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ARTICLE *in* JOURNAL OF INORGANIC BIOCHEMISTRY · NOVEMBER 1996

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Synthesis and Antitumor Activity of Platinum(II) Complexes with *trans*-3,4-Diamino-2,2,6,6-tetramethylpiperidine-1-oxyl

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ABSTRACT

Platinum complexes $Pt^{II}(DAPO)X_2$ with diaminonitroxyl radical—*trans*-3,4-diamino-2,2,6,6-tetramethylpiperidine-1-oxyl (DAPO)—were synthesized by the direct reaction of DAPO with K_2PtX_4 (X=Cl, I) or by the replacement of chloro ligands in $Pt^{II}(DAPO)Cl_2$ by bromo, nitrato, oxalato, malonato, and 1,1-cyclobutanedicarboxylato ligands. The complexes thus obtained were characterized by elemental analysis, infrared, electronic, electron paramagnetic resonance spectroscopic techniques, and high-performance liquid chromatography. The toxicity of compounds in terms of LD_{50} strongly depends on the nature of X-ligands, and varies between 11 mg/kg ($X=NO_3$) and 400 mg/kg (X_2=1,1-cyclobutanedicarboxylate). Up to 66% of mice bearing leukemia L1210 survive after the administration of these complexes. This effect is comparable to the effect of cisplatin (50% survive). An increase in the life span of the rest of the animals ranges from 158 to 383%. Complex $Pt^{II}(DAPO)Cl_2$ appears to be more efficient than cisplatin against adenocarcinoma 755. Cisplatin, cis-diamminedichloroplatinum(II); CBDCA, 1,1-cyclobutanedicarboxylic acid; DAPO, trans-3,4-diamino-2,2,6,6-tetramethylpiperidine-1-oxyl; Mal, malonic acid; Ox, oxalic acid; IR, infrared; EPR, electron paramagnetic resonance; HPLC, high-performance liquid chromatography; Ca755, adenocarcinoma 755; LD_{50} and LD_{100}, dose of compounds (mg/kg), causing a death of 50 or 100% of treated animals; ILS, increase in life span of mice.

INTRODUCTION

cis-Diamminedichloroplatinum(II) (cisplatin) exhibits considerable efficiency against a number of human tumors; however, it is highly toxic (neuro-, nephro-,

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Journal of Inorganic Biochemistry, **64**, 69–77 (1996)

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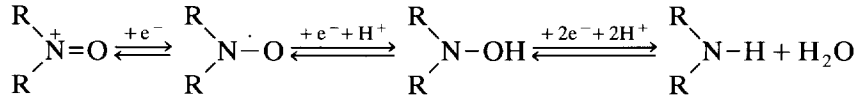
0162-0134/96/$15.00
PII S0162-0134(96)00029-3

and ototoxicity, bone marrow suppression, nausea, vomiting, allergic response) [1, 2]. Therefore, serious efforts are underway to find new platinum complexes exhibiting a combination of high potency and reduced toxicity [3]. Positive results were obtained by the addition of stable nitroxyl radicals to different known anticancer agents. Previously designed nitroxyl derivatives of ethyleniminethiophosphoramides [4], ethyleniminetriazines [5], 5-fluorouracil [6, 7], alkylnitrosoureas [8–10], and antibiotics [11] are less toxic, and some of them have shown higher chemotherapeutic indices than their analogs currently used in clinic practice [12]. Ruboxyl, the nitroxyl derivate of anthracycline antibiotic rubomicyn, recently has entered the second phase of clinical trials.

The biological activity of nitroxyl radicals arises primarily from their ability to participate in reversible redox reactions:



A considerable difference in the reactivity of the species participating in the reactions [13, 14] and the position of the equilibrium govern the effect of nitroxyl radicals on living systems.

Extending the work on the synthesis of nitroxyl derivatives of anticancer agents, we prepared and characterized [15, 16] DAPO—the first 1,2-diaminonitroxyl ligand. In this paper, we report the preparation conditions, characterization, toxicity, and anticancer activity of new platinum(II) complexes containing DAPO as a transport ligand.

EXPERIMENTAL

The synthesis of DAPO is described in [15]. (CBDCA)Ag_2 was prepared by agitation of aqueous solution of (CBDCA)Na_2 with an equivalent amount of $AgNO_3$ overnight. All other reagents were obtained from commercial suppliers and purified by the standard techniques.

