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Antileukemic Platinum(II)–Catecholamine Complexes

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The platinum complexes of L-norepinephrine, L-epinephrine, L-dopa, α-methyldopa, DL-dops, DL-isoprotenerol and adrenalone have been prepared and their mode of coordination characterized by elemental analysis, infrared and ^{1}*H NMR spectra. Preliminary screening tests of these complexes against mouse leukemia L 1210* in vitro *have been carried out.*

Introduction

In search of platinum complexes with specific pharmacological properties, we have lately focused our attention on functionalized transition metals coordinated to an *o*-catechol moiety [1–3]. We have already demonstrated the anchoring properties of these complexes by covalently coupling them to simple organic molecules [2] as well as elaborate organic structures such as derivatives of estrone, estradiol and testosterone [4]. Our interest in complexes bound to lypophilic carriers, such as hormonally active steroids, has been dictated by the possibility of providing antineoplastic drugs with enhanced selectivity towards hormone dependent tumors.

In addition to the fact that the overall geometry of *cis*-platinum(II)–*o*-catecholato complexes is in accordance with the basic structural and electronic requirements outlined for antitumor drugs [5], the importance of transition metals, coordinated to *o*-catechol derivatives, has been enhanced by early reports on the properties of catecholamines [6–8]. Within the huge armamentarium of pharmacological drugs, catecholamine derivatives have recently emerged as a new interesting class of antitumor agents. In addition to their marked neurological properties, a number of catecholamines have been found to possess antitumor activity, in particular against L 1210 and murine P 388 lymphatic leukemia, B16 melanoma and C1300 neuroblastoma [7, 8].

The possibility of combining the biological properties of a specific *o*-catechol ligand with the antitumor activity of the transition metal complex, has prompted us to extend our investigation to the preparation of novel bisphosphine–platinum(II)–catecholamine complexes. Here we describe the syntheses of platinum-coordinated L-norepinephrine, L-epinephrine, L-dopa, α-methyldopa, DL-dops, DL-isoprotenerol and adrenalone, and report some preliminary screening results of this class of complexes against mouse leukemia L 1210 in culture.

Experimental

Apparatus

All the reactions were performed in an argon atmosphere. The subsequent work-up was carried out in air. Infrared spectra were recorded with a Perkin Elmer 457 Grating Infrared Spectrophotometer, solid samples being run as KBr pellets. Proton NMR spectra were obtained using a WH-300 Bruker Spectrometer with deuteriochloroform as solvent and tetramethylsilane as internal standard.

Solvents and Chemicals

All solvents, purified as described in the literature [9], were deoxygenated prior to use and the transfers were carried out using the flexible needle or syringe technique. All the catechol derivatives were Aldrich reagent grade and were used throughout. The preparation of $Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}R)(PPh_3)_2$, $R{=}CO_2H$, CH_2CO_2H, $CH_2CH_2CO_2H$ [1], $CH_2CH_2NH_2$ [2], was improved by the modification given below.

Preparation of Compounds 1–11

In a typical preparation, to a suspension of 0.30 mmol of the appropriate catechol in 1 ml of benzene, was added 2 ml of methanol, containing potassium hydroxide (a two-fold amount for compounds *1–4, 6–9, 11,* or a three-fold amount for compounds *5, 10*). The benzene–methanol solution was syringed into 0.25 mmol of *cis*-dichlorobis(triphenylphosphine)platinum(II), suspended in 1 ml of benzene. The mixture was stirred at room temperature for 3.5 hr and filtered. The clear filtrate was evaporated to dryness. The crude product was thoroughly washed with water on a synthered filter, dried *in vacuo* and dissolved in methylene chloride. A small amount of unreacted metal halide complex was recovered by filtration. After removal of the solvent, the orange crystalline product was washed with ether and dried *in*

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vacuo. Compound *10* was crystallized from ether–hexane.

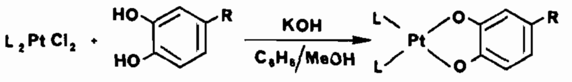
In Vitro Assay

The screening tests, carred out against L 1210 mouse leukemic cells in culture, were performed at the Department of Pharmacology, College of Medicine of the University of Vermont, 05405 Burlington, under the supervision of Prof. J. J. McCormack. The antitumor activity was determined as the level of the drug that produces 50% inhibition of growth of L 1210 cells in culture (ID_{50}). The compounds were added to the culture medium as suspensions and as DMSO solutions. The growth was measured 48–72 hr after the cells were inoculated into growth medium.

