ENDOR and EPR studies of highly isotopically ^{13}C-enriched ubiquinone radicals. Part 2 †

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ENDOR and EPR spectroscopy of ubiquinone ‡ (UQ)-0 and UQ-10 radicals, singly ^{13}C-labelled at the 1,6,5,5′ and 4(UQ-0) or the corresponding 1,2,3,3′ and 4(UQ-10) positions has been carried out in protic and aprotic solutions. The ^{13}C-hyperfine coupling constant for the various ^{13}C-positions in the semiquinone, neutral and cation radicals are presented.

The semi-empirical MO schemes AM1, PM3 and INDO have been used for calculating, in addition to the spin-density distribution, the geometry of isolated radicals and hydrogen-bonded radical–solvent complexes, and the results are compared.

Introduction

It is well known that in the reaction centre (RC) of the photosynthetic bacterium *Rhodobacter* (*Rb.*) *sphaeroides*, ubiquinone-10 is utilized as both the primary, Q_A, and the secondary, Q_B, acceptor. The difference in the redox characteristics, particularly in electron transfer rates and subsequent proton transfer, can be attributed to the difference in the specific interaction of the carbonyl groups of Q_A and Q_B with the nearest amino acids of the protein environment. Systematic studies of the electron structure of Q_A and Q_B acceptors[1,2] have been based on EPR and ENDOR measurements of the hyperfine interaction (hfi) constant with proton nuclei in the ubiquinone radical anions. It is difficult, however, to compare the values of proton hfi constants in the ENDOR spectra of the Q_A and Q_B radical anions in the RCs of *Rb. sphaeroides* R-26.1 and model systems, because of a great number of additional lines observed around the proton frequency, which are due to molecules (amino acids, water) surrounding Q_A and Q_B in RCs.

The application of chemically modified, in particular ^{13}C-labelled compounds, allows additional possibilities for studying quinone binding *in vivo*. The introduction of the ^{13}C-isotope as a spectroscopic label has no effect on the steric and electronic properties of ubiquinone; in fact, the natural RC complex contains this isotope in amounts of about 1%. However, the low natural content of ^{13}C makes it difficult to observe the additional ^{13}C-hfi in EPR spectra of the quinone anion. The development of selectively, highly enriched (up to 99%) ^{13}C-labelled compounds[3] has allowed us to start a systematic study of the electronic structure of ubiquinone radical molecules by analysing the hfi with a single ^{13}C nucleus. In an earlier communication, Part 1,[4] we analysed in detail the hfi values for carbonyl [1-^{13}C]-labelled radicals of ubiquinone-0. We have shown that the value of the hfi constant on [1-^{13}C] is sensitive to both the redox state of ubiquinone radicals and the character of the protonation of the [1-^{13}C] carbonyl carbon.

In the present contribution we are studying radicals of ubiquinone-0 (UQ-0) and ubiquinone-10 (UQ-10) in organic solvents, labelled with a single ^{13}C nucleus at different positions of the aromatic ring and at the methyl carbon. Values of the isotropic hfi constants are obtained by EPR and ENDOR spectroscopy and compared with values calculated with several semi-empirical quantum chemical methods. It is shown that the hfi constants are sensitive to the solvent properties, especially to hydrogen-bonding. This sensitivity is qualitatively well accounted for by the calculations.

Experimental

Materials

The synthesis of [2-^{13}C], [3-^{13}C], [4-^{13}C] and [3-^{13}C]methyl UQ-10 and the corresponding [6-^{13}C], [5-^{13}C], [4-^{13}C] and [5-^{13}C]methyl UQ-0 was carried out as described.[3] (Note that the IUPAC numbering scheme for the UQ-10 nuclei differs from that of UQ-0 and all other ubiquinones.)

Preparation of radical species

Radicals were prepared in high vacuum as described.[4] Radical anions were generated in protic (alcohol) and aprotic [hexamethylphosphoric triamide (HMPT)] solvents by chemical reduction with potassium butoxide (Bu^{t}OK). Diprotonated quinone radical cations were obtained by dissolving the corresponding quinones in a degassed mixture of trifluoromethanesulfonic (triflic) acid (CF_3SO_3H or CF_3SO_3D) with nitromethane. The ratio of triflic acid to nitromethane was 1:1. Neutral radicals were generated by UV-illumination of a quartz flat cell filled with a degassed alcohol solution of ubiquinone in the presence of acetic acid. The irradiation light from a high-pressure mercury lamp passed through a water filter and a glass cut-off filter (FS-6). The EPR spectra were measured on an ESP-300 Bruker or a JEOL (JES-RE2X) spectrometer. ENDOR measurements were carried out as in ref. 4, using frequency modulation of the RF. For display purposes, the spectra were numerically integrated.

