(1,2-Diaryl-1,2-ethylenediamine)platinum(II) complexes with sulfato and 3-sulfopropionato leaving groups: investigations with different types of coordination in the solid state, in solution and reactions with nucleophiles

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Abstract

The syntheses, solid state and solution chemistry, and the *in vitro* cytotoxic activities of water soluble (1,2-diaryl-1,2-ethylenediamine)platinum(II) complexes, having either the sulfato or the novel sulfopropionato anionic leaving group, are described. The compounds were prepared by allowing the (1,2-diaryl-1,2-ethylenediamine)-diiodoplatinum(II) complexes and either silver sulfate or silver sulfopropionate to react in water. IR studies of the complexes in the solid state indicate that the preparations are mixtures of platinum species, whereby the anionic leaving groups show varying forms of platinum coordination. These compositions vary depending on the procedure used to isolate the platinum complexes. The aqueous solution chemistry was investigated by means of a reversed-phase HPLC assay. The results of these studies show the presence of several platinum species in solution. Based on the column retention properties of the species as well as their behavior to changes in pH, it is concluded that these solution components are various monomers and oligomers of the parent platinum complex. These results are in agreement with previous ^{195}Pt and ^{15}N NMR studies from other diamineplatinum(II) complexes. The type of aromatic substitution has an influence on the qualitative composition of the aqueous platinum species; an additional platinum species is observed in aqueous solutions of platinum complexes possessing an *ortho* phenol group compared to the complexes possessing a *para* fluorine group. Aqueous reactions of the platinum complexes with chloride indicate that the sulfopropionato ligand is more stable-bound than the sulfato ligand, however, results from cell culture cytotoxicity tests show little difference between the cytotoxic activities of the complexes possessing a sulfato or a sulfopropionato leaving group.

Introduction

The antitumor activity of *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) was first reported in 1969 and today it is an effective chemotherapeutic agent for the treatment of several malignant tumors [1]. In an attempt to improve the therapeutic index and broaden the spectrum of antitumor activity of cisplatin, a wide variety of platinum complexes have been synthesized [2]. In recent publications we have shown that dichloro(1,2-diaryl-1,2-ethylenediamine)platinum(II) complexes having aromatic fluorine or hydroxy substituents can exhibit marked antitumor activity [3–5]. Since these compounds possess poor water solubility, a limiting factor for their clinical application, the more water soluble sulfatoplatinum(II) complexes were prepared. However, the highly reactive nature of the sulfatoplatinum(II) moiety is a disadvantage with respect to the bioavailability and toxicity of the drug. In order to overcome the problem of water solubility and at the same time avoid the highly electrophilic platinum species $Pt(H_2O)_2^{2+}$, the 3-sulfopropionato ligand was considered as an anionic leaving group. The resulting platinum complexes, which would be expected to possess a stable-bound carboxylate and a labile-bound sulfonate group, were envisioned to be less chemically reactive and hence less toxic than their sulfate analogues, while being sufficiently water soluble for clinical use.

The aqueous chemistry of platinum complexes is of central importance for the pharmacology of this class of antitumor agents [6]. It was expected that this chemistry would be quite complicated for the new compounds. Aqueous solutions of various sulfato- and nitratoplatinum(II) complexes have already been examined by means of ^{195}Pt and ^{15}N NMR spectroscopy as well as by potentiometric titration methods [7–10]. These compounds have been reported to hydrolyse immediately after dissolution in water and behave as

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diacids. The resulting diaquaplatinum(II) species reacted to form hydroxy-bridged dimers and trimers depending on the solution pH, platinum concentration and reaction time.

The following paper reports the synthesis and characterization of 1,2-diaryl-1,2-ethylenediamineplatinum(II) complexes possessing the novel 3-sulfopropionato leaving ligand, and compares them with their sulfatoplatinum(II) analogues. In order to investigate the aqueous solution chemistry of these complexes, a reversed-phase HPLC assay was developed. Through the use of this analytical method, the formation of oligomers, reactions with solvents (i.e. MeOH and DMSO), as well as the effects of added chloride were studied for the title compounds. The cytotoxic activities of the new compounds are also reported.

Experimental

Materials

The *meso*-1,2-bis(2-hydroxyphenyl)-1,2-ethylenediamine [11], and the *d/l*-[1,2-bis(4-fluorophenyl)-1,2-ethylenediamine]diiodo- and sulfatoplatinum(II) complexes [3] were synthesized as previously described. Unless mentioned otherwise, all reagents were of standard reagent quality. Water was deionized with a Millipore-Q-Water system and methanol (Baker) and DMF (Aldrich) were of HPLC grade. Na_2SO_4, $HClO_4$ and H_2SO_4 were of analytical quality. The elemental analyses of the prepared substances were performed in the microanalytical laboratory of the University of Regensburg. Based on the C, H and N analyses, all compounds were of acceptable quality (within 0.4% of the calculated values).

IR spectra were recorded on an Acculab 7 (Beckman) as Nujol mulls or KBr pellets. Proton NMR spectra (250 MHz) and ^{13}C NMR spectra (22.64 MHz) were recorded on Bruker WM 250 and WH 90 instruments, respectively. TMS was the internal standard. Proton NMR of compounds **1–4** are shown in Table 1. Fast atom bombardment mass spectra (FAB-MS) were obtained from a Varian MAT 311A instrument. Samples were dissolved in a glycerol/DMSO (1:1) matrix immediately prior to analysis.

The HPLC instrumentation consisted of two Altex 110 A pumps, an Altex 420 microprocessor controller and a Rheodyne 7125 sample injector, which was fitted with a 20 μl injection loop. Sample detection was made with a Kontron-Uvikon LC variable wavelength UV–Vis detector set at 269 nm for the analyses of **1** and **3** and at 276 nm for **2** and **4**. A 0.4×25 cm Nucleosil-100 RP-18 column with a 0.4×3 cm precolumn (Macherey-Nagel, Düren, FRG) was used for chromatography. Immediately before chromatography, the aqueous samples were diluted 1:1 in methanol. After being loaded onto the column, the sample (20 μl) was eluted at a flow rate of 0.6 ml/min with an isocratic system consisting of 3 parts MeOH to 2 parts of a 20 mM Na_2SO_4 (pH 3.0) solution. The void volume elution time (t_0) was 4.4 min.

