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Electrochemistry of β-lapachone and its diazoderivative: Relevance to their compared antimicrobial activities

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Abstract

A new diazo derivative (**2**) of β-lapachone (**1**) is synthesized in one step and good yield (72%). New and significant antimicrobial activity data toward several strains of resistant *Staphylococcus aureus* for β-lapachone are presented. For the new compound, compared with β-lapachone, the modification of the redox center decreased drastically the biological activity, a fact that could be rationalized by the electrochemical results. The redox properties of both compounds are investigated, in aprotic medium (DMF + 0.1 mol L^{-1} TBAP), using a glassy carbon electrode. For the diazoquinone, an unique irreversible monoelectronic reduction peak is observed. The reduction mechanism corresponds to an ECC process, where the first chemical reaction is the fast unimolecular loss of dinitrogen, leading to a transient carbene anion radical, which captures a hydrogen atom and furnishes a phenolate derivative. Addition of different proton sources (phenol, benzoic acid) does not affect at all the reduction wave, but leads to the disappearance of the corresponding anodic wave, due to the protonation of the phenolate anion. The reduction of **2** occurs at more negative potential and its anion radical is very short-lived, so this may be the main set of reasons, which prevents the generation of reactive oxygen species in situ and might be the reason for its lower antimicrobial activity.

Keywords: Diazo-β-lapachone; β-Lapachone; Cyclic voltammetry; Electrolysis; Carbene anion radical; Antibacterial; Resistant *Staphylococcus aureus*

1. Introduction

The drugs' arsenal for fighting against many tropical diseases includes many natural and synthetic naphthoquinone derivatives. One very interesting quinone is β-lapachone (**1**). It possesses a variety of pharmacological effects, including antibacterial, antifungal, and trypanocidal activities [1]. Nowadays, the largest interest in the chemistry of **1** elapses from its intensive investigation for clinical uses in cancer chemotherapy [2]. In vitro, it exhibits activity against various cancer cell lines and at low doses is a radiosensitizer upon several human cancer cell lines. It acts directly on the enzymes topoisomerases (Topos) I [3] and II [4]. In general, it is fully agreed that its mechanism of action as antibacterial, cytotoxic and

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trypanocidal is related to its redox properties, allowing the generation of reactive oxygen species (ROS) [5], which can damage, mainly, DNA topoisomerases by a mechanism distinct from that of other commonly known Topo inhibitors [6]. A ROS-related pathway was involved in β-lapachone-induced apoptosis program [6]. Tests on humans and clinical trials have not yet been performed but are being planned [2]. Despite the vast pharmacological studies on β-lapachone **1** [1–7] in vitro or in cell, only a few ones were addressed by in vivo assays, for which the results are less conclusive [7]. This may be ascribed in part to redox activation/inactivation mechanism operating in the whole mechanism [7]. A change in redox behavior may constitute a suitable approach to circumvent those problems, however, the number of reports on derivatives involving chemical modifications at the quinone redox center [8,9] and their biological and electrochemical studies [9] are less frequent.

Recently, new classes of naturally occurring quinones containing the diazo group in their structures, named kinamycins [10] and lomaiviticins [11], were shown to exhibit a very potent activity against a broad spectrum of cancer cell lines and also against Gram-positive bacteria [10,11]. Those compounds presented unique cytotoxicity profile compared to the known DNA-damaging anticancer drugs and were shown to cleave DNA under reducing conditions [11].

Along this line, we decided to prepare β-lapachone (**1**) derived diazoquinone (**2**), by reaction with tosyl hydrazide, [12,13] and to examine its antimicrobial activity and to measure MIC (minimum inhibitory concentration) for **1** and **2**, towards several strains of resistant Gram-positive *Staphylococcus aureus*. β-Lapachone was screened before against fungi and bacteria and showed activity towards strains of *S. aureus* in the range of 2–8 μg mL^{-1} [14]. In this paper, multiresistant strains of *S. aureus* were used.

