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ARTICLES

Two-Photon Transitions in CW-ENDOR Spectra of Radicals in Fluid Solution

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The radical anion of duroquinone (2,3,5,6-tetramethyl-1,4-benzoquinone) was used as a model compound to describe the low-frequency (LF) ENDOR lines observed in many radical systems. These LF lines are shown to arise from two-photon transitions, and they occur because of the rapid cross-relaxation rates and dynamical processes of the system. The LF lines occur when the radio frequency (RF) and microwave frequency match the energy of an adjacent EPR transition. In practical work, LF ENDOR lines disturb the determination of nuclei such as ^{2}H, ^{13}C, Na, and K, whose Larmor frequencies are in that region. Under certain conditions, two-photon transitions were also observed in the normal proton coupling region. A method for distinguishing normal ENDOR lines from two-photon transitions is given. The method is based on the different shape of an ENDOR-induced EPR spectrum when it is measured from two-photon-transition ENDOR lines.

1. Introduction

Electron−nuclear double resonance (ENDOR) spectroscopy has been used as an analytical tool for many years. Although the practical and theoretical aspects of ENDOR spectroscopy are well-known,[1−5] under appropriate conditions, it is possible to observe “unusual” signals that are not easily interpreted. Observations of such lines are possible for a variety of reasons that will be briefly described later. These unusual lines are usually detected in the low-frequency (LF) region, i.e., below 8 MHz, but under certain conditions, they can also be observed in the normal proton ENDOR region. The couplings of ^{2}H, alkali metals (Na, K), and ^{13}C, among others, are typically located in the LF region, and thus the analysis of these nuclei is complicated by these abnormal ENDOR lines. A practical method of distinguishing normal ENDOR lines from these, occasionally very prominent and easily misinterpreted lines, is needed.

The theories of saturation and line shapes are very well established in electron magnetic resonance (EMR).[6,7] In a steady-state ENDOR experiment, coherence effects seem to be the most important in generating signals that can be misinterpreted.[7] Dubinskii et al.[8] have analyzed and discussed the different mechanisms capable of generating LF ENDOR lines. Some LF lines arise because of different modulation mechanisms and are considered artificial in comparison with conventional ENDOR lines. Exceptional ENDOR features can also be produced by multiphoton transitions. It is well-known that if microwave field B_1 or radio-frequency field B_2 is not completely perpendicular to static magnetic field B_0, it is possible to observe EPR transitions in the ENDOR spectrum because of the interaction of the two fields.[9,10] Recently, Gromov et al.[10] explained the multiphoton transitions of an even number of photons in pulse EPR in terms of Floquet theory.

In our studies on ubiquinones,[11,12] we noticed strange lines in the LF region of the ENDOR spectrum. When certain EPR lines were radiated, prominent lines or line pairs (couplings) in the LF region were present that could not be assigned properly. Furthermore, the ENDOR-induced EPR (EIE) spectrum, which can be used to separate the EPR spectra of different radicals in a mixture, behaved curiously when these LF lines were investigated. The literature presents many cases of similar LF ENDOR lines.[13−15] LF lines are usually observed with radicals produced chemically by alkali metal reduction from quinones or other aromatic molecules. Kirste et al.[15] noticed that these lines are connected to the EPR transitions. Following up on this idea and applying the model for multiphoton transitions,[10] we can now explain the observed LF lines and we give an appropriate method for distinguishing these lines from the normal ENDOR transitions.

The LF ENDOR lines were observed to exist in the ENDOR spectrum of radical anions produced from different quinones. Duroquinone (2,3,5,6-tetramethyl-1,4-benzoquinone) was selected as the model compound to study the phenomenon because of its high symmetry. Furthermore, because of the four methyl groups attached to the ring, duroquinone is more resistant to different addition reactions as compared to the equally symmetrical 1,4-benzoquinone.[16,17] As assumed, the duroquinone showed very prominent LF ENDOR lines. In particular, under certain measurement conditions, similar transitions were observed in the frequency region where the proton couplings are normally detected. These lines can be very easily misinterpreted as normal proton couplings. During dynamical studies when experimental parameters are varied greatly, corresponding circumstances might arise in many other radical systems and might cause misinterpretations.

