Theoretical and electrochemical study of the mechanism of anthraquinone-mediated one-electron reduction of oxygen: the involvement of adducts of dioxygen species to anthraquinones

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Anthraquinone derivatives, which are an important class of anticancer drugs, possess the ability to mediate the transfer of one electron to molecular oxygen to form the superoxide anion radical, which results in their undesirable peroxidating and, further, cardiotoxic properties. In this paper one-electron reduction of dioxygen–anthraquinone systems was studied using electrochemical and theoretical methods. Cyclic voltammetry (CV) experiments performed on dimethyl sulfoxide and dimethylformamide solutions of selected anthraquinones suggest that anthraquinones bearing hydroxy groups or their semiquinones interact remarkably with molecular oxygen; this is manifested as a shift of anthraquinone-reduction potential towards more positive values in the presence of oxygen. This phenomenon can be explained by the assumption that anthraquinone reduction is accompanied with oxygen addition to form hydroperoxide anion radicals, which can be formed by anthraquinones possessing proton-donor (*e.g.* hydroxy) groups only; the calculated (using the *ab initio*, DFT and semiempirical PM3 methods, with 1-hydroxynaphthoquinone and model anthraquinone derivatives as model compounds) enthalpies of this process are greater than those of anthraquinone reduction alone.

Anthracycline derivatives constitute an important class of antitumour drugs which mainly act by intercalating the DNA of the cells of tumour tissues.[1] These compounds however, possess undesirable cardiotoxic properties, essentially related to their peroxidating activity, resulting from the mediation of the generation of superoxide anion radicals and other active oxygen species in the process of one-electron transfer from NAD(P)H to molecular oxygen.[2,3]

A widely accepted mechanism of anthraquinone-mediated superoxide generation, hereafter referred to as mechanism I, involves, in the first stage, one-electron reduction of an anthraquinone derivative by NAD(P)H (stage Ia), which is followed by the interaction of the semiquinone thus formed with molecular oxygen (stage Ib), leading ultimately to the superoxide on radical (stage Ic). This process is believed to occur at the ubiquinone-reducing site of the mitochondrial NADH dehydrogenase (complex I).[3–7]

$$A + e^- \longrightarrow A^{\cdot -} \qquad \text{(Ia)}$$

$$A^{\cdot -} + O_2 \longrightarrow AO_2^{\cdot -} \qquad \text{(Ib)}$$

$$AO_2^{\cdot -} \longrightarrow A + O_2^{\cdot -} \qquad \text{(Ic)}$$

A denotes the anthracycline derivative. The species $AO_2^{\cdot -}$ can be a non-covalent complex between semiquinone and molecular oxygen or a covalent adduct.

An alternative mechanism (hereafter referred to as mechanism II) assumes that in the first stage of the mediation covalent or non-covalent adducts of the singlet ($^1\Delta_g$) molecular oxygen to anthraquinones are formed (stage IIa), which then undergo one-electron reduction (stage IIb) the products of which could dissociate into anthraquinone and the superoxide anion radical (stage IIc).[8–12]

$$A + O_2(^1\Delta_g) \longrightarrow AO_2 \qquad \text{(IIa)}$$

$$AO_2 + e^- \longrightarrow AO_2^{\cdot -} \qquad \text{(IIb)}$$

$$AO_2^{\cdot -} \longrightarrow A + O_2^{\cdot -} \qquad \text{(IIc)}$$

Both mechanisms of superoxide production seem to be reasonable. The presence of semiquinone anion radicals upon incubation in anaerobic conditions of heart and liver mitochondrial preparations containing NADH dehydrogenase and cytochrome P-450 reductase, respectively, was evidenced by EPR spectroscopy.[5,13] On the other hand, singlet-oxygen addition to unsaturated and aromatic compounds is well known in organic chemistry (see *e.g.* ref. 14 for review). Specifically, the interaction of molecular oxygen with anthracyclines[15] or their iron(III) complexes[16,17] was shown to occur in oxygenated aqueous solutions of these compounds on visible-light irradiation, to give ultimately the superoxide anion radical and products of anthracycline oxidation.

In our earlier papers[8–12] we studied the mechanism of electron-transfer mediation to molecular oxygen by anthraquinones by means of electrochemical and theoretical methods. Cyclic voltammetry (CV) studies indicated the appearance of new reduction peaks that were attributed to the adducts of singlet oxygen to anthraquinones.[8,9] Such peaks were not observed for non-peroxidating anthraquinones, such as ametantrone.[8] *Ab initio* and semiempirical calculations of the structure and energetics of possible oxygen adducts to model anthraquinones (formed in stage IIa of mechanism II) had shown that such compounds probably have peroxide or hydroperoxide structure[8,10–12] (Fig. 1). Because hydroperoxide formation involves a proton transfer to the attached oxygen molecule, it is possible only for anthraquinones possessing proton-donor groups. The hydroperoxides were found to be lower in energy compared to peroxides, which is in agreement with the fact that hydroperoxides are the only oxygen adducts formed on the photooxygenation of aromatic compounds containing hydroxy groups, such

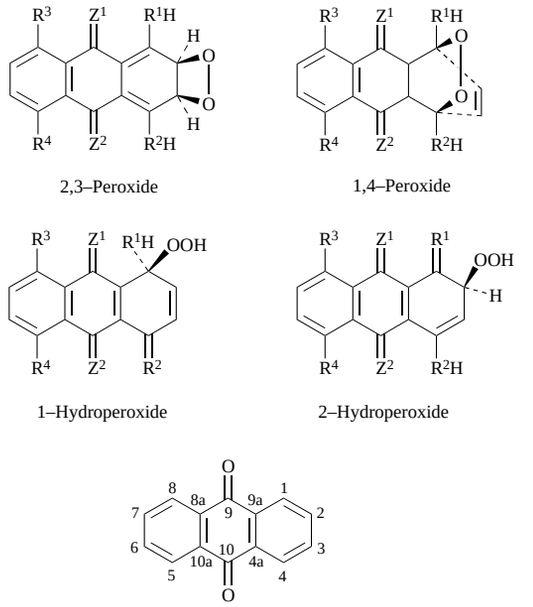

Fig. 1 Structures of low-energy peroxides and hydroperoxides of anthraquinones and the atom-numbering system of the anthraquinone moiety

as phenols[18–20] or naphthols.[21,22] So far, we have not investigated the energetics of the next two stages of mechanism II nor the energetics of mechanism I.

