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Interactions of Platinum(II) with Adenosine and its Acetyl Derivatives

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The synthesis of complexes of platinum with adenosine and tetracetyl-adenosine is reported.

*The products obtained can be represented by the general formula, PtL_2X_2, where L = purine base and X = Cl, Br. The structures of these complexes were investigated by means of nmr, ir and uv spectra, magnetic susceptibility, conductivity measurements and molecular weight determinations. The nmr data indicate that proton magnetic resonance broadening and shifts could be used to determine metal binding sites with purine bases.***

Introduction

The biological importance of purine bases, constituents of the nucleic acids, is well known. It was reported[1,2] that certain inorganic complexes of the transition platinum metals showed anti-tumor and antibacterial activity.

The reaction of metals with the purine bases is important in establishing the site of binding of the heterocyclic aromatic moiety and the stereochemistry of the complexes formed. Several publications[3-7] have appeared in recent years discussing the site of binding between purine derivatives and a number of bivalent metal ions of the transition series without conclusive evidence on the matter. Platinum(II) is known to form very stable complexes with nitrogen bases and its reaction with the purine bases could also serve as a model for the understanding of the reactions of other metal ions of biological interest. Consequently, a study of the interaction of platinum with adenosine and its acetyl derivatives seemed to us to be interesting. It was decided to start with the nucleoside adenosine instead of ATP as a complexing agent with platinum(II), because the former was a simpler ligand. Platinum seems to react preferentially and primarily with purine bases[1] rather than pyrimidine bases in the double helix of the Watson-Crick model[8] of DNA.

Experimental Section

Materials. Adenosine was purchased from Nitritional Biochemicals Corporation, and Raylo Chemicals Ltd. Potassium chloroplatinate(II) and potassium bromoplatinate(II) (10% aqueous solution) were purchased from Johnson Matthey and Mallory Ltd. Tetraethyl ammonium bromide was recrystallized from acetone before use. Tri- and tetracetyl-adenosine were synthesized as described in the literature.[9] Their purity was checked by TLC chromatography and by nitrogen analyses.

Preparation of the Complexes. Dichloro-bis-(adenosine)Platinate(II). Adenosine (1.9 g) was dissolved in 0.3 N hydrochloric acid (100 ml) and to this an aqueous solution of potassium chloroplatinate(II) (0.5 g) was added (molar ratio, adenosine: K_2PtCl_4 6:1). The initial red color of the reaction mixture changed into yellow in about 3 to 5 hours at room temperature. The solution was cooled and the precipitate was collected and thoroughly washed with water. The product was further recrystallized from DMF-H_2O, washed with alcohol and ether, and dried, *in vacuo*. Yellow powder, decomposed above 180°C without melting. Yield 30%.

Anl. Calcd for $PtCl_2C_{20}N_{10}H_{26}O_8 \cdot 3H_2O$, %C, 28.1; H, 3.7; N, 16.4; Cl, 8.3; Pt, 22.8; H_2O, 5.6. Found: C, 27.6; H, 3.6; N, 16.6; Cl, 8.1; Pt, 23.4; H_2O, 5.6 (In the hydrogen analyses, the water content is included).

Dibromo-bis(adenosine)Platinate(II). This complex was prepared by the above described procedure using HBr solution and potassium tetrabromoplatinate (II).

Orange powder, decomposed above 180°C without melting. Yield 43%.

Anal. Calcd for $PtBr_2C_{20}N_{10}H_{26}O_8 \cdot 3H_2O$, %C, 25.4; H, 3.4; N, 14.8; Br, 16.8; Pt, 20.7; H_2O, 5.1.

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Found: C, 25.3; H, 3.6; N, 14.9; Br, 17.2; Pt, 20.9; H_2O, 5.0.

In aqueous solutions (pH ~6) the change of color of the solution was completed in 15 minutes, instead of 3-5 hrs, as in the case of acid preparations. The neutral preparations gave the same complexes. This was confirmed by elemental analysis conductivity, MW measurements and nmr spectra.

Dichloro-bis-(tetracetyl-adenosine)Platinate(II). Tetracetyl-adenosine (5 g) was dissolved in acetonitrile (35 ml) and to this an aqueous solution of potassium chloroplatinate(II) (0.80 g) was added (Molar ratio, tetracetyl-adenosine: potassium tetrachloroplatinate (II) 6:1). The color of the reaction mixture changed to yellow i nabout 8 to 10 hrs at room temperature. The solution was concentrated to a small volume in vacuo at room temperature. The precipitate was filtered, washed with isopropanol, water, finally with ethanol, ether, and dried in vacuo. Yellow powder, m.p. 148-50°C. Yield 28%.

