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Sugar interaction with biologically active *cis*-$PtCl_2(NH_3)_2$ (anticancer) and its inactive *trans* isomer

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The interaction of D-glucuronic and D-gluconic acids with *cis*- and *trans*-$PtCl_2(NH_3)_2$ (cisplatin and transplatin) has been investigated in aqueous solution and solid complexes of the type *cis*-$[PtL(NH_3)_2]L \cdot H_2O$ and *trans*-$[PtL_2(NH_3)_2] \cdot H_2O$, where L = D-glucuronate or D-gluconate anions, are isolated and characterized by means of Fourier transform-infrared and ^1H-NMR spectroscopy, and molar conductivity and X-ray powder diffraction measurements. Spectroscopic and other evidence indicated that the sugar anions bind monodentately in *trans*-$[PtL_2(NH_3)_2] \cdot H_2O$ and bidentately in *cis*-$[PtL(NH_3)_2]L \cdot H_2O$ complexes through the carboxylate oxygen atoms and other sugar donor groups. The strong sugar intermolecular hydrogen-bonding network is altered to that of the sugar-$OH \cdots NH_3(H_2O) \cdots OH$-sugar, upon platinum-ammine interaction. The D-glucuronate anion has the β-anomer configuration both in the free salt and in these platinum-sugar complexes.

1. Introduction

During the past decade the anticancer activity of the compound *cis*-$[PtCl_2(NH_3)_2]$ has been the subject of extensive investigations [1,2]. A possible mode of action has been proposed [3] and the crystal structure of cisplatin with a DNA dodecamer (C-G-C-G-A-A-T-T-C-G-C-G) has been determined through X-ray diffraction measurements [4]. Owing to the problems of toxicity and insolubility of cisplatin in water, attention has been focused on the search for more soluble and less toxic platinum-amine compounds in order to enhance the antitumor activity of this class of platinum-amine compounds [5]. Recently, a new generation of *cis*-platinum-amine derivatives has been synthesized and tested against leukemia L1210 [6]. It has been concluded that the platinum-amine sugar complex [Pt(D-glucuronate)(*cis*-1-amcha)], where amcha denotes 2-(aminoethyl) cyclohexylamine, shows high solubility in H_2O and reduced toxicity with profound activity against leukemia L1210, which makes it a promising candidate for clinical trials [6]. It seems therefore that sugar binding to the *cis*-platinum-amine compounds improves the drug's solubility in water and possibly increases the antitumor activity of the platinum-amine complexes [6].

The aim of this work is the synthesis and characterization of several cis- and transplatin-sugar complexes containing D-glucuronate and D-gluconate anions in both solid and aqueous solutions, using Fourier transform-infrared and ^1H-NMR spectroscopy, and molar conductivity and X-ray powder diffraction measurements, that have not been previously reported. On the other hand, the spectroscopic properties of these complexes are compared with those of the structurally known Ca-glucuronate [7] and Mn-gluconate complexes [8]. This comparison allowed us to establish

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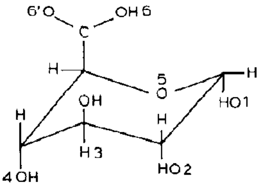
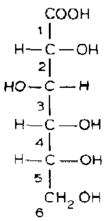
a correlation between the spectral changes and the sugar-binding modes. Furthermore, the effect of platinum binding on the anomeric forms of D-glucuronic acid is discussed here. The molecular structures of D-glucuronic and D-gluconic acids with the numbering of the atoms are shown below.



D-Glucuronic acid



D-Gluconic acid

2. Experimental

2.1. Materials and methods

D-Glucuronic acid and D-glucono-δ-lactone were from Aldrich and were converted to the sodium or potassium salt by the reaction of the alkali bicarbonate as reported earlier [9]. *cis*- and *trans*-$PtCl_2(NH_3)_2$ were from Sigma and used as supplied.