Because of the efficient hydration of the superoxide anion radical,[23] its reduction potential in aqueous solution in which our previous experiments on anthraquinone reduction in the presence of oxygen were carried out,[8,9] is less negative (−0.33 V for neutral and alkaline pH; ref. 23) than those of anthraquinones (typically −0.6 V; ref. 9), therefore the reduction of oxygen occurs prior to anthraquinone reduction. Because the superoxide anion radical easily yields such active oxygen species as the peroxyl and hydroxyl radical,[23,24] the possibility cannot be ruled out that the additional CV peaks observed in our previous experiments corresponded to the products of the reactions of active oxygen species with anthraquinones. In contrast, in aprotic solvents, such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF), the superoxide anions are solvated far less efficiently than in water and, in consequence, the reduction potential of oxygen is significantly more negative, taking a value of −0.86 and −0.95 V for DMSO and DMF, respectively.[23] Moreover, aprotic solvents mimic the nonpolar environment of the quinone-reducing centre of the mitochondrial NADH dehydrogenase.[25] All these points prompted us to re-examine, using CV, the one-electron reduction of selected anthraquinone derivatives in DMSO and DMF instead of water. The model compounds considered in our present study cover a wide range of anthraquinone-based anticancer drugs, such as demethoxy-daunorubicin, 5-iminodaunorubicin, ametantrone and novantrone;[2,3] their structures are shown in Fig. 2 (compounds **1**–**6**). Their peroxidating ability was measured previously *in vitro* as the NADH oxidation rate.[9]

We also extended the theoretical calculations of our earlier papers[10–12] to the energetics of all stages of mechanisms I and II, assuming low-energy hydroperoxides and peroxides obtained in earlier studies (Fig. 1) as intermediates. First, we considered 1-hydroxynaphthalene-5,8-dione (1-hydroxynaphthoquinone) as a model compound (it possesses all basic structural features of hydroxyanthraquinones), for which we carried out *ab initio* RHF/ROHF 4-31G, density functional (DFT), as well as semi-empirical PM3 calculations. The less expensive semiempirical PM3 calculation have been carried out on compounds **1**–**10** (Fig. 2), which are better models of anthraquinone-based anticancer drugs.

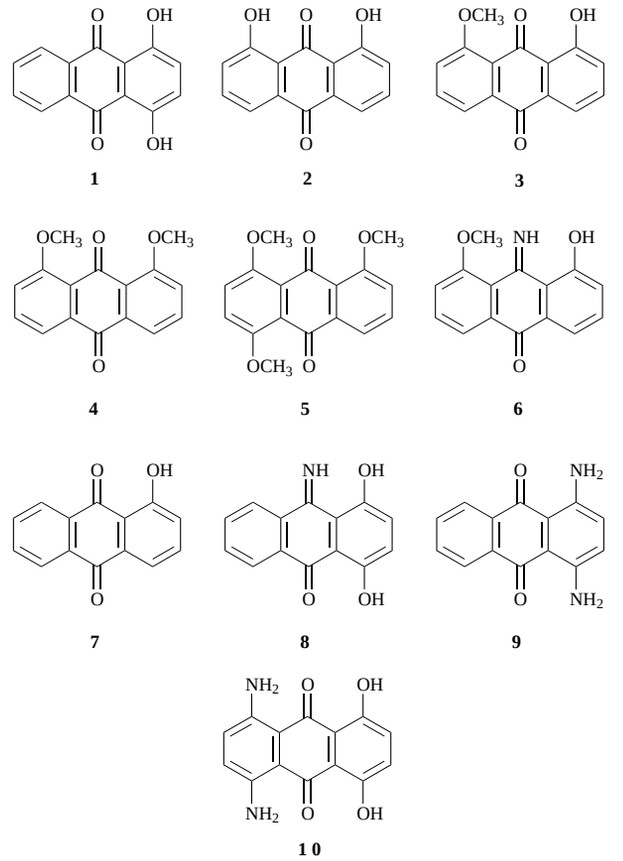

Fig. 2 Structures of anthraquinone derivatives considered in this study: 1,4-dihydroxy- (**1**), 1,8-dihydroxy- (**2**), 1-hydroxy-8-methoxy- (**3**), 1,8-dimethoxy- (**4**), 1,5,8-trimethoxyanthraquinone (**5**), 1-hydroxy-8-methoxy-9-iminoanthracene-10-one (**6**), 1-hydroxy- (**7**), 1,4-dihydroxy-9-iminoanthracene-10-one (**8**), 1,4-diamino- (**9**) and 1,4-diamino-5,8-dihydroxyanthraquinone (**10**).

Materials and methods

Chemicals

1,4-Dihydroxy- (**1**) and 1,8-dihydroxy-anthraquinone (**2**) were purchased from Aldrich and used without additional purification. 1-Hydroxy-8-methoxy- (**3**), 1,8-dimethoxy- (**4**), 1,5,8-trimethoxy-anthraquinone (**5**), and 1-hydroxy-8-methoxy-9-iminoanthracene-10-one (**6**), were a gift from Professor E. Borowski, Technical University of Gdańsk.

The solvents DMSO and DMF were purified according to procedures described in the literature.[26] DMSO was dried (24 h) with A4 molecular sieves, while DMF was dried with CaH_2 and subsequently A4 molecular sieves. Both solvents were distilled over the drying agents and used no later than 1 week after purification. The residual water content was less than 0.05% for DMSO and 0.04% for DMF, respectively, as assessed by the Fischer method.[27]

Tetraethylammonium perchlorate (TEAP) was crystallized twice from methanol–water (10 : 1 v/v) and dried for 48 h at 50 °C under reduced pressure before use.

Cyclic voltammetry measurements

The CV experiments were performed under dry argon in 0.1 M solution of TEAP at 25 °C in a three-electrode measuring cell (Laboratorny Prystroye, Czech Republic) with the static mercury drop electrode (SMDE) of 0.56 mm diameter used as the working electrode and a platinum wire used as the counter-electrode. The potential values were reported *versus* a NaCl saturated calomel electrode (SCE) which was connected to the measurement cell by a salt bridge filled with 0.1 M TEAP in DMF or DMSO, respectively. All measurements were made

using a potentiostat EP-20A (UNIPAN, Poland) and voltage generator EG-20 (UNIPAN, Poland). The XY-recorder 4100 from Laboratorny Prystroye (Czech Republic) was used to record CVs. The apparatus was equipped with an automatic feedback unit to eliminate the potential due to solution resistance (IR drop; ref. 28, p. 56). To test IR drop compensation, CV curves were recorded at 298 K for a standard ferricenium/ferrocene reversible system† in DMSO and DMF. The difference obtained between the peak and half-peak potential was $|E_{p/2} - E_p| = 0.056$ V, a value very close to the theoretical value of 0.0565 V for 298 K.[29] It can therefore be concluded that IR drop does not affect the results under the experimental conditions.

