Electrogeneration of nitranion species from nitrofuryl substituted 1,4-dihydropyridine derivatives

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Abstract

We have synthesized and studied the electroreduction of 1,4-dihydropyridine derivatives with a nitrofuryl substituent at 4-position. The one-electron reduction of these compounds in non-aqueous medium generates the corresponding nitro radical anion. In addition, the existence of an acidic proton on the 1,4-dihydropyridine ring triggered the appearance of father–son type reactions between the nitro radical anion and the parent compound, generating the corresponding nitranion.

The corresponding nitranion was exhaustively generated by controlled potential electrolysis. The nitranion voltammetric detection permitted a clear separation from the corresponding parent compound.

Keywords: Nitranion, Electrogeneration; Nitro radical; Nitrofuryl derivative; 1,4-Dihydropyridines; Voltammetric

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1. Introduction

5-Nitrofurans and 5-nitroimidazoles derivatives are the heterocyclic drugs most widely used as antibacterial, antiprotozoal and anticancer agents [1] but, it is also well established that these molecules present several adverse effects [2]. Both pharmacological and toxic effects are the result of the well documented in vivo reduction of the nitro group, producing the nitro radical anion species, which could produce damage by interaction with DNA or by generating more reactive species derived from oxygen by a specific redox cycling [3,4]. Consequently, the study of the one-electron reduction product has involved considerable attention, being a necessary intermediate in the nitro/hydroxylamine conversion. There are many articles where the electrochemistry of nitrofuran derivatives has been discussed [5–10] but there remain several unresolved aspects related mainly to the reactivity of the nitro radical anion.

On the other hand, 4-substituted Hantzsch 1,4-dihydropyridines (1,4-DHP) are analogues of NADH coenzymes and an important class of drugs which are potent blockers of calcium channels with relevant applications in various cardiovascular diseases [11–13]. Furthermore, it has recently been found that 1,4-DHP compounds such as nifedipine, nisoldipine, nicardipine, exhibit a potential trypanocide action, inhibiting culture growth and oxygen uptake in *Trypanosoma cruzi* epimastigotes [14,15], the parasite causing Chagas disease. This toxic effect, that could be associated with the reduction of the nitro group, is another way leading to new therapeutic alternatives for Chagas disease.

Based on the above evidences revealing that both nitrofuryl or substituted 1,4-DHP moieties turn to be pharmacophores with antichagasic effect, we have designed a synthetic strategy that implies including both moieties in the same molecule. Thus, we have synthesized new 1,4-DHP derivatives containing a nitrofuryl moiety as substituent at 4-position.

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In the present study we have centered our attention on the reduction of the nitrofuryl moiety by both microelectrolysis (cyclic voltammetry, CV) and macroelectrolysis (controlled potential electrolysis, CPE) in order to reveal the influence of the 1,4-DHP substitution on the reduction process of the nitrofuryl moiety.

2. Experimental

2.1. Compounds

All compounds (Fig. 1) were synthesized in our laboratory. General procedure: 10 mmol enamine (ethyl 3-aminocrotonate and/or 3-aminocrotononitrile) and 5 mmol aldehyde (5-nitro-2-furaldehyde, formaldehyde, acetaldehyde, 3-nitrobenzaldehyde) were dissolved in glacial acetic (30 ml). Water was added until precipitation of the dihydropyridine occurred. The crude solid product was filtered off and washed with ether. The compounds were recrystallized twice from absolute ethanol. Compounds were characterized by ^{1}H NMR, ^{13}C NMR spectroscopy using a 300 MHz spectrometer (Bruker, WM 300), infra red spectroscopy (FT-IR Paragon Spectrometer, 100PC) and an Elemental Analysis (Perkin–Elmer, 240 B).

2.1.1. Ethyl 4-(5′-nitro-2′-furyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (A)

IR (KBr): $\nu_{\max}$ 3346.7, 1704.3, 1656.0, 1517, 1487.9, 1400, 1355.0, 1211.9, 1114.4. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (dd, 6H, $J = 7.1$, $J = 7.1$); 2.36 (s, 6H); 4.15 (dq, 2H, $J = 10.9$, $J = 7.1$); 4.21 (dq, 2H, $J = 10.9$, $J = 7.1$); 5.27 (s, 1H); 6.19 (brs, 1H); 6.26 (d, 1H, $J = 3.4$), 7.21 (d, 1H, $J = 3.4$). ^{13}C NMR (75 MHz, $CDCl_3$): 166.8 (C2), 162.7 (C2), 146.4 (C2), 113.2, 108.9, 99.0 (C2), 60.2 (C2), 34.6, 19.6 (C2), 14.3 (C2) ppm. Anal. Calcd. for $C_{17}H_{20}N_2O_7$: C, 56.04; H, 5.53; N, 7.69. Found: C, 55.85; H, 5.54; N, 7.72%. M.p. 184.2 °C.