2.2. Preparation of cis- and transplatin-sugar complexes

Solutions of *cis*- and *trans*-diamminediaquoplatinum were prepared by stirring *cis*- or *trans*-$PtCl_2(NH_3)_2$ (1 mmol, 300 mg) with $AgNO_3$ (2 mmol, 335 mg) in water (50 ml) overnight in the dark. After removal of AgCl by filtration, the solution was checked for the absence of Ag^+ and Cl^- (excess Ag^+ should be avoided due to the strong reaction between Ag^+ and the sugar anion). The solutions of *cis*- and *trans*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ were reacted with sodium glucuronate or potassium gluconate (2 mmol) in H_2O (10 ml) and the mixtures heated to 50°C for 10 min. After cooling the solution to room temperature, it was filtered to remove insoluble materials and absolute ethanol (50 ml) was then used to bring down the precipitate. The white precipitate was filtered off, washed several times with acetone/ether and dried under vacuum. The analytical results showed the composition of the following complexes to be:

Complex		% Pt	% N
cis-$[PtL(NH_3)_2]L \cdot H_2O$	Calcd.	30.70	4.40
(L = D-glucuronate anion)	Found	29.90	4.30
trans-$[PtL_2(NH_3)_2] \cdot H_2O$	Calcd.	30.70	4.40
(L = D-glucuronate anion)	Found	30.10	4.25
cis-$[PtL(NH_3)_2]L \cdot H_2O$	Calcd.	30.62	4.39
(D-gluconate anion)	Found	29.90	4.32
trans-$[PtL_2(NH_3)_2] \cdot H_2O$	Calcd.	30.62	4.39
(D-gluconate anion)	Found	30.00	4.29

The platinum-ammine-sugar complexes synthesized here are very hygroscopic and should be kept in a desiccator over $CaCl_2$. The compounds are very soluble in water and slightly so in hot alcohol, but are insoluble in any other common organic solvents.

2.3. Physical measurements

Infrared spectra were recorded on a Nicolet 5DXB Fourier transform infrared instrument with DTGS detector. The spectra were taken as KBr pellets with a resolution of 2–4 cm^{-1}. ^1H-NMR spectra were recorded on a Bruker WH-90 MHz instrument in ^2H_2O solution containing DSS as reference. X-ray powder photographs were taken for comparative purposes, using a camera (Phillips, Debye-Scherre) with CuK_α radiation. Conductance measurements were carried out at room temperature in aqueous solution (10^{-3} M) with a conductivity meter (type CDM2e, Radiometer, Copenhagen).

3. Results and discussion

3.1. Fourier transform-infrared spectra

Infrared spectra of the cis- and transplatin-sugar complexes together with the free sugar salts have been studied in the region 4000–500 cm^{-1} and comparisons have been made with those of the structurally known Ca-glucuronate [7] and Mn-gluconate compounds [8], the results of the spectral analysis being described below:

3.2. Sugar OH and CH stretching vibrations

The OH stretching vibrations of the structurally related D-glucuronic and D-gluconic acids [7,11] appeared as several strong and broad absorption bands in the range 3500–3200 cm^{-1} and exhibited major changes (shifting and splitting) upon sugar ionization [9]. However, sugar OH stretching vibrations in the spectra of the sodium glucuronate and potassium gluconate salts (used as ligands) showed no major changes upon platinum-ammine interaction. The small changes observed for the free salt OH stretchings (small shifts) are due to rearrangements of the hydrogen-bonding network of the free salt to that of the sugar-OH $\cdots NH_3 \cdots$ OH-sugar system on platinum coordination and do not arise from direct Pt-OH (sugar) interaction. It should be noted that direct metal-sugar hydroxyl group bonding observed in the crystal structures of Ca(D-glucuronate)Br $\cdot 3H_2O$ [11] and Mn(D-gluconate)$_2$ $\cdot 2H_2O$ [8] caused considerable spectral changes (shifting and splitting) of the sugar OH stretching vibrations [7,8]. The free sugar CH and CH_2 stretching vibrations [7,8] were observed as several sharp and weak absorption bands at about 3000–2800 cm^{-1} and showed no changes upon sugar platination.

