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Synthesis and Toxicity Test of M/Zn(II) Complexes [M = Mn(II), Co(II)] with Pyridine-2,6-dicarboxylic Acid Ligand

F. MARTAK[1,*], W.P. UTOMO[1], Z.V. NUGRAHENI[1] and P. BUDI[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Hasanuddin, Makassar, Indonesia

*Corresponding author: Fax: +62 31 5928314; Tel: +62 81572535690; E-mail: fahima_riza@yahoo.com; fahimahm@chem.its.ac.id

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Heterodinuclear complexes of Co(II)/Zn(II) and Mn(II)/Zn(II) with pyridine-2,6-dicarboxylic acid as a ligand (H_2dipic, dipicolinate) have been synthesized yielding of reddish purple colour of Co(II)/Zn(II) dipicolinic crystal and reddish colour of Mn(II)/Zn(II) dipicolinic crystal. The determination of elements composition (%) of complex compound using atomic absorption spectroscopy and CHN elemental analyzer (C = 28.96; H = 3.47; N = 5.42; Co = 10.15 Mn = 9.47 and Zn = 11.26) showed that the molecular formula of Co(II) complex compound is $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$, while that of Mn(II) complex compounds is $[Zn(H_2O)_5Mn(dipic)_2]\cdot 2H_2O$. The IR spectrum of the Co(II) and Mn(II) complexes showed a typical vibration absorption in the wavenumber of 3082 cm^{-1} indicating of Co(II)/Zn(II) dipicolinic and Mn(II)/Zn(II) dipicolinic have been formed. A brine shrimp lethality test (BSLT), used to determine the toxicity of complexes showed that LC_{50} of Co(II) complex and Mn(II) complexes were 283.71 and 354.94 mg/L, respectively.

Keywords: Complex compounds, Toxicity, Paramagnetic, H_2dipic, Zn(II), Mn(II), Co(II), Heterodinuclear.

INTRODUCTION

Pyridine-2,6-dicarboxylic acid (dipicolinic, H_2dipic) is a material widely studied in inorganic chemistry. H_2dipic can be used as a chelating agent of N, O, which has a diversity in coordination models and has a biological function in the human metabolism [1]. Biological function of dipicolinic acid includes the ability to activate and to inactivate of several metal-containing enzymes, as well as to inhibit of electron transfer and to oxidize of low density lipids (LDL). This compound is a suitable ligand for pharmacologically active compounds due to their amphophilic and low toxicity properties [2]. The differences in coordination pattern of dipicolinic acid are caused by the relative positions of carboxylate group and nitrogen atom, which has a potential agent to act as a bidentate ligand, tridentate, or bridging ligand [3].

Various studies on metal complexes with H_2dipic ligand has been reported. A clear needle-shaped crystals has been formed from homonuclear dipicolinic complexes with manganese chloride as a metal source [4]. The formation of homodinuclear complex of $[Zn(H_2dipic)_2Zn_2(10\cdot H_2O)_5]Cl_4\cdot 2H_2O$ by using zinc chloride has also been reported [5]. The toxicity (LC_{50}) value of Zn metal complexes was 503.32 mg/L. The toxicity of Zn(II)-dipic homodinuclear complex has a smaller value than Mn(II)-dipic homonuclear complexes. Heteronuclear complexes of dipicolynic ligands have been also reported in previous studies; for example, the complex of $[Cu(H_2O)_5Zn(dipic)_2]\cdot 3H_2O$ and $[Zn(H_2O)_6Ni(dipic)_2]\cdot 3H_2O$ [6]. However, the toxicity from both previous studies have not been done.

Study on $[Pd(dipic)_2K(H_2O)_5\cdot 3H_2O]_n$ complex compounds (where dmp = 2,9-dimethyl-1,10-phenanthroline) using two different metals, Pd(II) and K(I) showed that the toxicity values of heteronuclear complexes are lower than the mononuclear complex $[Pd(dipic)_2](DMP)\cdot 6H_2O$ [7]. The biological activity of complex compounds is significantly affected by transition metal ions. The toxicity value (LC_{50}) of dinuclear complex of zinc(II) dipicolinic acid was 503.32 mg/L [6], whereas dinuclear complex of cobalt(II) was 5.38 mg/L [7]. Brine shrimp lethality test from cobalt complex showed that it has low toxicity. In other words, it has a high biological activity. The previous study from Fikriah and Lestari [8] revealed that if the toxicity value (LC_{50}) is less than 1000 mg/L, there won't be any biological activity exhibited, whereas compound with toxicity value (LC_{50}) less than 10 mg/L will have a potential application as anticancer [8].

