

Electrochemical oxidation of C4-vanillin- and C4-isovanillin-1,4-dihydropyridines in aprotic medium: Reactivity towards free radicals

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A B S T R A C T

This work reports the electrochemical oxidation of two new synthetic C4-vanillin and -isovanillin-1,4-dihydropyridines in aprotic medium. Its reactivity with alkylperoxyl radicals ABAP-derived at pH 7.4 is also studied. Voltammetry, coulometry, controlled-potential electrolysis, UV-visible spectroscopy and GC-MS techniques were employed to collect data that permitted us to study its oxidation. Effect of TBA-OH addition on the oxidation was electrochemically and spectroscopically followed. In aprotic medium, the oxidation mechanism involves the formation of the pyridine derivative, which was generated by controlled-potential electrolysis (CPE) at 1270 mV and identified by GC-MS technique as the final product of the electrolysis. Spectroelectrochemical experiments also support the formation of the pyridine derivative from the oxidation of both 1,4-dihydropyridines. Direct reactivity of synthesized compounds towards alkylperoxyl radicals ABAP-derived was determined. Results reveal that the inclusion of vanillin radical or its positional isomer, isovanillin in the 4-position of the dihydropyridine ring produced a significant positive effect on the reactivity towards alkylperoxyl radicals, even compared with commercial dihydropyridine drugs with a well-known antioxidant ability. Scavenging mechanism involves the electron-transfer and the formation of a pyridine derivative, which was identified by GC-MS.

Keywords:

C4-vanillin

C4-isovanillin-1,4-dihydropyridines

Oxidation

Voltammetry

GCE

GC-MS

Alkylperoxyl radicals

1. Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla, which is one of the most widely used and important flavoring material worldwide. In common with many other low-molecular weight phenolic compounds vanillin displays antioxidant properties [1–5]. Vanillin is an electroactive compound and it is possible to quantify its amount in vanilla and in the final products through the study of its oxidation. Thus, procedures based on electroanalytical methods, amperometry, differential pulse voltammetry (DPV) or square-wave voltammetry (SWV) for the detection of vanillin in food samples have been reported in the literature [6–8]. On the other hand, recently a disposable electrochemical sensor for vanillin detection based on screen-printed electrodes has been reported [9].

In the search of new 1,4-dihydropyridine derivatives (1,4-DHPs) with strengthened antioxidant effects, we have previously synthesized two new compounds, C4-vanillin-1,4-dihydropyridine and the corresponding positional isomer, C4-isovanillin-1,4-dihydropyridine [10].

Investigations on 1,4-DHP electrochemical oxidation have been previously performed in both aprotic and protic media. The more

detailed mechanistic information has been obtained in aprotic media [11–18]. These investigations were generally done 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 reaction, in particular, in an ECEC mechanism type. Also, our laboratory has previously contributed on the electrochemical characterization in aprotic and protic media of several commercial and new synthesized 1,4-DHPs [19–26]. In the present paper, the electrochemical oxidation of C4-vanillin-1,4-dihydropyridine and its positional isomer, C4-isovanillin-1,4-dihydropyridine in aprotic medium is reported. Results are compared with C4-phenyl-1,4-dihydropyridine. The reactivity of derivatives towards alkylperoxyl radicals ABAP-derived is also studied.

2. Experimental

2.1. Dihydropyridine derivatives

2.1.1. Synthesized compounds (Fig. 1)

They were synthesized in our laboratory according to a previously described synthesis procedure [10]. The following data correspond to physical and chemical characterization of the compounds.

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2.1.1.1. 2,6-Dimethyl-3,5-diethoxycarbonyl-4-phenyl-1,4-dihydropyridine (phenyl-DHP). This derivative was also synthesized and their physical and spectroscopic data were in accordance with previous results [27].

A mixture of 3.3 mmol of ethylacetoacetate and 5 mL concentrated ammonia hydroxide in 10 mL of ethyl alcohol was heated under reflux for 3 h. The resulting solution was added to a mixture of 3.3 mmol of ethylacetoacetate, 3.3 mmol of pure aldehydes (vanillin or isovanillin), 5 mL concentrated ammonia hydroxide and 5 mL ethyl alcohol and maintained under reflux for 15 h. The crude solid is filtered and recrystallized in ethyl alcohol.

2.1.1.2. 2,6-Dimethyl-3,5-diethoxycarbonyl-4-(4-hydroxy-3-methoxyphenyl)-1,4-dihydropyridine (V-DHP). Yield: 80.2%. m.p.: 161–163 °C. IR (KBr): ν_{\max} 3350.0; 2982.7; 1680.7; 1652.4; 1489.7; 1369.1; 1303.9; 1271.9; 1216.7; 1121.9; 753.3. ^1H NMR (300 MHz, DMSO- d_6): 1.1 (t, $J = 7.0$, 6H, 2x-CH₃); 2.2 (s, 6H, 2x-CH₃); 3.7 (s, 3H, OCH₃); 4.0 (q, $J = 7.0$, 4H, -CH₂); 4.82 (s, 1H, C-H); 6.60–7.19 (m, 3H, aromatic); 8.6 (s, 1H, N-H); 8.7 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO- d_6): 15.0 (-CH₃); 18.8 (-CH₃); 39.0 (Ar-CH₂); 56.2 (OCH₃); 60.0 (-CH₂); 103.3 (Cq); 113.2 (Cq); 116.0 (Cq); 120.5 (Cq); 131.0 (Cq); 140.5 (Cq); 145.7 (Cq); 147.6 (Cq); 169.0 (Cq) ppm. Anal. Calcd. for C₂₀H₂₅O₆N: C, 63.98; H, 6.71; N, 3.73. Found: C, 64.15; H, 6.73; N, 3.72.

