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Effect of the leaving group on the electrodic reduction mechanism of anti-*Helicobacter pylori* metronidazole derivatives, in aprotic and protic media

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Abstract

Because redox properties are central to bioreductive drug activity and selectivity, six 2-methyl-5-nitroimidazole, substituted at the N1-ethyl side chain with I, Br, Cl, OAc, OMs and NH_3^+ were synthesized and submitted to cyclic voltammetry and electrolyses, in order to define their electrodic reduction mechanism, in aprotic [dimethylsulphoxide (DMSO) + 0.1 mol l^{-1} tetrabuthylammonium perchlorate (TBAP)] and phosphate-buffered media, on glassy carbon electrode, in comparison with metronidazole. Three of these compounds, namely, the iodo, bromo and ammonium salt derivatives showed significant anti-*Helicobacter pylori* (strain resistant to metronidazole) activity. All the cyclic voltammograms (CV), in aprotic medium, are similar to the one for metronidazole, except for −I, -Br and -NH_3^+ derivatives. The CV of the N1-ethylhalide (-I, -Br) 5-nitroimidazole showed more intense and irreversible first waves, even at faster sweep rates ($v < 2$ V s^{-1}). The absence of the first wave anodic counterpart, along with analysis of the dependence of E_p, I_p and other parameters with v, and results from electrolysis (consumption of two electrons) showed the process to be an ECE system, with halide release, after uptake of two electrons. This behaviour represents a case of dissociative electron transfer (ET). For the ammonium salt, self-protonation mechanism was evident. The facility of reduction represented by the first wave potential and concerning the substituents is NH_3^+>Br>I>Cl>OMs>OH>OAc. In aqueous phosphate-buffered medium, the electrochemical behaviour of all the compounds is similar to the one of metronidazole, represented by a unique and irreversible $4e^-/4H^+$ wave. The order of reduction ease is NH_3^+>Br ∼ OMs>I>OH>OAc. Aprotic medium allows a better discrimination between the substituents. Concerning biological activity, despite the impossibility of establishing a correlation, it has been observed that the more electrophilic compounds showed better anti-*H. pylori* activity.

Keywords: 5-Nitroimidazole reduction; Leaving group effect; Reductive elimination; Anti-*Helicobacter pylori*; Self-protonation; Dissociative electron transfer

1. Introduction

Nitroimidazole derivatives are an extremely important class of compounds. They are extensively used in the treatment of anaerobic infections and are under continuing investigation regarding their use as hypoxic cell cytotoxins, radiation sensitizers and, more recently, as anti-*Helicobacter pylori* agents. It is well known that *H. pylori*, a Gram-negative microaerophilic spiral bacterium, is the major causative factor in peptic ulcer diseases. Highly effective treatments for *H. pylori* infection include a combination of antisecretory and antimicrobial agents, among them metronidazole [R = OH (**1**)] [1]. There is an urgent need for additional drugs due mainly to metronidazole-resistant *H. pylori* strains [2]. Therefore, the development of novel types of anti-*H. pylori* agents is an important medical need.

Redox properties are central to bioreductive drug activity and selectivity. Nitroarenes rely for their efficacy as biologically active and hypoxia selective agents on bioreduction to reactive intermediates. The activation of nitroaromatics could occur through a direct reduction and further reaction with oxygen, generating reactive oxygen species or alterna-

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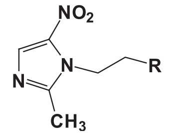

1 R = OH, **2** R = Br, **3** R = I, **4** R = Cl,

5 R = OMs, **6** R = OAc, **7** R = NH_2.HBr

Fig. 1. Analysed 5-nitroimidazoles.

tively, in lower oxygen concentration, by generation, after extensive reduction, of electrophilic species (iminoquinomethanes) [3] or by a trigger-effector's mechanism, with the break of the linking bond and thus release an effector, which is the bioactive agent [4]. 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 lipophilic systems (viz., in membranes, proteins, etc.) or in aqueous media corresponding to situations in most biological cytoplasmic fluids [5,6]. Studies in aprotic medium are also important, aiming the correlation of redox potentials with biological activities, because it also allows the obtention of potentials related to one-electron transfers [5,6]. Electrochemical methods have been used to verify the reduction process of nitroimidazoles, in dimethylformamide and acetonitrile [7] and a large number of studies are related to metronidazole (**1**), which has been studied on different electrodes [8,9], in dimethylsulphoxide (DMSO) [9], in protic [10] and mixed (DMF/H_2O) [8] media.

