Inorganic Chemistry, Vol. 16, No. 10, 1977 **2625**

Contribution from the Department of Chemistry,
Purdue University, West Lafayette, Indiana 47907

Heavy Metal–Nucleoside Interactions. 10. Binding of *cis*-Diammineplatinum(II) to Cytidine and Uridine in Aqueous Solution: Necessary Conditions for Formation of Platinum–Uridine "Blues"[1,2]

GRACE Y. H. CHU, ROBERT E. DUNCAN, and R. STUART TOBIAS*

Received October 23, 1976 AIC60769U

Both cytidine and uridine react with $(H_3N)_2Pt^{II}$ and $enPt^{II}$ in aqueous solution over a wide pH range. The reactions with cytidine are complete within a few hours, while those with uridine are considerably slower. In both cases mono and bis complexes are formed. In acidic solution, $(H_3N)_2Pt^{II}$ behaves as a bifunctional electrophile toward cytidine, while at pH 7 it acts as a unifunctional electrophile. The equilibrium constant for $[(H_3N)_2Pt(OH_2)_2]^{2+}$ + Cyd ⇄ $[(H_3N)_2PtCyd(OH_2)]^{2+}$ + H_2O was estimated from Raman spectra to have the value log K = *ca.* 2.9 (0.1 M ClO_4^-). The hydroxo ligand produced by hydrolysis of $[(H_3N)_2Pt(OH_2)_2]^{2+}$ is an effective blocking group. Raman and ^1H and ^{13}C NMR spectra all identify N(3) as the sole binding site. In particular, no interaction occurs at high pH with a deprotonated amine group. With uridine, the Raman and ^1H and ^{13}C NMR experiments all show that binding occurs at N(3) of the conjugate base $UrdH_{-1}^-$. No reaction takes place under comparable conditions with 3-methyluridine. The equilibrium constant for $[(H_3N)_2Pt(OH_2)_2]^{2+}$ + $UrdH_{-1}^-$ ⇄ $[(H_3N)_2PtUrdH_{-1}OH_2]^+$ has a value log $K \geq 9.6$ (0.1 M ClO_4^-), comparable to $H_3CHgOH_2^+$. The selectivity of the bases in a native polynucleotide for binding the substitutionally inert $(H_3N)_2Pt^{II}$ and related species is kinetic not thermodynamic in origin as also is the case with alkylating agents. Binding will occur preferentially at nonprotonated sites rather than N(3) of uridine or thymidine or N(1) of guanosine, the principal binding sites for labile heavy metal species. Kinetic and thermodynamic effects governing heavy metal binding are discussed. The binding behavior of labile species such as H^+ or CH_3Hg^+ is of little use in predicting the reactions of the inert platinum(II) complexes. At pH 6–7, the monouridine complex undergoes a change which causes small shifts in the ^1H NMR spectrum. Exposure of solutions containing equimolar $(H_3N)_2Pt^{II}$ and uridine with pH ≤8 to the atmosphere results in the characteristic platinum–uracil "blue". The Raman spectra suggest that the principal species in these solutions is the colorless $[(H_3N)_2PtUrdH_{-1}OH_2]^+$.

Introduction

The reactions between $(H_3N)_2Pt^{II}$ and cytidine should be relatively simple. This nucleoside has one good donor site, N(3) (pK—H^+, 4.2[3]), although there is evidence for coordination of CH_3Hg^{II} to the amino group[4] accompanied by proton loss, but only to a small extent and at high pH.[5] In a study of the effect of *cis*-$[PtCl_2(NH_3)_2]$ on the UV spectrum of cytidine, Mansy et al.[6] observed no reaction in strongly acidic solutions (pH ≤0.8), reaction between pH 5.6 and 10.5, and little reaction at higher pHs. Coordination to N(3) + $C(4)NH_2$ or C(2)O was suggested. The cytidine concentration was 10^{-4} M; and r, the $[(H_3N)_2PtCl_2]$:[Cyd] concentration ratio, was 10. A comparison of the UV difference spectra for cytidine in the presence of CH_3Hg^{II} [4] where coordination is at N(3)[4,5] and in the presence of $(H_3N)_2Pt^{II}$ [6] shows that they are identical within the experimental error. From studies of the UV spectrum at pH 6.5, Scovell and O'Connor[7] have calculated a value for the equilibrium constant of the reaction $[(H_3N)_2Pt(OH)OH_2]^+$ + Cyd ⇄ $[(H_3N)_2Pt(OH)Cyd]^+$, log K = 3.6. There was no evidence for formation of a bis complex under their experimental conditions, total cytidine 10^{-4} M, 1 ≤ r ≤ 8. Roos, Thomson, and Eagles[8] observed a reaction in which 1-methylcytosine replaced one chloride of *cis*-$[PtCl_2(NH_3)_2]$ to yield $[Pt(NH_3)_2Cl(1\text{-MeCyt})]Cl$. Kong and Theophanides[9] studied the reaction between equimolar $[Pt(dien)Cl]^+$ and cytidine monophosphate without pH control using ^1H NMR. Small shifts occurred upon reaction, and H(5) but not H(6) was reported to exhibit coupling to ^{195}Pt which would be consistent with coordination at N(3).

In the compound *trans*-dichloro(3-(diisopropyl sulfoxide)-1-methylcytosine-*N*)platinum(II),[10] the 1-methylcytosine ligand is coordinated through N(3). Bond lengths within the base are the same as those of 1-MeCytH^+, although the bond angles resemble those of 1-MeCyt more closely. The platinum electrophile appears to have considerably less effect on the ring geometry than the proton does.

In studies of reactions of platinum complexes with native DNA's, a number of workers have correlated the rate of reaction or the amount of platinum bound to the G-C base content. Mansy[6b] observed that the rate of reaction of $PtCl_2(NH_3)_2$ with DNA's increased with the G-C content. Robins[11] compared the rates of reaction of $Pt(en)Cl_2$ with several purines at 37 °C without pH control. From chromatographic profiles, it was found that the second-order rate constant for coordination to guanosine was over an order of magnitude larger than for cytidine. Only a 1:1 complex appeared to be formed with cytidine. This technique presupposes that the complexes are inert during the chromatographic separations. Stone, Kelman, and Sinex[12] observed large increases in buoyant density of DNA at pH 6.5 upon binding of platinum, and the increase was proportional to the G-C content as well as the ratio of total platinum:DNA phosphate. Munchausen and Rahn[13] found using radioactive ^{195m}Pt that bound platinum increased with the G-C content. Analysis of cyclobutadipyrimidine yields upon UV irradiation after acetophenone sensitization indicated that the platinum species was bound selectively to guanosine when $r < 0.1$. At higher r values, cytosine cyclobutadipyrimidine formation is enhanced, consistent with sensitization of the cytosine by platinum coordination. Examination of chromatograms of DNA subjected to mild formic acid hydrolysis, which depurinates the DNA, indicated that guanine but not adenine was complexed by platinum at low platinum:phosphate ratios. It must be assumed that the formic acid treatment has no effect on the platinum nucleotide complexes. Reaction of $PtCl_2$en with 1-β-D-arabinofuranosylcytosine gives a nonstoichiometric compound which appears to have one 1-β-D-arabinofuranosylcytosine per Pt^{II}en.[14]

It has been generally stated that uridine and thymidine do not react with the *cis*-$(H_3N)_2Pt^{II}$ species. No interaction of ca. 10^{-4} M uridine or thymidine with *cis*-$[PtCl_2(NH_3)_2]$ at 37 °C was observed in studies of the UV absorption.[6] In mass spectrometric studies of the products formed by reaction of *cis*-$[PtCl_2(NH_3)_2]$ in aqueous solution, no reaction was found with 1-methylthymine.[8] Kong and Theophanides[9] reported that ^1H NMR spectra of solutions of [Pt(dien)Cl]Cl (dien = diethylenetriamine) and uridine showed no evidence for any reaction. Munchausen and Rahn[13] could detect no platinum

binding in reactions of *cis*-$[PtCl_2(NH_3)_2]$ with poly(dT) at pH 7, using ^{195m}Pt and measurements of the thymidine triplet decay times.

In contrast to the reports described above implying nonreactivity of uridine and thymidine is the observation that *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ or its partially hydrolyzed products react with a number of uracil derivatives to yield "platinum blues".[15,16] Among these are uracil itself, 1-methyluracil, thymine, 1-methylthymine, uridine deoxyribose, thymidine,[15] and poly(U).[17] The production of these blue complexes also depends upon the presence of oxygen. Some initial studies of their antitumor activity have been reported.[18,19]

In a previous report,[20] we have examined the reactions between $(H_3N)_2Pt^{II}$ and the purine nucleoside inosine over a wide pH range. In this work, we have used Raman plus ^1H and ^{13}C NMR spectroscopy to identify the species formed with the pyrimidine nucleosides cytidine and uridine. Of particular interest to us was the chemical effect of platinum coordination to the bases. The reactions of $(H_3N)_2Pt^{II}$ and $enPt^{II}$ (en = ethylenediamine) over a wide range of pH have been examined to study the effect of hydrogen ion concentration. Comparison of these data with the spectra of the CH_3Hg^{II} complexes of cytidine and uridine also provides a test of the hypothesis that heavy metal coordination to a particular site on a nucleoside should give a Raman difference fingerprint applicable to binding reactions of other heavy metals. The $(H_3N)_2Pt^{II}$–uridine system also has been examined as a function of pH in both the presence and the absence of oxygen to study the reactions involved in the formation of the platinum blue.

Experimental Section

Platinum Complexes. Solutions of diaquodiammineplatinum(II) perchlorate were prepared as described previously.[20] Solutions of the trifluoromethanesulfonate for use in the NMR studies were prepared similarly from crystalline $[(D_3N)_2Pt(OD_2)_2](F_3CSO_3)_2$. This compound was synthesized by reacting *cis*-$[PtCl_2(NH_3)_2]$ with a solution of AgF_3CSO_3 in D_2O in the dark. The solvent was evaporated at ca. 35 °C, and the product was collected and dried in a desiccator over P_4O_{10}. Anal. Calcd for $PtN_2D_{10}F_6C_2S_2O_8$: N, 4.89; D, 3.51; F, 19.9; C, 4.19; S, 11.2; Pt, 34.0. Found: N, 4.91; D, 3.54; F, 20.0; C, 4.16; S, 11.3; Pt, 34.0. $[enPt(OD_2)_2](F_3CSO_3)$ was prepared in a similar way from $Pt(en)Cl_2$. Anal. Calcd: C, 8.04; H, 3.38; S, 10.7. Found: C, 8.30; H, 3.21; S, 10.8. The triflate salts were used in place of the perchlorates employed in the previous studies in order to avoid the explosion hazard. Solutions were prepared by dissolving weighed amounts in H_2O or D_2O (99.8%, Columbia Organic Chemicals, Columbia, S.C.).

Nucleoside Solutions. These solution were prepared as described earlier[20] using cytidine obtained from Cyclo-Chemical, Los Angeles, Calif., and Heterocyclic Chemical Corp., Harrisonville, Mo., uridine from Heterocyclic Chemical, and 3-methyluridine from Sigma Chemical Co., St. Louis, Mo.

