Inorganica Chimica Acta, *107* (1985) 259–267

Cisplatin Analogues. *cis*-Dichloroaminoacid-tert-butylamineplatinum(II) Complexes and their Adducts with Guanosine

ALESSANDRO PASINI* and ERIK BERSANETTI

Dipartimento di Chimica Inorganica e Metallorganica, The University, Via Venezian 21, 20133 Milan, Italy

Received February 14, 1985

Abstract

A series of compounds of formula *cis*-[$PtCl_2$(aaH)(tba)] (**1**) (aaH, N-coordinate amino acid; tba, tert-butylamine) were synthesized. The circular dichroism spectra of these compounds show that the phenylalanine and proline derivatives have an anomalous conformation in water solution. By reaction with guanosine (guo) compounds **1** give *cis*-[Pt(aaH)(tba)(guo)$_2$]Cl_2 (**2**), in which infrared and nuclear magnetic resonance evidence suggest N(7) coordination of guo. NMR and circular dichroism data suggest that in **2** the two guanosine ligands are arranged head-to-head and form a right-hand helix. The bulkiness of the other ligands make rotation around the Pt–N(7) bonds a slow process on the NMR time scale. The chiroptical properties of **2** are not greatly influenced by the absolute configuration of the amino acid, the right-hand screw probably arising by some guo-guo interaction since the derivatives of 9-methylguanine with chiral amino acids do not possess this conformation.

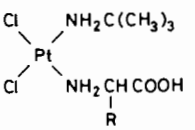
Preliminary results on the reaction between **1** and calf thymus DNA are also briefly reported. They show that the interaction of **1** with DNA is of a lower extent than in the case of cisplatin and its diamine analogues, and that it is independent on the configuration of the amino acids.

All these results are briefly discussed and tentatively correlated with the low antitumor activity of **1** reported in a previous paper.

Introduction

In the recent years a number of cisplatin** analogues have been proposed [1] in the hope of overcoming some of the toxic side effects of this powerful antitumor agent [2].

We have recently prepared and tested for antitumor activity a number of compounds in which the *cis*-$PtCl_2$ moiety (*i.e.* the reactive part of cisplatin) has been bound to carrier molecules as the inert (non-leaving) ligands, *viz.* some dichlorosulfadiazineplatinum(II) derivatives [3] and complexes of the type **1** in which the non-leaving ligands are tert-butylamine and N-coordinated amino acids [4]. These compounds have been prepared on the rationale that sulfadiazine has been reported to



1a, R = H
1b, R = CH_3 (*L*- and *D*-)
1c, R = $CH(CH_3)_2$ (*L*- and *D*-)
1d, R = $CH_2CH(CH_3)_2$ (*L*- and *D*-)
1e, R = $CH_2C_6H_5$ (*L*- and *D*-)
1f, R = CH_2OH (*L*- and *D*-)
1g, R = $CHOHCH_3$ (*L*-, threo)
1h, N-coordinate amino acid = proline (*L*-)

accumulate in the tumor tissues [5], whereas for compounds **1** tba was choosen to increase liposolubility and the amino acids because of the high requirements for nutrients of tumor tissues [6, 7]. Indeed, amino acid derivatives of alkylating agents [8] and of platinum(II) [9–11] have already been reported. Moreover, in the case of *cis*-dichlorobis-(ethylglycilglycinate)platinum(II) a preferential uptake by tumor tissues has been demonstrated [11].

Antitumor tests of most of these compounds gave, however, contrasting results. This was true also for **1**. In this series the antitumor activities (all lower than that of cisplatin) depend in an unpredictable way on the nature of the amino acid [4]. These results prompted us to undertake further studies on compounds **1** in the hope of obtaining informations

*Author to whom correspondence should be addressed.

**Abbreviations. Cisplatin, *cis*-dichlorodiamminoplatinum-(II); aaH, N-coordinate amino acid; guo, guanosine, 9-MeG, 9-methylguanine; guo(-H), N(1) deprotonated guanosine; diam, 1,2-diamine.

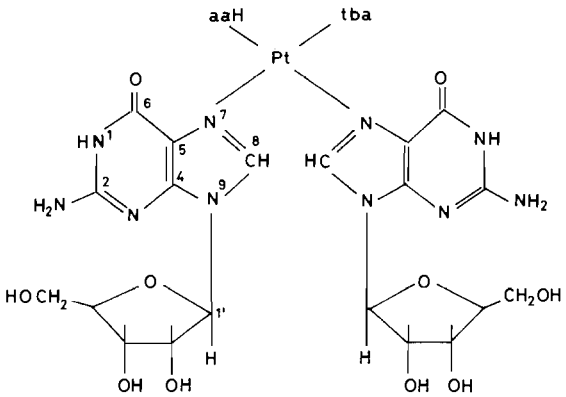
0020-1693/85/$3.30

on the role of the carrier ligands and clues on a better modulation of cisplatin analogues. The uptake of compounds **1** by tumor cells is currently under investigation. In this paper we wish to report some results obtained with model systems that mimic the molecular aspect of the mode of action.

It is widely accepted that the target of cisplatin is the chromosomal DNA [12]. More precisely it has been established that the preferential binding of platinum occurs at N(7) of guanine [13, 14], as has been found in the model reaction of cisplatin with nucleobases [15]. It is also likely that cisplatin binds to two guanine bases forming, as other bialkylating agents, inter- or intra-strand crosslinks [16–18], the letal lesion probably being the intra-strand one [17]. In any case, at least *in vitro*, the cisplatin-DNA interaction yields compounds in which Pt is bound to two guanine bases of the same strand [17, 18].

Two problems arise. First, whether DNA is the primary target of cisplatin has been questioned [19] (see also ref. [20]); moreover the mode of action of cisplatin analogues, particularly of the type **1**, may be different from that of cisplatin. We believe, however, that a study of the interaction of cisplatin analogues with DNA and nucleobases is of general validity because DNA is a target, even if not the primary one [19], and because in the absence of contrary evidence it is likely that the mechanisms of action of the analogues are similar. The results of these type of studies will help in elucidating the general problem of the interaction between heavy metal ions and biologically relevant ligands.

