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1003 — ELECTROCHEMICAL REACTIVITY OF BIOLOGICALLY ACTIVE QUINONE/HYDROQUINONE SESQUITERPENOIDS ON GLASSY CARBON ELECTRODES

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SUMMARY

The redox reactivity of avarone and avarol, a quinone/hydroquinone couple isolated from the marine sponge *Dysidea avara*, was investigated by cyclic voltammetry, using a glassy carbon electrode. Both oxidation of avarol and reduction of avarone in aqueous ethanol (1 : 1 *V/V*) take place by a 2 e^- process at a wide range of pH values; in acetonitrile, however, the reduction of avarone occurs as a stepwise electron transfer process. The mechanisms, as well as the scope and limitations of the method are discussed with reference to the biological activity of the two sesquiterpenoids.

INTRODUCTION

The involvement of many natural products containing a quinone/hydroquinone moiety in electron and proton transfer reactions in biological systems is well documented. There is also accumulating evidence that the biological activity of many such compounds can be attributed to the ability of the semiquinone intermediates, formed in the one-electron oxidation–reduction process, to generate reactive oxygen species with a pronounced detrimental effect on cells [1]. Such a mechanism of action was suggested for antitumor antibiotics of the anthracycline and mitosane class [2], although alternative mechanisms involving direct semiquinone participation in cell damage may also take place [3]. Therefore, considerable interest in the detection and characterization of reactive intermediates formed in redox reactions of biologically important quinone–hydroquinone systems gave

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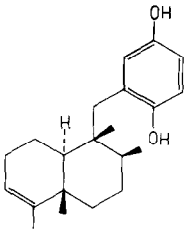
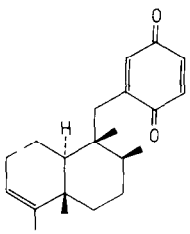


QH_2



Q

rise to studies of such species by different techniques, such as e.s.r., pulse radiolysis and electrochemical methods [4–6].

In our investigations of the biological activity of the sesquiterpenoids avarone (Q) and avarol (QH_2), a quinone/hydroquinone couple isolated from the marine sponge *Dysidea avara* [7], we have shown that these compounds display moderate antibacterial and antifungal activity, but strong antileukemic activity, both *in vitro* and *in vivo* [8–10].

Our recent results indicate that this activity is associated not only with interference in microtubule formation [11], but also with the effect of the two metabolites on the activity of the cell protective enzyme superoxide dismutase [12]. Consequently, in order to provide information which will contribute to the understanding of the initial redox reactions of the avarol/avarone couple, we have undertaken a study of their electrochemical reactivity in different media using a glassy carbon electrode.

EXPERIMENTAL

Chemicals and solutions

Analytically pure samples of avarol (QH_2) and avarone (Q), isolated and purified as described previously [8], were used as 1 m*M* solutions.

Acetonitrile (Koch Light Ltd) was purified by a procedure similar to that described earlier [13]. The supporting electrolyte was tetraethylammonium perchlorate (C. Erba, polarographic grade) as a 0.1 *M* solution.

The solutions for hydroalcoholic media were prepared by dissolving an accurately weighed quantity of material in an appropriate volume of absolute ethanol ($n_{D,25\,^\circ C} = 1.359$). Britton–Robinson modified universal buffers [14] (prepared from analytical grade A.R. chemicals) were used as supporting electrolyte. Because of the low solubility of the investigated compounds in water, 50% (*V*/*V*) ethanolic buffer mixtures were used.

Equipment and measurements

All electrochemical experiments were carried out with a Hi-Tek Instruments potentiostat, type DT 2101, a Chemical Electronics function generator, type RB 1,

and a Gould XY recorder and Gould digital storage oscilloscope OS 4020. The cell, electrodes and apparatus for voltammetric measurements were the same as described earlier [15]. The electrolytic solutions were handled under a nitrogen atmosphere. Measurements were made at 25 °C ± 1; pH values were determined by means of a Methrom-E 353 pH meter. In each experiment, a glassy carbon electrode (g.c.e.) was used as the working electrode, platinum as the counter electrode, and a s.c.e. as the reference electrode. Before each measurement, the g.c.e. was polished and cleaned for a few minutes in DMF, followed by rinsing with acetone, boiling in distilled water and drying with tissue paper. The area of the g.c.e. ($A = 0.1143$ cm^2) was determined electrochemically by using ferrocene as a standard for r.d.e. measurements in acetonitrile–tetraethylammonium perchlorate medium.

