

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF JYVÄSKYLÄ  
RESEARCH REPORT No. 33

ESR, ENDOR AND TRIPLE RESONANCE  
STUDY ON SUBSTITUTED  
9,10-ANTHRAQUINONE RADICALS IN SOLUTION

BY  
REIJO MÄKELÄ

Academic Dissertation  
for the Degree of  
Doctor of Philosophy



Jyväskylä, Finland  
ISBN 951-680-304-0  
ISSN 0357-346X

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF JYVÄSKYLÄ  
RESEARCH REPORT No. 33

ESR, ENDOR AND TRIPLE RESONANCE  
STUDY ON SUBSTITUTED  
9,10-ANTHRAQUINONE RADICALS IN SOLUTION

BY  
REIJO MÄKELÄ

Academic Dissertation  
for the Degree of  
Doctor of Philosophy

To be presented by permission of the Faculty of  
Mathematics and Natural Sciences of the University  
of Jyväskylä for public examination in Auditorium  
S-212 of the University on June 29, 1990,  
at 12 o'clock noon.



Jyväskylä, Finland  
ISBN 951-680-304-0  
ISSN 0357-346X

**URN:ISBN:978-952-86-0194-4**  
**ISBN 978-952-86-0194-4 (PDF)**  
**ISSN 0357-346X**

**University of Jyväskylä, 2024**

## CONTENTS

	ACKNOWLEDGEMENTS	3
	LIST OF ORIGINAL PUBLICATIONS	4
	ABSTRACT	5
1.	INTRODUCTION	6
2.	PROPERTIES AND GENERATION OF RADICALS OF 9,10-ANTHRAQUINONES	8
2.1.	General	8
2.2.	9,10-Anthraquinone	8
2.3.	Hydroxyanthraquinones	8
2.4.	Anthraquinone sulfonates	9
2.5.	Generation of anion radicals	10
2.6.	Generation of cation radicals	11
3.	THEORY OF ESR, ENDOR AND TRIPLE RESONANCE SPECTROSCOPY	12
3.1.	General	12
3.2.	Important instrumental parameters in ENDOR experiments	12
3.3.	Important experimental parameters in ENDOR experiments	14
3.4.	$^2\text{H}$ ENDOR spectroscopy	15
3.5.	The additivity relationship	15
4.	EXPERIMENTAL	16
4.1.	Equipment	16
4.2.	An ENDOR/TRIPLE experiment	16
4.3.	ENDOR/TRIPLE cavity	17
4.4.	Sample preparation	18
4.4.1.	Anion radicals	18
4.4.1.1.	Ethanol - water and ethanol- $\text{d}_1$ - $\text{D}_2\text{O}$ mixtures	18
4.4.1.2.	Tetrahydrofuran	19
4.4.1.3.	Dimethylsulfoxide	20
4.4.1.4.	Ammonia	20
4.4.2.	Cation radicals	20

5.	<b>RESULTS AND DISCUSSION</b>	20
5.1.	The ENDOR solvents	20
5.2.	Anion radicals	22
5.2.1.	9,10-Anthraquinone	22
5.2.2.	Hydroxyanthraquinones	23
5.2.3.	Anthraquinone sulfonates and 1,2-dihydroxy-9,10- anthraquinone-3-sulfonate	25
5.3.	Cation Radicals	26
5.4.	The additivity relationship applied to 9,10-anthraquinones	26
	SUMMARY	29
	REFERENCES	31
	ORIGINAL PUBLICATIONS	

## ACKNOWLEDGEMENTS

The present studies were carried out in the Department of Chemistry, University of Jyväskylä, during the years 1982-1990. I am indebted to the Department for placing facilities at my disposal and owe a debt of gratitude to many people, both in and outside the Department, for helping in innumerable ways.

I am very grateful to my teacher and the head of our ESR group, Professor Jorma Eloranta, for giving me the opportunity to work in his group. The subject of this investigation was proposed by acting Associate Professor Mikko Vuolle. His endless stream of new ideas, together with his interest, criticism and encouragement, have been of the greatest value and to him I extend special thanks.

Thanks are due to my research colleagues Doc. Heikki Joela and Dr. Esa Salo for advising on the data handling, and to them and Phil. Lic. Seppo Kasa and Mrs. Varpu Koski, M. Sc., for sound criticism, pleasant co-operation and patience. My thanks as well to Mrs. Sisko Paavola, Mrs. Eija Paananen and Mrs. Hilikka Heikkilä, M. Sc., for their assistance.

Dr. Kathleen Ahonen painstakingly improved the language of this manuscript and the original papers, Mr. Erkki Järvinen prepared the brass rings for r.f. coils and Mr. Reijo Kauppinen skilfully blew the special cuvette and closed-loop systems. To them my warm thanks as well.

Last but not least I owe a great debt of gratitude to my father, mother and sister for their understanding and support during these years.

Financial support of the Ellen and Artturi Nyysönen Foundation is gratefully acknowledged.

Jyväskylä, April 1990

Reijo Mäkelä

## LIST OF ORIGINAL PUBLICATIONS

This work is based on the following publications, which will be referred to in the text by their reference numbers:

1. R. Mäkelä and M. Vuolle: Electron Paramagnetic Resonance, ENDOR and TRIPLE Resonance of Some 9,10-Anthraquinone Radicals in Solution. Part 3. Hydroxyanthraquinones, *J. Chem. Soc., Faraday Trans. 1*, in press (Ref. 90).

<https://doi.org/10.1039/FT9908602569>

2. R. Mäkelä and M. Vuolle: Electron Spin Resonance, ENDOR and TRIPLE Resonance of Some 9,10-Anthraquinone Radicals in Solution. Part 2. - Anthraquinonesulfonates, *J. Chem. Soc., Faraday Trans. 1*, 1989, **85**, 4011-4017 (Ref. 91).

<https://doi.org/10.1039/F19898504011>

3. M. Vuolle and R. Mäkelä: Electron Spin Resonance, ENDOR and TRIPLE Resonance of Some 9,10-Anthraquinone and 9,10-Anthraquinol Radicals in Solution, *J. Chem. Soc., Faraday Trans. 1*, 1987, **83**, 51-55 (Ref. 92).

<https://doi.org/10.1039/F19878300051>

4. R. Mäkelä and M. Vuolle: Electron Spin Resonance, ENDOR and TRIPLE Resonance of Some 9,10-Anthraquinone Radicals in Solution. Part 4. - Anthraquinonesulfonates, *J. Chem. Soc., Faraday Trans. 1*, in press (Ref. 93).

<https://doi.org/10.1039/FT9908603257>

5. H. Joela and R. Mäkelä: The Additivity Relationship and Substituent Effects Studied by EPR and Multiresonance Spectroscopy, *J. Magn. Reson.*, 1989, **84**, 236-246 (Ref. 94).

[https://doi.org/10.1016/0022-2364\(89\)90367-3](https://doi.org/10.1016/0022-2364(89)90367-3)

## ABSTRACT

ESR, ENDOR and TRIPLE resonance spectra were recorded for 9,10-anthraquinone (I), 1,2-dihydroxy-9,10-anthraquinone (II), 1,4-dihydroxy-9,10-anthraquinone (III), 1,5-dihydroxy-9,10-anthraquinone (IV), 1,8-dihydroxy-9,10-anthraquinone (V), 1,2,5,8-tetrahydroxy-9,10-anthraquinone (VI), 2,6-dihydroxy-9,10-anthraquinone (VII), 9,10-anthraquinone-1-sodium sulfonate (VIII), 9,10-anthraquinone-1,5-disodium sulfonate (IX), 9,10-anthraquinone-2-sodium sulfonate (X), 9,10-anthraquinone-2,6-disodium sulfonate (XI), 1,2-dihydroxy-9,10-anthraquinone-3-sodium sulfonate (XII) and 2-methyl-9,10-anthraquinone (XIII) anion radicals and for 9,10-anthraquinol, 9,10-anthraquinol-1-sodium sulfonate, 9,10-anthraquinol-1,5-disodium sulfonate, 9,10-anthraquinol-2-sodium sulfonate, 9,10-anthraquinol-2,6-disodium sulfonate and 2-methyl-9,10-anthraquinol cation radicals under various chemical conditions. The hyperfine coupling constants and g-factors are given. Use of deuterated compounds and an additivity relationship allowed assignment of hyperfine coupling constants. New methods of radical generation and new solvent systems for ENDOR measurements are presented.



