New Indolium and Quinolinium Dyes Sensitive to Aqueous Halide Ions at Physiological Concentrations

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Twelve new highly fluorescent dyes have been produced by the reaction of two heterocyclic nitrogen bases with 8-bromooctanoic acid, 11-bromoundecanoic acid and 15-bromopentadecanoic acid. The bromide counter ions of the first six dyes have been replaced with the tetraphenylborate ion. Unlike the bases themselves, the quaternary salts are water soluble and have fluorescence characteristics independent of *pH* in the *pH* range 7-11. Both the fluorescence intensity and fluorescence lifetime of dyes **1-12** are reduced in the presence of aqueous halide ions allowing halide concentrations to be determined accurately at physiological levels. All the dyes have been characterised in terms of steady state fluorescence spectra and steady-state Stern-Volmer analysis.

Introduction.

Most biological fluids are complicated mixtures which include inorganic electrolytes. In plasma and muscle cells, electrolyte composition is normally fairly constant but in gastric/pancreatic juices, sweat, saliva, and urine this may vary considerably, particularly when affected by illness. A good example of this is cystic fibrosis. Few diseases can be so readily diagnosed as cystic fibrosis, which is characterised by a high chloride concentration in a patient's sweat and saliva. Accurately determining halide in blood [1], serum [2], plasma [3], and urine [4] is therefore of obvious importance to medicine. Physiological halide concentrations lie in the range 10^{-1}-10^{-4} mol dm^{-3}.

The fluorescence quenching of quinoline-type fluorophores was first described by Stokes [5] in 1869, when he observed that the fluorescence of quinine in dilute sulphuric acid was reduced after the addition of hydrochloric acid or halide ions. This process that he observed is now commonly referred to as 'dynamic fluorescence quenching' where both the lifetime and intensity of fluorescence are reduced in the presence of a quencher, Q. This process is known to follow Stern-Volmer kinetics [6] where:

$$I_0/I = 1 + k_q\tau^0[Q] \qquad \text{eq. 1.}$$

which can be used to obtain values of $k_q\tau^0$ (the Stern-Volmer constant) by plotting I_0/I as a function of [Q]. Here I_0 and I are the fluorescence intensities in the absence and presence of Q respectively, k_q is a specific constant describing bimolecular collisional deactivation of electronic energy and τ^0 the natural fluorescence lifetime.

In this paper the synthesis of some new highly fluorescent dyes, Figure 1, and their respective Stern-Volmer quenching constants, table [I], are presented.
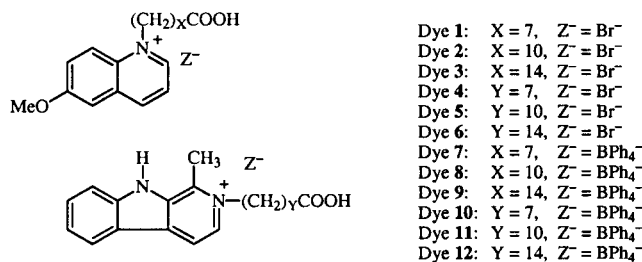

Dye 1:	X = 7,	Z$^-$ = Br$^-$
Dye 2:	X = 10,	Z$^-$ = Br$^-$
Dye 3:	X = 14,	Z$^-$ = Br$^-$
Dye 4:	Y = 7,	Z$^-$ = Br$^-$
Dye 5:	Y = 10,	Z$^-$ = Br$^-$
Dye 6:	Y = 14,	Z$^-$ = Br$^-$
Dye 7:	X = 7,	Z$^-$ = BPh$_4^-$
Dye 8:	X = 10,	Z$^-$ = BPh$_4^-$
Dye 9:	X = 14,	Z$^-$ = BPh$_4^-$
Dye 10:	Y = 7,	Z$^-$ = BPh$_4^-$
Dye 11:	Y = 10,	Z$^-$ = BPh$_4^-$
Dye 12:	Y = 14,	Z$^-$ = BPh$_4^-$

Figure 1. Fluorescent dyes **1-12**.

Results and Discussion.

