

# METAL ION-PROMOTED HYDROLYSIS OF URIDINE 2',3'-CYCLIC MONOPHOSPHATE: EFFECT OF METAL CHELATES AND UNCOMPLEXED AQUO IONS

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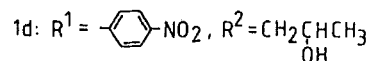
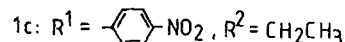
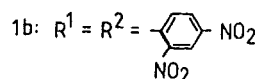
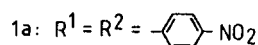
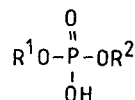
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The effect of a wide variety of metal ions and metal ion complexes on the hydrolysis of uridine 2',3'-cyclic monophosphate was studied over the pH range 4.5–8.0. The greatest rate accelerations observed were  $10^7$ – $10^8$ -fold. The kinetic data obtained are interpreted in terms of a mechanism involving a rapid monodentate binding of the metal ion to the monoanionic phosphodiester and a subsequent intracomplex participation of the metal-bound hydroxo ligand, either as a nucleophile attacking the tetracoordinated phosphorus or as a base facilitating an intermolecular attack of a water molecule. No effect on the product distribution between uridine 2'- and 3'-monophosphates was observed.

## INTRODUCTION

The effect of metal ions on the hydrolysis and transesterification of phosphodiester bonds of nucleic acids has recently received increasing interest for the reason that enzymes catalysing hydrolysis of phosphoesters frequently require metal ions as cofactors.<sup>1</sup> Extensive kinetic measurements on metal ion-promoted hydrolysis of simple diaryl monophosphates (**1**) have been carried out in order to elucidate the role of metal ions in enzyme catalysis, and to develop artificial catalysts for hydrolysis of phosphodiester bonds. In particular, the results obtained with substitution-inert Co(III)<sup>2–6</sup> and Ir(III) complexes<sup>7,8</sup> have provided valuable information on the mechanism of metal ion action. It has been shown that *cis*-diaquotetraazacobalt(III) complexes are highly reactive in promoting the hydrolysis of bis(4-nitrophenyl)phosphate (**1a**), the hydrolysis rate passing through a maximum at pH 7, i.e. under conditions where the aquohydroxo form prevails.<sup>5</sup> The rate enhancement, but not the phosphoester binding, appears to be sensitive to the geometry of the tetraaza ligand. For example, **1a** when bound to (trpn)Co(OH)(OH<sub>2</sub>)<sup>2+</sup> [trpn = tris(3-aminopropyl)amine] is hydrolysed 300 times more rapidly than when bound to (tren)Co(OH)(OH<sub>2</sub>) [tren = tris(2-aminoethyl)amine], in spite of comparable stabilities of the phosphodiester

complexes.<sup>3</sup> Compared with an unbound ester, the rate enhancement has been estimated<sup>9</sup> to be as high as  $10^{10}$ . Most likely the anionic phosphodiester undergoes a rapid initial monodentate binding to the metal ion, and subsequently a *cis*-hydroxo ligand performs a rate-limiting nucleophilic attack on the phosphorus atom, resulting in a trigonal bipyramidal intermediate that rapidly decomposes to products.<sup>9</sup> With more reactive diesters, such as bis(2,4-dinitrophenyl)phosphate (**1b**), the binding step may become partly rate limiting.<sup>4</sup> It has been questioned, however, whether this type of mechanism, involving the formation of a four-membered cyclic transition state, operates in biological



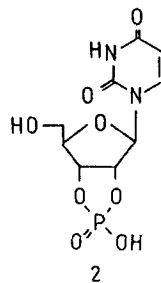
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systems.<sup>7</sup> Hydrolysis of ethyl 4-nitrophenyl phosphate (**1c**) and bis(4-nitrophenyl)phosphate (**1a**) coordinated to *cis*-(en)<sub>2</sub>Ir(OH)(OH<sub>2</sub>)<sup>2+</sup> (en = ethylenediamine) also proceeds by intracomplex attack of the hydroxo ligand on phosphorus, but the reaction rate is three orders of magnitude smaller than that of the corresponding Co(III) complex.<sup>7</sup> This reactivity difference has been ascribed to the larger size of Ir(III) ion.<sup>7</sup> Similarly, the (NH<sub>3</sub>)<sub>5</sub>Ir(III) complex of **1c** liberates 4-nitrophenol by intracomplex attack of the amido ligand much more slowly than the corresponding Co(III) complex.<sup>6,8</sup> Since the biologically important metal ions are even larger than Ir(III) ion, their intracomplex reactions may be considerably slower than those of Co(III) ion.<sup>7</sup>

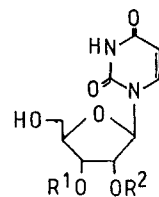
In addition to Co(III) and Ir(III) ions, several 3d transition metal ions (and Zn<sup>2+</sup>) and their aza complexes have been shown to catalyse the hydrolysis of simple phosphodiester derived from 4-nitrophenol.<sup>10-12</sup> The monohydroxo form of the complex has been identified as the catalytically active species, and an intracomplex hydroxide attack on a metal-bound phosphodiester has been regarded as the most plausible mechanism.<sup>10-12</sup> The hydrolysis rate of the metal-coordinated ester is under slightly alkaline conditions at least 10<sup>3</sup>-fold that of the unbound compound.<sup>10-12</sup> As with Co(III) complexes, the rate enhancement is sensitive to the ligand structure.<sup>12</sup>

Although the studies with **1a-c** have furthered considerably the understanding of metal ion-promoted hydrolysis of phosphodiester, it is worth noting that these compounds contain an exceptionally good leaving group, which is not the case with naturally occurring phosphodiester. Comparative investigations with real biomolecules would help to assess the general applicability of the mechanistic conclusions based on hydrolysis of aryl phosphates, but such data are scarce. Chin and Zou<sup>13</sup> reported on (trien)Co(OH)(OH<sub>2</sub>)-promoted hydrolysis of adenosine 3',5'-cyclic monophosphate. Moreover, several studies on hydrolysis of diribonucleoside monophosphates have been published.<sup>14-21</sup> The latter reaction, however, proceeds by an intramolecular participation of the neighbouring hydroxyl group, and is hence a transesterification reaction rather than a simple phosphodiester hydrolysis. We now report on metal ion-promoted hydrolysis of uridine 2',3'-cyclic monophosphate (**2**, 3'-cUMP, **2**).

