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9-(4-Dialkylaminostyryl)-Acridines – A New Class of Acidochromic Dyes

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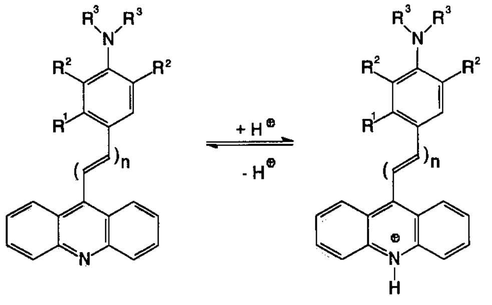
Abstract A series of new acidochromic 9-(4-dialkylamino-styryl)-acridines (**1**) were synthesized and characterized by means of elemental analysis, m.p., mass and ^{1}H-NMR-spectra. The colour change in the presence of perchloric acid and the pK$_a$ was investigated. Their photosensitizing ability was tested by oxygen consumption measurements.

Acidochromic dyes which absorb in the 600–700 nm region are interesting as sensitizers for the photodynamic therapy of cancer (PDT), in medical fluorescence diagnostics and in sensor techniques. Our main interest in these dyes results from their potential application in PDT. It has been argued that a slightly reduced pH in neoplastic tissue as compared to normal tissue may play a role in cell permeation and, consequently, in the selective retention of the photosensitizer hematoporphyrin derivative (HPD) [1]. Even if the sensitizer is equally distributed between normal and neoplastic tissue it should be possible to selectively achieve increased absorption of the neoplastic tissue if a sensitizer is used with a proper pK$_a$. The ratio of the absorbancies due to the protonated and unprotonated sensitizer is shifted, e.g., by a factor of 1.7 if the pH ist diminished by 0.5, thus a well matched pK$_a$ provided. It has been shown for experimental tumors that the pH difference can be artificially enhanced by infusion of glucose, hyperthermia and administration of certain drugs [2].

9-(4-Dialkylaminostyryl)-acridines (neutral form **1** and salt **2**) are promising candidates for that purpose because their protonated forms absorb in the red spectral region and their pK$_a$ should be tunable by a variation of the electron donor capability of the dialkylaminostyryl moiety [3, 4]. The acridine-based styrylcyanines **2** typically absorb at about 600 to 700 nm, thus absorbing at longer wavelengths than most other nitrogen-containing heterocyclic styryl dyes [5]. However, an examination of the influence of substituents on the acid/base and spectral behaviour is still lacking. In this paper, the synthesis of novel 9-(4-dialkylaminostyryl)-acridines **1**, their acid/base behavior and the visible absorption spectra of both forms **1** and **2** are reported.

As a preliminary test for their photosensitizing ability the rate of oxygen consumption of dye solutions under steady irradiation has been measured.



1 **2**

Scheme 1 Basic **1** and acid form **2** of 9-(4-dialkylaminostyryl)-acridines

1,2	a	b	c	d	e	f	g	h	i	k
n	1	2	1							
R^1	H			OCOCH$_3$	OH	OC$_2$H$_5$	OC$_{18}$H$_{37}$	H	OCOCH$_3$	OH
R^2	H							(CH$_2$)$_3$		
R^3	CH$_3$		C$_2$H$_5$							

Experimental

Apparatus

Melting points were determined on a Kofler apparatus and are uncorrected. Absorption spectra were recorded by means of spectrophotometers Specord M 40, Specord M 400 (Carl Zeiss Jena), and Lambda 16 (Perkin Elmer).

For recording NMR spectra a AMX-400 spectrometer (Bruker) has been used. Mass spectra were recorded by means of a quadrupol mass spectrometer "Trio 2000" (Fisons-Instruments).

Flash photolysis was performed with a self made conventional device. Time resolution was restricted to about 5 µs. Oxygen was removed from the solutions for flash photolysis by purging with nitrogen.

The consumption of dissolved oxygen was measured under irradiation by means of an oxygen electrode (Trioxmatic EO 200) and an microprocessor oximeter Oxi 196 (WTW GmbH Weilheim) in a 50 ml closed cell, similar as described in [6]. The cell was stirred during exposure. A halogen lamp with a color temperature of 2650 K was used as an illumination source with a cut-off filter OG3 (Schott) inserted. Tryptophane was used as singulet oxygen trap [7].