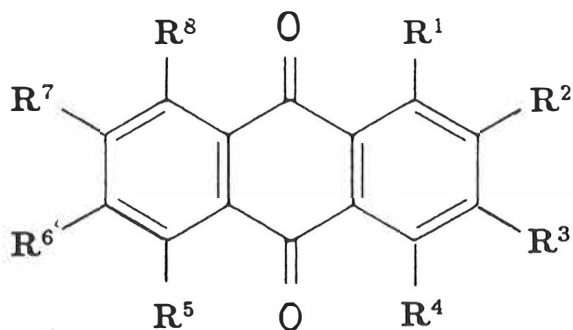
## 1. INTRODUCTION

Electron spin resonance (ESR) spectroscopy has been utilized in the study of properties and structures of a large number of naturally occurring paramagnetic quinones and quinols.<sup>1</sup> Despite the high selectivity and sensitivity of the ESR technique and the more accurate data analysis and easier extraction of hyperfine coupling (h.f.c.) constants made possible by digitized data processing, the identification of unknown radical species by this method continues to be difficult. If ESR spectroscopy is combined with ENDOR, general TRIPLE and special TRIPLE spectroscopy, a powerful tool for the investigation of paramagnetic species is available. The first published report of liquid state electron-nuclear-double-resonance (ENDOR) was that of Hyde and Maki<sup>2</sup> in 1964. An electron-nuclear-nuclear TRIPLE resonance was theoretically proposed by Freed<sup>3</sup> in 1969 and experimentally realized by Dinse et al.<sup>4</sup> in 1974. Relaxation times are shorter in liquid phase than in solid state, which means that there are extra technical and experimental problems to be solved.<sup>5-7</sup> The simulation of ESR spectra becomes more specific with the help of ENDOR data. The general TRIPLE technique makes it possible to determine the relative signs of the h.f.c. constants experimentally.<sup>8</sup>

In liquid state, 9,10-anthraquinones form at least three different ESR active species. At high pH, 9,10-anthraquinone anion radical, often called the 9,10-anthrasemiquinone radical in the literature,<sup>1</sup> is formed by one-electron reduction. In strongly acidic media, in turn, 9,10-anthraquinol cation radical<sup>1</sup> is generated by one-electron oxidation. The 9,10-anthraquinone neutral radical has been observed when the parent quinone is photolyzed in dioxane.<sup>9</sup> Many other radical products are possible as well, as will be shown below. In particular, deuterated compounds exist alongside the protonated ones.

This investigation comprises the generation and recording of spectra of anion radicals from compounds I-XIII and cation radicals from compounds I, VIII-XI, XIII (Scheme 1).

Scheme 1



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	R <sup>7</sup>	R <sup>8</sup>
I	H	H	H	H	H	H	H	H
II	OH	OH	H	H	H	H	H	H
III	OH	H	H	OH	H	H	H	H
IV	OH	H	H	H	OH	H	H	H
V	OH	H	H	H	H	H	H	OH
VI	OH	OH	H	H	OH	H	H	OH
VII	H	OH	H	H	H	OH	H	H
VIII	SO <sub>3</sub> Na	H	H	H	H	H	H	H
IX	SO <sub>3</sub> Na	H	H	H	SO <sub>3</sub> Na	H	H	H
X	H	SO <sub>3</sub> Na	H	H	H	H	H	H
XI	H	SO <sub>3</sub> Na	H	H	H	SO <sub>3</sub> Na	H	H
XII	OH	OH	SO <sub>3</sub> Na	H	H	H	H	H
XIII	H	CH <sub>3</sub>	H	H	H	H	H	H

## 2. PROPERTIES AND GENERATION OF RADICALS OF

### 9,10-ANTHRAQUINONES

#### 2.1. General

Anthraquinones (AQs) are widespread in biological systems,<sup>10</sup> where they have a vital role in electron transfer reactions.<sup>1,11-14</sup> They are by far the largest group of naturally occurring quinones.<sup>10</sup> Nearly half of them have been found in fungi and lichens, and a similar number in higher plants, where they are located in heartwood, bark, roots and occasionally in stems, seeds and fruit.<sup>15</sup>

Natural and synthetic AQs are employed as colorants in foods, drugs, cosmetics, hair dyes and textiles<sup>16</sup> and are widely used as chelating compounds and acid-base indicators.<sup>17</sup> Anthraquinones form part of the structure of anthracycline drugs, which are among the most important agents used in the treatment of human cancer.<sup>18-20</sup> And auto-oxidation of 1,8-dihydroxy-9-anthrone, which is an antipsoriatic drug, gives 1,8-dihydroxy-9,10-anthraquinone as one of the products.<sup>21</sup> AQ and its sulfonates also have significant commercial importance as accelerators for the alkaline pulping of wood.<sup>22</sup> Anthraquinone-2,6-disulfonate and anthraquinol-2,6-disulfonate are used as redox couples in layer-type semiconductor-based storage cells.<sup>23</sup> Recently, the sulfonated AQ derivatives have attracted a lot of attention because of their ready solubility in water and potential application in the storage of solar energy.<sup>24</sup>

#### 2.2. 9,10-Anthraquinone

9,10-Anthraquinone consists of two groups of four chemically and magnetically equivalent protons, which are referred to as the  $\alpha$ -protons (positions 1,4,5 and 8) and  $\beta$ -protons (positions 2,3,6 and 7). Anthraquinone forms pale yellow crystals and is only slightly soluble in cold alcohol or benzene. In general, it is a rather inert compound, but anthraquinol is a powerful reducing agent. With use of suitable reducing agents and conditions, AQ may be reduced partially or entirely. Complete reduction of the keto groups by zinc in ammonium hydroxide solution produces anthracene. Carefully controlled conditions are required to introduce one or more sulfonate groups into the anthraquinone ring.<sup>25</sup>

#### 2.3. Hydroxyanthraquinones

Most of the naturally occurring AQs are polyhydroxy and alkoxy derivatives.<sup>10</sup> Hydroxyanthraquinones are solids with high melting points. Though slightly or

not at all soluble in water, they dissolve in alkaline solutions and concentrated sulfuric acid.<sup>26</sup> Chelate formation, intramolecular hydrogen bonding<sup>26</sup> and keto-enol tautomerism<sup>27</sup> are observed for  $\alpha$ -hydroxyanthraquinones. Another characteristic reaction of the hydroxyanthraquinones is the removal of a hydroxyl group from  $\beta$ -position. Dissociation depends on the chemical conditions, as can be seen from the pK-values in table 1. 2-Hydroxyanthraquinones are also converted to dianthryl derivatives through treatment with zinc in alkaline solution.<sup>26</sup>

TABLE 1. The dissociation constants of selected substituted 9,10-anthraquinones in various solvents.

Substance	Solvent	pK <sub>a1</sub>	pK <sub>a2</sub>	pK <sub>a3</sub>	pK <sub>a4</sub>	Ref.
AQ	water, sulfuric acid	8.5				28
	aqueous alcohol	5.3				29
1,2-(diOH)-AQ	water	5.7	10.7			30
	ethanol	7.5	11.6			17
	alkaline water	8.8	10.2			31
1,4-(diOH)-AQ	ethanol	9.4	11.8			17
	alkaline water	9.2	11.7			31
1,5-(diOH)-AQ	alkaline water	9.6	11.2			31
1,2,5,8-(tetraOH)-AQ	water	5.6	9.6	10.5	11.4	30
	ethanol	5.3	9.2			17
1,2-(diOH)-AQ-3-sulfonate	water	5.7	11.0			17
	water	6.7	9.3			31
AQ-1-sulfonate	aqueous alcohol	5.4				29
AQ-2-sulfonate	water	8.2				32

Hydroxyanthraquinones can combine with metallic salts or exchange their OH groups for amino, alkylamino or arylamino groups, resulting in the formation of dyes. Acidic oxidation leads to polyhydroxyanthraquinones via the intermediate formation of acid esters.<sup>26</sup>

#### 2.4. Anthraquinone sulfonates

Anthraquinonesulfonic acids are rated as strong acids and they dissolve easily in water. In nature they usually appear as either sodium or potassium salts. The sodium salts of the monoacids are less soluble in water than those of the diacids. Sulfonic acids are relatively easily converted to hydroxy-, amino- and chloroanthraquinones. Substituents are more easily introduced to the  $\alpha$ -acids than the

$\beta$ -acids. Treatment of the  $\alpha$ -acids with concentrated sulfuric acid at 453 K leads to desulfonation of the compound. The sulfonic acid groups can also be removed by alkaline reduction, and conversion to the corresponding alkoxy derivatives can be effected by reaction with alcohols in alkaline solution.<sup>33</sup> 9,10-Anthraquinone-2- and 9,10-anthraquinone-2,6-disulfonates are strong sensitizers and 9,10-anthraquinone-1- and 9,10-anthraquinone-1,5-sulfonates weak sensitizers, corresponding to photochemically reactive and unreactive compounds, respectively.<sup>34</sup>

## 2.5. Generation of anion radicals

The anion radicals of 9,10-anthraquinones have been prepared and observed largely in alkaline alcoholic or aqueous alkaline alcoholic solutions.<sup>1</sup> At least the following methods of generation have been mentioned: pulse electron radiolysis,<sup>29</sup> photolysis,<sup>35</sup> electrolysis<sup>36-39</sup> and chemical reduction.<sup>40-50</sup> The reductants used were sodium dithionite, powdered zinc, sodium hydride, lithium, sodium and potassium metal, potassium superoxide, D-sorbitol, D-glucose and cellobiose. Organic solvents were methyl, ethyl, n-propyl, isopropyl and tert-butyl alcohols, dimethyl sulfoxide, dimethoxyethane, acetone, tetrahydrofuran, liquid crystals, hexamethylphosphoramide (HMPA) and pure dimethylformamide and its water mixtures.