Syntheses

meso-[1,2-Bis(2-hydroxyphenyl)-1,2-ethylenediamine]diiodoplatinum(II)

The synthesis of this compound was analogous to that of the previously described fluorinated diiodo complexes [3]. Briefly, *meso*-1,2-bis(2-hydroxyphenyl)-1,2-ethylenediamine and $K_2[PtI_4]$ were made to react in water, the resulting diiodoplatinum(II) complex was collected as a precipitate, washed with water and dried *in vacuo* over P_2O_5. Yield was 67% of a yellow powder.

IR (KBr): 3500, m (OH); 3280, m; 3230, m (NH_2).

^1H NMR (DMF-d_7): δ 4.80 (br m, 2H, benzylic); 5.13 (br m, 2H, NH_2); 6.65 (br m, 2H, NH_2); 6.67–6.73 (m, 4H, aromatic); 7.01–7.08 (m, 2H, aromatic); 8.09–8.18 (m, 2H, aromatic); 9.49 (s, 2H, OH).

Anal. Calc. for $C_{14}H_{16}I_2N_2O_2Pt$: C, H, N.

meso-[1,2-Bis(2-hydroxyphenyl)-1,2-ethylenediamine]sulfatoplatinum(II) (2)

The synthesis of **2** was identical to that described for **1** [3]. Briefly, the diiodoplatinum(II) complex was reacted with a molar equivalent of Ag_2SO_4 in water at 50 °C for 2 days in the dark. After filtering away the AgI, the filtrate was lyophilized to give a colorless powder in a yield of 96%.

IR (KBr): 1130, s; 1040, s; 950, m (ionic and coordinated sulfate).

Anal. Calc. for $C_{14}H_{16}N_2O_6SPt \cdot 3H_2O$: C, H, N.

Silver-3-sulfopropionate

3-Sulfopropionic acid anhydride (80 mmol) and $BaCO_3$ (75 mmol) were stirred in 140 ml water for 1 day at room temperature. After filtration, the filtrate was evaporated to dryness and the residue recrystallized from water. An aqueous solution of the resulting barium salt was stirred in the dark at 50 °C with an equimolar amount of Ag_2SO_4. After removing solid $BaSO_4$, the filtrate was evaporated to dryness and the crude product recrystallized from water. The yield was 27%.

Anal. Calc. for $C_3H_4Ag_2O_5S$: C, H.

d/l-[1,2-Bis(4-fluorophenyl)ethylenediamine](sulfopropionato)platinum(II) (3)

The diiodoplatinum(II) complex (1 mmol) was made to react with silver-3-sulfopropionate (0.95 mmol) by stirring in 100 ml water at 50 °C for 2 days in the dark. The AgI was removed by filtration, the filtrate

TABLE 1. ^{1}H NMR (250 MHz) of compounds **1–4**

Compound	Solvent	Aliphatic H	Benzylic H	NH+ArH	OH
1	d_6-DMSO		4.29, m, 1H 4.43, m, 1H	6.28–6.40, m, 2H(NH), 7.03–7.12, m, 4H(ArH) 6.68–6.70, m, 1H(NH), 7.38–7.47, m, 4H(ArH), 7.18–7.21, m, 1H(NH)	
2	d_6-DMSO		4.73, br m, 2H	5.84, br m, 1H(NH), 6.61–6.81, m, 5H(ArH+NH), 6.18, br m, 1H(NH), 7.01–7.04, m, 3H(ArH+NH), 7.58–7.69, m, 2H(ArH)	9.45, s, 2H
3	d_4-methanol	2.69–2.82, m, 2H 3.02–3.17, m, 2H	4.12–4.50, br m, 2H	5.97, br s, 2H(NH), 7.00–7.18, m, 4H(ArH), 6.74, br s, 2H(NH), 7.26–7.57, m, 4H(ArH)	
4	d_4-methanol	2.68–2.81, m, 2H 3.02–3.12, m, 2H	4.61–5.01, br m, 2H	5.57–5.81, m, 2H(NH), 6.34–7.34, m, 8H(ArH+NH), 7.67–8.17, m, 2H(ArH)	

Values are given in the following order: chemical shift (ppm), peak multiplicity (s, singlet; br s, broad single; m, multiplet; br m, broad multiplet), and relative peak area.

was lyophilized and subsequently dried *in vacuo* over silica gel to give a quantitative yield.

IR (KBr): 1730, m (C=O, monomer); 1580, m (C−O dimer).

^{13}C NMR (d_4-methanol): δ 30.97 (aliphatic-C sulfopropionate); 67.88 (benzylic-C, $J=3.3$ Hz); 116.74 (aromatic-C, β-position to F, $J=21.9$); 131.50 (aromatic-C, γ-position to F, $J=6.0$ Hz); 133.15 (aromatic-C, δ-position to F, $J=3.3$ Hz); 164.19 (aromatic-C, α-position to F, $J=248.1$ Hz); 173.97 (C−O); 175.29 (C−O).

Anal. Calc. for $C_{17}H_{18}N_2F_2O_5SPt\cdot 3H_2O$: C, H, N.

meso-[1,2-Bis(2-hydroxyphenyl)ethylenediamine] (sulfopropionato)platinum(II) (4)

The same procedure as described above for **3** gave a colorless powder. The yield was quantitative.

IR (KBr): 1720, w (C=O monomer); 1570, s (C−O dimer); 1390 (C−O symm.).

Anal. Calc. for $C_{17}H_{24}N_2O_9SPt\cdot 3H_2O$: C, H, N.

