MELDOLA MEDAL LECTURE*

N.M.R. Spectral Change as a Probe of Chlorophyll Chemistry

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1 Introduction

The shift reagent experiments which were so new and exciting in 1970 can be seen in retrospect to be part of a continuing trend in n.m.r. spectroscopy; it is often possible to derive more detailed information about molecular structure or reaction mechanism from induced changes in a spectrum than from the original spectrum itself. This article is concerned with various techniques of n.m.r. spectral change which have been used to study the chemistry of chlorophylls, but it also includes a section on shift reagents. The emphasis throughout is on the application of these techniques in Organic Chemistry as a whole.

In the early days, n.m.r. spectral parameters were regarded almost as fundamental and immutable. Chemical shifts and coupling constants were tabulated,[1] and organic chemists began to feel that n.m.r. spectroscopy had reached a useful but boring plateau as was apparently the case for u.v. and i.r.[2] Aromatic solvent-induced shifts were a nuisance until it was realized that they could not only improve spectral resolution but also give qualitative geometrical information.[3] However, these shifts were small and suffered a lack of selectivity. Paramagnetic effects had long been known, and had found some application[4] but were not generally useful. The breakthrough came in 1969.

2 Lanthanide-induced Changes

A. Shift Reagents.—In 1969, Hinckley discovered[5] a lanthanide shift reagent, the pyridine adduct of Eu(d.p.m.)₃.† This β-diketone complex was a Lewis acid

[1] L. M. Jackman and S. Sternhell, 'Applications of NMR Spectroscopy in Organic Chemistry,' Pergamon, Oxford 1969.
[2] Recently ^{13}C n.m.r. has added valuable detail, but has contributed little conceptually except in the area of biosynthesis: M. Tanabe in 'Biosynthesis,' ed. J. D. Bu'lock (Specialist Periodical Reports), The Chemical Society, London, 1976, Vol. 4, p. 204.
[3] J. Ronayne and D. H. Williams, *Annual Reviews of n.m.r. Spectroscopy*, 1969, **2**, 83.
[4] W. D. Phillips, C. E. Looney, and C. K. Ikeda, *J. Chem. Phys.*, 1957, **27**, 1435; W. D. Horrocks, R. C. Taylor and G. N. LaMar, *J. Amer. Chem. Soc.*, 1964, **86**, 3031.
[5] C. C. Hinckley, *J. Amer. Chem. Soc.*, 1969, **91**, 5160.

4

which formed adducts with basic lone pairs in organic molecules and which caused selective shifts with little broadening. For the first time it became possible to characterize a nucleus not only in terms of its immediate chemical environment but also by its geometrical relationship with one or more distant functional groups. We soon found that pyridine-free Eu(d.p.m.)₃ was an even better shift reagent and obtained a first order spectrum of n-hexanol (Figure 1),[6] equivalent to thousands of MHz in resolution (but not sensitivity!) for a trivial cost.
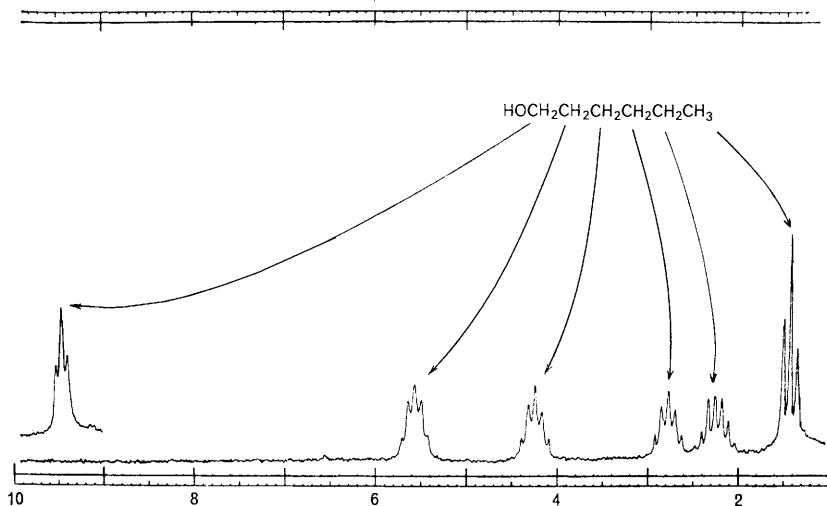

$HOCH_2CH_2CH_2CH_2CH_2CH_3$

Figure 1 *100 MHz ^{1}H n.m.r. spectrum of n-hexanol in* CCl_4 *after the addition of 0.29 equivalents of* Eu(d.p.m.)₃
(Reproduced from *Chem. Comm.*, 1970, 422)

This beautiful result is seen because the shifts are primarily caused by the pseudo-contact[7,8] mechanism. In effect the lanthanide ion, which is bound to the hydroxy group, exerts a magnetic field which decreases with distance. More precisely, the shift (ΔH) is given by equation 1, where R and ϕ are defined in Figure 2. C is a constant for any given adduct at a fixed temperature, but

$$\Delta H = C \cdot R^{-3}(3 \cos^2\phi - 1) \qquad (1)$$

both its sign and magnitude depend on the magnetic anisotropy of the metal ion; for Eu(d.p.m.)₃ shifts are normally downfield, but for Pr(d.p.m.)₃ they are upfield.[8,9] Note, however, that $(3 \cos^2\phi - 1)$ changes sign when $\phi = 54.7°$

[6] J. K. M. Sanders and D. H. Williams, *Chem. Comm.*, 1970, 422.
[7] E. de Boer and H. Van Willigen, *Prog. N.M.R. Spectroscopy*, 1967, **2**, 111.
[8] J. Reuben, *Prog. N.M.R. Spectroscopy*, 1973, **9**, 1.
[9] D. R. Crump, J. K. M. Sanders, and D. H. Williams, *Tetrahedron Letters*, 1970, 4419; J. Briggs, G. H. Frost, F. A. Hart, G. P. Moss, and M. L. Staniforth, *Chem. Comm.*, 1970, 749.
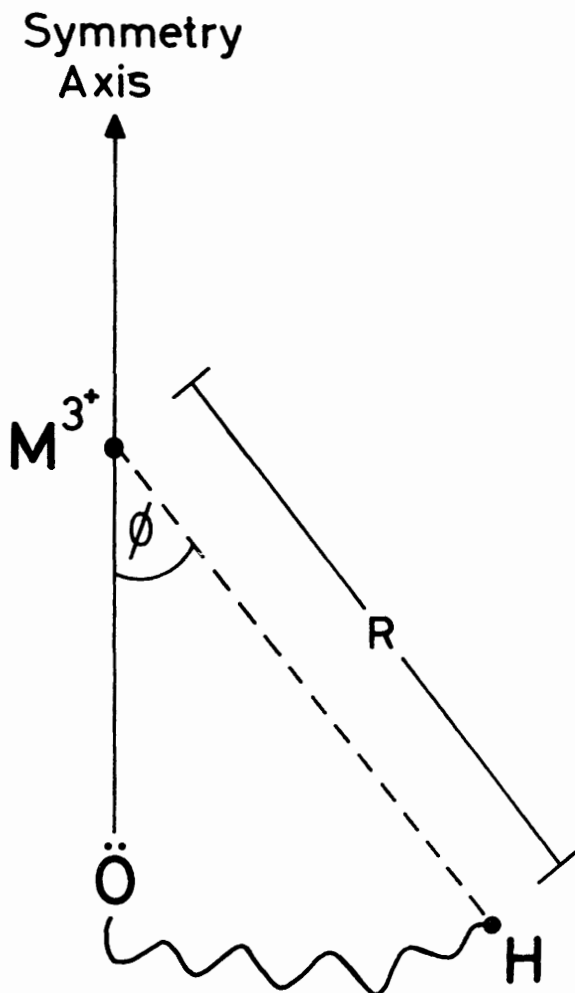
Figure 2 *Schematic view of a shift reagent–substrate adduct*

so that reversals in shift direction can be seen.[8,10]

