ELECTRON SPIN RESONANCE AND ELECTROCHEMICAL STUDIES OF OXIDATION PRODUCTS DERIVATES OF APOMORPHINE IN APROTIC SOLVENTS.

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RESUMEN

La apomorfina es una sustancia que presenta una acción contráctil del músculo liso, a través de un mecanismo dependiente de calcio extracelular. Esta función se manifiesta con un proceso de autoxidación, produciéndose la correspondiente o-quinona. Esta o-quinona se obtiene también por oxidación de la apomorfina en medio ácido, formándose una hidroxi-p-quinona por oxidación en condiciones básicas. Para caracterizar las especies derivadas de la apomorfina que se pueden generar ya sea por vía metabólicas o químicas y en especial las formas radicalarias producidas en estos procesos se realizó un estudio electroquímico y de Resonancia de Espín Electrónico para los metabolitos o y p-quinona en ausencia y presencia de iones Calcio. El estudio electroquímico utilizando voltametría cíclica de estos compuestos indicó que el mecanismo de reducción consta de dos pasos, la formación del radical semiquinónico y la producción finalmente de las hidroquinonas derivadas. No se observó ningún efecto de los iones calcio en las culplas de reducción de estos compuestos. Los radicales generados fueron caracterizados mediante la espectroscopia de resonancia de espín electrónica. Los cálculos teóricos de las constantes de acoplamiento estuvieron en concordancia con los obtenidos experimentales. Además, se detectó la existencia de especies radicalarias en el oxidación de la apomorfina a través de las técnicas de Spin Trapping. Finalmente, estos resultados estarían avalando la posible existencia de especies radicalarias en el mecanismo reductivo de las o y p-quinonas, así como también en la oxidación de la apomorfina las cuales podrían estar involucradas en la actividad contráctil.

PALABRAS CLAVES: ESR, apomorfina, quinonas, voltametría cíclica Spin Trapping.

SUMMARY

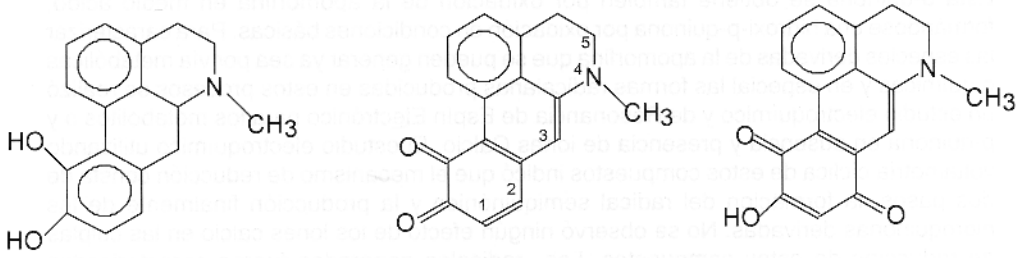
Apomorphine acts as contractile muscular agent through an extra cellular calcium mechanism dependent. This activity is accompanied with autoxidación process producing o-quinone derivates. o-Quinone is also obtained by apomorphine oxidation in acid medium; in basic conditions a hydroxi-p-quinone is formed. In order to characterize the apomorphine derivates species generated via metabolism or chemical mechanism, specially free radical forms, electrochemical and Electronic Spin Resonance studies were done to o and p-quinones metabolites in presence or absence of Calcium ions. The electrochemical study of these compounds using cyclic voltametric indicated that the reduction mechanism consists of two steps, the formation of semiquinonic radical and finally the production of hydroquinone derivatives. Calcium ions not modified the reduction couples of these compounds. Electronic Spin Resonance spectroscopy was used to characterize the radical

species generated. The hyperfine constants obtained by theoretical calculations were in agreement with the experimental values. Beside, the radical species generated through apomorphine oxidation were detected using Spin Trapping technique. Finally, these results would be indicating the possible existence of radicals species in the reduction mechanism of o and p-quinone as well oxidation process of apomorphine which could be involved in the contractile activity mechanism.

KEYWORK: ESR, apomorphine, quinones, Cyclic Voltammetry,Spin Trapping.

