Synthesis, molecular structure and solution chemistry of the iridium(III) complex imidazolium [trans(bisimidazole)tetrachloroiridate(III)] (IRIM)

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Abstract

The iridium(III) complex imidazolium [trans(bisimidazole)tetrachloroiridate(III)], (IRIM), isostructural to the well known ruthenium(III) analogue, (ICR), has been prepared and characterised, both in the solid state and in solution, by X-ray diffraction and by a variety of physico-chemical techniques. Single crystal X-ray diffraction studies point out that this complex is isomorphous with ICR and with the rhodium(III) analogue. IRIM is moderately soluble in water and within a physiological phosphate buffer. Electronic spectra in the visible, show that the complex is stable for days at pH 7.4; notably no significant chloride hydrolysis is observed over a period of 24 h at 25°C. Stability of IRIM within a physiological buffer was further tested and confirmed by 1H NMR spectra. The complex is stable toward treatment with either hydrogen peroxide or ascorbic acid or silver nitrate. The chemical behaviour in solution and the reactivity of IRIM are compared to those of ICR; implications of the present results for possible pharmacological applications of IRIM and for a better understanding of the mechanism of action of ICR are discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Iridium complexes; Imidazole complexes; Crystal structures; Possible anticancer complexes

1. Introduction

In recent years much interest has been focused on the design and the development of ruthenium(III) complexes as possible anticancer agents [1–3]. From a large number of investigations, mainly carried out by Keppler and his group, it clearly emerged that the monomeric complex [trans-bisimidazole(tetrachlororuthenate(III))] (ICR), which is far less cytotoxic than cisplatin, exhibits interesting antitumour and antimetastatic properties toward a number of experimental tumour models [3–5]. Thus, it may be inferred that the structure of ICR represents an interesting, new pharmacological ‘lead’ from which analogous complexes with similar biological properties may be derived. For instance, it is reasonable to assume that the biological properties of these complexes may critically depend on the kinetics of ligand substitution and on the occurrence of redox reactions at the metal centre. With this in mind we started preparing a number of complexes isostructural to ICR in order to...
evaluate their specific biological properties in relation to the chemical properties of the metal centre. A previous attempt in this direction was done by Mestroni et al., who prepared and described the rhodium(III) analogue of ICR [6]. We report here on the synthesis, the crystal structure and the solution properties of the imidazolium \([\text{trans-bisimidazoletetrachloroiridate(III)}]\) complex (IRIM hereafter), the iridium(III) analogue of ICR (Scheme 1). Iridium(III) complexes are known to be far more inert than ruthenium(III) analogues [7]; thus iridium was specifically selected to evaluate the biological effects arising from a net reduction in the rate of ligand exchange. Preliminary biological results for IRIM obtained in our laboratory have been reported elsewhere [8].

IRIM was prepared according to synthetic procedures reproducing those used for ICR [4] and for the rhodium analogue [6], but using less soft reaction conditions; its structure was determined by single crystal X-ray diffraction techniques. The solution properties of IRIM were investigated with the help of a number of physico-chemical techniques including absorption spectroscopy, \(^1\)H NMR and chloride-selective potentiometry. Overall, these results provide a quite detailed description of the solid state structure and the behaviour in solution of IRIM.

2. Experimental

2.1. Materials

All starting materials and solvents used were commercially available. The syntheses were carried out under nitrogen as described below.

2.2. First synthesis of \([\text{ImH}]\,[\text{IrCl}_4(\text{Im})_2]\), (IRIM), starting from \(\text{H}_2[\text{IrCl}_3]\)

\(\text{H}_2[\text{IrCl}_3] (0.5 \text{ g}, 1.2 \text{ mmol})\) was dissolved, by gentle warming, in a mixture of ethanol (3.2 ml) and 3.2 ml of 2 M HCl. Afterwards, 0.63 g of imidazole (9.3 mmol) dissolved in 0.65 ml of 6 M HCl was added with stirring. The red–brown mixture was reacted for 20 min and then left at room temperature (r.t.). After 1 week no crystals of the product precipitated. The mother liquor was then heated under reflux and after 2.5 h, a pink–red precipitate formed. The suspension was filtered and the solid washed with ethanol, ether and dried in vacuum. Yield 0.28 g (43.3\%) of pink–red microcrystals. \textit{Anal. Calc. for C}_9\text{H}_{13}\text{Cl}_4\text{IrN}_6: C, 20.04; H, 2.43; N, 15.59. Found: C, 20.01; H, 2.36; N, 15.47\%. From the mother liquor a good quantity of nice crystals formed.

