Synthesis, characterisation and antitumour activities of gallic acid hydrazone and its rare earth complexes

Ling Zhang[a]*, Jian-Hui Zhang[a] and Da-yuan Zhu[b]

[a]*College of Environmental and Chemical Engineering, Shanghai University, Shanghai, 201800, P. R. China*

[b]*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, P.R. China*

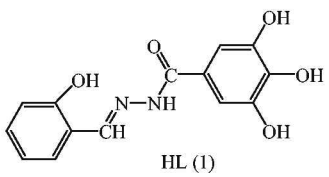
A new gallic acid hydrazone, 3,4,5-trihydroxybenzoyl salicylaldehyde hydrazone, and its four rare earth complexes, $[LnL(OAc)_2] \cdot H_2O$ (Ln = La^{3+}, Sm^{3+}, Tb^{3+}, Dy^{3+}), have been synthesised and characterised on the basis of elemental analyses, molar conductivity, IR and ^1H NMR spectra. The antitumour activities of the prepared compounds have also been evaluated. The results indicate that Gallic acid hydrazone and its four complexes synthesised possess antitumour activity to some extent, and the four complexes show higher antitumour activity against the human leukaemia cell line HL-60 than the ligand. The antitumour activity of the gallic acid hydrazone can be improved by the formation of the rare earth complexes.

Keywords: synthesis, hydrazone, rare earths, complexes, antitumour activity

Gallic acid and its related compounds are widely distributed in plants.[1] It has been reported to possess anticarcinogenic, antioxidative, antimutagenic, anti-allergic and anti-inflammatory activities. Gallic acid has been a building block of choice for different pharmaceutical leads due to the presence of this moiety in several bioactive natural products.

Hydrazones are an important class of organic compounds,[2,3] some of which show significant biological activities. Rare earth elements have been studied for their pharmaceutical activities. Moreover in many cases, it has been suggested that some biological activities of organic compounds are increased by the coordination with rare earths.[4]

Some gallic acid hydrazones have been synthesised in our research group.[5] In connection with our research programme directed toward the synthesis of novel hydrazones and their rare earth complexes with potential biological activity,[6,7] we describe the synthesis of a new chelating ligand, 3,4,5-trihydroxylbenzoyl salicylaldehyde hydrazone (**1**), and its four rare earth complexes, $[LnL(OAc)_2] \cdot H_2O$ (Ln = La^{3+}, Sm^{3+}, Tb^{3+}, Dy^{3+}). The antitumour activities of all the compounds synthesised were also evaluated against *HL-60* and *A-549*.



HL (1)

Experimental

All chemicals used in this work were of analytical reagent grade. Salicylaldehyde was freshly distilled before use. The rare earth acetate $Ln(OAc)_3 \cdot 5H_2O$ was prepared from Ln_2O_3 (> 99.95%) by the standard method.

The melting points of the compounds were determined on an X-4 microscopic melting point apparatus (made in China) and are uncorrected. Elemental analyses were carried out on a Vario EL elemental analyser. IR spectra were obtained in KBr disc on a Nicolet FT-IR 5DX spectrometer in the 4000–4400 cm^{-1} region. ^1H NMR spectra ($CHCl_3$-*d*) were recorded on a Bruker AC-80A instrument with TMS as an internal standard. Mass spectra were performed on a VG-7070E spectrometer (made in USA, EI at 70 eV). All conductivity measurements were performed in DMF with a DDS-11A conductometer (made in Shanghai, China) at 25 °C.

Preparation of the ligand (**1**)

3,4,5-Trihydroxybenzoylhydrazide was synthesised by refluxing methyl 3,4,5-trihydroxybenzoate (20 mmol, 3.68 g) with hydrazine hydrate (30 mmol, 15 ml) in ethanol (60 ml) for ~7 h. A white compound separated on standing over night. This was filtered and washed with distilled water and ethanol to give 3,4,5-trihydroxybenzoylhydrazide (2.98 g, 81% yield); Anal. Calcd for $C_7H_8N_2O_4$: C 45.30, H 4.81, N 15.14; found C 45.65, H 4.35, N 15.22%.

