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Complexes of 2,6-dihydroxybenzoic acid with divalent metal ions: Synthesis, crystal structure, spectral studies, and biological activity enhancement

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ABSTRACT

2,6-Dihydroxybenzoic acid or γ-resorcylic acid (DHBA) is a phenolic compound which is known to have poor biological performance such as DPPH scavenging activity and microbial growth inhibition. Combining DHBA with divalent metal ion to form complex is expected to improve its biological properties. The complexes of DHBA with divalent Ni/Co were synthesized and characterized in this study. The complex structures were determined by X-ray single crystal analysis and it was found that metal interacted with DHBA through H-bonds. The results on the biological properties indicate that the complexes have remarkable DPPH scavenging activities and microbial growth inhibition abilities. $IC_{0.5}$ value of DPPH is 35.68 mg/L (231.51 μM), 9.21 mg/L (18.09 μM) and 25.63 mg/L (50.33 μM) for DHBA, NiDHBA and CoDHBA respectively. The microbial growth inhibitory value (%) at a sample concentration of 400 mg/L is 2.9, 6.4 and 96.1 against *Escherichia coli* and 4.9, 16.8, 98.2 against *Staphylococcus aureus*, for DHBA, NiDHBA and CoDHBA, respectively.

1. Introduction

Dihydroxybenzoic acids are aromatic compounds containing two phenolic and a carboxylic acid functional groups [1]. 2,6-Dihydroxybenzoic acid or γ-resorcylic acid (DHBA) is one of the six isomerics of dihydroxybenzoic acids (Fig. 1) [2,3]. DHBA can be synthesized through the Kolbe-Schmitt carboxylation of resorcinol [4,5]. This compound also can be found as one of the phenolic compounds contained in white grape pomace [6]. There are few studies that reported the biological properties of DHBA since this compound possesses poor biological activity. Nishibe [7] reported that DHBA possesses poor DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity wherein <200 μM (30 mg/L) of DHBA was required to decrease by 0.200 ($IC_{0.2}$) in the absorbance after 30 min reaction with DPPH [7].

The synthesis of organic ligand and metal ion is an interesting research field since the product complex may enhance the biological properties of the ligand [8–12]. To the best of our knowledge there is no study utilize combination of DHBA and metal ions in order to enhance the biological properties of DHBA. In this work, DHBA and divalent metal ion (Ni/Co) were combined to form a metal-ligand complex. The structure of the complexes was determined by X-ray single crystal analysis. Several physical measurements such as SEM, UV–Vis, FTIR, TGA and ^1H NMR were conducted on the complexes. Biological activities of the complexes were tested, specifically the DPPH radical-scavenging activity and microbial inhibition growth activity against *Escherichia coli* and *Staphylococcus aureus*.

2. Experimental

2.1. Materials

Analytical grade DHBA ($C_7H_6O_4$, 98% purity), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and metal salts of cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$, 98% purity) were purchased from Sigma Aldrich (St. Louis, MO), nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$, 98% purity) was purchased from Alfa Aesar (Lancashire, UK). Ammonium hydroxide (NH_3, 30%) was obtained from Yakuri Pure Chemical (Kyoto, Japan).

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2.2. Physical property measurements

Acid dissociation constants of the ligand DHBA were spectroscopically determined by refining the spectrum as function of pH using HypSpec program [13]. Several measurements were done to characterize the physical properties of complexes. Surface topography analysis was done by using a JEOL JSM-639 scanning electron microscope at an accelerating voltage of 20 kV and Pt for sample coating. UV–Vis spectrum analysis was carried out using a JASCO V-550 spectrophotometer equipped with halogen and deuterium lamp. The sample was placed in a standard 10 mm quartz cell. FTIR spectrum analysis was recorded on a Bio-Rad FTS-3500 instrument on KBr disc with a spectra range of 400–4000 cm^{-1}. Thermogravimetric analyses (TGA) under N_2 atmosphere and a heating rate of 10 °C/min were done by using a Perkin Elmer Diamond TG/DTA. Elemental analysis on carbon and hydrogen atom was done by using an Elementar Vario EL III. ^{1}H NMR spectra analysis was measured on a Bruker AVIII-600 MHz FT-NMR in D_2O solution. Structure determination was performed by X-ray single crystal analysis using an Oxford Gemini Dual system Single-crystal XRD equipped with Cryojet.

2.3. Spectrophotometric method

The acidity constant of DHBA was determined spectroscopically in the range of 200–400 nm. The spectrum measurements were done using a solution containing DHBA at a concentration of 2×10^{-4} mol/L. The ligand solution was acidified by using 0.1 mol/L HCl until pH < 1.0. Subsequently spectra of the ligand were measured as a function of pH, where the pHs were sequentially adjusted by using 0.1 mol/L NaOH until pH 13.0. HCl and NaOH were standardized before use. The spectrum data were then used as input to the HypSpec program for determining acidity constant.

