J. Braz. Chem. Soc., Vol. 17, No. 8, 1627-1633, 2006.
Printed in Brazil - ©2006 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Three New Complexes of Platinum(II) with Doxycycline, Oxytetracycline and Chlortetracycline and their Antimicrobial Activity

Wendell Guerra,[a] Iara R. Silva,[a] Elaine A. Azevedo,[a] Ana R. de S. Monteiro,[b] Mônica Bucciarelli-Rodriguez,[b] Edmar Chartone-Souza,[b] Josianne N. Silveira,[c] Ana P. S. Fontes[d] and Elene C. Pereira-Maia[*,a]*

[a]*Departamento de Química, ICEx,* [b]*Departamento de Biologia Geral, ICB and*
[c]*Departamento de Análises Clínicas e Toxicológicas, FF,*
Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte - MG, Brazil
[d]*Departamento de Química - ICE, Universidade Federal de Juiz de Fora 36036-330, Juiz de Fora MG, Brazil.*

Este artigo descreve a síntese e a caracterização de três novos complexos de platina(II) com a oxitetraciclina, doxiciclina e clortetraciclina por análise elementar, espectroscopias IV e RMN de ^{195}Pt. As interações da doxiciclina com íons Pt^{II} em função do pH foram estudadas por RMN de ^{1}H. Todas as tetraciclinas investigadas formam complexos 1:1 com a Pt^{II} via oxigênio do grupo hidroxila e oxigênio do grupo amida do anel A. As concentrações mínimas inibitórias (MIC) dos ligantes e de seus complexos de Pt^{II} foram determinadas em duas cepas sensíveis (*E. coli* HB 101 and *E. coli* ATCC 25922) e em uma resistente (*E. coli* HB101/pBR322). O complexo de platina da doxiciclina é duas vezes mais potente do que o antibiótico livre na cepa resistente. Os coeficientes de partição dos complexos em octanol e água foram determinados. O aumento da lipofilia causa um aumento da atividade antimicrobiana na cepa resistente.

This paper reports on the synthesis and characterization of three new platinum(II) complexes with oxytetracycline, doxycycline and chlortetracycline by elemental analysis, IR and ^{195}Pt RMN spectroscopies. Doxycycline interactions with Pt^{II} ions as a function of the pH were studied by ^{1}H NMR. All the investigated tetracyclines form 1:1 complexes with Pt^{II} *via* the oxygen of the hydroxyl group and oxygen of the amide group at ring A. The minimal inhibitory concentrations (MIC) of the ligands and those of their Pt^{II} compounds were determined in two sensitive strains (*E. coli* HB 101 and *E. coli* ATCC 25922) and in a resistant one (*E. coli* HB101/pBR322). Platinum complex of doxycycline is twice as potent as the free antibiotic in the resistant strain. Octanol/water partition coefficients of the complexes were determined. Increasing lipophilicity enhances antimicrobial activity in the resistant strain.

Keywords: tetracycline derivatives, platinum complexes, antimicrobial activity, bacterial resistance

Introduction

Tetracyclines (Tc) were the first antimicrobial group of drugs for which the term "broad-spectrum" was used, *i.e*., an effective drug against a large variety of bacteria, including both Gram-positive and Gram-negative.[1] The main mechanism of tetracycline action is the inhibition of protein synthesis. The strong binding of a magnesium complex of tetracycline, $[MgTc]^+$, to the bacterial 30S ribosomal subunit leads to the inhibition of protein synthesis by causing the rupture of codon-anticodon interactions between tRNA and mRNA and consequent interruption of the bond between the aminoacyl-tRNA and the ribosomal acceptor site.[1,2] The pharmacokinetics and bioavailability of tetracyclines are affected by metal coordination. In blood plasma, the drug is transported as calcium complexes.[3] In the intracellular medium, magnesium complexes seem to be more important as they probably play a role in binding to ribosomes.[4]

The clinical use of tetracycline and analogues in the treatment of bacterial infections has declined because of the emergence of bacterial resistance to these drugs. Almost all pathogenic bacteria have acquired resistance against the presently known and used antibiotics. Resistance mechanisms against tetracycline involve proteins that either protect the ribosome from $[MgTc]^+$,

*e-mail: elene@qui.ufmg.br

or export $[MgTc]^+$ out of the bacterial cell. In order to enter the cell and bind to the ribosome, Tc must cross the cytoplasmic membrane. A membrane-associated protein, (Tet A(B)), that mediates an active efflux of tetracycline has been identified in the cytoplasmic membrane of resistant bacteria.[5-7] This protein acts as an antiporter by coupling the efflux of $[MgTc]^+$ with the influx of a proton, thereby preventing the attack of $[MgTc]^+$ at the ribosome.

