^{13}C Nuclear Magnetic Resonance Studies of Porphyrins and Related Compounds: Chlorophyll Derivatives

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^{13}C N.m.r. spectra of the methyl esters of deuteroporphyrin-IX, protoprophyrin-IX, chlorin-e_6, rhodin-g_7, and derivatives of pyromethylphaeophorbide-a have been measured in deuteriochloroform. The introduction of a *meso*-bromine substituent into the pyromethylphaeophorbide-a system causes large chemical shift changes in the neighbourhood of the substituent which are rationalised in terms of a distortion of the macrocycle. Shift differences found between chlorin-e_6 and rhodin-g_7 trimethyl esters are rationalised in terms of a preferred conformation of the formyl group in the latter. A comparison of our data with previously reported data on chlorophyll-a is made.

^1H N.M.R. spectra of porphyrins and porphyrin derivatives have been shown to be extremely useful for establishing structure and for studying the influence of substituents upon the basic macrocycle.[1] It is difficult to obtain information about chlorophyll derivatives, even at high magnetic fields, from ^1H spectra. Recent ^{13}C n.m.r. investigations of these macromolecules [2-5] and other related compounds [6] have shown that this is a powerful method for structural investigation, provided the resonances in the ^{13}C spectrum can be assigned. Although porphyrins and related compounds are suitable model compounds for, and biological precursors to, the chlorophylls few ^{13}C studies have been reported. All previous ^{13}C n.m.r. studies have dealt with porphyrins having substituent side chains in positions 1—8; no previous report has dealt with chlorin-e_6, rhodin-g_7, or derivatives of pyromethylphaeophorbide-a.

We have recorded natural abundance ^{13}C n.m.r. spectra of compounds (A)—(F) and have assigned all the signals belonging to protonated carbons and several signals of the non-protonated carbons with the aid of off-

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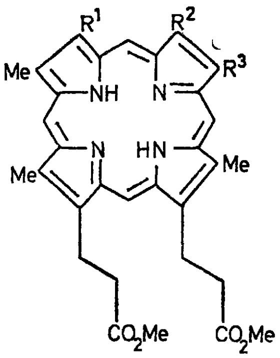
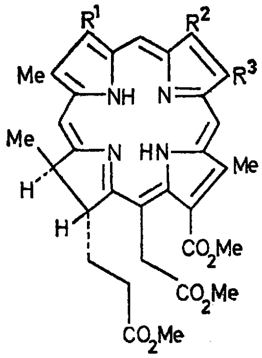
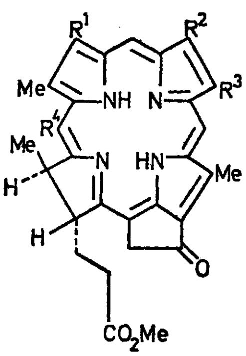


(A) Deuteroporphyrin-IX dimethyl ester, $R^1 = R^3 = H, R^2 = Me$
(B) Protoporphyrin-IX dimethyl ester, $R^1 = R^3 = CH{=}CH_2, R^2 = Me$

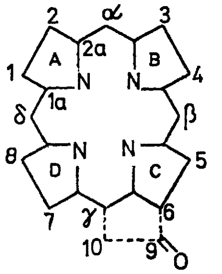


(C) Chlorin-e$_6$ trimethyl ester, (E) $R^1 = CH{=}CH_2, R^2 = Me, R^3 = Et$
(D) Rhodin-g$_7$ trimethyl ester, $R^1 = CH{=}CH_2, R^2 = CHO, R^3 = Et$



(E) Pyromethylphaeophorbide-a methyl ester, $R^1 = CH{=}CH_2, R^2 = Me, R^3 = Et, R^4 = H$
(F) *E*-δ,2″-Dibromopyromethylphaeophorbide-a methyl ester, $R^1 = CH{=}CHBr, R^2 = Me, R^3 = Et, R^4 = Br$

resonance proton spin decoupling and by comparison of our data with those of the literature. We have adopted the Fischer convention for numbering of the porphyrin ring and for brevity throughout this paper we refer to, say ' C-1,' by calling it simply ' 1.'



