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Synthesis of Platinum(II) Complexes of 4-Substituted *o*-Phenylenediamine Derivatives and Determination of Their Antitumor Activity

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Pt(II) complexes of 4-substituted *o*-phenylenediamine (opda-R; R=CH_3, OCH_3, H, Cl, COOH, NO_2, and SO_3H), PtX_2(opda-R), were synthesized and tested for antitumor activity against P-388 leukemia. The infrared spectra of those complexes were analyzed to assign the Pt-N stretching vibrations on the basis of the halogeno *trans* effect and Hammett's σ values. The assigned Pt-N strectching vibrations were related to the antitumor activities of the opda-R Pt(II) complexes.

Keywords——antitumor activity; Pt(II) complexes; 4-substituted *o*-phenylenediamine; P-388 leukemia; assignments of Pt-N stretching.

Since the discovery of the antitumor activity of *cis*-dichlorodiammineplatinum(II) by Rosenberg *et al.*,[2] various Pt complexes have been synthesized and tested for similar activity. Among them, *o*-phenylenediamine (opda-H) Pt(II) complex was reported to show antitumor activity.[3,4] We synthesized various Pt(II) complexes of opda-R, in which the 4-position is occupied by various substituting groups, CH_3, OCH_3, Cl, COOH, NO_2, and SO_3H, and examined the effects of these groups on the infrared spectra, and on the physiological activity.

Experimental

o-Phenylenediamine (Katayama Chemical Co., Ltd.), 4-methyl-, 4-chloro-, and 4-nitro-opda, and 3,4-diaminobenzoic acid (Tokyo Kasei Chemical Co. Ltd.) were converted to their hydrochlorides. 4-Methoxy-opda·2HCl was prepared by the reduction of 4-methoxy-2-nitroaniline, and 3,4-diaminobenzenesulfonic acid was prepared by the reduction of 2-nitroaniline-4-sulfonic acid. *p*-Aniline derivatives were purified before use by distillation or recrystallization.

Synthesis of Complexes——$PtCl_2$(opda-R) complexes were prepared according to the method of Connors *et al.*,[3] by the reaction of potassium tetrachloroplatinate with hydrochlorides of opda-R in a 1:1 ratio in 1N HCl solution for one day at 50°. The precipitates produced were collected and washed with water, acetone and ether, successively, then dried *in vacuo*. In the case of R=SO_3H, the reaction solution was concentrated, and the precipitates were collected. $PtBr_2$(opda-R) and PtI_2(opda-R) were prepared in the same way as the dichloro complexes, in the presence of excess KBr and KI. $PdCl_2$(opda-R) complexes were also prepared by the method used for Pt complexes, by reacting potassium tetrachloropalladate with opda-R. *cis*-$PtCl_2$-(*p*-R-an)$_2$ complexes, where *p*-R-an represents a *p*-substituted aniline derivative, were prepared by reacting potassium tetrachloroplatinate in water with 2 mol of p-R-an in aqueous acetone solution for one day, based on the method used for synthesizing *cis*-dichloro(dipyridine)platimun(II).[5] Elemental analyses of all the complexes obtained were satisfactory, and are shown in part in Table I.

Evaluation of Antitumor Activity——One million P-388 leukemia cells were transplanted intraperitoneally into CDF_1 mice on day 0. Treatment was given intraperitoneally twice (on days 1 and 5). The mean survival times of the treated (T) and control (C) groups (6 mice/group) were calculated, and the antitumor activity was expressed T/C%. Values of T/C% exceeding 120 were taken as indicating effectiveness.

Apparatus——Infrared spectra were measured with Jasco IRA-2 and ID-710G infrared spectrophotometers, by means of the KBr disc and nujol mull methods.

