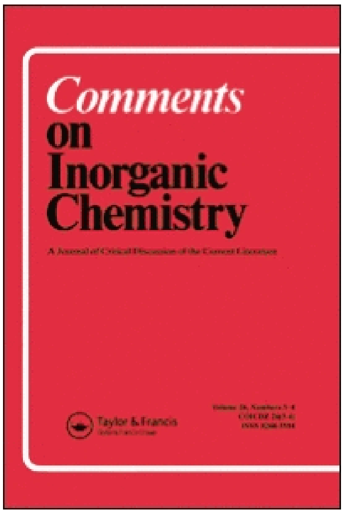

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On the Dichotomy of Metal Ion Binding in Adenosine Complexes

Helmut Sigel[a]; Nicolas A. Corfù[a]; Liang-Nian Ji[a]; R. Bruce Martin[b]
[a] Institute of Inorganic Chemistry, University of Basel, Basel, Switzerland [b] Chemistry Department, University of Virginia, Charlottesville, Virginia

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On the Dichotomy of Metal Ion Binding in Adenosine Complexes

HELMUT SIGEL, NICOLAS A. CORFÙ
and LIANG-NIAN JI[1]
Institute of Inorganic Chemistry,
University of Basel,
Spitalstrasse 51,
Ch-4056 Basel, Switzerland

R. BRUCE MARTIN
Chemistry Department,
University of Virginia,
Charlottesville, Virginia 22903

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The structural similarity of tubercidin (Tu) with adenosine (Ado) [in Tu the N-7 of Ado is replaced by a CH unit] and recently published results on the stability of $M(Tu)^{2+}$ complexes allow a quantification of the steric inhibitory effect of the *o*-amino group on the complexation tendency of the N-1 site in adenosine. This information together with an improved estimate for the pK_a value of the H(N-7) site in monoprotonated adenosine (which is not directly accessible by experiments) led to a reevaluation of the N-1 and N-7 dichotomy for metal ion binding as present in adenosine complexes. It is concluded that Ni^{2+} and Cu^{2+}, and most probably also Co^{2+} and Cd^{2+}, coordinate to adenosine preferably via the N-7 site; for Zn^{2+} a more even distribution between the N-1 and N-7 sites appears to occur, while Mn^{2+} possibly prefers the N-1 site, which definitely is strongly dominating for the binding of H^+. The possible formation of outer sphere complexes, i.e., a water molecule of the metal ion-coordination sphere is hydrogen bonded to an N-site of the adenine residue, is briefly discussed and some implications of the presented results for biological systems are indicated.

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Key Words: *adenosine complex dichotomy, basicity properties of adenosine, intramolecular equilibria, metal ion binding of adenosine, tubercidin complexes*

1. INTRODUCTION

The adenosine residue is an important metal ion binding site in adenine nucleotides,[2–4] including ATP^{4-},[5–7] and this site is also expected to interact with certain metal ions in single stranded nucleic acids.[3,8] The parent compound, i.e., adenosine itself (Fig. 1), offers metal ions two binding sites at the purine moiety, namely N-1 and N-7;[8] the affinity of N-3 appears to be insignificant in this case though it is known that in certain purine derivatives some interaction with metal ions occurs also at this site.[9–11]

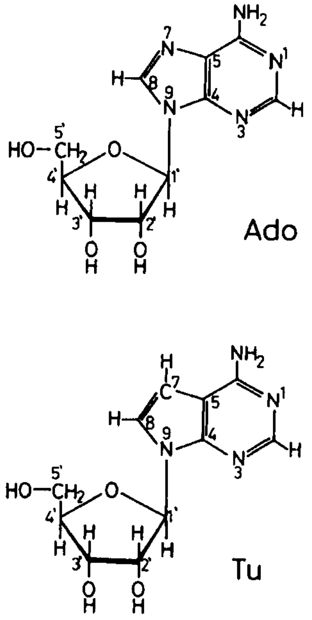

FIGURE 1 Chemical structures of adenosine (Ado) and tubercidin (Tu).

The indicated property of adenosine gives rise to a dichotomy in metal ion binding of N-1 versus that of N-7. This problem was first addressed by Kim and Martin[8,12] who showed that the logarithms of the stability constants for Ni^{2+}, Cu^{2+} and Zn^{2+} binding at pyridine or purine N-1 type nitrogens and imidazole or purine N-7 type nitrogens display a linear relationship with pK_a for the three metal ions (M^{2+}) and the two types of nitrogen ligating sites; this corresponds to the well-known linear relationship between log K^{M}_{ML} and pK^{H}_{HL} for many series of structurally related ligands (L).[13] As a result of this evaluation[8] it became clear that both metal ion binding sites of purines must be considered; in addition, ratios were given for the distribution of Ni^{2+}, Cu^{2+} and Zn^{2+} between N-1 and N-7 for adenosine.

In a study focusing on the evaluation of the metal ion binding dichotomy of xanthosine, Sigel and co-workers[14] provided evidence that an *ortho* amino group next to a pyridine-like nitrogen leads to a steric inhibition of the metal ion binding. As this structural situation is also present in the N-1/C(6)-NH_2 site of adenosine (Fig. 1) these authors[14] made estimates for the steric effect and reevaluated the N-1 to N-7 metal ion binding ratios given earlier[8] for some $M(adenosine)^{2+}$ complexes. Of course, such a steric effect on N-1 then favors, in a relative way, metal ion coordination at N-7.[14]

Comparison of the chemical structures shown in Fig. 1 for adenosine and tubercidin shows that a study of the metal ion complexing properties of the latter ligand should solve any ambiguity. Tubercidin differs from adenosine in the replacement of N-7 by a CH unit (see Fig. 1) and is therefore also known as 7-deaza-adenosine. It is evident (i) that for this molecule the dichotomy with N-7 does not exist and (ii) that the influence of the steric restriction on the metal ion binding properties of the N-1 site can be determined in a quantitative way. Indeed, this quantitative evaluation has very recently been carried out in a detailed study[15] of the metal ion coordinating properties of tubercidin.

In a joint effort, based on the indicated previous results,[8,12,14,15] we are now providing a sophisticated and detailed evaluation of the metal ion binding properties of adenosine.

2. CORRELATION BETWEEN Log K AND pK_a VALUES FOR PYRIDINE-LIKE (N-1) AND IMIDAZOLE-LIKE (N-7) BINDING SITES. SUMMARY OF THE STRAIGHT-LINE RELATIONSHIPS

The linear relationship between the logarithms of the stability constants for Ni^{2+}, Cu^{2+} and Zn^{2+} binding at pyridine or purine N-1 type nitrogens, as well as for imidazole or purine N-7 type nitrogens and the pK_a of the corresponding binding sites has already been established several years ago.[8,12] We summarize below the results of more recent efforts,[14,15] in which the inhibitory effect of an *o*-amino group next to a pyridine nitrogen has also been taken into account; i.e., with regard to purines we consider the N-1 site, the N-1 site with an *o*-amino group, designated as N-1,ortho, and the N-7 site:

(i) We begin with the last mentioned, simplest case. Use of log K^{M}_{ML}/pK^{H}_{HL} data pairs for the complexes of adenosine (1), 1-methylinosine (2), inosine (3), guanosine (4), imidazole (5), and 1-methylimidazole (6) [=L] with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} previously allowed the calculation[14] of the regression lines for plots of log K versus pK_a of imidazole-like or N-7 type ligands. The identification numbers given above with the names of the ligands are the same as used previously[14] and these numbers are also employed now in Fig. 3 (see Section 5.1). Equations (1) through (6) in Table I refer to the six mentioned metal ions. Not all data pairs of the ligands were available for each metal ion; this is reflected, at least in some cases, in the varying error limits given with the data in Table I (for details see Ref. 14).

