Tetrahedron Vol. 35, pp. 2275 to 2283
Pergamon Press Ltd., 1979. Printed in Great Britain

ELECTROPHILIC REACTIONS OF CHLORIN DERIVATIVES AND A COMPREHENSIVE COLLECTION OF ^{13}C DATA OF THESE PRODUCTS AND CLOSELY RELATED COMPOUNDS†

V. WRAY,* U. JÜRGENS and H. BROCKMANN, JR‡

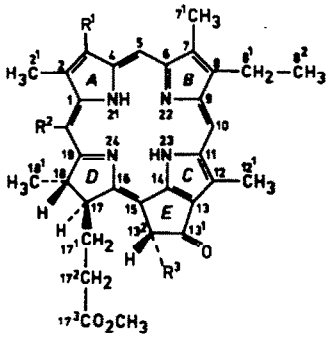
Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig, Federal Republic of Germany

(*Received in Germany* 16 *February* 1979)

Abstract—Vilsmeier-formylation of the copper(II) complex of chlorin-e_6 trimethyl ester (2), under mild conditions, gives selective substitution in the 3-vinyl group. In contrast chlorination of 2 is shown to lead to selective substitution at position 20 of the macrocycle. A similar result is found for [3-ethyl]-isochlorin-e_4 dimethyl ester (17) although further reaction leads to more highly chlorinated products which have been isolated and identified. ^{13}C NMR data for some of these compounds and several related chlorin derivatives are reported. In particular, after correction of the literature, many of the quaternary carbon signals of the macrocycle are assigned and substituent effects assessed. Consideration of the shifts of the α-pyrrolic carbons confirms that chlorin and its derivatives exist in a tautomeric form with the two inner H atoms on diagonally opposite pyrrole rings A and C. Such a form allows a satisfactory explanation of the substituent chemical shifts of the formyl group at positions 15 and 20 to be made.

In the present work we report the continued study of the reaction of various chlorin and phaeophorbide derivatives with electrophilic reagents. The ^{13}C data for these and previously prepared compounds are presented and rationalised.

During the partial synthesis[1,2] of [3-ethyl]-bacteriophaeophorbide-c methyl ester (1)§ (from *Chloroflexus aurantiacus*[3]) from chlorin-e_6 trimethyl ester (2) and [3-ethyl]-chlorin-e_6 trimethyl ester (3) several intermediates were prepared with various substituents at position 20 of the macrocycle. Substitution of the 20-H atom of 3 was initiated by either reaction with chloromethylmethyl ether[4] or by a Vilsmeier formylation[2] to give the 20-hydroxymethyl and 20-formyl products, respectively. These were reduced to the 20-Me compound and the ring was closed between C-13[1] and C-15[1]. Finally the 13[2]-methoxycarbonyl group of the resulting product was removed pyrolytically. Synthetic procedures and spectroscopic data, apart from the ^{13}C NMR data, are published elsewhere.[2,4]



	R^1	R^2	R^3
1 = 4	CH_2CH_3	CH_3	H
14	CH_2CH_3	CHO	CO_2CH_3
24	$CH{=}CH_2$	H	CO_2CH_3
25	CH_2CH_3	H	CO_2CH_3

Scheme 1.

The same work reported the preparation and identification of related compounds from chlorophyll-c of *Chlorobium limicola* that were needed to prove that the synthetic end product [3-ethyl]-20-methylpyrophaeophorbide-a methyl ester (4) was identical to 1. Enough compound was produced at each intermediate stage that ^{13}C NMR spectra could be recorded. Thus data for the following are reported here: 2, 3, [3-ethyl]-20-formylchlorin-e_6 trimethylester (5), [3-ethyl]-20-hydroxymethylchlorin-e_6 trimethyl ester (6), [3-ethyl]-20-methoxymethylchlorin-e_6 trimethyl ester (7) and [3-ethyl]-20-methylchlorin-e_6 trimethyl ester (8).

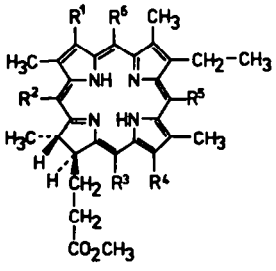
In the course of the above a series of interesting side products were isolated and identified, the precise origin of which were investigated in order to widen our knowledge of the behaviour of the different reaction centres of chlorin derivatives. In addition several reactions connected with this complex question were investigated. Quantities of many of these compounds were sufficient to allow ^{13}C NMR spectra to be taken. During the preparation of 2 and 3, from the raw phaeophytin-mixture isolated from spinach,[5] several side products were isolated and purified by chromatography. Thus those related to chlorophyll-a were rhodochlorin dimethyl ester (9), isochlorin-e_4 dimethyl ester (10) and purpurin-5 dimethyl ester (11).

The reactions summarised above of various chlorin derivatives with electrophilic reagents raised several questions regarding the reactivity of centres in the molecule. These were clarified by the reaction sequences described below.

†This paper should be considered as Part II in the series ^{13}C Nuclear Magnetic Resonance Studies of Porphyrins and Related Compounds. For Part I see REF. 14.

‡Present address: Fakultät für Chemie der Universität Bielefeld, Universitätsstraße, D-4800 Bielefeld, Federal Republic of Germany.

§The nomenclature used through out is according to the IUPAC convention (R. Bonnett, *Ann. N. Y. Acad. Sci.* **206**, 745 (1973)) except that the numbering is continued across the oxygen of the ester linkage for convenience with the ^{13}C NMR data. Only compounds of importance to the present work are numbered. An example of the numbering is given in Scheme 1.



	R^1	R^2	R^3	R^4
2	$CH{=}CH_2$	H	$CH_2CO_2CH_3$	CO_2CH_3
3	CH_2CH_3	H	$CH_2CO_2CH_3$	CO_2CH_3
5	CH_2CH_3	CHO	$CH_2CO_2CH_3$	CO_2CH_3
6	CH_2CH_3	CH_2OH	$CH_2CO_2CH_3$	CO_2CH_3
7	CH_2CH_3	CH_2OCH_3	$CH_2CO_2CH_3$	CO_2CH_3
8	CH_2CH_3	CH_3	$CH_2CO_2CH_3$	CO_2CH_3
9	$CH{=}CH_2$	H	H	CO_2CH_3
10	$CH{=}CH_2$	H	$CH_2CO_2CH_3$	H
11	$CH{=}CH_2$	H	CHO	CO_2CH_3
12	$CH{=}CHCHO$	H	$CH_2CO_2CH_3$	CO_2CH_3
13	$CH{=}CH_2$	Cl	$CH_2CO_2CH_3$	CO_2CH_3
15	$CH{=}CH_2$	H	CH_3	H
16	$CH{=}CH_2$	H	CH_3	CO_2CH_3
17	CH_2CH_3	H	$CH_2CO_2CH_3$	H
18	CH_2CH_3	Cl	$CH(OCOCH_3)CO_2CH_3$	H
19	CH_2CH_3	Cl	$CH(OCOCH_3)CO_2CH_3$	H
20	CH_2CH_3	Cl	$CH_2CO_2CH_3$	H
21	CH_2CH_3	Cl	$CH(OCOCH_3)CO_2CH_3$	Cl
22	CH_2CH_3	Cl	$CH_2CO_2CH_3$	H
23	CH_2CH_3	Cl	$CH_2CO_2CH_3$	CO_2CH_3

For **2**, **3**, **5** to **13**, **15** to **18**, **22** and **23**: $R^5{=}R^6{=}H$;
For **19**: $R^5{=}Cl$, $R^6{=}H$; For **20**: $R^5{=}Cl$, $R^6{=}H$;
For **21**: $R^5{=}Cl$, $R^6{=}Cl$.

Scheme 2.

