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Lawsone Dimerization in Cobalt(III) Complexes toward the Design of New Prototypes of Bioreductive Prodrugs

Francisco L. S. Bustamante,[†] Julia M. Metello,[†] Frederico A. V. de Castro,[‡] Carlos B. Pinheiro,[§] Marcos D. Pereira,[‡] and Mauricio Lanznaster*[,†]

[†]Instituto de Química, Universidade Federal Fluminense, Outeiro S. João Batista S/N, 24020-141 Niterói, RJ, Brazil

[‡]Instituto de Quimica, Departamento de Bioquímica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[§]Departamento de Física, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Supporting Information

ABSTRACT: Dimerization of lawsone occurs upon reaction with $Co(BF_4)_2{\cdot}6H_2O$ and *N,N′*-bis(pyridin-2-ylmethyl)ethylenediamine (py_2en) to produce the mononuclear complex $[Co^{III}(bhnq)(py_2en)]BF_4{\cdot}H_2O$ (**1**). This complex has been investigated as a prototype of a bioreductive prodrug, where the $bhnq^{2-}$ ligand acts as a model for cytotoxic naphthoquinones. Cyclic voltammetry data in aqueous solution have shown a *quasi*-reversible Co^{III}/Co^{II} process at $E_{1/2} = -0.26$ V vs Fc/Fc^+. Reactivity studies revealed the dissociation of $bhnq^{2-}$ from the complex upon reduction of **1** with ascorbic acid, and a dependence of the reaction rate on the oxygen concentration suggests the occurrence of redox cycling.

Bioreductive prodrugs have been evaluated as a promising strategy for the treatment of solid tumor cancer because of their ability to be selectively activated in the hypoxic environment of the tumor. The condition of hypoxia, which is considered an obstacle for conventional chemo- and radiotherapy, provides the selectivity that differentiates healthy and cancerous cells. Inert cobalt(III) complexes have been designed to release cytotoxic molecules inside hypoxic cells of solid tumors upon reduction to the labile cobalt(II) form.[1,2] This approach has been tested with promising results, although the conditions and mechanism of drug release are not yet clear.[1,2] In this scenario, we initiated an investigation on coordination of lawsone (2-hydroxy-1,4-naphthoquinone, HNQ)[3] to transition-metal ions, aiming to develop new prototypes of bioreductive prodrugs. The interest in the 1,4-naphthoquinone nucleus lies in the wide range of biological activities of its derivatives, including anticancer properties.[4] Lawsone, which is a commercially available nontoxic natural product, has been widely used for the synthesis of a variety of HNQs with pharmacological applications.[5] HNQs are also potential chelating agents, although their coordination chemistry remains poorly exploited.[3,6,7] In this work, we report the unexpected dimerization of lawsone upon reaction with $Co(BF_4)_2{\cdot}6H_2O$, *N,N′*-bis(pyridin-2-ylmethyl)ethylenediamine (py_2en), and Et_3N to produce the deprotonated form of 2,2′-bis(3-hydroxy-1,4-naphthoquinone) ($bhnq^{2-}$) coordinated to cobalt(III) in $[Co^{III}(bhnq)(py_2en)]BF_4{\cdot}H_2O$ (**1**). The lawsone dimer H_2bhnq works as a flexible hingelike ligand that is capable of adjusting itself sterically to coordinate two distinct metal centers, with known applications in the construction of metal−organic frameworks.[8] H_2bhnq can be prepared by irradiation of lawsone by UV light,[9] or from the reactions of lawsone either with potassium persulfate[10] or with vanadium(V) in perchloric acid.[11] To the best of our knowledge, however, the generation of $bhnq^{2-}$ during complex formation has only been reported for the reaction between lawsone and iron(III) chloride to form a coordination polymer.[12] Herein, we describe the synthesis and properties of **1** as a prototype for bioreductive prodrugs, with the $bhnq^{2-}$ ligand working as a model for cytotoxic naphthoquinones.[13,14]

The *one-pot* reaction between $Co(BF_4)_2{\cdot}6H_2O$, lawsone, py_2en, and Et_3N produced **1** as a brown crystalline precipitate, which was isolated after slow evaporation of the solvent. Formation of $bhnq^{2-}$ from lawsone and its coordination to the Co^{III} center was confirmed by a single-crystal X-ray diffraction study at 130 K (Figure 1). In order to better understand the factors that led to dimerization of lawsone in **1**, the *one-pot*