Anal. Calcd for $PtCl_2C_{36}N_{10}H_{42}O_{16}$, %C, 38.0; H, 3.7; Cl, 6.2; Pt, 17.2. Found: C, 37.3; H, 3.7; Cl, 6.2; Pt, 17.0.

Dibromo-bis-(tetracetyl-adenosine)Platinate(II). The above described procedure was aplied in this case using K_2PtBr_4. Orange powder, m.p. 145-48°C. Yield 25%.

Anal. Calcd for $PtBr_2C_{36}N_{10}H_{42}O_{16}$, %C, 35.3; H, 3.4; Br 13.0; Pt, 15.9. Found: C, 33.9; H, 3.6; Br, 13.2; Pt, 16.3.

Dichloro-bis-(adenosine)platinate(II) 0.22 g was dissolved in 2 ml DMF and a solution (2 ml) of $AgNO_3$ (0.09 gr.) in DMF was added. The reaction mixture was stirred for 15 min at 50°C. The precipitated silver chloride was filtered off. Ether was added to the filtrate and the precipitate was filtered and washed with a small amount of water. The ir spectra (KBr disc) gave a band at 1350 cm^{-1} (NO_3^-) and the chlorine analysis was negative.

Reactions of the above complex with NH_4NO_3, $LiClO_4$ and K_2HgI_4 carried out in DMF gave the initial product.

ND_2 and $3D_2O$ deuterations of the complexes. The $-ND_2$ and D_2O deuterated complexes were obtained by exposing the undeuterated complexes to D_2O in a desiccator for about 78 hrs. Deuteration was not complete in several cases, but longer periods of exposure permitted a 90% deuteration. The -ND-$COCH_3$ deuteration of the tetracetyladenosine complex was performed in DMSO-d_6-D_2O solution.

Microanalyses: (a). Schwarzkopf microanalytical Laboratory (U.S.A.), (b) Dr. Alfred Bernhard, Microanalytishes Laboratorium (Germany), (c) Dr. Daessle. Organic microanalysis (Canada).

Molecular weight determinations. The molecular weight determinations were performed using a Hewlet Packard 301 vapor pressure osmometer and the standard calibration curve was obtained by biphenyl in chloroform. The molecular weight of the dichloro-bis-(adenosine)platinate(II) was determined in DMF solutions by Dr. Alfred Bernhard's microanalytishes Laboratorium, Germany and the molecular weight of the dichloro-bis-(tetracetyl-adenosine)platinate(II) was determined in our Laboratory.

Conductivity measurements. The conductivity was determined by using an E365B conductoscope, Metrohm Ltd.

Magnetic susceptibility measurements. The Magnetic susceptibility values were obtained using the Faraday method. The R.G. Cahn electrobalance equipped with an ALPHA type 110 current regulator and an electromagnet No 9506 were employed. Calibration of the instrument was obtained by measuring the susceptibility of $HgCo(SCN)_4$. The measurements were made at room temperature.

Melting points. The melting points were determined on a Fisher-John's melting point apparatus and are uncorrected.

Spectroscopic measurements. Uv spectra were recorded using a Cary 14 Spectrophotometer with 1 cm quartz cells. The spectra were obtained in DMSO solutions.

Ir spectra were recorded using a Perkin-Elmer 621 spectrophotometer calibrated with polystyrene. The spectra were recorded in KBr, CsBr and CsCl disks or in nujol mulls. The spectrum of the dichloro-bis-(tetracetyl-adenosine)platinate(II) was also recorded in 3% chloroform solutions. The positions of the absorption are given within ± 3 cm^{-1}. Nmr spectra were recorded using a Varian T 60 high resolution spectrometer. TMS was employed as an external standard in DMSO-d_6.