The expression of Tet A(B) is tightly regulated by a repressor molecule, the tetracycline repressor. In the absence of tetracycline it is repressed because the resistance protein is toxic to the cell, as it interferes with the maintenance of the electrostatic potential across the cell membrane. In the absence of $[MgTc]^+$, the repressor is bound to the operator gene. This process switches it off and blocks the transcription of the structural gene that encodes for the resistance protein Tet A(B). If $[MgTc]^+$ is present, this complex binds to the tetracycline repressor, inducing conformational changes in it that weaken its affinity towards the operator gene. As a consequence, the operator gene is released and Tet A(B) is expressed.[8]

Many efforts have been made to control the propagation of multidrug resistance strains worldwide.[5-12] In a previous work, we described the syntheses of platinum(II) and palladium(II) tetracycline complexes. The former is six times and the later sixteen times as potent as tetracycline in inhibiting the growth of a resistant bacteria strain.[11,12]

Besides the pharmacological importance of tetracyclines, these molecules possess an interesting structure with many potential metal-binding sites: oxygens at the C_{10}-C_{12} phenolic β-diketone system, enolic oxygens and the nitrogens at C4 and at the carboxamide group in ring A.

In this work, we report a study of platinum(II) interactions with doxycycline (Dox), oxytetracycline (Oxy), and chlortetracycline (Chl) and their antimicrobial activity in three *E. coli* strains.

Experimental

Reagents

Oxytetracycline hydrochloride (Oxy), doxycycline hydrochloride (Dox) and chlortetracycline hydrochloride (Chl) were purchased from Sigma Co. Stock solutions were prepared just before use to avoid ligand degradation caused by oxygen and light. Potassium tetrachloroplatinate is commercially available (from Sigma Co).

Synthesis of the complexes

All the complexes were synthesized following the same general procedure and we describe below only the synthesis of the Pt^{II} complex of doxycycline. K_2PtCl_4 (0.2075 g, 0.5 mmol) was added to 5 mL of an aqueous solution of doxycycline (0.5 mmol) and the mixture was stirred for 120 min. The solid formed was separated by filtration, washed with water, and dried under vacuum.

*[Pt(Oxy)Cl$_2$]·2H$_2$O (**1**).* IR (KBr) ν_{max}/cm^{-1}: 3411, 3097, 1668, 1618, 1584, 1541, 1458, 1371, 1308, 1239, 1212, 1171, 1134, 1048, 1010, 914, 879, 844, 772, 725, 675, 609, 595, 570, 544, 516, 320, 311. ^{195}Pt NMR (86 MHz; DMF-d$_7$) δ –1462.8. Yield: 346.1 mg, 91%. Anal. Calc. for [Pt Cl$_2$($C_{22}H_{24}O_9N_2$)].2H$_2$O: C, 34.63; H, 3.67; N, 3.67; Pt 25.57. Found: C, 34.06; H, 3.66; N, 3.52; Pt 25.11%. Color: Pale yellow.

*[Pt(Dox)Cl$_2$]·2H$_2$O (**2**).* IR(KBr) ν_{max}/cm^{-1}: 3412, 3200, 2929, 1664, 1618, 1578,1457, 1324,1243, 1216, 1170, 1128, 1042, 1039, 1005, 987, 884, 803, 770, 663, 617, 587, 550, 515, 316, 307. ^{195}Pt NMR (86 MHz; DMF-d_7) δ –1469.5. Yield: 320.04 mg, 84%. Anal. Calc. for [PtCl$_2$($C_{22}H_{24}O_8N_2$)].2H$_2$O: C, 35.36; H, 3.75; N, 3.75 Pt, 26.12%. Found: C, 35.62; H, 3.71; N, 3.79; Pt, 25.61%. Color: Pale yellow.

*[Pt(Chl)Cl$_2$]·2H$_2$O (**3**).* IR(KBr) ν_{max}/cm^{-1}: 3403, 3070,1660, 1618, 1578,1542, 1497, 1447, 1353, 1311, 1239, 1223, 1202, 1123,1042, 1006, 944, 837, 798, 695, 591, 541, 515, 494, 320, 315. ^{195}Pt NMR (86 MHz; DMF-d_7) δ –1464.3. Yield: 159.31 mg, 39%. Anal. Calc. for [Pt Cl$_2$($C_{22}H_{24}O_8N_2$Cl)].2H$_2$O: C, 33.82; H, 3.45; N, 3.58; Pt, 24.98%. Found: C, 34.40; H, 3.99; N, 4.03; Pt 24.51. Color: Yellow.

Spectroscopic measurements

Infrared spectra were recorded over the 400-4000 cm^{-1} region with a Perkin Elmer 283 B spectrometer. The samples were examined in KBr pellets.

^{1}H NMR spectra were obtained using a Bruker Avance DRX 400 spectrometer in D$_2$O at pH 3.0, 5.0, 7.0 and 8.0 with tetramethylsilane as an internal standard. The ligand concentration used was 5.0×10^{-3} mol L^{-1} and, when indicated, that of Pt^{II} was 1×10^{-2} mol L^{-1}.

^{195}Pt NMR (86 MHz) spectra were obtained using a Bruker Avance DRX 400 spectrometer in DMF-d_7 with K_2PtCl_4 as the internal standard.

Conductivity measurements

Conductivity studies were carried out with a Digimed DM 31 conductivity meter using a cell of constant 1.013 cm^{-1}, spectroscopic grade dimethylformamide (Merck) (Λ_M = 1.22 μS cm^{-1}) and tetraethylammonium bromide (Λ_M = 79.56 μS cm^{-1}) as a standard.

