Synthesis and Toxicity Test Of Zinc (II) Pyridine-2,6-Dicarboxylate Complexes

Fahimah Martak and Tia Ayu Christanti[1]

Abstract*—Complexes of zinc (II) with pyridine-2,6-dicarboxylic acid were synthesized from a reaction between zinc(II) chloride and pyridine-2,6-dicarboxylic acid with two different methods. The first method was carried out by reflux method, mixing a certain mole ratio of metal and ligand in the mixed solution of methanol:water (1:1) as the solvent. The second method was carried out by employing a direct reaction of metal and ligand in a 1:2 mole ratio of the same solvent as it is of the first method. The first method yielded a small clear crystal grains shaped like parallelogram with the molecular formula ~~of~~ $[Zn(H_2dipic,dipic)Zn_2(10H_2O)]Cl_4{\cdot}5H_2O$ (Complex I-trinuclear). The second method yielded the colorless needle-like crystals and molecular formula $[Zn(H_2dipic)_2Zn(H_2O)_5]Cl_4{\cdot}2H_2O$ (Complex II-dinuclear). Both complex compounds were tested their toxicity using Brine Shrimp Lethality Test (BSLT) method and obtained LC50 values of 989.26 and 503.32 ppm, respectively.

Keyword—***complex compounds, ligands, metal ion zinc(II), acid pyridine-2,6 dicarboxylic acid, toxicity***

Abstrak*—Senyawa kompleks Seng(II) dengan asam piridin-2,6-diarboksilat telah disintesis dengan dua metode berbeda. Metode pertama dilakukan dengan metode refluks, mereaksikan larutan logam dan ligan dengan perbandingan mol tertentu dalam pelarut methanol: air (1:1). Metode kedua direaksikan larutan logam dan ligan dengan perbandingan mol 1:2, pelarut yang digunakan sama dengan metode pertama. Kristal jernih berbentuk persegi dengan formula molekul $[Zn(H_2dipic,dipic)Zn_2(10H_2O)]Cl_4{\cdot}5H_2O$ dihasilkan dari sintesis dengan metode pertama. Kristal seperti jarum dengan formula molekul $[Zn(H_2dipic)_2Zn(H_2O)_5]Cl_4{\cdot}2H_2O$ dihasilkan dari sintesis dengan metode kedua. Kedua senyawa kompleks yang dihasilkan diuji toksisitasnya dengan metode Brine Shrimp Lethality Test (BSLT), nilai LC_{50} untuk masing-masing senyawa berturut-turut adalah 989.26 dan 503.32 ppm.

Kata kunci—***senyawa kompleks, ligand, ion logam Zn(II), asam pyridine-2,6 dikarboksilat, toksisitas***

I. INTRODUCTION

Pyridine-2,6-dicarboxylic acid (dipicolinate) was first found to have relation with biological systems in 1936 and known as the largest component of the bacterial spores. H_2dipic is used at most as the enzyme inhibitors and plant maintenance. Currently, previous researches on H_2dipic show that the acid contained in it can prevent oxidation process in low density lipoprotein. Pyridine-2,6-dicarboxylic acid which is also one of the carboxylic acid derivatives has interesting coordination chemistry. H_2dipic is also potential to produce various forms of coordination.

Pyridine-2,6-dicarboxylic acid can interact with several metal ions that are owned by human body. One way to determine chemical properties and amino acid in dipicolinate is by investigating them in the form of complex compounds [1], and as in the complex between the Zn (Zinc) and chitosan is active as an anti-microbial [2]. Zinc, a metal ion which has the electroncon figuration of the $d^{10,}$ is a perfect construction of coordination polymers as well as networks (networks).

The metal ion of d^{10} allows a flexible configuration for the formation of complex geometries ranging from trigonal Zn bipyramidal (sp^3d) and square planar to octahedral (sp^3d^2) and many other distortions that may occur [3]. One example of this is the zinc complexes with 1-hydroxypyridine-2-thione in its application that can inhibit prostate cancer cells (A549 lung and PC3) in xenograft models [4].

The structure of the zinc complexes with ligands of dipicolinate has been figured out, but studies on the nature toxicity has still not assessed yet. Therefore, this study attempts to test the toxicity characteristics that would lead to anti-cancer. Then, further assessment of different methods of synthesis are expected to provide different crystal structures.

