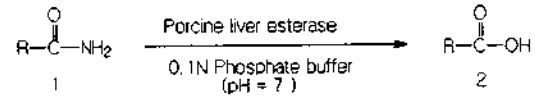
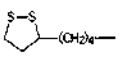
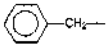
Table 2. Effects of pH on the hydrolysis of lipoamide with the porcine liver esterase

pH	reaction time (hr)	yield (%)
5	136	21
6	136	42
7	45	71
8	13	63
9	13	54

Table 3. Porcine liver esterase catalyzed hydrolysis of terminal amide



1 2

R=	Time(h)	Yield(%)
	40	71[a]
$CH_3(CH_2)_6$-	20	43[b]
$CH_3(CH_2)_6$-	160	20[b]
	90	95
	140	81

[a] The yield was based on methyl lipoate. [b] The unreacted starting material [octanoic amide (50%), decanoic amide (70%)] was recovered.

better than that of the lower pH. The reaction rate at pH 5 and 6 was very slow and the yield was 20-40%.

We expanded the utility of terminal amide hydrolysis by porcine liver esterase to the other amide derivatives in biphasic system. The broad substrate specificity of porcine liver esterase for the hydrolysis of terminal amide bond is shown in Table 3. The deamination of the carboxyamide group in aromatic amides was performed with facility giving the corresponding carboxylic acid. However, the hydrolyses of octanoic amide and decanoic amide were so slow and gave low yields of octanoic and decanoic acid. It is suggested the binding site of this catalyst may have strong affinity to an aromatic ring. These results indicated that the porcine liver esterase will be conveniently employed as a mild deprotecting method of terminal amide.

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Coordination Mode *vs.* Anticancer Activity of the Platinum(II) Complexes Involving Sulfur-Containing Ylidenemalonate Ligands

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Cisplatin, *cis*-diamminedichloroplatinum(II), is one of the most effective agents[1-3] against cancers of testis, ovary, bladder, and head and neck. However, its usefulness is limited due to its severe toxicities[4-6] such as nephrotoxicity, nausea, vomitting and myelosuppression along with development of resistance.[7,8] Therefore, there is a strong demand for the development of more efficient platinum anticancer drugs with lower toxicity and no cross-resistance. (Diamine)-platinum(II) complexes of sulfur-containing ylidenemalonate ligands were synthesized in our laboratory to display a variety of coordination modes depending on the anionic ligand structures, for example, (O,O)-chelation[9,10] for the 1,3-dithiol-2-ylidenemalonate (DTOYM) and 1,3-dithiolan-2-ylidenemalonate (DANYM) ligands, (O,S)-chelation[11,12] for the 1,3-dithian-2-ylidenemalonate (DTAYM) ligand and (S, S)-chelation[11-13] for the 1,3-dithiepane-2-ylidenemalonate (DTEYM), bismethylthiomethylenepropanedioate (BMTMP) and bisethylthiomethylenepropanedioate(BETMP) ligands.

(Diamine)platinum(II) complexes of DTOYM and DANYM which have (O,O)-chelation mode were reported[9] to exhibit fairly high anticancer activity. It can be conjectured that the anticancer activity of the platinum complexes of these sulfur-containing ylidenemalonate ligands may have a relation with their coordination modes. The coordination mode *vs.* anticancer activity relation is not easy to establish because the anticancer activity of these complexes also depends upon their carrier amine ligands. Therefore, in the present study, the carrier amine ligand was fixed to cyclopropylamine (CPA) and its platinum(II) complexes involving the anionic ligands of DTAYM, DTEYM, BMTMP and BETMP were prepared to compare their activity depending on their coordination modes. Among the complexes, $(CPA)_2Pt$(BMTMP) and $(CPA)_2Pt$(BETMP) were newly synthesized and characterized by various spectroscopic methods and X-ray crystallography in this study.

