Synthesis and Characterization of β-Diketone Isonicotinoylhydrazone—Rare Earth Complexes and their Elimination Effect on Biological Free Radicals

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Six new complexes, $RE(C_{16}H_{15}N_3O_2)_3$ and $RE(C_{20}H_{19}N_3O_2Fe)_3$ (RE = La, Sm, Y) have been synthesized and characterized by elemental analyses, IR and ^1H NMR spectra. It is proved that these β-diketones are tridentate ligands to the lanthanide ion. The coordination number of the central RE ion equals nine and we suggest a tricapped trigonal structure for these complexes. The inhibition rates of the complexes and the ligands to biological free radicals ($O_2^{-\cdot}$ and $OH^{\cdot}$) were determined.

Biological free radicals can destroy the biological membranes because of their oxidation ability. Experimental results of *Harman* and later researchers have supported the viewpoint that apolexis is the total result of the incessant destructive reactions by free radicals in cells and tissues [1—4]. To eliminate free radicals timely or keep their concentration at a relatively low level and their balance in the metabolic system, can prevent the damage of cells and tissues and therefore delay the apolexis. In recent years, the fields actively investigated include improving the concentration and effectiveness of the "protective enzyme" such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), deducing enzyme reaction which generates free radicals and selecting effective chemical scavengers for free radicals [5—7].

The "biological effects" and the "enzyme effects" of metals are well known. Hydrazone and acylhydrazone have many biological functions and also are chemical scavengers to lots of enzyme reactions [8], so, many researchers are interested in the metal complexes of hydrazone and acylhydrazone [9—11]. In this paper, we report on two ligands of acetylacetone isonicotinoylhydrazone (HL^I) and acetoacetylferrocene isonicotinoylhydrazone (HL^{II}), and six complexes REL_3^I, REL_3^{II} (RE = La, Sm, Y) and their inhibition rates to the free radicals $O_2^{-\cdot}$ and $OH^{\cdot}$ The results show that the good inhibition rates of complexes can be attributed to the accelerated effect of the ligands and the lanthanide ions.

EXPERIMENTAL

Reduced coenzyme I (NADH), phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), 3-methionpropionaldehyde (MPL), and benzoylacetone were purchased from Sigma. η^5-Cyclopentadienylironformacetone was prepared according to the literature [11]. Tris-HCl and NaH_2PO_4—Na_2HPO_4 buffer solutions were prepared using redistilled water. Ascorbic acid, EDTA, and $FeSO_4$ were of anal. grade.

Microanalyses were carried out in the Microanalytical Laboratory of Lanzhou University by an elemental analyzer, model Elemental Vario EL (Germany). IR spectra were obtained with a Nicolet-170SX spectrophotometer using KBr discs in the 200—400 cm^{-1} region. ^1H NMR spectra were recorded on an FT-80A spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. Melting points were determined using an RY-1 type apparatus, but the temperature was not calibrated. The inhibition rates of the complexes and the ligands were determined using a 721 type UV spectrophotometer for the free radical $O_2^{-\cdot}$ and using a GC-9A gas chromatography spectrometer with chromatographic column of poropack Q 3 m × 3 mm for the free radical $OH^{\cdot}$

Synthesis of the Ligands

The reactions of β-diketone with isonicotinic acid hydrazide were examined using anhydrous toluene as

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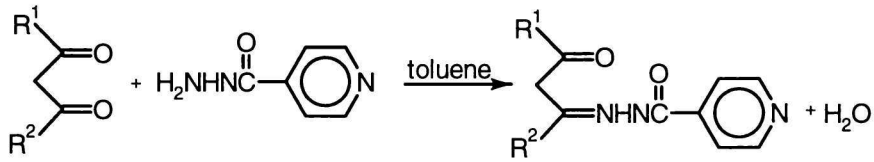


$$3\,HL + RECl_3 \xrightarrow{\text{ethanol}} REL_3 + 3\,HCl$$

HL^{I}: $R^1 = C_6H_5$, $R^2 = CH_3$ $\quad$ HL^{II}: $R^1 = \eta^5\text{-}C_5H_5FeC_5H_4$, $R^2 = CH_3$

Scheme 1

solvent (Scheme 1). After the reaction had been carried out under reflux for 6—7 h, the colour of solution changed from yellow or brown to orange or dark-red. The solution had been condensed to the appearance of precipitate using the distillation under reduced pressure. When the residue was maintained for 24 h at room temperature, the precipitate was filtered, washed and recrystallized from anhydrous ethanol.

