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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF RARE EARTH COMPLEXES OF 1-PHENYL-3-METHYL-5-HYDROXY-4-PYRAZOLYL PHENYL KETONE ISONICOTINOYL HYDRAZONE[a]

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ABSTRACT

The ligand, 1-phenyl-3-methyl-5-hydroxy-4-pyrazolyl phenyl ketone isonicotinoyl hydrazone (H_2L), was prepared by condensation of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone with isoniazid. Seven complexes of rare earths with H_2L have been synthesized and characterized on the basis of elemental analyses, IR, UV, ^1H NMR spectra and thermal analyses. The general formula of the complexes is $RE(HL)_3 \cdot 3.5H_2O$ (where RE = La^{3+} – Gd^{3+}). It has been discovered that the ligand and its complexes of the light rare earth metals possess antioxidative activity and inhibitory action on lipid peroxidation.

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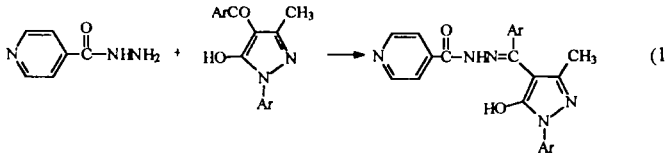
INTRODUCTION

Isoniazid is a good antituberculosis drug, because of its small size isoniazid has strong permeability and a high curing effect. It was reported that metal complexes of hydrazones derived from isoniazid and 2-oxopropionic acid possess apparent antitumor activity[1-3] as well as scavenger effects on $OH^{\cdot}$ and $O_2^{-\cdot}$ radicals[4]. Hydrazone ligands derived from isoniazid with heterocyclic β-diones have a certain inhibitory activity on bacteria[5], and in many cases it has been suggested that the biological activity of their complexes is superior compared to that of the ligands[6-8]. This idea led us to synthesize 1-phenyl-3-methyl-5-hydroxy-4-pyrazolyl phenyl ketone isonicotinoyl hydrazone (H_2L) and its rare earth complexes with a view to evaluating their biological activity. The ligand was prepared by the reaction of eq (1).



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EXPERIMENTAL

Materials

Nitroblue tetrazolium (NBT), methionine (MET), vitamin B_2 ($VitB_2$), tris (hydroxy-methyl)-aminomethane (Tris), thiobarbituric acid (TBA) and ascorbic acid were purchased from Sigma Chemical Co. 1-Phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP), isoniazid, trichloroacetic acid (TCA), and 3-methylthiopropionaldehyde (MTPA) were products of China. All chemicals used were of analytical grade. The hydrated rare earth(III) chlorides were prepared by dissolving RE_2O_3 in concentrated HCl, then crystallizing the products. Tris·HCl and KH_2PO_4–K_2HPO_4 buffers were prepared with deionized distilled H_2O.

Physical Measurements

Carbon, hydrogen and nitrogen were analyzed on a Carlo Erba 1106 elemental analyser. The metal contents of the complexes were determined by titration with

EDTA[9]. Infrared spectra (4000–400 cm^{-1}) were determined with KBr optics on a Nicolet 5DX IR spectrophotometer. Ultraviolet spectra were recorded on a Shimadzu UV-240 spectrophotometer. The thermal behavior was monitored on a PCT-2 differential thermal analyser. ^1H NMR spectra were measured on a Nicolet FT-80A NMR spectrophotometer, using TMS as a reference in DMSO-d_6. The molar conductance values were determined in ethanol on a model DSS-11A conductivity meter. Gas chromatography was performed using a Shimadzu GC-8A gas chromatographic instrument.

