

Full Paper

Electrochemical Study of 4-Substituted Analogues of Megazol

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Received: February 2, 2004

Final version: March 31, 2004

Abstract

The electrochemical behavior of three different megazol analogues substituted at position 4 and their comparison with the parent compound megazol in protic and aprotic media by cyclic voltammetry, Tast and differential pulse polarography was studied. All the compounds were electrochemically reducible in both media with the reduction of the nitroimidazole group the main voltammetric signal. The one-electron reduction couple due to the nitro radical anion formation was visualized only in aprotic media for all these compounds. By applying cyclic voltammetric methodology we have calculated the dimerization reaction decay constants (k_2) of the corresponding nitro radical anions in aprotic media. The nitro radical anion obtained from the synthesized nitroimidazole compound having a bromine substituent in 4-position (GC-141) was significantly more stable than the corresponding radical formed from the compound lacking of the substituent in 4-position, megazol.

Keywords: Nitroimidazole derivatives, Voltammetric reduction, Antichagasic drug

1. Introduction

The chemotherapy of Chagas' diseases is still an open field and supported by a few drugs which show toxicity and adverse side effects [1, 2]. Progress towards the development of novel therapeutics can be obtained through to rational drug design [3].

As part of the efforts to develop new compounds aimed at the therapy of parasitic infections, megazol (5-(1-methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine, CAS no. 19622-55-0) (Figure 1) represent a highly active compound used against several strains of *Trypanosoma cruzi* [4, 5], which essentially acts as a thiol scavenger particularly for trypanothione, the cofactor for trypanothione reductase, an essential enzyme in the detoxification process [6]. However, megazol has shown mutagenicity at least according to the Ames assay; for this reason it is necessary develop nitroimidazoles with similar trypanocidal activity but lower mutagenicity profiles.

Consequently a current challenge in this scope is the synthesis of analogues of megazol, in order to obtain substitutes analogues and minimize damage in host cells. According to previous studies [7] megazol analogues substituted at position 4 were synthesized to determine the specificity of the nitro reductase reducing megazol into the corresponding radical anion and eventually the importance of the substitution at position 4 to the half-life of the latter. These studies proved that the substitution at position 4 abolished or suppressed activity of nitroreductase.

In the scope of our investigations tending to contribute to find new therapeutic alternatives for the Chagas' disease, we have conducted several electrochemical studies of nitro compounds in order to characterize the redox activation

that implies the one electron transfer of the nitro group to produce the nitro radical anion [8–12] In such studies we characterized the reduction potential of the $\text{ArNO}_2/\text{ArNO}_2^-$ couple, a well recognized parameter as a very appropriate index to define the type of biological properties of the different nitrocompounds as was demonstrate by Olive [13] on a series of nitroheterocyclic compounds. Furthermore these previous studies revealed the voltammetric feasibility of quantitatively characterize the formation and stabilization of the nitro radical anion.

On the other hand, it is generally accepted that the redox properties of the one-electron couple $\text{ArNO}_2/\text{ArNO}_2^-$ provide only a limited approach on the biological properties of compounds that requires redox activation. In order to obtain a more complete relationship between in vitro behaviour of the molecules and physiological activity, many others factors as stereochemistry, lipid solubility, diffusion, enzymatic site binding, electron transfer reactions and kinetics also must be considered. Then in our works we were also focused in the electrochemical characteristics of the nitro compounds mainly to the feasibility of obtain stable nitro radical anions.

In the present paper we have studied three different megazol analogues substituted at position 4 (Figure 1). To get more information on how these different compounds may carry out reduction and with the aim to define in a further step other possible structures, the present work was undertaken by including in the same study and the same protocol a set of nitroimidazole derivatives related to megazol.

