Synthesis, Antitumor Activity, and Nephrotoxicity of the Optical Isomers of 2-Aminomethylpyrrolidine(1,1-cyclobutane-dicarboxylato)platinum(II)

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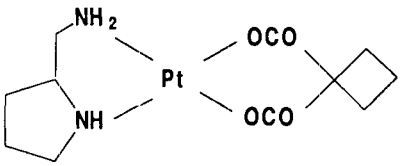
Abstract □ The optical isomers of 2-aminomethylpyrrolidine(1,1-cyclobutane-dicarboxylato)platinum(II) (DWA2114, **1**), which has potent antitumor activity against various tumors, were synthesized. They were examined for antitumor activity against Colon 26 carcinoma in a sc-iv system, and changes in urinary protein and sugar levels in drug-treated mice were used as an index of nephrotoxicity. In their effect on tumors, (+)-(*S*)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum (II) (**6b**) was more potent than the enantiomer **6a** in that the effective dose of **6b** was smaller than that of **6a**; but, both drugs exhibited potent antitumor activity. On the other hand, a distinct difference between **6a** and **6b** was shown in their nephrotoxicity. Isomer **6b** induced a great increase in urinary protein and sugar levels in mice, whereas **6a** caused no increase in these levels.

Since the discovery of the antitumor activity of *cis*-diaminedichloroplatinum(II) (CDDP),[1] many analogous antitumor platinum complexes have been studied vigorously. We have synthesized various platinum complexes having unsymmetrical bidentate diamines, such as 2-aminomethyl-*N*-heterocycles, as carrier ligands and screened them for antitumor activity.[2] Among these complexes, 2-aminomethylpyrrolidine(1,1-cyclobutane-dicarboxylato)platinum(II) (DWA2114; **1**) proved to be the most promising candidate for development as an antitumor agent with potent antitumor activity against various tumors with low nephrotoxicity, which is one of the serious side effects of CDDP.[3] Specifically, it was found that **1** exhibits potent antitumor activity against Colon 26 carcinoma in sc-ip, sc-iv, and ip-ip systems. The effects on the tumor are almost same as those following treatment with CDDP, although the effective dose of **1** is ~8 times higher than that of CDDP. Compound **1** was also found to have potent antitumor activity against other murine tumors such as P388 leukemia, L1210 leukemia, L5178Y lymphoma, M5076 ovarian tumor, Colon 38 carcinoma, and so on. It is of interest that **1** did not affect the blood urea nitrogen (BUN) level in mice at almost the maximum tolerated dose (MTD), whereas CDDP did cause an increase in the BUN level.

Examination of the optical isomers of **1** for biological activities is of great interest because different biological effects between optical isomers have often been reported.[4] In this paper, we report the synthesis of the optical isomers of **1**, their antitumor activity against Colon 26 carcinoma in mice (sc-iv system), and the changes in the urinary protein and sugar levels in intravenously drug-treated mice as an index of nephrotoxicity.



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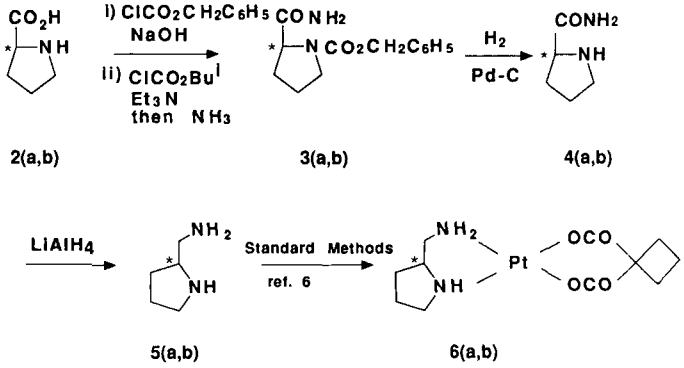
Results and Discussion

Chemistry—Both chiral 2-aminomethylpyrrolidines, which are the carrier ligands of the optical isomers of **1**, were synthesized from D- and L-proline by the general procedure[5] shown in Scheme I. Protection of both D- and L-proline (**2a** and **2b**, respectively) as the corresponding carbobenzyloxy derivatives, followed by mixed anhydride coupling with NH_3 gave the protected carboxyamides (**3a** and **3b**, respectively) in 81.0 and 81.7% yields, respectively. Deprotection by catalytic hydrogenation of **3a** and **3b**, followed by reduction with lithium aluminum hydride, gave (−)-(*R*)- and (+)-(*S*)-2-aminomethylpyrrolidine (**5a** and **5b**, respectively) in 40.2 and 32.5% yields, respectively.

