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Synthesis of Platinum 6-Thiopurine Riboside Complexes

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The reactions of 6-mercaptopurine riboside, (6-MPR = L) and 2-amino-6-mercaptopurine riboside (2-A-6-MPR = L'), with K_2PtX_4, where X = Cl, Br have been studied in neutral (H_2O) and strongly acidic media (3N HX). The obtained complexes have been characterized by chemical analyses, molecular weight determinations, conductivity measurements and ir and nmr spectra. In neutral solutions the inner complexes $Pt(L–H^+)_2$ are obtained, while in acidic solutions complexes of the formulae $PtLX_2$ and $PtL'X_2$ were isolated and characterized. The trans structure is attributed to the $Pt(L–H^+)_2$ complexes, whereas the complexes $PtLX_2$ are supposed to have polymeric structures, with a weak hydrogen bonding through the N_1 protonated purine nitrogen and the halogen of another molecule.

Introduction

The thio-analogs of the purine bases, 6-MP and 2-A-6-MP are among the most active antimetabolites[1,2]. Their ribose derivatives 6-MPR and 2-A-6-MPR are known to exhibit activity against certain types of tumours[3,4]. Recently, platinum inorganic compounds have been found to be powerful anticancer agents[5,6] and have been used in cancer chemotherapy. Kirschner *et al.*[7] reported complexes of Pt(IV), Pd(II), Bi(III) with 6-MP and found them to be more active against certain tumours than the initial 6-MP alone. Thus, our main objective in this study was to synthesise new mercaptopurine–platinum compounds with the hope to obtain even more powerful anticancer properties**. Although compounds of 6-MP with Pt(II) and Pd(II) have been synthesised and studied by Grinberg[8] and by Gel'fman and Kustova[9], to our knowledge complexes of 6-MPR and 2-A-6-MPR with metals have not yet been reported. The SH group reacts extremely fast with the heavy metals, in particular with platinum and forms five or four membered rings through the N_7 or N_1 atoms. Furthermore, the behavior *in vitro* of simple SH groups towards platinum(II) and other metals can be taken as a model for the understanding of more complex biochemical processes. It is known that sulfur containing nucleosides occur in nature[10,11]. Relatively few studies have been devoted to this type of investigation.

The 6-MP and 2-A-6-MP are known to form 1:1 and 2:1 complexes in solution with several metals[12–17]. Complexes of 6-MP with Co(II), Co(III), Cu(II), Ag(I) and Au(I) have been isolated and characterized[18–21]. Recently, the synthesis and crystal structure of bis-(6-mercapto-9-benzyl-purine)palladium(II)–dimethylacetamide has also been reported[22]. It has been reported that copper ions enhance the 6-MP binding to DNA in solution[23]. In addition, it has been found that these ions increase the inhibitory activity of the thio-IMP (anabolite of 6-MP) towards the enzyme adenylosuccinase[1].

Results

Two types of compounds have been isolated and characterized from the reaction of K_2PtX_4 with L = 6-MPR or L' = 2-A-6-MPR, having the general formulae $Pt(L–H^+)_2$ and $PtLX_2$, where X = Cl and Br. The first series of complexes, $Pt(L–H^+)_2$ are obtained in neutral media and the compounds formed are the inner bis-chelate complexes which result from deprotonation of the bases as is shown by the decrease in the pH value during the reaction (see Experimental). Similar compounds have been also obtained from the reaction of 6-MP with Pt(II) and Pd(II) in neutral media, although ring closure required excess of ligand[8]. The same has been obtained with 2-benzethiazolethiol[24]. Sulfur–nitrogen (N_7) chelates have also been obtained in 6-MP complexes with Cu(II), Ni(II), Cd(II), Ag(I) and Au(I)[18,20,21]. Brigando and Colaitis[19], however, have observed only S–M bonding in 6-MP-Co complexes. The 2-(2-mercapto-ethyl)-pyridine yields also analogous complexes with Ni(II), Pt(II) and Co(II) in ammoniacal solutions.

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** All the newly synthesised compounds will be sent to be screened for anti-cancer activity.

The general reaction with K_2PtCl_4 is as follows:

$$K_2PtX_4 + 2L \rightarrow Pt(L\text{–}H^+)_2 + 2KCl + 2HX \quad (1)$$

The yellow precipitates were insoluble in most organic solvents and sparingly soluble in DMF. They dissolved in DMSO with decomposition, which is due to reaction with the solvent and to the opening of the five membered chelate ring (see nmr spectra). The second type of complexes is obtained by a reaction of excess of K_2PtX_4 with L (Pt:L = 4:1) in strongly acidic media (3*N* HX). We have obtained 1:1 compounds with the following reaction:

$$K_2PtX_4 + L \xrightarrow{3N\ HX} PtLX_2 + 2KX \quad (2)$$

The orange compounds of the formulae $PtLX_2$ precipitate immediately with two water molecules which are eliminated on drying, under vacuum.

Reactions of the $PtLX_2$ Complexes

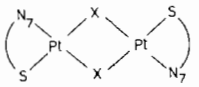
The $PtLX_2$ complexes are insoluble in DMSO and DMF, but they react slowly with DMSO as follows:

$$PtLX_2 + DMSO \rightarrow [Pt(L\text{–}H^+)(DMSO)X] + HX \quad (3)$$

The presence of small amounts of water seems to accelerate the reaction. The product of eq. (3) was isolated by adding an excess of water. In DMF the addition of excess water yielded a polymer, resulting from the removal of one HX molecule from $PtLX_2$ as follows:

$$nPtLX_2 \xrightarrow[+H_2O]{\text{in DMF}} [Pt(L\text{–}H^+)X]_n + nHX \quad (4)$$

The binuclear compound $[Pt(L\text{–}H^+)X]_2$ is shown below:



Similar polynuclear halogen bridged structures can be easily written.

Pyridine also displaces HX from the $PtLX_2$ complexes, however not definitive products were isolated.

The $PtLX_2$ complexes reacted with the bidentate chelate ligand $H_2NCH_2CH_2NH_2$ + en, in stoichiometric amounts in DMF solution at room temperature, as follows:

$$PtLX_2 + en \rightarrow [Pt(L\text{–}H^+)en]X \quad (5)$$

Addition of stoichiometric amounts of L in DMF solutions of $PtLX_2$ yielded the di-thio purine complexes $Pt(L\text{–}H^+)_2$ obtained also in neutral media. The bis-chelate $Pt(L\text{–}H^+)_2$ complexes precipitate immediately with the addition of a large excess of water:

Table I. Analytical Data of the Compounds[a].

