Heavy Metal–Nucleotide Interactions. 8. Binding of *cis*-Diammineplatinum(II) and Ethylenediamineplatinum(II) to Inosine, 1-Methylinosine, and 5′-Inosine Monophosphate in Aqueous Solution

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Abstract: The bifunctional electrophiles *cis*-diammineplatinum(II) and ethylenediamineplatinum(II) form stable complexes with inosine, 1-methylinosine, and 5′-inosine monophosphate over a wide range of pH. These reactions have been characterized primarily through Raman spectrophotometry and ^{1}H NMR spectroscopy of solutions containing 25 mM nucleoside or nucleotide and varying concentrations of the platinum complex in H_2O and D_2O. The reactions with 1-methylinosine and inosine below pH ~6 lead to the formation of mono and bis complexes where coordination occurs at N-7 of the base. The structure of the bis complex is discussed in terms of ring current effects observed in the ^{1}H NMR spectra. The perturbations of the ligand vibrations are very similar to those caused by coordination of CH_3Hg^{II} at N-7. Platination at N-7 is observed to increase the rate of exchange of H-8 with solvent protons. Above pH ~6, protons are dissociated from N-1, and the pK is 7.2 ± 0.3 in D_2O, 25°, for 25 mM inosine in the presence of an equimolar amount of *cis*-$(H_3N)_2Pt^{II}$. This is an increase of over two orders of magnitude compared to inosine alone. Under these conditions coordination occurs at both N-7 and N-1 with the formation of a polynuclear complex. A structure for the polymer is suggested on the basis of the Raman and ^{1}H NMR spectra, and this involves the stacking of a bipolar complex similar to the stacking of 7-methylinosine. The reactions of *cis*-$(H_3N)_2Pt^{II}$ with mononucleotides and with polynucleotides are discussed generally. Raman spectrophotometry provides an especially powerful technique for studying the interactions of ions or molecules with nucleotides in aqueous solution.

In 1969, Rosenberg and co-workers[2] discovered that *cis*-$[PtCl_2(NH_3)_2]$ and $[PtCl_2en]$ were potent antitumor agents against Sarcoma 180 in Swiss white mice. Consequently there have been many studies over the past 5 years on the cytotoxic effects of transition metal complexes.[3-6] Results from the initial clinical trials of *cis*-$[PtCl_2(NH_3)_2]$ have been reviewed by Carter and Slavik.[7] Tissue culture studies[8,9] indicated a selective and persistent inhibition of DNA synthesis. Phage inhibition studies showed binding of the platinum complex to single-stranded RNA and double-stranded DNA viruses and inhibition at platinum levels below those necessary to cross-link the two strands of the DNA duplex.[10] Studies on the biological inactivation of bacterial-transforming DNA by the bound complex indicate that inactivation results from interference with integration of the DNA into the recipient genome even when the bound platinum species are separated by as many as 500 base pairs.[11] Both cis and trans isomers inactivate transforming DNA, although the trans isomer is less efficient, indicating a different mode of binding.

Because the biological experiments suggest that binding of the platinum complex to nuclear DNA is responsible for the cytotoxic effect, a number of experiments have been carried out to study the nature of the binding reactions. Mansy et al.[12] used uv spectrophotometry to study the reaction of *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ *in tenfold excess* with nucleosides as a function of pH at 37°. They suggested that the cis isomer interacted bifunctionally with either the 6-NH_2 + N-7 or 6-NH_2 + N-1 of adenosine and 4-NH_2 + N-3 of cytidine. The trans isomer was reported to bind at N-7 or N-1 of adenosine. Both isomers were reported to bind monofunctionally to N-7 of guanosine and inosine, although it was suggested that N-1 might be metalated at higher pH; and neither isomer appeared to react with uridine or thymidine. Since relatively small changes occur in the base absorption spectrum when a metal is added, the uv spectra are difficult to interpret if more than one reaction is occurring. Roos et al.,[13] using mass spectrometry to probe the species formed in solution with nucleoside analogues at 1:1 metal-base ratio without pH control, reported that both 9-methyladenine and 1-methylcytosine reacted as unidentate ligands, 1-methylthymine did not react at all, while 9-methylguanine reacted as a bidentate ligand with the cis isomer but as a unidentate ligand with the trans isomer. Kong and Theophanides[14] used ^{1}H NMR to investigate the products of reactions of *cis*-$PtCl_2(NH_3)_2$ with excess guanosine and inosine at 65° without pH control. Only complexes of the type $[Pt(NH_3)_2Guo_2]Cl_2$ and $[Pt(NH_3)_2Ino_2]Cl_2$ were isolated. These exhibited ^{195}Pt–H spin–spin coupling to H-8 but not to H-2 of inosine indicating binding to N-7 of the neutral ligand. Similar studies with adenosine were structurally inconclusive but suggested multiple site binding and polynuclear complex formation.[15] The unifunctional $[Pt(dien)Cl]^+$ (dien = diethylenetriamine) was reported not to react with uridine, to bind to N-3 of cytidine, and to bind at both N-1 and N-7 of adenosine.[15] The generally low reactivity of uridine and thymidine in neutral aqueous solution is supported by the observation that *cis*-$[PtCl_2(NH_3)_2]$ will not precipitate polyuridylic acid.[16]

Reaction of *cis*-$[PtCl_2(NH_3)_2]$ with DNA causes a large increase in the bouyant density which is proportional to the G–C content of the DNA.[17] Goodgame et al.[18] have reported the preliminary crystal structure of a nonstoichiometric complex $Na_{2.88}[Pt(NH_3)_2]_{0.56}(5'\text{-IMP})_2 \cdot 16H_2O$ produced by reaction of Na_2[5′-IMP] with $(NH_3)_2Pt^{II}$ at pH 6.85. Two inosines are bound to platinum via N-7. They proposed a mechanism for the reaction of *cis*-$(H_3N)_2Pt^{II}$ with double-stranded DNA which involves the initial formation of a bond to one N-7 position, perhaps with a hydrogen bond between a coordinated water molecule and the O-6 position. It should be noted that $[Pt(NH_3)_2(OH_2)_2]^{2+}$ is extensively hydrolyzed at pH 7 and this makes the hydrogen bonding less plausible. It was suggested further that the water molecule could be lost giving a strong Pt–N-7 bond and a weak bond to the carbonyl O-6, although the crystal structure indicated no Pt–O-6 interaction. Upon denaturation of the DNA it was proposed additionally that platinum would form two strong bonds to N-7 of two bases giving the type of coordination found in their crystal structure determination. Recently, Macquet and Theophanides[19] have re-

ported evidence of proton release upon reaction of a series of platinum(II) complexes with salmon sperm DNA, presumably involving N-1 of guanosine and/or N-3 of thymidine.

To summarize the results with inosine and guanosine reported to date, the interaction in aqueous solution generally has been described as unifunctional with platinum(II) binding to N-7 of two guanine or hypoxanthine bases without proton loss from the base. The only compounds which have been isolated have been characterized as bis complexes with two neutral bases bound via N-7. Binding to the guanine base has been reported to be the most important reaction between $(H_3N)_2Pt^{II}$ and native DNA's.

In this work, we report a systematic study of the interaction of *cis*-$(H_3N)_2Pt^{II}$ with inosine, 1-methylinosine, and 5′-inosine monophosphate in moderately dilute aqueous solution using Raman and ^1H NMR spectroscopy. A preliminary account has been published.[20] Because platinum(II) complexes are substitutionally inert, it is not possible to make proton release measurements using electrochemical cells with sufficient accuracy to unravel complex equilibria. Consequently, spectroscopic measurements on solutions at equilibrium are most useful. In previous studies, we have systematically mapped the perturbations of nucleoside and nucleotide vibrations caused by coordination of the unifunctional probe ion CH_3Hg^{II} to different sites.[21-25] It will be shown that these data are very useful in the interpretation of heavy metal binding generally. A few measurements also were made with ethylenediamineplatinum(II) to verify that it reacts similarly to *cis*-$(H_3N)_2Pt^{II}$

Experimental Section

Diaquodiammineplatinum(II) Perchlorate Solutions. *cis*-Dichlorodiammineplatinum(II) was obtained from Matthey Bishop, Inc., Malvern, Pa. Anal. Calcd for $PtCl_2N_2H_6$: Cl, 23.6; N, 9.34; H, 2.00; Pt, 65.0. Found: Cl, 23.3; N, 9.53; H, 2.06; Pt, 65.0. Weighed amounts were allowed to react with aqueous $AgClO_4$ with stirring for 5 h, and the solution was evaporated under vacuum over P_4O_{10} to yield pale yellow [*cis*-$Pt(NH_3)_2(OH_2)_2](ClO_4)_2$. Anal. Calcd for $PtCl_2N_2O_{10}H_{10}$: Cl, 15.3; N, 6.04; H, 2.17; Pt, 42.0. Found: Cl, 15.5; N, 6.12; H, 2.25; Pt, 41.9. Note: this perchlorate and the ethylenediamine complex described below both are potentially explosive. Solutions were prepared by dissolving weighed amounts in H_2O or D_2O (99.8%, Columbia Organic Chemicals, Columbia, S.C.). Solutions of $(H_3N)_2Pt^{II}$ in D_2O were allowed to equilibrate for 1 day to ensure complete exchange of the ammine protons.