Experimental Section

Materials and Instrumentation. Cyclopropylamine (CPA) (Aldrich) was used as received. $(CPA)_2Pt$(O,S-DTAYM) and $(CPA)_2Pt$(S,S'-DTEYM) were prepared by our previous methods.[11,12]

Elemental analyses were performed at the Advanced Analysis Center at Korea Institute of Science and Technology. ^1H NMR spectra were recorded on a Varian Gemini-300 NMR spectrometer operating at 300 MHz. Infrared spectra were measured as KBr pellets on an MIDAC model 101025 FT-IR.

Synthesis of $(CPA)_2Pt$(BMTMP). This compound was synthesized according to our previous procedure[13] using CPA and BMTMP ligands in 69% yield. Cald for $C_{12}H_{20}N_2O_4S_2Pt\cdot 3H_2O$: C, 25.3; H, 4.60; N, 4.92. Found: C, 25.3; H, 4.51; N, 4.72. IR (KBr, cm^{-1}): 3472 (s), 3016 (m), 1621 (s), 1363 (s), 1319 (s), 1026 (m), 974 (m), 898 (m). ^1H NMR (δ, ppm): 3.18 and 3.03 (6H, s, methyl), 2.55 (2H, m), 0.70-0.88 (8H, m).

Synthesis of $(CPA)_2Pt$(S,S'-BETMP). This compound was synthesized according to our previous procedure[13] using CPA and BETMP ligands in 75% yield. Cald for $C_{14}H_{24}N_2O_4S_2Pt\cdot 3H_2O$: C, 28.1; H, 5.06; N, 4.69. Found: C, 28.4; H, 4.94; N, 4.73. IR (KBr, cm^{-1}): 3451 (s), 2995 (m), 1628 (s), 1368 (s), 1331 (s), 1122 (m), 1024 (m), 768 (m), 721 (m). ^1H NMR (δ, ppm): 3.2-3.4 (4H, m), 1.64 (6H, t), 2.49 (2H, br), 0.65-0.81 (8H, m).

In vitro assay. Murine leukemia L1210 cells were maintained in RPMI 1640 medium supplemented with 10% fatal bovine serum (Gibco). Cells were adjusted to 1×10^6 cells/mL and distributed to 24 well tissue culture plates (0.5 mL/well). Test compounds were serially diluted, and added to wells (0.5 mL/well). Following 48 hrs incubation in a 5% CO_2 atmosphere at 37 °C, cell counts were determined with a Coulter Model ZM cell counter. Cell growth in the presence of test compounds was expressed as a percentage of growth in untreated control wells and the concentration of compound producing 50% inhibition of cell growth was determined (ED_{50}).

In vivo assay. These tests were carried out using the ascites cell form of L1210 lymphoid leukemia, which was obtained from DBA/2 donor mice bearing 3-5 day tumour growth. L1210 leukemia cells (10^6) were inoculated i.p. in BDF mice (6-8 weeks old, 20-25 g, 8 mice per group), and 24 hrs later, compounds were administered i.p. on days 1, 5, 9. Mortality was recorded and the mean survival time was calculated for each group. The percentage of increased life span (ILS) was calculated as:

$$\%ILS = (T-C)/C \times 100$$

where T is the mean survival time of the drug treated mice and C is that of control mice.

Crystal Structure Determination and Refinement. All the X-ray data were collected on an Enraf-Nonius CAD4 automated diffractometer equipped with a Mo X-ray tube and a graphite crystal monochromator. The orientation matrix and unit cell dimensions were determined from 25 machine centered reflections. Intensities of three check reflections monitored every 1 h during the data collection period indicated no significant degradation. Absorption corrections were applied by an empirical psi scan method. The Patterson method was employed to locate a heavy atom (SHELXS-86[14]) and the subsequent cycle of the Fourier map and least square refinements located other atoms(SHELXL 97[15]). The C(12) atom was refined in disordered form of C (12A) and C(12B) with equal site occupancy factor of 0.5. All the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in the structure factor calculation using a riding model.

