The precise measurement of free radical g-values and their dependence upon structure

The precise measurement of free radical g-values and their dependence upon structure *

M. S. Blois, Jr., H. W. Brown and J. E. Maling

Biophysics Laboratory, Stanford University Stanford, California

Introduction.

It is customary to regard electron spin resonance (e.s.r.) techniques as belonging to the general field of spectroscopy. As such it would appear that the observable quantity of greatest interest should be one associated with the energy separation of the electronic levels involved in the transition. In the case of e.s.r. this is the g-value, but since for free radicals these values usually differ from the free electron value of 2.00229 by less than 1%, these measurements are generally considered to be unrewarding.[1] The greatest attention has therefore been focused upon the observation and interpretation of the hyperfine interaction.

It is our purpose here to consider the general problem of precisely measuring g-values, to describe experimental methods which we have used, and to indicate in certain specific instances the manner in which a free radicals g-value is affected by its structure. It will be shown that under appropriate conditions free radical g-values can be measured with the precision usually associated with spectroscopic measurements, and that in most instances the measurement of absolute g-values is limited by the accuracy with which the fundamental physical constants are known. Finally, the interpretation of g-values which, though not simply related to structure, will be shown to afford valuable additional information regarding the radical species under study.

---

* A preliminary report on this work was given at the Symposium on Free Radicals in Biological Systems, Stanford University, March 21-23, 1960, and appears in *Free Radicals in Biological Systems*, Academic Press, New York (1960) (in preparation). This work was supported by Grant A-2304 from the National Institutes of Health, U. S. Public Health Service, and by Contract AF 18 (600)-1511, Office of Scientific Research, U. S. Air Force.

Experimental.

The resonance condition of an electron spin undergoing a transition between its Zeeman levels is given by: $h\nu = g\mu_B H$, where $h\nu$ is the quantum of energy absorbed, g is the spectroscopic splitting factor, μ_B the Bohr magneton and H the magnetic field producing the Zeeman level separation. The g-value is therefore the ratio of microwave frequency to local magnetic field, at resonance, multiplied by a constant. The uncertainties and corrections in the measured g-value may therefore arise from the following sources:

A. *g-value measurement uncertainty*

1. *The uncertainty in determining the center of an absorption line.*

For the case of a free radical which shows a broad, weak absorption singlet, with a poor signal to noise ratio the determination of line center is the factor limiting the accuracy of the g-value measurement. It may be possible, under such highly unfavorable circumstances only to say the g-value is near 2, that is, close to that of the free electron. In a more favorable situation, for example, the p-benzosemiquinone radical at room temperature in alkaline alcohol one may observe the familiar quintet with the center component having a line width of about 200 milligauss and with a signal to noise ratio in excess of 100. It is easily possible to locate this line center to within a tenth of a line width which corresponds to an error in g-value of $\pm$ .00001. With more care it is possible to adjust the magnetic field to resonance at the line center to within nearly one hundredth of this line width. Figure 1, which shows the g-value of this radical as a function of temperature, shows that the average deviation from the mean due to all errors and for a number of samples is of the order of $\pm$ .00001.

Under *optimum conditions* encoutered by us, we consider that the error in selecting the center of a symmetrical line produces uncertainties in g-value of from $\pm$ .000001 to $\pm$ .00001, and this will be seen later to be essentially negligible when deriving absolute g-values, although it may set the limit in measuring relative g-values (i.e., g-value differences). In the case of an asymmetric line, due to anisotropy in g, it becomes of course extremely difficult to choose the line center. For the radicals studied herein this has been avoided by the averaging which occurs in solution.

A Varian e.p.r. spectrometer with a six-inch magnet was employed and data were obtained using two different methods. One was to record

the complete derivative absorption spectrum on a Leeds and Northrup Speedomax G potentiometer recorder while slowly scanning (1-10 gauss-min) with the magnetic field. During this time a series of proton resonance frequencies were obtained and a reference mark was placed on the chart paper at each corresponding field value. The center of the line and the corresponding value of ν_p were then obtained graphically. Small systematic errors were eliminated by taking spectra in successively increasing and decreasing fields and averaging an even number of such determinations. The other method was to set the magnetic field so that the potentiometer indicated zero deflection when compared with the base line of the spectrum. The latter was more rapid and at least as precise, but does not warn of changes in the constitution of the sample (e.g., accumulation of secondary radicals) so that in practice both methods were used.

