Substituent Effects on the Electrochemistry and Photostability of Model Compounds of Calcium Channel Antagonist Drugs

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We have synthesized three nitrocompounds in order to mimic the model structure of some relevant drugs such as nifedipine, nitrendipine, nisoldipine, nimodipine, and related ones, in order to investigate the effect of structural change on its electrochemical and photodegradation properties. We have found that ortho substitution with a nitro group produces a distortion of the coplanar arrangement, thus decreasing the resonance interaction between the nitro group and the aromatic system. A consequence of this fact is the observed cathodic peak potential shift toward negative values in the ortho-substituted isomer. It is observed that the position of the nitro substitution also produced differences in the photostability of the studied compounds. Stability studies of the compounds in buffered solutions (pH 7) stored in room light conditions show that only the ortho derivative was unstable in the time scale of the experiment (2 h). The instability of the ortho derivative may be explained if we consider that in this isomer, the photoexcited state of the nitro group is not stabilized by resonance as a consequence of the loss of coplanarity between the nitro group and the aromatic ring. Using molecular modeling, we have found that the configuration of minimal energy shows torsion angles of 70, 7.5, and 2.2° for ortho- meta- and para-derivatives, respectively, confirming the lost of coplanarity between the nitro group and the aromatic ring in the ortho derivative.

The 4-(nitrophenyl) substituted 1,4-dihydropyridines (1,4DHP) as nifedipine, nitrendipine, nisoldipine, nimodipine, and related compounds, have received major attention because of their relevant applications in various cardiovascular diseases.[1-3]

The electrochemistry of 1,4DHP has been extensively studied in the last few years. These molecules contain two different redox centers, a nitro group capable of electroreduction, and a dihydropyridine moiety capable of electro-oxidation. Investigations dealing with the electrochemical oxidation of some DHPs have previously been performed in both nonaqueous and aqueous[4-9] media but the investigations are mostly related with the electroreduction of the nitroaromatic group.[10-16] The presence of the nitro group in their structures is not essential for pharmacological activity but it confers to them relevant electronic and toxicological characteristics. From the biotransformation point of view, the nitro group can undergo several reactions and can lead to toxic metabolites, *i.e.*, nitro radical anion, hydroxylamine, and nitroso derivatives.

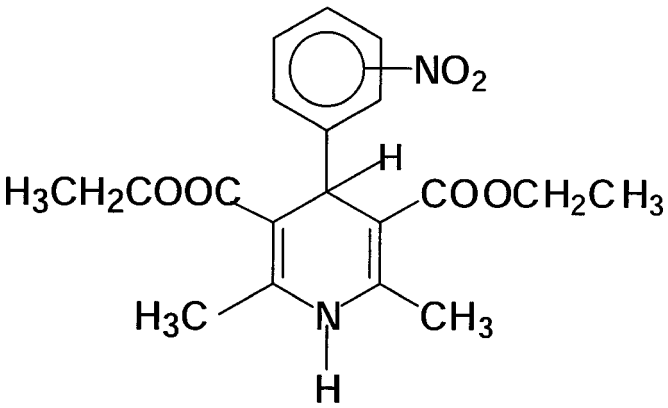
Some of the most used 1,4DHP differ on the position of the nitro group within the phenyl substituent, *i.e.*, nifedipine contains an ortho-nitrophenyl substituent at position 4 while nitrendipine posses a meta-nitrophenyl substituent in the same position. Moreover, it is a well-known matter[17] that the position of the phenyl ring substituent is of major importance for the drug potency, which decreases from ortho over meta to para substitution. Previous studies have demonstrated that the nitrophenyl substituent plays an important role in some properties such as radical anion formation and stability and also in the photostability of drug parent. Specifically, previous works, using different techniques such as cyclic voltammetry[11] and electron spin resonance,[18] concluded that orthonitrophenyl derivatives show a trend to give less stable radical anions. On the other hand, several photodecomposition studies on 1,4DHP[19-24] have revealed that ortho-nitrophenyl derivatives have shown lower stability to light exposure than do meta-nitrophenyl derivatives.

According to the state-of-the-art, the position of the nitrophenyl substitution is a relevant point affecting several aspects that need more research. Probably, the importance of the nitro group substitution is related with its electronic effects on the molecule, and consequently, the knowledge of the involved electrochemical aspects would contribute to an understanding of the phenomena.

