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Syntheses, Absorption and Fluorescence Spectra of 7-Hydroxy-3-pyridylcoumarins, their Esters, Ethers, and Quaternized Derivatives[1)]

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7-Hydroxycoumarins **4** with a 2-, 3- or 4-pyridyl substituent in position 3, their ethers (**3**), esters (**5, 6**), and quaternized derivatives (**7**–**9**) have been synthesized by a generally applicable method starting from 2-hydroxy-4-methoxybenzaldehyde (**1**). The absorption and fluorescence spectra in methanol and water of different pH values are reported, and the effects of substituents on spectra and pK_a values are discussed.

Synthesen, Absorptions- und Fluoreszenzspektren von 7-Hydroxy-3-pyridylcumarinen, deren Ethern, Estern und quartärisierten Derivaten[1)]

Es wurden 7-Hydroxycumarine **4** mit einem 2-, 3- oder 4-Pyridylsubstituenten in 3-Stellung sowie deren Ether (**3**), Ester (**5, 6**) und quartärisierten Derivate (**7**–**9**) mit Hilfe einer generell anwendbaren Methode hergestellt, welche von 2-Hydroxy-4-methoxybenzaldehyd (**1**) ausgeht. Die Absorptions- und Fluoreszenzspektren in Methanol sowie Wasser von verschiedenem pH-Wert werden angegeben und die Effekte der Substituenten auf Spektren und pK_a-Werte diskutiert.

Coumarins with an electron-donating substituent in position 7 along with an electron-withdrawing or electron-delocalizing substituent in position 3 are known to exhibit particularly strong fluorescence. Compounds of this type (with amino groups in position 7) have therefore found widespread applications as optical brighteners[2,3)]. In contrast, 7-hydroxycoumarins are not suitable for this purpose, since their emission maxima are to far red-shifted and strongly affected by alkalinity.

However, due to their pK_a values between 7 and 8, use has been made of 7-hydroxycoumarins as fluorescent indicators for measuring pH values in cases, where electrodes can be employed only with difficulty[4,5)].

There are only scarce reports available on the synthesis of the isomeric 7-hydroxy-3-pyridylcoumarins and their ethers. Thus, 7-hydroxy-3-(2-pyridyl)coumarin (**4a**) has been prepared via a Perkin reaction using sodium 2-pyridylacetate and 2,4-dihydroxybenzaldehyde, followed by acidic hydrolysis[6)]. The 3-pyridyl isomers were similarly prepared[7,8)].

Various 3- and 4-pyridylcoumarins have been obtained by modifications of the *von Pechmann, Knoevenagel,* and *Perkin* reactions, and their effects on the central nervous system of mice was studied[9)]. Some exhibit weak antifungal activity in vitro. The electronic spectra have not been reported so far.

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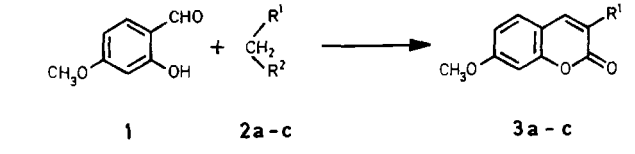
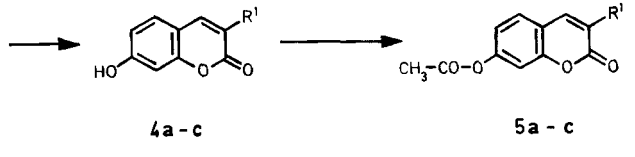
Syntheses

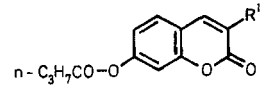
Coumarins **3a–c** were prepared by condensing 2-hydroxy-4-methoxybenzaldehyde (**1**) under Knoevenagel condensation conditions with an appropriate ethyl pyridylacetate (**2a, c**) or 3-pyridylacetonitrile (**2b**). The resulting 7-methoxycoumarins **3a–c** were transformed into the respective 7-hydroxy derivatives by ether cleavage using pyridine hydrochloride which we found to be much more suitable than hydrobromic or hydroiodic acid. This two-step synthesis gave much better yields than any other method starting with 2,4-dihydroxybenzaldehyde. In addition, it gives acceptable yields in case of all three isomers.