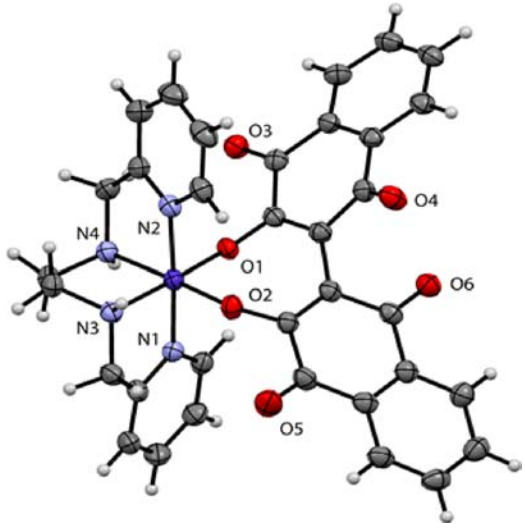

Figure 1. Representation of $[Co(bhnq)(py_2en)]^+$. Atomic displacement parameters at 50% probability.

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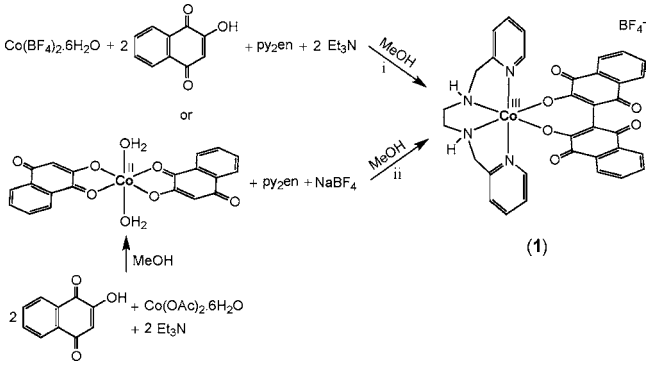
synthesis was split into a two-step reaction, as illustrated in Scheme 1. First, the reaction between $Co(OAc)_2{\cdot}4H_2O$,

Scheme 1. Synthesis of 1



(i) One-pot reaction between lawsone, $Co(BF_4)_2{\cdot}6H_2O$, py_2en, and Et_3N in MeOH. (ii) Reaction of $[Co(lawsonato)_2(H_2O)_2]$ with py_2en and $NaBF_4$ in MeOH.

lawsone, and Et_3N (1:2:2) was carried out in methanol (MeOH) to produce a red crystalline precipitate of *trans*-$[Co^{II}(lawsonato)_2(H_2O)_2]$. Preliminary single-crystal X-ray diffraction analysis (Figure S1 in the Supporting Information, SI) indicated that dimerization of lawsone does not occur under these conditions. *trans*-$[Co^{II}(lawsonate)_2(H_2O)_2]$ was then dissolved in hot MeOH and heated under reflux for 2 h in the presence of py_2en and $NaBF_4$. A brown crystalline precipitate was isolated, fully characterized, and identified as complex **1**. This experiment strongly suggests that coordination of two lawsonate molecules to Co^{II} is a necessary step prior to dimerization, which is then induced by the presence of the py_2en ligand. Because Co^{II} was the precursor for the reactions that ended with Co^{III} from both synthetic pathways shown in Scheme 1, we speculated on whether the Co^{II}/Co^{III} oxidation process was involved in formation of $bhnq^{2-}$. Therefore, the *one-pot* reaction was repeated using $Ga(NO_3)_3{\cdot}xH_2O$ instead of the Co^{II} salt because the Ga^{III} ion is known to be redox-inactive.[15,16] An orange crystalline precipitate was isolated and identified as $[Ga^{III}(bhnq)(py_2en)]NO_3{\cdot}4H_2O$ (**2**), thus indicating that formation of $bhnq^{2-}$ is not related to Co^{II}/Co^{III} oxidation. The possibility that dimerization of lawsone may be photoinduced was also tested by repeating the *one-pot* reaction in the dark, with the same result.