Results and discussion

Scheme 1 shows the structure of UQ-0 and UQ-10 in terms of the IUPAC numbering of atoms. The asterisk in the Scheme denotes the positions of the enriched ^{13}C-isotope.

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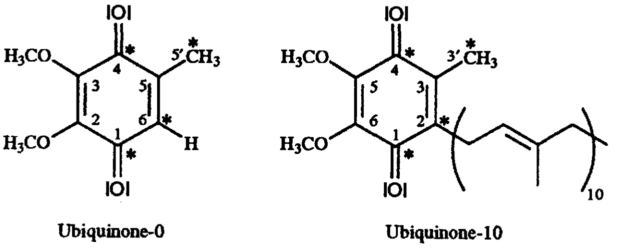
† For Part 1, see ref. 4.

‡ 2,3-Dimethoxy-5-methylbenzoquinone.

Table 1 Experimental and calculated values of [^{13}C] hfi (in gauss) for the ubiquinone radical anion and its complex with two methanol molecules

	Experimental UQ-10		Experimental UQ-0		Calculated UQ-0			
					INDO		PM3	
Carbon	HMPT	Methanol	HMPT	Methanol	Anion	Complex	Anion	Complex
1	1.51	0.62	1.5–1.6	0.63–0.68	−1.82	−1.51	−1.96	+0.16
2	<0.1	0.46			0.49	0.44	4.11	3.44
3	<0.1	0.52			0.32	0.27	3.82	3.44
4	1.2	0.98	1.25	0.8	−1.64	−1.41	−1.96	−1.1
5					0.47	0.45	4.16	5.16
6			0.65–0.8	1.4–1.5	0.76	0.61	3.20	1.13
Me	1.51	1.42	1.75	1.70	−0.29[a]	−0.31	−1.35	−1.50
OMe	<0.1		<0.1		0.08[a]	−0.08[a]	1.17	1.11

[a] The value of twist angle = 45°.



Ubiquinone-0 Ubiquinone-10

Scheme 1

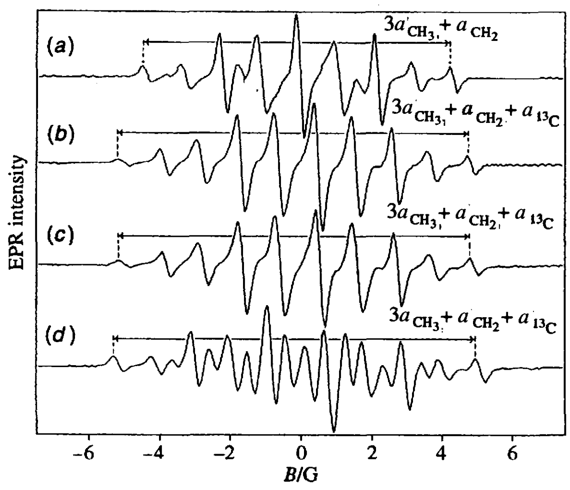


Fig. 1 EPR spectra of UQ-10 radical anions at room temperature in HMPT with Bu^{t}OK: (*a*) unlabelled; (*b*) [1-^{13}C]; (*c*) [4-^{13}C]; (*d*) [^{13}C]methyl

Radical anions

EPR spectra. As in our previous work,[4] the study was started with the radical anions, generated in protic and aprotic solvents. Fig. 1(*a*) gives the EPR spectrum at room temperature of UQ-10 in HMPT. The spectrum is similar to that reported in the literature.[5] Radical anions in HMPT of ubiquinone enriched with a single spin 1/2 ^{13}C nucleus, showed an additional splitting in the EPR spectra, Fig. 1(*b,c,d*). One more additional splitting is observed in protic solvents. However, the values of the ^{13}C-hfi for methanol (Table 1) are substantially decreased compared to those for HMPT, due to the formation of a hydrogen bond between the radical anion and the solvent *via* the carbonyl groups.