The effect of counterion interactions with chemically prepared radicals in liquid samples has been extensively studied. The Arrhenius parameters for such systems can usually be determined by EPR spectroscopy.[18] However, intramolecular cation

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exchange can also occur at such a rate that it is observed not in the EPR but only in the ENDOR spectrum.[19] Under typical measurement conditions, the cation exchange rate is on the order of 10^6 s^{-1}. For 2,5-di-*tert*-butyl-1,4-benzoquinone, the chemical rate constant for cation exchange was shown to vary from 3.0 × 10^3 to 1.5 × 10^7 s^{-1}.[18,19] The radical ion pairing in our model compound, duroquinone, is also well understood.[20−22]

The EPR spectrum of the radical anion of duroquinone consists of 13 lines on account of the 12 equivalent methyl protons. The outermost lines are not easily noticed because of the high intensity of the central line (intensity ratio 1:924). The radical anion, without perturbation of its surroundings (free radical), has one isotropic hyperfine coupling constant (ihfc), whereas the solvent-separated or tight ion pair has two proton couplings. Between the strong EPR lines belonging to the 12-proton multiplet of the free radical, there are a number of much smaller, although very clear, signals. Das et al.[23] identified these lines, so-called satellite lines, as belonging to three species with natural abundances of ^{13}C couplings of 2.02, 2.99, and 3.86 MHz.

In this report, we provide one explanation for the appearance of unconventional lines in the ENDOR spectrum and give a recipe for distinguishing these ENDOR lines from those belonging to normal ihfc's. The method is based on the behavior of the ENDOR-induced EPR (EIE)[24] spectrum under experimental conditions where the applied radio frequency is not chosen to correspond to a real transition of the system. For example, the previously uninterpreted transitions in the ENDOR spectrum of the *m*-dinitrobenzene radical anion measured in liquid ammonia[13] as well as the LF lines in the ENDOR spectrum of the radical anion of ubiquinone[12] can now be explained on the basis of the present study.

2. Results and Discussion

LF ENDOR lines can be observed in the liquid ENDOR spectra of the radical anions of different quinones when the radical is prepared by using alkali metal or alkali metal hydroxide as the reducing agent. The radical cations of the corresponding quinones do not show any unusual ENDOR features, and thus, it was assumed that these features are produced by the structure of the radical or by the interaction of the radical and the counterion. Because of the different degrees of ion pairing observed in duroquinone, the radical was studied in different solvents, namely, ethanol; 1,2-dimethoxyethane (1,2-DME); NH_3; and perdeuterated ethanol with NaOH, KOH, K, or Na as the reducing agent. The LF ENDOR lines were observed in every sample, but in certain cases, it was also possible to adjust the experimental conditions such that these LF ENDOR lines were no longer present. Additionally, in ethanol and in liquid ammonia, it was possible to achieve measurement conditions where unusual ENDOR features were also observed around the normal proton ENDOR lines.

The ENDOR spectra of the radical anion of duroquinone in ethanol and in 1,2-DME are shown in Figure 1. In ethanol at 253 K (Figure 1a), three lines were observed: the pair of lines of a proton coupling and an additional line in the LF region. The proton ihfc of 5.33 MHz is due to the equivalence of the methyl-group protons (12 H), and the broad (peak-to-peak width of 0.61 MHz) LF line is located at 5.33 MHz, i.e., at the position describing the absolute value of the ihfc. Additionally, around both proton ENDOR lines, two small peaks were observed. The origin of these lines is discussed in more detail below when the samples prepared in ethanol and NH_3 are compared.

In 1,2-DME, when potassium was used as the countercation (Figure 1b), two proton ihfc's (6H + 6H) of 4.06 and 6.69 MHz were observed at 253 K as a result of the removal of the degeneracy by distortion due to the potassium ion. In the LF region, there were three lines at 6.69, 4.06, and 2.63 MHz. As in the experiments in ethanol, the LF lines were located at the values of the ihfc's, the smallest being the difference between the two couplings.