(ii) The analogous comments as given in (i) also hold for the regression lines valid for pyridine-like or purine N-1 type ligands. These straight-line equations are based[14] on pyridine (7), 4-(2-thienyl)pyridine (8), 4-methylpyridine (9), 7-methylinosine (10), inosine (11), and ammonia (12, see Ref. 14); the results are summarized in Eqs. (7) through (12) of Table I.

(iii) The linear relationship between log K and pK_a for N-1 type ligands with an *ortho* (amino) substituent has so far only been established[15] for Cu^{2+} by using the ligands 2-phenylpyridine (13), tubercidin (14), 2-methylpyridine (15), 2-amino-pyridine (16), and 2-amino-3-methylpyridine (17). However, comparison of Eqs. (10)

TABLE I

Correlations between M^{2+} complex stability with imidazole-like (N-7) or pyridine-like (N-1) ligands or pyridine-like ligands with an *ortho* amino group (N-1,ortho) and the basicity of the ligand binding site.[a]

Eq. No.	M^{2+}	m	b	R	SD[b]
	(a) Regression Lines for Imidazole-like or N-7 Type Ligands[c]				
(1)	Mn^{2+}	0.30	−0.86		0.07[d]
(2)	Co^{2+}	0.315 ± 0.026	0.312 ± 0.135	0.993	0.057
(3)	Ni^{2+}	0.377 ± 0.020	0.519 ± 0.095	0.996	0.046
(4)	Cu^{2+}	0.499 ± 0.019	0.766 ± 0.084	0.997	0.053
(5)	Zn^{2+}	0.416 ± 0.016	−0.246 ± 0.069	0.997	0.045
(6)	Cd^{2+}	0.328 ± 0.020[d]	0.404 ± 0.070[d]	1.000	0.05[d]
	(b) Regression Lines for Pyridine-like or N-1 Type Ligands[c]				
(7)	Mn^{2+}	0.262 ± 0.021	−1.189 ± 0.145	0.997	0.029
(8)	Co^{2+}	0.204 ± 0.031	0.244 ± 0.236	0.977	0.045
(9)	Ni^{2+}	0.235 ± 0.025	0.633 ± 0.179	0.984	0.036
(10)	Cu^{2+}	0.415 ± 0.010	0.296 ± 0.065	0.999	0.013
(11)	Zn^{2+}	0.367 ± 0.025	−0.923 ± 0.178	0.991	0.034
(12)	Cd^{2+}	0.332 ± 0.013	−0.450 ± 0.094	0.999	0.015
	(c) Regression Lines for *o*-Amino Pyridine-like (N-1,ortho) Ligands				
(13[c])	Mn^{2+}	0.262	−1.159		0.067[g]
(14[c])	Co^{2+}	0.204	−0.856		0.075[g]
(15[c])	Ni^{2+}	0.235	−0.917		0.056[g]
(16[f])	Cu^{2+}	0.456 ± 0.029	−1.428 ± 0.175	0.994	0.024
(17[c])	Zn^{2+}	0.367	−1.613		0.058[g]
(18[c])	Cd^{2+}	0.332	−1.060		0.052[g]

[a] Slopes (m) and intercepts (b) are given for the straight base line plots of log K versus pK_a as calculated by the least-squares procedure from the experimental equilibrium constants of the systems listed in Section 2 (see also Ref. 14), together with the corresponding correlation coefficients (R). The errors given with m and b correspond to one standard deviation (1σ). Straight-line equation: $y = mx + b$; x may represent the pK_a value of any N-7, N-1 or N-1,ortho type ligand. The column at the far right lists the standard deviations (SD)[b] resulting from the differences between the experimental and calculated (from the straight-line equations) log K values of the individual systems (I = 0.1 – 1 M; t close to 25°C).[14]

[b] These SD values times 2 or 3 are considered as reasonable error limits for any stability constant calculation in the pK_a range of the experimental data employed (see Table III of Ref. 14 and also Fig. 3).

[c] From Table IV of Ref. 14.

[d] Estimated error limit.

[c] In these cases the slopes are taken from Eqs. (7) to (9), (11) and (12), and from the corresponding values for b in these equations the stability difference log $K^{M}_{M/N\text{-}1}$ − log $K^{M}_{M(Tu)}$ was subtracted to obtain the b values valid in Eqs. (13) to (15), (17) and (18); the mentioned stability differences are from Table II of Ref. 15 (see also text in Section 3.c of Ref. 15, as well as in Section 2 of this work).

[f] From Section 3.a of Ref. 15.

[g] These SD values contain also the contribution of the error limits of the stability differences, log $K^{M}_{M/N\text{-}1}$ − log $K^{M}_{M(Tu)}$ (the latter are given in the fourth column of Table II in Ref. 15).

and (16) in Table I shows that the slopes of the two regression lines agree nearly within a single standard deviation. This and the fact that the data pair for Cu^{2+}/tubercidin very well fits within the error limits on the straight line (see also Fig. 3, vide infra) means that the effect of the steric inhibition at a pyridine-like nitrogen or a purine N-1 type site by an *ortho* amino group is well represented by the stability differences, $\log K^{M}_{M/N\text{-}1} - \log K^{M}_{M(Tu)}$, calculated for the individual M(tubercidin)$^{2+}$ complexes (see also Ref. 15). Values of $\log K^{M}_{M/N\text{-}1}$ for the complexation at a sterically freely accessible N-1 site with Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} were obtained[15] with $pK^{H}_{H(Tu)} = 5.29$ and the straight-line equations (6) to (9), (11) and (12) of Table I; the values of the stability constants $\log K^{M}_{M(Tu)}$ are known.[15] Application of the mentioned differences to Eqs. (6)–(9), (11) and (12) gives the straight line equations (13)–(15), (17) and (18) listed in Table I which quantify the relationship between $\log K^{M}_{M/N\text{-}1,\text{ortho}}$ and the pK_a of the corresponding binding site.

Use of Eqs. (1) through (18) of Table I together with the pK_a values of the N-1 and N-7 sites for *monoprotonated* adenosine allows us now to calculate the metal ion affinity of the mentioned sites, and to compare these results with those obtained experimentally for the corresponding M(adenosine)$^{2+}$ complexes.

The reliability of the reference lines in Table I may be proven with some recently published data[16] for the Ni^{2+} and Cu^{2+} complexes of 8-substituted 9-methylpurine derivatives. For these complexes it was "assumed that protonation and complexation take place at N-1."[16] Indeed, the stability constants calculated with Eqs. (9) and (10) of Table I and the known pK_a values of the 9-methylpurine derivatives are identical within the error limits with the experimentally determined values (see Table II). This result is helpful in a two fold sense: (i) It confirms the above mentioned assumption[16] that binding occurs at N-1 in these purine derivatives; in other words, the substituents at position 8 prevent complex formation at N-7. (ii) The N-1 equations of Table I are based on experimental data valid for the pK_a range 5 to 9.5 (Ref. 14; see also Fig. 3 below); the fact that the data pairs for the 9-methylpurine derivatives fit on the corresponding straight lines (see Table II) proves that the equations are also valid in the pK_a range down to 2.5. This latter point is especially important for the conclusions

TABLE II

Stability constant comparisons between the experimental (exptl) and calculated (calcd) logarithms of the stability constants[a] for the $M(L)^{2+}$ complexes formed between Ni^{2+} or Cu^{2+} and an 8-substituted 9-methylpurine (L). The calculated stability constants are for a pure N-1 coordination and they are based on the straight-line equations (9) or (10) of Table I quantifying the relationship between complex stability and N-1 basicity and the negative logarithms of the acidity constants[a] for the monoprotonated 9-methylpurine derivatives.

