Substitutedcatecholato(1,2-diaminocyclohexane)-platinum(II) Compounds

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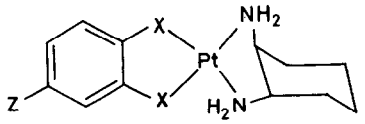
One approach to the preparation of nontoxic platinum drugs involves the noncovalent complexation of appropriate organoplatinum compounds by certain water-soluble polymers, principally, poly(*N*-vinylpyrrolidone) (PVP) [1]. For an initial effort, *cis*-dichloro(4-substituted *o*-phenylenediamine)platinum compounds were used for the preparation of such polymer complexes [2]. These compounds contain both the *cis*-dichlorodiamineplatinum moiety necessary for antitumor activity and a polar substituent necessary for the complexing interaction with the polymer. While these compounds are active in bacterial screens, organoplatinum compounds derived from aromatic amines as inert ligands generally display somewhat lower antitumor activity than to those generated from aliphatic amines [3]. Therefore, it is desirable to prepare polymer complexes of platinum compounds containing aliphatic amines as inert ligands. In particular, the most useful compounds might be those containing 1,2-diaminocyclohexane as the amine ligand [4]. However, this would then require that some aromatic leaving group of lability comparable to that of chloride be utilized to:

(1) maintain the proper hydrolytic reactivity for function as an antitumor agent;

(2) provide a polar aromatic group for complexation with the polymer.

Compounds of the following type where Z is a polar group and X is an atom capable of coordinating platinum with approximately the same effectiveness as chloride would be required. A set of ligands which might be utilized for the preparation of such compounds may be derived from catechol. That is, compounds containing the (1,2-diaminocyclohexane)-platinum moiety coordinated to 4-substituted-catecholato ligands to function as (1) polar handles for attachment to the carrier polymer and (2) labile leaving groups.



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Experimental

In general, the preparation of ligands was carried out in dry apparatus under a static atmosphere of dry nitrogen. Platinum compounds were prepared in the absence of light and oxygen at 55 °C. Melting points (m.p.) were measured using a Thomas-Hoover capillary melting point apparatus (m.p. < 250 °C) or a Mel-Temp melting point block (m.p. > 250 °C). Infrared (IR) spectra were obtained using 1% suspensions in potassium bromide discs and either a Perkin-Elmer 597 grating infrared spectrophotometer or a Nicolet 20DXB FT-IR spectrometer. Solutions in deuterochloroform ($CDCl_3$) or deuterodimethylsulfoxide ($DMSO\text{-}d_6$) and a Varian Associates T-60 or IBM NR-80 spectrometer were used to record proton nuclear magnetic resonance (NMR) spectra. Chemical shifts are reported relative to tetramethylsilane (δ 0.00) as internal reference.

Ligands

Nitration of the 2-butanone ketal of catechol with nitric acid in acetic acid afforded the 4-nitroketal while nitration with a mixture of nitric and sulfuric acids provided the 4,5-dinitroketal. Reduction of these compounds with sodium borohydride/water in the presence of 5% palladium-on-charcoal afforded the corresponding amino compounds. Hydrolysis of the substituted ketals with aqueous trifluoroacetic acid at 50 °C generated the free ligands. Other catechol ligands were obtained from commercial sources.

Substitutedcatecholato(1,2-diaminocyclohexane)-platinum(II) Compounds

These compounds were prepared from the appropriate ligands and *cis*-dinitrato(1,2-diaminocyclohexane)platinum(II) [4]. The procedures listed below are typical of those used for the preparation of all the compounds reported herein.

cis-Dichloro(1,2-diaminocyclohexane)platinum(II)

