ESR and electrochemical study of 5-nitroindazole derivatives with antiprotozoal activity

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Abstract

The electrochemistry of 3-alkoxy- and 3-hydroxy-1-[ω-(dialkylamino)alkyl]-5-nitroindazole derivatives were characterized using cyclic voltammetry in DMSO. The nitro reduction process was studied and this was affected by the acid moieties present in these compounds. A nitro anion self-protonation process was observed. This phenomenon was studied by cyclic voltammetry in presence of increasing amount of NaOH. The reactivity of the nitro anion radical of these derivatives with glutathione was also studied by cyclic voltammetry. The oxidizing effect of glutathione is supported by the parallel decrease of the anodic peak current and increase of the cathodic peak in the cyclic voltammograms, corresponding to the wave of the nitro anion radical from uncharged species with the addition of glutathione. Nitro anion radicals obtained by electrolytic reduction of these derivatives were measured and analyzed in DMSO using electron spin resonance spectroscopy.

Keywords: 5-Nitroindazole; Cyclic voltammetry; ESR; *Trypanosoma cruzi*

1. Introduction

Trypanosoma cruzi (*T. cruzi*) is the etiological agent of Chagas' disease (American Trypanosomiasis), affecting approximately 20 million people from Southern California to Argentina and Chile [1–3]. Currently, this pathology is treated with nitroheterocyclic agents such as Nifurtimox® (Nfx) and Benznidazole®, but this chemotherapy is still inadequate due to its undesired side effects [3].

Recently, we have synthesized a series of new 3-alkoxy- or 3-hydroxy-1-[ω-(dialkylamino)alkyl]-5-nitroindazole (Fig. 1) and their antiprotozoa properties and unspecific cytotoxicity against macrophages have been studied. Derivatives **2**, **3**, and **4** exhibited high activity against *T. cruzi* at 25 μM and their antiparasitic activity was not due to its unspecific cytotoxicity, since at the concentration evaluated they showed a slight unspecific cytotoxicity against macrophages [4].

The biological activity of nitroheterocycle antiparasitic drugs is dependent upon the nitro reduction process. The suggested mode of action is due to the intracellular nitro reduction followed by redox cycling yielding reactive oxygen species (ROS) and the formation of active intermediate species that can cause cellular damage directly by reaction with various biological macromolecules, or indirectly by generation of the highly reactive hydroxyl radical [3,5–9].

The electrochemical production of nitro anion radical has been the target of studies of some pharmacologically active compounds. However, until nowadays, the electrochemical behavior of 5-nitroindazole derivatives (5-NI) has not been reported. The reduction properties of a wide number of nitroaromatic drugs in presence of a biological relevant

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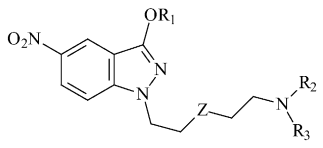
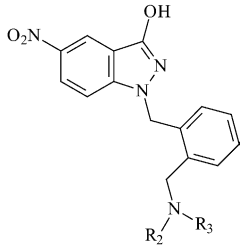


	R_1	R_2,R_3	Z
1	Bn	$(CH_2)_5$	O
2	Bn	Me,Me	O
3	Bn	$(CH_2)_5$	CH_2
4	Bn	Me,Me	CH_2
5*	CH_3	$(CH_2)_5$	O
6*	CH_3	Me,Me	O
7*	CH_3	Me,Me	CH_2
8**	H	$(CH_2)_5$	O
9**	H	Me,Me	O
10**	H	$(CH_2)_5$	CH_2
11**	H	Me,Me	CH_2

(*) Compounds isolated as the hydrochlorides
(**) Compounds isolated as the hydrobromides



	R_2,R_3
12*	$(CH_2)_5$
13*	Me,Me

Fig. 1. Chemical structure of studied 5-nitroindazole derivatives.

thiol, such as glutathione (GSH), have been studied using cyclic voltammetry (CV), to establish whether the thiol biologically acts as a radical scavenger, an oxidizing agent, or a reducing agent, or all three depending on the conditions [6,10–12].