2.3. Second synthesis of \([\text{ImH}]\,[\text{IrCl}_4(\text{Im})_2]\), (IRIM), starting from \(\text{H}_2[\text{IrCl}_3]\)

\(\text{H}_2[\text{IrCl}_3] (0.5 \text{ g}, 1.2 \text{ mmol})\) were dissolved, by gentle warming, in a mixture of ethanol (3.2 ml) and 3.2 ml of 2 M HCl. Afterwards, 0.63 g of imidazole (9.3 mmol) dissolved in 0.65 ml of 6 M HCl was added with stirring. This time the mixture was refluxed immediately and the reaction continued, as in the first reaction, for 2.5 h. An appreciable quantity of pink–red precipitate formed. The suspension was filtered and the solid washed with ethanol, ether and dried in vacuum. Yield 0.28 g (43.3\%) of pink–red microcrystals. \textit{Anal. Calc. for C}_9\text{H}_{13}\text{Cl}_4\text{IrN}_6: C, 20.04; H, 2.43; N, 15.59. Found: C, 20.01; H, 2.36; N, 15.47\%.

2.4. First synthesis of \([\text{ImH}]\,[\text{IrCl}_4(\text{Im})_2]\), (IRIM), starting from hydrated \(\text{IrCl}_3\)

Hydrated \(\text{IrCl}_3 (0.5 \text{ g}, 1.42 \text{ mmol})\) were dissolved, by gentle warming, in a mixture of ethanol (3.8 ml) and 3.8 ml of 2 M HCl. Afterwards, 0.77 g of imidazole (11.36 mmol) dissolved in 0.8 ml of 6 M HCl was added with stirring. The mixture was reacted for 5 min and left for three days at r.t.; no crystals precipitated from the mother liquor. The mixture was successively reacted, under reflux, for 1 h.; a pink–red precipitate formed. The suspension was filtered and the solid washed with ethanol, ether and dried in vacuum. Yield 0.29 g (37.9\%) of pink–red microcrystals. \textit{Anal. Calc. for C}_9\text{H}_{13}\text{Cl}_4\text{IrN}_6: C, 20.04; H, 2.43; N, 15.59. Found: C, 19.84; H, 2.51; N, 15.30\%.

2.5. Second synthesis of \([\text{ImH}]\,[\text{IrCl}_4(\text{Im})_2]\), (IRIM), starting from hydrated \(\text{IrCl}_3\)

Hydrated \(\text{IrCl}_3 (0.5 \text{ g}, 1.42 \text{ mmol})\) were dissolved, by gentle warming, in a mixture of ethanol (3.8 ml) and 3.8 ml of 2 M HCl. Afterwards, 0.77 g of imidazole (11.36 mmol) dissolved in 0.8 ml of 6 M HCl was added with stirring. This time the mixture was refluxed immediately and the reaction continued for 1 h., an appreciable quantity of pink–red precipitate formed. The suspension was filtered and the solid washed with ethanol, ether and dried in vacuum. Yield 0.28 g (36.6\%) of pink–red microcrystals. \textit{Anal. Calc. for C}_9\text{H}_{13}\text{Cl}_4\text{IrN}_6: C, 20.04; H, 2.37; N, 15.66\%.

\(^1\)Elemental analyses were made by “Servizio di Microanalisi” of Research Area of Rome.