In this paper we therefore wish to report on some properties of compounds **1a–h** and of their adducts



2a, aaH = glycine
2b, aaH = *L*- and *D*-alanine
2c, aaH = *L*- and *D*-valine
2d, aaH = *L*- and *D*-leucine
2e, aaH = *L*- and *D*-phenylalanine
2f, aaH = *L*- and *D*-serine
2g, aaH = *L*-threonine
2h, aaH = *L*-proline

TABLE I. Elemental Analyses.[a]

Compound		Found (calcd) %		
		C	H	N
cis-[$PtCl_2$(*L*-valH)(tba)]	(*L*-**1c**)	23.4(23.7)	4.6(4.8)	6.2(6.1)
cis-[$PtCl_2$(*D*-valH)(tba)]	(*D*-**1c**)	23.8(23.7)	4.7(4.8)	6.4(6.1)
cis-[$PtCl_2$(*L*-leuH)(tba)]	(*L*-**1d**)	25.4(25.5)	5.1(5.1)	5.8(6.0)
cis-[$PtCl_2$(*D*-leuH)(tba)]·H_2O	(*D*-**1d**)	24.8(24.6)	5.4(5.3)	5.7(5.7)
cis-[$PtCl_2$(*D*-pheH)(tba)]	(*D*-**1e**)	31.1(31.0)	4.5(4.4)	5.4(5.6)
cis-[$PtCl_2$(*D*-serH)(tba)]	(*D*-**1f**)	18.9(18.9)	4.3(4.1)	6.0(6.0)
cis-[$PtCl_2$(*L*-proH)(tba)]	(*L*-**1h**)	23.7(23.8)	4.4(4.4)	6.4(6.4)
cis-[Pt(glyH)(tba)(guo)$_2$]Cl_2	(**2a**)	31.9(31.8)	4.2(4.3)	17.0(17.1)
cis-[Pt(*L*-alaH)(tba)(guo)$_2$]Cl_2	(*L*-**2b**)	32.3(32.6)	4.6(4.4)	16.9(16.9)
cis-[Pt(*D*-alaH)(tba)(guo)$_2$]Cl_2	(*D*-**2b**)	32.6(32.6)	4.4(4.4)	16.5(16.9)
cis-[Pt(*L*-valH)(tba)(guo)$_2$]Cl_2	(*L*-**2c**)	34.5(34.1)	4.8(4.7)	16.1(16.4)
cis-[Pt(*D*-valH)(tba)(guo)$_2$]Cl_2	(*D*-**2c**)	33.9(34.1)	4.7(4.7)	16.5(16.4)
cis-[Pt(*L*-leuH)(tba)(guo)$_2$]Cl_2	(*L*-**2d**)	34.8(34.8)	4.2(4.8)	16.1(16.2)
cis-[Pt(*D*-leuH)(tba)(guo)$_2$]Cl_2	(*D*-**2d**)	34.7(34.8)	4.7(4.8)	16.2(16.2)
cis-[Pt(*L*-pheH)(tba)(guo)$_2$]Cl_2	(*L*-**2e**)	36.9(37.0)	4.6(4.5)	15.9(15.7)
cis-[Pt(*D*-pheH)(tba)(guo)$_2$]Cl_2	(*D*-**2e**)	37.3(37.0)	4.5(4.5)	15.7(15.7)
cis-[Pt(*L*-serH)(tba)(guo)$_2$]Cl_2	(*L*-**2f**)	31.9(32.1)	4.3(4.4)	16.4(16.6)
cis-[Pt(*D*-serH)(tba)(guo)$_2$]Cl_2·H_2O	(*D*-**2f**)	31.4(31.5)	4.6(4.5)	16.3(16.3)
cis-[Pt(*L*-thrH)(tba)(guo)$_2$]Cl_2	(*L*-**2g**)	32.7(32.8)	4.5(4.5)	16.5(16.4)
cis-[Pt(*L*-proH)(tba)(guo)$_2$]Cl_2·H_2O	(*L*-**2h**)	35.5(35.5)	4.7(4.6)	16.4(16.2)
cis-[Pt(*L*-valH)(tba)(9-MeG)$_2$]Cl_2		32.5(32.1)	4.1(4.1)	21.4(21.4)
cis-[Pt(*L*-serH)(tba)(9-MeG)$_2$]Cl_2		29.4(29.5)	4.0(3.9)	21.5(21.7)

[a]The analytical characterization of compounds **1a**, *L*-**1b**, *D*-**1b**, *L*-**1e**, *L*-**1f**, and *L*-**1g** has already been reported [4].

with two moles of guanosine (**2a–h**). Some very preliminary results on the interaction of **1** with DNA will also be briefly reported.

Experimental

Analyses (Table I) were from the microanalytical laboratory of the University of Milan.

The following spectrometers were used: Nicolet MX-1 FTIR for infrared spectra, Perkin-Elmer lambda 5 for electronic spectra, Jasco J 500 A for circular dichroism measurements, and a Bruker WP80 for ^{1}H and ^{13}C NMR spectra. All chemicals were reagent grade.

Trans-diamminedichloroplatinum(II) was prepared according to ref. [21].

cis-Aminoacid-tert-butylaminedichloroplatinum(II) (1a–h)

These complexes were obtained as already described [4], by reaction in water solution of $K[PtCl_3$-(tba)] and the proper amino acid in the molar ratio 1:1

cis-Aminoacidtert-butylaminebis(guanosine)platinum-(2+) Chloride

These complexes were obtained [22] by mixing in water compounds **1a–h** and guanosine in the ratio 1:2. The slurries heated at 60 °C for 20 h yielded colourless solutions which were filtered and concentrated to 2–3 ml. The white compounds were crystallized by addition of a large excess of acetone or methanol. The derivatives of 9-methylguanine were prepared in a similar way.

Reactions of 1 with DNA

Calf thymus DNA (43% G + C content) was a generous gift from Dr. A. Gambetta, Istituto Nazionale dei Tumori, Milan, and was prepared according to ref. [23]. DNA and the appropriate amounts of platinum complexes were mixed and diluted to 10 ml in a volumetric flask with a pH 7 phosphate buffer. The solutions were incubated in the dark for 48 h at 30 °C. No further spectral change was observed after this time. In all experiments the base concentration was 0.0974 10^{-3} mol dm^{-1}, corresponding to an optical density of 0.640 (1 cm cell path) for unreacted DNA. Circular dichroism spectra were recorded in 1 cm path cells.