Results and Discussion

The general reaction of the platinum(II) salts with adenosine (L) could be represented as follows:

$$K_2PtX_4 + 2L \longrightarrow 2KX + PtL_2X_2$$

The above reaction was completed in over 2½ hrs when performed in hydrochloric acid solution (pH = 1). Whereas, in water (pH = 6.5) it took place in 15 min. This is obviously due to the excess of halide ions in solution which slows down chloride substitution.[10] Protonation of the ligand may also retard the reaction.*

The compounds obtained were found to have the general formula, PtL_2X_2 (see figure 1). Adenosine[11] crystallizes with 1-1/2 H_2O and this could explain the three waters of crystallization in the complexes with adenosine. On drying the complexes in vacuo at 110°C it was found that they contained $3H_2O$ of crystallization per molecule (see Table I). The presence of water of crystallization was also shown in the ir

* We thank the referee for this suggestion.

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Table I. Physical data of the complex PtX_2L_2.

Compound	No of Water molecules lost	Molar [a] Conductance ohm^{-1} cm^2	Molecular Weight	Magnetic Susceptibility χg cgs $\times 10^{-6}$
$(PtCl_2(adenosine)_2) . 3H_2O$	3	2.8	812 [b] (theor. 800.4)	—0.65
$(PtBr_2(adenosine)_2) . 3H_2O$	2.9	4.1		—0.60
$PtCl_2$(ta-adenosine)$_2$ [d]	0	3.0	1161.5 [c] (theor. 1136.4)	—1.00
$PtBr_2$(ta-adenosine)$_2$[d]	0			

[a] Molar conductance was measured in a 2m mole DMF solutions at 20°. The standard products (Ph_4AsCl and Et_4NBr) under the same conditions gave 64.3 and 75.6 ohm^{-1} cm^2, respectively. [b] Obtained by Dr. Alfred Bernhard in DMF solution (an average of three measurements). [c] Obtained in 1.5m mole chloroform solution (an average of three measurements). [d] ta-adenosine = tetracetyl-adenosine.

spectra of the complexes in the water absorption regions. Deuteration experiments confirmed the water of crystallization.

The dichloro (or dibromo)-bis(tetracetyl-adenosine) platinate(II) complexes did not contain water of crystallization. This is obviously due to the absence of free hydroxyl and amino groups in the organic moiety. Tetracetyl-adenosine itself does not contain water of crystallization. The complexes obtained were soluble only in DMF, DMSO and pyridine. However, the complex of the tetracetyl-derivative was found to be soluble in chloroform. Increased solubility in less polar solvents of the tetracetyl analog could be attributed to increased lipophylicity of the ligand. The complexes were found to be zero-charged from conductivity measurements and this is also in agreement with their overall poor solubility in polar solvents.

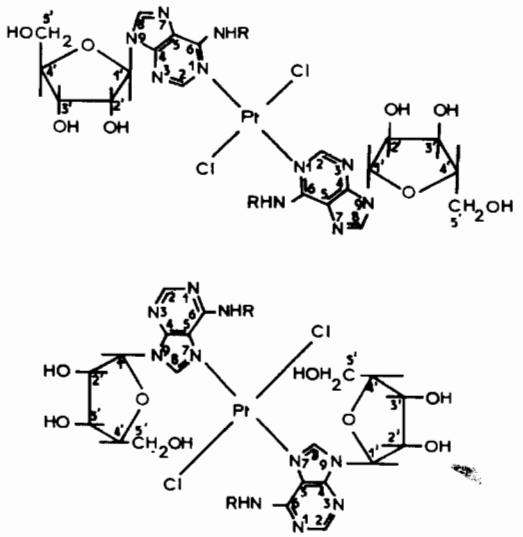

Figure 1. Two possible structures for the platinum-dipurine complexes with a linkage through either N-1 or N-7 positions is considered. R = H, $-COCH_3$.

In addition direct determination of the halogens of the complexes in aqueous solutions indicated the absence of free halide ions. However, back titations of the strongly heated (50°C) solutions for a period of one hour showed the liberation of halide ions corresponding to two halide atoms per complex. Furthermore, attempts to substitute the halides with common anions (NO_3^-, ClO_4^-), were not successful. This behavior is substantial evidence that the halides are in the first coordination sphere.

Magnetic susceptibility measurements were consistent with a planar configuration around the platinum atom and all the compounds measured were found to be diamagnetic (Table I).

The uv spectra of the platinum-adenosine complexes show absorptions in the region 255-300 nm with extinction coefficients ($\varepsilon\lambda_{max}$) of the order of $3.5 \times 10.^4$ Adenosine itself has a band at 262 nm (π - π) and $\varepsilon\lambda_{max}$ 18×10^3 and on complexation with platinum(II) a slight bathochromic effect was observed. This could be explained by the particpitaion of the purine ring to bonding.[12]

The complexes in the far infrared region showed only one sharp platinum-chlorine (340 cm^{-1}) absorption (solid and solution $CDCl_3$) and this could indicate a *trans*-configuration. This evidence, however is weak and dipole moment measurements were unsuccessful due to the low solubility of the compounds in suitable solvents.