When using the g.c.e., reproducible current-potential curves were obtained over a wide range of potentials at which electrolysis could be carried out.

RESULTS

Electrochemical reduction of avarone (Q)

Cyclic voltammograms were run at a series of potential scan rates at different pH values in Britton–Robinson buffers. A typical cyclic voltammogram of Q shows a single irreversible cathodic peak at −0.26 V *versus* s.c.e. at pH 7.07 (Fig. 1). No evidence of reversibility was noticed for sweep rates ranging from 0.1 to 80 V s^{-1}, which means that a one-electron reduction product of Q, the radical anion (semi-

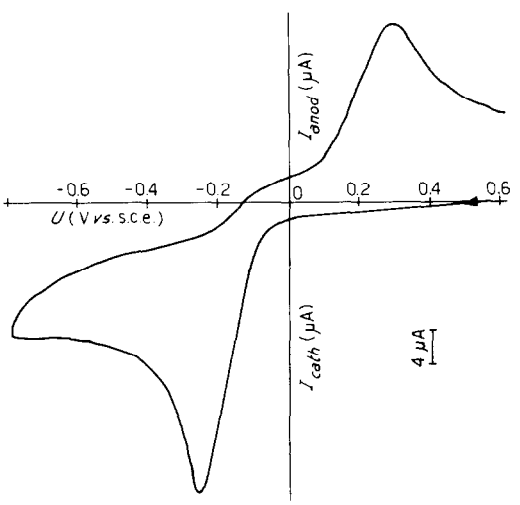


Fig. 1. Cyclic voltammogram of avarone (1 mM) at 100 mV s^{-1} in buffer pH 7.07 at the g.c.e.

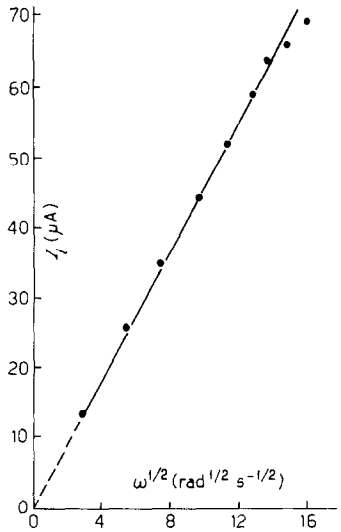


Fig. 2. Plots of I_{lim} *versus* $\omega^{1/2}$ for the reduction of avarone (1 mM) in buffer pH 7.07 at a g.c.e.-disc electrode. Scan rate 30 mV s^{-1}.

quinone), was not observed even in alkaline solutions. Sweep reversal from the cathodic to the anodic side caused the appearance of the oxidation peak at +0.27 V *versus* s.c.e., corresponding to oxidation of QH_2 formed at the reduction peak at −0.26 V *versus* s.c.e. Linear sweep voltammetry of QH_2, added to the solution of Q, run at 0.1 V s^{-1} in the range of potentials from 0.0 to 0.6 V at pH 7.07, showed the oxidation peak at +0.27 V *versus* s.c.e. The reduction peak at −0.26 V versus s.c.e. was broad ($U_p - U_{p/2} = 95$ mV) and the variation of U_p with log v is 125 ± 10 mV per decade of sweep rate, in the range from 0.1 to 1.0 V s^{-1}. The large activation energy in this case can be attributed to slow irreversible electron transfer [16]. Another possiblity which could explain the voltammetric behaviour is that the rate of chemical reaction following electron transfer is much faster than the electron transfer itself, so that a totally irreversible wave is observed [17]. For the first possibility, theory predicts $U_p - U_{p/2} = 48/\alpha n$ mV and the observed value of 95 mV fits well for $\alpha = 0.5$ and $n = 1$.