1) Location: *3-1 Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.*
2) B. Rosenberg, L. VanCamp, J.E. Trosko, and V.H. Mansour, *Nature* (London), **222**, 385 (1969).
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4) L.M. Hall, R.J. Speer, H.J. Ridgway, and J.M. Hill, *J. Clin. Hemat. and Oncol.*, **7**, 877 (1977).
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Table I. Elemental Analysis of $PtCl_2$(opda-R) Complexes and Their Yields

Pt(II) Complex	Yield (%)	Analysis (%) Calcd. C	H	N	Found. C	H	N
$PtCl_2$(opda-CH_3)	70.3	2.60	21.66	7.22	2.73	21.73	7.37
$PtCl_2$(opda-OCH_3)	84.6	2.49	20.80	6.94	2.54	21.14	6.65
$PtCl_2$(opda-H)	94.7	2.15	19.26	7.49	2.04	19.05	7.81
$PtCl_2$(opda-Cl)	93.7	1.73	17.63	6.86	1.63	17.87	6.78
$PtCl_2$(opda-COOH)	91.0	1.93	20.11	6.70	2.06	20.06	6.53
$PtCl_2$(opda-NO_2)	70.0	1.68	17.19	10.03	1.73	17.17	10.04
$PtCl_2$(opda-SO_3H)	60.0	1.77	15.86	6.17	2.33	15.71	6.31

Results and Discussion

$PtCl_2$ (opda-R) complexes were synthesized in high yields, as shown in Table I. They are hardly soluble in water, and the complexes and the ligands are both unstable in alkaline solutions. Therefore, the syntheses were performed in 1N HCl solution. This method is also useful in that it avoids 1: 2 opda-R complex formation.

IR Spectral Analysis

The main absorption bands due to the ligands of $PtCl_2$ (opda-R) are shown in Table II. The IR spectra of the Pt(II) complexes were analyzed mainly in the 700—400 cm^{-1} region, which contained the absorption bands due to Pt-ligand bonds. These bands indicate the nature of the coordination bond. We next attempted the assignment of Pt–N stretching vibrations.

Table II. Infrared Spectral Data for $PtCl_2$(opda-R) Complexes

Substituent group (R)	ν_{NH_2}	δ_{NH_2}	ρ_{NH_2}	Other bands (cm^{-1})
CH_3	3210, 3170	1541, 1534	709	
OCH_3	3205, 3175	1556, 1540	737	ν_{C-O-C} 1220, 1103, 1024
H	3210, 3160	1546, 1524	701	
Cl	3200, 3160	1535	703	
COOH	3250, 3190	1548	698	$\nu_{asy(CO_2H)}$ 1714, $\nu_{syn(CO_2H)}$ 1313
NO_2	3185, 3140	—	758	$\nu_{asy(NO_2)}$ 1546, $\nu_{syn(NO_2)}$ 1353, 1321
SO_3H	3195, 3165	1549, 1535	708	ν_{SO} 1135, 1037

Figure 1 shows the IR spectra of opda-CH_3 complexes together with that of the ligand. The band at 606 cm^{-1} of $PtCl_2$(opda-CH_3) was not observed in the ligand. When Cl^- was replaced with Br^- and I^-, the corresponding peaks were shifted to 601 and 589 cm^{-1}, respectively; these shifts toward lower wave number can be explained in terms of the *trans* effect of halogens. When the central metal ion of $PtCl_2$(opda-CH_3) was changed to Pd^{2+}, a peak was found at 582 cm^{-1} in $PdCl_2$(opda-CH_3). These results are shown in Fig. 1.

In the case of PtX_2(opda-H) complexes, the peaks at 628, 625, and 613 cm^{-1} for X=Cl, Br, and I, respectively, were the most sensitive to the *trans* effect of halogens. The peak at 628 cm^{-1} of $PtCl_2$(opda-H) was shifted to 613 cm^{-1} when Pt^{2+} was replaced with Pd^{2+}.