This cyclic phosphodiester was selected as a model compound for the following reasons. First, it is a biologically relevant compound; ribonuclease-catalysed hydrolysis of RNA proceeds by intermediate formation of a 2',3'-cyclic monophosphate structure at the 3'-end of the polynucleotide chain.<sup>22</sup> Second, hydrolysis of nucleoside 2',3'-cyclic monophosphates is fairly rapid,<sup>23-25</sup> in spite of the fact that these compounds do not contain a good leaving group. Hence extensive data on metal ion effects may be collected. Third, hydrolysis of a five-membered cyclic phosphodiester may be



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3a: R<sup>1</sup> = H, R<sup>2</sup> = PO(OH)<sub>2</sub>3b: R<sup>1</sup> = PO(OH)<sub>2</sub>, R<sup>2</sup> = H

expected to be sensitive to the structure of the metal ion catalyst. Most likely the driving force of the reaction is the relief of ring strain accompanying the formation of a pentacoordinated intermediate that the nucleophilic attack of water (or hydroxide ion) on the tetracoordinated phosphorus results in.<sup>26</sup> Binding of a metal ion to the negatively charged phosphate group and subsequent intracomplex attack of aquo or hydroxo ligand on phosphorus would result in a pentacoordinated transition state having two oxygen ligands coordinated to the metal ion. This kind of bidentate coordination undoubtedly affects the geometry and ring strain of the pentacoordinated transition state. Finally, the effect of metal ions on product distribution between 2'- and 3'-monophosphates (**3a** and **b**) elucidates the role that metal ions play in the breakdown of the pentacoordinated intermediate. The previous data on metal ion-promoted hydrolysis of nucleoside 2',3'-monophosphates are limited to the observations that Eu(III) ion promotes the hydrolysis much more effectively than Zn(II) ion,<sup>20</sup> or its macrocyclic triaza complex, Zn<sup>2+</sup> (1,4,7-triazacyclononane).<sup>19</sup>

## RESULTS AND DISCUSSION

Table 1 gives the first-order rate constants observed for the hydrolysis of 2',3'-cUMP (**2**) in the presence of various metal ions ([M<sup>2+</sup>] = 0.005 mol dm<sup>-3</sup>) in acetate (pH 4.7) and HEPES (pH 5.6) buffers. Neither of these buffers binds metal ions strongly. The low complexing tendency of HEPES has been well established.<sup>27</sup> Acetate ion forms relatively stable complexes with di- and trivalent metal ions,<sup>28</sup> but at the low buffer concentrations employed (0.005 mol dm<sup>-3</sup>) the proportion of the complexed metal ion never exceeds 30%. Accordingly, the rate enhancements in Table 1 mainly refer to metal aquo ions. The fact that these enhancements are invariably larger in HEPES than in acetate buffer, and virtually independent of buffer concentration at the low concentration range employed, suggests that the efficiency of metal ions in promoting the hydrolysis of 2',3'-cUMP is increased with increasing pH.

Table 1. Effect of metal ions on the hydrolysis of 2',3'-cUMP at 363.2 K

$M^{z+}$	$k(10^{-6}s^{-1})^a$		$x(3'-UMP)^b$	
	pH 4.7 <sup>c</sup>	pH 5.6 <sup>d</sup>	pH 4.7 <sup>c</sup>	pH 5.6 <sup>d</sup>
$Mg^{2+e}$	$0.86 \pm 0.03$	$1.02 \pm 0.02$	0.73	0.70
$Mn^{2+e}$	$1.06 \pm 0.02$	$1.88 \pm 0.03$	0.71	0.67
$Mn^{2+c}$	$2.70 \pm 0.06$	$16.4 \pm 0.1$	0.66	0.68
$Co^{2+}$	$2.55 \pm 0.03^e$	$10.6 \pm 0.2^f$	0.67	0.67
$Ni^{2+}$	$1.86 \pm 0.03^e$	$6.23 \pm 0.07^f$	0.70	0.66
$Cu^{2+e}$	$902 \pm 9$	— <sup>g</sup>	0.57	
$Zn^{2+}$	$13.3 \pm 0.2^e$	$33.9 \pm 0.5^f$	0.63	0.65
$Cd^{2+}$	$4.53 \pm 0.09^e$	$23.8 \pm 0.6^f$	0.66	0.65
$Eu^{3+e}$	$1070 \pm 9$	$26,200 \pm 100$	0.64	0.65
$Pb^{2+e}$	$434 \pm 5$	— <sup>g</sup>	0.64	

<sup>a</sup> The first-order rate constants refer to a metal ion concentration of  $0.005 \text{ mol dm}^{-3}$ .

<sup>b</sup> Mole fraction of 3'-UMP in the mixture of 2'- and 3'-UMP.

<sup>c</sup> Adjusted with acetic acid-sodium acetate buffer ( $[HA]/[A^-] = 0.005/0.005 \text{ mol dm}^{-3}$ ). Ionic strength adjusted to  $0.10 \text{ mol dm}^{-3}$  with sodium perchlorate.