To record CVs corresponding to the solutions of compounds **1**–**6** in the absence of oxygen, argon was passed through the measurement cell for 30 min before applying the voltage. DMSO and DMF solutions containing given concentrations of oxygen were obtained by injecting appropriate amounts of oxygen-saturated DMSO or DMF to the measurement cell, using the Hamilton syringe provided with a micrometric screw. The procedure as described in the literature was used to obtain oxygen-saturated solutions of the solvents.[30] The concentration of oxygen in oxygen-saturated DMSO at 298 K is 2.2×10^{-3} M.[30] For DMF the concentration of oxygen was estimated to be 4.8×10^{-3} M, based on literature data on the solubility of oxygen in this solvent.[23] The error in oxygen concentration can be estimated to be *ca*. 5%, as assessed by measurements of the height of the oxygen-reduction peak for a series of solutions prepared using the above-mentioned procedure. During the experiments with oxygen at fixed concentrations air was carefully excluded from the measurement cell.

Adding small amounts of water (up to a concentration of 1%) did not shift the peaks corresponding to the one-electron reduction and back-oxidation of anthraquinone derivatives or oxygen by more than 0.01 V, their height being virtually unchanged. It can therefore be concluded that small amounts of water inadvertently introduced into the system on preparing the solutions and manual operation did not affect the results of CV measurements.

Quantum-mechanical calculations

Ab initio 4-31G calculations on 1-hydroxynaphthoquinone and its peroxides and hydroperoxides, as well as their reduced forms, were carried out with the use of the GAMESS package.[31] The restricted Hartree–Fock (RHF) or restricted open-shell Hartree–Fock (ROHF) schemes were used, depending on whether the closed-shell systems or radicals were considered. The reference state of molecular oxygen was assumed to be the doubly degenerate $^1\Delta_g$ state approximated by a single configuration with one doubly occupied antibonding $\pi^*_{\pm 1}$ MO, or, equivalently, two singly occupied $\pi^*_{2p_x}$ and $\pi^*_{2p_y}$ MOs. Density functional (DFT) calculations were carried out with the BLYP functional and nonlocal Becke–Perdew (BP) correction using the DGAUSS software.[32] Two basis sets: 6-31G* and DZVP were implemented. The semiempirical PM3 calculations of 1-hydroxynaphthoquinone and compounds **1**–**10** and the species occurring in mechanisms I and II derived from them were carried out using the MOPAC 93 package.[33–35] In all cases geometry was optimized with respect to all degrees of freedom. The starting geometries for PM3 calculations were built with the use of the PCMODEL software,[36] based on standard bond lengths and angles. The MOPAC built-in EF minimizer was used and optimization was terminated after the Euclidean norm of energy gradient decreased below 0.01 kcal mol^{-1} Å. The PM3-optimized geometries of the species derived from 1-hydroxynaphthoquinone served as starting geometries for the *ab initio* and DFT calculations. The convergence criterion for *ab initio* and DFT optimization was the decrease of the maximum component of the gradient below 10^{-4} hartree bohr^{-1} and 10^{-3} eV bohr^{-1}, respectively. Unless indicated otherwise, calculations were carried out without including solvation, which mimics the conditions at the largely nonpolar ubiquinone-reducing site of the NADH dehydrogenase.[25] In the case of *ab initio* calculations, the partition–function contributions to enthalpy (PFC) at 298 K were calculated in the harmonic approximation from energy Hessian at minimum. The enthalpy of each species was then computed by adding up the total *ab initio* energy of this species and the relevant PFC. Because the PM3 methods was parametrized so as to reflect the heats of formation at 298 K, it was unnecessary to calculate the PFC contributions in this case. In the case of DFT calculations we did not compute the PFC either, because the results were too divergent from the *ab initio* results and were, therefore, not considered in subsequent discussion (see the Results section).

The energies and enthalpies of the reactions constituting mechanisms I and II and the total energetic effect of reductive oxygen addition to anthraquinone (which is common for mechanism I and II) were calculated using the Hess law from eqns. (1)–(5) (the equations contain enthalpies, but the same relations also hold for energies), where A denotes the anthraquinone

$$\Delta H_{red}(X) = H(X^{\bullet -}) - H(X) \quad (1)$$

$$\Delta H_{Ib} = H(AO_2^{\bullet -}) - [H(A^{\bullet -}) + H(O_2)] \quad (2)$$

$$\Delta H_{IIa} = H(AO_2) - [H(A) + H(O_2)] \quad (3)$$

$$\Delta H_{sum} = H(AO_2^{\bullet -}) - [H(A) + H(O_2)] \quad (4)$$

$$\Delta H_{Ic,IIc} = [H(A) + H(O_2^{\bullet -})] - H(AO_2^{\bullet -}) \quad (5)$$

derivative and AO_2 the corresponding peroxide or hydroperoxide, $H(X)$ is the enthalpy of species X at 298 K, $X^{\bullet -}$ is the one-electron-reduced form of the species X.

Results and discussion

Cyclic voltammetry studies

The potentials and assignment of all observed electric-current peaks are summarized in Table 1. As shown, the compounds studied fall into two classes: for compounds **4** and **5** the one-electron-reduction peaks are virtually insensitive to the presence of oxygen, while for compounds **1**–**3** and **6** the influence of oxygen on one-electron reduction of anthraquinone is apparent. The compounds for which oxygen influences the reduction process all contain hydroxy groups, while the compounds for which no influence is observed do not contain hydroxy groups. It should be noted that for compounds **1**–**3** the peak of oxygen reduction to the superoxide anion radical appears at a more negative potential than the peaks of the reduction of anthraquinones to the corresponding semiquinones. This implies that no remarkable amount of superoxide anion radical can be formed at a potential at which one-electron reduction of anthraquinone derivative takes place and, further, effectively excludes the possibility of the interaction of anthraquinones with superoxide radicals when reduction is carried out in aerobic conditions. It should also be noted that such reduction-peak ordering is opposite to that of the reduction peaks in aqueous solutions[8,9] and indicates that the superoxide anion radical is thermodynamically stabilized in protic solvents.