Procedures

The efficiencies of oxygen consumption in Table 3 (v.i.) are given relative to methylene blue:

$$\eta_{rel} = \frac{\eta_{O_2,dye}}{\eta_{O_{O_2},MB}} \tag{1}$$

If the efficiency of oxygen consumption is defined as

$$\eta_{O_2} = -\frac{const}{I_{abs}}\frac{dC_{O_2}}{dt} \tag{2}$$

with

$$I_{abs} = \int I_0(\lambda)T_f(\lambda)(1 - 10^{E(\lambda)})d\lambda \tag{3}$$

where I_0 represents the number of photons incident per unit time to the probe volume according to the Planck distribution. T_f is the transmission of the light filter and $E(\lambda)$ is the absorbance of the sensitizer at the wavelength λ. C_{O_2} is the concentration of molecular oxygen in ppm as measured by the oximeter. The integral was numerically evaluated between 550 to 900 nm. In all cases C_{O_2} showed a linear decrease with the irradiation time.

50 % water/i-propanol mixtures were used as a solvent. The pH was adjusted to 4.3 by means of a phosphate buffer prepared by mixing of varying amounts of 1/15 M KH_2PO_4 and 1/15 M Na_2HPO_4. Solutions were allowed to equilibrate 20 min by stirring under normal atmospheric conditions before measurement.

pK_a-Values were estimated by nonlinear regression of the absorbance at the maximum of the longest wavelength band as a function of pH according to the Henderson-Hasselbalch equation. The solvent was also a 50 % water/isopropanol mixture. The pH was varied by addition of acetic acid. It was measured with the help of a Schott pH-meter CG 825 and a glass electrode N 42 A.

Theoretical estimates of the position and strength of the electronic transitions and π-electron charge distributions were obtained from standard PPP-calculations using the Mataga Nishimoto approximation [8]. The geometry of the molecules was optimized by MM2 force field calculations [9].

Preparations

9-Methylacridine was synthesized according to reference [10]. With the exception of 4-N,N-diethylamino-2-octadecyloxybenzaldehyde all 4-dialkylamino-benzaldehydes used as starting materials are commercially available.

The 9-(4-dialkylaminostyryl)-acridines **1a–d** and **1f–i** were obtained by condensation of 9-methylacridine with the corresponding 4-dialkylamino-benzaldehydes or 4-dialkylaminocinnamaldehyde in acetic anhydrid according to the following general procedure:

9-(4-Dialkylaminostyryl)-acridines (**1a–d** and **1f–i**)

9-Methylacridine (1.93 g, 0.01 mol), the corresponding dialkylamino-benzaldehyde (0.01 mol) or 4-dimethylaminocinnamaldehyde (1.75 g, 0.01 mol), and acetic anhydride (30 ml) were refluxed for 3–4 h. After cooling, 100 ml of 5 % aqueous sodium hydroxide were added to the mixture. The resulting precipitate was filtered off and washed to neutrality with water. Then it was recrystallized from acetonitrile or ethanol. The yields were in the range from 76–96 %.

The 9-(4-dialkylamino-2-hydroxystyryl)-acridines **1e** and **1k** were prepared according to the procedure:

9-(4-Dialkylamino-2-hydroxystyryl)-acridines (**1e** and **1k**)

9-(4-Dialkylamino-2-acyloxystyryl)-acridine **1d** or **1i** (0.005 mol) was dissolved in a mixture of water (40 ml), ethanol (40 ml), and conc. sulphuric acid (4 ml). The resulting solution was refluxed with stirring for 30 min and then poured onto 10 % aqueous sodium hydroxide (90 ml). The crude product which precipitated in quantitative yield was filtered, washed with water, and recrystallized from acetonitrile or ethanol.

4-N,N-Diethylamino-2-octadecyloxy-benzaldehyde

4-N,N-Diethylamino-2-hydroxy-benzaldehyde (1.93 g; 0.01 mol), 1-octadecyl-iodide (3.80 g; 0.01 mol), sodium hydroxide (0.4 g; 0.01 mol) and tetrapropylammoniumbromide (0.27 g; 0.001 mol) were dissolved in 30 ml acetone and refluxed for 6 h. Water (200 ml) was added to the cooled reaction mixture and the pH was adjusted to 10–11. The precipitate was filtered off and dried. The yield was 1.56 g (35 %); m.p. 54–55 °C (ethanol).

$C_{29}H_{51}NO_2$	Found:	C 76.20	H 11.33	N 2.77
(445.73)	Calc.:	C 78.15	H 11.33	N 3.14

Results

The molecular formula, the molecular weight, the molecular peak of the mass spectrum (M^+) and the elemental analysis data of the dyes **1a–k** are given in table 1.