Results and Discussion

Synthesis and Properties. The $(CPA)_2Pt$(II) complexes of the BMTMP and BETMP ligands synthesized by our previous methods were obtained as fine crystals. A single crystal of $(CPA)_2Pt$(BETMP) prepared from a solvent pair of water and acetone was subjected to X-ray crystallographic study to analyze its structural properties.

$(CPA)_2Pt$(BMTMP) has two conformational isomers[13] of *syn* and *anti* forms when the central platinum atom is coordinated by both sulfur atoms. The *syn* isomer exhibits a resonance at 3.18 ppm and the *anti* isomer at 3.03 ppm in 1 : 1.1 integration ratio in its ^1H NMR spectrum. Both peaks are significantly shifted to downfield region compared to that (2.45 ppm) of the dipotassium salt of BMTMP, which excludes the possibility of any Pt-O coordination. $(CPA)_2Pt$-(S,S'-BETMP) exist predominantly as *anti* isomer in solution because of more crowded ethyl groups. The methylene protons of ethyl group in $(CPA)_2Pt$(S,S'-BETMP) display complex patterns in the ^1H NMR spectrum. Ethyl groups in the complex are elucidated to be rigid even in solution and their protons seem to couple with other geminal and vicinal protons.

Crystal Structure of $(CPA)_2Pt$(BETMP). An ORTEP drawing of the complex is shown in Figure 1. Its crystallographic data, and selected bond distances and angles are listed in Table 1 and Table 2, respectively. The BETMP ligand chelates the platinum(II) atom through two sulfur atoms with the two carboxylate groups uncoordinated. The coordination geometry around the platinum atom is a typical square planar structure. The distances of Pt-S(1) and Pt-S(2) bonds are 2.277(2) and 2.268(2) Å, respectively, which fall in the range of normal Pt-S bond.[16,17] Bond distances of Pt-N

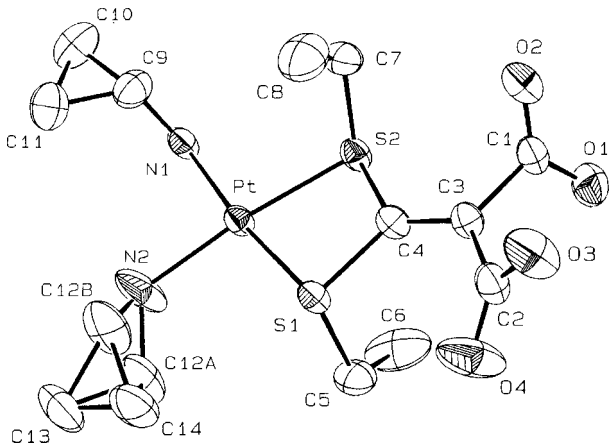

Figure 1. An ORTEP drawing of $(CPA)_2Pt(S,S'\text{-BETMP})$ with an atomic labeling scheme.

Table 1. Crystallographic Data for $(CPA)_2Pt(S,S'\text{-BETMP})\cdot 3H_2O$

Empirical formula	$C_{14}H_{24}N_2O_3Pt\ S_2\cdot 3H_2O$
Formula weight	597.61
Wavelength	0.71073 Å
Crystal system	triclinic
space group	$P\bar{1}$ (No. 2)
Unit cell dimensions	a=8.643(2) Å α=93.51(2)
	b=9.850(3) Å β=106.15(2)
	c=13.781(3) Å γ=106.79(2)
V	1066.0(5) Å^3
Z	2
Calculated density	1.862 g/cm^3
Absorption coefficient	6.812 mm^{-1}
$F(000)$	588
θ range for data collection	1.56 to 24.97°
Index ranges	$-10\le h\le 9$, $-11\le k\le 11$, $0\le l\le 16$
Reflections collected	3428
Refinement method	Full-matrix least-squares on F^2
Data/parameters	3274/253
Goodness-of-fit on F^2	1.032
Final R indices $[I>2\sigma(I)]$	R_1=0.0291, wR_2=0.0739
R indices (all data)	R_1=0.0298, wR_2=0.0745
Largest diff. peak and hole	1.288 and −1.008 e.Å^{-3}