The X-ray structure of **1** revealed a Co^{III} ion surrounded by the two ligands, $bhnq^{2-}$ and py_2en, in a distorted octahedral environment, with a BF_4^- counterion and a water molecule in the second coordination sphere. Figure 1 shows the $bhnq^{2-}$ anion coordinated to Co^{III} by the two oxygen atoms O1 and O2 in the equatorial plane, mutually trans to the two secondary amine nitrogen atoms N3 and N4 of py_2en, respectively. The pyridine nitrogen atoms N1 and N2 of the py_2en ligand occupy the remaining axial positions. To the best of our knowledge, this is the first example of a structure containing the $bhnq^{2-}$ ligand coordinated to a single metal ion in a mononuclear complex. Coordination of O1 and O2 to Co^{III} forms a seven-membered ring that is stabilized by the hingelike characteristics of the $bhnq^{2-}$ ligand. The planes defining the two naphthoquinones are turned by 57.0(1)° evidencing both the distortion suffered by the ligand to bind the metal in such an unusual way and the O4−O6 mutual repulsion. The Co−L distances (L = N and O) range from 1.897(2) to 1.952(2) Å, respectively, and are consistent with a low-spin Co^{III} ion.

The electrochemical response of **1** was evaluated by cyclic voltammetry (CV) in MeCN, revealing a *quasi*-reversible pair of waves at $E_{1/2}$ = −0.52 V vs Fc/Fc^+ (ΔE_p = 0.12 V; I_{pa}/I_{pc} = 1.1; Figure 2), assigned to the redox couple Co^{III}/Co^{II}.[17] This

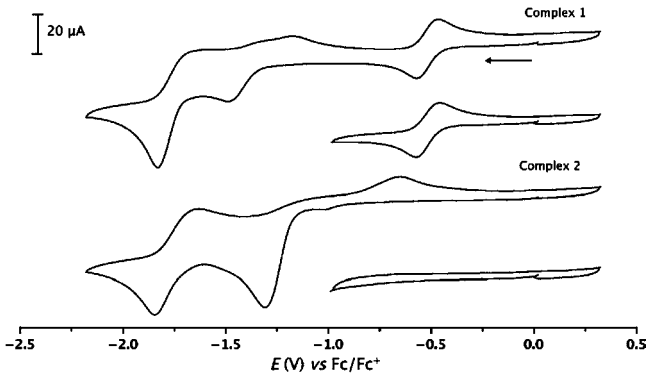


Figure 2. CVs of **1** (two on the top) and **2** (two on the bottom) in MeCN with 0.1 $mol{\cdot}L^{-1}$ $TBAClO_4$. Electrodes: reference, Ag/AgCl (organic); working, carbon; auxiliary, platinum (scan rate = 0.1 $V{\cdot}s^{-1}$); internal reference,[22] ferrocene.

assignment is supported by the absence of electrochemical response between 0.30 and −1.10 V in the CV of the gallium complex **2**.[15] Two other irreversible peaks were also observed at −1.49 and −1.83 V for **1** and at −1.31 and −1.84 V for **2**, respectively assigned to the successive reduction of the coordinated $bhnq^{2-}$ ligand.[3,18] The metal-centered process in **1** was also measured in an aqueous[19] solution to give $E_{1/2}$ = −0.26 V vs Fc/Fc^+ with ΔE_p = 0.12 V and I_{pa}/I_{pc} = 1.1. This potential is 0.26 V more positive than that measured in MeCN, which indicates stabilization of the 2+ oxidation state of the cobalt ion in water. The reversibility parameters in organic and aqueous[19] solutions are similar and indicate that ligand dissociation does not take place in the time scale of the experiment. To present selectivity for hypoxic cells, the reduced Co^{II} prodrug should be sufficiently stable to be reoxidized to the Co^{III} form in the presence of O_2. Indeed, **1** has a suitable potential to be reduced by biological reductants,[20,21] and the electrochemical reversibility of the Co^{III}/Co^{II} process indicates that redox cycling may compete with ligand dissociation in a well-oxygenated environment (Scheme 2).

