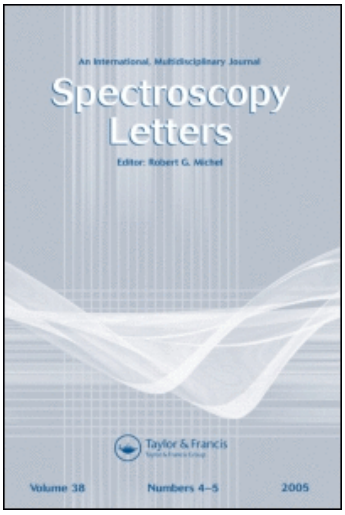

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Cyclic Voltammetry and Electron Paramagnetic Resonance Studies of Some Analogues of Nifurtimox.

C. Qlea-Azar[a]; Ana Maria Atria[a]; Fernando Mendizabal[b]; Rossana di Maio[c]; G. Seoane[c]; Hugo Cerecetto[c]
[a] Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile [b] Departamento de Química Facultad de Ciencias, Universidad de Chile, Santiago, Chile [c] Cátedra de Química Orgánica, Universidad de la República, Montevideo, Uruguay

To cite this Article Qlea-Azar, C. , Atria, Ana Maria , Mendizabal, Fernando , Maio, Rossana di , Seoane, G. and Cerecetto, Hugo(1998) 'Cyclic Voltammetry and Electron Paramagnetic Resonance Studies of Some Analogues of Nifurtimox.', Spectroscopy Letters, 31: 1, 99 — 109
To link to this Article: DOI: 10.1080/00387019808006764
URL: http://dx.doi.org/10.1080/00387019808006764

SPECTROSCOPY LETTERS, 31(1), 99-109 (1998)

CYCLIC VOLTAMMETRY AND ELECTRON PARAMAGNETIC RESONANCE STUDIES OF SOME ANALOGUES OF NIFURTIMOX.

C. Olea-Azar[1], Ana María Atria[1], Fernando Mendizabal[2], Rossana di Maio[3], G. Seoane[3] and Hugo Cerecetto[3]

1. Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, Santiago 1, Chile

2. Departamento de Química Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

3. Cátedra de Química Orgánica, Universidad de la República, Montevideo Uruguay

ABSTRACT

The EPR of radicals obtained by electrolytic reduction of some new analogues of the antiprotozoal drug nifurtimox were measured in DMSO and DMF. The electrochemistry of these compounds was studied using cyclic voltammetry. AM1 and INDO semiempirical molecular orbital calculations were performed to obtain the optimized geometries and spin distribution, respectively. Density functional theory was used to rationalize the reduction potentials of these compounds.

INTRODUCTION

Parasitic diseases in tropical and subtropical areas constitute a major health and economic problem. Chaga's disease, produced by several strain of *Trypanosoma cruzi*, affects approximately 24 million people from Southern of California to Argentina and Chile (1). Nifurtimox and benznidazole are currently used to treat this disease. A characteristic

EPR (electron paramagnetic resonance) signal corresponding to the nitro anion radical ($R\text{-}NO_2\cdot$) appears when nifurtimox is added to intact *T. cruzi* cells (2). This and other experiments (3-5) suggest that intracellular reduction of nifurtimox followed by redox cycling, yielding $O_2^{\cdot-}$ and H_2O_2, may be the major mode of action against *T. cruzi*. However, the use of nifurtimox has the disadvantage of its side effects (6). Mester et al. (7) synthesized new nifurtimox analogues in which the tetrahydrothiazine moiety was replaced by unsaturated five- and six-membered nitrogen heterocycles. Most of the new compounds proved to be more effective than nifurtimox (8).

In general, the biological effects of nitroheterocyclic compounds, especially in *T. cruzi*, are believed to involve redox cycling of these compounds and oxygen radical production, two processes in which the nitroanion radicals play an essential role. (9).

In the present work, we report electrochemical studies on four new semicarbazone derivatives of 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde (FIG.1) in dimetylsulfoxide (DMSO) and dimethylformamide (DMF). The formal one electron transfer potential for the new nitroheterocyclic compounds were compared with that of Nifurtimox. The anion radicals produced in the electrochemical process were characterized by EPR.

