J. Chem. Soc., Faraday Trans. 1, 1987, **83**, 107–112

Electron Spin Resonance Spectroscopy as an Analytical Tool

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Monomethoxy- and dimethoxy-*p*-benzoquinones have been investigated in alkaline aqueous methanol and ethanol by electron spin resonance spectroscopy. All compounds show solvent exchange of the methoxy group(s). 2,6-Dimethoxy-*p*-benzoquinone dissolved in aqueous ethanol exhibits spectra of three radicals, due to partial exchange of none, one and two methoxy groups. Computer simulations demonstrate that γ-protons of the ethoxy group(s) give rise to splittings comparable to the linewidths. Monomethoxy-*p*-benzoquinone undergoes alkoxylation solely leading to the 2,5 analogue.

Electron spin resonance (e.s.r.) spectroscopy has been relatively little applied as an analytical tool, in spite of its high selectivity and sensitivity. Identification of an unknown compound by way of its e.s.r. spectrum is often rendered difficult because radicals of compounds with differences remote from the radical centre may furnish nearly identical spectra, where others, with closely related structures may yield radicals the spectra of which appear rather different. A further nuisance is the frequent occurrence of composite spectra, when working with biological extracts or reaction mixtures. The application of computers for manipulating digitized spectra then becomes indispensable. Furthermore, data processing makes possible more accurate data analysis and extraction of hyperfine splittings in the mG range, *i.e.* of a magnitude comparable to the linewidths.

We have utilized e.s.r. analytically in a study of a large number of naturally occurring quinones/quinols.[1, 2] These compounds are easily extracted by aqueous alcohol and converted to semiquinone radicals by increasing the pH and adding a reducing agent in the case of quinones. Many compounds may be studied when still contained in the crude extract. Quinones and quinols, however, frequently participate in various reactions in alkaline alcoholic solutions, *e.g.* hydroxylations, alkoxylations and decarboxylations, in addition to solvent exchange. The chance of such reactions must be taken into account, whenever one is analysing unknown spectra.

In this paper we report the study of monomethoxy- and dimethoxy-*p*-benzoquinones in aqueous alcohols, with the aim of following solvent exchange and alkoxylation reactions. The structural study of suitable model compounds is of analytical interest and of interest for the understanding of how naturally occurring alkoxy-*p*-benzoquinones function, *e.g.* ubiquinone (coenzyme Q), which participates in electron transport and contains a 2,3-dimethoxy-*p*-benzoquinone structure, or the allergen primin (2-methoxy-6-pentyl-*p*-benzoquinone) which causes dermatitis.

Experimental

The *p*-benzosemiquinone radicals were generated in aqueous ethanol/methanol by reducing the corresponding *p*-benzoquinones with sodium dithionite, after adjustment

of the pH to the desired value with sodium hydroxide. The solvent composition was *ca.* 64% alcohol (v/v). ^{13}C methanol was enriched to 90%.

The spectra were recorded on a Bruker ER 200 spectrometer with a modulation frequency of 12.5 kHz and a modulation amplitude of 20 mG or less. To avoid saturation effects the microwave power was kept below 0.5 mW in all experiments. Spectra were stored digitally and transferred to a Vax 11/780 computer as 4k discreet signals for final data processing. The resolution was 2.5 mG per signal interval (scan width 10 G). Initially all spectra were simulated by means of a set of parameters obtained by the operator. 'Best-fit' parameters were then obtained by an iterative optimization procedure. By this procedure the splitting constants, offsets (*g*-factors), linewidths and relative intensities for each radical were adjusted by the computer and the sum of squares of the pointwise deviation between theoretical and experimental spectra calculated as a measure of fitness. The adjustments were continued with increments or decrements of each parameter until a minimum was reached in the sum of squares (best least-squares solution). For composite spectra different linewidths were usually obtained for each spectrum, *cf.* table 1. For each individual spectrum all the lines were simulated with the assumption of constant linewidth and an overall Lorentzian lineshape. Since semiquinone spectra usually exhibit alternating linewidth effects the assumption of constant linewidth may lead to minor discrepancies between the intensities of some of the lines of the observed spectra and the corresponding lines of the calculated ones.