$R_1=\Sigma||F_o|-|F_c||/\Sigma|F_o|$. $wR_2=\{\Sigma w(F_o^2-F_c^2)^2/\Sigma wF_o^4\}^{1/2}$, where $w=1/\{\sigma^2F_o^2+(aP)^2+bP\}$

Table 2. Selected Bond Lengths (Å) and Angles (°) for $(CPA)_2$-$Pt(S,S'\text{-BETMP})\cdot 3H_2O$

Pt-N(1)	2.072(6)	Pt-N(2)	2.054(7)
Pt-S(1)	2.277(2)	Pt-S(2)	2.268(2)
S(1)-C(4)	1.779(7)	S(1)-C(5)	1.830(8)
S(2)-C(4)	1.780(7)	S(2)-C(7)	1.816(8)
C(3)-C(4)	1.339(9)		
N(2)-Pt-N(1)	89.0(3)	N(2)-Pt-S(2)	173.7(2)
N(1)-Pt-S(2)	96.95(16)	N(2)-Pt-S(1)	97.4(2)
N(1)-Pt-S(1)	172.28(16)	S(2)-Pt-S(1)	76.75(6)
C(4)-S(1)-C(5)	103.0(4)	C(4)-S(1)-Pt	88.8(2)
C(5)-S(1)-Pt	107.6(3)	C(4)-S(2)-C(7)	105.6(4)
C(4)-S(2)-Pt	89.1(2)	C(7)-S(2)-Pt	109.6(3)
C(9)-N(1)-Pt	112.7(5)	S(2)-C(4)-S(1)	104.8(4)

(1) and Pt-N(2) are 2.072(6) and 2.057(7) Å, respectively, which are slightly longer than the Pt-N distances in $(CPA)_2$-$Pt(DTOYM)$ and $(CPA)_2Pt(DANYM)$ by 0.02-0.04 Å, presumably due to the stronger trans influence of sulfur than oxygen. Five atoms of Pt, N(1), N(2), S(1) and S(2) constitute a well defined least squares plane. Maximum deviation from the plane is −0.066(3) Å for S(1) atom. Six atoms of S(1), S(2), C(1), C(2), C(3) and C(4) also form a well defined sp^2 plane. The C(2) atom shows maximum deviation of 0.063(3) Å from the sp^2 plane. The angle between the sp^2 plane and the platinum coordination plane is 9.13(8)°. The two ethyl groups at two sulfur atoms are in *anti* position against the aforementioned planes, which is consistent with the result of the solution ^{1}H NMR study. The C(3)-C(4) distance is 1.339(9) Å, which fall in the range of normal C=C double bond[18] but is remarkably different from the distances of C=C in $(CPA)_2Pt(DOLYM)$ (1.385(10) Å)[9] and $(CPA)_2Pt(DANYM)$ (1.374(9) Å).[10] Such a result supports the explanation[11] on the variety of coordination mode in platinum complexes of sulfur containing ylidenemalonate ligands coordinated to platinum.

The (S,S)-coordinated complex, $(CPA)_2Pt(BETMP)$, has zwitterionic character, that is, a cationic character around the platinum atom and an anionic character around the uncoordinated carboxylate groups. In the crystal packing system, amine nitrogen atoms closely interact with neighbouring carboxylate oxygens through hydrogen bonding which is presumed to stabilize the energy of zwitterionic compound. A hydrogen bonded dimer formed between the molecules of symmetry code (x, y, z) and (-x, 1-y, -1-z) is shown in Figure 2. The intermolecular distance of N(1)---O(3) is 2.870(8) Å. A close distance of 3.462(3) Å which is within the van der Waals interaction range (3.6 Å)[19] was observed between neighbouring S(2) atoms in this dimer. This dimeric interaction was found to be broken in aqueous solution because the freezing point deppression study has shown a monomeric molecular weight. The zwitterionic complex seems to be stabilized also by hydrogen bonding with the solvent molecules in aqueous solution, implying that the complex is very hydrophilic, which, however, is not advantageous in affording anticancer activity, as will be discussed