1 **2a-c** **3a-c**



4a-c **5a-c**



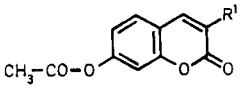
6a-c

2,3,4,5,6	R^1	R^2
a	2-pyridyl	$COOC_2H_5$
b	3-pyridyl	CN
c	4-pyridyl	$COOC_2H_5$

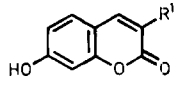
The hydroxy groups of coumarins **4a–c** were protected by acetylation with acetic anhydride to give the *O*-acetates **5a–c** in almost quantitative yields. After it had been recognized that butyrate **6a** is a more useful enzyme substrate than acetate **5a**, we also have prepared butyrates **6b, c** from **4b, c**.

Acetates **5a, c** were quaternized with methyl iodide to give the methoiodides **7a–c**, which were easily hydrolyzed with dilute hydroiodic acid, thus affording the desired quaternized umbelliferones **8a–c**. The respective methyl ethers **9a–c** were obtained by quaternization of compounds **3a–c**. **9a** was obtained independently by condensing the methoiodide of ethyl 2-pyridylacetate with 2-hydroxy-4-methoxybenzaldehyde (**1**).

Attempts to synthesize **7c** or **8c** according to a method involving condensation of 4-hydroxy- or 4-methoxysalicylaldehyde with 4-pyridylacetic acid thiomorpholide in the presence of a quaternizing agent such as dimethyl sulfate failed, despite of numerous variations of the described procedure[10]. However, the method gave acceptable results in the preparation of the corresponding 7-(dimethylamino)coumarins.

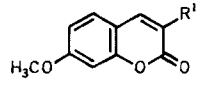


7a - c



8a - c

7,8,9	R¹
a	1-methyl-2-pyridinio
b	1-methyl-3-pyridinio
c	1-methyl-4-pyridinio



9a - c

Spectra

The absorption maxima of the methoxy- and hydroxycoumarins consist of a longest-wavelength transition between 340 and 355 nm and an intense fluorescence maximum between 424 and 429 nm (Table 1). Most notably, the spectra are practically not influenced by the nature of the pyridyl substituent: All three isomers exhibit very similar spectra, thus indicating the lack of strong conjugation between the coumarin and pyridine chromophores. The 7-acetoxy-3-pyridyl coumarins **5** have absorption maxima (but not fluorescence maxima) with considerably shorter wavelengths than the respective hydroxy- or methoxy compounds.

Quaternization of the pyridine nitrogen atom has a pronounced bathochromic effect upon the spectra of the 4-pyridyl isomers (**8c, 9c**), but much less so upon the other isomers. In case of the 2-isomers (**8a, 9a**) steric constraints may prevent the formation of a planar π-electron system, whilst for the 3-isomers (**8b, 9b**) no mesomeric resonance can be expected.

pH Effects

Changing to water as a solvent has several remarkable effects upon the spectra. The absorption maxima are shifted to shorter wavelenghts and the molar absorbances become slightly smaller (Table 1). The emission maxima suffer similar changes, albeit less expressed.

The 7-hydroxycoumarins are an exception in that their fluorescence suffers a large red shift when changing to water as a solvent. In fact, the fluorescence maxima in pH 5 solution are almost identical to those in alkaline solution. According to *Förster*'s model[11] this can be interpreted in terms of excited state dissociation due to a decrease in the pK_a value following photoexcitation.

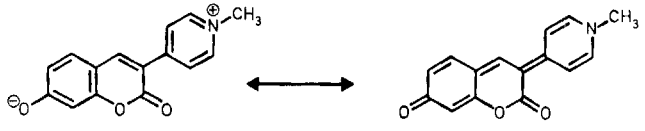
7-Hydroxycoumarins **4a**–**c** and **8a**–**c** as well as other coumarins with a non-quaternized pyridine substituent exhibit pH-dependent absorption and fluorescence spectra. Dissociation of the hydroxy group as well as protonation of the pyridine nucleus result in bathochromic spectral shifts. In general, fluorescence efficiencies are highest for the anion species, medium for the uncharged species, and low or zero for the protonated forms.

