Structure of Chloro(cyclopentadienone)-(cyclopentadienyl)ruthenium and its Cytotoxic Activity

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The structure of Ru(cpO)(cp)Cl complex (3), which was precipitated from aqueous solutions of Ru(cp)Cl2PF6 (1), was determined by X-ray crystallographic analysis. The Ru-halogen bond distance was shorter and the dihedral angle between the rings was larger than the values reported for the corresponding Br analogue, Ru(cpO)(cp)Br (4). The complex 3 (IC50 109 μM) was more cytotoxic than 1 (IC50 163 μM) against mouse sarcoma 180 cells, but less toxic than 4 (IC50 72 μM).

Introduction

Metal complexes are a promising class of compounds as anticancer agents [1]. Among them, chloroosmocenium ion is remarkably cytotoxic against several cancer cell lines, but its ruthenium analogue, Ru(cp)Cl2PF6 (1), has shown only weak cytotoxicities [2]. Aqueous solutions of 1 were colored green just after its dissolution, but the color gradually turned orange and, if the concentration of 1 was over about 10 mg/ml, red plates were precipitated together with colorless needles. All crystals were uncharged ring-oxidized derivatives, which was precipitated from aqueous solutions of Ru(cp)2Cl+PF6- (1) and Ru(cp)2Br+PF6- (2) were prepared by the published method [2,4].

The present study has aimed at clarifying the structure and cytotoxic activity of our red crystals, because the structure of 3 has not been published, and because we were interested in the relation between 1 and 3 concerning the cytotoxicity.

Experimental

Ru(cp)Cl+PF6- (1) and Ru(cp)Br+PF6- (2) were prepared by the published method [2,4]. The salt 1 (50 mg) was dissolved in 4 ml water at 50 °C. After keeping that temperature for 30 min, undissolved material was removed by filtration. The filtrate was kept at 4 °C for 2 days to give red plates and colorless needles. All crystals were collected by filtration, and then the needles were removed by washing with acetone and ether. Thus, the remaining red plates (Ru(cpO)(cp)Cl, 3) were collected, dried in vacuo, and weighed: 7.3 mg (21% yield).

Analysis for C10H9ClORu

Calcd C 42.64 H 3.22 Cl 12.59%.
Found C 42.54 H 3.16 Cl 13.22%.

IR (v/KBr): 3086, 1751, 1677 (C = 0), 1416, 1145, and 837 cm⁻¹. Ru(cpO)(cp)Br (4) was similarly prepared from 2 in 37 % yield.

Analysis for C10H9BrORu

Calcd C 36.83 H 2.78 Br 24.50%.
Found C 36.92 H 2.74 Br 25.09%.

IR (v/KBr): 3085, 1751, 1679 (C=O), 1414, and 1144 cm⁻¹.

X-ray crystallographic data for a crystal of 3 [0.2 x 0.3 x 0.15 mm, orthorhombic, space group Pnma, a (Å) = 13.699(1), b = 8.7309(4), c = 7.2681(4), V = 869.31(9) Å³, Z = 4] were collected with CuKα radiation (λ = 1.5418 Å), on Enraf-Nonius CAD4. Empirical absorption correction was applied. Structure was solved by direct method using MoLEN package (Enraf-Nonius, Delft). 931 unique reflections (|I|/σ(I) ≥ 3σ(|I|)) were used (120° ≥ 2θ ≥ 4°) for the full matrix least squares refinement. The non-hydrogen atoms were refined anisotropically and all parameters of the hydrated molecule to another haloruthenocenium molecule.

2 Ru(cp)X+ + H2O → Ru(cpO)(cp)X + Ru(cp)Cl+ + 3 H+ + X-
(cp, cpO, and X represent n-cyclopentadienyl, n-cyclopentadienone moiety, and halogen atom, respectively.)

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the hydrogen atoms were omitted. $R(Reo) = 0.091(0.099)$. The positional parameters are deposited to Cambridge Crystallographic Data Centre.

Cytotoxicity was assayed by the previous method [2]. Cyclic voltammograms were measured in CH$_2$Cl$_2$ containing 1 mM 3 or 4 and 0.1 M tetrabutylammonium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate at a scan rate of 100 mV/s.

