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Platinum Purine Nucleosides. II. Interaction of K_2PtX_4 (X = Cl, Br) with Inosine and Guanosine

N. HADJILIADIS and T. THEOPHANIDES*
Université de Montréal, Département de Chimie C.P. 6210, Montréal, P.Q., H3C 3Vl, Canada

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The reactions of K_2PtX_4, where X = Cl,Br with inosine and guanosine have been studied in acidic, neutral and basic aqueous solutions. The isolated solid adducts have been characterized by elemental analyses, conductivity measurements, nmr and ir spectra. Four types of complexes have been isolated, corresponding to the formula $Pt(Nucl)_2X_2$, $Pt(Nucl{-}H^+)_2$, $[Pt(Nucl{-}H^+)X]_n$ and $[Pt(Nucl)_4]X_2$, where Nucl = nucleoside, X = Cl,Br. The complexes of the formula $Pt(Nucl)_2X_2$ have the cis-configuration which implies a weaker trans-effect of the nucleosides compared to halogens. The binding sites are the N_7 and N_1 nitrogen atoms and the O_6 of the purine nucleoside depending on pH. Chelation is possible under certain conditions. It has been found that four purine nucleosides are bound to one platinum atom using the N_7 nitrogen as a coordinative site and form complexes similar to $[PtPy_4]X_2$.

Introduction

The binding of the transition metals to nucleic acids and their constituents has been of great interest in recent years[1,2]. Among the transition metals the platinum inorganic salts have recently been found to be active against several tumors[3,4]. It was suggested that platinum complexes attacked DNA[3]. However, due to the complexity of DNA, we have decided to investigate the behavior of each purine base towards platinum(II) in order to understand the reactivity of DNA with the same metal.

Inosine and guanosine are both purine bases with an O at the 6th position of the purine skeleton. The binding site of these nucleosides with metals has been the subject of many investigations[5–10]. The coordinative positions N_1, O_6 and N_7 have so far been proposed as active sites of bonding in aqueous solutions depending on pH[8,9].

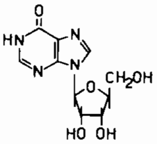
Furthermore, it was in our thoughts that the isolation of new platinum inorganic complexes with such ligands could help to discover new compounds of platinum with useful anti-tumor properties. The study of the general reactivity of these bases towards platinum(II) should be interesting in understanding the metal–molecule interactions in the biologically useful systems.

Results and Discussion

Inosine

Reactions in neutral aqueous solutions

Inosine is found from ir and Raman studies[11,12] to be predominantly in the keto structure in the solid state and in neutral and acidic aqueous solutions:



The proton attached to the N_1 nitrogen atom has a pK_a value of 8.96 in water.[2] The nmr measurements of solutions of mixtures of inosine and K_2PtX_4 = 2:1, X = Cl,Br in D_2O have shown that the H_8 proton resonance of the free base[13,14] upon complexation with platinum(II) shifted downfield. The formation of a platinum–N_7 bond changes the charge distribution of the ring[15] in the H_8 proton becomes more acidic. The nmr experiments showed two species in solution at the end of the reaction.

Conductivity measurements on the isolated products have indicated hydrolysis of the chlorine atom and the molar conductance of the complex in water increased with time and resulted in the formation of a precipitate after three days. It was therefore decided to carry out the experiments in the presence of NaCl in order to prevent hydrolysis and precipitation. Indeed, the nmr spectra in D_2O of mixtures of inosine:K_2PtCl_4 = 2:1 in the presence of 1 to 2*M* NaCl showed the formation of only one product in solution, which was isolated and characterized. The isolated complex had the formula $PtIn_2Cl_2$ with N_7–Pt(II) coordination (Figure

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* To whom reprint requests and correspondence should be addressed.

1a and Table I). Even in the presence of 1 to 2*M* NaCl precipitation due to chlorine hydrolysis did occur after about two weeks. However, a second compound before precipitation was detected in solution by nmr spectra (Figure 1b).

During the reaction of the formation of $PtIn_2Cl_2$ (see below) the presence of the intermediate $K[PtInCl_3]$ could not be detected in considerable amounts in solution. The intermediate $K[PtInCl_3]$ was observed in the initial nmr spectra in very small amounts, but it was converted immediately to the final product $PtIn_2Cl_2$ as was shown by the changes of the nmr spectra. The reaction proceeds through two steps as follows:

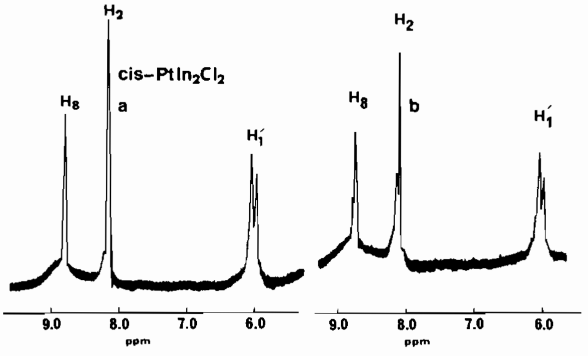


Figure 1. The nmr spectra of platinum–inosine complexes in D_2O solutions. (a) A mixture of 0.1 g inosine + 0.077 g K_2PtCl_4 (2 : 1 molar ratio) in 2 ml D_2O after 4 days of reaction and in the presence of 2*M* NaCl. (b) The same as above, two weeks later.

$$K_2PtCl_4 + In \xrightarrow{\text{slow}} K[PtInCl_3] \qquad (1)$$

$$K[PtInCl_3] + In \xrightarrow{\text{very fast}} PtIn_2Cl_2 \qquad (2)$$

The UV–Visible spectra of the above reactions have also shown an isosbestic point which supports the presence of the above intermediate (Figure 2).

The $PtIn_2Br_2$ analog was isolated in the same way. The compounds $PtIn_2X_2$ are shown to have a *cis*-configuration as in the case of the adenosine compounds. The addition of an excess of thiourea in the nmr tube of $PtIn_2Cl_2$ resulted in the immediate liberation of In. Atomic absorption for platinum determinations also supports the *cis*-configuration[16].

The ir spectra of the solids showed a strong band at about 1690 cm^{-1} which is assigned to the free C=O stretching vibration. In the free inosine this band occurs near 1700 cm^{-1} [5,6]. The 328 cm^{-1} broad band of *cis*-$[PtIn_2Cl_2]$ is assigned to a Pt–Cl stretching. The presence of one broad band instead of two for a *cis*-geometry in this region is not consistent with a *cis*-configuration of the complex. However, this may be

TABLE I. Analytical Data and Conductivity Measurements of Platinum–Inosine and Platinum–Guanosine Complexes.