With shift reagents available, the n.m.r. spectra of many compounds could be modified to yield a wealth of new information, and applications abounded.[8] The interpretation of results was relatively simple for monofunctional compounds, but we felt that an approach to polyfunctional cases was necessary. It was apparent, both qualitatively[11] and quantitatively,[10] that a group's affinity for

[10] J. K. M. Sanders, S. W. Hanson, and D. H. Williams, *J. Amer. Chem. Soc.*, 1972, **94**, 5325.
[11] J. K. M. Sanders and D. H. Williams, *J. Amer. Chem. Soc.*, 1971, **93**, 641.

a shift reagent was in part a measure of the basicity of its lone pair. Therefore, a functional group should be 'turned off' by suitable derivatization; *e.g.* hydroxy (—OH) can usually be converted to trifluoroacetate (—OCOCF$_3$) or tosylate.[12] Also, since lanthanides are 'hard' metals with little affinity for second row elements, ketones can be protected as ethylene thioketals.[12]

However, derivatization is not always possible and we must be able to study molecules with multiple binding sites of varying affinity. At low shift reagent concentration, the highest affinity site is partly complexed and its associated protons shift. As the shift reagent concentration increases, the high affinity site becomes saturated, the low affinity site becomes more complexed and the shifts reflect these changes. Figure 3 illustrates this process for the bicyclic
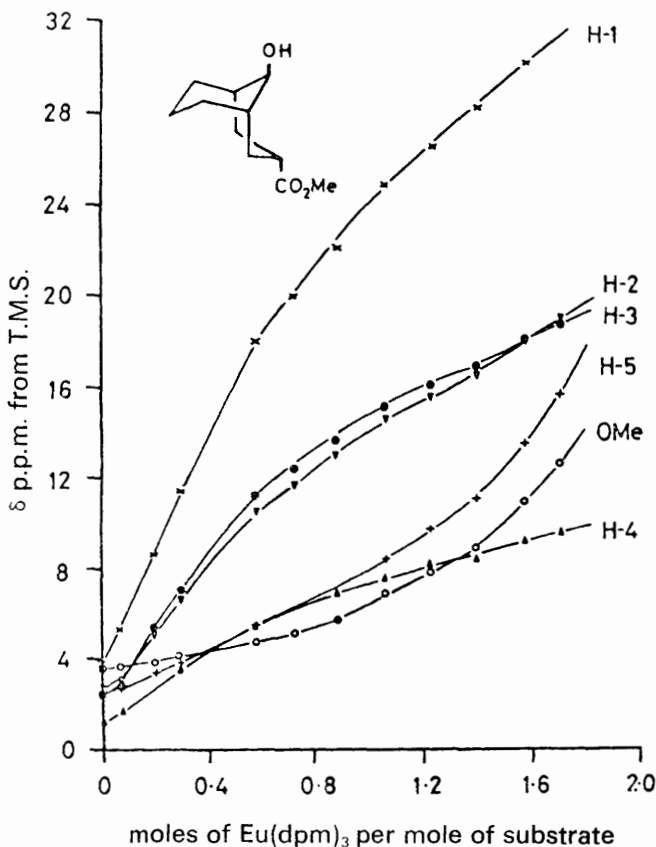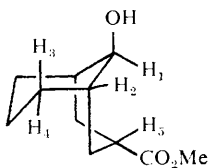

Figure 3 *The effect of added* Eu(d.p.m.)$_3$ *on the* [1]H *(n.m.r.) spectrum of* (1) (Reproduced by permission from *Tetrahedron Letters*. 1971, 3733)

[12] D. R. Crump, J. K. M. Sanders, and D. H. Williams, *Tetrahedron Letters*, 1970, 4949.

compound (1).[13] The same competition effect can be used to study subtle isotope effects on Lewis basicity.[14]

(1)

B. Aquo Ions.—Simple Lanthanide ions can be used in polar solvents as shift reagents for carboxylates[10,15] or $R_3\overset{+}{X}$—O^- (where X is N, P, or As).[10,16] The resulting shifts are normally *upfield* for Eu^{3+}, presumably due to a change in magnetic anisotropy. Figure 4 shows the effect of europium nitrate on a solution
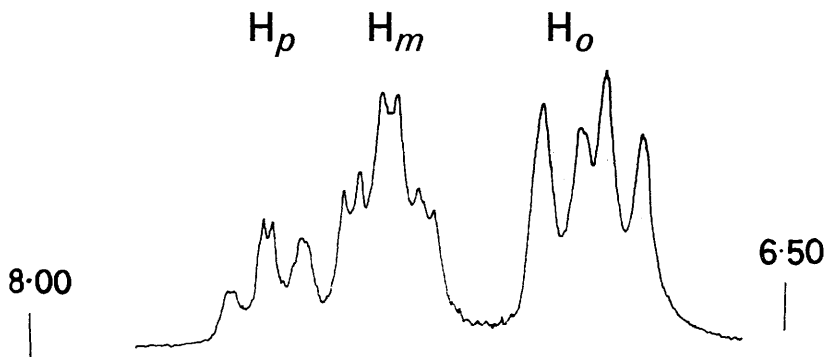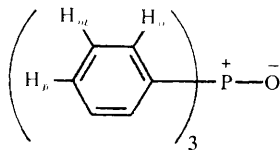

Figure 4 100 MHz ^1H *n.m.r. spectrum of* (2) *in* [$^2H_4$]*methanol after the addition of* 0.4 *equivalents of* $Eu(NO_3)_3$, 6^2H_2O

of triphenylphosphine oxide (2) in [$^2H_6$]acetone. The normally uninformative spectrum is rendered amenable to first order analysis: $J(P—H_o)$ = 11.4 Hz, $J(P—H_m)$ = 3.0 Hz.[10]

(2)

[13] I. Fleming, S. W. Hanson, and J. K. M. Sanders, *Tetrahedron Letters*, 1971, 3733.
[14] J. K. M. Sanders and D. H. Williams, *Chem. Comm.*, 1972, 436.
[15] F. A. Hart, G. P. Moss, and M. L. Staniforth, *Tetrahedron Letters*, 1971, 3389.
[16] J. K. M. Sanders and D. H. Williams, *Tetrahedron Letters*, 1971, 2813.

Lanthanide ion-induced shifts provide a powerful method of mapping metal binding sites in proteins.[17] Complexes such as Ln(edta)⁻ may also be useful.[18]

C. Relaxation Effects.—Lanthanides can also induce changes in relaxation times which can be used to probe molecular shape.[19] The principles of relaxation times are discussed in Section 4B.

