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Dependence of the Reduction Products of Platinum(IV) Prodrugs upon the Configuration of the Substrate, Bulk of the Carrier Ligands, and Nature of the Reducing Agent

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Supporting Information

ABSTRACT: Most evidence indicates that platinum(IV) prodrugs are rapidly reduced under physiological conditions by biologically relevant reducing agents, such as ascorbic acid and glutathione; however, the precise mechanisms of reduction are not fully understood, thus preventing rational design of compounds with better pharmacological properties. In the present study, reduction of three all-trans platinum(IV) compounds of formula $[PtCl_2(CH_3COO)_2LL']$ (LL′ = {*E*-HN═C(CH_3)OCH_3}$_2$, **1c**, (H_3N)(cyclohexylamine), **2c**, and (H_3N)(1-adamantylamine), **3c**) by two biologically relevant reductants (ascorbic acid and glutathione) and by a classical coordination chemistry reductant (triphenylphosphine) has been investigated. Reduction by triphenylphosphine and glutathione leads, in all cases examined, to loss of the two chlorides and formation of the diacetato species *trans*-$[Pt(CH_3COO)_2LL']$. This is in accord with an "inner-sphere" redox process in which a chlorido ligand bridges the reductant with the platinum(IV) center. In contrast, reduction by ascorbic acid/sodium ascorbate 1:1 leads, in addition to the diacetato complex, also to formation of a significant amount of dichlorido species, particularly in the case of **1c** (31%) and to a lesser extent of **3c** (16%). The latter results indicate that ascorbic acid is less efficient to promote an inner-sphere redox process (attack on a chlorido ligand), therefore allowing participation of an "outer-sphere" mechanism, ultimately leading to formation of the more stable dichlorido species. The dependence of the yield of diacetato species upon the steric hindrance of the carrier ligand (69%, 84%, and 95% for **1c**, **3c**, and **2c**, respectively) points to the possible participation of a second type of inner-sphere mechanism in which the interaction between the ascorbate and a chlorido ligand of the platinum(IV) substrate is mediated by a platinum(II) catalyst, the transition state resembling that of a platinum(II)-catalyzed ligand substitution at a platinum(IV) center. This investigation demonstrates that different species can be obtained by reduction of a platinum(IV) prodrug (depending upon the configuration of the substrate and the nature of the intervening reducing agent) and can explain some lack of correlation between prodrug and putative active species as well as contrasting literature results.

INTRODUCTION

Although the search for new antitumoral drugs has concerned mainly platinum(II) complexes, more recently attention has been directed also toward platinum(IV) analogs for which the two additional coordination sites may provide some advantages, such as (a) a lower reactivity toward biological substrates, due to their higher kinetic inertness which can prevent the incidence of side reactions, (b) a greater cellular uptake, due to higher lipophilicity stemming from the additional ligands arranged around the metal center,[1,2] and (c) the possibility to link pharmacological active molecules in the axial positions in order to obtain a synergistic effect.[3,4]

Significantly, the prototype platinum(IV) complex Satraplatin (JM216), *cis*,*trans*,*cis*-$[PtCl_2(CH_3COO)_2(NH_3)$(cyclohexylamine)], entered phase III clinical trials in 2001 as an orally active anticancer drug for treatment of hormone-refractory prostate cancer, and it remains under intensive clinical evaluation.[5,6]

Most evidence to date indicates that platinum(IV) complexes are rapidly reduced under physiological conditions by biologically relevant reducing agents, such as glutathione and ascorbic acid, to release two axial ligands and yield the cytotoxic platinum(II) species.[2] However, the correlation between structure and rate of reduction and the precise mechanisms of reduction are not fully understood, thus preventing a rational design of new compounds with better pharmacokinetic properties.[2]

trans-$[Pt(CN)_4X_2]^{2-}$ (X = Cl^- or Br^-) was used by Elding as a stable model for reduction of platinum(IV) anticancer drugs by L-methionine and L-cysteine.[10,11] Since platinum(IV) complexes adopt a substitution-inert octahedral geometry, direct coordination of the reductant to the platinum(IV) substrate was considered highly unlikely. Therefore, the proposed mechanism involved association of the reductant to

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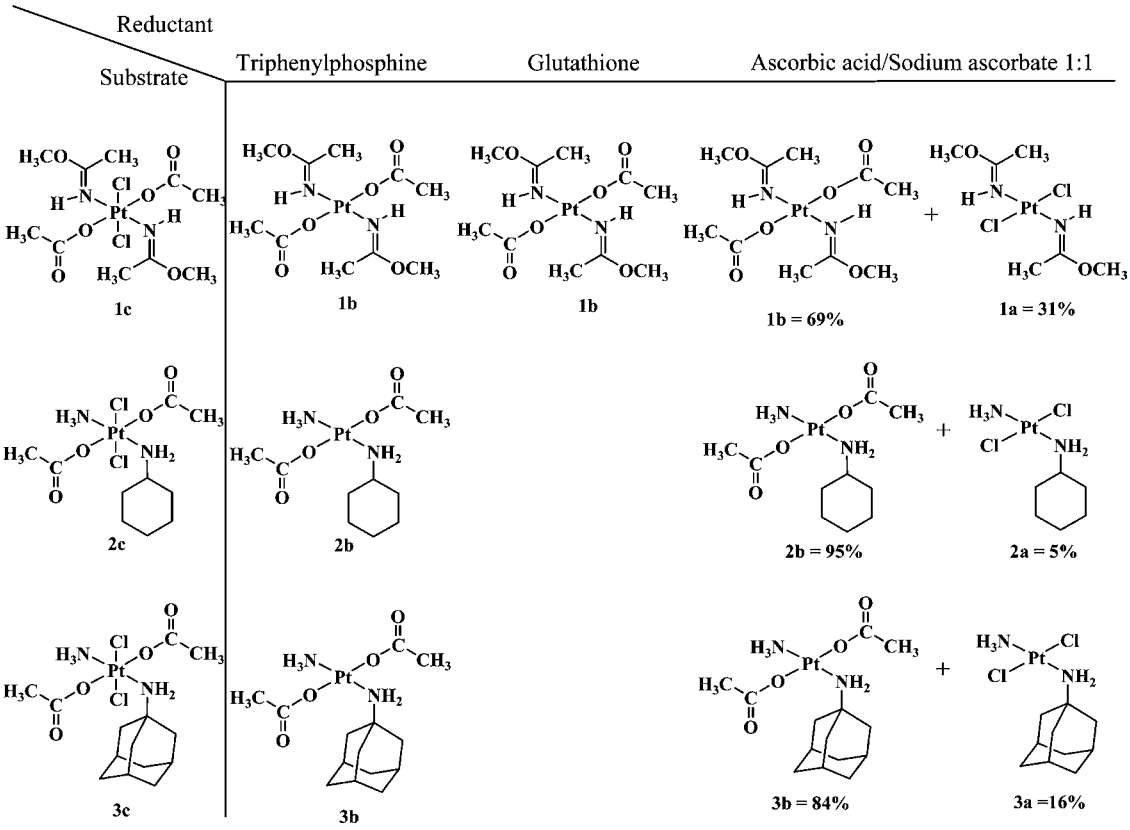
Chart 1



the platinum(IV) substrate through a halido ligand in a transition state and subsequent electron transfer.

Reduction by S-containing biomolecules of *cis,trans,cis*-$[PtCl_2(CH_3COO)_2(NH_3)_2]$, as a more soluble model of Satraplatin, was investigated by Randford.[9] For the latter complex, the rate constants were orders of magnitude lower than for *trans*-$[Pt(CN)_4Cl_2]^{2-}$, consistent with the observation that the cathodic potential for *cis,trans,cis*-$[PtCl_2(CH_3COO)_2(NH_3)_2]$ (−698 mV) is more negative than that for *trans*-$[Pt(CN)_4Cl_2]^{2-}$ (−399 mV). In principle, the S-containing reductant could attack one of the cis chlorides of *cis,trans,cis*-$[PtCl_2(CH_3COO)_2(NH_3)_2]$; however, the chloride-bridged pathway was considered energetically unfavorable, since the chlorides are coordinated trans to ammines, which are firmly bound to the metal center and dissociate with difficulty in the reduction step.[7] Thus, by analogy with halide-mediated reductive elimination reactions of platinum(IV) halogen complexes,[12,13] the reduction was suggested to take place via an attack of the S-containing reductant upon the coordinated oxygen of an acetato ligand, followed by electron transfer through the oxygen bridge. The different nature of the bridge (O instead of Cl) could account for the different reactivity. For both L-cysteine and L-methionine the rate of reduction depends also upon the state of protonation at the carboxylic and aminic groups, the less protonated species being the most reactive.[9] Moreover, for L-cysteine the second-order rate constant (k_{obs}/[reductant]) was ca. 2000 times bigger than for L-methionine, and this could be a consequence of the greater basicity of the thiol as compared to the thioether.[11] Given the typical concentrations of divalent sulfur compounds present in blood plasma and cells,[14,15] it was predicted that the predominant reductant would be glutathione.

