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Short Communication

High yield preparation of purpurin-18 from *Spirulina maxima*

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ABSTRACT

Purpurin-18, a derivative of chlorophyll, is an interesting dihydroporphyrin for generating photosensitizers such as purpurinimides which absorb light in the 700–850 nm range and which display efficient anti-tumor activity. To promote extensive utilization of Pp-18 as a starting material for the preparation of new photosensitizers, a simple and rapid method for obtaining this chlorin in high yield from *Spirulina maxima* is described. Optimized experimental conditions were determined using a Latin square design of experiments.

1. Introduction

Over the last years, natural dihydroporphyrins have attracted much interest as they could be used as efficient photosensitizers (PS) for mediating photodynamic therapy [1]. Indeed, the compounds exhibit high quantum yield of singlet oxygen formation, low dark toxicity and strong absorption in the 650–700 nm, near-infrared tissue transparency window [2]. Purpurin-18 (Pp-18), a starting material for the further synthesis of more sophisticated photosensitizers (Fig. 1) has been used in numerous studies [3–5].

This particular chlorin contains three distinct functional groups, namely the vinyl group, the fused anhydride ring and the propionic acid side chain, whose modification can generate a series of photosensitizers such as purpurinimides, with varying lipophilicity, UV–vis absorption, etc. [3,6]. Pp-18 is usually obtained from chlorophyll *a* extracted from spinach leaves using a method developed in 1988 by Hoober et al. [7]. This article describes the synthesis of Pp-18 from chlorophyll *a* extracted from the cyanobacterium *Spirulina maxima*. This starting material presents two advantages over higher plants: a high chlorophyll content (~1% dry weight *vs* 0.6% in spinach leaves) and the presence of only chlorophyll *a* (*vs* *a* + *b* in plants). Optimization of Pp-18 synthesis was investigated by experimental design, taking into account four reaction parameters: temperature, reaction time, alkali concentration and water/acetone ratio.

2. Experimental

2.1. Materials

Chemicals were obtained from the following providers: sodium hydroxide (98.5%) from Acros, hydrochloric acid (0.985 M in solution in water) from Sigma Aldrich, chloroform (99.95%) from SDS, methyl (99.5%) and ethyl (99.9%) alcohols from VWR. *S. maxima* was purchased from Claudine Vallée EURL (France). White standard filter paper was purchased from FischerBrand (Ø: 17–30 μm).

Flash chromatography was realized with a Combiflash companion/Ts, Teledyne Isco. UV–Vis spectra were recorded with a Perkin Elmer Lambda 25 spectrophotometer. ^{1}H NMR spectra were realized with a Brüker DPX-400 spectrometer.

2.2. Methods

All reactions and operations were carried out with protection from direct light.

2.2.1. Pigment extraction from S. maxima

Pigments were extracted twice from *S. maxima* dry powder (5 g) with acetone (2 × 100 ml) with stirring at 60 °C (reflux) for 2 × 30 min. The dark green extract was filtered and the filtrate

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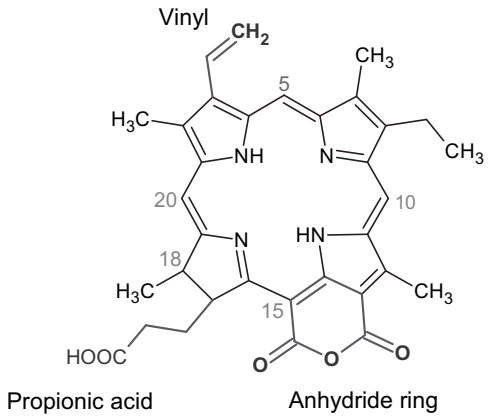

Fig. 1. Formula of Pp-18.

(200 ml) was reduced to 100 ml by partial evaporation in vacuo with a rotary evaporator.

2.2.2. Extract oxidation

20 ml of NaOH were added to the previous extract (100 ml); the mixture was vigorously stirred (1000 rpm) and oxygen was provided by air bubbling. The solution was then finally acidified by 10 ml of concentrated HCl and filtered on filter paper. Different NaOH concentrations, temperatures and reaction times have been used throughout this study.

2.3. Purification

The oxidized extract was evaporated to dryness. Carotenoids and part of xanthophylls were removed by extraction with petroleum ether (2x100 ml). The resulting residue was dissolved with minimum $CHCl_3$ and purified by flash chromatography (eluent gradient: from $CHCl_3$ to $CHCl_3$/EtOH: 8/2).

3. Results and discussion

The synthesis of Pp-18 from chlorophyll *a* is described in Fig. 2. Dioxygen oxidizes the cyclopentane ring [8] while the two ester functions are saponified by the alkali.

In order to optimize the experimental conditions and to identify the most important factors, we investigated a Latin square design of experiments. Mass yield of Pp-18 was studied as a function of temperature (25–60 °C), reaction time (1–6 h), NaOH concentration (3–9 M) and water/acetone ratio (0.1–0.4). After this screening, we then defined a new experimental field where the studied factors have the largest influence on Pp-18 mass yield. The choice of factors and their modes are given in Table 1.

Table 1
Choice of parameters.

Factors	Modes		
	1	2	3
Temperature (°C)	25	40	60
Time (h)	1	3	6
[NaOH] (mol.L^{-1})	3	6	9
Water/acetone	0.1	0.2	0.4

So, 3^4 (or 81) experiments should be done in order to explore the whole experimental field. In order to reduce the number of experiments, we built a Latin square design of experiments [9]. Hence, nine experiments were sufficient to determine optimized conditions and identify the influence of factors modes. The results have been analyzed according to Lochner and Matar (1990). Fig. 3 represents the average effect of the factors on Pp-18 mass yield. In the experimental field, Pp-18 yield did not seem to be linked to temperature and reaction times; on the contrary, influences of NaOH concentration and water/acetone ratio were found significant (0 mg of Pp-18 with [NaOH]: 3 M and water/acetone: 0.1, against 13.5 mg of Pp-18 with [NaOH]: 6 M and water/acetone: 0.2).