To an ethanol solution (25 ml) of 3,4,5-trihydroxylbenzoylhydrazide (10 mmol, 1.84 g), salicylaldehyde (10 mmol, 1.10 g) was added with stirring at room temperature. Then the mixture was refluxed on an oil bath for 6 h. After cooling to room temperature over night, the precipitated solid was filtered, washed with ethanol and recrystallised from absolute ethanol to give the ligand 3,4,5-trihydroxybenzoyl salicylaldehyde hydrazone (1), (2.16 g, 75% yield); m.p. 246–247 °C. ^1H NMR (DMSO-d_6, 300 MHz) δ: 11.85, 11.46, 9.28 (3H, s, OH), 8.96(1H, s, NH), 8.57 (1H, s, CH), 7.49–7.46 (2H, d, ArH), 7.30–7.26 (3H, t, ArH), 6.97-6.89 (2H, s, ArH), 3.40 (H_2O); MS (70 eV) *m/z* (%): 288 (M^+, 31), 169 (35), 153 (100), 136 (56), 125 (56), 79 (56); IR (cm^{-1}): 3220 (NH/OH), 1650 (C=O), 1601 (C=N), 1274 (C-O), 956 (N–N).

Preparation of the complexes

A powder of $Ln(OAc)_3 \cdot 5H_2O$ (0.2 mmol) was added to the solution of the ligand (**1**) (0.0576 g, 0.2 mmol) with stirring in hot anhydrous ethyl alcohol (15 ml). During the reaction, the solid salts dissolved and the colour of the solution changed from colourless to light yellow; then, after 30 min, a yellow solid appeared gradually. Subsequently, the reaction mixture was heated on an oil bath and refluxed. About 5 hours later, the solid complexes were obtained by hot filtration, washed several times with water, anhydrous ethyl alcohol and anhydrous diethyl ether, finally dried *in vacuo*.

Testing of the antitumour activity

All of the compounds synthesised were tested for their antitumour activities *in vitro* against the human leukaemia cell line *HL-60* and the human lung epithelial cell line *A-549* at concentrations of 10^{-4}, 10^{-5}, 10^{-6} μg ml^{-1} employing methyl-thiazol-tetrozolium (MTT) and sulforhodamine B (SRB) methods.[8]

Results and discussion

The elemental analyses and some physical properties of the ligand and the complexes are given in Table 1.

The analytical results show that the ligand coordinates to the central metal ions in the enol form of the hydrazone group, and the composition of its complexes may be expressed as $[Ln\ L(OAc)_2] \cdot H_2O$ (Ln = La^{3+}, Sm^{3+}, Tb^{3+}, Dy^{3+}).

The ligand is soluble in common organic solvents, such as ethanol, methanol, acetone and chloroform, while the complexes are insoluble in water and common organic solvents and only soluble in DMF and DMSO. The low molar conductance (0.78–1.29 $\Omega^{-1}cm^2mol^{-1}$) of the complexes suggests that they are non-electrolytes in DMSO solution.[9]

IR spectra

The spectral data of the compounds and their tentative assignments are shown in Table 2. The bonding of the ligand to the metal ions was investigated by comparing the IR spectra of the free ligand with its metal complexes.

The following conclusions in this regard can be drawn.

(i) The IR spectra of complexes show that the ν(C=O) band at 1650 cm^{-1} in the spectra of the free ligand disappeared, which

* Correspondent. E-mail: zhanglinglzu@163.com

Table 1 Elemental analysis data and physical properties of the compounds

Compound (Formula)	Colour	Yield (%)	Found (Calcd)/%			$\Lambda_m(\Omega^{-1}cm^2mol^{-1})$[a]
			C	H	N	
HL (1)	White	75	58.25 (58.33)	4.35 (4.17)	9.65 (9.72)	
La $L(OAc)_2 \cdot H_2O$	Yellow	55	38.62 (38.44)	3.59 (3.38)	4.76 (4.98)	0.95
Sm $L(OAc)_2 \cdot H_2O$	Yellow	70	37.66 (37.67)	3.48 (3.31)	4.51 (4.88)	0.78
Tb $L(OAc)_2 \cdot H_2O$	Yellow	65	37.09 (37.14)	3.36 (3.27)	4.64 (4.81)	0.85
Dy $L(OAc)_2 \cdot H_2O$	Brown	48	36.56 (36.89)	3.45 (3.25)	4.55 (4.78)	1.29

[a]Molar conductance, concentration of the solution in DMSO was about 10^{-3} mol dm^{-3}.

