Synthesis, Structure and Antitumor Activity of a New Water-Soluble Platinum Complex, (1*R*,2*R*-Cyclohexanediamine-*N*,*N*′)[2-hydroxy-4-oxo-2-pentenoato(2-)-O^2]platinum(II)

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The reaction of dihydroxo(1*R*,2*R*-cyclohexanediamine)platinum(II) with 2,4-dioxopentanoic acid gave a water-soluble complex, (1*R*,2*R*-cyclohexanediamine-*N*,*N*′)[2-hydroxy-4-oxo-2-pentenoato(2-)-O^2]platinum(II). The structure of the complex was determined by X-ray crystal analysis. The data indicated a chelation of the acetylacetonato part of 2,4-dioxo-pentanoic acid to platinum(II). The complex showed moderate antitumor activity against murine leukemia L1210 in mice (T/C=195% at a dose of 200 mg/kg) and high activity against cisplatin-resistant L1210 leukemia (T/C=275% at a dose of 25 mg/kg).

Keywords platinum(II) complex; 2,4-dioxo-pentanoic acid; X-ray analysis; antitumor activity; cisplatin resistance; MNDO-PM3 calculation

Since its discovery, *cis*-dichlorodiamineplatinum(II)(cisplatin)[1] has become a major marked drug used in the treatment of human solid tumors such as genito-urinary and gynecologic tumors as well as head, neck, and lung tumors.[2]

The usefulness of cisplatin is, however, compromised by its propensity to cause several severe dose-limiting toxicities, including nephrotoxicity, bone marrow toxicity, gastrointestinal toxicity, and neurotoxicity.[3] For the purpose of reducing or even eliminating the nephrotoxicity of cisplatin, new platinum coordination complexes, socalled second generation complexes, have been developed. The representatives of second-generation platinum complexes are carboplatin[4] and 254-S[5] (Chart 1).

1,2-Cyclohexanediamine platinum(II) complexes have been reported to indicate a lack of cross resistance to cisplatin against murine leukemia L1210.[6] Yet it is difficult to obtain 1,2-cyclohexanediamine platinum(II) complexes with high stability and solubility in water. We have attempted to synthesize a new water-soluble platinum(II) complex having the organic ligand 1,2-cyclohexanediamine as its carrier ligand.

In general, carrier ligands provide the spectrum of antitumor activity while "leaving groups" influence the stability and solubility of platinum complexes.[7] So our approach to development is to find new "leaving groups" to solve the above problems. Our synthetic plan is to design the "leaving groups" as bidentate ligands, which are expected to reduce toxicity and enhance stability in water, but may increase antitumor activity.

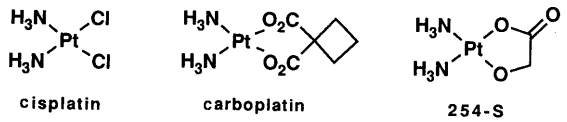

Chart 1

It is estimated that 2,4-dioxo-pentanoic acid (**3**) is equilibrated with the enol form (Chart 2). Accordingly, it is suggested that his compound acts as a bidentate leaving group. The metal complex having **3** as ligands has not been studied. In the binding form of platinum(II) atom to the leaving group, there are two possibilities: acetylacetonate form (I) and α-hydroxycarboxylate form (II) (Chart 3). Acetylacetonate form (I) is expected to have high water solubility by reason of the existence of a carboxylate ion. This paper reports the preparation, X-ray crystal structure determination and antitumor activity of water-soluble (1*R*,2*R*-cyclohexanediamine)platinum(II) complex with **3**.

