Electrochemistry of potential bioreductive alkylating quinones

Part 2. Electrochemical properties of 2,5-bis(1-aziridinyl)-3,6-bis(ethoxycarbonylamino)-1,4-benzoquinone and some model compounds

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ABSTRACT

The reduction mechanism of 2,5-bis(1-aziridinyl)-3,6-bis(ethoxycarbonylamino)-1,4-benzoquinone (Diaziquone, AZQ) and several model compounds of the mono- and bis(1-aziridinyl)quinone type at the dropping mercury electrode in aqueous solutions was studied. In addition, the influence of methyl substitution of the aziridinyl moiety at the 2-position on the protonation of the aziridine nitrogen was investigated. Substituent effects on quinone reduction and aziridine protonation prior to and following quinone reduction were studied qualitatively.

Keywords: Controlled potential electrolysis, Aziridinylquinones, Quinones, Bis(1-aziridinyl)bis(ethoxycarbonylamino)-benzoquinone

Aziridinylquinones, such as 2,5-bis(1-aziridinyl)-3,6-bis(ethoxycarbonylamino)-1,4-benzoquinone (Diaziquone, AZQ) (Fig. 1), are interesting antitumour agents. Aziridines are alkylating moieties, whereas the presence of a quinone function offers the possibility of bioreduction. Indeed, bioreductive activation of aziridinylquinones, followed by the formation of an alkylating species by protonation and opening of the aziridinyl rings on nucleophilic attack, has been proposed as the pos-

sible mode of action of aziridinylquinones [1–5]. However, alkylation of a nucleophilic particle (such as water, acetate or phosphate) may also proceed without a preceding reduction step [6], as was demonstrated in studies on the chemical sta-

Fig. 1 Structure of the cytostatic aziridinylquinone 2,5-bis(1-aziridinyl)-3,6-bis(ethoxycarbonylamino)-1,4-benzoquinone (Diaziquone, AZQ)

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bility of AZQ [7,8] and other aziridinyquinones [9–12].

The electrochemical properties of relatively simple quinones, as model compounds of aziridiny- and other more complex quinones, were reported in Part 1 [13]. This study was aimed at the elucidation of the mechanism of formation of the alkylating species of AZQ in aqueous solutions. The two aziridiny rings and ethoxycarbonylamino substituents of AZQ can undergo acid–base reactions. These reactions and the number of substituents complicate the interpretation of the electrochemical data for AZQ. To separate individual acid–base reactions and to distinguish the role of individual substituents, the electrochemical properties of simpler 1,4-benzoquinones and 1,4-naphthoquinones, bearing only one aziridine and only one ethoxycarbonylamino group and/or simpler amino substituents, were also studied. In addition, the influence of methyl substitution of the aziridiny moiety at the 2-position was investigated.

EXPERIMENTAL

Chemicals

The compounds studied are listed in Table 1. The synthesis or source of **I**, **II** and **XIII** has been

described elsewhere [13]. **III–VI** were synthesized according to [14]. Compound **VII** was prepared starting from 2-chloro-3-(ethoxycarbonylamino)-1,4-naphthoquinone [15] and aziridine in the presence of triethylamine in benzene analogously to the synthesis of 2-(chloroacetyl amino)-3-(substituted-1-aziridiny)-1,4-naphthoquinone described by Casini et al. [16]: m.p. 171°C (decomposition) (ethanol) {lit. [17] m.p. 171°C (decomposition)}. AZQ (**VIII**) was a gift from the Drug Synthesis and Chemistry Branch, National Cancer Institute, MD, U.S.A., and was used as received. The methyl-substituted analogues of **VII** and **VIII**, i.e., **IX** and **X**, were prepared as described in [15] and [18], respectively. Compounds **XI** and **XII**, viz., 2-[(2-hydroxy)ethylamino]-1,4-naphthoquinone and 2,5-bis[(2-hydroxy)ethylamino]-1,4-benzoquinone, were synthesized as described in [19] and [20], respectively. Compound **XIV** was obtained from Aldrich Europe (Janssen Chimica, Beerse, Belgium). Two additional compounds were synthesized, viz., 2-(ethoxycarbonylamino)-1,4-naphthoquinone (**XV**) and 2,5-bis(ethoxycarbonylamino)-1,4-benzoquinone (**XVI**). However, the poor solubility of these quinones made their electrochemical analysis in aqueous media impossible. It was therefore decided to omit the description of their relatively complicated pathways of synthesis in this paper.

TABLE 1

Structures of compounds **I–XVI**

Compound	2-	3-	Compound	2-	3-	5-	6-
I	NMe ₂	H	II	NMe ₂	H	NMe ₂	H
III	Az	H	IV	Az	H	Az	H
V	Az-Me	H	VI	Az-Me	H	Az-Me	H
VII	Az	NHCOOEt	VIII	Az	NHCOOEt	Az	NHCOOEt
IX	Az-Me	NHCOOEt	X	Az-Me	NHCOOEt	Az-Me	NHCOOEt
XI	NHCH ₂ CH ₂ OH	H	XII	NHCH ₂ CH ₂ OH	H	NHCH ₂ CH ₂ OH	H
XIII	OH	H	XIV	OH	H	OH	H
XV	NHCOOEt	H	XVI	NHCOOEt	H	NHCOOEt	H

Stock solutions (5 mM) of the compounds were freshly prepared by dissolving in acetonitrile or methanol (zur Analyse, Merck, Darmstadt) or *N,N*-dimethylformamide (reinst, Merck) and stored at 4°C. Other chemicals were of analytical-reagent grade. The composition of the buffers used is the same as reported previously [13], except that 2 M sodium nitrate or 1 M potassium sulphate was used to adjust the ionic strength to 0.1 M.

Apparatus

The apparatus used in direct current (DC) and differential-pulse polarography (DPP) has been described previously [13]. All potentials given here were measured vs. Ag/AgCl/3 M KCl, using the reduction of thallium as an external standard [13]. In addition, a computerized polarograph combined with a laboratory-made autosampler [21] was used to investigate the pH dependence of the half-wave potential ($E_{1/2}$) and the limiting current (i) by DC polarography. Logarithmic analysis of the waves observed and cyclic voltammetric (CV) studies were performed also with the computerized polarograph. pH values were measured with a Radiometer PHM 64 research pH meter, equipped with an Ingold LOT 401 combined glass/reference electrode. Controlled potential electrolysis (CPE) was carried out at a mercury pool electrode with a surface area of ca. 7 cm², using a Bruker E310 electrochemical system as the potentiostat. Stirring was done by bubbling nitrogen through the solution.

