Inorganica Chimica Acta, 25 (1977) 241–245
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Some Platinum(II) Complexes of DNA Bases and Nucleosides

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Received May 4, 1977

The syntheses of $[Pt(adenine)X_2]\cdot 2H_2O$ *(X = Cl, I),* $[Pt(adenosine)I_2]\cdot 2H_2O$, $[Pt(guanosine)_2I_2]$, $[Pt(guanine)I_2]$ *and* $[Pt(cytosine)I_2]$ *are reported. Infrared, electronic and proton n.m.r. spectra have been used to assign coordination numbers, denticity and binding sites of the ligands.*

Introduction

The unexpected antitumour properties of *cis*-$Pt(NH_3)_2Cl_2$, $Pt(en)Cl_2$ and related complexes have given rise to intensive investigation [1–3] of these species, including extremely promising clinical trials [3, 4]. Whilst it has been proposed that the antitumour activity could be related to enzyme inhibition [5–7], most workers have concentrated on and postulated that the activity of the compounds is related to their ability to interact with DNA, and several models have been proposed which involve the formation of platinum-bridged covalent cross-links [8–12]. These models frequently feature coordination to platinum of nitrogen or oxygen atoms from the purine or pyrimidine bases, and guanine has been singled out as especially available for coordination [11–14] since it is known that guanine derivatives react more rapidly with platinum complexes than do other nucleotide bases [14]. We here wish to report the synthesis and spectral properties of some platinum(II) complexes of some DNA bases, including guanine and guanosine.

Results and Discussion

The complexes isolated from reaction between the DNA bases and the tetrahaloplatinate(II) ions are listed in Table I, and the electronic spectra of these complexes (Table II) are all suggestive of a four-coordinate structure [15]. The spectra of the free ligands all exhibit an intense absorption in the 38–36 $\times 10^3$ cm^{-1} range assignable to π–π^* transitions; upon coordination a slight bathochromic shift of this band occurs, and this has been previously observed on coordination of purine rings [16]. All the iodo species exhibit an absorption at *ca.* 27 kK assignable to Pt ⟵ I charge-transfer, and in addition there is a further absorption in the visible region assignable to d–d transitions.

Having thus established the likelihood of tetracoordination, some preliminary remarks can be made about the denticity of the ligands. The reaction of adenine with both $K_2[PtCl_4]$ and $K_2[PtI_4]$ produces neutral complexes in which the ligand is bidentate. Adenosine reacts with $K_2[PtI_4]$ to produce an analogous complex, $[Pt(adenosine)I_2]\cdot 2H_2O$, con-

TABLE I. Colours and Analytical Data for DNA Base Complexes of Platinum(II).

Complex	Colour	% C[a]	% H[a]	% N[a]	% X[a]
$[Pt(adenine)Cl_2]\cdot 2H_2O$	Yellow	14.8(14.3)	2.2(1.7)	17.0(16.7)	15.9(16.9)
$[Pt(adenine)I_2]\cdot 2H_2O$	Pale Yellow	10.0 (9.7)	1.4(1.4)	11.4(11.3)	40.2(41.0)
$[Pt(adenosine)I_2]\cdot 2H_2O$	Deep Yellow	15.9(16.0)	2.3(2.3)	9.7 (9.3)	34.5(33.8)
$[Pt(guanosine)_2I_2]$	Pale Brown	23.3(23.6)	3.4(2.6)	14.0(13.8)	22.1(25.0)
$[Pt(guanine)_2I_2]$	Brown	16.1(16.3)	1.7(1.4)	18.6(19.0)	31.8(34.6)
$[Pt(cytosine)I_2]$	Brown	7.7 (8.6)	1.1(0.9	6.8 (7.5)	44.7(45.3)

[a]Found (calc.).

TABLE II. Electronic and Infrared Spectra of DNA Base Complexes of Platinum(II).

