SYNTH. REACT. INORG. MET.-ORG. CHEM., 28(6), 917-928 (1998)

SYNTHESIS, PROPERTIES AND ANTIOXIDATIVE BEHAVIOR OF SOME TRANSITION METAL COMPLEXES OF N-[(BENZOYLAMINO)THIOXOMETHYL]GLYCINE

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ABSTRACT

Two new solid complexes of N-[(benzoylamino) thioxomethyl]-glycine (HL) with transition metals [Cu(II), Zn(II)] have been synthesized. The complexes were characterized by elemental analyses, IR, TG-DTA, molar conductance measurements and ^1H NMR spectra. The general formulas of the complexes are $Cu_2L_2Cl_2$ and $Zn_2L_2(OH)_2$. The antioxidative activity of the complexes was tested. The result obtained showed that these complexes possess scavenging effects on $O_2^{-\cdot}$ radicals. Especially the complex of Cu(II) has an excellent scavenger effect for activated oxygen.

INTRODUCTION

Thiourea and thiourea derivatives and their metal complexes have aroused great interest due to their broad spectrum of biological activity,

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such as antitumor, antiviral activity, antioxidative and bacteriostatic activity[1,2].

It is well known that Cu(II) and Zn(II) ions have some biological activity[3]. Moreover, in many cases[4-6] it has been suggested that the biological activity of thiourea and thiourea derivatives can be increased by being coordinated or mixed with suitable metal ions. This idea led us to prepare N-[(benzoylamino)thioxomethyl]glycine (HL) metal complexes with a view to evaluate their biological properties.

HL is a well known plant growth accelerator[7] and has been investigated. But no paper concerning the study of transition metal complexes with HL has been seen. Therefore, we now wish to report the synthesis and characterization of Cu(II) and Zn(II) complexes with HL and discuss their biological properties.

This paper is one of a series of our investigation of metal complexes with HL. Further studies on the complexes of other transition metals and rare earths with HL are in progress.

RESULTS AND DISCUSSION

The elemental analyses of the Cu(II) and Zn(II) complexes (see Table I) show that the metal ions form complexes having the general formulas $Cu_2L_2Cl_2$ and $Zn_2L_2(OH)_2$. The complexes are stable at room temperature and soluble in dimethylformamide (DMF) and dimethylsulfoxide (DMSO), but insoluble in water, methanol and ethanol. The molar conductances of the complexes in DMF solution at room temperature vary from 4.5 to 14.3 S cm^2 mol^{-1}, indicating that they are non-electrolytes[8] and that the chloride ions and the hydroxide ions in the complexes $Cu_2L_2Cl_2$ and $Zn_2L_2(OH)_2$ coordinate to the copper(II) ion and the zinc(II) ion, respectively.

IR Spectra

The important IR spectra data of the ligand and its complexes are listed in Table II. The infrared spectra of the ligand show a band of

Table I. Analytical Data[a], Molar Conductances ($S\ cm^2\ mol^{-1}$) and Thermal Data of the Ligand and its Complexes

Compounds	$C_{10}H_{10}O_3N_2S$	$NaC_{10}H_9O_3N_2S$	$Cu_2C_{20}H_{18}O_6N_4S_2Cl_2$	$Zn_2C_{20}H_{20}O_8N_4S_2$
Analytical results	HL	NaL	$Cu_2L_2Cl_2$	$Zn_2L_2(OH)_2$
Formula weight	238.28	260.26	672.54	639.32
% M			18.76 (18.90)	20.75 (20.44)
% Cl			10.98 (10.54)	
% C	50.61 (50.40)	46.27 (46.15)	35.06 (35.72)	37.86 (37.57)
% H	4.12 (4.24)	3.34 (3.49)	2.63 (2.70)	2.98 (3.16)
% N	11.84 (11.76)	10.88 (10.77)	8.14 (8.32)	8.54 (8.77)
Color	yellow	yellow	yellow	white
Molar conductances		70.3	14.3	4.5
Yield (%)	75	98	98	96
M.p. (° C)	200	237		190
Decomposition temperature (° C)	483		173	442
% Weight loss (800° C)			23.70 (23.66)	25.00 (25.46)
Residue (800° C)			CuO	ZnO

[a] Calculated values in parentheses

relative medium intensity at 3232 cm^{-1} attributed to the $\nu(N-H)$ vibration. The bands in the 2510 − 3212 cm^{-1} region are assigned to the stretching vibration of the OH group of the carboxy group (−COOH). The four strong bands at 1708, 1669, 1556 and 721 cm^{-1} are attributed to $\nu(C=O)(COOH)$, $\nu(C=O)$, $\nu_{as}(N-C-N)$ and $\nu(C=S)$ vibrations, respectively[9]. But, no bands exist above 3500