Due to the importance of redox properties for bioreductive drugs, six N1-ethyl-substituted-2-methyl-5-nitroimidazoles [R=Br (**2**), R=I (**3**), R=Cl (**4**), R=OMs (**5**), R=OAc (**6**), R=NH_2HBr (**7**)] (Fig. 1) were synthesized and submitted to cyclic voltammetry (CV) and electrolyses, in order to define their electrodic reduction mechanism and the effect of the side chain substituent on it, in aprotic medium (DMSO + 0.1 mol l^{-1} TBAP), on glassy carbon electrode, in comparison with metronidazole (**1**). Those compounds were assayed against different strains of *H. pylori*. Compounds **2**, **3** [11] and **7** [12] showed significant antibacterial activity, superior to **1** and against metronidazole-resistant strains. As observed in Fig. 1, the substituents are joined to the 5-nitroheterocycle through a CH_2–CH_2 spacer, which prevents a direct conjugative interaction between the original nitro group or any reduced species from it.

2. Experimental

Electrochemical studies were carried out in dimethylsulphoxide (DMSO) containing 0.1 mol l^{-1} tetrabuthylammonium perchlorate (TBAP) and in a phosphate-buffered aqueous/ethanolic (4:1) solution (pH 7.2 ± 0.1) using a glassy carbon electrode BAS (diameter = 3.0 mm) as a working electrode, a platinum-wire electrode as a counter electrode, and a homebuilt Ag|AgCl|0.1 mol l^{-1} NaCl Luggin reference electrode, isolated from the solution by a Vycor® rod. In protic medium, the values of E_{pc} vs. NHE were obtained by adding 0.272 V to the measured values, in Ag/AgCl, as shown before [13]. The scan rate v was in the range 0.010–2 V s^{-1}. Metronidazole (**1**) is commercially available. Other compounds (**2–7**) were synthesized following reported procedures. More specifically 1-(2-ammoniumethyl)-2-methyl-5-nitroimidazol bromide (**7**) was recently reported [12]. All the derivatives show analytical and spectral data in full accord with the indicated structures. The electrolysis of compounds **1**, using two applied potentials ($E_{ap1} = -1.30$ V; $E_{ap2} = -2.40$ V) and **2** (40 mg, 0.17 mmol, $E_{ap} = -1.30$ V) were performed in the above conditions, using a divided cell and a reticulated vitreous carbon electrode, RVC 2000 from Le Carbone Lorraine, 26 × 13 × 5 mm, platinum and homebuilt Ag|AgCl|0.1 mol l^{-1} NaCl as the working, auxiliary and reference electrodes, respectively. The electrolysis of **1** was held initially at E_{ap} -1.30 V, with consumption of 1 mol electron mol^{-1} and at $E_{ap} = -2.40$ V, with the final charge of 3.15 mol electron mol^{-1} (10% of the initial current remained). For **2**, at E_{ap} close to the first reduction wave (-1.30 V), the final charge consumption was 2 mol electrons mol^{-1}. The work-up consisted of addition of water and extraction with ether and dichloromethane. During the electrolyses at the first wave potential, of **1** and **2**, the colour of the catholytes, in both cases, changed from colourless to light yellow and to purple. In the case of **2**, after work up, there was evidence of the presence of Br^- in the aqueous phase.

3. Results and discussion

The electrochemical reduction process of **1**, in dimethylsulphoxide, is typical of nitroaromatics in aprotic medium [7–9,14] and is represented by a diffusion-controlled ($I_{pIc} \propto v^{1/2}$) monoelectronic quasi-reversible first wave [ΔE_p ($E_{pIc} - E_{pIa}$) ~ 0.100 V; E_{pIc} varies slightly with v], related to the formation, in the timescale of the experiment, of a stable nitro anion radical, followed by a more intense second wave, leading, probably to an unstable hydroxylamine (Fig. 2A; Table 1). There is no evidence of the reversible hydroxylamine/nitroso couple, on the successive scan (figure not shown), as observed in case of stable derived-hydroxylamines. Compounds **4–6** behave similarly as **1**, with some differences in the height of the second wave. The CV of compound **6** is included, as an example (Fig. 2B).