In this paper, a family of 13 5-NI were electrochemically studied. The reduction pathway of these derivatives was studied in organic solvent using CV and the radical species were characterized using electron spin resonance (ESR) spectroscopy. Also, we examined the interaction between radical species generated from 5-NI and GSH. The electrochemical generation of nitro anion radical species from 5-NI by CV has been used to follow the interaction of radical species and GSH.

2. Experimental

2.1. Samples

The 5-NI derivatives (Fig. 1) were synthesized according to methods described earlier [4].

2.2. Cyclic voltammetry

Dimethylsulfoxide (DMSO) (spectroscopy grade) was obtained from Aldrich. Tetrabutylammonium perchlorate (TBAP), used as supporting electrolyte, was obtained from Fluka. CV was carried out using a Metrohm 693 VA instrument with a 694 VA Stand convertor and a 693 VA Processor, in DMSO (ca. 1.0×10^{-3} mol L^{-1}), under a nitrogen atmosphere at room temperature, with TBAP (ca. 0.1 mol L^{-1}), using a three-electrode cell. A hanging mercury drop electrode was used as the working electrode, a platinum wire as the auxiliary electrode, and saturated calomel as the reference electrode.

2.3. ESR spectroscopy

ESR spectra were recorded in the X band (9.7 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50 kHz field modulation. The hyperfine splitting constants were estimated to be accurate within 0.05 G. The anion radicals were generated by electrolytic reduction in situ under the same conditions of temperature, atmosphere and concentrations stated at the CV experiment. The ESR spectra were simulated using the program WINEPR Simphonia 1.25 version.

3. Results and discussion

3.1. Cyclic voltammetry

Fig. 2 shows typical voltammograms displayed by the 5-NI family when a solution of 5-NI derivatives (1 mM) and TBAP (100 mM) in DMSO was swept from 0.0 to −2.0 V. Clearly, it was noticed that 3-benzyloxy-5-nitroindazole derivatives, **1**–**4**, showed a one electron reversible transference process (peak IIc/IIa, around −1.2 V, Fig. 2a) corresponding to the generation of the nitro anion radical $RNO_2^{\bullet -}$, this typical voltammogram corresponds to the generation of the radical of 3-benzyloxy-5-nitroindazole derivatives (Fig. 3a). Fig. 2b shows the typical voltammogram of 3-methoxy-5-nitroindazole hydrochloride derivatives, **5**–**7**. Two reduction waves appeared, one due to electron transfer system (peak IIc/IIa, around −1.2 V) corresponding to nitro anion radical, $RNO_2^{\bullet -}$, generation and a new wave at lower negative potential (peak Ic, near to −1.1 V, Fig. 2b). This new wave, Ic, corresponds to the reduction of the nitro group in the presence of an internal proton donor, due the fact that these derivatives were obtained as the hydrochlorides. Fig. 3b shows this electroreduction process, where **C1** corresponds to an acid–base equilibrium in aprotic media, a typical behavior of a self-protonation phenomenon displayed by nitrocompounds with acidic moieties in its structure [13–15]. We can explain the pre-peak as the reduction of the positive charged species **E1** being reduced at less cathodic potentials, followed the self-protonation reactions **C1** and reduction of the uncharged species, **E2**. Fig. 2c shows the typical voltammogram of 3-hydroxy-5-nitroindazole hydrobromides or hydrochlorides derivatives, **8**–**13**. Three reduction waves appeared, two cathodic peaks Ic (around −1.1 V) and IIc (around −1.2 V) and a new wave at higher cathodic potential peak (IIIc/IIIa, around −1.5 V). The electroreduction processes for these derivatives are proposed in Fig. 3c. In a same manner that for the case of 5-methoxy-5-nitroindazole hydrochloride derivatives, we can explain the reduction process of these 3-hydroxy derivatives firstly as the generation of the nitro anion radical $RNO_2^{\bullet -}$ of the positive charged species, process **E′1**, at the lowest cathodic potential, followed by the self-protonation reactions, **C′1**, the generation of the nitro anion radical $RNO_2^{\bullet -}$ of the uncharged species, process **E′2**, the sec-

