Synthesis, Structure, and Antitumour Testing of Platinum(II) and Palladium(II) Complexes of 1,6-Diaminotetrahydropyrrolo[2,3-*b*]pyrrole-2,5(1*H*,4*H*)-dione ☆

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The syntheses of the platinum(II) and palladium(II) complexes of 1,6-diaminotetrahydropyrrolo[2,3-*b*]pyrrole-2,5(1*H*,4*H*)-dione (**3**) are described. These compounds contain a six-membered chelate ring with four nitrogen atoms. An X-ray diffraction study of the palladium(II) complex [Pd(**3**)Cl_2] shows a distorted "sofa" conformation for this chelate ring. Both complexes exhibited in the ^{1}H-NMR spectrum an AB*gem* system for the amino protons. In vitro ICL_{50} and ICT_{50} and in vivo antitumour activities have been determined for these products. The corresponding dicarboxylato complexes have been obtained by the reaction of the platinum(II) complex [Pt(**3**)Cl_2] with silver sulfate followed by the addition of barium hydroxide and oxalic, malonic, hydroxymalonic, and 1,1-cyclobutanedicarboxylic acid.

In 1965, Rosenberg et al. published their observations on the induction of filamentous growth in bacteria cells by platinum complexes[1]. These results led to the testing of complexes against animal tumour systems (solid Sarcoma 180 and the leukemia L1210), and *cis*-[Pt(NH_3)$_2Cl_2$], *cis*-diamminedichloroplatinum(II) or cisplatin (**1**), emerged as a potential antitumour agent[2]. The clinical trials initiated in 1971 showed a reasonable spectrum of activity against testicular, ovarian, head, and neck cancers, and also bladder and lung cancers[3]. However, the efficacy of cisplatin is reduced by the numerous toxicities which have been observed during its use. These side effects led to an intensive work in developing analogs of cisplatin to reduce the dose-limiting effects and to improve the spectrum of activity.

This work has involved a detailed study of structure-activity relationships among a large number of platinum complexes[4]. These relationships have shown that the primary requisite for antitumour activity is the presence of an inert amino group as the non-leaving ligand in complex of the general structure *cis*-[Pt(amine)$_2X_2$]. The range of amines tested includes normal and branched alkylamines such as isopropylamine, diaminoalkanes (ethylenediamine, propanediamine), mono- and disubstituted cycloalkylamines (cyclohexylamine, 1,2-diaminocyclohexane), and aromatic amines (*o*-phenyleneamine, 2-aminopyridine). Bidentate ligands form five- or six-membered chelate rings which usually contain two or three nitrogen atoms. The introduction of four nitrogen atoms has only been achieved, to the best of our knowledge, by using dipyrazolylalkanes (**2**) as bidentate ligands[5].

In connection with this, and as a part of our research in the field of masked 3-formylglutaric compounds[6], we have obtained 1,6-diaminotetrahydropyrrolo[2,3-*b*]pyrrole-2,5(1*H*,4*H*)-dione (**3**)[7]. This compound bears two amino groups on the same side of the molecule and fulfills the prerequisities to obtain *cis*-[M(amine)$_2X_2$] complexes with a six-membered chelate ring containing four nitrogen atoms. An X-ray diffraction study[8] of **3** reveals that the skeleton of the molecule can be described by the shape of a distorted tiled roof and that the distance between the amino groups is 3.226 Å. The present paper covers the results obtained during our complexation studies.

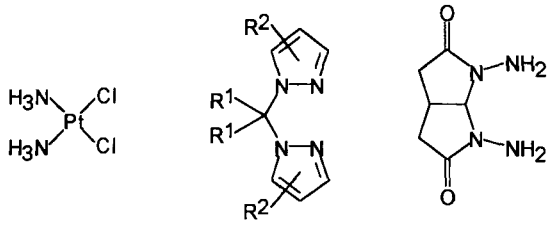
Results and Discussion

Synthesis of Platinum(II) and Palladium(II) Complexes of 1,6-Diaminotetrahydropyrrolo[2,3-*b*]pyrrole-2,5(1*H*,4*H*)-dione (3)

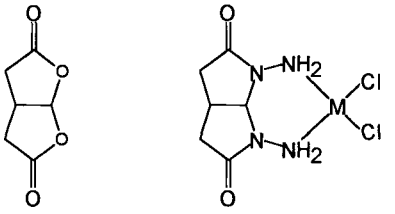
Compound **3** is obtained[7] in 63% yield by slow addition of 2,8-dioxabicyclo[3.3.0]octane-3,7-dione[9] (**4**) to boiling 80% hydrazine hydrate. In order to obtain the platinum(II) and palladium(II) complexes we have treated and aqueous solution of **3** with a K_2PtCl_4 or K_2PdCl_4 solution, respectively, in an inert atmosphere and with the exclusion of light.

Thus, dichloro{1,6-diaminotetrahydropyrrolo[2,3-*b*]pyrrole-2,5(1*H*,4*H*)-dione}platinum(II) [Pt(**3**)Cl_2] is obtained in 70% yield after 8 h at 50°C, while dichloro{1,6-diaminotetrahydropyrrolo[2,3-*b*]pyrrole-2,5(1*H*,4*H*)-dione}palladium(II) [Pd(**3**)Cl_2] is instantaneously formed at room temperature in 82% yield. Although both complexes can be recrystallized from 0.1 M HCl, such treatment causes great

losses of product. However, treatment of **3** with K_2PtCl_4 in 1 M HCl at room temperature for one week affords crystalline [Pt(**3**)Cl_2] in 82% yield.