Fischer nomenclature (side-chain carbons are referred to by their point of attachment on the macrocycle and are consecutively primed)

EXPERIMENTAL

All spectra were recorded on a Varian XL-100-12 spectrometer operating in the Fourier transform mode at 25·16 MHz and locked to the deuterium resonance (15·40 MHz) of the $CDCl_3$ solvent. The instrument was controlled with a Varian 620-L computer equipped with a moving head disc together with complementary software. Parameters were usually chosen to give a 16K transform which gave a resolution of 0·63 and 0·75 Hz per point for sweep widths of 5000 and 6000 Hz, respectively.

A sample of each porphyrin was dissolved in $CDCl_3$ at a concentration of 0·055 ± 0·005M, except for (F) which was at a concentration of 0·042M because of the low availability of material.[7] Noise decoupled and off-resonance proton decoupled spectra [8] were obtained at 37° with internal tetramethylsilane as standard in 10 mm sample tubes. Typically samples required 12 h to give a good signal-to-noise ratio; the spectral reproducibility was ±0·03 p.p.m.

RESULTS

The ^{13}C chemical shifts of all the compounds studied are shown in Table 1. The multiplicity of the resonances in the off-resonance proton decoupled spectra allowed the assignment of signals to either primary, secondary, tertiary, or quaternary carbons. Figures 1 and 2 show correlation diagrams of the ^{13}C shifts for the regions 0·00—60·00 and 80·00—180·00 p.p.m., respectively. The assignment of the various signals is discussed below.

(i) *Assignment of Side-chain Carbons*.—The signals from the side-chain carbons of (A) and (B) were assigned from chemical shift considerations [9] and previously reported results.[10,11] The signal that remains constant at *ca.* 11·5 p.p.m. was assigned to 5′ and 8′, while the remaining ring methyl signals [13·63 for (A) and 12·42 p.p.m. for (B)] were assigned to 1′ and 3′.

The assignments of the macrocyclic ring methyl groups in compounds (C)—(F) were made as follows. Comparison of the corresponding spectra with those of (A) and (B) indicates that the introduction of saturation in ring D causes a downfield shift of 8′ as expected for a methyl carbon attached to a saturated ring. This carbon may be distinguished from 4″ as the signal for the latter should be unaffected by introduction of the bromine in (E), while the signal of 8′ should suffer a shift. The high-field methyl signal is assigned to 3′, as this resonance is absent in (D), and an upfield shift relative to (B) may be expected because of increased steric crowding by the 4-ethyl substituent. The resonance that remains constant for compounds (C)—(F) at 11·98 ± 0·11 p.p.m. is assigned to the 5′-methyl carbon. The resonance in (C) and (D) at approximately 12·3 p.p.m. is assigned to 1′ by comparison with (B), while in (E) the signals of 1′ and 5′ overlap. The signals at 20·84 and 18·28 p.p.m. in (E) are assigned to 8′ and 1′, from their residual couplings in the off-resonance experiment. The remaining methyl signals belong to the ester methyls. By comparison with (A) and (B), the resonance at 51·65 ± 0·05 p.p.m. is assigned to the 7⁗-methyl and the resonances at 52 and 53 p.p.m. are assigned to γ‴ and 6″ of (C) and (D); unambiguous assignment of the latter without recourse to relaxation time measurement is impossible.

The signals for 7′ and 7″ are assigned by comparison with

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TABLE 1

^{13}C Chemical shifts of the methyl esters of compounds (A)—(F) with their assignments in parentheses

