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Studies on Synthesis, Structure, and Antitumor Activity of Pt(II) Complexes Containing 1,2-Diamino-1,2-dideoxy-D-glucitol

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An attempt was made to develop new water-soluble antitumor Pt(II) complexes by introducing hydroxyl groups into a carrier ligand, 1,2-diamino-1,2-dideoxy-D-glucitol (1,2-DAG), and their structures were determined by ^{13}C nuclear magnetic resonance and circular dicroism spectral analyses. Only [$Pt(NO_3)_2$(1,2-DAG)] and [$Pt(SO_4)$(1,2-DAG)] exhibited marginal effects against leukemia L1210 *in vivo*, among compounds with various leaving groups.

The unexpectedly low antitumor activity of Pt(II) complexes containing 1,2-DAG was investigated in *in vitro* experiments. The Pt(II) complexes of 1,2-DAG showed the same binding mode with calf-thymus deoxyribonucleic acid (DNA) as *cis*-diamminedichloroplatinum(II), but inhibition of DNA synthesis in L1210 cells *in vitro* was not observed even at the concentration of 100 μM [$PtCl_2$(1,2-DAG)] or [Pt(oxalato)(1,2-DAG)]. Consideration of the Pt contents taken into the cells indicated that the Pt(II) complexes have difficulty in passing through the cell membranes, which might account for the low antitumor effects observed *in vivo*.

Keywords——platinum(II) complex; membrane permeability; antitumor activity; Pt uptake; solubility; circular dichroism; ^{13}C-NMR

The discovery of antitumor activity of *cis*-diamminedichloroplatinum(II) (*cis*-DDP)[1] led us to look for antitumor-active Pt complexes which lack the severe renal toxicity of *cis*-DDP. In our laboratory, a variety of Pt(II) complexes has been synthesized and tested against leukemia L1210. Among them Pt(II) complexes containing 1,2-cyclohexanediamine (dach) isomers, especially 1*R*,2*R*-dach, exhibited the highest antitumor activity.[2–10] Many of the antitumor-active Pt(II) complexes are poorly soluble in water, but the difficulty has been overcome by substituting NO_3, SO_4 as leaving groups instead of halogens.

We have undertaken experiments to synthesize water-soluble Pt(II) complexes by introducing hydroxyl groups into carrier ligands. As the first step, we chose 1,2-diamino-1,2-dideoxy-D-glucitol (1,2-DAG) as a carrier ligand and synthesized a series of its Pt(II) complexes containing halogens, nitrate, sulfate, oxalate, and malonate as the leaving group. Their structures have been determined and their antitumor activity has been tested against L1210 *in vivo* and *in vitro*. The *in vitro* antitumor activity was correlated with cell membrane permeability.

Experimental

Reagents——α-D-Glucosamine hydrochloride, and phenylhydrazone were purchased from Nakarai Chemicals Ltd., and 5% palladium on carbon was purchased from Mitsuwa Chemical Ltd.; they were used without further purification.

Synthesis of 1,2-DAG——The ligand, 1,2-DAG was synthesized from α-D-glucosamine hydrochloride according to the method reported by Wolfrom[11] and its hydrochloride salt was obtained through the decomposition of the salicylaldehyde Schiff base.

Syntheses of Pt(II) Complexes——Dichloro (1,2-DAG) platinum(II): 1,2-Dideoxy-1,2-bis(salicylideneamino)D-glucitol (2.0 g, 5.5 mmol) was dissolved in 100 ml of EtOH by warming, then 1 ml of conc. HCl was added to decompose the Schiff base. The solution was left at room temperature and the hydrochloride salt of 1,2-DAG was deposited as a white precipitate. The supernatant was removed by decantation and the residue was dissolved in 20 ml of H_2O. The solution was filtered and to the filtrate was added 2.14 g (5.15 mmol) of K_2PtCl_4 dissolved in 20 ml of H_2O. The pH of the solution was adjusted to *ca.* 7 with a dil. KOH solution. After 24 h at room temperature, a pale yellow precipitate deposited, and this was recrystallized from H_2O. Yield: 57%.

Dinitrato (1,2-DAG) platinum(II): $PtCl_2$ (1,2-DAG) (1.0 g, 2.24 mmol) was suspended in 40 ml of H_2O, then 0.78 g (4.48 mmol) of $AgNO_3$ was added. The resultant suspension was left at room temperature, with protection from light, for 4 d with constant agitation. After the removal of AgCl by filtration, the filtrate was evaporated to dryness under reduced pressure at 50—60 °C. The residue obtained was dissolved in 10 ml of H_2O, and the solution was treated with charcoal then evaporated to give a white residue. Yield: 79%.