Reduction of the oral anticancer platinum(IV) drug Satraplatin (JM216) by ascorbic acid was investigated by Elding.[7] Satraplatin is reduced rather slowly to *cis*-$[PtCl_2(NH_3)(cyclohexylamine)]$ by a second-order rate law (first order in each of the two reactants) and was suggested to take place via an "outer-sphere" mechanism (absence of direct coordination or ligand bridge between the platinum(IV) center and the reducing agent). In contrast, the Satraplatin isomers *trans,cis,cis*-$[PtCl_2(CH_3COO)_2(NH_3)(cyclohexylamine)]$ (JM394) and *trans,trans,trans*-$[PtCl_2(CH_3COO)_2(NH_3)$-$(cyclohexylamine)]$ (JM576), both containing two trans chlorides, were reduced 3 orders of magnitude faster by ascorbic acid and lead to the diacetato rather than to the dichlorido platinum(II) species. An "inner-sphere" mechanism involving a reductive attack on one of the mutually trans chlorides by fully deprotonated ascorbic acid and, less efficiently, by monodeprotonated ascorbic acid, leading to formation of a chlorido-bridged activated complex, was proposed.

In summary, current data indicate that reduction by thiols and thioethers takes place always by attack of sulfur on a coordinated ligand (inner-sphere mechanism) and is much faster in the case of trans halides than in the case of trans carboxylates. In contrast, reduction by ascorbic acid takes place by direct attack on a coordinated ligand only in the case of trans halides, while in the case of trans carboxylates the reduction, much slower, is considered to take place by an outer-sphere mechanism.

The aim of the present study was to prepare a JM576 analog by adding two axial acetato groups to the iminoether complex *trans*-$[PtCl_2\{E\text{-}HN{=}C(CH_3)OCH_3\}_2]$ (**1a**), the first trans-geometry platinum(II) complex for which significant in vivo

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antitumor activity was established against leukemic and solid murine tumors,[16−19] and to investigate its redox behavior.

Reduction of *trans,trans,trans*-[$PtCl_2(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1c**) by two biologically relevant reductants (ascorbic acid and glutathione) and by a classical coordination-chemistry reductant (triphenylphosphine) was considered. In accord with literature data on analogous compounds containing two trans chlorides (as summarized in the previous paragraphs), we expected to observe, in all cases, loss of the two chlorides and formation of *trans*-[$Pt(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1b**) and hence a species different from the precursor complex **1a**, which would make the platinum(IV) complex *trans,trans,trans*-[$PtCl_2(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1c**) not a prodrug for **1a**. However, the diacetato platinum(II) species (**1b**) was the exclusive reduction product only in the case of reduction by glutathione and triphenylphosphine, while in the case of reduction by ascorbic acid, depending upon the acidity of the solution, a relevant amount of dichlorido species **1a** was also formed, indicative of participation of an "outer sphere" mechanism.

Moreover, by extending the investigation to the Satraplatin isomer *trans,trans,trans*-[$PtCl_2(CH_3COO)_2(NH_3)$(cyclohexylamine)] (JM576, **2c**) and to the analogous complex *trans,trans,trans*-[$PtCl_2(CH_3COO)_2(NH_3)$(1-adamantylamine)] (**3c**), it was found that, in the reduction by ascorbic acid/sodium ascorbate 1:1, the yield of the diacetato species (resulting from an inner-sphere mechanism) was inversely dependent upon the size of the carrier ligands (results summarized in Chart 1).

EXPERIMENTAL SECTION

Starting Materials. Commercial reagent-grade chemicals were used without further purification. *trans*-[$PtCl_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1a**),[20] *trans,trans,trans*-[$PtCl_2(CH_3COO)_2(NH_3)$(cyclohexylamine)] (**2c**),[21,22] and *trans,trans,trans*-[$PtCl_2(CH_3COO)_2(NH_3)$(1-adamantylamine)] (**3c**)[23] were prepared according to already reported procedures. Elemental analysis and spectroscopic features were fully consistent with the data reported in the literature.

Physical Measurements. NMR spectra were collected at 295 K on a Bruker AVANCE DPX 300 MHz instrument. ^{1}H chemical shifts were referenced to TMS using the residual protic peak of the solvent (D_2O, CD_3OD, $CDCl_3$) as internal reference. One-dimensional ^{195}Pt spectra were acquired using ^{1}H decoupling sequences. ^{195}Pt chemical shifts were referenced to K_2PtCl_4 (1 M in water, δ = −1614 ppm). Elemental analyses were carried out on a CHN Eurovector EA 3011 equipment. ESI-MS analyses were performed on a Agilent 1100 series LC-MSD Trap system VL.

Synthesis. *Preparation of trans-[Pt(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1b**).* A suspension of *trans*-[$PtCl_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1a**) (0.105 g, 0.25 mmol) in water was treated with a 2-fold excess of $AgNO_3$ (0.086 g, 0.51 mmol) previously solubilized in water (1 mL). The reaction mixture was kept under stirring at 25 °C for 17 h in the dark. During this time a white precipitate (AgCl) formed that was separated by filtration of the solution through Celite. The pale yellow filtrate was heated to 40 °C and treated with a 2-fold excess of $K(CH_3COO)$ (0.056 g, 0.57 mmol). The obtained yellow solution was stirred at 40 °C for 2 days; meanwhile a pale pink solid precipitated. The reaction mixture was filtered, and the mother liquor was taken to dryness by evaporation of the solvent under reduced pressure. Solid residue was extracted with acetone in order to separate KNO_3 and unreacted $K(CH_3COO)$. The soluble fraction in acetone was taken to dryness by evaporation of the solvent under reduced pressure, and the sticky residue was washed with diethyl ether and dried under vacuum. We obtained 0.070 g (0.15 mmol, 60% yield) of *trans*-[$Pt(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1b**). ESI-MS: calcd for $[C_{10}H_{20}N_2O_6PtNa]^+$ 482.34. Found: *m*/*z* (% relative to the base peak) 481.9(100), $[M + Na]^+$. ^{1}H NMR (D_2O) δ: 1.81 (6H, s), 2.39 (6H, s), 3.66 (6H, s) ppm. ^{195}Pt NMR (D_2O) δ: −1397 ppm.

*Preparation of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**).* *trans*-[$Pt(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1b**) (0.100 g, 0.218 mmol) was dissolved in CH_2Cl_2 (15 mL) and treated with ca. 4 mL of a saturated solution obtained by bubbling $Cl_{2(g)}$ in CCl_4. The obtained suspension was kept under stirring at 25 °C for 5 days; meanwhile a yellow precipitate formed that was removed by filtration of the mother liquor. The yellow filtrate was taken to dryness by evaporation of the solvent under reduced pressure and analyzed by TLC using dichloromethane/acetone 9:1 as eluant. TLC showed two spots corresponding (in order of increasing retention time) to *trans*-[$PtCl_4\{E$-HN=$C(CH_3)OCH_3\}_2$] and *trans*-[$PtCl_2(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1c**) in addition to some noneluting side products. The desired complex was obtained by chromatography on silica gel of the raw material using dichloromethane/acetone 9:1 as eluant. We obtained 0.06 g (0.108 mmol, 49.4% yield) of *trans*-[$PtCl_2(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1c**). Anal. Calcd for *trans,trans,trans*-[$PtCl_2(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] ($C_{10}H_{20}N_2O_6Cl_2Pt$): C, 22.64; H, 3.77; N, 5.28. Found: C, 22.45; H, 3.74; N, 5.11. ESI-MS: calcd for $[C_{10}H_{20}N_2O_6Cl_2PtNa]^+$ 553.25. Found: *m*/*z* (% relative to the base peak) 552.8(100), $[M + Na]^+$. ^{1}H NMR ($CDCl_3$) δ: 2.07 (6H, s), 2.46 (6H, s), 3.95 (6H, s), 11.49 (2H, b) ppm. ^{195}Pt NMR ($CDCl_3$) δ: 1376 ppm.

