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Dyes and Pigments 78 (2008) 1–7
DYES and PIGMENTS
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Synthesis and spectroscopic study of new biscoumarin dyes based on 7-(4-methylcoumarinyl) diesters

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Received 11 July 2007; received in revised form 21 September 2007; accepted 25 September 2007

Abstract

A liquid/liquid interfacial technique was used to prepare biscoumarin dyes by condensing 7-hydroxy-4-methylcoumarin with various diacyl chlorides in the presence of a phase-transfer catalyst such as triethylbenzylammonium chloride. The reaction conditions were optimized. Six new biscoumarin derivatives were thus prepared in high yield. Their optical properties were studied in chloroform by UV/vis absorption and fluorescence spectroscopies. It appeared that the shape of the absorption spectra varies with the nature of the tether between both chromophores. In contrast, the excitation and emission spectra were almost unchanged, although the compounds exhibited drastic differences in their fluorescence efficiency.

Keywords: Biscoumarin; Coumarin; Phase-transfer; Fluorescence

1. Introduction

In the well-known family of coumarin derivatives, dimeric coumarins (also called biscoumarins) occupy an interesting position. These compounds occur naturally in a large number of plants and micro-organisms [1,2]. They are often biologically active [3–6] and many of them have been tested in view of therapeutical use [7], as antimicrobial [8], cardiovascular [9], anticoagulant [10,11], anti-inflammatory [12], and antiproliferative [13–15] agents. Moreover, since they are related to the most widely used class of fluorescent dyes, biscoumarins also show original optical properties [16–19]. They are highly efficient laser dyes [20,21]. Their inclusion in zeolites could lead to artificial antenna systems [22]. They have also been studied as photosensitizers in resins [23–26], active material for organic light-emitting devices [27], dying stuff [28] and signaling units in fluorescent ion sensors [29,30]. In this context, we recently reported that dimeric coumarin derivatives, namely 3,3′-phenylene biscoumarin dyes, displayed excellent optical properties. Their molar absorption coefficient was much higher than that of monocoumarins, their absorption and emission spectra were shifted to long-wavelengths, while their fluorescence quantum yield was very high [31]. Biscoumarin compounds are therefore of high interest in the field of fluorescent dyes.

Like for simple coumarins, the optical properties of biscoumarin dyes are influenced by the nature and position of substituents [16–21]. Additionally, the way the two coumarin moieties are linked to each other, as well as the chemical nature of the bridge, play a determining role in the optical properties. As a continuation of our studies aimed at clarifying this point, and as a part of a program directed toward the synthesis of dimeric coumarins, we report here the synthesis of new molecules, made of two coumarin moieties linked by a diester bridge. Our goal is to see how the presence of the ester group affects the fluorescence properties of these compounds.

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doi:10.1016/j.dyepig.2007.09.008

To do so, 7-hydroxy-4-methylcoumarin (**1**) was condensed with various diacid chlorides in a biphasic liquid/liquid medium to yield several 7-(4-methyl)coumarinyl diesters (**2**–**7**). These dyes were then studied in chloroform by UV/vis absorption spectroscopy, and by steady-state and dynamic fluorescence spectroscopy. Their properties were compared to those of monomeric coumarin **1**.

2. Experimental

2.1. Materials

The dichloromethane used for synthesis was purified by distillation. The chloroform (Purex) used for spectroscopic measurement was from SDS and used as-received. The four phase-transfer catalysts tested in this work (triethylbenzylammonium chloride, TEBAC; tetrabutylammonium bromide, TBAB; tetrabutylammonium bisulphate, TBAS; and hexadecyltrimethyl ammonium bromide, HTAB) were purchased from Aldrich and employed as-received. Terephthaloyl, phthaloyl, oxalyl, succinyl, adipoyl, and sebacoyl chlorides were from Aldrich.

2.2. Apparatus

Compounds were examined by FTIR (KBr pellets) on a Jasco FT-IR 420 spectrometer. ^{1}H and ^{13}C NMR spectra of compounds **3**–**7** were recorded on a Bruker WP 200 spectrometer operating at 300 and 75 MHz, respectively, with TMS as internal standard. ^{1}H and ^{13}C NMR spectra of compound **2** were recorded on a Bruker Avance 400 spectrometer operating at 400 and 100 MHz, respectively. Melting points were taken using an Electrothermal 9100 apparatus. Mass spectra (CI) were performed on a TSQ7000 spectrometer from ThermoQuest using DCI/NH_3 as the ionization mode (positive mode).

