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THE NUCLEAR MAGNETIC RESONANCE SPECTRA OF PORPHYRINS—IX[1]

CARBON-13 NMR SPECTRA OF SOME CHLORINS AND OTHER CHLOROPHYLL DEGRADATION PRODUCTS

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Abstract—The ^{13}C NMR spectra of the methyl esters of phaeophorbides-*a* and -*b*, mesophaeophorbides-*a* and -*b*, pyrophaeophorbide-*a*, mesopyrophaeophorbide-*a*, chlorin-e_6, mesochlorin-e_6, chlorin-p_6, rhodin-g_7, mesorhodin-g_7, phaeoporphyrin-a_5, 2-vinylphaeoporphyrin-a_5, rhodoporphyrin-XV, and 2-vinylrhodoporphyrin-XV, and of *trans*-octaethylchlorin, in deuteriochloroform and/or trifluoroacetic acid solution are reported. On the basis of comparisons within this comprehensive series and proton off-resonance decoupled spectra, assignments of most resonances are made; complete assignment of the quaternary "pyrrole" ring carbons was difficult to accomplish. A downfield shift of the α- and β-*meso*-carbons of chlorins in trifluoroacetic acid relative to deuteriochloroform is used to confirm that the *Chlorobium* chlorophylls (660) from *Chloropseudomonas ethylicum* are *meso*-methylated at the δ-position.

Carbon-13 NMR spectroscopy has been widely used in studies of biosynthetic pathways to the pyrrole pigments and vitamin B_{12}.[2-6] In connection with our own studies on the biosynthesis of chlorophylls from various sources, we required specific assignments of carbon resonances, not only in the chlorophylls themselves (which have already been investigated[7-9]) but also in the common degradation products from the natural pigments, because these are usually easier to handle and the degradative chemistry of this class of compounds is efficient and well-understood.[10,11] We now report the ^{13}C NMR spectra and assignments of a number of chlorins and other degradation products from chlorophyll, in deuteriochloroform ($CDCl_3$) and/or trifluoroacetic acid (TFA) solution.† In some instances, specific deuteriation was used as an aid in assignment of resonances. Only tentative assignments of the "quaternary pyrrole" carbons could be made in most cases, but from the biosynthetic point of view this is not a serious drawback because the broad outline of the biosynthesis of the tetrapyrrole nucleus from porphobilinogen is now known; in the chlorophylls it is the subsequent transformations of the side-chains which are of particular interest in our investigations.

EXPERIMENTAL

Spectra were obtained with samples (50–100 mg) of the appropriate compound dissolved in 1·5 ml $CDCl_3$ and/or TFA (i.e. 0·05–0·1 M), with 5% TMS added as an internal standard. Proton noise decoupled spectra were obtained using a Varian XL-100-15 FT spectrometer with 8K computer memory, using 12 mm sample tubes carrying an inner 5 mm tube of D_2O for the field-frequency lock. Spectra were routinely recorded on 5120 Hz sweep widths at 25·197 MHz using the fast Fourier Transform technique. The accuracy of the ^{13}C chemical shifts with 2048 plot data points was ±2·5 Hz. Proton off-resonance decoupled spectra were also used for assignment.

trans-Octaethylchlorin was obtained[12] from the corresponding porphyrin and the chlorophyll degradation products were obtained from phaeophytins using literature methods[10] or variations of them.[11] Methyl d_5-pyrophaeophorbide-*a* was prepared according to Inhoffen's method[13] and then further deuteriated in the δ-position by heating in deuterioacetic acid.[14]

RESULTS AND DISCUSSION

The nomenclature used, and compounds investigated are shown in Fig 1. Tables 1 and 2 show the chemical shifts and assignments in $CDCl_3$ and TFA respectively.

1. *Aryl methyl substituents*

(a) $CDCl_3$ *spectra.* On the basis of single frequency off-resonance decoupled spectra, Katz[7] assigned the signal at 10·4 ppm in methyl phaeophorbide-*a* to C-3a, and that at 11·6 ppm to C-1a and C-5a. The shift differences were explained in terms of C-3a being electronically the most dissimilar, because it is the only methyl group attached to a "pyrrole" ring flanked by two other pyrrole rings. The spectra of methyl mesophaeophorbide-*a* and methyl mesopyrophaeophorbide-*a* possess signals at 10·8, 11·1, and 11·6 (±0·1) ppm; this suggests that there is an upfield shift of an aryl methyl carbon associated with the conversion of vinyl to ethyl (i.e. on passing from the normal to "meso" series). Throughout the chlorins investigated herein, a methyl adjacent to an ethyl group resonates in the range 11·0 ± 0·3 ppm. Comparison of methyl phaeophorbide-*a* with methyl phaeophorbide-*b* and rhodin-g_7 trimethyl ester (the last two having no signals upfield of 11·8 ppm) adds further confirmation. The signal

†Owing to solubility difficulties in $CDCl_3$, the spectra of the phaeoporphyrin and rhodoporphyrin esters were recorded in TFA alone.

