Electrochimica Acta 125 (2014) 457–464

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

# Synthesis and electrochemical oxidation of hybrid compounds: dihydropyridine-fused coumarins

V. Pardo-Jiménez [a,b], C. Barrientos [a,b], K. Pérez-Cruz [a], P.A. Navarrete-Encina [b], C. Olea-Azar [c,1], Luis J. Nuñez-Vergara [a,b,c,2], J.A. Squella [a,*]

[a] Laboratorio de Bioelectroquímica, Universidad de Chile, Sergio Livingstone P. 1007, Santiago, Chile
[b] Laboratorio de Síntesis Orgánica y Modelamiento Molecular, Universidad de Chile, Sergio Livingstone P. 1007, Santiago, Chile
[c] Departamento de Química Inorgánica y Analítica. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Sergio Livingstone P. 1007, Santiago, Chile

ARTICLE INFO

*Article history:*
Received 26 July 2013
Received in revised form 25 January 2014
Accepted 27 January 2014
Available online 10 February 2014

*Keywords:*
Dihydropyridine-fused coumarins
Cyclic voltammetry
Oxidation
Spectroelectrochemistry
ESR.

ABSTRACT

In this paper, a series of six dihydropyridine-fused coumarins were synthesized and electrochemically characterized in dimethylformamide (DMF).

Dihydropyridine ring oxidation on glassy carbon electrode (GCE) for condensed heterocyclic compounds revealed a single anodic peak. Oxidation potential values correlated fairly well with substituent effects at 9-position. The overall oxidation mechanism involved 2-electrons and 2-protons as determined by chronoamperometry.

Controlled-potential electrolysis followed by UV-Visible spectroscopy proves that dihydropyridine-fused coumarins are electrochemically oxidized in DMF giving rise to the aromatic pyridine derivative. ESR experimental spectra show a triplet, due to the C-centered dihydropyridyl radical trapped with N-tert-butylamine-α-phenylnitrone (PBN). Hyperfine coupling constant values (*a*N) of dihydropyridine-fused coumarins were higher than corresponding values for non-fused ones. These results could be due to the effect of the coupling of the dihydropyridine moiety with the coumarin ring over the splitting constant.



## 1. Introduction

Within the wide field of pharmaceuticals compounds, 1,4-dihydropyridines (DHPs) are a class of drugs based on a pyridine core. They have an ample spectrum of biological and pharmacological actions. Their clinical success in the treatment of cardiovascular diseases, mainly arterial hypertension, are supported by its calcium-channel modulating actions [1]. Moreover, it has been demonstrated that DHPs could prove to be highly important as multidrug-resistance-reversing agents in cancer chemotherapy [2].

In recent publications [3–5] the use of the DHP motif as a scaffold in the synthesis of other more complex heterocyclic compounds with promissory pharmacological applications has been reported.

Described 1,4-DHPs exhibit a wide variety of structural modifications, but they must have some essential features for exhibiting pharmacological activity, i.e., they must have a secondary nitrogen atom on the dihydropyridine ring and substituents, including aromatic ones at 4-position [6].

Oxidation of 1,4-DHPs either by chemical reactions, photochemical decomposition, or electrochemical techniques has received much attention, because the biological and pharmacological activity of these compounds lies in the integrity of the dihydropyridine moiety [7–9]. Furthermore, the main metabolic pathway of dihydropyridine drugs in human beings involves the oxidation of the dihydropyridine ring to the pyridine derivative mediated by the P450 enzymes [10].

Electrochemical oxidation of 1,4-DHPs has been extensively studied in aprotic medium by our laboratory [11–21] and others [22–27]. These investigations were generally carried out by using mainly rotating ring disk electrode (RRDE), linear and cyclic voltammetry and ESR spectroscopy, and have revealed that in non-aqueous solutions, 1,4-dihydropyridine derivatives are oxidized in a two-electron and 2-protons reaction, involving an ECE mechanism.

Safak and Simsek [28] have reviewed the chemistry of fused-dihydropyridines, showing that the condensation modifies the pharmacological activity of these types of compounds. Thus, dual effects have been found, i.e. antagonistic-agonistic activities on the

* Corresponding author.
*E-mail addresses:* asquella@ciq.uchile.cl, arturosquella@gmail.com (J.A. Squella).
[1] Tel: 56 2 9782812
[2] The authors dedicated this paper to one of us, Luis J. Núñez-Vergara, who passed away on October 25th 2013 when this paper was in the revision process.

0013-4686/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.electacta.2014.01.137

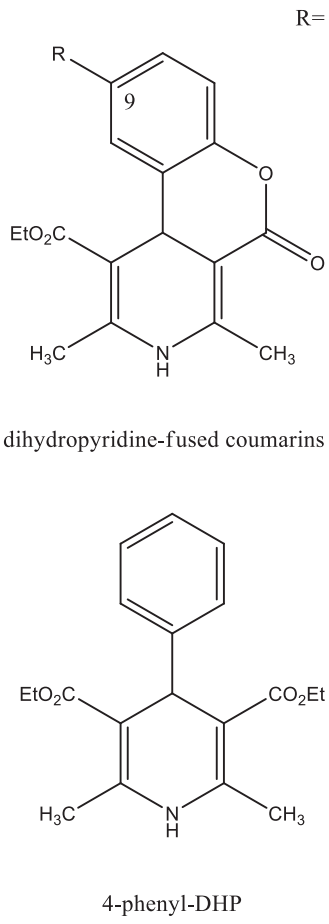


**Fig. 1.** - Chemical structures of studied compounds.

calcium channels, opening new avenues for its medical applications. To the best of our knowledge, this is the first study examining the effect of the condensation of dihydropyridines with coumarins on its electrochemical oxidation. Nevertheless, in a previous report [29], our laboratory established correlations between oxidation peak potential values and trypanosomicidal activity of some fused-dihydropyridines. Results demonstrated that these compounds had diminished inhibitory effects when compared with non-fused dihydropyridines.

In this paper we extended our interest on these types of molecules with the synthesis of a series of six dihydropyridine-fused coumarins with both electron withdrawing and electron-donor groups at 9-position which are electrochemically characterized on glassy carbon electrode in DMF.

Fig. 1

## 2. Experimental

### 2.1. Chemical structures of compounds are shown in Fig. 1.

All the new compounds were synthesized in accordance to previously reported procedures [30,31].

## 3. 4-phenyl-2,4-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine (4-ph-DHP)

$^1$HNMR (300 MHz, DMSO-d6): (δ) 1.16 (t, 6H, 2x -CH2CH3); 2.26 (s, 6H, 2x–CH3); 3.98 (q, 4H, 2x–OCH2CH3); 4.88 (s, 1H, ArCH); 7.18 (m, 3H, J= 6.975 Hz, 3x ArH) 7.22 (d, 2H, J= 8. 14 Hz, 2x ArH); 8.80 (s, 1H, NH). $^{13}$CNMR (75 MHz, DMSO-d6): 10.51; 9.26; 9.07; 7.88; 7.78; 6.18; 2.09; 2.05; 2.04; 1.97; 0.63. Elem. Anal. $C_{19}H_{23}NO_4$: Calc. C: 62.28; H: 7.04; N: 4.25. Found: C: 62.08; H: 6.98, N: 4.30. Yield: 58%.