Results and Discussion

Analytical data for the platinum(II)–catechol complexes (Table I) are consistent with the formation of 1:1 adducts. Owing to the presence of additional functional groups on the catecholamine drugs (*i.e.*, α-amino-carboxyl fragment for L-dopa, α-methyldopa, DL-dops; β-hydroxy-amine fragment for L-norepinephrine, L-epinephrine, DL-isoprotenerol; or β-keto-amine for adrenalone) different modes of complexation for the platinum(II)–catecholamine derivatives are conceivable.

In earlier communications [1–3] we demonstrated that the interaction between palladium(II), platinum(II) and iridium(III) halide complexes with carboxyl- or amine-substituted catechols, in the presence of a base, leads to the formation of O,O′-catecholate chelates. The possibility of metal–carboxylate or metal–nitrogen bond formation, due to the availability of extra binding sites on the catechols employed in this study, can be excluded on the basis of the observed IR and NMR spectra. Selected infrared bands and NMR data for the novel platinum(II)–catecholamine complexes are reported in Table II. They show two strong IR absorptions at ~1275 cm^{-1} and ~1485 cm^{-1}; the first being characteristic for an *o*-diolato skeletal vibration and the second for a ν(C–O) bending of a M–O–diolato moiety [1–3]. Thus the general scheme for the formation of *cis*-platinum(II)–catecholamine complexes can be formulated in accordance with eqn. 1:



(1)

$L = PPh_3$;

$R = COOH, \underline{1}; CH_2COOH, \underline{2}; CH_2CH_2COOH, \underline{3}; CH_2CH_2NH_2, \underline{4};$
$CH(OH)CH_2NH_2, \underline{5}; CH(OH)CH_2NHCH_3, \underline{6}; CH_2CH(NH_2)COOH, \underline{7};$
$CH_2C(CH_3)(NH_2)COOH, \underline{8}; CH(OH)CH(NH_2)COOH, \underline{9};$
$CH(OH)CH_2NHCH(CH_3)_2, \underline{10}; COCH_2NHCH_3, \underline{11}.$

The retention of half a molecule of methylene chloride by almost all the complexes, as shown by the analytical results (Table I), has been confirmed by the presence of an additional singlet at δ = 5.16 in the NMR spectra. All the products appeared chemically stable even when left for several days in solutions of methylene chloride, benzene, ethanol, THF or DMSO. On the other hand, all attempts to isolate the platinum(II)–*o*-catecholato complex with 6-hydroxydopamine were unsuccessful, owing to the instability of the product even under argon. We suggest that the presence in the catecholamine of an hydroxyl group in the para position may promote a *p*-quinonoid arrangement of the ligand, thus destabilizing the whole complex. Formation of unstable *o*-semiquinolate species with other related transition metal–*o*-catecho-

TABLE I. Analytical Data for the Platinum(II)–Catechol Complexes: $L = PPh_3$.

No.	Compound	Yield (%)	Found (Calcd) %	
			C	H
1	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CO_2H)L_2$ [a]	91	57.32 (57.84)	4.01 (3.94)
2	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CO_2H)L_2\cdot\frac{1}{2}CH_2Cl_2$ [a]	89	57.60 (57.55)	4.15 (4.03)
3	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2CO_2H)L_2\cdot\frac{1}{2}CH_2Cl_2$ [a]	73	59.12 (59.24)	4.23 (4.18)
4	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NH_2)L_2\cdot\frac{1}{2}CH_2Cl_2$ [a]	94	58.71 (58.50)	4.35 (4.42)
5	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH(OH)CH_2NH_2)L_2\cdot\frac{1}{2}CH_2Cl_2$	88	57.40 (57.51)	4.20 (4.35)
6	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH(OH)CH_2NHCH_3)L_2\cdot\frac{1}{2}CH_2Cl_2$	91	57.94 (58.00)	4.51 (4.50)
7	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH(NH_2)CO_2H)L_2\cdot\frac{1}{2}CH_2Cl_2$	83	57.26 (57.11)	4.32 (4.22)
8	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}C(CH_3)(NH_2)CO_2H)L_2\cdot\frac{1}{2}CH_2Cl_2$	84	57.90 (57.51)	4.63 (4.37)
9	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH(OH)CH(NH_2)CO_2H)L_2\cdot\frac{1}{2}CH_2Cl_2$	86	56.49 (56.17)	4.23 (4.15)
10	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH(OH)CH_2NHCH(CH_3)_2)L_2$	50	60.59 (60.80)	4.85 (4.89)
11	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}COCH_2NHCH_3)L_2\cdot\frac{1}{2}CH_2Cl_2$	87	58.28 (58.05)	4.69 (4.29)

[a] Ref. [2].