2.1.1.3. 2,6-Dimethyl-3,5-diethoxycarbonyl-4-(3-hydroxy-4-methoxyphenyl)-1,4-dihydropyridine (I-DHP). Yield: 61.6%. m.p.: 166–168 °C. IR (KBr): ν_{\max} 3316.4; 2957.6; 1669.5; 1592.4; 1484.2; 1371.1; 1270.7; 1209.2; 1116.1; 1018.3; 764.8. ^1H NMR (300 MHz, DMSO- d_6): 1.2 (t, $J = 7.0$, 6H, -CH₃); 2.3 (s, 6H, -CH₃); 3.6 (s, 3H, OCH₃); 4.0 (q, $J = 7.0$, 4H, -CH₂); 4.75 (s, 1H, C-H); 6.81–7.30 (m, 3H, aromatic); 8.70 (s, 1H, N-H); 8.72 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO- d_6): 15.1 (-CH₃); 19.5 (-CH₃); 38.6 (Ar-CH₂); 58.2 (OCH₃); 60.0 (-CH₂); 102.9 (Cq); 112.4 (Cq); 116.0 (Cq); 118.5 (Cq); 133.5 (Cq); 142.2 (Cq); 146.1 (Cq); 148.1 (Cq); 167.0 (Cq) ppm. Anal. Calcd. for C₂₀H₂₅O₆N: C, 63.98; H, 6.71; N, 3.73. Found: C, 63.90; H, 6.73; N, 3.74.

2.1.2. Commercial drug

Nisoldipine was obtained as a gift from Chile Laboratories (Santiago, Chile).

2.2. Electrolytic media

Aprotic medium. Dimethylsulfoxide containing 0.1 mol L⁻¹ tetrabutylammonium hexafluoro phosphate (TBAHFP). Working concentrations of 1,4-DHPs varied between 0.1 mmol L⁻¹ and 2 mmol L⁻¹.

2.3. Voltammetry

Differential pulse (dpv), cyclic (cv) and linear sweep voltammetry (lsv) were performed with a BAS-CV 100 assembly. All voltammetric experiments were carried out with 1,4-DHP 1 mM solutions. A stationary glassy carbon electrode was used as working electrode for dpv and cv experiments. For hydrodynamic experiments, a rotating disk glassy carbon electrode was employed. The surface of the disk was polished to a mirror finish with alumina powder (0.3 mm and 0.05 mm) before use and after each measurement. Platinum wire was used as auxiliary electrode and all potentials were measured against an Ag/AgCl electrode in saturated KCl.

2.4. Coulometric analyses

Studies on exhaustive electrolysis were carried out during 2 h out at constant electrode potential (+900 and +1270 mV) on glassy

carbon mesh electrode by using 30 mL of solutions 1 mmol L⁻¹ of the compounds. A three-electrode circuit with a reference electrode Ag/AgCl and platinum wire as counter electrode were used. A BAS-CV 100 assembly was used to electrolyze the 1,4-DHPs solutions. Net charge was calculated including correction for the estimated background current.

2.5. UV-visible spectrophotometry

UV-visible spectra were recorded in the 200–1000 nm range by using an Agilent spectrophotometer with diode array.

2.6. Reactivity towards alkylperoxyl ABAP-derived radicals

ABAP (2,2'-azobis (2-amidinopropane) dihydrochloride, Aldrich Chemical Company) was used as radical generator. Different series of 20 mmol L⁻¹ ABAP solutions in 0.04 mol L⁻¹ Britton–Robinson buffer/DMF 70/30 pH 7.4 at a constant ionic strength of 0.1 mol L⁻¹ adjusted with KCl for alkylperoxyl radicals were incubated with 0.1 mmol L⁻¹ solutions of each 1,4-DHP or NADH at 37 °C for 120 min with constant bubbling of oxygen. The rate of alkylperoxyl radical formation from this initiator is constant at a given temperature [28]. But, the rate of radicals formation from ABAP will not be constant as it depends upon the concentration of ABAP (rate = $k[\text{ABAP}]$). It appears that, over 120 min at 37 °C, only a small amount of the ABAP will decay, therefore the concentration may be considered constant under these conditions. In neutral aqueous solutions, the half-life of ABAP is about 175 h, and the generation rate of radicals is constant for the first few hours [29]. Control solutions containing either 1,4-DHP or NADH solutions were run in the same conditions as the above mixtures. The time dependence of the reactivity of the synthesized 1,4-DHP derivatives with generated alkylperoxyl radicals was followed by UV/visible spectroscopy and GC/MS technique.

Pseudo first-order kinetic condition was used to determine kinetic rate constants for the reactivity between the 1,4-DHPs derivatives and alkylperoxyl radicals.

$$V = k \times [1,4\text{-DHP}] \times [\text{ROO}\cdot]$$

In pseudo first-order kinetic condition: $[1,4\text{-DHP}] \ll [\text{ROO}\cdot]$, therefore

$$V = k' \times [1,4\text{-DHP}], \quad \text{where } k' = k[\text{ROO}\cdot]$$

Reactivity towards alkylperoxyl radicals was expressed in comparison either with NADH or commercial 1,4-DHPs using the following ratio (r):

$$r = \frac{\text{Slope value DHP tested}}{\text{Slope value NADH or commercial 1,4-DHP}}$$

where the slope value is obtained from concentration-time plots in presence of free radicals. Control solutions (in the absence of ABAP-derived radicals) revealed no changes either in their original UV/visible absorption bands or GC-MS mass fragmentation. Also, a possible photodecomposition of 1,4-dihydropyridines was assessed, but in the time-scale of the experiments this was negligible.

