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Preliminary Communication

The first isolated antineoplastic Ru(IV) complex: synthesis and structure of $[Cl_2(1,2\text{-cyclohexanediaminotetraacetate})Ru]\cdot 2H_2O$

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Abstract

Treatment of ruthenium(III) chloride with solid cyclohexanediaminetetraacetic acid (H_4cdta) affords the water soluble complex $[Ru(H_2cdta)Cl_2]\cdot 2H_2O$ (**1**), the first isolated Ru^{4+} compound showing cytostatic activity. Complex **1** has been characterized by X-ray diffraction, electronic spectroscopy and magnetic studies. The molecular structure indicates that the chelating ligand acts a tetradentate, showing two free carboxylic groups. The octahedral coordination around the metal ion is completed by two chlorine atoms.

Keywords: Antitumour activity; Ruthenium complexes; Octahedral complexes; Chelating ligand complexes

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1. Introduction

The potential use of ruthenium complexes in cancer chemotherapy has been recognized by researchers in different areas [1–5]. These substances have not only shown good antitumor activity in screening studies [5–8] but many also localize in tumor tissues [4,9]. Ru^{3+} compounds, as prodrugs, can be reduced in the hypoxic tumoral areas into more active species which bind rapidly to cellular DNA [10,11]. In this way ammine ruthenium complexes may be activated in vivo to coordinate the nucleobase appropriate atoms in a similar fashion to that of platinum anticancer drugs [12–16].

Ruthenium complexes with polyaminopolycarboxylic chelating ligands belong to a recently investigated new group of promising anticancer compounds [17,18], highly soluble in water in which their active forms are six-coordinate with octahedral structures. The ligands bear nitrogen donor atoms and labile carboxylate groups while the corresponding ruthenium complexes contain two additional halide ligands in *cis* positions. In this way, it is likely that these complexes are transported to the tumor site by transferrin, since the halides should be easily displaced at the transferrin binding site. Moreover, Ru^{4+} and Ru^{3+} with edta-type ligands often substitute rapidly owing to the labilizing effect of the pendant carboxylate groups [18,19].

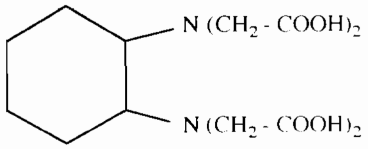

Fig. 1. Schematic structure of the polydentate ligand 1,2-cyclohexanediaminotetraacetic acid (H_4cdta).

We present here the synthesis and structural features of the complex *cis*-dichloro[cyclohexanediaminotetraacetate]ruthenium(IV) dihydrate (**1**), the first example of a promising antineoplastic compound that is active and non-toxic against several in vivo tumors. The synthesis of this complex involves the reaction between ruthenium(III) chloride and the ligand 1,2-cyclohexanediaminotetraacetic acid (H_4cdta, Fig. 1), in acid medium.

2. Experimental

2.1. Starting compounds

Hydrated ruthenium(III) chloride was used as obtained from Johnson Matthey and the ligand *trans*-1,2-

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cyclohexanediaminotetraacetic acid (H_4cdta) was used as purchased from Fluka.

2.2. Preparation of [Ru(H_2cdta)Cl_2]·$2H_2O$ (1)

To a solution of $RuCl_3 \cdot 3H_2O$ (0.5 g) in HCl (75 cm^3, 0.1 mol dm^{-3}), solid H_4cdta (2.294 g; Ru:H_4cdta, 1:1.1) was added. The deep red mixture was then introduced into a closed pressure reactor and heated at 140 °C (7.5 atm.). After cooling, the pink–red obtained solution was filtered and slowly concentrated to 5 cm^3 by evaporation at room temperature. Yellow–amber crystals of suitable size for X-ray analysis were selected. Yield 0.76 g (72%). *Anal.* Calc. for $C_{14}H_{20}N_2O_8Cl_2Ru \cdot 2H_2O$: C, 30.4; H, 4.5; N, 5.1; Cl, 12.8; Ru, 18.3. Found: C, 30.4; H, 4.4; N, 5.2; Cl, 12.6; Ru, 18.4%.