Ethylenediaminеplatinum(II) Perchlorate Solutions. Dichloroethylenediamineplatinum(II) was synthesized using the method of Hoeschele.[26] An aqueous solution of K_2PtCl_4 was allowed to react with KI to yield K_2PtI_4, ethylenediamine (en) was added, and dark yellow *cis*-$[PtI_2en]$ was collected. The iodide was allowed to react with aqueous $AgClO_4$, KCl was added, and the precipitate of $Pt(en)Cl_2$ which resulted was collected on a frit. Anal. Calcd for $PtC_2H_8N_2Cl_2$: C, 7.37; H, 2.47; N, 8.59; Cl, 21.7; Pt, 59.8. Found: C, 7.64; H, 2.17; N, 8.49; Cl, 21.8; Pt, 60.0. This was converted to the diaquo complex $[Pt(en)(OD_2)_2](ClO_4)_2$ as described above for the ammine. Anal. Calcd for $PtC_2H_4D_8N_2O_{10}Cl_2$: C, 4.82; N, 5.63; Cl, 14.2. Found: C, 4.85; N, 5.90; Cl, 14.3. Solutions were prepared by dissolving weighed amounts in H_2O or D_2O.

Ethylenediaminebis(inosine)platinum(II) Dichloride. This compound was synthesized as described by Kong and Theophanides.[14] Anal. Calcd for $PtC_{20}H_{30}N_{10}O_{10}Cl_2$: C, 30.0; H, 3.77; N, 17.5 Found: C, 30.2; H, 3.89; N, 16.2.

Nucleoside and Nucleotide Solutions. Inosine and 1-methylinosine were obtained from Sigma Chemical Co., St. Louis, Mo., and 5′-inosine monophosphate (99%+) was from the Aldrich Chemical Co., Milwaukee, Wis. The nucleoside or nucleotide solutions in D_2O were prepared shortly before a given experiment to minimize exchange of deuterium onto the C-8 position. The pH's (pD's) of the solutions were adjusted with $HClO_4$ ($DClO_4$) or NaOH (NaOD) solutions using a Radiometer PHM-4 meter. With D_2O solutions, the meter reading was corrected as described by Glascoe and Long.[27] After addition of the platinum complex, solutions were stored in the dark to prevent photochemical reactions, thermostated at 25 ± 0.5°.

Raman Spectra. The general procedures for Raman difference spectroscopy and Raman spectrophotometric titrations have been described in previous papers,[21-25,28] and a description of the instrument used for most of this work has been published.[28] Some of the latest data were obtained with a modified version of this instrument operating on-line to a Nova 2/10 minicomputer.[29] Solutions were contained in rectangular, 1.2-ml cells which were thermostated at 25°. Mirrors were deposited on the cell bottoms to double-pass the laser beam. Unless otherwise noted, all solutions contained 100 mM ClO_4^- as an internal frequency and intensity reference. Excitation was normally by the 5145-Å line of the Ar^+ laser with ca. 460 mW power, The normal slits were 7.7 cm^{-1}, and a photon counting time of 10 s per point was standard, one point per reciprocal centimeter. Spectra of crystalline solids were obtained in some cases using a Jarrell Ash 25-300 Raman spectrophotometer equipped with a Spectra Physics 125 He–Ne laser. Solid samples were sealed in capillaries, and the transillumination technique was employed.

Band frequencies were determined with the computer program RAMAN,[27] and the relative values are accurate to within 1 cm^{-1} for sharp bands. The spectra in the illustrations were reproduced directly from computer-generated plots. In general, the raw data were subjected to a 25-point quintic smoothing procedure.

Nuclear Magnetic Resonance Spectra. The general procedure employed has been described previously.[25] A Perkin-Elmer R-32 (90 MHz) spectrometer was used for survey spectra, and for the determination of chemical shifts and coupling constants, a Varian XL-100 (100 MHz) spectrometer was used to accumulate multiple sweeps. For the former, the internal reference, $N(CH_3)_4^+$, provided the lock signal, while the D_2O solvent served with the XL-100. Probe temperatures were approximately 35° (R-32) and 40° (XL-100).

Data and Results

Raman Spectra of Diammineplatinum(II) in Aqueous Solution. When *cis*-$[(H_3N)_2Pt(OD_2)_2]^{2+}$ is produced in D_2O solution, the ammine hydrogens undergo exchange with the solvent. Figure 1 illustrates the effect of exchange at 25° on the spectrum of the *cis*-diaquodiammineplatinum(II) cation. The Raman spectrum was scanned repetitively, and exchange of all protons at pD 4.5 was found to be complete within 1 h after the addition of crystalline $[(H_3N)_2Pt(OH_2)_2](ClO_4)_2$ to D_2O. Spectra recorded 5 days later were identical.

The spectrum of the cation is very similar in aqueous solution and in crystalline $[(H_3N)_2Pt(OH_2)_2](ClO_4)_2$ as illustrated in Figure 1. While the symmetric and asymmetric (Pt–N) stretching modes are well resolved with $(H_3N)_2PtCl_2$ and observed at 520 and 506 cm^{-1}, respectively, only a single broad band is found with the aquo cation, 560 (crystal) and 556 cm^{-1} (solution).

Changes in the spectra also are caused by proton transfer from the coordinated water molecules. There is reasonable agreement in the literature on the magnitude of the first acid dissociation constant, log $*K_1 = -5.56$,[30,31] -5.63,[32] but the values for log $*K_2$ differ considerably, -7.32,[30,31] -9.25.[32] Figure 1 also illustrates the effect of changing pH on the spectrum of *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$. Proton loss from the aquo cation causes a decrease in ν_s(Pt–N) of 15 cm^{-1} at pH 7.8 in H_2O. When *cis*-$[(H_3N)_2Pt(OH_2)_2](ClO_4)_2$ is dissolved in D_2O, pD 7.7, the spectrum showed bands at 522 and 497 cm^{-1}. The intensity of the former decreased slowly until, after 6.5 days, only a very weak shoulder remained. Spectra showing this change are illustrated in the microfilm edition. No analogous change occurred in H_2O where a single band at 541 cm^{-1} is observed at pH 7.8 shortly after mixing. The explanation of the two bands in D_2O at pD 7.7 is not clear. It is possible that the decrease in the acidity of the ammine groups caused by hydrolysis of the cation more than offsets the effect of increasing pH on

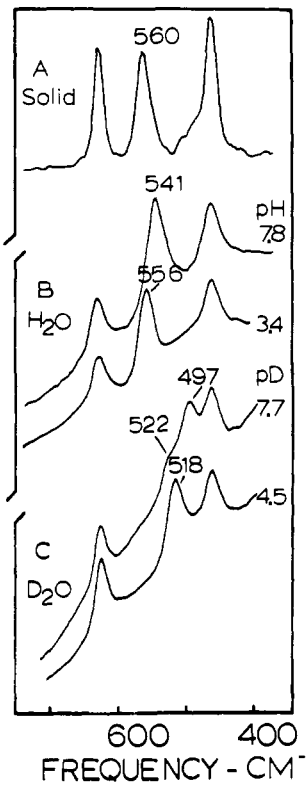


Figure 1. Raman spectra at 25° of $[(H_3N)_2Pt(OH_2)_2]^{2+}$ and of $[(D_3N)_2Pt(OD_2)_2]^{2+}$ in the (Pt-N) stretching region: A, crystalline $[(H_3N)_2Pt(OH_2)_2](ClO_4)_2$; B, 50 mM H_2O solution, pH 7.8, 3.4; C, 50 mM D_2O solution, pD 7.7, 4.5. Ordinate range in counts ($\times 10^3$): A, 0-11; B (pH 7.8), 17-108, (pH 3.4), 20-123; C (pD 7.7), 14-97, (pD 4.5), 13-106 counts.

the rate of exchange. The isotope effect on the symmetric (Pt-N) stretching mode is close to that predicted for a point mass ammine group vibrating against a large mass: theoretical 0.922; observed for the aquo cation 518/556 = 0.932; observed for the hydroxo species, pH ~7.8, 497/541 = 0.912.

There is some question about the long-term stability of *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ and its conjugate bases in solution. Perumareddi and Adamson[32] observed that acidified solutions of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ isomerized to the trans form upon photolysis. There was no spectroscopic evidence for isomerization in any of these experiments. Perumareddi and Adamson reported nonacidified solutions changed to dark yellow upon photolysis and the pH increased. It was suggested that decomposition with release of ammonia occurred upon irradiation. Grinberg et al.[31] converted *cis*-$[PtCl_2(NH_3)_2]$ to *cis*-$[Pt(OH)_2(NH_3)_2]$ by boiling with base, and it was reported that no decomposition occurred after boiling for more than 30 min. The fact that the integrated intensity in the (Pt-N) region remains approximately constant indicates there is no cleavage of Pt-N bonds in any of our experiments.