Raman and NMR Titrations. Solutions were prepared by mixing stock solutions of the aquoplatinum(II) complex and of the nucleoside in H_2O or D_2O to give the desired r = Pt(II):nucleoside ratio. The pH was adjusted using NaOH (NaOD) or $HClO_4$ ($DClO_4$) solutions. It was measured with a Radiometer PHM-4 meter, and the values for D_2O were corrected as described by Glascoe and Long.[21] To obtain correct pH values, a pH-stat type of experiment must be done. This is especially true with uridine where the reaction is rather slow and the pH changes over several days.

Raman Spectra. The general procedures for Raman difference spectroscopy and Raman spectrophotometric titrations have been described in previous papers.[20,22] The computerized Raman difference spectrophotometer used also has been described.[23] For the absorbing solutions of the platinum blue, the spinning ligand sample cell device manufactured by Cary-Varian, No. 8240400, was used. In all the Raman spectra, the ClO_4^- ν_1 band provided an internal frequency and intensity reference. Integrated intensities were obtained from large scale plots (20 × 40 in.) using a Gelman planimeter.

Nuclear Magnetic Resonance Spectra. The general procedure for NMR titrations has been described previously.[20,22] The ^1H spectra were determined with a Varian XL-100 (100 MHz) spectrometer using a CAT. The internal reference was $N(CH_3)_4^+$. The D_2O solvent provided the lock signal. The probe temperature was ca. 30 °C. The ^{13}C spectra were obtained with a Varian CFT-20 (20 MHz); the probe temperature was 35 °C. The $N(CH_3)_4^+$ cation also was used as the internal reference for the ^{13}C spectra. Coupling with ^{14}N, $I = 1$, causes this signal to be a distinct triplet with $^1J(^{13}C-^{14}N)$ = 4.0 Hz.

Data and Results

Diammineplatinum(II)–Cytidine System. Raman–pH Profile for the 1:1 System at 25 °C. Raman difference spectra (RADS) were recorded for solutions 25 mM in cytidine and 25 mM in $(H_3N)_2Pt^{II}$ (i.e., r = $(H_3N)_2Pt^{II}$:nucleoside = 1) in H_2O vs. 25 mM cytidine at pH values of 1.5, 3, 5, 7, 9, and 11 to establish the effect of pH on the reaction. Since the pK for the N(3) proton of $CydH^+$ is 4.22 at 25 °C,[3] the cytidine reference is protonated at pH 1.5 and 3 but not appreciably at pH 5. The extent of the reaction can be followed using the integrated intensity of the Cyd or $CydH^+$ band at ca. 780 cm^{-1} which shifts ca. 10 cm^{-1} higher in frequency upon complex formation. Above pH 5, the disappearance of the ca. 1290-cm^{-1} Cyd band with complex formation can be followed.

The spectra of the solutions with r = 1 indicate almost quantitative complexation has occurred at pH 5 and 7. Reaction at pH 9 and 11 is incomplete, although the difference spectra were almost identical with those for pH 5 and 7. At pH 3, the contour of the envelope in the 784–794-cm^{-1} region indicates ca. 50% of the cytidine is complexed. This gives an approximate value for the equilibrium constant of $[(H_3N)_2Pt(OH_2)_2]^{2+}$ + Cyd ⇌ $[(H_3N)_2PtCyd(OH_2)]^{2+}$ + H_2O, log K = ca. 2.9 (0.1 M ClO_4^-). This is somewhat smaller than the value of Scovell and O'Connor obtained from UV absorption measurements, log K = 3.6,[7] which should be more accurate. At pH 1.5, there was very little reaction, and the difference spectrum showed weak features suggesting a slight interaction between $[(H_3N)_2Pt(OH_2)_2]^{2+}$ and $CydH^+$ had occurred. The parent spectra are illustrated in Figure 1 of the supplementary material.

The Pt–N stretching vibrations occur in the 500–600-cm^{-1} region, and the difference spectra of $(H_3N)_2Pt^{II}$ + Cyd vs. Cyd, r = 1, from pH 3 to 11 are illustrated in Figure 1. The pH 3 spectrum has a band at 558 cm^{-1} due both to ν_s and ν_{as} of $[(H_3N)_2Pt(OH_2)_2]^{2+}$ [20] and probably also to the stretch of the Pt–N bond trans to H_2O in $[(H_3N)_2PtCyd(OH_2)]^{2+}$. The two spectra taken for solutions with pH < p*K_1 of $[(H_3N)_2Pt(OH_2)_2]^{2+}$ (log *K_1 = −5.6)[24,25] also exhibit a band at 530 cm^{-1} which probably arises mainly from stretching of the Pt–N bond trans to Cyd. Spectra for solutions with pH ≥7 show a single, broad band at 540 cm^{-1} that is assigned to $[(H_3N)_2PtCydOH]^+$. This is accidentally coincident with the band for $[(H_3N)_2Pt(OH)_2]$.[20] Although there are two distinct Raman-active Pt–N stretching modes, these appear to be accidentally degenerate. The bis complex observed at pH 5.4 and 6.3 exhibits one band at 533 cm^{-1}. These frequencies are consistent with an increasing ligand trans influence in the order H_2O < OH^- < Cyd.

The Raman frequencies are collected in Table I of the supplementary material.

Reaction Rate. The reaction is relatively rapid even at high pH, and 25 mM cytidine solutions with r = 0.5 and 1.0, pH 11, gave spectra which were essentially the same when recorded 3 h, 42 h, 6 d and 6 h, 45 h, and 6 d after mixing, respectively. Consequently, it may be assumed that all $(H_3N)_2Pt^{II}$–Cyd solutions described were substantially at equilibrium.

Raman Determinations of the Stoichiometry of the Reaction between Cytidine and *cis*-$(H_3N)_2Pt^{II}$ in H_2O (25 °C). pH 4.5. A Raman spectrophotometric titration of 25 mM Cyd with $(H_3N)_2Pt^{II}$ was carried out at pH 4.5, below p*K_1 of the platinum(II) complex, where it should be present primarily as $[(H_3N)_2Pt(OH_2)_2]^{2+}$ and complex formation should be

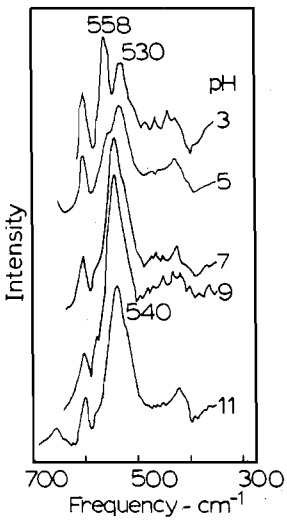


Figure 1. The Raman difference–pH profile of the ν(Pt–N) region for solutions of $(H_3N)_2Pt^{II}$ + Cyd, r = 1, in H_2O; total Cyd 25 mM vs. solvent (0.1 M $NaClO_4$).

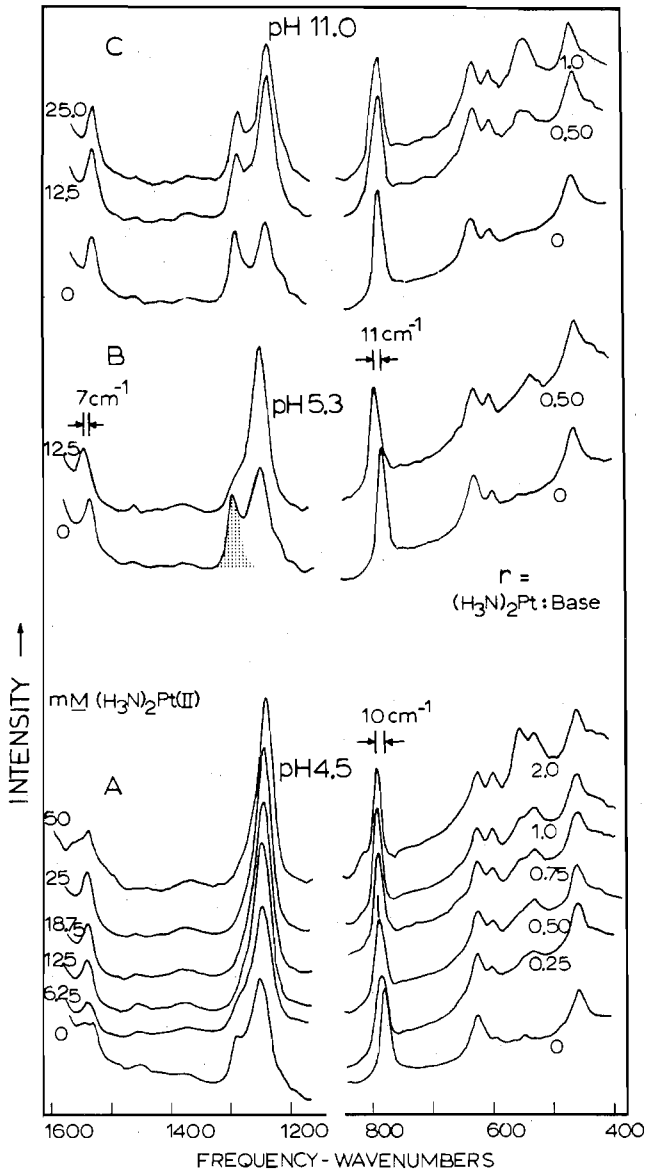


Figure 2. Raman spectrophotometric titration of 25 mM Cyd with $(H_3N)_2Pt^{II}$ in H_2O at 25 °C: A, pH 4.5; B, pH 5.3 (the shaded band can be used to monitor the reaction); C, pH 11.0.

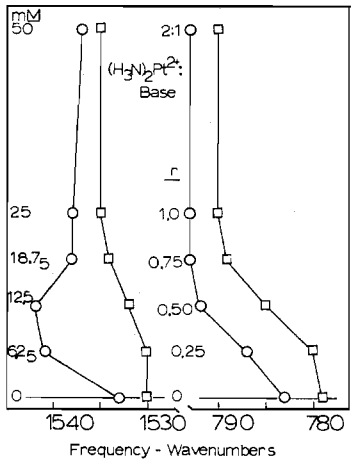


Figure 3. Variation in frequency of the ring modes of cytidine in H_2O solution, 25 °C, as a function of the $(H_3N)_2Pt^{II}$ concentration: ○, pH 4.5; □, pH 7.

extensive. Because the ligand is a mixture of Cyd and $CydH^+$ at this pH (pK_1(Cyd) = 4.3) and the spectra of the proton and $(H_3N)_2Pt^{II}$ complexes are very similar, the spectral changes upon the addition of the platinum complex are relatively small. The spectra are illustrated in Figure 2A. The band at 1292 cm^{-1} characteristic of Cyd disappears, the band at ca. 780 cm^{-1} due both to Cyd and $CydH^+$ increases 10 cm^{-1} in frequency, and the band at 604 cm^{-1} characteristic of the complex appears.

The $[(H_3N)_2Pt(OH_2)_2]^{2+}$ cation is a strong electrophile, and there is evidence for some reaction of it with $[(H_3N)_2PtCyd(OH_2)]^{2+}$. With r = 2, shoulders appear on the high-frequency side of the 790- and 1535-cm^{-1} bands, suggesting a secondary reaction at a site other than N(3).

The shifts in the band maxima in the 780-and 1540-cm^{-1} regions with increasing $(H_3N)_2Pt^{II}$ concentrations are illustrated in Figure 3. These indicate an essentially 2:1 stoichiometry. The frequency of the ca. 1540-cm^{-1} band decreases above r = 0.5 because the secondary reaction described above splits this into two components, the lower of which is the more intense.

