

Complexes of Tetraiodofluorescein with Some Transition Metals and their Antitumour Activity

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Received 16 November 2000

Cobalt(II), nickel(II), copper(II), and zinc(II) complexes of tetraiodofluorescein (H₂L) have been prepared and characterized by means of elemental analysis, molar conductivity, TG-DTA, IR, UV VIS, and ¹H NMR spectra. The general formula for the complexes is ML · nH₂O where L = tetraiodofluorescein (2,7-OH group deprotonated and the lactonic ring opened); M = Zn, Cu, Co, and Ni; n = 0, 4, 5, and 2. H₂L and its complexes have been studied for their possible antitumour activity against Hep-2 pharynx cancer cells *in vitro*.

Fluorescein and tetraiodofluorescein (H₂L) have been used in analysis of metal ions because of their ability to form complexes with metal ions in solution [1, 2]. Complexes of fluorescein with some metal ions have already been prepared, of which the cobalt(II), copper(II), and zinc(II) complexes have antibacterial activity and the mercury(II) complex has antiseptic function [3, 4]. Solid complexes of metals with H₂L, however, have rarely been reported. As H₂L itself exhibits antitumour activity [5, 6], and some transition metals play a vital role in a vast number of widely differing biological processes [7, 8], the study of the synthesis of transition metal complexes with H₂L and their antitumour activity is appealing.

In this paper, we report on the preparation and characterization of cobalt(II), nickel(II), copper(II), and zinc(II) complexes with H₂L and discuss their antitumour activity for the first time.

EXPERIMENTAL

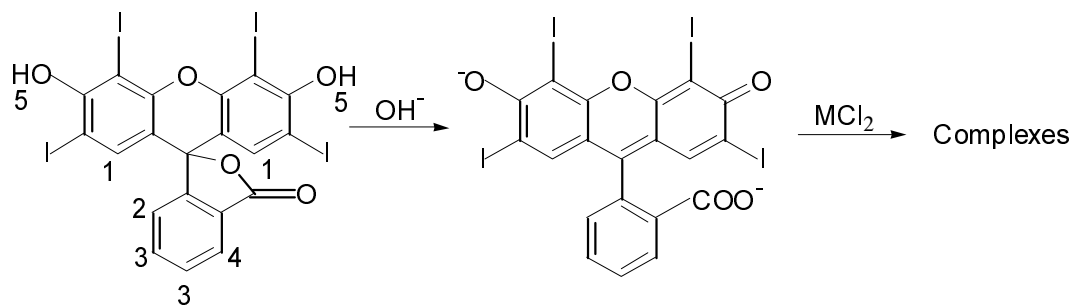
The starting compounds included cobalt(II) chloride hexahydrate, nickel(II) chloride hexahydrate, zinc(II) chloride, and copper(II) chloride dihydrate, H₂L (Merck, USA), RPMI1640 medium (Gibco, USA), inactive fetal calf serum (FCS, Lanzhou Institute of Biological Products, Ministry of Health, China), and Hep-2 human pharynx cancer cells (The Fourth Military Medical University, China). Solvents and reagents used were of anal. grade.

The elemental analyses of the complexes were obtained using a Varian EL analyzer. The metal content was determined by titration with EDTA. Electrolytic conductance measurements of compounds (*c* ≈ 10⁻³ mol dm⁻³) were made with a DDS-11A digital conductometer with DMSO as solvent at 25 °C. IR spectra were recorded on a Nicolet 170SX FTIR spectrophotometer using KBr discs in the range $\tilde{\nu}$ = 200–4000 cm⁻¹. The electronic spectra were recorded on a Shimadzu UV-240 spectrophotometer in the region of 190–450 nm and 400–900 nm. ¹H NMR spectra were measured with an FT-80A nuclear magnetic resonance instrument using DMSO-*d*₆ as solvent and TMS as internal reference. TG-DTA measurements were made in a nitrogen atmosphere between room temperature and 800 °C using a Dupont 1090-B thermal analyzer. The Gouy method was employed to measure magnetic moment.

