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Dyes and Pigments 57 (2003) 259–265

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# UV/vis absorption and fluorescence spectroscopic study of novel symmetrical biscoumarin dyes

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Received 29 November 2002; received in revised form 6 January 2003; accepted 31 January 2003

## Abstract

The optical properties of six new 3,3′-phenylenebiscoumarin dyes, differing by the substitution pattern of the central phenyl group and by the presence of methoxy substituents, were investigated in $CH_2Cl_2$ by UV/vis absorption and fluorescence spectroscopy. The spectroscopic behaviour of one of the dyes was also analysed in five other solvents. By comparison with monocoumarins, the absorption and emission spectra of the biscoumarin derivatives were shifted to high wavelengths. Most of them displayed high fluorescence quantum yields (0.7–0.8) and lifetimes in the nanosecond range, which can make these dyes very useful as new fluorescent probes.

*Keywords:* Fluorescence; Coumarin; Biscoumarin; Symmetrical dye

## 1. Introduction

There is a constant demand for new fluorescent dyes, especially for the high-technology industry and the biological sciences. Among those commonly available, coumarins are attractive molecules due to their extended spectral range, high emission quantum yields, photostability, and good solubility in the safest solvents. They constitute the largest class of laser dyes for the “blue–green” region [1–3]. They are also widely used as fluorescent labels and pigments [4,5], and as fluorescent probes for physiological [6,7] and enzymatic measurements [8]. Moreover, coumarin derivatives are frequently encountered as signalling units in sensors [9–12] and in sophisticated photophysical systems [13–15].

Recently, two of us reported the synthesis of new 3,3′-phenylenebiscoumarin derivatives [16,17] which had been rarely described until then in the literature [18,19]. These compounds were obtained by a Knoevenagel reaction taking place between various salicylaldehydes and phenylenediacetonitrile. It was shown that carrying out this reaction in a biphasic solid/liquid medium, using a strongly basic macroporous resin, allowed most of the biscoumarin derivatives to be obtained in high yields. The idea of linking two coumarin moieties by a phenyl ring originated from the will to extend the absorption and emission wavelength ranges, by increasing the electron conjugated system. It

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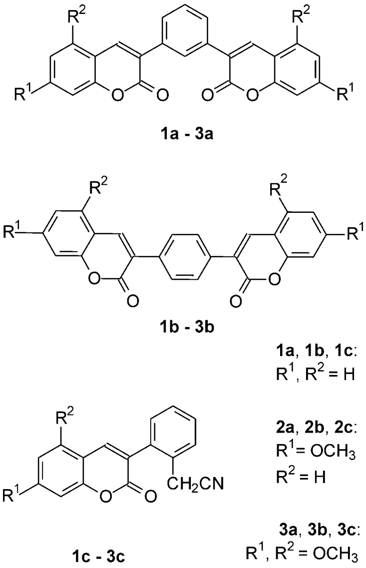
0143-7208/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0143-7208(03)00028-7

has also been reported that some biscoumarins display lasing properties [20]. Moreover, a great deal of interest is presently focused on symmetrical structures, which are liable to be the most efficient for two-photon excitation fluorescence. Potential applications encompass fluorescence microscopy [21], optical data storage [22,23] and microfabrication [23] to name just a few.

The aim of the present paper was to report the spectroscopic and photophysical properties of two series of symmetrical compounds. The dyes of series **a** (**1a–3a**, Scheme 1) are derivatives of *meta*-phenylenediacetonitrile. Those of series **b** derive from the *para* analogue (compounds **1b–3b**). The coumarin moieties were differently substituted by methoxy groups in the 7- and 5-positions. For the sake of comparison, the properties of unsymmetrical 3-phenylcoumarin derivatives (**1c–3c**) were also investigated. The latter compounds bear a $CH_2CN$ group on the *ortho*-position of the phenyl ring, but since the cyano group is not directly connected to the conjugated electron system, it was assumed that it has a minor effect on the spectroscopic behaviour.