The optical purity of **5a** and **5b** was examined by the following method. Compounds **5a** and **5b** were reacted with D-(+)-10-camphorsulfonyl chloride to give the corresponding sulfonamides **8** and **9**, respectively. The same treatment of racemate **7** gave the sulfonamides of **8** and **9** as a diastereomic mixture (Scheme II). Comparison of the ^1H NMR spectral data of **8** and **9** suggested that neither the **8** obtained from **5a** nor the **9** obtained from **5b** contain the corresponding diastereomer, and **5a** and **5b** were optically pure within the limits of NMR detection. Furthermore, **5a** and **5b** were converted to the desired platinum complexes (**6a** and **6b**, respectively) by a well-established method.[6] Compounds **6a** and **6b** were



a: R configuration at asymmetric carbon
b: S configuration at asymmetric carbon

Scheme I

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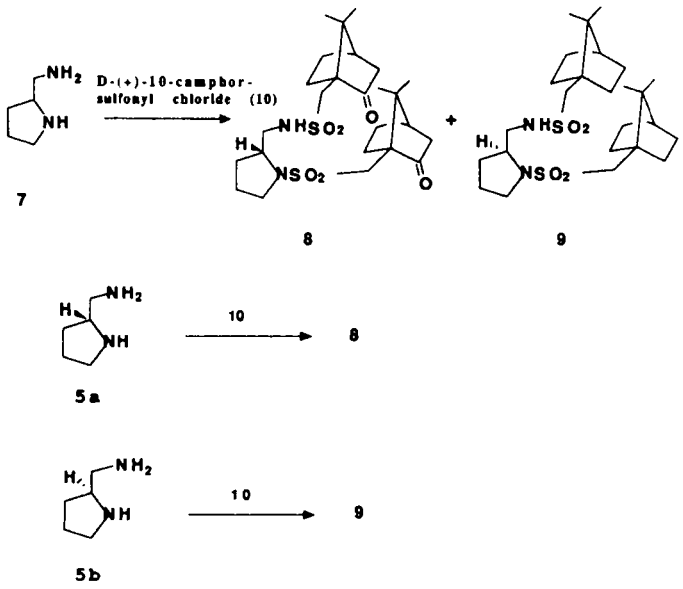

Scheme II

shown to be >99.9% enantiomerically pure by chiral HPLC analysis.

The CD spectra of **6a** and **6b** are illustrated in Figure 1. Compound **6a** gave a mirror-image spectra of **6b**. Compound **6a** has $\Delta\epsilon$ values of −0.04, 0.23, and −0.45 at 363, 313, and 266 nm, respectively. On the other hand, **6b** has $\Delta\epsilon$ values of 0.04, −0.23, and 0.45 at 363, 313, and 266 nm, respectively.

Biology—We examined the optical isomers (**6a** and **6b**) of **1** following single and triple iv administration for antitumor activity against Colon 26 carcinoma transplanted subcutaneously into male CDF1 mice. In Table I, the antitumor activity of these isomers and the racemate against Colon 26 carcinoma following single administration is shown as the growth inhibitory ratio (GIR). All compounds exhibited antitumor activity in a dose-dependent manner. Compound **6b** exhibited the most potent antitumor activity of these compounds at all doses (20, 40, and 80 mg/kg), and the order of the potency of their antitumor activity was **6a** < **1** < **6b**. However,

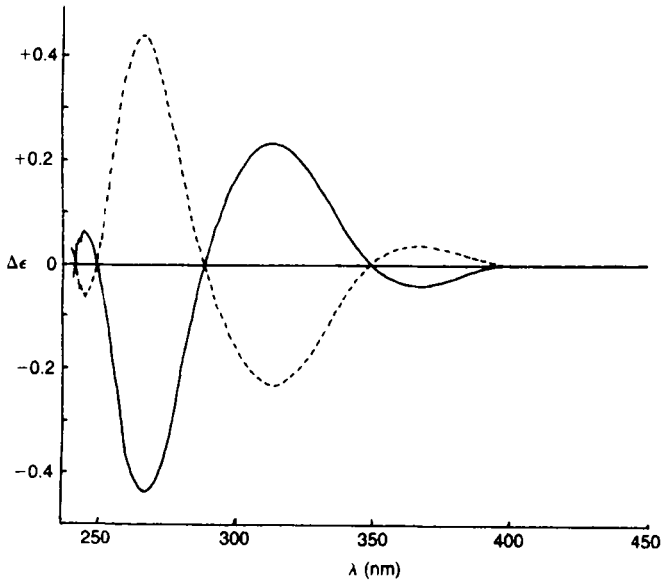

Figure 1—The CD spectra of **6a** (——) and **6b** (– – –) in water.

Table I—Antitumor Activities of 1, 6a, and 6b against Colon 26 Carcinoma in a sc-iv System following Single Administration

Compound	Dose, mg/kg	No. of Mice	GIR, %
1	20	7	22
	40	7	43
	80	7	86
6a	20	7	12
	40	8[a]	33
	80	7	67
	120	6	83
	140	6[a]	97
6b	20	7	25
	40	7	56
	80	6	93

[a] One mouse died before weighing tumor on the 14th day after tumor implantation.

