

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL PROPERTIES OF COPPER(II) COMPLEX OF HETEROCYCLIC LIGANDS

(Sintesis, Pencirian dan Ciri Antimikrob Kuprum(II) Kompleks dengan Ligan Heterosiklik)

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Received: 21 August 2016; Accepted: 27 July 2017

Abstract

Copper(II)- β -mangostin complex was prepared by 2:1 molar reaction of ligand, β -mangostin and copper(II) acetate in one-pot reaction. The resulting complex, $[\text{CuL}_2(\text{H}_2\text{O})_2]$ has been characterized by using spectroscopic techniques. Ligand and its metal complex were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Salmonella pneumoniae* bacteria to assess on their antibacterial properties using Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) method. The UV-Vis result showed that the complex has an octahedral geometry. The general formula $[\text{ML}_2(\text{H}_2\text{O})_2]$, in which L = β -mangostin, is determined from UV-Vis, FTIR and CHNS data. Ligands were completely inactive against bacteria whereas the copper(II) β -mangostin complex has significant action on bacteria, indicating that it has a good potential as bactericide.

Keywords: β -mangostin, copper(II) complex, antimicrobial, xanthenes

Abstrak

Kompleks kuprum(II)- β -mangostin disediakan dengan nisbah 2:1 mol antara ligan, β -mangostin dan kumprum asetat dalam tindak balas satu-bekas. Kompleks yang dihasilkan, $[\text{CuL}_2(\text{H}_2\text{O})_2]$ dicirikan dengan menggunakan teknik spektroskopik. Ligan dan logam kompleks diuji ke atas bakteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Salmonella pneumoniae* untuk menilai tindak balas bakteria menggunakan kaedah Kepekatan Perencatan Minimum (KPM) dan kaedah Kepekatan Bakteria Minimum (KBM). Keputusan UV-Vis menunjukkan kompleks mempunyai geometri oktahedron. Formula umum $[\text{ML}_2(\text{H}_2\text{O})_2]$, yang mana L = β -mangostin ditentukan dari data UV-Vis, FTIR dan CHNS. Ligan sepenuhnya tidak aktif ke atas bakteria, manakala kompleks kuprum(II) β -mangostin memberikan tindak balas yang signifikan ke atas bakteria, menunjukkan ia mempunyai potensi yang baik sebagai bactericide.

Kata kunci: β -mangostin, kuprum(II) kompleks, antimikrob, xanthenes

Introduction

Transition metal ions such as copper(II), iron(II), iron(III) and zinc(II) have been known for many years to exert wide biological activity, for example against tumor cells [1]. Also chromones, flavonoid and coumarins have been known for similar properties. These two facts have attracted the attention of researchers to check whether metal complexes are more effective against anticancer and antibacterial than free ligands.

Copper is an essential trace mineral present in all body tissues. Copper works with iron to help the body to form red blood cells and increase the iron absorption. It also helps to keep the blood vessels, nerves, immune system and bones healthy. Copper has a major role in the production of the very reactive hydroxyl radical through the Fenton and Haber-Weiss reaction. Copper(II) can binds and manifests large hyperchromic and bathochromic shifts in the molecular absorption spectra [2].

Another class of less explored naturally occurring ligands are xanthenes. These bioactive compounds are heterocyclic compounds originally isolated as secondary metabolites from plants and microorganisms. Previous phytochemical studies on *Garcinia mangostana* have resulted in isolation of more than 50 xanthenes. The major components in *Garcinia mangostana* are xanthenes, such as α -mangostin, β -mangostin, and γ -mangostin [3]. The xanthone derivatives has a remarkable effect on antiulcerogenic, antifungal activity, antibacterial activity, inhibitors of protein kinase, anticancer activity, antioxidant and anti-inflammatory [4], which depend on their wide range of structures modified by substituents on the ring [5].

Simultaneously, synthetic and medicinal chemistry studies of xanthenes derivative have been performed [6]. In contrast, there are only a few reports that deal with complexation of metal ions with xanthenes derivatives. So far, complexation having xanthenes as a ligand only involve synthetic xanthenes. The synthetic xanthone was prepared via reaction dihydroxyxanthone with crown ether [7] or piperidiny [8], respectively. The macrostructure of crown ether helps to stabilize the Cu(II) complexes formed whereas piperidiny structure contributed basicity properties to encourage formation of Cu(II) and Zn(II) complexes with simple oxygenated xanthenes.

In this work, the isolated β -mangostin from *Garcinia mangostana* will be used as a ligand to synthesize a new metal complex having copper(II) as the central metal. The newly synthesized compound will be tested for antimicrobial properties. Isolated β -mangostin was inactive against antimicrobial [9] and anticancer [10]. The inclusion of copper(II) could alter the overall structure of newly formed synthesized compound and thus can increase the biological properties of the compound.