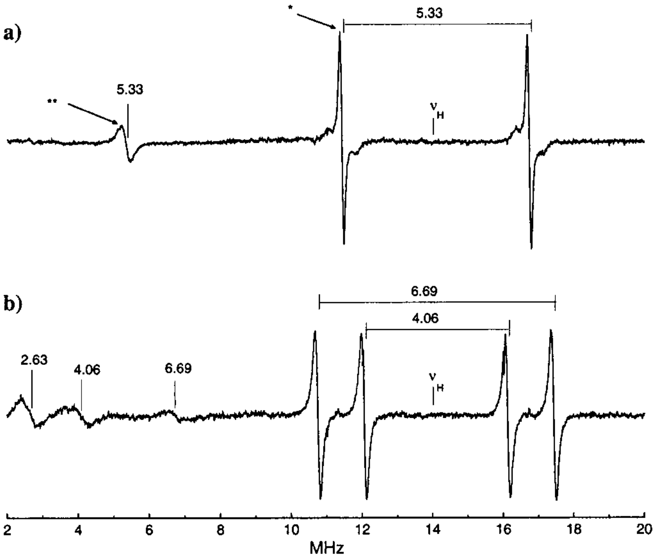

Figure 1. ENDOR spectrum of radical anion of duroquinone in (a) ethanol and (b) 1,2-DME. KOH is used as the reducing agent. The peaks in the LF region are at the frequencies of the ihfc's. In 1,2-DME, the separation peak of two couplings (at 2.63 MHz) is also visible. Both spectra were measured at 253 K. The stars indicate the positions where the EIE spectra in Figure 2 were measured: *, Figure 2c; **, Figure 2d. The observed LF line in spectrum a is explained in the transition diagram in Figure 3b.

When an ENDOR spectrum (Figure 2b, in ethanol) was measured from the ^{13}C satellite line, indicated by the arrow in Figure 2a, two LF lines were observed at 1.78 and 3.55 MHz. Two LF lines were observed from every satellite, but the positions of these lines varied such that the sum of the two frequencies was always the absolute value of the ihfc of 5.33 MHz. The LF lines were observed even if the positions for the ENDOR experiment were selected so as to not correspond any real EPR transition. The positions of the LF lines depended on the "distance" of the pumping position from the neighboring EPR lines of the main radical. In fact, these lines are actually double quantum transitions arising from the interaction of the microwave and the RF quanta. These two RF transitions (ν_3 and ν_4 in Figure 3), together with the microwave field, correspond to the EPR transitions whose resonance is next to the position selected for the ENDOR experiment, and we assume that they contribute to the ENDOR enhancement because of the fast cross-relaxation rates typical for quinone radicals.

In Figure 3, an example of a two-photon transition observed in the LF region of the ENDOR spectrum is shown. In the ENDOR experiment, static magnetic field B_0 is kept constant, and the two-photon resonance lines are observed when the sum of the MW field, B_1, and the RF field, B_2 (now ν_{aH}), corresponds to the next EPR transition. It is worth mentioning that these transitions are not the "forbidden" $\Delta m_s = 1$, $\Delta m_I = 1$ transitions but the "normal" EPR $\Delta m_s = 1$, $\Delta m_I = 0$ transitions from the states where the magnetic quantum numbers m_I differ by ±1. The LF transitions corresponding to the ihfc's (Figure 1a and b) and the LF lines, as in Figure 2b whose sum is the ihfc, can be explained as arising from the two-photon transitions. The resonance condition for the LF line located at the position of the absolute value of the ihfc is fulfilled simultaneously for the