Cytotoxicity testing

The *in vitro* cytotoxicity testing of the platinum complexes was done on exponentially dividing MDA-MB-231 human breast cancer cells (American Type Culture Collection, Rockville, MD, USA) according to a previously published microtiter assay [12]. Briefly, in 96-well microtiter plates (Costar), 100 μl of a cell suspension at 7000 cells/ml cell culture medium (McCoy's 5A Medium with 5% foetal calf serum) were plated into each well and incubated 48 h at 37 °C in a humidified atmosphere of 5% CO_2/air. From 100× concentrated aqueous seed solutions of **1–4**, 200 μl were diluted into 10 ml of medium. (Cisplatin was administered at 1000 times its final concentrations in DMF.) From this solution, 100 μl were immediately added to each well at 24 wells per concentration. Control wells received only medium with the corresponding amount of solvent (i.e. distilled water or DMF). After either 1 or 5 days, the medium was removed, the cells were fixed with a glutardialdehyde solution and stored at 4 °C. Cell biomass was determined by a crystal violet staining technique as described [12]. The effectiveness of the compounds is expressed as corrected T/C values according to the following equation: $T/C_{corr}=(T-C_0)/(C-C_0)\times 100$, where T and C are the density of cells in the microtiter wells either 1 or 5 days following the addition of platinum complex and solvent alone, respectively, and C_0 is the density of cells on the day of treatment.

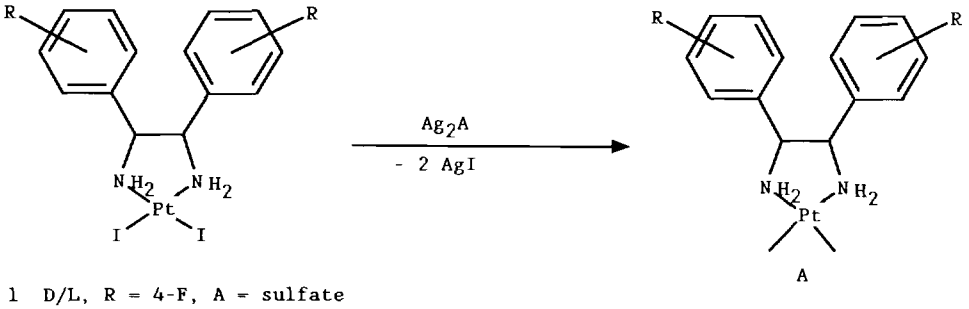
Results and discussion

Synthesis of the platinum complexes

The sulfato- and (3-sulfopropionato)platinum(II) complexes (**1–4**) are easily obtained through the reaction of the diiodoplatinum(II) compounds with the corresponding silver salt (Scheme 1).

Characterization of the sulfatoplatinum complexes in the solid state

The sulfate portion of the sulfatoplatinum(II) complexes can act either as a counterion, a monodentate ligand or as a bridging/chelating bidentate ligand. Ionic sulfate possesses T_d symmetry, and only two vibrational modes absorb in the IR spectrum, which appear at *c.* 1130 and 620 cm^{-1} [13]. Upon complexation with platinum, the symmetry is lowered and the degenerated vibrational modes split. For many years IR spectroscopy offered the only possibility to investigate the structure of sulfatoplatinum(II) complexes. However, an X-ray analysis of [Pt(SO_4)(*N*,*N*′-dimethyl-1,2-ethylenediamine)(H_2O)$\cdot x H_2O$ has provided additional insight into the structure of these types of compounds [14]. These



1 D/L, R = 4-F, A = sulfate

2 meso, R = 2-OH, A = sulfate

3 D/L, R = 4-F, A = 3-sulfopropionate

4 meso, R = 2-OH, A = 3-sulfopropionate

Scheme 1. Syntheses of the platinum complexes.

studies showed that sulfate coordinates as a monodentate ligand in the square planar complex with the remaining coordination position occupied by water.

In this work, it is found that the sulfate of the fluorinated complex **1** can coordinate with platinum in various ways depending on the isolation procedure. If the compound precipitates from a concentrated solution, a diaquaplatinum(II)sulfate is obtained. The vibrational modes at 1130 and 635 cm^{-1} are assigned to an ionic sulfate. On the other hand, if the compound is isolated by lyophilization, a higher number of vibrational modes (1170, 1130, 1040, 940 and 635 cm^{-1}) are observed. The additional vibrational modes have been assigned to a monodentate or a bridging bidentate sulfate [11]. The Pt–OH bending mode contributes little to the vibrational mode at 1040 cm^{-1}, as shown by deuteration of the complex [15].

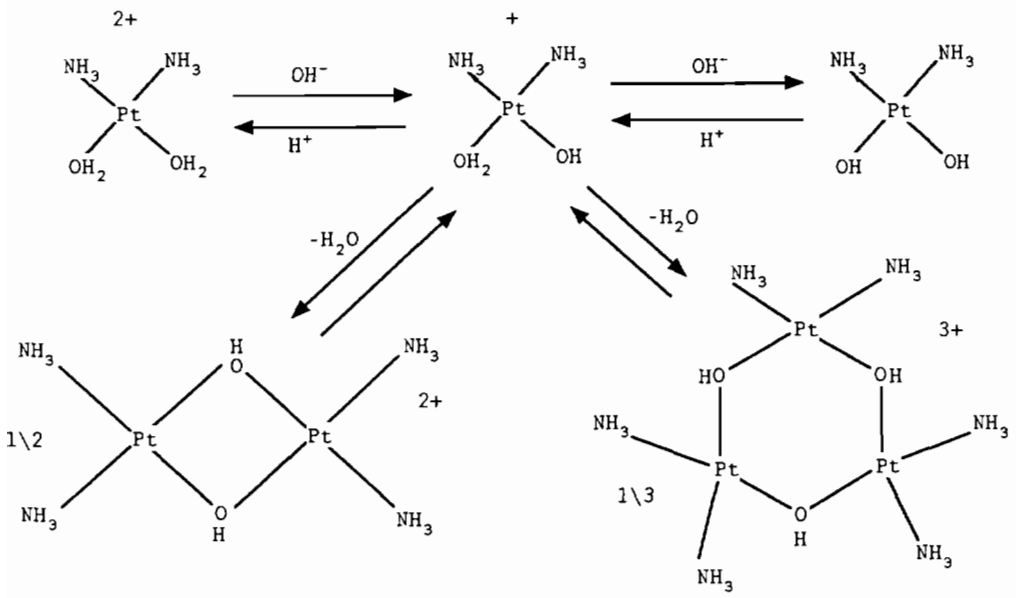
Aqueous solution chemistry of the sulfatoplatinum(II) complexes

