Ambivalent Metal Ion Binding Properties of Cytidine in Aqueous Solution

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Received May 27, 1992

The acidity constant of H(cytidine)$^+$ and the stability constants of the 1:1 complexes formed between Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} and cytidine (Cyd) were determined by potentiometric pH titration in aqueous solution (I = 0.5 M ($NaNO_3$); 25 °C); some of the equilibrium constants were also independently measured by UV spectrophotometry. Comparison of the results with previously established reference lines (log K versus pK_a) for *o*-aminopyridine-like ligands allows quantification of the coordination tendency, i.e. of the stability-enhancing effect of the 2-carbonyl group in these M(Cyd)$^{2+}$ complexes, which increases within the following series: Co^{2+} ~ Ni^{2+} (no effect) < Mn^{2+} ~ Zn^{2+} (ca. 0.25 log unit) < Cd^{2+} (~0.55) < Cu^{2+} (~1.05). A positive effect is also observable for the M(Cyd)$^{2+}$ complexes of Mg^{2+} and Ca^{2+}. However, in most instances the coordination tendency of the *o*-carbonyl group is *not* able to compensate completely for the steric inhibitory effect of the *o*-amino group; hence, the stability differences between the experimental values and the calculated ones for a corresponding simple pyridine-like binding site are negative in most cases. The solution structures of the various M(Cyd)$^{2+}$ complexes are discussed in connection with available crystal structures and it is concluded that in Co(Cyd)$^{2+}$ and Ni(Cyd)$^{2+}$ the 2-carbonyl group does not participate to any appreciable extent in metal ion binding; in the M(Cyd)$^{2+}$ systems with Zn^{2+}, Cd^{2+}, or Cu^{2+}, at least in equilibrium, chelates involving N-3 and a weaker bound O-2 are formed (these could be four-membered chelates such as in the solid state, but also a metal ion bound water molecule might participate in aqueous solution and then six-membered chelates would result from the partial outer-sphere coordination, a binding type sometimes also addressed as semichelation). In contrast, the stabilities of the M(Cyd)$^{2+}$ complexes with Mn^{2+}, Mg^{2+}, and Ca^{2+} are apparently to a large part determined by the metal ion affinity of O-2. It is further concluded that stability determinations of this type should eventually allow the mapping of the relative metal ion affinities of the various binding sites present in nucleic acids.

The cytosine residue occurs in nucleotides like CMP^{2-}, CDP^{3-}, and CTP^{4-},[2,3] as well as in DNA and RNA.[2] All these substances are also ligands for metal ions, which indeed affect their biological action. Therefore, an understanding of the interplay between metal ions and cytidine, i.e. 1-(β-D-ribofuranosyl)cytosine (Cyd), will help to rationalize the effects of metal ions on the mentioned polydentate ligands.

Regarding the coordinating properties of cytidine (see Figure 1),[4-6] at least three questions arise: (i) Does N-3 show the properties of a pyridine-like binding site? (ii) Does the neighboring *o*-amino group at C-4 exercise an inhibitory effect on the stability of M(Cyd)$^{n+}$ complexes? (iii) What is the role of the carbonyl oxygen at C-2? For different metal ions somewhat different answers are to be expected. To obtain material for comparisons we studied now the stability of the M(Cyd)$^{2+}$ complexes of Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}.

It is well known that for series of structurally related ligands (L) plots of log K^{M}_{ML} versus pK^{H}_{HL} result in straight lines.[7,8] Such reference or base lines have recently been established for pyridine-like ligands and most of the mentioned metal ions.[9] Moreover, our recent work with tubercidin (see Figure 1)[10] and adenosine[11] has led to a quantification of the inhibitory effect of an *o*-amino group next to a pyridine-like nitrogen for the complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}.

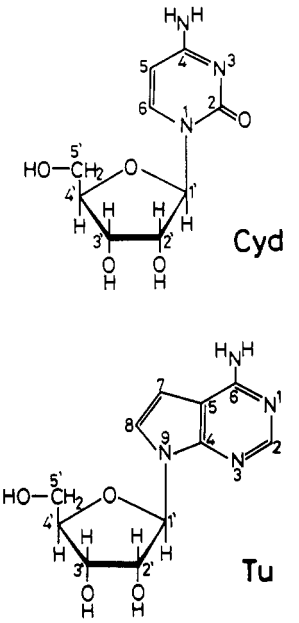

Figure 1. Chemical structures of cytidine (Cyd) and tubercidin (Tu = 7-deazaadenosine), shown in their dominating *anti* conformation.[4-6]

(1) Work done at the University of Basel during leaves from the University of the Ryukyus, Okinawa, Japan (Y.K.), and the Zhongshan University, Guangzhou, People's Republic of China (L.J.).
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(3) Abbreviations: A, optical absorption; Bpy, 2,2'-bipyridyl; CMP^{2-}, CDP^{3-}, and CTP^{4-}, cytidine 5'-mono-, -di-, and -triphosphate; Cyd, cytidine; L, general ligand; M, general metal ion; M^{2+}, divalent metal ion; Py, pyridine; Py-N, pyridine-like N ligand; Py-N,ortho, *o*-amino pyridine-like N ligand; Tu, tubercidin (=7-deazaadenosine).
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0020-1669/92/1331-5588$03.00/0

With the corresponding straight-line equations[9,11] for all of these log K^{M}_{ML} versus pK_a plots and the acidity constant, $K^{H}_{H(Cyd)}$, of monoprotonated cytidine, it will be possible to calculate the stability of the complexes for a simple pyridine-like coordination, as well as for a coordination in which the effect of the *o*-amino group is taken into account. Hence, comparison of these two calculated stability constants with the experimentally determined stability constant, $K^{M}_{M(Cyd)}$, should then for each metal ion allow conclusions regarding the effects of the *o*-amino group and the *o*-carbonyl oxygen on the stability of the complex formed with N-3 of cytidine. It is the aim of this study to carry out such an evaluation.

Experimental Section

Materials. Cytidine (Sigma Grade) was from Sigma Chemical Co., St. Louis, MO. The disodium salt of ethylene-*N,N,N',N'*-tetraacetic acid (Na_2H_2EDTA), potassium hydrogen phthalate, HNO_3, $HClO_4$, NaOH (Titrisol), $NaClO_4$, and the nitrate salts of Na^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} (all pro analysi) were from Merck AG, Darmstadt, FRG. The perchlorate salt of Cd^{2+} was from Johnson Matthey GmbH, Alfa Products, Karlsruhe, FRG, and that of Co^{2+} was from Fluka AG, Buchs, Switzerland. All solutions were prepared with distilled CO_2-free water.

The exact concentrations of the cytidine solutions were measured by titrations with NaOH. Its titer was determined with potassium hydrogen phthalate, and the concentrations of the stock solutions of the metal ions were established with EDTA.

Potentiometric pH Titrations. The pH titrations were carried out with a Metrohm E536 potentiograph, E655 dosimat, and 6.0202.100 (JC) combined, single-junction, macro glass electrodes. The buffer solutions (pH 4.64, 7.00, and 9.00; based on the scale of the U.S. National Bureau of Standards) used for calibration were also from Metrohm AG, Herisau, Switzerland. The direct pH meter readings were used in the calculations of the acidity constants; i.e., these constants are so-called practical, "mixed", or Brønsted constants.[12] Their negative logarithms given for aqueous solutions at I = 0.5 M and 25 °C may be converted into the corresponding concentration constants by subtracting 0.03 log unit;[12] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity coefficient.[12,13] No conversion is necessary for the stability constants of metal ion complexes.