The first ENDOR spectrum of AQ anion radical was recorded in ethanol by Atherton and Blackhurst.<sup>47</sup> Recently Kirste observed a strong temperature dependence in the smallest h.f.c. constant of AQ anion radical.<sup>50</sup> The magnitude of the same coupling constant decreases with decreasing polarity of the solvent.<sup>1,38</sup> The assignment of the two h.f.c. constants of the anion radical has been proposed on the basis of theoretical calculations<sup>38</sup> and confirmed experimentally by Pedersen.<sup>51</sup>

The anion radicals of hydroxyanthraquinones have mostly been generated in mixtures of water and alcohol with alkali hydroxide or alkaline sodium dithionite.<sup>1,41-55</sup> Some compounds have been deuterated and the ESR spectra scanned in the corresponding deuterated solvents.<sup>1,41,53-56</sup> Sodium hydride has been used as reducing agent in dimethylformamide.<sup>57</sup> Dimethylsulfoxide, dimethylformamide and mixtures of dimethylformamide and water or deuterium oxide, have been used as solvents with electrolysis,<sup>56</sup> and aqueous solutions have been used with ionizing radiation, uv photolysis and electrolysis.<sup>18</sup>

The anion radicals of 9,10-anthraquinonesulfonates have been produced in various alcoholic solvents by ionizing radiation, uv photolysis, or electrolysis or by chemical reduction with ascorbic acid,<sup>18,58</sup> D-sorbitol, D-glucose or cellobiose.<sup>35</sup>

Some unpublished laboratory work done in alkaline water and ethanol with sodium dithionite as reducing agent has also been described.<sup>1</sup>

Pedersen attempted to assign the ring proton h.f.c. constants of variously substituted anthraquinones with the help of an additivity rule<sup>1</sup>, but without the information on relative signs available from general TRIPLE experiments. His results disagreed with earlier assignments done by semiempirical methods.<sup>56</sup> He also studied the substitution mechanism for the hydroxyanthraquinones and remarked on the absence of the OH proton at the  $\beta$ -position. He proposed that  $\beta$ -oxido radicals are formed instead.<sup>41</sup> Only once has this OH proton been reported.<sup>53</sup> Intramolecular hydrogen bonding between carbonyl oxygen and OH proton at  $\alpha$ -position has been demonstrated by several workers.<sup>27,30,54-58</sup>

## 2.6. Generation of cation radicals

Aromatic cation radicals are obtained from the parent compounds by one-electron oxidation. Many oxidation methods have been presented, including electron impact, radiolysis, photo-ionization and anodic oxidation. Efficient chemical oxidants are strong organic or inorganic acids, alone or together with oxidizing agents such as  $\text{AlCl}_3$  and  $\text{SbCl}_5$ . Some chloro- and cyano-substituted 1,4-benzoquinones form cation radicals in strong acids and these further oxidize electron-rich aromatic molecules to their corresponding cation radicals.<sup>59</sup> Another example of this charge-transfer process is the generation of some sulfur-containing cation radicals with the help of 1,4-benzoquinone cation radical.<sup>60</sup>

Quinones form doubly protonated and stable cation radicals in strongly acidic media.<sup>61</sup> For generation of cation radicals of quinones the following general systems have been used: aluminium chloride and nitromethane,<sup>62</sup> sulfuric acid and nitromethane, trifluoroacetic acid, and trifluoroacetic anhydride with uv photolysis.<sup>63</sup>

Only a few ESR studies have been done on cation radicals generated from 9,10-anthraquinone and hydroxyanthraquinones. Radicals were produced with zinc dust in concentrated sulfuric acid and deuterated sulfuric acid.<sup>61,64</sup> The h.f.c. constants of 9,10-anthraquinol cation radical have been assigned with the help of MO calculations. Deuteration of the OH protons resulted in a cation with marked temperature dependence.<sup>61</sup>

### 3. THEORY OF ESR, ENDOR AND TRIPLE RESONANCE SPECTROSCOPY

#### 3.1. General

The theory of ESR, ENDOR and TRIPLE resonance spectroscopy has been well reviewed in several papers<sup>65,66</sup> and textbooks<sup>5-7,67-70</sup> and will be dealt with here in only a summary way. Analysis of the ESR spectrum of free radicals in solution reveals the magnitude and multiplicity of the h.f.c. constants but not always in an unambiguous way. When there are many overlapping lines with small h.f.c. constants almost equal in magnitude, insufficient resolution often becomes a problem. The ENDOR spectrum offers selectivity because each type of nucleus gives a pair of lines centred around the free nuclear frequency and separated by the h.f.c. constant. Another advantage of ENDOR spectroscopy is that it allows two or more radicals in a mixture to be distinguished if just one ESR line belonging uniquely to one type of radical can be identified.

The h.f.c. constants are more easily detected in the ENDOR spectrum than the ESR spectrum because of the lower spectral density. The h.f.c. constants are also more accurately measured in the ENDOR spectrum, the information on multiplicity is lost, however. According to theory, in the special TRIPLE the signal intensities reflect the ratio of numbers of equivalent nuclei in a molecule.<sup>66</sup> In practice, however, the spectroscopist can trust the result in only a very few cases. An important additional feature of the double ENDOR technique is the possibility of determining the relative signs of the h.f.c. constants, which is done by comparing the intensity differences of the corresponding ENDOR and general TRIPLE lines.<sup>66</sup>

If the intensity of a pair of lines is the same in the general TRIPLE spectrum as in the ENDOR spectrum, the lines may have been produced by different radical species or by protons having two h.f.c. constants of opposite sign. It is still possible to perform the ENDOR-induced ESR experiment when the ENDOR spectrum represents two or more radical species.<sup>65</sup> In this experiment the external magnetic field is swept over the range of ESR absorption and at the same time the ENDOR line is pumped by the appropriate NMR frequency. If two different radical species exist, a spectrum can be recorded from each of the two pumped NMR frequencies.

#### 3.2. Important instrumental parameters in ENDOR experiments

The Bruker ENDOR spectrometer is computer controlled, but the scanning parameters such as the critical coupling, detector current, microwave power and

field position have to be optimized. When all the microwave energy has been coupled into the cavity and there are no reflections from the iris, the cavity and the sample are said to be critically coupled.<sup>71</sup> Usually the best sample position is found by lifting or lowering the sample tube when the nearly coupled system is achieved. The usual definition of the quality factor of the cavity is<sup>67</sup>

$$Q = \frac{2\pi * \text{energy stored}}{\text{energy dissipated per cycle}} \quad (1)$$

In general, the  $Q_0$ -factor is defined

$$Q_0 \simeq \frac{\text{volume of the cavity}}{\delta * \text{surface area of the cavity}} \quad (2)$$

where  $\delta$  = skin depth in gaussian units.

ENDOR signals are generally observed if ESR transitions are saturable at the microwave power levels available, up to 30 mW, and if NMR transitions are saturable at a radio frequency (r.f.) power less than 200 mW. The use of very high r.f. powers is liable to cause a distortion of ENDOR line shape - the so-called coherence effect<sup>72</sup> - and problems with the baseline. Especially in the case of narrow ESR lines the sensitivity and the line shape of the ENDOR spectrum depend on the stability of the magnetic field and microwave frequency. Drift can be prevented by using a field frequency lock. The higher resolution needed to compensate for the line broadening due to the frequency modulation (FM) can be achieved by making the modulation depth of the r.f. field smaller than half of the peak to peak ENDOR line width.<sup>73</sup>



### 3.3. Important experimental parameters in ENDOR experiments

Usually the intensity of the ESR signal increases with increase in the effective sample volume and with increase in the Q-factor. The ENDOR experiment demands large sample volumes because the ENDOR intensity is only a few per cent of the ESR intensity.<sup>65</sup> The spin concentration can also be increased by preparing more concentrated samples, but this has the undesirable effect of increasing the probability of collisions between the radical species and its diamagnetic precursor, with transfer of the unpaired electron to the precursor. If this chemical exchange rate becomes larger than the electron spin relaxation rate, the hyperfine interaction will effectively be averaged out.<sup>74</sup> Also, with increasing concentration the spin-spin exchange between two colliding radicals increases. If this so-called Heisenberg exchange rate is much larger than the electron spin-lattice relaxation rate, the ESR transition between any two levels cannot be saturated and an ENDOR signal will no longer be detected.<sup>74</sup>

The ENDOR enhancement has been shown to depend critically on the relative magnitudes of rates of electron spin relaxation ( $W_e$ ), nuclear spin relaxation ( $W_n$ ) and cross relaxations. The relaxation rates can be changed by adjusting temperature and viscosity, because the electron spin relaxation rate is inversely proportional and nuclear spin relaxation rate directly proportional to the rotation correlation time,  $\tau_R$ . The desired ratio is  $W_n/W_e \cong 1$ . The rotational correlation time can be estimated from the Debye-Einstein relation

$$\tau_R = V_{eff} \frac{\eta}{k_B T} \quad (3)$$

where  $V_{eff} = 4/3\pi r^3$  is the effective tumbling volume of the molecule approximated as a sphere with radius  $r$ ,  $\eta$  is the dynamic viscosity of the solvent,  $T$  is the absolute solvent temperature and  $k_B$  is the Boltzman constant. The ENDOR enhancement is optimized near the freezing point of the solution with such solvents as DME, THF and toluene, but in solvents of high viscosity, like mineral oil, it may be found at room temperature.<sup>66</sup>

