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Dedicated to Professor Dr. ALEXANDRU T. BALABAN, member of the Roumanian Academy on the occasion of his 75th anniversary

4-(3-(PHTALHYDRAZIDE) AZO)N-PHENYLAZA-15-CROWN-5. SYNTESIS AND PROPERTIES

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By diazotization of luminol **1** and then coupling the so-formed diazonium salt with amine **2** (N-phenylaza-15-crown-5) resulted compound **3** (4-(3-(phtalhydrazide) azo) N-phenylaza-15-crown-5) whose physical properties were investigated by spectral analysis (NMR, IR and VIS). The ^{1}H-NMR spectra in the presence of TFA at different temperatures proved the quaternary character of the nitrogen from the macrocycle moiety in acidic medium, and allowed the determination of the free energy of the rotation barrier around the double bond that was formed (ΔG^* = 15.2 kcal mol^{-1}). Compound **3** is soluble in organic solvents only and the solution colour is red (a relative positive solvatochromy depending on the solvent polarity was noticed). Though compound **3** is insoluble in water, it is soluble in acidic and alkaline solutions with colour depending on the medium character (red to violet in acidic medium and yellow in alkaline medium). In contrast with compound **1**, compound **3** is not fluorescent. Compound **3** is a relatively weak acid with pK_a = 6.7 in water.

INTRODUCTION

Nowadays, combinatorial chemistry[1-3] offers elegant means to design and subsequently obtain by synthesis some chemical compounds having directed structural design in terms of their physico-chemical properties and application-oriented. Chemical organic reagents, by themselves, benefit from these kinds of specific strategies of combinatorial chemistry. Crown ethers moieties present in the structure of the chemical compounds enforce their known physico-chemical properties and typical application characteristics.[4] Different synthesis strategies are known based on anchoring crown ethers moieties on various molecules,[5,6] so that the whole assembly may exhibit directed physico-chemical properties (*e.g.*, reversibility of acidic-basic processes, solvatochromy, complexation of metallic ions, and so on).

Luminol **1** (5-amino-2,3-dihydro-1,4-phtalazine-dione or 3-aminophatalic hydrazide) is a fluorescent compound that has been known for a long time (ever since 1928) with important applications as a reagent for chemiluminescence in the determination of oxidant chemical species and some ions[7-13] (*e.g*, copper, iron). Linking of a crown ether to **1** is accomplished due to both the groups of **1** and the structure of the molecule that carries the crown ether moiety. In this respect, the azo group may represent an important arm of the bond between **1** and the crown ether, which gives chromogenic and ionophoric properties as a consequence of the molecular design. Such a strategy was pursued for anchoring different moieties[4,5] to crown ether **2** (N-phenylaza-15-crown-5 or N-phenyl-13-aza-1,4,7,10-tetraoxacyclopentadecan, respectively), which is known for its complexant properties.[14] By diazotization of compound **1,** subsequently followed by coupling with crown ether **2**, compound **3** (4-(3-(phtalhydrazide) azo) N-phenylaza-15-crown-5) was obtained.

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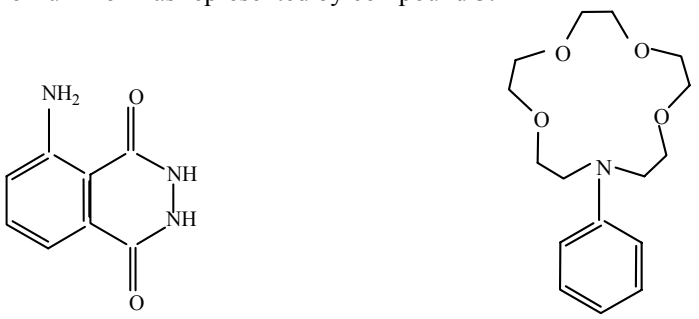
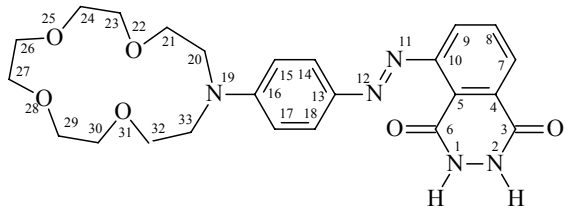
This study aims at presenting the results obtained in the synthesis and physico-chemical properties of the new derivate of luminol **1** as represented by compound **3**.



