98. Synthesis of Chlorophyll *a* Labeled at C(3^2) from Pheophorbide *a* Methyl Ester

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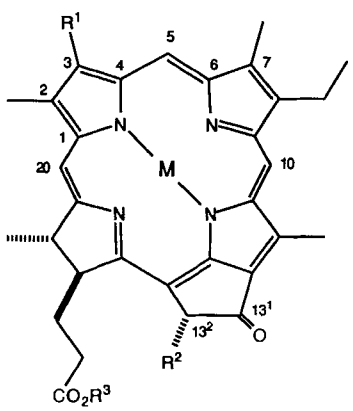
[3^2-^{14}C]Chlorophyll *a* (**10b**) was synthesized from pheophorbide *a* methyl ester (**5a**) in a seven-step partial synthesis. The key intermediate pheophorbide *d* methyl ester (**6**) was obtained by ozonolysis of the vinyl group of **5a** in 91% yield. Selective reduction of the CHO group of **6** gave the corresponding alcohol **7**, and conversion of the latter to the phosphonium bromide **8** yielded after *Wittig* reaction with [^{14}C]paraformaldehyde, [3^2-^{14}C]pheophorbide *a* methyl ester (**5b**). The final transformation to the title compound was achieved by acid hydrolysis of **5b**, esterification with natural phytol to [3^2-^{14}C]pheophytin *a* (**9b**), and eventual insertion of a Mg^{2+} ion.

1. Introduction. – Different endogenous chlorophyll *a* (**10a**) catabolites which are excreted by some photoautotrophic microorganisms into the culture medium, under particular growing conditions, were recently isolated and characterized as 19-formyl-1-oxobilin derivatives in our laboratory [1]. However, the fate of these primary products of chlorophyll breakdown in living organisms, which lack the faculty of getting rid of them, is still an enigma. Obviously, the study of the enzymatic degradation of chlorophyll would be facilitated by the availability of derivatives labeled with isotopes which could be used as substrates of both *in vivo* and *in vitro* transformations.

Though isotopic labels, which are not exchangeable under physiological conditions, can be introduced in chlorophyll derivatives by different procedures already reported in the literature, none of them proved to be suitable for our purpose. Thus, the (acidic) H-atoms of the Me(12^1) group can be exchanged by ^{2}H-atoms [2] and ^{18}O can by incorporated in the CO(13^1) group by enzymatic methods [3]. However, whereas the former



	R^1	R^2	R^3	M
1a, b[a])	$CH=CH_2$	H	Me	2 H
2	CHO	H	Me	2 H
3	CH_2OH	H	Me	2 H
4	$[CH_2P(Ph)_3]^+Br^-$	H	Me	2 H
5a, b[a])	$CH=CH_2$	CO_2Me	Me	2 H
6	CHO	CO_2Me	Me	2 H
7	CH_2OH	CO_2Me	Me	2 H
8	$[CH_2P(Ph)_3]^+Br^-$	CO_2Me	Me	2 H
9a, b[a])	$CH=CH_2$	CO_2Me	phytyl	2 H
10a, b[a])	$CH=CH_2$	CO_2Me	phytyl	Mg

[a]) [3^2-^{14}C]-enriched.

procedure is limited to labeling with H-isotopes, the latter is handicapped by the inaccuracy of the analytical methods available for the detection of the ^{18}O-isotope. On the other hand, the most commonly used enzymatic incorporation of 5-aminolaevulinic acid enables certainly to introduce isotopic labels in chlorophyll *a* *in situ* [4], but it is neither appropriated for large-scale preparations nor for specific labeling at predetermined positions [5].

The present work deals with a seven-step high-yield partial synthesis of chlorophyll *a* selectively enriched at C(3^2) with the ^{14}C-isotope. Obviously, the method is also applicable to the synthesis of the corresponding ^{13}C-labeled derivative. The starting material is pheophorbide *a* methyl ester (**5a**)[1]) which can be conveniently obtained from the commercially available spray-dried cyanobacterium *Spirulina geitlerii* [6] [7].