### 3.4. $^2\text{H}$ ENDOR spectroscopy

In ESR, deuteration of radical species that have relatively large h.f.c. constants is usually detected as a decrease in the spectral width and increase in the number of lines. In the case of small proton coupling constants, deuteron replacement is observed only in a sharpening of the ESR line widths. These effects are due to the different nuclear spin: deuteron has  $I=1$  and proton has  $I=1/2$ . In ENDOR spectroscopy the deuterium labelling is detected as an absence of the corresponding proton coupling, the free deuteron frequency being 6.5 times lower than the free proton frequency. The isotropic h.f.c. constant for proton is multiplied by the ratio  $\gamma_{2\text{H}}/\gamma_{1\text{H}} = 0.154$  when a proton is replaced by a deuteron at the same molecular position. The optimum conditions for detecting a deuteron ENDOR spectrum include stronger r.f. fields and lower temperatures or higher viscosities than for protons. This is because the rotational correlation time is decreased in the case of deuterium. The best detectable ratio of proton to deuteron ENDOR line amplitudes is theoretically predicted as 2:1; experimentally the ratio 3:1 has been found. Deuteron ENDOR lines are expected to be half as wide as those of protons.<sup>75</sup>

### 3.5. The additivity relationship

The additivity rule provides a useful method of assigning the h.f.c. constants of protons in radicals.<sup>76-82</sup> Introduction of a substituent to a molecule causes a small perturbation in the spin density on every atom, and every additional substituent causes a further perturbation. If the additivity relationship holds, the total effects on any atom can be calculated by summing the perturbations due to each of the substituents. In principle the perturbation must be small enough to be presented by first order perturbation theory. The method requires the unique assignment of the h.f.c. constants of the parent compound (here 9,10-anthraquinone), and measurement of the coupling constants, with correct relative signs, of several compounds differently substituted with the same substituent. The constants have to be measured accurately under exactly similar conditions as regards solvent, temperature and concentration of substrate and reducing or oxidizing agent.

The additivity rule was first published by Venkataraman et al.<sup>82</sup> for the h.f.c. constants of methyl- and chloro-substituted 1,4-benzosemiquinones. Since then it has been modified and applied to a wide variety of systems.<sup>76-81</sup> Pedersen was the first to apply it to 9,10-anthraquinones.<sup>1,41,51</sup>

## 4. EXPERIMENTAL

### 4.1. Equipment

The ESR spectra were obtained with a Varian E9 or a Bruker ESP-300 FT-ESR X-band spectrometer. The Varian E9 spectrometer was provided with a Varian E-500 NMR gaussmeter, a Takeda Riken TR5211D microwave counter, an Apple II EUROPLUS microcomputer and a Varian E257 variable temperature control unit controlled with the help of an electric thermometer (accuracy  $\pm 2$  °C). This system was used for g-value measurements and for collecting and simulating the ESR spectra. Better resolution was obtained with the Bruker ESP-300 spectrometer because of the variable field modulation facility (1.56- 100 kHz) and the more modern technique. The measuring temperature was controlled with a Bruker variable temperature unit ER4111VT (accuracy  $\pm 0.5$  °C). The ESP-1600 data system was used for data collecting and data handling.

The ENDOR and TRIPLE resonance spectra were measured with a Bruker ER-200D-SRC spectrometer equipped with 12" Varian magnets, a Varian E-272B field frequency lock and a Bruker variable temperature unit ER4111VT (accuracy  $\pm 0.5$  °C). The system operates at X-band with 12.5 kHz field modulation. Other facilities were a Bruker ENDOR cavity ER 200 ENB of TM<sub>110</sub> type with an internal laboratory-made NMR coil, an ENDOR unit EN 810 with two different frequency synthesizers and an A-500 r.f. power amplifier (500 W). An Aspect 2000 minicomputer controls the measurements, collects ENDOR/TRIPLE spectra and is capable of simulating the ESR spectra.

### 4.2. An ENDOR/TRIPLE experiment

The ESR signals are plotted on the x,y recorder of the Bruker console after phase-sensitive detection and amplification. The microwave power is increased until saturation of the ESR signal is achieved, and the magnetic field is adjusted to the zero passage of the first derivative of the selected ESR line by means of the field frequency lock. The field modulation is then switched off. In the ENDOR experiment a Wavetek synthesizer generates the 12.5 kHz square wave (FM) NMR frequency signal, which is amplified, passed through the NMR coil in the cavity and dissipated in a 50  $\Omega$  water cooled load. The change in the ESR absorption of the sample induced by the NMR transitions undergoes phase-sensitive detection, amplification and modulation and is stored in the minicomputer.

In the general TRIPLE resonance experiment, one ENDOR line is selected for irradiation with unmodulated radio frequency. The pumping frequency (PF),

which is generated by an independent TRIPLE oscillator, is positioned at the centre of the first derivative ENDOR signal. In the special TRIPLE resonance experiment the pumping frequency is positioned at the free nuclear frequency. Two frequency modulated sidebands are produced in such a way that the low and high frequency ENDOR transitions for all h.f.c. constants of the same type of nuclei are irradiated simultaneously.<sup>71</sup> The pumping frequency covers all h.f.c. constants smaller than 1 MHz.

### 4.3. ENDOR/TRIPLE cavity

The heart of an ENDOR spectrometer is the resonator, which forms the microwave and r.f. field and simultaneously functions as a sensitive device for the detection of changes in the ESR signal intensity. The desirable attributes of an ENDOR probe head are large r.f. bandwidth, high r.f. and microwave magnetic fields over the large r.f. bandwidths and the ability to support variable temperature dewars while still maintaining a large sample volume area. The Bruker ENDOR cavity with mode  $TM_{110}$  largely meets these requirements and furthermore allows the presence of an r.f. coil inside the resonator. This broadband arrangement is particularly well suited for liquid phase ENDOR and TRIPLE resonance investigations.<sup>71</sup>

The first ENDOR spectrometer in our laboratory was a low power (20 W) instrument, which challenged us to develop more powerful NMR coils. The r.f. field strength is increased by increasing the number of turns,<sup>65</sup> which is limited by the effect on the cavity filling factor. The new high power ENDOR (500 W) instrument has a thin-walled quartz Dewar, which is easily broken if the temperature changes too rapidly. The thickness of the walls of low temperature fingers varies, which means that the number of turns to be wrapped around the fingers varies too. Naturally the resonance frequency varies with the glassware and the number of turns of the NMR helix because the filling factor changes.

Our laboratory-made ENDOR coil was modelled after the Bruker coil.<sup>83</sup> Two brass rings exactly sized for the cavity and a thin-walled Wilmad quartz Dewar were positioned on the Dewar so as to be flush with the cavity casing and fastened with Tammer glue (elastic glue) at just one point. The end of a flattened silver (original diameter 0.3 mm) thread was soldered to one of the rings, wrapped by hand tightly and regularly around the quartz finger and soldered to the second brass ring. A tiny drop of cyanoacrylate was used to attach the helix at three points in every loop. Most of the work of this study was done with a 15-turn silver helix with a resonance frequency of 9.38 GHz and resonance field of 3285 G. Rapid temperature changes had to be avoided during experiments because quartz

and metal have different coefficients of thermal expansion. Gaseous nitrogen flow inside the cavity protects the coil against overheating.

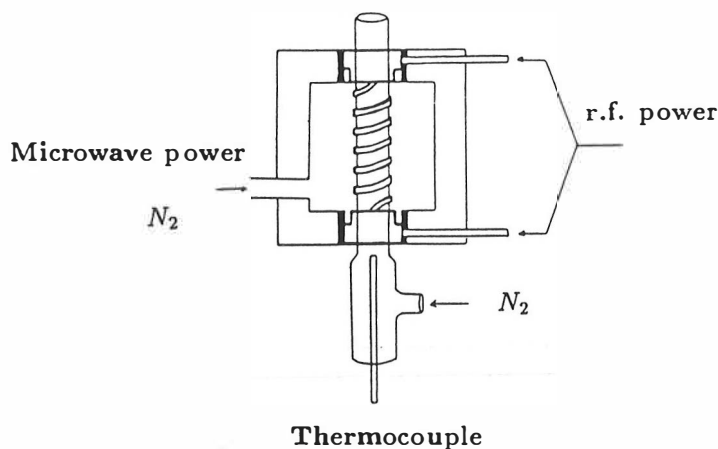


Fig. 1. The positioning of the r.f. coil inside the cavity.

#### 4.4. Sample preparation

##### 4.4.1. Anion radicals

The anion radicals for this study were generated with sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ), tetracyanoethylene (TCNE) and uv light, and ascorbic acid and uv light, in five different solvent systems: ethanol-water, ethanol- $\text{d}_1$ - $\text{D}_2\text{O}$ , tetrahydrofuran, dimethylsulfoxide and ammonia. In the case of TCNE, the anion radical that immediately formed from TCNE died during the uv illumination, producing the AQ anion radical. The mechanisms by which TCNE and uv light and ascorbic acid and uv light reduce AQ are not yet known. Black painted cuvettes were needed when the AQ had an OH-substituent at the 2-position.

##### 4.4.1.1. Ethanol - water and ethanol- $\text{d}_1$ - $\text{D}_2\text{O}$ mixtures

To find the optimum conditions for water and ethanol mixtures and proper concentrations of starting material and reducing agent at suitable pH, methods faster and more convenient than vacuum technique were developed. The special cuvette depicted in figure 2 was specially designed for the purpose.