Sulfato (1,2-DAG) platinum(II): The sulfato Pt(II) complex was obtained by the same method as used for the dinitrato Pt(II) complex but with the addition of Ag_2SO_4 instead of $AgNO_3$. Yield: 88%.

Dibromo- or Diiodo (1,2-DAG) platinum(II): $Pt(NO_3)_2$ (1,2-DAG) (0.8 g, 1.0 mmol) was dissolved in 15 ml of H_2O by warming, then an excess amount of KBr (or KI) was added to yield pale yellow crystals. Yield: 65% (70%).

Oxalato- or Malonato (1,2-DAG) platinum(II): $Pt(NO_3)_2$ (1,2-DAG) (2.0 g, 4.0 mmol) was dissolved in 20 ml of H_2O by warming, then 0.74 g (4.0 mmol) $K_2C_2O_4 \cdot H_2O$ (or 0.45 g of $Na_2C_3H_2O_4$) was added. The resultant solution was kept at room temperature for 24 h and the white precipitate formed was collected by filtration and recrystallized from H_2O, giving white crystals. Yield: 80% for both oxalato and malonato Pt(II) complexes.

Diammine (1,2-DAG) platinum(II) Chloride: $PtCl_2$ (1,2-DAG) (1.0 g, 2.24 mmol) was dissolved in 30 ml of H_2O by warming and then 6.2 ml of dil. NH_4OH (1→10) was added. The resultant solution was warmed on a water bath at 50 °C for 1 h with agitation, then evaporated to 2 ml under reduced pressure and filtered. Addition of 20 ml of EtOH to the filtrate gave a white precipitate, which was collected by filtration and washed with acetone.

Elemental analyses of the Pt(II) complexes thus obtained are shown in Table I.

Table I. Elemental Analyses of Pt(II) Complexes Containing 1,2-DAG

Complexes	Found (%)			Calcd (%)		
	C	H	N	C	H	N
$[PtCl_2(1,2\text{-DAG})]$	15.95	3.53	6.28	16.15	3.61	6.28
$[PtBr_2(1,2\text{-DAG})]$	13.74	2.95	5.28	13.47	3.01	5.24
$[PtI_2(1,2\text{-DAG})]$	11.33	2.52	4.33	11.46	2.56	4.45
$[Pt(NO_3)_2(1,2\text{-DAG})]$	14.55	3.57	10.99	14.43	3.23	11.22
$[Pt(SO_4)(1,2\text{-DAG})]$	14.59	3.78	5.64	14.72	3.71	5.72
[Pt(ox)(1,2-DAG)][a]	20.68	3.49	6.07	20.74	3.48	6.05
[Pt(mal)(1,2-DAG)][b]	22.47	3.82	5.87	22.65	3.80	5.87
$[Pt(NH_3)_2(1,2\text{-DAG})]Cl_2$	14.89	4.51	11.38	15.00	4.62	11.67

a) ox = oxalate. *b*) mal = malonate.

Measurements——The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were obtained on a JEOL NM-FX-100 spectrometer. Absorption and circular dichroism (CD) spectra were measured with a Shimadzu UV 200 spectrophotometer and a JASCO J-40 spectropolarimeter, respectively.

Evaluation of Antitumor Activity——According to the Platinum Analog Study Protocol recommended by the National Cancer Institute, L1210 cells (10^5) were transplanted intraperitoneally into CDF_1 mice (1 group consists of 6 mice) on day 0, and the complexes were given intraperitoneally on days 1, 5 and 9. From the mean survival times (days) of treated (T) and control (C) mice, T/C% values were calculated. Complexes with T/C% of over 125 were evaluated as antitumor-active.

Interaction between 1,2-DAG Pt(II) Complex and Calf-Thymus Deoxyribonucleic Acid (DNA)——Calf-thymus DNA type I purchased from Sigma Chemical Co. was dissolved in 10 mM $NaClO_4$ solution to give a concentration in the range of $10^{-4} - 2 \times 10^{-4}$ M (P). Mixed solutions of the DNA and Pt $(NO_3)_2$ (1,2-DAG) were prepared by adding suitable amounts of a stock solution of the Pt (II) complex to 5 ml of a DNA stock solution with adjustment of the Pt/P ratios. The resultant solution was incubated at 37 °C for 3 d and the CD spectra were measured at 37 °C.

Cell Cultivation——L1210 was kindly provided by Prof. M. Sasaki, School of Medicine, Nagoya City University. L1210 was cultivated in Roswell Park Memorial Institute's Medium 1640 (RPMI 1640) containing 10% fetal calf serum (FCS, Flow Laboratory) in a humidified atmosphere of 5% CO_2 at 37 °C. The medium was supplemented with 10% FCS, 40 μU/ml penicillin, and 200 μg/ml streptomycin, and was changed every 48 h.

