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Synthesis and Antitumor Activity of *cis*-Platinum Complexes of Aromatic Amines

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cis-Platinum complexes of aromatic amines were synthesized in water or at the interfacial layer between water and an organic solvent. The position of substitution of aniline derivatives had a marked effect on the synthesis of the complexes. The coordination of 2-substituted anilines needed a long time and the yields were low. That of 3- or 4-substituted anilines was fast, and the yields were high. Disubstituted or N-substituted anilines needed fairly long reaction times and the yields were lower than those of monosubstituted anilines; sometimes unidentified complexes were obtained or no reaction product was formed.

Six synthesized *cis*-dichloro platinum(II) complexes which coordinate bis(4-aminophenol), bis(3-toluidine), bis(2-aminobiphenyl), bis(4-sulfamidoaniline), bis(1-naphthylamine) and bis(4-phenylazo-1-naphthylamine) had high antitumor activities against Sarcoma 180 ascites in female ICR/JCL mice. The dose of platinum of these effective complexes given to the mice was in the range of 7.37—105.76 mg/kg (26.44%—39.96%). This result suggests that the toxicity of the platinum complexes might depend on their carrier ligands.

Keywords——platinum; platinum complex; *cis*-dichlorodiammineplatinum(II); antitumor activity; toxicity; interfacial reaction

Since Rosenberg *et al.*[1] reported the antitumor activities of *cis*-dichlorodiammineplatinum(II) {[$PtCl_2(NH_3)_2$], **1**}, many platinum complexes have been synthesized and their antitumor activities examined. However, a more effective organoplatinum complex with higher antitumor activity at a small dosage level and with lower toxicity than **1** has not yet been reported.

In a series of aliphatic amine complexes, the order of the antitumor activities was primary amine > secondary amine > tertiary amine.[2] In a series of cyclic amine complexes, cyclohexylamine complex showed the highest antitumor activity; complexes with higher or lower carbon number showed lower activity.[3]

The previous syntheses of complexes similar to **1** have been carried out in water or in organic solvents miscible with water. This procedure limits the kind of ligands that can be used, due to the need for the ligand to be soluble in the solvent. The present synthesis was carried out by using an interfacial reaction between water and organic solvent layers, allowing greater flexibility in the choice of ligands.

In this paper, we wish to report the synthesis and antitumor activity of platinum complexes which have aromatic amine derivatives as ligands.

Experimental

Various ligand compounds were dissolved in the following solvents and each solution thus prepared was added stoichiometrically to 2 ml of aqueous solution containing 0.207 g (0.5 mmol) K_2PtCl_4 (**2**): the solvent used was water

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(A), ethyl acetate (B), benzene (C) or 0.2 M (mol/dm^3) KOH aqueous solution (D). Complex formation in A or D was performed without agitation, whereas that in B or C was done with agitation. All reactions were carried out in a dark room and at room temperature. The resultant precipitates were filtered off, washed with a small amount of water and then dried in a $CaCl_2$ desiccator.

Tables I—IV summarize the reaction conditions (the kind of ligands, the amount of solvents, the reaction time, and the yield of products). The results of elemental analysis of the complexes synthesized are listed in Table V. The platinum complexes gave reproducible elemental analysis values consistent with the expected structures.

The antitumor activity of the platinum complexes was determined against ascites Sarcoma 180 tumor in mice. One million cells of Sarcoma 180 were transplanted intraperitoneally (i.p.) into female ICR/JCL mice on Day 0 of each test. On the next day (Day 1), a saline suspension of a platinum complex at a dosage of 10 ml/kg saline was injected i.p. into the mice, which were observed for 60 d. Antitumor activity was expressed as T/C (%), calculated by using the following equation.

$$T/C\ (\%) = L_T/L_C \times 100$$

L_T: average survival time of treated animals in days.
L_C: average survival time of untreated controls in days.

Five mice were used in each group. The antitumor activity was evaluated as (+): T/C 150—199%, (++): 200—299%, (+++): >300%, and (−): <150%. No evaluation was performed with the animal groups in which deaths occurred owing to toxicity (*i.e.*, death within 5 d of drug administration).

