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Spectroscopic Studies on the Interaction of Dichlorobis(cycloalkylamine)platinum(II) Complexes with DNA

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The interaction of cis- *and* trans-*dichlorobis(cyclohexylamine)-, cis-dichlorobis(cyclopentylamine)-, and cis-dichlorobis(cyclobutylamine)platinum(II) with DNA has been studied by means of circular dichroism and thermal denaturation–renaturation of the nucleid acid. Structural variations in DNA are seen to occur mainly with the cyclohexylamine complexes which are pH and base-sequence dependent. No systematic pattern of structure–activity relationship could be uncovered suggesting that the anti-tumour power of platinum(II) complexes probably requires additional mechanisms besides a direct interaction with DNA.*

Introduction

A number of complexes of platinum(II) have been prepared and their effectiveness in the treatment of certain human tumours has been tested [1–3]. The general conclusion was that the complexes should be uncharged, two amino groups should be present *cis* to one another, and the complexes are most effective when the other two sites are occupied by chloride: Among them, *cis*-dichlorodiamineplatinum(II) has received an extensive display of interest [2] owing to its high and broad activity [2, 5–7]. However, a number of analogues bearing cycloalkylamino groups have been synthesized by Connors *et al.* [1–3] which show higher activity against the PC 6 tumour than the original complex. The cyclopentylamine complex shows activity also against Walker and L1210 systems [3] and was effective on cell cultures [4]. The cyclohexylamino complex, on the other hand, although tremendously active against the PC 6 tumour, has no effect on different systems [3]. As already emphasized [3], this could argue against the implicit assumption that all the anti-tumour platinum complexes have the same mechanism of action. As a matter of fact, a direct interaction of Pt(II) complexes with DNA, observed by *in vitro* studies [8–10], has been claimed to explain the inhibition of DNA synthesis found *in vivo* [11, 12]. We have previously studied by circular dichroism (CD) the effect of *cis*- and *trans*-dichlorodiamineplatinum(II) complexation on the structure of DNAs from various sources [13]. In this paper we report studies by CD on the *cis*- and *trans*- cyclohexylamino, *cis*-cyclopentylamino-, and *cis*-cyclobutylamino complexes interacting with calf thymus and/or salmon sperm DNA. In addition, the effect on thermal hyper- and hypochromicity of DNA was also investigated (Abbreviations: CD, circular dichroism; A-T, adenine-thymine; G-C, guanine-cytosine).

Experimental

Materials

The cycloalkylamino platinum compounds were synthesized according to the literature [3]. The compounds gave the expected analytical values and showed IR bands in the Pt–Cl stretching region at 332 cm^{-1} (*trans*) and 320 and 328, 300 (shoulders) cm^{-1} (*cis*) for cyclohexylamino derivatives, 312 and 300 (shoulder) cm^{-1} for cyclopentyl, 310 and 300 (shoulder) cm^{-1} for cyclobutyl compounds. Samples of DNA were obtained from Sigma Chemical Co., St. Louis, Missouri, and were used without further purification. Protein content, according to the analytical values given by the manufacturer, was no higher than 2%. All other chemicals were of analytical grade.

Methods

Solutions were made by mixing DNA and platinum derivatives at concentrations approximating the published solubilities [3] (except for the cyclobutylamino derivative for which a lower concentration, *i.e.* 8.1 μg/ml, was used) under stirring for 20–24 h at room temperature. CD spectra were recorded on a Cary model 61 dichrograph. Cylindrical cells were used with 10 and 1 cm optical path. The usual instrumental precautions were taken to avoid artifacts. Samples of nucleic acids were examined at concentrations ranging from 6 to 32 mg/l. The data are expressed in terms of $[\theta]$ the mean residue molecular ellipticity, in units of degrees cm^2 $dmol^{-1}$. Temperature–absorbance profiles were automatically recorded

with a Beckman Tm Analyzer attached to a Beckman DB spectrophotometer, with a cell-block electrically heated, the temperature being programmed to change at a constant rate of about 1°/min. The temperature was read by a thermometer immersed in the sample. The concentration of the solutions of nucleic acid was determined spectrophotometrically at the wavelength of the maximum of absorption, around 260 nm.