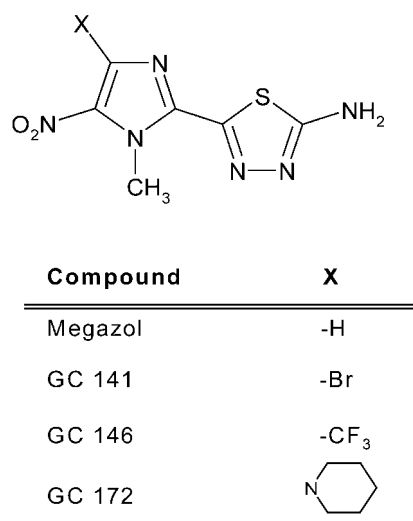


Fig. 1. Chemical structure of megazol and its 4 substitute derivatives.

2. Experimental

2.1. Reagents and Solutions

All the compounds were synthesized and characterized by one of us [7]. All the other reagents employed were of analytical grade.

Stock solutions of each compound were prepared at a constant concentration of 0.025 M in DMF. The polarographic and cyclic voltammetric working solutions were prepared by diluting the stock solution until to obtain final concentrations of 0.1 and 1 mM respectively. A mixture of 30/70: ethanol/Britton–Robinson buffer (KCl 0.3 M) for a protic medium was used. The pH was adjusted with little aliquots of concentrated NaOH or HCl, respectively.

Dimethylsulfoxide (DMSO) and tetrabutyl ammonium perchlorate (TBAP) as solvent and supporting electrolyte were used in aprotic media.

2.2. Apparatus

Electrochemical experiments were performed with a totally automated BAS CV-50W voltammetric analyzer. All experiments were carried out at a constant temperature of 25 ± 0.1 °C using a 10 mL thermostated cell. A mercury drop electrode (Controlling Growth Mercury Electrode, CGME stand of BAS) with a drop area of 0.42 mm^2 as working electrode and a platinum wire as a counter electrode were used. All potentials were measured against Ag/AgCl 3 M.

For differential pulse (DP) and Tast polarography the CGME stand was used in a CGME mode (controlling growth mercury electrode) and for cyclic voltammetric experiments the CGME stand was used as SMDE mode (static mercury drop electrode). Operating parameters: (a) DPP: scan rate 4 mV/s, pulse amplitude 50 mV, sample width 17 ms pulse width 50 ms, drop time 1000 ms. (b) Tast

Polarography : scan rate 4 mV/s, sample width 17 ms, drop time 1000 ms.

For the kinetic analysis carried out in aprotic media, the return-to-forward peak current ratio I_{pa}/I_{pc} for the reversible one electron couple ($\text{ArNO}_2/\text{ArNO}_2^-$) was measured. This measurement was carried out from each individual cyclic voltammogram according to the Nicholson's procedure [14]. The scan rate was varied between 0.1 to 10 V/s.

Using the theoretical approach of Olmstead et al. for dimerization or disproportionation [15, 16], the I_{pa}/I_{pc} values, experimentally measured at each scan rate, were inserted into a working curve to determine the ω parameter, which incorporates the effects of rate constant, drug concentration and scan rate. A plot of ω versus τ resulted in a linear relationship described by the equation

$$\omega = k_2 \times C_0 \times \tau$$

Where k_2 is the second-order rate constant for the decomposition of ArNO_2^- , C_0 is the nitrocompound concentration and $\tau = (E_\lambda - E_{1/2})/\nu$, where E_λ is the switching potential, $E_{1/2}$ is the half-wave potential and ν is the scan rate. Consequently we can obtain the second order rate constant for the decomposition of the nitro radical anion from the slope of the ω versus τ straight line. The assumption that the decomposition of ArNO_2^- follows second-order kinetics is supported by the obtained linearity between the kinetic parameters ω and τ .

3. Results and Discussion

All the compounds were electrochemically reduced at mercury electrodes (DME and HMDE) in protic and aprotic media. According to our previous works [8–12], the most easily electroreducible group in the molecules was the nitro group. However, the reduction of the imine moiety also is possible, but at higher potential than nitro group [8].