According to refs. 2, 6 and 7, the formation of a hydrogen bond with the radical anions and consequent redistribution of spin density, leads to a 2% decrease in the values of the ^{1}H-hfi constants in a protic solvent (EtOH) compared to an aprotic one (Et$_2$O). In our experiments we have almost the same change in the values of the hfi constant of the [^{13}C]methyl group, but the range of the change of ^{13}C-hfi for the carbonyl atoms, [1-^{13}C] and [4-^{13}C], is much larger, *viz.* 57 and 58% in UQ-0, and 36 and 18% in UQ-10. One would think that the isoprene chain in UQ-10 would screen the [1-^{13}C] atom in the radical anion, and therefore would decrease the effect of hydrogen bond formation on the hfi constant of this carbonyl carbon. However, our data shows that the effect is in fact larger for the [1-^{13}C] than for the [4-^{13}C] atom, although the latter is farther removed from the isoprene substituent.

For radical anions of [2-^{13}C]-labelled UQ-10 in HMPT, no ^{13}C-splitting is observed in the EPR spectrum, whereas for UQ-0 in HMPT, the value of the ^{13}C-hfi constant in the corresponding [6-^{13}C] position is about 0.65–0.8 G. The decrease in the value of the hfi constant of the [2-^{13}C]-atom in UQ-10 compared to UQ-0, is attributed to the influence of the isoprene substituent.

ENDOR spectra. The data on the isotropic hfi constants of ubiquinone radical anions could be obtained directly from the EPR spectra recorded at room temperature. There is, however, an interest in obtaining information about the hfi constants from ENDOR spectra of samples for which the EPR spectra are badly resolved, for example, because of low temperature, or because of immobilization by a protein matrix. It is known that as the temperature of measurement drops, the lines of ring protons broaden and become unobservable, because the contribution of the anisotropic hfi components for ring protons is about 50% of the value of the isotropic hfi constant. In contrast, for methyl protons this contribution is about 10%, and the methyl groups are highly mobile down to 77 K,[8] so that the lines of the methyl protons in ENDOR spectra remain narrow and strong.

Observation of ENDOR transitions of ^{13}C nuclei is more difficult than for protons, due to the increase of the anisotropic contribution, determined by electron–nuclear dipole interaction. The lower the unpaired spin density in the π-atomic orbital of a carbon atom is ($\rho_\pi \leq 0.1\%$), the more probable is the experimental observation of ^{13}C ENDOR.[9]

In previous work,[4] we have been unable to determine the hfi constant of the [1-^{13}C] atom of UQ-0 radical anions by ENDOR spectroscopy, because the optimum conditions for observing an ENDOR signal could only be achieved above room temperature. For the [^{13}C]methyl group, however, in which the carbon atom is sp^3-hybridized, the optimum condition for observing ENDOR is expected to be the same as for the ^{1}H-nucleus of the methyl group.

Fig. 2 gives the ENDOR spectra, observed upon reduction of [^{13}C]methyl-labelled UQ-0 in isopropyl alcohol at

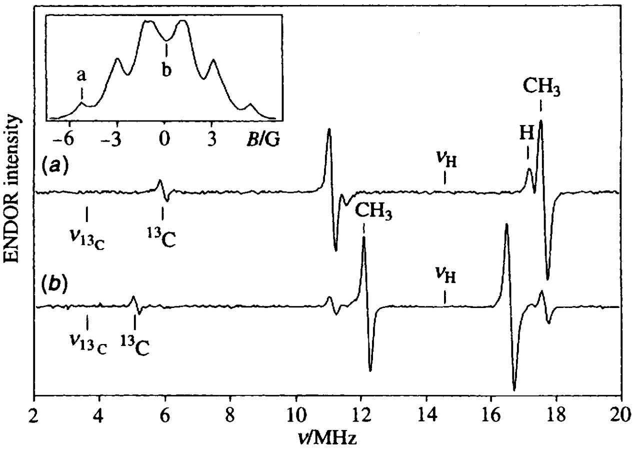


Fig. 2 ENDOR spectra of [^{13}C]methyl-labelled UQ-0 radical anions at room temperature in isopropyl alcohol taken with different field setting indicated by a and b in the EPR spectrum (inset)

$T \approx -80$ °C. The EPR spectrum (Fig. 2, inset) is badly resolved at this temperature. As follows from Fig. 2(*a*,*b*) ENDOR spectra obtained at the different field settings allow us to resolve individual spectra of a mixture of radical anions R^1 and R^2 in the sample, that is, of usual UQ-10 (R^1) and quinone, substituted in the sixth site by an alkoxy group (R^2). For radical anion R^1, the ENDOR lines are equally spaced about the free proton nuclear frequency ν_H and separated by the hfi with the methyl protons and the ring proton. When the ring proton is substituted, the ENDOR spectrum shows lines separated by the hfi with the methyl protons in radical R^2.

