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ELECTRON SPIN RESONANCE OF ELECTROCHEMICALLY GENERATED FREE RADICALS FROM DIAZIQUONE AND ITS DERIVATIVES

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The one-electron electrochemical reduction of diaziquone (AZQ) and 12 analogs is analyzed using ESR spectroscopy and cyclic voltammetry. The hyperfine coupling constants arising from the interaction of the unpaired electron with the aziridine nitrogen nuclei fall within 1.20 and 2.26 G. Smaller couplings are observed arising from the protons and nitrogens in the carboethoxyamino groups. The in vitro activity of AZQ and its analogs is examined. Methyl groups in the aziridine rings increase the activity of some analogs. In the absence of aziridines, a chloroquinone compound with only carboethoxyamino groups was surprisingly active. This compound has a more positive cathodic peak than diaziquone.

1. Introduction

Diaziquone (AZQ) (table 1) belongs to a family of quinone-containing antitumor agents that can be biologically reduced to their free radical anions by rat liver microsomes [1], rat liver nuclei [2], purified NADPH : cytochrome *c* reductase [3], xanthine oxidase [4,5] and in some cases by whole cells [6]. Anthracycline antibiotics (e.g., Adriamycin) have been the agents most widely studied in this manner (e.g., refs. 1–4). Recently, we have reported the enzymatic reduction of AZQ, a diaziridinyl quinone [7], and its analogs RQ2 and RQ14 (table 1) [8] to their free radical anions. Analogs RQ2 and RQ14 are of particular interest because they contain chlorine atoms substituting either the carboethoxyamino groups (RQ2) or the aziridine groups (RQ14). The rationale for studying the biological activation of antineoplastic quinone-containing agents to their free radicals is based on the hypothesis that the mechanism of action of these drugs is not only intercalation into DNA or alkylation to vital molecules, but that in addition to these, the drug's free radicals themselves as well as oxygen free radicals are involved in the agent's toxicity and mechanism of action [1]. For example, oxygen free radicals have been implicated in the cardiotoxicity of Adriamycin [9].

In this paper we study the free radicals of AZQ and 12 of its analogs by electrochemical reduction using controlled potential electrolysis. This technique allows one to control the number of electrons involved in the reduction process as well as to provide an environment free of proteins, metals and other chemicals. The spectra obtained from electrochemically reduced compounds have well defined lines which allow the identification of the free radical in terms of hyperfine coupling, *g* value, and indirectly, the estimation of the unpaired electron density in the quinone ring. In the

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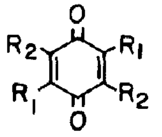
Table 1

Structure of AZQ and analogs



COMPOUND	R_1	R_2
AZQ	N◁	$NHCOOCH_2CH_3$
RQ 1	N◁	H
RQ 2	N◁	Cl
RQ 3	N◁	F
RQ 4	N◁	OC_2H_5
RQ 5	N◁	SC_2H_5
RQ 6	N◁	$NHCH_2CH_3$
RQ 7	N◁	$N(CH_3)_2$
RQ 10	N◁	N◯N-CH_2CH_2OH
RQ 11	N◁	N◯
RQ 12	N◁CH_3	$NHCOOCH_2CH_3$
RQ 13	N◁CH_3 CH_3	$NHCOOCH_2CH_3$
RQ 14	Cl	$NHCOOCH_2CH_3$

case of AZQ and its derivatives, electrochemical reduction and ESR analysis of free radicals thus produced is relevant in terms of enzymatically and in vitro produced free radicals. This is so because we have shown that for AZQ, RQ2 and RQ14, the free radicals obtained electrochemically are the same as those generated enzymatically [10]. The required aqueous solvent for the enzymatic reductions broadens the ESR spectral lines hiding additional hyperfine coupling which is resolved with the use of aprotic solvents and electrochemical reduction [10].

The physical parameters presented here are important not only because they contribute to the overall knowledge of aziridinyl quinones, but also because some of these compounds have antineoplastic activity.