For UV/vis absorption and steady-state fluorescence spectroscopies, the step between two measurements was 2 nm. The absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Corrected steady-state fluorescence spectra were recorded with a Photon Technology International (PTI) Quanta Master 1 spectrofluorometer. The measurements were conducted at 25 °C in a temperature-controlled cell, on solutions whose absorbance is equal or below 0.05 at the excitation wavelength. The fluorescence quantum yields were determined using the classical formula: $\Phi_x = (A_s \times F_x \times n_x^2 \times \Phi_s)/(A_x \times F_s \times n_s^2)$ 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 [32]. 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). Excitation was performed at 337 nm, and the fluorescence signal was collected at the maximum emission wavelength of each compound. Data were collected over 200 channels with a time-base of 0.1 ns per channel. Analysis of fluorescence decay was performed using the multi-exponential method software from PTI.

2.3. Condensation procedure

All biscoumarins **2**–**7** were prepared according to the following optimized interfacial procedure. An aliquot (10 mmol) of 7-hydroxy-4-methylcoumarin (**1**) was dissolved in the basic aqueous solution in 50 mL of 0.2 M aqueous sodium hydroxide, and the phase-transfer catalyst (0.12 mmol of triethylbenzylammonium chloride (TEBAC)) was added to the solution, just before mixing with the methylene chloride solution containing 5 mmol of appropriate diacid chloride under vigorous mechanical stirring (700 rpm). The reaction was carried out for 2 h at room temperature under stirring. Meanwhile, a fraction of the biscoumarin formed precipitated in the medium. After filtration the biscoumarin was washed with water until the filtrate gave a neutral pH value. The compound was dried under vacuum in a Büchi oven. This procedure gives compounds that contain about one half (**5**, **6**), one (**2**, **3**), two (**7**), and three (**4**) molecules of water according to elemental analysis.

2.4. Characterization of the biscoumarin derivatives

2.4.1. p-Phenylenedicarboxylic acid 7-(4-methylcoumarinyl) diester (2)

Yield: 95%. M.p. undetermined (decomposes around 330 °C). FTIR (ν cm^{-1}): 1619 (C═C); 1733 (C═O). ^{1}H NMR ($CDCl_3$, δ ppm): 2.49 (d, $J = 0.9$ Hz, 6H, $2 \times CH_3$); 6.33 (d, $J = 0.9$ Hz, 2H, $2 \times H_3$); 7.05 (dd, $J = 2.1$, 8.4 Hz, 2H, $2 \times H_6$); 7.33 (d, $J = 2.1$ Hz, 2H, $2 \times H_8$); 7.71 (d, $J = 8.4$ Hz, 2H, $2 \times H_5$); 8.09 (*s*, 4H, Ar). ^{13}C NMR (δ ppm): 18.7 (CH_3); 110.6 (C_8); 114.8 (C_3); 118.0 (C_6); 118.2 (C_{10}); 125.6 (C_5); 130.5–133.5 (Ar); 151.9 (C_7); 153.0 (C_4); 154.0 (C_9); 160.4 (C_2); 165.1 (C_{11}). MS: 483.4 ($M + H^+$); 500.3 ($M + NH_4^+$). Anal. calcd. for $C_{28}H_{18}O_8 + H_2O$: C, 67.19; H, 4.03%. Found: C, 66.68; H, 3.70%.

2.4.2. o-Phenylenedicarboxylic acid 7-(4-methylcoumarinyl) diester (3)

Yield: 82%. M.p. 200 °C. FTIR (ν cm^{-1}): 1615 (C═C); 1730, 1763 (C═O). ^{1}H NMR ($CDCl_3$, δ ppm): 2.45 (d, $J = 0.9$, 6H, $2 \times CH_3$); 6.28 (d, $J = 0.9$, 2H, $2 \times H_3$); 7.24 (dd, $J = 2.1$, 8.4 Hz, 2H, $2 \times H_6$); 7.25 (d, $J = 2.1$ Hz, 2H, $2 \times H_8$); 7.65 (d, $J = 8.4$ Hz, 2H, $2 \times H_5$); 7.74–7.78 (m, 2H, Ar); 8.00–8.04 (m, 2H, Ar). ^{13}C NMR (δ ppm): 18.7 (CH_3); 110.4 (C_8); 114.7 (C_3); 117.9 (C_6); 118.2 (C_{10}); 125.6 (C_5); 129.7–131.1–132.3 (Ar); 151.8 (C_7); 153.0 (C_4); 154.3 (C_9); 160.4 (C_2); 165.1 (C_{11}). MS: 483.4 ($M + H^+$); 500.3 ($M + NH_4^+$). Anal. calcd. for $C_{28}H_{18}O_8 + H_2O$: C, 67.19; H, 4.03%. Found: C, 67.86; H, 3.72%.