L	$pK^H_{H(L)}$[b]	$\log K^{Ni}_{Ni(L)}$ exptl[b]	$\log K^{Ni}_{Ni(L)}$ calcd	$\log K^{Cu}_{Cu(L)}$ exptl[b]	$\log K^{Cu}_{Cu(L)}$ calcd
8-Amino-9-methylpurine	4.77 ± 0.08	1.59 ± 0.28	1.75 ± 0.11	2.33 ± 0.24	2.28 ± 0.04
8-Chloro-9-methylpurine	2.55 ± 0.03	1.00 ± 0.17	1.23 ± 0.11	1.18 ± 0.17	1.35 ± 0.04
8-Methoxy-9-methlypurine	3.59 ± 0.03	1.36 ± 0.12	1.48 ± 0.11	1.69 ± 0.10	1.79 ± 0.04
8-Methylthio-9-methylpurine	3.39 ± 0.03	1.31 ± 0.13	1.43 ± 0.11	1.62 ± 0.11	1.70 ± 0.04

[a] $K^M_{M(L)} = [M(L)^{2+}]/([M^{2+}][L])$; $K^H_{H(L)} = [H^+][L]/[H(L)^+]$.

[b] All experimental values refer to 25°C and $I = 1.0$ M ($NaClO_4$). The values for $pK^H_{H(L)}$ are from Table I of Ref. 16. The above values for $\log K^M_{M(L)}$ were recalculated on the basis of the data ($pK^H_{H(L)}$ and $pK_{a(app)}$) listed in Table I of Ref. 16 with Eq. (2) of Ref. 16 (see also Ref. 17) to obtain unequivocal error limits, and these latter values were calculated according to the error propagation after Gauss (2σ); the constants given above are very close to those listed in Table II of Ref. 16, whereas for the error limits this is only partly true.

presented in Section 6.2 which are based on $pK_a = 3.61$ of $H(adenosine)^+$.

3. FACTORS GOVERNING THE STABILITY OF $M(ADENOSINE)^{2+}$ COMPLEXES

The overall stability constant measured in solution for a complex formed between a metal ion and adenosine (Ado) (Eq. (19a)) is defined by Eq. (19b):

$$M^{2+} + \text{Ado} \rightleftharpoons M(\text{Ado})^{2+}, \tag{19a}$$

$$K^M_{M(\text{Ado})} = [M(\text{Ado})^{2+}]/([M^{2+}][\text{Ado}]). \tag{19b}$$

Considering that $M(Ado)^{2+}$ may exist in two isomeric forms,[8,14] i.e., one species with N-1 binding and a second one with N-7 binding, one may rewrite Eq. (19b) in the form of Eq. (20):

$$K^{M}_{M(Ado)} = \frac{([M(N\text{-}1/Ado)^{2+}] + [M(N\text{-}7/Ado)^{2+}])}{[M^{2+}][Ado]} \tag{20a}$$

$$K^{M}_{M(Ado)} = \frac{[M(N\text{-}1/Ado)^{2+}]}{[M^{2+}][Ado]} + \frac{[M(N\text{-}7/Ado)^{2+}]}{[M^{2+}][Ado]}, \tag{20b}$$

$$K^{M}_{M(Ado)} = K^{M}_{M(N\text{-}1/Ado)} + K^{M}_{M(N\text{-}7/Ado)}. \tag{20c}$$

It is thus clear that the basicity of N-1 *and* of N-7 governs metal ion binding. From all metal ions studied so far with regard to their interacting properties with the N-1 or N-7 sites of purines, the information for Cu^{2+} is certainly most complete (see Section 2).

Similarly, the stability constant for $Cu(Ado)^{2+}$ has also been determined in four independent laboratories with a satisfactory agreement: i.e., log $K^{Cu}_{Cu(Ado)}$ = 0.71 (Ref. 18), 0.76 (Ref. 19), 0.84 (Ref. 20), and 0.96 (Ref. 21); the first and last mentioned values were measured via potentiometric pH titration and the second and third ones via UV spectrophotometry. These stability constants for the $Cu(Ado)^{2+}$ complex may be used to estimate in the way described below in Section 4.1 an acidity constant for the H^{+}(N-7) site of monoprotonated adenosine; i.e., for the micro acidity constant[22] $pk^{H}_{H(N\text{-}7/Ado)}$.

4. THE ACIDITY CONSTANT OF $H(Ado)^{+}$ AND THE PROTON AFFINITY OF THE N-1 AND N-7 SITES OF ADENOSINE

From the equations summarized in Section 3 it is apparent that resolution of the N-1 versus N-7 dichotomy of metal ion binding to adenosine rests not only on the linearity of the log K versus pK_a plots described in Section 2 (Table I) but also on the identification of the microscopic intrinsic pk_a values for proton binding at the N-1 and N-7 sites. Because N-1 of adenosine is much more basic than N-7, the acidity constant determined by potentiometric pH titration[23] equals the intrinsic $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}1/Ado)}}$ value, i.e., $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(Ado)}} = \mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}1/Ado)}} = 3.61 \pm 0.03$. What is also required is $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}7/Ado)}}$, the intrinsic micro acidity constant for deprotonation of N-7 when N-1 is *not* protonated.

4.1. Estimation of the Micro Acidity Constant for the H^+(N-7) Site of Monoprotonated Adenosine

The acidity constant, $k^{\mathrm{H}}_{\mathrm{H(N\text{-}7/Ado)}}$, which defines the deprotonation of the H^+(N-7) site in the presence of a *neutral*, *uncharged* N-1 site is not directly accessible to an experimental determination because N-1 is the more basic site, as indicated in the preceding paragraph, and therefore the first protonation step at adenosine overwhelmingly occurs at this site (see also Table V in Section 6.1).

By using the constant valid for the deprotonation of the H^+(N-1) site, $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}1/Ado)}} = 3.61\ (\pm 0.03)$,[23] and the reference line given in Eq. (16) of Table I for N-1 coordination in the presence of an *ortho* amino group, $K^{\mathrm{Cu}}_{\mathrm{Cu(N\text{-}1/Ado)}}$ may be calculated: log $K^{\mathrm{Cu}}_{\mathrm{Cu(N\text{-}1/Ado)}} = 0.22 \pm 0.07$ ($= 3 \times$ SD). With this constant, the four mentioned experimental values for $K^{\mathrm{Cu}}_{\mathrm{Cu(Ado)}}$ (see Section 3), and Eq. (20c) one may calculate values for $K^{\mathrm{Cu}}_{\mathrm{Cu(N\text{-}7/Ado)}}$ and obtains: log $K^{\mathrm{M}}_{\mathrm{M(N\text{-}7/Ado)}} = 0.54$ (based on 0.71), 0.61 (with 0.76), 0.72 (with 0.84), and 0.87 (with 0.96).