In order to simulate the reactivity of **1** in a biological environment, ascorbic acid was used to reduce the complex under different O_2 concentrations. The reaction was followed by UV−vis spectroscopy in order to evaluate the release of $bhnq^{2-}$ upon reduction of **1**. The spectrum of **1**, measured from 1.0×10^{-4} $mol{\cdot}L^{-1}$ aqueous[19] solution, revealed a series of absorptions in the 300−800 nm range (Figure S3 in the SI). These spectral features are quite distinct from those found in the spectrum of free $bhnq^{2-}$, which presents a broad band with λ_{max} at 476 nm (ε = 4300 $mol^{-1}{\cdot}L{\cdot}cm^{-1}$) under similar conditions (Figure S4 in the SI). The addition of ascorbic acid (1:1) to an aqueous[19] solution of **1**, under argon, led to the release of $bhnq^{2-}$, indicated by the appearance of a band at 476 nm (Figure S5 in the SI). Maximum absorbance at 476 nm was reached after 10 min, with a well-defined isosbestic point at 433 nm, indicating clean conversion from reagents to products. No further spectral changes were observed over the next 60 min. When the reaction was carried out in open air, the maximum

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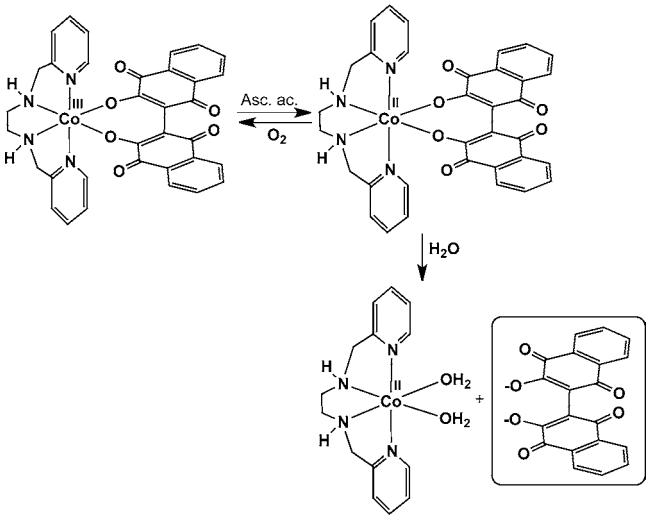
Scheme 2. Reactions of 1 with Ascorbic Acid and O_2



absorbance at 476 nm was reached after 30 min. Finally, under O_2 saturation conditions, 60 min was necessary for the reaction between **1** and ascorbic acid to reach the maximum absorbance at 476 nm. These results show that the reaction time depends on the O_2 concentration, as a consequence of either redox cycling, with Co^{II} being oxidized back to Co^{III} by O_2 prior to ligand dissociation, or competition between the cobalt(II) complex and the reducing agent for O_2. So, in the presence of O_2, the reducing agent may be depleted before total conversion of **1** to the products, accounting for the difference between the maximum absorbance reached (83% for O_2/air and 71% for O_2/saturated air) and expected for a 1.0×10^{-4} mol·L^{-1} solution of $bhnq^{2-}$.

In a preliminary study, the biological activity of **1** was assessed using wild-type strain of *Saccharomyces cerevisiae* BY4741.[23] Yeast cells growing on fermentative metabolism were exposed to **1** for survival rate determinations. Experiments with $Co(BF_4)_2{\cdot}6H_2O$, py_2en, and H_2bhnq were also performed. Analysis in the presence and absence of ascorbic acid (1:1) provided similar results, suggesting that the culture medium (YPD 2%) might afford the necessary reductive environment to reduce **1**. It was observed that survival rates decrease from 93% to 62%, from 100% to 51%, and from 96% to 56% respectively for **1**, py_2en, and $Co(BF_4)_2{\cdot}6H_2O$ when their concentrations changed from 0.5 to 1.0 mmol·L^{-1}, while a steady rate of 90% was measured for H_2bhnq at both concentrations. These results suggest that toxicity of **1** is not caused by the release of $bhnq^{2-}$ but may be related to the byproducts generated after complete dissociation of **1**.

In summary, dimerization of lawsone took place upon reaction with $Co(BF_4)_2{\cdot}6H_2O$ and py_2en, producing the $bhnq^{2-}$ ligand that ended up coordinated to the Co^{III} center in **1**. It was demonstrated that dimerization is neither related to Co^{II}/Co^{III} oxidation nor photoinduced. CV experiments revealed that **1** can be easily reduced in the biological environment, and its Co^{II} reduced form may be sufficiently inert to undergo redox cycling. Indeed, dissociation of $bhnq^{2-}$ after reduction with ascorbic acid is oxygen-dependent, suggesting that redox cycling is involved.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

X-ray crystallographic data in CIF format, synthetic procedures, and other additional information. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ml@id.uff.br or mlanz@vm.uff.br.

Notes

The authors declare no competing financial interest.

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