Inorganica Chimica Acta, *137* (1987) 45–51

Biological Activity of Platinum Complexes Containing Chiral Centers on the Nitrogen or Carbon Atoms of a Chelate Diamine Ring*

F. P. FANIZZI, F. P. INTINI, L. MARESCA, G. NATILE, R. QUARANTA

Dipartimento Farmaco-Chimico, Università, Via G. Amendola 173, 70126 Bari, Italy

M. COLUCCIA, L. DI BARI, D. GIORDANO and M. A. MARIGGIÓ

Istituto di Patologia Generale, Università, Piazza Giulio Cesare, 70124 Bari, Italy

Abstract

Platinum complexes with different chirality either at the coordinated nitrogens themselves or at the carbon atoms of the organic chain bridging the two nitrogens of a diamine have been tested for their mutagenic activity towards different strains of *Salmonella typhimurium.* In the case of [$PtCl_2$-(i-Pr_2-en)] the isomer with different absolute configuration at the two nitrogens (*R,S*) appears to be more active towards TA92, TA2410 and TA100 strains while those with equal configuration at the nitrogens (*R,R* or *S,S*) are more active towards the TA98 strain. In the case of [$PtCl_2$(1,2-diaminocyclohexane)], [$PtCl_2$(2,3-diaminobutane)] and [$PtCl_2$-(1,2-diaminopropane)] the isomers with *S* configuration at the asymmetric carbons are by far the most active towards all strains of bacteria.

Introduction

The initial discovery of the antitumor activity of *cis*-[$PtCl_2(NH_3)_2$] (cisplatin) and its subsequent clinical trial has inspired intensive research into the mechanism of action of this complex and the development of suitable analogs with better pharmacological properties [1–3]. Heterocyclic, alicyclic, straight and branched-chain alkyl amines, as well as chelating amines all give compounds with appreciable activity, moreover the nitrogen ligands appear to have a primary influence on the antitumor properties although all of them affect in a very similar way the reactivity of the *trans*-ligands [4].

We studied this very important and not well established role of the aminic ligands and started an investigation in which the conformation and the configuration of these ligands were systematically changed. The biological effect was evaluated by tests of mutagenesis on different strains of *Salmonella typhimurium* [5–11].

Substitution of primary amines for ammonia causes relevant changes in mutagenic activity, for instance the compound *cis*-[$PtCl_2(H_2NCHMePh)_2$] was found to be active only towards bacteria lacking in excision-repair systems (uvrB) in marked contrast with the behavior of *cis*-[$PtCl_2(NH_3)_2$] (cisplatin) which, although to a different degree, is mutagenic towards his-G46 bacteria having either efficient or defective repair systems [12].

The effect of bridging with an ethylene chain the two aminic nitrogens *cis*-coordinated to the metal was also investigated. The conclusion was that compounds with bidentate amines generally exhibit a weaker mutagenic activity than the parent compounds with monodentate amines [13]. In the case above, change of chirality of the alkyl substituents at the coordinated nitrogens had no effect whatsoever on the mutagenic activity of the compounds. The situation could be different in the case in which the platinum compounds have different chirality either at the coordinated nitrogens themselves or at a carbon atom which is restricted in its motion, such as those of the organic chain bridging the two nitrogens of a diamine.

With reference to the first of the two possibilities, an aminic nitrogen, in order to become asymmetric upon coordination, must carry two different substituents; moreover the inversion of configuration is also required to be slow [14, 15]. Both conditions appear to be fulfilled in the case of an *N,N'*-disubstituted ethylenediamine such as *N,N'*-di-isopropylethylenediamine (i-Pr_2en) which we shall refer to in the present paper.

Concerning the case of platinum complexes with an asymmetric carbon chain bridging the two nitrogens of a diamine, when we started this investigation there were already some reports of dependence of the mutagenic activity upon the configuration of 1,2-diaminocyclohexane in its complex with platinum. The *S,S* (*trans* +) form is more active than the

*Paper presented at the Symposium on Cisplatin and Inorganic Anticancer Drugs, Bari, Italy, November 6–7, 1986.