Compound		C%	H%	N%	Pt%	X%	M.P.[b]	Molar Conductance, ohm^{-1} cm^2 mol^{-1} [c]
$PtIn_2Cl_2$	Calc.	29.90	2.99	13.95	24.30	8.84	210–215° C	22–100 in H_2O
	Found	29.79	3.00	14.05	24.61	8.68		
$PtIn_2Br_2$	Calc.	26.92	2.69	12.56	21.88	17.92		
	Found	26.99	2.64	12.47	21.43	18.15		
$[Pt(In-H^+)Cl]_n$[a]	Calc.	24.10	2.20	–	39.19	–	185–190° C	
	Found	25.41	3.27	–	38.40	–		
$Pt(In-H^+)_2$[a]	Calc.	32.89	3.01	–	26.74	–	205–210° C	
	Found	29.51	3.66	–	27.95	–		
$[PtIn_4]Cl_2$	Calc.	–	–	–	14.55	–	190–200° C	183 in H_2O
	Found	–	–	–	14.42	–		
$[PtIn_2G_2]Cl_2$	Calc.	–	–	–	14.22	–	205–210° C	199 in H_2O
	Found	–	–	–	13.92	–		
$Pt(In-H^+)InCl$	Calc.	–	–	–	25.47	–	215–220° C	
	Found	–	–	–	25.69	–		
$Pt(G-H^+)_2$	Calc.	31.59	3.15	18.42	25.68	–	205–210° C	
	Found	31.19	3.17	16.89	25.94	–		
$PtG_2Cl_2 \cdot 2HCl$	Calc.	26.50	3.09	15.45	21.54	15.67	230–235° C	194 in H_2O
	Found	26.49	3.31	15.57	21.41	15.57		
PtG_2Cl_2	Calc.	28.82	3.12	–	23.42	–	230–235° C	7.7 in DMSO
	Found	29.06	3.08	–	23.52	–		
$[PtG_4]Cl_2$	Calc.	–	–	–	13.94	–	195–200° C	217 in H_2O
	Found	–	–	–	13.52	–		

[a] The C and Pt contents of these compounds are a little different from the theoretical, due to a mixture of the one into the other (see Experimental). [b] The observed temperatures are the decomposition points. [c] 1 m*M* solutions at 20° C.

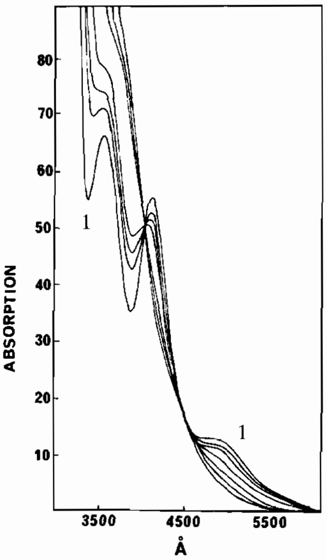
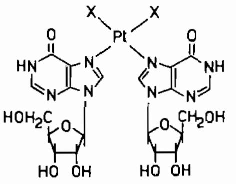


Figure 2. Changes of the uv–visible spectra of a mixture of $10^{-2}M$ K_2PtCl_4 $+2\times10^{-2}M$ *inosine in the presence of* 1.5*M* NaCl as a function of time. Temp. 26° C.

due to a coincidence of the sym. and asym. Pt–Cl stretchings[17]. In fact, the broadness of the above band suggests such a coincidence. The same was observed in the ir spectra of the adenosine complexes. This band was absent from the spectrum of the bromo analogs. The proposed *cis*-structure of the inosine–platinum complexes is shown below:

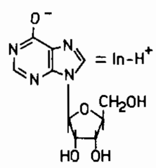


where X = Cl,Br.

The solubility of these compounds may be due to the absence of intramolecular hydrogen bonding as in the case of the adenosine complexes and to the low degree of molecular stacking of the base[14]. These compounds may be potential anticancer agents due to their *cis*-configuration and to their solubility in water*.

Reactions in basic aqueous solutions (pH ≧ 9)

In order to investigate the possibility of N_1 or O_6 bonding with the platinum atom, reactions of K_2PtCl_4 with In at 1:1 and 1:2 molar proportions have been carried out at pH>9. At this pH the imino protons are ionized and create a negative charge on the O^- atom at the 6th position[2]:



After 3 to 4 days at room temperature the obtained insoluble solid adducts were found to be a mixture of the empirical formula Pt(In–H$^+$)Cl and Pt(In–H$^+$)$_2$ (Table I). Unfortunately, their nmr spectra could not be taken because of their insolubility in all solvents.

In the ir spectra of the above compounds the band at 1700 cm^{-1} disappears and a new band is shown at about 1625 cm^{-1}. This indicates an O^- involvment in bonding with platinum. Recently, Ogawa and Sakaguchi[7] studied the metal adducts of IMP by ir and nmr spectra and proposed structures involving the N_7 atom and the phosphates in bonding. It was found that in the case of Hg complexes the C=O stretching was shifted to lower frequencies as was found here and a Hg–O bonding has been postulated. In the case of the other metals (Co(II), Ni(II), Cu(II), *etc.*) the C=O stretching did not change on complexation. In this latter case, the metal did not interact with oxygen. An X-ray single crystal structure determination on a Ni derivative of the above complexes has confirmed the N_7–metal interaction[18]. The same conclusion has also been reached by Fritzsche and collaborators[19]. Tu *et al.*[5] in the Ag(I)–inosine complex have proposed a structure in which a five membered chelate ring between the O_6^- and the N_7 atom of the base is formed. A five membered chelate enol structure was proposed from ir, uv and titration data[6] also in a Cu(II)–inosine complex. In these two cases the ~1700 cm^{-1} carbonyl stretching frequency of inosine was shifted to lower frequencies upon complexation. In the IMP–Cu(II) complexes Berger and Eichhorn[8] suggested O_6–N_7 chelation. The Cu(II) binds only to N_7 with inosine at low pH values (pH<3.5) and to the N_1 and/or O_6 positions which become active coordination sites as the pH increases[8]. Recently, Sletten[20] excluded O_6–N_7 chelation with Cu(II) on the basis of a crystal structure determination of bis(9-methyl-6-oxopurine) copper(II). It was argued that steric effects prevented chelation with O_6. Neumann *et al.*[9] have found similar results.