In summary these data indicate the following equilibria describe the system at pH 4.5 as the r value increases:

$$[(H_3N)_2Pt(OH_2)_2]^{2+} + 2Cyd \rightleftharpoons [(H_3N)_2Pt(Cyd)_2]^{2+} + 2H_2O \quad (1)$$

$$[(H_3N)_2Pt(Cyd)_2]^{2+} + [(H_3N)_2Pt(OH_2)_2]^{2+} \rightleftharpoons 2[(H_3N)_2PtCyd(OH_2)]^{2+} \quad (2)$$

$$[(H_3N)_2PtCyd(OH_2)]^{2+} + (n-1)[(H_3N)_2Pt(OH_2)_2]^{2+} \rightleftharpoons [[(H_3N)_2Pt(OH_2)]_nCyd]^{2n+} + (n-1)H_2O \quad (3)$$

pH 5.3. Complex formation was examined at this pH where cytidine is present almost entirely as the neutral base, and the platinum(II) complex is partially hydrolyzed. The spectral changes are considerably more pronounced and are illustrated in Figure 2B. The bis complex is formed almost quantitatively.

pH 7. Because there are marked differences between the spectra of cytidine and its platinum(II) complex, the reaction at pH 7 can be followed easily by a Raman titration as was the case at pH 5.3. This is illustrated in Figure 2 of the supplementary material. In this case, the platinum species should be mainly $[(H_3N)_2Pt(OH)(OH_2)]^+$. Characteristic shifts occur in the bands at ca. 780 and 1530 cm^{-1} and the band positions are not constant until $r \simeq 1$. This is illustrated in Figure 3. During complex formation, the band at ca. 1290 cm^{-1} disappears and the one at ca. 1240 cm^{-1} increases in intensity. Variation in the integrated intensity of these two bands with total $(H_3N)_2Pt^{II}$ concentration also indicates an approximately 1:1 reaction and this is illustrated in Figure 3

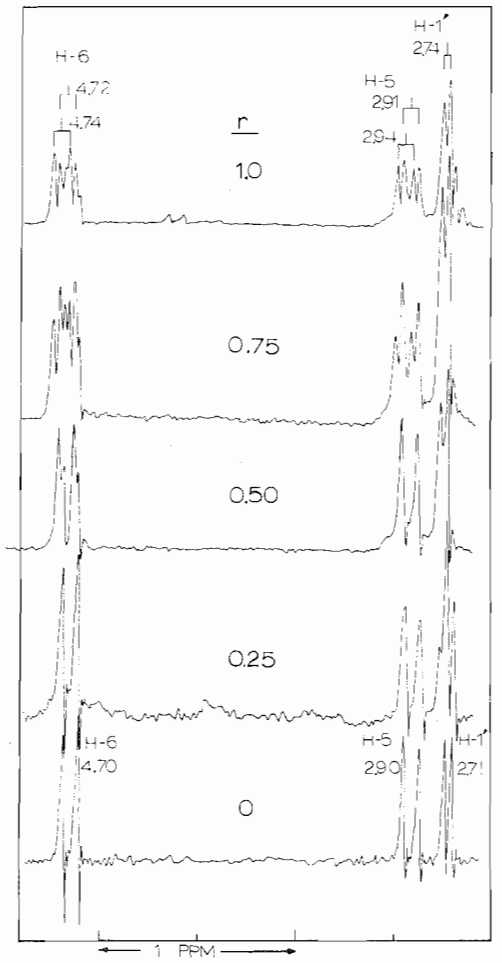


Figure 4. ^{1}H NMR (100 MHz) titration of 25 mM Cyd with enPt^{II} in D_2O, pD 5.4.

of the supplementary material. There is no evidence for a secondary reaction at high $(H_3N)_2Pt^{II}$ concentrations as was observed at pH 4.5. The only reaction important under these conditions at pH 7 is

$$[(H_3N)_2Pt(OH)(OH_2)]^+ + Cyd \rightleftharpoons [(H_3N)_2Pt(OH)Cyd]^+ + H_2O \quad (4)$$

pH 11. A Raman titration at pH 11 indicated that rather little reaction occurs. The data are illustrated in Figure 2C. Here the principal platinum species is $(H_3N)_2Pt(OH)_2$. The difference spectrum of 25 mM Cyd + 25 mM $(H_3N)_2Pt^{II}$ vs. 25 mM Cyd, pH 11.3, is identical with the corresponding pH 5 spectrum. This shows that the same binding site is occupied in acidic and alkaline solutions. Only reaction 5 is significant

$$(H_3N)_2Pt(OH)_2 + Cyd \rightleftharpoons [(H_3N)_2Pt(OH)Cyd]^+ + OH^- \quad (5)$$

at pH 11.

Nuclear Magnetic Resonance Studies of the Reaction of *cis*-$(H_3N)_2Pt^{II}$ or enPt^{II} and Cytidine in D_2O. ^{1}H NMR. Solutions 25 mM in $(D_3N)_2Pt^{II}$ and 25 mM in Cyd, i.e., concentrations corresponding to the Raman experiments, were prepared with pD 2.3, 3.5, 5.4, 6.4, 7.8, 9.0, and 10.0. The pD 2.3 spectrum was characteristic of $CydD^+$, and the solutions with pD from 3.5 to 7.8 gave evidence of complex formation, while the pD 9.0 and 10.0 solutions indicated little reaction had occurred.

Solutions 25 mM in cytidine and with enPt^{II} to give r from 0 to 1.0 were prepared with pD 5.4. This corresponds to the Raman titration, Figure 2B. Since the positions of the cytidine resonances are very pH dependent when N(3) protonation is occurring, it also is advantageous with ^{1}H NMR spectroscopy to work at a pH above this pK. The spectra are illustrated in Figure 4.

Table I. ^{1}H NMR Chemical Shifts (ppm)[a]

Species	H(5)	H(6)	H(1′)
$CydH^+$[b]	3.06	4.98	2.72
Cyd[c]	2.90	4.70	2.71
$[enPtCyd(OH_2)]^{2+}$[c]	2.94	4.74	2.74
$[enPt(Cyd)_2]^{2+}$[c]	2.91	4.72	2.74
Urd[d]	2.70	4.66	2.72
$UrdH_{-1}^-$[e]	2.62	4.50	2.74
$enPt(UrdH_{-1})_2$[d]	2.45	4.36	2.65
$[enPt(UrdH_{-1})OH_2]^+$[f]	2.61	4.51	2.76
$enPt(UrdH_{-1})OH$[d]	2.54	4.48	2.72

[a] Relative to internal $N(CH_3)_4^+$. These can be converted to shifts relative to 3-(trimethylsilyl)-1-propanesulfonate by adding 3.18 ppm. [b] 25 mM; measured at pH 2.3. [c] 25 mM total Cyd, measured at pH 5.4. [d] 25 mM total Urd; measured at pH 8.5. [e] 25 mM; measured at pH 11. [f] 25 mM; measured at pH ≤7.

With r = 0.25, signals due to free cytidine are observed plus those of a new species, complex "A", with the H(5) and H(6) resonances shifted downfield relative to cytidine. These occur at 2.91 and 4.72 ppm relative to internal $N(CH_3)_4^+$. With r = 0.5, the signal due to free cytidine is quite low in intensity. At r = 0.75, a trace of the free cytidine still persists, the signals due to complex "A" are the principal resonances, and a new set of resonances due to complexed cytidine, complex "B", appear in the H(5) and H(6) regions at 2.94 and 4.74 ppm. With r = 1, the signals due to "B" are somewhat more intense than those of "A".

The ^{1}H NMR data are consistent with the Raman data if complex "A" is $[enPtCyd_2]^{2+}$ and "B" is $[enPt(Cyd)(OD_2)]^{2+}$ and the pertinent reactions are analogous to (1) and (2). Since coordination of cytidine should decrease the aquo acidity of platinum slightly, it is unlikely that $[enPtCydOD]^+$ is a significant species at this pH. Chemical shift data are collected in Table I.

No coupling of ^{195}Pt to either H(5) or H(6) could be detected for either complex. Solutions 0.25 M in enPt^{II} and 0.50 M in cytidine also were examined, and still no coupling could be detected. The methylene resonance of enPt^{II} occurs as a sharp signal, −0.43 ppm (upfield) relative to $N(CH_3)_4^+$, and $^3J(^{195}Pt-^1H)$ is 48 Hz. The sharpness of the resonance indicates that it arises from a complex where both ethylenediamine methylene groups are equivalent on the ^{1}H NMR time scale, i.e., from $[Pt(en)(Cyd)_2]^{2+}$. This signal may be compared to that of $[Pt(en)(OD_2)_2]^{2+}$: δ = −0.62 ppm, $^3J(^{195}Pt-^1H)$ = 53 Hz.

^{13}C NMR. For comparison with the Raman and ^{1}H NMR spectra, natural-abundance, proton-decoupled ^{13}C spectra were obtained with D_2O solutions, pD 6.5. These were prepared with $[Pt(en)(OD_2)_2](F_3CSO_3)_2$. The spectra are illustrated in Figure 5, and the chemical shift data are collected in Table II. For these experiments it was necessary to use very concentrated solutions, total cytidine 0.5 M. Consequently, some caution must be used in comparing the results to those of the Raman and ^{1}H NMR experiments. In addition, with r > 0.5, pD <8, there was a slow decomposition. In a similar manner, cytidine reduces Ag^+ slowly to the metal.[26]

The solutions with r = 0.25 exhibit resonances due to both free and complexed cytidine. At r = 0.5, there is an essentially quantitative complexation of the cytidine corresponding to the formation of $[Pt(en)Cyd_2]^{2+}$. The C(6) and most of the ribose carbon resonances are two bands separated by only a few tenths of 1 ppm suggesting that the two ligands are not strictly equivalent. For the solutions with r = 0.25 and 0.50, the ethylenediamine carbons give a single sharp resonance, δ =

Table II. ^{13}C NMR Chemical Shifts (ppm)[a]

Species	C(2)	C(4)	C(5)	C(6)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	en
$CydH^{+}$[b]	92.9	103.8	39.7	89.0	35.0	18.7	13.8	29.0	5.1	
Cyd[c]	102.0	110.6	40.8	86.4	35.0	18.7	14.0	28.5	5.5	
$[enPt(Cyd)_2]^{2+}$[c,d]	99.3	109.9	40.1	86.3	35.8	19.1	13.6	28.6	5.2	−7.7
				86.9		18.8	13.9	28.9		
										−6.2
$[enPtCyd(OH_2)]^{2+}$[c]	99.0	110.1	39.9	86.6	35.9	18.8	13.8	28.7	5.3	−7.0
						19.1	13.6	28.5		−7.2
										−8.3
Urd[e]	96.5	111.0	47.0	86.5	34.1	18.4	14.2	28.9	5.5	
$UrdH_{-1}^{-}$[f]	104.1	121.3	47.6	85.2	34.5	18.2	14.4	28.5	5.8	
$enPt(UrdH_{-1})_2$[d,e]	101.6	118.2	46.1	84.0	35.1	18.3	14.2	28.5	5.7	−7.8
										−6.6
$enPt(UrdH_{-1})(OH)$[e]	102.0	118.5	46.4	84.6	35.0	18.5	14.2	28.5	5.7	−7.4
				84.3						−8.2
										−8.4

[a] Relative to $N(CH_3)_4^{+}$. [b] 0.2 M $CydH^{+}Cl^{-}$; measured at pH 2.1. [c] 0.5 M; measured at pH 6.5. [d] Taken from the spectrum with r = 0.5. [e] 0.5 M; measured at pH 8.5. [f] 0.5 M; measured at pH 11.1.