Table 2 IR spectra data of the ligand HL (**1**) and its complexes

Compound	ν_{H2O}	$\nu_{NH/OH}$	$\nu_{C=O}$	$\nu_{C=N}$	CH_3COO^-	
					vas	vs
HL (**1**)		3220_w	1650_s	1606_s		
$LaL(OAc)_2 \cdot H_2O$	3413_b	3211_w		1581_s	1473_m	1407_m
$SmI(OAc)_2 \cdot H_2O$	3432_b	3208_w		1572_s	1468_m	1405_m
$TbL(OAc)_2 \cdot H_2O$	3423_b	3210_w		1572_s	1469_m	1397_m
$DyL(OAc)_2 \cdot H_2O$	3431_b	3205_w		1572_s	1472_m	1388_m

*b = broad, w = weak, s = strong, m = middle

suggests the bonding of the ligand through the enol oxygen in the enol form of the hydrazone group.[10]

(ii) The spectra of the ligand exhibit a band at 3220 cm^{-1} of the ν(NH/OH) vibration, though in its complexes it shifted to lower frequency by 6–15 cm^{-1}. This indicates that the phenol OH group takes part in the coordination without deprotonation and breaks up the hydrogen bond between the OH (A) and NH groups.

(iii) The ν(C=N) band was observed at 1606 cm^{-1} in the spectra of the free ligand, while in the complexes the ν(C=N–N=CH–) bands due to the azomethine linkage were shifted to lower frequency by 20–29 cm^{-1}, indicating that the ligand coordinated to the metal ions via the azomethine nitrogen.[11]

(iv) The appearance of two new absorptions at ~1470 and ~1399 cm^{-1} in the spectra of the complexes corresponding to $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$, $\Delta\nu(\nu_{as}(COO^-) - \nu_s(COO^-))$ is 63–73 cm^{-1}, indicates that the acetate group has coordinated with the metal ions in the bidentate form.[12]

(v) In the complexes the bands at 3431 cm^{-1}, which are absent in the free ligand, are assigned to $\nu(H_2O)$ vibrations, showing that there are crystal water molecules in the complexes.

^1H NMR spectra

Since the majority of the coordination compounds are paramagnetic, only the ^1H NMR spectra of the ligand (HL) (**1**) and La $L(OAc)_2 \cdot H_2O$ were obtained. The ligand exhibits signals at δ (ppm) 11.85, 11.46, 9.28 (3H, s, OH), 8.96(1H, s, NH), 8.57 (1H, s, CH), 7.49–7.46 (2H, d, ArH), 7.30–7.26 (3H, t, ArH), 6.97–6.89 (2H, s, ArH), while in the La (III) complex the proton peak of the N-H group disappeared, which suggests that the hydrazone group coordinates to the metal ions through the enol form. The appearance of the signal at 1.76 (s, 6H) in the La (III) complex attributed to the acetate group proves that two acetate ions are coordinated to the metal ion through the carboxylic oxygen. The appearance of the signal at 3.43 ppm confirms the presence of water in the complexes. The proton peak of the OH group cannot be observed perhaps due to the exchange of active hydrogen with the water in the complexes. The downfield shift of the other signals is attributed to the increased conjugate system and the deshielding action.

Thus, the ^1H NMR studies reinforce the conclusions drawn from the IR spectra.

Antitumour activity

The method of MTT and SRB was used to test the preliminary *in vitro* antitumour activity of the synthesised compounds. The inhibitory rate data of the compounds on tumour cell line *HL-60* and *A-549* are sumarised in Table 3.

