

ENDOR and ESEEM Studies of Ion Radicals of Artificial Dimethoxy- or Halogen-1,4-benzoquinones with an Alkyl Side Chain of Differing Length

Andrei P. Spoyalov,^a Rimma I. Samoilova,^b Alexei M. Tyryshkin,^b Sergei A. Dikanov,^b Ben-Li Liu^c and Arnold J. Hoff^c

^a Physics Department, Novosibirsk State University, Novosibirsk 630090, Russian Federation

^b Institute of Chemical Kinetics and Combustion, Novosibirsk 630090, Russian Federation

^c Biophysics Department, University of Leiden, PO Box 9504, 2300 RA Leiden, The Netherlands

ENDOR measurements of anion radicals of artificial dimethoxy- or halo-1,4-benzoquinones with an alkyl side chain of differing length in solution yield a number of proton isotropic hyperfine constants. Analysis of the constants obtained gives the orientation of substituents relative to the quinone plane. ESEEM measurements of the ion radicals in frozen solutions of deuteriated solvents indicate that the cation, but not the anion, radicals experience a strong interaction with solvent deuterium, which is hydrogen bonded to the quinone oxygen.

Quinones are ubiquitous in biological systems where they play an important role in many redox processes involving the transfer of one or two electrons, such as photosynthesis, oxidative phosphorylation and blood coagulation.

The most important steps in photosynthesis are the so-called primary processes: charge separation and subsequent charge stabilization. These primary processes occur in a special pigment-protein complex, the reaction centre, which is embedded in the photosynthetic membrane and contains a group of co-factor molecules with a special spatial arrangement. Quinone, the only non-protein membranal electron carrier, plays an obligatory role as a redox carrier in almost all organisms with 'electron-transfer phosphorylation' except for methanogenic bacteria: ubiquinone in mitochondria and purple photosynthetic bacteria, plastoquinone in chloroplasts and cyanobacteria and vitamin K in purple and green photosynthetic bacteria and perhaps also in PS I of chloroplasts.¹

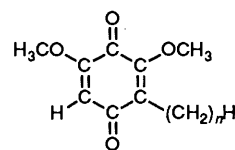
In order to mimic photosynthetic electron and energy transfer and translate knowledge of the photosynthetic events from a molecular scale to a technological scale, synthesis and spectroscopic studies of artificial photosynthetic systems have been carried out. One direction of research is the synthesis of artificial quinones, their reconstitution to the photosystems in the place of natural quinones, and finally the investigation of the influence of co-factor modification on photosynthetic electron transport.² During electron transport, quinone radicals are produced and therefore the problem arises of the characterization of different radical forms of quinones.^{1,3}

EPR and related high resolution methods offer the opportunity for distinguishing between different quinones, their different protonation states and their corresponding structures based on the spectral properties of the semiquinone radical states.^{1,3,4}

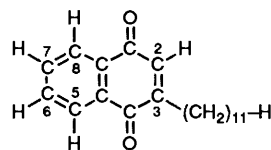
In this paper we report results from high resolution EPR experiments including electron-nuclear double resonance (ENDOR) and electron spin echo envelope modulation (ESEEM) of anion and cation radical forms of artificial dimethoxy- and halo-1,4-benzoquinones with an alkyl side chain of differing length.

Experimental

Experiments were performed with quinones of the following structures. (a) 2,6-Dimethoxy-3- R_n -1,4-benzoquinones (BQ- n) with $n = 1, 5, 9, 11, 13, 17$. (b) 3-Pentadecyl-5,6-dimethyl-1,4-benzoquinone (MBQ-15) and 2-tridecyl-3,6-dimethyl-1,4-benzo-



quinone (MBQ-13). (c) 2,5-Diundecyl-3,6-dimethyl-1,4-benzoquinone (U₂BQ). (d) 2,6-Dibromo-3- R_n -5-methyl-1,4-benzoquinones (Br-BQ- n) with $R_n = -(CH_2)_n - H$, $n = 7, 11, 13, 17$. (e) 3-Tridecyl-5-chloro-6-methyl-1,4-benzoquinone (Cl₃BQ-13). (g) 3-Undecyl-1,4-naphthoquinone (UNQ). The quinones were synthesized as described in refs. 2, 5 and 6.