Based on the above arguments, this paper will study about the heteronuclear complexes synthesized by the incorporation of zinc metal with manganese and cobalt supported by dipicolinate ligand. The complex compounds will be characterized using atomic absorption spectroscopy and CHN elemental analyzer, while the toxicity will be determined by brine shrimp lethality test method using *Artemia salina*. Since the complex combination of two metals with dipicolinate as ligand have been reported previously the complex compounds that have been obtained are expected to produce a new crystalline structure and exhibit a different level of toxicity.

EXPERIMENTAL

The materials used in this study are aquademin, $MnCl_2\cdot 4H_2O$, $ZnCl_2$, $Zn(NO_3)_2\cdot 4H_2O$, $Co(NO_3)_2\cdot 6H_2O$, pyridine-2,6-dicarboxylic acid, HNO_3, NaOH, absolute methanol, *Artemia salina* shrimp, seawater and dimethyl sulfoxide (DMSO).

The equipment used in this study used are glassware, micropipette, a set of 30 µL vial, magnetic stirrer and analytical balance. The complex compounds produced were characterized using atomic absorption spectrophotometer (AAS) ZEEnit 700, Fourier transform infrared (FTIR) Shimadzu, TGA/DTA METTLER, scanning electron microscope, elemental analyzer C, H, N, brine shrimp lethality test method and UV-visible spectrophotometer ECHCHOMP HITACHI type.

Synthesis of complexes Mn(II)/Zn(II) with pyridine-2,6-dicarboxylate: A total of 10 mL of solution consist of 5 mL of $MnCl_2\cdot 4H_2O$ (0.5 mmol) and 5 mL of $ZnCl_2$ (0.5 mmol) was prepared in a beaker. Then, 3 drops of HNO_3 (1.0 mmol), 1 g of pyridine-2,6-dicarboxylic acid and 2 mL of NaOH (80 g, 2 mmol) were added into beaker by stirring at room temperature for 4 h. The solution was filtered and evaporated at room temperature. The crystals will be formed for about 5 days and dried it in a desiccator. The crystals will be characterized FTIR, atomic absorption spectroscopy, elemental analyzer of C, H, N, SEM, TGA and brine shrimp lethality test method.

Synthesis of Co(II)/Zn(II) complexes with pyridine-2,6-dicarboxylate: The synthesis of zinc-cobalt complex with pyridine-2,6-dicarboxylic acid as a ligand has been carried out earlier. The previous study also reported that mononuclear complex has been synthesized using ninhydrin as a ligand and Zn(II) as a metal. In this study, the method was adapted from previous study [9] and the complex compounds were synthesized using H_2dipic as ligand and Co(II) and Zn(II) as metals to obtain heteronuclear complex.

A part of H_2dipic solid (1.67 g; 10 mmol) was dissolved in 25 mL of methanol and 25 mL of demineralized water. The solution was put into a round flask and then added $Zn(NO_3)_2\cdot 4H_2O$ (0.68 g, 5 mmol) and $Co(NO_3)_2\cdot 6H_2O$ (0.68 g, 5 mmol). The solution was refluxed by stirring using magnetic stirrer for 3 h at 70 °C. This process aimed to accelerate the reaction so that the metals and the ligand can bind perfectly. After this process finished, the solution then filtered and cooled at room temperatures, covered with plastic wrap and stored in a desiccator. The reddish purple parallelogram crystals formed after 3 days. The crystals filtered and dried in the desiccator before characterized and tested the toxicity.

Characterization of complex compound: Characterization of complex compounds was done by infrared spectroscopy in 4000-400 cm^{-1} range [10].

