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# FREE RADICAL FORMATION FROM ANTHRACYCLINE ANTITUMOUR AGENTS AND MODEL SYSTEMS—I MODEL NAPHTHOQUINONES AND ANTHRAQUINONES

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**Abstract**—Several naphthoquinones and anthraquinones were chosen as simple models of the anthracycline drugs and their semiquinone radical anions were generated by various methods. With the exception of 1,4-naphthoquinone, all of the quinones studied gave radicals that were highly reactive with oxygen, but which, in its absence, were stable over a limited pH range. The radicals were studied using electron spin resonance (ESR) spectroscopy and an examination was made of the effect on the distribution of the unpaired electron, of introducing various groups into the conjugated ring system. Hydroxyl groups capable of participating in strong intramolecular hydrogen bonding with neighbouring carbonyl groups had a marked influence on electron distribution and reduced the effects of intermolecular hydrogen bonding of the radicals with solvent molecules.

Endogenous quinones play a vital role in many biologically important processes, while quinone drugs such as adriamycin are amongst the most important agents used in the treatment of human cancers. Unresolved ESR spectra have been observed on incubation of adriamycin with microsomal systems [1] and it has been shown that the radicals readily form covalent complexes with DNA [2]. This reaction may be damaging, or damage may be produced by superoxide anions formed at a biologically important target through reaction of oxygen with the semiquinone radical anion:

$$Q^{\cdot-} + O_2 \rightarrow Q + O_2^{\cdot-}$$

The formation of superoxide in microsomal incubations containing quinone anticancer drugs has been demonstrated by spin trapping [3]. Spin trapping also provides evidence of hydroxyl radical production which is inhibited by catalase, but not by superoxide dismutase [4]. The proposed mechanism is:

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$Q^{\cdot-} + H_2O_2 \rightarrow Q + OH^- + OH^{\cdot}$$

Recently it was shown [5] that adriamycin can be actively cytotoxic without entering the cell, possible by inducing peroxidation of lipid membranes.

The anthracycline anticancer drugs contain both quinone and hydroquinone structures. The hydroxyl derivatives naphthazarin (5,8-dihydroxy-1,4-naphthoquinone), juglone (5-hydroxy-1,4-naphthoquinone) and quinizarin (1,4-dihydroxy-9,10-anthraquinone) have now been employed as models, together with some related quinones. Pulse radiolysis studies have shown that long-lived semiquinone radical anions of naphthazarin [6] and quinizarin [7] should be observed at about pH 8.5, where the equilibrium between the various forms of the quinones represented as $2Q^{\cdot-} \rightleftharpoons Q + Q^{2-}$ is significantly over to the left. The stability of the semiquinones has now been confirmed using ESR, and the formation and behaviour of radicals from naphthoquinone and anthraquinone have been compared with those of their hydroxy derivatives. ESR studies of radicals from adriamycin and other anthracycline drugs will be reported in a subsequent paper.

## MATERIALS AND METHODS

*Chemicals.* The sodium salt and methyl ester of quinizarin-2-sulphonate and the methyl ester of quinizarin-6-sulphonate were synthesized by Miss P. M. Hayes and Dr. J. M. Bruce, Manchester University. Naphthazarin was synthesized and purified from 1,5-dinitronaphthalene [8]. All other chemicals were analytical grade reagents. Sodium dodecyl sulphate (SDS), anthraquinone-1-,-2- and -2,6-sulphonates, juglone and quinizarin were purified by recrystallization. Anthraquinone was purified by sublimation and propan-2-ol was purified by refluxing with 2,4-dinitrophenylhydrazine and $H_2SO_4$ for 24 hr, followed by double-distillation.

*Radical formation.* Radicals were produced by the following methods: ionizing radiation, UV photolysis, electrolysis or chemical reduction using ascorbic acid. Unless otherwise stated, the radicals observed were not influenced by the method of generation and, with most of the quinones studied, all methods could be employed.

