*Inorganica Chimica Acta*, 78 (1983) 259–267



# Complexes of (ethylenediamine)Pd(II) with Uridine and Cytidine. Occurrence of Hydroxo Bridged and Anionic Cytosine Ring Bridged Complexes

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Received December 30, 1982

*In solutions containing a 1:2 mixture of $enPd^{2+}$ and either uridine or cytidine the expected 1:2 complexes BMB predominate over a wide pH range. A mixed complex with each base is more stable than expected statistically. In equimolar solutions five complexes are observed with uridine and seven with cytidine. There occurs with uridine the simple hydroxo complex BMOH and the hydroxo bridged complexes BM–OH–MB and BM–OH–MOH. With cytidine the hydroxo complexes occur to only a small extent as they are replaced by the more stable dibridged $(MA)_2$ complex where two anionic cytosine rings bridge through N3 and a deprotonated amino N4 nitrogen two $enPd^{2+}$. A planar complex with a single bridging anionic cytosine ring and a hydroxo bridge, MA(OH)M, also occurs. For both cytidine and 5′-CMP the dibridged anionic cytosine ring complex $(MA)_2$ has been observed and identified for the first time in three distinct isomeric forms. Two head-to-tail diastereomers and the head-to-head isomer exhibit different proton chemical shifts. When equilibrium is finally achieved, Pt(II) binds nucleic bases about 30 times more strongly than Pd(II).*

## Introduction

As more rapidly reacting prototypes for the antitumor *cis* diamine Pt(II) complexes we have been studying reactions of enPd(II) [1–3] Both metal ions form diamagnetic, planar complexes, possess similar radii, and strongly prefer nitrogen to oxygen donor atoms. Due to their very slow reactions equilibrium constants for *cis*-diaminePt(II) with nucleic bases are uncertain. It has been argued that published constants fail to reflect systems at equilibrium [4]. An alternative approach is to provide comparisons between enPd(II) and corresponding Pt(II) complex equilibrium constants [2, 5]. Though Pd(II) reacts much more rapidly than Pt(II), it still reacts slowly enough among nucleic base ligand sites so that in proton NMR spectra individual ligand resonances appear for each kind of Pd(II) binding site [6].

This paper reports reactions of enPd(II) with 1-methylcytosine, the ribonucleosides uridine and cytidine, and the nucleotides 5′-uridine monophosphate and 5′-cytidine monophosphate. The reactions turn out to be more complicated and interesting than anticipated. Not only hydroxo but also hydroxo bridged complexes occur with uridine. Without nucleic base a dihydroxo bridge links two enPd(II) in neutral solutions [5, 7]. With cytidine an entirely new feature appears due to displacement of an amino group proton at N4 by Pd(II). In a crystal structure determination important for this research, *cis*-$(NH_3)_2Pt(II)$ forms with 1-methylcytosine a dimer, $(MA)_2$, where an amino group N4 proton on each ligand is substituted by Pt(II) and each cytosine ring anion bridges by N3 and N4 two Pt(II) to give an 8-membered ring [8]. Since each Pt(II) is bound to the amino group N4 of one cytosine ring anion and to N3 of the other, there is a head to tail arrangement of the ligand pair. We propose a similar dimer structure for one of the complexes observed in solutions of cytidine with enPd(II).

## Experimental

Pyrimidine ribonucleosides and their 5′-monophosphates were purchased from Sigma or Aldrich Chemical Companies. $K_2PdCl_4$ was an Alfa Inorganics product. $Pd(en)Cl_2$ was synthesized according to a published procedure [9]. Some care is needed to eliminate all $Cl^-$ without introducing excess $Ag^+$ to make a solution of $enPd(H_2O)_2^{2+}$ with $NO_3^-$ counterion. To about 50 m*M* $enPdCl_2$ is added 1.98 eqs $AgNO_3$ and the solution is stirred at room temperature for at least 40 hours under argon in the dark. Do not try to prepare solutions with greater than 70 m*M* $enPd^{2+}$ or heat the solutions. The AgCl

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precipitate is filtered off and the solution used directly. The solution over the white precipitate should be absolutely clear and dark yellow. It may be stored in the refrigerator under argon for at least one week. For the NMR studies the $enPd(H_2O)^{2+}$ concentration was usually 50 m*M*.

Solutions were prepared at room temperature and most $^1H$ NMR spectra were recorded on a Varian EM-390 spectrometer at 90 MHz and 34 °C and a sweep width of 2 ppm. A few spectra were taken on a Nicolet 360 MHz spectrometer at 25 °C. A frequency counter was used for accurate determination of chemical shifts in NMR titration studies. Though t-butyl alcohol (about 0.2 m*M*) was employed as an internal reference all chemical shifts are reported downfield from DSS (2,2-dimethyl-2-silapentane-5-sulfonate) by adding 1.234 ppm. t-Butyl alcohol was confirmed as a suitable internal reference in these systems [6].

Standing of solutions for at least 45 minutes after mixing and a constant pH before and after taking NMR spectra supports attainment of equilibrium. In most cases $KNO_3$ was added to control the ionic strength at 0.5 *M*. There are perplexing dependencies of both NMR chemical shifts and peak areas on salt content in the cytidine system. Sharper peaks were obtained with $KNO_3$ and $NaNO_3$ than with $NaClO_4$ and $NaBF_4$. All experiments were performed in $D_2O$. The pH values are not corrected for $D_2O$ [Footnote 11 in ref. 6].

Acidity constants were determined from NMR spectra as already described [6]. For 5′-CMP in the absence of $enPd^{2+}$ virtually identical $pK_a$ values were obtained when referencing H6 against t-butyl alcohol, H5, or H1′ in CMP. A non-linear least squares program fitted chemical shift differences against pH by theoretical equations.