Results and Discussion

The preparation and characterization of compounds **1a**, *L*- and *D*-**1b**, *L*- and *D*-**1f**, and *L*-**1g** have already been described [4]. Compounds **1c–e** and **1h** were prepared in a similar way. Infrared data are in accordance with a *cis*-structure and with the presence of N-coordinate, non-ionized amino acids (for details see ref. [4]).

The circular dichroism spectra of some representative compounds are reported in Fig. 1 (water solu-

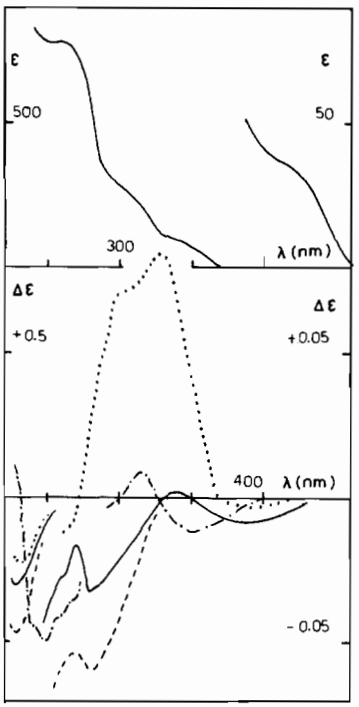


Fig. 1. Electronic spectrum in water solution (pH 5) of **1a** and circular dichroism spectra, under the same conditions of ———, *L*-**1b**; –·–·–, *L*-**1e**; ------, *L*-**1f**; and ·····, *L*-**1h**. The electronic spectra of these latter compounds are similar to that of **1a**.

tions; pH 5. No relevant change was observed in the pH range 1.5–7). The Cotton effects in the 260–460 nm region are of a rather low intensity, as expected for unidentate amino acids. The spectra of the derivatives of *L*-valH and *L*-leuH are almost superimposable to that of *L*-alaH. The spectra of the compounds with *L*-serH and *L*-thrH are also superimposable and are similar to those of the above mentioned compounds.

On the contrary the spectra of the complexes with *L*-pheH and *L*-proH display rather different patterns. A similar behaviour was observed for another series of N-coordinate amino acids platinum complexes, *trans*-$[Pt(aaH)_2(thio)_2]Cl_2$ (thio = thiocarbamide) [24], in which the derivative of the aromatic amino acid *L*-tyrosine (the phe derivative was not reported) displays a C.D. spectrum with a pattern opposite to those of the complexes with amino acids with aliphatic side chains of the same absolute configuration. This behaviour was attributed to some dominant role of

the bulky hydroxyphenyl group in determining the rotational strength of the d–d transitions [24].

For our compounds, however, this behaviour could have a conformational origin. Molecular models show that in type **1** complexes the tert-butyl group and the amino acid residue can rest either on the opposite sides (Fig. 2-a) or on the same side (2-b)

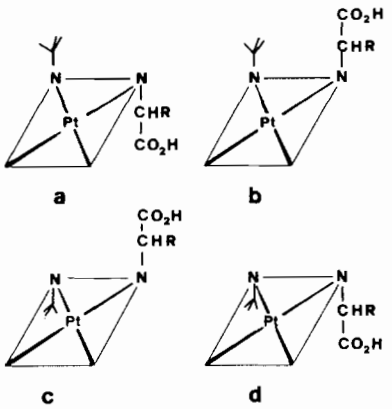


Fig. 2. The possible arrangements of tba and aaH with respect of the coordination plane.

of the coordination plane. The former structure (2-a) seems the more likely, being less sterically hindered. Moreover models show that there is the possibility of hydrogen bond formation between the carboxylic group of the amino acid and the amino group of tba in 2-a and not in 2-b. The chirality of molecules similar to 2-a has already been discussed by Cramer [25], but for a square planar molecule of formula *cis*-[PtX_2AB] (where A ≠ B) structure 2-b is also is disymmetric, 2-d being its enantiomer. The two enantiomeric pairs (2-a *vs.* 2-c and 2-b *vs.* 2-d) both acquire a diastereoisomeric relationship when the amino acid is chiral, and one can imagine some diastereoselectivity in the reaction between a chiral amino acid and K[$PtCl_3$(tba)]. At the present stage we have no evidence either in favour or against such a selectivity, but it could be that the observed differences of the C.D. spectra of Fig. 1 arise from different ratios of structures 2-a and 2-c (and presumably also 2-b and 2-d) of the various derivatives.

The highest optical activity is displayed by the complex with *L*-proH, an amino acid which on complexation acquires a new center of chirality (the secondary nitrogen atom). It is likely that the formation of the Pt–N(proH) bond is stereoselective. The high optical activity is in accordance with the presence of an asymmetric atom in the coordination sphere [24].

Work is in progress to elucidate the stereochemical problems posed by these compounds.

TABLE II. Infrared Spectra of some Representative Guanosine Derivatives.[a,b]

Compound	ν (cm^{-1})		
	band I	band II	band III
2a	1698	1585	1538, 1497
L-**2c**	1694	1585	1537, 1497
L-**2e**	1698	1587	1540, 1497
L-**2g**	1694	1588	1539, 1498
L-**2h**	1695	1588	1537, 1498
[Pt(en)(guo)$_2$]Cl_2[c]	1698	1587	1539, 1500
[Hg(Ph)(guo)]NO_3[d]	1690	1594	1538, 1508
[Hg(Ph)(guo(–H))][e]	1650sh	1579	1525, 1508

[a]KBr pellets; sh, shoulder. [b]For the assignments of bands I, II and III, see ref. [28]. [c]N(7) coordination [38]. [d]N(7) coordination [28]. [e]N(1) coordination [28].

