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Synthesis of (Diamine)platinum(II) and (Diamine)platinum(IV) Complexes of Isopropylidenmalonate Ligand and Their Interaction with Guanosine-5'-Monophosphate

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A series of (diamine)isopropylidenmalonatoplatinum(II) complexes and the oxidation products, (diamine)Pt$(OOC)_2C{=}C(CH_3)_2(X)_2$, (diamine=ethylenediamine(en), 1,2-diaminopropan(dap), N-methylethylenediamine(men); X=OH, $OCOCH_3$, $OCOCF_3$), have been prepared, and their interaction with guanosine-5'-monophosphate (5'-GMP) have been examined by means of ^1H NMR spectroscopy. The present platinum(II) complexes have shown to interact with 5'-GMP through N7 coordination in two concecutive steps in a similar way as with cisplatin, but no interaction between the present platinum(IV) complexes and 5'-GMP was observed. However, in the presence of ascorbic acid, the platinum(IV) complexes have been found to interact with 5'-GMP with the reaction rate depending on their reduction rate.

Introduction

cis-Diamminedichloroplatinum(II) (cisplatin) is one of the most widely used anticancer drugs, but its disadvantages are severe toxicities and acquired resistance. Efforts to overcome such disadvantages over the past two decades have resulted in clinical trials of a few second-generation drugs, such as diammine(1,1-cyclobutanedicarboxylato)platinum(II) and (1,2-diaminocyclohexane)-oxalatoplatinum(II).[1-5] Converting platinum(II) complexes to their platinum(IV) analogues is another approach to search for new platinum drugs. The antineoplastic activity of several platinum complexes has prompted intensive research on their working mechanism.[6-9] However, no systematic research for the platinum(IV) complexes of alkylidenmalonate ligand has been carried out. Nowadays, it is generally accepted that the interaction of these drugs with the nucleobases of the cellular DNA, and, in particular, with the kinetically preferred guanine, plays an important role in this process. Although we cannot exclude the fact that the active platinum(IV) drugs might exhibit a different mechanism compared to the corresponding platinum(II) complexes, it has been suggested that the former complexes might act as a type of "prodrug" which need activation by in vivo reduction before exerting their antitumor activity.[10-13] For reduction of the octahedral platinum(IV) complexes to the square planar platinum(II) complexes, several biomolecules, such as ascorbic acid and cystein-containing peptide, are presented to be utilized.

In order to gain more insight into the mode of the platinum(IV) complex-DNA interaction, we have prepared a series of platinum(IV) complexes, (diamine)Pt$(OOC)_2C{=}C(CH_3)_2(X)_2$ complexes (diamine=ethylenediamine(en), 1,2-diaminopropane(dap), N-methylethylenediamine(men); X=OH, $OCOCH_3$, $OCOCF_3$) by oxidation of the precurser platinum(II) complexes, (diamine)-Pt$(OOC)_2C{=}C(CH_3)_2$.

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Both of these platinum(II) and platinum(IV) complexes have been subjected to reaction with guanosine-5'-monophosphate (5'-GMP), which has been examined in detail by means of NMR spectroscopy, and here the results are reported.

Experimental

Instrumentation and Materials. Elemental analyses were performed by the Korea Basic Science Institute Seoul Branch. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 250MH/52MM spectrometer relative to TMS as an external standard. The infrared spectra in the 4000-400 cm^{-1} region were measured as KBr pellets on a Nicolet Impact 400 FT-IR spectrophotometer. Melting points were measured with a Mettler FP 82 Hot Stage and a Mettler FP 90 Central Processor.

Potassium tetrachloroplatinate(II) purchased from Kojima, and ethylenediamine(en), 1,2-propanediamine(dap), N-methylethylenediamine(men), acetic anhydride, trifluoroacetic anhydride, diethyl isopropylidenemalonate (IPM) and guanosine-5'-monophosphate (5'-GMP) obtained from Aldrich were used as received. Diethyl isopropylidenemalonate was converted to barium salt by the literature method,[14] and *cis*-diaminediiodoplatinum(II) was prepared also by the known procedure.[15]

Synthesis of (diamine)Pt(OOC)$_2$C=C(CH$_3$)$_2$ (diamine=en, dap, men). To a suspension of 3.0 mmol of (diamine)PtI_2 in 50 cm^3 of water was added 3.0 mmol of silver sulfate in 100 cm^3 of water. The reaction mixture was stirred for 6 h and then the precipitated silver iodide was filtered off. An equimolar solution of barium isopropylidenemalonate (Ba(IPM)) dihydrate in 50 cm^3 of water was dropped into the filtrate of (diamine)$PtSO_4$, and the reaction mixture was stirred further for 3 h. After the barium sulfate was filtered off, the filtrate was condensed to 5 cm^3, to which excess acetone was added to precipitate the solid product. The crude product was recrystallized from water to obtain crystals.

(en)Pt(IPM) (**1**). Yield 85%. mp 182 °C (dec.). Found (calc. for $C_8H_{14}N_2O_4Pt$): C, 23.8 (24.2); H, 3.3 (3.6); N, 6.7 (7.0). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1638, 1597; ν (C=O)$_{sym}$, 1365. ^{1}H NMR (D_2O, ppm): 2.53 (s, 4H, $^3J_{Pt\text{-}H}$, 39.6 Hz); 1.85 (s, 6H).