TABLE II. Selected IR Bands[a] and NMR Data[b] for the Platinum(II)–Catecholamine Complexes *5–11*.

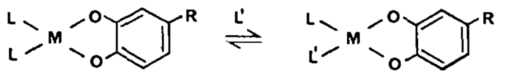
Compound	ν(catecholato) cm^{-1}	ν(C=O) cm^{-1}	ν(C–N–H) bending cm^{-1}	δ values					
				C_6H_5	$1,2\text{-}O_2C_6H_3$	CH–N	CH–O	NH	CH_3
5	1485vs, 1270vs		1565w	7.31(m)	6.31(m)		4.41(m)	2.24(br s)	
6	1480vs, 1270vs		1565w	7.28(m)	6.29(m)		4.57(t)	2.38(s)	2.26(s)
7	1480vs, 1275vs	1670s	1570w	7.31(m)	6.45(m)	4.84(m)		2.1(s)	
8	1480vs, 1275vs	1670s	1570w	7.30(m)	6.41(m)			1.99(s)	1.45(s)
9	1485vs, 1275vs	1650s	1570w	7.35(m)	6.55(m)	5.6(d)	4.64(m)	2.52(s)	
10	1485vs, 1275vs		1570w	7.35(m)	6.40(m)	2.75(d)	4.62(t)	1.9(br s)	1.03(s)
11	1480vs, 1270vs	1640s	1550m	7.33(m)	6.37(m)			2.75(s)	2.46(s)

[a] As KBr pellets. [b] In $CDCl_3$ with Me_4Si as internal standard.

lates leading to the cleavage of the *o*-catechol in a *o*-quinonoid form has been reported [10–13].

Antitumor Activity

As mentioned, the configuration of non-electrolytic *cis*-platinum(II)–*o*-catecholates has suggested a possible antitumor activity. It is now largely recognized that the mode of action of classical neutral *cis*-platinum(II) complexes is based on a primary attack on cellular DNA [5]. The active species, such as $[PtL_2X]^+$ and $[PtL_2]^{2+}$, where L is a neutral and inert carrier ligand and X^- is an anionic leaving group of intermediate lability, are formed in the cytosol by means of a chemical- or enzyme-assisted dissociative kinetic process [5, 14]. In the case of *cis*-platinum(II)-*o*-catecholates, the strongly bonded phosphines may well be considered as the neutral and inert carrier ligands. In contrast to palladium(II)–*o*-catecholato complexes [2], the platinum(II) analogs strongly retain the phosphine ligands, even after prolonged refluxing of the complexes in THF in the presence of an excess of neutral σ-donors such as amines, eqn. 2:

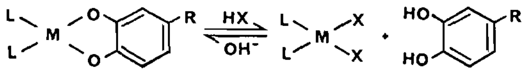


(2)

$L = L' = PPh_3$; M = Pt.

$L = PPh_3$; L' = amine ; M = Pd.

Moreover, from earlier observations [4] it emerged that the *o*-catecholate ligand can be displaced by other anionic nucleophiles, as depicted in eqn. 3.



(3)

M = Pd, Pt ; X = halogen ; $L = PPh_3$.

The generation of active $[L_2Pt]^{2+}$ species in the cytosol, as a consequence of the lability of the *o*-catecholate ligand is therefore feasible. In addition, the possibility of an enzyme-assisted cleavage of the anionic bidentate ligand is not excluded, as it has been suggested also for the chemically inert *cis*-platinum(II)–bidentate carboxylate complexes [14], with similar geometry. Our anticipation that the *cis*-platinum(II)–*o*-catecholates possess antitumor activity has been confirmed by the preliminary screening tests carried out against L 1210 mouse leukemic cells in culture. The results are listed in Table III.

TABLE III. Antitumor Activity against L 1210 Cells *in vitro* for the Platinum(II)–*o*-Catecholato Complexes *1–11*.