2. *The determination of microwave and proton resonance frequencies.*

The klystron frequency was measured by leading the microwave signal to a Hewlett-Packard 450-A transfer oscillator where it was heterodyned with the 47th harmonic of the transfer oscillator and then brought to zero beat. The fundamental frequency of the transfer oscillator was then measured with a Hewlett-Packard 524-B electronic counter which has a nominal stability of 1 part in 10^6.

As stated earlier, the value of H was obtained by observing a proton resonance simultaneous with the observation of the electron resonance. The oscillator driving the proton probe was monitored by a second HP-524-B electronic frequency counter and ν_p was obtained directly.

The absolute accuracy of our frequency determinations has not been studied extensively since we performed frequency measurements in such a way that absolute g-values were derived without knowing absolute frequencies. This was so, since the g-value is a ratio of frequencies and does not involve time as a dimension. Thus while the local crystal oscillators of the two counters can be adjusted to a standard frequency such as the National Bureau of Standards broadcast frequency (WWV) to within better than 1 part in 10^6, in order to put them on a common, absolute time base, this was not necessary. Instead, the local crystal oscillator of one was used to gate both counters so that they were on a common though perhaps erroneous time base. It is estimated that errors in measuring frequencies do not produce g-value errors in excess of $\pm$ .000003.

3. *Measurement of magnetic field intensity.*

The measurement of the proton resonance frequency has been discussed above; we here consider *(a)* how closely can the proton probe be set to resonance, and *(b)* proton probe oscillator instability.

The proton samples used consist of glass cylinders approximately 2 cm in length and 0.5 cm in diameter and containing a 0.01 M aqueous $Fe\ Cl_3$ solution. The proton resonance was displayed as a Lissajou figure on an oscilloscope and the proton Larmor frequency measured as described. The proton resonance line width was approximately 200 milligauss and it was possible to select the line center to better than a tenth of this or to less than a g-value uncertainty of $\pm$ .00001. Proton oscillator instability was a problem in the most accurate measurements. Flexing of chassis walls and leads, temperature variations, etc., led to noticeable shifts in frequency as the data were being taken. This effect was minimized by checking the proton resonances both before and after each counter reading. It did contribute singificantly to scatter in the data, without to our knowledge introducing any systematic error.

4. *Uncertainties in the fundamental physical constants.*

We have used the values given by Cohen, Crowe, and DuMond [2] as follows:

$$\text{Bohr magneton} = \mu_B = (0.92731 \pm .00002) \times 10^{-20} \text{ erg gauss}^{-1}$$

$$\frac{h}{2\pi} = (1.05443 \pm .00004) \times 10^{-27} \text{ erg sec.}$$

$$\text{gyromagnetic ratio} = \gamma_p = (2.67523 \quad .00006) \times 10^4 \text{ rads/sec-gauss of proton-uncorrected for diamagnetism}$$

Because of the uncertainty of the fundamental constants, the absolute g-values tabulated below will have uncertainties of at least $\pm$ .00008, but the accuracies of the relative g-values (g-value shifts) are not affected by this.

5. *Temperature variation.*

The g-value of parabenzosemiquinone in alkaline butanol showed a definite temperature variation (Fig. 1). At room temperature the g-value decreases with decreasing temperature at a rate of 3×10^{-6} per 10° C shift. Most measurements at room temperature on radical solutions were within 10° C of one another so that this uncertainty is almost negligible. All

g-values reported below were obtained at 30° C ± 5° C unless otherwise specified.

6. *Solvent differences.*

The g-value vs. temperature behavior of parabenzosemiquinone in alkaline ethanol is qualitatively similar to that in butanol. At room temperature the g-values are identical to within experimental uncertainty, but the former has a smaller temperature coefficient.

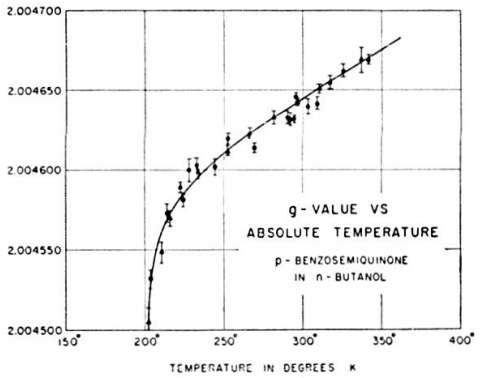

Fig. 1.

The g-value of p-benzosemiquinone in n-butanol as a function of temperature. The limits of error represent the mean deviation from the mean of ten separate measurements.