TABLE I. H6 Chemical Shifts for $enPd^{2+}$ Complexes of Uridine and Cytidine.[a]

| Species | Uridine | Cytidine |
|---|---|---|
| BH | 7.833 | 8.108 |
| B | 7.650 | 7.802 |
| BM | 7.652 | 7.886 |
| BMB | 7.525 | 7.877, 7.863 |
| BMOH | 7.451 | |
| BM–OH–MB | 7.634 | |
| BM–OH–MOH | 7.548 | |
| $(MA)_2$ (Head-head) | | 7.093, 7.080 |
| $(MA)_2$ (Head-tail) | | 7.110, 7.093 |
| MA(OH)M | | 7.230 |

[a]At 34 °C in $D_2O$ with 0.5 *M* $KNO_3$.

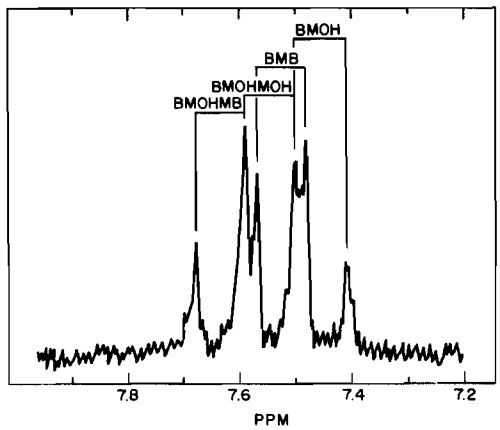


Fig. 1. H6 region of proton NMR spectrum of solution containing uridine and $enPd^{2+}$ both at 50 m*M* at pH 9.0. Spectrum was taken at 90 MHz and 34 °C.

## Results

Numerous $^1H$ NMR spectra were taken over a wide pH range and with $enPd(H_2O)_2^{2+}$ to pyrimidine ribonucleoside ratios of 1:2, 1:1, and 2:1. Five different complexes were observed for uridine and seven for cytidine. For both ligands, solutions containing a 1:2 ratio of $enPd^{2+}$ to nucleoside contained almost exclusively BMB complexes (with the BM complex with uridine in acidic solutions). Identification of complexes containing two metal ions per ligand were most evident in solutions containing a 2:1 mole ratio. Stoichiometries of complexes containing equivalent amounts of metal ion and ligand were deduced from their pH dependencies in equimolar solutions. Most of the analysis was performed with $^1H$ NMR chemical shifts of H6 which were more distinct than those of H5. Though more condensed, analysis of the peaks due to the carbon bound hydrogens of 1,2-diaminoethane in the complex often clarified and supported conclusions reached on the basis of the H6 resonance.

The H6 region of a proton NMR spectrum of a solution containing equimolar amounts of uridine and $enPd^{2+}$ at pH 9.0 is shown in Fig. 1. Due to coupling with H5, the H6 peak is a doublet. The proton coupling constant $J_{5-6}$ is about 7.6 Hz for both free ligand and complexes. Chemical shifts listed in Table I refer to the midpoint of the H6 doublet.

Table I lists nucleic base H6 peak assignments compatible with the many spectra. We designate the complex with a single nucleoside bound *via* N3 on the nucleic base to $enPd^{2+}$ as BM, and the 1:2 complex where all four planar sites are occupied by en and 2 nucleosides as BMB. The en ligand always

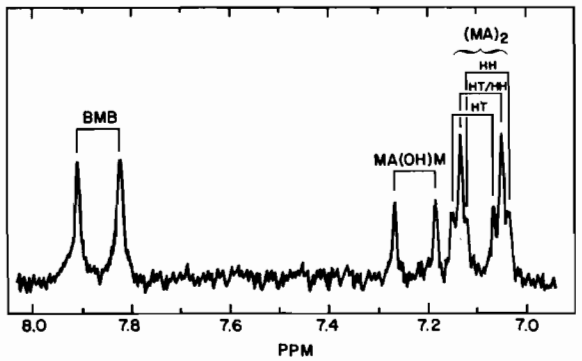


Fig. 2. H6 region of proton NMR spectrum of solution containing cytidine and $enPd^{2+}$ both at 50 m*M* at pH 6.0. Spectrum was taken at 90 MHz and 34 °C. The symbols HH and HT identify peaks due to head-to-head and head-to-tail isomers of $(MA)_2$.

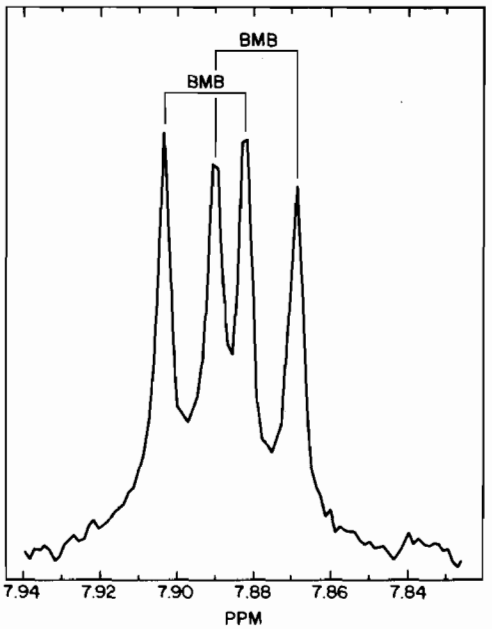


Fig. 3. Expanded BMB portion of H6 region in proton NMR spectrum of solution containing cytidine and $enPd^{2+}$ both at 50 m*M* and pH 6.2. In contrast to Figs. 1 and 2 this spectrum was taken at 360 MHz and 25 °C. The decrease in temperature causes a small downfield shift compared to chemical shifts reported in Tables and text at 34 °C.

remains bound to $Pd^{2+}$ in these experiments. Similarly the complex with *cis* nucleic base and $OH^-$ groups is represented as BMOH. The $OH^-$ group may also serve as a bridge to form BM–OH–MB, BM–OH–M, and BM–OH–MOH. In the last complex the second $enPd^{2+}$ bears *cis* $OH^-$ groups, one bridging to the first $enPd^{2+}$, which also binds a nucleic base at N3. The complex BM–OH–M was not of sufficient concentration to be observed in our experiments.

