Synthesis and Spectral Properties of 7-(*N*-Arylsulfonyl)aminocoumarins, A New Class of Fluorescent *p*H Indicators

Otto S. Wolfbeis* [a] and John H. Baustert [a,b]

[a] Institut für Organische Chemie, Karl-Franzens-University, A-8010 Graz, Austria

[b] Centre Universitaire de Luxembourg, Department des Sciences, Place Auguste Laurent, Luxembourg

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7-Arylsulfonylamino-coumarins **3** and **5**, with electron-delocalizing substituents in position 3 were obtained by condensation of 4-arylsulfonylamino-2-hydroxybenzaldehyde with various (hetero)arylacetic esters and by other methods. Oxidative cyanation using potassium cyanide and elemental bromine gave the corresponding 4-cyano derivatives **6a,b**.

The absorption and emission maxima of coumarins **3** and **5** in organic solvents and in water of different *p*H were determined. All showed bright blue fluorescence in organic or acidic aqueous solution, which is shifted to the green in alkaline solution. The 4-cyano derivatives **6a,b** exhibit green fluorescences, which change to yellow in alkaline solution. Simultaneously the color changes from yellow to orange.

The p*K*a values of compounds **3** and **5** were found to lie between 6.33 and 7.06, whereas for the 4-cyano derivatives they were distinctly lower (5.66-5.95). The spectral changes observed in the near neutral *p*H range may render the new compounds useful indicators for the measurement of physiological *p*H's.

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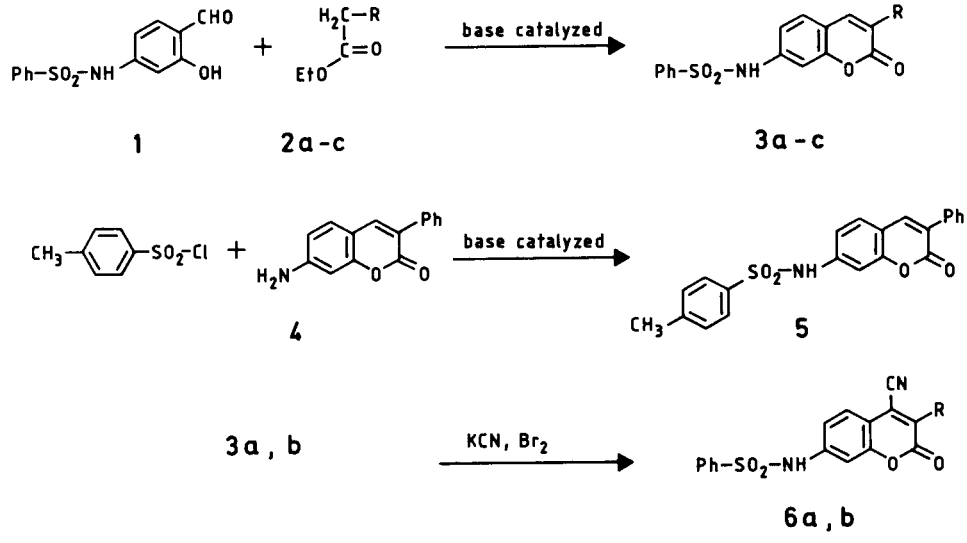
Coumarins with electron-donating substituents in position 7 are well known for their strong fluorescences. 7-Aminocoumarin derivatives have been used as optical brighteners [1], laser dyes [2,3], signal colors [4], and light collectors [5]. 7-Hydroxycoumarins, in contrast, have not been used as brighteners because of the *p*H dependence of their spectra which, on the other hand, can be utilized to measure physiological *p*H values by fluorimetry [6-8].

We have exploited several synthetic approaches for the synthesis of new *N*-substituted 7-aminocoumarins which we found to be potentially useful *p*H indicators. Their synthesis and properties will be described here.

Syntheses.