Table 1. ^{13}C NMR spectra[a] and

Compound	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7
Methyl phaeophorbide-*a*	11·8*		128·3*	121·8	10·7		19·0	17·1	11·8*				51·0
Methyl mesophaeophorbide-*a*	(11·1)		19·3*	16·7	(10·8)		19·3*	17·3	11·9				50·8
Methyl pyrophaeophorbide-*a*	11·8*		128·5	121·6	10·8		19·1	17·2	11·8*				51·4*
Methyl mesopyrophaeophorbide-*a*	(11·0)		19·2*	16·7	(10·7)		19·2*	17·3	11·7				51·3*
Chlorin-p_6 trimethyl ester	11·9		128·5*	121·6	11·0		19·4	17·5	12·4		172·2	52·5*	51·9
Chlorin-e_6 trimethyl ester	12·0		128·8*	121·0	11·1		19·4	17·6	12·3		172·4	52·8*	51·8
Mesochlorin-e_6 trimethyl ester	(10·9)		(19·2)	17·0	(11·3)		(19·6)	17·7	12·3		172·4	52·7*	51·9
Methyl phaeophorbide-*b*	11·8*		127·8	122·6	185·9		18·1	18·9	11·8*				51·2
Methyl mesophaeophorbide-*b*	10·7		19·0*	16·6	186·2		18·3	19·0*	11·8				51·1
Rhodin-g_7 trimethyl ester	11·9		128·2	122·0	186·5		(18·9)	(19·3)	12·3		172·1	52·9*	52·0
Mesorhodin-g_7 trimethyl ester	10·8		19·2*	16·9	186·9		19·2*	19·2*	12·3		172·1	52·8*	51·9
trans-Octaethylchlorin	(19·2)	(17·9)	(19·4)	(18·3)	(19·7)	(18·5)	†	†	†	†	†	†	54·5

Table 1

Compound	Centre of $CDCl_3$	122–6	126·1–132·0	132·1–136·0
Methyl phaeophorbide-*a*	76·7		128·3*, 128·3*, 131·1	135·3, 135·7*, 135·7*
Methyl mesophaeophorbide-*a*	76·7		128·0, 128·2, 130·9	135·1
Methyl pyrophaeophorbide-*a*	76·7		127·4, 129·7, 130·7	135·1, 135·2, 135·3
Methyl mesopyrophaeophorbide-*a*	76·7		127·0, 129·5, 130·5	134·8
Chlorin-p_6 trimethyl ester	76·7	122·0	128·5, 129·1, 130·3	135·1*, 135·1*, 135·1*
Chlorin-e_6 trimethyl ester	76·7	123·0	128·8*, 129·9	134·1, 134·7*, 134·7*, 135·3, 135·8
Mesochlorin-e_6 trimethyl ester	76·7	122·4	128·3, 129·8	134·6, 134·8, 135·3, 135·7
Methyl phaeophorbide-*b*	76·7		128·8, 131·3*, 131·3*, 131·3*	135·7, 135·9
Methyl mesophaeophorbide-*b*	76·7		128·8, 131·2*, 131·2*, 131·5	
Rhodin-g_7 trimethyl ester	76·8	123·8	128·8, 130·7, 131·8	135·9*, 135·9*
Mesorhodin-g_7 trimethyl ester	76·7	123·5	128·6, 130·4, 131·7	
trans-Octaethylchlorin	76·7		131·6*, 131·6*	133·6*, 133·6*

[a]Chemical shifts in ppm downfield from TMS.
*Coincident resonances.
†Owing to symmetry of OEC, only half of carbons listed.
Signals in parentheses are ambiguous, and those underlined were either absent or significantly reduced in

at 11·8 ppm in methyl pyrophaeophorbide-*a* is assigned to C-1a and C-5a because d_6-labelled methyl pyrophaeophorbide-*a*[14] showed considerable reduction in the intensity of this signal. Hence, throughout the series, an aryl methyl carbon adjacent to vinyl resonates at 11·9 ± 0·1 ppm, as does a methyl adjacent to the carbocyclic (isocyclic) ring. Rupture of the carbocyclic ring, as in the triesters of chlorin-e_6, chlorin-p_6, and rhodin-g_7, causes a downfield shift of an adjacent methyl carbon of 0·5 ± 0·1 ppm. The spectrum of protoporphyrin-IX dimethyl ester[3] also features two types of aryl methyl carbon resonance, one at 11·4 (adjacent to propionate) and one at 12·7 ppm (adjacent to vinyl); in contrast, the four coproporphyrin isomers show[1] only one kind of aryl methyl, resonating at 11·5 ± 0·3 ppm. This also implies an upfield shift of *ca* 0·5 ppm associated with the change from the porphyrin to chlorin macrocycle. An upfield shift of C-3a with respect to C-1a in methyl phaeophorbide-*a* and related compounds presumably reflects the differential steric compression suffered from sp^3 or sp^2 hydridised carbons on adjacent rings.