2. Materials and methods

AZQ, 2,5-diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone, was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD, U.S.A., and tested for purity by HPLC [11]. AZQ analogs were a gift from Dr. J. Driscoll, Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.

2.1. Cyclic voltammetry

Cyclic voltammetry was performed with a CV-1A voltammetry control unit (Bioanalytical Systems, Lafayette, IN) connected to an IBM electrochemical cell (IBM Instruments, Danbury, CT) at room temperature. Gold or glassy carbon was used as the working electrode. The IBM cell was equipped with a platinum wire counter electrode and an Ag/AgCl reference electrode. The voltage was monitored with a Fluke 8600A digital multimeter (John Fluke, Seattle, WA). Voltammograms were recorded on an Omnigraphic 2000 X-Y recorder (Houston Instrument, Austin, TX). Solutions of AZQ or analogs at a concentration of 5×10^{-4} M were prepared in spectrophotometric grade dimethyl sulfoxide (DMSO) containing 0.1 M tetraethylammonium perchlorate (TEAP) (Kodak, Rochester, NY) as supporting electrolyte. Each solution was purged with high-purity nitrogen (Air Products, Allentown, PA) for 5 min prior to measurement.

2.2. Controlled potential electrolysis

Electrochemical reductions were carried out in an ESR electrolytic flat cell (J.F. Scanlon, Solvang, CA) which was subsequently placed in the ESR spectrometer cavity. Cathode contact was provided by placing mercury in contact with a platinum wire at the bottom of the ESR cell. Another platinum wire was inserted through a side port of the cell and used as a counter electrode. The reference electrode was Ag/AgCl. An AMEL Model 500 potentiostat/galvanostat (ECO Instruments, Newton, MA) was used as a power supply and voltages were set using a Fluke 8600A digital multimeter. Solutions of AZQ or analogs (5×10^{-4} M) and TEAP (0.1 M) in DMSO were deoxygenated with nitrogen prior to electrolysis for 20 min. Reductions were carried out for 45 min at potentials determined by cyclic voltammetry. Immediately after electrolysis, the flat cell was placed in the ESR cavity and spectra were recorded at room temperature.

2.3. ESR

A Varian E-109 Century Series X-band (9.3 GHz) spectrometer (Varian Instrument Division, Palo Alto, CA) with a dual rectangular (TE_{104}) cavity was used. A strong pitch standard ($g = 2.0028$) was placed in the reference cavity and was used to determine g values. The magnetic field was calibrated relative to an alkaline solution of potassium peroxylamine sulfonate (Fremy's salt) at $g = 2.0055$ and $a_N = 13.0$ G [12]. A Nicolet 1180 data system (Nicolet Instrument, Madison, WI) was used to simulate spectra using the program ESRSIM.

2.4. Cytotoxic evaluation

The activity of AZQ and its analogs was evaluated in L1210 murine leukemia cells by determining the concentration which inhibits cell growth to 50% of controls (IC_{50}). L1210 murine leukemia cells were maintained in vitro by serial culture in RPMI medium 1640 (Flow Laboratories, McLean, VA) containing penicillin (50 U/ml), streptomycin (50 μg/ml), and 15% new born calf serum (NCS) (Flow Laboratories). Under these conditions, cells had a population doubling time of 14–18 h and achieved a maximum cell density of $1.5\text{–}2.0 \times 10^6$ cells/ml. Prior to incubation with drug or DMSO, cells were washed twice with 0.154 M NaCl and resuspended to a final cell concentration of 20×10^5 cells/ml in RPMI medium 1640 with penicillin, streptomycin, and NCS. Cell counts were performed on a Model ZBI Coulter Counter (Coulter Electronics, Hialeah, FL).

Incubations were performed in triplicate. 1.5-ml aliquots of cell suspension were placed into sterile 16 × 150 mm borosilicate glass culture tubes fitted with stainless-steel caps (Bellco Glass, Vineland, NJ). 1.5-ml volumes on RPMI median 1640 containing appropriate amounts of drug or DMSO were added to each culture tube. Cultures were incubated in slanted test tube racks at 37°C in an atmosphere of 95% air:5% CO_2 and 95% humidity. After 72 h incubation, the cells were counted, and IC_{50} values were calculated.