The fluorescence of many dyes is known to be quenched by aqueous halide, the sensitivity and hence concentration range detectable is shown by the magnitude of the Stern-Volmer constant. At present there is a need for water soluble dyes with large Stern-Volmer constants, many commercially available dyes having relatively small Stern-Volmer constants, *e.g.* Acridine orange: I$^-$ = 51, Br$^-$ = 1.2 mol^{-1} dm^3; Rhodamine B (a common laser dye): I$^-$ = 5 mol^{-1} dm^3, thus limiting halide detection limits. In comparison, aqueous solutions of dye 1, (the Stern-Volmer constant for I$^-$ ions is 634 mol^{-1} dm^3) are capable of determining halide concentrations of 0.001 mol dm^{-3} with ≈2-4% error.

Stern-Volmer constants for dyes **7-9** and **10-12** are smaller than those of dyes **1-3** and **4-6** respectively, showing that the counter ion has an effect on sensitivity. This may simply be a size effect, the larger counter ion sterically hindering halide diffusion to the dye. There is a decrease in halide sensitivity, for all dyes, as the alkyl chain lengths 'X' and 'Y' increase.

To ascertain dye selectivity, interferences were studied, 0.5 mol dm^{-3} solutions of sulphite and a pseudohalide,

Table 1

Stern-Volmer Constants for Dyes **1-12**, pH 10, 21°. Constants at other pH's are not shown but are $\approx$ constant between pH's 7-11. λ_{ex} – Excitation wavelength maxima, λ_{em} – Emission wavelength maxima. '-' indicates no sensitivity.

Dye	Steady-State Stern-Volmer Constants (mol^{-1} dm^3)			Wavelength maxima		Water solubility
	Cl$^-$	Br$^-$	I$^-$	λ_{ex} (nm)	λ_{em} (nm)	
1	52	225	634	360	460	VeryGood
2	34	150	480	360	460	VeryGood
3	34	127	398	360	460	VeryGood
4	-	3	191	390	460	Good
5	-	5	198	390	460	Good
6	-	3	192	390	460	Good
7	20	70	218	360	460	Good
8	14	55	165	360	460	Good
9	11	27	139	360	460	Good
10	-	1	139	390	460	Fair
11	-	1	143	390	460	Fair
12	-	1	141	390	460	Fair

isothiocyanate, were shown to be effective fluorescence quenchers with large Stern-Volmer constants. Whilst one would not normally expect to find these ions in serum and urine, other potential quencers, such as the anaesthetic 'Halothane' may be present and detectable by some of these dyes. No interferences were observed with nitrate, sulphate and phosphate ions.

All dyes **1-12** are highly fluorescent with emission wavelengths of $\approx$460 nm, some 70-100 nm Stokes shifted from the absorption maxima. The ease of discrimination between the excitation and emission wavelengths, their good water solubility and sensitivity towards halide ions offers good prospects for use in halide sensor devices.

EXPERIMENTAL

The ^{1}H nmr spectra were recorded on a Varian Unity plus (400 MHz) spectrometer. Chemical shifts are reported in δ ppm relative to tetramethylsilane in deuteriochloroform. All IR spectra were recorded on a Perkin-Elmer PE2000R NIR FT-Raman spectrometer. All absorption measurements were performed using a Philips Unicam PU8620 UV/VIS/NIR spectrophotometer. Steady-state emission and excitation maxima were recorded on a Jobin-Yvon JY3D spectrofluorimeter. Steady-state Stern-Volmer analysis of dyes **1-12** was carried out at 21°, pH 10 (borax buffer), using halide concentrations in the range $1 \rightarrow 10^{-4}$ mol dm^{-3}. Subsequently, the Stern-Volmer quenching constants for dyes **1-12** with aqueous halide ions were calculated using the Axum Graphics linear regression program. All reagents were purchased from the Aldrich Chemical Company and used as received.

6-Methoxy-1-(8-octanoic Acid)quinolinium Bromide, (**Dye 1**).

6-Methoxyquinoline (1.154 g, 7.2 mmoles) and 1.606 g (7.2 mmoles) 8-bromooctanoic acid were heated under reflux for 4 hours at 110°. The mixture was allowed to cool and 50 ml of acetone was added. The mixture was stirred for 1 hour and the acetone decanted. Continual washings of the product with diethyl ether gave 1.71 g (65%) of product. ^{1}H nmr (deuterium oxide):

(1.2-2.4 (m, 12H), 4.1 (s, 3H), 5.1 (t, 2H), 7.6-9.2 (m, 6H); ir (potassium bromide): 1696, 2856 and 2932 cm^{-1}.