Reduction Experiments. *Reduction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) by Triphenylphosphine.* *trans,trans,trans*-[$PtCl_2(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1c**) (2.0 mg, 3.77 μmol) was dissolved in a mixture of CD_3OD/D_2O 7.5:1 (750 μL of CD_3OD and 100 μL of D_2O), and the resulting one-phase solution was placed into a NMR tube. A solution of triphenylphosphine (1.0 mg, 3.77 μmol) in $CDCl_3$ (300 μL) was added into the NMR tube, and the resulting solution was monitored by recording ^{1}H NMR spectra at different time intervals while the sample was kept at 22 °C. The reduced platinum species, *trans*-[$Pt(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1b**), was identified by characteristic NMR resonance peaks and ESI-MS (*m*/*z* = 481.9, [**1b** + Na]$^+$).

*Reduction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) by Glutathione.* *trans,trans,trans*-[$PtCl_2(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1c**) (2.2 mg, 4.15 μmol) was dissolved in a mixture of $CD_3OD/CDCl_3$ 2.5:1 (750 μL of CD_3OD and 300 μL of $CDCl_3$) and placed into a NMR tube. A solution of a 2-fold excess of glutathione (2.6 mg, 8.3 μmol) in D_2O (100 μL) was added into the NMR tube, and the resulting solution was monitored by recording ^{1}H NMR spectra at different time intervals while the sample was kept at 22 °C. Also, in this case the reduction product, *trans*-[$Pt(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1b**), was identified by NMR and ESI-MS (*m*/*z* = 481.9, [**1b** + Na]$^+$).

*Reduction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) by Ascorbic Acid in Acidic Solution.* *trans,trans,trans*-[$PtCl_2(CH_3COO)_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1c**) (2.0 mg, 3.77 μmol) was dissolved in a mixture of $CD_3OD/CDCl_3$ 2.5:1 (750 μL of CD_3OD and 300 μL of $CDCl_3$) and placed into a NMR tube. A 3-fold excess of ascorbic acid (1.9 mg, 11.3 μmol) in D_2O (100 μL) was added into the NMR tube, and the resulting solution was monitored by recording ^{1}H NMR spectra at different time intervals while the sample was kept at 22 °C. The reduced platinum species, *trans*-[$PtCl_2\{E$-HN=$C(CH_3)OCH_3\}_2$] (**1a**), was identified by characteristic NMR resonances and ESI-MS (*m*/*z* = 434.8, [**1a** + Na]$^+$).

*Reduction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) by Ascorbic Acid/Sodium Ascorbate 1:1 Buffered Solution.* Since the reducing ability of ascorbic acid has been shown to depend upon its protonation state, the same experiment was performed using a 5-fold excess of ascorbic acid and sodium ascorbate. Therefore, **1c** (1.97 mg, 3.71 μmol) was dissolved in a mixture of $CD_3OD/CDCl_3$ 2.5:1 (750 μL of CD_3OD and 300 μL of $CDCl_3$) and placed into a NMR tube. A 5-fold excess of ascorbic acid (3.27 mg, 18.5 μmol) and sodium ascorbate (3.68 mg, 18.5 μmol) in D_2O (100 μL) was added into the NMR tube, and the resulting solution was

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monitored by recording ^{1}H NMR spectra at different time intervals while the sample was kept at 22 °C. The reduced platinum species, *trans*-[Pt(CH$_3$COO)$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (**1b**) and *trans*-[PtCl$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (**1a**), were identified by characteristic NMR resonances and ESI-MS (m/z = 481.9, [**1b** + Na]$^+$; 434.8, [**1a** + Na]$^+$).

*Reduction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2c**) by Triphenylphosphine.* *trans,trans,trans*-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2c**) (2.2 mg, 4.38 μmol) was solubilized in CD$_3$OD/D$_2$O 7.5:1 (750 μL of CD$_3$OD and 100 μL of D$_2$O) and placed into an NMR tube. A solution of triphenylphosphine (1.2 mg, 4.38 μmol) in CDCl$_3$ (300 μL) was added into the NMR tube, and the resulting solution was monitored by recording ^{1}H NMR spectra at different time intervals while the sample was kept at 22 °C. The reduced platinum species, *trans*-[Pt(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2b**), was identified by characteristic NMR resonance peaks and ESI-MS (m/z = 457.1, [**2b** + Na]$^+$).

*Reduction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2c**) by Ascorbic Acid/Sodium Ascorbate 1:1 Buffered Solution.* *trans,trans,trans*-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2c**) (2.25 mg, 4.52 μmol) was solubilized in CD$_3$OD/CDCl$_3$ 2.5:1 (750 μL of CD$_3$OD and 300 μL of CDCl$_3$) and placed into an NMR tube. A solution of a 5-fold excess of ascorbic acid (3.98 mg, 22.6 μmol) and sodium ascorbate (4.48 mg, 22.6 μmol) in D$_2$O (100 μL) was added into the NMR tube, and the resulting solution was monitored by recording ^{1}H NMR spectra at different time intervals while the sample was kept at 22 °C. The reduced platinum species, *trans*-[Pt(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2b**) and *trans*-[PtCl$_2$(NH$_3$)(cyclohexylamine)] (**2a**), were identified by characteristic NMR resonance peaks and ESI-MS (m/z = 457.1, [**2b** + Na]$^+$; 409.0, [**2a** + Na]$^+$).

*Reduction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(1-adamantylamine)] (**3c**) by Triphenylphosphine.* *trans,trans,trans*-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(1-adamantylamine)] (**3c**) (2.2 mg, 3.98 μmol) was dissolved in a mixture of CD$_3$OD/D$_2$O 7.5:1 (750 μL of CD$_3$OD and 100 μL of D$_2$O) and placed into a NMR tube. A solution of triphenylphosphine (1.04 mg, 3.98 μmol) in CDCl$_3$ (300 μL) was added into the NMR tube, and the resulting solution was monitored by recording ^{1}H NMR spectra at different time intervals while the sample was kept at 22 °C. The reduced platinum species, *trans*-[Pt(CH$_3$COO)$_2$(NH$_3$)(1-adamantylamine)] (**3b**), was identified by characteristic NMR resonances and ESI-MS (m/z = 508.0, [**3b** + Na]$^+$).

*Reduction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(1-adamantylamine)] (**3c**) by Ascorbic Acid/Sodium Ascorbate 1:1 Buffered Solution.* *trans,trans,trans*-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(1-adamantylamine)] (**3c**) (2.47 mg, 4.47 μmol) was dissolved in a mixture of CD$_3$OD/CDCl$_3$ 2.5:1 (750 μL of CD$_3$OD and 300 μL of CDCl$_3$) and placed into a NMR tube. A solution of a 5-fold excess of ascorbic acid (3.94 mg, 22.3 μmol) and sodium ascorbate (4.42 mg, 22.3 μmol) in D$_2$O (100 μL) was added into the NMR tube, and the resulting solution was monitored by recording ^{1}H NMR spectra at different time intervals while the sample was kept at 22 °C. The reduced platinum species, *trans*-[Pt(CH$_3$COO)$_2$(NH$_3$)(1-adamantylamine)] (**3b**) and *trans*-[PtCl$_2$(NH$_3$)(1-adamantylamine)] (**3a**), were identified by characteristic NMR resonances and ESI-MS (m/z = 508.0, [**3b** + Na]$^+$; m/z = 431.7, [**3a** − H]$^-$).

RESULTS

Synthesis and Characterization of *trans*-[Pt(CH$_3$COO)$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (1b) and *trans,trans,trans*-[PtCl$_2$(CH$_3$COO)$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (1c). The standard procedure for synthesizing dichlorido−diacetato−diamine−Pt(IV) complexes contemplates the initial oxidation of the dicholorido−diamine−Pt(II) complex to the corresponding dihydroxido platinum(IV) species by addition of hydrogen peroxide to an aqueous solution of the platinum(II) species. Subsequent acetylation of the hydroxido ligands, by reaction with acetic anhydride, leads to the desired product.[24] This procedure, however, cannot be applied to synthesis of complex **1c**, since oxidation of *trans*-[PtCl$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (**1a**) by hydrogen peroxide causes also hydrolysis of the iminoether ligands to the corresponding amide. To overcome this problem a different synthetic procedure was used.