2,4-dimethyl-coumarin[3,4-c]dihydropyridine-1-carboxylic acid ethyl ester (H-CDHP): $^1$HNMR (300 MHz, DMSO-d6): (δ) 1.10 (t, 3H, -CH3, J= 7.2); 2.00 (s, 3H, -CH3); 2.25 (s, 3H, -CH3); 4.09 (m, 2H, -CH2); 4.75 (s, 1H, -CH); 6.74-7.24 (m, 4H, -ArH); 8.97 (s, 1H, -NH). $^{13}$CNMR (75 MHz, DMSO-d6): (δ) 13.4; 15.7; 17.7; 32.6; 58.6; 95.; 96.6; 115.7; 123.1; 123.4; 126.5; 131.7; 145.0; 147.1; 149.4; 163.9; 166.7. Elem. Anal. $C_{17}H_{17}NO_4$: Calc. C: 68.22; H: 5.73; N: 4.68. Found: C: 68.49; H: 5.70; N: 4.70. Yield: 34%.

9-methoxy-2,4-dimethyl-coumarin[3,4-c]dihydropyridine-1-carboxylic acid ethyl ester (MeO-CDHP):

$^1$HNMR (300 MHz, DMSO-d6): (δ) 1.05 (t, 3H, -CH3, J= 7.2); 2.62 (s, 3H, -CH3); 2.68 (s, 3H, -CH3); 3.76 (s, 3H, -OCH3); 4.17 (q, 2H, -CH2, J= 7.2); 6.89-7.36 (m, 3H, -ArH); 8.27 (s, 1H, -NH). $^{13}$CNMR (75 MHz, DMSO-d6): (δ) 14.2; 19.4; 19.5; 47.5; 55.2; 60.1; 96.2; 101.4; 110.8; 11.9; 116.2; 117.7; 145.6; 146.7; 147.5; 155.3; 157.9; 165.3. Elem. Anal. $C_{18}H_{19}NO_5$: Calc. C: 65.64; H: 5.82; N: 4.25. Found: C: 65.90; H: 5.79; N: 4.24. Yield: 26%.

9-nitro-2,4-dimethyl-coumarin[3,4-c]dihydropyridine-1-carboxylic acid ethyl ester ($NO_2$-CDHP):

$^1$HNMR (300 MHz, DMSO-d6): (δ) 1.10 (t, 3H, -$CH_3$, J= 7.2); 2.04 (s, 3H, -$CH_3$); 2.30 (s, 3H, -$CH_3$); 4.12 (m, 2H, -$CH_2$); 4.87 (s, 1H, -CH); 7.32-8.17 (m, 3H, -ArH); 9.14 (s, 1H, -NH). $^{13}$CNMR (75 MHz, DMSO-d6): (δ) 14.5; 17.0; 19.0; 33.9; 60.0; 94.0; 96.8; 118.2; 120.2; 127.9; 133.3; 143.8; 147.9; 149.1; 155.2; 163.4; 167.3. Elem. Anal. $C_{17}H_{16}N_2O_6$: Calc. C: 59.30; H: 4.68; N: 8.14. Found: C: 59.54; H: 4.70; N: 8.10. Yield: 14%.

2,4,9-trimethyl-coumarin[3,4-c]dihydropyridine-1-carboxylic acid ethyl ester (Me-CDHP)

$^1$HNMR (DMSO-$d_6$): (δ) 1.10 (*t*, 3H, $CH_3$-$CH_2CO_2$, J= 6.8 Hz), 2.25 (*s*, 3H, $CH_3$-Arom), 2.0 (*s*, 3H, 2.24, $CH_3$-DHP), 2.23 (*s*, 3H, 2.24, $CH_3$-DHP), 4.15 (*m*, 2H, $CH_3$-$CH_2CO_2$), 4.70 (*s*, 1H, 4H-DHP), 8.85 (*s*, 1H, NH-DHP), 6.97 (*m*, 3H, H-Arom). $^{13}$CNMR (DMSO-$d_6$): 14.9, 17.16, 19.19, 21.46, 34.14, 60.00, 97.03, 97.90, 116.89, 125.19, 128.18, 132.77, 133.44, 146.10, 148.48, 148.78, 165.61, 168.07.Elem. Anal. $C_{18}H_{18}NO_3$: Calc. C: 72.95, H: 6.12, N: 4.73. Found: C: 72.85, H: 6.13, N: 4.75. Yield: 10%.

9-chloro-coumarin[3,4-c]dihydropyridine-1-carboxylic acid ethyl ester(Cl-CDHP)

$^1$HNMR (DMSO-$d_6$): (δ) 1.11 (*t*, 3H, $CH_3$-$CH_2CO_2$, J= 6.86 Hz), (δ) 2.0 (*s*, 3H, $CH_3$-DHP), 2.27 (*s*, 3H, $CH_3$-DHP), 4.10 (*m*, 2H, $CH_3$-$CH_2CO_2$), 4.75 (*s*, 1H, 4H-DHP), 9.04 (*s*, 1H, NH-DHP), 6.96 (*m*, 3H, H-Arom). $^{13}$CNMR (DMSO-$d_6$): (δ) 13.53, 15.93, 18.02, 32.93, 58.82, 94.44, 95.96, 117.86, 123.40, 126.52, 127.24, 133.70, 145.84, 148.01, 148.25, 163.49, 166.48. Elem. Anal. $C_{17}H_{15}NO_3Cl$: Calc. C: 64.46, H: 4.77, N: 4.42. Found: C: 64.60, H: 4.76, N: 4.44. Yield: 14%.

9-bromo-coumarin[3,4-c]-dihydropyridine -1-carboxylic acid ethyl ester (Br-CDHP)

$^1$HNMR (300 MHz, CDCl3-d1) 1.37 (t, 3H, -CH3, J = 7.13), 2.62 (s, 3H, -CH3), 2.73 (s, 3H, -CH3),4.42 (q, 2H, -CH2-, J = 7.15), 7.15 (m, 3H, Ar-H). $^{13}$CNMR (CDCl3-d1) d 13.2, 18.3, 22.7, 61.1, 112.8, 116.5, 117.3, 119.5, 127, 131.2, 134, 148.9, 149.9, 150.4, 158.2, 159.8, 168.5. Elem. Anal. $C_{17}H_{14}BrNO_4$: Calc. C: 54.27; H: 3.75; N: 3.72. Found: C: 54.20; H: 3.76; N: 3.71. Yield: 14%.