A *cis* form is less favored from steric considerations. The *trans*-geometry of these adenosine complexes is also in agreement with literature data[13-15] concerning similar complexes of heavy metals with purine bases.

The site of binding of the adenosine molecule with metals is of great biological importance and has been the subject of numerous studies.[13-20] In these studies (in solution) the N_7 was always reported as being the site of bonding with the metal. Although, in several cases in polynuclear complexes N_3 and N_9 (in adenine complexes) were also participating in bonding. Participation of the sugar moiety under the conditions of the experiments (acidic and neutral media) was also considered and it was excluded from several lines of evidence(ir and acetylation). In the present complexes reaction of the hydrogen of the amino

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group with platinum was not observed, since the $-NH_2$ group was clearly present in the ir spectra of the adenosine complexes in the regions of N-H stretching (3300 cm^{-1}) and $-NH_2$ deformation (1600 cm^{-1} vibrations. The tetracetyl complex also did not show the $-NH_2$ deformation vibration in the 1600 cm^{-1} region because one of the hydrogens is replaced by $-COCH_3$.

This was also confirmed by the absorptions in the regions of N-D stretching (2350 cm^{-1}) and $-ND_2$ deformation (1265 cm^{-1}) vibrations.[20] In addition the ability of tetracetyl-adenosine to form complexes with platinum is further evidence for the non-participation of NH_2 group in NH-Pt or $H_2N \rightarrow Pt$ bonding. The lone pair of electrons on the $-NH_2$ group is not available for bonding with the metal, because it par-

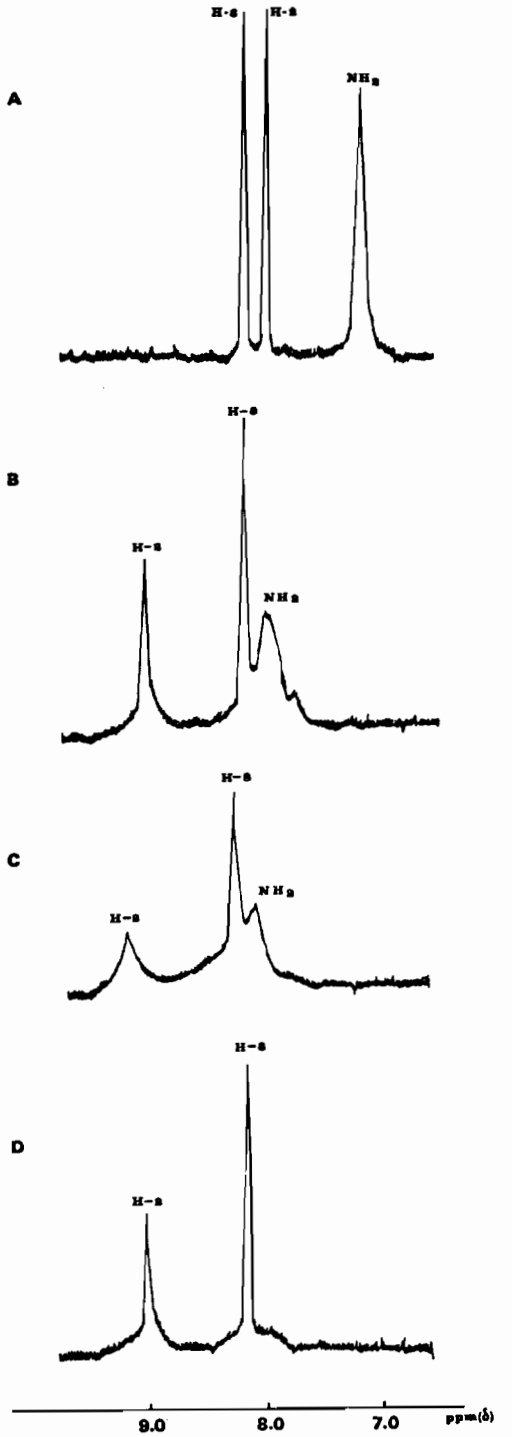

Figure 2. Proton magnetic resonance spectra of (A) adenosine, (B) *trans*-($PtCl_2$(adenosine)$_2$) . $3H_2O$, (C) *trans*-($PtBr_2$(adenosine)$_2$) . $3H_2O$ and (D) *trans*-($PtCl_2$(adenosine-ND_2)) $3D_2O$, in DMSO-d_6 solutions. The spectrum of the sugar is not shown.