3.3. Sugar carboxyl group stretching vibrations and binding modes

The carboxyl stretching vibration of each sugar anion exhibited major spectral changes upon platinum coordination which will be dealt with separately:

3.3.1. D-Glucuronate-anion-binding modes

In the infrared spectrum of D-glucuronic acid, the carbonyl group appears as a strong absorption band at 1709 cm^{-1} and shows shifting and splitting at 1600 and 1475 cm^{-1} in the spectrum of sodium glucuronate $\cdot 2H_2O$ [9]. The two absorption bands are assigned to the antisymmetric and symmetric stretchings of the carboxylate group, respectively [9]. Upon platinum-ammine interaction the two components of the carboxylate group appeared as broad and strong absorption bands at 1640 and 1413 cm^{-1} in the spectra of the *cis*-$[PtL(NH_3)_2]L \cdot H_2O$, and at 1600 and 1415 cm^{-1} in those of *trans*-$[PtL_2(NH_3)_2] \cdot H_2O$ (fig. 1). It has been suggested that, in a series of metal-carboxylate compounds, separation between the antisymmetric and symmetric carboxylate components is a measure of the chelation or bridging of the carboxylate group [12]. This separation was $\Delta\bar{\nu} = 125$ cm^{-1} for sodium glucuronate, where the sodium-carboxylate interaction is almost ionic [9], whereas for cis- and transplatin-glucuronate compounds these separations were $\Delta\bar{\nu} = 227$ cm^{-1} (*cis* isomer) and $\Delta\bar{\nu} = 185$ cm^{-1} (*trans* isomer) (fig. 1). The magnitude of these separations is indicative of chelation of the sugar anion in cisplatin-glucuronate and monodentate binding in the corresponding *trans* isomer. Similar spectral changes were observed for $[CoL(NH_3)_4]Cl_2 \cdot H_2O$ and $[CoL(NH_3)_5]Cl_2 \cdot H_2O$ complexes (L = D-glucuronate anion) where the sugar anion was found to be chelated in the cobalt-tetrammine and monodentately bonded in the cobalt-pentammine complexes [13]. It is interesting to note that, in the case of all the alkali metal-glucuronate salts (Na^+, K^+ and Rb^+) where the nature of the metal-carboxylate interaction was found to be largely ionic [14], the separations between the two carboxylate components were about 100–125 cm^{-1} [9], whereas separations of 150–200 cm^{-1} have been observed in the spectra of the Mg(II)-, Ca(II)-, Sr(II)- and Ba(II)-glucuronate compounds, consistent with chelation and bridging of the carboxylate group, in these series of metal-sugar complexes [7,11].

3.3.2. D-Gluconate anion-binding modes

The carbonyl stretching vibration of the free D-glucono-δ-lactone appeared as a strong absorption band at 1729 cm^{-1} [10] and exhibited shifting and splitting at 1600 and 1435 cm^{-1} in the spectrum of potassium gluconate (fig. 2), which are related to the antisymmetric and symmetric sugar carboxylate stretching vibrations [10]. It is noteworthy that, in the crystal structure of potassium gluconate [14], the K^+ is bonded to the sugar OH groups and showed no direct interaction with the carboxylate anion. Therefore, the separation of $\Delta\bar{\nu} = 165$ cm^{-1}, which was observed for the two

Fig. 1. Fourier transform-infrared spectra of D-glucuronate anion and its platinum-ammine complexes in the range 1900–1300 cm^{-1} for: (a) sodium D-glucuronate·2H$_2$O; (b) *cis*-[Pt(D-glucuronate)(NH$_3$)$_2$](D-glucuronate)·H$_2$O; (c) *trans*-[Pt(D-glucuronate)$_2$(NH$_3$)$_2$]·H$_2$O.

