Spectrochimica Acta, Vol. 47A, No. 2, pp. 187–192, 1991
Printed in Great Britain

0584-8539/91 $3.00+0.00
Pergamon Press plc

New highly fluorescent ketocyanine polarity probes

MANFRED A. KESSLER and OTTO S. WOLFBEIS*
Analytical Division, Institute of Organic Chemistry, Karl Franzens University, A-8010 Graz, Austria

(*Received* 10 *July* 1990; *accepted* 31 *July* 1990)

Abstract—The syntheses and spectral properties of three new and highly fluorescent solvent polarity probes are described. They are found to be extremely sensitive to solvent polarity in that spectral red shifts in both absorption and fluorescence spectra occur upon increasing solvent polarity. Excitation and emission data of the dyes in a set of different polar solvents are given. The emission data are compared with the standard ET^N values of solvent polarity and a linear correlation is obtained over a wide range. The origin of the unusual solvatochromic properties is discussed in terms of the resonance structures of this new group of molecular probes. Their outstanding features include high spectral sensitivity to polarity, high molar absorptivities, high fluorescence quantum yields, longwave excitation and emission, insignificant quenching by oxygen, and a sufficient stability in aqueous solution. Therefore, the new probes are considered to be advantageous over other polarity probes used so far in probing biochemical and biological systems.

INTRODUCTION

EMPIRICAL parameters of solvent polarity have been shown to be useful in examining a variety of physicochemical and analytical processes. Many of these parameters are based on polarity induced shifts in the absorption spectra of organic indicator dyes. Although a large number of organic dyes being influenced by solvent polarity are known so far [1], only a few of them have been applied in practice. Thus, KOSOWER *et al.* [2] used 1-ethyl-4-methoxycarbonylpyridinium iodide to establish an empirical scale of solvent polarity, the so-called Z-scale. DIMROTH and REICHARDT [3, 4] applied a large number of pyridinium-*N*-phenolate betaines to polarity investigations of solvents and the determination of water content in organic solvents. The extremely large spectral shifts in absorbance of these dyes, when dissolved in solvents of different polarity, make them the most polarity-sensitive probes known so far. Derivative ET(30) is now considered to be the standard indicator of solvent polarity [5].

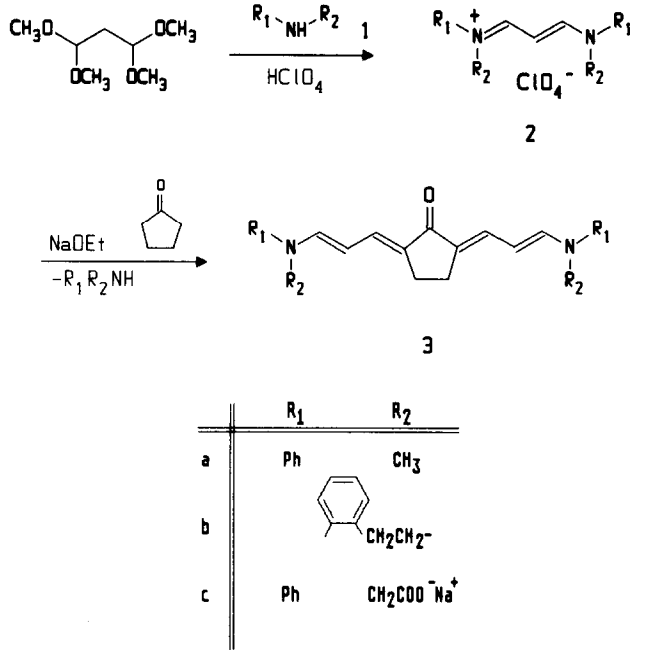
Also, the use of polarity probes for the evaluation of microenvironmental properties of biochemical and biological systems has found widespread application during the last couple of years [6]. In most cases, this technique is the only way to study the molecular structure of binding sites and dynamics of carrier proteins, lipid layers and natural membranes. Although the various applications of ET(30) have been extended by specially designed derivatives in order to get rid of poor solubility [7] or the high pK_a value [8], these dyes, unfortunately, are not all fluorescent. However, fluorescent polarity probes are advantageous over non-fluorescent probes in many cases, especially in biochemical studies, when working with highly inhomogeneous samples such as cell suspensions, and when applied in combination with fibre optics with their particular geometry [9]. Therefore, we focused our interest on polarity indicators which, on the one hand, are highly sensitive to solvent polarity and, on the other, exhibit strong fluorescence. Here, we present the synthesis as well as fluorescence excitation and emission properties of three species of an interesting group of ketocyanine dyes with unique solvatochromic properties in both absorption and fluorescence.