In a first step, the *trans*-[PtCl$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (**1a**) complex was converted to the *trans*-[Pt(H$_2$O)$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$]$^{2+}$ species by reaction with AgNO$_3$. Addition of a slight excess of K(CH$_3$COO) to the formed aqua species at 40 °C produces the platinum(II) diacetato species *trans*-[Pt(CH$_3$COO)$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (**1b**). ESI-MS data and NMR spectra of the obtained compound were in accord with the proposed formulation. The ^{1}H NMR spectrum of **1b** (Figure S1, Supporting Information), recorded in D$_2$O, consists of three singlets. The signal at 1.81 ppm is assigned to the acetate methyl protons, while the signals at 2.39 and 3.66 ppm are assigned to the C−Me and O−Me protons of the iminoether ligands, respectively. The ^{195}Pt NMR spectrum in D$_2$O (Figure S1, Supporting Information) gives a signal at −1397 ppm; this chemical shift is consistent with platinum(II) having a N$_2$O$_2$ set of donor atoms.[25] The ESI-MS spectrum of **1b** dissolved in CH$_3$OH shows the parent peak at 481.9 m/z corresponding to [M + Na]$^+$. The fragmentation spectrum (MS/MS) of the parent peak exhibits two signals at 408.9 and 348.9 m/z, corresponding to the species [M − HN=C(CH$_3$)OCH$_3$ + Na]$^+$ and [M − HN=C(CH$_3$)OCH$_3$ − CH$_3$COOH + Na]$^+$.

In a second step, oxidation of **1b** by chlorine leads to formation of the desired *trans,trans,trans*-[PtCl$_2$(CH$_3$COO)$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) complex together with *trans*-[PtCl$_4${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] as a side product. The two products can be separated by silica gel chromatography using dichloromethane/acetone 9:1 as eluant. Elemental analysis and spectroscopic features are in agreement with the given formulation. The ^{1}H NMR spectrum (Figure S2, Supporting Information) (CDCl$_3$ solvent) shows the presence of three singlets, of similar intensity, falling at 2.07, 2.46, and 3.95 ppm, which can be assigned to the acetato ligands and to the C−Me and O−Me protons of the iminoether ligands, respectively. Moreover, the spectrum shows a broad signal falling at 11.49 ppm and assigned to the iminic proton of the iminoether ligands. The strong downfield shift ($\Delta\delta$ = 4.34 ppm) of the iminic protons in the platinum(IV) species is noteworthy, as compared to the platinum(II) dichlorido species **1a**. A hydrogen-bond-type interaction between this iminic proton and the carbonyl oxygen of a cis acetato ligand can explain such a remarkable downfield shift. The ^{195}Pt spectrum (Figure S2, Supporting Information) consists of a signal at 1376 ppm. The chemical shift is in the range typical for a platinum(IV) in a N$_2$O$_2$Cl$_2$ coordination environment.[26,27] For full characterization of complex **1c**, an ESI-MS spectrum was also recorded. The parent peak is present in the positive ions current at 552.8 m/z and belongs to the species [M + Na]$^+$. The fragmentation spectrum of the parent peak consists of two major signals falling at 479.8 and 419.9 m/z and corresponding to species [M − HN=C(CH$_3$)OCH$_3$ + Na]$^+$ and [M − HN=C(CH$_3$)OCH$_3$ − CH$_3$COOH + Na]$^+$.

Reduction Reactions. Reduction of *trans,trans,trans*-[PtCl$_2$(CH$_3$COO)$_2${*E*-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) by three different reducing agents (triphenylphosphine, glutathione, and ascorbic acid) has been monitored by NMR spectroscopy and ESI-MS. Triphenylphosphine is a classical two-electron

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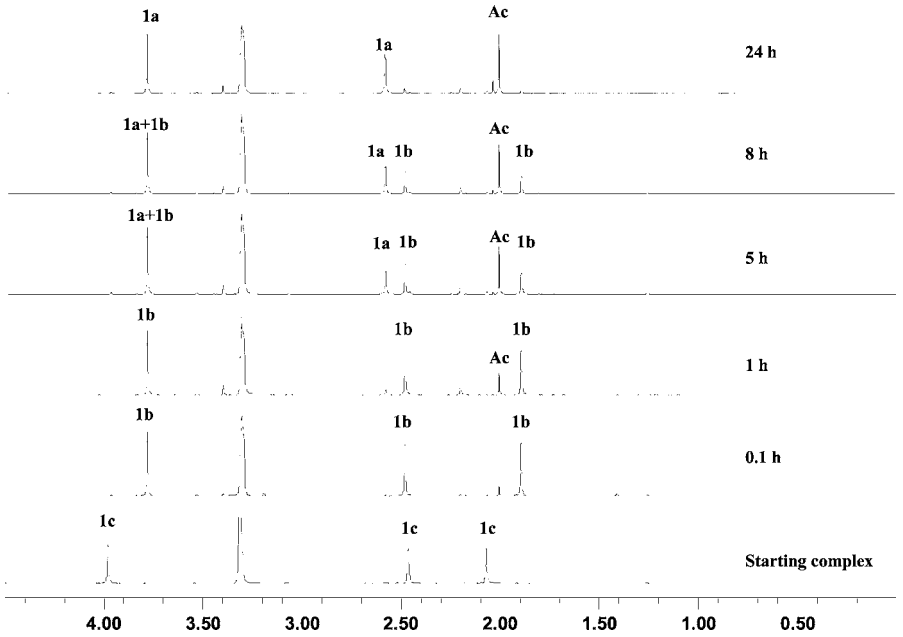


Figure 1. ^{1}H NMR spectra, at different time intervals, of complex **1c** treated with triphenylphosphine. Ac stands for free acetate.

reducing agent widely used in coordination and organometallic chemistry, while glutathione (a monoelectron reductant) and ascorbic acid (a two-electron reductant) are considered to be the major intra- and extracellular bioreductants, respectively.[2] In all cases a mixture of deuterated methanol/chloroform/water 7.5:3:1 was employed in order to favor complete dissolution of the reactants and reaction products.

*Reaction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) with Triphenylphosphine.* Reaction with triphenylphosphine was performed using a 1:1 Pt^{IV}/triphenylphosphine molar ratio. In this case reduction was instantaneous and produced the acetato species *trans*-[Pt(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1b**). However, with time, the acetato species **1b** was converted to the chlorido species **1a**. Such substitution reaction was complete in ca. 24 h.

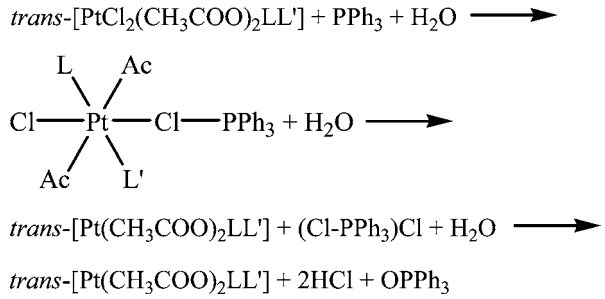
The ^{1}H NMR spectrum (Figure 1), recorded soon after mixing of the reactants, shows the near disappearance of the three singlets at 2.07, 2.46, and 3.97 ppm belonging to complex **1c** and the concomitant appearance of new signals at 1.90, 2.48, and 3.79 ppm, assigned to the coordinated acetate and iminoether C−Me and O−Me protons of **1b**, respectively. Subsequently, the signals at 1.90 and 2.48 ppm decreased, while new signals at 2.00 (free acetate released in the solution) and 2.58 ppm (iminoether C−Me protons belonging to **1a**) increased. The peak at 3.79 ppm remained constant since it belongs to the O−Me protons of both **1a** and **1b**.

In addition, ESI-MS provides evidence for formation of the diacetato species in the first step (peak falling at m/z 481.9 and corresponding to [**1b** + Na]$^+$, which, in the subsequent fragmentation, loses HN=C(CH$_3$)OCH$_3$ and CH$_3$COOH giving peaks at m/z 408.9 and 348.9, respectively).

Conversion of **1b** into **1a** is promoted by release, in the redox reaction (Scheme 1), of HCl, which provides a good ligand for platinum (Cl$^-$) and acidic conditions favoring protonation of the acetate.