1 **2** **3**



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M=Pt: [Pt(**3**)Cl_2]

M=Pd: [Pd(**3**)Cl_2]

The spectral data of [Pt(**3**)Cl_2], [Pd(**3**)Cl_2], and the starting diamine **3** are compiled in Table 1. The IR spectra of both complexes show that the C=O stretching band is shifted to higher wavenumbers with respect to **3** while the frequency of the N–H stretching bands decreases. These displacements are ascribed to a change in the electron density distribution of the CO–N–NH_2 subunit caused by the formation of the coordination bond between the amino groups and the metal atom. Finally, the presence of two metal–Cl stretching bands confirms the *cis* geometry of the complexes.

Table 1. IR (KBr), ^{1}H- and ^{13}C-NMR ([D_6]DMSO) data of **3**, [Pt(**3**)Cl_2], and [Pd(**3**)Cl_2]

	3	[Pt(**3**)Cl_2]	[Pd(**3**)Cl_2]
IR: ν [cm^{-1}]			
N-H	3300, 3200	3190, 3090	3210, 3080
C=O	1685	1715	1710
M-Cl	----	345, 335	345, 325
M-N	----	610	580
^{1}H NMR: δ			
NH_2	4.68 (br. s, 4H)	8.91, 8.99 (AB*gem*, 4H)	7.66, 7.35 (AB*gem*, 4H)
6a-H	4.95 (d, 1H)	5.34 (d, 1H)	5.17 (d, 1H)
3a-H	2.94 (m, 1H)	3.10 (m, 1H)	3.10 (m, 1H)
3-H_α, 4-H_α	2.59 (dd, 2H)	2.70 (dd, 2H)	2.75 (dd, 2H)
3-H_β, 4-H_β	2.18 (dd, 2H)	2.30 (dd, 2H)	2.28 (dd, 2H)
$J_{H\text{-}N\text{-}H}$ [Hz]	----	9.5	8.7
$J_{3a,6a}$ [Hz]	7.7	7.4	7.3
$J_{\alpha,\beta}$ [Hz]	17.2	17.6	17.7
$J_{\alpha,3a}$ [Hz]	10.0	10.3	10.2
$J_{\beta,3a}$ [Hz]	4.6	4.1	4.0
^{13}C-NMR: δ			
C-2, C-5	170.7	170.9	169.4
C-3, C-4	35.5	35.3	34.5
C-3a	24.1	24.6	23.8
C-6a	79.3	76.9	75.3

The ^{1}H-NMR spectra of [Pt(**3**)Cl_2] and [Pd(**3**)Cl_2] show all signals of the carbon-bound protons shifted to lower field with respect to **3**. However, most interesting in the ^{1}H-NMR spectrum of [Pd(**3**)Cl_2] (Figure 1) is the presence of the amino group hydrogen atoms as an AB*gem* system of $J_{AB} \approx 9$ Hz which are very slowly replaced by D_2O. This phenomenon, which has previously not been observed, is due to the high rigidity of the chelate ring which causes both amino protons to be chemically and magnetically nonequivalent. The coordination of the amino group is also the reason for the very slow exchange with deuterium because of the unavailability of the amino nitrogen lone pair. Such a difficult exchange has been previously reported with dichloro(ethylenediamine)platinum(II) complexes[10].

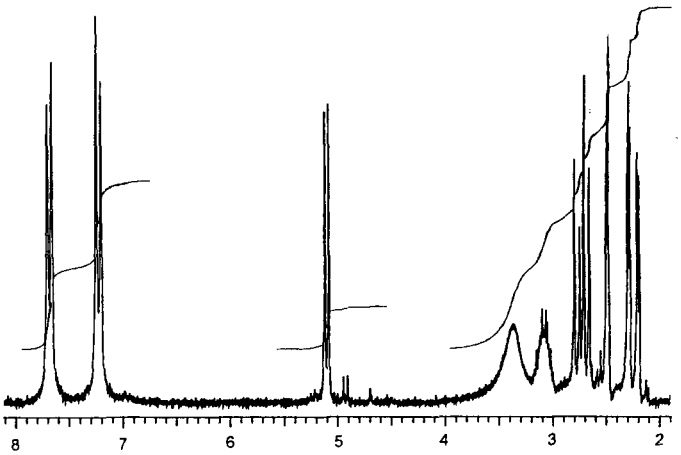


Figure 1. ^{1}H-NMR spectrum (200 MHz, δ scale, [D_6]DMSO) of [Pd(**3**)Cl_2]

In contrast, the ^{1}H-NMR spectrum of [Pt(**3**)Cl_2] shows the signal of the amino group hydrogen atoms either as an AB*gem* system or as a broad signal at $\delta \approx 8.30$ depending on the recording conditions (temperature, concentration, presence of H_2O in [D_6]DMSO); in some spectra both kinds of signals are even present. This fact suggests that, in contrast to the palladium complex, the exchange rate of the amino protons of [Pt(**3**)Cl_2] highly depends on the concentration of labile hydrogens present in solution. Nevertheless, this exchange rate seems to be high enough to inhibit a coupling of the amino hydrogen atoms with the magnetically active ^{195}Pt similar to that observed in dichloro (*N*,*N*′-dialkylethylenediamine)platinum(II) complexes[10a]. Such coupling has not been observed although the spectrum has been recorded at 60, 80, 200, and 500 MHz.