Table 1. Absorption and fluorescence maxima as well as pK_a values of coumarins **3**–**9**

Compound	Solvent[a]	Absorption Maximum (nm)	ε	Fluorescence Maximum (nm)	pK_a (22°C)
3a	A	348	27300	424	3.96
	B	347	24300	425	
	C	379	29200	446	
3b	A	341	21100	424	4.33
	B	340	20900	426	
	C	351	22300	442	
3c	A	347	27200	425	4.87
	B	345	25300	426	
	C	376	28200	461	
4a	A	353	22300	427	4.18; 7.58
	B	347	19800	468[b]	
	C	379	29900	455	
	D	394	29000	469	
4b	A	345	22200	427	4.24; 7.88
	B	341	21400	471[b]	
	C	351	23100	445	
	D	388	26800	470	
4c	A	351	21800	429	4.74; 7.64
	B	346	21100	470[b]	
	C	374	27500	466	
	D	398	31700	471	
5a	A	333	21100	410	
	B	326	19000	411	
5b	A	326	18800	426	
	B	323	18100	410	
5c	A	329	17500	420	
	B	326	17200	414	
7a	A	334	–	431	
	B	332	–	424	
7b	A	334	–	424	
	B	329	–	418	
7c	A	355	–	450	
	B	347	–	440	
8a	A	365	27700	455	6.62
	B	355	25200	480[b]	
	D	414	32700	477	
8b	A	365	24600	427	7.08
	B	353	23100	465[b]	
	D	409	29400	472	
8c	A	390	32000	477	6.68
	B	378	29100	523[b]	
	D	449	38000	521	
9a	A	356	25100	446	
	B	354	24800	456	
9b	A	351	23500	444	
	B	349	22900	430	
9c	A	385	31600	469	
	B	383	27100	463	

[a] A: methanol; B: water of pH 6; C: 1 N H_2SO_4; D: aqueous sodium hydrogen carbonate. – [b] Anion fluorescence due to excited state photodissociation.

The low fluorescence efficiency of the quaternized 7-hydroxycoumarins **8a–c** in water solution is rather unexpected and is in contrast to the much stronger fluorescence of **8a–c** in methanol, and of the methyl ethers **9a–c** in both water and methanol. We assume that the charge transfer excited states of **8a–c** are better described by a quinoid structure. In comparison to their parent aromatics, quinones are known to show intense phosphorescence at the expense of fluorescence[12]. The quinoid structure of the **8c** anion is shown in the formula, and a similar structure can be given for **8a**.



zwitterion quinone type

We have determined the pK_a values of several compounds by the spectrophotometric method in 6.67 mM phosphate buffer of ionic strength below 0.05. The results (Table 1) show that the pK_a values of 7-hydroxycoumarins are not strongly affected by a pyridyl substituent: Compound **4b** has practically the same pK_a as 7-hydroxycoumarin[13] and its 3-phenyl derivative[1]. The electron-withdrawing effect of 2- and 4-pyridyl substituents is more expressed, lowering the pK_a value of the phenol groups to 7.58 and 7.64, respectively.

As expected, quaternization has a strong effect in lowering the pK_a values by 0.8 to 1.0 units. Again, the quaternized 3-pyridyl substituent is least effective.

The pK_a values of the pyridine substituents were found to increase in the series 2-pyridyl, 3-pyridyl, and 4-pyridyl (**3a–c, 4a–c**). In compounds **4a–c** the two pK_a values are approximately 3–3.5 units apart, which makes these compounds useful pH indicators covering the pH range 3–9.

Acetates **5a–c** and butyrates **6a–c** suffer slow hydrolysis in neutral aqueous solution to form the highly fluorescent hydroxycoumarins, which partially dissociate by virtue of their low pK_a (7.6–7.9). Much faster hydrolysis is observed in the presence of the enzyme acetylcholine esterase. Preliminary results indicate that the acetates and, even more so, the butyrates are suitable substrates for the direct and continuous determination of esterases by either photometry or fluorimetry by following the increase in anion absorption (or fluorescence) with time.