Elemental analysis

Carbon, nitrogen and hydrogen were determined on a Perkin Elmer 2400 CHN. Atomic absorption analysis of the platinum content was carried out on a model 8200 Hitachi Atomic Absorption Spectrophotometer.

Partition coefficient

Partition coefficients for the platinum complexes were determined in duplicate in an *n*-octanol/water system. Each platinum complex was dissolved in water at 2×10^{-5} mol L^{-1} and subsequently an equal volume of *n*-octanol was added. The mixtures were shaken mechanically for 24 h to insure the distribution between the two solvent phases. The samples were centrifuged (13000 rpm, 5 min). Afterwards they were diluted 5-fold and the platinum concentration was determined in both phases by FAAS in a Varian model Zeeman 220 spectrophotometer equipped with a graphite tube atomizer and an autosampler. Results are expressed as apparent partition coefficients (P) done by the total platinum in *n*-octanol divided by the total platinum in the aqueous layer.[13]

Microbial strains, plasmids and growth conditions

The sensitive and resistant bacterial strains selected from the bacteria collection of the Laboratory of Microbial Molecular Genetics of the General Biology Department of ICB-UFMG to perform microbiological tests were: *E.coli* HB101 F- *hsdS*20 (r_B^-, m_B^-) *leu*B6 *supE*44 *ara*14 *recA*13 *pro*A2 *rpsL*20 (str^r) *lacY*1 *galK*2 *mtl*1.[14]

E.coli HB101/pBR322 is an *E.coli* HB101 harboring plasmid pBR322 that carries resistance genes to ampicillin (Ap) and tetracycline (Tc). It is a cloning vector derivative of pSC101.[15]

E. coli ATCC 25922: clinical isolate, susceptible to cefamandole, cephalexin, cephaloglycin, cephaloridine, cephalothin, chloramphenicol, colistin [colimycin], gentamicins, kanamycin, nalidixic acid, neomycin, tetracycline (collection ATCC).

Bacterial cultures were grown in the specified medium at 37 ºC. Culture stocks were performed on Lignières medium (0.8% m/v Difco Nutrient Broth, 0.5% m/v Sigma gelatin, 0.7% m/v Merck agar).

Determination of Minimal Inhibitory Concentration

Tetracyclines and their complexes were dissolved in N,N-dimethylformamide. Stock solutions were diluted accordingly and added to Mueller Hinton Agar (Merck) previously melted and cooled to 40 ºC for the preparation of plates. This agar was distributed onto Petri dishes so as to obtain antibiotic concentrations of 2.08, 4.16, 8.32, 16.60, 33.30, 66.50, 133.00, and 266.20 μmol L^{-1}. Dimethylformamide concentration did not exceed 2.7%. Plates for each concentration were prepared in duplicate. Control plates containing only the solvent (dimethylformamide), the metal salt (K_2PtCl_4), or Nutrient Agar without either drug or solvent were also prepared.

The bacterial strains selected were transferred to 4.0 mL of the Nutrient Broth (Difco) and incubated at 37 ºC for 24 h. The resulting cultures were inoculated onto the plates previously prepared with antibiotics by means of a multi-inoculation apparatus and were also incubated at 37 ºC for 24 h. Bacterial growth was recorded after this period. A resistance factor (RF) was obtained by dividing the Minimal Inhibitory Concentration (MIC) of the resistant *E. coli* HB101/pBR322 strain by the MIC of the sensitive *E. coli* HB101.

Results and Discussion

*Characterization of complexes $[Pt(Oxy)Cl_2]\cdot 2H_2O$ (**1**), $[Pt(Dox)Cl_2]\cdot 2H_2O$ (**2**), and $[Pt(Chl)Cl_2]\cdot 2H_2O$ (**3**)*

Oxy, Dox, and Chl contain an identical 4-ring carbocyclic structure and substituent variations at carbons 5, 6, and 7. The difference between oxytetracycline and tetracycline lies in the presence of an OH group at C5 in the first molecule. Doxycycline is also hydroxylated at C5 but it lacks the hydroxyl group at C6, though it has the same minimal formula as tetracycline. Chlortetracycline has one chlorine at C7.

The complexes were characterized by elemental analysis, conductivity measurements, vibrational spectroscopy and ^{195}Pt NMR. Interactions of Pt^{II} ions with Dox were also studied in aqueous solution by ^{1}H NMR.

The results of the elemental analyses are in accordance with the proposed structures.

The molar conductivity values of 10^{-3} mol L^{-1} solutions for the three complexes were far below that of the 1:1 standard electrolyte indicating that they are not charged.

The assignments of the infrared spectrum of the isolated complexes were made on the basis of a detailed study of IR and vibrational assignments for tetracycline and analogues made by Dziegielewski *et al.*[16]

The modifications observed in the complexes spectra compared those of the respective ligands are very similar and only the spectrum of the platinum complex of doxycycline, complex **2**, will be discussed.