On the other hand, the CV for the -Br and -I derivatives (**2** and **3**) showed a more intense and irreversible first wave (Fig. 2C–E). The absence of the first wave anodic counterpart, along with analysis of the dependence of E_p, I_p and other parameters with v, and results from electrolysis (consumption of 2 mol electrons mol^{-1}) showed the process to be an ECE system, with consumption of two electrons and halide release (result from electrolysis).

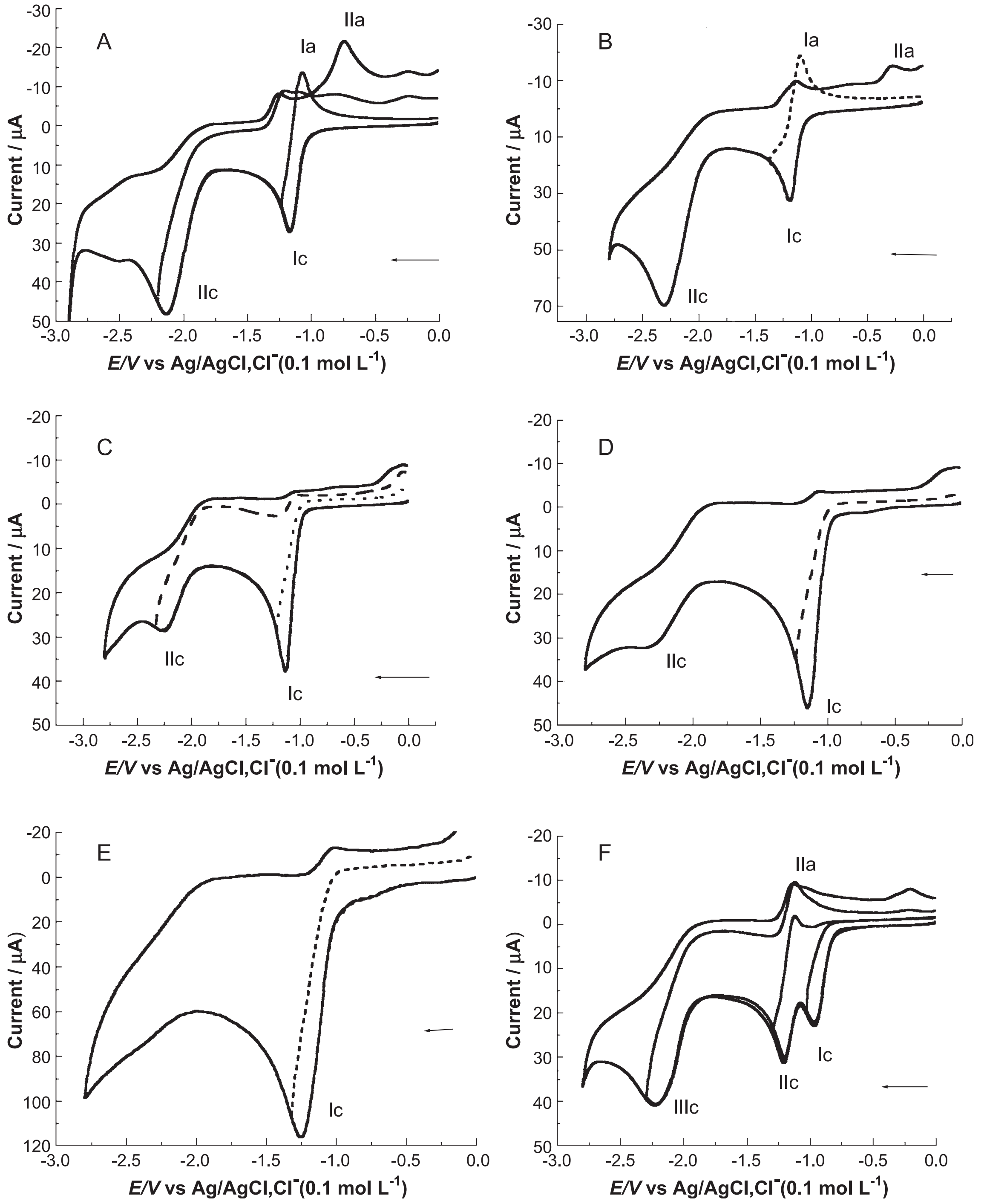

Fig. 2. Cyclic voltammograms (CV) of chosen 5-nitroimidazoles, in DMSO + 0.1 mol l^{-1}, glassy carbon electrode, $c1 = c2 = c3 = c6 = 2$ mmol l^{-1}, (A) CV of of **1**, $v = 0.100$ V s^{-1}. (B) CV of **6**, $v = 0.100$ V s^{-1}. (C) CV of **2**, $v = 0.100$ V s^{-1}. (D) CV of **3**, $v = 0.100$ V s^{-1}. (E) CV of **3**, $v = 1.00$ V s^{-1}. (F) CV of **7**, $v = 0.100$ V s^{-1}.

The CV of **7** is represented by three reduction waves, one of them at less negative potential ($E_{pIc} = -0.968$ V), when compared to the first wave of compound **1** (Fig. 2F vs. Fig. 2A; Table 1). There are evidences for a self-protonation mechanism [7]. Waves IIc and IIIc in the CV of **7**, are, however, very similar to Ic and IIc in the CV of **1** (Fig. 2F vs. Fig. 2A; Table 1).

The facility of reduction represented by the first wave potential (Table 1) is in the following order: **7** (NH_3^+)>**2** (Br)>**3** (I)>**4** (Cl)>**5** (OMs)>**1** (OH)>**6** (OAc).