Elemental analyzer was carried out to determine the content of C, H and N elements in the complex. The elemental analyzer is standardized using L-cystine as standard ($C_6H_{12}N_2O_4S_2$, C = 29.99 %, H = 5.03 %, N = 11.66 % and S = 26.69). The complex compounds (2.83 mg) were placed in aluminum foil and put in a perforated plate to burn using oxygen gas. The elemental analyzer was operated and the result of C, H, N composition will be appear on the screen.

Determination of metal content is carried out using atomic absorption spectroscopy. The sample solution was prepared in 100 ppm of concentration by weighing 0.0024 g of complex compound and 1 mL of concentrated HNO_3 and diluted with aqua DM in a 50 mL of volumetric flask. The solution was diluted to obtain 1 ppm of concentration. The solution then measured by atomic absorption spectrophotometer (ZEEnit 700).

To confirm the proposed structure, complexes were also analyzed using TGA. A total of 10-15 mg samples placed into aluminum cup then the mass was weighed during heating process. The temperature range between 25-600 °C with 10 °C/min of heating rate.

Toxicity test: Toxicity test was conducted to determine the value of LC_{50} (lethal concentration) of the complex compound. The samples have toxic effect when LC_{50} value is less than 1000 g/mL, whereas for pure compounds LC_{50} value is less than 200 g/mL [11]. The test solution was prepared in 10 µg/mL, 100 µg/mL, 500 µg/mL and 1000 µg/mL of concntration. A total of 15 mL solution was taken and put in a tube with 30 mL of capacity. After that, added 15 mL of seawater containing 10 shrimp pups. The solution allowed to stand for 96 h to observed and calculated the number of died shrimp pups visually. The test was done three times for each concentration [12].

RESULTS AND DISCUSSION

The number of metal ion [Mn(II), Co(II) and Zn(II)] in the each complex was measured by atomic absorption spectroscopy. The measurement showed that the content of Mn(II) and Zn(II) in $[Zn(H_2O)_5Mn(dipic)_2]\cdot 2H_2O$ were 9.47 and 11.30 %wt, respectively. In addition, the content of Co(II) and Zn(II) in $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ were 10.00 and 11.63 wt %.

The relative compositions of carbon, hydrogen and nitrogen in $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ were 29.68; 3.91 and 5.31 wt %, respectively. Analysis of cobalt and zinc ion contents and C, H and N contents using elemental analysis as shown in Table-1 showed the matching between the theoretical composition based on calculation with the experimental result of complex compound of $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$, which is similar with previous result [1]. From this result, it predicted that the complex has molecular structure as shown in Fig. 1.

DTA/TGA analysis: The use of DTA/TGA analysis is aimed to determine molecular formula by analyzing the step and amount of weight loss of the complex compound at a certain temperature. There were four steps of weight loss attributed to loosing of certain compounds in complex or decomposition of complex structure itself. The first weight loss was mainly caused by evaporation of water molecules with 9.33 % at 103.63-163.73 °C, which is according to Huang *et al.* [4], the water molecules at complex will be evaporated at 100-230 °C. The 33.30 % of weight loss at 227.09-278.63 °C

TABLE-1
CONTENT OF AN APPROACHING THEORETICAL COMPOSITION OF METAL ION COBALT, ZINC AND CHN ELEMENT FOR COMPLEX COMPOUNDS

Molecular structure	Content (%)				
	C	H	N	Co	Zn
Experimental	29.68	3.91	5.32	10.00	11.63
$[Zn(H_2O)_5Co(dipic)_2]$	30.88	2.96	5.14	10,82	12.01
$[Zn(H_2O)_5Co(dipic)_2]\cdot NO_3$	27.72	2.66	6.93	9.72	10.78
$[Zn(H_2O)_5Co(dipic)_2]\cdot H_2O$	29.89	3.22	4.98	10.47	11.62
$[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$	28.96	3.47	4.82	10.15	11.26
$[Zn(H_2O)_5Co(dipic)_2]\cdot 3H_2O$	28.09	3.70	4.68	9.84	10.92