Five membered chelation is known to take place in the 6-mercaptopurine analogs with metals[21]. Heitner and Lippard[22] from X-ray analysis concluded that a chelation can exist only when the ligand undergoes the proper disortion to accommodate the metal ion. It seems that the reason for the lack of formation of a chelate between the N_7 and the substituent at the 6th

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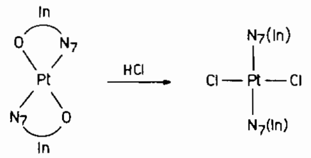
* The compounds are undergoing testing for screening purposes.

position is the tendency of the substituent to react with the particular metal. Evidence for the presence of four and five membered chelate rings involving the O_6^- the N_7 and N_1 atoms simultaneously during the above reaction at pH>9 is given by the reaction of $Pt(In{-}H^+)_2$ and 2–3 *N* HCl at 25° C reacting for 2 to 3 days. The initially insoluble compound enters slowly into solution according to the following reaction:

$$Pt(In{-}H^+)_2 + 2HCl \rightarrow PtIn_2Cl_2 \qquad (3)$$

The isolated compound $PtIn_2Cl_2$ has the same empirical formula as that taken from the direct reaction of K_2PtCl_4 and inosine (Table I). However, the nmr spectrum of this compound showed the presence of three peaks substantially shifted and three more slightly shifted to the up-field region. This is consistent with several species in solution (Table II and Figure 3).

The reaction can be represented as follows:



(4)

The same with compounds N_1–Pt–N_1 and N_1–Pt–N_7.

The formation of four (ON_1) and five (ON_7) membered chelate rings in the initial product $Pt(In{-}H^+)_2$ and the reappearance of the carbonyl (C=O) stretching band in the ir spectra at ~1690 cm^{-1}, as well as the absence of the Pt–Cl stretchings at ~330 cm^{-1} implies an opening of the chelate ring and the breaking of the Pt–O bonds.

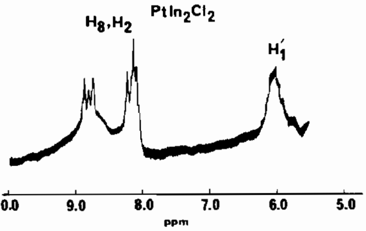


Figure 3. The nmr spectrum of $PtIn_2Cl_2$ prepared according to eq. (3).

In addition the imino proton is observed in the nmr spectrum of the compound $PtIn_2Cl_2$ taken in DMSO-d_6 after the opening of the rings. However, a polymeric structure for the initial compound cannot be excluded (see Figure 8).

Metal complexes similar to the above platinum complexes are known with 8-hydroxyquinoline used as an analytical reagent[23]. The oxygen in the complex $Pt(In{-}H^+)_2$ is suggested to be in *trans*-configuration. The same was found with metal chelates of 8-hydroxyquinoline and guanosine for which the Kurnakoff test

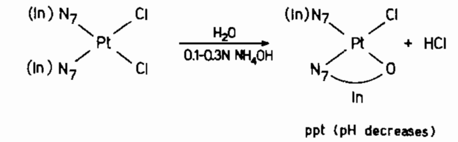
TABLE II. The Nmr Chemical Shifts of Pt(II)–Inosine Complexes in ppm (δ).

Compound		Inosine Protons			Guanosine Protons		Cytidine Protons		
		H_2	H_8	H_1'	H_8	H_1'	H_5	H_6	H_1'
Inosine		8.11	8.22	5.93 6.03[a]	–	–	–	–	–
$PtIn_2Cl_2$		8.20	8.84	6.10 6.17[a]	–	–	–	–	–
	N_7–Pt–N_7	8.17	8.93						
$PtIn_2Cl_2$	N_1–Pt–N_1[c]	8.80	8.30	M~6.00[b]	–	–	–	–	–
	N_1–Pt–N_7[d]	H_2 and H_8 8.88,8.22	H_2 and H_8 8.88, 8.22						
Guanosine					7.80	5.74 5.68			
$[PtIn_2G_2]Cl_2$		8.34	9.05	6.17 6.23[a]	8.67	5.96 6.03[a]	–	–	–
$[PtIn_4]Cl_2$		8.36	9.09	6.03 6.10[a]	–	–	–	–	–
$[PtIn_2en]Cl_2$		8.25	8.68	5.99 6.08[a]	–	–	–	–	–
$[PtIn_2C_2]Cl_2$		8.10	M~8.70[b]	5.90	–	–	7.72 7.84[a]	6.03	5.73 5.78[a]

[a] D = doublet of H_1'. [b] M = multiplet due to three species in solution. The mean value is reported. [c] Prepared from the reaction of $Pt(In{-}H^+)_2 + 2HCl \rightarrow PtIn_2Cl_2$. [d] For this compound eight peaks are expected for the H_2 and H_8 protons. It was assumed that the peak of H_8 of inosine coordinated through N_7 coincided with that of H_2, where the N_1 nitrogen of inosine is coordinated with platinum (8.88 ppm (δ)). They are both shifted. The same happens for the H_2 of the N_7–Pt and the H_8 of the N_1–Pt molecules (8.22 ppm(δ)).

also showed a *trans*-configuration (see under guanosine).

The conductivity of the *cis*-[$PtIn_2Cl_2$], prepared in neutral aqueous solutions changed with time from an initial Λ_m value of 22 ohm^{-1} cm^2 to the final value of ~100 ohm^{-1} cm^2 and at this point precipitation started. The precipitate shows a new band at ~1625 cm^{-1} in addition to the ~1690 cm^{-1} band and the νPt–Cl vibration at ~330 cm^{-1} which became less intense. From the above results it seems that the reaction of $PtIn_2Cl_2$ in solution proceeds through HCl liberation and formation of a five membered chelate ring as follows:



(5)

The precipitate [Pt(In–H^+)InCl] of equation (5) was also obtained in 0.1–0.3 *N* NH_4OH solutions of *cis*-[$PtIn_2Cl_2$] on standing for 2 to 3 hours, as is shown from ir spectra and platinum analyses (Table I). Strongly basic solutions (pH = 14) of *cis*-$PtIn_2Cl_2$ produced similar compounds as in the case of direct K_2PtCl_4–inosine interaction at pH>9, but chelation took place only through O_6–N_7.