3 Radical-anion-induced Changes

An amine-catalysed dimerization of 2-methyl-1,4-naphthoquinone (3), to give the pentacene derivative, (4), was discovered in this laboratory, but attempts to extend the reaction to quinones such as (5) failed.[20] The only dimeric product isolated was (6), the stereochemistry being determined using a shift reagent.[21]

(4)

Bu^tNH₂

(3) R = H
(5) R = Me or Ph

(6)
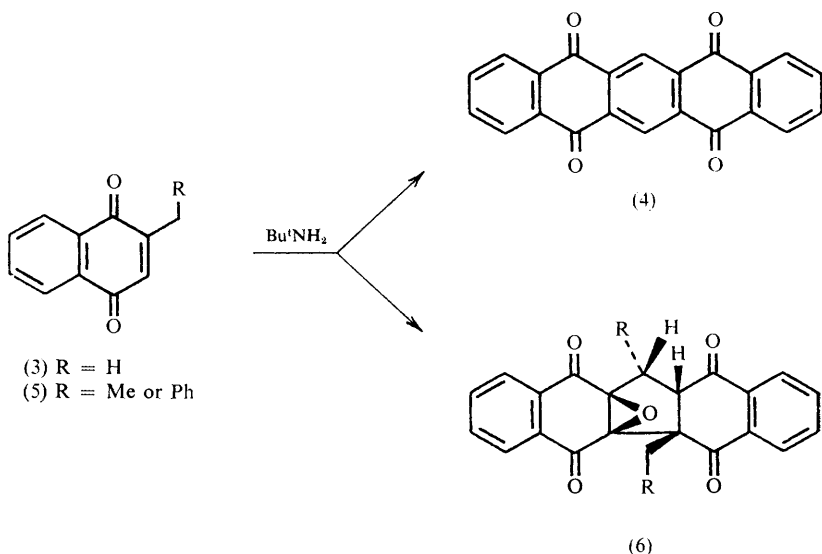
In an attempt to study the mechanisms of these dimerizations we ran n.m.r. spectra of the reaction mixtures. Addition of small amounts of t-butylamine to a solution of (3) in [²H₄]methanol caused selective broadening in the n.m.r. spectrum (Figure 5) without affecting solvent or reference signals. Various experiments[22] convinced us that the broadening was due to a small amount of semi-

[17] I. D. Campbell, C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *Annals New York, Acad. Sci.*, 1973, **222**, 163.
[18] G. A. Elgavish and J. Reuben, *J. Amer. Chem. Soc*, 1977, 99, 1762.
[19] J. W. Faller, M. A. Adams, and G. N. LaMar, *Tetrahedron Letters*, 1974, 699.
[20] I. Baxter, D. W. Cameron, and R. B. Titman, *J. Chem. Soc. (C)*, 1971, 1253.
[21] I. Baxter, D. W. Cameron, J. K. M. Sanders, and R. B. Titman, *J.C.S. Perkin I*, 1972, 2046; I. Baxter, J. K. M. Sanders, and G. E. Evans, *J.C.S. Perkin I*, 1974, 2574.
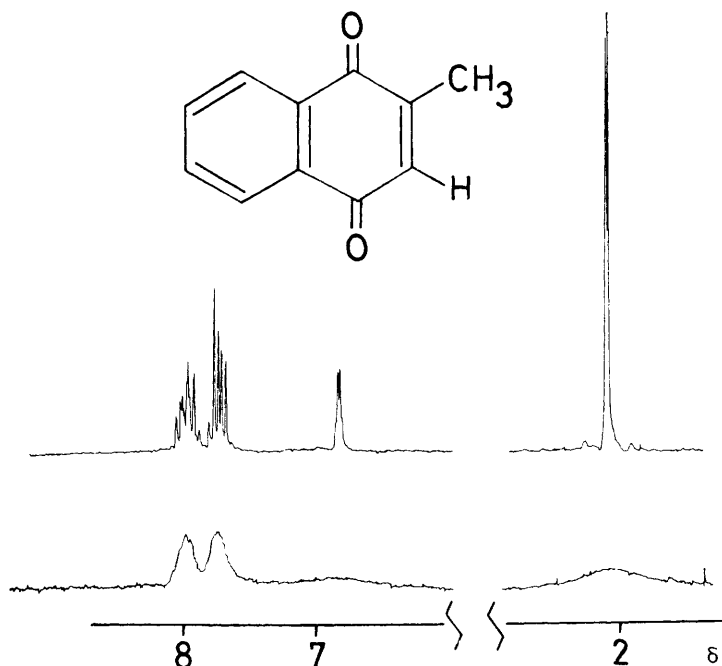[22] J. K. M. Sanders and I. Baxter, *J.C.S. Chem. Comm.*, 1974, 255.

Figure 5 100 MHz ¹H *n.m.r. spectrum of* (3) *in* [²H₄]*methanon* (*upper trace*) *and in the presence of a trace of* (3·) (*lower trace*)

quinone, (3⁻), undergoing fast electron exchange with (3). Similar effects occur with radical cations. The spectrum exhibits characteristics of both species, and the protons closest to unpaired spin density broaden most. Thus we had a beautifully *visual* way of mapping unpaired spin density and molecular orbitals in an organic molecule.[23]

This technique had been known for many years,[24] and the theory was well established.[25] Its use in assigning e.s.r. spectra had been advocated[26] but mostly ignored.[27] We used it successfully on a variety of small molecules[28] and then decided to look at a large molecule where e.s.r. fails, the chlorophyll radical cation. The remainder of the article is concerned with chlorophyll but I should

[23] The relationships between spin density, molecular orbital coefficients, and chemical reactivity are described in I. Fleming, 'Frontier Orbitals and Organic Chemical Reactions,' Wiley, London, New York, Sydney, 1976.

[24] C. R. Bruce, R. E. Norberg, and S. I. Weissman, *J. Chem. Phys.*, 1956, **24**, 473.

[25] E. de Boer and C. MacLean, *J. Chem. Phys.*, 1966, **44**, 1334.

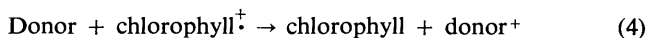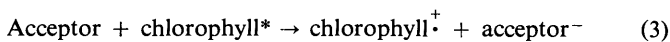[26] G. T. Jones and J. N. Murrell, *Chem. Comm.*, 1965, 28.

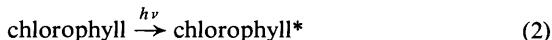[27] See however, C. S. Johnson and R. Chang, *J. Chem. Phys.*, 1965, **43**, 3183; H. Angad-Gaur, C. H. Wijtzes, and J. Smidt, *Mol. Phys.*, 1969, **17**, 179; J. Chaudhuri, S. Kume, J. Jagur-Grodzunski, and M. Swarc, *J. Amer. Chem. Soc.*, 1968, **90**, 6421.

[28] Quinone and nitro-aromatic radical anions and aminium radical cations: I. Baxter and J. K. M. Sanders, unpublished work.

point out here that we are still no wiser about the mechanisms of the quinone dimerizations!