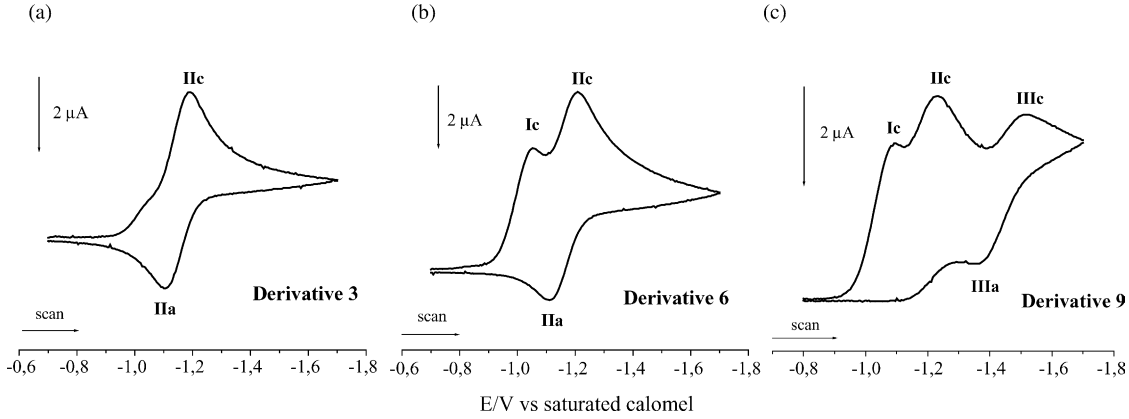


Fig. 2. Typical cyclic voltammograms of 1 mM 5-NI in 100% DMSO with 0.1 M TBAP. Sweep rate 2 V s^{-1}. (a) 3-benzyloxy-5-nitroindazole derivatives, (b) 3-methoxy-5-nitroindazole hydrochloride derivatives, and (c) 3-hydroxy-5-nitroindazole derivatives.

ond self-protonation reaction, **C′2**, generating 3-hydroxylate derivative and finally the generation of the nitro anion radical $RNO_2^{\bullet -}$ of negative charged 3-hydroxylate species at higher potential, **E′3**. The high negative potential for 3-hydroxylate derivatives corresponds to a less capacity to accept electrons from species with negative charge. Table 1 lists the values of voltammetric cathodic peaks for all compounds and Nfx [16]. All derivatives exhibited more negative potential values than Nfx (−0.91 V) showing lesser capacity to be reduced.

In order to obtain non-distorted cyclic voltammograms for 3-methoxy and 3-hydroxy-5-nitroindazole derivatives and verify the above mechanism proposed, we have worked in presence of increasing amounts of aqueous NaOH (0.1 M). Fig. 4 shows the typical voltammograms obtained for 3-methoxy and 3-hydroxy-5-nitroindazole derivatives in the presence of different quantities of base. The electroreduction wave Ic gradually disappears with the increase of NaOH concentration from 0 to 1 mM (Fig. 4a) and IIc for 3-hydroxy-5-nitroindazole derivatives with the increase of NaOH concentration from 1 to 2 mM (Fig. 4b). The calculated ipa/ipc ratio with the Nicholson and Shain equation [17,18] increases to 1 with the addition of NaOH for peak IIc/IIa in the case of 3-methoxy-5-nitroindazole derivatives and for peak IIIc/IIIa in the case of 3-hydroxy-5-nitroindazole derivatives (results not shown). This study allows to confirm the presence of acid protons in the chemical structure of these derivatives, which were neutralized with the gradual addition of NaOH. On the other hand, we confirm the mechanism ECErev proposed for these 3-methoxy-5-nitroindazole derivatives and ECECErev for the 3-hydroxy-5-nitroindazole derivatives given by the increment in the ipa/ipc ratio toward the reversibility of it final peak.