Since sulfate is only a weak ligand in aqueous solutions, a rapid hydrolysis of the sulfatoplatinum(II) complexes is known to occur and the diaquaplatinum(II) complexes are formed. Following dissociation of a proton, the resulting aquahydroxyplatinum(II) species can react in condensation reactions with one another. At pH > 6, hydroxy-bridged dimers and trimers are formed. The $[Pt_2(\mu\text{-}OH)_2(NH_3)_4]^{2+}$ and $[Pt_3(\mu\text{-}OH)_3(NH_3)_6]^{3+}$ species have been isolated from such aqueous solutions and characterized with X-ray crystallography [15, 16]. A pH-dependent equilibrium has been formulated for these reactions (Scheme 2) [17].

Additional evidence for these species in aqueous solutions was obtained by means of ^{195}Pt NMR spectroscopy [7–10]. In our case, the water solubility of diaquaplatinum(II) complexes with the lipophilic 1,2-diaryl-1,2-ethylenediamine ligands is too low to allow for the recording of ^{195}Pt NMR spectra, and an alternative method for the detection of the equilibrium species was sought. We have found reversed-phase HPLC to be quite useful for studying the chemistry of dilute aqueous solutions of dichloroplatinum(II) complexes [18]. For this work, a reversed-phase HPLC assay was developed by which the aqueous reaction dynamics of platinum complexes **1–4** could be analyzed.

Charged platinum complexes can be eluted with H_2O/MeOH from C_{18}-bonded reversed-phase columns. However, the low elution recovery of the platinum hydrolysis species from the column is often a problem. (A likely explanation is that a nucleophilic displacement of the aqua ligand by residual silanol groups of the column packing material takes place.) The following measures are taken to overcome this: (i) the use of an end-capped Nucleosil C_{18} column; (ii) the pH of the mobile phase is adjusted to 3; (iii) the use of Na_2SO_4 as a supporting electrolyte in the mobile phase; and (iv) the aqueous solution is diluted 1:1 with methanol immediately prior to injection. Similar conditions have already been reported for the chromatographic analysis of aqua[1,2-bis(aminomethyl)cyclohexane]sulfatoplatinum(II) and its derivatives [19]. A quantitative recovery of the injected material can then be achieved if the above steps are taken.

In a chromatogram of *d,l*-[1,2-bis(4-fluorophenyl)-1,2-ethylenediamine]sulfatoplatinum(II) (**1**), three peaks are apparent (Fig. 1(a)). Molecular size and polarity are known to influence the mobility of a solute on a hydrophobic column; smaller, more polar species elute sooner that larger, non-polar ones [20]. Therefore, the first peak (retention time (t_r): 7.2 min), the major component of the solution mixture, is attributed to the diaquaplatinum(II) species. Accordingly, the com-



Scheme 2. pH dependent equilibria.

pounds eluting at 13.0 and 14.9 min are assigned to the hydroxy-bridged dimer and trimer, respectively. With these tentative assignments in mind, it is possible by means of HPLC to study the behavior of these three platinum species to changes in solution pH, and then to relate these finding back to the proposed structures of the solution mixture components.

When **1** is dissolved in 0.1 M $HClO_4$, the species eluting at 13.0 and 14.9 min have completely disappeared from the chromatogram by 2.5 h (Fig. 1(b)). On the other hand, the quantity of substance eluting at 7.2 min grows in a proportional relationship to the lost substances. As discussed in the literature, lowering the solution pH to 1 results in a complete hydrolysis of the oligomeric species [21]. Thus, it is consistent that the early peak be assigned to the diaquaplatinum(II) species.

At pH 6.4 the dimer, $[Pt_2(\mu\text{-}OH)_2(NH_3)_4](NO_3)_2$, was isolated [15]. We find that after a one day incubation of **1** at a pH of 6.4, the peak at 13.0 min increases significantly and grows larger over the next two days (Fig. 1(c)). The elemental analysis of the compound, obtained after lyophilization of the solution mixture and removal of the Na_2SO_4, agrees with the hydroxy-bridged dimer. Therefore, the assignment of the species eluting at 13.0 min to the dimer is consistent with the observed chemistry of that compound.

For aqueous solutions of *d,l*-(1,2-cyclohexanediamine)dinitratoplatinum(II), the formation of the corresponding trimer was reported to be enhanced under basic conditions [10]. In the case of **1** at pH 9.3, only a slight increase of the trimer is observed after one day, but after three days the area under the peak at 14.9 increases significantly. The chemistry of this species appears consistent with it being assigned the trimer structure.

It should be mentioned that all of these processes are reversible; that is the single species can be interconverted when the solution pH is readjusted. This is evidence for the presence of equilibria of the type shown in Scheme 2. The solution chemistry of *d,l*-[1,2-bis(4-fluorophenyl)-1,2-ethylenediamine]sulfatoplatinum(II) reported in this work is in agreement with the ^{195}Pt NMR findings for other sulfato- and nitratoplatinum(II) complexes having neutral amine ligands [9, 10, 22].

The HPLC analysis of an aqueous solution of *meso*-[1,2-bis(2-hydroxyphenyl)-1,2-ethylenediamine]sulfatoplatinum(II) (**2**) gives results comparable to **1** with one interesting exception: in the region of the diaquaplatinum(II) species two peaks are observed (t_r: 5.6 and 6.6 min). After a two-day incubation, the area of the first peak increases and the addition of $HClO_4$ (0.1 M final concentration) brings about the complete loss of the second peak at t_r 6.6 min. It is known that under aqueous conditions, decreases in solution pH favor the formation of diaquaplatinum(II) complexes [23], and for this reason the first peak is attributed to this species. The second peak most likely corresponds to the aquasulfatoplatinum(II) species, since the observed conversion to the diaquaplatinum(II) species is slowed in a 40 mM aqueous Na_2SO_4 solution.