(dap)Pt(IPM) (**2**) Yield 90%. mp 179 °C (dec.). Found (calc. for $C_9H_{16}N_2O_4Pt$): C, 26.0 (26.3); H, 3.4 (3.9); N, 6.5 (6.8). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1634, 1576; ν (C=O)$_{sym}$, 1385. ^{1}H NMR (D_2O, ppm): 2.9 (m, 1); 2.49 (m, 1H); 2.26 (m, 1H); 1.83 (s, 6H); 1.17 (d, J=6.25 Hz, 3H).

(men)Pt(IPM) (**3**). Yield 92%. mp 180 °C (dec.). Found (calc. for $C_9H_{16}N_2O_4Pt$): C, 26.1 (26.3); H, 3.5 (3.9); N, 6.4 (6.8). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1634, 1576; ν (C=O)$_{sym}$, 1385. ^{1}H NMR (D_2O, ppm): 2.4 (m, 7H); 1.85 (s, 6H).

Synthesis of (diamine)Pt((OOC)$_2$C=C(CH$_3$)$_2$)(OH)$_2$ (diamine=en, dap, men). To a suspension of 2 mmol of (diamine)Pt(OOC)$_2$C=C(CH$_3$)$_2$ in 20 cm^3 of water was added 20 cm^3 of H_2O_2 (30%), and the solution was stirred for 2 h. The resulting pale yellow solution was concentrated to 5 cm^3 in a rotavapor at 40 °C, to which 100 cm^3 of acetone was added. The light yellow precipitate was filtered and washed with ethyl ether. The crude product was recrystallized from water to obtain the crystalline solid product.

(en)Pt(IPM)(OH)$_2$ (**4**). Yield 70%. mp 168 °C (dec.). Found (calc. for $C_8H_{16}N_2O_6Pt$): C, 22.1 (22.3); H, 3.5 (3.7); N, 6.4 (6.5). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1630; ν (C=O)$_{sym}$, 1358, 1330. ^{1}H NMR (D_2O, ppm): 2.92 (s, 4H, $^3J_{Pt\text{-}H}$, 30.4 Hz); 1.96 (s, 6H), ^{13}C NMR (D_2O, ppm): 173, 157, 130, 47, 23.

(dap)Pt(IPM)(OH)$_2$ (**5**). Yield 79%. mp 165 °C (dec.). Found (calc. for $C_9H_{18}N_2O_6Pt$): C, 24.5 (24.3); H, 3.6 (4.1); N, 6.5 (6.3). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1646; ν (C=O)$_{sym}$, 1372, 1252. ^{1}H NMR (D_2O, ppm): 3.4 (m, 1H); 2.6-2.9 (m, 2H); 1.92 (s, 6H); 1.36 (d, J=6.50 Hz, 3H). ^{13}C NMR (D_2O): 174, 157, 127, 56, 52, 23, 14.

(men)Pt(IPM)(OH)$_2$ (**6**). Yield 80%. mp 167 °C (dec.). Found (calc. for $C_9H_{18}N_2O_6Pt$): C, 24.1 (24.3); H, 3.6 (4.1); N, 6.2 (6.3). IR (KBr cm $^{-1}$): ν (C=O)$_{asym}$, 1646; ν (C=O)$_{sym}$, 1372, 1252. ^{1}H NMR (D_2O, ppm): 2.6-2.9 (m, 3H); 2.52 (s, 4H, $^3J_{Pt\text{-}H}$, 25.4 Hz); 1.94 (s, 6H). ^{13}C NMR (D_2O): 173, 157, 130, 47, 27, 23.

Synthesis of (diamine)Pt((OOC)$_2$C=C(CH$_3$)$_2$)X$_2$ (diamine=en, dap, men X=CH$_3$COO, CF$_3$COO). To a suspension of 0.7 mmol of (diamine)Pt((OOC)$_2$C=C(CH$_3$)$_2$)(OH)$_2$ in 50 cm^3 of CH_2Cl_2 was added 5 cm^3 of acetic anhydride or trifluoroacetic anhydride, and the mixture was stirred until the solution became clear. The solvent was removed by evaporation under reduced pressure and the residue was recrystallized from methanol.

(en)Pt(IPM)(OCOCH$_3$)$_2$ (**7**). Yield 65%. mp 172 °C (dec.). Found (calc. for $C_{12}H_{20}N_2O_8Pt$): C, 27.8 (28.0); H, 4.2 (3.9); N, 5.0 (5.4). IR (KBr cm^{-1}): ν (C=O)$_{asy}$, 1640; ν (C=O)$_{sy}$, 1368, 1330. ^{1}H NMR (D_2O, ppm): 2.92 (s, 4H, $^3J_{Pt\text{-}H}$, 18.7 Hz); 2.01 (s, 6H); 1.95 (s, 6H). ^{13}C NMR (D_2O, ppm): 181, 175, 23, 21.

(dap)Pt(IPM)(OCOCH$_3$)$_2$ (**8**). Yield 60%. mp 176 °C (dec.). Found (calc. for $C_{13}H_{22}N_2O_8Pt$): C, 29.2 (29.5); H, 4.0 (4.2); N, 5.1 (5.3). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1646; ν (C=O)$_{sym}$, 1372, 1252. ^{1}H NMR (D_2O, ppm): 3.4 (m, 1H); 2.7-2.9 (m, 2H); 1.99 (d, J=5.0 Hz, 6H); 1.93 (d, J=3.3 Hz, 6H); 1.33 (d, J=6.35 Hz, 3H). ^{13}C NMR (D_2O): 173, 157, 127, 55, 52, 23, 14.