Anion Radicals.—(i) KOH was dissolved in isopropanol. Approximately 100 mm³ of a cooled solution of alkaline isopropanol and triethylamine (1:1) was mixed with *ca.* 3 μg of quinone, placed in a 5 mm OD X-band EPR tube, frozen, degassed with several freeze-pump-thaw cycles and sealed.

(ii) KOH was dissolved in MTHF. About 50 mm³ alkaline MTHF and a small amount of Na:K (30:70) liquid alloy was placed in one arm of a two-horned ampoule. Quinone (3 μg) was dissolved in MTHF and put into the other arm. Degassing was performed as described above. Then the ampoule was sealed. The cooled solutions from the arms were mixed and frozen in liquid nitrogen.

Anion samples for electron spin echo measurements were made in a similar manner using NaOD and [²H₈]isopropanol.

Cation Radicals.—Two parts of concentrated H₂SO₄ (D₂-SO₄) were added to one part H₂O (D₂O), followed by a small amount of Mg powder and quinone (*ca.* 3 μg). The solutions were transferred to X-band EPR tubes and frozen.

Deuteriated compounds were obtained from the firm Isotope (USSR). All other chemicals were reagent grade or better.

An ESP-300 Bruker EPR-ENDOR spectrometer was used for X-band EPR and ENDOR. The electron spin echo spectrometer used for ESEEM measurements has been described previously.⁷

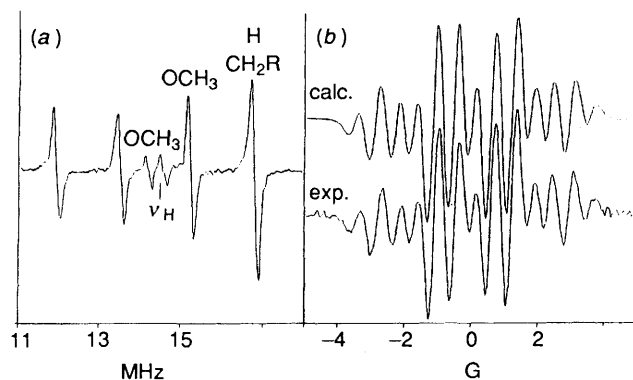


Fig. 1 (a) Experimental ENDOR spectrum of the anion radical of BQ-9 at $T = 203$ K; (b) experimental and calculated EPR spectra of the anion radical of BQ-9. The isotropic hfi constants of the protons used in the calculations are listed in Table 1.

Table 1 Isotropic hyperfine constants of protons in anion radicals of 1,4-benzoquinones (± 0.03 G)

Quinone	Method of preparation	Proton isotropic hfi constants (G) at different ring positions			
		2	3	5	6
BQ-1	(i)	—	3.16	1.59	0.64
BQ-5	(i)	0.21	1.69	1.69	0.62
BQ-9	(i)	0.21	1.74	1.74	0.62
BQ-11	(i)	0.22	1.75	1.75	0.62
BQ-13	(i)	0.22	1.75	1.75	0.62
BQ-17	(i)	0.22	1.75	1.75	0.62
BQ ^a		0.77	1.50	1.50	0.77
BQ ^b		0.61	1.83	1.83	0.61
BQ-2 ^b		—	1.35	1.35	0.61
U ₂ BQ	(i)	0.91	1.91	0.91	1.91
MBQ-15	(i)	2.31	0.89	1.81	1.81
M ₄ BQ ^c		1.90	1.90	1.90	1.90
Vit. E ^d		0.91	1.90	1.90	1.90
UNQ	(i)	2.27	2.27	(5,8)	(6,7)
MNQ ^e		2.51	2.8	0.44	0.71
ENQ ^f		2.64	2.28	0.40	0.69
BrBQ-7	(i) ^g	—	1.32/1.63	2.81	—
	(i) ^h	—	1.41	2.94	—
	(ii)	—	1.54	3.12	—
BrBQ-11	(i) ^g	—	1.30/1.59	2.85	—
	(i) ^h	—	1.38	2.88	—
BrBQ-15	(i) ^g	—	1.36/1.55	2.89	—
	(i) ^h	—	1.43	2.95	—
	(ii)	—	1.53	3.12	—
Cl ₅ BQ-13	(i)	1.35	2.07	—	2.90
	(ii)	1.33	2.07	—	3.04

^a 2,6-Dimethoxy-1,4-benzoquinone in 50% water-Bu^tOH, taken from ref. 8. ^b In DMF, taken from ref. 9. ^c 2,3,5,6-Tetramethyl-1,4-benzoquinone, taken from ref. 10. ^d Vitamin E, taken from ref. 11. ^e 3-Methyl-1,4-naphthoquinone, taken from ref. 4. ^f 3-Ethyl-1,4-naphthoquinone, taken from ref. 12. ^g $T = 200$ K. ^h $T = 215$ K.