B. *Corrections to the measured g-values.*

1. *Magnetic field inhomogeneity.*

(a) A significant magnetic field difference existed between the electron sample and the proton sample because of cavity shielding and field variation across the gap. The proton and radical samples were separated by 2 or 3 cm and the field difference ΔH, between the two points, measured for different cavities and probes, led to corrections in g-value of from .00004 to .00010. This number is a constant correction for a particular experimental configuration, and has the same maximum uncertainty associated with its measurement as that of the free radical g-values. This correction was very carefully measured and the uncertainty was less than ± .00001.

(b) Field inhomogeneity over the proton and electron sample tubes should lead to an effective field difference between proton and electrons because of a difference in the shape of the r.f. field distributions over the proton sample and the electron sample. A difference between the micro-

wave r.f. field distribution, and the proton coil field distribution leads to a difference in weighting factor between comparable volumes of the two samples. Assuming an inhomogeneity of 20 milligauss over both samples a correction of $\pm$ 0.000006 in g-value is obtained. This has been neglected.

2. *Chemical shielding of protons in water.*

The chemical shift for protons in water [3] is given by Dickinson as -2.66×10^{-5} which leads to a g-value correction of $-$ 0.000053. This effect is automatically accounted for in the constant multiplier since the gyromagnetic ratio of the proton uncorrected for diamagnetism of the water molecule has been used.

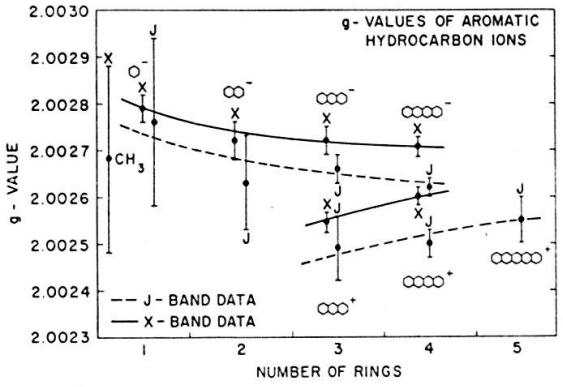

Fig. 2.

The g-values of + and — hydrocarbon ions at X and J-band and the methyl radical (at X-band). The limits of error include the maximum deviation between results on different samples plus the mean scatter of the most divergent sample.

3. *Bulk diamagnetic shielding of proton sample and of the free radical by the solvent.*

Both samples are cylindrical in shape, the former consisting of water with added Fe Cl_3, and the latter ordinarily in an organic solvent (alcohol, benzene, etc.). The difference between the diamagnetic susceptibility of the two is of the order of -0.2×10^{-6} which is negligible.

4. *Static paramagnetic fields due to protons, free radical odd electrons, and paramagnetic ions.*

The paramagnetic susceptibility of protons in water is about $+ 3 \times 10^{-10}$ and is therefore negligible. The proton sample is a solution of .01 M $FeCl_3$ which has a paramagnetic susceptibility of $+ 5 \times 10^{-9}$. This also may

be neglected. Since this sample has a much higher density of unpaired electrons than the radical sample, this also shows that the bulk paramagnetism of the latter sample gives a negligible shift.

Dickinson [3] reports a shift in the proton resonance in H_2O as a function of concentration of Fe^{++} ions. Assuming Fe^{+++} will have a comparable effect, a .01 M Fe^{+++} solution may be expected to give a fractional shift of -2×10^{-7} which can be neglected.

5. *Demagnetizing fields.*

Such fields, due to the finite size and the shape of the samples are of the same order as the bulk susceptibilities and can be neglected.

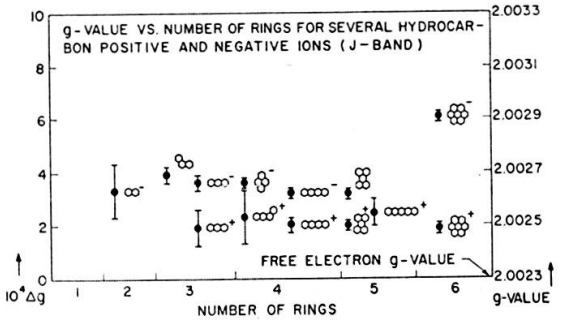

Fig. 3.
The g-values of + and — hydrocarbon ions taken at J-band.
Experimental errors determined as in figure 2.

6. *Rotational moments* [4].

These are known to be of the same magnitude as the nuclear moments and can therefore be neglected.