Procedures

Procedures to obtain $E_{1/2}$ -pH and i -pH plots of DC curves, to calculate the number of electrons transferred (n) and to correct $E_{1/2}$ and E_p values for day-to-day variations in the reference electrode were the same as reported previously [13].

In DPP analysis, the pulse amplitude was varied over a range from 5 to 100 mV, using a scan rate of 2 mV s⁻¹ and a drop time of 2 s. CPE was carried out in 10.0 ml of a 0.05 mM solution of the compound in 0.1 M phosphate buffer (pH 7.0). Before the electrolysis was started, a complete DC curve was recorded; the electrolysis potential was selected in the plateau of the curve.

The course of the electrolysis was monitored at appropriate time intervals by means of DC polarography. In CV experiments, the chemical reversibility at pH 7.0 was examined, using a variable scan range and a scan rate of 115 mV s⁻¹.

RESULTS

Electrochemistry of 2-(1-aziridinyl)-1,4-naphthoquinone (III) and 2,5-bis(1-aziridinyl)-1,4-benzoquinone (IV)

Being the simplest mono- and bis(1-aziridinyl)quinones of the present series of compounds, quinones **III** and **IV** were examined in detail. The results will be presented extensively here. Results for compounds **V**, **VII** and **IX** are summarized in Fig. 4 and Table 2 and those of **VI**, **VIII** and **X** in Fig. 5 and Table 2.

DC polarography. The electrochemical properties of 2-(dimethylamino)-1,4-naphthoquinone (**I**) and 2,5-bis(dimethylamino)-1,4-benzoquinone (**II**) have been discussed in detail [13]. Figures 2a and 3a show the well developed polarographic waves i_1 at pH 7.0 of **I** and **II**, respectively, corresponding to the reversible two-electron reduction of the quinone function to the hydroquinone. When a dimethylamino function reacts to form an aziridinyl ring, yielding **III** and **IV**, respectively, significant differences in the polarographic curves are observed (Figs. 2b and 3b): a second wave at more negative potentials appears, which in DC polarography at pH 7.0 is about equal in height to wave i_1 , but is in DPP at pH 7.0 much lower than i_1 (Figs. 2c and 3c).

Several waves can be observed in the polarographic recordings of **III** and **IV**, all having a characteristic dependence of half-wave potential on pH (Figs. 4 and 5), whereas the limiting current depends on pH in addition to the time scale of the experiment (Figs. 4–7).

The current of wave i_2 of **III** equals that of i_1 at pH 7, whereas that of **IV** is approximately double the height of wave i_1 at pH 7 (Figs. 4a and 5a, respectively). The current decreases rapidly at pH > 6.5 and, as was proved by repeated polarographic scans, independently of time. This decrease is in the shape of a dissociation curve with

Fig 2 Direct current polarographic recordings of a 0.05 mM solution of (A) 2-(dimethylamino)-1,4-naphthoquinone (I) and (B) 2-(1-azirindinyl)-1,4-naphthoquinone (III) in 0.1 M phosphate buffer (pH 7.0) at a scan rate of 2 mV s^{-1} and a drop time of 2 s, correction of $E + 73 \text{ mV}$ (see Experimental) (C) Differential pulse polarographic recording of a 0.05 mM solution of 2-(1-azirindinyl)-1,4-naphthoquinone (III) under identical experimental conditions, using a pulse amplitude of 100 mV

an inflection point at a pH of about 8.0 (III) and 7.5 (IV), respectively. In the pH range 7–5 the height of i_2 of III and IV decreases gradually, which was also proved to be independent of time. At $\text{pH} < 5$, the current of wave i_2 decreases rapidly and curves recorded at more acidic pH no longer show this wave. The addition of a few drops of

Triton X to a buffered solution of pH 7.0 results in the complete absence of wave i_2 .

The composition of the buffered solution influences the height of all waves. In acetate buffers, waves i_1 and i_2 are smaller and waves i_5 and i_6 are larger, in comparison with phosphate buffers of equal pH and ionic strength. In phosphate

Fig 3 Direct current polarographic recordings of a 0.05 mM solution of (A) 2,5-bis(dimethylamino)-1,4-benzoquinone (II) and (B) 2,5-bis(1-aziridinyl)-1,4-benzoquinone (IV) in 0.1 M phosphate buffer (pH 7.0) at scan rate of 2 mV s^{-1} and a drop time of 2 s, correction of $E + 55 \text{ mV}$ (see Experimental) (C) Differential pulse polarographic recording of a 0.05 mM solution of 2,5-bis(1-aziridinyl)-1,4-benzoquinone (IV) under identical conditions, using a pulse amplitude of 100 mV

buffers of alkaline pH, waves I_7 and I_8 are larger and I_1 is smaller than in sodium hydroxide solutions of equal pH and ionic strength.

A small pre-wave could be observed in front of

wave I_1 for all the quinones examined and in the whole pH range studied.

A strong dependence of the $E_{1/2}$ values on pH can be observed in the $E_{1/2}$ -pH plots of quinones

III (Fig. 4a) and **IV** (Fig. 5a). Comparison of the half-wave potentials of wave i_1 of 2-(dimethylamino)-1,4-naphthoquinone (**I**) (Fig. 2a, $E_{1/2} = -374$ mV) and of **III** (Fig. 2b, $E_{1/2} = -259$ mV) at pH 7.0 shows that "closure" of the dimethylamino group to an aziridinyl ring facilitates reduction of the compound (Table 2).

Comparison of the half-wave potentials of wave i_1 of 2,5-bis(dimethylamino)-1,4-benzoquinone (**II**) (Fig. 3a, $E_{1/2} = -320$ mV) and of **IV** (Fig. 3b, $E_{1/2} = -105$ mV) at pH 7.0 also shows that reduction of the compound is facilitated in the case of the aziridinylquinone **IV**.