Results and Discussion

Anion Radicals.—*Quinones without halogen substituents.* Samples of quinones prepared using methods (i) and (ii) were studied by ENDOR and EPR spectroscopies in the liquid state at temperatures $T > 190$ K (i) and $T > 220$ K (ii).

The ENDOR spectra of samples with BQ- n , U₂BQ, MBQ-15 and UNQ usually show a few pairs of lines located symmetrically around the proton Zeeman frequency ν_H [Fig. 1(a)],

corresponding to groups of protons with different isotropic hfi constants. These lines were present in the spectrum in liquid solution at all temperatures until freezing.

The proton couplings from the ENDOR spectra were assigned to the different ring positions of benzoquinones as shown in Table 1 on the basis of computer simulation of the EPR spectra [Fig. 1(b)] taking into account the number of protons at each substituent.

Comparison of the isotropic hfi constants obtained for different ring positions with the ones known from the literature on anion radicals of related chemical analogues⁸⁻¹² (Table 1) confirms our assignment and confirms the formation of anion radicals as paramagnetic products.

The comparison of ENDOR data for BQ- n quinones shows that the length of the alkyl side chain has almost no influence on the spin-density distribution of the semiquinone moiety. This conclusion helps to explain, as first done by Das *et al.*,¹¹ the approximately twofold decrease of the isotropic hfi constant of β -methylene protons relative to the methyl protons (compare, for example the hfi constants of BQ-1 with all other BQ- n , M₄BQ with U₂BQ and MBQ-15) when an alkyl side chain is introduced to the quinone structure instead of a methyl group. The isotropic hfi constant of β -protons is well described by the relation $\alpha_\beta = \rho^\pi B \cos^2 \theta$ where $B = ca. 50$ G is a constant and θ is the angle between the CH bond and the axis of the p_z carbon orbital, both projected in a plane orthogonal to the C-C bond. For each proton of a freely rotating methyl group $\langle \cos^2 \theta \rangle = 1/2$. Therefore a ratio of 1/2 for a β -proton splitting to a methyl proton splitting from positions of nearly equal spin density readily suggests a preferred conformation with $\cos^2 \theta = 1/4$ or $\theta = \pm 60^\circ$.

Our experimental data testify to a substantial difference in the proton isotropic hfi constants in the methoxy groups of BQ- n . The different splittings of methoxy protons in the BQ- n series of compounds can be explained by recourse to a simple steric argument as has been done in ref. 9. The p_x orbital of oxygen in the OCH₃ group must overlap with the π -system of the ring for effective proton splitting to take place. In this case the O-C bond lies in the nodal plane of the π -system. Sterically this geometry can be realised most easily when the substituent at the neighbouring carbon atom of the ring is a hydrogen. If another, more voluminous substituent is adjacent to the methoxy group the result would be to twist the methoxy group out of the plane, giving a decreased π -orbital overlap and smaller hfi constant. The same suggestion about one planar and one twisted methoxy group has been recently put forward to explain the ENDOR results for the BQ-1 anion radical.¹³

To a first approximation the spin density on the oxygen atom should decrease by $\cos^2 \theta$ where θ is the out-of-plane twist angle. Therefore, on the assumption that the larger constant for the methoxy protons corresponds to the methoxy group in the nodal plane with $\theta = 0^\circ$, one can estimate the twist angle for the methoxy group with the smaller proton constant. This gives $\cos^2 \theta = 0.35$ and hence $\theta = \pm 53^\circ$.