Similar shifts to lower wave number on replacing Pt^{2+} with Pd^{2+} or on changing the halogen were reported for *cis*-dichloro platinum(II) complexes of diammine, ethylenediamine, and dipyridine.[6]

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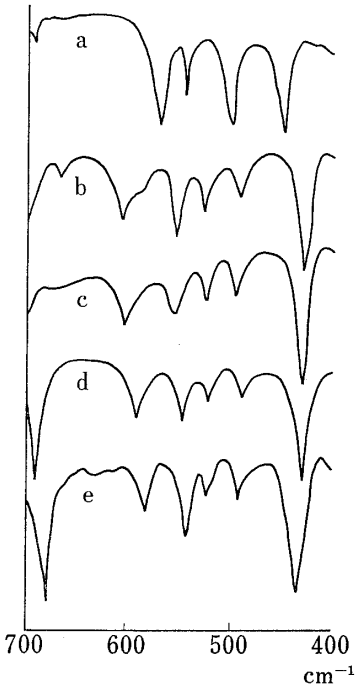

Fig. 1. Infrared Spectra of Pt(II) and Pd(II) Complexes of 4-Methyl-*o*-phenylenediamine

a: opda-CH_3·2HCl. b: $PtCl_2$ (opda-CH_3). c: $PtBr_2$ (opda-CH_3). d: PtI_2 (opda-CH_3). e: $PdCl_2$ (opda-CH_3).

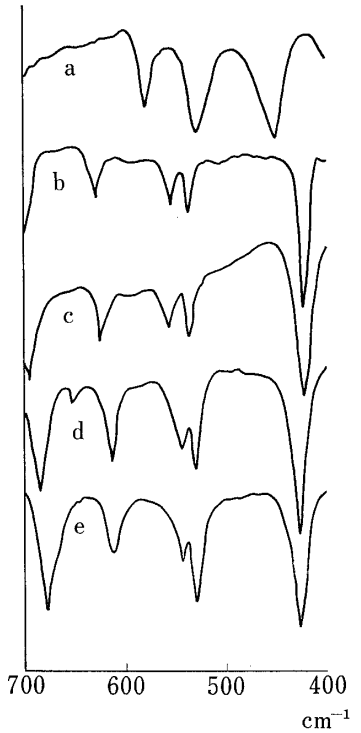

Fig. 2. Infrared Spectra of Pt(II) and Pd(II) Complexes of *o*-Phenylenediamine

a: opda-H. b: $PtCl_2$(opda-H). c: $PtBr_2$ (opda-H). d: PtI_2 (opda-H). e: $PdCl_2$ (opda-H).

For the Pt(II) complexes of other opda derivatives, the Pt–N stretching vibrations were assigned on the basis of similar considerations, and the results are tabulated in Table III. However, in the case of $PtCl_2$(opda-SO_3H), many bands appeared in the region of 700—400 cm^{-1} and the assignment of the Pt–N stretching vibration was difficult. In order to confirm the validity of these assignments, Pt(II) and Pd(II) complexes of aniline derivatives with substituting groups at the *para* position, abbreviated as p-R-an, were synthesized in order to compare their IR spectra with those of the opda-R Pt(II) complexes.

Figure 3 shows the IR spectra of Pt(II) and Pd(II) complexes of the aniline derivatives with R=CH_3 and NO_2 together with those of the ligands. The peaks at 567 and 561 cm^{-1} of *cis*-$PtCl_2$(p-CH_3-an)$_2$ and *cis*-$PtCl_2$(p-NO_2-an)$_2$, respectively, were not obserbed in the spectra of the ligands, and these peaks shifted to lower wave number, *i. e.* 557 and 550 cm^{-1}, when Pt^{2+} was replaced with Pd^{2+}. We therefore assigned the bands to the Pt–N stretching

Table III. Assigned Pt–N and Pt–Cl Stretching Vibrations and Hammett's Sigma Values