Figure 2 displays the H6 region of a proton NMR spectrum of an equimolar solution of cytidine and $enPd^{2+}$ at pH 6.0. Once again, the H6 peaks appear as doublets and the proton coupling constant $J_{5-6}$ = 7.6 Hz is unaffected by ligand environment. The lowest field doublet pair is due to the 1:2 complex BMB. The most interesting aspects of Fig. 2 are the sets of H6 peaks strongly shifted to fields higher than 7.3 ppm. The H5 peaks (not shown in Fig. 2) undergo a corresponding large upfield shift while the ribose peaks are relatively unshifted. We account for the high field shift, which occurs only on the nucleic base, as due to Pd(II) substitution for a hydrogen on the amino group at C4. The three highest field H6 doublet pairs appear as an apparent triplet with a 1:2:1 intensity ratio as shown in Fig. 2. For the cytidine complex the area under the center peak equals the area under the two side peaks, the areas of which are not always identical. Most experiments were conducted by starting in acidic solutions and obtaining NMR spectra upon successive additions of base. If, however, base is added promptly to an equimolar cytidine solution to bring the pH > 6, instead of the doublet of apparent 1:2:1 triplets only a doublet of doublets appears with chemical shifts corresponding to the most low field and center peaks in the triplet. We assign these peaks to head-to-tail and head-to-head isomers of a complex $(MA)_2$ where two anionic N4 deprotonated cytosine rings, A, bridge two $enPd^{2+}$ *via* N3 and N4 nitrogens. H6 chemical shift assignments reported as the midpoint of the doublets are collected in Table I and the structures are discussed more fully in the Discussion section.

The H6 doublet centered at 7.23 ppm in Fig. 2 undergoes an intensity increase compared to the three highest field H6 doublets in the presence of excess $enPd^{2+}$. We assign this 7.23 ppm doublet to a new dibridged structure MA(OH)M where one anionic cytidine and one hydroxide ion bridge two $enPd^{2+}$.

A 360 MHz proton NMR spectrum of the BMB portion of the H6 region of a solution containing equimolar amounts of $enPd^{2+}$ and cytidine at pH 6.2 is shown in Fig. 3. The broad doublet at lowest field at 90 MHz in Fig. 2 has become a pair of doublets at 360 MHz in Fig. 3. (The doublet pair also appears in some spectra taken at 90 MHz). The doublet pair is interpreted as due to two isomers of the complex BMB. Structure elaboration appears in the Discussion section.

Interpretations concerning assignments and structures of cytidine complexes are supported by results obtained with 1-methylcytosine as a ligand. Equimolar solutions with $enPd^{2+}$ yield a distribution pattern similar to that of cytidine (Fig. 5).

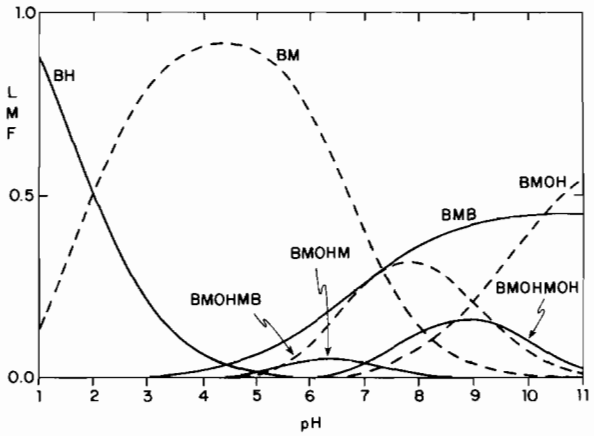


Fig. 4. Ligand mole fraction *versus* pH for solutions containing equimolar (0.05 *M*) amounts of uridine and $enPd^{2+}$.

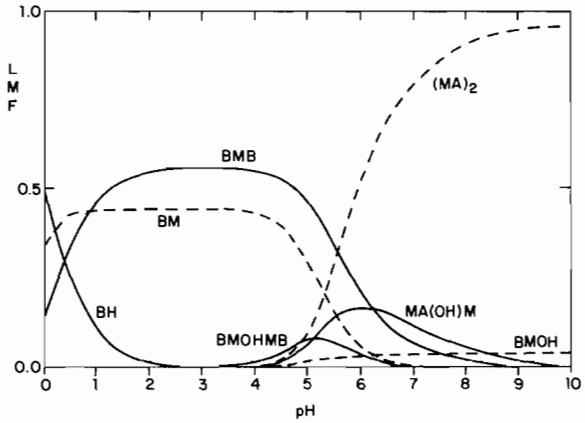


Fig. 5. Ligand mole fraction *versus* pH for solutions containing equimolar (0.05 *M*) amounts of cytidine and $enPd^{2+}$. The curve labelled $(MA)_2$ represents the sum of mole fractions for all three isomers of the dibridged dimer.

Simple doublet peaks are observed for H6 and a singlet peak for the $CH_3$ group for BM, BMB, and MA(OH)M complexes. For the $(MA)_2$ dimer the H5 and $CH_3$ (but not the H6) peaks split into a more intense lower field peak that was larger than the less intense higher field peak. By analogy with cytidine we assign the respective peaks to head-to-tail and head-to-head isomers.