0020-1693/87/$3.50

© Elsevier Sequoia/Printed in Switzerland

TABLE I. Analytical and Spectroscopic ($[\alpha]_D$) Data for Diamine·2HCl[a]

Diamine	C	H	N	$[\alpha]_D$	Reference
R-DAP	24.4 (24.5)	8.0 (8.2)	18.6 (19.0)	+3.93[b]	20
S-DAP	24.3 (24.5)	7.9 (8.2)	18.7 (19.0)	−3.95[b]	20
R,S-DAB	30.2 (29.8)	9.0 (8.8)	18.0 (17.4)		
R,R-DAB	29.7 (29.8)	8.9 (8.8)	17.4 (17.4)	+12.08[b]	21
S,S-DAB	29.7 (29.8)	8.9 (8.8)	17.5 (17.4)	−11.53[b]	21
R,S-DAC	39.6 (38.5)	8.8 (8.6)	15.2 (15.0)		
R,R-DAC	39.0 (38.5)	9.1 (8.6)	15.4 (15.0)	−16.15 (+12.12)[c]	19
S,S-DAC	38.9 (38.5)	9.3 (8.6)	15.2 (15.0)	+16.05(−11.92)[c]	19

[a]Calculated values in brackets. $[\alpha]_D$ were measured at 25 °C. [b]Solution 10% in water. [c]Solution 1% in water. The values in brackets refer to (*R,R*-DAC)(H-*S,S*-tartrate)$_2$ and (*S,S*-DAC)(H-*R,R*-tartrate)$_2$.

R,R (*trans* –) and *R,S* (‘*meso*’) forms [16]. We have extended this investigation to other diamines (1,2-diaminopropane and 2,3-diaminobutane) and to a wider variety of bacteria strains, and the results of this investigation are also reported.

Experimental

Chemicals

Commercial reagent grade products i-Pr$_2$en (ICN Pharmaceuticals), 1,2-diaminocyclohexane (DAC) and 1,2-diaminopropane (DAP) (Aldrich) were used without further purification. 2,3-Diaminobutane (DAB) was obtained from dimethylglyoxime (Merck) by reduction with Raney nickel [17].

Separation of Isomers

The ‘*meso*’ form (*R,S*-DAC) and the racemic mixture (*R,R*-DAC + *S,S*-DAC) were isolated from the commercial product, containing all different isomers, using the method of Saito and Kidani [18]. The *meso* form (*R,S*-DAB) and the racemic mixture (*R,R*-DAB + *S,S*-DAB) were separated by taking advantage of the different solubilities of their hydrochlorides in methanol [17].

The separation of (*R,R*-DAC) and (*S,S*-DAC) was accomplished by reacting the racemate with (*S,S*) and (*R,R*) tartaric acid respectively according to the reported procedures [19]. The diastereoisomers formed (*R,R*-DAC)(H-*S,S*-tartrate)$_2$ and (*S,S*-DAC)(H-*R,R*-tartrate)$_2$ were recrystallized several times until they gave an $[\alpha]_D$ value in agreement with the literature data [19].

The racemic mixtures (*R,R*-DAB + *S,S*-DAB) and (*R*-DAP + *S*-DAP) were resolved using the method described for DAC. The final products were converted to hydrochloride salts by standard procedures.

Analytical and spectroscopic data are given in Table I.

Platinum Complexes

The platinum complexes [PtCl$_2$(diamine)] were prepared by decomposition of the ionic species, [PtCl(diamine)(DMSO)] Cl (DMSO = dimethylsulfoxide), obtained by the method of Romeo *et al.* [22]. Details for typical experiments are given below, yields and analyses are given in Table II.

TABLE II. Analytical Data for [PtCl$_2$(diamine)]

Diamine	C	H	N	Yield (%)
R-DAP	10.4 (10.6)	2.9 (3.0)	7.7 (8.2)	57
S-DAP	10.4 (10.6)	2.9 (3.0)	7.7 (8.2)	56
R,S-DAB	13.3 (13.6)	3.4 (3.4)	7.7 (7.9)	41
R,R-DAB	13.2 (13.6)	3.3 (3.4)	7.7 (7.9)	56
S,S-DAB	13.3 (13.6)	3.3 (3.4)	7.6 (7.9)	68
R,S-DAC	18.9 (19.0)	3.7 (3.7)	7.3 (7.4)	67
R,R-DAC	18.8 (19.0)	3.7 (3.7)	7.1 (7.4)	60
S,S-DAC	18.9 (19.0)	3.7 (3.7)	7.1 (7.4)	56
i-Pr$_2$en (*cis*)	23.3 (23.4)	5.1 (4.9)	6.7 (6.8)	30
i-Pr$_2$en (*trans*)	22.8 (23.4)	4.8 (4.9)	6.5 (6.8)	35

