

# Oxidation of Hantzsch 1,4-Dihydropyridines of Pharmacological Significance by Electrogenerated Superoxide

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**Purpose.** To study the reaction of a series of Hantzsch dihydropyridines with pharmacological significance such as, nifedipine, nitrendipine, nisoldipine, nimodipine, isradipine and felodipine, with electrogenerated superoxide in order to identify products and postulate a mechanism.

**Methods.** The final pyridine derivatives were separated and identified by gas chromatography/mass spectrometry (GC-MS). The intermediates, anion dihydropyridine and the  $\text{HO}_2^\bullet/\text{HO}_2^-$  species, were observed from voltammetric studies and controlled potential electrolysis was used to electrogenerate  $\text{O}_2^\bullet$ .

**Results.** The current work reveals that electrogenerated superoxide can quantitatively oxidize Hantzsch dihydropyridines to produce the corresponding aromatized pyridine derivatives.

**Conclusions.** Our results indicate that the aromatization of Hantzsch dihydropyridines by superoxide is initiated by proton transfer from the N1-position on the 1,4-dihydropyridine ring to give the corresponding anion dihydropyridine, which readily undergoes further homogeneous oxidations to provide the final aromatized products. The oxidation of the anionic species of the dihydropyridine is more easily oxidized than the parent compound.

**KEY WORDS:** cyclic voltammetry; 1,4-dihydropyridine; mass spectrometry; superoxide.

## INTRODUCTION

Active oxygen species like superoxide radical anion ( $\text{O}_2^\bullet$ ), hydroxyl radical ( $\text{HO}\cdot$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) are involved in the etiology of several human diseases because of their great destructive effects (1). Special attention has been directed to superoxide anion, because it is the first species produced from the reduction of oxygen. Many studies have been done on the organic chemistry of superoxide anion, and many of them are related to its interaction with different organic substances. Depending on the nature of the substance and the medium characteristics, superoxide anion may interact as a base (2,3) a nucleophile (4–7), a reductant (8,9), or as an oxidant (9,10). Although all the reports that discuss this property have involved systems with proton sources, the real oxidizing property is attributed to the intermediates and products of the proton-induced dismutation reaction ( $\text{HO}_2^\bullet$ ,  $\text{O}_2$ ,  $\text{HO}_2^-$ , and  $\text{H}_2\text{O}_2$ ) (11).

Some Hantzsch 1,4-dihydropyridine (1,4-DHP) derivatives are part of classical clinical drugs used for the treatment of hypertension and other cardiovascular diseases because of

their calcium channel blocker characteristics. Previous studies have suggested (12–17) that these types of drugs also provide an antioxidant protective effect that may contribute to their pharmacological activity. This effect is not due to the  $\text{Ca}^{2+}$  antagonist effect, but it is related to the reactivity of these compounds toward radical species (13). Moreover, the oxidation of 1,4-DHP is the main metabolic route for these compounds.

The oxidation of 1,4-DHP is one of the ubiquitous problems in organic chemistry, and several researchers have reported oxidation methods including chemical oxidation with Pd/C in acetic acid (18), oxidation with ceric ammonium nitrate (19), ultrasound-promoted oxidation by clay-supported cupric nitrate (20), oxidation with pyridinium chlorochromate (21), and oxidation with nitric acid (22). More recently, the oxidation of 1,4-DHP derivatives has attracted more attention from chemists because of its oxidative reactivity with endobiotics such as nitric oxide (NO) or its donor *N*-methyl-*N*-nitrosotoluene-*p*-sulfonamide (MNTS) (23,24) and superoxide ( $\text{O}_2^\bullet$ ) (25,26). At the best of our knowledge, the oxidation of 1,4-DHP derivatives by superoxide is a totally unexplored matter.

The current paper is a part of the work developed on the study of the interaction between superoxide radical anion and a group of 1,4-DHP derivatives in aprotic media. As was shown previously (25), nisoldipine, one of these 1,4-DHP compounds, reacts directly with  $\text{O}_2^\bullet$ . The latter acts as a Brønsted base deprotonating nisoldipine, and nisoldipine acts by scavenging  $\text{O}_2^\bullet$ . Moreover, we have developed a very easy and direct voltammetric procedure for studying the relative reactivity of different 1,4-DHP with  $\text{O}_2^\bullet$  (26) being the study of the reaction mechanism a current challenge.

Here, we report the behavior of the reaction between the commercial drugs nifedipine, nisoldipine, nimodipine, nitrendipine, isradipine, and felodipine (Fig. 1) with superoxide employing electrochemical techniques. Furthermore, the oxidation product generated after the reaction of the 1,4-DHP with electrogenerated superoxide has been separated and identified by gas chromatography/mass spectrometry. A mechanism for the oxidation of 1,4-DHP with superoxide is postulated.