Falcon flasks (75 cm^3, No. 3024) were used to maintain the cells, culture test tubes (10 × 70 mm, No. 547601, Assit Trading Co., Ltd.) to examine cell growth and DNA synthesis, and Petri dishes (dia. 15 cm, Lux No. 2080543) to determine the platinum contents in the cells.

After the cells had grown to a monolayer, they were homogeneously suspended by treatment with 0.25% trypsin for 5 min at 37 °C. A portion of the suspension was gently pipetted in a saline solution, and cell numbers were counted in a hemocytometer.

Drug Treatment——The stock solutions of $PtCl_2$ (1,2-DAG) (1600 μM) were prepared in 0.02 M Tris–HCl saline buffer, pH 7.4, and sterilized by passing them through a Millipore filter (pore size, 0.22 μm) immediately prior to experiments. The cultured L1210 cells were treated as follows: (1) 2×10^5 cells were plated in a culture test bute, (2) after 48 h of incubation the medium was replaced by fresh RPMI containing 10% FCS with or without the drug and (3) every 48 h the medium was replaced by a fresh one with or without the drug.

Assay of DNA Synthesis——DNA synthesis of the cells was determined by a modification of the McLeester method. Two hundred thousand cells were seeded in a test tube (2 ml of RPMI 1640 medium). After 48 h of incubation, the medium was replaced with fresh RPMI 1640 containing 10% FCS with or without the drug, and [methyl-^{3}H]thymidine (New England Nuclear) was added. L1210 cells were labeled with 0.1 μCi of [methyl-^{3}H]thymidine for a 24 h period. After removal of the medium, the cells were harvested by trypsinization for 5 min and pelleted in a micro-test-tube by centrifugation (Microlagofuge, type M-15, Sakuma Seisakusho Ltd.) at 3000 rpm. The pellet was solubilized by the addition of 50 μl of 0.5% sodium dodecyl sulfate followed by sonification for 5 s with a micro-ultrasonic cell disrupter (Kontes, K-881440) equipped with a tip set at 8 W power output. A portion (20 μl) of the cell solution was deposited on a Whatman filter paper (dai. 1.9 cm, No. 3MM) which had previously been acidified with 40 μl of 20% trichloroacetic acid. The paper disc was washed by immersing it in cold 5% trichloroacetic acid solution for 10 min and subsequently in a cold absolute ethanol for another 10 min. The radioactivity in the paper disc was determined with a liquid scintilation counter (Packard TRI-CARB).

Determination of Platinum Contents in the Cells——One million L1210 cells were plated in each of three Petri dishes. Every 48 h, the medium was replaced with a fresh one. After 8 d, the media of two Petri dishes were replaced with a fresh one containing 10 μM *cis*-diamminedichloroplatinum(II) (*cis*-DDP) or $PtCl_2$ (1,2-DAG). The medium in the third Petri dish was replaced with a fresh one without the drug and used as a blank. After a 24 h incubation, the same number of cells was pelleted from each Petri dish and the cells were washed twice with 0.02 M Tris–HCl saline buffer. The pellets were solubilized by the addition of 0.5 ml of 1% sodium dodecyl sulfate, followed by sonification for 10 s with an ultrasonic cell disrupter equipped with a tip set at 15 W power output. The platinum content of each sample was determined with a flameless atomic absorption spectrophotometer (Jarrel-Ash A8200) under the following conditions: drying time 15 s at 40A, ashing time 45 s at 100 A, and atomization 10 s at 300 A.

Results and Discussion

Solubility of 1,2-DAG Pt(II) Complexes

Since 1,2-DAG was introduced as a carrier ligand in order to increase the solubility of Pt-(II) complexes in water, solubility was measured by suspending the Pt(II) complexes containing 1,2-DAG in 5 ml of water at 37 °C for 48 h with continuous stirring. Pt contents in the supernatant solutions were determined by atomic absorption spectrophotometry. The results are summarized in Table II. Compared with the solubility of [$PtCl_2$(1*R*,2*R*-cyclohexanediamine)], *i.e.*, 0.26 mg/ml, [$PtCl_2$ (1,2-DAG)] showed a higher solubility of 2.4 mg/ml, achieving the initial objective. Since the solubility of *cis*-DDP is about 1 mg/ml, the solubility of 1,2-DAG Pt(II) complexes is in a practical range.

Structures of 1,2-DAG Pt(II) Complexes

In order to examine the structures of Pt(II) complexes containing 1,2-DAG, stable and water-soluble [$Pt(NH_3)_2$ (1,2-DAG)]Cl_2 was prepared. Its ^{13}C-NMR, absorption and circular dichroism spectra were measured and analysed. Based upon these data, the structures of other Pt(II) complexes of 1,2-DAG were also deduced.