The current-potential curves for the reduction of Q, obtained with the rotating disc electrode, showed one single wave. The limiting current dependence on the square root of the rotation speed (in the range 10.46–282.6 rad s^{-1}) was linear at −0.6 V *versus* s.c.e., which corresponds to the plateau on the steady-state current–potential curves, indicating that the cathodic process is first order in Q and diffusion controlled (Fig. 2). Plots of I_{lim} *versus* $\omega^{1/2}$ at different pH values were also linear. The diffusion coefficient was calculated ($D = 1.54 \times 10^{-6}$ cm^2 s^{-1}) using the Levich equation and assuming $n = 2$ for the reduction of Q.

Coulometry at −0.6 V *versus* s.c.e. of 10^{-3} M solution of Q in Britton–Robinson buffer at pH 7.07, performed at a platinum gauze electrode, gave values for n of 1.8

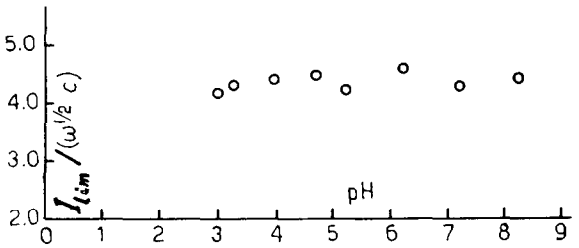


Fig. 3. Plots of current functions, $I_{lim}/\omega^{1/2}c$, with pH for the reduction of avarone (1 mM).

and 1.9, respectively, as expected for an overall reaction leading to QH_2 as the final product.

The current functions $I_{lim}/\omega^{1/2}c$, obtained at different pH values (Fig. 3) were constant with the square root of the rotation rate in the range from 10.46 to 282.6 rad s^{-1} and showed a two-electron overall reduction of Q.

The pH dependence of the peak potential, U_p, was linear up to pH 6.2 with a slope of 60 mV per pH unit (Fig. 4). The pH dependence of U_p in solution of high pH tends to zero.

The cyclic voltammogram of Q at pH 10 shows two irreversible cathodic peaks at $U_{p,I} = -0.33$ V and $U_{p,II} = -0.43$ V *versus* s.c.e. (Fig. 5). When the direction of the potential scan was reversed, an oxidation peak around 0.07 versus s.c.e. could be observed, corresponding to the oxidation of avarol (QH_2) formed.

The cyclic voltammogram of 1 mM solution of Q in acetonitrile–tetraethylammonium perchlorate medium using the g.c.e. as cathode showed two well defined peaks at -0.50 V and -0.78 V *versus* s.c.e., respectively (Fig. 6). Based on peak-potential separation (60 mV), peak-current ratio ($I_{anod}/I_{cath} = 1$) and peak-current dependence on scan rate (0.1–1 V s^{-1}), the first wave was found to represent a reversible one-electron reduction leading to the stable radical anion (semiquinone) and the second, an irreversible reduction leading to the dianion. The position of the second cathodic peak is dependent on the presence of water in the acetonitrile solution which causes a pronounced shift of the potential to more

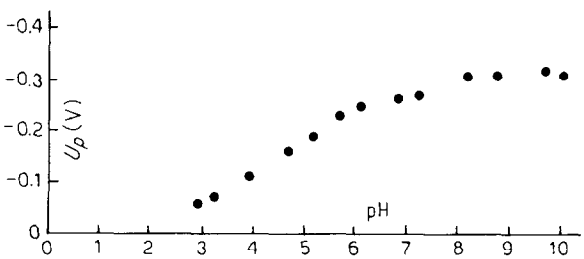


Fig. 4. Variation of peak potentials with pH for the reduction of avarone (1 mM) at 100 mV s^{-1} in buffer solutions.

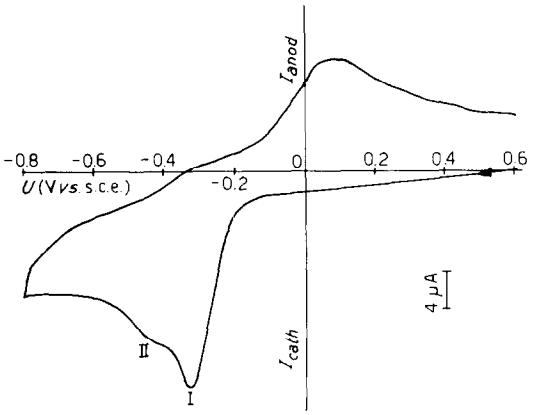


Fig. 5. Cyclic voltammogram of avarone (1 mM) at 100 mV s^{-1} in buffer pH 10 at the g.c.e.

positive values [18]. The irreversibility of the second wave is the result of the fast protonation of the dianion formed, following the second electron transfer; in the presence of a weak acid, such as phenol, the second wave disappears while the first wave increases.