2. Results and Discussion. – As chlorophyll derivatives bearing a COOMe group at C(13^2) are prone to oxidation at this position (so-called allomerization reaction [8a]), the reaction sequence used for the synthesis of isotopically enriched chlorophyll *a* was previously elaborated with pyropheophorbide *a* methyl ester [6] (**1a**)[1]), which is considerably less sensitive to O_2. Thus, following the procedure previously described for the transformation of protoporphyrin IX dimethyl ester into 3,8-diformyldeuteroporphyrin IX dimethyl ester [9], ozonolytic cleavage of the vinyl group of **1a** led to pyropheophorbide *d* methyl ester (**2**) in 92% yield.

Attempts to reconstruct the vinyl group of **1a** by *Wittig* reaction of aldehyde **2** with an excess of triphenylphosphonium methylide according to the procedure described for the synthesis of 7-demethyl-7-vinylchlorophyll *a* from chlorophyll *b* [10] afforded the desired pyropheophorbide *a* methyl ester in only 16% yield, along with several uncharacterized by-products. Under similar conditions, the Zn complex of **2** gave (pyropheophorbidato *a* methyl ester)zinc(II) in 32% yield.

Better results were obtained, however, using the reverse strategy for the formation of the vinyl C=C bond. Thus, reconstruction of the vinyl group of **1a** was achieved in three steps: *i*) reduction of the aldehyde group of **2** with tetrabutylammonium triacetoxyborhydride [11], a reagent which did not affect the carbonyl group at C(13^1), *ii*) transformation of the resulting primary alcohol **3** into the corresponding phosphonium salt **4** by reaction with PPh_3/CBr_4 [12], and *iii*) *Wittig* reaction of **4** with paraformaldehyde at 90° in the presence of 1,2-epoxypropane as base [13]. The overall yield of the whole reaction cycle amounted to 65%. A similar series of transformations was used by *Wasielewski et al.* [14] for the synthesis of a pyrochlorophyllide *a* dimer.

By the same procedure, the labeled [3^2-^{14}C]pheophorbide *a* methyl ester (**5b**) was obtained *via* **6–8** from **5a** in 36% overall yield using ^{14}C-enriched paraformaldehyde. Some loss of material was due to de(methoxycarbonylation) of **5b** in the last step of the reaction sequence, which led to **1b** as by-product. Moreover, as during the reaction cycle, epimerization at C(13^2) took place to some extent (*cf.* [8b]), the obtained product **5b** consisted of a mixture of both epimers in a ratio of 85:15, which were easily determined by NMR spectroscopy. After crystallization, the (13^2R)-isomer was usually enriched, depending on the solvent used.

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[1]) As the first step of the synthesis is carried out in $MeOH/CHCl_3$ which contains H_2SO_4, esterification takes place during the reaction so that the parent carboxylic acid can be used instead of the methyl ester.

Both intermediates **3** and **6** are potential precursors of naturally occurring tetrapyrrolic pigments. Thus, the Ni complex of **3** was isolated, as the free propanoic acid (Tunichlorin), from the Caribbean tunicate *Trididemnum solidum* [15], and aldehyde **6** is the chromophore of chlorophyll *d*, an accessory pigment of some *Rhodophyceae* [16].

After acid-catalyzed hydrolysis of the methyl propanoate group of **5b**, the propanionic acid chain was esterified with natural (7*R*,11*R*,*E*)-phytol in the presence of [(1*H*-benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (BOP) [17] to yield [3^2-^{14}C]pheophytin *a* (**9b**). The required phytol was obtained by saponification of crude chlorophyll extracts from *Spirulina* according to *Willstätter*'s procedure [18]. Magnesium insertion into **9b** using [2,6-di(*tert*-butyl)-4-methylphenoxy]magnesium iodide [19] afforded [3^2-^{14}C]chlorophyll *a* (**10b**) in 61% overall yield from **5b**.

