Synthesis Under Solvent Free Conditions of Some Unsymmetrically Substituted Porphyrinic Compounds

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Unsymmetrical substituted porphyrinic compounds, 5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)–21,23-H porphine and 5-(3-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)–21,23-H porphine, were synthesized with superior yields using microwave irradiation under solvent-free conditions. The structures of the compounds were confirmed using elemental analysis, NMR, FT-IR and UV-Vis spectroscopy. In addition, a study of the solubility and their spectral behaviour in environments with different polarities was performed.

Keywords: solvent free synthesis; unsymmetrical porphyrins; microwave irradiation

Over the last decades, tetrapyrrolic compounds have attracted increasing interest in bioinorganic chemistry and medicinal chemistry due to their diagnostic and therapeutic potential [1–11]. A wide variety of the pharmaceutical forms which include as active substances tetrapyrrolic compounds have been clinically approved for use in diagnosis and treatment of cancer. These include photosensitizers such as: Photofrin, Foscan, Radachlorin, Visudyne, Purytin, Lutrin and many more [1-3].

The first pharmaceutical form approved for used in photodynamic therapy of esophageal cancer, bladder cancer, early stage cervical cancer, endobronchial lesions and in Barrett’s esophagus with high grade dysplasia and also for detection head and neck tumors, cervical cancer and lung cancer by fluorescence bronchoscopy was Photofrin in 1993 [1-3].

Foscan has been approved for the treatment of prostate cancer, breast cancer, pancreatic cancer, head and neck cancer as well as for detection of bladder cancer, brain tumors and ovarian cancer [1-3, 12, 13]. Also, the recent clinical studies have revealed efficacy photodynamic therapy with Foscan in antibacterial treatment [14-17].

Radachlorin is another example of photosensitizer used in fluorescent diagnosis and photodynamic therapy of superficial tumors of the skin [1]. Visudyne is used in treatment subfoveal choroidal neovascularisation due to age-related macular degeneration [1, 3].

Porphyrinic structures can be use as fluorescent markers in cancer diagnosis due to their favorable photophysical characteristics such as emissions in the spectral range 600-800nm, large Stokes shift and high fluorescence lifetime [4-6]. In addition, their absorption coefficients in the spectral range 600–680 nm, acceptable solubility in biologic fluids, great selectivity for the malignant or other targeted tissue, photostability, low in vivo toxicity in the absence of the exciting light as well as their ability for the generation of singlet oxygen are specific characteristics of these compounds that allow their use in photodynamic therapy (PDT) of cancers and for photoinactivation of viruses and bacteria [1-3]. Both in diagnostic and therapeutic applications, efficiency of the porphyrinic compounds are influenced by its localization at subcellular level, directly correlated with the polarity degree of the molecule [18–20]. Therefore our researches were focused on obtaining by microwave irradiation under solvent free conditions of some porphyrinic compounds with a various degrees of hydrophobic/hydrophilic substitutions that favours the transport of these structures to the cellular targets [21-27].

As a continuation of our researches in this paper is described synthesis and spectral evaluation of some unsymmetrical porphyrins, namely 5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)–21,23-H porphine (TMAPOHo), 5-(3-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)–21,23-H porphine (TMAPOHm) (fig. 1).

Fig. 1. Structure of 5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)–21,23-H porphine (a) and 5-(3-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)–21,23-H porphine (b)
Experimental part

Materials and methods

Commercially available chemicals and solvents were used as received from Sigma-Aldrich and Merck. For the microwave assisted synthesis a domestic microwave oven with power and temperature controlled microwave oven was used. The elemental analysis of C, H and N was performed with an automatic Carlo Erba 1108 analyzer. IR spectra were recorded with a FT-IR Bruker Tensor 27 spectrometer. The spectra were recorded in the 4,000–500 cm⁻¹ spectral range. The NMR spectra of the porphyrinic compounds were recorded with a 400 MHz Bruker NMR Spectrometer. The UV-Vis spectra of the porphyrinic compounds were recorded with a Lambda 35 Perkin-Elmer spectrophotometer and fluorescence spectra were recorded with a Jasco FP 6500 spectro fluorimeter. The porphyrin solutions in different solvents (ethanol, isopropyl alcohol, dimethylformamide, dichloromethane, dimethyl sulfoxide) were freshly prepared in the spectrally pure solvents at the concentration 2.5×10⁻⁶ M and kept in dark until the measurement to prevent photodegradation.