Synthesis of the Complexes

To a solution of NaL (sodium metal and equivalent of HL) in anhydrous ethanol with concentration of 0.05 mol dm^{-3}, an anhydrous ethanol solution of $RECl_3$ was added dropwise under stirring at 40—60 °C. After 4 h, the precipitate was filtered, washed with hot anhydrous ethanol and diethyl ether (each three times) and dried *in vacuo*.

Elimination of the Superoxide Anion $O_2^{-\cdot}$ [12]

$O_2^{-\cdot}$ released from the reaction of NADH and PMS can react quantitatively with NBT to give a blue solution, and the absorbance of the solution has a linear relationship with concentration of $O_2^{-\cdot}$ in the range of 0—1 × 10^{-4} mol dm^{-3}, which can be used to detect $O_2^{-\cdot}$ The capability of biological free radical scavenger on $O_2^{-\cdot}$ can be expressed by the inhibition rate

$$\text{Ih} = \frac{(A_0 - A)}{A_0} \times 100\ \%$$

where A_0 is the absorbance of blank solution and A absorbance of solution containing free radical scavenger with different dose.

The compounds HL^{I}, REL_3^{I}, HL^{II}, and REL_3^{II} were dissolved in acetone—DMF (1:2 v/v) to give a respective solution D (ρ = 0.5 mg cm^{-3}) for determination.

NADH, PMS, and NBT were dissolved in tris-HCl (pH 8.0 and c = 0.01 mol dm^{-3}) buffer, respectively to give a solution of $c = 3.0 \times 10^{-5}$ mol dm^{-3} for use. The solutions of NADH (1.0 cm^3), PMS (1.0 cm^3), and NBT (1.0 cm^3) were added to the solution of 0.0 cm^3, 0.25 cm^3, 0.50 cm^3, 0.75 cm^3, 1.00 cm^3 of D, respectively. After diluting them to 5.0 cm^3 with tris-HCl buffer and keeping them under 37 ± 1 °C for 5 min the absorbance of the solutions was determined at the wavelength of 560 nm with a reference solution of acetone—DMF (1:2 v/v).

Elimination of Hydroxyl Radical OH· [13, 14]

The hydroxyl radical OH· generated by the reaction of H_2O_2 with ascorbic acid using Fe^{2+}—EDTA as catalyst reacts with MPL to release ethylene that can be detected by gas chromatography. The inhibition rate of free radical scavenger is as follows

$$\text{Ih}' = \frac{(a_0 - a)}{a_0} \times 100\ \%$$

where a_0 is the yield of ethylene without free radical scavenger and a the yield of ethylene with free radical scavenger.

$FeSO_4$ and an equimolar quantity of EDTA were dissolved in NaH_2PO_4—Na_2HPO_4 (pH 7.4 and c = 0.01 mol dm^{-3}) buffer to give a solution of $c = 3.0 \times 10^{-5}$ mol dm^{-3} The MPL and ascorbic acid were dissolved in this buffer solution respectively to give a solution of $c = 3.0 \times 10^{-5}$ mol dm^{-3} for use. The solutions of Fe^{2+}—EDTA (1.0 cm^3), MPL (1.0 cm^3), and ascorbic acid (2.0 cm^3) were added to the solution of 0.0 cm^3, 0.25 cm^3, 0.50 cm^3, 0.75 cm^3, 1.00 cm^3 of D, respectively. After diluting them to 5.0 cm^3 with NaH_2PO_4—Na_2HPO_4 buffer, and keeping them under 37 ± 1 °C for 30 min the yields of ethylene were determined by the gas chromatography.