3.1. Protic Medium

In protic medium (ethanol + Britton–Robinson buffer : 30/70, 0.3 M KCl) all the compounds were reduced, producing well-resolved signals. Figure 2A summarizes the polarographic study conducted at different pHs (2, 6 and 10). By DPP, at pH 2 one reduction peak around 0 V (peak I) for GC-141 and GC-146 derivatives was observed; which is moved to more negative potentials when pH was increased (-500 mV at pH 10). On the other hand two peaks (peak I and II) are observed for GC-172. Moreover in the first two compounds beside peak I a small peak is observed. On the other hand all the compound exhibit a third reduction signal (peak III) around -800 mV for pH 2 which shifts to more negative potential when pH increases, disappearing around pH 10. All the signals observed by DPP are also observed by Tast polarography showing the faradaic character of the response (Figure 2B).

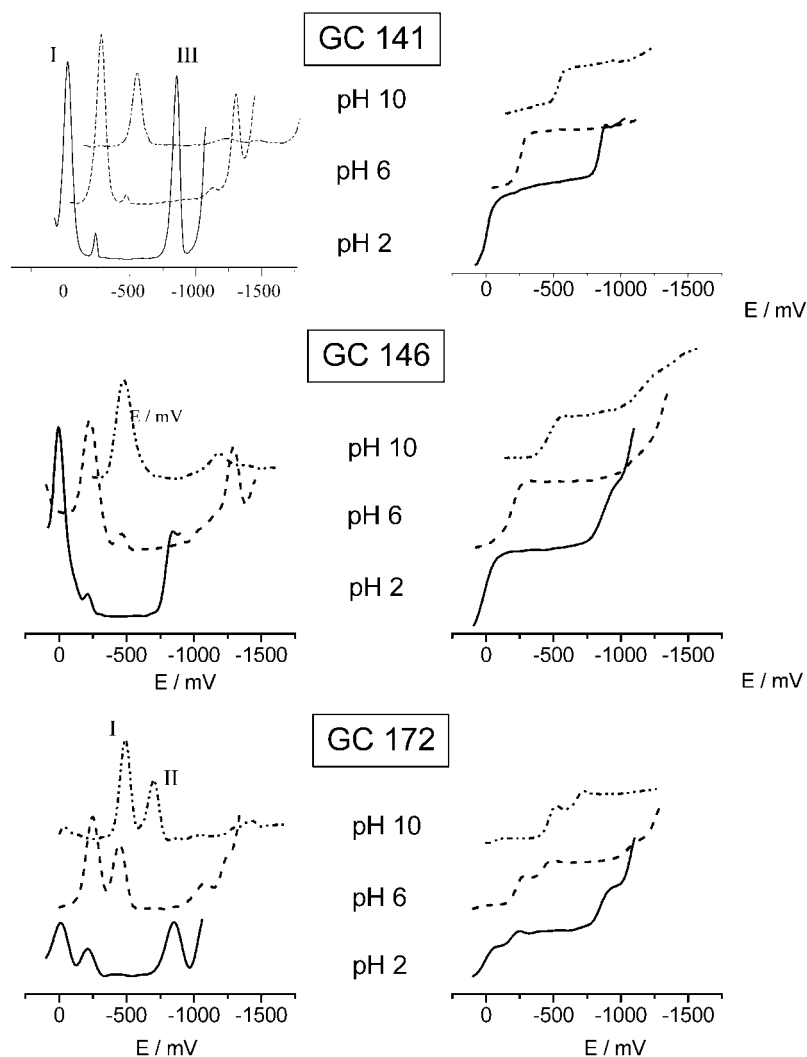


Fig. 2. Differential pulse and Tast polarograms at different pH values of 0.1 mM of GC 141, GC 146 and GC 172 in protic medium, 30/70 : ethanol/Britton–Robinson buffer (KCl 0.3 M) at pH 2 (—), 6 (- - - -), and 10 (- · - · - ·)

In previous work we studied the electroreduction of megazol [8], the parent nitro compound of GC-141, GC-146 and GC-172, and observed the signal I and III. In this work we concluded that the first signal is due to the nitro group reduction, by 4 electron and 4 protons, and the second one correspond to the electroreduction of the imine group.