Results

CD Studies

Shown in Fig. 1 are the CD spectra of calf thymus DNA at different input ratios of Pt(II) to nucleotide residues (expressed as Pt/P), in unbuffered aqueous solution. Surprisingly, the observed trends are opposite to those found for *cis* and *trans*-$[Pt(NH_3)_2Cl_2]$ interacting with DNA [13]. As a matter of fact, we observed for the *cis*-compound a very small decrease of the longer wavelength positive band, even at highest Pt/P ratios, a much stronger decrease of the negative band and an increase of the positive band at shorter wavelengths. The *trans* isomer seems to be less effective in inducing these spectral variations which are in both cases similar to those found for DNA at the earliest steps of the thermal denaturation [14].

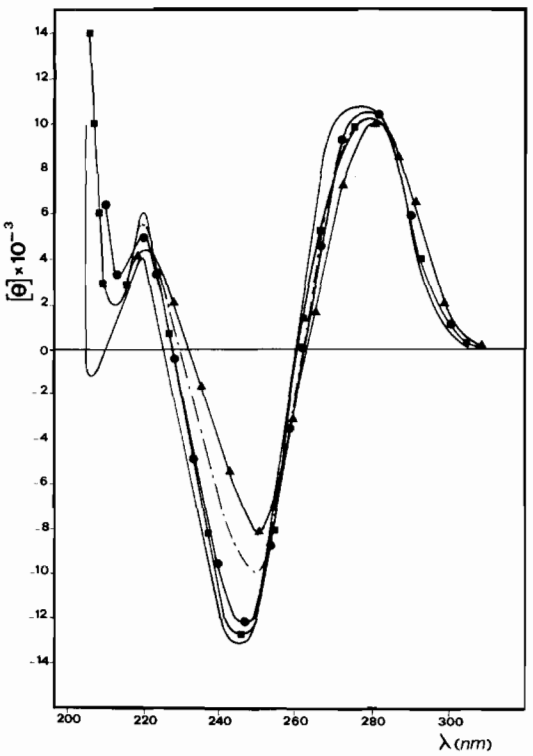


Figure 1. CD spectra of calf thymus DNA in water in the presence of *cis*- and *trans*-dichlorobis(cyclohexylamine)-platinum(II). *trans*: (●–●) Pt/P = 0.25; (■–■) Pt/P = 0.5; *cis*: (– –) Pt/P = 0.25; (▲–▲) Pt/P = 0.5; (——) Pt/P = 0.

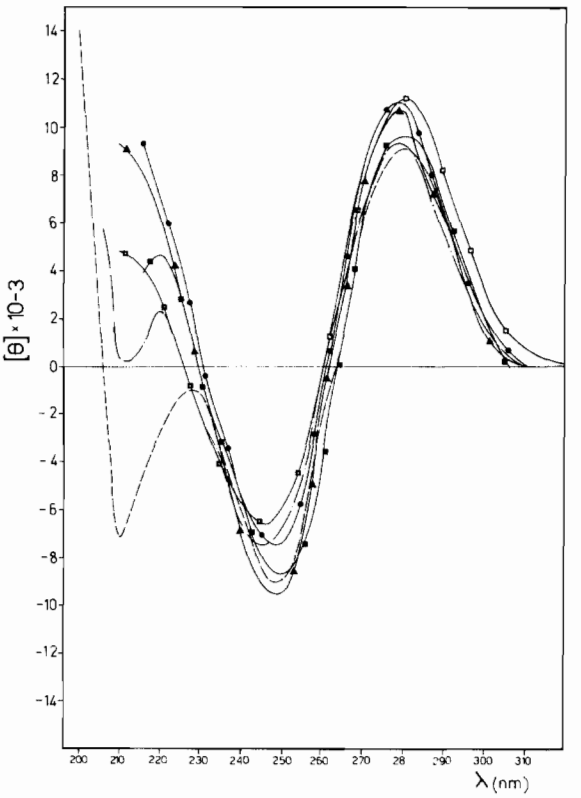


Figure 2. CD spectra of salmon sperm DNA in water in the presence of *cis*- and *trans*-dichlorobis(cyclohexylamine)-platinum(II). *trans*: (●–●) Pt/P = 0.2; (■–■) Pt/P = 0.4; *cis*: (▲–▲) Pt/P = 0.2; (– –) Pt/P = 0.4; (– – –) Pt/P = 1.2; (□–□) Pt/P = 0.