Table 1
Cyclic voltammetric parameters in DMSO corresponding to cathodic peaks vs. saturated calomel electrode (sweep rate 2 V s^{-1})

Derivative	E_{pIc} (V)	E_{pIIc} (V)	E_{pIIIc} (V)
1	–	−1.21	–
2	–	−1.21	–
3	–	−1.19	–
4	−1.07	−1.19	–
5	−1.05	−1.21	–
6	−1.05	−1.21	–
7	−1.08	−1.22	–
8	−1.07	−1.21	−1.50
9	−1.09	−1.23	−1.51
10	−1.11	−1.22	−1.52
11	−1.09	−1.19	−1.49
12	−1.06	−1.20	−1.49
13	−1.07	−1.23	−1.50
Nfx	−0.91[a]	–	–

[a] [16].

We studied the stability of the radical intermediates in presence of NaOH (peak IIc/IIa or IIIc/IIIa) by changing the electrochemical conditions, i.e. the scan rate, while keeping the chemical conditions of the solution unaltered. We observed that the calculated ipa/ipc ratio [17] increased slightly as the scan rate increase (from 100 to 2000 mV/s) (results not shown), this is typical for a reversible charge transfer process [18]. Table 2 lists the values of voltammetric peaks and anodic and cathodic currents for all compounds. The 5-NI derivatives exhibited more negative $E_{1/2}$ ($E_{1/2} = (E_a + E_c)/2$) values than Nfx (−0.88 V [16]) showing a worse ability to generate the radical species.

3.2. Reactivity of the nitro anion radical anion electrochemically generated from 5-NI with GSH

We studied the reactivity of GSH with the nitro anion radical of 5-NI by cyclic voltammetry, adding increasing amounts of aqueous GSH (0.1 M in buffer phosphate pH 7.4) solution to the media. Fig. 5 shows the typical CV behavior of 3-benzyloxy derivatives in DMSO solutions in absence and in presence of GSH, curve (a) illustrates the cyclic voltammograms involving 1-electron transfer process corresponding to the nitro anion radical formation then when GSH was added the anodic peak practically disappeared concomitantly with a significant increment of the cathodic peak (curve (b)). In the case of 3-methoxy and 3-hydroxy derivatives, when GSH