The acidity constant $K^{H}_{H(Cyd)}$ of N-3 protonated cytidine was determined by titrating 50 mL of aqueous 1.80 mM HNO_3 (I = 0.5 M ($NaNO_3$); 25 °C) in the presence and absence of 1 mM cytidine under N_2 with 1 mL of 0.10 M NaOH and by using the differences in NaOH consumption between such a pair of titrations for the calculations. $K^{H}_{H(Cyd)}$ was calculated from 24 independent titration pairs by taking into account the species H^+, Cyd, and $H(Cyd)^+$ and by using a curve-fit procedure with a Newton-Gauss nonlinear-least-squares program within the pH range determined by about 3% and 97% neutralization for the equilibrium $H(Cyd)^+$/Cyd with a Hewlett-Packard Vectra 60PC MS-DOS desk-computer connected with a Brother M-1509 printer and a Graphtec MP 3100 plotter.[14]

The stability constants $K^{M}_{M(Cyd)}$ of the $M(Cyd)^{2+}$ complexes were measured under the same conditions used for the pairwise acidity-constant titrations, but $NaNO_3$ was partially or fully replaced by $M(NO_3)_2$. For most M^{2+} systems (i.e., Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}) titrations were made with $[M^{2+}]$ = 0.1667 M (i.e., Cyd:M^{2+} = 1:167) and 0.1333 M (Ni^{2+}, Cu^{2+}, Zn^{2+}/Cyd:M^{2+} = 1:133); for Cu^{2+} and Cd^{2+} also conditions with $[M^{2+}]$ = 0.0833 M (i.e., Cyd:M^{2+} = 1:83) were used. Each final value for $K^{M}_{M(Cyd)}$ is the result of at least eight independent pairs of titrations which were carried out independently (about half and half) by two persons.

Several of the $M(Cyd)^{2+}$ complexes are rather unstable; i.e., the depression of the buffer region of $H(Cyd)^+$/Cyd by the addition of M^{2+} is very small. Therefore,[10] the stability constants $K^{M}_{M(Cyd)}$ were calculated in the following way: The curve-fitting procedure described above for $K^{H}_{H(Cyd)}$ was also used to determine an *apparent* acidity constant, K'_a, for the deprotonation of $H(Cyd)^+$ in the presence of a large (and hence during the titration constant) excess of M^{2+}. The data used for the calculation of K'_a again covered the pH range between about 3% and 97% neutralization where possible; of course, the data collection was not carried into the pH range in which hydrolysis of $M(aq)^{2+}$ occurs, which was evident from the titrations in the absence of cytidine. Values for $K^{M}_{M(Cyd)}$ (Table II) were calculated[10,15] with eq 1.

$$K^{M}_{M(Cyd)} = (K'_a - K^{H}_{H(Cyd)})/(K^{H}_{H(Cyd)}[M^{2+}]_{tot}) \quad (1)$$

Spectrophotometric Measurements. The acidity constant $K^{H}_{H(Cyd)}$ was also determined by spectrophotometry. The UV spectra ([Cyd] = 0.025 mM) were recorded between 190 and 350 nm with Perkin Elmer Lambda 2 and Cary 219 instruments in aqueous solutions at 25 °C and I = 0.5 M ($NaClO_4$) with 1-cm quartz cells. The pH of the solution was adjusted by dotting with relatively concentrated $HClO_4$ or NaOH and measured with a Metrohm 605 digital pH meter using a Metrohm 6.0202.100 (JC) glass electrode. The spectrophotometric data were analyzed with the above-mentioned Hewlett-Packard Vectra desk-computer as described earlier[10] (following also the procedure given for NMR data[6]). The spectra measured for solutions with [Cyd] = 0.025 mM were evaluated at 240 and 280 nm where $H(Cyd)^+$ has a minimum and a maximum in its absorption spectrum, respectively; in addition the absorption difference between 280 and 240 nm was evaluated. An example of an experimental series with its evaluated data is shown in Figure 2 (vide infra). The final result (Table I) is the average of four independent series of experiments with, in total, 12 evaluations at the wavelengths or absorption differences mentioned above.

The stability constants $K^{M}_{M(Cyd)}$ of $Co(Cyd)^{2+}$ and $Cd(Cyd)^{2+}$ were also determined by recording UV difference spectra between 190 and 350 nm on the two mentioned instruments. For each series of experiments the pH was set close to 6 by adjusting the cytidine solution first to such an alkaline pH that after mixing with the metal perchlorates a pH somewhat higher than the desired one was reached; then, by using (only) $HClO_4$ the final pH was adjusted (which was identical in both cells used in a measurement). In the Cd^{2+}/Cyd system, the 1-cm quartz cell in the reference beam contained [Cyd] = 0.1 mM; the cell in the sample beam contained in addition $[Cd(ClO_4)_2]$ = 0.05–0.36 M. To both cells $NaClO_4$ was added to maintain I at least at 0.5 M (25 °C); it is evident that in some instances I was larger, i.e. up to 1.1 M. In the Co^{2+}/Cyd system the same experimental arrangement was employed but 1-mm quartz cells were used with [Cyd] = 1 mM and $[Co(ClO_4)_2]$ = 0.1–0.6 M (i.e., I = 0.5–1.8 M). The difference spectra for Cd^{2+}/Cyd showed a maximum at 285 nm and a minimum at 250 nm; both were independently evaluated, as well as their difference, i.e. $\Delta\Delta A = \Delta A_{285} - \Delta A_{250}$. The difference spectra for Co^{2+}/Cyd had a maximum at 267 nm and minima at 247 and 290 nm; evaluated were the differences $\Delta\Delta A = \Delta A_{267} - \Delta A_{247}$ and $\Delta A_{267} - \Delta A_{290}$. The perchlorate salts have practically no absorption at these wavelengths. The stability constants were calculated by plotting the measured UV-absorption differences in dependence on $[M^{2+}]_{tot}$ (cf. Figure 3; vide infra). These spectrophotometric data were analyzed for the corresponding apparent constant, K_{app} (and ΔA_{max} or $\Delta\Delta A_{max}$; Figure 3), with the above-mentioned desk-computer by a curve-fit procedure with a Newton–Gauss nonlinear-least-squares program in a manner analogous to that described previously[16] for ^{1}H-NMR shift data.

The constants determined in this way for the M^{2+}/Cyd systems are *apparent* constants, K_{app}, which are valid *only* at the pH of the experiment (see Figure 3). They become independent of pH if one considers the protonation of N-3 of cytidine, which is different for each set of experiments. The competition of the proton is taken into account[10,17] with eq 2 (by using $pK^{H}_{H(Cyd)}$ = 4.24). In the present instances the corrections according to eq 2 are very small because all experiments were done at pH ≥ 5.90 where Cyd is largely deprotonated. The final results

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(14) In the described calculation procedure the ionic product of water (K_w) and the hydrogen ion activity (γ) [to be more exact: the mentioned "combined" term of 0.03 log unit for converting the measured data into H^+ concentration] do *not* enter into the calculations, because we evaluate the *differences* in NaOH consumption between two corresponding solutions; i.e., always solutions with and without ligand are titrated (see above). The advantage of this procedure is (aside from not needing K_w and "γ" values) that impurities in the solvent or in the salts, as well as systematic errors, etc., cancel (for details see ref 12).
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$$\log K^{M}_{M(Cyd)} = \log K_{app} + \log (1 + [H^+]/K^{H}_{H(Cyd)}) \quad (2)$$

given in the Results, section 2, are the weighted means with their relative errors (3σ) of three independent series of experiments with, in total, 10 evaluations for $Cd(Cyd)^{2+}$ and of four series with, in total, eight evaluations for $Co(Cyd)^{2+}$. The experiments for these M^{2+}/Cyd systems were difficult to carry out, as the spectral alterations are small and therefore the error limits of the results are rather large.

Results and Discussion

Self-stacking is a well-known phenomenon for purines and pyrimidines[18] and must therefore be considered in any measurements designed for the quantification of monomeric species.[19] Fortunately, the self-association tendency of cytidine is low; for equilibrium 3 the association constant $K = 1.4 \pm 0.5$ M^{-1}.[20] As

$$(Cyd)_n + Cyd \rightleftharpoons (Cyd)_{n+1} \quad (3)$$

the cytidine concentrations used in this study were throughout $\leq 10^{-3}$ M, any formation of dimers or larger associates is negligible;[18-20] in fact, monomeric cytidine dominates with 99.7% or more, and therefore all the following results apply to monomeric cytidine.