Aqueous solutions were prepared containing $10^{-4}$–$10^{-3}$ M quinone. When the solubility of the

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quinone was too low, propan-2-ol was added to concentrations of 0.5–4 M. The pH was adjusted to the required value and the solutions were thoroughly purged with argon or nitrogen. Samples were transferred anoxically to a flat quartz ESR cell or 1 mm i.d. glass capillary. In some cases, solutions were similarly prepared using deuterium oxide. Radiation doses were given in a single pulse of between 10 and 400 Gy, using a 10 MeV electron accelerator. Where propan-2-ol was not necessary to increase solubility, as with naphthazarin solutions, $10^{-1}$ M sodium formate was added. UV irradiation of aqueous solutions containing approximately 2 M propan-2-ol was carried out in the microwave cavity of the ESR spectrometer, using unfiltered light from a mercury lamp. Formation of the semiquinone was generally slow, taking 5–10 min, but once formed the radicals were stable, even in the absence of illumination. In an alternative procedure, aqueous micellar solutions of the quinones were photolysed [9]. SDS, Triton X-100 or cetyltrimethylammonium chloride (CTAC) was used at a concentration much greater than the critical micellar concentration. Electrolytic generation of the radicals was carried out *in situ* in a standard flat quartz ESR cell using aqueous solutions containing $10^{-1}$ M tetraethylammonium *p*-toluenesulphonate as supporting electrolyte. In certain cases, dimethylformamide (DMF) or dimethyl sulphoxide (DMSO) solutions were used, with the same supporting electrolyte. Chemical reduction of quinones was achieved in alkaline solution using an excess of ascorbic acid. In this case, deoxygenation was not essential.

*ESR measurements.* Spectra were recorded at room temperature, using a Varian E-9 X-band spectrometer in conjunction with a Nicolet 1170 signal averager and Hewlett Packard HP-85 microcomputer. Spectra were obtained at an incident microwave power of 5 mW and the magnetic field was modulated at 100 kHz with an amplitude of 0.05 G or less. The field was calibrated using a $Mn^{2+}$ standard in an $H_{014}$ dual cavity. Spectra were stored on magnetic discs as 1000 data points.

In several cases, analysis of the ESR spectrum was greatly facilitated by ENDOR measurements, kindly made by Drs. C. C. Rowlands and J. C. Evans at University College, Cardiff. Details of these measurements will be published elsewhere.

## RESULTS AND DISCUSSION

Delivery of single pulses of electrons to dilute aqueous solutions of quinones containing a high concentration of formate ions produces semiquinone radical anions by the following reactions:

$$H_2O \rightsquigarrow H^{\cdot}, e^-_{aq}, {}^{\cdot}OH$$

$${}^{\cdot}OH + HCO_2^- \rightarrow H_2O + CO_2^-$$

$$H^{\cdot} + HCO_2^- \rightarrow H_2 + CO_2^-$$

$$e^-_{aq} + Q \rightarrow Q^-$$

$$CO_2^- + Q \rightarrow Q^- + CO_2$$

Propan-2-ol similarly scavenges the radiolysis products of water giving radicals [$CH_3\dot{C}(OH)CH_3$] that can react with the quinones. The mechanism of photochemical production of semiquinones in aqueous solutions containing propan-2-ol is believed to occur [10] as follows:

$$Q + h\nu \rightarrow Q^*(S) \rightarrow Q^*(T)$$

$$Q^*(T) + CH_3CH(OH)CH_3 \rightarrow CH_3\dot{C}(OH)CH_3 + QH^{\cdot}$$

$$QH^{\cdot} \rightarrow Q^- + H^+$$

Photochemical generation of the radicals in micellar systems is believed to occur in a similar manner, the excited triplet state of the quinone abstracting a hydrogen atom from the surfactant and the semiquinone radical anion being stabilized by the micelle [9].

### 1,4-*Naphthoquinone*

The radical anion of naphthoquinone formed spontaneously in aqueous alkaline solution and gave a spectrum showing a triplet of quintets where $a_2^H = a_3^H = 3.0$ G and $a_5^H = a_6^H = a_7^H = a_8^H = 0.6$ G. This is in agreement with previous reports of the semiquinone in aqueous alkaline medium [11, 12], but differs markedly from the spectrum obtained by electrolytic reduction of 1,4-naphthoquinone in DMF [13], where the hyperfine splitting of the protons on $C_5$ and $C_8$ was only half the splitting of those on $C_6$ and $C_7$ giving a triplet of septets. The possibility that hydroxylation might have occurred in alkaline solution could be discounted since the spectrum was unaffected when water was replaced by deuterium oxide and differed from the reported spectrum of 2-hydroxy-naphthosemiquinone [14]. Addition of 1% water to DMF significantly altered the hyperfine pattern of the electrolytically generated radical, while in 50% water the spectrum corresponded to that observed in aqueous alkali, confirming that the differences between spectra in water and DMF are due to solvent effects as reported previously [15].