Analytical and Spectral Measurements

Platinum content was measured by the atomic absorption spectroscopy technique (accuracy ± 3 rel.%) with an AAS-3 spectrometer. The HPLC analysis was carried out using a Millichrom chromatograph with a 2×64 mm column packed with Separone C18 (5 μm), at $\lambda = 240$ nm. A buffer mixture containing 0.05 M of KH_2PO_4 and 0.005 M H_3PO_4 was used as eluent in the analysis of DAPO. DAPO purified by distillation exhibits one peak with a retention volume of 380 μL. In the analysis of the dicarboxylate $Pt^{II}(DAPO)X_2$ complexes, the same buffer mixture with the addition of 5 vol.% of acetonitrile was used. The chromatograms of these complexes show a single peak with the following retention volumes: 450 μL for Pt^{II}(DAPO)Ox and for its dihydrate, 445 μL for $Pt^{II}(DAPO)Mal \cdot H_2O$, and 1100 μL for Pt^{II}(DAPO)CBDCA. At equal molar doses, the areas under the peaks for Pt^{II}(DAPO)Ox and its dihydrate are equal within the experimental error ($\pm 5\%$). IR spectra were taken in vaseline oil in the range of 400–4000 cm^{-1} with a Specord 75-IR spectrometer. Electronic

spectra were recorded in the 200–800 nm range with a Specord UV-VIS spectrophotometer. The EPR spectra were taken at room temperature with an SE/X 2544 device, at a microwave power of 2 mW, and at a modulation frequency of 0.32 mT.

Preparation of Platinum Complexes

Dichloro(trans-3,4-diamino-2,2,6,6-tetramethylpiperidine-1-oxyl-N^3,N^4)platinum(II), $Pt^{II}(DAPO)Cl_2$. A solution of DAPO (1.86 g, 10 mmol) in H_2O (10 mL) was added dropwise to a stirred solution of K_2PtCl_4 (4.15 g, 10 mmol) in 50 mL of water. After stirring for 2 h, the mixture was left at room temperature overnight. The yellow precipitate was filtered, washed sequentially with water, ethanol, and diethyl ether, and dried in vacuum. The yield of $Pt^{II}(DAPO)Cl_2$ was 4.38 g (97%).

$Pt^{II}(DAPO)I_2$ was prepared similarly using K_2PtI_4 and DAPO with a yield of 98%.

Dinitrato(trans-3,4-diamino-2,2,6,6-tetramethylpiperidine-1-oxyl-N^3,N^4)platinum (II), $Pt^{II}(DAPO)(NO_3)_2$. $Pt^{II}(DAPO)Cl_2$ (1.131 g, 2.5 mmol) was mixed with $AgNO_3$ (0.841 g, 4.95 mmol) in 50 mL of H_2O for 24 h in a dark place. The resulting AgCl was centrifuged. The solution was filtered through a dense filter and concentrated under reduced pressure to ~ 3 mL. Ethanol (5 mL) was added upon stirring, and the mixture was left in a refrigerator (5°C) overnight. The pale pink precipitate was filtered, washed with cold water and ethanol, and dried in vacuum. The yield of $Pt^{II}(DAPO)(NO_3)_2$ was 0.80 g (61%).

(Oxalato)(trans-3,4-diamino-2,2,6,6-tetramethylpiperidine-1-oxyl-N^3,N^4)platinum (II), $Pt^{II}(DAPO)Ox$. A solution of $Pt^{II}(DAPO)(NO_3)_2$ in 150 mL of H_2O was prepared from $Pt^{II}(DAPO)Cl_2$ (4.523 g, 10 mmol) and $AgNO_3$ (3.383 g, 19.9 mmol) as described above. Dipotassium oxalate monohydrate (2.76 g, 15 mmol) in 15 mL of H_2O was added to this solution. The mixture was stirred for 2 h, and then left overnight at ~ 20°C. The orange precipitate was filtered, washed with cold water, ethanol, and dried in air. The yield of crude $Pt^{II}(DAPO)Ox \cdot 2H_2O$ was 3.69 g (73%). The product was recrystallized from 165 mL hot water. Red prisms of anhydrous $Pt^{II}(DAPO)Ox$ were allowed to crystallize from a warm solution for 20 min, and then were filtered, washed with cold water, ethanol, and dried in vacuum. The yield of $Pt^{II}(DAPO)Ox$ was 1.36 g. The remaining filtrate was evaporated to 30 mL under reduced pressure, left in a refrigerator (5°C) overnight. The precipitated orange prisms of dihydrate were separated, washed with cold H_2O, ethanol, and dried in vacuum. The yield of recrystallized $Pt^{II}(DAPO)Ox \cdot 2H_2O$ was 1.97 g.

