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The Modification of Exocyclic Ketone on Methyl (Pyro)pheophorbide-a and Influence with Visible Spectra

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The methyl pheophorbide-a (MP-a) and methyl pyropheophorbide-a (MPP-a) were modified by reaction of exocyclic ketone in E-ring with nucleophilic reagent and several chlorin derivatives were synthesized. The change of the structure in E-ring served an expanding conjugation region and introduction of electron-withdrawing group, which strongly influenced the visible spectra. The Qy bands of synthesized compounds were affected by the substituents on the Qy axis (N_{21}-N_{23}).

Key words: Chlorin-based porphyrin, methyl pheophorbide- a, methyl pyropheophorbide- a, Photodynamic therapy (PDT), tumor necrosis

INTRODUCTION

Photodynamic therapy (PDT) involves the administration of a photosensitizing agent, allowing time for its accumulation in the target tissue (tumor). The sensitizer is then activated by non-thermal light of a wavelength matched to its absorption characteristics. In the presence of molecular oxygen, a photochemical interaction result in the production of highly reactive species, in particular singlet oxygen, which are toxic to the cells in which they are produced [1]. Due to a degree of preferential accumulation of the photosenstizing agent in tumor tissue, coupled with the targeting afforded by highly directional illumination, some selectivity in PDT effect can be obtained although it is seldom possible to get selective tumor necrosis when both tumor and adjacent normal tissue are exposed to the same light dose [2]. PDT has been used as an experimental clinical modality for some years and has recently been approved for clinical use in Canada, Japan, United States, The Netherlands, France etc. in specific malignant and premalignant condition. Most experience has been shown to be benefit in a range of clinical situation [3].

This first generation photosensitizer has, however, a number of limitations. It is composed of a complex and variable mixture of porphyrins, with a weak absorption band in the red region of the spectrum (630 nm) and causes cutaneous photosensitivity for up to 3 months. Considerable effort has been put into the development of new photosensitizer, with the aim of overcoming some of these disadvantages. The ideal photosensitizer should be a single compound, and should have improved tumor selectivity [4] and increased absorption in the red region for deeper tissue penetration of light and minimal skin photosensitivity to sunlight [5].

Pheophorbide- and pyropheophorbide- photosensitizers as chlorin analogues are a kind of promising new compounds because the chlorin analogues are activated with much longer red light at ~670 nm and may produce less long-term normal tissue phototoxicity than Photofrin II®. In this present study, we used methyl pheophorbide-a (MP-a) and methyl pyropheophorbide-a (MPP-a) as starting materials which were obtained from *Spirulina Maxima*, a kind of alga to produce a mixture of natural chlorin. Several chlorin derivatives were synthesized by modifying exocyclic ketone in E-ring and change of visible spectra of these compounds were discussed.

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MATERIALS AND METHODS

Instruments. Ultra-violet (UV) spectra were taken on a Varian model Cary-1 Spectrophotometer. ^{1}H-nuclear magnetic resonance spectra were recorded on a Bruker 300 MHz NMR spectrometer.

Methyl pheophorbide-a (1) from Spirulina Maxima (1) Approximately 500g of dried *Spirulina maxima* alga was slurried in 2 L of acetone and then was heated to reflux under nitrogen with mechanical stirring for 2 h. The supernatant was then filtered through Whatman filter paper on Buchner funnel and the alga was washed with an extra acetone. The extraction and filtration process was repeated 2 more times. The green filtrate was evaporated and then purified by flash chromatography on silica gel column to obtain pheophytin a which was converted to compound **1**(1.8 g) by treating with 500 mL of 5% Sulfuric acid in methanol for 12 h. mp 206°C; NMR: δ 9.52, 9.49, 8.58(each 1 H, s, meso-H), 8.03(1 H, m, 2a-H), 6.29, 6.16(each 1 H, d, 2b-H), 6.26(1 H, s, 10-H), 4.46(1 H, q, 8-H), 4.20(1 H, d, 7-H),

3.88(3 H, s, 10-OMe) 3.70(1 H, q, J = 7.6 Hz, 4a-H), 3.68(3 H, s, 7d-OMe), 3.57(3 H, s, 5-Me), 3.41(3 H, s, 1-Me), 3.25(3 H, s, 3-Me), 2.64(1 H, m, 7a-H), 2.32(1 H, m, 7a'-H), 2.52(1 H, m, 7b-H), 2.23(1H, m, 7b'-H), 1.69(3H, t, J = 7.6 Hz, 4b-Me), 1.81(3 H, d, J = 7.3 Hz, 8-Me), 0.53, -1.67(each br s, NH). Vis(CH_2CI_2) λ_{max} = 666.0 nm (relative intensity, 0.71), 610.4(0.23), 537.2(0.25), 506.6(0.27), 476.6(0.18), 411.8(1.52).

