Pergamon

S0277-5387(97)00225-8

Polyhedron Vol. 16, No. 24, pp. 4297–4303, 1997

0277-5387/97 $17.00+0.00

Platinum(II) substituted pyrrolidine complexes. Crystal structure of dichloride 1,2 diethyl 3 aminopyrrolidine Pt^{II}. Reactions with DNA and dinucleotides

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(Received 4 April 1997; accepted 22 May 1997)

Abstract—The effect of five derivatives of Pt^{II}, *cis* dichloro-(1,2-dimethyl-3-aminopyrrolidine)platinum(II), *cis* dichloro-(1-methyl, 2-ethyl-3-aminopyrrolidine)platinum(II), *cis* dichloro-(1-ethyl, 2-methyl-3-aminopyrrolidine)platinum(II), *cis* dichloro-(1,2-diethyl-3-aminopyrrolidine)platinum(II), and *cis* dichloro-(1 propyl, 2 methyl-3 aminopyrrolidine)platinum(II) on platination of DNA was studied by CD and melting temperature determination. Reactions with the nucleotides d(ApG) and d(ApA) were also followed by ^1H NMR and CD, indicating binding *via* N(7) and formation mainly of bifunctional, in the case of d(ApG), or monofunctional adducts, in the case of d(ApA). The crystal structure of *cis* dichloro-(1 ethyl, 2 methyl-3 aminopyrrolidine)platinum(II) shows the analogues *cisplatin* structure of these active antitumour complexes. This compound is racemic. © 1997 Elsevier Science Ltd

Keywords: Substituted pyrrolidine Pt complexes; dichloride 1,2 diethyl-3-aminopyrrolidine Pt^{II}; analogues *cisplatin*; antitumour activity Pt complexes; crystal structure analogues *cisplatin*.

INTRODUCTION

The search for soluble and less toxic analogues of *cisplatin* is one of the main goals in the synthesis of new platinum(II) complexes [1–3]. The establishment of a relationship between the antitumour activity and structure, and the possibility of binding to the cellular target, the DNA, are also the main objectives of chemical research in this field [4,5].

The substituted aminopyrrolidines *cis* dichloro-(1,2 dimethyl-3-aminopyrrolidine)platinum(II), (MMpyrr), *cis* dichloro-(1 methyl, 2 ethyl-3 aminopyrrolidine)platinum(II). (MEpyrr), *cis* dichloro-(1 ethyl, 2 methyl-3 aminopyrrolidine)platinum(II), (EMpyrr), *cis* dichloro-(1,2 diethyl-3 aminopyrrolidine)platinum(II), (EEpyrr) and *cis* dichloro-(1 propyl, 2 methyl-3 aminopyrrolidine)platinum(II), (PMpyrr), (Fig. 1) were first synthesized [6] and tested for antitumour activity by Sampedro *et al.* [7], but it was not possible to determine their molecular structure. We have achieved adequate crystals for X-ray diffraction of the *cis* dichloro-(1 ethyl, 2 methyl-3 aminopyrrolidine)platinum(II) complex, and we have also studied the behaviour of the five analogous derivatives with DNA and the reactions of *cis* dichloro-(1 ethyl, 2 methyl-3 aminopyrrolidine) platinum(II) with d(ApG) and d(ApA) we can now

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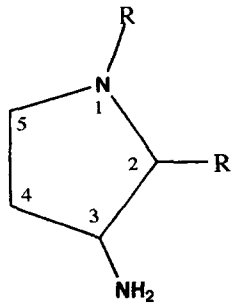

Fig. 1. 1,2-disubstituted 3 aminopyrrolidine.

confirm that the activity is due to the binding to DNA *via* N(7) sites of the nucleotides, as found for the case of *cisplatin*.

MATERIALS AND METHODS

Chemicals

The five substituted aminopyrrolidines were synthesized as described [8] and their respective platinum compounds were synthesized by reaction with $K_2[PtCl_4]$ 1:1 in aqueous medium [6]. The starting material $K_2[PtCl_4]$ was supplied by Johnson Matthey and all the other reagents used were from Aldrich and were used without further purification. The deoxydinucleotides sodium salts d(ApG) and d(ApA) were obtained from Sigma Chemicals and used without further purification.