1. Acidity Constants of Monoprotonated Cytidine. Cytidine (see Figure 1) has only N-3 as a basic site,[4] which may be protonated to $H(Cyd)^+$. No indication for any further protonation was observed down to pH 2.7. Neutral cytidine may release a proton, i.e. from the ribose residue,[21] but this reaction occurs only with pK_a 12.5 and does not play a role in the physiological pH range. Hence, in the present context and in the pH range 2.5 to 11.5, only equilibrium 4 needs to be considered.

$$H(Cyd)^+ \rightleftharpoons Cyd + H^+ \quad (4a)$$

$$K^{H}_{H(Cyd)} = [Cyd][H^+]/[H(Cyd)^+] \quad (4b)$$

The acidity constant for $H(Cyd)^+$ was determined by potentiometric titrations and UV spectrophotometry; Figure 2 shows an example with its evaluation for the latter method. The results are summarized in Table I, together with the corresponding data for $H(tubercidin)^+$ and $H(pyridine)^+$.[22,23]

The constants obtained by both methods at $I = 0.5$ M agree well (Table I), despite the large difference in the concentrations of cytidine employed in the experiments, i.e. 10^{-3} and 2.5×10^{-5} M, respectively. This confirms the above conclusion that any self-association is negligible. The present values for $pK^{H}_{H(Cyd)}$ also agree excellently with various previous results: e.g., 4.15 ± 0.02 ($I = 0.1$ M; 25 °C),[24] 4.13 ± 0.03 ($I = 0.1$ M; 25 °C),[25] 4.23 ± 0.01 ($I = 1$ M ($NaNO_3$); 20 °C),[26] and 4.21 ± 0.04 ($I = 1$ M; 25 °C).[24]

Comparison of the results in Table I shows that the deprotonation of $H(Cyd)^+$ is affected by the ionic strength as is common for nitrogen sites: a change from $I = 0.1$ to $I = 0.5$ M increases the basicity for the three examples on average by about 0.08 log unit. The difference of about 0.19 log unit between the acidity

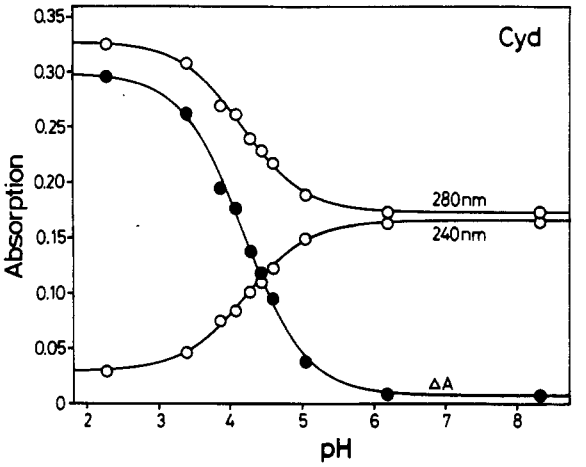

Figure 2. Evaluation of the dependence of the UV absorption of cytidine at 240 and 280 nm and of the UV absorption difference $\Delta A = A_{280} - A_{240}$ on pH in aqueous solution (measured in 1-cm cells; [Cyd] = 0.025 mM; $I = 0.5$ M ($NaClO_4$); 25 °C) by plotting the absorption versus pH. The solid curves represent the computer-calculated best fits of the experimental data points at pH 2.270, 3.389, 3.869, 4.078, 4.278, 4.434, 4.592, 5.045, 6.186, and 8.316 (from left to right), which leads for this experimental series to $pK^{H}_{H(Cyd)} = 4.23 \pm 0.08$ (3σ), 4.17 ± 0.09, and 4.20 ± 0.08 for the evaluations of 240 nm, 280 nm, and ΔA, respectively, for the deprotonation of $H(Cyd)^+$ (see also Experimental Section).

Table I. Negative Logarithms of the Acidity Constants (Eq 4) in Aqueous Solution for Monoprotonated Cytidine (Cyd),[a,b] Tubercidin (Tu = 7-Deazaadenosine),[c] and Pyridine (Py)[d,e] at 25 °C and Various Ionic Strengths (I)

I, M	$pK^{H}_{H(Cyd)}$	$pK^{H}_{H(Tu)}$	$pK^{H}_{H(Py)}$
0.1	$4.14 \pm 0.02^{b,f}$	$5.21 \pm 0.03^{c,f}$	$5.26 \pm 0.02^{d,e}$
0.5	$4.24 \pm 0.02^{a,f}$	$5.29 \pm 0.02^{c,f}$	5.34 ± 0.02^{d}
0.5	$4.20 \pm 0.04^{a,g}$	$5.30 \pm 0.04^{c,g}$	

[a] This work. Here and in refs 9 and 10 so-called "practical" constants are given; see Experimental Section. For all these constants the error limits given are *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] From ref 9. [c] From ref 10. [d] The concentration constants listed in ref 23, $pK^{H}_{H(Py)} = 5.24 \pm 0.02$ at $I = 0.1$ M and 5.31 ± 0.02 at $I = 0.5$ M, were transformed into the "practical" acidity constants given above by adding 0.02 and 0.03 log units, respectively; for details see ref 12. [e] From ref 22. [f] Determined by potentiometric pH titrations with $NaNO_3$ as background electrolyte. [g] Determined by UV spectrophotometry (see also Figure 2) with $NaClO_4$ as background electrolyte.

constant of the zwitterionic $H_2(CMP)^{\pm}$ species ($pK^{H}_{H_2(CMP)} = 4.33 \pm 0.04$; $I = 0.1$ M ($NaNO_3$); 25 °C)[27] for deprotonation at the H^+(N-3) site and the value for $H(Cyd)^+$ ($pK^{H}_{H(Cyd)} = 4.14 \pm 0.02$; cf. Table I) is also on the order expected[10] for the inhibitory effect of a relatively distant negatively charged 5′-$PO_2(OH)^-$ group as present in $H_2(CMP)^{\pm}$.

2. Stability Constants of Some M(Cytidine)$^{2+}$ Complexes. The experimental data from the potentiometric pH titrations may be completely described by considering equilibria 4 and 5,

$$M^{2+} + Cyd \rightleftharpoons M(Cyd)^{2+} \quad (5a)$$

$$K^{M}_{M(Cyd)} = [M(Cyd)^{2+}]/([M^{2+}][Cyd]) \quad (5b)$$

provided the evaluation of the data is not carried into the pH range where hydroxo complexes form. The stability constants determined according to equilibrium 5a are collected in column 2 of Table II; columns 3 and 4 contain the constants for the related M(tubercidin)$^{2+}$ and M(pyridine)$^{2+}$ complexes.[28-31] The

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Table II. Logarithms of the Stability Constants, log $K^{M}_{M(Cyd)}$, of Some $M(Cyd)^{2+}$ Complexes (Eq 5) As Determined by Potentiometric pH Titrations in Aqueous Solutions at 25 °C and I = 0.5 M ($NaNO_3$),[a,b] Together with the Stability Constants log $K^{M}_{M(Tu)}$ and log $K^{M}_{M(Py)}$ of the Corresponding Complexes of Tubercidin (Tu)[c] and Pyridine (Py), Respectively[b]

M^{2+}	log $K^{M}_{M(Cyd)}$ [a,b]	log $K^{M}_{M(Tu)}$ [b,c]	log $K^{M}_{M(Py)}$
Ca^{2+}	0.18 ± 0.06	0.09 ± 0.14	<0.1[d]
Mg^{2+}	0.12 ± 0.04	−0.01 ± 0.22	<0.4[d]
Mn^{2+}	0.19 ± 0.08	0.23 ± 0.16	0.19 ± 0.10[e]
Co^{2+}	0.03 ± 0.08	0.22 ± 0.12	1.25 ± 0.02[f,g]
Ni^{2+}	0.14 ± 0.12	0.33 ± 0.07	1.87 ± 0.01[f,g]
Cu^{2+}	1.56 ± 0.06	1.06 ± 0.08	2.49 ± 0.02[f,g]
Zn^{2+}	0.20 ± 0.11	0.33 ± 0.10	1.00 ± 0.03[f,g]
Cd^{2+}	0.91 ± 0.07	0.70 ± 0.14	1.32 ± 0.10[h]

