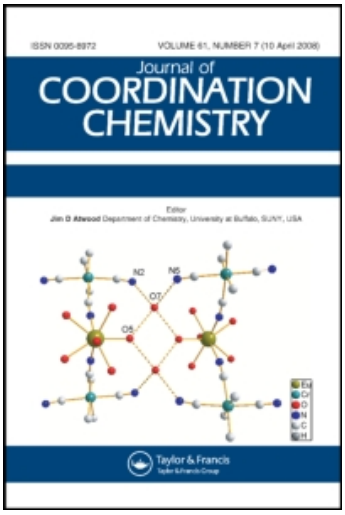

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Stability and Structure of Binary and Ternary Metal Ion Complexes of Orotidinate 5'-Monophosphate (OMP^{3-}) in Aqueous Solution

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To cite this Article Bastian, Matthias and Sigel, Helmut(1991) 'Stability and Structure of Binary and Ternary Metal Ion Complexes of Orotidinate 5'-Monophosphate (OMP^{3-}) in Aqueous Solution', Journal of Coordination Chemistry, 23: 1, 137 — 154
To link to this Article: DOI: 10.1080/00958979109408247
URL: http://dx.doi.org/10.1080/00958979109408247

J. Coord. Chem. 1991, Vol. 23, pp. 137–154
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STABILITY AND STRUCTURE OF BINARY AND TERNARY METAL ION COMPLEXES OF OROTIDINATE 5'-MONOPHOSPHATE (OMP^{3-}) IN AQUEOUS SOLUTION

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(Received July 20, 1990)

The stability constants of the 1:1 complexes formed between Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} or Cd^{2+} and orotidinate 5'-monophosphate (OMP^{3-}) were determined by potentiometric pH titrations in aqueous solution (I = 0.1 M, $NaNO_3$; 25°C). In addition to the stability constants of these $M(OMP)^-$ complexes, for several cases also the corresponding acidity constants for the release of the proton from the H(N-3) site were calculated; *i.e.*, the formation of $M(OMP-H)^{2-}$ complexes was quantified. On the basis of recent measurements for simple phosphate monoesters [$R\text{-}MP^{2-}$; R is a noncoordinating residue; S.S. Massoud and H. Sigel, *Inorg. Chem.*, **27**, 1447–1453 (1988)], evidence is provided that the somewhat increased stability of all the mentioned $M(OMP)^-$ complexes is mainly the result of a charge effect of the carboxylate group (in position 6 of OMP^{3-}) and *not* of a direct participation in complex formation; *i.e.*, there are no indications for the formation of significant amounts of macrochelates involving the phosphate and the carboxylate groups. This is different for the $M(OMP-H)^{2-}$ complexes of Co^{2+}, Ni^{2+} and Cd^{2+}: in these cases significant amounts of macrochelates form; *i.e.*, the metal ion is not only coordinated to the phosphate group but also (in part) to the ionized $^-$(N-3) site, which is placed in the neighbourhood of the phosphate residue in the dominating *syn* conformation of this nucleotide. For the metal ions Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} and Mn^{2+}, which have in general a rather low affinity for N binding sites, no evidence for the formation of macrochelates is detected. In addition, the stability constants of the ternary $Cu(Arm)(OMP)^-$ complexes, where Arm = 2,2'-bipyridyl or 1,10-phenanthroline, were determined by potentiometric pH titrations. Evaluation of the stability data shows that an equilibrium betweeen an 'open' isomer and a $Cu(Arm)(OMP)^-$ species with an intramolecular stack exists; the formation degree of these aromatic ring stacks reaches about 40 percent. Overall it is quite evident that OMP^{3-} is a versatile ligand with remarkable properties which may be utilized by nature in recognition reactions during the intricate metabolic processes in which this nucleotide is involved.

Keywords: Macrochelates, mixed-ligand complexes, orotidine, orotidinate 5'-monophosphate, stability constants, stacking interactions

1. INTRODUCTION

The initial stages of the biosynthesis of pyrimidine derivatives involve carbamoyl phosphate and *L*-aspartate leading *via* dihydroorotate and orotate to orotidinate 5'-monophosphate (OMP^{3-}; Fig. 1),[1] which is then decarboxylated to give uridine 5'-monophosphate (UMP^{2-});[2] this nucleotide may be further transformed, *e.g.*, into UTP^{4-} or CMP^{2-}. The indicated metabolic formation of OMP^{3-} involves metal ion dependent enzymic reactions,[3] and therefore the metal ion coordinating properties of this nucleotide are of interest. We decided to deal with this problem in the course of our studies on nucleotide complexes.[4,5]

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Dedicated with best wishes to Professor Dr. Arthur E. Martell on the occasion of his 75th birthday

Figure 1 shows the chemical structure of OMP^{3-} in its *syn* conformation. Orotidine and its derivatives,[6,7] including OMP^{3-},[8] exist in solution predominantly in this form; *i.e.*, the carbonyl O-2 and N-3 are above the ribose ring,[7,9] while the carboxylate group at position 6 is pointing away. As the carboxylate group, which is also highly solvated due to its negative charge, is much larger than the carbonyl group at position 2, this *syn* conformation is in fact expected. The other three nucleoside 5'-monophosphates which are additionally shown in Figure 1, and which will be used in comparative discussions, all predominantly exist in the *anti* conformation.[9,10] It should especially be pointed out that this *anti* conformation also holds for UMP^{2-}, which differs from OMP^{3-} solely by the absence of the carboxylate group; *i.e.*, now the carbonyl O-2 and N-3 are no longer above the ribose ring, but are pointing away from it.

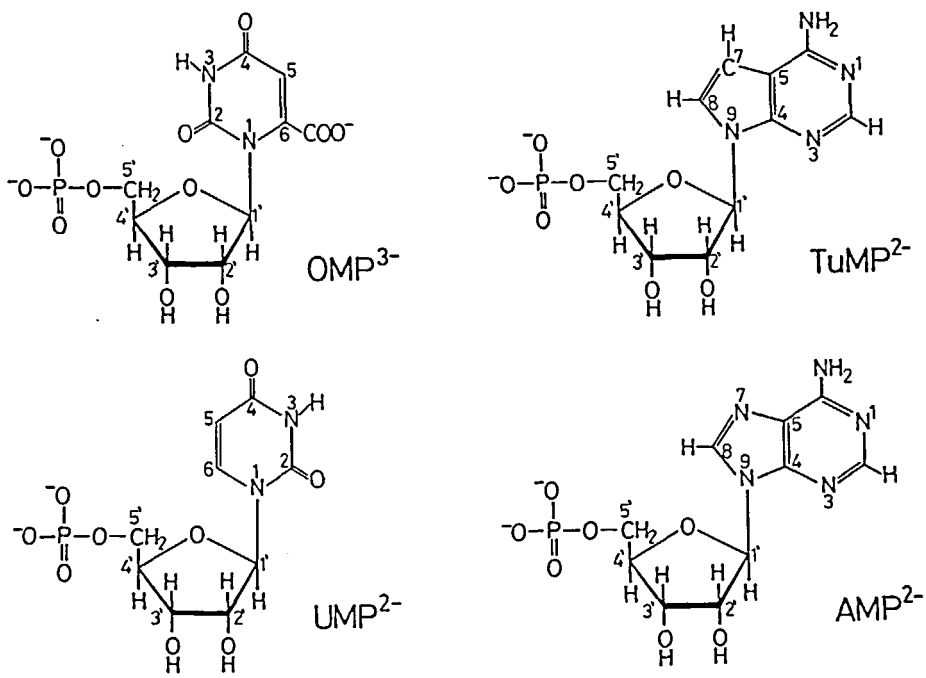


FIGURE 1 Chemical structures of the nucleoside 5'-monophosphates (NMPs) considered in this study.[1] OMP^{3-} is shown in its dominating *syn* conformation,[8] and UMP^{2-}, $TuMP^{2-}$ and AMP^{2-} in their dominating *anti* conformation.[9,10]

Considering the chemical structure of OMP^{3-} (Fig. 1), one of the main questions regarding the coordination of metal ions obviously is whether a metal ion bound to the phosphate residue would also interact with the carboxylate group. It is evident that such a twofold coordination would require a transformation of the nucleotide from the *syn* to the *anti* conformation. Is the driving force for the formation of a macrochelate involving the phosphate and carboxylate groups large enough to enforce such a structural change?