### 2-*Methyl*-1,4-*naphthoquinone*

The 2-methyl derivative of naphthoquinone did not form the semiquinone spontaneously in alkaline

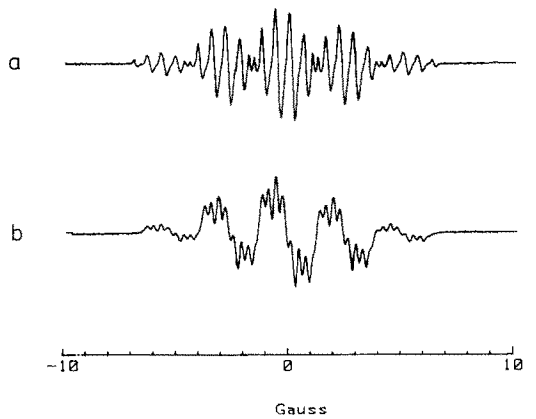



Fig. 1. ESR spectrum of the semiquinone radical anion produced from 2-methyl-1,4-naphthoquinone by (a) electron irradiation in anoxic, aqueous 2 M propan-2-ol at pH 8.3; (b) electrolysis in DMF.

solution, but irradiation of an anoxic, aqueous solution at pH 8.3, containing 2 M propan-2-ol, gave a stable radical with a 5 × 5 spectral pattern (Fig. 1a). This was interpreted as $a_2^{CH_3} = 3.02$ G, $a_3^H = 2.37$ G and $a_5^H = a_6^H = a_7^H = a_8^H = 0.66$ G. A similar spectrum was obtained on electrolysis of an alkaline, aqueous solution, while electrolysis of a DMSO or DMF solution gave a spectrum (Fig. 1b) showing inequivalence of the two pairs of protons on $C_{5,8}$ and $C_{6,7}$, where $a_2^{CH_3} \approx 2.70$ G, $a_3^H \approx 3.04$ G, $a_6 = a_7 = 0.68$ G and $a_5 = a_8 = 0.34$ G, the difference from the aqueous case being again attributed to a solvent effect.

*Juglone*

Semiquinone radicals were not detected by ESR in anoxic aqueous solutions of juglone, either following electron irradiation or during *in situ* UV irradiation in the presence of propan-2-ol. The radical could be detected in aqueous, alkaline solutions containing ascorbic acid and a very weak signal was detected during *in situ* electrolysis of aqueous, alkaline solutions of juglone. The semiquinone radical anions of juglone were readily detected in anoxic, aqueous micellar systems using SDS, Triton X-100 or CTAC exposed to UV light. Under these conditions, the radical population was stable, but disappeared on exposure to air. The optimum pH for radical formation was found to be 12 and radicals were not detected at a pH below 6.

The spectrum of the juglone radical in $D_2O$ was identical to that obtained in water, with the exception of the loss of a small doublet splitting (Fig. 2a, b), which identifies the hydroxyl proton. The hyperfine splittings were assigned as follows:

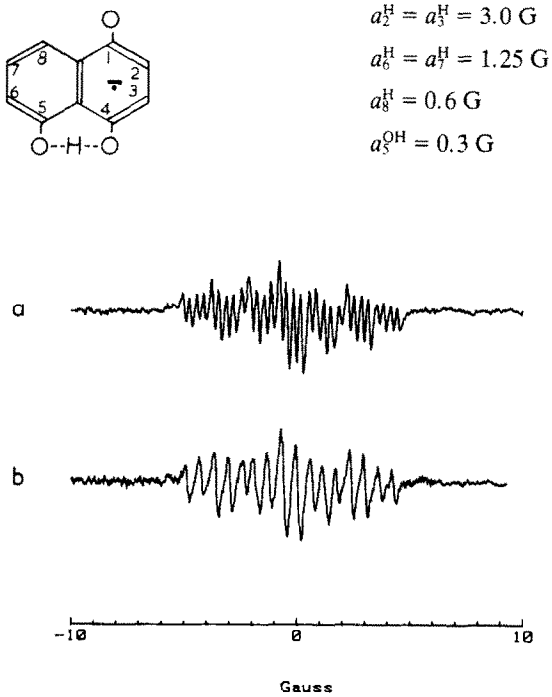
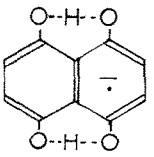



Fig. 2. ESR spectrum of the semiquinone produced by UV irradiation of juglone in SDS micelles at pH 11.7 in (a) $H_2O$; (b) $D_2O$.