[a] This work. [b] The error limits given are *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger, if nothing else is mentioned. [c] From ref 10; I = 0.5 M ($NaNO_3$); 25 °C. [d] These values are close to the stability constants of the M(2,2′-bipyridyl)$^{2+}$ complexes[31] (cf. also ref 24) of Mg^{2+} (log $K^{Mg}_{Mg(Bpy)}$ = 0.32) and Ca^{2+} (log $K^{Ca}_{Ca(Bpy)}$ = −0.05) at I = 0.25 M and 25 °C, and they are considered as the upper limits for the stability constants of the corresponding M(pyridine)$^{2+}$ complexes. It may be added that the acidity constant of H(2,2′-bipyridyl)$^{+}$ (p$K^{H}_{H(Bpy)}$ = 4.53 ± 0.05; I = 0.5 M; 25 °C)[24] is between those for $H(Cyd)^{+}$ and $H(Py)^{+}$ (cf. Table I). [e] Average of two values listed in refs 28 and 29 (see also ref 9); the error limit is an estimate; I = 0.5 M; 25 °C. [f] From ref 22; I = 0.1 M ($NaNO_3$); 25 °C. [g] A view on the constants given in the literature[23,28-30] shows that the influence of ionic strength (and temperature) is small; this agrees also with the conclusion given in the last paragraph of section 3. [h] Average (with 3σ) of 14 values[9] listed in refs 23 and 28–30; I = 0.1–1 M; 20–30 °C.[g]

relatively large error limits for the log $K^{M}_{M(Cyd)}$ values listed are not surprising as the stability of the $M(Cyd)^{2+}$ complexes is low and therefore the depression of the buffer region of $H(Cyd)^{+}$/Cyd upon complex formation is very small: e.g., with Co^{2+} it corresponds only to about 0.07 log unit while in the most favorable case with Cu^{2+} a depression of about 0.77 log unit is reached. From eq 1 it is clear that any experimental error will become the more serious the more similar the acidity constants K'_a and $K^{H}_{H(Cyd)}$ are (see Experimental Section).

The stability of $Co(Cyd)^{2+}$ and $Cd(Cyd)^{2+}$ has also been determined by recording difference spectra (see Experimental Section) as the UV absorption spectrum of cytidine is altered by the presence of divalent metal ions (Figure 3). The weighted means (3σ) of several experiments are as follows (I = 0.5 M ($NaClO_4$); 25 °C): log $K^{Co}_{Co(Cyd)}$ = 0.05 ± 0.28, and log $K^{Cd}_{Cd(Cyd)}$ = 0.80 ± 0.24. The error limits of these two values are large because the spectral alterations due to coordination of M^{2+} to N-3 of cytidine are small (see Figure 3), and therefore this type of measurement is rather prone to errors. However, the constants for the two $M(Cyd)^{2+}$ complexes studied independently by spectrophotometry and potentiometric pH titrations (Table II) agree within their error limits; in particular, the *difference* in stability between $Co(Cyd)^{2+}$ and $Cd(Cyd)^{2+}$ is confirmed, an observation important for the interpretation of these results (see sections 6 and 7).

3. Comparison of the Present M(Cytidine)$^{2+}$ Complex Stabilities with Previous Determinations and Other Related Data. From column 2 of Table II it is evident that the Irving–Williams

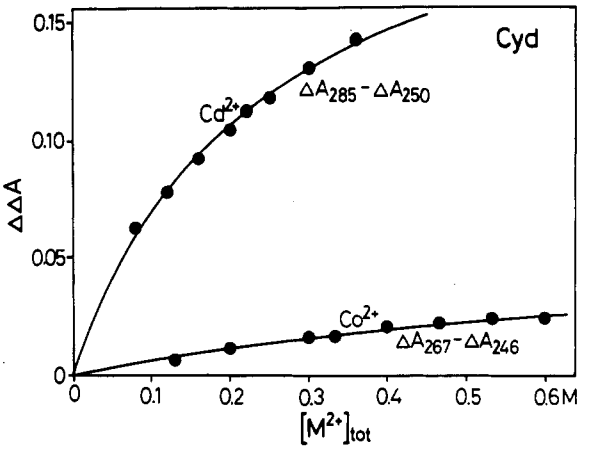

Figure 3. Evaluation of the UV absorption difference spectra for cytidine ([Cyd] = 0.1 mM; 1-cm cells) via the differences $\Delta\Delta A = \Delta A_{285} - \Delta A_{250}$ in dependence on the total concentration of $Cd(ClO_4)_2$ in aqueous solution at pH 5.93 (I = 0.5–1.1 M; 25 °C) for log K_{app} and $\Delta\Delta A_{max}$. The solid curve labeled with Cd^{2+} represents the computer-calculated best fit of the experimental data points (see Experimental Section) which leads to K_{app} = 4.23 ± 0.331 (1σ) and $\Delta\Delta A_{max}$ = 0.234 ± 0.009 (1σ); hence, according to eq 2, log $K^{Cd}_{Cd(Cyd)}$ = (0.63 ± 0.034) + 0.009 = 0.64 ± 0.10 (3σ) follows. A corresponding series of experiments is shown for the cytidine/$Co(ClO_4)_2$ system ([Cyd] = 1 mM; 1-mm cells) at pH 5.90 (I = 0.5–1.8 M; 25 °C) which was evaluated for $\Delta\Delta A = \Delta A_{267} - \Delta A_{246}$. The computer-calculated best fit of the data leads to K_{app} = 1.11 ± 0.325 (1σ) and $\Delta\Delta A_{max}$ = 0.064 ± 0.013 (1σ); hence, according to eq 2, log $K^{Co}_{Co(Cyd)}$ = (0.05 ± 0.128) + 0.009 = 0.06 ± 0.38 (3σ) follows. The final results given in the text in section 2 for log $K^{Cd}_{Cd(Cyd)}$ and log $K^{Co}_{Co(Cyd)}$ are the weighted means (including the relative errors) of several experiments and their evaluations (see Experimental Section).

series[17,32] is not strictly followed: the stability of the $M(Cyd)^{2+}$ complexes with Ca^{2+}, Mn^{2+}, and Mn^{2+} is somewhat too high,[17] an observation which may be related to the presence of the 2-carbonyl group (see section 7). However, overall the characteristic trends[17] of nitrogen donor ligands are observed; i.e., there are relatively large stability differences between the $M(Cyd)^{2+}$ complexes with Cu^{2+} and Mn^{2+} or Cu^{2+} and Zn^{2+} (each about 1.35 log units).

Comparison of the structures shown in Figure 1 for cytidine and tubercidin shows *o*-amino pyridine-type binding sites for both ligands, except that cytidine contains in addition an *o*-carbonyl group. The influence of this latter group is evident because in some instances the $M(Cyd)^{2+}$ complexes are by about 0.1–0.5 log unit more stable than the corresponding $M(Tu)^{2+}$ complexes (Table II) despite the lower basicity of cytidine by about 1 log unit (see Table I). Further details of the effect of this *o*-carbonyl group on complex stability are discussed in sections 5–7.

Regarding the following discussion on the reliability of several published stability constants for $M(Cyd)^{2+}$ complexes, it is important to note that the $M(Cyd)^{2+}$ complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} are by about 0.4–1.7 log units *less* stable than the corresponding $M(Py)^{2+}$ complexes (see Table II). This observation is expected because cytidine is by about 1 log unit less basic than pyridine and the access to the N site in the latter simple ligand is also not inhibited by an ortho substituent. In the case of the low-stability complexes of Mn^{2+}, Mg^{2+}, and Ca^{2+}, the stabilities of $M(Py)^{2+}$ and $M(Cyd)^{2+}$ are of about the same order.