Comparisons of areas found under assigned peaks in solutions of different pH and with 1:1 and 1:2 $enPd^{2+}$ to nucleoside molar ratios permit estimation of equilibrium constants for the several complex species. In association with the following reactions we define the related equilibrium constants. Coordinated $H_2O$ and en ligands are not indicated; the symbol M represents enPd(II).

| | |
|---|---|
| $HB \rightleftarrows H^+ + B$ | $K_a = (H)[B]/[HB]$ |
| $B + M \rightleftarrows BM$ | $K_1 = [BM]/([B][M])$ |
| $BM + B \rightleftarrows BMB$ | $K_2 = [BMB]/([B][BM])$ |
| $BM \rightleftarrows BMOH + H^+$ | $K_3 = (H)[BMOH]/[BM]$ |
| $BMOH + BM \rightleftarrows$ BM–OH–MB | $K_4 = [BMOHMB]/([BMOH][BM])$ |
| $BMOH + M \rightleftarrows$ BM–OH–M | $K_5 = [BMOHM]/([BMOH][M])$ |
| BM–OH–M $\rightleftarrows$ BM–OH–MOH + $H^+$ | $K_6 = (H)[BMOHMOH]/[BMOHM]$ |

By considering cyclic systems it is possible to derive from the above equations other equilibrium constants relating the species in solution.

In addition to the above ligand reactions the following reactions involving only the $enPd^{2+}$ species are included in the analysis.

| | |
|---|---|
| $M \rightleftarrows MOH + H^+$ | $K_M = (H)[MOH]/[M]$ |
| $MOH \rightleftarrows M(OH)_2 + H^+$ | $K_N = (H)[M(OH)_2]/[MOH]$ |
| $2MOH \rightleftarrows$ dimer | $K_D = [dimer]/[MOH]^2$ |
| $3MOH \rightleftarrows$ trimer | $K_T = [trimer]/[MOH]^3$ |

The dimer and trimer species contain 2 and 3 hydroxy bridges, respectively. From an earlier analysis [5] we use $pK_M = 6.2$, $\log K_D = 3.7$ ($M^{-1}$), and $\log K_T = 6.5$ ($M^{-2}$). For the related $enPt^{2+}$ species the $pK'_M = 5.8$ and $pK'_N = 7.6$ [5]. We apply the same difference of 1.8 log units to the $enPd^{2+}$ species and estimate $pK_N = 8.0$. The dimer and trimer species are most important in neutral solutions but even in this region are of little significance. The dihydroxo species $enPd(OH)_2$ may withdraw Pd from solution at high pH; it accounts for the observed plateau rather than a decrease in BMB concentration in equimolar solutions with uridine at pH > 10 (Fig. 4).

In the cytidine system it is necessary to include additional equilibria due to formation of anionic, N4 deprotonated cytidine which bridges two $enPd^{2+}$ via N3 and N4. In our system deprotonation at N4 occurs only in association with $enPd^{2+}$ substitution for a N4 proton. However we formulate complex formation of anionic cytidine in terms of deprotonation of an amino proton and subsequent metalation. We write

$$B \rightleftarrows A + H^+ \qquad K_b = (H)[A]/[B]$$

where A represents anionic, N4 deprotonated cytidine. For a modified ribosyl 1-substituted cytosine, $pK_b = 14.8$ [10], which provides an estimate

TABLE II. Equilibrium Constant Logarithms for $enPd^{2+}$ Complexes of Uridine and Cytidine.[a]

| Constant | Uridine | Cytidine |
|---|---|---|
| $K_a$ | −9.53 | −4.62 |
| $K_1$ | 9.1 | 6.0 |
| $K_2$ | 6.1 | 5.6 |
| $K_3$ | −8.2 | (−6.4) |
| $K_4$ | 2.4 | (2.3) |
| $K_5$ | 4.4 | (2.5) |
| $K_6$ | (−7.0) | (−6.7) |
| $K_E$ | | 3.1 |
| $K_F$ | | 3.7 |

[a]At 34 °C in $D_2O$ with 0.5 *M* $KNO_3$. Values in parentheses are assumed.

of the amino group acidity constant for cytidine. Metal ion reacts at the anionic N4 amino group nitrogen.

$$M + A \rightleftarrows MA \qquad K_7 = [MA]/[M][A]$$

followed by dimerization of MA to give a dibridged complex

$$MA + MA \rightleftarrows \begin{matrix} & A & \\ M & & M \\ & A & \end{matrix} \qquad K_8 = [(MA)_2]/[MA]^2$$

or formation of a mixed hydroxo dibridged complex

$$MA + MOH \rightleftarrows \begin{matrix} & A & \\ M & & M \\ & OH & \end{matrix} \qquad K_9 = [MA(OH)M]/[MA][MOH]$$

Since the intermediate species A and MA do not occur to a significant extent, only equilibrium constants associated with two overall reactions to give dibridged complexes are determined.

$$2M + 2B \rightleftarrows 2H^+ + (MA)_2 \qquad K_E$$

$$M + B + MOH \rightleftarrows H^+ + MA(OH)M \qquad K_F$$

From equations already given it may be shown that $K_E = K_b^2 K_7^2 K_8$ and $K_F = K_b K_7 K_9$, The equilibrium constants $K_8$ and $K_E$ refer to the summed equilibrium constants for all isomers of $(MA)_2$.

All of the above equilibria were considered simultaneously in conjunction with mass balance equations in fitting experimental results to distribution curves generated by computer. An iterative procedure was used to achieve an exact solution; no approximations were made. The results are presented as equilibrium constants in Table II and as distribution curves for equimolar mixtures in Figs. 4 and 5. The distribution curves show ligand mole fraction as seen in an NMR experiment *versus* pH. Due to the difficulty of evaluating areas under overlapping peaks in some situations, the equilibrium constants listed in Table II should be viewed as approximate. Values in parentheses in Table II are assumed values for species present in low concentration. We consider all likely species. The resulting distribution curves confirm that the assumed low concentration species are present in amounts too small to be observed.