These stability constants together with Eq. (4) of Table I, which defines the dependence between log $K^{\mathrm{Cu}}_{\mathrm{Cu/N\text{-}7}}$ and the basicity of N-7, offer the possibility to calculate values for $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}7)/Ado)}}$. Estimates for this value exist,[8,14] and based on several arguments it was concluded[14]: "A precise value for $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}7/Ado)}}$ is evidently difficult to assess, but we believe it to be (in its extremes) between $\mathrm{p}K_a = -0.5$ and $+0.8$ and estimate $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}7/Ado)}} = 0.2 \pm 0.5$." Indeed, based on Eq. (4) and the mentioned values for log $K^{\mathrm{Cu}}_{\mathrm{Cu(N\text{-}7/Ado)}}$ we now calculate $\mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}7/Ado)}} = -0.45$ (from 0.54), -0.31 (from 0.61), -0.09 (from 0.72), and 0.21 (from 0.87). The average of these values leads to

$$\mathrm{p}k^{\mathrm{H}}_{\mathrm{H(N\text{-}7/Ado)}} = -0.2 \pm 0.3\ (2\sigma),$$

as the best estimate presently available for this micro acidity constant.[24]

4.2. Some Support for the Present Value of $pk^{H}_{H(N\text{-}7/Ado)}$

The following observation also provides evidence for the approximate validity of $pk^{H}_{H(N\text{-}7/Ado)} = -0.2$: The purine-nucleotides adenosine 5′-monophosphate (AMP^{2-}), inosine 5′-monophosphate (IMP^{2-}), and guanosine 5′-monophosphate (GMP^{2-}) form with divalent 3d metal ions, including Zn^{2+} and Cd^{2+}, complexes in which the metal ion is not only coordinated to the phosphate group but to some extent also to N-7 of the purine moiety. Of course, this extent of base-backbinding varies with the NMP^{2-} ($=AMP^{2-}$, IMP^{2-}, GMP^{2-})[3,29] and also the metal ion,[3,29,30] but it is in all instances reflected by an increased complex stability,[3,30] as one would expect:[13]

$$\log \Delta_{M(NMP)} = \log K^{M}_{M(NMP)} - \log K^{M}_{M(NMP)_{op}}. \qquad (21)$$

The stability constant, $K^{M}_{M(NMP)}$, quantifies the overall stability of the M(NMP) complexes and is experimentally measured,[30] while $K^{M}_{M(NMP)_{op}}$ describes the stability of the complex with a sole phosphate coordination; this latter value is only indirectly accessible.[31] Some examples of such data are listed in Table III.[3,29–34]

As metal ion backbinding occurs to N-7 in these M(NMP) complexes, one would expect that its extent depends on the basicity of this site. In other words, a plot of the stability increase $\log \Delta_{M(NMP)}$ versus the pK_a of the N-7 site in these three NMPs should result in a straight line. In a relative sense the basicity of the N-7 site in AMP^{2-}, IMP^{2-}, and GMP^{2-} should be reflected by the pK_a values of the corresponding nucleosides, adenosine (Ado), inosine (Ino), and guanosine (Guo), i.e., by $pk^{H}_{H(N\text{-}7/Ns)}$. The values $pK^{H}_{H(Ino)} = pk^{H}_{H(N\text{-}7/Ino)} = 1.06 \pm 0.04$ and $pK^{H}_{H(Guo)} = pk^{H}_{H(N\text{-}7/Guo)} = 2.11 \pm 0.04$ were measured[35] and the one for $pk^{H}_{H(N\text{-}7/Ado)} = -0.2$ has now been estimated in Section 4.1.

Indeed, for the Cu(NMP) and Cd(NMP) systems, as well as for other related M(NMP) systems, the plots of $\log \Delta_{M(NMP)}$ versus the mentioned $pk^{H}_{H(N\text{-}7/Ns)}$ values result in straight lines as may be seen in Fig. 2. This observation is interesting in a twofold sense:

TABLE III

Comparison of the measured stability, $K^{M}_{M(NMP)}$, of the Cu^{2+} and Cd^{2+} 1:1 complexes of AMP^{2-}, IMP^{2-} and GMP^{2-} with the calculated stability, $K^{M}_{M(NMP)_{op}}$, for an isomer with only a phosphate-metal ion coordination, and extent of the stability increase as defined by log $\Delta_{M(NMP)}$ (Eq. (21)). All constants refer to aqueous solutions at 25°C and I = 0.1 M (NaNO$_3$)[a].

M(NMP)	log $K^{M}_{M(NMP)}$	log $K^{M}_{M(NMP)_{op}}$[b]	log $\Delta_{M(NMP)}$
Cu(AMP)[c]	3.14 ± 0.01	2.87 ± 0.08	0.27 ± 0.08
Cu(IMP)[d,e]	3.38 ± 0.02	2.87 ± 0.08	0.51 ± 0.08
Cu(GMP)[d]	3.61 ± 0.04	2.89 ± 0.08	0.72 ± 0.09[f]
Cd(AMP)[c]	2.68 ± 0.02	2.44 ± 0.06	0.24 ± 0.06
Cd(IMP)[d,e]	2.88 ± 0.02	2.44 ± 0.06	0.44 ± 0.06
Cd(GMP)[g]	2.98 ± 0.02	2.45 ± 0.06	0.53 ± 0.06[f]

[a] Acidity constants for $H_2(NMP)$: $pK^{H}_{H_2(AMP)} = 3.84 \pm 0.02$, $pK^{H}_{H(AMP)} = 6.21 \pm 0.01$ (Ref. 30); $pK^{H}_{H_2(IMP)} = 1.30 \pm 0.10$, $pK^{H}_{H(IMP)} = 6.22 \pm 0.01$ (Ref. 34); $pK^{H}_{H_2(GMP)} = 2.48 \pm 0.04$ (Ref. 34), $pK^{H}_{H(GMP)} = 6.25 \pm 0.02$ (Ref. 29). All error limits in this table correspond to three times the standard errors (3σ).

[b] Calculated with the straight-line equations listed in Table V of Ref. 31 and the above[a] values for $pK^{H}_{H(NMP)}$.

[c] From Ref. 30.

[d] From Ref. 34.

[e] From Ref. 32.

[f] From Ref. 33.

[g] From Ref. 29.

(i) It confirms the mentioned expectation that the extent of N-7 backbinding in M(NMP) complexes depends on the basicity of N-7, and (ii) the fact that the data pairs with $pk^{H}_{H(N\text{-}7/Ado)}$ fit within the error limits well on the straight lines allows the conclusion that the estimate $pk^{H}_{H(N\text{-}7/Ado)} = -0.2 \pm 0.3$ is of the correct order.

5. STABILITY CONSTANTS OF M(ADENOSINE)$^{2+}$ COMPLEXES

5.1. Calculated Stabilities via the Straight-Line Relationships

With the two acidity constants for $H(Ado)^+$, i.e., $pk^{H}_{H(N\text{-}1/Ado)} = 3.61\ (\pm 0.03)$[23] and $pk^{H}_{H(N\text{-}7/Ado)} = -0.2\ (\pm 0.3)$ (Section 4.1), and the known correlations between log K and pK_a for N-1,ortho and

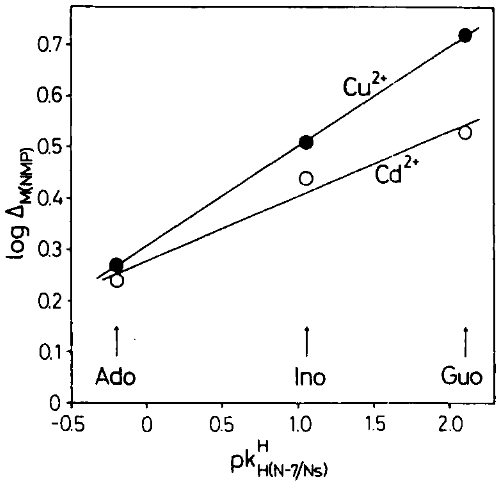

FIGURE 2 Relationship between log $\Delta_{M(NMP)}$ (Eq. (21)) for the Cu^{2+} (●) and Cd^{2+} (○) 1:1 complexes of AMP^{2-}, IMP^{2-} or GMP^{2-} and $pk^{H}_{H(N\text{-}7/Ns)}$ of the corresponding nucleosides (Ns), adenosine (Ado), inosine (Ino) and guanosine (Guo). The values for log $\Delta_{M(NMP)}$ are from Table III and those for $pk^{H}_{H(N\text{-}7/Ns)}$ from the text in Section 4.2 (25°C; I = 0.1 M, $NaNO_3$).