4-(*N*-Phenylsulfonyl)amino-2-hydroxybenzaldehyde (**1**) was reacted with benzazolyl acetic acid esters **2a,b** in ethanol/piperidine to give coumarins **3a,b** in good yield. **3c** was obtained by reaction of **1** with ethyl 2-pyridylacetate in the presence of pyridine, which turned out to be a much worse catalyst in giving a 22% yield only. It was also attempted to prepare 7-(*N*-arylsulfonyl)aminocoumarins by *N*-sulfonation of various 7-aminocoumarins with arylsulfochlorides. However, it was found that 3-benzazolyl-7-aminocoumarins do not easily react with acid chlorides including carboxylic acid chlorides. Under more drastic con-



1 2a-c 3a-c

4 5

3a, b 6a, b

2a, 3a and 6a: R = benzoxazolyl, 2b, 3b and 6b: R = benzthiazolyl, 2c and 3c: R = 2-pyridyl

ditions several reaction products were obtained along with the hydrochlorides of starting materials. Compound **5** was the only product that was obtained by this route in sufficient yield.

It has been noticed [5,9] that introducing a cyano group into coumarins causes a considerable bathochromic shift in absorption and emission, an effect that is very desirable in order to obtain strongly colored dyes and indicators. Oxidative cyanation in position 4 of coumarins **3a,b** was accomplished by treatment with potassium cyanide in dimethylformamide, followed by oxidation with elemental bromine. Compounds **6a,b** precipitate from the solution as orange-colored crystals.

Their structures were confirmed by elemental analyses, ir and ^{1}H nmr spectra. Interestingly, the CN absorption band in the ir spectra of the cyanocoumarins is very weak. In the ^{1}H nmr spectra the proton in position 4 is a most characteristic signal consisting of a sharp singlet which considerably shifts to lower field with increasing electronegativity of the substituent in position 3.

Electronic Spectra.

The absorption spectra of coumarins **3a-c** and **5** in organic solvents are characterized by a fairly strong transition between 341 and 389 nm (log ϵ 4.34-4.62). Introducing a 4-cyano group causes a longwave shift in absorption spectra, with typical maxima ranging from 406 to 429 nm. Simultaneously, log ϵ values become somewhat smaller (*ca.* 4.4).

The fluorescence emission intensities of 7-sulfonamidocoumarins are rather intense. Again the spectra of coumarins **3a,b** become dramatically longwave-shifted by cyanation, with emission bands maximizing at around 525 nm. The spectral data are compiled in Table 1.

*p*H Effects.

During the synthetic work it was observed that the visible spectra of the compounds were highly dependent on the acidity of the solution. A more detailed investigation of *p*H effects on the spectra revealed that 7-(*N*-arylsulfonyl)aminocoumarins have unusual low p*K*a values ranging from 6.3 to 7.1, which is even lower than the p*K*a values of the corresponding phenols (7-hydroxycoumarins) [9]. Although sulfonamides are known to exhibit weak acidic properties and to be soluble in alkali hydroxide solution (which, for instance, is utilized for the Hinsberg separation), sulfonamides with p*K* values as low as those reported here have not come to our attention yet. The values are

Table 1

Longestwave Absorptions, Fluorescence Maxima and p*K*a Values of 7-Sulfonamidocoumarins

Compound	Solvent	abs max (in nm)	log ϵ	flu max (in nm)	p*K*a Value (23 C)
3a	A	-	-	471	6.33 +/- 0.02
	B	375	4.61	457	
	C	373	4.55	485, 450 [d]	
	D	417	4.67	493	
3b	A	389	-	471	6.47 +/- 0.02
	B	383	4.62	471	
	C	385	4.59	497	
	D	428	4.63	508	
3c	B	349	4.34	4.30	6.96 +/- 0.03
	E	342	4.27	482, 430 [d]	3.72 +/- 0.03
	F	372	4.37	460	
	D	382	4.40	485	
5	A	341	-	424	7.06 +/- 0.05
	B	341	4.43	432	
	C	343	4.42	472, 435 [d]	
	D	373	4.50	483	
6a	A	406	-	520	5.95 +/- 0.03
	B	407	4.44	522	
	C	408	4.43	606, 520 [d]	
	D	477	4.51	606	
6b	A	429	-	525	5.66 +/- 0.07
	B	422	4.43	525	
	C	423	4.40	619	
	C	490	.46	617	

[a] A: dioxane, B: methanol, C: glycine buffer of *p*H 3, D: glycine buffer of *p*H 9, E: citrate buffer of *p*H 5, F: sulfuric acid, 1 *N*. [b] Containing 50% methanol. [c] Very broad signal. [d] Shoulder.

further lowered by around 0.4-0.6 units in cyanocoumarins **6** (Table 1).

