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Antitumor Activity of Pt(II) Complexes Containing Diaminocarboxylates and Their Ester Derivatives

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Antitumor Pt(II) complexes containing ester derivatives of DL-2,3-diaminopropionate and DL-2,4-diaminobutyrate were synthesized and their structures were determined from their infrared and ultraviolet absorption spectral data. The antitumor activity of these Pt(II) complexes was tested *in vivo* against leukemia L1210 according to the NCI Pt Analog Protocol. [Pt(Malonato)(DL-2,3-diaminopropionate ethyl ester)] exhibited the highest antitumor effect with a T/C % value of 364 at an administration dose of 100 mg.

The partition coefficients between water and *n*-octanol were measured, but no clear correlation with antitumor effects was found.

Keywords——platinum(II) complex; antitumor activity; diaminocarboxylate; partition coefficient

In the previous paper[1] we reported the syntheses and antitumor activities of Pt(II) complexes containing diaminocarboxylic acids and their ethyl ester derivatives. The diaminocarboxylic acids used as carrier ligands were *meso*-1,2-diaminosuccinic acid, L-1,2-diaminopropionic acid, and L-1,3-diaminobutyric acid, whose Pt(II) complexes did not show any antitumor activity against leukemia P388. However, Pt(II) complexes of their ethyl esters were found to be antitumor-active, being neutral forms that should be able to permeate through the cell membrane.

In order to obtain more antitumor-active Pt(II) complexes, we have newly prepared Pt(II) complexes of DL-2,3-diaminopropionic acid (dapaH), DL-2,4-diaminobutyric acid (dabaH), and their ester derivatives, and tested their antitumor activity against leukemia L1210. The results are discussed in relation to the partition coefficients between *n*-octanol and water.

Experimental

Reagents——DL-2,3-Diaminopropionic acid monohydrochloride and DL-2,4-diaminobutyric acid dihydrochloride were purchased from Sigma Chemical Co., Ltd. and were used without further purification.

Syntheses of Ester Derivatives——Ethyl (or butyl) ester of DL-2,3-diaminopropionate or DL-diaminobutyrate was synthesized by bubbling Cl_2 gas through a suspension of the appropriate diaminocarboxylic acid (3.0 g) in EtOH (or *n*-butanol) (1.5 l) followed by evaporation of the resultant ethanol solution under reduced pressure.

Syntheses of Pt(II) Complexes——Diiodo(ethyl DL-2,3-diaminopropionate)platinum(II) [PtI_2 (dataEt)]: Solid KI (17.6 g) was added to a solution containing 4.4 g of K_2PtCl_4 in 40 ml of H_2O, and the resultant solution was stirred for 1 h at room temperature, then evaporated to dryness under reduced pressure. The residue was dissolved in 140 ml of EtOH, and 2.17 g of ethyl diaminopropionate and 0.8 g of NaOH were added. This solution was stirred for several

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hours at room temperature. The resultant yellow precipitates were collected by filtration, and recrystallized from an acetone–H_2O solution. Yield: 4.45 g (72.5%).

Dichloro(ethyl DL-2,3-diaminopropionate)platinum(II) [$PtCl_2$ (dapaEt)]: Solid $AgNO_3$ (0.47 g) was added to 50 ml of an aqueous suspension containing 0.8 g of PtI_2 (dapaEt), and the mixture was stirred overnight at room temperature in the dark. Silver iodide deposited was filtered off, and 1.0 g of NaCl was added to the filtrate. The

TABLE I. Elemental Analyses of Pt(II) Complexes Containing Diaminopropionate Derivatives