(men)Pt(IPM)(OCOCH$_3$)$_2$ (**9**). Yield 60%. mp 170 °C (dec.). Found (calc. for $C_{13}H_{22}N_2O_8Pt$): C, 29.1 (29.5); H, 4.1 (4.2); N, 5.3 (5.3). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1646; ν (C=O)$_{sym}$, 1372, 1252. ^{1}H NMR (D_2O, ppm): 3.0-2.7 (m, 3H); 2.4 (s, 4H, $^3J_{Pt\text{-}H}$, 22.8 Hz); 1.97 (d, J=7.10 Hz, 6H); 1.93 (d, J=9.75 Hz, 6H). ^{13}C NMR (CD_3OD): 173, 160, 27, 23.

(en)Pt(IPM)(OCOCF$_3$)$_2$ (**10**). Yield 60%. mp 177 °C (dec.). Found (calc. for $C_{12}H_{14}N_2O_8PtF_6$): C, 22.8 (23.1); H, 2.5 (2.3); N, 4.1 (4.5). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1714, 1630; ν (C=O)$_{sym}$, 1358, 1330. ^{1}H NMR (CD_3OD, ppm): 2.92 (s, 4H, $^3J_{Pt\text{-}H}$, 28.7 Hz); 2.0 (s, 6H). ^{13}C NMR (CD_3OD, ppm): 172, 163, 22.

(dap)Pt(IPM)(OCOCF$_3$)$_2$ (11). Yield 60%. mp 180 °C (dec.). Found (calc. for $C_{13}H_{16}N_2O_8PtF_6$): C, 24.2 (24.5); H, 2.6 (2.5); N, 4.7 (4.4). IR (KBr cm^{-1}): ν (C=O)$_{asym}$, 1715, 1646; ν (C=O)$_{sym}$, 1372, 1252. ^{1}H NMR (CD_3OD, ppm): 3.3 (m, 1H); 2.7 (m, 2H); 1.90 (d, J=3.6 Hz, 6H); 1.40 (d, J=6.5 Hz, 3H). ^{13}C NMR (CD_3OD): 181(d), 173(d), 156, 153, 128, 56, 52, 23(d), 14.

(men)Pt(IPM)(OCOCF$_3$)$_2$ (**12**). Yield 65%. mp 178 °C

(dec.). Found (calc. for $C_{13}H_{16}N_2O_8PtF_6$): C, 24.4 (24.5); H, 2.7 (2.5); N, 4.5 (4.4). IR (KBr cm^{-1}): ν $(C=O)_{asym}$, 1715, 1646; ν $(C=O)_{sym}$, 1372, 1252. ^1H NMR (D_2O, ppm): 3.0-2.7 (m, 3H); 2.4 (s, 4H, $^3J_{Pt-H}$, 22.8 Hz); 1.90 (d, J=6.5 Hz, 6H). ^{13}C NMR (CD_3OD): 180, 173, 28, 24.

Reduction of Pt(IV) Complexes with Ascorbic Acid. The progress of the reaction of platinum(IV) complexes with ascorbic acid in 1 : 1 and 1 : 5 mole ratios in neutral D_2O solution were monitored at 37 °C by means of ^1H NMR spectroscopy.

Interactions of Platinum Complexes with 5'-GMP. Interactions of platinum(II) and platinum(IV) complexes with 5'-GMP in a mole ratio of 1 : 2.5 in neutral D_2O solution were observed in NMR tubes. In addition, interaction between platinum(IV) complexes and 5'-GMP in the same mole ratios was examined in the presence of 50 mM ascorbic acid. All these reactions were monitored at 37 °C by ^1H NMR spectroscopy.

Results and Discussion

Synthesis and Spectroscopic Properties. All the platinum(II) complexes of isopropylidenemalonate ligand, (diamine)-$Pt(OOC)_2C=C(CH_3)_2$ (diamine=en, dap, men) and their oxidized derivatives (diamine)$Pt(OOC)_2C=C(CH_3)_2(X)_2$ (X=OH, CH_3COO, CF_3COO) have been prepared according to the general methods.[14,15] The complexes have been characterized by elemental analysis, IR and NMR spectroscopy. All the complexes obtained as white or yellow solids are air stable. The complexes are soluble and fairly stable in water at room temperature. The water-solubility of the trans-dicarboxylatoplatinum(IV) complexes (20 mg/cm^3 H_2O) is less than that of the *trans*-dihydroxoplatinum(IV) complexes (>50 mg/cm^3 H_2O).