Materials and Methods

Materials

All the chemicals used were Analar grade. Copper(II) acetate and ethanol were used as received.

Extraction, Isolation and Purification of β -mangostin

Around 5-6 kg barks samples of *Garcinia mangostana* were obtained from Sarawak. 2.3 kg of powdered air-dried stem barks samples from *Garcinia mangostana* was macerated with chloroform for 48 hours at room temperature. The solvent was removed under reduced pressure using a rotavapor. The crude chloroform extract was isolated using Liquid Vacuum Chromatography and afforded 27 fractions. Same R_f values on the TLC plates of β -mangostin were combined. The purity of β -mangostin was determined using Agilent HPLC Series 1260 Infinity.

Synthesis of Cu(II) complexes

The procedure to obtain metal complex was adopted from established method by Bukhari et al. [11]. 0.589 mmol of copper(II) acetate was dissolved in ethanol. Then, 0.1178 mmol of β -mangostin was added and the colour of the solution was olive green. The mixture was refluxed for 3 hours. After refluxed, the mixture solution was filtered and washed with ethanol for few times. The filtrate was evaporated at room temperature. The resulting complex has been characterized using UV-VIS, Fourier Transformation Infrared (FTIR) and CHNS.

Instrumentations

Ligand, β -mangostin and its metal complex were determined using Gas Chromatography – Mass Spectrometry (GCMS), Nuclear Magnetic Resonance (NMR), UV-Visible, Fourier Transformation Infrared (FTIR), High Performance Liquid Chromatography (HPLC) and CHNS analyser.

Elemental analyses (C, H and N) was performed on elemental analyser Model vario MICRO cube. Infrared spectra of β -mangostin and its metal complex were recorded on a Perkin-Elmer Frontier FTIR using KBr pellet in the

spectral range of 4000-400 cm^{-1} . Electronic spectra of the ligands and its metal complexes will be carried out by using Perkin-Elmer Model Lambda 25.

^1H and ^{13}C NMR measurements were obtained on Bruker at 400 MHz, a 5 mm probe head in acetone- d_6 at room temperature. HPLC analysis for β -mangostin was carried out on Agilent G1316A (150 mm x 4.6 mm, 5 μm) column, the mobile phase was (80:20, v/v) acetonitrile/water mixture at room temperature with 1 mL min^{-1} flow rate and a 5 μl injection loop. Detection was performed with a UV detector in HPLC at 320 nm. The xanthone scaffold having good absorption at this wavelength and only a few number of other compounds absorb making 320 nm wavelength for xanthone detection [12].

Antimicrobial activities

Antibacterial test for ligand and synthesized compound were carried out using Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) method. The following bacteria were used as test organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Salmonella pneumoniae*.

Results and Discussion

Structural elucidation of β -mangostin

Fraction 11 until 13 from previous vacuum column chromatography were fractionated using gravity column chromatography yielded β -mangostin. Recrystallization from chloroform yielded yellow needle crystals with melting point of 174 – 175 $^{\circ}\text{C}$ (Lit. 175 – 176 $^{\circ}\text{C}$ [13]). The R_f value was 0.575 using a solvent system of chloroform. The molecular ion peak was found at m/z 424 indicating a molecular formula of $\text{C}_{25}\text{H}_{28}\text{O}_6$. Figure 1 showed the structure of β -mangostin. The UV-Visible spectrum exhibited characteristic absorption bands of xanthone at λ_{max} (ethanol) 244, 259, 315 and 355 nm. The IR spectrum showed the presence of hydroxyl group at 3399 cm^{-1} and a chelated carbonyl group at 1648 cm^{-1} . The absorption band situated at 1600, 1571, 1458 and 1278 cm^{-1} are related to carbon vibration in benzene rings. The purity of isolated β -mangostin was 98% determined from HPLC analysis.

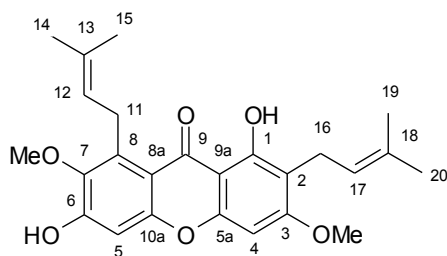


Figure 1. β -mangostin

^1H -NMR and ^{13}C -NMR data were compared with reported data by Shen et al. [15]; Al-Massarani et al. [16] and Syam et al. [17]. The ^1H NMR spectrum showed the presence of one chelated hydroxyl group at δ 13.66. In addition, typical signals of four allylic methyl groups at δ_{H} 1.67 (3H, s, H-14), 1.84 (3H, s, H-15), 1.65 (3H, s, H-19) and 1.79 (3H, s, H-20); two pairs of methylene proton at δ_{H} 4.15 (2H, d, J = 6.6 Hz, H-11) and 3.34 (2H, d, J = 7.2 Hz, H-16); and two olefinic protons at 5.22 (2H, t, J = 7.3 Hz, H-12) and 5.29 (2H, t, J = 6.7 Hz, H-17) were observed in the ^1H NMR spectrum.