Results and Discussion

Synthesis of (1*R*,2*R*-Cyclohexanediamine-*N*,*N*′)[2-hydroxy-4-oxo-2-pentenoato(2-)-O^2]platinum(II) Formulated with 3 as the "Leaving Group" Compound **3** was easily obtained from acetone (**1**) in two steps (Chart 4). The

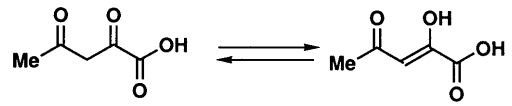

Chart 2

Chart 3

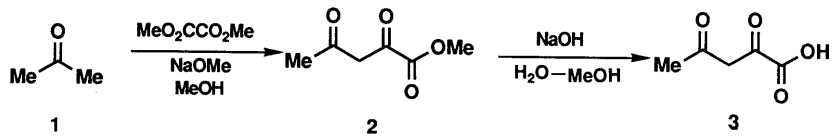

Chart 4

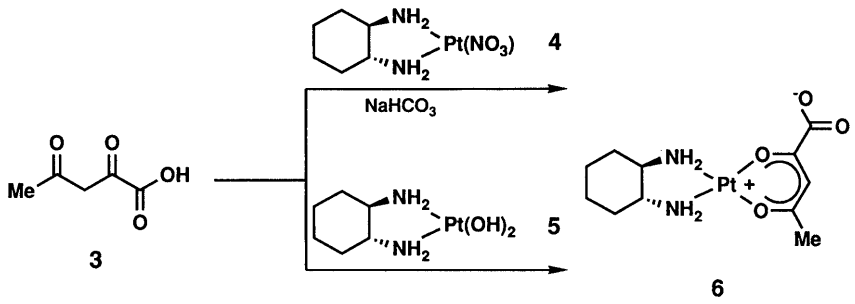

Chart 5

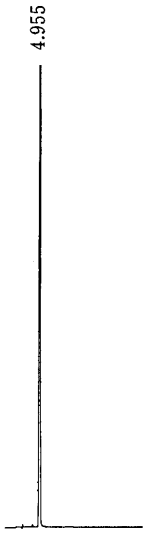

Fig. 1. Chromatogram of **6** on Reverse-Phase HPLC

Reverse-phase HPLC was carried out on a 4.6 mm × 250 mm Shiseido Capcell Pak C18 SG-120 column. Buffer was 10% methanol in water at a flow rate of 1 ml/min. The detection wavelength was 230 nm.

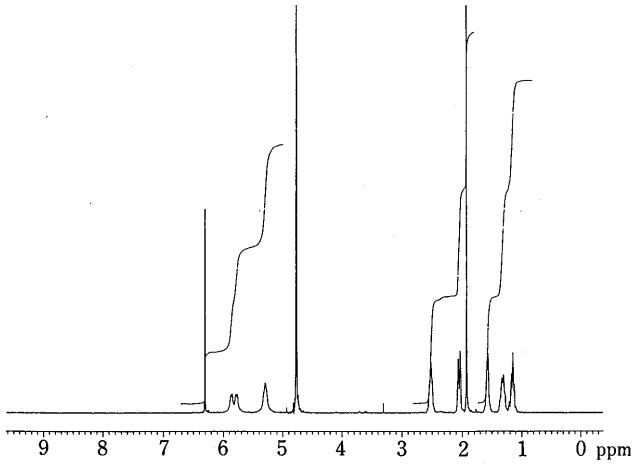

Fig. 2. ^{1}H-NMR (400 MHz, D_2O) Spectra of **6**

reaction of **1** with dimethyl oxalate gave the Aldol condensation product **2**.[8] Treatment with sodium hydroxide, methyl ester **2** afforded the corresponding carboxylic acid.

The desired platinum complex was prepared by treatment of carboxylic acid derivative **3** with dinitrato (1*R*,2*R*-cyclohexanediamine)platinum(II) (**4**)[9] or dihydroxo(1*R*,2*R*-cyclohexanediamine)platinum(II) (**5**)[10] in aqueous solution (Chart 5).

The obtained complex was purified by recrystallization from water and/or gel chromatography.

Figure 1 shows the high performance liquid chromatography (HPLC) chromatogram of the reactant of **4** and **3**.[11] On HPLC, the peak of the above platinum complex **6** was one. Further ^{1}H-NMR spectrum (Fig. 2) was found to be identical with the product formed from **4** with an equimolar **3**. These facts revealed that the obtained complex was stable,[12] and **3** combined to the platinum(II) atom as a good bidentate ligand.