Preparation of the Ligand

A quanlity of 0.549 g of isoniazid (4 mmol) and 1.113 g of PMBP (4 mmol) were dissolved in ethanol (150 mL) and refluxed on a water bath for 1 h. The color of the solution changed from initial light yellow to deep red. After cooling to room temperature, a large amount of red precipitate separated out. The product was purified by recrytallizing it in anhydrous ethanol; yield, 1.256 g (79%); m.p. 244–245° C. Anal. Found: C, 69.43; H, 4.89; N, 17.08. Calc. for $C_{23}H_{19}N_5O_2$ (397.44): C, 69.50; H, 4.82; N, 17.26%. ^1H NMR (DMSO-d_6), δ (ppm): 1.50 (3H, singlet, CH_3); 7.10–7.90 (11H, multiplet, 2 phenyl,-NH-); 8.20 (2H, doublet, J = 6Hz, H^3, H^5 of pyridine ring); 8.76 (2H, broad, H^2, H^6 of pyridine ring).

Preparation of the Complexes

A quanlity of 0.79 g of the ligand H_2L (2 mmol) was added to water (40 mL), the pH of which was adjusted to 7 by the addition of an aqueous solution of NaOH (10%); the solution turned light-yellow. Then a solution of $RECl_3 \cdot 6H_2O$ (1 mmol) in water (10 mL) was added dropwise to the system. After stirring at 50° C for 0.5 h, a yellow precipitate was formed which was seperated by filtration, and washed several time with water and hot ethanol, respectively, and dried in a vacuum.

Scavenger Test of Superoxide Radicals

The superoxide radicals ($O_2^{-\cdot}$) were produced by the system of MET/VitB_2/-NBT[10–11]. Because NBT can be reduced quantitatively to blue formazan by $O_2^{-\cdot}$, the amount of $O_2^{-\cdot}$ and the suppression ratio for $O_2^{-\cdot}$ can be calculated by measuring the absorbance at 560 nm[10–12].

The solutions of MET, $VitB_2$ and NBT were prepared with 0.01 M Tris·HCl buffer (pH = 8) avoiding light. The reaction mixture (4.5 mL) included MET (13.5 μM), NBT (1.0 μM), $VitB_2$ (0.1 μM) and the tested compound (0.056 μM). After illuminating with a fluorescent lamp at room temperature for 10 min the absorbance (Ai) of the mixture was measured at 560 nm on a UV-240 spectrophotometer. The sample without the tested compound was used as the control, its absorbance is A_0. The suppression ratio was calculated on the basis of $(A_0 - A_i)/A_0$.

Scavenger Test of Hydroxyl Radicals

Hydroxyl radicals (OH^*) were produced by the system H_2O_2/Fe^{2+}–EDTA/ascorbic acid[11], its amount was calculated by measuring the amount of ethene produced from OH^* reacting with MTPA[11,12]. The reaction mixtures (4.5 mL) involved MTPA (50.0 μM), 1:2 Fe^{++} – EDTA (83.0 μM), ascorbic acid (100 μM), H_2O_2 (0.2 μM) and the tested compound (0.2 μM) and were prepared with 0.01 M of KH_2PO_4–K_2HPO_4 buffer (pH = 7.4). The tested compound was not added to the control group. The reaction was carried out at a constant 37° C for 30 min, then the amount of ethene produced was detected by gas chromatography. The suppression ratio was calculated on the basis of $(S_0 - S)/S_0$, where S and S_0 are the peak areas given by the gas chromatography for the ethene content of the sample (S) and the control group (S_0).

Inhibitory Action for Lipid Peroxidation

Malondialdehyde (MDA) is one of the products formed in lipid peroxidation. The measurement of lipid peroxidation is on the basis of a TBA test[13-15] based on a spectrophotometric quantitation of a red-violet (λ_{max} = 532nm) complex formed with MDA.

1 g of fresh rabbit's liver was weighed into a glass homogenizer, 10 mL of physiological saline solution was added, and homogenized in an ice bath. The sample (3 mL) containing the tested compound (0.2 μM) and control group without the tested compound were vibrated at a constant 37° C for 2 h, then a 20% aqueous solution of TCA (3 mL) was immediately added to every sample and the control group, respectively. After 15 min the reaction mixtures were centrifuged at 4000 cycles per min for 10 min. The clear solution of the samples (2 mL) was mixed in a screw capped tube with 2 mL TBA reagent, and heated in a boiling water bath for 30 min, then

cooled in tap water. The absorbances (A_i, A_0) were measured at 532 nm, using 20% TCA as a reference. The inhibitory ratio was calculated on the basis of inhibition = $(A_0 - A_i)/A_0$.

