Electrochemical and Electron Spin Resonance Studies of Selected Benzazolo[3,2-*a*]quinolinium Salts

Osvaldo Cox* [a],[b], José A. Dumas [a], Luis A. Rivera [a], Carmelo García [c] and Antonio E. Alegria [c]

[a] Department of Chemistry, University of Puerto Rico, Rio Piedras Campus
PO Box 23346, San Juan, Puerto Rico 00931-3346
[c] Department of Chemistry, University of Puerto Rico, Humacao campus
CUH Station, Humacao, Puerto Rico 00791

Received July 20, 1998
Revised April 25,1999

The electrochemical reduction of the chloride or perchlorate salts of benzazolo[3,2-*a*]quinolinium ion and several of its analogues is reported. The compounds studied are the perchlorate salt of 3-nitrobenzothiazolo- and 3-nitro-9-methoxybenzothiazolo[3,2-*a*]quinolinium, and the chloride salts of 7-ethyl-, 3-nitro-7-methyl-, 3-nitro-7-ethyl-, 3-nitro-7-isopropyl-, 3-nitro-7-butyl- and 3-nitro-7-benzylbenzimidazolo[3,2-*a*]-quinolinium, respectively. Cyclic voltammetry of the corresponding 3-nitrobenzothiazolo[3,2-*a*]quinolinium derivatives in *N,N*-dimethylformamide shows an irreversible peak potential at -0.6 and a quasi-reversible peak at -(1.2-1.3) volts, respectively, relative to the standard calomel electrode. In contrast, the corresponding 3-nitrobenzimidazolo[3,2-*a*]quinolinium derivatives show, in general, reversible peaks at near -0.8 and -(1.2-1.4) volts, respectively. Upon electrolytic reduction, only the nitro-substituted derivatives produced observable electron paramagnetic resonance electron spin resonance spectra. This observation is explained in terms of the stabilization of the radicals produced by the nitro group. Theoretical MM+/AM1/UHF calculations support the idea that the larger nitrogen splitting is caused by N-12 and the minor splittings by N-7 in the benzimidazolo[3,2-*a*]quinolinium ion series.

J. Heterocyclic Chem., **36**, 943 (1999).

Introduction.

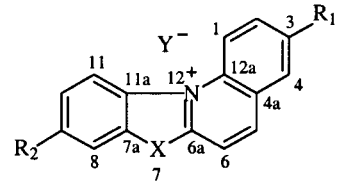
Several years ago Cox, *et al.* [1] reported for the first time the synthesis of 3-nitrobenzothiazolo- **2**, see Scheme 1, and 3-nitrobenzoxazolo[3,2-*a*]quinolinium perchlorates *via* the photochemically induced cyclization of the corresponding 2-(2-chloro-5-nitrostyryl)benzothiazole and 2-(2-chloro-5-nitrostyryl)benzoxazole. Compound **2** displays good antitumor activity against Erhlich ascites tumor and P 388 leukemia cells in mice [1]. Further studies have shown that **2** inhibits deoxyribonucleic acid, ribonucleic acid and protein synthesis [2], interacts with deoxyribonucleic acid by the intercalation mechanism [3], stimulates the *in vivo* regeneration of the lens of adult newt *Notophthalmus viridescens* [4,5], induces the differentiation of HL-60 leukemia cells [6] and inhibits topoisomerase II activity [7]. More recently it has been shown that other benzazolo[3,2-*a*]quinolinium salts also inhibit topoisomerase II activity [8,9].

A complete chemical shift assignment of the ^{1}H and ^{13}C nmr spectra of selected benzazolo[3,2-*a*]quinolinium salts and related systems using two-dimensional techniques was reported by Cox and coworkers [10]. Additionally, in order to obtain more insight into the structure of these compounds, the single crystal X-ray structure of 1-acetoxy-1,2-dihydrothiazolo[3,2-*a*]quinolinium perchlorate was determined [11].