The ^{13}C NMR spectrum gave a total of twenty five carbons which was validated for the molecular formula, $\text{C}_{25}\text{H}_{28}\text{O}_6$. A signal at δ_{C} 182.0 indicated a carbonyl group in the middle ring. Meanwhile, the methoxyl carbons signals were observed at δ_{C} 55.7 and δ_{C} 60.5. The locations of the two prenyl moieties were located at the C-2 (δ_{C} 110.9) and C-8 (δ_{C} 137.3). In the COSY spectrum the nature of the allylic and homoallylic coupling systems within the isoprene unit was clearly demonstrated. It showed correlation between the olefinic proton of C-12 and benzylic proton of C-11, and the germinal dimethyl group of C-14 and C-15. The same correlations above were also

observed for the other prenyl moiety.

Physical properties of Cu(II) complexes

Metal complexes involving β -mangostin and copper metal produced green complex which was stable at room temperature. The ligand, β -mangostin (L) on interaction with copper(II) acetate yields complex corresponding to the general formula $[ML_2(H_2O)_2]$. The analytical data showed that the metal to ligand ratio is 1:2. (Yield 72%) Analytical calculation for $CuL_2(H_2O)_2$ (MW = 946 $gmol^{-1}$): C=62.6%, H=5.4%. Found: C=63.4%, H=6.1%.

Spectroscopic study of Cu(II) complexes

IR spectra of β -mangostin has shifted in $\nu(C=O)$ peak from 1648 cm^{-1} to 1615 cm^{-1} and the $\Delta\nu$ (ligand – complex) is 33 [7, 8]. These absorption frequencies demonstrated that the group has lost its original characteristics and formed a coordinative bond with Cu(II) ion. Thus, this shows that the electron density in the carbonyl is slightly decreased probably due to back bonding process. Free OH stretching at 3399 cm^{-1} was absent and replaced with a broad $\nu(OH)$ which was observed at 3438 cm^{-1} indicating coordination of water with β -mangostin. These data suggested that the oxygen of the carbonyl and hydroxyl have formed a coordination bond with the Cu^{2+} cation.

Another significant difference between the ligand and its metal complex was observed from UV spectrum. The UV spectrum of copper(II) complex shows a broad band at 22 000 cm^{-1} (λ_{max} , 450 nm) which presumably corresponded to $d-d$ transition with octahedral arrangement [18]. The chelation of Cu(II)- β -mangostin complex with bidentate ligand occurred with hydroxyl and carbonyl group at C_9 and C_1 respectively. The proposed structure of the complex was shown in Figure 2. The chelation formation in this study was similar to Farhan [19] and Wang et al. [7].

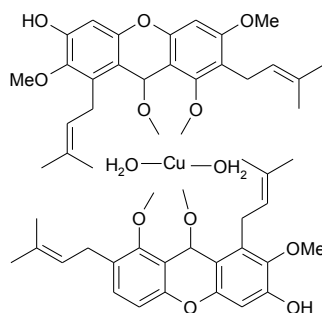


Figure 2. The proposed structure of Cu(II)- β -mangostin

Antimicrobial properties

β -mangostin was classify as inactive against the five bacteria tested. This antimicrobial result of β -mangostin was similar to Angia et al. [9]. Whereas the copper(II) complex showed weak inhibition with MIC value of 900 $\mu g/ml$ to *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Salmonella pneumoniae* but moderate inhibition with MIC value of 450 $\mu g/ml$ towards *Escherichia coli*. The MIC value for positive control, streptomycin sulphate is 7.03 $\mu g/ml$ for all bacteria used.

Conclusion

Copper(II)- β -mangostin complex, $[CuL_2(H_2O)_2]$ was successfully synthesized with an octahedral geometry. The copper(II) complex showed more potent antimicrobial activity compared to ligand itself. This finding could lead to the development of new metal based bioactive compounds with enhanced antimicrobial properties, suitable for medical application and cosmetic products.

Acknowledgment

Authors are highly thankful to the Faculty of Applied Sciences, Universiti Teknologi MARA, Campus Samarahan 2, Sarawak and Ministry of Higher Education for given Fundamental Research Grant Scheme (FRGS) grant (FRGS/1/2014/ST01/UiTM/02/3) to support this study.

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