Results and Discussion

The generalized structure of platinum complexes reported in this paper can be expressed as *cis*-$[PtCl_2L_2]$ (L=ligand). The formation of the *cis* configuration of Pt complexes is due to the *trans* effect originating from the platinum metal: *cis* complexes are usually stable at room temperature, but at higher temperature, the *cis* form isomerizes to the *trans* one.[4] The infrared spectra of the synthesized Pt complexes showed a weak absorption at 430—565 cm^{-1} (Pt–N stretching). This absorption is due to the *cis*-form of platinum complex.

The position of substitution in aniline derivatives as ligands of **1** greatly affected the synthesis. As shown in Table I, the *cis*-platinum complexes coordinating 2-substituted anilines

TABLE I. Reaction Conditions and Yield of Coordination Products of Monosubstituted Anilines with **2**[a]

No.	R	Position of substituent group	Solvent[b]	(ml)	Reaction time (d)	Yield (%)
1	OH	2	A	15	3	26
2	OH	3	A	4	8	83
3	OH	4	A	17	4	47
4	COOH	2	A	10	10	82
5	COOH	3	A	20	2	66
6	COOH	4	B	2	2	69
7	CH_3	2	C	2	6	91
8	CH_3	3	C	2	2	94
9	CH_3	4	B	2	1	68
10	NH_2	3	A	2	7	—
11	NH_2	4	A	5	1	86
12	$COOCH_3$	2	B	2	3	45
13	C_6H_5	2	B	2	4	58
14	$CONH_2$	4	A	5	1	85
15	SO_2NH_2	4	A	5	1	70
16	C_6H_4-4-NH_2	4	B	5	1	100
17	NNC_6H_5	2	C	4	14	13
18	SO_3H	4	A	7	16	—

a) **2** (0.5 mmol) was dissolved in 2 ml of H_2O. *b*) A, H_2O; B, ethyl acetate; C, benzene.

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TABLE II. Reaction Conditions and Yield of Coordination Products of Disubstituted or Trisubstituted Anilines with **2**[a]

No.	R_1 R_2	Position of substituent group	Solvent[b]	(ml)	Reaction time (d)	Yield (%)
19	NH_2	2	A	4	23	—
	NH_2	4				
20	NO_2	2	B	2	16	57
	CH_3	4				
21	NO_2	3	B	2	14	46
	CH_3	4				
22	NO_2	2	B	2	10	26
	Cl	4				
23	NO_2	2	B	4	15	—
	NO_2	4				
24	COOH	2	D	4	9	—
	NO_2	5				
25	COOH	2	D	2	3	9
	CH_3	4				
26	CH_3	2	A	10	3	98
	NC_6H_4-4-$N(CH_3)_2Cl$	4				
	NH_2	5				

a) **2** (0.5 mmol) was dissolved in 2 ml of H_2O. *b*) A, H_2O; B, ethyl acetate; D, 0.2 M KOH.

TABLE III. Reaction Conditions and Yield of Coordination Products of N-Substituted, N-Substituted-2-, or N-Substituted-4-Anilines with **2**[a]

No.	R_1[b] R_2	R_3[b]	Position of substituent group	Solvent[c]	(ml)	Reaction time (d)	Yield (%)
27	$C_6H_4NNC_6H_5$			C	2	8	—
28	CH_3	$COOCH_3$	2	B	1	17	—
29	CH_3	CH_3	4	B	2	9	24
	CH_3						
30	CH_3	NNC_6H_5	4	C	2	13	—
	CH_3						
31[d]	CH_3	$NC_{10}H_6O$	4	B	21	—	—
	CH_3						
32	CH_3	NNC_6H_4-4-SO_3H	4	A	10	—	—
	CH_3						
33	CH_3	NNC_6H_4-4-COOH	4	D	10	—	—
	CH_3						

a) **2** (0.5 mmol) was dissolved in 2 ml of H_2O. *b*) R_1, R_2, N-substituent group; R_3, ring substituent group. *c*) A, H_2O; B, ethyl acetate; C, benzene; D, 0.2 M KOH. *d*) The ligand is indophenol.