Diammineplatinum(II) + Inosine. Raman pH Profile for the 1:1 System at 25°. Raman difference spectra (RADS) were recorded for solutions with 25 mM inosine, 25 mM $(D_3N)_2Pt^{II}$ in D_2O from pD 3 to 9 to determine when reaction occurs and the effect of hydrogen ion concentration on it. From these spectra which are illustrated in the microfilm edition (see paragraph at end of paper regarding supplementary material) it was clear that reaction occurred in all these solutions. These difference spectra could be separated into three groups: (a) pD 6 and below, (b) pD 7-8, (c) above pD 8. By analogy with the effect of CH_3Hg^{II} on inosine vibrations,[25] the following interpretation can be made of

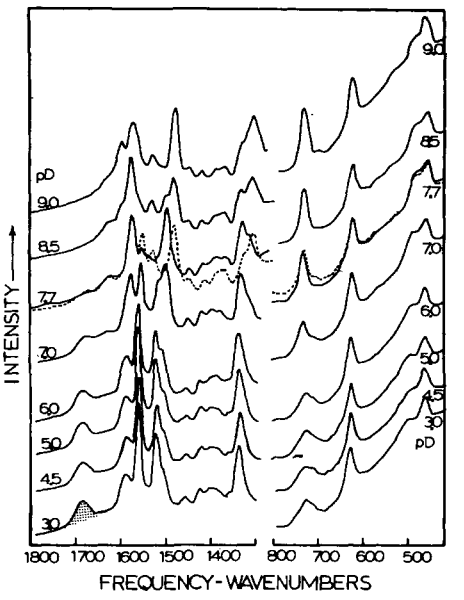


Figure 2. Raman spectra of D_2O solutions 25 mM in inosine, 25 mM in $(D_3N)_2Pt^{II}$ as a function of pD. The broken curve is for a solution 25 mM in [8-^{2}H]inosine, 25 mM in $(D_3N)_2Pt^{II}$, pD 7.7, to illustrate the effect of deuteration at C(8) of inosine on the spectrum. Ordinate range ca. 0-141 $\times 10^3$ counts.

these spectra. Below pD 6, inosine has coordinated without loss of the proton on N-1. This is indicated by (i) increases in all frequencies above 1500 cm^{-1} indicated by derivative features in the difference spectra, especially the shift in the 1676-cm^{-1} band to 1683 cm^{-1}, and (ii) the increase in frequency and decrease in intensity with broadening of the 721-cm^{-1} band. At pD 7 and above the proton on N-1 is displaced. The evidence for this includes (i) the disappearance of the 1676-cm^{-1} band and the appearance of a new band at 1632 cm^{-1} and (ii) the shift of the 721-cm^{-1} band to 735 cm^{-1} with no significant change in intensity. In the pD 9 spectrum, the 1505 band has shifted to 1482 cm^{-1}, a change characteristic of deuterium substitution onto C-8.[25] It is clear from the difference spectra that reaction is quantitative in the acidic 1:1 solutions because no scattering due to $[(D_3N)_2Pt(OD_2)_2]^{2+}$ could be detected. The ν_s(Pt-N) mode decreases from 518 to ca. 496 cm^{-1}.

The parent spectra for the $(D_3N)_2Pt^{II}$-inosine solutions are illustrated in Figure 2. The effect of pD on the 1676- and 721-cm^{-1} bands can be seen clearly. For comparison, the spectrum of a solution at pD 7.7 containing 25 mM [8-^{2}H]inosine + 25 mM $(D_3N)_2Pt^{II}$ is given. This illustrates the effect of deuterium substitution onto the C-8 of the inosine. It has been observed previously that the rate of this exchange is increased greatly by heavy metal coordination.[25] It should be noted that the 25 mM inosine reference solution spectra all appeared identical throughout the entire pD 3-9 range, showing neither evidence for proton transfer nor deuterium exchange. With these reference solutions, there is probably considerable inaccuracy in the pD values, because they were unbuffered.

Since the inosine band at 1676 cm^{-1} disappears when the proton is transferred from N-1,[25] the intensity of the band in the 1650-1700-cm^{-1} region can be used to follow proton loss from the complex. Figure 3 illustrates the variation of the integrated intensity for the 1:1 $(D_3N)_2Pt^{II}$-inosine solutions as a function of pH. For this determination, large plots of the spectra, 30 × 100 cm, were generated scaling them so the integrated intensity of the ν_1 ClO_4^- internal reference mode was the same in all plots. The band area in the 1650-1700-cm^{-1} region was measured with a Gelman pla-

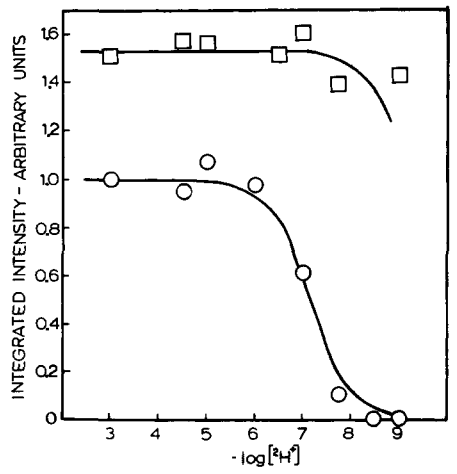


Figure 3. The pH profile for the 1:1 $(D_3N)_2Pt^{II}$-inosine reaction in D_2O at 25° determined by the integrated intensity (arbitrary units) of the Raman band in the 1650–1700-cm^{-1} region of inosine: □, 25 mM inosine; ○, 25 mM inosine + 25 mM $(D_3N)_2Pt^{II}$. The curve is calculated for pK = 7.2.

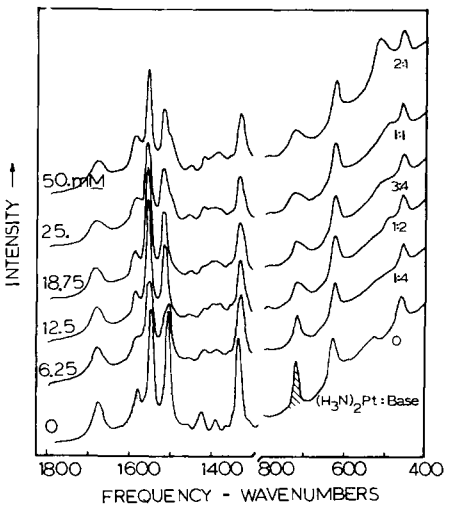


Figure 4. Raman spectrophotometric titration of 25 mM inosine in D_2O at 25°, pD 4.5, with *cis*-$(D_3N)_2Pt^{II}$. The intensities of the shaded bands can be used to monitor the reactions. Ordinate range ca. 0–120 × 10^3 counts.

nimeter, a straightforward procedure since the band is rather well isolated. For comparison, the intensity of the 1676-cm^{-1} inosine band in the reference solutions also is given in Figure 3. From these data, the pK in D_2O of N-1-H in the presence of an equal concentration of *cis*-$(D_3N)_2Pt^{II}$ is 7.2 ± 0.3.

Stoichiometry of the Reaction between Inosine and *cis*-$(D_3N)_2Pt^{II}$ in D_2O, 25°, pD 4.5. A Raman spectrophotometric titration was carried out to study the reaction under conditions where the proton is not lost from N(1). The data are illustrated in Figure 4. The frequency of the 1676-cm^{-1} inosine band increases to 1683 cm^{-1} in the solution containing 12.5 mM $(D_3N)_2Pt^{II}$ and shows no further shift at higher platinum concentrations. Similar shifts are observed in all the other bands above 1500 cm^{-1}, and these are illustrated in Figure 5 as a function of the $(D_3N)_2Pt^{II}$ concentration. Clearly, the reaction which is responsible for these shifts in the hypoxanthanine ring modes has 2:1 inosine-$(D_3N)_2Pt^{II}$ stoichiometry.

The intensity of the scattering between 1650 and 1700 cm^{-1} shows an odd variation as $(D_3N)_2Pt^{II}$ is added as illustrated in Figure 6. As the band shifts from 1676 to 1683 cm^{-1}, the intensity falls to ca. two-thirds of the inosine

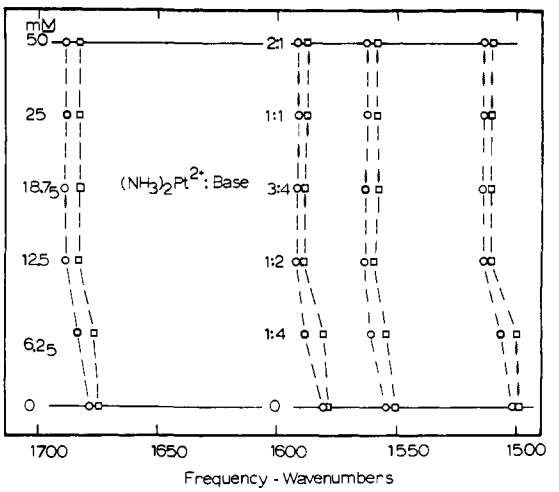


Figure 5. Variation of the ring modes in the double bond region of inosine and 1-methylinosine in D_2O solution, 25°, as a function of the $(D_3N)_2Pt^{II}$ concentration: □, inosine; ○, 1-methylinosine.

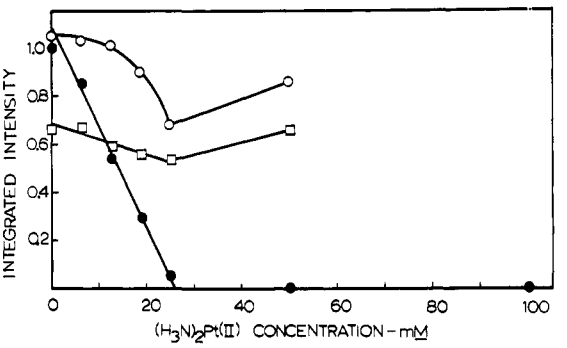


Figure 6. Variation of the integrated intensity (arbitrary units) of the Raman band of inosine in the 1650–1700-cm^{-1} region, D_2O, 25°, as a function of the $(D_3N)_2Pt^{II}$ concentration: □, inosine, pD 4.5; ●, pD 7.6; ○, 1-methylinosine, pD 7.7.

value and then increases slightly with higher concentrations of $(D_3N)_2Pt^{II}$. This decrease in intensity with coordination but not deprotonation of inosine also is shown by the data from pH 3 to 6 in Figure 3. The basis for these changes is not clear.

The ca. 720-cm^{-1} inosine band which generally is sensitive to metal coordination broadens in the solutions with $(D_3N)_2Pt^{II}$. The sharp ligand band appears to be replaced by two overlapping bands. A spectrum of crystalline $[(H_3N)_2Pt(Ino)_2]Cl_2$ shows two bands at 720 and 726 cm^{-1}. Little further change occurs at inosine-platinum ratios below 2:1, again indicating a reaction with 2:1 stoichiometry.