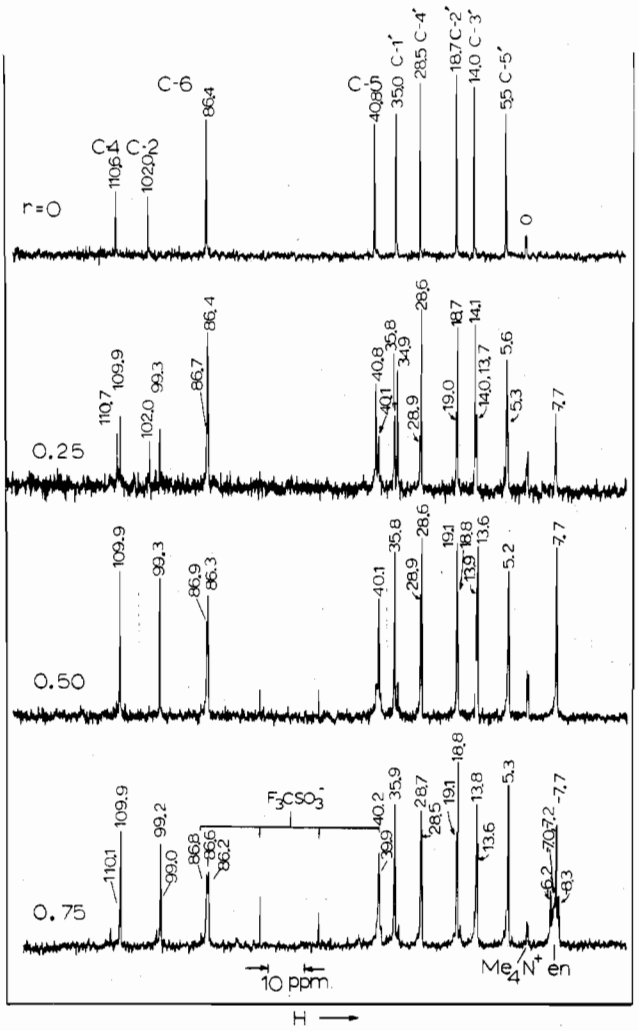


Figure 5. ^{13}C NMR (20 MHz) titration of 0.5 M Cyd with $enPt^{II}$ in D_2O, pD 6.5.

−7.8 ppm (upfield) from $N(CH_3)_4^{+}$, consistent with the formation of the bis complex where the two carbons are approximately equivalent. The en resonance may be compared to that observed for $[Pt(en)(OH_2)_2]^{2+}$, −6.7 ppm. With r = 0.75, new weak signals appeared for the cytidine carbons, and the en resonance showed two signals in addition to that due to $[Pt(en)(Cyd)_2]^{2+}$. Because of the thermodynamic instability of these concentrated solutions, no further studies were made of them.

Diammineplatinum(II)–Uridine System. Reaction Rate in D_2O at 25 °C. In contrast to the reaction with cytidine in which equilibrium was attained at 25 °C within a few hours, the reaction with uridine is considerably slower. Except at high pH, the pH also falls as reaction occurs, and it is necessary to prepare the solutions by the pH-stat procedure. Figure 4 of the supplementary material illustrates changes with time in the Raman spectra for a solution with r = 1, pH 8.5. The extent of the reaction can be monitored using the shift in the 779-cm^{-1} band to 795 cm^{-1} or the decrease in scattering at 1299 or 1692 cm^{-1} as complex formation occurs. Reaction is approximately half complete after 40 h and essentially complete after 9 d. These solutions were saturated with N_2 and carefully protected from atmospheric oxygen. Another sample saturated with O_2 behaved in an identical fashion, and reaction was almost complete at the end of 6.5 d.

Raman–pH Profile for the 1:1 $(H_3N)_2Pt^{II}$–Urd in H_2O System at 25 °C. Spectra were obtained with solutions 25 mM in total Urd, r = 1, at pH values of 3, 7, and 11, and the solutions were maintained under a N_2 atmosphere. Since the pK for the N(3) proton of uridine is ca. 9.5 in H_2O at 25 °C[3] and is probably about 0.5 unit larger in D_2O, the ligand is present as the undissociated base in all but the pH 11 solution.

The difference spectra of $(H_3N)_2Pt^{II}$ + Urd vs. Urd showed that extensive reaction occurred at pH 3 and 7, while the pH 11 spectrum was just a superposition of the spectra of $(H_3N)_2Pt(OH)_2$ and $UrdH_{-1}^{-}$. (Since Urd is the normal abbreviation for uridine, we have used $UrdH_{-1}^{-}$ for the conjugate base deprotonated at N(3).) These data indicate that reactions 6 and 7 occur at low and neutral pH, respectively.

$$[(H_3N)_2Pt(OH_2)_2]^{2+} + Urd \rightleftharpoons [(H_3N)_2Pt(UrdH_{-1})(OH_2)]^{+} + H_3O^{+} \quad (6)$$

$$OH^{-} + [(H_3N)_2Pt(OH)OH_2]^{+} + Urd \rightleftharpoons (H_3N)_2Pt(UrdH_{-1})(OH) + 2H_2O \quad (7)$$

^1H NMR–pH Profile for the $enPt^{II}$–Urd System in D_2O. Solutions 25 mM in uridine and 25 mM in $[Pt(en)(OD_2)_2](F_3CSO_3)_2$, r = 1, were examined at pD values of 2.1, 3.0, 4.1, 5.1, 6.0, 7.0, 8.0, 9.1, 9.9, and 11.1. These were maintained under an N_2 atmosphere, heated to 40 °C for 3 h in a water bath, and the pH was maintained at the stated value by addition of NaOD over a period of several days. The spectra are illustrated in Figure 6. Reaction was extensive even at pD 2.1 where signals due to free uridine plus a complexed species were observed. For this new species, complex "A", the H(5) and H(6) resonances occurred upfield relative to free uridine at 2.61 and 4.51 ppm, respectively (internal $N(CH_3)_4^{+}$). The H(1′) resonance shows only a very

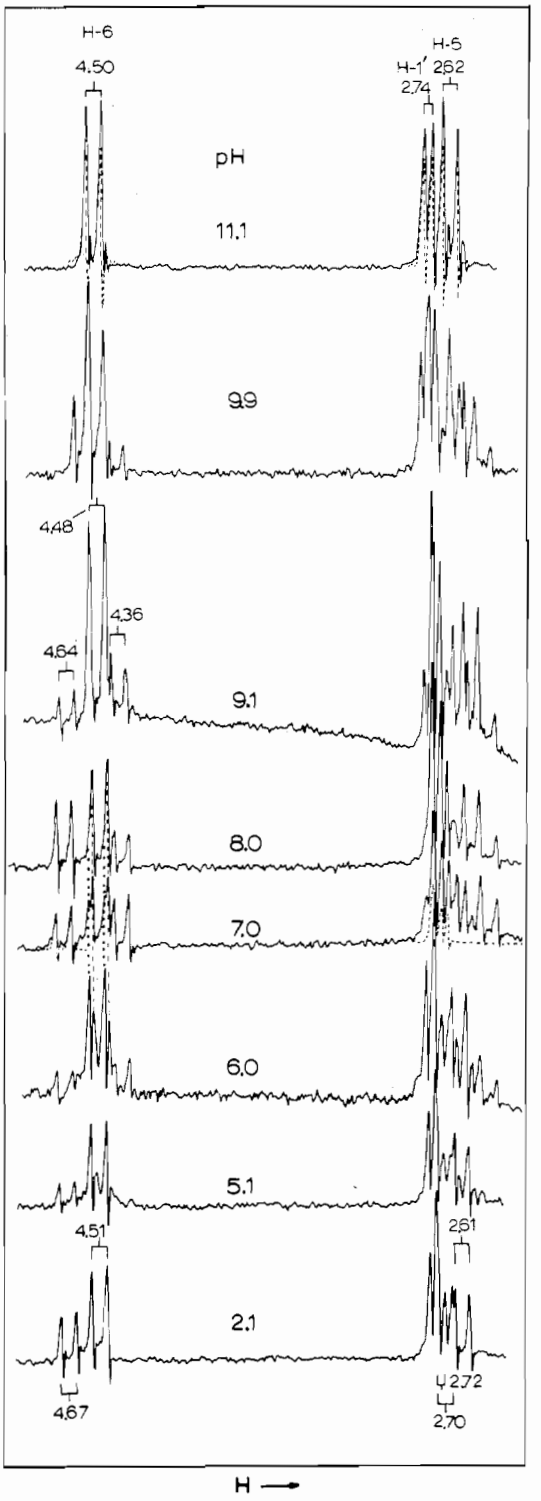


Figure 6. ^{1}H NMR (100 MHz) spectra of 25 mM Urd + 25 mM enPt^{II} in D_2O as a function of pH. The broken line at pH 11.1 is the spectrum of the conjugate base $UrdH_{-1}^-$, while that at pH 7 gives the spectrum of uridine.

small downfield shift and occurs at 2.76 ppm. The spectra show no significant change as the pD increases until above pD 5.

At pD 6, the resonances in the H(5), H(1′) region are quite complex, while four distinct H(6) doublets can be recognized. Two are due to a small amount of unreacted uridine and to complex "A". A new resonance occurs 0.03 ppm upfield from that due to "A", assigned to complex "B", and a weak resonance occurs further upfield at 4.36 ppm, complex "C". As the pH is increased to 7, the intensity of the "A" resonance

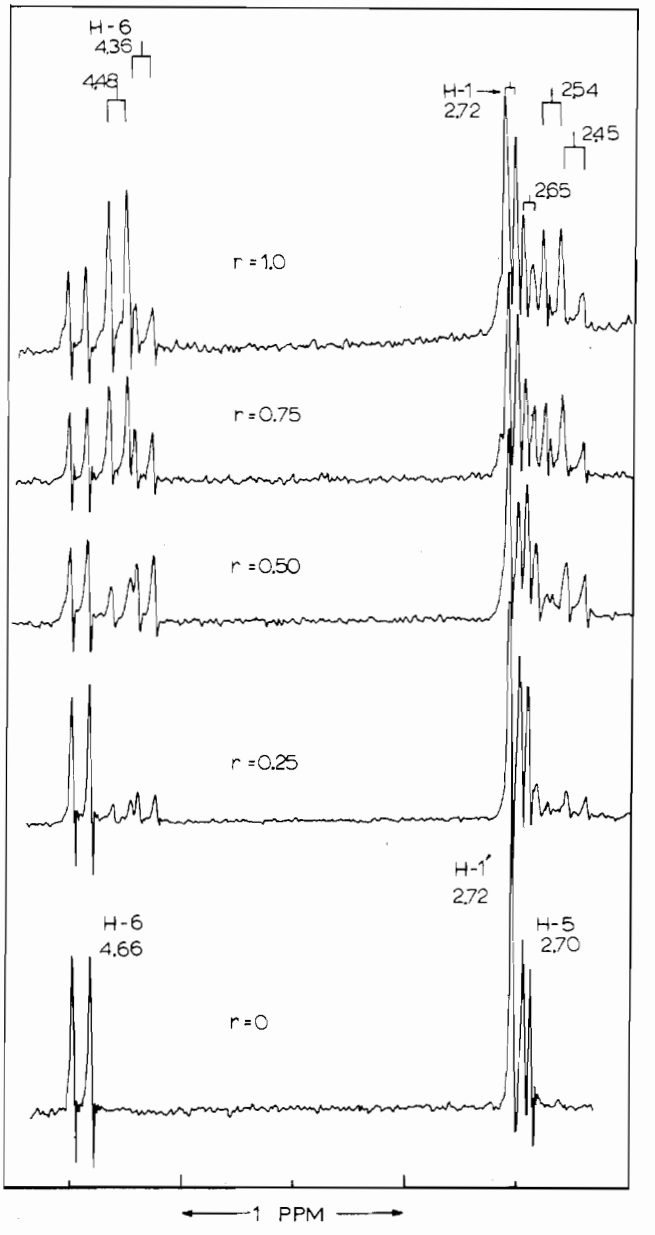


Figure 7. ^{1}H NMR (100 MHz) titration of 25 mM Urd with enPt^{II} in D_2O, pD 8.5.

decreases, that of "B" increases, and the resonances due to uridine and complex "C" are unshifted. The $^3J(^1H(5)^1H(6))$ values for all four H(6) resonances are the same, 8 Hz.

