Solvent effect on the UV/Vis absorption and fluorescence spectroscopic properties of berberine†

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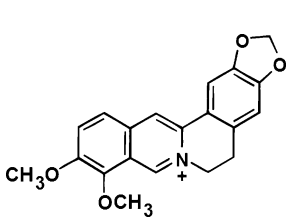
Received 12th December 2008, Accepted 9th April 2009
First published as an Advance Article on the web 27th April 2009
DOI: 10.1039/b822363g

Spectral and photophysical properties of the alkaloid berberine (**B**) were studied in solvents with different solvent parameters, using UV/Vis absorption, emission and excitation spectroscopy. The absorption and emission maxima were found to be between 421–431 nm and 514–555 nm, respectively, leading to Stokes' shifts between 4099 and 5735 cm^{-1}. The fluorescence quantum yields varied between 10^{-2}–10^{-4}, depending on the solvent. Different solvent scales have been used to study the solvatochromism of **B**. Linear solvation energy relationships (LSER) proposed by Kamlet–Taft suggest that **B** is a molecule attractive as a probe for solvent polarity and hydrogen bonding properties.

1. Introduction

Alkaloids with chemical structures related to protoberberines produced by plant cells exhibit diverse activities. Roots and barks of *Berberis* species, plants that accumulate protoberberines, have been used for the treatment of different diseases. Indian people in the south of Argentina used the roots of the local species as a yellow dye.[1]

A major representative of the protoberberines, berberine (**B**, Scheme 1), while being relatively non-toxic to man, has various biochemical and pharmacological, *e.g.* antimicrobial,[1–2] antiparasitic,[3] anti-inflammatory[4] and cytotoxic[5–6] activities. Furthermore, **B** binds to nucleic acids with high affinity, forming fluorescent compounds, which could be used in biological studies.[7] The photochemistry of **B** has been investigated,[8–13] showing that the spectroscopic properties of **B** are strongly dependent on its environment. Although the absorption and fluorescence properties of **B** have been studied in a small number of solvents, a systematic study is useful to establish a relationship between the environment and the photophysical properties of a fluorescent probe. The solvatochromic response of **B** dissolved in solvents of different properties has been measured in order to obtain information into the nature of the solute-solvent interactions. The Vis spectroscopic absorption ($\bar{\nu}_a$) and emission ($\bar{\nu}_f$) maxima and Stokes' shifts ($\Delta\bar{\nu} = \bar{\nu}_a - \bar{\nu}_f$) were analysed using the solvent polarity parameter $\Delta f(\varepsilon,n)$, the normalized solvent polarity parameter E_T^N and the empirical Kamlet–Taft solvent parameters π^* (dipolarity/polarizability), α (hydrogen-bond donating (HBD) or electron-pair donation ability) and β (hydrogen-bond accepting (HBA) ability) in terms of the well-established linear solvation energy relationship (LSER).[14,15] The solvent independent coefficients **p**, **a**, and **b** and $(\nu_{max})_0$ have been determined.



Scheme 1 Chemical structure of berberine.

2. Experimental

2.1. Chemicals

Berberine chloride and fluorescein were from Sigma Chem. Co. and were used as received. All solvents, 1,4-dioxane (1), chloroform (2), ethyl acetate (3), tetrahydrofuran (4), acetone (5), acetonitrile (6), dimethylsulfoxide (7), *n*-butanol (8), 2-propanol (9), ethanol (10), methanol (11), were of HPLC grade. Doubled distilled water (12) was used for aqueous solutions.

2.2. Absorption spectra

Ground state absorption spectra were measured at room temperature on an Agilent 8453 diode array spectrophotometer. Absorption maxima were recorded with an accuracy of 1 nm. For measurement of the extinction coefficients, **B** (5–10 mg) was dissolved in 10 ml of the solvent. The stock solution was diluted and the absorbance was measured in a 1 cm standard quartz cell. All dye concentrations were in the range 1×10^{-5}–2×10^{-4} M. The plots of absorbance *versus* **B** concentration yield a straight line ($R^2 > 0.999$). The extinction coefficients were determined with a reproducibility of 100 M^{-1} cm^{-1} according to Lambert–Beer's law from two independently weighed stock solutions.