4 Chlorophyll Radical Cations

A Introduction.—The fundamentally important step in photosynthesis is light-induced generation of chemical oxidizing and reducing power. This is achieved by a chlorophyll-mediated electron transfer in the following manner

$$\text{chlorophyll} \xrightarrow{h\nu} \text{chlorophyll*} \tag{2}$$

$$\text{Acceptor} + \text{chlorophyll*} \rightarrow \text{chlorophyll}^{\overset{+}{\cdot}} + \text{acceptor}^- \tag{3}$$

$$\text{Donor} + \text{chlorophyll}^{\overset{+}{\cdot}} \rightarrow \text{chlorophyll} + \text{donor}^+ \tag{4}$$

Summation of equations (2)—(4) gives (5)

$$\text{Donor} + \text{Acceptor} \xrightarrow{h\nu} \text{Donor}^+ + \text{Acceptor}^- \tag{5}$$

Clearly donor$^+$ is an oxidizing agent and acceptor$^-$ is a reducing agent but their exact chemical nature remains unclear.[29] These reactions take place in a photosynthetic reaction centre and involve only a small proportion of the chlorophyll *a*, (7). The bulk of chlorophyll *a*, and all the chlorophyll *b*, (8), is simply used as an antenna, harvesting incident sunlight and transferring it to the reaction centre. Many photosynthetic bacteria use the closely related bacteriochlorophyll (9) in photosynthesis.
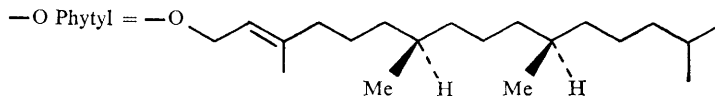

(7) R = Me

(8) R = CHO

(9)

—O Phytyl = —O
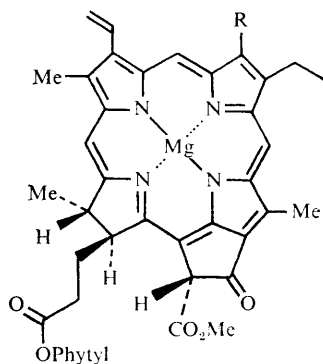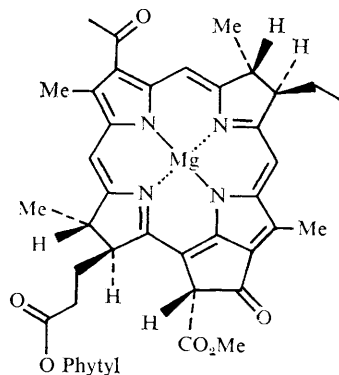
[29] A. J. Bearden and R. Malkin, *Quart, Rev. Biophysics*, 1975, **7**, 131.

Equation (3) above, shows that the radical cation $(7\overset{+}{\cdot})$ is a crucial intermediate, but until very recently little was known of its properties; the e.s.r. spectra of $(7\overset{+}{\cdot})$ and $(9\overset{+}{\cdot})$ are single signals containing over 10^9 unresolved lines! ENDOR, a double resonance technique, has very recently[30,31] yielded detailed spin distributions but when we began our work ENDOR was rather limited.[32] ENDOR and e.s.r. suffer the fundamental disadvantage that peak assignments must be made chemically whereas n.m.r. spectra can be assigned spectroscopically (Sections 4B and 4C).[33] We set out, therefore, to study $(7\overset{+}{\cdot})$ *via* its effect on the n.m.r. spectrum of (7) which is rich in information (Figure 6).

The very richness of the spectrum poses the first problem—assignment of resonances. The spectrum had been assigned previously using various analogues and chemical shift arguments, and aggregation-induced shifts had been most informative.[34] We decided to develop more general spectroscopic methods for assignment which would be useful for a wide range of porphyrins and chlorophylls. Note that these methods are based on spectral changes which yield structural information.

B. Spin-lattice Relaxation Times (T_1).—In the n.m.r. experiment, we observe transition from a lower to an upper nuclear energy level, which requires electromagnetic radiation at the correct frequency. The radiationless return to the lower level is spin-lattice relaxation and requires fluctuating magnetic fields of the same frequency. These fields can arise from many sources,[35] but for organic compounds, in practice they are due to the tumbling of other protons in the same molecule; this is the dipole–dipole interaction.[36] The intensity of fields of the correct frequency at the relaxing proton depends both on its distances from other protons and on their tumbling times. This is illustrated in Figure 7 and expressed mathematically in equation (6)

$$T_1^{-1} \propto \tau \ \Sigma r^{-6} \tag{6}$$

T_1^{-1} is the relaxation rate and is the reciprocal of the relaxation time (T_1), τ is an effective correlation time,[37] and r is the distance from another proton.

Clearly, if two protons are close in space they will relax each other efficiently and have short T_1's. A proton attached to a rigid framework (porphyrin, steroid,

[30] H. Scheer, J. J. Katz, and J. R. Norris, *J. Amer. Chem. Soc.*, 1977, 99, 1372.

[31] D. C. Borg, A. Forman, and J. Fajer, *J. Amer. Chem. Soc.*, 1976, **98**, 6889.

[32] J. R. Norris, H. Scheer, M. E. Druyan, and J. J. Katz, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 4897.
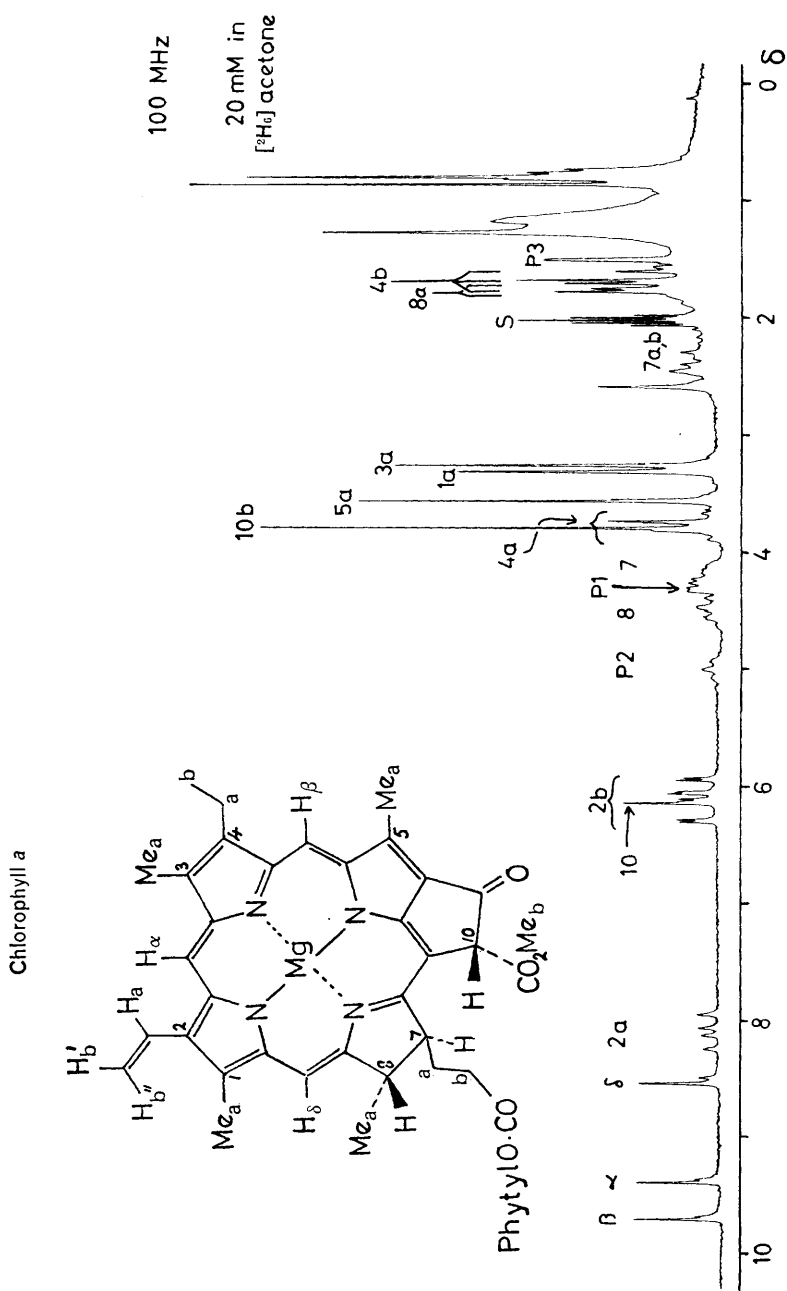
[33] The n.m.r. experiments yield only *relative* spin densities and ENDOR is necessary to provide absolute values.