(b) TFA *spectra.* Most of the effects in $CDCl_3$ solution are mirrored in TFA; change from porphyrin to chlorin macrocycle causes an upfield shift of about 0·5 ppm in all types of aryl methyl carbon, and an aryl methyl adjacent to ethyl resonates upfield from one adjacent to vinyl. However, the degeneracy of C-1a and C-5a in methyl phaeophorbide-*a* and similar compounds is lost in TFA solution; C-5a is shifted about 1 ppm downfield of C-1a, probably due to the presence of the adjacent carbonyl function. The assignment of this signal to C-5a was again confirmed by reference to the spectrum of methyl d_6-pyrophaeophorbide-*a*. Rupture of the carbocyclic ring (as in rhodoporphyrin-XV dimethyl ester) causes a further downfield shift of 1·4 ppm of this signal. This effect also occurs, though to a less marked extent, in $CDCl_3$ solution, and may be due to the carbocyclic ring reducing conjugation of the carbonyl group with the macrocycle.

2. *Aryl ethyl substituents*

(a) $CDCl_3$ *spectra.* In the "*a*" series, the methyl carbon (C-4b) of the ethyl groups resonates at 17·2 ± 0·5 ppm (*cf*

assignments of chlorins in $CDCl_3$ solution

Assigned carbon resonances																	
7a	7b	7c	7d	8	8a	8b	9	10	10a	10b	γa	γb	γc	α	β	γ	δ
31·0	29·8	172·6	51·4	49·9	22·9		188·7	64·5	168·9	52 6				96·4	103·6	104·8	92·6
31 0	29·8	172·6	51·4	50·0	22·8		188·8	64·4	168·9	52·5				95·8	103·9	104·7	92·1
30·9	29·7	172·8	51·4*	49·7	22·9		195·2	47·8						96·4	103·2	105·4	92·4
30·8	29 6	172·7	51·3*	49 8	22·8		195·2	47·7						95·4	103·4	105·2	91·8
31·3*	31·3*	172·8	51·2	49 2	23·4						(166·6)	52·5*		99·8	104·2	102·8	93 3
31·0	29 5	172·8	51·4	49·2	22·8						38·4	168·8*	52·8*	98·2	101·7*	101·7*	93·2
31 1	29·6	172·9	51·4	49 4	22·7						38 5	169·0	52·7*	97·0	102 0	101·7	92·6
31·2	29·6	172·6	51·5	49·9	23·0		188·5	64·3	168·6	52 8				100·4	105·5	104·3	92·7
31·1	29 6	172·4	51·4	50·0	22·9		188·6	64·3	168·7	52 7				99·7	105·9	104·2	92·3
31·0	29·5	172·8	51·5	49 3	22·8						38·2	168·3	52·9*	102·1	103·7	101·9	93·3
31 1	29·6	172·7	51·5	49·3	22·7						38 1	168·5*	52 8*	101·1	104 2	101·6	92 7
30·8	10·9			†	†	†								98·3	†	92·5	†

—Cont.

Unassigned macrocyclic carbons (Ranges in ppm)				
136·1–138 0	138·1–142·0	142·1–146·0	146·1–159·0	Carbons 17 and 18
137·2	141·3	144·2	149·0, 150·0, 155·0	160·5, 171·4
136·7, 137·0	141·4	142 3, 144·6	149·1, 149·9, 155·0	160·3, 171·9
136·9	140·7	144·0	148·2, 149·7, 154·1	159 5, 170·4
136 2, 136·7	140·9, 141·6	144·2	148·3, 149·6, 154·5	159·0, 170·8
137·2	140·6	144·6	148·0, 154·1	(166·4), 170·0
	138·8	144·4	148·2, 154·1	166·2, 168·8*
	139·9, 140·4	144·6	147·9, 154·3	165·7, 169·4
136·4, 136·6		142·6, 145·6	149·6, 157·8	163·2, 173 0
136·8*, 136·8*, 136·8*		143·2, 143 4, 145·6	150·0, 158·1	163·0, 173·4
137·3	138·3, 141·0	144·1	149·9, 157·6	168·8, 171·3
136·8, 137·3	138·1, 142·0	142·6, 144·0	150·2, 157·7	168·5*, 171·8
137·0*, 137·0*	138·5*, 138·5*, 141·7*, 141·7*		148·7*, 148·7*	167 1*, 167·1*

intensity in the spectrum of methyl d_6-pyrophaeophorbide-*a*.

trans-octaethylchlorin, OEC, at 18·2 ± 0·3 ppm). In the "*b*" series, the methyl carbon of the ethyls experiences a downfield shift to 19·1 ± 0·2 ppm due to the adjacent formyl group. The methylene signals (C-4a) in the "*a*" series are more constant at 19·2 ± 0·2 ppm, and correspond more nearly to the methylene signals in OEC (19·4 ± 0·2 ppm). In the "*b*" series, this methylene moves upfield by about 1 ppm in methyl phaeophorbide-*b* and methyl mesophaeophorbide-*b*, but rupture of the carbocyclic ring seems to negate this effect, since the upfield shift in rhodin-g_7 trimethyl ester is only 0·3 ppm and it is 0 ppm in mesorhodin-g_7 trimethyl ester.

(b) TFA *spectra.* The methyl carbon of the ethyl groups resonates at 16·8 ± 0·2 ppm in both chlorins and porphyrins. However, the methylene carbon, which resonates at 21·1 ± 0·1 ppm in chlorins, is shifted downfield in porphyrins by about 0·5 ppm (*cf* comparable shift in aryl methyl carbons).