### 3.1. Electrochemical Characterization

#### 3.1.1. Electrolytic medium

Dimethylformamide (DMF) containing 0.1 M tetrabutylammonium hexafluorophosphate (TBAHFP).

*3.1.2. Voltammetry.*

Cyclic voltammetry (CV) and dp voltammetry were performed with a CH Instrument 760 C assembly. A stationary glassy carbon electrode was used as working electrode (0.07 $cm^2$). The surface of the electrode was polished to a mirror finish with alumina powder (0.3 μm and 0.05 μm) and washed with Milli-Q water before use and after each measurement. Platinum wire was used as auxiliary electrode and all potentials were measured against a non-aqueous Ag/AgCl (Filling solution: DMF and 0.1 M tetrabutylammonium hexafluorophosphate, TBAHFP) in saturated KCl. Potentials *vs* SCE (-0.045 V).

Current concentration of studied compounds was 5 mM solutions.

*3.1.3. Chronoamperometry*

Measurements were carried out on a CH Instrument 760 C assembly. 3 mM concentration of derivatives in DMF + 0.1 M TBAHFP were used for the experiments. A glassy carbon ultramicrolectrode (10 μm diameter) was employed as working electrode. The number of transferred electrons (n) was determined from the Cottrell equation by the ultramicroelectrode technique based on Baranski work [28]. Derivatives were dissolved in DMF + 0.1 M TBAHFP and the net charges were calculated and corrected for the background current.

*3.2. Spectroelectrochemistry*

These experiments were conducted using 1-5 mM solutions of compounds in DMF to characterize the intermediates produced during the electrolysis of the electroactive groups. All UV–Vis spectra were recorded in the 200–1000 nm range by using an Agilent spectrophotometer with diode array. A 0.1 ml quartz spectroelectrochemical cell (140A CH Instrument, path length: 1 mm) with three electrodes: Pt gauze as a working electrode (CH Instrument 011547), non-aqueous Ag/AgCl (Filling solution: DMF and 0.1 M tetrabutylammonium hexafluorophosphate, TBAHFP) in saturated KCl (CH Instrument 010249) and Pt wire as counter electrode (CH Instrument 011548) were used.

*3.3. ESR characterization*

ESR spectra were recorded in the X band (9.85 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. ESR spectra of the radical derivatives were obtained in the electrolysis solution. The ESR spectra were simulated using the program WINEPR Simphonia 1.25 Version. Electrolysis was performed in the ESR cell by using a platinum mesh electrode at 1 mM dihydropyridine solutions in the presence of 100 mM N-tert-butylamine-α-phenylnitrone (PBN, Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA), which was used as spin trap.

## 4. Results and Discussion

The electrochemical oxidation in DMF of a series of dihydropyridine-fused coumarins substituted at 9-position was studied to establish both the effects of the condensation of dihydropyridine (DHP) moiety with a coumarin and substituent effects on the oxidation process. The mechanism of the oxidation of the DHP moiety to the pyridine derivative will be further supported by different experimental approach such as spectroelectrochemistry and ESR experiments.

*4.1. Cyclic voltammetry*

Cyclic voltammograms (CV) corresponding to the studied compounds at a sweep rate of 0.1 $Vs^{-1}$ are shown in Fig. 2A. As can be seen, the oxidation of the DHP moiety is of irreversible character, exhibiting a single oxidation signal (I) probably due to the well-known oxidation of DHP to the corresponding pyridine derivative. In the reverse sweep, all compounds exhibited a well defined cathodic peak (II) at approximately–0.8 V. For compounds Cl-CDHP and MeO-CDHP a very little signal (II′) appears before the cathodic peak II. The presence of peaks II and II′ is a consequence of the previous oxidation corresponding to peak I because when the cathodic sweep was started at potentials more negative than 1.0 V no reduction peaks II and/or II′were observed. Consequently peaks II and II′ are due to the reduction of the previously formed pyridine derivative.

It is a well known chemical fact that reduction of pyridine derivatives to dihydropyridines yields both 1,2-DHP and 1,4-DHP where 1,2-DHP is the kinetically favored product whereas in the equilibrium the 1,4 DHP is the predominant form because of their greater stability [32a,b]. Therefore we assumed that both DHPs could be formed in the electrochemical reversal sweep starting from pyridine derivative and we assigned the peaks in consequence. Peak II is formed due to pyridine reduction to 1,2-dihydropyridine whereas II′ is formed due to pyridine reduction to the 1,4-dihydropyridine. As the formation of 1,2-DHP (peak II) is kinetically favored the formed quantity of 1,4-DHP (signal II′) is much smaller and difficultly or not detectable by cyclic voltammetry. Signal III (corresponding to the oxidation of 1,2-DHPs derivatives to pyridine) appears at less positive potential than the original signal I (corresponding to the oxidation of 1,4-DHPs to pyridine). This result is in agreement with a previous work of Ogle *et al.* [23]. They have described that 1,2-dihydropyridine derivatives were oxidized more easily (by 200–250 mV) than the corresponding 1,4-dihydropyridines.

Furthermore, the $NO_2$-CDHP derivative exhibits a cathodic peak at -0.47 V in the same anodic region of potential (Fig. 2B), which corresponds to the reduction of the nitro group from the nitropyridine derivative (peak IV) previously generated by the oxidation of the initial nitro-dihydropyridine.

On the other hand, peaks II and II' are related to peak I for all the derivatives excluding $NO_2$-CDHP derivative, since scans from 0 V to negative potentials up to -1.5 V did not show any peaks in this cathodic region (Fig. 2C, I). Cathodic scans in the -1.0 V to -2.25 V region for the compounds are thoroughly described in reference [33]. However, in the case of $NO_2$-CDHP derivative a cathodic scan in this same region shows a reversible couple at -1.0 V (Fig. 2 C, II). This corresponds to the reduction of the nitro group to the nitro radical anion and its further oxidation.The difference between the reduction potentials for the nitro group of the nitro-pyridine (-0.47 V) and the nitro-DHP (-1.0 V) is due to the electronic delocalization towards the pyridine ring, diminishing the electronic density on the nitro group and therefore facilitating its reduction.

On the other hand, in all the cases, log *Ivs* log $\nu$ plots exhibited slopes close to 0.5, indicating that no adsorption processes are involved. Peak potentials values (Ep) were dependent on the sweep rates confirming the irreversibility of the oxidation process. In Table 1 the peak potential values according to CV of Fig. 2 A are thoroughly described for each compound.