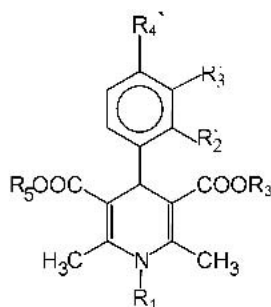
## MATERIALS AND METHODS

### Reagents

The aprotic solvent dimethylsulfoxide (DMSO) used in electrochemical experiments was purchased from Merck and was dried with 3 Å molecular sieves. Nisoldipine was provided by Laboratorio Chile (Santiago, Chile), nifedipine by Laboratorio Mintlab (Santiago, Chile), nitrendipine by Laboratorio Sanitas (Santiago, Chile), isradipine by Laboratorio Sandoz (Santiago, Chile), and nimodipine and felodipine were provided by Laboratorio Saval (Santiago, Chile). All the drugs were 100% chromatographically pure. Compound I was synthesized and checked for purity in our laboratory according to a previously described procedure (27). Unless some different condition will be mentioned, all the experiments were made in aprotic (100% DMSO) media with 0.1 M tetrabutylammonium perchlorate (TBAP, Fluka, Santiago, Chile). Oxygen gas (99.8% pure) and nitrogen (99.9% pure)

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1,4-DIHP	R <sub>1</sub>	R <sub>3</sub>	R <sub>5</sub>	R <sub>2</sub> '	R <sub>3</sub> '	R <sub>4</sub> '
Nifedipine	H	CH <sub>3</sub>	CH <sub>3</sub>	NO <sub>2</sub>	H	H
Nisoldipine	II	CH <sub>2</sub> CH(CIT <sub>3</sub> ) <sub>2</sub>	CIT <sub>3</sub>	NO <sub>2</sub>	II	II
Nimodipine	H	(CH <sub>2</sub> ) <sub>2</sub> OCH <sub>3</sub>	CH(CH <sub>3</sub> ) <sub>2</sub>	H	NO <sub>2</sub>	H
Nitrendipine	II	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	II	NO <sub>2</sub>	II
Isradipine	H	CH(CH <sub>3</sub> ) <sub>2</sub>	CH <sub>3</sub>			H
Felodipine	H	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	Cl	Cl	H
Compound I	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	OCH <sub>3</sub>

Fig. 1. Chemical structure of Hantzsch 1,4-dihydropyridine derivatives.

were purchased from AGA (Santiago, Chile). Potassium superoxide (KO<sub>2</sub>) (97% pure) and crown ether/18-crown-6 (>98% pure) were purchased from Sigma and Merck (Santiago, Chile), respectively.

#### Apparatus and Procedures

Cyclic voltammetric (CV) and differential pulse voltammetric (DPV) experiments were performed with a BAS (West Lafayette, IN, USA) CV-50W voltammetric analyzer. All the measurements were carried out in a three-electrode measuring cell. A hanging mercury drop electrode (HMDE), a platinum wire counter electrode, and a Ag | AgCl | NaCl(sat) reference electrode were used for the measurements. Alternatively, a glassy carbon (GC) disk electrode with area of 0.071 cm<sup>2</sup> was also used. For measurements in oxygen media, O<sub>2</sub> gas was bubbled directly into the cell in order to obtain fixed concentrations of oxygen, and during the measurement, O<sub>2</sub> gas was flushed over the cell solution. In order to maintain fixed oxygen concentration in the measurement cell, an apparatus consisting of two flow-meters (Cole Palmer, Vernon Hills, IL, USA, 316SS) for oxygen and nitrogen, respectively, equipped with needle valves were used. The oxygen concentration in the gas passing through the measurement cell was determined taking into account the given oxygen solubility in DMSO containing 0.1 M TEAP (28) and by establishing oxygen and nitrogen flow rates (29). All cyclic voltammograms were carried out at a constant temperature of 25°C. The return-to-forward peak current ratio,  $I_{pa}/I_{pc}$ , for the oxygen/superoxide couple was measured for each cyclic voltammogram varying the scan rate from 0.05 Vs<sup>-1</sup> up to 50 Vs<sup>-1</sup> according to the procedure described by Nicholson (30).

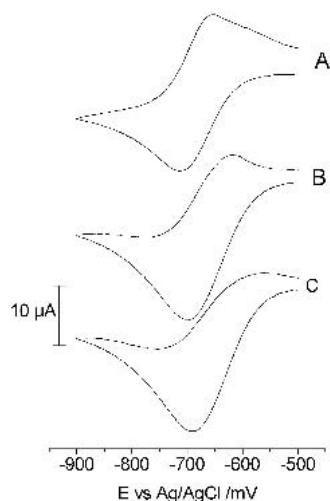
#### Controlled Potential Electrolysis

Controlled potential electrolysis (CPE) was carried out using a mercury pool cathode (area, 10.18 cm<sup>2</sup>). The applied potential (−1000 mV) was obtained using a WENKING POS 88 (Clausthal-Zellersel, Germany) potentiostat as a source. The CPE was carried out in a two-compartment cell with the counterelectrode separate from the pool electrode. In the three-electrode system, an Ag | AgCl | NaCl(sat) electrode was used as the reference electrode. Before each experiment, the solutions were first degassed with nitrogen and then saturated with oxygen. The solution was continuously stirred during 10 min of electrolysis. Nitrogen was continually passed over the solution during the electrolysis and later on to purge all possible nonreacted dissolved oxygen. CPE was tracked by DPV method in order to prove the generation of superoxide radical anion. Nitrogen was continually passed over the solution during the DPV experiment.