Some reactions of the cis-[$PtIn_2Cl_2$] complex

The reactions of *cis*-[$PtIn_2Cl_2$] with other nucleosides have been further investigated in order to prepare complexes with two different bases on a platinum atom and to study their properties. We therefore attempted the reaction of the above compound with guanosine cytidine, *etc.* Wang and Li[15,26] have reported ternary complexes of Zn with nucleosides in solution, of the type adenosine–Zn–guanosine, adenosine–Zn–cytidine, guanosine–Zn–imidazole, cytidine–Zn–imidazole and imidazole–Zn–purine. In all cases formation of the ternary complexes in solution was assumed, involving two ligands with a metal atom. The purine nucleosides in the above work[15,26] were assumed to act as bidentate ligands. We have attempted to isolate similar complexes using the *cis*-[$PtIn_2Cl_2$] complex as a starting material by refluxing it with excess guanosine which yielded the mixed complex [$PtIn_2G_2$]Cl_2, according to the reaction:

$$cis\text{-}[PtIn_2Cl_2] + 2G \xrightarrow{\text{reflux}} cis\text{-}[PtIn_2G_2]Cl_2 \qquad (6)$$

The analytical data for the platinum content are given in Table I. The molar conductance of the compound in water was ~199.0 ohm^{-1} cm^2 mol^{-1} at 20° C which is consistent with three ions in solution. The nmr spectrum of the compound showed the aromatic proton resonance peaks of both bases shifted (Figure 4 and Table II).

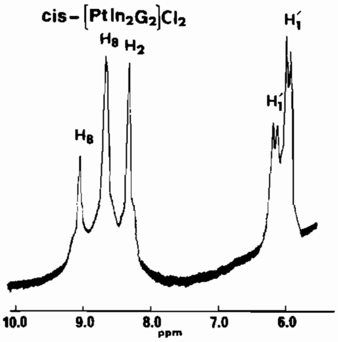


Figure 4. Nmr spectrum of *cis*-[$PtIn_2G_2$]Cl_2 in D_2O.

The H_8 resonance of inosine is shifted more downfield (9.05) than that of guanosine (8.67 ppm). The H_2 is also shifted slightly (8.34 ppm) compared to the free ligand (Table II). The H_1' protons of the sugar of both nucleosides are also shown distinctly in the nmr spectrum. This behavior of the aromatic hydrogens indicates N_7 coordination with Pt(II) and the ligands do not act as bidentate chelate agents under these conditions[10]. These experiments further demonstrate that it is possible to have four such ligands around the central platinum atom, coordinated through the N_7 site. Molecular models show that this is possible from steric considerations. In this manner the compound [$PtIn_4$]Cl_2 was prepared in two ways: (i) by reacting *cis*-[$PtIn_2Cl_2$] and inosine in stoichiometric amounts at 55° C and (ii) by direct reaction of K_2PtCl_4 with inosine at the same temperature, as follows:

$$K_2PtCl_4 + 2In \xrightarrow[25^\circ C]{2M\ NaCl} cis\text{-}[PtIn_2Cl_2]$$
$$\xrightarrow[55^\circ C]{+2In} [PtIn_4]Cl_2 \qquad (7)$$

$$K_2PtCl_4 + 4In \xrightarrow{55^\circ C} [PtIn_4]Cl_2 + 2KCl \qquad (8)$$

Platinum analyses, conductivity data, and nmr spectra confirmed the existence of the compound [$PtIn_4$]Cl_2 (Tables I, II and Figure 5).

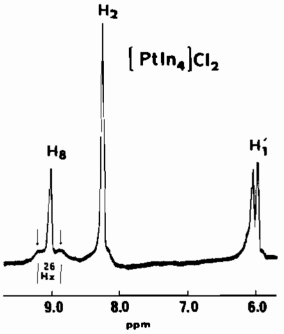


Figure 5. The nmr spectrum of [$PtIn_4$]Cl_2 in D_2O.

After 2 to 3 days of reaction in both cases (eqs. 7 and 8) the nmr spectra became simple and at the end showed two resonances at 8.36 and 9.09 ppm (δ) due to the H_8 and H_2 protons, respectively. This clearly indicates N_7 coordination of the base. In the ir spectrum of the compound $[PtIn_4]Cl_2$ the band at 1690 cm^{-1} indicates free C=O and the absence of the 330 cm^{-1} band is consistent with the change from covalent Pt–Cl bonding to ionic bonding. Furthermore, reactions of *cis*-$[PtIn_2Cl_2]$ with ethylenediamine (en) at room temperature and with cytidine at room temperature and at 55° C did not yield pure and stable compounds. The ionic compounds however, exist in solution. The reaction may take place as follows:

$$cis\text{-}[PtIn_2Cl_2] + en \rightarrow cis\text{-}[PtIn_2en]Cl_2 \qquad (9)$$

Their nmr spectra clearly show the formation of a ionic complex $[PtIn_2en]Cl_2$, previously obtained by Kong and Theophanides[10] using *cis*-$[PtenCl_2]$ as the starting material. In the above reaction ethylenediamine tends to replace inosine being a stronger reagent. This reaction produces more evidence of the *cis*-configuration for the initial $PtIn_2Cl_2$ compound. The nmr spectrum also clearly shows the ^{195}Pt–H_8 spin–spin coupling satellites[10] (Figure 6):

Cytidine also reacts with *cis*-$[PtIn_2Cl_2]$ in the same manner as above:

$$cis\text{-}[PtIn_2Cl_2] + 2C \xrightarrow{55°\,C} cis\text{-}[PtIn_2C_2]Cl_2 \qquad (10)$$

However the reaction was not complete, as is shown by the nmr spectra (Figure 7a, b) the slight shift of H_5 of cytidine shows that it interacts with platinum at the N_3 nitrogen atom[15,26,27].

The overall reactions of inosine with K_2PtCl_4 can be summarized as follows:

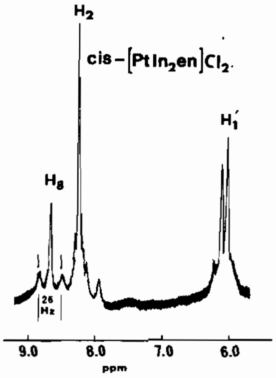


Figure 6. The nmr spectrum of $[PtIn_2en]Cl_2$ in D_2O prepared according to eq. (9).