<sup>d</sup> Adjusted with HEPES buffer ( $[HA]/[A^-] = 0.10/0.010 \text{ mol dm}^{-3}$ ). Ionic strength adjusted to  $0.10 \text{ mol dm}^{-3}$  with sodium perchlorate. The  $pK_a$  value of HEPES extrapolated to 363.2 K is 6.6.<sup>27</sup>

<sup>e</sup> Added as nitrate.

<sup>f</sup> Added as perchlorate.

<sup>g</sup> Precipitated.

The results of more extensive studies with  $Zn^{2+}$  and  $Eu^{3+}$  ions are depicted in Figure 1. The rate of metal ion-promoted hydrolysis continuously increases as a function of pH. With  $Zn^{2+}$  the reaction is first order in hydroxide ion concentration, whereas with  $Eu^{3+}$  the reaction order continuously increases with increasing pH. In particular, on approaching the conditions where precipitation of europium hydroxide takes place ( $pH > 7$ ), the plot of the logarithmic rate constant vs pH shows a marked upward curvature.

The rate-accelerating effects of various metal ions differ considerably. Among divalent cations, the effect of  $Mg^{2+}$  is hardly noticeable, whereas  $Cu^{2+}$  ion results in a  $10^3$ -fold acceleration.  $Pb^{2+}$  and trivalent lanthanide ions are approximately as effective promoters as  $Cu^{2+}$ .  $Zn^{2+}$  and  $Cd^{2+}$  ions appreciably enhance the hydrolysis of 2',3'-cUMP, but much less than  $Cu^{2+}$ . Accordingly, those metal ions which are known to cleave RNA<sup>14,29-32</sup> also promote the hydrolysis of 2',3'-cUMP most efficiently.

Figure 2 shows as an illustrative example the dependence of the hydrolysis rate on the concentration of  $Zn^{2+}$ ,  $Eu^{3+}$  and  $Zn^{2+}$  (1,5,9-triazacyclododecane) (4) ions. The rate acceleration is almost linearly related to the metal ion concentration at  $[M^{z+}] < 0.01 \text{ mol dm}^{-3}$ . In other words, only one metal ion is involved in the pre-equilibrium and/or rate-limiting stage of the hydrolysis reaction. Bearing this in mind, four alternative mechanisms may be formulated for the

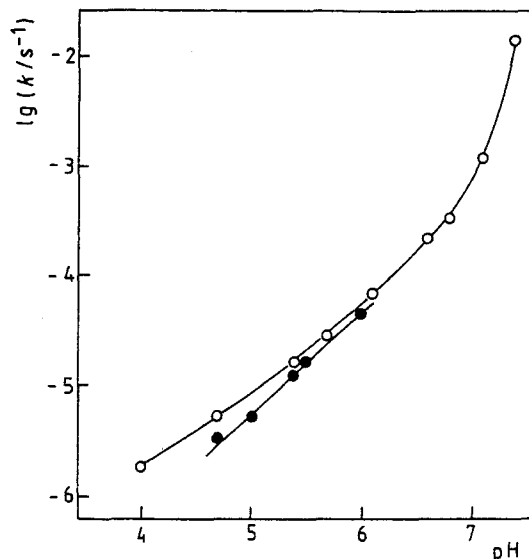


Figure 1. Effect of hydronium ion concentration on  $Zn^{2+}$ - and  $Eu^{3+}$ -promoted hydrolysis of 2',3'-cUMP (2). The hydronium ion concentration was adjusted with acetate and HEPES buffers ( $I = 0.1 \text{ mol dm}^{-3}$  with  $NaClO_4$ ). The first-order rate constants were obtained at  $[Eu^{3+}] = 3.0 \times 10^{-3} \text{ mol dm}^{-3}$  ( $T = 303.2 \text{ K}$ ) ( $\circ$ ) and at  $[Zn^{2+}] = 2.0 \times 10^{-3} \text{ mol dm}^{-3}$  ( $T = 363.2 \text{ K}$ ) ( $\bullet$ ).