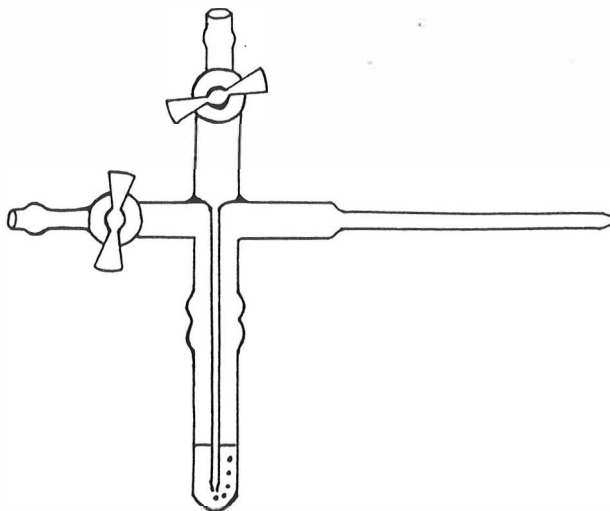


Fig. 2. The special cuvette with two vacuum stopcocks.

After the system was evacuated, the substances needed were added one by one, argon was bubbled through the mixture and gaseous ammonia was added until the desired pH was reached. Before measurement of the sample, argon was bubbled for 20 minutes more through the solution to remove the residual oxygen. The special cuvette sample gave nearly as sharp lines as a vacuum sample but the lifetime of radicals was shorter.

Another useful system for searching for the best radical yield was a closed-loop flow system; this required larger amounts of chemicals and worked only for ESR measurements at room temperature, however. Once the optimal conditions were found, the classical vacuum samples gave the best results. The special cuvette and closed-loop flow system were equally useful for investigating heavy water and ethanol- $d_1$  systems ( $D_2O$  and  $CH_3CH_2OD$ ).

#### 4.4.1.2. Tetrahydrofuran (THF)

The samples were prepared at the port of a high vacuum line through which residual oxygen could be removed in several freeze-pump-thaw cycles. THF was distilled over liquid Na-K alloy and potassium mirror was used as reducing surface.

#### 4.4.1.3. Dimethylsulfoxide (DMSO)

The classical vacuum samples were prepared from starting material,  $\text{KO}_2$  and DMSO. Uv light generated the corresponding anion radical.

#### 4.4.1.4. Ammonia ( $\text{NH}_3$ )

Liquid ammonia with alkali metal forms a well-known reductive solvent system.<sup>84</sup> Gaseous ammonia was bubbled into a cuvette in a cold bath and a small piece of sodium metal was dropped in along with the parent compound. This was done under nitrogen atmosphere. Finally the cuvette was evacuated with a high vacuum line. Because of the high pressure inside the ampoule the samples had to be stored in a refrigerator. Except for the radical anion of the 9,10-anthraquinone, the ammonia system was found unsuitable for radical generation because of the many substitution products.

#### 4.4.2. Cation radicals

Cation radicals were successfully generated from 9,10-anthraquinone, 2-methyl-9,10-anthraquinone and anthraquinone sulfonates in strongly acidic media under uv illumination. Radical yield was good when chemicals and ampoules were very dry. Oxygen had to be removed carefully using a high vacuum line and several freeze-pump-thaw cycles. Trifluoromethanesulfonic acid ( $\text{CF}_3\text{SO}_3\text{H}$ ), trifluoroacetic acid ( $\text{CF}_3\text{COOH}$ ), fluorosulfonic acid ( $\text{FSO}_3\text{H}$ ), sulfuric acid ( $\text{H}_2\text{SO}_4$ ), dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) and sulfonylchlorofluoride ( $\text{SO}_2\text{ClF}$ ) and their mixtures were used as solvents. The OH protons were assigned by using deuterated sulfuric acid ( $\text{D}_2\text{SO}_4$ ) and deuterated trifluoroacetic acid ( $\text{CF}_3\text{COOD}$ ).

### 5. RESULTS AND DISCUSSION

#### 5.1. The ENDOR solvents

It is useful to know how different solvents behave during ENDOR measurements. Especially important properties are melting and boiling points, viscosities and dielectric constants. The viscosities of ethanol, methanol and sulfuric acid increase exceptionally quickly with decreasing temperature. Likewise the dielectric constants of ethanol, methanol and water increase dramatically when the temperature drops (see table 2). Trifluoromethanesulfonic and fluorosulfonic acid, which

are two of the strongest simple protic acids known, must be handled with special care.<sup>85,86</sup>

Table 2. The melting and boiling points, viscosities and dielectric constants of the solvents.

Solvent	Melting point (°C)	Boiling point (°C)	Viscosity (cp) (°C)		Dielectric constant (°C)		Ref.
H <sub>2</sub> O	0.0	100	1.770	0	87.74	0	87
			0.284	100	55.72	100	87
D <sub>2</sub> O	3.82	101.43	1.098	25	78.25	25	87
CH <sub>3</sub> OH	-97.7	64.7	0.547	25	32.63	25	87,88
			13.9	-98.3	64	-113	89,88
CH <sub>3</sub> CH <sub>2</sub> OH	-114	78.3	1.078	25	24.30	25	87,88
			44	-98.11	41.0	-60	89,88
CH <sub>3</sub> CH <sub>2</sub> OD		78.8					87
CF <sub>3</sub> COOH	-15.3	71.8	0.926		8.55		87
CF <sub>3</sub> COOD		75					87
CF <sub>3</sub> SO <sub>3</sub> H	34	162	2.87				87,86
CH <sub>2</sub> Cl <sub>2</sub>	-96.7	40.5	0.449	15	9.08	20	87,88
DMSO	18.5	189.0	1.996	25	46.6	25	87
THF	-108.5	66	0.55		7.58	25	87
NH <sub>3</sub>	-77.75	-33.42	0.254	-33.5	25	-77.7	87,88
			0.475	-69	16.9	25	89,88
H <sub>2</sub> SO <sub>4</sub>			48.4	0			89
	10.38	280	24.54	25	100	25	87
			5.19	80			89
D <sub>2</sub> SO <sub>4</sub>	14.35						87
FSO <sub>3</sub> H	-87.3	165.5	1.56	25	120	25	87
SO <sub>2</sub> ClF	-124.7	7.1					87

Optimum ENDOR enhancement requires that temperature be carefully selected for each solvent separately. In THF and DMSO the ENDOR signal was best near the freezing point. Lowering the temperature increases viscosity and decreases microwave and r.f. powers and improves signal to noise ratio. A single solvent seldom offers a wide enough temperature range to ensure sharp ENDOR



lines. Trifluoromethanesulfonic acid was found to be an exception to this, and particularly wide ranges were available with the mixed solvents water and ethanol and fluorosulfonic acid and sulfurylchlorofluoride (table 3). If the value of the dielectric constant of the solvent is greater than 40, the inside diameter of the sample tube must be smaller than 1 mm.

Table 3. The optimum temperature range for the ENDOR experiment in different solvents.

Solvent	ENDOR temperature (°C)
THF	-95 - -70
DMSO	+10 - +40
NH <sub>3</sub>	-77 - -20
H <sub>2</sub> O and CH <sub>3</sub> CH <sub>2</sub> OH	-60 - +10
D <sub>2</sub> O and CH <sub>3</sub> CH <sub>2</sub> OD	-13 - +17
FSO <sub>3</sub> H and SO <sub>2</sub> ClF	-80 - +5
CF <sub>3</sub> SO <sub>3</sub> H	-40 - +40
FSO <sub>3</sub> H and D <sub>2</sub> SO <sub>4</sub>	-10 - +20
CF <sub>3</sub> SO <sub>3</sub> H and D <sub>2</sub> SO <sub>4</sub>	-15 - +20
D <sub>2</sub> SO <sub>4</sub> and SO <sub>2</sub> ClF	-15 - +20

## 5.2. Anion radicals

### 5.2.1. 9,10-Anthraquinone

Strong and stable anion radical solutions of 9,10-anthraquinone was easily produced in all five solvent systems. The ENDOR spectra showed a clear decrease in the  $\alpha$ -proton h.f.c. constant from 0.056 mT to 0.030 mT when the solvent was changed from ethanol to DMSO, but the  $\beta$ -proton stayed unchanged. Anion radicals deuterated at positions 1 and 2 were synthesized from 1- and 2-chloro-9,10-anthraquinone. Deuterium replacement was especially clearly detected by ESR in the case of 2-(<sup>2</sup>H)-9,10-anthraquinone anion radical.<sup>90</sup>

The deuterium and proton ENDOR spectra were recorded with the same scanning parameter sets at  $-15\text{ }^{\circ}\text{C}$  (Fig. 3).

