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Preparation and Photophysics of 2-(1-Pyrenyl)acrylic Acid and Its Methyl and 2′,2′,6′,6′-Tetramethyl-4′-Piperidyl Esters

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Novel probes represented connection of pyrene as chromophore and sterically hindered amine stabilizers (HAS) in the form of esters of 2-(1-pyrenyl)acrylic acid were synthesized. HAS was in the form of parent amine (PAP) as well as stable nitroxyl radical form (PAP-NO$^{\cdot}$). Photophysics of these probes were compared with their precursor as 2-(1-pyrenyl)acrylic acid (PAA) and its methyl ester (PAM). The fluorescence spectrum of PAA strongly depends on the acidity of the solution. The spectrum in neutral methanol indicates that it originates from the anionic form –COO$^-$. Changes of acidity or basicity of methanol solution resulted in the changes of shape, position as well as the intensity of fluorescence band. This is due to the presence of protolytic equilibria, either in the ground state or in the singlet excited state, leading to the formation of molecular form –COOH and the cationic form –COOH$_2{}^+$. The ester analogues did not show any changes in various pH conditions. Fluorescence of all probes depends on the polarity of solvents and the presence of oxygen. Intermolecular quenching was studied with external quenchers TEMPO and oxygen and the data were compared with the intramolecular quenching using 1′-oxo-2′,2′,6′,6′-tetramethyl-4′-piperidinyl-2-(1-pyrenyl)acrylate (PAP-NO$^{\cdot}$).

KEY WORDS: Fluorescence probes; 2-(1-pyrenyl)acrylic acid; 2′,2′,6′,6′-tetramethyl-4′-piperidinyl-2-(1-pyrenyl)acrylate; quenching; stable nitroxyl radicals.

INTRODUCTION

Fluorescence probes based on chromophores of different types are widely used for monitoring various processes and functions on the microscopic as well as the macroscopic levels [1]. The photo-induced separation of charge in electron donor–acceptor molecules plays a crucial role in photochemistry as well as in photobiology. A change in either the strength of the donor or acceptor group and a change in the polarity of the medium allow the modification of the excited state features of these systems in a well-controlled manner.

The unique photophysical properties of pyrene and its derivatives in different media enable this chromophore to play an important role in the characterization of microenvironments [2,3]. Pyrene fluorescence exhibits a change in the relative intensities of its vibrational bands, which depends on the polarity of the solvent [3,4]. Another feature of the pyrene derivatives concerns their ability to form inter- or intramolecular homo- and heterodimer in the excited states (excimers, exciplexes) and their long lifetimes in non-polar media (ca. 400 ns) [5]. Vinyl pyrene derivatives represent probes with conjugation of the chromophore and the vinyl double bond. They are precursors of monomers in order to obtain specific polymers with pyrene fluorophores attached to the polymer backbones [6,7]. Pyrene acrylic acid and its methyl ester were used as probes to monitor polymerization and surface properties by spectroscopic method [8].

Stable free nitroxyl radicals attract attention for several reasons. They play an important role in the

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mechanism of light and partially heat stabilization of polymers, mainly polyolefins by hindered amine stabilizers (HAS) [9]. Stable free nitroxyl radicals are produced in the course of stabilization from their precursor parent amine–HAS. Nitroxyl radicals are able to react with alkyl radicals under production of amino ether, which are able to convert to starting nitroxyl radical by reaction with alkylperoxyl radicals. Each molecule is able to break several radical chains behaving as "inhibition catalyst". The free nitroxyl radicals influence the photophysical and photochemical processes due to their paramagnetic effect [10–12]. Quenching of singlet and triplet states by nitroxyl radicals was studied in detail.

Lately fluorescent probes in which simple aromatic chromophores were combined with free radicals of *N*-oxyl type were prepared. The presence of the paramagnetic moiety was proved by ESR measurements [15–18]. Formation or decay of the free radical is connected with switching off or on the chromophore emission as a result of intramolecular quenching [13–18].

Derivatives from 2-(1-pyrenyl)acrylic acid were chosen in this study because the molecules consist of a pyrene chromophore, a vinyl double bond and four different derivatives of carboxylic acid. We compared the spectroscopic features of free acid and its methyl ester as well as 2,2,6,6-tertramethyl-4-piperidyl ester in the form of parent amine and stable nitroxyl radical. All this functionalities contribute to the knowledge of photophysical and photochemical properties, which are influenced by the polarity of solvent.

EXPERIMENTAL

Methods

FT-IR spectra were recorded on Impact 400 (Nicolet, USA). ESR spectrum was measured with X-band spectrometer E-4 Varian (USA) interfaced on PC with program Symphonia Bruker. ^{1}H NMR spectra were measured with Bruker 300 MHz spectrometer and MALDI-TOF measurements were performed on Kratos Compact MALDI-TOF II (Shimadzu).

Absorption spectra were measured with UV 160 (Shimadzu) spectrophotometer. Fluorescence spectra were recorded either on a Perkin-Elmer MPF-3L spectroflurometer (slits 4 nm for excitation and 6 nm for emission, 1P28 multiplier) or Perkin-Elmer LS 55 (slits 5 nm for excitation and 5 nm for emission, R928 multiplier). Contrary to the former, the latter spectrofluorometer permits to obtain corrected spectra. The MPF-3L is connected through interface and A/D converter to an ISA slot of PC using ORPHY GTI program for data collection. Spectra were taken at an excitation wavelength of 360 nm. Measurements in argon atmosphere were performed in septum-closed cell after 20 min bubbling. For quenching experiments, a solution of quencher was added with micro syringe. The pH of the solutions was adjusted by adding either concentrated hydrochloric acid or sodium hydroxide. It is important to note that since the pH scale in methanol is completely different from that obtained in water [19,20], the pH ($-\log [H^+]$) values are given as indication for the acidic and basic character of the solution.

Synthesis

The pyrene derivatives were prepared according to Scheme 1.