Selective Vilsmeier-formylation of the 3-vinyl group of **2**. Nichol[6] has carried out a Vilsmeier reaction on **2** in which he heated the iron(III) complex with $POCl_3$ in dimethyl formamide for 90 sec at 100°. He obtained a monoformyl compound in which the 3-vinyl group had reacted together with a second diformyl compound in which the 3-vinyl group had reacted and a formyl group had been introduced at position 20. Demetalation with concentrated sulphuric acid of the corresponding copper(II) and nickel(II) complexes of related formylated compounds caused loss of the formyl groups in the products.[6] However as the formylation of the copper(II) complex of **3** gave the 20-formyl product[2] we were interested to see if the copper(II) complex of **2** could be formylated using the same conditions, and if through the use of a lower reaction temperature a selective formylation of **2** was possible. The reaction was carried out at 20° and only 3^2-(E)-formylchlorin-e_6 trimethyl ester (**12**) was obtained. The configuration of the side chain was determined from a comparison of the proton coupling constants of this fragment with those of *trans* crotonaldehyde.[7]

Chlorination of **2**. Compound **2** was reacted by the method of Jeckel[8] with hydrogen peroxide (30%) and hydrochloric acid in order to see if the 3-vinyl group would be again attacked first. The only product isolated, however, was 20-chlorochlorin-e_6 trimethyl ester (**13**) with the vinyl group intact. Thus there is not a general preference of electrophilic reagents for the 3-vinyl group as suggested by the formylation reaction. The basis for the selective formation of **12** must lie primarily in the greater steric requirement of the Vilsmeier complex as the 3-vinyl group is less hindered than the 20 methine-bridge position.

Preparation of a phaeophorbide-a derivative with a 20-formyl substituent. Previous attempts at formylation of pyrophaeophorbide-a methyl ester had caused reaction to occur exclusively in the isocyclic 5-membered ring rather than at position 20. An alternative way to generate a 20-formyl substituted phaeophorbide-a derivative was to effect ring closure[9] of **5** to give [3-ethyl]-20-formylphaeophorbide-a methyl ester (**14**). The amount produced, however, precluded any attempt at synthesising the 20-Me compound **4** from this intermediate.

Separation of a mixture of chlorin derivatives with very small differences in polarity. For further completion of our ^{13}C NMR data collection of chlorin derivatives we would like to thank Prof. H. H. Inhoffen for supplying us with a mixture containing **9**, **10**, phyllochlorin methyl ester (**15**) and chlorin-e_4 dimethyl ester (**16**). Tlc of the mixture showed a long, unresolved spot that contained several compounds as can be seen from its ^1H NMR spectrum. Preparative low pressure chromatography[10] at 6 bar, through a column packed with 10 μ silica gel, gave a quantitative separation of all four components upon elution with *n*-hexane/acetone (10:1); the order of elution being **15**, **9**, **16** and **10**.

Preparation and chlorination of [3-*ethyl*]-*isochlorin-e_4 dimethyl ester* (**17**).[5] Compound **10** was hydrogenated to **17** using a palladium-activated charcoal catalyst. As expected[8] the chlorination of **17** gave a mixture of

chlorinated products. It was already known that porphyrin derivatives react with sulphuryl chloride to give substitution at the methine bridge C atoms.[11] Similar reactions of the methine bridge protons occurred in **17**. In addition a 15^1 H atom of the 15-acetic acid residue was replaced even though it is not in direct contact with the ring current of the macrocycle. Work up in sodium acetate solution gave exchange to the acetyl derivative. Thus the first chlorination reaction lead to the following products (in the order of their polarity): 15^1 - acetoxy - 20 - chloro - [3 - ethyl] - isochlorin - e_4 dimethyl ester (**18**), 15^1 - acetoxy - 10,20 - dichloro - [3 - ethyl] - isochlorin - e_4 dimethyl ester (**19**), 10,20 - dichloro - [3 - ethyl] - isochlorin - e_4 dimethyl ester (**20**) and 15^1 - acetoxy - 5,10,13,20 - tetrachloro - [3 - ethyl] - isochlorin - e_4 dimethyl ester (**21**). The desired product, 20 - chloro - [3 - ethyl] - isochlorin - e_4 dimethyl ester (**22**), was not isolated which suggested that it had reacted further during the reaction. This was shown to be the case by performing the chlorination under milder conditions (shortening the reaction time and decreasing the amount of reagent). Thus a selective preparation of **22** was performed in which the reaction was stopped when 50% of **17** had been converted.

A further question of interest was which compound was produced after **22**. A second modification of the reaction conditions allowed isolation of only the mono and dichloro compounds, **22** and **20** respectively. That more highly chlorinated products, together with the 15^1-acetoxy compound **18**, had formed under longer reaction conditions suggests that the methine-bridge position 20 is first attacked, followed by methine-bridge position 10. Concurrent with the latter attack substitution must take place at the 15^1-H atom inorder to give **18**, as well as reaction of **18** or **20** or both to give **19** after a longer reaction time.

Using a similar method to the above **3** was selectively chlorinated to 20 - chloro - [3 - ethyl] - chlorin - e_6 trimethyl ester (**23**).

^{13}C NMR investigation of the chlorin derivatives. The quantities of material produced for many of the compounds in this and related synthetic studies[1,2,4] of chlorin derivatives made it possible to run ^{13}C NMR spectra. In all cases these data confirmed the nature of the reaction products and have enabled us to assemble a comprehensive collection here. In doing so it has been possible to assign many of the signals belonging to the quaternary pyrrolic carbons as well as those of the hydrogen bearing carbons. In order to do this, however, it was necessary to make reference to the literature.

Boxer *et al.*[12] have reported the important assignment of the quaternary pyrrolic carbon signals in the ^{13}C spectra of chlorphyll-a and its magnesium-free derivative, phaeophorbide-a methyl ester (**24**), by the use of heteronuclear INDOR experiments. The only difficulties arose in the assignment of the signals of carbons 13, 14 and 16. Recent reports of the ^{13}C spectra of **24**, the results of an extensive study of many metal-free derivatives of various bacteriochlorophylls and data from the present study indicate that these latter three assignments are incorrect. Thus Smith and Unsworth[13] report for **24** two overlapping quaternary signals at 128.3 ppm and only one signal at 172.6 ppm (Boxer *et al.* require one and two signals at these positions respectively). The signal at 128.3 ppm also overlaps with the signal of the vinyl group, carbon 3^1. That this is correct is shown by the observation of two distinct quaternary carbon signals at 128.0 and 128.2 ppm for [3-ethyl]-phaeophorbide-a methyl ester (**25**).[13] Thus the signal at 172.6 ppm (173.3 ppm Boxer *et al.*) belongs to only one C atom. *In order to be consistent with the INDOR responses* only the signal belonging to C 13 could be at 128.3 ppm, moreover such a high field signal for an α-pyrrolic carbon is unlikely. This assignment also indicates that the signal of C 16 has been incorrectly assigned. Recent work suggests that the signal of 16 is to low field of 14 and this will be assumed here. Thus the corrected assignments of the quaternary carbon signals of **24** are shown in Table 1. For comparison purposes we have rerecorded the spectra of **24** and **25** and report here the assignment of the carbon signals of **2** to **11**, **15** to **17** and **22** to **25**.

Assignments and discussion of ^{13}C data. All the data and assignments are reported in Table 2.

Compounds **2**,**3**, **24** *and* **25**

Hydrogen bearing carbons. These signals of **2**,**24** and **25** have been assigned previously.[13,14] The assignments for **3** follow immediately from these and from the SFORD experiments. The only notable difference between **2** and **3**, apart from the changes in the signals associated with the 3-substituent, is the upfield shift of C 2^1 and downfield shift of C 5 caused by the substituent change at C 3 with C 7^1 being unchanged. The same occurs on comparing **25** with **24**. The assignment reversal for C 17^1 and C 17^2 is noted below.

Quaternary carbons. These were made by comparison with the above revised literature assignment of **24**. All the lowest field signals belonging to the carbonyl carbons and carbons 16 and 19 were assigned by comparison with **24** and for internal consistency. The signal of C 19 in **3** was identified from that of $C13^1$ by the small downfield shift on going from **2** to **3**, as found on going from **24** to **26**.

Comparison of **25**, **2** and **3** with **24** immediately allows the assignment of the signals for C 6, C 9, C 2 and C 12. The signal for C 13 in **2** and **3** was the highest field signal. The differences between the effects of vinyl and ethyl substituents in aromatic systems[15] immediately allows the remaining assignments in **25** to be made. For consistency in the comparison of **24** with **25** and of **2** with **3** the signals of C 1 and C 3 need to be assigned as shown. The remaining signals for **2** and **3** could not be unambiguously identified and lie in the region 135.6 ± 0.8 ppm.