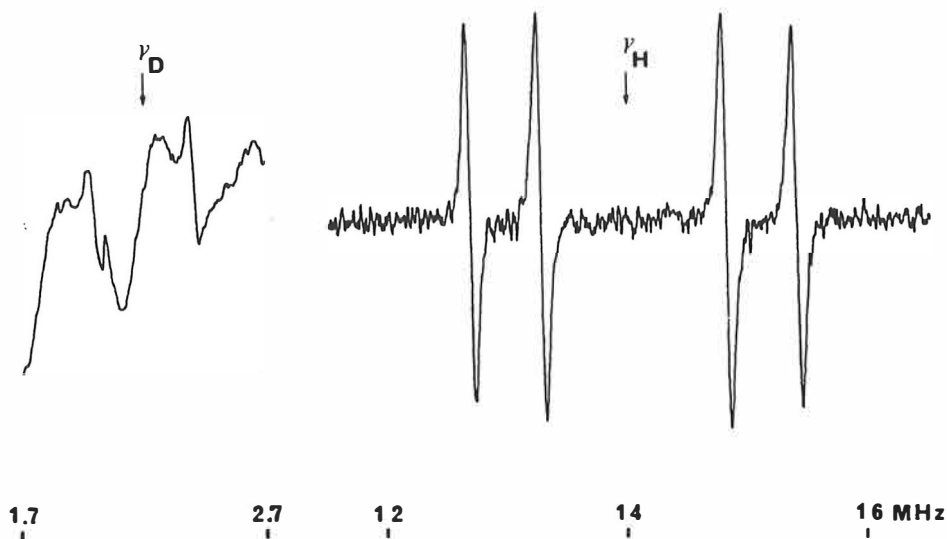


Fig. 3. The ENDOR spectrum of 2-( $^2\text{H}$ )-9,10-anthraquinone anion radical in a mixture of  $\text{D}_2\text{O}$  and  $\text{CH}_3\text{CH}_2\text{OD}$  (1:2) at  $-15\text{ }^{\circ}\text{C}$ .

The microwave and r.f. powers needed were 12 mW and 100 W. Free proton and deuterium frequencies were 14.040 and 2.172 MHz, and linewidths 80-100 and 50 kHz, respectively. Distortion of the baseline due to the relatively high r.f. power complicated the analysis of spectra.

### 5.2.2. Hydroxyanthraquinones

$\alpha$ -Hydroxyanthraquinones easily produced strong and long-lived anion radicals in all solvent systems except ammonia, but the magnitude of the h.f.c. constants varied with solvent and temperature. The intensity of the signals for OH protons decreased when temperature was decreased, while that of other protons

remained unchanged. This effect was clearly recorded for 1,4-dihydroxy-, 1,5-dihydroxy- and 1,8-dihydroxy- 9,10-anthraquinone anion radicals.<sup>90</sup>

$\beta$ -Hydroxyanthraquinones were very sensitive to chemical conditions. They dissolved in water better than  $\alpha$ -hydroxyanthraquinones. At least 20% water was needed in alcoholic solution. Side reactions often occurred in the presence of light, high pH and an excess of reducing agent. Optimal conditions for all studied  $\alpha$ - and  $\beta$ -hydroxyanthraquinones were found in alkaline aqueous ethanol (25%/75%) (v/v) at pH 9. Although couplings due to from  $\beta$ -hydroxyl protons were not detected with certainty, the ESR linewidths were considerably sharper when deuterated solvents were used.

The radical concentrations were optimized for ENDOR measurements so that the r.f. and microwave powers needed were 100 W and 2-20 mW. In general the spectra of hydroxyanthraquinones consisted of accidentally magnetically equivalent protons, a finding that was confirmed by simulation of the ESR spectra. A deuteron ENDOR spectrum was obtained only from deuterated 1,2,5,8-tetrahydroxy-9,10-anthraquinone anion radical in which three accidentally equivalent OH protons were replaced with deuterons (Fig. 4). One of the h.f.c. constants of the ring protons had an opposite sign according to a general TRIPLE experiment.

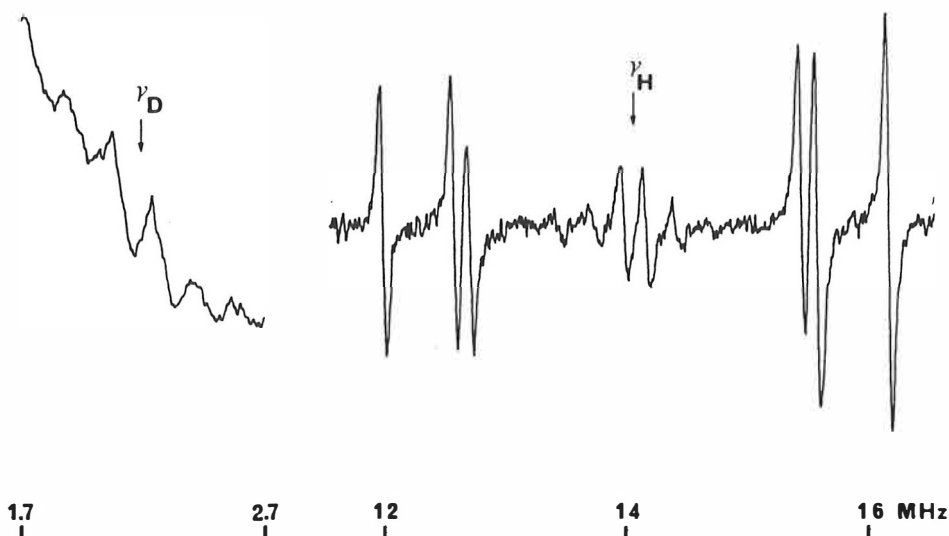


Fig. 4. The ENDOR spectrum generated from 1,2,5,8-tetrahydroxy-9,10-anthraquinone with sodium dithionite in a mixture of  $D_2O$  and  $CH_3CH_2OD$  (1:2) at  $-10^\circ C$ .

### 5.2.3. Anthraquinone sulfonates and 1,2-dihydroxy-9,10-anthraquinone-3-sodium sulfonate

Chemical conditions played an essential role in the anion radical generation in alkaline aqueous ethanol, as discussed in the original papers.<sup>91,92</sup> Strong and stable solutions of anion radicals could be produced from 9,10-anthraquinone-2-sodium sulfonate and 9,10-anthraquinone-2,6-disodium sulfonate but not from 9,10-anthraquinone-1-sodium or 9,10-anthraquinone-1,5-disodium sulfonate. The  $\alpha$ -substituted sulfonates dissolved only sparingly in the solvent mixtures, even when the sample was heated and the pH elevated. Moreover, accidental magnetic equivalency and the missing of some h.f.c. constants sometimes made spectral analysis tedious. The same problems were observed in the spectra of 1,2-dihydroxy-9,10-anthraquinone-3-sodium sulfonate anion radical. The h.f.c. constant of the 2-hydroxyl proton was assumed to remain within a broad ESR line.

### 5.3. Cation radicals

As described in the original papers<sup>92,93</sup> cation radicals were produced from 9,10-anthraquinone, 2-methyl-9,10-anthraquinone and the sulfonated anthraquinones in strong organic fluoroacids or sulfuric acid and their mixtures, with uv light. The increase in radical concentration was followed by ESR by generating radicals with uv light directly in the cavity. Mixtures of deuterated and protonated solvents gave mixtures of partially deuterated cation radicals. When cation radicals were generated in fully deuterated solvents, hydroxyl protons were replaced with deuterons, except for 1-sulfonate which was deuterated only partly and 1,5-disulfonate which was not deuterated at all. The ENDOR measurement of unstable radicals required that all scanning parameters and concentrations be optimized beforehand. Radicals cannot be generated during ENDOR detection but have to be generated outside the cavity. In the best cases, a lifetime of just half an hour was sufficient for the experiment.

### 5.4. The additivity relationship applied to 9,10-anthraquinones

The ESR spectrum of 9,10-anthraquinone anion radical, which has  $D_{2h}$  symmetry, can be simulated with two quintets arising from the four  $\alpha$ -protons and four  $\beta$ -protons. Deuterium labelling allows assignment of the h.f.c. constants and picks up the OH protons in the case of hydroxy-substituted AQs. If a substituent is introduced to position  $i$ , it causes perturbation  $d_{ij}$  at position  $j$ ; and if there are more substituents than one, the h.f.c. coupling constant at position  $j$  can be calculated by summing the separate perturbations due to each substituent.

$$a_j = a_k + \sum_{i,j=1, i \neq j}^8 d_{ij}, \quad k = 1, 2 \quad (4)$$

Consider 1,2,5,8-tetrahydroxy-9,10-anthraquinone anion radical, for example. The coupling constant of the proton at position 4 can be calculated with the help of the perturbation parameters

$$a_4 = a_1 + d_{14} + d_{24} + d_{18}(=d_{54}) + d_{15}(=d_{84}) \quad (5)$$

and correspondingly

$$a_3 = a_2 + d_{13} + d_{23} + d_{17}(=d_{53}) + d_{16}(=d_{83}) \quad (6)$$

$$a_6 = a_2 + d_{16} + d_{26} + d_{12}(=d_{56}) + d_{13}(=d_{86}) \quad (7)$$

$$a_7 = a_2 + d_{17} + d_{27} + d_{13}(=d_{57}) + d_{12}(=d_{87}) \quad (8)$$

The symmetry is used to minimize the subscript j. The method developed to explore these perturbation parameters is described in detail in the original paper.<sup>94</sup> The factor of perturbation (=additivity) parameter  $d_{ij}$  is 0 if there is no substituent at position i and 1 if there is. Thus linear equations of the following form are solved for protons at positions 3,6 and 7.

$$\begin{pmatrix} 0 & 1 & 1 & 1 & 1 & 0 & 0 & 1 \\ 1 & 1 & 0 & 1 & 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 1 & 0 & 0 & 1 & 1 \end{pmatrix} \begin{pmatrix} d_{12} \\ a_2 \\ d_{23} \\ d_{13} \\ d_{16} \\ d_{26} \\ d_{27} \\ d_{17} \end{pmatrix} = \begin{pmatrix} a_3 \\ a_6 \\ a_7 \end{pmatrix}$$

For the present set of hydroxy compounds, 13 equations are formed for protons at positions 1,4,5 and 8 and nine for compounds with protons at positions 2,3,6 and 7. The computer program solves these two groups of equations and gives the h.f.c. constants  $a_1$  and  $a_2$  of 9,10-anthraquinone anion radical and 14 different perturbation parameters. The goodness of fit can be followed in two ways: by looking at the R factor<sup>77</sup>

$$R = \sqrt{\frac{\sum_{n=1}^N (O_n - C_n)^2}{N - k}} \quad (9)$$

where  $O_n$  are the  $N$  observed coupling constants,  $C_n$  are the  $N$  calculated coupling constants and  $k$  is the number of compounds, and at the differences between the calculated and experimental h.f.c. constants of 9,10-anthraquinone anion radical.