Another potential binding site at the base moiety of OMP^{3-} which could be reached from a phosphate-coordinated metal ion is N-3. After release of the proton, which is expected to occur in the alkaline pH range, this negatively charged nitrogen

should be well suited for the coordination of (especially transition) metal ions. The main advantage of this site is that the indicated macrochelate could be formed in the *syn* conformation of the ligand. However, the question as to whether macrochelates involving the phosphate group and the $^-$(N-3) site actually form still remains.

The indicated questions are now addressed in this study in a consecutive way. In addition, some mixed-ligand complexes involving OMP^{3-} and 2,2'-bipyridyl or 1,10-phenanthroline are also considered.

2. EXPERIMENTAL SECTION

2.1 Materials

The trisodium salt of orotidine (=3-β-*D*-ribofuranosyl orotic acid) 5'-monophosphoric acid was purchased from Sigma-Aldrich Co., St. Louis, MO, U.S.A. The disodium salt of ethylene-*N,N,N',N'*-tetraacetic acid (Na_2H_2EDTA), potassium hydrogen phthalate, HNO_3, NaOH (Titrisol), 2,2'-bipyridyl, 1,10-phenanthroline monohydrate and the nitrate salts of Na^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} (all pro analysi) were from Merck AG, Darmstadt, FRG.

The titre of the NaOH used for the titrations was determined with potassium hydrogen phthalate; the exact concentrations of the OMP^{3-} solutions used in the titrations with the metal ions (titrated in the presence of an excess of HNO_3; see below) were measured by titrations with NaOH. The concentrations of all stock solutions of the divalent metal ions were established with EDTA.

2.2 Potentiometric pH Titrations

The pH titrations were carried out with a Metrohm E 536 potentiograph equipped with a E 655 dosimat, and 6.0202.100 (JC) combined macro glass electrodes. The buffer solutions (pH 4.64, 7.00, and 9.00) used for calibration were also from Metrohm AG, Herisau, Switzerland; the buffers of this company are based on the scale of the U.S. National Bureau of Standards.[11,12] The direct pH meter readings were used in the calculations of the acidity constants for $H_2(OMP)^-$; *i.e.*, these constants are so-called "mixed" or Brønsted constants. The negative logarithms of these acidity constants given for aqueous solutions of $H_2(OMP)^-$ at I = 0.1 M ($NaNO_3$) and 25°C may be converted into the corresponding concentration constants by subtracting 0.02 log unit;[13] this conversion term, which contains the junction potential of the glass electrode and the activity coefficient of H^+ (see, *e.g.*, ref. 14), is close to that given by others: *e.g.*, 0.036 log unit (25°C; I = 0.1 M, KNO_3)[15] or 0.039 log unit (25°C; I = 0.5 M, KCl).[16] It should be noted that for stability constants of metal ion complexes no conversion is necessary.

A further point to be emphasized is that in our calculation procedures the ionic product of water (K_w) and the hydrogen ion activity (γ) [to be more exact: the mentioned 'combined' term for converting the measured data into H^+ concentration] do *not* enter into the calculations because we evaluate *differences* in NaOH consumption between two corresponding solutions; *i.e.*, always solutions with and without ligand are titrated (see below). The advantage of this procedure is (aside from not needing K_w or γ values) that impurities in the solvent or in the salts, as well as systematic errors, *etc.*, cancel to a large part.

The calculations for the equilibrium constants were carried out with a Hewlett Packard Vectra 60 PC desk computer connected with a Brother M 1509 printer and a Hewlett-Packard 7475 A plotter.

2.3 Conditions for the Determination of the Acidity Constants

The acidity constants $K^{H}_{H_2(OMP)}$ and $K^{H}_{H(OMP)}$ of $H_2(OMP)^-$ were determined by titrating 15 cm^3 of aqueous 0.032 M HNO_3 and $NaNO_3$ (I = 0.1 M; 25°C) in the presence and absence of 6 mM OMP^{3-} under N_2 with 2.5 cm^3 of 0.2 M NaOH and by using the differences in NaOH consumption between two such titrations for the calculations. The two acidity constants were calculated with the abovementioned computer by a curve-fitting procedure using a Newton-Gauss nonlinear-least-squares program within the pH range of about 2 to 6.7, *i.e.* between the highest point of protonation reached by the experimental conditions (about 25% protonation) for the equilibrium $H_2(OMP)^-/H(OMP)^{2-}$ and the highest point of neutralization (about 65%) for the equilibrium $H(OMP)^{2-}/OMP^{3-}$. Three independent pairs of such titrations were carried out leading by average to the final result for $K^{H}_{H_2(OMP)}$.

In another set of experiments (again) $K^{H}_{H(OMP)}$ and K^{H}_{OMP} of $H(OMP)^-$ were determined by titrating 50 cm^3 of aqueous 0.54 mM HNO_3 and $NaNO_3$ in the presence and absence of 0.3 mM OMP^{3-} with 1 cm^3 of 0.03 M NaOH as described in the preceding paragraph. In this case 16 pairs of independent titrations were made and evaluated between pH 4.7 and 9.6, *i.e.* between about 2% of neutralization for the equilibrium $H(OMP)^{2-}/OMP^{3-}$ and about 64% for the equilibrium $OMP^{3-}/(OMP–H)^{4-}$. Hence, the final results for $K^{H}_{H(OMP)}$ and K^{H}_{OMP} are the averages of 19 and 16 pairs of titrations, respectively.

2.4 Conditions for the Determination of the Stability Constants

The conditions for the determination of the stability constants $K^{M}_{M(OMP)}$ of the binary $M(OMP)^-$ complexes (I = 0.1 M; 25°C) were the same as given in the last paragraph for the acidity constants $K^{H}_{H(OMP)}$ and K^{H}_{OMP} (*i.e.*, with 0.3 mM OMP^{3-}), except $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$. With Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} [$M(NO_3)_2$] was 0.0333 M or 0.0267 M, *i.e.* $OMP:M^{2+}$ = 1:111 or 1:89, respectively. For Mn^{2+}, Co^{2+}, Zn^{2+}, and Cd^{2+} [$M(NO_3)_2$] was 0.0167 M (OMP: M^{2+} = 1:56); for Ni^{2+} and also Co^{2+} [$M(NO_3)_2$] was 0.0133 M (1:44), and for Mn^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} [$M(NO_3)_2$] was also 0.0083 M (1:28). For Cu^{2+} [$M(NO_3)_2$] was 3.33 and 1.67 mM, *i.e.* the OMP to Cu^{2+} ratios were 1:11 and 1:5.6. For each metal ion system at least 4, usually 5, independent pairs of titrations were made.

The stability constants $K^{M}_{M(OMP)}$ were computed for each pair of titrations by taking into account the species H^+, $H_2(OMP)^-$, $H(OMP)^{2-}$, OMP^{3-}, M^{2+}, and $M(OMP)^-$.[17] Throughout the data were collected (every 0.1 pH unit) from about 5% complex formation to a neutralization degree of about 85% or to the beginning of the formation of $M(OMP–H)^{2-}$ species or of the hydrolysis of $M(aq)^{2+}$; the latter was evident from the titrations without OMP. The values calculated individually for $\log K^{M}_{M(OMP)}$ showed no dependence on pH or on the excess amount of M^{2+}.

In those cases where $M(aq)^{2+}$ did not hydrolyze before the onset of the formation of $M(OMP–H)^{2-}$ (i.e., for Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+}), the experimental data were also analyzed with a curve-fitting pro-

cedure[18] by taking into account in addition to the species already mentioned $M(OMP-H)^{2-}$ and $(OMP-H)^{4-}$. In this way again the stability constant $K^{M}_{M(OMP)}$ was determined, but this time together with the acidity constant $K^{H}_{M(OMP)}$ of the $M(OMP)^{-}$ species. It may be emphasized that the agreement of the values obtained for $K^{M}_{M(OMP)}$ in the two evaluation procedures was excellent.