EXPERIMENTAL

Ketocyanine dyes

The synthetic pathway and the chemical structures of dyes **3a–c** are shown in Scheme 1. Dyes **3a–c** have been synthesized according to a general procedure [10] with slight modification.

* Author to whom correspondence should be addressed.



Scheme 1. Synthetic pathway and chemical structures of dyes **3a–c**.

Compounds **2a–c** have been prepared by analogy to a published method [11]. All dyes are now commercially available.

1,5-*Diaza*-1,5-*diphenyl*-1,5-*dimethyl*-1H-*pentadienium perchlorate* (**2a**)

Perchloric acid (60%, 10 ml) is added to a solution of 1,1,3,3-tetramethoxypropane (5.5 g, 25 mmol) and *N*-methylaniline (5.35 g, 50 mmol) in ethanol (10 ml). The mixture is kept at 50°C for 30 min and stored overnight in a refrigerator to yield 7.2 g (82%) yellow needles, m.p. 164–165°C. $C_{17}H_{19}N_2O_4Cl$ calc.; C 58.21, H 5.46, N 7.99, Cl 10.11 (350.80). Found; C 58.10, H 5.44, N 7.91, Cl 10.27. i.r. (KBr); 1610, 1575, 1490, 1380 cm^{-1}. ^1H NMR (DMSO-d_6); $\delta = 8.60$ (d, 2 H), 7.3–7.7 (m, 10 H), 6.27 (t, 1 H), 3.70 (s, 6 H) ppm.

2b is prepared similarly from perchloric acid, indoline and 1,1,3,3-tetramethoxypropane by heating the mixture in ethanol to 70°C for 1.5 h, followed by addition of diethyl ether to yield 6.2 g (66%) of orange crystals. Upon heating, material decomposition starts at 230°C without a defined melting point. $C_{19}H_{19}N_2O_4Cl$ calc.; C 60.88, H 5.11, N 7.47, Cl 9.46 (374.82). Found; C 60.46, H 5.12, N 7.11, Cl 8.96. i.r. (KBr); 1610, 1570, 1495, 1470, 1420 cm^{-1}.

2c is prepared similarly from perchloric acid, *N*-phenylglycine sodium salt and 1,1,3,3-tetramethoxypropane by heating the mixture in ethanol to 60–70°C for 1 h. The solvent is removed *in vacuo* to obtain a resin which solidifies overnight. The product can be used directly in the next step.

1,9-*Di*-(N-*phenyl*-N-*methyl*)-4,6-*dimethylene-nona*-1,3,6,8-*tetraen*-5-*on* (**3a**)

To a previously prepared solution of sodium (3.7 g) in anhydrous methanol (80 ml) is added **2a** (10 g, 28.5 mmol) and cyclopentanone (1.2 g, 14 mmol). The mixture is refluxed for 2 h and the orange precipitate obtained is sucked off, washed with water, methanol and diethylether to yield 4.2 g (81%) of an orange powder, m.p. not defined, 230–255°C (dec.). $C_{25}H_{26}N_2O$ calc.; C 81.05, H 7.07, N 7.56 (370.49). Found; C 80.53, H 6.93, N 7.48. i.r. (KBr); 1610, 1570, 1490 cm^{-1}.

In the same way, **3b** is obtained from **2b** (2.0 g, 5.3 mmol), cyclopentanone (0.20 g, 2.4 mmol), methanol (10 ml) and sodium (1.10 g). Yield: 0.33 g (42%) of a red-brown powder, m.p. not defined, 230–285°C (dec.). $C_{27}H_{26}N_2O$ calc.; C 82.20, H 6.64, N 7.10 (394.52). Found; C 81.82, H 6.79, N 6.78. i.r. (KBr); 1620, 1580, 1485, 1400 cm^{-1}

In the same way, **3c** is obtained from **2c** (2.0 g), cyclopentanone (0.20 g), methanol (10 ml) and sodium (1.10 g). The crude product precipitates upon addition of diethylether and is collected, dissolved in 100 ml methanol and filtered. The solvent is removed *in vacuo* to yield 0.78 g (65%) of

a red-brown powder, m.p. not defined, decomposition starts at 235°C. $C_{27}H_{24}N_2O_5Na_2$ calc.; C 64.54, H 4.81, N 5.58 (502.48). Found; C 64.23, H 4.99, N 5.43. i.r. (KBr); 1590, 1490, 1400 cm^{-1}.