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

Finally, we have applied the approximate density functional theory (DFT) (10) using the amsterdam density functional (ADF) (11) program for obtain the electron affinities of these compounds.

EXPERIMENTAL SECTION AND THEORETICAL METHODS

Reagents

The DMSO and DMF (spectroscopy grade) were obtained from Aldrich. The tetrabutylammonium perchlorate (TBAP) used as the supporting electrolyte was obtained from Fluka.

The new semicarbazone derivatives were prepared using a three step synthetic route from 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde.

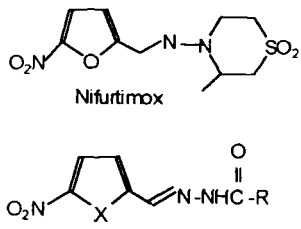

FIGURE 1

	X	R
Molec.1	O	$NH(CH_2)_5CH_3$
Molec.2	S	$NH(CH_2)_5CH_3$
Molec.3	O	Morfoline
Molec.4	S	Morfoline

Electrochemical and EPR Measurement

Cyclic voltammetry was carried out using a Weenking POS 88 instrument with a Kipp Zenen BD93 recorder, in DMSO or DMF (ca. $1.0x10^{-2}$ moles dm^{-3}), under a nitrogen atmosphere, with TBAP (ca. 0,1 moles 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 were generated by eletrolyltical reduction *in situ* at room temperature. EPR spectra were recorder in the x band (9.85 GHz) on the Bruker ECS 106 spectrometer using a rectangular cavity with a 50 KHz field modulation. The hyperfine splitting constants were estimated to be accurate within 0.05 G.

THEORETICAL CALCULATION

Full geometry optimizations of these nitrocompounds in spin-paired and free radical forms were carried out by AM1 methods (12). INDO calculations were done employing the open shell UHF option. The electron affinities were calculated using ADF calculations. This

approach solves the Kohn-Sham equations within the local density approximation (LDA) with gradient corrections for the exchange and correlation potentials. The ADF basis set used was a doublet-ξ plus polarization functions. The LDA exchange correlations suggested by Vosko (13) were used. The nonlocal gradient corrections for exchanges proposed by Becke and the nonlocal correction for correlation proposed by Perdew (14) were used. The 3d orbitals were incorporated in the calculations corresponding to the nitrothiophenes derivated.

RESULTS AND DISCUSSION

CYCLIC VOLTAMMETRY

Table 1 lists the values of the voltammetric peaks and the anodic and cathodic currents for all compounds. All nitroheterocyclic compounds display comparable voltammetric behavior, showing two well-defined reduction waves in DMSO and DMF.

The first wave for all compounds studied corresponds to a reversible one-electron transfer. The reverse scan showed the anodic counterpart of the reduction waves. The breadth of the 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 (FIG. 2). According to the standard reversibility criteria this couple corresponds to a reversible diffusion-controlled one-electron transfer. It is attributable to the reduction of $R\text{-}NO_2$ to $RNO_2^{\cdot-}$, a stable anion radical at room temperature. The second cathodic peak is irreversible in the whole range of sweep rates used (50-1000 mV/s). We can attribute this wave to the production of the hydroxylamine derivative.

EPR SPECTRA

The electrochemical reductions to the radical forms (*in situ*) in DMSO were carried out applying the potential corresponding to the first wave for the nitroheterocyclic compounds, as obtained from the cyclic voltammetry experiments.

The interpretation of the EPR spectra by means of a simulation process has led to the determination of the coupling constants for all magnetic nuclei.