The experimental conditions for the determination of the stability constants $K^{Cu}_{Cu(Arm)(OMP)}$ of the ternary $Cu(Arm)(OMP)^{-}$ complexes correspond to those given above for the binary Cu^{2+} system; *i.e.*, $OMP:Cu^{2+}:Arm = 1:5.6:5.6$. Four independent pairs of titrations were carried out each for the 2,2'-bipyridyl and 1,10-phenanthroline (=Arm) systems. In the pH range (4.7–5.3) used for the calculation of the stability constants of the ternary $Cu(Arm)(OMP)^{-}$ species, complex formation between Cu^{2+} and Bpy or Phen is already complete due to the large stability of $Cu(Arm)^{2+}$;[19] this was also evident from the identity of the titration curves obtained from a pair of solutions, one that contained HNO_3 only and another that contained Cu^{2+}/Arm (=1:1) in addition. Hence, in the calculations only the complex formation between $Cu(Arm)^{2+}$ and OMP^{3-} had to be considered,[20] and each of these two systems could be treated as a binary one (see above) by considering the species H^{+}, $H_2(OMP)^{-}$, $H(OMP)^{2-}$, OMP^{3-}, $Cu(Arm)^{2+}$, and $Cu(Arm)(OMP)^{-}$.

3. RESULTS

The experiments were carried out in water under conditions where the self-association of OMP is expected to be negligible, due to previous experience with related pyrimidine derivatives.[21-23] Most of the potentiometric pH titrations for the determination of the acidity constants of $H_2(OMP)^{-}$ and all of the titrations for the stability constants of the binary species $M(OMP)^{-}$ or $M(OMP-H)^{2-}$ and the ternary $Cu(Arm)(OMP)^{-}$ complexes were made with [OMP] = 0.3 mM. Using this concentration and assuming for the self-stacking tendency of OMP (according to the isodesmic model of indefinite noncooperative self-association) the upper limit $K < 2\,M^{-1}$ for the equilibrium constant,[21-23] one calculates that more than 99% of the OMP species are present in their monomeric form (in fact under the given conditions this is still true even if K would equal $16\,M^{-1}$); hence, the results presented in the following sections apply to OMP monomers.

3.1 Acidity Constants for $H_3(OMP)$

From the chemical structure shown in Figure 1 for OMP^{3-} it is evident that this nucleotide may accept three protons, two at the phosphate group and one at the carboxylate. Accordingly, at least the following three deprotonation equilibria have to be considered:

$$H_3(OMP) \rightleftharpoons H_2(OMP)^{-} + H^{+} \qquad (1a)$$

$$K^{H}_{H_3(OMP)} = [H^{+}][H_2(OMP)^{-}]/[H_3(OMP)] \qquad (1b)$$

$$H_2(OMP)^{-} \rightleftharpoons H(OMP)^{2-} + H^{+} \qquad (2a)$$

$$K^{H}_{H_2(OMP)} = [H^{+}][H(OMP)^{2-}]/[H_2(OMP)^{-}] \qquad (2b)$$

$$H(OMP)^{2-} \rightleftharpoons OMP^{3-} + H^{+} \qquad (3a)$$

$$K^{H}_{H(OMP)} = [H^{+}][OMP^{3-}]/[H(OMP)^{2-}] \qquad (3b)$$

The release of the first proton (*1*) from monoesterified derivatives of phosphoric acid occurs at a very low pH and no attempt was made to determine this pK_a value. However, it is safe to assume that the acidities of $H_3(OMP)$ and $H_2(UMP)$ are very similar due to their near structural identity (see Fig. 1); *i.e.*, $pK^H_{H_3(OMP)} \simeq pK^H_{H_2(UMP)} = 0.7 \pm 0.3$.[24] The release of the next proton in $H_2(OMP)^-$ (*2*) occurs from the carboxylic acid group in position 6, and then follows in $H(OMP)^{2-}$ the final proton from the phosphate residue (*3*).

For UMP^{2-} it is well-known that the H(N-3) site of the nucleic base moiety may release a further proton,[24] and consequently the following reaction has to be expected for OMP^{3-}:

$$OMP^{3-} \rightleftharpoons (OMP\text{–}H)^{4-} + H^+ \qquad (4a)$$

$$K^H_{OMP} = [H^+][(OMP\text{–}H)^{4-}]/[OMP^{3-}] \qquad (4b)$$

Finally, the ribose residue of nucleosides and nucleotides may also be ionized[25,26] but this reaction occurs only at pH > 12 and does not play a role in the physiological pH range; hence, this reaction was not studied in the present context. The results obtained *via* potentiometric pH titrations for the various acidity constants of the three-proton-donor $H_2(OMP)^{2-}$ (*2–4*) are listed in Table I, together with the pK_a values for the related compounds H(Or) and $H_2(UMP)$.[1,24,27] All the acidity constants given in Table I are in the expected order.

TABLE I

Negative logarithms of the acidity constants (eqs. (*1*)–(*4*)) in aqueous solution for orotidine 5'-monophosphoric acid, $H_3(OMP)$, as well as for some related species at 25°C and I = 0.1 M ($NaNO_3$) as determined by potentiometric pH titrations.[a]

	pK_a for the sites			
Acid	–$PO(OH)_2$	–COOH	–$PO(O^-)(OH)$	> N(3)H
$H_3(OMP)$	~0.7 (eq. (*1*))	1.46 ± 0.10 (eq. (*2*))	6.40 ± 0.02 (eq. (*3*))	9.35 ± 0.02 (eq. (*4*))
H(Or)		0.5 ± 0.3		9.12 ± 0.02
$H_2(UMP)$	0.7 ± 0.3		6.15 ± 0.01	9.45 ± 0.02

[a] The errors given are *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The acidity constants for H(Or) and $H_2(UMP)$ are from references 27 and 24, respectively.

3.2 Stability and Acidity Constants of M(OMP)⁻ Complexes

The experimental data of the potentiometric pH titrations of $H(OMP)^{2-}$ in the presence of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} may be completely described by considering equilibria (*2*), which actually plays only a very minor role, (*3*) and (*5*) as long as the evaluation is not carried into the pH range where formation of $M(OMP\text{–}H)^{2-}$ species or of hydroxo complexes occurs (see Section 2.4).

$$M^{2+} + OMP^{3-} \rightleftharpoons M(OMP)^- \qquad (5a)$$

$$K^M_{M(OMP)} = [M(OMP)^-]/([M^{2+}][OMP^{3-}]) \qquad (5b)$$

In those systems where deprotonation of the H(N-3) site in $M(OMP)^-$ takes place before the onset of the hydrolysis of $M(aq)^{2+}$, the equilibrium constant for the following reaction could be determined as well.

$$M(OMP)^- \rightleftharpoons M(OMP\text{–}H)^{2-} + H^+ \qquad (6a)$$

$$K^H_{M(OMP)} = [M(OMP\text{–}H)^{2-}][H^+]/[M(OMP)^-] \qquad (6b)$$

The results are summarized in Table II. The values for $\log K^M_{M(OMP)}$ are in the order previously observed for other nucleoside monophosphate complexes;[24,28,29] for the acidity constants $pK^H_{M(OMP)}$ (*6*) no comparable values are available. The stabilities of the $M(OMP)^-$ species show the usual trends: Complex stability with the alkaline–earth ions is lower than that with the divalent 3d metal ions; for the latter the long-standing experience (see ref. 24) is confirmed that the stabilities of metal ion-phosphate complexes do not strictly correspond to the Irving–Williams series.[30]

TABLE II

Logarithms of the stability constants of binary $M(OMP)^-$ (eq. (*5*)) and ternary $Cu(Arm)(OMP)^-$ complexes (eq. (*7*)) together with the negative logarithms of the acidity constants for some of the binary $M(OMP)^-$ complexes (eq. (*6*)), as determined by potentiometric pH titrations in aqueous solution at 25°C and I = 0.1 M ($NaNO_3$).[a]

M^{2+}	$\log K^M_{M(OMP)}$	$pK^H_{M(OMP)}$
Mg^{2+}	1.93 ± 0.02	8.89 ± 0.02
Ca^{2+}	1.76 ± 0.02	8.77 ± 0.02
Sr^{2+}	1.56 ± 0.02	8.78 ± 0.04
Ba^{2+}	1.62 ± 0.02	8.78 ± 0.02
Mn^{2+}	2.49 ± 0.02	8.91 ± 0.04
Co^{2+}	2.37 ± 0.02	8.40 ± 0.05
Ni^{2+}	2.30 ± 0.03	8.24 ± 0.08
Cu^{2+}	3.29 ± 0.05	
Zn^{2+}	2.50 ± 0.04	
Cd^{2+}	2.91 ± 0.03	7.66 ± 0.10
$Cu(Bpy)^{2+}$	3.50 ± 0.04	
$Cu(Phen)^{2+}$	3.55 ± 0.05	

[a] The error limits are *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger.