Unfortunately, the agreement between the present results (Table II) and most previously published data is poor: the constants given in ref 33 for the $M(Cyd)^{2+}$ complexes of Mg^{2+} (log $K^{M}_{M(Cyd)}$ = 2.53), Ca^{2+} (2.56), Mn^{2+} (2.60), Co^{2+} (2.69), Ni^{2+} (3.03), Cu^{2+} (3.26), and Zn^{2+} (2.82) are about 1.7–2.9 log

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units too large compared to our results. These values[33] exceed even those for $M(Py)^{2+}$ complexes (Table II) by about 0.8–2.4 log units, and this is clearly *not* possible. These enormous discrepancies cannot be attributed to the different experimental conditions (45 °C; I = 0.1 M (KNO_3));[33] instead it appears, on the basis of the experimental part in ref 33, that the hydrolysis of $M(aq)^{2+}$ ions was neglected. In addition at $[M^{2+}] = [Cyd] = 10^{-3}$ M, as used in ref 33, no significant depression of the buffer region of $H(Cyd)^+/Cyd$ can be obtained (see section 2), and therefore measurements result in artifacts. Similarly, the constants given in refs 34 and 35 (35 °C, I = 0.1 M (KNO_3)) for the same $M(Cyd)^{2+}$ complexes are about 1.6–2.8 log units too large compared to our results. Consequently, all the information and conclusions provided in refs 34–36 about mixed ligand complexes involving the mentioned metal ions and cytidine are obscure and have to be rejected; most probably this applies also to the binary and ternary complexes involving lanthanide ions and cytidine.[36,37]

The stability constants given in ref 38 for the $M(Cyd)^{2+}$ complexes with Co^{2+} (log $K^{M}_{M(Cyd)}$ = 0.88), Ni^{2+} (0.97), Cu^{2+} (2.06), and Zn^{2+} (0.76) (35 °C; I = 0.1 M (KNO_3)) are partially similar to values in ref 39 (21 °C; I = 0.1 M ($NaClO_4$)) and are closer to the present results (Table II) than those mentioned above, but they are still about 0.5–0.85 log units too large. This is confirmed by a comparison with the stability constants of the corresponding $M(Tu)^{2+}$ and $M(Py)^{2+}$ complexes in Table II if one takes into account the higher basicity of tubercidin and pyridine compared with that of cytidine (about 1 log unit; see Table I); this conclusion is further supported by an application of the reference-line equations (Table III) discussed in sections 4–6.[40–45] The only stability constants that are available in the literature to our knowledge and which agree well with the present results are from early work by Martin[46] and by Fiskin and Beer,[26] as well as from our own laboratory.[9] All regard the stability of the $Cu(Cyd)^{2+}$ complex;[23,29] the values for log $K^{Cu}_{Cu(Cyd)}$ are 1.4 (I = 0.16 M; 25 °C),[46] 1.59 ± 0.07 (I = 1 M ($NaNO_3$); 20 °C),[26] and 1.56 ± 0.06 (I = 0.1 M ($NaNO_3$); 25 °C).[9] The last two values together with the result given in Table II confirm the expectation for uncharged ligands[47] that the stability of their complexes is rather unaffected by a change in ionic strength from 0.1 to 1 M. There is also agreement between the present results and the two conclusions that "the interaction of zinc ion was very weak" with cytidine[46] and that for the $Ni(Cyd)^{2+}$ complex log $K^{Ni}_{Ni(Cyd)} < 0.6$ holds.[9]

Table III. Correlations between M^{2+} Complex Stability and Basicity of the Ligand Binding Site[a] for Pyridine-like Ligands and Pyridine-like Ligands with an *o*-Amino Group

eq	M^{2+}	m	b	R	SD[b]
	(a) Regression Lines for Pyridine-like Ligands[c]				
6	Mn^{2+}	0.262 ± 0.021	−1.189 ± 0.145	0.997	0.029
7	Co^{2+}	0.204 ± 0.031	0.244 ± 0.236	0.977	0.045
8	Ni^{2+}	0.235 ± 0.025	0.633 ± 0.179	0.984	0.036
9	Cu^{2+}	0.415 ± 0.010	0.296 ± 0.065	0.999	0.013
10	Zn^{2+}	0.367 ± 0.025	−0.923 ± 0.178	0.991	0.034
11	Cd^{2+}	0.332 ± 0.013	−0.450 ± 0.094	0.999	0.015
	(b) Regression Lines for *o*-Aminopyridine-like Ligands[d]				
12	Mn^{2+}	0.262	−1.159		0.067[e]
13	Co^{2+}	0.204	−0.856		0.075[e]
14	Ni^{2+}	0.235	−0.917		0.056[e]
15	Cu^{2+}	0.456 ± 0.029	−1.428 ± 0.175	0.994	0.024
16	Zn^{2+}	0.367	−1.613		0.058[e]
17	Cd^{2+}	0.332	−1.060		0.052[e]

[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 4 (for details see refs 9 and 11), together with the corresponding correlation coefficients (R). The errors given with m and b correspond to one standard deviation (1σ). Straight line equation: $y = mx + b$ = log K^{M}_{ML}; x may represent the pK_a value of any pyridine or *o*-aminopyridine-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).[9,11] [b] These SD values multiplied by 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 section 4, refs 9 and 11, and also Figure 4). [c] From Table IV of ref 9. [d] From Table I of ref 11. [e] These SD values[11] contain also the contribution of the error limits of the stability differences, log $K^{Tu}_{M/Py\text{-}N}$ − log $K^{M}_{M(Tu)}$ (see Table 2 in ref 10).

4. Evaluation of the Substituent Effects on N-3 of Cytidine via Reference-Line Plots. It was already mentioned (Introduction) that an amino group in the ortho position of a pyridine nitrogen, as present in *o*-aminopyridine or tubercidin, inhibits the metal ion affinity of the aromatic N site. Therefore, such an inhibition is also expected in $M(Cyd)^{2+}$ complexes, but in addition an influence of the second *o*-substituent, i.e. of the carbonyl group (see Figure 1), might occur as well. Both these assumptions are correct (section 3; paragraphs 1 and 2); however, prior to a quantitative evaluation (see sections 5 and 6), the known correlations[9–11] between the stability of M^{2+} complexes with pyridine-like or *o*-aminopyridine-like ligands and the basicity of the corresponding ligand binding site have to be recalled.

On the basis of the log K^{M}_{ML}/pK^{H}_{HL} data pairs for the M^{2+} complexes of pyridine (**7**), 4-(2-thienyl)pyridine (**8**), 4-methylpyridine (**9**), 7-methylinosine (**10**), inosine (**11**), and ammonia (**12**; see ref 9) the straight-line equations 6 to 11 listed in Table III for plots of log K versus pK_a have previously been calculated for pyridine-like ligands.[9] The listed ligands are identified by the same numbers as previously[9–11] (see also Figure 4 in section 6); numbers **1**–**6** refer to imidazole-like ligands, needed for evaluations of metal ion–purine systems,[9] which are not of relevance in the present context; in addition, for a given metal ion all of the stability constants for the whole set of ligands were not always available (see, e.g., Figure 4).[9] Evidently with eqs 6–11 and the pK_a value of any pyridine-like ligand, the corresponding value for log K^{M}_{ML} may be calculated within the pK_a range of the experimental data employed.[9] For Ni^{2+} and Cu^{2+} it has been proven[11] that the reference lines are valid down to a pK_a value of about 2.5 (i.e., significantly beyond the pK_a range shown in Figure 4).

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(39) Kim, S.-H.; Martin, R. B. *Inorg. Chim. Acta* **1984**, *91*, 19–24.
(40) The reference line equations of Table III[9–11] (see also Figure 4 and sections 4–6) may also be used to judge the validity of other published stability constants of nucleoside complexes. For example, the constants given in ref 41 (35 °C; I = 0.1 M, KNO_3) for the M(uridine-H)$^+$ complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} are about 0.8–2.4 log units too large; this holds most probably also for the constants given[41] for Mg(uridine-H)$^+$ and Ca(uridine-H)$^+$. Similarly, the values provided in ref 42 (25 °C; I = 0.1 M (KNO_3)) for the M(uridine-H)$^+$ complexes with the same metal ions also have to be rejected as well as most probably the values given in ref 36 for the corresponding lanthanide complexes. Moreover, all the conclusions given in connection with related mixed ligand complexes[36,41,42] need to be ignored. In contrast, the constants given in ref 38 for the binary M(uridine-H)$^+$ complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} appear to be of a reasonable order, as is the value for Cu(uridine-H)$^+$ in ref 26. An analogous evaluation regarding M(xanthosine-H)$^+$ complexes was recently made;[9] it may be added that the constants and conclusions published in refs 43 and 44 should be viewed with some suspicion, while those given in ref 45 for several M(xanthine-H)$^+$ complexes may be recommended.
(41) Reddy, P. R.; Rao, V. B. M. *J. Chem. Soc., Dalton Trans.* **1986**, 2331–2334.
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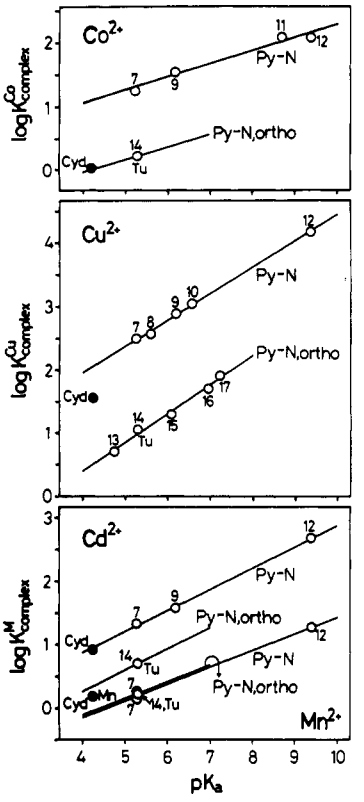

