Reaction Intermediates of *cis*-Diammineaqua(hydroxo)platinum(II) with Guanosine 5'-Monophosphate characterized by Proton Nuclear Magnetic Resonance Spectroscopy

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The reaction between guanosine 5'-monophosphate (GMP) and *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ at pD 6.8 (I = 0.10 mol dm^{-3} $NaNO_3$, 37 °C) has been examined by ^1H n.m.r. spectroscopy. The reaction proceeds with stoicheiometry GMP: Pt of 1.8:1.0 yielding N(7) bound *cis*-$[Pt(ND_3)_2(GMP)_2]^{2+}$ as the predominant product. Proton n.m.r. spectra recorded as a function of time at various concentration ratios show two kinetically allowed intermediates along the reaction course. The first is a N(7) bound complex, *cis*-$[Pt(ND_3)_2(GMP)(OD)]^+$, and the second can be best interpreted as *cis*-$[\{Pt(ND_3)_2\}_2(GMP)_2]^{4+}$ in which each platinum atom is co-ordinated to N(7) of one GMP molecule and to O(6) of the other. In parallel experiments using *trans*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ and GMP the second intermediate is not formed.

The interactions of *cis*-diamminedichloroplatinum(II) and its hydrolysis products with nucleotides, nucleosides, and their constituent bases have received much attention because of their significance in elucidating the mechanisms of the anti-tumor activity of cisplatin.[1] It is well documented that the addition of *cis*-$[Pt(NH_3)_2Cl_2]$ or its hydrolysis products to an aqueous solution of guanosine nucleotide yields complexes in which the platinum is bound to N(7) of the nucleotide.[2] More recently, the reactions of *cis*-$[Pt(NH_3)_2Cl_2]$ with ortho-, pyro-, and tri-phosphate have been explored.[3] These may have implications in the understanding of the chelation by the phosphate ligands of nucleotides.[4] A bidentate N(7),O(6) chelate complex which has provoked much debate has been proposed by Rosenberg,[5] who suggested that the interaction of platinum at O(6) of guanosine 5'-monophosphate* (GMP) would promote deprotonation at N(1) and such deprotonation could lead to the mispairing of guanosine with thymine.[5] However, in spite of some indirect evidence,[6] hard chemical evidence for the N(7),O(6) chelate complex has not been forthcoming, indeed many investigators have argued against this mode of chelation on stereochemical grounds.[7] Rosenberg has pointed out the importance of examining the kinetically allowed intermediates for evidence of the N(7),O(6) chelate complex.[5] In this regard, n.m.r. perhaps is the most suitable method.

In this paper, I present a ^1H n.m.r examination of the interaction between guanosine 5'-monophosphate and *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ at pD 6.8. Attention is focused on the characterization of the reaction intermediates. An interesting feature of this study is that it provides results which may represent a N(7),O(6) intermediate. The reaction of *trans*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ with GMP does not generate such an intermediate.

Experimental

Materials.—The complexes *cis*- and *trans*-diamminedichloroplatinum(II) were obtained from D. F. Goldsmith, Inc. and used as received. The diamminediaquaplatinum(II) nitrate solutions were prepared according to the literature procedures.[8] A mixture of diamminedichloroplatinum(II) and $AgNO_3$ solutions in a 1:2 mol ratio was stirred for 24 h in the dark. The silver chloride precipitate was filtered off and a small aliquot of the supernatant liquid was tested for silver ion with 0.10 mol dm^{-3} HCl. The absence of silver ion indicated that the chloroplatinum complexes were quantitatively converted into the diaqua complexes. Stock solutions were kept in the dark at 5 °C and were never stored for more than one week. The formation of dimers, trimers, and tetramers of platinum–hydroxo complexes from this preparation procedure has been documented recently.[2,5] In this paper I do not address the issue of the complexity of the platinum hydrolysis products, but I assume that this complexity would not be expected to affect significantly the conclusions of this work. However, note should be made that the dimerisation and trimerisation reactions of the monomeric hydroxo-complexes would certainly greatly complicate the kinetics, which I did not attempt to analyse in detail. Guanosine 5'-monophosphate was obtained from the Sigma Chemical Co. Its concentration in aqueous solution was determined from its molar absorptivity (Sigma). All other chemicals were reagent grade and used as received.

