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## The spectroscopic characterisation of proline derivatives of tolyl-porphyrins and their iron and cobalt complexes

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#### Abstract

The new tolylporphyrin-prolin derivatives: TTP-CONH-Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> and TTP-NHCO-Pro-Cbz and their iron and cobalt complexes have been synthesised and characterised by MS, IR, UV-vis, EPR and LIOAS spectroscopy studies. The EPR investigations demonstrate that Fe(III) and Co(II) ions are located in the site of distorted axial symmetry. Besides, for ferric porphyrins a thermally induced spin crossover was observed. The possibility of the applications in photodynamic therapy or diagnostics of cancerous tissues of the porphyrins obtained was studied. In the light of the experiments performed, from the group of investigated compounds, it seems that the metal-free TTP-NHCO-Pro-Cbz is well suited for PDT.

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## 1. Introduction

The porphyrin compounds form an important class of materials considering their application, e.g. in the photodynamic therapy (PDT) in the diagnosis and treatment of cancer. For example *meso*-tetra-4-hydroxyphenylporphyrin has for several years now been used as a photosensitizer, approved for clinical use [1]. Apart from that, the complexes of the 3d metal (above all cobalt and iron) with porphyrins, when fixed on various carriers, can be used as efficient catalysts [2].

The subject of our investigations was *meso*-derivatives of tolyl-porphyrins with proline. We decided to investigate these porphyrins as a result of their chemical properties. Carboxylic or an amine group allows forming an amide bond, e.g. with amino acids, what is characteristic for pro-

\* Corresponding author. Tel./fax: +48 322 588 431. *E-mail address:* dskrzyp@us.edu.pl (D. Skrzypek). teins. Proline derivatives, e.g. proline peptidases are involved in regulation of lifetime biologically active peptides [3].

In this paper, the results of study (by using EPR method) of metalloporphyrins with this new substituent are also presented. The EPR spectroscopy is useful method for determining electronic and magnetic properties of the paramagnetic complexes. The aim of this work is to analyse the influence of the porphyrin peripheral substituents and the temperature effect on the electronic structure of ferric and cobalt 5-(4-carboxyphenyl)-10,15,20-tritolyl- and 5-(4-aminophenyl)-10,15,20-tritolyl-porphyrins with proline.

The information on the dye singlet states is obtained from the absorption and fluorescence spectra recorded for dyes in solutions. On the basis of these experiments, the photophysical properties and spectral parameters of dyes, essential for the further analysis of the LASER induced optoacoustic spectroscopy (LIOAS) results, were estimated.

The introduction of the metals to the porphyrin ring changes drastically their photochemical properties.

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## 2. Experimental

## 2.1. Synthesis of the porphyrins

## 2.1.1. Procedure for preparation of 5-(4-carboxyphenyl)-10,15,20-tritolylporphyrin

5-(4-Carboxyphenyl)-10,15,20-tritolylporphyrin was prepared basically according to the Adler method [4], with some modifications. The procedure was as follows: in a 500-mL round-bottom flask, equipped with an efficient mechanical stirrer, were placed: propionic acid (300 mL), 4-tolylaldehyde (1.8 g, 15 mmol) and 4-carboxybenzaaldehyde (0.75 g, 5 mmol). The mixture was heated to 140 °C and then pyrrole (3.4 g, 20 mmol) was added dropwise, for 30 min with stirring. Heating was continued for 0.5 h and the mixture cooled. The mixture was left for 24 h and a separated product was filtered off, washed with water and hen with a mixture of water and methanol (v/v,8:2), several times. Washing was continued until the filtrate becomes colourless and odourless. The product was dried to give a dark solid (it was a mixture of six porphyrins; m = 1.1, 30%). The mixture was separated by column chromatography (silica gel 60-230 mesh, eluent:chloroform-methanol; v/v, 9:1), collecting the eluate containing the second, purple band with the desired product. After evaporation of the solvent, a dark-violet product was obtained. Yield: 250 mg (5%). MS: ESI + Q1MS, 701 (M+1).

## 2.1.2. Procedure for preparation of 5-(4-aminophenyl)-10,15,20-tritolylporphyrin

Procedure for preparation is similar like above. 4-Tolylaldehyde (1.8 g, 15 mmol), 4-acetamidobenzaldehyde (0.85 g, 5 mmol) and pyrrole (3.4 g, 20 mmol) were heated to 140 °C in 500 mL of propionic acid for 1.5 h. The mixture was left for 24 h and separated on chromatography column (silica gel 60–230mesh, as an eluent was used chloroform), and the second band was collected, which was the desired product. After evaporation of the solvent, 250 mg 5-(4-acetamidophenyl)-10,15,20-tritolylporphyrin was obtained. Yield: approx. 3%. The conversion of acetamide to amine group was conducted by heating at 80 °C in the presence of the mixture of trifluoric acid and hydrochloric acid (v/v, 1:1) during 12 h. Next, the reaction mixture was neutralised by saturated solution of sodium dicarbonate. The final product was extracted with chloroform. After evaporation of solvent, 220 mg of 5-(4-aminophenyl)-10,15,20-tritolylporphyrin was obtained. Yield: approx.: 80%. MS: ESI = Q1MS, 671 (M+1).

