UV/vis absorption and fluorescence spectroscopic study of some new 4-hydroxy-7-methoxycoumarin derivatives. Part I: Effect of substitution by a benzo-1,4-dioxanyl or an ethyl furoate group in the 3-position

Régine Dondon,[a] Vladimir P. Khilya,[b] Alexander D. Roshal[a] and Suzanne Fery-Forgues*[a]

[a] *Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique, (CNRS UMR 5623), Université Paul Sabatier, 118, Route de Narbonne, 31062 Toulouse cedex, France. E-mail: sff@gdp.ups-tlse.fr*

[b] *Chemistry Department, National Taras Shevchenko University, Volodymyrska 64, Kiev 252017, Ukraine*

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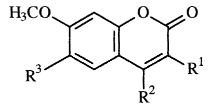
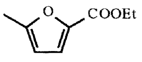
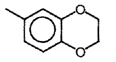
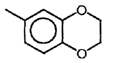
The UV/vis spectroscopic behaviour of some new 4-hydroxy-7-methoxycoumarins was investigated with the aim of analysing the influence of the substituent in the 3-position. In comparison with the unsubstituted analogue, the ethyl furoate and benzo-1,4-dioxanyl derivatives exhibited UV/vis absorption and emission spectra strongly shifted to the red. The highest fluorescence quantum yields and longest lifetimes were encountered for the ethyl furoate derivatives, which also showed little sensitivity to environmental factors. In contrast, substitution by a benzo-1,4-dioxanyl group induced the appearance of positive solvatochromism together with a drastic decrease of the fluorescence efficiency. Emission properties were particularly sensitive to viscosity and temperature variations. The involvement of a non-radiative rotatory decay mechanism was discussed, based on the analysis of the photophysical constants. AM1 MOPAC calculations pointed out some differences in the molecular geometry and energy levels, which allowed the substituent effect to be better identified.

Etude par spectroscopie d'absorption UV/vis et de fluorescence de nouveaux dérivés de la 4-hydroxy-7-méthoxycoumarine. Partie 1. Effet de la substitution par un groupement benzo-1,4-dioxanyle ou furoate d'éthyle en position 3. Le comportement spectroscopique de nouveaux dérivés de la 4-hydroxy-7-méthoxycoumarine a été étudié de manière à connaître l'influence du substituant en position 3. Par comparaison avec l'analogue non-substitué, les dérivés porteurs d'un groupement furoate d'éthyle ou benzo-1,4-dioxanyle montrent un fort déplacement vers le rouge des spectres d'absorption et d'émission. Le rendement quantique de fluorescence le plus élevé et la durée de vie la plus longue sont observés avec le dérivé furoate d'éthyle, qui est également peu sensible aux variations de l'environnement. Au contraire, la substitution par un groupement benzo-1,4-dioxanyle induit l'apparition d'un solvatochromisme positif et un effondrement de l'efficacité de fluorescence. Les propriétés d'émission sont particulièrement sensibles aux variations de viscosité et de température. La possibilité de désactivation non-radiative par rotation du substituant est discutée au vu de l'analyse des constantes photophysiques. Des calculs AM1 MOPAC mettent en évidence des différences dans la géométrie des deux molécules et dans leurs niveaux d'énergie et permettent de mieux cerner l'effet du substituant.

There is a constant demand for fluorescent dyes for new applications, many of which concern the biological sciences or high technology industry. Among the dyes commonly available, coumarins are widely used owing to their high emission yields, photostability, extended spectral range, and good solubility in the safer solvents. These compounds constitute the largest class of laser dyes for the blue–green region.[1–3] They also are currently used as fluorescent labels or pigments,[4,5] as well as for measuring physiological pH values[6,7] and enzyme activities[8,9] by fluorimetry.