The solubility of prepared platinum complex **6** in water was high as expected (more than 20 mg/ml at room temperature) compared to the solubility of cisplatin in water (1 mg/ml).

The Quantum Chemistry Calculation of 3 Yu. S. Bogachev *et al.* carried out CNDO/2 and CNDW/BW calculations of the energetics profiles of **3**.[13] Since **2** plays the role of bidentate ligand, we had to calculate the dianion of **3**. Figure 3 shows the optimized geometry of **3** dianion, which was calculated using the MNDO-PM3 molecular model. The acetylacetonate part is perpendicular to the carboxylate part. It is predicted that the platinum(II) atom is bound to **3** as an acetylacetone-form(I).

Structure of (1*R*,2*R*-Cyclohexanediamine-*N*,*N*')[2-hydroxy-4-oxo-2-pentenoato(2-)-O^2]platinum(II) (6). X-Ray Crystal Structure Analysis The reaction of **3** and **4** gave a yellow plate-shaped crystal. In order to elucidate the absolute structure of this complex **6**, a single crystal of the complex was subject to X-ray diffraction analysis.

The presence of three molecules of hydrated water is consistent with the result of elemental analysis. Figure 4 shows the chelation of the Pt(II) atom to **3** as an acetylacetonate-form ligand. The carboxy group in the leaving group has nothing to do with the binding to the Pt atom. Complex **6**, which has zwitter ionic character, is a new type of platinum(II) complex, while cisplatin, carboplatin and 254-S (Chart 1) are non-ionic platinum(II) complexes. It is expected that the cationic platinum(II) complex reacts more rapidly with nucleophilic reagents like DNA and RNA than a non-ionic platinum(II) complex, and shows higher antitumor activity. We consider that the carboxylate ion in the leaving group and the cationic Pt atom bring about high solubility in water. The Pt atom has a square planar geometry. (1*R*,2*R*-Cyclohexanediamine)platinum(II) fragment forms a six-membered (Pt–O–C–C–C–O) ring, which is nearly planar. This result is compatible with optimized geometry by MNDO-PM3 calculation.

Intermolecular hydrogen bonding was observed between

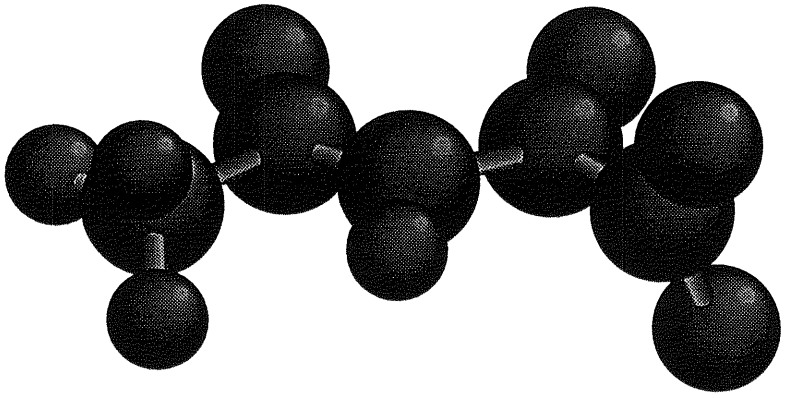

Fig. 3. The Optimized Geometry of **3**

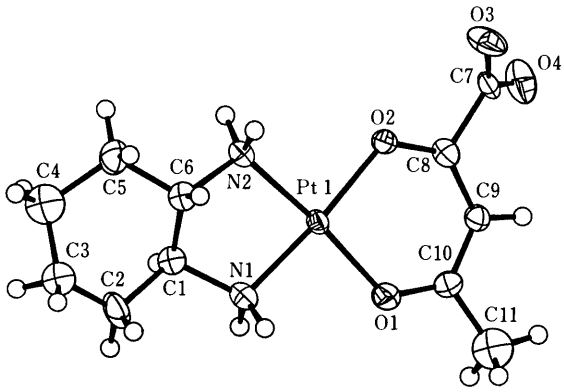

Fig. 4. ORTEP View of **6**

TABLE I. Selected Intramolecular Distances (Å) and Angles (deg) of Platinum Complex **6**