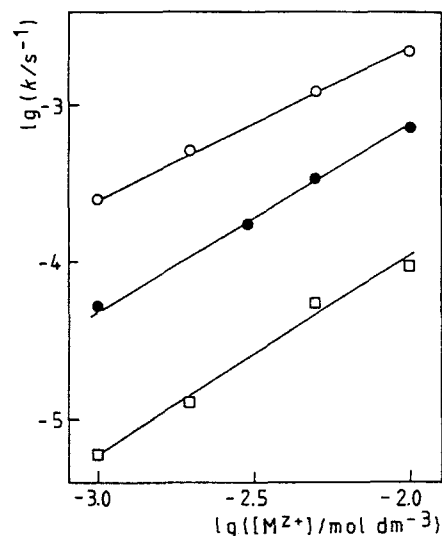


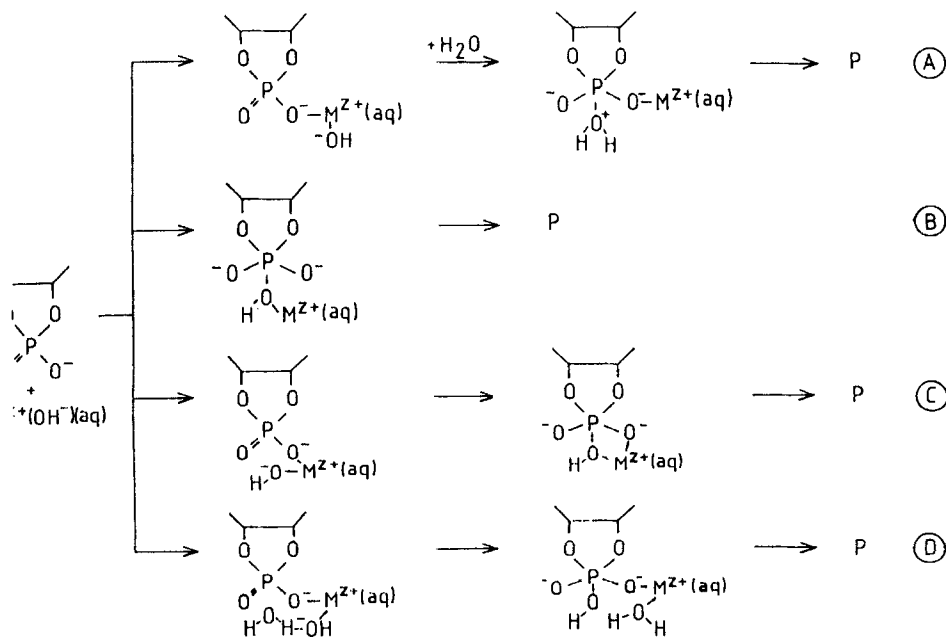
Figure 2. First-order rate constants for the metal ion-promoted hydrolysis of 2',3'-cUMP (2) at different metal ion concentrations ( $I = 0.1 \text{ mol dm}^{-3}$  with  $NaClO_4$ ). ( $\circ$ )  $Zn^{2+}$  (1,5,9-triazacyclododecane) at  $pH 6.1$ ,  $T = 363.2 \text{ K}$ ; ( $\bullet$ )  $Eu^{3+}$  at  $pH 6.7$ ,  $T = 303.2 \text{ K}$ ; ( $\square$ )  $Zn^{2+}$  at  $pH 5.3$ ,  $T = 363.2 \text{ K}$ .

metal ion-promoted reaction. In the absence of metal ions, the reaction is independent of pH in the range 4.5–6.0, and most likely proceeds by an intermolecular nucleophilic attack of a water molecule on the phosphorus atom of the monoanionic phosphodiester, giving a pentacoordinated phosphorane intermediate that is subsequently decomposed to a 1:2 mixture of the 2'- and 3'-monophosphates.<sup>23–25</sup>

Metal ions may accelerate this process by one of the following mechanisms: (i) a metal ion binds to the phosphodiester monoanion, and hence electrostatically facilitates the intermolecular attack of a water molecule on phosphorus (route A in Scheme 1); (ii) a metal ion acts in its monohydroxo form as a source of intermolecular nucleophile, hydroxide ion (route B); (iii) a metal ion binds to the phosphodiester monoanion, and its hydroxo ligand performs an intracomplex nucleophilic attack on phosphorus (route C); or (iv) a metal ion binds to the phosphodiester monoanion, and its hydroxo ligand abstracts a proton from a water molecule attacking the phosphorus (route D). Of these four alternatives, route A appears unlikely. The efficiency of various metal ions in promoting the hydrolysis of 2',3'-cUMP differs more than could be expected on the basis of their binding affinities to the starting material. For example, the  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  complexes of dihydrogenphosphate ion, mimicking the phosphodiester monoanion, are only one order of magnitude more stable than the  $\text{Mg}^{2+}$  complex.<sup>33</sup> It therefore appears improbable that the more than  $10^3$ -fold rate-accelerating effect

of  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  compared with  $\text{Mg}^{2+}$  could result entirely from a stronger complexing of these ions with 2',3'-cUMP. For the following reasons it seems likely that the acidity of the metal aquo ion plays a more decisive role: (i) the rate enhancing-effect is increased with increasing pH; (ii) among divalent metal ions, the most acidic ones<sup>34</sup> ( $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ) exhibit the greatest rate accelerations; and (iii) with lanthanide ions (and  $\text{Y}^{3+}$ ) the rate enhancement correlates with the  $\text{pK}_a$  value of the aquo ion (Figure 3). To distinguish between the inter- and intramolecular participation of metal hydroxo aquo ions (route B vs routes C and D) the effect of ligand structure on the rate-accelerating ability of metal ions is considered in the following.

Figure 4 shows the first-order rate constants for the hydrolysis of 2',3'-cUMP in the presence of various tri- and tetraaza complexes of  $\text{Zn}^{2+}$  (4–9). Complexing with these ligands markedly affects the acidity of  $\text{Zn}^{2+}$  aquo ion. The  $\text{pK}_a$  values increase in order of decreasing acidity:  $\text{Zn}^{2+}$  [12]aneN<sub>3</sub> (4) 7.51 ( $T = 298.2 \text{ K}$ ,  $I = 0.1 \text{ mol dm}^{-3}$ ),<sup>35</sup>  $\text{Zn}^{2+}$  [12]aneN<sub>4</sub> (5) 8.02 ( $T = 298.2 \text{ K}$ ,  $I = 0.1 \text{ mol dm}^{-3}$ ),<sup>36</sup>  $\text{Zn}^{2+}$  8.96 ( $T = 298.2 \text{ K}$ ,  $I = 0$ ),<sup>37</sup>  $\text{Zn}^{2+}$  (tren) (9) 9.07 ( $T = 293.2 \text{ K}$ ,  $I = 0.4 \text{ mol dm}^{-3}$ ),<sup>38</sup>  $\text{Zn}^{2+}$  [14]aneN<sub>4</sub> (6) 9.77 ( $T = 298.2 \text{ K}$ ,  $I = 0.1 \text{ mol dm}^{-3}$ ),<sup>36</sup> and  $\text{Zn}^{2+}$  (tren) (8) 10.59 ( $T = 293.2 \text{ K}$ ,  $I = 0.4 \text{ mol dm}^{-3}$ ).<sup>38</sup> The  $\text{pK}_a$  value of  $\text{Zn}^{2+}$  [15]aneN<sub>4</sub> (7) is unknown, but in all likelihood it is of the same order of magnitude as the one reported for  $\text{Zn}^{2+}$  [14]aneN<sub>4</sub>. As seen from Figure 4, with each complex the rate-



Scheme 1.

