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

PASVO H. HYNNINEN*, Simo LOTJONEN

Departments of Biochemistry* and Chemistry, University of Kuopio, P.O. Box 138, SF-70101 Kuopio 10, Finland

Application of our two-phase extraction method, followed by precipitation of chlorophyll a (1a; R¹ = CH₃) and b (1b; R¹ = 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
Table. Preparation and Spectroscopic Properties of Phorbin Derivatives

<table>
<thead>
<tr>
<th>Product</th>
<th>R¹</th>
<th>R²</th>
<th>Yield [%]</th>
<th>Molecular Formula</th>
<th>U.V./Vis. (THF)</th>
<th>1H-N.M.R. (60 MHz, TMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Mol. weight)</td>
<td>λmax [nm] (ε x 10⁻³)</td>
<td>δ [ppm]</td>
</tr>
<tr>
<td>2a</td>
<td>CH₃</td>
<td>H</td>
<td>79</td>
<td>C₁₄H₁₀N₂O₄</td>
<td>668.0 (49.3), 609</td>
<td>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, 2H-α-H); 6.28 (dd, 1H, J = 11 Hz, 17 Hz, 2H-α-H); 6.13 (dd, 1H, J = 11 Hz, 17 Hz, 2H-β-H); 6.34 (s, 1H, 10-H); 4.65 (m, 1H, J = 7 Hz, 2H-β-H); 4.20 (m, 1H, J = 7 Hz, 7-H); 3.88 (s, 3H, 10b-CH₃); 3.61 (s, 3H, 5a-CH₃); 3.55 (q, 2H, J = 7 Hz, 4a-CH₂); 3.40 (m, 3H, 1a-CH₂); 3.07 (s, 3H, 3a-CH₂); 2.46 (m, 1H, 7a, 7b-CH); 1.84 (d, 3H, J = 7 Hz, 8a-CH₃); 1.59 (t, 3H, J = 7 Hz, 4b-CH₂); 0.904 (s, broad, 1H, NH); 1.82 (s, broad, 1H, NH)</td>
</tr>
<tr>
<td>2b</td>
<td>CH₃</td>
<td>CH₃</td>
<td>88</td>
<td>C₁₄H₁₀N₂O₄</td>
<td>667.5 (51.0), 609</td>
<td>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, 17 Hz, 2H-α-H); 6.32 (dd, 1H, J = 11 Hz, 17 Hz, 2H-β-H); 6.16 (dd, 1H, J = 2 Hz, 18 Hz, 2b-α-H); 6.04 (dd, 1H, J = 2 Hz, 18 Hz, 2b-β-H); 6.32 (s, 1H, 10-H); 4.64 (m, 1H, J = 7 Hz, 7-H); 4.18 (m, 1H, J = 8 Hz, 8-H); 3.87 (s, 3H, 10b-CH₃); 3.62 (s, 3H, 5a-CH₃); 3.56 (q, 2H, J = 7 Hz, 4a-CH₂); 3.49 (s, 3H, 7d-CH₃); 3.43 (s, 3H, 1a-CH₃); 3.13 (s, 3H, 3a-CH₂); 2.53–2.35 (m, 4H, 7a, 7b-CH₂); 1.53 (d, 3H, J = 7 Hz, 8a-CH₃); 1.62 (t, 3H, J = 7 Hz, 4b-CH₂)</td>
</tr>
<tr>
<td>2c</td>
<td>CHO</td>
<td>CH₃</td>
<td>90</td>
<td>C₁₄H₁₂N₂O₄</td>
<td>654.5 (30.8), 599</td>
<td>10.99 (s, 1H, 1a-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-α-H); 6.21 (dd, 1H, J = 1 Hz, 18 Hz, 2b-α-H); 5.96 (dd, 1H, J = 1 Hz, 11 Hz, 2b-β-H); 6.30 (dd, 1H, J = 10 Hz, 4a-CH₂); 3.93–3.99 (m, 2H, 7, 8-H); 3.55 (s, 3H, 10b-CH₃); 3.53 (s, 3H, 7d-CH₃); 3.26 (s, 3H, 5a-CH₃); 2.95 (s, 3H, 1a-CH₃); 2.31–2.43 (m, 4H, 7a, 7b-CH₂); 1.56 (d, 3H, J = 7 Hz, 8a-CH₃); 1.42 (t, 3H, J = 8 Hz, 4b-CH₂)</td>
</tr>
<tr>
<td>2d</td>
<td>CHO</td>
<td>C₂H₅</td>
<td>100</td>
<td>C₁₆H₂₀N₂O₄</td>
<td>654.0 (43.9), 599</td>
<td>11.01 (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-α-H); 6.19 (dd, 1H, J = 2 Hz, 18 Hz, 2b-α-H); 6.08 (dd, 1H, J = 2 Hz, 11 Hz, 2b-β-H); 6.30 (s, 1H, 10-H); 5.06 (t, 1H, J = 7 Hz, 2-H); 4.70–4.21 (m, 4H, 8-H, 1'-CH₂); 3.96 (s, 3H, 10b-CH₃); 3.45 (s, 3H, 5a-CH₃); 3.29 (s, 3H, 1a-CH₃); 2.19 (q, 2H, 4a-CH₂); 2.58–2.44 (m, 4H, 7a, 7b-CH₂); 1.92 (d, 3H, J = 7 Hz, 8a-CH₂); 1.37 (t, 3H, J = 7 Hz, 4b-CH₂); 1.54 (s, 3H, 3a-CH₂); 1.09 (s, broad, 1H, 4'-15'-CH); 0.83 (74, s, 12H, 7a, 16'-CH₃)</td>
</tr>
</tbody>
</table>

a The purity was checked by T.L.C. and by comparing the spectroscopic values of the Table with those given in literature⁶, ¹², ¹⁴.
b In acetone-δ₆.
c In C₆D₆.

The purity was checked by T.L.C. and by comparing the spectroscopic values of the Table with those given in literature⁶, ¹², ¹⁴.

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 phytol esters (2e; R¹ = CH₃, R² = C₁₃H₂₅, 2d; R¹ = CHO, R² = C₁₃H₂₅) for the separation of the a and b series derivatives and observed that phophytin a (2e) is completely hydrolyzed to phophorbide a (2a; R¹ = CH₃, R² = H) in 1 h at room temperature when 30% (w/w) aqueous hydrochloric acid/diethyl ether is used as a solvent system whereas phophytin b (2d) remains unaffected. As 2a possesses an nMCl value of 15, it goes almost completely into the acid phase while 2d (nMCl = 25) 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 phophytin mixture can be used as a starting material.

The simple hydrolysis - partition procedure yields phophorbide a (2a) and phophytin b (2d) both in high purity¹², ¹³, ¹⁴. Derivative 2a may be easily esterified with metha-
Methylphosphoribidene \( b (2 c; R^1 = \text{CHO}, R^2 = \text{CH}_3) \): Compound 2d (50 mg) is stirred with 5% (v/v) sulfuric acid/methanol (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 2e is extracted into the organic phase which is washed with water (2 × 300 ml) and evaporated to dryness. Crystallization from tetrahydrofuran/heptane yields 2c; yield: 28 mg. T.L.C. analysis\(^{11}\) shows the presence of only one component in the product.

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1. Address for correspondence.