3. Results and discussion

The electrochemical reduction of AZQ and its analogs (table 1) in DMSO occurred in two one-electron steps corresponding to the formation of anion radicals and diamagnetic dianions, respectively (fig. 1). Evidence that the first waves involve the quinone/one-electron anion couple (eq. 1) was obtained by ESR and by the presence of the first one-electron wave (fig. 1). Reduction at potentials

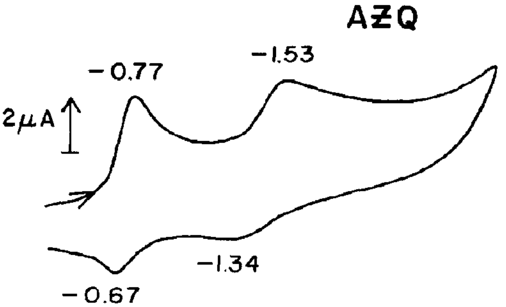
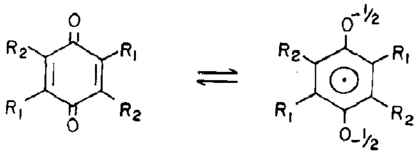


Fig. 1. Cyclic voltammogram at 60 mV/s of diaziquone (AZQ) (0.5 mM) in DMSO at 25°C containing 0.1 M tetraethylammonium perchlorate as electrolyte. A glassy carbon electrode was used as the working electrode.



(I)

more negative than those of the first cyclic voltammetric cathodic peak potentials, E_p^c, but less than those of the second, yielded species that exhibited well-resolved ESR spectra (table 2, figs. 2–4). These spectra are described below and are consistent with the interaction of an unpaired electron with magnetic nuclei in these anions. The formation of the diamagnetic two-electron reduced anions upon reduction at potentials beyond the E_p^c values of the second waves was evidenced by the disappearance of the ESR spectra.

The first waves in the cyclic voltamograms were quasi-reversible (fig. 1, table 2) while the shape of the second waves were slightly deformed probably due to adsorption effects. The separation of cathodic and anodic peak potentials ($E_p^c - E_p^a$) given in table 2 varied for most compounds between 60 and 100 mV for the first waves. A value of 59 mV is expected for a reversible one-electron process [13].

The presence of electron-withdrawing substituents such as chlorine and fluorine in RQ2 and RQ3 respectively, changes the cathodic peak potential values of the two waves to less negative potentials with respect to the unsubstituted RQ1 (table 2). For example, for RQ2 one has −0.42 and −1.19 V and for RQ3 −0.41 and −1.23 V as compared to −0.66 and −1.34 V for RQ1. On the other hand, RQ6, RQ7, RQ10 and RQ11 which contain electron-donating substituents (amino groups) at C_3 and C_6 are reduced at much more negative potentials with respect to RQ1 (e.g., approx. −0.9 and −1.6 V for the first and second waves, respectively) (table 2). AZQ, RQ12, RQ13 and RQ14 form a special group among themselves because they all contain the carboethoxyamino groups at C_3 and C_6. As with the other halogenated compounds, RQ14 which has electron-withdrawing chlorine substituents exhibits voltammetric peaks at potentials that are more positive than those of RQ12, RQ13, or AZQ (table 2). RQ12 and R13 contain methyl groups in the aziridine rings that shift the cathodic peak potential values to less negative potentials with respect to AZQ.

The one-electron reduction of the AZQ derivatives studied here produces radical anions that exhibit ESR spectra (figs. 2–4) composed of lines with defined intensity ratios and patterns relatively easy to understand. The spectra of these analogs allowed for the interpretation of the more complex spectrum observed for the AZQ free radical anion (fig. 4).