Bis guanosine Derivatives of 1

Compounds **2** were prepared by reaction of **1** with two moles of guanosine. The white, water-soluble compounds were precipitated by addition of guo (*cf.* Table II). Coordination through N(1) infrared spectra (KBr pellets, see Table II) the bands due to the guanosine moiety are similar to those found in *cis*-[$Pt(NH_3)_2(guo)_2$]Cl_2 and [M(diam)-$(guo)_2$]Cl_2 (M = Pt, Pd) [26, 27]. This spectral pattern seems representative of N(7) coordination of guo (*cf.* Table II). Coordination through N(1) is kinetically unfavourable under neutral or slightly acidic conditions [31], as it should occur with deprotonated guanosine, and it should give rise to different stoichiometries of **2**, in contrast with the analytical data. Moreover the N(7) mode of coordination seems the most common in the Pt(II)–guo interaction [15, 31]: it has been found in a number of crystal structures of Pt guanosine complexes [15, 32–35], and it is relevant since this type of binding occurs in the reaction of cisplatin with DNA [12].

N(7) coordination to platinum should give rise to the coupling of the resonances of H(8) and C(8) with the ^{195}Pt nucleus [22], but the complexity of the spectra makes such an observation rather unreliable (see Figs. 3 and 4 and below). NMR spectroscopy, however, provides other evidence for such a binding. These are the downfield shifts of the resonances of H(1), H(2) and H(8) and the shifts of the resonances of C(5) (upfield) and C(8) (downfield), with respect to free guanosine or to N(1) coordinated guanosine [28, 31, 36]. These shifts have been proposed to indicate N(7) binding [31, 36]. Interestingly even the C(1′) resonance is shifted upfield by such a binding, as already observed in other compounds [31, 36]. This fact may be related to a change of the ribose conformation (from C(2′)-*endo* to C(3′)-*endo*) which occurs upon

TABLE III. ^{1}H and ^{13}C NMR Data for some Representative Guanosine Derivatives.[a]

Compound	H(1)	H(2)	H(8)	C(5)	C(8)	C(1')
guo	10.7	6.5	7.95	116.5	135.6	86.3
D-2c[b]	11.2–11.6	7.2	8.2–8.6	113.3–113.5	136–138	87.2–87.8
$[Hg(CH_3)(guo)]NO_3$[c]	11.4	6.9	8.6	113.2	138.8	87.8
$[Hg(CH_3)(guo(-H))]$[d]		6.5	7.9	118.0	135.8	86.4
Pt-guo[e]				113.0	138.8	87.6
$[Pt(en)(guo)_2]Cl_2$[f]	11.6	7.2	8.5			

[a]DMSO solutions. δ values in ppm from TMS as external standard. [b]Most of the nuclei give rise to multiple resonances, see text. The spectra of the other compounds of type **2** are similar. [c]N(7) coordination, see ref. [36]. [d]N(1) coordination [36]. [e]DMSO solution of $[PtCl_2(DMSO)_2]$ and guanosine, see ref. [31]. [f]Prepared according to ref. [38].

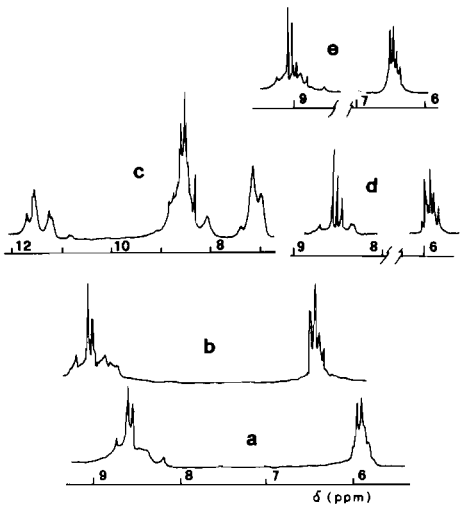


Fig. 3. Parts of the ^{1}H NMR spectra, at 80 MHz, of *L*-2c in D_2O solution (pD 6.5) at 25 °C (a); 80 °C (b); *L*-2c in DMSO solution at 25 °C (c); *L*-2d in D_2O solution (pD 6.5) at 25 °C (d) and at 80 °C (e). δ values are in ppm relative to TMS as external reference.

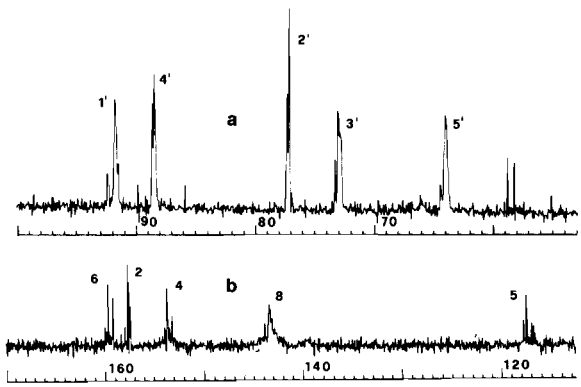


Fig. 4. The ribose (a) and guanine (b) regions in the ^{13}C NMR spectrum at 20.148 MHz of *L*-2c (D_2O solution, pD 6.5) at room temperature. δ values are in ppm relative to TMS as external standard.

N(7) coordination [32, 33]. The data in Table III summarize the above discussion.

During the preparation of **2** we observed a pH raise from 3 to 6, presumably because of a lower acidity of the carboxylic group in **2**. In fact in the IR spectra of **2** the bands of the carboxylic groups, although partly obscured by the absorptions of the guo moiety, appear at 1750 and 1250 cm^{-1} in agreement with the formulation $[Pt(aaH)(tba)(guo)_2]Cl_2$. The alternative zwitterionic structure (with the same elemental analysis) $[Pt(aa^{-})(tba)(guo)(guoH^{+})]Cl_2$ should in fact show a different IR spectrum and is therefore unlikely, because both N(3) and $N(2)H_2$ are rather weak basic sites. In compounds of the type $[PtCl_2(aaH)(guo)]$ the N-coordinate amino acid has been reported to be non-ionized at pH 7 [37]. Interestingly, **1** compounds are partly ionized at pH 6 [4].

The inert nature of the Pt–N bonds should ensure the retention of the *cis*-configuration during the reaction **1** → **2**. Proof of the *cis*-configuration of **2** is given by the C.D. spectra, which are different from that of *trans*-$[Pt(NH_3)_2(guo)_2]^{2+}$ and are similar to those of compounds with two guo bound to Pt in the *cis* position [38] (see below).

