

PROTON NMR STUDY OF THE CONFORMATION OF RING D AND ITS SIDE CHAINS IN CHLOROPHYLL DERIVATIVES

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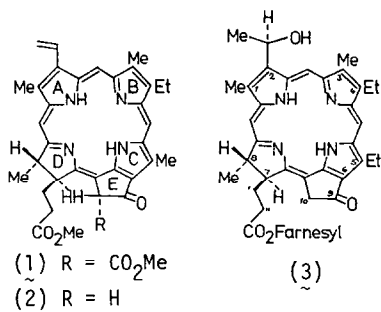
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Abstract: The complex multiplet proton NMR pattern at 360 MHz of the propionic side chains in methyl pheophorbides and a pheofarnesin derived from bacteriochlorophyll-d is completely analyzed; the major preferred orientation in solution is found to be different from that determined by single crystal X-ray studies.

The molecular organization of chlorophylls and pheophytins in the chloroplasts of green plants and in photosynthetic bacteria is of crucial importance in the transmission and primary light conversion events in photosynthesis.¹ To this end, the precise conformation of the chlorin ring D, and its associated side chains in the matrix has been the subject of a number of single crystal X-ray investigations of chlorophyll derivatives, with and without the carbocyclic ring E.² Such studies include ethyl chlorophyllide-a dihydrate,^{2a} ethyl chlorophyllide-b,^{2b} methyl pheophorbide-a,^{2c} and zinc(II) tetraphenylchlorin.^{2d}

However, no precise determination of the reduced ring and its side chain conformations in solution has ever been obtained, though this is in principle given from the proton-proton couplings around ring D. In this letter we present the complete analysis of ring D and associated side chain NMR spectra at 360 MHz for a series of derivatives of chlorophyll-a and bacteriochlorophyll-d, and derive from the observed couplings the conformations of the 7-substituents in these compounds. Previous NMR studies of these molecules were either at such low fields that the C₇ and C₇'' protons were unresolved,³ or made only intuitive assignments.⁴

The proton NMR spectrum of the propionic side chain of methyl pheophorbide-a (1) is a complex four spin multiplet since all of the proton chemical shifts differ, due to chiral centers



at C₇, C₈, and C₁₀, in which two of the protons are further coupled to the C₇ proton. Comparison of the spectrum obtained by decoupling the C₇-H (Figure 1C) with the full spectrum (Figure 1A) shows that, quite unexpectedly,⁴ the closely coupled multiplets are vicinal rather than geminal pairs. This was the key to the analysis of the spectrum which eventually converged smoothly⁵ to a well defined solution for the decoupled four-spin (Figure 1D) and full