7. *Finite r.f. field.*

The r.f. field reduces the effective magnetic field H_0 by a term in $(Hrf/H_0)^2$. This is a shift of 10^{-7} and may be neglected.

These corrections are all small compared to the experimental uncertainty under the best of conditions and except for field inhomogeneity and the chemical shift for protons in water are therefore all neglected. For purposes of comparison, purified α, α diphenyl β picrylhydrazyl (DPPH) in dilute benzene solution at room temperature, gives in our apparatus a g-value of $2.00354 \pm .00003$ at 9.5 kmc of $2.00352 \pm .00010$ at 6.0 kmc.

TABLE 1.

g-values of Hydrocarbon Ions

Positive hydrocarbon ions (J-band)	
Anthracene"	2.00249 ± .00007
Tetracene"	2.00250 ± .00003
1,2 benzanthracene"	2.00253 ± .00010
Pentacene"	2.00255 ± .00005
Perylene"	2.00250 ± ,00002
Coronene"	2.00249 ± .00002
Negative hydrocarbon ions (J-band)	
Benzene"	2.00276 ± .00014
Naphthalene"	2.00263 ± .00010
Anthracene"	2.00266 ± .00003
Phenanthrene"	2.00269 ± .00003
Tetracene"	2.00262 ± .00002
Pyrene"	2.00266 ± .00002
Perylene"	2.00262 ± .00002
Coronene"	2.00291 ± .00002
Miscellaneous hydrocarbon ions (X-band)	
Transtilbene.	2.00285 ± .00002
Truxene"	2.00343 ± .00002
Triphenyl benzene"	2.00276 ± .00004
Benzophenone"	2.00359 ± .00002
2, fluorobenzophenone"	2.00363 ± .00004

EXPERIMENTAL RESULTS.

The hydrocarbon monopositive and mononegative ions, as prepared by the methods of Weissman et al.,[5] and de Boer et al.,[6] have the g-values shown in Table 1. These data are plotted in Figure 9 where the monotonic change in g-value for a given homologous series is shown. Measurements on several of these compounds were made with both X-band (9.5 kmc) and J-band (6.0 kmc) instruments in the hope of determining whether the g-value depended upon the magnetic field. After all corrections were made, there appears to be a small, systematic shift of g-value with frequency (or field) but the sensitivity of the J-band instrument was so much poorer than the X-band, that the experimental uncertainty with the former is considerably greater. The g-value for the benzene negative ion, though obtained on a sample prepared in the same manner as the other negative ions, may be questioned because of the anomalous hyperfine spectrum of this sample. A value for the methyl radical is also shown in this figure

and was obtained from the observation of u.v. irradiated methanol at liquid nitrogen temperature, and its identity confirmed from its hyperfine spectrum.

TABLE 2.

g-values of Semiquinone Free Radicals (X-band)

1,4 benzosemiquinone	2.00468 ± .00002
2 methyl—1,4 benzosemiquinone . . .	2.00463 ± .00002
2 chloro— " . . .	2.00486 ± .00003
2 bromo— " . . .	2.00512 ± .00004
2,3 dichloro— " . . .	2.00516 ± .00002
2,3,5,6 tetrachloro— " . . .	2.00568 ± .00002
2,3,5,6 tetrabromo— " . . .	2.00875 ± .00002
2,3,5,6 tetraiodo— F . . .	2.01217 ± .00013
1,4 naphthasemiquinone	2.00437 ± .00003
2 methyl—1,4 naphthasemiquinone. . .	2.00432 ± .00002
2,3 dichloro— . . .	2.00507 ± .00003
9,10 anthrosemiquinone	2.00413 ± .00002
2 methyl—9,10 anthrosemiquinone . . .	2.00408 ± .00002
2 chloro— " . . .	2.00431 ± .00002

Th eg-values of the semiquinones and the substituted semiquinones were observed on reaction mixtures of the corresponding quinols undergoing air oxidation in alkaline ethanol. These free radicals were measured as quickly as practicable in order to avoid the effects of the polymeric oxidation products. The appropriate hyperfine structure was observed in each instance, and the measured g-values are listed in Table 2 and shown in Figures 4, 5 and 6.

Several qualitative features of the g-value dependence upon molecular structure become apparent upon examination of these data.

1. As a group, the hydrocarbon ions (and the methyl radical) lie within a relatively narrow range of values.

2. With the exception of the positive hydrocarbon ions, all molecules studied show a lowering of g-value as one progresses along a homologous series. The positive hydrocarbon ions appear to have g-values that increase asymptotically.