Substituent group (R)	ν_{Pt-N}[a] (cm^{-1})	ν_{Pt-N}[b] (cm^{-1})	ν_{Pt-Cl}[b] (cm^{-1})	σ_m	$\sigma_p^{(-)}$	$\sigma_m+\sigma_p^{(-)}$	pK_{a2}
CH_3	606	567	325, 317	−0.07	−0.13	−0.20	4.68
OCH_3	609	563	330, 325	+0.08	−0.11	−0.03	4.83
H	628	573	330, 318	0.00	0.00	0.00	4.47
Cl	588	568	325, 316	+0.37	+0.24	+0.61	3.83
COOH	580	566	333	+0.36	+0.73	+1.09	3.49
NO_2	578	561	330	+0.71	+1.27	+1.98	2.61

a) Pt-N stretching vibrations of $PtCl_2$ (opda-R).
b) Pt-N and Pt-Cl stretching vibrations of $PtCl_2$ (p-R-an)$_2$.

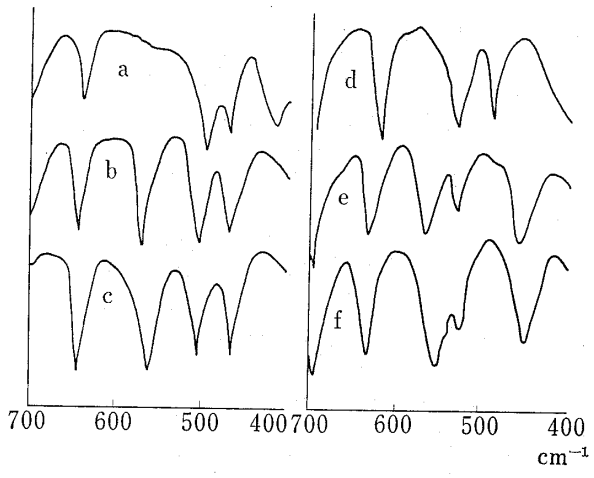

Fig. 3. Infrared Spectra of Pt(II) and Pd(II) Complexes of *p*-Substituted Aniline Derivatives

a: *p*-methylaniline. b: *cis*-$PtCl_2$(p-CH_3-an)$_2$.
c: *cis*-$PdCl_2$(p-CH_3-an)$_2$.
d: *p*-nitroaniline. e: *cis*-$PtCl_2$(p-NO_2-an)$_2$.
f: *cis*-$PdCl_2$(p-NO_2-an)

vibration. The *cis* configuration of $PtCl_2$-(p-R-an)$_2$ was confirmed by the characteristic absorption bands due to symmetric and asymmetric stretching vibrations of Pt–Cl,[7] as summarized in Table III. The Pt–N stretching vibrations of Pt(II) complexes of other p-R-an derivatives were assigned on the basis of similar considerations (Table III).

The Pt–N bands of *cis*-$PtCl_2$(p-R-an)$_2$ were observed at 561—573 cm^{-1}, while those of $PtCl_2$(opda-R) were observed in a higher wave number region, 578—628 cm^{-1}. This indicates that the latter complexes are stabilized by the formation of a chelate ring.

Relationship between the Hammett Values and Pt-N Stretching Vibrations

Since the pK_a values of the ligands affect the stability of the complexes, substitution effects on the assigned Pt–N stretching were studied. Hammett's σ values (σ_m, σ_p, total values of $\sigma_m+\sigma_p$) for opda-R,[8] the wave numbers of the Pt–N stretching vibrations and the pK_{a2} values[9] are shown in Table III. A plot of the pK_{a2} values against total values of $\sigma_m+\sigma_p$ was linear, but no linear relation was found between pK_{a1} and σ values. pK_a values are closely related to the stability constants of the complexes. If we postulate that larger stability constants correspond to stronger metal-ligand bonds, then a proportionality should exist between Pt–N and total values of $\sigma_m+\sigma_p$. This was indeed the case, as shown in Fig. 5.