An electrochemically formed nitro radical anion can suffer, in aprotic medium, different ways of decay, the second order chemical reactions of disproportionation and/or dimerization, or in the presence of a leaving group

Table 1
Main electrochemical parameters for the analysed compounds, DMSO+0.1 mol l^{-1} TBAP, c=2 mmol l^{-1}, v=0.100 V and 1 V s^{-1}, glassy carbon electrode

Compound	Sweep rate (V s^{-1})	$-E_{pIc}$ (V)	$-E_{pIa}$ (V)	I_{pa}/I_{pc} (V)	$-E_{pIIc}$ (V)	$-E_{pIIa}$ (V)	$-E_{pIIIc}$ (V)
1	0.100	1.169	1.067	0.95	2.139	–	
	1	1.205	1.030	1.05	2.303	–	
2	0.100	1.132	–	–	2.215	–	
	1	1.185	–	–	2.387	–	
3	0.100	1.149	–	–	2.373	–	
	1	1.254	–	–	–	–	
4	0.100	1.158	1.052	0.84	–	–	
	1	1.186	1.027	1.01	–	–	
5	0.100	1.168	–	–	2.378	–	
	1	1.215	1.018	0.96	–	–	
6	0.100	1.189	1.084	1.0	2.306	–	
	1	1.251	1.018	1.0	–	–	
7	0.100	0.968	–	–	1.213	1.113	2.229
	1	1.044	–	–	1.247	1.092	2.475

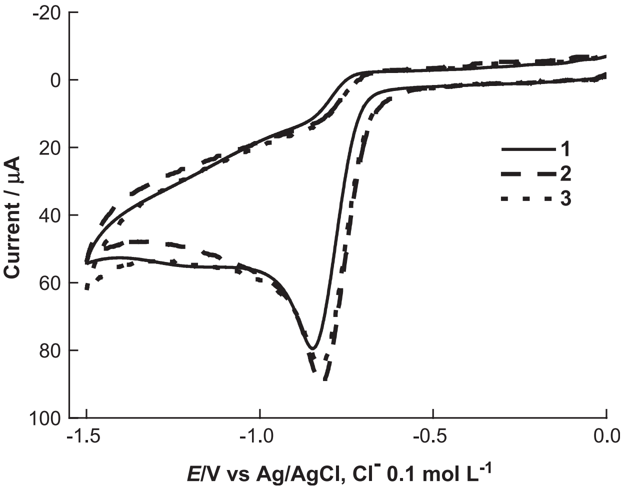

Fig. 3. Cyclic voltammograms (CV) of chosen 5-nitroimidazoles (1, 2 and 3) in aqueous–ethanolic (4:1) phosphate-buffered medium, (pH 7.2 + 0.1), c = 2 mmol l^{-1}, v = 0.100 V, glassy carbon electrode.

adequately located, a primary order reductive elimination. Additionally, for substrates carrying acidic protons, a primary decay of the anion radical is caused by a fast protonation reaction by the starting nitroimidazole (a father–son type of reaction) [7].