An X-ray diffraction study of a yellow monocrystal of [Pd(**3**)Cl_2] obtained by recrystallization from 1 M HCl confirmed the proposed structure (Figure 2). The principal bond distances and angles of [Pd(**3**)Cl_2] and of the ligand **3** are listed in Tables 2 and 3. This analysis reveals that, in comparison with ligand **3**, the skeleton of the carrier ligand can be described by the shape of a much more symmetrically tiled roof, from which the ridge is the C3–C6 bond. Complexation causes the dihedral angle H–C3–C6–H to change from −18.2(0.2)° in **3** to 0.2(0.6)° in [Pd(**3**)Cl_2] allowing the

eclipsed conformation necessary for the roof to be symmetrical. Such conformation has not been achieved by **3** due to the steric strain of H–C3 and H–C6.

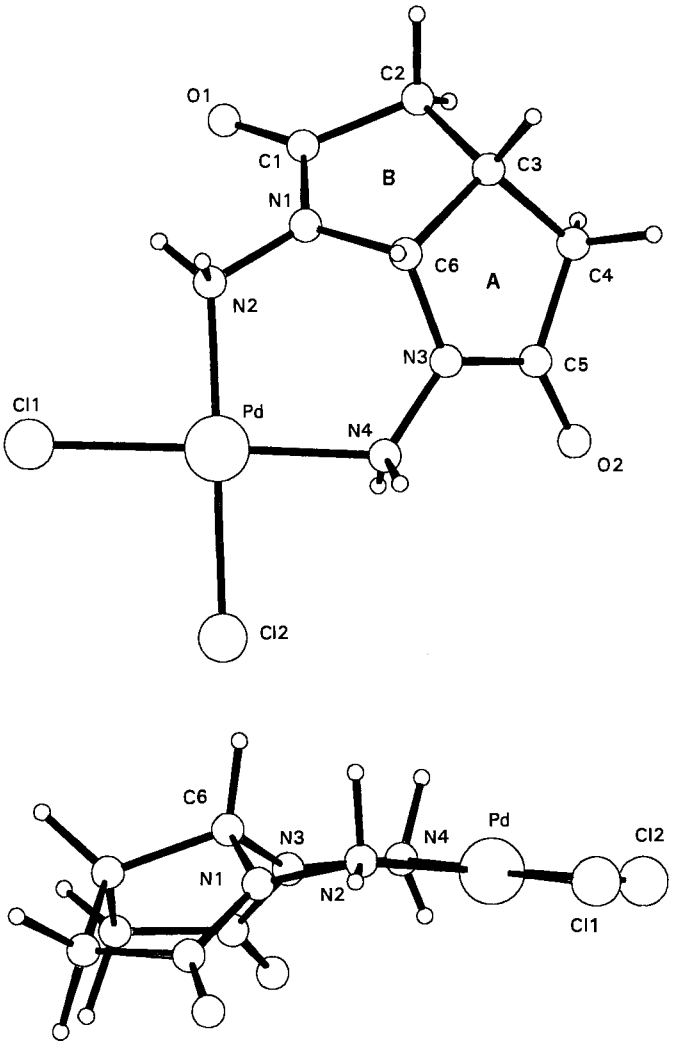


Figure 2. Molecular structure of [Pd(**3**)Cl_2], showing the "sofa" conformation of the chelate ring

The first slope of the roof is defined by the ring C6–N3–C5–C4–C3 (ring A) and the second one by the ring C3–C2–C1–N1–C6 (ring B). Both rings deviate significantly from planarity, and the dihedral angle formed by the mean planes of rings A and B is 116.6(0.2)°. Complexation causes small changes in the bond distances and angles of both rings but the most significant difference is found in the case of the C=O bonds which are clearly shortened. This fact correlates with the aforementioned shift of the C=O stretching band to a higher frequency in comparison with **3**.

The formation of the chelation ring also causes the distances N3–N1 and N4–N2 [2.386(3) and 3.010(5) Å, respectively] to be shortened in comparison with those in **3** [2.423(5) and 3.226(8) Å]; it also closes the angle formed by lines N3–N4 and N1–N2 [26.6(0.3)° for [Pd(**3**)Cl_2] in front of 34.4(0.2)° for **3**].

Finally, the six-membered chelate ring shows standard N–Pd and Pd–Cl bond distances and approximately right angles for N4–Pd–N2 and Cl1–Pd–Cl2. The whole ring adopts a distorted "sofa" conformation (asymmetry parameter[11] $\Delta C_s^{Pd} = 12.3°$).

Table 2. Selected bond lengths [Å] for [Pd(**3**)Cl_2] in comparison with **3**

	[Pd(**3**)Cl_2]	**3**
N3-N4	1.402 (6)	1.412 (5)
N1-N2	1.423 (6)	1.396 (6)
N3-C5	1.357 (5)	1.335 (5)
C5-O2	1.195 (5)	1.232 (5)
C5-C4	1.499 (7)	1.498 (6)
C4-C3	1.547 (6)	1.525 (6)
C3-C6	1.577 (5)	1.525 (6)
C3-C2	1.550 (5)	1.532 (6)
C2-C1	1.527 (5)	1.496 (6)
C1-O1	1.204 (3)	1.234 (5)
C1-N1	1.342 (5)	1.341 (6)
N1-C6	1.417 (3)	1.445 (5)
N3-C6	1.449 (4)	1.450 (5)
N2-Pd	2.018 (4)	----
N4-Pd	2.073 (3)	----
Pd-Cl1	2.230 (1)	----
Pd-Cl2	2.291 (1)	----