2.3. Emission spectra and quantum yields

Corrected fluorescence and excitation spectra were obtained with a Jasco FP6200 spectrofluorimeter. All measurements were done repeatedly and reproducible results were obtained. All the solutions were excited at 421 nm with an excitation and emission slit width of 5 nm. The fluorescence quantum yield of **B** in different solvents was measured using fluorescein in ethanol ($\Phi_S = 0.97$)[16]

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† This article was published as part of the themed issue in honour of Esther Oliveros.

as the reference compound. Fluorescence quantum yields of **B** (Φ_f) under N_2-saturated solutions were determined according to eqn (1):[17]

$$\Phi_f = \frac{A_S F_B n_B^2}{A_B F_S n_S^2}\Phi_S \quad (1)$$

where A, F and n denote absorption at the excitation wavelength, integrated area underneath the fluorescence emission spectrum corrected after solvent blank subtraction and refractive index of the solvent, respectively. Optically matched solutions of the sample and reference were used. The sample concentration of **B** was *ca.* 10^{-5} M (it refers to absorbances < 0.05 at excitation wavelength in a 1 cm cell).

2.4. Photostability

Static photolysis was carried out with one home-made photolysis line for non-monochromatic irradiation (150 W quartz–halogen lamp and cut-off filters) already described.[18] Stock solutions of **B** were prepared and irradiated with magnetic stirring in the presence of oxygen or under an atmosphere of nitrogen. The absorption and emission spectra of the solutions were measured before and after one hour of irradiation.

All measurements were made at room temperature.

2.5. Data analysis

The correlation constants and the molar absorption coefficients of **B** were determined on a personal computer with MINITAB™ (Minitab Inc.) Release 14.20 statistical package.

The values of the refractive index (n) and the static dielectric constant (ε) of the solvents and of the microscopic solvent parameters were taken from the data compiled by Reichardt[14] and from the review by Marcus,[19] respectively.

3. Results and discussion

Absorption and corrected emission and excitation spectra were recorded in several solvents in which **B** is soluble. The data obtained are listed in Table 1. After one hour irradiation no changes in the absorption and fluorescence spectrum of **B** could be observed.

Table 1 UV-vis absorption and fluorescence data of **B** in different solvents

Solvent	Maximal wavelength/nm				Stokes' shift/cm^{-1}
	Absorption (ε/M^{-1} cm^{-1})			Emission	
1,4-Dioxane	270	352	430 (5328)	522	4100
Chloroform	268	355	429 (4254)	523	4190
Ethyl acetate	266	347	427	520	4190
THF[a]	269	349	424	514	4130
Acetone	—	350	427 (4278)	533	4660
Acetonitrile	266	350	430 (4488)	537	4630
DMSO	266	349	425	553	5500
1-Butanol	267	351	430 (4529)	534	4530
2-Propanol	267	351	431 (5290)	534	4480
Ethanol	236	345	430 (4436)	536	4600
Methanol	257	340	429 (4570)	540	4790
Water	257	340	421 (4209)	555	5740

[a] Low solubility.

Fig. 1 shows representative UV-Vis absorption spectra of **B** in solution. In a dilute solution (*ca.* 10^{-5} M), the absorption spectrum exhibits three bands with maxima around 250 and 350 nm, which are strong in nature, and another relatively weak band at about 430 nm with extinction coefficients between 4200 and 5300 M^{-1} cm^{-1} for water and 1,4-dioxane, respectively. A relative high molar extinction coefficient (~10^4 M^{-1} cm^{-1}) in all solvents indicates a character of $\pi \rightarrow \pi^*$ transition for the absorption bands in the 250–350 range.

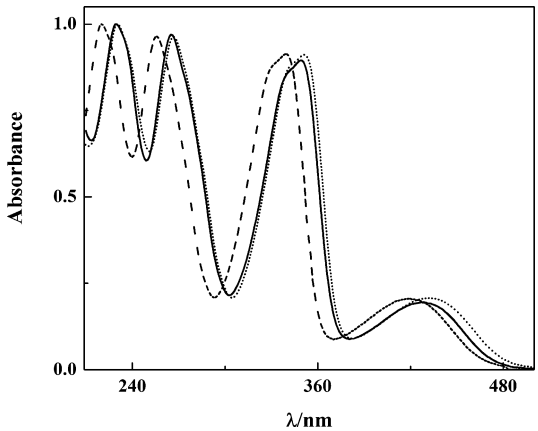

Fig. 1 Normalized absorption spectra of **B** in water (dashed line), acetonitrile (solid line) and 2-propanol (dotted line).