Methods.—Proton (250.1 MHz) n.m.r. spectra of the reactions of GMP with *cis*- and *trans*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ at pD 6.8 (maintained with 0.010 mol dm^{-3} potassium phosphate), I = 0.10 mol dm^{-3} $NaNO_3$, and 37 ± 0.3 °C were recorded using a Bruker WM 250 spectrometer locked to the resonance of internal D_2O. Peak positions were determined by computer examination of the final Fourier-transformed spectra and the chemical shifts were measured with respect to an internal sodium tetradeuterio-3-(trimethylsilyl)propionate (tsp) standard. Intensity measurements performed on the spectra were recorded with a 2-s pulse delay. For the ^1H study presented in this paper, it was found that the most optimum condition was to keep [GMP] at 4.0 mmol dm^{-3} to vary the platinum complex concentrations at appropriate molar ratios. Outside this range the GMP–platinum complexes would either precipitate out within the experimental time-scale, or the concentrations would be too small to allow clear n.m.r. signals.

Results

The ^1H n.m.r. spectrum of GMP alone shows a singlet at 8.18 p.p.m. and a doublet ($J_{1'2'}$ = 6.35 Hz) at 5.94 p.p.m. corresponding to the protons at C(8) and C(1') respectively.[9] Upon addition of *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ to a solution of 4.0

* The abbreviation used is that recommended by the I.U.P.A.C.-I.U.B. Commission on Biochemical Nomenclature (*Eur. J. Biochem.*, 1970, **15**, 203).

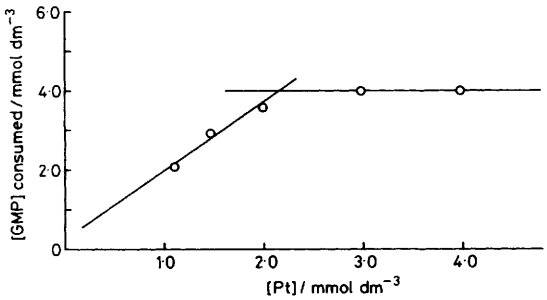

Figure 1. Stoicheiometry for the reaction between GMP and *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$; [GMP] = 4.0 mmol dm^{-3}

mmol dm^{-3} GMP (48 h were allowed for the reaction to reach completion), H(8) is shifted downfield to 8.65 p.p.m. while H(1′) is shifted upfield to 5.92 p.p.m. ($J_{1'2'}$ = 4.75 Hz). Chemical shifts of the rest of the proton resonances are relatively unaffected by the presence of the platinum complex. Only one GMP–platinum product was readily detected in the [GMP]:[*cis*-$Pt(ND_3)_2(D_2O)(OD)^+$] ($=r$) range $1 \leqslant r \leqslant 10$. It should be noted that a 1:1 mixture would form a blue platinum precipitate if allowed to stand at room temperature for one week. Spectroscopic titration in this range (Figure 1) demonstrates that the stoicheiometry for the reaction of GMP with *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ is *ca.* 1.8:1.0, suggesting that the predominant product is a bis-GMP complex, *i.e.* *cis*-$[Pt(ND_3)_2(GMP)_2]^{2+}$. This complex has been previously prepared and characterized by Kong and Theophanides.[10] The *X*-ray diffraction studies by Gellert and Bau[11] have shown that this bis-GMP product is N(7) bound.