Complexes	Found (%)			Calcd (%)		
	H	C	N	H	C	N
dapaEt·2HCl	7.12	29.15	13.69	6.83	29.30	13.66
dapaBu·2HCl	7.51	35.92	12.09	7.73	36.05	12.02
$PtCl_2$(dapaH)	2.07	9.73	7.61	2.16	9.73	7.51
$PtCl_2$(dapaEt)	3.01	14.90	6.96	3.01	15.06	7.04
PtI_2(dapaEt)	1.93	10.20	4.60	2.07	10.33	4.82
$Pt(SO_4)$(dapaEt)·$2H_2O$	3.15	12.97	6.17	3.49	13.07	6.10
Pt(oxalato)(dapaEt)	2.98	20.17	6.60	2.89	20.24	6.75
Pt(malonato)(dapaEt)	3.27	22.19	6.59	3.26	22.38	6.53
$PtCl_2$(dapaBu)	3.64	19.60	6.51	3.76	19.72	6.57
PtI_2(dapaBu)	2.44	13.74	4.37	2.63	13.79	4.60
$Pt(SO_4)$(dapaBu)·$2H_2O$	3.85	17.28	5.80	4.22	17.25	5.75
Pt(oxalato)(dapaBu)·$1/2\,H_2O$	3.44	23.83	6.10	3.76	23.89	6.19
Pt(malonato)(dapaBu)	3.77	25.72	6.31	3.97	26.26	6.13

dapaEt = 2,3-diaminopropionate ethyl ester. dapaBu = 2,3-diaminopropionate butyl ester. dapaH = 2,3-diaminopropionic acid.

TABLE II. Elemental Analyses of Pt(II) Complexes Containing 2,3-Diaminobutyrate Derivatives

Complexes	Found (%)			Calcd (%)		
	H	C	N	H	C	N
dabaEt·2HCl	7.44	32.68	12.70	7.31	32.88	12.79
dabaBu·2HCl	7.93	38.18	11.44	7.97	38.25	11.16
$PtCl_2$(dabaH)	2.90	12.70	7.50	2.60	12.50	7.29
$PtCl_2$(dabaEt)	3.43	17.24	6.87	3.40	17.48	6.80
PtI_2(dabaEt)	2.32	12.20	4.91	2.35	12.10	4.71
$Pt(SO_4)$(dabaEt)·$2H_2O$	3.53	15.26	5.97	3.80	15.22	5.92
Pt(oxalato)(dabaEt)·$1/2\,H_2O$	3.38	21.73	6.40	3.42	21.91	6.39
Pt(malonato)(dabaEt)	3.46	23.91	6.14	3.76	23.89	6.19
$PtCl_2$(dabaBu)	3.86	21.37	6.44	4.16	21.59	6.30
PtI_2(dabaBu)	2.77	15.31	4.38	2.89	15.41	4.49
$Pt(SO_4)$(dabaBu)·$3/2\,H_2O$	3.91	19.02	5.65	4.27	19.51	5.65
Pt(oxalato)(dabaBu)·$1/2\,H_2O$	3.81	25.46	6.14	4.08	25.75	6.01
Pt(malonato)(dabaBu)	4.12	27.90	6.05	4.25	28.03	5.94

dabaEt = 2,4-diaminobutyrate ethyl ester. dabaBu = 2,4-diaminobutyrate butyl ester. dabaH = 2,4-diaminobutyric acid.

TABLE III. Analytical Conditions for Flameless Atomic Absorption Spectrometry

Wavelength	265.9 nm	Drying	40 A	20 s
Lamp current	10 mA	Ashing	100 A	25 s
Argon flow rate	3.0 l/min	Atomizing	300 A	10 s

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solution was agitated for 1 h on a water bath at *ca.* 60 °C. The yellow precipitates were collected, recrystallized from 0.1 N NaCl solution. Yield: 0.44 g (81%).

Sulfato(ethyl DL-2,3-diaminopropionate)platinum(II) [Pt(SO_4) (dapaEt)]: Solid Ag_2SO_4 (1.02 g) was added to 50 ml of an aqueous suspension of 2.0 g of PtI_2 (dapaEt), and the mixture was agitated for 24 h at room temperature, with protection from light. The AgI deposited was filtered off and the filtrate was evaporated to dryness under reduced pressure. The residue thus obtained was washed with a small amount of H_2O and then ether. Yield: 1.25 g (86%).

Oxalato or Malonato(ethyl DL-2,3-diaminopropionate)platinum(II) [Pt(ox) (dapaEt)] or [Pt(mal) (dapaEt)]: Pt(SO_4) (dapaEt) (0.23 g) was dissolved in 5 ml of H_2O, and 1.0 g of sodium oxalate (or potassium malonate) was added to the solution. The mixture was stirred overnight at room temperature, and the resultant white precipitates were collected by filtration, recrystallized from H_2O. Yields: 0.12 g (57%) and 0.16 g (65%) for the oxalato and malonato Pt(II) complexes, respectively.