Although investigation of the detailed reaction mechanism was beyond the scope of this work, formation of the acetato species **1b**, in the first step of the reaction, is in accord with an inner-sphere redox mechanism that involves attack by the

Scheme 1. Reduction of All-Trans Platinum(IV) Complexes by Triphenylphosphine[a]



[a]L and L′ stand for N-donor ligands, PPh$_3$ stands for triphenylphosphine, and Ac stands for CH$_3$COO$^-$.

phosphine on one coordinated chloride leading to formation of a chlorido-bridged activated complex. A chloride bound to the highly oxidizing platinum(IV) center can have appreciable Cl$^+$ character and therefore be susceptible to attack by the phosphine base. It follows a concerted two-electron transfer from the phosphine to the platinum(IV) center, leading to a platinum(II) complex and phosphonium chloride, (Ph$_3$PCl)Cl. The phosphonium chloride then undergoes hydrolysis, forming phosphine oxide and hydrochloric acid. This inner-sphere mechanism is well known for reduction of platinum(IV) complexes with several reductants.[8,10,11,28]

*Reaction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) with Glutathione (RSH).* Reduction of complex **1c** by glutathione was carried out using a 1:2 molar ratio (Pt^{IV}/RSH). ^{1}H NMR spectra (Figure 2), taken at different time intervals, show a decrease of the resonances belonging to glutathione (HOOC−CH$_2^B$−NH−CO−CH^A(CH^{D}H^E−SH)−NH−CO−CH$_2^F$−CH^{G}H^H−CH^C(NH$_2$)−COOH: peaks at 2.15, 2.55, 2.88, 3.75, 3.92, and 4.54 ppm assigned to H^{G,H}, H^F, H^{D,E}, H^C, H^B, and H^A, respectively) and to the platinum(IV) complex (2.07, 2.46, and 3.97 ppm) and an increase of a new set of signals belonging to the oxidized glutathione (peaks at 2.20, 2.58, 2.95, 3.22, 3.92,

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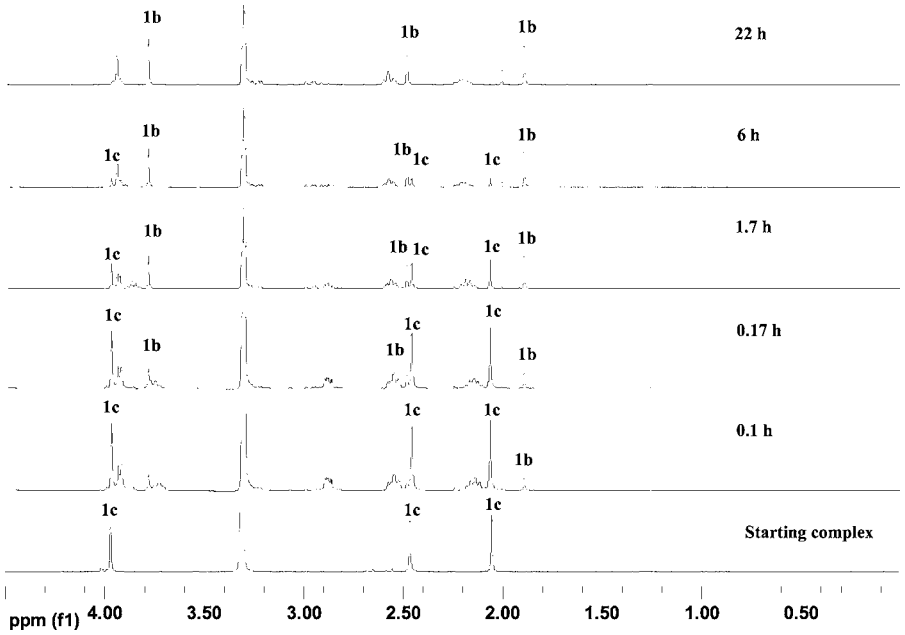


Figure 2. ^{1}H NMR spectra, at different time intervals, of complex **1c** treated with glutathione.

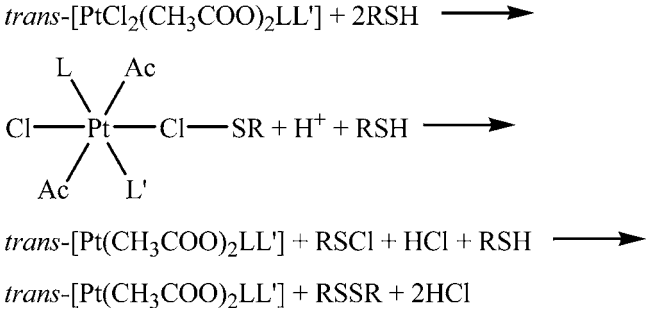
3.94, and 4.75 ppm assigned to $H^{G,H}$, H^F, H^D, H^E, H^C, H^B, and H^A, respectively) and three signals falling at 1.90, 2.48, and 3.79 ppm belonging to the coordinated acetates and the C−Me and O−Me groups of the coordinated iminoethers in *trans*-$[Pt(CH_3COO)_2\{E\text{-}HN{=}C(CH_3)OCH_3\}_2]$ (**1b**). Reduction of the platinum(IV) complex was complete in ca. 1 day.

The identity of the reduction product was confirmed by ESI-MS. In the positive ions current the parent peak at 481.9 *m*/*z*, corresponding to the cation $[\mathbf{1b} + Na]^+$, generates a peak at 408.9 *m*/*z* by loss of a neutral moiety of 73 Da, corresponding to $HN{=}C(CH_3)OCH_3$. In turn, the latter peak, by loss of a fragment of 60 Da corresponding to CH_3COOH, generates the peak at 348.9 *m*/*z*. Similar to the previous case of reduction by triphenylphosphine, reduction of complex **1c** by glutathione could involve a chloride-mediated two-electron transfer from the reductant to platinum. The mechanism would be entirely similar to that proposed by Elding for reduction of the model compound *trans*-$[Pt(CN)_4Cl_2]^{2-}$ by L-cysteine or L-methionine.[10,11] The reaction would lead initially to the platinum(II) complex, a sulfenyl chloride, and hydrochloric acid. The sulfenyl chloride would then rapidly react with another molecule of glutathione, affording glutathione disulfide and a second molecule of hydrochloric acid (Scheme 2). Therefore, at the end of the reaction, each glutathione has contributed one electron to the platinum(IV) substrate.

For sake of completeness, the proposal made by Bose and co-workers for reduction of *cis,trans,cis*-$[PtCl_2(OH)_2(isopropylamine)_2]$ (iproplatin) by glutathione should also be mentioned. According to this proposal, each glutathione molecule contributes one electron and is oxidized to a glutathiyl radical while a transient platinum(III) species is formed. It should be mentioned, however, that in the case of iproplatin there are axial hydroxido ligands and not axial chlorides as in our case.[28]

*Reaction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2${E-HN=C(CH$_3$)OCH$_3$}$_2$] (**1c**) with Ascorbic Acid.* Initially, reduction of complex **1c** by ascorbic acid was carried out using a 3-fold excess of reductant. In 3 h, loss of the acetato ligands and complete conversion of complex **1c** into the dichlorido derivative *trans*-$[PtCl_2\{E\text{-}HN{=}C(CH_3)OCH_3\}_2]$ (**1a**) was observed.

Scheme 2. Reduction of All-Trans Platinum(IV) complexes by Glutathione[a]



[a] L and L′ stand for N-donor ligands, RSH stands for glutathione, and Ac stands for CH_3COO^-.

A time-course series of ^{1}H NMR spectra (Figure 3) showed a decrease of the resonances belonging to the platinum(IV) complex (singlets falling at 2.07, 2.46, and 3.97 ppm) and to the ascorbic acid ($\overline{O{-}C(=O){-}C(OH){=}C(OH){-}}CH^D{-}CH^B(OH){-}CH^FH^G{-}OH$: signals at 3.70, 3.9 and 4.66 ppm assigned to $H^{F,G}$, H^B, and H^D, respectively) with concomitant increase of three peaks falling at 2.00, 2.58, and 3.79 ppm belonging to free acetate and to the iminoether C−Me and O−Me protons of **1a**, respectively, and four signals between 4 and 4.6 ppm assigned to dehydroascorbic acid.

Formation of the dichlorido species **1a** was also confirmed by ESI-MS taken in the reaction course. The parent peak at 434.8 *m*/*z* corresponds to the species $[\mathbf{1a} + Na]^+$, and the fragmentation spectrum (MS/MS) of the parent peak consists of two major signals falling at 399 and 326 *m*/*z*, corresponding to species $[\mathbf{1a} - HCl + Na]^+$ and $[\mathbf{1a} - HCl - HN{=}C(CH_3)OCH_3 + Na]^+$, respectively. It is to be noted that the intensity of the peak of the diacetato species, if present, is generally much more intense than that of the dichlorido species.