*4.2. Differential Pulse Voltammetry (DPV).*

It is a well known matter that the selectivity of voltammetric method depends on the difference of peak potential, with a minimum difference of 0.2–0.3 V for a linear potential scan

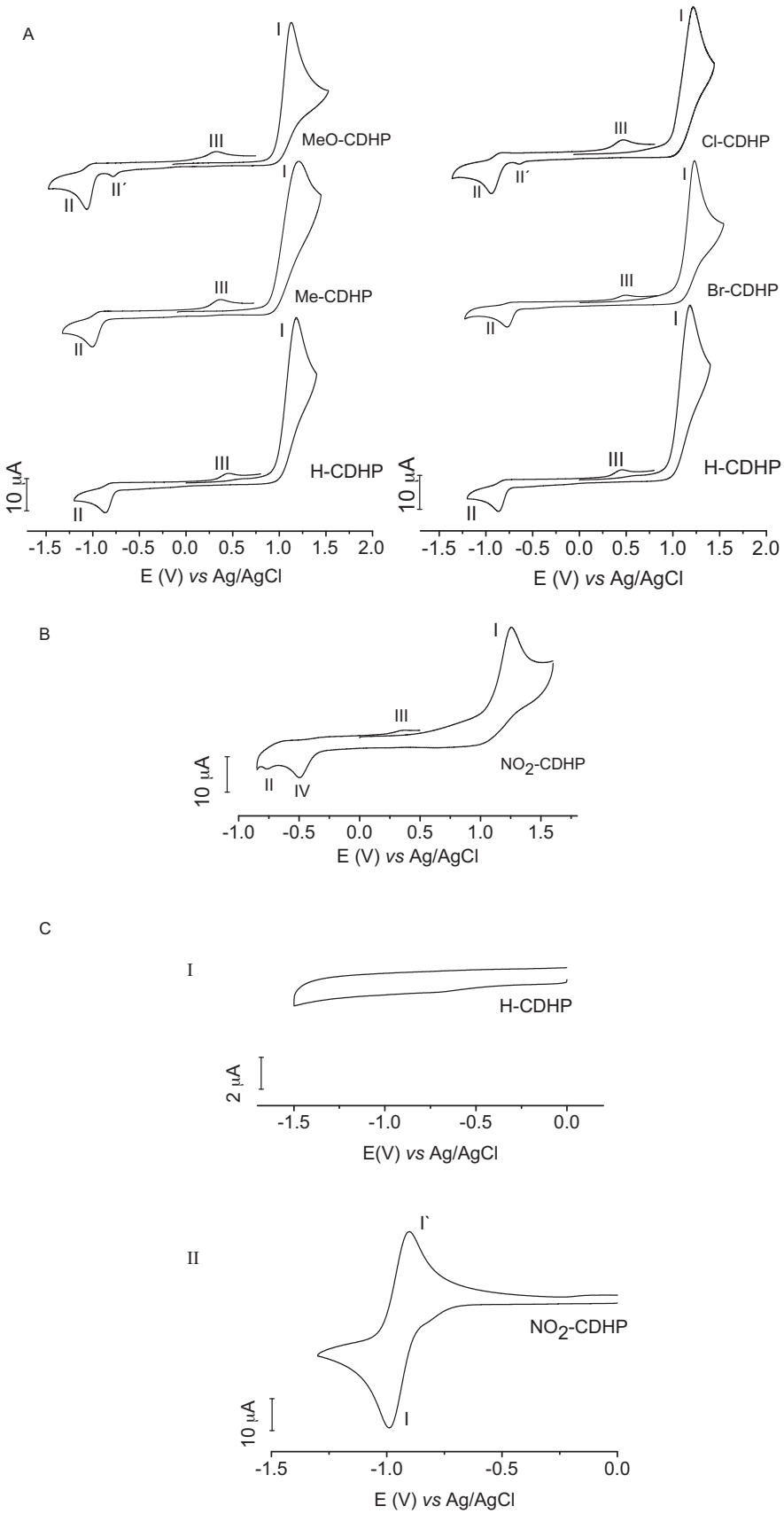


**Fig. 2.** - Cyclic voltammograms in DMF + 0.1 M TBAHFP of: **(A)** H-CDHP, Cl-CDHP, Br-CDHP, Me-CDHP, MeO-CDHP derivatives at 0.1 V/s sweep rate. **(B)** $NO_2$-CDHP derivative at a sweep rate of 0.3 V/s. Concentration: 5 mM. **(C).-** Cyclic voltammograms in DMF + 0.1 M TBAHFP at a sweep rate of 0.1 V/s of: (I) H-CDHP at sweep rate of 0.1 V/s. (II) $NO_2$-CDHP derivatives. Concentration: 5 mM.

**Table 1**
Potential values (V) *vs* Ag/AgCl obtained from cyclic voltammetric experiments for dihydropyridine-fused coumarins measured at a 0.1 $Vs^{-1}$ sweep rate.

| Derivative | $E_I$ | $E_{II}$ | $E_{II}'$ | $E_{III}$ |
|---|---|---|---|---|
| $NO_2$-CDHP | 1.14 | ----- | ---- | ---- |
| Br-CDHP | 1.19 | -0.76 | ---- | 0.48 |
| Cl-CDHP | 1.16 | -0.83 | -0.54 | 0.52 |
| H-CDHP | 1.18 | -0.86 | ---- | 0.46 |
| Me-CDHP | 1.18 | -0.86 | ---- | 0.41 |
| MeO-CDHP | 1.13 | -0.83 | -0.57 | 0.42 |
| 4-ph-DHP[1] | 1.15 | -1.08 | ----- | 0.43 |

[1] Non-fused dihydropyridine

**Table 2**
Oxidation peak potential values determined by dp voltammetry on glassy carbon electrode in dimethylformamide + 0.1 M TBAHFP.

| Derivative | + Ep/(V)[1] | ΔEp/(mV)[2] |
|---|---|---|
| $NO_2$-CDHP | 1.08 | +50 |
| Br-CDHP | 1.09 | +63 |
| Cl-CDHP | 1.05 | +16 |
| H-CDHP | 1.03 | ---- |
| Me-CDHP | 1.01 | -20 |
| MeO-CDHP | 1.00 | -30 |
| 4-ph-DHP | 1.08 | +50 |

[1] Values *vs* Ag/AgCl
[2] Potential value differences respect the H-CDHP derivative

and 0.04–0.05 V for DPV. Consequently, accurate oxidation potential values were obtained with this technique using a glassy carbon electrode as working electrode. In Table 2, anodic peak potential values are shown for the studied compounds. Electron-withdrawing groups (-Cl, -Br, -$NO_2$) shifted the oxidation potentials values towards more positive potentials compared with H-CDHP derivative. In contrast, electron-donating groups (-$CH_3$, -$OCH_3$) facilitates the oxidation process. In consequence, the easiness oxidation rank order is as follows: MeO-CDHP > Me-CDHP > H-CDHP > Cl-CDHP > $NO_2$-CHDP > Br-CDHP. We realized that Br-CDHP has an oxidation value greater than expected. Up to this moment we have no clear explanation for this fact. Moreover there is a fairly good correlation between Hammett $\sigma_p$ values with signal I oxidation potentials when excluding the bromine substituted compound as is shown in Fig. 3.