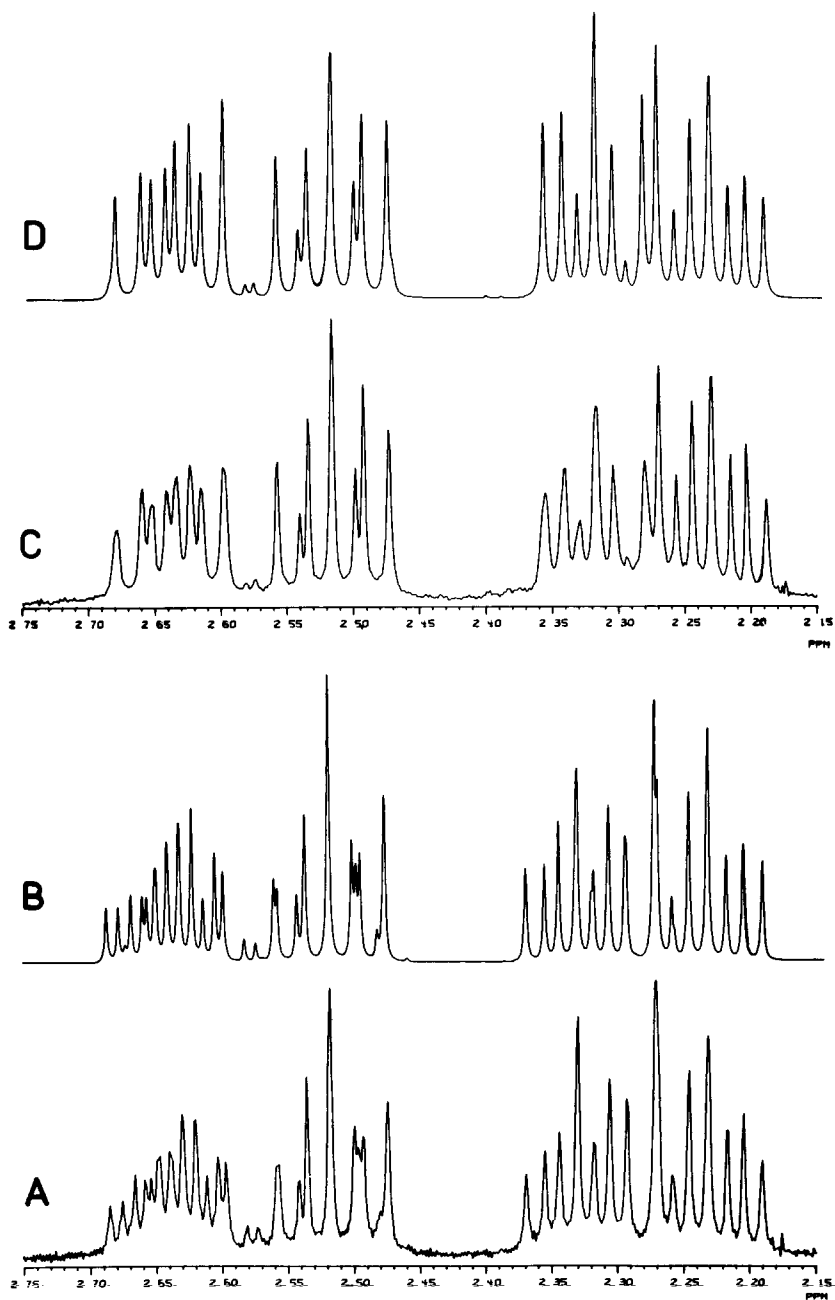


Figure 1: The 360 MHz proton NMR spectra (Nicolet NT-360) of the propionic side chain of methyl pheophorbide-a (1), 8.1 mM in CDCl_3 : A, normal (5-spin) spectrum. B, simulated spectrum.⁵ C, spectrum (4-spin) with $\text{C}_7\text{-H}$ decoupled. D, simulated 4-spin spectrum.⁵

TABLE: Side Chain Proton Chemical Shifts (δ) and Couplings (Hz) in Chlorophyll Derivatives^a

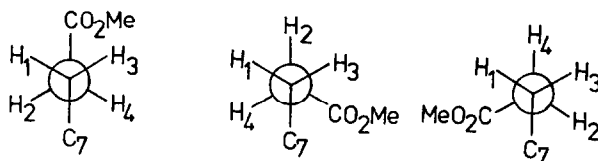
| | (1) ^b | (2) ^c | (3) ^d |
|--|------------------|------------------|------------------|
| δ_1 (C ₇ -H) | 2.636 | 2.703 | 2.675 |
| δ_2 (C _{7_n} -H) | 2.515 | 2.562 | 2.539 |
| δ_3 (C ₇ -H) | 2.319 | 2.313 | 2.279 |
| δ_4 (C _{7_n} -H) | 2.233 | 2.286 | 2.250 |
| δ_5 (C ₇ -H) | 4.204 | 4.298 | 4.270 |
| J ₁₂ | 6.7 | 7.1 | 7.0 |
| J ₁₃ | -14.0 | -14.2 | -14.0 |
| J ₁₄ | 9.9 | 9.5 | 9.7 |
| J ₁₅ | 3.2 | 2.8 | 3.1 |
| J ₂₃ | 9.3 | 9.4 | 9.6 |
| J ₂₄ | -15.7 | -15.8 | -16.0 |
| J ₂₅ | -0.2 | 0.0 | 0.2 |
| J ₃₄ | 5.2 | 5.0 | 5.0 |
| J ₃₅ | 9.0 | 9.0 | 8.5 |
| J ₄₅ | -0.2 | -0.2 | -0.2 |
| J ₇₈ | 1.6 | 2.1 | 1.9 |