Electrochemical oxidation of avarol (QH_2)

A typical cyclic voltammogram of QH_2 shows a single irreversible anodic peak at +0.22 V *versus* s.c.e. at pH 7.32 (Fig. 7), corresponding to the formation of Q which is reduced in the cathodic sweep at −0.18 V *versus* s.c.e. No evidence of reversibility

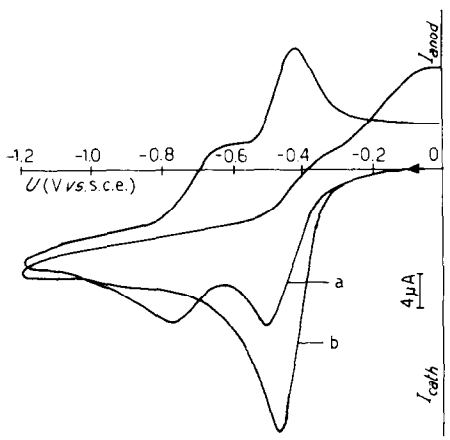


Fig. 6. Cyclic voltammograms of avarone (1 mM) at the g.c.e. at 100 mV s^{-1}: (a) CH_3CH–01. M Et_4NClO_4; (b) CH_3CN–0.1 M Et_4NClO_4, phenol (10 mM).

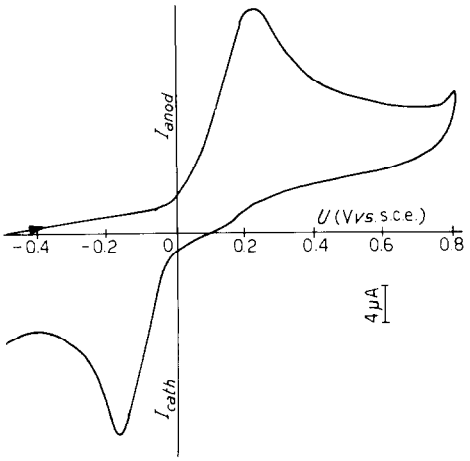


Fig. 7. Cyclic voltammogram of avarol (1 mM) at 100 mV s^{-1} in buffer pH 7.32 at the g.c.e.

for the oxidation of QH_2 was noticed for sweep rates from 0.1 to 80 V s^{-1}. The cyclic voltammogram exhibits a single irreversible oxidation wave ($U_p - U_{p/2} = 110$ mV; $dU/d\log v = 110 \pm 10$ mV).

The current–potential curves, obtained with the rotating disc electrode, were run at different pH values for the oxidation of QH_2 (rotation rates 10.46 to 282.6 rad s^{-1}). The limiting currents, I_{lim}, were linearly dependent on the square root of the rotation rate, $\omega^{1/2}$, at +0.5 V *versus* s.c.e., corresponding to the plateau on the steady-state current–potential curves and indicating that the overall process is diffusion controlled.

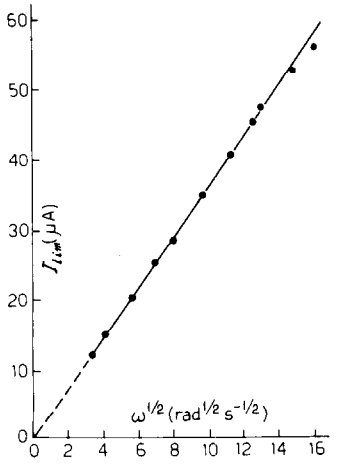


Fig. 8. Plots of I_{lim} *versus* $\omega^{1/2}$ for the oxidation of avarol (1 mM) in buffer pH 7.32 at the g.c.e.-disc electrode; scan rate 30 mV s^{-1}.