[PtCl(diamine)(DMSO)] Cl

1 mmol (0.422 g) of [PtCl$_2$(DMSO)$_2$] was suspended in 40 cm^3 of methanol in a 100 cm^3 round bottomed flask with magnetic stirrer. A solution containing 1 mmol of free amine (or 1 mmol of hydrochloride salt stoichiometrically neutralized with LiOH), dissolved in 20 cm^3 of methanol, was slowly added and the suspension was left under stirring at room temperature until a clear, colorless solution was formed. This was left overnight at 5 °C, filtered, and concentrated to small volume (5 cm^3). The white salt [PtCl(diamine)(DMSO)] Cl was precipitated by adding diethyl ether, separated by filtration of the mother liquor, washed twice with diethyl ether and dried. In all cases the yields were above 90%.

[PtCl$_2$(diamine)]

A solution containing 1 mmol of [PtCl(diamine)-(DMSO)] Cl and 5 mmol of LiCl in 10 cm^3 of water was refluxed at 80 °C for 2 h. The yellow precipitate of the neutral compound separated out. After cooling, the solid was separated, washed with water,

TABLE III. Strains of *Salmonella typhimurium*

Strain	Relevant genotype	Reference
TA92	hisG46, pKM101	23
TA2410	hisG46, uvrB, pKM101	23
TA100	hisG46, uvrB, rfa, pKM101	23
TA98	hisD3052, uvrB, rfa, pKM101	23

and dried. The decomposition of [PtCl(i-Pr_2en)-(DMSO)]Cl gave a 1:1 mixture of the complexes with *cis* and *trans* conformation of the ligand corresponding to different and equal absolute configuration at the coordinated nitrogens. These were separated taking advantage of their different solubilities in dimethylformamide (DMF). The *trans*-form separated out as a pale yellow powder when the crude product was treated with 10 cm^3 of DMF. It was washed twice with DMF, then with diethyl ether and dried. The *cis*-form separated out as yellow–green crystals by addition of diethyl ether to the DMF mother liquor. The crystals were washed with diethyl ether and dried.

Mutagenic Assays

The bacterial strains used are listed in Table III [23]. The mutagenic activity of the platinum compounds was measured according to the protocol of Ames *et al.* [24] without metabolic activation. Briefly, multiple 2.0 cm^3 aliquots of top agar containing 5×10^{-5} mol dm^{-3} histidine were prepared and kept at 45 °C. 0.1 cm^3 of the solution of the compound to be tested and 0.1 cm^3 of fresh overnight bacterial culture were added rapidly to the top agar, mixed, and poured onto the Vogel-Bonner minimal media plate [25]. After 2 days incubation at 37 °C, the number of colonies per plate (histidine revertants) were counted.

The number of revertant colonies per plate, expressed as the mean value of three different experiments are reported in Table IV for [$PtCl_2$(i-Pr_2en)] and in Figs. 3–5 for [$PtCl_2$(DAC)] [$PtCl_2$(DAB)] and [$PtCl_2$(DAP)]. In the case of Figs. 3–5 the number of spontaneous revertants (TA92, 41 ± 7; TA2410, 140 ± 20; TA100, 116 ± 21; and TA98, 18 ± 6) have been subtracted from those of the experimental tests.

Results and Discussion

It is generally understood that the platinum drug *cis*-[$PtCl_2(NH_3)_2$] (cisplatin) in its way to the target loses the two chloride ions which are replaced by other nucleophiles, and keeps the amminic ligands. Therefore although the anionic ligands are likely to play an important role in determining the solubility of the complex and its transport throughout the living organism, on the contrary the aminic ligands are likely to be more responsible for the drug–receptor interaction. Therefore it is of some interest to see how a different conformation or configuration of the aminic ligands can influence the biological activity of the complex.