Table 3. Selected bond angles [°] for [Pd(**3**)Cl_2] in comparison with **3**

	[Pd(**3**)Cl_2]	**3**
N4-N3-C6	120.1 (3)	121.1 (3)
N2-N1-C6	119.3 (3)	120.3 (4)
N3-C5-C4	107.5 (3)	108.6 (4)
C5-C4-C3	107.4 (3)	104.6 (3)
C4-C3-C6	104.4 (3)	104.8 (3)
C6-N3-C5	116.8 (3)	114.5 (3)
C6-C3-C2	104.6 (3)	104.0 (3)
C3-C2-C1	106.4 (3)	105.5 (3)
C2-C1-N1	106.1 (3)	108.3 (4)
C1-N1-C6	119.3 (3)	114.3 (4)
N1-C6-C3	103.2 (3)	104.4 (3)
N3-C6-N1	112.8 (3)	113.7 (3)
N3-C5-O2	124.1 (4)	124.1 (4)
N1-C1-O1	124.9 (3)	124.1 (4)
C4-C3-C2	114.7 (3)	114.3 (4)
N4-Pd-N2	94.7 (1)	----
Cl2-Pd-Cl1	92.8 (1)	----

The presence of such a ring has prompted us to consider the possible existence of a conformational equilibrium between the "sofa" and boat conformations. Consequently, the ^{1}H-NMR spectra of [Pd(**3**)Cl_2] and [Pt(**3**)Cl_2] have been recorded by varying the temperature. This study shows that the signals of the amino group protons of both complexes change on raising the temperature from the AB*gem* system to a broad signal. Simultaneously, the signals are shifted to higher field. This phenomenon seems to exclude the existence of any conformational equilibrium. However, decom-

position is observed on recording the ^{1}H-NMR spectrum of [Pt(**3**)Cl$_2$] due to the replacement of the chloride ligands by [D$_6$]DMSO in a process comparable to the solvolysis reaction of cisplatin to form *cis*-[Pt(NH$_3$)$_2$(Me$_2$SO)Cl][12].

In view of the high water solubility of the carrier ligand **3** we have expected good solubilities of the complexes. However, the solubilities of [Pt(**3**)Cl$_2$] and [Pd(**3**)Cl$_2$] are rather poor (0.8 and 2.0 mg/ml, respectively) in H$_2$O at room temperature. In contrast, these complexes are very soluble in DMSO (110 and 40.2 mg/ml).

In order to increase the water solubility of the platinum complex we have decided to introduce dicarboxylate anions as leaving groups. Complexes with these ligands usually show an activity comparable or superior to those containing chloride groups exhibiting a considerable reduced toxicity[3b].

Synthesis of (Alkanedicarboxylato){1,6-diaminotetrahydropyrrolo[2,3-*b*]pyrrole-2,5(1*H*,4*H*)-dione}platinum(II) Complexes

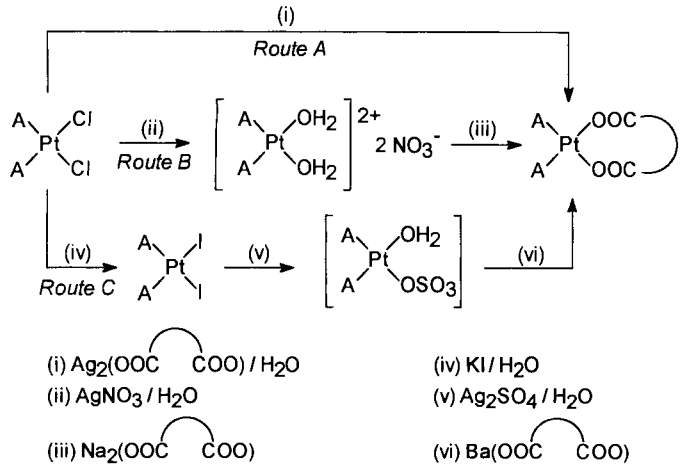
The replacement of the chloride ligands by the alkanedicarboxylate ions was assayed according to three different strategies usually described in the literature (Scheme 1):

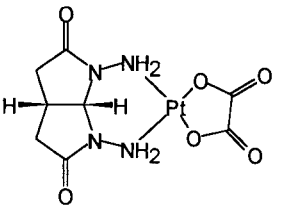
Route A: Reaction of the platinum complex with the silver salt of the diacid. Silver chloride precipitates and the supernatant solution is concentrated in vacuo to yield the product[13].

Route B: Reaction of the platinum complex with silver nitrate. The water-soluble nitrato complex is filtered off from the precipitated AgCl, and the sodium salt of the dicarboxylic acid is added[14]. This method is only effective when the alkanedicarboxylato complex is insoluble in the aqueous medium and precipitates. On the other hand, when a concentration of the mother liquor is necessary to afford the complex, it is usually contaminated with sodium nitrate.

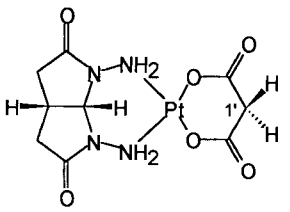
Route C: Addition of silver sulfate to a solution of the diiodo complex. After filtration of the AgI formed, addition of the barium salt of the dicarboxylic acid. The dicarboxylato complex is obtained after filtration of the barium sulfate and concentration of the solution in vacuo[15]. This method allows the dicarboxylato complex to be obtained in a neutral medium, free of inorganic ions, from which the complex is obtained by evaporation of the solvent.