A value of $a_7^H$ greater than that of $a_8^H$ is consistent with the splittings previously proposed [16] for 1-hydroxyanthraquinone, although the values may be reversed. The results can be compared with those of Piette *et al.* [13], who generated the radical electrolytically, in DMF, when $a_2^H = 4.01$ G, $a_3^H = 2.64$ G, $a_6^H = 1.05$ G, $a_7 = a_8 = 0.78$ G, and $a_5^{OH} = 0.34$ G. Once again, the solvent has a marked effect on the splittings.

*Naphthazarin*

Semiquinone radicals of naphthazarin were readily produced by any of the methods tried. The radicals were stable in deoxygenated, aqueous solution, but decayed rapidly in the presence of air. The magnitude of the ESR signal was dependent on the pH of the solution, being maximal at pH 8.5 and undetected below pH 7 or above pH 11 (Fig. 3) in agreement with the stability of the radical as measured by pulse radiolysis [6]. No change in the nature of the radical was detectable over this range and from its ESR spectrum the radical could be positively identified as the monoanion:



When naphthazarin was reduced with ascorbic acid at pH 8.5, a relatively weak spectrum of the ascorbyl radical was also detected and by comparison of the separation of the centres of the two spectra and the reported *g*-value of 2.0052 for the ascorbyl radical

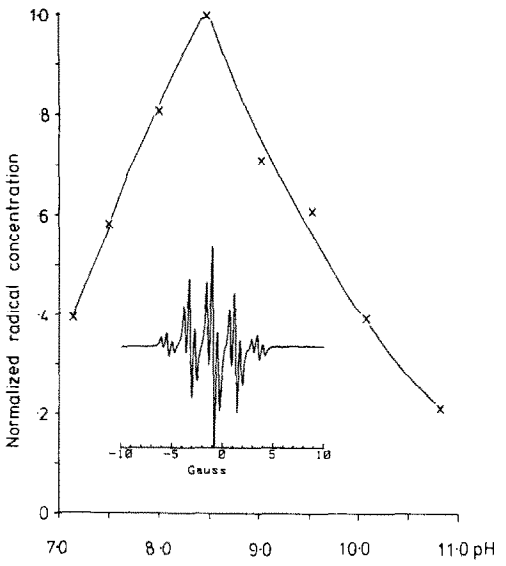



Fig. 3. Effect of pH on the relative concentration of naphthazarin radical anions observed in anoxic, aqueous solutions containing $10^{-4}$ M naphthazarin, $10^{-1}$ M sodium formate, $10^{-3}$ M buffer and given a radiation dose of 280 Gy with a single pulse of electrons. Inset: ESR spectrum of the radical.

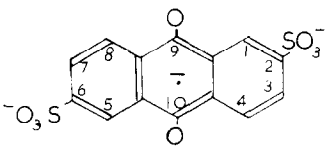
[17], a $g$-value of 2.0040 was obtained for the naphthazarin radical. The spectrum was a quintet of triplets, intramolecular hydrogen bonding producing equivalence of all the ring protons. The measured hyperfine splittings were $a_2^H = a_3^H = a_6^H = a_7^H = 2.36$ G and $a_1^{OH} = a_4^{OH} = 0.58$ G. The assignment of the triplet splitting to the hydroxyl protons was confirmed by irradiation of naphthazarin in $D_2O$, when the spectrum collapsed to a 1:4:6:4:1 quintet. The spectrum of the naphthazarin radical anion produced by electron irradiation of naphthazarin in water gave splitting constants which agreed closely with those reported previously for the radical produced by air oxidation of an alkaline, aqueous or alcoholic solution of 1,4,5,8-tetrahydroxy-naphthalene [18]. The lack of stability noted in the earlier report is no doubt due to the presence of oxygen. Production of the radical by electrolysis in aprotic solvents [13, 19, 20] caused little change in the hyperfine splittings compared with those measured in aqueous media. From INDO calculations it has been inferred [20] that the strong intramolecular O–H–O hydrogen bond is linear.

### 9,10-*Anthraquinone*

UV photolysis of an anoxic solution of anthraquinone in 2 M propan-2-ol in water at pH 13 gave a spectrum that could be analysed as a quintet of quintets, where $a_2^H = a_3^H = a_6^H = a_7^H = 0.95$ G and $a_1^H = a_4^H = a_5^H = a_8^H = 0.58$ G. The radical was also produced at pH 13 by reduction of the quinone with ascorbic acid. At this pH ascorbyl radicals are unstable and are not detected. The spectrum of the anthrasemiquinone radical anion is reported to show a marked solvent effect [15, 21], the hyperfine splitting of protons at $C_1$, $C_4$, $C_5$ and $C_8$ being reduced to 0.30 G in DMSO, although that at $C_2$, $C_3$, $C_6$ and $C_7$ is only slightly affected.