In this paper, we have studied the electrochemical behavior of some compounds containing a structural pattern similar to that of nifedipine or nitrendipine where the only difference among them was the position of the nitro group (ortho, meta, and para) in the phenyl substituent of the 1,4DHP derivative. In a previous recent paper,[13] Baumane *et al.* studied several nitrophenyl substituted 1,4DHP, but his study was restricted to aprotic media (anhydrous dimethylformamide).

Experimental

Reagents and solutions.—According to a general procedure[25] for the synthesis of nitro-DHP derivatives, we have synthesized the fol-



Nitro substituent

Ortho = *2-NDHP*
Meta = *3-NDHP*
Para = *4-NDHP*

Figure 1. Molecular structures of 4-(2-nitrophenyl)-, 4-(3-nitrophenyl)-, and 4-(4-nitrophenyl)-substituted 1,4-dihypropyridines.

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lowing already known compounds (Fig. 1): 4-(2-nitrophenyl)-2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine (2-NDHP), 4-(3-nitrophenyl) -2,6 -dimethyl -3,5-diethoxycarbonyl -1,4-dihydropyridine (3-NDHP), and 4-(4-nitrophenyl)-2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine (4-NDHP).

A mixture of 10 mL (0.079 mol) of ethyl acetoacetate and 10 mL of concentrated ammonia hydroxide in 20 mL of ethyl alcohol was heated under reflux for 2.5 h. The resulting clear solution was added to a mixture of 10 mL of (0.079 mol) of ethylacetoacetate, 2.5 g. (0.0165 mol) of nitrobenzaldehyde, 25 mL of concentrated ammonia hydroxide and 20 mL of ethyl alcohol and maintained under reflux for 15 h. The crude solid product is filtered and recrystallized in ethyl alcohol. The yields are in the 80-90% range depending upon the derivative.

All the compounds were characterized according to the following physical and chemical characteristics:

1. 2-NHDP.

mp 124.8°C. IR (KBr): ν_{max} 3433, 1695, 1528, 1489, 1280, 1212 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.16 (t, 6H, J = 7.1 Hz, $-CH_2CH_3$), 2.33 (s, 6H, $-CH_3$), 4.07 (m, 4H,$-CH_2CH_3$), 5.85 (s, 1H, Ar-CH<), 5.70 (s, 1H, -NH-), 7.49 (m, 4H, Ar-H).

Analysis calculated for $C_{19}H_{22}O_6N_2$: C, 60.94; H, 5.93; N, 7.49. Found: C, 60.81; H, 6.00; N, 7.81.

2. 3-NDHP.

mp 163.0 °C. IR (KBr): ν_{max} 3345, 1705, 1646, 1526, 1488, 1348, 1214 cm^{-1}.^1H NMR (300 MHz, $CDCL_3$): δ 1.22 (t, 6H, J = 7.1 Hz, -CH2CH3), 2.36 (s, 6 H, $-CH_3$), 4.07 (m, 4 H,$-CH_2CH_3$), 5.06 (s, 1H, Ar-CH<), 5.90 (s, 1H, -NH-), 7.8 (m, 4H, Ar-H).

Analysis calculated for $C_{19}H_{22}O_6N_2$: C, 60.94; H, 5.93; N, 7.49. Found: C, 60.69; H, 5.98; N, 7.78.

3. 4-NDHP.

mp 130.8°C. IR (KBr): ν_{max} 3427, 1690, 1518, 1489, 1346, 1215 cm^{-1}. ^1H NMR (300 MHz,$CDCl_3$): δ 1.16 (t, 6 H, J= 6.7 Hz, $-CH_2CH_3$), 2.33 (s, 6 H, $-CH_3$), 4.06 (m, 4 H,$-CH_2CH_3$), 5.85 (s, 1H, Ar-CH<), 5.70 (s, 1H, -NH-), 7.55 (m, 4H, Ar-H).

Analysis calculated for $C_{19}H_{22}O_6N_2$: C, 60.94; H, 5.93; N, 7.49. Found C, 60.92; H, 5.67; N, 7.71.

All the reagents employed were of analytical grade.

Stock solutions of each compound (0.01 M in ethanol) were prepared. The polarographic working solutions were prepared by diluting the stock solution to obtain a final substrate concentration of 0.1 mM. Solutions for cyclic voltammetry (CV) were prepared by weighing an adequate quantity of each compound in order to obtain a final substrate concentration of 1 mM. Stock and working solutions were handled and stored avoiding exposure to light.