Table 2

Cyclic voltammetric peak potentials (V) *for AZQ and analogues

Compound	Wave I			Wave II		
	E_p^c	E_p^a	($E_p^c - E_p^a$)	E_p^c	E_p^a	($E_p^c - E_p^a$)
RQ1	−0.66	−0.58	0.08	−1.34	−1.28	0.06
RQ2	−0.42	−0.32	0.09	−1.19	−1.11	0.08
RQ3	−0.41	−0.32	0.09	−1.23	−1.13	0.10
RQ4	−0.71	−0.61	0.10	−1.45	−1.35	0.10
RQ5	−0.64	−0.55	0.09	−1.40	−1.31	0.09
RQ6	−0.94	−0.85	0.09	−1.61	−1.51	0.10
RQ7	−0.91	−0.81	0.10	−1.61	−1.51	0.10
RQ10	−0.87	−0.78	0.09	−1.57	−1.50	0.07
RQ11	−0.90	−0.81	0.09	−1.56	−1.44	0.12
RQ12	−0.42	−0.34	0.08	−1.19	−0.91	0.28
RQ13	−0.48	−0.39	0.09	−1.26	−1.18	0.08
RQ14	−0.15	−0.06	0.09	−0.71	−0.51	0.20
AZQ	−0.77	−0.67	0.10	−1.53	−1.34	0.19

* vs. Ag/AgCl in DMSO using 0.1 M TEAP as a supporting electrolyte.

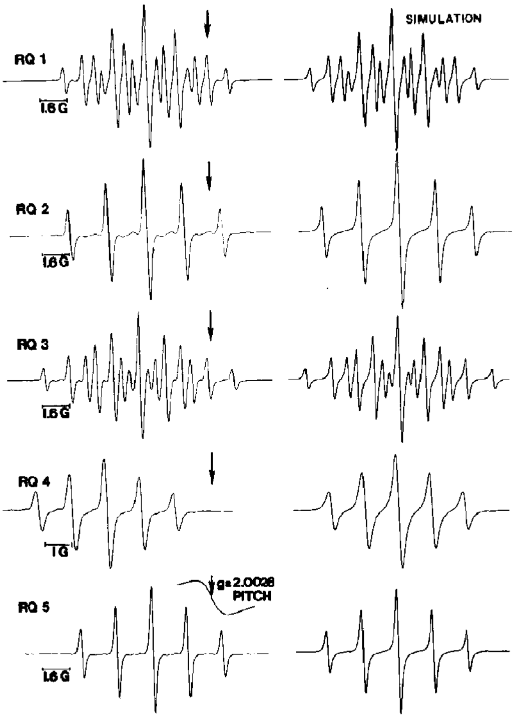


Fig. 2. ESR spectra of electrochemicaly reduced AZQ analogs RQ1–RQ5 (0.5 mM) in DMSO using 0.1 M tetraethylammonium perchlorate as electrolyte. The control potential was between the first and second cathodic peak potentials (E_p^c) of their respective cyclic voltammograms. The ESR conditions at room temperature were: 9.3 GHz microwave frequency, 10 mW incident microwave power and 0.1–0.25 G modulation amplitude. Spectral simulations were performed in a Nicolet 1180 computer using Lorentzian linewidths.

RQ1 free radical anion gave rise to an ESR spectrum consisting of a 1 : 2 : 3 : 2 : 1 quintet of 1 : 2 : 1 triplets due to the coupling of the unpaired electron to two equivalent aziridine nitrogens (^{14}N, $I = 1$) at C_2 and C_5 and to the two equivalent ring protons at C_3 and C_6 (fig. 2, table 3). The ESR spectrum obtained for RQ2 radical anion exhibited the same aziridine nitrogen quintet as RQ1 with ^{13}C satellites ($I = 1/2$, natural abundance 1.11%). The presence of ^{13}C at either C_2 or C_5 can further split the quintet lines into doublets. The intensity of each of the two satellite lines to its major line is approx. 1% as expected, since 1.98% is the probability of having a ^{13}C at either C_2 or C_5. This phenomenon was also clearly observed for spectra with narrow lines (e.g., RQ5). RQ3 free radical anion exhibited a relatively large coupling (2.40 G) to the two fluorine nuclei (^{19}F, natural abundance 100%, $I = 1/2$) at C_3 and C_6 and a relatively smaller one (1.44 G) to the aziridine nitrogens. RQ4 and RQ5 free radicals also exhibited the aziridine nitrogen quintet with no measurable contribution from the substituents at C_3 and C_6.