Complex	$E_{max}(\epsilon_{mol})$, kK (l mol^{-1} cm^{-1})[a]	E_{max}[b]	ν(O–H) and ν(N–H)	ν(C=N)	δ(N–H)	ν(Pt–N)	ν(C=O)
$[Pt(adenine)Cl_2]\cdot 2H_2O$	36.1	24.2	3380sh, 3300br, 3130sh	1645br	1592sh		
$[Pt(adenine)I_2]\cdot 2H_2O$	37.3(8669), 27.7(713)	23.4	3410br, 3280br	1650sh 1635br	1578sh	560br	
$[Pt(adenosine)I_2]\cdot 2H_2O$	37.5(9370), 32.5(3349)sh, 26.5(604)	24.0	3370br, 3520sh, 3110sh	1659sh 1648s	1569m	545w	
$[Pt(guanosine)_2I_2]$	36.9(200&), 35.6(1695), 22.0(180)	23.0	3450m, 3315w, 3195br	1629br	1532m		1729m 1680br
$[Pt(guanine)_2I_2]$	36.4, 26.0, 22.1	21.7	3330m, 3100m, 3055sh	1615br	1540br		1683br
$[Pt(cytosine)I_2]$	35.7(21938), 25.9(1249), 22.0(409)	23.8		1635br	1595m	479w	1700br

[a]Solution in N,N-dimethylformamide. [b]Solid reflectance.

taining bidentate adenosine. The complex [Pt(cytosine)I_2] is produced by reaction of $K_2[PtI_4]$ with cytosine in either a 1:1 or 1:2 metal to ligand ratio. In contrast guanine and guanosine act only as monodentate ligands in the complexes isolated in this study. Reaction of the ligands with $K_2[PtI_4]$ in a ratio of 1.8:1 produces [Pt(ligand)$_2I_2$] complexes. In this study no complex was isolable from similar reaction between thymine and $K_2[PtI_4]$.

All of the complexes are non-electrolytes in nitromethane and are also soluble in N,N-dimethylformamide (DMF) and dimethylsulphoxide (DMSO). Additionally, when aqueous suspensions of the iodo complexes are stirred with two mol of silver nitrate, no precipitate of silver iodide is produced which is substantial evidence that the iodides are strongly bound to the metal in these species.

Adenine and $[Pt(adenine)X_2]\cdot 2H_2O$ *(X = Cl, I)*

Two signals are observed in the 60 MHz n.m.r. spectrum of free adenine in d_6-DMSO (Table III). The resonances due to H_2 and H_8 apparently coincide at 8.06 p.p.m.* and the NH_2 signal is assigned to a broad multiplet at 7.01 p.p.m., assignments supported by integration. The spectrum of adenine in D_2O has been reported to have two resonances due to H_8 and H_2 at 8.20 and 8.32 p.p.m., but Hadjiliadis *et al.* [18] report similar observations to ours with tetracetyladenosine where the H_8 and H_2 resonances coincide in d_6-DMSO but are resolved in $CDCl_3$.

On complexation to form $[Pt(adenine)I_2]\cdot 2H_2O$ both bands are shifted to lower field, the resonances due to H_2 and H_8 again coincide at 8.45 p.p.m. and that due to NH_2 occurs at 8.26 p.p.m. It is probable that the proton undergoing the greater shift is nearer to the platinum–nitrogen bond and it thus appears that bidentate coordination occurs either *via* NH_2 + N_1 or NH_2 + N_7 sites. Since the H_2 and H_8 resonances are not resolved on complexation it is to be assumed that bidentate binding occurs *via* $NH_2 + N_1$ and also *via* $NH_2 + N_7$ in this complex. Mansy *et al.* [19] have shown that in the reaction of adenosine with *cis*-$[Pt(NH_3)_2Cl_2]$ bidentate binding occurs at both these sites in the pH range 3–9. The possibility of bidentate binding at $N_3 + N_9$ cannot be ruled out but is not supported by the available spectroscopic data.

In the complex $[Pt(adenine)Cl_2]\cdot 2H_2O$ however, the H_8 and H_2 proton resonances are resolved, and signals at 8.36 and 8.26 p.p.m. are assigned to them. Differentiation between the two is not possible; the greater shift should be assigned to the proton nearer the metal–nitrogen bond and so if binding occurs at the $NH_2 + N_7$ site then H_8 must be assigned the

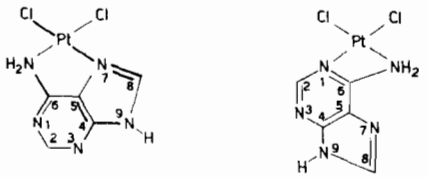
*referenced externally to p-$C_6H_4Cl_2$, values quoted downfield from TMS.