RESULTS AND DISCUSSION

All complexes are yellow, soluble in DMSO and ethanol, insoluble in water, benzene and diethyl ether, and can be kept in air for a long time. The molar conductiveties of the complexes are around 2.97–10.6 $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$ in ethanol (see Table I), this shows that all complexes are non-electrolytes in ethanol[16]. The elemental analyses (see Table I) show the formula of the complexes as $RE(HL)_3 \cdot 3.5H_2O$ (RE = La^{3+} – Gd^{3+}), with three and a half H_2O and three ligands (HL^-) for each complex and was confirmed by ^1H NMR and thermal analyses.

Thermal Analysis

Some data of thermal analyses are listed in Table II. The DTA curves of the complexes have an endothermic peak between 83 and 93° C, the corresponding TG curves show that the weight loss is equal to three and a half water molecules. These results are in accordance with the compositions of the complexes determined by elemental analyses. Two exothermic peaks appear around 442–469° C and 501–547° C, respectively. Initial temperature of decomposition is about 450° C or so, this indicates that the complexes have a high thermal stability.

Ultraviolet Spectra

UV spectra data of complexes are listed in Table III. The free ligand exhibits a strong absorption at 298 nm and a shoulder peak at 286 nm. The complexes yield two new peaks at approximately 366 and 383 nm, respectively. This shows that there is a conjugate system formed by enolization of the ligand in the complexes. Therefore, the absorption bands of two carbonyl $\pi \rightarrow \pi^*$ transitions appear at the region observed. The complexes should be a chelate containing both five and six-membered rings. This is the reason why the complexes exhibit high thermal stability. This is consistent with the thermal analysis results.

Table I. Elemental Analysis and Molar Conductivity

Formula	M.w.	M.p.[a] (°C)	Color	Yield (%)	Elemental Analysis (Calc.) C	H	N	% RE	Λ_M (U)[b]
H_2L $C_{23}H_{19}N_5O_2$	397.44	244	Red	79	69.43 (69.50)	4.89 (4.82)	17.08 (17.26)		
$La(HL)_3 \cdot 3.5H_2O$ $LaC_{69}H_{61}N_{15}O_{9.5}$	1390.4	458	Yellow	64	59.36 (59.57)	4.39 (4.42)	15.08 (15.10)	10.02 (9.98)	3.24
$Ce(HL)_3 \cdot 3.5H_2O$ $CeC_{69}H_{61}N_{15}O_{9.5}$	1391.4	422	Yellow	67	59.64 (59.52)	4.32 (4.42)	14.95 (15.09)	10.18 (10.06)	5.10
$Pr(HL)_3 \cdot 3.5H_2O$ $PrC_{69}H_{61}N_{15}O_{9.5}$	1392.4	468	Yellow	62	59.49 (59.48)	4.40 (4.41)	15.01 (15.08)	10.32 (10.11)	10.6
$Nd(HL)_3 \cdot 3.5H_2O$ $NdC_{69}H_{61}N_{15}O_{9.5}$	1393.4	447	Yellow	69	59.48 (59.34)	4.38 (4.40)	15.11 (15.04)	10.46 (10.33)	4.81
$Sm(HL)_3 \cdot 3.5H_2O$ $SmC_{69}H_{61}N_{15}O_{9.5}$	1403.4	449	Yellow	71	59.13 (59.08)	4.14 (4.38)	14.80 (14.98)	10.96 (10.72)	2.97
$Eu(HL)_3 \cdot 3.5H_2O$ $EuC_{69}H_{61}N_{15}O_{9.5}$	1404.4	473	Yellow	65	59.24 (59.02)	4.24 (4.38)	14.91 (14.96)	11.01 (10.82)	3.75
$Gd(HL)_3 \cdot 3.5H_2O$ $GdC_{69}H_{61}N_{15}O_{9.5}$	1409.4	469	Yellow	68	58.86 (58.79)	4.32 (4.36)	14.93 (14.90)	11.21 (11.16)	8.51

a: M.p.for the complexes is the decomposition point; b: U=ohm^{-1}·cm^2·mol^{-1}.