The structural features present in benzazolo[3,2-*a*]quinolinium salts include a planar tetracyclic heteroaromatic system incorporating a positive quaternary nitrogen atom and a fused benzazole nucleus as shown in Scheme 1. These

Scheme 1

Structure and Numbering System of Benzazolo[3,2-*a*]quinolinium Salts



Compound	X	R_1	R_2
1	S	H	H
2	S	NO_2	H
3	S	NO_2	MeO
4	N-CH_2CH_3	H	H
5	N-CH_3	NO_2	H
6	N-CH_2CH_3	NO_2	H
7	N-$CH(CH_3)_2$	NO_2	H
8	N-$(CH_2)_3CH_3$	NO_2	H
9	N-$CH_2C_6H_5$	NO_2	H

systems include an iminium ion species and most of them also include a nitro moiety, both of which are capable of undergoing redox reactions [12]. In fact, the reductive activation of these salts in water as evidenced by the benzazolo[3,2-*a*]quinolinium salts-mediated reduction of ferricytochrome in the presence of xanthine/xanthine oxidase has been reported [13]. This process is proposed to occur *via* the formation of the corresponding benzazolo[3,2-*a*]quinolinium radical intermediates [13]. However, direct evidence for the observation of these radical intermediates as well as their characterization has not

yet been presented. In addition, it is important to compare our results with those determined in studies involving other nitroaromatic compounds [14-16]. In this work we present electrochemical data which characterize the redox behavior of a series of selected benzazolo[3,2-*a*]quinolinium salts. The electrochemically-generated radical intermediates are studied using electron spin resonance spectroscopy.

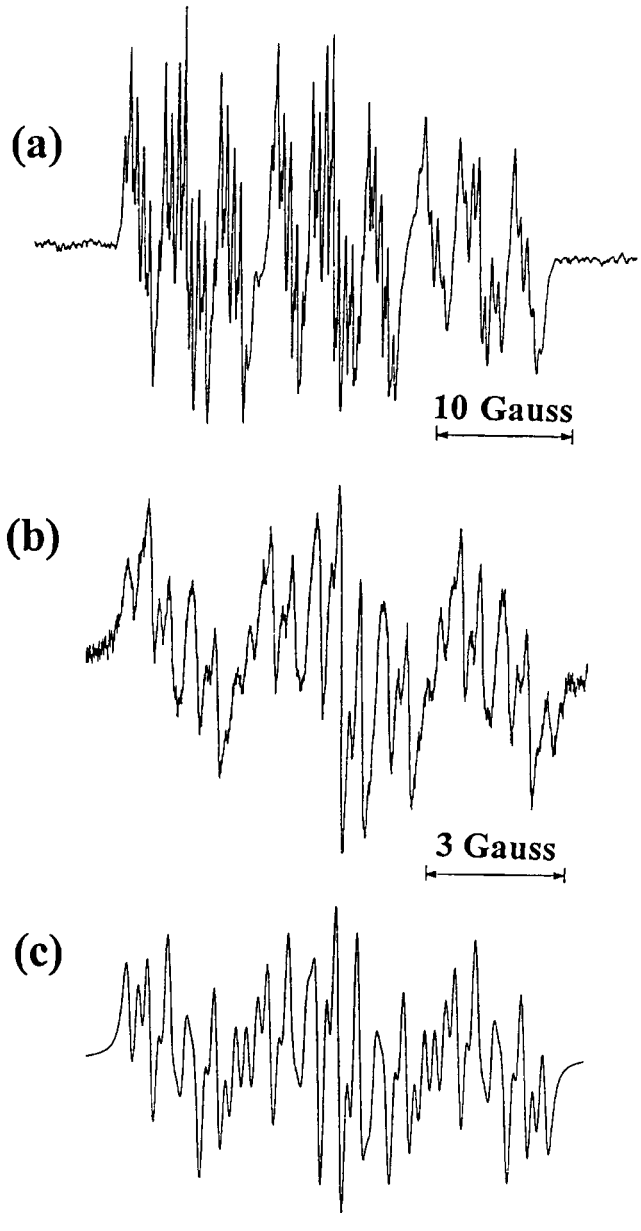


Figure 1. (a) Electron spin resonance spectrum obtained during the electrolytic reduction of **2** in nitrogen-saturated *N,N*-dimethylformamide containing 1 m*M* of tetrabutilammonium perchlorate. (b) Resolved group of peaks corresponding to the lowest field 1/3 fraction of the spectrum shown in (a). (c) Simulated Electron spin resonance spectra corresponding to the groups of peaks depicted in (b) using a_N (1N) = 0.41 G, a_N (1N) = 0.28 G, a_H (1H) = 4.09 G, a_H (1H) = 2.62 G and a_H (1H) = 1.04 G.