\(^2\)Some time, when using different batches of hydrated \(\text{IrCl}_3\), the reproducibility of the synthesis is not good. We cannot give an account for this problem.
Table 1
Crystal data for [ImH][IrCl₄(Im)₂] (IRIM)

<table>
<thead>
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<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
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<tr>
<td>Formula</td>
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</tr>
<tr>
<td>Molecular weight</td>
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<tr>
<td>Crystal dimensions (mm)</td>
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<tr>
<td>Space group</td>
<td>C2/c</td>
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<tr>
<td>Cell dimensions a</td>
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</tr>
<tr>
<td>Cell dimensions b</td>
<td>16.757(2)  12.197(1)  114.13(1)</td>
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<tr>
<td>V (Å³)</td>
<td>1618.8(3)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
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<td>Dcalc (g cm⁻³)</td>
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<tr>
<td>Scan method</td>
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<tr>
<td>2θ Range (°)</td>
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<tr>
<td>Octants collected</td>
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<tr>
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</tr>
<tr>
<td>Variable scan speed (° min⁻¹)</td>
<td>4–16</td>
</tr>
<tr>
<td>Number of observed data</td>
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</tr>
<tr>
<td>(I_o &gt; 3σ(I_o))</td>
<td></td>
</tr>
<tr>
<td>R value for equivalent reflections</td>
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</tr>
<tr>
<td>ρ(cm⁻³)</td>
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</tr>
<tr>
<td>F(000)</td>
<td>1016.0</td>
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<tr>
<td>Final residuals (for 1200 data)</td>
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</tr>
<tr>
<td>a,b,c values in the weight function</td>
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</tr>
<tr>
<td>w = 1.0(a+bF_w+cF_w²)</td>
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<tr>
<td>Goodness-of-fit for last cycle</td>
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<tr>
<td>Max. Δ/σ for last cycle</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Lattice parameters calculated from 25 high-angle reflections measured at ±2θ (2θ interval 32.43–45.30°).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>R</td>
<td>0.0384</td>
</tr>
<tr>
<td>Rw</td>
<td>0.0527</td>
</tr>
</tbody>
</table>

2.6. Physico chemical measurements

Infrared spectra were obtained with a Perkin–Elmer 16PC Fourier transform spectrometer. Solution ¹H NMR spectra were recorded on a Varian Gemini 2000 spectrometer. Electronic absorption spectra were measured by a Perkin–Elmer Bio 20 instrument equipped with thermostated cuvettes. Chloride selective potentiometric measurements were carried out with a chloride selective electrode interfaced to a standard potentiometer. The instruments were calibrated with a series of sodium chloride samples of known concentration.

2.7. X-ray structure solution and refinement of [ImH][IrCl₄(Im)₂], (IRIM)

Pink–brown crystals of IRIM were obtained from the mother liquor of the reaction. Data collection was performed using a Rigaku AFC5 diffractometer (r.t.); no decay correction was applied. Data were corrected for Lorenz and polarisation effects. An empirical absorption correction, based on azimuthal scans of several reflections, was applied to intensities [9]. The structure was solved by heavy-atom techniques and refined as full matrix in the least-square calculation using local programs [10]. The number of the observations was 1200 (I_o > 3σ(I_o)), and the number of variable parameters was 115 (10.4 observations for each parameter). The full matrix refinement, after introduction of the fixed contribution of 9 H atoms of the imidazoles gave R = 0.0384 and Rw = 0.0527. Additional details of the crystallographic experiments concerning (IRIM) are given in Table 1.

3. Results

3.1. Preparation and characterisation of [ImH][IrCl₄(Im)₂] (IRIM)

IRIM was obtained as the imidazolium salt using a synthetic approach similar to that adopted for the ruthenium(III), ICR, [4] and the rhodium(III) analogues [6]. However, the mild reaction conditions reported for the ruthenium(III) and rhodium analogues, as described above, were not sufficient to obtain formation of the corresponding iridium(III) product even after several days incubation; only refluxing conditions produced appreciable amounts of IRIM. Two different starting materials were used for this synthesis, i.e. H₂[IrCl₄] and hydrated IrCl₃; both materials gave rise to IRIM in similar yields. All IRIM samples obtained from the above described syntheses gave the same elemental analyses. IR spectra and X-ray powder spectra were the same; of course, cell parameters were identical to those obtained for the monocystal. Infrared spectra of [ImH][IrCl₄(Im)₂], (IRIM), show two bands at 326 (ms) and 274 cm⁻¹ (m) attributed to Ir–Cl stretching vibrations and a band at 232 cm⁻¹ (w) tentatively attributed to νIr–N [4]. Additional bands at 1064 (s) and 614 cm⁻¹ (s) are attributed to co-ordinated imidazole whereas other two bands at 1048 (m) and 624 cm⁻¹ (s) are attributed to ImH⁺ [6].