The biological test carried out throughout this work was mutagenicity towards four different strains of *Salmonella typhimurium* [23]. All strains are unable to synthesize histidine and will die unless, by interaction with the platinum complex, they undergo a mutation which enables them to synthesize histidine again. The four strains differ in the mechanism by which they undergo mutation (base substitution or frame shift), the efficiency of the excision-repair systems, and finally the permeability of the cell membrane.

The absence of any significant difference in the mutagenic activity of enantiomeric species in which the chiral groups (*N*-alkyl substituents) are free to rotate about the C–N bond [13] led us to the conclusion that in order to see any effect of chirality

TABLE IV. Mutagenic Activity of [$PtCl_2$(i-Pr_2 en)]: *cis* (A) and *trans* (B) Isomers

Amount (μg/plate)	Revertants on tester strains[a]							
	TA92 (41 ± 7)[b]		TA2410 (140 ± 20)[b]		TA100 (116 ± 21)[b]		TA98 (18 ± 6)[b]	
	A	B	A	B	A	B	A	B
1600	300	161	1091	482	569	540	i[c]	285
800	200	83	1687	400	1650	460	83	370
400	117	60	1240	298	1168	310	165	300
200	62	45	890	216	800	210	153	161
100	44	44	462	180	480	150	117	101
50	35	34	350	140	277	100	55	50

[a]The reported values are the mean of three different experiments; the spontaneous revertants have not been subtracted, for other conditions see Experimental. [b]Number of spontaneous revertants. [c]i = inhibition of background growth.

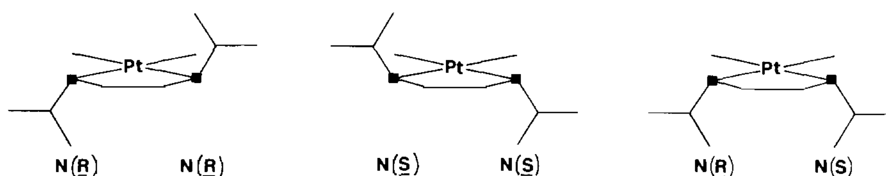

Fig. 1. Schematic drawing of isomeric $[PtCl_2(i\text{-}Pr_2en)]$ complexes differing in the absolute configuration of coordinated nitrogens (bold squares).

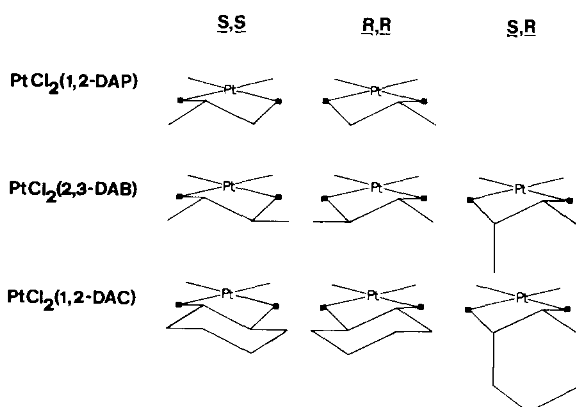

Fig. 2. Schematic drawing of isomeric $[PtCl_2(N\text{-}N)]$ complexes [(N–N) = 1,2-diaminopropane (1,2-DAP), 2,3-diaminobutane (2,3-DAB), and 1,2-diaminocyclohexane (1,2-DAC)] differing in the absolute configuration of the carbon atoms bridging the two nitrogens (bold squares) of the diamine.