Compound		C%	H%	N%	S%	Pt%	X%	Color	M.p. with decomp.
$Pt(L\text{–}H^+)_2$[b]	Calc.	31.50	2.88	14.70	8.40	25.50		deep	
	Found	31.48	3.02	14.36	8.39	25.50		yellow	250–55° C
$PtLCl_2$	Calc.	21.80	2.18	10.17	5.81	35.45	12.90	orange	210–15° C
	Found	21.85	2.25	10.75	5.71	35.35	12.06		
$PtLBr_2$	Calc.	18.77	1.88	8.76	5.00	30.52	25.00	orange	200–5° C
	Found	18.57	1.77	9.12	5.14	30.20	24.74		
$Pt(L'\text{–}H^+)_2$[c]	Calc.	30.31	3.03	17.68	8.08	24.64		deep	200–5° C
	Found	29.85	3.34	17.61	8.06	24.64		yellow	
$PtL'Cl_2$[d]	Calc.	21.22	2.29	12.38	5.65	34.50	12.55	orange	
	Found	23.16	2.97	13.64	6.22	33.07	7.35		
$PtL'Br_2$	Calc.	18.34	1.98	10.70	4.89	29.82	24.42	orange	230–5° C
	Found	19.03	2.11	10.28	4.89	30.32	24.83		
$Pt(L\text{–}H^+)(DMSO)Cl$	Calc.	24.32	2.87	9.45		32.95		light	210–5° C
	Found	24.15	3.06	9.31		32.70		yellow	
$[Pt(L\text{–}H^+)en]Cl$	Calc.	25.09	3.31	14.63	5.57	33.99	6.18	light	220–5° C
	Found	24.76	4.15	14.90	5.12	34.12	6.37	orange	
$Pt(L\text{–}H^+)_2 \cdot HCl$	Calc.	30.06	2.63			24.44		light	260–65° C
	Found	29.17	3.39			24.71		yellow	
$Pt(L\text{–}H^+)_2 \cdot 2HCl$	Calc.	28.75	2.67	13.41		23.37	8.50	light	230–5° C
	Found	28.73	2.40	13.12		23.13	8.46	yellow	
$[Pt(L\text{–}H^+)X]_n$	Calc.					37.97		orange	230° C
	Found					37.95			

[a] All the compounds were dried at 110° C under vacuum in the presence of P_2O_5 for 2–5 hours.
[b] L = 6-mercaptopurine riboside. [c] L' = 2-amino-6-mercaptopurine riboside. [d] This compound gave slightly different analytical values from the calculated and the results were somewhat variable when the L:Pt ratio was varied from 1:1 to 1:10 and the acidity of the solution from 1*N* to 4N HCl.

$$PtLX_2 + L \xrightarrow[+H_2O]{\text{in DMF}} Pt(L\text{–}H^+)_2 + 2HX \qquad (6)$$

In view of the strong *trans* influence of the sulfur atom[8, 24] the compounds $Pt(L\text{–}H^+)_2$ are assumed to be *trans*.

It has been found that the following reaction takes place in the presence of a large excess of acetone:

$$PtLX_2 + L \xrightarrow[+\text{ acetone}]{\text{in DMF}} Pt(L\text{–}H^+)_2 \cdot HX \qquad (7)$$

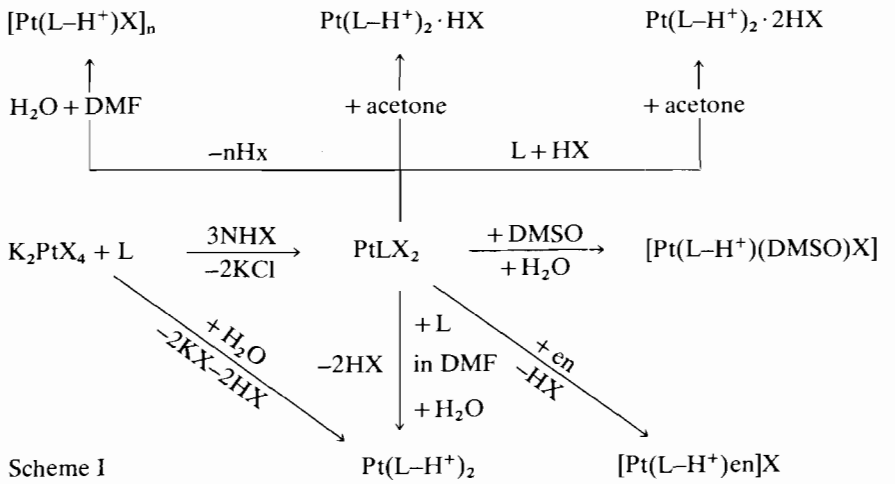
When L was previously dissolved in 3*N* HX and equal volumes of L and $PtLX_2$ solutions were used the addition of excess acetone produced $Pt(L\text{–}H^+)_2 \cdot 2HX$. The compounds $Pt(L\text{–}H^+)_2$ precipitated out in water. The compounds $Pt(L\text{–}H^+)_2$ or $Pt(L'\text{–}H^+)_2$ were also obtained from the reaction in water of *cis*-$[Pt(Nucl)_2Cl_2]$ with L and L′ where Nucl = Adenosine and Inosine.

This is similar to the reaction of *cis*-$(Pt(NH_3)Cl_2$ with 6-MP, which also gives the *trans* bis-inner complexes[8].

The analytical data for these compounds are given in Table I and the conductivity measurements in Table II.

From the data in Table II it is shown that the compounds $Pt(L\text{–}H^+)_2$, $Pt(L'\text{–}H^+)_2$ and $[Pt(L\text{–}H^+)(DMSO)Cl]$ are non-electrolytes. However, the compound $[Pt(L\text{–}H^+)en]Cl$ is an 1:1 electrolyte in H_2O. It is also shown from Table II that the conductivity of the compounds containing HX is due only to the ionization of the acid in DMF or DMSO solutions. A $10^{-3}M$ solution of HCl in DMF gave similar molar conductances as those obtained for $Pt(L\text{–}H^+)_2 \cdot nHX$ or $PtLX_2$ complexes under similar conditions (see Table II). Molecular weight determinations in DMF for the compounds $Pt(L\text{–}H^+)_2$ and $PtLCl_2$ indicate monomeric forms even though the calculated values of 762 and 550 for the above compounds were higher by 20–30% than the experimental ones, *i.e.*, 603 and 386. This difficulty may be due to HX elimination of $PtLX_2$ in DMF.

The compounds obtained in the present synthetic work the methods of production and their reactions can be summarized as follows:



Scheme I

Table II. Molar Conductances of the Complexes at 20° C in ohm^{-1} cm^2.