Figure 4. Relationship between log K and pK_a for the 1:1 complexes of Co^{2+}, Cu^{2+}, Cd^{2+}, and Mn^{2+} (from top to bottom) with pyridine-like N ligands (Py-N; O) and sterically less accessible *o*-aminopyridine-like N ligands (Py-N,ortho; O). The least-squares lines are drawn according to eqs 7 and 13, 9 and 15, 11 and 17, and 6 and 12 of Table III for the Co^{2+}, Cu^{2+}, Cd^{2+}, and Mn^{2+} systems, respectively; the inserted numbers correspond to the ligand numbers[9-11] given in the text of section 4; the points due to the M(tubercidin)$^{2+}$ complexes (Tables I and II) are in addition identified (Tu). The points due to the complexes formed between Co^{2+}, Cu^{2+}, Cd^{2+}, or Mn^{2+} and cytidine (Cyd) are inserted for comparison (●) (Tables I and II); see also the text in section 6.

The corresponding facts also hold for the regression lines of *o*-aminopyridine-like ligands. For Cu^{2+} the straight-line equation, eq 15, of Table III is based[10] on 2-phenylpyridine (**13**), tubercidin (**14**), 2-methylpyridine (**15**), 2-aminopyridine (**16**), and 2-amino-3-methylpyridine (**17**) (see also Figure 4). Comparison of eqs 9 and 15 shows that the slopes of the two straight lines for Cu^{2+} complexes agree nearly within a single standard deviation (1σ). This observation has allowed us to use the stability constants of M(tubercidin)$^{2+}$ complexes[10] to also construct reference lines for other M^{2+} complexes;[11] these corresponding straight-line equations for ligands with an *o*-amino group next to the pyridine-like nitrogen undergoing metal ion coordination are listed in Table III under the numbers 12–14, 16, and 17.

5. Quantification of the Effects of the *o*-Amino and the *o*-Carbonyl Substituents on the Metal Ion Affinity of N-3 in Cytidine. Application of eqs 6–11 in Table III allows, with $pK^{H}_{H(Cyd)} = 4.24$ (Table I), the calculation of stability constants for a hypothetical N-3 site in a cytidine ligand without any substituents at C-2 and C-4; these results are listed in the third column of Table IV (log $K^{Cyd}_{M/Py\text{-}N}$). The same type of calculation with eqs 12–17 gives the results of column 4 in Table IV which reflect the stabilities of complexes with an also hypothetical cytidine ligand that has an *o*-amino group next to N-3 but *no* carbonyl group at position 2 (log $K^{Cyd}_{M/Py\text{-}N,ortho}$). The differences between the values in columns 4 and 3 reflect the inhibitory effect of the *o*-amino group on the metal ion affinity of N-3 toward the various metal ions in the *absence* of the 2-carbonyl substituent (see column 5). Evidently there is no inhibition toward Mn^{2+} but a quite pronounced one toward Cu^{2+} and Ni^{2+} (about −1.55 log units); hence, the following series reflects the decreasing inhibitory effect of the *o*-amino group on metal ion coordination: $Cu^{2+} \sim Ni^{2+}$ (ca. −1.55 log units) > Co^{2+} (−1.1) > Zn^{2+} (−0.7) ~ Cd^{2+} (−0.6) > Mn^{2+} (about 0).

The difference between the experimentally measured stability constants (column 2 in Table IV) and those calculated for the *o*-amino inhibited hypothetical cytidine ligand (column 4) gives the values listed in column 6 of Table IV. They quantify the influence of the 2-carbonyl substituent showing that this group has no remarkable effect on the stability of the M(Cyd)$^{2+}$ complexes with Co^{2+} and Ni^{2+}; their stability is governed by the *o*-aminopyridine-type binding site. In contrast, Cu(Cyd)$^{2+}$ is by about 1 log unit more stable than expected for an *o*-aminopyridine-type coordination. Hence, the stability-*increasing* effect of the *o*-carbonyl group on the *o*-aminopyridine-type binding site of cytidine is reflected by following the series: $Co^{2+} \sim Ni^{2+}$ (ca. 0 log unit) < $Mn^{2+} \sim Zn^{2+}$ (0.25) < Cd^{2+} (0.55) < Cu^{2+} (about 1.05).

It is remarkable that Cu^{2+} and Ni^{2+} experience in their coordination tendency toward a pyridine N a comparable steric inhibitory effect by an *o*-amino substituent (column 5 in Table IV) and that this effect is not at all offset in a comparable way by the *o*-carbonyl group as a further substituent (column 6). Co^{2+} behaves here similarly to Ni^{2+}: the inhibition by an *o*-amino group is also very significant (column 5) while no stability promotion occurs by a further *o*-carbonyl substituent (column 6) (see also sections 6 and 7). These observations are a reflection of the relatively "low" affinity of Co^{2+} and Ni^{2+} toward O donors, e.g. phosphate groups,[27] compared to Mn^{2+} and Cu^{2+}; i.e., the Irving-Williams series[32] is *not* strictly followed.[17,27] Mn^{2+} shows in accord herewith an enhanced complex stability in the presence of an *o*-carbonyl group (column 6) (see also the following paragraph).

Finally, the stability differences (column 7 of Table IV) between the experimentally determined stability constants and those calculated for a simple pyridine-like coordination of a metal ion to N-3 (i.e., in the absence of any substituents) reflect the combined effects of the *o*-amino and the *o*-carbonyl groups on a pyridine-like binding site; they correspond therefore to the sum of the values listed in columns 5 and 6. Most values in column 7 have a negative sign because the positive effect of the *o*-carbonyl group (column 6) does not completely offset the inhibitory effect of the *o*-amino group (column 5). Only in the case of Cd^{2+} the two effects cancel, while for Mn^{2+} the *o*-amino *o*-carbonyl N-3 site of cytidine is even somewhat more attractive than a simple pyridine-like site of the same basicity (see also sections 6 and 7). Adding these differences to the b values (intercepts) of eqs 6–11 in Table IV leads to straight-line equations which quantify the metal ion affinity of *o*-amino *o*-carbonyl pyridine-like binding sites in dependence on the basicity of the pyridine nitrogen. In doing this, the assumption is made that the slope of a straight line is not affected for a given metal ion if an *o*-carbonyl substituent is introduced into ligands **7–11** (**12**); in fact, this has already been proven for the *o*-amino substituent (cf. eqs 9 and 15, and section 4).[10,11]

6. Classification of the *o*-Carbonyl-Substituent Effect on Pyridine-Type Complexes for Various Metal Ions. Several of the above conclusions are best reinforced with the aid of Figure 4, where the log $K^{M}_{M(Cyd)}/pK^{H}_{H(Cyd)}$ data pairs for representative M(Cyd)$^{2+}$ cases are shown in their relations to the straight-line plots defined by log K versus pK_a for pyridine-like (Py-N) and *o*-aminopyridine-like (Py-N,ortho) N ligands. The numbers inserted at the data points refer to the ligands listed in section 4 (for further details see refs 9 and 11). The following four categories, each representing a certain effect of the carbonyl substituent, become apparent:

(i) No Evident Effect. The data pair for Co(Cyd)$^{2+}$ (top of Figure 4) fits exactly on the reference line quantifying the stability of Co^{2+} complexes with *o*-amino pyridine-type ligands. This

