Journal of Inorganic Biochemistry 189 (2018) 103–114

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Equilibrium studies of new bis-hydroxypyrone derivatives with Fe^{3+}, Al^{3+}, Cu^{2+} and Zn^{2+}

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ARTICLE INFO

Keywords:
Hydroxypyrones
Metal complex stability
Potentiometry
UV–vis spectrophotometry
NMR spectroscopy

ABSTRACT

This paper presents an easy and low cost synthesis of chelating agents for potential medical and environmental applications, and the evaluation of the stability of their complexes with Fe^{3+}, Al^{3+}, Cu^{2+} and Zn^{2+}. In the last years, we synthesized and characterized effective iron chelators based on two kojic acid units joined by different linkers in position 6. In this study, we preserved kojic acid (a cheap and non-toxic molecule) as the basic unit but joined the two kojic acid units through ethylene diamine, propylene diamine and butylene diamine by reacting them with the OH groups in position 2. The different anchoring position of the linker, as well as the linker length, can affect both protonation and complex formation equilibria. A thorough study of the protonation and complex formation equilibria of the three ligands toward the metal ions is presented based on combined potentiometric and spectroscopic studies, and ^1H NMR. The obtained results allow remarking that the orientation of the oxygen atoms in the kojic acid units, related to the anchoring position of the linker, strongly affects the protonation constants, while the chelating ability is practically unaffected. The trivalent metal ions form stable complexes with a 2:3 metal to ligand stoichiometry through the oxygen donor atoms of the ligands, whereas divalent metal ions form binuclear complexes for which the nitrogen atoms from the linker might be involved in the coordination sphere. The stability of the complexes decreases with linker length, and the selectivity of the ligands toward metal ions grows in the order $Zn^{2+} < Cu^{2+} < Al^{3+} < Fe^{3+}$.

1. Introduction

Metal ion toxicity is nowadays a primary topic in medicinal, environmental and agricultural chemistry, and the research of new metal chelators is still a leading motif in scientific research. Effective metal chelating agents, in addition to their recognized medical uses [1–3], find also an increasing number of applications in environmental remediation [4–6] and in agriculture for supplying essential elements to plants in an easily available form, or making accessible those elements already present in the soil [7–9]. Besides the formation of stable metal complexes, the lack of toxicity, the biodegradability and the low cost are the fundamental requisites of metal chelating agents for these applications. With these aims in mind, we designed and synthesized the three new ligands object of the present paper as iron and aluminium chelating agents. Since 2010, our research group has been studying a number of kojic acid (KA) (Fig. 1) derivatives, formed by two KA units joined through variable linkers always attached in position 6 (Fig. 2) [10–13].

In the same years, other research groups have been working on KA derivatives [14–17]. The main features of these molecules are the low toxicity, the versatility for metal complex formation, and in some cases tyrosinase inhibitory activity. In a recent review regarding these ligands [18], it was reported that the length of the linker plays a basic role in determining the stoichiometry of the formed iron complexes (Fe_2L_2 and Fe_2L_3 stoichiometries). In the 2:3 complexes the mode of coordination completely fits that in $Fe(KA)_3$, remarking that no strain is introduced with the length of the linker. The entropic and enthalpic contributions were also qualitatively discussed to explain the gain in pFe[1] passing from kojic acid to the bis-kojic derivatives [18]. In the following, when

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[1] pM, the parameter generally used to evaluate the overall stability of a chelating agent toward a given metal ion M, is defined as the concentration, expressed as negative logarithm, of free metal ion in a solution at pH 7.4 that is 1 μM in the metal ion and 10 μM in the ligand.

https://doi.org/10.1016/j.jinorgbio.2018.09.013
Received 8 November 2017; Received in revised form 11 September 2018; Accepted 15 September 2018

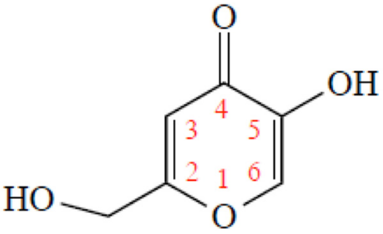

Fig. 1. Molecular structure of kojic acid, and numbering scheme.

mentioning the KA derivatives linked in position 6, we will refer to them as L ligands.