2.4.3. Dicarboxylic acid 7-(4-methylcoumarinyl) diester (4)

Yield: 84%. M.p. 185 °C. FTIR (ν cm^{-1}): 1608 (C═C); 1730, 1763 (C═O). ^{1}H NMR (DMSO, δ ppm): 2.48 (d,

$J = 1.2$ Hz, 6H, $2 \times CH_3$); 6.10 (d, $J = 1.2$ Hz, 2H, $2 \times H_3$); 6.69 (d, $J = 2.4$ Hz, 2H, $2 \times H_8$); 6.79 (dd, $J = 2.4$, 8.7 Hz, 2H, $2 \times H_6$); 7.57 (d, $J = 8.7$ Hz, 2H, $2 \times H_5$). ^{13}C NMR (δ ppm): 18.5 (CH_3); 102.6 (C_8); 110.7 (C_3); 113.3 (C_6); 112.5 (C_{10}); 127.0 (C_5); 160.7 (C_7); 154.0 (C_4); 155.3 (C_9); 161.6 (C_2); 161.6 (C_{11}). MS: 424.3 ($M + NH_4^+$). Anal. calcd. for $C_{22}H_{14}O_8 + 3H_2O$: C, 57.19; H, 4.38%. Found: C, 56.40; H, 4.69%.

2.4.4. Dimethylene dicarboxylic acid 7-(4-methylcoumarinyl) diester (**5**)

Yield: 68%. M.p. 225 °C. FTIR (ν cm^{-1}): 1621 (C═C); 1734 (C═O). ^1H NMR ($CDCl_3$, δ ppm): 2.44 (s, 6H, $2 \times CH_3$); 3.05 (s, 4H, $2 \times CH_2$); 6.28 (s, 2H, $2 \times H_3$); 7.09 (dd, $J = 2.1$, 8.4 Hz, 2H, $2 \times H_6$); 7.14 (d, $J = 2.1$ Hz, 2H, $2 \times H_8$); 7.62 (d, $J = 8.4$ Hz, 2H, $2 \times H_5$). ^{13}C NMR (δ ppm): 19.0 (CH_3); 29.5 (CH_2); 110.7 (C_8); 115.0 (C_3); 118.3 (C_6); 125.8 (C_5). MS: 435.4 ($M + H^+$); 452.3 ($M + NH_4^+$). Anal. calcd. for $C_{24}H_{18}O_8 + (1/2)H_2O$: C, 65.01; H, 4.32%. Found: C, 64.71; H, 4.14%.

2.4.5. Tetramethylenedicarboxylic acid 7-(4-methylcoumarinyl) diester (**6**)

Yield: 82%. M.p. 172 °C. FTIR (ν cm^{-1}): 1618 (C═C); 1730 (C═O). ^1H NMR ($CDCl_3$, δ ppm): 1.93 (m, 4H, $2 \times CH_2$); 2.43 (d, $J = 1.2$ Hz, 6H, $2 \times CH_3$); 2.70 (m, 4H, $2 \times CH_2$); 6.27 (d, $J = 1.2$ Hz, 2H, $2 \times H_3$); 7.08 (dd, $J = 2.1$, 8.4 Hz, 2H, $2 \times H_6$); 7.12 (d, $J = 2.1$ Hz, 2H, $2 \times H_8$); 7.61 (d, $J = 8.4$ Hz, 2H, $2 \times H_5$). ^{13}C NMR (δ ppm): 18.7 (CH_3); 24.1 (CH_2); 33.9 (CH_2); 110.4 (C_8); 114.5 (C_3); 118.0 (C_6); 117.9 (C_{10}); 125.6 (C_5); 151.8 (C_7); 153.0 (C_4); 154.4 (C_9); 160.4 (C_2); 171.0 (C_{11}). MS: 463.4 ($M + H^+$); 480.3 ($M + NH_4^+$). Anal. calcd. for $C_{26}H_{22}O_8 + (1/2)H_2O$: C, 66.23; H, 4.91%. Found: C, 65.77; H, 4.86%.

