

Aspartato(1,2-cyclohexanediamine)platinum(II) complexes: synthesis and characterization; effects of minor impurities on antitumor activity

Abdol H. Talebian*, Dennis Bensely, Alem Ghiorghis, Charles F. Hammer

Reiss Science Building, Department of Chemistry, Georgetown University, Washington, DC 20057 (U.S.A.)

Philip S. Schein** and Dianna Green

Vincent T. Lombardi Cancer Research Center, Georgetown University, School of Medicine, Washington, DC 20007 (U.S.A.)

(Received May 21, 1990)

Abstract

Aspartato(1,2-cyclohexanediamine)platinum(II) (ADP), where 1,2-cyclohexanediamine (DAC) is either *trans-RR*-, *trans-SS*-, *meso-RS*-, or a mixture of the three isomers (ADP mixture), has been synthesized and evaluated for antitumor activity. The structures of the complexes have been characterized by various spectroscopic techniques (IR, ^1H , ^{13}C , ^{195}Pt and 2D-COSY ($^1\text{H}\{^1\text{H}\}$) and 2D-HETCOSY ($^1\text{H}\{^{13}\text{C}\}$) NMR). Purification and murine antitumor activity of the individual ADP isomers indicate that minor impurities in the ADP mixture have a significant effect on the potency of the platinum complexes.

Introduction

cis-Diamminedichloroplatinum(II), *cis*-DDP, is a potent antitumor agent with clinical efficacy for several human carcinomas [1]. Its usefulness, however, is compromised by its dose-limiting toxicities (nephrotoxicity, nausea and vomiting, and myelosuppression) [2, 3].

To obtain platinum compounds with reduced toxicity, better efficacy, and higher water solubility than the parent *cis*-DDP, several thousand new platinum analogues have been prepared and evaluated in preclinical animal studies [1, 4–6]. Complexes containing 1,2-cyclohexanediamine (DAC; DAC has three isomeric forms, *RR*, *SS* and *meso-RS*) as their non-leaving ligand have received special attention because of their high antitumor activity, lower nephrotoxicity, and lack of cross resistance with *cis*-DDP [7–10]. *R,R*-DAC is the most potent while *S,S*-DAC is the most toxic isomer [11, 12].

We have synthesized a platinum(II) complex of type PtA_2X_2 in which A_2 is a mixture of *cis*- and *trans*-1,2-cyclohexanediamine and X_2 is L-aspartic acid (ADP mixture, structure 1). In preclinical studies, the ADP mixture was highly effective against several murine tumor models. However, the purified

individual ADP isomers (>98% by HPLC) were substantially less potent than the ADP mixture. Doses of the individual isomers up to three-fold the LD_{10} dose (dose lethal to 10% of the mice) of the mixture produced no deaths due to drug toxicity, and P388 antitumor activity for the isomers did not equal that of the ADP mixture. This significant loss of potency of the ADP isomers raised questions about the purity of the ADP mixture.

We report here on the synthesis, chemical characterization and biological evaluation of the ADP mixture and its individual isomers. Suggestions are also made to avoid purification problems in the synthesis of platinum analogues.

Experimental

Decomposition points, uncorrected, were determined on a Gallenkamp MF-370. IR spectra were run as KBr pellets on a Nicolet-FT spectrometer model 170SX. NMR spectra were recorded with a Bruker AM-300 WB spectrometer using a 10-mm tunable probe for ^{195}Pt or a 5-mm probe for ^1H and ^{13}C . An internal lock on the deuterium solvent, D_2O , was used. Chemical shifts are reported in ppm units, and are relative to internal sodium 3-(trimethylsilyl)propionate-2,2,3,3- d_4 (Tier salt) for ^1H and ^{13}C , and to an external sample of Na_2PtCl_6 (0.5 g in 3 ml of D_2O) for ^{195}Pt . The ^{195}Pt (64.520 MHz) spectra

*Author to whom correspondence should be addressed.