Sample CVs corresponding to the experiments with quinizarin **1** in DMSO are shown in Figs. 3 and 4. These runs were carried out while keeping the potential within the range of the

† A CV process is considered reversible, if the difference of the peak potential and half-current peak potential $|E_{p/2} - E_p| = 0.0565$ V at 298 K[29] and the ratio of the cathodic to the anodic current is close to 1.0 (p. 43 of ref. 28). Practically, peaks with $|E_{p/2} - E_p| < 0.06$ V can still be considered reversible (p. 43 of ref. 28).

Table 1 Potentials (V) and assignment of electric-current peaks in CV runs for compounds **1**–**6** (see Fig. 2 for compound numbering)

		Peak potential and assignment[b]								
		A		O_2		Other		A		O_2
System[a]	Solvent	E_{pc}^1	E_{pa}^1	E_{pc}^1	E_{pa}^1	E_{pc}^1	E_{pa}^1	E_{pc}^2	E_{pa}^2	E_{pc}^2
O_2	DMSO			−0.870	−0.795					−2.16
1	DMSO	−0.625	−0.565					−1.180	−1.120	
1 + O_2	DMSO	−0.500	irv	−0.830	−0.735			−1.140	irv	
2	DMSO	−0.630	−0.565					−1.260	−1.190	
2 + O_2	DMSO	−0.460	irv	−0.860	−0.780		−0.580	−1.210	irv	
3	DMSO	−0.765	−0.700					−1.267	−1.192	
3 + O_2	DMSO	−0.734	irv	−0.837	−0.622			−1.374	−1.242	
4	DMSO	−1.050	−0.978					−1.605	irv	
4 + O_2	DMSO	−1.050	−0.975	−0.850	−0.780			−1.695	irv	
5	DMSO	−1.145	−1.080					−1.588	irv	
5 + O_2	DMSO	−1.130	−1.065	−0.840	−0.775			−1.610	irv	
6	DMSO	−0.945	−0.870					−1.436	−1.270	
6 + O_2	DMSO	−0.9	ads	−0.830	ads	−0.590	−0.360	−1.6	−1.270	
O_2	DMF			−0.945	−0.870					−1.91
1	DMF	−0.645	−0.590					−1.1245	−1.170	
1 + O_2	DMF	−0.620	irv	−0.890	−0.820		−0.640	−1.200	irv	
2	DMF	−0.625	−0.555					−1.200	−1.125	
2 + O_2	DMF	−0.450	irv	−0.880	−0.780		−0.58	−1.380	−1.230	

[a] The peaks reported here that were obtained in the presence of oxygen were recorded for oxygen-saturated solvents and oxygen-saturated solutions of anthraquinones; see text for details. [b] E_{pc}^1, E_{pc}^2—the potentials of the cathode (reduction) peaks corresponding to one- and two-electron reduction, respectively, of anthraquinones, molecular oxygen and the putative anthraquinone–oxygen complexes (listed in the 'other peaks' column); E_{pa}^1, E_{pa}^2 denote the potential of the anode (back-oxidation) peaks; irv in the field of an anode peak indicates that the anode peak did not appear, so the reduction process was irreversible; ads marks a series of adsorption peaks the position of which cannot be clearly established.

one-electron reduction processes of the species under study. As shown [Fig. 3(*a*) and 3(*b*)], the reduction of **1** under anaerobic conditions and the reduction of oxygen dissolved in DMSO are reversible processes which occur at potentials of −0.870 and −0.625 V, respectively. For oxygenated solutions of **1** the CV process becomes clearly irreversible, with the anodic peak corresponding to semiquinone oxidation disappearing [Fig. 3(*c*)]. The process is irreversible even when the potential does not reach the value of the oxygen-reduction potential [dashed curve in Fig. 3(*c*)]. For compounds **3** and **4** that do not have hydroxy groups the reduction process remains reversible after introducing oxygen and the corresponding CV curves are superpositions of the curves corresponding to anthraquinone and oxygen reduction (data not shown). At the same time the cathode reduction peak of **1** shifts towards less negative potentials, reaching the value of −0.500 V for long-oxygenated solutions (Table 1). This is indicative of an E_rC_i sequence of processes (*i.e.* reversible electrochemical reduction followed by irreversible chemical reaction; see *e.g.* Fig. 2–14 of ref. 28). This conforms with the two first stages of mechanism I, Ia corresponding to the E_r and Ib to the *C* process. The diminishing of the cathodic peak corresponding to the back-oxidation of the anthraquinone anion radical suggests that process Ib is irreversible, so that anthraquinone anion radical cannot be restored quickly. However, from the results presented in Table 1 and Figs. 3 and 4, it cannot be inferred whether the adduct $AO_2^{\bullet -}$ dissociates into A and $O_2^{\bullet -}$ (stage Ic), remains unchanged or rearranges to give further products. In order to find out which of these processes can occur, we carried out a CV experiment on the oxygenated solution of compound **1**, starting from a potential more negative than that of oxygen reduction and reversing the sweep direction (Fig. 4). Under such conditions, compound **1** should be reduced to the corresponding semiquinone and the latter react with oxygen, before the anthraquinone-reduction peak can be observed. Fig. 4 shows that the anthraquinone-reduction peak disappears. The same behaviour is observed for the solutions of **1** in DMF and for the solutions of **2** in both solvents. The absence of anthraquinone-reduction peak (Fig. 4) suggests that in the solvents studied either the stage Ic that would restore free anthraquinone is slow compared to the voltage-scan rate or the adduct $AO_2^{\bullet -}$ undergoes further irreversible reactions leading to the formation of saturated hydroxy derivatives, as in the case of acyclic unsaturated compounds[37] and/or the breaking of anthraquinone moiety.