The IR spectrum of doxycycline shows characteristic absorptions at 3400 and 3200 cm^{-1}, corresponding to νOH and νNH, respectively. The first band is broader in the complex due to the presence of water.

The amide I band at 1670 cm^{-1}, ν(C=O), appears at 1661 cm^{-1} in the complex and decreases in intensity. Two very strong bands at 1618 and 1578 cm^{-1} are assigned to carbonyl stretching ν(C=O) on rings A and C, respectively. These bands appear for complex **2** at the same wavenumber, ruling out the participation of carbonyl oxygens in the coordination.

Two new absorptions at 307 and 316 cm^{-1} may be assigned to ν(Pt-Cl) stretching in accordance to the *cis* geometry.[11]

These results suggest that the oxygen of the amide group is involved in the coordination sphere.

The interactions of doxycycline with Pt^{II} ions were also studied in aqueous solution as a function of the pH, by ^1H NMR spectroscopy.

Doxycycline possesses three ionizable protons: the first one in the C_1-C_3 tricarbonyl system in ring A, the second in C_{10} and C_{12} ketophenolic system, and the third one in the C_4 dimethylammonium group, with pKa values of 3.10, 7.41, and 8.68, respectively.[17] The presence of an OH group at C5 render its protons more acidic as compared to tetracycline, especially the one in the dimethylammonium group, whose pKa is 0.61 units lower.

The ^1H NMR spectra of Dox and Tc have already been assigned.[17-23] Two regions in Dox ^1H NMR spectrum are of special interest for the attribution of metal coordination sites: the D ring aromatics and the dimethylamino, C4, C4a, and C6 protons on ring A. These protons are not labile and their chemical shift, which is very sensitive to metal coordination, can be distinguished if the coordination occurs through O10 and O12 sites or through A ring sites.[11,12] The O-H protons and the A-ring amide protons cannot be observed because they are exchanged for deuterium.

Figure 1 presents a section of the ^1H NMR spectrum of a solution containing 5.0×10^{-3} mol L^{-1} of Dox at pH 3.0, 5.0, 7.0, and 8.0. The resonance frequencies of the most affected protons, their assignments and the coupling constants are shown in Table 1. In the free ligand, the

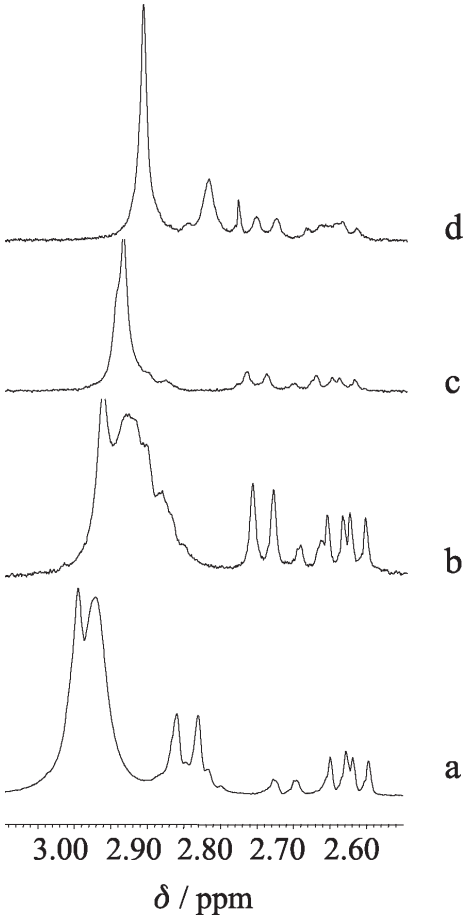


Figure 1. Sections of ^1H NMR spectra of solutions containing 5×10^{-3} mol L^{-1} Dox at pH = 3 (a); 5 (b); 7 (c) and 8 (d).

protons of group $N(CH_3)_2$ give rise to two sets of resonances at pH 3 (centered at δ 2.93) and 5 (centered at δ 2.89). The presence of a proton in the N-methyl group can prevent free rotation of the CH_3 groups and render them non-equivalent.

Table 1. ^1H NMR data for doxycycline and complex **2** in D_2O at different pH. Coupling constants, *J*(Hz), are even in parenthesis, following chemical shifts

	pH			
	3	5	7	8
H_{4a} Dox	2.79 (11.56)	2.68 (11.32)	2.68 (10.28)	2.69 (11.04)
H_{4a} complex **2**	2.81 (11.15)	2.70 (11.60)	2.69 (10.76)	–
H_{5a} Dox	2.57 (8.52;12.56)	2.57 (8.52;12.56)	(8.80;12.28)	-
H_{5a} complex **2**	2.62 (8.52;12.32)	2.59 (8.86;12.80)	2.60	-
$N(CH_3)_2$ Dox	2.93	2.89	2.87	2.86
$N(CH_3)_2$ complex **2**	2.92	2.88	2.87	2.84

By increasing the pH, N-methylenic protons at C4 are shielded, as it was expected due to the deprotonation in N4. Above pH 7, these two sets of signals are no longer present and one observes a singlet at δ 2.87. The presence of two sets of resonances is due to the fact that the methylenic protons are not chemical equivalent when the dimethylammonium group is protonated (pKa 8.68).