**Present address: US Bioscience, Blue Bell, PA 19422, U.S.A.

were collected using a spectral width of 10–20 KHz, relaxation delay of 0.2 s, pulse width of 10 μ s, acquisition time of 0.034 ms, and 10 000 to 20 000 scans. HPLC assays were carried out with a Waters 6000 high-pressure liquid chromatograph operating at 230 nm using: system a; a Dupont Zorbax cyano column (3.9 $\times$ 250 mm, 10 μ m packing) in the reverse phase mode, a mobile phase (isocratic elution) of 5 mM hexanesulfonic acid sodium salt, 8 mM triethylamine, 4 mM phosphoric acid, and 10% acetonitrile at a flow rate of 1 ml/min, or system b; a Waters Microbond pack C₁₈ column (3.9 $\times$ 300 mm) and a mobile phase (isocratic elution) of 5 mM hexanesulfonic acid sodium salt at a flow rate of 1.5 ml/min, or system c; Waters C₁₈ semipreparative column (10 $\times$ 250 mm) and a mobile phase (isocratic elution) of 5 mM hexanesulfonic acid sodium salt at a flow rate of 3 ml/min. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

Materials

K₂PtCl₄ and *cis*-1,2-cyclohexanediamine were purchased from Strem Chemicals (Newburyport, MA); 1*R*,2*R*-, and 1*S*,2*S*-cyclohexanediamine were obtained from Alfa Chemicals (Danvers, MA) and used as received; The *cis*- and *trans*-1,2-cyclohexanediamine mixture and L-aspartic acid (referred to as aspartic acid in the text) were purchased from Aldrich (Milwaukee, WI). All solvents, reagent grade, were used without further purification.

Syntheses

Preparation of the platinum complexes

General procedure, preparation of ADP mixture. To a freshly prepared solution of K₂PtCl₄ (13.867 g, 33.406 mmol) in distilled degassed water was added 1,2-cyclohexanediamine (mixture of *cis* and *trans*; 4.09 ml, 33.346 mmol) and the mixture was allowed to stir at room temperature, protected from light, for 8 h. The yellow *cis*-dichloro(1,2-cyclohexanediamine-*N,N'*)platinum(II), (DAC)PtCl₂, precipitate was collected and washed successively with 10% HCl, H₂O, ethanol, acetone and ether (9.024 g, 71% yield). A solution of (DAC)PtCl₂ (8.928 g, 23.483 mmol) was reacted with silver sulfate (6.6 g, 21.167 mmol) in distilled, degassed water (800 ml) under a nitrogen atmosphere for 24 h in the dark. Silver chloride precipitate was filtered off and the filtrate was then freeze dried to give 7.72 g (90% yield) of *cis*-sulfato–DAC–platinum(II) as a faintly yellow powder. Addition of *cis*-sulfato–DAC–platinum(II) (7.056 g, 17.40 mmol) to a solution of barium aspartate (4.67 g, 17.40 mmol; prepared *in situ* from aspartic acid and Ba(OH)₂·8H₂O) in distilled water (300 ml)

resulted in the immediate precipitation of BaSO₄. After completion of the coupling (monitored by HPLC), BaSO₄ was filtered and the resulting filtrate was concentrated to about 2 ml under reduced pressure. Addition of ethanol to the concentrated solution yielded 4.75 g (62% yield) of the crude ADP mixture as a white solid. The solid was redissolved in the minimum amount of water and reprecipitated with methanol and washed several times with ethanol, acetone, ether, and dried over P₂O₅ in a vacuum desiccator (> 95% pure by HPLC; system a and b). Further purification by means of redissolving the compound in water and precipitating with various solvents (ethanol, methanol, acetone) resulted in loss of yield and did not improve the purity of the product (95–96% by HPLC).

Preparation of individual ADP isomers. Except for crystallization, *R,R*-ADP, *S,S*-ADP and *meso-R,S*-ADP were all prepared in the same manner as described above for the ADP mixture. *S,S*-ADP and *meso-R,S*-ADP were crystallized from water–ethanol (98–100% pure by HPLC). *R,R*-ADP was crystallized from water–acetone (vapor diffusion method) as colorless needles (> 99% pure by HPLC). Preliminary examination of these crystals revealed that they are twinned. Attempts to grow crystals suitable for X-ray studies have been unsuccessful.