Besides the lines aligned at the proton Larmor frequency, lines are observed at low frequency. Assuming the lower-frequency line is one component of the doublet originating from hfi with the ^{13}C nucleus (Larmor frequency $\nu = 3.7$ MHz in a field of 3500 G), it appears that the value of the hfi constant for [^{13}C]methyl in radical anion R^1 is 4.6 MHz, and for R^2 it is 2.88 MHz; these values coincide with the data obtained from the EPR spectra. Using ENDOR-induced EPR experiments [9] one can obtain the isolated EPR spectra of the two radicals R^1 and R^2 by recording the changes in the intensity of an ENDOR line, as the external magnetic field is swept over the range of EPR absorptions and the NMR frequency is positioned at the top of the ENDOR line.

The analysis of the data of our study of the radical anions of the UQ-0 and UQ-10 compounds, confirms that the isoprene chain has only a weak influence on the spin density distribution. A direct measurement of the ^{13}C-hfi constant shows conclusively that the addition of the isoprene chain decreases the values of constants on the ring carbon atom (site 6 in UQ-0 and site 2 in UQ-10) in the radical anions with 0.8 G to 0.1 G. The standard calculations of the values of spin densities on the ring carbon atoms,[10] performed using the standard bond lengths and the hybrid orbitals equivalent to sp^2, fail to account for the experimental results. As we shall see later, it is necessary to obtain more detailed information about hybridization and the change of the bond lengths, and to perform more up-to-date calculations.

Radical cations

The maximum effect of the influence of protons on the redistribution of spin density in ubiquinone is observed for diprotonated radical cations, when the protons covalently bind to the oxygens of the carbonyl groups. In our previous work we have measured the hfi parameters for the [1-^{13}C] atom in the diprotonated radical cation of UQ-0.

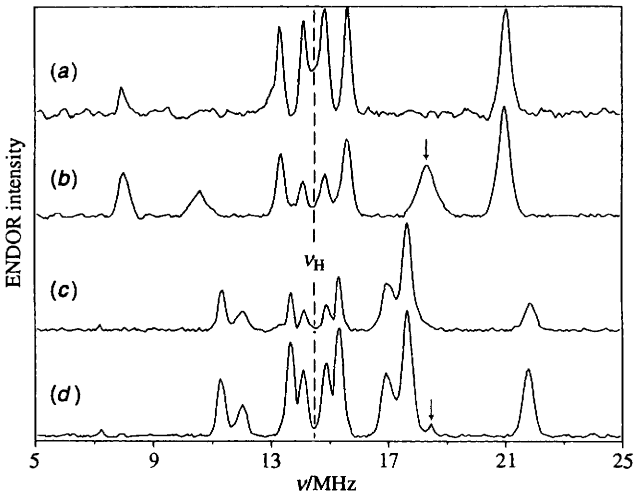
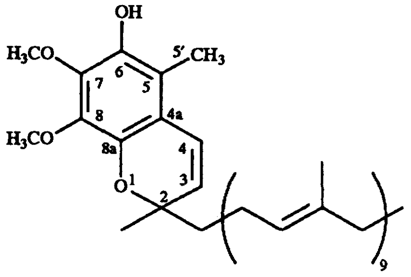


Fig. 3 ENDOR spectra of the UQ-0 radical cation (*a*,*b*) and the chromenoxyl radical (*c*,*d*) in D-triflic acid with nitromethane (*a*,*c*) and H-triflic acid with nitromethane (*b*,*d*)

In this work we have generated radicals using deuterium trifluoromethanesulfonic acid (CF_3SO_3D), to more accurately discriminate the lines of the protons of ring and hydroxy groups. For suppressing the secondary radical formation,[11] we have used a mixture of two solvents, triflic acid and nitromethane.