Synthesis of porphyrinic compounds

A mixture of 4-acetoxy-3-methoxybenzaldehyde (0.03mol), 2-hydroxybenzaldehyde (0.01mol), fresh distilled pyrrole (0.04mol) and 2–3 g of Kieselgel 60 (200–500 μm, 35–70 mesh) was subject to microwave irradiation at 475W for 10 min. The extraction of samples for monitoring of synthesis reaction was performed after every 2 min of irradiation. The presence of porphyrin compound in the reaction mixture was monitored by thin layer chromatography and UV-Vis spectroscopy. The reaction product was extracted with dichloromethane/diethyl ether (25:1, v/v) as eluent. The solutions of the porphyrinic compounds were concentrated by simple distillation. The final porphyrinic compound was obtained by preparative column chromatography, using silica gel (100–200 mesh size) as stationary phase and dichloromethane/diethyl ether (25:1, v/v) as eluent. The solubility of the porphyrinic compounds were concentrated by simple distillation. The final porphyrinic compound was obtained by preparative TLC (2 mm, silicagel 60 plates were used).

RESULTS AND DISCUSSIONS

The choice solvent free synthesis by microwave irradiation for obtaining the porphyrinic compounds was justified by its major advantages: shorter reaction times, absence of solvent in the reaction mixtures with effect on decreased interaction time between reactant molecules and improves the reaction yield, absence of acidic medium that prevent the formation of chlorins (reduced forms of the porphyrins) and allow for an easy purification of the main compound [28-31]. The synthesis reactions have been repeated several times with identical results and then the porphyrinic compounds were evaluated by spectral analysis. Furthermore, the study of the solubility and their spectral behaviour in environments with different polarities was performed taking in account the fact that tetrapterylic compounds exhibit photoactivity only after the cell membrane permeation.

Infrared spectra

The IR spectra recorded for the synthesized compounds include typical vibration modes of both porphyrinic macrocycle and phenyl substituents (table 1) and infrared spectral assignments are generally in agreement with those previously reported for similar structures [21, 25]. The obtained data confirm presence of the –OH functional group in the structure of TMAPOH₀ and TMAPOHₘ by appearance in the IR spectra of a large band at about 3,394 cm⁻¹. Also, the corresponding band to N-H vibration is observed in the IR spectra recorded for the synthesized compounds with a yield of 33%. Elemental analysis for CₓHᵧNₓOₜₙ (TMAPOH₀) and CₓHᵧNₓOₜₙ (TMAPOHₘ): calculated C 71.14, H 4.69, N 6.26; found C 70.93, H 4.53, N 6.10. The chemical shifts of the NMR signals for the TMAPOH₀ are: 1H-NMR (CDCl₃), δ, ppm: -2.76 (s, 2H), 3.90 (s, 9H), 4.13 (s, 9H), 6.2 (s, 1H), 7.15 (s, 1H), 7.28 (s, 3H), 7.45 (d, 3H), 7.68 (m, 1H), 7.75 (d, 3H), 8.83 (d, 8H). 13C-NMR (CDCl₃), δ, ppm: 55.4, 75.8, 77.0, 114.6, 116.6, 118.0, 121.3, 123.0, 126.1, 128.8, 130.0 132.6, 137.0, 143.5, 145.5, 148.7. The procedure was adopted in the preparation of 5-(3-hydroxyphenyl)-10,15,20-tris-(4-acetoxy-3-methoxyphenyl)-21,23-H porphine (yield of 30%) and the following results were obtaining: elemental analysis - calculated C 71.14, H 4.69, N 6.10; found C 70.93, H 4.53, N 6.10. The chemical shifts of the NMR signals for the TMAPOHₘ are: 1H-NMR (CDCl₃), δ, ppm: 2.80 (s, 2H), 3.90 (s, 9H), 4.13 (s, 9H), 6.2 (s, 1H), 7.15 (s, 1H), 7.28 (s, 3H), 7.45 (d, 3H), 7.68 (m, 1H), 7.75 (d, 3H), 8.83 (d, 8H). The following results were obtained: elemental analysis - calculated C 70.93, H 4.53, N 6.10; found C 70.93, H 4.53, N 6.10. The chemical shifts of the NMR signals for the TMAPOHₘ are: 1H-NMR (CDCl₃), δ, ppm: -2.76 (s, 2H), 3.90 (s, 9H), 4.13 (s, 9H), 6.2 (s, 1H), 7.15 (s, 1H), 7.28 (s, 3H), 7.45 (d, 3H), 7.68 (m, 1H), 7.75 (d, 3H), 8.83 (d, 8H). 13C-NMR (CDCl₃), δ, ppm: 55.4, 75.8, 77.0, 114.6, 116.6, 118.0, 121.3, 123.0, 126.1, 128.8, 130.0 132.6, 137.0, 143.5, 145.5, 148.7.