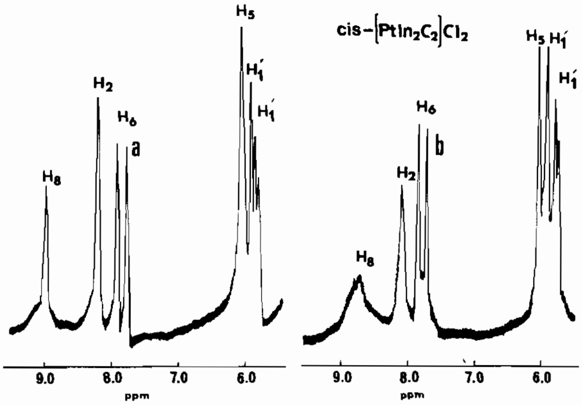
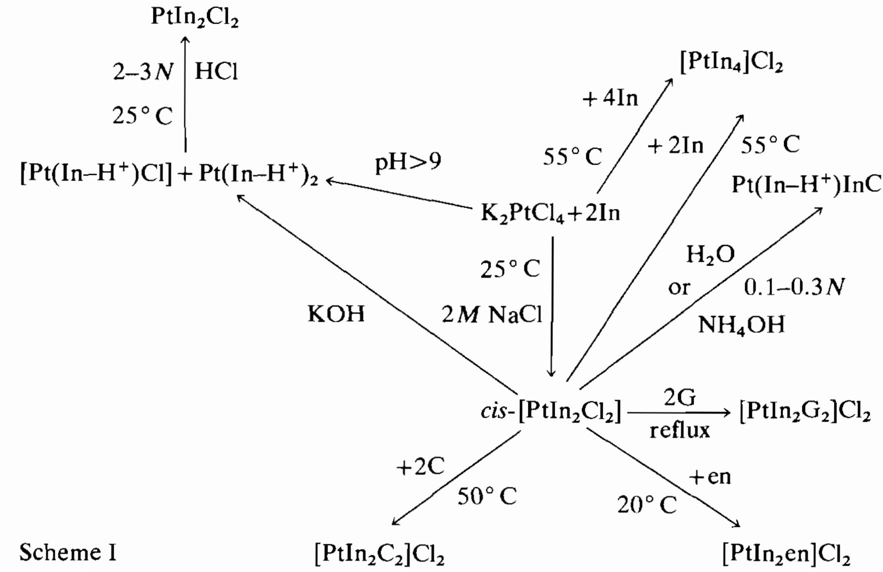


Figure 7. The nmr spectra of (a) Cytidine, (b) *cis*-$[PtIn_2C_2]Cl_2$ in D_2O.

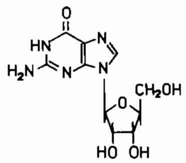


Scheme I

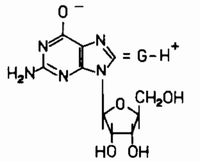
Guanosine

Reactions in basic aqueous solutions, pH ≧ 9.5

The keto structure is attributed to this nucleoside from ir, uv, nmr and X-ray data[2,28]:



The N_1H proton has a pK_a value of 9.2–9.5[28] and at pH ≧ 9.5 guanosine exists mainly in the ionic form[2,28] with a negative charge on the O^- at the 6th position:



Reaction of guanosine with K_2PtCl_4 in aqueous alkaline solutions (pH ~9.5) at 40° C or at room temperature gave a white product of the empirical formula $Pt(G–H^+)_2$ (Table I). This compound is similar to the analogous inosine complex $Pt(In–H^+)_2$. The imino proton is removed and a four or five membered chelate ring between O_6^- and N_7 or O_6^- and N_1 could be formed. The possibility of a polymeric structure cannot be excluded (see Figure 8).

It has been shown that the N_7 nitrogen atom of guanosine is a potential binding site with metals[5,6,10,26,27,29,30]. It was also found by uv and titration studies that Hg(II) affected the ionization of N_1–H proton in guanosine[29] and that N_1 and/or O_6^- were involved in bonding. Studies with Cu(II)[8,9] also show that the metal binds with N_1 or O_6^- of inosine in aqueous solutions, as the pH increases.

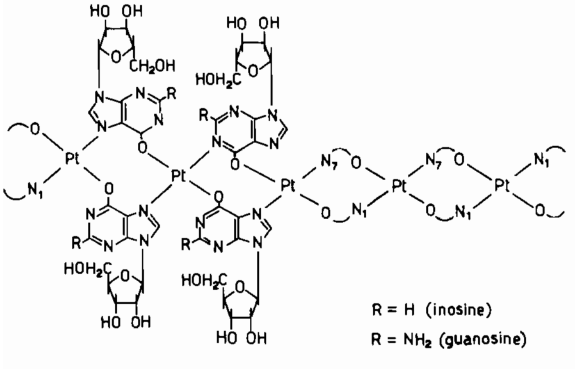


Figure 8. A possible polymeric structure of the complexes $Pt(In–H^+)_2$ and $Pt(G–H^+)_2$ is shown. Two sites of each base are always involved in bonding with platinum in a random sequence.

In the ir spectrum of free guanosine there are three strong bands in the region 1750–1550 cm^{-1}. The first band at 1721 cm^{-1} is shifted to 1678 cm^1 in the complex $Pt(G–H^+)_2$ and is shown in the spectra as a shoulder. Similar ir spectral changes were observed with guanosine in basic media[31]. The second band at 1680 cm^{-1} becomes also a shoulder at 1615 cm^{-1} in the complex, while the third band at 1630 cm^{-1} remains as a strong band in the complex and it shifts to 1578 cm^{-1}. Similar ir spectral behavior was observed in the Ag(I)–guanosine complex to which a five membered chelate structure was attributed[5] involving the O_6^- and N_7 sites with the metal. The same changes were observed in the Cu(II)–guanosine complex, where the oxygen reacted in the enol form with the metal[6]. Craciunescu and Fruma[32] observed similar changes in the ir spectra of copper–guanine complexes with analogous structures. The ir spectra therefore, strongly suggest O_6–Pt interaction. Due to the insolubility of the product in all solvents no nmr data or other measurements in solution could be reported.

The $Pt(G–H^+)_2$ complex when treated with 2–3 *N* HCl at room temperature for 2 to 3 days gave a yellow product of the formula $PtG_2Cl_2 \cdot 2HCl$ (Table I). During the reaction, the O_6–Pt bond slowly opens and the initial N_1H bonds are reformed. This behavior is similar to the analogous inosine complexes. In the far ir spectrum of the compound a new weak and broad band appears at ~330 cm^{-1}, which was missing from the spectrum of the starting material. This is obviously the Pt–Cl stretching vibration. At the same time the ir absorptions in the 1750–1550 cm^{-1} region regained in intensity and the three bands appeared at ~1690, ~1630 and ~1590 cm^{-1}. This indicates the regeneration of a free C=O group (~1690 cm^{-1}). From chemical analyses it was found that two HCl molecules were held in the formula. The compound $PtG_2Cl_2 \cdot 2HCl$ when treated with KOH until the strongly acid solution became neutral (pH ≃ 6) yielded PtG_2Cl_2, as the analytical results show (Table I). The reaction could be reversed back to $PtG_2Cl_2 \cdot 2HCl$ with HCl. The ir spectra of $PtG_2Cl_2 \cdot 2HCl$ and PtG_2Cl_2 appeared identical in the region 4000–200 cm^{-1}.