2-(1-Pyrenyl)acrylic Acid (PAA)

To stirred mixture of 1-pyrene-carboxaldehyde (4 g, 17.4 mmol), pyridine (4 mL) and piperidine (0.3 mL) malonic acid (1.7 g, 16.4 mmol) was added at 70°C. The reaction mixture was then refluxed for 2 hr (becoming inhomogeneous). After this time the temperature was increased to 150°C for 5 min. Crystals formed after cooling were filtered, washed with 0.5 M HCl (50 mL) and dried. The crude product was crystallized from EtOAc to yield 3.1 g (65%) of yellow crystals with melting point (m.p.) 271–275°C (lit [8] m.p. 279–281°C).

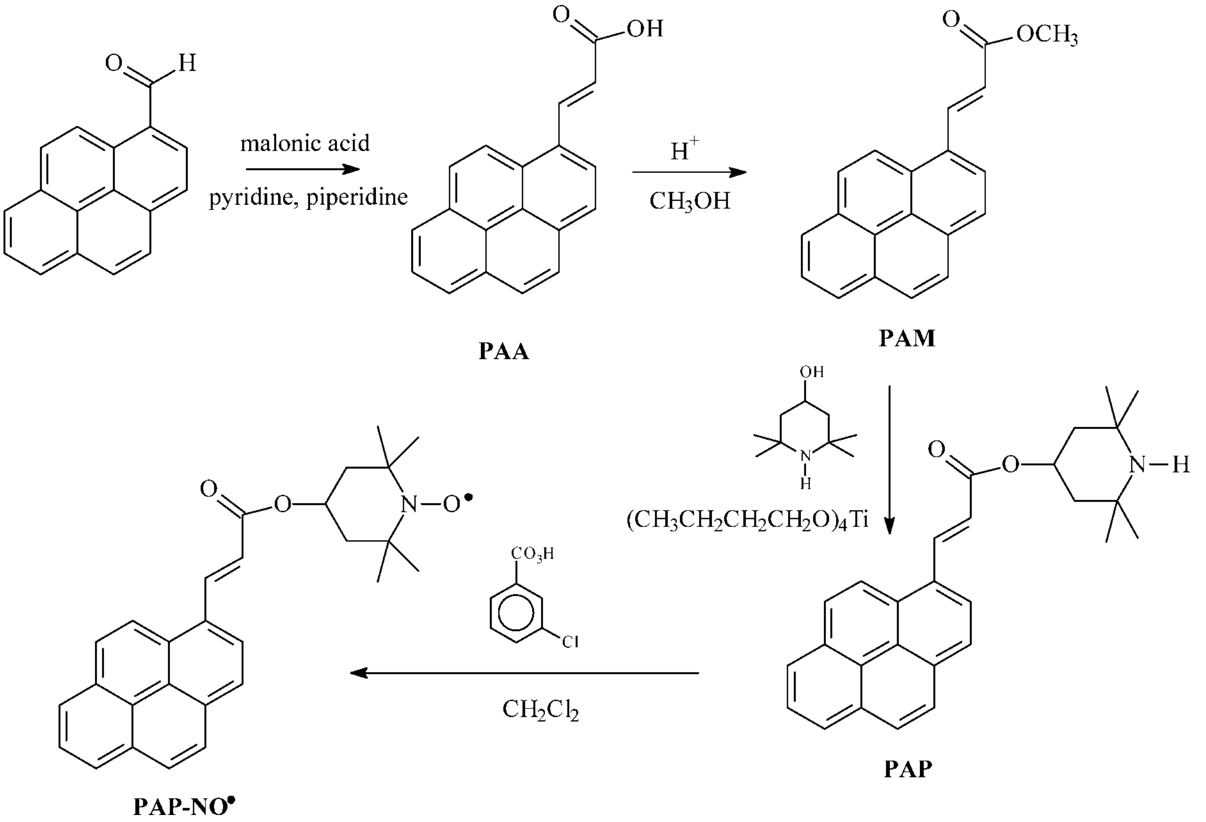
^{1}H NMR (DMSO) δ: 6.85 (d, 1H, $=$CH$-$COO, $J = 15.7$ Hz), 8.10–8.60 (m, 9H, pyrene), 8.70 (d, 1H, $=$CH-pyrene, $J = 15.8$ Hz).

FTIR (KBr), (cm^{-1}): 1613 (C$=$C), 1687 (C$=$O), 845 (pyrene).

Methyl-2-(1-Pyrenyl)acrylate (PAM)

A gaseous hydrochloride was bubbled through the stirred solution of 2-(1-pyrenyl) acrylic acid (2 g, 7.4 mmol) in MeOH (50 mL) under reflux during 3 hr. The mixture was then cooled, water was added and the mixture was extracted with diethyl ether. The organic portion was dried over Na_2SO_4 and the solvent was removed. Crude product was purified by column chromatography (silica gel/toluene). The eluent was than evaporated to give 1.78 g (70%) of yellow final product with m.p. 141–144°C (lit [8] m.p. 144–145°C).

^{1}H NMR ($CDCl_3$) δ: 3.80 (s, 3H, CH_3–O–CO), 6.65 (d, 1H, $=$CH–COO, $J = 15.8$ Hz), 8.00–8.45 (m, 9H, pyrene), 8.80 (d, 1H, $=$CH-pyrene, $J = 15.7$ Hz).



Scheme 1.

MALDI-TOF (Matrix 1,8,9-THA): 286 (100%), 277, 248, 242, 211, 176, 84.

FTIR (KBr) (cm^{-1}): 1707 (C=O), 1618 (C=C), 1437 (CH$_3$), 1177 (C–O), 845 (pyrene).