INTRODUCTION

In isolated rat aorta, apomorphine induces a contractile response depending on its autoxidation. This response implies calcium entry from the extracelular space because it can not be elicited in Ca^{2+}-free medium and could be due to the contractile activity of autoxidation products or radical intermediates in the oxidation process (1-2). In order to clarify the mechanism implicated in this mechanical response in the present work we report electrochemical studies on two oxidation derivatives of apomorphine (Figure 1), o-quinone 1 and p-quinone 2 in dimetylsulfoxide (DMSO), dimethylformamide (DMF) and Acetonitrile (ACN). The anion radicals produced in the electrochemical process were characterised by EPR. Also, the radical species generated through apomorhine oxidation were detected using DMPO.



Apomorphine 1 2

Fig.1 Apomorphine and o-quinone 1 and p-quinones derivated.

To estimate the theoretical hyperfine constants, INDO-SCF calculations were carried out. The geometry of each quinone in both spin-paired and free radical forms was fully optimized by AM1 methodology

EXPERIMENTAL

Samples. The compound 1 and 2 were synthesized and supplied as described in references (3-5).

Cyclic voltammetry. The DMSO, DMF and ACN (spectroscopy grade) and DMPO were supplied by Aldrich. The tetrabutylammonium perchlorate (TBAP) used as supporting electrolyte was purchased from Fluka. Cyclic voltammetry was carried out using a Weenking POS 88 instrument with a Kipp Zenen BD93 recorder, in DMSO or DMF (*ca* 1.0 x 10^{-2} mol dm^{-3}), under a nitrogen atmosphere, with TBAP (*ca* 0.1 mol dm^{-3}), using three-electrode cells. A mercury-dropping electrode was used as the working electrode, a platinum wire as the auxiliary electrode, and saturated calomel as the reference electrode. The radicals of compounds 1 and 2 were generated by electrolytic reduction *in situ* at room temperature.

ESR Spectroscopy. ESR spectra were recorded in 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.

Theoretical Calculations. Full geometry optimizations of these quinones in spin-paired and free radical forms were carried out by AM1 methods (6). INDO calculations were done employing the open shell UHF option.

RESULTS AND DISCUSSION

CYCLIC VOLTAMMETRY

Table I lists the values of voltammetric peaks and the anodic and cathodic currents for all compounds. The two quinones display comparable voltammetric behaviour, showing two well-defined reduction waves in DMSO DMF and ACN.

The first wave for both compounds studied corresponds to a reversible one-electron transfer in DMSO and DMF, however, in ACN were cuasireversible process. The reverse scan showed the anodic counterpart of the reduction waves. The breadth of cathodic wave at its half intensity has a relatively constant value of 60 mV. The intensity ratio ipa/ipc has a value close to one. According to the standard reversibility criteria this couple corresponds to a reversible diffusion-controlled one-electron transfer (7-8). This is attributable to the reduction of a quinone to a semiquinone stable anion radical at room temperature. The second cathodic peak is cuasirreversible in the whole range of sweep rates used (50-2000 mV/s). The results show that the scan rate increases, the ipa/ipc ratio not increases, indicating that this couple is a typical for cuasireversible charger transference (7-8). We can attribute this wave to the production of the corresponding hydroquinones. Figure 2 show the Voltamogram of p-quinone in ACN solvent.

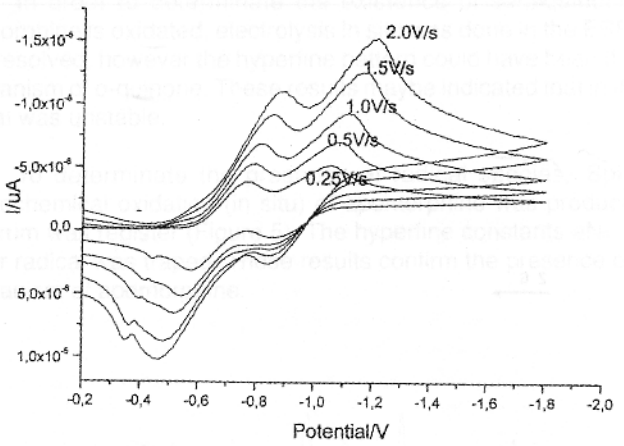


Fig.2 Cyclic Voltammetry of p-quinone in ACN at different sweep rates

Table I. Cyclic Voltammetric parameters vs saturated calomel electrode, sweep rate 1.0V/s