Changes in the acid-base equilibra are accompanied by spectral changes. Thus, a yellow solution of compound **6b** in acetone, which shows green fluorescence, turns to deep red with orange-yellow fluorescence after addition of base. Unfortunately, the cyanocoumarins are not stable in strongly alkaline solution, a fact that renders them useless for *p*H's outside the physiological range.

Coumarins **3a,b**, in contrast, are stable over weeks and have p*K*a values which are most suitable for fluorimetric measurement of physiological *p*H values at longwave excitation and emission wavelengths. In contrast to the frequently used 4-methylumbelliferone [8] their fluorescences are distinctly outside the intrinsic fluorescence of biological matter.

The emission of coumarins **3**, **5** and **6** in aqueous solution is from the anion form in the *p*H 3-10 range, despite of exciting the undissociated molecule at *p*H's below the p*K*a. The phenomenon is attributed to an excited state dissociation at the sulfonamide group. While photodissociations have been shown to occur with phenols, thiols and aromatic amines [10], such a decrease in p*K*a as a result of photoexcitation has not been described for sulfonamides yet. Sulfonic acids, on the other hand, are known to become weaker acids in their first excited singlet state [10]. Thus, the excited state acid-base equilibria of these sulfonamides are governed by the aniline part rather than by the sulfonic acid part.

EXPERIMENTAL

Melting points are uncorrected. The ir spectra were obtained using a Perkin Elmer 421, and the ^{1}H nmr spectra on a Varian A 60 A instrument. Chemical shifts are given in delta units. Absorption spectra were run on a Perkin Elmer Lambda 5 spectrophotometer, fluorescence spectra at room temperature on an Aminco SPF 500 fluorimeter.

3-(2-Benzoxazolyl)-7-(*N*-phenylsulfonyl)aminocoumarin (**3a**).

4-Phenylsulfonylamino-2-hydroxybenzaldehyde (1.39 g, 5 mmoles) and ethyl 2-benzoxazolylacetate (1.03 g, 5 mmoles) were dissolved in 5 ml of boiling ethanol. After addition of 0.1 ml of piperidine, the mixture was kept at 50-60° for 24 hours in a closed flask. The resulting precipitate was collected by suction and recrystallized from acetic acid to give a 63% yield of yellow crystals, mp 268°; ir (potassium bromide): 3100 br, 1740, 1620 and 1575 cm^{-1}; ^{1}H nmr (DMSO-d$_6$): 6.9-8.1 (m, 12H), 8.83 (s, 1H).

Anal. Calcd. for $C_{22}H_{14}N_2O_5S$ (418.42): C, 63.16; H, 3.35; N, 6.70. Found: C, 62.96; H, 3.26; N, 6.45.

3-(2-Benzothiazolyl)-7-(*N*-phenylsulfonyl)aminocoumarin (**3b**).

To a solution of 4-phenylsulfonylamino-2-hydroxybenzaldehyde (1.39 g, 5 mmoles) and ethyl 2-benzthiazolylacetate (1.10 g, 5 mmoles) in absolute ethanol, 0.1 ml of piperidine was added. The mixture was kept in a closed reaction flask at room temperature for 24 hours. A precipitate was formed which was collected and recrystallized from acetic acid, yield 69% (1.5 g) of yellow crystals, mp 292°; ir (potassium bromide): 3240, 1710, 1625 and 1565 cm^{-1}; ^{1}H nmr (DMSO-d$_6$): 6.9-8.1 (m, 12H), 8.95 (s, 1H).

Anal. Calcd. for $C_{22}H_{14}N_2O_4S_2$ (434.48): C, 60.82; H, 3.23; N, 6.45. Found: C, 60.77; H, 3.22; N, 6.35.

7-(*N*-Phenylsulfonyl)amino-3-(2-pyridyl)coumarin (**3c**).