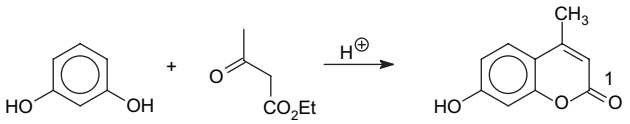
2.4.6. Octamethylenedicarboxylic acid 7-(4-methylcoumarinyl) diester (**7**)

Yield: 92%. M.p. 109 °C. FTIR (ν cm^{-1}): 1621 (C═C); 1698, 1735 (C═O). ^1H NMR (DMSO, δ ppm): 1.47 (m, 4H, $2 \times CH_2$); 1.65 (m, 4H, $2 \times CH_2$); 2.17 (m, 4H, $2 \times CH_2$); 2.43 (d, $J = 1.2$ Hz, 6H, $2 \times CH_3$); 2.60 (t, 4H, $2 \times CH_2$); 6.36 (d, $J = 1.2$ Hz, 2H, $2 \times H_3$); 7.15 (dd, $J = 2.4$, 8.7 Hz, 2H, $2 \times H_6$); 7.23 (d, $J = 2.4$ Hz, 2H, $2 \times H_8$); 7.79 (d, $J = 8.7$ Hz, 2H, $2 \times H_5$). ^{13}C NMR (δ ppm): 18.7 (CH_3); 24.6 (CH_2); 24.9 (CH_2); 28.7 (CH_2); 28.9 (CH_2); 29.0 (CH_2); 33.9 (CH_2); 34.1 (CH_2); 110.5 (C_8); 114.2 (C_3); 118.8 (C_6); 117.9 (C_{10}); 126.8 (C_5); 160.0 (C_7); 153.4 (C_4); 154.0 (C_9); 171.9 (C_2); 174.9 (C_{11}). MS: 519.5 ($M + H^+$); 536.4 ($M + NH_4^+$). Anal. calcd. for $C_{30}H_{30}O_8 + 2H_2O$: C, 64.97; H, 6.18%. Found: C, 65.62; H, 6.85%.

3. Results and discussion

3.1. Synthesis of biscoumarin dyes

7-Hydroxy-4-methylcoumarin (**1**) was first prepared in good yield (Scheme 1) using a Pechman procedure by condensation of ethylacetoacetate with an equimolecular amount of resorcinol in the presence of Amberlyst 15 as the catalyst [33].



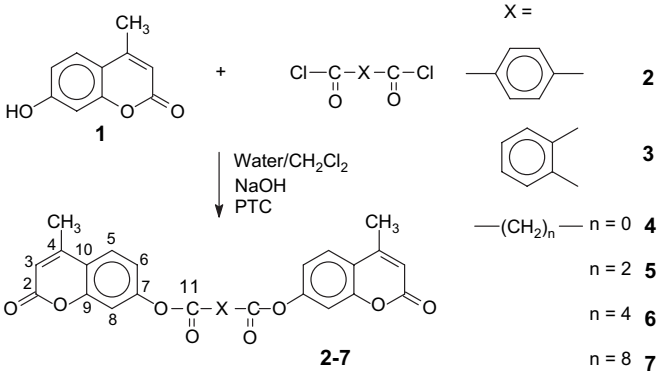
Scheme 1. Synthesis of 7-hydroxy-4-methylcoumarin (**1**).

Then, the biscoumarins were synthesized by condensation of **1** with various diacyl chlorides according to the following global reaction scheme (Scheme 2).

The interfacial technique was chosen for preparing biscoumarins **2**–**7**, because we have shown that carrying out this type of reaction in a biphasic liquid/liquid medium rather than in a homogeneous system presents distinct advantages, such as simplicity of operation and ease of product isolation from the reaction medium [34]. According to this technique, 7-hydroxyl-4-methylcoumarin **1** is first dissolved in the aqueous phase where it reacts with a base such as NaOH to give the corresponding phenolate ion. The latter reacts in water with the catalyst (an ammonium salt) to form an ion pair, which becomes soluble in the organic phase. There, the ion pair reacts with the acid chloride, giving the expected biscoumarin derivative, and regenerating the catalyst that returns to the aqueous phase. The condensation product precipitates in the aqueous phase. It is filtered, dried and obtained as a hydrated form (see Section 2). It must be noted that in the following work, the yields are calculated with respect to the hydrated compounds.