The substituent changes associated with the change of an ethyl for a vinyl group are only short range. The cleavage of the E ring are longer range and can be seen to have effects in ring A. These probably arise from an increase in planarity of the whole system and change in the mesomeric interaction of the 13^1 carbonyl with the delocalised ring system. The changes in shifts of the signals of C 16 and C 14 support the view that C 14 is to high field of C 16. The largest change being associated with C 14 which could interact conjugatively with the planar carbonyl of ring E in **24** and **25**.

Compounds **5** *to* **8** *and* **23** (*the 20-substituted chlorin-e_6 trimethyl ester derivatives*)

Hydrogen-bearing carbons. Comparison with **3** and the results of the SFORD spectra allowed the assignment of all the signals in the region 27–110 ppm. The constancy of the Me signals of the carbomethoxy groups allowed unambiguous assignment of the signals of C 17 and C 18. It is interesting to note that, on the reasonable assumption that the signals of C 17^1 and C 17^2 are not inter-

Table 1. Corrected assignment of the quaternary carbon signals of phaeophorbide-a methyl ester (24)

Carbon	Boxer et al[12]	Smith & Unsworth[13]	Present work
1	141.9	141.3	142.04
2	131.6	131.1	131.79
3	136.3	135.7	136.47
4	135.9	135.7	136.16
6	155.3	155.0.	155.58
7	135.9	135.3	136.16
8	144;9	144.2	145.11
9	150.7	150.0	150.95
11	137.8	137.2	137.96
12	128.8	128.3	129.02
13	161.2	128.3	129.02
14	149.6	160.5	149.70
16	173.3	149.0	161.23
17^3	173.3	172.6	173.34
13^3	169.7	168.9	169.61
19	172.0	171.4	172.15

Table 2. ^{13}C chemical shifts of the chlorin derivatives with their assignments in parentheses§

24	25	2	3	5	6	7	8
189.59 (13^1)	189.66 (13^1)	173.54 (17^3)	173.53 (17^3)	192.12 (20^1)	174.13 (17^3)	173.68 (17^3)	173.59 (17^3)
173.34 (17^3)	173.41 (17^3)	173.00 (15^2)	173.09 (15^2)	173.55 (17^3)	172.93 (15^2)	173.04 (15^2)	173.08 (15^2)
172.15 (19)	172.58 (19)	169.50 (19)	170.13 (19)	172.65 (15^2)	169.37 (19)	169.91 (19)	169.56 (13^1)
169.61 (13^3)	169.73 (13^3)	169.50 (13^1)	169.58 (13^1)	171.31 (19)	171.32 (13^1)	171.91 (13^1)	169.56 (19)
161.23 (16)	160.89 (16)	166.84 (16)	166.36 (16)	168.80 (13^1)	165.91 (16)	166.06 (16)	165.14 (16)
155.58 (6)	155.70 (6)	154.80 (6)	154.83 (6)	167.44 (16)	153.76 (6)	153.80 (6)	153.41 (6)
150.95 (9)	150.63 (9)	148.91 (9)	148.48 (9)	154.02 (6)	149.76 (9)	149.60 (9)	149.94 (9)
149.70 (14)	149.79 (14)	144.91 (8)	145.11 (8)	149.77 (9)	144.81 (8)	144.81 (8)	144.48 (8)
145.11 (8)	145.12 (8)	139.47 (1)	140.93 (3)	145.10 (8)	143.66 (3)	143.54 (3)	142.69 (3)
142.04 (1)	142.88 (1)	136.38 (14)	140.48 (1)	144.97 (3)	141.33 (1)	141.73 (1)	139.98 (1)
137.96 (11)	141.94 (3)	135.84 (11)	136.28 (14)	144.46 (1)	136.31 (14)	136.26 (14)	136.16 (14)
136.47 (3)	137.57 (11)	135.40 (7)	135.89 (11)	138.00 (14)	136.14 (11)	135.98 (11)	135.81 (11)
136.16 (4)	137.27 (4)	135.40 (4)	135.35 (7)	137.63 (11)	135.08 (7)	135.13 (7)	134.36 (7)
136.16 (7)	135.75 (7)	134.72 (3)	135.25 (4)	136.01 (7)	133.76 (4)	133.97 (4)	133.35 (4)
131.79 (2)	131.48 (2)	130.46 (2)	130.32 (2)	134.03 (4)	129.90 (12)	129.73 (12)	130.11 (12)
129.02 (12)	128.69 (13)	129.38 (12)	128.90 (12)	130.41 (12)	129.46 (2)	129.66 (2)	129.89 (2)
129.02 (13)	128.46 (12)	129.29 (3^1)	122.95 (13)	129.00 (2)	123.56 (13)	123.48 (13)	123.58 (13)
129.02 (3^1)	105.07 (15)	123.48 (13)	102.44 (10)	124.01 (13)	106.93 (20)	104.14 (20)	104.73 (20)
122.64 (3^2)	104.34 (10)	121.53 (3^2)	102.12 (15)	106.16 (10)	102.52 (10)	102.53 (10)	101.32 (15)
105.28 (15)	96.17 (5)	102.24 (15)	97.37 (5)	105.98 (20)	101.63 (15)	101.61 (15)	101.07 (10)
104.33 (10)	92.50 (20)	102.08 (10)	92.93 (20)	103.33 (15)	98.72 (5)	98.69 (5)	97.61 (5)
97.47 (5)	64.72 (13^2)	98.64 (5)	52.93 (17)	102.98 (5)	61.30 (20^1)	71.14 (20^1)	53.42 (17)
93.09 (20)	52.88 (13^4)	93.49 (20)	52.93 (13^2)	53.76 (17)	54.05 (17)	58.57 (20^2)	52.94 (13^2)
64.77 (13^2)	51.64 (17^4)	53.04 (17)	52.01 (15^3)	52.98 (13^2)	52.92 (13^2)	53.75 (17)	52.02 (15^3)
52.81 (13^4)	51.07 (17)	52.97 (13^2)	51.56 (17^4)	52.01 (15^3)	52.04 (15^3)	52.93 (13^2)	51.59 (17^4)

Table 2 (*Contd*)

24	25	2	3	5	6	7	8
51.64 (17^4)	50.27 (18)	52.01 (15^3)	49.55 (18)	51.64 (17^4)	51.61 (17^4)	52.05 (15^3)	47.56 (18)
51.21 (17)	31.12 (17^2)	51.57 (17^4)	38.60 (15^1)	47.37 (18)	45.93 (18)	51.60 (17^4)	39.68 (15^1)
50.17 (18)	29.91 (17^1)	49.42 (18)	31.11 (17^2)	39.03 (15^1)	39.42 (15^1)	45.79 (18)	31.54 (17^2)
31.12 (17^2)	23.04 (18^1)	38.56 (15^1)	29.64 (17^1)	31.60 (17^2)	31.47 (17^2)	39.37 (15^1)	28.37 (17^1)
29.92 (17^1)	19.27 (3^1)	31.14 (17^2)	22.85 (18^1)	28.52 (17^1)	27.48 (17^1)	31.47 (17^2)	20.89 (18^1)
23.08 (18^1)	19.27 (8^1)	29.63 (17^1)	19.67 (8^1)	20.64 (18^1)	20.78 (18^1)	27.82 (17^1)	19.66 (8^1)
19.34 (8^1)	16.84 (8^2)	22.95 (18^1)	19.32 (3^1)	19.25 (8^1)	19.52 (8^1)	20.60 (18^1)	19.66 (3^1)
17.32 (8^2)	16.78 (3^2)	19.55 (8^1)	17.68 (8^2)	19.25 (3^1)	19.37 (3^1)	19.58 (8^1)	19.41 (20^1)
12.01 (12^1)	12.05 (12^1)	17.58 (8^2)	17.01 (3^2)	17.40 (8^2)	17.53 (8^2)	19.38 (3^1)	17.61 (8^2)
12.01 (2^1)	11.03 (7^1)	12.36 (12^1)	12.31 (12^1)	16.68 (3^2)	16.93 (3^2)	17.61 (8^2)	17.13 (3^2)
11.08 (7^1)	10.95 (2^1)	11.99 (2^1)	11.28 (7^1)	15.73 (2^1)	13.91 (2^1)	16.95 (3^2)	16.35 (2^1)
		11.16 (7^1)	10.84 (2^1)	12.08 (12^1)	12.24 (12^1)	13.44 (2^1)	12.29 (12^1)
				10.94 (7^1)	11.27 (7^1)	12.25 (12^1)	11.32 (7^1)
						11.28 (7^1)	