The shape of the absorption spectrum is not changed and a slight blue shift of the absorption bands upon increasing solvent polarity is observed (Fig. 1).

Upon excitation at the longest absorption maximum, the emission spectra of solutions of **B** exhibit identical intensities regardless of whether they are purged with nitrogen or air. The emission spectra are structureless with a maximum around 530 nm (Fig. 2). The band position and shape are found to be independent of excitation wavelength, indicating that in each solvent the transition in the **B** molecule occurred between the same electronic states. The corrected excitation spectra (data not shown) monitoring at the fluorescence peak agree reasonably well with the

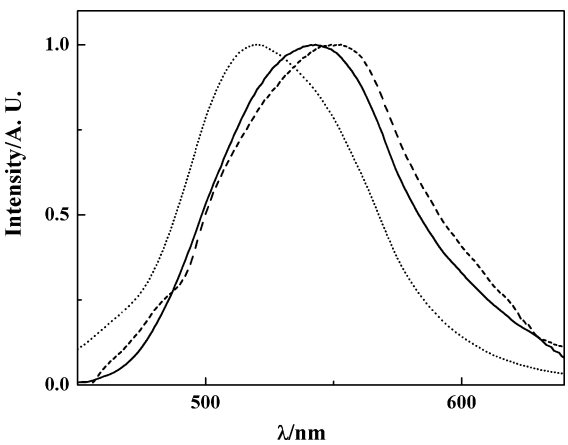

Fig. 2 Normalized emission spectra of **B** in water (dashed line), acetonitrile (solid line) and ethyl acetate (dotted line).

Table 2 Fluorescence quantum yields (Φ_f) of **B** in different solvents

Solvent	Φ_f (±15%)
1,4-Dioxane	0.030
Chloroform	0.041
Ethyl acetate	0.031
Acetone	0.023
Acetonitrile	0.021
DMSO	0.0016
1-Butanol	0.057
2-Propanol	0.045
Ethanol	0.028
Methanol	0.014
Water	0.00045

absorption spectra showing that the fluorescence originates from the main absorbing species in the ground state.[20]

The emission is weak in polar solvents. The presence of less polar solvents results in a fluorescence enhancement accompanied by a blue shift of the fluorescence peak, as shown in Fig. 2. **B** exhibits a relative large Stokes' shift, with the largest shift (134 nm) being observed in water.

The fluorescence quantum yields of **B**, evaluated according to eqn (1), are low and decrease significantly when solvent polarity increases (Table 2). Such a dependence of Φ_f with the media has already been reported, for example fluorescence quantum yields of 4.7×10^{-4} and 0.025 were found by Inbaraj *et al.*[9] in deuterated water and acetonitrile, respectively, in agreement with our results.

Interactions between the solute and the solvent can occur in either the ground state or the excited state. In order to determine the behaviour of **B**, its spectroscopic properties were correlated with relevant solvent polarity scales. The energies of absorption ($\bar{\nu}_a$) and emission transitions ($\bar{\nu}_f$), $\Delta\bar{\nu}$ and Φ_f were plotted as a function of the polarity parameter (or orientation polarizability) $\Delta f(\varepsilon,n)$ expressed as:[21]

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \quad (2)$$

where n and ε are the refractive index and the static dielectric constant of the solvent, respectively.

Non-linear plots against the polarity parameter $\Delta f(\varepsilon,n)$ were obtained, implying that the solvatochromic properties of **B** cannot be explained solely in terms of the change in permanent dipole moments and other characteristics of the specific solvent effect should be considered.[20]

The spectroscopic properties of **B** were also correlated with the solvent polarity parameter E_T^N.[14] The poor correlation obtained for $\bar{\nu}_a$ ($r = 0.128$, $n = 12$) suggest that absorbance is almost independent of E_T^N. The least square correlation analysis gave a moderate correlation for $\bar{\nu}_f$ and $\Delta\bar{\nu}$:

$$\bar{\nu}_f = 19\,408 - 1380E_T^N,\ r = 0.636,\ n = 12 \quad (3)$$

$$\Delta\bar{\nu} = 3898 + 1535E_T^N,\ r = 0.574,\ n = 12 \quad (4)$$

where r is the regression coefficient and n is the number of solvents used.