A solution of 6.01 g (0.015 mol) of potassium tetrachloroplatinate in 80 ml of 1.0 N aqueous hydrochloric acid was placed in a 250 ml, round-bottomed flask. 1,2-Diaminocyclohexane (5.3 ml, 4.655 g, 0.0407 mol) was added in a single portion and the contents of the flask were swirled to effect thorough mixing. The flask was flushed with nitrogen, closed with a glass stopper and wrapped with aluminum foil to exclude light. The contents of the flask were held at 55 °C for 72 h and then at 7 °C (refrigerator) for 10 h. The yellow crystals which had formed were collected by filtration at reduced pressure, washed repeatedly with water and dried at reduced pressure over Drierite (4.71 g, 82% yield): IR (cm^{-1}, KBr)

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3272(s), 3199(s) (N–H), 2947(s), 2866(m) (aliphatic C–H), 1564(s), 757(m) (Pt–Cl), 603(w), 574(w) (Pt–N); proton NMR (δ, DMSO-d_6) 0.68–3.24 (m-broad, 10H, cyclohexyl protons), 4.62–6.93 (m-broad, 4H, amino protons).

cis-Dinitrato(1,2-diaminocyclohexane)platinum(II)

A solution of *cis*-dichloro(1,2-diaminocyclohexane)platinum(II) (0.845 g, 0.0024 mol) and silver nitrate (0.425 g, 0.0022 mol) in 150 ml of water was placed in a 250 ml, round-bottomed flask containing a magnetic stirring bar. The flask was flushed with nitrogen, closed with a glass stopper and wrapped with aluminum foil to exclude light. The mixture was stirred at room temperature for 2 h. The solution was freed of silver chloride which had precipitated by filtration at reduced pressure through a sintered glass filter. The absence of silver nitrate in the filtrate was confirmed by treating a small aliquot with 1.0 N aqueous hydrochloric acid solution; no precipitate was formed. The clear colorless aqueous solution of *cis*-dinitrato(1,2-diaminocyclohexane)platinum(II) was utilized for the preparation of catecholato compounds containing the (1,2-diaminocyclohexane)-platinum(II) moiety.

Catecholato(1,2-diaminocyclohexane)platinum(II)

A portion of the aqueous solution of *cis*-dinitrato-(1,2-diaminocyclohexane)platinum(II) previously prepared (40.0 ml, 0.0147 mmol/ml, 0.587 mmol) and 2.50 g (22.71 mmol) of catechol were placed in a 100 ml, round-bottomed flask. The flask was flushed with nitrogen, closed with a glass stopper and wrapped with aluminum foil to exclude light. The contents of the flask were maintained at 55 °C for 12 h and then at 7 °C (refrigerator) for 3 h. The dark crystalline solid which had formed was collected by filtration at reduced pressure, washed repeatedly with cold water and dried over Drierite at reduced pressure to afford the desired catecholato compound (0.135 g, 56.2% yield), m.p. > 250 °C: IR (cm^{-1}, KBr) 3226 (s-broad) (N–H), 2947(s), 2864(m) (aliphatic C–H), 1581(s), 1483(s) (aromatic nucleus), 1257(s) (phenoxy C–O), 517(w), 461(w), 441(w) (Pt–N), 363-(w), 322(w) (Pt–O); proton NMR (δ, DMSO-d_6) 0.68–2.18 (m, 10H, cyclohexyl protons), 4.42–6.99 (m-broad, 4H, amino protons), 6.67 (m, 4H, aromatic protons).

Spectrophotometric Determination of Salmon DNA Interstrand Binding

A solution of 50% methanol/50% phosphate buffer (pH 7.0) containing the desired platinum compound was prepared. A 0.05 ml aliquot of this solution was then transferred to a teflon tube containing 5.0 ml of 50% methanol/50% phosphate buffer and 5.0 ml of 400 μg/ml salmon DNA (Sigma) in phosphate buffer solution. The final working concentrations of molecular species was 200 μg/ml DNA and 40 μg/ml of platinum compound. These solutions were allowed to incubate at 24 °C for the respective time intervals. Incubation times were 0, 0.5, 1, 2, 4, and 24 h. After the desired time, a 0.25 ml aliquot was transferred to a tube containing 5.0 ml of 50% methanol/50% phosphate buffer and immediately placed in a 45 °C water bath. After 0.25 h the tubes were removed and held at 0 °C for 0.25 h. The solutions were allowed to warm to room temperature (0.25 h) and analyzed using a Perkin-Elmer 55 UV–Vis spectrophotometer. Absorbance was recorded at a wavelength of 260 nm. Each determination was conducted in triplicate. Blank tubes containing only 50% methanol/50% phosphate buffer solution were used to calibrate the spectrophotometer at each interval.

