Preparation of Phorbin Derivatives from Chlorophyll Mixture Utilizing the Principle of Selective Hydrolysis

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Application of our two-phase extraction method[1], followed by precipitation of chlorophyll *a* (**1a**; $R^1 = CH_3$) and *b* (**1b**; $R^1 = CHO$), permits the isolation of large amounts of relatively pure chlorophyll mixture containing ~80% of **1a** and 20% of **1b**. We have recently developed methods for the preparation of metal-free chlorophyll derivatives of the *a* and *b* series such as pheophytins, pheophorbides, and methylpheophorbides, directly from this mixture. The methods previously used for this purpose include the conventional partition between aqueous hydrochloric acid and

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Table. Preparation and Spectroscopic Properties of Phorbin Derivatives

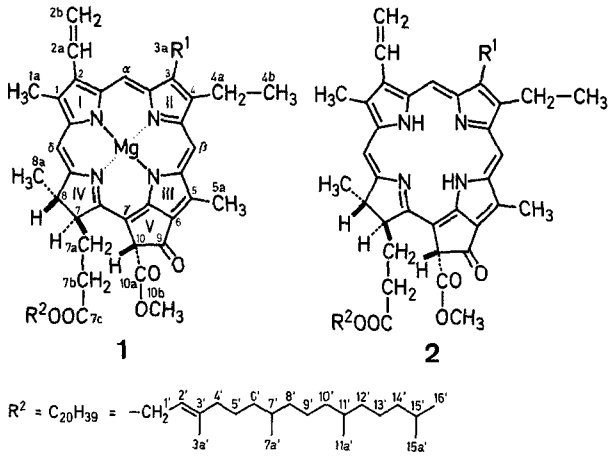
Product	R^1	R^2	Yield [%]	Molecular Formula[a] (Mol. weight)	U.V./Vis. (THF) λ_{max} [nm] ($\varepsilon \cdot 10^{-3}$)	^{1}H-N.M.R. (60 MHz, TMS_{int}) δ [ppm]
2a	CH_3	H	79	$C_{35}H_{36}N_4O_5$ (592.7)	668.0 (49.3), 609 (8.08), 558 (3.15), 535 (10.0), 506 (11.5), 471 (4.23), 411.0 (104.7), 322 (19.6), 275 (17.2)	9.60 (s, 1H, β-H); 9.29 (s, 1H, α-H); 8.86 (s, 1H, δ-H); 8.04 (dd, 1H, J=11 Hz, 18 Hz, 2a-H_X); 6.28 (dd, 1H, J=2 Hz, 18 Hz, 2b-H_B); 6.13 (dd, 1H, J=2 Hz, 11 Hz, 2b-H_A); 6.34 (s, 1H, 10-H); 4.65 (m, 1H, J=7 Hz, 8-H); 4.20 (m, 1H, J=7 Hz, 7-H); 3.88 (s, 3H, 10b-CH_3); 3.61 (s, 3H, 5a-CH_3); 3.55 (q, 2H, J=7 Hz, 4a-CH_2); 3.40 (s, 3H, 1a-CH_3); 3.07 (s, 3H, 3a-CH_3); 2.46 (m, 4H, 7a, 7b-CH_2); 1.84 (d, 3H, J=7 Hz, 8a-CH_3); 1.59 (t, 3H, J=7 Hz, 4b-CH_3); 0.904 (s, broad, 1H, NH); −1.82 (s, broad, 1H, NH)[b]
2b	CH_3	CH_3	88	$C_{36}H_{38}N_4O_5$ (606.9)	667.5 (51.0), 609 (8.26), 559 (3.07), 535 (10.5), 506 (11.9), 470 (4.31), 411.0 (106.1), 321 (19.9), 273 (15.8)	9.67 (s, 1H, β-H); 9.36 (s, 1H, α-H); 8.88 (s, 1H, δ-H); 8.10 (dd, 1H, J=11 Hz, 18 Hz, 2a-H_X); 6.32 (dd, 1H, J=2 Hz, 18 Hz, 2b-H_B); 6.16 (dd, 1H, J=2 Hz, 11 Hz, 2b-H_A); 6.32 (s, 1H, 10-H); 4.64 (m, 1H, J=7 Hz, 8-H); 4.18 (m, 1H, J=8 Hz, 7-H); 3.87 (s, 3H, 10b-CH_3); 3.63 (s, 3H, 5a-CH_3); 3.56 (q, 2H, J=7 Hz, 4a-CH_2); 3.49 (s, 3H, 7d-CH_3); 3.43 (s, 3H, 1a-CH_3); 3.13 (s, 3H, 3a-CH_3); 2.51–2.35 (m, 4H, 7a, 7b-CH_2); 1.83 (d, 3H, J=7 Hz, 8a-CH_3); 1.62 (t, 3H, J=7 Hz, 4b-CH_3)[b]
2c	CHO	CH_3	80	$C_{36}H_{36}N_4O_6$ (620.9)	654.5 (30.8), 599 (7.45), 554 (6.78), 525 (10.9), 436.0 (151.4), 414 (61.6), 370 (24.3), 325 (24.3)	10.99 (s, 1H, 3a-H); 10.79 (s, 1H, β-H); 9.16 (s, 1H, α-H); 8.23 (s, 1H, δ-H); 7.69 (dd, 1H, J=11 Hz, 18 Hz, 2a-H_X); 6.21 (dd, 1H, J=1 Hz, 18 Hz, 2b-H_B); 5.96 (dd, 1H, J=1 Hz, 11 Hz, 2b-H_A); 6.30 (s, 1H, 10-H); 4.30–3.93 (m, 2H, 7, 8-H); 3.55 (s, 3H, 10b-CH_3); 3.35 (s, 3H, 7d-CH_3); 3.26 (s, 3H, 5a-CH_3); 2.95 (s, 3H, 1a-CH_3); ~2.31 (m, 4H, 7a, 7b-CH_2); 1.56 (d, 3H, J=7 Hz, 8a-CH_3); 1.42 (t, 3H, J=8 Hz, 4b-CH_3)[c]
2d	CHO	$C_{20}H_{39}$	100	$C_{55}H_{72}N_4O_6$ (885.2)	654.0 (43.9), 599 (10.4), 554 (9.65), 525 (15.6), 435.0 (215.9), 414 (87.8), 369 (34.6), 325 (34.6)	10.41 (s, 1H, 3a-H); 9.39 (s, 1H, β-H); 9.02 (s, 1H, α-H); 8.73 (s, 1H, δ-H); 7.68 (dd, 1H, J=11 Hz, 18 Hz, 2a-H_X); 6.19 (dd, 1H, J=2 Hz, 18 Hz, 2b-H_B); 6.08 (dd, 1H, J=2 Hz, 11 Hz, 2b-H_A); 6.30 (s, 1H, 10-H); 5.06 (t, 1H, J=7 Hz, 2′-H); 4.70–4.21 (m, 4H, 8-H, 1′-CH_2); 3.96 (s, 3H, 10b-CH_3); 3.45 (s, 3H, 5a-CH_3); 3.29 (s, 3H, 1a-CH_3); ~3.19 (q, 2H, 4a-CH_2); 2.58–2.44 (m, 4H, 7a, 7b-CH_2); 1.92 (d, 3H, J=7 Hz, 8a-CH_3); 1.87 (t, 3H, J=7 Hz, 4b-CH_3); 1.54 (s, 3H, 3a′-CH_3); 1.09 (s, broad, 19H, 4′–15′-$CH_{(2)}$); 0.83, 0.74 (s, s, 12H, 7a′–16′-CH_3)[b]