[34] H. Scheer and J. J. Katz in 'Porphyrins and Metalloporphyrins,' ed. K. M. Smith, Elsevier, Amsterdam 1975, p. 399.

[35] T. C. Ferrar and E. D. Becker, 'Pulse and Fourier Transform NMR,' Academic Press, London, New York, 1971, Chapter 4.

[36] For details of when this is true, and for a good review of proton relaxation in general see L. D. Hall, *Chem. Soc. Rev.*, 1975, 401.

[37] τ Contains contributions both from the overall rate of molecular reorientation and from internal rotation: H. B. Coates, K. A. McLauchlan, I. D. Campbell, and C. E. McColl, *Biochem. Biophys. Acta*, 1973, **310**, 1.
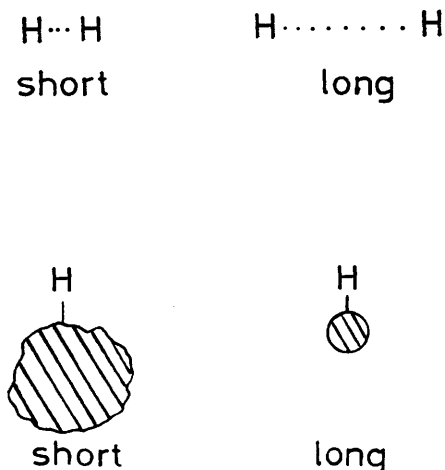
Figure 6 100 MHz ^{1}H *n.m.r. spectrum of chlorophyll a, 20 mM in* [^{2}H$_6$]*acetone*

H···H H········H
short   long

H   H

short long

Figure 7 *The dependence of proton* T_1 *on environment*

or alkaloid) will tumble slowly and have a short T_1, whereas if it is part of a small molecule or it has motional freedom within a large one then τ will be short and T_1 long. Steric hindrance of C—Me bond rotation will also lead to short T_1's. It is not always easy to separate the τ and r contributions. However, within methyl groups each proton relaxation is dominated by interaction with the other protons on the carbon to which they are attached. The Σr^{-6} term is therefore constant and T_1 is simply a measure of τ. Thus, we can predict that as we move along a flexible alkyl chain away from a rigid framework, T_1 should increase smoothly.

Our results (Table 1) show that this prediction is correct,[38] and that T_1 measure-

Table 1 *Typical* T_1 *values for metalloporphyrins in co-ordinating solvents at* 310 K

	T_1 (sec)	
Substituent	CH$_2$	CH$_3$
—CH$_3$	—	0.5
—CH$_2$CH$_3$	0.3	0.7
—CH$_2$CH$_2$CO$_2$Me	α 0.3	1.1
α β	β 0.5	

ments could provide a method of sorting protons into groups according to their distance from a macrocycle. Absolute T_1 values are heavily dependent on solvent, temperature, and aggregation state but relative values within a molecule have been reliable (so far!).

Relaxation times are measured[36] by applying a '180° pulse' to the sample,

[38] I. S. Denniss, J. K. M. Sanders, and J. C. Waterton, *J.C.S. Chem. Comm.*, 1976, 1049; 1977, 192.

which inverts all the spins, and monitoring their return to equilibrium. A normal spectrum taken with a 90° pulse immediately after the 180° pulse shows all peaks to be negative. As the spins relax towards equilibrium the negative-going peaks decrease in intensity, pass through a null point (at roughly 0.69 T_1) and grow in a positive sense. It is therefore possible to measure T_1 by observing 'partially-relaxed' spectra at various times after the 180° pulse. Figure 8 illustrates
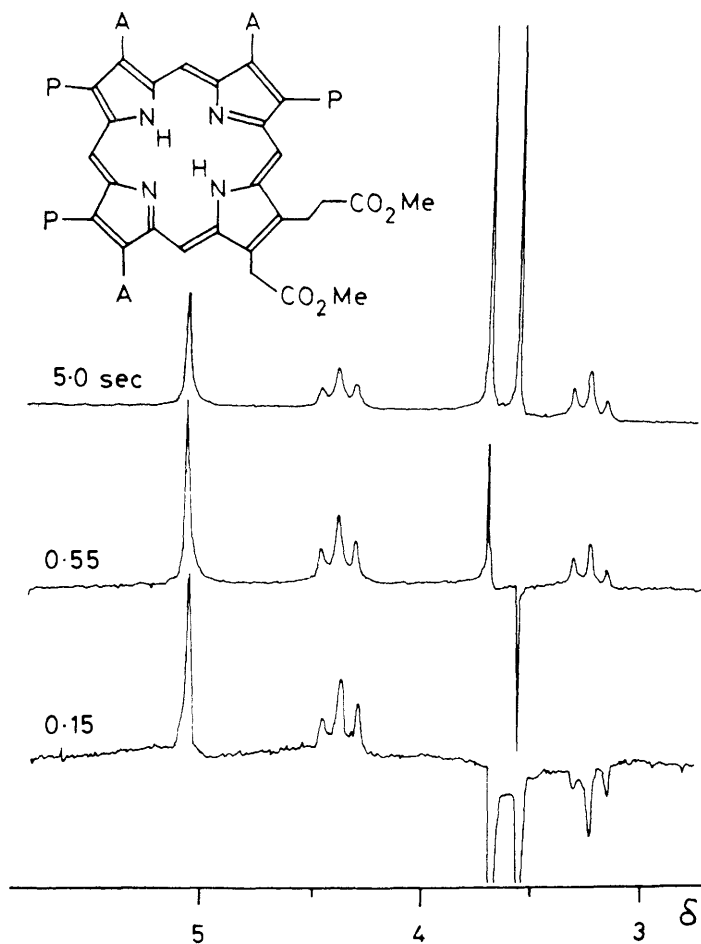

Figure 8 *Partially-relaxed* 100 MHz ¹H *n.m.r. spectra of uroporphyrin-II-octamethyl ester, 6 mM in CDCl₃. Each spectrum is displayed with a different vertical scale.*
A = $-CH_2CO_2Me$, P = $-CH_2CH_2CO_2Me$

results for a typical porphyrin. 0.15 Seconds after the pulse only the protons directly attached to the macrocycle are up. After 0.55 seconds, only the pro-

pionate methyl signal is still inverted as it has the longest T_1. Therefore, solely on this distance basis we can assign not only the α and β methylenes of the propionate side chain but also the acetate and propionate methyl groups. This is a useful technique for unravelling new natural product structures, partly through the sorting of groups by distance, and also because partially relaxed spectra with some nulled peaks are simpler to interpret.

T_1 measurements on chlorophylls give the expected results[38] but do not allow distinction between methyl groups in similar positions, and these assignments require Nuclear Overhauser Effects (see next section). Note, however, that all the *meso* protons in (9) have the same τ but H_χ has few proton neighbours (due to the acetyl group). It therefore has a much longer T_1 than H_β or H_δ and can be confidently assigned.[38] Relaxation times also tell us something of the conformational mobility of the phytyl group.[39]

As deuterium has a smaller magnetogyric ratio than protium, it is much less effective in causing proton or ^{13}C relaxation and leads to lengthening of T_1's in its vicinity. This can be a useful technique for locating the position[40] and stereochemistry[39] of deuterium in a molecule. On the other hand, paramagnetic ions generate intense magnetic fields, causing T_1 to shorten by an amount proportional to r^{-6}. This is the basis of the mapping method mentioned in Section 2C.