1 **2**

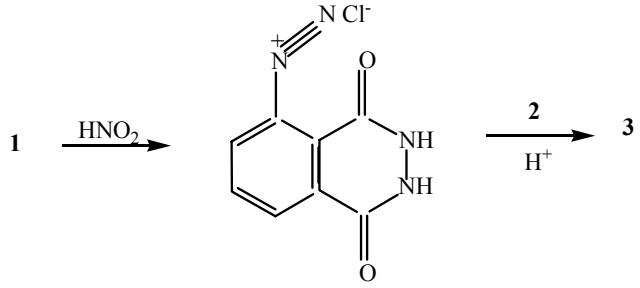


3

RESULTS AND DISCUSSION

Synthesis of compound 3

The synthesis of compound **3** was carried out by diazotization of **1**, followed by coupling in acidic medium of the so-formed diazonium salt with amine **2** (Scheme 1). Compound **3** precipitated from the reaction mixture, then it was isolated and purified by preparative thin layer chromatography (TLC) with a reasonable yield (61%). The structure of compound **3** was proved by IR and NMR, whereas its properties were investigated also by electronic spectra in the VIS domain.



Scheme 1– Synthesis of compound **3.**

IR spectra of compound 3

The FTIR (ATR-PIKE) spectrum for compound **3** proves the proposed structure for compound **3** ($\nu_{C\text{-}O\text{-}C}$ 1100 cm^{-1}, $\nu_{C=O}$ 1653 cm^{-1}).

NMR spectra of compound 3

NMR data of compound **3** are shown in Table1.

Table 1

The ^{1}H- and ^{13}C-NMR data of compound **3**

NMR-spectra in $CDCl_3$[a] (δ, ppm, *J* Hz)
^{1}H-NMR: 12.41(bl, 1H, deuterable, H-1 or H-2); 9.81(bs, 1H, deuterable, H-1 or H-2); 8.44(dd, 1H, H-9, 1.2, 7.8); 8.26(dd, 1H, H-7, 1.2, 7.8); 7.83(t, 1H, H-8, 7.8); 7.64(d, 2H, H-14-18, 9.1); 6.74(d, 2H, H-15-17, 9.1); 3.89(t, 4H, H-20-33, 6.6); 3.75(m, 12H, H-21-32, H-23-30, H-24-29); 3.70(s, 4H, H-26 **^{13}C-NMR**: 158.73(Cq); 152.61(Cq); 152.17(Cq); 147.66(Cq); 142.24(Cq); 131.94(CH); 130.39(Cq); 128.52(CH); 126.92(CH); 126.81(Cq); 119.43(CH); 112.16(CH); 71.33(CH_2); 70.29(CH_2); 69.85(CH_2); 68.17(CH_2); 53.54(CH_2).
NMR-spectra in $CDCl_3$+TFA (δ, ppm, *J* Hz)
^{1}H-NMR($CDCl_3$+TFA, δ ppm, *J* Hz): 8.53(dd, 1H, H-9, 1.0, 8.2); 8.28(bd, 1H, H-7, 8.2); 8.12(t, 1H, H-8, 8.2); 7.85(bd, 1H, H-14-18, 9.6); 7.38(vbd, 1H, H-15 or H-17); 7.18(vbd, 1H, H-15 or H-17); 4.16(bt, 4H,H-20-33, 5.8); 4.01(bt, 4H, H-21-32, 5.8); 3.81(s, 8H, H-23-24-29-30); 3.80(s, 4H, H-26-27).

[a] At about 295 K and with TMS as internal standard.

The ^{1}H- and ^{13}C-NMR spectra of compound **3** (Table 1) prove the presence of the phtalhydrazide and phenylaza-15-crown-5 moieties, respectively. Uneven H-1 and H-2 indicates different unshielding of the two H by the substitutes. Addition of trifluoroacetic acid (TFA) leads to changes in the signals of the protons H-15 and H-17, which show up in this case as larger doublets and have different chemical shift (δ) values (Table 1). This behavior indicates the quaternary character of N^{19}, and the process of equivalence for positions 15 and 17 is realized by thermo-activation. In this case, the free energy of activation (ΔG) of rotation around the C^{16}-N^{19} bond can be calculated by recording the ^{1}H-NMR spectra at various temperature values (Table 2). This procedure allows the estimation of the coalescence temperature, T_C=37.5°C (310.5 K), as shown in Fig. 1.