3. *Carbons associated with the partially reduced ring*

(a) $CDCl_3$ *spectra.* The methyl group attached to the reduced ring (C-8a) resonates at 22·9 ± 0·2 ppm throughout the chlorins, excepting chlorin-p_6 trimethyl ester, where a downfield shift of 0·5 ppm is observed. Katz[7] was unable to differentiate C-7a and C-7b in methyl phaeophorbide-*a* using single frequency off-resonance decoupling, but he tentatively assigned C-7a to the low-field signal. Measurements of relaxation times enabled Allerhand[9] to confirm these tentative assignments. Further corroboration comes from the spectrum of OEC, where C-7a(8a) resonates at 30·8 ppm, corresponding well with C-7a in the series (with the exception of chlorin-p_6 trimethyl ester), resonating at 31·0 ± 0·2 ppm; C-7b appears at 29·6 ± 0·2 ppm throughout the series. In chlorin-p_6 trimethyl ester, C-7a and C-7b are degenerate at 31·3 ppm. Throughout the series, C-7 and C-8 are constant at 51·1 ± 0·3 and 49·8 ± 0·2 ppm respectively whilst the carbocyclic ring is intact; its rupture causes an upfield shift in C-8 of about 0·6 ppm and a downfield shift in C-7 of about 0·7 ppm. The equivalent signal in OEC for C-7(8) occurs downfield of both of these lines, at 54·5 ppm.

Table 2. ^{13}C NMR spectra[a] and assignments

Compound	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b
Methyl phaeophorbide-*a*	12·2		127·8	129·7	11·3		21·2	16·7	13·2	
Methyl pyrophaeophorbide-*a*	12·1		127·7	129·4	11·2		21·0	16·6	13·1	
trans-Octaethylchlorin	(20·6)	(17·1)	(20·9)	(17·7)	(21·1)	(17·9)	†	†	†	†
Rhodoporphyrin-XV dimethyl ester	12·0*		21·4*	16·8*	12·0*		21·4*	16·8*	14·9	
2-Vinylrhodoporphyrin-XV dimethyl ester	13·1		128·4	130·0	12·2		21·6	17·0	15·1	
Phaeoporphyrin-a_5 dimethyl ester	12·1*		21·6*	16·9*	12·1*		21·6*	16·9*	13·6	
2-Vinylphaeoporphyrin-a_5 dimethyl ester	13·0		128·2	130·2	12·1		21·6	16·9	13·6	

Table 2

Compound	centres TFA	1	2	3	4	5
Methyl phaeophorbide-*a*	115·9 162·7	‡	133·4	138·5	139·4	140·2
Methyl pyrophaeophorbide-*a*	115·8 162·5	‡	134·7	138·3	139·1	139·8
trans-Octaethylchlorin	116·2 163·5	139·0	†	139·3	†	141·8
Rhodoporphyrin-XV dimethyl ester	115·8 162·3	127·2	140·3	140·9	141·5*	141·5*
2-Vinylrhodoporphyrin-XV dimethyl ester	116·1 162·5	128·0	140·8	141·5	142·0*	142·0*
Phaeoporphyrin-a_5 dimethyl ester	116·0 162·4	136·8	139·9	141·9*	141·9*	142·2
2-Vinylphaeoporphyrin-a_5 dimethyl ester	116·0 162·4	136·8	140·2	142·0*	142·0*	142·0*

[a]Chemical shifts in δ ppm downfield from TMS.
*Coincident resonances.
†Owing to symmetry of OEC, only half of carbons are listed.
Signals in parentheses are ambiguous, and those underlined were either absent or significantly
‡One macrocyclic Carbon signal not observed.

(b) TFA *spectra.* Carbons 7a and 7b can be assigned for the chlorins at 32·0 and 30·2 ppm respectively on the basis of the OEC C-7a(8a) resonance at 31·7 ppm. Change from the chlorin to porphyrin macrocycle shifts C-7b to 36·7 ± 0·6 and C-7a to 23·5 ± 0·4 ppm. C-7 and C-8 are assigned from the $CDCl_3$ spectrum, C-8 being shifted upfield (by 1·2 ppm) and C-7 downfield (1·9 ppm) with respect to the $CDCl_3$ spectrum. Again, both signals are upfield of the equivalent C-7(8) in OEC.

4. *Other signals in the aliphatic region*

(a) $CDCl_3$ *spectra.* Katz[7] used randomly distributed 15% ^{13}C enriched chlorophyll and methyl phaeophorbide-*a* for his assignments; because the methyl ester carbon was introduced synthetically into the phaeophorbide, it possessed ^{13}C at natural abundance only, and no signal was observed. C-7d occurs at 51·4 ± 0·2 ppm throughout the series of chlorins (*cf* 51·6 ± 0·2 in the coproporphyrin methyl esters[1] and protoporphyrin-IX dimethyl ester[3]). The other methyl ester carbons (carbocyclic ester, γ-acetate, γ-carboxylate, nuclear peripheral carboxylate) resonate within the range 52·7 ± 0·2 ppm.

The assignment of the C-10 methylene in the pyrophaeophorbides was confirmed in the spectrum of methyl d_6-pyrophaeophorbide-*a*. The methylenes of the triesters of rhodin-g_7 and chlorin-e_6 were assigned as indicated in the Table, by elimination.