With the aim of studying the UV/vis spectral properties of new coumarins and their possible applications, we became interested in 4-hydroxy-7-methoxycoumarins. Many compounds in this series have been known for a long time.[10] They are precursors in the synthesis of biologically active compounds and their arylation in the 3-position is well-documented,[11,12] giving rise to important isoflavonoid derivatives. The ease of preparation and purification of these compounds prompted us to think that they could find interesting applications in UV/vis spectroscopy. Knierzinger and Wolfbeis[13] reported that 4-hydroxy-7-methoxycoumarin is fluorescent, its emission maximum being $\lambda_{em} = 349$ nm in 95% ethanol. However, compounds emitting further to the red would be more suitable for applications in biology and organized media, measurements being less affected by any self-fluorescence of these environments. In an attempt to do this, we modified the chemical structure of 4-hydroxy-7-methoxycoumarin. To our knowledge, no systematic study has been undertaken to elucidate the effect of substitution on the UV/vis spectroscopic and photophysical behaviour of this compound. By introducing an electron-withdrawing substituent in the 3-position, we hoped to shift the excitation and emission maxima to longer wavelengths. The bathochromic effect of an ethyl substituent in the 6-position was also postu-



R^1	R^2	R^3	Compound
	OH	C_2H_5	**1a**
"	OCOPh	C_2H_5	**1b**
	OH	C_2H_5	**2a**
"	OAc	C_2H_5	**2b**
H	OH	H	**3a**
	OAc	H	**4b**

Scheme 1 Molecular structure of the coumarin derivatives.

lated. Consequently, molecules **1a** and **2a** were synthesized (Scheme 1) and their UV/vis spectroscopic and photophysical behaviour investigated.

A comparison was made with 4-hydroxy-7-methoxycoumarin, **3a**, as the basic model compound. Compounds **1a** and **2a** bear a reactive hydroxyl group in the 4-position. This group can be esterified, allowing the coumarin residue to be covalently linked to another organic molecule. This is the reason why the benzoyl and acyloxy derivatives **1b** and **2b** were studied as models, as well as the hydroxy compounds **1a** and **2a**.

Results and discussion

UV/vis absorption spectra

Coumarins **1a**, **2a**, **2b**, and **3a** were obtained as white solids whereas coumarin **1b** appeared as bright yellow crystals. The UV/vis absorption spectra of these compounds in solution showed an intense unresolved band in every solvent investigated. For a given coumarin, the peak position hardly varied with the nature of the solvent (Table 1). The analysis of the absorption wavelength maxima showed that substitution in the 3-position led to a marked red-shift, which was stronger for the ethyl furoate derivatives than for the benzo-1,4-dioxanyl ones. Esterification also caused the absorption band to shift by $\Delta\lambda = 10$ to 20 nm towards the red, as compared to the corresponding hydroxycoumarins. Similar trends were observed for the molar absorption coefficients. They passed from 10 000 L mol^{-1} cm^{-1} for **3a** to 28 400, 32 000, 22 000, and 23 000 L mol^{-1} cm^{-1} for **1a**, **1b**, **2a**, and **2b**, respectively.

Emission properties

In ethyl acetate, the excitation spectrum of **1b**, **2a**, **2b**, and **3a** was similar to the absorption one, whatever the emission wavelength. For **1a**, the absorption and excitation spectra displayed a difference, which was not encountered in acetonitrile. This difference observed in ethyl acetate was attributed to the

Table 1 Spectroscopic and photophysical characteristics of coumarins **1a**, **1b**, **2a**, **2b**, and **3a** in six different solvents (except for **3a**, which is insoluble in dichloromethane): maximum absorption wavelength (λ_{abs}), maximum emission wavelength (λ_{em}), fluorescence quantum yield (Φ_f), fluorescence lifetime (τ), and rate constants of radiative (k_r) and non-radiative (k_{nr}) deactivation