Compounds	R_1	R_2	$-E_{PC}$ (mV)	ΔE_P(mV)
A	$-CO_2Et$	$-CO_2Et$	1044	63 ± 6
B	-CN	-CN	929	58 ± 9
C	$-CO_2Et$	-CN	998	64 ± 8

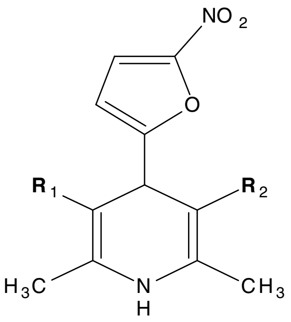


Fig. 1. Chemical structures and voltammetric parameters of synthesized nitrofuryl derivatives.

2.1.2. 3,5-Dicyano-4-(2′-furyl-5′-nitro)-2,6-dimethyl-1,4-dihydropyridine (B)

IR (KBr): $\nu_{\max}$ 3369.2, 2205.9, 1665.8, 1536.3, 1505.1, 1393.1, 1358.8. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.07 (s, 6H); 4.92 (s, 1H); 9.81 (brs, 1H); 6.82 (d, 1H, $J = 3.8$), 7.70 (d, 1H, $J = 3.8$). ^{13}C NMR (75 MHz, DMSO-d_6): 159.7 (C2), 158.0 (C2), 121.8 (C2), 114.9, 111.9, 73.8 (C2), 33.9, 20.8 (C2) ppm. Anal. Calcd. for $C_{13}H_{10}N_4O_3$: C, 57.78; H, 3.73; N, 20.73. Found: C, 57.75; H, 3.73; N, 20.65%. M.p. 225.2 °C.

2.1.3. Ethyl 3-cyano-4-(5′-nitro-2′-furyl)-2,6-dimethyl-1,4-dihydropyridine-5-carboxylate (C)

IR (KBr): $\nu_{\max}$ 3317.9, 2199.4, 1664.3, 1638.5, 1527.1, 1493.1, 1383.0, 1355.5, 1257.0, 1115.7. ^{1}H NMR (300 MHz, $CDCl_3$): δ1.29 (dd, 3H, $J = 7.1$, $J = 7.2$); 2.18 (s, 3H); 2.38 (s, 3H); 4.12 (dq, 1H, $J_3 = 10.9$, $J = 7.1$); 4.16 (dq, 1H, $J = 10.9$, $J = 7.2$); 4.90 (s, 1H); 6.04 (brs, 1H); 6.38 (d, 1H, $J = 3.7$), 7.24 (d, 1H, $J = 3.7$). ^{13}C NMR (75 MHz, $CDCl_3$): 166.2, 160.0 (C2), 147.6, 146.3, 118,6, 113.0, 109.5, 97.6, 81.8, 60.5, 35.9, 19.7, 18.5, 14.2 ppm. Anal. Calcd. for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.24. Found: C, 56.56; H, 4.77; N, 13.19%. M.p. 161.9 °C.

2.2. Electrolytic media

Solutions contained 1 mM of 1,4-DHPs derivatives in 100% *N*,*N*-dimethylformamide (DMF) and 0.1 M tetrabutyl ammonium perchlorate (TBAP) were prepared.