Determination of the type of current of waves i_1 and i_2 . In order to examine the type of waves i_1 and i_2 , the dependence of i on the concentration of **III** (c) and on the mercury pressure (h) was investigated. Wave i_1 shows a linear dependence on the concentration of **III** at pH 7 and wave i_2 reveals a more or less parabolic dependence (Fig. 8a). A linear dependence of the current of both waves on $\sqrt{h}$ at pH 7 can be observed (Fig. 8b). However, only wave i_1 shows a linear dependence on $\sqrt{h}$ at pH 9. Wave i_2 , at about 10% of its maximum height, is independent of the mercury pressure under these conditions (Fig. 8c). These

Fig 4

Fig 4 (continued) Dependence of half-wave potentials ($E_{1/2}$) and wave current (i) on pH for the polarographic reduction waves 1, 2, 5 and 7 obtained at a dropping mercury electrode for 0.05 mM solutions of (a) 2-(1-aziridinyl)-1,4-naphthoquinone (**III**), (b) 2-(2-methyl-1-aziridinyl)-1,4-naphthoquinone (**V**), (c) 2-(1-aziridinyl)-3-(ethoxycarbonylamino)-1,4-naphthoquinone (**VII**) and (d) 2-(2-methyl-1-aziridinyl)-3-(ethoxycarbonylamino)-1,4-naphthoquinone (**IX**), obtained by direct current polarography from +200 to -1500 mV at a scan rate of 2 mV s⁻¹ and a drop time of 2 s

results point to a diffusion-controlled behaviour of i_1 , independent of pH, whereas that of i_2 is kinetically controlled, limited by the rate of a chemical reaction, partly at pH 7 and completely at pH 9 [22]. The chemical process involved is strongly dependent on pH, as can be concluded from the pronounced dependence of i_2 on pH.

Controlled-potential electrolysis. CPE of **III** was carried out at -400 mV, a potential just in the beginning of the diffusion plateau of wave i_1 at

pH 7 (Fig. 9). After 15 min of electrolysis, a DC curve was recorded, showing a new oxidation wave i'_1 and a smaller reduction wave i_1 ($E_{1/2} = -226$ and -259 mV, respectively), accompanied by a very small post-wave. Wave i_2 was hardly affected, the current remaining at about its original value.

CPE of **IV** at pH 7 yielded similar results. After 15 min of electrolysis at -250 mV, a decrease in the current of i_1 , to about 50% of its original

height is observed, which is accompanied by the appearance of an oxidation wave i_1' of equal height ($E_{1/2} = -70$ mV). Wave i_2 is hardly affected during the course of this experiment and remains virtually at its original height.

Differential pulse polarography. DPP analysis of **III** and **IV** at pH 7 also shows two steps, peak i_1 and peak i_2 (**III**, Fig. 2c, $E_{p1} = -212$ mV, $E_{p2} = -900$ mV; **IV**, Fig. 3c, $E_{p1} = -60$ mV, $E_{p2} = -820$ mV). In DPP at pH 7, peak i_2 is much smaller than peak i_1 , in contrast to DC polarography, where i_1 and i_2 of **III** are of approximately equal height and even a ratio of i_2/i_1 of 1.7 was determined for **IV**. A plot of the amplitude ap-

plied against $W_{1/2}$ (width of the peak current at half its maximum height) is presented in Fig. 10. Peak i_1 becomes relatively broad on increasing the amplitude, approaching a value of $W_{1/2}$ (in mV) equal to the amplitude applied, suggesting a reversible two-electron reduction process [23]. Peak i_2 , however, becomes relatively sharp on increasing the amplitude.

Cyclic voltammetry. Information on the (electro)chemical reversibility of the electrode processes of waves i_1 and i_2 was obtained from cyclic voltammetric (CV) experiments. CV curves of **III** and **IV** at pH 7 are plotted in Fig. 11. The influence of variation of the switching potential on

Fig 5

Fig. 5 (continued) Dependence of half-wave potentials ($E_{1/2}$) and wave current (i) on pH for the polarographic reduction waves 1, 2, 5, 6, 7 and 8 (and 9 in the case of compounds **VIII** and **X**) obtained at a dropping mercury electrode for 0.05 mM solutions of (a) 2,5-bis(1-aziridinyl)-1,4-benzoquinone (**IV**), (b) 2,5-bis(2-methyl-1-aziridinyl)-1,4-benzoquinone (**VI**), (c) 2,5-bis(1-aziridinyl)-3,6-bis(ethoxycarbonylamino)-1,4-benzoquinone (AZQ, **VIII**) and (d) 2,5-bis(2-methyl-1-aziridinyl)-3,6-bis(ethoxycarbonylamino)-1,4-benzoquinone (**X**), obtained by direct current polarography from +200 to -1500 mV at a scan rate of 2 mV s^{-1} and a drop time of 2 s

the number and height of peaks in the back scan is clearly demonstrated: when in scan 1 the cathodic sweep is reversed at -500 mV, shortly after peak i_1 ($E_{p1} = -289 \text{ mV}$), only peak i'_1 ($E'_{p1} = -237 \text{ mV}$) is observed on the CV curves of **III**. However, on reversing the potential scan at -1100 mV, shortly after peak i_2 (scan 2), a new peak i'_3 appears at ca. -310 mV on the curves of **III**. A second reductive sweep on the same mercury drop

confirms the reversibility of the couple i_3/i'_3 (not indicated in Fig. 11). Analogously, when the cathodic scan is reversed at -500 mV, shortly after peak i_2 (scan 1) ($E_{p1} = -135 \text{ mV}$), only peak i'_1 ($E'_{p1} = -69 \text{ mV}$) is observed on the CV curves of **IV**. However, on reversing the potential scan at -1300 mV, shortly after peak i_2 (scan 2) ($E_{p2} = -980 \text{ mV}$), two new peaks i'_3 and i'_4 appear on the curves of **IV** and a second reductive

Fig 6 (A) Repeated direct current polarographic recordings of a 0.05 mM solution of 2-(1-aziridinyl)-1,4-naphthoquinone (**III**) in 0.1 M acetate buffer (pH 4.0) at a scan rate of 2 mV s^{-1} and a drop time of 2 s, correction of $E + 63 \text{ mV}$. The first scan was started after 90 s, the second after 13 min and the third after 24 min. (B) Direct current polarographic curve under identical conditions of a 0.05 mM solution of 2-[(2-hydroxy)ethylamino]-1,4-naphthoquinone (**XI**)

sweep on the same mercury drop (not indicated in Fig. 11) confirms the chemical and electrochemical reversibility of the couples i_3/i'_3 and i_4/i'_4 .