Toxicity tests of the compound are commonly performed by BSLT (Brine Shrimp Lethality Test). BSLT uses *Artemia salina* shrimp larvae as tested animals. This method, in addition, is easy, fast and accurate enough, and also frequently used in the searching for new anticancer-research, generally derived from plants. The results of toxicity tests with Brine Shrimp Lethality Test methods have been shown to have a correlation with the power of cytotoksis anticancer compounds. Toxicity test results expressed in percent of the LC_{50} (Lethal Consentratios) [5,6].

II. METHOD

A. Synthesis of Complex I, Zn (II)- pyridine-2,6-dicarboxylic acid by reflux method

A total amount of H_2dipic 1.6712 (10 mmol) was dissolved in a solvent of methanol:water (1:1) each 25 mL and then put in a three neck flask. 0.68145 gram (5 mmol) of $ZnCl_2$ was sealed into a three neck flask containing H_2dipic solution [7]. Then the mixture was refluxed in the temperature of 70 °C while stirring at 400 rpm for 3 hours; as soon as the mixture was cooled to

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room temperature, the colorless needle-like crystals were obtained. The obtained crystal was characterized and tested by bioactivity.

B. Synthesis of Complex II, Zn (II)- Pyridine-2, 6 Dicarboxylic Acid using Non-Reflux Method

A total amount of H_2dipic 1.6712 (10 mmol) was dissolved in methanol:water (1:1). 0.68145 gram (5 mmol) $ZnCl_2$ was dissolved in water. $ZnCl_2$ solution was stirred at 400 rpm to room temperature, and was added to H_2dipic solution [8]. The mixture was heated at 60 °C for 24 hours, then it was colled to room temperature; after 5 days, the colorless needle-like crystals were obtained. Crystals was collected on filter and washed with a small amount of water.

C. Determination of Metal Ion Content

Solution was prepared by weighing samples of complex I as much as 0.0046 gram, adding two drops of concentrated HNO_3 and dissolved in distilled water at 10 mL volumetric flask. The addition of distilled water to mark boundaries in order to obtain a solution of 100 ppm concentration. Solution was then diluted to obtain a solution with a concentration of 2 ppm. The same treatment performed on complex II with a weighing of 0.0056 gram. Final solution with concentration of 2 ppm was then aspirated on the appliance.

D. Determination of the Content of C, H and N

Elemental analysis for C, H, N were carried out with Perkin Elmer Thermo Finnigan EA 1112 elemental analyser. Elemental analyser was standardized with L-Cistina Standard ($C_6H_{12}N_2O_4S_2$, C = 29.99%, H = 5.03%, N = 11.66%, S = 26.69% and O = 26.63%) before it was used. 2.83 mg was placed in an aluminum foil and sealed in a perforated plate for an oxygen-burning process. Element of micro tool was run and the elemental composition of C, H, N and S compounds were made legible on computer screen.

E. Determination of Functional Groups by Infrared Spectroscopy

Functional groups coordinated in the metal ion ~~is~~ were determined by an infrared spectrophotometer. The materials used were in the form of complex solids. Infrared spectra was recorded in KBr pellet in the range of 4000-300 cm^{-1} on Shimadzu FTIR Prestige-21 spectrometer.

F. Analysis of Magnetic Moment

Magnetic moment measurements performed at room temperature were observed by weighing the empty tube and its mass and expressed as an m_o. The next tube was inserted into the tool and the value of magnetic susceptibility (R) that appears recorded as Ro, the next with footage filling the tube. The tubes were weighed again and its mass is expressed as M and also measured the magnetic susceptibility footage afterwards expressed as R_1. Height in the tube and the temperature (in Kelvin) were recorded. Data variable obtained were calculated to obtain magnetic moment.

G. Analysis of ^{1}H NMR and ^{13}C NMR

^{1}H NMR and ^{13}C NMR were recorded on NMR (JEOL JNM-ECA) 500 Mhz in DMSO-d_6. The chemical shifts are referred to TMS (the accuracy is 0.01 ppm). Measurement of ^{1}H NMR and ^{13}C NMR chemical research was carried out in the center-LIPI. However, before measuring NMR, the test on the solubility of the ligand and the two complexes were performed at 5 different solvents, namely the solvent DMSO, acetone, methanol, chloroform, and H_2O [9]. Solubility test was performed on a vial of clear bottles, by taking 1 mg of sample with 1 mL of solvent. Samples were dissolved in a deuterium solvent (D_2O), which previously had been determined by solubility, and then inserted into the NMR tube and then analyzed.