Scheme 1

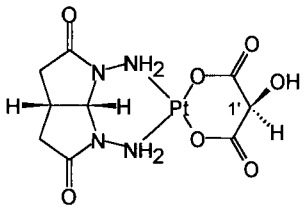




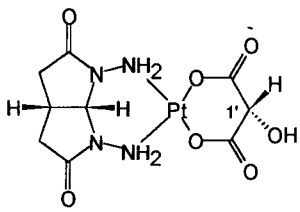
[Pt(**3**)ox]



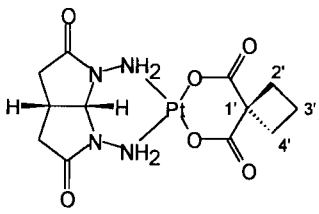
[Pt(**3**)mal]



cis-[Pt(**3**)(malOH)]



trans-[Pt(**3**)(malOH)]



[Pt(**3**)CBDC]

In our case, although these three methods have been tested, the best results are obtained by a slight modification of route C consisting of the treatment of [Pt(**3**)Cl$_2$] with an equivalent amount of Ag$_2$SO$_4$, filtration of the AgCl formed, and simultaneous addition of barium hydroxide and the corresponding dicarboxylic acid. After filtration of the BaSO$_4$ formed and concentration of the filtrate in vacuo, the oxalato [Pt(**3**)(ox)], malonato [Pt(**3**)(mal)], hydroxymalonato [Pt(**3**)(malOH)], and 1,1-cyclobutanedicarboxylato [Pt(**3**)(CBDC)] complexes are obtained in 50–60% yield. The purity of these compounds has been confirmed by HPLC which shows a sole peak for each compound tested. The absence of Ag and Ba in the complexes has been ensured by gravimetric analysis of the AgCl and BaSO$_4$ formed.

The IR spectra of the white solids obtained show the absorption frequencies of the dicarboxylato complexes (N–H stretching bands at 3120 ± 20 and 3020 cm^{-1}, C=O stretching bands at 1710 ± 10 and 1650 ± 30 cm^{-1}) superimposed to the O–H stretching (3300 cm^{-1}) and bending (1600 cm^{-1}) vibrations of the water molecule. The presence of water of crystallization in the complexes has been confirmed by the elemental analyses which give the following hydrates: [Pt(**3**)(Ox)] · 2.5 H$_2$O, [Pt(**3**)(mal)] · 0.5 H$_2$O, [Pt(**3**)(malOH)] · H$_2$O, and [Pt(**3**)(CBDC)] · 1.5 H$_2$O. The good water solubility of these complexes disappears when these are dried in a high vacuum, an effect which has been reported earlier[16].

Table 4. ^{1}H-NMR spectral data of [Pt(**3**)(ox)], [Pt(**3**)(mal)], [Pt(**3**)(malOH)], and [Pt(**3**)(CBDC)], δ values ([D$_6$]DMSO)

	[Pt(**3**)(ox)]	[Pt(**3**)(mal)]	[Pt(**3**)(malOH)]	[Pt(**3**)(CBDC)]
NH_2	8.90, 9.10 (br. s, 4H)	8.60, 8.95 (br. s, 4H)	8.70, 9.05 (br. s, 4H)	8.58, 8.91 (AB*gem*, 4H)
6a-H	5.40 (d, 1H)	5.35 (d, 1H)	5.45 (d, ½H), 5.31 (d, ½H)	5.34 (d, 1H)
3a-H	3.10 (m, 1H)	3.05 (m, 1H)	3.12 (m, 1H)	3.10 (m, 1H)
3-H$_\alpha$, 4-H$_\alpha$	2.74 (dd, 2H)	2.70 (dd, 2H)	2.72 (dd, 2H)	2.71 (dd, 2H)
3-H$_\beta$, 4-H$_\beta$	2.30 (dd, 2H)	2.32 (dd, 2H)	2.31 (dd, 2H)	2.31 (dd, 2H)
1'-H	----	3.15, 3.35 (AB*gem*, 2H)	5.04 (s, ½H), 5.08 (s, ½H)	----
2'-H	----	----	----	2.87 (t, 2H)
3'-H	----	----	----	1.65 (m, 2H)
4'-H	----	----	----	2.67 (t, 2H)
$J_{H\text{-}N\text{-}H}$ [Hz]	----	----	----	7.0
$J_{3a,6a}$ [Hz]	6.6	7.4	7.4	7.6
$J_{\alpha,\beta}$ [Hz]	16.5	16.0	17.6	17.6
$J_{\alpha,3a}$ [Hz]	10.0	11.0	10.2	10.5
$J_{\beta,3a}$ [Hz]	4.1	4.0	4.2	4.0
$J_{1',1'}$ [Hz]	----	14.0	----	----
$J_{2',3'}$ [Hz]	----	----	----	7.6
$J_{3',4'}$ [Hz]	----	----	----	7.6

The ^{1}H-NMR (Table 4) and ^{13}C-NMR spectral data of the complexes confirm their structures. Thus, the ^{1}H-NMR spectra show both the proton signals of the dicarboxylato ligands and the signals of the carbon-bound proton of the carrier ligand. The latter are shifted to lower field with respect to **3** as in [Pt(**3**)Cl$_2$]. The signal of the amino group hydrogen atoms appears either as an AB*gem* system ([Pt(**3**)(CBDC)]) or as two broad signals ([Pt(**3**)(ox)], [Pt(**3**)(mal)], and [Pt(**3**)(malOH)]).

The high rigidity and symmetry of the complex cause 1'-hydrogen atoms of the malonato ligand of [Pt(**3**)(mal)] to be diastereotopic and, consequently, their signal to appear as an AB*gem* system at δ = 3.15 and 3.35 with J_{AB} = 14.0 Hz.