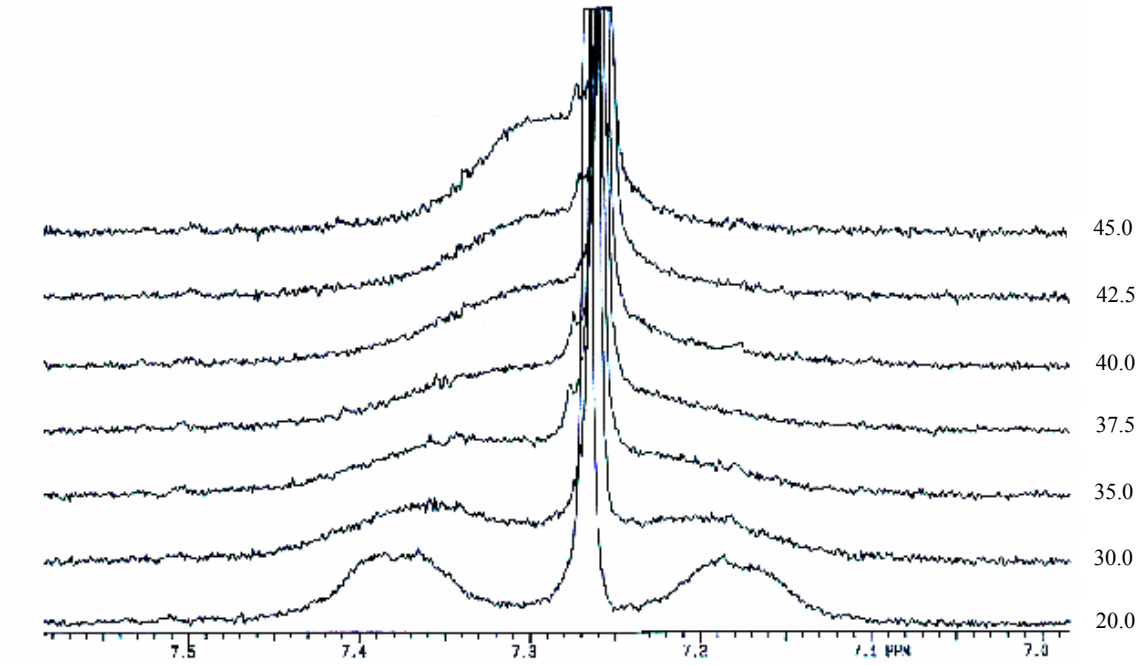


Fig. 1 – ^{1}H-NMR spectra (for the H-15 and H-17 protons) of compound **3** in the presence of TFA at different temperatures (°C).

By recording the ^{1}H-NMR spectra in the presence of TFA in the range of 20 to 90 °C, we first determined the coalescence temperature for the H-15 and H-17 protons ($H^{15} \Leftrightarrow H^{17}$), T_C =37.5°C (H^{15}=7.38 ppm, ν^{15}=2214 Hz; H^{17}=7.18 ppm, ν^{17}=2152 Hz; Δν= 62 Hz). At T_C, the signal of these protons is completely flat, whereas for higher temperature values, one single signal pops up representing the average of the two signals recorded at lower temperature value (20°C), yet larger

due to a limited cycle mobility. At 64°C sharp triplet signals pop up for H-20-33 (δ=4.15 ppm) and H-21-32 (δ=3.99 ppm) with vicinal coupling constant ^3J=5.6 Hz. Likewise, at the same temperature value, singlet signals pop up corresponding to the protons of the methylene groups of the macrocycle (the equivalence is due to molecule mobility at this temperature) having δ=3.78 ppm (H-23-24-29-30) and δ=3.75 ppm (H-26-28) values, respectively.