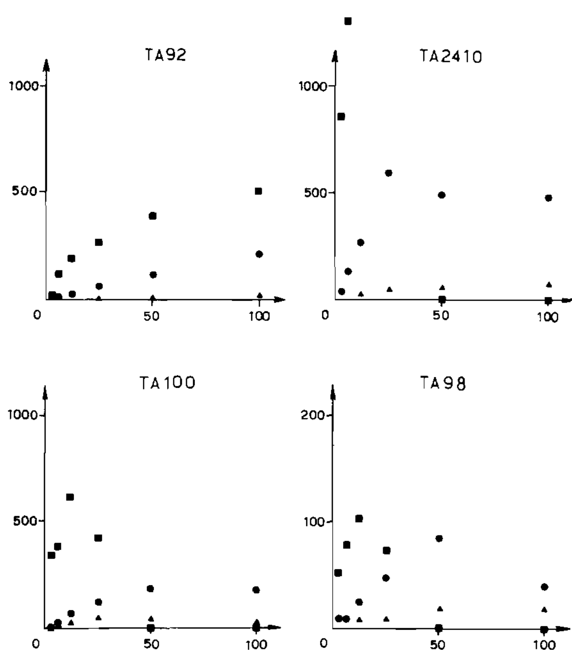

Fig. 3. Mutagenic activity [expressed as number of colonies (ordinate) per µg of complex (abscissa) per plate] of $[PtCl_2$-(1,2-DAC)] [isomers *S,S* (■), *R,R* (●), and *R,S* (▲)] towards different strains (TA92, TA2410, TA100 and TA98) of *Salmonella typhimurium*.

it is necessary to move the chiral center either to the coordinate nitrogens themselves or to a carbon atom of the chain bridging the two nitrogens of a diamine.

With reference to the first of the two possibilities we have prepared the platinum complexes with *N,N'*-di-isopropylethylenediamine ($i\text{-}Pr_2en$). By coordination to platinum two enantiomers and a '*meso*' form are obtained, see Fig. 1. In the two enantiomers the two nitrogens have equal configuration (either *R,R* or *S,S*) and the ligand is said to have a *trans*-conformation. The complex, as a whole, is rather flat with the i-Pr substituents in a bis-equatorial position. In the '*meso*' form the two nitrogens have different configurations (*R,S*), the ligand is said to have a *cis*-conformation and the complex extends outwards in the coordination plane with one equatorial and one axial i-Pr group.

The *cis*- and *trans*-isomers could be separated by fractional crystallization and appeared to be quite stable under the experimental conditions of our mutagenic tests, the results of which are shown in Table IV. The *cis*-isomer appeared to be more active towards his-G46 strains which suffer mutation through a base-substitution mechanism. The *trans*-isomer, on the contrary, appeared to be more active towards his-D3052 strains which suffer mutation by a base-shift mechanism. From these data it could be inferred that the *cis*-isomer gives preferentially crosslinks by chloride substitution while the *trans*-isomers prefer to form intercalation compounds. The overall mutagenic activity was, however, rather small and this fact coupled with a very low water solubility of these species and some uncertainty in the long-range stability of their configuration in solution prevented further investigation of these compounds.

The possibility of having an unwinding chiral center is that it resides on a carbon atom of a chain bridging the two nitrogens of a diamine. Some examples are shown in Fig. 2. When the ligand contains a single chiral center, as in 1,2-diaminopropane (1,2-DAP), two enantiomeric complexes are obtained. When instead the ligand contains two chiral centers three isomeric species are formed, two of these are mirror images and constitute a couple of enantiomers, the third isomer is the '*meso*' form.

When we started this investigation there were already some reports on the dependence of the mutagenic and anticancer activity of the platinum complexes with 1,2-diaminocyclohexane (1,2-DAC) upon the absolute configuration of the ligand [16, 26–28] but it was not clear if this applied to all amines of this kind and if all types of bacteria (in the case of mutagenic tests) or all kinds of tumors (in the case of anticancer activity) would be more affected by the same isomer. Moreover some experimental results were susceptible to different interpretations [29–31] and, as a result of this, biological and clinical investigations have continued without paying much attention to the different chirality of the complexed amines [32–36].

In this paper we have investigated, under strictly analogous conditions, the mutagenic activity towards four different strains of *Salmonella typhimurium* of all different isomers of [$PtCl_2$(1,2-DAC)], [$PtCl_2$-(1,2-DAB)], and [$PtCl_2$(1,2-DAP). The results are plotted in Figs. 3, 4 and 5 for the three complexes, respectively.