Complexes I–IV

H₂L (2 g; 2.4 mmol) was dissolved in 20 cm³ of absolute ethanol and mixed with 10 cm³ of aqueous solution containing NaOH (0.072 g; 1.8 mmol), then with stirring the solution was added dropwise to 20 cm³ of an aqueous solution containing MCl₂ (1 mmol). Along with quick formation of precipitates the solution was stirred continuously for 2 h. The precipitates were isolated by filtration, washed several times with 95 % ethanol, dried in vacuum to constant mass. Yield: ≥ 85 %.

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Scheme 1

Table 1. Characterization of the Complexes

Compound	Formula	$w_i(\text{calc.})/\%$ $w_i(\text{found})/\%$			Decomp. temp. °C	Molar conductivity $\Omega^{-1}\text{cm}^2 \text{mol}^{-1}$	Yield %	μ_{eff} B.M.	Colour
		Metal	C	H					
I	$\text{Zn}(\text{C}_{20}\text{H}_6\text{I}_4\text{O}_5) \cdot 2\text{H}_2\text{O}$	6.99	25.68	1.08	441.4	2.11	87	Diamagnetic	Bright red
		7.15	26.00	1.22					
II	$\text{Cu}(\text{C}_{20}\text{H}_6\text{I}_4\text{O}_5)$	7.08	26.77	0.67	466.5	5.69	86	1.81	Dark red
		7.12	26.72	0.68					
III	$\text{Co}(\text{C}_{20}\text{H}_6\text{I}_4\text{O}_5) \cdot 4\text{H}_2\text{O}$	6.11	24.90	1.46	382.1	1.99	88	4.70	Red
		6.21	25.36	1.64					
IV	$\text{Ni}(\text{C}_{20}\text{H}_6\text{I}_4\text{O}_5) \cdot 5\text{H}_2\text{O}$	5.97	24.45	1.64	365.7	3.50	88	Diamagnetic	Yellow-red
		6.17	24.58	1.81					

Antitumour Activity Tests

In vitro, the antitumour activity tests of H_2L and its complexes against Hep-2 pharynx cancer cells were performed using the micropore plate method [9, 10]. Hep-2 pharynx cancer cells were propagated in RPMI1640 medium supplemented with 10 % FCS and antibiotics at 37°C in air with 5 % CO_2 and a culture medium containing $3 \times 10^5 \text{ cm}^{-3}$ suspension of cultivated tumour cells was prepared. 0.05 m^3 of the cells suspension was put into each well of the plate, 0.05 cm^3 of ethanol solution of the tested chemicals was also added to give final concentration $c/(\mu\text{g cm}^{-3}) = 0.1, 1.0, 10, \text{ and } 100$. An equal volume of the corresponding solvent ethanol was used as a control. The plates were cultivated for 48 h at 37°C under saturated humidity and 5 % CO_2 atmosphere and then dyed with trypan-blue, the living cells were counted under a microscope. The inhibitory ratios (IR) were calculated as follows

$$\text{Inhibitory ratio} = 100 \times \frac{n_C - n_T}{n_C}$$

where n_C is the number of living cells of control and n_T is the average number of living cells of dosed group. The logarithm of concentration numerical values of the compounds and IR were treated by linear regression analysis and IC_{50} values were obtained from the equations. IC_{50} was defined as drug concentration required for reducing the number of living cells by 50 %.

RESULTS AND DISCUSSION

Syntheses of the complexes are shown in Scheme 1. Elemental compositions, colour, molar conductance, molecular formulae, and magnetic susceptibilities of the newly prepared compounds I–IV are listed in Table 1. The elemental analyses data show that the complexes have the general formula $\text{ML} \cdot n\text{H}_2\text{O}$ where L = tetraiodofluorescein (2,7-OH group deprotonated and the lactonic ring opened); M = Zn, Cu, Co, and Ni; $n = 0, 4, 5, \text{ and } 2$. The complexes are air-stable and soluble in methanol, ethanol, DMSO, THF, and acetone, but not in benzene, ether, and water. The molar conductivity values of the complexes in DMSO at 25°C vary from 1.99 to $5.69 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$, indicating that they are nonelectrolytes in DMSO [11].