Bearing in mind that our aim herein is to obtain the highest mass yield, optimal conditions were found related with low temperature (25 °C), medium reaction time (3 h), medium NaOH concentration (6 M) and medium water/acetone ratio (0.2). An experiment taking into account this optimized combination of factors gave 31.5 mg of Pp-18 (0.63% of initial *spirulina* mass) (about three times more than previously observed yields). Since *S. maxima* contains about 1% of chlorophyll *a* [10], and given the molecular weights of chlorophyll *a* and Pp-18 (893.5 and 564.6 respectively), this yield corresponds to an essentially quantitative conversion of chlorophyll *a* into Pp-18.

4. Characterizations

Pp-18: CCM: Rf = 0.27 ($CHCl_3$/EtOH: 96/4); UV–Vis: ($CHCl_3$): λ_{nm} ($\varepsilon.10^{-3}$): 360 (22.9); 413 (44.6); 481 (1.9); 510 (3.9); 548 (10.9); 646 (4.7); 701 (23.2); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 9.54, 9.33, 8.55 s (1H, 5-H, 10-H, 20-H); 7.86 dd (J = 17.0, 11.4 Hz, 1H, 3^1-CH); 6.28 dd (J = 17.0, 1.0 Hz, 1H,trans-3^2-CH_2); 6.19 dd (J = 11.4, 1.0 Hz, 1H, cis-3^2-CH_2); 5.15 m (1H, 17H); 4.39 q (J = 6.0 Hz, 1H, 18-H); 3.72 s (3H, 12-CH_3); 3.59 q (J = 6.6 Hz, 2H, 8^1-CH_2); 3.32 et 3.15 s (3H, 2-CH_3 et

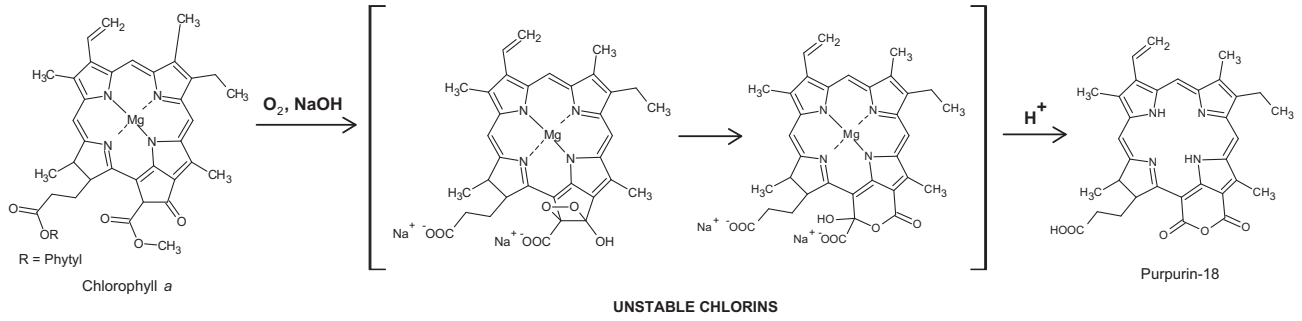

Fig. 2. Synthesis of Pp-18 from Chlorophyll.

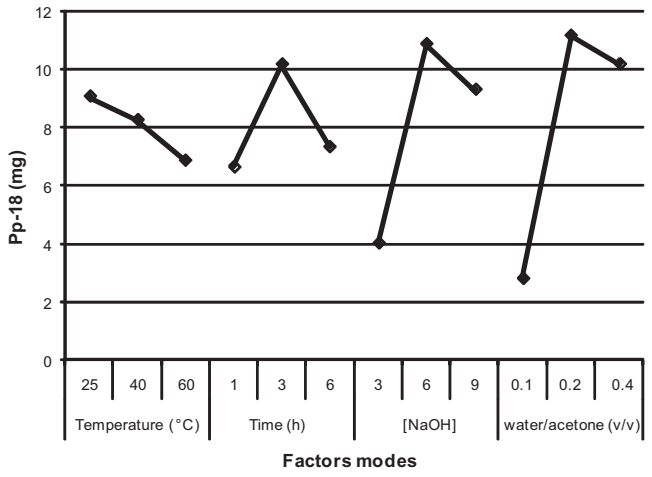

Fig. 3. Average effect of temperature, reaction time, NaOH concentration and water/acetone volume ratio on collected Pp-18.

7-CH_3); 2.70, 2.46, 2.35, 1.97 m (1H, 2×17^1-H and 2×17^2-H); 1.73 d ($J = 6.0$ Hz, 3H, 18-CH_3); 1.65 t ($J = 6.8$ Hz, 3H, 8^2-CH_3).

5. Conclusion

We have developed an efficient transformation of chlorophyll *a* into Pp-18, starting from *S. maxima* crude dry powder. To improve Pp-18 yields, a statistical experimental design was investigated which led to an optimized reaction. This method makes Pp-18 an inexpensive compound compared to other commercially available chlorins or porphyrins. Studies on Pp-18 coupled to cell-targeting agents are currently in progress with the purpose to provide water-soluble tissue-specific photosensitizers.

Acknowledgements

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