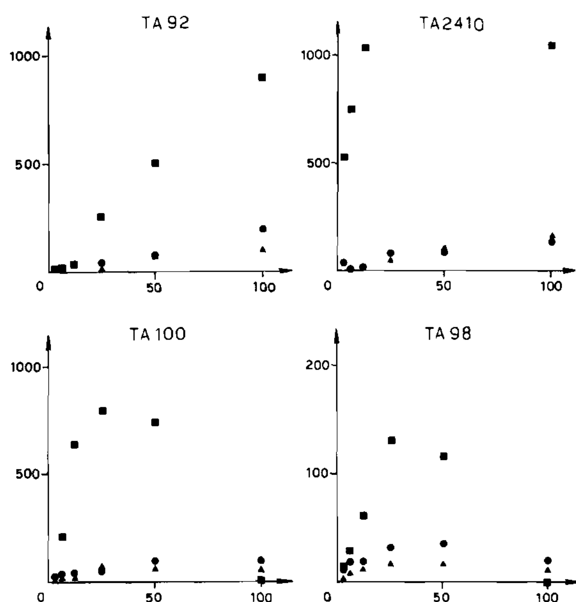

Fig. 4. Mutagenic activity [expressed as number of colonies (ordinate) per μg of complex (abscissa) per plate] of [$PtCl_2$-(1,2-DAB)] [isomers *S,S* (■), *R,R* (●), and *R,S* (▲)] towards different strains (TA92, TA2410, TA100 and TA98) of *Salmonella typhimurium.*

In Fig. 3 we have reported for each strain of *Salmonella* the different mutagenic activity (expressed as number of colonies per μg of complex per plate) of the three isomers of [$PtCl_2$(1,2-DAC)]. The results indicate that the *S,S*-isomer is nearly ten times more active than its *R,R*-enantiomer. The '*meso*' isomer is still less active and can be considered not mutagenic at all. The same trend is observed towards all different strains of bacteria whatever is the efficiency of their repair systems, the permeability of the cell membrane, or the mechanism by which they undergo mutation. It is also to be noted that, contrary to the '*meso*' form, the two enantiomers have similar chemical properties such as solubility in different solvents, partition coefficients, etc. and therefore the different behavior can only be ascribed to a different degree of interaction with asymmetric substrates.

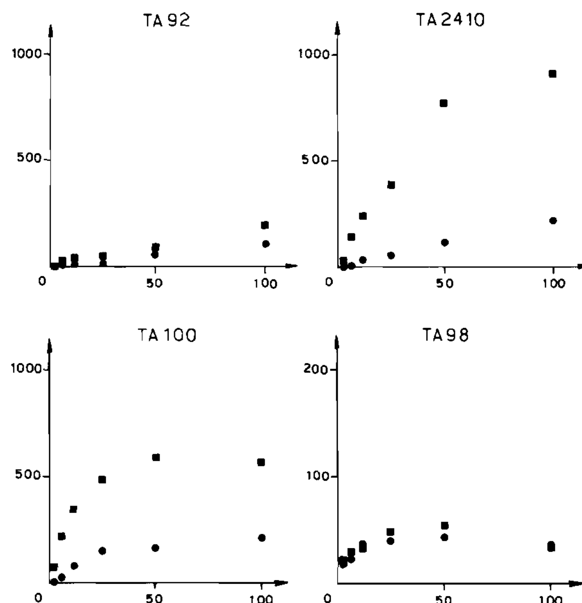

Fig. 5. Mutagenic activity [expressed as number of colonies (ordinate) per μg of complex (abscissa) per plate] of [$PtCl_2$-(1,2-DAP], isomers *S* (■) and *R* (●)] towards different strains (TA92, TA2410, TA100 and TA98) of *Salmonella typhimurium.*

The same investigation, extended to the platinum complexes with 2,3-DAB, has given similar results (Fig. 4). The *S,S*-isomer is by far the most active while the *R,R*-isomer is now a very weak mutagen and resembles the behavior of the '*meso*' form (*R,S*-isomer).

Finally, the behavior of the two enantiomers of [$PtCl_2$(1,2-DAP] is shown in Fig. 5. Once again the *S* absolute configuration results to be the more active although the differences between the two isomers appear to be less pronounced than in the previous cases, particularly with respect to TA92 and TA98 strains.