Footnotes: ^a Consideration of the rms error of ca. 0.2 Hz with the broad lines obtained and the digitization limits (0.3 Hz) suggests that the couplings are accurate to 0.2-0.3 Hz. Concentrations: ^b, 8.1 mM; ^c, 7.0 mM; ^d, 5.0 mM in CDCl₃.

five-spin (Figure 1B) spectra, with rms errors of 0.11 and 0.22 Hz, respectively. The assignments were independently confirmed by deuteration of the C_{7_n}-methylene with MeOD/MeO⁻.

Results of these analyses for methyl pheophorbide-a (1), methyl pyropheophorbide-a (2), and the band 5 pheofarnesin (3) derived from the bacteriochlorophyll-d of *Chlorobium vibrioforme forma thiosulfatophilum* are given in the Table. The propionic methylenes in pheophytin-a were partially obscured by the phytyl resonances, but the fact that there are no significant variations in the couplings for the methyl pheophorbides (1,2) and the farnesyl ester (3) suggests that there is a basic conformational similarity which would probably extend also to phytyl esters. The value of J₇₈ (which is a first-order coupling) changes rather more than our estimated limits of error.

The vicinal couplings around ring D and the propionic side chain can be used to deduce the rotamer populations and dihedral angles in this section of the molecule, once the couplings in the discrete rotamers have been estimated. The general approach of Feeny⁶ was used in which the anti H-H coupling is given by recourse to a cyclohexane fragment with similar substituents, and the gauche couplings may simply be estimated by the equation of Abraham and Loftus.⁷ The assignments, rotamer couplings, and populations obtained for the C.CH₂.CH₂.C fragment of the C₇ propionic side chain are shown below. These results are of interest as only the g₋ conformer is observed in the crystal studies.^{2a-c} In solution, the predominant anti conformer reflects the steric interactions of the bulky end groups and the solid state conformer is a minor, but populated form. The other conformation (g₊) may be neglected.



| | anti | g ₊ | g ₋ |
|-------------------|------|----------------|----------------|
| J ₁₂ | 4.4 | 2.8 | 13.2 |
| J ₁₄ | 13.2 | 3.6 | 3.6 |
| J ₂₃ | 13.2 | 3.6 | 3.6 |
| J ₃₄ | 4.4 | 13.2 | 2.8 |
| <u>Population</u> | 0.63 | 0.10 | 0.27 |

The couplings in the C₇-H.C₇-H₂ fragment (J₁₅ J₃₅) may be used to probe the conformation of this moiety, though here the distorted non-tetrahedral angles about C₇ make such analyses less rigorous. A similar procedure gives rotamer populations of ca. ²/₃ : ¹/₃ in favor of the conformation found in the crystal (C_{7a}.C₇.C₇.C₇ anti). The only other populated conformer is the H₇.C₇.C₇.C₇ anti conformer, i.e. with the propionic side chain over the chlorin ring. These results show that although the major rotamer has the expected lateral side chain conformation, there is an appreciable proportion with the carbomethoxy group over the chlorin ring.

Finally, the C₇-H.C₈-H coupling provides some information on the conformation of the reduced ring D. The crystal investigations showed that this ring is appreciably less buckled in the pheophorbide and chlorophyllide than in tetraphenylchlorin,² the conformation being almost C₂ with C₇ out of the plane of the other atoms. Calculations based on the crystal geometry of Fischer et al.^{2c} give H_{7,8} dihedral angles of 110°, and this is consistent with the observed coupling of ca. 2 Hz.

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