Solution chemistry of the sulfatoplatinum complexes in organic solvents

(1,2-Diaryl-1,2-ethylenediamine)sulfatoplatinum(II) compounds dissolve not only in water but also in polar organic solvents such as alcohols, acetone, DMF and

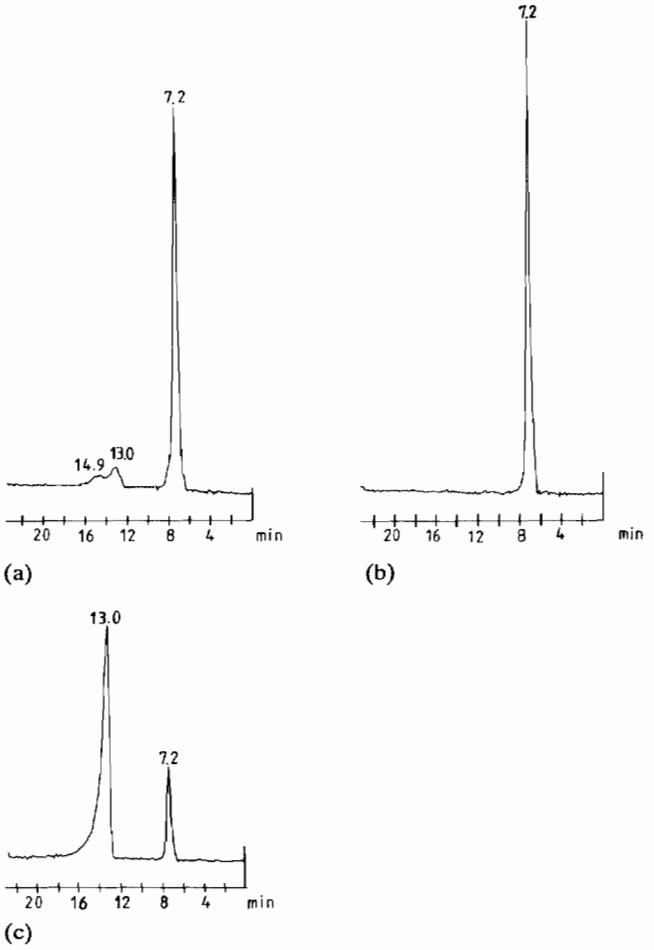


Fig. 1. The HPLC chromatograms of the sulfatoplatinum complex **1** were obtained under the following conditions: (a) immediately after dissolving 2.5 mg (4.2 μmol) in 1 ml deionized water (pH 6.4); (b) 2.5 h after dissolving 2.5 mg of **1** in 1 ml 0.1 $HClO_4$; and (c) 3 days after dissolving in deionized water. All solutions were held at room temperature. See 'Experimental' for details about the HPLC assay.

DMSO. These solvents are frequently used in biochemical assays, although often little is known about the interaction between the sulfatoplatinum(II) complexes and these solvents. In order to clarify the effects of some commonly used organic solvents on the stability of the sulfatoplatinum(II) complexes, the following investigations were done.

It was found that from a solution of **1** in MeOH (50 mg/ml), a new compound precipitates shortly after dissolving. This new compound is insoluble in the above mentioned solvents except DMSO. In the IR spectrum of the compound, significant changes in the region of the sulfate bands occur. The absorption at 1230 cm^{-1} is indicative of sulfate coordinating as a chelating ligand [7, 24]. The elemental analysis is unchanged when compared with **1**, and hence no complexation with MeOH is inferred. This phenomenon can be explained as follows. In the solid state **1** consists of a mixture of diaquaplatinum(II), sulfatoplatinum(II) and hydroxy-bridged platinum oligomers. In aqueous solution, hydrolysis is irreversible because of the large excess of water. In methanolic solutions of **1** (the concentrations of water and sulfate are in about the same order of magnitude) the sulfate ion can displace water from the platinum atom and a sulfatoplatinum chelate is formed. The same phenomenon is observed when **1** is dissolved in DMF (50 mg/ml).

In contrast to MeOH and DMF, DMSO represents a good ligand for the platinum atom. IR studies have previously shown that coordination of platinum(II) with one or two DMSO molecules occurs exclusively through the sulfur atom [25]. Bis(DMSO)platinum(II) complexes are only isolated if the solution contains no other possible ligands [26]. The addition of DMSO (10% final concentration) to an aqueous solution of **1** brings about the rapid formation (<2.5 h) of the $[Pt(DMSO\text{-}S)(H_2O)]^{2+}$ species, which appears as the peak with retention time of 8.0 min in the HPLC chromatogram shown in Fig. 2. In addition, the sulfatochelate complex of **1**, as well as the hydroxy-bridged dimer of **1**, form the same DMSO adduct in an aqueous solution of 10% DMSO.