In addition to the Raman titration, a ^1H NMR titration of 25 mM inosine was carried out. These data are illustrated in Figure 7. With 6.25 mM $(D_3N)_2Pt^{II}$, signals due to bound and free inosine are observed, but at 12.5 mM $(D_3N)_2Pt^{II}$, only bound inosine is present. The 0.55-ppm downfield shift of H-8 relative to H-2 is essentially that observed by Kong and Theophanides for *cis*-$[(H_3N)_2Pt(Ino)_2]Cl_2$ dissolved in D_2O.[14] The 26-Hz ^{195}Pt-H-8 coupling[14] can only be detected with somewhat more concentrated solutions. Chemical shift data are collected in Table I.

As the $(D_3N)_2Pt^{II}$-inosine ratio is increased beyond 1:2, a new set of bound inosine resonances occurs together with those due to the bis complex. All of the signals including those due to the ribose protons in this new species are shifted ca. 0.1 ppm downfield relative to the bis complex. Based

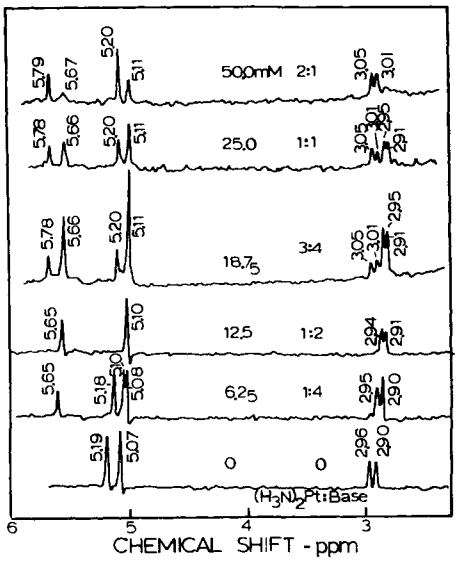


Figure 7. Proton magnetic resonance spectra (100 MHz) of 25 mM inosine solutions in D_2O, pD 4.5, 40°, containing varying concentrations of *cis*-$(D_3N)_2Pt^{II}$.

Table I. Proton Chemical Shifts of Inosine and 1-Methylinosine and Their Complexes in D_2O Solution at 40° [a,b]

Species[c]	pH	δ, ppm H-8	H-2	H-1′	CH_3
Inosine	4.5	5.19	5.07	2.93	
cis-$[(H_3N)_2Pt(OH_2)Ino]^{2+}$	4	5.78	5.20	3.03	
cis-$[(H_3N)_2Pt(Ino)_2]^{2+}$	4.5	5.65	5.10	2.92	
1-Methylinosine	7.5	5.20	5.25	2.95	0.53
cis$[(H_3N)_2Pt(OH_2)$(1-MeIno)$]^{2+}$	4.5[d]	5.77	5.38	3.02	0.53
cis-$[(H_3N)_2Pt(1\text{-MeIno})_2]^{2+}$	7.5	5.63	5.26	2.91	0.48

[a] Data obtained at 100 MHz. [b] All shifts are measured from an internal $(CH_3)_4N^+$ standard. [c] All solutions are 25 mM in total inosine or 1-methylinosine. [d] Reported for acid solution because H(8) exchanges at pH 7.5.

on the solution stoichiometry this new species could be either $[(D_3N)_2Pt(Ino)(OD_2)]^{2+}$ or some sort of a cluster with both inosine and $(D_3N)_2Pt^{II}$ bifunctional. The sharpness and simplicity of this new spectrum rule out the latter, vide infra.

In summary, these data indicate the reaction sequence:

$$\text{Ino} + [(D_3N)_2Pt(OD_2)_2]^{2+} \rightleftarrows [(D_3N)_2Pt(Ino)(OD_2)]^{2+} + D_2O \quad (1)$$

$$\text{Ino} + [(D_3N)_2Pt(Ino)(OD_2)]^{2+} \rightleftarrows [(D_3N)_2Pt(Ino)_2]^{2+} + D_2O \quad (2)$$

Raman frequencies for the mono and bis complexes are tabulated in the microfilm edition.

Reaction between Inosine and *cis*-$(H_3N)_2Pt^{II}$ or $enPt^{II}$ in H_2O and D_2O, 25°, pH (pD) 7–8. Raman difference spectra recorded for the complex vs. inosine differ for H_2O and D_2O, because exchange of the N-1 hydrogen alters the inosine reference. A Raman spectrophotometric titration of 25 mM inosine in D_2O, pD 7.6, is illustrated in Figure 8. As was observed when CH_3Hg^{II} bound at N-1 of inosine displacing the proton,[25] the 1676-cm^{-1} band disappears entirely as the $(D_3N)_2Pt^{II}$ concentration increases. The behavior is quite different from that at pD 4.5. A plot of the integrated intensity of the 1676-cm^{-1} band vs. the *cis*-$(D_3N)_2Pt^{II}$ concentration is illustrated in Figure 6, and this shows that a reaction with 1:1 stoichiometry occurs in which the proton is displaced from N-1. Similarly, the variation of the integrated intensity of the 720-cm^{-1} band indicates a 1:1 reaction. The replacement of this band with one of comparable intensity but higher frequency, here 739 cm^{-1}, also is consistent with loss of the N-1 proton by analogy with the CH_3Hg^{II} case.[25]

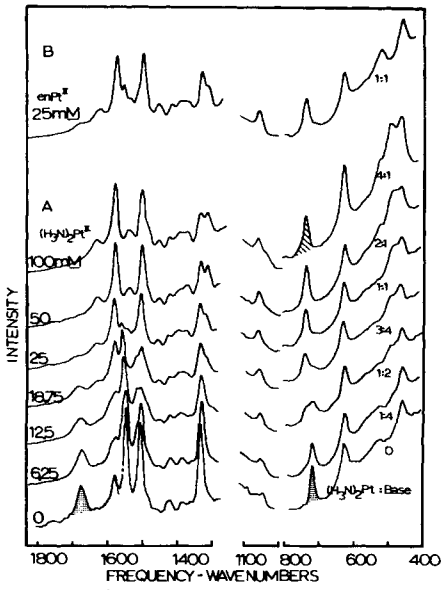


Figure 8. Raman spectrophotometric titration of 25 mM inosine in D_2O at 25°, pD 7.6, with (A) *cis*-$(D_3N)_2Pt^{II}$, (B) $enPt^{II}$. Ordinate scale ca. 0–108 × 10^3 counts. Shaded bands can be used to monitor the extent of the reactions.

Spectra were obtained for solutions of inosine and $enPt^{II}$ at pD ~7.5 with $enPt^{II}$–Ino ratios of 0.5, 1, and 2. The ligand vibrations were indistinguishable from those of the corresponding $(H_3N)_2Pt^{II}$ systems. The spectrum of a 25 mM $enPt^{II}$–25 mM Ino solution, pD 7.5, is illustrated in Figure 8 for comparison with the *cis*-$(H_3N)_2Pt^{II}$ spectra.

Examination of the frequencies of the inosine ring modes in the 1500–1600-cm^{-1} region indicates that the reaction sequence is more complex than just a simple 1:1 reaction. The inosine band at 1580 cm^{-1} decreases in intensity as $(D_3N)_2Pt^{II}$ is added, a minimum is reached with 12.5 mM $(D_3N)_2Pt^{II}$, and at higher concentrations the intensity increases to give a much stronger band, again at 1580 cm^{-1}. The 1550-cm^{-1} band shifts initially to ca. 1558 cm^{-1} with 12.5 mM $(D_3N)_2Pt^{II}$ and then decreases in intensity rapidly at higher platinum concentrations. The 1508-cm^{-1} band splits into two components at ca. 1512 and 1525 cm^{-1} with 12.5 mM $(D_3N)_2Pt^{II}$ and at higher platinum concentrations collapses to a single band at ca. 1503 cm^{-1}. These changes clearly indicate a 2:1 reaction, different from the process in which the proton is lost, occurs with excess inosine.

The ^1H NMR spectra of these solutions also were examined. These are illustrated in Figure 9. With only 6.25 mM $(D_3N)_2Pt^{II}$, the spectrum is essentially a superposition of spectra of complexed and free inosine, although a weak new signal also appears in the ribose H-1′ region at δ 2.86 ppm from $N(CH_3)_4^+$. The spectrum at 1:2 $(D_3N)_2Pt^{II}$–inosine is similar to that of the corresponding solution at pD 4.5, except the ring proton signals are upfield by 0.05–0.10 ppm. The Raman spectra indicate partial proton loss from inosine in this solution. Since proton transfer is fast, only averaged

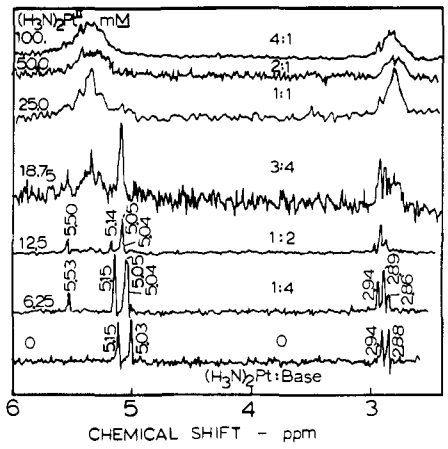


Figure 9. Proton magnetic resonance spectra (100 MHz) of 25 mM inosine in D_2O, pD 7.6, containing varying concentrations of $(D_3N)_2Pt^{II}$.