Scheme 1. Chemical structure of the bis- and monocoumarin dyes.

## 2. Experimental

### 2.1. Materials

The synthesis of the dyes was described in previous papers [16,17]. Spectroscopic grade solvents were purchased from SDS and Aldrich and used without further purification.

### 2.2. Apparatus

Spectrophotometric measurements were conducted in a thermostatted cell at 23 °C. Absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. The estimated experimental error was 1 nm on the band maximum, 5% on the molar extinction coefficient. Steady-state fluorescence work was performed on a Photon Technology International (PTI) Quanta Master 1 spectrofluorometer. All fluorescence spectra were corrected. The fluorescence quantum yields ($\Phi$) were determined using the classical formula: $\Phi_x = \left(A_s \times F_x \times n_x^2 \times \Phi_s\right)/\left(A_x \times F_s \times n_s^2\right)$ where $A$ is the absorbance at the excitation wavelength, $F$ the area under the fluorescence curve and $n$ the refraction index. Subscripts s and $x$ refer to the standard and to the sample of unknown quantum yield, respectively. Coumarin 6 in ethanol ($\Phi = 0.78$) was taken as the standard [24]. Fluorescence decay was measured with the stroboscopic technique utilising 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). Data were collected over 200 channels with a time-base of 0.1 ns per channel. Fluorescence decay was analysed using the monoexponential method software from PTI.

## 3. Results

### 3.1. UV/vis absorption spectra

The dyes were dissolved in dichloromethane, their concentration being in the $10^{-5}$ M range. They were firstly studied by UV/vis absorption spectroscopy. The spectrum of unsubstituted dyes **1a** and **1c** showed two maxima around 300–330 nm.

In contrast, compound **1b** and all the methoxy-substituted compounds displayed an intense unresolved band, with a weak shoulder on its short-wavelength side. The absorption maximum was situated between 336 and 372 nm. All spectral characteristics are collected in Table 1.

It was noted that the band maximum shifted to the red when increasing the number of methoxy groups. On passing from a monocoumarin (series **c**) to a biscoumarin, the maximum shifted to higher wavelengths, this effect being stronger when the coumarin moieties occupied the *para*-positions on the phenyl ring (series **b**).

Regarding the molar absorption coefficient, it increased for the methoxy-substituted compounds, with respect to the unsubstituted ones. The value measured for **3b** seems to be a little low, but this may be attributed to the very poor solubility of this compound in $CH_2Cl_2$.

### 3.2. Excitation spectra

For fluorescence spectroscopy, the absorbance at the excitation wavelength was around 0.05, i.e. the dye concentration was in the $10^{-6}$ M range. For all the compounds that bear methoxy groups, and also for **1b**, the excitation spectrum was similar to the absorption spectrum, and did not vary with the emission wavelength. This indicates that the emitting species is the one responsible for the absorption spectrum.

For dye **1c**, the excitation spectrum displayed two bands, the maxima of which were identical to those of the absorption spectrum. However, the respective proportions of the two excitation bands varied with the emission wavelength, the band at 318 nm becoming more intense when the emission was gathered at higher wavelengths.

For dye **1a**, the intensity of the shoulder at 308 nm decreased, accompanied by a slight red shift of the whole excitation spectrum, when the emission was collected at high wavelengths (Fig. 1).

### 3.3. Steady-state emission spectra

The methoxy-substituted dyes **2a**, **3a**, **2c** and **3c** showed only one structureless emission band. The compounds of series **b** displayed an emission spectrum where two intense bands and many

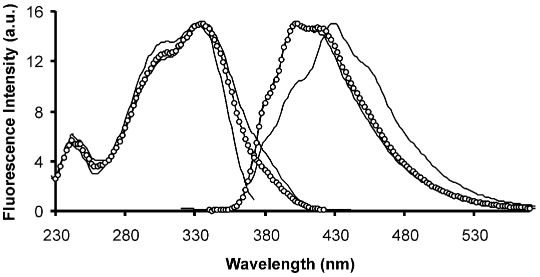


Fig. 1. Fluorescence spectra of **1a** ($1.6\times10^{-6}$ M) in $CH_2Cl_2$. From left to right: Excitation spectrum, $\lambda_{em} = 380$, 430 and 450 nm; Emission spectrum, $\lambda_{ex} = 280$, 330 and 360 nm.