2′,2′,6′,6′-Tetramethyl-4′-Piperidinyl-2-(1-Pyrenyl)acrylate (PAP)

To the solution of methyl-2-(1-pyrenyl)acrylate (0.5 g, 1.8 mmol) in dried toluene (25 mL). 4-Hydroxy-2,2,6,6-tetramethylpiperidine (0.3 g, 1.9 mmol) and two drops of titanium(IV)-*n*-butoxide as a catalyst were added. The mixture was refluxed for 30 hr. After this time another portion of 2,2,6,6-tetramethylpiperidine-4-ol (0.3 g, 1.9 mmol) was added and the reaction mixture was then refluxed for additional 100 hr. Dichloromethane (20 mL) was added to cooled reaction mixture and the organic layer was washed with water (3 x 20 mL), dried over Na_2SO_4 and the solvent was removed by using the vacuum rotary evaporator. Yellow solid was purified by column chromatography using a mixture of benzene and methanol 4:1 to give, after removing the solvents, 0.46 g (64%) yellow powder with m.p. 179–182°C.

^{1}H NMR (CDCl$_3$,) δ: 1.20 (s, 6H, 2CH$_3$ 2 + 6 axial), 1.30 (s, 6H, 2CH$_3$ 2 + 6 equat.) 1.40 (m, 2H, CH$_2$ 3 + 5 axial.), 2.05 (m, 2H, CH$_2$ 3 + 5 equat.), 5.35 (m, 1H, CH—O), 6.65 (d, 1H, =CH—COO, J = 15.7 Hz), 8.00–8.45 (m, 9H, pyrene), 8.80 (d, 1H, =CH-pyrene, J = 15.7 Hz).

MALDI-TOF (Matrix nitroaniline): 412 (100%), 371, 299, 269, 229, 200.

FTIR (KBr, ν/cm^{-1}): 1697 (C=O), 1616 (C=C), 1179 (C—O), 844 (pyrene).

1′-Oxo-2′,2′,6′,6′-Tetramethyl-4′-Piperidinyl-2-(1-Pyrenyl)acrylate (PAP-NO$^{\cdot}$)

A solution of 2′,2′,6′,6′-tetramethyl-4′-piperidinyl-2-(1-pyrenyl)acrylate (0.8 g, 2 mmol) in CH$_2$Cl$_2$ (20 mL) was cooled to 0°C. Under vigorous stirring, 3-chloroperbenzoic acid (1.4 g, 8 mmol) was added slowly in very small portions over about 10 min. The reaction mixture was stirred for 4 hr at room temperature. The reaction mixture was then washed with water (2 x 20 mL), 5% NaHCO$_3$ (2 x 20 mL) and dried over Na_2SO_4. Solvent was removed by the vacuum rotary evaporation. The crude product was purified by column chromatography using a mixture of toluene and methanol 5:1 to give the orange crystals. Yield was 0.61 g (74%), m.p. 176–181°C.

The purity has been proved by TL chromatography and ESR spectroscopy.

RESULTS AND DISCUSSION

Synthesis

The route to the synthesis of 1-pyrenylacrylic acid (PAA), its methyl ester (PAM) and 2′,2′,6′,6′-tetramethyl-4′-piperidyl ester (PAP) in the form of parent amine and stable *N*-oxyl radical (PAP-NO$^{\cdot}$) involves four steps (Scheme 1). PAA was synthesized by the reaction of 1-pyrene-carboxaldehyde and malonic acid in pyridine in the presence of small amount of piperidine. The product was crystallized from ethyl acetate. Methyl-2-(1-pyrenyl)acrylate (PAM) was prepared by esterification of the PAA in methanol under the catalysis of HCl. Crude product was purified by column chromatography and final product was obtained with a high purity and a good yield.

The sensitivity of sterically hindered amine as 4-hydroxy-2,2,6,6-tetramethyl-piperidine to acids excluded the preparation of the respective ester by the standard method used for preparation of methyl ester. Therefore, the 2′,2′,6′,6′-tetramethyl-4′-piperidinyl-2-(1-pyrenyl)acrylate (PAP) was prepared by reesterification of methyl ester in the presence of excess of 4-hydroxy-2,2,6,6-tetramethylpiperidine. The reaction is catalyzed by titanium(IV)-*n*-butoxide. Stable nitroxyl radical 1′-oxo-2′,2′,6′,6′-tetramethyl-4′-piperidinyl-2-(1-pyrenyl)acrylate (PAP-NO$^{\cdot}$) was prepared by careful oxidation of parent amine PAP with 3-chloroperoxybenzoic acid. The formation of PAP-NO$^{\cdot}$ was checked by ESR spectroscopy. Quantitative measurements were performed in benzene solutions ($c = 1.0 \times 10^{-3}$ mol L^{-1}). Integral of ESR spectra for the PAP-NO$^{\cdot}$ was compared with the integral of standard measured under the same conditions. As the standard 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl was used. The values of integrals appear to be proportional to the number of radicals indicating that the relative concentration c_r provides information about the amount of radical in the PAP-NO$^{\cdot}$. By assuming that the concentration of radicals in the standard is 100%, the value c_r found for PAP-NO$^{\cdot}$ was 95%. ESR spectrum of the PAP-NO$^{\cdot}$ as well as of the standard was triplet with equal line intensities. Substitution in position 4 of piperidine ring does not influence the paramagnetic centre [17]. The hyperfine splitting constants were evaluated as 1.53 mT for both radicals. To our knowledge the parent amine PAP and its oxidized derivative PAP-NO$^{\cdot}$ are newly prepared bifunctional probes.