Somewhat different effects are observed with salmon sperm DNA (Fig. 2). Actually, the negative band around 245 nm is increased by the binding of the Pt(II) derivatives, whereas opposite trends are shown by *cis* and *trans* compounds, as a function of Pt/P ratio, at shorter wavelengths. It is worth noting that, in the presence of an input ratio Pt/P = 1.2 of *cis*-compound, the resulting spectrum is resembling that of G-C rich DNAs, such as *micrococcus lysode ikticus* DNA [15]. Quite different behaviour is observed when complexation is carried out in 0.05*M* Tris-Cl buffer, pH = 7.2 (Figs. 3–4). The spectra of calf thymus DNA maintain the original conservative character, although a significant reduction of the positive and negative bands at longer wavelengths is evident. Although not reported, quite similar variations are shown by salmon sperm DNA.

A similar effect (Fig. 5) is shown by the *cis*-cyclobutylamino derivative when complexed with salmon sperm DNA. The effect however is much less pronounced than in the case of the above mentioned cyclohexylamino compound, indicating only slight structural perturbations in DNA. In the case of the cyclopentylamino derivative only a very small increase, if any, of the positive band is observed while the negative one remains unchanged.

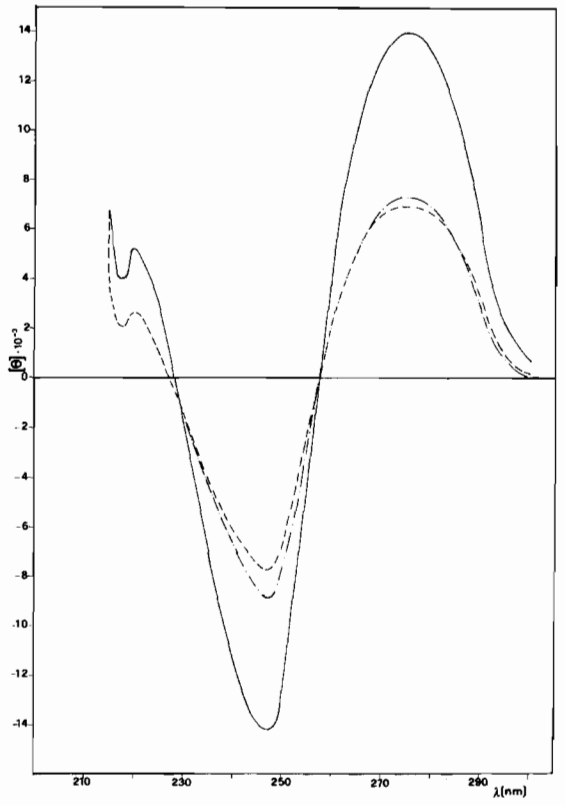


Figure 3. CD spectra of calf thymus DNA in 0.05*M* Tris-Cl buffer (pH 7.2) in the presence of *cis*-dichlorobis(cyclohexylamine)platinum(II). (——) Pt/P = 0; (– – –) Pt/P = 0.25; (–·–) Pt/P = 0.5.

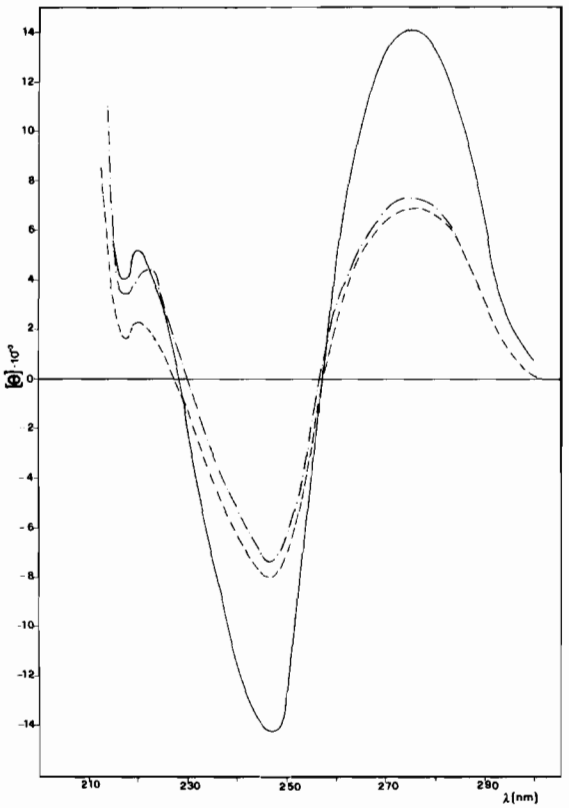


Figure 4. CD spectra of calf thymus DNA in 0.05*M* Tris-Cl buffer (pH 7.2) in the presence of *trans*-dichlorobis(cyclohexylamine)platinum(II). Symbols as in Fig. 3.

