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The Interaction of Deprotonated Guanosine with en-Pt(II) in the Ratio 1:1

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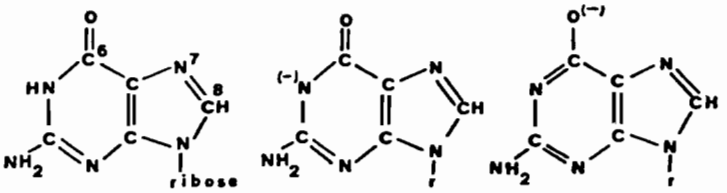
The widely accepted mechanism of action of the antitumor complexes [1] of the type *cis*-$[(NH_3)_2PtCl_2]$ or $[enPtCl_2]$* (1) is the interaction between the platinum atom and the guanine group of DNA [1, 2]. Many model compounds have been described, for instance the *cis*-diamine platinum moiety reacts easily with an excess of guanosine (I) to give complexes as $[enPtguo_2]Cl_2$ (2), with two guanosine molecules coordinated *via* N(7) [3, 4]. Monoguanosinato platinum derivatives have also been reported [5–9], and in some cases N(7)–O(6) chelation has been proposed [5, 6, 9] as a possible explanation that only platinum compounds with two *cis* leaving groups possess antitumor activity.

Since the interaction between DNA and *cis*-Pt(II), or the analogous enPt(II) group has been reported to give, at a certain stage of the reaction, liberation of protons [10], we have investigated the reaction between enPt(II) and guo in the presence of bases, because, under these conditions, the reactive species (III), which is the tautomeric form of (II) [8, 11], could easily lead to the formation of chelates.



(I) (II) (III)

If a water slurry of a 1:1 mixture of (1) and guo is heated at 50 °C for 24 h, a clear solution will result, which probably contains a 1:1 enPt–guo complex. When this solution is reduced in volume the low solubility of (1) yields disproportionation of the 1:1 complex to (1) and (2) [3]. If, however, the solution is treated with one equivalent of NaOH, and run down a column of Sephadex G 10 to remove NaCl, addition of ethanol produces the grey, electrolyte, compound (3) of formula $[enPtguo(-H)]Cl\cdot 2H_2O$.** In a similar way the following compounds have been obtained** $[bnPtguo(-H)]Cl\cdot 3H_2O$ (4) and $[enPtguo(-H)]I\cdot 3H_2O$ (5) ($\Lambda_{o,e}$ = 85 $ohm^{-1}\cdot cm^2\cdot mol^{-1}$, in water at 18 °C). The corresponding perchlorates** (obtained by metathesis reaction) all show the presence of ionic ClO_4^- in the IR spectrum (ν_{ClO_4} *ca.* 1100 cm^{-1}, nujol mulls).

In order to obtain the 1:1 complex of neutral guanosine, we reacted $[enPt(H_2O)_2](NO_3)_2$ and guo in the ratio 1:1, in water. During this reaction (about one day at 40 °C) there is no change of pH (*ca.* 3.5). Addition of ethanol precipitates $[enPtguo](NO_3)_2\cdot 2H_2O$** (6) ($\nu_{NO_3}$ = 1380 cm^{-1}, and ν_{ClO_4} ~ 1100 cm^{-1} for the corresponding perchlorate** (7), nujol mulls). Onsager [12] like plots of both compounds, in water solution, are in agreement with the presence of a 1:2 electrolyte (B = 206 [12], $\Lambda_{o,e}$ = 90 $ohm^{-1}\cdot cm^2\cdot mol^{-1}$ for the nitrate).

When (6), or (7) are treated, in water, with one equivalent of NaOH the pH initially rises to 7.2 and then decreases to 6.9. Addition of ethanol precipitates $[enPtguo(-H)]NO_3\cdot 2H_2O$** (8) or $[enPtguo(-H)]ClO_4\cdot 2H_2O$** (9) ($\nu_{NO_3}$ = 1380 and ν_{ClO_4} ~ 1100 cm^{-1}, nujol mulls. The molar conductivity of these compounds increases rapidly with dilution, showing that, in concentrated solution, some molecular aggregation occurs; only at low concentrations B approaches the correct value of a 1:1 electrolyte (*ca.* 100 [12], $\Lambda_{o,e}$ = 85 $ohm^{-1}\cdot cm^2\cdot mol^{-1}$ at 18 °C, in water, for compound (9)).

The low conductivity of all the compounds here described are in agreement with the presence of heavy cations of low mobility.

The stretching frequency, KBr pellets, of the carbonyl group in free guanosine (I) and in (2) lies at about 1680 cm^{-1} [13], (Fig. 1). In (6) and in (7) this absorption is shifted and it appears as a new shoulder at about 1660 cm^{-1} (Fig. 1). No band

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*en, ethylenediamine; bn, butane-2,3-diamine; guo, guanosine; guo(–H), deprotonated guanosine.

**Satisfactory elemental analyses (C, H, N, Pt) were obtained for these compounds.

Fig. 1. Infrared spectra, KBr pellets, of (**2**), (**6**) and (**8**).