6a also exhibited potent antitumor activity when administered at much higher doses (120 and 140 mg/kg). In Table II, these platinum complexes were examined for antitumor activity against Colon 26 carcinoma following triple administrations. All compounds exhibited antitumor activity in a dose-dependent manner. Compound **6b** exhibited the most potent antitumor activity of these compounds at all doses (10, 20, and 40 mg/kg/day), and the order of the potency of their antitumor activity was **6a** < **1** < **6b**. However, **6a** also exhibited potent antitumor activity at a much higher dose. Compound **6a** showed 99% GIR at 80 mg/kg/day, and **6b** showed 99% GIR at 60 mg/kg/day.

Next, the changes of the urinary protein and urinary sugar levels following iv administration of **1** (80 mg/kg), **6a** (120 and 140 mg/kg), and **6b** (80 mg/kg) in normal male BDF1 mice (four mice per group) were examined. Compound **6a** was shown to not increase the urinary protein level at doses of 120 and 140 mg/kg. On the other hand, **6b** greatly increased the urinary protein level, which reached the maximum level on the 3rd day after **6b** administration. Three out of four **6b**-treated mice showed high urinary protein levels of >500 mg/dL. The urinary protein level of mice treated with **1** increased to the middle range of the levels following treatment with **6a** and **6b** (Figure 2). Similarly, **6a** was shown to not increase the urinary sugar level at doses of 120 and 140 mg/kg, and **6b** greatly increased the urinary sugar level, which reached the maximum level on the 3rd day after **6b**

Table II—Antitumor Activities of Platinum Complexes against Colon 26 Carcinoma in a sc-iv System following Triple Administration

Compound	Day of Treatment	Dose, mg/kg/day	No. of Mice	GIR, %
1	4, 6, 8	10	6	39
		20	6	52
		40	6	85
6a	4, 6, 8	10	6	25
		20	6	32
		40	6	83
		60	6	92
		80	6	99
6b	4, 6, 8	10	6	44
		20	6	53
		40	6	94
		60	6[a]	99
CDDP	4, 6, 8	4	6	85
		6	6	94
		8	6	97

[a] Three mice died before weighing tumor on the 14th day after tumor implantation.

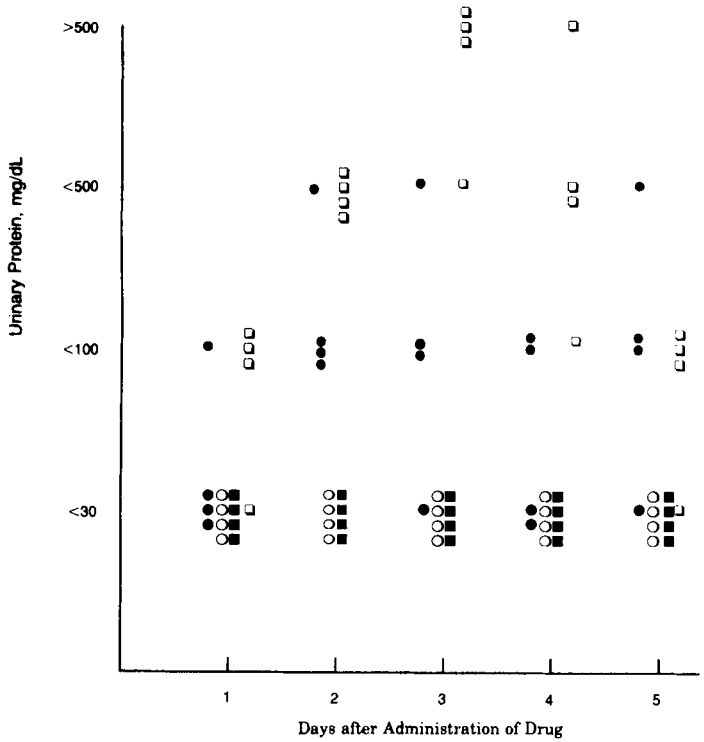

Figure 2—Change of urinary protein level following iv administration of **1** (80 mg/kg, ●), **6a** (120 mg/kg, ○); 140 mg/kg, ■), and **6b** (80 mg/kg, □) in four normal BDF1 mice. Four mice administered saline were used as a control group. All control urinary protein levels were <30 mg/dL.

administration at a dose of 80 mg/kg. Also, three out of four **6b**-treated mice showed high urinary sugar levels of >1000 mg/dL (Figure 3).

Finally, the changes of the urinary protein and urinary sugar levels in tumor-bearing CDF1 mice (six mice per group) following iv administration of **1**, **6a**, and **6b** were examined. Drugs were administered three times in mice on the 4th, 6th, and 8th days after tumor implantation, and urinalysis was performed every other day after the first drug administration. Compound **6a** was shown to not increase the urinary protein level, even at a dose of 80 mg/kg/day, a dose which showed strong antitumor activity (99% GIR). On the other hand, **6b** greatly increased the urinary protein level at doses of both 40 and 60 mg/kg/day, and three mice in the **6b**-treated group at a dose of 60 mg/kg/day died before the 4th day after the third drug administration. The urinary protein level of mice treated with **1** at a dose of 40 mg/kg/day was almost the same as that of the control mice (Figure 4). Similarly, **6a** was shown to not increase the urinary sugar level at dose of 80 mg/kg/day. On the other hand, **6b** increased the urinary sugar level at doses of both 40 and 60 mg/kg/day. In particular, all **6b**-treated mice at 60 mg/kg/day showed high urinary sugar levels of >1000 mg/dL on the 4th day after the first drug administration. Compound **1** did not increase the urinary sugar level at a dose of 40 mg/kg/day (Figure 5). The decrease in urinary protein and sugar levels after the third **6b** administration is probably due to the decreasing food intake of the mice.