### 9,10-*Anthraquinone*-2,6-*disulphonate*

Reduction of an aqueous solution of anthraquinone-2,6-disulphonate with ascorbic acid at pH 13 gave an 11-line spectrum (Fig. 4a), interpreted as a triplet of quintets. Closer analysis showed that the quintets resulted from overlap of two almost equivalent triplet splittings. This was confirmed by ENDOR measurements. The probable assignment of the splittings is:



$a_3^H = a_7^H = 1.23$ G

$a_{4,8}^H$ or $a_{1,5}^H = 0.40$ G

$a_{1,5}^H$ or $a_{4,8}^H = 0.39$ G

The radical was also produced, under anoxic conditions, by electron or UV irradiation of solutions in 2 M propan-2-ol at pH 13. Electrolysis of anthraquinone-2,6-disulphonate in DMSO gave a 13-line spectrum (Fig. 4b) due to less overlap of the quintets. Under these conditions, the triplet and quintet splittings were 1.06 and 0.26 G, respectively.

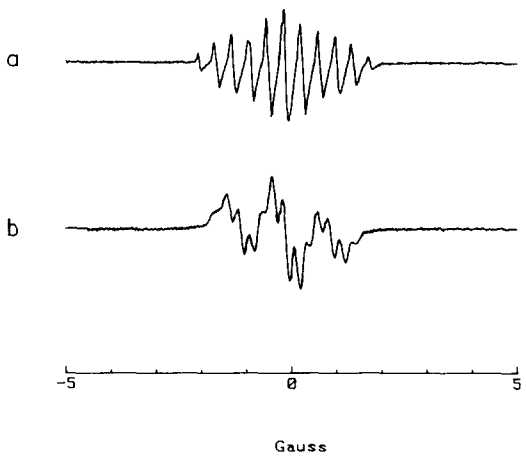



Fig. 4. ESR spectrum of the semiquinone produced from 9,10-anthraquinone-2,6-disulphonate by (a) irradiation in 2 M propan-2-ol at pH 13 with a dose of 400 Gy; (b) electrolytic reduction in DMSO.

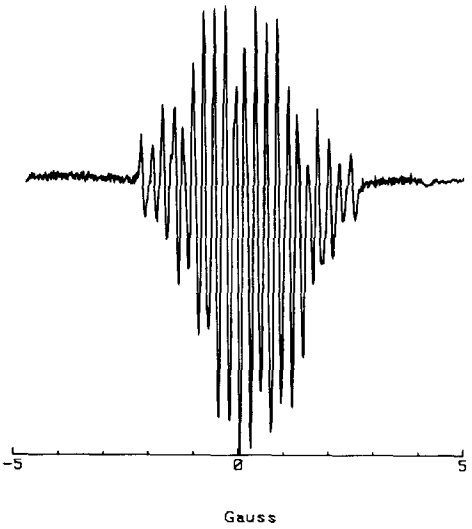
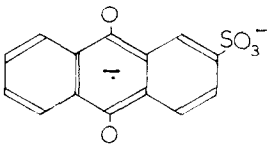



Fig. 5. ESR spectrum of the semiquinone produced by irradiation of 9,10-anthraquinone-2-sulphonate in anoxic, aqueous 2 M propan-2-ol at pH 13.

### 9,10-*Anthraquinone*-2-*sulphonate*

Electron or UV irradiation of the quinone in anoxic, aqueous 2 M propan-2-ol at pH 13, or reduction of the quinone with ascorbic acid, gave a 21-line spectrum due to extensive overlap (Fig. 5). ENDOR measurements showed six different splitting constants and the ESR spectrum was analysed as a $2 \times 2 \times 2 \times 2 \times 3 \times 2$ pattern. A probable assignment of the splittings is as follows:



$a_5^H = 1.24$ G, $a_6^H = 0.97$ G

$a_7^H = 0.79$ G, $a_1^H = 0.75$ G

$a_5^H = a_8^H = 0.53$ G, $a_4^H = 0.28$ G

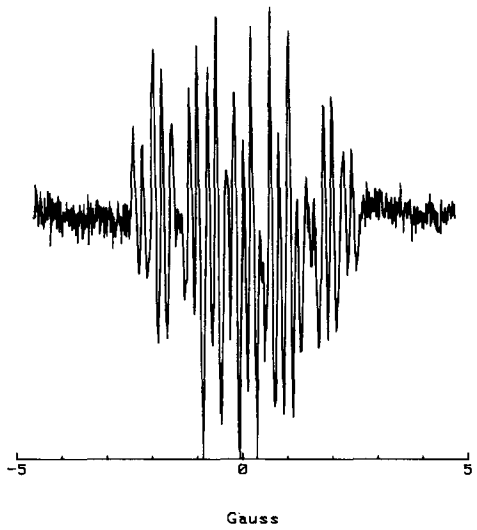
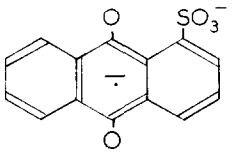