In table 2 the characteristical spectral data and the pKa-values of the 9-(4-dialkylaminostyryl)-acridines **1** and their corresponding salts are recorded. The unprotonated dyes **1** absorb between 430 to 470 nm and exhibit considerably lower molar extinction coefficients than their protonated counterparts **2**. A typical set of absorption spectra as a function of pH is shown in figure 1. The pK_a-values vary from 4.62 to 5.66.

It was impossible to detect triplet-triplet absorptions by means of conventional flash photolysis in oxygen free solutions. Such attempts revealed reversible depletion in the region of the longest wavelength absorption band but no transient absorption. Whereas with the protonated acridines recovery times in the millisecond time scale have been found, the photoinduced depletion of the unprotonated dyes reverts thermally at room temperature within tens of minutes. In a subsequent paper the photophysical desactivation behaviour and the photoisomerization of dyes **1** will be reported.

Table 1 Analytical data of 9-(4-dialkylaminostyryl)-acridines

Dye	Molecular formula (Mol. weight)	Mass spectrum[a] (M^+)	M.p.[°C]	C Calc./ Found	H Calc./ Found	N Calc./ Found
1a	$C_{23}H_{20}N_2$	324.1	240	85.15	6.21	8.63
	(324.43)		241–242 [3]	84.19	6.05	8.62
1b	$C_{25}H_{22}N_2$	349.9	163–167	81.78[c]	7.12	7.06
	(350.46)			81.16	7.05	7.34
1c[b]	$C_{25}H_{24}N_2$	352.2	188–189	83.16[c]	7.25	7.46
	(352.48)		188–189 [3]	83.75	6.89	7.99
1d	$C_{27}H_{26}N_2O_2$	410.1	170–174	79.00	6.38	6.82
	(410.52)			79.49	6.25	7.07
1e	$C_{25}H_{24}N_2O$	368.0	272–274	81.49	6.56	7.60
	(368.48)			81.59	6.45	7.64
1f	$C_{27}H_{28}N_2O$	396.3	180–182	81.78	7.12	7.06
	(396.53)			81.72	7.15	6.97
1g	$C_{43}H_{60}N_2O$	620.9	33–40	79.16[c]	10.18	3.93
	(620.96)			79.86	9.72	3.99
1h	$C_{27}H_{24}N_2$	376.1	183–187	86.13	6.43	7.44
	(376.50)			86.43	6.45	7.88
1i	$C_{29}H_{26}N_2O_2$	434.1	214–220	80.16	6.03	6.45
	(434.54)			79.97	6.13	6.48
1k	$C_{27}H_{24}N_2O$	392.4	235	82.62	6.16	7.14
	(392.50)			81.43	6.22	6.99

[a] **1a** and **1c–1k** : 204 (M^+ – dialkylaminophenyl); **1b** : 230 (M^+ – dialkylaminophenyl).

[b] **1c**: ^{1}H-NMR (DMF-d_7) [ppm]: 1.16 (t, CH_3, 6H); 3.45 (q, CH_2, 4H); 6.79 and 7.71 (AA′ and XX′, aryl, 4H); 7.08 and 7.94 (d and d, ethylen, 2H); 8.16 and 8.47 (d and d, acridyl, 4H); 7.60 and 7.84 (m and m, acridyl 4H).

[c] The calculated elemental analysis data are given for **1b** · C_2H_5OH (396.53), **1c** · 0.5 C_2H_5OH (375.52) and **1g** · 2 C_2H_5OH (713.10).

Table 2 Absorption maxima, molar extinction coefficients and pK_a-values for **1a–j** and **2a–j** in ethanol, counterion of the hydronium form is acetate.

Dye	λ_{max} (ethanol) [nm]	ε [1 mol^{-1}cm^{-1}]	pK_a
1a	436	15800	4.87
2a	618	28400	±0.08
1b	440	10350	4.62
2b	642	24100	±0.06
1c	448	16400	5.12
2c	639	32900	±0.05
1d	435	17400	4.78
2d	614	29300	±0.08
1e	472	16130	5.41
2e	679	52800	±0.10
1f	464	16600	5.50
2f	670	47000	±0.03
1g	464	12600	5.27
2g	668	31000	±0.06
1h	469	13470	5.06
2h	698	39600	±0.07
1i	456	15300	4.85
2i	675	35000	±0.04
1k	475	n.d.[a]	5.66
2k	717	n.d.[a]	±0.01

[a] Not determined due to poor solubility in ethanol

As compared to the efficient photosensitizer methylene blue the styrylacridinium salts **2**, as can be seen from the η-values given in table 3, are much less efficient.