Elemental analysis and spectroscopic characterization

All the compounds were characterized by elemental analysis, melting point, IR and ^{1}H NMR spectroscopy. The compounds are soluble in water. The analyses of carbon, hydrogen, nitrogen were carried out using a Carlo Erba model 1106 microanalyzer at the Serveis Científico-Tècnics from University of Barcelona. Chlorine was determined by conventional chemical methods. The infrared spectra were recorded in solid state (KBr pellets) on an FT-IR Nicolet 5DZ spectrometer.

The pH values were adjusted by addition of DCl or NaOD solutions. Values of pH (hereafter denoted pH*) have not been corrected for deuterium isotope effects. All the reactions, 2–3 millimolar concentrations of d(ApG) and d(ApA) were carried out in the NMR tube using D_2O as solvent, pH* 7, at room temperature were followed by ^{1}H NMR spectroscopy as a function of time. 1 equivalent of Pt complex was used.

The ^{1}H NMR and ^{13}C NMR spectra were recorded with a Brucker 250 MHz spectrometer. D_2O was used as a solvent and TMS as internal reference.

Circular dichroism

The CD spectra were recorded at room temperature on a Jasco J720 spectropolarimeter with a 450 W xenon lamp, from solutions in the same conditions (using H_2O as dissolvent, and pH 7) and concentrations as those used in the determination by NMR at final time considered for the formation of the adducts.

Melting temperature determination

UV spectra were recorded at room temperature on a double-beam Shimadzu UV-2101-PC spectrometer. The measurement of the absorbance change in the process of DNA denaturalization (Tm) was carried out on the same Shimadzu UV-2101-PC spectrometer with thermostatic cells by connection to an automatic temperature regulator Neslab RTE-110 bath. The heating rate was of 1.5°C/min The absorbance was measured at $\lambda = 260$ nm.

X-ray diffraction

A prismatic crystal ($0.1 \times 0.1 \times 0.2$ mm) was selected and mounted on an Enraf-Nonius CAD4 four-circle diffractometer. Unit cell parameters were determined from automatic centering of 25 reflections ($12 \leqslant \theta \leqslant 21°$) and refined by least-squares method. Intensities were collected with graphite monochromatized Mo K_α radiation, using $w/2\theta$ scan technique. 3584 reflections were measured in the range $1.94 \leqslant \theta \leqslant 30.00$, 3447 of which were non-equivalent by symmetry (R_{int} (on I) = 0.022). 2882 reflections were assumed as observed applying the condition $I > 2\sigma(I)$. Three reflections were measured every two hours as orientation and intensity control, significant intensity decay was not observed. Lorentz-polarization, but not absorption corrections were applied.

The structure has a pseudo-C orthorhombic cell, with cell parameters $a = 12.957$; $b = 17.988$ and $c = 10.1542$ Å, but the Laue group was 112/m, which does not justify the orthorhombic cell. The structure was solved by Patterson synthesis, using SHELXS computer program [9] and refined by full-matrix least-squares method with SHELX93 computer program [10]. The function minimized was $\Sigma w[(F_o)^2-(F_c)^2]^2$, where $w = [\sigma^2(I)+(0.1153\ P)^2+2.8128\ P]^{-1}$, and $P = [(F_o)^2+2(F_c)^2]/3$, f, f' and f'' were taken from International Tables of X-Ray Crystallography [11]. 16 H atoms were located from a difference synthesis, all were refined with an overall isotropic temperature factor, using a riding model for the 14H linked to C atoms. Max. shift/e.s.d. = 1.8, Mean shift/e.s.d. = 0.14. Final atomic coordinates as well as anisotropic thermal parameters are included as supplementary material.

RESULTS AND DISCUSSION

Crystallographic study

A perspective of the molecular structure of *cis* dichloro-(1 ethyl, 2 methyl-3 aminopyrrolidine) platinum(II), Pt-EMpyrr, is shown in Fig. 2. Crystal and refinement data are summarized in Table 1. Bond lengths and angles are presented in Table 2.