Other Pt(II) complexes containing DL-2,4-diaminobutyrate ethyl ester (dabaEt), DL-2,3-diaminopropionate butyl ester (dapaBu), and DL-2,4-diaminobutyrate butyl ester (dabaBu) were synthesized by procedures similar to those described for dapaEt Pt(II) complexes.

The results of elemental analyses of Pt(II) complexes thus obtained are shown in Tables I and II.

Measurements——Infrared (IR) and ultraviolet (UV) absorption spectra were measured with a Shimadzu IR 400 infrared spectrophotometer and a Hitachi 577 spectrophotometer, respectively. Determination of Pt contents was done with a Shimadzu AA-630-12 flame atomic absorption spectrophotometer or a Jarrel Ash AA 8200 flameless atomic absorption spectrometer under the conditions shown in Table III.

Evaluation of Antitumor Activity——Antitumor activities of the platinum complexes were tested by means of the protocols for routine screening at the National Cancer Institute (Bethesda, Md.). L1210 cells (10^5) were transplanted intraperitoneally into CDF_1 mice on day 0, and the samples were given intraperitoneally on days 1, 5, and 9. From the mean survival times (d) of treated (T) and control (C) mice, T/C% values were calculated. Samples with T/C% values that exceeded 125 were evaluated as antitumor-active. A dose at which the T/C% value was less than 85% was designated as a toxic dose.

Measurement of Partition Coefficients——Aqueous sample concentrations were adjusted to be in the range of 10^{-3}—2×10^{-4} M. After 30 ml of the sample solution and 30 ml of *n*-octanol had been mixed and incubated at 25 °C with constant agitation for 48 h, the Pt content of each phase was determined by flameless atomic absorption spectrometry and the apparent partition coefficients of the Pt(II) complexes were calculated according to the following equation.

$$P = C_o/C_w$$

P: apparent partition coefficient

C_o: Pt concentration in *n*-octanol phase

C_w: Pt concentration in aqueous phase

Results and Discussion

Structures of Pt(II) Complexes

Table IV summarizes IR data for Pt(II) complexes containing 1,2-diaminopropionic acid (dapaH), 1,3-diaminobutyric acid (dabaH) and their ethyl ester derivatives. The frequency range of 3070—3250 cm^{-1} showed asymmetric and symmetric stretching frequencies of the coordinated amino groups, being shifted to the higher frequency side compared with those of the hydrochloride salts of dapaEt and dabaEt. Stretching frequencies of carbonyl groups were observed in the range of 1757—1730 cm^{-1}, whereas the oxalato and malonato Pt(II) complexes exhibited two absorption peaks in the frequency range of 1620—1690 cm^{-1} due to the carbonyl stretching, clearly reflecting their coordination to the central Pt(II) ions.

These IR data indicated that dapaEt and dabaEt bind to Pt(II) ions through the amino groups. Similar IR spectral behavior was also observed for Pt(II) complexes containing dapaBu and dabaBu.

Table V lists the UV spectral data for dichloro-, and oxalato- and sulfato Pt(II) complexes containing dapaEt. The first complex showed absorption maxima at 370, 297 and 272 nm, resembling the absorption spectrum of $PtCl_2$ (1-pn). $PtCl_2$ (1-pn) exhibited absorption maxima at 366, 302 and 270 nm, which were assigned to bands II ($^1A_1$—^3E), III ($^1A_1$—

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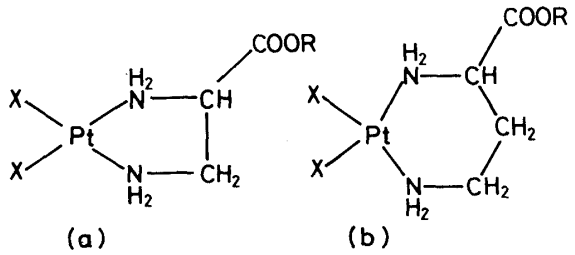
TABLE IV. Infrared Spectral Data for Pt(II) Complexes Containing Ethyl Ester Diaminocarboxylate