Biological assays

Except for cisplatin, each complex was dissolved in sterile distilled water or 5% dextrose at 4 °C immediately before use. Cisplatin was dissolved in 0.9% sterile saline (NaCl). Male CD2F₁ mice were used to evaluate antitumor activity. Each drug was administered intraperitoneally (i.p.) on day 1 or days 1, 5 and 9 after implantation of 1 $\times$ 10⁶ P388 or 1 $\times$ 10⁵ L1210 leukemia cells i.p. Percentage increase in life span (%*ILS*) was calculated as follows

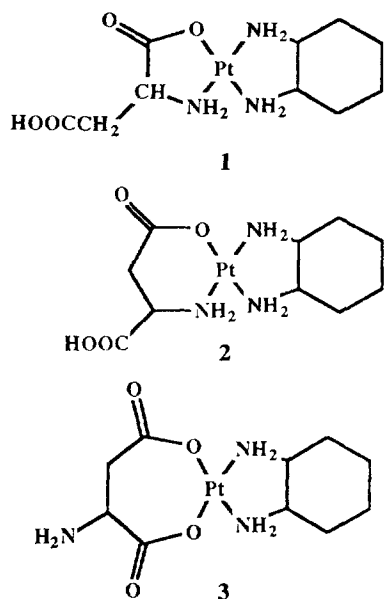
$$\%ILS = (T - C)/C \times 100$$

where *T* is the mean survival days of the drug treated mice, and *C* is the mean survival days of the vehicle treated mice.

Results and discussion

Chemistry

There are two different ways in which aspartic acid can coordinate to the platinum(II) nuclei: binding through the amino group and one of the carboxylic acid groups, a process which is thermodynamically more favorable (structures 1 and 2), or coordination through the two carboxylic acid groups (structure 3), a process which is kinetically more favorable [13].



Analytical data for the platinum complexes are listed in Table 1 and are consistent with the chemical formulation of the proposed structure 1. Selected IR bands for the platinum analogues are presented in Table 2. Each platinum complex exhibits two different asymmetric (C–O) stretching frequencies in the 1605–1665 cm^{-1} region. The absorption band at 1640 cm^{-1} in the IR spectrum of *R,R*-ADP is assigned to the coordinated carboxyl group and the peak at 1629 cm^{-1} to the uncoordinated (COO) group. These two bands overlap and appear as a strong single broad peak at 1610 cm^{-1} in the IR spectrum of *S,S*-ADP. The asymmetric C–O stretch-

ing absorptions of the chelated and non-chelated carboxyl groups are observed at 1654 and 1619 cm^{-1} for *meso-R,S*-ADP, respectively. This difference of over 220 cm^{-1} is consistent with monodentate coordination of the carboxylate group. The symmetric C–O stretching frequencies of the metal coordinated carboxyl groups in the ADP isomers appear between 1385–1395 cm^{-1} .

The ^{13}C spectra of the ADP isomers (Table 3) show the same number of carbon signals as that expected for only one monomeric complex. The ^{13}C spectra of barium aspartate and free aspartic acid are tabulated for comparison (Table 3). Two different carbonyl peaks are observed for each isomeric complex around 180 and 190 ppm. The resonance signals around 180 ppm are similar in chemical shift to the carboxyl carbon nucleus of barium aspartate and they are located several ppm downfield from those of free aspartic acid. Hence, the peaks around 180 ppm are assigned to the uncoordinated carboxyl groups and the carbonyl signals around 190 ppm to the coordinated carboxyl groups. This large downfield chemical shift of the chelated carboxyls carbonyl is attributed to the deshielding effect of platinum.

The ^{195}Pt NMR chemical shift is very sensitive to the donor atoms to the platinum nuclei [14]. Therefore, structural changes in the complexes should result in different platinum chemical shifts. The ^{195}Pt NMR for each isomer shows only one broad peak from –2219 to –2419 ppm (Table 3), a region which is characteristic of a PtN_3X complex [15]. The broadening of the platinum signals is due to the

TABLE 1. Elemental analyses and decomposition points for platinum(II) complexes

Compound	Molecular formula	Decomposition point ($^{\circ}\text{C}$)	Found (calc.) (%)		
			C	H	N
ADP mixture	$\text{C}_{10}\text{H}_{20}\text{N}_3\text{O}_4\text{Pt} \cdot 2\text{H}_2\text{O}$	> 223	24.92(25.21)	4.44(4.86)	8.42(8.82)
<i>R,R</i> -ADP	$\text{C}_{10}\text{H}_{20}\text{N}_3\text{O}_4\text{Pt} \cdot \frac{1}{2}\text{H}_2\text{O}$	> 280	27.21(27.00)	4.56(4.18)	9.51(9.10)
<i>S,S</i> -ADP	$\text{C}_{10}\text{H}_{20}\text{N}_3\text{O}_4\text{Pt}$	> 280	26.14(26.41)	4.82(4.53)	9.14(9.01)
<i>meso-S,R</i> -ADP	$\text{C}_{10}\text{H}_{20}\text{N}_3\text{O}_4\text{Pt} \cdot \frac{1}{2}\text{H}_2\text{O}$	> 280	27.21(27.08)	4.56(4.64)	9.51(9.35)

