Bacteriochlorophyll *a*: Assignment of the Natural Abundance ^{13}C N.M.R. Spectrum. Use of Power Spectra

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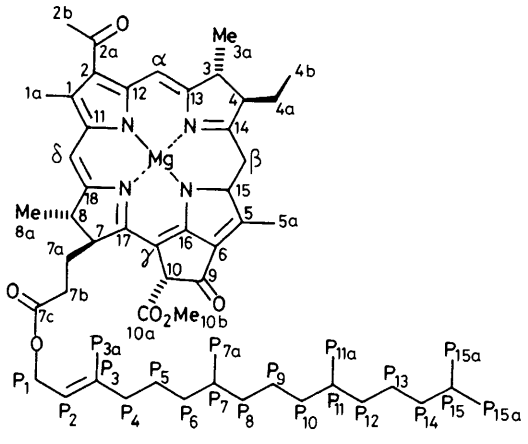
A mixed solvent system was devised which gave highly concentrated but stable solutions of bacteriochlorophyll suitable for ^{13}C n.m.r. spectroscopy. Most of the resonances were unambiguously assigned using conventional methods augmented by the use of the power spectrum display mode.

The ^{13}C n.m.r. spectrum of chlorophyll *a* was first investigated using samples which were uniformly ^{13}C-enriched biosynthetically.[1,2] The enrichment, to 90 and 15% ^{13}C levels respectively, not only improved sensitivity but also gave valuable assignment information in the form of C–C couplings. In contrast, natural abundance spectroscopy of chlorophylls presents considerable experimental problems, not least of which is that their low solution stability makes conditions for long term data accumulation elusive. We now report the natural abundance ^{13}C n.m.r. spectrum of bacteriochlorophyll *a* (1), using our new information [3] on the influence of co-ordination state on bacteriochlorophyll chemistry to obtain stable solutions approaching 1 molal concentration in 1.7 mm capillary n.m.r. tubes. These solutions allowed ready observation of proton *coupled* ^{13}C spectra. Assignments were made mainly by conventional methods enhanced by the use of the power spectrum display mode. The power spectrum [4,5] is the square of the real spectrum plus the square of the imaginary spectrum.† It has the advantages over normal phased spectra that (i) because intensities are squared, the apparent signal to noise ratio is improved (although this is a largely cosmetic change) and linewidth variations are exaggerated, and (ii) because the square of the absolute value (rather than phased) spectrum is displayed, smaller couplings can be resolved.[4] The power display mode has been used for proton spectra, but the effect of squaring is to cause distortions, *e.g.* a 1 : 2 : 1 triplet becomes 1 : 4 : 1.[5] This is not a problem in the present work where only doublet splittings are relevant. All spectra in this paper are displayed in the power mode.



(1) BChl

Results and Discussion

It is clear from the results of the preceding paper that stable concentrated solutions of BChl could only be expected in six-co-ordinating solvents, such as methanol or pyridine. BChl is only sparingly soluble in methanol (and pyridine is unsuitable for several reasons) so we acquired spectra in a mixture of [$^2H_6$]acetone and [$^2H_4$]methanol (*ca.* 4 : 1). Under these conditions (1) is highly soluble but largely six-co-ordinate; furthermore the proton spectrum is essentially identical to that which had already been rigorously assigned in pure [$^2H_6$]acetone.[6] These solutions of BChl were highly viscous and best prepared *in situ* in a capillary n.m.r. tube as described in the Experimental section.

Figure 1 shows the 25.2 MHz proton noise decoupled spectrum obtained with a micro-insert. Most noteworthy is the large linewidth variations between different carbon signals:

Table ^{13}C Chemical shifts in bacteriochlorophyll *a*

Chemical shift (p.p.m.)	Assignment	Chemical shift (p.p.m.)	Assignment
197.6	2a	49.0	7
187.5	9	48.0	8
171.7	7c	46.7	3
170.7	10a	38.5	
166.1	17, 18 [a]	38.2	P4, P6, P8,
164.9		36.2	P10, P12, P14 [a]
156.6	6	35.3	
150.2	11	31.6	
148.8	16	31.4	P7, P11, P15 [a]
147.9	15, 12	31.1	
141.2	P3	31—27	7a, 7b, 4a, solvent
140.6	13, 14	23.8	
135.1	5	23.6	P5, P9, P13
127.6	1	23.2	
122.3	2	21.6	3a, 8a [a]
117.2	P2	21.2	
107.1	γ	21.2	P7a, P11a, P15a
99.9	β	18.3	
97.6	α	14.1	P3a
94.2	δ	12.0	1a
65 [b]	10	10.3	5a
59.8	P1	9.1	4b
54.0	4		
50.8	10b		

[a] Assignment unknown within brackets. [b] Variable shift.