$Pt^{II}(DAPO)Br_2$ and $Pt^{II}(DAPO)Mal \cdot H_2O$ were prepared similarly from $Pt^{II}(DAPO)(NO_3)_2$, with yields of 89 and 67% respectively.

(Cyclobutane-1, 1-dicarboxylato)(trans-3, 4-diamino-2,2, 6,6-tetramethylpiperidine-1-oxyl-N^3,N^4)platinum(II), $Pt^{II}(DAPO)CBDCA$. $Pt^{II}(DAPO)Cl_2$ (1.53 g, 3.38 mmol) was mixed with $(CBDCA)Ag_2$ (1.21 g, 3.38 mmol) in H_2O (75 mL) for 24 h in a dark place at ~ 20°C. The resulting silver chloride was centrifuged. The solution was filtered with a dense glass filter and concentrated under reduced pressure to ~ 15 mL. The suspension of orange crystals, presumably unstable $Pt^{II}(DAPO)CBDCA \cdot nH_2O$, was obtained. Upon heating for 5 min in a water

bath (50°C), the orange crystals changed their color to pale pink, thus indicating transformation to anhydrous Pt^{II}(DAPO)CBDCA. The crystals were separated, washed with H_2O, ethanol, and dried in vacuum. The yield of Pt^{II}(DAPO) CBDCA was 1.42 g (80%).

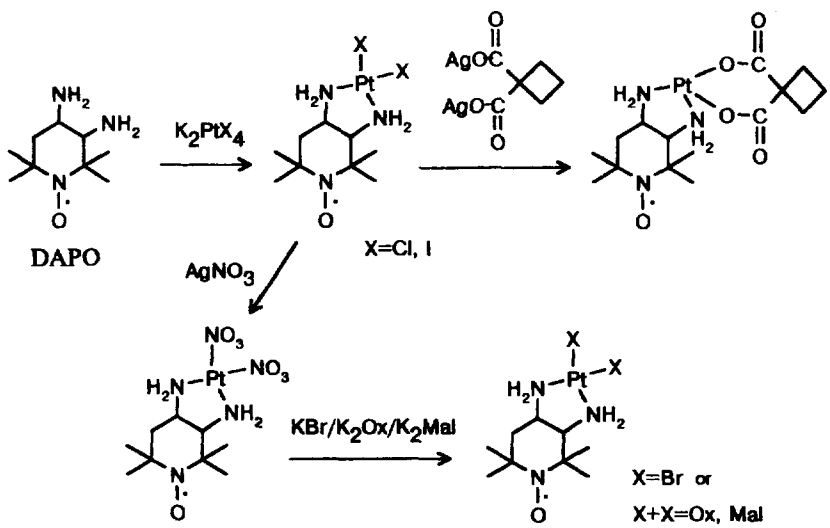
Toxicity and Antitumor Evaluation

The nitrato and dicarboxylato complexes were dissolved in water for injection. The halogeno complexes were administered as suspensions in a mixture of tween-80 and H_2O (1:9). The overall toxicity of the complexes was determined at single injections to BDF_1 mice. Antitumor activity was determined against leukemias L1210 and P388 and adenocarcinoma 755. BDF_1 female mice were inoculated intraperitoneally with 0.2 mL saline containing 10^6 cells of leukemia L1210 or P388. A percent increase in the life span of treated animals over control animals [100(T/C-1)] and the number of survivors was used for the estimation of antitumor activity. Ca755 was transplanted subcutaneously into the flank of C57B1 mice with 0.3 mL of the 1:1 diluted tumor tissue. The antitumor efficiency against Ca755 was evaluated by an inhibition of growth of tumor diameter (D).

RESULTS AND DISCUSSION

Chemistry

Synthesis of $Pt^{II}(DAPO)X_2$ Complexes. Starting $Pt^{II}(DAPO)X_2$ complexes (X=Cl or I) were synthesized in almost quantitative yields by the reaction of DAPO with K_2PtX_4 in water (see Scheme 1). The reaction proceeds smoothly with



SCHEME 1.