(cm^{-1})

Complexes	ν_{NH_2}		$\nu_{C=O}$		
dapaEt·2HCl	2850	2760	1740		
dabaEt·2HCl	2960	2870	1725		
$PtCl_2$(dapaH)	3180	3120	1757		
$PtCl_2$(dabaH)	3240	3190	1715		
$PtCl_2$(dapaEt)	3240	3180	1735		
$PtCl_2$(dabaEt)	3170	3120	1735		
$Pt(SO_4)$(dapaEt)	3250	3200	1730		
$Pt(SO_4)$(dabaEt)	3210	3100	1735		
Pt(oxalato)(dapaEt)	3150	3080	1740	1690	1660
Pt(oxalato)(dabaEt)	3180	3070	1738	1690	1650
Pt(malonato)(dapaEt)	3160	3100	1750	1660	1620
Pt(malonato)(dabaEt)	3220	3130	1740	1670	1635



R = H, C_2H_5, C_4H_9
X = leaving group

Fig. 1. Proposed Structures of Pt(II) Complexes Containing dapaH, dabaH, and Their Ester Derivatives

TABLE V. Ultraviolet Absorption Spectral Data for Pt(II) Complexes Containing Ethyl Ester Diaminocarboxylate

Complexes	Band II		Band III		Band IV	
	λ/nm	($\log\varepsilon$)	λ/nm	($\log\varepsilon$)	λ/nm	($\log\varepsilon$)
$PtCl_2$(dapaEt)	370	(1.62)	297	(2.34)	272	(2.24)
$PtCl_2$(dabaEt)	357	(1.72)	301	(2.06)	260	(2.26)
$Pt(SO_4)$(dapaEt)	324	(2.00)			260	(2.36)
$Pt(SO_4)$(dabaEt)	313	(1.79)			250	(sh)
Pt(oxalato)(dapaEt)	313	(2.42)			256	(sh)
Pt(oxalato)(dabaEt)	312	(2.54)			248	(3.4)
Pt(malonato)(dapaEt)	322	(1.60)	270	(sh)	242	(sh)
Pt(malonato)(dabaEt)	318	(1.68)	270	(sh)	238	(3.5)

sh = shoulder.

$^1A_2$) and IV ($^1A_1$—^1E), respectively.[2]

The other two complexes showed absorption maxima shifted toward the shorter wavelength side, and bands II and IV were seen around 320—310 and 260—250 nm. These shorter wavelength shifts compared with those of the dichloro Pt(II) complex can be explained by the higher position of oxygen atoms than chloride ions in the spectrochemical series.[3] These absorption spectral data resemble those of sulfato and oxalato Pt(II) complexes of 1*R*,2*R*-cyclohexanediamine, absorption maxima of which were observed around 330—315 and 260—245 nm assigned to bands II and IV based upon the circular dichroism (CD) spectral data.[4] Similar UV spectra were also observed for Pt(II) complexes of dapaBu, dabaEt, and dabaBu.

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TABLE VI. Antitumor Activity of Dihalogeno Pt(II) Complexes Containing 2,3-Diaminopropionate, 2,4-Diaminobutyrate and Their Ester Derivatives

Complexes	Dose (mg/kg)					
	12.5	25	50	100	200	400
	T/C%					
$PtCl_2$(dapaH)	115	117	108			
$PtCl_2$(dapaEt)	153	175	112			
$PtCl_2$(dapaBu)	116	135	180 (1)			
PtI_2(dapaEt)	103	103	106	116	113	76
PtI_2(dapaBu)		109	103	103		
$PtCl_2$(dabaH)	107	121	115			
$PtCl_2$(dabaEt)	182	210	93			
$PtCl_2$(dabaBu)	111	125	113			
PtI_2(dabaEt)		100	91	97		
PtI_2(dabaBu)		103	93	0		

Underlining indicates positive effects (T/C%≧125). Numbers in parentheses indicate 30-d survivors out of 6 mice.