† The square root of the power spectrum is the absolute value spectrum. Confusingly, the absolute value display is erroneously called the power spectrum in some publications.

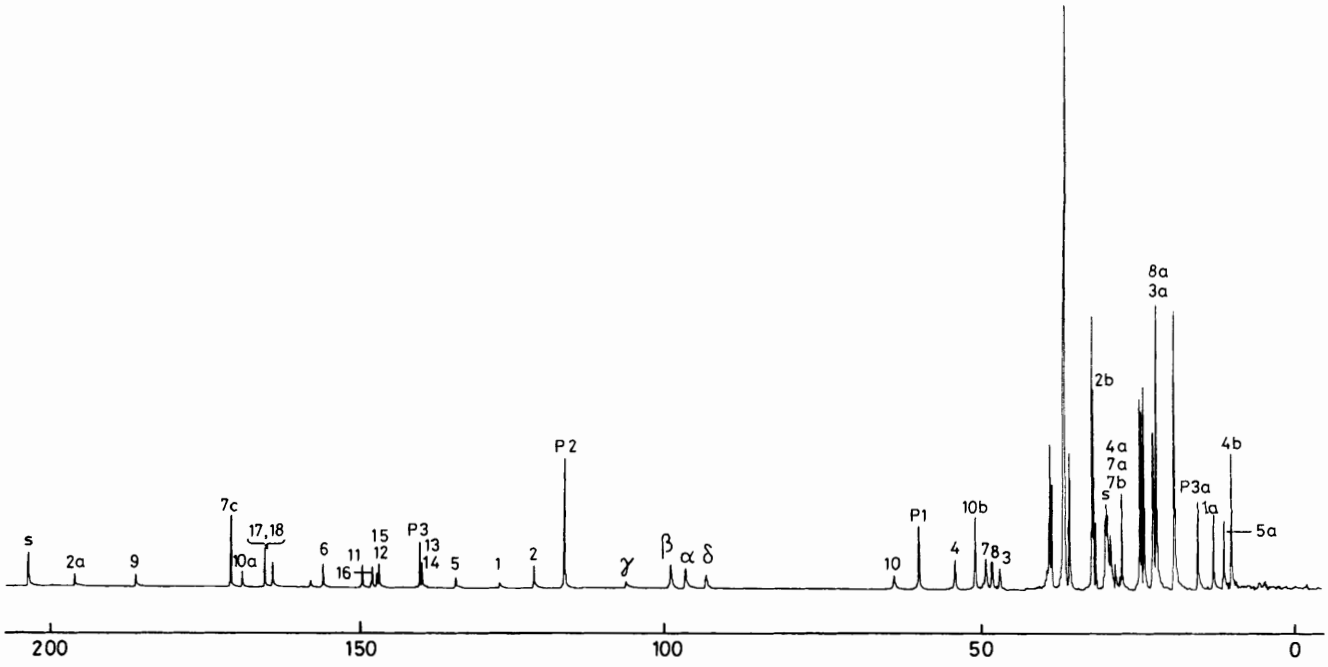


Figure 1. 25.2 MHz ^{13}C n.m.r. noise decoupled spectrum of bacteriochlorophyll *a*, *ca.* 1 molal in [$^2H_6$]acetone: [$^2H_4$]methanol (4 : 1). Signal marked s at δ 207 and 29 are due to solvent