3. Oxygen addition to an aromatic ring (as in hydroxylation) produces a large increase in g-value; methylation produces a small lowering.

4. Halogenation produces a g-value increase in the order: I > Br > Cl > F and this is further increased on polyhalogenation.

5. In the case of the polynuclear aromatic hydrocarbons, there is a suggestion that the non-linear isomers increase or decrease the g-value for the (—) and (+) ions respectively.

Theory of g-values.

McConnell and Robertson [7] have presented a qualitative theory of g-values of aromatic radicals which has proved very useful in the interpretation of our results. First, it will be remembered that g is actually a tensor quantity and what one measures for aromatic radicals in solution is

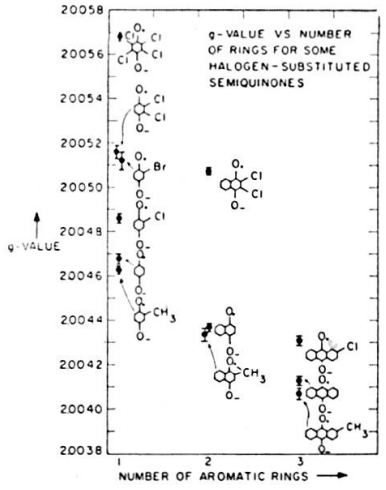

Fig. 4.
The g-values of semiquinones. The limits of error determined as in figure 2.

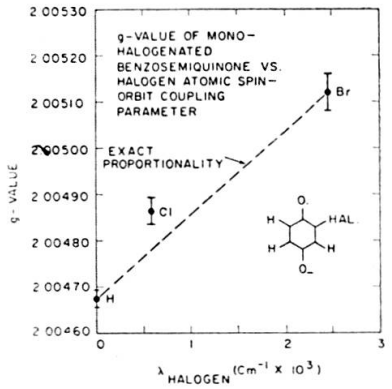

Fig. 5.
The g-values of singly halogenated p-benzosemiquinones. Limits of error determined as in figure 2.

the average of the values along the three molecular axes. Shifts from the free spin value (2.00229) can be explained in terms of spin-orbit interaction through configurational mixing of σ bonding and antibonding excited states with the ground state of the radical. Taking the spin-orbit coupling parameter of carbon to be 28 cm^{-1} [8] and assuming reasonable energies for the excited states, McConnell and Robertson predict a small positive shift from the free spin value in the case of hydrocarbon ions. This is concordant with our findings. Presumably, if one knew the energy levels the differences observed in the series of hydrocarbon ions (Figs. 5 and 6) could be explained.

The g-values we have observed for the semiquinone radicals may be similarly explained. Oxygen has a much larger spin-orbit coupling parameter (152 cm^{-1}) and therefore one might expect a larger shift. Furthermore, there are low energy $n \rightarrow \pi$ transitions which allow effective mixing of this angular momentum with the odd electron. This gives rise to a shift which is an order of magnitude larger than was observed for the hydrocarbons. We have measured the $n \rightarrow \pi$ optical transitions in benzo-, naphtha-, anthraquinone and their 2-methyl derivatives, and have found

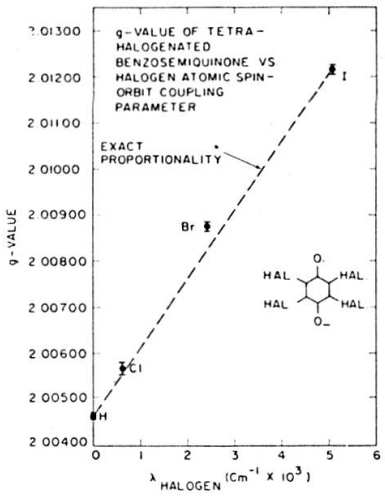

Fig. 6.
The g-values of tetrahalogenated p-benzosemiquinones.
Limits of error determined as in figure 2.

that as this transition shifts to higher energies the g-value of the corresponding radical decreases, in agreement with theory. It should be noted, however, that these relative weak $n \rightarrow \pi$ transitions in most cases were overlapped by an intense band to the blue which made measurement of the band center very difficult.

The halogens have very large spin-orbit coupling parameters;[8] *viz.*, F(272 cm^{1}), Cl (587), Br (2460) and I (5060) and indeed, the halogenated semiquinones have the largest g-values of any organic radical that we have studied. Figures 5 and 6 show plots of g for mono and tetra substituted benzosemiquinones *versus* the spin-orbit coupling parameter of the halogen. These plots differ markedly from linearity, which is not surprising, for we have neglected variations in transition energy. In addition, as McConnell and Robertson have indicated, this shift is pro-

portional to the odd electron density on these atoms, so we should include the effect of variations in the electonegativity of the halogens. Clearly, however, the major effect in this series is the change in the spin-orbit parameter.