From the above results, the optical isomers **6a** and **6b** of **1**

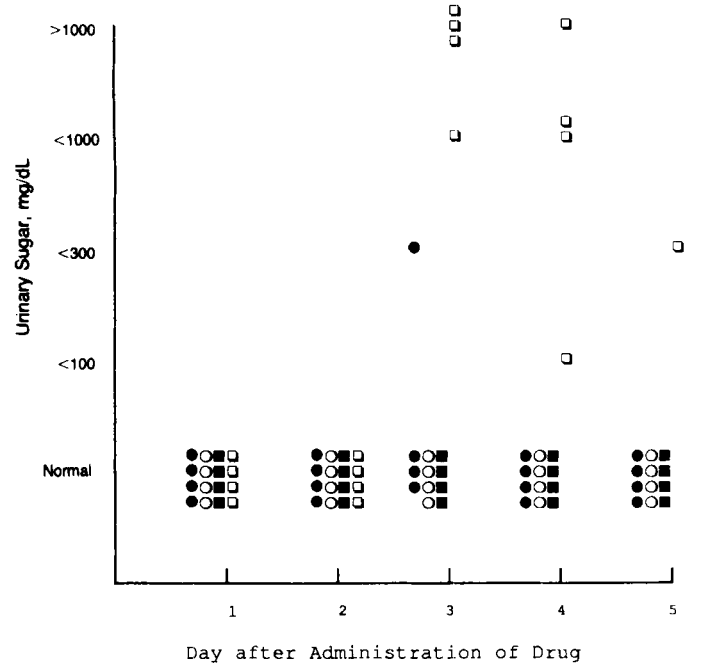

Figure 3—Change of urinary sugar level following iv administration of **1** (80 mg/kg, ●), **6a** (120 mg/kg, ○); 140 mg/kg, ■), and **6b** (80 mg/kg, □) in four BDF1 mice. Four mice administered saline were used as a control group. All control urinary sugar levels were normal.

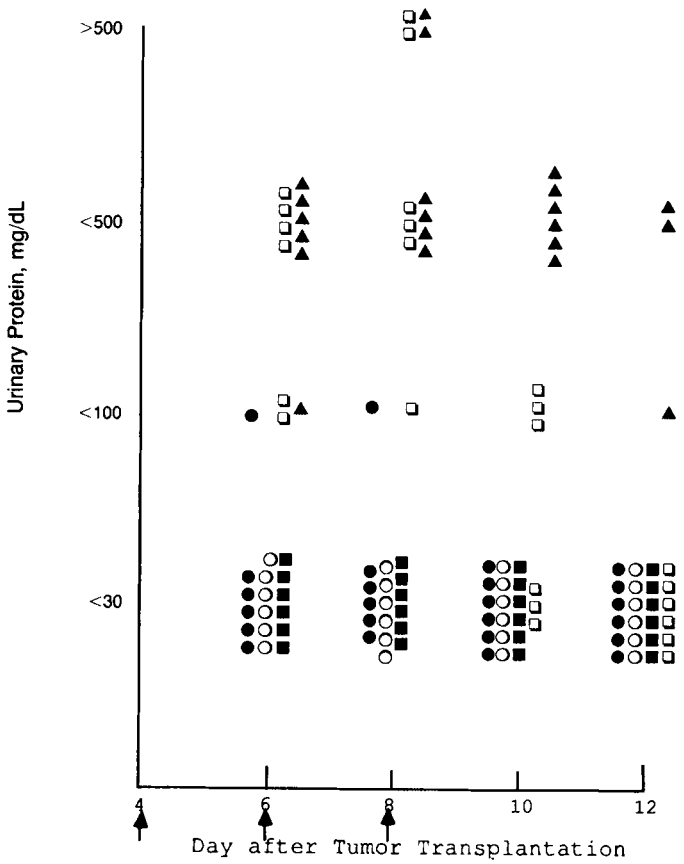

Figure 4—Change of urinary protein level in tumor-bearing CDF1 mice administered the platinum complexes iv. Drugs were administered in mice (six mice per group) on days 4, 6, and 8 after sc tumor implantation on day 0 at the following doses: **1** (40 mg/kg/day, ●), **6a** (60 mg/kg/day, ○; 80 mg/kg/day, ■), and **6b** (40 mg/kg/day, □; 60 mg/kg/day, ▲). The urinalyses on days 6 and 8 were done before drug administration. In the **6b**-treated (60 mg/kg/day) mice, two mice died on day 11 and one mouse died on day 12. The data were obtained from two experiments. Twelve mice (six mice per experiment) administered saline were used as a control group. In the control group, one mouse showed a urinary protein level of <100 mg/dL on days 6 and 12, and the other control urinary protein levels were <30 mg/dL.