2.4. Synthesis of complexes

DHBA (0.39 g, 2.5 mmol) was dissolved in distilled water (15 mL). The solution was heated to 70 °C and a few drops of 2 M ammonia solution were added until DHBA was completely dissolved. Five milliliters of metal salt solution (0.61 g (2.5 mmol) of $NiCl_2 \cdot 6H_2O$ or 0.74 g (2.5 mmol) of $Co(NO_3)_2 \cdot 6H_2O$) were added into the DHBA solution. The pH of the mixture was adjusted to ~4 by adding a few drops of 2 M ammonia solution. The reaction was carried out at 70 °C for 4 h with constant stirring, then the solution was cooled to room temperature and left to stand until crystal was formed. The crystal complex was isolated by vacuum filter, washed several times with distilled water and air-dried.

Small needle green crystals of NiDHBA complex were formed between DHBA and Ni^{2+}. The complex was formed after the solution was kept overnight with a yield of 46.6%. *Anal.* Calcd for $C_{14}H_{26}NiO_{16}$: C, 33.03; H, 5.15. Found: C, 32.66; H, 5.19%. *Form. Weight* 509.06 g/mol. ^{1}H NMR (methanol-d_4, ppm): 7.41, 9.02.

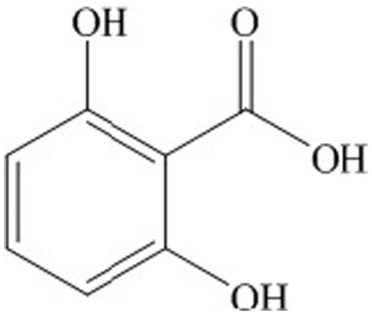

Fig. 1. Structure of 2,6-dihydroxybenzoic acid.

Cubic red crystals (CoDHBA) were formed after DHBA and Co^{2+} solution was kept for 3 days (yield 59.1%). *Anal.* Calcd for $C_{14}H_{26}NiO_{16}$: C, 33.02; H, 5.15. Found: C, 32.90; H, 5.27%. *Form. Weight* 509.28 g/mol. ^{1}H NMR (methanol-d_4, ppm): 6.18, 6.82.

2.5. Biological property measurement

2.5.1. Radical scavenging activity

Radical scavenging activity of complex was tested against the stable radical DPPH [14]. The tested compound was dissolved in methanol at different concentrations. DPPH solution (0.2 mL, 5×10^{-4} mol/L in methanol) was added to the prepared tested compound solution (0.8 mL). The tested complex was incubated at 37 °C for 30 min and the absorbance was measured at 517 nm against methanol as the blank. DPPH (0.2 mL) in methanol (0.8 mL) without any tested complex was used as the control. Percent DPPH scavenging activity was calculated as:

$$\%\text{DPPH scavenging activity} = \frac{Ac - As}{Ac} \times 100$$

where *Ac* is the absorbance of control and *As* is the absorbance of sample at 517 nm. Ascorbic acid was used as the positive control.

2.5.2. Microbial growth inhibitory activity

Broth macro dilution method [15] was used to determine the microbial growth inhibitory activity of the complexes against the gram negative microorganism *Escherichia coli* and gram positive microorganism *Staphylococcus aureus*. Ampicilin (895.5 μg/mL potency) was used as the antibiotic reference. Lysogeny Broth (LB) media prepared from the mixture of tryptone, yeast extract and sodium chloride with ratio 2:1:2 w/w was used as the medium.

The assay was performed in tubes containing different concentrations of the tested complex dissolved in LB medium with a total volume of 3 mL for each tube. The prepared bacteria suspension (15 μL, 1×10^8 cfu/mL) was injected into each test tube. All test tubes were incubated for 24 h at 37 °C. The OD_{600} of each tube was measured after incubation. As the control, 15 μL of bacteria suspension was injected into a test tube containing no tested compound. The antimicrobial activity was expressed as %inhibition, calculated as:

$$\%\text{inhibition} = \frac{Ic - Is}{Ic} \times 100$$

where *Ic* is the absorbance of control and *Is* is the absorbance of sample at 600 nm.

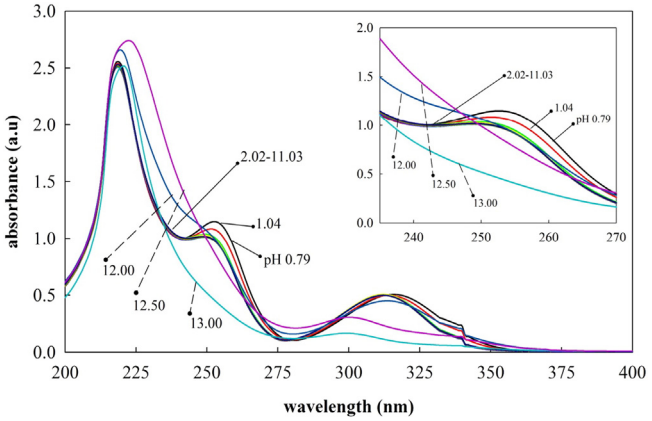

Fig. 2. Spectra of ligand DHBA.