In addition, the stability of the mixed-ligand complexes formed between OMP^{3-}, Cu^{2+}, and an aromatic amine (Arm), i.e. 2,2′-bipyridyl (Bpy) or 1,10-phenanthroline (Phen), was determined (see Section 2.4). The stability constants according to (*7*),

$$Cu(Arm)^{2+} + OMP^{3-} \rightleftharpoons Cu(Arm)(OMP)^- \qquad (7a)$$

$$K^{Cu(Arm)}_{Cu(Arm)(OMP)} = [Cu(Arm)(OMP)^-]/([Cu(Arm)^{2+}][OMP^{3-}]) \qquad (7b)$$

are also listed in Table II and discussed in Section 4.5.

4. DISCUSSION

4.1 Comparison of the Acidity Constants for $H_2(OMP)^-$ with Those of Relate Species

Comparison of the constants listed in Table I shows that deprotonation of th carboxylic acid group in H(Or) occurs at a lower pH than that of the same group i the negatively charged $H_2(OMP)^-$ species. This result is expected and corresponds t the observations made for the deprotonation of the H(N-3) site, *i.e.* $pK^H_{Or} < pK^H_{OM}$

The fact that the H(N-3) site in OMP^{3-} is deprotonated at a somewhat lower pH ($pK^H_{OMP} = 9.35$) than the same site in UMP^{2-} ($pK^H_{UMP} = 9.45$) may appear a surprising at first sight because OMP^{3-} carries overall more negative charges tha UMP^{2-}. However, in this case a further effect is operating: the carboxylat substituent at the pyrimidine residue is a so-called second order substituent[31a] wit electron-withdrawing qualities and this facilitates the release of the proton from th H(N-3) site; this property of a carboxylate group is also evident from the observa tions[31b] made with the nitrogen-protonated species of pyridine ($pK_a = 5.21$) an pyridine-4-carboxylate ($pK_a = 4.90$).

Regarding the discussion in Section 4.3 on the effect of the carboxylate grou on the stability of the $M(OMP)^-$ complexes, the following comparison is of specia interest: $pK^H_{H(OMP)} = 6.40\ (\pm 0.02) > pK^H_{H(UMP)} = 6.15\ (\pm 0.01)$ (*c.f.* Table I). Thi means the negative charge of the carboxylate group at position 6 inhibits throug space the release of the proton from the $-PO(O^-)(OH)$ group by 0.25 (± 0.02) lo units. It is interesting to note that the same observation (though with opposite sign) i made for the release of the proton from the H^+(N-1) sites in $H(Ado)^+$ an $H_2(AMP)^{\pm}$; removal of the $-PO(O^-)(OH)$ group facilitates the deprotonatio reaction at the H^+(N-1) site of the adenine residue by 0.23 (± 0.04) log unit $pK^H_{H(Ado)} = 3.61\ (\pm 0.03) < pK^H_{H_2(AMP)} = 3.84\ (\pm 0.02)$.[10,28] Comparison of the corres ponding nucleotide structures in Figure 1 indicates that the through space distanc between the sites involved is comparable in the two pairs of compounds considered A similar result is also obtained for the H^+(N-3) sites of $H_2(CMP)^{\pm}$ ($pK^H_{H_2(CMP)} = 4.3 \pm 0.04$)[24] and $H(cytidine)^+$ ($pK^H_{H(Cyd)} = 4.14 \pm 0.02$);[26] *i.e.*, $\Delta pK_a = 0.19 \pm 0.05$.

4.2 Partial Evaluation of the Stabilities of the $M(OMP)^-$ Complexes

The existence of a linear relationship between log K^M_{ML} and pK^H_{HL} is well-known fo many series of structurally related ligands.[32] The corresponding relationship betwee metal ion-phosphate coordination and phosphate group basicity was recently estab lished by plotting log $K^M_{M(R\text{-}MP)}$ *versus* $pK^H_{H(R\text{-}MP)}$ for a series of simple phosphat monoesters ($R\text{–}MP^{2-}$). The parameters of the resulting straight reference line (least-squares) for phosphate-ester complexes of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+} Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} are summarized in Table V of ref. 24 (*c.f.* als Table I in ref. 28). This achievement[24] now allows the calculation of the stabilit constant for a pure phosphate coordination with the known acidity constant of an monoprotonated phosphate residue.

Two examples for plots of log $K^M_{M(R\text{-}MP)}$ versus $pK^H_{H(R\text{-}MP)}$ are shown in Figure 2. I is evident that for both cases, i.e. $Mg(OMP)^-$ and $Cd(OMP)^-$, the data pairs do no fit on the reference lines; though not shown, the same is true also for the othe $M(OMP)^-$ complexes. In all 10 cases studied (Table II) the points due to th $M(OMP)^-$ complexes are above the reference lines indicating an increased comple

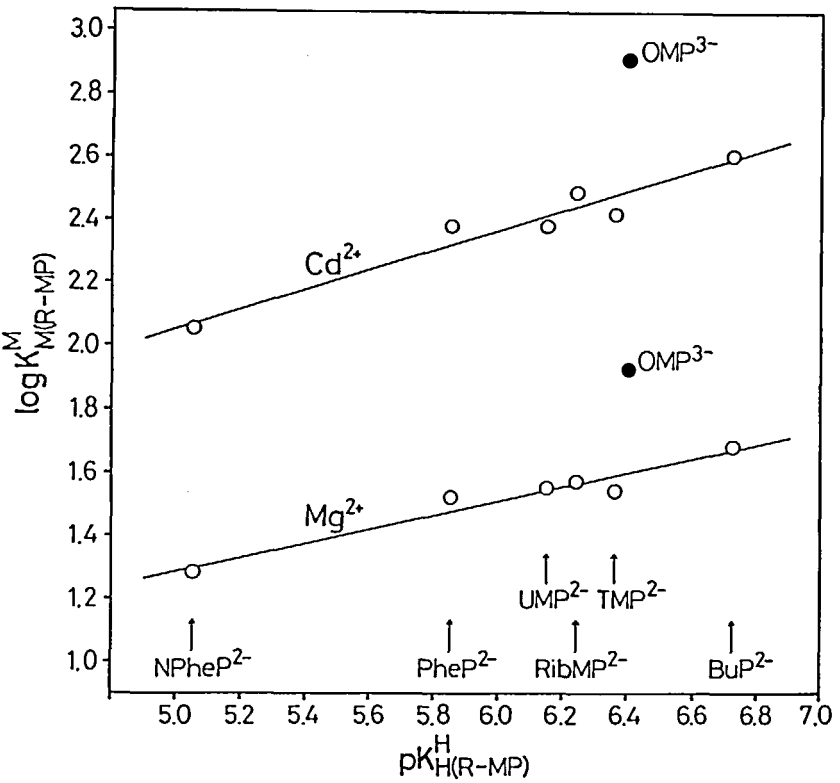
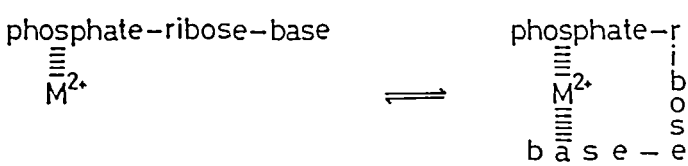