In summary, during CV at pH 7, reversible couples i_1/i'_1 , i_3/i'_3 and i_4/i'_4 are formed whereas generation of i_3 and i_4 depends on the irreversible reduction process of peak i_2 .

Electrochemistry of compounds **XI–XVI**

Compounds **XI–XIV** were included in this study because they are potential degradation products of **III** and **IV**. Only some of the experimental data are presented here. DC polarograms of quinones **XI** and **XII** show only one, well developed reduction wave i_1 , corresponding to a two-electron reduction process in the entire pH range (Fig. 12).

The DC polarographic behaviour of **XIII** has been discussed previously [13]. Polarographic analysis of quinone **XIV** indicates the occurrence of new, complicated phenomena. Pre-waves, post-waves and maxima can be observed on the polarographic curves of **XIV**, which will not be discussed here, however.

The poor solubility of 2-(ethoxycarbonylamino)-1,4-naphthoquinone (**XIV**) and 2,5-bis(ethoxycarbonylamino)-1,4-benzoquinone (**XV**) in water made their electrochemical analysis in aqueous media virtually impossible.

Some results of the electrochemical analysis of all aziridinylquinones and of the two most relevant model compounds are summarized in Table 2. In addition, the observed rate constants, k_{obs} ,

Fig 7 (A) Repeated direct current polarographic recordings of a 0.05 mM solution of 2,5-bis(1-aziridinyl)-1,4-benzoquinone (IV) in 0.1 M acetate buffer (pH 4.0) at a scan rate of 2 mV s^{-1} and a drop time of 2 s, correction of $E + 63 \text{ mV}$. The first scan was started after 60 s, the second after 12 min and the third after 22 min. (B) Direct current polarographic recording of a 0.05 mM solution of 2,5-bis[(2-hydroxy)ethylamino]-1,4-benzoquinone (XII) under identical conditions.

Fig 8 (A) Dependence of wave current (i) on concentration of 2-(1-aziridinyl)-1,4-naphthoquinone (III) in 0.1 M phosphate buffer (pH 7.0) for the polarographic reduction waves (○) 1 and (●) 2, and (B, C) dependence of wave current on mercury pressure h for the polarographic reduction waves (B) 1 and (C) 2 of 0.05 mM solutions of 2-(1-aziridinyl)-1,4-naphthoquinone (III) in phosphate buffer (pH 7.0) (○ and ●, respectively) and borate buffer (pH 9.0) (Δ and ▲, respectively), obtained at a dropping mercury electrode by direct current polarography from +200 mV to -1500 mV at a scan rate of 2 mV s^{-1} and a drop time of 2 s.

Fig 9 Direct current polarographic recordings of a 0.05 mM solution of 2-(1-aziridinyl)-1,4-naphthoquinone (III) before electrolysis at -400 mV in 0.1 M phosphate buffer (pH 7.0) (scan 1) and after 15 min of electrolysis (scan 2)

DISCUSSION

of the acid-catalysed opening of the (first) aziridinyl ring, determined at pH 4.0, are listed, as a parameter describing the ease of protonation of the aziridine prior to quinone reduction (see below).

General mechanism of reduction of mono(1-aziridinyl)quinones

It is evident that wave i_1 can be attributed to the reversible two-proton, two-electron reduction of the quinone moiety to the corresponding hydroquinone (Scheme 1, Eqn. 1). The sequence of proton and electron uptake probably takes place as outlined previously [13]. Wave i_2 corresponds to an irreversible two-electron reduction process, which is strongly dependent on pH and is kinetically controlled. Further, a new reversible couple is formed during this process. Taking into account the fact that the dimethylamino analogue (I) does not show this wave, it can be concluded that i_2 can be attributed to an irreversible reduction of the aziridine (Scheme 1, Eqns. 2 and 3). This reduction process involves a two-proton, two-electron opening of the aziridine ring to the corresponding ethylaminohydroquinone, as has been described for some aziridinylquinones [24–30]. The product formed is responsible for the appearance of peaks i_3/i_3' in CV (Scheme 1, Eqn. 4, Fig. 8a). The kinetic character of wave i_2 is caused by the fact that the aziridine has to be in protonated form (Eqn. 2) before reduction can take place. This also explains the strong dependence of i_2 on pH. Because this reduction takes place at relatively negative potentials, the quinone is already reduced to the hydroquinone (Eqn. 1). Hence, the current of i_2 is limited by the rate of protonation of the aziridine attached to the hydroquinone

Fig 10 Variation of the peak half-width, $W_{1/2}$, with pulse amplitude applied for differential pulse polarography of a 0.05 mM solution of 2-(1-aziridinyl)-1,4-naphthoquinone (III) in 0.1 M phosphate buffer (pH 7.0) scanning from 0 to -1500 mV at a scan rate of 2 mV s^{-1} and a drop time of 2 s. Bottom curve theoretical curve for $n = 2$.