Fused DHPs have similar oxidation potential values than non-fused 4-phenyl substituted DHP. In this latter compound, the phenyl ring bounded at 4-position lies in a plane that is bisecting the DHP ring (Fig. 4A). On the other hand, the synthesized

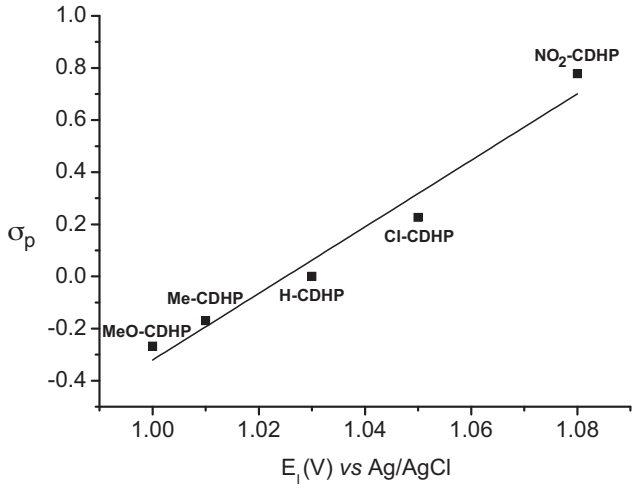


**Fig. 3.** - Hammett $\sigma_p$ constants correlation with $E_I$ (V) *vs* Ag/AgCl values.

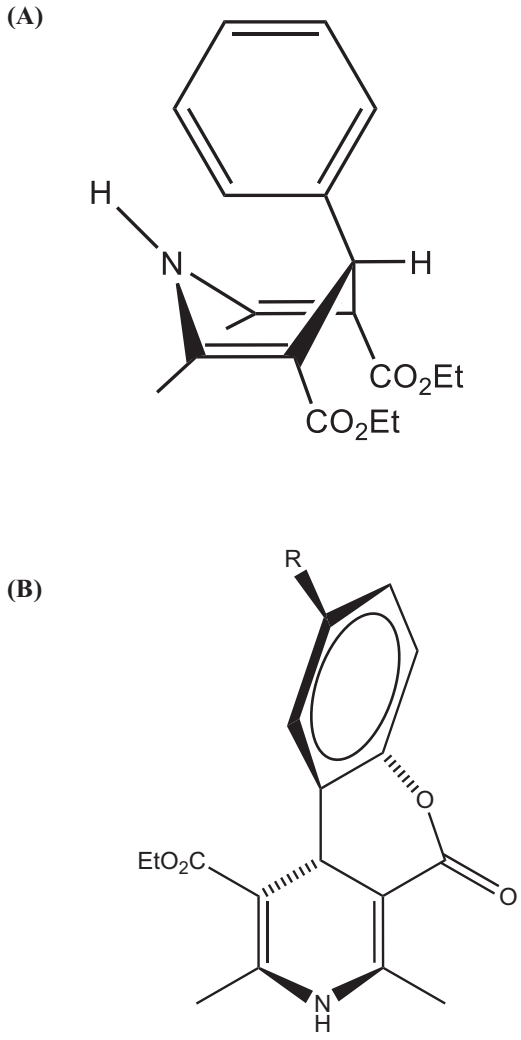


**Fig. 4.** - Molecular geometry of: (A) phenyl-substituted dihydropyridines (B) dihydropyridine-fused coumarins.

fused-DHPs adopt more or less the same conformation having the plane of the phenyl ring close to the plane bisecting the DHP ring (Fig. 4B) [34–38]. This particular conformation permits that only inductive effects of the phenyl ring acting as a whole affect oxidation potentials values of the DHP ring. Only small variations on the oxidation potential values were observed for both kinds of compounds (Table 1).

By the way and interestingly, the calcium channel antagonist activity of this type of compounds was found to increase as the rings approached the perpendicular orientation [39].

### 4.3. Chronoamperometry

Results obtained from this technique for the number of electrons transferred in the oxidation process and diffusion coefficients are shown in Table 3. The average of the number of electrons transferred was $1.98 \pm 0.01$ confirming that the overall oxidation process involved two-electrons. Concerning with the diffusion coefficient values it is observed that are within the same-scale, but showing higher values for compounds containing electron-donor groups at 9-position.

### 4.4. Spectroelectrochemistry

Controlled-potential electrolysis of different dihydropyridine-fused coumarins was carried out in anhydrous acetonitrile in a UV

**Table 3**
Diffusion coefficients and number of electrons (n) dihydropyridine-fused coumarins calculated by chronoamperommetry technique.

| Derivative | Diff. Coeff. [$cm^2s^{-1}$] | n |
|---|---|---|
| H-CDHP | $5.78{\cdot}10^{-6} \pm 1.11{\cdot}10^{-7}$ | 1.8 |
| Me-CDHP | $4.90 \cdot 10^{-6} \pm 0.90{\cdot}10^{-7}$ | 1.8 |
| MeO-DHP | $5.18{\cdot}\ 10^{-6} \pm 2.11{\cdot}10^{-7}$ | 2.2 |
| Br-CDHP | $0.74{\cdot}10^{-6} \pm 1.8{\cdot}10^{-8}$ | 1.9 |
| Cl-CDHP | $0.91{\cdot}\ 10^{-6} \pm 1.08{\cdot}10^{-7}$ | 2.1 |
| $NO_2$-CDHP | $1.38 \cdot 10^{-6} \pm 1.07{\cdot}10^{-7}$ | 2.1 |

cell on a platinum mesh electrode and spectra were recorded at short time intervals during the electrolysis.

Electrolysis of compounds was performed at a potential of +1.35 V *vs* Ag/AgCl. Changes in the UV-Visible spectra can be summarized as follows: a) The original UV-Visible band at 378-379 nm decreased in parallel with the electrolysis time supporting that the compounds concentration is decreasing to give product(s). b) The appearance of two new UV-Visible bands at 262-265 nm and a second one at 313-327 nm was noted. c) An isosbestic point varying between 329-343 nm was observed in all spectra, indicating that the reaction is a simple transformation without concurrent reactions. In Fig. 5, differential UV-Visible spectra corresponding to Me-CDHP and Cl-CDHP derivatives are shown. From these data, we can conclude that the UV-Visible band at 262-265 nm could be assigned to the pyridine derivative formation as was previously