Platinum atom shows a distorted square plane environment. The four atoms Pt, Cl(1), N(1) and N(2) are in a plane (largest deviation from the mean plant 0.006(1) in Pt), but the Cl(2) atom is deviated −0.112(3) Å from this plane. This is explained by the steric hindrance between the different molecules.

The five-membered cyclometallated ring has an envelope form with the C(1) atom out of plane defined by the remaining four atoms, and the pyrrolidine ring also has an envelope from with C(1) out of plane. C(5) methyl group occupies the syn-endo position which is due to steric hindrance produced by the platinum and N(2) atoms on the pyrrolidine ring. These two atoms occupy an equatorial position with respect to pyrrolidine ring in the opposite side that the C(5) methyl. The ethyl group occupy an equatorial site (angle with the mean plane 70.3(6)°). The shortest intermolecular bond lengths are N(2)···Cl(1) (3.401(10) Å), N(2)···Cl(2) (3.504(10) Å) and the weak hydrogen bond N(2)···O(1) (3.029(13) Å). In the molecule there are three asymmetric centers, the C(1) and C(2) already present in the metal free ligand and the new chiral center N(1) created by its coordination to the metal atom. In the $P2_1/C$ crystal structure we observe two correlated enantiomeric forms that correspond to R,S,R for C(1), C(2) and N(1) atoms and S,R,S for C′(1), C′(2) and N′(1) atoms.

Table 1. Crystallographic data for Pt-EMpyrr

Empirical formula	$C_7H_{18}Cl_2N_2OPt$
Formula weight	412.22
Crystal system	Monoclinic
Space group	$P2_1/a$
a (Å)	11.084(6)
b (Å)	10.1542(15)
c (Å)	11.084(6)
α (°)	90
β (°)	108.47(5)
γ (°)	90
V (Å^3)	1183.2(9)
Z	4
F(000)	776
Density (g/cm^3)	2.314
Absorption coefficient (mm^{-1})	12.278
Temperature (K)	293(2)
Data/restraints/parameters	3446/0/126
Goodness-of-fit on F^2	1.108
Final $R[I > 2\sigma(I)]$	$R1 = 0.0549$, $wR2 = 0.153$
R indices (all data)	$R1 = 0.0684$, $wR2 = 0.164$
Extinction coefficient	0.0067(8)
Largest diff. peak and hole	0.497, −0.443 e Å^{-3}

It was not possible to isolate crystals suitable for X-ray study of other compounds of this family, but they may have analogue structures. The antitumour behaviour and the results from studies with other techniques are similar [7].

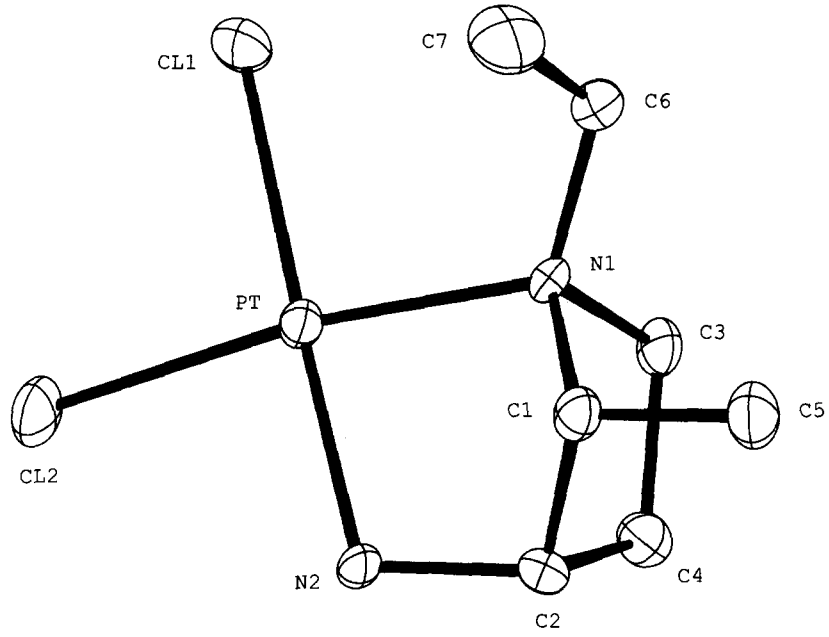

Fig. 2. Molecular structure of *cis* dichloro-(1-ethyl, 2-methyl-3-aminopyrrolidine)Pt^{II}, Pt-EMpyrr.

