from cyclohexane to give 10 g (52%) of **8g**: mp 135 °C; IR (KBr) 1630 (CO), 3280 and 3340 (NH) cm^{-1}. Anal. ($C_{19}H_{29}N_3O_5$) C, H, N.

Method F. *N*-[4,7-Dimethoxy-6-[2-(dimethylamino)ethoxy]-5-benzofuranyl]-*N'*-methylurea Hydrate (8e). Compound **2f** (10 g, 34 mmol) was dissolved in dry toluene and treated dropwise with methyl isocyanate (**caution**: lachrymatory) (2.4 mL, 40 mmol) at room temperature. The solution was stirred for 5 h, the solvent was distilled off under vacuum at 50 °C, and the residue was recrystallized from AcOEt to give 8 g (66%) of **8e**: mp 132 °C; IR 1620 (CO), 3320 (NH) cm^{-1}. Anal. ($C_{17}H_{25}N_3O_5 \cdot 0.5H_2O$) C, H, N.

Synthesis of Seleno- and Thioguanine–Platinum(II) Complexes and Their Antitumor Activity in Mice

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Selenoguanine–, selenoguanosine–, thioguanine–, and thioxanthine–platinum(II) complexes were synthesized, and their antitumor activities were studied against L1210 cells in mice and in an in vitro system. These compounds exhibited antitumor activity of medium strength and showed very low toxicity. The effect of the selenoguanine–platinum(II) complex in mice was retained longer than that of the parent compound, selenoguanine, because the selenoguanine–platinum(II) complex very slowly released selenoguanine into the blood.

Mautner et al.[1] have shown that selenoguanine (SeG) is as effective an inhibitor as thioguanine (TG) to the growth of several experimental tumors, is less toxic to the host, and shows a somewhat superior therapeutic index than thioguanine.

Many platinum complexes, such as *cis*-dichlorodiammine–platinum(II), have been tested as antitumor agents.[2-8] Several nucleosides and nucleoside bases complexed with *cis*-diaminoplatinum(II) have been shown to be good antitumor agents in experimental animals.[9]

In this article, we describe the antitumor activity of some selenoguanine–platinum(II) [SeG–Pt(II)] and thioguanine–platinum(II) [TG–Pt(II)] complexes and their structural assignments.

Structure Assignment. The UV spectrum of SeG–Pt(II) shows absorption maxima at 297, 340, and 368 nm in aqueous base. These maxima are not observed in the parent compound under the same conditions. These values are closed to those of the protonated form of selenoguanine and indicate the formation of a SeG–Pt(II) complex. This implies that the N^7 position of selenoguanine, which is a protonation site in the molecule, chelates to platinum. The similarity of the UV spectrum of the selenoguanosine–platinum(II) complex with that of SeG–Pt(II) [237, 273 (sh), 303, and 370 nm] also supports N^7 as the chelation site in the complexes.

^{13}C nuclear magnetic resonance spectra of the complexes were obtained with rather poor resolution due to their limited solubility, even in aqueous base. When compared with the parent SeG, the spectra of SeG–Pt(II) shows a marked downfield shift (3.03–3.07 ppm) for two carbons, positions 2 and 8, and an upfield shift (0.54–4.19 ppm) for three carbons, positions 4, 5, and 6. Other platinum complexes, such as TG–Pt(II), TGR–Pt(II), TX–Pt(II), and SeGR–Pt(II), show similar shifts.

Selenoguanine, similarly to thioguanine, is predominantly in the selenocarboxamide, (thiocarboxamide) rather than in iminoselenol (iminothiol) form in aqueous solution or in the solid state at room temperature.[10-16] ^{13}C NMR has shown that carbon 6 shifts upfield when the thiocarboxamide converts to the iminothiol (selenocarboxamide to iminoselenol).[17,18] The fact that the carbon 6 signals of the SeG–Pt(II) and TG–Pt(II) complexes were shifted upfield by 2.41–4.19 ppm indicates that selenium is bound to platinum as a seleno ether type compound.