In the IR spectra of the platinum(II) and *trans*-dihydroxoplatinum(IV) complexes, the differences ($\Delta\nu$) between the asymmetric and symmetric carbonyl stretching frequencies are larger than 200 cm^{-1}, which suggests that the two carboxylate groups in the isopropylidenemalonate ligand act as a monodentate. The oxidation products of the platinum(II) complexes would be readily identified by their characteristic PtO-H and Pt-O stretches at 3450 and 3430 cm^{-1}, and at 556 and 562 cm^{-1}, respectively, which disappeared after carboxylation. One broad carbonyl stretching absorption was observed in the range of 1636-1640 cm^{-1} for the *trans*-diacetatoplatinum(IV) complexes due to overlapping of the stretching frequencies of the different carboxylate groups, while two carbonyl stretching bands appeared in the *trans*-bis(trifluoroacetato) platinum(IV) analogs. The carbonyl stretching frequencies of the trifluoroacetate ligand in the platinum(IV) complexes appeared in the region at 1713-1723 cm^{-1} blue-shifted by approximately 100 cm^{-1} compared with that (1601-1630 cm^{-1}) of other carboxylate ligands.[16-19] Such a blue shift is in accordance with the data reported for other metal complexs with carboxylate ligands.[19]

^1H and ^{13}C NMR spectral data of the octahedral platinum (IV) complexes are consistent with the structure in the solid state. For the platinum(II) complexes, the methyl protons of the IPM ligand resonate at 1.83-1.85 ppm as a singlet, which shifted downfield to 1.90-2.01 ppm in their corresponding octahedral platinum(IV) complexes. This result suggests that the symmetrical coordination of both carboxylate groups of the IPM ligand to the platinum atom are retained in aqueous solution. The α-carbon proton peak of the amine ligand exhibits satellites from coupling with ^{195}Pt(I=½, 33% abundance). For example, the α-carbon proton of the en ligand of (en)Pt(ipm) resonates as a singlet at 2.53 ppm with satellites of $^3J_{Pt-H}$=39.6 Hz. For (en)Pt(ipm)$(OH)_2$, the corresponding protons appear as a singlet at 2.92 ppm with satellites of $^3J_{Pt-H}$=30.4 Hz. Also, the β-carbon proton peak of the (dap) ligand resonate at 1.17 ppm as a doublet, which shifted downfield to 1.33-1.40 ppm as a doublet in their corresponding octahedral platinum(IV) complexes. Thus, ^3J(Pt-H) coupling constants of the present complexes and β-carbon proton downfield shift are characteristic of oxidation state of the present platinum complexes. However, no distinct influence could be noticied from the axial ligands of platinum(IV) complexes.

Reduction Rates. Since previous studies[20,21] suggest that the platinum(IV) complexes should be reduced to the platinum(II) complexes for antitumor activity, the reduction properties of the platinum(IV) complexes prepared in this work have been examined. The reaction of the platinum(IV) complexes with equimolar ascorbic acid in the neutral buffer solution at 37 °C have been monitored by ^1H NMR spectroscopy (Figure 1). As the reaction progressed, the proton peaks corresponding to the intact complexes disappeared with increasing intensity of the resonances for the corresponding platinum(II) complexes. As shown in Table 1, the platinum(IV) complexes have been reduced with variable rates to the corresponding platinum(II) complexes by ascorbic acid. The reduction rates of the platinum(IV) complexes seem to be largely dependent on

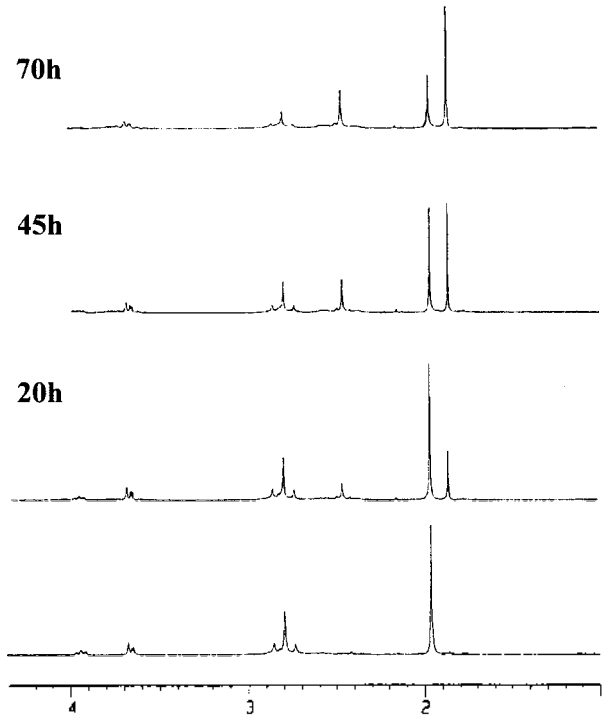

Figure 1. Time dependence of the ^1H NMR spectra for reduction of (en)Pt(IPM)-$(OH)_2$ by ascorbic acid in the neutral aqueous solution at 310 K.

Table 1. Reduction rates of (diamine)Pt(IPM)X_2 complexes by ascorbic acid

Complexes	$t_{1/2}$(h)	
	10 mM	50 mM
en-OH(**4**)	44	13
dap-OH(**5**)	40	10
men-OH(**6**)	50	15
en-$OCOCH_3$(**7**)	20	6
dap-$OCOCH_3$(**8**)	23	8
men-$OCOCH_3$(**9**)	25	8
en-$OCOCF_3$(**10**)	4	>1
dap-$OCOCF_3$(**11**)	3	>1
men-$OCOCF_3$(**12**)	5	>1

the type of the axial ligands but nearly independent of the carrier amine ligand (Figure 2) as was reported earlier.[14] The trifluoroacetate analogs were reduced more readily than any other platinum(IV) complexes, and the mole ratio of platinum(IV) and platinum(II) complexes was about one after 5 h. When the other hydroxo and acetato analogs were exposed to equimolar ascorbic acid at 37 °C for 50 h, the mole ratio of platinum(IV) and platinum(II) complexes was about one.