23	9	10	11	15	16	17	22
173.48 (17^3)	173.81 (17^3)	173.68 (17^3)	191.60 (15^1)	173.88 (17^3)	173.78 (17^3)	173.76 (15^2)	173.75 (15^2)
172.82 (15^2)	171.16 (19)	173.48 (15^2)	173.37 (17^3)	167.63 (19)	170.11 (13^1)	173.62 (17)	173.50 (17^3)
169.28 (13^1)	167.04 (13^1)	168.29 (19)	173.15 (19)	165.29 (16)	168.94 (19)	168.84 (19)	167.18 (19)
168.26 (19)	166.62 (16)*	166.06 (16)	171.32 (16)	152.89 (6)	165.45 (16)	165.43 (16)	166.52 (16)
166.38 (16)	154.19 (6)	152.88 (6)	167.33 (13^1)	149.04 (9)	154.68 (6)	152.77 (6)	151.76 (6)
153.69 (6)	149.54 (9)	149.21 (9)	154.81 (6)	144.01 (8)	148.83 (9)	148.56 (9)	150.81 (9)
150.41 (9)	144.75 (8)	144.04 (8)	149.00 (9)	139.00 (14)	144.81 (8)	144.16 (8)	144.00 (8)
144.81 (8)	140.21 (14)	139.06 (14)	145.23 (8)	137.79 (12)	138.96 (1)	140.27 (3)	142.38 (3)
143.11 (3)	139.83 (11)	138.23 (12)	142.67 (14)	136.28 (11)	135.73 (14)	139.25 (14)	138.82 (14)
136.68 (14)	137.18 (7)	136.88 (11)	138.10 (11)	135.80 (7)	135.39 (11)	138.72 (12)	137.31 (12)
136.61 (11)	135.98 (11)	135.91 (7)	136.72 (7)	133.73 (4)	135.39 (7)	136.46 (11)	136.71 (11)
136.51 (7)	134.97 (3)	134.07 (4)	136.56 (4)	133.73 (3)	135.16 (4)	135.37 (7)	135.31 (7)
134.93 (4)	134.63 (1)	133.63 (3)	135.95 (3)	132.63 (2)	134.41 (3)	134.45 (4)	134.24 (4)
133.35 (1)	130.00 (12)	132.76 (2)	135.73 (1)	129.16 (1)	130.38 (2)	132.16 (2)	131.79 (2)
130.84 (12)	130.11 (2)	129.15 (1)	130.74 (12)	129.87 (3^1)	129.47 (3^1)	129.13 (1)	129.63 (1)
130.55 (2)	129.39 (3^1)	129.67 (3^1)	129.71 (2)	121.00 (3^2)	129.39 (12)	118.72 (13)	120.28 (13)
124.31 (13)	121.68 (3^2)	121.08 (3^2)	128.72 (3^1)	119.53 (13)	124.21 (13)	101.84 (15)	105.54 (20)
105.52 (20)	119.03 (13)	119.23 (13)	122.58 (13)	104.98 (15)	121.42 (3^2)	101.11 (10)	102.35 (15)
102.43 (15)	101.98 (10)	101.99 (15)	122.58 (3^2)	100.16 (10)	105.45 (15)	97.79 (5)	100.35 (10)
101.75 (10)	98.85 (5)	100.73 (10)	106.80 (15)	98.24 (5)	101.47 (10)	92.63 (20)	99.42 (5)
98.92 (5)	96.29 (15)	98.96 (5)	106.80 (10)	93.12 (20)	97.94 (5)	52.80 (17)	53.11 (17)
53.14 (17)	92.84 (20)	93.23 (20)	102.11 (5)	53.44 (17)	93.41 (20)	52.14 (15^3)	52.18 (15^3)
53.02 (13^2)	54.78 (17)	52.94 (17)	93.99 (20)	51.43 (17^4)	53.33 (17)	51.46 (17^4)	51.58 (17^4)
52.05 (15^3)	51.78 (13^2)	52.10 (15^3)	52.51 (17)	48.79 (18)	52.79 (13^2)	49.16 (18)	48.77 (18)
51.56 (17^4)	51.53 (17^4)	51.45 (17^4)	52.32 (13^2)	30.97 (17^2)	51.50 (17^4)	39.06 (15^1)	39.61 (15^1)
49.28 (18)	48.67 (18)	49.03 (18)	51.57 (17^4)	30.02 (17^1)	48.97 (18)	30.91 (17^2)	31.19 (17^2)
39.37 (15^1)	32.46 (17^2)	39.00 (15^1)	49.24 (18)	23.78 (18^1)	31.12 (17^2)	30.28 (17^1)	29.27 (17^1)
31.29 (17^2)	31.14 (17^1)	30.92 (17^2)	32.57 (17^2)	19.75 (15^1)	29.84 (17^1)	23.29 (18^1)	19.93 (18^1)
28.77 (17^1)	23.32 (18^1)	30.18 (17^1)	31.58 (17^1)	19.38 (8^1)	23.43 (18^1)	19.72 (8^1)	19.68 (8^1)
19.58 (18^1)	19.58 (8^1)	23.32 (18^1)	23.45 (18^1)	17.73 (8^2)	19.88 (15^1)	19.44 (3^1)	19.60 (3^1)
19.58 (8^1)	17.59 (8^2)	19.63 (8^1)	19.35 (8^1)	13.70 (12^1)	19.64 (8^1)	17.76 (8^2)	17.61 (8^2)
19.42 (3^1)	13.56 (12^1)	17.67 (8^2)	17.43 (8^2)	12.17 (2^1)	17.61 (8^2)	17.17 (3^2)	17.27 (3^2)
17.54 (8^2)	12.02 (2^1)	13.62 (12^1)	12.22 (12^1)	11.37 (7^1)	12.04 (12^1)	13.67 (12^1)	16.18 (2^1)
17.08 (3^2)	11.22 (7^1)	12.06 (2^1)	11.84 (2^1)		12.04 (2^1)	11.37 (7^1)	13.55 (12^1)
16.25 (2^1)		11.26 (7^1)	10.94 (7^1)		11.23 (7^1)	10.94 (2^1)	11.41 (7^1)
12.25 (12^1)							
11.23 (7^1)							

§ The shifts are bracketed where the assignments are uncertain and the relevant carbons are given in descending numerical order. For the assignment of $C17^1$ and $C17^2$ see the text.

* C16 is either at 167.04 or 166.62 ppm.

changed in compound 3 and these compounds, the higher field signal shows the greater substituent shift variation (2.16 ppm compared to 0.49 ppm for the lower field signal). As a monotonic fall off of substituent effects would be expected and is observed for C 17, C 17^3 and C 17^4, the higher field signal is tentatively assigned to C 17^1 and the lower field one to C 17^2. This is contrary to previous assignments which have relied on assumed protonation shifts[13] or upon comparisons with chlorophyll-a T_1 measurements.[13,14] Of the remaining high field signals those of C 7^1, C 12^1, C 3^2, C 8^2, C 3^1, C 8^1 and C 20 (in 8) were assigned from their relative constancy and/or multiplicity in the SFORD spectra. The two signals that show pronounced substituent shifts belong to C 2^1 and C 18^1 with the latter to lowfield of the former.

Quaternary carbons. Comparison with 3 and the expected relative constancy of the CO signals allowed unambiguous assignment of C 17^3, C 15^2, C 13^1, C 19, C 16, C 6, C 9, C 8, C 3, C 1, C 12 and C 13. In 23 the signals of C 8 and C 3 should be similar to those of 6–8 while only that of C 1 is expected to be different and hence is to high field. The signals of C 2 and C 12 were very close and no attempt to separate them has been made. Similarly the remaining signals can not be unambiguously assigned.

The multiplicity of the methin-bridge (meso)carbon signals in the SFORD spectra showed that meso substitution had taken place in these compounds and the shift changes were only compatible with these being at the meso position between rings A and D. The 20-Me substituent in 8 has effects upon the carbon signals of C 2^1 and C 18^1 similar to those of a bromo substituent in pyrophaeophorbide-a methyl ester investigated previously.[14] Arguments have been presented to suggest that the major cause of these effects was distortion of the macrocycle with concomitant loss of the planarity of the aromatic system. Thus the decreased shielding upon 2^1 is similar to that found on going from 1-methylnaphthalene to 1,8-dimethylnaphthalene and is in the opposite direction to the usual steric crowding effects which cause upfield shifts. Previously the quaternary carbons could not be assigned hence distortion effects which should manifest themselves elsewhere in the molecule, in particular in the macrocycle carbons, could not be observed. That long range effects are present is clearly seen in this case where signals of C 6 and C 9 show pronounced shift changes (> 1 ppm).