# 2.1.3. General procedure for preparation of the tolylporphyrin-prolin derivatives [3,5,6]

The properly protected proline (as benzyl ester or carbobenzyloxyproline) was coupled with 5-(4-aminophenyl)-10,15,20-tritolylporphyrin or 5-(4-carboxyphenyl)-10,15, 20-tritolylporphyrin, respectively. As a coupling agent was used 1,3-dicyclohexylocarbodiimide (DCC).

To a solution of 5-(4-carboxyphenyl)-10.15.20-tritolylporphyrin (35 mg, 0.05 mmol) in dry methylene chloride (10 mL), 11 mg (0.05 mmol) of DCC was added. Next a solution of 12 mg (0.05 mmol) of proline benzyl ester hydrochloride in dry methylene chloride and 11 mg triethylamine was introduced. The mixture was stirred for 24 h at room temperature and then a solvent was evaporated to dryness. Formed 1,3-dicyclohexylurea (DCU) was removed by treating with ethyl acetate and filtrated. The solution of product was washed twice with 1.0 M ag. sodium bicarbonate, water and dried over sodium sulphate. The crude product of coupling (TTP- $CONH-Pro-COOCH_2C_6H_5$ ) was additionally purified by column chromatography (silica gel 60-230 mesh, eluent; chloroform). Yield: 37 mg, approx. 75%. MS: ESI = Q1MS, 889 (M+1).

The coupling product of 5-(4-aminophenyl)-10,15,20-tritolylporphyrin and carbobenzyloxyproline (TTP–NHCO– Pro–Cbz) was prepared like above. Yield: 40 mg, approx. 80%. MS: ESI = Q1MS, 902 (M+1). Fig. 1 presents the molecular structure of these compounds.

# 2.1.4. General procedure for metalation of the tolylporphyrin-prolin derivatives [7]

Iron and Cobalt derivatives of TTP–CONH–Pro– COOCH $_2C_6H_5$  and TTP–NHCO–Pro–Cbz were conducted by standard dimethylformamide (DMF) method.

Threefold excess of Iron (III) chloride or Cobalt (II) acetate with 0.05 mmol of tolylporphyrin-prolin derivatives was refluxed during 2 h in 50 mL of DMF. The crude reaction product crystallises by adding 200 mL of water and cooling. Excess of used salts was removed by washing with water. Yield was approx. 70–80%.

The crystalline structure of the compounds under investigations was studied by powder X-ray diffraction. We obtained the amorphous products for ferric complexes and weakly crystallised cobalt complexes.



Fig. 1. The molecular structure of base porphyrins.

## 2.2. Methods

MS spectra were measured on MS Spectrometer TSQ 700 Finigan-Mat using ESI technique. Molecular peaks proved molecular masses of investigated porphyrins:

- (a) TTP-CONH-Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>-compound 1: m/e = 888.5.
- (b) TTP–NHCO–Pro–Cbz–compound **2**: m/e = 902.
- (c) Fe(III) TTP-CONH-Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>-compound 3: m/e = 977.5.
- (d) Fe(III) TTP-NHCO-Pro-Cbz-compound 4: m/e = 991.
- (e) Co(II) TTP-CONH-Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>-compound **5**: m/e = 945.5.
- (f) Co(II) TTP-NHCO-Pro-Cbz-compound **6**: m/e = 959.

The IR spectra were measured on IR 560 Magma Nicolet spectrometer as KBr pellets in the spectral range of 450– 4000 cm<sup>-1</sup>. The UV–vis spectra of compounds were measured at normal incidence at room temperature in the range of 350–700 nm by a Jasco UV-Spectrometer in dimethylformamide solutions (where concentrations were  $10^{-6}$ and  $10^{-4}$  M).

The EPR spectra were recorded on Radiopan SE/X spectrometer with  $TE_{102}$  rectangular cavity and 100 kHz field modulation, equipped with an Oxford Instruments ESR 910 helium flow cryostat. The microwave frequency was measured using Hewlett–Packard 534 microwave frequency counter and the magnetic field strength was monitored by a NMR teslameter. The temperature dependence measurements were performed in the temperature range from 3 to 300 K. The EPR parameters were confirmed by simulation using Bruker-Symphonia software package.