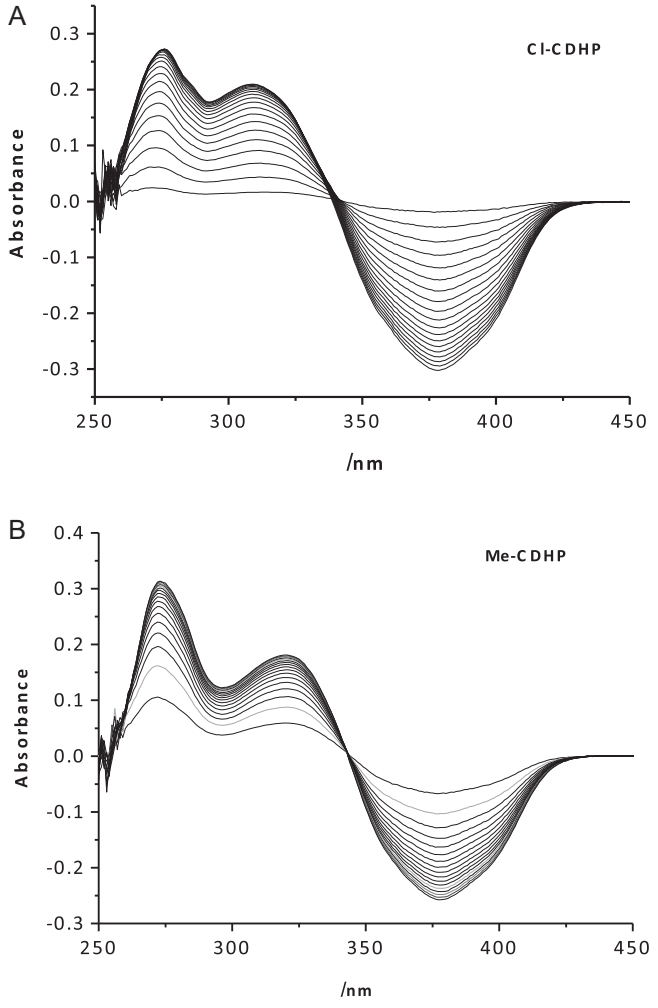


**Fig. 5. -** Differential UV-Visible spectra for the CPE corresponding to some 1 mM dihydropyridine-fused coumarins. Total time: 180 s. Electrolysis potential: +1.35 V. Electrolytic medium: DMF + 0.1 M TBAHFP.

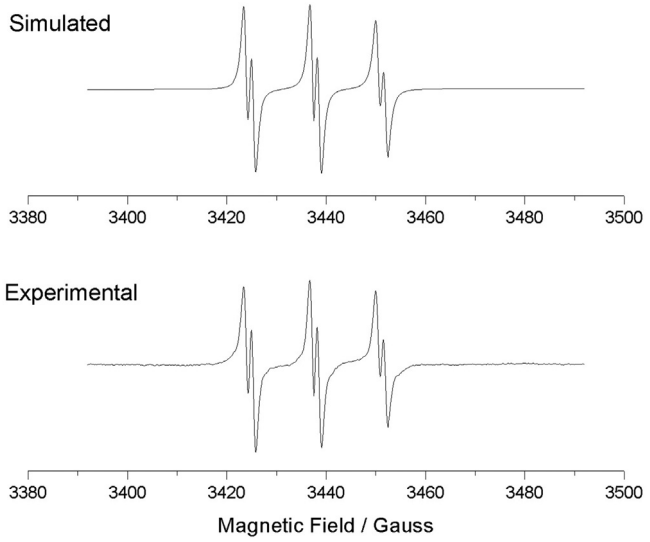


**Fig. 6. -** Simulated and experimental ESR spectra of adduct PBN/dihydropyridyl radical electrochemically generated from 1 mM H-CDHP solution in DMF/0.1 M TBAHFP. PBN concentration: 100 mM.

demonstrated by Nuñez-Vergara *et al.* [40–42] and Labudzinska *et al.* [43].

### 4.5. ESR results

Spin trapping experiments were done to characterize the intermediates produced by the electrolysis of dihydropyridines-fused coumarins. ESR spectra show a characteristic nitrogen triplet and their splitting into doublets due to vicinal hydrogen. The corresponding aN values varied between 13.2-17.6 G. aH splitting constant values for the same compounds are in the range 2.6-3.5 G. Fig. 6 shows the experimental and simulated ESR spectrum corresponding to H-CDHP derivative. Simulated spectra are in good agreement with the corresponding experimental ones. *a*N values, obtained through PBN capture of the radicals, for dihydropyridine-fused coumarins are higher than that of non-fused ones (see Table 4). This fact may be explained because radicals captured by PBN have lesser freedom degree and therefore their spin density are mainly located on the nitrogen atom of the spin trap used.

In conclusion, even in the case of dihydropyridine-fused coumarin derivatives, the results correspond well to the classic mechanism of electrochemical oxidation of dihydropyridines, which can be exemplified by the following simple ECEC scheme:

$$H_2Py\text{-}e^- = H_2Py^{*+}$$

$$H_2Py^{*+}\text{-}H^+ = HPy^*$$

**Table 4**
Experimental hyperfine coupling constant ($a_N$, $a_H$) for the radicals C-centered corresponding dihydropyridine-fused coumarins and some non-fused coumarins.[1].

| Derivative | $a_N$, Gauss | $a_H$, Gauss | *g*-value |
|---|---|---|---|
| Cl-CDHP | 17.6 | 2.60 | 2.018 |
| Br-CDHP | 17.0 | 3.50 | 2.013 |
| $NO_2$-CDHP | 16.2 | 3.40 | 2.015 |
| H-CDHP | 13.5 | 2.60 | 2.023 |
| MeO-CDHP | 14.1 | 3.10 | 2.019 |
| Me-CDHP | 13.2 | 2.80 | 2.023 |
| Amlodipine[1] | 13.5 | 2.60 | 2.030 |
| 4-(3-hydroxyphenyl)-DHP[1,2] | 12.1 | 2.80 | 2.028 |
| 4-phenyl-DHP[1,2] | 13.2 | 3.50 | 2.045 |

[2]Ref. 18.

$HPy^{*}\text{-}e^{-} = HPy^{+}$

$HPy^{+}\text{-}H^{+} = Py$

*Concluding Remarks*

- A series of dihydropyridine-fused coumarins with both electron-attracting and electron-withdrawing groups at 9-position were synthesized.
- Regarding with the electronic effect on the potentials, the rank order of easiness oxidation is as follows: MeO-CDHP > Me-CDHP > H-CDHP > Cl-CDHP > $NO_2$-CHDP > Br-CDHP.
- The electrochemical oxidation of dihydropyridine-fused compounds in DMF occurs involving 2-protons and 2-electrons with formation of the dihydropyridyl radical intermediate, which was trapped with PBN. These results were supported by chronoamperometry and ESR experiments, respectively.
- The restrained rotation of the structure of synthesized fused-dihydropyridines, influenced the *a*N hyperfine splitting constant values. Thus, experimental values were higher than that of non-fused dihydropyridines. In contrast, *a*H hyperfine splitting constant values did not show significant changes compared with non-fused dihydropyridines.
- The pyridines obtained as oxidation products were evidenced by the UV-Visible absorption at 260-265 nm after spectroelectrochemical experiments.