Due to the importance of the bioreduction for the majority of the already-mentioned biological activities of β-lapachone (**1**) [5–7] and also for kinamycins and lomaiviticins [10,11], the electrochemical behavior, in DMF + 0.1 mol L^{-1} TBAP, using cyclic voltammetry and controlled-potential electrolysis, on a glassy carbon electrode, of **1** and **2** were also performed. Despite its importance in the understanding of the biological mechanism of action and for the modification of the redox center, by electron capture, detailed electrochemical studies of β-lapachone (**1**) are restricted to few experiments, by some of us [15–17], mainly in aqueous media. Depending on the cell compartment, the environment of the cells could be hydrophilic or lipophilic and, in order to mimic biological conditions, the reduction/oxidation processes can be carried out in non-aqueous media resembling the situation in hydrophobic systems (viz., in the cytosol, at the membranes of endoplasmic reticulum and mitochondria, at proteins, etc.) or in aqueous media corresponding to situations in most biological cytoplasmic fluids [18]. The electrochemical method was considered to have extra value concerning the prodrug approach [9,18], hence the relevance of these combined electrochemical/biological studies.

2. Experimental

2.1. Synthesis of 6-diazo-2,2-dimethyl-5-oxa-2,3,4,6-tetrahydro-2H-benzo[h]chromene (2)

To a stirred solution of the **1** (200 mg, 0.83 mmol) in 12 mL of distilled methanol was added an excess of *p*-toluenesulfonyl hydrazide (230 mg, 1.24 mmol) at room temperature. The solution was stirred for 3 h and concentrated under reduced pressure. The residue was extracted with hexane (4×25 mL) and the resulting solution was evaporated giving a second residue, which was chromatographed on silica gel, using *n*-hexane-ethyl acetate (9:1 to 9:3) as the eluent giving **2** as a yellow solid (144 mg, 72%). IR (film) ν_{max} (cm^{-1}): 1616 (C=O), 2100 (C=N_2); UV ($CHCl_3$) λ_{max} (ε) 233 (1.06×10^4), 290 (2.70×10^3), 335 (2.50×10^3) nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 6H, $2CH_3$); 1.84 (t, 2H, $J = 6.9$ Hz, CH_2); 2.65 (t, 2H, $J = 6.9$ Hz, CH_2); 7.22 (1H, d, $J = 8.1$ Hz, Ar-H); 7.28 (1H, t, $J = 8.4$ Hz, Ar-H); 7.48 (1H, td, $J = 8.1$; 0.9 Hz, Ar-H); 8.01 (1H, d, $J = 7.5$ Hz, Ar-H) ppm; ^{13}C NMR ($CDCl_3$) δ 16.9 (C_4); 26.6 (CH_3–C_2); 26.6 (CH_3–C_2); 31.6 (C_3); 72.9 (C_6-diazo); 75.9 (C_2); 111.0 (C_{4a}); 119.1 (C_7); 122.2 (C_{6a}); 124.1 (C_8); 124.1 (C_9); 129.4 (C_{10}); 126.0 (C_{10a}); 157.6 (C_{10b}); 179.2 (C_5, C=O) ppm; LRMS (*m*/*z*) (relative intensity): 142 (100), 114 (79), 170 (56), 241 (24), 184 (11); HRMS $[M]^+$ calcd. for $C_{15}H_{14}N_2O_2$: 254.1055, found 254.1052. Toluene-4-thiosulfonic acid *S*-*p*-tolyl ester (**3**) [12] was also obtained as a deep purple solid, m.p. 74–76 °C [lit. 76 °C] [13].

2.2. Antimicrobial tests

MIC against seven resistant *Staphylococcus* strains was estimated in vitro for **1** and **2**. The following strains were used: *S. aureus* CI 133; *S. aureus* CI 138 (from vaginal secretion); *S. aureus* CI 139 (from skin lesion); *S. aureus* CI 311; *S. aureus* CI 155; *S. aureus* C 27 (from vaginal secretion, multiresistant); *S. aureus* ATTC 6538. It is worthy to mention that *S. aureus* CI 133, CI 138 and CI 139 are resistant to several antibiotics, among them, amicacine, gentamicine, sulfonamides, cefotaxime, erythromycine and also to chloramphenicol. *S. aureus* CI 311 is even more resistant, with additional resistance to cephalotine, tetracycline and peniciline. Those strains came from the collection of the Department of Antibiotics (UFPE, Recife, Pernambuco, Brazil). Bacterial