The concentration of superoxide anion generated by CPE of an oxygen-saturated solution in DMSO was determined using a calibration curve constructed with O<sub>2</sub><sup>•−</sup> chemically generated (KO<sub>2</sub>) as a reference standard (26).

#### Gas Chromatography/Mass Spectrometry

A gas chromatograph/mass selective detector (5890/5972) combination (Hewlett, Palo Alto, CA, USA) and a Hewlett Packard 7673 autosampler were used for the analyses. A Hewlett Packard data system based on a Pentium II processor printer was used to control instrumentation and for data processing. The  $m/z$  range monitored was 40–550 with a scan rate of 1.5 scan/s; the nominal electron energy was 70 eV. A Hewlett Packard Ultra-1 column, 25 m × 0.2 mm i.d. × 0.11



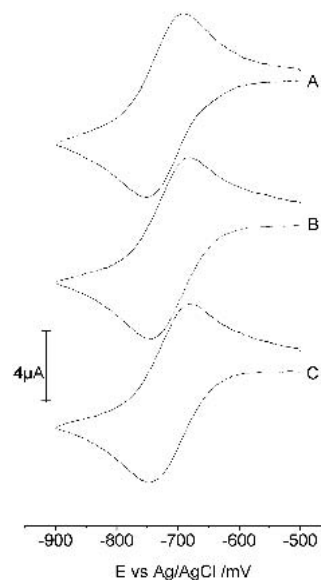
**Fig. 2.** CVs of the  $O_2/O_2^{\bullet-}$  redox couple in the presence of different nifedipine concentrations: (A) without nifedipine, (B)  $5 \times 10^{-3}$  M, (C)  $1 \times 10^{-2}$  M (sweep rate,  $1 \text{ Vs}^{-1}$ ).

$\mu\text{m}$  film thickness (Little Falls, Wilmington, DE, USA) was used.

The chromatographic conditions were as follows: detector temperature,  $300^\circ\text{C}$ ; injector temperature,  $250^\circ\text{C}$ ; split ratio, 1:10; pressure, 13 psi; purge flow, 40 ml/min; purge time, 0.5 ml/min. For the temperature program, the oven temperature was programmed from  $130$  to  $305^\circ\text{C}$  (hold for 5 min) at  $15^\circ\text{C}/\text{min}$ ; run time was 16.67 min. Helium was used as carrier gas with an inlet pressure of 35 kPa. The identification of the samples was based on the analyses of the mass spectra (full scan).

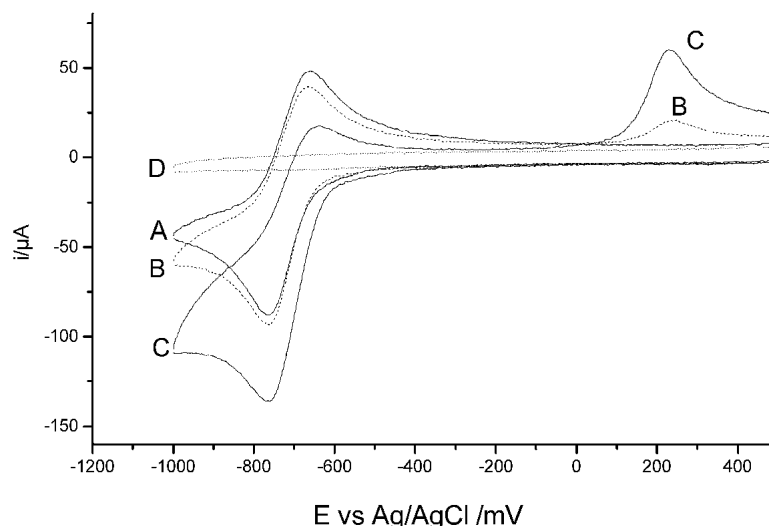
## RESULTS AND DISCUSSION

In a previous paper (25), the optimal conditions for generating and studying the superoxide redox couple in HMDE were presented. Moreover, this couple was used to reveal an interaction between superoxide with a 1,4-DHP such as nifedipine. In the current study, we have applied the previously