In the present study, a different strategy in the synthesis is proposed: we preserved kojic acid, a cheap molecule without toxic effects, as the basic unit for the new chelators, but adopted a different synthetic strategy joining the 2 KA units through simple linear diamines of variable length by reacting them with the OH groups in position 2. The simplicity of synthesis allows an easy and low cost preparation. The different mutual position of the binding oxygen groups, related to the anchoring position of the linker, as well as the linker length, can affect both the protonation and complex formation equilibria. In particular, we present a thorough study of the ligands obtained using as a linker the three basic linear ethylene diamine, propylene diamine and butylene diamine. This strategy has the aims of evaluating how the anchoring position and the length of the linker affect both the protonation constants, and the complex formation constants; of introducing coordinating nitrogen atoms in the linker, that could be involved in the coordination to Cu^{2+} and Zn^{2+} ions [19], and to use a synthetic method transferable to analogous pyridinone molecules.

The complex formation and coordination mode of S2, S3 and S4 ligands (Fig. 3) toward Fe^{3+} and Al^{3+} ions, studied by combined potentiometric and spectroscopic techniques, has been extended to the bivalent essential metal ions Zn^{2+} and Cu^{2+}. In fact, a recent speciation study showed that iron chelation could imply the depletion or dislocation of essential metal ions, and stated “that a thorough understanding of the copper and zinc complexation models is of paramount importance and must always be presented for any ligand intended to be used in therapy” [19].

2. Experimental

2.1. Reagents

HCl, NaCl, NaOH, D_2O, ethanol, DCl, NaOD, $FeCl_3$, $AlCl_3$, $ZnCl_2$, $CuCl_2$, 5-hydroxy-2-hydroxymethyl-pyran-4-one (kojic acid, KA; purity 99%), ethylene diamine, propane-1,3-diamine, and butane-1,4-diamine were Aldrich products. Carbonate free sodium hydroxide solutions were prepared according to Albert and Serjeant [20].

The metal ion standard solutions were prepared by dissolving the required amount of chloride salts in pure double distilled water and adding a stoichiometric amount of HCl to prevent hydrolysis. Fe^{3+} solution was standardized by spectrophotometric analysis of the Fe^{3+}–desferal complex (λ_{max} 433 nm, ε 2765 $M^{-1}\,cm^{-1}$) using a method developed in our laboratory [21], while Al^{3+}, Cu^{2+} and Zn^{2+} solutions were standardized by EDTA titration.

2.2. Synthesis

The general procedure for the synthesis of the three ligands was: 1.0 g (7 mmol) of KA was dissolved in 20 mL of ethanol (96%) and 3.5 mmol of the proper diamine were added. After stirring the reaction mixture at room temperature for 1 h, the precipitate was filtered and washed with ethyl acetate. The solubility of the three compounds has been estimated > 0.4–0.6 M. Sample identity and purity were confirmed by NMR and elemental analyses. NMR analyses were carried out with a Bruker Ascend™ 400 MHz spectrometer. The melting points were measured with an Electrothermal Melting Point Apparatus. The elemental analyses were performed using a CHNS/O analyzer Perkin Elmer 2400 Series II.

2.2.1. 2,2′-[ethane-1,2-diylbis(iminomethanediyl)]bis(5-hydroxy-4H-pyran-4-one) (S2)

Yield 98%, Melting Point 138–139 °C, Elemental Analysis: $C_{14}H_{16}N_2O_6$ (MW 308,29) calculated C, 54.54%; H, 5.23%; N, 9.09, found C, 54.93%; H, 5.52%; N, 8.99%.

^1H NMR (400 MHz, H_2O-D_2O, 90%–10%, pH 2.71–12.25), *δ* (ppm): 8.016–7.635 (s, 2H, G), 6.509–6.343 (s, 2H, F), 4.435–4.361 (br s, 4H, E), 3.280–2.561 (br s, 4H, D).