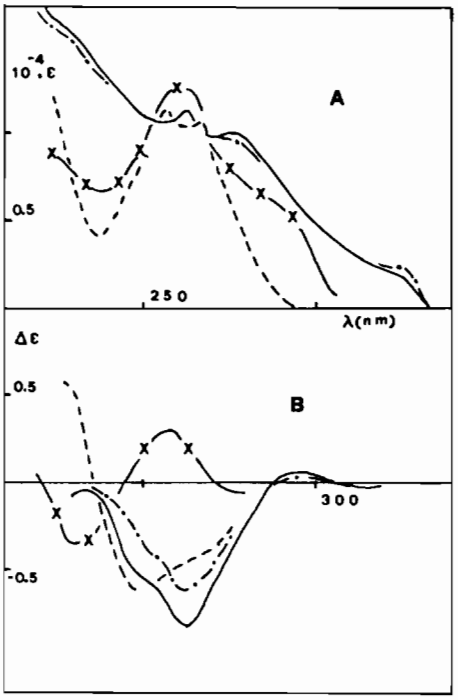


Fig. 2. Electronic (A) and circular dichroism (B) spectra in water solution, of (**2**) (—X—); (**6**) (———); (**8**) (-·-); and guo(–H) (------). The C.D. spectrum of guo is very similar to that of the latter compound. The intensities of the spectra of (**2**) are referred only to one guanosine for comparison purposes.

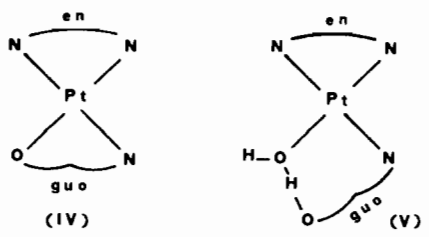
attributable to the C(6)=O stretching can be found in the compounds prepared in the presence of bases, as expected for derivatives of tautomer (III) [8, 11].

The ^{1}H NMR spectra (D_2O solutions) of all the compounds here reported show broad bands, as already observed in similar cases [8, 11]. This fact has been attributed to the presence, in solution, of polymeric species containing non equivalent guanosine ligands [11]. Although the presence, at high concentrations, of some amount of polymeric species cannot be ruled out, it must be noted that also (**6**) and (**7**) show broad NMR resonances, despite the linear Onsager plot of these compounds shows their monomeric nature even at relatively high (10^{-2} *M*) concentrations. It could be that such a broadening can be originated by some at yet undetected paramagnetic impurity. We are currently investigating this point. In all cases the presence of an ill defined coupling of H(8) with ^{195}Pt ($J \sim 27$–30 Hz) is in agreement with coordination of N(7) to platinum [3].

A piece of evidence which stands for the presence, at least in dilute (10^{-4} *M*) solutions of monomers as the major species is given by circular dichroism (C.D.) spectroscopy. In fact polymeric guanosinato complexes should give rise, in the C.D. spectra to a biphasic signal due to the coupling of the transitions of adjacent guanosines, as it is found in the case of (**2**) [14]. On the contrary the observed C.D. spectra of compounds (**3**) to (**9**) are similar to the spectra of guo or guo(–H) (Fig. 2).

The presence of monomeric species suggests chelation of guo for (**6**) and (**7**), and guo(–H) in the other cases. The lowering of the C(6)=O vibration in (**6**) and (**7**) is in agreement with a N(7)–O(6) chelation [6], whereas for the other compounds the absence of the carbonyl stretching is not a proof for such a chelation, since it simply shows that tautomer (III) has formed. Since, however, (III) derives from (I) by addition of sodium hydroxide, it is likely that during the reaction (**6**) + NaOH → (**8**) chelation is retained.

Two types of N(7)–O(6) chelation have been proposed, either directly, as in (IV), or *via* an outer sphere interaction with a coordinated water molecule as in (V), as it has been found, for instance in the case of Co(II) [15], Cu(II) [16] and Ni(II) [17]



guanosine complexes. The occurrence of direct N(7)–O(6) chelates of guo (type (IV)) has been the subject of many discussions [6–9, 11], and although early rejected on the grounds of orbital geometry [16], it has recently been proposed on the basis of MO calculations [18], and found, through X-ray investigations, in a copper complex of theophylline, a 6-oxopurine similar to guanine [19]. Finally in a recent X-ray investigation the *cis*-$(NH_3)_2Pt(II)$ group has been found to lie near the N(7) and O(6) atoms of guanine of a synthetic DNA model [20].

The results here reported add more evidence that the *cis*-platinum moiety, after an initial attack to N(7) of guo [3], can form chelates of the type (IV) or (V), involving the carbonyl oxygen in position 6. We have also shown that these chelates can be easily deprotonated at neutral pH (reaction **(6)** → **(8)** + H^+) thus accounting for a published observation [10]. It could be that chelation, followed by deprotonation can be related to some mechanism of DNA damage involving the hydrogen bond of O(6) of guanosine.

Acknowledgement

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