Interaction of Platinum(II) Complexes with 5'-GMP. Figure 3 shows the schematic structure of 5'-GMP and (dap)PtIPM. The interactions of various platinum(II) complexes with 5'-GMP in a mole ratio of 1 : 2.5 have been monitored for 45 h by ^{1}H NMR spectroscopy. Figure 4 shows the time dependent NMR spectra for the mixture of (dap)Pt(II)IPM and 5'-GMP. New signals appearing in the H8 region of the spectra after 1 h, beside those of the free nucleotide, indicate that platinum-GMP adducts are formed; A new H8 signal at 8.40 ppm (Figure 4(a)) appears and grows in intensity for 9 h and then decreases in intensity as the reaction proceeds, which is assigned to the intermediate (dap)Pt(IPM-O)(5'-GMP).[10,13] At the same time another

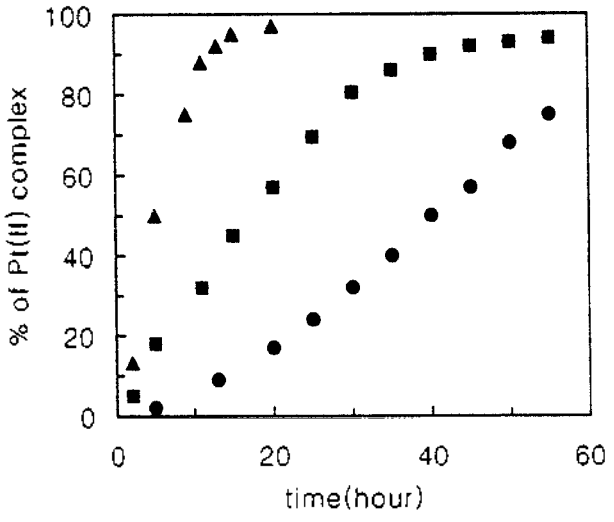

Figure 2. Plot of concentrations (as a percentage of the Pt(II) species formed) versus time for the reaction of (dap)Pt(IPM)$(X)_2$ with ascorbic acid in the neutral buffer solution at 310 K. (X= OH (●), $OCOCH_3$ (■), $OCOCF_3$ (▲))

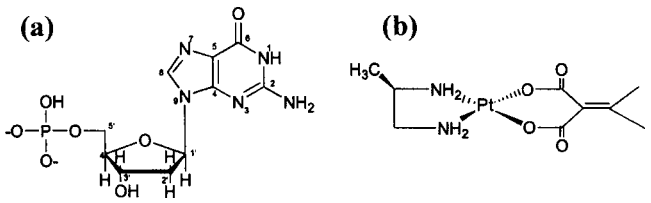

Figure 3. Schematic representation of nucleotide 5'-guanosine monophosphate (5'-GMP, (a)) and (dap)Pt(IPM) (b).

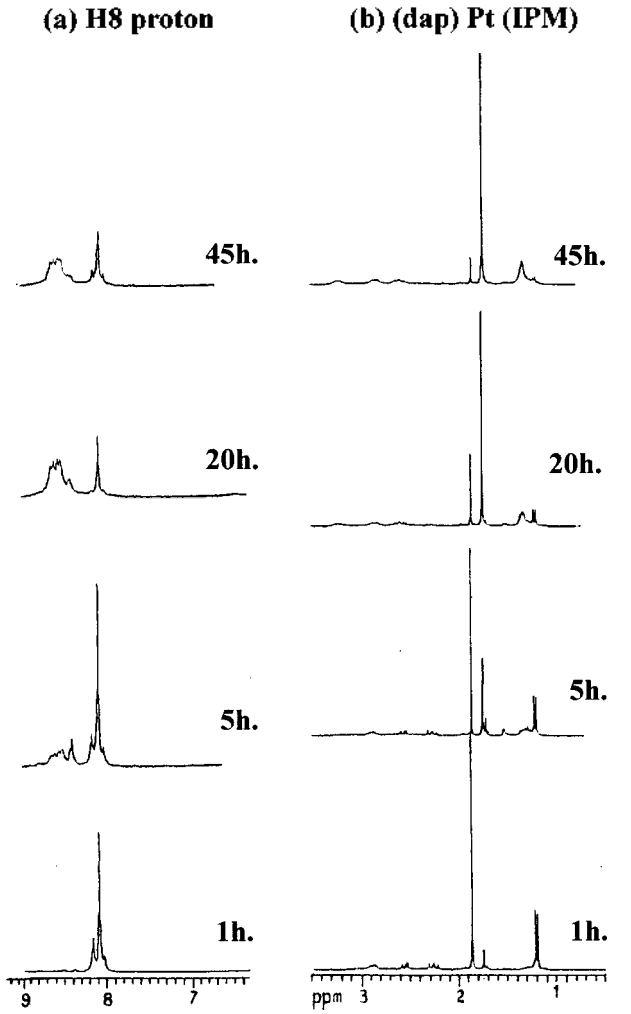

Figure 4. Time dependent ^{1}H NMR spectra for the reaction of (dap)Pt(IPM) with 5'-GMP in the neutral buffer solution at 310 K. (a) H8 proton, (b) (dap)Pt(IPM)

broad H8 resonance at 8.56 ppm is formed and increases in intensity as the reaction proceeds, while the concomitant decreases in intensity both of the resonance of the free 5'-GMP (H8, 8.09 ppm) and the intermediate (dap)Pt(IPM-O)-(5'-GMP)(H8, 8.40 ppm). Such results suggest that the growing signal at 8.56 ppm seems to be originated from the bis(nucleotide) platinum(II) complex, and the N7 coordination of 5'-GMP is retained in the complex, since the H8 proton of the complexes is shifted downfield by ~0.47 ppm.[23,24] In parallel with this result, a singlet for free IPM^{2-} appears and increases in intensity with time, with a concomitant decrease in the intensity of the peak for IPM of (dap)Pt(II)IPM (Figure 4(b)). The high field shift of the