In Figure 2 the spectra of solutions containing 5.0×10^{-3} mol L^{-1} of doxycycline and 1.0×10^{-2} mol L^{-1} Pt^{II} at pH 3.0, 5.0, 7.0, and 8.0 are shown. The main modification concerns the N-methyl protons at ring A. In the presence of Pt^{II} there are two sets of resonances related to N-methyl protons from pH 3 to 8. At pH 7 these protons appear as a singlet in the spectrum of Dox, and, in the presence of Pt^{II} ions, they are splitted in two signals at δ 2.89 and 2.86, since the methyl groups are no longer equivalent in the complex. Same effect is observed at low pH values, in which the dimethylammonium group, also at C4, is protonated. The presence of a proton at N4 or of a metal bound at ring A prevents free rotation of the two methyl groups. These results are indicative of complexation of the metal at ring A (see Figure 3).

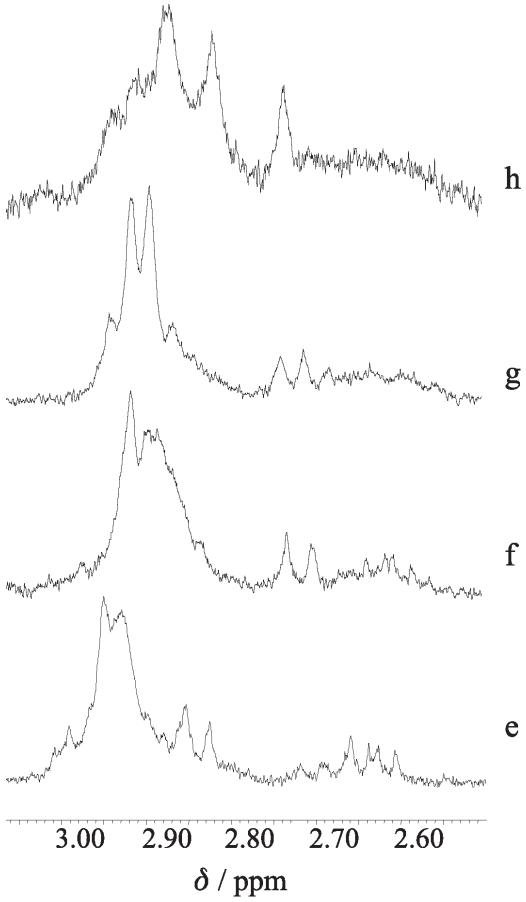


Figure 2. Sections of ^{1}H NMR spectra of solutions containing 5×10^{-3} mol L^{-1} Dox and 1×10^{-2} mol L^{-1} Pt^{II} at pH = 3 (a); 5 (b); 7 (c) and 8 (d).

A double doublet at δ 2.57 was attributed to the C5a proton. C4a and C5a are deshielded in the presence of Pt^{II} ions.

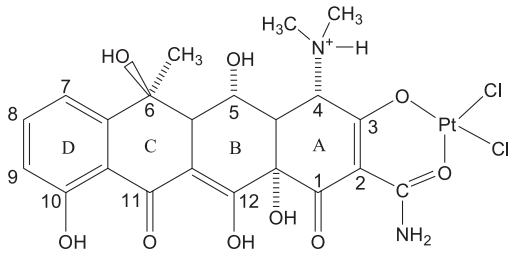
The resonances related to the aromatic protons on the D ring are not affected by addition of the ion Pt^{II}. The involvement of the phenolic β-diketone system in the coordination sphere would lead to a chemical shift change of the D-ring protons, and this does not occur.

This pattern confirms that binding occurs at A-ring sites.

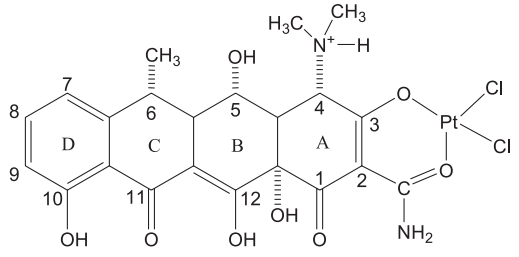
All the complexes were characterized by ^{195}Pt NMR spectroscopy. The purity of the complexes can be verified by this technique, since each species will generate a different signal. In the ^{195}Pt NMR spectra were observed signals at δ –1462.8, –1469.5 and –1464.5 for complexes **1**, **2**, and **3**, respectively. ^{195}Pt chemical shifts are sensitive to the nature of the ligands in the coordination sphere, and are a useful tool to predict the metal coordination sphere. The coordination sphere (PtO_2Cl_2) is not very common and ^{195}Pt chemical shifts relative to it are scarce in the literature. For $[Pt(Ox)Cl_2]^{2-}$ the reported δ(Pt) is –1005 and for $[Pt(2\text{-methymalonato})Cl_2]^{2-}$ –995.[24] Due to the low solubility of the compounds in water, ^{195}Pt NMR spectra were measured in a 1×10^{-2} mol L^{-1} solution in dimethylformamide (DMF). It is possible that the species present in DMF and in water are not the same. In aqueous solution, at neutral pH, Dox is present as a zwitterion, in which the dimmethylammonium group is protonated and the oxygen at C3 deprotonated. In lower dielectric media, the more stable species is a neutral tautomer, in which the proton of the dimethylammonium group is transferred to the oxygen at carbon 3. In the neutral tautomer the most basic site is the amino nitrogen at C4. It is possible that in DMF, Pt^{II} ions are coordinated to the nitrogen of the dimethylamino group, to the oxygen at C3 and to two chlorides. Quite recently, dos Santos *et al.*[25] reported a theoretical analysis for a complex of Pt^{II} with anhydrotetracycline. The global minimum in the gas phase corresponded to the coordination of Pt^{II} to the N4 and O3, which is the mode we propose to be present in a lower dielectric media. Calculations taking into account the solvation energy pointed to two different coordination sites: O3 and amide oxygen or amide oxygen and O1. The authors compared their calculated ^{1}H NMR spectrum to our previous experimental results for the Pt^{II} complex of tetracycline and concluded that in aqueous solution Pt^{II} ion might be bound to O3 and the amide oxygen.[25]