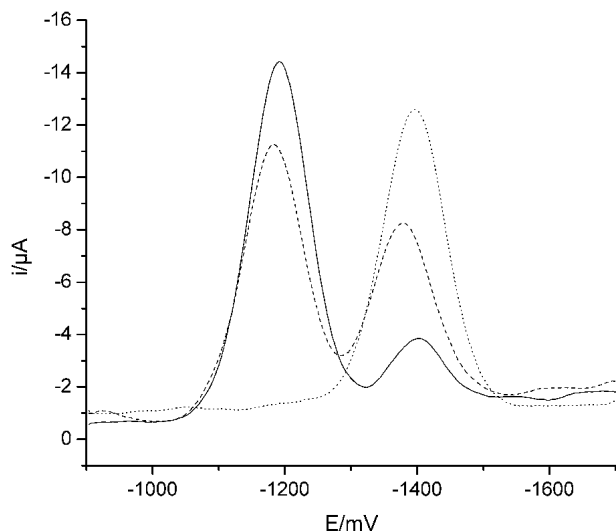


**Fig. 3.** CVs of the  $O_2/O_2^{\bullet-}$  redox couple in the presence of different concentrations of compound I: (A) without compound, (B)  $1 \times 10^{-2}$  M, (C)  $2 \times 10^{-2}$  M (sweep rate,  $1 \text{ Vs}^{-1}$ ).

developed methodology in order to study the reaction of a large series of 1,4-DHP derivatives with superoxide. All of these molecules are of current pharmacological interest. In order to study the reaction of the xenobiotics with superoxide, we have examined for all tested compounds the effect on the cyclic voltammetric response for the  $O_2/O_2^{\bullet-}$  couple by adding 1,4-DHP. Figure 2 shows the cyclic voltammograms at various concentrations of nifedipine solutions in oxygenated DMSO containing 0.1 M TBAP. With the addition of nifedipine, the oxidation peak current of  $O_2^{\bullet-}$  (anodic current, oxygen regeneration) decreases whereas the reduction current (cathodic current,  $O_2^{\bullet-}$  formation) increases. These data suggest that nifedipine reacts with  $O_2^{\bullet-}$ , that is, it scavenges  $O_2^{\bullet-}$  in DMSO, in a concentration-dependent way. Furthermore, if the concentration of added 1,4-DHP was higher than  $1 \times 10^{-2}$  M, the oxidation peak current of  $O_2^{\bullet-}$  completely disappeared.



**Fig. 4.** CVs of the  $O_2/O_2^{\bullet-}$  redox couple at the GC electrode in  $O_2$ -saturated DMSO solution in the (A) absence and in the presence of different nifedipine concentrations (B)  $3 \times 10^{-3}$  M and (C)  $2 \times 10^{-2}$  M. Curve (D) shows the CV of 3 mM nifedipine without oxygen (sweep rate,  $1 \text{ Vs}^{-1}$ ).



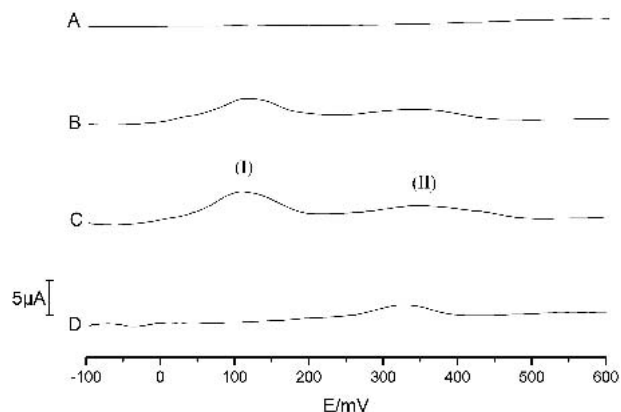
**Fig. 5.** DPV of 1 mM nifedipine in DMSO, 0.1 M TBAP at different concentrations of electrogenerated superoxide: (—) without superoxide, (-----)  $2.8 \times 10^{-5}$  M, (.....)  $8.1 \times 10^{-5}$  M.

We have obtained the same results for all the other 1,4-DHP derivatives tested; that is, furnidipine, nimodipine, isradipine, and felodipine. From these data, we can infer that all the studied 1,4-DHP derivatives react with superoxide anion in a similar way to that previously described for nisoldipine. In fact, superoxide acts as a Brønsted base deprotonating 1,4-DHP whereas 1,4-DHP acts scavenging  $O_2^{\cdot-}$ . In a previous paper (25), it was demonstrated that the hydrogen at the N-position of nisoldipine could be released as a proton in the presence of  $O_2^{\cdot-}$ , and consequently acts as a scavenger of  $O_2^{\cdot-}$ . In the current case, a similar effect with all the other members of the 1,4-DHP family was observed, and consequently the scavenging of  $O_2^{\cdot-}$  can be attributed to the hydrogen at the N-position. Similar effects were reported by other authors wherein the hydrogen at the N-position of indole compounds (31) acts as a proton donor to  $O_2^{\cdot-}$ . In order to confirm the hypothesis that the hydrogen at the N-position in 1,4-DHP compounds is crucial for the interaction with  $O_2^{\cdot-}$ , a new 1,4-DHP derivative (named as compound I in Fig. 1) lacking the hydrogen at the N-position was synthesized. This compound has been tested with electrogenerated superoxide, and no change in the voltammetric response of the superoxide oxidation was obtained (26). In the current case when different concentrations of compound I were added to oxygenated DMSO solution containing 0.1 M TBAP, no diminution of the oxidation peak current of superoxide was observed (Fig. 3) showing that this 1,4-DHP derivative does not scavenge superoxide. Consequently, these experiments strongly support our hypothesis in the sense that hydrogen at the N-position is crucial in scavenging superoxide.