EXPERIMENTAL

The synthesis of **1** [13], **2** [1], **4**[13] and **6** [13] were reported previously. The synthesis of **3, 7, 8** and **9** will be the subject of a forthcoming paper from this laboratory [17].

Radical Generation and Characterization.

Free radicals derived from benzazolo[3,2-*a*]quinolinium salts were generated by electrolytic reduction, using a Jameco potentiostat, in nitrogen-purged *N,N*-dimethylformamide containing 0.1 *M* tetrabutylammonium perchlorate. The transient formation of red-colored reduction products was observed. Reduction occurred on a mercury cathode localized inside the flat portion of an electron paramagnetic resonance quartz cell (60 x 10 x 0.25 mm) in the electron spin resonance instrument cavity. The electron spin resonance spectra were recorded on an X-band Varian E-9 spectrometer. Coupling constants were calibrated using a value of $a_N = a_H = 14.92$ Gauss for the hydroxyl radical adduct of 5,5-dimethyl-1-pyrroline 1-oxide in aqueous solution [18]. Electron spin resonance spectral simulations were performed using WINSIM [19].

Cyclic Voltammetry Study.

Cyclic voltammograms were made with a system consisting of a PARC Model 175 Universal Programmer, a PARC Model 173 potentiostat provided with current-to-voltage converter Model 176 and a Soltec Model 6431 X-Y Recorder. A 5.0 ml polarographic cell was used with platinum wire as working and auxiliary electrodes. A saturated calomel electrode was used as reference. The platinum working electrode was cleaned and pretreated by immersing it in sulfochromic solution, washing with distilled water and with acetone. It was then immersed in supporting electrolyte and maintained for 1 minute at -1.4 V. All the compounds were dissolved in 5.0 ml of argon-saturated *N,N*-dimethylformamide containing 0.1 *M* tetrabutylammonium perchlorate. Measurements were made under an argon stream. All voltammograms were measured at three different scan rates. The formation of red-colored transient products was also observed in the second reduction peak of benzazolo[3,2-*a*]quinolinium salts.

Semiempirical Theoretical Calculations.

Geometry optimization using combined molecular mechanics (MM+) and open shell unrestricted SCF quantum chemical calculations (AM1/UHF, Convergence limit 0.01, RMS gradient 0.0001 Kcal [Å mol], no charge and Polak-Ribiere conjugated gradient) were done with Hyperchem 5.1 (Hypercube Inc, Miami, Fl.). All molecular parameters were obtained from a single point calculation of the AM1 optimized structures. The probability of having other local minima with lower energies near the considered minimum was decreased by using different starting structures. The spin density (ρ) was obtained from the difference betweeen α- and β-electron population. The isotropic contribution of the spin density is calculated from the unpair electron population of s-atomic orbitals centered on each nucleous. The corresponding anisotropic part is calculated using the p-orbitals.

Results and Discussion.

Electron Paramagnetic Resonance Spectroscopy.

All electrolytic reductions produced a steady state concentration of the corresponding benzazolo[3,2-*a*]quinolinium-derived radical, as detected by electron spin resonance spectroscopy. The

Table 1

Electron spin resonance Hyperfine Coupling Constants Corresponding to Spectra Obtained During the Electrochemical Reduction of Benzazolo-[3,2-*a*]quinolinium Salts in *N,N*-Dimethylformamide at 25°C.

Compound	a_N/Gauss	a_H/Gauss
2[a]	10.17 (1N)	3.29 (2H's)
3	10.29 (1N), 0.43 (1N), 0.30 (1N)	3.89 (1H), 3.57 (1H), 1.14(1H)
5	10.30 (1N), 0.42 (1N), 0.30 (1N)	4.32 (1H), 2.75(1H), 1.03(1H)
6	10.88 (1N), 0.41 (1N), 0.28 (1N)	4.09 (1H), 2.62(1H), 1.04(1H)
7	10.62 (1N), 0.43 (1N), 0.26 (1N)	4.03 (1H), 2.61(1H), 1.06(1H)
8	10.87 (1N), 0.40 (1N), 0.25 (1N)	4.23 (1H), 3.36 (1H), 0.90 (1H)
9	10.20 (1N), 0.37 (1N), 0.27 (1N)	4.02 (1H), 3.11 (1H), 0.91 (1H)