H. Electrical Conductivity Analysis

Standard solution of KCl and $MgCl_2{\cdot}6H_2O$ with concentration of 0.001 M was prepared in advance in methanol solvent. Complex I and complex II with concentration of 0.001 M were prepared in the same solvent. Electrical conductivity was measured with conductometer.

I. Toxicity Test

Toxicity tests was conducted to determine the LC_{50} (Lethal Concentration) of $ZnCl_2$, H_2dipic, complex (I) and complex (II). Concentration of test solutions was made with 62.5 g / ml, 125 µg / ml, 250 µg / ml, 500 µg / ml and 1000 µg / ml and then ~~were~~ was taken and put in a 15 mL tube and then inserted into the tube with a capacity of 30 mL. 15 mL of sea water was filled with 10 of shrimp pups [10]. The tube was then allowed to stand for 24 hours and then to count~~ed~~ the number of shrimp pups which visually died. The tests were performed three times for each concentration. *Artemia salina* larvae were said to be died if it did not show any movement during the observation. When there were deaths in the control, it was corrected by Abbot's formula [11].

III. Result and Discussion

Prediction of crystal structure I can start with the measurement of zinc levels by AAS instrument (atomic absorption spectroscopy). The method was used to determine the levels of zinc in complex synthesis. These measurements can be supplemented with data from the elemental analyzer elements of C, H, and N. Measurement of zinc levels in the complex I shows the results of 19.77%. If the results of these measurements are combined with data C, H, N then obtained a complex variety of possible formulas shown in Table 1.

Table 1 shows that the complex with formula of $[Zn(H_2dipic,dipic)Zn_2(10H_2O)]{\cdot}5H_2O{\cdot}Cl_4$ has good content compatibility of Zn, C, H and N based on the results of theoretical calculations of trinuclear complex with the experimental data.

The results of infrared spectroscopic measurements on the wave number of 4000-300 cm^{-1} produces a spectrum as shown in Figure 1.

The spectrum shows a broad peak at 3410.15 cm^{-1} region, corresponding to an OH group from the ligand bound to the metal and the OH hydrate water. The next major peak appears at 1635.64 cm^{-1} corresponding to C = O carboxylic group, which originated from the carbonyl of the ligand that binds to the metal. IR spectrum of the other peaks of the complex I found the peak at 416.62

and 540.07 cm^{-1} that for each could be identified as the vibrations of Zn-N and Zn-O.

Before employing NMR analysis, the magnetization of the complexes was analyzed in order to prevent the magnetic field affected by the complex compounds, namely paramagnetic and ferromagnetic, in terms of its characterization [12]. Chemical shift data are shown in Table 2.

Table 2 shows that there is a shift for the ligand at 8.35 which is the aromatic shift. Furthermore, for compound I, there is a shift in the region of 8.40 ppm. This indicates the complex environment of aromatic H, and a shift when compared with the ligand, is also an indication that the complex is formed [13]. More analysis can actually be supported by the C environment, but because the ligand ^{13}C analysis is not done, then it can not be compared like any other type of carbon that can be determined by ^{13}CNMR. Each of which appears in the shift of 167.7: 146.3: 143.5: 126.7. These shifts can be divided to be 4 C of the dominant environmental group that is ≈ 167, ≈ 146, ≈ 143 and ≈ 126. Each of these shifts can be explained in the Figure 2. The shift to the environment 1 ≈ 167, ≈ 146 for C2 and C6, C3 and C5 ≈ 143 and ≈ 128 for C4.

Further analysis is the delivery, where delivery is later than the standards that have been made earlier, namely the conducting KCl (+1) in water at 157.32 and $MgCl_2 \cdot 6H_2O$ (+2) at 310.02 ($S.cm^2.mol^{-1}$). Complex I has a conductivity of 621.02, so it can be concluded that the complex has a charge of 4+. Of the overall characterization performed, it can be suggested that the structure shown in Figure 3.

Table 3 shows that the complex II is almost the same as dinuclear complex, so it can be concluded that this complex is a dinuclear complex.