The ^{1}H and ^{13}C NMR spectra (D_2O solutions) are rather complicated, since most of the nuclei give rise to a set of resonances which vary little with temperature (Figs. 3 and 4. Assignments were made according to literature data [22, 31, 36, 39, 40]). This behaviour has been observed also in $(CD_3)_2SO$ solutions, and therefore it does not arise from intermolecular stacking of the guanine rings, since DMSO has been reported to minimize these particular interactions [41]. Moreover in this solvent H(1) and H(2) (not observable in D_2O) also give rise to complex patterns (see Fig. 3).

A similar behaviour has already been reported for compounds of the type $[Pt(diam)(guo)_2]^{2+}$ [25, 38, 42] and other *cis*-bis(oxopurine) complexes [43], and attributed to the presence of unequivalent purine moieties with hindered (*i.e.* slow on the NMR time

scale) rotation around the Pt–N(7) bonds [25, 38, 42, 43]. In **2** compounds, however, the two guo are in different chemical environments and may not be equivalent even in the case of fast rotation. However the NMR spectra of compounds **2** usually show, for a given nucleus, two intense resonances (of different intensities) and a number of ill-resolved peaks. This pattern could arise from the presence of all (or some of) the possible rotamers slowly interconverting. These rotamers originate from structures 2-a to 2-d and from the various relative dispositions (*i.e.* head-to-head and head-to-tail) of the guanine rings in each isomer of Fig. 2. Interestingly, the non-equivalence of the guo moieties is also reflected on the non-equivalence of the H(1′) and to the carbon atoms of the ribose rings (Figs. 3 and 4). Similar findings have already been observed [38, 42], but to our knowledge this is the first case of *cis*-bis(guo) compounds in which all the ribose carbon atoms are non-equivalent.

Circular Dichroism Spectra

The representative CD spectra of **2** compounds in water solutions (pH 6.5) in the range 220–320 nm are reported in Figs. 5 and 6. The spectra consist of a broad negative band in the 270–320 nm range, usually with a shoulder at 275 nm, a positive band

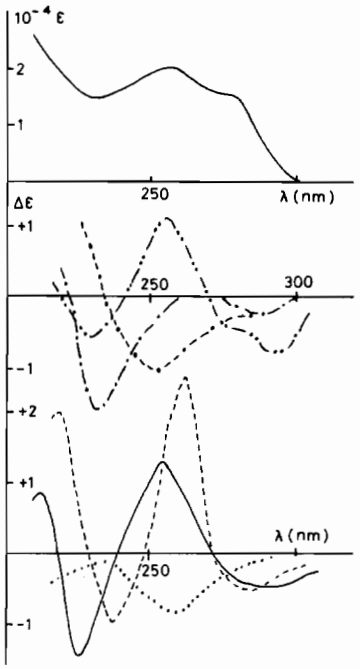


Fig. 5. Electronic spectrum (Water solution, pH 6.5) of **2a** and circular dichroism spectra of: ———, [Pt(diam)(guo)$_2$]-Cl$_2$ (diam = S,S-2,3-diaminobutane, the spectra of the derivatives of other diamines are all similar [38]); -------, GpG [45]; ·-·-·-·-, guanosine [38]; ·····, [Pt(en)(guo)]-(NO$_3$)$_2$ [26]; —·—·—, *trans*-[Pt(NH$_3$)$_2$(guo)$_2$]Cl$_2$; and —··—··—, **2a**.

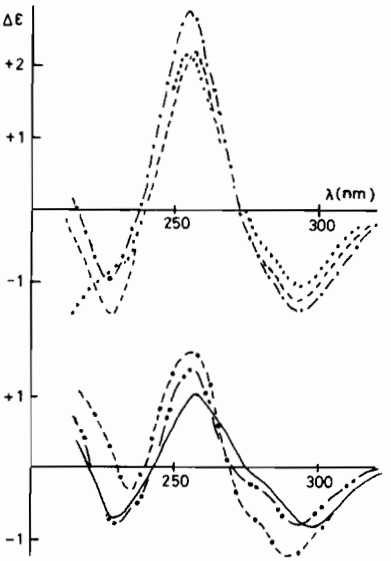


Fig. 6. Circular dichroism spectra (water solutions, pH 6.5) of: ———, *L*-**2c**; —·—·—, *D*-**2c**; -·-·-·-; *L*-**2e**; —·—·— *L*-**2f**; -------, *D*-**2f**; and ·····, *L*-**2h**.

at 255 nm and a negative band at 230 nm. These spectra are all alike, except for the higher intensity (especially of the 255 nm band) of the spectra of the compounds with the hydroxyamino acids and proline. The derivatives of 9-methylguanine are inactive in the 220–300 nm region and their spectra are not reported here. The absolute configuration of the amino acids has no influence on the sign of the dominant Cotton effects in the range studied. The phe and pro derivatives do not show any anomaly, in contrast to what we observed with **1** compounds.

The general trends of these spectra resemble those of [Pt(diam)(guo)$_2$]$^{2+}$, *i.e.* complexes with two guo moieties bound to the metal ion via N(7) and in the *cis*-configuration [38], and bear no resemblance to the spectra of guanosine itself, to complexes with only one guanosine [26], or to two guanosines in the *trans*-position (see Fig. 5). This difference has been attributed to interaction of the electronic transitions responsible for the Cotton effects, giving rise to a characteristic positive-negative exciton splitting [38]. Such an interaction can only occur if the two guanine planes are arranged in a helical fashion, a structure clearly unattainable for the *trans*-configuration. We can therefore assume that the presence of such a splitting is proof for the *cis*-configuration of a bis guo compound.

In the case of [Pt(diam)(guo)$_2$]$^{2+}$ analysis of the spectra and the knowledge of the direction of polarizations of the transitions localized on the guanine planes [44] allowed us to determine the 'time averaged' orientation, in solution, of the purine planes as that of a right-hand propeller, with the two

guanine arranged head-to-tail [38]. In the spectra of **2** compounds the positive component of the doublet centered at about 240 nm is also at higher wavelengths, suggesting a helical arrangement of the guanine planes of the same handedness as the diamine derivatives discussed above, but the two components of the doublets have different intensities, the positive one being more intense (see Fig. 5). The overall shape and the intensity ratio of the bands in the spectra of **2** resemble those of GpG in the B DNA conformation [45] and those of IpI and its platinum complex $[Pt(NH_3)_2(IpI)]^+$ [46, 47], for which a stacked head-to-head arrangement of the dinucleotide has been proposed [47].