The proposed structures for complexes **1**, **2**, and **3** are presented in Figure 3. The same coordination mode has been observed for an $HgCl_2$ complex of oxytetracycline, which had its crystal structure determined.[26]

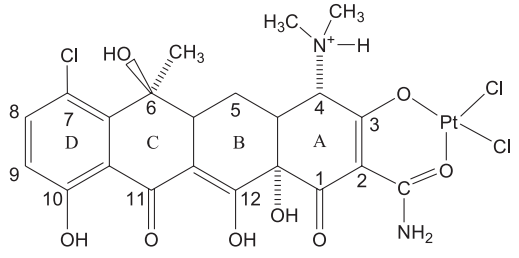
Other coordination possibilities would involve the O12-O10 system. However, the participation of the phenolic



Complex **1**



Complex **2**



Complex **3**

Figure 3. Proposed structures for platinum complexes **1**, **2** and **3**.

β-diketone system in the coordination sphere would lead to a frequency shift of the D-ring protons, and this does not occur. Moreover, in the IR spectrum of the complex, the νC=O of the carbonyl at C11 appears at the same wavenumber as the ligand, 1578 cm^{-1}. Therefore, we ruled out an involvement of these sites in platinum coordination.

Antimicrobial activity

The minimal inhibitory concentrations (MIC) of the ligands and those of their Pt^{II} compounds were investigated in two sensitive strains (*E. coli* HB 101 and *E. coli* ATCC 25922) and in a resistant one *(E. coli* HB101/ pBR322). The resistant strain *E. coli* HB101/pBR322 express Tet A(B) protein encoded on the pBR3222 plasmid. A resistance factor (RF) was calculated by dividing the MIC of *E. coli* HB101/pBR322 by that of the parental sensitive strain *E. coli* HB 101. Neither the solvent nor the platinum salt used in the synthesis affected the bacterial growth in the concentrations used.

As one can see in Table 2, the RF of Oxy is twofold higher than that of Dox and Chl. The RF of complexes **2** and **3** is 4 times higher than that of complex **1**. i.e., the resistant strain exhibits the highest RF to Oxy and its Pt^{II} complex.

Pt^{II} coordination to Chl and to Oxy does not improve their activity against the *E. coli* HB101/pBR322 strain while complex **2** is twice as potent as free Dox (Table 2). Previously, we had observed that a Pd^{II} complex of Chl had the same activity as the free ligand whereas Pd^{II} complexes of Tc and Dox were more potent than the respective ligands.[12] The different effect observed for the complexes of Chl and Oxy as compared to that of Dox is a puzzling question as their structures differ only in substituents at carbons 5, 6, and 7.

Bioavailability of tetracyclines is affected by metal coordination and the different substituents present in this series affect the lipophilicity of the antibiotics.[27] In order to seek a structure/activity relation, we have investigated the lipophilicity of **1**, **2**, and, **3**. Partition coefficient in octanol/water is often used to assess the lipophilic character of drugs: hydrophobic compounds will have a high *P* value whereas hydrophilic compounds will have a low *P* value. In Table 2, one can observe that as *P* increases MIC values decrease in the resistant strain *E. coli* HB101/ pBR322. This correlation between antimicrobial activity and partition coefficients suggest that increasing complexes hydrophobicity causes an increase in the antimicrobial activity in the resistant strain.

