An Electron Nuclear Double Resonance and Electron Spin Resonance Study of Semiquinones Related to Vitamins K and E[1a]

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Abstract: The electron nuclear double resonance (endor) spectra of ethanolic solutions of several semiquinones of biological interest have been observed, and splitting constants as well as per cent enhancement measurements have been made. The endor results, which are especially useful in obtaining unequivocal analyses of the more complex esr spectra, also showed greatly increased resolution by resolving small splitting constant differences unobservable in the esr. This latter result was due to a factor of ~5 decrease in the endor as compared to the esr widths. These results were used to evaluate spin densities. MO calculations which were performed were found to be in satisfactory agreement with the "experimental" spin densities, including those at positions of small density. It is found that the introduction of a long side chain in the vitamin semiquinones in place of a methyl group has very little effect on the spin density distribution, including that at the ring carbon to which the side chain is attached. Alternating line width effects were observed in the esr of the semiquinones derived from vitamin K_1 and coenzyme Q-10 (ubisemiquinone, USQ) with associated line width effects in the endor. They are due to rotation of the aromatic ring relative to the attached methylene protons of the side chain. At $-50°$, this motion is sufficiently slow in USQ in dimethoxyethane (DME) that two inequivalent methylene protons are observed in the endor and esr. Comparable activation energies and preexponential factors were found for USQ in DME and ethanol with similar results for vitamin K_1.

The first electron nuclear double resonance (endor) signal of a free radical in solution was obtained by Hyde and Maki[2a] from Coppingers radical. Hyde has since shown[2b] experimentally that endor signals could be obtained from a variety of organic radicals, and a large number of derivatives of triphenylmethyl have been investigated[3-8] in great detail using endor and electron spin resonance (esr) techniques. Meanwhile, a detailed analysis of the theory of endor in liquids has been developed by Freed and coworkers.[9-11]

We have had a twofold interest in the investigation of semiquinone anions using endor. First, we wished to use endor studies on those semiquinones yielding simple and well-resolved spectra[12-26] to compare with the theoretical predictions of endor spectra in an effort to understand the relevant spin–relaxation phenomena. In addition, we wished to apply the endor technique to study semiquinones with complex spectra, in particular those of biological interest. We hoped to take advantage of this technique to resolve out splitting constants, especially in those cases where the esr spectra are not amenable to an unequivocal interpretation.

We have restricted our attention to the quinones of the vitamin K group, ubiquinone, and α-tocopherol quinone (vitamin E) in the present investigation. Identification of vitamins K with the blood coagulation mechanism is well established and the importance of vitamins K, ubiquinone, and vitamin E in biological electron transport and oxidative phosphorylation has been well recognized in recent years.[27] In addition, there is considerable interest in determining the specific role of these quinones in biological reactions. Detailed information on the molecular electronic structure of these quinones and the geometry of the molecules should be of use in furthering this goal and the present investigation had this in mind.

Several investigators have conducted esr studies of a variety of vitamin semiquinones.[15,17,28-32] Because, in the earlier work there was the problem of highly overlapped esr spectra, not much reliable detailed information could be obtained. Although the more re-

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cent work of Fritsch, *et al.*,[32] displayed considerable spectral resolution, as a consequence of the complex nature of the esr spectra, it was not possible to assign splitting constants to the esr spectrum of even the simplest vitamin quinone, *viz.*, menadione (see Figure 1) without relying heavily on computer simulations of experimental spectra. Furthermore, as our work reported here shows, all the inequivalent splittings of the vitamin semiquinones in ethanol solvent cannot be obtained by this procedure.

We have been able to obtain strong and well-resolved esr spectra from a number of vitamin semiquinones in ethanolic solvent by modified methods of generating the free radicals, and this included the semiquinone anion of ubiquinone (coenzyme Q-10), for the first time. Further, by using endor techniques we have been able to obtain accurate and unequivocal splitting constants from all the vitamin semiquinones that were investigated. In some cases, as will be discussed later, it was also possible to distinguish splitting constants that were so close to each other that the difference could not even be suspected from the esr results alone. Thus, it is our objective to show that accurate, detailed, and convincing information can be obtained on complex but highly interesting radicals, such as biologically important semiquinones, using a combination of endor and esr techniques together with modified methods of their generation. In this investigation we have restricted our attention to studying semiquinones in ethanol, rather than aqueous solutions which are more characteristic of *in vivo* biological reactions, because of the instability of the radical anions in water. However, little difference is expected in the characteristics of the radicals in these two solvents especially as compared to solvents like acetonitrile, dimethoxyethane (DME), dimethyl sulfoxide (DMSO), or dimethylformamide (DMF), the other common solvents that have been used in the investigation of semiquinones.[32] This is evidenced in the very similar spin-density distributions of semiquinones in aqueous and ethanolic solvents compared to the somewhat different values obtained in these other solvents.[15,20,23,30]

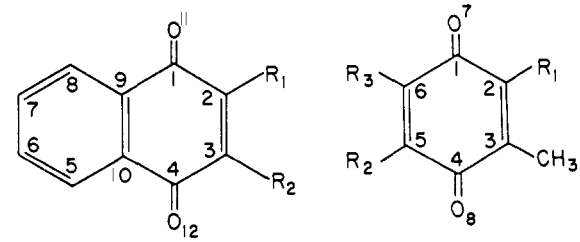
Our results on the detailed relaxation investigations on *p*-benzo- and durosemiquinones will be given elsewhere.[33]

Experimental Section

We have employed a Varian endor accessory and a Varian V-4502-14 esr spectrometer in the present studies. The details of experimental techniques employed are discussed elsewhere.[2,33] The temperature of the sample under investigation was maintained at the desired value using a Varian V-4540 temperature control unit.

1,4-Naphthoquinone was obtained from Eastman Organic Chemicals. Duroquinone used in the present experiments was from Aldrich Chemical Co., Inc. and menadione and α-tocopherol quinone from City Chemical Corporation. Phytonodione and ubiquinone were obtained from Mann Research Laboratories and were used without further purification. 2,3-Dimethyl-1,4-naphthoquinone was synthesized from 2,3-dimethylnaphthalene (Aldrich) according to the method of Smith and Webster.[34]

Most of the endor signals were obtained from ethanolic solutions of the semiquinones. The samples were made by first reducing the parent quinones in DME[24-26] by potassium metal *in vacuo* using



(a) (b)

Figure 1. (a) Naphthoquinone and related vitamin quinones: (i) $R_1 = R_2 = H$, 1,4-naphthosemiquinone; (ii) $R_1 = CH_3$, $R_2 = H$, menadione (vitamin K_3); (iii) $R_1 = R_2 = CH_3$, 2,3-dimethyl-1,4-naphthosemiquinone; (iv) $R_1 = CH_3$, $R_2 = -CH_2CH{=}C(CH_3)-(CH_2CH_2CH_2CH(CH_3))_3CH_3$, 2-methyl-3-phytyl-1,4-naphthosemiquinone (vitamin K_1). (b) Duroquinone and other biological important quinones: (i) $R_1 = R_2 = R_3 = CH_3$, duroquinone; (ii) $R_2 = R_3 = CH_3$, $R_1 = CH_2CH_2C(OH)(CH_3)(CH_2CH_2CH_2CH-(CH_3))_3CH_3$, α-tocopherol quinone (vitamin E quinone); (iii) $R_2 = R_3 = OCH_3$, $R_1 = (CH_2CH{=}C(CH_3)CH_2)_{10}H$, ubiquinone.

well-known techniques.[35-37] After allowing the quinone in DME to react with the potassium mirror for 12–18 hr at room temperature, the DME solution was removed into a different compartment of the sample tube. The solvent was distilled back into the compartment containing the unreacted potassium by cooling this portion under liquid nitrogen. This portion was then sealed off and removed. The dry residue left behind from the DME solution was dissolved in absolute ethanol, which was stored under vacuum in a third limb of the sample tube. The semiquinone samples made using this procedure are stable for several weeks if they are stored in the freezer compartment of the refrigerator. Using the present procedure, the semiquinones can be obtained in any desired solvent by storing the required solvent in place of ethanol in the beginning of the experiment.