We can therefore conclude that there is a definite dependence of the mutagenic activity upon the absolute configuration of the amine and in the cases examined the *S*-configuration appears to be by far the more active. Now the question arises of how to relate mutagenic activity and drug–receptor interaction. A mutation generally stems from an interaction between a drug and DNA; moreover, in the case of platinum complexes an intrastrand crosslink

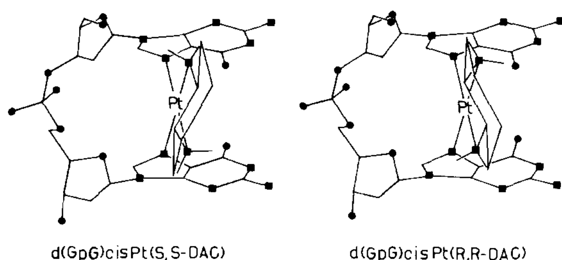

Fig. 6. Schematic drawing of d(GpG)*cis*Pt(*S,S*-DAC) (left) and d(GpG)*cis*Pt(*R,R*-DAC) (right). Nitrogen and oxygen atoms are indicated with squares and circles respectively. Only N–H bonds of coordinated aminic groups are shown.

between two adjacent guanine bases appears to be the preferred interaction [37, 38].

In Fig. 6 we have sketched such an interaction between a dinucleotide and the two enantiomers of 1,2-DAC as is found from molecular models [39]. It can be seen how in each case one hydrogen of an aminic group points towards the oxygen atom of a guanine, but in one case (*R,R*-DAC) the base involved is that of the C3′-guanosine side while in the other case (*S,S*-DAC) that of the C5′-guanosine side. In the past much emphasis has been put on the role of such an interaction in the appearance of antitumor activity [40], however the role of the aminic ligand is not confined to an interaction with adjacent bases, it can also interact with other groups such as phosphate units which in coiled DNA could come across to it [41–44]. A definite answer must wait direct studies of the interactions between enantiomeric complexes of platinum and DNA.

Acknowledgements

The authors are grateful to the Ministero della Pubblica Istruzione (M.P.I.) and Consiglio Nazionale delle Ricerche (C.N.R.), Rome, for financial support (P.F. Oncologia, Grant No. 852183-44).