A double linear correlation of Φ_f with E_T^N is observed for protic solvents ($r = 0.829$, $n = 5$) and for aprotic solvents ($r = 0.532$, $n = 6$), suggesting that hydrogen bond specific interactions on the photophysical properties of **B** may be significant.

In order to get information about the individual contributions of the solvent hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) abilities on the spectroscopic properties of **B**, the Kamlet–Taft solvatochromic comparison method[15] was applied. By the use of this approach, it is possible to separate hydrogen bonding from polarity effects. A regression using all three solvatochromic parameters gave the highest adjusted multiple correlation for **B** in the selected solvents. A poor correlation was obtained for $\bar{\nu}_a$ ($r = 0.368$, $n = 12$). Thus, solvent properties do not significantly affect the ground state of **B**. The results of the multiple regression and the analysis of variance for $\bar{\nu}_f$ and $\Delta\bar{\nu}$ are given in Table 3. The high F-statistic and p-value (0.000), obtained by analysis of variance, show that the model estimated by regression is significant at a confidence level of 0.95.

The negative signs for both α and β coefficients are consistent with the shifts of the fluorescence spectrum to lower energies. Although the influence of solvent acidity and basicity cannot be neglected, the dipolarity/polarizability interaction is mainly responsible for the positive solvatochromism observed in the fluorescence spectra.

A good correlation between $\Delta\bar{\nu}$ experimental ($\Delta\bar{\nu}_E$) and theoretical ($\Delta\bar{\nu}_T$) calculated with eqn (5) was obtained (Fig. 3).

$$\Delta\bar{\nu} = 2682 + 241\alpha + 755\beta + 2179\pi^*,\ r = 0.966,\ n = 12 \quad (5)$$

The regression data given for each coefficient in Table 3, the small standard deviations, the high t-ratios and low p-values for the estimated coefficients indicate that the three parameters are significantly related to the Stokes' shift.

A moderate correlation between Φ_f and the Kamlet–Taft parameters has been obtained ($r = 0.641$, $n = 11$). The estimated

Table 3 Regression and analysis of variance results of the multiparametric correlation

		$(\nu_{max})_0$	a	b	p	n^a	R^b	SD^c	F^d
$\bar{\nu}_f$	Coef.	20 338.3	−276.7	−737.4	−1618.8	12	0.89	173.5	20.5
	SE^e	237.3	130.9	270.2	257.9				
	T^f	85.69	−2.11	−2.73	−6.28				
	p^g	0.000	0.07	0.03	0.000				
$\Delta\bar{\nu}$	Coef.	2681.7	240.9	754.5	2178.7	12	0.97	110.1	76.2
	SE^e	150.7	83.1	171.6	163.8				
	T^f	17.79	2.90	4.40	13.30				
	p^g	0.000	0.02	0.002	0.000				

[a] Number of solvents. [b] Correlation coefficient. [c] Standard deviation of the regression. [d] F-test. [e] Standard error of the coefficient. [f] t-test. [g] p-value.

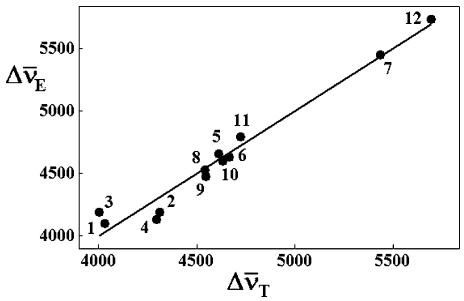

Fig. 3 Kamlet–Taft multiparameter solvation energy relationship for the Stokes' shift of **B** in: (1) 1,4-dioxane, (2) chloroform, (3) ethyl acetate, (4) tetrahydrofuran, (5) acetone, (6) acetonitrile, (7) dimethylsulfoxide, (8) *n*-butanol, (9) 2-propanol, (10) ethanol, (11) methanol and (12) water.

coefficients suggests that Φ_f mainly depends on solvent dipolarity/polarizability.