Absorption and Fluorescence Spectra

Most pyrene derivatives exhibit different fluorescence spectra in various solvents. These changes refer to the ratio of intensities of vibrational bands, to the loss of vibrational structure or to the shift in position of emission bands. Two solvents with respect to the polarity were chosen, namely the polar methanol and non-polar

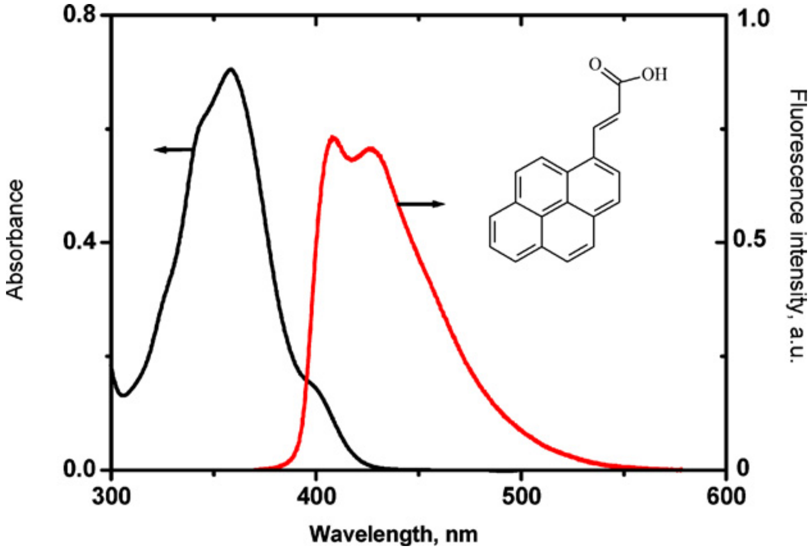


Fig. 1. Absorption and fluorescence spectra (non-corrected spectra, 2.0×10^{-6} mol L^{-1}, excitation at 360 nm, $A_{360} = 0.1$) of 2-(1-pyrenyl)acrylic acid (PAA) in neutral methanol solution.

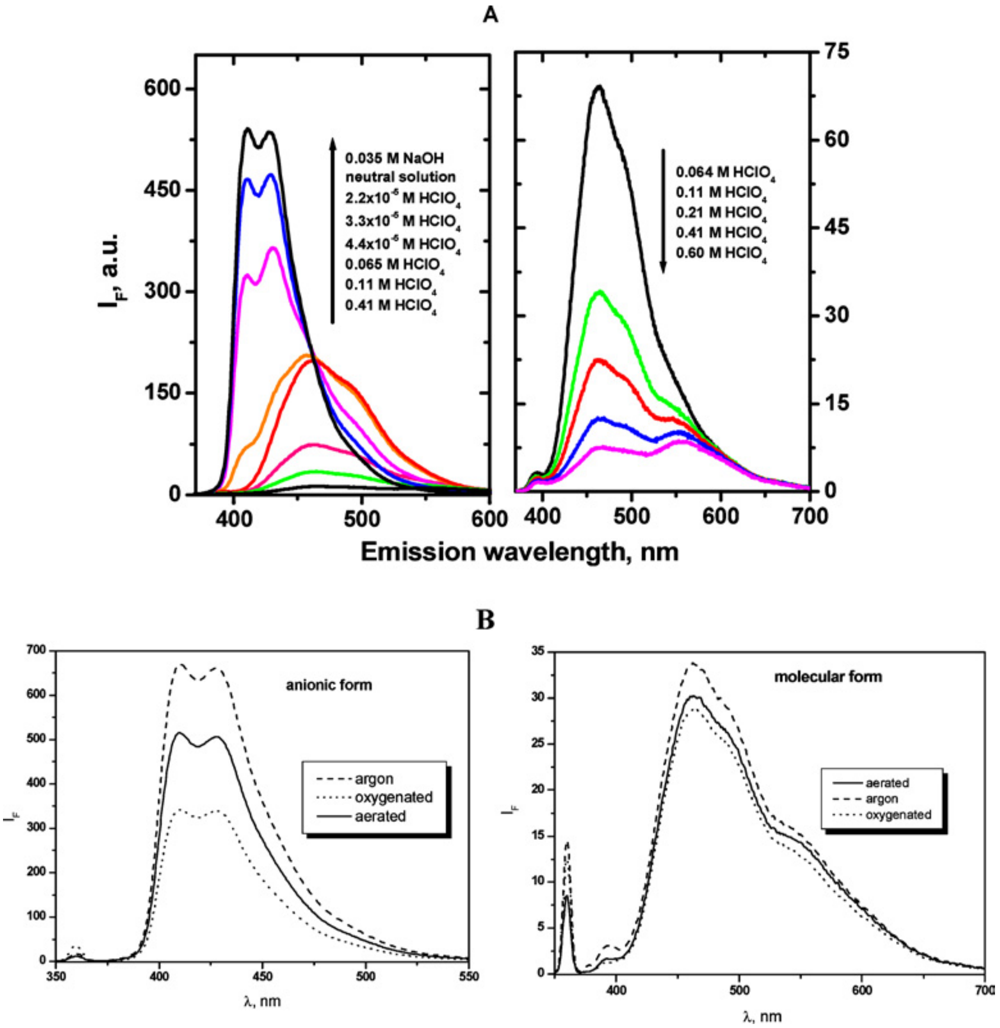


Fig. 2. (A) Evolution of the fluorescence spectra (corrected) of 2-(1-pyrenyl)acrylic acid (PAA, 2×10^{-6} mol L^{-1}) in methanol as a function of various pH values with excitation at 360 nm ($A_{360} = 0.1$). Left: basic and mildly acidic solutions. Right: acidic solutions. (B) Oxygen quenching of the fluorescence of the molecular and anionic forms (corrected spectra).

cyclohexane. Since PAA was already studied in cyclohexane solution [8], we undertook the study of its spectral features in methanolic solutions. Figure 1 presents the absorption and emission spectra of PAA in methanol and in neutral condition. Fluorescence spectrum with a maximum at 420 nm does not present a well-resolved vibrational structure as in the case of non-substituted and alkyl-substituted pyrenes [18]. The emitting state is more likely the mixture of π–π^* and n--π^* character states. The interaction between a polar solvent and the acid emphasized the contribution of the π–π^* state. This is the case of PAA in neutral methanolic solution (see Experimental section). As clearly observed in Fig. 2A, the addition of NaOH does not give significant change in the shape of the 420 nm fluorescence band when compared to that of the neutral solution. However, the addition of increasing amount of $HClO_4$ leads to important changes listed as follows.