2.7. GC-MS

A gas chromatograph/mass selective detector (5890/5972) combination (Hewlett-Packard, Palo Alto, CA, USA) and a Hewlett-Packard 7673 autosampler were used for the analyses. The m/z range monitored was 45–550 with a scan rate of 1 scan/s; the normal energy electron was set at 70 eV. A Hewlett-Packard Ultra-1 column, 25 cm \times 0.2 mm i.d. \times 0.11 mm film thickness (Little Falls, Wilmington, DE, USA), was used.

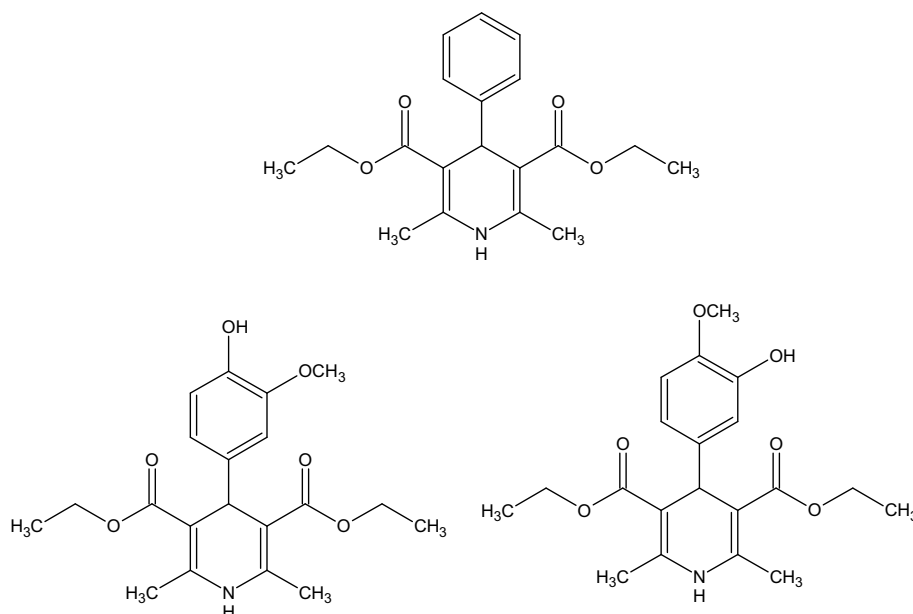


Fig. 1. Chemical structures of C4 vanillin-1,4-dihydropyridine (V-DHP), C4-isovanillin-1,4-dihydropyridine (IV-DHP) and 4-phenyl-1,4-dihydropyridine (4-Ph-DHP).

3. Results and discussion

3.1. Electrochemical oxidation in aprotic medium (DMSO + 0.1 mol L⁻¹ tetrabutylammonium hexafluorophosphate)

3.1.1. Voltammetry

Differential pulse voltammetry (dpv) results revealed that V-DHP exhibited two well-defined anodic peaks at potentials of +824 mV and +1118 mV (Fig. 2, Table 1). Oxidation peak potential values corresponding to I-DHP were shifted towards more positive values compared with V-DHP, i.e. $E_{p1} = +884$ mV and $E_{p2} = +1174$ mV, respectively (Table 1). These oxidation peaks can be assigned based on the following considerations: (a) 4-phenyl-DHP derivative, having no OH or -OCH₃ groups, experiments only the oxidation of the dihydropyridine ring, which occurs at +1064 mV in this aprotic medium (Fig. 2, insert, Table 1) and (b) 2-methoxyphenol having no DHP ring, was oxidized at +950 mV,

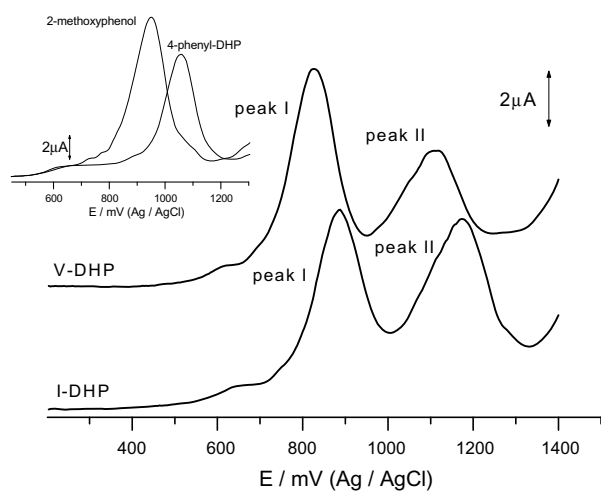


Fig. 2. Differential pulse voltammograms (DPV) of 1 mmol L⁻¹ solutions V-DHP and I-DHP in dimethylsulfoxide + 0.1 mol L⁻¹ TBAHFP at insert: dpv of 1 mmol L⁻¹ 4-phenyl-1,4-DHP (I) and 2-methoxyphenol.

which corresponds to the oxidation of the OH group (Fig. 2, insert, Table 1). Consequently, the oxidation peaks shown in Fig. 2 can be assigned as follows: those appearing at lower potential values belong to the oxidation of the OH group and the remaining ones correspond to the oxidation of DHP ring.