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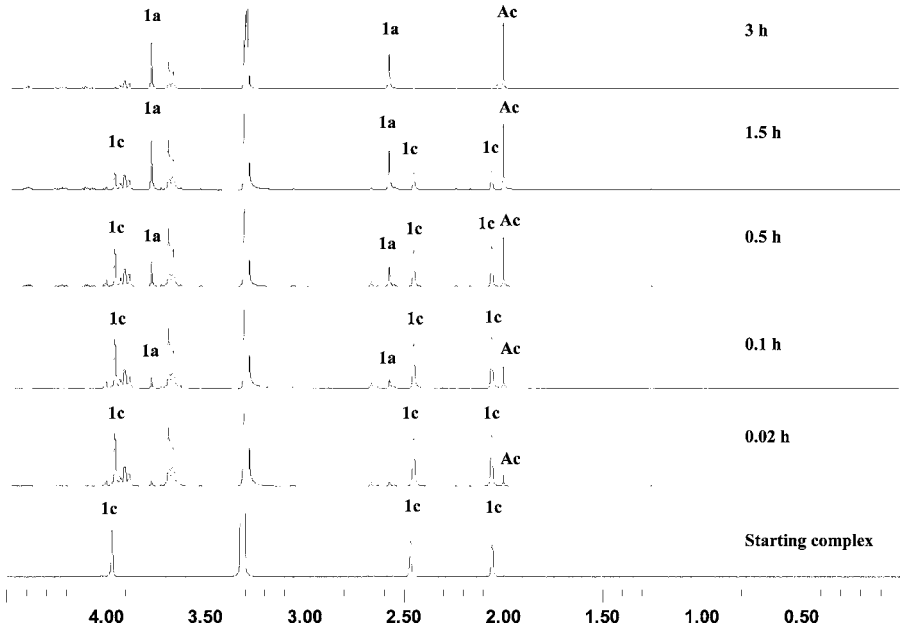
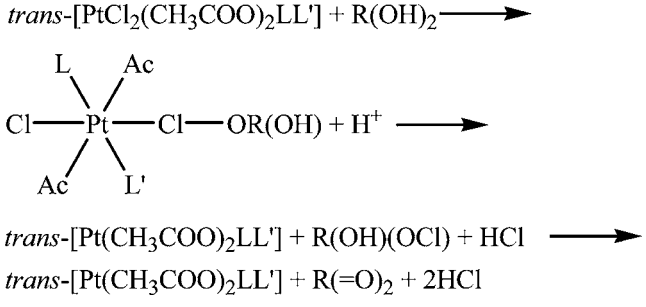


Figure 3. ^{1}H NMR spectra, at different time intervals, of complex **1c** treated with ascorbic acid. Ac stands for free acetate.

At first, this result could appear rather unusual since the analogous compound *trans,trans,trans*-[$PtCl_2(CH_3COO)_2(NH_3)$(cyclohexylamine)] (JM576, compound **2c** in the present investigation), an isomer of Satraplatin (JM216), was reported to be reduced to the acetato species *trans*-[$Pt(CH_3COO)_2(NH_3)$(cyclohexylamine)] and not to the chlorido species by reaction with ascorbic acid.[7] The mechanism was proposed to be inner-sphere and to contemplate nucleophilic attack of ascorbate on one of the trans chlorides followed by concerted two-electron transfer from the ascorbate to the platinum(IV) center. The reaction products would be a platinum(II) diacetato species, an ascorbyl ipochlorite, and hydrochloric acid. The ascorbyl ipochlorite would then undergo an internal redox process with formation of dehydroascorbic acid and a second molecule of hydrochloric acid (Scheme 3).

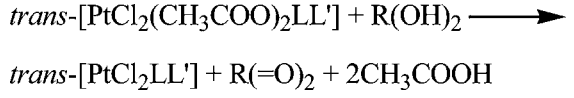
However, the reason for the different reduced product obtained in our investigation on compound **1c** (dichlorido species) and in that reported by Elding for compound JM576 (diacetato species)[7] could have been the different reaction conditions: strongly acidic conditions in our case, buffered solution in the case of ref 7. It was already pointed out in ref 7 that the reducing ability of ascorbic acid was strongly dependent upon the degree of protonation. Fully protonated ascorbic acid was considered unable to carry on the reduction by an inner-sphere mechanism; therefore, under conditions of an unfavorable chlorido-bridged inner-sphere pathway, an outer-sphere mechanism could intervene (Scheme 4).[7]

Scheme 3. Reduction of All-Trans Platinum(IV) Complexes by Ascorbic Acid According to an Inner-Sphere Mechanism[a]



[a]L and L′ stand for N-donor ligands, $R(OH)_2$ stands for ascorbic acid, and $R(=O)_2$ stands for dehydroascorbic acid.

Scheme 4. Reduction of All-Trans Platinum(IV) Complexes by Ascorbic Acid According to an Outer-Sphere Mechanism[a]



[a]L and L′ stand for N-donor ligands, $R(OH)_2$ stands for ascorbic acid, and $R(=O)_2$ stands for dehydroascorbic acid.

This prompted us to perform the reaction with ascorbic acid under buffered conditions. Since the solvent used in our experiments (methanol, chloroform, and water 7.5:3:1 v/v ratio) is not suitable for direct control of pH, it was decided to use an excess of ascorbic acid/sodium ascorbate 1:1 buffering system. Therefore, reduction of **1c** was performed using a 5-fold greater concentration of ascorbic acid and a 5-fold greater concentration of sodium ascorbate. The reaction, monitored by NMR (Figure 4), revealed, in the mixing time, complete disappearance of the signals of the starting compound (signals at 2.07, 2.46, and 3.97 ppm) and the appearance of two sets of signals belonging to the diacetato **1b** (singlets at 1.90, 2.48, and 3.79 ppm) and to the dichlorido **1a** (singlets at 2.58 and 3.79 ppm) platinum(II) species formed in 69% and 31% yields, respectively.

The ratio remains constant for the following hours in accord with a slow rate of displacement of the acetato by the chlorido ligand.

We can conclude that in buffered conditions both an inner-sphere and an outer-sphere mechanism contribute to the reduction process and the contribution of the former increases

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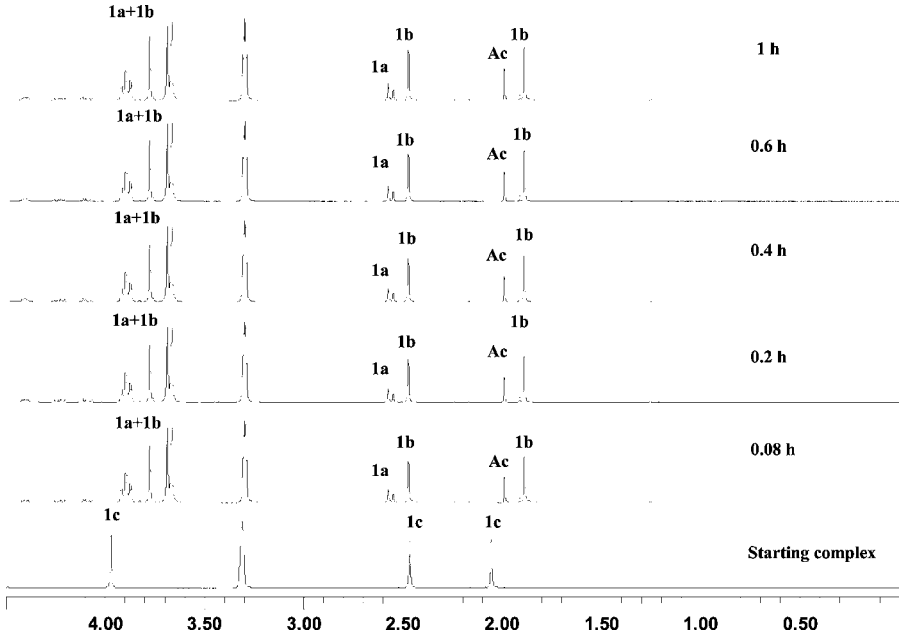

Figure 4. ^{1}H NMR spectra, at different time intervals, of complex **1c** treated with excess ascorbic acid/sodium ascorbate 1:1. Ac stands for free acetate.