N-7 coordination (Table I in Section 2), the individual stability constants of Eq. (20c) may be calculated. For Cu^{2+} and Zn^{2+} this procedure is also graphically indicated in Fig. 3: the vertical arrows at the x axis mark the position of the negative logarithms of the two acidity constants of $H(Ado)^{+}$; the vertical extention of these arrows leads to intercepts with the straight lines, and these intercepts correspond to the logarithms of the stability constants. The mathematical evaluation leads to the results summarized in columns three and four of Table IV, where (see Eq. (20c)) $K^{M}_{M(N\text{-}1/Ado)} = K^{Ado}_{M/N\text{-}1,ortho}$ and $K^{M}_{M(N\text{-}7/Ado)} = K^{Ado}_{M/N\text{-}7}$.

Column two of Table IV contains values for log $K^{Ado}_{M/N\text{-}1}$, i.e., for the coordination of a metal ion to a hypothetical adenosine in which the *o*-amino group does *not* exist or at least does *not* affect the metal ion coordination at N-1. The difference log $K^{Ado}_{M/N\text{-}1}$ − log $K^{Ado}_{M/N\text{-}1,ortho}$ reflects the steric inhibition of the *o*-amino group as discussed already in Section 2. In the case of Cu^{2+} this difference corresponds in Fig. 3 closely to the vertical distance between the straight lines representing N-1 and N-1,ortho complexation; for

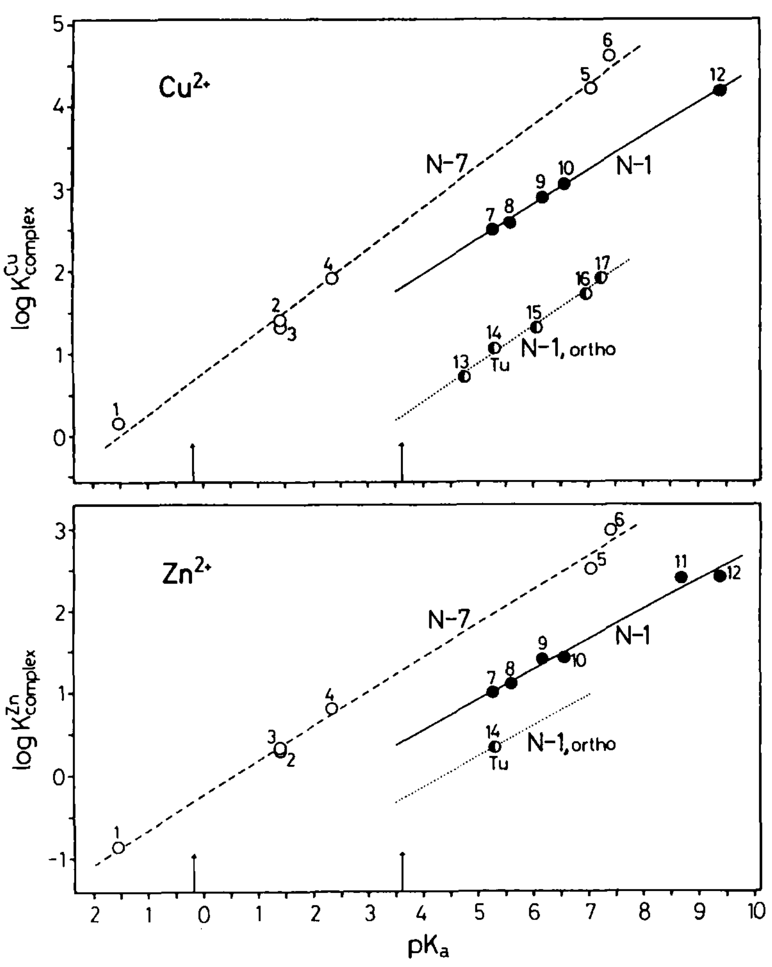

FIGURE 3 Relationship between log K and pK_a for the 1 : 1 complexes of Cu^{2+} and Zn^{2+} with imidazole-like or purine N-7 type ligands (○, broken line), pyridine-like or N-1 type ligands (●, full line), and pyridine-like or N-1 type ligands with an *ortho* amino substituent (◐, dotted line). The least squares lines are drawn according to Eqs. (4), (10) and (16), and (5), (11) and (17) of Table I for the Cu^{2+} and Zn^{2+} systems, respectively; the inserted numbers correspond to the ligand numbers given in the text of Section 2; only the points due to Cu(tubercidin)$^{2+}$ or Zn(tubercidin)$^{2+}$ are in addition identified (Tu). The two arrows placed on each pK_a axis correspond to the acidity constants of *mono*protonated adenosine; i.e., $pk^{H}_{H(N\text{-}7/Ado)} = -0.2 \pm 0.3$ and $pk^{H}_{H(N\text{-}1/Ado)} = 3.61 \pm 0.03$ (see text in Section 4.1 and also the bottom row in Table V).

TABLE IV

Logarithms of the stability constants for metal ion coordination at N-1 (log $K^{Ado}_{M/N\text{-}1,ortho}$) or N-7 (log $K^{Ado}_{M/N\text{-}7}$) of neutral adenosine and of the resulting calculated overall stability constant log $K^{M}_{M(Ado)calc}$, together with the stability constant measured for $M(Ado)^{2+}$ complexes by various experimental procedures, log $K^{M}_{M(Ado)exp}$ (t is close to 25°C; I varies).[a,b]

M^{2+}	log $K^{Ado}_{M/N\text{-}1}$ [c]	log $K^{Ado}_{M/N\text{-}1,ortho}$ [d]	log $K^{Ado}_{M/N\text{-}7}$ [c]	log $K^{M}_{M(Ado)calc}$ = log ($K^{Ado}_{M/N\text{-}1,ortho}$ + $K^{Ado}_{M/N\text{-}7}$)[f]	log $K^{M}_{M(ado)exp}$	
					individual results	weighted mean (2σ)
Mn^{2+}	−0.24 ± 0.09	−0.21 ± 0.20	−0.92 ± 0.23	−0.13 ± 0.17	−0.82 ± 0.4[g]	
Co^{2+}	0.98 ± 0.14	−0.12 ± 0.23	0.25 ± 0.19	0.40 ± 0.15	−0.30 ± 0.4[g] 0.17 ± 0.34[h]	−0.03 ± 0.52
Ni^{2+}	1.48 ± 0.11	−0.07 ± 0.17	0.44 ± 0.18	0.56 ± 0.14	−0.17 ± 0.4[g] 0.34 ± 0.10[i] 0.30 ± 0.13[j] 0.32 ± 0.10[k] 0.41 ± 0.22[h]	0.32 ± 0.12
Cu^{2+}	1.79 ± 0.04	0.22 ± 0.07	0.67 ± 0.22	0.80 ± 0.16	0.84 ± 0.1[g] 0.76 ± 0.05[l] 0.71 ± 0.05[m] 0.96 ± 0.06[h]	0.80 ± 0.12
Zn^{2+}	0.40 ± 0.10	−0.29 ± 0.17	−0.33 ± 0.18	−0.01 ± 0.12	−0.28 ± 0.3[g] −0.3 ± 0.2[n] 0.24 ± 0.30[h]	−0.17 ± 0.33
Cd^{2+}	0.75 ± 0.05	0.14 ± 0.16	0.34 ± 0.18	0.55 ± 0.13	0.11 ± 0.06[n]	

[a] As adenosine is a neutral ligand the binding constants should not show a pronounced ionic strength dependence and indeed, for $Ni(Ado)^{2+}$ this has been verified.[36]

[b] The error limits are based in general on *three* times the standard deviations (see also Table I) and they were calculated according to the error propagation after Gauss. The error limits given with log $K^{M}_{M(Ado)exp}$ are those from the literature if nothing else is specified.