Distribution curves of solutions containing 1:2 mixtures of $enPd^{2+}$ and nucleoside (not shown) are dominated by the 1:2 complex BMB. This complex is the dominant species in the uridine system from $4 < pH < 11$ (the limit of our experiments). At lower pH the BM complex and free ligand predominate. For cytidine the BMB complex prevails from $1 < pH < 8$. At higher pH the dibridged $(MA)_2$ complex begins to appear even in 1:2 mixtures.

Experiments were also performed on solutions 50 m*M* in each of the three components: uridine, cytidine, and $enPd^{2+}$. In addition to peaks for the complexes BMB for both uridine, U, and cytidine, C, a new mixed complex UMC appears with H6 peaks at 7.627 ppm for uridine and 7.791 ppm for cytidine. Analysis of the peak heights at 5 pH values from pH 6.7 to 8.9 in terms of the equilibrium constant $X = [UMC]^2/([CMC][UMU])$ yields log $X = 1.79 \pm 0.03$. Singlet en peaks appear at 2.780, 2.805, and 2.817 ppm for the UMU, UMC, and CMC complexes, respectively.

Investigations were also conducted on the 1:2 mixtures of $enPd^{2+}$ and 5′-UMP or 5′-CMP. For 5′-UMP addition of base to deprotonate the phosphate group in the BMB complex gives a H6 chemical shift change from 7.632 to 7.721 ppm with $pK_a = 6.29 \pm 0.04$. For unbound 5′-UMP the change is from 7.927 to 8.073 ppm with $pK_a = 6.16 \pm 0.01$ for the phosphate group.

As for cytidine there are two isomeric BMB complexes for $enPd^{2+}$ with 5′-CMP. The pair of isomeric complexes possess virtually identical H6 chemical shifts at 7.956 ppm and peak areas in the phosphate protonated complexes. Upon addition of base to deprotonate the phosphate group one isomer gives a limiting H6 chemical shift of 8.128 ppm with $pK_a = 6.09 \pm 0.01$ and the second isomer a limiting shift of 8.094 ppm with $pK_a = 6.14 \pm 0.02$. For unbound 5′-CMP, phosphate group deprotonation yields a H6

downfield chemical shift change from 7.911 to 8.036 ppm with $pK_a = 6.17 \pm 0.03$.

In equimolar (0.05 *M*) mixtures of 5′-CMP and $enPd^{2+}$ at pH > 5 there are also three $(MA)_2$ isomers. In this case the most intense peak, instead of being centered between the two smaller peaks as for cytidine, appears at lowest field at 7.310 ppm (pH 6.9). The two smaller peaks appear at 7.300 and 7.276 ppm. Associated with the phosphate deprotonation, H6 chemical shifts of all three peaks move downfield to the same extent. For the most prominent peak a non-linear least squares analysis yields a limiting chemical shift of 7.21 ppm in the acidic form and 7.33 ppm in the basic form with $pK_a = 6.2$. The last value corresponds to that expected for titration of an unbound phosphate group.

## Discussion

Solutions containing a 1:2 mole ratio of $enPd^{2+}$ and either uridine or cytidine exhibit the expected predominant BMB complex. In the case of cytidine two separate resonances are associated with the BMB complex. Neutral and basic solutions with equimolar amounts of $enPd^{2+}$ and either uridine or cytidine show complex species distributions. As shown in Fig. 4 for the uridine case, hydroxo complexes are important species in neutral solutions and make up most of the species present at pH > 8. There occur both simple, BMOH, and bridged hydroxo species, BM–OH–MB and BM–OH–MOH. In equimolar solutions with cytidine, hydroxo complexes occur to only a small extent as they are replaced by the more stable dibridged $(MA)_2$ complex. Bridging occurs through N3 and N4 of the cytosine ring anion. The lack of an amino group at C4 precludes similar strong bridging with uridine.

The first three equilibrium constants for uridine in Table II are in excellent agreement with those found from potentiometric titrations [11] in $H_2O$ rather than $D_2O$ and at 25 °C rather than the 34 °C of this study: $pK_a = 9.20$, $\log K_1 = 8.65$, and $\log K_2 = 5.92$. The $\log K_1$ values in Table II for $enPd^{2+}$ binding may be compared with the 0.5 and 0.6 log unit smaller $\log K_1$ values of 8.6 and 5.4 for binding of $dienPd^{2+}$ to uridine and cytidine, respectively, under similar conditions [6]. In these complexes uridine binds as an anionic base and cytidine as a neutral base.

One of the purposes of this research, as indicated in the introduction, is to relate results of $enPd^{2+}$ interactions to those of $enPt^{2+}$ and *cis*-$(NH_3)_2Pt^{2+}$. Sometime ago it was found that the equilibrium constant for $dienPt^{2+}$ binding to uridine is at least ten times greater than that of $dienPd^{2+}$ [2]. This early conclusion is supported by more recent studies which report thymidine $\log K_1$ values of 8.8 for $enPd^{2+}$ [11], and 10.3 for $enPt^{2+}$ and 10.4 for *cis*-$(NH_3)_2Pt^{2+}$ [12]. The last two *cis* amine species yield virtually identical equilibrium constants. A corresponding increase of about 1.5 log units is expected for interactions with cytidine, so that we anticipate $\log K_1$ for $enPt^{2+}$ and *cis*-$(NH_3)_2Pt^{2+}$ to be about 7.5 compared to $\log K_1 = 6.0$ for $enPd^{2+}$ from Table II. The expected $\log K_1 = 7.5$ value for *cis*-$(NH_3)_2Pt^{2+}$ and cytidine is 4 log units greater than that reported from UV absorption spectra studies [13], which may be due to use of excess Pt(II) and omission of allowance for dihydroxo bridge formation [4]. An even lower estimate of $\log K_1 \cong 2.9$ [14] is presumably due to the inability of Raman difference spectra to identify the species present. Instead of only 50% of the cytidine being complexed in equimolar solutions at pH 3 [14], Fig. 5 shows complete complexation with comparable amounts of BM and BMB complexes.

