Electrochimica Acta 140 (2014) 557–563

Contents lists available at ScienceDirect

Electrochimica Acta

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

# Electrochemical and computational studies, in protic medium, of Morita-Baylis-Hillman adducts and correlation with leishmanicidal activity

Yen G. de Paiva[a], Waldomiro Pinho Júnior[a], Antonio A. de Souza[b], Cícero O. Costa[c], Fábio P.L. Silva[d], Cláudio G. Lima-Junior[d], Mario L.A.A. Vasconcellos[d,1], Marília O.F. Goulart[a,*]

[a] Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Maceió, AL, 57072-970, Brazil
[b] Instituto Federal de Educação, Ciência e Tecnologia de Alagoas, IFAL, 57020-600 Maceió, AL, Brazil
[c] Instituto Federal de Educação, Ciência e Tecnologia de Alagoas, IFAL, Satuba, AL, Brazil
[d] Departamento de Química, Universidade Federal da Paraíba, Campus I, 58059-900 João Pessoa-PB, Brazil

ARTICLE INFO

*Article history:*
Received 11 January 2014
Received in revised form 12 May 2014
Accepted 13 May 2014
Available online 22 May 2014

*Keywords:*
Morita-Baylis-Hillman adducts
electrochemical parameters
aromatic nitrocompounds
protic media
leishmanicidal activity

ABSTRACT

Enzymatic bioreduction of nitro groups plays an important role on the activity of biologically active nitroaromatic compounds. Electrochemical methods are useful tools to simulate *in vivo* metabolic processes. This work presents electrochemical studies, in protic media (EtOH + phosphate buffer 4:6), using cyclic voltammetry (CV) of twelve Morita-Baylis-Hillman adducts (MBHA) with significant leishmanicidal activity. To facilitate the analysis, the molecules were grouped in four classes according to their side chains. Cyclic voltammograms display, in all cases, only one cathodic wave related to the formation of the correspondent hydroxylamines, which suffer further oxidation generating the nitroso derivatives in a sequential cycle. *Ortho* compounds exhibit more negative reduction potentials compared to the other isomers, in the same chemical class. This phenomenon could be related not only to structural effects but also to the presence of solvation spheres during the electroreduction process and/or stabilization of the resulting hydroxylamine. A proposal to explain the higher leishmanicidal activity of the *ortho* compounds compared with the *meta* and *para* compounds was suggested based on theoretical calculations (HF/6-31 + G */PCM, water, as a calculation level) that indicated lower thermodynamic stability for the *ortho*, in comparison to the corresponding *meta* and *para* hydroxylamines, fact that may suggest the easier transformation of the electrogenerated compounds into reactive electrophilic intermediates or final products, able to react with physiological important endobiotics.



## 1. Introduction

Leishmaniasis is a poverty-related vector-borne disease endemic in 98 countries caused by parasite of the genus *Leishmania* [1]. It represents a worldwide public health problem with 350 million people being at risk [2–4]. There are three different clinical manifestations: cutaneous, the most common, mucocutaneous (CL) and visceral leishmaniasis (VL) (also known as black fever or kalazar), a potentially lethal systemic infection. Approximately 0.2 to 0.4 cases and 0.7 to 1.2 million VL and CL cases, respectively, occur each year. More than 90% of global VL cases occur in six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil. Cutaneous leishmaniasis (CL) is more widely distributed, with about one-third of cases occurring in each of three epidemiological regions, the Americas, the Mediterranean basin, and western Asia from the Middle East to Central Asia, with a tentative estimate of 20,000 to 40,000 leishmaniasis-caused deaths per year [3]. Up to now, no vaccine approved for human use is available [5], despite several prototypes, in different states of product and clinical development [6]. The current treatment is unsatisfactory due to its high cost, toxic side effects and drug resistance of the parasite [7,8]. Thus, many efforts have been made for the development of new

* Corresponding author. Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, 57072-970 Maceió, AL, Brazil, Tel.: +55 82 3214-1393; fax: +55 19 3214-1393.
*E-mail addresses:* mlaav@quimica.ufpb.br (M.L.A.A. Vasconcellos), mariliaofg@gmail.com (M.O.F. Goulart).
[1] Departamento de Química, Universidade Federal da Paraíba, Campus I, 58059-900 João Pessoa-PB, Brazil, Tel.: +55 83 32167589; fax: +55 83 32167433.

http://dx.doi.org/10.1016/j.electacta.2014.05.066
0013-4686/

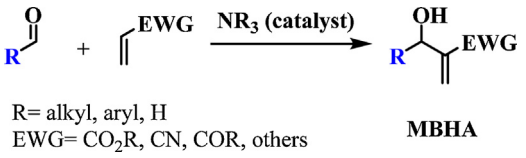


**Fig. 1.** General scheme for Morita-Baylis-Hillman (MBH) reactions.

effective drugs for the treatment of this neglected disease, which discovery is a pressing concern for global health programs. Some nitrocompounds are still in investigation toward this disease, using a hybrid approach, with a promising result [9].

The Morita-Baylis-Hillman (MBH) reaction is a carbon-carbon bond-forming reaction. It has all interesting basic properties of an efficient synthetic method – it is selective, atom economical, requires mild conditions and generates poly-functionalized molecules [10,11]. It involves coupling of alkenes containing electron-withdrawing groups (EWG) with aldehydes, imines or ketones, among other starting materials. Most of these reactions are normally catalyzed by tertiary amines as 1,4-diazabicyclo[2.2.2]octane (DABCO) [12,13] (Fig. 1).