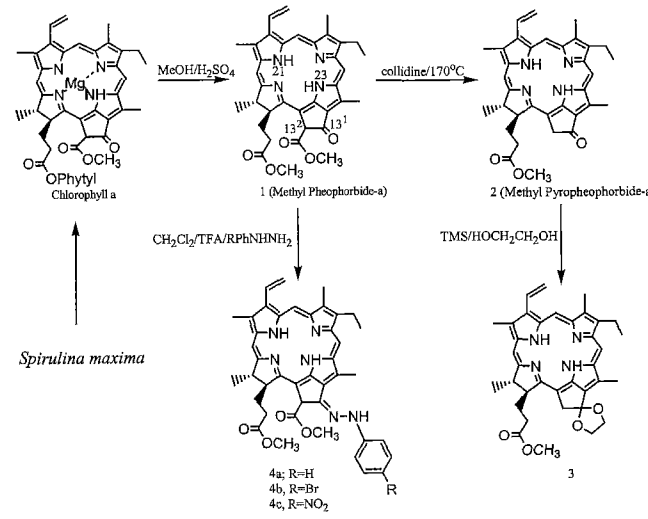
Methyl pyropheophorbide-a (2) Methyl pheophorbide- a **1** (0.821 g) was dissolved in 80 mL of collidine and stirred at reflux for 5 h under nitrogen in dark. The collidine was removed by reduced distillation. The residue was crystallized from dichloromethane / methanol to give methyl pyropheophorbide-a **2a** (0.741 g, 93%). Mp 217~219 °C; NMR: δ 9.52, 9.40, 8.56(each 1 H, s, meso-H), 8.02(1 H, m, 2a-H), 6.29, 6.28(each 1 H, d, 2b-H), 5.27, 5.11 (each 1 H, d, J = 20.0 Hz, 10-H), 4.49(1 H, m, 8-H), 4.30(1 H, m, 7-H), 3.70(2 H, q, 4a-H), 3.61(3 H, s, 7d-OMe), 3.68(3 H, s, 5-Me), 3.41(3 H, s, 1-Me), 3.25(3 H, s, 3-Me), 2.70, 2.31(each 1 H, m, 7a-H), 2.56, 2.29(each 1 H, m, 7b-H), 1.70(3 H, t, J = 7.6 Hz, 4b-Me), 1.81(3 H, d, J = 7.3 Hz, 8-Me), 0.48, -1.67(each br s, NH). Vis(CH_2CI_2) λ_{max} = 668.0 nm (relative intensity, 0.42), 610.0 (0.09), 538.2(0.10), 507.4(0.12), 413.2(1.09).

Methyl 13^1-ethylenedioxypyropheophorbide-a (3) Ethylene glycol (25 mL) and trimethylsilyl chlorde (2 mL) were added to a stirred solution of compound **2** (1.0 g) in dry dichloromethane (200 mL). The mixture was stirred at room temperature for 24 h and then poured into ice-cooled ammonia water. The organic layer was seperated, washed, dried and evaporated to dryness. The residue was chromatographed on Alumina Grade III with dichloromethane as eluent to give the title compound as bright green crystals (700 mg, 65%). mp 180-182°C; NMR: δ 9.82, 9.68, 8.89(1 H, each s, meso-H), 8.21(1 H, dd, 2a-H), 6.35, 6.18 (1 H, each dd, 2b-H), 5.12(2 H, q, 10-H), 4.80-5.50(5 H, m, OCH_2CH_2O+7-H), 4.42(1 H, m, 8-H), 3.84(2 H, q, 4a-H), 3.64, 3.60, 3.55, 3.40(3 H, each s, Me+OMe), 2.80-2.20(4 H, m, 7a, 7b-H), 1.81(3 H, d, 8-Me), 1.76(3 H, t, 4b-H), -1.22, -3.06(1 H, each br s, NH). Vis(CH_2CI_2) λ_{max} = 652.0 nm (relative intensity, 0.46), 598.1(0.09), 550.5(0.10), 500.4(0.12), 400.2(1.12).

