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Synthesis of Pt(II) Complexes Containing D-Glucuronate, D-Gluconate, or Their Acetyl Derivatives and Evaluation of Antitumor Activity against Murine Leukemia L1210

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New antitumor-active Pt(II) complexes of (1*R*,2*R*)-cyclohexanediamine (=(1*R*,2*R*)-dach) and 2-(aminomethyl)cyclohexylamine (=amcha) isomers containing D-glucuronate, D-gluconate, tetra-*O*-acetylglucuronate (=Ac_4-glucuronate) or tetra-*O*-acetylgluconate (=Ac_4-gluconate) as a leaving group were synthesized. The structures of the Pt(II) complexes were determined by analyzing the infrared and ^{13}C-nuclear magnetic resonance spectra and it was concluded that these leaving groups coordinate to the central Pt(II) ions through the carboxyl groups. Antitumor activities of the Pt(II) complexes were tested against murine leukemia L1210 according to the protocol of the National Cancer Institute (Bethesda, Md.). All the Pt(II) complexes synthesized were antitumor-active. In particular, water-soluble Pt(gluconato)(NO_3)(*cis*-amcha) and lipo-soluble Pt(Ac_4-β-glucuronato)$_2$-((1*R*,2*R*)-dach) exhibited excellent antitumor activity, giving T/C% values of 317 and 388, respectively, each with four cured mice out of six at a dose of 50 mg/kg. These two Pt(II) complexes are considered to be worthy of further development.

Keywords——platinum(II) complex; antitumor activity; 1,2-cyclohexanediamine; 2-(aminomethyl)cyclohexylamine; D-glucuronate; D-gluconate; tetra-*O*-acetyl-D-glucuronate; tetra-*O*-acetyl-D-gluconate; L1210

cis-Diamminedichloroplatinum (II) (=*cis*-DDP) was reported for the first time to have antitumor activity by Rosenberg *et al.* in 1969[1] and it was approved by FDA in 1979. It has became one of the most widely used anticancer drugs in the United States, Europe and Japan. It has been successful in the treatment of testicular and ovarian cancers, although severe side effects accompany and sometimes limit the clinical usages of the drug. Efforts to develop *cis*-DDP analogues with reduced side effects and enhanced antitumor activity led to the release of diammine (1,1-cyclobutanedicarboxylato)platinum (II), carboplatin,[2-6] which was approved for clinical use in the United Kingdom. Another second-generation drug, *cis*-dichloro-*trans*-dihydroxo-bis(isopropylamine)platinum (IV), is currently undergoing clinical trials.[4-8] One of the Pt(II) complexes synthesized in our laboratory, (1*R*,2*R*)-cyclohexanediamine-(oxalato)platinum(II), has completed the phase I trial[9] and is now undergoing phase II trial.

In our laboratory a variety of Pt(II) complexes containing 1,2-cyclohexanediamine (=dach) and 2-(aminomethyl)cyclohexylamine (=amcha) isomers has been synthesized and tested against murine leukemia L1210. Among the Pt(II) complexes of three dach isomers, Pt(II) complexes containing (1*R*,2*R*)-dach exhibited the highest antitumor activities[10,11] and in this experiment only (1*R*,2*R*)-dach was used as a carrier ligand. Since no isomer-dependency was found in the antitumor activities of Pt(II) complexes containing optical

isomers of amcha,[12,13] we adopted two geometrical isomers, *i.e.*, racemic *trans*- and *cis*-amcha.

We prepared highly antitumor-active water-soluble or lipo-soluble Pt(II) complexes of (1*R*,2*R*)-dach or amcha isomers. Leaving groups adopted were D-glucuronate, D-gluconate, 1,2,3,4-tetra-*O*-acetyl-α or β-D-glucuronate (=Ac_4-α or β-glucuronate), or 2,3,4,6-tetra-*O*-acetyl-D-gluconate (=Ac_4-gluconate). The former two leaving groups afforded water-soluble Pt(II) complexes and the latter two, lipophilic complexes.