(b) TFA *spectra.* The methyl ester C-7d resonates in both chlorins and porphyrins in the range 54·6 ± 0·1 ppm, and the carbocyclic methyl ester (C-10b) at 56·0 ± 0·2 ppm in both macrocycles. Again, the assignment of the C-10 methylene in the pyro-compounds rests on the spectrum of the d_6-labelled material.

5. *Aromatic-olefinic region*

(A) *Vinyl substituents*

In $CDCl_3$, the methylene C-2b resonates at 122·1 ± 0·5 and the methine C-2a at 128·3 ± 0·5 ppm for all the chlorins (*cf* 120·3 ± 0·1 and 129·8 ± 0·1 ppm respectively

of chlorins and porphyrins in TFA solution

Assigned carbon resonances																	
6a	6b	7	7a	7b	7c	7d	8	8a	8b	9	10	10a	10b	α	β	γ	δ
		52·7	32·0	30·2	178·3	54·6	48·7	22·8		190·6	66·0	170·7	55·8	105·7	113·0	104·9	94·1
		52·4	31·9	30·2	178·4	54·5	48·4	22·5		198·8	48·9			105·3	112·8	106·5	93·9
†	†	53·9	31·7	10·7			†	†	†					106·1	†	93·5	†
168·4	55 1		23·1	37·1	178·2	54·4		12·3						(100·2)	102·9*	102·9*	(101·0)
168·7	55·4		23·3	37·3	178·5	54·7		12·5						(101·1)	102·9*	102·9*	(102·3)
			23·9	36·2	177·7	54·6		12·6		189·6	68·7	171·3	56·0	(101·1)	105·6	112·1	(101·6)
			23·8	36·1	177·6	54·6		12·6		189·6	68·7	171·3	56·2	(101·9)	105·4	111·9	(102·6)

—*Cont.*

Unassigned macrocyclic carbon resonances (Signal numbers)										
6	7	8	9	10	11	12	13	14	15	16
142·6	143·2	143·8	144·7	146·6	150·6	153·4	154·0	158·4		
142·6	143·0	143·0	144·7	146·2	150·2	151·4	153·6	158·7		
†	143·3	†	144·9	†	150·2	†	150·9	†		
141·8	142·3	143·6*	143·6*	144·4	145·8*	145·8*	145·8*	148·6*	148·6*	149·0
142·2	142·8	143·6	143·8	144·1	145·1	145·7	146·3	149·0*	149·0*	‡
142·7	144·1	144·7	145·3	146·4	146·9	147·2	147·9	149·0	149·8	153·7
142·3	142·5	144·3	144·9	145·1	145·3	145·9	147·3	149·1	149·3	153·6

reduced in intensity in the spectrum of methyl d_6-pyrophaeophorbide-*a*.

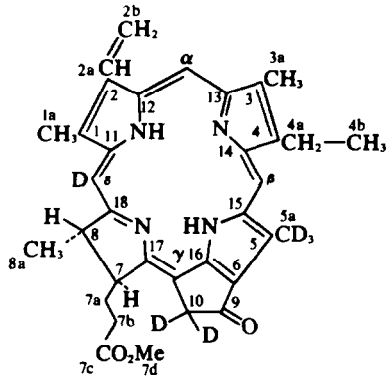
in protoporphyrin-IX dimethyl ester[3]). Thus, the methylene carbon is shifted upfield by 1·8 and the methine carbon downfield by 1·5 ppm in going from chlorin to porphyrin. Changing solvent to TFA causes a large downfield shift of the methylene C-2b of 8 ± 1 ppm, but the shift of C-2a is unaltered.

(B) Meso-*carbon atoms*

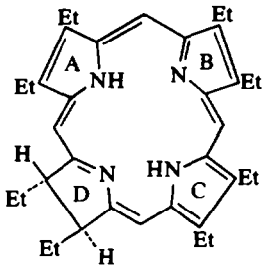
(a) $CDCl_3$ *spectra.* In the series of chlorins, the quaternary γ-*meso*-carbon is easily identified; it is the only singlet in the *meso*-carbon region of the undecoupled spectra and has lower intensity in the decoupled spectra. The δ-carbon is assigned from its chemical shift and this was unambiguously confirmed using methyl d_6-pyrophaeophorbide-*a*. The main difficulty was in differentiation of the α- and β-*meso* carbon atoms.

Four structural changes in the series of chlorins were examined (see Table 3). These were the differences between the same chlorins of (1) the -*a* and -*b* series, (2) the normal and "meso" series, (3) the normal and "pyro" series, and (4) the normal series of phaeophorbides and those with the cleaved carbocyclic ring (as in chlorin-e_6 trimethyl ester). It was found that these structural changes correlated closely with changes in chemical shift of individual *meso*-carbons in different chlorins. Reversal of any of the α and β assignments shown in Table 3 gave no correlation in either direction or magnitude of the shift change. These assignments correspond with those of Katz[15,16] for methyl phaeophorbide-*a*.