Fig. 6. ESR spectrum of the semiquinone produced by reduction of aqueous 9,10-anthraquinone-1-sulphonate with ascorbic acid at pH 13.

Electrolysis in DMF gave a spectrum, which, although not well-resolved, appeared to be a quartet of quintets, interpreted as due to $a_3^H \approx a_6^H \approx a_7^H \approx 1.1$ G and $a_1^H \approx a_4^H \approx a_5^H \approx a_8^H = 0.2$ G.

9,10-*Anthraquinone*-1-*sulphonate*

Electron or UV irradiation of anthraquinone-1-sulphonate in anoxic, aqueous 2 M propan-2-ol at pH 13 gave a spectrum closely resembling that of the 9,10-anthrasemiquinone radical anion, with possible contributions from another radical. In contrast, reduction with ascorbic acid produced a radical giving a 12-line spectrum, which could be further resolved and clearly showed a small doublet splitting (Fig. 6). The spectrum appeared to be a $2 \times 4 \times 3 \times 2$ pattern and the splittings were confirmed by ENDOR. Consequently, the most probable assignment appears to be:



$a_2^H = 1.32$ G

$a_3^H = a_6^H = a_7^H = 0.92$ G

$a_5^H = a_8^H = 0.47$ G

$a_4^H = 0.18$ G

The poorly resolved 12-line spectrum corresponds with a previously published spectrum [22]. However, these authors also report a 19-line spectrum which does not correspond with our more highly resolved spectrum. The large central peak of the published spectrum suggests at least partial loss of the sulphonate group. From our results it appears that both photolysis and ionizing radiation cause loss of the sulphonate group and formation of the anthrasemiquinone radical anion. ENDOR measurements of irradiated samples of anthraquinone-1-sulphonate show the presence of two additional lines identical to those of the 9,10-anthrasemiquinone radical. These lines are generally absent in samples of the sulphonate reduced with ascorbic acid. In such mixtures, the ascorbyl radical can be detected at pH 9, but not the semiquinone, while at pH 10 neither radical is detected. A marked effect of solvent on the hyperfine splittings of the semiquinone was observed when the radical was generated by electrolytic reduction in DMF.

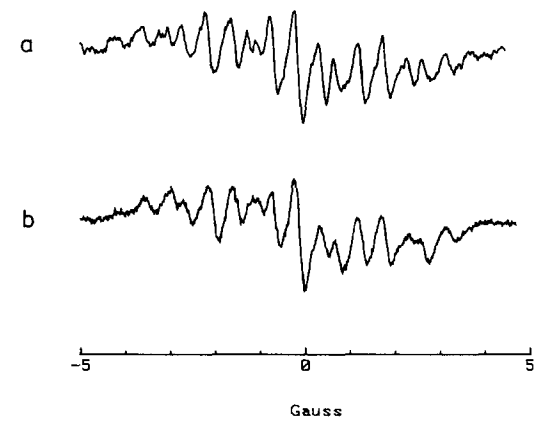
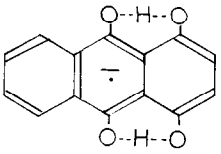



Fig. 7. ESR spectrum of the semiquinone produced by UV irradiation of quinizarin in anoxic solution at pH 9 containing 4 M propan-2-ol in (a) $H_2O$; (b) $D_2O$.