Table 2

The ^{1}H- and ^{13}C-NMR data of compound **3** in the presence of TFA at variable temperature[a]

NMR-spectra in DMSO-D_6 (δ, ppm, *J* Hz, **T=90^{0}C**)
^{1}H-NMR: 11.57 (s, 2H, H-1-2);8.24(bd, 1H, H-9, 7.7); 8.07 (bs, 1H, H-7); 7.90 (t, 1H, H-8, 7.7); 7.77 (d, 2H, H-14-18, 9.3); 6.81(d, 2H, H-15-17, 9.3); 3.74 (m, 8H, H-20-21-32-33); 3.59 (m, 8H, H-23-24-29-30); 3.53 (s, 4H, H-26-27)
NMR-spectra in DMSO-D_6 (δ, ppm, *J* Hz, **T=70^{0}C**)
^{1}H-NMR: 11.66 (s, 2H, H-1-2); 8.24(bd, 1H, H-9, 7.2); 8.09(vbs, 1H, H-7); 7.91 (t, 1H, H-8, 7.9); 7.77 (d, 2H, H-14-18, 9.3); 6.94(d, 2H, H-15-17, 9.3); 3.73 (m, 8H, H-20-21-32-33); 3.58 (m, 8H, H-23-24-29-30); 3.53 (s, 4H, H-26-27)
NMR-spectra in DMSO-D_6 (δ, ppm, *J* Hz, **T=50^{0}C**)
^{1}H-NMR: 11.77 (bs, 2H, H-1-2); 8.15-8-24(vbs, 2H, H-7-9); 7.92 (t, 1H, H-8, 7.9); 7.77 (d, 2H, H-14-18, 8.8); 6.95(d, 2H, H-15-17, 8.8); 3.73 (m, 8H, H-20-21-32-33); 3.58 (m, 8H, H-23-24-29-30); 3.52 (s, 4H, H-26-27)
NMR-spectra in DMSO-D_6 (δ, ppm, *J* Hz, **T=20^{0}C**)
^{1}H-NMR: 11.98 (s, 1H, H-1 or HY-2, deuterable); 11.90 (1, 1H, H-2 or H-1, deuterable); 8.27(bs, 1H, H-9); 8.20 (vbd, 1H, H-7);7.94 (t, 1H, H-8, 8.1); 7.78 (d, 2H, H-14-18, 8.8); 6.96(bd, 2H, H-15-17, 8.8); 3.72 (bs, 8H, H-20-21-32-33); 3.57 (m, 8H, H-23-24-29-30); 3.52 (s, 4H, H-26-27)

[a] With TMS as internal standard.

Eyring's formula[15] eq(s) was used to calculate the energy barrier at T_C:

$$k = \chi \frac{k_B T}{h} e^{-\Delta G^* / RT} \quad (1)$$

where: k_B = Boltzmann's constant (3.2995×10 $^{-24}$ [cal K^{-1}]);

χ= the transmission coefficient (usually 1);

h = Planck's constant (1.5836×10^{-34} [cal s] = 6.6256×10^{-34} [J s]);

ΔG^* = 4.575 T(10.319 + logT/k_C) [cal/mol]

ΔG^*= 15.2 kcal mol^{-1}.

Electronic spectra of compound 3

Compound **3** is insoluble in water, though soluble in organic solvents. In contrast with luminol **1**, compound **3** is not fluorescent but it is dark coloured. Thus, in the solid state, it has a red-brick colour, while in organic solvents, its colour turns from red to violet. Compound **3** is solved either in acidic or alkaline water; its colour turns to red-violet in the first case, and to yellow in the latter case. Such a behavior required supplementary investigations reported hereafter.

In Table 3, the spectral behavior (λ_{max} and ε) is presented in the visible domain (VIS) of compound **3** in five solvents having different values of the Dimroth-Reichardt parameter[16] E_T(30).

Table 3

VIS spectral data of compound **3**

Solvent	E_T(30)[kcal/mol][a]	λ[nm]	ε ×105 (logε)
Methanol	55.5	510	0.271
Dimethyl-Sulfoxide	45.0	521	0.197
1,2-Dichloroethane	41.9	511	0.177
Dichloromethane	41.1	509	0.343
Chloroform	39.1	507	0.265

[a]Dimroth-Reichardt parameters[16]

The experimental results presented in Table 3 show a relative correlation between the polarity of the solvent, the $E_T(30)$ parameter, and a positive solvatochromy along with an increase in the solvent polarity. The maximum value of λ_{max} is attained in DMSO.