Molecules	E_{pc1}/V	E_{pa1}/V	DE/V	ip_a/ip_c	E_{pc2}/V	E_{pa2}/V	DE/V	ip_a/ip_c
O-quinone1								
DMSO	-0.68	-0.62	0.06	0.90	-1.10	-0.95	0.15	1.2
DMF	-0.58	-0.50	0.08	0.85	-1.20	-1.10	0.10	0.9
ACN	-0.75	-0.65	0.10	0.80	-1.25	-1.15	0.10	0.85
p-quinone 2								
DMSO	-0.50	-0.43	0.07	1.03	-1.00	-0.90	0.10	1.1
DMF	-0.55	-0.46	0.09	0.80	-1.15	-0.95	0.20	1.3
ACN	-0.63	-0.52	0.11	0.75	-1.20	-1.05	0.15	0.85

ELECTRON SPIN RESONANCE

The (in situ) electrochemical reductions to the radical forms in DMSO were carried out applying the potential corresponding to the first wave from the cyclic voltammetry experiments to each quinone.

The interpretation of the ESR spectra by means of a simulation process has led to the determination of the coupling constants for all magnetic nuclei and comparison with theoretical calculation.

O-quinone **1** radical was analyzed and simulated in terms of two doublets due to hydrogens of carbons 1 and 2 two triplets due to hydrogens of carbons 3 and 5, and one triplet due to one heterocyclic nitrogen atom (FIG. 3). The hyperfine constants are listed in Table II.

Table II. Experimental (DMSO) and calculated INDO hyperfine splitting (Gauss) for the quinone anion radicals

Molecules	aH (1)	aH (2)	aH (3)	aN (4)	aH (5)	aH(OH)
0-quinone 1						
EXP	5.20	2.20	1.60	0.60	0.80	-
INDO	5.85	3.45	1.50	0.45	0.69	-
p-quinone 2						
EXP	2.60	-	1.00	0.80	0.30	1.90
INDO	2.40	-	0.89	0.50	0.19	2.20

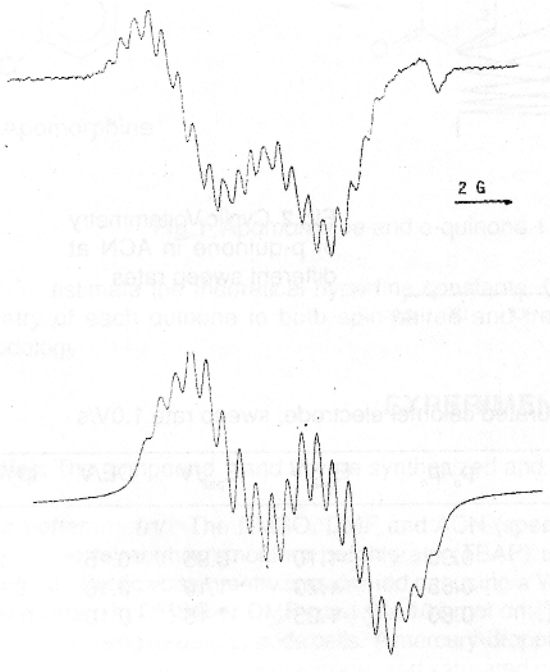


Fig.3 Experimental and simulated ESR spectra of o-quinone derivated.

The EPR spectrum of p-quinone **2** radical was simulated in terms of two doublets due to hydrogens of carbon numerated 1 and hydrogen of hydroxil group, two triplets due to hydrogens of carbons 3 and 5 and one triplets due to and heterocyclic nitrogen atom (FIG. 4). The hyperfine constants are listed in Table II.