	Solvent	λ_{abs}/nm	λ_{em}/nm	ϕ_f	τ/ns	$k_r/10^8$ s^{-1}	$k_{nr}/10^8$ s^{-1}
1a	1,4-Dioxane	368 (sh 345, 384)	433	0.66	2.3 ± 0.2	2.9 ± 0.6	1.5 ± 0.5
	Ethyl acetate	366 (sh 354, 384)	429	0.79	2.2 ± 0.2	3.6 ± 0.7	0.9 ± 0.4
	Dichloromethane	368 (sh 354, 384)	425	0.17	1.1 ± 0.1	1.5 ± 0.3	7.5 ± 0.9
	Acetonitrile	366 (sh 352, 382)	427	0.38	1.8 ± 0.2	2.1 ± 0.4	3.4 ± 0.6
	N,*N*-Dimethylformamide	370 (sh 386)	428	0.62	2.1 ± 0.2	2.9 ± 0.6	1.8 ± 0.5
	Tripropionin	368 (sh 354, 386)	426	0.48	2.0 ± 0.2	1.0 ± 0.3	4.0 ± 0.6
1b	1,4-Dioxane	378	441	0.46	1.8 ± 0.2	2.5 ± 0.5	3.0 ± 0.6
	Ethyl acetate	378	440	0.48	1.5 ± 0.1	3.2 ± 0.6	3.5 ± 0.7
	Dichloromethane	380	444	0.58	2.0 ± 0.2	2.9 ± 0.6	2.1 ± 0.5
	Acetonitrile	376	444	0.38	1.6 ± 0.2	2.4 ± 0.5	3.9 ± 0.6
	N,*N*-Dimethylformamide	380	447	0.47	1.8 ± 0.2	2.6 ± 0.5	2.9 ± 0.6
	Tripropionin	380	442	0.55	2.2 ± 0.2	2.5 ± 0.5	2.0 ± 0.4
2a	1,4-Dioxane	324	414[a]	3.3 x 10^{-3}	1.0 ± 0.1	0.03 ± 0.01	10 ± 1
	Ethyl acetate	322	424[b]	1.6 x 10^{-3}	1.2 ± 0.1	0.01 ± 0.01	8.3 ± 0.8
	Dichloromethane	328	414[a]	2.3 x 10^{-3}	0.7* ± 0.07	0.035 ± 0.01	15 ± 1
	Acetonitrile	322	434[b]	7.4 x 10^{-3}	⩽0.7*	—	—
	N,*N*-Dimethylformamide	334	462[c]	3.9 x 10^{-3}	⩽0.7*	—	—
	Tripropionin	326	423[c]	5.7 x 10^{-3}	2.0 ± 0.2	0.03 ± 0.01	5.0 ± 0.5
2b	1,4-Dioxane	340	432	8.0 x 10^{-2}	0.7 ± 0.1	1.1 ± 0.2	13.1 ± 1.4
	Ethyl acetate	342	428	7.2 x 10^{-2}	⩽0.7*	—	—
	Dichloromethane	344	440	9.5 x 10^{-2}	0.8 ± 0.1	1.2 ± 0.2	11.3 ± 1.2
	Acetonitrile	340	450	8.7 x 10^{-2}	⩽0.7*	—	—
	N,*N*-Dimethylformamide	344	454	1.4 x 10^{-1}	1.0 ± 0.1	1.4 ± 0.3	8.6 ± 0.9
	Tripropionin	342	432	2.0 x 10^{-1}	1.7 ± 0.2	1.2 ± 0.4	4.7 ± 0.5
3a	1,4-Dioxane	310 (sh 298, 324)	347 (sh 359)	1.3 x 10^{-2}	—	—	—
	Ethyl acetate	310 (sh 298, 322)	359 (sh 347)	1.5 x 10^{-2}	—	—	—
	Acetonitrile	308	360	2.0 x 10^{-2}	—	—	—
	N,*N*-Dimethylformamide	304	360	2.0 x 10^{-2}	—	—	—
	Tripropionin	307	361	1.9 x 10^{-2}	—	—	—

For fluorescence measurements, unless specified $\lambda_{ex} = \lambda_{abs}$. The error on the fluorescence quantum yields is estimated to be about 10%. [a] λ_{ex} = 350 nm. [b] λ_{ex} = 340 nm. [c] λ_{ex} = 360 nm. * Detection limit of the spectrofluorometer.