Protic medium working solutions were prepared by diluting the stock solution to obtain final concentrations of 0.1 or 1 mM in a mixture of 30/70: ethanol/0.1 M Britton-Robinson buffer (KCl 0.3 M). The pH was adjusted with aliquots of concentrated NaOH or HCl, respectively.

All experiments were carried out at a constant temperature of 25 ± 0.1°C and the solutions were purged with pure nitrogen for 10 min prior to the cathodic voltammetric runs.

Apparatus.—Electrochemical experiments, differential pulse polarography (DPP), differential pulse voltammetry (DPV) tast polarography, and cyclic voltammetry were performed with a totally automated BAS CV-50 W voltammetric analyzer. A 10 mL thermostated measuring cell, with three electrodes, was used. A dropping mercury electrode (for DPP and tast polarography), hanging mercury electrode (for CV) and glassy carbon electrode (GCE) (for anodic DPV and CV) as a working electrode were used. A platinum wire counter electrode, and a saturated calomel reference electrode (SCE), respectively, was used for the measurements.

Spectrophotometric measurements were carried out with an UV-vis spectrophotometer ATI Unicam model UV3, using a 1 cm quartz cell and equipped with a 486 computer with Vision acquisition and data treatment software.

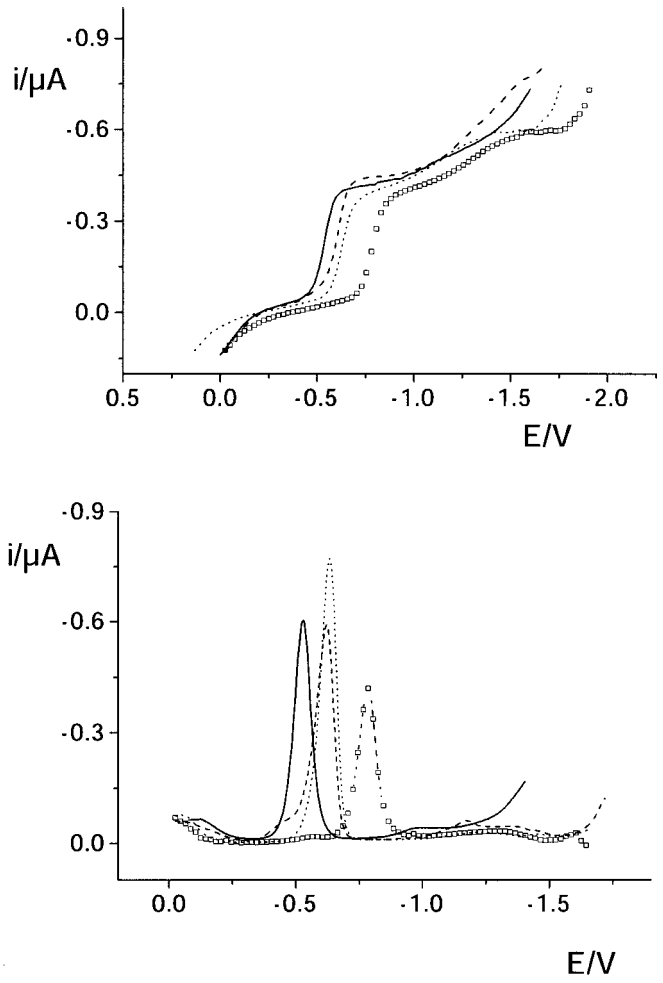

Figure 2. Tast and differential pulse polarograms for 1 mM solutions of (solid line) nitrobenzene, (□-□-□-□) 2-NDHP, (.....) 3-NDHP, and (------) 4-NDHP in ethanol/0.1 M Britton Robinson buffer: 30/70, pH 7.

Theoretical calculations to optimize the configuration of the derivatives were carried out using HyperChem 6.0 software, thanks to HyperCube for the demonstrative software available in the web.

Results and Discussion

In the present paper we include an exhaustive study devoted to the electrochemistry behavior of the 4-(2-nitrophenyl)-, 4-(3-nitrophenyl)-, 4-(4-nitrophenyl)-, 1,4-dihydropyridine derivatives (2-NDHP, 3-NDHP, and 4-NDHP) in protic media. The objective is to reveal some consequences of the different position of the nitro substitution in these compounds. As mentioned above these compounds have a structural pattern that is similar to the pattern of several important drugs such as nifedipine, nitrendipine, nisoldipine, nimodipine, furnidipine, and related ones.