Results and Discussion

Solvent Exchange

When 2,6-dimethoxy-*p*-benzoquinone is dissolved in alkaline aqueous ethanol at a pH of *ca.* 13.3, a complete solvent exchange takes place in the course of minutes. The only e.s.r. spectrum observed is from 2,6-diethoxy-*p*-benzosemiquinone. At a pH of *ca.* 12.3, a partial exchange takes place leading to the simultaneous occurrence of three semiquinone radicals, I, II and III in nearly equal amounts. Fig. 1 shows the superpositioned spectra of the radicals. A 'best-fit' simulation obtained from parameter optimizations reveals the three spectra to be derived from 2,6-dimethoxy- (I), 2-ethoxy-6-methoxy- (II) and 2,6-diethoxy-*p*-benzosemiquinone (III). This is substantiated in the data of table 1, exhibiting a clear consistency among the three radicals. Thus the β-methyl proton splitting (0.79 G) and the β-methylene proton splitting (0.95 G) are virtually unchanged when going from I to II and from II to III, respectively. On the other hand, a_3 is reduced by 27–28 mG each time a methoxy group is exchanged. This reduction must occur *via* the oxygen at C(1), rather than at C(2), since no asymmetry appears for radical II ($a_3 = a_5$).

If we look at fig. 1 an interesting linewidth increase is observed for the six outermost lines of the spectra of I, II and III. We have marked the lines 1, 2 and 3 and observe an increase in the order $1 < 2 < 3$. One might expect some broadening to be introduced, when methoxy groups are exchanged with the more bulky ethoxy groups. However, by adding a small hyperfine splitting in the simulation from the methyl protons of the ethoxy group(s), the computer responds with a reduced linewidth (L_{W_2}) for II and III, and reveals a final γ-splitting of *ca.* 30 mG (see table 1). In addition one observes an improved simulation, visualized by a reduced figure for the sum of squares from the least-squares procedure applied (see Experimental). Notice, L_{W_2} increases by 13 mG for each ethoxy group incorporated. Fig. 2 shows the simulation of the three spectra of fig. 1.

In order to get an estimate of the relative concentration of the involved radicals the computer calculates the following relative intensity for radical i:

$$\text{intensity} = h(i)\, N(i)\, L^2_{W(i)}$$

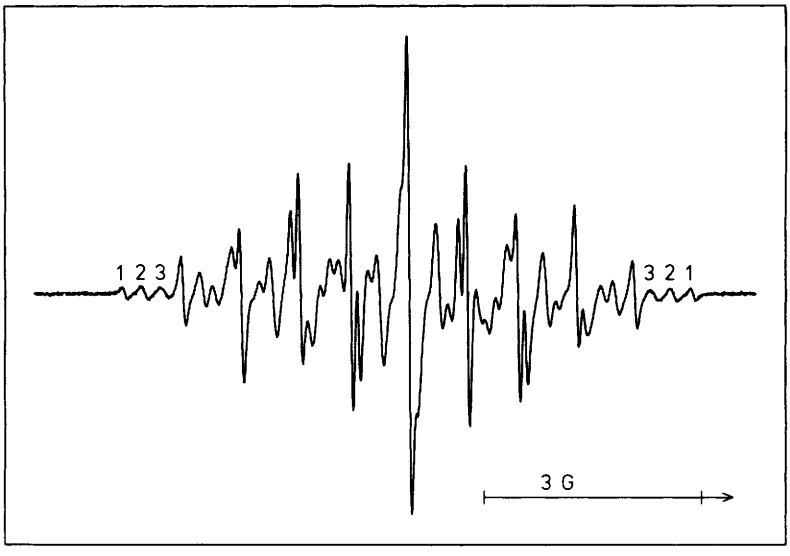


Fig. 1. E.s.r. spectra of 2,6-dimethoxy- (I), 2-ethoxy-6-methoxy- (II), and 2,6-diethoxy-*p*-benzosemiquinone (III). Radicals obtained from 2,6-dimethoxy-*p*-benzoquinone by solvent exchange in aqueous ethanol.

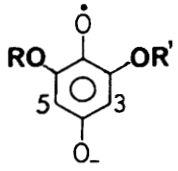
where h is the relative height of a single non-degenerate hyperfine component [$h(1)$ is set arbitrarily equal to one], N the sum of the degeneracies of all the components (lines) and L_w the linewidth, assumed to be constant for all the lines of the radical. The intensities obtained are shown in table 1.