2.3. Voltammetry

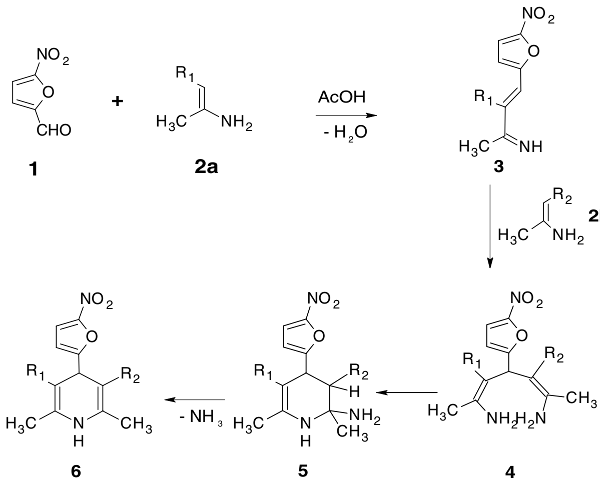
Differential Pulse (DPV), cyclic (CV) and linear sweep voltammetry (LSV) were performed with BAS-CV 50 assembly. A glassy carbon stationary electrode as working electrode (BAS model MF-2012, 3 mm diameter) for DPV and CV experiments was used. A platinum wire was used as auxiliary electrode and all potentials were measured against an Ag/AgCl electrode in saturated KCl.

Potential controlled electrolysis (CPE): these studies were carried on exhaustive electrolysis in a 75 ml cell at constant electrode potential (500–1200 mV) on a glassy carbon mesh electrode (BAS, model MF-2077). Oxygen was removed with pure and dry pre-saturated nitrogen. A three electrode circuit with an Ag/AgCl electrode was used as reference and platinum wire as a counter electrode. A BAS-CV 50 assembly was used to electrolyze the 1,4-DHPs solutions. The net charge was calculated by correction for the estimated background current.

3. Results and discussion

We have synthesized three new nitrofuryl substituted 1,4-DHP derivatives (Fig. 1) according to the mechanism displayed in Scheme 1. Formation of compound 6 is assumed to be via initial condensation of aldehyde 1 with one molecule of enamine 2a to yield the unstable ylidene derivative 3 that, in turn, reacts with another molecule of enamine 2b giving 4 which cyclizes into 5, this intermediary then loses NH_3 to yield 6. The obtaining of the specific nitrofuryl 1,4-dihydropyridine derivative depends on the nature of enamine 2a and 2b.

Fig. 2 shows the cyclic voltammogram of one of the synthesized nitrofuryl derivatives in DMF and TBAP as solvent and supporting electrolyte, respectively. We have observed that all the compounds under study follow a similar pattern, displaying voltammograms wherever three main peaks are detected. We have focused our attention only on the first reversible reduction couple (peak I) which corresponds to the nitro radical anion formation due to the one electron reduction of the nitro group according to the following reduction equation (Scheme 2). The obtained ΔE_P values (Fig. 1) are consistent with the one-electron and reversible character of the nitro/nitro radical anion couple. From these values it follows that more electron-attracting groups in R_1 and R_2 favor nitrofuryl moiety reduction indicating an inductive effect of the 1,4-DHP moiety on the nitrofuryl electroactive group. In fact, the 1,4-DHP moiety acts by diminishing the electron density on the nitrofuryl moiety, favoring nitro reduction.



6 a : R1 = R2 = - CO2Et
6 b : R1 = R2 = - CN
6 c : R1 = -CN , R2 = -CO2Et

Scheme 1. Mechanistic reaction scheme for the synthesized nitrofuryl 1,4-dihydropyridine derivatives.

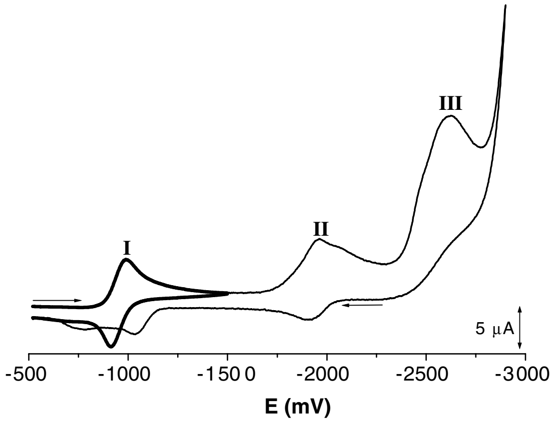
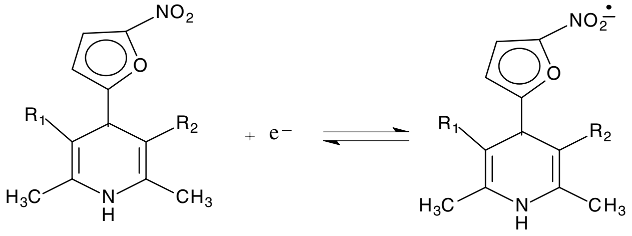


Fig. 2. Cyclic voltammograms of Compound C in DMF, 0.1 M TBAP. Darker line represents a short sweep. Sweep rate 1 V/s.