K_2PtCl_4, but slight oxidation of I^- to I_2 was observed in the case of the reaction of K_2PtI_4 derived *in situ* from K_2PtCl_4 and KI. This result can be explained by the reduction of the nitroxyl group by the I^- ion [17]. For this reason, the replacement of X-ligands was performed using $Pt^{II}(DAPO)Cl_2$. The suspension of $Pt^{II}(DAPO)Cl_2$ in H_2O was treated with two equivalents of $AgNO_3$ for 24 h. The insoluble silver chloride was thoroughly removed, and soluble $Pt^{II}(DAPO)(NO_3)_2$ was either separated from its concentrated solution or converted into other complexes (X = Br, X + X = Ox, Mal). Water-soluble $Pt^{II}(DAPO)CBDCA$ was prepared by the reaction of $Pt^{II}(DAPO)Cl_2$ with $(CBDCA)Ag_2$.

Analysis and Spectroscopic Characterization. All $Pt^{II}(DAPO)X_2$ complexes are yellow, pink, or red crystalline substances which gradually decompose (turn dark) without melting at 220–250°C. The halogeno complexes have very low solubility in water (≤ 0.1 mg/mL). The nitrato and dicarboxylato complexes are moderately soluble (1–3 mg/mL), but several times higher concentration can be achieved by evaporation of their diluted solutions. Their tendency to form hydrates is probably the reason for the formation of supersaturated solutions in H_2O.

The composition of each complex was determined by elemental analysis. There is good agreement between the calculated and the found values. The results are presented in Table 1. The EPR spectra of dilute aqueous solutions of $Pt^{II}(DAPO)X_2$ exhibit three lines with the intensity ratio 100:100:86. The hyperfine splitting constants $a_N = 1.667 \pm 0.001$ mT and g-factors ($g = 2.00590 \pm 1 \cdot 10^{-5}$) are the same for all of these complexes within experimental error. Electronic spectra of water-soluble $Pt^{II}(DAPO)X_2$ complexes (X = nitrato or X_2 = dicarboxylato ligands) in the range 200–800 nm contain bands of the nitroxyl and Pt(II) chromophores (see Table 2). In the visible region, the band at ~440 nm is due to the $n \rightarrow \pi^*$-transition in the $>N-O^{\cdot}$ group. In the ultraviolet region, the band of the $\pi \rightarrow \pi^*$-transition in the $>N-O^{\cdot}$ (for piperidinoxyls $\lambda_{max} \sim 240$ nm, $\varepsilon \sim 2000$ L/mol·cm) is overlapped by the band of Pt(II) chromophore. The transitions in the last chromophore depend, in turn, on the properties of the X ligands involved [18].

TABLE 1. Elemental Analysis of $Pt^{II}(DAPO)X_2$

Complex	Found(%)				Calc.(%)			
	C	H	N	Pt	C	H	N	Pt
$Pt^{II}(DAPO)Cl_2$[a]	23.93	4.42	9.15	42.4	23.90	4.46	9.29	43.1
$Pt^{II}(DAPO)Br_2$	19.76	3.66	7.58	34.7	19.97	3.73	7.76	36.0
$Pt^{II}(DAPO)I_2$	17.16	3.27	6.60	30.8	17.02	3.17	6.62	30.7
$Pt^{II}(DAPO)(NO_3)_2$	21.51	4.12	13.73	37.5	21.39	3.99	13.86	38.6
$Pt^{II}(DAPO)Ox$	28.16	4.28	8.90	41.2	28.15	4.28	8.95	41.6
$Pt^{II}(DAPO)Ox \cdot 2H_2O$	26.16	4.85	8.23	37.8	26.14	4.79	8.31	38.6
$Pt^{II}(DAPO)Mal \cdot H_2O$	28.60	4.86	8.26	38.0	28.74	4.82	8.38	38.9
$Pt^{II}(DAPO)CBDCA$	34.32	5.00	7.96	36.8	34.41	5.01	8.03	37.3

[a] Found: Cl, 15.67; calc.: Cl, 15.68.