At pD 9.9, the uridine resonances shift upfield as N(3) is deprotonated and averaging over the resonances of Urd and $UrdH_{-1}^-$ occurs. The pD 9.9 spectrum shows, in addition, signals due to "B" and "C". At pD 11.1, only the spectrum of $UrdH_{-1}^-$ occurs. The anion resonances are almost identical with those of complex "A" produced in strongly acidic solution.

^{1}H NMR Studies of enPt^{II}–Urd Solutions with Varying Stoichiometry in D_2O (pD 8.5). Solutions were prepared with total uridine 25 mM plus $[Pt(en)(OD_2)_2]^{2+}$ in D_2O, 25 °C, to give r values from 0 to 1, pD 8.5. These were maintained under a N_2 atmosphere. The spectra are illustrated in Figure 7. With $r = 0.25$, signals in the H(6) region due to unreacted uridine are observed plus signals corresponding to the complexes "B" and "C". Of these the resonance of "C" is the more intense. With $r = 0.5$, the concentration of free uridine has decreased, while that of complexed uridine has increased. "B" and "C" are present in approximately the same ratio as when $r = 0.25$. With $r = 0.75$ and 1.0, the concentration of free

uridine has decreased further and the signals due to complex "B" increase progressively in intensity.

From these data, it can be concluded that complex "B" is the mono complex which, at pD 8.5, should be Pt(en)-$UrdD_{-1}(OD)$, while "C" is the bis complex $Pt(en)(UrdD_{-1})_2$.

By analogy with the spectrum of complex "B", "A" should be a mono complex. These have H(5), H(6), and H(1′) resonances at 2.54, 4.48, and 2.72 and at 2.61, 4.51, and 2.76 ppm, respectively, downfield from $N(CH_3)_4^+$. The $^3J(^1H(5)^1H(6))$ coupling is 8 Hz throughout. The change from "A" to "B" occurs in the pD 6–7 range and has characteristics of a proton-transfer reaction with a pK of ca. 6.5. Since signal averaging does not occur on the ^1H NMR time scale—both species give signals at pD 6 and 7—this cannot be a simple proton transfer but must be accompanied by some slow process. Chemical shift data are collected in Table I.

The sharp signals in the H(5) region, in general, seem to be less intense than the corresponding ones in the H(6) region. This is not due to deuteration of C(5), because the H(6) resonances are doublets in all spectra. It may be due to unresolved satellites due to ^{195}Pt coupling with H(5).

These data indicate that the bis complex also is produced to a small extent, reaction 8.

$$(H_3N)_2Pt(UrdH_{-1})OD + Urd \rightleftharpoons (H_3N)_2Pt(UrdH_{-1})_2 + D_2O \qquad (8)$$

^{13}C NMR Studies on $enPt^{II}$–Urd Solutions with Different Stoichiometry in D_2O (pD 8.5). Solutions with total uridine 0.5 M, r from 0 to 1.0, were prepared and maintained under N_2. The spectra are illustrated in Figure 8. The solution with r = 0.25 exhibits resonances due to unreacted uridine plus those due to complexed uridine. The en resonance is a sharp signal at −7.8 ppm (upfield) relative to $N(CH_3)_4^+$. This indicates that the complex has two essentially equivalent ligands bound to $enPt^{II}$; i.e., it is complex "C", the bis complex. With r = 0.5, only traces of unreacted uridine remain. C(2), C(4), and C(6) all give two resonances separated by a few tenths of 1 ppm with one member much more intense than the other and these logically are due to mono and bis complexes. In addition to the sharp en resonance at −7.8 ppm, weak additional resonances occur at −6.7 and −8.5 ppm suggesting the presence of an unsymmetrical complex. At r = 1, the signals assigned to mono and bis complexes are of roughly comparable intensity, and the en resonance has become quite complex.

In the sugar, the C(1′) and C(4′) resonances shifted measurably upon formation of the bis complex at low r, while the shifts of C(2′), C(3′), and C(5′) were too small to determine. As the mono complex is produced at high r values, two C(3′) resonances occur. The chemical shift data are collected in Table II.

Raman Study of the $(H_3N)_2Pt^{II}$–3-Methyluridine System. A Raman spectrum of a solution in H_2O with total 3-MeUrd 25 mM, r = 1, pH 8, was obtained. This was just a superposition of the spectrum of $(H_3N)_2Pt^{II}$ and that of 3-MeUrd. No measurable reaction had occurred after 4 d, a time in which reaction in the comparable uridine system was almost complete. A difference spectrum was obtained for $(H_3N)_2Pt^{II}$, 25 mM, + 3-MeUrd, 25 mM, vs. 25 mM 3-MeUrd at pH 3 to search for a weak binding process. None was observed.

Raman Study of the Formation of the $(H_3N)_2Pt^{II}$–Uridine "Blue". Solutions were made up in pairs. One member was prepared from solutions that had been saturated with N_2, and the solution was sealed under a nitrogen atmosphere. This served as the control. The other solution was either oxygenated or exposed to the atmosphere.

Solutions with 25 mM uridine, r = 1, pH from 3 to 11, remained colorless when maintained under a nitrogen atmosphere. Solutions with pH ≥8 remained colorless even when oxygenated by bubbling O_2 through them. The oxygen has no effect on the Raman spectrum as can be seen by comparison of the spectra in Figure 4 of the supplementary material for solutions saturated with N_2 and O_2.

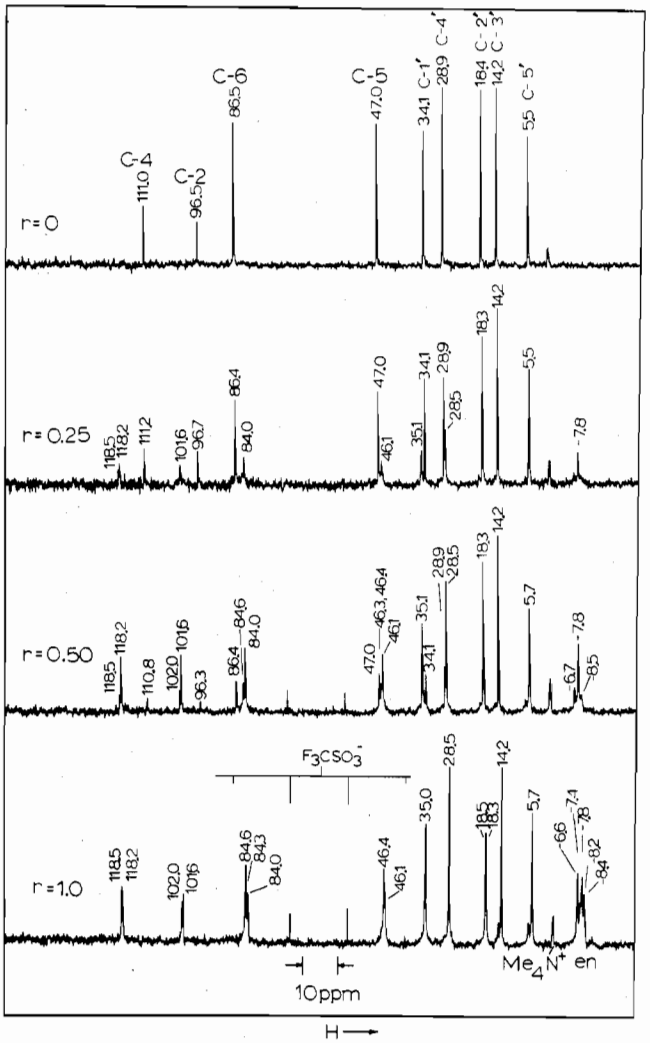


Figure 8. ^{13}C NMR (20 MHz) titration of 0.5 M Urd with $enPt^{II}$ in D_2O, pD 8.5.

The spectra of solutions with r = 1 with pHs of 3 and 8.5 show only one significant difference; at low pH the intensity of the ca. 793-cm^{-1} band decreases and a shoulder appears at 816 cm^{-1}. This is illustrated in Figure 9.

Below pH 7, the solutions react readily with molecular oxygen giving the typical blue solutions which have broad absorptions at ca. 560 and 620 nm. Figure 9 illustrates Raman spectra of $(H_3N)_2Pt^{II}$–Urd solutions, r = 1, initially at pH 7. As reaction occurs, the pH falls. Solutions maintained under nitrogen remained colorless; those exposed to oxygen turned green-blue and then deep blue. All these solutions show the characteristic shifts in the uridine vibrations characteristic of the complexation reaction discussed above. The only significant differences between the spectra of the colorless and blue solutions are that the latter have new bands at 1526, 1485 (H_2O) and 1527, 1487 cm^{-1} (D_2O). The similarity of the spectra in H_2O and D_2O indicates that the complex is essentially identical in these two solvents. Exchange of the hydroxyl protons of the ribose causes only slight changes in the free uridine spectrum, since the vibrations involving the sugar have very low Raman intensity: (H_2O) 1472, 873; (D_2O) 1460, 840 cm^{-1}.[27]

The blue color is rapidly discharged by the addition of $Fe(CN)_6^{4-}$ or simply by making the solutions alkaline. No irreversible attack on the uridine ring has occurred, because

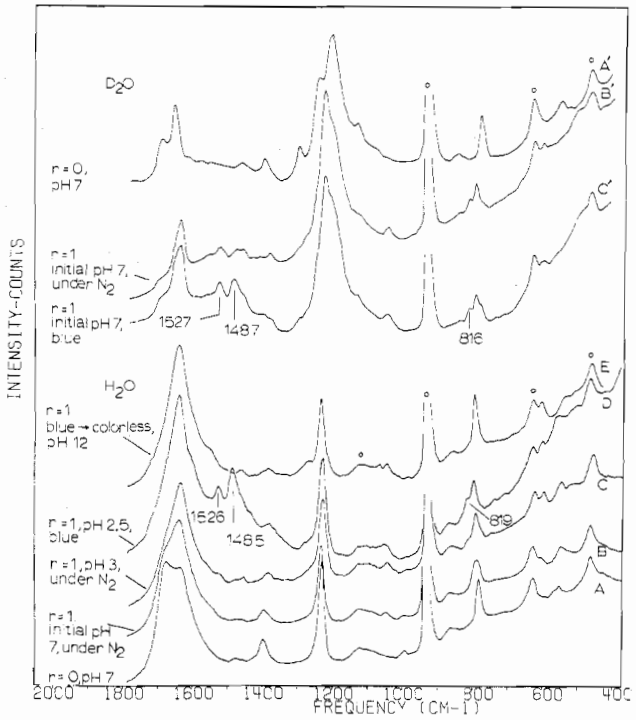


Figure 9. Raman spectra of "platinum uridine blues": A, 25 mM uridine, pH 7; B, 25 mM $(H_3N)_2Pt^{II}$ + 25 mM Urd, initial pH 7, under N_2; C, 25 mM $(H_3N)_2Pt^{II}$ + 25 mM Urd, pH 3, under N_2; D, same as C, exposed to atmosphere, blue; E, solution D, raised to pH 12.1, colorless; A′, 25 mM uridine, pD 7; B′, 25 mM $(H_3N)_2Pt^{II}$ + 25 mM Urd, under N_2, initial pH 7; C′, same as B, blue, exposed to atmosphere.