TABLE IV. Reaction Conditions and Yield of Coordination Products of 1-Naphthylamines, 2-Aminoanthracene or 3-Aminopyrene with **2**[a]

No.	Ligand	Solvent[b]	(ml)	Reaction time (d)	Yield (%)
34	1-Naphthylamine	B	2	3	56
35	4-Phenylazo-1-naphthylamine	C	3	3	52
36	2-Aminoanthracene	C	6	38	—
37	3-Aminopyrene	C	2	2	40

a) **2** (0.5 mmol) was dissolved in 2 ml of H_2O. *b*) B, ethyl acetate; C, benzene.

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TABLE V. Elemental Analysis of Obtained Complexes

No.	Formula	Analysis (%) Calcd (Found)					
		C	H	Cl	N	Pt	S
1	$[PtCl(C_6H_6NO)(C_6H_7NO)]$	32.18	2.93	7.92	6.26	43.57	
	$C_{12}H_{13}ClN_2O_2Pt$	(31.83	2.74	8.08	6.29	42.95)	
2	$[PtCl_2(C_6H_7NO)_2]$	29.76	2.92	14.64[a]	5.79	40.29	
	$C_{12}H_{14}Cl_2N_2O_2Pt$	(29.55	2.83	12.34	5.84	41.13)	
3	$[PtCl_2(C_6H_7NO)_2]$	29.76	2.92	14.64[a]	5.79	40.29	
	$C_{12}H_{14}Cl_2N_2O_2Pt$	(29.20	2.88	12.96	5.83	39.96)	
4	$[PtCl_2(C_7H_7NO_2)_2]$	31.12	2.62	13.12[a]	5.19	36.11[a]	
	$C_{14}H_{14}Cl_2N_2O_4Pt$	(31.64	2.69	11.02	5.22	34.27)	
5	$[PtCl_2(C_7H_7NO_2)_2]\cdot H_2O$	30.12	2.89	12.70	5.02	34.94[a]	
	$C_{14}H_{16}Cl_2N_2O_5Pt$	(30.60	2.64	12.79	5.24	33.50)	
6	$[PtCl_2(C_7H_7NO_2)_2]\cdot H_2O$	30.12	2.89	12.70	5.02	34.94	
	$C_{14}H_{16}Cl_2N_2O_5Pt$	(29.18	2.46	12.92	5.18	35.59)	
7	$[PtCl_2(C_7H_9N)_2]$	35.01[a]	3.78	14.76	5.83	40.62[a]	
	$C_{14}H_{18}Cl_2N_2Pt$	(37.42	3.89	14.38	5.51	36.97)	
8	$[PtCl_2(C_7H_9N)_2]$	35.01[a]	3.78	14.76	5.83	40.62[a]	
	$C_{14}H_{18}Cl_2N_2Pt$	(37.72	3.89	14.16	5.77	36.24)	
9	$[PtCl_2(C_7H_9N)_2]$	35.01	3.78	14.76	5.83	40.62	
	$C_{14}H_{18}Cl_2N_2Pt$	(34.62	3.55	15.14	5.85	39.86)	
11	$[Pt_4Cl_8(H_2O)(C_6H_8N_2)_7]\cdot 2H_2O$	26.90	3.34	15.12	10.46	41.62	
	$C_{42}H_{62}Cl_8N_{14}O_3Pt_4$	(26.69	3.02	15.22	10.46	41.77)	
12	$[PtCl_2(C_8H_9NO_2)_2]$	33.81	3.20	12.47	4.93	34.33[a]	
	$C_{16}H_{18}Cl_2N_2O_4Pt$	(33.45	2.91	12.82	4.81	31.61)	
13	$[PtCl_2(C_{12}H_{11}N)_2]$	47.68	3.68	11.73[a]	4.64	32.27[a]	
	$C_{24}H_{22}Cl_2N_2Pt$	(48.42	3.85	10.14	4.38	29.47)	
14	$[PtCl_2(C_8H_{10}N_2O)_2]\cdot H_2O$	32.88	3.80	12.13	9.59	33.38[a]	
	$C_{16}H_{22}Cl_2N_4O_3Pt$	(32.47	3.56	12.69	9.57	30.42)	
15	$[PtCl_2(C_6H_8N_2O_2S)_2]$	23.61[a]	2.65	11.61[a]	9.18	31.96[a]	10.50
	$C_{12}H_{16}Cl_2N_4O_4PtS_2$	(24.69	2.53	10.18	9.81	28.83	10.30)
16	$[PtCl_2(C_{12}H_{12}N_2)_2]$	45.43	3.82	11.17	8.83	30.75[a]	
	$C_{24}H_{24}Cl_2N_4Pt$	(45.42	3.81	11.47	8.92	27.93)	
17	$[PtCl_2(C_{14}H_{15}N_3)_2]\cdot 2H_2O$	44.68	4.56	9.42	11.17	25.92[a]	
	$C_{28}H_{34}Cl_2N_6O_2Pt$	(45.24	4.36	9.91	11.26	23.80)	
20	$[PtCl_2(C_7H_8N_2O_2)_2]$	29.48	2.83	12.43	9.83	34.21	
	$C_{14}H_{16}Cl_2N_4O_4Pt$	(29.38	2.61	12.41	9.81	34.28)	
21	$[PtCl_2(C_7H_8N_2O_2)_2]$	29.48	2.83	12.43	9.83	34.21	
	$C_{14}H_{16}Cl_2N_4O_4Pt$	(28.97	2.91	12.78	9.75	33.82)	
22	$[PtCl_2(C_6H_5N_2O_2Cl)_2]$	23.58	1.65	23.20	9.17	31.92[a]	
	$C_{12}H_{10}Cl_4N_4O_4Pt$	(23.59	1.47	22.85	9.27	30.64)	
29	$[PtCl_2(C_9H_{13}N)_2]$	40.30[a]	4.90	13.22	5.22	36.37[a]	
	$C_{18}H_{26}Cl_2N_2Pt$	(37.91	4.37	13.63	5.14	38.38)	
34	$[PtCl_2(C_{10}H_9N)_2]$	43.48	3.29	12.84	5.07	35.32[a]	
	$C_{20}H_{18}Cl_2N_2Pt$	(44.14	3.33	12.68	5.09	34.13)	
35	$[PtCl_2(C_{16}H_{13}N_3)_2$	50.53[a]	3.45	9.32	11.05	25.65	
	$C_{32}H_{26}Cl_2N_6Pt$	(49.42	3.30	10.16	10.40	26.44)	
37	$[PtCl_2(C_{16}H_{11}N)_2]$	54.86[a]	3.17	10.12	4.00	27.85	
	$C_{32}H_{22}Cl_2N_2Pt$	(56.40	3.32	10.72	3.64	28.67)	