A problem in characterizing (1,2-diaryl-1,2-ethylenediamine)sulfatoplatinum(II) complexes has been the acquisition of ^1H NMR spectra of these compounds. As discussed above, these compounds precipitate during NMR measurements in most organic solvents. Furthermore, their solubility in water is much too poor to obtain well resolved spectra. DMSO, on the other hand, proved to be an excellent solvent for acquiring NMR spectra of the sulfatoplatinum complexes **1** and **2**; the DMSO adducts, [1,2-diaryl-1,2-ethylenedi-

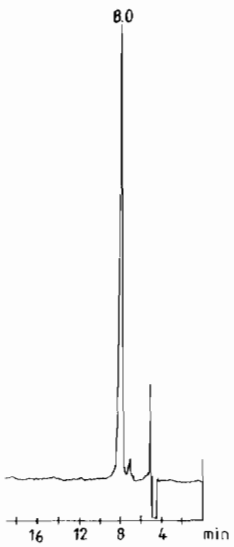


Fig. 2. The HPLC chromatogram of **1** (0.8 mg, 1.3 μmol) that had been dissolved in 400 μl of a 10% aqueous DMSO solution for 2.5 h.

amine](DMSO-d_6-*S*)sulfatoplatinum(II), are stable enough to allow the recording of well resolved ^{1}H NMR spectra (Table 1). Due to the coordination of the DMSO and SO_4^{2-} ligands, unsymmetrically substituted platinum complexes are formed, which give rise to four NH signals. (In contrast, a symmetrically substituted complex, i.e. dichloro[*d*/*l*-(1,2-bis(4-fluorophenyl)-1,2-ethylenediamine)]platinum(II) showed only two NH signals, which are assigned to the axial and equatorial amine protons [3].) The ^{1}H NMR spectrum of **1** in deuterated DMSO clearly shows the unsymmetrical environment of the platinum(II) atom (Table 1). By chance, two of the four non-equivalent amine protons have the same chemical shift and for this reason only three absorptions are observed (Table 1). Furthermore, the benzylic protons have become diastereotopic and are split into two signals (δ 4.29 and 4.43). The signals of the amine protons are broadened because of geminal, vicinal and ^{195}Pt coupling, and because of coupling with the ^{14}N nucleus, which rapidly relaxes by the quadrupolar effect.

Characterization of the (3-sulfopropionato)platinum(II) complexes in the solid state

The solid state chemistry of the new (3-sulfopropionato)platinum complexes was also investigated by means of IR spectroscopy. For compounds of this type several types of coordination are possible. Following complexation with platinum, the two oxygen atoms of the carboxylate group become non-equivalent, and as a consequence the wave numbers of ν_{asym} in the IR spectrum increase and the wave numbers of ν_{sym} decrease, causing an increase in the Δ value (Δ:$\nu_{asym} - \nu_{sym}$). Deacon and Philips [27] have correlated the Δ value with the type of coordination for many (acetato)metal complexes. Based on these assignments, the following can be said about the platinum complexes **3** and **4**. In the IR spectra of these compounds, two different wave numbers for the asymmetric C–O vibrational mode at 1730 and 1580 cm^{-1} are observed. One likely reason for this is that the substances consist of a mixture of two species. The symmetric vibrational modes appeared between 1380 and 1440 cm^{-1}. A high wave number of 1730 cm^{-1} for a coordinated carboxylate group has never been described in the literature to our knowledge. Only the asymmetric C–O absorption of the structurally similar complex *meso*-[1,2-bis(4-chlorophenyl)-1,2-ethylenediamine]malonato)platinum(II) comes in this same region (1700 cm^{-1}) [28]. Therefore, this species can be assigned to the complex with a monodentate 3-sulfopropionato ligand. The remaining fourth coordination position is most likely occupied by water. In the IR spectra of **3**, the stretching modes of the sulfonate group appear at 1200, 1180 and 1140 cm^{-1}. These results are in accordance with data obtained from IR spectra of sodium alkylsulfonates, in which the sulfonate group does not coordinate [29], but are not consistent with a coordinated sulfonate [30]. The identification of the second species is more difficult since the Δ value lies between 140 and 200. This makes a distinction between the remaining coordination types impossible.

In the IR spectrum of **4**, the region between 1200 and 1300 cm^{-1} where the absorption of a coordinated sulfonate should come [30], is strongly obscured by the vibrational modes of the 1,2-bis(2-hydroxyphenyl)-1,2-ethylenediamine ligand and hence no information about the state of coordination can be obtained.

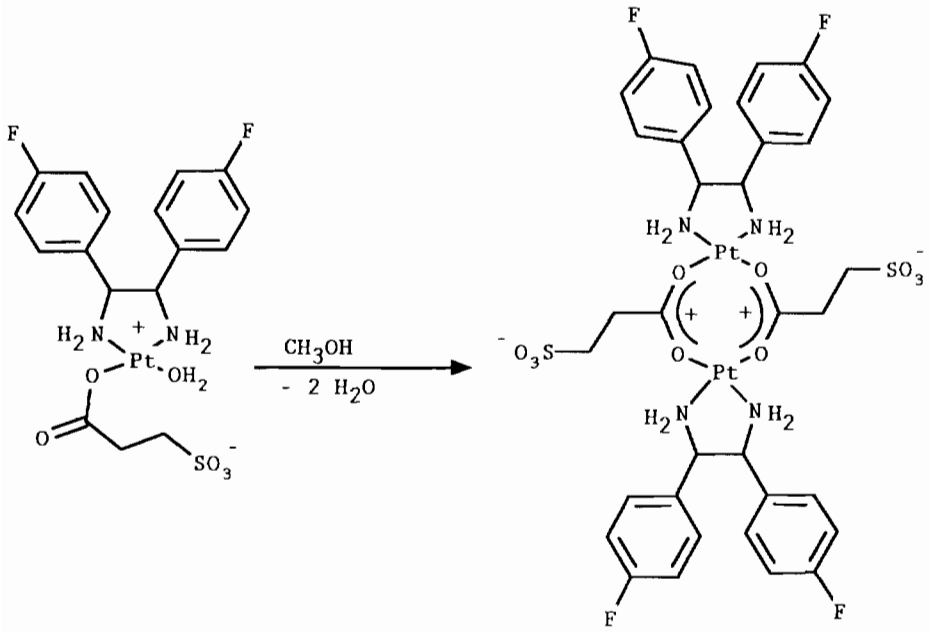
Solution chemistry of the (3-sulfopropionato)platinum complexes in methanol

In deuterated methanol, a well resolved ^{13}C NMR spectrum of **3** is obtained, and because of coupling between ^{13}C and ^{19}F, each aromatic carbon can be assigned. Upon complexation of platinum with 3-sulfopropionate, two new peaks can be assigned to the carbon atom of the coordinated carboxylate group, and this indicates the existence of two different coordinated species. During the recording of the ^{13}C NMR of **3**, a precipitate began to build. The IR of this precipitate shows only the ν_{asym} at 1580 cm^{-1}; the species with the monodentate coordinated sulfopropionatoplatinum group has disappeared. In the FAB-MS spectrum of **3** a small dimer peak is always present. This peak increases in intensity in the FAB-MS of the precipitate. The most likely dimer structure that would account for this is the carboxylato-bridged di-(μ-3-sulfopropionato-O^1,$O^{1'}$)-bis{[*d*/*l*-bis(4-fluorophenyl)-1,2-ethylenediamine]platinum(II)}. After dissolving **3** in methanol, the aqua ligand of the monomer is apparently labilized, and as a consequence the carboxylate group can act as a bridging ligand (Scheme 3). The resulting dimer precipitates.