Fig 11 (A) Cyclic voltammograms at a hanging mercury drop electrode of a 0.05 mM solution of 2-(1-aziridinyl)-1,4-naphthoquinone (III) in 0.1 M phosphate buffer (pH 7.0) scanning from 0 to -500 mV (scan 1) and from 0 to -1100 mV (scan 2) at a scan rate of 400 mV s^{-1} (B) Same for a 0.05 mM solution of 2,5-bis(1-aziridinyl)-1,4-benzoquinone (IV) in 0.1 M phosphate buffer (pH 7.0) scanning from $+200$ to -500 mV (scan 1) and from $+200$ to -1300 mV (scan 2) at a scan rate of 500 mV s^{-1}

function and will therefore decrease when the pH of the buffered solution becomes too high to protonate the aziridine of the hydroquinone. Conse-

quently, the decrease in i_2 with increasing pH has the shape of a dissociation curve, reflecting the amount of protonated and unprotonated species

TABLE 2

Electrochemical data for AZQ (VII) and several model compounds

Compound	Wave i_1				Wave i_2					
	Slope ^a	At pH	n ^b	$E_{1/2}$ ^c	Slope ^a	At pH	n ^b	$E_{1/2}$ ^c	$pK_{\text{red}(2)}$ ^d	k_{obs} ^e
I	65	5.5–11.5	2.0	-374	—	—	—	—	—	—
II	62	6.0–10.5	2.2	-319	—	—	—	—	—	—
III	61	3.0–11.5	2.0	-259	151	< 7.5	1.9	-902	8.0	1.42
IV	64	3.0–11.0	2.0	-105	158	< 8.2	3.4	-849	7.5 ^f	2.69
V	60	5.3–11.8	2.0	-252	144	< 7.8	2.0	-897	8.3	13.4
VI	55	6.0–11.0	1.9	-115	148	< 8.3	3.8	-803	8.1 ^f	28.2
VII	61	2.0–9.0	2.0	-285	138	< 7.8	2.0	-921	8.3	0.05
VIII	59	3.0–11.0	2.0	-149	138	< 8.2	3.2	-865	8.3 ^f	0.06
IX	61	2.0–11.5	1.8	-261	136	< 8.0	1.7	-970	8.1	0.18
X	59	3.3–10.8	2.0	-145	149	< 8.3	3.0	-921	8.5 ^f	0.27

^a Slope: $dE_{1/2}/dpH$ in mV at indicated pH range ^b n , at pH 7.0 ^c $E_{1/2}$, in mV at pH 7.0 ^d For definition of pK_{red} see Scheme 1 and of $pK_{\text{red}(2)}$ see Scheme 2 ^e k_{obs} values $\times 10^{-3} \text{ s}^{-1}$ at pH 4.0 ^f $pK_{\text{red}(2)}$ values

at the surface of the mercury electrode; the pH at the point where the DC current is half its maximum height corresponds to a 1 : 1 ratio of protonated and unprotonated species and therefore to the pK_a value of the aziridine of the hydroquinone. Consequently, at $pH > pK_a$ a kinetic DC wave is observed and at $pH < pK_a$, when protonation can take place easily and is no longer rate

controlling, a combination of diffusion and kinetically controlled behaviour can be expected.

The data currently available show that adsorption phenomena cannot be excluded. The small pre-wave observed in DC polarography of all quinones may be caused by adsorption of the quinone at the mercury surface, whereas wave i_2 may be affected by adsorption of the hydro-

Fig. 12 Dependence of half-wave potentials ($E_{1/2}$) and wave current (i) on pH for the polarographic reduction wave 1 obtained at a dropping mercury electrode for 0.05 mM solutions of (a) 2-[(2-hydroxy)ethylamino]-1,4-naphthoquinone (XI) and (b) 2,5-bis[(2-hydroxy)ethylamino]-1,4-benzoquinone (XII), obtained by direct current polarography from +200 to -1500 mV at a scan rate of 2 mV s^{-1} and a drop time of 2 s

quinone. Indications for the latter process were found in the absence of wave i_2 at pH 7.0 in the presence of Triton X-100 and in CV experiments,

where the current of peak i_1' is larger than the current of peak i_1' . This may point to adsorption of the hydroquinone formed. Protonation of the

Scheme 1

(Continued on p 300)

Scheme 1 General, simplified mechanism of reduction of 2-(1-aziridinyl)-3-(substituted)-1,4-naphthoquinones

aziridinyl ring at the mercury surface, either prior to or following quinone reduction, may be favoured by these adsorption processes. For this reason, pK_{ox} and pK_{red} are defined, which include a possible interaction of the compound and/or the product of electrolysis with the electrode surface, influencing protonation of the aziridines. Both pK_{ox} and pK_{red} , determined from the ι -pH plots of wave ι_1 and wave ι_2 , respectively, may be higher than the true pK_a of the aziridinyl ring of the quinone and the hydroquinone (pK_3^Q and pK_3^{HQ} , respectively; see [13]).

The gradual decrease in ι_2 from pH 7 to 5 may be caused by hydrolysis of the protonated aziridine to an ethanolamino function (Scheme 1, Eqn. 5) before reduction can take place. Hence the protonated aziridinyl ring is subjected to two processes: first reductive opening of the ring and second hydrolytic opening of the ring. These processes will always compete for the aziridinium ion at the electrode surface and the current of ι_2 will therefore only reach its theoretical value when hydrolysis is negligible during the drop time.

Although, following quinone reduction, protonation of the aziridinyl ring takes place (wave ι_2)

and a pK_{red} value of 8 can be determined from the ι -pH plot for **III**, no inflection point can be observed in the $E_{1/2}$ -pH plot of wave ι_1 at pH 8. An explanation for this observation might be the fact that, after quinone reduction, subsequent protonation of a functional group will only attribute to an increasing slope in the $E_{1/2}$ -pH plot when the protonated form is stable and is, as such, present in the electrode double layer or at the electrode surface. Protonated aziridines, however, are known to be extremely unstable [6] and, therefore, protonation will be immediately followed by either hydrolytic cleavage of the ring by nucleophilic attack of a buffer component which opens the ring, or by reduction of the ring by uptake of a second proton and two electrons. The ratio of protons and electrons (m/n) of the electrode process involving the quinone reduction will remain 1 and, since the process was proved to be reversible, a slope of 60 mV pH⁻¹ will be observed in the $E_{1/2}$ -pH plot for wave ι_1 , despite subsequent protonation of the aziridine.

The pK_{red} value can also be determined from the $E_{1/2}$ -pH plot for wave ι_2 , where it corresponds to the pH where $E_{1/2}$ becomes independent of pH. However, $E_{1/2}$ values are difficult to determine in this pH region, owing to low values and a broad shape of the current of wave ι_2 . Therefore, the pK_{red} value can be determined more accurately from the ι -pH plot. Because an accurate measurement of the height of the wave is thwarted, the estimated error is 0.2–0.3 units.