In the IR spectra of $Pt^{II}(DAPO)X_2$ (see Table 2), N-H stretching and deformation vibrations appeared at 3050–3295 and 1553–~1600 cm^{-1}, respectively. Dicarboxylato complexes are distinguished by their tendency to form crystallohydrates. Depending on the temperature of crystallization, the oxalate complex can be obtained as red crystals of anhydrous $Pt^{II}(DAPO)Ox$ or orange crystals which, according to the IR spectrum (bands at 3410, 3445, and 3560 cm^{-1}), contain crystallization water. According to the data of elemental analysis, they have a structure of $Pt^{II}(DAPO)Ox \cdot 2H_2O$. This dihydrate is fairly stable, and loses crystallization water only partially upon heating in vacuum (56°C, 2 Pa) for 4 h. The HPLC data (see Experimental) also confirm the assumed difference in the structure of these oxalate complexes. At equal molar doses, $Pt^{II}(DAPO)Ox$ and $Pt^{II}(DAPO)Ox \cdot 2H_2O$ show peaks with equal retention volumes and equal areas. Bands relating to crystallization water (3380 and 3440 cm^{-1}) are seen in the IR spectrum of the pink malonato complex. For this complex, the structure of monohydrate $Pt^{II}(DAPO)Mal \cdot H_2O$ fits best to the data of elemental analysis. Presumably, the hydrate form of 1,1-cuclobutanedicarboxylato complex precipitates initially from its concentrated aqueous solution as orange crystals. On heating or treating with ethanol, they transform into anhydrous pink $Pt^{II}(DAPO)CBDCA$. In the IR spectra of hydrates, the N–H and hydrate water deformation vibrations and C=O stretching vibration are close, so that the corresponding absorption bands overlap in the region of 1600–1700 cm^{-1} and cannot be unambiguously assigned.

TABLE 2. IR and Electronic Spectroscopy of $Pt^{II}(DAPO)X_2$[a]

Complex	IR		Electronic	
	ν (cm^{-1})	Group	λ_{max} (nm)	ε (L/mol·cm)
$Pt^{II}(DAPO)Cl_2$	1560, 3190, 3240	N–H	—	—
$Pt^{II}(DAPO)Br_2$	1557, 3185, 3256	N–H	—	—
$Pt^{II}(DAPO)I_2$	1553, 3167, 3230	N–H	—	—
$Pt^{II}(DAPO)(NO_3)_2$	1578, 3135, 3215,		437	14
	3272	N–H	333 sh[b]	80
	967, 1262, 1488	NO_3	246 sh	2100
$Pt^{II}(DAPO)Ox$	1578, 3108, 3175,		440	13
	3200, 3295	N–H	312 sh	360
	1668, 1698, 1716	C=O	242	4800
$Pt^{II}(DAPO)Ox \cdot 2H_2O$	1602, 1656, 1678,	N–H,C=O,	441	14
	1698	H_2O	312 sh	360
	3100, 3217	N–H	241	5000
	3410, 3445, 3560	H_2O		
$Pt^{II}(DAPO)Mal \cdot H_2O$	1608, 1678	N–H,C=O,	442	13
		H_2O	327 sh	44
	3075, 3150, 3235	N–H	254 sh	2100
	3380, 3440	H_2O	227 sh	5300
$Pt^{II}(DAPO)CBDCA$	1563, 3052, 3155,		442	14
	3200	N–H	333 sh	40
	1603, 1618	C=O	229 sh	5200

[a] IR spectra were taken in vaseline oil, electronic spectra in H_2O.
[b] Shoulder.

Toxicity and Antitumor Activity

The data on overall toxicity of $Pt^{II}(DAPO)X_2$ complexes are given in Table 3. As found earlier [19, 20], the toxicity of platinum complexes containing the same transport ligands correlates rather well with the hydrolysis rate of leaving X-ligands. Qualitatively, $Pt^{II}(DAPO)X_2$ complexes show the same relationship. Easily hydrolyzed $Pt^{II}(DAPO)(NO_3)_2$ is more toxic than even cisplatin. Pt^{II}(DAPO)CBDCA is probably the most stable to hydrolysis and has low toxicity (LD_{50} = 400 mg/kg). The LD_{50} values for $Pt^{II}(DAPO)Cl_2$ (54 mg/kg) and its 1,2-diaminocyclohexane analog $Pt^{II}(DACH)Cl_2$ (25 mg/kg) [20] suggest that the lower toxicity of $Pt^{II}(DAPO)Cl_2$ arises from the influence of the nitroxyl group. The data of antileukemia activity for $Pt^{II}(DAPO)X_2$ complexes and for cisplatin are shown in Table 3. At the examined regime of administration, the new complexes cure (life span > 60 days) up to 66% of mice bearing leukemia L1210. This is comparable to the effect of cisplatin (50%). An increase in the life span of the rest of the animals ranges from 158 to 383% (383% for cisplatin). Leukemia P388 was sensitive only to $Pt^{II}(DAPO)Br_2$ and Pt^{II}(DAPO)CBDCA. $Pt^{II}(DAPO)Cl_2$ showed the greatest activity against Ca 755. Growth curves of Ca 755 shown in Figure 1 confirm that $Pt^{II}(DAPO)Cl_2$ suppresses tumor growth more efficiently than cisplatin.