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Experimental Part

Elemental analyses: Carlo Erba Model 1106 Analyzer. – Melting points: Büchi apparatus, uncorrected. – IR: Perkin Elmer 421. – ^{1}H NMR: Varian A 60 A. – MS: Varian MAT 111. – UV/VIS: Perkin Elmer Lambda 5. – Fluorescence: Amino SPF 500.

7-Methoxy-3-(2-pyridinyl)-2H-1-benzopyran-2-one (**3a**): A solution of 1.00 g (6.6 mmol) of 2-hydroxy-4-methoxybenzaldehyde (**1**), 0.66 g (4.0 mmol) of ethyl 2-pyridylacetate, and

0.1 ml of piperidine in 20 ml of isopropyl alcohol was heated under reflux for 8 h. When the mixture was left at room temperature for ca. 15 h, the product crystallized as colorless needles, which were filtered off. Yield 0.65 g (65%). Recrystallization from isopropyl alcohol gave a product of m. p. 148°C. For physicochemical and spectral data see Table 2.

7-Methoxy-3-(3-pyridinyl)-2H-1-benzopyran-2-one (**3b**): 3-Pyridylacetonitrile (1.77 g, 15 mmol), **1** (2.4 g, 16 mmol), and 0.1 ml of piperidine were heated in 10 ml of ethanol (95%) under reflux for 7 h. After addition of 5 ml of 2 N HCl **3b** precipitates after cooling. It is collected by filtration, washed with 1% aqueous sodium hydrogen carbonate solution, and recrystallized from ethanol. Data in Table 2.

7-Methoxy-3-(4-pyridinyl)-2H-1-benzopyran-2-one (**3c**): Ethyl 4-pyridylacetate (2.0 g, 12 mmol), **1** (2.8 g, 18.0 mmol), and 0.1 ml of piperidine were reacted as described for **3a**, except that before crystallization the reaction solution was reduced in vacuo to half of its volume. Data in Table 2.

7-Hydroxy-3-pyridinyl-2H-1-benzopyran-2-ones (**4a–c**), *General Procedure:* The respective 3-pyridylcoumarin **3a–c** (1.0 g) was carefully mixed with dry pyridine hydrochloride (4.0 g) and heated under reflux (bath temperature 220–240°C) for 1 h. After cooling, the mixture was suspended in 25 ml of water and stirred at ca. 30°C. The insoluble part consists of a fairly pure product (**4a–c**), which may be used directly in the next step. Recrystallization solvents, physicochemical and spectral data are given in Table 2.

7-Acetoxy- and 7-(Butanoyloxy)-3-pyridinyl-2H-1-benzopyran-2-ones (**5a–c, 6a–c**), *General Procedure:* The corresponding 7-hydroxycoumarin **4a–c** (1.0 g, 4.2 mmol) was refluxed in acetic anhydride (40 ml) or in a mixture of 1.5 g of butyric anhydride (9.4 mmol) and 15 ml of pyridine[14] until a clear solution was obtained. The products crystallized from the solution after slow cooling. A second fraction of the butyrates may be obtained from the filtrates by removing the pyridine *in vacuo*. Data for compounds **5** and **6** are given in Table 2.

2-,3-, or 4-(7-Acetoxy-2-oxo-2H-1-benzopyran-3-yl)-1-methylpyridinium Iodides (**7a–c**), *General Procedure:* The appropriate 7-acetoxy-3-pyridylcoumarin **5a–c** (100 mg) was dissolved in a mixture of 10 ml of nitromethane and 0.5 ml (8 mmol) of methyl iodide and kept at 80°C for 14 h in a tightly closed flask. After removing the solvent, the residue was treated with ether (5 ml) to induce crystallization. Yellow crystals, yield 90–98%. Data in Table 2.

2-,3-, or 4-(7-Hydroxy-2-oxo-2H-1-benzopyran-3-yl)-1-methylpyridinium Iodides (**8a–c**), *General Procedure:* The appropriate 7-acetoxycoumarin **7a–c** (500 mg, 1.2 mmol) was added under stirring to 15 ml of 3% aqueous hydroiodic acid at 70°C. The compound dissolved and after 5–20 min the product began to separate. After cooling to room temperature the yellow material was collected by filtration and recrystallized from methanol or ethanol. Data in Table 2.