Table IV. Comparison of the Experimentally Determined Stability Constants, log $K^{M}_{M(Cyd)}$, of Some $M(Cyd)^{2+}$ Complexes (Eq 5) (in Aqueous Solutions at 25 °C and I = 0.5 M ($NaNO_3$)) with the Stability Constants log $K^{Cyd}_{M/Py\text{-}N}$ Calculated for a *Noninhibited* Coordination of the Metal Ions to a Pyridine-Type N Ligand (Py-N) with the Same Basicity as N-3 in Cytidine but without the 4-Amino and 2-Carbonyl Groups, as Well as with the Stability Constants log $K^{Cyd}_{M/Py\text{-}N,ortho}$ Calculated for an *o*-Amino-Inhibited Coordination of the Metal Ions to an *o*-Aminopyridine-Type N Ligand (Py-N,ortho) with the Same Basicity as N-3 in Cytidine but without the 2-Carbonyl Group (Figure 1) Where the Effects Are Expressed by the Differences between the Various log K Values (See Also Text in Section 5)[a]

M^{2+}	log $K^{M}_{M(Cyd)}$	log $K^{Cyd}_{M/Py\text{-}N}$	log $K^{Cyd}_{M/Py\text{-}N,ortho}$	log $K^{Cyd}_{M/Py\text{-}N,ortho}$ − log $K^{Cyd}_{M/Py\text{-}N}$	log $K^{M}_{M(Cyd)}$ − log $K^{Cyd}_{M/Py\text{-}N,ortho}$	log $K^{M}_{M(Cyd)}$ − log $K^{Cyd}_{M/Py\text{-}N}$
Mn^{2+}	0.19 ± 0.08	−0.08 ± 0.09	−0.05 ± 0.20	0.03 ± 0.22	0.24 ± 0.22	0.27 ± 0.12
Co^{2+}	0.03 ± 0.08	1.11 ± 0.14	0.01 ± 0.23	−1.10 ± 0.27	0.02 ± 0.24	−1.08 ± 0.16
Ni^{2+}	0.14 ± 0.12	1.63 ± 0.11	0.08 ± 0.17	−1.55 ± 0.20	0.06 ± 0.21	−1.49 ± 0.16
Cu^{2+}	1.56 ± 0.06	2.06 ± 0.04	0.51 ± 0.07	−1.55 ± 0.08	1.05 ± 0.09	−0.50 ± 0.07
Zn^{2+}	0.20 ± 0.11	0.63 ± 0.10	−0.06 ± 0.17	−0.69 ± 0.20	0.26 ± 0.20	−0.43 ± 0.15
Cd^{2+}	0.91 ± 0.07	0.96 ± 0.05	0.35 ± 0.16	−0.61 ± 0.17	0.56 ± 0.17	−0.05 ± 0.09

[a] The values of the second column are from Table II; those of columns 3 and 4 were calculated with the straight-line equations 6–11 and 12–17, respectively, listed in Table III and $pK^{H}_{H(Cyd)}$ = 4.24 of Table I. The error limits are based on *3 times* the standard deviations (see also Tables I–III) and they were calculated according to the error propagation after Gauss (columns 5–7).

remarkable observation allows the following conclusion: the *o*-carbonyl group exercises no steric effect as observed for the *o*-amino group because there is no further decrease in complex stability! Indeed, it was already concluded earlier[9,48] that the steric influence of an *o*-carbonyl oxygen is smaller than that of an amino group; e.g., the data pairs for inosine and 7-methylinosine complexes fit well on the Py-N reference lines.[9] Hence, if there is any steric influence of the carbonyl group on the pyridine nitrogen, i.e. on N-3 of cytidine, it is offset by an oxo–metal ion interaction, which (if at all) might also occur via a water molecule (cf. also section 7). Next to Co^{2+}, Ni^{2+} also belongs in this category (compare the data in column 6 with those of columns 5 and 7 in Table IV).

(ii) A Positive Effect. The data pair for $Cu(Cyd)^{2+}$ is situated between the two reference lines (Figure 4, middle) proving that the steric inhibition of the 4-amino group is partly offset by a Cu^{2+}–oxo interaction (see section 7) with the 2-carbonyl group. Zn^{2+} also belongs into this category (cf. columns 5–7 in Table IV).

(iii) Two Effects That Cancel. The data pair for $Cd(Cyd)^{2+}$ fits within the error limits exactly on the reference line valid for a simple pyridine-like coordination (see lower part of Figure 4). Without other information one would conclude that the *o*-amino *o*-carbonyl N-3 site of cytidine shows the properties of a simple pyridine-like ligand. Yet, the available reference line for *o*-amino pyridine-like ligands proves that the mentioned conclusion is accidental: there is defintely an inhibitory effect of the 4-amino group on the coordination tendency of Cd^{2+} to N-3, but this is cancelled by an equally pronounced "positive" effect due to a Cd^{2+}–oxo interaction (section 7) with the 2-carbonyl group. It is obvious that this is a fortuitous situation between the preceding category (ii) and the following one (iv).

(iv) A Stability Promoting Effect. From the lowest part in Figure 4 it is evident that the effect of an *o*-amino group on the affinity of pyridine-like ligands for Mn^{2+} is negligible (see section 7): the two reference lines are identical within their error limits (cf. eqs 6, 12 in Table III). However, the data pair due to the $Mn(Cyd)^{2+}$ complex is about 0.25 log unit above the reference lines (see also columns 6 and 7 in Table IV). Hence, in this case the Mn^{2+}–oxo interaction promotes the complex stability beyond that observed for simple pyridine-type Mn^{2+} complexes. Comparison of the stability constants listed in Table II for $M(Cyd)^{2+}$ complexes of Ca^{2+} and Mg^{2+} with the also listed related constants for $M(Tu)^{2+}$ and $M(Py)^{2+}$ complexes indicates that such a stability promotion also occurs for these two alkaline earth ion complexes. In other words, metal ions showing a preference for O donor ligands belong in this category (see also section 7).

Overall, categories i–iv show a gradual increasing intensity of the metal ion–oxo interaction in the $M(Cyd)^{2+}$ complexes. This interaction with the 2-carbonyl group promotes a stability increase from the Py-N,ortho reference line (Co^{2+}) to one beyond that of the Py-N reference line (Mn^{2+}). Clearly, the various metal ions behave rather individually in the way they interact with the *o*-amino *o*-carbonyl N-3 binding site of cytidine.

7. Structural Considerations on Metal Ion Binding to an *o*-Amino *o*-Carbonyl Pyridine-Type Site. From the stability differences listed in column 5 of Table IV as well as from Figure 4 (Py-N versus Py-N,ortho reference lines) it is evident that the inhibitory effect of an *o*-amino group on the metal ion affinity of a pyridine nitrogen differs for different metal ions: e.g., the inhibition is strong for Cu^{2+} and Ni^{2+}, intermediate for Zn^{2+} and Cd^{2+}, and nonexistent for Mn^{2+}. What are the reasons for this observation? An explanation based on the differences in the geometry of the coordination sphere of these metal ions is obviously not possible but may be offered if one assumes that part of the M^{2+}–(Py-N) interaction occurs in an outer-sphere manner,[10] especially with metal ions prefering O donors, like Mn^{2+} or the alkaline earth ions. If the outersphere interaction occurs with the pyridine nitrogen via a coordinated water molecule by hydrogen bonding, the steric effect of the *o*-amino group is expected to be minor. In addition, the exocyclic amino group could even participate in the interaction by hydrogen bonding to a water molecule in the first or second coordination sphere of the metal ion; hydrogen bonding of the 4-amino group of the cytosine residue to a water molecule is known from X-ray structure analysis.[49] In contrast, Cu^{2+} or Ni^{2+} are expected to interact with a Py-N site predominantly by an inner-sphere bond (cf. also ref 50) and such an interaction should be sterically affected in a negative way by an ortho substituent, like an amino group.