Similarly, 2'-H and 4'-H of the 1,1-cyclobutanedicarboxylato ligand of [Pt(**3**)(CBDC)] are not magnetically equivalent, and their signals appear as triplets coupled with 3'-H (J = 7.6 Hz) at δ = 2.87 and 2.67, respectively. In addition, the ^{13}C-NMR spectrum of [Pt(**3**)(CBDC)] shows the signals of C-2' and C-4' at δ = 30.7 and 29.3.

Finally, the ^{1}H-NMR spectrum of [Pt(**3**)(malOH)] reveals that this complex is obtained as an equimolecular mixture of the *cis* and *trans* isomers due to the chirality center C-1'. Thus, the signal of 1'-H of both isomers appears as two singlets at δ = 5.04 and 5.08. Moreover, the spectrum shows the signal of 6a-H as two doublets at δ = 5.31 and 5.45 due to the proximity of C-1'. This duplicity is also present in the ^{13}C-NMR spectrum.

In vitro and in vivo Antitumour Activities of [Pt(3)Cl$_2$] and [Pd(3)Cl$_2$]

Cytolytic and cytostatic effects of cisplatin, **3**, [Pt(**3**)Cl$_2$], and [Pd(**3**)Cl$_2$] were determined in vitro in P388.D1 leukemia, B16.F10 melanoma, and Hela human carcinoma by a modification of the procedure described by Ruff and Gifford[17]. Thus, ICL_{50} (the drug concentration in μg/μl at which 50% of cells die) and ICT_{50} (the drug concentration in μg/μl at which 50% of cell growth is inhibited) are obtained (Table 5). A low ICT_{50} and a $ICL_{50} \gg ICT_{50}$ are considered necessary for the complex to demonstrate activity. The results show that both **3** and [Pt(**3**)Cl$_2$] are not active against the cell lines assayed. As for [Pd(**3**)Cl$_2$], the proximity of the cytostatic and cytolytic indexes discards it as a possible antitumour agent.

Table 5. In vitro ICL_{50} and ICT_{50} for **3**, [Pt(**3**)Cl$_2$], [Pd(**3**)Cl$_2$], and cisplatin with P388.D1, B16.F10, and Hela

Compound	Parameter[a]	P388D1	B16.F10	HeLa
3	ICL_{50}	≥ 500	≥ 500	≥ 500
	ICT_{50}	≥ 500	≥ 500	≥ 500
[Pt(3)Cl$_2$]	ICL_{50}	≥ 250[b]	≥ 250[b]	≥ 250[b]
	ICT_{50}	≥ 250[b]	≥ 250[b]	≥ 250[b]
[Pd(3)Cl$_2$]	ICL_{50}	250[b]	200-100[b]	500[b]
	ICT_{50}	100[b]	230-90[b]	300-200[b]
Cisplatin	ICL_{50}	≥ 10	≥ 10	≥ 10
	ICT_{50}	0.5-2.2	1.2-4.0	0.9-2.8

[a] Concentrations expressed in μg/μl. — [b] Administered in DMSO solution.

Table 6. In vitro antitumour activity of [Pt(**3**)Cl$_2$] against leukemia L1210

Dose [mg/kg]	T/C%	Dose [mg/kg]	T/C%
400	Toxic	25	101
200	130	12.5	101
100	117	6.25	103
50	113	3.12	100

On the other hand, the in vivo antitumour activity of [Pt(**3**)Cl$_2$] (NSC 620247) has been estimated according to the protocols[18] from the National Cancer Institute in CDF$_1$

mice-bearing L1210 leukemia at several dose levels using the intraperitoneal treatment route, but no significant activity has been observed as compared with the controls (Table 6).

We are indebted to the *Laboratorios Andrómaco S.A.* (Madrid) for the determination of in vitro antitumour activities. In vivo antitumour activities are the results of screening performed under the auspices of the *Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute,* Bethesda, Maryland.

Experimental

IR (KBr pellets): Perkin Elmer 683 and Bomem-Michelson 100 FTIR. – ^{1}H- and ^{13}C-NMR: Varian XL-200/F19 at 200 and 50.3 MHz, respectively. – Elemental Analyses: Carlo-Erba CHNS-O/EA 1108; Cl was determined by using the Schöniger apparatus and titrating the resulting solution with mercury perchlorate in the presence of diphenylcarbazone as indicator, Pt and Pd were determined as PtO_2 and PdO_2 by thermogravimetry in a Mettler TG50. – HPLC: Teknokroma Hypersil SAS C-1 RP2 column, with acetonitrile/water (3:2) mobile-phase flow rates at 1.0 ml/min and UV detection at 210 nm.

Dichloro{1,6-diaminotetrahydropyrrolo[2,3-b]pyrrole-2,5(1H,4H)-dione}platinum(II) ([Pt(**3**)Cl$_2$]): A solution of 2.87 g (17 mmol) of 2,8-dioxabicyclo[3.3.0]octane-3,7-dione[9] in 70 ml of water was added to a solution of 7.00 g (17 mmol) of K_2PtCl_4 (prepared by starting from Pt according to the procedure described by Kauffmann and Cowen[19]) in 70 ml of water. The resulting mixture was stirred at 50°C for 8 h with the exclusion of light in an inert atmosphere. After filtration, the solid obtained was washed with water, ethanol, and ether. The product was dried over P_2O_5 to give 5.10 g (70%) of [Pt(**3**)Cl$_2$] which was recrystallized from 1 M HCl to afford a yellow powder. – IR, ^{1}H- and ^{13}C-NMR data see Table 1. – UV (ethanol): λ_{max} (lg ε) = 225 nm (3.845) sh. – $C_6H_{10}Cl_2N_4O_2Pt$ (436.1): calcd. C 16.52, H 2.31, Cl 16.26, N 12.85, Pt 44.73; found C 16.80, H 2.08, Cl 15.70, N 12.73, Pt 44.08.