The hydrophobic character of a drug is crucial to how easily it crosses cell membranes and may also be important in receptor interactions. The resistance conferred by the

Table 2. MIC of free tetracyclines and their platinum(II) compounds[a]

	MIC / (μM)					
Bacterial strains	Oxy	**1**	Dox	**2**	Chl	**3**
ATCC 25922	8.32	8.32	8.32	4.16	2.08	2.08
HB 101	4.16	4.16	2.08	2.08	2.08	4.16
HB101/ pBR322	266.20	266.20	66.50	33.30	66.50	66.50
RF	63.99	63.99	31.97	16.01	31.97	15.99
P[b]	-	0.01	-	1.76	-	0.68

[a] MIC is the minimal drug concentration required to inhibit bacterial growth. RF is the MIC of HB101/ pBR322 divided by the MIC of HB 101. Number of essays = 4. [b]*P* is the total platinum in *n*-octanol divided by total platinum in the aqueous layer.

presence of Tet A(B) is due to a decrease of the intracellular drug concentration to a level inferior to the one necessary for the activity. A possible explanation for the antimicrobial activity of these Pt^{II} compounds is that by increasing their lipophilicity the intracellular drug concentration inside resistant bacterial cell increases.

The most important result is that Pt^{II} coordination to Dox improves the antibiotic activity in the resistant strain. In a previous work, we have found that a platinum(II) complex of tetracycline (Tc), $[Pt(Tc)Cl_2]$, was six times as potent as Tc in inhibiting the bacterial growth of the strain resistant to tetracycline.[11] The mechanism by which complex **2** can overcome tetracycline resistance is still unclear. We can speculate that either it does not bind to the repressor molecule and thus TetA(B) is not expressed in its presence, or that even if TetA(B) is expressed it cannot effectively transport the platinum complex out of the bacterial cell. Finding agents that are not recognized by the resistance mechanisms is very important because the emergence of bacterial resistance is the main obstacle for tetracycline derivatives use in the treatment of bacterial infections.

Supplementary Information

^{195}Pt NMR spectra of compounds **1**, **2**, and **3** are available free of charge at http://jbcs.sbq.org.br, as a PDF file.

Acknowledgments

This work was supported by grants of CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil) and FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais, Brazil).

References

1. Chopra, I.; Hawkey, P. M.; Hinton, M. J.; *Antimicrob. Chemother.* **1992,** *29*, 245.
2. Chartone-Souza, E.; *Ciencia Hoy* (in Spanish) **1999,** *9*, 30.
3. Lambs, L.; Brion, M.; Berthon, G.; *Inorg. Chim. Acta* **1983,** *106*, 151.
4. Brion, M.; Berthon, G.; Fourtellan, J. B.; *Inorg. Chim. Acta* **1981**, *55*, 47.
5. Salyers, A. A.; Speer, B. S.; Shoemaker, N. B.; *Mol. Microbiol.***1990**, *4*, 151.
6. Davies, J.; *Science* **1994**, *264*, 375.
7. Nikaido, H.; *Science* **1994**, *264*, 382.
8. Saenger, W; Orth, P. K. C.; Hillen, W.; Hinrichs, W.; *Angew. Chem. Int. Ed*. **2000**, *39*, 2042.
9. McMurry, L.; Petrucci, R. E.; Levy, S. B.; *Proc. Natl. Acad. Sci. USA* **1980**, *77,* 3974.
10. Speer, B. S.; Shoemaker, N. B.; Salyers, A. A.; *Clin. Microbiol. Rev*. **1992**, *5*, 387.
11. Chartone-Souza, E.; Loyola, T. L.; Bucciarelli-Rodriguez, M.; Menezes, M. A.; Rey, N. A.; Pereira-Maia, E. C.; *J. Inorg. Biochem*. **2005**, *99*, 1001**.**
12. Guerra, W.; Azevedo, E. A.; Monteiro, A. R. S.; Chartone-Souza, E.; Nascimento, A. M. A.; Bucciarelli-Rodriguez, M.; Fontes, A. P. S.; Le Moyec, L.; Pereira-Maia, E. C.; *J. Inorg. Biochem*. **2005**, *99*, 2348.
13. Robillard, M. S.; Galanski, M.; Zimmermann, W.; Keppler, K. B.; Reedijk, J.; *J. Inorg. Biochem.* **2002**, *88*, 254.
14. Lacks, S.; Greenberg, B.; *J. Mol. Biol.* **1977**, *114*, 153.
15. Rodriguez, R. L.; Tait, R.; Shine, J.; Bolivar, F.; Heyneker, H.; Betlach, M.; Boyer, H. W.; In *Molecular Cloning of Recombinant DNA*; Scott, W. A.; Werner, R., eds.; Winter Symposia: Miami, **1977**, *13*, 73.
16. Dziegielewski J.; Hanuza J.; Jezowska-Trzebiatowska, B.; *Bull. Acad. Pol. Sci., Ser. Sci. Chim*. **1976**, *24*, 307.
17. Lambs,L.; Decock-Le Révérend, B.; Kozlowski, H.; Berthon, G.; *Inorg. Chem*. **1988**, *27* 3001.
18. Casy, A. F.; Yasin, A.; *J. Pharm. Biomed. Anal*. **1983**, *1*, 281.
19. Casy, A. F.; Yasin, A.; *Magn. Res. Chem.* **1985**, *23*, 767.
20. Willianson, D. E.; Everett, G. W.; *J. Am. Chem. Soc*. **1975**, *97*, 2397.
21. Asleson, G. L.; Stoel, L. J.; Newton, E. C.; Frank, C. W.; *J. Pharm. Sci*. **1974**, *63*, 1144.
22. Rigler, N. E.; Bag, S. P.; Leyden, E. D.; Sudmeier, J. L.; Reilley, C. M; *Anal. Chem.* **1965**, *37*, 872.
23. Yang, W.; Moore, K. I.; Koteva, P. K.; Barei, C. D.; Hurghes, W. D.; Wrigth, D. G.; *J. Biol. Chem.* **2004**, *279*, 52346.
24. Dunham,S. O.; Larsen, R. D.; Abbott,E. H.; *Inorg. Chem.* **1991**, *30*, 4328.
25. Dos Santos, H. F.; Marcial, B. L.; De Miranda, C. F.; Costa, L. A. S.; De Almeida, W. B.; *J. Inorg. Biochem*. **2006**, *100*, 1594.
26. Jogun, K. H.; Stezowski, J. J.; *J. Chem. Soc*. **1976**, *98*, 6018.
27. Brown, J. R.; Ireland, D. S.; *Adv. Pharmacol. Chemother*. **1978**, *15*, 161.