FIGURE 2 Relationship between log $K^{M}_{M(R-MP)}$ and $pK^{H}_{H(R-MP)}$ for the 1 : 1 complexes of Mg^{2+} and Cd^{2+} with some simple phosphate monoester ligands ($R-MP^{2-}$): 4-nitrophenyl phosphate ($NPheP^{2-}$), phenyl phosphate ($PheP^{2-}$), uridine 5'-monophosphate (UMP^{2-}), *D*-ribose 5'-monophosphate ($RibMP^{2-}$), thymidine 5'-monophosphate (TMP^{2-}), and *n*-butyl phosphate (BuP^{2-}) (from left to right) (○).[24] The least-squares lines are drawn through the corresponding six data sets; the equations for these reference lines are given in Table V of ref. 24. The points due to the complexes formed with OMP^{3-} (●) are inserted for comparison; the corresponding equilibrium constants are taken from Tables I and II. All plotted equilibrium constant values refer to aqueous solutions at 25°C and I = 0.1 M ($NaNO_3$).

stability, and this might be taken as a hint that the intramolecular equilibrium (*8*) is existing because participation of a further binding site should lead to an increased complex stability.[33]



(*8*)

However, such a conclusion in this simple way is in the present context *not* justified. It should be recalled from Section 4.1 that the negative charge of the carboxylate group in position 6 of the pyrimidine ring increases the affinity of the phosphate group in OMP^{3-} for a proton by 0.25 log units *without* any direct interaction between this proton and the carboxylate group; here simply a charge

effect is operating. Hence, a corresponding effect must also be expected for the binding of metal ions to the phosphate group in OMP^{3-} and this will be evaluated in the next section.

However, before doing so, another related aspect should be indicated, which might be put forward with the argument that the charge effect of the carboxylate group in OMP^{3-} is already compensated for in calculations with the mentioned reference lines (Fig. 2) for a pure metal ion-phosphate coordination by using the acidity constant of $H(OMP)^{2-}$, $pK^{H}_{H(OMP)} = 6.40$; a pK_a value which is 0.25 log units higher than in the absence of the carboxylate group (Section 4.1). On this basis one would then attribute the increased stability of the $M(OMP)^{-}$ complexes, which amounts in average to 0.34 ± 0.06 log units (see also Fig. 2) for the 10 complexes studied, to a carboxylate coordination. Yet, such a procedure is *not* justified because the charge effect of the carboxylate group is expected to be different for a (monovalent) proton and a divalent metal ion. Therefore we have chosen to evaluate the stability data (Table II) by considering the charge effect of the carboxylate group on a divalent metal ion in the independent manner described in the next section.

4.3 Does the Carboxylate Group Directly Participate in Metal Ion Binding in $M(OMP)^{-}$ Complexes?

Comparison of the structures shown in Figure 1 for OMP^{3-} and UMP^{2-} shows that they differ (aside from their preferred conformations) only by the presence of the carboxylate group in position 6. Hence, the acidity constant for the monoprotonated phosphate group in $H(UMP)^{-}$ reflects certainly the properties of the same group in $H(OMP)^{2-}$ in the *absence* of any carboxylate influence; in other words, the negative logarithm of this micro acidity constant $pk^{OMP}_{OMP\cdot H}$ (*c.f.* ref. 28) is well represented by $pK^{H}_{H(UMP)} = 6.15$ (Table I). With this acidity constant and the abovementioned reference line equations for the $\log K^{M}_{M(R-MP)}$ *versus* $pK^{H}_{H(R-MP)}$ plots (see also Fig. 2),[24] the logarithms of the stability constants for $M(OMP)^{-}$ complexes *without* the influence of the carboxylate group may be calculated, and the following stability difference with the experimentally determined values may then be defined:

$$\log \Delta = \log K^{M}_{M(OMP)/exptl} - \log K^{M}_{M(OMP)/calcd} \qquad (9)$$

The results of these calculations are presented in Table III. An argument against this procedure could be that the experimentally determined values for $\log K^{M}_{M(OMP)/exptl}$ are based on the macro acidity constant $pK^{H}_{H(OMP)} = 6.40$. However, a decreased basicity of $\Delta pK_a = 0.25$ lowers the $\log K^{M}_{M(OMP)/exptl}$ values on average only by 0.06 log units and thus our conclusions remain unaffected. Moreover, for completeness, these basicity adjusted 'experimental' values are given in parentheses with the second column of Table III and the connected values for $\log \Delta$, also in parentheses, with the fourth column at the right. Again it is evident that the result of the interpretation is not significantly influenced by the small changes in these data; in fact, the lower parenthesized values for $\log \Delta$ are even more in favour of our conclusions given below.

In screening the data of Table III it is surprising to observe that the kind of metal ion has no significant influence on the extent of the increased complex stability and that all the values for $\log \Delta$ are very similar; indeed, the average of the ten differences is 0.40 ± 0.06 (3σ) log unit (or 0.34 ± 0.06 for the numbers in parentheses), and this

value overlaps within the error limits with all log Δ values. As a somewhat larger effect of the negatively charged carboxylate group is expected for a divalent metal ion than for the monovalent proton (log $\Delta = 0.25 \pm 0.02$; Section 4.1), this result indicates that the carboxylate group does *not* directly bind to a significant extent to the phosphate-coordinated metal ions.[33]

TABLE III

Stability constant comparisons (eq. (9)) for the $M(OMP)^-$ complexes formed between the measured stability constants (exptl.) from Table II and the calculated stability constants for a pure metal ion-phosphate coordination (calcd.) based on the straight line equations[a] quantifying the relationship between complex stability and phosphate group basicity (see Figure 2) and the micro acidity constant $k^{OMP}_{OMP\,II}$,[b] not affected by the negatively charged carboxylate group in position 6 (see Figure 1).[c,d]

	$\log K^{M}_{M(OMP)}$		$\log \Delta = \log K_{exptl} - \log K_{calcd}$
M^{2+}	exptl	calcd	
Mg^{2+}	1.93 ± 0.02 (1.87)[d]	1.55 ± 0.04	0.38 ± 0.04 (0.32)[d]
Ca^{2+}	1.76 ± 0.02 (1.72)	1.45 ± 0.05	0.31 ± 0.05 (0.27)
Sr^{2+}	1.56 ± 0.02 (1.54)	1.24 ± 0.05	0.32 ± 0.05 (0.30)
Ba^{2+}	1.62 ± 0.02 (1.60)	1.15 ± 0.05	0.47 ± 0.05 (0.45)
Mn^{2+}	2.49 ± 0.02 (2.42)	2.14 ± 0.07	0.35 ± 0.07 (0.28)
Co^{2+}	2.37 ± 0.02 (2.31)	1.92 ± 0.07	0.45 ± 0.07 (0.39)
Ni^{2+}	2.30 ± 0.03 (2.23)	1.94 ± 0.06	0.36 ± 0.07 (0.29)
Cu^{2+}	3.29 ± 0.05 (3.18)	2.84 ± 0.08	0.45 ± 0.09 (0.34)
Zn^{2+}	2.50 ± 0.04 (2.42)	2.10 ± 0.08	0.40 ± 0.09 (0.32)
Cd^{2+}	2.91 ± 0.03 (2.83)	2.42 ± 0.06	0.49 ± 0.07 (0.41)

[a] The corresponding parameters are listed in Tables V and VI of ref. 24 (see also Table I in ref. 28). [b] $pk^{OMP}_{OMP\,II} = pK^{H}_{H(UMP)} = 6.15$ (see Section 4.2 and Table I). [c] The error limits (see also Table II) for the differences log Δ were calculated according to the error propagation after Gauss. [d] The data in parentheses are basicity corrected values (*via* the straight line equations); see text. The error limits of these values are the same as those given to the left.