Table 2. Bond lengths (Å) and angles (°)

Pt—N(2)	2.043(7)	N(2)—Pt—N(1)	81.9(3)
Pt—N(1)	2.082(7)	N(2)—Pt—C(2)	90.7(3)
Pt—Cl(2)	2.299(3)	N(1)—Pt—Cl(2)	172.1(2)
Pt—Cl(1)	2.323(3)	N(2)—Pt—Cl(1)	178.6(2)
N(1)—C(6)	1.473(11)	N(1)—Pt—Cl(1)	96.7(2)
N(1)—C(3)	1.512(11)	Cl(2)—Pt—Cl(1)	90.62(11)
N(1)—C(1)	1.528(11)	C(6)—N(1)—C(3)	111.8(7)
N(2)—C(2)	1.486(12)	C(6)—N(1)—C(1)	114.0(7)
C(1)—C(2)	1.510(12)	C(3)—N(1)—C(1)	102.1(6)
C(1)—C(5)	1.520(14)	C(6)—N(1)—Pt	118.5(6)
C(2)—C(4)	1.539(14)	C(3)—N(1)—Pt	104.9(5)
C(3)—C(4)	1.519(14)	C(1)—N(1)—Pt	103.7(5)
C(6)—C(7)	1.49(2)	C(2)—N(2)—Pt	107.6(5)
		C(2)—C(1)—C(5)	112.5(8)
		C(2)—C(1)—N(1)	98.7(7)
		C(5)—C(1)—N(1)	115.7(7)
		N(2)—C(2)—C(1)	105.6(7)
		N(2)—C(2)—C(4)	108.6(8)
		C(1)—C(2)—C(4)	103.1(7)
		N(1)—C(3)—C(4)	104.3(7)
		C(3)—C(4)—C(2)	105.2(7)
		N(1)—C(6)—C(7)	114.0(9)

Melting temperature determination

In Table 3 the ΔT_m values at 0 and 72 h and $ri = 0.10$ or $ri = 0.50$ for *cisplatin* and pyrrolidine derivative Pt^{II} complexes are summarized. (ri = molar ratio complex/DNA).

All the complexes caused slight changes at low values of ri and short incubation times, but induced decreases in the T_m at $ri = 0.50$ and periods of 72 h. The maximum change was produced by the compound Pt-MEpyrr. These changes indicate destabilization of the double helix, probably due to an interaction with the bases similar to that caused by *cisplatin*. The structure of Pt-pyrrolidine derivatives are suitable for an interaction in *cis* with two positions of DNA. No decrease in absorbance at high temperatures, in comparison with DNA control, was observed at time zero and there was only a slight decrease at 72 h, which indicated that DNA aggregation processes are scarcely detectable for these compounds and that, if they occur, it would be at longer incubation times. The maximum modification occurs for Pt-EMpyrr and the minimum for Pt-EEpyrr.

The changes observed in UV spectra of DNA incubated with the compounds are less marked, especially at $t = 48$ h. For $t = 24$ h a slight change was observed and the ratio $\Delta A_{270}/\Delta A_{290}$ increased. This, together with the bathochromic effect observed, may indicate DNA interaction especially for $ri = 0.50$.

Circular dichroism study

The θ_{max} and θ_{min} for λ_{max} and λ_{min} values from the circular dichroism spectra of Calf Thymus DNA and Calf Thymus DNA incubated with the pyrrolidines compounds for several times and several ri values are summarized in Tables 4, 5 and 6.

The changes caused by the Pt-EMpyrr complex in the secondary structure of DNA are slight, but in the same direction as those caused by *cisplatin*. The

Table 3. DNA melting temperature[a] variation for complexes at 0 and 72 h and at different ri

Complex	ri	ΔT_m (°C) 0 h	72 h
DNA : *cisplatin*	0.10		−0.8
	0.50	0.0	−3.3
DNA-Pt-MMpyrr	0.10		0.4
	0.50	−0.5	−1.0
DNA-Pt-MEpyrr	0.50	0.2	−1.8
DNA-Pt-EMpyrr	0.50	−0.8	−1.1
DNA-Pt-EEpyrr	0.50	−0.7	−0.5

[a] T_m in reference to DNA control.