The balance between specific and non-specific interactions is responsible of either an increase or a decrease in fluorescence intensity[12] and larger Stokes' shifts in protic solvents is typical of specific solvent–fluorophore interactions and have been seen for other fluorophores.[20]

The intensity enhancement and the hypsochromic displacement of the fluorescence maximum of **B** with decreasing solvent polarity were already reported.[9,12] Similar results were obtained with **B** bound to surfactants,[10,13] DNA and RNA[7] and keratinocyte cells,[9] impregnated onto silica gel plates in the presence of *n*-alkanes[22,23] and confined in the cavity of host molecules, like β-cyclodextrin,[11] calixarenes[24] and cucurbiturils.[25] Computational analysis performed by Cossío *et al.*[22,23] suggests that there is a weak electrostatic interaction between the electron-deficient π-system of **B** and *n*-alkanes and that the fluorescence enhancement is due to an ion-induced dipole. Megyesi *et al.*[25] proposed that the partial immersion of **B** in the cavity of the guest, which reduces the interaction with water and hinders alternative relaxation mechanisms of the excited **B** is responsible of the observed effects. The existence of ion pairs was postulated to explain the enhancement of the fluorescence intensity of **B** in the presence of the surfactant SDS below its critical micelle concentration.[10] On the other hand, polar molecules, like some aminoacids and proteins were found to quench **B** fluorescence.[12] Megyesi and Biczók[26] postulated that the formation of weak fluorescent ion pairs between $\mathbf{B}^+$ and chloride anions is responsible of the decrease in the fluorescence intensity observed in dichloromethane.

To understand the characteristics of **B** in different solvents further, radiative lifetimes (τ_0) were calculated from the absorption spectra using the Strickler-Berg relation:[17]

$$\frac{1}{\tau_0} \approx 2.88 \times 10^{-9} \frac{(\bar{\nu}_{max})^2 n^2}{cl} \int A(\bar{\nu})\, d\bar{\nu} \qquad (6)$$

where $\int A(\bar{\nu})\, d\bar{\nu}$, $\bar{\nu}_{max}$, c and l are the area under the absorption spectrum plotted against wave number $\bar{\nu}$, wave number of the maximum of the absorption band, concentration of the sample and optical length, respectively. An almost constant radiative lifetime of about 20 ns was obtained in all the solvents, suggesting that absorption of light populates the same excited state. Megyesi and Biczók[13] reported fluorescence lifetimes (τ_f) of 40 ps in water, 1.24 ns in ethanol and 0.53 ns in 1,2-ethanediol, suggesting that the viscosity of the solvent has a minor effect on the fluorescence lifetime of **B**. The values of the fluorescence lifetimes are solvent dependent and much lower than the radiative ones, therefore relatively small values of Φ_f are expected, as observed in Table 2. In the range of concentrations employed, no evidence was found of association between $\mathbf{B}^+$ and chloride anions. It is unlikely that ion pairing is the responsible of the lower Φ_f obtained in polar solvents, for univalent ions and solvents with ε higher than 30, the existence of ion pairs could not be unambiguously established.[27] Changes in Φ_f can be attributed to changes in the rates of non-radiative decay (k_{nr}),[20] an apolar environment improves the fluorescence quantum yield and a polar one enhances relaxation. The short τ_f compared to τ_0 and also the small Φ_f clearly suggest that a rapid non-radiative transition is occurring, enhanced by hydrogen bonding interactions as was proposed by Inbaraj *et al.*[9] They found that **B** has no phosphorescence and no singlet molecular oxygen ($^1O_2$) is generated in water, suggesting that the deactivation of the singlet state to ground state occurs *via* radiationless processes. On the other hand, phosphorescence was observed in ethanol and $^1O_2$ was generated in less polar aprotic solvents, like dioxane, as a result of a rapid intersystem crossing from the singlet to the triplet sate.[9]

The non-radiative rate constant seems to control the solvent dependence of the spectroscopic properties of **B** with the solvent properties. Our results confirm the importance of hydrogen bonding and polarity of solvents in the fluorescence properties of **B**.

4. Conclusions

The determination of Stokes' shifts when **B** is incorporated into several solvents may provide a valuable tool for the interpretation of its spectroscopic properties. In the present investigation the linear solvation energy relationship has been found to give better results when compared to other solvatochromic single parametric equations.

The results obtained imply that the reduction in fluorescence quantum yields can be due to the competing radiationless processes, enhanced by hydrogen bonding between **B** and the solvent. The observed solvatochromism makes **B** a potential probe for polarity and also for hydrogen bonding properties of the local microenvironment.

Acknowledgements

The authors thank the Secretaría de Ciencia y Técnica (SCyT) at the Universidad Nacional de la Patagonia San Juan Bosco for funding this research.

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