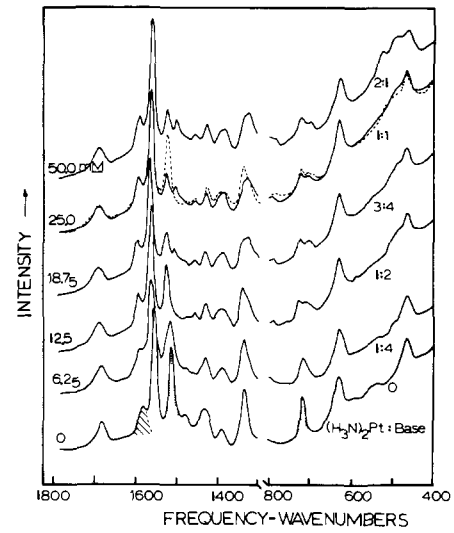


Figure 10. Raman spectrophotometric titration of 25 mM 1-methylinosine in D_2O at 25°, pD 7.7, with *cis*-$(D_3N)_2Pt^{II}$. Ordinate range ca. 13–126 × 10^3 counts. Shaded bands can be used to monitor the extent of the reactions. Broken line, pD 4.5.

chemical shifts are observed for the two kinds of bound inosine.

At higher $(D_3N)_2Pt^{II}$–inosine ratios, the ^1H NMR spectrum is complicated by two factors. Since the proton is lost from N-1, inosine has two very good nucleophilic centers N-7 and N-1, and the negative ligand charge should reduce the repulsion between the positive platinum centers. This leads to polynuclear complex formation. Because platinum(II) is substitutionally inert, all of the resonances become very broad. Secondly, H-8 exchanges with the deuterium of the solvent. This reaction is not very fast at 25°, because the Raman spectra at high $(D_3N)_2Pt^{II}$ concentrations only show a weak shoulder at ca. 1480 cm^{-1} where deuterium exchange produces an intense band. If the pH is increased to 8.2 with 100 mM $(D_3N)_2Pt^{II}$, a spectrum identical with 25 mM $(D_3N)_2Pt^{II}$ + 25 mM [8-^2H]inosine is obtained. The latter frequencies are given in the microfilm edition.

The Raman and ^1H NMR spectra show no significant changes at inosine-$(D_3N)_2Pt^{II}$ ratios above 1:1 indicating that the polymeric complex is not broken down to a monomer in the presence of excess platinum. The solution with 100 mM *cis*-$(H_3N)_2Pt^{II}$ shows the band characteristic of the unreacted platinum complex at 498 cm^{-1}, while the (Pt–N) stretching vibration for the 1:1 solution occurs at ca. 487 cm^{-1}. This behavior indicates considerable stability for the polynuclear complex.

To summarize, these data indicate a sequence of reactions.[33]

$$\text{Ino} + [(D_3N)_2Pt(OD)(OD_2)]^+ \rightleftarrows [(D_3N)_2Pt(OD_2)(\text{InoD}_{-1})]^+ + D_2O \quad (3)$$

$$\text{Ino} + [(D_3N)_2Pt(OD_2)(\text{InoD}_{-1})]^+ \rightleftarrows [(D_3N)_2Pt(\text{Ino})(\text{InoD}_{-1})]^+ + D_2O \quad (4)$$

$$[(D_3N)_2Pt(\text{Ino})(\text{InoD}_{-1})]^+ + [(D_3N)_2Pt(OD)(OD_2)]^+ \rightleftarrows 2/n[(D_3N)_2Pt(\text{InoD}_{-1})]_n^+ + 2D_2O \quad (5)$$

Raman frequencies for the 1:1 solution, pD 7.6, are listed in the microfilm edition. These correspond to $[(D_3N)_2Pt(\text{InoD}_{-1})]_n^+$.

Reaction between 1-Methylinosine and *cis*-$(H_3N)_2Pt^{II}$ in D_2O, 25°, pD 7.7. In order to test the conclusions drawn above, the reactions of 1-methylinosine were studied at pD 7.7. Since the N-1 position is blocked by methylation, the changes attributed to proton transfer from this position of inosine at pD 7.7 should not occur. In addition, since only N-7 appears to be a good donor site besides N-1 of the conjugate base, no polynuclear complex formation should occur. If, on the other hand, polymerization occurred via hydroxo bridges between platinum centers, this would be expected to occur with 1-methylinosine as well.

The data for the Raman spectrophotometric titration of 25 mM 1-methylinosine at pD 7.7 are illustrated in Figure 10. It was noted that Raman difference spectra for 1:1 1-methylinosine-$(D_3N)_2Pt^{II}$ at pD 7.7 were almost indistinguishable from the corresponding spectrum of inosine at pD 4.5. Comparison of Figures 4 and 10 shows that the Raman spectra are almost identical throughout. Figure 5 shows that the shifts in ligand vibrations are almost identical with those which occur when inosine binds without loss of the N-1 bound hydrogen. These data clearly show the reaction has 2:1 stoichiometry. The spectral changes which accompany the reactions of 1-methylinosine at pD 4.5 also are very similar, and one spectrum is illustrated in Figure 10 for comparison.

The only significant difference between the reactions of 1-methylinosine at pD 7.7 and inosine or 1-methylinosine at pD 4.5 is the growth of a new band at 1502 cm^{-1} with the higher $(D_3N)_2Pt^{II}$ concentrations at pD 7.7. This is caused by exchange of solvent deuterium onto the C(8) position.

The ^1H NMR titration data are illustrated in Figure 11. Chemical shifts are collected in Table I. They are very similar to the corresponding data for inosine, pD 4.5, Figure 7. With 1:2 solutions, the H-8 signal occurs at 5.63 ppm compared to 5.65 ppm with inosine, pD 4.5. The H-2 signal shifts only 0.01 ppm compared to the free ligand; the corresponding shift with inosine, pD 4.5, is 0.03 ppm. The methylation at N-1 causes a downfield shift of 0.18 ppm for H-2 of inosine and 0.16 ppm for H-2 of the $(D_3N)_2Pt^{II}$ complex.

At 1-methylinosine-$(D_3N)_2Pt^{II}$ ratios below 2:1, a second set of signals occurs. At pD 4.5, they are virtually identical with the corresponding inosine spectrum, except the H-2 signals are shifted downfield by 0.16–0.18 ppm because of the N-1 methylation. The signals in the H-8 region are weak in the pD 7.7 solutions because of exchange, and the signal at 5.77 ppm which, by analogy with inosine at pD 4.5, is assigned to H-8 of $[(D_3N)_2Pt(\text{Ino})(OD_2)]^{2+}$ is absent.

The indicated reactions are the following.

$$\text{1-MeIno} + [(D_3N)_2Pt(OD)(OD_2)]^+ \rightleftarrows [(D_3N)_2Pt(OD)(\text{1-MeIno})]^+ + D_2O \quad (6)$$

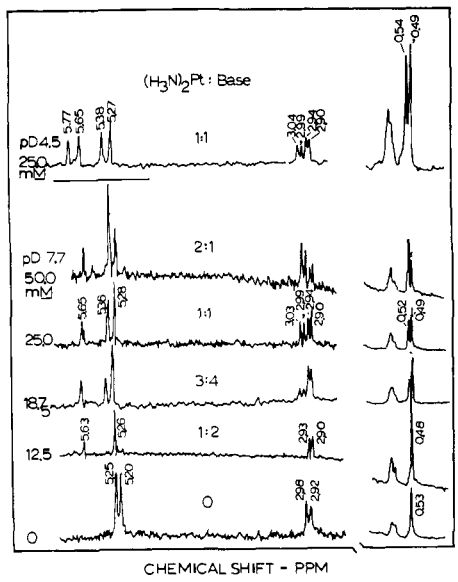


Figure 11. Proton magnetic resonance spectra (100 MHz) of 25 mM 1-methylinosine in D_2O, pD 7.5, containing varying concentrations of $(D_3N)_2Pt^{II}$.

$$\text{1-MeIno} + [(D_3N)_2Pt(OD)(\text{1-MeIno})]^+ \rightleftarrows [(D_3N)_2Pt(\text{1-MeIno})_2]^{2+} + OD^- \quad (7)$$

Reaction between Inosine Monophosphate and $(H_3N)_2Pt^{II}$ in H_2O, 25°, pH 6.85. Difference spectra were recorded for solutions containing 25 mM inosine and 12.5 or 25.0 mM $(H_3N)_2Pt^{II}$. The pH was adjusted to the value which was reported by Goodgame et al.[18] for the solution from which they crystallized $Na_{2.88}[Pt(NH_3)_2]_{0.56}(5'\text{-IMP})_2 \cdot 16H_2O$. In addition, to correspond to the experiment of Goodgame et al., H_2O rather than D_2O was used as the solvent. Since the water scattering is very intense from 1500 to 1700 cm^{-1} (δ OH_2) and below 850 cm^{-1} (librational motion), all solutions were run vs. solvent (0.1 M $NaClO_4$). These difference spectra as well as the complex vs. IMP difference spectrum for the 1:2 solution are illustrated in Figure 12. The corresponding spectra for the 1:1 solution are given in the microfilm edition.

It is clear that 5'-IMP and inosine react alike. For comparison, a difference spectrum for a 1:1 $(H_3N)_2Pt^{II}$-inosine solution, H_2O, pH 7.2, is given with the corresponding 5'-IMP spectrum in the microfilm edition. They are almost identical. In particular, the IMP-PO_3 stretching mode at 976 cm^{-1} shows no significant alteration in the presence of equimolar $(H_3N)_2Pt^{II}$. This is the same behavior observed previously for CH_3Hg^{II} with nucleotides.[22] The heavy metals interact only weakly with the phosphate.

The difference spectra of the 1:2 and 1:1 $(H_3N)_2Pt^{II}$-5'-IMP solutions are not the same. The changes in the 1650-1700- and 700-750-cm^{-1} regions indicate that in the 1:2 solution coordination of inosine occurs without appreciable proton transfer from N-1, vide supra. On the other hand, the 1:1 solution spectrum indicates most of the inosine has been deprotonated. This is what would be anticipated from the data in Figure 3, recognizing that the pK of inosine will be ca. 0.5 units smaller in H_2O than D_2O because of the isotope effect. Clearly 5'-IMP reacts just as does inosine.