All the tested compounds were reduced at the dropping mercury electrode (DME) in ethanol/0.1 M Britton Robinson Buffer (0.3 M KCl): 30/70 rendering well-defined waves (tast polarography) or peaks (differential pulse polarography). Although all the compounds have a similar behavior, there are some differences depending on the position of the nitro group. All the compounds produced one main cathodic peak in the 2-12 pH range (Fig. 2). The comparison of the limiting currents of the studied compounds with limiting currents of an equimolar solution of nitrobenzene (a classical four-electron re-

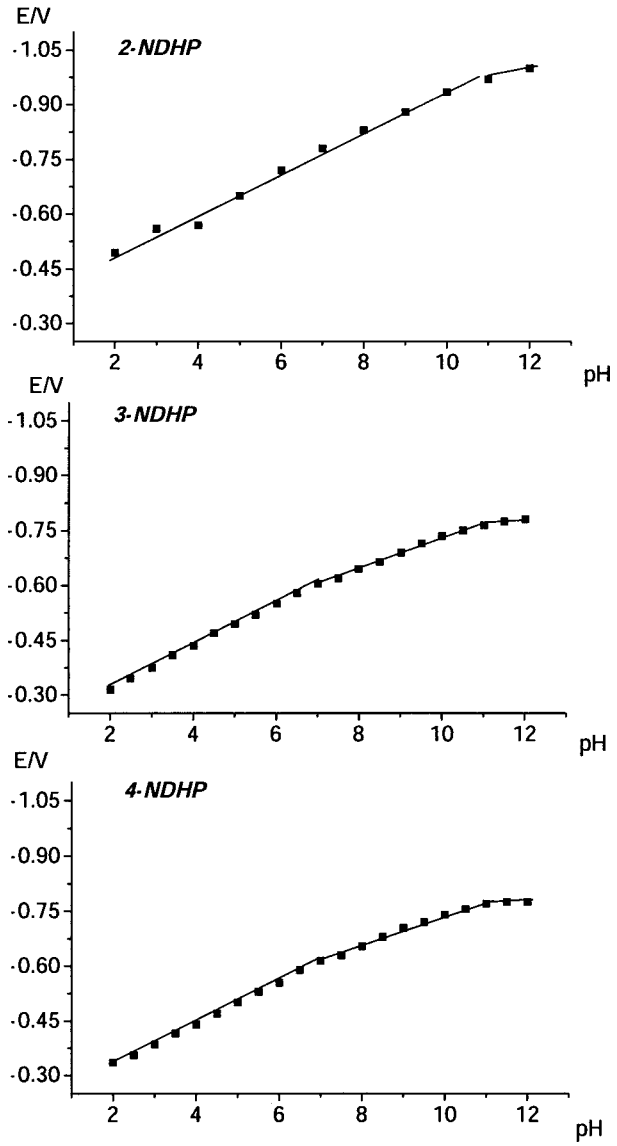

Figure 3. Cathodic peak potential dependence with pH for the 4-(2-nitrophenyl)-, 4-(3-nitrophenyl)-, and 4-(4-nitrophenyl)-substituted 1,4-dihypropyridines.

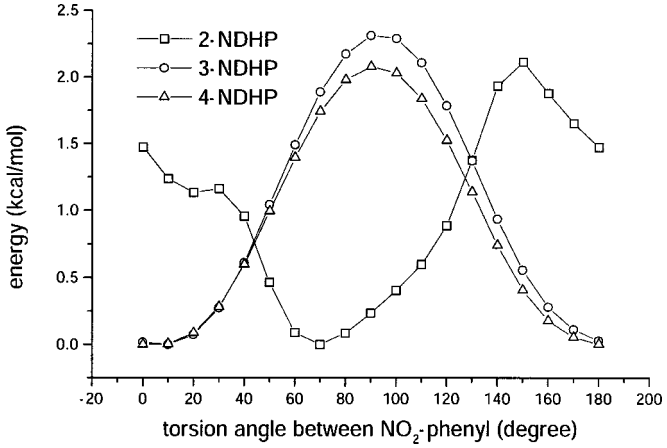

Figure 4. Energy plot showing the torsion angle between the nitro group and the phenyl ring for the 4-(2-nitrophenyl)-, 4-(3-nitrophenyl)-, and 4-(4-nitrophenyl)-substituted 1,4-dihypropyridines.