The nmr spectra of the above compounds (Figure 9 a–c and Table III) show the presence of three different species in solution. The three species are differentiated only by the coordination sites, *i.e.* N_1–Pt–N_1, N_7–Pt–N_7' and N_1–Pt–N_7 species. The situation is similar to the inosine complexes. The H_8 nmr resonances have been observed at 8.41 ppm, 8.80 ppm and 8.57 ppm for the three species, respectively. This implies four and five coordination at the same time for the initial product. Coordination of two metals with one base is excluded from the analytical results (Table I). When the N_1 nitrogen atom is linked to Pt(II) the proton of each

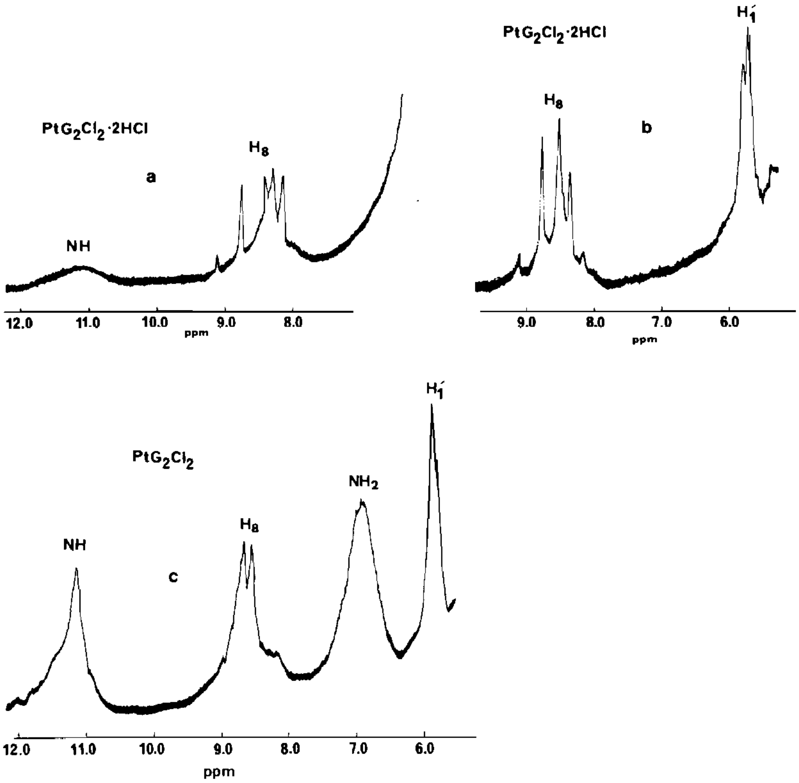


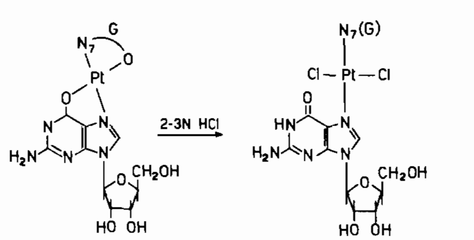
Figure 9. The nmr spectra of platinum–guanosine complexes. (a) The nmr spectrum of $PtG_2Cl_2 \cdot 2HCl$ in DMSO-d_6. (b) The same in DMSO-d_6 + 2 drops of D_2O. (c) PtG_2Cl_2 in DMSO-d_6.

TABLE III. The Nmr Chemical Shifts of Pt(II)–Guanosine Complexes in ppm (δ).

Compound	H_8	H_1'	NH	NH_2	Solvent
$PtG_2Cl_2 \cdot 2HCl$					DMSO-d_6
N_7–Pt–N_7	8.80				
a N_1–Pt–N_1	8.21	c	11.19	c	
N_1–Pt–N_7	8.37, 8.53				
N_7–Pt–N_7	8.80				
b N_1–Pt–N_1	8.41	5.76 5.86[e]	–	–	DMSO-d_6 + 2 drops D_2O
N_1–Pt–N_7	8.57				
PtG_2Cl_2					
N_7–Pt–N_7	8.66				
d N_1–Pt–N_1	8.54	5.76[f]	11.14	6.81	DMSO-d_6
N_1–Pt–N_7	8.66, 8.54				
$[PtG_4]Cl_2$	9.11	6.20 6.48	–	–	D_2O

[a] In the species N_1–Pt–N_7, the 8.37 ppm peak is assigned to the guanosine coordinated through N_1, and the 8.53 ppm peak to that coordinated through N_7. [b] The 8.57 ppm peak is assigned to the species coordinated through N_1 and N_7 coinciding after the addition of a drop of D_2O. [c] It was not determined due to interference (see spectra). [d] The 8.66 ppm(δ) peak is attributed to the species N_7–Pt–N_7 and N_1–Pt–N_7 with the H_8 of the guanosine molecule coordinated through N_7 and the 8.54 ppm(δ) peak to the N_1–Pt–N_1 and N_1–Pt–N_7. [e] D = doublet of H_1'. [f] M = multiplet due to three species in solution. The mean value is reported.

HCl molecule added can go either to N_3 or to N_7 nitrogen atoms, since the NH_2 group is not very basic in guanosine[28] and the N_7 is the preferred protonation site[28,33]. The compounds are *trans* with respect to oxygens as in 8-hydroxyquinoline complexes[23–25]. A Kurnakoff test on the complex $PtG_2Cl_2\cdot 2HCl$ is consistent with this interpretation. The reaction of the formation of $PtG_2Cl_2\cdot 2HCl$ from $Pt(G–H^+)2$ is shown below:



(11)

Reactions in acidic or neutral aqueous solutions ($pH \leqq 6$)

Direct interaction of K_2PtCl_4 with guanosine failed to give pure compounds of the formula PtG_2Cl_2 or $PtG_2Cl_2\cdot 2HCl$. Pure *trans*-compounds could be obtained only by the action of HCl on the ionic complexes $[Pt(Nucl)_4]Cl_2$ where Nucl = nucleoside, with four nucleosides in the inner coordination sphere similar to the $[Pt(py)_4]Cl_2$ complex[34]. Reflux of K_2PtCl_4 in H_2O with excess of guanosine yielded the compound $[PtG_4]Cl_2$ which is extremely soluble in water. The molar conductance of a $2\times10^{-3}M$ solution of this compound in water at 20° C was ~217 ohm^{-1} cm^2 consistent with the presence of three ions in solution. In the nmr spectrum of this complex (Figure 10) there is only one peak at the aromatic proton region (~9.00 ppm), broad at the base, due obviously to the ^{195}Pt–H coupling satellites[10,27] which could not be clearly observed. In agreement with previous studies in solution[10,27,30] the N_7 nitrogen atom in this case is the only binding site with the metal in neutral aqueous solutions. The ir spectrum of this guanosine complex was similar to the ir spectra of $PtG_2Cl_2\cdot 2HCl$ and PtG_2Cl_2, with the difference that the band at ~330 cm^{-1} was absent. This evidence confirms the non-involvment of the O in bonding with the metal (presence of free C=O) and the replacement of all chlorine atoms from the first coordination sphere. The high similarities in the ir spectra of the guanosine complexes may be due to the great tendency of the ligand to aggregate, adsorp and bind, and to form gels and viscous solutions[28].