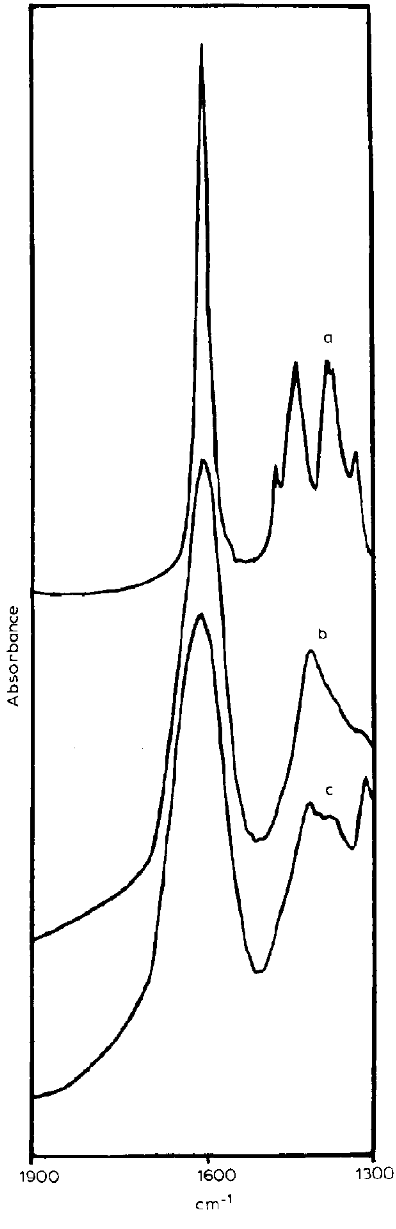


Fig. 2. Fourier transform-infrared spectra of D-gluconate anion and its platinum-ammine complexes in the range 1900–1300 cm^{-1} for: (a) potassium D-gluconate·H$_2$O; (b) *cis*-[Pt(D-gluconate)(NH$_3$)$_2$](D-gluconate)·H$_2$O; (c) *trans*-[Pt(D-gluconate)$_2$(NH$_3$)$_2$]·H$_2$O.

carboxylate components in the infrared spectrum of the potassium salt (fig. 2), is related to the purely ionic nature of the potassium-carboxylate interaction. On the other hand, a separation of about $\Delta\bar{\nu} = 190$ cm^{-1} was observed in the infrared spectrum of the structurally identified Mn(D-gluconate)$_2 \cdot 2H_2O$ compound, where direct metal-carboxylate binding occurred [8]. Upon cis- and transplatin interaction, antisymmetric and symmetric stretching vibrations of the D-gluconate anion was observed at about 1600 and 1410 cm^{-1}, respectively (fig. 2). Therefore, the separations of $\Delta\bar{\nu} = 190$ cm^{-1} observed between the two carboxylate components, in spectra of the cis- and transplatin-gluconate complexes (fig. 2), are indicative of chelation and monodentately bonded carboxylate in these platinum-sugar complexes. Similar spectral changes were observed for carboxylate stretching vibrations in the infrared spectra of cobalt-sugar complexes $[CoL(NH_3)_4]Cl_2 \cdot H_2O$ and $[CoL(NH_3)_5]Cl_2 \cdot H_2O$ (L = D-gluconate anion), in which the sugar anion was found to be chelated and monodentately bonded in these cobalt (III)-ammine sugar complexes [13].

A sharp absorption band with medium intensity at 1645 cm^{-1} observed in the spectrum of sodium glucuronate $\cdot 2H_2O$ salt is assigned to the bending mode of the bonded H_2O molecule (fig. 1). An absorption band at 1625 cm^{-1} in the spectrum of *trans*-$[PtL_2(NH_3)_2] \cdot H_2O$ is also related to bending vibrations of the H_2O or NH_3 group (fig. 1). In the spectra of *cis*-$[PtL(NH_3)_2]L \cdot H_2O$ (L = D-glucuronate) and the corresponding cis- and transplatin complexes of D-gluconate anion, the absorption bands related to the bending modes of the H_2O or NH_3 group in the region 1600–1650 cm^{-1} were obscured by the strong and broad carboxylate antisymmetric stretching vibrations (figs. 1 and 2). Similarly, the antisymmetric and symmetric stretchings of NH_3 and H_2O were overlapped by the strong sugar OH stretching frequencies (3500–3200 cm^{-1}) in all these platinum-sugar complexes.

The absence of any broad and strong absorption band at about 1400 cm^{-1} in the spectra of these platinum-sugar complexes related to the ionic nitrate group [15] is indicative of the complete replacement of NO_3^- from *cis*- or *trans*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ by the sugar anions (figs. 1 and 2).