Experimental

1,2-Cyclohexanediamine was purchased from Aldrich Chemical Co. Inc. Separation of dach isomers was done according to the reported method.[14] The *cis*- and *trans*-2-(aminomethyl)cyclohexylamines were prepared from the corresponding *cis*- and *trans*-1,2-cyclohexanedicarboxylic acids according to the method reported by Armarego and Kobayashi.[15]

1,2,3,4-Tetra-*O*-acetyl-α or β-D-glucuronic acid was synthesized from D-glucuronolactone according to the method reported by Fry.[16] 2,3,4,6-Tetra-*O*-acetyl-D-gluconic acid was synthesized from D-glucono-δ-lactone according to the method reported by Major and Cook.[17]

Syntheses of Pt(II) Complexes——(1*R*,2*R*)-Cyclohexanediaminebis(1,2,3,4-tetra-*O*-acetyl-α or β-D-glucuronato)-platinum(II): $Pt(NO_3)_2$((1*R*,2*R*)-dach) (2.0 g) was dissolved in 20 ml of H_2O by heating and mixed with Ac_4-α or β-glucuronic acid (3.35 g) in 100 ml of EtOH. Next, 9.2 ml of 1.00 N NaOH was added and the resultant solution was kept at room temperature for 3 d protected from light. The solution was evaporated to dryness under reduced pressure at *ca.* 50 °C and the resultant residue was extracted twice with 60 ml of benzene. Removal of benzene from the combined extracts gave a pale yellow residue, which was dried *in vacuo* at 60 °C for 2 h to yield 4.25 g (89%) and

TABLE I. Elemental Analyses of Pt(II) Complexes Containing (1*R*,2*R*)-dach and amcha

Complexes	Found (%)			Calcd (%)		
	H	C	N	H	C	N
Pt(glucuronato)$_2$((1*R*,2*R*)-dach)·$2H_2O$	4.96	29.52	3.76	4.92	29.54	3.83
Pt(glucuronato)(NO_3)((1*R*,2*R*)-dach)·$1.5H_2O$	4.38	24.53	6.93	4.43	24.37	7.11
Pt(gluconato)$_2$((1*R*,2*R*)-dach)·H_2O	5.42	30.22	3.74	5.35	30.14	3.91
Pt(gluconato)(NO_3)((1*R*,2*R*)-dach)·$1.5H_2O$	4.38	24.53	6.83	4.76	24.29	7.08
Pt(Ac_4-α-glucuronato)$_2$((1*R*,2*R*)-dach)	4.79	39.76	2.81	4.70	39.57	2.72
Pt(Ac_4-β-glucuronato)$_2$((1*R*,2*R*)-dach)	4.89	39.79	2.81			
Pt(Ac_4-α-glucuronato)(NO_3)((1*R*,2*R*)-dach)·H_2O	4.23	32.01	5.69	4.44	32.00	5.60
Pt(Ac_4-β-glucuronato)(NO_3)((1*R*,2*R*)-dach)·H_2O	4.27	31.80	5.89			
Pt(Ac_4-gluconato)$_2$((1*R*,2*R*)-dach)	5.07	40.42	2.70	5.10	40.62	2.59
Pt(Ac_4-gluconato)(NO_3)((1*R*,2*R*)-dach)·H_2O	4.47	31.74	5.46	4.69	31.92	5.58
Pt(glucuronato)$_2$(*trans*-amcha)·$3H_2O$	5.26	29.74	3.64	5.24	29.88	3.67
Pt(glucuronato)$_2$(*cis*-amcha)·$2H_2O$	5.02	3.054	3.68	5.10	30.60	3.76
Pt(glucuronato)(NO_3)(*trans*-amcha)·H_2O	4.11	25.81	6.80	4.53	26.17	7.05
Pt(glucuronato)(NO_3)(*cis*-amcha)·H_2O	4.42	26.06	6.81			
Pt(gluconato)$_2$(*cis*-amcha)	5.38	31.39	4.02	5.33	31.98	3.93
Pt(Ac_4-α-glucuronato)$_2$(*trans*-amcha)·$1.5H_2O$	5.14	39.21	2.57	4.94	39.18	2.61
Pt(Ac_4-α-glucuronato)$_2$(*cis*-amcha)	4.77	40.26	2.88	4.78	40.19	2.68
Pt(Ac_4-β-glucuronato)$_2$(*trans*-amcha)	4.90	39.64	2.86	4.78	40.19	2.68
Pt(Ac_4-β-glucuronato)$_2$(*cis*-amcha)	4.77	40.14	2.77			
Pt(Ac_4-α-glucuronato)(NO_3)(*trans*-amcha)·$2H_2O$	4.43	32.36	5.11	4.73	32.36	5.37
Pt(Ac_4-α-glucuronato)(NO_3)(*cis*-amcha)	4.48	33.36	5.71	4.22	33.78	5.68
Pt(Ac_4-β-glucuronato)(NO_3)(*trans*-amcha)·$2H_2O$	4.33	32.06	5.40	4.73	32.23	5.37
Pt(Ac_4-β-glucuronato)(NO_3)(*cis*-amcha)·$1.5H_2O$	4.46	32.33	5.42	4.66	32.60	5.43
Pt(Ac_4-gluconato)$_2$(*trans*-amcha)	5.36	40.45	2.80	5.15	40.04	2.67
Pt(Ac_4-gluconato)$_2$(*cis*-amcha)	5.29	39.67	2.41			
Pt(Ac_4-gluconato)(NO_3)(*trans*-amcha)	4.70	33.20	5.00	4.83	32.90	5.48
Pt(Ac_4-gluconato)(NO_3)(*cis*-amcha)	4.60	32.60	5.08			