Some MBH adducts (MBHA) are nitrocompounds of whose biological activity is related to aromatic nitro group reduction generating $ArNO_2^{-\bullet}$ or more reduced intermediates [14,15], depending on external conditions. As interesting biological electrophiles, some studies have been reported, using these adducts, against *Biomphalaria glabrata* [16], *Plasmodium*, *Leishmania*, *Trypanosoma* [17–23], bacterias [23], fungi [24] and some human cancer cells [14,15,24], displaying significant activity [25]. Concerning the biotransformation point of view, the bioactive metabolites (*i.e.* nitro radical anion and/or hydroxylamine and/or nitroso derivatives) formed through biological reactions, have several cell components (for instance enzymes, DNA, membranes) as potential targets [26–28]. The redox chemistry of different nitro compounds of biological significance is focused to understand how the reduction of the nitro group can play an active role in several aspects, especially in free radicals generation, in stability and reactivity [28]. As bioreductive prodrugs, they are designed to be activated by a metabolic reaction of reduction in target tissues or organisms, with different oxygen concentrations. Only organisms with an appropriate redox machinery will produce the biologically active form of the compound. In other words, cellular toxicity will depend first and foremost on oxygen levels in the cells and the molecular mechanism of action can vary accordingly [26].

Electrochemical techniques have been extensively used for providing important insights about the mechanisms of action of a variety of drugs. This understanding may inspire the design of next-generation more effective therapeutic compounds [28–30]. Redox potentials are considered the main physicochemical parameter that determines the effectiveness of nitrocompounds providing information about the electron transfer (ET) process, with thermodynamic and kinetic informations [27–30]. However, to obtain more information about the mechanisms of molecular action, it is interesting to study not only the electronic properties of the bioactive nitrocompounds, but also their molecular environment. Changes of the intermolecular interactions (hydrogen-bond ability, dipolar moment, etc.) are clearly capable of affecting the structures, reactivity, biological activities, equilibria, reaction rate constants, and a host of other aspects that are of central interest to chemistry and biology. Thus, specific shifts in the redox potentials reflect the extent of stabilization that the molecular ground and electrogenerated states experience due to their own nature as well as their solvent-solute interactions.

The present work aims to investigate the electrochemical studies in protic media (EtOH + phosphate buffer, 4:6), using cyclic voltammetry (CV) of twelve Morita-Baylis-Hillman adducts (MBHA) with significant leishmanicidal activity, to complement the studies already published in aprotic medium [31]. A proposal for the higher bioactivity of the compounds was suggested based on theoretical calculations (HF/6-31 + G*/PCM, water) of the corresponding hydroxylamines.

## 2. Experimental

### 2.1. Chemicals

According to the general procedures [13,21] already published, we have synthesized 12 MBHA (Fig. 2). The purity of all the compounds was assessed by gas chromatography and their structures confirmed by usual physicochemical methods.

### 2.2. Apparatus and procedures

All experiments were performed at room temperature ($25 \pm 2\,^{\circ}C$) and degassed by pure and inert gas (Argon). Cyclic voltammetry (CV) experiments were performed with a conventional three-electrode cell in an Autolab PGSTAT-30 potentiostat (Echo Chemie, Utrecht, the Netherlands) coupled to a PC microcomputer, using GPES 4.9 software. The working electrode was a Metrohm GC electrode of 2 mm diameter, the counter electrode was a platinum coil, and the reference electrode was Ag|AgCl, KCl (0.1 mol $L^{-1}$), all contained in an one-compartment electrochemical cell with a volumetric capacity of 10 mL, using a volume of 5 mL. The glassy carbon electrode was previously polished with alumina on a polishing felt until getting a mirror-like surface appearance. All the reagents employed for the preparation of the buffer solution (pH = 7.4) were of analytical grade. The phosphate buffer (pH 7.4) (ionic strength of 0.2 mol $L^{-1}$) was prepared as following: 0.434 g of $NaH_2PO_4$ and 1.747 g of $Na_2HPO_4$ dissolved in milliQ water in a volumetric flask of 200 mL.The pH value was monitored in a pHmeter, previously calibrated. Stock solutions of each compound in EtOH + buffer (4:6) were prepared. The aqueous solutions were prepared by diluting the stock solution in order to obtain a final substrate concentration of $1 \times 10^{-3}$ mol $L^{-1}$. Stock and working solutions were handled and stored avoiding exposure to light during all experiments. The solutions were purged with pure argon for five minutes before the voltammetric runs and covered with a argon blanket during the experiments.

### 2.3. Computational Details

Computational analyses of hydroxylamines **Ia'–IVc'** were performed using GAUSSIAN09W® package version for Linux [32]. Initially, relaxed potential energy surfaces scan (RPESS) was performed in a semi-empirical PM6 level, considering the relevant rotational degrees of freedom for the reduced compounds (sigma bonds). Dihedral angles were frozen in steps of 10°, while the remaining portion of the molecule was optimized. At the end, potential energy curves for each dihedral angle were obtained, and the conformation of minimum energy for each compound was selected. They were, then, subjected to calculation at HF/6-31G+(d) level, considering a polarized continuum model (PCM) by introducing water dielectric constant ($\varepsilon = 78.39$). HOMO, LUMO and *N* values were obtained from the most stable conformations of the hydroxylamines. The molecular hardness (*N*) was calculated from $N = [(\text{LUMO} - \text{HOMO})]/2]$ equation.

## 3. Results and Discussion

The nitrocompounds were classified into four classes (**I, II, III** and **IV**) based on the side chain as nitrile, methyl acrylate,