The ENDOR spectra of anion radicals of BQ- n also show that within experimental accuracy, dependent on the ENDOR linewidth (*ca.* 0.03 G), the isotropic hfi constants of the α -proton in the 5-position and the methylene β -protons accidentally coincide. For methylene protons in the conformation with $\theta = \pm 60^\circ$, $\alpha_\beta = \frac{1}{4} B \rho^\pi$. The constant of isotropic hfi with an α -proton is $\alpha_\alpha = Q \rho^\pi$, where for radical-anions $Q = -(27 \pm 3)$ G.^{11,14} This means that in the quinones with a voluminous substituent the spin densities on the carbon atoms in the 3- and 5-ring positions differ by almost twofold (relative to symmetric BQ), independently of its length. Comparing the π -spin densities on the 3- and 5-carbon atoms in the anion radicals (calculated using proton hfi constants taken from ref. 4 with $B = 50$ G and $|Q| = 27$ G) of the series 1,4-benzoquinone, 3-methyl-1,4-benzoquin-

Table 2 Comparison of the experimental and calculated spin densities on carbons of ring positions 3 and 5 in anion radicals of 1,4-benzoquinones

Quinone	Experimental		Calculated	
	ρ_3^π	ρ_5^π	ρ_3^π	ρ_5^π
1,4-Benzoquinone	0.085	0.085	0.078	0.078
3-Methyl-1,4-benzoquinone	0.084	0.088	0.075	0.079
2-Methoxy-1,4-benzoquinone	0.018	0.135	0.041	0.099
2,6-Dimethoxy-1,4-benzoquinone	0.055	0.055	0.058	0.058
BQ- <i>n</i>	0.128	0.062	^a 0.088	0.045
			^b 0.094	0.042

^a Twist angle of methoxy oxygen in the 2-position, $\theta = 50^\circ$. ^b $\theta = 90^\circ$.

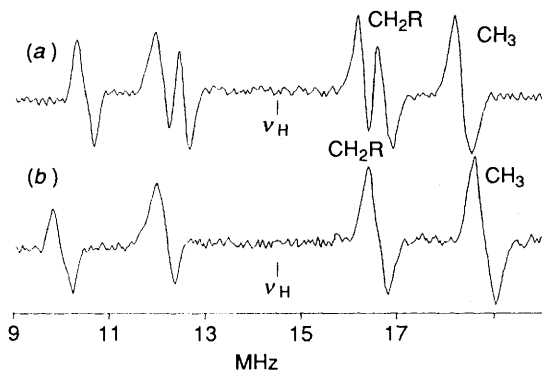


Fig. 2 ENDOR spectra of the BrBQ-7 anion radical at $T = 200$ K (a) and $T = 215$ K (b)

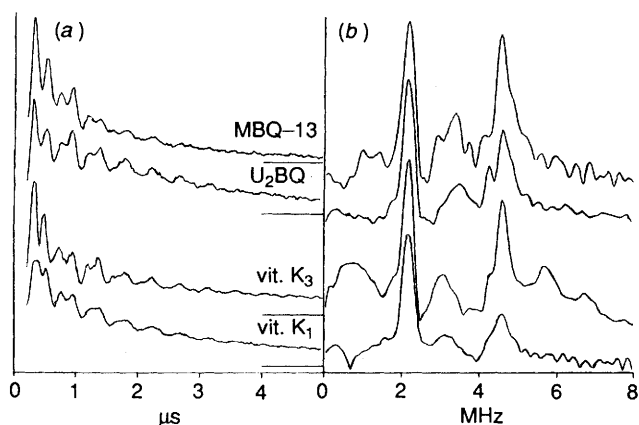


Fig. 3 (a) Primary ESEEM of cation radicals of MBQ-13, U₂BQ, vitamin K₁ and K₃, prepared in deuteriated solution. Data for vitamins taken from ref. 19. (b) Corresponding ESEEM spectra.

one, 2-methoxy-1,4-benzoquinone, 2,6-dimethoxy-1,4-benzoquinone and BQ-*n* (Table 2), it is seen that the difference appears to be mainly due to the decrease of π -orbital overlap of the oxygen in the 2-position and, to a lesser degree, to an inductive influence of the substituents. This conclusion was confirmed by simple HMO calculations performed with the following parameters: $h_o = 2$, $k_{oc} = 0.8$ for methoxy oxygen and $h_o = 1$ and $k_{oc} = 1$ for carbonyl oxygen.¹⁵ The calculated spin densities completely reproduce the tendency of the experimental spin density changes on the 3- and 5-carbons in the series discussed.