Fig. 3 displays the ENDOR spectra of the UQ-0 radical cation, obtained using protonated and deuteriated triflic acid. The complete width of the EPR spectrum of radical cations in deuteriated acid decreases by 5.6 G, which corresponds to the substitution of two protons with hfi constant of 2.8 G by deuterium. A simulated EPR spectrum with the constants obtained from the ENDOR spectrum fully agrees with the experimental one. Generating diprotonated UQ-10 radicals under the same conditions gives quite unexpected results. When deuteriated acid was used, the full width of the EPR spectrum decreases by ~ 2.8 G owing to the substitution by deuterium of either one proton with 2.8 G hfi constant or two protons with a two-fold lower constant. As follows from Fig. 3(*c*,*d*), in the ENDOR spectrum the lines at 18.4 MHz disappear, which corresponds to a proton hfi of 2.8 G (~ 8 MHz). Thus, the radical formed from UQ-10 has one proton that can be substituted by deuterium, with hfi constant close to that of the protons of the hydroxy groups in the UQ-0 radical cation. For the UQ-10 radical, the ENDOR spectrum shows substantial changes in the values of the hfi constants of the protons of other groups. The EPR and ENDOR spectra suggest that in the case of UQ-10, another radical can form due to the presence of the isoprene substituent in the ring. One of the possibilities is the formation of the chromenoxyl radical, first obtained by chromenol oxidation.[12]

The possibility of chromenoxyl formation due to intramolecular cyclization of the isoprene substituent into a pyran cycle under the action of acids or base is known from the literature.[13,14] For the molecule depicted in Scheme 2 several conformers are possible. At room temperature, however, the pattern observed in the ENDOR spectrum does not allow us to distinguish them. Fig. 4 displays the experimental and simulated spectra of the UQ-0 cation and chromenoxyl radicals obtained in protonated and deuteriated acids. For the simulation it was assumed that the constants on the two protons of the heterocycle are substantially unequivalent. The ^{13}C and proton hfi constants for the two radicals are listed in Table 2.



Chromenol

Scheme 2

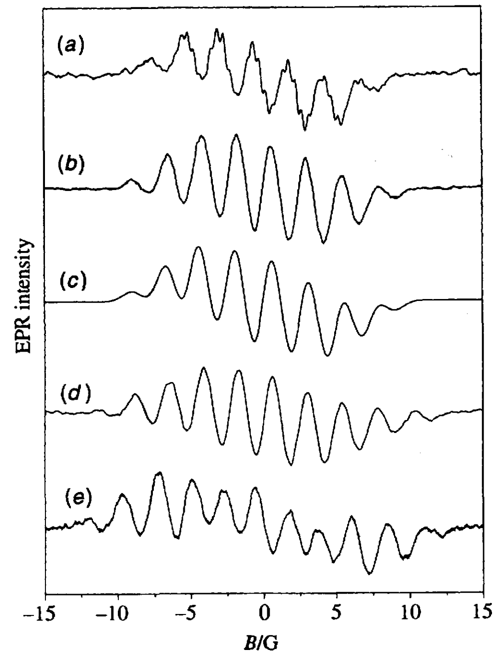


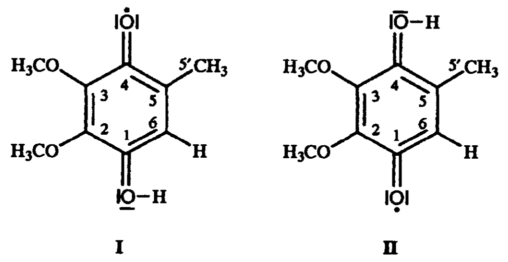
Fig. 4 EPR spectra of the chromenoxyl radical observed after dissolving unlabelled (*a*,*b*), and [1-^{13}C] (*d*), [4-^{13}C] (*e*) labelled UQ-10 in D-triflic acid (*a*) and H-triflic acid (*b*,*d*,*e*) with nitromethane. Computer simulation (*c*) of the spectrum of (*b*) with the data from Table 2 and a linewidth of 0.01 mT.