C. Nuclear Overhauser Effect (NOE).—If proton A causes proton B to relax, then saturation of the A resonance will cause the B resonance intensity to increase by up to 50%;[41] this is the NOE. It arises through the dipole–dipole interaction, and if several protons are close to that being observed then their relative NOE contributions are proportional to each r^{-6}. The sum of their NOE's cannot exceed 50%.

The rigidity of the porphyrin macrocycle makes it ideal for these experiments, and the positive observation of an NOE is powerful evidence that two nuclei are close to each other. Table 2 confirms the value of the technique for

Table 2   *Nuclear Overhauser Effects for* $(10)^{a,b}$

| Signal irradiated		% increase		
	H_α	H_β	H_γ	H_δ
vinyl—CH	15	15	—	—
—CH_2	—	—	30	—
—CH_3	20	20	—	30

[a] J. K. M. Sanders, unpublished results; [b] In $COCl_3/[^2H_5]$ pyridine

the model compound (10). Extension to chlorophylls is in principle simple; the unambiguous signals of (7) are the 8-methyl doublet at 1.75 δ and the 2a-vinyl double doublet at 8 δ (see Figure 6). Using these starting points it is possible to 'walk' round most of the macrocycle, obtaining connectivities from successive

[39] J. C. Waterton and J. K. M. Sanders, unpublished results.
[40] L. M. Jackman and J. C. Trewella, *J. Amer. Chem. Soc.*, 1976, **98**, 5712.
[41] J. H. Noggle and R. E. Schirmer, 'The Nuclear Overhauser Effect,' Academic Press, London, New York, 1971.
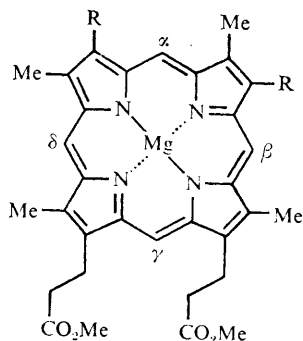
$(10)\ R = -CH=CH_2$

$(11)\ R = H$

NOE experiments.[39] In addition, these experiments, when carried out with utmost care, reveal in (9) small long-range couplings which were previously unsuspected and which are invaluable in making assignments.[39]

The experiments are, however, quite difficult to do, and in the long run it may be that NOE methods will be replaced by Hall's selective T_1 method, which is based on the same relaxation phenomenon but is numerically more reliable.[42]

D. Distribution of Unpaired Spin Density.—With assigned spectra available, we could interpret the line broadening effects of $(7\overset{+}{\cdot})$, generated by oxidation of (7). This can be done electrochemically,[31] or with iodine[30] but for our work the most convenient method is with the crystalline radical cation (12).[43,44] Addition of (12) to a chlorophyll solution sets up an equilibrium (equation 7), the spectroscopic result of which is shown in Figure 9.

$(12)\ X^- = SbCl_6^-$

$(13)\ X^- = ClO_4^-$

$$R_3N^+ + \text{chlorophyll} \rightleftharpoons R_3N + \text{chlorophyll}\overset{+}{\cdot} \qquad (7)$$
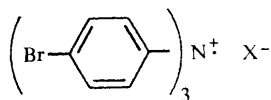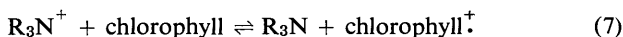
It is immediately obvious that

[41] K. Bock, R. Burton, and L. D. Hall, *Canad. J. Chem.*, 1977, **55**, 1045; L. D. Hall and H. D. W. Hill, *J. Amer. Chem. Soc.*, 1976, **98**, 1269.

[42] F. A. Bell, A. Ledwith, and D. C. Sherrington, *J. Chem. Soc.* (C), 1969, 2719.

[44] We have also used (13) and the perchlorate salt of (tetraphenylporphyrin) $\overset{+}{\cdot}$;[31,43] these give essentially the same results as (12).

270 MHz

Chlorophyll *a*

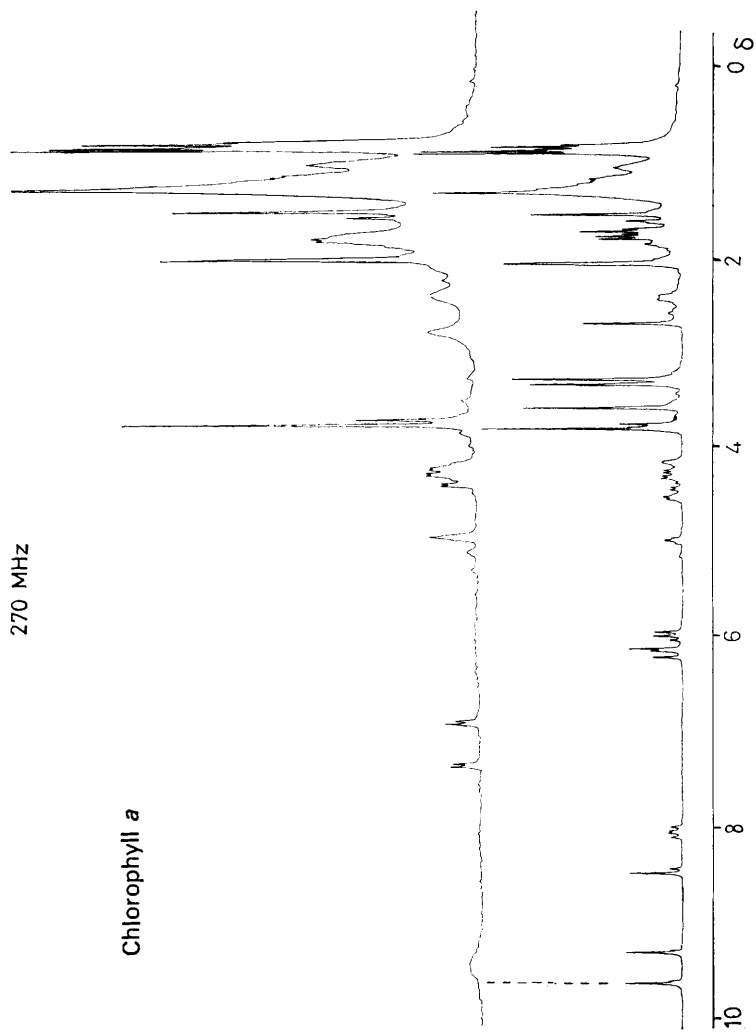**Figure 9** 270 H *n.m.r. spectra of chlorophyll a, (7), in [²H₆]acetone (lower trace) and after partial oxidation with* (12) *(upper trace)*

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(i) the amine signals ($\sim 7\ \delta$) are sharp, confirming that the equilibrium lies to the right.

(ii) some chlorophyll signals have disappeared, some are broadened, and others unaffected.