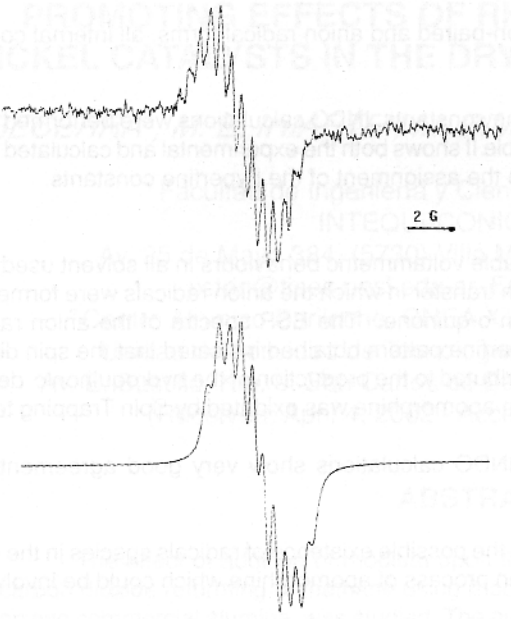


Fig.4 Experimental and simulated ESR spectra of p-quinone derivated.

In order to determinate the existence of semiquinonic radical specie generated when the apomorphine is oxidated, electrolysis in situ was done in the ESR spectrometer. The ESR spectrum was poor resolved, however the hyperfine pattern could have been the same which was obtained in reduction mechanism of o-quinone. These results maybe indicated that in the oxidation conditions the semiquinonic radical was unstable.

To determinate the presence of radical species Spin trapping technique was using. The electrochemical oxidation (in situ) of apomorphine was produce in presence of DMPO, a typical ESR spectrum was register (Figure 5). The hyperfine constants aN= 14.2 G and aH=21.5 G indicated that C center radical was traped. These results confirm the presence of o-semiquinone radical in the oxidation mechanism of apomorphine.

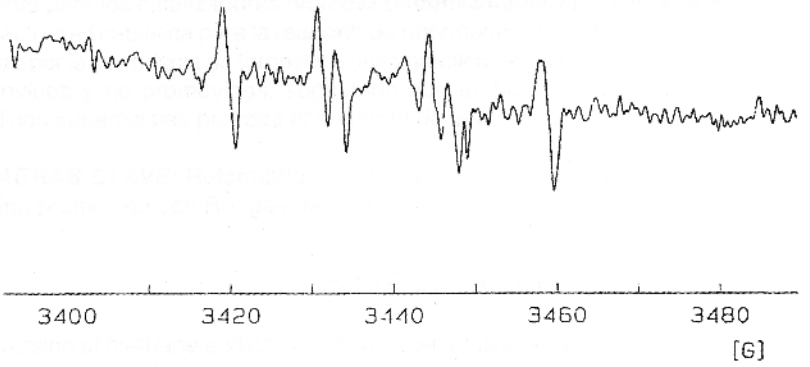


Fig.5 ESR spectra of apomorphine radical generated by electrochemical oxidation in presence of 100 mM of DMPO.

THEORETICAL CALCULATIONS

For the AM1 calculations of both electron-paired and anion radical forms, all internal coordinates were completely optimised.

In order to obtain the theoretical hyperfine constants, INDO calculations were performed using the geometries obtained using AM1 calculations. Table II shows both the experimental and calculated hyperfine constants. These results are in agreement with the assignment of the hyperfine constants.

CONCLUDING REMARKS

Both quinones studied showed comparable voltammetric behaviours in all solvent used. The first wave corresponded to a reversible one-electron transfer in which the anion radicals were formed. The p-quinone showed lower reduction potential than o-quinone. The ESR spectra of the anion radicals for molecules **1** and **2** were well resolved. The hyperfine pattern obtained indicated that the spin distribution is not homogeneous. The second peak was attributed to the production of the hydroquinonic derivatives. Also, o-semiquinone radical was detected when apomorphine was oxidated by Spin Trapping technique.

The hyperfine constants obtained by INDO calculations show very good agreement with the experimental ones.

Finally, these results would be indicating the possible existence of radicals species in the reduction mechanism of o and p-quinone as well oxidation process of apomorphine which could be involved in the contractile activity mechanism.

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