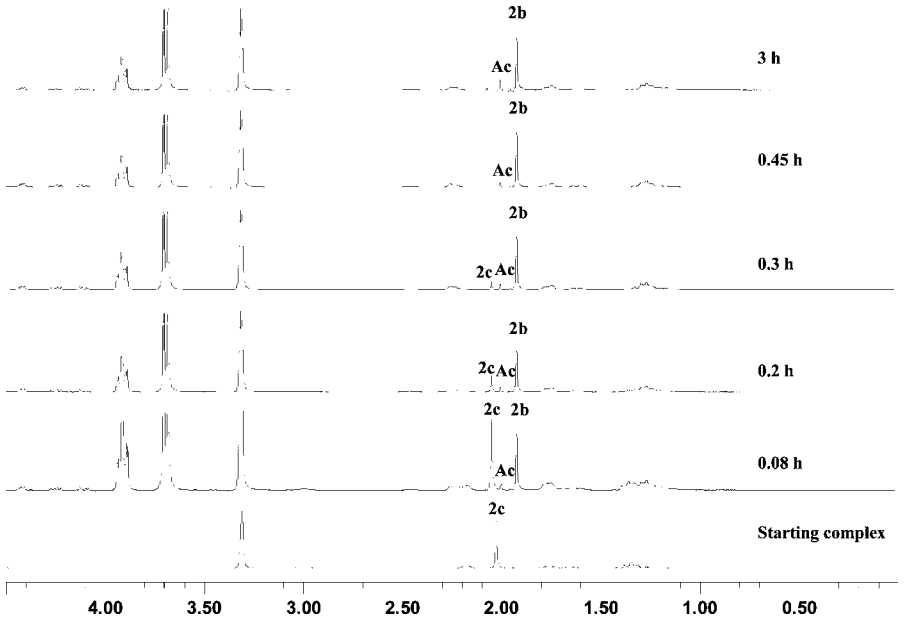

Figure 5. ^{1}H NMR spectra, at different time intervals, of complex **2c** treated with excess ascorbic acid/sodium ascorbate 1:1. Ac stands for free acetate.

as the concentration of the deprotonated ascorbate species increases, as suggested by Elding.[7]

*Reaction of trans,trans,trans-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2c**, JM576) with Excess Ascorbic Acid/Sodium Ascorbate 1:1.* Reduction by ascorbic acid of *trans,trans,trans*-[PtCl$_2$(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2c**), already investigated by Elding in buffered conditions,[7] was investigated under our experimental conditions of a 5-fold excess of ascorbic acid/sodium ascorbate 1:1. The reaction was monitored by ^{1}H NMR (Figure 5) and revealed, in the mixing time of the reactants, complete disappearance of the signals of the starting complex (coordinated acetates at 2.04 ppm) and appearance of the signals of *trans*-[Pt(CH$_3$COO)$_2$(NH$_3$)(cyclohexylamine)] (**2b**) (coordinated acetates at 1.92 ppm) and *trans*-[PtCl$_2$(NH$_3$)(cyclohexylamine)] (**2a**) (free acetate at 2.00 ppm) in a 95:5 ratio.

Formation of the diacetato species was also confirmed by ESI-MS. The parent peak at 454 *m*/*z* corresponds to the species [**2b** + Na]$^+$, and its fragmentation spectrum (MS/MS) exhibits two signals at 436 and 376 *m*/*z* corresponding to sequential loss of NH$_3$ and CH$_3$COOH ligands. The dichlorido species **2a** not only is formed in much smaller yield (NMR data) but is also much less volatile in the conditions of ESI-MS experiments; therefore, its peak is hardly seen at 409 *m*/*z* corresponding to species [**2a** + Na$^+$].

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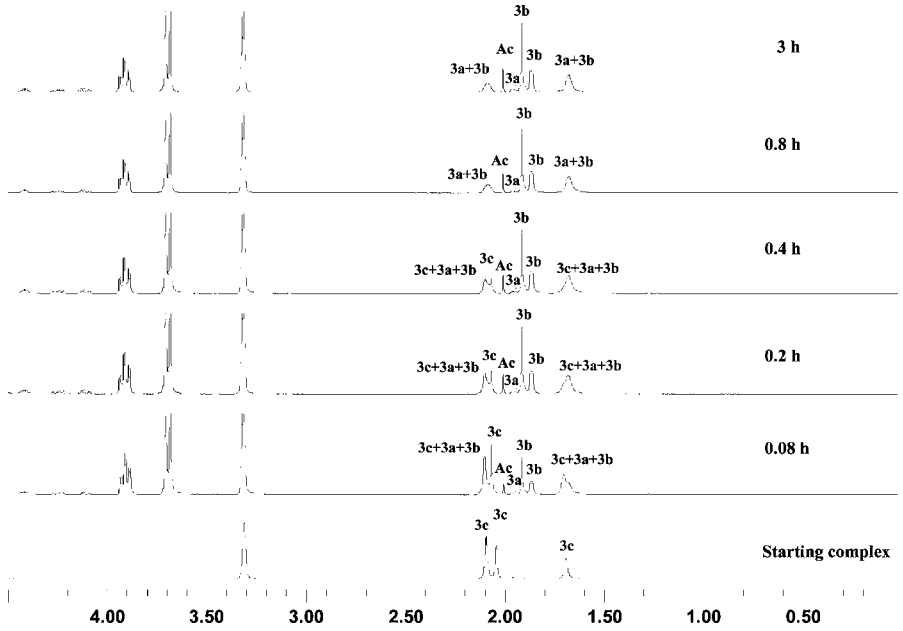

Figure 6. ^{1}H NMR spectra, at different time intervals, of complex **3c** treated with excess ascorbic acid/sodium ascorbate 1:1. Ac stands for free acetate.

It is clear from the previous experiments that the different yields of products observed in the reduction of all-trans complexes **1c** and **2c** by ascorbic acid in buffered conditions (diacetato/dichlorido ratio = 2:1 in the case of **1c** and 20:1 in the case of **2c**) stems from their intrinsic reactivity and not from differences in the experimental conditions, thus suggesting that the N-donor ligands are not innocent and can play a role in the redox process of platinum(IV) complexes with trans geometry.

We hypothesized that the different behavior of the two compounds could be determined by the different steric hindrance of the two iminoether ligands present in **1c** as compared to that of the ammine and cyclohexylamine ligands present in **2c**. Therefore, we decided to extend the investigation to another compound, strictly analogous to **2c** but having a bulkier amine, i.e., *trans,trans,trans*-[$PtCl_2(CH_3COO)_2(NH_3)$-(1-adamantylamine)] (**3c**). This latter compound also has been reported to have potential as an antitumor drug.[23]

*Reaction of trans,trans,trans-[$PtCl_2(CH_3COO)_2(NH_3)$(1-adamantylamine)] (**3c**) with Excess Ascorbic Acid/Sodium Ascorbate 1:1.* Complex **3c** was treated with a 5-fold excess of ascorbic acid/sodium ascorbate 1:1, and ^{1}H NMR spectra were recorded at various time intervals (Figure 6). In the mixing time of the reactants, the signals of complex **3c** disappeared (resonances at 2.04, 2.09, and 1.69 ppm assigned to the methyl protons of coordinated acetates, to the three methinic and six methylenic protons closer to the nitrogen of adamantylamine, and to the six methylenic protons farer from nitrogen of adamantylamine, respectively) with simultaneous appearance of two set of signals. One set contemplates the signal of free acetate (2.00 ppm) and those of compound **3a** (*trans*-[$PtCl_2(NH_3)$(1-adamantylamine)]) (signals at 2.08, 1.94, and 1.64 ppm assigned to the three methinic protons, to the six methylenic protons closer to nitrogen, and to the six methylenic protons farer from nitrogen of adamantylamine, respectively). The second set corresponds to the signals of compound **3b** (*trans*-[$Pt(CH_3COO)_2(NH_3)$(1-adamantylamine)]) (signals at 2.08, 1.85, and 1.66 ppm). Compounds **3b** and **3a** are formed in about 5:1 ratio.

The identity of the final products was also confirmed by ESI-MS (parent peaks at 508 *m*/*z*, corresponding to species [**3b** + Na]$^+$ in the positive ions current, and at 431.7 *m*/*z*, corresponding to species [**3a** − H]$^-$, in the negative ions current).

The latter experiment clearly shows that reduction by ascorbic acid can take place by a dual mechanism: loss of the acetato ligands is in accord with an outer-sphere mechanism as outlined in Scheme 4. In contrast, loss of the chlorido ligands implies direct involvement of the chlorido ligands in the redox mechanism and can be accounted for by an inner-sphere process in which a chloride bridges the reductant with the platinum(IV) center (as proposed by Elding and outlined in Scheme 3). Such a dual mechanism is not observed in the reduction by triphenylphosphine or glutathione which, in all cases, leads to formation of the diacetato species, as expected for an inner-sphere mechanism. Reduction of compound **1c** by triphenylphosphine and glutathione has already been described in the previous paragraphs; reduction of compounds **2c** and **3c** by triphenylphosphine is described in the following paragraph.

*Reactions of trans,trans,trans-[$PtCl_2(CH_3COO)_2(NH_3)$-(cyclohexylamine)] (**2c**) and trans,trans,trans-[$PtCl_2(CH_3COO)_2(NH_3)$(1-adamantylamine)] (**3c**) with Triphenylphosphine.* Reduction of complexes **2c** and **3c** by triphenylphosphine (1:1 Pt^{IV}/triphenylphosphine molar ratio), under experimental conditions analogous to those used for the other experiments, was very fast and produced, as expected, the diacetato species **2b** and **3b**, respectively. In a following step, the diacetato species react slowly with hydrochloric acid formed in the redox process (Scheme 1), yielding the dichlorido species **2a** and **3a** and acetic acid.