In order to study the pH influence on the nitro reduction we have evaluated the behavior of the peak potential, (E_p) obtained by DPP, and the limiting current (I_{lim}) obtained by Tast polarography, at different pH between 2 and 12. In Figure 3A, the E_p vs. pH plots show that the peak potential of signal I (○) is pH-dependent shifting to more negative potentials when pH increased up to pH 10. The slope values for E_p /pH plots were 65.8, 63.5 and 69.6 mV/pH for GC 141, GC 146 and GC 172, respectively. But up to pH 10 the peak potential remained constant for all the compounds.

The limiting currents remain practically pH independent between pH 2–8, according to a diffusion controlled process (Figure 3B). The limiting current values obtained at the same concentration for each nitro compound were very

close and similar to megazol, and then we can conclude that in the electroreduction of the three nitroimidazoles there is the same number of electrons involved. As the process for the nitroreduction of megazol is well described as a four-electron process [8, 10], we can conclude that the reduction process for all the studied compounds produces the hydroxylamine derivative according to the well-known Equation 1:



As the main goal of this paper is to evaluate the influence of the different substitutions in position 4 on the nitro group reduction a series of peak potential values for the nitro reduction are presented in Table 1. From these values it is possible to observe that the trifluoromethyl substituted nitrocompound (GC-146) is easier to be reduced, except in acidic pH where all the compounds displayed the same peak potential. This easiness of GC-146 to be reduced can be attributed to an electronic effect of the group in position 4,

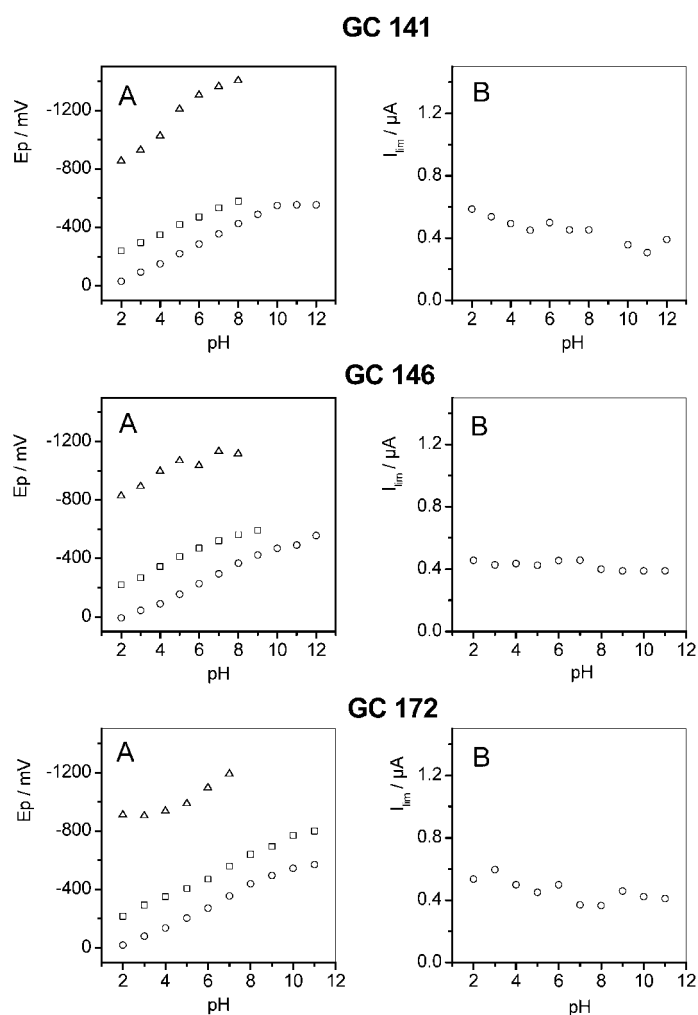


Fig. 3. Peak potential (A) and limiting current (B) dependence with pH for the nitro reduction of nitroimidazole derivatives in protic medium.