Studies on the interaction of nucleosides or nucleotides with *cis*-dichlorodiammineplatinum(II) have indicated that the N^7 and O^6 atoms of guanosine and inosine are the sites of bond formation with platinum.[19-21]

The selenium atom, the strongest nucleophilic center in selenoguanine, could be the site of binding to the tetrachloroplatinate ion to produce *cis*- or *trans*-dichlorobis(selenoguanin-6-yl)platinum(II). The results of the following kinetic study support this view. Subsequent intramolecular displacement will afford the SeG–Pt(II) complex. Owing to the strong trans influences of sulfur or selenium in the molecule,[22] the intermediates are con-

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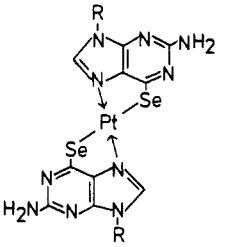
sidered to be the trans isomers. Accordingly, the final products, such as SeG–Pt(II), TG–Pt(II), and SeGR–Pt(II), are assumed to be trans.

The time course for the UV spectral change of the reaction of thioguanine with potassium tetrachloroplatinate(II) was monitored at various pHs in Kolthoff's Borax phosphate buffer solution at 28 °C. It was observed that the absorption maximum of thioguanine at 345 nm decreased, while that at 370 nm increased, during the reaction. When the reaction time was longer than 60 min, there was no isosbestic point under the condition used. An inflection point was observed in the pH–rate profile at pH 8.3, which is almost the same as the pK_a value of thioguanine. The profile was flat in the region of pH 6.0–7.5. This indicates that it is the neutral form of thioguanine reacting rapidly rather than the dissociated form. This is due to a repulsion between tetrachloroplatinate anion and thioguanine anion. Similar results were observed for the reaction of selenoguanine with potassium tetrachloroplatinate(II) with small differences; that is, the inflection point in the pH–rate profile shifted to lower pH, which is close to the pK_a value (7.62)[26] of selenoguanine. These phenomena indicate that the selenocarboxamide or iminoselenol moiety (thiocarboxamide or iminothiol) must be the initial attacking position in the molecule, because the N^1 proton in selenoguanine (thioguanine) is the first dissociation position to produce the monoanionic form. The result of elemental analysis shows a 1:2 molar ratio of platinum to selenoguanine in the complex. A possible structure of the platinum complex is shown.



R = H or β-D-ribofuranosyl

Bioassay Results. The present bioassay results indicated that SeG was less toxic than TG as previously reported.[1,23] The same order of toxicity was found for their platinum complexes; however, the toxicity of the platinum complexes was substantially lower. The effective dose range of the platinum complexes was wider than that of the parent compound, as shown in Table I. In some cases, mice treated with the platinum complexes survived for more than 30 days. The antitumor activity of SeGR–Pt(II) was less than that of SeG–Pt(II).

To examine whether the platinum complex has a direct antitumor effect or whether it is effective after hydrolysis to SeG, the concentrations of SeG in the blood and of SeG–Pt(II) in the injected locus of the mice were measured. The results showed that very small amounts of SeG were gradually released into the blood over a 10-day period (0.04–0.1 OD per mL of blood), whereas more than 98% of SeG clears from the blood within 24 h. This is in accord with Hitchings' result on thioguanine metabolism in mice.[24] SeG–Pt(II) was observed in the intraperitoneal cavity of the mice by visual detection, even 10 days after the drug injection. Thin-layer chromatography of 8% aqueous ammonia extracts from the intraperitoneal cavity of mice revealed the presence of only SeG–Pt(II). this means that SeG–Pt(II) is stable during stagnation in the locally injected site.