[a] Too broad to resolve other couplings

Table 2

Total Spin Density (ρ) for some Atoms of the Reduced Form of Nitro Substituted Quinolinium Derivatives

Compound	Torsion Angle [a] (Degree)	Atomic Spin Density C-1 [b]	C-2 [c]	C-5	C-6	N-7	C-8 [d]	C-9 [e]	N-12	C-6a
2	9.7	-0.17	0.21	0.64	-0.49	0.02	-0.05	0.07	0.13	0.58
5	6.1	-0.38	0.40	0.69	-0.50	0.03	-0.19	0.21	0.12	0.58
6	5.1	-0.37	0.41	0.69	-0.49	0.02	-0.27	0.30	0.12	0.56
7	4.4	-0.33	0.36	0.68	-0.50	0.03	-0.16	0.18	0.12	0.57

[a] measured for atoms 11-11a-12-12a. [b] similar values were obtained for atoms C-3 and C-4a. [c] similar values were obtained for atoms C-4 and C-12a. [d] similar values were obtained for atoms C-10 and C-11a. [e] similar values were obtained for atoms C-11 and C-7a.

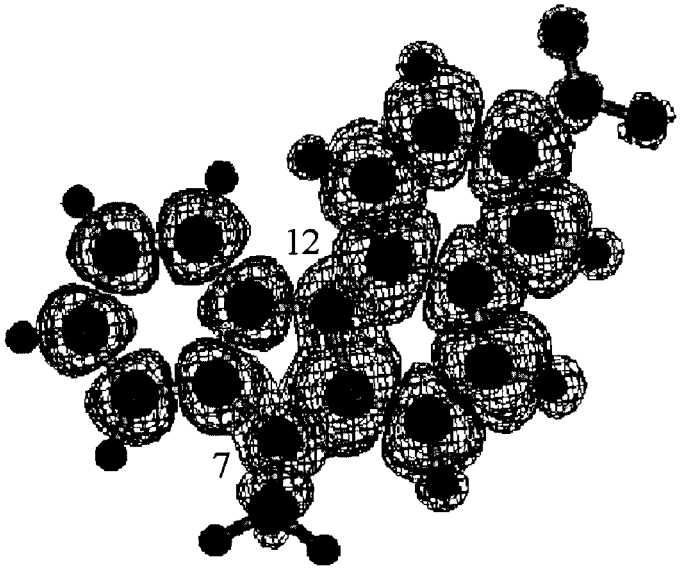


Figure 2. Calculated total spin density distribution using MM+/AM1/UHF for reduced 3-nitro-7-methylbenzylbenzimidazolo-[3,2-*a*]quinolinium (**5**).

radical concentration slowly decayed if the current flow was stopped, with the exception of the radicals corresponding to **2** and **4**. No electron paramagnetic resonance signal could be obtained for **2** and **4** indicating that the presence of a nitro group is essential for the formation of a relative persistent radical. These observations are consistent with the highly irreversible cyclic voltammogram obtained for these two compounds as compared to other quinolinium derivatives, see below. An example of a resolved electron spin resonance spectrum is shown on Figure 1. The corresponding electron paramagnetic resonance hyperfine coupling constants are depicted on Table 1.

We first assumed that the largest nitrogen hyperfine coupling constant corresponded to the nitro group, in analogy to what is observed for other nitroaromatic anion radicals [16]. However, this assumption was disproved by molecular orbital calculations. The MM+/AM1/UHF- calculated spin density must correlate to the electron spin resonance hyperfine constants according to the general McConnell relationship [20]. As expected, only a small s-orbital isotropic contribution to the total spin density was found, since the quinolinium derivatives are almost planar with a conjugated π-system (see Table 2). For C-atoms and most of the *N*-atoms the *S*-contribution to the total spin density is only <6%, but for N-7 it is 18%. This is due to the fact that this atom is at the center of the torsion center inducing a higher contribution of s-orbitals to the molecular orbitals. Nevertheless, this atom has an average total spin density of only 0.12. Table 2 contains the AM1 single point calculated density parameters for several atoms of the studied compounds. These values indicate that the spin density is distributed within the quinolinium moiety containing the nitro group where the higher values reside at C-5 and C-6a. The 6-membered rings show a symmetric alternate distribution of spin density with the exception of that containing the N-12 atom. The highest spin densities in this ring occurs at carbon atoms positioned ortho and para relative to the N-12 atom. In terms of the nitrogen atoms, the largest spin density corresponds to the N-12 iminium nitrogen, followed by N-7 and almost no spin density is associated with the nitro group (with an average spin density of only 0.03). This nitrogen atom at the 12-position, is *para* relative

to the NO_2 group. Same theoretical calculations on unprotonated and protonated *p*-aminonitrobenzene radical ions demonstrated that the spin density concentrates at the nitro group for the NH_2-substituted compound, while a very small spin density is assigned to the nitro group when the amino group is quaternated, as it appears in all compounds of this work (See Table 3).