Received: June 9, 2006
Published on the web: December 1, 2006

J. Braz. Chem. Soc., Vol. 17, No. 8, S1, 2006.
Printed in Brazil - ©2006 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Three new Complexes of Platinum(II) with Doxycycline, Oxytetracycline and Chlortetracycline and their Antimicrobial Activity

Wendell Guerra,[a] Iara R. Silva,[a] Elaine A. Azevedo,[a] Ana R. de S. Monteiro,[b] Mônica Bucciarelli-Rodriguez,[b] Edmar Chartone-Souza,[b] Josianne N. Silveira,[c] Ana P. S. Fontes[d] and Elene C. Pereira-Maia*[,a]

[a] Departamento de Química, ICEx, [b] Departamento de Biologia Geral, ICB and [c] Departamento de Análises Clínicas e Toxicológicas, FF, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte - MG, Brazil

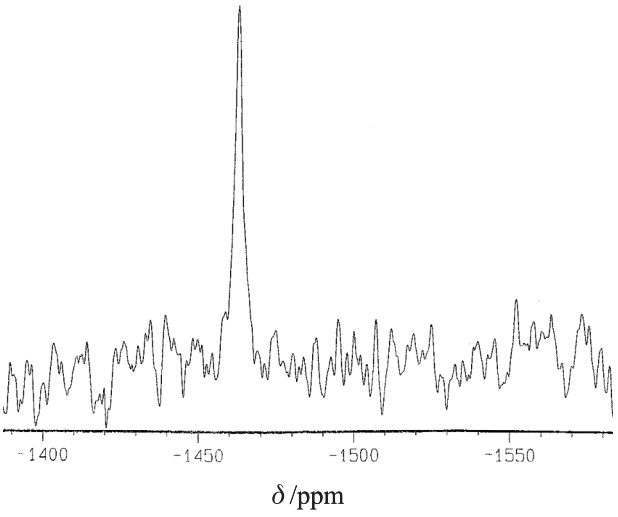


Figure S1. [195]Pt NMR spectra of complex **1**.

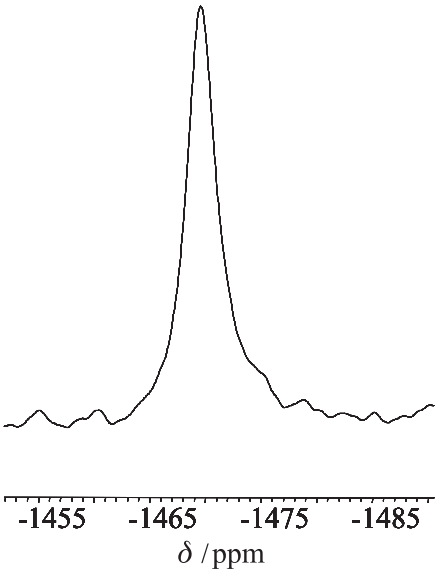


Figure S2. [195]Pt NMR spectra of complex **2**.

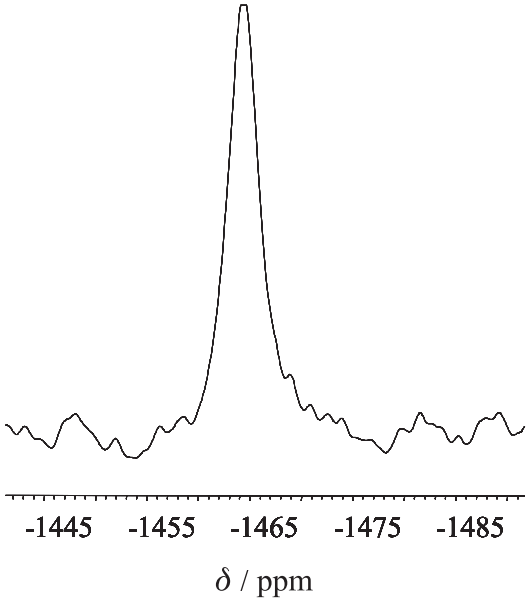


Figure S3. [195]Pt NMR spectra of complex **3**.

*e-mail: elene@qui.ufmg.br