Absorbance–Temperature Studies

Shown in Fig. 6a are the thermal chromicity changes of calf thymus DNA incubated with *cis*- and *trans*-cyclohexylamino compounds at the selected Pt/P ratio of 0.25. Upon heating, some denaturation has occured in Pt(II)-treated DNA as evidentiated by the lowering of the temperature at which the thermal transition starts and by a slight drop in Tm, the temperature at which the hyperchromicity was half its maximum value. Tm values were obtained, as shown in Fig. 6b, by plotting the expression

$$\frac{A^{260}(t_2) - A^{260}(t_1)}{A^{260}(90°) - A^{260}(30°)}$$

as a function of $(t_1 + t_2)/2$ were t_1 and t_2 are two sequential temperatures differing by 1 °C.

Platinum- induced renaturation was also studied as one parameter of interstrand cross-linking [16]. However, it is seen in Figure 6a that upon cooling no renaturation is observed at the Pt/P input ratio used. This compares well with the results of Harder [16] who found for *cis*-dichlorodiamineplatinum(II) that the extent of renaturation dropped to zero at a Pt/P ratio of about 0.15–0.20. Essentially similar results are obtained with cyclopentyl- and cyclobutylamino derivatives (Fig. 7), interacting with salmon sperm DNA. By comparing Figs. 6b and 7b it is seen that all Pt(II) derivatives have the same effect by slightly depressing the melting point of DNA (about 2 °C). However, the cyclohexylamino compounds show an additional effect in that a broadening of DNA melting zone is observed. This will be further considered in the following discussion.

Discussion

As a first result of our spectroscopic investigations it can be said that cyclohexylamino derivatives affect more deeply the DNA conformation than the other terms of the family. Actually, both CD spectra and absorbance–temperature profiles in buffered solutions give evidence of similar qualitative variations for all derivatives, the quantitative differences being minimal between cyclopentyl- and cyclobutylamino derivatives and very pronounced between these two compounds and the cyclohexylamino derivatives. As a matter of fact, the CD spectra are compatible with some extent of opening and/or rotation of stacked

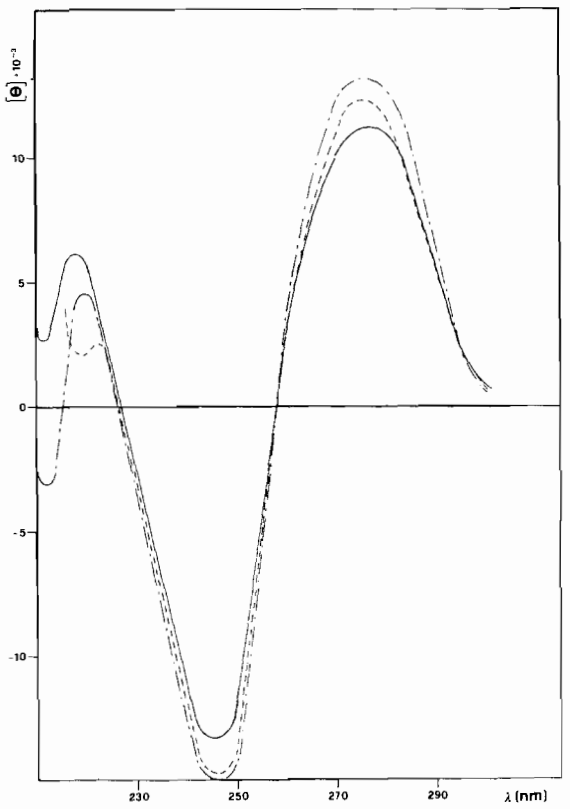
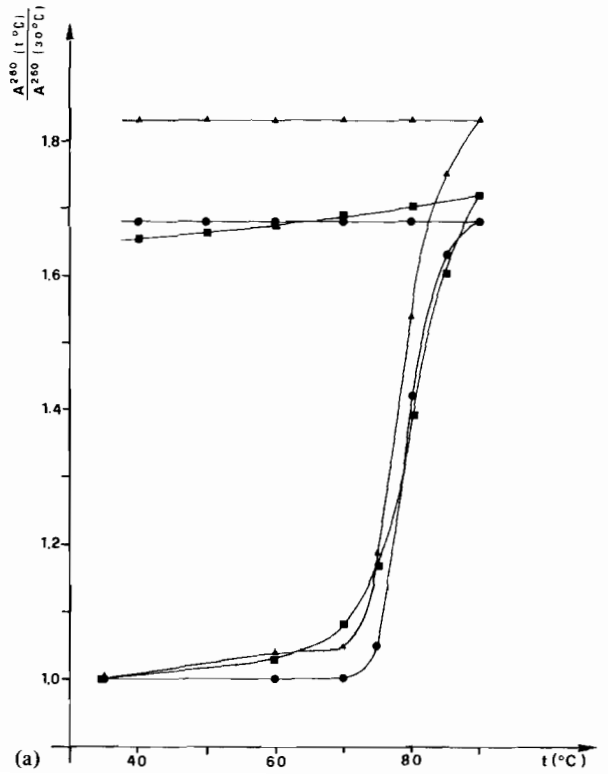