Table II. Some Characteristic IR Data (cm^{-1}) of the Ligand and its Complexes

Ligand		Complexes		Assignment
HL	NaL	$Cu_2L_2Cl_2$	$Zn_2L_2(OH)_2$	
1708				$\nu(C=O)$ free carboxyl group
3212 – 2510				$\nu(OH)(COOH)$
3232	3289	3179	3224	$\nu(NH)$
1669	1664	1624		$\nu(C=O)$ free carbonyl group
			1129	$\nu(C-OH)$
	1605	1574	1576	$\nu_{as}(COO^-)$
	1404	1387	1394	$\nu_s(COO^-)$
	201	187	192	$\Delta\nu$
1556	1551	1508		$\nu_{as}(N-C-N)$
			1600	$\nu(C=N)$
721	714	740	743	$\nu(C=S)$
		479, 493	454	$\nu(M-O)$
		449	428	$\nu(M-N)$
		290		$\nu(M-Cl)$
			3394	$\nu(OH)$
			1102	$\delta(M-OH)$

cm^{-1} or in the 2500 – 2600 cm^{-1} region which would be due to $\nu(OH)$ and $\nu(SH)$ vibrations, respectively. The absence of these bands indicates the presence of HL in the thione form[9].

The infrared spectra of the complexes differ from the free ligand. The characteristic $\nu(C=O)$ mode of the ligand carboxyl group (1708 cm^{-1}) disappeared upon complexation. However, the complexes displayed both asymmetric and symmetric stretching vibrations of COO^- (ν_{as}: 1574 – 1576, ν_s: 1387 – 1394 cm^{-1}), compared with 1605 [$\nu_{as}(COO^-)$], 1404 [$\nu_s(COO^-)$] cm^{-1} of sodium N-[(benzoylamino)thioxomethyl]glycinate (NaL), $\Delta\nu$[$\Delta\nu = \nu_{as}(COO^-) - \nu_s(COO^-)$] of the complexes is quite close to that of NaL. This fact strongly suggests coordination of the ligand carboxyl group with copper(II) or zinc(II) ion[10].

In the complex $Cu_2L_2Cl_2$, the band due to $\nu(C=O)$ shifts to lower frequency by 45 cm^{-1}. This shows that the carbonyl oxygen ($C=O$) of the benzoyl moiety of the ligand bonds to the copper(II) ion in the keto form. In addition, the band due to $\nu_{as}(N-C-N)$ shifts to lower frequency by 43 cm^{-1} and the band due to $\nu(C=S)$ shifts to higher frequency by 19 cm^{-1}. This shows that the nitrogen atom of the $-CH_2NHC(S)-$ group also coordinates to the Cu(II) ion. By comparison with the free ligand, the appearance of $\nu(C \doteq O-Cu)$ at 479 cm^{-1}, $\nu(C=O-Cu)$ at 493 cm^{-1} and $\nu(Cu-N)$[9] at 449 cm^{-1} in the complex further proves the coordination of HL with the Cu(II) ion. Another new band appeared at 290 cm^{-1} in the spectra of the Cu (II) complex which is assigned to the $\nu(Cu-Cl)$ mode. This indicates the coordination of the chloride ion with the Cu(II) ion as a monodentate ligand[9], which is confirmed by the study of the molar conductance. Because of the volume effect of a bridging structure of the carboxyl group of HL with Cu(II) ion, chloride ion could not coordinate to Cu(II) ion via a bridging structure.

Comparing the IR spectrum of the ligand and the copper complex with that of the zinc complex, $\nu(C=O)$ of the benzoyl moiety disappeared and new bands at 1129 and 1600 cm^{-1} assigned to $\nu(C-OH)$ and $\nu(C=N)$ are seen. This shows that the enolic carbonyl oxygen ($=C-OH$) of the benzoyl moiety coordinates to Zn(II). In addition, the band of $\nu(C=S)$ shifts to higher frequency by 22 cm^{-1}. This shows that the nitrogen atom of the $-CH_2NHC(S)-$ group coordinates to Zn(II) ion. By comparison with the free ligand, the appearance of $\nu(Zn-O)$ at 454 cm^{-1} and $\nu(Zn-N)$ at 428 cm^{-1} in the