Methyl pheophorbide-a phenylhydrazone (4) Compound **1** (40 mg) and phenylhydrzine (35 mg) were dissolved in methylenechloride (20 mL), and then several drops of TFA was added. This mixture was refluxed for 24 h and was poured into water (30 mL). The separated organic layer was evaporated, dried over anhydrous Sodium Sulfate and chromatographed on silica gel column to give the title compound in 78~80% yield. **4a** : mp 211~213°C; NMR: δ 9.74, 9.59, 8.81(1 H, each s, meso-H), 9.56(1 H, s, NH), 8.11(1 H, dd, J = 17.9, 11.3 Hz, 2a-H), 6.81 ~7.46(5 H, m, Ph-H), 6.70(1 H, s, 10-H), 6.27(1 H, dd, J = 17.9, 1.5 Hz, 2b-H), 6.11(1 H, dd, J = 11.6, 1.5 Hz, 2b'-H), 4.53 (1 H, m, 8-H), 4.18(1 H, m, 7-H), 3.76(2 H, q, J = 7.6 Hz, 4a-H), 3.71, 3.64, 3.53, 3.48, 3.30(each 3 H, s, Me+OMe), 2.28~2.65, 2.01~2.27(each 2 H, m, 7a, 7b-H), 1.80(3 H, d, J = 7.2 Hz, 8-Me), 1.76(3 H, t, J = 7.6 Hz, 4b-Me), -0.91(1 H, br, NH), -0.89(1H, br, NH); Vis(CH_2CI_2) λ_{max} = 671.2 nm (relative intensity, 0.89), 649.4(0.23), 540.0(0.12), 509.2(0.27), 403.2(1.66). **4b**: mp 234~236 °C; NMR: 9.79, 9.64, 8.86(1 H, each s, meso-H), 8.15(1 H, dd, J = 17.9, 11.5Hz, 2a-H), 7.29~7.71(5 H, m, Ph-H), 6.73(1 H, s, 10-H), 6.70(1 H, s, NH), 6.32(1 H, dd, J = 17.9, 1.5 Hz, 2b-H), 6.15(1 H, dd, J = 11.5, 1.5 Hz, 2b'-H), 4.57(1 H, m, 8-H), 4.22(1 H, m, 7-H), 3.78(2 H, q, J = 7.6 Hz, 4a-H), 3.73, 3.68, 3.56, 3.51, 3.33 (each 3 H, s, Me+OMe), 2.51~2.78, 2.11~2.23(each 2 H, m, 7a, 7b-H), 1.79(3 H, d, J = 7.2 Hz, 8-Me), 1.76(3 H, t, J = 7.6 Hz, 4b-Me), -2.85(2 H, br, NH); Vis(CH_2CI_2) λ_{max} = 681.4 nm (relative intensity, 1.06), 623.6(0.12, 567.6(0.07), 538.6(0.12), 509.6(0.30), 405.2(1.63). **4c**: mp 199~202°C; NMR: 9.74, 9.59, 8.81(1 H, each s, meso-H), 9.56(1 H, s, NH), 8.15(1 H, dd, J = 17.9, 11.5 Hz, 2a-H), 6.95~7.71(4 H, m, ph-H), 6.70(1 H, s, 10-H), 6.32(1 H, dd, J = 17.9, 1.5 Hz, 2b-H), 6.15(1 H, dd, J = 11.5, 1.5 Hz, 2b'-H), 4.57 (1 H, m, 8-H), 4.22(1 H, m, 7-H), 3.78(2 H, q, J = 7.6 Hz, 4a-H), 3.71, 3.65, 3.53, 3.48, 3.30(each 3 H, s, Me+OMe), 2.51~2.78, 2.11~2.23(each 2 H, m, 7a, 7b-H), 1.79(3 H, d, J = 7.2 Hz, 8-Me), 1.76(3 H, t, J = 7.6 Hz, 4b-Me), -2.90(2 H, br, NH); Vis(CH_2CI_2) λ_{max} = 682.4 nm (relative intensity, 1.09), 625.0(0.16), 549.2(0.11), 516.7(0.16), 414.0(1.30).