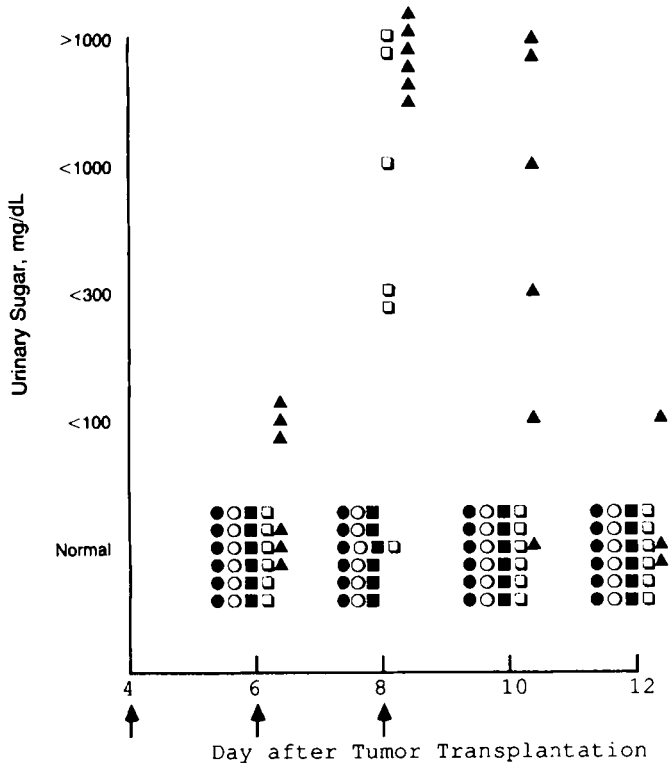

Figure 5—Change of urinary sugar level in tumor-bearing CDF1 mice administered the platinum complexes iv. Drugs were administered in mice (six mice per group) on days 4, 6, and 8 after sc tumor implantation on day 0 at the following doses: **1** (40 mg/kg/day, ●), **6a** (60 mg/kg/day, ○; 80 mg/kg/day, ■), and **6b** (40 mg/kg/day, □; 60 mg/kg/day, ▲). The urinalyses on days 6 and 8 were done before drug administration. In the **6b**-treated (60 mg/kg/day) mice, two mice died on day 11 and one mouse died on day 12. The data were obtained from two experiments. Twelve mice (six mice per experiment) administered saline were used as a control group. In the control group, one mouse showed a urinary sugar level of <300 mg/dL on days 6 and 12, and all urinary sugar levels of the control mice were normal.

were shown to have some different biological activities. The difference in nephrotoxicity was particularly great in consideration of the facts that **6b** greatly increased the urinary protein level and the urinary sugar level in mice and **6a** did not effect these levels, even at doses sufficiently effective to act on tumors. The differences of biological activity between **6a** and **6b** as shown here were observed in screens of drugs administered ip to mice.[7] Compound **6b** was shown to accumulate in the kidney in greater quantities than **6a** in experiment examining distribution of these drugs in mice,[7] and it is thought that this property is closely related to the nephrotoxicity of **6b**. The X-ray crystallographic analysis of **6a** showed that its molecular structure is folded and that the cyclobutane ring is in a puckered conformation, closed to the pyrrolidine ring.[8] This analysis indicates that **6a** and **6b** are largely different in three-dimensional structure, and such a large structural difference may influence biological activity.

Compound **6a** exhibited potent antitumor activity when administered at a higher dose, although **6a** has a little weaker antitumor activity than **6b**. Moreover, **6a** has no nephrotoxicity. We were able to find the desirable antitumor platinum complex **6a** by the optical division of **1**. A preclinical phase III study of **6a** as DWA2114R has been reported[9] and a clinical phase III study of **6a** as DWA 2114R is in progress.

Experimental Section

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra were recorded with a Hitachi 260-30 spectrophotometer, and ^1H NMR spectra were recorded on a Hitachi R-24B (60 Hz) or a Jeol FX-200 (199.5 MHz) spectrometer, with Me_4Si ($CDCl_3$) used as the internal standard. Chemical shifts are reported on the scale with peak multiplicities as follows: s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet. The ^{13}C NMR spectra were recorded on a Jeol FX-200 (50.1 MHz) spectrometer, with 1,4-dioxane (D_2O) used as the internal standard. Mass spectra were recorded on a Shimadzu LKB-9000 instrument. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Circular dichroism spectra were measured in H_2O with a JASCO J-500A spectropolarimeter. Elemental analyses were performed by the Analytical Research Laboratory of Chugai Pharmaceutical Company, Ltd., or the Toray Research Center. All analyses (C,H,N) are within ±0.4% of the theoretical values. The TLC was done on a silica gel 60 Merck F254 (0.25 mm). High-pressure liquid chromatography was performed on a Waters 510 liquid chromatograph operating at 0.6 mL/min, using a SUMIPAX OA-4100 column, with UV monitoring by a Waters 481 detector at 250 nm. A Shimadzu C-R2AX integrator was used for peak data. The solvent was $CHCl_3$:CH_3OH:H_2O (35:15:2, v/v/v).