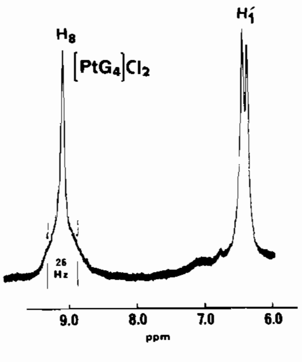


Figure 10. The nmr spectrum of $[PtG_4]Cl_2$ in D_2O.

Attempts to prepare the *trans*-$[PtG_2Cl_2]$ isomer from $[PtG_4]Cl_2$ by the action of HCl as in the case of $[Pt(py)_4]Cl_2$ failed, due to decomposition of guanosine on heating in the presence of HCl[34].

The overall reactions of guanosine with K_2PtCl_4 are given below:

$$\begin{tikzcd} K_2PtCl_4 + 2G \arrow[r, "pH \simeq 10"] \arrow[ddr, "pH = 5.5"', "reflux"] & Pt[G\text{–}H^+]_2 \arrow[d, "2\text{–}3N"', "HCl"] & \\ & PtG_2Cl_2\cdot 2HCl \arrow[r, shift left, "KOH\ pH = 6"] & PtG_2Cl_2 \arrow[l, shift left, "2N\ HCl"] \\ & {[PtG_4]Cl_2} & \end{tikzcd} \qquad (12)$$

Experimental

Materials

Inosine and guanosine were purchased from Raylo Chemicals Ltd and used without further purification.

Potassium chloroplatinate(II) and potassium bromoplatinate(II) (20% aqueous solution) were from Johnson Matthey and Mallory Ltd. The aqueous or acid solutions of the platinum salts were filtered before use.

Preparation of the Complexes

(i) Cis-bis(inosine)dichloroplatinum(II), cis-$[PtIn_2Cl_2]$

To prepare this complex 0.645 g (2.4×10^{-3} mol) of inosine were dissolved in 10 ml of water and to this a filtered solution of 5 ml of water containing 0.500 g (1.2×10^{-3} mol) of K_2PtCl_4 were added (1.755 g). The color of the solution became slowly yellow from red within 4–5 days. The water then was evaporated to dryness and the residue was washed with 5 ml of DMF. The filtrate was separated from the insoluble KCl and NaCl and an excess of alcohol was added to precipitate the product quantitatively. This was then filtered off by suction and washed with ether. It was then dried at 110° C under vacuum.

In preliminary experiments the reaction of K_2PtCl_4 with inosine was followed by nmr by disolving 0.100 g of inosine and 0.077 g of K_2PtCl_4 (2:1) in 2 ml D_2O, without NaCl or in the presence of 0.5, 1 and 2*M*

NaCl. The best results were obtained in the presence of 1–2*M* NaCl.

(ii) Cis-bis(inosine)dibromoplatinum(II), cis-[(PtIn$_2$Br$_2$)]

0.452 g (1.68×10^{-3} mol) of inosine and 0.5 g (0.84×10^{-3} mol) of K_2PtBr_4 reacted as in procedure (i) in the presence of KBr. The final product was isolated in a quantitative yield.

(iii) Tetrakis(inosine)platinum(II) dichloride, [PtIn$_4$]Cl$_2$

(a) 0.200 g of *cis*-[$PtIn_2Cl_2$] (2.4×10^{-4} mol) and 0.1336 g of inosine (4.8×10^{-4} mol) were mixed in 2 ml of D_2O at 55° C. The nmr spectra of the reaction mixture were followed for 3–4 days, until a simple spectrum was obtained. Then the complex formed was precipitated by addition of excess of acetone. It was finally recrystallized in hot CH_3OH and dried first at room temperature and then at 110° C under vacuum. Yield 20–30%.

(b) The same complex was also obtained by direct reaction of inosine (0.200 g or 7.4×10^{-4} mol) with K_2PtCl_4 (0.077 g or 1.85×10^{-4} mol) at 55° C for 3–4 days. The mixture dissolved in 2 ml of D_2O allowed the reaction to be followed by taking nmr spectra at certain intervals (one every 4–5 hours). Then the same procedure as in (iii) (a) was followed.

(iv) Diinosine(diguanosine)platinum(II) dichloride, [PtIn$_2$G$_2$]Cl$_2$

0.500 g (6.2×10^{-4} mol) of *cis*-[$PtIn_2Cl_2$] prepared according to procedure (i) were dissolved in 15–20 ml of H_2O and to this 0.500 g (1.76×10^{-3} mol) of guanosine (excess) were added. The mixture was refluxed for 2 to 4 hours until the color of the solution became white. It was left in a refrigerator to precipitate the excess of guanosine. The filtrate was then reduced to a small volume of 2–3 ml and the complex was precipitated with excess of acetone, filtered and washed with ether. The white product was then recrystallized in hot CH_3OH and dried under vacuum at 110° C. The compound lost four molecules of water on drying. The yield was 40–50%.

*(v) Ethylenediaminebis(inosine)platinum(II) dichloride, [PtIn$_2$*en]

0.350 g of *cis*-[$PtIn_2Cl_2$] (4.3×10^{-4} mol) were dissolved in 2 ml of D_2O and to this 0.29 ml of a 10% solution of concentrated en in D_2O (d = 0.896) were added (4.3×10^{-4} mol). The mixture was kept at 18–20° C and the reaction was followed by taking nmr spectra for 2–3 days, until the spectrum of Figure 6 was obtained. The compound was not completely pure as the spectrum showed, possibly due to some inosine liberation or the formation of the complex [PtInenCl] Cl. Attempts to purify the complex by recrystallization in CH_3OH or C_2H_5OH failed to give a pure compound (decomposition).