For the IR spectra of complex II is shown in Figure.4 is not far different from complex I. Wide peak at 3410.15 cm^{-1} region which also shows that there is an OH group either as a ligand bound to the Zn(II) metal ion or OH from the water hydrates that bound chemically to the complex as the complex I. The peak at 1705.07 cm^{-1} correspond to C=O vibrational states, and for the vibration of Zn(II) metal ion with O and N atoms appeared at 524.64 cm^{-1} and 455.2 cm^{-1}, respectively [14].

Another test was employed for the NMR of complex II, in which 1H NMR was also shifted when compared with the ligand The shift of complex II occurred at 8.38 (s) while the ligand occurred at 8:35 (d), it also supports that complex II is formed. 13C NMR shifts occur at 167.1: 146.4: 142, 8, and 127.5; are also almost the same as previous complex I [15].

In accordance with the characterization of the power delivery (Λm) on complex II conductivity of 562.02 ($S.cm^2.mol^{-1}$), it can also be concluded that this complex has a charge of +4. Structure characterization was performed; therefore, it may be advisable that the structure is shown in Figure 4.

A.Characterization Of Complex II

Prediction of the crystal structure of complex II is also similar to previous measurements of zinc which is begun with the instrument data ofAAS and C, H and N of the elemental analyzer. Measurement of zinc levels in the complex II shows the results of 15.94%. Various possible structures are shown in Table 3.

Table 3 shows that the complex is almost the same as dinuclear complex, so it can be concluded that this complex is a dinuclear complex.

B. Toxicity Test

Characterization of complex (I) and (II) demonstrate the suitability of the complex of zinc (II) pyridine-2,6-dicarboxylic acid. Both complexes are expected to have great potential in terms of toxicity. The method used for toxicity testing is BSLT (Brine Shrimp Lethality Test), in which the test will be obtained from the LC_{50} (Lethal Concentration) [16].

This study uses an experimental design with treatments of giving the concentration of 2000 ppm, 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 62.5 ppm, respectively, of zinc(II) chloride and H_2dipic as negative gram, and complex (I) and complex (II) as positive gram, by the number of repetition of three times (triplo). The value of LC_{50} is calculated by equation of the log of concentration with percentage (%) of shrimp pups mortality [17]. LC_{50} values of each test solution was then calculated. The LC_{50} values are obtained for $ZnCl_2$, H_2Dipic, Zinc Complex I and Zinc Complex II are at 29.54 ppm; 263.66 ppm; 989.26 ppm and 503.32 ppm, respectively.

CONCLUSION

Complex compound (I) [Zn(H_2dipic, dipic) $Zn_2(10H_2O)] \cdot 5H_2O \cdot Cl_4$ and complex (II) [Zn(H_2dipic) $2Zn(H_2O)_5] \cdot 2H_2O \cdot Cl_4$ can be synthesized using acid ligands of pyridine-2,6- dicarboxylic acid and zinc(II) metal ion chloride by the method of heating under reflux and with stirring. Finally, the present study has yielded the results of 1H NMR shift of the ligand to complex the support for complex formation, and LC50 values for complex I is obtained at 989.26 ppm and 503.32 ppm for complex II.

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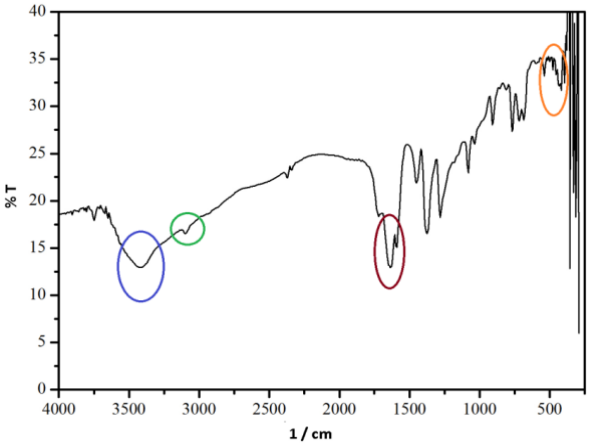