Figure 2 illustrates the ^{1}H n.m.r. spectra in the downfield region for a solution of GMP (4.0 mmol dm^{-3}) treated with *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ (2.0 mmol dm^{-3}) at r = 2. In this reaction mixture an intermediate (**I**), with H(8) at 8.87 p.p.m. and H(1′) at 6.05 p.p.m. ($J_{1'2'}$ = 3.25 Hz), was readily detected. This intermediate almost certainly is a mono-GMP complex involving N(7) of the nucleotide, *i.e.* *cis*-$[Pt(ND_3)_2(GMP)(OD)]^+$. This conclusion is based on a previous study by Kong and Theophanides[12] in which it is shown that the H(8) resonance of GMP shifts downfield with $\Delta\delta$ = 0.73 p.p.m. when GMP is treated with $[Pt(dien)Cl]^+$ [dien = $NH_2(CH_2)_2NH(CH_2)_2NH_2$]. In their study, the pseudo-triplet peaks with *ca.* 1:4:1 intensity ratio unequivocally demonstrated that the platinum atom was bound to N(7) of the nucleotide. In the present study the H(8) resonance of GMP is shifted downfield with $\Delta\delta$ = 0.69 p.p.m., identical to that reported by Kong and Theophanides within experimental error. It should be noted that the pseudo-triplet peaks observed by Kong and Theophanides are not observed in the present study because relatively small initial concentrations of GMP and the platinum complexes were used in order to avoid the precipitation of the GMP–platinum complexes. A similar intermediate has been observed by Marcelis *et al.*[13] in the reaction of GMP with *cis*-$[Pt(ND_3)_2Cl_2]$ or *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ (neutral pD, 35 or 5 °C respectively), and by Clore and Fronenhorn[14] in the reaction of GMP with *cis*-$[Pt(ND_3)_2Cl_2]$ (pD 6.5, 80 °C, 0.50 mol dm^{-3} KCl). It should be noted that Figure 2 shows a very complex kinetic pattern. Most of the *cis*-$[Pt(ND_3)_2(GMP)(OD)]^+$ intermediate (**I**) disappeared in 2 h, but nearly half of the initial GMP remained in the unco-ordinated state. The free GMP then gradually transformed into the final product, *cis*-$[Pt(ND_3)_2(GMP)_2]^{2+}$, without detectable intermediates. After 20 h, all the GMP detected was in the product form. It is not feasible to derive a rate expression for the kinetic pattern with the data in hand.

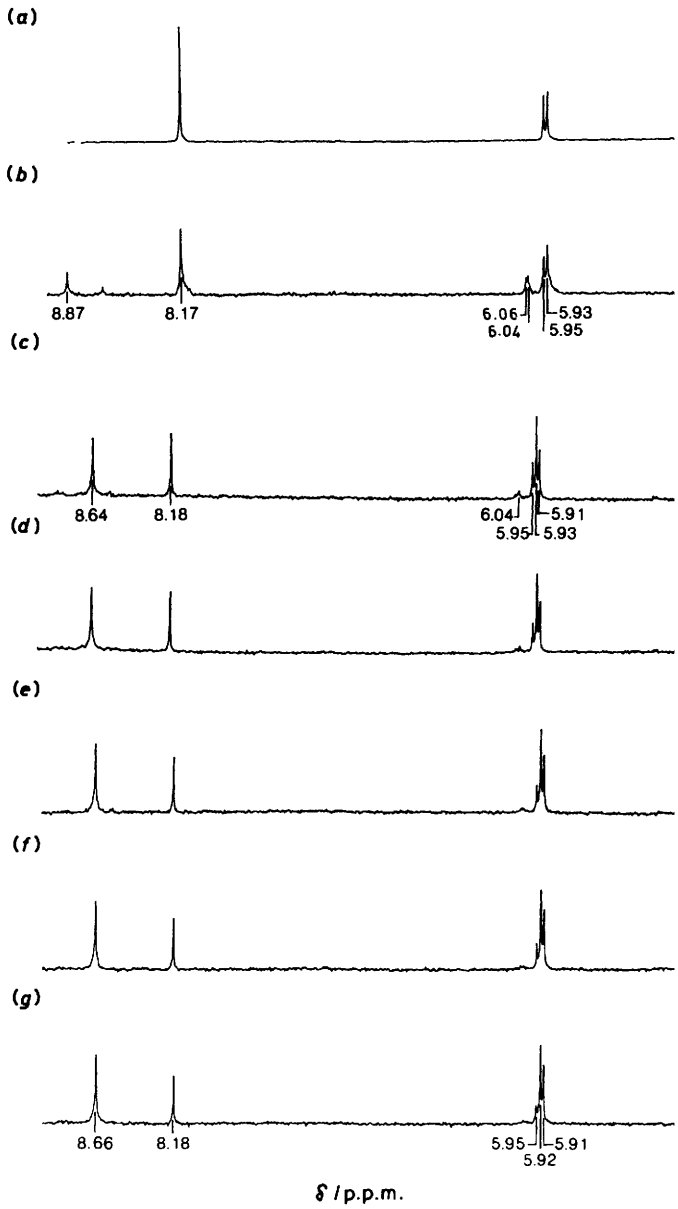

Figure 2. Interaction between GMP (4.0 mmol dm^{-3}) and *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ (2.0 mmol dm^{-3}) determined by ^{1}H n.m.r. Only the downfield region (>5 p.p.m.) of spectrum is shown. (*a*) Free GMP, (*b*) 5 min after incubation, (*c*) 1 h, (*d*) 2 h, (*e*) 3 h, (*f*) 4 h, and (*g*) 5 h