Quinones with halogen substituents. Quinones of the BrBQ-*n* type have just two substituents containing protons. Therefore, at temperatures $T > 220$ K their ENDOR spectra show two pairs of the lines centred on the proton Zeeman frequency. Computer simulations of the EPR spectra with the determined isotropic hfi constants allow one to attribute the smaller of them

to the methylene protons and the larger to the methyl protons. For these anion radicals also, the isotropic hfi constants of methylene protons are with great accuracy (3–5%) twice as low as those of the methyl protons, indicating a symmetrical distribution of spin density over the ring and a conformation of methylene protons with $\theta = \pm 60^\circ$.

In the samples prepared using method (i) the decrease of the temperature leads to spectral changes (Fig. 2). As the temperature is lowered from $T = 215$ K to $T = 200$ K the ENDOR lines of the methylene protons broaden and finally split into two distinct lines corresponding to different coupling constants for the two methylene protons (Table 1). The shape of the ENDOR lines of the methyl protons depends slightly on the temperature. The temperature dependence of the methylene protons can be explained by a freezing-in of the internal rotation of the alkyl side chain. It has been observed earlier in ENDOR spectra of quinones of other types containing long side chains.¹¹ The temperature-induced changes are accompanied by slight changes of the absolute values of the isotropic hfi constants (Fig. 2, Table 1).

With the help of the value of the hfi constant of methyl protons in 5-positions it is possible to estimate the change of the orientation of the CH bonds in the static conformation assuming the spin densities at positions 3 and 5 are nearly the same. Using this procedure the splittings of methylene proton lines are found to correspond to a deviation $\varphi = 2\text{--}3^\circ$ from the equilibrium conformation with $\theta = \pm 60^\circ$. This inequivalence in angle compared to the BQ-*n* quinones undoubtedly arises from the substitution of the OCH₃ group by the Br atom next to the methylene group. One can suggest that the larger angle $60^\circ + \varphi$ corresponds to the proton closer to oxygen, due to a slight hydrogen-bonding pull effect.

The ESEEM experiments with samples of anion radicals in deuteriated solvents, carried out to study the interaction with matrix nuclei, showed only modulation with the deuterium Zeeman frequency. There is nothing in the ESEEM spectra to suggest strong hydrogen bonding with transfer of significant spin density to matrix deuterium nuclei or proton-deuteron exchange. This result agrees with the solid state ENDOR data for 1,4-benzoquinone anion radical, indicating that hydrogen bonding of the carboxyl group to the alcohol hydroxy group of the solvent is characterized by a purely dipolar interaction.¹⁶

Cation Radicals.—The cation radicals of quinones have featureless X-band EPR spectra in the frozen state. Deep ESEEM was obtained from samples when prepared in deuteriated solvents (Fig. 3). The ESEEM spectra in all cases contain a strong component at the deuterium Zeeman frequency $\nu_1 = 2.2$ MHz from weakly-coupled matrix deuterium nuclei (Fig. 3). Additionally, the spectra from these envelopes exhibit lines with maxima at frequencies 3.2–3.4 and 4.7 MHz. The line at 3.2–3.4 MHz has a positive amplitude in cosine FT spectra, whereas the line at 4.7 MHz has a negative amplitude. Similar ESEEM spectra have been observed previously for some other paramagnetic centres stabilized in matrices containing deuterium nuclei, including the tyrosyl radical in photosystem II^{17,18} and cation radicals of vitamin K₁ and K₃¹⁹ (Fig. 3).

As in ref. 19 we assign these additional lines to deuterons of the –OD groups of the respective quinones. Quinone oxygens are expected to be bonded to deuterons (hydrogens) when prepared in strongly acidic solvents.

Similarly to the results of previous works, the line at 3.2–3.4 MHz is assigned to the $\nu_{\alpha(\beta)}^\pm$ frequency of the hydroxy OD deuterons strongly (relative to the matrix nuclei) coupled to the unpaired electron. The line with maximum at 4.7 MHz is the $\nu_\alpha + \nu_\beta$ harmonic of these nuclei shifted from the position $2\nu_1$ to high frequency, mainly due to the influence of the anisotropic hfi. Using the formulae^{20–22} of eqn. (1) one can determine an

Table 3 Hyperfine parameters (G) of hydroxy deuterium (proton) in cation radicals of 1,4-benzoquinones^a