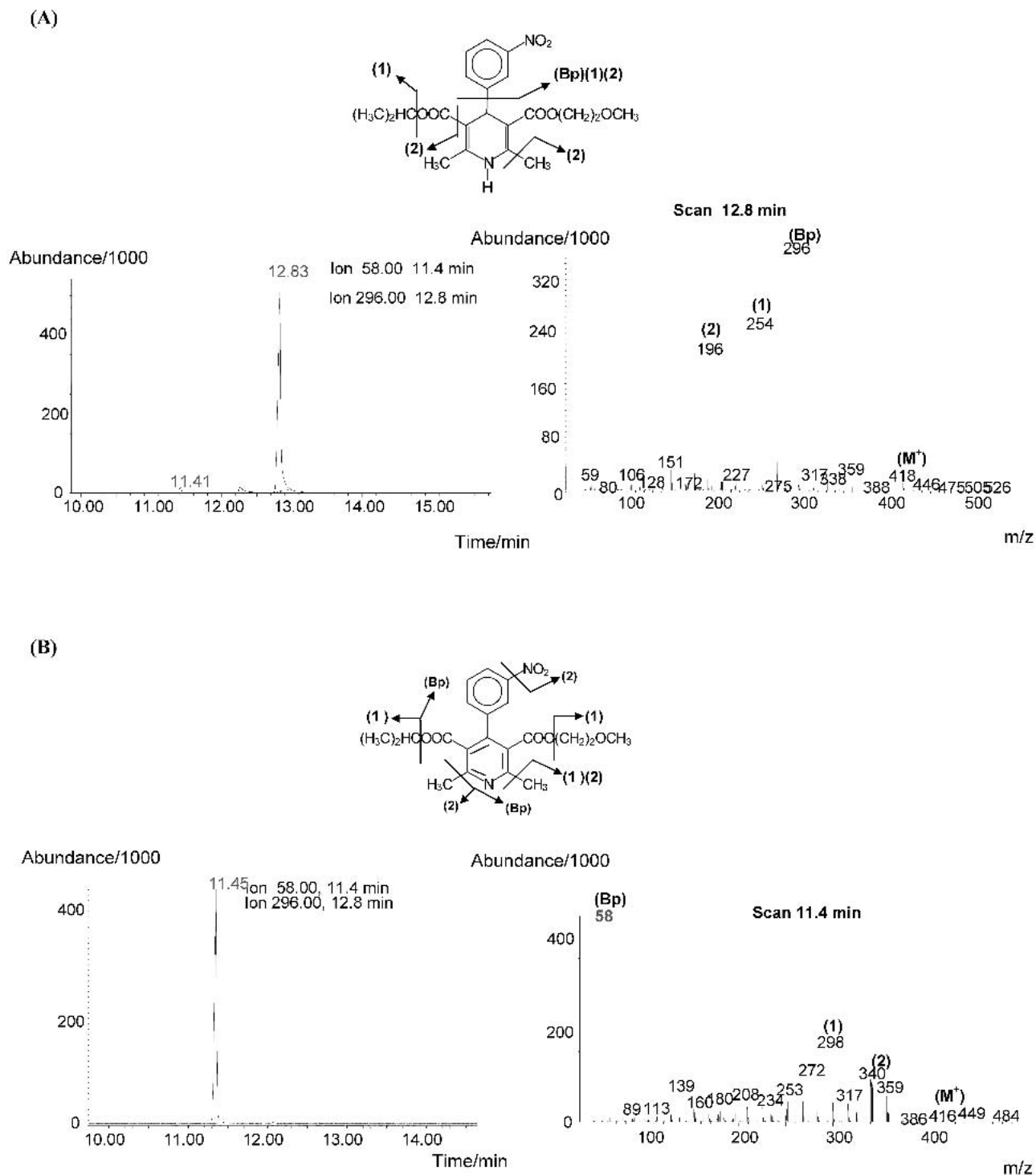
In order to obtain a different voltammetric view (i.e., extension of the anodic window) of the above reactivity between 1,4-DHP and superoxide, we also tracked the reaction using a glassy carbon electrode (GCE) as the working electrode. Surprisingly, we have observed that when an oxygenated DMSO solution was combined with an excess of 1,4-DHP, a new peak appeared at approximately 230 mV. Representative voltammograms obtained with GCE for an oxygenated DMSO solution at different concentrations of ni-

fedipine are shown in Fig. 4. In these voltammograms, we can observe that when nifedipine was added, the voltammetric signal at 230 mV increased as a consequence of adding the drug. A similar behavior was observed with all the 1,4-DHP commercial derivatives tested. This anodic peak can be ascribed to the oxidation of the anionic form of the 1,4-DHP derivative produced by deprotonation with superoxide. A similar effect was found when moderately weak acids, such as phenol (32) or indole compounds (31), were present in excess. In each case, the conjugate base produced by the reaction of the conjugate acid with  $O_2^{\cdot-}$  showed a similar oxidation peak at positive potential. As expected, when compound I was added to the solution, no anodic peak appeared, reaffirming the idea that the proton at the N-position is crucial for the observed reactivity between 1,4-DHP and superoxide. It is interesting to emphasize that according to the above voltammetric evidences, the reaction of superoxide with 1,4-DHP derivatives implies the deprotonation (loss of the hydrogen on the N-position) of the 1,4-DHP derivative. This process produces the corresponding conjugate base that is considerably more easily oxidizable than the parent 1,4-DHP derivative, thus making the overall oxidation process for the 1,4-DHP more expeditious.