No.	Compound	ID_{50} (μg/ml)[a]
1	Pt–[3,4-Dihydroxybenzoic acid]	>10 (2.2)
2	Pt–[3,4-Dihydroxyphenylacetic acid]	3.4
3	Pt–[3,4-Dihydroxycinnamic acid]	>10 (10)
4	Pt–[Dopamine]	>10 (2.3)
5	Pt–[L-Norepinephrine]	10 (2.8)
6	Pt–[L-Epinephrine]	7.5 (2.4)
7	Pt–[L-Dopa]	>10 (>10)
8	Pt–[α-Methyl-dopa]	>10
9	Pt–[DL-Dops]	>10 (7.8)
10	Pt–[DL-Isoprotenerol]	3 (2.3)
11	Pt–[Adrenalone]	>10 (2.2)

[a] Level of drug that produces 50% inhibition of growth of L 1210 cells in culture; growth measured 48–72 hours after cells inoculated into growth medium. Figures in parentheses for compounds solubilized in DMSO before addition to culture medium.

In contrast to their parent bis(triphenylphosphine) platinum(II) halide complexes, which have been reported to have a marginal antitumor activity [5], the bis(triphenylphosphine)platinum(II)–*o*-catecholates exhibited a marked cytotoxicity against L 1210 cells. In spite of the presence of phosphine ligands, which are responsible for the unfavorable aqueous solubility of the complexes, some of the platinum(II)–*o*-catecholate derivatives (*e.g.*, Pt–[dihydroxyphenylacetic acid] (*2*), Pt–[L-epinephrine] (*6*) and Pt–[DL-isoprotenerol] (*10*) complexes) exhibited antitumor activity even when suspended in water. Apparently, this can be attributed to the presence of the polar substituents on the catecholate ligand. It is noteworthy that evaluation against L 1210 mouse leukemic cells in culture is a very sensitive test to 'classical' platinum complexes and that activity against leukemia is a highly predictive indication of the clinical usefulness of a drug.

It is interesting that the ID_{50} values given in Table III, being in the range of 10^{-3} m*M*, are lower than those obtained in the same tumor system with uncoordinated catecholamines [7]. Although a full understanding of the mode of action within the cell is still premature, the important biological results achieved with *cis*-platinum(II)–*o*-catecholato complexes deserve some further comments.

Transition metal complexes of *o*-benzoquinones have been known for many years. Three possible coordination modes are now recognized, each one depending on the oxidation state of the ligand (Fig. 1).

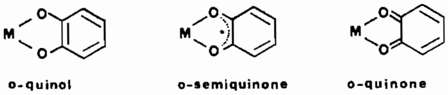


Fig. 1.

We have also mentioned the ability of transition metal–*o*-catecholato complexes to acquire the less stable *o*-semiquinonoid geometry [10–13, 15, 16]. Therefore, it appears feasible that displacement of the *o*-catechol ligand inside the cell may occur also as a result of the formation of a paramagnetic *o*-semiquinolate species. Such an activation mechanism may help to explain the unexpectedly high antileukemic properties of the *cis*-platinum(II)–*o*-catecholato complexes. Graham [17] and Vogel [18] have shown that mammalian DNA polymerase is the specific target of preformed 1,2-benzoquinones. Recent results [7] on the inhibition of leukemia cells by catecholamine derivatives have confirmed that antitumor activity can be achieved also by administration of *o*-quinol species, the latter undergoing oxidation inside the cell. Although the mechanism of intracellular conversion of *o*-catechols into *o*-quinones is not yet clear, the presence of peroxidases in many cells has been reported [19]. The possibility that the neutral lypophilic structure of a *cis*-platinum(II)–*o*-catecholato complex may facilitate the diffusion of the catechol drug into the cell and consequently catalyze the liberation of the corresponding active *o*-quinonoid structure of the catechol, is therefore suggested.

Upon these considerations it may be postulated that the inhibitory effects of the *cis*-platinum(II)–*o*-catecholato complexes are the result of two major contributions, both synergically correlated:

1. Intracellular formation of the $[L_2Pt]^{2+}$ active species.
2. Catalytic conversion of the coordinated *o*-catechol into the corresponding active *o*-quinonoid form.

This postulation is represented schematically in Fig. 2:

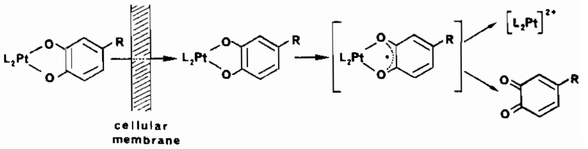


Fig. 2.

Finally, it is noteworthy that some of the platinum(II)–*o*-catecholato complexes can be coupled to biologically active steroids. Preliminary screening tests of some of our steroidal platinum(II)–*o*-catecholato complexes against a human breast cancer cell line, MCF-7, which produces estrogen, progesterone, glucocorticoid and insuline receptors, have been carried out and will be the subject of a separate report [20].

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