The properties of the electronic singlet states of the porphyrins were determined by means of the steady-state absorption and fluorescence techniques using a Varian Cary 4000 and a Hitachi F4500 spectrophotometers, respectively. Time-resolved laser-induced optoacoustic spectroscopy (LIOAS) signal, in the time range limited up to 5  $\mu$ s, was recorded by means of the setup described earlier in [8,9]. For the LIOAS measurements, the 415 nm (288.35 kJ/mol) laser flash wavelengths were used. In the optoacoustic experiments, the bromocresol purple (BCP) in toluene was used as a reference, because for this dye the total excitation energy is promptly thermally deactivated (in time shorter than  $\tau_1$  – the time resolution of the setup used estimated to be about 0.5 µs). The investigated porphyrins were dissolved in toluene with concentrations ranged from about  $10^{-6}$ – $10^{-3}$  M.

## 3. Results and discussion

## 3.1. IR and electronic spectra measurements

The principal infrared absorption bands for the proline derivatives of tolylporphyrin presented in this work are tabulated in Table 1. Our assignments are in general in agreement with those previously reported in literature [10–12]. The absence of representative N–H stretching vibrations of free base porphyrins in the region of 3300–3360 cm<sup>-1</sup> or sharp peaks at 740 and 680 cm<sup>-1</sup> associated with N–H out-of-plane deformations indicates a successful cobalt and iron insertion into porphyrin ring. The presence of substituents of phenyl ring in *p*-position is observed. We were able to assign the characteristic absorptions originated from properly protected amino acid molecules, as stretching and bending vibrations of C=O in amide's and ester's carboxylic groups.

The obtained UV-vis spectra of the studied porphyrins are typical [13,14]. Absorption spectra of our two free base are characterised by very intense Soret band in the near UV region and four O bands in the visible region with about 10 times smaller intensity. In the case of our metalo-derivatives of formed compounds, disappearance of two Q bands is observed (instead of the I, III and II,  $IV - \alpha$  and  $\beta$  are only present), what is connected with symmetry changing from  $D_{2h}$  to  $D_{4h}$ . The selected optical parameters are listened in Table 2. Practically all transition metal ions with open shells produce abnormal spectral types for the porphyrin. Cobalt derivatives of porphyrins belong to "hypso spectrum" class (blue shifted spectra, what is observed here in the Q region) and iron derivatives represent "hyper" type of spectrum. From the absorption spectra of cobalt derivatives of studied porphyrins can be observed that the second Q bands at about 600 nm has much smaller intensity or has been almost disappeared. The explanation suggested by Kaizu et al. [15] was that metal ion position is out of plane of the porphyrin ring, and the attachment the solvent molecule in axial position disturbs the symmetry of the system.

Table 1

The	optical	parameters	of studied	compounds
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Compounds	Soret band (nm)	Q bands (nm)	$\varepsilon(v) \times 10^5$	$(\text{mol}^{-1}\text{cm}^{-1})$
TTP-NHCO-Pro-Cbz	420	516; 552; 593; 649	1.63	0.07; 0.047; 0.026; 0.028
TTP-NHCO-Pro-Cbz-Fe(III)	417	508; 573; 614	0.26	0.029; 0.019; 0.013
TTP-NHCO-Pro-Cbz-Co(II)	433	548; 593	0.76	0.057; 0.025
TTP-CONH-Pro-COOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	417	513; 557; 592; 646	1.02	0.088; 0.037; 0.027; 0.027
TTP-CONH-Pro-COOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> -Fe(III)	417	514; 572; 612	0.88	0.03; 0.04; 0.026
TTP-CONH-Pro-COOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> -Co(II)	433	547; 564	0.8	0.067; 0.041

 Table 2

 Infrared absorption bands of formed compounds

TTP- NHCO- Pro-Cbz (cm <sup>-1</sup> )	TTP-NHCO- Pro-Cbz- Fe(III) (cm <sup>-1</sup> )	TTP-NHCO- Pro-Cbz- Co(II) (cm <sup>-1</sup> )	TTP-CONH-Pro- COOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> (cm <sup>-1</sup> )	$\begin{array}{l} TTP-CONH-Pro-\\ COOCH_2C_6H_{5}-\\ Fe(III) \ (cm^{-1}) \end{array}$	$\begin{array}{l} TTP-CONH-Pro-\\ COOCH_2C_6H_{5^-}\\ Co(II) \ (cm^{-1}) \end{array}$	Types of vibrations
3325	_	_	3325	_	_	N-H stretch in porphyrin ring
2928	2925	2927	2929	2928	2929	C-H stretch in methyl groups
2851	2852	2853	2851	2849	2851	C-H stretch in proline (and
						aliphatic groups)
1740	1740	1741	1749	1743	1740	C=O stretch in ester's carbonyl
						group
1627	1622	1638	1628	1627	1628	C=O stretch in amide's carbonyl group
1575	1572	1573	_	_	_	N-H bend in amide
1535	1543	1545	_	—	_	C=O bend in amide's carbonyl group
1470	1471	1473	1471	1471	1470	C = C stretch in porphyrin ring
1448	1448	1449	1447	1450	1448	C—H bend in phenyl
1406	1413	1419	1410	1411	1408	C—H bend in porphyring ring
1366	1350	1351	1348	1349	1350	C—H bend in phenyl
1311	1311	1310	1312	1312	1311	C=O stretch in ester's carbonyl
						group
1245	1245	1249	1245	1245	1245	C—H bend, C—N stretch in porphyrin ring
1182	1179	1180	1184	1182	1182	C—H bend in phenyl
1088	1077	1086	1090	1090	1088	Stretch in phenyl
	1002	1003	_	1000	1001	In plane bending in porphyrin ring
967	_	_	967	_	_	In plane bending in porphyrin ring (ring vibration)
801	800	799	800	799	799	C—H out-of-plane <i>p</i> -substituted phenyl
740	—	—	734	_	—	Out-of-plane N—H bending in porphyrin ring
640	660	660	640	640	640	In-plane vibration deformation porphyrin