	(A)	(B)	(C) *	(D)	(E) *	(F)
1	11·58 (5′, 8′)	11·47 (5′, 8′)	11·24 (3′)	11·93 (5′)	11·12 (3′)	11·12 (3′)
2	13·64 (1′, 3′)	12·42 (1′, 3′)	12·07 (5′)	12·31 (1′)	11·97 {5′	12·09 (5′)
3	21·84 (7′)	21·71 (7′)	12·36 (1′)	19·05 (4′)	12·02 1′}	17·33 (4″)
4	36·96 (7″)	36·86 (7′)	17·64 (4″)	19·37 (4″)	17·37 (4″)	18·28 (1′)
5	51·68 (7⁗)	51·65 (7⁗)	19·64 (4′)	22·87 (8′)	19·36 (4′)	19·37 (4′)
6	95·90 {γ	95·62 (γ)	22·92 (8′)	29·62 (7″)	23·11 (8′)	20·84 (8′)
7	97·02 δ}	96·53 (δ)	29·63 (7″)	31·17 (7′)	29·86 (7″)	29·88 (7″)
8	99·40 {α	96·88 (β)	31·14 (7′)	38·28 (γ′)	30·97 (7′)	30·99 (7′)
9	100·16 β}	97·42 (α)	38·59 (γ′)	49·50 (8)	48·04 (10)	48·60 (10)
10	128·72 (2, 4)	120·23 (2″)	49·44 (8)	51·68 (7⁗)	49·98 (8)	51·69 (7, 7⁗)
11	136·60	130·17 (2′)	51·60 (7⁗)	52·14 {γ‴	51·70 (7, 7⁗)	51·97 (8)
12	136·69	137·0—138·0	52·06 {γ‴ (7)	53·10 6″} (7)	92·91 (δ)	95·03 (δ)
13	138·32	142·9—144·3	53·05 6″}	93·57 (δ)	97·10 (α)	98·82 (α)
14	138·52	173·54 (7‴)	93·55 (δ)	102·33 (γ)	103·90 (β)	103·68 (β)
15	140·12		98·70 (α)	102·61 (β)	105·99 (γ)	106·97 (γ)
16	140·32		102·09 (β)	104·26 (α)	122·37 (2″)	113·55 (2″)
17	143·8—145·2		102·26 (γ)	122·62 (2″)	128·18	129·85 (2′)
18	173·55 (7‴)		121·62 (2″)	124·33	129·17 (2′)	130·10
19			123·46	128·78 (2′)	131·45	132·02
20			129·39 (2′)	129·40	135·75	132·14
21			130·50	131·21	135·93	132·40
22			134·76	132·45	136·10	137·00
23			135·40	136·50	137·78	137·66
24			135·44	136·62	141·47	140·09
25			135·90	137·90	144·84	144·63
26			136·41	138·83	148·94	147·95
27			139·47	141·63	150·67	152·23
28			144·99	144·89	155·08	153·05
29			148·94	150·68	160·18	153·71
30			154·58	158·33	171·26	161·37
31			166·86	168·96	173·45 (7″)′	171·24
32			169·51	169·45 (γ″ or 6′)	196·06 (9)	173·33 (7‴)
33			169·55 {γ″	172·10		195·84 (9)
34			173·01 6′}	172·86 (6′ or γ″)		
35			173·55 (7‴)	173·45 (7‴)		
36				187·44 (3′)		

CDCl$_3$ resonances
(A) 75·73 77·00 78·27
(B) 75·75 76·93 78·28
(C) 75·75 77·01 78·29
(D) 75·76 77·03 78·29
(E) 75·75 77·02 78·28
(F) 75·75 77·02 78·29

* One non-protonated macrocycle carbon could not be identified.

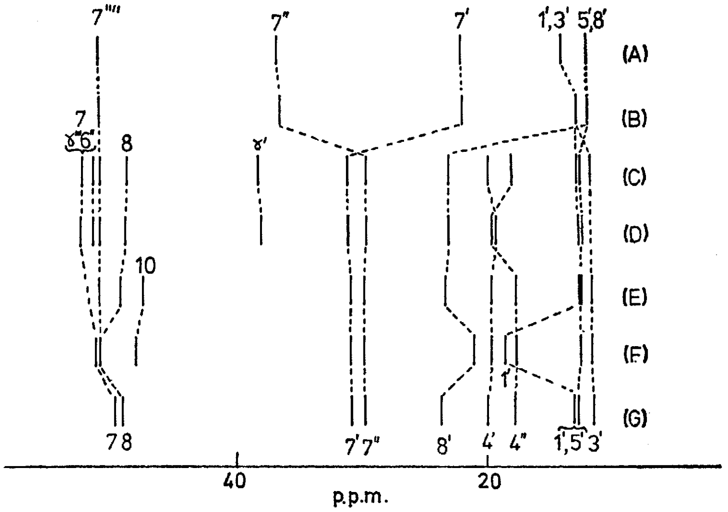

FIGURE 1 Correlation diagram of chemical shifts (0·00—60·00 p.p.m.) for compounds (A)—(F) and chlorophyll-α (G)