A well-resolved ESR spectrum with equidistant lines (0.42 G) results from the free radical anion of RQ6 (fig. 3). A 0.42 G coupling was extracted from the experimental spectrum, and assigned to the nitrogens of the carboethoxyamino groups by comparison to RQ14 (vide infra). The value of 1.28 G was obtained by computer simulation for the two aziridine nitrogens and the two imide protons (table 4). This value is a multiple of the 0.42 G coupling as expected in highly symmetric spectra such as that of RQ6 radical anion.

Table 3

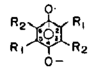
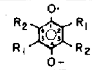
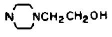
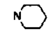
			ESR Parameters of AZQ^{-} Derivatives		
			Hyperfine Coupling Constants (Gauss)		
RQ	R_1	R_2	$a_N(R_1)$	$a(R_2)$	g-values
1	N◁	H	1.75 (2N)	1.07 (2H)	2.0049
2	N◁	Cl	2.10 (2N)		2.0049
3	N◁	F	1.44 (2N)	2.40 (2F)	2.0052
4	N◁	OCH_2CH_3	1.20 (2N)		2.0051
5	N◁	SCH_2CH_3	1.97 (2N)		2.0048

Table 4

			ESR Parameters of AZQ$^{\mp}$ Derivatives			
			Hyperfine Coupling Constants (Gauss)			
RQ	R_1	R_2	$a_N(R_1)$	$a_N(R_2)$	$a_H(R_2)$	g-values
6	N◁	$NHCH_2CH_3$	1.28 (2N)	0.42 (2N)	1.28 (2H)	2.0046
7	N◁	$N(CH_3)_2$	1.36 (2N)	*	*	2.0049
10	N◁		1.43 (2N)	*	*	2.0049
11	N◁		1.40 (2N)	*	*	2.0048

* THESE COUPLINGS WERE UNRESOLVED EVEN AT SMALL MODULATION AMPLITUDES (e.g. 0.01 G)

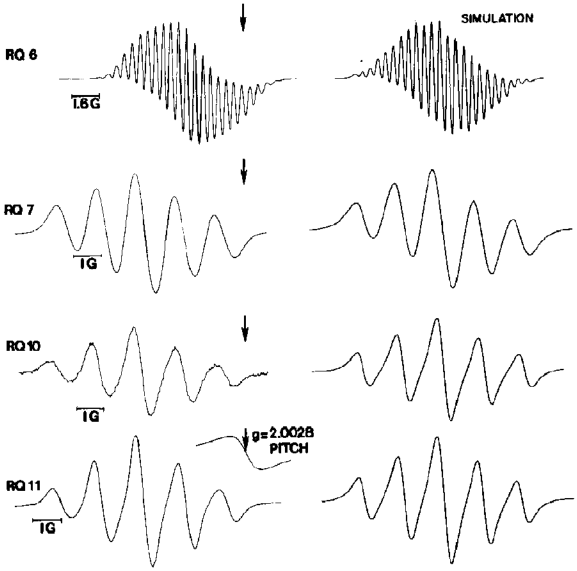
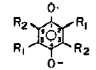
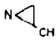
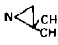
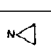


Fig. 3. ESR spectra of electrochemically reduced AZQ analogs RQ6, RQ7, RQ10, and RQ11 (0.5 mM) in DMSO using 0.1 M tetraethylammonium perchlorate as electrolyte. The control potential was between the first and second cathodic peak potentials (E_p^c) of their respective cyclic voltammograms. The ESR conditions at room temperature were: 9.3 GHz microwave frequency, 10 mW incident microwave power and 0.1 G modulation amplitude. Spectral simulations were performed in a Nicolet 1180 computer using Lorentzian linewidths.