Scheme 2. Conversion of nitrofuryl 1-4-DHP derivative to the corresponding nitro radical anion by electroreduction.

On the other hand, the observation of the cyclic voltammogram in Fig. 2 permits to detect a difference in the nitro/nitro radical anion couple depending on whether a wide or shortened sweep was applied. A very good resolved reversible couple was obtained when the shortened sweep was applied; however, when the sweep was enlarged to a potential switching of about −2800 mV the oxidation peak of the original couple was notoriously different. This could be indicative of different species being oxidized in the reverse sweep depending on whether a wide or shortened sweep is applied. In the case of the wide sweep voltammogram, probably the elapsed time between the formation of the nitro radical anion (cathodic peak I) and the corresponding oxidation in the reverse sweep permit the occurrence of a fast coupled chemical reaction of the radical species thus forming a different oxidizable specie. In order to explain this effect we have hypothesized the formation of a nitranion on the N-1 of the 1,4-DHP ring as a consequence of the deprotonation of the N-1. The formation of the nitranion from different nitrobenzene substituted 1,4-DHP compounds in aprotic media as a consequence of alkali addition [16,17] or reaction with electrogenerated superoxide [18] has been previously reported. Probably, in this case, the weakly acid character of the dihydropyridine and the sparseness of protons in aprotic media favored an intermolecular acid–base reaction between the nitro radical anion and the proton in the N-1 position of the dihydropyridine ring, thus producing the corresponding nitranion according to the father–son type intermolecular reaction showed in Scheme 3. Consequently, the nitro radical anion on the nitrofuryl moiety would act as a base deprotonating the N1 of the dihydropyridine ring. In order to validate this hypothesis, we replaced the hypothetic nitro radical anion base with a well-known base as alcoholic NaOH; the effect on the nitro/nitro radical anion couple is shown in Fig. 3. As shown in Fig. 3, the effect of adding a base to a non-aqueous solution containing the 1,4-DHP derivative produced a shifting in the $\mathrm{RNO_2/RNO_2^{\bullet-}}$ couple, displacing both cathodic and anodic peaks to more negative potentials. This shifting in potential peaks is in accord with our hypothesis since the nitranion specie has to be more difficult to reduce than the parent compound as a consequence of its increased electron density. On the other hand, the nitranion specie must be more easily oxidized than the parent compound.

In order to deepen into the above findings we also used controlled potential electrolysis (CPE) to exhaustively electrogenerate the nitranion species. Solutions containing 1 mM of 4-(5′-nitro-2′-furyl)-1,4-dihydropyridine derivatives were electrolyzed at controlled potentials between −1.4 to −1.5 V in order to exhaustively generate the corresponding nitro radical anion. When the electrolysis was running, we observed, at the beginning, a similar phenomenon that occurred when a base was added to the solution. By comparing Figs. 3 and 4 we can observe a similar evolution for the cyclic voltammograms, showing a displacement of the signal towards more negative potentials. We can notice that the nitro/nitro radical anion couple has completely disappeared after 7 min of the electrolysis; however, a new reversible couple became visible at a little more negative potential. The new couple was also consumed when the electrolysis was continued, but more slowly, at the same potential. By adequately selecting the controlled potential and the time for the electrolytic process, it is possible to convert all 1,4-DHP derivatives in the corresponding nitranion derivative without adding a base to the reaction. In this figure we can observe that the change from 1,4-DHP derivative to the corresponding nitranion means about extra 100 mV in energy requirements to carry out the reduction making possible the perfect separation by the voltammetric measurements. Moreover the detection of the nitranion species obtained from the nitrofuryl derivatives was possible because it is very well stabilized in this medium.

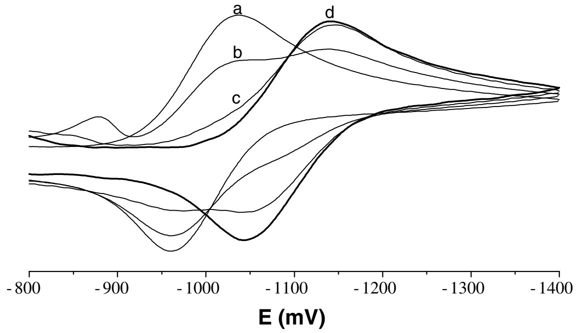


Fig. 3. Cyclic voltammograms of Compound C in DMF, 0.1 M TBAP at different concentrations of base added: *a* = 0, *b* = 0.3 M, *c* = 0.6 M, *d* = 1.0 M, of NaOH respectively. Sweep rate 1 V/s.