The shift changes found for 6 and 7 compared with 8 are relatively short range and occur in the vicinity of the substituent, arising presumably mainly from the polarity of the substituent. Long range changes are only small suggesting changes due to the bulk of the substituent are small. The introduction of the formyl substituent in 5 causes additional changes to those associated with the bulk of the substituent. Thus the substituent changes at the non-substituted meso carbons (+5.61 (C 5), +3.72 (C 10) and +1.21 (C 15) ppm) must arise primarily from a mesomeric interaction of the substituent with the macrocycle.

Compounds 9 to 11, 15 to 17 and 22

These compounds have the basic structures of 2, 3 and 23 with modifications at carbons 13 and 15. ^{13}C NMR has been used to identify these and only one structure in each case was compatible with the spectra.

Hydrogen-bearing carbons. Compounds 9 to 11, 15 and 16 were compared with 2, 17 with 3 and 22 with 23. These, together with the multiplicity of signals from SFORD spectra, allowed straight forward assignment of the signals. Only those signals of 17^1 and 17^2 in 9 and 11 could not be unambiguously assigned. For 11 the meso carbons were assigned as follows. From a comparison with 2 and the SFORD experiment the signals of C 20 and C 15 were immediately assigned. The remaining two signals were assigned to give reasonable shifts for both resonances compared to 2 (C 5 = +3.47 and C 10 = +4.72 ppm). The alternative assignment would give substituent shifts (C 5 = +8.16 and C 10 = +0.03 ppm) that imply substitution at C 5, a situation incompatible with the rest of the spectrum.

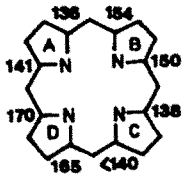
Quaternary carbons. The above comparisons allowed the assignment of C 17^3, C 6, C 9, C 8, C 13 and the signals to low field of 160 ppm apart from those of 9 and 11. Of the remaining signals only C 1 in 16, C 3 in 17 and 22, C 2 and C 12 in 16, 9 and 11 could be unambigously assigned.

The chlorin and phaeophorbide ring systems have the possibility of existing in several tautomeric forms with the two H atoms bonding to any of the pyrrolic N atoms. The non-equivalence of the four pyrrole ring requires that the energies of the various possible forms are unequal. The sharpness of the resonance lines and the absence of differential broadening in the present ^{13}C spectra indicate that either only one tautomeric form is present or that on the NMR time scale the exchange between the various forms is fast. Evidence from ^1H NMR spectra indicates that chlorin exists predominantly in one tautomeric form with exchange between the two protons having a coalescence temperature near to room temperature.[16] For the phaeophorbide system only one form, without exchange at room temperature was observed.[17] The nature of these preferred tautomeric forms may be inferred from the present ^{13}C data.

In all the compounds studied the α pyrrolic carbons (1,4,6, etc.), in those compounds where they have been assigned, have shifts over relatively narrow ranges and these are shown in Table 3. The effects of substituents on the β pyrrolic carbons (2,3,7, etc.) may be assumed to be constant to a first approximation. The only problem is the 15-substituent and the direct attachment of the CO function to C 13. Compound 9, with a hydrogen on C 15, indicates that the average shift for the α pyrrolic C 14 is

Table 3. ^{13}C chemical shift ranges for the quaternary pyrrolic carbons that have been assigned

Carbon	Range	Average	No. of compounds
1	138.96 - 142.88	140.86	8
2	130.38 - 131.79	130.89	5
3	136.47 - 143.66	141.67	9
4	136.14	136.14	1
6	151.76 - 155.70	153.97	16
7	136.16 - 135.75	135.96	2
8	144.00 - 145.23	144.68	15
9	148.48 - 150.95	149.59	16
11	137.96	137.96	1
12	128.69 - 129.39	129.08	5
13	118.72 - 129.02	122.92	15
14	149.70 - 149.79	149.74	2
16	160.89 - 167.44	165.36	14
19	167.63 - 172.58	169.55	14

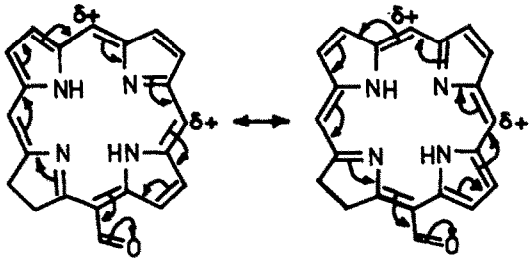


Scheme 3.

unusually low and in the absence of these substituents would be to high field of 140 ppm. Thus the averaged α pyrrolic carbon shifts are summarised in Fig. 1. It is immediately apparent that the carbons associated with rings A and C are considerably more shielded than those of rings B and D. The shifts of ring D carbons are the lowest field and are in the region associated with imino carbon (C=N) resonances.[18]

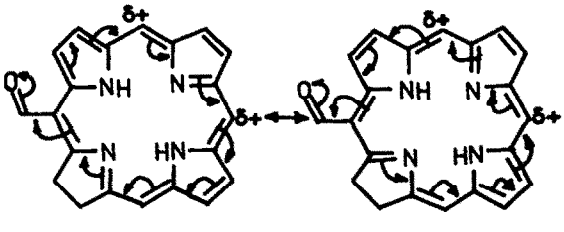
Thus the predominant tautomeric form has the H atoms on the diagonally opposite pyrrole rings A and C. This condusion is in keeping with MO calculations,[19] with the ^{1}H NMR studies mentioned above and with the results of the crystal structure of phaeophorbide-a methyl ester.[20] The latter found electron density greatest at sites for H atoms on rings A and C although disorder for these atoms was apparent.

The long-range substituent effects upon the meso carbons in 11 and 5 with the strongly electron withdrawing formyl group on C 15 and C 20 respectively can be rationalised in terms of this tautomeric form. Thus mesomeric electron withdrawl at C 15 (see Scheme 4) reduces electron density only at carbons 5 and 10 (not



Scheme 4.

20) as is found on comparing 11 and 9 (C 5 = +3.26; C 10 = +4.22; C 20 = +1.15 ppm). Similarly mesomeric electron withdrawl at carbon 20 reduces electron density at carbons 5 and 10 (not 15) as is found on comparing 5 and 3 (C 5 = +5.61; C 10 = +3.72; C 15 = +1.21 ppm). A preponderance of alternative tautomeric forms does not furnish a satisfactory explanation of these considerable shifts.



Scheme 5.

†In all the spectra the 17-CH_2 and 17^1-CH_2 were poorly resolved multiplets in the region 2–3 ppm and consequently they are not reported for any of the compounds described here.

The long-range shifts associated with the weaker electron withdrawing group—CO_2CH_3 at C 13 are much smaller than the above and probably arise from changes in the macrocyde distortion as steric interactions with the substituent at C 15 can occur.

CONCLUSION

The electron density distribution at the methine bridge C atoms has been probed by investigation of electrophilic attack on 2, 3 and 17. Chlorination indicates attack occurs first at position 20, followed in the case of 17 at position 10. Vilsmeier-formylation of the copper(II) complex of 2 gives selective attack in the 3-vinyl group. Literature data,[6] however, indicate that under more vigorous conditions attack also occurs at position 20. These data are in keeping with the important generalisation, first stated by Woodward,[21] that methine bridges of chlorins neighbouring reduced pyrrole rings are more susceptible to electrophillic attack than those between nonreduced pyrrolic rings.

The ^{13}C NMR data for the various chlorin derivatives, in particular those for the quaternary pyrrolic carbons, gives direct evidence for a preferred tautomeric form with hydrogens on the nitrogens of rings A and C. Such a form allows a rationalisation of the shift changes observed upon the introduction of a strong electron withdrawing group at various positions on the macrocycle. Other groups show long-range shift changes that arise from distortions of the macrocycle.