In order to confirm the existence of the conjugate base, we have detected it by an alternative procedure that implies tracking the voltammetric signal of the aromatic nitro group reduction. For all the nitro aromatic 1,4-DHP compounds tested by CPE, we have studied the effect of adding increasing quantities of electrogenerated superoxide. The response of the nitro aromatic 1,4-DHP signal was followed by using differential pulse voltammetry (DPV). For compounds with electro-reducible nitro groups in ortho-position, we have found two voltammetric peaks, assigned to the ionized and unionized forms of the 4-(nitrophenyl) substituted 1,4-dihydropyridine compounds which are in equilibrium. The differential pulse voltammograms of nifedipine at different superoxide concentrations, displaying peaks due to the ionized form ( $E_p \approx -1400$  mV) and unionized form ( $E_p \approx -1200$  mV), are shown in Fig. 5. The ratio between both peaks was displaced to the ionized form as a consequence of adding electrogenerated superoxide, and, as expected, the reduction of the negative charged molecule (unionized form) is more



**Fig. 6.** DPV of 1 mM isradipine in DMSO, 0.1 M TBAP at different concentrations of electrogenerated superoxide: (A) without superoxide, (B)  $2.7 \times 10^{-4}$  M, (C)  $3.5 \times 10^{-4}$  M, (D) compound I with 3 mM of superoxide.



**Fig. 7.** Extracted ion chromatograms and mass spectra of nimodipine in DMSO, 0.1 M TBAP: (A) without  $O_2^{\cdot-}$ ; (B) with electrogenerated  $O_2^{\cdot-}$ .

difficult to reduce. The same phenomena was previously described (33) but using an alcoholic NaOH base instead of superoxide to displace the equilibrium. In our case, superoxide acts as a base deprotonating the 1,4-DHP derivative. As well as nifedipine, isradipine displayed the same behavior. However, in the case of meta-nitro substituted compounds such as nimodipine and nitrendipine, the signals corresponding to ionized and unionized forms are overlapped, and consequently no change is appreciated.

On the other hand, the effect of adding electrogenerated superoxide in the anodic voltammograms of the 1,4-DHP compounds was studied. In Fig. 6, A, the voltammogram corresponding to an isradipine solution (without added superoxide) showing no peaks in the anodic range 0–600 mV is displayed. From Fig. 6, B and C, we can observe that when superoxide is added, two new voltammetric signals appear, at 108 mV (peak I) and at 350 mV peak (II). The gradual appearance of peaks I and II are directly related with the quan-

**Table I.** Retention Times and Abundances of the 1,4-DHP Compounds in Absence and Presence of Superoxide

1,4-DHP derivative	Retention time/min	Abundance/%	
		Without $O_2^{\bullet-}$	With $O_2^{\bullet-}$
Nisoldipine	11.7	63.3	0
	10.3 (Oxidized derivative)	36.7	100
Nifedipine	10.9	70	5
	9.2 (Oxidized derivative)	30	95
Nimodipine	12.8	98.3	0
	11.4 (Oxidized derivative)	1.7	100
Felodipine	11.4	99.4	38.7
	9.2 (Oxidized derivative)	0.6	61.3
Nitrendipine	11.7	99.6	80.5
	9.9 (Oxidized derivative)	0.4	19.5
Isradipine	11.2	99.2	82.2
	9.8 (Oxidized derivative)	0.8	17.8

1,4-OHP, 1,4-dihydropyridine.