[c] Calculated with $pk^{H}_{H(N\text{-}1/Ado)} = 3.61\ (\pm 0.03)$[23] and the straight-line equations (7)–(12) in Table I for *freely accessible* N-1 binding sites.

[d] Calculated with $pk^{H}_{H(N\text{-}1/Ado)} = 3.61\ (\pm 0.03)$[23] and the straight-line equations (13)–(18) in Table I.

[e] Calculated with $pk^{H}_{H(N\text{-}7/Ado)} = -0.2 \pm 0.3$ (Section 4.1) and the straight-line equations (1)–(6) in Table I; the error limits given in this column contain also the contribution resulting from the ± 0.3 log unit of the pK_a value.

[f] See Eq. (20c).[b]

[g] The stability constants are from Ref. 20, while the error limits are estimates based on our reevaluation as indicated under (i) in Section 5.2.

[h] These constants and their error limits are the results of the re-calculation described under (ii) in Section 5.2 for the data published in Ref. 21.

[i] Interpolation towards 25°C of the stability constants listed in Table V of Ref. 37 gives the above value. The given error is estimated by the present authors.

[j] Ref. 36.

[k] Ref. 38; the given error limit is estimated by the present authors.

[l] From Ref. 19.

[m] Ref. 18.

[n] Ref. 5.

Zn^{2+} (lower part of Fig. 3) the difference and the vertical distance are identical (see Section 2).

A comparison of the constants listed in the third and fourth column of Table IV indicates that both binding sites of adenosine, i.e., N-1 and N-7, may contribute to the overall stability of the $M(Ado)^{2+}$ complexes. The calculated overall stability constant (see Eq. (20c)) is listed in the fifth column of Table IV.

5.2. Experimentally Measured Stability Constants

Column six of Table IV contains the available experimentally determined stability constants of $M(Ado)^{2+}$ complexes;[5,18–21,36–38] the average values are listed in column seven.

Two of these experimental data sets given in the sixth column need some special comments:

(i) For all the $M(Ado)^{2+}$ complexes discussed here, except for $Cd(Ado)^{2+}$, Schneider *et al.*[20] provide stability constants based on UV spectrophotometric measurements, but considering that these workers had to carry out their evaluations nearly 30 years ago without modern computer facilities, it is not surprising that no error limits are given. On the other hand, for the present context, knowledge of approximate error limits is clearly desirable. Fortunately, Schneider *et al.*[20] published their differenc spectra (ΔA) in Fig. 4 and further experimental data ($1/\Delta A$) in Fig. 5 of Ref. 20, respectively; we evaluated these experimental data in various ways and thus estimated error limits. Hence, the stability constants published by Schneider *et al.*[20] are listed in the sixth column of Table IV togehter with our estimates for the error limits.

(ii) Another relatively comprehensive set of stability constants for $M(Ado)^{2+}$ complexes was published by Lönnberg and Arpalahti.[21] These authors determined by potentiometric pH titrations the stabilities of the corresponding Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} complexes, yet most log stability constants are given with only a single digit after the decimal point and the error limits are not clearly defined. However, these authors also list more principal experimental data (i.e., $pK^{H}_{H(Ado)}$ and $pK_{a(app)}$ plus the corresponding M^{2+} concentration) in Table I of their paper;[21] we now used these data with their error limits, evaluated them with Eq. (2) of

Ref. 16 (see also Ref. 17) and also calculated the error limits according to the error propagation after Gauss. As expected, the values for log $K^{M}_{M(Ado)}$ agree well with those given in Table II of Ref. 21; comparison of the error limits calculated now (2σ) with those given by the authors shows close agreement for the values of the Ni^{2+} and Cu^{2+} complexes; therefore, in all cases the error limits with the data of Ref. 21 now correspond to 2σ (Table IV, column six) according to our calculations.

5.3. Comparison of the Calculated Stability for $M(Ado)^{2+}$ Complexes with the Experimentally Determined Stability Constants

The error limits for the calculated equilibrium constants listed in columns three and four, and consequently also in row five of Table IV are relatively large. However, for the first time, values calculated on the basis of log K versus pK_a plots lead to results which are on the order of experimentally determined stability constants. Previous estimations for the stability constants of the adenosine complexes with Ni^{2+}, Cu^{2+} and Zn^{2+} were by far too high;[8,12] even though this problem had been recognized, the revised values (obtained by taking into account an estimate for the steric effect) generally were still somewhat too large.[14]

A more detailed comparison of the experimentally determined values for log $K^{M}_{M(Ado)}$ (last two columns of Table IV) with the calculated values (fifth column) shows that the constants for the $Co(Ado)^{2+}$, $Ni(Ado)^{2+}$, $Cu(Ado)^{2+}$, and $Zn(Ado)^{2+}$ complexes agree within the error limits. Despite the large error limits there is only poor agreement for the values of $Mn(Ado)^{2+}$ and $Cd(Ado)^{2+}$.

To conclude, evaluation of further, finer details has to await the advent of more precise stability data; e.g., the reference lines available for Mn^{2+} (cf. Table I) need to be based on more experimental data (see Ref. 14) and the stabilities of $Mn(Ado)^{2+}$ and $Cd(Ado)^{2+}$ should be redetermined. However, it is evident that the principle factors governing the stability of $M(Ado)^{2+}$ complexes are now understood. Indeed, the available data allow some further conclusions, also of a principle nature, as shown below.

6. ON THE DICHOTOMY OF METAL ION BINDING TO N-1 VERSUS N-7 IN METAL ION COMPLEXES OF ADENOSINE

6.1. Intrinsic N-1 to N-7 Binding Ratios

From the equilibrium constants listed in Table IV for metal ion binding to N-1 and N-7, i.e., log $K^{\mathrm{Ado}}_{\mathrm{M/N\text{-}1,ortho}}$ and log $K^{\mathrm{Ado}}_{\mathrm{M/N\text{-}7}}$, it should be possible to calculate the distribution of metal ions between the two binding sites of adenosine. Indeed, from Eq. (20) it is evident that the ratio R (or the dimensionless equilibrium constant K_I) for the intramolecular equilibrium (22)

$$\mathrm{M(N\text{-}7/Ado)^{2+}} \rightleftharpoons \mathrm{M(N\text{-}1/Ado)^{2+}} \tag{22}$$

may be calculated with Eq. (23):

$$R = K_I = \frac{[\mathrm{M(N\text{-}1/Ado)^{2+}}]}{[\mathrm{M(N\text{-}7/Ado)^{2+}}]} = \frac{K^{\mathrm{M}}_{\mathrm{M(N\text{-}1/Ado)}}}{K^{\mathrm{M}}_{\mathrm{M(N\text{-}7/Ado)}}} = \frac{K^{\mathrm{Ado}}_{\mathrm{M/N\text{-}1,ortho}}}{K^{\mathrm{Ado}}_{\mathrm{M/N\text{-}7}}}. \tag{23}$$

Considering the error limits of the available data (Table IV), the results listed in Table V can only be viewed as estimates. To mediate a feeling how the ratios of column four and the percentages in column five depend on the errors of the values listed in columns two and three, we used the lower and upper limits of the log $K^{\mathrm{Ado}}_{\mathrm{M/N\text{-}7}}$ values to again calculate with these "limiting constants" the values for columns four and five; these results are given in parentheses in the mentioned two columns.