A change of substituent means that another group of equations must be solved. Ten equations each can be written for the  $\alpha$ -ring protons and  $\beta$ -ring protons of the four 9,10-anthraquinonesulfonates under study. To obtain a better solution for equations arising from the anion radicals, 9,10-anthraquinone-1,8-disulfonate and 1,2-dihydroxy-9,10-anthraquinone-3-sodium sulfonate were included in the data set. Because the values for 9,10-anthraquinone-1,8-disulfonate were taken from the literature and 1,2-dihydroxy-9,10-anthraquinone-3-sodium sulfonate does not properly belong to the set, the solution should be considered tentative. The h.f.c. constants of the cation radicals of 9,10-anthraquinonesulfonates can be assigned only partly with the data available.

## SUMMARY

The anion radicals were generated from every compound of the study in at least two different ways. The most suitable solvent system overall was alkaline aqueous ethanol. The optimal conditions for the water and ethanol mixture and for the D<sub>2</sub>O and ethanol-d<sub>1</sub> mixture were the same. The formation of secondary radical species disturbed the production of anion radicals from hydroxyanthraquinones hydroxylated at position 2.

The cation radicals were tedious to generate. Best radical yields were obtained from anthraquinone sulfonates, with uv light, in trifluoromethanesulfonic acid and the mixtures of strong acids. Hydroxyl protons were deuterated in the deuterated acids, but the radical concentrations were usually very small. Concentrations of the cation radicals generated from hydroxyanthraquinones were weak owing to the poor solubility of the starting material, and secondary radicals interfered with the measurement. Better methods for the generation of cation radicals of hydroxyanthraquinones are being developed.

The best resolved ENDOR spectra of anion and cation radicals were scanned from 2-methyl-9,10-anthraquinone. Accidentally magnetically equivalent protons were frequently observed with other compounds. Sometimes a change of solvent or method of radical generation helped to separate the h.f.c. constants of the protons. Another problem was the absence of the coupling constants of the 2-hydroxyl proton. Lines were usually broader in the ESR spectra of the anion radicals of 2-hydroxyl compounds than of  $\alpha$ -hydroxyl compounds and the lines of the corresponding deuterated species were sharper than those of the protonated ones. These findings suggested that the h.f.c. constant of the 2-hydroxyl proton might be below the detection limit. The smallest detected h.f.c. constant was 0.006 mT, while the experimental accuracy was estimated to be  $\pm 0.002$  mT for ENDOR measurements and  $\pm 0.004$  mT for ESR measurements.

The assignment of h.f.c. constants can be done by several methods. The most correct but also the most tedious way is selective deuteration of protons position by position. Results obtained with INDO calculations have so far not been in good agreement with the experimental data. The additivity relationship method could be applied because a way was found of measuring the h.f.c. constants of the different compounds under identical chemical conditions. In the case of hydroxyanthraquinones the set of compounds investigated was large enough for correct assignment of the ring protons. The method assumes the unambiguous assignment of the protons of 9,10-anthraquinone radical and hydroxyl protons. The assignment



of the anion radicals of anthraquinone sulfonates was uncertain, on the other hand, owing to the use of literature values for 9,10-anthraquinone-1,8-disulfonate and the supplementation of the set with 1,2-dihydroxy-9,10-anthraquinone-3-sodium sulfonate in calculations. Moreover the number of cation radicals was so small that only a few positions of the ring protons could be fixed.

The radicals studied in this work can be used as models for analyzing complicated biological radical systems. New anion radicals of hydroxyanthraquinones and anthraquinone sulfonates can be assigned on the basis of the perturbation parameters calculated here. The new generation methods, especially those developed for cation radicals, appear to be suitable for other types of starting materials.

## REFERENCES

1. J.A. Pedersen, *Handbook of EPR Spectra from Quinones and Quinols* (CRC Press, Boca Raton, Florida, 1984) and references therein
2. J.S. Hyde and A.H. Maki, *J. Chem. Phys.*, 1964, **40**, 3117
3. J.H. Freed, *J. Chem. Phys.*, 1969, **50**, 2271
4. K.P. Dinse, R. Biehl and K. Möbius, *J. Chem. Phys.*, 1974, **61**, 4335
5. C.P. Poole, Jr., *Electron Spin Resonance, A Comprehensive Treatise on Experimental Techniques* (John Wiley, New York, 2nd edn, 1963)
6. H. Kurreck, B. Kirste and W. Lubitz, *Electron Nuclear Double Resonance Spectroscopy of Radicals in Solution, Application to Organic and Biological Chemistry*, (VCH Publishers, New York, 1988) and references therein
7. L. Kevan and L.D. Kispert, *Electron Spin Double Resonance Spectroscopy* (John Wiley, New York, 1976)
8. Ref. (6.) pp. 20-26
9. S.K. Wong, W. Sytnyk and J.K.S. Wan, *Can. J. Chem.*, 1972, **50**, 3052
10. R.H. Thomson, *Naturally Occurring Quinones*, (Academic Press, London, 1971)
11. L.E. Echevoyen, H.K. Yoo, V.J. Gatto, G.W. Gokel and L. Echevoyen, *J. Am. Chem. Soc.*, 1989, **111**, 2440
12. M. Delgado, D.A. Gustowski, H.K. Yoo, V.J. Gatto, G.W. Gokel and L. Echevoyen, *J. Am. Chem. Soc.*, 1988, **110**, 119
13. E. Ozeki, S. Kimura and Y. Imanishi, *J. Chem. Soc., Chem. Commun.*, 1988, 1353
14. V.A. Kuzmin, A.K. Chibisov and A.V. Karyakin, *International J. Chem. Kinetics*, 1972, **4**, 639
15. Ref. (10.) pp. 1-2
16. J.P. Brown, *Mutation Research*, 1980, **75**, 243
17. J. Barbosa, E. Bosch and R. Carrera, *Talanta*, 1985, **32**, 1077

18. N.J.F. Dodd and T. Mukherjee, *Biochem. Pharmac.*, 1984, **33**, 379
19. K. Reszka and J.W. Lown, *Photochem. and Photobiol.*, 1989, **50**, 297
20. T. Julich, K. Scheffler, P. Schuler and H.B. Stegmann, *Magn. Reson. Chem.*, 1988, **26**, 701
21. J.M. Bruce, C.W. Kerr and N.J.F. Dodd, *J. Chem. Soc., Faraday Trans. 1.*, 1987, **83**, 85
22. M.B. Hocking, H.I. Bolker and B.I. Fleming, *Can. J. Chem.*, 1980, **58**, 1983
23. B. Keita and L. Nadjo, *J. Electroanal. Chem.*, 1983, **151**, 283
24. B. Keita and L. Nadjo, *J. Electroanal. Chem.*, 1984, **163**, 171
25. Kirk-Othmer, *Encyclopedia of Chemical Technology* (Interscience Publishers, New York, Vol. 2., 2nd edn, 1967) pp. 431-435
26. Ref. (19.) pp. 465-475
27. S.R. Flom and P.F. Barbara, *J. Phys. Chem.* 1985, **89**, 4489
28. K. Kratochvil and M. Nepras, *Collect. Czech. Chem. Commun*, 1972, **37**, 1533
29. P.S. Rao and E. Haynon, *J. Phys. Chem.* , 1973, **77**, 2274
30. V.V. Palchevskii, E.K. Skaletskii, A.A. Talub and E.E. Yudovich , *Zh. Fiz. Khim.*, 1979, **53**, 1233
31. R.M. Issa, M.S. El-Ezaby and A.H. Zewail, *Z. Phys. Chem. Leipzig*, 1970, **244**, 155
32. A. Roy and S. Aditya, *J. Photochem.*, 1983, **22**, 361
33. Ref. (25.) pp. 439-447
34. J.N. Moore, D. Phillips and R.E. Hester, *J. Phys. Chem.*, 1988, **92**, 5619
35. P.J. Baugh, G.O. Phillips and J.C. Arthur, Jr., *J. Phys. Chem.*, 1966, **70**, 3061
36. C. Siero, A. Sanchez and P. Crouigneau, *Spectrochim. Acta*, 1984, **40A**, 453