To conclude, a number of promising platinum complexes containing 1,2-diaminonitroxyl radical as a transport ligand were synthesized and characterized. Their toxicity is shown to be influenced predominantly by the nature of leaving X-ligands. Low $Pt^{II}(DAPO)Cl_2$ toxicity (compared to that of its 1,2-diaminocyclohexane analog) can be explained by the influence of the nitroxyl group, which is capable of controlling redox processes in cells. New platinum complexes show high antitumor activity against a number of experimental animal tumors. Besides, they can be used as spin labels in exploring biological targets by EPR. The reaction of $Pt^{II}(DAPO)X_2$ complexes with DNA is under investigation.

TABLE 3. Toxicity and Antileukemic Activity of Complexes $Pt^{II}(DAPO)X_2$

Complex	Toxicity		L1210[a]		P388[b]	
	LD_{50} (mg/kg)	LD_{100} (mg/kg)	Dose (mg/kg)	ILS[c] (%)	Dose (mg/kg)	ILS[c] (%)
Cisplatin	12	16	1.0	383(50)	4.0	147(50)
$Pt^{II}(DAPO)Cl_2$	54	80	4.0	158(66)	16	189(0)
$Pt^{II}(DAPO)Br_2$	120	175	10	274(66)	40	135(33)
$Pt^{II}(DAPO)I_2$	96	140	8	160(0)	32	71(0)
$Pt^{II}(DAPO)(NO_3)_2$	11	12	NT[d]	—	NT	—
Pt^{II}(DAPO)Ox	30	50	2.5	226(33)	10	133(0)
$Pt^{II}(DAPO)Ox \cdot 2H_2O$	33	50	2.8	383(33)	11	132(0)
$Pt^{II}(DAPO)Mal \cdot H_2O$	225	350	19	267(16)	75	133(0)
Pt^{II}(DAPO)CBDCA	400	500	NT	—	133	202(33)

[a] Intraperitonel daily treatment from day 1 to day 7.
[b] Intraperitonel treatment on days 1, 5,'and 9.
[c] Percent of cured animal (> 60-day survivors) is shown in parentheses.
[d] NT—not tested.

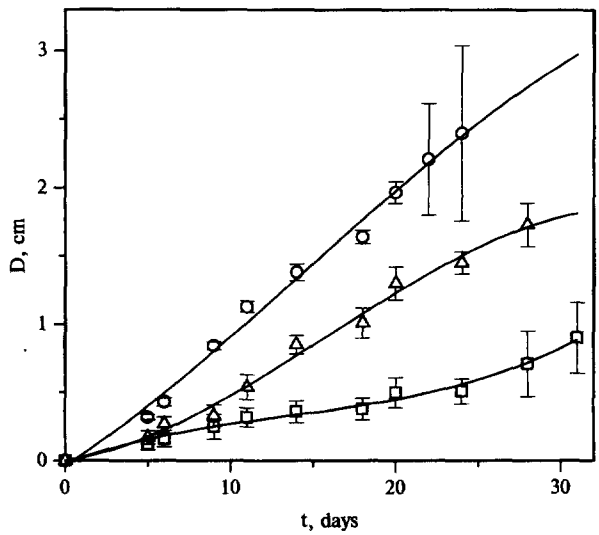


FIGURE 1. Growth curves for Ca755 in control (o), at daily treatment from day 2 to day 8 with 1.88 mg/kg of cisplatin (Δ), and with 4.75 mg/kg of $Pt^{II}(DAPO)Cl_2$(□).

This work was supported by the International Science and Technology Centre, Project 123-94.

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Received December 6, 1995; accepted January 4, 1996