4.21 g (88%) of the α- and β-anomers, respectively.

(1*R*,2*R*)-Cyclohexanediaminenitrato(tetra-*O*-acetyl-α or β-D-glucuronato)platinum(II): $Pt(NO_3)_2$((1*R*,2*R*)-dach) (0.5 g) was dissolved in 5 ml of H_2O by heating and mixed with a solution of tetra-*O*-acetyl-α or β-D-glucuronic acid (0.42 g) in 20 ml of EtOH. Next, 1.5 ml of 1.00 N NaOH was added and the resultant solution was kept at room temperature for 3 d protected from light. The solution was evaporated to dryness at 50 °C under reduced pressure and the resultant residue was extracted with 10 ml of EtOH. The ethanol solution was filtered and the filtrate was evaporated at 50 °C under reduced pressure to give a pale yellow residue. The residue was washed twice with 10 ml of benzene to remove $Pt(Ac_4$-α or β-glucuronato$)_2$((1*R*,2*R*)-dach) and extracted with 10 ml of $CHCl_3$. The extract was evaporated to dryness under reduced pressure to give a pale yellow residue which was dried *in vacuo* at 100 °C for 3 h to yield 0.64 g (74%) and 0.52 g (60%) of the α- and β-anomers, respectively.

(1*R*,2*R*)-Cyclohexanediaminebis(D-gluconato)platinum(II) and (1*R*,2*R*)-Cyclohexanediamine(D-gluconato)-nitratoplatinum(II): Sodium D-gluconate (6.54 g) was dissolved in 100 ml of H_2O and mixed with 50 ml of an aqueous solution containing $AgNO_3$ (5.1 g). The resultant solution was stirred at room temperature for 1 d protected from light. Silver gluconate was precipitated by addition of 100 ml of EtOH as needle-like crystals. An aqueous suspension containing $PtCl_2$((1*R*,2*R*)-dach) (0.83 g) in 200 ml of H_2O wa added to the silver gluconate (1.41 or 0.71 g for mono-gluconato) dissolved in 50 ml of H_2O and the mixture was stirred overnight at room temperature in the dark. After the removal of AgCl by filtration, the filtrate was evaporated to dryness under reduced pressure at 60 °C to yield 1.3 g (84%) and 0.87 g (70%) of the mono- and bis-gluconato complexes, respectively.

Synthesis of mono- and bis-glucuronato Pt(II) complexes of (1*R*,2*R*)-dach was described in a previous paper.[18] The results of elemental analyses of Pt(II) complexes thus obtained are shown in Table I.

Measurements——Infrared (IR) spectra were measured with a Shimadzu IR 400 infrared spectrometer by the KBr disk method. FT ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra were obtained on a JEOL JNM-FX-100 spectrometer. All NMR spectra were measured in $CDCl_3$ solutions with tetramethylsilane as an external reference.