Figure 1. FTIR spectrum of complex I

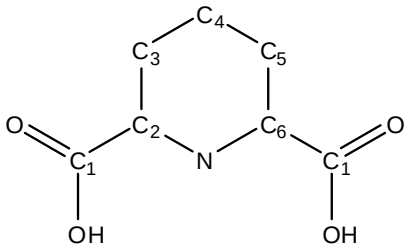

Figure 2. Prediction of complex structures I and II on the aromatic

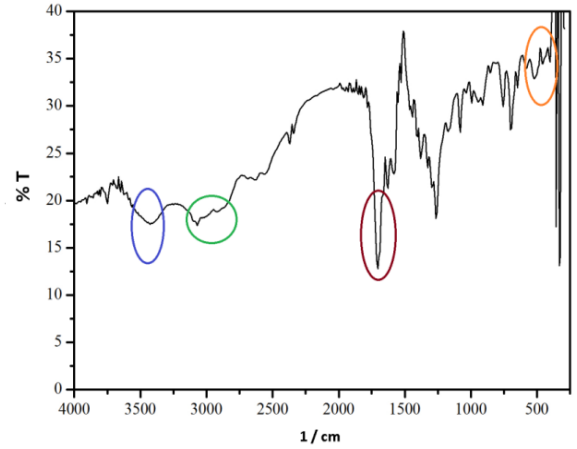

Figure. 3. FTIR spectrum of complex II

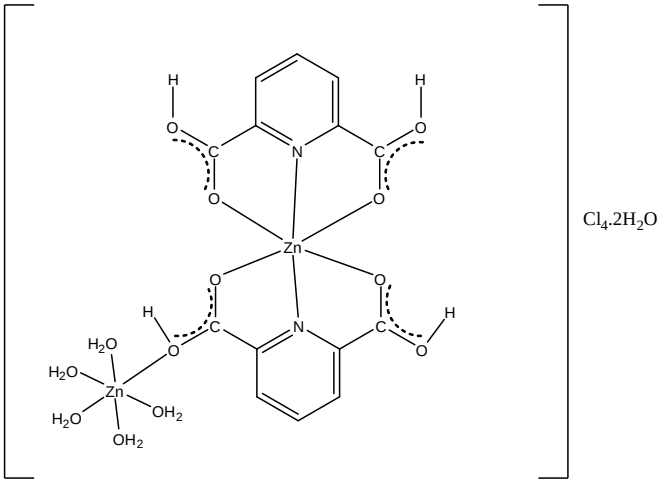

Figure. 4. Predicted structures of complex compounds II [Zn(H_2dipic) 2Zn(H_2O)]·2H_2O·Cl_4

TABLE 1.
CONTENT ZN, C, H AND N IN THE COMPLEX I THEORETICALLY AND EXPERIMENT

Molecular Formula	% Zn	%C	%H	%N
Experiment	19.77	17.679	4.374	2.945
[$Zn(L)_2Cl_2$·5 H_2O] (mononuclear)	11.66	29.99	3.56	4.99
[$Zn(L)_2$ $Zn(5H_2O)Cl_4$·5 H_2O] (dinuclear)	16.60	21.36	3.81	3.56
[($Zn(L)_2$/$Zn_2(10H_2O)$)Cl_4·5 H_2O] (trinuclear)	20.86	17.88	4.04	2.98

TABLE 2.
LIGAND NMR DATA, COMPLEX I AND COMPLEX II

NMR	H_2dipic (ppm)	Complex I (ppm)	Complex II (ppm)
^{1}H	8.35 (d)	8.40 (m)	8.38 (s)
^{13}C	-	167.7 146.3 143.5 126.7	167.1 146.4 142.8 127.5
^{1}H	8.35 (d)	8.40 (m)	8.38 (s)
^{13}C	-	167.7 146.3 143.5 126.7	167.1 146.4 142.8 127.5

TABLE 3.
CONTEND ZN, C, H, N IN THE COMPLEX II THEORETICALLY AND EXPERIMENT

Molecular Formula	% Zn	%C	%H	%N
Experiment	15.94	21.457	3.689	3.983
$[Zn(L)_2Cl_2 \cdot 2H_2O]$ (mononuclear)	12.90	33.18	2.76	5.53
$[Zn(L)_2Zn(5H_2O)Cl_4 \cdot 2H_2O]$ (dinuclear)	17.84	22.94	3.27	3.82
$[(Zn(L)_2/Zn_2(10H_2O))Cl_4 \cdot 2H_2O]$ (trinuclear)	22.08	18.92	3.60	3.16

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