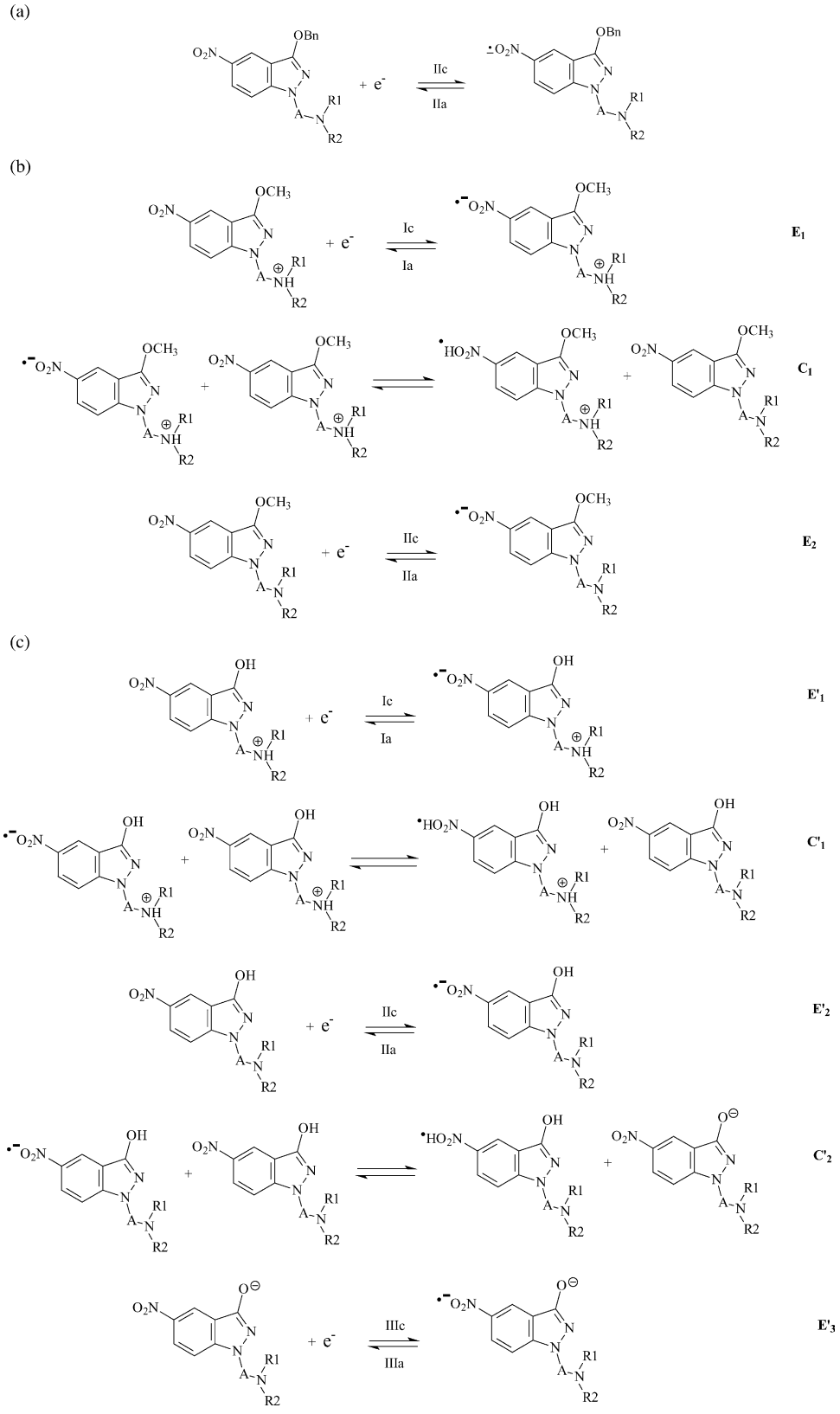


Fig. 3. Reduction mechanism of studied 5-NI derivatives.

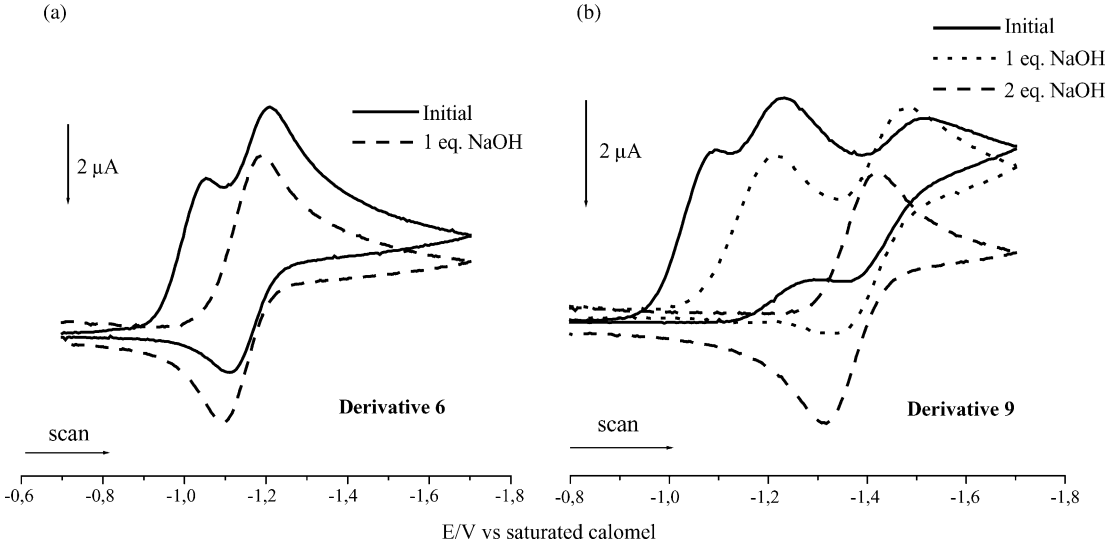


Fig. 4. Typical cyclic voltammogram of 1 mM 5-NI in the presence of different amount of aqueous NaOH (0.1 M), sweep rate 2 V s^{-1}: (a) 3-methoxy-5-nitroindazole derivatives, and (b) 3-hydroxy-5-nitroindazole derivatives.