EXPERIMENTAL

All ^{1}H and ^{13}C NMR spectra were recorded on either a Varian XL-100-12 (^{1}H and ^{13}C) or a Varian CFT-20 (^{13}C) spectrometer operating in the Fourier transform mode and locked to the deuterium resonance of the solvent, $CDCl_3$. Proton noise decoupled and single frequency off-resonance proton decoupled (SFORD) ^{13}C spectra were obtained at ambient temperature, ~36°, with internal TMS as standard. The use of a centroid routine ensured that shifts were accurate to better than ±0.5 Hz (0.03 ppm).

UV/VIS-spectra were recorded on a Beckmann Spectrophotometer model 25 and IR-spectra on a Perkin–Elmer Spectrophotometer IR-521. Mass spectra were taken on AEI MS-9 and MS-30 spectrometers.

For thin layer chromatography tlc-plates SI F silica gel from Riedel-De Haën and tlc-aluminium foil silica gel 60 from Merck were used, while for preparative plate chromatography psc-ready plates of silica gel 60 F from Merck were employed. For normal column chromatography silica gel S (0.032–0.063 mm) from Riedel–De Haën and neutral aluminium oxide W 200 from ICN Pharmaceuticals Ltd. & CO. were used, while for the low pressure chromatography silica gel LiChrosorb Si 100 (10 μ) from Merck was employed. The eluant for this was transported with a regulated dispensing pump from Chemie und Filter, and the elution diagram was followed with a Uvicord II photometer from LKB Products coupled to a horizontal plotter Servogor S from Metrawatt. The preparation of the low-pressure chromatography column is described elsewhere.[10]

Rhodochlorin dimethyl ester (9), *isochlorin-e_4 dimethyl ester* (10) *and purpurin-5 dimethyl ester* (11). These were isolated during the preparation of 2 from the raw phaeophytin mixture produced from spinach.[2] During alkaline hydrolysis ring E was opened[22] and the above byproducts were formed. These were separated from 2 and rhodin-g_7 trimethyl ester by chromatography.[2]

Rhodochlorin dimethyl ester (9). ^{1}H NMR: δ† 9.81 (15-H), 9.76 (10-H), 9.62 (5-H), 8.74 (20-H), 8.04 (3^1-H_x), 6.31 (3^2-H_B), 6.14 (3^2-H_A), 4.49 (18-H), 4.45 (17-H), 4.35 (13^1-OCH_3), 3.70 (8-CH_2), 3.63 (17^3-OCH_3), 3.81 (12-CH_3), 3.47 (2-CH_3), 3.30 (7-CH_3), 1.87 (18-CH_3), 1.72 ppm (8^1-CH_3). MS (70 eV, 290°): *m/e* 566 (M^+), 479 ($[M-87]^+$). IR: ν_{max}^{KBr} 3320 (NH), 2950/2920/2860 (CH), 1735/1695

(ester-CO), 1605 cm^{-1} ("chlorin band").[23] UV: λ_{max}^{Dioxan} (rel. int.) 665 (34), 610 (3), 527 (37), 497 (8), 399 nm (100). Isochlorin-e_4 dimethyl ester (**10**). ^1H NMR: δ 9.74 (10-H), 9.74 (5-H), 8.86 (20-H), 8.81 (13-H), 8.14 (3^1-H_x), 6.35 (3^2-H_B), 6.13 (3^2-H_A), 5.39 (15-CH_2), 4.65 (17-H), 4.52 (18-H), 3.83 (8-CH_2), 3.74 (15^2-OCH_3), 3.62 (17^3-OCH_3), 3.62 (12-CH_3), 3.52 (2-CH_3), 3.35 (7-CH_3), 1.75 (18-CH_3), 1.75 ppm (8^1-CH_3). MS (70 eV, 270°): *m/e* 580 (M^+), 493 ([M-87]$^+$). IR: ν_{max}^{KBr} 3310 (NH), 2940/2910/2850 (CH), 1725 (ester-CO), 1590 cm^{-1} (chlorin band). UV: λ_{max}^{Dioxan} (rel. int.) 659 (31), 602 (3), 525(3), 499(9); 399 nm (100). Purpurin-5 dimethyl ester (**11**). ^1H NMR: δ 11.47 (15-CHO), 9.55 (10-H), 9.31 (5-H), 8.51 (20-H), 7.86 (3^1-H_x), 6.32 (3^2-H_A) 6.09 (3^2-H_B), 5.04 (17-H), 4.40 (18-H), 4.18 (13^1-OCH_3), 3.62 (8-CH_2), 3.59 (17^3-OCH_3), 3.55 (12-CH_3), 3.32 (2-CH_3), 3.14 (7-CH_3), 1.78 (18-CH_3), 1.64 ppm (8^1-CH_3). MS (70 eV, 300°): *m/e* 594 (M^+), 565 ([M-29]$^+$). IR: ν_{max}^{KBr} 3320 (NH), 2950/2920/2855 (CH), 1715 (ester-CO), 1655 (formyl-CO), 1590 cm^{-1} (chlorin band). UV: λ_{max}^{Dioxan} (rel. int.) 691 (27), 630 (shoulder), 540 (9), 503 (7), 406 nm (100).

3^2-(E)-Formyl chlorin-e_6 trimethyl ester (**12**). Copper(II) acetate (200 mg) in MeOH (20 ml) was added to 2 (12.8 mg) dissolved in CH_2Cl_2 (20 ml). The solvents were removed by vacuum distillation at 60°, the residue taken up in MeOH and partitioned between water and CH_2Cl_2 in order to remove the excess copper(II) salt. The organic phase was washed with water and evaporated to dryness under high vacuum. Distilled phosphoryl chloride (0.6 ml) was added dropwise with stirring at 0° to the resulting copper(II) complex dissolved in freshly distilled dimethylformamide (5 ml). The mixture was stirred for 10 min at 0° and 30 min at 20°, hydrolysed with ice (100 mg) and neutralised with NaOAc soln. The metal complex was extracted with CH_2Cl_2 and demetallated after removal of the solvent. Conc. H_2SO_4 (10 ml) was added slowly with cooling and the soln was stirred for 30 min at 45°. After cooling to 0° the mixture was poured on to ice (30 g) and ammonium acetate soln (30 g as a sat soln) and brought to pH 8 by further addition of ammonium acetate. The soln was extracted with CH_2Cl_2 and the extracts were washed, dried and the solvent removed to give the formylated raw product which was esterified with methanolic H_2SO_4 (4 vol.%). After standing for 15 hr at 20° the soln was neutralised and worked up as above. The crude product was purified by column chromatograph on silica gel (eluant: hexane/acetone 4:1) to yield 12 (4.1 mg). ^1H NMR: δ 10.14 (3^2-CHO), 9.70 (10-H), 9.60 (5-H), 8.91 (3^1-H), 8.88 (20-H), 7.40 (3^2-H), 5.31 (15-CH_2), 4.47 (18-H), 4.43 (17-H), 4.28 (13^1-OCH_3), 3.77 (8-CH_2), 3.77 (15^2-OCH_3), 3.64 (17^3-OCH_3), 3.58 (12-CH_3), 3.58 (2-CH_3), 3.31 (7-CH_3), 1.75 (18-CH_3), 1.72 ppm (8^1-CH_3). MS (70 eV, 360°): *m/e* 666 (M^+), 607 ([M-59]$^+$), 593 ([M-73]$^+$), 579 ([M-87]$^+$). IR: $\nu_{max}^{CCl_4}$ 3290 (NH), 2950/2920/2860 (CH), 1790 (ester-CO), 1670 (formyl-CO), 1615/1595 cm^{-1} (chlorin band). UV: λ_{max}^{Dioxan} (rel. int.) 686 (55), 629 (5), 544 (11), 507 (10), 416 nm (100).

20-Chlorochlorin-e_6 trimethyl ester (**13**). H_2O_2 (30%; 0.4 ml) and conc HCl (0.5 ml) were added to 2 (100 mg) in dioxan (40 ml). After stirring for 5 min the soln was poured into water (300 ml), neutralised with NaOAc soln and worked up as described for 12 above. Chromatography on silica gel (eluant: acetone/hexane 4:1) yielded 49 mg starting material and 45 mg 13. The conditions had to be chosen such that only half of the starting material was reacted inorder to avoid further reaction of the product (compare with the further chlorination of 17 below). ^1H NMR: δ 9.56 (10-H), 9.54 (5-H), 7.96 (3^1-H_x), 6.60 (3^2-H_B), 6.44 (3^2-H_A), 5.20 (15-CH_2), 4.87 (18-H), 4.26 (17-H), 4.25 (13^1-OCH_3), 3.82 (15^2-OCH_3), 3.70(8-CH_3), 3.62(17^3-OCH_3), 3.56(12-CH_3), 3.52(2-CH_3), 3.22 (7-CH_3), 1.67 (8^1-CH_3), 1.63 ppm (18-CH_3). MS (70 eV, 330°): *m/e* 672 (M^+), 638 ([M-34]$^+$), 565 ([M-34-73]$^+$).