^{13}C NMR (100 MHz, D_2O, pD ~ 8.2), *δ* (ppm): 179.51 (2C, 3), 166.65 (2C, 2), 149.23 (2C, 1), 142.24 (2C, F), 110.25 (2C, G), 60.05 (2C, E), 39.37 (2C, D).

^{13}C spectra in D_2O for S2, S3 and S4 are reported in Fig. 1S.

2.2.2. 2,2′-[propane-1,3-diylbis(iminomethanediyl)]bis(5-hydroxy-4H-pyran-4-one) (S3)

Yield 91%, Melting Point 145–146 °C, Elemental Analysis: $C_{15}H_{18}N_2O_6$ (MW 322,32) calculated C, 55.90%; H, 5.63%; N, 8.69, found C, 56.03%; H, 5.92%; N, 8.58%.

^1H NMR (400 MHz, H_2O-D_2O, 90%–10%, pH 2.96–12.17), *δ* (ppm): 8.009–7.635 (s, 2H, G), 6.504–6.343 (s, 2H, F), 4.429–4.361 (s, 4H, E), 3.014–2.552 (t, J = 8.1 Hz, 4H, D), 1.969–1.475 (qt, J = 7.9 Hz, 2H, C).

^{13}CN MR (100 MHz, D_2O, pD ~ 8.8), δ (ppm): 180.43 (2C, 3), 165.41 (2C, 2), 152.12 (2C, 1), 142.27 (2C, F), 110.03 (2C, G), 60.11 (2C, E), 39.32 (2C, D), 21.21 (1C, C).

2.2.3. 2,2′-[butane-1,4-diylbis(iminomethanediyl)]bis(5-hydroxy-4H-pyran-4-one) (S4)

Yield 94%, Melting Point 168–169 °C, Elemental Analysis: $C_{16}H_{20}N_2O_6$ (MW 336.34) calculated C, 57.14%; H, 5.99%; N, 8.33, found C, 57.54%; H, 6.26%; N, 8.16%.

^1H NMR (400 MHz, H_2O-D_2O, 90%–10%, pH 2.15–12.24), *δ* (ppm): 8.003–7.637 (s, 2H, G), 6.497–6.344 (s, 2H, F), 4.424–4.362 (s, 4H, E), 2.950–2.524 (br t, 4H, D), 1.652–1.349 (br qt, 4H, C).

^{13}C NMR (100 MHz, D_2O, pD ~ 9.2), *δ* (ppm): 181.50 (2C, 3), 165.31 (2C, 2), 152.58 (2C, 1), 142.31 (2C, F), 109.98 (2C, G), 60.17 (2C, E), 38.96 (2C, D), 24.29 (2C, C).

2.3. Potentiometric-spectrophotometric measurements

Potentiometric, or combined potentiometric spectrophotometric measurements for protonation and complex formation equilibria were performed in a thermostatted glass cell equipped with a magnetic stirrer, a Metrohm LL UNITRODE glass electrode connected to a Metrohm 691 pH-meter, a microburet delivery tube connected to a Dosimat 665 Metrohm titrator, an inlet–outlet tube for Argon and a fibre optic dip probe connected to a Varian Cary 50 UV–vis spectrophotometer. Accuracy and precision of this equipment were previously discussed [22]. Protonation and Fe^{3+} complex formation constants were determined by simultaneous potentiometric and spectrophotometric titrations, while Al^{3+}, Cu^{2+} and Zn^{2+} complex formation constants were potentiometrically determined. Solutions (20 mL at working ligand concentration 5.0×10^{-4} M) were titrated with 0.1 M NaOH at 25.0 °C, and 0.1 M NaCl ionic strength. The electrode was daily calibrated for hydrogen ion concentration by titrating HCl with NaOH in the above experimental conditions and the results were analyzed with Gran procedure [23]. The electrode potential and pKw were calculated by linear regression from the two linear arms of the Gran plot. If the calculated pK_w did not agree with the literature value at 25 °C and 0.1 ionic strength, the calibration was rejected and the experimental system was checked for faults in the temperature control or in the ionic strength. 120 points are generally acquired for each