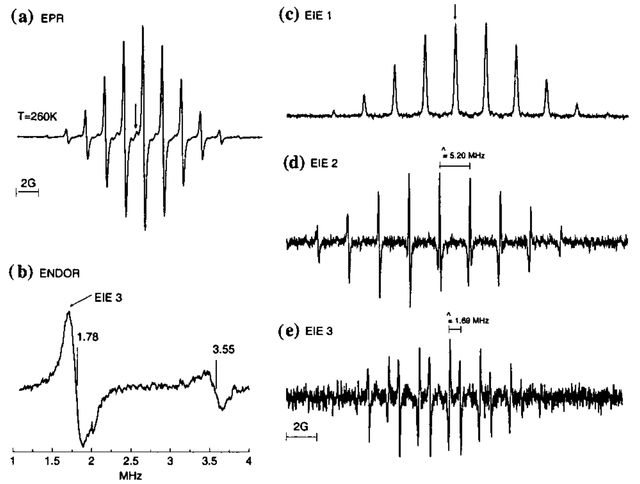

Figure 2. Radical anion of duroquinone in ethanol. (a) The arrow in the EPR spectrum indicates the position from which the ENDOR spectrum in b was measured. (b) In the LF region, two lines are observed at 1.78 and 3.55 MHz. The frequencies correspond to the distances from the nearest EPR transitions, and the sum of those frequencies is the ihfc, 5.33 MHz. The actual transitions for these lines are shown in Figure 3c. Three EIE spectra were measured using different pumping frequencies (PFs): (c) 11.34 MHz (* in Figure 1a), (d) 5.20 MHz (** in Figure 1a), and (e) 1.69 MHz (2b). Spectrum EIE1 (c) was measured from the top of the line belonging to the normal proton coupling, whereas spectra EIE2 and EIE3 (d,e) were measured from the top of the LF ENDOR lines. The arrow in c indicates the position of the central line of the EPR spectrum. Because of the method of detection, the EIE spectrum is normally in the absorption mode. However, if the EIE spectrum is measured from the LF line, its appearance resembles the normal first-derivative EPR spectrum. In addition to the normal EPR spectrum, one extra coupling corresponding to the pumping frequency is observed, and the phase of the spectrum changed in the middle of the spectrum. All spectra were measured at 260 K.