Figure 5. CD spectra of salmon sperm DNA in 0.05*M* Tris-Cl buffer (pH 7.2) in the presence of *cis*-dichlorobis(cyclopentylamine)platinum(II), Pt/P = 0.25 (– –) and of *cis*-dichlorobis(cyclobutylamine)platinum(II); Pt/P = 0.25 (——); (– – –) Pt/P = 0.



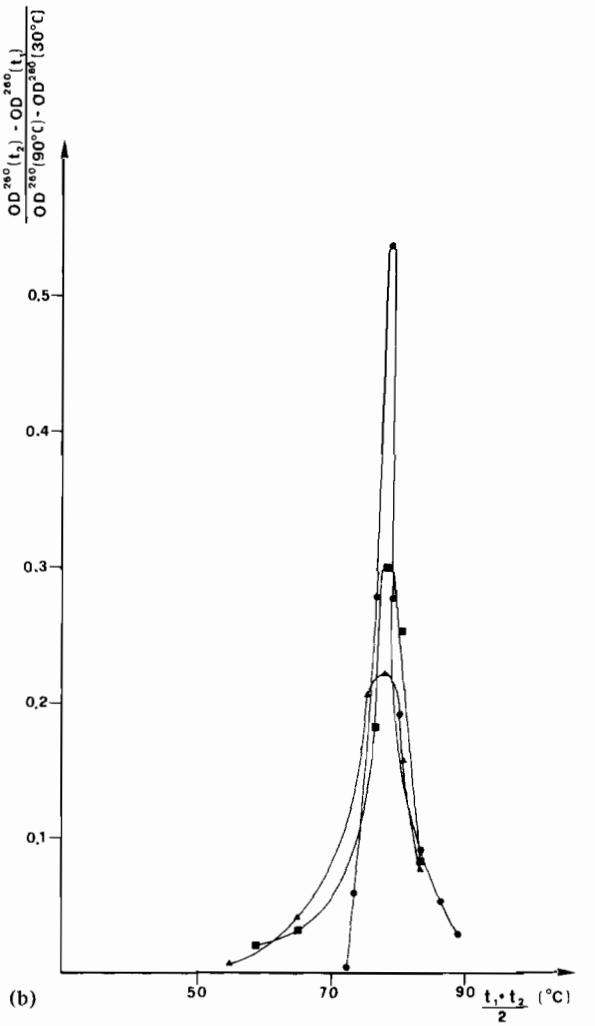
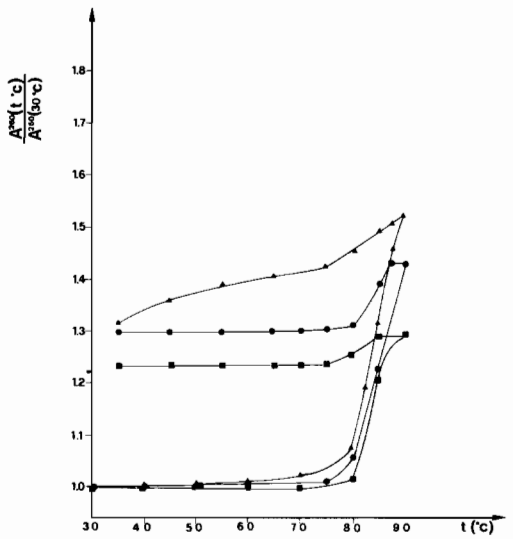
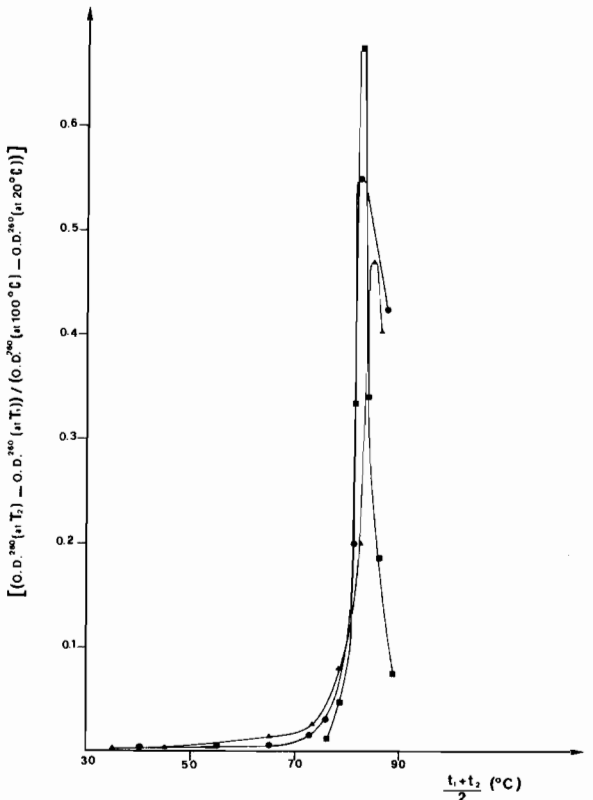