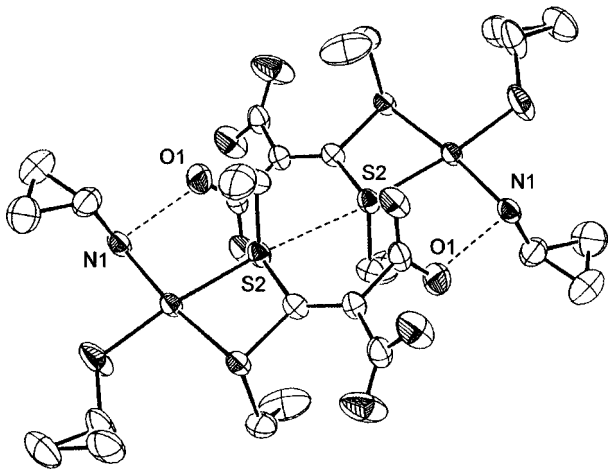

Figure 2. Intermolecular interactions present in the crystal packing system between the molecules of symmetry code (x, y, z) and (-x, 1-y, -1-z). The disordered C(12B) atom was omitted.

Table 3. Antitumor Activity of Pt(II) Complexes against Leukemia L1210

Compounds	*in vitro*	*in vivo*		
	ED_{50} (μg/mL)	Dose (mg/Kg)	ILS (%)	No. of survivors at day 45
$(CPA)_2Pt(DTOYM)^9$	1.05	4	>199	2/8
$(CPA)_2Pt(DANYM)^9$	5.36	10	>133	1/8
$(CPA)_2Pt(DTAYM)$	>40.0	20	0	0
$(CPA)_2Pt(DTEYM)$	>40.0	20	0	0
$(CPA)_2Pt(BMTMP)$	>40.0	20	0	0
$(CPA)_2Pt(BETMP)$	>40.0	20	0	0
Cisplatin	0.33	4	74	0
Carboplatin	3.80	40	68	0

later. In particular, a short distance (3.480(12) Å) was observed between C(6) and neighbouring C(8) of symmetry code (1+x, y, z), which is comparable to the shortest distance (3.57 Å)[19] observed in gear-like packing of aliphatic compounds. This unique interaction seems to occur presumably because the hydrophobic methyl groups are surrounded by a hydrophilic environment.

Anticancer Activity. The *in vitro* and *in vivo* anticancer activities of the title complexes with CPA as a carrier ligand are listed in Table 3. The $(CPA)_2Pt(II)$ complexes of the anionic leaving groups DTOYM and DANYM affording (O,O) chelation to the platinum atom exhibit fairly high *in vivo* activity, but the platinum(II) complexes of other anionic ligands affording (O,S)- or (S,S')-chelation show no or only marginal activity. The reason of the loss of biological activity of the platinum complexes involving the Pt-S bond may be explained based on the structure-activity relationships described in the previously reported reference.[20] The Pt-S bond formed by a soft acid and a soft base is so strong that it may not be labile enough to dissociate in the cytoplasma to interact with DNA. However, some platinum (II) complexes with a Pt-S bond were reported[21~23] to have some anticancer activity, and therefore, this cannot be the sole reason. The title platinum(II) complexes are zwitterionic when they have one or two Pt-S bonds and are very hydrophilic as was discussed in the aforementioned structural analysis. This polar zwitterionic complexes may not be easy to diffuse into the hydrophobic cell membrane, which may be another important reason for the loss of anticancer activity.

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Supporting Information Available. Tables of crystallographic details, non-hydrogen positional parameters, bond distances and angles, anisotropic thermal parameters of non-hydrogen atoms, and hydrogen coordinates and isotropic thermal parameters (7 pages). Ordering information is given on any current mastheadpage.

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