because $-\text{CF}_3$ is an electron withdrawing group. On the other hand bromine and piperidine, are groups that increase the peak potential for the nitro reduction of GC-141 and GC-172. When we compare the peak potential values with that previously obtained for megazol, we can appreciate that the three compounds present higher peak potential values than megazol under the same experimental conditions. This effect is emphasized in the case of GC-141 and GC-172, where the increase in the peak potential respect to the molecule with an $-\text{H}$ in position 4 was expected, due to the electron donor nature of the 4-substituent groups. But for GC-146, the molecule with an electro withdrawing group, the increment in the peak potential respect to megazol was surprising. An explanation can be attributed to a steric hindrance between the groups at position 4 and the nitro group at position 5; with a final result wherein the nitro group loses its coplanarity with the imidazole ring. This loss of coplanarity was also reported in a previous work [17] with different kind of molecules, which produces a final effect of an increased energetic for the nitro reduction, i.e., a higher

Table 1. Peak potential values for the nitro group reduction at different pHs in protic medium.

	pH			
	3	7.4	9	10.5
GC 141	-95	-355	-490	-550
GC 146	-89	-296	-424	-492
GC 172	-80	-355	-495	-545
Megazol [a]	-60	-290	-390	-475

[a] data obtained from [8].

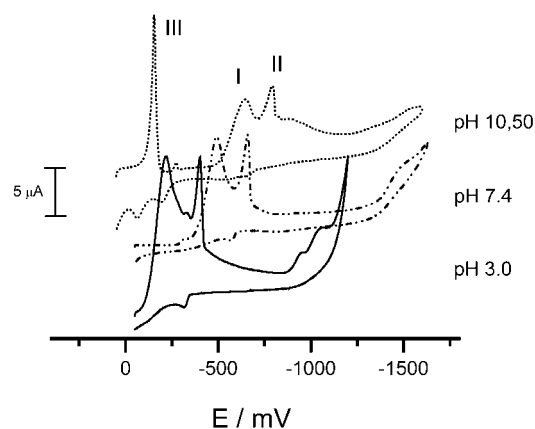


Fig. 4. Cyclic voltammograms of 1 mM GC-146 solution in protic media 30/70: ethanol/Britton-Robinson buffer (KCl 0.3 M) at different pH. Sweep rate: 1 V/s.

peak potential value for the process. In the case of GC-146 the effect is less intense probably due to the $-\text{CF}_3$ group which also exerts some electron withdrawing effect.

From the cyclic voltammetric experiments we obtained very distorted signals due to a strong adsorptive phenomenon that produces pre and post peaks modifying the shape of the reduction signals. In Figure 4 a cyclic voltammogram of GC-146 is shown, where it is possible to observe reductive signals that are in concordance with that observed by differential pulse polarography. Analyzing the corresponding $\log I_p$ vs. $\log v$ plot it is clear that signals II and III are adsorptives, with slope values of 0.80 and 0.72. On the other hand, signal I shows a slope value of 0.51 according to a pure diffusion control of the electrode process. A similar result was found for the other two nitroimidazoles, being that signal I corresponding to the nitro reduction is controlled by a diffusion process. The slope found for GC-141 and GC-172 was 0.43 and 0.49, respectively. In conclusion in protic media a strong adsorption phenomenon is present which hindered a more exhaustive study in this media.

3.2. Aprotic Medium

In aprotic medium the one-electron couple due to the nitro radical anion formation was isolated for the three com-

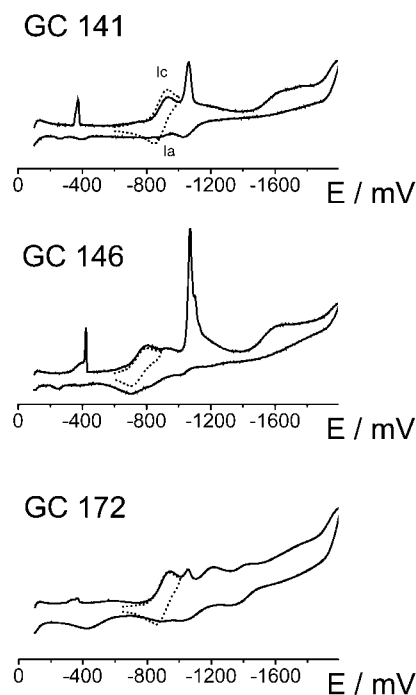


Fig. 5. Cyclic voltammograms GC 141, GC 146 and GC 172 (1 mM solution) in aprotic medium (100 % DMSO) at different potential amplitude. Dashed lines correspond to the reversible $R-NO_2/RNO_2^-$ couples. Sweep rate: 1 V/s.