2-,3-, or 4-(7-Methoxy-2-oxo-2H-1-benzopyran-3-yl)-1-methylpyridinium Iodides (**9a–c**): General procedure as with **7a–c**, starting from **3a–c**. The slightly yellow products are obtained in almost quantitative yields and may be recrystallized from 95% ethanol. Data in Table 2.

2-(7-Methoxy-2-oxo-2H-1-benzopyran-3-yl)-1-methylpyridinium Iodide (**9a**): 2-Hydroxy-4-methoxybenzaldehyde (**1**) (0.90 g, 6.1 mmol), ethyl 2-pyridylacetate methoiodide, and piperidine (2 drops) were heated in isopropyl alcohol (40 ml) for 75–90 min under reflux. In case the product did not crystallize upon cooling, the solvent was removed and the residue

Table 2. Physicochemical and spectral data of coumarins 3–9

	Formula (mol. mass)		Analysis C	H	N	m.p. (°C) (cryst. from)	Yield (%)	IR (cm^{-1})	^1H NMR Data[a]	Mass Spectrum *m/z* (70 eV)
3a	$C_{15}H_{11}NO_3$ (253.3)	Calc.	71.14	4.38	5.53	148	65	1728, 1610	($CDCl_3$) 3.95 (s, 3H);	253 (M^+, 100%)
		Found	70.91	4.35	5.44	(2-PrOH)		1580, 1500	6.75–8.00 (m, 8H)	
3b	$C_{15}H_{11}NO_3$ (253.3)	Calc.	71.14	4.38	5.53	188[b]	42	–	–	–
		Found	71.23	4.44	5.41	(EtOH)				
3c	$C_{15}H_{11}NO_3$ (253.3)	Calc.	71.14	4.38	5.53	195–196	44	1710, 1590	($CDCl_3$) 3.90 (s, 3H);	253 (M^+, 100%)
		Found	71.34	4.27	5.34	(EtOH)		1500, 1448	6.80–8.80 (m, 8H)	
4a	$C_{14}H_9NO_3$ (239.2)	Calc.	70.29	3.79	5.86	254–255[c]	87	1675, 1532	([D_6]DMSO)	239 (M^+, 100%)
		Found	70.54	3.80	5.68	(H_2O)		1525, 1450	6.68–8.80 (m, 8H)	
4b	$C_{14}H_9NO_3$ (239.2)	Calc.	–	–	5.86	322[d]	90	–	–	–
		Found	–	–	5.77	(20% acetic acid)				
4c	$C_{14}H_9NO_3 \cdot HCl$ (275.7)	Calc.	61.14	3.67	5.08	330[e] (dec.)	86	1705, 1600	([D_6]DMSO) 6.80 (s,	239 (M^+, 100%)
		Found	61.32	3.52	5.01	(2 N HCl)		1560, 1495	1H); 6.95 (d, 1H); 7.70 (d, 1H); 7.97 (d, 2H); 8.50 (s, 1H); 8.72 (d, 2H)	
5a	$C_{16}H_{11}NO_4$ (281.3)	Calc.	–	–	4.98	177–178[f]	95	–	–	–
		Found	–	–	4.87	(MeOH/benzene, 1:1)				
5b	$C_{16}H_{11}NO_4$ (281.3)	Calc.	–	–	4.98	177[g]	95	–	–	–
		Found	–	–	4.79	(acetic anhydride)				
5c	$C_{16}H_{11}NO_4$ (281.3)	Calc.	68.32	3.94	4.98	206	95	1730, 1612	([D_6]DMSO) 2.35 (s,	281 (M^+, 44%);
		Found	68.22	3.74	4.91	(2-PrOH)		1595, 1500	3H); 7.10–8.75 (m, 8H)	239 (100)
6a	$C_{18}H_{15}NO_4$ (309.3)	Calc.	69.89	4.89	4.53	158	95	1768, 1730	–	309 (M^+, 19%);
		Found	70.08	4.90	4.36	(2-PrOH)		1618, 1589		239 (100)
6b	$C_{18}H_{15}NO_4$ (309.3)	Calc.	69.89	4.89	4.53	178–179	95	1760, 1724	–	309 (M^+, 21%);
		Found	69.62	4.89	4.55	(2-PrOH)		1620, 1482		239 (100)
6c	$C_{18}H_{15}NO_4$ (309.3)	Calc.	69.89	4.89	4.53	199–200	95	1760, 1724	–	309 (M^+, 25%)
		Found	69.65	5.00	4.60	(2-PrOH)		1620, 1482		239 (100)
7a	$C_{17}H_{14}INO_4$ (423.2)	Calc.	48.25	3.33	3.31	219–221 (dec.)	90	1745, 1715	([D_6]DMSO) 2.32 (s,	–
		Found	48.51	3.34	3.24	(MeOH)		1610, 1578	3H); 4.25 (s, 3H); 7.12–9.23 (m, 8H)	