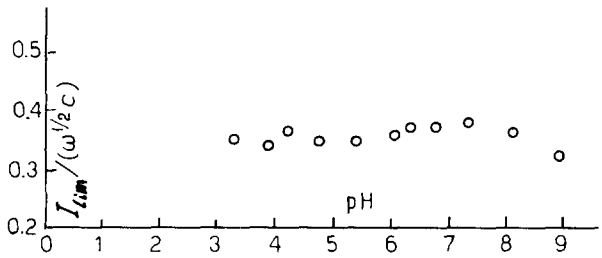


Fig. 9. Plots of current functions, $I_{lim}/\omega^{1/2}c$, with pH for the oxidation of avarol (1 mM).

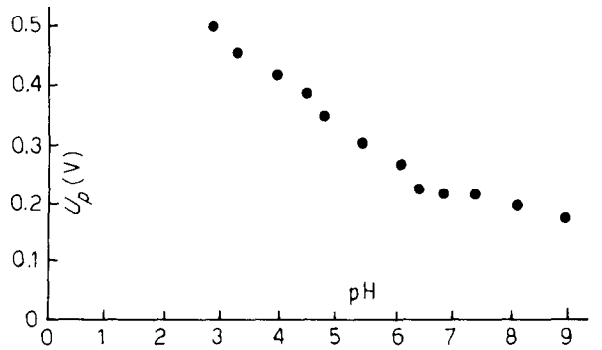


Fig. 10. Variation of peak potential with pH for the oxidation of avarol (1 mM) at 100 mV s^{-1} in buffer solutions.

For illustration, the plot at pH 7.32 is presented in Fig. 8. The diffusion coefficient of QH_2 ($D = 0.95 \times 10^{-6}$ cm^2 s^{-1}) was calculated using the Levich equation, assuming $n = 2$ for the oxidation of QH_2.

The current functions, $I_{lim}/\omega^{1/2}c$, obtained at different pH values (Fig. 9), were constant with the square root of the rotation rate and showed virtually a two-electron oxidation of QH_2 in the pH range examined.

The pH dependence of the oxidation peak potential of QH_2, illustrated in Fig. 10, tended to zero with the intercept at a pH value of around 6.4.

DISCUSSION

The following redox reaction describes formally the electrochemical behaviour of the avarone–avarol (Q–QH_2) system in aqueous media:

$$Q + 2\,H^+ + 2\,e^- \rightleftharpoons QH_2$$

In general, the results of investigations of the quinone/hydroquinone system suggest that this system is complex because both the reduction and oxidation proceed in a number of steps combined with the exchange of protons and electrons, involving the

species presented in the corresponding nine-membered square scheme [19]. However, important progress has been achieved by theoretical investigations of the kinetics of the *p*-benzoquinone/hydroquinone couple on a platinum electrode [20]. Following these considerations and our results, which indicate that a two-electron reduction of Q occurs at a wide pH range, the overall reduction in the pH range from 2.8 to 7.0 might be taking place by a H^+eH^+e, eH^+H^+e and eH^+eH^+ process.

At higher pH, the pH dependence of U_p disappears, indicating that no protons are involved. Under these conditions, the voltammetric wave becomes narrower ($U_p - U_{p/2} = 50$ mV), and it may be suggested that $n = 2$, provided that the initial electron transfer is irreversible and that $\alpha = 0.5$. The current function, $I_{lim}/\omega^{1/2}c$, was 4.0 at pH 10 (4.2 at pH 7.1), corresponding to the two-electron wave. On the other hand, if the small peak, $U_{p,II}$ (Fig. 5) is attributed to the further reduction of the intermediate semiquinone $Q^{\cdot -}$, the peak difference ($U_{p,II} - U_{p,I} = 100$ mV) suggests that the second electron transfer occurs mainly through a homogeneous disproportionation reaction of the semiquinone. Thus, the reaction could follow an edHH mechanism, where d designates a homogeneous electron transfer. Our explanations are in accordance with a recently published study on the disproportionation of quinone radical anions in protic solvents at high pH [21].

