Synthesis and Spectroscopic Characterization of Meso-tetra(3-hydroxyphenyl)Porphyrin

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The preparation of compounds where functionality in the meso-position of the tetrapyrrolic ring is introduced during the formation of the porphyrin represents the main purpose of this work. The synthesis of 5,10,15,20-tetakis(3-hydroxyphenyl)porphyrin (3-OHPP) by Adler method, implying reaction between m-hydroxybenzaldehyde/ pyrrole/ propionic anhydride at a molar ratio of 1/ 1/ 1, in large excess of propionic acid, as solvent, is presented. The hydroxy-porphyrin was isolated from the reaction mixture by precipitation with hexane and it was purified by column chromatography. The purity of the compound (3-OHPP) was certified by HPLC on polar KROMASIL SI 100 column, using as eluent hexane. The structure was elucidated by H-NMR, FT-IR, fluorescence and UV-vis analysis. Photophysical properties were studied by UV-vis, emission and excitation spectra performed in different solvents and in various acid pH-media. The obtained compound represents a precursor for new materials exhibiting opto-electronic properties, useful in PDT therapy and for further applications in solar energy conversion and catalysis.

Keywords: porphyrin, HPLC, 1H-NMR, FT-IR, UV-Vis analysis

Experimental part
Materials and methods

1H-NMR spectrum was registered on 400 MHz Bruker spectrometer in CDCl₃, and chemical shifts are reported relative to internal TMS (0.0 ppm). FT-IR spectrum was registered on a 4200 JASCO apparatus, as KBr pellets. The HPLC analysis were performed on MD 1510 JASCO apparatus equipped with KROMASIL SI 100 5 µm polar column, 240. 4 mm, at ambient temperature. The samples were subjected to analysis (20 µL) at a flow rate of 1 mL/min with hexane as eluent for polar column. UV-visible spectra were recorded on a UV/VIS PERKIN ELMER, LAMBDA 12 spectrometer. Fluorescence spectra were recorded in CHCl₃ on a PERKIN ELMER Model LS 55 apparatus in a 1 cm cuvette. For MS analysis a 212 V ariano apparatus in a 1 cm cuvette. For MS analysis a 212 V apparatus equipped with KROMASIL SI 100 5 µm polar column, 240. 4 mm, at ambient temperature. The samples were subjected to analysis (20 µL) at a flow rate of 1 mL/min with hexane as eluent for polar column. UV-visible spectra were recorded on a UV/VIS PERKIN ELMER, LAMBDA 12 spectrometer. Fluorescence spectra were recorded in CHCl₃ on a PERKIN ELMER Model LS 55 apparatus in a 1 cm cuvette. For MS analysis a 212 Varian Finnigan Mat mass spectrometer was used. Reagents were p.a. grade and were purchased from Fluka, Aldrich and Merck and used as received, excepting pyrrole distilled prior to use. Acid media were made by adding of 0.1 M HCl stock solutions.

Results and discussions
The compound was subjected to 1H-NMR, UV-vis, HPLC [8], TLC, FT-IR and the main results are listed below: 5,10,15,20-tetakis(3-hydroxyphenyl)-21H,23H-porphine, (3-OHPP) by Adler method. The porphyrin (3-OHPP) was prepared according to modified literature methods [5-7]. A mixture consisting of m-hydroxybenzaldehyde (9.2 g, 75 mmol) and propionic anhydride (9.9 mL, 75 mmol), dissolved in 125 mL propionic acid was vigorously stirred and heated at reflux. A propionic acid (25 mL) solution of freshly distilled pyrrole (5.25 mL, 75 mmol) was added within 45 min. After an additional 2 h of reflux under vigorous stirring, the reaction mixture was cooled and allowed to stand overnight. An amount of 150 mL ethanol 95% was added to the reaction mixture. The precipitation was finished by adding hexane. The system was filtrated and washed several times with hot water, to give a dark purple powder, dried in a vacuum oven at 110°C for 2 h, then purified by column chromatography using as eluent a mixture of: chloroform/ethylic ether in the ratio of 2/2/1.

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State of the art studies explored photosensitizing efficacy of glucoconjugated tri and tetra(meta-hydroxyphenyl) chlorins, in order to elucidate how glucoconjugation of the macrocycle affects the photoactivity of the molecule [1]. In connection to previous researches [2-4] and in order to prepare further functionalization of the hydroxy group, this work is concerned with the synthesis (equation 1) and characterization of meso-tetra (3-hydroxyphenyl) porphyrin.