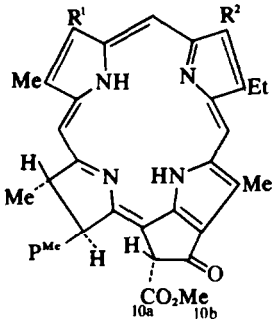
In chlorophyll-*a* the α and β *meso*-carbons resonate at 100 and 107 ppm respectively;[7-9] they are both 3 ppm downfield of the corresponding atoms in methyl phaeophorbide-*a*.[7] Application of the substituent shift differences in Table 3 (1) to chlorophyll-*b* predicts the α and β carbons to resonate at 104 and 109 ppm respectively. Resonances at 102 and 109 ppm are observed by Matwiyoff *et al.*[8] for chlorophyll-*b*, but these are assigned, with the help of single frequency off-resonance decoupling, to the β and α *meso*-carbons respectively. Using



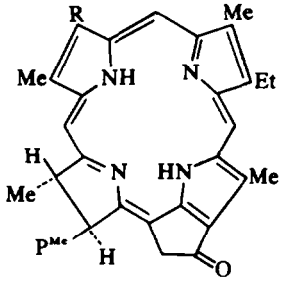
Methyl d_6-pyrophaeophorbide-*a*
(showing nomenclature)



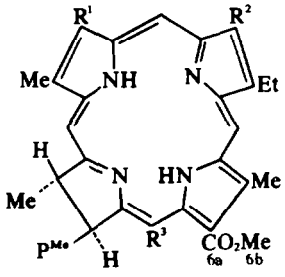
trans-Octaethylchlorin



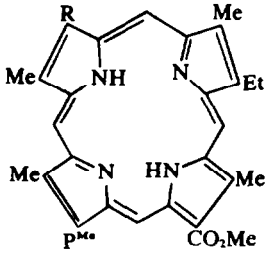
Methyl phaeophorbide-*a*: $R^1 = V$, $R^2 = Me$
Methyl phaeophorbide-*b*: $R^1 = V$, $R^2 = CHO$
Methyl mesophaeophorbide-*a*: $R^1 = Et$, $R^2 = Me$
Methyl mesophaeophorbide-*b*: $R^1 = Et$, $R^2 = CHO$



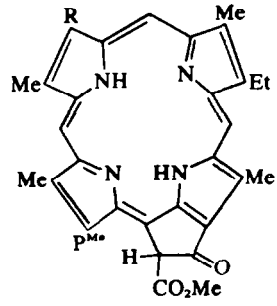
Methyl pyrophaeophorbide-*a*: R = V
Methyl mesopyrophaeophorbide-*a*: R = Et



Chlorin-e_6 trimethyl ester: $R^1 = V$, $R^2 = Me$, $R^3 = CH_2CO_2Me$
Mesochlorin-e_6 trimethyl ester: $R^1 = Et$, $R^2 = Me$, $R^3 = CH_2CO_2Me$
Chlorin-p_6 trimethyl ester: $R^1 = V$, $R^2 = Me$, $R^3 = CO_2Me$
Rhodin-g_7 trimethyl ester: $R^1 = V$, $R^2 = CHO$, $R^3 = CH_2CO_2Me$
Mesorhodin-g_7 trimethyl ester: $R^1 = Et$, $R^2 = CHO$, $R^3 = CH_2CO_2Me$



Rhodoporphyrin-XV dimethyl ester: R = Et
2-Vinylrhodoporphyrin-XV dimethyl ester: R = V
$P^{Me} = CH_2CH_2CO_2Me$



Phaeoporphyrin-a_5 dimethyl ester: R = Et
2-Vinylphaeoporphyrin-a_5 dimethyl ester: R = V
V = CH:CH$_2$

Fig 1.

Table 3. Correlation of *meso*-carbon shift differences with structural changes in the methyl esters of chlorophyll degradation products

	Shift difference[a]			
	α	β	γ	δ
(1) *3-Methyl with respect to 3-formyl*				
Phaeophorbide-*a* to phaeophorbide-*b*	+4·0	+1·9	−0·5	+0·1
Mesophaeophorbide-*a* to mesophaeophorbide-*b*	+3·9	+2·0	−0·5	+0·2
Chlorin-e_6 to rhodin-g_7	+3·9	+2·0	+0·2	+0·1
Mesochlorin-e_6 to mesorhodin-g_7	+4·1	+2·2	−0·1	+0·1
Mean value	+4·0	+2·0	−0·1	+0·1
Range of values	±0·1	±0·2	±0·4	±0·1
(2) *2-Vinyl with respect to 2-ethyl*				
Phaeophorbide-*a* to mesophaeophorbide-*a*	−0·6	+0·3	−0·1	−0·5
Pyrophaeophorbide-*a* to mesopyrophaeophorbide-*a*	−1·0	+0·2	−0·2	−0·6
Phaeophorbide-*b* to mesophaeophorbide-*b*	−0·7	+0·4	−0·1	−0·4
Chlorin-e_6 to mesochlorin-e_6	−1·2	+0·3	0·0	−0·6
Rhodin-g_7 to mesorhodin-g_7	−1·0	+0·5	−0·3	−0·6
Mean value	−0·9	+0·4	−0·2	−0·5
Range of values	±0·3	±0·2	±0·2	±0·2
(3) *"Normal" carbocyclic ring with respect to "pyro" ring*				
Phaeophorbide-*a* to pyrophaeophorbide-*a*	0·0	−0·4	+0·6	−0·2
Mesophaeophorbide-*a* to mesopyrophaeophorbide-*a*	−0·4	−0·5	+0·5	−0·3
Mean value	−0·2	−0·4	+0·5	−0·2
Range of values	±0·2	±0·1	±0·1	±0·1
(4) *"Normal" carbocyclic ring with respect to 6-methoxycarbonyl or γ-methoxycarbonylmethyl*				
Phaeophorbide-*a* to chlorin-e_6	+1·8	−1·9	−3·1	+0·6
Mesophaeophorbide-*a* to mesochlorin-e_6	+1·2	−1·9	−3·0	+0·5
Phaeophorbide-*b* to rhodin-g_7	+1·7	−1·8	−2·4	+0·6
Mesophaeophorbide-*b* to mesorhodin-g_7	+1·4	−1·7	−2·6	+0·4
Mean value	+1·5	−1·8	−2·7	+0·5
Range of values	±0·3	±0·1	±0·4	±0·1