Furthermore, the direct cytocidal effects, of SeG–Pt(II), TG–Pt(II), and TX–Pt(II) on L1210 cells in vitro were much smaller than those of the parent compounds, as shown in Table II. The order of the cytocidal effect of the platinum complexes are as follows: TG–Pt(II) > TX–

Table I. Effect of Seleno- and Thioguanine and Their Platinum Complexes on the Survival Time of Mice Bearing L1210 Cells

sample[a]	dose: mg/kg × days	dose: mmol/kg × days	median survival time, days	T/C,[b] %	30th day survivors/total
SeG–Pt	1000 × 2	1.41 × 2	15	166.7	0/5
	800 × 2	1.13 × 2	15	166.7	0/5
	600 × 2	0.85 × 2	15	166.7	1/5
	400 × 2	0.56 × 2	16	177.8	1/5
	200 × 2	0.28 × 2	14	155.6	0/5
	100 × 2	0.14 × 2	12	133.3	1/5
	50 × 2	0.07 × 2	12	133.3	0/5
	10 × 2	0.01 × 2	10	111.1	0/5
SeG	200 × 2	0.93 × 2	toxic		
	100 × 2	0.47 × 2	>30	>300	3/5
	50 × 2	0.23 × 2	18	200	1/5
control			9		0/9
TG–Pt	1000 × 2	1.62 × 2	14	140	0/5
	800 × 2	1.30 × 2	14	140	0/5
	600 × 2	0.97 × 2	15	150	0/5
	400 × 2	0.65 × 2	19	190	0/5
	200 × 2	0.32 × 2	15	150	0/5
	100 × 2	0.16 × 2	17	170	1/5
	50 × 2	0.08 × 2	14	140	0/5
TG	200 × 2	1.20 × 2	toxic		
	100 × 2	0.60 × 2	10	100	0/5
	50 × 2	0.30 × 2	12	120	0/5
	25 × 2	0.15 × 2	>30	>300	4/5
control			10		0/6
SeGR–Pt	800 × 2	0.81 × 2	14	155.6	0/5
	400 × 2	0.40 × 2	12	133.3	0/5
	100 × 2	0.10 × 2	11	111.1	0/5
	50 × 2	0.05 × 2	12	133.3	0/5
SeGR	75 × 2	0.20 × 2	13	144.4	0/5
	50 × 2	0.13 × 2	13	144.4	0/5
	25 × 2	0.07 × 2	15	166.7	0/5
control			9		0/6

[a] The sample was injected on days 1 and 5. [b] Ratio of the median survival time of the treated (T) vs. control (C) animals.

Table II. ID_{50} Values of SeG, TG, TX, and Their Platinum Complexes on L1210 Cell Growth in Vitro

compd	ID_{50},[a] M
TG	2.68×10^{-12} [c]
SeG	4.16×10^{-9}
TX	1.06×10^{-5}
TG–Pt(II)[b]	4.07×10^{-10}
SeG–Pt(II)[b]	2.51×10^{-4}
TX–Pt(II)[b]	1.87×10^{-3}

[a] The ID_{50} values (the concentration required for 50% inhibition of L1210 cell growth) were determined from the log probit dose-response curve. [b] These compounds were used as suspension in medium, because of their insolubility. [c] This value was estimated by extrapolation to be approximate ID_{50} from log probit dose-response curve, because the curve did not across 50% inhibition under the condition used. Accordingly, the value was somewhat less than that (1.50×10^{-10} to 1.92×10^{-11} M) accurately obtained.[29]

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Table III. Effect of Pretreatment with Platinum Complexes on Survival Time of Mice

day of sample injn[a]	T/C,[b] % SeG (50 mg/kg)	SeG-Pt(II) (150 mg/kg)	TG (50 mg/kg)	TG-Pt(II) (150 mg/kg)
−14		125		167
−7		150		144
−3	100	138	111	144

[a] L1210 cells were transplanted on day 0. [b] Ratio of the median survival time of the treated (T) vs. control (C) animals.