Ubisemiquinone and the semiquinone derived from vitamin K_1 were generated electrolytically also using methods described elsewhere.[21]

The present endor experiments were conducted at a magnetic field of about 3250 G, which corresponds to a precession frequency of 13.84 MHz for the free proton. The spectra were usually obtained by monitoring the endor effects on the most intense line of the saturated esr spectrum. We have examined the endor lines in the frequency range 8–22 MHz. In the present experiments, the largest proton splitting (from naphthosemiquinone, NSQ) has a value of 3.23 G, so we were in a position to observe all the pairs of endor lines occurring on either side of the free proton precession frequency. The spacing between each pair of line centers was measured accurately to obtain the splitting constant. (This procedure eliminates any error arising from the second-order frequency shifts,[7] which of course is very small for the radicals we studied.) In Figures 2, 4, 5, 8, 10, 12, and 14 we have shown only the endor lines on the high-frequency side of the true proton precession frequency. The low-frequency lines are, indeed, similar to those shown in these figures but are smaller in amplitude.

Most of the endor experiments described below have been performed at −50°, as this was found to be the optimum temperature[10,11] for several of the semiquinones we have investigated. However, results obtained at different temperatures are reported for ubisemiquinone and vitamin K_1 semiquinone.

Results

1. Naphthosemiquinone (NSQ). The esr spectrum of NSQ, which serves as the model system for the vitamin K quinones, was examined by earlier investigators and accurate values of splitting constants were also obtained[18,20] from its simple esr spectrum. We show in Figure 2 the endor spectrum corresponding to the two small splittings a_5^H and a_6^H, which differ by only

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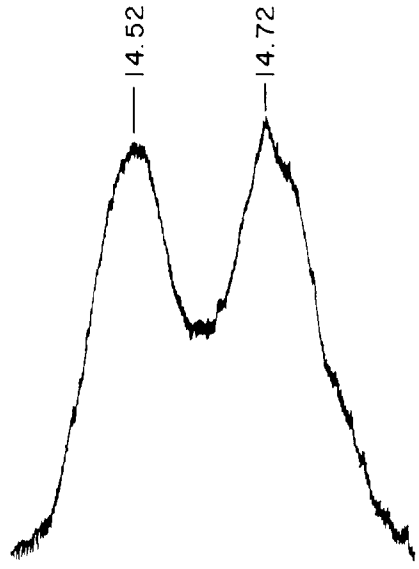

Figure 2. Endor spectrum from protons in the 5 and 8 (left) and 6 and 7 (right) positions in NSQ (see text).

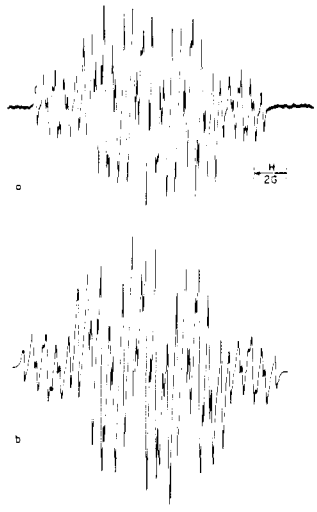

Figure 3. (a) First-derivative esr spectrum from vitamin K_3 in ethanol. (b) Computer simulation of the esr spectrum in Figure 3a using splitting constants obtained with the help of endor data.

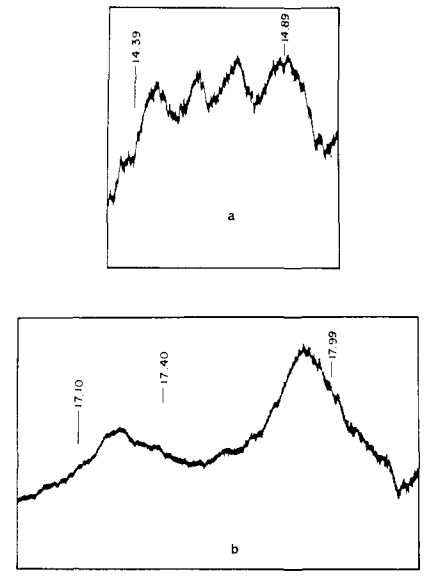

Figure 4. (a) Endor spectrum corresponding to the protons in positions 5, 6, 7, and 8 in vitamin K_3. (b) Endor spectrum corresponding to the ring proton at position 3 (left) and the methyl proton (right) in vitamin K_3.

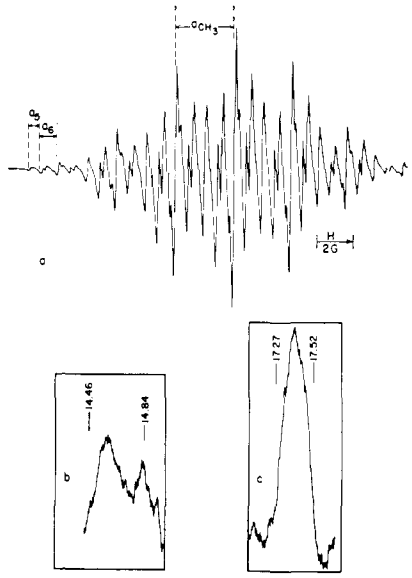

Figure 5. (a) First-derivative esr spectrum of 2,3-DMNSQ in ethanol. (b) Endor lines corresponding to protons at positions 5 and 8 (left) and 6 and 7 (right) in 2,3-DMNSQ. (c) Endor lines corresponding to the methyl protons at positions 2 and 3 in 2,3-DMNSQ.

142 mG, to demonstrate how this is easily distinguished in an endor spectrum.

2. Vitamin K_3 (Menadione). We have obtained improved resolution of the esr spectrum of menadione in ethanol, as compared to earlier studies.[15,17,28-32] Our results are shown in Figure 3a. Except for the three protons in the methyl group, none of the other protons are symmetrically equivalent and one expects as many as six different splittings from this molecule. However, from the esr spectrum, at best only four splitting constants could be discerned. This would normally be interpreted as an indistinguishable difference between protons from positions 5 and 8, and between those from 6 and 7.

The endor spectrum from the same sample as the one giving rise to Figure 3a is shown in Figures 4a and b. The line at the extreme right in Figure 4b corresponds to the methyl protons, and the one next to it arises from the proton at position 3. The four-finger pattern in Figure 4a arises from the four inequivalent protons at the 5, 6, 7, and 8 positions in the ring. Figure 3b shows the computer simulation of the esr spectrum using the splitting constants obtained with the help of the endor spectrum, and it can be noticed that the agreement between the computed and experimental spectra is excellent.

3. 2,3-Dimethyl-1,4-naphthoquinone. Low-resolution spectra for the semiquinone arising from 2,3-methyl-1,4-naphthoquinone (DMNSQ) have been reported by Wertz and Vivo[29] and also by Adams, Blois, and Sands.[15] The esr spectrum from DMNSQ obtained in the present experiments is shown in Figure 5a. Unlike the earlier studies, splitting constants from the three different groups of protons may be assigned from

Table I. Proton Hyperfine Splittings in the Esr Spectra of Naphthosemiquinone and Vitamin K Semiquinones

Compound	Solvent	Hfs at different proton positions, G					
		2	3	5	6	7	8
NSQ	EtOH[a]	3.32	3.23	0.513	0.655	0.655	0.513
K_3, menadione	EtOH[b]	2.911[c]	2.467	0.480	0.780	0.560	0.700
	EtOH–H_2O[d]	3.01[c]	2.38	0.64	0.64	0.64	0.64
2,3-DMNSQ	EtOH[b]	2.573[c]	2.573[c]	0.561	0.740	0.740	0.561
K_1, phytonodione	EtOH[b]	2.541	1.269	0.521	0.698	0.698	0.521
	Alkaline EtOH–H_2O[d]	2.2	1.1	0.55	0.55	0.55	0.55

[a] From ref 19. [b] Present results. [c] Methyl proton splitting. [d] From ref 15.

this work. The endor spectrum corresponding to these splittings is shown in Figures 5b and c and the splitting constants are given in Table I. Figure 5c corresponds to the endor from methyl protons and 5b corresponds to the splittings from the unsubstituted ring.