Table 2 (*Continued*)

	Formula (mol. mass)		Analysis C	H	N	m.p. (°C) (cryst. from)	Yield (%)	IR (cm^{-1})	^1H NMR Data[a]
7b	$C_{17}H_{14}INO_4$ (423.2)	Calc.	48.25	3.33	3.31	206 (dec.) (MeOH)	95	1775, 1730 1612, 1503	([D$_6$]DMSO) 2.37 (s, 3H); 4.42 (s, 3H); 7.22 (d, 1H); 7.36 (s, 1H); 7.82 (d, 1H); 8.18 (t, 1H); 8.60 (s, 1H); 8.90 (dd, 2H); 9.34 (s, 1H)
		Found	47.99	3.38	3.24				
7c	$C_{17}H_{14}INO_4$ (423.2)	Calc.	48.25	3.33	3.31	215 (dec.) (EtOH)	98	1765, 1745 1703, 1610	([D$_6$]DMSO) 2.37 (s, 3H); 4.37 (s, 3H); 7.10–7.35 (s + d, 2H); 7.93 (d, 1H); 8.50 (d, 2H); 8.90 (s, 1H); 9.05 (d, 2H)
		Found	48.05	3.41	3.17				
8a	$C_{15}H_{12}INO_3$ (381.2)	Calc.	47.27	3.17	3.68	251–254 (dec.) (MeOH)	92	1710, 1690 1570, 1510	([D$_6$]DMSO) 4.28 (s, 3H); 6.82–7.00 (s + d, 2H); 7.74 (d, 1H); 8.22 (d, 2H); 8.43 (s, 1H); 8.71 (t, 1H); 9.19 (d, 1H)
		Found	47.07	3.18	3.54				
8b	$C_{15}H_{12}INO_3$ (381.2)	Calc.	47.27	3.17	3.68	285 (dec.) (MeOH)	89	1720, 1615 1570, 1510	([D$_6$]DMSO) 4.46 (s, 3H); 6.80–7.06 (s + d, 2H); 7.72 (d, 1H); 8.24 (t, 1H); 8.62 (s, 1H), 8.98 (dd, 2H); 9.24 (s, 1H)
		Found	47.00	3.25	3.69				
8c	$C_{15}H_{12}INO_3$ (381.2)	Calc.	47.27	3.17	3.68	270 (dec.) (MeOH)	94	1705, 1640 1595, 1555	([D$_6$]DMSO) 4.37 (s, 3H); 6.74 (s, 1H); 6.92 (d, 1H); 7.75 (d, 1H); 8.05 (d, 2H); 8.93 (s, 1H); 9.03 (d, 2H)
		Found	47.28	3.18	3.51				
9a	$C_{16}H_{14}INO_3$ (395.2)	Calc.	48.63	3.64	3.55	233 (dec.) (EtOH)	85	1715, 1610 1570, 1500	(D_2O) 3.24 (s, 3H); 4.48 (s, 3H); 5.78 (s + d, 3H); 6.29 (d, 1H); 6.58 (br. d, 1H); 6.79 (s, 1H); 6.97 (t, 1H); 7.25 (d, 1H)
		Found	48.45	3.64	3.49				
9b	$C_{16}H_{14}INO_3$ (395.2)	Calc.	48.63	3.57	3.55	210 (dec.) (EtOH)	88	1690, 1600 1562, 1505	([D$_6$]DMSO) 3.96 (s, 3H); 4.46 (s, 3H); 7.04–7.20 (m, 2H); 7.78 (d, 1H); 8.18–8.32 (dd, 1H); 8.66 (s, 1H); 8.92 (d, 1H); 9.05 (d, 1H); 9.44 (s, 1H)
		Found	48.70	3.60	3.49				
9c	$C_{16}H_{14}INO_3$ (395.2)	Calc.	48.63	3.57	3.55	252 (dec.) (EtOH)	92	1722, 1650 1560, 1525	([D$_6$]DMSO) 3.80 (s, 3H); 4.21 (s, 3H); 6.70–9.10 (m, 8H)
		Found	48.59	3.63	3.50				