In order to find the optimum esterification conditions applicable to our context, we carried out a series of trials using the reactive combination of 7-hydroxy-4-methylcoumarin and terephthaloyl chloride in biphasic liquid medium as a model system. The production of compound **2** was measured. Four parameters were allowed to vary, namely the nature of the organic medium, the base concentration in the aqueous phase, the volume ratio between organic and aqueous phases, and the nature of the phase-transfer catalyst (PTC).

Experiments were carried out in five different organic solvents. Table 1 shows that there is no direct link between



Scheme 2. General pathway for the synthesis of biscoumarins **2**–**7**.

Table 1
Influence of the nature of the organic solvent upon the yield of **2**

Solvent	CH_2Cl_2	$C_2H_2Cl_4$	THF	$C_6H_5NO_2$	CCl_4
ϵ_r	8.93 [a]	7.06 [b]	7.58 [a]	34.78 [a]	2.23 [a]
Yield (%)	55	53	32	41	45

V (solvent) = V (H_2O) = 25 mL; T = 25 °C; **1**: 10 mmol; terephthaloyl chloride: 5 mmol; NaOH: 10 mmol; TEBAC: 0.12 mmol. ϵ_r = Dielectric constant for the pure liquid.
[a] At 25 °C from Ref [35].
[b] At 35 °C from Ref. [36].

solvent polarity (expressed by the dielectric constant) and the reaction yield, at least for the solvents considered. However, it can be noticed that the synthesis of biscoumarin **2** was particularly successful in chlorinated solvents such as dichloromethane and 1,1,2,2-tetrachloroethane.

The role of the base is to transform 7-hydroxyl-4-methylcoumarin into its associated anion, in order to enhance its reactivity. The influence of the base concentration was investigated by rising the amount of NaOH in the aqueous phase from 0.25 to 40 mmol, while keeping constant the volume of water and organic solvent (25 mL each), as well as the concentration of reactants and catalyst (**1**: 10 mmol; terephthaloyl chloride: 5 mmol; TEBAC: 0.12 mmol). Fig. 1 shows that the best result was obtained for a molar ratio [OH^-]/[**1**] equal to 1. Lower amounts of NaOH gave poor results and higher amounts induced the hydrolysis of the diester.

Since the condensation reactions associated with these heterogeneous systems occur at the interface or within the organic medium, but close to the aqueous phase [37,38], it is essential to take into account the phenomena associated with the diffusion of the reagents [39,40], and more particularly the availability of the reactant that is dissolved in the aqueous phase. The reaction yield will therefore depend on the actual concentration ratio of the reactants at the interface, and consequently on the reactant concentration in each phase. This can be expressed, indirectly, by the volume ratio between the organic and aqueous phases, while keeping constant the total amount of reactants in the system. For the present system, the volume of dichloromethane was fixed at 25 mL, and the volume of water was allowed to vary. The reactants and catalyst concentrations were the same as before, with an amount of

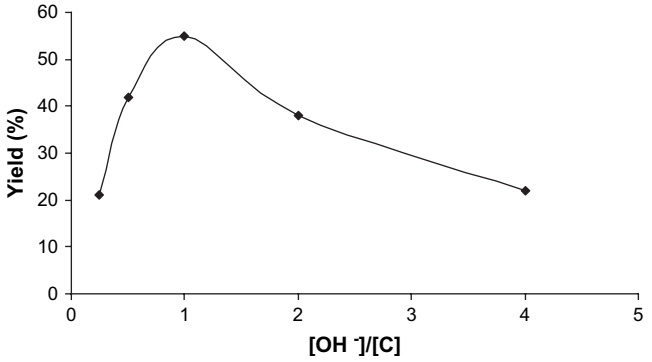


Fig. 1. Influence of the molar ratio [OH^-]/[**1**] on the reaction yield of **2**. V (CH_2Cl_2) = V (H_2O) = 25 mL; **1**: 10 mmol; terephthaloyl chloride: 5 mmol; NaOH: from 0.25 to 40 mmol; TEBAC: 0.12 mmol; T = 25 °C.