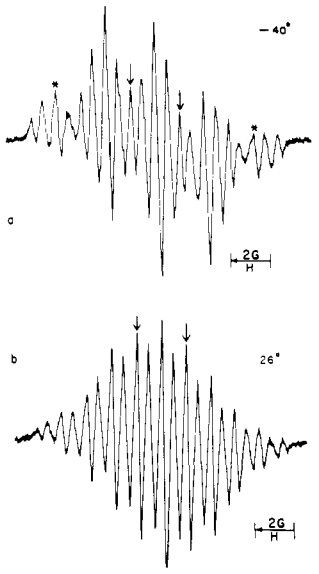

Figure 6. (a) First-derivative esr spectrum of vitamin K_1 in ethanol at −40°. The lines indicated by arrows correspond to $M_{CH_3} = \pm 1/2$, $M_{CH_2} = 0$, $M_{H_{5-8}} = 0$ (see text). The lines indicated with asterisks correspond to (left) $M_{CH_3} = 3/2$, $M_{CH_2} = 1$, $M_{H_{5-8}} = 0$, and (right) $M_{CH_3} = -3/2$, $M_{CH_2} = -1$, $M_{H_{5-8}} = 0$. (b) First-derivative esr spectrum of vitamin K_1 at 26°. The lines indicated by arrows correspond to the same lines indicated by arrows in Figure 6a. These lines have sharpened at the higher temperature.

4. Vitamin K_1 (Phytonodione). Figures 6a and b show the esr spectra obtained from vitamin K_1 in ethanol at −40 and 26°. There are clearly significant differences between these two spectra. We are able to attribute this to a significant alternating line width effect at the lower temperature (discussed later). Figure 7 shows the computer simulation for the esr spectrum, at −40° using the splitting constants reported in Table I and using widths of 0.300 G for lines with $M_{CH_2} = \pm 1$ and 0.500 G for lines with $M_{CH_2} = 0$ (see following discussion). It may be noted that the amplitudes of the lines shown with arrows in Figures 6a and b are very sensitive to the widths used for simulation. The splitting constants have been obtained with the help of the endor data, and the endor spectrum is displayed in Figure 8.

It could be noticed that the signal-to-noise ratio in this endor spectrum is quite low. The endor line arising from the methylene protons (see later text for assignments) is unusually broad, and of very low amplitude;

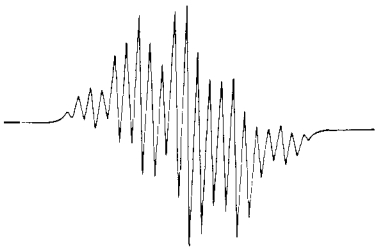

Figure 7. Computer simulation of the esr spectrum of vitamin K_1 in ethanol (see text).

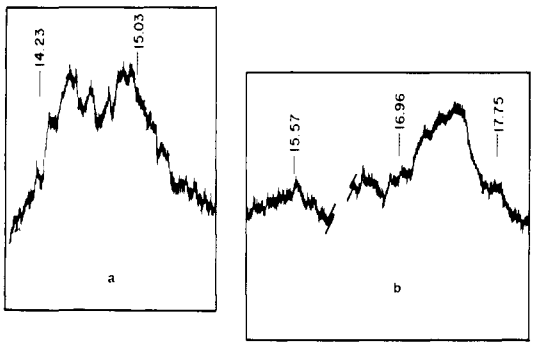

Figure 8. (a) Endor lines corresponding to protons in the unsubstituted ring in vitamin K_1. (b) Endor line corresponding to CH_2 protons in vitamin K_1 (left, see text) and endor line corresponding to the methyl protons from position 2 in vitamin K_1 (right).

thus it is barely discernible above the noise level. The reasons for this are discussed later. We have tried to vary the radical concentration and the temperature of the experiment in order to improve the overall signal-to-noise ratio for this spectrum, but without success.[38]

5. Duroquinone and α-Tocopherol Quinone (Vitamin E). Durosemiquinone in ethanol gives a simple and strong endor signal consisting of one pair of lines corresponding to a single splitting of 1.900 G. The details of the spectrum and the quantitative relaxation measurements made on the endor of this radical will be published elsewhere.[33] Figures 9a and b, and 10 show the esr spectrum, the computer-simulated esr spectrum, and the

(38) We have also produced the radical anion electrolytically in ethanol and also in DME using tetra-*n*-butylammonium perchlorate as supporting electrolyte. The ethanol spectrum was identical with the ones shown in Figure 6. The signal-to-noise ratio in the endor spectrum was not improved by using the electrolytic sample in ethanol, while the endor signal from the DME solution was very weak.

Table II. Proton Hyperfine Splittings in the Esr Spectra of Durosemiquinone, α-Tocopherol Semiquinone, and Ubisemiquinone (Coenzyme Q-10)

Compound	Solvent	Splittings, G						
		a_2^H	a_3^H	a_5^H	a_6^H	a_1^C	a_2^C	a_{Me}^C
DSQ[a]	EtOH	1.900	1.900	1.900	1.900	1.067	−0.721	±1.377
Vitamin E[b]	EtOH	0.910	1.905	1.905	1.905			
USQ[b,c]	EtOH	1.018	2.037					
USQ[b,c]	DME[d]	1.044	2.089					
USQ[b,c]	DME[e]	1.342[f]	2.089					
		0.736[f]						

[a] From ref 48. [b] Present work. [c] No splittings have been observed from the methoxy protons. [d] Splittings constants at 40°. [e] Splittings constants at −50°. [f] Inequivalent methylene proton splitting.

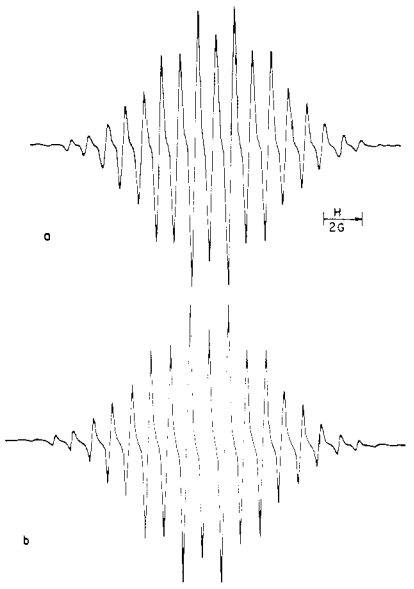

Figure 9. (a) First-derivative esr spectrum of vitamin E at 25°. (b) Computer simulation of the spectrum in Figure 9a using splitting constants reported in Table II.

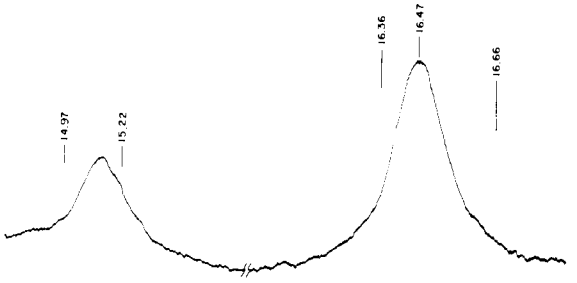

Figure 10. Endor lines corresponding to the methylene (left) and methyl (right) proton in vitamin E.

endor spectrum from α-tocopherol semiquinone. The splittings are assigned as shown in Table II.