(vi) Diinosine(dicytidine)platinum(II) dichloride, [PtIn$_2$Cy$_2$]Cl$_2$

0.350 g (4.3×10^{-4} mol) of *cis*-[$PtIn_2Cl_2$] were mixed with 0.2121 g of cytidine (8.6×10^{-4} mol) in 2 ml of D_2O, and the reaction was followed by nmr at 50° C or at room temperature. The reaction was not complete even after a week from the preparation of the mixture, but attempts to purify the complex failed to yield the pure [$PtIn_2C_2$]Cl_2 due to decomposition in solvents CH_3OH or C_2H_5OH. No other solvents could be used. The presence of the intermediate [$PtIn_2$CCl]Cl and free cytidine was apparent (see Figure 7a, b).

(vii) Bis(inosinato)platinum(II), Pt(In–H$^+$)$_2$

0.645 g of inosine (2.4×10^{-3} mol) were mixed with 0.500 g (1.2×10^{-4} mol) of K_2PtCl_4 in 30 ml of water at pH = 9.5–10 by the addition of the appropriate amount of 0.1*M* KOH. The mixture was left to evaporate slowly at room temperature for 3 days to a week. The pH of the mixture tended to decrease during the reaction, but it was always kept constant at 9.5–10 by the addition of 0.1*M* KOH. At the end of this period an insoluble white solid was obtained which was washed with 0.1*M* KOH and water until neutral reaction, then with DMF and small quantities of alcohol and ether. Dried first at room temperature and at 110° C under vacuum, it yielded ~80% of the theoretical amount. This complex contained a small amount of [Pt(In–H$^+$)Cl] as indicated by analysis.

(viii) (Inosinato)platinum(II) chloride, [Pt(In–H$^+$)Cl]

0.250 g of K_2PtCl_4 (6×10^{-4} mol) were mixed with 0.161 g of inosine (6×10^{-4} mol) in 30 ml of water at pH = 9.5–10. Following the same procedure as in (vii), after 3–7 days a white insoluble solid was obtained in 70–80% yield. This compound contained also a small amount of Pt(In–H$^+$)$_2$ as an impurity.

(ix) Bis(inosinato)dichloroplatinum(II), PtIn$_2$Cl$_2$ prepared from (vii) by the action of HCl

0.500 g of the compound (vii) were left to react with 200 ml of 2–3*N* HCl at room temperature for 2–3 days. It was then filtered from the insoluble residue and the filtrate was evaporated to a small volume (~5 ml). To this an excess of acetone was added and the precipitate was filtered and washed with ether. Dried at room temperature and 110° C under vacuum, it yielded 60–70%.

(x) Inosinato(inosine)chloroplatinum(II), Pt(In–H$^+$)InCl

0.250 g (3.1×10^{-4} mol) of *cis*-[$PtIn_2Cl_2$] were dissolved in 0.1–0.3*M* NH_4OH (3 ml). After 2–3 hours at 50° C a yellow solid was separated by filtration. It was washed with water, alcohol and ether. It was then dried at room temperature and under vacuum at 110° C. Yield = 30–40%.

The same complex was also obtained from *cis*-$[PtIn_2Cl_2]$ in water at room temperature for longer times (precipitation started after 3–4 days) with lower yields.

cis-$[PtIn_2Cl_2]$ in 0.1–1*M* KOH was also decomposed after standing at room temperature for a week to yield compound (vii).

(xi) Bis(guanosinato)platinum(II), $Pt(G\text{–}H^+)_2$

1 g $(2.4 \times 10^{-3}$ mol) of K_2PtCl_4 was dissolved in 200 ml of H_2O with 2 g $(7 \times 10^{-3}$ mol) of guanosine (excess) and the pH was brought to 9.5–10 by the addition of 0.1*N* KOH. Standing at room temperature for 2 to 3 days and with a constant pH an almost white insoluble complex was obtained. It was washed with 0.1*N* KOH, water and DMF and dried at 110° C under vacuum. Yield more than 70%.

(xii) Bis(guanosine)chloroplatinum(II) dihydrochloride, $PtG_2Cl_2 \cdot 2HCl$

0.200 g $(2.6 \times 10^{-4}$ mol) of the compound Pt $(G\text{–}H^+)_2$ were treated with 100 ml of 2–3*N* HCl for 2 to 3 days at room temperature. The compound initially was not soluble in HCl, but slowly it went into solution. Then this was filtered off from the insoluble material and the filtrate was evaporated to a small volume (~5 ml). The final product was obtained by precipitation with addition of excess of acetone. The product was filtered off by suction, washed with ether and dried under vacuum at 110° C in the presence of NaOH. The light yellow complex lost four molecules of water on drying. Yield ~80%.

(xiii) Bis(guanosine)chloroplatinum(II), PtG_2Cl_2

0.500 g of $PtG_2Cl_2 \cdot 2HCl$ $(5.5 \times 10^{-4}$ mol) in 100 ml of water showed a pH $\simeq 3$. The solution was neutralized with 0.1*N* KOH to pH ≃ 5–6 and left in the refrigerator for precipitation. The subsequent precipitates during a week were collected by filtration, washed with alcohol and ether and dried under vacuum at 110° C. Yield ≃ 70%.

(xiv) Tetrakis(guanosine)platinum(II) dichloride, $[PtG_4]Cl_2$

0.5 g $(1.2 \times 10^{-3}$ mol) of K_2PtCl_4 and 2 g $(7 \times 10^{-3}$ mol) of guanosine were stirred in 100 ml of water and the mixture was refluxed for 5–6 hours. It was then left in a refrigerator to precipitate the excess of guanosine. The filtrate was evaporated to dryness and redissolved in 5 ml of DMF to separate the insoluble KCl. The white product was then precipitated with excess of acetone, recrystallized in hot CH_3OH and dried under vacuum at 110° C. Yield ~70%.

Microanalyses

(a) SCHWARZKOPF microanalytical Laboratory (U.S.A.); (b) CHEMALYTICS, Inc. (U.S.A.).

Conductivity Measurements

The conductivity of the compounds was obtained by using an E365B conductoscope, Metrohm Ltd, Herisau, Switzerland.

Melting Points

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

Nmr Spectra

The nmr spectra were taken with a Varian T60 high resolution spectrometer. TMS was used as internal reference.

Ir Spectra

The ir spectra were recorded using a Perkin–Elmer 621 spectrophotometer calibrated with polystyrene. The spectra were recorded in KBr disks. The positions of the absorptions are given within ± 2 cm^{-1}.

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