Preliminary bioassays indicate that all the synthesised compounds were active against *HL-60* and *A-549* at the concentration of 10^{-4} μg ml^{-1}. The four rare earth complexes show higher antitumour activity against *HL-60* than the ligand (1), though only Sm(III), Tb(III) and Dy(III) complexes showed higher antitumour activity against *A-549* cell line. It is very interesting that the Dy(III) complex possesses strong antitumour activity against *HL-60* compared to all the other compounds, and its inhibition rate was 62.0% at concentration of 10^{-6} μg ml^{-1}.

Conclusions

From the above discussions, the following conclusions can be drawn:

(1) The metal ions are seven-coordinated by the ligand (**1**) with O, O and N donors from the phenolic OH via deprotonation of the enol oxygen of the hydrazone group (–N=C–O) and the azomethine (CH=N) groups, respectively, and by the four carboxylic oxygens from two acetate groups (CH_3COO^-) in the bidentate form.

(2) The ligand HL (**1**) and its four rare earth complexes showed antitumour activity against HL-60 and A-549, especially La(III), Sm(III), Tb(III) and Dy(III) complexes exhibited higher antitumour activity against HL-60 cell line than the ligand. The antitumour activity of the ligand (**1**) can be improved by the formation of the complexes with rare earth metals.

Table 3 Inhibitory effect (%) of the compounds on HL-60 and A-549 tumour cell line

Compound	HL-60[a]			A-549[b]		
	Concentration/μg ml^{-1}			Concentration/μg ml^{-1}		
	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
HL (1)	48.1	48.4	30.5	38.2	0	9.4
$LaL(OAc)_2 \cdot H_2O$	100	53.1	0	37.0	0.3	0
$SmI(OAc)_2 \cdot H_2O$	57.6	14.2	10.5	46.6	14.2	10.5
$TbL(OAc)_2 \cdot H_2O$	76.2	0	0	82.0	0.6	0
$DyL(OAc)_2 \cdot H_2O$	68.1	76.9	62.0	53.0	0	7.5

[a]72 h, MTT method; [b]72 h, SRB method.

We thank the Education Committee Foundation of Shanghai (Grant No. 2006AZ060, Grant No.37-0111-07-015) for financial support.

Received 7 July 2008; accepted 11 September 2008
Paper 08/0041 doi:10.3184/030823408X371326
Published online: 10 November 2008

References

1 F.A.M. Silva, F. Borges and C. Guimaraes, *J Sci Food Agric.,* 2000, **48**, 2122.
2 T.C. Maria, C. Cenzo and L. Valentina, *Bioorg. Med. Chem.,* 2006, **14**, 366.
3 W. Xiao, H.-Y. Zhang, Z.-L. Lu, C.-Y. Su and B.-S. Kang, *Chem. Abstr.*, 135: 5250x (2001).
4 K. Nehru, P. Athappan and G. Rajagopal, *Trans. Met. Chem.,* 2001, **26**, 652.
5 D.-Y. Zhu and L. Zhang, *Chem. Abstr.,* 2007, **146**, 521546.
6 L. Zhang, N. Tang, J.-G. Fang and M.-Y. Tan, *J. Chin. Rare Earth Soc.,* 2003, **21**, 595.
7 L. Zhang, N. Tang, J-G. Fang and M-Y. Tan, *Syn.React. Inorg. Met-org. Chem.,* 2004, **34**, 163.
8 Z.-Q. Situ and J.-Z. Wu, *Culture of cell.* Beijing; World Book Press Company, 1996(in Chinese).
9 W.J. Geary, *Coord. Chem. Rev.*, 1971, **7**, 81.
10 K.K. Narang and V.P. Singh, *Trans. Met. Chem.*, 1993, **18**, 287.
11 L. El-Sayed and M.F. Iskander, *J. Inorg. Nucl. Chem.*, 1971, **33**, 435.
12 K. Nakamoto, *Infrared and Raman spectra of inorganic and coordination compounds*. John Wiley, New York, 1976, Chap. 3.