(*R*)-1-Carbobenzyloxypyrrolidine-2-carboxamide (3a)—D-Proline (**2a**; 200 g, 1.74 mol) and sodium hydroxide (73.3 g, 1.74 mol) were dissolved in water (1.2 L) and cooled to 5 °C in an ice bath. A solution of benzyl chloroformate (348 g, 2.04 mol) and sodium hydroxide (87.7 g, 2.32 mol) in water (500 mL) were then added alternately over a 30-min period in five equal portions to the magnetically stirred solution. After allowing the solution to stand overnight at room temperature, excess benzylchloroformate was extracted with chloroform (1 L) three times. The aqueous solution was slowly acidified to Congo Red with 5M HCl. The product which separated was extracted into chloroform (4L). The organic layer was dried over anhydrous magnesium sulfate. To the above solution was added triethylamine (172 g, 1.70 mol) and isobutyl chloroformate (232 g, 1.70 mol) at 0 °C, with stirring. After 2 h, anhydrous ammonia gas was passed through the cooled solution for 4 h. The reaction mixture was then allowed to warm to room temperature and stand overnight. The white precipitate was filtered off and the filtrate was washed with water, dried over anhydrous magnesium sulfate, and evaporated. The residue was recrystallized from methanol to give **3a** (349.3 g, 81.0%) as a colorless crystal: mp 92–93 °C; ^1H NMR (60 MHz, $CDCL_3$): δ 1.55–2.40 (4H, m), 3.48 (t, J = 6 Hz, 2H, NCH_2), 4.28 (t, J = 6 Hz, 1H, NCH), 5.05 (s, 2H, $C_6H_5CH_2$), 5.90–6.80 (br. s, 2H, NH_2).

L-Proline (**2b**; 6.8 g, 0.059 mol) was converted into (*S*)-1-carbobenzyloxypyrrolidine-2-carboxamide (**3b**; 12.0 g, 81.7%) as a colorless solid in the same manner as described for **3a**: ^1H NMR (60 MHz, $CDCl_3$): δ 1.55–2.40 (4H, m), 3.48 (t, J = 6 Hz, 2H, NCH_2), 4.28 (t, J = 6 Hz, 1H, NCH), 5.05 (s, 2H, $C_6H_5CH_2$), 5.90–6.80 (br. s, 2H, NH_2).

(*R*)-Pyrrolidine-2-carboxamide (4a)—To a solution of **3a** (361 g, 1.46 mol) in methanol (2.5 L) was added 10% palladium on carbon catalyst (50 g); this solution was hydrogenolyzed at room temperature for 9 h. The catalyst was filtered off and the filtrate was evaporated. The residue was washed with *n*-hexane (1 L) to give **4a** (144.8 g, 87.3%) as a colorless crystal: mp 97–98 °C; ^1H NMR (60 MHz, $CDCl_3$): δ 1.42–2.35(m, 4H), 2.58 (s, 1H, NH), 2.95 (t, J = 6 Hz, 2H, NCH_2), 3.56–3.90 (m, 1H, NCH), 5.90–6.70 and 7.00–7.80 (br.s × 2, 2H, NH_2).

Compound **3b** (12.0 g, 0.048 mol) was converted into (*S*)-pyrrolidine-2-carboxamide (**4b**; 6.2 g, 88.4%) as a colorless crystal in the same manner as described for **4a**: mp 97–98 °C (lit.[10] mp, 97 °C); ^1H NMR (60 MHz, $CDCl_3$): δ 1.42–2.35(m, 4H), 2.58 (s, 1H, NH), 2.95 (t, J = 6 Hz, 2H, NCH_2), 3.56–3.90 (m, 1H, NCH), 5.90–6.70 and 7.00–7.80 (br.s × 2, 2H, NH_2).

(−)-(*R*)-2-Aminomethylpyrrolidine (5a)—To a suspension of **4a** (144.8 g, 1.27 mol) in tetrahydrofurane (7 L) was added lithium aluminum hydride (260.6 g, 6.87 mol) in small portions, and then the reaction mixture was refluxed for 33 h. After cooling to room temperature, the reaction mixture was treated with water (436.5 mL) and then filtered. The collected solid was suspended in tetrahydrofuran (9 L) and refluxed for 3 h. The above operation was performed again and the filtrate was gathered. The filtrate was concentrated, and the residue was distilled to give **5a** (58.5 g, 46.1%) as a colorless oil: bp 79–85 °C (20 mmHg); $[\alpha]_D$ −8.67° (c = 1.1, H_2O); ^1H NMR (60 MHz, $CDCl_3$): δ 1.00–2.10 (m, 7H), 2.30–3.30 (m, 5H).