We have made a few preliminary studies of other variables affecting g-values. Radical concentration, type of solvent and temperature all have detectable effect, but in general these effects are an order of magnitude smaller than those considered above. Though very small, the variation of g with temperature is of considerable interest. On hydrogen bonding, $n \rightarrow \pi$ transitions in aromatic molecules are known to shift to higher

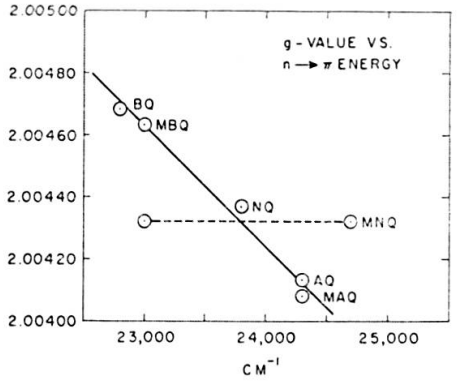

Fig. 7.

The g-values of several semiquinones as a function of the $n \quad \pi$ energy separation. (BQ = 1,4 benzosemiquinone, MBQ = 2 methyl, 1-4 benzosemiquinone, NQ = 1,4 naphthosemiquinone, MNQ = 2 methyl, 1-4 naphthosemiquinone, AQ = 9,10 anthrasemiquinone, MAQ = 2 methyl, 9-10 anthrasemiquinone)

energies [9], which in turn would result in a corresponding decrease in the g-value of the radical. Lowering the temperature increases the amount of hydrogen bonding and one might, therefore expect a lowering of the g-value. We have observed such an effect; however, another explanation may lie in the reduced rate of tumbling at lower temperatures.

In summary, our measurements are in gratifying agreement with the qualitative theory of McConnell and Robertson. The three series of compounds considered demonstrate that *(a)* unsubstituted aromatic hydrocarbon radicals all have g-values only slightly greater than the free spin value, *(b)* the benzo-, naphtha-, anthraquinone and their methyl derivatives have a monotonic, probably linear dependence of g-value upon energy level shifts ($\Delta E n \rightarrow \pi$) when the spin-orbit parameter is the same, and *(c)*

the halogenated semiquinone series shows the effect of varying the spin-orbit parameter. Though the theory is in good qualitative agreement with our data, one is not yet in a position to predict g-values accurately, and it is apparent that additional optical data are needed in order to attain this goal.

It would appear that the free radical g-value, depending as it does upon spin-orbit coupling, energy separation of the $n \rightarrow \pi$ transition, and electronegativity of substituent atoms, all of which in turn depend upon molecular structure, is a parameter of considerable value in describing a given free radical. Because of its dependence upon these several factors it is not unique, and in its measurement the slight shifts due to environmental factors must be accounted for. While it is not as diagnostic of structure as a resolved hyperfine spectrum, it has the advantage of being accessible to measurement in many instances when the h.f.s. cannot be resolved; e.g., as with many biological free radicals. Finally, it is much less sensitive to environmental factors (temperature, concentration, solvent) than are line width or line shape.

[1] Ingram, D.J.E., *Free Radicals as Studied by Electron Spin Resonance*, Butterworths, London, 1958.

[2] Cohen, E. R., Crowe, K. M., and DuMond, J. W. M., *Fundamental Constants of Physics*, Interscience, New York, 1957.

[3] Dickinson, W. C., *Phys. Rev.*, *51*, 717 (1951).

[4] Ramsey, N. F., *Nuclear Moments*, p. 121, John Wiley, New York (1953).

[5] Weissman, S. I., Paul, D. E., and Lipkin, D., *J. Am. Chem. Soc.*, *78*, 116 (1956).

[6] de Boer, E., and Weissman, S. I., *J. Am. Chem. Soc.*, *80*, 4549 (1956).

[7] McConnell, H. M. and Robertson, R. E., *J. Phys. Chem.*, *61*, 1018 (1957).

[8] McClure, D. S., *J. Chem. Phys.*, *20*, 682 (1952); *17*, 905 (1949).

[9] Pimentel, G. C., and McClellan, A. L., *The Hydrogen Bond* (W. H. Freeman and Company, San Francisco and London, 1960), p. 158.