Further on, the VIS spectrum of compound **3** in aqueous alkaline or acidic solutions was investigated (Fig.1) and compared with its counterpart in methylene chloride (λ_{max}=511 nm). In both cases, a change in colour occurred: hypsochromatic in alkaline medium (λ_{max}=426 nm), and bathochromatic in acidic medium (λ_{max}=534 nm).

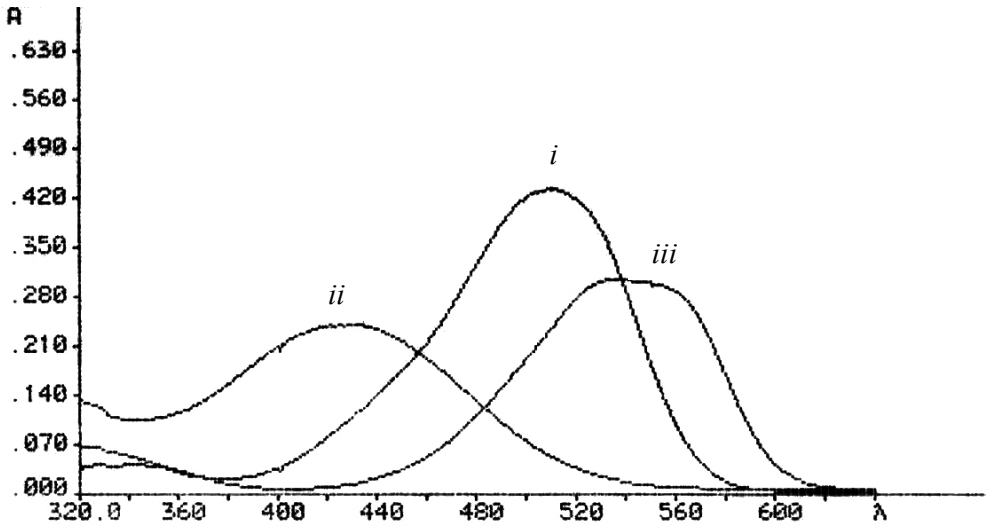
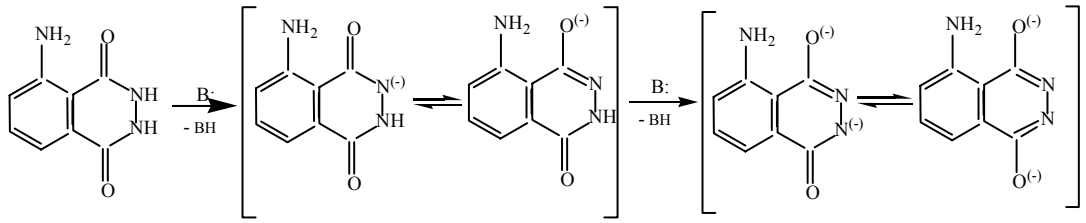


Fig. 2 – The VIS spectra of compound **3**: *i*= in CH_2Cl_2 (λ_{max}=511 nm) ; *ii*= in NaOH 1 N ; (λ_{max}=426 nm) ; *iii*= in HCl 1N (λ_{max}=534 nm).

As already known,[9,17,18] luminol **1** is a bibasic acid: the monoanion **4** is formed in water, and the dianion is formed in aprotic medium.[9] The anions **4** and **5**, due to their structure, may exhibit the corresponding forms[9] presented in Scheme 2. In acidic medium, luminol **1**, due to the presence of the NH_2 group, goes through in the form of chlorhidrate.