RESULTS AND DISCUSSION

The data of elemental analyses in Table 1 indicate that the formed ligands and complexes correspond to the given formulae. All complexes obtained are stable solids at ambient conditions. They are soluble in

Table 1. Physical Properties and Elemental Analyses of the Compounds

Compound	Formula M_r	w_i(calc.)/% w_i(found)/%			Yield	Colour
		C	H	N	%	
HL^I	$C_{16}H_{15}N_3O_2$	68.32	5.37	14.94	82	pale-
	281.31	68.20	5.38	14.88		yellow
LaL_3^I	$LaC_{48}H_{45}N_9O_6$	58.66	4.62	12.83	88	pale-
	982.85	58.68	4.36	12.69		yellow
SmL_3^I	$SmC_{48}H_{45}N_9O_6$	57.98	4.56	12.68	82	deep-
	994.30	58.02	4.32	12.55		yellow
YL_3^I	$YC_{48}H_{45}N_9O_6$	61.80	4.86	13.51	85	pale-
	932.85	62.15	4.37	13.02		yellow
HL^{II}	$C_{20}H_{19}N_3O_2Fe$	61.72	4.92	10.80	76	orange
	389.24	62.06	5.06	10.91		
LaL_3^{II}	$LaC_{60}H_{57}N_9O_6Fe_3$	55.15	4.40	9.65	88	brown
	1306.62	55.40	4.19	9.69		
SmL_3^{II}	$SmC_{60}H_{57}N_9O_6Fe_3$	54.68	4.36	9.56	82	dark-
	1318.07	54.82	4.17	9.71		brown
YL_3^{II}	$YC_{60}H_{57}N_9O_6Fe_3$	57.35	4.57	10.03	72	brown
	1256.62	57.47	4.26	10.12		

Table 2. ^{1}H NMR Chemical Shifts of Ligands and Complexes

Compound	Chemical shift, δ_i				
	R^2(—CH_3)	—CH_2— or =CH—	R^1		=NNHCO
HL^I	2.01 (s, 3H)	4.76 (s, 2H)	6.96—7.21 (m, 5H)	7.75—8.79 (m, 4H)	11.66 (s, 1H)
SmL_3^I	2.02 (s, 3H)	4.86 (s, 1H)	6.94—7.20 (m, 5H)	7.75—8.80 (m, 4H)	11.64 (s, 1H)
HL^{II}	2.31 (s, 3H)		4.24 (s, 5H) 4.51—4.7 (m, 4H)	7.66—8.70 (m, 4H)	11.90 (s, 1H)
SmL_3^{II}	2.30 (s, 3H)		4.17 (s, 5H), 4.3 (m, 2H), 4.7 (m, 2H)	7.90—8.50 (m, 4H)	11.90 (s, 1H)

Table 3. Important IR Spectral Data of Ligands and the Complexes

Compound	$\tilde{\nu}_i$/cm^{-1}					
	ν(N—H)	ν(C=O)	δ(N—H)	ν(C—O)	ν(C=O, C=N, C=C)	ν(RE—O)
HL^I	3246 w	1670 m	1522 m	1282 m	1600 vs, 1540 s	
LaL_3^I	3319 w	1648 m	1540 m	1266 m	1580 vs, 1550 s	486 m
SmL_3^I	3320 w	1652 m	1535 m	1265 m	1580 vs, 1552 s	480 m
YL_3^I	3320 w	1652 m	1540 m	1262 m	1580 vs, 1556 s	480 m
HL^{II}	3269 w	1666 m	1532 m	1279 m	1595 vs, 1567 s, 1377 m	
LaL_3^{II}	3386 w	1642 m	1534 m	1251 m	1586 vs, 1528 s	490 m
SmL_3^{II}	3392 w	1644 m	1534 m	1250 m	1579 vs, 1529 s	490 m
YL_3^{II}	3402 w	1644 m	1534 m	1250 m	1574 vs, 1523 s	496 m

vs = very strong, s = strong, m = medium, w = weak.

organic solvents such as DMF, DMSO, ethanol, acetone, and tetrahydrofuran, but insoluble in hexane, petroleum ether, and water.