Compound	Molar Conductance	Concentration, *M*	Solvent
$Pt(L\text{–}H^+)_2$	4.77	2×10^{-3}	DMF
$Pt(L'\text{–}H^+)_2$	6.95	2×10^{-3}	DMF
$PtLCl_2$	~27.00	10^{-3}	DMSO and DMF
$Pt(L\text{–}H^+)(DMSO)Cl$	7.55	10^{-3}	DMF
$Pt(L\text{–}H^+)_2 \cdot HCl$	28.70	1.6×10^{-3}	DMF
$Pt(L\text{–}H^+)_2 \cdot 2HCl$	44.32	1.6×10^{-3}	DMF
$[Pt(L\text{–}H^+)en]Cl$	90.50	0.5×10^{-3}	H_2O
Solution of HCl in DMF	36.50	10^{-3}	DMF

Table III. Nmr Chemicals Shifts of Aromatic and Other Protons of 6-mercaptopurine Riboside and 2-amino-6-mercaptopurine Riboside and Their Platinum(II) Complexes in ppm (δ).

Compound	NH^+	SH	H_8	H_2	N^+H	H_1'[c]	NH_2	Solvent
6-MPR[a]	13.76[c] 13.83	–	8.50	8.10[c] 8.16		5.79 5.88	–	DMSO-d_6
6-MPR[b]	14.31	5.43	8.90	8.59	–	6.14 6.23	–	DMSO-d_6
2-A-6-MPR[a]	12.40	5.30	8.47	–	–	5.94 6.02	7.08	DMSO-d_6
6-MPR	–	–	9.45	8.45	–	6.20 6.26	–	1N DCl
[Pt(L–H^+)en]Cl	–	–	8.85	8.40	–	6.10 6.17	–	D_2O
$Pt(L–H^+)_2$	–	–	9.41[e] 9.25	8.48[e] 8.61		5.97 6.04	–	DMSO-d_6
$Pt(L'–H^+)_2$			9.00[e] 8.80 8.47			5.80 5.90	6.23	DMSO-d_6
$PtLCl_2 \cdot 2H_2O$[a]			9.17	8.69	5.4–4.25	6.01 6.09	–	DMSO-d_6
$PtLCl_2$ (dissolved by heating)[b]	–	–	9.28	8.79	6.28	[f]	–	DMSO-d_6
$Pt(L–H^+)Cl(DMSO)$	–	–	9.21	8.68	–	5.99 6.07	–	DMSO-d_6
$Pt(L–H^+)_2 \cdot 2HCl$	–		8.97[e] 9.34	8.84		6.10 6.18	–	DMSO-d_6
$Pt(L–H^+)_2 \cdot HCl$	–	–	8.90[e] 9.30	8.75	6.47	6.10[d]	–	DMSO-d_6

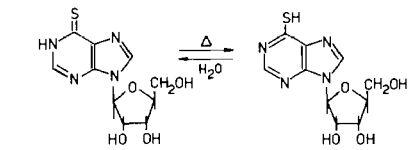
[a] The spectra were taken without drying the sample. [b] The spectra were taken after drying at 110° C for 3–5 hours. Concentration was ~0.2*M* in all cases. L = 6-mercapto-purine-riboside, L' = 2-amino-6-mercaptopurine-riboside. [c] D = Doublet. [d] M = Multiplet due to several species in solution. The mean value is given here. [e] These two or three resonances are due to two or three species in solution. [f] Obscurred because of NH^+.

Nmr Spectra

The nmr chemical shifts of the aromatic protons of 6-MPR and 2-A-6-MPR and their platinum complexes are given in Table III.

Thiopurines, generally, exist in aqueous solution or in the solid state at room temperature as thiones rather than thiols[26–29]. The same is true for thiopurine ribonucleosides[28d,28c,29]. In the nmr spectra of 6-MPR and 2-A-6-MPR the signals at 8.50 and 8.47 ppm(δ) are attributed to the H_8 aromatic protons of these two thioribonucleosides, respectively. The assignment of the aromatic protons of 6-MPR has been reported[30]. The nmr of 2-A-6-MPR has not been reported previously. It shows only one resonnance at 8.47 ppm(δ), the position 2 being occupied. The nmr spectrum of 6-MPR (obtained from Raylo and was used without further purification) in DMSO-d_6 shows a doublet at 13.76, 13.83 ppm(δ) with a coupling constant of 0.07 ppm(δ) (see Figure 1a). This is assigned to the N_1 imino proton, coupled with the adjacent H_2 proton. The H_2 proton is also shown as a doublet with a coupling constant of $^3JN_1H–H_2 = 0.4$ Hz (0.07 ppm). The doublet at ~13.8 ppm disappears by adding one drop of D_2O whereas the doublet at ~8.10 ppm becomes a singlet and moves to 8.30 ppm(δ) as is shown in Figure 1b. This behavior suggests that the proton of N_1H is tightly bound to the N_1 nitrogen and that the imino form is predominant in DMSO-d_6 solutions. Self association of 6-MPR through N_1H in DMSO-d_6 obviously does not occur, allowing the proton to be predominantly located at the N_1 position and coupled with H_2. The SH resonance was not observed in the spectra of 6-MPR (Figure 1a). The same behavior was also observed in imidazole[31]. The nmr spectra of imidazole in DMSO-d_6 solutions, when complex formation occured in the presence of Zn showed the proton located near one of the two nitrogen atoms of imidazole and coupled with the adjacent carbon proton$_{31}$. By heating the 6-MPR at 110° C in vacuum for a few hours and recording its nmr spectrum resulted in the disappearance of the $^3JN_1H–H_2$ coupling. The N_1H signal of 6-MPR becomes almost invisible and another signal appears at about 5.40 ppm(δ) attributable to the SH protons (Figure 1c). This probably shows an equilibrium between the thiol and the thione

forms, which is perturbed by heating and by the presence of water, as is shown below:



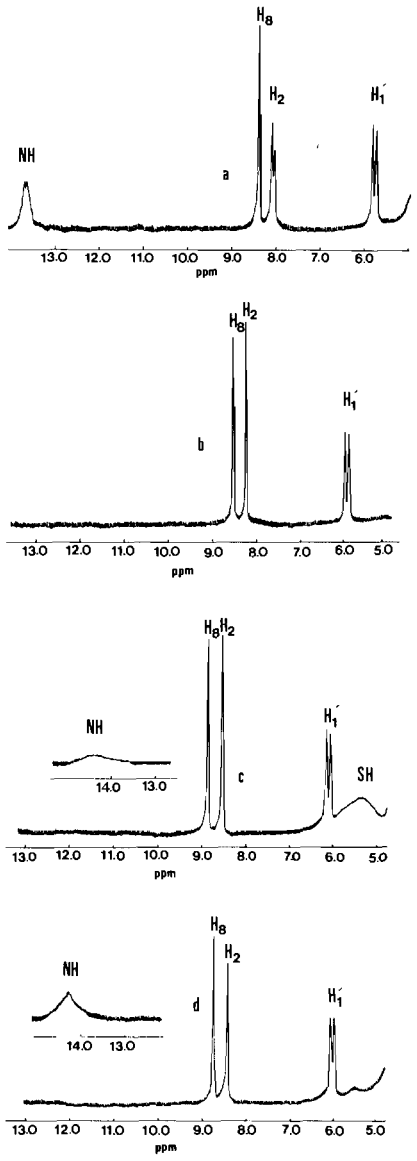


Figure 1. (a) NMR spectrum of 6-mercaptopurine riboside (bought from Raylo) in DMSO-d_6; (b) The same in DMSO-d_6 with two drops of D_2O; (c) The same as in (a) after heating at 110° C under vacuum; (d) The same as in (c) with two drops of H_2O. Region of aromatic protons.

Heating favors the thiol form and addition of water favors the thione form. Shefter[27d] reported a 20% contribution of the zwitterionic amidic form, $^+$NH=C(–S$^-$)– in the crystal structure of 6-MPR, with the thione form predominant, –NH–C(=S)–. Although 6-MP monohydrate has the thione structure in the solid state it may exist in equilibrium between thiol and thione forms in solution[27a]. Recently[32] it was reported that the 2-phenyl-4-mercapto-pyrimidine also exists in equilibrium between the thiol and the thione forms in DMSO-d_6, the thiol form being favored. However, when a methyl group was attached to the mercaptopyrimidine the thione form only was observed. Upon addition of a drop of water in DMSO-d_6 solutions of 6-MPR the signal attributed to the SH group disappeared and the signal of the NH group increased, proportionally (Figure 1d). The transfer of hydrogen could be explained by the higher proton acceptor properties of H_2O and the higher hydrogen bonding ability of the NH group as compared to the SH group[33].

Transformation of the thiol (–SH) to the thione (C=S) form and subsequent participation of the imino (NH) hydrogen in hydrogen bonding with water is favored. The N_1H–H_2 coupling has not been observed in the nmr spectra, which may be due to a rapid proton exchange and it was also found that the H_2 proton signal was less intense. On deuteration all exchangable protons disappear (NH, SH and NH_2).

Complex formation of thiopurines with metals should cause downfield shifts[34] of the aromatic ring protons nearest to the metal. This has been attributed to extensive π-electron redistribution on protonation or complexation[34,35]. The technique has been widely used to determine metal binding sites in purine bases and their derivatives[31,34–36]. The N_7 nitrogen atom has in many cases proven to be the preferred site of coordination in numerous purine nucleoside–metal complexes[34,37]. In addition, the ^{195}Pt–H coupling constant and the satellite bands due to the coupling are extremely interesting and provide conclusive evidence of bonding sites between the platinum atom and the purine base[37]. In the nmr spectra of all the compounds here the H_8 protons of the thio-bases are shifted downfield with respect to H_2 and they become less intense upon complexation. Very often the signal is broadened, due probably to ^{195}Pt–H_8 spin–spin coupling satellites[37] not resolved in this case. In all synthesized complexes no nmr signals attributable to NH or SH protons were observed. Therefore, the formation of a five membered chelate ring between the sulfur atom and the N_7 nitrogen atom is proposed here (see Discussion). The H_8 proton shifts downfield in the protonated form of 6-MPR, as is shown in Figure 2. The preferred site of protonation in 6-MP and 6-MPR has been found to be the N_7 nitrogen atom[28,29]. The same conclusion is reached also here. The complex formation of a four membered ring involving the N_1

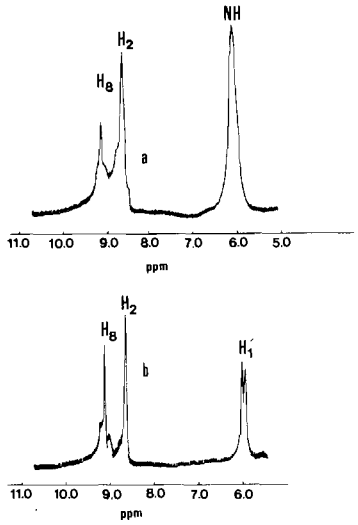
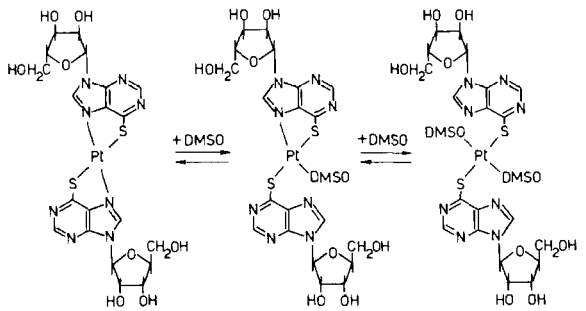


Figure 2. NMR spectra of (a) The compound $PtLCl_2$ dried at 110° C in vacuum and disolved in DMSO-d_6 by slight heating; (b) The same with drops of D_2O. Aromatic proton region.

nitrogen and the sulfur is unlikely because the H_2 proton signal shifts only slightly downfield in the nmr spectra of the complexes (see Figure 3). The compounds $Pt(L–H^+)_2$, $Pt(L'–H^+)_2$, $Pt(L–H^+)_2 \cdot HCl$ and $Pt(L–H^+)_2 \cdot 2HCl$ reacted in DMSO, probably by opening the five membered ring at the N_7 binding site and forming the following type of complexes with DMSO.