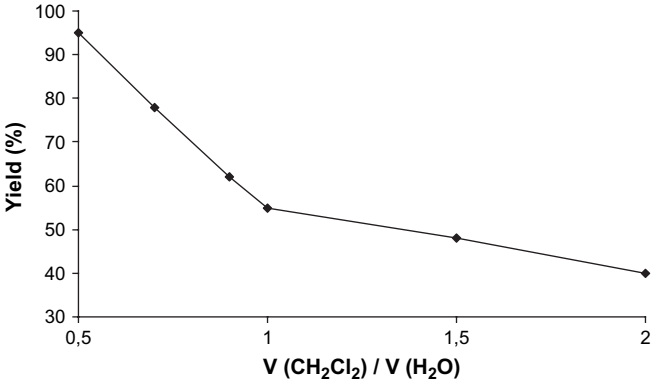


Fig. 2. Influence of the ratio V (CH_2Cl_2)/V (H_2O) upon the yield of **2**. V (H_2O): from 12.5 to 50 mL; V (CH_2Cl_2) = 25 mL; T = 25 °C; **1**: 10 mmol; terephthaloyl chloride: 5 mmol; NaOH: 10 mmol; TEBAC: 0.12 mmol.

NaOH equivalent to that of **1**. In these conditions, we found that the best result was obtained with V (CH_2Cl_2)/V (H_2O) = 0.5 as shown in Fig. 2.

The last variable examined in this investigation was the presence or absence of a phase-transfer catalyst, and the nature of the latter. Table 2 shows the paramount role of such an agent, since reactions conducted without it gave very poor results. As for the chemical structure of the agent, four ammonium salts (triethylbenzylammonium chloride (TEBAC), tetrabutylammonium bromide (TBAB), tetrabutylammonium bisulphate (TBAS), and hexadecyltrimethyl ammonium bromide (HTAB)) were tested at the same concentration. The best results were obtained with TEBAC. When comparing the results obtained with TBAB and TBAS, it appears that bromide should be preferred to bisulphate as a counterion. The long alkyl chains of HTAB decrease the efficiency of the catalyst.

The optimized conditions established above for the combination **1** + terephthaloyl chloride allowed pure compound **2** to be obtained with a yield of 95%. These conditions were used to prepare the other five biscoumarins **3**–**7** by the interfacial condensation of 7-hydroxy-4-methylcoumarin (**1**) with the corresponding diacid chloride. All compounds were obtained in good yields (from 62 to 92%) and with high selectivity, which confirms the interest of the interfacial condensation approach. It can be noted that the characteristics of **6** are in agreement with those given in bibliography [22,41].

3.2. Spectroscopic study of biscoumarins

The optical properties of the six compounds were first studied by UV/vis absorption spectroscopy, then by

Table 2
Influence of the presence and structure of the phase-transfer catalyst (PTC) on the interfacial synthesis of **2**

PTC	TEBAC	TBAB	TBAS	HTAB	None
Yield [%]	95	94	82	63	41

V (CH_2Cl_2) = 25 mL; V (H_2O) = 50 mL; **1**: 10 mmol; terephthaloyl chloride: 5 mmol; NaOH: 10 mmol; PTA: 0.12 mmol. T = 25 °C.

fluorescence spectroscopy. These studies were performed in chloroform with dye concentrations ranging from 2.8×10^{-5} to 7×10^{-5} M for absorption, and from 2.2×10^{-6} to 3.7×10^{-6} M for fluorescence. For the sake of comparison, 7-hydroxy-4-methylcoumarin (**1**) was also studied. Since chloroform is an aprotic solvent, we thought that specific H-bonding interaction with the hydroxyl group of **1** would be limited, and that this compound could be compared to the biscoumarin derivatives. The results are gathered in Table 3.

All the compounds considered absorb in the same spectral region, but the shape of their absorption spectra differs with the nature of the tether. The absorption spectrum of biscoumarin derivative **2** is the only one to exhibit three bands of rather similar intensity, as shown in Fig. 3. The spectra of **3** and **5**–**7** display two bands centred at around 314 and 280 nm, which is in line with the data reported in the bibliography for compound **6** [41]. The spectrum of **4** shows only one band at 320 nm, with a shoulder at short wavelengths (Fig. 4). This compound, in which both chromophores are directly condensed without any spacer between them, has therefore an absorption spectrum similar to that of coumarin **1**. For all dyes, the magnitude of the molar absorption coefficients ϵ is typical of $\pi-\pi^*$ transitions. The ϵ value varies weakly between biscoumarins and coumarin **1**, which confirms that there is no noticeable extension of the electron conjugated system in the dimers.