## Acknowledgements

This work was supported by Grant from Project Fondecyt 1110039.

## References

[1] J. David, Triggle, Calcium channel antagonists: Clinical uses, past, present and future, Biochem. Pharmacol 74 (1-9) (2007).
[2] N. Edraki, A.R. Mehdipour, M. Khoshneviszadeh, R. Miri, Dihydropyridines: evaluation of their current and future pharmacological applications, Drug Dis. To Day 14 (2009) 1058–1066.
[3] R. Lavilla, Recent developments in the chemistry of dihydropyridines, J. Chem. Soc. Perkin Trans 2 (2002) 1141–1144.
[4] H. Wu, W. Lin, Y. Wan, R. Yuan, H. Xin, R. Bo, D. Shi, W. Yin, Silica Gel-Catalyzed One-Pot Syntheses in Water and Fluorescence Properties Studies of 5-Amino-2-aryl-3H-chromeno[4,3,2-de]-[1,6]naphthyridine-4-carbonitriles and 5-Amino-2-aryl-3H-quinolino-[4,3,2-de][1,6]naphthyridine-4-carbonitriles, J. Comb. Chem 12 (2010) 31–34.
[5] W. Zhang, X. Yang, W. Chen, X. Xu, L. Li, H. Zhai, Z.Li. Design, Multicomponent Synthesis, and Bioactivities of Novel Neonicotinoid Analogues with 1,4-Dihydropyridine Scaffold, J. Agric. Food Chem 58 (2010) 2741–2745.
[6] G.C. Rovnyak, S.D. Kimball, B. Beyer, G. Cucinotta, J.D. DiMarco, J. Gougoutas, A. Hedberg, M. Malley, J.P. McCarthy, R. Zhang, S. Moreland, Calcium Entry Blockers and Activators: Conformationaland StructuralDeterminants of Dihydropyrimidine Calcium Channel Modulators, J. Med. Chem 38 (1996) 119–129.
[7] D.J. Triggle, D.A. Langs, R.A. Jamis, Ca2+ channel ligands: structure-function relationships of the 1,4-dihydropyridines, Med. Res. Rev 9 (1989) 123–180.
[8] M. Morad, Y.E. Goldman, D.R. Trentham, Rapid photochemical inactivation of Ca2 + -antagonists shows that Ca2+ entry directly activates contraction in frog heart, Nature (London) 304 (1983) 635–638.
[9] B. Loev, M.M. Goodman, K.M. Snader, R. Tedeschi, E. Macko, Hantzsch-type dihydropyridines hypotensive agents, J. Med. Chem 17 (1974) 156–165.
[10] R. Böcker, F. Guenguerich, Oxidation of 4-Aryl- and 4-Alkyl-Substituted 2,6-Dimethyl-3,5-bis(alkoxycarbony1)-1,4-dihydropyridines by Human Liver Microsomes and Immunochemical Evidence for the Involvement of a Form of Cytochrome P-450, J. Med. Chem 29 (1986) 1596–1603.
[11] A. Alvarez-Lueje, L.J. Núñez-Vergara, J.A. Squella, Voltammetric Behaviour of 1,4-Dihydropyridines Calcium Antagonist, Electroanalysis 6 (1994) 259–264.
[12] L.J. Núñez-Vergara, C. López-Alarcón, P.A. Navarrete-Encina, A.M. Atria, C. Camargo, J.A. Squella, Electrochemical and EPR characterization of 1,4-dihydropyridines, Reactivity towards alkyl radicals. Free Radical Res 37 (2003) 109–120.
[13] L.J. Núñez-Vergara, J.C. Sturm, A. Alvarez-Lueje, C. Olea-Azar, C. Sunkel, J.A. Squella, Electrochemical oxidation of 4-methyl-1,4-dihydropyridines in protic and aprotic media - Spin trapping studies, J. Electrochem. Soc 146 (1999) 1478–1485.
[14] C. López-Alarcón, L.J. Núñez-Vergara, J.A. Squella, Voltammetric oxidation of Hantzsch 1,4-dihydropyridines in protic and aprotic media: relevance of the substitution on N position, Electrochimica Acta 48 (2003) 2505–2516.
[15] R. Salazar, P.A. Navarrete-Encina, J.A. Squella, C. Barrientos, V. Pardo-Jiménez, L.J. Núñez-Vergara, Study on the oxidation of C4-phenolic-1,4-dihydropyridines and its reactivity towards superoxide radical anion in dimethylsulfoxide, Electrochimica Acta 56 (2010) 841–852.
[16] R. Salazar, P.A. Navarrete-Encina, J.A. Squella, J. Luis, L.J. Núñez-Vergara, Electrochemical oxidation of C4-vanillin- and C4-isovanillin-1,4-dihydropyridines in aprotic medium: Reactivity towards free radicals, J. Electroanal. Chem 622 (2008) 29–36.
[17] R. Salazar, P.A. Navarrete-Encina, C. Camargo, J.A. Squella, L.J. Núñez-Vergara, Oxidation of 4-(3-Indolyl)- and 4-(5-Indolyl)-1,4-dihydropyridines in Aprotic and Protic Media: Reactivity toward Alkylperoxyl Radicals, J. Electrochem. Soc 155 (2008) 103–108.
[18] L.J. Núñez-Vergara, R. Salazar, C. Camargo, J. Carbajo, B. Conde, P.A. Navarrete-Encina, J.A. Squella, Oxidation of C4-hydroxyphenyl 1,4-dihydropyridines in dimethylsulfoxide and its reactivity towards alkylperoxyl radicals in aqueous médium, Bioorg. Med. Chem 15 (2007) 4318–4326.
[19] L.J. Núñez-Vergara, R. Salazar, P.A. Navarrete-Encina, C. Camargo, C. Yáñez, J.A. Squella, Electrolytic oxidation of C4-nitrofuryl 1,4-dihydropyridines in non-aqueous médium, J. Electrochem. Soc 154 (2007) F25–F30.
[20] J. Arguello Da Silva, L.J. Núñez-Vergara, S. Bollo, J.A. Squella, Voltammetric redox behaviour of nitrofuryl 1,4-dihydropyridine derivatives: interdependence between two redox centers, J. Electroanal. Chem 591 (2006) 94–104.
[21] C. López-Alarcón, J.A. Squella, D. Miranda-Wilson, J. Luis, Núñez-Vergara, Spectroelectrochemical study on the electro-oxidation in aqueous media of some 1,4-dihydropyridines: effects of substitution in 1-position and 4-position, Electroanalysis 16 (2004) 539–546.
[22] J. Stradins, J. Ogle, V. Kadish, L. Baumane, R. Gavars, G. Duburs, Electrochemical oxidation of N-substituted 1,4- and 1,2-dihydropyridine derivatives: Studies using the rotating ring-disk electrode and ESR, J. Electroanal. Chem 226 (1987) 103–116.
[23] J. Ogle, J. Stradins, L. Baumane, Formation and decay of free-cation radicals in the course of electrooxidation of 1,2- and 1,4-dihydropyridines (Hantzsch esters), Electrochim. Acta 39 (1994) 73–79.
[24] J. Klima, J. Ludvik, J. Volke, M. Krikava, V. Skala, J. Kuthan, Spin trapping in electrochemical processes: Trapping of radical intermediates in the electrooxidation of substituted 1,4-dihydropyridines, J. Electroanal. Chem 161 (1984) 205–211.
[25] R. Toniolo, F. Di Narda, G. Bomtenpelli, F. Ursini, An electroanalytical investigation on the redox properties of lacidipine supporting its anti-oxidant effect, Bioelectrochemistry 51 (2000) 193–200.
[26] J. Ludvik, J. Klima, J. Volke, A. Kurdurst, J. Kuthan, Electrochemical oxidation of substituted 1,4-dihydropyridines in non-aqueous acetonitrile, J. Electroanal. Chem 138 (1982) 131–138.
[27] A. El Jammal, J.C. Vire, G.J. Patriarche, O. Nieto Palmeiro, Cyclic voltammetric study of some calcium antagonist dihydropyridines in aqueous médium, Electroanalysis 4 (1992) 57–64.
[28] C. Safak, R. Simsek, Fused 1,4-dihydropyridines as potential calcium modulatory compounds Mini-Rev, Med. Chem 6 (7) (2006) 747–755.
[29] J.D. Maya, A. Morello, Y. Repetto, R. Tellez, A. Rodríguez, U. Zelada, P. Puebla, E. Caballero, M. Medarde, L.J. Núñez-Vergara, J.A. Squella, M. Bontá, S. Bollo, A. San, Feliciano, Effects of 3-chloro-phenyl-1,4-dihydropyridine derivatives on Trypanosome cruziepimastigotes, Com. Biochem. Physiol 125C (2000) 103–109.
[30] R. Salazar, P.A. Navarrete-Encina, C. Camargo, J.A. Squella, J. Luis, L.J. Núñez-Vergara, Oxidation of 4-(3-indolyl)- and 4-(5-indolyl)-1,4-dihydropyridines in aprotic and protic media, Reactivity towards alkylperoxyl radicals. J. Electrochem. Soc 155 (2008) 103–108.
[31] P.A. Navarrete-Encina, R. Salazar, Ch. Vega-Retter, K. Pérez, J.A. Squella, J. Luis, L.J. Núñez-Vergara, On the One-Pot Syntheses of Chromeno[4,3-b]pyridine-3-carboxylate and Chromeno[3,4-c]pyridine-3-carboxylate and Dihydropyridines, J. Braz. Chem. Soc 21 (2009) 413–418.
[32] (a) Pyridine and its derivatives. Supplement part one. Edited by R.A. Abramovitch, University of Alabama, John Wiley & Sons. New York, London, Sydney, Toronto. Page 145 (1974);
(b) E.M.P. Silva, P.A.M. Varandas, A.M.S. Silva, Developments in the synthesis of 1,2-dihydropyridines (Review), Synthesis 45 (2013), A-AK.
[33] L.J. Nuñez-Vergara, V. Pardo-Jiménez, C. Barrientos, C.A. Olea-Azar, P.A. Navarrete-Encina, J.A. Squella, Dihydropyridine-fused and pyridine-fused coumarins: Reduction on a glassy carbon electrode in dimethyl formamide, Electrochimica Acta 85 (2012) 336–344.
[34] S.P. Gupta, QSAR Studies on Calcium Channel Blockers, Top Heterocycl. Chem. 4 (2006) 251–289.
[35] A.C. Gaudio, A. Korolkovas, Y. Takahata, Quantitative structure-activity relationships for 1,4-dihydropyridine calcium channel antagonists (nifedipine analogues): A quantum chemical/classical approach, J. Pharm. Sci. 83 (1994) 1110–1115.
[36] A.M. Triggle, E. Shefter, D.J. Triggle, Crystal Structures of Calcium Channel Antagonists: 2,6-Dimethyl-3,5-dicarbomethoxy-4-2[-nitro-, 3-cyano-, 4-(dimethylamino)-,and 2,3,4,5,6-pentafluorophenyl-1,4-dihydropyridine, J. Med. Chem. 23 (1980) 1442–1445.
[37] R. Fossheim, K. Svarteng, A. Mustad, C. Romming, E. Shefter, D.J. Triggle, Crystal structures of calcium channel antagonists: 2,6-dimethyl-3,5-dicarbomethoxy-4-[2-nitro-,3-cyano, 4-(dimethylamino)-, and 2,3,4,5,6-pentafluorophenyl]-1,4-dihydropyridine, J. Med. Chem. 25 (1982) 126–131.