[a] In ppm, positive values referring to a downfield shift.

Matwiyoff's assignments, the α carbon would be shifted downfield by 9 ppm and the β carbon upfield by 3 ppm in chlorophyll-*b* compared with methyl phaeophorbide-*b*; reversal of Matwiyoff's assignments leads to downfield shifts of both the α and β *meso*-carbons of 2 and 4 ppm respectively, which correlates more satisfactorily with the effects observed in the "*a*" series. These data suggest that the α and β methine assignments in the ^{13}C spectrum of chlorophyll-*b*[8] should be reversed.

As would be expected, examination of the mean values of the chemical shift difference accompanying a specific structural change shows the largest values for the *meso*-carbons adjacent to the site of the structural modification. For example, the α and β carbons are shifted downfield by 4·0 and 2·0 ppm respectively on going from the "*a*" to "*b*" series, whilst the γ and δ carbons each shift by only about 0·1 ppm.

Comparison of the spectrum of chlorin-e_6 trimethyl ester with that of OEC gives an indication of the magnitude of the shift at γ-C caused by introduction of a bulky *meso*-acetate substituent (*viz* downfield by 9·2 ppm).

(b) TFA *spectra*. Use of TFA as solvent causes a downfield shift of 1·1 ppm for the $\gamma(\delta)$-C in OEC, and one of 7·8 ppm for $\alpha(\beta)$. Similar shifts occur in methyl phaeophorbide-*a* and methyl pyrophaeophorbide-*a*. The γ and δ-carbons experience small downfield shifts, whereas the α-C signal moves downfield by 9·1 ± 0·2 and β-C by 9·5 ± 0·1 ppm. A spectrum of methyl phaeophorbide-*a* in $CDCl_3$ containing 6 equiv. of TFA is intermediate between those of the two pure solvents and help to confirm the TFA assignments. The chemical shifts of this protonated methyl phaeophorbide-*a* in $CDCl_3$ are quite close to those of chlorophyll-*a* itself in $CDCl_3$ solution (*cf* 100·1, 107·1, 106·2 and 92·8 ppm for the α, β, γ and δ-carbons of chlorophyll-*a*[15,16] and 101·9, 108·5, 104·2 and 93·5 ppm for the same carbons in the partially acidified sample of methyl phaeophorbide-*a*). This indicates an interesting correlation between metallation and protonation upon chemical shifts.

δ-C is unambiguously assigned using methyl d_6-pyrophaeophorbide-*a* (see Table 4).

Partial assignment of the *meso*-carbon signals in rhodoporphyrins and phaeoporphyrins could be achieved; as in chlorins in $CDCl_3$ (Table 3), conversion of 2-vinyl into ethyl caused small upfield shifts in the α- and δ-carbons. Here, two *meso*-signals in both rhodo- and phaeoporphyrins are shifted upfield by hydrogenation of the 2-vinyl group, and these are assigned to α- and δ. The other two *meso*-carbons are unaltered. The γ-carbon in the phaeoporphyrins can be further identified from the undecoupled spectrum.

Table 4. *Meso*-Carbon resonances of chlorins in $CDCl_3$ and TFA[a]

Compound (Solvent)		*meso*-carbons α	β	γ	δ
OEC	($CDCl_3$)	98·3		92·5	
	(TFA)	106·1		93·5	
Methyl phaeophorbide-*a*	($CDCl_3$)	96·4	103·6	104·8	92·6
	($CDCl_3$ + 6 eq. TFA)	101·9	108·5	104·2	93·5
	(TFA)	105·7	113·0	104·9	94·1
Methyl pyrophaeophorbide-*a*	($CDCl_3$)	96·4	103·2	105·4	92·4
	(TFA)	105·3	112·8	106·5	93·9
Methyl mesophaeophorbides from *C. ethylicum.*	($CDCl_3$)	95·1(d)[b]	102·3(d)	105·3(s)	105·0(s)
	(TFA)	105·8(d)	111·4(d)	105·4(s)	105·4(s)

[a] In δ (ppm), downfield from TMS. Multiplicity of undecoupled spectrum is shown in parentheses.
[b] Signal absent in sample pre-treated[14] with deuterioacetic acid.