Instrumentation

Absorption spectra were run on a Perkin–Elmer Lambda 5 spectrophotometer. Uncorrected fluorescence excitation and emission spectra were obtained with an Aminco SPF 500 spectrofluorometer linked to a HP 9815 A desk computer. All spectroscopic measurements were performed at 22°C in 1 × 1 cm glass cuvettes. Solutions were not degassed since molecular oxygen does not significantly quench fluorescence.

Solvents

All solvents for polarity studies were of commercially available reagent grade quality and were stored over a molecular sieve 0.3 nm (Merck, Darmstadt, F.R.G.).

Results

The fluorescence excitation and emission spectra of dye **3b** recorded in toluene and water are shown in Fig. 1. Depending on the solvent, dyes **3a–c** are bright yellow to purple and exhibit a strong green, yellow, orange or red fluorescence. The effects of solvents are demonstrated by the fluorescence excitation and emission data given in Table 1. Excitation maxima are practically identical with the respective absorption peaks.

Both the excitation and emission maxima are red shifted by about 100 nm in going from toluene to water. Also, the fluorescence intensity and the Stokes' shift significantly increase in going from apolar to polar solvents. We also have recorded the absorption spectra and find them to be quite similar to the respective fluorescence excitation spectra. The molar absorptivities (at the respective absorption peaks in ethanol) are 95 000 l mol^{-1} cm^{-1} at 494 nm (**3a**), and 65 500 l mol^{-1} cm^{-1} at 524 nm (**3b**). The respective value of **3c** could not be determined because this compound could not be purified to a sufficient extent. For estimation of the relation between solvent polarity and spectral properties we have compared the wavenumbers of the emission maximum with the standard ET^N scale of solvents [1] (Table 1) which is based on the well known [3] polarity indicator ET(30). In this scale, tetramethylsilane is defined as the most apolar solvent ($ET^N = 0.000$), whereas water is the most polar one ($ET^N = 1.000$). Other solvents have

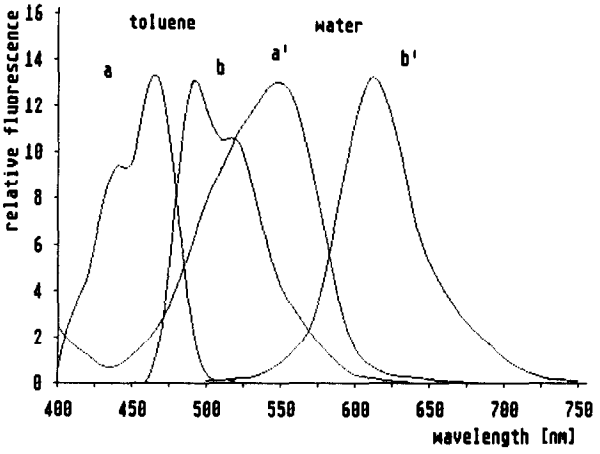


Fig. 1. Fluorescence excitation (a, a') and emission spectra (b, b') of **3a** in toluene and water. The respective peak wavelengths are at 452/491 nm in toluene and at 552/610 nm in water. Spectra are normalized to same height.

Table 1. Excitation and emission maxima (in nm) of probes **3a–c** in solvents of different polarity along with the standard ET^N values of solvent polarity (from Ref. [1]). The excitation maxima are practically the same as the respective absorption maxima

Solvent	ET^N	Emission (excitation) maximum (nm)		
		3a	**3b**	**3c**
Toluene	0.099	491 (452)	517 (496)	*
THF†	0.207	508 (468)	532 (493)	*
Acetone	0.355	527 (473)	557 (500)	522 (460)
DMF‡	0.404	538 (480)	565 (510)	540 (480)
Isopropanol	0.546	567 (498)	600 (530)	574 (505)
Ethanol	0.654	590 (505)	622 (555)	595 (515)
Methanol	0.762	598 (510)	635 (555)	605 (520)
Water	1.000	610 (552)	648 (570)	614 (550)

* Insoluble.
† Tetrahydrofurane.
‡ Dimethylformamide.

ET^N values located in between, depending on the respective absorption maximum of ET(30).