Table 1
Spectral and photophysical characteristics of coumarin derivatives **1a–3c**: maximum absorption wavelength ($\lambda_{abs}$), molar extinction coefficient at the absorption maximum ($\varepsilon$), maximum emission wavelength ($\lambda_{em}$, underlined is the peak of major intensity), fluorescence quantum yield ($\Phi$, with excitation at $\lambda_{abs}$), fluorescence lifetime ($\tau$), radiative ($k_r$) and non-radiative ($k_{nr}$) deactivation constants

| Compound | $\lambda_{abs}$ (nm) | $\varepsilon$ ($M^{-1}$ $cm^{-1}$) | $\lambda_{em}$ (nm) | $\Phi$ | $\tau$ (ns) | $k_r$ ($10^8$ $s^{-1}$) | $k_{nr}$ ($10^8$ $s^{-1}$) |
|---|---|---|---|---|---|---|---|
| **1a** | 308 | 27 400 | 419 | $0.098\pm3\times10^{-3}$ | 0.4±0.02 | 2.45±0.20 | 22.5±1.2 |
| | 330 | 29 800 | | | | | |
| **2a** | 344 | 44 700 | 426 | $0.80\pm2\times10^{-3}$ | 2.5±0.01 | 3.20±0.03 | 0.80±0.01 |
| **3a** | 352 | 44 000 | 438 | $0.70\pm5\times10^{-3}$ | 2.6±0.01 | 2.69±0.03 | 1.15±0.03 |
| **1b** | 346 | 36 500 | 410, $\underline{430}$ | $0.73\pm7\times10^{-3}$ | 1.4±0.01 | 5.21±0.01 | 1.93±0.06 |
| **2b** | 362 | 50 000 | $\underline{434}$, 454 | $0.72\pm5\times10^{-3}$ | 1.4±0.01 | 5.14±0.01 | 2.0±0.05 |
| **3b** | 372 | 39 700 | $\underline{446}$, 469 | $0.80\pm8\times10^{-3}$ | 1.8±0.02 | 4.44±0.01 | 1.11±0.06 |
| **1c** | 292 | 16 700 | 404 | $0.055\pm2\times10^{-3}$ | 1.4±0.04 | 0.39±0.03 | 6.75±0.2 |
| | 318 | 13 300 | | | | | |
| **2c** | 336 | 19 700 | 409 | $0.81\pm7\times10^{-3}$ | 2.4±0.01 | 3.38±0.04 | 0.79±0.04 |
| **3c** | 342 | 23 100 | 422 | $0.82\pm5\times10^{-3}$ | 2.8±0.01 | 2.93±0.03 | 0.64±0.03 |

shoulders can be distinguished (Fig. 2). In every case, neither the shape nor the position of these spectra were affected by a change in the excitation wavelength. In contrast, for unsubstituted dyes **1a** and **1c**, exciting in the red of the absorption spectrum led to the distortion of the emission spectrum which moved to higher wavelengths (Fig. 1).

Let us now compare the position of the emission maximum for all the compounds excited at their absorption maximum. The emission spectrum was shifted to the red on increasing the number of methoxy groups on the coumarin moieties. The biscoumarin derivatives, and especially those of series **b**, also emitted at higher wavelengths than the monocoumarins.

### 3.4. Fluorescence quantum yields and lifetimes

The fluorescence quantum yield was determined by exciting at the absorption maximum for each dye (Table 1). It was lower than 0.1 for unsubstituted compounds **1a** and **1c**. However, it was drastically increased in series **a** and **c** by the presence of a methoxy group on the coumarin heterocycle, since it was then between 0.7 and 0.8. The presence of a second methoxy group did not lead to subsequent enhancement. In series **b**, the coumarin heterocycle being substituted or not, no difference was observed in the value of the quantum yield, which was high in every case.