*Quinizarin*

Due to the very low solubility of quinizarin in water (*ca* $2 \times 10^{-6}$ M), a 4 M propan-2-ol solution was used. The radical produced by electron or UV irradiation was stable under anoxic conditions, but disappeared in air: maximum radical yield was observed at pH 9. The spectrum (Fig. 7a) approximated to a $3 \times 3 \times 5$ pattern due to four pairs of protons, two of which gave similar splittings. The radical was assigned to a radical anion with strong intramolecular hydrogen bonding between the hydroxyl protons and the carbonyl groups:



$a_2^H = a_3^H = 2.05$ G

$a_6^H = a_7^H = 0.90$ G

$a_5^H = a_8^H = a_1^{OH} = a_4^{OH} = 0.58$ G

Assignment of the hydroxyl proton splittings was confirmed by formation of the radical in $D_2O$, when the quintet collapsed to a triplet, with no significant changes in the other hyperfine splittings. This produced only minor changes in the centre of the spectrum, but was noticeable as a loss of two lines in the wings of the spectrum (Fig. 7b). Other authors report spectra of quinizarin semiquinone produced by reduction of quinizarin with zinc in DMF containing 5% aqueous KOH [23], reduction with sodium dithionite in 60% (v/v) ethanol in water [16], electrolytic reduction in DMF [19] or aerobic oxidation of 1,4,9,10-tetrahydroxyanthracene in alkaline ethanol [18]. These differ only slightly from the spectrum presented here, showing that solvent has little effect in this case. In another paper [24] the radical was reported to be produced by reduction of quinizarin in water, using borohydride, with free

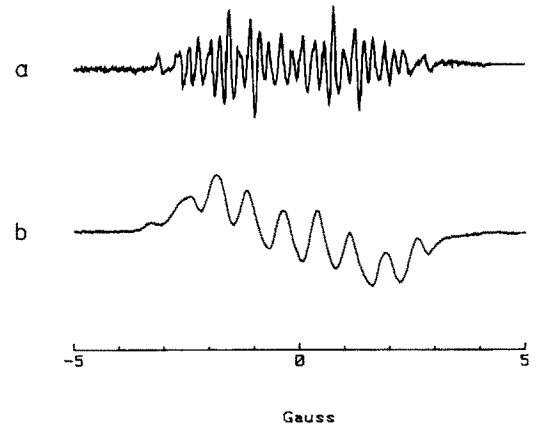



Fig. 8. ESR spectrum of the semiquinone produced by electron irradiation of quinizarin-2-sulphonate in anoxic solution at pH 9 containing 2 M propan-2-ol in (a) $H_2O$; (b) $D_2O$.

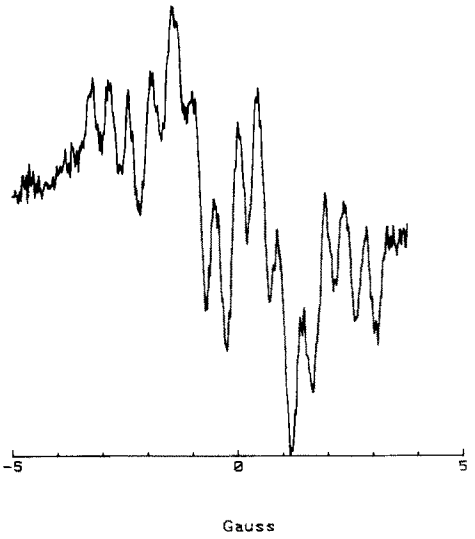
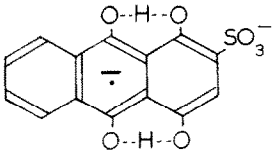



Fig. 9. ESR spectrum of the semiquinone produced by UV irradiation of methyl quinizarin-6-sulphonate in anoxic solution at pH 11 containing 2 M propan-2-ol in $H_2O$.

access to air. The high solubility in water of quinizarin reported by these authors, the apparent stability of the radical in air, and the lack of a deuterium effect lead us to question the purity of their sample. Moreover, their interpretation of the spectrum is inconsistent with that published by others and with the p$K_a$ of the radical, which will be in anionic form at pH 7.4.

*Quinizarin-2-sulphonate*

The sodium sulphonate is more soluble than quinizarin and spectra were obtained by electron or UV irradiation in 2 M propan-2-ol in water or $D_2O$ at pH 9, but not on UV irradiation of a SDS micellar system. The radical gave a complex ESR spectrum in water (Fig. 8a) which changed in $D_2O$ (Fig. 8b). With the aid of ENDOR measurements on a $D_2O$ solution, four hyperfine splittings were found. The spectrum of the radical in water showed an additional triplet splitting from two equivalent hydroxyl protons. A tentative assignment of hyperfine couplings was made as follows:




$a_3^H = 2.54$ G

$a_6^H = 0.95$ G

$a_7^H = 0.73$ G

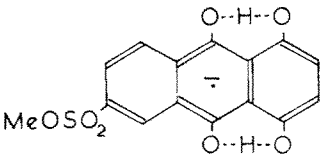
$a_5^H = a_8^H = 0.52$ G

$a_1^{OH} = a_4^{OH} = 0.43$ G

The spectrum obtained from the methyl sulphonate in aqueous solution was indistinguishable from that of the sodium salt.