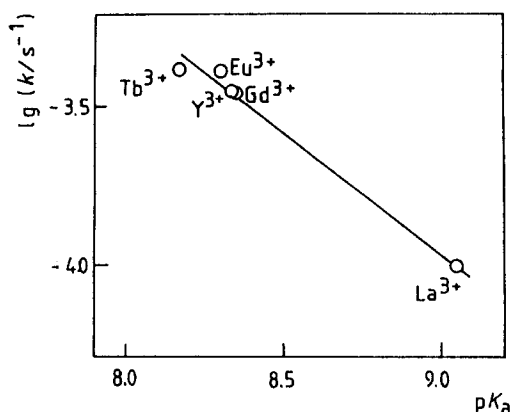


Figure 3. Logarithmic first-order rate constants for the lanthanide ion- (and  $\text{Y}^{3+}$ )-promoted hydrolysis of 2',3'-cUMP (2) plotted against the  $\text{pK}_a$  value of the metal aquo ion ( $[\text{M}^{3+}] = 0.005 \text{ mol dm}^{-3}$ ,  $\text{pH} = 6.7$  with HEPES,  $T = 303.2 \text{ K}$ ,  $I = 0.1 \text{ mol dm}^{-3}$  with  $\text{NaClO}_4$ ). For the  $\text{pK}_a$  values, see Ref. 34

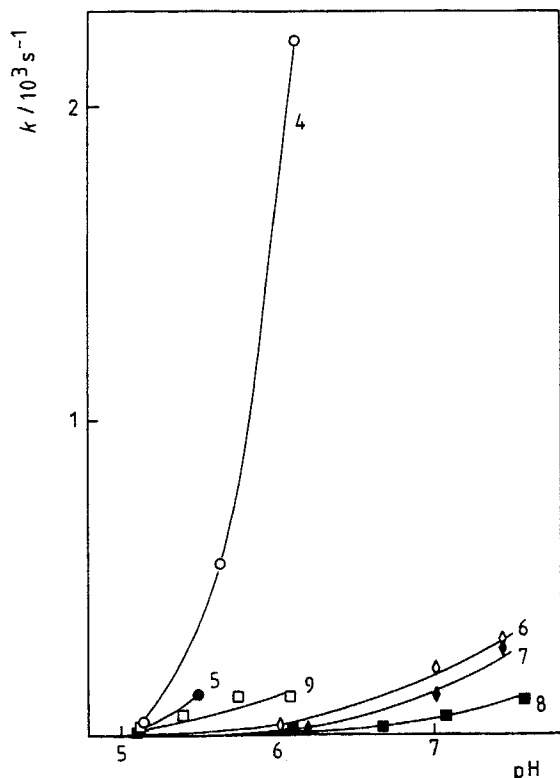
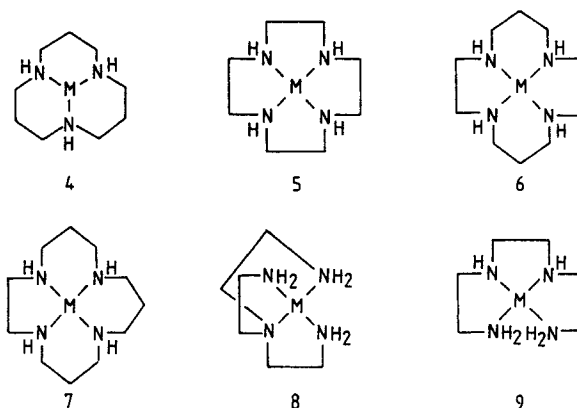


Figure 4. First-order rate constants at different pH values (adjusted with HEPES buffer) for the hydrolysis of 2',3'-cUMP (2) in the presence of various tri- and tetraaza complexes of  $\text{Zn}^{2+}$  ( $[\text{Zn}^{2+}] = [\text{L}] = 0.010 \text{ mol dm}^{-3}$ ,  $T = 363.2 \text{ K}$ ,  $I = 0.10 \text{ mol dm}^{-3}$  with  $\text{NaClO}_4$ ). For 4–9 see structural formulae



accelerating effect becomes prominent on approaching the  $\text{pK}_a$  value of the complex, i.e. under conditions where the mole fraction of the hydroxo form becomes appreciable. In other words, the complexes become catalytically active only on deprotonation. Consistent with this conclusion, the rate enhancements observed under neutral conditions correlate fairly well with the  $\text{pK}_a$  value of the complex (Figure 5). Accordingly, as suggested above, the metal ion-promoted hydrolysis proceeds by either inter- or intramolecular participation of the metal hydroxo aquo ion (routes B–D), and hence the acidity of the metal aquo ion plays an important role.

The results obtained with the  $\text{Ni}^{2+}$  complexes, 4–9, are analogous to those described above for the  $\text{Zn}^{2+}$  complexes: a marked rate acceleration takes place under conditions where the complex may be expected to undergo deprotonation (Figure 6). Since the  $\text{Ni}^{2+}$  complexes are less acidic than the corresponding  $\text{Zn}^{2+}$  species, the rate accelerations take place at higher pH. The following  $\text{pK}_a$  values have been reported for the  $\text{Ni}^{2+}$  complexes:  $\text{Ni}^{2+}$  [14]ane $\text{N}_4$  (6) 13.0 ( $T = 298.2 \text{ K}$ ,  $I = 0.1 \text{ mol dm}^{-3}$ ),<sup>39</sup>  $\text{Ni}^{2+}$  [15]ane $\text{N}_4$  (7) 11.7 ( $T$  not indicated,  $I = 1.0 \text{ mol dm}^{-3}$ ),<sup>40</sup>  $\text{Ni}^{2+}$  (tren) (8) 11.8 ( $T = 298.2 \text{ K}$ ,  $I = 0.1 \text{ mol dm}^{-3}$ ),<sup>41</sup> and  $\text{Ni}^{2+}$  (trien) (9) 11.8 ( $T = 298.2 \text{ K}$ ,  $I = 0.1 \text{ mol dm}^{-3}$ ).<sup>41</sup> The effect of some  $\text{Cu}^{2+}$  complexes on the hydrolysis of 2',3'-cUMP was determined at pH 4.7 (Table 2). All the complexes studied were catalytically considerably less efficient than the uncomplexed aquo ion. On going to higher pH, the rate-enhancing influence was drastically diminished, possibly owing to the tendency of  $\text{Cu}^{2+}$  to form dimers.<sup>35</sup>