presence in this solvent of two species, with and without an intramolecular hydrogen bond. Conversely, it was checked in ethyl acetate that for every compound the shape of the emission spectra did not depend on the excitation wavelength. The fluorescence data are collected in Table 1. The emission spectra were recorded in solution by exciting at the absorption maximum (unless specified) and they showed only one band (as an example, the spectra of coumarins **1a** and **1b** in ethyl acetate are given in Fig. 1). As expected, substitution in the 3-position induced a shift of the emission maxima towards the red. The shift was around $\Delta\lambda = 70$ nm in ethyl acetate for hydroxycoumarins **1a** and **2a**, as compared to the reference compound, **3a**. For both series, esterification again induced a red-shift of the spectrum.

For ethyl furoate derivatives **1a** and **1b**, the emission spectrum showed little variation with the type of solvent. Moreover, the spectrum of **1a** in acetonitrile hardly varied after addition of triethylamine or acetic acid. This behaviour has been reported by Arora *et al.*[6] for 4-hydroxycoumarin, the emission spectrum of which is very close to that of the conjugated anion in methanol.

In contrast, for the esterified benzo-1,4-dioxanylcoumarin **2b**, the emission spectrum underwent a moderate but clear positive solvatochromism. The maximum passed from $\lambda_{em} = 432$ nm in the low polarity solvent 1,4-dioxane (dielectric constant $\varepsilon = 2.21$ [14]) to $\lambda_{em} = 454$ nm in the strongly polar ($\varepsilon = 36.7$ [14]) solvent *N*,*N*-dimethylformamide (DMF). This solvatochromic behaviour is reminiscent of that of several mono- and disubstituted coumarins, among which unsubstituted 4-hydroxycoumarins.[15,16] A spectacular red-shift of the emission spectra was also observed for hydroxycoumarin **2a** in *N*,*N*-dimethylformamide, although no particular shift was encountered in the corresponding absorption spectra. Care must be taken in assigning this shift to solvatochromism. It could more likely be attributed to the formation of a hydroxycoumarin phenate in the excited state. It is widely recognized that the acidity of hydroxyl derivatives increases in the excited state, making these compounds more sensitive to the presence of basic residues which may be present at quite high concentrations in organic solvents.[17,18] In fact, adding triethylamine to the acetonitrile solution of **2a** induced a shift of the emission spectrum from 434 nm to 462 nm, which is the same value as that found in DMF. In contrast, traces of acetic acid in acetonitrile shift the spectrum back towards the blue (402 nm).

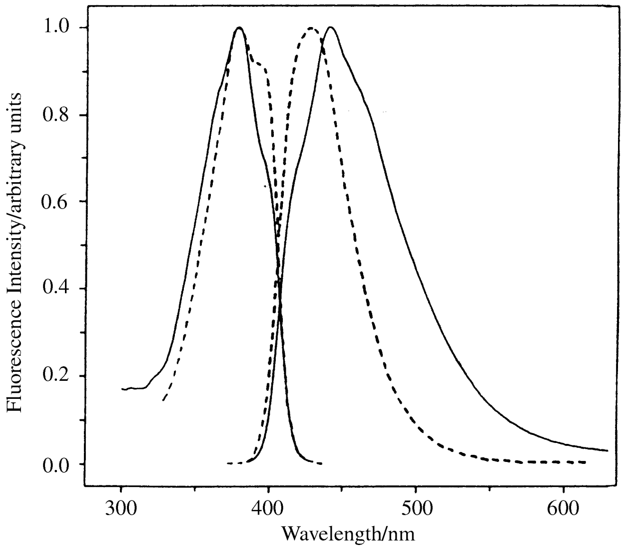


Fig. 1 Normalized excitation (left) and emission (right) spectra of coumarins **1a** (broken line) and **1b** (solid line), measured in ethyl acetate.

The behaviour of compound **3a** seems to be intermediate between those of **1a** and **2a**.

Concerning the fluorescence quantum yield, substitution by an ethyl furoate group led to an enhancement by one order of magnitude as compared to the unsubstituted coumarin. In contrast, the quantum yield collapsed for the benzo-1,4-dioxanyl series and especially for hydroxycoumarin **2a**.