Bond distances					
Pt(1)–O(1)	1.992 (5)	Pt(1)–O(2)	2.004 (4)	Pt(1)–N(1)	2.017 (5)
Pt(1)–N(2)	2.035 (5)	O(1)–C(10)	1.273 (9)	O(2)–C(8)	1.280 (8)
O(3)–C(7)	1.235 (8)	O(4)–O(7)	1.246 (8)	N(1)–C(1)	1.46 (1)
N(2)–C(6)	1.50 (1)	C(1)–C(6)	1.39 (2)	C(7)–C(8)	1.538 (9)
C(8)–C(9)	1.386 (9)	C(9)–C(10)	1.40 (1)	C(10)–C(11)	1.51 (1)

Bond angles			
O(1)–Pt(1)–O(2)	94.5 (2)	O(1)–Pt(1)–N(1)	89.1 (2)
O(1)–Pt(1)–N(2)	172.9 (2)	O(2)–Pt(1)–N(1)	175.0 (2)
O(2)–Pt(1)–N(2)	92.0 (2)	N(1)–Pt(1)–N(2)	84.5 (2)
Pt(1)–O(1)–C(10)	123.6 (4)	Pt(1)–O(2)–C(8)	121.6 (4)
Pt(1)–N(1)–C(1)	110.5 (6)	Pt(1)–N(2)–C(6)	109.3 (6)
N(1)–C(1)–C(6)	118 (1)	N(2)–C(6)–C(1)	115 (1)
O(3)–C(7)–O(4)	128.3 (6)	O(3)–C(7)–C(8)	115.4 (6)
O(4)–C(7)–C(8)	116.4 (6)	O(2)–C(8)–C(7)	114.3 (5)
O(2)–C(8)–C(9)	127.7 (6)	C(7)–C(8)–C(9)	118.0 (6)
C(8)–C(9)–C(10)	126.7 (6)	O(1)–C(10)–C(9)	125.7 (6)
O(1)–C(10)–C(11)	115.1 (6)	C(9)–C(10)–C(1)	119.2 (6)

O(3) and H(1), O(4) and H(2), O(4) and H(7), and O(5) and H(6) with the oxgen–hydrogen distances of 1.919, 1.875, 1.835, and 1.964 Å, respectively. The O(4)–N(1) distance is 2.885(7) Å.

Table I lists some important bond distances and bond angles for complex **6**.

Antitumor Activity The antitumor activity of the prepared platinum complex **6** was tested against murine leukemia L1210 in mice.[14] The results are listed in Table II. This platinum complex **6** showed moderate activity at relatively high doses ($T/C = 195\%$ at a dose of 200 mg/kg). The potency of carboplatin having a bidentate ligand was lower than that of complex **6**. The cationic character of complex **6** may contribute to its increase in activity. On the other hand, the platinum compound **6** did not exhibit toxicity indicated by a decrease in T/C value, event at the highest dose of 200 mg/kg. Furthermore, complex **6** indicated potent antitumor activity against cisplatin-resistant leukemia L1210 (L1210/CDDP) *in vivo*, while cisplatin and carboplatin did not show any antitumor activity against

TABLE II. *In Vivo* Antitumor Activity of Pt Complexes against L1210

Compound	Dose (mg/kg)	T/C (%)	Survival mice on day 30
6	25	122	0/6
	50	126	0/6
	100	161	0/6
	200	195	0/6
Carboplatin	25	130	0/6
	50	143	0/6
	100	154	0/6
	200	130	0/6
Cisplatin	5.0	230	0/6
	7.5	152	1/6

