

Absorption and Fluorescence Properties of Benzo[*a*]phenoxazinium Salts

Vânia H. J. Frade, M. Sameiro T. Gonçalves* and João C.V.P. Moura

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Centro de Química, Universidade do Minho, Gualtar, 4710-057 Braga, Portugal

msameiro@quimica.uminho.pt

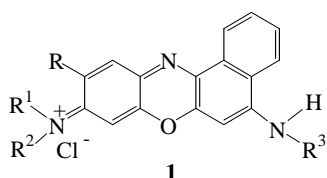
Introduction

During the last years, long-wavelength fluorescent dyes have attracted considerable attention on account of their advantages over conventional fluorescent dyes, such as background interferences and photodecomposition.^{1,2} Among others, long-wavelength fluorochromophores have been used as labels of proteins,³ in chromatography studies⁴ and in DNA analyses.⁵

Following our previous work on the synthesis and characterisation of benzo[*a*]phenoxazinium chlorides⁶ we decided to study their absorption and fluorescence properties in other solvents in addition to ethanol and water (pH 7.4).

Results and Discussion

Benzo[*a*]phenoxazinium chloride **1a** was prepared by the reaction of 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride with *N*-phenyl-1-naphthylamine in acidic medium. After purification by dry chromatography, compound **1a** was obtained as a blue solid in 54% yield and was fully characterised by high resolution mass spectrometry, IR and NMR (¹H and ¹³C) spectroscopy. Compounds **1b-d** were obtained previously by a similar procedure.⁶



a R = CH₃, R¹ = H, R² = CH₂CH₃, R³ = Ph

b R = CH₃, R¹ = H, R² = CH₂CH₃, R³ = CH₂CH₂CO₂CH₂CH₃

c R = H, R¹ = R² = CH₃, R³ = CH₂CH₂CO₂CH₂CH₃

d R = H, R¹ = R² = CH₂CH₃, R³ = CH₂CH₂CO₂H

Absorption spectra of compounds **1a-d** were run in eleven solvents of different polarity and proton donor ability, such as 1,4-dioxane, chloroform, methanol and water (pH 7.4). The wavelength maxima (λ_{\max}) and wavenumber maxima (ν_{\max}) of these compounds are listed in Table 1. For all carbonyl dyes, the highest energy transitions were found with 1,4-dioxane, the least polar aprotic solvent. More polar protic solvents such as water (pH 7.4) (**1c** and **1d**) or ethanol (**1b**) result in lower energy transitions, thus indicating a positive solvatochromic response (between $\Delta\nu_{\max} = 4909 \text{ cm}^{-1}$ for **1c** and $\Delta\nu_{\max} = 4404 \text{ cm}^{-1}$ for **1b**), which is associated to a greater stabilisation of the excited state relative to the ground state with increasing polarity of the solvent. Compound **1a** showed the highest ν_{\max} in tetrahydrofuran and the lowest in 1,4-dioxane.

Table 1. The absorption spectra data of compounds **1a-d** in various solvents.

Solvent	ϵ	1a		1b		1c		1d	
		λ_{\max}	ν_{\max}	λ_{\max}	ν_{\max}	λ_{\max}	ν_{\max}	λ_{\max}	ν_{\max}
1,4-Dioxane	2.20	502	19920	489	20202	487	20534	497	20121
Chloroform	4.80	506	19763	426	16393	616	16234	628	15924
Ethyl acetate	6.08	509	19646	497	16367	611	16367	499	20040
Tetrahydrofuran	7.52	637	15699	641	16000	637	15699	630	15873
Dichloromethane	8.93	510	19608	634	16129	638	15674	635	15748
Acetone	21.01	514	19455	505	20161	497	20121	505	19802
Ethanol ⁶	25.3	523	19121	634	15798	633	15798	638	15674
Methanol	33.0	527	18975	632	16000	629	15898	637	15699
DMF	38.25	527	18975	511	19724	501	19960	511	19569
Acetonitrile	36.60	517	19343	524	16129	495	20202	640	15625
Water (pH 7.4) ^a	80.1 ^b	--	--	645	16000	640	15625	650	15385

Units: λ_{\max} , nm; ν_{\max} , cm^{-1} . ϵ , dielectric constant. ^aData previously reported ⁶ for compounds **1b**, **1c** and **1d**. ^bValue for distilled water.

Fluorescent molecules, whose emission spectra and quantum yields are markedly sensitive to solvent polarity, are widely used as reporter probes for investigating chemical, biochemical and biological phenomena.⁷

Previous studies⁶ on the fluorescence of compounds **1a-d** showed that compound **1b** gave the highest fluorescence quantum yields (for example, for compound **1d** in ethanol, $\phi = 0.23$).

Bearing this in mind, a fluorescence study was carried out with the most interesting fluorescent compound presented (**1b**), in solvents of different polarity. These studies were carried out using Oxazine 1 as standard and are summarised in Table 2.

Table 2. The fluorescence data of compound **1b** in various solvents.

Solvent	λ_{exc} [nm]	λ_{em} [nm]	ϕ	Stokes' shift [nm]
Chloroform	580	629	0.36	49
Ethyl acetate	590	644	0.40	54
Acetone	510	598	0.15	88
Ethanol ⁶	590	643	0.50	53
DMF	490	611	0.017	121
Acetonitrile	590	644	0.043	54
Water (pH 7.4) ⁶	600	654	0.32	54

Compound **1b** exhibits fluorescence in all solvents, having in ethanol the best quantum yield ($\phi = 0.50$), and show good to high Stokes' shift (the lowest 49 nm in chloroform and the highest 121 nm in DMF). The results also showed that the fluorescence is red shifted when going from acetone (λ_{em} 598 nm) to water (pH 7.4) (λ_{em} 654 nm) *via* acetone, DMF, chloroform, ethanol, ethyl acetate or acetonitrile and water (pH 7.4).

These preliminary results suggested that these cationic fluorophores are good candidates as reporter probes in a variety of important studies.

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