6. Ubiquinone (Coenzyme Q-10). The esr spectrum obtained from ubisemiquinone (USQ) in ethanol (see Figure 1) is shown in Figures 11a and b. The spectrum shows considerable change in its appearance with temperature, as can be seen from the results obtained at −20° and +40°. At −50°, in addition to the alternating line width effect (as seen in the −20° spectrum) there is a decrease in the intensity of the spectrum. At still lower temperatures it becomes difficult to obtain an esr signal. The radical precipitates from solution as a result of cooling below −50°. Further the esr lines are broader as the temperature is decreased. Ubisemiquinone in ethanol exhibited a very strong endor signal

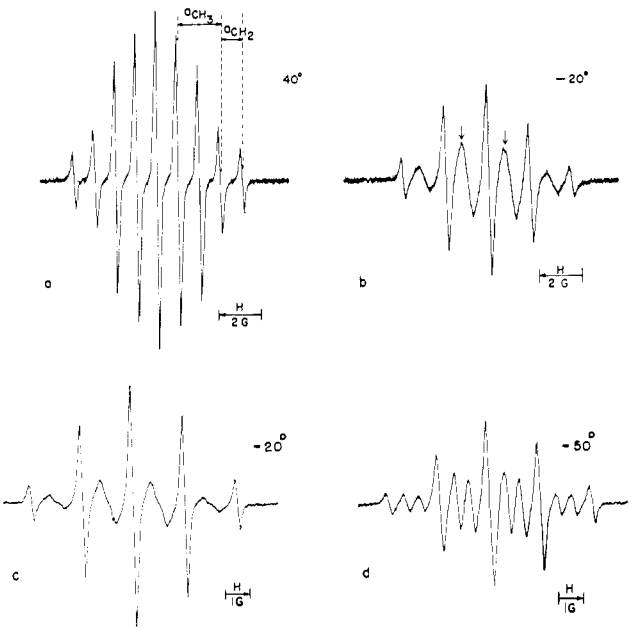

Figure 11. (a) First-derivative esr spectrum of USQ in ethanol at +40°. (b) First-derivative esr spectrum of USQ in ethanol at −20°. The lines indicated by arrows correspond to $M_{CH_3} = \pm 1/2$, $M_{CH_2} = 0$. (c) First-derivative esr spectrum of USQ in DME at −20°. (d) First-derivative esr spectrum of USQ in DME at −50°.

and it is shown in Figure 12a (see text and Table II for assignments).

Ubisemiquinone was also generated using electrolysis in DME. At +40° the appearance of the esr spectrum is the same as that obtained from the ethanolic sample (Figure 11a). The spectrum obtained at −20° is shown in Figure 11c. However, on cooling the DME sample to −50° (unlike results with the ethanol sample) a well-resolved spectrum is obtained, and it is shown in Figure 11d. The endor spectra from USQ in DME between −43 to −50° are shown in Figures 12b–d. At higher temperatures (*ca.* −15°) the endor spectrum from the DME sample has the same appearance as the spectrum from ethanol shown in Figure 12a.

7. Percentage Endor Enhancements and Widths. Percentage endor enhancement is defined by

$$\% \text{ enhancement} = [(Z''_{endor} - Z''_{esr})/Z''_{esr}]100 \quad (1)$$

where Z''_{esr} is the esr signal amplitude in the absence of double resonance, while Z''_{endor} is the esr signal amplitude in the presence of double resonance.[9-11] Measurements of per cent enhancements for the different semiquinones were made at −50° for all compounds investigated, except for USQ at −20°. The results for

the methyl lines in vitamin K_3, 2,3-DMNSQ, vitamin K_1, and vitamin E semiquinones were all in the range 2–4% with an experimental precision of about 40%. The value obtained for USQ was somewhat higher, about 6–8%, which is similar to the value for DSQ. These results imply that for most of the vitamin semiquinones the spin–relaxation mechanisms which determine the per cent enhancements must be quite similar. While this is of interest in terms of understanding relevant relaxation mechanisms, we have not found per cent enhancement measurements very useful for analytical purposes, especially in view of the large errors in their measurements by current techniques. This result of similar per cent enhancements might at first appear to contradict the fact that for vitamin K_1 the endor signal-to-noise ratio (S/N) was quite poor while vitamin E, for example, exhibited a very good S/N. We note, however, that S/N is directly related to the *absolute* endor enhancement ($Z''_{endor} - Z''_{esr}$), and this is directly proportional to the actual intensity of the original esr signal (Z''_{esr}). The main difference between vitamins K_1 and E is that the former has many more hyperfine lines. Thus, when a particular hyperfine line is being saturated in an endor experiment, a significantly smaller fraction of the spin systems is involved; hence the S/N must necessarily be smaller.

We have also observed endor signals from electrolytically generated semiquinones in DME. However, these signals are weak compared to the ones obtained in ethanol. We found it still more difficult to obtain endor signals from semiquinones generated by alkali metal reduction in DME. The system K^+DSQ^- in DME did give a signal at $-70°$ corresponding to two different methyl proton splittings from the ion-paired species[24, 25] (2.41 and 1.43 G). However, the per cent enhancements in this case is only 2–3% as compared to 8% in ethanol[33] and 4–6% in electrolytically generated DSQ in DME. The per cent enhancement of K^+DSQ^- in DME is very sensitive to temperature, the optimum being about $-70°$, At $-60°$, although the signals are weak, we have observed the lines corresponding to the two inequivalent methyl groups from the tight ion pair, and also a broad line corresponding to a loose ion pair[24,25] ($a^H = 1.91$ G). Our more detailed analysis of spin–relaxation effects is consistent with the existence of less favorable spin–relaxation characteristics for endor enhancements for the DME solutions as compared to the ethanol solutions.[39] The reason for the difference between the electrolytic and alkali metal reduced samples is currently being explored.

We note that the signals obtained from the same compounds in ethanolic and DME solutions did not show any measurable difference in the widths of their endor lines. However, while the methyl proton endor in vitamin E had the same width as the methyl lines, the methylene endor for USQ exhibited variation in widths corresponding to the width variation arising from the alternating line width effect in the esr spectrum (see later text).

(39) For most free radicals optimum enhancement is determined by the magnitude of the electron–nuclear dipolar (END) interation.[9-11] At any given temperature its value is directly proportional to the solvent viscosity, and since ethanol is more viscous than DME, we expect greater enhancements in ethanol. Fluorine-containing compounds are an exception because their END interaction is too large—here DME would be the better choice.

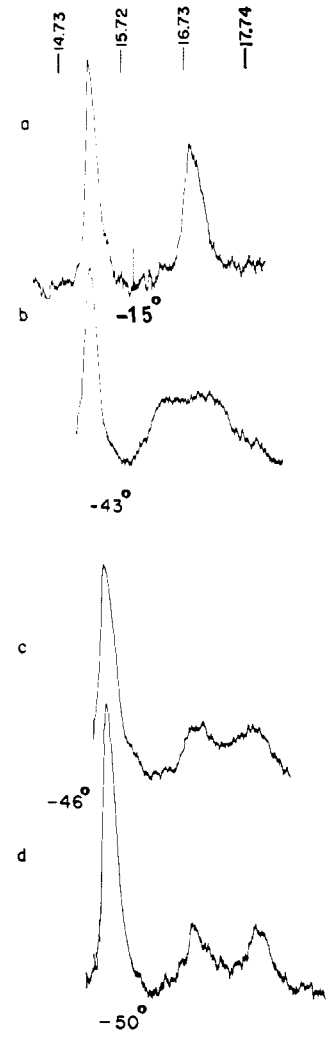

Figure 12. (a) Endor spectrum of USQ in ethanol at $-20°$. The line on the left corresponds to the methyl protons and the one on the right corresponds to the methylene protons. (b) Endor spectrum of USQ in DME at $-43°$. (c) Endor spectrum of USQ in DME at $-46°$. (d) Endor spectrum of USQ in DME at $-50°$.

The esr and endor widths of the different samples we have investigated are given in Table III. The ratio of the esr to endor widths is of the order of five for all the samples. The significantly narrower endor widths are responsible for the greater resolution of slightly different splitting constants (*e.g.*, vitamin K_3) in this work.

The endor and esr widths reported in Table III are not the minimum widths for the radicals studied. Both widths could be reduced by using more dilute solutions and the endor width should be more significantly affected.[40] The endor width would also be reduced by using lower rf powers.[41] Unfortunately these conditions also significantly reduce the S/N, so we did not make any attempt to obtain the minimum endor widths.