The fluorescence lifetime, which was monoexponential, was in the range of 2 ns for compounds **1a** and **1b**, decreased below 1 ns for compounds **2a** and **2b**, and was even shorter for **3a**. Note that lifetimes below 1 ns should be regarded with circumspection, since the detection limit of our apparatus was of this order.

From the values obtained for the fluorescence quantum yield Φ_f and the radiative lifetime τ (when reliable measurements could be made), radiative (k_r) and non-radiative (k_{nr}) deactivation constants were calculated by the classical photophysical equations: $k_r = \Phi_f/\tau$ and $k_{nr} = (1 - \Phi_f)/\tau$. The values obtained are listed in Table 1. It was observed that for the ethyl furoate series, constants k_r and k_{nr} were around 1 to 4×10^8 s^{-1} and hardly varied from one solvent to another, with the exception of the particular behaviour encountered for **1a** in dichloromethane. In contrast, for the benzo-1,4-dioxanyl series, the very low value found for k_r indicated reduced fluorescence efficiency, whereas high values for k_{nr} suggested that thermal deactivation of the excited state may also take place.

Effect of substitution in the 6-position by an ethyl group

It is assumed in this work that esters bearing a benzoyl or an acetyl residue should be quite similar from a spectroscopic viewpoint. On the contrary, a molecule substituted in the 6-position by an electron-donating ethyl group should behave differently from the unsubstituted compound. In order to measure this effect, the spectroscopic properties of **2b** were compared to those of the analogue **4b** which does not bear an ethyl group in the 6-position. In this case, the UV/vis absorption spectrum of **2b** was red-shifted by $\Delta\lambda = 10$ nm in ethyl acetate. The position of the emission spectrum was hardly changed but the fluorescence efficiency of **2b** was twice as high as that of **4b**.

Viscosity effects

Concerning the photophysical behaviour, the presence of substituents introduces a new possibility for deactivation. In particular, deactivation by substituent rotation is likely to occur. To test this hypothesis, the viscosity effect was investigated using tripropionin (glycerol tripropionate, see Table 1). The polarity of this solvent is identical to that of ethyl acetate, although its viscosity is 7.1 cP instead of 0.45 cP. For the benzo-1,4-dioxanyl derivatives, **2a** and **2b**, the fluorescence lifetime was markedly increased and the quantum yield was found to be 3 times higher in tripropionin. Calculations revealed that it was the constant k_{nr} that was mainly affected, showing that viscosity had an influence upon the deactivation process. A far weaker effect was observed with compounds **1b** and **3a**. For hydroxycoumarin **1a**, the fluorescence quantum yield was reduced in the presence of tripropionin compared to ethyl acetate. This unusual effect was not explained.

Temperature effects

If substituent rotation is involved in the deactivation of the excited state, the latter should be influenced by temperature. The temperature effect was only studied on esterified coumarins **1b** and **2b**, because their lifetimes were higher than those of the hydroxylated derivatives and allowed variations to be measured accurately. When passing from 5 °C to 47 °C, the fluorescence intensity was decreased by 50 and 65%, for

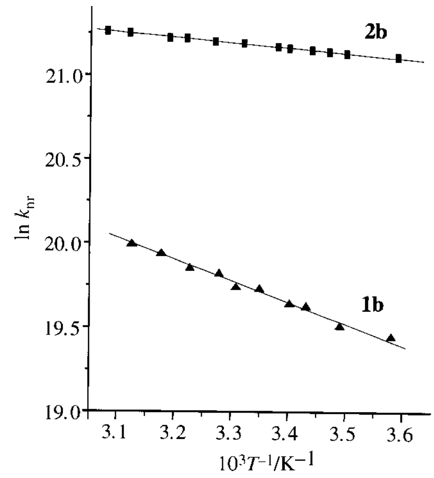


Fig. 2 Arrhenius plots for **1b** and **2b** in ethyl acetate.

coumarins **1b** and **2b**, respectively, in ethyl acetate. The lifetime was measured and found to be shortened with rising temperature. Again, the radiative and non-radiative constants were calculated. For both compounds, k_r was found to be unchanged in the temperature range investigated whereas k_{nr} was strongly increased.