2,5-Dimethoxy-*p*-benzoquinone undergoes solvent exchange as outlined for the 2,6-disubstituted quinone above. In concert with a marked linewidth decrease (157 to 82 mG), we obtain by means of the computer optimization a hyperfine splitting of 50 mG from the six methyl protons of the ethoxy groups (table 1). Unfortunately, we have not been able to obtain a clear-cut spectrum of 2-ethoxy-5-methoxy-*p*-benzosemiquinone.

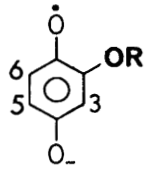
2,3-Dimethoxy-*p*-benzosemiquinone gives rise to a triplet with a splitting of 2.69 G from the two protons at C(5) and C(6). The lack of visual splittings from the methoxy group protons has been explained as steric hindrance, giving the 2,3 analogue a structure which does not correspond with the structures of the other disubstituted analogues.[3] The observed lines of the triplet (linewidths of 147 mG in aqueous ethanol), probably hide further splittings. Whether solvent exchange has taken place and these splittings are from four methylene protons, has not yet been determined from computer studies. Additions of splittings from four or from six protons in the simulations furnish linewidths of 107 and 105 mG and splittings of 46 and 39 mG in the two cases, respectively.

2-Methoxy-*p*-benzoquinone undergoes slow solvent exchange when dissolved in alkaline aqueous ethanol at a pH of *ca.* 12.3. The splitting constants obtained from the radicals of the methoxy (IV) and ethoxy (V) analogues are shown in table 1. A simulation of (V) yields an additional γ-splitting of 27 mG from the methyl protons of the ethoxy group and an accompanying linewidth decrease (from 125 to 111 mG). The magnitude of this splitting might be incorrect ($27 \ll 111$). At higher pH, methoxy-*p*-benzoquinone is attacked by the solvent and the spectra of 2,5-dimethoxy-(VI) and 2,5-diethoxy-*p*-benzosemiquinone (VII) appear in methanol and ethanol, respectively. There is no indication that other dialkoxy-*p*-benzosemiquinones are formed, in line with the rule that nucleophilic attack is likely to take place at quinone positions with highest spin density in the corresponding semiquinone, *i.e.* at C(5).

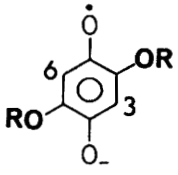
Table 1. Splitting constants and linewidths (Gauss) from e.s.r. spectra of monoalkoxy-*p*-benzosemiquinone and 2,5- and 2,6-dialkoxy-*p*-benzosemiquinone



	R	R′	$a_3 = a_5$	a^{β}_{R}	a^{γ}_{R}	$a^{\beta}_{R'}$	$a^{\gamma}_{R'}$	L_{w_1}[a]	L_{w_2}	relative intensity
I[b]	CH_3	CH_3	1.478	0.790	—	0.790	—	0.066	0.066	36%
II	CH_3	CH_2CH_3	1.451[c]	0.787	—	0.950	0.030	0.101	0.079	38%
III	CH_2CH_3	CH_2CH_3	1.423	0.945	0.028	0.945	0.028	0.124	0.092	26%



		a_3	a_5	a_6	a^{β}_{R}	a^{γ}_{R}			
IV	R = CH_3	0.600	3.652	2.046	0.806	—	0.078	0.078	82%
V	R = CH_2CH_3	0.570	3.618	2.077	0.966	0.027	0.125	0.111	18%



		$a_3 = a_6$	$a^{\beta}_{2} = a^{\beta}_{5}$	$a^{\gamma}_{2} = a^{\gamma}_{5}$			
VI	R = CH_3	0.172	1.033	—	0.052	—	—
VII	R = CH_2CH_3	0.213	1.179	0.050	0.157	0.082	—

[a] L_{w_1}/L_{w_2} linewidth before/after addition of hyperfine splittings in the simulation from the γ-protons of the ethyl groups. [b] I, II and III simultaneously obtained in aqueous ethanol, IV and V similarly obtained, VI and VII obtained in aqueous methanol and ethanol, respectively. [c] The simulation reveals the figures 1.449 and 1.452 for the two splittings at position 3 and 5.