Changes in the oxidation potential values of V-DHP and I-DHP compared with the unsubstituted compounds just mentioned, evidence a shift to lower oxidation values for the OH group, and, higher ones for the oxidation of the DHP ring. The results above-summarized are consistent with the combined electron-donating effects of both the methoxyl group and the dihydropyridine ring on the OH group oxidation. A plausible explanation in the case of I-DHP for the shifting of the oxidation potential corresponding to the OH group towards more anodic values, could reside in the hydrogen-bonding formation between OH group and carbonyl group of ester. In the case of V-DHP, the position the OH group does not allow this possibility. In Fig. 2 this effect is illustrated, V-DHP exhibits a lower oxidation potential than I-DHP ($\Delta E_p = 60$ mV). On the other hand, in the first step the hydroxyl group is oxidized to give rise to a phenoxyl radical and further a quinone derivative [30,31] which affects the subsequent oxidation of the DHP ring, producing a shift toward higher oxidation potential values. Coulometric studies on the first oxidation peak revealed an average number of transferred electrons of 1.3 ± 0.2 . In Table 1 oxidation peak potential values and ΔE_p of different derivatives in aprotic medium are summarized.

Table 1
Oxidation peak potential values on glassy carbon electrode in aprotic medium (DMSO + 0.1 M HFPTBA)

Derivative	E (mV) (Ag/AgCl)			
	Peak I	ΔE_p -OH ^a	Peak II	ΔE_p -NH ^b
V-DHP	824	126	1118	54
I-DHP	884	66	1174	110
4-Phenyl-DHP	-	-	1064	-
2-Methoxyphenol	950	-	-	-

^a Differences of oxidation peak potential values of OH group with respect to 2-methoxyphenol derivative.

^b Differences of oxidation peak potential values of 1,4-dihydropyridine ring with respect to 4-phenyl-DHP derivative.

3.2. Cyclic voltammetry

These experiments were carried out at different sweep rates ranging from 0.1 up to 4 V/s. Under these conditions anodic signals were irreversible as can be seen in Fig. 3. Plots of $\log i_p$ vs. $\log v$ exhibited slopes close to 0.5 for both signals indicating that currents were diffusion-controlled. On the other hand, oxidation potential values were dependent on the sweep rates supporting the irreversible character of the oxidation of the compounds in this medium [32].

3.3. Hydrodynamic voltammetry

In Fig. 4, typical voltammograms on graphite rotating disk electrode corresponding to 1 mM solutions of V-DHP and I-DHP are shown. Both derivatives exhibit two oxidation waves (I and II). The half-wave oxidation potential values at 500 rpm for V-DHP were I = +848 mV, II = +1,160 mV; and for I-DHP derivative were I = +851 mV, II = +1157 mV. 4-Phenyl-DHP exhibits only a single oxidation wave at +1051 mV, which corresponded to the oxidation of the dihydropyridine ring. In consequence, wave I corresponds to the oxidation of the hydroxyl group and wave II to the oxidation of the dihydropyridine ring.

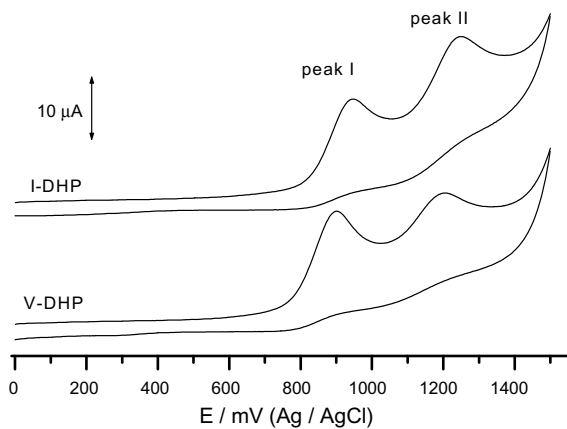


Fig. 3. Cyclic voltammograms of 1 mmol L⁻¹ V-DHP and I-DHP in dimethylsulfoxide + 0.1 M TBAHFP.

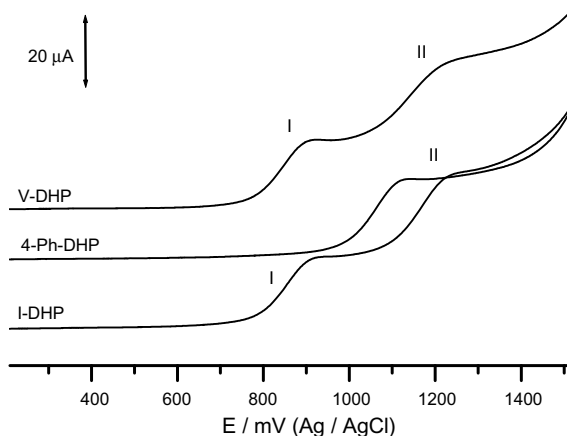


Fig. 4. Typical hydrodynamic voltammetry on glassy carbon rotating disk electrode in aprotic medium of 1 mmol L⁻¹ solutions of: I-DHP, V-DHP and 4-phenyl-DHP.

Furthermore, comparison of the height of limiting currents corresponding to wave II for 1 mM solutions of 4-phenyl-DHP, V-DHP and I-DHP did not show significant differences between them. This supports the fact that the oxidation process involved a similar number of transferred electrons, and also that it was diffusion-controlled one.