Only broad quintets were found for RQ7, RQ10, and RQ11 probably due to unresolved coupling to the nitrogens of the substituents at C_3 and C_6. These unresolved couplings cannot be precisely found by simulation because the values fall in a relatively broad range. We could easily simulate these spectra using relatively large line-widths such as 0.70, 0.65 and 0.58 G for RQ7, RQ10 and RQ11, respectively (fig. 3).

Table 5

			ESR Parameters of AZQ$^{\overline{\cdot}}$ Derivatives			
			Hyperfine Coupling Constants (Gauss)			
RQ	R_1	R_2	$a_N(R_1)$	$a_N(R_2)$	$a_H(R_2)$	g-values
12		$NHC(O)OCH_2CH_3$	2.11 (2N)	*	*	2.0048
13		$NHC(O)OCH_2CH_3$	2.26 (N) 2.17 (N)	0.15 (2N)	0.68 (H) 0.58 (H)	2.0049
14	Cl	$NHC(O)OCH_2CH_3$	—	0.30 (2N)	1.64 (2H)	2.0055
AZQ		$NHC(O)OCH_2CH_3$	1.88 (N) 1.78 (N)	0.15 (2N)	0.87 (H) 0.73 (H)	2.0049

* THESE COUPLINGS WERE TOO SMALL TO DETECT

The free radical anions generated from analogs RQ13, and RQ14 which have, as AZQ, the carboethoxyamino substituent ($NHC(O)OCH_2CH_3$), exhibited spectra which revealed that the coupling from the carboethoxyamino groups is due to imide protons and nitrogens, and that the coupling from the imide protons is larger than that arising from the nitrogens. This was best demonstrated in the case of RQ14 which lacks the aziridine rings. The spectrum of the RQ14 free radical anion exhibited a 1.64 G 1 : 2 : 1 triplet that was further split by a 0.30 G 1 : 2 : 3 : 2 : 1 quintet (fig. 4, table 5). The spectrum of RQ13 free radical anion was simulated by assuming magnetic inequivalence in the pair of imide protons and the pair of the aziridine nitrogens. Only by knowing these two results was it possible to simulate successfully the complex AZQ free radical anion spectrum which consists of 11 lines with different linewidths and heights (fig. 4, table 5). It appears that methyl groups in the aziridine rings tend to push spin density onto C_2 and C_5, the site of the aziridine rings (RQ12 and RQ13). This results in a larger coupling (vis a vis AZQ) of the unpaired electron to the nitrogen in the aziridine rings and a corresponding decrease in the coupling to the carboethoxyamino imide protons (table 5). In the case of RQ12 free radical (one methyl), the hyperfine couplings to the carboethoxyamino group were too small to detect while they were detectable for RQ13 free radical (two methyls). This suggests a molecular geometry whereby the imide groups at C_3 and C_6 of RQ12 are in planes at which they may not couple to the unpaired electron in the aromatic ring. Two methyl groups in the aziridines as in RQ13 reverse this geometry and somewhat restore symmetry so as to allow for the observed hyperfine coupling. (Space-filling models give a crude approximation of this geometry.)

The relatively large coupling to the imide protons as compared to the imide nitrogens in the AZQ free radical as well as in the free radicals from RQ13, and RQ14, is consistent with the presence of intramolecular hydrogen bonding of