The principal IR bands of H_2L and its complexes are listed in Table 2, along with those of disodium salt of H_2L (Na_2L). The $\nu(\text{OH})$ frequency of H_2L was not observed in the complexes, which indicates coordination of the —OH group of H_2L after deprotonation to form the M—O bond in the complexes. The $\nu(\text{C}=\text{O})$ band of lactone of H_2L disappears upon complexation. However, two new bands corresponding to $\nu_{\text{as}}(\text{COO}^-)$ and $\nu_{\text{s}}(\text{COO}^-)$ vibrations are observed at $1540\text{--}1546 \text{ cm}^{-1}$ and $1332\text{--}1345 \text{ cm}^{-1}$. This shows that the carboxylate group of H_2L coordinated to the metal ion after the lactonic ring was opened. Since $\Delta\tilde{\nu}$ ($\Delta\tilde{\nu} = \tilde{\nu}(\nu_{\text{as}}(\text{COO}^-)) - \tilde{\nu}(\nu_{\text{s}}(\text{COO}^-))$) of the com-

Table 2. IR Data of H₂L and its Complexes

Compound	$\tilde{\nu}/\text{cm}^{-1}$									
	$\nu(\text{OH})$	$\nu(\text{OH})(\text{H}_2\text{O})$	$\nu(\text{C}=\text{O})^a$	$\nu(\text{C}=\text{O})^b$	$\nu_{\text{as}}(\text{COO}^-)$	$\nu_{\text{s}}(\text{COO}^-)$	$\nu(\text{C}-\text{O}-\text{C})$	$\rho_{\text{r}}(\text{H}_2\text{O})$	$\rho_{\text{w}}(\text{H}_2\text{O})$	$\nu(\text{M}-\text{O})$
H ₂ L	3424		1755				1207			
Na ₂ L				1600	1540	1340	1233			
<i>I</i>		3381		1606	1545	1332	1241			482
<i>II</i>		3343		1608	1540	1340	1246			506, 468
<i>III</i>		3346		1601	1544	1345	1229	768	619	474
<i>IV</i>		3330		1604	1546	1340	1240			474

a) $\nu(\text{C}=\text{O})$ of lactone; *b*) $\nu(\text{C}=\text{O})$ of the quinoid carbonyl.

Table 3. Thermal Data of Complexes

Compound	Water loss		Decomp. temp. °C	Residue	
	Temp./°C	$-\Delta w/\%$		$-\Delta w/\%$	Formula
<i>I</i>	82.5	3.36	441.4	9.28	ZnI ₂
<i>II</i>			466.5	7.54	CuI ₂
<i>III</i>	73.4	8.60	382.1	8.91	CoI ₂
<i>IV</i>	71.2	9.76	365.7	10.64	NiI ₂

plexes are close to that of Na₂L, the carboxylate group is coordinated to the metal ion with bridging bidentate mode [12]. The complexes display lower frequency $\nu(\text{C}=\text{O})$ of the quinoid carbonyl at 1600–1608 cm⁻¹, Na₂L at 1600 cm⁻¹ (overlapped with the aromatic skeletal carbon–carbon stretch), indicating that the oxygen atom in the quinoid carbonyl participated in the bond formation with the metal ions. The stretching vibration of the ligand ring C–O–C (1207 cm⁻¹) shifts to higher frequency in the complex (1229–1246 cm⁻¹), excluding the possibility of the coordination of this oxygen to metal [13]. The broad feature at *ca.* 3330–3381 cm⁻¹ in the spectra of the complexes is attributed to the hydroxyl stretching mode of water molecules. In addition, two bands at 768 cm⁻¹ and 619 cm⁻¹ of the complex *III* suggest that some of the water molecules are coordinated to Co(II) ion. By comparison of the far-IR spectra of the complexes with that of H₂L, new peaks appear at 468–506 cm⁻¹, indicating the formation of the M–O bond.

The ¹H NMR spectrum of H₂L exhibits five singlets at $\delta = 7.40, 7.00, 7.61, 7.99,$ and 10.20 assigned to H-1, H-2, H-3, H-4, and H-5, respectively. When coordinated to the metal ion in the complexes, the singlet of the ligand OH hydrogen atom at $\delta = 10.20$ disappeared, *i.e.* that ligand OH hydrogen atom is replaced by metal ion on complex formation. The δ values of hydrogens 2, 3, and 4 were shifted by 0.34, 0.08, 0.14 to lower field in the complexes, which is due to the decreasing of the electron density of the benzene ring where the hydrogens 2, 3, and 4 are located. Because of the coordination of ligand OH after deprotonation, the electron density of the corresponding benzene rings increases and therefore the δ values of hydrogen 1 are

shifted by 0.22 to higher field.