Curiously, the excitation spectrum of compounds **2**–**3** and **5**–**7** was red-shifted with respect to the absorption spectrum (Fig. 3). Above all, it displayed only one band situated around 323 nm, and so it was markedly different from the absorption spectrum. Since the compounds were checked to be pure, this unusual behaviour could be explained by the fact that part of the absorption spectrum essentially situated at short wavelengths arises from the diester bridge, and that the latter does not contribute to fluorescence. Consequently, the excitation spectrum would only show the long-wavelengths band that arises from transitions centred on the chromophores, which are responsible for fluorescence. Regarding compound **4** that does not bear a tether, the excitation spectrum is identical to the absorption spectrum (Fig. 4), as it is also the case for coumarin **1**.

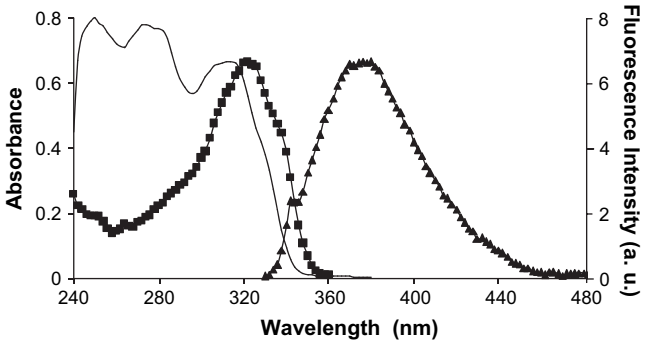


Fig. 3. Absorption spectrum (plain line) for compound **2** (2.8×10^{-5} M) in chloroform. Excitation (squares, $\lambda_{em} = 379$ nm) and emission (triangles, $\lambda_{ex} = 312$ nm) spectra normalized with respect to the absorption spectrum. Dye concentration for fluorescence measurements: 2.2×10^{-6} M.

For all compounds, the shape and position of the excitation spectrum were independent of the emission wavelength. Conversely, the emission spectrum did not vary with excitation wavelength. This indicates the presence of only one type of fluorophore in the medium.

The emission spectrum of the dyes showed only one band with no vibronic structure. The maximum was situated between 370 and 379 nm (Figs. 3 and 4), molecules **2** and **3** emitting at slightly longer wavelengths than the others. Thus, the central link only has a small influence upon the position of the emission spectrum. In contrast, spectacular differences were found for the fluorescence quantum yield that varies by two orders of magnitude according to the compound considered. Compounds **2** and **3**, which bear an aromatic tether, are very poorly fluorescent. Amongst the biscoumarin derivatives that bear an aliphatic tether, the compound that bears the shorter chain is less fluorescent than the others. Biscoumarin **4**

Table 3
Spectroscopic characteristics of biscoumarin derivatives **2**–**7** and 7-hydroxy-4-methylcoumarin (**1**) in chloroform

Dye	λ_{abs} (nm)	ϵ ($M^{-1}\,cm^{-1}$)	λ_{ex} (nm)	λ_{em} (nm)	Φ	τ (ns)	k_r ($10^8\,s^{-1}$)	k_{nr} ($10^8\,s^{-1}$)
2	314	22 600	323	379	5.2×10^{-3}	0.37	0.14 ± 0.05	26.9 ± 6.7
	274	27 600						
	250	27 700						
3	314	20 400	320	379	3.0×10^{-3}	0.50	0.06 ± 0.02	19.9 ± 4.9
	274	24 100						
4	320	21 700	323	377	1.1×10^{-1}	0.75	1.46 ± 0.55	11.9 ± 3.1
	294 (sh)	12 000						
5	312	18 200	323	370	3.4×10^{-3}	0.80	0.042 ± 0.016	12.5 ± 3.1
	274	20 500						
6	314	18 900	323	376	1.9×10^{-2}	0.54	0.35 ± 0.13	18.2 ± 4.5
	274	20 500						
7	314	13 500	323	376	1.6×10^{-2}	0.49	0.32 ± 0.13	20.1 ± 5.0
	274	14 500						
1	322	17 400	325	377	1.1×10^{-1}	0.71	0.15 ± 0.05	12.5 ± 3.3
	294 (sh)	9200						

λ_{abs}: maximum absorption wavelength; ϵ: molar absorption coefficient; λ_{ex}: maximum excitation wavelength; λ_{em}: maximum emission wavelength; Φ_f: fluorescence quantum yield with excitation at the maximum absorption wavelength; τ: fluorescence lifetime; k_r: radiative deactivation constant; k_{nr}: non-radiative deactivation constant; sh: shoulder. The error is estimated to be 10% on the quantum yields and 20% on the lifetimes.