Anal.—($C_5H_{12}N_2$) C, H, N.

Compound **4b** (18.8 g, 0.165 mol) was converted into (−)-(*S*)-2-aminomethylpyrrolidine (**5b**; 6.1 g, 36.8%) as a colorless oil in the

same manner as described for **5a**: bp 80–85 °C [20 mmHg; lit.[10,11] bp 65 °C (11 mmHg), bp 50 °C (7 mmHg)]; $[\alpha]_D$ +10.02° (c = 1.1, H_2O) lit.[10] **5b** · 2HCl: $[\alpha]_D$ −1.2° (H_2O); ^1H NMR (60 MHz, $CDCl_3$): δ 1.00–2.10 (m, 7H), 2.30–3.30 (m, 5H).

Anal.—($C_5H_{12}N_2$) C, H, N.

***N,N'-bis*-[(*S*)-10-Camphorsulfonyl]-2-aminomethylpyrrolidine (Mixure of 8 and 9)**—To a solution of 2-aminomethylpyrrolidine[2b] (0.20 g, 2.0 mmol) and triethylamine (2.19 g, 21.7 mmol) in dichloromethane (3 mL), in an ice bath, a solution of D-(+)=10-camphorsulfonylchloride (1.03 g, 4.1 mmol) in dichloromethane (2 mL) was added in a dropwise manner. The mixture was refluxed for 1 h and cooled in an ice bath. The reaction mixture was quenched with 10% HCl. The organic layer was washed with water, dried over anhydrous potassium carbonate, and evaporated. The residue was chromatographed on silica gel. Elution with chloroform gave the mixture of diastereomeres of *N,N-bis*-[(*S*)-10-camphorsulfonyl]-(*R*)-2-aminomethylpyrrolidine (8) and *N,N-bis*-[(*S*)-10-camphorsulfonyl]-(*S*)-2-aminomethylpyrrolidine (9; 0.93 g, 88.5%) as a colorless solid; MS (m/z 313): [M^+ −(10-camphorsulfone)]; ^1H NMR (199.5 MHz, $CDCl_3$): δ 0.89 (s, 9H, CH_3 × 3), 0.9 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 1.13 (s, 6H, CH_3 × 2), 1.35–2.60 (m, 36H), 2.81–2.98 (m, 4H), 3.24–3.52 (m, 12H), 3.93–4.02 (m, 2H), 5.63 (t, *J* = 6.3 Hz, 1H), 5.73 (t, *J* = 6.1 Hz, 1H).

Anal.—($C_{25}H_{40}N_2O_6S_2$) C, H, N.

Compound **5a** (210 mg, 2.09 mmol) prepared from D-proline and **5b** (200 mg, 1.99 mmol) prepared from L-proline were converted into 8 (940 mg, 85.2%) and 9 (920 mg, 87.5%), respectively, as a colorless solid in the same manner as described for the above mixtures of 8 and 9.

Compounds 8 and 9 had the same R_f (0.2) on TLC by developing 10 times in chloroform. Compound 8; ^1H NMR (199.5 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.35–2.60 (m, 18H), 2.87 (dd, *J* = 14.4 Hz, *J* = 14.4 Hz, 2H, $NHCH_2$), 3.24–3.52 (m, 6H, SO_2NH_2 × 2 and NCH_2), 3.94–4.02 (m, 1H, NCH), 5.73 (t, *J* = 6.1 Hz, 1H, $NHSO_2$).

Anal.—($C_{25}H_{40}N_2O_6S_2$) C, H, N.

Compound 9; ^1H NMR (199.5 MHz, $CDCl_3$): δ 0.89 (s, 6H, CH_3 × 2), 1.04 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.37–2.60 (m, 18H), 2.92 (dd, *J* = 14.6 Hz, *J* = 5.2 Hz, 2H, $NHCH_2$), 3.25–3.49 (m, 6H, SO_2NH_2 × 2 and NCH_2), 3.93–3.96 (m, 1H, NCH), 5.63 (t, *J* = 6.3 Hz, 1H, $NHSO_2$).

Anal.—($C_{25}H_{40}N_2O_6S_2$) C, H, N.

(*R*)-2-Aminomethylpyrrolidinedichloroplatinum(II) (7a)—To a solution of potassium tetrachloroplatinate(II) (2.32 g, 5.59 mmol) in water (80 mL) was added **5a** (0.56 g, 5.60 mmol), and the reaction mixture was stirred at room temperature for 3.5 h. The resulting precipitate was collected by filtration, washed with water, and dried under reduced pressure to give **7a** (1.55 g, 75.8%) as a pale yellow crystal: mp 282–286 °C (decomp.); 1R (KBr): 3220, 3150, 2440, 2360, 1570, 1530, 1450, 1400, 1280, 1180, 1140, 1080, 1040, 1020, 975, 930, 780, and 580 cm^{-1}.