related compounds from the literature [(A) and (B);[10-12] (C)—(F) [3]]; the assignment of the remaining protonated carbons of the side chains was from chemical shift considerations.[9] The carbonyl resonances of (C)—(F) at 173·4 ± 0·2 p.p.m. are assigned to 7‴ by comparison with (A) and (B) while the remaining ester carbonyl resonances of (C) and (D) are assigned to the unique signals that remain constant at 172·9 ± 0·1 and 169·5 ± 0·05 p.p.m.; unambiguous

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assignment is impossible at present. The lowest field signals in the spectra of (E) and (F) are assigned to the ring carbonyls.

(ii) *Assignment of the Protonated and* meso-*Carbons of the Macrocycle.*—The tertiary carbon signals at 49·7 ± 0·3 p.p.m. in (C)—(E) are assigned to 8 while 7 gives rise to the signal that overlaps with an ester methyl signal at 53 in (C) and (D), and 51·7 p.p.m. in (E). This latter signal is the one we expect to be affected the most by ring closure on going from (C) and (D) to (E) and (F). The signals of 7 and 8 in (F) can not be unambiguously assigned and occur as shown in Table 1 and Figure 1.

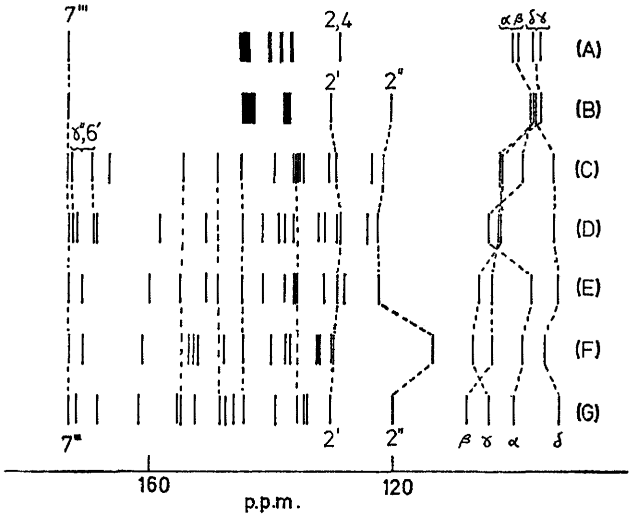

FIGURE 2 Correlation diagram of chemical shifts (80·00—180·00 p.p.m.) for compounds (A)—(F) and chlorophyll-α (G)

TABLE 2

^{1}H Resonance positions and residual couplings in the ^{13}C off-resonance proton decoupled spectra for the protonated *meso*-carbons of compounds (C)—(F)

	Resonance position in ^{1}H spectrum (Hz) *			Residual coupling in ^{13}C spectrum (Hz) †		
	α	β	δ	α	β	δ
(C)	949	965	872	53·8	53·1	49·7
(D)	1019	948	858	59·6	53·6	50·1
(E)	917	926	848	64·9	65·5	61·5
(F)	947	926		62·5	64·9	

* ^{1}H Spectra were recorded at 100·06 MHz for the solutions used for the ^{13}C experiment and assignments are from comparison with refs. 1*d* and 7. † The decoupling frequency was positioned at 2 p.p.m. to high field of tetramethylsilane in the proton spectrum.