pounds despite the adsorption phenomenon that is also present (Figure 5). In this reaction media, GC-146 is the most easily reducible derivative ($E_{pc} = -755$ mV), and GC-141 and GC-172 there have no significant differences between them (-900 and -890 mV respectively). Megazol under the same conditions presents a peak potential value of -950 mV (Table 2). These results are in concordance with the electron withdrawing character of $-CF_3$ group present in GC-146. In fact the presence of the $-CF_3$ group in 4-position produces a nitroreduction process ca. 200 mV more easy. In the case of GC-141 and GC-172, its nitro groups were reduced ca. 50 mV easier than megazol, an effect that is not in agreement with the electron donors effect of the groups at position 4.

In Figure 5, we can appreciate that although strong adsorption is present in the electroreduction process, it is possible to obtain adequate cyclic voltammograms for the isolated $ArNO_2/ArNO_2^-$ couple. Consequently we can apply a methodology to characterize the ability of formation and stabilization of the nitro radical anion in this medium.

In Figure 6, the voltammograms of the isolated couple at different sweep rates are shown, and the information that we obtained from these experiments is as follows: (a) ΔE_p ($E_{p1c} - E_{p1a}$) was ≈ 60 mV for all the sweep rates studied (0.1–10 V/s), thus the one-electron transfer and nitro radical anion formation was verified, (b) the peak potentials were sweep rate independent, which agree with a reversible electron transfer process. (c) The current ratio (I_{pa}/I_{pc}) V/s sweep rate plot (Figure 7) reveals an EC_{irrev} mechanism (Irreversible chemical reaction following reversible charge

Table 2. Peak potential, decay constant and half time values for the nitro radical anion electrochemically formed in aprotic medium (100 % DMSO).

	E_p (mV)	k_2 ($M^{-1}s^{-1}$) [a]	$t_{1/2}$ (s) [b]
GC 141	-900	$(3.71 \pm 0.51) \times 10^3$	0.27
GC 146	-755	$(5.88 \pm 0.51) \times 10^3$	0.17
GC 172	-890	$(17.69 \pm 2.59) \times 10^3$	0.06
Megazol	-950	$(9.73 \pm 1.16) \times 10^3$	0.10

[a] $n = 4$; [b] estimated for 1 mM.

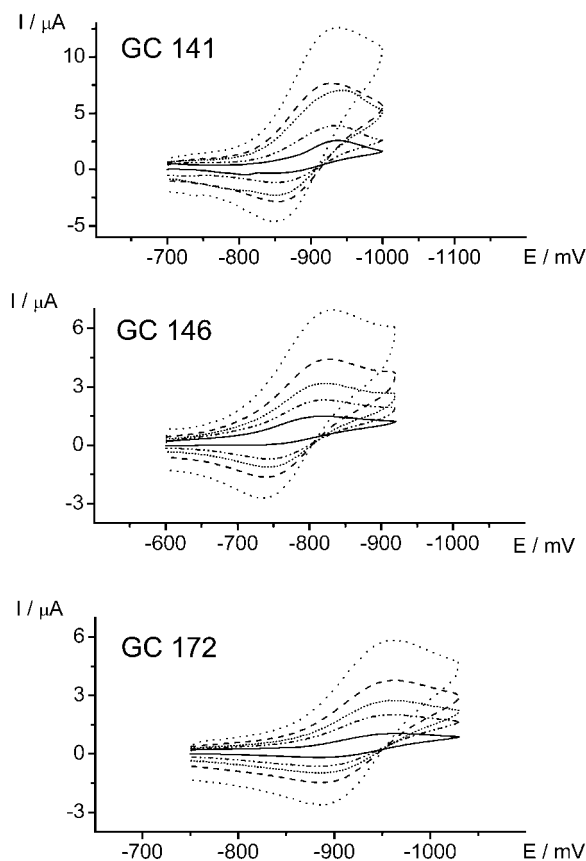


Fig. 6. Cyclic voltammograms of the electrochemically formed $R-NO_2/RNO_2^-$ couple from GC 141, GC 146 and GC 172 (1 mM solution) at different sweep rates in aprotic medium (100 % DMSO).