Pt(II) > SeG–Pt(II). It is worth noting that the order of TX–Pt(II) and SeG–Pt(II) is inverted compared with that of the parent compounds. However, neither TX–Pt(II) nor TX showed antitumor activity on L1210 cells in vivo. Accordingly, it is required for the appearance of the in vivo antitumor activity of the complex that the ligand possesses an even weak antitumor effect.

A single injection of 150 mg/kg of SeG–Pt(II) on days 3, 7, and 14 before transplantation of L1210 cells produced the significant T/C values shown in Table III. The maximum T/C values were obtained on day 7 for SeG–Pt(II) and on day 14 for TG–Pt(II) before tumor inoculation. This difference of the effectiveness on the injection schedule may be due to the stability of the complex in vivo.

From these studies, it appears that SeG–Pt(II) is effective after gradual hydrolysis to SeG. However, from the results of pretreatment of mice with SeG–Pt(II) we do not exclude the possibility that SeG–Pt(II) could also have immunological antitumor effects. These questions are currently under investigation. Recent results have shown that hypersensitivity reactions in mice feet are enhanced by treatment with SeG–Pt(II).[25]

Experimental Section

Chemistry. Thioguanine, thioguanosine (TGR), 6-chloroguanine, 6-chloroguanosine, and 6-thioxanthine were purchased from Sigma Chemical Co. (St. Louis, Mo.), potassium tetrachloroplatinate(II) was purchased from Kojima Chemical Co. (Tokyo). Thin-layer chromatography (TLC) was carried out with Avicel SF cellulose precoated glass plates purchased form Funakoshi Co. Ltd. (Tokyo). The solvent systems used were as follows: A, 1-butanol–acetic acid–H_2O (3:1:1); B, 5% NH_4OH; C, 2-propanol–concentrated NH_4OH–H_2O (7:1:2). IR spectra were measured with JASCO-IRA-1 spectrophotometer in KBr disks. UV spectra were measured with Cary 14 spectrophotometer, and ^{13}C NMR spectra were measured with a JEOL-PS-FT-100 spectrometer in 2 N NaOH solution, operated at 25 MHz with or without irradiation of the proton region.

UV Kinetic Studies. The pseudo-first-order rate constant of the reaction of thioguanine ($2.87–5.74 \times 10^{-5}$ M) or selenoguanine ($3.03–7.27 \times 10^{-5}$ M) with freshly prepared potassium tetrachloroplatinate(II) (8.34×10^{-4} M) was determined from the hight of the 345-nm peak of thioguanine (358 nm for selenoguanine) in 0.05 M Kolthoff's Borax phosphate buffer solution at various pH. During incubation of the solution at 28 °C, the absorption spectrum was redetermined at 5-min intervals. The pseudo-first-order rate constant was plotted as a function of pH on semilogalithmic graph paper to draw up the pH–rate profile.

Preparation of Selenoguanine and Its Riboside. SeG and SeGR were synthesized from 6-chloroguanine and 6-chloroguanosine according to Mautner et al.[1] and Chu,[26] respectively.

Preparation of Thioguanine–Platinum(II) Complexes. TG–Pt(II) and TGR–Pt(II) were prepared by the method previously reported.[27] These complexes were chromatographically pure on cellulose TLC with solvent B.