This conclusion is further supported by the two following comparisons where the effect of a positive charge (and not a negative one as above) is seen, and hence the effects are of opposite sign: (i) the same divalent metal ions as considered above, if coordinated to the phosphate group of $TuMP^{2-}$ (see Fig. 1), lead to an acidification of the $^{+}H(N\text{-}1)$ site by 0.4 ± 0.2 log units; *i.e.* $pK^{H}_{M(H\cdot TuMP)} - pk^{TuMP}_{H\cdot TuMP} = (5.2 \pm 0.2)$ [average of the values given in Table III of ref. 28] $-(5.61 \pm 0.06)$ [average of the values in Fig. 2 of ref. 28] $= -0.4 \pm 0.2$; (ii) similarly, the release of the proton from the $^{+}H(N\text{-}1)$ site in tubercidin ($pK^{H}_{H(Tu)} = 5.21 \pm 0.03$)[34] is inhibited by 0.40 ± 0.07 log units by a doubly negatively charged 5′-monophosphate group ($pk^{TuMP}_{H\cdot TuMP} = 5.61 \pm 0.06$; see above).

In all the examples discussed above, the electrostatic effects result in stability differences of about 0.4 log unit. As the distance between the phosphate group and the $^{+}H(N\text{-}1)$ site in TuMP is similar to the distance between the phosphate residue and the carboxylate group in OMP (see structures in Fig. 1), these observations together provide further evidence that in the $M(OMP)^-$ complexes mainly an electrostatic charge effect of the carboxylate group is operating and that this group does *not* directly interact to a significant extent with the phosphate-coordinated metal ions; hence, for the $M(OMP)^-$ complexes considered here equilibrium (8) lies to the left.

In conclusion, the energy barrier between the *syn* and *anti* conformation OMP^{3-} (Fig. 1) is evidently too large to be overcome in the $M(OMP)^{-}$ complex and to allow to a significant extent a direct coordination of the carboxylate group aqueous solution. It is interesting to note in this context that for orotidinate the *a* population has been estimated[7] to occur only to about 14 (±2)%; the remaini 86% being in the *syn* conformation. Hence, for OMP^{3-} an even smaller value for t *anti* population must be expected (<14%) due to charge repulsion between t phosphate and carboxylate groups, while the *syn* population will thus be higher th the mentioned 86%. It should be recalled that in this *syn* conformation simultaneo binding of the metal ion to the phosphate and carboxylate groups is *not* possible.

Furthermore, considering that the basicity of the carboxylate group is very lo (Table I) it is not surprising that also the metal ion affinity of this group is very sm in aqueous solution, as was shown recently[27] in studies with orotidine. Hence, t stability gain by carboxylate coordination is evidently not enough to overcome t *syn–anti* energy barrier. It should be added that formation of 10% of a macrochela in equilibrium (*8*) means only a stability increase (compared to the stability of t open form) of 0.05 log units; such a small stability increase would remain unnotic in the present evaluation. A formation degree of 20% for the macrochelate corre ponds to a stability enhancement of 0.1 log units and this appears to be close to t limit that would have been recognized (beyond the charge effect). Hence, we m conclude for aqueous solutions that of the $M(OMP)^{-}$ complexes, if at all, not mo than 20% form a macrochelate involving simultaneous metal ion coordination to t 5′-phosphate group and the 6-carboxylate residue (Fig. 1). However, it might be th in solvents with a reduced polarity compared to water (*e.g.*, in 1,4-dioxane–wat mixtures) the 'effective' dielectric constant may be reduced so far that the affinity the carboxylate group for metal ions increases enough to allow a transformation the *syn* into the *anti* conformation and thus the formation of larger amounts macrochelates; this aspect clearly warrants further studies.

TABLE IV

Extent of intramolecular macrochelate formation (eq. (*8*)) in several $M(OMP-H)^{2-}$ comple as expressed by the dimensionless equilibrium constant K_I (eqs (*12*), (*13*)) and the percentage $M(OMP-H)^{2-}_{cl}$ (eq. (*14*)) in aqueous solution at 25°C and I = 0.1 M ($NaNO_3$).[a]

No.	M^{2+} in $M(OMP-H)^{2-}$	$pK^{H}_{OMP} - pK^{H}_{M(OMP)}$[b]	log Δ* (eq. *10*)[c]	K_I (eqs. *12,13*)	%M(OMP-H) (eqs. *8,14*)
1	Mg^{2+}	0.46 ± 0.03			~0
2	Ca^{2+}	0.58 ± 0.03			~0
3	Sr^{2+}	0.57 ± 0.04	0.52 ± 0.09[d]		~0
4	Ba^{2+}	0.57 ± 0.03			~0
5	Mn^{2+}	0.44 ± 0.04			~0
6	Co^{2+}	0.95 ± 0.05	0.43 ± 0.10	1.69 ± 0.64	63 ± 9
7	Ni^{2+}	1.11 ± 0.08	0.59 ± 0.12	2.89 ± 1.08	74 ± 7
8	Cd^{2+}	1.69 ± 0.10	1.17 ± 0.13	13.8 ± 4.6	93 ± 2

[a] The error limits (3σ) were calculated according to the error propagation after Gauss. [b] These values w calculated from the constants listed in Tables I and II. [c] See Section 4.4. [d] Arithmetic mean of entries 1 1–5, together with *3 times* the standard error of the mean value. This value quantifies the charge effect a divalent metal ion on the deprotonation of the H(N-3) site without its coordination to the result $^{-}$(N-3); *i.e.*, this value represents the "stability" of the open isomer and is therefore taken as ΔpK (see eq. (*10e*) and text).

4.4 Evidence for the Formation of Macrochelates Involving the N-3 Site in Several M(OMP–H)$^{2-}$ Complexes

We have already seen that the H(N-3) site of OMP^{3-} releases its proton with $pK^{H}_{OMP} = 9.35$ (Table I). In the *syn* conformation of $(OMP–H)^{4-}$ this negatively charged N-3 is placed in the neighbourhood of the phosphate group (Fig. 1) and space-filling molecular models indicate that the formation of a macrochelate by simultaneous coordination of a metal ion to the phosphate group and the $^{-}$(N-3) site is possible. Hence, the question again arises about the position of equilibrium (*8*), which describes the situation in a general way.

Any kind of metal ion interaction that occurs in addition to the coordination of the phosphate group must be reflected in an increased complex stability.[32b] Therefore, it has to be the aim to determine the difference between experimentally increased (overall) stability constants and (usually) estimated or calculated constants, which quantify the stability of the 'open' species in equilibrium (*8*). This is expressed in a general form in equation (*10a*) (which is analogous to (*9*)) and for the present case of the $M(OMP–H)^{2-}$ complexes in equation (*10b*).

$$\log \Delta^* = \log K_{exptl} - \log K_{calcd} \qquad (10a)$$

$$= \log K^{M}_{M(OMP-H)} - \log K^{M}_{M(OMP-H)op} \qquad (10b)$$

$$= pK^{H}_{M(OMP)op} - pK^{H}_{M(OMP)} \qquad (10c)$$

$$= (pK^{H}_{OMP} - pK^{H}_{M(OMP)}) - (pK^{H}_{OMP} - pK^{H}_{M(OMP)op}) \qquad (10d)$$

$$= \Delta pK_a - \Delta pK_{a/op} \qquad (10e)$$

Since $\log K^{M}_{M(OMP-H)}$ is connected with $pK^{H}_{M(OMP)}$ (*6*) *via* equation (*11*),

$$\log K^{M}_{M(OMP-H)} = \log K^{M}_{M(OMP)} + pK^{H}_{OMP} - pK^{H}_{M(OMP)} \qquad (11)$$

it is evident that $\log \Delta^*$ can also be expressed by equation (*10c*), and this can easily be transformed into (*10d*), as well as into the corresponding ΔpK_a-differences given in equation (*10e*).