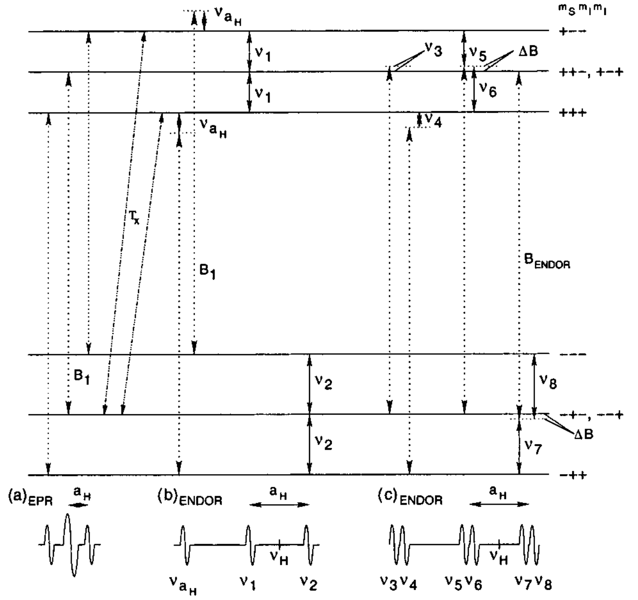

Figure 3. Transition diagram for the system having two equivalent protons ($S = {}^1/_2$, $I = {}^1/_2$, $I_{TOT} = 1$) for an ENDOR experiment. The simplified diagram represents the different transitions observed in the case of 12 equivalent protons in the radical anion of duroquinone ($a_H = 5.33$ MHz). The quantum numbers $|m_S, m_I, m_I\rangle$ for each level are shown on the right ($+ = {}^1/_2$, $- = -{}^1/_2$). The microwave transitions are indicated by dotted (· · ·) lines, the RF transitions by a solid (—) lines, and examples of the cross-relaxation transitions by dash−dotted (- · · · -) lines. The transition arrows are placed such that the spectra below the diagram show the spectral position of each transition. The normal EPR transitions ($\Delta m_S = 1$, $\Delta m_I = 0$) are in part a. Part b shows the ENDOR transitions as in Figure 1a. The ENDOR experiment is performed from the central EPR line (B_1). The frequencies ν_1 and ν_2 are the normal ($\Delta m_S = 0$, $\Delta m_I = 1$) ENDOR transitions located around the proton Larmor frequency ν_H. The ENDOR spectrum is measured as an enhancement in the observed MW transition. When the RF is equal to the coupling constant ν_{aH}, two two-photon transitions corresponding to the EPR transitions $|-++\rangle \rightarrow |+++\rangle$ and $|---\rangle \rightarrow |+--\rangle$ can occur. If the cross-relaxation ($\Delta m_S = 1$, $\Delta m_I = 1$) rates are dominant, both of these two-photon transitions increase the populations of states $|-+-\rangle$ and $|--+\rangle$ and thus produce the ENDOR enhancement. The frequencies ν_3 and ν_4 in part c correspond to the situation in the ENDOR spectrum shown in Figure 2b when the pumping position for the ENDOR experiment, B_{ENDOR}, differs from the resonance conditions for the main EPR lines by ΔB. If ΔB is small, the two-photon transition to the nearest EPR line, ν_3, goes to very low frequencies and contributes to the problems almost routinely observed in the baseline at very low frequencies (<0.8 MHz). When ΔB is small, the two two-photon transitions contributing to the line at ν_{aH} are no longer equal, and the line is split accordingly. Similarly, the real ENDOR lines around ν_H are split corresponding to ΔB. Common to all of the two-phonon transitions is that they all end at a real level corresponding to $\Delta m_S = 1$, $\Delta m_I = 1$ compared to the starting level. Again, the rapid cross-relaxation processes give rise to the ENDOR enhancement.

two EPR transitions ($m_I = \pm 1$), and thus, the intensity of the line is twice the intensity of the single line (ENDOR1 and ENDOR2 in Figure 5). However, to observe such multiphoton transitions, the RF field should not be perpendicular to the static magnetic field as it supposed to be in the normal ENDOR spectrometer. The possible reasons for this will be given below.

Generally, the extent of ion pair formation depends greatly on the solvent, temperature, and the countercation. Whereas, in 1,2-DME, the two different ihfc's of the ion pair are observed at 253 K, at 300 K, the exchange of a counterion is so rapid ($k > 2 \times 10^7$ s^{-1}) that only the average value of the couplings is detected. Thus, the EPR spectrum in 1,2-DME at 300 K is similar to the EPR spectrum measured in ethanol or in NH_3 for the temperature range used here. Because potassium has such a small magnetic moment, the alkali metal coupling is not observed when potassium is used as the counterion even if the ion pairing is so strong that two different proton ihfc's are detected. However, if the radical is prepared in 1,2-DME using metallic sodium as the reducing agent, the ihfc of Na is also observed.[22,23] When the tight ion pair was formed, one coupling of 0.62 MHz for Na was observed in addition to the two proton ihfc's of 3.73 and 7.10 MHz (Figure 4b). The formation of a tight ion pair is also seen in the EPR spectrum (Figure 4a, simulation done with the above ihfc's). In the LF region of the ENDOR spectrum, there were again very prominent EPR satellite lines, now separated by the amount of the Na coupling.

The EPR spectrum measured at 300 K in ethanol is identical to that measured at 206 K in NH_3. Similarly, all of the ENDOR features observed in ethanol at 300 K are also seen in liquid ammonia at temperatures ~100 K lower. This is not surprising because the rotational correlation time, τ_R, in liquid ammonia at ~200 K is similar to that in ethanol at ~300 K ($\tau_R \approx 4 \times 10^{-11}$ s).[1]

In ethanol at temperatures above 290 K (and in NH_3 near 200 K), additional ENDOR lines were detected around the lines of the proton coupling of the 12 equivalent protons. At 296 K, these additional lines arose so that ihfc's of 4.95, 5.31, and 5.69 MHz were observed. These values closely resemble the ihfc's measured in NH_3 at 206 K (4.91, 5.41, and 5.91 MHz, Figure 5, ENDOR1 and ENDOR2). Nevertheless, these new "couplings" do not belong to any real radical system but are again two-photon transitions. However, the mechanism is now slightly different from that of the lines observed in the LF region.