![Equation 1](image)

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The UV-vis spectrum of 5,10,15,20-tetrakis(3-hydroxyphenyl)porphyrin (3-OHPP) performed in CHCl₃ has the etio type allure (fig. 1). The band at 421 nm was assigned to the Soret band which arose from transition of $a_1 \pi - e_g^* \pi$, and the other four absorption maxima situated at 517, 554, 591, and 649 nm could be attributed to the Q bands corresponding to $a_1 \pi - e_g^* \pi$. In CHCl₃, the UV-vis spectrum of 3-OHPP has the aspect of an aggregate absorption spectrum in the Soret region, because of the broadened band at 421 nm and of the presence of the shoulder around 430 nm, corresponding to an initiation J type association process [2].

In comparison with the UV-vis spectrum registered in CHCl₃, the spectrum in THF is a non-associated one (fig. 2). The spectrum contains the high intensity Soret band, with maximum at 418 nm, followed by four Q bands, Y-polarized bands IV $[Q_x(0,1)]$ and III $[Q_y(0,0)]$ at 513 and 547 nm, respectively, and X-polarized bands II $[Q_x(0,1)]$ and I $[Q_x(0,0)]$ at 591 and 649 nm, respectively.

In order to understand the nature of biological potential behaviour of porphyrins, a detailed understanding of their behaviour in aqueous electrolyte solutions is required, especially in acid environment. According to figures 3 and 4, a study regarding the pH influence upon the allure of the absorption bands was done, by using THF-water system. The splitting of Soret band into two bands at pH=3.5 (fig. 3), one shifted batochromic around 449 nm, and the other one of weaker intensity corresponding to the monomer and preserving its position at 421 nm, both of them having Lorentzian shape, are the proof of an equilibrium process between a monomeric form and an associated one. The second band located at 449 nm could be attributed to J type aggregate. The pronounced shoulder at 421 nm is attributed unequivocally to the monomer species. With increasing acidity, equilibrium between the monomer and the aggregated porphyrin is to be noticed in aqueous electrolyte solutions (fig. 4). Another important transformation, in acid media, is the reducing of the four Q bands to only one, red-shifted to 660 nm and accompanied by major hyperchromic effect. These changes in spectral allure might be attributed to the dication (H⁺,3-OHPP) generation.

The acid type spectrum is generated by the added protons which attach themselves to the two pyridine-type nitrogen atoms in the centre of the porphyrin ring. The overcrowding thus created in the centre is responsible for the distortion of the ring structure of the planar porphyrin. This increased charge density in the core region transforms the spectrum to acid type expected for D₄h symmetry, associated to the enhancement intensity for band QI which is forbidden otherwise [9].

The FT-IR spectrum is in agreement with the structure. In figure 5, the strong band observed at about 3425 cm⁻¹ is assigned to the overlapped OH stretching vibration with NH vibration, while the absorption at 782 cm⁻¹ is attributed to the rocking vibration of N-H, respectively, which are the characteristic absorptions of porphyrin free base. The bands around 1600 and 1500 cm⁻¹ are due to stretching vibration of C = C in the aromatic benzene.

Regarding the emission spectrum of compound 3-OHPP (fig. 6), it is to be mentioned that two bands are present: one around 660 nm, accompanied by a second one of much diminished intensity, located around 720 nm. The excitation spectra registered in pure CHCl₃ has the same allure as the UV-vis spectra, but the spectra performed in CHCl₃-water systems in acid media (pH=3.5), revealed an equilibrium process between two associated
species. The Soret band is splitted into two bands, one band hypsochromic shifted belonging to H aggregates present in 400 nm range, and the other band bathochromic shifted at 431 nm, assigned to J aggregates, as it can be seen in figure 7.

Conclusions

The synthesis of 5,10,15,20-tetrakis(3-hydroxyphenyl)porphyrin (3-OHPP), by Adler method, and the characterization by HPLC, TLC, 1H-NMR, FT-IR, fluorescence and UV-vis analysis, were presented. Porphyrins can be considered as amphoteric compounds having both acidic (NH-acids) and basic (N-base) properties at the same time (nitrogen atoms of imine groups (-N=) are capable to accept protons). The photophysical behaviour of the 5,10,15,20-tetrakis(3-hydroxyphenyl)porphyrin depends a lot on the pH of the studied solution. In acid media, equilibrium between the monomer and the aggregated porphyrin is to be noticed and is accompanied by another important transformation, namely the reducing of the four Q bands to only one. These changes in spectral allure might be attributed to the dication (H2*3-OHPP) generation.

References

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