^{13}C-NMR Spectra

The halogeno, oxalato and malonato Pt(II) complexes of 1,2-DAG are hardly soluble in

TABLE II. Solubility of 1,2-DAG Pt(II) Complexes in Water at 37 °C

Complexes	Solubility (mg/ml)
$PtCl_2$(1,2-DAG)	2.4
$PtBr_2$(1,2-DAG)	1.7
PtI_2(1,2-DAG)	1.5
$Pt(NO_3)_2$(1,2-DAG)	>100
$Pt(SO_4)$(1,2-DAG)	>100
Pt(oxalato)(1,2-DAG)	>5.8
Pt(malonato)(1,2-DAG)	4.4
$PtCl_2$(1*R*,2*R*-dach)[a]	0.26

a) 1*R*,2*R*-dach = 1*R*,2*R*-cyclohexanediamine.

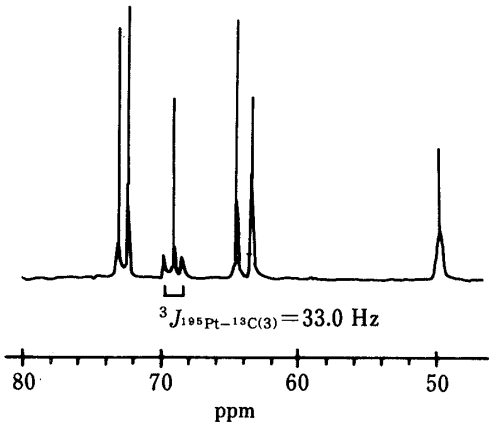

Fig. 1. ^{13}C-NMR Spectrum of $[Pt(NH_3)_2$(1,2-DAG)$]Cl_2$ in D_2O

TABLE III. ^{13}C-NMR Chemical Shifts and Coupling Constants of $[Pt(NH_3)_2$(1,2-DAG)]

	C(5)	C(4)	C(3)	C(6)	C(2)	C(1)
1,2-DAG·2HCl	73.30	71.79	66.87	63.78	53.51	40.55
$[Pt(NH_3)_2$(1,2-DAG)$]Cl_2$	72.62	71.96	68.60 (33.0)	64.07	62.97	49.47

* ^{13}C-NMR chemical shifts were measured in ppm from external TMS. The value in parenthesis is the coupling constant of ^{195}Pt–^{13}C.

water, making it difficult to obtain their ^{13}C-NMR spectra. The sulfato and nitrato Pt(II) complexes are soluble in water, but they are unstable, giving aqua and hydroxo Pt(II) complexes.

In order to overcome these difficulties $[Pt(NH_3)_2$ (1,2-DAG)$]Cl_2$, which is soluble and stable in water, was synthesized and its ^{13}C-NMR spectrum was measured to elucidate the conformation of the chelate ring formed between 1,2-DAG and Pt (II) ions.

Figure 1 shows the ^{13}C-NMR spectrum of $[Pt(NH_3)_2$ (1,2-DAG)$]Cl_2$ in D_2O. The peaks were assigned to C(1), C(2), C(6), C(3), C(4), and C(5) of 1,2-DAG·2HCl from higher magnetic field.[12-14] As a result of chelate ring formation through the amino groups, the resonance peaks of C(1) and C(2) were shifted *ca.* 9 ppm toward lower magnetic field, appearing resonance peaks at 49.77 and 62.97 ppm, respectively. Furthermore, the complex exhibited a pair of satellite peaks at the resonance peak of 68.60 ppm with a coupling constant of 33.0 Hz.

In general, it has been pointed out that coupling constants between ^{195}Pt and ^{13}C nuclei offer valuable information for determining the conformations of Pt(II) complexes. As regards the coupling constants between ^{195}Pt and ^{13}C nuclei, a series of five-membered Pt(II) chelates of diamines has been studied, and it has been reported that $^3J_{Pt-C}$ values follow a Karplus-type equation.[15-18] Erickson *et al.*[16] reported a $^3J_{Pt-C}$ value of 52 Hz for an equatorial carbon atom and predicted a very small value for an axial orientation, perhaps approaching zero. Consequently, intermediate values would be expected when interconversion between axial and equatorial orientations takes place. Therefore, the peak at 68.6 ppm was assigned to C(3), being accompanied with $^3J_{Pt-C}$ satellite peaks. Coupling constants between ^{195}Pt and C(1) or C(2) were not observed due to cancellation of $^2J_{Pt-C}$ and $^3J_{Pt-C}$. The observed coupling consant of 33.0 Hz indicates that the chelate ring interconverts rapidly between λ and

δ conformations, the former of which is preferred.