- Decrease of the fluorescence intensity for the band with a maximum at 420 nm when the concentration of $HClO_4$ increases within 0 to 4.4×10^{-5} mol L^{-1} domain.
- As clearly shown in Fig. 2A (left) the fluorescence spectrum at a concentration of 4.4×10^{-5} mol L^{-1} presents a well-defined band with a maximum at 460 nm and without resolved vibrational structure is observed. The presence of a nice isobestic point permits to conclude that a protolytic equilibrium

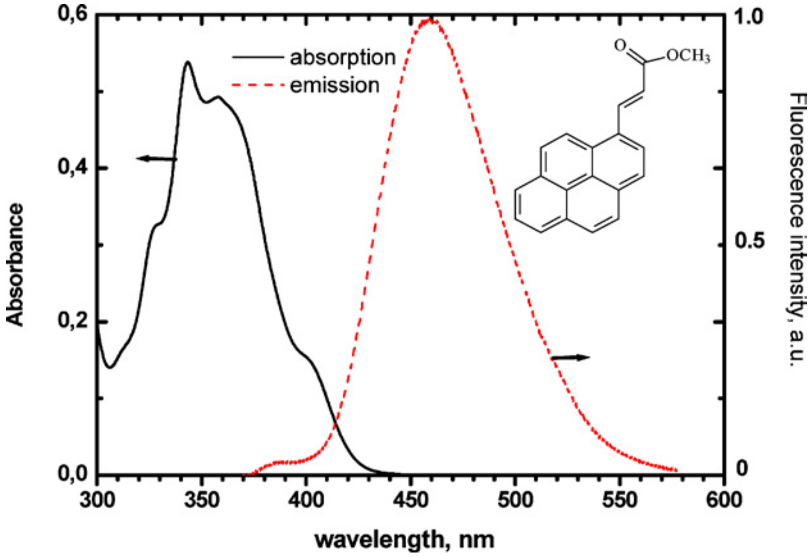


Fig. 3. Absorption and fluorescence spectrum (non-corrected) of methyl-2-(1-pyrenyl) acrylate (PAM, 2.0 × 10^{-6} mol L^{-1}) in methanol with excitation at 360 nm ($A_{360} = 0.1$).

occurs. The pKa for this observed equilibrium, obtained from the evolution of the fluorescence intensity as a function of pH, was found equal to 4.8 ± 0.5.

- For higher concentrations of perchloric acid (Fig. 2A, right), we observed an important decrease of the fluorescence band at 460 nm with a simultaneous increase of the fluorescence intensity at 555 nm. It has to be pointed out that this latter fluorescence shows a very weak response.

All esters of 2-(1-pyrenyl)acrylic acid revealed the same features of fluorescence spectra as can be seen in Figs. 3 and 4 for methyl ester of 2-(1-pyrenyl)acrylic

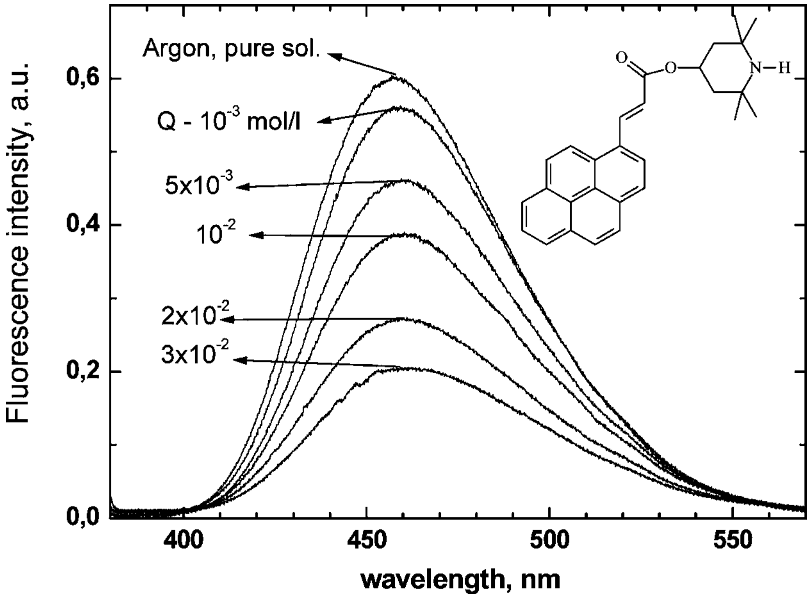


Fig. 4. Changes of fluorescence spectra (non-corrected) of 2′,2′,6′,6′-tetramethyl-4′-piperidinyl-2-(1-pyrenyl)acrylate (PAP, 2.0 × 10^{-6} mol L^{-1}) in deaerated methanol at various quencher (TEMPO) concentrations with excitation at 360 nm ($A_{360} = 0.1$).

Table I. Emission Characteristics and Quenching in Methanol

Compound	Emission intensity[a] (I_e) Air	Argon	Ratio (argon/air)	Quenching Oxygen[b] (K_{SV}) (L mol^{-1})	Q^c (K_{SV}) (L mol^{-1})
PAA (pH = 11.3)	513	669	1.30	184	180
PAA (pH = 2.4)	32	34	1.06	14	75
PAM (pH = 2.4)	143	155	1.08	38	35
PAP (pH = 2.4)	130	143	1.1	45	65
PAP-NO$^·$ (pH = 2.4)	52	52	1	0	60

[a] Integral intensity.
[b] Oxygen concentration $c = 2.2 \times 10^{-3}$ mol L^{-1} [22].
[c] TEMPO 1.0 mol dm^{-3} solution in methanol.