Comparison of the rate-enhancing effects of the  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  complexes (Figures 4 and 6) suggests that the acidity of the equated complex is not the only factor affecting the catalytic efficiency. As seen from Figure 5, the points referring to  $\text{Ni}^{2+}$  complexes fall above the straight correlation line,  $\log[k(\text{s}^{-1})]$  vs  $\text{pK}_a$  that the  $\text{Zn}^{2+}$  complexes yield. In particular,  $\text{Ni}^{2+}$  (tren) is con-

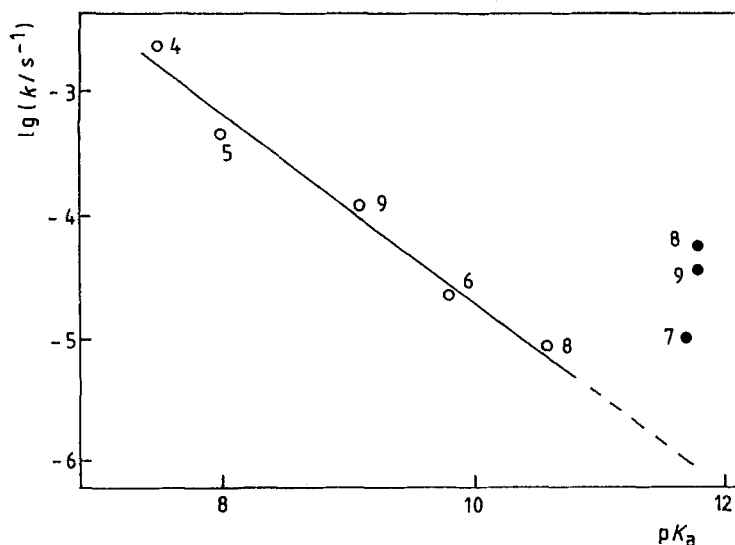


Figure 5. (○) logarithmic first-order rate constants for  $\text{Zn}^{2+}$  complex-promoted hydrolysis of 2',3'-cUMP (2) at pH 6.1 (adjusted with HEPES buffer) plotted against the  $\text{pK}_a$  value of the complex (kinetics refer to  $[\text{Zn}^{2+}] = [\text{L}] = 0.010 \text{ mol dm}^{-3}$ ,  $T = 363.2 \text{ K}$ ,  $I = 0.10 \text{ mol dm}^{-3}$  with  $\text{NaClO}_4$ ;  $\text{pK}_a$  values refer to  $T = 298.2 \text{ K}$ ). (●)  $\text{Ni}^{2+}$  complexes. For 4–9 see structural formulae

siderably less acidic than  $\text{Zn}^{2+}(\text{tren})$  ( $\text{pK}_a$  values 11.8<sup>41</sup> and 10.6,<sup>38</sup> respectively), but still it is a more effective catalyst, the deviation from the correlation line of  $\text{Zn}^{2+}$  complexes being larger than 1.5 log units. A similar observation has previously been made for the hydrolysis of bis(4-nitrophenyl)phosphate.<sup>11</sup> Accordingly, the nature of the central ion and/or the coordination geometry of the complex also appear to have a profound effect on the catalytic efficiency. This strongly suggests that the metal aquo complex is not a mere intermolecular source of hydroxide ions (route B), but it interacts with the phosphodiester. The geometry of  $\text{Ni}^{2+}(\text{tren})$ , for example, differs from that of  $\text{Zn}^{2+}(\text{tren})$ , and this may explain its unexpectedly high catalytic activity. The tren ligand is known to adopt a *cis* structure around an octahedral  $\text{Ni}^{2+}$  ion,<sup>42</sup> whereas a trigonal bipyramidal structure (four azaligands and one water molecule) has been suggested for  $\text{Zn}^{2+}(\text{tren})$ .<sup>43</sup> Replacement of the aquo ligand of  $\text{Ni}^{2+}(\text{tren})$  with the negatively charged oxygen atom of the cyclic phosphodiester would give a metal–substrate complex having the hydroxo in a *cis* position to the substrate molecule. Accordingly, an intracomplex participation of the hydroxo ligand might occur without any change in the coordination geometry. With  $\text{Zn}^{2+}(\text{tren})$  utilization of a similar mechanism would require a change of coordination number from 5 to 6, which may be expected to lower the catalytic efficiency. Comparison of the rate-accelerating effects of the  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  complexes of [14]aneN<sub>4</sub> (6) lends some additional support to this reasoning. Both of these com-

plexes most likely have *trans*-octahedral configurations with the four N atoms in-plane,<sup>44,45</sup> the deprotonated forms probably having a five-coordinated structure.<sup>46</sup> Therefore, both complexes have to undergo a similar structural change before an intracomplex participation of the hydroxo ligand is possible. In contrast to tren complexes, the less acidic  $\text{Ni}^{2+}$  [14]aneN<sub>4</sub> is now also a less efficient catalyst, although it still exhibits a positive deviation from the correlation line of the  $\text{Zn}^{2+}$  complexes. In conclusion, mechanisms C and D, involving a pre-equilibrium binding of metal ion to substrate and intracomplex participation of the hydroxo ligand, either as a nucleophile or Brønsted base, seem more attractive than mechanism B.