Table II. Thermal Analyses Data of the Complexes

Complexes	Dehydration (°C)	Process	W. loss (Calc.) %	Decomp.(°C) t_1	t_2	Process	Residue
La-comp.	87	Endothermic	5.1 (4.53)	458	528	Exothermic	La_2O_3
Ce-comp.	93	Endothermic	4.9 (4.53)	422	515	Exothermic	CeO_2
Pr-comp.	83	Endothermic	4.9 (4.52)	468	537	Exothermic	Pr_6O_{11}
Nd-comp.	85	Endothermic	4.6 (4.52)	447	518	Exothermic	Nd_2O_3
Sm-comp.	87	Endothermic	4.6 (4.50)	449	501	Exothermic	Sm_2O_3
Eu-comp.	90	Endothermic	4.5 (4.49)	473	542	Exothermic	Eu_2O_3
Gd-comp.	89	Endothermic	4.5 (4.47)	469	547	Exothermic	Gd_2O_3

Table III. Ultraviolet Spectral Data

Complexes	λ_{max} (nm) and $\varepsilon \times 10^{-3}$ ($L \cdot mol^{-1} \cdot cm^{-1}$)									
	λ_I	ε_I	λ_{II}	ε_{II}	λ_{III}	ε_{III}	λ_{IV}	ε_{IV}	λ_V	ε_V
H_2L	198	1.23	286	5.81	298	6.34				
La-comp.	200	4.49	290	6.10	297	30.17	365	12.57	391	7.82
Ce-comp.	208	4.87	289	31.01	306	27.21	369	11.46	395	6.49
Pr-comp.	210	4.32	297	24.8	310	25.91	376	8.10	397	9.54
Nd-comp.	200	5.40	283	24.18	298	25.12	366	10.33	383	7.98
Sm-comp.	200	5.67	287	30.01	298	31.43	365	12.76	382	10.63
Eu-comp.	199	6.81	286	31.36	297	32.55	366	13.61	383	10.95
Gd-comp.	198	7.14	285	29.36	297	30.55	368	14.28	383	11.50

Infrared Spectra

The main stretching frequencies of the IR spectra of the ligand (H_2L) and its complexes are tabulated in Table IV. The aqueous υ(OH) for the complexes appears at 3350 cm^{-1}, υ(C^7=O) and υ(C^5=O) vibration of the free ligand are at 1616 and 1560 cm^{-1}, respectively; for the complexes these peaks shift to 1602, 1434 cm^{-1} or so, Δυ is equal to 14–16, and 119–126 cm^{-1}. The band at 460 cm^{-1} is assigned to υ(M–O)[17]. This demonstrates that the oxygen of the carbonyl has formed a coordinative bond with the rare earth ions, one of which (C^5=O) is in the enolic form. The band at 1588 cm^{-1} for the free ligand is assigned to the υ(C=N) stretch, it shifts to 1571 cm^{-1} or so for its complexes, weak bands at 410 cm^{-1} are assigned to υ(M–N). This further confirms that the nitrogen of the imino-group bonds to the rare earth ions. All of these data confirm the fact that a conjugate chelate ring formed by ligand enolazition exists in the complexes.

^{1}H Nuclear Magnetic Resonance Spectra

The ^{1}H NMR spectra of $La(HL)_3 \cdot 3.5H_2O$ only are discussed in the paper. $La(HL)_3 \cdot 3.5H_2O$, δ (ppm): 1.18 (3H, singlet, CH_3), 1.36 (3H, singlet, CH_3), 8.13 (4H, doublet, J = 6Hz, $2H^3$, $2H^5$ of pyridine ring), 8.51 (4H, broad, $2H^2$, $2H^6$ of pyridine ring), 6.80 - 7.86 (20 H, multiplet, 4 phenyl), 3 - 3.5 (H_2O). Two methyls of the

Table IV. Some Main IR Data of Ligand and its Complexes (cm^{-1})