The chemical stability of **III** is also strongly affected by the introduction of the aziridinyl ring. Whereas decomposition processes of a simple aminoquinone were observed only in extremely alkaline conditions over a long period of time [13], the aziridinylquinone **III** shows these processes in a relatively wide pH range over much shorter time periods. Degradation of the parent compound prior to quinone reduction leads to a decrease in the concentration of **III** with time and to an increase in that of the degradation product formed. The parent compound and degradation product could be distinguished electrochemically (Fig. 4a) and the polarographic curves of the finally formed product, wave ι_5 , correspond well with those of the pure ethanolaminoquinone **XI**, recorded under

identical conditions (Fig. 6). Consequently, wave i_5 can be attributed to the reduction of 2-(ethanol-amino)-1,4-naphthoquinone **XI**, as a degradation

product of **III** (Scheme 1, Eqns. 6–8). The total current ($i_1 + i_5$) remains at the original level of i_1 at pH 7, indicating that the total amount of

Scheme 2

(Continued on p 302)

Scheme 2

Scheme 2. General, simplified mechanism of reduction of 2,5-bis(1-aziridinyl)-3,6-(substituted)-1,4-benzoquinones

quinone does not change. The inflection point in the i -pH plot of wave i_1 at pH 3.3 corresponds roughly with the pK_{ox} value of the aziridinyl ring of the quinone (Scheme 1, Eqn. 6). The inflection point in the $E_{1/2}$ -pH plot of wave i_5 at pH 3.5 corresponds to the pK_a value of the aminoethanol function of the hydroquinone (pK_3^{HQ} ; see [13]).

Analogously, wave i_7 can be attributed to the reduction of 2-hydroxy-1,4-naphthoquinone (XIII), again as a degradation product of III under alkaline conditions (Scheme 1, Eqns. 9–11). Both types of degradation processes of aziridinylquinones have been reported [7–12].

The polarographic curves of III (waves i_1 and i_7), XI (wave i_1) and XIII (wave i_1) show a less steep slope of the wave and sometimes even minor wave splitting into two separate one-electron waves at alkaline pH can be observed. This indicates a

slightly increased stability of the radical anion formed by the first electron uptake. A change in the reduction mechanism from eeHH to eeH and finally ee is observed for all compounds, as demonstrated by a decrease in the slope of the $E_{1/2}$ -pH plot until it becomes independent of pH. Inflection points correspond to the pK_2^{HQ} values, as discussed previously [13].

General mechanism of reduction of bis(1-aziridinyl)quinones

The reduction mechanism of bis(1-aziridinyl)quinones (Scheme 2) is roughly similar to that of mono(1-aziridinyl)quinones, but some extra waves due to other degradation products are observed. The general reduction mechanism of symmetrical bis(1-aziridinyl)quinones with equivalent aziridine groups, i.e., with equal substituents at positions 3

and 6 (such as **IV**), is simpler than that presented in Scheme 2; in some equations, several structures are redundant.

Quinone reduction can be followed by protonation of only one (Scheme 2, Eqn. 2) or of both aziridines (Eqn. 3), accompanied by the irreversible, reductive opening of the ring, consuming two electrons for each aziridine (Eqns. 4 and 5). The resulting ethylaminohydroquinones are responsible for the peaks i_3/i'_3 (one aziridine opened) and i_4/i'_4 (both aziridines opened) in the CV of **IV** (Scheme 2, Eqns. 6 and 7; Fig. 11b). Again the aziridine has to be in the protonated form (described by pK_{red1} and pK_{red2} in Eqns. 2 and 3) before reduction can take place.

The aziridines can be protonated and subsequently reduced one at a time (Eqns. 2 and 4, followed by protonation and reduction of the second ring, which is not indicated in the scheme) or the aziridines can be protonated and reduced simultaneously. In the DC polarographic, CV and DPP study of **IV**, one broad wave (peak) i_2 is observed, indicating that under the conditions used both aziridines seem to be protonated and reduced almost simultaneously. In CV experiments on **IV**, however, it is demonstrated that when the switching potential was set just in front of peak i_2 , two reversible couples i_3/i'_3 and i_4/i'_4 were formed, (hydro)quinones with one and two aziridines reductively opened, respectively. Hence a small difference in the pK_{red} value of the aziridinyl rings can be observed, which is, however, too small to separate wave i_2 into two single waves of one aziridine reduction each. This difference in the pK_a value of the aziridinyl rings may be larger when the substituents in positions 3 and 6 are no longer identical but have significant different electronic and/or steric properties.

The current of i_2 of **IV** is limited by the rate of protonation of the aziridines attached to the hydroquinone function and will, therefore, decrease when the pH of the buffered solution becomes higher than the pK_{red} of the aziridines of the hydroquinone. The decrease in i_2 on increasing pH again has the shape of a dissociation curve. Although a current of i_2 lower than the theoretical value of four electrons can be frequently observed [as a result of hydrolysis of the protonated aziri-

dine(s) to the ethanolaminohydroquinones at the electrode before reduction can take place, Scheme 2, Eqns. 8 and 9], a relatively accurate value of pK_{red2} (7.5 ± 0.1) can be found by using the point of intersection of wave i_2 with wave i_1 (which corresponds to a two-electron uptake).

Because the protonated aziridinyl ring is unstable and either reductively or hydrolytically opened, no inflection point can be observed in the $E_{1/2}$ -pH plot of wave i_1 of **IV** (see above).