At r = 1, *i.e.* [GMP] = [*cis*-$Pt(ND_3)_2(D_2O)(OD)^+$] = 4.0 mmol dm^{-3} (Figure 3), intermediate (**I**) [H(8), 8.87; H(1′), 6.05 p.p.m.] is again observed. However, a second intermediate, (**II**), with H(8) at 8.56 p.p.m. and H(1′) at 5.21 p.p.m. ($J_{1'2'}$ = 4.88 Hz) accumulates and diminishes in *ca.* 10 h. The free GMP was no longer detectable with n.m.r. after *ca.* 5 min. It is important at this point to note that intermediate (**II**) is unlikely to be a blue platinum species because: (*a*) at the time that appreciable amounts of intermediate (**II**) accumulated, the reaction solution was colourless, (*b*) intermediate (**II**) accumulated and then disappeared, in contrast to the known formation of blue platinum complexes, and (*c*) if intermediate (**II**) was a blue platinum species, then it would be expected that the paramagnetic centre would affect the chemical shift of H(8) much

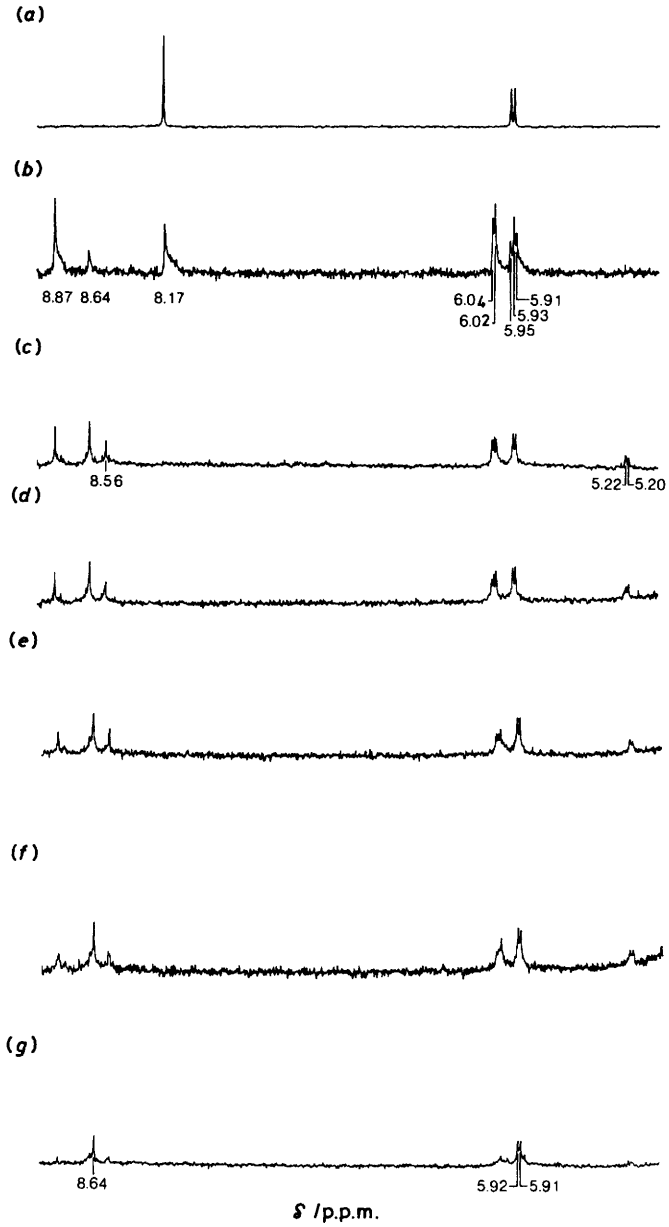

Figure 3. Interaction between GMP (4.0 mmol dm^{-3}) and *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ (4.0 mmol dm^{-3}) determined by ^1H n.m.r. (*a*) Free GMP, (*b*) 5 min after incubation, (*c*) 1 h, (*d*) 2 h, (*e*) 3 h, (*f*) 4 h, and (*g*) 17 h