Dichloro{1,6-diaminotetrahydropyrrolo[2,3-b]pyrrole-2,5(1H,4H)-dione}palladium(II) ([Pd(**3**)Cl$_2$]): A solution of 2.87 g (17 mmol) of 2,8-dioxabicyclo[3.3.0]octane-3,7-dione[9] in 70 ml of water was added to a solution of 5.52 g (17 mmol) of K_2PdCl_4 (prepared by starting from Pd according to the procedure described by Kauffmann and Hwa-san Tsai[20]) in 70 ml of water with the exclusion of light in an inert atmosphere. The precipitate formed was filtered, washed with water, ethanol, and ether. The product was dried over P_2O_5 to give 4.84 g (82%) of [Pd(**3**)Cl$_2$] which was recrystallized from 1 M HCl to furnish a yellow powder. – IR, ^{1}H- and ^{13}C-NMR data see Table 1. – UV (ethanol): λ_{max} (lg ε) = 225 nm (4.191), 274 (3.861). – $C_6H_{10}Cl_2N_4O_2Pd$ (347.5): calcd. C 20.74, H 2.90, Cl 20.40, N 16.12, Pd 30.62; found C 20.75, H 2.87, Cl 20.59, N 16.13, Pd 31.57.

X-Ray Data of Dichloro{1,6-diaminotetrahydropyrrolo[2,3-b]pyrrole-2,5-(1H,4H)-dione}palladium(II): $C_6H_{10}Cl_2N_4O_2Pd$; mol. mass 347.47; yellow monocrystal (0.4 × 0.5 × 0.1 mm) was grown by recrystallization from 1 M HCl. Triclinic, space group $P\bar{1}$ (No. 2), $a = 6.836(2)$, $b = 9.112(1)$, $c = 9.522(1)$ Å, $\alpha = 110.29(1)$, $\beta = 100.01(2)$, $\gamma = 103.62(2)°$, $V = 519.01$ Å^3, $Z = 2$, $D_{calcd.} = 2.223$ g · cm^{-3}, μ(Mo-K_α) = 22.69 cm^{-1}; Enraf-Nonius CAD-4 diffractometer; graphite-monochromated Mo-K_α radiation; room temperature; $\omega/2\Theta$ scans, 1925 collected reflections ($2\Theta_{max} = 50°$); range of *hkl*: $-8 < h < 8$, $-10 < k < 10$, $0 < l < 11$, 1887 reflections considered observed [$I > 2.5\sigma(I)$]; Lorentz and polarization corrections applied; absorption correction according to the empirical Ψ-scan method[21] (minimum transmission 0.652, maximum transmission 0.999, average transmission 0.864). Structure solution according to the Patterson method[22]; refinement by the full-matrix least-squares method[23] to $R = 0.0268$, $R_w = 0.0351$ [$w = 1/(\sigma^2(F) + 0.0016F^2$]; all non-hydrogen atoms refined anisotropically, hydrogen atoms in calculated positions (except the hydrogen bound to nitrogens) with a common isotropic temperature factor; 153 parameters refined[24].

Synthesis of (Alkanedicarboxylato){1,6-diaminotetrahydropyrrolo[2,3-b]pyrrole-2,5(1H,4H)-dione}platinum(II) Complexes – General Procedure: A mixture of 0.714 g (1.64 mmol) of [Pt(**3**)Cl$_2$] and 0.510 g (1.64 mmol) of silver sulfate in 40 ml of water was stirred at room temp. for 3 h with the exclusion of light in an inert atmosphere. At the end of this period, the silver chloride formed was separated by centrifugation and filtration through a 0.45-µm Millipore nylon filter. Then 0.516 g (1.64 mmol) of barium hydroxide octahydrate and 1.64 mmol of the corresponding dicarboxylic acid were added to the resulting solution. The mixture was stirred for 4 h at room temp. with the exclusion of light in an inert atmosphere. The barium sulfate precipitated was separated by centrifugation and filtration through a 0.45-µm Millipore nylon filter. The resulting solution was concentrated in vacuo at room temp. to about 10 ml. The white precipitate obtained was collected by filtration, washed with cool water, ethanol and ether, and dried over P_2O_5 to afford the corresponding dicarboxylato complex.

{1,6-Diaminotetrahydropyrrolo[2,3-b]pyrrole-2,5(1H,4H)-dione}(oxalato)platinum(II) ([Pt(**3**)(ox)]): Starting from 0.147 g of oxalic acid, we obtained 0.453 g (55%) of [Pt(**3**)(ox)] · 2.5 H_2O as a white powder. – IR: $\tilde{\nu}$ = 3130 cm^{-1}, 3020 (NH), 1700, 1670, and 1625 (C=O). – ^{1}H-NMR see Table 4. – ^{13}C-NMR ([D$_6$]DMSO): δ = 169.4 (C-2 and -5), 165.4 (carboxylato), 23.5 (C-3a), 35.0 (C-3 and -4), 76.8 (C-6a). – $C_8H_{10}N_4O_6Pt$ · 2.5 H_2O (498.3): calcd. C 19.28, H 3.03, N 11.24; found C 19.13, H 3.20, N 11.63.