RESULTS AND DISCUSSION

In this paper, the structure of chlorin at 13^1- and 13^2-position were modified, and all compounds showed the same phyllo-type visible spectra in dichloromethane, but the peaks in λ_{max} of Qy and Soret bands are different. The Soret band and Qy band are the most intense band and the longest absorption band which were regarded as characteristics of this macrocylic conjugation. In the chlorin, Qy band in visible region is very prominent and shifts about 25 nm to longer wavelength than in porphyrins. The Soret: Qy band ratio is only about 5 (about 50 in porphyrins). The Qy band of chlorin compound were strongly affected by the substituents on the Qy axis (N_{21} - N_{23}, see Scheme 1), i.e., functional groups at 3-



Scheme 1.

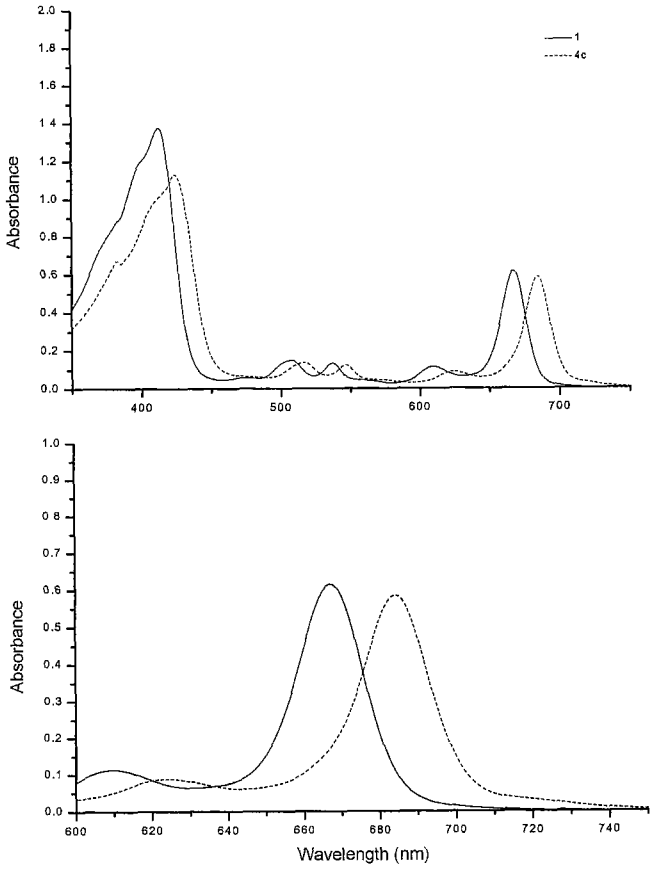


Figure 1. The Visible spectra of compound **1** and **4c** in dichloromethane, normalized at Qy peak.

and 13-position.

Methyl pheophorbide-a **1** and methyl pyropheophorbide-a **2** having the same electron-withdrawing groups at the 13-position, the Qy peak at 668 nm of **2** was red-shifted in comparison with 666 nm of **1**. This difference in visible spectra was explained by the fact that methoxycarbonyl group in **1** at 13^2-positopn is more sterically bulky than a hydrogen atom in **2** and influences the contribution of conjugation between exocyclic ketone and macrocycle. So compound **1** possesses the less conjugation of the carbon-oxygen double bond at 13^1 with the chlorin chromophore because of steric repulsion between the methoxy-cabonyl at 13^2 and exocyclic ketone group. The construction of hydrazone at 13^1-position induces red-shifted effects due to the introducing auxochrome and expanding conjugation region. The order in the Qy peaks are 671 nm in **4a** < 681 nm in **4b** < 682 nm in **4c** shown in Fig. 1 can be explained by electronic and conjugating effects of substituted groups at benzene ring. The compound **3** shows blue-shifted in visible spectra from the compound **2** because the conjugation between C = O with chlorin chromophore was deconstructed after ketone structure was changed into spiro-type.

From our result, we suggest the different substituted group on 13^1-position in MP-a or MPP-a will change visible spectra of molecule. The enlarging the conjugation system in chlorin chromophore and introducing electron-withdrawing groups will generate red-shifted in visible spectra.

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