However, despite the evident shortcomings several facts are clear: (i) For the first time values are listed for the $\mathrm{Mn^{2+}}$, $\mathrm{Co^{2+}}$, and $\mathrm{Cd^{2+}}$ systems; (ii) all the values given now are certainly more exact than those provided previously (for the $\mathrm{Ni^{2+}}$, $\mathrm{Cu^{2+}}$, and $\mathrm{Zn^{2+}}$ systems)[8,14]; (iii) there can be no doubt that equilibrium (22) truly exists.

6.2. Evaluation of Intrinsic Binding Ratios by the Use of Experimentally Measured Stability Constants

At this point one should mention that arguments against the above procedure and the results of Table V could be based on the fol-

TABLE V

Estimates for the intrinsic N-1 to N-7 binding ratios for $M(Ado)^{2+}$ complexes and percentages of the (N-7)-coordinated isomers, $M(N\text{-}7/Ado)^{2+}$, formed in the corresponding intramolecular equilibria.[a] It should be noted that the species $M(N\text{-}1/Ado)^{2+}$ and $M(N\text{-}7/Ado)^{2+}$ represent the complex formation occurring at N-1 or N-7, respectively, without a distinction between possibly occurring inner sphere and outer sphere coordination (see Section 7).[a]

M^{2+} or H^+	$\log K^{Ado}_{M/N\text{-}1,\text{ortho}}$ [b]	$\log K^{Ado}_{M/N\text{-}7}$ [c]	$R = \frac{[M(N\text{-}1/Ado)^{2+}]}{[M(N\text{-}7/Ado)^{2+}]}$ [d,e]	$\%M(N\text{-}7/Ado)^{2+}$ [a,e]
Mn^{2+}	−0.21 ± 0.20	−0.92 ± 0.23	5.13(8.71/3.02)	16(10/25)
Co^{2+}	−0.12 ± 0.23	0.25 ± 0.19	0.43(0.66/0.28)	70(60/78)
Ni^{2+}	−0.07 ± 0.17	0.44 ± 0.18	0.31(0.47/0.20)	76(68/83)
Cu^{2+}	0.22 ± 0.07	0.67 ± 0.22	0.35(0.59/0.21)	74(63/82)
Zn^{2+}	−0.29 ± 0.17	−0.33 ± 0.18	1.10(1.66/0.72)	48(38/58)
Cd^{2+}	0.14 ± 0.16	0.34 ± 0.18	0.63(0.95/0.42)	61(51/71)
H^+	3.61 ± 0.03[f]	−0.2 ± 0.3[g]	~6500	~0.02

[a] The related data for $H(Ado)^+$ are given for comparison. The percentages for the $M(N\text{-}1/Ado)^{2+}$ species follow from 100 − $\%M(N\text{-}7/Ado)^{2+}$.

[b] Values from the third column of Table IV.

[c] Values from the fourth column of Table IV.

[d] See Eq. (23).

[e] To provide a feeling for the error limits, the first value in the parentheses is calculated with the lower limit of $\log K^{Ado}_{M/N\text{-}7}$ and the second one with the corresponding upper limit.

[f] Ref. 23.

[g] See text in Section 4.1

lowing reasonings: The accuracy of the N-1 to N-7 ratios for binding of metal ions to adenosine depends on the legitimacy and accuracy of the log K versus pK_a plots (cf. Table I and Fig. 3). This in turn rests on both the reliability of the stability constants and upon the appropriateness of the plotted ligands as models for metal ion binding to adenosine (see Section 2 and Ref. 14), and this ligand appropriateness may further depend upon the metal ion. For example, crystal structures reveal hydrogen bond donation to the 6-oxo group in purines from water in the coordination sphere of a metal ion bound at N-7.[39] Indeed, heavy use is made of inosine and guanosine as representative model ligands in our analysis, especially for the baselines of N-7 type ligands (Section 2), and this analysis rests on the assumption that such hydrogen bonding with the 6-oxo group does not exist in aqueous solution or at least does not significantly strengthen complex stability. (In fact, to some extent this assumption is confirmed by the results shown in Fig. 2 and discussed in Section 4.2).

With this problem in mind we carried out a further analysis, which is analogous to a procedure employed previously for xanthosine complexes.[14] This analysis rests (i) only on the baseline of N-1 ligands, and as we have seen in the last paragraph of Section 2 in connection with the 9-methylpurine derivatives (Table II) this baseline is very well supported; (ii) the steric effect of the 6-NH$_2$ group on an N-1 metal ion coordination is also well defined by the results for the tubercidin complexes; hence, reliable N-1,ortho baselines exist (Section 2; Table II); and (iii) only p$K^{H}_{H(Ado)}$ = p$k^{H}_{H(N\text{-}1/Ado)}$ = 3.61 ($\pm$0.03) is needed, which is again well defined (Section 4). The crux of this analysis is that the experimentally measured values for $K^{M}_{M(Ado)}$ have to be employed; therefore, we restrict ourselves now to the adenosine systems with Ni^{2+}, Cu^{2+}, and Zn^{2+}, because only for these three metal ions three or more independent experimental values from different research groups exist allowing the calculation of a reasonably exact value for log $K^{M}_{M(Ado)exp}$ (see last column in Table IV).

It is evident that with Eqs. (13) through (18) of Table I and p$k^{H}_{H(N\text{-}1/Ado)}$ the stability constant log $K^{Ado}_{M/N\text{-}1,ortho}$ can be calculated; with this value and log $K^{M}_{M(Ado)exp}$ via Eq. (20c) values for log $K^{Ado}_{M/N\text{-}7}$ are obtained, and hence also the ratio R of Eq. (23) can again be calculated. These results are summarized in Table VI.

TABLE VI

Estimates for the intrinsic N-1 to N-7 binding ratios (Eq. (23)) for $M(Ado)^{2+}$ complexes and percentages of the (N-7)-coordinated isomers, $M(N\text{-}7/Ado)^{2+}$ (Eq. (22)), as calculated from the experimentally measured stability constants for the $M(Ado)^{2+}$ complexes and the baseline for N-1,ortho ligands (Table I) (see also Section 6.2 and Table IV)

M^{2+}	$\log K^{M}_{M(Ado)exp}$[a]	$\log K^{Ado}_{M/N\text{-}1,ortho}$[b]	$\log K^{Ado}_{M/N\text{-}7}$[c]	$R = \frac{[M(N\text{-}1/Ado)^{2+}]}{[M(N\text{-}7/Ado)^{2+}]}$[d]	$\%M(N\text{-}7/Ado)^{2+}$[d]
Ni^{2+}	0.32 ± 0.12	−0.07 ± 0.17	0.09 ± 0.23	0.69(0.41/1.17)	59(46/71)
Cu^{2+}	0.80 ± 0.12	0.22 ± 0.07	0.67 ± 0.16	0.35(0.25/0.51)	74(66/80)
Zn^{2+}	−0.17 ± 0.33	−0.29 ± 0.17	−0.79 ± 1.47	3.16(0.11/93.3)	24(1.1/90)

[a] From column 7 of Table IV.