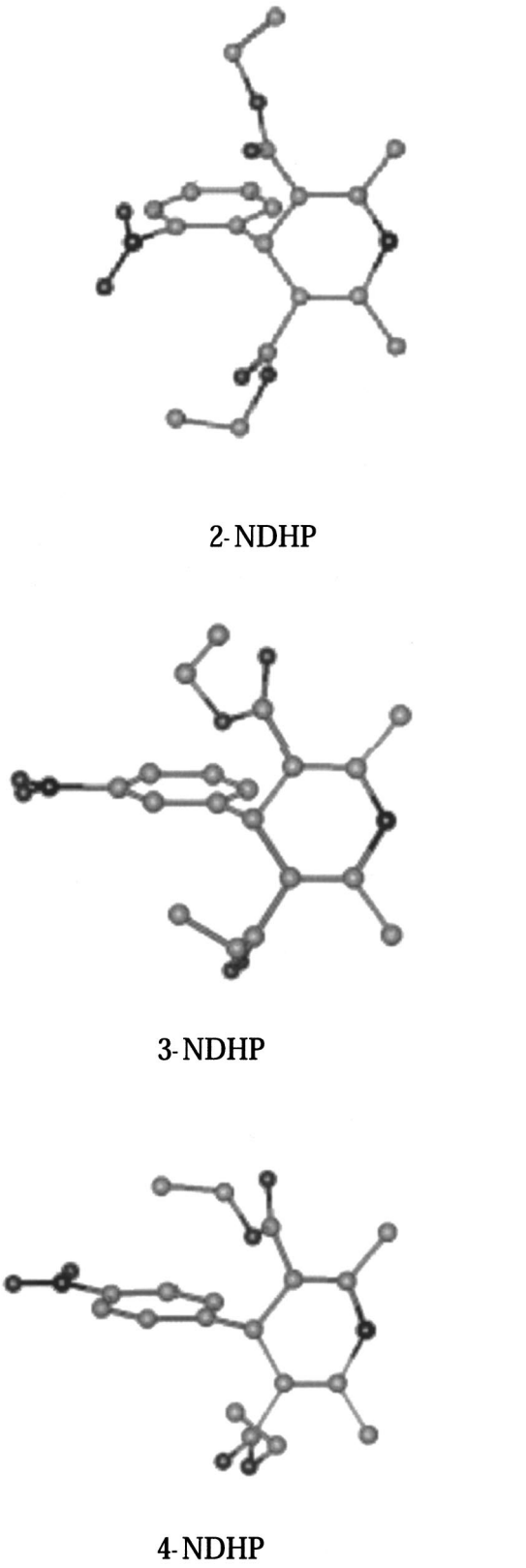

Figure 5. Optimized molecular structures obtained by molecular modeling for the 4-(2-nitrophenyl)-, 4-(3-nitrophenyl)-, and 4-(4-nitrophenyl)-substituted 1,4-dihypropyridines.

ducible compound) is shown in Fig. 2. From this observed similarity we can affirm that the main cathodic wave is due to the four-electron

reduction of the nitro group to produce the hydroxylamine derivative according to the well-studied behavior of the nitro aromatic compounds[26]

$$ArNO_2 + 4e^- + 4H^+ \rightarrow ArNHOH + H_2O \quad [1]$$

A second peak appearing only at acidic pH values is due to the two-electron reduction of the protonated hydroxylamine

$$ArNHOH_2^+ + 2e^- + H^+ \rightarrow ArNH_2 + H_2O \quad [2]$$

Both peaks are shifted to more cathodic potentials when the pH increases, but we have focused our study on the main cathodic peak. The peak potential dependence with pH is showed in Fig. 3. From this figure, we can observe a linear dependence in the pH range between 2 and 11. A break at pH 7 is observed in the case of 3 and 4 substituted compounds, but in the 2-NDHP, this break was not observed. Beyond pH 11, the peak potential was pH-independent for all the derivatives. Furthermore from the UV-vis spectra of the compounds, at different pH values between 2 and 12, was not possible to find evidences of some p*K*, consequently, we cannot ascribe the above breaks in the *E*p *vs.* pH plot to possible protonation equilibrium in the molecules.