Table 3

Total Spin Density (ρ) for *para*-Isomers of Nitrobencene [a]

		Atomic Spin Density						
R	C-1	C-2	C-3	C-4	N-7	O-8	O-9	N-12
H	0.01	0.44	-0.39	0.59	0.12	0.11	0.11	--
		0.14	-0.03	0.21	0.12			
CN	0.20	0.27	-0.23	0.50	0.04	0.08	0.08	0.18
		0.11	-0.01		0.08			0.07
	0.14	0.10	-0.01	0.16	0.17	0.16		0.03
NH_2	-0.01	0.45	-0.43	0.61	0.11	0.10	0.10	0.01
		0.25	-0.05		0.14			0.02
NH_3^+	-0.24	0.23	-0.24	0.70	0.01	-0.01	-0.01	0.36

[a] For each substituent, first row contains the results of our calculation, second row is from reference 23 and third row from reference 24.

Figure 2 shows a 3-D representation of the total spin density for the reduced form of **5**. Similar plots were obtained for all nitro derivatives. For compounds having a singly occupied orbitals with π-character, the hyperfine interactions only occur due to the different local α- and β-electron densities, which generate different electronic environments for the σ-system [21]. All the compounds studied here display similar hyperfine splitting constants independent of the type of X, R_1 and R_2 substitution. This is consistent with the similar spin densities obtained for the corresponding atoms in Table 2.

Cyclic Voltammetry.

Reduction of Benzothiazolo[3,2-*a*]quinolinium Salts.

The electrochemical behavior of several benzazolo[3,2-a]quinolinium salts is summarized in Table 4. The reduction of **2** and **3**, produce major peaks at voltages near -0.6 and -1.2 V. The ratio $i_p / v^{1/2}$ was constant indicating a diffusion-controlled reduction process [22], Figure 3. A typical voltammogram is shown on Figure 4(a). The peak at -0.6 V is irreversible and is displaced with the scan rate indicating electrochemical irreversibility. The peak at *ca.* -1.2 V is quasi-reversible. A third irreversible poorly defined peak at -1.09 V was not always present. The width of the peaks, as evaluated by the criterion Ep -Ep/2 = 2.2RT/nF was shown to correspond to n = 1, that is a single electron process [22].

Reduction of Benzimidazolo[3,2-*a*]quinolinium Salts.

The behavior of benzimidazolo[3,2-*a*]quinolinium salts is different from the previously discussed group, as they show mostly two consecutive reversible reductions. A typical voltammogram can be seen in Figure 4(b). The results are summarized in Table 3. The first reduction peak in these benzimidazolo[3,2-*a*]quinolinium salts occurs at a more negative peak potential than those observed for the benzothiazolo[3,2-*a*]quinolinium salts indicating a more thermodynamically stable radical when a sulfur atom is located at position 7 as opposed to a nitrogen atom. However, the reduced species are less kinetically stable when sulfur is present as observed from the irreversibility of the corresponding voltammograms. As the 7-substituent increases in size, while the first reduction is little affected, its reverse peak becomes smaller and less reversible. The fact that the first reduction is not affected

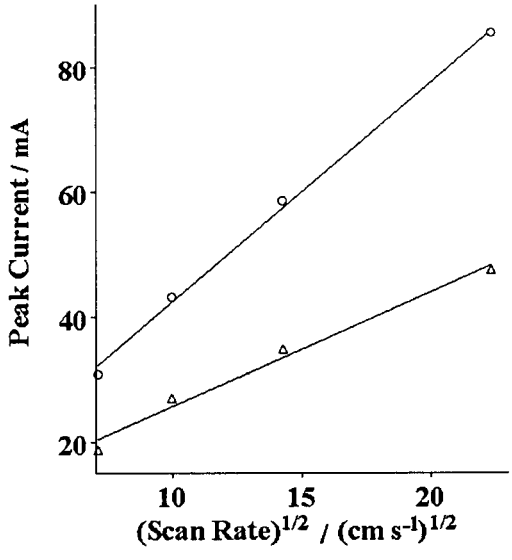


Figure 3. Variation in the peak current at -0.6 (Δ) and -1.27 volts (O) with scan rate observed during the cyclic voltammetry of **2** in *N,N*-dimethylformamide containing 0.1 *M* tetrabutilammonium perchlorate. A similar type of dependance is observed for all of the compounds studied in this work.