Anal.—($C_5H_{12}Cl_2N_2Pt$) C, H, N.

Compound **5b** (1.39 g, 13.9 mmol) was converted into (*S*)-2-aminomethylpyrrolidinedichloroplatinum(II) (**7b**; 4.15 g, 81.6%) as a pale yellow crystal in the same manner as described for **7a**; mp 267–272 °C (decomp.); IR (KBr): 3220, 3150, 2440, 2360, 1570, 1530, 1450, 1400, 1280, 1180, 1140, 1080, 1040, 1020, 975, 930, 780, and 580 cm^{-1}.

Anal.—($C_5H_{12}Cl_2N_2Pt$) C, H, N.

(−)-(*R*)-2-Aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) (6a)—To a suspension of **7a** (732 mg, 2.0 mmol) in water (150 mL) was added silver nitrate (680 mg, 4.0 mmol), and the reaction mixture was stirred at room temperature under light shielding for 4 days. The resulting white precipitate of silver chloride was removed by filtration using a millipore filter (0.22 μm). The filtrate, which contained the unreacted silver nitrate, was treated with an aqueous solution of NaCl (12 mg, 0.2 mmol). The resulting silver chloride was removed by filtration and the filtrate was concentrated into 14 mL at <40 °C under reduced pressure. To the solution was added disodium 1,1-cyclobutanedicarboxylate (338 mg, 1.8 mmol). The reaction mixture was stirred at room temperature for 15 h and concentrated into 1.5 mL at <40 °C under reduced pressure. The solution was stirred at room temperature for 3 h. The resulting precipitate was collected by filtration and recrystallized from water to give **6a**[8] (670 mg, 73.6%) as a colorless crystal: mp 248–254 °C (decomp.); $[\alpha]_D$ −40.1° (c = 0.4, H_2O); HPLC, single peak (t_R = 9.9 min); IR (KBr): 3480, 3420, 3190, 3100, 3050, 2870, 1635, 1590, 1380, 1220, 1120, 1020, 910, 780, 710, 540, and 470 cm^{-1}; ^{13}C NMR (50.1 MHz, D_2O): δ 16.3, 25.2, 25.5, 31.2, 32.7, 51.2, 52.3, 57.2, 67.1, 182.6 (two carbonyl C).

Anal.—($C_{11}H_{18}N_2O_4Pt \cdot H_2O$) C, H, N.

Compound **7b** (3.0 g, 8.2 mol) was converted into **6b** (2.87 g, 76.9%) as a colorless crystal: mp 247–252 °C (decomp.); $[\alpha]_D$ +39.2° (c = 0.4, H_2O); HPLC, single peak (t_R = 8.3 min); IR (KBr): 3480, 3420, 3190, 3100, 3050, 2870, 1635, 1590, 1380, 1220, 1120, 1020, 910, 780, 710, 540, and 470 cm^{-1}; ^{13}C NMR (50.1 MHz, D_2O): δ 16.3, 25.2, 25.5, 31.2, 32.7, 51.2, 52.3, 57.2, 67.1, 182.6 (two carbonyl C).

Anal.—($C_{11}H_{18}N_2O_4Pt \cdot H_2O$) C, H, N.

Biological Evaluation—*Antitumor Activity against Colon 26 Carcinoma*—Colon 26 carcinoma was maintained by continuous passage in syngeneic mice. This tumor was transplanted subcutaneously in a 2-mm^3 fragment by trocar into male CDF1 mice. Four days after the implantation, mice with almost the same size tumors were divided into groups of 6–8 members each, and the drugs were administered iv once on the 4th day or three times on the 4th, 6th, and 8th days after tumor implantation. Drugs were prepared in a vehicle of 0.25 mL of saline. Tumor-bearing mice administered 0.25 mL of saline were used as a control group. The tumors were removed and weighed on the 14th day after tumor implantation. Drug efficacy was expressed as GIR (growth inhibitory ratio):

$$\text{GIR (\%)} = \left(1 - \frac{\text{mean tumor weight of treated group}}{\text{mean tumor weight of control group}}\right) \times 100 \quad (1)$$

The criteria for significant therapeutic response, according to the NCI, is 50% GIR.

Urinary Protein and Sugar Level Test—*Effect of Drugs in Normal Mice*—Male BDF1 mice (four mice per group) were used. Drugs were administered once iv. Drugs were prepared in a vehicle of 0.25 mL of saline. Mice administered 0.25 mL of saline were used as a control group. Urinary protein and sugar levels were determined by BM Test 8-III (Boehring Mannheim) every day for 5 days after drug administration.

Effect of the Drugs in Tumor-Bearing Mice—Male CDF1 mice (six mice per group) with subcutaneously transplanted Colon 26 carcinoma, which were employed in the above antitumor activity experiment, were used. Drugs were administered three times iv on the 4th, 6th, and 8th days after tumor implantation. The urinalyses were done every other day after the first drug administration using BM TEST 8-III. The urinalyses on the 6th and 8th day after tumor implantation were done before drug administration.

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