Preparation of Thioxanthine–Platinum(II) Complex. TX–Pt(II) was prepared by the method used for TG–Pt(II). The yield was 1.47 g (93%) of a yellow solid from 1 g (5.95 mmol) of thioxanthine and 1.24 g (2.98 mmol) of K_2PtCl_4. The complex was chromatographically pure on cellulose TLC with solvent B. An analytical sample was further purified to remove a trace amount of free thioxanthine by extraction with 10% $NaHCO_3$ solution containing 5% Na_2CO_3 in suspension for 2 h. The solid was filtered, resuspended in water, and acidified by the addition of acetic acid. The solid was filtered, washed with water, ethanol, and ether, and dried over P_2O_5 in vacuo: UV (pH 13) λ_{max} 250 nm (sh), 295, 343; IR 1702 (br s), 1648 (s), 1610 (s), 1350 (w), 1220 (m), 1162 (w), 1048 (m), 984 (m) cm^{-1}. Anal. Calcd for $C_{10}H_4N_8O_2S_2Pt \cdot 2HCl$: C, 20.00; H, 1.00; N, 18.65; S, 10.67; Pt, 32.50. Found: C, 20.06; H, 1.15; N, 19.11; S, 11.09; Pt, 32.90.

Selenoguanine–Platinum(II) Complex. A solution of 1.04 g (2.51 mmol) of potassium tetrachloroplatinate(II) in 30 mL of water was added to a suspension of 1 g (5.03 mmol) of finely powdered selenoguanine in 30 mL of ethanol. The mixture was heated in a water bath at 80 °C for 30 min with vigorous stirring. The resulting yellow precipitate was filtered, washed with cold water, ethanol, and ether, and then dried in vacuo over P_2O_5 to yield 1.74 g (97% yield based on K_2PtCl_4). An analytical sample was suspended in CS_2 and stirred for 2 h to remove a small amount of impurity, such as selenium. The precipitate was filtered, throughly washed with ethanol and ether, and dried over P_2O_5: UV (pH 13) λ_{max} 297 nm, 340, 368; IR 1610 (br s), 1510 (m), 1420 (m), 1265 (m) cm^{-1}; IR spectrum of SeG–Pt(II) showed new absorption bands at 1510, 1480, 1420, and 1265 cm^{-1}. Anal. Calcd for $C_{10}H_6N_{10}Se_2Pt \cdot 2HCl \cdot H_2O$: C, 16.90; H, 1.41; N, 19.72; Se, 22.25; Pt, 27.46. Found: C, 16.89; H, 1.35; N, 20.10; Se, 22.13; Pt, 27.69.

Selenoguanosine–Platinum(II) Complex. The SeGR–Pt(II) complex was prepared by the method described above. The yield was 990 mg (93%) from 750 mg (2.38 mmol) of selenoguanosine and 494 mg (1.19 mmol) of K_2PtCl_4 as a yellow solid. An analytical sample was purified as described for SeG–Pt(II): UV (pH 13) λ_{max} 297 nm, 340, 368 nm; IR 1615 (s), 1518 (s), 1480 (m), 1426 (m), 1395 (m), 1233 (m), 1110 (m), 1095 (m) cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_{10}Se_2O_8Pt \cdot 2Cl \cdot 2H_2O$: C, 24.19; H, 2.82; N, 14.11; Se, 15.93; Pt, 19.66. Found: C, 24.32; H, 2.91; N, 14.48; Se, 16.15; Pt, 19.83.

Assay of Antitumor Activity. (BALB/c × DBA/2)F_1 mice weighing 20 g were used for the antitumor assay. L1210 cells (10^4) were transplanted intraperitoneally into mice. The test samples (injection volume 0.4 mL), homogenized with 7 times Nikkol in ethanol by the method of Aoshima et al.,[28] were intraperitoneally injected on days 1 and 5 after tumor inoculation. Antitumor activity was determined by comparing the median survival time of the treated group with those of the control group.

In Vitro Culture of L1210 Cells. L1210 cells (8×10^4) were incubated in a CO_2 gas incubator at 37 °C for 48 h in 1 mL of medium containing various concentrations of the drugs. Their viability, estimated by staining with 0.17% Trypan blue, was compared with that of control cells incubated in the same medium without drugs. Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum was used for culture. Platinum complexes, after heating at 110 °C for 10 h, were used as suspension in medium and taken in volume from well done suspension to give various concentrations of drugs. The assay was carried out using at least six different concentrations, to give the log probit dose–response curve.

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