TABLE III. *In Vivo* Efficacy of Pt Complexes against L1210/CDDP

Compound	Dose (mg/kg)	T/C (%)	Survival mice on day 30
6	3.2	126	0/6
	6.3	207	3/6
	12.5	245	4/6
	25	275	5/6
	50	182	2/6
	100	96	0/6
Carboplatin	3.2	107	0/5
	6.3	104	0/6
	12.5	99	0/6
	25	101	0/6
	50	109	0/6
	100	114	0/6
Cisplatin	1.3	106	0/6
	2.5	104	0/6
	5	101	0/6

this resistant tumor in mice, as expected. (Table III). The maximum T/C value obtained with complex **6** was 275% at a dose of 25 mg/kg. Complex **6** was more effective against cisplatin-resistant L1210 than against L1210 cells. To assess the *in vitro* cytotoxicity of complex **6**, the potency towards the L1210 cells was lower than that of cisplatin-resistant L1210 cells; the IC_{50} values were 0.62 μM to L1210 cells and 0.50 μM to cisplatin-resistant L1210 cells. The tendency of the *in vitro* assay was similar to that of the *in vivo* assay. We have investigated the cause of the marked difference of the antitumor activity *in vivo*.

The cytotoxicity of complex **6** towards the L1210 and cisplatin-resistant L1210 cell line was determined by the colony formation assay. The assay was carried out following 96 h exposure to the platinum drug.

In conclusion, (1*R*,2*R*-cyclohexanediamine)platinum(II) complex with **3** was found to have high solubility in water and good antitumor activity.

Experimental

Chemicals Potassium tetrachloroplatinite(II) was purchased from Kojima Chemical Co., Ltd. l-1,2-Diaminocyclohexane was supplied by Toray Finechemical Co., Ltd. All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO FT/IR-5000 spectrophotometer. ^{1}H-NMR spectra were recorded on a GSX500 or GX400 spectrometer with tetramethylsilane ($CDCl_3$) or 3-trimethylsilyl-1-propanesulfonic acid sodium salt (DSS) (D_2O) as an internal standard.

2,4-Dioxo-pentanoic Acid Methyl Ester (2)[11] Acetone (8.5 ml) and dimethyl oxalate (13.7 g, 0.116 mmol) was gradually added to a stirred dry methanol solution (100 ml) of sodium methoxide (9.41 g, 0.174 mol), and the reaction mixture was refluxed for 3 h. After being cooled to room temperature, the mixture was concentrated under reduced pressure. After being acidified with a dilute HCl solution, the mixture was extracted with ethyl acetate three times. The combined extracts were washed with water and brine. The organic layers were combined, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by recrystallization from ethanol–*n*-hexane to afford **2** (33.3 g, 68%). **2**, colorless plate, mp 59.2—61.0 °C (lit. 98 °C[11]). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.26 (3H, s) 3.90 (3H, s), 6.38 (1H, s), 14.43 (1H, br ds).

2,4-Dioxo-pentanoic Acid (3) Methyl 2,4-dioxo-pentanate (25.69 g, 0.162 mol) was dissolved in methanol (300 ml), and then 2 N sodium hydroxide solution (200 ml) was added at room temperature. The reaction mixture was allowed to stir at room temperature for 5 h. After evaporation, the mixture was acidified with 9 N sulfulic acid solution, continuously extracted with ether, and evaporated *in vacuo*. The residue was recrystallized from carbon tetrachloride to afford **3** (5.2 g, 25%). **3**, colorless crystals, mp 107.6—282.0 °C (dec.). IR (KBr): 3256, 1686, 1278, 663, 480 cm^{-1}. MS *m/z*: 131 (M^+).