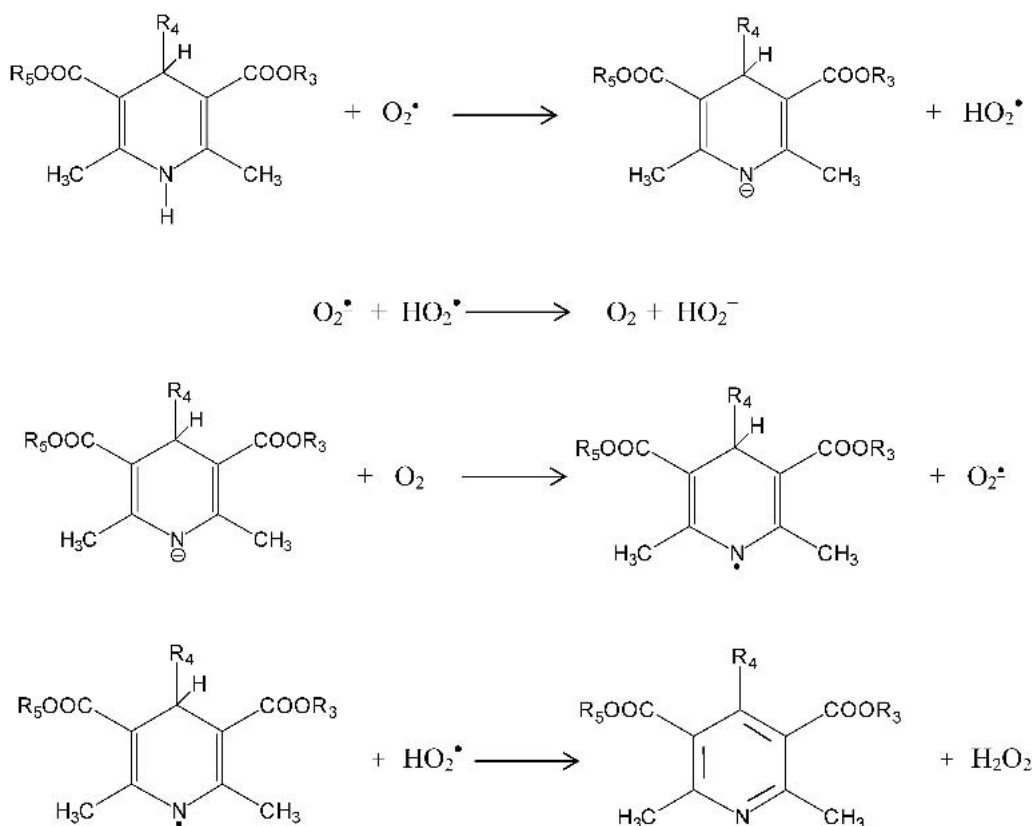
tity of superoxide added. This result suggests that peak I would be attributed to the oxidation of the anionic form of the 1,4-DHP derivative. Considering that the only redox active species produced during the primary proton abstraction process is  $HO_2^{\bullet}$  (34), a possible  $HO_2^{\bullet}/HO_2^-$  couple can be assumed. Consequently, the peak (II) would correspond to the oxidation of  $HO_2^-$ . It is a well-known fact that redox potential values may vary depending on the electrolyte and the electrode material; however, the obtained value correlates very well with other published values. For instance, an  $E_{1/2}$  value of 0.4 V vs. SCE for the couple  $HO_2^{\bullet}/HO_2^-$  in pyridine

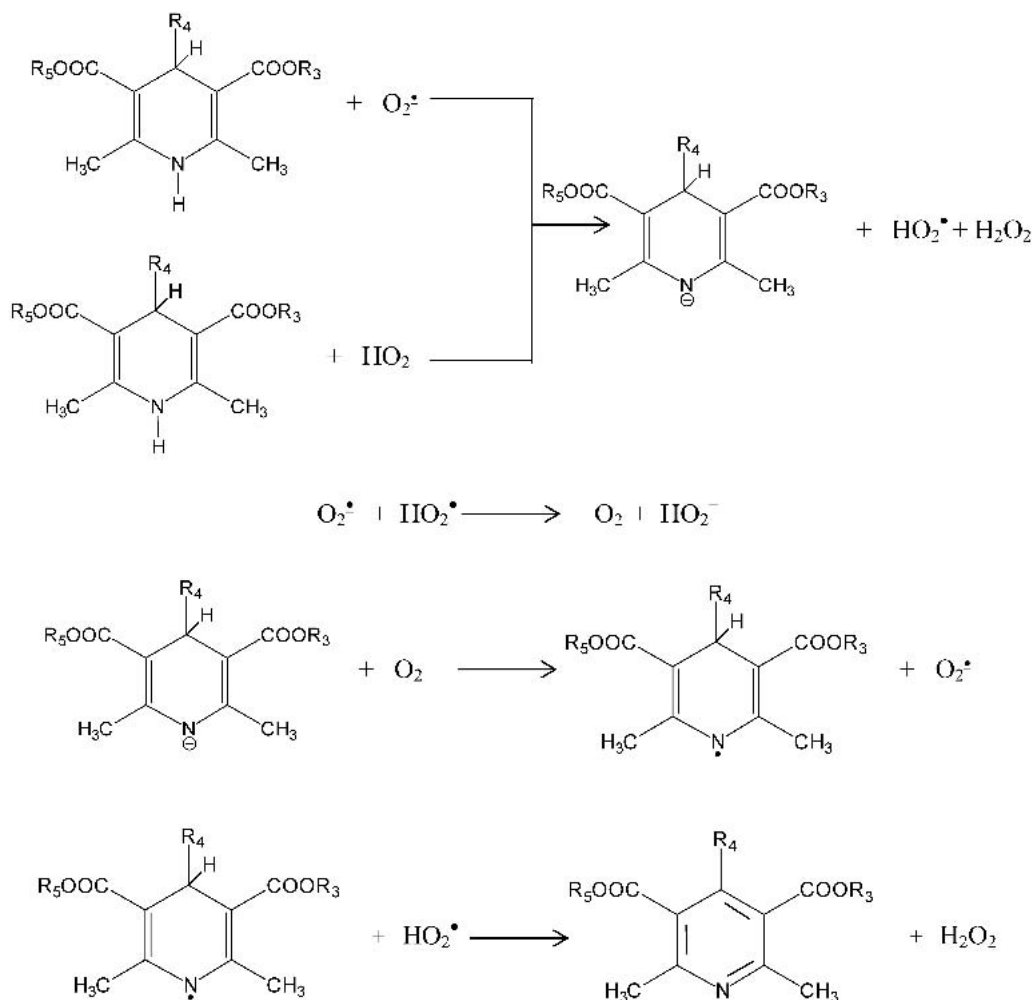
has been reported (35). The presence of this species indicates to us that the reaction must involve a proton transfer from the 1,4-DHP to  $O_2^{\bullet-}$ , followed by further chemistry of the resulting  $HO_2^{\bullet}$  species (11).