The final H_8 proton signal is shown to be upfield with respect to the initial product $Pt(L–H^+)_2$ which indicates the breaking of the $Pt–N_7$ bond. The intensity of the H_8 signal increased with time and an equilibrium of several species in solution was whown from the spectra (Figure 3). The reaction of DMSO has been found to be faster with the compounds $Pt(L–H^+)_2 \cdot HCl$ and $Pt(L–H^+)_2 \cdot 2HCl$. These two compounds as well as $PtLCl_2$ show a signal at about 6.5 ppm(δ) assigned to $N–H^+$, which disappears on deuteration. The signal of the H_2 proton of the compound $PtLCl_2$ is observed

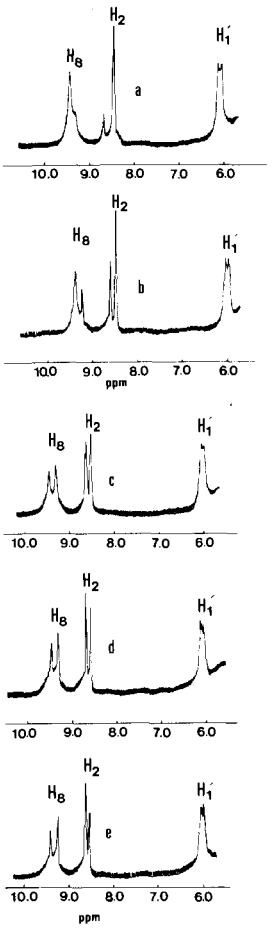


Figure 3. NMR spectra of the compound $Pt(L–H^+)_2$ in DMSO-d_6. (a) Immediately after sample preparation; (b) One day later; (c) Three days later; (d) Six days later; (e) Twelve days later.

up field as compared to the H_2 of $Pt(L–H^+)_2$ (Table III), possibly due to the $N_1–H^+$ protonation.

The compound $PtLCl_2$ reacts with DMSO in the presence of H_2O and forms $Pt(L–H^+)(DMSO)Cl$ (see eq. (3)). The $N–H^+$ signal at about 6.28 ppm was shown in the spectra when the DMSO solution of $PtLCl_2$ was heated. It was rapidly removed upon addition of one to two drops of water to the solution. The reaction of K_2PtCl_4 with L = 6-MPR in 1N DCl in 1:2 molar ratio, as well as that of $PtLCl_2$ with L (1:1) in DMSO was recorded in order to compare the nmr spectra with those of $Pt(L–H^+)_2$ in 1*N* DCl and of $Pt(L–H^+)_2 \cdot HCl$ in DMSO. The spectra were similar which may indicate some ring opening consistent with equation [8], *i.e.*, reaction with DMSO. Grinberg *et al.*[8] have isolated a complex of 6-MP and Pt(II) in 1*N* HCl with a chlorine atom bound to Pt(II) indicating partial closure of the chelate ring. In the present

case a partial or complete closure of one or two purine rings was observed from the nmr spectra and ring opening occured when $Pt(L–H^+)_2$ was dissolved in 1*N* DCl. However, protonation of the purines cannot be excluded. Reaction of K_2PtCl_4 with L (1:2) carried out in less acidic solutions gave a precipitate which when washed with water proved to be the compound $Pt(L–H^+)_2$ from analytical data and ir measurements (see Experimental).

Ir Spectra

The detailed interpretation of the ir spectra is difficult and will be discussed in a later publication, when more specific experiments are available. However, the structures of the complexes can be deduced from a few characteristic ir bands of the complexes.

Ring protonation was shown in the nmr spectra of the compounds $PtLX_2$, $Pt(L–H^+)_2 \cdot HCl$ and $Pt(L–H^+)_2 \cdot 2HCl$. The ir spectra confirm these observations from the following remarks: (i) In the region 3500–2500 cm^{-1} there is shown a strong absorption in the spectra of all the compounds and the ligands, which is assigned to NH_2, OH, CH and NH stretching motions and their inter-molecular hydrogen bondings[26]. In the above compounds this absorption is more pronounced and extents to about 2500 cm^{-1}. This band is assigned to the presence of weak $NH^+ \cdots Cl^-$ intermolecular hydrogen bonds of the ring protonated N_1 nitrogen atom and a chlorine atom of another $PtLCl_2$ molecule, which agrees with observations on the mono and diprotonated purine derivatives[38b]. In these systems the existence of a strong hydrogen bonding gave rise to the strong absorption near 2400 cm^{-1}, while the weak hydrogen bonding obtained by replacement of Cl^- with ClO_4^- gave the same absorption at 3200 cm^{-1} [38b]. Similar results were also observed in the protonated forms of pyridine with frequencies diminishing in the order, $Br^- < Cl^-$ [39]. In the compounds $PtLBr_2$ and $PtL'Br_2$ the broad band extends to 2750 cm^{-1} which is consistent with a weaker inter-molecular hydrogen bonding $NH^+ \cdots X^-$ in the order $X = Cl^- > Br^-$. The absence of this band in the spectra of the other complexes indicates the non protonation of their rings, or their deprotonation on passing for example from $PtLCl_2$ to $Pt(L–H^+)(DMSO)Cl$ (see eq. (3)). No absorption attributable to SH stretching was observed in the ligands and in their platinum complexes. The coordinated DMSO in the compound $Pt(L–H^+)(DMSO\text{-}d_6)Cl$ is recognized from the asymmetric and symmetric C–D stretching motions occuring at 2252 and 2118 cm^{-1}. (ii) It is observed that the 1582 cm^{-1} band of 6-MPR, assigned to a skeletal stretching motion in purine[38a] increases in energy upon complexation or protonation[38b,39]. It appears at 1613 cm^{-1} in the complex $Pt(L–H^+)_2$, while in the protonated compound $PtLCl_2$ it is observed at 1649 cm^{-1}. In the $PtLBr_2$ it is at 1642 cm^{-1} and in the $Pt(L–H^+)_2 \cdot HCl$ it appears as a doublet at 1649 and 1645 cm^{-1} with a shoulder at 1623 cm^{-1}. The shoulder at 1623 cm^{-1} disappears in the compound $Pt(L–H^+)_2 \cdot 2HCl$. On complecation of the ligand 6-MPR to form the non protonated Pt $(L–H^+)_2$ the 1582 cm^{-1} band increases in energy by 31 cm^{-1} indicating the non availability of the lone pair electrons on the sulfur atom to participate in the ring resonance. Thus the ring C=N bond acquires more localized double bond character and its frequency increases. On protonation we observe a further increase by 29–36 cm^{-1} (Figure 4).