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Experimental Part

General. All commercially available chemicals were reagent grade, solvents for chemical reactions and chromatography were generally dried and distilled prior use. Spray-dried *Spirulina geitlerii* was purchased from Dr. *W. Behr*, D–53225 Bonn, and [^{14}C]paraformaldehyde (1 mCi, 490 mCi/g) from *ICN Radiochemicals*. Ozone generator: model *502* from *Fischer Labor und Verfahrenstechnik AG*. TLC Monitoring: *E. Merck* silica gel *60* (0.2 mm) precoated aluminium foils. Prep. TLC: silica gel *60* $PF_{254+366}$ plates (1.25 mm thick, 20 × 20 cm) from *E. Merck*. Scintillation counter: *Beckmann LS 1800*. M.p.'s: *Kofler* hot-stage apparatus from *Thermovar, C. Reichert AG*, Vienna; uncorrected. UV/VIS (CH_2Cl_2): *Hewlett-Packard-8452A* diode-array spectrophotometer; λ_{max} in nm and ε, in $l \cdot mol^{-1} \cdot cm^{-1}$, in parentheses. IR (KBr): *Mattson-5000*. FT-IR Spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H-NMR ($CDCl_3$): *Bruker-AM-360* equipped with a data system *Aspect 3000*; chemical shifts δ in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz; assignments, if necessary, confirmed by $^1H\{^1H\}$-NOE difference correlations. FAB-MS: *Vacuum Generator Micromass 7070 E* equipped with a data system *DS 11-250* from *VG Micromass Ltd.*, Manchester, UK; 6 kV acceleration voltage under Xe bombardment and NBA (3-nitrobenzyl alcohol) as matrix; *m/z* (rel. peak intensity).

Pyropheophorbide a *Methyl Ester* (= *Methyl (3S,4S)-9-Ethenyl-14-ethyl-4,8,13,18-tetramethyl-20-oxophorbine-3-propanoate*; **1a**). Air was displaced by Ar from a suspension of phosphonium salt **4** (59.7 mg, 68 μmol) and paraformaldehyde (2.05 mg, 68 μmol) in THF (5 ml), contained in a sealable *Pyrex* glass tube. Then the tube was immersed in an ultrasonic bath for 5 min, 1,2-epoxypropane (0.1 ml) added, and the mixture heated at 90° for 3 h. After cooling to r.t., the solvent was evaporated and the remaining residue submitted to prep. TLC (hexane/acetone 4:3): 24.3 mg (65%) of **1a** (R_f 0.67). ^{1}H-NMR: identical with reported data [20].

Pyropheophorbide d *Methyl Ester* (= *3-Devinyl-3-formylpyropheophorbide* a *Methyl Ester* = *Methyl (3S,4S)-14-Ethyl-9-formyl-4,8,13,18-tetramethyl-20-oxophorbine-3-propanoate*; **2**). A stirred soln. of **1a** (1.037 g, 1.89 mmol) in $CHCl_3$/MeOH 1:1 (60 ml) containing conc. H_2SO_4 (2.1 ml) was chilled to −70° before a stream of O_3/O_2 (0.34 mmol/l O_3) was bubbled through a sintered glass disc (*G4*) with a flow rate of 35 l/h. After 13 min, Me_2S (590 μl, 8 mmol) was added and stirring continued in the dark for 12 h at r.t. Thereafter, the mixture was diluted with $CHCl_3$ (150 ml) and shaken successively with H_2O and 6% aq. HCl soln. (3 × 60 ml; hydrolysis of the dimethyl acetal at C(3^1) formed during ozonolysis). Finally, the org. layer was washed with H_2O, dried (Na_2SO_4), and evaporated and the residue crystallized from CH_2Cl_2/hexane: 957 mg (92%) of **2**. Spectroscopic data: identical with those reported in [21].