TABLE III. N.m.r. Data of DNA Bases and Their Complexes of Platinum(II).[a]

DNA Base	H_8	H_2	NH_2	Complex	H_8	H_2	NH_2
Adenine	8.06	8.06	7.01	$[Pt(adenine)Cl_2]\cdot 2H_2O$	8.36	8.26	7.89
				$[Pt(adenine)I_2]\cdot 2H_2O$	8.45	8.45	8.26
Adenosine	8.28	8.09	7.25	$[Pt(adenosine)I_2]\cdot 2H_2O$	8.60	8.36	8.10
Guanosine	7.84		6.38	$[Pt(guanosine)_2I_2]$	7.96		6.44
Guanine	7.68[b]			$[Pt(guanine)I_2]$	8.41		6.51–6.66
Cytosine	7.25[c]	5.54[d]	3.26	$[Pt(cytosine)I_2]$	8.48[c]	5.78–5.65[d]	7.38–7.33

[a]Spectra obtained in d_6-DMSO and referenced externally to p-$C_6H_4Cl_2$. Values are quoted downfield from TMS (the signal due to p-$C_6H_4Cl_2$ was measured at 7.26 p.p.m. downfield from TMS). [b]Value in D_2O (reference 21). [c]Value for H_6 proton resonance. [d]Value for H_5 proton resonance.

greater shift, but if at $NH_2 + N_1$ then the H_2 proton will be shifted further downfield. The two possible structures (A) and (B) are shown



A

B

The ring formed in (A) ($NH_2 + N_7$) is a five-membered ring and consequently will have less internal strain than the four-membered ring in (B) ($NH_2 + N_1$). On this basis it might be tentatively suggested that structure (A) is adopted and thus to assign the signal at lowest field to the H_8 resonance. It is interesting to note that the dichloro complex contains only one type of ligand coordination, whereas two modes of coordination exist in $[Pt(adenine)I_2]\cdot 2H_2O$.

Adenosine and $[Pt(adenosine)I_2]\cdot 2H_2O$

Assignment of the resonances in the proton n.m.r. spectrum of free adenosine are in agreement with those of Hadjiliadis *et al.* [18] and show the H_8 proton resonance at lower field than H_2. When complexation to produce $[Pt(adenosine)I_2]\cdot 2H_2O$ occurs, shifts of the three signals due to H_8, H_2, and NH_2 are observed. As a bidentate ligand, adenosine has three possible binding sites, $N_1 + NH_2$, $N_7 + NH_2$ and $N_3 + N_9$. Of these, the $N_3 + N_9$ sites would seem very unlikely because of interactions with the bulky ribose group attached to N_9. For the reasons outlined previously, binding at $NH_2 + N_7$ would appear to be more favoured than $NH_2 + N_1$ on ring strain grounds; but here, if the proton resonance at lowest field is assigned to H_8, then the shifts in the H_8 and H_2 protons are very similar, being 0.32 and 0.27 p.p.m. respectively. These results suggest that interaction with platinum(II) occurs *via* both N_7 and N_1 with binding at $NH_2 + N_7$ site slightly more favoured, which is in agreement with the findings of Mansy *et al.* [19] for the binding of adenosine with *cis*-$[Pt(NH_3)_2Cl_2]$.

Guanosine and $[Pt(guanosine)_2I_2]$

The signals due to the H_8 and NH_2 protons of free guanosine in d_6-DMSO are assigned at 7.86 and 6.38 p.p.m. respectively in agreement with Kong and Theophanides [20]. No signal is observed corresponding to the N_1-H proton in the range of the instrument (0–10 p.p.m.). In the complex $[Pt(guanosine)_2I_2]$ the guanosine acts as a monodentate ligand, there being five probable binding sites – N_1, N_3, NH_2, N_7 and N_9. The resonance due to H_8 shifts only slightly on complexation to 7.96 p.p.m. and that due to NH_2 shifts to 6.44 p.p.m. The H_8 proton resonance appears as a triplet due to spin coupling with ^{195}Pt and a spin coupling constant, $J_{Pt-H} = 30$Hz. Since the coupling constants in pyridine complexes are $J_{Pt-H_o} = 33$ Hz and $J_{Pt-H_m} = 10$ Hz for *ortho* protons (H_o) and *meta* protons (H_m), respectively [21], the indication is that N_7 is the binding site.