This conclusion is supported by the CD spectral behavior of 1,2-DAG Pt(II) complexes, which will be described in detail in the next section.

Absorption and CD Spectra

The absorption spectrum (AB) of $[Pt(NH_3)_2$ (1,2-DAG)$]Cl_2$ resembles that of N_4 type Pt(II) complexes, giving AB bands at 280 and 220 nm which were assigned to bands II and IV.[18] The CD spectrum exhibited two positive bands at 285 and 227 nm with $\Delta\varepsilon$ values of +0.188 and +0.406, respectively. The positive signs of the CD bands indicate that *λ-gauche* conformation of the chelate ring is prevailing.[7-10,18]

The AB spectra of $[Pt(NO_3)_2$ (1,2-DAG)], $[Pt(SO_4)$ (1,2-DAG)] and [Pt(oxalato) (1,2-

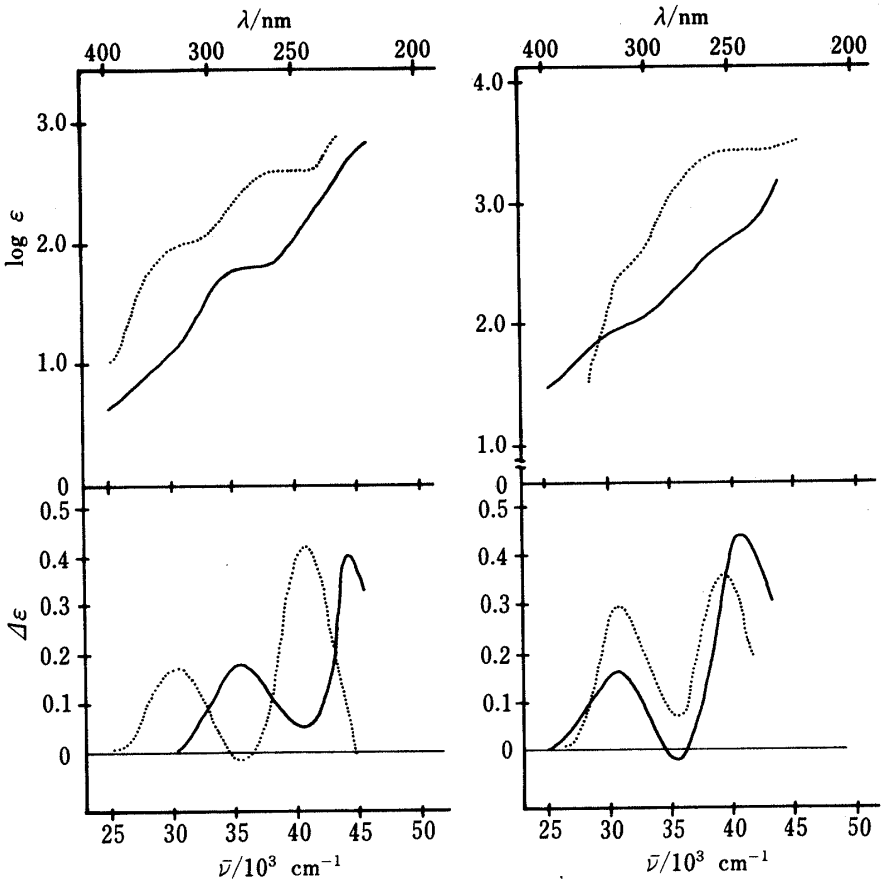

Fig. 2. Absorption and CD Spectra of 1,2-DAG Pt(II) Complexes

(a) ——, $[Pt(NH_3)_2(1,2\text{-DAG})]$; ------, $[Pt(NO_3)_2(1,2\text{-DAG})]$. (b) ——, $[Pt(SO_4)(1,2\text{-DAG})]$; ------, [Pt(oxalato)(1,2-DAG)].

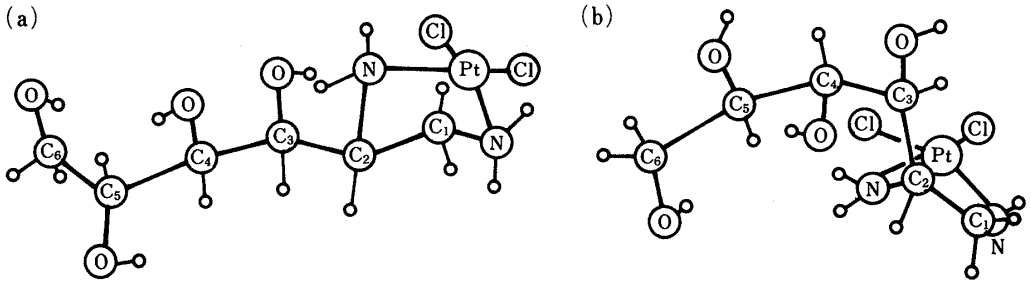

Fig. 3. The Conformational Structures of $[PtCl_2(1,2\text{-DAG})]$

(a) *λ-gauche* conformation. (b) *δ-gauche* conformation.

