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Iron(III) and aluminum(III) complexes with hydroxypyrone ligands aimed to design kojic acid derivatives with new perspectives

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ABSTRACT

With the aim to design new chelators for the clinical treatment of different diseases involving the trivalent metal ions Fe(III) and Al(III), we present the equilibria of kojic acid and its derivative 6-[5-hydroxy-2-hydroxymethyl-pyran-4-one]-5-hydroxy-2-hydroxymethyl-pyran-4-one with these two metal ions. Potentiometric and spectrophotometric techniques for iron, and potentiometry and ^{1}H NMR for aluminum were used, supported by X-ray, electrospray ionization-mass spectrometry (ESI-MS), calorimetry and quantum chemical calculations. In this work, evidence is given on the formation of MeL, MeL_2, and MeL_3 complexes of both metal ions with kojic acid, confirmed by the X-ray structure of the FeL_3 complex, and of variously protonated Me_2L_2 and MeL_2 complexes of 6-[5-hydroxy-2-hydroxymethyl-pyran-4-one]-5-hydroxy-2-hydroxymethyl-pyran-4-one. The extremely good pFe value for this second ligand gives confidence to, and opens perspectives for, the search of new kojic acid derivatives.

1. Introduction

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone, HKa) is a natural γ-pyrone derivative, closely related to maltol, produced by many species of *Aspergillus* and *Penicillium* moulds, especially *Aspergillus oryzae* which has the Japanese common name *koji*. A comprehensive review on kojic acid, and a critical review on metal complexes of hydroxypyrones in medicinal inorganic chemistry have been recently published [1,2]. HKa, recognized at first as an antibiotic substance, has antibacterial and antifungal properties, and inhibits the rate of the formation of pigmented products in plant and in animal tissue, as well as the rate of oxygen uptake when *o*-dihydroxy- and trihydroxy phenols are oxidized by tyrosinase [3–6]. It is used in food and cosmetics to preserve or "ameliorate" colors of substances: on cut fruits it prevents oxidative browning, in seafood preserves pink and red colors and in cosmetics lightens the skin. HKa, studied as a metal chelator since the forties, may serve as a template for new biologically active derivatives for iron(III) chelation [7,8]. Research on iron chelators in medicine has considerably increased in the last two decades because of its social relevance. In clinical practice, in order to protect patients from iron overload, chelating agents are largely used, even though the ideal chelator for humans has not been identified yet [9–12]. The ligands for iron also find application as aluminum chelators. In fact, this metal ion is involved in a variety of neurodegenerative diseases as Parkinson, Alzheimer and dialysis encephalopathy [13–16]. Recently, Fox and Taylor [17] proved that 6-[5-hydroxy-2-hydroxy methyl-pyran-4-one]-5-hydroxy-2-hydroxy methyl-pyran-4-one is an efficient ligand for the *in vitro* mobilization of ferritin-bound iron. Despite the interesting iron mobilization prerogatives of this ligand, neither its chemical properties, nor its complex formation equilibria with Fe(III) have been thoroughly studied. The knowledge of acido-base properties of chelators, as well as kind and stability of formed complexes, is of paramount importance to rationalize the biological action and

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the design of new molecules with improved properties. In this context, since our research attention has been devoted in these last years to iron chelators [9,18–22], we present here a study on Fe(III) and Al(III) complexation equilibria with the ligand proposed by Fox and Taylor. The study of complex formation equilibria between HKa and the two trivalent metal ions together to the X-ray structure of the complex $Fe(Ka)_3$ is also presented as the base to interpret the behavior of 6-[5-hydroxy-2-hydroxy methyl-pyran-4-one]-5-hydroxy-2-hydroxy methyl-pyran-4-one.

2. Experimental

2.1. Reagents

$Al(NO_3)_3{\cdot}9H_2O$, $FeCl_3{\cdot}6H_2O$, HCl, KCl, KOH, D_2O, ethanol, DCl and NaOD were Aldrich products, formaldehyde was a Sigma product, 5-hydroxy-2-hydroxymethyl-pyran-4-one and dimethylamine were Fluka products. Carbonate free potassium hydroxide solutions were prepared according to Albert and Serjant [23]. The ligand 6-[5-hydroxy-2-hydroxymethyl-pyran-4-one]-5-hydroxy-2-hydroxymethyl-pyran-4-one was synthesized according to Fox and Taylor [17] and its purity checked by CHN analysis and MS spectra. The formulae, names and acronyms of these two ligands are shown in Scheme 1.

2.2. Tyrosinase activity

2.2.1. Tyrosinase from agaricus bisporus

Mushroom tyrosinase was partially purified as previously described [24]. Enzyme activity was estimated in presence of given units of enzyme, 50 mM potassium phosphate buffer (pH 6.5) and 0.33 mM L-tyrosine, in a final volume of 1 mL. We assume the amount that increases the absorbance at 305 nm by 0.001 per min in the assay conditions to be one enzyme unit [25]. Since tyrosinase catalyzes a reaction between two substrates, molecular oxygen and L-tyrosine, the assay was carried out in air-saturated solutions.