TABLE 2. Selected IR bands of platinum(II) complexes

Compound	ν_{NH} (cm^{-1})	$\nu_{\text{CH}_2\text{CH}_2}$ (cm^{-1})	$\nu_{\text{a(C-O)}}_{\text{c}}$ (cm^{-1})	$\nu_{\text{a(C-O)}}_{\text{u}}$ (cm^{-1})	$\nu_{\text{s(C-O)}}_{\text{c}}$ (cm^{-1})
ADP mixture	3190, 3108	2944, 2862		1560–1650	1389
<i>R,R</i> -ADP	3418, 3205	2928, 2864	1640	1629	1394
<i>S,S</i> -ADP	3420, 3283	2930, 2859		1605–1664	1389
<i>R,S</i> -, <i>S,R</i> -ADP	3204, 3113	2930, 2866	1654	1619	1387

a: asymmetric, c: coordinated, u: uncoordinated.

TABLE 3. ^{13}C and ^{195}Pt NMR chemical shifts (ppm) of the platinum complexes

Compound	C ₂	C ₁	C _{3,6}	C _{4,5}	C _{1'}	C _{4'}	C _{3'}	C _{2'}	^{195}Pt
Aspartic acid					175.66	177.19	37.45	53.57	
Ba aspartate					182.8	184.30	44.69	56.38	
<i>R,R</i> -DAC-PtCl ₂	64.02		32.40	25.20					
<i>R,R</i> -DAC-SO ₄	65.97		34.22	26.56					
			34.08						
<i>R,R</i> -DAC-(OH) ₂	65.58		35.09 ^a	26.88					
	65.17								
ADP mixture	65.44	63.42	34.62 ^b	28.59	190.67	180.62	42.04	57.53	
	61.37	59.16		26.63 ^a					
				23.19 ^a					
<i>R,R</i> -ADP	65.44	63.28	34.74	26.76	190.95	180.54	41.97	57.51	-2218.9
			34.65	26.66					
<i>S,S</i> -ADP	65.39	63.34	34.70	26.68 ^a	190.02	180.12	41.85	57.60	-2419.1
<i>R,S</i> -, <i>S,R</i> -ADP	61.32	59.16	28.53	23.00	190.23	180.33	41.83	57.40	-2393.3
				22.84					

^aTwo overlapping resonances. ^bThree overlapping resonances.

partial decoupling and the quadrupole relaxation effect of the nitrogens on platinum [16]. The appearance of platinum resonances above -2100 ppm, together with the manifestation of two different carbonyl resonances in the ^{13}C NMR and two asymmetric C-O stretching frequencies in the IR spectra, suggest that aspartic acid is coordinated to the platinum nuclei through its amino group and one of its carboxylic acid groups. Chelation of aspartic acid through its α -carboxylic group gives rise to structure 1, while coordination through its β -carboxylate produces structure 2.

The appearance of ^{13}C chemical shifts of the chelated carboxyl carbons around 190 ppm strongly suggest that the α -carboxyl group of aspartic acid is coordinated to the platinum ion. This is in excellent agreement with the chemical shifts reported for dicarboxylic acids that form a five-membered ring with platinum(II) metal. For example, the carboxyl carbon in $\text{Pt}(\text{NH}_3)_2(\text{gly-}N,O)^+$ which forms a five-membered ring is reported at 190.0 ppm, while the carboxyl carbon in the six-membered ring of $\text{Pt}(\text{NH}_3)_2(\text{ala-}N,O)^+$ occurs at 181.1 ppm [17]. The carboxyl carbon in the $\text{Pt}(\text{NH}_3)_2(\text{glycolato-}O,O')$, with a five-membered chelate ring is observed at 195.9 ppm [18].