The resonance assigned to H_1, a proton in the ribose group of guanosine moves downfield from 5.59–5.68 (doublet) to 6.82 p.p.m. (triplet), suggesting N_9 as a possible binding site. A Pt–N_9 interaction seems rather unlikely on steric grounds because of the presence of the bulky sugar at N_9 and also the coupling constant, $J_{Pt-H} = 51$ Hz. Other workers have found binding to copper at N_9 in both guanine [22] and adenine [23] where no sugar group is attached to the purine base, but in guanosine the evidence suggests a Cu–N_7 linkage [16, 24].

The infrared spectrum shows ν(O–H), ν(N–H), δ(N–H) and ν(C=O) bands shifted only slightly from the positions in free guanosine, suggesting that binding does not occur at NH_2 or O_6. It is concluded, therefore, that binding of platinum(II) to guanosine in the complex $[Pt(guanosine)_2I_2]$ is *via* the N_7 of the nucleoside. This is in agreement with Kong and Theophanides' suggestions for the complex $[Pt(en)(guanosine)_2]Cl_2\cdot 2H_2O$ [180], and which has recently

been confirmed by X-ray analysis [12]. Our own results thus reinforces the growing conclusion that N_7 is the preferred site for binding of guanosine to platinum(II). This is perhaps to be expected, since the N_7 atom is not involved in Watson–Crick base pairing, but occupies an exposed position on the surface of DNA and is thus readily available to an electrophilic complexing species.

Guanine and $[Pt(guanine)_2I_2]$

Due to the limited solubility of guanine in d_6-DMSO no n.m.r. spectrum was obtained for this base. The resonance of the H_8 proton in D_2O has been assigned at 7.68 p.p.m., however [25]. The proton n.m.r. spectrum of $[Pt(guanine)_2I_2]$ shows a triplet centred at 8.41 p.p.m. assigned to H_8 with J_{Pt-H} = 33 Hz, and a signal at 6.51–6.66 p.p.m. due to NH_2. The infrared spectrum of this complex exhibits bands due to ν(O–H), ν(N–H) and δ(N–H) modes little changed on coordination and thus it is unlikely that coordination occurs *via* the NH_2 group. No band can be unambiguously assigned to ν(Pt–N), since strong ligand vibrations occur in the 560–450 cm^{-1} region.

The evidence derived from the shift and Pt–H coupling constant of the H_8 resonance suggests coordination to platinum at either N_7 or N_9. In this ligand there is no bulky sugar group to discourage $Pt-N_9$ coordination, and such binding has been found in copper–guanine complexes [22].

Cytosine and $[Pt(cytosine)I_2]$

The proton n.m.r. spectrum of cytosine in d_6-DMSO shows two doublets and a broad multiplet which are in the ratio 1:1:3. Kokko *et al.* [26] have reported a similar result in this solvent and attribute it to a preponderance of the zwitterion structure (D).

enol zwitterion

C D

These workers suggest that the free amine protons should appear as a broad triplet due to coupling with the ^{15}N nucleus. The three signals observed are centred at 7.25, 5.54 and 3.26 p.p.m. and are assigned to H_6, H_5 and NH_3 resonances. The infrared spectrum of the free base indicates it is present mainly in the enol form and bands at 3364 and 3124 cm^{-1} are assignable to ν(O–H) and ν(N–H) respectively.