Figure 6. a) Thermal denaturation–renaturation profiles of calf thymus DNA in the absence (●–●) and in the presence of *cis*-dichlorobis(cyclohexylamine)platinum(II), Pt/P = 0.25 (▲–▲), and of the *trans* isomer, Pt/P = 0.25 (■–■). Solvent 0.05*M* Tris-Cl buffer, pH 7.2. b) Thermal denaturation profiles plotted in differential form. See text for details.

bases in DNA [17–18]. However, the double helical conformation should be largely maintained as indicated by the retainment of the conservative nature of the CD spectra and by the small effect on the midpoint of the thermal transitions. On the other hand, on binding of cyclohexylamino compounds, the width of the DNA melting zone is broadened. As a A-T rich segment is considerably broader than a G-C rich section owing to the difference in base pair stability [19], it is tempting to suggest a preferential binding of these Pt(II) complexes to G-C rich regions. It is to be recalled that the interaction of *cis*-[Pt-$(NH_3)_2Cl_2$] with DNA has been found to be dependent on heterocyclic base composition, guanine being



(a)



(b)

Figure 7. a) Thermal denaturation–renaturation profiles of salmon sperm DNA in the absence (▲–▲) and in the presence of *cis*-dichlorobis(cyclopentylamine)platinum(II), Pt/P = 0.25 (■–■) and of *cis*-dichlorobis(cyclobutylamine)platinum(II), Pt/P = 0.25 (●–●). Solvent 0.05*M* Tris-Cl buffer, pH 7.2. b) Thermal denaturation profiles plotted in differential form.

the preferred site of attact at least at low complex–DNA ratios [20–21].

The effects promoted by the cyclohexylamine compounds also show a pH-dependence in that the CD spectra in unbuffered solution (acidic pH) differ from those obtained when the solution is buffered at the neutrality. In addition, in the former case the alterations in the DNA structure are not simply dependent on base composition (which is approximately the same for calf thymus and salmon sperm DNA) but probably depend on base sequence. The exact nature of these DNA structural perturbations are of difficult detection. However, one can see from Figures 1 and 2 that the spectral trends are toward a positive non conservative CD spectrum in the case of calf thymus DNA and toward a negative non conservative spectrum in the case of salmon sperm DNA. Theoretical calculations of Moore and Wagner [22] have convincingly shown that such differences arise as a consequence of variations in opposite directions of the distance of the paired bases along the positive or negative dyad axis away from the original position.

In any event, it is to be noted that the DNA structural changes induced by all cycloalkylamino derivatives of platinum(II), herein reported, are different from those induced by *cis*-$[Pt(NH_3)_2Cl_2]$ and interpreted as localized changes [23] probably involving a B to C conformational transition [13].