The CD spectra of the cisplatin adducts of GpG and d(GpG) (which should have a similar conformation) are different from that of the dinucleotide [48, 49]. These differences have not been discussed in detail, but it could be that in these particular cases there is some de-stabilization of the helical arrangement. In the case of our complexes, CD spectra suggest that it is the formation of the complex that stabilizes the helical head-to-head conformation.

CD spectroscopy suggests that the most abundant conformers in solution are those in which the two guo moieties are head-to-head and arranged as a right-hand helix. In such a conformation the two guanosines are not magnetically equivalent, thus also accounting for the NMR results. Moreover, this conformation does not depend on the absolute configuration of the amino acids or on the possible spatial orientations of aaH and tba of Fig. 2, but arises only from some interaction of the two guanosine moieties, presumably via the ribose groups. In agreement with these points the derivatives of 9-MeG present no optical activity in the 220–300 nm region in complexes of chiral amino acids.

For the head-to-head arrangement it is worth noting that the majority of the *cis*-(guanine)$_2$ complexes so far reported are in the head-to-tail conformation [15, 31–35], but head-to-head dispositions have been described both in solution [50], and more recently also in the solid state [51]. The head-to-head rotamer is of interest because it is a more realistic model of the intra-strand crosslink, G–Pt–G, in DNA-cisplatin adducts [51].

Interaction of 1 with DNA

Compounds **1a**, *L*-**1b**, *D*-**1b**, *L*-**1d**, *D*-**1d**, *L*-**1f**, and *D*-**1f** were incubated with calf thymus DNA at pH 7 in Pt/G ratios of 0.4 and 1.0 for 36 h at 30 °C. After this time no further spectral change could be detected. Under these conditions and at these Pt/G ratios in the case of cisplatin and its diamine analogues, reaction with DNA is complete, *i.e.* all the charged platinum complex is essentially bound to DNA [52, 53]. This may not be the case for **1** compounds, but no analytical technique is as yet available to check this point. These results are therefore preliminary, but we wish to report them briefly because they are relevant to the overall discussion.

In Fig. 7 we have reported only the spectra obtained upon reaction of DNA with **1a**, since they are

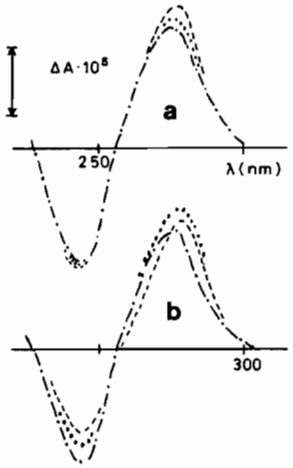


Fig. 7. Circular dichroism spectra of solutions of calf thymus DNA and **1a** (a) and $[PtCl_2(en)]$ (b), at Pt/G ratios; –·–·–, 0; ··,····, 0.4; and -------, 1. Cell path = 1 cm, for other conditions see text.

exactly superimposable to the spectra obtained with the other Pt complexes. The spectral changes are therefore independent on the nature and the absolute configuration of the amino acid bound to platinum. Such a lack of chiral recognition between DNA and chiral cisplatin analogues have already been described in other instances [54–56].

The changes of the DNA spectrum observed upon reaction with compounds **1** consist of a rise of the positive part of the bisignated curve at 275 nm (with a slight batochromic shift) and in a decrease of the positive band at 220 nm whereas the negative band is practically unaffected. This behaviour is different from what is found in the case of cisplatin [57] and its diamine analogues [55], *i.e.* a relevant increase of the positive band at 275 nm until Pt/G ≃ 0.4, followed by a decrease at higher Pt/G ratios (see Fig. 7). As we have no means to check how much platinum is bound to DNA we do not know whether this difference is due to a lower reactivity of **1** or if the secondary structure of DNA is less altered by **1** than by cisplatin. Whichever the explanation it is tempting to correlate these results with the fact that the antitumor activities of compounds **1** are lower than those of cisplatin [4].

If the letal lesion produced by cisplatin to DNA is the formation of intrastrand crosslinks between two guanine bases [17], it has been proposed that an important step of the formation of such a cross-

link is the rotation around the Pt–N(7) bond in the intermediate $(NH_3)_2PtGCl$ (cisplatin bound monofunctionally to DNA) in order to reach a second base and give rise to the bifunctionally bound species $(NH_3)_2PtG_2$ [42]. The hindered rotation observed in compounds **2** can therefore correlate with the small variation of the DNA structure when reacted with **1** and/or with their low reactivity towards DNA.

Conclusions

This work was undertaken with the hope of finding some relationship between the antitumor activities of compounds **1** and some of their chemical properties, but, within the series, the different antitumor activites [4] do not correlate with any of the properties reported in this paper, such as the chiroptical properties of **1**, or those of the DNA and guo derivatives. It seems therefore that the techniques used in this work yield results that contribute to the general knowledge of the chymism of cisplatin analogues, but are not sensitive to the fine chemical modulations of such closely-related compounds as **1a–h**. More detailed studies on cellular aspects, such as membrane transport and cell uptake of **1**, are currently underway with the hope of obtaining more insight into the mechanism of action and clues for a better modulation of the activity of cisplatin analogues.

It appears that the results obtained with the techniques here reported allow only some comparison between different classes of compounds such as **1**, cisplatin, and other analogues. For instance we feel that this paper adds new evidence to Reedijk's proposal [42] that free rotation around the Pt–N(7) (guanine) bond is important in the mechanism of formation of the intra-strand cisplatin–DNA adducts and, therefore [17], ultimately of the antitumor activity. Type **1** compounds, as a series, show only marginal (or no) antitumor activity [4] and in their guo adducts (**2**) rotation around the Pt–N(7) bond is slow on the NMR time scale. Cisplatin is highly active and the guanine bases rotate freely in $[Pt(NH_3)_2(guo)_2]^{2+}$ [25].

