Assignment of Metalloporphyrin and Chlorophyll Nuclear Magnetic Resonance Spectra *via* Spin–Lattice Relaxation Times

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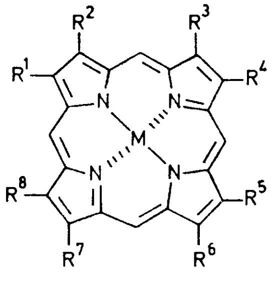
Summary Proton spin–lattice relaxation times may be used to assign the n.m.r. spectra of the title compound types; the technique is particularly valuable for methyl groups.

The n.m.r. spectra of porphyrins and chlorophylls are generally assigned using chemical shift arguments and by comparison with related compounds.[1] Problems can arise, especially when several types of methyl group resonate with similar chemical shifts and deuteriation may be necessary.[2] We report here that spin-lattice relaxation times (T_1) are highly characteristic of environment and may be useful in this assignment problem.

The T_1 for a proton depends[3] principally on its distances (r) from all neighbouring protons and its effective correlation time (τ)† [equation (1)]. The porphyrin macrocycle is heavy and rigid so that groups attached directly to it should have long τ and short T_1, but protons in extended side chains should have more motional freedom leading to shorter τ and longer T_1 if the r^{-6} term remains constant.

$$1/T_1 \propto \tau r \Sigma^{-6} \qquad (1)$$



(1) R^1–R^8 = Et, M = Mg
(2) $R^1 = R^3 = R^5 = R^8$ = Me,
$R^2 = R^4$ = H,
$R^6 = R^7 = CH_2CH_2CO_2Me$,
M = Mg
(3) As (2) but $R^2 = R^4 = CH{=}CH_2$
(4) As (3) but M = Zn

The latter condition is normally fulfilled by methyl protons because their relaxation is usually dominated by inter-

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† This is a simplified treatment in which correlation time contains contributions both from the overall rate of molecular reorientation and from internal rotation (see ref. 4). T_1 values in conformationally mobile molecules are often difficult to interpret owing to the poorly defined r^{-6} term.

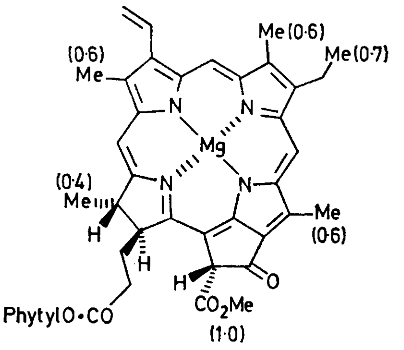
action with the other protons on the carbon atom to which they are attached.

TABLE. T_1 values for metalloporphyrins[a]

Substituent	T_1/s H	T_1/s CH_2	T_1/s Me
H	0·8—1·0	—	—
Me	—	—	0·5—0·6
CH_2Me	—	0·25—0·35	0·7—0·8
$CH_2CH_2CO_2Me$	—	α0·25—0·35	1·1
α β	—	β0·4—0·6	

[a] Determined at 310 K by 180°–t–90° method (ref. 3); 80 or 100 MHz.

The Table summarises the T_1 values for compounds (**1**—**4**) in dilute solution [10—20 mM in $(CD_3)_2CO$ or 10:1 $CDCl_3$–C_5D_5N]. As expected, methyl T_1 values increase with distance from the macrocycle and provide a good method for partial assignment.



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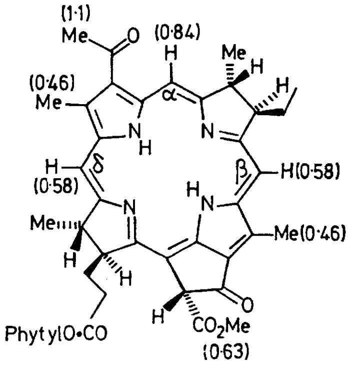

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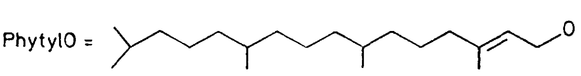

The Figure shows part of the normal and 'partially relaxed' spectra of (**2**); 0·7 s after a 180° pulse the ring methyl groups (T_1 *ca.* 0·6 s) have substantially relaxed towards equilibrium, whereas the ester methyl signal (T_1 *ca.* 1·1 s) is still inverted. Assignment is therefore a trivial matter even though all these resonances fall in a range of < 0·2 p.p.m.

These effects are most important for the interpretation of the spectra of complex molecules; we illustrate results for chlorophyll-a [**5**; 25 mM in $(CD_3)_2CO$] and bacteriopheophytin (**6**; 25 mM in $CDCl_3$) where the ester methyl [and, in (**6**), the 2b-Me] are readily distinguished from the ring methyl groups. If the phytyl group of (**5**) is replaced by methyl, then all T_1 values increase slightly owing to the decrease in molecular weight, and the new methyl group has a T_1 of 1·7 s, reflecting its mobility. In (**6**) all the *meso* protons have the same τ but H_α has fewer near neighbours and thus it has a longer T_1 (0·84 s) than H_β and H_δ (0·58 s).

All assignments have been confirmed by deuteriation or nuclear Overhauser effect experiments and agree with those previously published.[1]

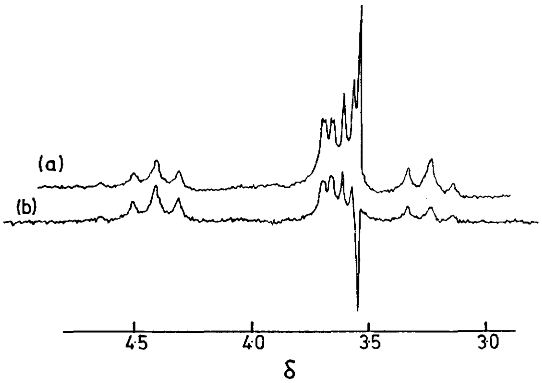

FIGURE. Partial 80 MHz n.m.r. spectra of Mg-deuteroporphyrin-IX-di-Me ester (**2**) [13 mM in $(CD_3)_2CO$]. (a) Normal spectrum after 50 90° transients. (b) Spectrum of 170 180°–0·7 s–90° transients.

Where overlapping resonances have substantially different relaxation times, they may be separately observed in partially relaxed spectra when either signal is nulled.[3] This facilitates determination of multiplicities or line widths which are otherwise obscured.

It is important to note that absolute T_1 values are dependent on solvent, concentration, and temperature but relative values within a molecule have proved reliable in all cases we have studied to date. The same relative values are found in metal-free porphyrins and pheophytins (*e.g.* **6**), with absolute values tending to be shorter due to aggregation.

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