It can also be noted that the oxygen-reduction peak is shifted towards a less negative potential. This can be explained by direct electron transfer from semiquinone to the oxygen molecule, as outlined by eqn. (6). This phenomenon has been observed

$$A^{\bullet -} + O_2 \longrightarrow A + O_2^{\bullet -} \qquad (6)$$

even for the unsubstituted anthracene-9,10-dione.[38]

A different picture of CV runs is observed for compound **6** (Fig. 5). Instead of shifting the reduction peak of **6** towards less negative potentials, a new peak appears at a potential much less negative than that of the iminoanthraquinone. The intensity of this peak gradually increases as more oxygen is dissolved in the solution. The reduction peak of **6** occurs at almost the same potential as the oxygen-reduction peak and therefore the second peak in Fig. 5 should be regarded as the overlap of the reduction peaks of these two species. Although in this case the reduction peak of **6** occurs at an even more negative potential than that of oxygen, it is unlikely that the new peak is formed on the interaction of superoxide ion with **6**, because it appears long before any oxygen can be reduced to superoxide. Thus, the new peak should be attributed to the formation of a new species prior to reduction (the *CE* mechanism[28]), which conforms with stages IIa (*C*) and IIb (*E*) of mechanism II.

Because only one-electron reduction of anthraquinones and oxygen was the subject of this work we did not analyse the peaks corresponding to the second stage of reduction, leading to the formation of anthraquinol or peroxide dianions. The potential values corresponding to this stage are summarized in Table 1 only for the sake of completeness.

Model calculations of electron-transfer mediation by 1-hydroxynaphthoquinone

The total energies (4-31G and DFT) or heats of formation (PM3) of 1-hydroxynaphthoquinone, its peroxides and hydroperoxides, as well as their reduced forms are summarized in Table 2 (see Fig. 6 for the atom-designation system). The enthalpies (4-31G and PM3) or energies (DFT) of the reactions Ia, Ib, IIa, IIb, Ic (IIc) and the total enthalpies/energies of

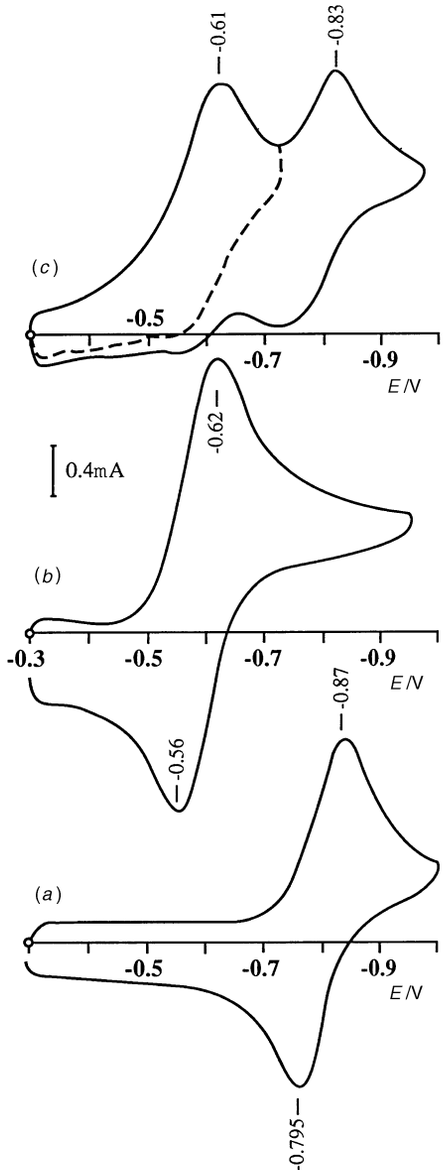

Fig. 3 CVs of DMSO containing oxygen at a concentration of 4.0×10^{-4} M (*a*), deoxygenated 8.0×10^{-4} M solution of quinizarin (**1**) in DMSO (*b*), and 8.0×10^{-4} M solution of quinizarin in DMSO containing oxygen at a concentration of 4.0×10^{-4} M (*c*). The rate of voltage change was 100 mV s^{-1}. The solid line in graph (*c*) corresponds to changing the voltage from −0.30 to −1.00 V, *i.e.* beyond the oxygen-reduction potential, the broken line corresponds to the experiment in which the voltage was changed from −0.30 to −0.70 V, not reaching the value of −0.83 V at which one-electron reduction of oxygen takes place.

reductive oxygen binding (ΔH_{sum} and ΔE_{sum}) calculated by *ab initio*, DFT and PM3 methods are shown in Table 3. It should be noted that the absolute reaction enthalpies, especially those involving charged species, will depend on the reaction environment and, if reduction is involved, also on the redox potential of the reducing agent. Therefore only the difference between the reaction enthalpies involving alternative intermediates are meaningful and will be a subject of further discussion.

As shown, both PM3 and RHF/ROHF results predict the hydroperoxides and their anion radicals to have a lower energy than the peroxides and their anion radicals (Table 2). However, the *ab initio* results indicate that the 4-hydroperoxide should form rather than the 2-hydroperoxide (though the enthalpy difference is not big), which is consistent with the structure data of experimentally observed hydroperoxides of phenols [18–20] and naphthols.[21,22] Conversely, PM3 predicts the 4-hydroperoxide to have a comparatively high energy. Because *ab initio* 4-31G calculations produce the valence geometry of the peroxide/hydroperoxide moiety consistent with the experimental data,

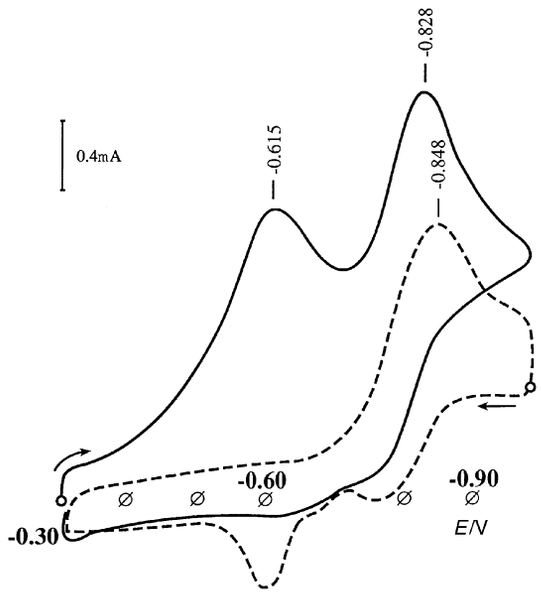

Fig. 4 Comparison of the CVs of a 8.0×10^{-4} M solution of quinizarin in DMSO containing oxygen at a concentration of 5.5×10^{-4} M registered for two different values of starting potential. For the solid curve the starting potential was −0.30 V and the initial direction of voltage change was towards more negative values. For the dashed curve the starting potential was −1.10 V and the initial direction of voltage change was towards less negative values. The rate of voltage change was 100 mV s^{-1} and the initial delay time was **5** s; no changes in curve shape were observed after increasing the delay time beyond this value.