Antitumor Activity——Antitumor activities of the platinum(II) complexes were tested by means of the protocols used for routine screening at the National Cancer Institute (Bethesda, Md.). L1210 cells (10^5) were transplanted intraperitoneally into CDF_1 mice (1 group consisted of six mice) on day 0, and the complexes were given intraperitoneally on days 1, 5 and 9. From the mean survival times (d) of treated (T) and control (C) mice, T/C% values were calculated. Samples with T/C% values that exceeded 125 were evaluated as antitumor-active. A dose at which the T/C% value was less than 85% or the body weight of a mouse decreased by more than 4 g was designated as a toxic dose.

Results and Discussion

IR Spectra

IR spectral data for water-soluble (1*R*,2*R*)-dach Pt(II) complexes containing glucuronate

TABLE II. IR Spectral Data for dach Pt(II) Complexes Containing D-Glucuronate, D-Gluconate or Their Acetyl Derivatives

Complexes	ν_{NH_2}	ν_{CH}	$\nu_{C=O}$	$\nu_{CO_2(asym)}$	$\nu_{CO_2(sym)}$	ν_{NO_3}	$\nu_{C-O-C(asym)}$	$\nu_{C-O-C(sym)}$
Pt(glucuronato)$_2$-((1*R*,2*R*)-dach)	3250 s	2930 m 2860 m	—	1620 s	1390 s	—	—	—
Pt(glucuronato)-(NO_3)((1*R*,2*R*)-dach)	3250 s	2930 m 2860 m	—	1620 s	1380[a] s	1380[a] s	—	—
Pt(gluconato)$_2$-((1*R*,2*R*)-dach)	3250 s	2930 m 2860 m	—	1610 s	1380 s	—	—	—
Pt(gluconato)(NO_3)-((1*R*,2*R*)-dach)	3250 s	2930 m 2860 m	—	1610 s	1380[a] s	1380[a] s	—	—
Pt(Ac_4-α-glucuronato)$_2$-((1*R*,2*R*)-dach)	3260 m 3230 m	2930 m 2860 m	1750 s	1650 s	1370 s	—	1220 s	1040 s
Pt(Ac_4-α-glucuronato)-(NO_3)((1*R*,2*R*)-dach)	3260 m 3230 m	2930 m 2860 m	1750 s	1650 s	1380[a] s	1380[a] s	1220 s	1040 s
Pt(Ac_4-β-glucuronato)$_2$-((1*R*,2*R*)-dach)	3260 m 3230 m	2930 m 2860 m	1750 s	1650 s	1370 s	—	1220 s	1070 s 1040 s
Pt(Ac_4-β-glucuronato)-(NO_3)((1*R*,2*R*)-dach)	3260 m 3230 m	2930 m 2860 m	1750 s	1650 s	1380[a] s	1380[a] s	1220 s	1070 s 1040 s

In cm^{-1}. *a*) Overlapping between $\nu_{CO_2(sym)}$ and ν_{NO_3}.

are summarized in Table II. The absorptions at around 3250 cm^{-1} are due to stretching of the amino groups, and partially overlapped with those of H_2O in both mono- and bis-glucuronato Pt(II) complexes. The methylene groups in the cyclohexane ring gave two characteristic absorption peaks at 2930 and 2860 cm^{-1}. Carboxyl groups of glucuronates in the bis-glucuronato Pt(II) complex gave asymmetric and symmetric stretching absorptions at 1620 and 1390 cm^{-1}, respectively. The mono-glucuronato Pt(II) complex contained nitrate, the stretching absorption of which was observed at 1380 cm^{-1} as a strong peak due to overlapping with the symmetric stretching absorption of the carboxyl group.

Lipo-soluble Pt(II) complexes containing Ac_4-α or β-glucuronato showed absorptions due to asymmetric and symmetric stretching of the amino groups at 3260 and 3230 cm^{-1} respectively. Absorption due to stretching of the carbonyl moieties of acetyl groups was observed at 1750 cm^{-1} as a very strong peak and that due to asymmetric stretching of the carboxyl group at 1650 cm^{-1}. The IR spectra of Ac_4-α and β-glucuronato Pt(II) complexes showed a difference in the symmetric stretching frequencies of C–O–C, in the former a single peak was observed at 1040 cm^{-1} which was split into two peaks at 1040 and 1070 cm^{-1} in the latter. In mono-Ac_4-glucuronato Pt(II) complexes a peak due to stretching of NO_3 was observed at 1380 cm^{-1}, overlapped with the peak due to symmetric stretching of the carboxyl group.