Table 4. CD of DNA : Pt-EMpyrr for $ri = 0.50$ and different times

Complex	θ_{max}[a]	λ_{max}[b]	θ_{min}	λ_{min}
DNA	9.92	277.0	−11.85	245.4
16 h	10.63	274.6	−10.80	247.0
24 h	10.75	275.8	−10.78	245.8
48 h	11.32	277.6	−11.70	246.6

[a] °C cm^2 dmol^{-1} 10^3.

[b] nm.

Table 5. CD of DNA : Pt-EEpyrr for $ri = 0.50$ and different times

Complex	θ_{max}[a]	λ_{max}[b]	θ_{min}	λ_{min}
DNA	8.22	276.8	−9.89	245.8
16 h	8.75	277.8	−9.31	245.6
24 h	8.86	278.6	−9.51	246.0
48 h	9.33	276.4	−9.20	246.4

[a] °C cm^2 dmol^{-1} 10^3.
[b] nm.

Table 6. CD of DNA : Pt-MMpyrr, DNA : Pt-MEpyrr, and DNA : Pt-PMpyrr compounds for $ri = 0.20$ and 24 h

Complex	θ_{max}[a]	λ_{max}[b]	θ_{min}	λ_{min}
DNA	5.71	269.0	−5.36	240.4
MMpyrr	6.56	271.0	−5.12	241.0
MEpyrr	5.60	272.2	−4.09	242.2
PMpyrr	6.01	269.8	−4.79	241.0

[a] °C cm^2 dmol^{-1} 10^3.
[b] nm.

elipticity of the positive band decreases for $ri = 0.01$ and increases for the other values of *ri*. The elipticity of the negative band decreases for all the values of *ri*. A downshift in the λ_{max} and upshift in the λ_{min} are also produced. These modifications are probably due to opening of the helix and to changes in the base stacking on the formation of DNA intrastrain adducts [12]. The spectrum registered at 24 h is similar to that at 16 h. However, the most noticeable modification occurs at 48 h.

In the case of the Pt-EMpyrr derivative a similar variation in the secondary structure of DNA can be observed. The main changes occur at 48 h and for $ri = 0.5$. The type of structure for these complexes, Pt-EMpyrr and Pt-EEpyrr, similar to that of *cisplatin*, with two Cl in *cis* and a bidentate ligand by means of two amine groups, is appropriate for a covalent interaction between two close intrastrain sites from the DNA double helix [13].

Spectra for DNA incubated with Pt-MMpyrr, Pt-MEpyrr and Pt-PMpyrr showed that the compound Pt-MEpyrr presents a different behaviour to the other two complexes. PT-MEpyrr decreases the elipticity of the positive and negative bands below that corresponding to DNA at 16 and 24 h, while the other compounds increase the elipticity. So, Pt-MEpyrr at 16 and 24 h of incubation causes uncoiling of the helix, as does *transplatin*. In contrast, the other two compounds stabilize the B form of DNA. The three compounds produce a bathochromic effect in the positive band, but the greatest shift is produced by Pt-MEpyrr. After 40 h of incubation the behaviour observed for this compound changes and the elipticity of the positive band increases, while the elipticity of the negative band decreases as does *cisplatin*. It is possible that the first adduct formed changes to another type of adduct at longer incubation times. The three compounds modify considerably the CD spectrum of DNA. The changes in the elipticity and in the wavelengths, after 40 h, correspond to a stabilization of the DNA B, probably due to a better compaction of the double helix.

Reactions with the dinucleotides d(ApG) and d(ApA)

The reaction between Pt-EMpyrr and dinucleotides d(ApG) and d(ApA) was carried out in D_2O medium in a NMR tube, and studied by ^{1}H NMR spectroscopy. The pH of the solutions was monitored to 6.8–7.2. The ^{1}H NMR shifts, zone of aromatic protons, for a 0.439 μM solution of d(ApG) in D_2O and for the reaction of d(ApG) with 1 equivalent of Pt-EMpyrr over time are summarized in Table 7.