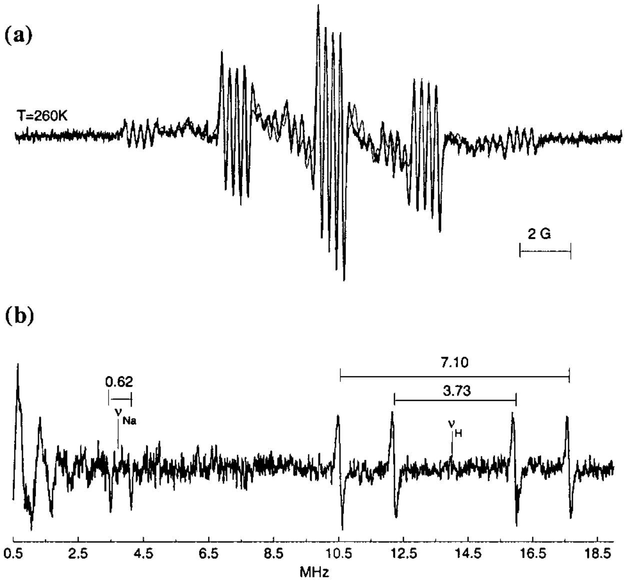

Figure 4. (a) Measured and simulated EPR spectra and (b) ENDOR spectrum of duroquinone in 1,2-DME. The tight ion pair is formed in 1,2-DME when metallic sodium is used as the reducing agent. The simulation of the EPR spectrum was done with the couplings shown in b. The LF ENDOR lines that do not belong to the normal couplings are again very prominent compared to the Na lines. However, the former can be identified as two-photon transitions by using EIE, as shown in Figure 2.

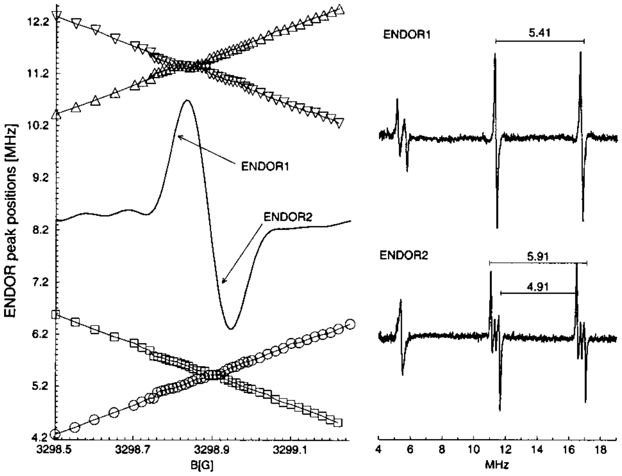

Figure 5. Central EPR line of the radical anion of duroquinone in liquid NH_3 at 206 K and the positions of the ENDOR lines as the spectral position for the ENDOR experiment is varied. Only the lower-frequency line of the proton coupling lines is shown. If the EPR line is very narrow, even a small deviation from the exact resonance has a noticeable effect on the ENDOR spectrum. The splitting of the LF and lower-field proton ENDOR line depends linearly on the pumping position. The spectral positions for the two ENDOR spectra are indicated by arrows. ENDOR1 shows the real ihfc, whereas in ENDOR2, the two-photon transitions are very prominent even if the ENDOR spectrum is measured quite close to the point of resonance. The risk of misinterpreting these new lines as two sets of unequal ihfc's, such as in ion pair 1b or 4b, is substantial. The origin of these lines becomes clear if the EIE experiment is performed as reported in this paper.

Whereas those LF lines presented adjacent EPR transitions, these lines correspond to the ENDOR transitions from the virtual state generated by the MW field to the same energy level as the real ENDOR transition between those lines (ν_5, ν_6, ν_7, and ν_8 in Figure 3c). The ENDOR-type two-photon transition lines were almost inert in the general TRIPLE experiment. Furthermore, the deviating origin of these lines was ascertained in the EIE experiment.