From this experiment, we can conclude that the conjugate base that is produced by dissociating hydrogen at the N-position as a proton in the presence of  $O_2^{\bullet-}$  is oxidized easily at more negative potential than the conjugate acid (i.e., the 1,4-DHP). In this case, the anionic form is oxidized approximately 1000 mV before the corresponding 1,4-DHP. The same behavior was obtained for the other compounds of the series.

Consistently, when the above experiment was repeated using compound I as the 1,4-DHP derivative, no anionic response attributed to the conjugate base was obtained as can be observed in Fig. 6, D.

In order to determinate the final products present in solution after the reaction between electrogenerated superoxide and the 1,4-DHP derivatives, a gas chromatography/mass spectrometry (GC/MS) method was used. Figure 7 shows typical extracted ion chromatograms and mass spectra corresponding to nimodipine in DMSO in absence and presence of superoxide. In the chromatogram (Fig. 7A), nimodipine shows a mean peak at 12.8 min that corresponds to the 1,4-DHP derivative. In presence of electrogenerated superoxide, nimodipine shows a different chromatogram: the peak at 12.8 min disappears, and a peak at 11.4 min appears. This peak corresponds to the pyridine derivative. The other 1,4-DHP derivatives have shown a similar behavior, varying only the product's abundance (Table I). In all the compounds, the retention times of the oxidized derivatives were lower than

**Fig. 8.** Proposed mechanism for the oxidation of Hantzsch dihydropyridines by electrogenerated superoxide in DMSO.



**Fig. 9.** Alternative proposed mechanism for the oxidation of Hantzsch dihydropyridines by electrogenerated superoxide in DMSO.

those of the original drugs, and the mass spectral fragmentation pattern was different from that of the original drugs. In particular, the substituents in the C-4 were not expelled, probably as a consequence of the aromatization of the 1,4-dihydropyridine rings. From these results, we can conclude that in the presence of superoxide, all the 1,4-DHP derivatives produce the pyridine derivatives as a final product. Furthermore, using the specific peroxid-test Merckoquant,  $\text{H}_2\text{O}_2$  was also detected as a final product of the reaction between 1,4-DHP and superoxide.

According to our results and literature data for similar reactions (34), a mechanism for the interaction between 1,4-DHP derivatives bearing an H substituent at the N1-position and superoxide can be postulated (Fig. 8). The postulated mechanism is supported by the identification of both products; that is, pyridine derivative and  $\text{H}_2\text{O}_2$  and intermediates (i.e., anion DHP and  $\text{HO}_2^{\bullet}/\text{HO}_2^-$  species). If we take into account that more probably the  $\text{HO}_2^-$  species is initially also present, an alternative mechanism can be proposed (Fig. 9).

## CONCLUSIONS

The current work reveals that superoxide can quantitatively oxidize Hantzsch dihydropyridines to produce the corresponding aromatized pyridine derivatives.

Our results indicate that the aromatization of Hantzsch dihydropyridines by superoxide are initiated by proton transfer from the N1-position on the 1,4-dihydropyridine ring to give the corresponding anion dihydropyridine, which readily undergoes further homogeneous oxidations to provide the final aromatized products. The oxidation of the anionic species of the dihydropyridine is easier oxidized than the parent compound.

If we take into account that 1,4-DHP can be accumulated in membranes, due to its lipophilic nature (14), the aprotic medium can mimic adequately this condition. Consequently, our findings are of pharmacological significance because they give an answer, from a molecular point of view, for the previously reported (12–17) antilipoperoxidant effect of these extensively used type of drugs. Having this information, we can affirm that possible radical scavenging properties, in combination with a lipophilic character and high affinity for the DHP calcium channel receptors, could be the basis for the protective activity in free radical-involved pathologies that appears to accompany many instances of hypertension and also CNS disorders (36).

On the other hand, though the electrochemical technology used in this paper would appear rather strange to pharmaceutical scientists, we claim that this study is a good example wherein electrochemical techniques provides

excellent advantages in the scope of redox metabolic chemistry.

## ACKNOWLEDGMENTS

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