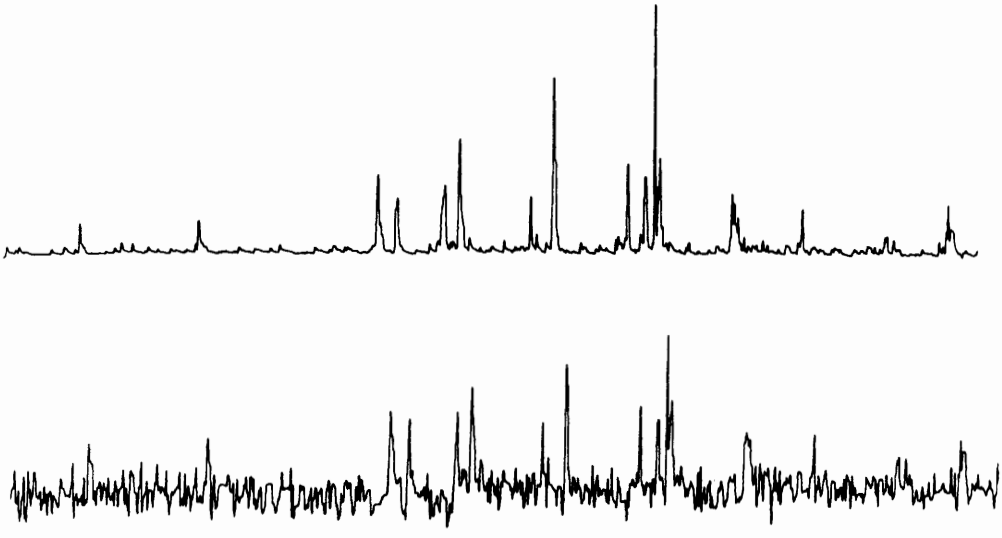


Figure 2. The effect of power spectral display mode on a portion of the bacteriochlorophyll ^{13}C n.m.r. spectrum. The normal phased display is below, and the power spectrum display is above

this reflects the rapid relaxation of carbons which are directly attached to the macrocycle, and proved useful in assignment. This effect has been previously observed in proton spectra of chlorophylls and porphyrins [6] but is exaggerated in these very concentrated viscous solutions. The presentation value of the power display mode is demonstrated in Figure 2.

Virtually all 55 carbons were resolved; their shifts and assignments are given in the Table. Most of the phytyl carbons were assigned by comparison with published data,[2] and the remaining non-quaternary carbons were mostly assigned by specific off-resonance proton decoupling. C-3a and C-8a Resonate at 21.6 and 21.2 p.p.m. but could not be distinguished as the corresponding protons are virtually coincident. Similarly, C-4a, -7a, and -7b are partially obscured by solvent acetone signals and could not be separately assigned.

With a few exceptions the quaternary carbons presented more difficulty, assignments being based on reproducible line sharpening on removal of unresolved proton couplings. C-γ Was straightforward on chemical shift, multiplicity, and low intensity grounds (when rapidly pulsing), C-16 is so far removed from protons that its linewidth was uniquely invariant in all decoupling experiments. C-2a Was assigned on chemical-shift grounds; this was confirmed by observing it sharpen when 2b-H was decoupled; C-7c, C-6, and C-10a are assigned by analogy with chlorophyll *a* and, for C-6, by decoupling 10-H and 5a-Me. C-1, -2 and -5 Were identified

by specific decoupling of their attached methyl groups and C-12 by decoupling α-H. Further decoupling experiments and analogies with chlorophyll *a* show that C-13 and -14 are effectively coincident at 140.6 p.p.m. This leaves two pairs of ambiguous signals: C-17 and -18 resonate at 166.1 and 164.9 p.p.m., and C-11 and -15 at 150.2 and 147.9 p.p.m., but we do not have reliable assignments within each pair.

The ^{13}C chemical shifts in BChl are mostly similar to those in chlorophyll *a*[1,2] except where there are substantial chemical differences in the northern half. There are, however, significant shift differences (up to *ca.* 10 p.p.m.) in the identical southern halves of the two compounds. We take these to reflect electronic differences imposed by the northern half but do not attempt to interpret the shift differences in detail.

Experimental

Microcrystalline BChl, isolated as described previously,[3] was tightly packed into a 1.7 mm (o.d.) capillary n.m.r. tube (Wilmad). Small quantities of [$^2H_6$]acetone were added and the mixture was warmed to complete dissolution. A little [$^2H_4$]methanol was then added and the sample tube was flame sealed. The solutions, which approached 1 molal concentration, were too viscous to be transferred into capillary tubes directly.

100 MHz ^1H N.m.r. spectra were recorded in the FT mode at ambient probe temperature on a Varian XL100A spectrometer. Assignments were carried out as described previously.[3,6] 25.2 MHz ^{13}C N.m.r. spectra were obtained with the same instrument using a microprobe. We employed 30° pulse width, 6 000 Hz spectral width, and 0.3 s acquisition time. FIDs were zero-filled to 32K data points prior to Fourier transformation. Two series of ^{13}C spectra with off-resonance ^1H decoupling were acquired: in one, constant decoupler power and different irradiation frequencies were employed and in the other constant frequency and variable decoupler powers were employed. For experiments designed principally to assign non-quaternary carbons we accumulated *ca.* 10 000 transients (50 min) and for experiments designed principally to assign quaternary carbons *ca.* 100 000 transients (10 h 25 min). Between each decoupling experiment we accumulated a fully broadband noise-decoupled spectrum to ensure linewidths and purity remained the same.

Acknowledgements

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