Aqueous solution chemistry of the (3-sulfopropionato)platinum(II) compounds

The results of the HPLC investigations of the aqueous chemistry of **3** are consistent with what had been found by IR, ^{13}C NMR and FAB-MS methods. In aqueous solution, two peaks are observed in the HPLC chromatogram for **3**. The monomer elutes at a retention time of 7.6 min. The signal at 16.4 min is assigned to the dimer (Fig. 3). This assignment is supported by the fact that the dimer peak decreases when left to stand in aqueous solutions, while the first peak increased in a proportional way over the same time period. No diaquaplatinum(II) species are observed even after **3** is allowed to stand in water for one month at room temperature.

The HPLC chromatogram of **4** is more complicated than the chromatogram of **3** (Fig. 4(a)). This is reminiscent of the differences in the aqueous chemistry



Scheme 3. Dimer formation reaction.

Fig. 3. The HPLC chromatogram of the 3-sulfopropionatoplatinum complex **3** that had been dissolved in a 60% aqueous methanol solution to a concentration of 1.5 mM and immediately chromatographed.

observed between the sulfatoplatinum(II) complexes **1** and **2**. Based on the results obtained from the investigations with **2** and **3**, we suggest the following assignments for the five peaks observed in the chromatogram of **4**: (1) t_r: 5.6 min, the diaquaplatinum(II) species; (2) t_r: 6.6 min, the aqua(3-sulfopropionato-O^1)platinum(II) species; (3) t_r: 9.6 min, the (3-sulfopropionato-O^1,O^3)platinum(II) species; (4) t_r: 16.8 min, carboxylato-bridged dimer; (5) t_r: 18.0 min carboxylato-bridged trimer.

It is surprising that the substitution of a hydroxy group in the *ortho* position of the aromatic ring as opposed to a fluorine atom in the *para* position can have such a striking influence on the type of coordination of a sulfate and a sulfonate group. Space filling models show that a distance of *c.* 2 Å between an oxygen of the coordinated sulfate and a phenolic proton in the *ortho* aromatic position is possible. Therefore, it is theorized that the *ortho* phenol group can stabilize a coordinated sulfate or sulfonate group through hydrogen bonding.

Reactions of the platinum complexes with chloride

Aqueous reactions of the platinum complexes with nucleophiles can be followed clearly with HPLC. For instance, an aqueous solution of **4** with a four-fold excess of chloride leads to a new peak at t_r 7.2 min after an incubation time of 0.5 h (Fig. 4(b)). This signal is attributed to the chloro(3-sulfopropionato-O^1)platinum(II) species. The other peaks decrease proportionally. After 1.5 h, the dichloroplatinum(II) complex (t_r 9.2 min) is first observed (Fig. 4(c)). Following a one-day incubation, all of the starting peaks have disappeared and only the chloro(3-sulfopropionato-O^1)platinum(II) and dichloroplatinum(II) species are present (Fig. 4(d)). The further addition of a six-fold excess of chloride gives almost exclusively the dichloroplatinum species after two days.

A similar experiment was done with an aqueous solution of **2**. In the first anation reaction, the aquachloroplatinum(II) species is formed (Table 2). A second reaction with chloride gives the dichloroplatinum(II) complex. Although both **2** and **4** react with chloride to give the same dichloroplatinum complex, the overall reaction kinetics starting from **4** are five times slower than from **2**. This is evidence of the improved chemical stability of the 3-sulfopropionatoplatinum(II) complex over the sulfatoplatinum(II) complex. Whether these differences actually mean an improvement in the stability