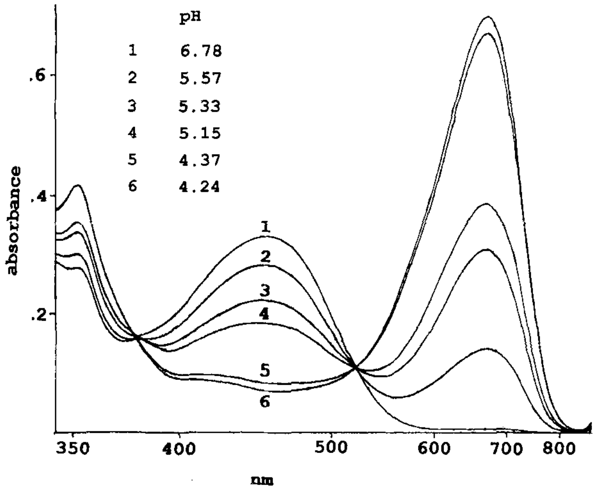


Fig. 1 Set of absorption spectra of **1h/2h** under a variation of pH

Table 3 Efficencies of oxygen consumption relative to that of methylene blue

Dye	**2b**	**2d**	**2e**	**2f**	**2h**	**2i**
η_{rel}	0.04	0.02	0.04	0.03	0.02	0.02

Discussion

As obvious from table 3, an additional donor substituent at the dialkylamino-styrene moiety increases the wavelength of the lowest energy transition with the neutral bases as well as with the cationic species. The acceptor substituent -OCOMe causes a small hypsochromic shift of about 20 nm (**2d** and **2i**). If expressed in wavenumbers the spectral shifts in the unprotonated dyes **1** parallel those in the acid dyes **2**. The extinction coefficients are enhanced roughly by a factor of two upon fixation of the dialkylamino substituent (**1h–1k**) and donor-substitution at the phenyl ring (**1e–1g**).

PPP-calculations predict the wavelength of the lowest energy transition of **1a**, **1c–1k** and **2a**, **2c–2k** reasonably well: values from 435 to 469 nm ($1.0 < f < 1.1$) and 674 to 695 nm ($1.5 < f < 1.6$) are calculated for the basic (**1**) and the acid form (**2**) of the E-isomers, resp.. (From the PPP-calculations the corresponding Z-isomers are predicted to absorb at 10 to 20 nm longer wavelengths. The oscillator strengths f of the Z-isomers amount to about 2/3 of those of the E-forms.) As compared to all other dyes reported in this work compound **2b** exhibits a chromophoric system which is enlarged by one vinylene group. Surprisingly the enlargement of the chromophore in **2b** causes only a very small vinylene shift contradictory to the prediction of the PPP calculation: $\lambda_{max} = 884$ nm. Though it is known that vinylene shifts are smaller with asymmetric cyanins as compared to symmetric ones [11] the shift exhibited by **2b** seems unusually small. For **1b** we calculated $\lambda_{max} = 404$ nm in crude agreement with the experiment. PPP-calculations with **1a** also confirm a small charge shift (0.05 electron charge) from the dimethylaminophenyl moiety to the acridine part which is increased to 0.34 in the first excited state. With **2a** the calculated charge shift amounts to 0.19 and 0.68 for the ground and S_1 states, resp.. It is obvious from the calculated charge shifts that a charge transfer the dialkylamino (donor) moiety to the acridine (acceptor) part occurs upon excitation. The direction of the charge transfer is the same for type **1** and type **2** molecules, however, it is more pronounced in the hemicyanines **2**. The π-electron densities alternate along the chain connecting the heteroatoms and this alternation is reversed upon excitation.

Thus, the protonated species may be considered as styrylcyanine chromophores with donor/acceptor behaviour.

The pK_a varies with the donating ability of the dialkylaminostyryl moiety. From the data in table 2 (dyes **c, d, e, f**) the following pK_a correlation is derived:

$$pK_a = (5.04 \pm 0.04) - (1.36 \pm 0.17)\sigma_p$$

(σ_p is the Hammett substituent constant, the values were taken from reference [12]). With $\sigma_p = -0.6$ for $R^1 = NMe_2$ a $pK_a = 5.8 \pm 0.3$ is predicted from the correlation. The same result, ($pK_a = 5.8 \pm 0.1$), is obtained if σ_p^+-values are used for the correlation. Significantly stronger donor substituents do not exist. Thus, we conclude that it will probably be impossible to synthesize dyes of type **1** and **2**, resp., which will exhibit a pK_a in the desired region between 6.5 and 7.5. However, one has to take into consideration that the position of an acid-base equilibrium need not be the same in water/isopropanol mixtures and in the interstitial liquor or with the dyes adsorbed on a cell membrane.

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