^{13}C Studies

One of the drawbacks of quinone/quinol compounds in relation to e.s.r. studies is that most of their atoms (carbon and oxygen) form 'blind' spots on the spectroscopic mapping. Since the spectral information derives solely from the protons, it is desirable to extend studies of quinones to ^{13}C and ^{17}O enriched ones. Data of only a few enriched quinones have been reported, probably due to costly and time-consuming synthesis.[4] We have used ^{13}C enriched methanol in solvent exchange in order to obtain additional information about the carbon atom of the methoxy groups. We observe splittings of 0.39

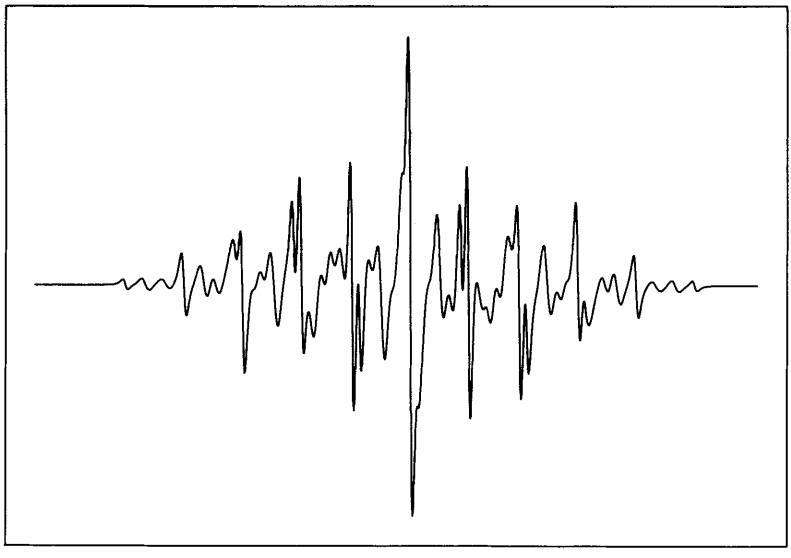


Fig. 2. Simulated spectra of I, II and III from data in table 1.

and 0.37 G from the methoxy group carbon of 2,5- and 2,6-dimethoxy-*p*-benzosemiquinone, respectively. The ^{13}C experiments are complicated, however, by the appearance of at least three spectra from any of the disubstituted *p*-benzoquinones, *viz.* those from *p*-benzosemiquinones with none, with one and with two ^{13}C atoms.

2,3-Dimethoxy-*p*-benzoquinone is easily attacked at C(5) and C(6) by the solvents and a number of unknown reaction products results. The use of ^{13}C enriched alcohols renders valuable information for the identification of these unknowns and should imply the possibility of obtaining a final proof of solvent exchange in the case of the 2,3 analogue. The complexity of the reactions, with superpositioned spectra and additional ^{13}C splittings, makes extensive computer simulations indispensable and precludes immediate results. We are presently engaged in studies with ^{13}C and ^{17}O isotopes, the results of which will be published in a subsequent paper.

Conclusion

E.s.r. spectroscopy is a convenient tool for the analytical study of alkoxybenzoquinones. Structural studies of several alkoxy exchanged derivatives can be made with ease from a few model compounds and extended to ^{13}C and ^{17}O studies by using isotopically enriched alcohols. Simulations of superpositioned spectra, by means of fast computers, give reliable data for all radicals, including γ-hyperfine splittings of magnitude comparable to the linewidths. The digitized spectra must, in this connection, contain a sufficient number of data points (2.5 mG between points in the present study). It should be emphasized, however, that splitting constants of most semiquinones exhibit strong solvent and pH dependence.[4] Care should be taken in comparing hyperfine splitting data not recorded from radicals exposed to absolutely identical conditions, *e.g.* aqueous solutions with different alcohols. In the present study the spectra of I, II and III were obtained with the radicals contained in one and the same sample as were the spectra of IV and V, thereby exposed to identical experimental conditions in either of the two cases.

The authors thank Dr J. M. Bruce for samples of 2,5- and 2,6-dimethoxy-*p*-benzoquinone.

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Paper 6/1002; *Received* 22*nd May*, 1986