3.2. Molecular structure of [ImH][IrCl₄(Im)₂] (IRIM)

Fig. 1 shows a perspective view of IRIM, and defines the atom numbering scheme for the heavy atoms. Selected bond lengths and angles are given in Table 2. Final positional parameters for non-hydrogen atoms, thermal parameters, hydrogen atomic parameters, and observed and calculated structure factors are available as supplementary material. IRIM is isomorphous with the ruthenium analogue ICR [4]. The asymmetric unit contains half of the complex with the Ir, Cl(1) and Cl(2) atoms lying on a twofold rotation axis; half of the ImH⁺ cation is disordered around a crystallographic inversion centre, resulting into an eight-membered ring and interpreted as a superposition of two five-mem-
3.2.1. Solution chemistry of IRIM

IRIM is sufficiently soluble in water and in the reference physiological buffer (buffer phosphate 50 mM, pH 7.4). Aqueous solutions of IRIM at mM concentration are pale yellow. The electronic spectrum of IRIM is characterised by the presence of two relatively intense bands in the visible, respectively located at 360 (ε = 105 M⁻¹ cm⁻¹) and 410 nm (ε = 87.5 M⁻¹ cm⁻¹), plus a stronger band at 290 nm (ε = 185 M⁻¹ cm⁻¹) and a weaker one at 550 nm (ε = 30 M⁻¹ cm⁻¹). The two former bands are tentatively assigned as chloride-to-iridium charge transfer transitions whereas the band at 290 nm is assigned as an imidazole-to-iridium(III) charge transfer transition and the one at 550 nm as a d–d transition of the iridium(III) ion. The same spectrum is obtained upon dissolving IRIM in the physiological buffer (Fig. 2(a)). Notably, the visible spectrum of IRIM in the buffer, at variance with the case of ICR (Fig. 2(b)), does not change with time over a period of several h at room temperature (Fig. 2(a)).

The 300 MHz ¹H NMR spectrum of IRIM, dissolved in D₂O, (pH 5) is shown in Fig. 3. The spectrum is easily assigned to protons of free and iridium(III) coordinated imidazoles: signals at 8.63 and 7.45 ppm are, respectively assigned to 2H and 4/5H protons of the free imidazolium ion by comparison with the spectrum of [ImH₃][IrCl₆] (data not shown) while signals at 8.21, 7.51 ad 7.20 ppm are assigned to the 2H, 4H and 5H protons of iridium(III) co-ordinated imidazoles. Notably the ¹H NMR spectrum does not undergo significant changes when monitored over 24 h at room temperature (Fig. 3) implying that the [bisimidazole-tetrachloroiridate(III)] anion remains the dominant species in solution for quite some time; this also means that no significant chloride hydrolysis takes place at least during the first hour after dissolution. In nice agreement with this interpretation, the proton spectra of IRIM in D₂O, (pH 5) is shown in Fig. 3. The spectrum is not show any significant change.

This view was further confirmed by potentiometric studies, carried out by use of a chloride-selective electrode. Such showed no time dependent changes of chloride concentration in solution within 24 h implying that chloride is not released by IRIM under physiological conditions.

To further address the stability of the bisimidazole tetrachloroiridate anion in water solutions some additional experiments were carried out. In particular to monitor the redox properties of the iridium(III) centre, freshly prepared solutions of IRIM were treated with oxidizing or reducing agents such as hydrogen peroxide and ascorbic acid. Remarkably, the addition of a stoichiometric amount of either compound did not bring about any change of the visible spectrum of IRIM suggesting that the oxidation state +3 of the iridium centre is highly stable. In addition, the occurrence of possible ligand exchange reactions was monitored analysing the visible spectrum of IRIM upon addition of increasing amounts of sodium thiocyanate. Again,
no spectral change was observed within a few h confirming the high stability of the \([\text{IrCl}_4\text{Im}_2]^-\) chromophore toward ligand substitution reactions. The latter point was further checked by monitoring the effects of the addition of silver nitrate on freshly prepared water solutions of IRIM. Remarkably, the addition of increasing amounts of silver nitrate results into the progressive disappearance of the visible spectrum of IRIM and the appearance of a yellow precipitate. This precipitate corresponds to the poorly soluble \(\text{Ag(IrCl}_4\text{Im}_2)\) species. The \(\text{Ag(IrCl}_4\text{Im}_2)\) pellet may be redissolved by treatment with excess thiocyanate that sequesters the
silver(I) ion as the [Ag(SCN)₂]⁻ complex. Following redissolution, the characteristic visible spectrum of IRIM is observed again (Fig. 4).