To assign all of the protons and carbons in the ADP complexes, ^1H homonuclear chemical-shift correlated (COSY) (Fig. 1) and $^1\text{H}\{^{13}\text{C}\}$ chemical-shift correlated (HETCOSY) (Fig. 2) spectra of the *R,R*-ADP, as a complex representative, were carried out. The proton resonances of α - and β -carbons of free

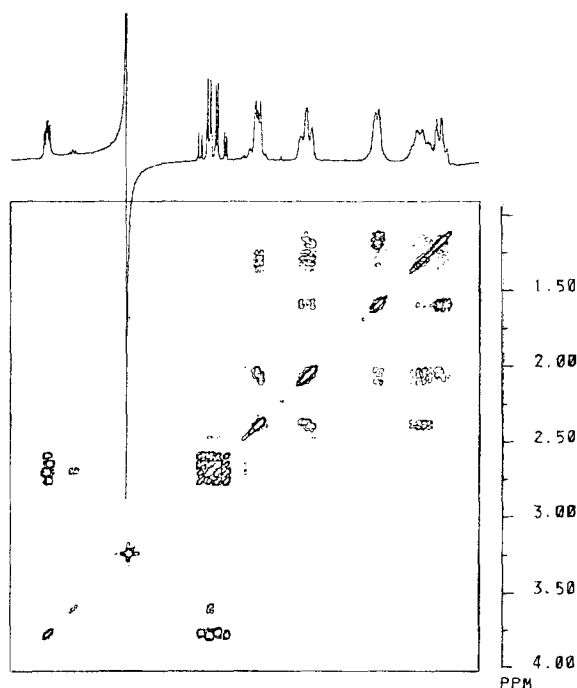


Fig. 1. $^1\text{H}\{^1\text{H}\}$ homonuclear chemical-shift correlated (COSY) of *R,R*-ADP.

aspartic acid, barium aspartate and the ADP complexes display an ABX pattern (Table 4). As expected, the AB coupling constants of the methylene group for the platinum complexes are more similar to that of the free aspartic acid than to the barium aspartate. This is consistent with the more freely rotating methylene groups in both the ADP complexes (struc-

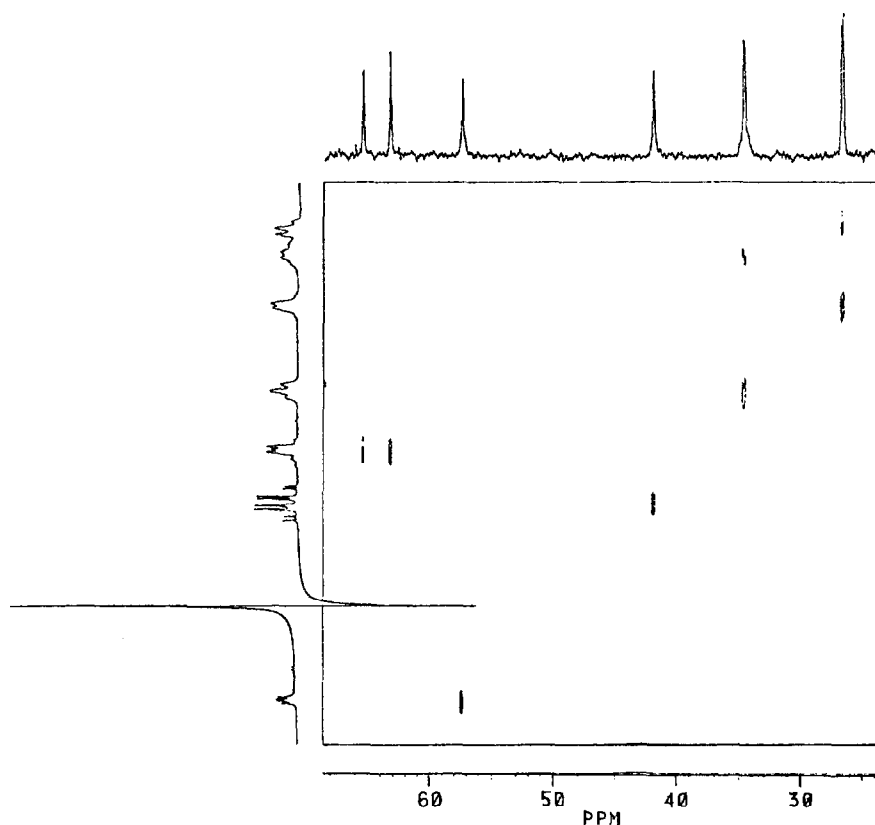


Fig. 2. $^1\text{H}\{^{13}\text{C}\}$ heteronuclear chemical-shift correlated (HETCOSY) of R,R -ADP.