2.3. X-ray structure determination

A prismatic crystal was mounted on an Enraf-Nonius CAD-4 diffractometer and 2388 unique reflections were measured. The cell dimensions were refined by least-squares fitting of the 2θ values of 25 reflections. Intensities of 3123 reflections within $2° < 2\theta < 50°$ were measured at 295 K with monochromatic Mo Kα radiation and $\omega/2\theta$ scan. There was 2% intensity variation of three standard reflections monitored periodically. Intensities were corrected for Lorentz and polarization effects and 2388 ($I > 2\sigma(I)$) were considered as observed. Scattering factors for neutral atoms and anomalous dispersion corrections for Ru and Cl were taken from Ref. [20a]. The structure was solved from a three-dimensional Patterson map and Fourier synthesis [20b]. An empirical absorption correction [20c] was applied at the end of the isotropic refinement. In order to prevent bias on $\langle F \rangle$ versus $\langle F_o \rangle$ or $\langle \sin \theta/\lambda \rangle$, the last steps of the refinement were carried out with weights $\omega = \omega_1 \omega_2$, where $\omega_1 = 1/[a + b|F_o|]^2$ and $\omega_2 = 1/[c + d(\sin \theta/\lambda)]$ with coefficients calculated by PESOS [20d]. A final mixed refinement was undertaken with anisotropic thermal parameters for all atoms except O(9), O(10) and hydrogen atoms that have been refined isotropically. The hydrogen atoms were included with fixed isotropic contributions at their calculated positions. Final difference synthesis showed no significant electron density. Most calculations were carried out with XRAY 80 [20e]. See also Section 4.

2.4. Electronic spectroscopy and voltammetry

UV and visible spectra were recorded with a Jasco UVidec 610 spectrophotometer. Cyclic voltammetries were carried out using a Princeton Applied Research (PAR) 175 universal programmer coupled with a PAR 174A analyzer. A glassy carbon electrode was used as working electrode. Potentials were measured against a saturated (NaCl) calomel electrode (SSCE) as reference electrode.

3. Results and discussion

The molecular structure obtained by X-ray diffraction analysis is shown in Fig. 2 along with the numbering of atoms in the molecule. Crystal and refinement data for compound **1** are summarized in Table 1.

The coordinated ligand is tetradentate [21], with two glycinate chelate rings and two free carboxylic groups. The coordination sphere of the metal ion is formed by two nitrogen atoms from the H_4cdta molecule and two chloride atoms *cis* to each other, all of them in the equatorial plane. The axial octahedral positions are occupied by two oxygen atoms from coordinated carboxylate groups.

An important feature of the complex molecule is the Ru–Cl bond distances (2.37 Å); much longer than other Ru–Cl distances reported for similar compounds [22,23] and, as expected, much further from the ruthenium ion than the two coordinated nitrogen atoms (Ru–N 2.12 Å). On the other hand, Ru–O(3) and Ru–O(8) distances (2.03 and 2.07 Å, respectively) are of the same order, this fact indicating the absence of tetragonal distortion.

As a consequence, the ruthenium coordination structure is a slightly distorted octahedron with angles significantly reduced from ideal octahedral values, i.e. N(1)–Ru–N(2), 84.6(2)°; N(2)–Ru–O(8), 79.1(2)°.

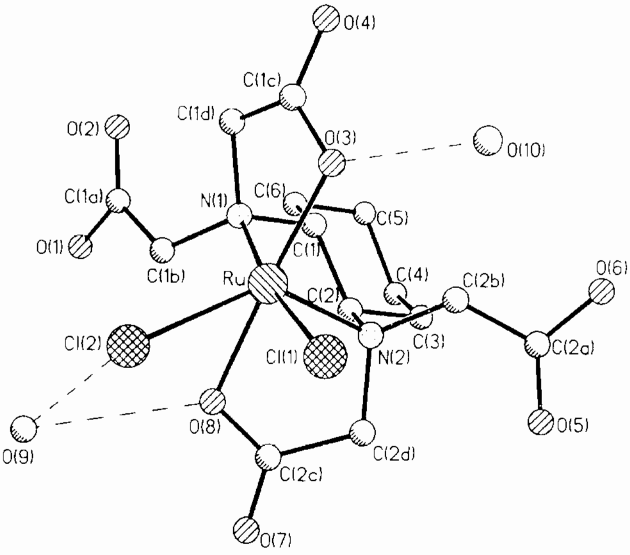

Fig. 2. Molecular structure of **1** showing the crystallographic atomic numbering scheme and the oxygens from water hydration. First coordination sphere main bond distances (Å) and angles (°): Ru–Cl(1) 2.372(2), Ru–Cl(2) 2.366(3), Ru–N(1) 2.107(5), Ru–N(2) 2.130(7), Ru–O(3) 2.027(6), Ru–O(8) 2.070(6), Cl(1)–Ru–Cl(2) 91.4(1), Cl(1)–Ru–N(2) 92.5(2), N(1)–Ru–N(2) 84.6(2), Cl(2)–Ru–N(1) 92.7(2), O(3)–Ru–O(8) 173.5(2). Hydrogen bonding interactions with water hydration are indicated by dashed lines.