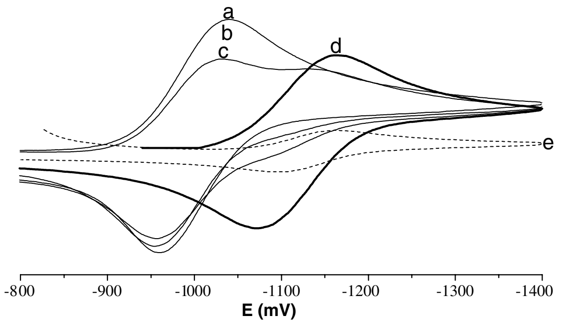
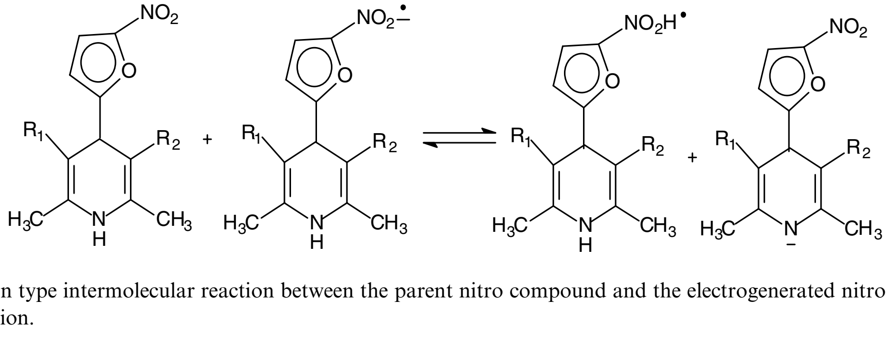


Fig. 4. Cyclic voltammograms of Compound A in DMF, 0.1 M TBAP tracking the time course of the CPE. Sweep rate 1 V/s. Electrolysis times: *a* = 0, *b* = 0.5, *c* = 1.0, *d* = 7.0 and *e* = 120 min, respectively.

On the other hand by comparison of the cyclic voltammograms after total conversion (Figs. 3(d) and 4(d)) we can observe that in the case of base mediated conversion (Fig. 3) the peaks of the voltammograms for parent compound and nitranion are the same height. However in the



Scheme 3. The father–son type intermolecular reaction between the parent nitro compound and the electrogenerated nitro radical anion generating the corresponding nitranion.

case of the electrochemical conversion (Fig. 4) the peaks height ratio between nitranion and parent compound reach a value of 0.6, near to a theoretical 0.5 value according to the proposed mechanism in Scheme 3.

In previous studies [16,17] a nitranion species was also postulated as a product of a father–son type reaction from some nitrobenzene derivatives, but never was macroscopically isolated and detected as with these nitrofuryl derivatives. Considering the previous studies and the above results obtained with this new synthesized nitrofuryl 1,4-DHP derivatives, is possible to extrapolate this type of reactivity to all type of compounds containing, in the same molecule, a group capable to be electroreducible to a radical anion and a mildly acid hydrogen.

4. Conclusions

Our results reveal that the presence of the 1,4-DHP substituent strongly affects the reduction of the nitrofuryl group. In fact more attracting groups in 3,5 positions of the 1,4-DHP favour the nitrofuryl moiety reduction indicating an inductive effect of the 1,4-DHP moiety on the nitrofuryl electroactive group. Furthermore the weakly acid character of the proton in the N1 position of the 1,4-DHP triggered the appearance of father–son type reactions between the electrogenerated nitro radical anion and the parent compound generating the corresponding nitranion.

The nitranion can be exhaustively produced by controlled potential electrolysis thus generating important synthetic and pharmacological consequences. From the synthetic point of view the generation of an stabilized nitranion open new possibilities of synthetic routes for obtaining N-substituted 1,4-DHP derivatives. On the other hand, from the pharmacological point of view this type of reactivity will affect both the generation and reactivity of the nitro radical anion with relevant consequences for the action mechanism of these potential new bioactive molecules.

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