Table 2. ^{1}H Chemical shifts(ppm) for (diamine)Pt(II)IPM complexes at pH 7 and 310 K

Complexes	IPM	*cis*-diamine	5'-GMP(H8)
5'-GMP			8.09
(en)Pt(IPM)	1.85	2.46	
(en)Pt(5'-GMP)$_2$	1.74	2.77	8.64
(dap)Pt(IPM)	1.86	2.9(m), 2.6(d-d), 1.1(d)	
(dap)Pt(5'-GMP)^2	1.74	3.2(m), 2.8(d), 1.32(d)	8.62(d), 8.56(d)
(men)Pt(IPM)	1.86	2.6-2.3(m)	
(men)Pt(5'-GMP)^2	1.74	3.0-2.5(m), 2.35(s)	8.5-8.7
IPM^{2-}	1.74		

s; singlet, d; doublet, t; triplet, m; multiplet.

IPM proton of (dap)Pt(IPM-O)(5'-GMP) is explicable by their proximity to the purine ring of N7-coordinated 5'-GMP in the *cis*-position.[10] Also, the resonance for the proton on the β-carbon of the amine ligand in (dap)PtIPM shifted down field to 1.17 ppm as a doublet which slowly disappeared as the new resonance for the amine ligand cis to 5'-GMP in (dap)Pt(5'-GMP)$_2$ is grow to 1.31 ppm.[23] Therefore, it seems likely that the low field shift is caused by hydrogen-bonding between the dap amine and the 5'-phosphate of GMP.[10] Such hydrogen-bonding is known to be important in stabilizing *cis*-[Pt(diamine)DNA].[10,23] The signals of the H8 resonances for other diamine complexes appear similarly in the range of 8.56-8.70 ppm (Table 2). The profiles of time dependent concentrations of free 5'-GMP, the intermediate (dap)Pt(IPM-O)(5'-GMP) and the final product (dap)Pt(5'-GMP)$_2$ are shown in Figure 5 for the reaction of (dap)Pt(II)IPM with 5'-GMP in the neutral buffer solution. The concentration of the monodentate intermediate increased to a maximum of approximately 15%

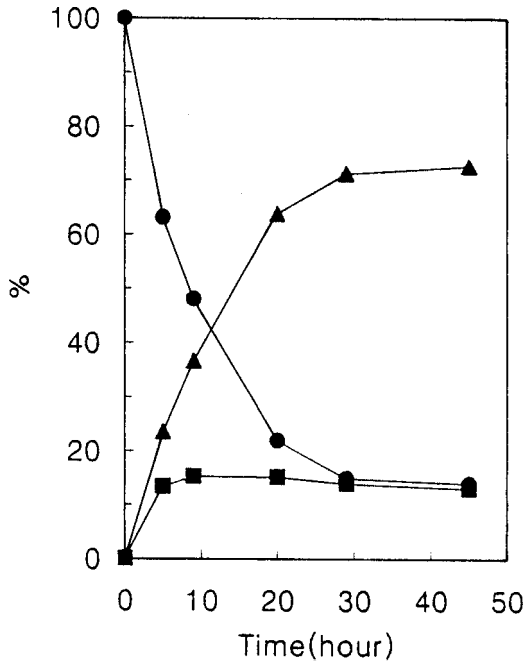

Figure 5. Plot of concentrations of 5'-GMP (●), (dap)Pt(IPM-O)(5'GMP) (■) and (dap)Pt(5'GMP)2 (▲) versus time during the reaction of (dap)Pt(IPM) with 5'-GMP in the neutral buffer solution at 310 K.

Table 3. Observed first-order rate constants for the displacement of IPM from (diamine)Pt(IPM) complexes by 5'-GMP

Complexes	k_{obs1}/s^{-1}	k_{obs2}/s^{-1}
(en)Pt(IPM)	7.0×10^{-6} ($\pm$0.2)	2.0×10^{-5} ($\pm$0.3)
(dap)Pt(IPM)	9.1×10^{-6} ($\pm$0.2)	3.1×10^{-5} ($\pm$0.4)
(men)Pt(IPM)	8.2×10^{-6} ($\pm$0.1)	2.5×10^{-5} ($\pm$0.3)
carboplatin	4.1×10^{-6} ($\pm$0.1)	3.3×10^{-5} ($\pm$0.4)

of the total platinum concentration at 9 h. These data suggest that the reaction of (dap)Pt(II)IPM with 5'-GMP occurs in two consecutive steps as shown in Eq. (1) and (2), and these data were fitted to the first order rate equation to obtain the rate constants, $k_{obs1}=(9.1\pm0.3)\times10^{-6}$ s^{-1} and $k_{obs2}=(3.1\pm0.2)\times10^{-5}$ s^{-1} (Table 3).