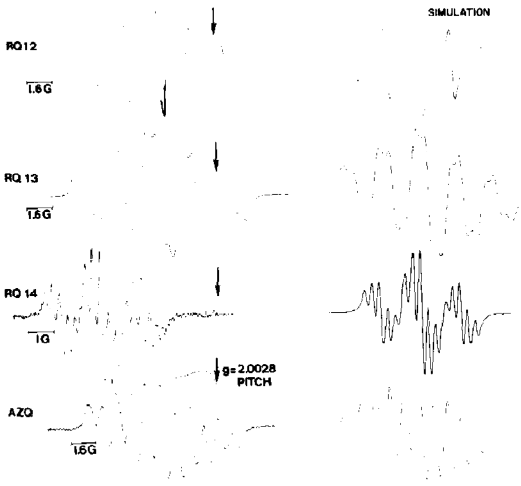


Fig. 4. ESR spectra of electrochemically reduced AZQ and analogs RQ12, RQ13, and RQ14 (0.5 mM) in DMSO using 0.1 M tetraethylammonium perchlorate as electrolyte. The control potential was between the first and second cathodic peak potentials (E_p^c) of their respective cyclic voltammograms. The ESR conditions at room temperature were: 9.3 GHz microwave frequency, 10 mW incident microwave power and 0.1–0.25 G modulation amplitude. Spectral simulations were performed in a Nicolet 1180 computer using Lorentzian linewidth.

Table 6

Comparison of IC_{50}, aziridine nitrogen couplings (a_N^{Az}) and cathodic peak potentials (E_p^c) for AZQ and analogs

RQ	IC_{50} (μM) [a]	a_N^{Az} (G)	E_p^c (V) [b]
AZQ	1.00	1.88 (N), 1.78 (N)	−0.77
RQ1	0.01	1.75 (2N)	−0.66
RQ2	0.20	2.10 (2N)	−0.42
RQ3	0.20	1.44 (2N)	−0.41
RQ4	1.00	1.20 (2N)	−0.71
RQ5	1.10	1.97 (2N)	−0.64
RQ6	n.a. [c]	1.28 (2N)	−0.94
RQ7	4.5	1.36 (2N)	−0.91
RQ10	0.25	1.43 (2N)	−0.87
RQ11	8.8	1.40 (2N)	−0.90
RQ12	0.48	2.11 (2N)	−0.42
RQ13	0.07	2.26 (N), 2.17 (N)	−0.48
RQ14	0.38	–	−0.15

[a] The dose require to inhibit growth to 50% of control growth by 72 h after drug addition (low IC_{50} means high drug activity). The cells used were L1210. Points represent the results of triplicate measurements. S.E. was less or equal to 20%.

[b] Measured vs Ag/AgCl. See fig. 1 and text for details.

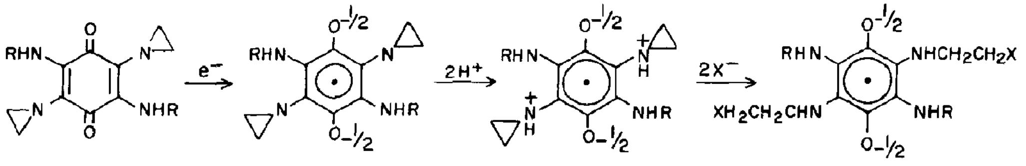
[c] Not available.

the type CO ··· HN which takes place between the oxygens on C_1 and C_4 and the imide protons. It has been demonstrated that in quinones where suitable proton donor groups such as NH or OH are present, strong hydrogen bonding occurs [14].

In general, our data show that the unpaired electron delocalized on the quinone ring is mostly on the carbons adjacent to the aziridine groups (table 3–5). The coupling constants arising from the interaction of the unpaired electron with the aziridine nitrogen nuclei all fall within the range 1.20–2.26 G.