The strongly coupled COH, CCH and CH_2 bending modes [16,17] of the sugar moiety appeared in the range 1470–1100 cm^{-1}, and exhibited some changes (lesser than the carbonyl stretching region) upon platinum-ammine complexation. The observed changes are related to rearrangements of the strong sugar hydrogen-bonding systems on sugar platination [13]. Similarly, the sugar ring C-O stretching vibrations [7,10] observed at about 1100–900 cm^{-1} showed shifts towards higher frequencies on complexation. The shifts in C-O stretching vibrations towards higher frequencies are due to changes in the sugar hydrogen-bonding network around the C-O group. Similar types of behaviour were observed in the spectra of silver glucuronate and gluconate [10,18], in which Ag^+ binding was found to be via the carboxylate oxygen atoms only and not the sugar OH or C-O groups. Since the major spectral changes observed here are for the carboxylate groups, therefore, platinum-ammine-sugar binding is through the carboxylate group and the possibility of direct Pt-OH (sugar) interaction cannot be excluded.

The sugar C-O-C and C-C-C skeletal deformation modes [16,17] in the region 1000–500 cm^{-1} exhibited minor changes in the spectra of platinum-ammine-sugar complexes. Since the ring vibrational frequencies are strongly coupled, platination of the sugar moiety largely perturbs the electron distributions within the ring system, where the vibrations are mostly localized and bring about ring distortion [10,18].

3.4. ^1H-NMR spectra and D-glucuronate anion anomeric configurations

In aqueous solutions, the β-anomer of D-glucuronic acid is predominant (β/α; 80/20%), whereas the α-anomer is preferred by the alkaline earth metal ion complexation [7,19]. Upon ionization, the β-anomer is favored by the alkali metal ion salts [9]. In this work, the β-anomeric (H_1) of sodium glucuronate appeared at 5.25 ppm in ^2H_2O solution (β-anomer; 100%) and exhibited no changes in the ^1H-NMR spectra of *cis*-

$[PtL(NH_3)_2]L \cdot H_2O$, whereas it showed a small upfield shift in spectra of the corresponding *trans* isomer. The absence of a downfield shift of the anomeric (H_1) proton in spectra of cis- and trans-platin-glucuronate complexes is indicative of non-participation of the sugar OH groups in metal-ligand binding, which is consistent with the infrared results discussed above. The small upfield shift of the anomeric proton (H_1), in the spectra of transplatin-glucuronate is related to rearrangements of the sugar anion hydrogen-bonding network, upon platinum-ammine coordination (possible hydrogen bonding of the sugar OH group with the NH_3 groups). The β-anomer configuration of this sugar moiety is predominant in these platinum sugar complexes (β-anomer; 100%).

3.5. X-ray powder diffraction and molar conductivity measurements

The X-ray powder diagrams of the platinum-ammine-sugar complexes were almost identical, indicating similar coordination geometry around the Pt(II) ion in these metal-sugar complexes. On the other hand, the X-ray powder patterns of the platinum-sugar complexes exhibited no marked similarities with those of the structurally characterized eight-coordinate Ca(D-glucuronate) $Br \cdot 3H_2O$ [11] and six-coordinate Mn(D-gluconate)$_2 \cdot 2H_2O$ [8]. The dissimilarities observed are due to the smaller coordination numbers exhibited by the Pt(II) ion (four-coordinate) with respect to those of Ca(II) and Mn(II).

The high molar conductivities (150–190 Ω^{-1} cm^2 mol^{-1}) observed for the platinum-ammine-sugar complexes are indicative of considerable dissociation of these metal-sugar complexes in H_2O solutions.

4. Conclusion

On the basis of the spectroscopic and structural properties of the *cis*- and *trans*-platinum-ammine-sugar complexes studied here and comparisons made with those of structurally characterized metal-glucuronate and metal-gluconate compounds, the following remarks can be made:

(1) Platinum-sugar coordination occurs monodentately via the negatively charged carboxylate oxygen atom in *trans*-$[PtL_2(NH_3)_2] \cdot H_2O$ and bidentately in *cis*-$[PtL(NH_3)_2]L \cdot H_2O$ through carboxylate oxygen atoms or carboxylate O(6) and sugar ring O(5) in D-glucuronate anion and O(1) and O(2) of the D-gluconate anion, forming a five-membered chelate ring around the platinum ion;

(2) Upon platinum-ammine interaction, the sugar hydrogen-bonding network alters to that of sugar-$OH \cdots NH_3(H_2O) \cdots OH$-sugar;

(3) The D-glucuronate anion shows the β-anomer configuration in both the free sodium salt and in these platinum-amine-sugar complexes;

(4) The platinum-ammine-sugar complexes are more soluble in water than the parent cis- and transplatin.

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