From the above results we can observe that 2-NDHP was reduced at considerable higher cathodic potentials (about 150 mV negative) than 3-NDHP and 4-NDHP. The obtained half-wave potentials at pH 7 of 2-NDHP, 3-NDHP, and 4-NDHP were −0.79, −0.63, and −0.62 V *vs.* SCE, respectively. From analysis of the electrochemical reduction potentials at different pH values (Fig. 3) of the three substituted nitrobenzenes it merges that the same order is maintained in all the pH range. The observed differences in the reduction potentials may be ascribed as a consequence of one main factor. In fact we can explain the reduction potential differences by the displacement of the nitro group from coplanarity, with respect to the benzene ring, as a consequence of the steric effect due to a 1,4-DHP substituent in an ortho-position in the 2-NDHP compound. The distortion of the coplanar arrangement decreases the resonance interaction between the nitro group and the aromatic system, and the observed shift toward negative potentials in the 2-NDHP isomer corresponds to this decrease. This type of steric effects was also described in a different series of substituted nitrobenzene in a previous paper by Geske *et al.*[27] Furthermore, in order to corroborate the above assumption we have used HyperChem 6.0 software to calculate the torsion angles between the nitro group and the phenyl ring. The methodology of the optimization involved the following steps. The nitrocompounds were first optimized using MM+. Next, with the structures obtained in the first step, a PM3 optimization was carried out. Finally, the energy barrier for the torsion angle between NO_2 and phenyl ring was performed using the PM3 method. The configuration of minimal energy shows torsion angles of 70.0, 7.5, and 2.2° for 2-NDHP, 3-NDHP, and 4-NDHP derivatives, respec-

Table I. Anodic peak potential dependence with pH for the 4-(2-nitrophenyl)-, 4-(3-nitrophenyl)-, and 4-(4-nitrophenyl)-substituted 1,4-dihydropyridine derivatives obtained by DPV.

Compound	E_P (pH 7)/mV *vs.* SCE	pH range	Slope (mV/pH)
2-NDHP	752	2-5	−10.2
		5-9	−38.5
		9-12	−56.6
3-NDHP	723	2-5	−7.6
		5-9	−36.8
		9-12	−61.5
4-NDHP	720	2-5	−2.2
		5-9	−37.4
		9-12	−65.5

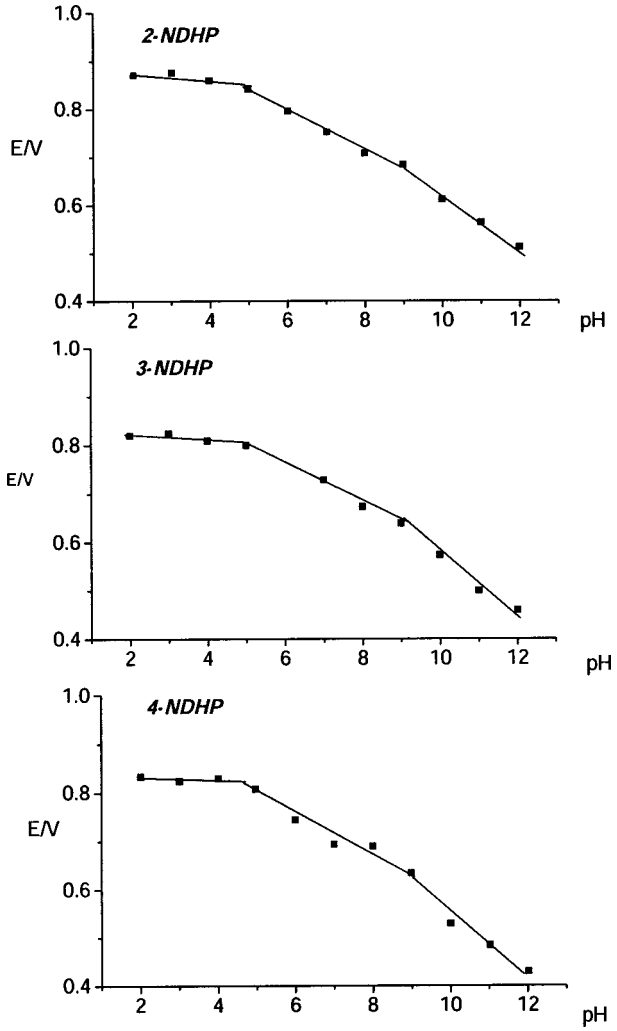

Figure 6. Anodic peak potential dependence with pH for the 4-(2-nitrophenyl)-, 4-(3-nitrophenyl)-, and 4-(4-nitrophenyl)-substituted 1,4-dihypropyridines.