3-Devinyl-3-(hydroxymethyl)pyropheophorbide a *Methyl Ester* (= *Methyl (3S,4S)-14-Ethyl-9-(hydroxymethyl)-4,8,13,18-tetramethyl-20-oxophorbine-3-propanoate*; **3**). To a stirred soln. of tetrabutylammonium borohydride (512 mg, 2.0 mmol) in dry CH_2Cl_2 (10 ml), AcOH (0.34 ml, 6.0 mmol) was slowly added under Ar. Stirring was continued for 1 h at r.t. before the mixture was cooled to 4° and added to an ice-cooled soln. of **2** (220 mg, 0.4 mmol) in CH_2Cl_2 (10 ml). After 3 h at 4°, the mixture was poured into 5% (*v/v*) aq. AcOH (60 ml) and shaken with $CHCl_3$. The org. layer was washed twice with H_2O, dried (Na_2SO_4), and evaporated. Purification of the residue by prep. TLC (hexane/acetone 4:3) yielded the product (R_f 0.39), which was dissolved in CH_2Cl_2,

precipitated by addition of hexane, and finally recrystallized from acetone: 181 mg (82%) of **3**. M.p. 234–235°. Spectroscopic data: in agreement with those reported in [15].

{3-Devinyl-3-[(triphenylphosphonio)methyl]pyropheophorbide a *Methyl Ester} Bromide* (= *{Methyl (3S,4S)-14-Ethyl-4,8,13,18-tetramethyl-20-oxo-9-[(triphenylphosphonio)methyl]phorbine-3-propanoate} Bromide*; **4**). CBr_4 (230 mg, 0.69 mmol) and PPh_3 (217 mg, 0.83 mmol) were added under Ar in the dark to a stirred soln. of **3** (170 mg, 0.308 mmol) in dry THF (40 ml). After 2 d at r.t., anal. TLC (acetone/hexane 1:1) showed complete transformation of **3** to the corresponding bromide (R_f 0.54). More PPh_3 (408 mg, 1.23 mmol) was added at once and the mixture refluxed for 4 h. After evaporation the residue was purified by prep. TLC (CH_2Cl_2/MeOH 9:1) to afford the product (R_f 0.65) which was recrystallized from CH_2Cl_2/hexane: 256 mg (95%) of **4**. M.p. > 300°. ^{1}H-NMR ($5.2 \cdot 10^{-3}$ M): 9.32 (*s*, CH(10)); 8.86 (*s*, CH(20)); 8.25 (*s*, CH(5)); 7.79–7.71 (*m*, 6 H_o of Ph); 7.67–7.62 (*m*, 3 H_p of Ph); 7.47–7.41 (*m*, 6 H_m of Ph); 6.81, 6.64 (*ABX*, J = 15.3, 14,5, $CH_2(3^1)$); 5.26, 5.11 (*AB*, J = 19.9, $CH_2(13^2)$); 4.36 (*qd*, J = 7.3, 2.0, CH(18)); 4.27–4.23 (*m*, CH(17)); 3.65 (*s*, MeO); 3.61 (*s*, $Me(12^1)$); 3.48 (*q*, J = 7.6, $CH_2(8^1)$); 2.92 (*s*, $Me(7^1)$); 2.71 (*d*, J = 2.7, $Me(2^1)$); 2.70–2.53 (*m*, $CH_2(17^1)$); 2.38–2.16 (*m*, $CH_2(17^2)$); 1.72 (*d*, J = 7.3, $Me(18^1)$); 1.56 (*t*, J = 7.6, $Me(8^2)$); −1.43, −3.24 (2 br. *s*, 2 NH). FAB-MS: 797 (27, $[M - Br]^+$), 535 (100).

[3²-^{14}C]Pheophorbide a *Methyl Ester* (= *Methyl (3S,4S,21R)-9-[2-^{14}C]Ethenyl-14-ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxophorbine-3-propanoate*; **5b**). Following the procedure described for **1a**, **5b** (20.6 mg, 50%; 14.7 mCi/mmol) and **1b** (3.3 mg, 9%; 14.7 mCi/mmol) were obtained from the reaction of **8** (64 mg, 68 μmol) with [^{14}C]paraformaldehyde (2.05 mg; 1 mCi). Both **5b** and **1b** (R_f 0.62 and 0.67, resp.) were separated by prep. TLC (hexane/acetone 4:3). ^{1}H-NMR of **5b**: identical with data given in [7].