The experimentally based differences $pK^{H}_{OMP} - pK^{H}_{M(OMP)}$ (see equations (*4*) and (*6*)) are listed in the third column of Table IV. A view of these data reveals that the acidification of the H(N-3) site by Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, or Mn^{2+} coordinated to the phosphate group of OMP^{3-} is evidently very similar. In fact, the arithmetic mean of these values and its error limit, *i.e.* 0.52 ± 0.09 log units, is exactly of the order one would expect for the charge effect between a phosphate-coordinated metal ion and the H(N-3) site, because the distance between these two sites is somewhat shorter than the distance to the carboxylate group in position 6 for which a corresponding effect of 0.40 ± 0.06 log units was derived (Section 4.3). Considering that the affinity of the alkaline–earth ions and of Mn^{2+} for nitrogen sites is low, these are exactly the ions among those studied, for which one would expect that their $M(OMP–H)^{2-}$ complexes exist in the 'open' form, *i.e.* that equilibrium (*8*) is to the left. Hence, we conclude that the 0.52 ± 0.09 log units represent well the $\Delta pK_{a/op}$ value in equation (*10e*).

The metal ions Co^{2+}, Ni^{2+}, and Cd^{2+} are known for their pronounced affinity towards N sites and indeed for the corresponding $M(OMP–H)^{2-}$ complexes an increased complex stability beyond the electrostatic effect is observed as can be seen from the $\log \Delta^*$ values listed in the fourth column of Table IV. We attribute this

increased complex stability to the existence of equilibrium (*8*) and define the 'open' isomer as $M(OMP-H)^{2-}_{op}$ and the 'closed' species in which M^{2+} is macrochelated to the phosphate group and the $^{-}$(N-3) site, as $M(OMP-H)^{2-}_{cl}$. The intramolecular and thus dimensionless equilibrium constant K_I is then given by equation (*12*).

$$K_I = [M(OMP-H)^{2-}_{cl}]/[M(OMP-H)^{2-}_{op}] \qquad (12)$$

The observed increased complex stability is linked[22,28,32b] to K_I by equation (*13*).

$$K_I = 10^{\log \Delta^*} - 1 \qquad (13)$$

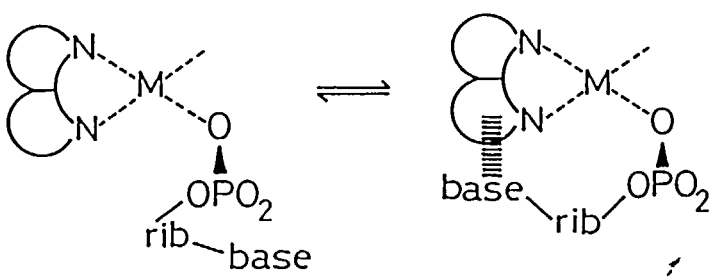
Knowledge of K_I allows calculation of the percentage of the macrochelated form according to equation (*14*).

$$\%M(OMP-H)^{2-}_{cl} = 100 \cdot K_I/(1 + K_I) \qquad (14)$$

The results of the calculations based on the log Δ^* values are summarized in columns 5 and 6 of Table IV. These data show that equilibrium (*8*) is truly in operation for the $M(OMP-H)^{2-}$ complexes of Co^{2+}, Ni^{2+} and Cd^{2+}. In addition, these results fit also into the general picture existing for nucleotide complexes:[4,5,28,29] in many instances, the extent of base backbinding of phosphate-coordinated divalent later-3d metal ions, as well as of Zn^{2+} or Cd^{2+}, to a base-nitrogen is rather pronounced.

4.5 Evidence for the Formation of Intramolecular Stacks in Cu(Arm)(OMP)$^{-}$ Complexes

Comparison of the stability constants for the Cu(Bpy)(OMP)$^{-}$ and Cu(Phen)-(OMP)$^{-}$ complexes in Table II with the corresponding values for Cu(OMP)$^{-}$ indicates an increased stability of the mixed-ligand species. As it is well-known for a number of Cu(Arm)(NMP) complexes,[4,5b,35-37] including pyrimidine-nucleoside 5'-monophosphates,[38] that an increased complex stability is connected with the formation of intramolecular stacks between the aromatic ring systems of 2,2'-bipyridyl or 1,10-phenanthroline and the nucleic base residue, the following intramolecular equilibrium (*15*) has to be considered.



(*15*)

For a detailed evaluation it is necessary to know the log $K^{Cu(Arm)}_{Cu(Arm)(R-MP)}$ *versus* $pK^{H}_{H(R-MP)}$ relationship for simple phosphate monoesters ($R-MP^{2-}$) that do not undergo any intramolecular interactions. This relationship has recently been established,[38] and the results are summarized in the following two straight reference line equations.

$$\log K^{Cu(Bpy)}_{Cu(Bpy)(R-MP)} = 0.453 \cdot pK^{H}_{H(R-MP)} + 0.103 \quad (16)$$

$$\log K^{Cu(Phen)}_{Cu(Phen)(R-MP)} = 0.453 \cdot pK^{H}_{H(R-MP)} + 0.090 \quad (17)$$

The error limits of log stability constants calculated with given $pK^{H}_{H(R-MP)}$ values and these equations are ± 0.027 log units (1σ) in the pK_a range of about 5 to 7.

The value for the micro acidity constant for a $H(OMP)^{2-}$ species in which the charge effect of the carboxylate group is *not* operating, *i.e.* $pk^{OMP}_{OMP\cdot H} = 6.15$, should now be recalled (Section 4.3). With this pK_a value and equations (*16*) and (*17*) we can calculate the stabilities for $Cu(Bpy)(OMP)^{-}$ and $Cu(Phen)(OMP)^{-}$ complexes in which *no* charge effect and *no* intramolecular ligand–ligand interaction is occurring: $\log K^{Cu(Bpy)}_{Cu(Bpy)(OMP)/calcd} = 2.89 \pm 0.08$ (3σ) and $\log K^{Cu(Phen)}_{Cu(Phen)(OMP)/calcd} = 2.88 \pm 0.08$. These stability constants need now to be corrected for the charge effect that is exercised by the carboxylate group in position 6 of OMP^{3-} (Fig. 1) on a divalent metal ion coordinating to the phosphate group; this correction amounts to 0.40 ± 0.06 log units (see Section 4.3). Hence, we obtain for the stability constants of the 'open' isomers in equilibrium (*15*) the following results: $\log K^{Cu(Bpy)}_{Cu(Bpy)(OMP)op} = 3.29 \pm 0.10$ and $\log K^{Cu(Phen)}_{Cu(Phen)(OMP)op} = 3.28 \pm 0.10$.

The difference, if it exists, between these last mentioned constants and the experimentally determined constants listed in Table II is to be attributed to a possible intramolecular ligand–ligand stacking interaction in the $Cu(Arm)(OMP)^{-}$ complexes. As previously,[35] this difference is defined by equation (*18*).

$$\log \Delta_{Arm} = \log K^{Cu(Arm)}_{Cu(Arm)(OMP)} - \log K^{Cu(Arm)}_{Cu(Arm)(OMP)op} \quad (18)$$

Hence, one obtains for $Cu(Bpy)(OMP)^{-}$ $\log \Delta_{Bpy} = (3.50 \pm 0.04) - (3.29 \pm 0.10) = 0.21 \pm 0.11$ and for $Cu(Phen)(OMP)^{-}$ $\log \Delta_{Phen} = (3.55 \pm 0.05) - (3.28 \pm 0.10) = 0.27 \pm 0.11$. These $\log \Delta_{Arm}$ values are listed in the third column in Table V.

By analogy to equations (*12*) and (*13*), equation (*19*) may now be defined[32b] (see also ref. 38), where $Cu(Arm)(OMP)^{-}_{st}$ represents the species with the intramolecular stack; its percentage-wise formation degree is calculated by analogy to equation (*14*).

$$K_I = \frac{[Cu(Arm)(OMP)^{-}_{st}]}{[Cu(Arm)(OMP)^{-}_{op}]} = 10^{\log \Delta_{Arm}} - 1 \quad (19)$$

The results of these calculations are summarized in Table V together with some previously obtained data for related Cu(Arm)(NMP) complexes.