TABLE IV. Absorption and Circular Dichroism Spectral Data for 1,2-DAG Pt(II) Complexes

Complexes	Band II		Band III		Band IV	
	AB λ_{max}/nm (log ε)	CD λ_{max}/nm ($\Delta\varepsilon$)	AB λ_{max}/nm (log ε)	CD λ_{max}/nm ($\Delta\varepsilon$)	AB λ_{max}/nm (log ε)	CD λ_{max}/nm ($\Delta\varepsilon$)
$[Pt(NH_3)_2(1,2\text{-DAG})]Cl_2$	280 (1.76)	285 (+0.188)	240 sh	—	220 (2.72)	227 (+0.406)
$[Pt(NO_3)_2(1,2\text{-DAG})]$	330 (1.90)	328 (+0.164)	280 sh	280 (−0.025)	250 (2.64)	245 (+0.443)
$[Pt(SO_4)(1,2\text{-DAG})]$	330 (1.95)	328 (+0.182)	290 sh	283 (−0.02)	255 (2.56)	246 (+0.431)
[Pt(oxalato)(1,2-DAG)]	320 (2.42)	325 (+0.297)	280 sh		250 (3.39)	255 (+0.364)

DAG)] showed absorption maxima around 320—330 nm and 250—255 nm, which were assigned to bands II and IV, respectively, from the longer wavelength side. The longer wavelength shifts of AB bands in these Pt(II) complexes can be explained by taking account of the lower position of O,O-coordination in the spectrochemical series as compared with N,N-coordination. The CD spectra of these N_2O_2–Pt(II) complexes were also shifted to the longer wavelength side, exhibiting CD bands at 328 and 245 nm, which correspond to bands II and IV in the AB spectra, for $[Pt(NO_3)_2$ (1,2-DAG)] and $[Pt(SO_4)$ (1,2-DAG)]. For these two Pt(II) complexes, small negative CD bands were observed around 280 nm, the signs of which can not be correlated to the conformations of the Pt(II) complexes. The same phenomenon was also reported for $[PdCl_2(1\text{-pn})]$.[18] The CD spectrum of [Pt(oxalato) (1,2-DAG)] also exhibited two positive CD bands at 325 and 240 nm with values of +0.297 and +0.364, respectively, which were assigned to bands II and IV from the longer wavelength side.

These positive CD bands assigned to bands II and IV indicate that a *λ-gauche* conformation is predominant in the Pt(II) complexes of 1,2-DAG, being consistent with the result of ^{13}C-NMR analyses. The molecular models of 1,2-DAG Pt(II) complexes, which are illustrated in Fig. 3, suggest that in order for the chelate ring between 1,2-DAG and Pt(II) to take a *λ-gauche* conformation, the configuration around C(2) has to be S with an equatorial C(3) atom, while in a *δ-gauche* conformations C(3) has to take an unstable axial orientation.

Antitumor Activity

Antitumor activity of the Pt(II) complexes of 1,2-DAG was tested *in vivo* against leukemia L1210 according the NCI protocol. The results are shown in Table V. Although a variety of leaving groups had been introduced, the antitumor activity did not change much; that is, the solubility of the Pt(II) complexes in water was not directly related to the activity.

Only marginal effects were observed for $[PtBr_2$ (1,2-DAG)], $[Pt(NO_3)_2$ (1,2-DAG)] and $[Pt(SO_4)$ (1,2-DAG)] at doses of 100, 50, and 25 mg/kg, respectively, while $[PtCl_2$ (1,2-DAG)], $[PtI_2$ (1,2-DAG)], [Pt(oxalato) (1,2-DAG)] and [Pt(malonato) (1,2-DAG)] exhibited almost no activity.[19]

Interactions between Calf-Thymus DNA and $[Pt(NO_3)_2$ (1,2-DAG)]

In order to clarify the unexpectedly low antitumor activity, the interactions between calf-thymus DNA and $[Pt(NO_3)_2$ (1,2-DAG)] (or $[Pt(SO_4)$ (1,2-DAG)]) were studied by the CD spectral method, since it has been pointed out by Macquet and Butour,[20] and Srivasta *et al.*[21] that antitumor-active *cis*-DDP reacts with DNAs to give enhanced $\Delta\varepsilon$ values around 280 nm at low Pt/P ratios and that *trans*-DDP does not show such a phenomenon. The enhancement