(40) It was found that, for samples of the order of 10^{-3} *M*, Heisenberg exchange made a significant contribution to the line widths of both the esr and endor spectra. Working with more dilute solutions narrows the lines, but also decreases the endor signal intensity. Since the endor line widths contribute information concerning spin relaxation processes,[33] not interpretation of esr spectra, it was decided to forego narrow lines and obtain maximum signals.

(41) The endor line width is dependent upon the degree of saturation of the nuclear spin system by the rf field. Because of saturation broadening, it is necessary to extrapolate to zero rf power in order to obtain the true nuclear (endor) line widths.

Table III. Esr and Endor Widths in Semiquinones in Ethanol

Compound	T_2^{-1} (esr)[a] × 10^6 sec^{-1}	T_2^{-1} (endor)[b,c] × 10^5 sec^{-1}	Esr width/endor width
NSQ	2.4	5.0	4.8
Vitamin K_3[d]	2.1	3.8	5.5
Vitamin K_3[e]	2.3	5.5	4.2
2,3-DMNSQ	2.4	5.5	4.4
Vitamin K_1	4.6	7.9	5.9
DSQ	2.2	3.8	5.8
Vitamin E	2.3	4.7	4.9
USQ[f]	2.6	5.0	5.2
USQ[g]	8.4	10.7	7.8

[a] Calculated from peak-to-peak derivative width. [b] Calculated from width at half-maximum intensity. [c] The widths reported are not the minimum widths attainable. In many cases by using lower rf powers and making use of more dilute samples it is possible to obtain smaller widths. [d] Concentration 2×10^{-4} M, see footnote c. [e] Concentration 1×10^{-3} M, see footnote c. [f] Methyl proton endor line at $-20°$ (see text). [g] Methylene proton endor line at $-20°$ (see text).

Assignments of Splitting Constants

In assigning the splitting constants in the esr spectra of the vitamin semiquinones we make use of endor results, esr results, and MO calculations. Although endor results give accurate splitting constants, the relative intensities of the endor lines do not necessarily bear a simple relationship to the relative numbers of protons they represent. This is believed to be largely due to the strong dependence of endor enhancements on the spin–relaxation processes which can differ for the different inequivalent protons. However, over small frequency ranges it appears from our results on the semiquinones that a comparison of the areas under unoverlapped endor lines can sometimes yield a fairly good estimate of the relative number of equivalent protons giving rise to the endor lines (see below, *e.g.*, the assignment for vitamin E, vitamin K_3, and USQ).

With the exception of vitamins K_3 and K_1, it was possible to assign all the splitting constants from just the experimental results. For vitamins K_3 and K_1, assignment of the proton splitting to specific positions can be made only with the help of MO results and we will take this up later.

In the case of vitamin E the esr spectra could easily be explained in terms of two splittings, one arising from nine equivalent methyl protons and another from two equivalent β methylene protons of the side chain. The endor results with their much greater resolution, by yielding only two splittings, demonstrate even more clearly the equivalence of all the methyl protons. It should be noticed also that the ratio of the areas under the two different endor peaks is $\approx 1:4$ or close to the statistical ratio of 1:4.5. The analysis of the USQ esr spectra in terms of considerable accidental degeneracy, is easily made with confidence utilizing the endor results. The spectra at the higher temperatures are interpreted in terms of one splitting from the methyl protons and another from the β methylene protons of the side chain at position 2. In Figure 10, the areas under the peaks corresponding to the two splittings are in the ratio 3:2 in agreement with the statistical ratio. We do not observe any splittings from the methoxy protons from positions 5 and 6.

We interpret the alternating line width effect seen in the spectra of USQ at intermediate temperatures as arising from a hindered rotation around the bond connecting the β carbon atom of the alkyl side chain and the aromatic ring. As expected,[42] it is the lines corresponding to $M_H = 0$ for two nuclei with spins $1/2$ (the methylene protons) that are broadened, (*cf.* Figures 11a and b). Note also that the endor line from the methylene protons is significantly broader than the one from the methyl protons. When the DME samples of USQ are cooled to $-50°$, the rotatory motion of the side chain is slowed sufficiently that esr and endor spectra corresponding to the slow-motion limit are obtained. In fact, the dramatic difference in the nature of the simple endor spectra (see Figures 12a–d) corresponding to all the situations described above demonstrates how the endor technique can be used as a powerful analytical tool in faithfully following such intramolecular processes.

For vitamins K_3 and K_1 the splittings shown in Table I have been assigned with the help of endor spectra and the MO calculations to be discussed in the following section. The 3:2 ratio of the areas for the peaks corresponding to the methyl and methylene protons could be seen in the endor spectra from vitamin K_3. We note that the interpretation of the alternating line width for vitamin K_1 is entirely equivalent to that for USQ. It is the $M_H = 0$ methylene lines which are broadened. The principal lines with $M_H = 0$ (methylene) and $M_H = \pm 1/2$ (methyl) are indicated with arrows in Figure 6, and one may note how they sharpen at the higher temperature.

Molecular Orbital Calculations

It was of interest to see how our results could be rationalized in terms of MO calculations. Since those calculations performed in the earlier work on these molecules[17,31,32] were based on significantly cruder experimental results, they were not detailed enough for comparison with our experimental data. Further, the procedure adopted for treating an alkyl side chain by Fritsch, *et al.*,[32] was also found to be unjustified from our results.

We have carried out MO calculations employing the McLachlan perturbation correction[43] to the Hückel LCAO–MO method, using a value of 1.20 for λ. For the methyl group, the inductive model of Lazdins and Karplus[44] was employed and the same value of the parameters as for the methyl group in toluene[44] was used.

In order to obtain reasonable values for spin densities in the vitamin K quinones we have used the following procedure. Naphthosemiquinone was taken as the model compound for vitamins K_1 and K_3, and MO calculations were done for NSQ in which the Coulomb integral parameter for the oxygen, δ_O, and the resonance integral parameter for the carbonyl group, γ_{CO}, were varied over a wide range (δ_O from 0.5 to 2.0 and γ_{CO} from 0.5 to 1.8) using an interval of 0.01 unit. The best fit was obtained using a value of $\delta_O = 1.20$ and $\gamma_{CO} = 1.30$. The "experimental" spin densities using a value of $Q_{CH}^H = -27$ G for ring protons in the McConnell

(42) J. H. Freed and G. K. Fraenkel, *J. Chem. Phys.*, **39**, 326 (1963).
(43) A. D. McLachlan, *Mol. Phys.*, **3**, 233 (1960).
(44) D. Lazdins and M. Karplus, *J. Amer. Chem. Soc.*, **87**, 920 (1965).

Table IV. Spin Density Distribution in Naphthosemiquinone and Vitamin K Semiquinones[a]

Atom position	NSQ		Vitamin K_3		2,3-DMNSQ		Vitamin K_1	
	Exptl[b]	Calcd	Exptl[b]	Calcd	Exptl[b]	Calcd	Exptl[b]	Calcd
2	0.1196	0.1221	0.1386[c]	0.1324[c]	0.1224[c]	0.1034[c]	0.0605[c]	0.0614[c]
3	0.1196	0.1221	0.0914	0.0914	0.1224[c]	0.1034[c]	0.1210	0.1205
5	0.0190	0.0215	0.0178	0.0190	0.0208	0.0216	0.0220	0.0257
6	0.0242	0.0250	0.0289	0.0290	0.0274	0.0279	0.0263	0.0260
7	0.0242	0.0250	0.0207	0.0239	0.0274	0.0279	0.0289	0.0342
8	0.0190	0.0215	0.0259	0.0241	0.0208	0.0216	0.0200	0.0174

[a] See text for MO parameters used in the calculation. [b] Experimental spin densities obtained using McConnell's relationship with $Q_{CH}^{H} = -27$ G and $Q_{ME}^{H} = 21$ G (see text). [c] Methyl position.

equation,[45] and the computed spin densities using the

$$a_{CH}^{H} = Q_{CH}^{H}\rho_{C}^{\pi} \quad (2)$$

above parameters are shown in Table IV. It may be noticed that the agreement obtained between experimental and calculated spin densities in NSQ by Gendell, Freed, and Fraenkel[19] using the McLachlan method is also excellent. However, in that work a value of $Q_{CH}^{H} = 23.7$ G was employed. It has been observed in later experimental work[21,23,26,46-48] that in aromatic hydrocarbon and semiquinones it is preferable to use a value of $Q_{CH}^{H} \approx -27$ G as we have done in the present study.