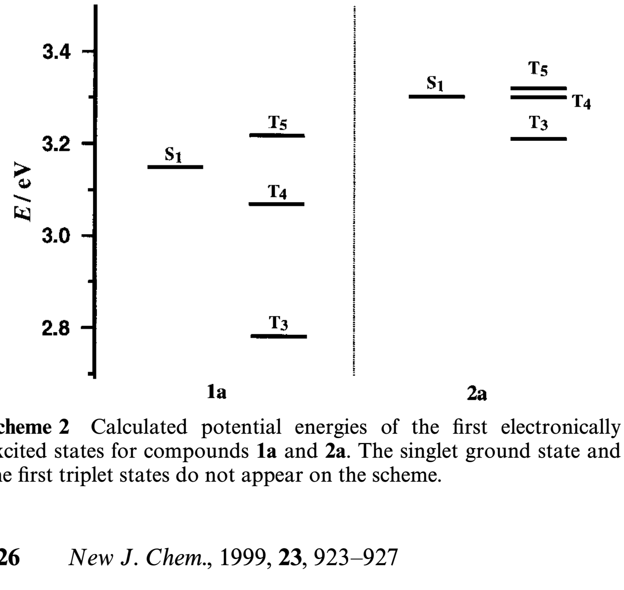
Assuming that non-radiative deactivation is a thermally activated process, the variation of k_{nr} should obey the Arrhenius law:

$$k_{nr} = A \exp(-E_a/RT)$$

where E_a is the apparent activation energy, accounting for both the temperature and viscosity effects. By plotting $\ln(k_{nr})$ *vs.* $1/T$, plots (Fig. 2) with excellent correlation coefficients ($r^2 \geqslant 0.995$) were obtained. The slope directly provides the ratio $-E_a/R$ and hence the value of E_a, which was found to be equal to 10.8 and 2.5 kJ mol^{-1} for **1b** and **2b**, respectively. This means that for **1b**, the value of the energy barrier corresponds to one to two quanta of vibrational energy, while for **2b** the low value of E_a lies in the range of rotational energy.

AM1 calculations

In order to help understand the behaviour of the polysubstituted compounds, AM1 MOPAC calculations were performed for hydroxycoumarins **1a** and **2a** (Table 2). The ground state was considered first. The sum of the charges Σq appearing on the coumaryl radical after removal of the substituent in the 3-position was found to be +0.034 and +0.052 for **1a** and **2a**, respectively. The positive signs confirmed that both substituents act as electron-withdrawing groups but the electrostatic effect was very weak, as indicated by the values. This is in line with the structure of the substituents. On the benzo-1,4-dioxanyl group, the oxygen atom in the para position induces a donor effect while the second oxygen atom in the meta position is an electron acceptor. On the ethyl furoate group, the electron-withdrawing effect of the ester carbonyl group is balanced by the donor ethoxy group.



Scheme 2 Calculated potential energies of the first electronically excited states for compounds **1a** and **2a**. The singlet ground state and the first triplet states do not appear on the scheme.

Table 2 Calculated data for hydroxycoumarins **1a** and **2a**

			S_1 energy		
	Σq	Torsion angle/°	λ/nm	E/eV	ν/cm^{-1}
1a	+0.034	13.2	393	3.15	25 390
2a	+0.052	39.5	374	3.31	26 689

Strong geometrical differences between the two compounds were demonstrated. In molecule **2a**, the benzo-1,4-dioxanyl group displayed an angle of about 40° with respect to the plane of the coumaryl cycle, which suggests poor conjugation between the two electron systems. Compound **1a**, however, was found to be almost planar, the angle between the coumarin residue and the ethyl furoate substituent being only 13.2°. The flatness of **1a** could be related to the existence of a hydrogen bond between the oxygen atom of the ethyl furoate group and the hydroxyl moiety of the coumarin. This is consistent with the fact that the strongest auxochromic effect was encountered for the ethyl furoate series.