TABLE 4. ^1H NMR chemical shifts (ppm) of platinum(II) complexes

Compound	$\text{H}_{1,2}$	$\text{H}_{3,6}$	$\text{H}_{4,5}$	$\text{H}_2(\text{X})$	$\text{H}_3(\text{AB})$
Aspartic acid				4.10(dd) ($J_{\text{AX}} = 6.80$; $J_{\text{BX}} = 4.8$; $J_{\text{AB}} = 18.0$)	3.05 ^a
Ba aspartate				3.69(dd) ($J_{\text{AX}} = 8.90$; $J_{\text{BX}} = 3.90$; $J_{\text{AB}} = 16.0$)	2.71(dd), 2.44(dd)
R,R -ADP	2.37(m)	2.05(bt) 1.30(m)	1.6(bd) 1.13(bdd)	3.76(dd) ($J_{\text{AX}} = 6.30$; $J_{\text{BX}} = 3.9$; $J_{\text{AB}} = 17.40$)	2.74(dd), 2.61(dd)
S,S -ADP	2.39(m)	2.05(bt) 1.31(m)	1.59(bd) 1.20(m)	3.75(dd) ($J_{\text{AX}} = 6.27$; $J_{\text{BX}} = 3.8$; $J_{\text{AB}} = 17.35$)	2.70(dd), 2.61(dd)
R,S -, S,R -ADP	2.40(m)	2.09(bt) 1.32(m)	1.62(bd) 1.20(dd)	3.80(dd) ($J_{\text{AX}} = 6.30$; $J_{\text{BX}} = 3.96$; $J_{\text{AB}} = 17.30$)	2.75(dd), 2.65(dd)

b: broad; dd: doublet of doublets; m: multiplet; t: triplet.

^aTwo overlapping doublet of doublets.

ture 1) and the free aspartic acid. For example, $J_{\text{AX}} = 6.30$, $J_{\text{BX}} = 3.90$ and $J_{\text{AB}} = 17.40$ Hz in R,R -ADP, and $J_{\text{AX}} = 6.80$, $J_{\text{BX}} = 4.8$ and $J_{\text{AB}} = 18.0$ Hz in the free aspartic acid, while in the barium aspartate, they are 6.00, 3.90 and 16.00 Hz, respectively (Table 4).

The HPLC of *cis-meso*-ADP shows two peaks in the ratio of 56/44 (Fig. 3(a) and (b)). These absorptions are attributed to the R,S -ADP and the S,R -ADP. However, the ^1H (Table 4) and ^{13}C NMR

(Table 3) spectra of the *cis-meso*-ADP isomer shows no evidence for the presence of more than one species. This indicates that the ^1H and ^{13}C chemical shifts are not sufficiently sensitive to discriminate between these two platinum complex diastereomers. To assure that the absorptions in the chromatograms (Fig. 3(a) and (b) are associated with *cis-meso*-ADP, the peaks were isolated by semi-preparative HPLC (system c; see 'Experimental'). Fractions from several runs containing the two peaks were combined and

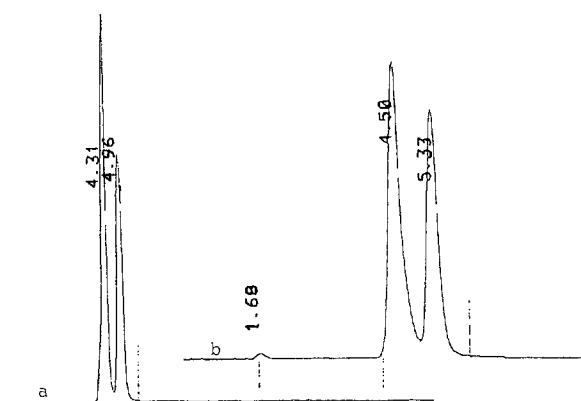


Fig. 3. High pressure liquid chromatogram of *meso*-*RS*-ADP.