TABLE VII. Antitumor Activity of Pt(II) Complexes Containing dapa and daba Derivatives

Complexes	Dose (mg/kg)						
	1.56	3.12	6.25	12.5	25	50	100
				T/C%			
$Pt(SO_4)$(dapaEt)	96	102	125	T107	93		
$Pt(SO_4)$(dapaBu)				117	91	T75	
Pt(oxalato)(dapaEt)				126	118		
Pt(oxalato)(dapaBu)				118	145	97	
Pt(malonato)(dapaEt)				113	122	130	
Pt(malonato)(dapaBu)					101	117	T140
$Pt(SO_4)$(dabaEt)				182	156		
$Pt(SO_4)$(dabaBu)				105	124	105	
Pt(oxalato)(dabaEt)				97	111	137	
Pt(oxalato)(dabaBu)			149	225	168		
Pt(malonato)(dabaEt)					138	209 (1)	362 (3)
Pt(malonato)(dabaBu)					106	115	125

Underlining indicates positive effects (T/C%≧125). T indicates toxicity. Numbers in parentheses indicate 30-d survivors out of six mice.

These UV spectral data showed that the carrier ligands bind to Pt(II) ions through amino groups. Based upon these data, the structures of the Pt(II) complexes containing dapaH, dabaH, and their ester derivatives are deduced to involve a 5- or 6-membered chelate ring as shown in Fig. 1.

Antitumor Activity

Table VI lists the antitumor activities of halogeno Pt(II) complexes containing dapa and daba ester derivatives against leukemia L1210. The Pt(II) complexes of dapaH and dabaH did not exhibit any antitumor activity. Since deprotonation of the carboxyl groups takes place at physiological pH, they are present as negatively charged species, which are considered to have difficulty in penetrating cell membranes. This explanation was supported by results obtained

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TABLE VIII. Aqueous Solubility of Pt(II) Complexes Containing Diaminocarboxylate Derivatives

Complexes	Solubility (mg/ml)	Complexes	Solubility (mg/ml)
$PtCl_2$(dapaEt)	0.61	$PtCl_2$(dabaEt)	0.67
PtI_2(dapaEt)	0.21	PtI_2(dabaEt)	0.10
Pt(oxalato)(dapaEt)	9.13	Pt(oxalato)(dabaEt)	21.0
Pt(malonato)(dapaEt)	1.73	Pt(malonato)(dabaEt)	2.02
$PtCl_2$(dapaBu)	0.55	$PtCl_2$(dabaBu)	0.32
PtI_2(dapaBu)	0.03	PtI_2(dabaBu)	<0.02
Pt(oxalato)(dapaBu)	6.11	Pt(oxalato)(dabaBu)	12.6
Pt(malonato)(dapaBu)	0.35	Pt(malonato)(dabaBu)	0.26

TABLE IX. Partition Coefficients of Pt(II) Complexes between Water and *n*-Octanol (25 °C)

Complexes	P	log P	Complexes	P	log P
$PtCl_2$(dapaH)	1.35×10^{-3}	−2.87	$PtCl_2$(dabaH)	7.76×10^{-4}	−3.11
$PtCl_2$(dapaEt)	3.09×10^{-3}	−2.51	$PtCl_2$(dabaEt)	3.20×10^{-3}	−2.50
$Pt(SO_4)$(dapaEt)	4.67×10^{-3}	−2.34	$Pt(SO_4)$(dabaEt)	4.51×10^{-3}	−2.35
Pt(ox)(dapaEt)	5.18×10^{-3}	−2.29	Pt(ox)(dabaEt)	6.13×10^{-3}	−2.21
Pt(mal)(dapaEt)	2.52×10^{-3}	−2.60	Pt(mal)(dabaEt)	2.92×10^{-3}	−2.53
$PtCl_2$(dapaBu)	3.24×10^{-2}	−1.49	$PtCl_2$(dabaBu)	2.60×10^{-2}	−1.59
$Pt(SO_4)$(dapaBu)	2.18×10^{-2}	−1.66	$Pt(SO_4)$(dabaBu)	1.94×10^{-2}	−1.71
Pt(ox)(dapaBu)	3.81×10^{-2}	−1.42	Pt(ox)(dabaBu)	3.55×10^{-2}	−1.45
Pt(mal)(dapaBu)	3.87×10^{-2}	−1.41	Pt(mal)(dabaBu)	3.26×10^{-2}	−1.44

ox = oxalato, mal = malonato.

with the ester derivatives.