4. Discussion

4.1. Synthesis and solid state chemistry of IRIM

Interest in this iridium(III) complex was raised by the observation that the isostructural ruthenium(III) complex, ICR, developed by Keppler and his group, exhibits promising anticancer and antimetastatic properties. So we prepared the iridium(III) analogue of ICR and carried out its chemical characterisation in the solid state and in solution. The above described synthetic approaches, starting from two different materials, namely H₂[IrCl₆] and hydrated IrCl₃, gave rise to IRIM with nearly the same yield. As regard to the reaction conditions employed in both syntheses, they are more drastic compared to those used for the ruthenium and rhodium analogues [4,6]. The inertness of iridium(III) compounds, revealed by the above syntheses, reflects quite well into the extreme inertness of chloride hydrolysis in solution.

As expected, the iridium(III) complex displays structural features that strictly resemble those of the ruthenium(III) complex. The bond lengths are almost the same as in ICR and fall in any case within the range reported in the literature both for Ir–Cl [11,12a,b] and Ir–N [12] bond distances. Thus the two complexes exhibit an almost identical three-dimensional structure and bear the same charge; in this case, they do not undergo chemical transformations in vivo, they should not be recognised as distinct by their potential biological targets.

4.2. Solution chemistry of IRIM

Despite their strict structural relatedness, IRIM and ICR show a far different solution behaviour. Infact, whereas ICR undergoes slow but progressive hydrolysis of the metal co-ordinated chlorides (the half life is about 6 h (at 25°C) under physiological conditions) this process is extremely slower in the case of IRIM so that after 24 h virtually no chloride has dissociated. The inertness of the ligand exchange process makes the solution chemistry and the reactivity of IRIM substantially different from those of ICR and may have important consequences on the biological properties. The biological effects observed for ICR were mainly ascribed to its hydrolysis products and to their covalent binding to biomolecules [13]. In contrast the bisimidazole tetrachloroiridate centre is stable for days, and is resistant towards either oxidation or reduction, or ligand exchange. According to these results, the biological properties of IRIM should be primarily ascribed to the effects that the bisimidazole tetrachloroiridate moiety could produce on biologically relevant targets.

4.3. Implications for the pharmacological activity and conclusions

We have shown above that the iridium(III) analogue of ICR can be prepared and obtained with relatively high yield and purity. Notably, this compound is sufficiently soluble both in water and in buffer solutions so that its biological effects may be conveniently studied. The information obtained so far on the chemical properties of IRIM in solution allow us to state that IRIM exists under physiological conditions as the bisimidazolotetrachloroiridate anion and that such species is stable.

![Fig. 4. Electronic spectrum of IRIM (2 mM) in physiological buffer (NaH₂PO₄ 50 mM pH 7.4) before (a) and after addition (b) of a stoichiometric amount of AgNO₃.](image-url)
for several days at room temperature. We recently described some in vitro biological effects of IRIM in strict comparison to ICR [8]. It was found that both complexes slightly modify DNA structure in solution and exhibit nearly negligible cytotoxic properties toward the reference A2780 tumour cell line. In spite of its modest in vitro cytotoxic effects, it is known that ICR shows a promising profile of antitumour activity towards selected in vivo models. Now, it would be extremely interesting to establish whether IRIM in vivo retains, at least partially, the promising biological properties of ICR. If this is true, it is straightforward to propose that the monoanionic bisimidazolotetra-
chlorometallo(III) species is intrinsically biologically active; in the opposite case, it is inferred that chloride hydrolysis and the consequent ability to form covalent bonds with biomolecules represent an essential requirement for the biological effects of ICR. From this reasoning it also emerges that IRIM, apart from its intrinsic biological properties, may offer good opportunities to clarify the mechanism of action of ICR.

5. Supplementary material

The listing of final positional parameters, anisotropic thermal parameters, hydrogen atoms positions and parameters and structure factors tables for IRIM are available from the authors.

Acknowledgements

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References