Based upon analyses of the IR spectral data of water-soluble Pt(II) complexes it was concluded that the carboxylate ions of the leaving groups coordinate to the central Pt(II) ions, since the asymmetric stretching absorption of the carboxylates shifted to 1600—1620 cm^{-1} from 1700 cm^{-1} for the free ligand.

The situation is clearer in the case of Ac_4-glucuronato Pt(II) complexes since the asymmetric stretching absorption of the carboxylates was found at 1650 cm^{-1}, being shifted to the higher frequency side than those of the water-soluble Pt(II) complexes. Sodium D-glucuronate and D-glucuronic acid showed asymmetric stretching of carboxyl groups at 1595 and 1700 cm^{-1}, respectively, reflecting covalent character of the bond between the carboxylate and sodium or hydrogen ions. The stronger the covalency of the bond between Pt(II) and carboxylate, the higher the frequency of the stretching absorption of the carboxylate.

Since gluconato and Ac_4-gluconato Pt(II) complexes of 1*R*,2*R*-dach showed the same IR spectra as those of glucuronato and Ac_4-glucuronato Pt(II) complexes, their IR spectral data were omitted from the table. Amcha Pt(II) complexes showed the same IR spectra as those of the corresponding (1*R*,2*R*)-dach Pt(II) complexes and their data were also omitted.

^{13}C-NMR Spectra

Table III gives ^{13}C-NMR spectral data for Pt(Ac_4-α or β-glucuronato)$_2$((1*R*,2*R*)-dach) together with those for 1,2,3,4-tetra-*O*-acetyl-D-glucuronic acid. The assignments of Ac_4-α or β-glucuronic acid in Table III were made based upon those of glucuronic acid reported by Jaques *et al.*[19] and those of 1,2,3,4,5-penta-*O*-acetyl-D-glucose by Dorman and Roberts.[20] That is, chemical shifts produced by the introduction of acetyl groups on the carbon atoms of glucose were calculated from the latter data and the shifts obtained were used to calculate the chemical shifts of Ac_4-α or β-D-glucuronic acid. In both α- and β-anomers, the observed chemical shifts coincided well with the calculated values within 1 ppm, except for C(4) atom of the β-anomer, the chemical shift of which was 69.89 ppm and differed by more than 1 ppm from the calculated value of 68.54 ppm. The good agreements may support the correctness of the assignments.

The new resonance lines that appeared at around 174—175 ppm upon coordination of Ac_4-glucuronato to Pt(II) ions were assigned to the carboxylate carbon atoms of (1*R*,2*R*)-dach and *cis*-amcha Pt(II) complexes.

Pt(Ac_4-α-glucuronato)$_2$((1*R*,2*R*)-dach) gave a resonance line at 89.92 ppm due to the C(1)

TABLE III. ^{13}C-NMR Data for Ac_4-Glucuronato Pt(II) Complexes Containing (1*R*,2*R*)-dach and *cis*-amcha in $CDCl_3$

Complexes	Methyl carbons	Carbon number						Carbonyl carbons
		1	2	3	4	5	6	
Ac_4-α-D-glucuronic acid	21.88	89.87	70.08	70.43	71.11	70.28	171.41	170.97
	21.74							170.05
	21.59							169.95
	21.49							
Pt(Ac_4-α-glucuronato)$_2$((1*R*,2*R*)-dach)	22.28	89.92	70.82	71.30	72.38	70.43	174.92	171.95
	21.98							171.17
	21.79							170.68
	21.64							169.95
Pt(Ac_4-α-glucuronato)$_2$(*cis*-amcha)	22.10	90.02	70.77	71.74	73.50	71.15	174.63	171.60
	21.83							171.10
	21.70							170.20
	21.64							
Ac_4-β-D-glucuronic acid	21.83	92.46	71.35	73.54	69.89	73.05	171.36	171.22
	21.64							170.68
								170.39
								169.00
Pt(Ac_4-β-glucuronato)$_2$((1*R*,2*R*)-dach)	22.13	92.65	71.55	74.86	71.01	73.64	174.58	171.22
	21.98							170.63
	21.79							170.34
								170.19
Pt(Ac_4-β-glucuronato)$_2$(*cis*-amcha)	22.37	92.35	71.16	73.50	70.77	71.74	174.60	171.07
	21.88							170.53
								170.24