Table 2 Experimental values of ^{13}C and H hfi (in gauss) for the ubiquinone-0 radical cation and the chromenoxyl radical

	Ubiquinone-0 diprotonated radical cation	Chromenoxyl radical	
C-1	4.7 ± 0.2	C-8a	4.8
C-4	4.0 ± 0.2	C-6	6
Methyl-C	2.6 ± 0.2		2.4
a_{AH}	2.6		2.7
a_{OCH_3}	0.27		< 0.1
	0.8		0.58
a_{CH_3}	4.6		2.3
a_H	0.8		5.2
			1.7

Neutral radicals

From ref. 15, in which detailed studies were made of hydrogen atom abstraction from the solvent by the excited triplet state of methyl-substitued quinones, it is known that the decay of two neutral radical isomers during photolysis leads to the accumulation of one radical in which a hydrogen atom is added to the carbonyl group in the *meta*-position to the methyl substituent. In our previous paper,[4] the EPR spectrum observed during photolysis of [1-^{13}C]-labelled ubiquinone-0 has been interpreted as the superposition of the spectra of two conformers (Scheme 3). In conformer I the proton is bound to the carbonyl that is in the *meta*-position with respect to the methyl group. In conformer II the proton is bound to the carbonyl carbon in the *ortho*-position with respect to the methyl group. The hfi constant of the isotopically labelled carbon, [1-^{13}C], differed substantially for the two conformers. It is expected that also upon ubiquinone photolysis, one of the neutral radical isomers will selectively be accumulated.



I II

Scheme 3

We have studied the formation of neutral radicals in [^{13}C]methyl-labelled UQ-0. Neutral radicals were generated in protonated and deuteriated isopropyl alcohol with acid additive. Studying EPR spectra of radicals in deuteriated solvent considerably simplifies the identification of the radicals formed by photolysis.

Fig. 5 shows the EPR spectra observed under UV irradiation of alcoholic solutions of [^{13}C]methyl-labelled UQ-0. In deuteriated alcohol the appearance of a doublet splitting due to the ^{13}C nucleus of the methyl group is clearly visible. The large value of the hfi constant of the ^{13}C carbon (about 3 G) conclusively shows that the spectrum belongs to conformer I of the neutral radical. The relative intensities of the lines in the spectrum of Fig. 5(*a*) coincides with those obtained theoretically for a spectrum with hf splitting determined by three equivalent protons of the methyl group, leaving no room for a contribution of conformer II to this spectrum.

Calculations

In our previous paper,[4] the experimental data have been interpreted by preliminary quantum-chemical calculations using the AM1 method[16] based on a modified MNDO-85 program.[17] The geometry and distribution of the π-electron spin density for neutral, cation and anion ubiquinone radicals have been obtained by the restricted Hartree-Fock (RHF) technique in a 'half-electron' approximation.[18] It is known that RHF is unsuitable for obtaining a correct picture of the spin density distribution, and it is necessary to take into account additional spin-polarization effects.[19] An alternative procedure, also implemented in the program of ref. 17, has been used here for molecular orbital (MO) calculations with the unrestricted Hartree-Fock (UHF) method.[20]

One of the aims of the MO calculations was the interpretation of a very strong influence of the medium on the hfi in the ubiquinone radical anion (Table 1). We have optimized the geometry, not only of the isolated radicals, but also of their complexes with one or two alcohol (methanol) molecules. From the literature[21,22] it is known that the AM1 method is not very good at reproducing the geometry and energy of the complexes with a hydrogen bond. A later modification (PM3 method) is better suited for describing the geometric parameters of the complexes with a hydrogen bond.[21,23]

The PM3 method has been parametrized so as to reproduce as well as possible the enthalpy of complex formation and the molecular geometry,[21,23] but it is not well suited for calculating the spin density distribution. The INDO

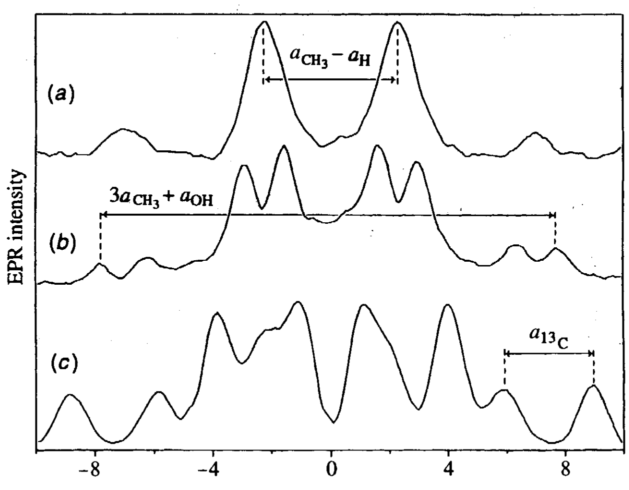


Fig. 5 Integrated EPR spectra observed during photolysis of UQ-0 solutions: (*a*) [$^2H_8$]IPA with [$^2H_5$]acetic acid; (*b*) IPA with acetic acid; (*c*) [^{13}C]methyl-labelled UQ-0 in [$^2H_8$]IPA with [$^2H_8$]acetic acid

method[24] has long been successfully employed[19] for calculating the spin-density distribution. We have therefore applied the INDO (UHF) method to geometries calculated with the PM3 technique for accurately reproducing the hfi constants of the ^{13}C and H nuclei. The calculations have been performed with the program 'Spin Hamiltonian'.[25] In all cases the procedure of quartet component annihilation has been used.[19]