In Figure 5, the frequencies of the ENDOR features are plotted against the value of the magnetic field used in the ENDOR experiment. The two-photon transition in the LF region as well as the normal proton ENDOR lines (only the lower-frequency line is shown in Figure 5) are split if the ENDOR pumping frequency differs from the exact resonance. The dependence of the ENDOR transition on the pumping position is linear with a slope of an electron magnetogyric ratio of ±2.8 MHz/G. That these "ENDOR" lines are obtained even if the spectrum is not measured from the EPR transition is noteworthy. The small offset from the exact resonance might produce ENDOR effects that can be misinterpreted if the EPR lines are very narrow or if the EPR lines of two different radical species overlap almost completely. The fact that these double-quantum transitions are observed only in certain circumstances suggests that some kind of dynamic process is behind the phenomenon. In ethanol, the new couplings can be raised either by increasing the temperature or by increasing the countercation concentration. The increased number of alkali metal cations around each radical might affect the relaxation rates quite drastically. The interaction between the radical and counterion might even lead to the formation of insoluble alkali metal salt. In our earlier study,[26] in the case of menadione (vitamin K_3), we managed to measure the EPR spectrum of a paramagnetic potassium salt of the corresponding radical.

A radical was also prepared in perdeuterated ethanol, CD_3-CD_2OD, to determine whether the couplings around the methyl proton lines are due to solvent protons, i.e., the neutral semiquinone radical. Both the EPR and ENDOR spectra were similar to those found in nondeuterated ethanol.

Leniart et al.[25] have extensively analyzed the effects of different ENDOR parameters for quinone radical systems. To exclude any artifacts from the measurement conditions, several variables were studied systematically. LF ENDOR lines were detected at every concentration where normal lines were also observed and from all of the EPR lines of the multiplet of the 12 protons. The effects of MW and RF power and RF modulation were studied systematically. MW power did not have any effect on the peaks appearing around the ENDOR lines of the free radical. However, RF power had a very noticeable effect: at 100 W, these proton lines were easily recognized, and at 25 W, the lines could be detected only very weakly although at the same positions. Lowering of the RF modulation achieved a better resolution of the ENDOR lines. The analysis of the effects of the RF and MW powers on the line intensities and positions excluded different coherence effects as the origin of observed transitions. Also, the possibility of modulation sidebands was excluded.

The requirement for the observation of the two-photon transition, namely, that RF field B_2 no longer be perpendicular to static magnetic field B_0, might arise from the sample or some technical origin (e.g., misalignment of the RF coil). The dielectric constant of the medium, the presence of ionic species, or dynamic processes in the sample can change the orientation of the effective magnetic field in the sample. Because we have observed these kinds of transitions only with the radical anions of different quinones (not, for example, radical cations of quinones or radical anions of tocopherols prepared using same methods), we assume that the efficient cross-relaxation processes of the system have a crucial role in the generation of these two-photon transitions. We believe that, under appropriate conditions, even a very small deviation from the perpendicularity of B_0 and

B_2 (<0.5°) might be enough to create the observed effect. It is also possible that the sample might increase the deviation of the magnetic fields. If the orientation of the RF coil were the only reason for the removal of the perpendicularity of the fields, the necessary misalignment would be so small that it can be assumed to be present in every spectrometer. All of the observations were reproduced in another laboratory,[28] and to the best of our knowledge, the most probable reason for the misalignment of the magnetic field is dynamical processes of the sample.

ENDOR-induced EPR spectroscopy proved to be a very valuable tool for determining the nature of the observed ENDOR transitions. EIE is normally used to measure the EPR spectrum of one particular radical from a mixture of radicals. We observed another, very powerful application for this method. From the shape of the EIE spectrum, it is possible to determine whether or not the observed ENDOR transition is real. Because the EIE spectrum is measured by detecting the change in the first-derivative ENDOR line, it is normally obtained in the absorption mode (Figure 2c). However, the EIE spectrum measured from any of the ENDOR peaks that did not arise from the real ENDOR transition was acquired in the derivative mode (Figure 2d and e). Additionally, the phase of the spectrum changed at the electron Zeeman frequency, and an extra splitting of twice the RF pumping frequency (ν_{RF}) appeared in the EIE spectrum. This type of behavior is always observed if the selected RF does not match the real ENDOR transition, and thus, EIE spectroscopy can be easily used to distinguish normal ENDOR lines from lines that are due to the two-photon transition.