Increase in frequency of C=N ring stretchings was also observed in the protonated forms of adenosine and cytidine[40]. In the compounds $Pt(L–H^+)_2 \cdot HCl$ and $Pt(L–H^+)_2 \cdot 2HCl$ the splitting of this band may be due to a crystal effect or to fermi resonance[38b], while the presence of the shoulder for the first compound at 1623 cm^{-1} indicates the protonation of only one purine ring. In agreement again with eqs. (3–4), when deprotonation occurs the band at about 1650 cm^{-1} of $PtLCl_2$ diminishes in energy in the compounds Pt $(L–H^+)(DMSO)Cl$ and $[Pt(L–H^+)Cl]_n$ down to about 1625 cm^{-1} (Figure 4). The C=S stretching vibration in the ligand 6-MPR is assigned to the band at 1171 cm^{-1} which is absent in the spectra of the complexes, in agreement with platinum–sulfur bonding and with what is found in a number of pyrimidine thiones studied by Spinner[41]. This again indicates a thione structure for the ligand 6-MPR in the solid state[29]. (iii) The ir spectra support a *trans* configuration for the compound $Pt(L–H^+)_2$ by the presence of only one νPt–S band at 388 cm^{-1} and by the absence of any band in the region 400–500 cm^{-1}. The presence of a band in this region has been interpreted as an indication of

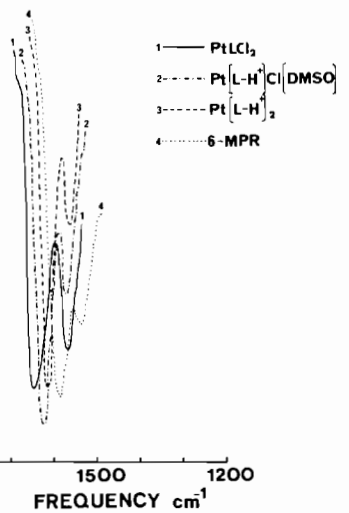


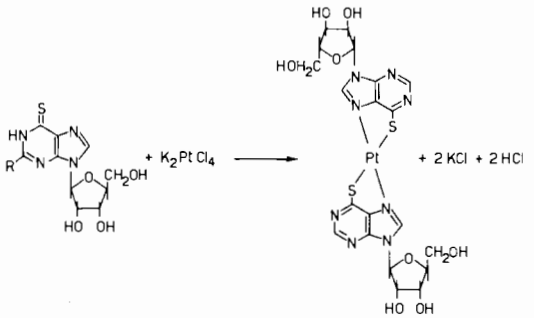
Figure 4. Variation in the frequency of the first C=N ring stretching vibration in some Pt-6-mercaptopurine riboside complexes.

deviation from a square-planar configuration in metal–thio-oxine complexes of the formulae $M(L\text{–}H^+)_2$[42]. (iv) Finally the νPt–Cl and νPt–Cl---H^+ vibrations of $PtLCl_2$ occur at 337 and 312 cm^{-1}, respectively and these bands are absent in the spectra of the bromo derivatives and the compounds $Pt(L\text{–}H^+)_2$, $Pt(L\text{–}H^+)_2 \cdot HCl$ and $Pt(L\text{–}H^+)_2 \cdot 2HCl$ indicating also that in the last two compounds the chlorine is not directly bound to platinum and can be also represented as $[Pt(L\text{–}H^+)_2 H]^+Cl^-$ and $[Pt(L\text{–}H^+)_2H_2]^{2+}2Cl^-$.

Discussion

The 6-MPR and 2-A-6-MPR seem to have similar coordinative abilities to the chelating agent 8-mercaptoquinoline, which has been widely used as an analytical reagent[43,44]. Both ligands are mercapto-derivatives of heterocyclic systems and contain a sulfur and a nitrogen atom in a suitable geometric arrangement to form a five membered chelate ring with metal ions. Acid dissociation measurements[43] indicate that 8-hydroxyquinoline forms more stable 1:1 metal chelate complexes than 8-mercaptoquinoline, but this is not the case here. The explanation given was based on the greater electron withdrawing ability of the thiol group, as compared to the hydroxyl group. The sulfur platinum bond possesses a greater degree of covalent character than the oxygen platinum bond. This is due to the stronger π-bonding ability of sulfur[43]. For all the above reasons 8-mercaptoquinoline is used analytically in a substantially lower pH region than 8-hydroxyquinoline[43]. Furthermore, the thiocarbonyl (C=S) group possesses a greater polarizability than the carbonyl (C=O) as is revealed from the nmr spectra of a number of 6-thiopurines and hypoxanthines[28,30] (the aromatic hydrogen signals are deshielded to a greater extent in 6-thiopurines). It is also found that the substitution of a C=O by a C=S group in the 6th position of a purine ring causes an increase in the acidity of the hydrogen H_2 adjacent to N_1–H group (compare the pK_a values of the N_1H group in Table IV).

Therefore, 6-thiopurines are stronger acids than hypoxanthines[28]. The above acid dissociation properties of the 6-MP ribosides are very useful in controlling their reactions with platinum. In neutral media the strong tendency of sulfur to react with platinum ionizes the adjacent imino protons and the pH of the initial neutral solution decreases during the reaction as is shown below:



where R = H, NH_2 and X = Cl, Br; pH decreases.

The di-thiopurine complex should have the *trans* configuration, because of the high *trans* effect of the sulfur atom. This is also consistent with the preparation of the same complex from $PtLX_2$ and L, as well as the reactions of $PtLX_2$ with *cis*-$Pt(Nucl)_2X_2$, and supported further by ir data. Unfortunately, due to solubility restrictions, no dipole moment measurements could be obtained to confirm the *trans* isomerism.

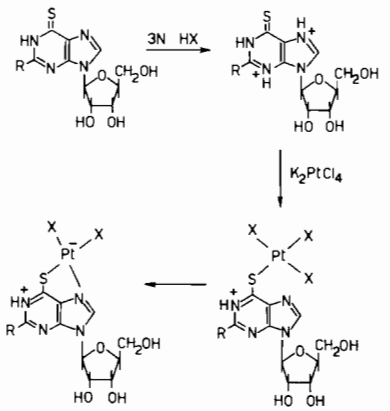
The crystal structure of bis-(6-mercapto-9-benzylpurine)palladium(II)–dimethylacetamide revealed a *cis* configuration[22]. However. heating these complexes during the preparation may play an important role in obtaining the *cis* or *trans* isomers. Grinberg *et al.*[8] agree also with a *trans* configuration for the Pt(II) and Pd(II)–(6-MP) complexes. The above reactions proceeds most likely through platinum–sulfur interaction first, followed by ring closure with the N_7 nitrogen atom, which is the most reactive nitrogen in purine nucleosides[37]. This behavior was not observed in the case of adenosine–platinum complexes[46]. Sletten[47] states that steric factors do not allow the formation of a five membered chelate ring in a copper–(9-methyl-6-oxopurine) analog. However, Heitner and Lippard[22] reached the conclusion that a sulfur N_7 chelate can exist, when the ligand undergoes the proper distortion to accommodate the metal ion. Oxygen–N_7–Pt(II) chelates have also been found with Inosine and Guanosine platinum(II) complexes under proper pH conditions[48].