Results and Discussion

Earlier studies have utilized dichloro(4-substituted *o*-phenylenediamine)platinum(II) compounds for complexation by poly(*N*-vinylpyrrolidone) to generate potentially useful time-release antitumor formulations [1, 2]. In this case, chloride served as the labile ligand to permit the *in vivo* formation of the *cis* diaquo species required for antitumor activity [3]. The substituted *o*-phenylenediamine served as the inert ligand and contained the polar aromatic group required for complexation with the polymer. However, platinum compounds containing aromatic amine ligands do not display optimum antitumor activity. To design compounds with enhanced activity, *i.e.* containing an aliphatic amine as the inert ligand (the complexing interaction must now occur via the leaving group rather than the inert ligand), it is necessary to include a labile ligand which contains a polar aromatic group and at the same time displays hydrolytic activity comparable to that of chloride. Substituted catechols might function as such labile ligands in these compounds. Accordingly, several 4-substitutedcatecholato(1,2-diaminocyclohexane)platinum(II) compounds have been synthesized (see Table I).

In general, ligands were prepared by the action of an appropriate electrophilic reagent on the 2-butanone ketal of commercially available catechol followed by suitable modification of the functionality introduced and hydrolysis of the ketal. In cases in which the substituent was either hydrogen or an electron-withdrawing group, treatment of *cis*-dinitrato(1,2-diaminocyclohexane)platinum(II) with the catechol ligand afforded the corresponding catecholato(1,2-diaminocyclohexane)platinum(II) compounds. These compounds were obtained by precipitation from aqueous solution and characterized

TABLE I. Metal–Ligand Absorption Bands in the Infrared Spectra of Catecholato(1,2-diaminocyclohexane)platinum(II) Compounds

Catechol ligand	Absorption (cm^{-1})	
	Pt–N	Pt–O
Catechol	537, 482	383, 342
4-Nitrocatechol	506, 421	383, 338
4,5-Dinitrocatechol	504, 439	399, 344
4-Acetylcatechol	510, 440	360
4-Formylcatechol	510, 440	340
2-Sulfo-6,7-dihydroxynaphthalene	495, 439	390
3,6-Disulfo-1-nitroso-2-hydroxynaphthalene	486, 442	387

spectroscopically. The platinum–nitrogen and platinum–oxygen infrared stretching frequencies for some of these compounds are listed in Table I. Treatment of *cis*-dinitrato(1,2-diaminocyclohexane)platinum(II) with a catechol ligand bearing an electron-releasing substituent in the 4-position led to the rapid formation of the corresponding quinones and platinum(0) which appeared as a mirror on the walls of the reaction flask.

The inhibition of DNA crosslinking may be used to evaluate the potential antitumor activity of organoplatinum compounds. In this procedure, the platinum compound is incubated with DNA under a set of standard conditions. The DNA is then denatured and examined by ultraviolet spectroscopy to establish the extent of interstrand crosslinking, *i.e.* inhibition of denaturation which has occurred. Two of the compounds generated in this work, catecholato(1,2-diaminocyclohexane)platinum(II) and 4,5-dinitrocatecholato(1,2-diaminocyclohexane)platinum(II), were examined by this technique. Both brought about interstrand crosslinking much more rapidly than did cisplatin used as a control. This suggests that catecholato and 4,5-dinitrocatecholato are considerably better leaving groups than is chloride. In fact, the rate of hydrolysis of the catecholato ligands may be so great as to diminish the effectiveness of organoplatinum compounds derived from these ligands as antitumor compounds.

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