Table 1
Crystallographic data for $[Ru(H_2cdta)Cl_2]\cdot 2H_2O$

Formula	$C_{14}H_{20}N_2O_8Cl_2Ru\cdot 2H_2O$
Molecular weight	552.34
Crystal system	hexagonal
a (Å)	13.567(2)
b (Å)	13.567(2)
c (Å)	22.286(6)
α (°)	90.00(0)
β (°)	90.00(0)
γ (°)	120.00(0)
V (Å^3)	3552(1)
Space group	$P6_5$
Z	6
D_{calc} (g cm^{-3})	1.53
F(000)	1644
λ (Mo Kα) (Å)	0.71069
μ (Mo Kα) (cm^{-1})	9.2
Reflections measured	3123
Scan method	$\omega/2\theta$
Reflections ($I \geqslant 2\sigma(I)$)	2388
R	0.051
R_w	0.049

The coordinate oxygen atoms, O(3) and O(8), are associated to the water molecules, O(10) and O(9), through intramolecular bonding (i.e., O(3)...O(10) 2.86(2); O(8)...O(9) 2.98(3) Å). Furthermore, the coordinated atom Cl(2) is simultaneously bonded to the water molecule O(9). On the other hand, two strong intermolecular hydrogen bondings have been detected involving the two pairs of oxygen atoms O(1)...O(5) 2.64(2) Å, and O(2)...O(6) 2.65(1) Å, as found previously in similar H_4cdta complexes [24], i.e. the hydroxyl groups of the organic ion act as hydrogen atom donors and the carbonyl groups act as hydrogen atom acceptors in these associations.

The electronic spectra shows three absorption maximums at 28 410 ($\epsilon = 11\,310$ dm^3 mol^{-1} cm^{-1}), 31 056 ($\epsilon = 15\,600$ dm^3 mol^{-1} cm^{-1}) and 35 336 ($\epsilon = 4070$ dm^3 mol^{-1} cm^{-1}) cm^{-1}. The two first peaks may be attributed to the electronic transitions $^3E_g \leftarrow {}^3T_{1g}$ and $^3T_{2g} \leftarrow {}^3T_{1g}$, respectively, while the shoulder registered at $\lambda = 283$ nm could be due to the $^3A_{1g} \leftarrow {}^3T_{1g}$ transition.

Magnetic susceptibility measurements show that the ruthenium ion is paramagnetic with a magnetic moment (spin only) characteristic of two unpaired electrons ranging between 2.87 (298 K) and 1.97 (80 K) μ_B which is expected for an oxidation state of +4 for ruthenium with a low-spin $4d^4$ configuration.

The voltammogram of a just prepared 4.0 mM aqueous solution of the complex ($\mu = 0.15$ M, $NaClO_4$; pH 2.0) shows two characteristic reversible/quasi-reversible couples at −0.25 and 0.54 V, corresponding to the one-electron reduction processes Ru^{3+}/Ru^{2+} and Ru^{4+}/Ru^{3+}, respectively, in agreement with previous results [25,26].

Compound **1** exhibits important antitumoral activity against Ehrlich ascitic tumour (*T*/*C* (%) of 350 at 25 mg kg^{-1}), P388 lymphocytic leukemia tumor (*T*/*C* (%) of 140 at 60–20 mg kg^{-1}) and a MX-1 transplanted human mammary carcinoma (*T*/*C* (%) of 16 at 240 mg kg^{-1}). (*T*/*C* values are expressed as 100 times the ratio of the lifetime of animals treated with the ruthenium drug to that for the untreated animals. Experiments were performed at NIH, Bethesda, USA and ONC, Madrid, Spain.)

The solid compound is stable to air at room temperature and soluble in water, ethanol and diluted acids.

4. Supplementary material

Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

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