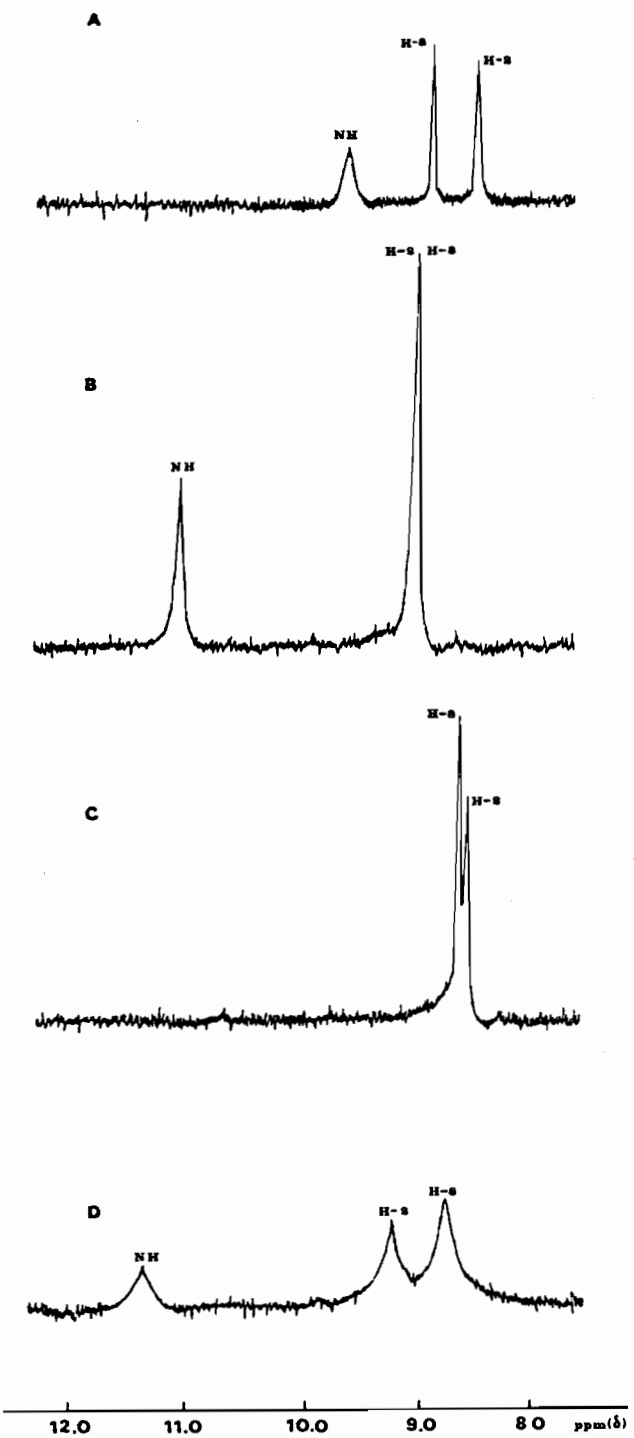

Figure 3. Proton magnetic resonance spectra of (A) tetra-acetyladenosine in $CDCl_3$, (B) tetra-acetyladenosine in DMSO-d_6, (C) tetra-acetyladenosine in DMSO-d_6 with 2 drops D_2O, and (D) *trans*-$PtCl_2$ (tetra-acetyladenosine)$_2$ in DMSO-d_6. The spectrum of the sugar is not shown.

Table II. Chemical shifts (ppm) of adenosine and tetracetyladenosine and their complexes with platinum(II) in DMSO-d_6 solutions.