The error limits of the results given for the $Cu(Arm)(OMP)^{-}$ complexes in Table V are rather large; however, considering the described complicated pathway necessary to obtain these data, this is no surprise. In fact, it is amazing and quite satisfying to note that the extent of intramolecular stack formation in the two $Cu(Arm)(OMP)^{-}$ complexes is so similar to that in the corresponding Cu(Arm)(UMP) species. This result is reassuring because the aromatic nucleic base fragments of UMP^{2-} and OMP^{3-} are structurally identical (Fig. 1) except for the carboxylate substituent in position 6 of OMP^{3-} and for this group no significant steric influence is expected. A simplified structure for such a stacked species according to equilibrium (*15*) is shown in Figure 3 for the ternary Cu^{2+} complex with 2,2'-bipyridyl and OMP^{3-}.

TABLE V

Extent of intramolecular stack formation in ternary Cu(Arm)(NMP) complexes as calculated from stability constants (eq. (7)): Intramolecular and dimensionless equilibrium constant K_I (eqs. (15), (19)) and percentage of stacked Cu(Arm)$(NMP)_{st}$ species in aqueous solution at 25°C and I = 0.1 M ($NaNO_3$).[a]

No.	Cu(Arm)(NMP)	log Δ_{Arm}[b]	K_I	% Cu(Arm)(NMP)
1a	Cu(Bpy)$(OMP)^-$	0.21 ± 0.11	0.62 ± 0.41	38 ± 16
1b	Cu(Phen)$(OMP)^-$	0.27 ± 0.11	0.86 ± 0.47	46 ± 14
2a	Cu(Bpy)(UMP)	0.23 ± 0.07	0.70 ± 0.26	41 ± 9
2b	Cu(Phen)(UMP)	0.33 ± 0.07	1.14 ± 0.34	53 ± 7
3a	Cu(Bpy)(AMP)	0.73 ± 0.08	4.37 ± 1.02	81 ± 4
3b	Cu(Phen)(AMP)	0.99 ± 0.08	8.77 ± 1.81	90 ± 2

[a] The error limits were calculated according to the error propagation after Gauss. The results for entries No. 2 and 3 are from refs. 38 and 35, respectively. [b] See eq. (18) and text.

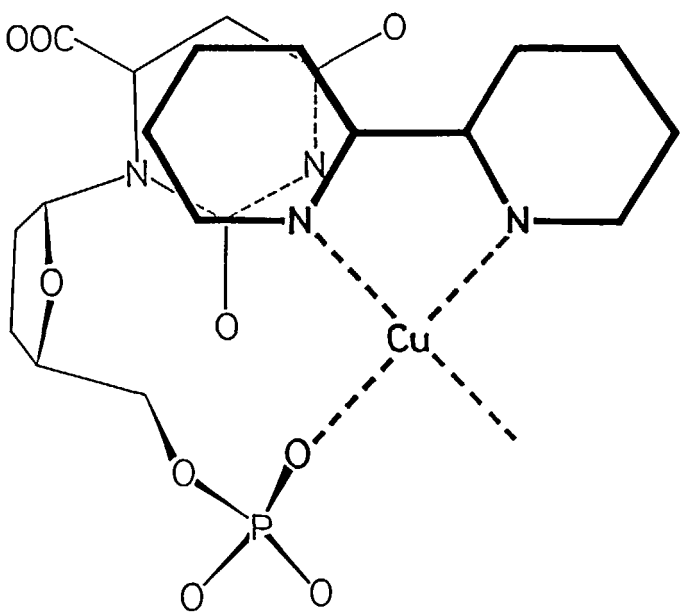


FIGURE 3 Probable (schematic) structure of the species with an intramolecular stack for Cu(Bpy)$(OMP)^-$ in solution.

Comparison of entries No. 3 in Table V with the other entries reveals a much higher stacking tendency for the mixed-ligand complexes with AMP^{2-}. Taking into account that the purine moiety is much larger than the pyrimidine residue this result also fits into the general picture. Similarly, the indicated trend of a higher stacking tendency in the Cu(Phen)(NMP) complexes compared with that in the Cu(Bpy)(NMP) species is also along these lines. Based on previous experience with other nucleotides[5b,37] it is to be expected that the pyrimidine system of OMP^{3-} may interact also with other aromatic ring moieties of importance in nature, like the indole residue of tryptophanate or the phenyl side chain of phenylalaninate.

5. CONCLUSIONS

The results described in this study show that OMP^{3-} is a very versatile ligand. Due to the dominating *syn* conformation in aqueous solution hardly any macrochelates are formed in the $M(OMP)^-$ complexes; *i.e.*, the carboxylate group exercises mainly a charge effect on the stability of the complexes and it does not directly bind to the phosphate-coordinated metal ions to a significant extent (Section 4.3). In other words, the energy difference between the *syn*/*anti* conformations is too large and the metal ion affinity of the carboxylate group too low[27] to allow a transformation of significant amounts of OMP^{3-} into the *anti* conformation; only in this *anti* conformation is a simultaneous binding of a metal ion to the phosphate and carboxylate groups possible.

It has to be further emphasized that the basicity of the carboxylate group in aqueous solution is very low (Section 3.1) and consequently this also applies for the coordinating properties of this group;[27] in aqueous-organic solvents this is different,[27] and it appears quite possible that in the active site cavity of an enzyme with a low 'effective' dielectric constant[37,39,40] the carboxylate group develops into a suitable metal ion ligator. It is evident that depending on the conformation of OMP^{3-} under such conditions a macrochelate could be formed in the *anti* conformation, but in the *syn* conformation also two independent metal ion or other kinds of ionic interactions (*e.g.*, with positively charged side-chain groups of amino acid residues) could occur.

Another interesting situation exists in the alkaline pH range; here $M(OMP-H)^{2-}$ complexes are formed which contain an ionized and thus negatively charged N-3 site. This fact allows with some metal ions, *e.g.* with Co^{2+}, Ni^{2+}, and Cd^{2+}, the formation of macrochelates, the metal ion being coordinated to the phosphate group and also to the $^-$(N-3) site. For this type of interaction the *syn* conformation is evidently ideal, as it places the phosphate group close to the N-3 site (Fig. 1).

A further way for OMP^{3-} to participate in recognition reactions is *via* the formation of stacks with the pyrimidine-ring system. In the mixed–ligand Cu(Arm)-$(OMP)^-$ complexes, where Arm = 2,2′-bipyridyl or 1,10-phenanthroline, intramolecular stacks are formed. Certainly, the formation degree of these stacks is not very large (about 40%), but still remarkable enough that one may predict with confidence that corresponding interactions are also possible, *e.g.*, with imidazole,[41] phenyl or tryptophanyl residues[37] of the appropriate amino acids. The versatility of OMP^{3-} is impressive, but most probably also necessary for the intricate metabolic processes in which this nucleotide is participating.

ACKNOWLEDGEMENTS

The support of this work by research grants from the Swiss National Science Foundation and the 'Stiftung der Portlandcementfabrik Laufen' is gratefully acknowledged.

REFERENCES AND NOTES

1. Abbreviations used: AMP^{2-}, adenosine 5′-monophosphate; Arm, heteroaromatic *N* base, (= Bpy or Phen); Bpy, 2,2′-bipyridyl; CMP^{2-}, cytidine 5′-monophosphate; $H_3(OMP)$, orotidine 5′-monophosphoric acid; H(Or), orotidine (=3-β-*D*-ribofuranosyl orotic acid); L, general ligand; M^{2+}, divalent metal ion; NMP^{2-}, nucleoside 5′-monophosphate; OMP^{3-}, orotidinate 5′-monophosphate; Or^-, orotidinate; Phen, 1,10-phenanthroline; $R\text{-}MP^{2-}$, phosphate monoester (R = noncoordinating organic residue); Tu, tubercidin; $TuMP^{2-}$, tubercidin 5′-monophosphate (=7-deaza-AMP^{2-}); UMP^{2-}, uridine 5′-monophosphate.

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