For vitamin K_3 and 2,3-DMNSQ the carbonyl group parameters were taken as the best NSQ parameters, and the Lazdins–Karplus[41] parameters were employed for the methyl group. The agreement between the theoretical and experimental spin densities is excellent for vitamin K_3 and also quite good for 2,3-DMNSQ as seen in Table IV. This is especially true for the small spin densities in the unsubstituted ring. In obtaining the experimental spin density at the carbon atom attached to a methyl group we have employed a value of $Q_{CH_3}^{H} = 20$ G. Although Sullivan and Bolton[47] have used a value of 27 G based probably on the fact that the methyl proton splitting in ethyl radical ≈ 27 G,[49] a lower value, near 20 G, is suggested by Barton and Fraenkel,[50] who have analyzed experimental results from a large number of methyl-substituted pyrazines and also semiquinones. As can be seen from Table I, there are not very significant differences between the splitting constants in 2,3-DMNSQ and vitamin K_1 and no separate MO calculation was done for this molecule.

The methylene proton splitting in vitamins K_1 and E and USQ are discussed separately in the following section. However, we note at this stage that the use of a larger value for the Coulomb integral parameter for C_3 by Fritsch, *et al.*,[32] taking care of the inductive effect by a large side chain, is not justified, since the smaller splitting from the methylene protons can be shown to be simply due to effects of orientation.

We adopted a similar procedure to the above for studying DSQ and its related derivatives, vitamin E semiquinone and USQ. In NSQ and its derivatives there are several different inequivalent proton positions which could be used for comparisons with calculated spin densities. For DSQ, on the other hand, there is only one proton splitting. Thus, one can expect that several combinations of δ_O and γ_{CO} parameters in the McLachlan framework can give a good fit. As a consequence, even a sophisticated calculation on this molecule, such as the one by Claxton and McWilliams[48] designed to analyze solvent effects, does not provide a correct answer so long as the comparison is so restricted. It is also known[21,23] that such a restriction can lead to erroneous results because in the semiquinone system, the major perturbation by the solvent takes place in the vicinity of the carbonyl group, and the spin density variations on other carbon atoms are not very sensitive to those interactions.[21,23] We therefore made use of the ^{13}C splittings.

When an electrolytically reduced sample of DSQ in ethanol was used with tetra-*n*-butylammonium perchlorate as supporting electrolyte, strong signals with well-resolved ^{13}C satellites were observed.[51] It was also possible to assign the signs of the ^{13}C splittings using the well-known procedure of observing the variations in line widths between the high-field and low-field ^{13}C components arising from a given splitting.[21,26,52-54] The ^{13}C splittings of DSQ in ethanol are $a_1^{C} = 1.067 \pm 0.007$, $a_2^{C} = 0.721 \pm 0.005$, and $a_{CH_3}^{C} = 1.377 \pm 0.005$ G. Using the theory of Karplus and Fraenkel,[55] we are now in a position to evaluate experimentally the spin densities in DSQ. However, among the three ^{13}C splittings available, the only one that can be easily treated is a_1^{C}. For methyl ^{13}C splittings a proper treatment has not yet been developed. Analysis of a_2^{C} involves the use of parameters $Q_{C-CH_3}^{C}$ and $Q_{(CH_3)-C}^{C}$ in the general Karplus–Fraenkel equation.[55] These values involving a methyl carbon are not available from either theoretical calculations or experimental evaluations. However, we can make use of the value of a_1^{C} using the following procedure. The spin density at position 2 can be obtained using eq 1, by making use of $Q_{C-CH_3}^{H}$ in place of Q_{CH}^{H}, while a_1^{C} can be expressed in terms of the Karplus–Fraenkel equation, which upon using the values of the carbonyl ^{13}C parameters obtained by Das and Fraenkel[21] becomes

$$a_1^{C} = 33.8\rho_1^{\pi} - 27.8\rho_2^{\pi} - 27.1\rho_7^{\pi} \quad (3)$$

In addition, one has the spin density conservation relation

$$\sum_{\text{all } i}\rho_i^{\pi} = 1 \quad (4)$$

(45) H. M. McConnell, *J. Chem. Phys.*, **24**, 632, 764 (1956); H. M. McConnell and H. H. Dearman, *ibid.*, **28**, 51 (1958); H. M. McConnell, and D. B. Chesnut, *ibid.*, **28**, 107 (1958).
(46) J. R. Bolton and G. K. Fraenkel, *ibid.*, **40**, 3307 (1964).
(47) P. D. Sullivan and J. R. Bolton, *J. Amer. Chem. Soc.*, **90**, 5366 (1968).
(48) C. A. Claxton and D. McWilliams, *Trans. Faraday Soc.*, **64**, 2593 (1968).
(49) R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963).
(50) B. L. Barton and G. K. Fraenkel, *ibid.*, **41**, 1455 (1964).
(51) M. R. Das and G. K. Fraenkel, unpublished results
(52) E. deBoer and E. L. Mackor, *J. Chem. Phys.*, **38**, 1450 (1963).
(53) J. H. Freed and G. K. Fraenkel, *ibid.*, **40**, 1815 (1964).
(54) B. L. Barton and G. K. Fraenkel, *ibid.*, **41**, 695 (1964).
(55) M. Karplus and G. K. Fraenkel, *ibid.*, **35**, 1312 (1961).

These equations completely define ρ_1, ρ_2, and ρ_7 with the assumption that the spin density on the methyl group is small and can be neglected. These results may be checked by employing the linear relation between ρ_{CO}^{π} and ρ_O^{π}, which was found to be valid for *p*-benzosemiquinone ion[26]

$$\rho_{CO}^{\pi} = -1.115\rho_O^{\pi} + 0.3406 \quad (5)$$

Using eq 2, with $Q_{C-CH_3}^{H} = 21$ G, and eq 3 and 4 we obtain the results in column a of Table V while using eq 5

Table V. Experimental and Calculated Spin Densities in Durosemiquinone Ion in Ethanol[a]

	Experimental			Calculated[b]		
	a	b	c	d	e	f
ρ_1	0.2012	0.2045	0.1949	0.1239	0.1902	0.1974
ρ_2	0.0903	0.0903	0.0704	0.0811	0.0776	0.0782
ρ_7	0.1178	0.1221	0.1307	0.2055	0.1455	0.1368

[a] Column a is evaluated using eq 2, 3, and 4, with $Q_{CCH_3}^{C} = 21$ G (see text); column b using eq 2, 3, and 5 with $Q_{CCH_3}^{C} = 21$ G (see text); and column c using eq 2, 3, and 5 with $Q_{CCH_3}^{C} = 27$ G (see text). Column d values are calculated using best NSQ parameters (see text); those of column e using best PBSQ parameters (see text); and those of column f using $\delta_O = 1.67$ and $\gamma_{CO} = 1.21$ (see text). [b] Methyl group parameters from ref 48 are used in all calculations.

instead of 4 we obtain the results in column b of Table V, which agree quite well.

The results of an MO calculation using the carbonyl group parameters which were employed for NSQ and vitamins K with the Lazdins–Karplus parameters[44] for the methyl are given in column d of Table V. This result disagrees markedly with the experimental spin densities.