TABLE V. Antitumor Activity of Pt(II) Complexes of 1,2-DAG against L1210

Complexes	Dose (mg/kg)								
	400	200	100	50	25	12.5	6.25		
				T/C (%)					
$PtCl_2$(1,2-DAG)		83[T]	103	113					
$PtBr_2$(1,2-DAG)		91	129	117					
PtI_2(1,2-DAG)		0	0	103	100				
$Pt(NO_3)_2$(1,2-DAG)			103[T]	135	124	116	113		
$Pt(SO_4)$(1,2-DAG)		0	0	104	129	107	107		
Pt(oxalato)(1,2-DAG)		0	0	0		97 (10)		103 (5)	110 (2.5)
Pt(malonato)(1,2-DAG)	112	98	102	96					

Underlining indicates antitumor activity (T/C % ≧ 125). T, indicates toxicity. The numbers in parentheses under oxalato Pt(II) indicate the doses (mg/kg) used. 10^5 cells/mouse were transplanted i.p. into CDF_1 mice (6 mice/group) and the samples were administered i.p. on days 1, 5, and 9.

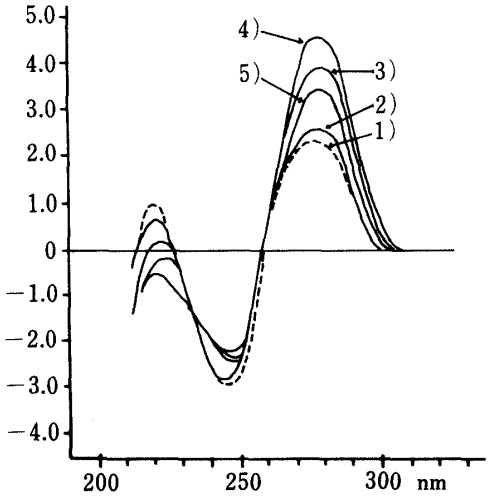

Fig. 4. CD Spectra of Calf-Thymus DNA–[Pt-$(NO_3)_2$(1,2-DAG)] Mixtures at Various Pt/P Ratios

1) Calf-thymus DNA (1.39×10^{-4} M). 2) Pt/P = 0.029. 3) Pt/P = 0.058. 4) Pt/P = 0.101. 5) Pt/P = 0.173.

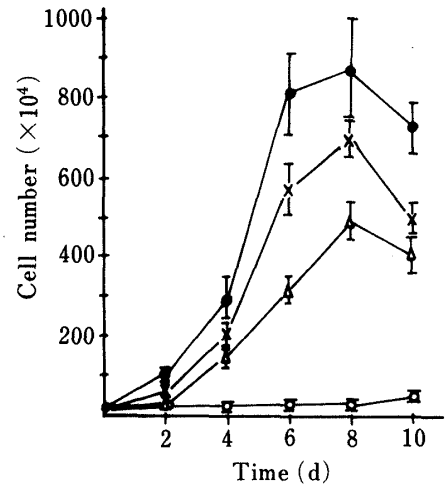

Fig. 5. Growth Curves for L1210 Cells Treated with [$PtCl_2$(1,2-DAG)]

●—●, control; ×—×, 10 μM; △—△, 20 μM; ○—○, 40 μM.

is considered to be characteristic of antitumor-active Pt (II) complexes, reflecting a special binding mode between DNA and *cis*-DDP. The exact binding mode is open to discussion, but at present *cis*-DDP is considered to produce an intrastrand crosslink between adjacent guanine bases in DNA.[22-26]

The results are illustrated in Fig. 4; in the Pt/P range of 0.1—0.3, $\Delta\varepsilon$ enhancements were observed around 280 nm indicating the same binding modes between the DNA and 1,2-DAG Pt(II) complexes as in the case of *cis*-DDP. The interaction of [$Pt(SO_4)$ (1,2-DAG)] with calf-thymus DNA also produced the similar $\Delta\varepsilon$ enhancements.