## 3.2. The EPR studies

## 3.2.1. The ferric complexes

Figs. 2 and 3 show the temperature evolution from the room temperature to T = 3 K of EPR spectrum of Fe(III) TTP-CONH-Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> and Fe(III) TTP-NHCO-Pro-Cbz, respectively. For both compounds EPR spectra are complex and contain the group lines around g = 2 (denoted as A) and low-field signal with g-factor near 6 (denoted as B). Additionally, when the temperature is lowered the EPR spectra of compound 4 exhibit the signal with g = 4.3 (denoted as C). The appearance of these features suggested the presence of different paramagnetic species: (i) A-type signals are associated with the low-spin state (S = 1/2) of Fe(III) complexes in the rhombic symmetry crystal field; (ii) B-type signal is due to the high-spin state (S = 5/2) of Fe(III) ions in the tetragonal symmetry crystal field [16-21]; (iii) C-type signal is, as a rule, observed in Fe<sup>3+</sup>-doped disordered media [22-24] and it is generally assigned to  $Fe^{3+}$  in a weak crystal field environment.

The A-type signal may be described by the rhombic spin Hamiltonian including only the Zeeman interaction:

$$H = \mu_{\mathbf{B}} \left( g_x B_x S_x + g_y B_y S_y + g_z B_z S_z \right) \tag{1}$$

The exemplary simulation referring only to A-type species (with EPR parameters listed in Table 3) is presented in Fig. 3.

The spin Hamiltonian for a paramagnetic ion with S = 5/2 in tetragonal symmetry may be written as [25]:

$$H = D\{S_z^2 - 1/3 \ S(S+1)\} + g_{\parallel} \mid \mu_{\rm B} B_z S_z \cos \theta + g_{\perp} \mu_{\rm B} B_x S_x \sin \theta$$
(2)

where the magnetic field *B* forms an angle  $\theta$  with the *z*-tetragonal axis and *D* is zero-field splitting parameter. For a large *D* value ( $D \gg hv$ ), the EPR signal that can be observed is the transition from  $m_{\rm S} = -1/2$  to +1/2 states. The resonance condition for the ground-state doublet is described with an effective *g* value so that:

$$hv = g_{\rm eff}(\theta)\mu_{\rm B}B\tag{3}$$

When the magnetic field is applied along the z-axis, one has

$$g_{\parallel_{\text{eff}}} = g_{\parallel} \tag{4}$$

and when the magnetic field is applied perpendicularly to the *z*-axis

$$g_{\perp \rm eff} = 3g_{\perp} \left[ 1 - 2(g_{\perp}\mu_{\rm B}B)^2 (2D)^{-2} \right]$$
(5)



Fig. 2. The temperature evolution of the EPR spectra of Fe(III) TTP– CONH–Pro–COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>. The comparison of the experimental and simulated signals B-type at T = 3 K is also shown (simulated spectrum for the parameters , which are listed in Table 3).



Fig. 3. The temperature evolution of the EPR spectra of Fe(III) TTP– NHCO–Pro–Cbz. The comparison of the experimental and simulated signals A-type at T = 300 K is also shown (simulated spectrum for the parameters, which are listed in Table 3).

From the Eq. (5) we can see that when a zero-field-parameter |2D| is much greater than the Zeeman splitting, the signal with highly anisotropic g-factor  $(g_{\parallel_{\text{eff}}} = 2; g_{\perp \text{eff}} = 6)$  is observed. It was found for many high-spin ferric heme proteins [16,17].