2.2.2. Spectrophotometric measurements

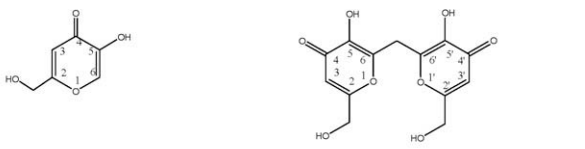
Kinetic measurements were carried out with an *Ultrospec 2110 pro* UV–vis spectrophotometer (Amersham Biosciences, Milan, Italy). In the kinetic measurements of enzyme inhibition, L-tyrosine concentration ranged from 0.021 to 1.630 mM, starting from a stock solution of 2 mM L-tyrosine.

2.2.3. Statistical analysis and software

All experiments were run at least in triplicate. Origin 7.0 was used for statistical analysis (Origin Corporation, Northampton, MA, USA). Lineweaver–Burk data were analysed with GraFit 4.0.21, Erithacus Software Ltd., UK.

2.3. Synthesis of $[Fe(Ka)_3]$

A water solution of $Fe(NO_3)_3{\cdot}9H_2O$ (2.01 g, 4.97 mmol) (20 mL) was added drop wise to the same volume of water solution containing HKa (2.24 g, 15.76 mmol) and NaOH (0.64 g, 16 mmol). After stirring for 5 h at room temperature, the solvent was removed under reduced pressure and the residue was dissolved in a 1:1 methanol:water mixture. Yield: 65%. Crystals suitable for X-ray analysis were collected from the solution left at room temperature, after 7 days.



Kojic acid
5-hydroxy-2-hydroxymethyl-pyran-4-one
HKa / **L1**

6-[5-hydroxy-2-hydroxymethyl-pyran-4-one]-5-hydroxy-2-hydroxymethyl-pyran-4-one
H_2BK / **L2**

Scheme 1. Chemical structures, names and acronyms of studied ligands.

2.4. Spectrophotometric–potentiometric measurements

Protonation and complex formation equilibria were studied 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 [26]. Protonation and Fe(III) complex formation constants were determined by simultaneous potentiometric and spectrophotometric titrations, while Al(III) complex formation constants were potentiometrically determined. To take into account the low complex formation rate with Al(III), a suitable procedure was used: the titrations started 1 h after the mixing of the reagents, and long delay times between two subsequent additions were used (2–7 min). Solutions were titrated with 0.1 M KOH at 25.0 °C, and 0.1 M KCl ionic strength. The electrode was daily calibrated for hydrogen ion concentration by titrating HCl with KOH in the above experimental conditions and the results were analysed with Gran procedure [27]. Ligand concentrations ranged from 2×10^{-4} M to 2.5×10^{-3} M, depending on absorptivity values. Spectra of pure ligands were recorded in the 200–400 nm spectral range with 0.2 cm path length, while those of iron(III) complexes in the 300–700 nm range with 0.5 cm path length. The complex formation constants were studied using constant ligand concentration and 1:1, 1:2 and 1:3 metal/ligand molar ratios for **L1**, and 1:1, 1:2, 1:3 and 2:1 for **L2**. Potentiometric data were processed with PSEQUAD [28] and HYPERQUAD [29] programs, while spectrophotometric data with SPECFIT [30–32] and HYPERQUAD programs. Protonation equilibria of **L2** were studied by HCl titrations of a solution containing **L2** 2×10^{-3} M and KOH 4×10^{-3} M (the base addition was necessary because of **L2** low solubility). In the study of complex formation equilibria, the ligand–metal solutions were prepared by adding the metal solution to the solid ligand and keeping the mixture in a 25 °C thermostatted sonic bath till complete solubilization. Reported log β values refer to the overall equilibria: $pM + qH + rL = M_pH_qL_r$ (the charges are omitted), where p might also be 0, in the case of protonation equilibria, and q can be negative.

2.5. NMR measurements

^{1}H NMR spectra were collected on a Bruker Advance 300 spectrometer at 300.13 MHz in D_2O with a 5 mm sample tube at 25 °C; chemical shifts were referenced to residual solvent signal (4.8 ppm). The NMR spectra of free ligand and Al(III)/L solutions (L – 0.002 M and Al/L ratios 1:1.8 for **L1** and 1:3 for **L2**) were recorded at different pH values. The pD was adjusted by adding DCl or NaOD, and pD was calculated as $pD = pH_{[pHmeter\ reading]} + 0.4$ [33].

2.6. Electrospray ionization-mass spectrometry analysis of complexes

High-resolution mass spectra were obtained on a Bruker MicrOTOF-Q spectrometer (Bruker Daltonik, Bremen, Germany), equipped with an Apollo II electrospray ionization source with an ion funnel. The mass spectrometer was operated in the positive ion mode. Instrumental parameters were as follows: scan range