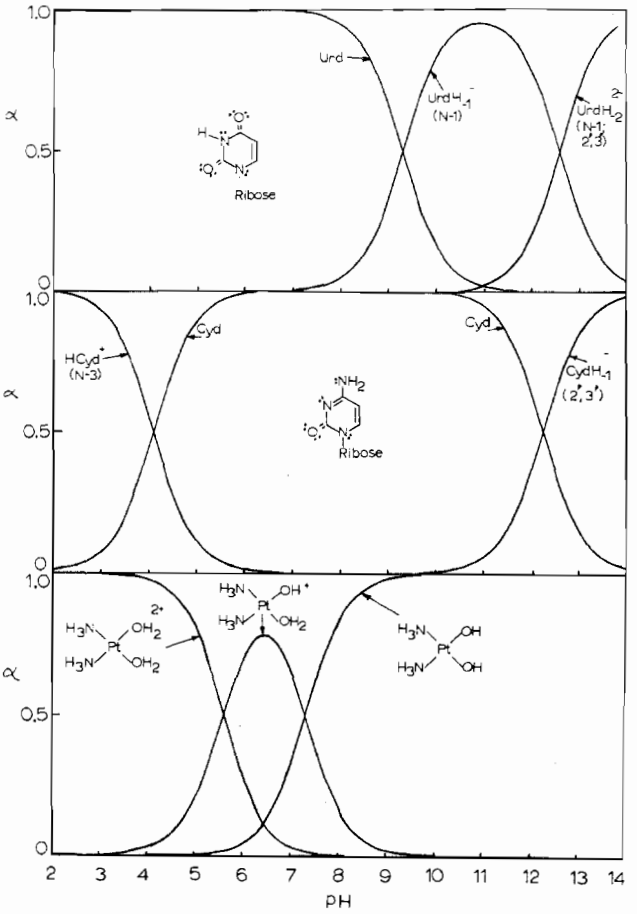


Figure 10. Species distribution in the $(H_3N)_2Pt^{II}$, cytidine, and uridine systems as a function of pH. The equilibrium constants of Jensen[24] were used for $(H_3N)_2Pt^{II}$; those of Izatt et al.,[3] for cytidine and uridine.

the colorless solution produced from the blue has the same Raman spectrum as the colorless complex above pH 7. This also is illustrated in Figure 9.

Discussion

Reaction with Cytidine. The reaction of $(H_3N)_2Pt^{II}$ or $enPt^{II}$ is quite rapid with 25 mM reagents at 25 °C, and equilibrium was attained within, at most, a few hours. Reaction is quantitative with equimolar cytidine and $(H_3N)_2Pt^{II}$ for pH ca. 4–8. With solutions which have pH ≥5.5, this reaction should increase the pH, because the $(H_3N)_2Pt^{II}$ aquo acid is removed. This can be seen from Figure 10 which describes the aquo acidity of the platinum complex. The Raman difference spectrum of the complexed cytidine measured with solutions that had $r = 1$ from pH 3 to 11 indicated only one type of cytidine binding is involved. This generally has been assumed to involve coordination at N(3), and the Raman spectrum is in accord with this. The species distribution as a function of pH is summarized in Figure 11. In Figure 12 it can be seen that the Raman difference spectrum for the $(H_3N)_2Pt^{II}$–Cyd reaction at pH 5 is very similar to the difference spectrum for the H_3CHg^{II}–Cyd system at pH 5 where only N(3) binding is important.[28] These two heavy metal electrophiles seem to cause very similar perturbations in the cytidine electron distribution. In particular it is noteworthy that the pH 11 spectrum shows no evidence for attack at the C(4) NH_2 as was observed to occur with CH_3Hg^{II} at high pH[5] and had been suggested as a possibility in the platinum reaction.[6]

The $(H_3N)_2Pt(OH_2)_2^{+}$ cation present at pH <5 is a strong bifunctional electrophile and forms $[(H_3N)_2PtCyd(OH_2)]^{2+}$ and $[(H_3N)_2PtCyd_2]^{2+}$ the latter in the presence of excess cytidine. In the presence of excess $(H_3N)_2Pt^{II}$, there is evidence for a weak additional interaction besides the binding at N(3). The nature of this interaction is unknown, but logically it would

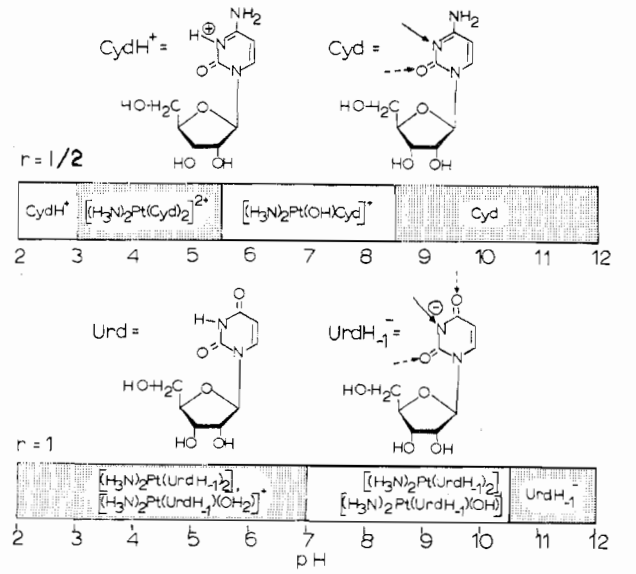


Figure 11. Ligand distribution as a function of pH for *cis*-$(H_3N)_2Pt^{II}$ + 25 mM cytidine (top) or uridine (bottom).

involve electrophilic attack at the next most basic site, the C(2) oxygen. The isopotential map for the interaction of cytosine with an external point charge has rather deep minima at both N(3) and the carbonyl oxygen.[29] This oxygen characteristically occupies an axial position in Cu(II)–cytosine or –cytidine complexes,[30,31] and recently a weak C(2)=O···Hg interaction has been observed in the crystal structure of Hg(1-MeCyd)Cl_2.[32] The Hg–N(3) and Hg–O distances are 2.17

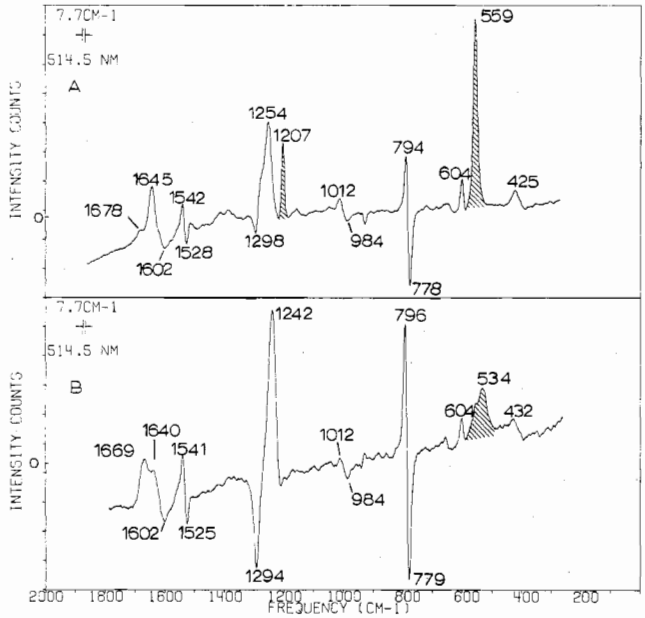


Figure 12. Raman difference spectra: A, 50 mM Cyd + 50 mM CH_3Hg^{II} vs. 50 mM Cyd, H_2O, pH 5, −18 to +47 kHz; B, 25 mM Cyd + 25 mM $(H_3N)_2Pt^{II}$ vs. 25 mM Cyd, H_2O, pH 5, −16 to 21 kHz. Shaded bands are those due to the CH_3Hg^{II} or $(NH_3)_2Pt^{II}$ internal vibrations.

(1) and 2.84 (1) Å, respectively. Because of the weakness of the metal–oxygen interaction, a bifunctional attack on a single platinum is less likely, especially in alkaline solution where the much stronger base OH^- would have to be replaced.

As the pH is raised to 7, the stoichiometry changes from 2:1 to 1:1, and at pH 7 $(H_3N)_2Pt^{II}$ acts as a unifunctional electrophile forming $[(H_3N)_2Pt(OH)Cyd]^+$. There is no evidence for interaction at other than N(3) even with excess platinum complex. The complex distribution is summarized in Figure 11.

The effects on cytidine due to platination appear to be similar to those caused by protonation although smaller in magnitude as observed in the crystal structure of $PtCl_2((i\text{-}Pr)_2SO)$(1-methylcytosine).[10] The Raman spectra of cytidine bound to $(H_3N)_2Pt^{II}$ and of $CydH^+$ are similar in both band position and intensity and differ significantly from the spectrum of cytidine itself. Alterations in the 1600–1700-cm^{-1} region indicate particularly a perturbation of the C(2)=O group. Protonation causes the ^1H resonances of H(5) and H(6) to shift downfield by 0.16 and 0.28 ppm, respectively. In $[enPtCyd(OH_2)]^{2+}$, the corresponding downfield shifts are much smaller, 0.04, 0.04 ppm, respectively. In the ^{13}C NMR spectra, only C(2) and C(4) undergo appreciable shifts upon protonation, and these move upfield by ca. 9.1 and 6.8 ppm, relatively. The ligand shifts are similar in both $[enPtCyd(OH_2)]^{2+}$ and $[enPtCyd_2]^{2+}$, and only the C(2) resonance is shifted appreciably, 2.7 ppm upfield. Rather similar behavior was observed in the reaction of cytosine and $PtCl_2$ in Me_2SO-d_6.[33] The largest shift was that of the C(2) resonance which moved upfield by 3.1 ppm. C(4) shifted upfield by 1.3 ppm and C(5) and C(6) shifted downfield by 1.1 ppm. In the reaction of $HgCl_2$ with cytidine in Me_2SO,[34] the C(2) resonance shifts upfield by 3 ppm, C(4) shifts upfield by 2.5 Hz, and C(5) and C(6) move downfield by 1.0 and 1.1 ppm. In general, all these effects suggest that heavy metal binding at N(3) has a very similar effect and does not alter the charge distribution in the cytosine ring greatly. The main effect appears to be localized in the N(3)–C(2)=O region.