[a] The purity was checked by T.L.C. and by comparing the spectroscopic values of the Table with those given in literature[5-9, 12-14].
[b] In acetone-d_6.
[c] In C_6D_6.

diethyl ether[2,3] and liquid chromatography (L.C.) on alumina[4,5] or silica gel[6-9]. The hydrochloric acid – diethyl ether fractionation is based on different "hydrochloric acid numbers" (n_{HCl}) of the derivatives. The Δn_{HCl} of the *a* and *b* series derivatives is not, however, large enough to afford complete separation of the derivatives. L.C. yields quite easily pure *a* series derivative, but the purification of the corresponding *b* derivative usually requires another chromatographic separation and/or a chemical reaction specific of the formyl group. Kenner et al.[5] utilized the reaction of the *b* derivative with Girard's reagent 'T'[10] prior to chromatography. Isenring[9] used dimethyl acetal formation of the formyl group for purification of the *b* derivative.

The chromatographic methods are rather time-consuming and require large amounts of organic solvents. We have investigated the utilization of the difference in the hydrolyzability of the phytyl esters (**2e**; $R^1=CH_3$, $R^2=C_{20}H_{39}$; **2d**; $R^1=CHO$, $R^2=C_{20}H_{39}$) for the separation of the *a* and *b* series derivatives and observed that pheophytin *a* (**2e**) is completely hydrolyzed to pheophorbide *a* (**2a**; $R^1=CH_3$, $R^2=H$) in 1 h at room temperature when 30% (w/w) aqueous hydrochloric acid/diethyl ether is used as a solvent system whereas pheophytin *b* (**2d**) remains unaffected. As **2a** posesses an n_{HCl} value of 15, it goes almost completely into the acid phase while **2d** ($n_{HCl}=35$) stays completely in the ether phase. Based on their different hydrolyzability, the derivatives of the *a* and *b* series may thus be easily separated in a separatory funnel. Chlorophyll or pheophytin mixture can be used as a starting material.