The spin Hamiltonian (2) with the assumption that  $D \gg hv$  was used to simulate the low-field part of the experimental spectrum. Fig. 2 shows the exemplary simula-

Table 3The EPR parameters of obtained metalloporphyrins

Compound	Type of spectrum		
	A	В	С
3 (Fe(III)TTP-CONH-Pro- COOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> )	$T = 3 \text{ K}$ $g_{\perp} = 5.80$ $g_{\parallel} = 2.00$	T = 300  K $g_x = 1.79$ $g_y = 2.03$ $g_z = 2.35$	Absent
4 (Fe(III) TTP–NHCO–Pro– Cbz)	$T = 3 \text{ K}$ $g_{\perp} = 5.80$ $g_{\parallel} = 2.00$	T = 300  K $g_x = 1.96$ $g_y = 2.10$ $g_z = 2.28$	$T = 3 \text{ K}$ $g_{\text{eff}} = 4.3$
	<b>S</b> 1	S2	S3
5 (Co(II) TTP–CONH–Pro– COOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> )	T = 3 K $g_{\perp} = 3.22,$ $A_{\perp} = 390$ $g_{\parallel} = 1.87,$ $A_{\parallel} = 122$	$T = 300 \text{ K}$ $g_{\perp} = 2.21$ $g_{\parallel} = 2.29$	$g_{\rm eff} = 2.002$
6 (Co(II) TTP–NHCO–Pro– Cbz)	T = 3  K $g_{\perp} = 3.22,$ $A_{\perp} = 390$ $g_{\parallel} = 1.87,$ $A_{\parallel} = 122$	$T = 300 \text{ K}$ $g_{\perp} = 2.28$ $g_{\parallel} = 2.22$	$g_{\rm eff} = 2.002$

 $A \text{ in } 10^{-4} \text{ cm}^{-1}.$ 

tion obtained for compound 3. The values of g-factors for both compounds are the same and are listed in Table 3.

The C-signal with g = 4.3 implies the presence in examined compounds the high-spin ferric species in the rhombic symmetry crystal field. In this system, the <sup>6</sup>A<sub>1</sub> ground state is split into three Kramers' doublets due to spin–orbit mixing with excited states. These doublets are split by an applied magnetic field and the near isotropic g-factor of 4.3 is assigned to a transition within one of them [23]. Many authors [26] have discussed the matter from different points of view. Kliava [22] analysed the EPR spectra of glasses on the basis of a spin Hamiltonian equivalent to:

$$H = g\mu_{\rm B}BS + D(S_z^2 - 35/12) + (1/2)E(S_+^2 - S_-^2)$$
(6)

with S = 5/2 and g = 2. Here, *D* and *E* are the axial and rhombic *fs* parameters. This Hamiltonian contains no quartic crystal field terms. An isotropic *g* of 30/7 from a high-spin (HS)  $d^5$  ion in a site of rhombic symmetry can only appear if the symmetry is completely rhombic  $(\lambda = E/D = 1/3)$ , making quartic crystal field terms are insignificant. Such interpretation was supported also by successful simulation of the spectra by Yahiaoui and Kliava et al. [27,28].

The temperature evolution from the room temperature to T = 3 K of EPR spectrum of ferric complexes shows a sequential build-up of the high-spin species. The LS and HS paramagnetic species are in different proportions at different temperatures. The low-spin component over the lowest temperature range is still present but much less intense than at room temperature. It is known that the ferric ion in several materials [17,29,30] exhibits a thermally induced change of spin-state. Additionally, for explanation of the character of observed EPR spectra we estimated the temperature dependence of the spectrum intensity. In Fig. 4 temperature evolution of intensity of EPR spectrum (calculated as double integration of the spectrum – DI) for compounds 3 and 4 is shown. This defined intensity should be proportional to the spin susceptibility of the sample. It was established that the intensity does not follow the Curie law.

We suppose that the examined porphyrins obtained by us as disordered solids contain iron complexes with the different local symmetry around them. Additionally, when temperature is lowered the EPR spectra exhibit predominant contribution high-spin state of ferric ions. It means the change of ferric ion position and stiffness of Fe(III) complexes. It is worth noting, that observed effects appear under solvent-free conditions.

#### 3.2.2. The cobalt complexes

The powder EPR spectra of: Co(II) TTP-CONH-Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> and Co(II) TTP-NHCO-Pro-



Fig. 4. The plot of spin susceptibility vs. temperature calculated as double integration of the EPR spectrum for: (a) Fe(III) TTP–CONH–Pro–COOCH $_2C_6H_5$ ; (b) Fe(III) TTP–NHCO–Pro–Cbz.

Cbz and their temperature evolution are shown in Figs. 5 and 6. For both compounds the spectra are complex and contain the groups of lines denoted as S1, S2, and S3.

The signal S1 consists of a series of eight nuclear hyperfine lines. Its best simulation (T = 3 K) with parameters listed in Table 3 is shown in Fig. 6. This feature with  $g_{\perp}$ around 3.2 is weak, broad and unresolved at ambient temperature. The signal S2 of the EPR spectrum is characterised by single asymmetrical line, which was simulated with parameters in Table 3. The signal S2 was detectable at whole temperature range (from 300 to 3 K) and at low temperatures this line is partially covered by EPR signal labelled as S1. The line S3 shows unresolved feature with  $g_{\rm eff} = 2.002$ .



Fig. 5. The experimental EPR spectrum of Co(II) TTP-CONH-Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> at T = 3 K (the inset shows the experimental spectrum at T = 300 K).