We conclude that equilibrium constants for Pt(II) binding to nucleic bases and other nitrogen containing molecules are about 30 times stronger than for the corresponding Pd(II) complexes. Weaker values, often much weaker values, reported for Pt(II) systems are ascribed to failure to attain equilibrium and to misinterpretation of spectral information. Equilibrium is difficult to achieve with *cis*-Pt(II) amines in neutral solutions due to dihydroxo bridge formation and in basic solutions due to formation of inert dihydroxo complexes [5, 7]. Formation of either of these species markedly reduces the amount of Pt(II) available for coordination. If the ligand site to be platinated is also protonated a further reduction in rate occurs. Finally $Cl^-$ is a poorer leaving group than water, but better than a hydroxo bridge or a hydroxo group, so its presence may slow or speed attainment of equilibrium depending upon conditions.

Though there are many areas of general agreement between this research and a lengthy paper of Tobias and coworkers on binding of *cis*-$(NH_3)_2Pt^{2+}$ and $enPt^{2+}$ to uridine and cytidine [14], there are also numerous points of disagreement. Their almost $10^5$ times too weak an equilibrium constant for Pt(II) binding to cytidine is mentioned above. Some of the other differences are mentioned now. No bridging complexes of any kind with either cytidine or uridine were identified in their work. In the uridine–enPt(II) system the complex assigned as BMOH by Tobias we identify by comparative chemical shift analysis as BM–OH–MB. This new identification also accounts naturally for the reported slow process occurring between pH 5–7 with the monouridine complex BM. Simple ionization to give BMOH occurs rapidly while formation of the binuclear complex BM–OH–MB is slow. All $^1H$ NMR spectra in the uridine-$enPt^{2+}$ system displayed by Tobias *et al.* in their Figs. 6 and 7 show too

much uncomplexed ligand due either to incomplete reactions or insufficient $enPt^{2+}$. Tobias suggested greater uridine anion ring current anisotropy and closer interaction between uridine bases (their Fig. 15) to account for a greater upfield shift in the uridine than in the cytidine system upon binding to BM a second ligand to give BMB. These proposals are unnecessary, however, as binding of an anionic uridine *versus* a neutral cytidine alone is capable of accounting for the chemical shift difference. The mixed complex of cytidine and uridine, CMU, investigated in this research, exhibits only a small upfield uridine shift and a larger upfield cytidine shift compared to the respective BM complexes.

Formation of the mixed complex of uridine and cytidine, UMC, may be compared with that for the pure 2:1 complexes UMU and CMC with the addition of the following two reactions [15, 16].

$$UM + C \rightleftarrows UMC \qquad K_{uc} = [UMC]/([UM][C])$$

$$MC + U \rightleftarrows UMC \qquad K_{cu} = [UMC]/([MC][U])$$

Since UMC is made by two pathways there is a cyclic system for which $K_u K_{uc} = K_c K_{cu}$. The values of $K_u$ and $K_c$ refer to the $K_1$, and $K_{uu}$ and $K_{cc}$ to the $K_2$ constants for uridine and cytidine tabulated in Table II. For the equilibrium constant X defined in the Results section we may write:

$$1.8 = \log X = \log(K_{uc}K_{cu}) - \log(K_{uu}K_{cc})$$

The statistically expected value is 4 in X or 0.6 in log X. Thus the observed log X = 1.8 is 1.2 log units greater than statistical. The pronounced favoring of the mixed UMC complex over the pure UMU and CMC complexes may be ascribed for the most part to the large difference $\log(K_u/K_{uu}) = 3.0$ due to resistance to binding of a second anionic uridine ligand.

Insertion of the values for $K_{uu}K_{cc}$ into the log X equation yields $\log K_{uc}K_{cu} = 13.5$. For the cyclic system $K_{uc}/K_{cu} = K_c/K_u = 10^{-3.1}$. Since both the product and quotient of $K_{uc}$ and $K_{cu}$ are known we may calculate $\log K_{uc} = 5.2$ and $\log K_{cu} = 8.3$.

As shown in Fig. 2 in an equimolar mixture of cytidine and $enPd^{2+}$ some peaks are shifted strongly upfield to 7.2 to 7.1 ppm. We assign these peaks to complexes in which a Pd(II) has substituted for a proton at the amino group nitrogen. Upfield shifts are expected for this kind of substitution. The amino group is not a metal ion binding site without deprotonation [17]. For proton loss from the amino N4 group of a modified ribose substituted cytosine, $pK_a = 14.8$ [10]. In contrast deprotonation of the amino group N4 occurs with $pK_a = 8.7$ in 3-methylcytidine [18] and in this range with N3 protonated cyclic CMP [19]. Thus methylation and protonation at N3 acidifies the N4 amino group by $10^6$. Though metalation at N3 is not expected to be nearly as effective as protonation in acidifying an N4 proton, the comparison supports the proposal of a N3 metalated, N4 deprotonated species, MA, as an intermediate in formation of dimer $(MA)_2$. Substitution of Pd(II) for an amino group proton occurs as low as pH 5, aided by formation of the dibridged $(MA)_2$ complex. This promotion of amino group deprotonation by 10 log units by Pd(II) substitution and dimerization is less than the 13 log unit promotion of amide deprotonation by Pd(II) substitution for an amide hydrogen and chelation to form a tridentate complex of glycylglycine [20].