Discussion

The reactions of the bifunctional electrophile *cis*-$(H_3N)_2Pt^{II}$ with the purines inosine or 5'-inosine monophosphate are relatively straightforward, although they are somewhat more complex than has been suggested on the basis of preparative[14] or uv spectrophotometric[12] studies.

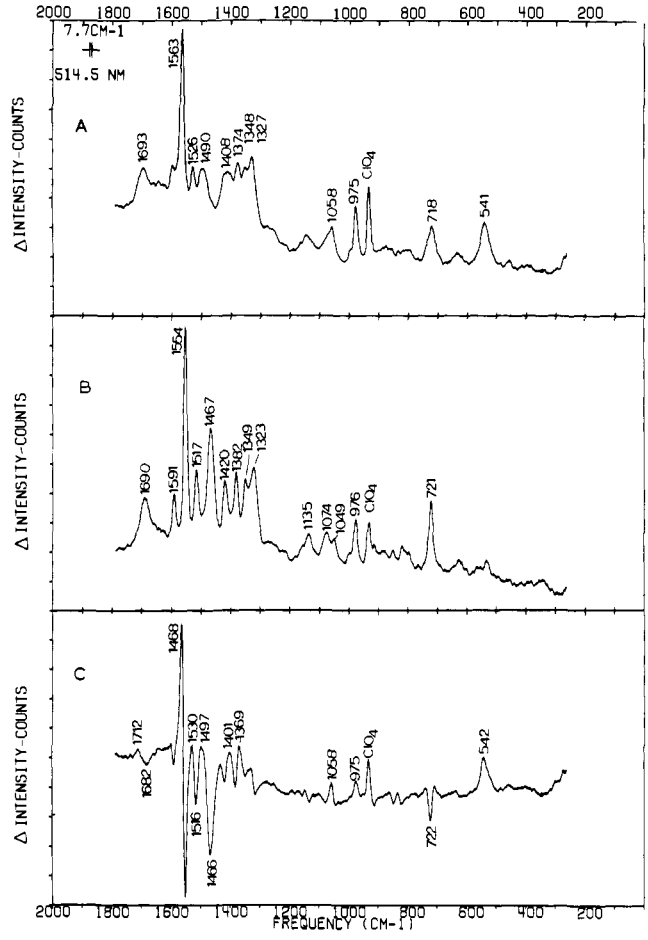
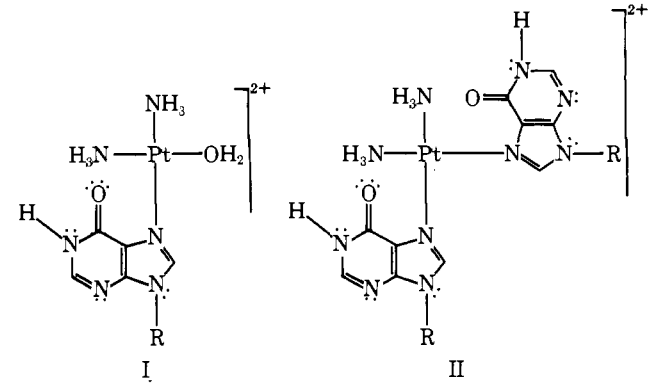


Figure 12. Raman difference spectra of 5'-inosine monophosphate and of its 2:1 complex with *cis*-$(H_3N)_2Pt^{II}$ in H_2O at 25°, pH 6.85: A, 25 mM IMP + 12.5 mM $(H_3N)_2Pt^{II}$ vs. solvent (0.1 M $NaClO_4$), ordinate range 0-40 × 10^3; B, 25 mM IMP vs. 0.1 M $NaClO_4$, ordinate range 0-34 × 10^3; C, (A-B), ordinate range −12 to +27 × 10^3 counts.

Since the uv absorption spectra were examined in the presence of a large excess of *cis*-$(H_3N)_2Pt^{II}$, while the preparative studies were made with 2[18] or 2.5[14] inosine-$(H_3N)_2Pt^{II}$, we have studied the reactions over a rather wide concentration range. In the discussions which follow, $(H_3N)_2Pt^{II}$ will be used for the cis platinum species in both H_2O and D_2O. In the later case, complete exchange of the ammine hydrogens occurred.

Reactions with Neutral Inosine in Acidic Solution or with 1-Methylinosine. In acid solution below pH 5 where $[(H_3N)_2Pt(OH_2)_2]^{2+}$ predominates, simple mono, I, and bis, II, inosine complexes are formed by coordination to N(7) of the neutral nucleoside.



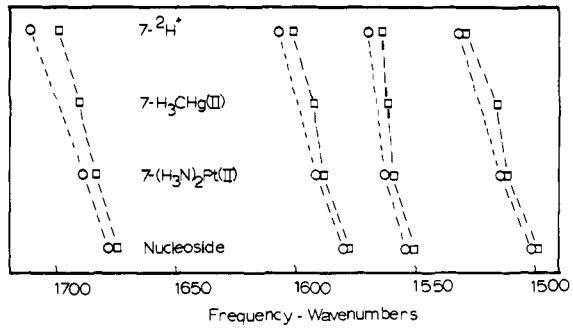


Figure 13. Comparison of the effect of cation binding at N-7 on the inosine ring modes in the double bond region: □, inosine; ○, 1-methylinosine.

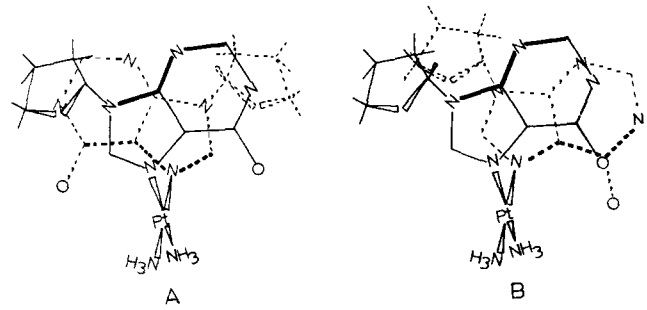


Figure 14. Models for the arrangements for two inosine ligands in the bis complex. A minimizes the steric hindrance of the ribosyl groups but the hydrophobic bonding should be less effective than with B.

When inosine is titrated with $(H_3N)_2Pt^{II}$ in D_2O, pD 4.5, the reaction for the formation of II is essentially quantitative. The inosine vibrations observed by the Raman scattering are the same within 1 cm^{-1} for I and II, although shifted significantly from the vibrations of free inosine. Thus, the vibrations are sensitive to coordination of the inosine but not to the overall structure of the complex. In Figure 13, the effects of coordination of $(H_3N)_2Pt^{II}$, CH_3Hg^{II}, and $^2H^+$ [34] at N(7) of inosine are compared. The shifts upon platination and mercuriation are similar, although somewhat larger in the latter case. Deuteration at N-7 causes shifts which are in the same direction but even larger. All of these shifts are consistent with a decrease in electron delocalization upon binding of a cation at N-7.

The decrease in intensity of the highest frequency ring mode upon platination is consistent with a decrease in inosine polarizability as first II and then I is formed as $(H_3N)_2Pt^{II}$ is added, but the basis for the slight increase at higher metal concentrations is not obvious.

While the changes in the vibrational frequencies primarily reflect electronic changes in the ring system, the ^1H NMR spectrum is very sensitive to the overall environment of the coordinated inosine. Coordination of $(H_3N)_2Pt^{II}$ at N-7 in the bis complex II causes a shift downfield, relative to free inosine, of the H-8 resonance by 0.46 ppm but only 0.03 ppm for the H-2 resonance. As discussed by Kong and Theophanides,[14] this is consistent with N-7 coordination of $(H_3N)_2Pt^{II}$. At higher concentrations than those studied here, the ^{195}Pt-H-8 spin-spin coupling of 26 Hz[14] also can be seen, but none is observed to H-2. This, too, indicates N-7 binding. The shifts are still farther downfield with the monoaquomonoinosine complex I, 0.59 ppm for H-8 and 0.13 ppm for H-2, relative to free inosine.

Coordination of $(H_3N)_2Pt^{II}$ to inosine causes a significant decrease in ν_s(Pt-N) from 518 to 501 cm^{-1} for D_2O solutions containing 2:1 inosine-$(D_3N)_2Pt^{II}$, i.e., the bis complex II. The band is broad and, within the experimental error, there was no difference in frequency with a 1:1 solution. Above 1:1 $(H_3N)_2Pt^{II}$-inosine, the scattering is centered at 516 cm^{-1}, approximately the value of $[(H_3N)_2Pt(OH_2)_2]^{2+}$ indicating the presence of unreacted diaquodiammineplatinum(II).

There is one peculiar feature of this reaction scheme. Solutions with 2:1 inosine-$(H_3N)_2Pt$ contain only the bis complex II, i.e., the equilibrium constant for formation of the bis complex is large; but solutions with a 1:1 stoichiometry contain I, II, and unreacted $[(H_3N)_2Pt(OH_2)_2]^{2+}$. For this to be true, some effect must stabilize the bis complex. One possibility is the normal stacking process of the nucleoside itself. The "bite" of the platinum(II) complex is ca. 3.4 Å, and this is the normal stacking distance for purine nucleosides. Possible structures for II are illustrated in Figure 14. The magnetic equivalence of the two ligands requires that they be exchanged by some symmetry operation, a C_2 axis passing through platinum in A and σ containing the platinum atom in B.