Pheophorbide d *Methyl Ester* (= *3-Devinyl-3-formylpheophorbide* a *Methyl Ester* = *Methyl (3S,4S,21R)-14-Ethyl-9-formyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxophorbine-3-propanoate*; **6**). A soln. of **5a** (349 mg, 0.575 mmol) in $CHCl_3$/MeOH 1:1 (30 ml) containing conc. H_2SO_4 (1.05 ml) was ozonolyzed for 8 min as described for **2**. The product was recrystallized from CH_2Cl_2/hexane: 318 mg (91%) of **6**. M.p. > 256° (dec.). UV/VIS (CH_2Cl_2): 696 (56100), 634 (6700), 580 (sh), 552 (12200), 520 (10800), 484 (sh), 426 (70000), 386 (64300), 332 (sh), 310 (22200). IR: 3379*w*, 2957*m*, 2927*m*, 2867*m*, 1737*s*, 1700*s*, 1677*s*, 1618*m*. ^{1}H-NMR ($6.5 \cdot 10^{-3}$ M): 11.54 (*s*, CHO); 10.33 (*s*, CH(5)); 9.65 (*s*, CH(10)); 8.85 (*s*, CH(20)); 6.33 (*s*, $CH(13^2)$); 4.54 (*qd*, J = 7.3, 1.8, CH(18)); 4.31–4.27 (*m*, CH(17)); 3.90 (*s*, $Me(13^5)O$); 3.78 (*s*, $Me(2^1)$); 3.77 (*q*, J = 7.6, $CH_2(8^1)$); 3.75 (*s*, $Me(12^1)$); 3.57 (*s*, $Me(17^5)O$); 3.33 (*s*, $Me(7^1)$); 2.71–2.51 (*m*, $CH_2(17^1)$); 2.36–2.17 (*m*, $CH_2(17^2)$); 1.85 (*d*, J = 7.3, $Me(18^1)$); 1.73 (*t*, J = 7.6, $Me(8^2)$); −0.06, −1.98 (2 br. *s*, 2 NH). FAB-MS: 608 (95, M^+), 609 (100).

3-Devinyl-3-(hydroxymethyl)pheophorbide a *Methyl Ester* (= *Methyl (3S,4S,21R)-14-Ethyl-9-(hydroxymethyl)-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxophorbine-3-propanoate*; **7**). As described for **3**, from **6** (300 mg, 0.49 mmol). Recrystallization from acetone yielded 265 mg (88%) of **7**. M.p. > 235° (dec.). UV/VIS (CH_2Cl_2): 664 (42500), 604 (8300), 556 (sh), 534 (9600), 504 (10200), 470 (sh), 410 (99200), 376 (59000), 332 (21900). IR (KBr): 3452*m*, 3398*w*, 2955*m*, 2925*m*, 2869*m*, 1738*s*, 1679*s*, 1618*s*. ^{1}H-NMR ($5.7 \cdot 10^{-3}$ M): 9.45 (*s*, CH(10)); 9.39 (*s*, CH(5)); 8.54 (*s*, CH(20)); 6.21 (*s*, $CH(13^2)$); 5.85 (*s*, $CH_2(3^1)$); 4.44 (*qd*, J = 7.3, 2.0, CH(18)); 4.20–4.16 (*m*, CH(17)); 3.87 (*s*, $Me(13^5)O$); 3.64 (*s*, $Me(12^1)$); 3.63 (*q*, J = 7.6, $CH_2(8^1)$); 3.58 (*s*, $Me(17^5)O$); 3.38 (*s*, $Me(2^1)$); 3.21 (*s*, $Me(7^1)$); 2.65–2.48 (*m*, $CH_2(17^1)$); 2.32–2.16 (*m*, $CH_2(17^2)$); 1.79 (*d*, J = 7.3, $Me(18^1)$); 1.67 (*t*, J = 7.6, $Me(8^2)$); 0.32, −1.76 (2 br. *s*, 2 NH). FAB-MS: 610 (100, M^+), 611 (87).