3. Experimental Section

Radical anions were prepared in various solvents, including ethanol, perdeuterated ethanol, 1,2-dimethoxyethane (1,2-DME), and ammonia, with different alkali metals (Na and K) or KOH as the reducing agent. All samples were prepared under high-vacuum conditions (ca. 5×10^{-6} Torr). The sample cuvette with a 3.0/1.7-mm (outside/inside diameter) EPR tube was sealed with a flame after the freeze−pump−thaw cycle showed constant pressure. The cuvette and EPR tube were Pyrex-type glass because of the high explosion risk of liquid NH_3 samples in the quartz cuvette. Ammonia was trapped in the cuvette by an alcohol bath cooled with liquid nitrogen, and when sealed, it stayed in the liquid phase even at room temperature because of the high pressure in the cuvette.

Duroquinone (97%) was obtained from Aldrich and further purified by vacuum sublimation or recrystallization from ethanol. Ethanol for spectroscopic purposes (>99.5%) was prepared by Primalco Oy (Finland) and perdeuterated ethanol (CD_3CD_2OD, $HDO + D_2O < 0.3\%$) by Euriso-top (France). 1,2-Dimethoxyethane (1,2-DME) was obtained from Fluka, and NH_3 (99.95%) from AGA Specialgas AB (Finland). Sodium and potassium were from Riedel de Haën, and KOH pellets (86.4%) were from Baker Analyzed; all reducing agents were used without further purification. Spectra were recorded on a Bruker ER 200 D-SRC X-band spectrometer equipped with a Varian E-12 magnet, Bruker ER 033M FF lock and Bruker EN 810 ENDOR unit. In the ENDOR experiments, the applied microwave power varied from 3 to 10 mW. The radio frequency power used in the experiments varied from 25 to 100 W ($B_2 < 10$G rotating frame). All spectra were collected and analyzed using the *xemr* program.[27]

4. Conclusions

We have presented an explanation for the observation of different LF ENDOR lines in quinone samples. The rapid cross-relaxation rates compared to the electron and nuclear relaxation rates enable the detection of two-photon transitions caused by the small misalignment of the effective transversal ENDOR field. This misalignment can arise either from technical reasons or, for example, from dynamical exchange processes that change the local magnetic field in the vicinity of single radicals. Similar spectral features can be observed in many other radical systems (e.g., some neutral radicals or triplet-state radicals both in liquid solutions and in liquid crystals) in addition to the radical anions of different quinones if the above prerequisites are fulfilled. The significant temperature dependence of the occurrence of the LF ENDOR lines confirms the role of the properties of the sample in the effect. This was further verified by the observation that the spectral features in liquid ammonia at around 200 K were similar to those in ethanol at 300 K, under which conditions the rotational correlation times of the solvents are similar.

The importance of acquiring knowledge of the fundamental reasons for the different phenomena that might create effects in the ENDOR spectrum is obvious. Many interesting nuclei have their Larmor frequencies in the LF region, and thus, it is extremely valuable to be aware of possible misleading spectral information. It should be remembered that it is possible to select the ENDOR pumping position by accident such that the difference between the split LF lines is twice the Larmor frequency of some nuclei and thus to identify this erroneously as a coupling (for example, two lines in the LF region in Figure 2b). The problem arises mainly when studying other radicals that are present in the sample in much smaller concentrations than the main radical. This can often be the situation in samples of biological interest.

A method for distinguishing real ENDOR transitions from the other spectral features is given. If the RF corresponds to the real ENDOR transition, the EIE spectrum will be in the absorption mode; otherwise, the spectrum will resemble the normal first-derivative EPR spectrum except for an additional splitting corresponding to the RF applied and the change in the phase in the middle of the EIE spectrum. Thus, the shape of the EIE spectrum reveals the origin of the ENDOR transition.

When samples of biological interest are measured, the radical under scrutiny might be present in only a very low concentration and in a mixture containing other radicals. It is then extremely important to have a method by which to distinguish actual ihfc's from the other spectral features that might possibly arise from the sample.

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