Table 1Cyclic Voltammetric parameters vs saturated calomel electrode, sweep rate 0.20V/s

Molecules	E_{pc1}/V	E_{pa1}/V	ΔE/V	ip_a/ip_c	E_{pc2}/V	E_{pa2}/V
1						
DMSO	-0.83	-0.76	0.06	0.80	-1.38	-
DMF	-0.84	-0.78	0.06	0.85	-1.47	-
2						
DMSO	-0.78	-0.68	0.1	1.03	-1.38	-
DMF	-0.79	-0.69	0.1	0.97	-1.38	-
3						
DMSO	-0.81	-0.75	0.06	1.00	-1.63	-
DMF	-0.85	-0.79	0.06	1.10	-1.75	-
4						
DMSO	-0.79	-0.72	0.07	0.96	-1.38	-
DMF	-1.00	-0.93	0.07	0.97	-1.30	-
Nifurtimox						
DMSO	-0.91	-0.85	0.06	1.01	-1,60	-
DMF	-0.89	-0.84	0.05	0.83	-1.30	-

Molecules **1** and **2** presented the same hyperfine pattern, complete resolved into 36 lines. These spectra were simulated in terms of one triplet due to the nitrogen nucleus of the nitro group, two doublets due to hydrogens 3 and 4 which are not equivalent, and one triplet due to the nitrogen of the azomethine bond (FIG. 3). The hyperfine constants are listed in Table 2.

The EPR spectrum of molecule 4 was simulated in terms of one triplet due to the nitrogen nucleus of the nitro group and two doublets due to hydrogens 3 and 4 which are not equivalent. This hyperfine pattern indicated that the unpaired electron is delocalized only in the heterocyclic ring (FIG. 4). The hyperfine constants are listed in Table 2. The anion radical of the molecule **3** could not be detected by EPR.

THEORETICAL CALCULATIONS

For the AM1 calculations of both electron-paired and anion radical forms, all internal coordinates were completely optimized. In both electron-paired and anion radical structures the extended conformations of the side chain are the most stable. In addition, in all molecules in both neutral and anion radical forms the nitro group lies in the ring plane.

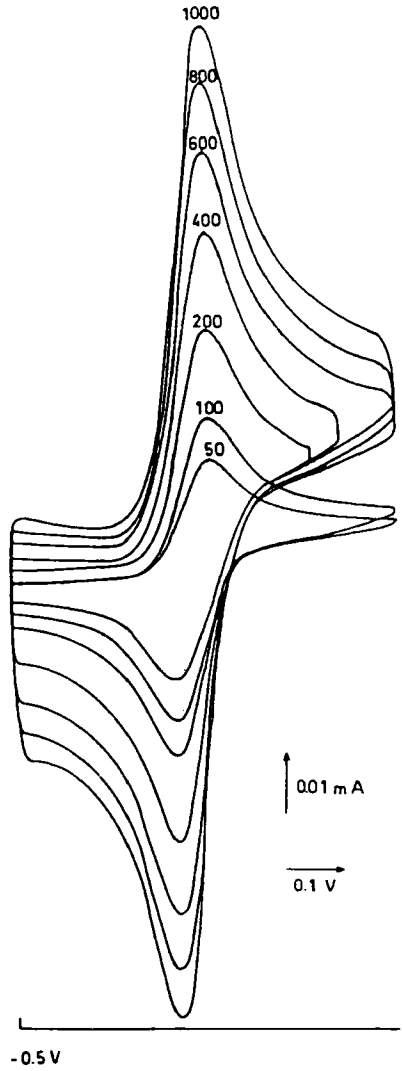

FIGURE 2

First couple obtained at differents sweep rates used of molecule 1 in DMSO.