(C) *Carbonyl carbons*

(a) $CDCl_3$ *spectra.* The C-10a and C-7c signals in methyl phaeophorbide-*a* were assigned by Katz[7] at 168·9 and 172·6 ppm, on the basis of similar intensities. A third signal, assigned as a macrocyclic carbon appears at 171·4 ppm. Comparison with methyl pyrophaeophorbide-*a* and methyl mesopyrophaeophorbide-*a* shows no signal around 169 ppm, whereas one is present in methyl phaeophorbide-*b* and methyl mesophaeophorbide-*a*; thus, this signal is assigned to C-10a. The line which is constant at 172·6 ± 0·2 throughout is assigned to C-7c, and the lowest field carbon resonance in chlorins bearing a carbocyclic ring is assigned to C-9. The formyl carbon in the "*b*" series is readily identified from the undecoupled spectrum.

(b) TFA *spectra.* The carbonyl carbons are more easily assigned by comparison than in the case of the $CDCl_3$ spectra, due to the upfield shift of the low field macrocyclic carbon atoms. C-10a resonates at 170·7 in methyl phaeophorbide-*a* compared with 171·3 ppm in the phaeoporphyrin esters, a shift downfield of *ca* 3 ppm relative to the $CDCl_3$ spectrum. In both chlorins and porphyrins, C-7c appears at 178·1 ± 0·5 ppm, indicating a similar downfield shift of *ca* 5 ppm. Downfield shifts also occur at the carbocyclic ring carbonyls and these are compatible with a small proportion of protonation of these groups.

(D) *Macrocycle carbon atoms*

There are far too many variables to allow any accurate assignments of these carbons in either $CDCl_3$ or TFA. However, no broadening of the carbons adjacent to nitrogen occurs in chlorins in $CDCl_3$ or TFA, or in porphyrins in TFA, unlike the cases of porphyrins in $CDCl_3$.[1,3,17] This has been shown to be due to NH tautomerism in the porphyrin ring[1,17] which is excluded in acid solution by diprotonation. The broadness is presumably not observed in chlorins in $CDCl_3$ because of differing exchange rates. In TFA the low field macrocyclic carbon atoms are shifted upfield considerably.

Correlation between the shifts of macrocyclic carbon atoms and identical structural changes in different chlorins, as achieved with the *meso*-carbons, was impossible to interpret fully. A characteristic feature of conversion of a normal (2-vinyl) chlorin to its meso (2-ethyl) analogue is downfield shifts of two resonances in the 134–140 ppm region—one downfield by about 7 and the other by about 1 ppm, and assigned to carbons 2 and 1 respectively (e.g. in methyl phaeophorbide-*a*, the two signals at 135·7 ppm are shifted downfield in methyl mesophaeophorbide-*a* to 136·7 and 142·3 ppm). The remaining twelve signals are more or less constant.

In the spectra of the methyl esters of chlorin-e_6, chlorin-p_6, and rhodin-g_7, and their meso-derivatives, a high-field resonance at *ca* 122–124 ppm is observed; this is presumably a carbon in ring C. Comparison of the spectra of methyl phaeophorbide-*a* with methyl pyrophaeophorbide-*a*, and that of methyl mesophaeophorbide-*a* with methyl mesopyrophaeophorbide-*a* shows only four signals which shift more than 1·0 ppm (*viz* for methyl phaeophorbide-*a*, those at 160·5, 171·4, and the two at 128·3 ppm). The significant shift differences of the high field pair suggests that they are carbons from ring C. The low field pair are assigned to carbons 17 and 18 by comparison with the spectra of OEC, which exhibits a low field carbon [presumably C-17(18)]. This is further confirmed by comparison with the spectrum of cyanocobalamin,[18] in which the five unsaturated carbons of the corrin ring resonate in the region 180–190 ppm downfield from HMDS. Rupture of the carbocyclic ring, as in chlorin-e_6 trimethyl ester, shifts both of these signals very close to the value of 167·1 ppm found in OEC.

Diagnostic use of the meso-*carbon* TFA *shift* (see Table 4)

The downfield shift of the α- and β-*meso*-carbon resonances of chlorins in TFA relative to $CDCl_3$ can be used as a diagnostic effect for identification of the site of *meso*-methylation in the methyl mesophaeophorbides (*Chlorobium* mesophaeophorbides (660)) from *Chloropseudomonas ethylicum.*[19] There has been some argument over the position of the novel *meso*-methyl group in these compounds,[20,21] though the δ-position was strongly favoured by synthetic and degradative work.[22,23] Examination of the ^{13}C NMR spectrum of the methyl mesophaeophorbides (*ca* 70% fraction 5) showed the signals indicated in Table 4. Substantial downfield shifts of the two *meso*-carbons bearing hydrogens, in TFA

solution, confirmed them as α- and β-*meso*-carbons, proving that the methyl substitution is at the δ-position in *Chlorobium* chlorophyll (660) band 5, and therefore in all of the (660) series.[23] Thus, methyl substitution at the δ-position in these mesophaeophorbides shows a downfield shift of 12·6 ppm relative to OEC, compared with a downfield shift of 9·2 ppm due to *meso*-acetate substitution which occurs in the γ-position in chlorin-e_6 trimethyl ester.

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