The signals at 8.14, 8.09 and 7.93 ppm in the spectrum of the free dinucleotide d(ApG) can be assigned

Table 7. ^{1}H NMR shifts (in ppm) for d(ApG) and d(ApG) with Pt-EMpyrr

		H8Ap	H2Ap	H8pG
d(ApG)		8.14	8.09	7.93
d(ApG) + Pt-EMpyrr				
$t = 0$		8.12	8.07	7.91
$t = 9$ h	8.49[a]	8.18, 8.15	8.10	7.92
$t = 24$ h	8.50	8.19[a], 8.16[b]	8.12	8.06[a], 7.93[b]
$t = 72$ h	8.52	8.21	8.14	8.08
$t = 6$ d	8.54	8.23	8.16[c]	8.11
$t = 8$ d	8.54	8.24	8.17[c]	8.12
$t = 28$ d	8.55	8.25	8.19[c]	8.13

[a] New signal increasing in intensity.
[b] Signal that disappears progressively.
[c] Signal decreasing slightly in intensity.

to the H8 Ap, H2 Ap and H8 pG respectively [14]. Addition of Pt-EMpyrr produced considerable changes in H8 pG. It decreased in intensity over time, and simultaneously, a new signal at 8.06 ppm appeared and increased in intensity over time. Modifications were also observed for the Ap protons: the signal assigned to H2 shifted downfield and decreased in intensity. After 24 h the signal assigned to H8 Ap disappeared and a new signal at 8.55 ppm was observed.

The CD spectrum of the solution registered at final time, Fig. 3(a), shows interesting changes. The inversion of the sign and shift of the band at 270 nm, elipticity $\theta = -0.51 \times 10^3$°C cm^2 dmol^{-1} for the free dinucleotide to 288 nm, $\theta = +0.75 \times 10^3$°C cm^2 dmol^{-1} for the dinucleotide after addition of Pt-EMpyrr, indicate a serious modification of the conformation when the platinum complex binds. The band at 248.8 nm, $\theta = +1.09 \times 10^3$°C cm^2 dmol^{-1} observed in the spectrum of free d(ApG) also changed to 268 nm, $\theta = -0.95 \times 10^3$°C cm^2 dmol^{-1} in the presence of the complex. We can conclude that the platinum compound binds to the dinucleotide mainly by N7 positions of adenine and guanine bases, giving a bifunctional adduct Pt-EMpyrr-d(ApG)N7(1)N7(2). The structure of the adduct has a head–head arrangement and the bases adopt an anti–anti configuration [15]. This hypothesis is in good agreement with the ^{1}H NMR results. The presence of other minor species in equilibrium, such as the monofunctional adduct Pt-EMpyrr-d(ApG)N7(2) cannot be ruled out and would be justified on the basis of the changes observed in ^{1}H NMR.

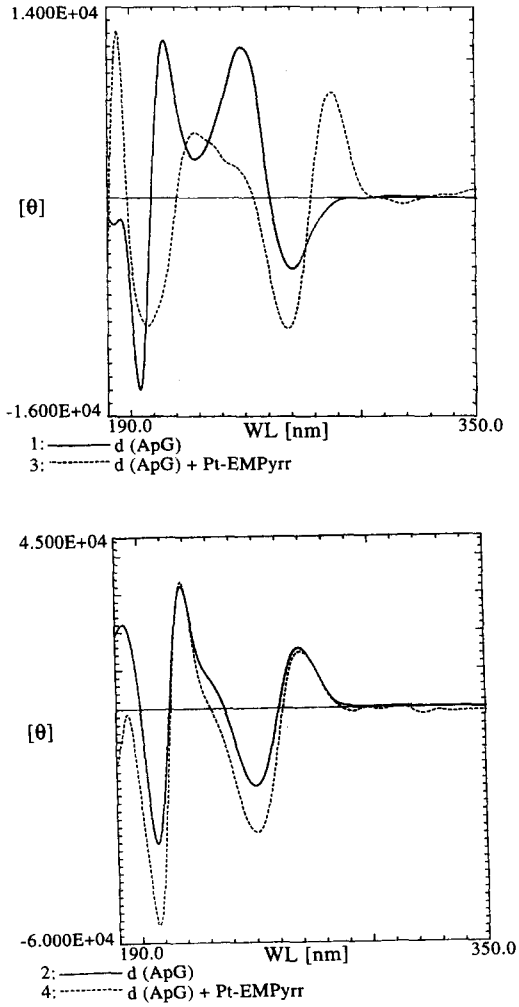

Fig. 3. (a) CD spectra of d(ApG) and d(ApG) with Pt-EMpyrr; (b) CD spectra of d(ApA) and d(ApA) with Pt-EMpyrr.