The indicated explanation satisfies all observations: Mn^{2+}, Mg^{2+}, and Ca^{2+} form only very unstable complexes, e.g. with the *o*-aminopyridine-type ligand tubercidin (Figure 1) (see Table II), and their interaction is proposed to be largely of an outer-sphere nature. The inhibition of the *o*-amino group on complex stability is most pronounced for Cu^{2+} and Ni^{2+} (column 5 in Table IV), and for these ions inner-sphere binding to Py-N sites is expected to dominate. An intermediate inhibitory effect is observed for Zn^{2+} and Cd^{2+} and here inner-sphere–outer-sphere equilibria may be surmised; though in the case of Cd^{2+} a part of the steric inhibition may also have disappeared due to the longer Cd^{2+}–N bond length (about 0.3-Å more compared with those for Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}).[51]

In certain instances much of the *o*-amino inhibition on complex stability may be reversed by the additional presence of a further ortho substituent (see section 6, Figure 4, and column 6 of Table

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IV). By which mode may this *o*-carbonyl group offset the steric inhibition of an *o*-amino substituent? On the basis of X-ray crystal structure studies, it has long been concluded[4,52] that (N-3)–M–(O-2) chelation may play a role in complexes of cytosine and its derivatives. Recent compilations by Aoki[51,53] of crystal structure studies of nucleoside[53] and nucleotide[51,53] complexes are even more helpful in elucidating the various properties of metal ions in their interaction with cytosine derivatives. In all those cases where in the solid state the four-membered chelate resulting from an (N-3)-M-(O-2) coordination is observed, in aqueous solution much of the steric strain could be removed by including into the chelate (at least in equilibrium) a metal ion coordinated water molecule forming a hydrogen bond either with O-2 or with N-3, depending on the metal ion's preference for N or O coordination; if the hydrogen atom of the water molecule is counted, this partial outer-sphere coordination then would lead to a six-membered chelate, a situation sometimes addressed as semichelation. Hydrogen bonding, of course, is well-known for both O-2 and N-3 of the cytosine residue.[51] Several of the crystal structure studies compiled in refs 51 and 53 are devoted to complexes of the general composition $M(CMP)(H_2O)_n$; these complexes will be abbreviated as M(CMP) in the following.

There are five examples of different Pt^{2+} coordination spheres containing cytidine or derivatives;[51,53] all of them contain a strong Pt–(N-3) bond with no indication of a Pt–(O-2) interaction. Similarly, Pd^{2+} is also coordinated to cytidine via N-3.[53,54] However, in the present context the result for Co(CMP) is most revealing:[49,51,55] the distorted tetrahedral coordination sphere of Co^{2+} contains a strong Co–(N-3) bond (1.987 Å) with no further interaction to the base residue. This observation is in line with the fact that in aqueous solution there is *no* indication for an increased stability of $Co(Cyd)^{2+}$ via a 2-carbonyl participation (section 6). For $Ni(Cyd)^{2+}$ also a sole Ni^{2+}–(N-3) interaction is surmised from the stability data.

Solid Zn(CMP) contains a metal ion coordination sphere[51] related to that in Co(CMP), but the metal ion is now 5-fold coordinated (distorted trigonal-bipyramidal)[51] with a strong bond to N-3 (2.04 Å) and a rather weak one to O-2 (2.69 Å).[51,56] In accord herewith are the results obtained in solution (Table IV): the medium inhibition of the 4-amino group (–0.69 log unit; column 5) is only partially offset (+0.26 log unit; column 6) by a Zn^{2+}–oxo interaction in $Zn(Cyd)^{2+}$ (see section 5). It may be emphasized that in all cases discussed here monocoordinated species are expected to occur to various extents in intramolecular equilibria with chelates, including also various types of outer-sphere binding (see also above). From the crystal structure studies of Cd(CMP) and of related complexes,[51] it is clear from the available bond lengths that the two interaction modes with the cytosine residue can be more equivalent despite the fact that from the seven examples[51] two show only a Cd–(N-3) coordination (average 2.33 Å) to the base residue,[57] as the other five contain Cd–(N-3) (average also 2.33 Å) and Cd-(O-2) bonds (average 2.71 Å), which are of a more comparable length. Indeed, for $Cd(Cyd)^{2+}$ in aqueous solution the steric *o*-amino inhibition (–0.61 log unit; column 5 in Table IV) is completely compensated within the error limits by the positive Cd^{2+}–oxo interaction (+0.56 log unit; column 6), which places $Cd(Cyd)^{2+}$ into category iii of section 6 (see also Figure 4).

Four crystal structure studies[58-61] of mixed-ligand Cu^{2+} complexes containing cytosine[58-60] or cytidine[61] show for the observed six different Cu^{2+} coordination spheres (N-3)–Cu–(O-2) chelates; N-3 is always placed in an equatorial position (Cu–N: 1.99 Å)[62] and O-2 in an axial one (2.78 Å).[62] Such axial interactions should not be underestimated,[8,63] though in $Cu(Cyd)^{2+}$ in aqueous solution both sites, N-3 and O-2, could well occupy equatorial Cu^{2+} positions. The inhibitory effect of the *o*-amino group is large (–1.55 log units; column 5 in Table IV) and not completely offset by the significant Cu^{2+}–oxo interaction (+1.05 log units; column 6); hence, $Cu(Cyd)^{2+}$ is still 0.5 log unit less stable than the corresponding simple pyridine-type complex (see Figure 4), and this places $Cu(Cyd)^{2+}$ into category ii of section 6.

A most interesting case is Mn(CMP):[51,64] in the solid state the Mn-base interaction occurs *only* via O-2 (2.08 Å); N-3 is *not* involved. This relatively strong Mn^{2+}–oxo interaction is clearly responsible for the increased stability of $Mn(Cyd)^{2+}$ (ca. 0.25 log unit; Table IV) compared with a pyridine-type or Py-N,ortho-type interaction (see Figure 4), though it does of course not exclude in aqueous solution a further interaction with N-3, be it outer sphere or direct as discussed above. Along these lines also the alkaline earth ion complexes of cytidine and their increased stabilities in aqueous solution (Table II) have to be viewed. On the basis of Raman and ^{13}C NMR spectroscopy, an O-2 interaction in dimethyl sulfoxide as solvent has already been proposed for cytidine,[65] and it is well-known that, e.g., Ca^{2+}–carbonyl interactions are legion and also often relatively strong (2.2–2.3 Å).[66] Finally, the crystals of Ba(CMP) are composed of three kinds of Ba^{2+} coordination spheres[51,67] which all contain only O atoms and one of these is an octacoordinated Ba^{2+} with a Ba–(O-2) distance of 2.59 Å; the seven other Ba–O distances range from 2.78 to 3.03 Å.

Conclusions

The presented results and their discussion show that the apparently simple cytidine ligand is actually truly ambivalent. Depending on the kind of metal ion cytidine may behave as (i) a simple pyridine-like ligand, (ii) an *o*-aminopyridine-like ligand, or—completely in its own right—as (iii) a ligand with its own category. Consequently, the number of reference lines needed (see Figure 4) to describe the binding properties of *o*-amino and/or *o*-carbonyl substituted pyridines varies from metal ion to metal ion. For Mn^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+} two lines are sufficient, but the $M(Cyd)^{2+}$ complexes appear in different categories: for Co^{2+} and Ni^{2+}, the *o*-aminopyridine-type reference lines quantify the binding properties of cytidine; for Cd^{2+}, the simple pyridine-type line is appropriate, while for $Mn(Cyd)^{2+}$ a new reference line is needed despite the identity of the Py-N and Py-N,ortho reference

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lines for Mn^{2+} (Figure 4). Such a new reference line is also compulsory for the quantification of the binding properties of the *o*-amino *o*-carbonyl N site toward Cu^{2+} and Zn^{2+}; hence, for these two ions *three* reference lines exist: the one last mentioned, one for simple pyridine-type sites, and one for *o*-aminopyridine-like ligands.

It is evident that the *o*-amino *o*-carbonyl N binding site as present in cytidine also occurs in N-1 deprotonated guanosine; hence, these sites, as well as those of adenosine,[10,11] are now quantified. We intend to continue these efforts, because eventually they should allow us to map for the different metal ions the relative binding affinities of the various sites present in nucleic acids.

Acknowledgment. The support of this research by a grant from the Swiss National Science Foundation (H.S.) and a fellowship within the exchange program between the National Natural Science Foundation of the People's Republic of China and the Swiss National Science Foundation (L.J.) and support for a leave of absence by the Swiss National Science Foundation and the University of the Ryukyus (Y.K.) are gratefully acknowledged.