The use of 8-hydroxyquinoline and 8-thioquinoline as chelating agents in analytical chemistry is a further

Table IV. pK_a Values of the N_1–H Imino Protons in Some 6-Thiopurines and Hypoxanthines.

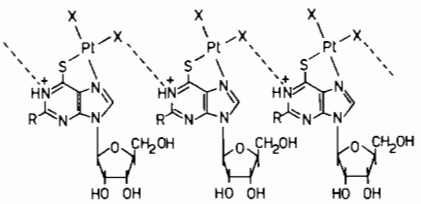
Hypoxanthine pK_a		6-Thiopurine pK_a		Reference
Hypoxanthine	9.4	6-MP	7.9	28
9-methyl-hypoxanthine	10.3	9-methyl-6MP	7.8	28
Inosine	8.96	6-MPR	7.56–7.71	50, 45
Guanosine	9.2–9.5	2-A-6-MPR	8.33–8.35	51, 45

evidence for the possible formation of this type of chelates under proper conditions. The formation or not of a five chelate ring in 6-substituted purines seems to be due mainly to the basic properties of the substituent at the 6th position. In strongly acidic media the tendency to Pt–S bond formation ionizes the imino proton, which is immediately replaced by protons present in strongly acidic solutions.



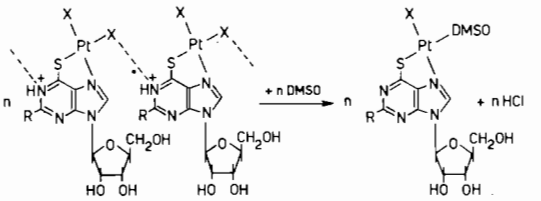
where R = H, NH_2 and X = Cl, Br.

Protonation of 6-MP always place first at the N_7 position[28], similarly with 6-MPR (see nmr section). However, platinum seems to compete favorably with N_7 protonation (N_7–H^+) and when the Pt–S bond is formed, it is followed by ring closure of the five membered ring with N_7. The protons near N_1 are kept in the complex, forming a zwitterionic structure. As was shown in the ir spectra a weak hydrogen bonding does exist in the spectra of these complexes. The hydrogen bonding is most likely inter-molecular, involving the protons at N_1 and the chlorines *trans* to the sulfur atom (N_1H^+----Cl^-) forming polymeric chains as is shown below:



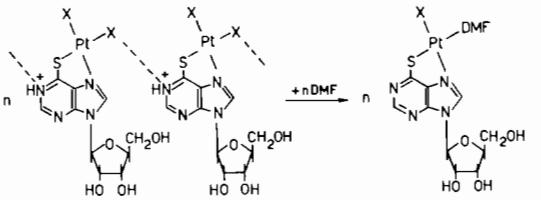
R = H, NH_2, X = Cl, Br.

Hydrogen bonding within the same molecule is not possible from molecular models and the square arrangement around platinum. The formation of the above complex takes place only in strongly acidic media, where ring closure, removal of X^- and ionization of the imino group are more difficult. This also provides the inter-molecular hydrogen bonding. In addition, the excess of K_2PtX_4 prevents the reaction of a second molecule of 6-MPR with the same platinum *trans* to the sulfur. The 1:1 compound precipitates as soon as it is formed. The above chain structure can also explain the easy removal of one HX molecule in DMF–H_2O, or DMSO–H_2O solutions with subsequent replacement of one chlorine atom by DMSO as is shown below:



R = H, NH_2 and X = Cl, Br.

The DMSO was shown to react with platinum complexes with a large *trans* effect[49]. The final product of the above reaction does not whow the presence of NH^+ groups in the ir and nmr spectra. The removal of one proton in acidic media from 6-MP was reported by Grinberg *et al.*[8], in the reactions of this ligand with platinum(II) and palladium(II). However, the 6-MP was retained its full chemical composition with immediate protonation at the N_1 position. Hydrogen bonding Through N_3 seems less probable, since the N_1 nitrogen atom is found to be the most basic in almost all purine derivatives[2]. The formation of the complex $Pt(L–H^+)_2$ from $Pt(L–H^+)_2 \cdot nHX$ (n = 1,2) in water dissolution and subsequent ionization is consistent with the lower basic strength of the N_1 nitrogen in the complex compared to the free base. The influence of the positive charge on the central atom and the high negative charge of the ionic complex were reported[8] as being responsible for the lower basicity of the coordinated thio-purines. The molecular weight determinations have shown a monomer character for the compound $PtLX_2$ in DMF solution (M.W. 386). This can be explained by the rupture of the inter-molecular hydrogen bonding in solution, forming monomers of the formula:



However, the DMF molecule was not retained in the final isolated compound from DMF solutions and the final product, $[Pt(L–H^+)X]_n$, was obtained (see eq. (7)).

Experimental

Materials

6-mercaptopurine riboside and 2-amino-6-mercaptopurine riboside were purchased from Raylo Chemicals Ltd and were 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

*Bis-(6-mercaptopurine riboside–H^+)*platinate(II)

A solution of 0.137 g (4.8×10^{-4} mol) of 6-MPR was mixed 0.1 g (2.4×10^{-4} mol) of K_2PtCl_4 in 100 ml of water with continous stirring. The initial pH value of the solution mixture was 5.3 and decreased to 2.4 after 2 hours at room temperature. The color of the solution changed rapidly from red to yellow and precipitation started after 10 minutes. The deep yellow product was filtered off by suction and washed thoroughly with water and small quantities of alcohol and ether. It was then dried at 110° C under vacuum in the presence of NaOH or P_2O_5. The yield was quantitative. The same compound is obtained when K_2PtBr_4 is used instead of K_2PtCl_4.

Dichloro-(6-mercaptopurine riboside)platinate(II)

0.1 g (3.5×10^{-4} mol) of 6-MPR were mixed with 0.584 (14×10^{-4} mol) of K_2PtCl_4 in 25 ml of 3*N* HCl. Precipitation of the final product started after 15 minutes at room temperature. The orange powder was filtered off by suction and washed with small quantities of water, alcohol and ether and it was dried at 110° C under vacuum in the presence of P_2O_5. Yield: 90%.

Dibromo-(6-mercaptopurine riboside)platinate(II)

Using 0.1 g (3.5×10^{-4} mol) of the base and 0.42 ml of a 20% water solution of K_2PtBr_4 (0.84 g or 14×10^{-4} mol) in 30 ml of 3*N* HBr, the same procedure as in 2 was followed in this reaction. Precipitation of the product started only 5 minutes later. Yield 85%.