The proposed identification of the dibridged dimer, $(MA)_2$, with two cytosine ring anions bridging through N3 and N4 has precedent in the crystal structure determination described in the introduction. No such precedent exists for our suggestion that the H6 doublet centered at 7.23 ppm is due to a mixed cytosine ring anion and hydroxo bridged dimer, MA(OH)M. This complex is more prevalent in a 2:1 $enPd^{2+}$ to cytosine mixture as indicated by its composition. The observed relative mole fractions follow closely the distribution curve indicated in Fig. 5. Finally the Pt–Pt bond distance of 2.98 Å in the dicytosinate bridged dimer [8] is similar to that of 3.09 Å found in dihydroxo bridged dimers [21]. The similar bond lengths suggests compatibility of a cytosine ring anion bridge and a hydroxo bridge linking two metal ions.

The overall equilibrium constants, $K_E$ and $K_F$, for dibridged dimer formation in the cytidine system both consist of equilibrium constants for individual reactions the values of which are difficult to estimate accurately. It is possible, however, to evaluate the combination of constants corresponding to disproportionation of the mixed dibridged complex to give the two pure dibridged species.

$$2\,MA(OH)M \rightleftarrows (MOH)_2 + (MA)_2$$

For this reaction the overall equilibrium constant $K_E K_D/K_F^2$ is about 0.25. Hence the equilibrium lies to the left and the mixed dibridged complex is more stable than a mixture of the two pure dibridged species. This result may be due to strain in the 4-membered ring dihydroxo bridged species.

In the structures of the $(MA)_2$ and BMB complexes the *cis* relationship of the two nucleic bases demands that they be oriented in a non-parallel or nearly perpendicular orientation with respect to the coordination plane about Pd(II). Ignoring the ribose in both kinds of complexes the second nucleic base may be related to the first in either a head-to-head or head-to-tail arrangement. The complex with head-to-head ligands contains a plane of symmetry and hence is not chiral. The head-to-tail complex con-

tains only a $C_2$ axis without a rotation-reflection axis, the mirror image is non-superimposable, and hence the complex is chiral. When the D-ribose of the nucleoside is also considered the head-to-tail complex gives rise to two diastereomers [22]. The statistical distribution of isomers without any steric considerations is 50% head-to-head and 25% each for the pair of head-to-tail diastereomers. In the head-to-tail diastereomers both of the cytidines within a complex are related by a $C_2$ axis and are chemical shift equivalent. Since both diastereomers are present in solution there is a doubling of peaks. In the head-to-head complex (with slow rotation about the metal-N3 bond in BMB) no symmetry element relates the two cytidines, they are chemical shift inequivalent and again, but for a different reason, doubling of peaks is expected. Thus in a solution with a statistical distribution of the three isomers there are two pairs of doublets of equal height. In our $(MA)_2$ complex with cytidine one peak from each of the doublets falls at the same chemical shift producing the apparent triplet shown in Fig. 2. This interpretation is supported by occurrence of only two peaks in the $(MA)_2$ region of spectra for the 1-methylcytosine complex. Since there are no diastereomers there is only a single head-to-tail peak. In the head-to-head complex the two cytosine rings are now related by a symmetry plane, they are chemical shift equivalent, and only a singlet appears.

The proton NMR spectrum of a cytidine–$enPd^{2+}$ mixture displayed in Fig. 2 shows at high field for $(MA)_2$ a doublet pair of peaks in a 1:2:1 intensity ratio. This ratio corresponds to that expected statistically with 50% each of head-to-tail and head-to-head isomers. Of several ways to resolve the apparent 1:2:1 triplet one is suggested by the result that only the most low field and half the center resonances appear upon prompt addition of base to attain pH > 6. The easiest way to visualize formation of $(MA)_2$ is by dimerization of MA, which contains a N3 metalated and N4 deprotonated cytosine ring. Dimerization of two MA complexes in this configuration leads directly to only the head-to-tail diastereomeric pair. Therefore we suggest that the complexes made upon prompt addition of base, with the two lowest field peaks of the apparent 1:2:1 cytidine triplet, are the head-to-tail diastereomeric pair. The other half of the center and the highest field peaks are then left for the head-to-head complex with its magnetically inequivalent cytidines. This complex requires slow addition of base or time near pH 5 to form in equilibrium amounts. A possible mechanism for formation of the $(MA)_2$ head-to-head isomer that accounts for its appearance in less basic solutions is rate limiting reaction of a neutral cytosine ring with MAM, in which an anionic cytosine ring binds metal ions at both N3 and N4.

The cytidine and 5′-CMP $(MA)_2$ complexes of $enPd^{2+}$ described here represent the first time all three isomers have been observed and identified. The dimer structure with each cytosine ring anion bridging through N3 and N4 the same two $enPd^{2+}$ to give a 8-membered ring yields a stable, inert structure that locks in the three isomers. Similar structures occur in the equimolar 1-methylcytosine complexes, but without the D-ribose the head-to-tail complex is enantiomeric.

While three distinct isomers are observed in the proton NMR spectrum of cytidine $(MA)_2$ complexes only two peaks appear in the BMB complex of Fig. 3. In the BMB complex a neutral cytosine base is bound only through N3. Rapid rotation about the Pd–N3 bond would interconvert the three isomers. However, at 360 MHz a pair of doublets of approximately equal height are present. If the pair of interligand-intracomplex hydrogen bonds from the amino group N4 hydrogen of a head cytosine to O2 of a tail cytosine that appear in the crystal structure of *cis*-$(NH_3)_2Pt(1\text{-methylcytosine})_2^{2+}$ [21] also occur in solution with $enPd(cytidine)_2^{2+}$, then the head-to-tail diastereomers may be sufficiently stabilized with respect to the head-to-head isomer so that rapid rotation does not take place. In some acidic $D_2O$ solutions containing primarily the BMB complex a broad proton resonance was observed at about 8.33 ppm which might be due to unexchanged hydrogen bound to N4. The slowed exchange is consistent with hydrogen bonding. Also only two isomeric BMB peaks of approximately equal height are observed with 5′-CMP before and after phosphate deprotonation. Assignment to the diastereomeric pair with little head-to-head isomer present is again suggested. There is a difference of 0.05 log units between the phosphate $pK_a$ values in the 5′-CMP diastereomeric BMB pair. In the case of a bis guanosine complex of Pt(II) it was necessary to use, N,N,N′,N′-tetramethylethylenediamine to slow isomer interconversion and observe two H8 peaks in the proton NMR spectrum [22]. The two peaks were assigned to the two head-to-tail diastereomers and the head-to-head isomer was assumed not to occur. In this research distinguishable peaks assignable to separate isomers were not apparent at 90 MHz for either the uridine or 5′-UMP BMB complexes. Presumably rapid interconversion occurs among all three isomers. Uracil lacks the amino group so that the stabilizing hydrogen bonding interactions suggested to occur between cytosine rings cannot take place. The mixed UMC complex of uridine and cytidine also exhibits only a single set of peaks indicating that a single cytidine N4 to uridine O2 hydrogen bond is inadequate to stabilize the head-to-tail isomers.