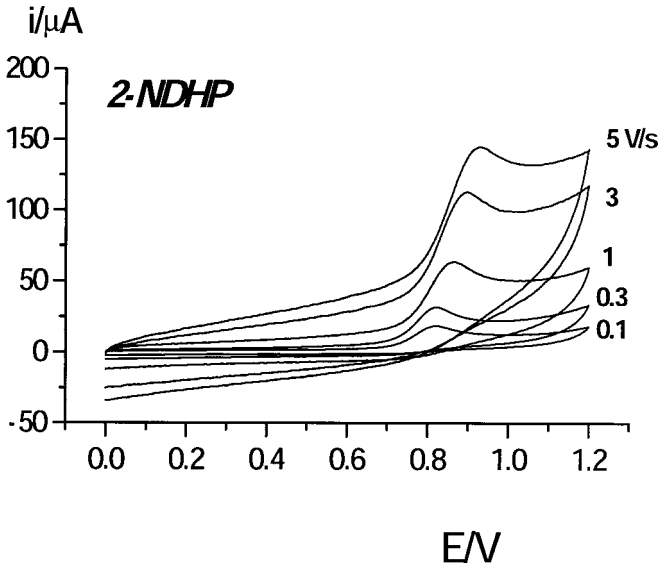

Figure 7. Cyclic voltammograms on GCE of 1×10^{-3} M 2-NDHP, pH 7 at different sweep rates.

tively. A barrier of about 2.3 kcal/mol was obtained for the rotation on all the derivatives. These results are shown in the plot of Fig. 4. Also we have included a Fig. 5 with the optimized molecules wherein the above mentioned lost of planarity in the 2-NDHP is clearly appreciated.

On the other hand, there is a secondary factor explaining the observed differences in the reduction potentials, *i.e.*, the inductive electron-donating effect of the 1,4-DHP produces an enhanced electron density on the nitro group hindering the reduction. This effect decay with the distance being bigger in the 2-NDHP isomer. The electron-donating effect of 1,4-DHP substituent is proved by comparison with nitrobenzene wherein the reference substituent is hydrogen. As can be seen in Fig. 2, all of the compounds results are more difficult to reduce than nitrobenzene showing that the 1,4-DHP substituent is more strongly electron-donating than hydrogen.

As the studied compounds have two possible redox centers in the molecule, *i.e.*, the electroreducible nitro group and the electrooxidable 1,4-DHP, we have also studied the anodic behavior. We have found that in Britton-Robinson/EtOH:70/30 solutions, all the 1,4-DHP compounds can be oxidized at the GCE producing only one anodic peak which is best resolved using differential pulse voltammetry. The peak potential (Ep) *vs.* pH plots (Fig. 6) shows three linear segments for each studied compound. In the acidic range ($pH < 5$), it is close to pH-independent. This behavior would indicate that the transfer of the first electron to produce a highly reactive cation radical is the rate-determining step.[28-30] The voltammetric peak follows a pH-dependent behavior at $pH > 5$, with slopes of ~37 mV/pH between pH 5 and 9 and ~61 mV/pH between pH 9 and 12, respectively, as detailed in Table I. On the other hand, the peak current, i_p, was not affected with pH changes, suggesting that the limiting current is controlled by the diffusion of the electroactive species to the electrode surface.

Furthermore, CV experiments on GCE show also only one anodic peak up to 5 V/s for all the studied isomers. In Fig. 7, the cyclic voltammogram of 1×10^{-3} M 2-NDHP, pH 7 at different sweep rates is shown. The observed irreversible peak was similar for all the isomers. This totally irreversible peak shows a linear relation of the peak current, i_p, with the square root of the sweep rate, $v^{1/2}$, producing a $\delta \log i_p/\delta \log v$ value of about 0.5, thus supporting a diffusion-controlled mechanism. The above described anodic behavior was very similar to the previously described for a series of 1,4-DHP calcium antagonists[30] wherein the global oxidation process is given by the following equation

$$\text{dihydropyridine} \rightarrow \text{pyridine} + 2H^+ + 2e^- \qquad [3]$$

As a consequence of the above study, we can conclude that, in the case of anodic behavior, we have not observed a marked difference between the oxidation potentials of the three derivatives as it was observed in the case of the reduction potential values. Oxidation potential values obtained by DPV at pH 7 were 0.75, 0.72, and 0.72 V *vs.* SCE for 2-NDHP, 3-NDHP, and 4-NDHP, respectively. Consequently, we can affirm that the nitro position of the substituent on the aromatic ring did not affect the electron density in the dihydropyridine moiety.