Secondly, calculations were performed for the excited state. The difference between the excited and ground state dipole moments $\Delta\mu = \mu^* - \mu$ was calculated to be 3.9 and 1.1 D for **1a** and **2a**, respectively. It should be accompanied by a weak positive solvatochromic effect, which was actually the case for the benzo-1,4-dioxanyl series. The lack of solvatochromic behaviour encountered for the ethyl furoate series remains unexplained. Note that the values found for $\Delta\mu$ are close to those reported by Parkanyi *et al.*[16] for other hydroxycoumarins.

The potential energy of the first excited states was calculated. For the first singlet state, the energy was higher for **2a** than for **1a** (Table 2), and the values were in line with the spectral data. Let us now compare the energy of the excited triplet states closest to the singlet state. For the benzo-1,4-dioxanyl derivative, the first singlet state was found to be very close to the triplet state, which lies immediately below it (Scheme 2). A loss of fluorescence energy by intersystem crossing can therefore be expected compared with the ethyl furoate compound, the fluorescence efficiency decreasing when the gap between the excited states is small. This is in total accordance with experiment and explains why compounds **2a** and **2b** were poorly emissive. On the other hand, substitution by the benzo-1,4-dioxanyl group also makes the first singlet state closer to the triplet above it. This agrees with the fact that the non-radiative deactivation pathway was favoured by rising temperatures. These results are in line with the data in the literature concerning the energy levels of substituted 4-hydroxycoumarins.[19]

Conclusions

According to Wolfbeis and Uray,[20] 4-hydroxycoumarins are fluorescent when they bear an electron-donating group in the 7-position, which is in fact the case for 4-hydroxy-7-methoxycoumarin, as reported in the literature[10] and confirmed in this work. However, the present study indicates that the fluorescence properties are also critically dependent on the nature of the substituent borne in the 3-position.

The compounds of the ethyl furoate series showed more interesting fluorescence properties than those of the unsubstituted analogue: higher fluorescence quantum yield, longer lifetimes, and above all the photophysical behaviour was almost unaffected by the environment. Therefore, these compounds can be suitable fluorescent probes to label molecules that must be analysed in different media. As for the benzo-1,4-dioxanyl derivatives, the fluorescence properties were very sensitive to any restriction of substituent rotation. We are now planning to investigate the use of these products as fluorescent microviscosity probes in organized media.

Experimental

Materials

Spectroscopic grade solvents (Merck and SDS) were used as received for UV/vis absorption and fluorescence measurements. The 4-hydroxy-7-methoxycoumarin derivatives studied in this work were synthesized by Claisen condensation of the corresponding acetophenone with diethyl carbonate in the presence of base. The preparation of polysubstituted 4-hydroxycoumarins **1a** and **2a** was previously described.[21,22] Boiling **1a** with benzoyl chloride and **2a** with acetic anhydride led to derivatives **1b** and **2b**, respectively. Compound **4b** was obtained from the corresponding hydroxycoumarin (**4a**) using the same procedure as for **2b**.[21,22] 4-Hydroxy-7-methoxycoumarin **3a** was prepared according to ref. 10. The compounds were obtained with good yields and characterized by the usual methods.

Apparatus

Absorbance spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer. Steady-state fluorescence studies were performed on a Photon Technology International (PTI) Quanta Master 1 spectrofluorometer. All fluorescence spectra were corrected. The fluorescence quantum yields were determined using coumarin 6 in ethanol ($\Phi_f = 0.78$) as standard.[23] Fluorescence decay was measured with the stroboscopic technique utilizing a Strobe Master fluorescence lifetime spectrometer from PTI. The excitation source was a flash lamp filled with a mixture of nitrogen and helium (30 : 70). Samples were excited at $\lambda_{ex} = 357$ nm and the decay monitored at the emission maximum. Data were collected over 200 channels with a time base of 0.2 ns per channel. The measurements were conducted in a thermostatted cell. Analysis of fluorescence decay was performed using the monoexponential method software from PTI.

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