References

1 B. Rosenberg, *Biochimie, 60,* 859 (1978).
2 M. Hacker, E. Douple and I. Krakoff (eds.), 'Platinum Coordination Complexes in Cancer Chemotherapy', Martinus Nijhoff, Boston, 1984.
3 J. Reedijk and P. H. M. Lohman, *Pharm. Weekbl. Sci. Ed., 7,* 173 (1985).
4 J. F. Holland, H. W. Bruckner, R. C. Wallach, S. B. Gusberg, E. M. Greenspan and J. Goldberg, 'Cisplatin: Current Status and New Developments', Academic Press, New York, 1980.
5 D. J. Beck and R. R. Brubaker, *Mutat. Res., 27,* 181 (1975).
6 P. Lecointe, J. P. Macquet, J. L. Butour and C. Paoletti, *Mutat. Res., 48,* 139 (1977).
7 L. Cocchiarella, I. E. Mattern and C. G. van Cralingen, *Mutat. Res., 74,* 252 (1980).
8 L. A. Zuelling, M. O. Bradley, N. A. Sharkey, T. Andersen and K. W. Kohn, *Mutat. Res., 67,* 271 (1979).
9 A. C. M. Plooy and P. H. M. Lohman, *Toxicology, 17,* 169 (1980).
10 N. P. Johnson, J. D. Hoeschele, R. O. Rahn, J. P. O'Neill and A. W. Hsie, *Cancer Res., 40,* 1463 (1980).
11 J. Brouwer, P. Van de Putte, A. M. J. Fichtinger-Schepman and J. Reedijk, *Proc. Natl. Acad. Sci. U.S.A., 78,* 7010 (1981).
12 M. Coluccia, M. Correale, F. P. Fanizzi, D. Giordano, L. Maresca, M. A. Mariggiò, G. Natile and M. Tamaro, *Toxicol. Environ. Chem., 8,* 1 (1984).
13 M. Coluccia, M. Correale, D. Giordano, M. A. Mariggiò, S. Moscelli, F. P. Fanizzi, G. Natile and L. Maresca, *Inorg. Chim. Acta, 123,* 225 (1986).
14 L. E. Erickson, H. L. Fritz, R. J. May and D A. Wright, *J. Am. Chem. Soc., 91,* 2513 (1969).
15 D. A. Buckingham, L. G. Marzilli and A. M. Sargeson, *J. Am. Chem. Soc., 91,* 5227 (1969).
16 W. R. Leopold, R. P. Batzinger, E. C. Miller, J. A. Miller and R. H. Earhart, *Cancer Res., 41,* 4368 (1981).
17 F. H. Dickey, W. Fickett and H. J. Lucas, *J. Am. Chem. Soc., 74,* 944 (1951).
18 R. Saito and Y. Kidani, *Chem. Lett.,* 123 (1976).
19 P. E. Reimbold and K. H. Pearson, *Talanta, 17,* 391 (1970).
20 L. Tschugajew and W. Sokoloff, *Berichte, 40,* 3461 (1907).
21 E. Strack and H. Schwaneberg, *Berichte, 67,* 1006 (1934).
22 R. Romeo, D. Minniti, S. Lanza and M. L. Tobe, *Inorg. Chim. Acta, 22,* 87 (1977).
23 J. McCann, N. E. Spinhgarn, J. Kobori and B. N. Ames, *Proc. Nat. Acad. Sci. U.S.A., 72,* 979 (1975).
24 B. N. Ames, F. D. Lee and W. E. Durston, *Proc. Nat. Acad. Sci. U.S.A., 70,* 782 (1973).
25 M. J. Vogel and D. M. Bonner, *J. Biol. Chem., 218,* 97 (1956).
26 Y. Kidani, K. Inagaki and S. Tsukagoshi, *J. Clin. Hematol. Oncol., 7,* 197 (1977); *Gann, 67,* 921 (1976).
27 Y. Kidani, M. Noji, S. Tsukagoshi and T. Tashiro, *Gann. 69,* 263 (1978).
28 R. J. Speer, L. M. Hall, D. P. Stewart, H. J. Ridgway, J. M. Hill, Y. Kidani, K. Inagaki, M. Noji and S. Tsukagoshi, *J. Clin. Hematol. Oncol., 8,* 44 (1978).
29 A. Pasini, A. Velcich and A. Mariani, *Chem. Biol. Interact., 42,* 311 (1982).
30 M. Gullotti, G. Pacchioni, A. Pasini and R. Ugo, *Inorg. Chem., 21,* 2006 (1982).
31 M. Gullotti, A. Pasini and R. Ugo, *Inorg. Chim. Acta, 91,* 223 (1984).
32 M. C. Strandberg, E. Bresnick and A. Eastman, *Biochim. Biophys. Acta, 128,* 698 (1982).
33 J. P. Macquet and J. L. Butour, *J. Natl. Cancer Inst., 70,* 899 (1983).
34 J. P. Macquet, S. Cros and J. P. Armand, *Cancer Res., 44,* 3736 (1984).
35 D. G. Craciunescu, A. Doadrio, A. C. Furlani, U. Scarcia and C. Ghirvu, *Eur. J. Med. Chem., 19,* 353 (1984).
36 J. H. Burchenal, G. Irani, K. Kern, L. Lokys and J. Turkevich, *Recent Results Cancer Res., 74,* 146 (1980).
37 A. L. Pinto and S. J. Lippard, *J. Biochem. Biophys. Acta, 780,* 167 (1985).
38 A. M. J. Fichtinger-Schepman, J. L. van der Veer, J. H. J. den Hartog, P. H. M. Lohman and J. Reedijk, *Biochemistry, 24,* 707 (1985).
39 K. Inagaki and Y. Kidani, *Inorg. Chem., 25,* 1325 (1986).
40 H. K. V. Leh and W. Wolf, *J. Pharm. Sci., 65,* 315 (1976).

41 J. Kozelka, G. A. Petsko, G. J. Quigley and S. J. Lippard, *Inorg. Chem.*, *25*, 1075 (1986).
42 J. Kozelka, G. A. Petsko, S. J. Lippard and G. J. Quigley, *J. Am. Chem. Soc.*, *107*, 4079 (1985).
43 S. E. Sherman, D. Gibson, A. H. J. Wang and S. J. Lippard, *Science*, *230*, 412 (1985).
44 M. D. Reily and L. G. Marzilli, *J. Am. Chem. Soc.*, *108*, 6785 (1986).