Effect of 1,2-DAG Pt(II) on L1210 *in Vitro*

In vitro, some of the 1,2-DAG Pt(II) complexes showed marginal antitumor activity against L1210, but in the study of the interaction between the DNA and [$Pt(NO_3)_2$ (1,2-DAG)], they bound to DNA in the same way as *cis*-DDP. Therefore, there is a possibility that the membrane permeability of L1210 cells for [$PtCl_2$ (1,2-DAG)] is very low. On this

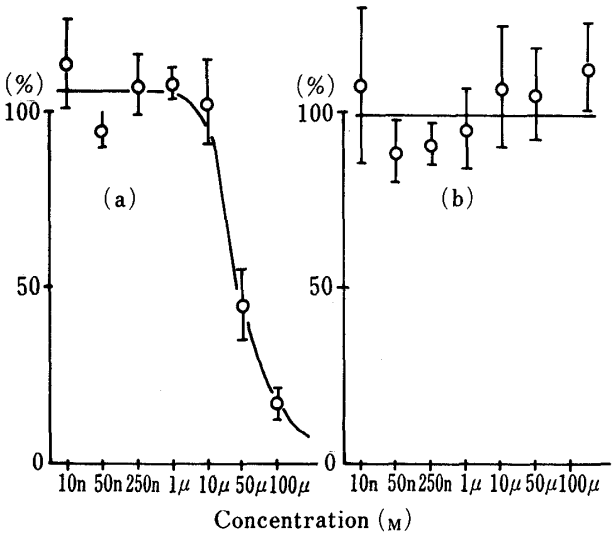

Fig. 6. Inhibition of Deoxyribonucleic Acid Synthesis in L1210 Cells

(a) *cis*-$[PtCl_2(NH_3)_2]$. (b) $[PtCl_2(1,2\text{-DAG})]$.

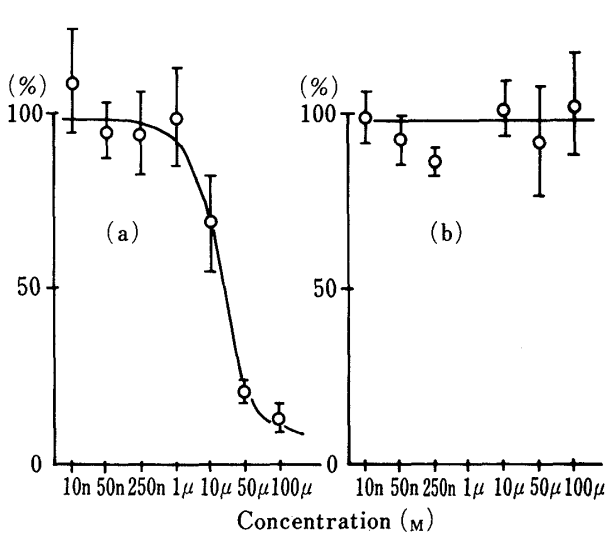

Fig. 7. Inhibition of Deoxyribonucleic Acid Synthesis in L1210 Cells

(a) [Pt(oxalato)(1*R*,2*R*-cyclohexanediamine)]. (b) [Pt(oxalato)(1,2-DAG)].

TABLE VI. Pt Uptake in L1210 Cells Treated with 20 μM $[PtCl_2(1,2\text{-DAG})]$ or *cis*-DDP

Complexes	Amounts taken up ($ng/10^5$ cells)
$PtCl_2(1,2\text{-DAG})$	0.3
cis-DDP	1.6

assumption, the effect of $[PtCl_2$ (1,2-DAG)] on L1210 *in vitro* was studied in more detail.

At the first step, the inhibition of cell growth of L1210 by $[PtCl_2$ (1,2-DAG)] was studied and is shown in Fig. 5. The inhibition of the cell growth increased with increasing concentration of the Pt(II) complex. At 40 μM complex, the cell growth was completely inhibited. However, this result did not show whether the inhibition of cell growth depends on cytotoxicity or on inhibition of DNA synthesis by the Pt(II) complex. Therefore, the inhibition of DNA synthesis was studied.

cis-DDP and [Pt(oxalato) (1*R*,2*R*-dach)], which have antitumor activity against L1210, inhibited the DNA synthesis but $[PtCl_2$ (1,2-DAG)] did not show inhibition, even at the drug concentrations at which the former complexes completely inhibited DNA synthesis of the cells (Fig. 6). This suggests that the Pt(II) complex does not pass through the cell membranes.

In order to ascertain the membrane permeability, L1210 cells were contacted with 10 μM $[PtCl_2$ (1,2-DAG)] for 24 h, and the platinum content taken into the cells was determined by flameless atomic absorption spectrometry. The result is shown in Table VI. Platinum content inside L1210 cells treated with $[PtCl_2$ (1,2-DAG)] was 0.3 $ng/10^5$ cells, while that of *cis*-DDP was 1.6 $ng/10^5$ cells. This result indicates that the permeability of L1210 cell membranes to $[PtCl_2$ (1,2-DAG)] was low, which would account for the lack of antitumor activity in the *in vivo* experiments.

In conclusion, these experiments confirm the importance of considering cell membrane permeability in designing antitumor Pt(II) complexes.

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