$$\text{(diamine)Pt(II)IPM + 5'-GMP} \xrightarrow{k_{obs1}} \text{(diamine)Pt(IPM-O)(5'-GMP)} \quad (1)$$

$$\text{(diamine)Pt(IPM-O)(5'-GMP) + 5'-GMP} \xrightarrow{k_{obs1}} \text{(diamine)Pt(5'-GMP)}_2 \quad (2)$$

This corresponds to the reaction between 5'-GMP and other (diamin)PtIPM. Plots of the concentration of each species versus time have also been fitted to the first order rate equation to obtain the rate constants, as shown is Table 3. The rate constants are almost no difference among the amine ligands. All the second step of the reaction, formation of the 5'-GMP adduct, are three times faster than the first step, and the observed rate constant is comparable with that carboplatin.[10] The value of k_{obs1}, k_{obs2} have been obtained from NMR measurements of the time dependence of the reaction containing 5'-GMP and (diamine)PtIPM. The concentration of (diamine)Pt(IPM-O)(5'-GMP), (diamine)-Pt(5'-GMP)$_2$ and unreacted 5'-GMP have been determined by integration of their H8 signals.

When the amine ligand has no bulky substituents, the complexes [(*cis*-diamine)Pt(6-Opur-N7)$_2$] (6-Opur=6-oxopurine) are known[23,24] to exhibit fast rotation about the Pt-N7 bond on the NMR time scale. In fact the NMR spectra of the 1 : 1 and 1 : 2 adducts of (en)Pt(II) and 5'-GMP show only a single H8 resonance, suggesting the fast rotation of 5'-GMP about the Pt-N7 bond on the NMR time scale. However, the methyl group in (dap)Pt(II) and (men) Pt(II) complexes creates different chemical environments for the platinum coordination plane and shows slightly different H8 resonances (Figure 6).

Interaction of Platinum(IV) Complexes with 5'-GMP. It has been found in this work that without a reducing agent the platinum(IV) complexes react at a far slower rate or practically does not react with 5'-GMP under the present conditions. Therefore, it has been decided to perform this reaction in the presence of a cellular reducing agent, and the reaction of platinum(IV) complexes with ascorbic acid and 5'-GMP has been monitored by ^{1}H NMR spectroscopy. The platinum(IV) complex was treated with a solution containing both 50 mM of ascorbic acid and 25 mM of 5'-GMP. Figure 7(a) shows the time dependent H8 proton resonance for the reaction between the (dap)Pt(IPM)(OH)$_2$ and 5'-GMP carried out in NMR tube. As expected, the platinum(IV) complexes were reduced to the

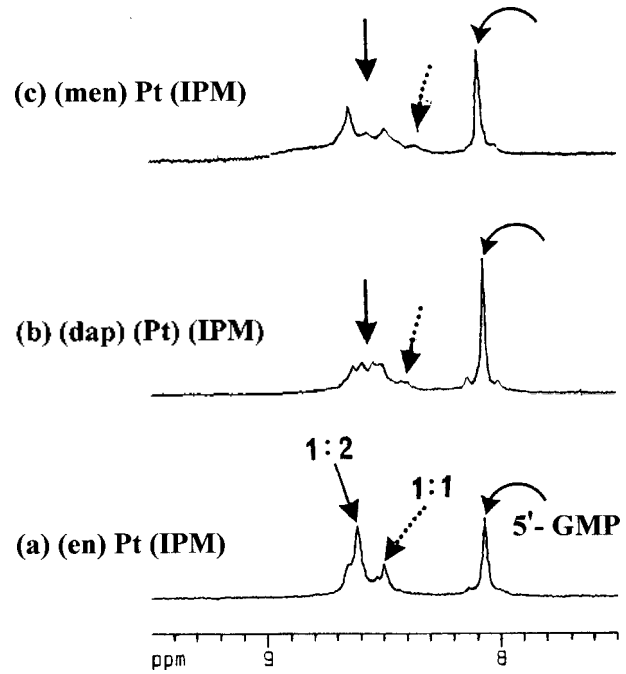

Figure 6. Downfield regions of the NMR spectra for the reaction mixture of (diamine)Pt(IPM) with 5'-GMP in the neutral buffer solution at 310 K. (a) (en)Pt(IPM), (b) (dap)Pt(IPM), (c) (men)Pt(IPM).

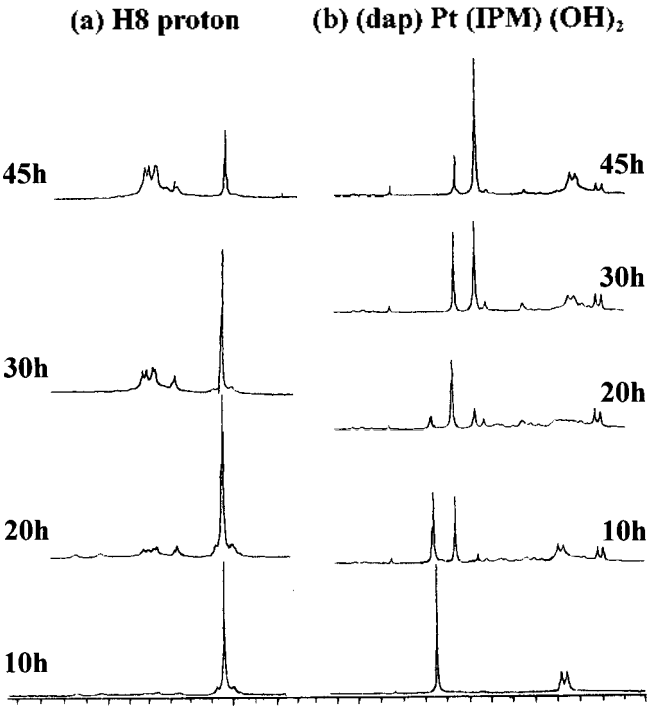

Figure 7. Time dependent ^{1}H NMR spectra of the reaction mixture of (dap)Pt-(IPM)(OH)$_2$ with 5'-GMP in the neutral buffer solution at 310 K. (a) H8 p roton, (b) (dap)Pt(IPM)(OH)$_2$.