For compounds **1** and **4–6**, the reduction pathway is typical for well-behaved nitrocompounds, where disproportionation occurs. For **7**, which carries an acidic ammonium salt ($HRNO_2^+$), the electrochemical behaviour is explained by a self-protonation mechanism [7]. The primary electrogenerated radical ($HRNO_2$) is protonated by the starting salt generating the conjugate base (RNO_2) and $H_2RNO_2^+\bullet$, which suffers further reductions (wave Ic, Fig. 2F) and protonations, giving rise, as the final reduction product, to the hydroxylamino derivative. The second step is assigned to the standard quasi-reversible reduction of a neutral nitro group to the anion-radical (waves IIc/IIa, Fig. 2F; Table 1).

The breaking of an existing bond and/or the formation of a new bond very often accompany electron transfer (ET) to molecules. The dissociation of the fragile bond may follow or be even concerted to the ET itself [15]. Everett et al. [16] reported on reductive elimination of a leaving group from a reduced 5-methyl-substituted 2-nitroimidazole. Reaction kinetics revealed that bromide, at the α-methyl-heteroaromatic position, was eliminated from a one-electron reduced species, i.e., the radical anion [16]. Halide release was also reported in the literature, using 1-haloalkyl-2-nitroimidazoles [17]. For **2** and **3**, this kind of process could be operating, despite the longer distance (two-carbon chain) between the leaving group and the aromatic ring. The participation of the nonbonding electrons of the heterocyclic nitrogen, after partial reduction on the nitro group, in an SNi-type reaction, giving an intermediate aziridinic nucleus [18] or an attack of the partially reduced nitro to the C–X (Br, I) or even an intramolecular electron transfer from the nitro anion radical to the C–Br bond [19] are possible and explain the release of those halides, generating a radical prone to receive a second electron, in an ECE process. The full understanding of the reduction mechanism is out of the scope of the present work. Kinetic measurements related to the stability of the anion-radicals and detailed experiments, concerning a definite proof for the proposed mechanisms are under way.

In aqueous phosphate-buffered medium, the electrochemical behaviour of all the compounds is similar to the one of metronidazole (**1**), represented by a unique and irreversible $4e^-$ wave (Fig. 3). The E_{pc} values are listed in Table 2. The order of reduction ease is NH_3^+>Br ~ OMs>I>OH>OAc.

Despite the small number of compounds analysed and such slight differences on E_{pIc} values (Table 1), which ranged from − 0.968 V (**7**) to − 1.189 V (**6**), the biologically more active compounds against *H. pylori* (**7**, **2**, **3**) were shown to have the more positive E_{pIc} values and less stable nitro anion-radicals, in aprotic medium. In protic medium, the E_{pc} values (vs. NHE) ranged from − 0.508 V (**7**) to − 0.587 V (**6**) (Table 2), all of them in the favorable range to be reduced in vivo [13]. This may suggest that reductive activation is important for the activity against *H. pylori*, but undoubtedly involves more than reduction characteristics. The biological activity in a live host is always a complex outcome not

Table 2
Main electrochemical parameters for the analysed compounds, aqueous–ethanolic (4:1) phosphate-buffered medium, (pH 7.2 ± 0.1), c = 2 mmol l^{-1}, v = 0.100 V, glassy carbon electrode

Compound	$-E_{pIc}$ (V) vs. Ag/AgCl	$-E_{pIc}$ (V) vs. NHE[a]
1	− 0.847	− 0.575
2	− 0.824	− 0.552
3	− 0.827	− 0.555
5	− 0.824	− 0.552
6	− 0.859	− 0.587
7	− 0.780	− 0.508

[a] Values obtained by adding 0.272 V to the values vs. Ag/AgCl [13].

usually dominated by one parameter [13]. The results here obtained prompt us to investigate further this class of compounds and their electrochemistry.

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