Results of the calculations

Radical anions. The geometric parameters of the optimal structures obtained by the AM1 and PM3 methods, are similar. The methyls of the two methoxy groups of UQ-0 in positions 2 and 3 are above and below the quinone plane: the twist angle is about 70° in the AM1 and about 80° in the PM3 calculations. The values of the hfi constants of ^{13}C calculated for these geometries by the INDO method (UHF) are similar. In this case, reasonable agreement is observed between calculated and experimental values (Table 1). In agreement with experiment, the values of hfi constants with ring atoms [2-^{13}C], [3-^{13}C] and [5-^{13}C] are substantially smaller than those with the [1-^{13}C], [4-^{13}C] and [6-^{13}C] atoms. The hfi with the ^{13}C atoms of the methoxy groups is overestimated when using the above-mentioned twist angles. Agreement with experiment is obtained for a twist angle below 45°, for which the hfi with the methoxy-^{13}C becomes less than 0.1 G. This value of the twist angle was chosen in ref. 1 for the calculations of the UQ-10 anion hfi.

In contrast to the results obtained with the INDO method, the s-spin density distribution and, accordingly the hfi calculated by the PM3 method deviate substantially from experiment (Table 1). This confirms that the INDO method is much better than the PM3 method for calculating the s-spin density distribution.

The substantial decrease of the hf coupling with the [1-^{13}C] and [4-^{13}C] nuclei for the quinone radical anions in alcoholic solution is most probably caused by complexation of the ubiquinone anion with two alcohol molecules. The formation of quite strong (binding energies 15–30 kcal mol^{-1}) hydrogen-bonded ion–molecule complexes has been described in the literature.[26–28]

Table 3 lists the calculated values of the enthalpy of the formation of the complexes with two methanol molecules, and the optimal lengths of the hydrogen bonds. According to the PM3 calculations, the arrangement of the O–H • • • O atoms is almost linear, with the hydrogen bond lying in the ring plane. The lengths of the hydrogen bonds are close to the values calculated for other complexes with *ab initio* methods[22] and to the values experimentally estimated for the ubiquinone-10 anion.[1] Fig. 6 depicts the geometry of the ubiquinone anion complexed with two methanol molecules calculated by the PM3 method. The AM1 method (as mentioned above) is less suitable for calculating hydrogen bonds and leads to a non-linear arrangement of O–H • • • O atoms, and a substantially larger O • • • H distance. Table 1 shows the calculated data for the hfi with the [1-^{13}C] nucleus for the isolated ubiquinone anion and its complex with two methanol molecules. The INDO calculations show a decrease of the hf couplings with [1-^{13}C] and [4-^{13}C] for UQ-0 in methanol compared to HMPT, but the calculated decrease is much less than that experimentally observed.

The situation is complicated by the fact that the actions of the two methanol molecules are oppositely directed, and compensate each other. Therefore, the resulting effect manifests itself as the difference of two comparatively weak actions of hydrogen bond formation. When the complex is formed with one methanol molecule (Table 4), hfi with a carbon atom of a carbonyl group participating in the formation of a hydrogen bond considerably decreases (INDO calculations). For the carbonyl group that does not take part in hydrogen bonding, the hfi with the carbonyl carbon then substantially increases (Table 4). When two hydrogen bonds form, the opposing effects lead to a decrease in hfi with the carbon atoms of both carbonyl groups. Experimentally, the change with hfi with [1-^{13}C] is more important than that with [4-^{13}C]. We conclude that the INDO method accurately gives the distribution of s-spin density in molecules, but accounts insufficiently for the effects of spin-density redistribution resulting from complex formation.