The ^{1}H NMR shifts, zone of aromatic protons, for a 0.101 μM solution of d(Apa) in D_2O and for the reaction of d(ApA) with 1 equivalent of Pt-EMpyrr over time are summarized in Table 8. The spectrum of the dinucleotide d(ApA) presents two signals at 8.13 and 7.83 ppm that can be assigned to the H8Ap and H8pA respectively [16,17]. The H2Ap and H2pA appear at 7.87 and 7.73 ppm respectively.

When Pt-EMpyrr was added to the dinucleotide a few modifications in the aromatic proton zone of the spectrum were observed. The H8pA, at 7.83 ppm, decreased in intensity after 14 days and simultaneously a new signal appeared at 8.05 ppm and increased in intensity over time. No changes were observed for the other signal corresponding to H2 and H8Ap.

The CD spectrum of the solution registered at final time, Fig. 3(b), shows only changes in the elipticity. In contrast with the d(ApG), no inversion of the sign of the band was observed. The band at 269 nm, elipticity $\theta = +1.54 \times 10^3$°C cm^2 dmol^{-1} for the dinucleotide free remains positive at 270 nm, without appreciable change in elipticity, $\theta = +1.45 \times 10^3$°C cm^2 dmol^{-1} for the dinucleotide after addition of Pt-EMpyrr. The minimum at 250.4 nm, $\theta = -1.98 \times 10^3$°C cm^2 dmol^{-1} observed in the spectrum of free d(ApA) appeared at 251 nm, but with a value for $\theta = -3.16 \times 10^3$°C cm^2 dmol^{-1} in the presence of the complex. We can conclude that the platinum compound binds to the dinucleotide forming only a monofunctional adduct Pt-EMpyrr-d(ApA)N7(2) *via* N7 position of adenine from pA. The structure of the adduct has the same arrangement as that of the free dinucleotide. This hypothesis is in good agreement with the ^{1}H NMR results.

These result suggest that the structure of the Pt-EMpyrr complex is suitable to bind two intrastrand adjacent bases in DNA structure. However, N7 from guanine are preferentially sites for platination. The binding *via* N7 from adenine is also produced after longer time, preferential in pA sites. The chelate can be formed only in the case of d(ApG) *via* N7 from the Ap site, although a small amount of chelate *via* N3 from the Ap has also been detected. The d(ApA) does not form chelates, in spite of the long time of reaction.

Table 8. ^{1}H NMR shifts (in ppm) for d(ApA) and d(ApA) with Pt-EMpyrr

	H8Ap	H2Ap	H8pA	H2pA
d(ApA)	8.13	7.87	7.83	7.73
d(ApA) + Pt-EMpyrr				
$t = 0$	8.12	7.88	7.83	7.72
$t = 7$ h	8.14[a]	7.88	7.87	7.73
$t = 72$ h	8.14	7.88	7.85	7.74
$t = 14$ d	8.13	7.87	7.83[c]	7.72
$t = 16$ d	8.13	7.88	8.05[a], 7.84[b]	7.73
$t = 24$ d	8.13	7.87	8.03[a], 7.82[b]	7.71
$t = 60$ d	8.06	7.79	7.96[a], 7.74[b]	7.62
$t = 150$ d	8.08	7.82	7.95[a], 7.76[b]	7.64

[a] New signal increasing in intensity;
[b] Signal that disappears progressively.

Acknowledgements—We are grateful to DGICYT (PB91-0806) and to the European Community HCM, (ERBCHRXCT 920016) for financial support, and to Johnson Matthey for supplying K_2PtCl_4.

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