(1*R*,2*R*-Cyclohexanediamine-*N*,*N*')[2-hydroxy-4-oxo-2-pentenoato(2-)-O^2]platinum(II) (6) Sodium hydrogen carbonate (523 mg, 6.2 mmol) was added to the tetrahydrofuran (THF) solution of **3** (810 mg, 6.2 mmol) at 0 °C. Dinitrato (1*R*,2*R*-diaminocyclohexane)platinum(II) (2.69 g, 6.2 mmol) in water (81 ml) was added dropwise, and the reaction mixture was stirred at room tempeature for 12 h. The mixture was concentrated, and methanol (20 ml) was added to filter the insoluble products. After evaporation, the residue was reprecipitated from ethanol–ethyl acetate, and washed with THF. The resulting products were purified by chromatography using MCI GEL (CHP-20P) and water–methanol (1 : 0—10 : 1) as the eluent to give pale yellow plates, **6** (1.5 g, 53%). mp 230.0—231.9 °C (dec.) *Anal.* Calcd for $C_{11}H_{20}N_2O_5Pt$: C, 27.90; H, 4.04; N, 5.92. Found: C, 28.10; H, 4.10; N, 5.90. IR (KBr): 3430, 3200, 2940, 1565, 1380, 1165 cm^{-1}. ^{1}H-NMR (400 MHz, D_2O) δ: 1.15 (2H, m), 1.33 (2H, m), 1.56 (2H, m), 1.93 (3H, s), 2.04 (2H, d, J=12.2 Hz), 2.52 (2H, m), 5.82 (2H, dd, J=9.2, 35.2 Hz), 6.30 (1H, s). ^{13}C-NMR (D_2O) δ: 192.44, 175.58, 104.28, 65.07, 65.04, 51.59, 34.68, 28.95, 26.58.

Calculation Method The calculations were carried out using the MNDO-PM3 molecular model as implemented within version 5.0 of the quantum chemistry program package MOPAC. For all of the calculations, the geometries of the models were fully optimized.

X-Ray Study of 6 Data were collected at 23±1 °C on a Rigaku AFC6R diffractometer with graphite-monochromated Mo K_α radiation (I=0.71069 Å) and a 12 kW rotating anode generator. A total of 2966 reflections were collected, 2765 of which were unique. Equivalent were merged. The intensities of three representative reflections measured after every 150 reflections remained constant throughout the data collection, indicating crystal and electronic stability. No decay collection was applied. An experimental absorption correction was applied. The data were collected for Lorenz-polarization absorption effects. The Pt atom was located by using the Patterson method. All other atoms were located in different Fourier maps. The final refinement was based on 2386 observed reflections ($I>3.00\sigma(I)$); a full-matrix least-squares refinement was done by TEXSAN. All non-hydrogen atoms were anomalous dispersion. Crystal data for **6**: a=8.781(2) Å, b=12.016(2) Å, c=8.249(2) Å, α=102.24(2)°, β=106.93(2)°, γ=72.86(2)°, V=788.0(3) Å^3, space group=$p1$(#2), Z=2, D_{calc}=2.079 g/cm^3, F_{000}=480, μ=90.26 cm^{-1}.

Antitumor Activity *in Vivo* L1210 Murine Leukemia: L1210 leukemia had been maintained by continious passage in DBA/2 mice. This tumor was inoculated (10^6 cells/mouse) intraperitoneally into male CDF_1 mice (6—7 weeks of age) on day 0. On the next day after inoculation, mice were randomly divided into groups of 6 members each, and the drugs were intraperitoneally administered three times on day 1, 5 and 9. Drugs were dissolved in a vehicle of distilled water. Drug efficacy was expressed as T/C value calculated by following equation.

$$T/C(\%) = \frac{\text{mean survival time of treated group}}{\text{mean survial time of control group}} \times 100$$

The criteria for siginificant therapeutic response is considered as 125% in T/C value according to the protocol of the National Cancer Institute.[15]

Cisplatin-Resistant Murine Leukemia L1210 (L1210/CDDP): A cisplatin-resistant murine leukemia cell line L1210 was used for assay of the antitumor activity of the complex. The assay procedure is the same as that used against L1210 murine leukemia.

Antitumor Activity *in Vitro* Cells were maintained in RPMI 1640 medium supplemented with 10% fetal boving serum, 2-mercaptoethanol, and antibiotics. 10^4 cells/0.5 ml were added to culture tubes, and the appropriate concentration of test compound was then added to the cultures. The cell concentration of the control and drug-treated cultures was determined 96 h later using a Model ZB Coulter Counter. The percentage of growth inhibition was calculated, and IC_{50} was determined from linear regression analysis of the growth inhibition data.

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