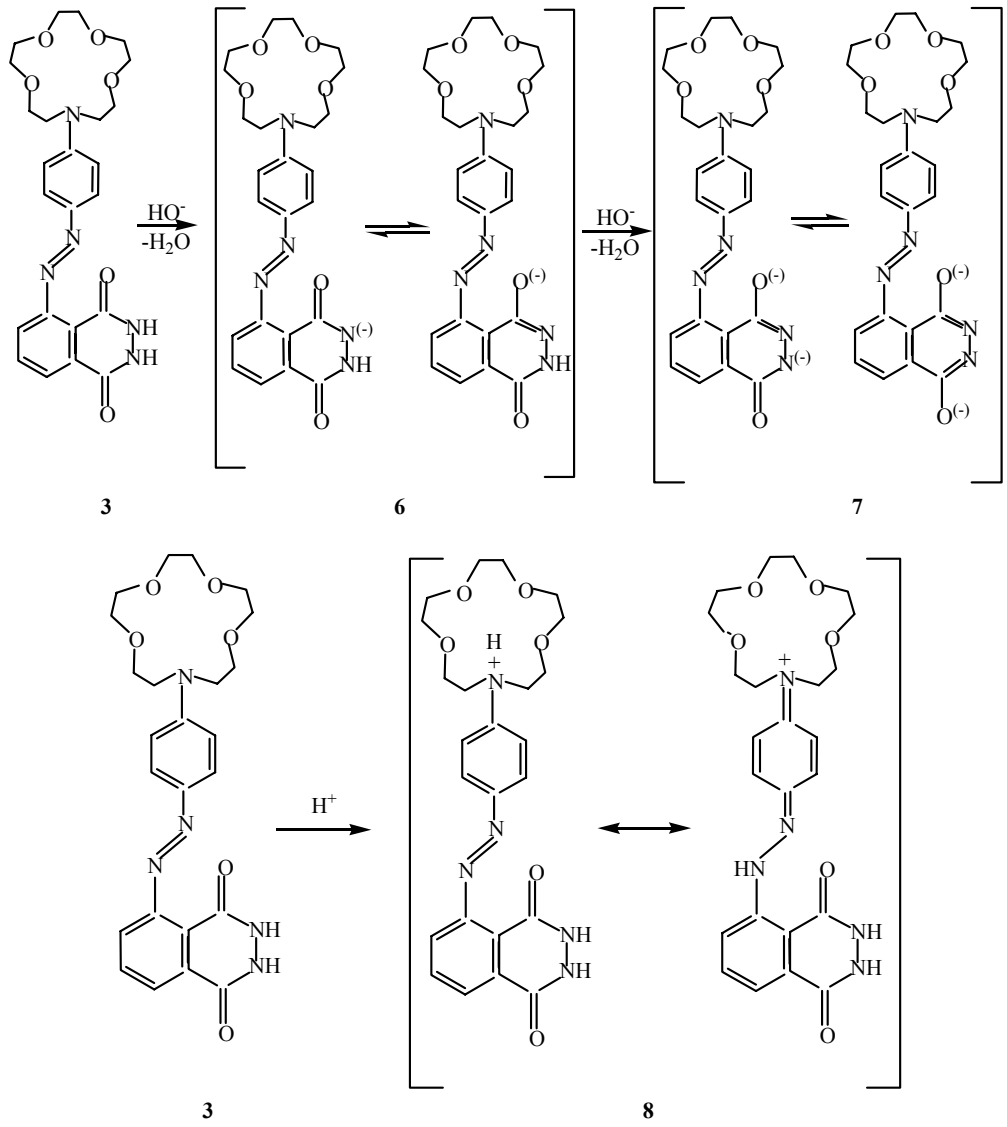
Compound **3** that contains the aza-15-C-5 moiety, is structurally similar with a series of known colorants (azoderivatives) like methylorange or methyl red, which contain the diethylamine group. Likewise, the structure of compound **3** is expected to present acidic-basic properties like compound **1**, and also properties enforced by the aza-15-crown-5 moiety, and similarities to the azo-colorants mentioned above.



1 **4** **5**

Scheme 2 – The behavior of compound **1** in alkaline medium.

The colour change of compound **3** in acidic or alkaline media (Fig. 2) can be explained by: (*a*) the cationic species **8** formed in acidic medium; (*b*) the two anionic forms **6** and **7** formed in alkaline medium (Scheme 3). The cationic form **8** may lead to other species by extended electronic conjugation (n+π) with phtalhydrazide moiety, which explains the bathochromic shift in acidic medium (λ_{max}=534 nm). Similarly to compound **1**, the anionic species **6** and **7**, may present corresponding forms (Scheme 3).



Scheme 3 – The behavior of compound **3** in alkaline (formation of anions **6** and **7**) and in acidic media (formation of cation **8**), respectively.

The acidity of compound **3** was estimated by spectrophotometric titrations in water for the first step of ionization (monoanion **6**), giving $pK_a = 6.7$.

The chromogenic properties of compound **3** in acidic and alkaline media may confer analytical applications first as an indicator, whereas the presence of the crown ether moiety may induce new properties which will constitute the subject of our future contributions in the field.