{3-Devinyl-3-[(triphenylphosphonio)methyl]pheophorbide a *Methyl Ester} Bromide* (= *{Methyl (3S,4S,21R)-14-Ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-9-[(triphenylphosphonio)methyl]phorbine-3-propanoate} Bromide*; **8**). As described for **4**, from **7** (250 mg, 0.409 mmol). The product (R_f 0.62) was recrystallized from CH_2Cl_2/hexane: 344 mg (90%) of **8**. M.p. > 200° (dec.). UV/VIS (CH_2Cl_2): 674 (46300), 616 (6300), 564 (sh), 536 (7700), 508 (9400), 470 (sh), 412 (79800), 380 (51700), 328 (16600). IR (KBr): 3390*w*, 3056*w*, 2955*m*, 2925*m*, 2867*m*, 1736*s*, 1699*s*, 1618*m*. ^{1}H-NMR ($4.3 \cdot 10^{-3}$ M): 9.32 (*s*, CH(10)); 8.90 (*s*, CH(20)); 8.27 (*s*, CH(5)); 7.79–7.71 (*m*, 6 H_o of Ph); 7.67–7.62 (*m*, 3 H_p of Ph); 7.47–7.41 (*m*, 6 H_m of Ph); 6.81, 6.61 (*ABX*, J = 15.3, 14.5, $CH_2(3^1)$); 6.27 (*s*, $CH(13^2)$); 4.35 (*qd*, J = 7.3, 2.0, CH(18)); 4.19–4.15 (*m*, CH(17)); 3.90 (*s*, $Me(13^5)O$); 3.62 (*s*, $Me(17^5)O$); 3.61 (*s*, $Me(12^1)$); 3.40 (*q*, J = 7.6, $CH_2(8^1)$); 2.90 (*s*, $Me(7^1)$); 2.68 (*d*, J = 3.0, $Me(2^1)$); 2.72–2.51 (*m*, $CH_2(17^1)$); 2.33–2.21 (*m*, $CH_2(17^2)$); 1.73 (*d*, J = 7.3, $Me(18^1)$); 1.53 (*t*, J = 7.6, $Me(8^2)$); −0.06, −3.62 (2 br. *s*, 2 NH). FAB-MS: 855 (32, $[M - Br]^+$), 593 (100).

[3²-^{14}C]Pheophytin a (= *(7R,11R,E)-3,7,11,15-Tetramethylhexadec-2-en-1-yl (3S,4S,21R)-9-[2-^{14}C]-Ethenyl-14-ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxophorbine-3-propanoate*; **9b**). A soln. of **5b** (20.6 mg, 34 μmol, 0.54 mCi) in 24% aq. HCl soln. (60 ml) was stirred at r.t. for 90 min. The soln. was then diluted with H_2O (100 ml) and extracted with CH_2Cl_2. The org. phase was washed with H_2O, dried (Na_2SO_4), and evaporated. To the remaining residue in CH_2Cl_2 (5 ml), [(1*H*-benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (164 mg, 0.37 mmol), Et_3N (75 mg, 0.74 mmol), and (7*R*,11*R*,*E*)-phytol (100 mg,

0.34 mmol) were added. The mixture was stirred for 24 h at r.t. and the product isolated by prep. TLC (hexane/acetone 4:3): 25.7 mg (87%) of **9b** (R_f 0.69, 14.7 mCi/mmol).

[3²-¹⁴C]Chlorophyll a (= {(7R,11R,E)-3,7,11,15-Tetramethylhexadec-2-en-1-yl (3S,4S,21R)-9-[2-¹⁴C]-Ethenyl-14-ethyl-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxophorbine-3-propanoato(2 –)-N^{23},N^{24},N^{25},N^{26}}*magnesium*; **10b**). A mixture of **9b** (25.7 mg) and unlabeled pheophytin *a* (**9a**; 25 mg) was converted to **10b** according to [19]: 36.3 mg (70%, 7.5 mCi/mmol) of **10b**.

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