On the other hand, the different position nitro substitution produces another relevant effect in this type of molecules. Specifically, this effect is related with the photostability of these compounds. Stability of buffered solutions (pH 7) of 2-NDHP, 3-NDHP, and 4-NDHP stored in room light conditions was determined by differential pulse (dp) polarography. In Fig. 8 we can observe the change in the dp polarograms with the light exposure time for all the studied compounds. In the inset of this figure, the time course of the photodegradation, based in the decay of the peak current of the nitro group, is observed. The results show that 2-NDHP was highly unstable, but the other isomers were stable in the time scale of the experiment. Furthermore, from the decay of the peak current of the nitro group in the 2-NDHP compound, we can deduce that the photodegradation implied the consumption of the nitro group by pho-

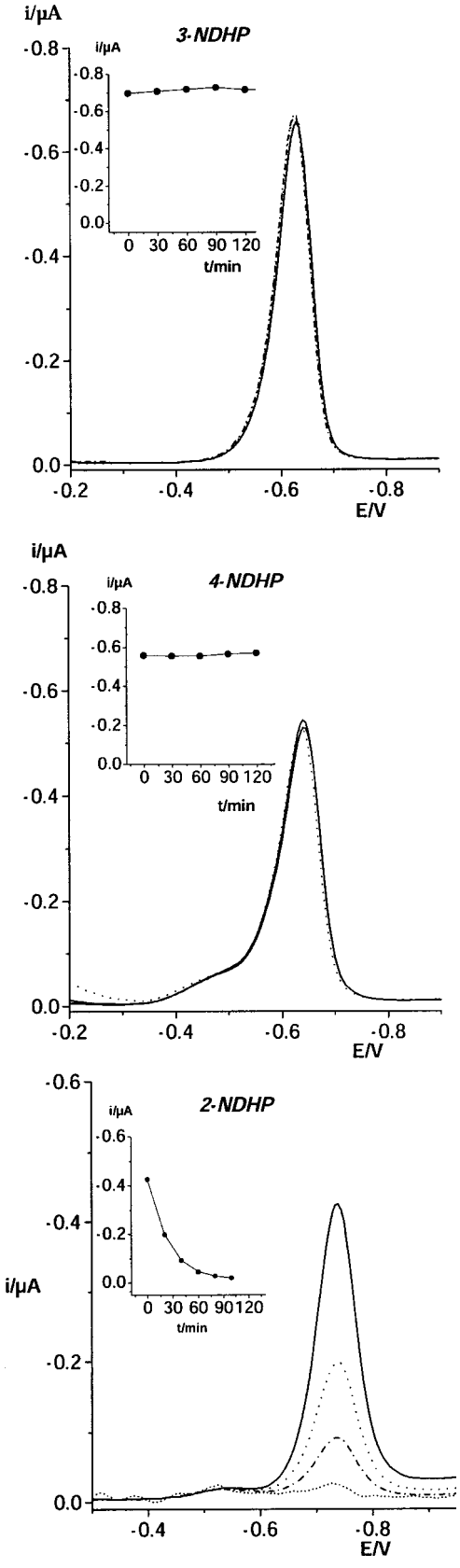

Figure 8. Effect of room light exposure time on the differential pulse polarograms of the 4-(2-nitrophenyl)-, 4-(3-nitrophenyl)-, and 4-(4-nitrophenyl)-substituted 1,4-dihypropyridines showing the instability of 2-NDHP.

toreduction. According to previous experiences with related compounds[19-24] photoreduction would produce the corresponding nitroso derivative. Probably the instability of 2-NDHP is due to that in this isomer the photoexcited state in the nitro group cannot be stabilized by resonance as a consequence of the above mentioned loss of coplanarity between the nitro group and the aromatic ring.

The above result is very important because permits one to generalize the fact that in compounds such as 4-nitrophenyl substituted 1,4-dihydropyridines the ortho derivatives are more unstable than the corresponding meta and para derivatives. In fact, a search in the literature (Ref. 19-24, 31; *i.e.*, Table II in Ref. 24) reveals that ortho-nitrophenyl derivatives such as nifedipine, furnidipine, and nisoldipine have shown lower stability to light exposure than meta-nitrophenyl derivatives such as nitrendipine and nimodipine. Consequently we can affirm that the photodegradation process in 4-nitrophenyl substituted 1,4-dihydropyridines is favored by the loss of planarity between the nitro group and the aromatic ring.

Acknowledgments

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