Since the $^3J(^1H(5)–^1H(6))$ coupling of ca. 8 Hz always was observed for the complexed cytidine resonances, it can be concluded that no attack occurred at the C(5) position under these mild experimental conditions. This has been observed to take place rapidly with mercuric acetate, perchlorate, and nitrate at 50 °C.[35] No ^{195}Pt–H(5) coupling could be detected even with solutions which had total cytidine 0.5 M, $r = 1$, and by using a 100-MHz spectrometer. Such coupling had been reported[9] and cited as evidence for N(3) coordination of platinum.

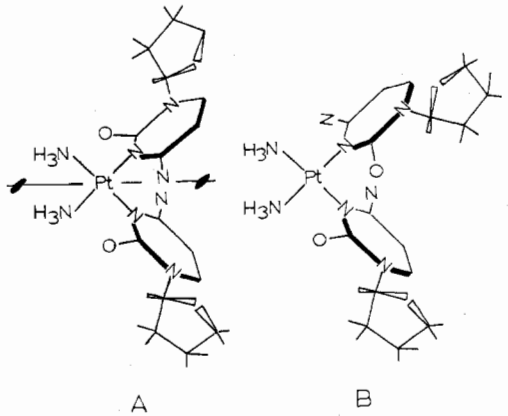


Figure 13. Models for the arrangement of two cytidine molecules in the bis complex.

While the Raman and ^{13}C NMR spectra are essentially the same for cytidine in the mono and the bis complexes, the ^1H resonances are slightly different. The ^1H resonances assigned to the bis complex $[enPt(Cyd)_2]^{2+}$ are 0.03 (H(5)) and 0.02 (H(6)) ppm upfield from those of $[enPtCyd(OH_2)]^{2+}$. These small differences are accurate, since both sets of resonances can be observed in the same spectrum. A shift in this direction would be expected from any ring current diamagnetic anisotropy in the bis complex. The downfield shift due to platination and the upfield shift of the bis relative to the mono complex are what was observed with the $(H_3N)_2Pt^{II}$–inosine system, except the magnitudes with cytidine are much smaller as expected for pyrimidines relative to purines. The H(5) and H(6) protons of pyrimidines do not show a significant concentration dependence, apparently because of the minimal ring current effect.[36] Also the platinum would be expected to be approximately in the least-squares plane of a given cytidine requiring the rings to be relatively far apart. Reasonable structures for the bis complex are illustrated in Figure 13. The ^{13}C NMR spectra suggest that both species or a structure such as B in Figure 13 with nonequivalent riboses is present in solution, since the C(6), C(2′), C(3′), and C(4′) resonances all occur as two signals separated by 0.3–0.5 ppm. Interconversion of structures A and B of Figure 13 would be expected to be slow on the NMR time scale.

Reaction with Uridine. The reaction of $(H_3N)_2Pt^{II}$ or $enPt^{II}$ with uridine is much slower than the reaction with cytidine, inosine,[20] or guanosine.[37] This accounts for the discrepancy between the reports stating that uridine and thymidine do not react[6,8,13] and the discussions concerning the syntheses of $(H_3N)_2Pt^{II}$–uracil and –uridine blues. In general, the former studies were carried out with uridine concentrations $\leq 10^{-4}$ M, while the latter generally recommend reagent concentrations above 0.1 M.[18] It is likely that the reaction is so slow with the dilute solutions that no significant complex formation occurred during the time of the experiments. Although the ^1H NMR study of the [Pt(dien)Cl]Cl–uridine system,[9] which indicated no reaction, appears to have been carried out with rather concentrated reagents, the chloride is a poorer leaving group than water, and reaction undoubtedly is slow.

Uridine actually reacts with $(H_3N)_2Pt^{II}$ and $enPt^{II}$ over a wider pH range than cytidine does. Appreciable complex formation occurs with $r = 1$, for $3 \leq pH \leq 9$. The species

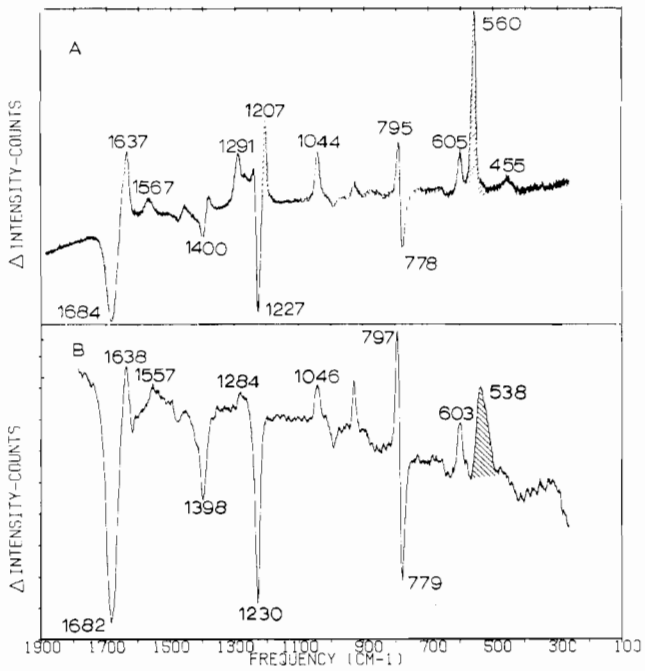


Figure 14. Raman difference spectra: A, 50 mM Urd + 50 mM H_3CHg^{II} vs. 50 mM Urd, H_2O, pH 7; B, 25 mM Urd + 25 mM $(H_3N)_2Pt^{II}$ vs. 25 mM Urd, H_2O, pH 7. Shaded bands are those due to the CH_3Hg^{II} of $(H_3N)_2Pt^{II}$ internal vibrations.

distribution is summarized in Figure 11. There is little doubt that binding is to N(3) with displacement of the proton. The difference spectrum for the $(H_3N)_2Pt^{II}$–Urd reaction at pH 7 is very much like the difference spectrum for the H_3CHg^{II}–Urd reaction at the same pH where binding is to N(3) of the conjugate base.[28] The complex spectrum is the same in H_2O and D_2O, indicating displacement of the proton at N(3). See Figure 14. In addition, if N(3) is blocked by methylation, i.e., 3-methyluridine is the reactant, no complexation occurs under conditions that give extensive reaction with uridine. Both mono and bis complexes are formed at pH 8.5. Above pH 8, these reactions should have little effect on the solution pH; below pH 8, they will cause a pH decrease.

Some deprotonation of the ligand is involved in the $(H_3N)_2Pt^{II}$– and $enPt^{II}$–uridine binding reactions; greater changes in the ligand electron distribution occur than in the reaction with cytidine. As was noted in the Raman studies on the binding of CH_3Hg^{II} to uridine[28,38] and thymidine,[39] the spectrum of the coordinated ligand is rather like that of the conjugate base indicating somewhat similar electron distributions in the two. Upon deprotonation of Urd, the base ^1H NMR signals move upfield by 0.08 (H(5)) and 0.16 (H(6)) ppm. The H(1′) signal is affected little and moves only 0.02 ppm downfield. The corresponding shifts upon formation of $enPt(UrdH_{-1})OH$ are 0.16, 0.18, and 0.00 ppm, respectively. It is to be noted that coordination of platinum(II) to uridine results in upfield shifts of H(5) and H(6) relative to the ligand, while binding to cytidine causes smaller downfield shifts. This is to be expected if proton loss is involved in the reaction with uridine.

In the ^{13}C NMR, the largest shifts upon platination again occur for the C(2) and C(4) resonances, 5.1 and 7.2 ppm downfield. All other resonances change by ≤1.5 ppm. Deprotonation of uridine also causes downfield shifts that are, however, larger: C(2), 7.6; C(4), 10.3 ppm. In a study of the reaction of $HgCl_2$ with uridine in Me_2SO where proton transfer *cannot* occur and only binding to the neutral ligand is possible, it was observed that all the uridine ^{13}C resonances were essentially the same in the presence or absence of the $HgCl_2$.[34] Consequently, the Raman and ^1H and ^{13}C NMR spectra in this study all indicate binding to the conjugate base over a very wide pH range.

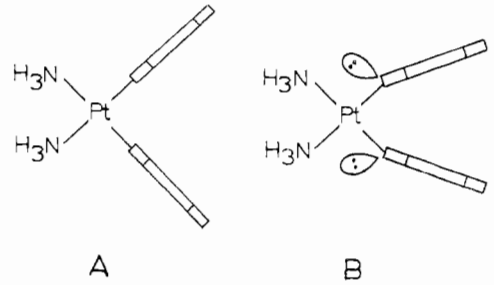


Figure 15. Comparison of the geometries expected for coordination of (A) two cytidine molecules and (B) two uridine conjugate bases.

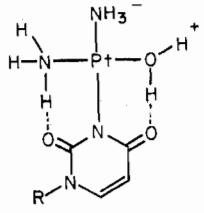
It is tempting to attribute the larger shifts of the ^{13}C resonances at C(2) and C(4) of both cytidine and uridine to the fact that platination takes place at N(3). However, upon platination at N(7) of inosine, a large shift occurs at C(2).[40] Apparently greater changes in electron delocalization occur upon metalation at those carbons bearing electronegative substituents.

The ^1H spectra always exhibit the $^3J(^1H(5)–^1H(6))$ coupling of ca. 8 Hz, so no attack occurs at the C(5) position. This position of uridine also can be mercuriated.[35] The ^1H spectra for the mono and bis uridine complexes also differ, while the Raman and ^{13}C NMR spectra were essentially the same. Clearly, these latter two experiments are most sensitive to electronic changes within the ligand brought about by complexation. The ^1H resonances assigned to the bis complex are 0.09 (H(5)) and 0.12 (H(6)) ppm upfield relative to the mono complex. This is a considerably greater effect than observed with cytidine and is consistent with a greater ring current diamagnetic anisotropy arising from $UrdH_{-1}^-$ compared to Cyd^0 and, probably, with a closer interaction between the bases in the uridine case as illustrated in Figure 15. The metal is usually significantly out of the least-squares plane of the uridine or thymidine ring. Since completion of this work, chemical shifts for the mono and bis complexes of $enPt^{II}$ have been reported by Lim and Martin and are essentially the same as the values reported here for $enPt(OH)UrdH_{-1}$ and $enPt(UrdH_{-1})_2$.[41] These authors added base to solutions of $[enPt(OH_2)_2]^{2+}$ plus uridine and heated them to bring about reaction.

The most peculiar spectroscopic effect observed in the reaction with uridine is the downfield shifts of the H(5), H(6), and H(1′) resonances of the mono complex that occur between pH 7 and 5. This occurs only with the mono and not with the bis complex. Since both resonances are observed in the intermediate pH range, a process slow on the NMR time scale must be involved. This rules out a simple proton transfer, although the fact that it occurs only with the monouridine complex over a pH range approximately that involved in the ionization $[(H_3N)_2Pt(OH_2)_2]^{2+} \rightleftarrows H^+ + [(H_3N)_2Pt(OH)(OH_2)]^+$ (see Figure 12) suggests that proton transfer to the coordinated hydroxo occurs as the pH is decreased. A slow reaction could result if a secondary binding reaction involving another site besides N(3) occurred with $[enPt(OH_2)UrdH_{-1}]^+$ but not with $enPt(OH)UrdH_{-1}$. This could involve displacement of the coordinated water molecule by the uridine ligand bound to this platinum center or displacement by uridine coordinated to another platinum(II). Such a process would be both thermodynamically and kinetically favorable compared to displacement of the hydroxo ligand of $enPt(OH)UrdH_{-1}$.