was added to the medium, a large increase in the 1-electron RNO_2 reduction step corresponding to the electroreduction of uncharged species and the absence of the return oxidation step are observed, on the other hand, disappeared the other cathodic and anodic peaks (data not shown). These changes were evidenced immediately after the addition of GSH (GSH:5-NI = 8:1). GSH signals did not interfere the corresponding nitro anion radical detection at the studied concentrations [10–12]. These results indicated that the nitro anion radical $RNO_2^{\bullet -}$ electrochemically obtained is immediately re-oxidized to the original material by the action of GSH. The effect is essentially catalytic, the nitro voltammetric changes was virtually complete after the octave thiol addition. The species responsible for redox cycling has not been identified, but it is possible that the $-S^{\bullet}$ radical (produced via the 1 electron oxidation of GSH) is the oxidizing agent for the $RNO_2^{\bullet -}$.

The fact that in biological medium it is present high thiol levels this kind of process could take place into the parasite explaining the observed biological activity for 5-NI derivatives against *T. cruzi*.

Table 2
Characteristic CV parameters in DMSO in presence of amount of NaOH to neutralized vs. saturated calomel electrode (sweep rate 2 V s^{-1})

Derivative	E_{pc} [a] (V)	E_{pa} [b] (V)	ΔE (V)	$E_{1/2}$ [c] (V)	ip_a/ip_c
1[d]	−1.21	−1.10	0.11	−1.16	0.86
2[d]	−1.19	−1.10	0.09	−1.15	0.90
3[d]	−1.19	−1.11	0.08	−1.15	0.93
4	−1.13	−1.04	0.09	−1.08	1.18
5	−1.19	−1.09	0.10	−1.14	0.99
6	−1.19	−1.09	0.10	−1.14	0.76
7	−1.18	−1.09	0.09	−1.14	1.02
8	−1.43	−1.32	0.11	−1.38	1.02
9	−1.42	−1.32	0.10	−1.37	1.03
10	−1.42	−1.32	0.10	−1.37	1.01
11	−1.42	−1.31	0.11	−1.37	1.03
12	−1.42	−1.31	0.11	−1.37	1.03
13	−1.40	−1.29	0.11	−1.34	1.02
Nfx	−0.91[e]	−0.85	0.06	−0.88	1.01

[a] $E_{pc} = E_{pc}$ (IIc) for derivatives **1**–**7** and E_{pc} (IIIc) for derivatives **8**–**13**.
[b] $E_{pa} = E_{pa}$ (IIa) for derivatives **1**–**7** and E_{pa} (IIIa) for derivatives **8**–**13**.
[c] $E_{1/2} = (E_{pc} + E_{pa})/2$.
[d] Derivatives not treated with NaOH.
[e] [16].

3.3. Electron spin resonance

5-NI free radicals characterized by ESR were prepared in situ by electrochemical reductions in DMSO, applying the potential corresponding to peak IIc obtained from the CV experiments. All the studied structures produced stable paramagnetic intermediates at that first reduction step. The interpretation of the ESR by means of a simulation process confirmed

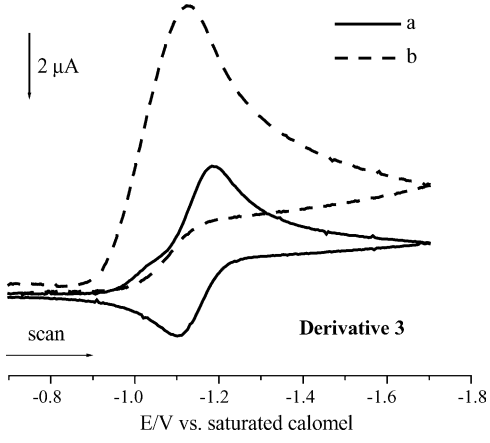


Fig. 5. Cyclic voltammogram in DMSO: (a) derivative **3**, and (b) GSH:derivative **3** (8:1).