Fig. 6. The comparison of the experimental and simulated signals S1-type for Co(II) TTP–NHCO–Pro–Cbz at T = 3 K (simulated spectrum for the parameters which are listed in Table 3). The inset shows the experimental spectrum at T = 300 K.

The characteristic feature of Co(II)-EPR spectrum is its spectroscopic complexity with respect to the orbital degeneracy of the ground state and coupling of excited state and also with respect to influence of changes in the coordination environment [25,31-34].

The reviews of the typical results for frozen solutions of the metalloporphyrins with symmetric  $D_{4h}$  or  $C_4$  square planar symmetry were done by Subramanian [35] and Walker [17]. These data imply that S1 and S2 the EPR features are consistent with the d<sup>7</sup>-configuration in the lowspin state, leading to the S' = 1/2 system in which the single unpaired d electron is localised on the  $d_z^2$  orbital. The axially symmetrical spin Hamiltonian [25]

$$H = \mu_{\rm B}[g_{\parallel}B_zS_z + g_{\perp}(B_xS_x + B_yS_y)] + A_{\parallel}S_zI_z + A_{\perp}(S_xI_x + S_yI_y)$$
(7)

[in which S = 1/2 and I = 7/2 (for <sup>59</sup>Co - 100% abundance)] generates the computer spectrum of signal S1-type with the parameters A and g (see Table 3) similar to those reported by several authors [25,31,35–37]. These data mean the formation of square-planar LS Co(II) species.

The signal denoted as S2 is consistent with the formation of a five-coordinate Co(II) species [38–42]. The addition of a fifth ligand to a square planar complex is known to have a strong destabilising effect on its  $d_z^2$  orbital [32]. Mc Garvey's theory [43] has shown that to adequately explain the EPR data on low spin cobalt complexes, it is necessary to include the spin–orbit mixing of excited quartet states into the doublet ground state. The set of equation for g and A parameters reproduced in the simplified versions by Barzaghi et al. [44] has been obtained on the basis of two possible ground states:

$${}^{2}A_{1} = (x^{2} - y^{2})^{2} (xz \text{ or } yz)^{2} (yz \text{ or } xz)^{2} (z^{2})$$
  
$${}^{2}B_{1} = (x^{2} - y^{2})^{2} (z^{2})^{2} (yz \text{ or } xz)^{2} (xz \text{ or } yz)$$
(8)

It was shown that for low symmetry complexes the large anisotropy in g and A values may result from the extensive mixing of  $d_z^2$  and  $d_{x^2-y^2}$  orbitals which is allowed in lower symmetries. The separation between the excited and ground states is a very sensitive function of an axial ligands. For example, the results of EPR investigations of tetraarylporphyrin complexes of Co(II) in the presence of a number of solutes are as follows [31]: for the Lewis base donor, piperidine,  $g_{\perp}$  is smallest (2.214); for  $\pi$  donors  $-g_{\perp}$ ranges from 2.4 to 2.7; for strong  $\pi$  acceptors  $-g_{\perp} \ge 3.0$ . Therefore, in our opinion, the presence of the five-coordinated cobalt (II) species in both compounds obtained suggests the possibility of coordination by eluent molecules during preparation.

The appearance of feature type S3 in the EPR spectra of examined compounds means that these porphyrins are partially oxygenated. This signal is associated with  $Co(III) - O_2^-$  complexes [45–47]. If the superoxide has several different conformations, each will have slightly different coupling to cobalt, resulting in smearing out of the hyperfine structure, what is observed in our experiment.

#### 3.3. The photophysical studies

The photophysical studies for: (i) (TTP–CONH–Pro– COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>; (ii) (TTP–NHCO–Pro–Cbz; (iii) Fe(III) TTP–NHCO–Pro–Cbz; (iv) Co(II) TTP–NHCO–Pro–Cbz were performed On the basis of the LIOAS measurements performed in air- and N<sub>2</sub>-saturated solvent it is possible to conclude about the population and oxygen quenching efficiency of the dye triplet state [48]. Assuming that in N<sub>2</sub>-saturated solution [49], the pigments studied are photochemically stable, the value of the yield of triplet state generation ( $\Phi_{\rm T}$ ) can be obtained from the equation:

$$\Phi_{\rm T} = \frac{(1-\alpha)E_{hv} - \Phi_{\rm F}E_{\rm F}}{E_{\rm T}} \tag{9}$$

where,  $E_{\rm T}$ , the energy of the tetraphenylporphyrin (TPP) triplet state (taken from literature [50]);  $E_{hv}$ , the molar energy of the incident photons;  $E_{\rm F}$ , the energy of the dye singlet state;  $\alpha(=k_1)$ , the part of the energy exchanged promptly into heat, i.e. in a time shorter than  $\tau_1$ , the values were obtained by Marti et al. [51] and deconvolution [52] methods, respectively.