a) These complexes are not pure judging from the elemental analysis data.

were generally obtained in low yields even at a long reaction time, perhaps due to steric hindrance at the position *ortho* to the substitution position. On the other hand, the complexes coordinating either 3- or 4-substituted anilines were obtained within a short time in high

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TABLE VI. Antitumor Activity of Platinum Complexes against Sarcoma 180

No.	Dose (mg/kg)	*T/C* (%)	Deaths (toxicity)	Evaluation
2	50	282	0	++
	100	255	0	++
	200	—	4	Toxic
3	50	255	0	++
	100	309	0	+++
	200	—	4	Toxic
4	50	120	0	−
	100	120	0	−
	200	82	0	−
5	50	100	0	−
	100	136	0	−
	200	—	5	Toxic
6	100	136	0	−
	200	—	5	Toxic
7	12.5	206	0	++
	25	182	0	+
	50	—	2	Toxic
	100	—	1	Toxic
	200	—	4	Toxic
8	50	309	0	+++
	100	318	0	+++
	200	—	4	Toxic
9	25	148	0	−
	50	205	0	++
	100	57	0	−
	200	—	4	Toxic
11	200	273	0	++
12	100	238	0	++
	200	—	5	Toxic
13	25	330	0	+++
	50	—	3	Toxic
	100	—	4	Toxic
	200	—	4	Toxic
14	50	200	0	+
	100	227	0	++
	200	133	0	−
15	100	318	0	+++
	200	—	5	Toxic
16	25	73	0	−
	50	145	0	−
	100	164	0	+
	200	100	0	−
20	50	210	0	++
	100	210	0	++
	200	—	5	Toxic
21	50	255	0	++
	100	—	3	Toxic
	200	—	5	Toxic
34	25	178	0	+
	50	264	0	++
	100	292	0	++
	200	428	0	+++
35	200	120	0	−
	400	336	0	+++
37	200	224	0	++

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Table VII. Antitumor Activity