Thus, the ^{1}H NMR spectrum (Figures S3 and S4, Supporting Information) recorded soon after mixing of the reactants shows the disappearance of the resonance characteristic of the coordinated acetates in complexes **2c** and **3c** (2.04 ppm) and appearance of the resonance typical of the coordinated acetates

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in compounds **2b** and **3b** (1.92/1.91 ppm). With time, the intensity of the peak falling at 1.92/1.91 ppm decreases with concomitant increase of a peak falling at 2.00 ppm and belonging to free acetate. Conversion of **2b**/**3b** in **2a**/**3a** was complete in ca. 1 day. In addition, ESI-MS provides evidence for formation of the diacetato species in the first step (peak falling at m/z 457.1/508.0 and corresponding to [**2b**/**3b** + Na]$^+$, which, in subsequent fragmentation, loses ND_3 and CH_3COOH, giving peaks at m/z 437/488 and 377/428, respectively).

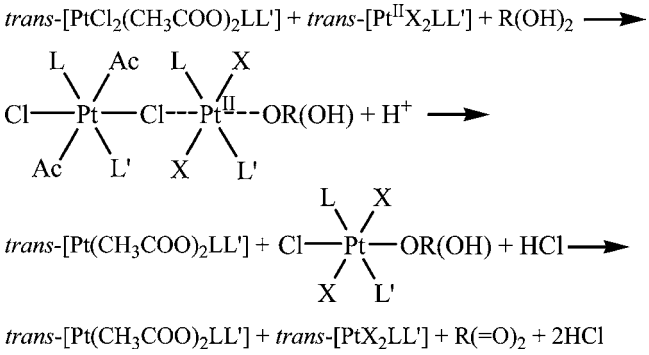
The latter experiments fully confirm that all platinum(IV) complexes containing two trans chlorido ligands are reduced by triphenylphosphine through an inner-sphere mechanism, involving attack of phosphine on a coordinated chloride and formation of a chlorido-bridged activated complex.

DISCUSSION

A significant difference between ascorbate and triphenylphosphine or glutathione is that while in the latter cases the reduction mechanism appears to be exclusively inner sphere with a chloride bridging the reductant with the platinum(IV) center, in the case of ascorbic acid/sodium ascorbate 1:1 the reaction can take place by both inner-sphere and outer-sphere mechanisms. The latter mechanism becomes dominant in strongly acidic conditions.

Moreover, the contribution of the inner-sphere mechanism appears to depend upon the bulkiness of the carrier ligand, it is lowest in the case of more sterically demanding iminoether ligands (**1b**/**1a** ratio of ∼2:1) and highest in the case of least sterically demanding ammine/cyclohexylamine ligands (**2b**/**2a** ratio of ∼20:1), while an intermediate value is found in the case of ammine/adamantylamine (**3b**/**3a** ratio of ∼5:1). In order to account for such a dependence upon the size of the carrier ligand, it is possible to hypothesize the intervention of a second type of inner-sphere mechanism (as outlined in Scheme 5) in which the interaction between the ascorbate and a chloride of the platinum(IV) substrate is mediated by a platinum(II) "catalyst". In other words the transition state should contain a platinum(II) catalyst having on one apical position a chlorido ligand of the platinum(IV) substrate and on the opposite side

Scheme 5. Reduction of All-Trans Platinum(IV) complexes by Ascorbic Acid According to a Second Type of an Inner-Sphere Mechanism[a]



[a]L and L′ stand for N-donor ligands; Ac stands for CH_3COO^-; X stands for anionic ligand which, in our context of catalyst generated by reduction of the platinum(IV) precursor, can be either CH_3COO^- or Cl^-; $R(OH)_2$ stands for ascorbic acid; $R(=O)_2$ stands for dehydroascorbic acid.

an ascorbate anion. Such a transition state is strongly reminiscent of that intervening in the Pt(II)-catalyzed Pt(IV) substitution mechanism established by Basolo and co-workers.[29,30] In the above-mentioned transition state, as a result of a two-electron transfer between the divalent and the tetravalent metal ions, the formerly Pt(IV) species loses the two axial chlorido ligands, affording the reduced diacetato platinum(II) species. On the other hand, the platinum(II) catalyst gets oxidized to a platinum(IV) species having an ascorbate and a chloride in axial positions. This intermediate species can decay by a first-order internal electron transfer between the coordinated ascorbate and the platinum(IV) center, affording dehydroacorbic acid, the initial platinum(II) catalyst, and hydrochloric acid.

Such a mechanism has already been proposed by Bose and co-workers for reduction by ascorbic acid of some platinum(IV) complexes including tetraplatin, *cis*-$[Pt(NH_3)_2Cl_4]$, and iproplatin, *cis,trans,cis*-$[PtCl_2(OH)_2(isopropylamine)_2]$. They also noted an initial slow uncatalyzed reduction, yielding the platinum(II) product, which served as catalyst for the following reaction; addition of the platinum(II) catalyst abrogated the induction period. The platinum(II) catalyst was shown to generate an ascorbate-bound platinum(IV) species which undergoes an internal electron transfer process, leading to back formation of the platinum(II) catalyst and dehydroascorbic acid.[31] However, we wish to point out that our deductions about the possible involvement of this second type of inner-sphere mechanism are based on analysis of the reaction products and their yields, rather than upon a kinetic investigation. The stability of a transition state in which one chloride of the platinum(IV) substrate interacts, from an apical position, with a platinum(II) substrate (the catalyst) which, on the opposite apical site, interacts with an ascorbate anion, is expected to be very much dependent upon the steric hindrance of the equatorial ligands at the two platinum centers and could explain why more bulky adamantylamine (compound **3c**) or iminoether ligands (compound **1c**) give smaller yield of diacetato species.

CONCLUSIONS

This investigation has shown that when platinum(IV) substrates contain two trans chlorido ligands, reduction by sulfur-containing reductants (glutathione) or phosphines (triphenylphosphine) invariably takes place by an inner-sphere mechanism with a chlorido ligand bridging the reductant with the platinum(IV) center and mediating the flow of electrons. This does not appear to be the case for ascorbic acid, which, depending upon the degree of protonation, can induce reduction by both outer-sphere and inner-sphere mechanisms. Moreover, the degree of involvement of the inner-sphere mechanism appears to depend upon the bulkiness of the carrier ligands, is lowest in the case of the most bulky iminoether ligands, while it is highest in the case of the least bulky ammine/cyclohexylamine ligands. To account for such an influence of the size of the carrier ligand, it is possible to hypothesize the participation of a second type of inner-sphere mechanism, as outlined in Scheme 5. The key feature of the latter mechanism is a platinum(II) catalyst mediating the interaction between a chlorido ligand of the platinum(IV) substrate and the ascorbate. Two-electron flow from platinum(II) to platinum(IV), mediated by the chlorido bridge, would lead to reduction of the platinum(IV) substrate and oxidation of the catalyst to a platinum(IV) species in which an ascorbate

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is directly bound to platinum trans to a chloride. An internal redox process would then restore the platinum(II) catalyst, while ascorbate gets oxidized to dehydroascorbic acid. Formation of the initial platinum(II) complex, needed for catalysis, could be provided by the concomitant occurrence of an outer-sphere mechanism and/or of a classical chlorido-bridging inner-sphere mechanism.[32]

The stability of the transition state in the type of mechanism proposed by Bose[31] would be very much dependent upon the steric hindrance of the equatorial ligands in the platinum(IV) substrate and in the platinum(II) catalyst. Destabilization of such a transition state would favor reduction by the alternative outer-sphere mechanism with increasing yield of platinum(II) dichlorido species. It should also be noted that the build-up of Pt(II) concentrations under physiological conditions (in blood) might never be great enough for Pt(II) to serve as a catalyst.

We wish to emphasize the different faith of Pt(IV) complexes with axial carboxylato (or hydroxido) ligands derived from $[PtCl_2LL']$ substrates having cis or trans geometry. In the case of cis geometry, reduction by low molecular weight reducing agent is likely to be outer sphere and afford the starting Pt(II) substrate. In contrast, in the case of trans geometry, as in the case of compound **1c** presently investigated, reduction is likely to be, exclusively or partially, inner sphere and afford a Pt(II) derivative (*trans*-$[Pt(carboxylato)_2LL']$) different from the starting substrate.

We are confident that the new insights in the redox mechanism of platinum compounds can also serve to explain contrasting results so far appearing in the literature. We also plan to investigate the reduction reaction using high molecular weight reducing agents (such as cytochrome *c*), which could play a major role in cellular deactivation of Pt(IV) complexes.[33,34]

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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