Ethyl 2-pyridylacetate (2.4 g, 14.6 mmoles) and 5.5 g, (20 mmoles) 4-phenylsulfonyl-2-hydroxybenzaldehyde were heated for 48 hours in a solution of 3 ml of pyridine and 50 ml of 2-propanol. After removing volatile parts under vacuum, the remaining oil was extracted with ether, dissolved in hot 2-propanol and filtered. After standing at room temperatur, an amorphous precipitate was formed which was removed. After several days **3c** crystallized in off-white needles, yield 22% (1.7 g), mp 223° (from chlorobenzene); ir (potassium bromide): 3230, 1725, 1615 and 1580 cm^{-1}; ^{1}H nmr (DMSO-d$_6$): 7.15 (t, 3H), 7.38 (t, 1H), 7.6-8.0 (m, 7H), 8.23 (d, 1H), 8.66 (d, 1H), 8.74 (s, 1H), 11.17 (s, 1H); ms: (70 eV) m/e = 378 (M$^+$, 63%), 227 (100%).

Anal. Calcd. for $C_{20}H_{14}N_2O_4S$ (378.40): C, 63.48; H, 3.73; N, 7.40; S, 8.74. Found: C, 63.66; H, 3.76; N, 7.28; S, 8.67.

3-Phenyl-7-(*N*-tolylsulfonyl)aminocoumarin (**5**).

A mixture of *p*-toluenesulfonic acid chloride (0.19 g, 1 mmole) and 3-phenyl-7-aminocoumarin (**4a**, 0.237 g, 1 mmole) in 7 ml of pyridine was heated to reflux for 30 minutes. Compound **5** was precipitated by addition of 10 ml of water. The precipitate was collected by suction and recrystallized from ethanol, yield 74% (0.29 g) of white needles, mp 241°; ir (potassium bromide): 3220, 1710 and 1625 cm^{-1}; ^{1}H nmr (DMSO-d$_6$): 2.3 (s, 3H), 6.9-7.8 (m, 12H), 8.1 (s, 1H).

Anal. Calcd. for $C_{22}H_{17}NO_4S$ (391.44): C, 67.52; H, 4.35; N, 3.58. Found: C, 67.80; H, 4.38; N, 3.54.

General Procedure for the Preparation of 4-Cyano-7-sulfonamidocoumarins **6a,6b**.

Potassium cyanide (0.26 g, 4 mmoles) dissolved in 0.5 ml water was added to a solution of the respective sulfonamidocoumarin (**3a,3b**) (2 mmoles) in 6 ml of *N,N*-dimethylformamide. The mixture was stirred for 1 hour at 40°. After filtration, 0.1 ml (2 mmoles) of bromine was slowly added at 0°. The mixture was then stirred for 1 hour at this temperature to give a precipitate that was collected, washed with water and purified by double recrystallization from acetic acid.

3-(2-Benzoxazolyl)-4-cyano-7-(*N*-phenylsulfonyl)aminocoumarin (**6a**).

This compound was obtained in 53% yield (0.47 g) as orange-red crystals, mp 270° dec; ir (potassium bromide): 3240, 2240 (very weak), 1760, 1620 and 1550 cm^{-1}; ^{1}H nmr (DMSO-d$_6$): 7.1-8.1 (m, 12H).

Anal. Calcd. for $C_{23}H_{13}N_3O_5S$ (443.43): C, 62.30; H, 2.93; N, 9.48. Found: C, 62.12; H, 2.93; N, 9.48.

3-(2-Benzthiazolyl)-4-cyano-7-(*N*-phenylsulfonyl)aminocoumarin (**6b**).

This compound was obtained in 48% yield (0.45 g) as orange-red crystals, mp 294° dec; ir (potassium bromide): 3260, 2230 (very weak), 1725, 1620 and 1540 cm^{-1}; ^{1}H nmr (DMSO-d$_6$): 6.9-8.2 (m, 12H).

Anal. Calcd. for $C_{23}H_{13}N_3O_4S_2$ (459.50): C, 60.13; H, 2.83; N, 9.15. Found: C, 59.92; H, 2.69; N, 8.91.

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