This correlation holds also for other compounds. $[PtCl_2(CH_3)_2NCH_2CH_2N(CH_3)_2]$ shows a very poor anticancer activity [58], and in $[Pt(CH_3)_2NCH_2CH_2N(CH_3)_2(guo)_2]^{2+}$ the guanosine ligands have been shown by Cramer to rotate slowly on the NMR time scale [25]. This also applies for $[PtCl_2(dipy)]$ (dipy = 2,2′-dipyridine) (inactive [59]) and its guanosine adduct $[Pt(dipy)(guo)_2]^{2+}$ (slow rotation [42]), but not for $[PtCl_2(\alpha\text{-picoline})_2]$ and $[PtCl_2(CH_3NHCH_2CH_2CH_2NHCH_3)]$ and their oxopurine adducts (*cf.* the data of refs. [42] and [59]). It appears therefore that such a correlation must be considered more as suggestion for further experiments than as a criterion.

In compounds **1a–f** the absolute configuration of the amino acid plays no relevant role in the interaction with both guanosine (since all compounds **2a–f** possess the same overall conformation) and DNA. This absence of chiral recognition appears to be a general rule for cisplatin analogues, since it has also been found with $[PtCl_2(\text{chiral diamine})]$ [54–56, 60, 61]. The damage induced by cisplatin and its analogues to DNA may be such that it destroys the stereochemical feature of DNA, at least in the neighbourhood of the site of attack, in a way that makes any chiral recognition impossible [55, 61].

This is probably a reflection of the kind of interaction of this type of compounds with DNA (*i.e.* covalent, bifunctional, binding) since, with chiral intercalating compounds, which do not destroy the helical structure of DNA, a high degree of chiral recognition is usually observed [62–64].

Acknowledgements

We wish to thank Dr. Vecchio, Istituto di Chimica degli Ormoni del CNR (Milano) for the use of the JASCO J 500 spectrophotometer for CD measurements, and Dr. Zunino, Istituto Nazionale dei Tumori, Milan, for helpful discussion. This work was supported by the Italian Ministry of Education.

References

1 P. C. Hydes, in M. P. Hacker, E. B. Douple and I. H. Krakoff (eds.), 'Platinum Coordination Complexes in Cancer Chemotherapy', Nijoff, Boston, 1984, p. 216.
2 J. J. Roberts, in H. M. Pinedo (ed.), 'Cancer Chemotherapy 1982', Excerpta Medica, Amsterdam, 1982, p. 95.
3 A. Pasini, E. Bersanetti, F. Zunino and G. Savi, *Inorg. Chim. Acta, 80,* 99 (1983).
4 E. Bersanetti, A. Pasini, G. Pezzoni, G. Pratesi, G. Savi, R. Supino and F. Zunino, *Inorg. Chim. Acta, 93,* 167 (1984).
5 C. Abel, T. A. Connors, W. C. Ross, N. H. Namm, H. Hoellinger and L. Pichot, *Eur. J. Cancer, 9,* 49 (1973).
6 D. F. H. Wallach, *J. Mol. Med., 1,* 97 (1976).
7 D. R. Williams, *Chem. Rev., 72,* 203 (1972).
8 M. Szekerke, *Cancer Treat. Rep., 60,* 347 (1976).
9 A. J. Charlson, R. J. Banner, R. P. Gale, N. T. McArdle, K. E. Trainor and E. C. Watton, *J. Clin. Emat. Oncol., 7,* 293 (1977).
10 A. J. Charlson and W. A. Shorland, *Inorg. Chim. Acta, 93,* L67 (1984).
11 W. A. Beck, in A. Mueller and E. Dueman (eds.), 'Transition Metal Chemistry, Current Problems and the Biological as well as the Catalytical Relevance', Verlag Chemie, Weinheim, 1981, p. 3, and refs. therein.
12 J. J. Roberts and F. M. Pera, Jr., in S. J. Lippard (ed.), 'Platinum, Gold and other Metal Chemotherapeutic