[a] Shifts in δ-units; TMS = 0. – [b] Ref.[7]: m. p. 188°C. – [c] Ref.[6]: m. p. 254–260°C (MeOH). – [d] Ref.[8]: m. p. 337°C. – [e] Recrystallization from 2 N HCl affords the well crystallizing hydrochloride. – [f] Ref.[6]: m. p. 176–177°C. – [g] Ref.[7]: m. p. 176°C.

treated with 20 ml of 95% ethanol. **9a** crystallized in slightly yellow crystals of m. p. 233 °C (dec.); yield 1.0 g (43%). Spectral data in Table 2.

2-[(Ethoxycarbonyl)methyl]-1-methylpyridinium Iodide: Ethyl 2-pyridylacetate (3.3 g, 20 mmol) was quaternized with methyl iodide (4 ml, 64 mmol) in nitromethane solution (30 ml) for 16 h at 80 °C (closed flask). After removing volatile parts *in vacuo*, the remaining oil was treated with ether (50 ml) to cause crystallization. The product was obtained as brownish-yellow needles in about 90% yield. M. p. 88–89 °C (from isopropyl alcohol).

$C_{10}H_{14}INO_2$ (307.1) Calc. C 39.11 H 4.59 N 4.56 Found C 38.90 H 4.51 N 4.47

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[1] Syntheses of Fluorescent Dyes, part 18. Part 17: *O. S. Wolfbeis, E. Koller,* and *P. Hochmuth,* Bull. Chem. Soc. Jpn. **58**, 731 (1985).

[2] *H. Gold,* Fluorescent Whitening Agents (*F. Coulson* and *F. Korte,* eds.), Georg Thieme Verlag, Stuttgart 1975.

[3] *A. Dorlars, C.-W. Schellhammer,* and *J. Schroeder,* Angew. Chem. **87**, 693 (1975); Angew. Chem., Int. Ed. Engl. **14**, 665 (1975).

[4] *D. W. Lübbers, N. Opitz, P. P. Speiser,* and *H. J. Bisson,* Z. Naturforsch., Teil C **32**, 133 (1975).

[5] *H. Alpes* and *W. G. Pohl,* Naturwissenschaften **65**, 652 (1978).

[6] *P. R. Bhandari,* Arch. Pharm. (Weinheim, Ger.) **297**, 698 (1964).

[7] *B. Sreenivasulu, V. Sundaramurthy,* and *N. V. Subba Rao,* Proc. Indian Acad. Sci., Sect. A **79**, 41 (1974).

[8] *N. P. Buu-Hoi, G. Saint-Ruf,* and *B. Lobert,* J. Chem. Soc. C **1969**, 2069.

[9] *R. B. Moffett,* J. Med. Chem. **7**, 446 (1964).

[10] *Ciba Geigy AG* (*S. Koller, R. Zink, D. Reichel,* and *J. Voltz*), D.O.S. 2.142.411 (March 2, 1972) [Chem. Abstr. **77**, 36393 (1972)].

[11] For a review, see: *S. G. Schulman,* Acid-Base Chemistry of Excited Singlet States, in: Modern Fluorescence Spectroscopy (*E. L. Wehry,* ed.), p. 239, Heyden & Sons, London-New York-Rheine 1976.

[12] *M. Zander,* Fluorimetrie, p. 28ff., Springer Verlag, Berlin-Heidelberg-New York 1981.

[13] *J. Yakatan, R. J. Juneau,* and *S. G. Schulman,* Anal. Chem. **44**, 1044 (1972).

[14] The presence of pyridine seems to be essential.

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