Neutral radicals. The generation of neutral radicals is possible only in proton-donor solvents. Detailed calculations performed by us show that the neutral conformer I radical is thermodynamically more favourable (by 0.9 kcal mol^{-1}) than conformer II. When taking into account the possibility of the formation of a hydrogen bond between the free oxygen and alcohol, the complex of conformer I with alcohol is calculated to be more stable than the complex of conformer II by 0.8 kcal mol^{-1}. Radicals I and II will then form in a ratio of 20% of conformer II to 80% of conformer I. We have also analysed the influence of hydrogen bond formation on the spin density distribution in neutral radicals. As might be expected, the heat of formation of the complex between the neutral radical and alcohol molecules is much lower than that for the corresponding complex with the radical anion. The heat of formation of the complex between methanol and the oxygen of the carbonyl group in the neutral radical is about 3.5 kcal mol^{-1}, somewhat lower than that of complex formation involving the hydrogen atom of the radical OH-group (5.8 kcal mol^{-1}, PM3 calculations). The influence of complex formation on the hfi value is minor and does not exceed 20% in the INDO calculations. Only the hfi with ^{1}H of the hydroxy group increased by 10% when it is involved in hydrogen-bonding.

Table 5 gives the calculated hfi of the methyl ^{13}C for two conformers of the neutral radical. The hfi value for conformer I is negative and closer to the experimental value than that of conformer II, supporting our assignment of the recorded EPR spectrum to conformer I.

Summary and conclusions

Experimental and MO-calculated values of ^{13}C-hfi have been obtained for a variety of ^{13}C-labelled ubiquinone anion, neutral and radical cations. The parameters of the EPR spectra of the

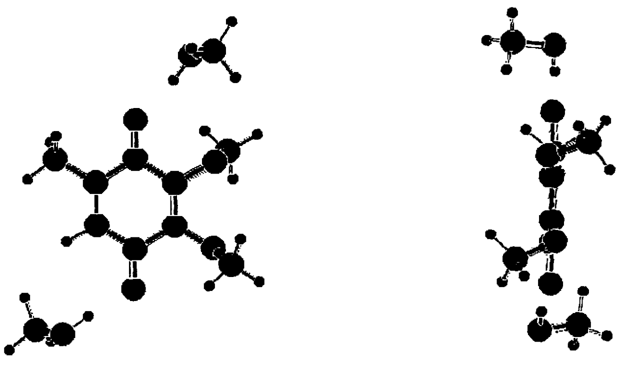


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Fig. 6 Geometry of the ubiquinone anion complexed with two methanol molecules as calculated by the PM3 method [front (1) and side (2) view]

Table 3 Enthalpy of complex formation between UQ-0 radical anion and two methanol molecules (ΔH), and calculated hydrogen bond distances

Method	ΔH/ kcal mol^{-1}	R(O···O)/Å		R(O···H)/Å	
		C^1=O	C^4=O	C^1=O	C^4=O
AM1	−15	2.91	2.91	2.10	2.10
PM3	−18.6	2.73	2.74	1.76	1.79

Table 4 Change of the calculated ^{13}C hfi values (in gauss) upon complexation of the radical anion with one methanol molecule

	INDO method		
		H-bonded anion	
Carbon	Anion	C^1=O···H	C^4=O···H
1	−1.82	−1.2	−2.2
4	−1.64	−2.0	−1.0
6	−0.76	0.3	1.1

Table 5 Experimental and calculated (INDO method) methyl ^{13}C hfi values (in gauss) for two conformers of the neutral ubiquinone radical

Exp	I	II
~3	−1.6	−0.8

radical anions are sensitive to the solvent properties mainly through hydrogen bond formation.

Augmenting a previous report,[4] two semi-empirical MO-schemes have been used to calculate the geometry and spin-density distribution of isolated radicals and of hydrogen-bonded radical–solvent complexes. The calculated hfi values are in good agreement with experiment and qualitatively reflect the influence of hydrogen bond formation on the spin-density distribution.

Comparing the data for UQ-0 and UQ-10, we show that for radical anions, the addition of the isoprene chain changes the values of the hf couplings of the ring carbon atoms. When generating the diprotonated UQ-10 radical cation in the presence of acids, intramolecular cyclization involving the isoprene chain was observed, leading to the formation of the chromenoxyl radical.

The hf couplings of the radical anion of ubiquinone enriched in the [^{13}C]methyl group, which has a poorly resolved EPR spectrum, were successfully determined with ENDOR spectroscopy.

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