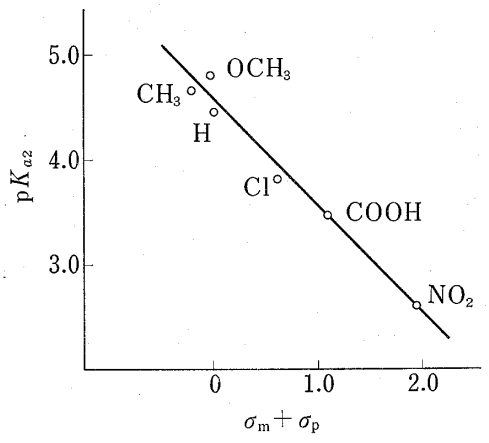

Fig. 4. Relationship between the Values of pK_{a_2} and the Sum of Hammett's Sigma Values for *para*-Substituted *o*-Phenylenediamine Derivatives

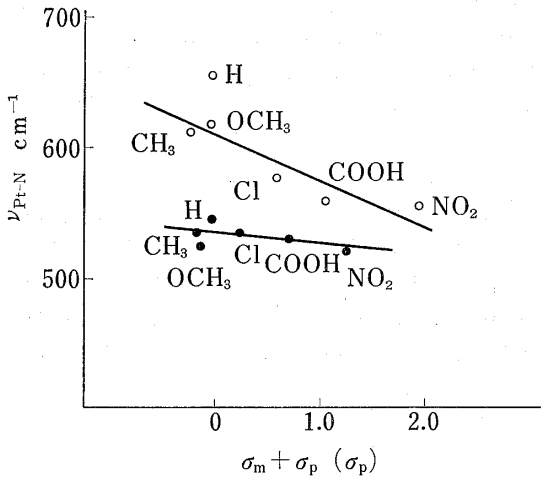

Fig. 5. Relationship between the Pt–N stretching Vibrations and Hammett's Sigma Values

○: $PtCl_2$(opda-R). ●: *cis*-$PtCl_2$(p-R-an)$_2$.

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Thus we can predict the relative stability of the Pt(II) complexes for various substituting groups. The stability of opda-Cl is lower than those of opda-H and opda-CH_3, and opda-NO_2 is the least stable. This is reminiscent of the relationship between the stability constants of Cu(II)(opda-R) complexes and the total values of $\sigma_m+\sigma_p$, measured by Kina.[10]

In the case of *p*-substituted aniline derivatives, no marked differences in Pt–N stretching vibrations were observed, but a similar relationship was observed between σ_p and Pt–N stretching, shown in Fig. 5. These results also support the validity of the assignments of Pt–N stretching vibrations of $PtCl_2$(opda-R).

Table IV. Antitumor Screening Results for $PtCl_2$(opda-R) Complexes against P-388 Leukemia

Substituent group (R)	Dose (mg/kg) 100	50	25 (T/C %)	12.5	6.25
H	—	—	110	193	163
CH_3	—	—	150	171	153
Cl	—	—	168	157	150
NO_2	—	—	137	118	110
OCH_3	—	—	131	144	132
COOH	120	110	100	102	100
SO_3H	143	124	119	111	102

Underlining indicates a positive effect ($T/C \leqq 120\%$).

Antitumor Activity

The results of antitumor screening tests of $PtCl_2$(opda-R) complexes against P-388 leukemia are shown in Table IV. The complex must maintain the N,N-coordination structures, which is required for activity, until it reaches the target DNA (the presumed site of action). Therefore, the higher the Pt–N stretching vibration, the stronger the chelate ring of the complex, and the more likely it is that antitumor activity will appear. This may be why opda-H and opda-CH_3 complexes showed higher activity. In the case of opda-R, with R=COOH and SO_3H, very low activity was seen. This may be explained by the fact that they both exist as ionic species, $[PtCl_2(opda\text{-}COO]^-$ and $[PtCl_2(opda\text{-}SO_3)]^-$, which would be unfavorable for penetration of the cell membranes.

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