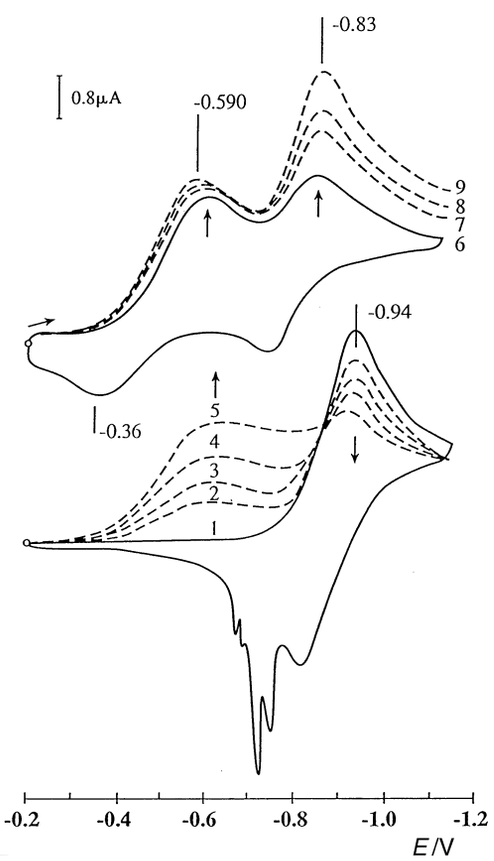

Fig. 5 A series of CVs of 6.0×10^{-3} M DMSO solutions of 1-hydroxy-8-methoxy-9-iminoanthracene-10-one (**6**) containing oxygen at gradually increasing concentration: 0, 1.4×10^{-4}, 2.1×10^{-4}, 3.6×10^{-4}, 5.4×10^{-4}, 6.3×10^{-4}, 9.2×10^{-4}, 12.0×10^{-4} and 20.1×10^{-4} M for curves **1**–**9**, respectively

Table 2 *Ab initio* and DFT-calculated total energies (hartree) and PFC contributions to enthalpies (kcal mol^{-1}) and PM3-calculated heats of formation (kcal mol^{-1}) of 1-hydroxynaphthoquinone, its peroxides and hydroperoxides and their reduced and protonated forms. A denotes 1-hydroxynaphthoquinone, the peroxides and hydroperoxides are designated by the places of the peroxy or hydroperoxy group attachment to the naphthoquinone moiety (see Fig. 6 for atom-numbering system)

	4-31G		DFT 6-31G*	DFT DZVP	PM3
Species[a]	E_{tot}	PFC	E_{tot}	E_{tot}	HOF
A	−605.886 617	100.2	−610.257 395	−610.501 642	−70.6
2,3-O_2	−755.201 583	106.8	−760.516 183	−760.848 068	−64.0
1,4-O_2	−755.195 214	106.0	—[a]	—[a]	−57.6
2-OOH	−755.215 034	105.5	−760.504 724	−760.835 742	−68.0
4-OOH	−755.220 546	106.1	−760.512 133	−760.842 303	−61.6
$A^{\bullet -}$	−605.921 476	98.6	−610.333 531	−610.583 721	−120.2
2,3-$O_2^{\bullet -}$	−755.235 104	105.1	−760.608 984	−760.943 452	−110.9
1,4-$O_2^{\bullet -}$	−755.258 737	104.6	—[a]	—[a]	−120.7
2-$OOH^{\bullet -}$	−755.286 330	104.8	−760.622 393	−760.956 244	−127.7
4-$OOH^{\bullet -}$	−755.287 663	104.9	−760.621 695	−760.954 134	−116.5
4-OOH(H-OH)$^{\bullet}$[b]	−755.815 546	113.1	—[c]	—[c]	−68.4
4-OOH(H-O1)$^{\bullet}$	−755.815 603	113.7	—[c]	—[c]	−70.7
4-OOH(H-O2)$^{\bullet}$	−755.835 946	113.7	—[c]	—[c]	−64.5
$O_2(^1\Delta_g)$	−149.328 154	4.6	−150.233 723	−150.315 785	10.9
$O_2^{\bullet -}$	−149.361 616	3.9	−150.293 363	−150.382 301	−13.3
$OOH^{\bullet}$	−149.960 021	11.6	—[c]	—[c]	−2.0

[a] The oxygen molecule dissociated from 1-hydroxynaphthoquinone during the course of minimization. [b] Protonated forms of the 4-hydroperoxide anion radical; OX denotes the protonation site (refer to Fig. 6 for atom designation). [c] The protonated forms were not considered in the case of DFT calculations.

Table 3 Upper part: 4-31G and PM3-calculated enthalpies of oxygen addition to 1-hydroxynaphthoquinone (ΔH_{IIa}), reduction of 1-hydroxynaphthoquinone and its peroxides and hydroperoxides (ΔH_{red}), oxygen addition to semiquinone (ΔH_{Ib}), and total energies of (hydro)peroxide anion radical formation (ΔH_{sum}), and superoxide elimination from (hydro)peroxide anion radical ($\Delta H_{Ic/IIc}$). Lower part: DFT-calculated energies of same reactions. See Fig. 6 for atom numbering

	ΔH_{IIa}		ΔH_{red}		ΔH_{Ib}		ΔH_{sum}		$\Delta H_{Ic/IIc}$	
Species	4-31G	PM3	4-31G	PM3	4-31G	PM3	4-31G	PM3	4-31G	PM3
A			−23.5	−49.6						
2,3-O_2	10.3	−4.2	−23.3	−46.9	11.0	−1.5	−12.5	−51.2	−9.2	27.0
1,4-O_2	13.5	2.1	−41.3	−63.1	−4.3	−11.3	−27.8	−61.0	6.1	36.8
2-OOH	0.5	−8.2	−45.4	−59.7	−21.4	−18.3	−44.9	−67.9	23.2	43.8
4-OOH	−2.3	−1.8	−43.3	−54.9	−22.2	−7.1	−45.8	−56.8	24.0	32.6
	ΔE_{IIa}		ΔE_{red}		ΔE_{Ib}		ΔE_{sum}		$\Delta E_{Ic/IIc}$	
	6-31G*	DZVP	6-31G*	DZVP	6-31G*	DZVP	6-31G*	DZVP	6-31G*	DZVP
A			−47.8	−51.5						
2,3-O_2	−15.7	−19.2	−58.3	−59.9	−26.2	−37.4	−74.0	−79.1	36.6	37.4
2-OOH	−8.5	−11.5	−73.9	−75.6	−34.6	−45.3	−82.4	−87.1	45.0	45.4
4-OOH	−13.2	−15.6	−68.8	−70.2	−34.2	−44.1	−82.0	−85.8	44.5	44.1

while the PM3 method overestimates the O–O bond length,[10,12] the 4-31G results should be considered more reliable than the PM3 results.