CONCLUSION

A new derivate of luminol **1** was synthesized, namely, the azoderivative **3** (4-(3-(phtalhydrazide) azo) N-phenylaza-15-crown-5). Its structure was confirmed by IR and NMR investigations. The NMR measurements performed at controlled values of temperature in the presence of TFA proved the quaternary character of the macrocylic nitrogen in acidic medium, and allowed the estimation of the free energy value ($\Delta G^* = 15.2$ kcal mol^{-1}), of the rotation of the C^{16}-N^{19} bond. In contrast to luminol **1**, which is fluorescent, compound **3** is not fluorescent, but it is intensely coloured in red-brick. Compound **3**, soluble in organic

solvents only, exhibits a relative positive solvatochromy, depending on the solvent polarity. In acidic and alkaline aqueous solutions, compound **3** is soluble, its colour turning from red to violet and yellow, respectively. The acidity of compound **3** was found to be $pK_a = 6.7$ in water.

EXPERIMENTAL

Starting compounds for synthesis and materials: luminol **1** (Aldrich), crown ether **2** and silica gel plates 60 GF_{254} for TLC (Merck).

^1H- and ^{13}C-NMR Spectra were recorded with a Varian Gemini 300 BB spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C, respectively); VIS spectra were recorded with Double Beam UV-VIS Specord M400 Carl Zeiss Jena; IR spectra were recorded with FTIR Spectrofotometer Bruker – Model Vector 33, using the ATR-PIKE technique.

Synthesis of compound 3 *(4-(3-(phtalhydrazide) azo) N-phenylaza-15-crown-5)*

Luminol **1** (0,108 g, 67 $\times 10^{-5}$ moles) was dissolved in 10 mL HCl 1N by stirring at 50-60^{0}C. After cooling down, the obtained solution was further cooled down at the exterior by means of ice and salt. While intensively stirring and cooling down externally (by ice and salt), an aqueous solution of $NaNO_2$ (0.0466 g, 67.5 $\times 10^{-5}$ moles in 10 mL water) was dropwise added for 1 hour. The reaction mixture gradually turned to red. After other 20 minutes of stirring and cooling, small amounts of amonium sulphamate in the solid state were added until the effervescence ceased. Finally, the pH of the solution was acidic. Further on, solid sodium acetate (in excess) was gradually added until the color became dark red-brick colour. Then 0.2 g (67.7×10^{-5} moles) of crown ether **2** dissolved in 10 mL HCl 1 N were added under strong stirring and external cooling, followed by addition of solid sodium acetate (in excess). The reaction mixture was saturated while stirring by solid NaCl at room temperature, resulting a suspension in solution, and left during the night in the fridge. Next, the mixture was filtered (G3), the precipitate was washed part by part by water on the filter, and subsequently dried ($CaCl_2$ anhydrous). A crude compound **3** resulted (66% yield), which was purified by preparative TLC (silica gel GF254, methylene chloride-methanol, 10:0,4, v/v), resulting pure compound **3** (61% yield): red-brick colour solid material, m.p. 121-123^{0}C; Anal. Calcd for $C_{24}H_{29}N_5O_6$: C, 59.61; H, 6.04; N, 14.48%; found C, 59.34; H, 5.84; N, 14.23 %; FTIR (ATR-PIKE) : 1100 cm^{-1} (ν_{C-O-C}), 1653 cm^{-1}($\nu_{C=O}$); for ^1H-and ^{13}C-NMR data see Tables 1 and 2 ; for the VIS spectra data see Table 3.

VIS spectral measurements

The VIS spectra for compound **3** were carried out in organic solvents (Table 3) and in alkaline and acidic solutions (Fig. 1).

The pKa value was determined by spectrophotometric measurements in aqueous solution so that compound 3 was dissolved in NaOH 1N ($c = 1.2 \times 10^{-5}$ M) during spectral monitoring with pH value determination. The obtained values for both absorbance and pH were employed for computing the pKa value by cq (2).[19]

$$pK_a = pH - \log \frac{A_b - A}{A - A_a} \quad (2)$$

where: A = absorbance in methylene chloride;
A_a = absorbance in acidic medium;
A_b = absorbance in alkaline medium.

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