Anal. Calcd. for $C_{18}H_{24}NO_3Br$: C, 56.55; H, 6.33; N, 3.66. Found: C, 56.61; H, 6.30; N, 3.65.

6-Methoxy-1-(11-undecanoic Acid)quinolinium Bromide, (**Dye 2**).

6-Methoxyquinoline (3 g, 18.8 mmoles) and 4.998 g (18.8 mmoles) of 11-bromoundecanoic acid were heated under reflux for 4 hours at 110°. The brown material, whilst hot, was stirred with 50 ml acetone for 12 hours. Decanting the acetone and continual washings with diethyl ether gave 3.3 g (41%) of product; ^{1}H nmr (methyl-d$_3$ alcohol-d): δ 1-2.4 (m, 18H), 4.0 (s, 3H), 4.8(s, 1H), 5.0 (t, 2H), 7.6-9.2 (m, 6H); ir (potassium bromide): 1697, 2853, 2929 and 3293 cm^{-1}.

Anal. Calcd. for $C_{21}H_{30}NO_3Br$: C, 59.44; H, 7.13; N, 3.30. Found: C, 59.43; H, 7.14; N, 3.28.

6-Methoxy-1-(15-pentadecanoic Acid)quinolinium Bromide, (**Dye 3**).

6-Methoxyquinoline (5 g, 31.4 mmoles) and 10.08 g (31.4 mmoles) of 15-bromopentadecanoic acid were heated under reflux for 4 hours at 110°. The brown material, whilst hot, was stirred with 50 ml acetone for 12 hours. Decanting the acetone and continual washings with diethyl ether gave 12.01 g (80%) of product; ^{1}H nmr (dimethylformamide): δ 1.5 (m, 26H), 2.3 (t, 2H), 4.1 (2, 3H), 5.3 (t, 2H), 7.8-9.8 (m, 6H); ir (potassium bromide): 1696, 2849 and 2919 cm^{-1}.

Anal. Calcd. for $C_{25}H_{38}NO_3Br$: C, 62.49; H, 7.97; N, 2.92. Found: C, 62.13; H, 7.98; N, 3.01.

1-Methyl-2-(8-octanoic Acid)-2H-pyrido[3,4-b]indolium Bromide, (**Dye 4**).

Harmane (0.3 g, 1.65 mmoles) was fused together with 0.367 g (1.65 mmoles) 8-bromooctanoic acid in a round bottomed flask at 120° for 1 hour. After cooling for 1 additional hour the yellow solid was treated with 50 ml of diethyl ether and vigorously stirred for 1 additional hour. The resulting yellow precipitate was recovered and recrystallised from methanol to give 0.37g (55% yield) of yellow powder; ^{1}H nmr (methyl-d$_3$ alcohol-d): δ 1.2-1.8 (m, 12H), 3.1 (s, 3H), 4.7 (t, 3H), 4.7-8.4 (m, 6H), 8.5 (s, 1H); ir (potassium bromide): 1627, 1726, 2851 and 2928 cm^{-1}.

Anal. Calcd. for $C_{20}H_{25}N_2O_2Br$: C, 59.26; H, 6.22; N, 6.91. Found: C, 59.30; H, 6.24; N, 6.95.

1-Methyl-2-(11-undecanoic acid)-2H-pyrido[3,4-b]indolium Bromide, (**Dye 5**).

Harmane (0.5 g, 2.7 mmoles) was fused with 0.728 g (2.74 mmoles) 11-bromoundecanoic acid in a round bottomed flask at 120° for 1 hour. The fused yellow solid was extracted into excess acetone and stirred continuously for 12 hours. Decanting the acetone and recrystallisation from ethanol gave 0.861g (70% yield); ^{1}H nmr (dimethyl-d$_6$ sulphoxide): δ 1.2-1.5 (m,18H), 3.3 (s,3H), 4.7 (t,2H), 7.0-8.7 (m,6H); ir (potassium bromide): 1339, 1734, 2852, 2919 and 3074 cm^{-1}.

Anal. Calcd. for $C_{23}H_{31}N_2O_2Br$: C, 61.74; H, 6.98; N, 6.26. Found: C, 61.67; H, 7.00; N, 6.25.

1-Methyl-2-(15-pentadecanoic Acid)-2H-pyrido[3,4-b]indolium Bromide, (**Dye 6**).