Agents', ACS Symposium Series 209, American Chemical Society, Washington, D.C., 1983, p. 3.
13 S. Mansy, G. Y. H. Chu, R. E. Duncan and R. S. Tobias, *J. Am. Chem. Soc., 100,* 607 (1978).
14 D. E. Hathway and G. F. Kolar, *Chem. Soc. Rev., 9,* 241 (1980).
15 L. G. Marzilli, in G. L. Eichorn and L. G. Marzilli (eds.) 'Advances in Inorganic Biochemistry, Vol. 3', Elsevier, New York, 1981, p. 48, and refs. therein.
16 K. W. Kohn, in A. C. Sartorelli, J. S. Lazo and J. R. Bertino (eds.), 'Molecular Action and Targets for Cancer Chemotherapeutic Agents', Academic Press, New York, 1981, p. 3.
17 J. P. Caradonna and S. J. Lippard, in P. M. Hacker, E. B. Douple and I. H. Krakoff (eds.), 'Platinum Coordination Complexes in Cancer Chemotherapy', Nijoff, Boston, 1984, p. 14.
18 J. Reedijk, J. H. J. den Hartog, A. M. J. Fichtinger-Schepman and A. T. M. Marcelis, in P. M. Hacker, E. B. Douple and I. H. Krakoff (eds.), 'Platinum Coordination Complexes in Cancer Chemotherapy', Nijoff, Boston, 1984, p. 39.
19 J. P. Macquet, J. L. Butour, N. P. Johnson, H. Razaka, B. Salles, C. Vieussens and M. Wright, in P. M. Hacker, E. B. Douple and I. H. Krakoff (eds.) 'Platinum Coordination Complexes in Cancer Chemotherapy', Nijoff, Boston, 1984, p. 27.
20 K. J. Scanlon, R. L. Safirstein, H. Thies, R. B. Gross, S. Waxman and J. B. Guttenplan, *Cancer Res., 43,* 4211 (1983).
21 G. B. Kaufman and D. O. Cowan, *Inorg. Synth., 7,* 239 (1963).
22 P. C. Kong and T. Theophanides, *Inorg. Chem., 13,* 1167 (1974).
23 F. Zunino, A. Di Marco, A. Zaccara and R. A. Gambetta, *Biochim. Biophys. Acta, 607,* 206 (1980).
24 O. P. Slyudkin and B. J. F. Norden, *Inorg. Chem., 22,* 2637 (1983).
25 R. E. Cramer and P. L. Dahlstrom, *J. Am. Chem. Soc., 101,* 3679 (1979).
26 A. Pasini and R. Mena, *Inorg. Chim. Acta, 56,* L17 (1981).
27 G. Pneumatikakis, *Inorg. Chim. Acta, 66,* 131 (1982).
28 A. J. Canty and R. S. Tobias, *Inorg. Chem., 18,* 413 (1979).
29 W. Beck and N. Kottmair, *Chem. Ber., 109,* 970 (1976).
30 G. Pneumatikakis, N. Hadjiliadis and T. Theophanides, *Inorg. Chem., 17,* 915 (1978).
31 L. G. Marzilli, B. de Castro and C. Solorzano, *J. Am. Chem. Soc., 104,* 461 (1982).
32 R. W. Gellert and R. Bau, *J. Am. Chem. Soc., 97,* 7379 (1975).
33 R. E. Cramer, P. L. Dahlstrom, M. J. T. Seu, T. Norton and M. Kashiwagi, *Inorg. Chem., 19,* 148 (1980).
34 T. J. Kistenmacher, J. D. Orbell and L. G. Marzilli, in S. J. Lippard (ed.), 'Platinum, Gold and other Metal Chemotherapeutic Agents', ACS Symposium Series, 209, American Chemical Society, Washington, D.C., 1983, p. 191.
35 A. P. Hitchcock, C. J. L. Lock, W. M. Pratt and B. Lippert, in S. J. Lippard (ed.), 'Platinum, Gold and other Metal Chemotherapeutic Agents', ACS Symposium Series, 209, American Chemical Society, Washington, D.C., 1983, p. 209.
36 E. Buncel, A. R. Norris, W. J. Racz and S. E. Taylor, *Inorg. Chem., 20,* 98 (1981).
37 B. Taqui Khan, S. Vijaya Kumari and G. Narsa Goud, *J. Coord. Chem., 12,* 19 (1982).
38 M. Gullotti, G. Pacchioni, A. Pasini and R.Ugo, *Inorg. Chem., 21,* 2006 (1982).
39 A. J. Jones, D. M. Grant, M. W. Winkley and R. K. Robins, *J. Am. Chem. Soc., 93,* 4079 (1970).
40 G. V. Fazakerley and K. R. Koch, *Inorg. Chim. Acta, 36,* 13 (1979).
41 S. M. Wang and N. C. Li, *J. Am. Chem. Soc., 90,* 5069 (1968).
42 A. T. M. Marcelis, J. L. Van der Veer, J. C. M. Zwetsloot and J. Reedijk, *Inorg. Chim. Acta, 78,* 195 (1983).
43 A. T. M. Marcelis, H. J. Korte, B. Krebs and J. Reedijk, *Inorg. Chem., 21,* 4059 (1982).
44 L. B. Clark, *J. Am. Chem. Soc., 99,* 3934 (1977).
45 N. P. Johnson and E. Switkes, *Biopolymers, 16,* 857 (1977).
46 J. C. Chottard, J. P. Girault, G. Chottard, J. Y. Lallemand and D. Mansuy, *Nouv. J. Chim., 2,* 551 (1978).
47 J. C. Chottard, J. P. Girault, G. Chottard, J. Y. Lallemand and D. Mansuy, *J. Am. Chem. Soc., 102,* 5565 (1980).
48 A. M. J. Fichtinger-Schepman, P. H. M. Lohman and J. Reedijk, *Nucl. Acids Res., 10,* 5345 (1982).
49 J. P. Girault, G. Chottard, J. Y. Lallemand and J. C. Chottard, *Biochemistry, 21,* 1352 (1982).
50 J. H. J. den Hartog, C. Altona, J. C. Chottard, J. P. Girault, J. Y. Lallemand, F. A. A. M. de Leeuw, A. T. M. Marcelis and J. Reedijk, *Nucl. Acids Res., 10,* 4715 (1982).
51 B. Lippert, G. Raudaschl, C. J. L. Lock and P. Pilon, *Inorg. Chim. Acta, 93,* 43 (1984).
52 J. P. Macquet and T. Theophanides, *Biopolymers, 14,* 781 (1975).
53 N. P. Johnson, J. D. Hoeschele and R. O. Rahn, *Chem.-Biol. Interact., 30,* 151 (1980).
54 K. Inagaki and Y. Kidani, *Inorg. Chim. Acta, 46,* 35 (1980).
55 A. Pasini, A. Velcich and A. Mariani, *Chem.-Biol. Interact., 42,* 311 (1982).
56 B. Wappes, M. Jennerwein, E. v. Angerer, J. Engel, H. Schoenenberger, H. Brunner, M. Schmidt, M. Berger, D. Schmal and S. Seeber, *J. Cancer Res. Clin. Oncol., 107,* 15 (1984).
57 J. P. Macquet and J. L. Butour, *Eur. J. Biochem., 83,* 375 (1978).
58 M. J. Cleare and J. D. Hoeschele, *Bioinorg. Chem., 2,* 187 (1973).
59 S. J. Meischen, G. R. Gale, L. M. Lake, C. J. Frangakis, M. G. Rosenblum, E. M. Walker, L. M. Atkins and A. B. Smith, *J. Nat. Cancer Inst., 57,* 841 (1976).
60 W. R. Leopold, R. P. Batzinger, E. C. Miller, J. A. Miller and R. H. Earhart, *Cancer Res., 41,* 4368 (1981).
61 M. Gullotti, A. Pasini, R. Ugo, S. Filippeschi, L. Marmonti and F. Spreafico, *Inorg. Chim. Acta, 91,* 223 (1984).
62 J. K. Barton, J. J. Dannenberg and A. L. Raphael, *J. Am. Chem. Soc., 104,* 4967 (1982).
63 A. Yamagishi, *J. Chem. Soc., Chem. Commun.,* 572 (1983).
64 J. K. Barton, A. T. Danishefsky and J. M. Goldberg, *J. Am. Chem. Soc., 106,* 2172 (1984).