The structures suggested are not inconsistent with the effects observed in the ^1H NMR spectra. The shifts downfield of 0.59 ppm for H-8, 0.13 ppm for H-2, and 0.10 ppm for H-1′ assigned to I relative to inosine itself would represent the deshielding effect due to coordination of the platinum electrophile. The ring current diamagnetic anisotropy in the bis complex II would be expected to cause an upfield shift relative to the mono complex as is observed: 0.13 ppm for H-8, 0.10 ppm for H-2, and 0.11 ppm for H-1′. For comparison, stacking of inosine alone has been reported to give upfield shifts of 0.09 ppm for H-8, 0.11 ppm for H-2, and 0.12 ppm for H-1′ attributed to ring current effects.[35] The effects might be expected to be less in the platinum complex, since the expected 90° (N-Pt-N) angle should prevent the planes of the two base moieties from being parallel. Another comparison of the ring current effect can be made by considering the separation of the resonances in a dinucleoside monophosphate. With A3′p5′A, the values at 4° are H-8, 0.13; H-2, 0.220; H-1′, 0.095 ppm.[36]

The reactions of 1-methylinosine at pD 7.7 are almost identical with those described above for inosine at pD 4.5. The effect of coordination of $(D_3N)_2Pt^{II}$ and $^2H^+$ on the vibrational frequencies of 1-methylinosine also is illustrated in Figure 13. The frequency shifts upon platination are virtually the same as for inosine reacting in acid solution. Again formation of the bis complex is almost quantitative, while solutions with 1:1 $(H_3N)_2Pt^{II}$-1-methylinosine contain $[(H_3N)_2Pt(OH)(OH_2)]^+$ and/or $[(H_3N)_2Pt(OH)_2]$, the monoaquo-1-methylinosine complex, and the bis complex with structures analogous to I and II, respectively. Again it seems that the hydrophobic interaction of the two inosine ligands stabilizes the bis complex.

The ^1H NMR spectra are consistent with this picture. If the resonances of the mono complex are taken as characteristic of 1-methylinosine bound to the electrophilic $(D_3N)_2Pt^{II}$, the downfield shifts caused by platination are 0.57 ppm for H-8, 0.13 ppm for H-2, 0.07 ppm for H-1′, and 0.00 ppm (CH_3), relative to the free ligand. The values for acid solution are used since H-8 exchanges rapidly at pD 7.7. The resonances of the bis complex are all shifted upfield relative to the mono complex as would be expected from the ring current effect: 0.14 ppm for H-8, 0.12 ppm for H-2, 0.11 ppm for H-1′, and 0.05 ppm (CH_3). These all are of the same order as the upfield shifts caused by 1-methylinosine stacking: 0.11 ppm for H-8, 0.15 ppm for H-2, 0.11 ppm for H-1′, and 0.09 ppm (CH_3).[34]

As noted above, there is partial exchange of H(8) in the $(H_3N)_2Pt^{II}$-1-methylinosine solutions in D_2O at pD 7.7. This is seen in the Raman spectra, Figure 10, by a new

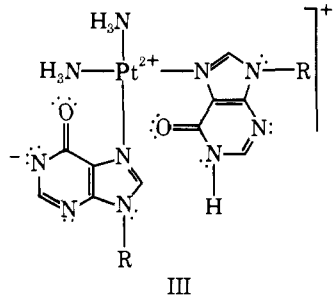
band at 1502 cm^{-1} for solutions 18.75 mM and higher in $(D_3N)_2Pt^{II}$ as well as by a decrease in the relative intensity of the ^1H NMR signals assigned to H-8 of complexed 1-methylinosine. On the basis of the assignments made above, H-8 exchanges more rapidly for the mono than the bis complex. It appears that $(H_3N)_2PtOH_2^{2+}$ is more electrophilic than $(H_3N)_2Pt(1\text{-MeIno})^{2+}$ which is not unreasonable. Although a coordinated water molecule has been written in I, the extent of hydrolysis at pD 7.7 of the 1-methylinosine complex is unknown. We have observed previously that coordination of CH_3Hg^{II} to N(7) of inosine, placing a positive charge on the five-membered ring, greatly increases the exchange of H(8) with solvent protons.[24] Alkylation has a similar effect.

The very broad (Pt-N) stretching band is observed at ca. 500 cm^{-1} with both the complex solutions as well as with the hydrolyzed $(H_3N)_2Pt^{II}$ solution in D_2O, pD 7.7. As the pH is decreased, only a very slight shift to higher frequency is observed for the 1:1 solution, but the aquo cation gives a Raman band at 518 cm^{-1}. The effect of hydrolysis and 1-methylinosine binding on ν_s(Pt-N) of $(H_3N)_2Pt^{II}$ is very similar.

Reactions with Inosine in Neutral Solution and at Higher pH. The most complex sequence of reactions takes place at pH values above 6. The pK of the N-1 bound hydrogen of inosine is ca. 8.8[37] (it will be somewhat larger in D_2O, perhaps ca. 9.3). In the presence of an equimolar concentration of $(H_3N)_2Pt^{II}$ this constant decreases to 7.2, D_2O, 25°. Consequently the species which predominate around the neutral point are very dependent upon the particular experimental conditions. At pD 7.6, solutions with an inosine-$(H_3N)_2Pt^{II}$ ratio greater than 2:1 clearly show ^1H NMR signals due to free inosine and the bis complex. The complex signals are shifted upfield slightly compared to the values discussed above for the bis complex in acidic solution II.

Although certain features of the Raman spectra, e.g., the variation with stoichiometry of the intensity of the band at 1580 cm^{-1} and the changes in the bands initially at 1550 and 1508 cm^{-1}, indicate a 2:1 inosine-$(H_3N)_2Pt^{II}$ reaction, they tend to be overshadowed by other changes which result clearly from a 1:1 reaction. These are the changes resulting from proton loss from N-1 of inosine, e.g., the shift of the broad band at 1676 to ca. 1630 cm^{-1} and of the sharp band at 720 to 739 cm^{-1}.

With excess inosine, the bis complex II is produced. As the pH increases a proton is lost from N(1) to give III. The



III

deprotonation reaction passes unnoticed in the ^1H NMR measurements, because rapid exchange of the proton averages the resonances for the two kinds of coordinated inosine present in the complex. Proton transfer also has, surprisingly, only a small effect on the chemical shifts of the inosine protons, and the same is true for complexed inosine. In addition, the uv spectra[12] show no significant changes when the proton is transferred.

Since the time scale of the Raman experiment is very short, bands due to both kinds of inosine are observed.

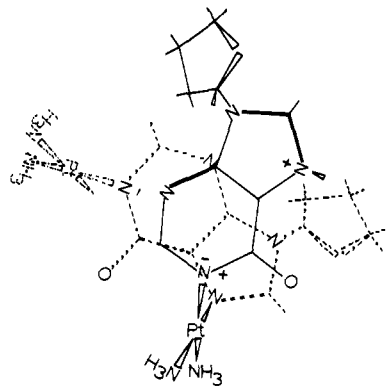
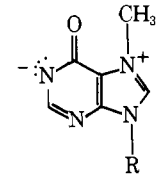


Figure 15. The proposed model for the structure of the polynuclear complex $[(H_3N)_2PtInoH_{-1}]_n^{+}$. Only two adjacent bases in the stack are illustrated for clarity.

When N-1 is deprotonated, a second very good nucleophilic site is generated. Solutions which do not have inosine-$(H_3N)_2Pt^{II}$ ratios of ca. 4:1 or higher in inosine show additional broad ^1H NMR signals and only complex multiplets are observed at ratios lower than 2:1. Interpretation of these changes is complicated further by exchange of H-8 with the deuterium of the solvent, and the total intensity of the resonances in the ring proton region approaches that of the H-1′ multiplet at the higher $(H_3N)_2Pt^{II}$ concentrations.

The complexity of the ^1H NMR spectra, particularly the appearance of multiplets for the ribose protons, suggests polynuclear complex formation, perhaps resembling the normal stacking of inosine. Bridging with the substitutionally inert $(H_3N)_2Pt^{II}$ would not allow rapid exchange as takes place in the normal base stacking. An observation by Dale et al.[38] that 0.1 M 5-mercuriuridine 5′-phosphate gave no ^1H NMR signals for any of the protons including those on ribose is consistent with this. Mercury appears to bridge two bases causing polymerization. Addition of mercaptoethanol blocked polymer formation, and sharp resonances then were obtained for the mononuclear mercuriated uridine.

A proposed structure for the polynuclear complex present in the solutions with 1:1 stoichiometry is illustrated in Figure 15. Coordination of the positive platinum center at N-7 and proton loss from N-1 should result in a highly polar complex with a deficiency of electrons in the five-membered ring and an excess in the six-membered ring. Consequently there should be considerable tendency for these to stack in an orientation which would be favorable to platinum bridging adjacent nucleosides via N(1) of one and N(7) of the other. Again, the normal stacking distance, ca. 3.4 Å, corresponds to the bite of the *cis*-$(H_3N)_2Pt^{II}$ complex, and it seems likely that this stacking is stabilized by platinum bridges. The suggested arrangement for the nucleosides is essentially that proposed for 7-methylinosine which has the polar betaine structure IV in neutral solution.[39]



IV

The Raman spectra are consistent with platination both at N-7 and N-1 in the solutions with 1:1 stoichiometry. The frequencies and intensities observed for the bound inosine are quite different from those observed for [CH_3Hg-$InoH_{-1}$] mercuriated only at N-1[25] but they are very like

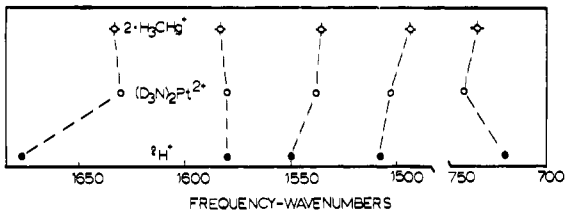


Figure 16. Comparison of the effect of cation binding at both N-1 and N-7 of inosine. There are either two deuterions, two CH_3Hg^+, or one $(H_3N)_2Pt^{2+}$ bridging nucleoside in the polynuclear complex.

the values determined for $[(CH_3Hg)_2InoH_{-1}]^+$ mercuriated at *both* N-1 and N-7.[25] This is illustrated in Figure 16, where the frequencies of neutral inosine in D_2O, the 1:1 $(H_3N)_2Pt^{II}$-inosine, and the 2:1 $CH_3Hg(II)$-inosine values are compared.