Harmane (2 g, 10 mmoles) was fused with 3.523 g (10 mmoles) of 15-bromopentadecanoic acid at 120° for 1 hour. The fused yellow

solid was extracted into excess acetone and stirred continuously for 12 hours. Decanting the acetone and recrystallisation from ethanol gave 4.31 g (78% yield); ^{1}H nmr (dimethylformamide): δ 1.4 (m, 26H), 3.4 (s, 3H), 4.9 (t, 2H), 7.4-8.8 (m, 6H); ir (potassium bromide); 1335, 1732, 1900, 2849, 2917 and 3075 cm^{-1}.

Anal. Calcd. for $C_{27}H_{39}N_2O_2Br$: C, 64.41; H, 7.81; N, 5.56. Found: C, 64.42; H, 7.85; N, 5.49.

Dyes 7-9: Counter Ion Exchange of Dyes **1-3**.

The quinolinium salts were dissolved in a minimum amount of hot water and pipetted into a saturated solution of sodium tetraphenylborate. The white precipitate was collected by filtration, washed with distilled water, to remove sodium bromide, and dried in a vacuum oven at 40°.

6-Methoxy-1-(8-octanoic Acid)quinolinium Tetraphenylborate, **(Dye 7)**.

Dye **7** was obtained in 96% yield (1.57 g, 2.53 mmoles), from 1 g (2.62 mmoles) of starting material.

Anal. Calcd. for $C_{42}H_{48}NO_3B$: C, 81.15; H, 7.08; N, 2.25. Found: C, 80.15; H, 6.93; N, 2.15.

6-Methoxy-1-(11-undecanoic Acid)quinolinium Tetraphenylborate, **(Dye 8)**.

Dye **8** was obtained in 99% yield (4.66 g, 7.03 mmoles) from 3 g (7.09 mmoles) of starting material.

Anal. Calcd. for $C_{45}H_{54}NO_3B$: C, 81.44; H, 7.54; N, 2.11. Found: C, 81.12; H, 7.51; N, 2.06.

6-Methoxy-1-(15-pentadecanoic Acid)quinolinium Tetraphenylborate, **(Dye 9)**.

Dye **9** was obtained in 89% yield (1.33 g, 1.85 mmoles) from 1 g (2.09 mmoles) of starting material.

Anal. Calcd. for $C_{49}H_{62}NO_3B$: C, 81.78; H, 8.07; N, 1.95. Found: C, 81.52; H, 7.99; N, 1.63.

Dyes 10-12: Counter Ion Exchange of Dyes **4-6**.

The yellow indolium salts were dissolved in a 50:50 mixture of warm methanol:water and pipetted into a saturated solution of sodium tetraphenylborate. The white precipitate that instantly formed was collected by filtration, washed with excess water to remove sodium bromide, and dried under vacuum at 40°.

1-Methyl-2-(8-octanoic Acid)-2*H*-pyrido[3,4-*b*]indolium Tetraphenylborate, **(Dye 10)**.

Dye **10** was obtained in 90% yield (1.43 g, 2.22 mmoles), from 1 g (2.48 mmoles) of starting material.

Anal. Calcd. for $C_{44}H_{49}N_2O_2B$: C, 82.0; H, 6.99; N, 4.35. Found: C, 80.07; H, 6.89; N, 4.36.

1-Methyl-2-(11-undecanoic Acid)-2*H*-pyrido[3,4-*b*]indolium Tetraphenylborate, **(Dye 11)**.

Dye **11** was obtained in 85% yield (2.61 g, 3.80 mmoles) from 2 g (4.47 mmoles) of starting material.

Anal. Calcd. for $C_{47}H_{55}N_2O_2B$: C, 82.21; H, 7.43; N, 4.08. Found: C, 79.82; H, 7.12; N, 4.31.

1-Methyl-2-(15-pentadecanoic Acid)-2*H*-pyrido(3,4-*b*)indolium Tetraphenylborate, **(Dye 12)**.

Dye **12** was obtained in 69% yield (2.03 g, 2.74 mmoles), from 2 g (3.98 mmoles) of starting material.

Anal. Calcd. for $C_{51}H_{63}N_2O_2B$: C, 82.48; H, 7.95; N, 3.77. Found: C, 79.13; H, 7.69; N, 3.69.

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