Quinone	<i>a</i>	<i>T</i> ₁₁	<i>T</i> ₂₂ / <i>T</i> ₁₁	Conformation
BQ-1	-0.44 (2.9)	-0.46 (2.55)	0.3	<i>cis</i>
MBQ-13	-0.41 (2.67)	-0.46 (3.0)	0.3	<i>cis</i>
U ₂ BQ	-0.43 (2.78)	-0.46 (3.0)	0.3	<i>trans</i>
UNQ	-0.36 (2.32)	-0.39 (2.55)	0.1 ± 0.05	<i>trans</i>
Vitamin K ₃	-0.39 (2.55)	-0.42 (2.78)	0.3	<i>cis</i>
	-0.36 (2.3) ^b	-0.3 (1.97)	1	
Vitamin K ₁	-0.32 (2.09)	-0.42 (2.78)	0.1 ± 0.05	<i>trans</i>
	-0.36 (2.3) ^b	-0.3 (1.97)	1	

^a The sign of the isotropic hfi was chosen according to experimental and theoretical results in liquid solutions. ^b Taken from ref. 19.

$$v_{\alpha(\beta)}^{\pm} = \left| v_1 \pm \frac{a}{2} \pm \frac{T_{\perp}}{2} \right| \quad (1)$$

$$(v_{\alpha} + v_{\beta})_{\max} = 2v_1 + \frac{9}{16} \frac{T_{\perp}^2}{v_1}$$

isotropic hfi constant *a* and a perpendicular component *T*_⊥ of the axially anisotropic hfi tensor. These parameters can be used as starting values in numerical calculations of the line shape of ESEEM spectra of strongly coupled deuterons, taking into account, in the general case, also the non-axial hfi and nuclear quadrupole interactions.

To determine the number of strongly coupled deuterons one has to compare the experimental and simulated modulation in the time domain. To perform such a comparison for the primary ESE, it is necessary to eliminate from the experimental envelopes the contribution of the distant matrix nuclei represented mainly by the line at 2.2 MHz. An adaptive digital filter is used to remove this contribution.^{20,21} This approach has worked well in the past to reveal modulation features from more strongly interacting nuclei and was applied for cation radicals of vitamin K₁ (phyloquinone or 2-methyl-3-phytyl-1,4-naphthoquinone) and K₃ (menadione or 2-methyl-1,4-naphthoquinone).¹⁹ As mentioned, ESEEM spectra of these radicals have shown positions of lines from hydroxy deuterons close to those of the quinones used in this work, indicating a similar interaction in both cases (Fig. 3). The hfi parameters for these deuterons (determined in the approximation of axial hfi and neglecting *nqj*¹⁹) coincide for vitamin K₁ and K₃ (Table 3). However, a comparison in the time domain of calculated modulation envelopes with the experimental ones after filtration of the matrix contribution has shown two deuterons for vitamin K₃, and only one strongly coupled deuteron for vitamin K₁ rather than the two expected for the cation radical. As an explanation of this result it has been suggested that steric hindrance from the phytol chain could cause the adjacent OD group to rotate away from its normal position and drastically change the deuterium isotropic coupling, which strongly affects the modulation amplitude or, alternatively, that hydrogen bonding of the ethylenic proton on the phytol chain to the carbonyl group would give one -OH, and thus only one -OD group contributing significantly to the ESEEM.¹⁹ But there is no real evidence in the ESEEM spectra for the existence of hydroxy deuterium nuclei with different hfi parameters, or for a proton with large isotropic hfi constant in solid state proton ENDOR spectra.¹⁹

Therefore we decided to offer another explanation of the differences obtained. This explanation is based on spatial correlation of hydroxy nuclei in the quinone molecule.

EPR spectra of quinone cation radicals have previously been studied in great detail and it has been shown that quinones can

exist as *cis* and *trans* isomers in liquid solutions for which the isotropic hfi constants of the protons of ring substituents are somewhat different. The hydroxy proton splitting shows a small, approximately linear temperature dependence in solution (*ca.* -0.08 G deg⁻¹).⁴ This temperature dependence has been attributed to temperature dependent torsional oscillations. The experimental results for the cation radical of the 1,4-benzoquinones⁴ and theoretical analysis²³ (*|a| ca.* 2.3-3 G for *cis* and *trans* isomers, with negative sign) have indicated that in those species the hydroxy group is very close to the nodal plane of the π-system in solution around room temperature and somewhat below. Values of the isotropic hfi constant obtained in ref. 19 and in this work (see below and Table 3) indicate that the hydroxy group is very close to the nodal plane in frozen solution also.