The relationship between the limiting currents and the rotation rate square root was linear for both V-DHP and I-DHP, according to Bard and Faulkner [33]. From these plots, the following diffusion coefficients were calculated: V-DHP = $3.7 \pm 0.1 \times 10^{-6}$ (cm² s⁻¹) and I-DHP = $3.2 \pm 0.2 \times 10^{-6}$ (cm² s⁻¹). As can be seen, no significant differences were found between the derivatives.

3.4. Influence of acid-base equilibrium on the electrochemical oxidation of the V-DHP and I-DHP

The effect of acidity or basicity on the electrochemical response is summarized in Fig. 5. A typical dp voltammogram corresponding to the oxidation of V-DHP derivative in the aprotic medium is shown in Fig. 5a. As can be seen, this derivative shows two oxidation signals at +884 mV and +1174 mV. The addition of a 1 mmol L⁻¹ solution of tetrabutylammonium hydroxide (TBA-OH) on the oxidation of V-DHP is depicted in Fig. 5b. The original signal at +1174 mV did not change but in parallel the signal at +884 mV decreased and a new signal appears at approximately 0 mV. This latter signal would correspond to the oxidation of the phenolate anion (R-O⁻) generated by the addition of TBA-OH, which is oxidized easier than the original phenol (R-OH) due to its negative charge density. As expected increasing the amount of TBA-OH (Fig. 5c-e) produce the complete disappearance of signal +887 mV concomitantly with an intensity maximum of the signal corresponding to the phenolate anion at 0 mV (Fig. 5e).

Finally, addition of 4 mmol L⁻¹ HClO₄ to solution (e) (Fig. 5f) completely reversed the electrochemical response, i.e. the original signal at +884 mV was recovered. On the other hand, if 4 mmol L⁻¹ HClO₄ solution is added to the original solution of V-DHP, no changes are evidenced. A similar result was obtained in the case of I-DHP. These effects can be explained by changes in the acid-base equilibrium of phenol in 4-position, according to the following scheme for V-DHP:

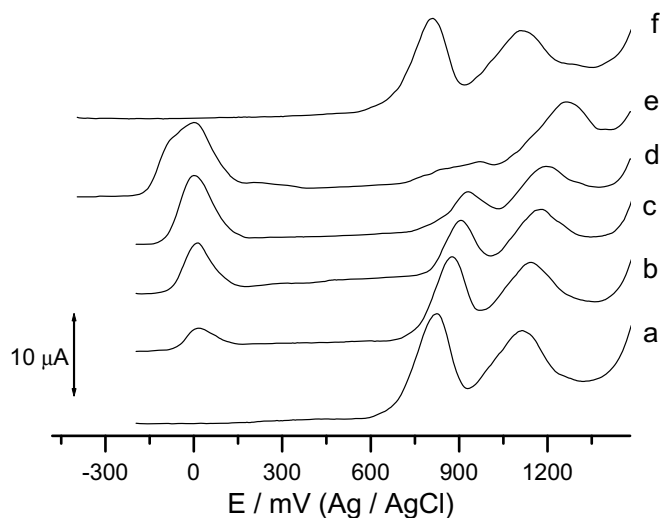
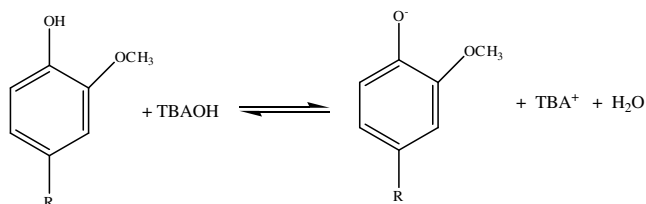


Fig. 5. (a) DPV of 1 mmol L⁻¹ V-DHP in DMSO + 0.1 mol L⁻¹ TBAHFP (b) solution (a) + 1 mmol L⁻¹ TBA-OH (c) solution (a) + 2 mmol L⁻¹ TBA-OH (d) solution (a) + 3 mmol L⁻¹ TBA-OH (e) solution (a) + 4 mmol L⁻¹ TBA-OH (f) solution (e) + 4 mmol L⁻¹ HClO₄.



3.5. UV-visible spectroscopic studies

In non-aqueous medium, V-DHP and I-DHP exhibit two absorption bands at $\lambda = 291$ nm and at $\lambda = 354$ nm (Fig. 6A). In order to investigate the effect of the acid–base equilibria on the UV-visible spectra of the molecules, TBA-OH was also employed. From these experiments, we calculated the molar absorption coefficients of the phenol (R-OH) and phenolate anion (R-O⁻), which appear in Table 2. Thus, the addition of 0.7 mmol L⁻¹ TBA-OH on the solution of 0.1 mmol L⁻¹ of I-DHP produced the disappearance of the original UV band at 354 nm in parallel with the appearance of a new visible band at $\lambda = 443$ nm, which corresponds to the phenolate anion (Fig. 6B). The addition of 0.7 mmol L⁻¹ non-aqueous HClO₄ produced the completely recovery of the original band at $\lambda = 354$ nm as shown in Fig. 6C. Similar results were obtained for V-DHP. In consequence, this result supports the contention that the UV-visible absorption changes observed after the addition of the base TBA-OH corresponds to changes in the acid–base equilibrium of the phenol group. This result is consistent with the previous interpretation on the effects of acid–base equilibrium on the electrochemical response.