From the LIOAS data obtained for dye dissolved in airsaturated solutions [49], it is possible to calculate the singlet oxygen production yield  $(\Phi_{\Delta})$  using the equation:

$$\Phi_{\Delta} = \Phi_{\rm T} S_{\Delta} = \frac{(\Phi_{\rm T} E_{\rm T} - E_{h\nu} k_2)}{E_{\Delta}} \tag{10}$$

where,  $E_{\Delta}$ , the electronic energy of singlet oxygen  $(E_{\Delta} = 94 \text{ kJ/mol})$ ;  $S_{\Delta}$ , represents the fraction of dye triplet states quenched by oxygen;  $k_2$ , a pre-exponential factor (the fraction of thermal energy released in  $\tau_2$ -lifetime) followed from deconvolution of LIOAS signals [52].

It has been shown lately [9,53] that a complementary methods of the molecular (optical) and photothermal spectroscopy can provide valuable information helpful for selection of dyes useful in photomedicine. The compounds that would be apply as photosensitizer for the therapeutic application (PDT) are different from those serve as photomarkers for diagnostic mode (PDD). Essential photophysparameters that for PDT, determine ical the photosensitizing capability of a compound are the quantum yields of triplet state ( $\Phi_{\rm T}$ ) and the singlet oxygen  $(\Phi_{\Lambda})$  [53]. For use in PDD, the compound's ability for fluorescence needs to be appreciable which is quantified as its fluorescence quantum yield ( $\Phi_{\rm F}$ ).

In order to study the dye excited state properties and its formation and depopulation as well as the decay times of thermal deactivation process, TTP dye derivatives were investigated by means of the optical and time-resolved thermal spectroscopy (LIOAS).

The obtained photophysical parameters gathered in Table 4, can help to estimate the ability of the investigated porphyrin to perform the photodynamic reaction. From the comparison of data in Table 4, for both basic metal-free porphyrins (compounds 1 and 2), it follows that these dyes show almost the same efficiency of fluorescence emis-

Table 4

Some photophysical parameters estimated for tetratoryporphysical parameters estimated for tetratoryporphysical parameters $(\lambda_{las} - 415 \text{ mm})$									
Compound	Atmosphere	$\Phi_{\rm F}(\pm 0.02)$	$\alpha$ (= $k_1$ ) (±0.01)	$k_2$	$k_3$	$\sum k_i$	$\tau_2 \; (\mu s)$	$\Phi_{\mathrm{T}}$	$\Phi_{\Delta}$
1 (TTP-CONH-Pro-COOCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> )	Air	0.032	0.82	0.08	0.09	0.99	2.25	0.37	0.30
	$N_2$	0.034	0.80	0.13		0.92	3.53		
2 (TTP–NHCO–Pro–Cbz)	Air	0.031	0.65	0.12	0.23	1.00	1.64	0.72	0.69
	$N_2$	0.038	0.63	0.20	_	0.82	2.21		
4 (Fe(III)–TTP–NHCO–Pro–Cbz)	Air	$\approx 0$	0.92	0.06	0.01	1.00	1.02	0.23	0.15
	$N_2$	$\approx 0$	0.89	0.02	_	0.91	1.73		
6 (Co(II)–TTP–NHCO–Pro–Cbz)	Air	0.003	0.98	0.01	0.01	1.00	0.98	0.06	0.05
	$N_2$	0.004	0.97	0.02		0.99	1.31		

Some photophysical parameters estimated for tetratolylporphyrins in toluene ( $\lambda_{las} = 415 \text{ nm}$ )

 $\Phi_{\rm F}$ , fluorescence yield;  $\Phi_{\rm F} = \Phi_{\rm R} \frac{I}{I_{\rm R}} \frac{OD_{\rm R}}{D_{\rm R}} \frac{n^2}{n_{\rm R}^2}$ , where  $\Phi_{\rm R}$ , the fluorescence quantum yield of the reference; I,  $I_{\rm R}$ ; and OD, OD<sub>R</sub> and n,  $n_{\rm R}$ , the areas under the fluorescence curves, the absorption intensities, refractive indices of the sample and reference, respectively,  $\alpha$ , a fraction of excitation energy exchange into heat promptly (in shorter time than time resolution of apparatus),  $k_i$ , pre-exponential factors (the fractions of thermal energy released in *i*th lifetime);  $\tau_2$ , decay times for time range 0.5–5.0 µs,  $\Phi_{\rm T}$ , yield of triplet state formation, Eq. (9),  $\Phi_{\Delta}$ , yield of singlet molecular oxygen production estimated based on Eq. (10);  $S_{\Delta}$ , the fraction of triplet states quenched by oxygen,  $S\Delta = \Phi_{\Delta}/\Phi_{\rm T}$ .

sion but they differ significantly in a deactivation of excitation energy by radiationless processes. The TTP-CONH-Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> sample exchanges quickly (in a time range of about  $0-0.5 \,\mu s$ ) about 80-82% of excitation energy into heat and as a consequence its triplet state is not very efficiently occupied. The molecular structure of TTP-NHCO-Pro-Cbz is not so much different than that of TTP-CONH- Pro-COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> but in some degree it influences the distribution of energy absorbed by dye. This dye (compound 2) shows much higher than TTP-CONH- $Pro-COOCH_2C_6H_5$  dye (compound 1) formation of triplet state as well as ability to generate the singlet oxygen. It seems that the photosensitising potential of TTP-NHCO-Pro-Cbz as a base porphyrin is sufficient to be taken into consideration as a candidate for further investigation for PDT treatment. Because of the TTP-NHCO-Pro-Cbz promising photodynamic properties it was also interesting to examine its metal derivatives (cobalt and iron complexes).