Mechanisms C and D cannot be rigorously distinguished on the basis of the data available. It is interesting, however, that the rate-accelerating effects of various metal aquo ions on the hydrolysis of 2',3'-cUMP closely resemble those reported recently<sup>47</sup> for the hydrolysis of 4-nitrophenyl 1-(2-hydroxypropyl)phosphate (1d; Figure 7). The latter reaction (Scheme 2) cannot proceed by an intracomplex nucleophilic attack of the metal-bound hydroxide ion, but the attacking nucleophile must be the 2-hydroxyl group. The hydroxo ligand of a phosphate-coordinated metal ion may only act as a base, deprotonating the 2-hydroxyl group concerted with its nucleophilic attack. Accordingly, one may speculate that the observed similarity of the effects of metal ions on hydrolysis of 2',3'-cUMP and 1d lends support to analogous mechanisms. In other words, the hydrolysis

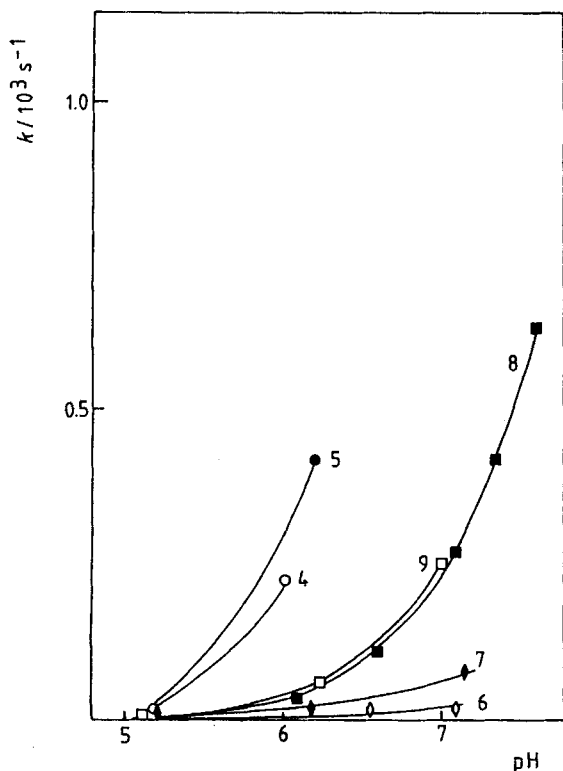


Figure 6. First-order rate constants at different pH values for the hydrolysis of 2',3'-cUMP (2) in the presence of various tri- and tetraaza complexes of  $\text{Ni}^{2+}$  ( $[\text{Ni}^{2+}] = [\text{L}] = 0.010 \text{ mol dm}^{-3}$ ,  $T = 363.2 \text{ K}$ ,  $I = 0.10 \text{ mol dm}^{-3}$  with  $\text{NaClO}_4$ ). For 4-9 see structural formulae

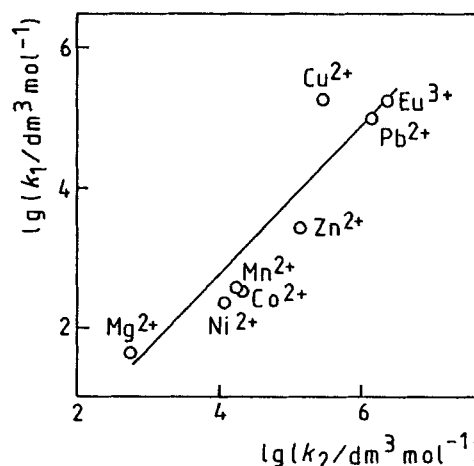
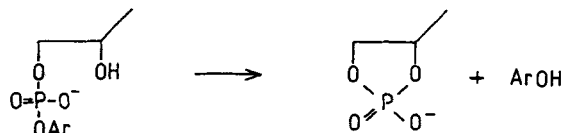


Figure 7. Comparison of the rates of metal ion-promoted hydrolysis of 2',3'-cUMP (2) and 4-nitrophenyl 1-(2-hydroxypropyl)phosphate (1d).  $k_1 = k_M/k_0$ , where  $k_M$  is the second-order rate constant for the metal ion-promoted hydrolysis of 2',3'-cUMP (pH 4.7,  $T = 363.2 \text{ K}$ ) and  $k_0$  is the first-order rate constant obtained in the absence of metal ions;  $k_2$  is the corresponding value for the hydrolysis of 1d (pH 6.85,  $T = 310 \text{ K}$ ). The latter values are taken from Ref. 47



Scheme 2.

of 2',3'-cUMP would proceed by route D. However, mechanism C can by no means be strictly excluded.

It is also worth noting that the uncomplexed lanthanide ions exhibit in their hydroxo form by far the largest rate-accelerating effects of all the metal ions and metal ion complexes studied. As seen from Figure 1, the rate-accelerating effect of  $\text{Eu}^{3+}$  ion, for example, is dramatically increased on approaching the conditions where precipitation takes place. Under such conditions the reaction order with respect to hydroxide ion is almost 3, and the rate acceleration reaches a value of  $10^8$  at  $[\text{Eu}^{3+}] = 0.01 \text{ mol dm}^{-3}$ . Binding of lanthanide ions to organic ligands, however, markedly reduces their catalytic efficiency (Table 3), the effect of polyanionic ligands, citrate and  $\text{EDTA}^{4-}$  being much larger than that of acetylacetone. Most likely the catalytically active species formed on approaching the pH of precipitation is no longer an aquohydroxo ion, but a more complex gel-like structure. In fact, according to the early finding of Butcher and Westheimer,<sup>48</sup> lanthanum hydroxide gel very effectively promotes the hydrolysis of phosphate esters.