Table 4

Cyclic Voltammetry Data of Benzazolo[3,2-*a*]quinolinium Chlorides in *N,N*-Dimethylformamide at 25°C

Compound	Peak Voltages (negative) [a]		
1	0.77/IRR		
2	0.60/IRR	1.09/IRR	1.27/1.07
3	0.58/IRR	0.97/IRR	1.23/1.07
4	1.2/IRR		
5	0.84/0.76		1.26/1.19
6	0.85/0.77		1.26/1.18
7	0.82/0.52		1.19/1.18
8	0.86/IRR		1.42/1.06
9	0.85/IRR		1.29/1.16

[a] Scan rate = 100 mV/s; IRR = irreversible.

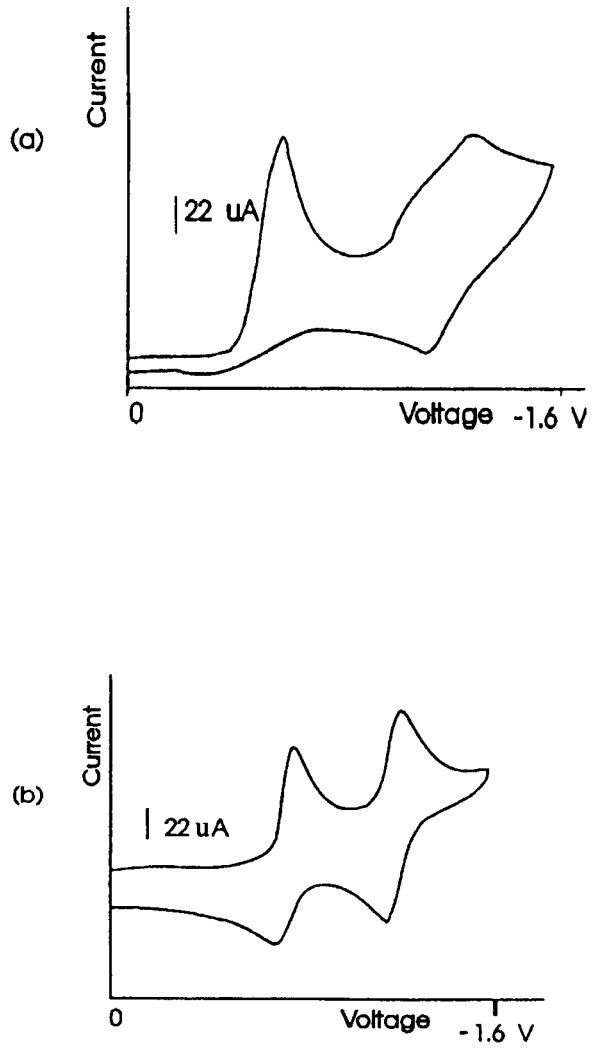


Figure 4. Typical cyclic voltammograms observed for benzothiazolo- and for benzimidazolo[3,2-*a*]quinolinium salts corresponding to (a) **2** and (b) **6** in nitrogen- saturated *N,N*-dimethylformamide containing 0.1 *M* tetrabutilammonium perchlorate. The standard calomel electrode is used as reference.

by substitutions at position 7, indicates that the first electron reduction step is not located there, but probably at the iminium or the nitro site. At slow scan rates, the isopropyl- and *n*-butyl-substituted compounds show reversal curves with smaller rate-dependent peaks indicating an electrochemical/chemical sequence. Other features of the voltammogram indicate the probability of an electolytic/ chemical sequence. All peaks were shown to be diffusion-controlled one-electron processes (*i.e.* Ep - Ep/2 = 2.2RT/nF, n = 1).

Acknowledgments.

The authors express appreciation to NIGMS-MBRS for grants SO6-GM08216 and 5S06-GM08224.