**Results and Discussion**

The elemental analysis and IR spectrum of our red crystals were consistent with the structure of Ru(cpO)(cp)Cl, which was confirmed by X-ray diffraction study (Fig. 1). It was isostructural with that reported for the Br analogue (4) [3], but several distinctions are also found between them: (1) the Ru-Cl bond distance ($2.422\,\text{Å}$) is shorter than the Ru-Br bond ($2.556\,\text{Å}$); (2) the dihedral angle between two rings of 3 ($38.4°$) is larger than that for 4 ($36.2°$); and (3) the angle between the line formed by the C=O group and the butadiene plane for 3 ($16.2°$) is smaller than that for 4 ($20.6°$). The above fact (1) should reflect the difference between the electronic structures of Cl and Br atoms and this would result in the second structural feature by steric repulsion between the Cl and the ring carbon and hydrogen atoms.

At the start, we surmised that the cytotoxicity of complex 1 was weak for inactivity of the automatically converted cyclopentadienone complex (3). Its cytotoxicity against cancer and normal embryonic cell lines was assayed in vitro together with the related compounds and cisplatin (Table 1). All the Ru compounds showed only weak cytotoxicities against both cell lines, while the following facts are worthy of notation. First, the ring-oxidized compounds showed stronger cytotoxicity than the corresponding haloruthenocenium salt. Since the latter is gradually converted to the former and ruthenocene, and since ruthenocene itself is not cytotoxic in that experimental condition (data not shown), the cytotoxicities of the ruthenocenium salts might be due to the ring-oxidized compounds. Secondly, cytotoxicity of the Cl-containing compounds (3 and 1) were weaker than that of the corresponding Br compounds (4 and 2). This fact is in contrast with the cytotoxicities of halosmocenium salts, the strength of which was in the order of Os(cp)$_2$Cl$^+$ $>$ Os(cp)$_2$Br$^+$ $>$ Os(cp)$_2$I$^+$ against every cell line [2] being parallel with cell respiration inhibition and redox potential. This fact about the Os complexes suggested that their cytotoxicity might be caused by interruption of intracellular electron transfer. Cyclic voltammetry was attempted for 3 and 4, but only oxidation half waves were observed, presumably because of their instability in electrochemically oxidized state. However, the half wave potential for 3 ($+1.41\,\text{V vs. s.c.e.}$) was higher than that for 4 ($+1.35\,\text{V vs. s.c.e.}$), implying that 3 would have a

![Image of ORTEP drawing of 3](image-url)

**Table I. Cytotoxicity of the Ru compounds against mouse sarcoma S180 and mouse embryo NIH/3T3 cells. Values are given in mean ± standard deviation ($n = 5$).**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$ ($\mu$M)</th>
<th>S180</th>
<th>NIH/3T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru(cpO)(cp)Cl (3)</td>
<td>109 ± 16</td>
<td>159 ± 29</td>
<td></td>
</tr>
<tr>
<td>Ru(cpO)(cp)Br (4)</td>
<td>72 ± 7</td>
<td>70 ± 7</td>
<td></td>
</tr>
<tr>
<td>Ru(cp)$_2$Cl$^+$PF$_6^-$ (1)</td>
<td>163 ± 16</td>
<td>160 ± 16</td>
<td></td>
</tr>
<tr>
<td>Ru(cp)$_2$Br$^+$PF$_6^-$ (2)</td>
<td>91 ± 13</td>
<td>128 ± 30</td>
<td></td>
</tr>
<tr>
<td>cisplatin</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. ORTEP drawing of 3. Selected bond lengths (Å) and angles (°): Ru-Cl 2.422 (3), Ru-C1 2.59(1), Ru-C2 2.253(8), Ru-C3 2.161(8), Ru-C4 2.17(1), Ru-C5 2.216(8), Ru-C6 2.214(8), Cl-O 1.21(1), C1-C2 1.51(1), C2-C3 1.42(1), C3-C3A 1.45(1), Cl-Ru-C1 71.0(3), Cl-Ru-C2 88.5(2), Cl-Ru-C3 125.4(2), Cl-Ru-C4 145.3(3), Cl-Ru-C5 117.7(2), Cl-Ru-C6 84.8(2), Cl-Ru-C2 35.3(2), Cl-Ru-C3 59.1(4), Cl-Ru-C4 143.8(4), Cl-Ru-C5 145.4(2), Cl-Ru-C6 149.5(3), O-C1-C2 128.9(5), C1-C2-C3 108.2(7), and Ru-C1-O 141.0(9). The subscripts A indicate symmetry operation code (x, 1/2-y, z).
stronger oxidation ability than 4. This is contrary to the order of their cytotoxic strength. These facts seem to suggest that the mechanism for cytotoxicity would be different between the haloosmocenium salts and the present haloruthenium complexes.