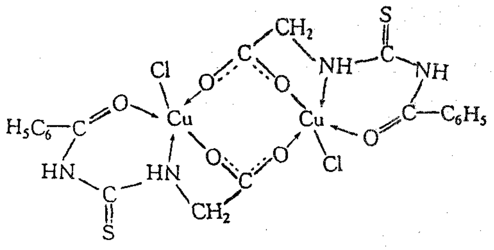

Fig. 1. The Proposed Structure of $Cu_2L_2Cl_2$

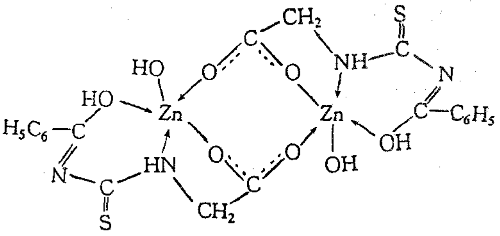

Fig. 2. The Proposed Structure of $Zn_2L_2(OH)_2$

complex further proves the coordination of the ligand with the Zn(II) ion. Moreover, the bands of ν(OH), δ(Zn − OH) (3394 cm^{-1}, 1102 cm^{-1}) show coordination of the oxygen atom of the OH^- group with the Zn(II) ion in an unidentate fashion[10], which is in agreement with the non-electrolytic behaviour of the complex.

On the basis of the above discussion, the probable structures of the complexes are proposed as shown in Figs. 1 and 2.

The structures of the Cu(II) and Zn(II) complexes cannot be assigned with certainty in the absence of X-ray analyses.

Thermal Analyses

The results of thermal analyses suggest that the Zn(II) complex has a characteristic melting point (see Table I), which indicates that the complex is a crystalline compound. The decomposition of the complexes starts around 173° C for the Cu(II) complex, or 442° C for the Zn(II) complex and occurs through more than one stage. On heating to about 800° C, the residue weights correspond to values calculated for CuO and ZnO. The thermal analytical data are listed in Table I.

^{1}H NMR Spectra

The ^{1}H NMR spectra of the ligand and the complex $Zn_2L_2(OH)_2$ were determined in DMSO – d_6 solution. The ^{1}H NMR spectrum of HL exhibits a singlet at 11.43 ppm, a triplet, doublet and multiplet in the region 11.15 – 11.23, 4.31 – 4.35 and 7.45 – 7.95 ppm assigned to $-C(O)NH-$, $-NH-CH_2-$, $-CH_2-$ and C_6H_5-, respectively. When coordinated to the zinc(II) ion in the complex, the δ value of $-CH_2-$ shifted 0.35 ppm to higher field. This is due to the electron withdrawing capacity of Zn(II) ion in the complex which is less than that of carboxyl proton in HL, which gave further evidence that the metal ion is bonded to the oxygen atom of the carboxyl group of the ligand. The peak of the $-C(O)NH-$ proton in the complex disappears. The reason is that the carbonyl oxygen coordinates to Zn(II) ion in the enol form ($N=C-OH$). There is a new peak at 3.85 ppm ascribed to OH^- in the complex, which indicates the coordination of OH^- with Zn(II) ion. This is in agreement with the analytical results and the IR spectra.

Antioxidative Activity

The suppression percentage of the ligand and its complexes for $O_2^{-\cdot}$ radicals and lipid peroxidation are summarized in Table III. The results show that the ligand and its complexes have inhibiting action for the superoxide radical $O_2^{-\cdot}$. Their activity is greatly enhanced at higher concentration. The reason for scavenging effects on superoxide radicals $O_2^{-\cdot}$ of the compounds may be due to the presence of the $C=S$ group which is present in the ligand as well as in its complexes[11].

Table III. Data of Scavenging Effect on $O_2^{-\cdot}$ and Inhibiting Results of Lipid Peroxidation of the Ligand and its Complexes

Compound	Concentration of compound (μM)	Suppression percentage for $O_2^{-\cdot}$ (%)	Concentration of compound (μM)	Suppression percentage for the lipid peroxidation (%)
	0.30	47.6		
HL	0.60	64.6	0.40	−87.2
	1.20	74.8		
	0.30	55.8		
$Cu_2L_2Cl_2$	0.60	81.0	0.40	67.0
	1.20	87.4		
	0.30	75.7		
$Zn_2L_2(OH)_2$	0.60	76.0	0.40	−62.4
	1.20	78.6		

As seen from Table III, the copper(II) complex possesses inhibiting action for the lipid peroxidation of homogenates of mouse liver, but the ligand and its zinc(II) complex accelerate the lipid peroxidation of the homogenates of mouse liver. Currently, the best interpretation of the fact that the zinc(II) complex behaves anomalously is uncertain. Further studies on the detailed mechanism for the reaction with superoxide of the complexes are in progress.