{1,6-Diaminotetrahydropyrrolo[2,3-b]pyrrole-2,5(1H,4H)-dione}(malonato)platinum(II) ([Pt(**3**)(mal)]): Starting from 0.171 g of malonic acid, we obtained 0.444 g (57%) of [Pt(**3**)(mal)] · 0.5 H_2O as a white powder. – IR: $\tilde{\nu}$ = 3120 cm^{-1}, 3020 (NH), 1715, 1680, and 1640 (C=O). – ^{1}H-NMR see Table 4. – ^{13}C-NMR ([D$_6$]DMSO): δ = 169.2 (C-2 and -5), 173.6 (carboxylato), 23.4 (C-3a), 34.9 (C-3 and -4), 76.8 (C-6a), 50.3 (C-1′). – $C_9H_{12}N_4O_6Pt$ · 0.5 H_2O (476.3): calcd. C 22.69, H 2.75, N 11.76; found C 22.91, H 2.84, N 12.08.

{1,6-Diaminotetrahydropyrrolo[2,3-b]pyrrole-2,5(1H,4H)-dione}(hydroxymalonato)platinum(II) ([Pt(**3**)(malOH)]): Starting from 0.197 g of hydroxymalonic acid, we isolated 0.514 g (63%) of [Pt(**3**)(malOH)] · H_2O as a white powder. – IR: $\tilde{\nu}$ = 3140 cm^{-1}, 3020 (NH and OH), 1710, 1655, and 1615 (C=O). – ^{1}H-NMR see Table 4. – ^{13}C-NMR ([D$_6$]DMSO): δ = 169.1, 169.5 (C-2 and -5), 173.7 (carboxylato), 23.3, 23.5 (C-3a), 35.0, 34.8 (C-3 and -4), 76.7, 77.1 (C-6a), 72.8, 72.6 (C-1′). – $C_9H_{12}N_4O_7Pt$ · H_2O (501.3): calcd. C 21.56, H 2.82, N 11.18; found C 21.52, H 2.82, N 10.81.

(1,1-Cyclobutanedicarboxylato){1,6-diaminotetrahydropyrrolo[2,3-b]pyrrole-2,5(1H,4H)-dione}platinum(II) ([Pt(**3**)(CBDC)]): Starting from 0.236 g of 1,1-cyclobutanedicarboxylic acid, we obtained 0.432 g (49%) of [Pt(**3**)(CBDC)] · 1.5 H_2O as a white powder. – IR: $\tilde{\nu}$ = 3120 cm^{-1} (NH), 1710 and 1650 (C=O). – ^{1}H-NMR see Table 4. – ^{13}C-NMR ([D$_6$]DMSO): δ = 169.3 (C-2 and -5), 176.9 (carboxylato), 23.3 (C-3a), 35.0 (C-3 and -4), 76.9 (C-6a), 55.5 (C-1′), 30.7 (C-2′), 14.8 (C-3′), 29.3 (C-4′). – $C_{12}H_{16}N_4O_6Pt$ · 1.5 H_2O (534.3): calcd. C 26.97, H 3.59, N 10.48; found C 27.07, H 3.83, N 11.00.

In Vitro Antitumour Activity of [Pt(**3**)Cl$_2$] *and* [Pd(**3**)Cl$_2$]: P388.D1 leukemia, B16.F10 melanoma, and Hela human carcinoma cells

were cultured at an initial density of $8 \cdot 10^4$ cells/0.2 ml/well in 96 well culture plates (Microtest II, Falcon Plastics, Oxnard, CA) for cytolytic assays or at $5 \cdot 10^3$ ccls/0.2 ml/well for cytostatic assays. Cisplatin, **3**, [Pt(**3**)Cl_2], and [Pd(**3**)Cl_2] were added, in aqueous or DMSO solution, until final concentrations ranging from 0.16 to 500 μg/ml were achieved. Plates were incubated in cytolytic assays for 18 h at 37°C in a humidified 5% CO_2 atmosphere, and for 72 h in cytostatic assays. At the end of the culture period, the wells were drained of the culture medium and washed with phosphate-buffered saline solution (PBS). Then 100 μl of 0.25% glutaraldehyde in PBS was added, and the plates were incubated at room temp. for 15 min. The glutaraldehyde solution was drained and 100 μl of crystal violet (2% w/v in 2% ethanol/water solution) was added. After incubation for 10 min at 37°C, the wells were washed with water and allowed to dry. After the addition of 100 μl of 1% sodium lauryl sulfate, absorbance of wells were measured at 550 nm with a Titertek Flow spectrophotometer. The values of ICL_{50} (the drug concentration at which 50% of cells died) and ICT_{50} (the drug concentration at which 50% of cell growth were inhibited) were calculated from the lg-probability graphs. Results are summarized in Table 5.

In Vivo Antitumour Activity: The in vivo antitumour activity test of [Pt(**3**)Cl_2] (NSC 620247) was carried out by National Cancer Institute (Bethesda, MD, USA). 10^5 L1210 leukemia cells were transplanted intraperitoneally in CDF_1 female mice on day 0. Compound NSC 620247 was suspended in saline solution with Tween-80 and administered i.p. injection once a day for 4 consecutive days after transplantation. The mean survival time of the treated (T) and control (C) groups was calculated and the results were expressed as T/C% (Table 6).

☆ Dedicated to Professor Dr. *Pedro Victory* on the occasion of his 65th birthday.

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