Carbon-13 shifts in parts per million from external Me_4Si.

atom, while that of Pt(Ac_4-β-glucuronato)$_2$((1*R*,2*R*)-dach) was found at 92.65 ppm. In the ^{13}C-NMR spectrum of tetra-*O*-acetyl-D-glucuronic acid, four resonance lines due to the carbon atoms of the carboxyl and acetyl groups were found in the region of 169.95—171.41 ppm, while in that of Pt(Ac_4-α-glucuronato)$_2$((1*R*,2*R*)-dach) a peak at 174.92 ppm was assigned to the carboxyl carbon atom, being shifted downfield upon coordination to Pt(II) ion. The carbon atoms of the acetyl groups in the complex gave four peaks around 170—172 ppm, being little affected, and four methyl carbons gave four peaks around 21.64—22.10 ppm, indicating that the two Ac_4-α-glucuronate moieties are chemically equivalent in the complex. Three resonance lines at 63.75, 33.29, and 25.54 ppm were assigned to C(α), C(β), and C(γ) of the (1*R*,2*R*)-dach.[11]

In the ^{13}C-NMR spectrum of Pt(Ac_4-β-glucuronato)$_2$((1*R*,2*R*)-dach), the C(6) atom of the carboxyl group gave a peak at 174.58 ppm shifted downfield compared with that of the free β-anomer, indicating the coordination of the carboxyl ions to Pt(II) ions. A resonance line at 92.65 ppm was assigned to the C(1) atom of the β-anomer.

The analyses of the ^{13}C-NMR spectra of Pt(Ac_4-α or β-glucuronato)$_2$(*cis*-amcha) both supported the same conclusion concerning the coordination of the carboxyl group to the central Pt(II) ion. In addition, signals of carbon atoms in *cis*-amcha were observed at 55.07, 36.89, 29.68, 25.24, 22.76, 29.68, and 44.94 ppm, which were assigned to C(1), C(2), C(3), C(4), C(5), C(6), and the aminomethyl carbon, respectively.[11]

However, ^{13}C-NMR spectra of bis-Ac_4-gluconato Pt(II) complexes were not obtainable due to their low solubility in $CDCl_3$ or other solvents.

Antitumor Activities

As shown in Table IV, water- or lipo-soluble Pt(II) complexes of (1*R*,2*R*)-dach exhibited high antitumor activities against murine leukemia L1210.

As for water-soluble Pt(II) complexes containing glucuronate or gluconate, mono-glucuronato (or gluconato) Pt(II) complex exhibited higher activity than bis-glucuronato (or gluconato) Pt(II) complex. For example, Pt(glucuronato)(NO_3)((1*R*,2*R*)-dach) showed the T/C% value of 301 at a dose of 12.5 mg/kg with three cured mice out of six, while Pt(glucuronato)$_2$((1*R*,2*R*)-dach) gave T/C% of 280 with only one cured mouse. Pt(gluconato)(NO_3)((1*R*,2*R*)-dach) also exhibited a high T/C% value of 314 with three cured mice.

Lipo-soluble (1*R*,2*R*)-dach Pt(II) complexes containing acetyl derivatives showed higher antitumor activities than the corresponding water-soluble Pt(II) complexes, except mono-Ac_4-α-glucuronato Pt(II) complex. For both mono- and bis-Ac_4-α or β-glucuronato Pt(II) complexes, Pt(II) complexes containing β-anomer as a leaving group exhibited higher activities than those of α-anomer. Bis-Ac_4-glucuronato Pt(II) complexes showed lower toxicity than mono-Ac_4-glucuronato Pt(II) complexes. For example, Pt(Ac_4-β-glucuro)$_2$-((1*R*,2*R*)-dach) showed a T/C% value of 382 at a dose of 50 mg/kg with four cured mice and at a dose of 100 mg/kg no decrease of body weight due to toxicity was observed. Pt(Ac_4-β-glucuronato)(NO_3)((1*R*,2*R*)-dach) showed toxicity at a dose of 50 mg/kg, although its antitumor activity was excellent, giving a T/C% value of 375 at a dose of 25 mg/kg with five cured mice.