[b] From column 3 of Table IV.

[c] Calculated via Eq. (20c).

[d] To provide a feeling for the error limits, the first value in the parentheses in column 5 is calculated with the upper limit of $\log K^{Ado}_{M/N\text{-}7}$ and the second one with the corresponding lower limit. In column 6 the first value in parentheses results from the lower and the second value from the upper limit.

As in part differences between rather similar values (which already carry a considerable error limit, especially in the case of the Zn^{2+} system) enter into the calculations, the error limits are rather large. However, despite this shortcoming it is reassuring to see that there is a reasonably close agreement with the values listed in Table V for R and % $M(N\text{-}7/Ado)^{2+}$.

6.3. Some Comments Regarding the N-1 to N-7 Binding Ratios

Considering the results of Tables V and VI, several conclusions may be drawn despite the crudeness of the results: It is clear that Ni^{2+} and Cu^{2+}, and most probably also Co^{2+} and Cd^{2+}, coordinate preferably to the N-7 site of adenosine; for Zn^{2+} a more even distribution, with a slight preference for N-1, appears to occur, while Mn^{2+} apparently prefers the N-1 site, which clearly is also overwhelmingly preferred by the proton.

Interestingly, the last two mentioned metal ions, i.e., Mn^{2+} and Zn^{2+}, form the lowest amount of macrochelates[6,7] with ATP^{4-} from all the transition metal ions considered here, including Zn^{2+} and Cd^{2+}. The formation degree of the closed species of $M(ATP)^{2-}$ equals 17 ± 10% for Mn^{2+} and 28 ± 7% for Zn^{2+}, and it should be recalled again that these macrochelates can only be formed with the N-7 site. Moreover, in each case about half of these macrochelates exist in the inner sphere and about half in the outer sphere form (see also Section 7).

7. CONCLUSIONS

In the whole discussion up to now the possible existence of outer sphere and inner sphere equilibria for the $M(Ado)^{2+}$ complexes has been ignored. In fact, there is evidence that both types of species may form at N-1 (cf. Ref. 15) as well as at N-7.[6,7] In connection with the N-1 coordination it was concluded[15] that outer sphere complexation, i.e., a water molecule of the metal ion coordination sphere is hydrogen bonded to the N-site, is especially favored for large metal ions and/or those with a relatively low affinity for N donor sites; this would favour outer sphere species for Cd^{2+}, which is large, and for Mn^{2+}, which prefers O donors.

For Cu^{2+} low amounts of outer sphere species are expected, and this is actually concluded for its N-1 as well as for its N-7 coordination.[6,7,15] It is clear that for a verification of such outer sphere–inner sphere equilibria more studies are needed, though for $Ni(Ado)^{2+}$ some information exists.[36] However, it should be emphasized that the N-1 to N-7 dichotomy described in this study is real and not influenced by the possible occurrence of outer sphere species, because in all cases considered here "overall" stability constants were employed which encompass *any* complex species, be it inner sphere or outer sphere.

Another point of interest in the present context is the recent evidence[14] that in certain $M(cytidine)^{2+}$ complexes the inhibitory effect of the *o*-amino group next to the metal ion binding site, i.e., N-3, is partly compensated for by a metal ion interaction with the *o*-carbonyl group, which is present on the other side of N-3 in the cytosine residue. Further studies on $M(cytidine)^{2+}$ complexes are on the way[40] and it appears that the size of the "inhibition and compensation" effects depends much on the kind of metal ion considered (3d ions, Zn^{2+} or Cd^{2+}).

Finally, it may be emphasized that binding of labile metal ions to single stranded nucleic acids can only be understood or predicted with reasonable security if more nucleoside systems are studied along the lines described here: Only in this way is the necessary background information becoming available which will allow judgment about the preferred binding sites at nucleic acids for each individual metal ion.

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24. In this connection one should mention that previously it had been concluded[12] that an interaction between the N-1 and N-7 sites in purines is reciprocal and depends upon whether a metal ion or proton is at the other site. For example, for guanosine the presence of a proton at N-1 or N-7 reduces the basicity of the other site by 2.0 log units. For inosine the corresponding proton–proton interaction is 2.2 log units.[12] For inosine the (Dien)Pd^{2+}–proton interaction difference is 1.5 log units and the (Dien)Pd^{2+}–(Dien)Pd^{2+} interaction difference is 1.0 log unit (Dien = diethylenetriamine = 1,4,7-triazaheptane). For adenosine the (Dien)Pd^{2+}–proton interaction difference is 1.9 log units and the (Dien)Pd^{2+}–(Dien)Pd^{2+} interaction difference is 1.0 log unit.[25] These latter values suggest that the N-1/N-7 interaction is apparently more sensitive in adenosine than in inosine. These mentioned interaction differences have been used earlier to estimate a value for $pk^{H}_{H(N\text{-}7/Ado)}$ in the following way.[12] Based on the $1.9 - 1.5 = 0.4$ larger (Dien)Pd^{2+}–proton interaction difference in adenosine, 0.4 log unit was added to the inosine proton–proton interaction, $0.4 + 2.2 = 2.6$, to obtain 2.6 log units as an estimate for the proton–proton interaction difference in adenosine. To estimate $pk^{H}_{H(N\text{-}7/Ado)}$ for protonation

of N-7 when N-1 is *not* protonated, the mentioned interaction difference was added to the $pK^{H}_{H_2(Ado)}$ value for protonation of N-7 with N-1 protonated. Two independent determinations by ultraviolet difference spectroscopy in sulfuric acid solutions yielded[12,26] $pK^{H}_{H_2(Ado)} = -1.6$, leading to the intrinsic acidity of the H^+(N-7) site in $H(Ado)^+$ as $pk^{H}_{H(N\text{-}7/Ado)} = 2.6 - 1.6 = 1.0$ (or 1.1 with later rounding).[12] Unfortunately, this result is in poor agreement with $pk^{H}_{H(N\text{-}7/Ado} = -0.2 \pm 0.3$ estimated above in Section 4.1. Moreover, use of $pk^{H}_{H(N\text{-}7/Ado)} = 1.1$ for the intrinsic acidity of the H^+(N-7) site in $H(Ado)^+$ in log K versus pK_a plots leads to predictions of stability constants that are significantly larger than the experimentally observed values.[8,12] Since this result is unacceptable, there must be a flaw in the line of reasoning. Most probably the proton–proton interaction difference of 2.6 log units in adenosine is overestimated. As in the above reasonings much rests on comparisons with the $(Dien)Pd^{2+}$ unit, it appears that this is not a suitable basis, possibly because of π interactions of Pd^{2+}. Finally one may add that use of $pK^{H}_{H_2(Ado)} = -1.6$ (Refs. 12 and 26) and of $pk^{H}_{H(N\text{-}7/Ado)} = -0.2$ (Section 4.1) gives an N-1 to N-7 proton–proton interaction difference of 1.4 log units. Hence, by also taking into account the results for xanthosine[14] this interaction difference *decreases* in the series, xanthosine (2.3) $\gtrsim$ inosine (2.2) $\gtrsim$ guanosine (2.0) > adenosine (1.4). In approximately the same order *increases* the aromatic character of the purine system in these nucleosides, which is especially pronounced in the pyrimidine ring of the adenine residue,[27] and in accord herewith has the guanosine moiety a considerably stronger dipole moment than the adenine residue.[28]

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