A correlation plot of the wavenumber of the emission maximum of **3a–b** vs the ET^N value of the respective solvent (data from Table 1) is shown in Fig. 2. The data for probe **3c** are not plotted in Fig. 2 but are quite similar to **3a** for polar solvents. This ionic derivative has been designed especially for aqueous systems. It is very slightly soluble in acetone and not at all in less polar solvents like tetrahydrofurane and hydrocarbons. On the other hand, the lipophilic derivatives **3a** and **3b** are hardly soluble in water but are very good in apolar systems, a fact that makes them useful for studies of lipid phase–water interfaces. All dyes are sufficiently stable in aqueous solution.

DISCUSSION

The ketocyanine dyes covered in this work are found to exhibit a large positive solvatochromism. The effect can be interpreted in terms of a merocyanine type structure of the ketocyanines. Merocyanines are known [1] to exhibit positive solvatochromism

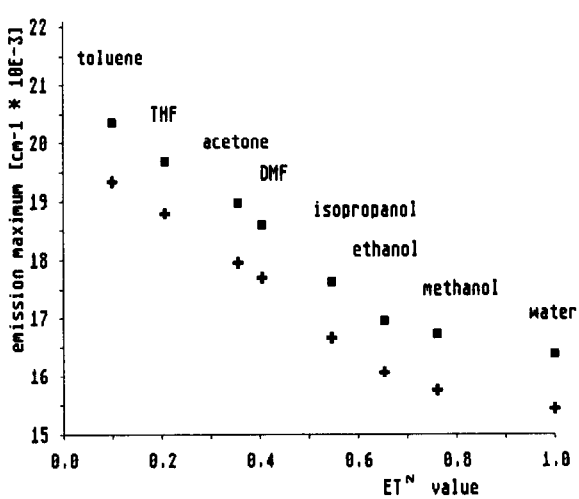


Fig. 2. Correlation of the standard ET^N values [1] of solvent polarity vs the wavenumber of the emission maximum of **3a** (■) and **3b** (+). The data of **3c** from acetone to water are almost identical to those of **3a**.

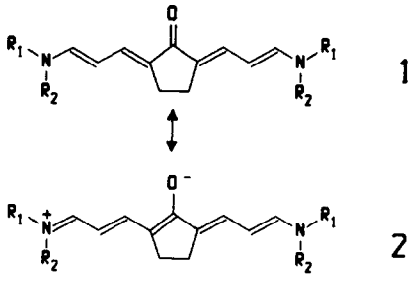


Fig. 3. Mesomer resonance structures of compounds **3a–c**. The shortwave non-ionic ketocyanine structure (1) is predominant in apolar solvents while the longwave zwitterionic polymethine cyanine structure (2) is favoured in polar solvents.

because of a transition from a polyene-like structure to a polymethine-like structure when the polarity of the solvent is increased. The lowest transition energy of these dyes is achieved when both the uncharged and the zwitterionic forms make equal contributions to the mesomer system [1]. In this particular case, the observed effects can also be interpreted in terms of a change of the mesomer system from two isolated polyene-like chromophores (which is predominant in apolar solvents) to a fully conjugated polymethine-like structure (which is stabilized in polar solvents), as shown in Fig. 3. We conclude that in this case the latter effect is mainly responsible for the positive solvatochromism because of the longwave absorption and fluorescence of **3a–c** that is due to a C_9 rather than a C_5 conjugated π-system.

For a correlation of the emission data with ET^N solvent polarity values, we have chosen the wavenumber of the respective emission maximum of the probes so to obtain a scale proportional to the energy of transition. The plot, shown in Fig. 2, shows that there is a wide range of linear correlation. Only in polar alcohols and water is the correlation curve nonlinear. This can be explained by the assumption that the mesomeric system of **3a–c** when dissolved in polar solvents is mainly described by the zwitterionic structure (Fig. 3) and that only small changes in the mesomer equilibrium of the system occur upon further increasing the solvent polarity. Also, hydrogen bonding effects of alcohols and water may be assumed that are different from those covered by the ET^N-scale.

Conclusion

The new polarity probes described here offer a number of interesting features. They are both highly sensitive to solvent polarity and strongly fluorescent. Their longwave absorption and fluorescence makes them advantageous over u.v.-excitable probes because of the low background fluorescence of most biological material in the longwave region as compared to the u.v. The high molar absorptivities together with strong fluorescence provide a further advantage, especially when applied in thin layers such as Langmuir–Blodgett films. Finally, the chemical and physical properties of this class of probes can be modified by substituents and the sensitivity to solvent polarity is unaffected by substituents attached to the nitrogen atoms. Reports on the use of such probes in fibre optic polarity sensors and in polarity studies on biochemical and biological systems will be published separately.

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