Table 2. First-order rate constants for the hydrolysis of 2',3'-cUMP in the presence of tri- and tetraaza complexes of  $\text{Cu}^{2+}$  ion (4-9) at pH 4.7 ( $T = 363.2 \text{ K}$ ,  $I = 0.1 \text{ mol dm}^{-3}$  with  $\text{NaClO}_4$ )<sup>a</sup>

Ligand	$k(10^{-5} \text{ s}^{-1})$	$x(3'-\text{UMP})^b$
[14]aneN <sub>4</sub> (6)	$1.90 \pm 0.03$	0.67
[15]aneN <sub>4</sub> (7)	$4.90 \pm 0.04$	0.63
tren (8)	$1.53 \pm 0.06$	0.63

<sup>a</sup> pH adjusted with acetic acid-sodium acetate buffer ( $[\text{HA}]/[\text{A}^-] = 0.005/0.005 \text{ mol dm}^{-3}$ ).

$[\text{Cu}^{2+}] = [\text{L}] = 0.010 \text{ mol dm}^{-3}$ .

<sup>b</sup> Mole fraction of 3'-UMP in the mixture of 2'- and 3'-UMP.

Table 3. Effect of ligands on the lanthanide ion-promoted hydrolysis of 2',3'-cUMP

M <sup>3+</sup>	Ligand	[M <sup>3+</sup> ] : [L] <sup>a</sup>	T(K)	pH	k(10 <sup>-3</sup> s <sup>-1</sup> )		x(3'-UMP) <sup>b</sup>
Eu <sup>3+</sup>	—	—	363.2	5.2	20.0	±0.1	0.65
	—	—	303.2	6.4	0.420	0.001	0.67
	Citrate	1:1	363.2	5.2	0.054	0.001	0.67
		1:1	363.2	6.2	0.130	0.001	0.67
		1:1	363.2	7.2	0.357	0.002	0.67
		1:2	363.2	7.2	0.070	0.001	0.65
		1:3	363.2	7.2	0.041	0.001	0.65
		1:1	363.2	8.1	0.641	0.015	0.66
	EDTA AA <sup>c</sup>	1:1	363.2	6.1	0.012	0.001	0.71
		1:1	303.2	6.4	0.108	0.002	0.67
		1:2	303.2	6.4	0.052	0.001	0.69
		1:4	303.2	6.4	0.012	0.001	0.70
	Tb <sup>3+</sup>	1:1	363.2	6.1	0.279	0.001	0.66
		1:1	363.2	7.2	1.02	0.01	0.65
		1:2	363.2	7.2	0.546	0.001	0.64
		1:1	363.2	8.1	2.37	0.04	0.63

<sup>a</sup> [Eu<sup>3+</sup>] = 7.6 × 10<sup>-3</sup> mol dm<sup>-3</sup>; [Tb<sup>3+</sup>] = 0.010 mol dm<sup>-3</sup>.<sup>b</sup> Mole fraction of 3'-UMP in the mixture of 2'- and 3'-UMP.<sup>c</sup> Acetylacetone.

Finally, attention should be drawn to the fact that although several of the metal ions and metal ion complexes studied markedly promote the hydrolysis of 2',3'-cUMP, none of them has any effect on the concentration ratio of the 2'- and 3'-monophosphates produced (Table 1). Since 2'- and 3'-UMP are formed by the rupture of the apical P—O-3' and P—O-2' bond of the phosphorane intermediate, respectively,<sup>23-25</sup> this means that the presence of metal ions does not affect the relative stability of the intermediates having either the O-2' or O-3' in an apical position. Whether the metal ions equally accelerate the breakdown of both species by stabilizing the developing phosphomonoesters via a bidentate coordination cannot be deduced on the basis of the data available. In summary, metal ions and metal ion complexes accelerate in their hydroxo form the hydrolysis of 2',3'-cUMP by several orders of magnitude without affecting the product distribution. Most likely the reaction involves a rapid initial binding of the metal ion to the negatively charged oxygen atom of the phosphodiester monoanion and subsequent intracomplex participation of the metal-bound hydroxo ligand, either as a base abstracting a proton from the attacking water molecule or as a nucleophile attacking the tetracoordinated phosphorus.

#### EXPERIMENTAL

**Materials.** 2',3'-cUMP (**2**) and also 2'-UMP (**3a**), 3'-UMP (**3b**) and uridine used as reference materials, were obtained from Sigma. They were used as received, after checking of purity by HPLC. The ligands **4**–**9** were products of Aldrich. The metal salts, buffer con-

stituents, EDTA, citric acid and acetylacetone were of reagent grade.

**Kinetic measurements.** Kinetic measurements were carried out by the HPLC technique described previously.<sup>25</sup> The pH of the reaction solutions were measured at 298.2 K and extrapolated to the temperature of kinetic measurements with the aid of the known temperature dependence of the pK<sub>a</sub> values of the buffer acids.<sup>27,49</sup> During the course of kinetic runs the pH remained constant within ±0.1 unit. The initial substrate concentration was 5 × 10<sup>-4</sup> mol dm<sup>-3</sup>. The progress of the reaction was stopped by cooling the aliquots rapidly in an ice-bath and adjusting their pH to 4. Chromatographic separations were carried out on a Hypersil RP-18 column (250 × 4 mm i.d., 5 μm film thickness) using a mixture of acetate buffer (0.025 mol dm<sup>-3</sup>, pH 4.3, containing 0.1 mol dm<sup>-3</sup> ammonium chloride) and acetonitrile (3%, v/v) as eluent. Before analysis the metal ions were removed by shaking the aliquots with Chelex.

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