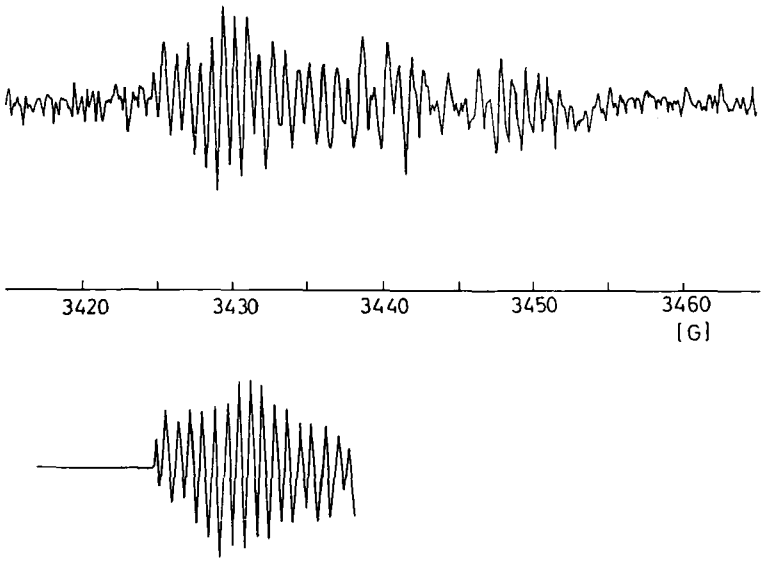

FIGURE 3

Top: EPR experimental Spectrum of the radical-anion of molecule 2 in DMSO.

Bottom: Computer Simulation of the Same Spectrum.

Table 2 Experimental (DMSO) and calculated INDO hyperfine splitting (Gauss) for the anion radicals investigate

Molecule	aN (NO2)	aH (3)	aH (4)	aN (C=N)	aN (N=N)
1					
EXP	8.97	5.34	1.38	2.03	0.53
INDO	7.90	4.95	1.05	2.40	0.34
2					
EXP	7.39	5.65	2.39	1.52	0.87
INDO	6.33	5.02	1.95	1.34	0.48
3					
EXP	-	-	-	-	-
INDO	-	-	-	-	-
4					
EXP	8.38	5.35	1.28	-	-
INDO	7.35	4.83	0.95	-	-

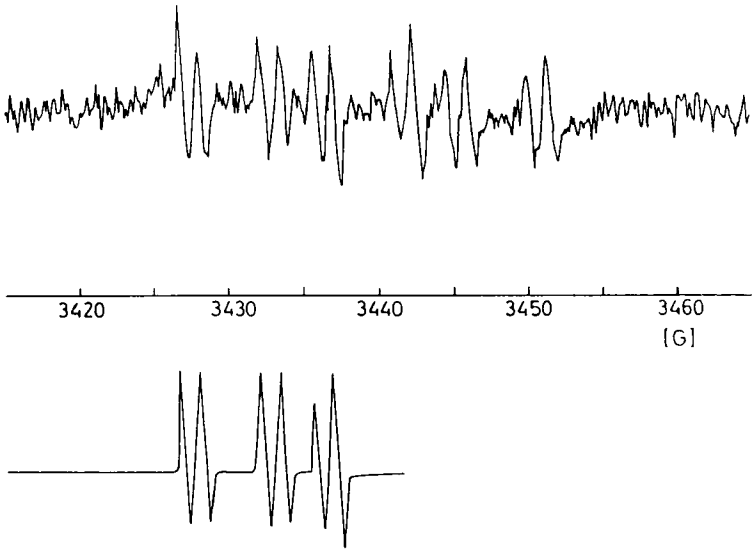

FIGURE 4

Top: EPR experimental Spectrum of the radical-anion of molecule 4 in DMSO.
Bottom: Computer Simulation of the Same Spectrum.

The examination of the MO coefficients indicated that the SOMOs of the anion radical forms have antibonding pz characteristics and is mainly localized on the nitro group. However, the unpaired electron was partially localized on the two nitrogens of the side chain.

In order to obtain the theoretical hyperfine constants, INDO calculations were performed using the geometries obtained using AM1 calculations. Table 2 shows both the experimental and calculated hyperfine constants. Comparison of these results shows in agreement with the assignment of the hyperfine constants.

In order to estimate the ability of the molecules to accept electrons, the electron affinities were calculated using ADF and compared with the formal reduction potentials of the anion radical forms (Table 3). The calculations show that the electron affinities of these compounds increase from the nitrothiophenes to the nitrofuran derivatives, in agreement

Table 3 Electron affinities (kcal/mol) and reduction potential (in DMSO)

Molecule	LDA	LDA +NL(Becke)	LDA+NL x C (BeckePerdew)	Reduction pontential
1	-35.2501	-39.6952	-35.5924	-0.83
2	-38.4684	-42.5885	-38.6695	-0.78
3	-34.2105	-37.8665	-34.1324	-0.81
4	-36.7721	-39.9965	-36.504	-0.79

with the experimental results which indicated that the nitrothiophene derivatives are more easily reduced.