corresponding platinum(II) complexes by ascorbic acid and new signals in the H8 region were observed at 8.50 and 8.64 ppm, the latter increasing in intensity with respect to the former and to the H8 peak of unreacted 5'-GMP during the reaction. In parallel with this result, a singlet for the free IPM^{2-} appeared and increased in intensity with time, with a concomitant decrease in intensity of the peak for the complexed IPM. In Table 4, the results of the reaction of platinum(IV) complexes with 5'-GMP are compiled. The reactions of the platinum(IV) complexes with 5'-GMP, as detected by shifts in the H8 proton resonances of 5'-GMP, were complete at 310 K in about 5 days. This result suggests that the final product of the platinum(II) complexes is the same as the precurser platinum(II) complexes. Thus, the reaction of platinum(IV) complexes with 5'-GMP seems to be dependent on the reduction rate of the platinum(IV) complexes, and it seems that the platinum(IV) complexes are likely the prodrugs of the active platinum(II) species.

Table 4. ^{1}H Chemical shifts(ppm) of the mixture of (diamine)Pt (IPM)(X)$_2$ with 5'-GMP with in the presence of Ascorbic Acid at 310 K. pH 7

Complexes diamine-X$_2$	5'-GMP H8 proton (ppm)	
	Ligand	(diamine)Pt(5'-GMP)$_2$
en-OH(**4**)	8.09	8.62
dap-OH(**5**)	8.08	8.54, 8.60
men-OH(**6**)	8.08	8.55-8.70
en-OCOCH$_3$(**7**)	8.09	8.61
dap-OCOCH$_3$(**8**)	8.08	8.54, 8.60
men-OCOCH$_3$(**9**)	8.08	8.55-8.67
en-OCOCF$_3$(**10**)	8.08	8.40
dap-OCOCF$_3$(**11**)	8.07	8.46
men-OCOCF$_3$(**12**)	8.07	8.42-8.67

In conclusion, the (diamine)platinum(IV) complexes prepared via oxidation of (diamine)platinum(II) complexes react with 5'-GMP only in the presence of ascorbic acid. The reaction rates depend on the reduction rates of platinum (IV) complexes by ascorbic acid. Such results suggest that the platinum(IV) complexes of alkylidenmalonate ligand are likely the prodrugs of the active platinum(II) species. Platinum(II) species react with 5'-GMP via ring-opened species, (diamine)Pt(IPM-O) (5'-GMP), which are reactive towards attack on the DNA base, and the final products of the reaction seem to be (diamine)Pt(5'-GMP)$_2$. The reaction of the ring-opened 5'-GMP intermediate with a second 5'-GMP is three times faster than with the first 5'-GMP. This suggest that formation of G-G cross-links on DNA will be facile.

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Liquid Chromatographic Resolution of Racemic α-Amino Acid Derivatives on an Improved π-Acidic Chiral Stationary Phase Derived from (S)-Leucine

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A chiral stationary phase derived from (S)-N-(3,5-dinitrobenzoyl)leucine N-phenyl N-alkyl amide (CSP **2**) was applied in separating the two enantiomers of various π-basic aromatic derivatives of leucine N-propyl amide in order to evaluate π-basic aromatic groups as an effective derivatizing group for the resolution of α-amino acids. Subsequently N-(3,5-dimethoxybenzoyl) group was found to be very effective as a π-basic aromatic derivatizing group. Based on these results, N-(3,5-dimethoxybenzoyl) derivatives of various α-amino N-propyl amides, N,N-diethyl amides and esters were resolved on the CSP derived from (S)-N-(3,5-dinitrobenzoyl) leucine N-phenyl N-alkyl amide (CSP **2**) and the resolution results were compared with those on the CSP derived from (S)-N-(3,5-dinitrobenzoyl)leucine N-alkyl amide (CSP **1**). The enantioselectivities exerted by CSP **2** were much greater than those exerted by CSP **1**. In addition, racemic N-(3,5-dimethoxybenzoyl)-α-mino N,N-diethyl amides were resolved much better than the corresponding N-(3,5-dimethoxybenzoyl)-α-mino N-propyl amides and esters on both CSPs. Based on these results, a chiral recognition mechanism utilizing the π-π donor-acceptor interaction and the two hydrogen bondings between the CSP and the analyte was proposed.

Introduction

Liquid chromatographic resolution of enantiomers on chiral stationary phases (CSPs) has been known as one of the most convenient and accurate means in evaluating enantiomeric purity of chiral compounds. Consequently, various CSPs for the liquid chromatographic resolution of enantiomers have been developed.[1] Among others, Pirkle-type CSPs have been known to separate two enantiomers by forming energetically different two transient diastereomeric π-π donor-acceptor complexes with two enantiomers.[2] For the effective formation of π-π donor-