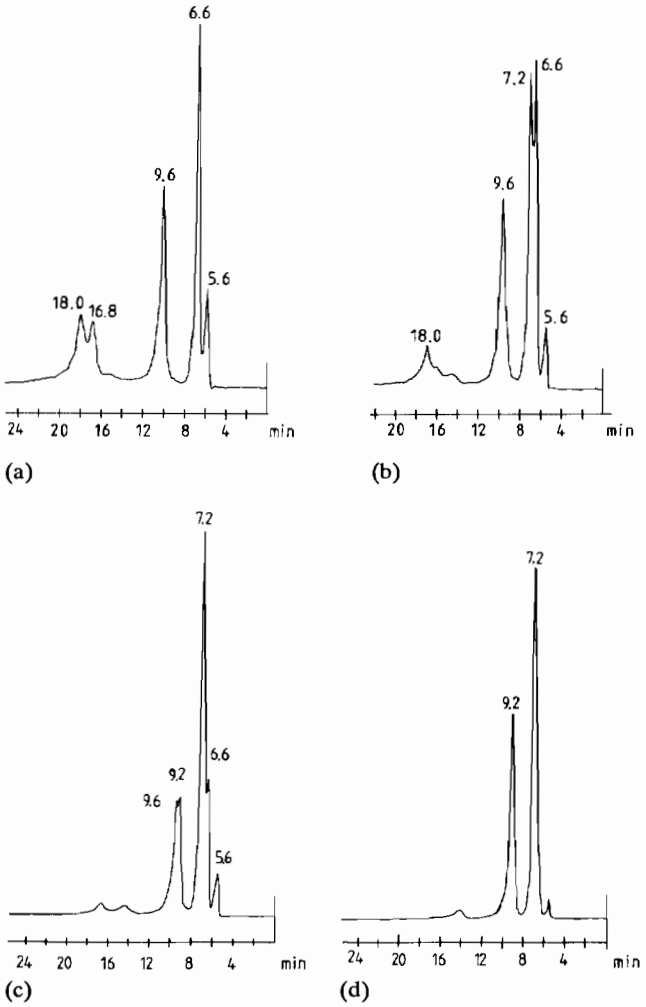


Fig. 4. The HPLC chromatograms of the 3-sulfopropionatoplatinum complex **4** were obtained under the following conditions: (a) immediately after dissolving 2 mg (3.1 μmol) in 1 ml deionized water; (b) 0.5 h following the addition of a four-fold excess of chloride to the solution in 4(a); (c) 1 additional h later; (d) 1 day later. All reactions were done at room temperature.

of the platinum complexes under biological conditions (i.e. in serum), and hence an improvement of the bioavailability of the compounds, remains to be investigated.

Cytotoxicity studies

Several dichloro(1,2-diaryl-1,2-ethylenediamine)-platinum(II) compounds show striking differences in their antitumor activity depending on the type and position of the aromatic ring substituents [31]. The configuration of the aromatic rings is also known to have an important effect on the pharmacological activity [3, 31]. When the aromatic ring is substituted with fluorine and the stereochemistry of the benzylic carbons is *d/l*-configured, potent *in vitro* cytotoxic effects were observed [3, 31, 32]. *d/l*-[1,2-Bis(2-hydroxyphenyl)-1,2-ethylenediamine]dichloroplatinum(II) also markedly inhibited tumor growth in various cisplatin-resistant tumor models, while structurally related compounds were without activity [33].

Compounds **1–4** were tested *in vitro* for cytotoxicity on the MBA-MB 231 human mammary carcinoma cell line (Table 3). From our experience, platinum(II) complexes with labile leaving groups (i.e. sulfato and nitrato) display maximal cytotoxic activity in cell culture assays. On the other hand, complexes with more tightly bound

TABLE 2. HPLC retention times (t_r) of the various aqueous platinum species from **1–4**[a]

Platinum species	Retention times (min)	
	1 and **3**	**2** and **4**
Aquachloro	6.6	6.6
Diaqua	7.2	5.6
Aquasulfato	NF	6.6
Aqua(3-sulfopropionato-O^1)	7.6	6.6
Chloro(3-sulfopropionato-O^1)	ND	7.2
Aqua(DMSO-*S*)	8.0	ND
Dichloro	8.2	9.2
3-Sulfopropionato-O^1,O^3	NF	9.6
Hydroxy-bridged dimer	13.0	12.0
Hydroxy-bridged trimer	14.9	14.6
3-Sulfopropionato-bridged dimer	16.4	16.8
3-Sulfopropionato-bridged trimer	NF	18.0

[a]See 'Experimental' for chromatographic conditions. NF: not found. ND: not determined.

TABLE 3. The *in vitro* cytotoxicity of **1–4** and cisplatin in the human mammary carcinoma cell line MDA-MB-231 determined by a microtiter assay. Cells were exposed to the platinum complexes continuously for either 1 (d_1) or 5 (d_5) days

Compound	Conc. (μM)	T/C_{corr}[a] (d_1)	T/C_{corr} (d_5)
1	0.5	73±9[b]	20±7
	1	56±18	8±1
	2	45±9	1±0
2	0.5	98±13	92±6
	1	92±11	76±11
	2	80±12	30±9
3	0.5	74±13	29±7
	1	69±12	14±9
	2	34±13	1±7
4	0.5	88±19	97±8
	1	92±19	76±8
	2	78±13	36±8
cisplatin	0.63	99±29	86±8
	1.25	93±29	68±7
	2.5	82±33	40±8

[a]$T/C_{corr} = (T-C_0)/C-C_0) \times 100$ (see 'Experimental' for abbreviations). [b]Mean ±standard error.

leaving groups (i.e. chloro and malonato) often show a diminished cytotoxic activity *in vitro*. However, these compounds show less systemic toxicity than the sulfatoplatinum complexes *in vivo* [34]. The exchange of the labile sulfato leaving group (**1** and **3**) for the more stable 3-sulfopropionato group (**2** and **4**) leads to no reduction in the *in vitro* cytotoxic activity (Table 3). Thus, the 3-sulfopropionato ligand appears to be a useful leaving group for antitumor platinum complexes. While this work was in progress, a report on the synthesis and testing of cisplatin analogues possessing various phosphonocarboxylato leaving groups appeared [35].

Conclusions

These results show that reversed-phase HPLC can complement ^{195}Pt NMR in investigating the aqueous solution compositions of platinum(II) complexes. Assuming that a sensitive and convenient detection method can be employed, HPLC would appear to be an attractive alternative to the more sophisticated NMR methods, especially when the limited water solubility of the platinum complex prevents the preparation of the concentrated aqueous solutions normally needed for NMR experiments. Another interesting aspect of the present work is the observation that the type and position of aromatic substituents in the compound class (1,2-diaryl-1,2-ethylenediamine)platinum(II) can have subtle but perhaps important influences on the nature of the platinum coordination chemistry. For example, a comparison of the composition of aqueous platinum species from **1** (*para*-fluorine aromatic substitutent) with those from **2** (*ortho*-hydroxy aromatic substituent) shows an additional species, tentatively assigned as the aqua-sulfatoplatinum(II) complex, appearing in the HPLC chromatogram of the latter but not of the former compound. It might be possible to take advantage of such intramolecular interactions to design more effective, antitumor platinum complexes. Finally, the sulfopropionato ligand has been introduced as an effective leaving group of intermediate reactivity and acceptable water solubility (**3**: ~0.5 mM; **4**: ~3 mM). The antitumor activity of **3** and of **4** is presently being investigated.

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