The *meso*-carbons (α, β, γ, δ) of (C)—(F) were assigned unambiguously and the assignments for (A) and (B) were taken from the literature.[10,13] The resonances of the γ-carbons in (C)—(F) and of the δ-carbon in (F) appear as singlets in the off-resonance experiments for (C)—(F). The effects of bromine on the γ- and α-*meso*-carbons would be expected to be similar and hence the assignment is as shown. Of the protonated *meso*-carbons all those of (D) together with the δ-carbons of (C) and (E) are assigned from the residual couplings in the off-resonance experiments (Table 2). The environment of the β-*meso*-carbon is affected little on going from (C) to (E) and hence is assigned as shown; the α-*meso*-carbon is assigned by default.

(iii) *Assignment of the Non-protonated Carbons of the Macrocycle.*—The signals of (1a)—(8a) of (A) and (B) and the signals of 1—8 in (B) appear as broad bands,* whereas the signals of 1—8 of (A) appear as six resolved resonances. The signals of the non-protonated macrocyclic carbons of (C)—(F) appear as discrete singlets in the range 124—173 p.p.m. We assign it to a carbon in either ring B or C as inspection of the structure suggests that only certain carbons in these rings would remain unaffected by the substituent changes that occur. No other signal in this region remains constant throughout the series. However, it is worth noting that, on going from (C) to (E), only certain resonances appear to remain constant at 148·9 ± 0·0, 154·8 ± 0·3, and 135·9 ± 0·0 p.p.m. These may be associated with carbons in rings A and B; further assignment at the moment is impossible.

DISCUSSION

^{13}C N.m.r. spectroscopy has been shown to be an extremely useful method for the investigation of molecular conformation. In the present work the preferred conformation of the formyl side chain in (D) and the effect of *meso*-substitution on the macrocycle were studied.

As ^{1}H n.m.r. studies have indicated, substitution at the *meso*-position of a porphyrin causes a change in the ring current and distortion of the macrocycle with concomitant loss of planarity of the aromatic system.[1b,d,15,16] This effect has also been observed through o.r.d. measurements[17] and i.r. spectroscopy;[18] Woodward has discussed this phenomenon.[19] In the present case the introduction of bromine in (F) causes several pronounced chemical shift changes compared to (E) which are shown in Figure 3. The effect of the introduction of bromine

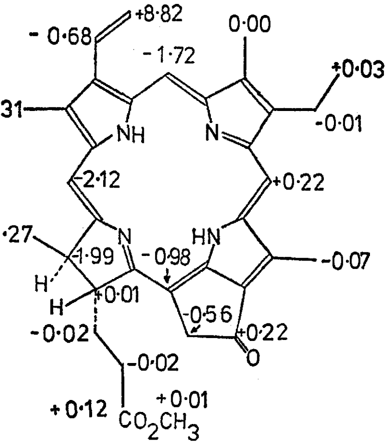

FIGURE 3 Chemical shift differences between compounds (E) and (F)

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* The resolution of the signals associated with these broad bands, that occurs on increasing the temperature in CDCl$_2$CDCl$_2$ as solvent, has been reported elsewhere.[14]

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into the vinyl group has the expected effect of causing a 9 p.p.m. upfield shift of the brominated carbon signal and *only* a small effect upon that of the carbon two bonds removed.[9] Longer range effects should be small as the side chain can adopt a conformation in which steric interactions are small. The introduction of bromine in the *meso*-position should result in a similar upfield shift; thus the observed downfield shift is unexpected. This shift may arise from a change in the ring current and/or a distortion of the macrocycle.[1b] The former effect is expected to be small as the δ-carbon is located directly in the macrocycle and hence lies in a position between the shielding and deshielding regions normally associated with ring currents.[10,20] The distortion will have pronounced effects upon the chemical shift of neighbouring carbons. Electric field effects associated with the carbon–bromine bond would be expected to give rise to chemical shift changes in the same direction for $1'$ and $8'$. This clearly is not the case; hence, although these may play some part in determining the observed effects, the different sign of these shift changes must arise from a distortion of the macrocycle. The constancy of the shifts of the side chain carbons of rings B and C suggests that the observed effect is caused by distortion of the molecule from planarity principally in the region of the δ-bromine substituent.