Bis-(2-amino-6-mercaptopurine riboside–H^+)$_2$ platinate(II)

0.252 g (8.4×10^{-4} mol) of the base were dissolved in 100 ml of water at 40° C. To this, 0.25 g (4.2×10^{-4} mol) of K_2PtBr_4 were added. The same procedure as in the first preparation was followed. The gelly-like precipitate was separated by centrifugation. The yield was quantitative.

Dichloro-(2-amino-6-mercaptopurine riboside)platinate(II)

0.1 g (3.3×10^{-4} mol) of the base was mixed with 0.555 g (13.2×10^{-4} mol) of K_2PtCl_4 in 30 ml of 3*N* HCl. Following the same procedure as in the second preparation the product was isolated and characterized. The results of the elemental analyses for the product were slightly different from the theoretical values, corresponding to the above formula (see Table I). This compound is probably a mixture with the previous one. Also the yield was lower, 70–80%.

In experiments with different acid normalities (1–4*N* HCl) and with excess of K_2PtCl_4 up to 10-fold the same products were obtained.

Dibromo-(2-amino-6-mercaptopurine riboside)platinate(II)

0.1 g (3.3×10^{-4} mol) of the base and 0.8 g (13.2×10^{-4} mol) of K_2PtBr_4 (or 4 ml of a solution of 20% of the salt in water) were mixed in 30 ml of 3*N* HBr. The same procedure as in the second preparation was followed. Yield: 80–85%.

Chloro-(6-mercaptopurine riboside–H^+)–dimethyl sulfoxide–platinate(II)

0.1 g (1.8×10^{-4} mol) of the compound Pt(6-MPR)Cl_2 was dissolved in 2 ml of DMSO at room temperature. After stirring the mixture for 15 minutes, 10-fold excess of water was added and the precipitate was filtered off by suction and washed with small quantities of alcohol and ether. Then it was dried at 110° C under vacuum. The yield was quantitative. The above procedure was also followed with DMSO-d_6 for the preparation of the compound Pt(6-MPR–H^+)(DMSO-d_6)Cl.

Chloro-(6-mercaptopurine riboside–H^+)-ethylenediamine-platinate(II)

0.491 g (8.9×10^{-4} mol) of Pt(6-MPR)Cl_2 was dissolved in 5 ml DMF. To this solution, 0.61 ml (8.9×10^{-4} mol) of a solution of 10% of en (d = 0.896) in water was added with continuous stirring. The mixture was stirred at room temperature until addition of excess of water yielded no nore precipitate. An excess of acetone was then added and the product was precipitated. It was filtered off by suction and washed with a small quantity of ether and dried at 110° C under vacuum in the presence of P_2O_5. Yield 0.413 g (86%).

Preparation of the first compound from the second

0.1 g (1.8×10^{-4} mol) of Pt(6-MPR)Cl_2 was mixed with an equimolar amount of 6-MPR (0.0516 g) in a mixture of 10 ml of DMF with $H_2O = 2:1$. The mixture was stirred for 15 minutes at room temperature and an excess of water was added. The resulting yellow precipitate was isolated and dried as above. Yield: 90%. Analysis for Pt of the first compound: Calculated 25.50%. Found 25.76%. The ir spectrum of this compound was identical to that of the second compound.

Preparation of the fourth compound from the sixth

0 1 g ($1\,5 \times 10^{-4}$ mol) of the compound Pt(2-A 6-MPR)Br_2 was mixed by stirring with an equimolar amount of 2 A-6-MPR in 5 ml of DMF for 20 minutes Then the same procedure as for the nenth compound was followed The precipitate was washed and dried after centrifugation Yield 82% Analysis for Pt of the fourth compound Calc 24 64% Found 24 33% The ir spectrum of this compound was identical to the ir of the fourth compound

Bis (6 mercaptopurine riboside–H^+)platinate(II) hydrochloride

0 273 g ($4\,9 \times 10^{-4}$ mol) of Pt(6 MPR)Cl_2 was mixed in 10 ml of DMF with 0 141 g ($4\,9 \times 10^{-4}$ mol) of 6-MPR After stirring for 1 hour at room tempera ture an excess of acetone was added to the reaction mixture The precipitate was filtered and washed with alcohol and ether It was then dried for one day in vacuum at room temperature in the presence of $CaCl_2$ for 3–4 hours at 60° C and finally at 110° C for 3–4 hours under vacuum in the presence of P_2O_5 Yield 90% The compound had the formula Pt(6-MPR–H^+)$_2$ HCl

Bis (6 mercaptopurine riboside–H^+)platinate(II) dihydrochloride

0 268 g ($4\,8 \times 10^{-4}$ mol) of Pt(6 MPR)Cl_2 was dissolved in 5 ml of DMF and 0 138 of 6 MPR ($4\,8 \times 10^{-4}$ mol) in 3 ml of 3*N* HCl The two solutions were mixed by continuous stirring for 1 hour at room tem perature Using the same procedure as for the eleventh compound and excess of acetone the yield of 85–90% was obtained

[Pt(6 MPR–H^+)Cl]$_n$

The polymer product [Pt(6 MPR–H^+)Cl]$_n$ was ob tained by the following procedure 0 1 g ($1\,8 \times 10^{-4}$ mol) of Pt(6 MPR)Cl_2 was dissolved in 3 ml DMF at room temperature and stirred for 15 minutes Then an excess of water was added and precipitation was completed upon standing in a refrigerator for 3–5 hours The yield was quantitative

Reactions of cis Pt(Adenosine)$_2Cl_2$ and cis Pt(Inosine)$_2Cl_2$ with 6 MPR and 2 A 6 MPR

Equimolar amounts of *cis* Pt(Adenosine)$_2Cl_2$ and *cis* Pt(Inosine)$_2Cl_2$ were mixed with 6 MPR and 2 A 6 MPR in DMF solutions with the adenosine complex and in H_2O solutions with the inosine com plex at 0 C The color of the solutions became instan taneously deep yellow and insoluble particles appeared in the water solution which increased on heating The DMF solution on heating and by addition of excess of water gave an insoluble compound The compounds were finally identified as being Pt(6-MPR–H^+)$_2$ and Pt(2 A 6-MPR–H^+)$_2$ respectively from platinum analyses and ir spectra

Microanalyses

Microanalyses were carried out by SCHWARZKOPF microanalytical Laboratory (U S A) Dr Alfred BERNHARD Microanalytisches Laboratorium (West Germany) CHEMALYTICS Inc (U S A)

Molecular Weights

These were determined by the above BERNHARD laboratory in DMF solutions

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 in all cases

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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