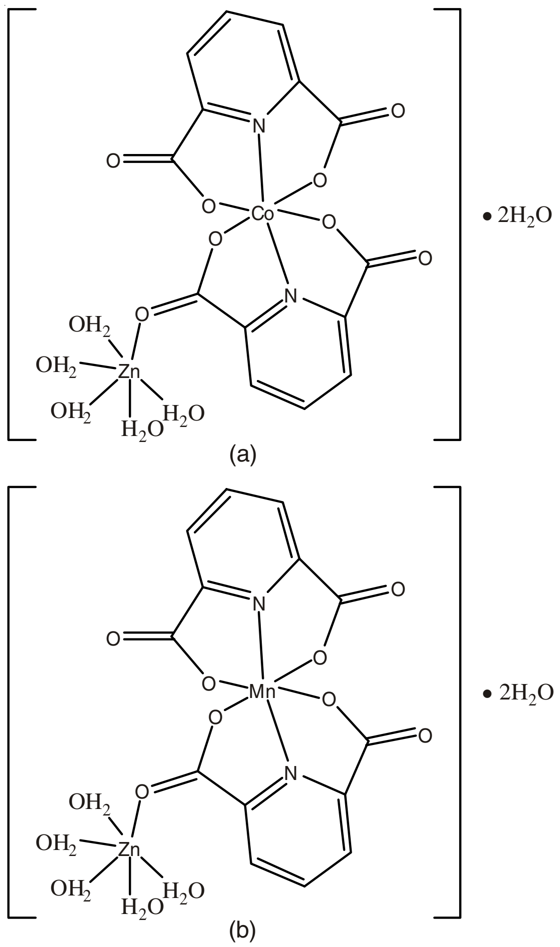

Fig. 1. Proposed structure of (a) $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ and (b) $[Zn(H_2O)_5Mn(dipic)_2]\cdot 2H_2O$

(step 2) was caused by the decomposition of dipicolinic ligand. This is consistent with previous research performed by Saravanan and Govindarajan [13] showing that the dipicolinic ligand decomposed at 230-540 °C. The third step was attributed to zinc ion loss at 429.20-487.47 °C with 22.52 % of weight loss. The weight loss continues until temperature of 575.15 °C, corresponding to the decomposition of the ligand bound to the cobalt ion.

The first weight loss, 9.15 % or 0.5857 mg, occured in the temperature range of 60-140 °C. This loss was caused by the decomposition of the hydrate compound, H_2O, in accordance with the prediction of complex molecular formula. The evaporation of water vapour adsorbed in crystal generally occurred at a temperature range of 100-200 °C [14]. At temperatures of 141-269 °C, weight loss as much as 19.61 % (1.2551 mg) occurred where the water ligand was expected to begin decomposing. The highest weight loss was appeared at the temperature of range 270-295 °C as much as 21.73 % (1.3912 mg). The weight loss at above 200 °C was predicted as the beginning of dipicolynic ligand decomposition [15,16] and the results shown in Table-2.

TABLE-2
DATA ANALYSIS ON DECOMPOSITION OF COMPLEX COMPOUNDS

Decomposition temp. (°C)	Residue weight (%)	Complex	Theoretical weight (%)
0	100	$[Co(C_{14}H_{20}N_2O_{15})Zn]$	100
0-163.73	90.67	$[Co(C_{14}H_{16}N_2O_{13})Zn]$	9.79
163.73-278.63	60.32	$[Co(C_7H_9NO_7)Zn]$	59.15
278.63-487.47	36.98	$[Mn(C_7H_3NO_4)Zn]$	38.59
487.47-575.15	14.16	$[Co(C_7H_3NO_4)]$	14.25

Structure determination of complex: The IR spectra of $[Zn(H_2O)_5Mn(dipic)_2]\cdot 2H_2O$ indicates the presence of a vibration from C=O carboxylic which indicated that the coordination occurred through the carboxylate groups. The spectra of $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ complex, shown by pyridine-2,6-dicarboxylic spectra, exhibits the vibration band at 3070.68 cm^{-1} corresponding to O-H carboxylic. The vibration band at 1705.07 cm^{-1} corresponds to C=O carboxylic, vibrational spectra at 1465.9 and 1265.3 cm^{-1} denotes to C=C aromatic and C-O, respectively.