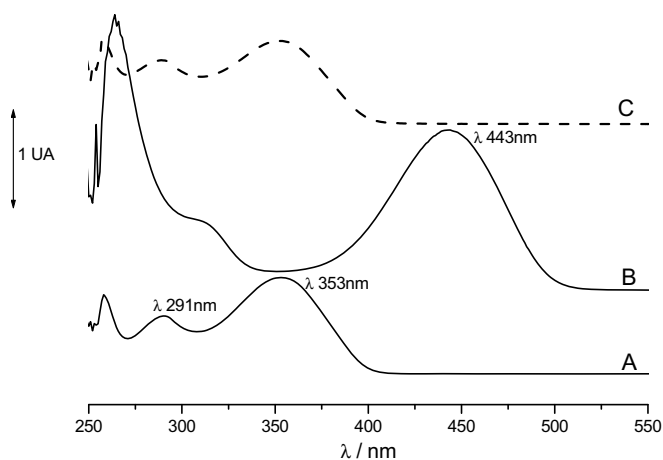


Fig. 6. UV-visible spectra of 0.1 mmol L⁻¹ solution of I-DHP (A) original signals in DMSO (B) solution in (A) +0.7 mmol L⁻¹ TBA-OH (C) solution in (B) +0.7 mmol L⁻¹ HClO₄.

Table 2
Absorption molar coefficients (ϵ) of synthesized derivatives in DMSO

Derivative	ϵ (cm ⁻¹ M ⁻¹)	
	Protonated R-OH ($\lambda = 354$ nm)	Deprotonated R-O ⁻ ($\lambda = 443$ nm)
2-Methoxyphenol	4629 ± 7 ($\lambda = 279$ nm)	8442 ± 11 ($\lambda = 315$ nm)
V-DHP	6701 ± 18	12,920 ± 155
I-DHP	7103 ± 3	11,095 ± 140

3.6. Controlled-potential electrolysis (CPE) experiments

Exhaustive controlled-potential electrolysis on reticulated carbon electrode to oxidize the 1,4-DHP solutions to generate the final products was used. The potential applied for V-DHP and I-DHP were +900 for the first anodic signal and +1270 mV for the oxidation of both electroactive groups. For 4-phenyl DHP, the potential applied were +1200 mV. The electrolyzed solutions were analyzed by GC-MS in order to identify such oxidation product (s). Also, spectroelectrochemical experiments were carried out to follow the electrolysis.

3.7. GC-MS studies

The fragmentation patterns of both synthesized V-DHP and I-DHP were previously described by us [34].

For the chromatographic analyses of electrolyzed 1,4-DHP solution products, it was essential to derivatize them with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) previous to the injection into the chromatograph to improve their chromatographic characteristics. The results obtained after 120 min of CPE are illustrated in Fig. 7 for I-DHP. A typical mass spectrum of this compound is displayed in this figure. Three most abundant mass fragments containing the pyridine nucleus were found. These fragments had m/z 445, m/z 415 and m/z 384, respectively. Consistently, the most abundant fragment was m/z 445 (M⁺), which corresponds to the oxidation of the 1,4-dihydropyridine moiety to give rise to the pyridine derivative. The mass fragment with m/z 415 corresponds to the cleavage to the methoxy group of the aromatic ring and m/z 384 to the simultaneous cleavage of methoxy group and an ethyl group corresponding to the lateral branch. The percentage of formed pyridine during the electrolysis for both compounds was around 90%. Summarizing, the results obtained by the GC-MS technique allow us to do the identification of the pyridine derivatives as the final products of the electrolytic oxidation of 1,4-dihydropyridines by applying an oxidation potential of +1270 mV. Coulometric studies on the second oxidation peak revealed an average of transferred electrons of 3.5 ± 0.3 . In conclusion, the oxidation of the second redox center (dihydropyridine ring) involves 2-electrons and 2-protons giving pyridine as the final product of oxidation.

3.8. Spectroelectrochemical experiments

These experiments were conducted to determine either unstable intermediates or final products during the *in situ* controlled-potential electrolysis by changes on the UV-visible spectra during 120 min.

The application of an oxidation potential of +900 mV close to the first anodic peak did not produce any noticeable change in the spectrum of I-DHP. In contrast, applying an anodic potential close to the second oxidation peak, i.e. +1270 mV (Fig. 8) produces a significant decrease in the absorption of the original band at 354 nm with electrolysis time. In parallel, an increase in the absorptions at 270 nm and 303 nm is observed. Insert of Fig. 8 shows the differential UV-visible spectra of I-DHP as a function of electrolysis time. As can be seen, the appearance of two new bands at approximately 270 nm and 303 nm were found. The UV band absorption at 270 nm corresponds to the oxidized derivative, i.e. the pyridine derivative, which is in agreement with previous observations [27,34–36]. The results of the spectroelectrochemical studies for V-DHP were very similar. Clearly from these results, during controlled-potential electrolysis no unstable intermediates were detected. However, the new signal at 270 nm corresponds to the pyridine derivative formation.