For the energy-optimized DFT structure of the 1,4-peroxide, the oxygen molecule was separated from naphthoquinone, with the C–O distance exceeding 3 Å. For other neutral oxygen adducts, the results of the DFT and the RHF calculations are consistent, but the DFT method greatly overestimates the energy of the reduction of the 2,3-peroxide. Thus, the DFT method does not seem to be superior over the semiempirical PM3 method, as far as the species studied here are concerned. Therefore we do not refer to the DFT results in subsequent discussion. Also for this reason, we did not consider worthwhile to calculate the PFC and then ΔH values corresponding to the DFT results.

The quantity of most general interest is ΔH_{sum}, *i.e.* the enthalpy of the formation of anion radicals from 1-hydroxynaphthoquinone and molecular oxygen upon one-electron reduction, because it does not depend on whether the reaction proceeds *via* route I or *via* route II. The data of Table 3 clearly show that for the hydroperoxide anion radicals this energy is almost two times lower than the energy of one-electron reduction of 1-hydroxynaphthoquinone (ΔH_{Ia}). Thus, reduction with simultaneous oxygen binding is highly favourable, if the hydroperoxide anion radicals are intermediates.

For the 1,4-peroxides ΔH_{sum} is not much lower than ΔH_{Ia} and for the 2,3-peroxide it is higher than ΔH_{Ia}. The analysis of the energetics of the other stages of mechanisms I and II leads to similar conclusions. For mechanism I, the enthalpy of oxygen addition to 1-hydroxyseminaphthoquinone (ΔH_{Ib}) is much lower when the hydroperoxide anion radical is formed than for the formation of the peroxide anion radical. For mechanism II, the enthalpies of oxygen addition to the neutral compound (ΔH_{IIa}) are more negative when hydroperoxides are formed and the electron affinities (ΔH_{IIb}) of hydroperoxides are substantially more negative than the electron affinities of the peroxides. Concluding, of all possible covalent intermediates in the electron-transfer process the hydroperoxide anion radicals are the most probable. This conclusion is valid for both of the proposed mechanisms. This conclusion is in perfect agreement with the results of our CV study, because oxygen influences one-electron reduction of anthraquinones only for the hydroxy-containing compounds, for which the proton transfer necessary to form hydroperoxides and their anion radicals can occur.

It should be noted that the energies of the formation of the hydroperoxides of 1,4-dihydroxybenzene obtained in our earl-

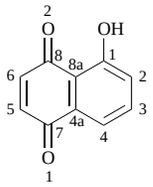

Fig. 6 Numbering of atoms in 1-hydroxynaphthoquinone

ier *ab initio* calculations[12] are *ca.* 20 kcal mol^{-1} lower than the energies of the formation of the hydroperoxides of 1-hydroxynaphthoquinone. As the half-lives of the hydroperoxides of hydroxybenzenes and hydroxynaphthalenes at room temperature are usually shorter than one hour,[18–20] the hydroperoxides of anthraquinones should be considered as possible intermediates in the electron transfer process, rather than stable compounds that could be isolated.

From Table 3 it follows that the dissociation of superoxide anion radical from the hydroperoxide anion radicals involves energy loss; this results from the calculations at all levels of theory applied in this study. This agrees with the results of CV experiments which suggest that the $AO_2^{\bullet -}$ anion radicals, if formed, are probably very reluctant to release superoxide anion radicals in aprotic solvents. Bearing in mind that superoxide is stabilized by hydration, we first carried out PM3 calculations of the reaction of the dissociation of 4-hydroperoxide anion radical of 1-hydroxynaphthoquinone (the lowest-energy hydroperoxide anion radical) solvating it by 11 explicit water molecules and representing the farther solvation sphere as a continuous dielectric with the relative permittivity $D = 78.4$ corresponding to water, the energy of interaction with the supermolecule was calculated using the conductor-like screening model (COSMO)[39] approximation. The superoxide anion radical and 1-hydroxynaphthoquinone (the products of the dissociation) were solvated by six and five water molecules, respectively (using COSMO to represent the farther solvation sphere). The geometry of all solvated molecules was then optimized. The estimated enthalpy of the reaction $AO_2^{\bullet -}(H_2O)_{11} \rightleftharpoons A(H_2O)_5 + O_2^{\bullet -}(H_2O)_6$ was -3.5 kcal mol^{-1}, *i.e.* the process should be energetically favourable, which is in agreement with the CV data in water.[8,9] Next, we estimated the energetics of the dissociation of the hydroperoxyl radical from the protonated form of the hydroperoxide anion radical. Table 4 shows the protonation energies of the 4-hydroperoxide anion radical and the enthalpies of superoxide abstraction from the protonated form. The lowest-energy protonated form appears to be the H-O2 hydroperoxide radical (see Fig. 6 for atom labelling). As shown, the dissociation of the hydroperoxide radical into the hydroperoxyl radical and 1-hydroxynaphthoquinone is favourable energetically. The COSMO-estimated solvation effect on hydroperoxyl release from the hydroperoxide radicals was less than 1 kcal mol^{-1} which is understandable, because all the species involved in this process are uncharged. The above results suggest that the hydroperoxide anion radical formed at the quinone-reducing site of the mitochondrial NADH dehydrogenase (complex I) which is largely nonpolar[25] can release the hydroperoxyl radical (which is an active oxygen species) after protonation or the superoxide anion radical after being transferred from membrane to aqueous solution.