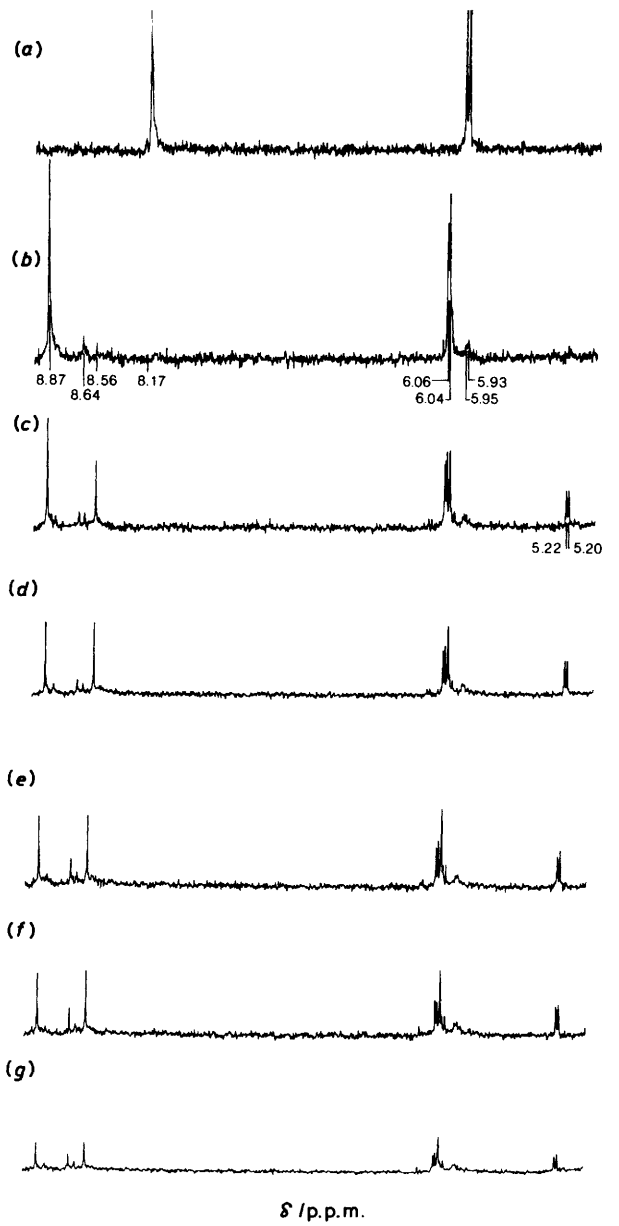

Figure 4. Interaction between GMP (4.0 mmol dm^{-3}) and *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ (8.0 mmol dm^{-3}) determined by ^1H n.m.r. (*a*) Free GMP, (*b*) 5 min after incubation, (*c*) 1 h, (*d*) 2 h, (*e*) 3 h, (*f*) 4 h, and (*g*) 6 h

more than that of H(1′), in contrast to what was observed. Again, the kinetic pattern is very complex at $r = 1$. The signals due to intermediate (**II**) appeared after the signals of *cis*-$[Pt(ND_3)_2(GMP)_2]^{2+}$, and although only the final bis-GMP product was readily detectable after 30 h, small amounts of *cis*-$[Pt(ND_3)_2(GMP)(OD)]^+$ and intermediate (**II**) persisted even after 17 h of incubation.

Figure 4 illustrates the ^1H n.m.r. spectra of a solution of 4.0 mmol dm^{-3} GMP treated with 8.0 mmol dm^{-3} *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ ($r = 0.5$). At this ratio, both the mono-GMP complex [H(8), 8.87; H(1′), 6.05 p.p.m.] and intermediate (**II**) [H(8), 8.56; H(1′), 5.21 p.p.m.] were observed. However, experiments at this ratio were complicated by the formation of a blue platinum complex, which eventually precipitated out of solution. This blue platinum complex introduces several additional proton resonances and obviates a ^1H n.m.r. study at a [GMP]:[*cis*-$Pt(ND_3)_2(D_2O)(OD)^+$] ratio < 1. It should be noted that the blue platinum species began to form within several hours at $r = 0.5$, in contrast to that at $r = 1$ which required one week to develop the blue colour. Also, the blue platinum compounds are not likely to contain phosphate, as reported by Appleton *et al.*[15] The 'platinum phosphate blue' is known to occur only in the pH range 1.5—4.5, with rather high concentrations of aquated platinum complexes and phosphate. If the pH is lower than 1.5, or higher than 4.5, no blue colour appeared under these conditions.[15] Co-ordination of higher phosphates to *cis*-$[Pt(NH_3)_2Cl_2]$ or its hydrolysis products does occur, however.[3]