The bromine atom has a similar van der Waals radius to a methyl group [21] and hence the steric effects caused by the introduction of bromine may be assessed from the ^{13}C data available for 1-methylnaphthalene [22] and 1,8-dimethylnaphthalene.[20] The introdution of the 8-methyl group causes a 6·7 p.p.m. downfield shift for the 1-methyl resonance, which arises mainly from molecular distortion [22] and not from the usual steric crowding effects which cause upfield shifts.[23] Since a similar downfield shift of 6·3 p.p.m. is observed here, we infer that molecular distortion is the major cause of this shift. Again, by analogy with the naphthalene shifts, the smaller downfield shifts for 8 and δ probably arise from steric interactions rather than from ring current effects.[24]

As with previous work,[1b] it is difficult to assess the details of this distortion. The exact nature of the folding of rings A and D and its correlation with the shifts of 10, δ, and $8'$ must await further ^{13}C work and the result of an *X*-ray analysis in progress.[25]

There are interesting changes observed on substitution of a formyl group for a methyl group [compounds (D) and (C), respectively]. The effects of this substitution can not be rationalised by simple considerations of mesomeric or inductive type interactions. The former would give rise to shifts of all the *meso*-carbon signals while the latter would cause similar shift changes for α and $4'$. There are two orientations for the formyl group in (D) (Figure 4) which are preferred because conjugation is maintained.

By analogy with ^{1}H studies [26-28] the anisotropy and field effects of the carbonyl group should give rise to pronounced chemical shift differences between the two conformations. In a comparison of conformation (i) with (C), the *meso*-α-carbon should be strongly deshielded while little change would be expected for $4'$ and $4''$, whereas in the alternative conformation (ii) the reverse should hold. The data reported in Table 1 are consistent only with a situation in which (i) is the predominant conformer. This conclusion agrees with the result of a previous ^{1}H study of these systems [1d] and is compatible with the results found for the preferred conformations of acetyl side chains in 2,4-diacetyldeuteroporphyrin-IX dimethyl ester.[10]

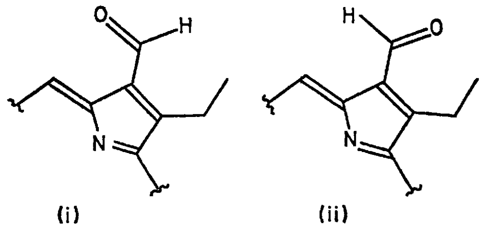

FIGURE 4 Preferred orientations for the formyl group on ring B of (D)

Our results are now compared with the reported ^{13}C shifts of the nonphytyl carbons of chlorophyll-a, which are included in the correlation diagrams. Figure 1 shows, in the region 0—51 p.p.m., that almost exact correlations are obtained and our assignments of these lines are in agreement with those of Allerhand *et al.*[3] In the region 90—175 p.p.m. differences are to be expected although certain signals still correlate. A comparison of the data for chlorophyll-a with (E) shows that the signals of the *meso*-carbons γ and β in chlorophyll-a have reversed their positions. In both our assignment and that of Allerhand these should be unambiguous as γ is a non-protonated carbon. Similarly the signal of $2''$ appears at *ca.* 2 p.p.m. to higher field than that in chlorophyll-a. These differences may arise from the introduction of the metal atom or from differences in the conditions at which the measurements were carried out. Even when these points are considered there are signals in this region that are common to both chlorophyll-a and our series. Thus the signal at 173·5 p.p.m. was assigned to $7''''$ (7c in Allerhand's nomenclature), the signal at 144·5 was assigned as above, while the signals at 148·9, 154·8, and 135·9 that did not change on going from (C) to (E) also appear (to ±0·3 p.p.m.) in the chlorophyll-a spectrum.

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As this study has shown, the comparison of ^{13}C shifts of chlorophyll-a with those of porphyrin-like derivatives is a powerful method of assigning the signals arising from the non-protonated macrocycle carbons. This, at present, is the only method of assigning these, although the study of many more metal-free and metal-containing derivatives is necessary. Work along these lines is continuing.

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