TABLE 5. Antitumor activity of ADP mixture and its isomers against P388 leukemia^a

Compound	Single dose (i.p.) (mg/kg)	%ILS	Deaths due to drug toxicity
<i>R,R</i> -ADP	125	66	
	200	87	
	250	82	
	350	91	
<i>S,S</i> -ADP	125	78	
	300	102	
<i>R,S</i> -, <i>S,R</i> -ADP	150	67	
	250	91	
	350	94	
ADP mixture	125	118	1/10

^a1 × 10⁶ P388 cells were implanted i.p. into male CD2F₁ mice on day 0; treatment on day 1 only.

lyophilized. The lyophilized material was redissolved in D₂O and examined by ¹⁹⁵Pt NMR. The ¹⁹⁵Pt NMR of the HPLC isolated sample was identical with that of the crystallized *cis-meso*-ADP (see 'Experimental' and Table 3).

Antitumor activity

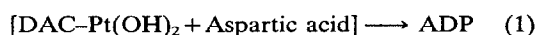
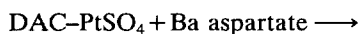
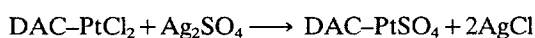
Generally, triamine platinum(II) complexes are known to be biologically inactive. As summarized in Table 5, the purified ADP isomers, although significantly less potent than the ADP mixture, demonstrated some P388 antitumor activity. This is not surprising, since Hollis *et al.* [19] have recently reported on several triamine platinum(II) complexes with moderate murine antitumor activity. However, at doses three times the LD₁₀ dose of the ADP mixture (95–96% pure), the individual ADP isomers (>98% pure) were not lethal to the mice and their efficacy did not fully match the efficacy of the ADP mixture. This significant loss of potency of individual

ADP isomers as compared to the ADP mixture suggested the presence of minor potent impurity(ies) in the ADP mixture. Efforts to isolate and identify the minor impurity(ies) in the ADP mixture were unsuccessful due to the low percentage of contaminant(s) (<5%) and the small differences in the retention time of the impurity(ies) (as a shoulder) and the main peak.

Individual DAC–Pt isomers are expected to vary in their degree of potency and toxicity, but the differences are usually small [20]. Furthermore, it has been shown that the efficacy and the potency of each DAC–Pt isomer is comparable to that of the parent mixture [20]. Therefore, the loss of potency in the individual ADP isomers as compared to the ADP mixture must be due to factors other than mere separation of the isomers.

Problems with the purification of platinum complexes, and in particular, the mixture of isomers, is known in the literature [21–23]. Several new series of dicarboxylic DAC platinum(II) complexes in which the dicarboxylic anions are either aminomalonic acid or *N*-(iminodiacetic acid) derivatives have been reported [24–26]. Preliminary biological results of these compounds demonstrated good to excellent murine antitumor activity. However, upon further purification, these compounds were not as active as originally observed [21, 22].

The preparation of carboxylato–DAC–platinum(II) compounds, and the ADP complexes in this investigation, via eqn. (1) requires the use of the DAC–Pt dichloride, the DAC–Pt sulfate, and the generation of the DAC–Pt dihydroxide intermediate, all potent platinum species.



The significantly higher efficacy and potency of the ADP mixture (Tables 5 and 6) as compared to its isomers (Table 5) is attributed to the presence of trace amounts of one, a combination of two or more, and/or polymeric form(s) of these species in the ADP mixture. Whatever the contaminant(s), careful crystallization of the individual isomeric platinum complexes must be allowed for the removal of the minor impurity(ies).

To meaningfully assess the antitumor activity of newly synthesized platinum complexes, careful purification and analysis of the compounds is essential particularly if their synthetic route requires potent platinum intermediates such as DAC–Pt(OH)₂ or DAC–Pt(SO₄). HPLC should be an integrated part of the routine analysis protocol. In the case of the

TABLE 6. Antitumor activity of platinum(II) complexes against P388 and L1210 Leukemia^a

Compound	Dose (i.p.) (mg/kg) (day)	%ILS		Deaths due to drug toxicity (%)
		P388	L1210	
ADP mixture	100 (1)	108		
	125 (1)	118		10
	150 (1)	> 135		25
	50 (1, 5, 9)	> 130	68	10
	75 (1, 5, 9)	> 135	90	40
	100 (1, 5, 9)	> 135	143	50
Carboplatin	100 (1)	78		10
	60 (1, 5, 9)	132	57	
Cisplatin	10 (1)	96		10
	5 (1, 5, 9)	100	129	10

^a 1×10^6 P388 and 1×10^5 L1210 cells were implanted i.p. into male CD2F₁ mice on day 0; treatment on day 1 or 1, 5, 9.

incorporation of 1,2-cyclohexanediamine as the non-leaving ligand, preparation and thorough analysis and testing of at least one of the individual isomers is strongly recommended.