In aprotic medium, the reduction of avarone points clearly to a stepwise two-electron process leading to the dianion of avarol. However, in the presence of proton donors (HA), the following simplified mechanism which explains the single two-electron wave, could be envisaged:

$$Q + e^- \rightleftharpoons Q^{\cdot -}$$

$$Q^{\cdot -} + HA \rightarrow QH^{\cdot} + A^-$$

$$QH^{\cdot} + e^- \rightleftharpoons QH^-$$

$$QH^- + HA \rightarrow QH_2 + A^-$$

On the basis of the above results one can conclude that, on the voltammetric time-scale, the oxidation of avarol in aqueous medium is a two-electron process leading to avarone at all pH values investigated; oxidation to avarone presumably occurs by reverse mechanistic pathways from those for the electrochemical reduction of avarone to avarol. The difference between the anodic and the cathodic potential in the oxidation of QH_2 and the reduction of Q (Figs. 1 and 7) is probably due to the formation of a polymeric film, which changes the conditions for the electron transfer at the electrode, as already noticed during the anodic detection of phenolic compounds [22,23]. It is noteworthy to point out that the second sweep in the cyclic voltammogram of Q, run at 0.1 V s^{-1} in the range of potentials from 0.0 to -0.8 V, showed a shift of the reduction peak of 0.02 V, *i,e.* a shift from -0.26 V to -0.24 V *versus* s.c.e., at pH 7.07. A similar phenomenon was observed in the cyclic voltammogram of QH_2, run at 0.1 V s^{-1} in the range of potentials from -0.1 to 0.8 V, in which an anodic shift of the oxidation peak of 0.03 V ($+0.22$ V to $+0.25$ V *versus* s.c.e.) was observed.

It should be pointed out that the formation of radical intermediates in avarol oxidation is supported by our biochemical experiments related to the effect of avarol on both mitochondrial Mn- and Cu/Zn-superoxide dismutase activity [12]. Thus, avarol showed an apparent decrease in superoxide dismutase activity, probably due to toxic superoxide radical formation *via* avarol/avarone radical intermediates. In an attempt to clarify the mechamism of this and other biological effects, we carried out a preliminary e.s.r. experiment indicating the formation of a paramagnetic species in a methanol/water solution of avarol (air oxidation) [24]; such intermediates are considered to be obligatory in the production of toxic oxygen radicals which have many deteriorating effects on cell membranes and cellular components, such as lipid peroxidation and macromolecule depolymerization [25].

The cyclic voltammetry experiments failed to show the formation of a one-electron product of avarol oxidation, *i.e.* the radical cation ($QH_2^{\cdot+}$) or radical ($QH^{\cdot}$), even at high sweep rates in aqueous ethanol or acetonitrile solution [26]. Radical cations of this type are strong acids which lose a proton, yielding radicals as intermediates at pH corresponding to physiological conditions. For the equilibrium constant of the reaction $QH_2^{\cdot+} \rightleftharpoons QH^{\cdot} + H^+$, pK_a of *ca.* -5 was determined [27]. If formed, the phenoxy radical $QH^{\cdot}$ is oxidized at the applied potential, leading to avarone through a loss of an electron and a proton. It is to be expected that the formal potential for the oxidation of the phenoxy radical ($QH^{\cdot}$) is less anodic than the potential for the oxidation of avarol [28] and consequently, could not be observed.

It is apparent that the results of studies related to the electrochemical behaviour of quinone/hydroquinone couples frequently lead to different conclusions regarding the nature and number of intermediates involved in these processes. The inconsistency of some of the interpretations, as well as the resulting controversies, should be ascribed to the differences in applied experimental procedures and, in particular, to severe time-scale limitations of the voltammetric experiments. However, it appears that the major problem still lies in the incomplete understanding of the complexity of the reactions of these structural types in different media. As for the biological system, it is quite obvious that the quinone head group can participate in the electron transfer processes by different mechanisms, depending on its location in such a heterogeneous environment. Thus, the reaction may take place either by a stepwise 2 e^- process in the lipid portion of the bilayer, or by a more complex process involving multiple electron and proton transfer reactions in a hydrophylic environment. The complexity of the process becomes even more pronounced if the conformational flexibility of the quinone component is taken into account, as a consequence of quinone–enzyme complex formation [29]. In such an arrangement, distortion of the planarity of the quinone ring affects charge distribution, resulting in a substantial change of reactivity of such sysytems. Our experiments in micellar systems, which are currently under way, may help in clarifying this problem.

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