self-associates or monomers of 5′-GMP. The gel state spectra did only differ from the solution spectra by a drastically increased intensity of the band at ca. 115 cm^{-1}, indicating that hydrogen-bonded self-associates are the dominant species in the gel state. The effect of deuterium substitution (N–D and O–D) upon the spectra was insignificant.

We compared[1,2] low-frequency Raman scattering (Rayleigh-wing scattering) to far-infrared absorption for a number of molecular liquids by calculation of $R(\bar{\nu})$ from the scattered intensity in Stoke's side. The absorption coefficient for water is very high in the far-IR region, and absorption spectra of water and aqueous solutions are thus difficult to obtain. The high intensity of the central (exciting) line makes it in practice nearly impossible to investigate Raman spectra of aqueous solutions below 200 cm^{-1}. The main advantage of the $R(\bar{\nu})$ technique is that the central line in a Raman spectrum is suppressed and the low-frequency vibrations thus much easier observed. This fact was demonstrated in our low-frequency studies of water[3] and of aqueous solutions

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