The ^{1}H NMR spectra of ligands and the Sm complexes are shown in Table 2. It can be seen that the chemical shifts of the methylene protons of the complexes are observed as singlets (1H) which are different from those of the ligands (2H). This shows that all the ligands in complexes are in conjugated form.

Infrared Spectra

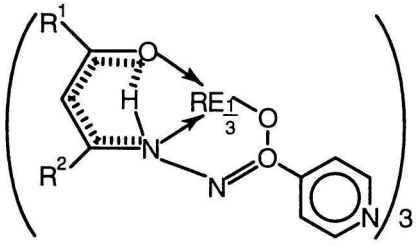
The important IR frequencies of ligands and the complexes are given in Table 3. The characteristic

Table 4. Data for Antioxidative Action of HL^{I}, HL^{II}, REL_3^{I}, REL_3^{II}, and $RECl_3$

Compound	Ih (Inhibition rate of $O_2^{-\cdot}$ radical/%)					Ih′ (Inhibition rate of $OH^{\cdot}$ radical/%)				
	Dose/mg					Dose/mg				
	0.0	0.125	0.250	0.375	0.500	0.0	0.125	0.250	0.375	0.500
HL^{I}	0	3.88	16.0	22.4	25.5	0	42.5	48.6	52.7	58.0
LaL_3^{I}	0	7.21	21.2	25.8	28.1	0	50.1	59.3	63.0	65.8
SmL_3^{I}	0	6.50	18.1	23.6	25.8	0	44.1	52.6	56.1	60.6
YL_3^{I}	0	5.72	17.8	23.0	25.2	0	43.8	52.2	55.6	59.8
HL^{II}	0	34.8	64.2	85.5	89.8	0	63.3	78.4	82.2	85.4
LaL_3^{II}	0	38.8	71.7	89.8	92.1	0	67.6	84.0	86.4	88.6
SmL_3^{II}	0	37.4	69.5	88.2	91.2	0	66.4	81.6	84.9	87.3
YL_3^{II}	0	35.6	67.9	85.8	88.6	0	64.6	78.8	81.4	84.1
$LaCl_3$	0	2.18	3.46	5.67	6.82	0	14.2	17.6	21.8	24.4
$SmCl_3$	0	0.26	0.21	0.28	0.31	0	1.21	1.48	1.56	0.88
YCl_3	0	0.11	0.22	0.18	0.27	0	1.04	1.24	1.23	0.93

absorption bands of complexes, SmL_3^{I} as example, appearing at $\tilde{\nu}$ = 3320 cm^{-1} (ν(N—H) stretching), 1535 cm^{-1} (δ(N—H) bending), 1652 cm^{-1} (ν(C=O) vibration), and 1265 cm^{-1} (ν(C—O) vibration), respectively, are shifted in comparison with their ligands. The absorption band at 480 cm^{-1} is attributed to ν(RE—O) vibration. This shows that the complexes are formed and the oxygen atoms of acyl are coordinated to the central ion. The bands occurring around 1580 cm^{-1}, 1552 cm^{-1} have been assigned to ν(C=O), ν(C=N), ν(C=C) vibrations, respectively in which the band ν(C=O) is shifted from 1600 to 1580 cm^{-1}. This result shows that all the ligands are coordinated to lanthanide ion in a conjugated form.

On the basis of elemental analyses and IR and ^1H NMR spectra the following structure *I* was suggested for the complexes which was similar to that of literature [15].



The results of inhibition rate of the ligands and the complexes (Table 4) show that all ligands and $RECl_3$ have somewhat antioxidative effect (Ih > 0, Ih′ > 0). The inhibition rates of the complexes REL_3^{I}, REL_3^{II} are higher than those of the ligands HL^{I} and HL^{II}, respectively. On the other hand, the inhibition rates of the HL^{I}, REL_3^{I} are lower than those of the HL^{II}, REL_3^{II} containing the group η^5-$C_5H_5FeC_5H_4$. We can see that the combination of ligand and lanthanide ions RE(III) produces an obvious synergistic effect, and that ferrocenyl group has more antioxidative effect. It may be attributed to the increase of interaction force between the complexes and the cellular membrane in which the arrangement of electron was changed. This gives us a valuable information for future study.

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