acid (PAM) and 2′,2′,6′,6′-tetramethyl-4′-piperidinyl-2-(1-pyrenyl)acrylate (PAP). The fluorescence band observed with a maximum at 460 nm for PAP and PAM is similar to that obtained with PAA in mildly acidic solutions. It is worth to note that for both compounds, PAP and PAM, there is no effect of pH within the range 2–14 on the fluorescence spectra. Hence the —COOH group of PAA is clearly responsible for the effects observed in Fig. 2A. From these results, we can assign the fluorescence band at 420 nm to the anionic form —COO$^-$ and that at 460 nm to the molecular form —COOH. The estimated value of pKa (namely 4.8) is roughly similar to that reported in the literature for pyrene-3-carboxylic acid (pKa = 3.8) [21]. Moreover, as clearly shown in Fig. 2A (right), decreasing the pH of the solution permits the observation of a third fluorescence band at 555 nm which corresponds more likely to the cationic form —COOH$_2{}^+$. These assignments are similar to those suggested by Thomas *et al.* for PAA in their study on the effect of pH on the fluorescence spectrum in water solution [8]. They clearly reported that the fluorescence of anionic form (—COO$^-$) presents a maximum at 450 nm, that of the molecular form (—COOH) at 500 nm and finally 530 nm for the cationic form (—COOH$_2{}^+$).

As shown in Fig. 2B, the excited states of anionic and molecular forms revealed different sensitivity to the molecular oxygen. Contrary to PAM and PAP the intensity of the emission of PAA in molecular form (—COOH) is slightly influenced by oxygen. From integral values of emission intensities on air atmosphere, under argon and by using oxygen as a quencher ([O$_2$] = 2.2×10^{-3} mol L^{-1} [22]) the Stern-Volmer constant value, K_{SV}, for PAA in acidic medium is only 14 L mol^{-1} (Table I). On the contrary, as can be seen with the evolution of the fluorescence intensity of the anionic form (Fig. 2B), the quenching is very efficient with oxygen. The Stern-Volmer constant, K_{SV}, was evaluated to 184 L mol^{-1} in neutral or basic medium. Efficiency of anionic form quenching by oxygen appears more than one order of magnitude higher than that of molecular form.

The intensity of emission for methyl ester (PAM) and 2′,2′,6′,6′-tetramethyl-4′-piperidinyl ester (PAP) is similar to the intensity of free acid PAA in its molecular form —COOH. Size and type of substituent do not play any substantial role. However, the ability of oxygen to quench excited states of PAM and PAP is about three times higher than in the case of PAA (Table I). As it has been mentioned earlier, stable nitroxyl radicals are able to quench excited singlet as well as triplet states. The compound PAP-NO$^·$ represents a molecule where the chromophore (pyrene) is covalently linked to the quencher and an intramolecular quenching is then possible. The comparison of the data in Table I for PAP and PAP-NO$^·$ reveals two conclusions. First, the emission intensity in aerated solution is ca. 2.5 times higher for parent amine PAP ($I_e = 130$) than for stable nitroxyl radical PAP-NO$^·$ ($I_e = 52$). In this case, there is a possibility for higher efficiency of quenching of PAP-NO$^·$ excited state by oxygen when compared to PAP ($K_{SV} = 45$ L mol^{-1}). Second, the emission intensity of PAP-NO$^·$ in argon atmosphere is the same as in air. This highlights the fact that the lower emission intensity of PAP-NO$^·$ in comparison with that of PAP is not due to the presence of oxygen but due to intramolecular quenching by nitroxyl radical centres (very low concentration 2.0×10^{-6} mol L^{-1}). Actually oxygen is not able to quench excited state of pyrene in the PAP-NO$^·$ ($K_{SV} = 0$) while for the parent amine PAP the K_{SV} value obtained with oxygen is 45 L mol^{-1}. Intramolecular quenching in PAP-NO$^·$ by radical centre is faster than the quenching by oxygen. This is most likely due to the very close proximity of the excited chromophore and the quencher.

Excited states of all four compounds used in this study in methanol can be quenched by addition of free nitroxyl radical type [10–12]. We have used TEMPO (1-oxo-2,2,6,6-tetramethyl-1-piperidine) and 4-hydroxy-TEMPO (1-oxo-2,2,6,6-tetramethyl-4-hydroxy-1-piperidine). Due to the much higher solubility of TEMPO (it was possible to prepare 1.0 mol L^{-1} stock solution), all the quenching experiments were performed with this latter quencher. Addition of 10–150 μL of

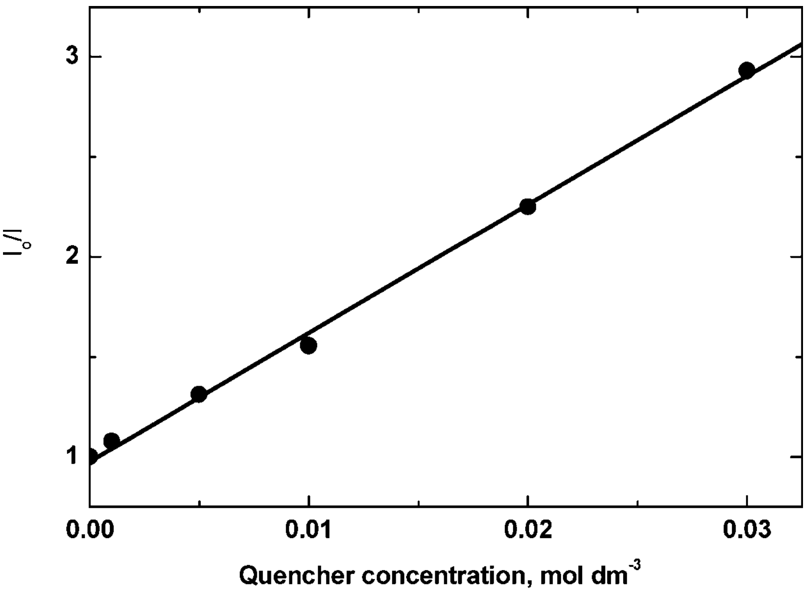


Fig. 5. Stern-Volmer plot for quenching of excited state of 2′,2′,6′,6′-tetramethyl-4′-piperidinyl-2-(1-pyrenyl) acrylate (PAP) in methanol by TEMPO.