(iii) the very broad H_β resonance has moved upfield.[45]

The broadened spectrum of Figure 9 arises from less than 1 % oxidation of (7). Solutions containing even less radical cation show highly selective effects. The various proton resonances broaden in the following order, covering a range of *ca.* 340 fold between 7 and 4b at ambient temperature[46]

$$7 > 8 > 5a > 1a, 3a > \delta > \alpha > 4a > 10 > 2b > 2a > \beta > 4b$$
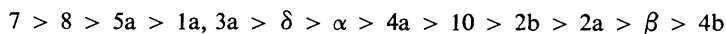
The broadening arises through the electron transfer reaction in equation 8. In the radical cation, protons are coupled to the unpaired electron with

$$(7) + (7\overset{+}{\cdot}) \underset{k}{\overset{k}{\rightleftharpoons}} (7\overset{\cdot}{\cdot}) + (7) \tag{8}$$
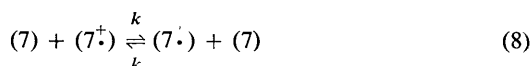
hyperfine coupling constants, a_H, of up to 110 MHz. At ambient temperatures the exchange rate k is fast compared with a_H and each line is broadened by an amount proportional to the square of a_H.[25] From relative broadenings we can therefore get relative values for all significant hyperfine couplings in $(7\overset{+}{\cdot})$, $(8\overset{+}{\cdot})$, $(9\overset{+}{\cdot})$ and their derivatives.[39,46]

The hyperfine coupling can be related to spin density, ρ, by the McConnell relation (equation 9), where Q is a 'constant' of notorious variability having a value of roughly 70 MHz* for

$$a_H = Q \cdot \rho \tag{9}$$

a proton attached to a π-system (14) and 75 MHz* for a methyl group attached to a π-system (15).[47] Due to some confusion in porphyrins[48] we synthesized (11) in order to actually measure Q values.[49] If the alkyl substituents have a negligible effect on the spin distribution, then ρ should be the same for each position and relative a_H's would reflect relative Q values. Thus from observed relative broadenings in $(11\overset{+}{\cdot})$ we find that

$$Q_H : Q_{Me} : Q_{CH_2} = 1.0 : 1.28 : 0.54$$

The very low figure for Q_{CH_2} indicates that the propionate side chains are out of

*2.8 MHz = 1 G

[45] This shift is unobservable at 100 MHz. Access to the 270 MHz instruments at Oxford and Portsmouth is gratefully acknowledged.

[46] J. K. M. Sanders and J. C. Waterton, *J.C.S. Chem. Comm.*, 1976, 247.

[47] J. R. Bolton in 'Radical Ions,' ed. E. T. Kaiser and L. Kevan, Wiley, London, New York, Sydney, 1968, p. 1.

[48] K. Wuthrich, B. J. Wyluda, and W. S. Caughey, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **62**, 636; R. G. Shulman, S. H. Glarum, and M. Karplus, *J. Mol. Biol.*, 1971, **57**, 93.

[49] C. G. Newton and J. K. M. Sanders, unpublished work.

plane leading to minimal overlap of the C—H σ-bonds with the π-system (16).
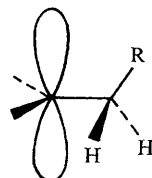
Armed with a_H and Q we can compare our observed spin densities with MO calculations. The agreement is qualitatively reasonable but calculations entirely fail to predict the highly asymmetric distribution that we[46] and Katz[30] find. Spin distributions for ($8\overset{+}{\cdot}$) and ($9\overset{+}{\cdot}$) and various derivatives are also poorly predicted.[39]
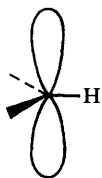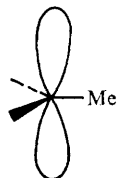
(14)   (15)   (16)

Solutions containing radical cations are reasonably stable, although in some cases specific decomposition products can be isolated.[50] The great amplification of radical effects given by the electron transfer process (equation 8) means that we can detect the paramagnetic species at concentrations as low as 10^{-8} mol l^{-1}.

E. Electron Transfer Rates.—When chlorophyll solutions containing radical cation are cooled, the hyperfine broadening increases. On further lowering of the temperature, we observe sharpening,[39] which is caused by the electron transfer rate, k, becoming slow on the NMR time scale so that we are no longer seeing an averaged spectrum. Using standard equations[25] we can calculate the expected dependence of broadening on k (Figure 10) and compare the results with experimental observations. Particularly diagnostic are the coalescence temperatures (point of maximum broadening) which are dependent on the hyperfine coupling.

From these results we can derive electron transfer rates and activation energies and investigate how they, and therefore the mechanism, depend on structure, solvent, and other factors. This work is at an early stage, but looks very promising.

F. Radical-induced Pseudocontact Shifts.—The upfield shift of H_β in (7$^+$) (Figure 9) is too large to be entirely due to the contact mechanism,[25] and we decided to test whether a shift-reagent type pseudocontact effect could operate. We chose the capped porphyrin (17) as the protons of the alkyl 'cap' are shifted upfield by the ring current.[51]

[50] R. G. Brereton and J. K. M. Sanders, unpublished work.
[51] M. Turnbull and A. R. Battersby generously provided the magnesium-free porphyrin: A. R. Battersby, D. G. Buckley, S. G. Hartley, and M. D. Turnbull, *J.C.S. Chem. Comm.*, 1976, 879.
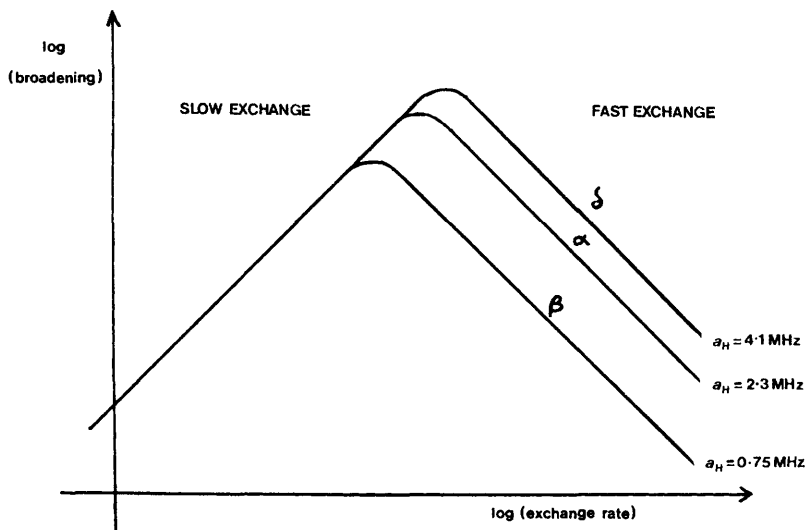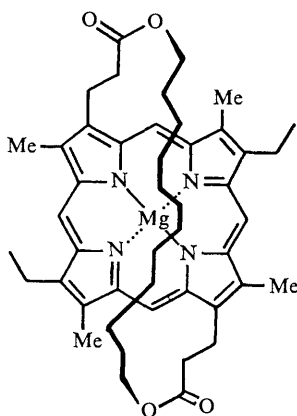
5   483

Figure 10 *The dependence of broadening on electron transfer rate for* meso *protons of chlorophyll* a

(17)

Slight oxidation of (17) using (12) ($\ll 1\%$) gave exchange broadening of the hyperfine-coupled peripheral protons in the usual way. Further oxidation causes the alkyl cap protons to shift strongly downfield;[52] the highest field resonance at -0.6 δ is paramagnetically shifted downfield by an amount corresponding to at least 21 p.p.m. in pure (17$\overset{+}{.}$). Other cap resonances are similarly shifted

[52] J. K. M. Sanders and J. C. Waterton, submitted for publication.

downfield by an amount proportional to their original upfield ring current shift.