The fluorescence lifetimes were measured by exciting at 337 nm and collecting the signal at the maximum emission wavelength for each compound. Monoexponential decay was obtained in every case. A very low value (0.4 ns) was measured for **1a**, but must be considered with circumspection, since the measurement limit of our apparatus was then attained. Besides, in these conditions, it was impossible to distinguish between two short lifetimes. For all the other compounds, the fluorescence lifetimes ranged between 1.4 and 2.8 ns. For series **a** and **c**, the lifetime was increased in the presence of at least one methoxy group on the coumarin moieties. For series **b**, the lifetime was almost independent of substitution.

### 3.5. Deactivation constants

The determination of the fluorescence quantum yields and lifetimes gives access to the calculation of the radiative ($k_r$) and non-radiative ($k_{nr}$) deactivation constants, classically defined as $k_r = \frac{\Phi}{\tau}$ and $k_{nr} = \frac{(1-\Phi)}{\tau}$. The highest values of $k_r$ were encountered for the compounds of series **b**. Interestingly, a value lower by one order of magnitude was found for **1c**. Regarding the $k_{nr}$ constant, a very high value was found for **1a** and, to a lesser extent, for **1c** (Table 1).

### 3.6. Solvatochromic behaviour

The spectroscopic characteristics of **1b** were analysed in five different solvents (Table 2). Very weak variations of the absorption and emission spectra were observed. In contrast, the quantum yield was decreased in very polar solvents, such as dimethylsulphoxide. No particular behaviour was detected when using a protic solvent (ethanol).

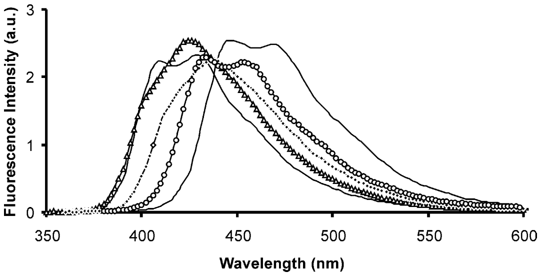


Fig. 2. Emission spectrum of dyes **1b**, **2a**, **3a**, **2b** and **3b** (from bottom to top, at 500 nm), 1 to 1.4×$10^{-6}$ M in $CH_2Cl_2$. Excitation at the maximum absorption wavelength.

Table 2
Spectral characteristics of coumarin derivatives **1b**: maximum absorption wavelength ($\lambda_{abs}$), maximum emission wavelength ($\lambda_{em}$), and fluorescence quantum yield ($\Phi$, with excitation at the absorption maximum)

| Solvent | $\lambda_{abs}$ (nm) | $\lambda_{em}$ (nm) | $\Phi$ |
|---|---|---|---|
| Toluene | 346 | 424 | 0.73 |
| Ethylacetate | 342 | 426 | 0.70 |
| Acetonitrile | 342 | 426 | 0.60 |
| Ethanol | 346 | 426 | 0.53 |
| Dimethylsulphoxide | 346 | 430 | 0.41 |

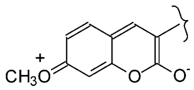
## 4. Discussion

It is known that the substitution of a coumarin heterocycle by a phenyl ring in the 3-position enhances the emission yield [25]. Interesting fluorescence properties were therefore expected for our compounds.

The spectroscopic behaviour found here for the monocoumarins is very close to that reported for phenylcoumarin [25,26]. Regarding the biscoumarin derivatives, they absorbed and emitted at higher wavelengths than the monocoumarins, and their molar extinction coefficient was markedly increased. This is in line with the extension of the electron conjugated system. These effects are particularly strong for the dyes of series **b**, because the *para* substitution pattern favours electron delocalization over the whole structure. It is also possible that in dyes of series **a**, for reasons of steric hindrance, the two coumarin moieties are twisted with respect to the phenyl plane, which usually reduces conjugation.