Table. Chemical shifts[a] and coupling constants[b] of H(8) and H(1′) for the GMP species involved in the reactions with *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$

	GMP	Intermediate (I)	Intermediate (II)	Bis-GMP product
H(8)	8.18	8.87	8.56	8.65
H(1′)	5.94 (6.35)	6.05 (3.25)	5.21 (4.88)	5.92 (4.75)

[a] Chemical shifts (±0.020 p.p.m.) in p.p.m. downfield with respect to internal tsp. [b] Coupling constants in parentheses (±0.10 Hz) in Hz.

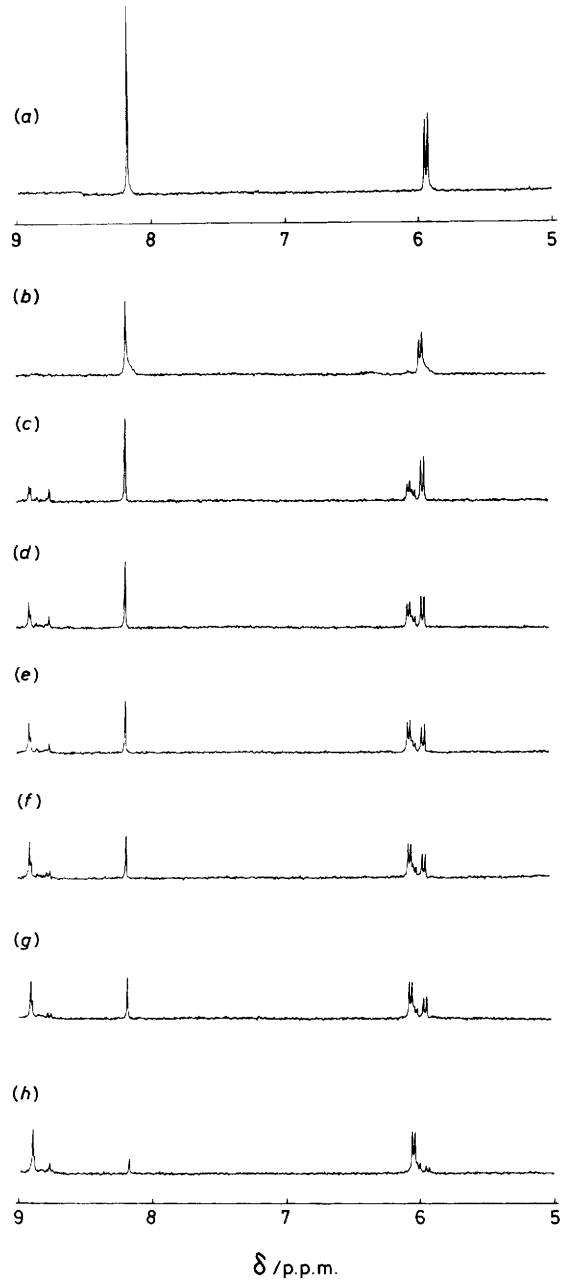

Figure 5. Interaction between GMP (4.0 mmol dm^{-3}) and *trans*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ (4.0 mmol dm^{-3}) determined by ^1H n.m.r. (*a*) Free GMP, (*b*) 5 min after incubation, (*c*) 1 h, (*d*) 2 h, (*e*) 3 h, (*f*) 4 h, (*g*) 5 h, and (*h*) 19 h

The chemical shifts for H(8) and H(1′) and the spin–spin coupling constants ($J_{1'2'}$) for free GMP and the three GMP–platinum complexes are presented in the Table.