Therapeutic indexes (T. I.) were obtained for (1*R*,2*R*)-dach Pt(II) complexes of glucuronate and its acetyl derivative. They are shown in Table V, together with that of *cis*-DDP used as a positive control. All of the Pt(II) complexes showed higher T. I. values than *cis*-

TABLE IV. Antitumor Activity of (1*R*,2*R*)-dach Pt(II) Complexes of D-Glucuronate, D-Gluconate and Their Acetyl Derivatives against Murine Leukemia L1210

Complexes	Dose (mg/kg)							
	200	100	50	25	12.5	6.2	3.1	1.5
	T/C%							
Pt(glucuronato)$_2$-((1*R*,2*R*)-dach)			156	280 (1)	168	184	134	118
Pt(glucuronato)(NO_3)-((1*R*,2*R*)-dach)			50	231	301 (3)	280 (3)	150	126
Pt(Ac_4-α-glucuronato)$_2$-((1*R*,2*R*)-dach)		279 (1)	382 (5)	188 (1)	171 (1)	114	128	
Pt(Ac_4-α-glucuronato)(NO_3)-((1*R*,2*R*)-dach)		T72	T229 (1)	288 (1) (1)	273 (2)	170	184 (1)	116
Pt(Ac_4-β-glucuronato)$_2$-((1*R*,2*R*)-dach)		219 (2)	382 (4)	382 (3)	225 (2)	128		
Pt(Ac_4-β-glucuronato)(NO_3)-((1*R*,2*R*)-dach)		0	T173 (1)	375 (5)	258 (1)	285 (2)	144	132
Pt(gluconato)$_2$((1*R*,2*R*)-dach)		T306 (2)	281 (1) (1)	304 (1)	166	139		
Pt(gluconato)(NO_3)-((1*R*,2*R*)-dach)				314 (3)	277 (1) (1)	290 (2) (1)	172	
Pt(Ac_4-gluconato)$_2$-((1*R*,2*R*)-dach)	0	188	322 (1)					
Pt(Ac_4-gluconato)(NO_3)-((1*R*,2*R*)-dach)			0	325 (1)	341 (2) (1)	301 (2)		

Underlining indicates positive effects (T/C% ≧ 125). T indicates toxicity. Numbers in parentheses indicate cured mice (or mouse) out of six mice and underlining here indicates survival at 30 d, but in a tumor-bearing state.

TABLE V. Therapeutic Indexes of (1*R*,2*R*)-dach Pt(II) Complexes Containing D-Glucuronate and Its Acetyl Derivatives against Leukemia L1210

Complexes	Toxic dose mg/kg	Optimal dose		MED[a]		T.I.[b]
		mg/kg	T/C%	mg/kg	T/C%	
Pt(glucuronato)$_2$((1*R*,2*R*)-dach)	≧100	25	280 (1)	3.1	134	8
Pt(glucuronato)(NO$_3$)((1*R*,2*R*)-dach)	≧50	12.5	301 (3)	1.5	126	8
Pt(Ac$_4$-α-glucuronato)$_2$((1*R*,2*R*)-dach)	≧200	50	382 (5)	3.1	128	16
Pt(Ac$_4$-α-glucuronato)(NO$_3$)((1*R*,2*R*)-dach)	100	25	288 (1)	3.1	184 (1)	≧8
Pt(Ac$_4$-β-glucuronato)$_2$((1*R*,2*R*)-dach)	≧200	50	382 (4)	6.2	128	8
Pt(Ac$_4$-β-glucuronato)(NO$_3$)((1*R*,2*R*)-dach)	100	25	375 (5)	1.5	132	16
cis-PtCl$_2$(NH$_3$)$_2$	25	6.2	246 (1)	1.5	146	4

a) MED: minimum effective dose. *b*) T.I.: therapeutic index (=optimal dose/MED).