Clearly these protons experience a magnetic field which has the same geometrical properties as the aromatic ring current but which is ten times larger and has the opposite sense. This we believe to be the first observation of a large radical-induced pseudocontact shift. The reasons why such shifts have not previously been seen are instructive and are discussed elsewhere.[52] In principle these results may allow the chlorophyll radical cation to be used as a natural spin label which, through its broadening and shift powers, could be used to map photosynthetic reaction centres but in practice the difficulties will be formidable.

Exhaustive experiments[39] on the concentration dependence of the H_β shift in $(7\overset{+}{\cdot})$ unfortunately are not consistent with contact, pseudocontact or a combination of these mechanisms, and its causes remain a mystery.

G. Modified Chlorophylls.—Spectroscopic evidence suggests that in the photosynthetic reaction centre a *pair* of chlorophyll molecules, held together by water as a non-covalently-bound dimer, is the true catalyst of equations (2)—(4).[31,53] We therefore synthesized a dimer (18) in which two chlorophylls were joined
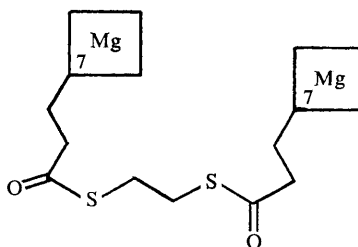
(18) [substitution pattern is same as in (7)]

via the carbonyls normally carrying the phytyl group.[54] Chlorophyll is sensitive to acid, base, heat, light, and air so the synthesis (Scheme 1) avoided all these reagents. The corresponding dimer using an ethylene glycol bridge can only be synthesized in the magnesium-free series followed by metal replacement.[53–55] Our shorter sequence gives a much better overall yield of dimer.

The dimers fold up in the presence of water to give a reaction centre model. The folding is accompanied[53–55] by dramatic changes in n.m.r., e.s.r. and electronic spectral properties which should yield detailed information on molecular conformation. Electron transfer studies on $(18\overset{+}{\cdot})$ should also prove illuminating.

We have also made (Scheme 2) the tailed chlorophyll (21) in which the imida-

[53] M. R. Wasielewski, M. H. Studier, and J. J. Katz, *Proc. Nat. Acad. Sci., U.S.A.*, 1976, **73**, 4282.
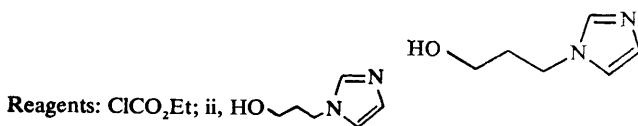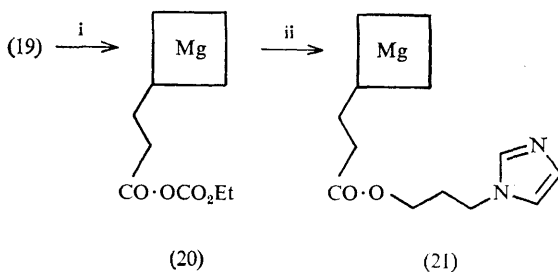[54] I. S. Denniss and J. K. M. Sanders, unpublished results.
[55] S. G. Boxer and G. L. Closs, *J. Amer. Chem. Soc.*, 1976, **98**, 5406.

$$(7) \xrightarrow{\;i\;} \boxed{Mg} \xrightarrow{\;ii\;} \boxed{Mg} \xrightarrow{\;iii\;} (18)$$

(19)
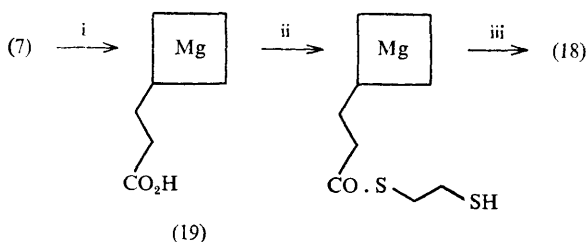
Reagents: i, spinach beet chlorophyllase; ii, dicyclohexylcarbodi-imide (DCC)/HS⌄⌃SH;
iii, DCC/*19*

Scheme 1

$$(19) \xrightarrow{\;i\;} \boxed{Mg} \xrightarrow{\;ii\;} \boxed{Mg}$$

(20)   (21)

Reagents: ClCO$_2$Et; ii, HO⌄⌃N

Scheme 2

zole can bind intramolecularly to the magnesium.[54] This binding is accompanied by large upfield shifts of the 'tail protons which sit over the macrocycle. An added ligand such as pyridine or *N*-methyl imidazole can either displace the tail or bind to the metal ion from the other side of the ring (Figure 11). Simultaneous n.m.r. monitoring of the ligand and 'tail' resonances enables the relative import- ance of these processes to be determined.[54] In principle, the temperature depend- ence of the equilibria allows separation of the enthalpy and entropy contributions to the inter- and intra-molecular reactions but the detailed molecular interpreta- tion of these results is difficult.

5 Conclusions
N.m.r. spectral change induced by complexation, double irradiation or pulse methods can give much detail about the structure, stereochemistry and mobility
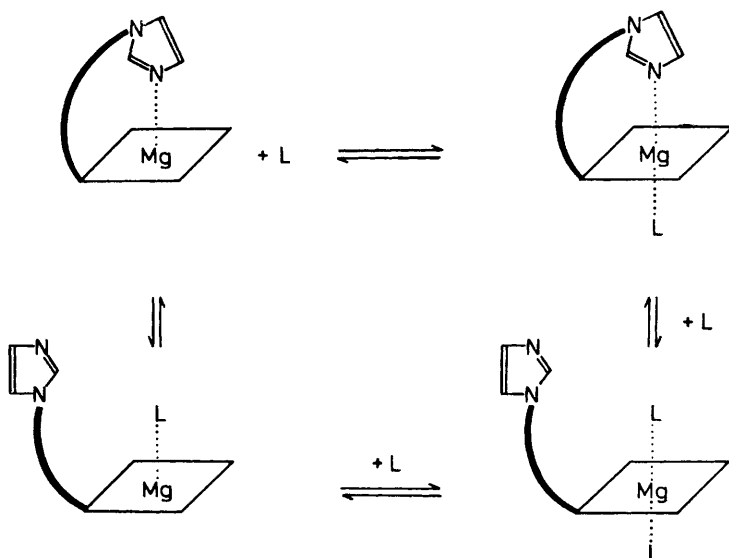
Figure 11 *The binding of ligands to* (21)

of molecules. More exciting in the long run is actually watching chemistry in the n.m.r. tube indirectly *via* electron transfer broadening, CIDNP,[56] and related effects,[57] or directly *via* flow n.m.r.[58]

I am grateful to the Society of Maccabaeans and the Royal Institute of Chemistry for the award of the Meldola Medal, and to the Science Research Council for generous support.

I am deeply indebted to Iain Denniss and John Waterton for their dedication, ideas and enthusiasm in the chlorophyll work and to Dudley Williams who was my supervisor and is now my most effective critic.

[56] 'Chemically Induced Magnetic Polarisation,' ed. A. R. Lepley and G. L. Closs, Wiley, London, New York, Sydney, 1976.

[57] S. G. Boxer and G. L. Closs, *J. Amer. Chem. Soc.*, 1975, 97, 3268.

[58] C. A. Fyfe, A. Koll, S. W. H. Danuji, C. D. Malkiewich, and P. A. Forte, *J.C.S. Chem. Comm.*, 1977, 335.