The sharp signals in the ^1H NMR spectra tend to rule out a binuclear or polynuclear species. Because of the deprotonation at N(3) and the concomitant increase in negative charge on the two carbonyl oxygens, they should have some

donor character, more for example than the C(2)=O oxygen of cytosine which is observed to coordinate.[30-32] A significant axial interaction with Pt(II) seems unlikely, and a 90° rotation of the ligand from the position illustrated in Figure 15 with displacement of H_2O would give a rather unfavorable chelate geometry. One explanation, but by no means a unique one, would be a strong intramolecular hydrogen bond from the coordinated water and/or ammonia molecule to either the C(2) or C(4) carbonyl oxygens or to both, e.g., I. The possible



I

significance of this interaction will be discussed below in the consideration of the platinum "blue" formation.

General Observations on Heavy Metal–Nucleotide Reactions. From the results discussed above, it is clear that much of the selectivity exhibited by the bases of polynucleotides upon reaction with $PtCl_2(NH_3)_2$ or $[(H_3N)_2Pt(OH_2)_2]^{2+}$ and the hydrolysis products is kinetic rather than thermodynamic. Mercurials such as Hg^{2+} or CH_3Hg^+ bind most strongly at N(3) on the Ura or Thy bases and then to N(1) of Gua upon reaction with native polynucleotides. The equilibrium constant for reaction 9 is log $K = 9.0$.[4] From estimates of the extent

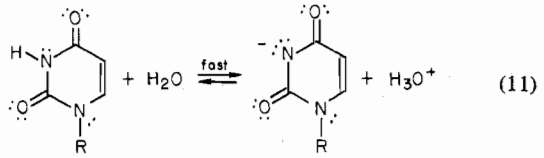
$$H_3CHgOH_2^+ + UrdH_{-1}^- \rightleftharpoons H_3CHgUrdH_{-1} + H_2O \quad (9)$$

of complexation of $(H_3N)_2Pt(OH_2)_2^{2+}$ by uridine at pH 3 from the Raman spectrum and at pH 2.3 from the ^1H NMR, the equilibrium constant for (10) must be log $K \geq 9.6$; i.e., it is

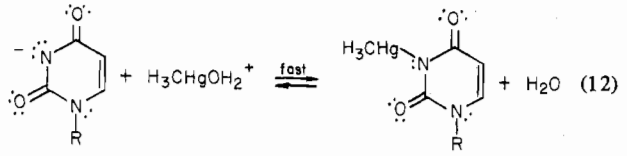
$$[(H_3N)_2Pt(OH_2)_2]^{2+} + UrdH_{-1}^- \rightleftharpoons [(H_3N)_2Pt(UrdH_{-1})OH_2]^+ + H_2O \quad (10)$$

comparable to the value for H_3CHg^{II}. Nevertheless binding to thymidine in DNA is not the major reaction with $(H_3N)_2Pt^{III}$.[6b,12,13]

The Hg^{2+} or H_3CHg^+ cations are substitutionally labile, and the reactions at either protonated or unprotonated sites are fast. It seems reasonable to assume that the reaction in solution involves electrophilic attack on the conjugate base in equilibrium with uridine, reactions 11 and 12. With sub-

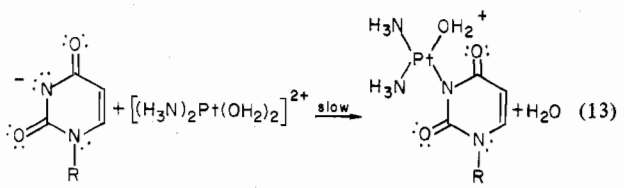


(11)



(12)

stitutionally inert platinum(II) complexes, the reaction analogous to (12), (13), will be very slow in acidic or neutral



(13)

solution because of the low concentration of $UrdH_{-1}^-$. Decreasing the pH will tend to slow the reaction because of the decrease in the $UrdH_{-1}^-$ concentration, but the protonation of hydrolyzed platinum species will tend to increase the rate because water is a better leaving group than hydroxide. Although bridge splitting reactions should be thermodynamically favorable, the presence of polycondensed species such as $[(H_3N)_2Pt(OH)_2Pt(NH_3)_2]^{2+}$[42,43] also will tend to decrease the rate of reaction at pH ca. 7.

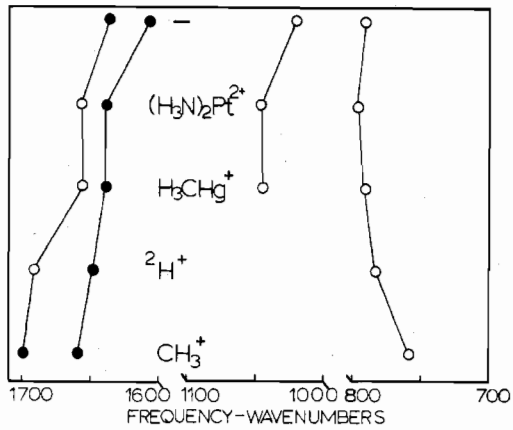


Figure 16. Effect on several electrophiles on the frequencies of the uridine conjugate base, $UrdH_{-1}^-$, upon coordination at N(3). The top set of points is for $UrdH_{-1}^-$ itself.

The principal consequence of the slowness of substitution at platinum(II) is that the reactions will be kinetically controlled as suggested earlier.[44] Platinum(II) complexes will bind preferentially to the unprotonated sites N(3) of cytidine, N(7) of guanosine, N(7) of inosine, and N(1) and N(7) of adenosine, rather than to the protonated sites N(3) of thymidine or uridine or N(1) of guanosine or inosine. Although this probably results in a nonequilibrium situation, rearrangement to produce the stable isomer will be very slow. Because of this factor, the behavior of the proton or H_3CHg^{II} cannot be used to predict the binding sites involved in platination of polynucleotides where the behavior is more like that with an alkylating agent.

Figure 16 illustrates the perturbing effect of four electrophiles CH_3^+, $^2H^+$, H_3CHg^+, and $(H_3N)_2PtOH^+$ on several vibrations of the uridine conjugate base $UrdH_{-1}^-$. The vibrations chosen are ones where the shifts appear to reflect electronic charges in the nucleotide rather than mechanical effects. For this reason, the deuteron was chosen instead of the proton. It can be seen that the effects of the two heavy metals are very similar, and the frequencies indicate that there is more electron delocalization than in the protonated species. The carbonium electrophile appears to give even less electron delocalization than present in the protonated species; i.e., it behaves quite differently from the heavy metals.

Necessary Conditions for Formation of the $(H_3N)_2Pt^{II}$–Uridine Blue. Much has been written about the synthesis of the platinum–uracil blues[15,16,18] and even their clinical use as an antitumor drug.[18,19] The preparations reported have been found to be mixtures of four or five components.[18] From the spectroscopic studies on the reactions of $(H_3N)_2Pt^{II}$ and $enPt^{II}$ with uridine, it is clear that the conditions of the "blue" syntheses lead to the formation of complexes of $UrdH_{-1}^-$. Since the standard synthesis[18] is carried out at pH 6.5 with $r = 1$ where the 25 mM solutions in the absence of oxygen contained three platinum–uridine complexes plus small amounts of unreacted uridine, it is not suprising that mixtures result.

If the $(H_3N)_2Pt^{II}$ solutions are maintained at pH >8, no blue color develops even when oxygen is bubbled through the solution. The species which is very readily oxidized to the paramagnetic blue complex is the cation $[(H_3N)_2PtUrdH_{-1}]^+$

which may contain a water molecule in the first coordination sphere of the platinum(II). The oxidation does not involve any major attack on the uridine ring, since the blue complex can be converted back to the colorless $(H_3N)_2Pt(OH)UrdH_{-1}$ simply by increasing the pH. The N(3)-bound uridine is necessary for the ready formation of the blue species; for example, acidic solutions of $(H_3N)_2Pt^{II}$ and 3-methyluridine remain colorless upon oxygenation. In addition, the same ready formation of the blue species was not observed for solutions containing $[enPtUrdH_{-1}]^+$. The high frequency and intensity of the scattering at ca. 1485 and 1526 cm^{-1} characteristic of the blue species indicate that the coordinates that participate in these vibrations involve multiple bonds, i.e., that they arise from the uridine. There also may be resonance enhancement of the intensity since the solutions are absorbing. Since it seems likely that oligomeric species are formed, this may occur by bridging involving both N(3) and the two oxygens. Recently Barton et al.[45] have determined the crystal structure of a blue species $[Pt_2(NH_3)_4(C_5H_4NO)_2]_2(NO_3)_5$ prepared by reaction of *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ and α-pyridone. This contains platinum with an average oxidation state of 2.25 and Pt–Pt interactions. One *cis*-$(H_3N)_2Pt^{II}$ has two nitrogens from two α-pyridonate ligands which bridge to a second *cis*-$(H_3N)_2Pt^{II}$ via the exocyclic oxygens. The Pt–Pt distance is 2.779 Å. Two of these binuclear complexes are linked by a second kind of Pt–Pt bond. It appears that the formation of Pt–Pt bands upon oxidation probably forces bridging by the α-pyridonates rather than vice versa. The sharpness of the ^1H NMR signals of the complexes of the conjugate base of uridine observed in this work are in marked contrast to the broad signals observed for analogous complexes of purine nucleotides where polynuclear complexes certainly exist.[20,46] It is possible that the Raman scattering characteristic of the blue solutions at 1485, 1526 cm^{-1} is caused by a further perturbation of the uridine conjugate base caused by C(2)=O and/or C(4)=O coordination.

Acknowledgment. We thank Engelhard Industries and Matthey Bishop, Inc., for loans of platinum and Dr. Henry Peresie for helpful discussions.

Registry No. $[(D_3N)_2Pt(OD_2)_2](F_3CSO_3)_2$, 63609-27-8; $[enPt(OD_2)_2](F_3CSO_3)_2$, 63609-30-3; $CydH^+$, 63600-30-6; Cyd, 65-46-3; $[enPtCyd(OH_2)]^{2+}$, 63609-24-5; $[enPt(Cyd)_2]^{2+}$, 63609-25-6; Urd, 58-96-8; $UrdH_{-1}^-$, 63609-72-3; $enPt(UrdH_{-1})_2$, 63609-15-4; $[enPt(UrdH_{-1})OH_2]^+$, 63609-16-5; $enPt(UrdH_{-1})OH$, 63609-17-6; $[(H_3N)_2Pt(Cyd)_2]^{2+}$, 63609-18-7; $[(H_3N)_2Pt(OH)Cyd]^+$, 63609-28-9; $[(H_3N)_2Pt(UrdH_{-1})_2]$, 63609-19-8; $[(H_3N)_2Pt(UrdH_{-1})(OH_2)]^+$, 63609-20-1; $[(H_3N)_2Pt(UrdH_{-1})(OH)]$, 63609-21-2; $(H_3N)_2Pt^{II}$, 20115-64-4; $enPt^{II}$, 50475-23-5; CH_3Hg^{II}, 18042-02-9; *cis*-$(D_3N)_2Pt(UrdH_{-1})(OD)$, 63609-22-3; [*cis*-$(H_3N)_2PtCyd(OH_2)]^{2+}$, 63609-23-4; ^{13}C, 14762-74-4.

Supplementary Material Available: Raman spectra (5 pages). Ordering information is given on any current masthead page.

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