EXPERIMENTAL

Reagents

The chemicals used included copper chloride ($CuCl_2$), zinc chloride ($ZnCl_2$), glycine (NH_2CH_2COOH), benzoylchloride (C_6H_5COCl), ammonium thiocyanate (NH_4SCN), nitroblue tetrazolium (NBT), 2-thiobarbituric acid (TBA), trimethylaminomethane (IRIS), L-methionine (MET), trichloroacetic acid (TCA) and riboflavine (VB_2). All chemicals used were of AR grade.

Apparatus

The contents of carbon, hydrogen and nitrogen were determined on a Carlo Erba 1106 elemental analyser. The metal and chloride contents were analysed by standard procedures[12]. The IR spectra were recorded on a Nicolet-170SX FT-IR spectrophotometer with a beamsplitter of germanium-plated CsI (using KBr discs) in the range 4000-200 cm^{-1}. Thermal analyses were carried out with a Du Pont 1090 thermal analyser. ^1H NMR spectra were recorded on a FT-80A NMR spectrometer in DSMO-d_6 solution using TMS as an internal reference. The absorbances of blue formazane, the reduced product of NBT, were determined on a 751G spectrophotometer from Shanghai Analytical Equipment Factory at 560 nm.

Synthesis of N-[(Benzoylamino)thioxomethyl]glycine

An improved literature[13] method was used. It has the advantages of a higher rate of transformation, a nearly 75% yield, and one-step reaction.

10 mmole (1.40 g) benzoylchloride was poured into a freshly prepared solution of ammonium thiocyanate (11 mmole, 0.84 g) solution in acetone (20 mL), and then refluxed for 30 min. Glycine (10 mmole, 0.75 g) and acetone (20 mL) were added as rapidly as possible to maintain vigorous refluxing. After refluxing for 6 h, excess cracked ice was poured into the mixture with vigorous stirring. The resulting solid was collected, liberally washed with water, followed by acetone and recrystallized from ethanol (95%) to give the pure product; yield, 1.8 g (75%), m.p. 200-201° C (lit.[13], m.p. 201-203° C; lit.[14], m.p. 199-200° C).

The purity of the ligand obtained was confirmed by elemental analyses (see Table I), ^1H NMR spectra and mass spectra.

Synthesis of Sodium N-[(Benzoylamino)thioxomethyl]glycine

N-[(Benzoylamino)thioxomethyl]glycine (2 mmole, 0.48 g) was dissolved with an equimolar amount of sodium hydroxide (2 mmole, 0.08 g) in 20 mL methanol. The reaction mixture was stirred until clear, then the solution was put on a water bath to evaporate it until a crystalline film appears. The product obtained was separated by filtration and dried over molecular sieves under vacuum, yield, 0.51 g (98%). The purity of the product obtained was also confirmed by elemental analysis (see Table I).

Synthesis of the Complexes

N-[(Benzoylamino)thioxomethyl]glycine (2 mmole, 0.48 g) and sodium hydroxide (2 mmole, 0.08 g) were dissolved in 20 mL methanol. With stirring, the metal chloride (1 mmole) in 20 mL methanol was added to the mixture and a precipitate appeared immediately. After stirring for 2 h at room temperature, the product was filtered, washed with methanol three times and dried in a vacuum desiccator to constant weight. Both complexes were prepared by the same method.

Test for Scavenger Effects on $O_2^{-\cdot}$

The superoxide radicals ($O_2^{-\cdot}$) were produced by a system of VB_2/MET/NBT[15] and measured by the amount of NBT reduced by $O_2^{-\cdot}$. The reaction mixture (3 mL) contained VB_2 (18 μM), MET (3000 μM), NBT (255 μM), tris-HCl buffer (0.05 M) (pH = 7.5) and the tested compound (30 - 500 μM). The reaction was left for 30 min under the irradiation of a fluorescent lamp (40 W). The amount of reduced NBT was detected by the absorbance at 560 nm, since the reduced product, blue formazane ($\overset{\overset{N:NH_2}{|}}{HC}=NNH_2$), absorbs at this wave length. The suppression percentage for $O_2^{-\cdot}$ was calculated from the following expression:

$$\text{Suppression percentage} = 100 \times \frac{A_0 - A}{A_0}$$

where A_0 = the absorbance in the absence of the ligand or its complexes and A = the absorbance in the presence of the ligand or its complexes.

The inhibiting activity for lipid peroxidation of the homogenates of mouse liver by the complexes was determined by a literature method[16].

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Received: 10 April 1997
Accepted: 29 January 1998

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