*Methyl quinizarin-6-sulphonate*

A radical was observed on electron or UV irradiation of a solution of methyl quinizarin-6-sulphonate in aqueous propan-2-ol at pH 11. The spectrum (Fig. 9a) was complex and differed from that obtained in a $D_2O$ solution. ENDOR measurements on the radical in $D_2O$ showed five different hyperfine splittings from the five ring protons. Spectra in water have not been sufficiently well-resolved to determine the splitting constants of the two hydroxyl protons, but a value of *ca.* 0.4 G would be expected by comparison with the other quinizarin derivatives. A possible assignment of the hyperfine couplings is as follows:




$a_2 = 2.03$ G

$a_3 = 1.82$ G

$a_7 = 1.18$ G

$a_5 = 0.51$ G

$a_8 = 0.30$ G

In $\pi$-electron radicals the unpaired-electron density $\rho_i$ on a carbon atom $C_i$ can be represented by the approximate relationship [25]:

$$a_i^H = Q\rho_i$$

where $a_i^H$ is the hyperfine coupling of the proton on $C_i$ and $Q$ is a constant for a particular radical. For the semiquinones, $Q$ has an approximate value of 23.7 G [26] and the relationship can be used to estimate the distribution of the unpaired electron in the carbo-skeleton of the semiquinone radical anions reported above. In aqueous solution, the electron density in the quinoid ring of 1,4-naphthosemiquinone is higher than that in the unsubstituted ring. The unpaired electron density on $C_2$ or $C_3$ is 10–15% while that on $C_6$ or $C_7$ is only 2–3%. Introduction of a hydroxyl group in the 5-position doubles the electron density at $C_6$ and $C_7$ while having little effect on the density at $C_2$, $C_3$ or $C_8$. This strongly suggests transfer of unpaired-electron density from the oxygen atoms of the semiquinone structure by means of intramolecular hydrogen bonding. Naphthazarin contains hydroxyl groups at both $C_5$ and $C_8$ positions and hydrogen bonding leads to complete equivalence of the two rings. Consequently the electron density

on $C_6$ and $C_7$ of the naphthazarin semiquinone is increased 4-fold compared with that of naphthosemiquinone, while the electron density on $C_2$ and $C_3$ is reduced by only 20%. Similar effects of intramolecular hydrogen bonding are observed in aprotic solvents, but the distribution of the unpaired-electron density is also affected by complexes between the solvent and polar substituents in the radical. In the 1,4-naphthosemiquinone radical anion the electron density on $C_{5,8}$ is *ca.* 2.5% in aqueous media, while in DMF it is reduced to 1.4%. A similar effect is observed in 2-methyl-1,4-naphthosemiquinone. In this case an aprotic solvent also reduces the hyperfine coupling of the methyl protons and increases the coupling of the proton on $C_3$. In juglone semiquinone radicals a greater delocalization of electron density onto the benzenoid ring is observed in aqueous media due to strong radical solvent interactions. In naphthazarin semiquinone, the presence of strong intramolecular hydrogen bonding even in aprotic solvents reduces the effect of intermolecular hydrogen bonding associated with the solvent. Similar effects of solvent and hydroxyl substituents on unpaired-electron distribution were observed in the anthrasemiquinones studied. Introduction of hydroxyl groups at $C_1$ and $C_4$ approximately double the electron density at $C_2$ and $C_3$ compared with that in anthrasemiquinone, while the electron density at $C_{5,6,7}$ or $C_8$ is little affected. Introduction of a sulphonate group into the ring in either anthraquinone or quinizarin produces considerable distortion of the electron distribution within that ring without apparently changing, to any great extent, the overall electron density in the ring.

All of the semiquinones studied, with the exception of 1,4-naphthosemiquinone, are highly reactive towards oxygen, but in its absence are stable over a limited pH range. The optimum pH for stability is lowered by the presence of the hydroxyl groups capable of hydrogen bonding with the carbonyl groups. This may be of significance for the action of the anthracycline drugs at physiological pH.

Analysis of the ESR spectra of semiquinone radical anions for model compounds provides a valuable basis on which to interpret the spectra of radicals from the anthracycline antitumour agents. Furthermore, it demonstrates how the unpaired-electron distribution and hence the reactivity of specific sites in the aromatic rings of the radicals are influenced by the proton availability of the surrounding medium and by substitution of certain groups into the molecules, in particular those leading to intramolecular hydrogen bonding.

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