The thermal analytical data are given in Table 3. The endothermic peaks of complexes *I* and *IV* corresponding to water loss in the DTA curve (at 82.5°C and 71.2°C, respectively) suggest that the water molecules are present as crystal water. Two endothermic peaks of complex *III* corresponding to water loss in the DTA curve (at 73.4°C and 130°C, respectively) suggest that the water molecules are either coordinated to cobalt(II) ion or are present as crystal water. The mass loss of complex *II* appears at 191°C, indicating that it does not contain water molecules. The exothermic peaks of the complexes show the higher decomposition temperature (around 365–466°C) than that of the ligand (303°C), indicating the former are more stable than the latter. The complexes decompose completely at around 500°C. The residues are metal iodides, consistent with that of Na₂L [14].

The important electronic absorption bands of H₂L and the complexes were recorded in DMSO and in nujol mull, two absorption bands at 32 790 cm⁻¹ and 20 830 cm⁻¹ were seen in the spectrum of the H₂L and were attributed to $\pi \rightarrow \pi^*$ transition of the benzene ring and $n \rightarrow \pi^*$ transition of the C=O group, respectively. In the spectra of complexes, red shifts were observed and the two bands appeared at 30 490 cm⁻¹ and 19 600 cm⁻¹, which suggests the coordination of H₂L to the metals. A broad band with a maximum at 16 640 cm⁻¹ for the copper(II) complex in nujol mull may be assigned to ${}^2B_{1g} \rightarrow {}^2A_{1g}$, suggesting the square-planar geometry around the copper(II) ion [15]. Also, the magnetic moments of 1.81 B.M. can be taken as additional evidence for the square-

Table 4. Data of Inhibitory Ratio of H₂L and its Complexes Against Hep-2 Cells

Compound	Concentration	Inhibitory ratio/%	IC ₅₀
	$\mu\text{g dm}^{-3}$		
H ₂ L	0.1	5.5	23.10
	1.0	12.0	
	10	30.2	
	100	74.5	
I	0.1	8.5	6.94
	1.0	22.1	
	10	52.3	
	100	82.6	
IV	0.1	8.1	8.31
	1.0	25.2	
	10	49.7	
	100	77.8	

planar configuration [16]. The cobalt(II) complex exhibits a magnetic moment of 4.70 B.M., suggesting that it is a paramagnetic high-spin complex with octahedral geometry [17]. The electronic spectrum of cobalt(II) complex also gives two bands corresponding to octahedral geometry of the cobalt(II) ion, the bands appear at $15\,380\text{ cm}^{-1}$ and $19\,400\text{ cm}^{-1}$, which can be assigned to the ${}^4T_{1g}(\text{F}) \rightarrow {}^4A_{2g}(\text{F})$ and ${}^4T_{1g}(\text{F}) \rightarrow {}^4T_{1g}(\text{P})$ transition, respectively [18]. The electronic spectrum of the nickel(II) complex shows two bands at $15\,400\text{ cm}^{-1}$ and $22\,220\text{ cm}^{-1}$ assigned to the transitions of ${}^1A_{1g} \rightarrow {}^1B_{1g}$ and ${}^1A_{1g} \rightarrow {}^1B_{3g}$ in a square-planar geometry [19]. The diamagnetic nature provides strong evidence for the square-planar geometry [20].

Data on effects of H₂L and its complexes against Hep-2 cells are listed in Table 4. The results show that H₂L and its complexes have dose-dependent inhibitory activity against Hep-2 cells. Their inhibitory ratios are greatly enhanced at higher concentration. Also, it has been observed that the complexes show obviously stronger effect than the ligand.

Acknowledgements. This project was supported by the Natural Science Foundation of Gansu Province (ZS991-A23-059-Y) and China Innovation Centre for Life Science (JB 00980462).

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