Reactions of 5′-Inosine Monophosphate. Goodgame et al. crystallized the compound $Na_{2.88}[(H_3N)_2Pt]_{0.56}(5'\text{-IMP})_2 \cdot 16H_2O$ from a solution at pH 6.85 which had a Pt-5′-IMP mole ratio of 1:2. The Raman spectrum of a solution with this stoichiometry and pH, 25 mM in 5′-IMP, indicates that the 5′-IMP coordinates mainly without dissociation of the N-1 proton. When the $(H_3N)_2Pt^{II}$ concentration is increased to 25 mM, almost complete dissociation of the N-1 proton takes place. There appears to be little involvement of the phosphate, because the 5′-IMP ν_s PO_3 mode at 976 cm^{-1} cancels completely in the difference spectrum of the 1:1 solution; i.e., there is no detectable change. There appears to be a slight hyperchromic effect upon reaction in the 1:2 solution. This may be caused by very slight differences in hydrogen ion concentration between sample and reference, since pH 6.85 is in the middle of the 5′-IMP phosphate buffer region, pK = 6.66.[37]

Based on this evidence, the compound crystallized by Goodgame et al. contains the platinum complex which predominates in solution under their experimental conditions. Slight increases in either pH or $(H_3N)_2Pt^{II}$ concentrations will alter the main species in solution. The structure determined by them for *cis*-$[Pt(NH_3)_2(5'\text{-IMP})_2]^{2-}$ is essentially that proposed in Figure 14, A. The ^1H NMR data suggest that there are hydrophobic bonds between the inosine rings in solution, and it is interesting to note that while the N(7)-Pt-N(7) angle is 89°, the angle between the two bases is only 43°. The platinum atom lies considerably out of the plane of the base.

Observations on the Reactions of Heavy Metals with Nucleotides and Polynucleotides. On the basis of preliminary studies of interactions of cytidine,[40] uridine,[40] and here of inosine and 5′-IMP with *cis*-$(H_3N)_2Pt^{II}$ together with previous studies on the reactions with CH_3Hg^{II}, some general observations can be made on heavy metal nucleotide reactions.

First, it should be noted that substitution reactions of CH_3Hg^{II} occur very rapidly,[41,42] so its binding, like that of the proton, is thermodynamically controlled. The reactions of the substitutionally inert platinum(II) complexes are different, and the binding sites may be controlled kinetically. In this respect they might resemble the alkylating agents.[43] The results previously reported for the platinum(II) complexes suggested a marked difference in the binding reactions from those of mercury(II), the heavy metal which has been most thoroughly studied. With mercury, the most important reaction with the bases of DNA or RNA is with thymidine or uridine, and it involves coordination to the conjugate base with substitution for H-3.[44,45] In accord with this is the observation that the concentration of CH_3Hg^{II} necessary to denature a native DNA varies inversely with the A-T content.[45] With cytidine, binding is much less extensive at pH 7.[21] Studies of competitive reactions between CH_3Hg^{II} and TMP, GMP, AMP, CMP[46] at pH 7 using Raman difference spectrophotometry show preferential reaction with TMP at N(3).

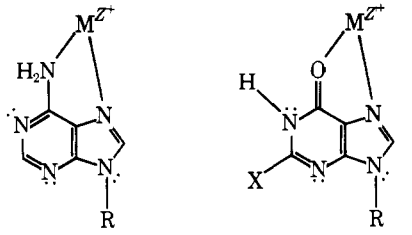
With platinum(II) complexes, it generally is stated that no reaction occurs with uridine or thymidine[12,13,15,16] while reaction is extensive with cytidine. Cytidine is good nucleophile above pH ca. 4.5 using N-3, and coordination of $(H_3N)_2Pt^{II}$ to cytidine gives an almost identical perturbation of the nucleoside Raman spectrum to that caused by CH_3Hg^{II}.[40] Uridine should be a rather poor nucleophile, because N-3 is protonated below pH ca. 8.8,[36] and no sites are comparable in basicity to N-3 of cytidine. Nevertheless, it reacts quantitatively with CH_3Hg^{II} at pH 7. Attack at mercury may occur via some other position besides N-3, but if this is the case proton transfer occurs accompanied by a rapid isomerization to the N-3 bound species. The poor nucleophilicity shows up in the much slower reaction observed between the substitutionally inert $(H_3N)_2Pt^{II}$ and uridine compared to cytidine. The study of this slow reaction is complicated by the development of a blue color in the solutions, the so-called platinum blue.[47]

By analogy with the reactions of cytidine and uridine, it can be reasoned that nucleophilic attack at platinum(II) by guanosine or inosine below pH 8 should involve N-7. Isopotential curves for guanine indicate the point of greatest attraction for an electrophile lies between O-6 and N-7.[48] Reaction of the platinum(II) complexes at N-1 should be slow under conditions where it is protonated. Reaction of CH_3Hg^{II} with inosine at pH 7 occurs in two steps with mercury binding first to N-1 quantitatively with displacement of the proton and then to N-7.[25] Although the initial attack at mercury may occur via N-7, the N-1 proton is lost in neutral solution, and a very rapid isomerization then would be expected to occur. With $(H_3N)_2Pt^{II}$, such an isomerization should be much slower. In addition, N-7 of inosine seems to compete much more effectively with solvent for $(H_3N)_2Pt^{II}$ than CH_3Hg^{II}. In neutral solution, 1-methylinosine reacts quantitatively with $(H_3N)_2Pt^{II}$ but only very slightly with CH_3Hg^{II}

A rather general effect of coordination of a heavy metal electrophile at N-7 of a purine is to increase the rate of exchange of the C(8)-H with solvent. This is observed both with CH_3Hg^{II} and $(H_3N)_2Pt^{II}$, and the mechanism has been discussed previously for CH_3Hg^{II}.[25]

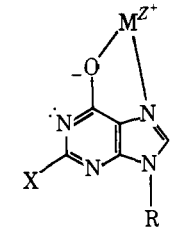
For many years, chelate interactions have been suggested for metal binding to adenosine, inosine, and guanosine as illustrated in VII-IX. Goodgame et al. suggested VIII as a possible mode of initial binding of $(H_3N)_2Pt^{II}$ to DNA. On the basis of this work, there is definite evidence against such a bidentate nucleotide interaction. If any interaction such as VIII took place, it should stabilize $[(H_3N)_2PtInoH_{-1}]^+$ against polymerization. The ^1H NMR spectra should be similar for inosine and 1-methylinosine at pH 7.5 and sharp signals would be expected in both cases. This is not observed. Evidently, energetics favor formation of the polynuclear complex.

Polynuclear complex formation with guanosine or inosine will depend upon proton loss from N-1 of the neutral base. With adenosine, there is no such restriction, and the pK for protonation of N-1 is ca. 3.5.[36] Consequently, stacked structures similar to that illustrated in Figure 15 would be expected over a wider pH range. Kong and Theophanides[15] observed that solutions of $[Pt(en)(Ado)_2]Cl_2 \cdot 4H_2O$ in D_2O gave very complicated ^1H NMR spectra with multiplets for H-2, H-8, and H-1′, and no assignments could be made. Undoubtedly similar stacking occurred with the adenosine complex.



V

$+H^+ \rightleftharpoons -H^+$



VI, X = H, Ino
VII, X = NH_2, Guo

Circular dichroism spectroscopy has indicated the presence of an interbase link formed by reaction of *cis*-$(H_3N)_2Pt^{II}$ with the dinucleotides A3′p5′A and A2′p5′A.[49] This originally was suggested to involve the formation of a bidentate complex either with the 6-NH_2(5′) and N-1 (3′) or N-7 (5′), 6-NH_2(3′). Since recent studies indicate little tendency of adenosine amino groups to interact with heavy metals except at high pH,[23] it is more likely that this involves only the N-7 and/or N-1 positions. The conformational model for A3′p5′A[36] has essentially the arrangement of bases illustrated in Figure 14, B. On the basis of these measurements and those of Roos et al. there is little doubt that *cis*-$(H_3N)_2Pt^{II}$ has a pronounced tendency to bind to two adjacent stacked bases. The N-7 position of the purine nucleotides is accessible in the large groove of DNA, and this site binds the platinum complex effectively over a wide pH range. If this interaction occurs at N-7 of the guanine base, it can be expected to affect the interbase hydrogen bonding since the acidity of H-1 will be increased considerably. With coordination to adenine, the interbase hydrogen bonding also should be altered by a decrease in the basicity of N-1 upon platination at N-7. The electrophilic effect of $(H_3N)_2Pt^{II}$ also is reflected in the increase of the acidity of H-8 of the purines which manifests itself in a greatly increased rate of exchange of this hydrogen with the solvent water protons. The primary difference in the reactions of *cis*-$(H_3N)_2Pt^{II}$ and H_3CHg^{II} is the pronounced tendency of the platinum complex to coordinate to two essentially stacked bases.

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Supplementary Material Available: Raman spectra, 6 pages. Ordering information is given on any current masthead page.

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