No.	Dose (mg/kg)	Platinum weight (mg/kg)	(%)	Evaluation of antitumor activity
3	50	20	39.96	++
	100	40	39.96	+++
	200	80	39.96	Toxic
8	50	18	36.24	+++
	100	36	36.24	+++
	200	72	36.24	Toxic
13	25	7	29.47	+++
	50	15	29.47	Toxic
	100	29	29.47	Toxic
	200	59	29.47	Toxic
15	100	29	28.83	+++
	200	58	28.83	Toxic
34	25	9	34.13	+
	50	17	34.13	++
	100	34	34.13	++
	200	68	34.13	+++
35	200	53	26.44	−
	400	106	26.44	+++
cis-DDP	7	5	65.00	+++

cis-DDP: *cis*-dichlorodiamineplatinum.

yields. The latter reactions may be favored by low steric hindrance at the *meta* and *para* positions.

As shown in Table II, the *cis*-platinum complexes coordinating disubstituted anilines were, in general, formed after a fairly long reaction time, and in lower yields than those of monosubstituted anilines. The platinum complexes of diamino- (No. 19) and dinitro- (No. 23) substituted anilines were not obtained even after reaction times of 23 and 16 d, respectively.

Table III shows the result of the reaction between **2** and N-substituted aniline or N-substituted-2- or N-substituted-4-anilines. In this series of reactions, only *N*,*N*-dimethyl-4-methylaniline (No. 29, yield 24%) reacted with **2** to yield the expected complex; The other anilines reacted to yield unidentified Pt complexes (Nos. 10, 18, 19, 23, 24, 27, 28, 30 and 36) or did not react (Nos. 31, 32 and 33).

Other aromatic amines such as 1-naphthylamine (No. 34), 4-phenylazo-1-naphthylamine (No. 35), and 3-aminopyrene (No. 37) reacted with **2** to produce their *cis*-Pt complexes in yields of 56, 52 and 40%, respectively, as indicated in Table IV. 2-Aminoanthracene reacted with **2**, but the product differed from the expected one as judged from the result of elemental analysis. The steric hindrance of these ligands may not be significant.

As shown in Table V, there was good coincidence between the elemental analysis data for most Pt complexes and the calculated values. The exceptions are marked by *a*).

Some complexes (Nos. 6—9, 12, 13, 16, 17, 20—23, 27—31) were synthesized by the use of an interfacial reaction. Since these ligand compounds were only soluble in organic solvents or were more soluble in organic solvents than water, the use of ethyl acetate (B) or benzene (C) was effective in the coordination reaction. The appropriate choice of solvent can increase the yield of Pt complexes. Some surfactants were tried as phase transfer catalysts to promote the reaction. The details will be reported elsewhere.

The antitumor activities of the synthesized *cis*-Pt complexes evaluated in terms of *T*/*C* (%) against ascites Sarcoma 180 tumor are listed in Table VI. Some complexes (Nos. 3, 8, 13, 15, 34 and 35) had high antitumor activities. No clear correlation was observed between

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antitumor activities and the kind or substitution position of ligands. However, we speculate that *cis*-Pt complexes coordinating i) lipophilic ligands, or ii) *para*-substituted anilines and 1-naphthylamine tend to show higher antitumor activity. These complexes are also easy to synthesize in high yields.

The antitumor activity of these effective complexes (Nos. 3, 8, 13, 15, 34 and 35) and the platinum weight (mg/kg) at each dosage are shown in Table VII. The platinum weights of the administered dosed complexes were more than 8 times larger than that of **1** to get (+++) evaluation. For example, the weights required were *ca.* 40 mg/kg for Nos. 3 and 8, 68 mg/kg for No. 34, and 106 mg/kg for No. 35. However, Nos. 8 and 13 showed antitumor activity at smaller platinum weights than the above complexes. In particular, No. 13 showed (+++) evaluation at a platinum weight of 7 mg/kg, which is only twice the dosage level of **1** required for the same effectiveness. As the toxic dose of **1** has been reported to be 10 mg/kg,[5] the toxicity of platinum complexes might depend markedly on their ligands. Complex No. 8 was effective over a wide range of platinum weight. These findings suggest that it will be possible to prepare complexes having lower toxicity and higher antitumor activity than those of **1**.

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