Semiempirical PM3 studies of the energetics of the formation of the peroxides and hydroperoxides and their anion radicals for model anthraquinones

Although, as follows from the preceding section, the semiempirical PM3 method does not reflect fully correctly the energetics of various stages of mechanism I and II as far as different hydroperoxide and peroxide intermediates are concerned, our earlier works[10–12] suggest that PM3 results can be trusted when comparing the variation of energetics on anthraquinone structure, provided that the same type of oxygen adduct is considered for all the compounds. Therefore, we undertook a PM3 study of the energetics of electron-transfer mediation for model compounds **1**–**10**, assuming that the 1,4-peroxides or 2-hydroperoxides can be formed (this abbreviated notation stands for all equivalent positions of the anthraquinone moiety, *i.e.* the 5,8-peroxides or 3-, 6- or 7-hydroperoxides, depending on which of them has a covalent structure or is lower in energy). The dioxethane-type 2,3- and 6,7-peroxides are not included, because according to the results of model calculations of the energetics of electron-transfer mediation by 1-hydroxynaphthoquinone, they cannot be considered putative intermediates in this process. The calculated enthalpies of the reactions corresponding to mechanism I and II are summarized in Table 5.

Table 4 4-31G and PM3-calculated enthalpies of protonation of the 4-hydroperoxide anion radical (ΔH_{prot}) and subsequent elimination of the OOH$^{\cdot}$ radical from the protonated forms of 4-hydroperoxide anion radical ($\Delta H'_{Ic/IIc}$)

	ΔH_{prot}		$\Delta H'_{Ic/IIc}$	
Species	4-31G	PM3	4-31G	PM3
4-OOH(H-OH)	−323.5	−305.5	−20.8	−4.2
4-OOH(H-O1)	−322.7	−307.8	−21.4	−2.0
4-OOH(H-O2)	−336.3	−301.6	−8.6	−8.1

The comparison of the reduction potentials of compounds **1**–**6** measured in DMSO with their reduction enthalpies provides some means of the assessment of the quality of the PM3 results, eqn. (7), with the correlation coefficient $R = -0.9938$

$$V_{red} = 0.0382(0.0021)\Delta H_{red} - 2.527(0.094) \qquad (7)$$

(the standard deviations of the slope and the intercept are in parentheses). The slope in eqn. (7) is not much different from the conversion factor from electronvolts to kcal mol^{-1} (0.043 349).

Based on the results summarized in Table 5 some relationships between the structure and the enthalpies of the processes that involve one-electron reduction can be found. The reduction enthalpies of both free anthraquinones and their oxygen adducts as well as the total enthalpies of reductive oxygen binding (ΔH_{sum}) increase with the number of hydroxy groups. The reduction enthalpies are lower for the amino, compared with those of the hydroxy compounds and the reduction enthalpies of the iminoanthraquinones are lower than the reduction enthalpies of the corresponding anthraquinones; this also agrees with the variation of the reduction potential measured by CV (Table 1). The latter features probably result from the fact that the nitrogen atom is less electronegative than the oxygen atom. It should be noted that this differentiates between the imino and non-imino derivatives and can explain why the latter compounds are generally poor stimulators of peroxidation processes,[3] a fact that cannot be explained taking into account only the enthalpies of oxygen addition to these compounds. Specifically, for compound **6** the stimulated NADH oxidation rate is $V_{max} = 22.2$ μM min^{-1} (ref. 9), as compared with $V_{max} = 25.6$, 37.0 and 51.0 μM min^{-1} for compound **1**–**3**, respectively.[9]

Conclusions

The results of CV measurements reported in this paper provide conclusive evidence that the presence of oxygen influences the one-electron reduction of the hydroxy, but not the non-hydroxy anthraquinone derivatives studied in this work. Theoretical calculations show that this interaction can be explained in terms of the formation of hydroperoxide anion radicals, which can be formed only for anthraquinones bearing the hydroxy groups. These species can be formed either by one-electron reduction of anthraquinone to semiquinone followed by oxygen addition (route I) or by singlet-oxygen addition to the neutral anthra-

Table 5 PM3-calculated enthalpies of subsequent reactions constituting mechanisms I and II (kcal mol^{-1}) for compounds **1**–**10** assuming that the hydroperoxides or 1,4-peroxides, respectively, and their anion radicals are intermediates in the electron-transfer process

	5,8-O_2/1,4-O_2[b]					2-OOH/6-OOH[c]			
	ΔH_{Ia}	ΔH_{Ib}	ΔH_{IIa}[a]	ΔH_{IIb}	ΔH_{sum}	ΔH_{Ib}	ΔH_{IIa}[a]	ΔH_{IIb}	ΔH_{sum}
1	−49.8	−14.4	−3.0	−61.2	−64.2	−22.3	−8.1	−64.0	−72.1
2	−50.2	−12.3	2.0	−64.5	−62.5	−22.4	−9.8	−62.8	−72.6
3	−45.0	−11.1	4.3	−60.5	−56.1	−23.7	−12.5	−58.0	−68.9
4	−38.3	−14.7	−2.0	−35.8	−53.0	—	—	—	—
5	−36.7	−12.8	−2.3	−37.5	−49.6	—	—	—	—
6	−41.7	−14.7	−4.6	−51.8	−56.4	−22.7	−13.3	−51.1	−64.4
7	−45.9	−14.5	−3.1	−57.2	−60.3	−24.8	−10.9	−59.7	−70.7
8	−46.6	−14.1	−3.3	−57.5	−60.7	−21.3	−9.8	−58.1	−67.9
9	−43.3	−14.8	−3.7	−54.4	−58.1	−18.3	−4.5	−57.1	−61.6
10	−51.0	−11.5	5.8	−68.3	−62.5	−22.4	−7.9	−65.5	−73.4

[a] Peroxide and hydroperoxide-formation enthalpies were calculated from values of refs. 11 and 12, expressing the oxygen-adduct enthalpies relative to the $^1\Delta_g$ singlet-oxygen state. [b] The energies of the peroxides lower in energy are listed; for all compounds except **3** these are the 5,8-peroxides, for compound **3** 1,4-peroxide. [c] 6-Hydroperoxide for compound **10**; in other cases the 2-hydroperoxides are lower in energy.

quinone-derivative molecule followed by one-electron reduction (route II). Hydrated and/or protonated, the hydroperoxide anion radicals can release superoxide (as $OO^{\bullet -}$ or $HOO^{\bullet}$, respectively) and therefore the hydroperoxide anion radicals are also likely to be intermediates in the process of electron-transfer mediation to molecular oxygen by anthraquinones. Because of the comparatively low heat of formation as predicted by quantum-mechanical methods, hydroperoxides and their anion radicals should be considered as short-living intermediates rather than stable chemical species.

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