The introduction of the methoxy groups allowed the position of the absorption and emission spectra to be tuned. The wavelength shift observed for the three series of dyes can be easily explained by the electron donor effect of the methoxy substituent borne on the 7- and 5-positions. Within each coumarin moiety, this boosts the internal charge transfer toward the electron withdrawing group, that is the carbonyl group of the lactone (Scheme 2). This is the reason why the electron donor groups are classically introduced in these positions.

A particular behaviour pattern was noted for unsubstituted dyes **1a** and **1c**. On the excitation spectrum, the intensity of the two bands varied when changing the emission wavelengths, and conversely the emission spectrum depended on the excitation wavelength. This phenomenon is frequently encountered when two emitting species are found in solution. Since the dyes used in this work were carefully purified, one hypothesis is the existence of rotational isomers. Actually, a torsion can occur around the bond which links the coumarin moiety and the phenyl ring, and isomers can differ by the angle formed between the two sub-units. The study of the deactivation constants agrees with this hypothesis. For molecules **1c** and **1a**, the $k_{nr}$ value was found to be particularly high. The non-radiative deactivation constant is related to all the phenomena which can deactivate fluorescence, and in this case, the most likely process is movements and torsions within the structure. Actually, the dependence of the fluorescence quantum yield on temperature and viscosity has been reported in the literature for 3-phenylcoumarin [27] and other coumarins differently substituted in the 3-position [27,28]. It has been shown that the radiationless process was related to the rotation of the group in the 3-position. It is interesting to see that this phenomenon was not observed after the introduction of the methoxy groups. It was not encountered either for the dyes of series **b**. Therefore, it seems to be prevented by the extension of the conjugated electron system.



Scheme 2. Resonance structure of a 7-methoxycoumarin moiety.

Substitution can also have a direct effect on the energy levels of the molecule. Let us consider the deactivation constants again. The $k_r$ constant, which measures the intrinsic fluorescence efficiency of the molecule, is particularly low for **1c**. This constant was much higher for the methoxy-substituted compounds. This behaviour is reminiscent of other studies, in which it was shown that in some coumarins, the first singlet state lies close to another singlet state [29] or to a triplet state [28,30], leading to a strong loss of the excitation energy by internal conversion or intersystem crossing. The presence of certain substituents widens the gap between these levels, causing a marked increase in the fluorescence efficiency.

Our solvatochromic approach showed that the absorption and emission spectra of **1b** were almost unchanged on varying the solvent. This indicates that only very moderate charge transfer takes place in the excited state. The decrease of the fluorescence quantum yield in polar solvents has been evoked in the literature for many coumarins, and often attributed to the deactivation of the excited state by a twisted internal charge transfer

(TICT) mechanism. This consists of the appearance of localized charges in the excited state, accompanied by the rotation of a molecular moiety to reach a right angle. However, this mechanism has been reported to take place in coumarins with a strong donor group (for example a dialkylamino group in the 7-position) and a strong acceptor group (often in the 3-, 4- and 6-positions [25,31–33]). This is not the case for compound **1b**. Therefore, the TICT hypothesis can be ruled out here, in total agreement with the fact that no TICT process has been encountered in phenylcoumarin either [25]. The decrease in fluorescence quantum yield observed here can more likely be related to the effect of the solvent on the molecular energy levels.

## 5. Conclusions

Most of the biscoumarin dyes investigated displayed excellent optical properties. Their molar absorption coefficients were much higher than those of the monocoumarins. Except for **1a**, the fluorescence quantum yield was particularly high, and the lifetime in the range of 1–2 ns allows this parameter to be monitored without using an ultra-fast measurement apparatus. The introduction of methoxy substituents moved the absorption and emission spectra to high wavelengths. The dyes of series **b** are especially interesting, since the position of their spectra can be controlled in this way, while their optimal photophysical behaviour remains unchanged. These biscoumarin dyes could be useful as new fluorescent probes, in a wide range of solvents. They could also be very interesting for two-photon excitation. We hope our compounds will be tested soon in this respect.

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