It is of interest to relate some of the physical parameters described above to AZQ drug activity. The activity of the compounds in table 6 was evaluated against L1210 cells in culture in terms of IC_{50} value as described in section 2. IC_{50} is the concentration required to arrest the growth of cells in culture by 50%. The least active compound (highest IC_{50}) is RQ11, while the most active one (lowest IC_{50}) is RQ1 (table 6). Adams et al. [15] showed a correlation between aerobic cytotoxicity and the reduction potential of three quinones (anthraquinone, duroquinone and menadione). In general, our data show no correlation either between IC_{50} values and cathode peak potentials or between IC_{50} values and ESR hyperfine coupling constants of the aziridine nitrogens (table 6). This coupling is a measure of the spin densities on carbons C_2 and C_5 of the quinone ring. Further examination of table 6, however, reveals some trends in the overall effect of specific structural changes on the aziridine ring and compound activity. If one considers AZQ, RQ12 and RQ13 which have carboethoxyamino groups, methylation of the aziridines influences the compounds activity. That is, RQ13 has two methyl groups in the aziridines, and possesses an IC_{50} value of 0.07 μM, AZQ with no methyl groups has an IC_{50} value of 1.0 μM, while RQ12, with one methyl group has an intermediate value of 0.48 μM. The aziridine nitrogen couplings (a_N^{Az}) also increase with increasing number of methyl groups. Thus, AZQ, the least active of the three, has coupling constants of 1.88 and 1.78 G from inequivalent nitrogens, while RQ13, the most active, has couplings of 2.25 and 2.17 G from inequivalent nitrogens. RQ12 has intermediate activity and an intermediate hyperfine coupling constant of 2.11 G from equivalent nitrogens. (The values of these couplings are with ±2%.) Similarly, although not as clearcut, AZQ, the least active, has the lowest reduction potential (−0.77 V), but RQ13, the most active, has a value close to RQ12 (−0.48 V), the quinone with intermediate activity.

It is difficult to separate the influence of the methyl groups on the compound's activity from that of the free radical spin densities at C_2 and C_5, which, based on aziridine nitrogen hyperfine constants, is highest for RQ13. It is known that methyl groups offer a high degree of stability to an opened aziridine ring [16], the required species for alkylation [17,18]. It could be that the increase in the spin density on carbons C_2 and C_5 facilities the protonation of the aziridine ring, leading to the aziridinium ion (eq. 2). A combination of these two effects is the most probable. In biological systems, the reaction depicted in eq. 2 can occur if these quinones are first reduced enzymatically. We have shown that biological reduction is possible for AZQ, RQ2, and RQ14 [8], and other investigators have shown that AZQ activity is increased when activated (to a free radical) by rat liver microsomes [19] or NADPH [20] and when reduced with sodium borohydride [21]. The last observation argues for hydroquinone cytotoxicity, since sodium borohydride fully reduces AZQ to the hydroquinone [21] by a two-electron transfer. Hence, the correlation of IC_{50} and reduction potentials in the three compounds mentioned above is feasible on the grounds that a compound with more positive reduction potential than others is most easily reduced. Our data, however, are not very clearcut at this point (i.e., −0.77 V for AZQ and −0.48 and −0.42 V for RQ13 and RQ12, respectively).



(2)

It is of interest to compare the activity of RQ14 and RQ2 because in these two compounds, the two groups of AZQ can be studied separately. The IC_{50} values for RQ2 and RQ14 are relatively close at 0.20 and 0.38 μM, respectively (table 6). An IC_{50} of 0.38 μM for RQ14 (table 6), is a relatively low value (high activity) for a compound with no aziridine moieties. This is of particular interest because the carboethoxyamino groups are not alkylating groups by themselves. It is possible that RQ14 acts directly as an electrophile, a statement which is in harmony with RQ14's low reduction potential (−0.15 V) and its fast reduction kinetics by NADPH: cytochrome *c* reductase [8].

These findings suggest that AZQ has several modes of action, and that these modes may be influenced by a particular chemical or biochemical environment. For instance, AZQ may act directly as an electrophile, or through the aziridines as an alkylating agent whose activity may be accentuated by its reduction to a semiquinone [8,19]. Finally, AZQ's activity can also be mediated through the hydroquinone [20]. Kohn et al. [22] have recently reported observations that are in agreement with this conclusion. They propose that DNA strand scissions and DNA cross-links observed in human cell lines are due to two different mechanisms. The former to the reduction-oxidation cycle of the quinone function of AZQ. The latter to alkylation through the aziridine rings.

In general, correlations between molecular physical parameters such as nitrogen ESR coupling constants and reduction potentials, while they describe the molecule well, may represent too large a gap between a molecule and its pharmacological activity. Further experiments relating quinone semiquinone reaction rates with biological nucleophiles are underway and may narrow the gap between these agents and their pharmacology.

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