Dichloro Pt(II) complexes of dapaEt and dapaBu showed relatively high antitumor effects against L1210, as shown in Table VI, and the same tendency was also observed for dichloro Pt(II) complexes of dabaEt and dabaBu. However, diiodo Pt(II) complexes of these ester derivatives were inactive, perhaps due to their low water solubility (Table VIII).

Water-soluble sulfato Pt(II) complex of dabaEt exhibited some antitumor activity, but complexes of the other ester derivatives did not show any appreciable activity. The oxalato and malonato Pt(II) complexes were moderately water-soluble and all of them were antitumor active. Pt(Mal) (dabaEt) exhibited the highest activity among the complexes examined in the present paper, giving a T/C% value of 364 at a dose of 100 mg/kg with three cured mice out of 6 mice.

Partition Coefficient

There are some reports on structure–activity correlations for antitumor Pt(II) complexes,[5,6] and Braddock *et al.*[7] studied the relation between physicochemical properties and antitumor activity of Pt(II) complexes containing alicyclic amines. The partition coefficients of the Pt(II) complexes between $CHCl_3$ and H_2O were used as an indicator of membrane permeability.

We have measured the solubilities of the Pt(II) complexes prepared in water and the partition coefficients between *n*-octanol and water in order to see whether there is any correlation with the antitumor activities.

These data are shown in Table IX, where the partition coefficients (P) are expressed as $\log P = C_o/C_w$ (C_o: Pt concentration in the *n*-octanol phase; C_w: Pt concentration in the

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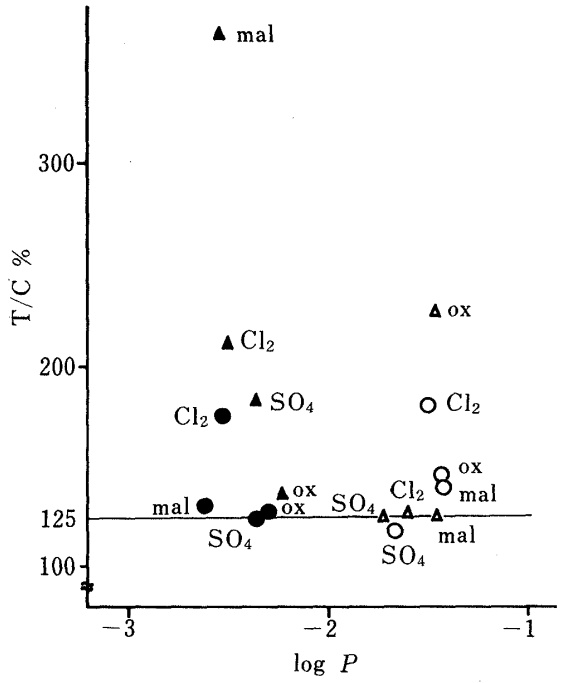

Fig. 2. Relationship between Partition Coefficients and Optimal T/C% Values of Pt(II) Complexes Containing dapa and daba Ester Derivatives

●, PtX_2(dapaEt); ○, PtX_2(dapaBu); ▲, PtX_2(dabaEt); △, PtX_2(dabaBu).

aqueous phase).

The ethyl ester derivatives gave partition coefficients in the range of 10^{-1}—10^{-2}, while those of butyl ester derivatives were in the 10^{-2}—10^{-3} range. These coefficients were plotted against the maximum T/C% values as illustrated in Fig. 2. There was no clear correlation between them. However, the moderately soluble Pt(II) complexes, *i.e.*, Pt(mal) (dabaEt), Pt(ox) (dabaBu), and $PtCl_2$ (dabaEt), having solubilities of 1—12 mg/ml, showed higher antitumor activities. The poorly soluble diiodo Pt(II) complexes, whose solubilities were less than 0.2 mg/ml, did not show any activity.

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