transfer) for all the compounds. An EC_{irrev} mechanism implies a chemical reaction that occurs after the electron transfer and is evidenced by I_{pa}/I_{pc} values increasing and tending to 1 with the increase of sweep rate. According with the literature information [8, 10], this chemical reaction corresponds to the decay of nitro radical anion electrochemically formed wherein for aprotic media corresponds to a dimerization reaction:



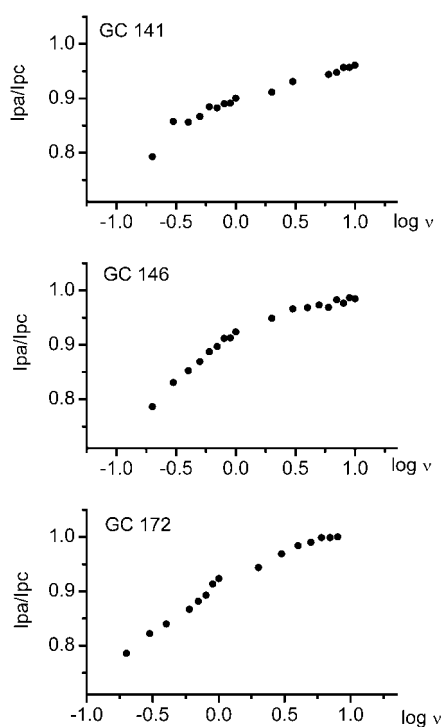


Fig. 7. Current ratio, I_{pa}/I_{pc} , dependence on sweep rate for the cyclic voltammograms of the isolated couple $\text{ArNO}_2/\text{ArNO}_2^-$ of GC 141, GC 146 and GC 172 (1 mM solution) in aprotic media (100% DMF).

According to the Olmstead's methodology [16] for a dimerization coupled reaction, from the slope of ω vs. τ plot the decay rate constant ($k_{2,dim}$) values for each nitro radical anion can be obtained. In Table 2 the $k_{2,dim}$ values are presented, being possible to conclude that the nitro radical anion formed from GC-172 (with a piperidine group) is the more unstable, with a constant value 3 times higher than the other two studied radicals. The nitro radical anion rate constants for the other two nitroimidazole compounds were very similar. The synthesized compound, GC 141, with bromine as substituent in 4-position produces a nitro radical anion that is significantly more stable than the nitro radical anion from the parent compound, megalzol. Furthermore if we consider that in aprotic media GC-141 is also more easily reducible than megalzol this compound could result in being a promising alternative as an antichagasic from the point of view of the electrochemical parameters. Our electrochemical results in aprotic media are contradictory with the previously observed in biological media [7] in the sense that the substitution at position 4 abolished or suppressed the activity of nitroreductase. If we consider the energetic necessary by the enzyme to reduce the nitro group to the nitro radical anion, the electrochemical results indicate that this fact is favored with the inclusion of the substituent in position 4, because the peak potential values are lower by at least 50 mV less than megalzol. Then, the difficulty of the

enzyme to form the radical anion is related to difficulties of the molecules to reach the active site of the enzyme more than electronic or electrochemical requirements. This fact is reasonable if we consider that all the substituents in 4-position are bulky and considerably change the surface activity of the molecule.

Finally, these results show that there is no correlation between the reduction peak potential necessary to form the nitro radical anion and its stability, since GC-172 is ca. 150 mV easier to be reduced than GC-146 or GC-141, however, it is three times more unstable.

4. Acknowledgements

The authors gratefully acknowledge the financial support of FONDECYT (grant 8000016). Also, the financial support from ECOS-Conicyt, for travel expenses and maintenance of Dr. Chauviere and Dra. Bollo is recognized.

5. References

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