The study of thermal depopulation processes of these metaloporphyrins excited states help in full description of the fate of the energy absorbed by these dyes and its lost through non-radiative channels. Some results obtained, for the metal substituted TTP, from LIOAS signal analysis are shown in Table 4. The metalation of porphyrin ring influences the  $\pi$ -electronic system in dye molecular skeleton and caused some modification of photophysical features of both singlet and triplet states as well as light-induced molecular processes responsible for a possible dye application in therapy or diagnosis. For Co(II)- TTP-NHCO-Pro-Cbz the yield of fluorescence emission decrease about 10-times whereas for Fe-porphyrin, the fluorescence is almost not observed. Both metal-porphyrin complexes convert quickly (mainly the singlet excited states energy) in radiationless processes about 30% more energy than their metal-free analogue. Especially Co-TTP exchange even more excitation energy into heat than Fe-TTP in a short time (see  $\alpha$ ,  $k_1$ , in Table 4). It indicated that the complexation of TTP ring with metal ions caused an decrease in the total energy dissipation through radiative processes (e.g. Fe-TTP) but also an increase in fast non-radiative

processes (e.g. Co–TTP) as well as the shortened of  $\tau_2$  for thermal deactivation process (Table 4).

 $\frac{S_{\Delta}}{0.81}$ 

0.96

0.83

## 4. Conclusions

The new tolylporphyrin-prolin derivatives and their iron and cobalt complexes have been synthesised.

(a) The observed EPR spectra of metal-complexes are complex. The EPR data of compounds **3** and **4** indicate that the character of EPR spectrum is determined by amorphous form of these samples. The characteristic features of disordered solids namely, distribution of the short-range order parameters: bond lengths and bond angles lead to the broadening of EPR lines and the statistical distribution of EPR parameters. Because of that for Fe(III) complexes the features of rhombic spectrum are smeared out and different values of g parameters for both examined compounds were obtained. Besides, for compounds **3** and **4** a thermally induced spin crossover was observed. When temperature is lowered the EPR spectra exhibit predominant contribution of high-spin state of ferric ions. It means the change of ferric ion position and stiffness of Fe(III) complexes.

The data in Table 3 for obtained Co(II)-porphyrins show that EPR spectra of examined compounds are consistent with the formation of square-planar LS Co(II) species (S1) and the five-coordinate LS Co(II) (S2) species. All the low-spin cobalt (II) complexes have very similar g and A components, whose values are typical and observed for other cobalt (II) porphyrins. Therefore, these results do not exclude the possibility of coordination by solvent and eluent molecules (axial ligand) during preparation. Besides, EPR spectra of both porphyrins indicate that these compounds are partially oxygenated and exhibit the typical signal Co(III)-O<sub>2</sub><sup>-</sup> around g = 2.

(b) In the light of the experiments performed, from the group of investigated porphyrins, it seem that the metal-free TTP-NHCO-Pro-Cbz is well suited for PDT because it shows the most effective triplet state formation, which are than efficiently quenched by energy transfer to molecular oxygen, what yields in the production of reactive singlet oxygen species. The results concern the dyes in toluene

imply that synthetic porphyrin (such as investigated TTP) exhibit lower  $\Phi_{\rm F}$  values (Table 4) than earlier investigated natural porphyrin [54,55], hence they rather cannot be a promising candidate as photomarkers for diagnosis.

It is known that the yield of singlet oxygen formation  $(\Phi_{\Delta})$  as a result of dye triplet interaction with oxygen can be given by the relation:  $\Phi_{\Delta} = \Phi_{T}S_{\Delta}$ . The value of  $S_{\Delta}$  coefficient is close to 1 for TTP–NHCO–Pro–Cbz and indicate that the very efficient excitation energy transfer between dye and molecular oxygen caused the singlet oxygen formation. It was also found out that for TTP–CONH–Pro– COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> the  $S_{\Delta}$  value is about 0.81 confirmed that the triplet state of this dye could be still able to participate directly (not only mediated by singlet oxygen) in photodynamic reaction. The result shows as small structural differences in porphyrins provide to the differentiations in their spectroscopic behaviour and therefore can affect the dye's effectiveness of photodynamic ability.

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