As far as the nature of the interaction is concerned, renaturation experiments clearly demonstrate that no significant interstrand crosslinking is present, at least at the Pt/P ratio used. Furthermore, some specificity toward G-C pairs is probably shown by the cyclohexylamine derivatives. However, the differences noted between the *cis*- and *trans* isomers are subtle and rather small if one takes into consideration the fact that the former compound is antitumoral and the other one has no activity. Coming to the antitumour activity of the platinum complexes, it is hard to uncover a systematic pattern of structure–activity relationship from the effects observed in the present study. As a matter of fact, although toxicity increases as the ring size of the ligand decreases, the potency follows the order cyclopentylamine>cyclobutylamine>cyclohexylamine [3]. According to our study the effectiveness of interaction with DNA seems to be cyclohexylamine>cyclopentylamine, cyclobutylamine. Therefore, although available evidence suggest DNA as the main and direct target of *cis*-$[Pt(NH_3)_2Cl_2]$, alternative or additional explanations should be considered. An example would be the inhibition of the cyclic 3′,5′-nucleotide fosfodiesterase with consequent elevation of cyclic AMP levels and blocking of the mitosis, as shown by Tisdale and Phillips for *cis*-$[Pt(NH_3)_2Cl_2]$ itself as well as for some alkylating agents [24].

References

1 T. A. Connors, M. Jones, W. C. J. Ross, P. D. Braddock, A. R. Khokhar and M. L. Tobe, *Chem.-Biol. Interact., 5,* 415 (1972).
2 M. J. Cleare, *Coord. Chem. Rev., 12,* 349 (1974).
3 P. D. Braddock, T. A. Connors, M. Jones, A. R. Khokhar, D. H. Melzack and M. L. Tobe, *Chem.-Biol. Interact., 11,* 145 (1975).
4 I. Szumiel and A. H. W. Nias, *Chem.-Biol. Interact., 14,* 217 (1976).
5 E. Wiltshaw and B. Carr, *Recent Results in Cancer Research, 48,* 178 (1974).
6 J. M. Hill, E. Loeb, A. S. MacLellan, N. O. Hill, A. Khan and J. Kogler, *Recent Results in Cancer Research, 48,* 145 (1974).
7 H. J. Wallace, Jr. and D. R. Higby, *Recent Results in Cancer Research, 48,* 167 (1974).
8 A. J. Thomson in "Recent Results in Cancer Research: Platinum Coordination Complexes in Cancer Chemotherapy", T. A. Connors and J. J. Roberts, Eds., p. 38, Springer-Verlag, Heidelberg (1974).
9 A. B. Robins in "Recent Results in Cancer Research: Platinum Coordination Complexes in Cancer Chemotherapy", T. A. Connors and J. J. Roberts, Eds., p. 63, Springer-Verlag, Heidelberg (1974).
10 I. A. G. Roos, *Chem.-Biol. Interact., 16,* 39 (1977).
11 H. C. Harder and B. Rosenberg, *Int. J. Cancer, 6,* 207 (1970).
12 L. L. Münchausen, *Proc. Nat. Acad. Sci. USA, 71,* 4519 (1974).
13 A. M. Tamburro, L. Celotti, D. Furlan and V. Guantieri, *Chem. Biol. Interact., 16,* 1 (1977).
14 A. F. Usatyi and L. S. Shlyakhtenko, *Biopolymers, 12,* 45 (1973).
15 G. Luck, C. Zimmer, G. Snatzke and G. Söngerath, *Eur. J. Biochem., 17,* 514 (1970).
16 H. Harder, *Chem.-Biol. Interact., 10,* 27 (1975).
17 W. C. Johnson, Jr. and I. Tinoco, Jr., *Biopolymers, 7,* 247 (1969).
18 W. C. Johnson, Jr., M. S. Itzkowitz and I. Tinoco, Jr., *Biopolymers, 11,* 225 (1972).
19 V. Bloomfield, *Biopolymers, 6,* 1368 (1968).
20 J. P. Macquet and T. Théophanides, *Biopolymers, 14,* 781 (1975).
21 M. M. Millard, J. P. Macquet and T. Théophanides, *Biochim. Biophys. Acta, 402,* 166 (1975).
22 D. S. Moore and T. E. Wagner, *Biopolymers, 12,* 201 (1973).
23 L. L. Münchausen and R. O. Rahn, *Biochim. Biophys. Acta, 414,* 242 (1975).
24 T. M. Tisdale and B. J. Phillips, *Biochem. Pharmacol., 24,* 1271 (1975).