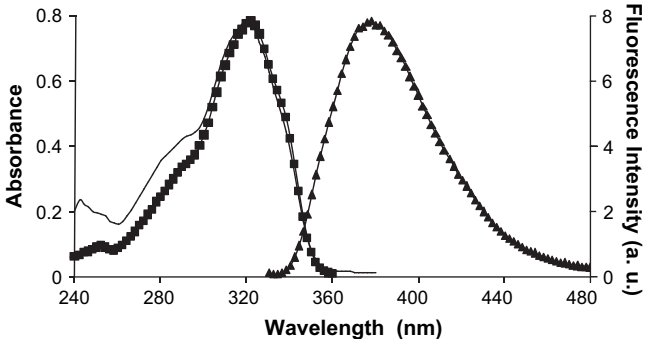


Fig. 4. Absorption spectrum (plain line) for compound **4** (3.6×10^{-5} M) in chloroform. Excitation (squares, $\lambda_{em} = 377$ nm) and emission (triangles, $\lambda_{ex} = 320$ nm) spectra normalized with respect to the absorption spectrum. Dye concentration for fluorescence measurements: 2.3×10^{-6} M.

that bears no tether exhibits the best fluorescence efficiency, together with reference coumarin **1**.

Finally, the lifetimes were found to be monoexponential and very short (below 1 ns) for all dyes, as it is generally the case for coumarin derivatives that do not bear a good electron donor group in the 7-position. Our spectrofluorometer does not allow precise measurements on lifetimes below 0.7 ns, which must be considered with circumspection. However, the lifetime values were used for the calculation of the photophysical constants, to get a general trend of the behaviour of the compounds investigated. Let us recall briefly that the radiative deactivation constant, classically defined as $k_r = \Phi/\tau$, is related to the intrinsic efficiency of the molecule to emit fluorescence, according to the distribution of its energy levels. The non-radiative deactivation constant, defined as $k_{nr} = (1 - \Phi)/\tau$, measures the ability of the molecule to waste its excitation energy in processes other than fluorescence, for example torsions. In the present case, it is interesting to see that the radiative deactivation constant varies by two orders of magnitude, while the non-radiative deactivation constant undergoes little variations.

In view of these results, two hypotheses at least can be made to explain the differences of fluorescence behaviour observed between the compounds. The first one is that the presence of the tether perturbs the energy levels of the chromophores. The gap between excited triplet and singlet states could be reduced, thus opening a non-radiative deactivation pathway. Such a mechanism could very well take place for dyes **2** and **3** that bear an aromatic bridge, and it would explain their very weak fluorescence efficiency with respect to the other compounds. However, this mechanism seems to be much less relevant for the dyes that bear an aliphatic tether, and in particular it cannot account for the differences observed between **5** and its two analogues **6** and **7**. Actually, the influence of the tether on the electron conjugated system is likely to be weak and almost identical in the three cases. This observation could be explained by the second hypothesis, considering that the conformation of some biscoumarin dyes allows the chromophores to overlap. This phenomenon has been shown to take place for dye **6**, since it induces photochemical intramolecular addition between both chromophores [41–44]. When such an overlapping occurs, the excitation energy can be lost after formation of a non-fluorescent excimer. It is possible that for some of our dyes, both hypotheses have to be considered simultaneously.

4. Conclusions

This work showed the usefulness of the interfacial technique to prepare biscoumarin derivatives. The products form in high amounts. Since they are poorly soluble in the reaction medium, they can easily be isolated and so the yield is very satisfying. The compounds investigated are not really interesting as fluorescent materials. It is well known that in the coumarin series substitution by an electron-donating group in the 7-position is favourable to fluorescence efficiency [45]. Our compounds are not built according to this substitution pattern, and coumarin **1** itself is poorly fluorescent. The presence of a tether induces additional deactivation pathways, and from a general viewpoint these biscoumarin derivatives are not an improvement with respect to the corresponding monomeric coumarin dye. However, this work allowed the tether influence to be clarified, and the spectroscopic properties of biscoumarins to be better known. In this respect, it provides some bases for the design of new structures. Besides, our biscoumarin derivatives can be useful for other applications. Their biological activity is presently under study.

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