TABLE VI. Antitumor Activity of amcha Pt(II) Complexes of D-Glucuronate, D-Gluconate and Their Acetyl Derivatives against Murine Leukemia L1210

Complexes	Dose (mg/kg)						
	200	100	50	25	12.5	6.3	3.1
	T/C%						
Pt(glucuronato)$_2$(*trans*-amcha)		144	178	149			
Pt(glucuronato)$_2$(*cis*-amcha)		T162	197	167			
Pt(glucuronato)(NO$_3$)(*trans*-amcha)			T250 (2)	241 (2)	156		
Pt(glucuronato)(NO$_3$)(*cis*-amcha)			0	T137	187 (1)		
Pt(Ac$_4$-α-glucuronato)$_2$(*trans*-amcha)		145	119	127			
Pt(Ac$_4$-β-glucuronato)$_2$(*trans*-amcha)		215	135	114			
Pt(Ac$_4$-α-glucuronato)(NO$_3$)(*trans*-amcha)		0	134	114	114		
Pt(Ac$_4$-β-glucuronato)(NO$_3$)(*trans*-amcha)	0	T206 (2)	253 (1)	183 (1)	201 (1)	198	
Pt(Ac$_4$-α-glucuronato)$_2$(*cis*-amcha)	T153	302 (2)	223 (1)	157	104		
Pt(Ac$_4$-β-glucuronato)$_2$(*cis*-amcha)		171	159	132			
Pt(Ac$_4$-α-glucuronato)(NO$_3$)(*cis*-amcha)		120	T402 (3)	286 (3)	240	228 (1)	134
Pt(Ac$_4$-β-glucuronato)(NO$_3$)(*cis*-amcha)		T152	271 (2)	328 (1) (2)	246 (1) (1)	140	
Pt(gluconato)$_2$(*cis*-amcha)			170	146	140		
Pt(gluconato)$_2$(*trans*-amcha)			280 (3)	195 (1)	182		
Pt(gluconato)(NO$_3$)(*cis*-amcha)			317 (4)	308 (2)	302 (2)		
Pt(Ac$_4$-gluconato)$_2$(*trans*-amcha)	142	208	186	177	142		
Pt(Ac$_4$-gluconato)$_2$(*cis*-amcha)	0	T205 (1)	190	207	144	111	
Pt(Ac$_4$-gluconato)(NO$_3$)(*trans*-amcha)		0	218	244	197 (1)	156	
Pt(Ac$_4$-gluconato)(NO$_3$)(*cis*-amcha)		T94	T208 (1)	202 (1)	172	156	102

For significance of underlining, see the footnote to Table IV.

DDP. In particular, bis-Ac$_4$-α-glucuronato and mono-Ac$_4$-β-glucuronato Pt(II) complexes exhibited T. I. values of 16.

All the water-soluble amcha Pt(II) complexes containing glucuronate or gluconate were antitumor-active against L1210 as shown in Table VI.

Among mono- and bis-glucuronato Pt(II) complexes, the antitumor activities were not dependent upon the amcha isomers involved and were lower than those of the corresponding (1*R*,2*R*)-dach Pt(II) complexes. However, Pt(gluconato)(NO$_3$)(*cis*-amcha) exhibited excellent activity, giving T/C% values of more than 300 at doses of 50, 25, and 12.5 mg/kg with 4, 2, and 2 cured mice, respectively.

Among the lipo-soluble amcha Pt(II) complexes, Ac_4-glucuronato Pt(II) complexes exhibited higher activities than Ac_4-gluconato Pt(II) complexes. When the antitumor activities of mono- and bis-Ac_4-glucuronato Pt(II) complexes are compared, the former complexes showed better results, but no definite difference was found between α- and β-anomers, as in the case of (1*R*,2*R*)-dach Pt(II) complexes. As far as lipo-soluble Pt(II) complexes are concerned, (1*R*,2*R*)-dach Pt(II) complexes showed higher activities than amcha Pt(II) complexes.

It is rather difficult to choose candidates for further development since many of the Pt(II) complexes synthesized exhibited rather high antitumor activities against L1210. Judging from T/C% values, numbers of cured mice and decreases of body weight due to toxicity, water-soluble Pt(gluconato)(NO_3)(*cis*-amcha) and lipo-soluble Pt(Ac_4-β-glucuronato)((1*R*,2*R*)-dach) seem worthy of further screening tests.

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