20-Chloro-[3-ethyl]-chlorin-e_6 trimethyl ester (**23**). This was prepared from 3 using the same conditions as above for 13. ^1H NMR: δ 9.62 (10-H), 9.51 (5-H), 5.19 (15-CH_2), 4.87 (18-H), 4.31 (17-H), 4.26 (13^1-OCH_3), 3.90 (3-CH_2), 3.82 (15^2-OCH_3), 3.74 (8-CH_2), 3.63 (17^3-OCH_3), 3.55 (12-CH_3), 3.55 (2-CH_3), 3.30 (7-CH_3), 1.71 (3^1-CH_3), 1.69 (8^1-CH_3), 1.64 ppm (18-CH_3). MS (70 eV, 150°): *m/e* 674 (M^+), 640 ([M-34]$^+$). IR: $\nu_{max}^{CCl_4}$ 3320 (NH), 2960/2940/2920/2860 (CH), 1725 (ester-CO), 1550 cm^{-1} (chlorin band). UV: λ_{max}^{Dioxan} (rel. int.) 664 (29), 610 (3), 534 (6), 506 (8), 403 nm (100).

[3-Ethyl]-20-formylphaeophorbide-a methyl ester (**14**). Compound 5 (58 mg) was taken up in dry pyridine (12 ml) and heated to boiling in an inert atmosphere (N_2). 10% Methanolic KOH (0.1 ml) was added with stirring and the soln was stirred for 10 min. The reaction was terminated by cooling quickly to 20° and mixing with 3% HCl (100 ml). The crude product was extracted with CH_2Cl_2 and worked up as for 12 above. Chromatographic purification on a column of silica gel (eluant hexane/acetone 4:1) gave 14 (6 mg). ^1H NMR: δ 11.82 (20-CHO), 9.30 (10-H), 9.21 (5-H), 5.13 (18-H), 5.02 (13^2-H), 4.21 (17-H), 3.86 (13^3-OCH_3), 3.81 (3-CH_2), 3.66 (8-CH_2), 3.56 (17^3-OCH_3), 3.54 (12-CH_3), 3.23 (2-CH_3), 3.13 (7-CH_3), 1.67 (3^1-CH_3), 1.64 (8^1-CH_3), 1.42 ppm (18-CH_3). MS (70 eV, 300°): *m/e* 636 (M^+), 604 ([M-32]$^+$), 578 ([M-58]$^+$), 549 ([M-87]$^+$) (Found: 636.2948. $C_{37}H_{40}O_6N_4$ requires: 636.2948). UV: λ_{max}^{Dioxan} (rel. int.) 687 (32), 629 (7), 546 (11), 510 (6), 412 nm (100).

Phyllochlorin methyl ester (**15**) *and chlorin-e_4 dimethyl ester* (**16**). The mixture of 9, 10, 15 and 16 was separated on a low pressure column,[10] packed with silica gel of particle size of 10 μ, and eluted with *n*-hexane/acetone (10:1) at a pressure of 6 bar. The elution order was 15, 9, 16 and 10. The spectroscopic data for 9 and 10 have been given above. Phyllochlorin methyl ester (15): ^1H NMR: δ 9.71 (10-H), 9.71 (5-H), 8.84 (20-H), 8.84 (13-H), 8.18 (3^1-H_x), 6.38 (3^2-H_B), 6.15 (3^2-H_A), 4.59 (17-H), 4.54 (18-H), 3.98 (15-CH_3), 3.88 (8-CH_2), 3.63 and 3.58 (12-CH_3 and 17^3-OCH_3), 3.53 (2-CH_3), 3.36 (7-CH_3), 1.83 (18-CH_3), 1.80 ppm (8^1-CH_3). MS (70 eV, 180°): *m/e* 522 (M^+), 435 ([M-87]$^+$). IR: ν_{max}^{KBr} 2950/2910/2840 (CH), 1725 (ester-CO), 1590 cm^{-1} (chlorin band). UV: λ_{max}^{Dioxan} (rel. int.) 658 (30), 602 (3), 527 (3), 500 (9), 399 nm (100). Chlorin-e_4 dimethyl ester (16): ^1H NMR: δ 9.67 (10-H), 9.54 (5-H), 8.74 (20-H), 8.05 (3^1-H_x), 6.33 (3^2-H_B), 6.05 (3^2-H_A), 4.49 (17-H), 4.38 (18-H), 4.31 (13^1-OCH_3), 3.83 (15-CH_3), 3.77 (8-CH_2), 3.62 (17^3-OCH_3), 3.59 (12-CH_3), 3.47 (2-CH_3), 3.29 (7-CH_3), 1.76 (18-CH_3), 1.72 ppm (8^1-CH_3). MS (70 eV, 255°): *m/e* 580 (M^+), 566 ([M-14]$^+$), 493 ([M-87]$^+$), 479 ([M-14-87]$^+$). IR: ν_{max}^{KBr} 3325 (NH), 2950/2930/2870 (CH), 1725 (ester-CO), 1595 cm^{-1} (chlorin band). UV: λ_{max}^{Dioxan} (rel. int.) 662 (31), 606 (4), 528 (4), 500 (9), 400 nm (100).

[3-Ethyl]-isochlorin-e_4 dimethyl ester (**17**). Compound 10 (600 mg) dissolved in acetone was mixed with palladium activated charcoal (350 mg) and formic acid (50 ml), with methacrylic acid methyl ester[5] (1 ml), was added. The mixture was hydrogenated with hydrogen at 1.6 atm for 15 min, diluted with acetone and filtered. The solid was washed with acetone until the filtrate was colourless. After evaporation under vacuum (the formic acid was distilled after addition of toluene) the crude product was purified by column chromatography on silica gel (eluant: hexane/acetone 3:1) to give 17 (463 mg). ^1H NMR: δ 9.74 (10-H), 9.57 (5-H), 8.78 (20-H), 8.78 (13-H), 5.38 (15-CH_2), 4.65 (17-H), 4.51 (18-H), 3.94 (3-CH_2), 3.85 (8-CH_2), 3.74 (15^2-OCH_3), 3.63 (12-CH_3), 3.62 (17^3-OCH_3), 3.42 (2-CH_3), 3.88 (7-CH_3), 1.77 (3^1-CH_3), 1.75 (8^1-CH_3), 1.74 ppm (18-CH_3). UV: λ_{max}^{Dioxan} (rel. int.) 646 (27), 593 (3), 545 (2), 495 (8), 393 nm (100).

20-Chloro-[3-ethyl]-isochlorin-e_4 dimethyl ester (**22**). H_2O_2 (30%, 0.4 ml) and conc HCl (0.5 ml) were added with stirring to 17 (130 mg) dissolved in dioxan (40 ml). The reaction was stopped after 5 min by neutralising with NaOAc soln and extracted with CH_2Cl_2. The product was worked up as above for 12. Low pressure column chromatography, on 10 μ silica-gel at a pressure of ~8 bar with hexane/acetone (5:1) as eluant, gave the starting material (60 mg) and 22 (57 mg) (see discussion in the first section). ^1H NMR: δ 9.66 (10-H), 9.62 (5-H), 8.79 (13-H), 5.34 (15-CH_2), 4.92 (18-H), 4.50 (17-H), 3.96 (3-CH_2), 3.79 (8-CH_2), 3.75 (15^2-OCH_3), 3.62 (17^3-OCH_3), 3.58 (12-CH_3), 3.58 (2-CH_3), 3.36 (7-CH_3), 1.73 (3^1-CH_3), 1.72 (8^1-CH_3), 1.58 ppm (18-CH_3). MS (70 eV, 220°): *m/e* 616 (M^+), 582 ([M-34]$^+$), 557 ([M-59]$^+$), 529 ([M-87]$^+$). UV: λ_{max}^{Dioxan} (rel. int.) 655 (28), 602 (3), 527 (4), 502 (8), 399 nm (100).