At this stage it is possible to argue that perhaps the "best" carbonyl parameters obtained for NSQ need not be the best ones for DSQ. It has been observed earlier[21] that for a good fit at all the positions, different carbonyl parameters had to be employed for two similar compounds like *p*-benzosemiquinone (PBSQ) and 9,10-anthrasemiquinone (ASQ) even when the same solvent was employed in the experiments. The fact that larger values of δ_O were required for the best fit for PBSQ compared to ASQ in any given solvent has been interpreted[21] in terms of a greater degree of solvent interaction for the former. If we assume that for DSQ one should use the best carbonyl parameters obtained for PBSQ, $\delta_O = 1.60$ and $\gamma_{CO} = 1.20$,[21] the results given in column e of Table V are obtained for DSQ. It may be noticed that the agreement between calculated and experimental spin densities at positions 1 and 7 is better in this case. However, the agreement is not as satisfactory as that obtained for the NSQ radicals. We have also tried varying the carbonyl parameters around the best PBSQ values and the best fit was obtained by using $\delta_O = 1.67$ and $\gamma_{CO} = 1.21$. The results using these parameters are given in column f of Table V. It can be noticed that when a better fit for the spin densities at the 1 and 7 positions is achieved using the above procedure, the agreement at position 2 is not so good. However, if one uses a value of $Q_{C-CH_3}^{H} = 27$ G and solves for the experimental spin densities using eq 4 and 5, the values given in column c of Table V are obtained. They compare favorably with the spin densities calculated using $\delta_O = 1.67$ and $\gamma_{CO} = 1.21$, but use of $Q_{C-CH_3}^{H} = 27$ G leads to wide disagreements in spin densities in the NSQ and vitamin K systems. We thus conclude that in the McLachlan framework, it may be necessary to use different carbonyl parameters for semiquinones involving a different ring system. Further, it may be necessary to use values of $Q_{CH_3}^{H}$ varying in a range similar to those used for Q_{CH}^{H} (*ie.*, 22–30 G).[56]

A comparison of the splittings in DSQ and vitamin E shows that the replacement of the methyl group by the long side chain in vitamin E does not cause any significant spin density redistribution. The situation is similar to that between 2,3-DMNSQ and vitamin K_1. In USQ also, the methyl proton splitting has a value which is not considerably different from that of either DSQ or vitamin E. It thus appears that the substitution of the methyl groups at the 5 and 6 positions by methoxyl groups does not greatly change the spin density distribution in the DSQ skeleton. Consequently, we have not attempted any MO calculations on vitamin E and USQ to reproduce the rather small spin density changes.

β-Methylene Proton Splittings in Vitamin Semiquinones and Alternating Line Width Effects in USQ and Vitamin K_1

In each of the radicals from vitamins K_1 and E and USQ, the methyl group and the alkyl side chain should be attached to positions of comparable spin density in the ring (*i.e.*, we have found no significant changes by replacing a methyl with a long side chain). Further, in all these molecules the methylene proton splitting is one-half the methyl proton splitting at room temperature and above. This observation can be readily rationalized on the basis of the well-known $\cos^2\theta$ dependence[57–61] of a β proton splitting, given as

$$a_\beta = \langle Q \rangle \rho^{\pi} \quad (6)$$

where $Q = B_0 + B\cos^2\theta$. In eq 6, ρ^{π} is the spin density on the carbon atom to which an alkyl group is attached 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. B_0 is expected to be small and is usually taken as zero. A ratio of 0.5 for β-proton splitting to methyl proton splitting from positions of nearly equal spin density readily suggests a preferred conformation which places the β-methylene protons at $\theta = \pm 60°$. The $\cos^2\theta$ dependence of β-proton splitting in a number of radicals has been well documented,[61] and there are other examples like ethyl- and propylcyclooctatetraene,[62] 4-isopropylphenoxyl,[63] and 9-ethyl- and 9-benzylxanthyl,[64] where a preferred conformation at $\theta = 60°$ has been observed.

Our experiments go a step further in providing a demonstration that this preferred conformation is not a static one in the vitamin quinones. At room temperature and above our results indicate a fast jump between two thermodynamically equivalent structures. The

(56) See, for example, the discussion on Q_{CH}^{H} in ref 43.
(57) C. Heller and H. M. McConnell, *J. Chem. Phys.*, **32**, 1935 (1960).
(58) A. D. McLachlan, *Mol. Phys.*, **1**, 233 (1960).
(59) P. G. Lykos, *J. Chem. Phys.*, **32**, 625 (1960).
(60) E. W. Stone and A. H. Maki, *ibid.*, **37**, 1326 (1962).
(61) D. H. Geske, "Progress in Physical Organic Chemistry," Vol. 4, Interscience Publishers, New York, N. Y., 1967, p 125.
(62) A. Carrington and P. F. Todd, *Mol. Phys.*, **8**, 299 (1964).
(63) T. J. Stone and W. A. Waters, *J. Chem. Soc.*, 213 (1964).
(64) M. D. Sevilla and G. Vincow, *J. Phys. Chem.*, **72**, 3647 (1968).

interconversion may be thought of as resulting from the relative rotation of the aromatic "head" around the single bond which joins the long side chain to the ring. When this rotational motion is slowed down by lowering the temperature, one observes the alternating line width effects in the esr spectra of vitamin K_1 and USQ. As noted before, for USQ in DME it was possible to freeze out the rotation and obtain the inequivalent methylene proton splittings.

Orientation of CH Bonds in USQ

From the limiting value of the splitting constants obtained from USQ in DME at $-50°$ it is possible to estimate the relative orientation of the CH bonds in the static conformation. Knowing the methyl splitting from position 3 in USQ for which $\langle\cos^2\theta\rangle = {}^1/_2$, the values for $\cos^2\theta$ for the two methylene proton splittings can be estimated by assuming that the spin densities at positions 2 and 3 are nearly the same. Using this procedure the splittings of 1.342 and 0.736 G are found to correspond respectively to angles of 54° 30′ and 65° 10′, which add up very nearly to 120°. Note that for the two values of $\pm 60° + \alpha$, where α is small, $\langle\cos^2\theta\rangle = {}^1/_4[1 + 2\sin^2\alpha] \cong {}^1/_4(1 + 2\alpha^2)$, which for $\alpha = 5°$ is a 1.5% increase from ${}^1/_4$, or a negligible amount.

This inequivalence in angle undoubtedly arises from the different groups on either side of the methylene, *viz.*, a methyl group *vs.* a quinonoid oxygen. We might guess that the 65° angle corresponds to the proton closer to oxygen due to slight hydrogen-bonding attractive effects. The existence of these different groups on either side of the methylene is argument against proposing that the alternating line width is due to torsional oscillations of about ±5° of the methylene protons *without* a complete rotation of the aromatic head around the single bond. This is because one would expect different probabilities for the two thermodynamically *inequivalent* conformations, a condition which would *not* lead to a single average line as the temperature was raised, but rather to two splittings at $p_A a_I + p_B a_{II}$ and $p_B a_I + p_A a_{II}$, where $p_A \neq p_B$ are the probabilities of the two conformers and a_I and a_{II} are the two static splittings.

Alternating Line Width Effects and Kinetic Information

In a two-jump dynamic process, in which two nuclear spins of $I = {}^1/_2$ exchange their different hyperfine splittings, the contribution to the esr width of the $M_H = 0$ hyperfine line in the fast jump region is given by[42,65]

$$T_2^{-1}(M_H = 0) = \frac{1}{8}\gamma_e^2\tau_0(a_I - a_{II})^2 \qquad (7)$$

where τ_0 is the mean jump time.

With the aid of this relation we have obtained τ_0 at different temperatures for USQ in DME and also in ethanol. The first-order rate constant $k_1 = \tau_0^{-1}$ *vs.* $1/T$ is shown in a semilog plot in Figure 13 for both DME and ethanol. Since for the radical in ethanol solvent we could not experimentally observe the splittings corresponding to the slow motion limit, we have assumed that the orientations of the CH bonds are the same as for DME solvent. [The value of the activation energy that is discussed below is independent of this assumption, but the preexponential factor is not.]