In parallel experiments using *trans*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ and GMP, intermediate (**II**) was not formed. For the purpose of comparison, the interaction between *trans*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ (4.0 mmol dm^{-3}) and GMP (4.0 mmol dm^{-3}) is shown in Figure 5. Based on the assignments of H(8) resonances for the complexation products of *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$, the resonances in 8—9 p.p.m. region shown in Figure 5 are tentatively assigned for H(8) as follows: 8.90 p.p.m., *trans*-$[Pt(ND_3)_2(GMP)(OD)]^+$; 8.74 p.p.m., *trans*-$[Pt(ND_3)_2(GMP)_2]^{2+}$. However, the interaction between GMP and *trans*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ was complicated by additional minor peaks in this region. Two points should be noted. First, the complication does not occur at the H(1′) resonances. Secondly, the additional peaks disappeared after 19 h of incubation. I did not identify the exact cause for this complication, although a conceivable reason is that the *trans* compound might have been impure. No attempts to purify the *trans* complex were made, because the signals for intermediate (**II**) are also missing in a reaction mixture of a two-fold excess of *trans*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ over GMP. This indicates that the absence of intermediate (**II**) is not likely to be caused by the impurity of the *trans* complex.

Discussion

Nuclear magnetic resonance has been increasingly used to probe the reactions between guanosine or related nucleosides and platinum complexes. For examples, Theophanides and co-workers,[10,16] Marcelis *et al.*,[13] Clore and Fronenhorn,[14] and Lippert[17] have used ^1H n.m.r. to examine the interactions of guanosine nucleosides and platinum complexes under various conditions. Marzilli *et al.*[18] used ^{13}C n.m.r. to study the binding of metal ions to guanosine in solution. Nitrogen-15[19] and ^{195}Pt[14] n.m.r. were used to examine the reaction modes between nucleosides and platinum complexes. Proton n.m.r. has also been used to study the interactions of platinum complexes with a variety of short oligonucleotides containing GpG or d(GpG) sequences.[20] This study presents, as far as I am aware, an intermediate of the reaction between GMP and the *cis*-diammineplatinum(II) complex [intermediate (**II**)] that has not been reported before. A striking feature of the ^1H n.m.r. spectrum of this complex is that the chemical shift of H(1′) is shifted upfield by 0.74 p.p.m. As I have pointed out earlier, intermediate (**II**) is unlikely to be a blue platinum complex, which rules out the possibility that the 0.74 p.p.m. upfield shift is due to the presence of paramagnetic species.

A possible structure for intermediate (**II**) is one in which two platinum atoms are independently bound to O(6) and N(7) of one GMP molecule, or one in which two platinum atoms are bound to N(1) and N(7) simultaneously. The latter is particularly attractive in the light of the recent study of Dijt *et al.*[21] on the reaction products of *cis*-diammineplatinum(II) compounds with GMP. They have assigned a GMP–platinum complex with a signal at 8.43 p.p.m. at pH 6.5 (with respect to sodium 4,4-dimethyl-4-silapentanesulphonate; this signal in fact consists of two ^1H n.m.r. signals separated only by 0.006 p.p.m.) to a product in which one platinum is bound to GMP at N(1), and a second platinum at N(7). This structure could not be ruled out especially if one considers the sequence of H(8) signals in Figure 3, *i.e.* signal due to intermediate (**II**) appears after the signal of *cis*-$[Pt(ND_3)_2(GMP)_2]^{2+}$. An obvious experiment would be a pH titration of the ^1H n.m.r. signals of intermediate (**II**). Unfortunately, this experiment may not be feasible for the diaquaplatinum complexes.

I propose an alternative structure in which the strong upfield

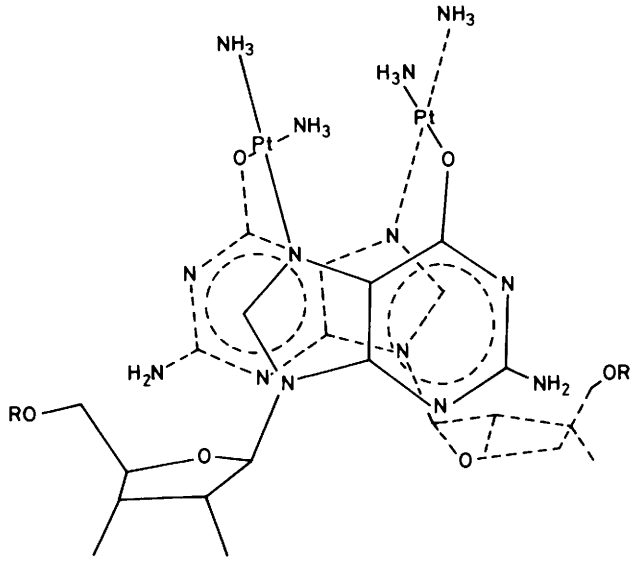

Figure 6. Proposed structure of intermediate (**II**)