The simple hydrolysis – partition procedure yields pheophorbide *a* (**2a**) and pheophytin *b* (**2d**) both in high purity[12,13,14]. Derivative **2a** may be easily esterified with metha-



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$R^2 = C_{20}H_{39} = -CH_2$

nol in pyridine/tetrahydrofuran using methyl or ethyl carbonochloridate for carboxy-group activation[15]. According to our experience, esterification is essentially complete within 10 min at room temperature. Methylpheophorbide *a* (**2b**; $R^1 = CH_3$, $R^2 = CH_3$) is thus available by a rapid and simple procedure. The esterification of **2a** with phytol and phosgene[2] yields pheophytin *a* (**2e**). Some of the more recent "mild" esterification methods[15,16,17] might also be suitable for this purpose. Methylpheophorbide *b* (**2c**; $R^1 = CHO$, $R^2 = CH_3$) can be conveniently prepared from pheophytin *b* (**2d**) by transesterification using 5% (v/v) methanolic sulfuric acid[5].

In summary, the present rapid and simple method makes possible the preparation of metal-free chlorophyll derivatives of the *a* and *b* series directly from the chlorophyll mixture without chromatographic separations. The products are obtained in high purity and high yield. The method is also amenable to larger-scale preparations.

Pheophorbide *a* (2a; $R^1 = CH_3$, $R^2 = H$); Typical Procedure:
Chlorophyll mixture[1] (250 mg) is dissolved in cold diethyl ether (500 ml) in a separatory funnel. To this is added precooled (0 °C) 30% (w/w) hydrochloric acid (148 ml). The system is then equilibrated by shaking, and allowed to stand for 1 h at room temperature with occasional agitation. The phases are then separated and the ether phase is extracted with additional amounts of 30% hydrochloric acid until the acid extract has accepted a yellow-green colour and T.L.C. on cellulose[11] shows the presence of only pheophytin *b* (**2d**) in the red-brown ether phase. The hydrochloric acid solutions are combined and an equal volume of ether is added. The pigments are transferred from the acid to the ether phase by the addition of distilled water (~500 ml) and by neutralization with ammonia. Pheophorbide *a* (**2a**) is extracted from the ether solution with 16% (w/w) hydrochloric acid (~300 ml) and the extraction is repeated. It is then transferred from the combined acid extracts to fresh ether by the addition of distilled water (~500 ml). After the removal of residual hydrochloric acid by washing with water, the ether solution of **2a** is evaporated to dryness. The derivative can be crystallized from tetrahydrofuran/heptane; yield: 105 mg. T.L.C. on cellulose[11] shows only one spot.

Methylpheophorbide *a* (2b; $R^1 = CH_3$, $R^2 = CH_3$):
Compound **2a** (95 mg) is esterified with methanol in pyridine/tetrahydrofuran using ethyl carbonochloridate for activation[15]. After the addition of methanol (in excess), the reaction mixture is allowed to stand for 10 min. The clear solution is separated from the precipitate and evaporated to dryness. The derivative is crystallized from tetrahydrofuran/heptane; yield: 86 mg. T.L.C. analysis[11] shows no impurities.

Pheophytin *b* (2d; $R^1 = CHO$, $R^2 = C_{20}H_{39}$):
The red-brown ether solution of **2d**, obtained from the preparation of **2a**, is washed with water and evaporated to dryness. The residue is dissolved in a small amount of acetone and light petroleum (b.p. 60–80 °C; 100 ml) is added. The mixture is shaken with water whereupon compound **2d** precipitates. The suspension is allowed to stand overnight at −30 °C and the precipitate then collected by centrifugation. The product can be crystallized from tetrahydrofuran/heptane; yield: 50 mg. T.L.C. analysis[11] shows no impurities.

Methylpheophorbide *b* (2c; $R^1 = CHO$, $R^2 = CH_3$):
Compound **2d** (50 mg) is stirred with 5% (v/v) sulfuric acid/methanol[5] (100 ml) for 15 h in the dark. The resultant mixture is diluted with chloroform or dichloromethane (200 ml) and water (~500 ml). Upon agitation, compound **2c** is extracted into the organic phase which is washed with water (2 × 300 ml) and evaporated to dryness. Crystallization from tetrahydrofuran/heptane gives **2c**; yield: 28 mg. T.L.C. analysis[11] shows the presence of only one component in the product.

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