37. K. Umemoto, *Bull. Chem. Soc. Jpn*, 1967, **40**, 1058
38. M.R. Das and G.K. Fraenkel, *J. Chem. Phys.*, 1965, **42**, 1350
39. B. Kirste and H. Kurreck, *J. Am. Chem. Soc.*, 1980, **102**, 6180
40. M.B. Hocking and S.M. Mattar, *J. Magn. Reson.*, 1982, **47**, 187
41. J.A. Pedersen and R.H. Thomson, *J. Magn. Reson.*, 1981, **43**, 373
42. G.R. Stevenson, Z.Y. Wang, R.C. Reiter and S.J. Peters, *J. Am. Chem. Soc.*, 1988, **110**, 6581
43. W.T. Dixon, P.M. Kok and D. Murphy, *Tetrahedron Lett.*, 1976, 623
44. G.R. Stevenson, A.E. Algeria and A. McB. Block, *J. Am. Chem. Soc.*, 1975, **97**, 4859
45. K.S. Chen and N. Hirota, *J. Am. Chem. Soc.*, 1972, **94**, 5550
46. T.E. Gough and P.R. Hindle, *Trans. Faraday Soc.*, 1970, **66**, 2420
47. N.M. Atherton and A.J. Blackhurst, *J. Chem. Soc., Faraday 2.*, 1972, **68** , 470
48. M. Adams, M.S. Blois, Jr. and R.H. Sands, *J. Chem. Phys.*, 1958, **28**, 774
49. R. Poupko and I. Rosenthal, *J. Phys. Chem.*, 1973, **77**, 1722
50. B. Kirste, *Magn. Reson. Chem.*, 1987, **25**, 166
51. J.A. Pedersen, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 3223
52. D. Lunney, J. Bailes and J.D. Memory, *J. Chem. Phys.*, 1970, **53**, 3387
53. J.A. Pedersen, *J. Magn. Reson.*, 1984, **60**, 136
54. (a) A.E. Lutskii, I.S. Romodanov and Yu.A. Kruglyak, *Teor. Eksp. Khim. Akad. Nauk. Ukr. SSR.*, 1966, **2**, 456
55. (b) I.S. Romodanov, N.V. Arshava and A.E. Lutskii, *Teor. Eksp. Khim. Akad. Nauk. Ukr. SSR*, 1973, **9**, 68
56. J. Gendell, W.R. Miller, Jr. and G.K. Fraenkel, *J. Am. Chem. Soc.*, 1969, **91**, 4369

57. A.V. Bulatov, A.T. Nikitaev and M.L.Khidekel, *Izv. Akad. Nauk. SSR, Ser. Khim.*, 1982, 437
58. I.M. Ahmed, A. Hudson and A. Alberti, *J. Organomet. Chem.*, 1988, **350**, 9
59. W.J. Sep, J.W. Verhoeven and TH.J. de Boer, *Tetrahedron*, 1979, **35**, 2161
60. M.C. Depew, L. Zhongli and J.K.S. Wan, *J. Am. Chem. Soc.*, 1983, **105**, 2480
61. J.R. Bolton, A. Carrington and J. dos Santos-Veiga, *Mol. Phys.*, 1962, **5**, 465
62. P.D. Sullivan, *J. Phys. Chem.*, 1969, **73**, 2790
63. Z-L. Liu, L-M. Wu and Y-C. Liu, *Magn. Reson. Chem.*, 1988, **26**, 577
64. N.V. Arshava, *Ukr. Khim. Zh.*, 1980, **46**, 863
65. A. Schweiger, in *Electron Spin Resonance, A Specialist Periodical Reports*, (The Chemical Society, London, 1986); vol. 10B, chap.4.
66. K. Möbius, M. Plato and W. Lubitz, *Phys. Reports*, 1982, **87**, 171
67. R.S. Alger, *Electron Paramagnetic Resonance: Techniques and Applications* , (Interscience Publishers, New York, 1968) pp. 106-115
68. *Multiple Electron Resonance Spectroscopy*, ed. M.M. Dorio and J.H. Freed, (Plenum press, New York, 1979)
69. J.E. Wertz and J.R. Bolton, *Electron Spin Resonance, Elementary Theory and Practical Applications* (Mc Graw-Hill, New York, 1972)
70. W. Gordy, *Theory Applications of Electron Spin Resonance* (John Wiley, New York, 1980)
71. Bruker Analytische Messtechnik GMBH, EPR Division, ER Series User s Manual (1983)
72. K.P. Dinsc, K. Möbius and R. Biehl, *Z. Naturforsch., Teil A*, 1973, **28**, 1069
73. Rcf. (6.) pp. 79 80
74. Ref. (6.) pp. 51-52
75. Ref. (6.) pp. 86-90

76. M.G. Bakker, R.F.C. Claridge, B.M. Peake and J.A. Weil, *J. Magn. Reson.*, 1988, **78**, 193
77. M.G. Bakker, R.F.C. Claridge and C.M. Kirk, *J. Magn. Reson.*, 1987, **74**, 503
78. I.B. Goldberg and B.M. Peake, *J. Phys. Chem.*, 1977, **81**, 571
79. P. Ashworth, *Mol. Phys.*, 1975, **30**, 313
80. J.A. Pedersen, *Mol. Phys.*, 1974, **28**, 1031
81. R.E. Moss, N.A. Ashford, R.G. Lawler and G.K. Fraenkel, *J. Chem. Phys.*, 1969, **51**, 1765
82. B. Venkataraman, B.G. Segal and G.K. Fraenkel, *J. Chem. Phys.*, 1959, **30**, 1006
83. G. Hurst, K. Kraft, R. Schultz and R. Kreilick, *J. Magn. Reson.*, 1982, **49**, 159
84. E. Salo, *Research Report 22, Ph.D. Thesis* (Department of Chemistry, University of Jyväskylä, ISBN 951-679-370-3, 1985) and references therein
85. Kirk-Othmer, *Encyclopedia of Chemical Technology* (John Wiley, New York, Vol 10., 1972) pp. 952,953, 812,813
86. R.D. Howells and J.D. Mc Cown, *Chem. Rev.*, 1977, **77**, 69
87. *Langes Handbook of Chemistry*, ed. A.J. Dean, (Mc Graw-Hill, New York, 13rd edn, 1985) pp. 10-(95-116), 7-(82-689), 4-(14-133)
88. *CRC, Handbook of Chemistry and Physics*, ed. R.C. Weast, (CRC Press, Boca Raton, Florida 67rd edn., 1987) pp. E-(49-52)
89. *CRC, Handbook of Chemistry and Physics*, ed. R.C. Weast, (CRC Press, Boca Raton, Florida 55rd edn.,1975) pp. F-(52-55)
90. R. Mäkelä and M. Vuolle, *J. Chem. Soc., Faraday Trans. 1*, in press.
91. R. Mäkelä and M. Vuolle, *J. Chem. Soc., Faraday Trans. 1*, 1989, **85**, 4011
92. M. Vuolle and R. Mäkelä, *J. Chem. Soc., Faraday Trans. 1*, 1987, **83**, 51
93. R. Mäkelä and M. Vuolle, *J. Chem. Soc., Faraday Trans. 1*, in press
94. H. Joela and R. Mäkelä, *J. Magn. Reson.*, 1989, **84**, 236

ERRATUM

Page	is:	should be:
13	200 mW	200 W
16	ESP-300	ESP-380 with cw option
26	be optimized	have to be optimized
26	h.f.c. coupling	h.f.c.
Paper		
p.1	do not know	do not know
p.2	Fig.1(a) (b)	remove Fig.1(b)
p.2	Fig.2	Fig.3
p.2	Fig.3	Fig.2
p.3	the cation	the anion
p.3	1,4-dihydroxy-	The 1,4-dihydroxy-
p.3	constants (0.055 mT)	constant (0.055 mT)
Table 2.	1,2,5,8-Tetra(OD)	1,2,5,8-Tetra(OD)AQ
Table 2.	1,5-di(OH)AQ	1,5-di(OH)AQ
	1,5-di(OH)AQ	1,5-di(OD)AQ
p.5	in table 3.	in table 2.
Paper II		
Table 1(a)	- 0.072 0.112 0.057	0.057 0.112 0.072 -
	- 0.071 0.104 0.055	0.055 0.104 0.071 -
Paper III		
p.51	(h.f.s.)	(h.f.c.s)
Paper IV		
Table 2.	4 or 8 0.190	4 or 8 { 0.190
	0.087	0.087
	0.275	not { 0.275
	0.153	0.153
	0.037	assigned { 0.037
	0.025	0.025
Table 3.	2 or 3 0.173	2 or 3 { 0.173
	0.079	0.079

**ORIGINAL PUBLICATIONS**



PAPER 1

*J. Chem. Soc., Faraday Trans. 1,*

1990, **86**, 000-000

(proof)

**<https://doi.org/10.1039/FT9908602569>**

**PAPER 2**

*J. Chem. Soc., Faraday Trans. 1,*

1989, **85**, 4011-4017

(reprint)

**<https://doi.org/10.1039/F19898504011>**

**PAPER 3**

*J. Chem. Soc., Faraday Trans. 1,*

1987, **83**, 51-55

(reprint)

**<https://doi.org/10.1039/F19878300051>**

PAPER 4

*J. Chem. Soc., Faraday Trans. 1,*  
1990, **86**, 000-000  
(proof)

<https://doi.org/10.1039/FT9908603257>

PAPER 5

*J. Magn. Reson.*,

1989, **84**, 236-246

(reprint)

[https://doi.org/10.1016/0022-2364\(89\)90367-3](https://doi.org/10.1016/0022-2364(89)90367-3)