The comparison between ligand and $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ sample spectra showed the broadening band at area of 3369.41 and 3101.32 cm^{-1} corresponding to the vibration of water molecules [1]. The OH band from carboxylic group of the complex at area of 3070.68 cm^{-1} shifted towards the lower value compared to ligand spectra. This shifting was also observed for C=O carboxylic band. The band was appeared at 1656.74 cm^{-1} for the complex, however, it was appeared at 1705.07 cm^{-1} for the ligand. The shifting in the spectra clarify that the carboxylic acid was not in the free condition but it was bound to the metal ion. This result confirmed the complex. The vibration band at 1450.37 cm^{-1} corresponds to C=C aromatic, band at 1284.50 cm^{-1} denotes to the CO group derived from a carboxylic bond in the ligand [17]. The presence of Zn metal ion was supported by the presence of peaks at wavenumber of 536.17 cm^{-1} showing the vibration of Zn-O. The presence of Co metal ion was indicated by the band at wavenumber 433.95 cm^{-1} representing the vibration absorption Co-N. This result is consistent with previous research reported by Colak *et al.* [5,18] where the Zn-O vibration would show at the wavenumbers between 550-450 cm^{-1} while the Co-N vibration would appear at the wavenumbers of 420-450 cm^{-1} [19].

Magnetic characterization: Magnetic property of the $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ complex was calculated and yielding the effective magnetic moment (μ_{eff}) of 3.78 BM at room temperature (27 °C). The value of the magnetic susceptibility of the complex indicates that the complex is paramagnetic.

Toxicity test: Toxicity test was carried out to determine the toxicity of $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ and

$[Zn(H_2O)_5Mn(dipic)_2]\cdot 2H_2O$ compounds toward normal cells. The toxicity properties of the complex are associated with the toxicity properties of several types of human's tissues. This toxicity test was carried out using brine shrimp lethality test using *Artemia salina* shrimp's larvae as tested animals.

The test was carried out using various concentration of complex solutions starting from 10, 100, 500 and 1000 ppm, consecutively in which each concentration was measured three times (triplo). The result of this brine shrimp lethality test is illustrated in Table-3. The table shows that the more concentrated the solution, the higher amount of mortality of shrimp larvae. The concentrations of 1-500 ppm did not cause 50 % of mortality of the total population of shrimp larvae. However at 1000 ppm of concentration, shrimp larvae mortality increased more than 50 %.

TABLE-3
TEST RESULTS BRINE SHRIMP LETHALITY TEST COMPLEX COMPOUNDS

Conc. (mg/L)	log conc.	Mortality accumulation	Natality accumulation	Mortality (%)
1000	3.00	1	112	100
500	2.70	7	82	72.881
250	2.40	14	57	45.902
100	2.00	28	33	19.718
50	1.70	43	16	7.865
25	1.40	73	0	0.885

The determination of LC_{50} was calculated using a linear regression of log concentration with % mortality to develop a regression equation resulting the equation of y = 39.496 x - 50.269. From this equation, the LC_{50} values of complex compounds was 283.71 mg/L for $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ complex and 354.94 ppm for $[Zn(H_2O)_5Mn(dipic)_2]\cdot 2H_2O$ complex.

Conclusion

Heterodinuclear complex of Zn(II)/Co(II) and Zn(II)/Mn(II) with acid ligand pyridine-2,6-dicarboxylic acid has been synthesized by reflux method. Based on the data obtained from the atomic absorption spectroscopy analysis, elemental analysis of C, H, N and TGA, it can be concluded that molecular formula of the complex compounds are $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ and $[Zn(H_2O)_5Mn(dipic)_2]\cdot 2H_2O$, respectively. The FTIR exhibited characteristic vibration bands at 536.17 and 433.95 cm^{-1} indicating the presence of Zn-O bond and Co-N as also supported by the TGA measurement as demonstrated by the decomposition of water molecules hydrate. The toxicity test revealed that the LC_{50} value was 283.71 mg/L for $[Zn(H_2O)_5Co(dipic)_2]\cdot 2H_2O$ complex and 354,94 mg/L for $[Zn(H_2O)_5Mn(dipic)_2]\cdot 2H_2O$ complex. The value of Mn(II) complex compounds is higher than Co(II) heterodinuclear complex.

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