However, many examples exist when only one conformation of hydroxy groups is preferred, depending on the position of substituents relative to the -OH group. For example, it has been argued that the preferred conformation for the hydroxy proton is *anti* to a methyl substituent, therefore this leads to the existence of only the *cis* form for the 2,3-dimethyl- and the *trans* isomer for 2,5-dimethyl-1,4-benzoquinone cation radicals.²⁴

The spatial nuclear environment of the electron can readily be taken into account when the nuclei belong to the same molecule with a well-defined geometry, such as a quinone. In the *trans* conformation of a quinone two hydroxy deuterium nuclei with similar hfi parameters are located on one straight line passing through the origin of the coordinates. These nuclei are magnetically equivalent and the ESEEM calculations in disordered systems are performed using the relation (2):

$$v_N(\tau) = \langle [V(\tau, \theta, \varphi)]^2 \rangle_{\theta, \varphi} \quad (2)$$

The angles θ and φ determine the *H*₀ direction in the arbitrary coordinate system.

In the *cis* conformation two hydroxy deuterons are located at the angle α *ca.* 140° relative to the origin of the coordinates. ESEEM in this case is calculated as the product (3):

$$v(\tau) = \langle V_1(\tau, \theta, \varphi) V_2(\tau, \theta, \varphi) \rangle \quad (3)$$

taking into account the different orientation of the nuclei relative to the external field.

In the ESEEM calculations performed in ref. 19 it was assumed that the hydroxy nuclei surrounding the unpaired electron fail to form a regular structure and occupy positions relative to one another that are not orientationally correlated. In this case the primary ESEEM from *N* nuclei with the same hfi parameters in an orientationally disordered sample is calculated using the relation (4).

$$V(\tau) = \langle V(\tau, \theta) \rangle_N^N \quad (4)$$

This approximation is often labelled as the spherical model.

In accordance with previous results²⁵ our model calculations show that the magnetically equivalent nuclei give the smallest modulation amplitude relative to all the other spatial geometries of the nuclei interacting with the electron and relative to the approximation of uncorrelated nuclei.

Using estimations of *a* and *T*_⊥, with the help of eqn. (1), as starting values we performed numerical calculations of the line shape of ESEEM spectra of strongly coupled spatially correlated deuterons for cation radicals of BQ-1, MBQ-13, UNQ and U₂BQ, taking into account the non-axial hfi and nuclear quadrupole interactions with quadrupole coupling constant 0.215 MHz. Two of them, namely BQ-1 and MBQ-13, have a lower modulation amplitude due to strongly coupled deuterons after filtration of their matrix contribution than the two others (UNQ and U₂BQ), similar to the observations for the cation radicals of vitamin K₁ and K₃. Fig. 3 clearly shows the decrease

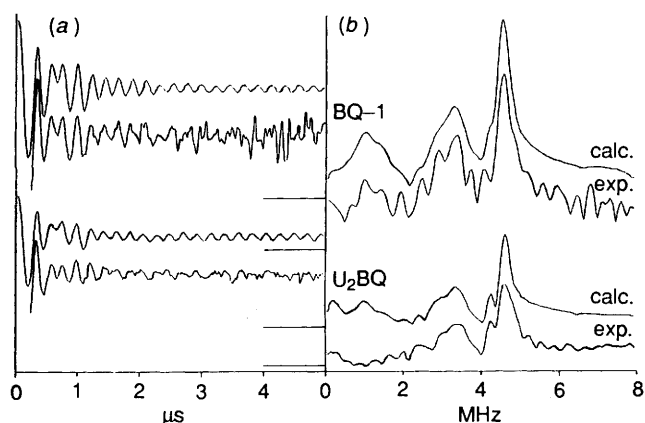


Fig. 4 Comparison of calculated and experimental ESEEM in the time- (a) and frequency (b) domain after filtration of the matrix contribution for cation radicals of BQ-1 and U₂BQ