[38] J.J. Baldwin, D.A. Clareman, P.K. Lumma, D.E. McClure, S.A. Rosentha, R.J. Linguist, E.P. Faison, G.J. Kaczorowski, M.J. Trumble, M.J.G.M. Smith, Diethyl 3,6-dihydro-2,4-dimethyl-2,6-methano-1,3-benzothiazocine-5,11-dicarboxylates as calcium entry antagonists: new conformationally restrained analogues of Hantzsch 1,4-dihydropyridines related to nitrendipine as probes for receptor-site conformation, J. Med. Chem. 30 (1987) 690–695.
[39] R.A. Janis, P. Silver, D.J. Triggle, Drug action and cellular calcium regulation, Adv. Drug. Res. 16 (1987) 309–591.
[40] L.J. Núñez-Vergara, R. Salazar, C. Camargo, J. Carbajo, B. Conde, P.A. Navarrete-Encina, J.A. Squella, Electrochemical characterization of C-4 hydroxyphenyl 1,4-dihydropyridines in aprotic medium and its reactivity towards alkylperoxyl radicals, Bioorg. Med. Chem. 15 (2007) 4318–4326.
[41] L.J. Núñez-Vergara, P.A. Navarrete-Encina, S. Salas, B. Conde, J. Carbajo, C. Camargo, J.A. Squella, Analyses by GC/MS and GC/MS-MS of the Hantzsch synthesis products using hydroxyl and methoxy-aldehydes, J. Pharm. Biomed. Anal. 44 (2007) 236–242.
[42] C. López-Alarcón, J.A. Squella, D. Miranda-Wilson, L.J. Núñez-Vergara, Spectroelectrochemical study on the electro-oxidation in aqueous medium of some 1,4-dihydropyridines: effects of substitution in 1-position and 4-position, Electroanalysis 16 (2004) 539–546.
[43] A. Labudzinska, K. Gorczynska, The UV difference spectra as a characteristic feature of phenols and aromatic amines, J. Molec. Struct 349 (1995) 469–472.