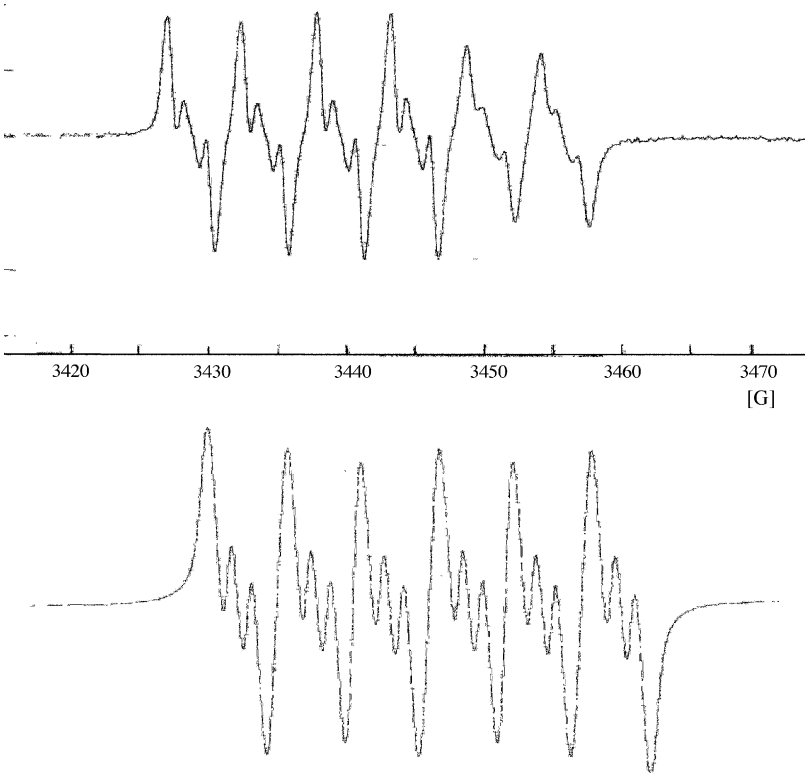


Fig. 6. (a) Experimental ESR spectrum of derivative **3** nitro anion radical produced by electrochemical generation in DMSO, 5-NI (1 mM) and TBAP (100 mM). Spectrometer conditions: microwave frequency 9.71 GHz microwave power 20 mW, modulation amplitude 0.2 G, scan rate 1.25 G/s, time constant 0.5 s, number of scan 10. (b) Computer simulation of the same spectrum.

the stabilities of these radical species due to the delocalization of the unpaired electron. All derivatives studied presented similar hyperfine pattern. For example, 5-NI derivative 3 was analyzed and simulated in terms of two triplets that could be assigned to one nitrogen atom of nitro group and one nitrogen of the indazole ring and one doublet that was accounted for one benzo hydrogen atom (Fig. 6).

4. Concluding remarks

We have studied the electrochemistry of 3-alkoxy- or 3-hydroxy-1-[ω-(dialkylamino)alkyl]-5-nitroindazole derivatives by CV, in DMSO as solvent. The reduction mechanism depends on the acidic moieties in their structures. A self-protonation process involving the protonation of the nitro group was observed. The 3-benzyloxy-5-nitroindazole derivatives presented a one electron reversible transfer corresponding to the generation of the nitro anion radical by a Erev mechanism. The reduction mechanism proposed for 3-methoxy-5-nitroindazole derivatives is a ECErev corresponding to the generation of the nitro anion radical from positive charged species, self-protonation process and the generation of a nitro anion radical from uncharged species. The reduction mechanism proposed to 3-hydroxy-5-nitroindazole derivatives is a ECECErev corresponding to the generation of the nitro anion radical from positive charged species, self-protonation process, generation of nitro anion radical from uncharged species, other self-protonation process from hydroxyl moiety and generation of nitro anion radical from negative charged species. The electrochemistry of these derivatives in presence of NaOH by CV allows to confirm the presence of acidic protons in the chemical structure of 5-NI derivatives and the proposed reduction mechanism. On other hand, GSH was capable to act as an oxidizing agent for the 5-NI regenerating the starting material from the nitro anion radical. The oxidizing effect of GSH was supported by the parallel decrease of the anodic peak current and the increase of the cathodic peak in the cyclic voltammograms, corresponding to the nitro anion radical to wave from uncharged species with the addition of GSH. Stable free radicals were generated using electrochemical reductions at potentials corresponding to the first wave and characterized by ESR spectroscopy. The 5-NI studied presented similar spectrum, due to its structural similarity.

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