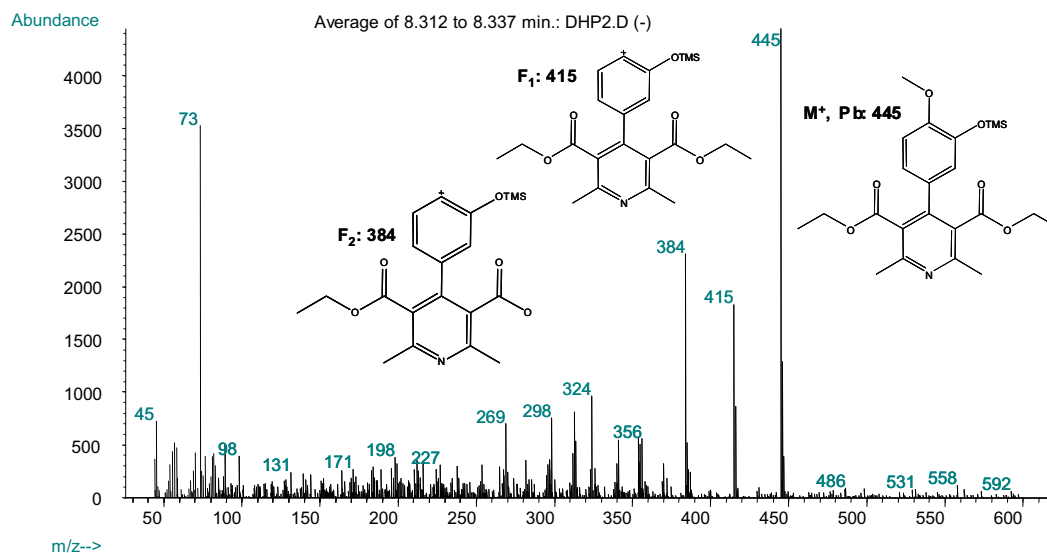


Fig. 7. Mass fragmentation spectrum corresponding to the oxidized I-DHP after 120 min of electrolysis. Applied potential = +1270 mV

3.9. Reactivity of vanillin-DHP and isovanillin-DHP towards alkylperoxyl radicals ABAP-derived in aqueous buffer at pH 7.4

Several authors have documented both *in vivo* [37,38] and *in vitro* [39,40] experiments towards the antioxidant effects of some 1,4-dihydropyridines in addition to their cardiovascular effects.

Based on these antecedents we decide to perform the synthesis of new 1,4-dihydropyridines containing vanillin and isovanillin at 4-position, which are well-known as antioxidants [41,42,2].

The coexistence of two redox centers in the same molecule could produce mutual interactions both in the electrochemical response and reactivity towards radical species. The electrochemical results above-described partially confirm this assumption. Both substituents vanillin and isovanillin were easier oxidized than the unsubstituted compounds on a glassy carbon electrode. In contrast, the oxidation of the dihydropyridine ring occurred at more anodic potential values compared with the unsubstituted moiety. Thus, the participation of both groups in the reactivity toward free radicals is expected.

Below we summarized the results on the reactivity of the compounds with alkylperoxyl radicals ABAP-derived. This model must be conducted in aqueous medium to resemble some of the experimental conditions in which the reaction occurs in the organism.

Oxidative stress is involved in a number of pathologies including the two major causes of death, namely cancer and atherosclerosis. Lipid and DNA oxidation and their protection by antioxidants have received much interest. On the other hand, oxidative damage to proteins may be of particular importance *in vivo*, since the loss of protein function may affect the activity of enzymes [43], receptors, and membrane transporters, among others [44]. In the past years, it has become evident that oxidative processes also take part in Alzheimer disease [45] and other neurodegenerative diseases [46,47]. Within oxygen-derived free radicals, peroxy radicals play an important role in the development of several pathologies, being involved in many radical chain reactions, for example lipid peroxidation and protein damage [48]. In addition, in the present paper we test the reactivity of V-DHP and I-DHP with alkylperoxyl radicals ABAP-derived in aqueous medium pH 7.4. The reactivity was followed by UV-visible spectroscopy and GC-MS techniques.

Time dependence on the reaction of alkylperoxyl radicals and 1,4-DHP derivatives was followed by changes in the original UV absorption band at $\lambda = 360$ nm. The results revealed that after the

addition of 1,4-DHP derivatives to an aqueous mixture containing alkylperoxyl radicals, the absorption band decreased along time (Fig. 9A and B), parallel with the appearance of new bands at 280 nm (V-DHP) and 276 nm (I-DHP). These bands correspond to the oxidized derivative, i.e. the pyridine derivative, which agrees with previous observations [27,35,36]. Reaction rate exhibited a linear dependence with concentration in the range $20\text{--}120 \times 10^{-6} \text{ mol L}^{-1}$ concentration of 1,4-DHPs. Analysis of reaction solutions by GC-MS after 120 min revealed that the most abundant fragments were m/z 445, m/z 415 and m/z 384 thus supporting the fact that after the reaction, the 1,4-DHPs were oxidized to the corresponding pyridine derivative.

To compare the reactivity, kinetic rate constants of tested 1,4-DHP derivatives and kinetic rate constants of the corresponding reference compounds (NADH and nisoldipine) were used

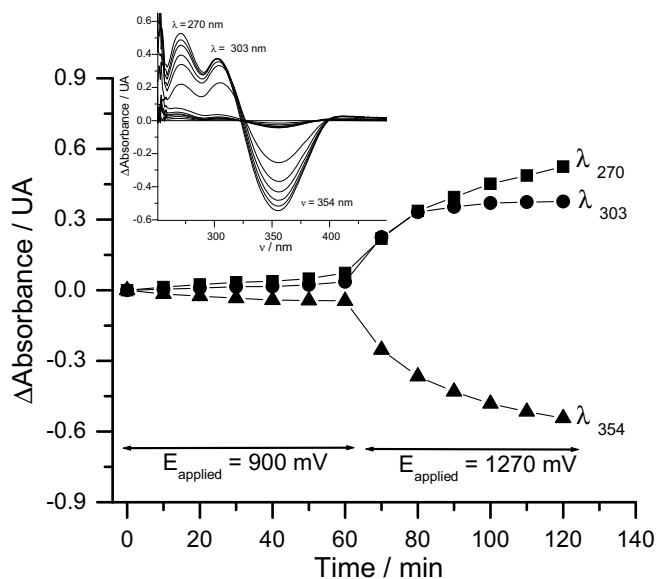


Fig. 8. UV-visible differential spectra of the time dependence on controlled-potential electrolysis at an applied potential of +1270 mV of a $1 \times 10^{-4} \text{ mmol L}^{-1}$ V-DHP solution followed during 120 min. Inset: Electrolysis time-course of the species in solution at two different oxidation potentials.