10,20-Dichloro-[3-ethyl]-isochlorin-e_4 dimethyl ester (**20**). Reaction procedure: as for 22; reactant: 17 (60 mg); reagents: dioxan (40 ml), H_2O_2 (30%, 0.4 ml) and conc HCl (0.5 ml); reaction time 7 min; products: 20 (12.3 mg) and 22 (24 mg). ^1H NMR: δ 9.52 (5-H), 8.70 (13-H), 5.23 (15-CH_2), 4.78 (18-H), 4.56 (17-H), 4.29 (8-CH_2), 3.88 (3-CH_2), 3.79 (15^2-OCH_3), 3.65 (12-CH_3), 3.62

(17^3-OCH_3), 3.49 (2-CH_3), 3.27 (7-CH_3), 1.70 (3^1-CH_3), 1.65 (8^1-CH_3), 1.63 ppm (18-CH_3). MS(70 eV, 200°): *m/e* 650(M^+), 616([M-34]$^+$), 591 ([M-59]$^+$). UV: λ_{max}^{Dioxan} (rel. int.) 658 (22), 605 (3), 539 (5), 507 (7), 402 nm (100).

Further chlorination of **17**. Reaction procedure: as for **22**; reactant: **17** (200 mg); reagents: dioxan (50 ml), H_2O_2 (30%, 0.6 ml) and conc HCl (0.8 ml); reaction time: 1 hr, product separation: after an initial purification on a silica gel column, separation on thin layer chromatography plates (0.5 mm) with an eluant of carbon tetrachloride/acetone 10:1; products: **18** (12 mg), **19** (5 mg), **20** (19 mg) and **21** (13 mg). Compound **18**: ^{1}H NMR: δ 9.63 (10-H), 9.62 (5-H), 8.94 (13-H), 7.99 (15^1-H), 4.93 (18-H), 4.81 (17-H), 3.93 (3-CH_2), 3.68 (8-CH_2), 3.56 (12-CH_3), 3.56 (17^3-OCH_3), 3.56 (2-CH_3), 3.51 (15^2-OCH_3), 3.32 (7-CH_3), 2.40 (15^1-$OCOCH_3$), 1.72 (3^1-CH_2), 1.70 (8^1-CH_3), 1.58 ppm (18-CH_3). MS (70 eV, 180°): *m/e* 674 (M^+), 616 ([M-58]$^+$). UV: λ_{max}^{Dioxan} (rel. int.) 657 (28), 605 (2), 527 (3), 502 (7), 400 nm (100). 15^1-Acetoxy-10,20-dichloro-[3-ethyl]-isochlorin-e_4 dimethyl ester (**19**): ^{1}H NMR: δ 9.29 (5-H), 8.74 (13-H), 7.68 (15^1-H), 4.80 (18-H), 4.51 (17-H), 4.27 (8-CH_2), 3.92 (3-CH_2), 3.68 (17^3-OCH_3), 3.50 (15^2-OCH_3), 3.41 (12-CH_3), 3.36 (2-CH_3), 3.28 (7-CH_3), 2.39 (15^1-$OCOCH_3$), 1.62 (3^1-CH_3), 1.59 (8^1-CH_3), 1.57 ppm (18-CH_3). MS (70 eV, 210°): *m/e* 708(M^+), 674([M-34]$^+$), 650[[M-58]$^+$], 616([M-34-58]$^+$), 582 ([M-34-34-58]$^+$). UV: λ_{max}^{Dioxan} (rel. int.) 661 (25), 608 (4), 546 (5), 506 (6), 406 nm (100). 15^1 - Acetoxy - 5,10,13,20 - tetrachloro - [3 - ethyl] - isochlorin - e_4 dimethyl ester (**21**): ^{1}H NMR: δ 8.32 (15^1-H), 4.90 (3-CH_2), 4.60 (18-H), 4.24 (8-CH_2), 3.98 (17-H), 3.74 (15^2-OCH_3), 3.64 (12-CH_3), 3.64 (17^3-OCH_3), 3.43 (2-CH_3), 3.22 (7-CH_3), 3.02 (15^1-$OCOCH_3$), 1.58 (3^1-CH_3), 1.51 (8^1-CH_3), 1.41 ppm (18-CH_3). MS (70 eV, 240°): *m/e* M^+ not detected, 742 ([M-34]$^+$), 708 [M-34-34]$^+$), 674 ([M-34-34-34]$^+$), 650 ([M-34-34-58]$^+$), 640 ([M-34-34-34-34]$^+$), 616 ([M-34-34-34-58]$^+$), 582 ([M-34-34-34-34-58]$^+$).

Phaeophorbide-a methyl ester (**24**) *and* [*3-ethyl*]-*phaeophorbide-a methyl ester* (**25**). These were prepared by the ring closure reaction[9,5] on **2**, and **3**, respectively, by the method described above for **14** from **5**. Their physical properties were identical with those in the literature.[24]

Acknowledgements—We would like to thank the following for their technical assistance: B. Eckert and D. Kapalla (NMR), D. Döring, H. Steinert and Dr. L. Grotjahn (MS), G. Hoffmann (IR) and E. Hoffmann and Dr. T. Kemmer (low-pressure chromatography).

REFERENCES

[1]H. Brockmann, Jr., U. Jürgens and M. Thomas, *Tetrahedron Letters* in press.
[2]U. Jürgens and H. Brockmann, Jr., *Liebigs Ann.* in press.
[3]A. Gloe and N. Risch, *Arch. Microbiol.* **118**, 153 (1978).
[4]U. Jürgens, L. Runte and H. Brockmann, Jr., *Liebigs Ann.* in press.
[5]U. Jürgens, Dissertation, Technical University of Braunschweig, West Germany (1978).
[6]A. W. Nicol, *J. Chem. Soc.* (C), 903 (1970).
[7]J. A. Pople and T. Schaefer, *Mol. Phys.* **3**, 547 (1960).
[8]G. Jeckel, Dissertation, Technical University of Braunschweig, West Germany (1967).
[9]H. Fischer and W. Lautsch, *Liebigs Ann.* **528**, 265 (1937).
[10]N. Risch, T. Kemmer and H. Brockmann, Jr., *Ibid.* 585 (1978).
[11]H. Voigt, Dissertation, Technical University of Braunschweig, West Germany (1964).
[12]S. G. Boxer, G. L. Closs and J. J. Katz, *J. Am. Chem. Soc.* **96**, 7058 (1974).
[13]K. M. Smith and J. F. Unsworth, *Tetrahedron* **31**, 367 (1975).
[14]D. N. Lincoln, V. Wray, H. Brockmann, Jr. and W. Trowitzsch, *J. Chem. Soc.* Perkin II, 1920 (1974).
[15]J. B. Stothers, *Carbon-13 NMR Spectroscopy*. Academic Press, London (1972).
[16]C. B. Storm and Y. Teklu, *J. Am. Chem. Soc.* **94**, 1745 (1972); C. B. Storm, Y. Teklu and E. A. Sokolski, *Ann. N.Y. Acad. Sci.* **206**, 631 (1973); H. Scheer and J. J. Katz, *Porphyrins and Metalloporphyrins* (Edited by K. M. Smith), p. 501. Elsevier, Amsterdam (1975).
[17]G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas and H. H. Stain, *J. Am. Chem. Soc.* **85**, 3809 (1963).
[18]A. Gossauer, B. Grüning, L. Ernst, W. Becker and W. S. Sheldrick, *Angew. Chem.* Internat. Edn. **16**, 481 (1977); F. W. Wehrli and T. Wirthlin *Interpretation of Carbon-13 NMR Spectra*, Heyden, London (1976).
[19]C. Weiss, H. Kobayashi and M. Gouterman, *J. Mol. Spectrosc.* **16**, 415 (1965).
[20]M. S. Fischer, D. H. Templeton, A. Zalkin and M. Calvin, *J. Am. Chem. Soc.* **94**, 3613 (1972).
[21]R. B. Woodward and V. Skaric, *Ibid.*, **83**, 4676 (1961).
[22]A. Stoll and E. Wiedemann, *Fortscher. chem. Forsch.* Vol. 2, No. 3, 538 (1952).
[23]H. R. Wetherell, M. J. Hendrickson and A. R. McIntyre, *J. Am. Chem. Soc.* **81**, 4517 (1959).
[24]G. W. Kenner, S. W. McCombie and K. M. Smith, *J. Chem. Soc.* Perkin I, 2517, (1973) and 527 (1974).