1.0 mol L^{-1} quencher of TEMPO to 20 mL of probes solution did not change the concentration of probes. There were no changes concerning the shape, intensity and position of the longest wavelength band of absorption so the emission light originates from the same species for all concentrations of quencher. Only PAA behaved in a different way as it will be discussed later. The changes of spectra intensities due to the addition of quencher for PAP are shown in Fig. 4. The Stern-Volmer plot describes the fluorescence quenching (Fig. 5). The ratio I_0/I, where I_0 and I are the emission intensities in the absence and in the presence of quencher respectively, is related to the quenching rate constant (k_q), the fluorescence lifetime (τ_f) and the quencher concentration ($[Q]$) as follows:

$$\frac{I_0}{I} = 1 + k_q\tau_f[Q] = 1 + K_{SV}[Q]$$

The linear dependence with an intercept of unity is a proof of a Stern-Volmer behaviour. The K_{SV} values for methanol solution are given in Table I. Stern-Volmer constant K_{SV} for quenching of PAM (35 L mol^{-1}) by TEMPO is similar to the quenching by oxygen (38 L mol^{-1}). TEMPO is more efficient than oxygen in the case of quenching of PAP excited state. The features of fluorescence spectra of PAM and PAP did not change after addition of quencher and just the intensities decreased (see Fig. 4 for PAP) and Stern-Volmer plots were linear. In the case of PAA in methanol the behaviour was somehow completely different after addition of the quencher. It has to be pointed out that the analysis of the absorption spectrum did not show any interaction between TEMPO and PAA. As shown in Fig. 6, the addition of the quencher at a concentration of 1.0×10^{-3} mol L^{-1} to a solution of PAA in neutral conditions (pH ≈ 6) leads to the decrease of the fluorescence intensity at 420 nm reflecting the quenching of the anionic form (—COO$^-$) by the quencher. However, by increasing the concentration of TEMPO in the solution, we observed an important shift with the appearance of a fluorescence band with a maximum at 460 nm corresponding, as stated above, to the molecular form of PAA (–COOH). This behaviour indicates, that under our pH conditions, both forms of PAA are present (pKa = 4.8) and they are quenched with different rate constants. The anionic form seems to be quenched more efficiently than the molecular form. In order to study this effect, we undertook the study of these quenchings in acidic and basic solutions (Table I). The anionic form was much more efficiently quenched by TEMPO than the molecular form. The Stern-Volmer rate constants K_{SV} = 180 and 75 L mol^{-1} were found respectively for the two forms. For this reason, in the presence of high concentrations of TEMPO the emission of the anionic form is quenched while that of the molecular form is left in the solution.

The ability of TEMPO to quench fluorescence more effectively than oxygen as it was mentioned for PAP could be seen in the case of PAP-NO$^{\cdot}$ in methanol solution even more clearly. As it was already mentioned, the intermolecular quenching by oxygen is not able to compete with intramolecular quenching by nitroxyl radical which is part of PAP-NO$^{\cdot}$ molecule. But added

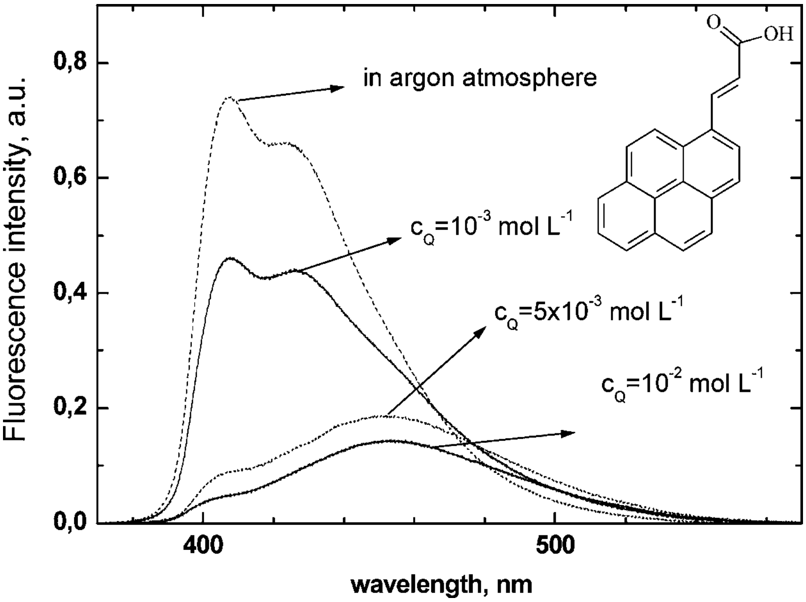


Fig. 6. Changes of fluorescence spectra (non-corrected) of 2-(1-pyrenyl)acrylic acid (PAA, 2×10^{-6} mol L^{-1}) in methanol at different TEMPO concentrations with excitation at 360 nm ($A_{360} = 0.1$).

TEMPO is able to quench the rest of excited states of PAP-NO$^{\cdot}$, which were not quenched by internal nitroxyl radical of complex molecule. This behaviour depends on the nature of the solvent.