Acknowledgements

This investigation was supported in part by US Bioscience and NIH Grant 5RO1CA17583.

References

- 1 A. W. Prestayko, S. T. Crooke, and S. K. Carter (eds.) *Cisplatin Current Status and New Developments*, Academic Press, New York, 1980.
- 2 J. B. Vermorken and H. M. Pinedo, *Neth. J. Med.*, **25** (1982) 270.
- 3 D. D. Von Hoff, R. Schilsky and C. M. Reichert, *Cancer Treatment Rep.*, **63** (1979) 1527.
- 4 M. J. Cleare, P. C. Hydes, B. W. Malerbi and D. M. Watkins, *Biochimie*, **60** (1978) 835.
- 5 M. F. Pera and H. C. Harder, *Cancer Res.*, **39** (1979) 1279.
- 6 M. Noji, K. Suzuki, T. Tashiro, M. Suzuki, K. Harada, K. K. Masuda and Y. Kidani, *Chem. Pharm. Bull.*, **35** (1987) 221.
- 7 M. Noji, K. Okamoto, Y. Kidani and T. Tashiro, *J. Med. Chem.*, **24** (1981) 508.
- 8 P. D. Braddock, T. A. Connors, M. Jones, A. R. Khokhar, D. H. Melzack and M. L. Tobe, *Chem.-Biol. Interact.*, **11** (1975) 145.
- 9 R. J. Speer, C. J. Storey, L. M. Hall and H. J. Ridgway, *J. Clin. Hematol. Oncol.*, **11** (1981) 47.
- 10 J. H. Burchenal, K. Kalaher, K. Dew and L. Lokys, *Cancer Treatment Rep.*, **63** (1979), 1493 and refs. therein.
- 11 R. J. Speer, H. Ridgway, D. P. Stewart, L. M. Hall, A. Zapata and J. M. Hill, *J. Clin. Hematol. Oncol.*, **7** (1977) 210.
- 12 Y. Kidani, M. Noji and T. Tashiro, *Gann*, **71** (1980) 637.
- 13 T. G. Appleton, J. R. Hall and S. F. Ralph, *Aust. J. Chem.*, **39** (1986) 1347.
- 14 C. J. Boreham, J. A. Broomhead, and D. P. Fairlie, *Aust. J. Chem.*, **34** (1981) 659.
- 15 T. G. Appleton, J. R. Hall and S. F. Ralph, *Inorg. Chem.*, **24** (1985) 4685.
- 16 I. M. Ismail, S. J. S. Kerrison and P. J. Sadler, *Polyhedron*, **1** (1982) 57.
- 17 T. G. Appleton, J. R. Hall and P. D. Prenzler, *Inorg. Chem.*, **28** (1989) 815.
- 18 T. Totani, K. Aono, M. Komura and Y. Adachi, *Chem. Lett.*, (1986) 429.
- 19 L. S. Hollis, A. R. Amundsen and E. W. Stern, *J. Med. Chem.*, **32** (1989) 128.
- 20 Y. Kidani, K. Inagaki, M. Iigo, A. Hoshi and K. Kuretan, *J. Med. Chem.*, **21** (1978) 1315.
- 21 J. D. Hoeschele, N. Farrell, W. R. Turner and C. D. Rithner, *Inorg. Chem.*, **27** (1988) 4106.
- 22 J. D. Roberts, W. J. Schmidt, W. P. Tong and M. P. Hacker, *Inorg. Chim. Acta.*, **153** (1988) 123.
- 23 R. J. Speer, H. Ridgway, L. M. Hall, D. P. Stewart, K. E. Howe, D. Z. Lieberman, A. D. Newman and J. M. Hill, *Cancer Chemother. Rep. Part 1*, **59** (1975) 629.
- 24 M. P. Hacker, A. R. Khokhar, I. H. Krakoff, D. B. Brown and J. J. McCormack, *Cancer Res.*, **46** (1986) 6250.
- 25 O. Gandolfi, H. C. Apfelbaum and J. Blum, *Inorg. Chim. Acta.*, **135** (1987) 27.
- 26 J. Lautersztain, R. Perez-Soler, A. R. Khokhar, R. A. Newman and G. Lopez-Berestein, *Cancer Chemother. Pharmacol.*, **18** (1986) 93.