Table 1

INFRARED SPECTRAL ASSIGNMENTS OF THE STUDIED COMPOUNDS (cm⁻¹)

<table>
<thead>
<tr>
<th>Assignments</th>
<th>TMAPOH₀</th>
<th>TMAPOHₘ</th>
</tr>
</thead>
<tbody>
<tr>
<td>vO-H</td>
<td>3394 m</td>
<td>3392 m</td>
</tr>
<tr>
<td>vN-H</td>
<td>3186 m</td>
<td>3191 m</td>
</tr>
<tr>
<td>vC-H</td>
<td>2921 m</td>
<td>2920 m</td>
</tr>
<tr>
<td>vC=O</td>
<td>2852 m</td>
<td>2850 m</td>
</tr>
<tr>
<td>vC=N</td>
<td>1733 m</td>
<td>1720 m</td>
</tr>
<tr>
<td>vC=N</td>
<td>1586 m</td>
<td>1586 m</td>
</tr>
<tr>
<td>vC=O</td>
<td>1500 m</td>
<td>1500 m</td>
</tr>
<tr>
<td>vC=O pyrrole</td>
<td>1462 m</td>
<td>1464 m</td>
</tr>
<tr>
<td>vC=O</td>
<td>1260 s</td>
<td>1274 s</td>
</tr>
<tr>
<td>δC-H</td>
<td>1082 m</td>
<td>1112 m</td>
</tr>
<tr>
<td>γC</td>
<td>1018 w</td>
<td>997 w</td>
</tr>
<tr>
<td>γC-N pyrrole</td>
<td>797 m</td>
<td>796 m</td>
</tr>
<tr>
<td>γC-H</td>
<td>716 m</td>
<td>719 m</td>
</tr>
</tbody>
</table>

The intensities of the signals are described as weak (w), medium (m), strong (s).
The IR spectra of the TMAPOH\textsubscript{0} and TMAPOH\textsubscript{m} indicate the presence of a medium band at \(\sim 2,852\ \text{cm}^{-1}\), band which corresponding to \(\text{C-H}\) vibration frequencies from the \(-\text{O-CH}_3\) group. Also, for both unsymmetric porphyrinic compounds the IR bands identified in the spectral ranges \(1,720–1,733\ \text{cm}^{-1}\) and \(1,260–1,274\ \text{cm}^{-1}\) can be assigned to the stretching vibration \(\text{C=O}\) and \(\text{C-O}\).

Another bands observed in the infrared spectra of the porphyrinic compounds are due to the stretching vibration \(\text{C=N}\) and \(\text{C-N}\) and were identified \(1,500–1,511\ \text{cm}^{-1}\) and \(1,586–1,606\ \text{cm}^{-1}\).

Absorption and fluorescence spectra

A series of spectral studies were carried out for the newly synthesized porphyrins in order to prove their structures and to determine their absorption and fluorescence characteristics. The UV-Vis spectral analysis of porphyrinic compounds is an efficient method used to confirm their structure because their molecular absorption spectra are typical. These contain a Soret (B) band situated in the spectral range \(400–440\ \text{nm}\) and four Q bands located between 500 and 670 nm.

The analysis of UV-Vis spectral data obtained for the synthesized porphyrins in this paper reveals in molecular absorption spectrum a intense Soret band at \(418-423\ \text{nm}\) and less intense four Q bands situated in the spectral range \(513-648\ \text{nm}\) depending on the environmental polarity. In addition, maximum absorptions are located in the spectral range required of a good photosensitizer.

Also, the obtained results by study of the spectral behaviour in environments with different polarities show the small shifts of the absorption peaks with increasing solvent polarity, changes which can be ascribed of the physical interaction between the solvent molecules and the functional groups in the meso positions of the porphyrin macrocycle.

The fluorescence measurements were performed on the synthesized porphyrinic compounds in different solvents (ethanol, isopropyl alcohol, dimethyl formamide, dichloromethane, dimethyl sulfoxide) for concentration \(2.5 \times 10^{-6}\ \text{M}\) and their fluorescence spectral data are presented in table 2.

The fluorescence spectra of 5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)–21,23-H porphine in different solvents is presented in figure 3.

For both porphyrins, under the experimental conditions used, the fluorescence spectral data shows two emission bands located in the spectral range \(598–656\ \text{nm}\) and smaller shifts of the emmision maxima as a consequence of the physical interactions that occur between the solvent molecules and porphyrinic compounds.
Conclusions
The paper describes the synthesis and spectral behaviour in environments with different polarities of new unsymmetrical tetrapyrrolic compounds. 5-(2-hydroxyphenyl)-10,15,20-tris-(4-acetoxy-3-methoxyphenyl)-21,23-H porphine and 5-(3-hydroxyphenyl)-10,15,20-tris-(4-acetoxy-3-methoxyphenyl)-21,23-H porphine were obtaining by microwave irradiation under solvent-free conditions and their structures were confirmed by elemental analysis, NMR, FT-IR and UV-Vis spectroscopy. The influence solvent polarity on spectral properties of the porphyrinic compounds was tested. The spectral changes that occur by increasing solvent polarity were ascribed to the physical interaction between the solvent molecules and the functional groups in the meso positions of the tetrapyrrolic ring.

References

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