REFERENCES AND NOTES

* Corresponding author: [b] Present Address: Department of Science and Technology, Universidad Metropolitana, PO Box 21150, San Juan, PR 00928-1150; Tel. 787-766-1717 ext 6014; FAX: 787-751-5386; E_MAIL: um_ocox@suagm4.suagm.edu.

[1] O. Cox, H. Jackson, V. A. Vargas, A. Báez, J. T. Colón, B. C. González and M. de León, *J. Med. Chem.*, **25**,1378 (1982).

[2] C. A. Casiano-Torres and A. Báez, *Biochem. Pharmacol.*, **35**, 679(1986).

[3] A. Báez, F. A. González, D. Vázquez and M. J. Waring, *Biochem. Pharmacol.*, **32**, 2089 (1983).

[4] F. A. González, M. Llende, J. R. Ortíz and A. Báez, *Differentiation*, **36**,125(1987).

[5] D. Herreño-Sanz, J. R. Ortiz and A. Báez, *Differentiation*, **55**,169 (1994).

[6] A. Báez, and J. Sepúlveda, *Leukemia Res.*, **16**, 363(1992).

[7] A. Báez, J. F. Riou, J. B. Le Pecq and G. Riou,. *Mol. Pharmacol.*, **37**, 377 (1990).

[8] P. Vivas-Mejía, J. L. Rodríguez-Cabán, M. Díaz-Velázquez, M. G. Hernández-Pérez, O. Cox and F. A. Gónzalez, *Molec. Cell Biochem.*, **177**, 69 (1997).

[9] P. Vivas-Mejía, O. Cox and F. A. González, *Molec. Cell Biochem.*, **178**, 203 (1998).

[10] O. Cox, J. A. Prieto and M. Rodríguez, *Magn. Reson. Chem.*, **27**, 1094 (1989).

[11] C. L. Barnes, O. Cox, L. Ramírez and L. A. Bernard, *Acta Crystallogr.*, C43, 1739 (1986).

[12] P. Kovacic, J. R. Ames, P. Lumme, H. Elo, O. Cox, H. Jackson, L. A. Rivera, L. Ramírez and M. D. Ryan, *Anti-Cancer Drug Design*, **1**, 197 (1986).

[13] A. E. Alegría, O. Cox, V. Santiago, M. Colón, Z. Reyes, L. Zayas, L. A. Rivera and J. A. Dumas, *Free Rad. Biol. Med.*, **15**, 49 (1993)

[14] R. P. Mason and P. D. Josephy in The Toxicity of Nitro Aromatic Compounds, D. E. Rickert, ed, Hemisphere Publishing Co. New York, 1985, Chapter 8, p 121.

[15] M. C. R. Symons and W. R. Bowman, *J. Chem. Soc., Perkin Trans. II*, 1077 (1988).

[16] B. H. J. Bielski and J. M. Gebicki, Atlas of Electron Spin Resonance, Academic Press, NY, 1967.

[17] O. Cox, L. A. Rivera, L. Ramírez and M. Cordero, manuscript in preparation.

[18] A. E. Alegría, O. Cox, J. A. Dumas, L. A. Rivera and P. Riesz, *Biochim. Biophys. Acta*, **967**, 1 (1988).

[19] D. R. Duling, Simulation of Multiple Isotropic Spin-trap EPR Spectra, *J. Magn. Reson., Ser. B*, **104**, 105 (1994).

[20] M. M. McConell, C, Heller, T. Cole and R. W. Fessenden, *J. Am. Chem. Soc.*, **82**, 766 (1960).

[21] J. A. Pople and L. Beveridge, Approximate Molecular Orbital Theory, McGraw Hill Book Co., New York, 1970.

[22] A. J. Bard and L. R. Faulkner, Electrochemical Methods: Fundamentals and Applications, John Wiley and Sons, Inc. NY, 1980.

[23] A. Hinchliffe, Semi empirical calculations of the spin density distributions in some niro substituted. Conjugated anions, *Theoret. Chim. Acta (Berlin.)* **5**, 451 (1966).

[24] J. Subramanian, M. S. Gopinathan and P. T. Narasimhan, Line-Width effect in the electron spin resonance spectra of electro-generated radical anions of nitrobenzonitriles, *J. Magn. Reson.*, **7**, 388 (1972).