These results were obtained utilizing the differences in width of the two extreme lines on either end of the USQ spectrum. The results of least-squares fits, in terms of activation energies and preexponential factors, are given in Table VI. Both direct width measurements of the broadened and unbroadened lines as well as measurements of the relative derivative amplitudes were used and are found to agree well. As expected, the precision is better for the data from amplitude measurements. Since alternating line widths can occur in complex spectra of highly overlapped lines, it was of interest to see whether the greater resolution and simplicity of endor spectra could be used to determine τ_0. A theoretical analysis of endor widths[9] has shown that the methylene proton endor line should have a width contribution in the fast jump region for a two-jump model of

$$T_2^{-1}(\text{endor}) = \frac{1}{4}T_2^{-1}(M_H = 0) = \frac{1}{64}\gamma_e^2\tau_0(a_I - a_{II})^2 \qquad (8)$$

Unfortunately, in the endor spectrum, there is no direct index of the width contribution in the absence of this broadening mechanism. However, it is reasonable to assume that the methyl proton endor line is an approximation to this intrinsic width (*cf.* previous discussion) so we have estimated τ_0 from eq 8 and this assumption. It is important in such experiments to extrapolate the endor widths to zero rf power to avoid any saturation effects.[41] The results, also given in Figure 13 and

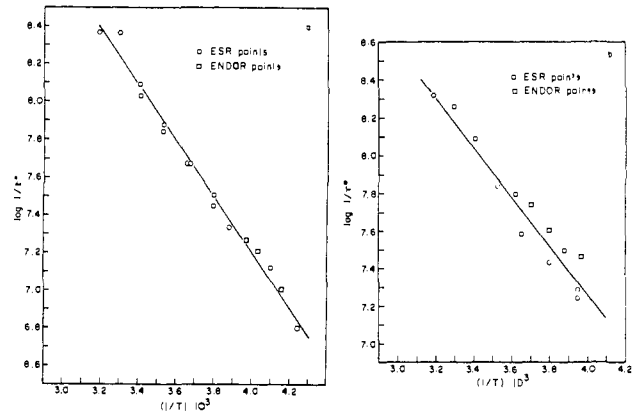

Figure 13. (a) Least-squares fit for a plot of log $1/\tau_0$ *vs.* $1/T$ for USQ in DME. (b) Least-squares fit for a plot of log $1/\tau_0$ *vs.* $1/T$ for USQ in ethanol.

Table VI. Activation Energies and Preexponential Factors for Internal Rotation of Side Chain in USQ and Vitamin K_1

Compound	Method of analysis	Activation energy, kcal/mol	Preexponential factor, sec^{-1}
USQ in DME	Amplitude	7.14 ± 0.32	$1.23 \times 10^{13.0\pm0.25}$
	Width	7.51 ± 1.28	$3.66 \times 10^{13.0\pm0.61}$
	Endor	8.15 ± 0.92	$2.36 \times 10^{14.0\pm0.82}$
USQ in EtOH	Amplitude	6.77 ± 0.32	$2.67 \times 10^{13.0\pm0.26}$
	Width	7.37 ± 0.78	$7.58 \times 10^{13.0\pm1.0}$
	Endor	5.38 ± 0.60	$3.72 \times 10^{12.0\pm0.5}$
Vitamin K_1 in EtOH	Amplitude	5.22 ± 0.30	*a*

[a] Preexponential factor not calculated as the inequivalent methylene splittings are not known (see text).

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Table VI, are in reasonable agreement with those obtained from the esr widths, and thus appear to justify this potentially useful method.

We note that for the activation energies and preexponential factors (obtained from relative amplitudes) given Table VI, there is some solvent dependence noted for USQ. The preexponential factors of $1-2 \times 10^{13}$ sec^{-1} for USQ lead to values of entropy of activation $\Delta S^{\ddagger} \sim 0$ at 25° (−0.6 and 0.9 cal deg^{-1} in ethanol and DME, respectively) as expected.

In vitamin K_1, although we cannot freeze out the internal rotation of the side chain, it is possible to obtain a value for the activation energy from the alternating line width effect. For the unbroadened line widths we made use of the lines shown with asterisks (lines with $M_{CH_3} = {}^3/_2$, $M_{CH_2} = +1$, $M_{H5-8} = 0$ and $M_{CH_3} = {}^3/_2$, $M_{CH_2} = -1$, $M_{H5-8} = 0$) in Figure 6, and for the broadened widths we used the lines indicated with the arrows ($M_{CH_3} = \pm {}^1/_2$, $M_{CH_2} = 0$, $M_{H5-8} = 0$). Despite some problems of overlap, we do obtain a good straight-line fit for the data, and the activation energy is close to but somewhat smaller than that obtained for USQ in ethanol.

In comparing the above results with those on other methylene-containing systems such as vitamin E, we recall that (1) the asymmetry of the radical about the plane containing the methylene-carbon to ring-carbon bond and perpendicular to the benzene ring is the obvious reason for the static inequivalence of the two methylene protons, but (2) the *average* angle from this plane is 60°. Thus, in other cases, like vitamin E, where the asymmetry and average angle are similar, there most likely is a similar rapid dynamical averaging process.

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Chemistry of Radical Anions and Dianions of Diphenylacetylene

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Abstract: Diphenylacetylene (DPA) reacts instantly with sodium biphenylide ($B\cdot^-,Na^+$) in hexamethylphosphoramide (HMPA) and forms the radical ions $DPA\cdot^-$. No further reduction takes place in HMPA even if $B\cdot^-$ is in excess. The free $DPA\cdot^-$ ions do not dimerize; their optical and esr spectra were recorded. Electron affinity of DPA was determined by two methods and found to be 0.27 V higher than that of biphenyl. At very low temperatures ($\lesssim -78°$) DPA in tetrahydrofuran (THF) reacts with metallic lithium giving first $DPA\cdot^-,Li^+$ and eventually insoluble $DPA^{2-},2Li^+$. Protonation of the latter at −78° yields quantitatively *cis*-stilbene, indicating the *cis* structure of the dilithium salt. Above −78° $DPA^{2-},2Li^+$ becomes protonated by THF giving $PhCH{:}CPh,Li^+$ whose optical spectrum was recorded. Protonation of $PhCH{:}\bar{C}Ph,Li^+$ with CH_3OD gives *trans*-PhCH:CDPh. Reduction of DPA in THF by metallic sodium at −78 or −92° yields mixtures of $DPA\cdot^-,Na^+$ and the soluble $DPA^{2-},2Na^+$. The spectrum of the latter was determined. The disodium salt very slowly reacts with THF at −60° and faster at higher temperatures giving again the salt of the $PhCH{:}\bar{C}Ph$ carbanion. The protonation of $DPA^{2-},2Na^+$ or $DPA\cdot^-,Na^+$ by methanol at −78 or −40° yields *trans*-stilbene, bibenzyl, and the parent hydrocarbon. It was shown that these results can be rationalized by assuming agglomeration of these salts and a rapid intra-aggregate electron transfer which competes with the protonation. At low temperatures $DPA\cdot^-,Na^+$ in THF reacts slowly with $B\cdot^-,Na^+$. It was proved that this process involves two steps: (I) rapid electron transfer yielding a minute equilibrium concentration of $DPA^{2-},2Na^+$ and (II) slow protonation of the latter by THF (k_p). The overall bimolecular rate constant is therefore K_1k_p and not, as mistakenly concluded by Evans, a rate of a slow electron-transfer process.

Continuing our studies of the chemistry of radical ions and dianions, we have investigated now the electron-transfer processes involving diphenylacetylene, DPA. This hydrocarbon forms radical ions, $DPA\cdot^-$, and dianions, DPA^{2-}. Their behavior is rather complex and influenced by the choice of cation and of solvent whose nature affects the state of aggregation of the reacting species. Not only free ions and ion pairs, but also some larger aggregates are present in the solutions of $DPA\cdot^-$ and DPA^{2-} and such aggregates seem to be responsible for some peculiar reactions which have been observed in our studies.

Reactions in Hexamethylphosphoramide

HMPA is a powerful solvating agent of alkali ions and many alkali salts of carbanions and radical ions are fully dissociated in this medium.[1,2] Hence, the reactions observed in HMPA are of free ions and not ion pairs. The free radical anions, $DPA\cdot^-$, may be formed in HMPA through electron transfer from

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(2) A. Cserhegyi, J. Jagur-Grodzinski, and M. Szwarc, *ibid.*, **91**, 1892 (1969).