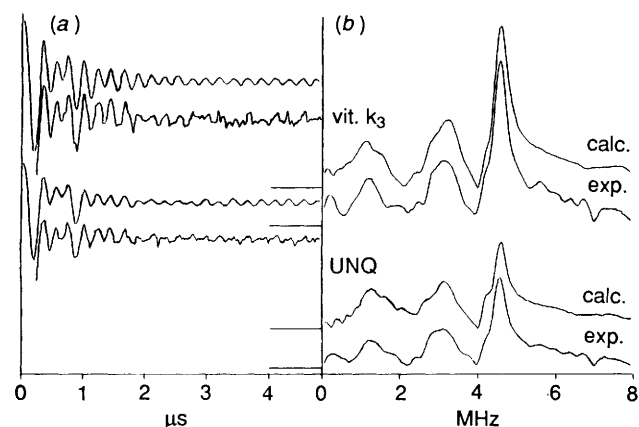
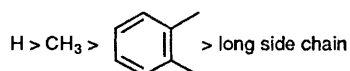


Fig. 5 The same as in Fig. 4 for cation radicals of vitamin K₃ and UNQ

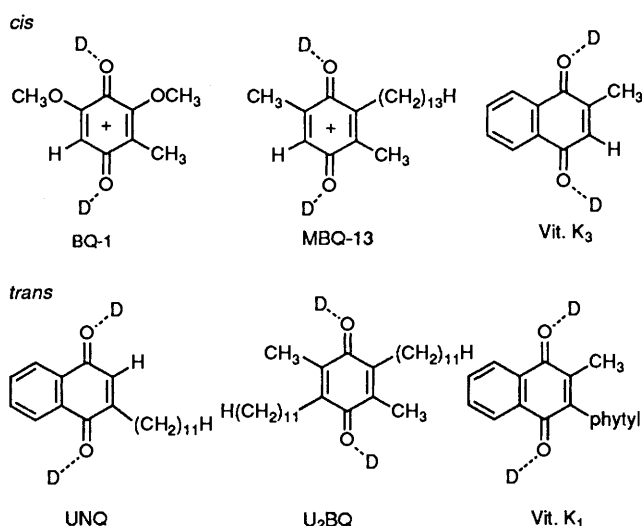
of intensity of the lines at 3.2–3.4 and 4.7 MHz relative to the matrix line for U₂BQ and vitamin K₁, compared with MBQ-13 and vitamin K₃.

Calculations were performed assuming the *cis* conformation for quinones with a larger modulation amplitude from hydroxy deuterium and a *trans* conformation for quinones with a small modulation amplitude. Table 3 lists the isotropic hfi constant, the T_{11} component of the anisotropic hfi tensor and the ratio $s = T_{22}/T_{11}$ for the hydroxy deuterium that correspond to the best agreement between experimental and simulated spectra with respect to line positions and relative intensities (Figs. 4, 5). Comparing the calculated time domain envelopes with the experimental ones after filtration of the matrix contribution (Figs. 4, 5) shows a good correspondence. We also performed new calculations for vitamin K₁ and K₃ (Table 3) and obtained good agreement in modulation amplitude for two hydroxy deuteriums in both cases, assuming a *trans* conformation for vitamin K₁ and *cis* for vitamin K₃. This result explains the different number of nuclei obtained for cation radicals of vitamin K₁ and K₃ when the calculations were performed in the approximation of uncorrelated nuclei.

Our result that in the cation radicals of BQ-1, UBQ-13 and vitamin K₃ the two hydrogen-bonded deuterium nuclei are in the *cis* conformation, whereas for the cation radicals of UNQ, U₂BQ and vitamin K₁ they are in the *trans* conformation, can be explained by invoking steric hindrance. We can formulate the empirical rule:



where the O...D hydrogen bond is oriented to the side of the substituent that is furthest to the left in the above sequence as illustrated below.



Our rule serves well as an empirical guide for the interpretation of the ESEEM spectra of quinone cation radicals. Of course, detailed calculations are necessary to put the rule on a firm theoretical foundation.

Acknowledgements

Part of this work was supported by the Dutch Ministry of Education and Science, under the grant for the joint research program between the Department of Biophysics, Huygens Laboratory, Leiden University and the Institute of Organic Chemistry, Central China Normal University, Wuhan, P.R.C. and by the Netherlands Foundation for Chemical Research (SON), financed by the Netherlands Organization for Scientific Research (NWO). S. A. D. and R. I. S. thank the NWO for financially supporting their sojourns at Leiden University (in 1990 and 1992, respectively), and the Department of Biophysics for its hospitality. The authors are grateful to Dr. A. V. Astashkin for writing the indispensable multipurpose data processing and graphics representation program used in this work.

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Paper 2/01953A

Received 14th April 1992

Accepted 12th May 1992