Compounds	$\upsilon(H_2O)$	$\upsilon(NH)$	$\upsilon(C^7=O)$	$\upsilon(C=N)$	$\delta(NNH)$	$\upsilon(C^5=O)$	$\delta(H_2O)$	$\upsilon(M-O)$	$\upsilon(M-N)$
H_2L		3018 m	1616 s	1588 s	1532 m	1560 s	1356 m		
La-comp.	3353 m	3051 m	1602 s	1572 s	1539 s	1434 s	1638 s	456 w	405 w
Ce-comp.	3353 m	3051 m	1602 s	1572 s	1546 s	1441 s	1631 s	456 w	405 w
Pr-comp.	3353 m	3051 m	1602 s	1572 s	1546 s	1434 s	1645 s	456 w	410 w
Nd-comp.	3353 m	3068 m	1602 s	1568 s	1546 s	1441 s	1638 s	456 w	410 w
Sm-comp.	3360 m	3065 m	1602 s	1574 s	1546 s	1434 s	1638 s	456 w	410 w
Eu-comp.	3360 m	3058 m	1602 s	1574 s	1546 s	1434 s	1638 s	456 w	410 w
Gd-comp.	3353 m	3058 m	1602 s	1574 s	1546 s	1434 s	1638 s	456 w	405 w

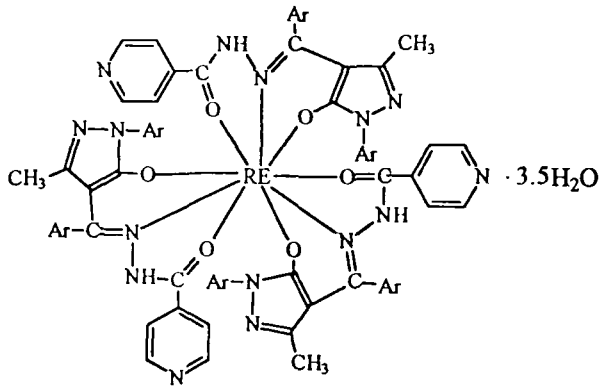

Fig.1. The Probable Structure of the Complexes

complexes, compared with the free ligand, shift upfield by 0.06, 0.24 ppm, respectively. The hydrogen atoms of the pyrazole ring are not seen in the ^1H NMR spectra of the complexes, implying that the 5-pyrazolone group in H_2L exists in the enolic form in the complexes. The probable structure of the complexes is shown in Fig.1 and is derivefrom the above results of IR, UV, and ^1H NMR.

Biological Activity

The data of biological activity are listed in Table V. The results show that H_2L and its light rare earth complexes possess a certain scavenger effect on $OH^{\cdot}$ and $O_2^{\cdot -}$

Table V. Biological Activity Data

Complexes	Dose (mg)	Average suppression ratio (%) for $OH^{\cdot}$	Average suppression ratio (%) for $O_2^{-\cdot}$	Average inhibitory ratio (%) for lipid peroxidation
H_2L	0.079	38.5	45.2	41.5
La-comp.	0.186	75.4	73.9	80.1
Ce-comp.	0.186	72.8	58.4	66.8
Pr-comp.	0.186	46.7	57.6	62.0
Nd-comp.	0.187	41.1	48.3	50.1
Sm-comp.	0.188	59.8	54.1	72.9
Eu-comp.	0.190	49.2	58.0	65.2
Gd-comp.	0.190	68.6	61.2	77.3
$Cu(HSal)_2$	3.10[a]		59	
$Na_2Cu(Sal)_2$	3.10[a]		30	

a: μ mol of Cu (see reference 18).

and can inhibit lipid peroxidation. The biological activity of the ligand is less than that of its rare earth complexes. It is clear that coordination enhances the biological activity, and that the scavenger effects on $OH^{\cdot}$ and $O_2^{-\cdot}$ as well as the inhibitory action on lipid peroxidation of the lanthanum and gadolinium complexes are the strongest in all complexes studied. Perhaps this is related to the valence electron arrangement of La(III)$4f^0$ and Gd(III)$4f^7$, the scavenger effects on $O_2^{-\cdot}$ of the two complexes is superior compared to that of the transition metal Cu(II) salicylate complexes[18] (see Table V).

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