CONCLUDING REMARKS

All nitrocompounds studied presented comparable voltammetric behaviors in both solvent used. The first wave corresponded to a reversible one-electron transfer in which the anion radicals were formed. The EPR spectra of the anion radical for molecules **1** and **2** presented very good resolution. The hyperfine splittings in these spectra indicated that the electron delocalization involved the side chain nitrogens. However, the EPR spectrum for molecule **4** indicated that in this case the delocalization was limited to the heterocyclic ring. The second peak was attributed to the production of the hidorxylamine derivated.

The hyperfine constants obtained by INDO calculations show very good agreement with the experimental ones. Finally, the electron affinities calculated by ADF methodology show a very good correlation with the first wave potentials.

References

1.- Schofield C.J., Control of Chagas' disease vectors. Br. Med. Bull (1985), 41, 187-194.

2.- Docampo R and Stoppani A.O.M., Generation of oxygen-reduction derivates induced by

nifurtimox and other nitrocompounds in *Trypanosoma cruzi*. Medicina (1980), 40 (Suppl.1) 10-16.

3.- Docampo R. and Moreno S.N.J. Free radical intermediates in the trypanocidal action of drugs and phagocytic cells. Free radicals in Biology (Edited by W.A. Pryor) (1984)Vol VI pp 243-288. Academic Press, New York.

4.- Docampo R. and Moreno S.N.J. Free radical intermediates in the trypanocidal action of drugs and phagocytic cells. Oxygen radicals in Chemistry and Biology (Edited by W.Bors) (1984) pp 749-751.

5.- Docampo R. Moreno S.N.J., Stoppani A.O.M, Leon W., Cruz F.S., Villalta F. and Muniz R.P.A. Mechanism of nifurtimox toxicity in different forms of *Tripanosoma cruzi*. Biological Pharmacology (1981), 30, 1947-1951.

6.- Goodman Gilman A., Goodman L.S., Rall T.W. Las bases farmacologicas y terapeuticas, Octava edicion. Editorial Medica Panamericana, Buenos Aires, 1991 pp 984.

7.- B. Mester, J. Elguero. R.M. Claramunt, S. Castanys, M.L. Mascaró A. Vilaplana and P. Molina, Activity against *Tripanosoma cruzi* of new analogues of nifurtimox. Archives in Pharmacy (1987), 320, 115-120.

8.- M. Dubin, S.H. Fernandez Villamil, M. Paulino de Blumenfeld and A.O.M. Stoppani, Inhibition of microsomal lipid peroxidation and cytochrome P-450-catalyzed reactions by nitrofuran compounds, Free Radical Research Communications, (1991), 14, 419-431.

9.- S.G. Goijman and A.O.M Stoppani, Oxygen radical and macromolecule turnover in *Tripanosoma Cruzi*, Oxidative Damage and Related Enzymes (Eds. G. Rotilio and J.V. Bannister) Harwood Academic Publisher, London, New York, 1984, pp 216-221.

10.- R.G. Parr and W. Rang, Density Functional Theory of Atoms and Molecules, Oxford University Press, New York, 1989.

11.- E.J. Baerendes, D.E., Allis and P. Ros, Self.Consistent molecular Hartree-Fock-Slater Calculations. The Computational procedure, Chem. Phys. (1990), 2, 41.

12.- M.J.S. Dewar, E.G. Zoebish, E.F. Healy. J.J.P Steward, AM1: A New General Porpose Quantum Mechanical Molecular Model, J. Am. Chem Soc., (1985), 107, 3902-3909.

13 - S.H. Vosko and L. Wilk, Accurate spin-dependent electron liquid correlation enrgies for local spin density calculations a critical analysis, J. Phys. (1983), B16, 3687.

14.- J. Perdew, Density-Functional approximation for the correlation energy of the inhomegeous electron gas, Phys. Rev., (1986), B33, 8822.

Date Received:June 4, 1997
Date Accepted: July 28, 1997