Wave i_6 can be attributed to the reduction of 2,5-bis(ethanolamino)-1,4-benzoquinone as the final degradation product of **IV** in acidic media (Scheme 2, Eqns. 10–15). The mono(1-aziridinyl) quinones **18** and/or **19** (Scheme 2) are the intermediate products formed and responsible for wave i_5 in the polarographic curves. Wave i_7 can be attributed to the reduction of an intermediate, viz., 2-(1-aziridinyl)-5-hydroxy-1,4-benzoquinone (Scheme 2, Eqns. 16–18), whereas wave i_8 corresponds to the reduction of 2,5-dihydroxy-1,4-benzoquinone (Eqns. 19–21). Similar indications of an increased stability of the radical anion formed by the first electron uptake, as found for **III**, have been observed for the waves i_1 , i_7 and i_8 of **IV**. Inflection points in the $E_{1/2}$ -pH plot correspond to pK_2^{HQ} values of **IV** and its degradation products. In alkaline media an additional degradation product of AZQ (**VIII**) can be formed, its reduction corresponding to wave i_9 . This wave was not observed with **X**. Further, a small anodic pre-wave in the polarographic curves of **VII**, **VIII**, **IX** and **X** at pH > 8 has been observed, in addition to the cathodic pre-wave mentioned previously. This phenomenon may be related to the dissociation of the amino hydrogen of (one of) the ethoxycarbonylamino side-chain(s), since this proton has weakly acidic properties owing to the electron-withdrawing properties of the quinone function and the carbonyl function of the ethoxycarbonylamino side-chain. The lone pair of electrons of the basic form might be delocalized over the quinone function, potentially yielding an oxidizable species. No further attempts have been made to clarify the processes responsible for wave i_9 and the pre-waves observed with the quinones examined in this study.

The inflection point in the i -pH plot of wave i_1

at pH 4 corresponds roughly to the pK_{ox1} value of the first aziridine of **IV** (Scheme 2, Eqn. 10) and that in the ι -pH plot of wave ι_6 at pH 3.2 roughly to the pK_{ox2} value of **IV** (Eqn. 11). The inflection point in the $E_{1/2}$ -pH plot of wave ι_6 at ca. pH 3.2 is in fairly good agreement with the inflection point of 3.0, calculated from the $E_{1/2}$ -pH plot of **XII** (Fig. 12), and can be attributed to the pK_3^{HQ} value of the second aminoethanol function of **VI**. The pK_4^{HQ} value of the first aminoethanol function can be estimated to be ca. 5.5.

For reasons of simplicity, deviations from Schemes 1 and 2, due to the presence of a methyl group at position 2 of the aziridinyl ring(s), have not been included. The overall pattern of reduction of the other compounds is qualitatively the same as observed for the model compounds **III** and **IV**. There are, however, considerable quantitative differences between the chemical and electrochemical properties of **III** and **IV** and their substituted analogues (Table 2), which will be discussed briefly.

Substituent effects in quinone reduction

Before comparison of half-wave potentials at a particular pH is allowed and substituent effects within a series of structurally related compounds can be examined, reduction mechanisms of all compounds have to be elucidated and similarity in the mechanisms finding expression in the parallelism in the $E_{1/2}$ -pH plots at a particular pH has to be verified [31]. In this study, comparison of half-wave potentials of wave ι_1 at pH 7.0 is allowed, since very similar slopes in the $E_{1/2}$ -pH plot of ι_1 are observed (Table 2). Comparison of the half-wave potential of wave ι_1 at pH 7.0 of **III** and **IV** with their dimethylamino analogues **I** and **II** shows that the ring-opened compound is much more difficult to reduce. This can also be concluded from comparison of the half-wave potentials of parent compound and decomposition product(s) having the aziridine(s) hydrolytically opened (e.g., **XI** and **XII**). These results can be explained by considering the fact that aminoquinones are known to behave as dipolar or quadrupolar compounds, owing to delocalization of the lone pair over the quinone moiety [32]. The electron density of the quinone function is in-

creased by the electron-donating character of the dimethylamino substituent (the latter reflected by its σ_p value of -0.83 [33]). Hence reduction of the quinone will become more difficult and a shift of $E_{1/2}$ to more negative values is observed [31]. Aziridines seem less electron-donating: the lone pair seems to be more fixed on the nitrogen, which may be required to stabilize the strained three-membered ring [6].

Compounds **XV** and **XVI**, having only the ethoxycarbonylamino function(s), could not be studied in aqueous media. However, it can be deduced from the present data [e.g., by comparison of **III** and **VII** (-26 mV), **V** and **IX** (-9 mV), or **IV** and **VIII** (-44 mV) and **VI** and **X** (-30 mV)] that the introduction of an ethoxycarbonylamino substituent into the quinone function only slightly affects quinone reduction, which is in accordance with the relatively small electron-donating character of such a substituent ($\sigma_p = -0.15$ [32]).

Substituent effects in aziridine protonation

It can be seen in Schemes 1 and 2 that several parameters give information on the ease of protonation of the aziridinyl moiety prior to and following quinone reduction. Protonation of the aziridinylquinone can be described by pK_{ox} and k_{obs} , both based on the acid-catalysed hydrolysis of the aziridinyl ring to an aminoethanol function, whereas protonation of the hydroquinone can be described by pK_{red} and pK_{red2} . Considering the hypothesis of an alkylative mechanism of action of aziridinylquinones, k_{obs} , pK_{red} and pK_{red2} may serve as parameters describing the ease of formation of the alkylating species. From these parameters, pK_{red} is most difficult to determine accurately, as outlined previously. It is clear from the data in Table 2 that no definite conclusions can be drawn with regard to the mono(1-aziridinyl) quinones presented in this paper.

For AZQ (**VIII**), an increase in pK_{red2} from 7.5 to 8.3 can be observed as compared with **IV**, indicating an increased ease of protonation of the aziridines when the quinone function has been reduced. A similar but smaller shift of 0.4 unit can be observed by comparison of the data for compounds **VI** and **X**. When not reduced to quinone,

however, the chemical stability of the aziridines is increased on introduction of the ethoxycarbonylamino functions, as can be observed by a decrease in k_{obs} and a change in the t -pH plots of waves i_1 and i_5 of **IV** and **VIII**. Hence protonation of the aziridines after quinone reduction is favoured, whereas protonation prior to quinone reduction is not. In addition, it can be observed from the k_{obs} values in Table 2 that **III**–**VI**, lacking a substituent at the position adjacent to the aziridinyl ring, are the most sensitive towards the acid-catalysed hydrolysis of the aziridinyl moiety.