shift of the anomeric proton is due to a platinum interaction at O(6) of the guanosine base. The platinum binding at O(6) would certainly promote lactam–lactim tautomerization of the purine ring, thus introducing an aromatic ring current effect on the anomeric proton. However, as pointed out by several investigators,[7] projections from the lone pairs of electrons associated with the O(6) and N(7) intersect at an angle of approximately 37°. Thus simultaneous binding of the platinum atom to both centres would produce considerable strain energy. Moreover, the 0.74 p.p.m. upfield shift is almost twice as much as a single aromatic ring current effect would allow.[22] Therefore, I further propose that intermediate (**II**) is *cis*-$[\{Pt(ND_3)_2\}_2(GMP)_2]^{4+}$ in which each platinum atom is coordinated to N(7) of one GMP molecule and to O(6) of the other (Figure 6). The structure in Figure 6 not only explains the enormous upfield shift of H(1′), but is also consistent with the stereochemistry. However, note should be made that this structure must remain tentative until more evidence is found.

Polissiou *et al.*[16] have used the spin–spin coupling constants of the sugar proton resonances to study the conformation of the platinum complexation products of GMP by K_2PtCl_4. It was found that the anomeric proton coupling constants ($J_{1'2'}$) for free GMP, $[PtCl_3(GMP)]^-$, and *cis*-$[PtCl_2(GMP)_2]$ are 6.1, 4.9, and 5.5 Hz, respectively. It can be seen from the Table that the trend for the $J_{1'2'}$ coupling constants for free GMP, *cis*-$[Pt(ND_3)_2(GMP)(OD)]^+$, and *cis*-$[Pt(ND_3)_2(GMP)_2]^{2+}$ is similar to that of the $[PtCl_4]^{2-}$ complexation products, although the changes in magnitudes are somewhat larger. It is thus of interest to note that $J_{1'2'}$ for the proposed *cis*-$[\{Pt(ND_3)_2\}_2(GMP)_2]^{4+}$ is very close to that of *cis*-$[Pt(ND_3)_2(GMP)_2]^{2+}$, rather than that of *cis*-$[Pt(ND_3)_2(GMP)(OD)]^+$.

$$\text{GMP} + \textit{cis}\text{-}[Pt(ND_3)_2(D_2O)(OD)]^+ \rightleftharpoons \textit{cis}\text{-}[Pt(ND_3)_2(GMP)(OD)]^+ \quad \textbf{(I)}$$

$$\tfrac{1}{2}\,\textit{cis}\text{-}[\{Pt(ND_3)_2\}_2(GMP)_2]^{4+} \quad \textbf{(II)}$$

$$\text{GMP} + \textit{cis}\text{-}[Pt(ND_3)_2(GMP)(OD)]^+ \rightleftharpoons \textit{cis}\text{-}[Pt(ND_3)_2(GMP)_2]^{2+}$$

Scheme.

Overall, the ^1H n.m.r. study shows that the reaction of GMP with *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ at pD 6.8 can be plausibly described by the Scheme. It should be noted that in this Scheme intermediate (**II**), *i.e.* *cis*-$[\{Pt(ND_3)_2\}_2(GMP)_2]^{4+}$ is an unproductive intermediate (it does not lead to the final product).

In summary, this ^1H n.m.r. study on the reaction of GMP with *cis*-$[Pt(ND_3)_2(D_2O)(OD)]^+$ at neutral pH demonstrates the formation of a kinetically allowed intermediate which can be interpreted as a N(7) and O(6) binding species, along with a N(7) bound intermediate *cis*-$[Pt(ND_3)_2(GMP)(OD)]^+$. Significantly, reaction with the *trans* isomer does not generate the N(7),O(6) intermediate. The platinum interaction at O(6) is feasible in view of the new understanding concerning the nature of Pt–O bonds,[2] and the recent report on the platinum binding at the carbonyl oxygen atom of the reactions between *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and amides.[23]

Acknowledgements

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