Absorption and emission spectra of PAM in cyclohexane are shown in Fig. 7. Absorption spectrum is similar to that in methanol (Fig. 3). However, the fluorescence spectrum in cyclohexane is different with well-defined vibrational structures : peaks at 407, 431 and a shoulder at 458 nm while a broad band with a maximum at 460 nm is present in methanol. Similar differences are observed with the two other esters PAP and PAP-NO$^{\cdot}$. When the

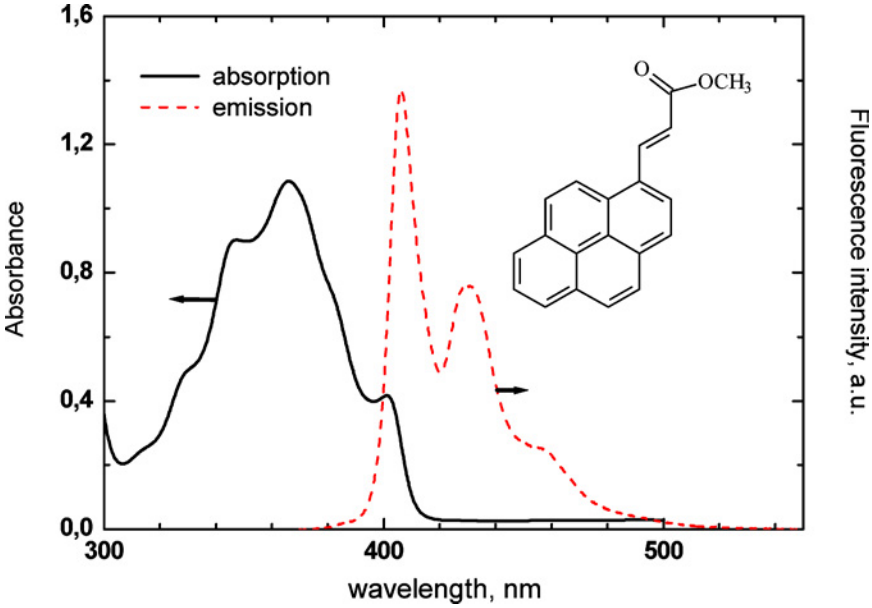


Fig. 7. Absorption spectrum and fluorescence spectrum (non-corrected) of methyl-2-(1-pyrenyl) acrylate (PAM, 2.0×10^{-6} mol L^{-1}) in cyclohexane with excitation at 360 nm ($A_{360} = 0.1$).

Table II. Emission Characteristics and Quenching in Cyclohexane

	Emission intensity[a] (I_e)			Quenching	
Compounds	Air	Argon	Ratio (argon/air)	Oxygen[b] (K_{SV}) (L mol^{-1})	Q[c] (K_{SV}) (L mol^{-1})
PAM	420	515	1.23	96	84
PAP	516	568	1.1	42	180
PAP-NO˙	59	111	1.88	366	1600

[a] Integral intensity.
[b] Oxygen concentration $c = 2.4 \times 10^{-3}$ mol L^{-1} [22].
[c] TEMPO 1.0 mol dm^{-3} solution in cyclohexane.

polarity of the solvent increases, the emission spectra of these derivatives loose their vibrational structure and the fluorescence peak maximum is shifted to the longer wavelength. This is more likely due to the fact that the lowest excited state becomes more of π–π^* character [21]. Emission intensities of PAM and PAP in aerated as well as in deaerated cyclohexane solutions are several times higher (three times for PAM and four times for PAP) than in methanol (Tables I and II). But for PAP-NO˙ the value in aerated cyclohexane solution is almost the same as in methanol. Presence of nitroxyl radical structure in the molecule of PAP-NO˙ is one reason for these differences.

The extent of intramolecular quenching of excited states of pyrene by nitroxyl radical structure in the molecule of PAP-NO˙ can be estimated by the ratio of integrated intensity of parent amine PAP with those of PAP-NO˙. The value is lower in aerated and deaerated methanol (2.5 and 2.8, respectively) than in aerated and deaerated cyclohexane (8.7 and 5.1, respectively). Contrary to methanol where oxygen was not able to quench excited singlet states of PAP-NO˙, in cyclohexane oxygen can quench excited states even to a higher extent ($K_{SV} = 366$ L mol^{-1}) and to quench excited states of PAM ($K_{SV} = 96$ L mol^{-1}) or PAP ($K_{SV} = 42$ L mol^{-1}) (Table II). Due to the fact that oxygen concentration is very similar in both solvents (oxygen concentration $c = 2.4 \times 10^{-3}$ mol L^{-1} in cyclohexane and $c = 2.2 \times 10^{-3}$ mol L^{-1} in methanol) [22] some other factors have to play crucial role in the quenching process. Methanol could stabilize more favourable conformation of PAP-NO˙ molecule for internal quenching so all excited states, which could be quenched by oxygen, are quenched by the –NO˙ part of the molecule. On the other hand, it could be a similar case in cyclohexane but due to the much higher population of excited states in cyclohexane there are still some excited molecules that can be quenched by oxygen. This effect of solvent on emission intensity as well as on quenching depends on the type of substituent on pyrene ring as was reported in the literature [18]. In some cases the fluorescence intensity in methanol is higher than in cyclohexane for example in the case of —COOR and —CH$_2$—CH$_2$—CH$_2$—COOR substituted pyrenes (where R is 2,2,6,6-tetramethyl-4-piperidyl), while in other studies it is the opposite, namely the intensity in cyclohexane is higher. The latter statement was observed for substituents such as —CO—CH$_2$—CH$_2$—COOR and —CH$_2$—COOR.

The efficiency of intermolecular quenching of excited states by TEMPO as an external quencher is higher in cyclohexane than in methanol. For PAM and PAP it is increasing by factor 2.4 and 2.8, respectively. For PAP-NO˙ this factor is even one order of magnitude higher (roughly 27). However in this case the value of $K_{SV} = 1600$ L mol^{-1} was calculated just from the first change of emission intensity after adding first portion of TEMPO to reach quencher concentration $c_Q = 1.0 \times 10^{-3}$ mol L^{-1} but at higher concentrations no more quenching was observed. This could reveal the presence of two different excited states, which are quenched with two different K_{SV} constant.

In conclusion, the main features of fluorescence of bifunctional probes and their model compounds strongly depend on polarity of the solvents and the presence of the oxygen. The intramolecular quenching by linked *N*-oxyl is less effective than intermolecular quenching by TEMPO.

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