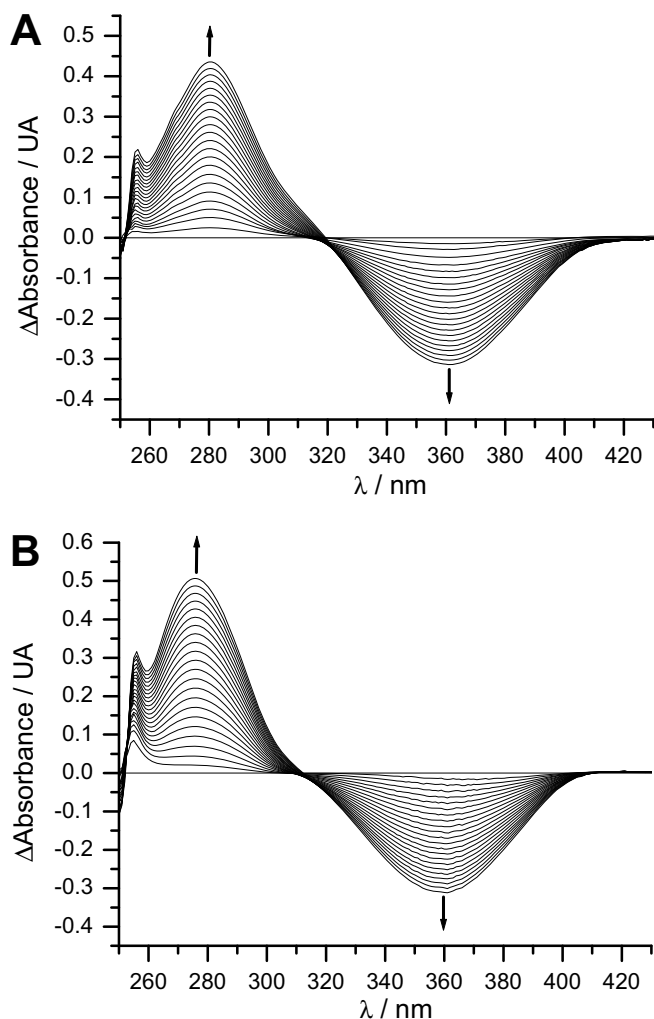


Fig. 9. UV-visible differential spectra of V-DHP (A) and I-DHP (B) for the reaction between alkylperoxyl radicals ABAP-derived followed during 120 min.

Table 3

Kinetic rate constants for the reaction between C4-substituted 1,4-DHPs and alkylperoxyl radicals ABAP-derived in Britton-Robinson buffer 0.04 M/ethanol; 70/30 at pH 7.4 at 37 °C

Derivative	$k/10^6/s^{-1}$ ^a	$r/\text{Nisoldipine}$ ^b	r/NADH ^c
4-Phenyl-DHP	5.1 ± 0.3	2.4	0.17
V-DHP	5.7 ± 0.1	2.7	0.15
I-DHP	5.1 ± 0.2	2.4	0.13
Nisoldipine	2.1 ± 0.1	1	0.06
NADH	38.0 ± 0.1	18.1	1

^a Kinetic rate constants assuming a first-order kinetic for the reaction between 1,4-DHPs and alkylperoxyl radicals.

^b Ratio between the kinetic rate constants for 1,4-DHPs/kinetic rate constant for nisoldipine for the reactivity with alkylperoxyl radicals.

^c Ratio between the kinetic rate constants for 1,4-DHPs/kinetic rate constant for NADH for the reactivity with alkylperoxyl radicals.

(Table 3). Clearly, the synthesized 1,4-DHP derivatives were significantly more reactive than nisoldipine, a commercial 1,4-DHP used in therapeutics. In conclusion, all the synthesized compounds were at least 2.4 times more reactive than this well-known antioxidant drug (Table 3). However, the most reactive compound was NADH, an endogenous compound structurally related with 1,4-DHPs.

At present, despite the lack of experimental evidences on the participation of the OH group in the scavenging reaction, this can-

not be discarded. Furthermore, the electrochemical results that show the easiness of oxidation of OH group support a significant role in the reactivity.

4. Concluding remarks

1. Electrochemical oxidation of V-DHP and I-DHP in aprotic medium occurs in both redox centers, i.e. the one-electron oxidation involving the phenol at dihydropyridine 4-position oxidation to give the corresponding phenoxyl radical and derived products. Oxidation of the dihydropyridine ring involves 2-protons and 2-electrons to give the pyridine derivative as a final product.
2. A direct quenching of alkylperoxyl radicals ABAP-derived by the new synthesized 1,4-DHPs (vanillin-DHP and isovanillin-DHP) was found.
3. The pyridine derivative was identified as the final product of the electrolysis at an applied potential of 1270 mV. Also, the pyridine derivative was found as product of the reaction between alkylperoxyl radicals and the tested 1,4-DHPs.

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