Proton	L	$(PtL_2Cl_2) \cdot 3H_2O$ δ	Δδ	$(PtL_2Br_2) \cdot 3H_2O$ δ	Δδ	L′	PtL'_2Cl_2 δ	Δδ	L′	PtL'_2Cl_2* δ	Δδ
H_2	7.95 [a]	9.12	1.17	9.15	1.20	8.93 [c]	9.22	0.29	8.58	9.22	0.64
H_8	8.17 [a]	8.26	0.09	8.22	0.05	8.93 [c]	8.69	—0.24	8.64	8.69	0.05
NH_2	7.10 [a]	7.85	0.75	8.06	0.86	10.98 [d]	11.32 [d]	0.34	*	—	—
H_2	7.97 [b]	8.83	0.86	8.89	0.92						

[a] Adenosine(L) [b] 8-bromoadenosine [c] Integrates for two protons. [d] The NH proton of $NHCOCH_3$. * After deuteration ($-NDCOCH_3$) L′ = ta-adenosine DMSO-d_6 with 2 drops D_2O.

ticipates in the ring.[21] This is shown by the high C-N stretching frequency of the C-NH_2 vibration (1215 cm^{-1}) also the availability of this lone pair is further reduced by the acetyl group.

The infrared measurements of the complexes in the solid state showed that the hydroxyl groups of the sugar moiety do not interact with the metal atom. The O-H stretching vibrations of the ligand (3410 cm^{-1}) did not shift on complexation[20] and the -OH group is not deuterated.

The nmr studies of adenosine and its complexes with platinum, as well as those of 8-bromo-adenosine indicate that the platinum atom is linked at the N_1 or at the N_7 position of the adenosine molecule. The results of the nmr spectra are shown in Table II and Figure 2. The chemical shifts of H_2 and H_8 protons have been explained by assigning the greater shift to the proton which is nearer the metal-nitrogen bond (N_1). The NH_2 resonance peaks were easily detected by deuteration experiments (Figure 2C, 2D). The shift of NH_2 protons on complexation is obviously related to the platinum N_1 linkage and probably to the presence of the nearby chlorine atoms (Figure 1). The nmr results of the 8-bromo-adenosine complexes which were prepared in solution but were not characterized, because they were photosensitive and decomposed readily are given in Table II. The spectra show the shift of the only ring proton H_2.

In Table II is shown the assignment which is in accordance with a platinum N_1 interaction. This assignment is based also on the fact that the proton which is remote from the binding site be less affected. The nmr spectra of tetracetyl-adenosine and its complexes with platinum(II) are shown in Figure 3. The assignment of the peaks is given in Table II. The nmr resonances of H_2 and H_8 protons in DMSO-d_6 apparently coincide (8.93 ppm), as is shown by integration. The nmr spectrum of tetracetyl-adenosine in $CDCl_3$ is also shown in the same figure, as a reference. The nmr spectrum of tetracetyl-adenosine in DMSO-d_6 after deuteration of the group $-NHCOCH_3$ ($-NDCOCH_3$) confirms the above assignment and slight split of the single peak for H_2 and H_8 in DMSO-d_6 (Figure 3B) solution could probably be explained by a stronger solvent-H_2 than solvent-H_8 interaction taking as reference the nmr spectrum of the tetracetyl-adenosine in $CDCl_3$ solution (Figure 3A). The nmr spectrum of the corresponding platinum(II) chloro complex (Figure 3D, Table II) shows shifts of the H_2 and H_8 protons comparable to those of adenosine complexes. It is interesting to note that a negative shift is observed for the H_8 proton in Figure 3D in comparison with that in Figure 3B. At present we have no rational explanation for this behavior.

Binding with the N_1 position of the adenosine is further supported by literature data, both theoretical[22] and experimental, in which protonation,[23] n-oxide formation[24] and N-methylation[25] predominantly take place at the N_1 position, although there is no complete agreement on this point.[20-26]

We have considered binding of the metal at the N_7 position of the adenosine, but this seems less likely, because it would imply a substantial shift of both the H_2 and the H_8 protons on complexation. It is also possible that one purine is linked through N_1 and the other through N_7 in these dipurine complexes. In this case we should have four signals in the H_2 and H_8 regions in the nmr spectra of the complexes which is not observed. The possibility of having a water molecule coordinated to the platinum(II) is excluded by comparison of the adenosine and tetracetyl-adenosine complexes. In the latter case, we do not have water of crystallization in the complexes. The given assignment (Table II) is consistent with a large shift of the neighboring proton, H_2, and a small influence on the H-8 proton which is far from the metal binding site. The H_2 shift is also observed with the platinum complex of 8-bromo-adenosine. The magnitude of the shift (Δδ : 0.90-1.20) indicates a fairly strong interaction between the purine N_1 position and the platinum atom. Therefore, in coordinated complexes of purine bases with metals, proton magnetic resonance shifts could be used to determine metal binding sites.

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