On complexation to form [Pt(cytosine)I_2] a considerable difference in both the proton n.m.r. and the infrared spectrum is observed. No bands are observed in the infrared spectrum in the ν(O–H) and ν(N–H) regions, although the band assignable to δ(N–H) is present at 1595 cm^{-1} (*cf.* 1605 cm^{-1} in cytosine) and a band at 479 cm^{-1} is assigned to ν(Pt–N). The n.m.r. spectrum is weak but shows resonances at 8.48, 7.38–7.33 (multiplet) and 5.78–5.65 (doublet) p.p.m. in the ratio 1:2:1. These are tentatively assigned to signals due to H_6, NH_2 and H_5 and are not incompatible with binding to platinum at both NH_2 and N_1. However, since these positions are too far apart to allow bidentate binding with platinum(II), it is more likely, as the I.R. spectra suggests, that binding occurs *via* the NH_2 + N_3 and N_1 + O^- sites.

Experimental

$[Pt(adenine)Cl_2] \cdot 2H_2O$

Potassium tetrachloroplatinate(II) (1.04 g, 2.5 mmol) was dissolved in distilled water (20 cm^3), filtered and adenine (0.34 g, 2.5 mmol) added. The mixture was stirred for 24 h and the yellow precipitate washed with water, ethanol and diethyl ether and dried. Yield 90%.

$[Pt(adenine)I_2] \cdot 2H_2O$

Potassium tetrachloroplatinate(II), (1.0 g, 2.4 mmol) was dissolved in distilled water (20 cm^3), filtered, potassium iodide (1.60 g, 9.8 mmol) added and the solution stirred for 0.25 h. Adenine (0.33 g, 2.4 mmol) was added in methanol (30 cm^3) and the solution stirred for 24 h. The light brown precipitate was filled off, washed with water, methanol and diethyl ether and dried. Yield 82%.

$[Pt(adenosine)I_2] \cdot 2H_2O$

To a filtered solution of potassium tetrachloroplatinate(II) (1.0 g, 2.4 mmol) in distilled water (20 cm^3) potassium iodide (1.60 g, 9.8 mmol) was added and the solution stirred for 0.25 h. Adenosine (0.64 g, 2.4 mmol) was added and the solution stirred for 48 h. A deep yellow solid was filtered off, washed with water, methanol and diethyl ether and dried. Yield 88%.

$[Pt(guanine)_2I_2]$

Potassium tetrachloroplatinate(II) (0.33 g, 0.8 mmol) was dissolved in water (20 cm^3), filtered, potassium iodide (0.53 g, 3.2 mmol) added and the solution stirred for 0.25 h. Guanine (0.24 g, 1.6 mmol) was then added and the solution stirred for 36 h. The brown solid formed was refrigerated in contact with the solution for *ca.* 144 h and then filtered, washed with water, methanol and diethyl ether and dried. Yield 71%.

$[Pt(guanosine)_2I_2]$

To a filtered solution of potassium tetrachloroplatinate(II) (1.0 g, 2.4 mmol) in water (20 cm^3)

potassium iodide (1.60 g, 9.8 mmol) was added and the solution stirred for 0.25 h. Guanosine (1.22 g, 4.2 mmol) was added and the solution stirred for 72 h. The brown solid formed was filtered off, washed with water, methanol and diethyl ether and dried. Yield 67% (based on guanosine).

[Pt(cytosine)I_2]

To a filtered solution of potassium tetrachloroplatinate(II) (1.0 g, 2.4 mmol) in water (20 cm^3) was added potassium iodide (1.60 g, 9.8 mmol) and the solution stirred for 0.25 h. Cytosine (0.54 g, 2.4 mmol) was added and the solution stirred for 24 h. The brown solid formed was filtered off, washed with water, methanol and diethyl ether and dried. Yield 62%.

Attempted Preparations of Other DNA Base Complexes

No reaction occurred between $K_2[PtI_4]$ and thymine in a 1:1 molar ratio. No pure complex was obtained from the reactions between $K_2[PtI_4]$ and guanine (1:1 ratio), $K_2[PtI_4]$ and guanosine (1:1 ratio) or $K_2[PtI_4]$ and adenosine (1:2 ratio).

Physical Measurements were obtained as previously described [27]

Proton n.m.r. spectra were obtained in saturated d_6-DMSO solutions on a Hitachi–Perkin Elmer R20 spectrometer, *para*-dichlorobenzene being used as an external standard (measured as 7.26 p.p.m. downfield from tetramethylsilane).

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