These results may be explained as follows: prior to quinone reduction, aziridine protonation seems to be virtually unaffected by the electronic character of the substituents and may be mainly governed by their steric properties and by the presence of a methyl function at the 2-position of the aziridinyl ring, as will be discussed below. The strongly electron-withdrawing influence of the quinone moiety on protonation of the aziridine(s) is probably superior to the electronic effect of the quinone substituents. Once reduced to the more electron-donating hydroquinone, however, electronic effects of quinone substituents become more important and influence protonation of the aziridines. Further, in addition to their electronic character, the steric properties of the substituents and their ability to form a hydrogen bond with the aziridinyl ring may also affect aziridine protonation, as demonstrated by the electrochemical analysis of a series of aziridinylquinoid analogues of AZQ, having quinone substituents of different electronic and steric character. The results will be presented in another paper.

Influence of methyl substitution at the 2-position on the ease of protonation of the aziridine(s)

The influence of methyl substitution at the 2-position on the ease of protonation of the aziridine(s) can best be examined by comparing compounds **III** and **V**, and **IV** and **VI**. No definite conclusion can be drawn from the pK_{red} values of **III** and **V**. Their k_{obs} and pK_{ox} values, however, clearly reveal an increased lability towards acid-catalysed hydrolysis on methyl substitution of the aziridine. These effects are even better demonstrated by comparison of the data obtained for **IV**

and **VI**, revealing a significant increase in $pK_{\text{red}2}$ from 7.5 to 8.1, accompanied by an increase in k_{obs} and pK_{ox} .

The results of this work on the electrochemistry of AZQ are in agreement with previous reports on the electrochemistry of aziridinylquinones [24–30]. They are not in agreement, however, with a recent report on the electrochemistry of AZQ [4]. The difference in the working electrodes used (mercury electrode in this study vs. a glassy carbon electrode in [4]) may be partly responsible for this.

To investigate substituent effects on quinone reduction and aziridine protonation quantitatively, a series of 92 quinones, including 46 of the aziridinylquinone type, was investigated by DC polarography in aqueous media. Results of quantitative structure–electrochemistry relationships will be presented in another paper.

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REFERENCES

- 1 P L Gutierrez, B M Fox, M M Mossoba and N R Bachur, *Mol Pharmacol*, 26 (1984) 582
- 2 P L Gutierrez, B M Fox, M M Mossoba, M J Egorin, H Nakazawa and N R Bachur, *Biophys Chem*, 22 (1985) 115
- 3 M S B Nayar, M Mossoba and P Gutierrez, *Proc Am Assoc Cancer Res*, 26 (1985) 214
- 4 M M Mossoba, M Alizadeh and P L Gutierrez, *J Pharm Sci*, 74 (1985) 1249
- 5 M Akhtar, A Begleiter, D Johnson, J W Lown, L McLaughlin and S-K Sim, *Can J Chem*, 53 (1975) 2891
- 6 O C Dermer and G E Ham, in O C Dermer and G E Ham (Eds), *Ethylenimine and Other Aziridines*, Academic Press, New York, 1969, pp 106–314
- 7 G K Poochikian and J C Craddock, *J Pharm Sci*, 70 (1981) 159
- 8 G K Poochikian and J A Kelley, *J Pharm Sci*, 70 (1981) 163
- 9 A G Bosanquet and S B McLoughlin, *Invest New Drugs*, 3 (1985) 43
- 10 A Kusai, S Tanaka and S Ueda, *Chem Pharm Bull*, 29 (1981) 3671
- 11 A Kusai, S Tanaka and S Ueda, *Chem Pharm Bull*, 30 (1982) 2534
- 12 A Kusai and S Ueda, *Chem Pharm Bull*, 32 (1984) 2406

- 13 R J Driebergen, J den Hartigh, J.J.M Holthuis, A. Hulshoff, W.J van Oort, S.J Postma Kelder, W Verboom, D N Reinhoudt, M Bos and W E. van der Linden, *Anal Chim Acta*, 233 (1990) 251
- 14 Farbenfabrieken Bayer, *Br Pat.*, 762 723, 1956, *Chem. Abstr.*, 51 (1957) 12 153h
- 15 Shenley Industries, *U S. Pat.* 2 913 453, 1959, *Chem. Abstr.*, 54 (1960) 8097c
- 16 G Casini, F Claudi, M Grifantini and S. Martelli, *J. Heterocycl Chem.*, 11 (1974) 377.
- 17 Farbenfabrieken Bayer, *Br Pat.*, 866 434, 1961; *Chem Abstr.*, 56 (1962) 11 757f
- 18 A H. Khan and J S. Driscoll, *J Med. Chem.*, 19 (1976) 313
- 19 A. Inoue, N Kuroki and K Konishi, *Bull Univ Osaka Prefect Ser.*, Ser A, 8 (1959) 31
- 20 K Konig, *Chem Ber.*, 92 (1959) 257
- 21 H H J L. Ploegmakers, M.J.M Mertens, R.J Driebergen, W.J van Oort, A Dirksen, W van der Horst, A C C van Til and R. van Trigt, *J Autom. Chem.*, 9 (1987) 15
- 22 P Zuman, in P Zuman and J M Kolthoff (Eds), *Progress in Polarography*, Vol II, 1962, pp 583–600
- 23 E P Parry and R.A Osteryoung, *Anal Chem.*, 37 (1965) 1634
- 24 H Berg and K.-H. Konig, *Z. Krebsforsch.*, 62 (1958) 495
- 25 H Wagner and H Berg, *J Electroanal. Chem.*, 1 (1959/60) 61
- 26 H Wagner and H Berg, *J Electroanal Chem.*, 2 (1961) 452
- 27 A Anhalt and H Berg, *J. Electroanal Chem.*, 4 (1962) 218
- 28 H. Berg, *Z Chem.*, 8 (1962) 237
- 29 H. Berg, *Chem Zvesti.*, 16 (1962) 342
- 30 E Bauer and H Berg, *Chem Zvesti.*, 18 (1964) 454.
- 31 P Zuman, in P. Zuman (Ed), *Substituent Effects in Organic Polarography*, Plenum, New York, 1967, pp 273–308
- 32 S Dahne, J Rauf and H Paul, *Tetrahedron Lett.*, (1964) 3355
- 33 C Hansch, A. Leo, S.H Unger, K.H Kim, D Nikaitani and E J Lien, *J Med Chem.*, 16 (1973) 1207