The cytidine H6 doublet with sharp peaks centered at 7.23 ppm is assigned to a new dibridged MA(OH)M complex. Since only one set of NMR

peaks is observed, the planes of the two enPd chelate rings should be in the same plane as the cytosine ring. Only one isomer then exists. The hydroxo bridge forms part of a 6-membered chelate ring which enforces the structure with all rings in a common plane. If the cytosine ring were perpendicular to the enPd rings the coordination center would be chiral, and with D-ribose diastereomers would occur and two sets of peaks appear. Only a single set of peaks does appear and this sterically improbable structure does not occur.

The distribution curves in Fig. 5 show that in neutral solutions dibridged $(MA)_2$ and MA(OH)M complexes are the predominant species with $enPd^{2+}$ when ligand is not in excess. They should also be the thermodynamically favored forms with *cis*-$(amine)_2$-Pt(II). With Pt(II), however, equilibrium may be difficult to achieve, though the crystal structure determination [8] proves that it is possible to make the $(MA)_2$ complex. Adenosine and guanosine also contain the $-N=C-NH_2$ disposition of donor atoms. Together with cytidine all three nucleic bases are potentially capable of making pure or mixed dimer complexes with two different A type ligands in $(MA)_2$. Thus this mode of chelation is potentially capable of occurring with three of the four nucleic bases and antitumor *cis*-$(amine)_2$Pt(II).

## Acknowledgement

This research was supported by a grant from the National Science Foundation.

## References

1 D. J. Nelson, P. L. Yeagle, T. L. Miller and R. B. Martin, *Bioinorg. Chem.*, *5*, 353 (1976).
2 M. C. Lim and R. B. Martin, *J. Inorg. Nucl. Chem.*, *38*, 1915 (1976).
3 I. Sovago and R. B. Martin, *Inorg. Chem.*, *19*, 2868 (1980).
4 P. I. Vestues and R. B. Martin, *J. Am. Chem. Soc.*, *103*, 806 (1981).
5 R. B. Martin, Chapter 11, pp. 231–244 in 'Platinum, Gold, and Other Metal Chemotherapeutic Agents', S. J. Lippard, ed., ACS Symposium Series, No. 209, American Chemical Society, Washington, D.C., 1983.
6 K. H. Scheller, V. Scheller-Krattiger and R. B. Martin, *J. Am. Chem. Soc.*, *103*, 6833 (1981).
7 M. C. Lim and R. B. Martin, *J. Inorg. Nucl. Chem.*, *38*, 1911 (1976).
8 R. Faggiani, B. Lippert, C. J. L. Lock and R. A. Speranzini, *J. Am. Chem. Soc.*, *103*, 1111 (1981).
9 B. J. McCormick, E. N. Jaynes, Jr. and R. I. Kaplan, *Inorg. Synth. XXIII*, 216 (1972).
10 R. Stewart and M. G. Harris, *Can. J. Chem.*, *55*, 3807 (1977).
11 M. C. Lim, *J. Inorg. Nucl. Chem.*, *43*, 221 (1981).
12 K. Inagaki and Y. Kidani, *Bioinorg. Chem.*, *9*, 333 (1978).
13 W. M. Scovell and T. O'Connor, *J. Am. Chem. Soc.*, *99*, 120 (1977).
14 G. Y. H. Chu, R. E. Duncan and R. S. Tobias, *Inorg. Chem.*, *16*, 2625 (1977).
15 R. B. Martin and R. Prados, *J. Inorg. Nucl. Chem.*, *36*, 1665 (1974).
16 H. Sigel, *Angew. Chem. Int. Ed. Engl.*, *14*, 394 (1975).
17 R. B. Martin and Y. H. Mariam, *Metal Ions Biol. Syst.*, *8*, 57 (1979).
18 P. Brooks and P. D. Lawley, *J. Chem. Soc.*, 1348 (1962).
19 B. McConnell, *Biochem.*, *17*, 3168 (1978).
20 H. Sigel and R. B. Martin, *Chem. Rev.*, *82*, 385 (1982).
21 R. Faggiani, B. Lippert, C. J. L. Lock and B. Rosenberg, *J. Am. Chem. Soc.*, *99*, 77 (1977); B. Lippert, C. J. L. Lock, B. Rosenberg and M. Zvagulis, *Inorg. Chem.*, *17*, 2971 (1978).
22 R. E. Cramer and P. L. Dahlstrom, *J. Am. Chem. Soc.*, *101*, 3679 (1979). See also A. T. M. Marcelis, H.-J. Korte, B. Krebs and J. Reedijk, *Inorg. Chem.*, *21*, 4059 (1982).
23 J. D. Orbell, L. G. Marzilli and T. J. Kistenmacher, *J. Am. Chem. Soc.*, *103*, 5126 (1981).