2,8-Bis[4-(diethylamino)phenyl]-3,7-dihydroxy-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione − A New Liquid-Phase-Sensitive Fluorescent Probe Utilising Intramolecular One- or Two-Proton Transfer Phenomena

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2,8-Bis[4-(diethylamino)phenyl]-3,7-dihydroxy-4*H*,6*H*-pyrano-[3,2-*g*]chromene-4,6-dione is a symmetric fluorescent dye that contains two cross-conjugated chromophores which are potential sites of tautomerisation in the excited state. This unique constitution of the compound is reflected in the steady-state absorption and emission spectra in which the positions and intensities of the bands depend markedly on the properties of the solvents, i.e. their polarity, as well as ability for electron donor–acceptor interactions or hydrogen-bond formation. The observed phenomena can be attributed to the transfer of one or two protons between hydroxy and oxo groups whose consequence is the occurrence of three excited states of different emission features. This unique behaviour opens up possibilities for the use of the compound as a spectral probe sensitive to the properties of liquid phases.

Introduction

It is well known that Excited-State Intramolecular Proton-Transfer (ESIPT) gives rise to two bands in the fluorescence spectra of 3-hydroxy-2-phenyl-4*H*-chromen-4-one (flavonol) (**1**) and its derivatives. Sengupta and Kasha were the first to identify and describe phototautomerism in these compounds.[1] The short-wavelength band in the flavonol spectra has been ascribed to the emission of a normal form (**1**, **N**), and the long-wavelength one to the emission of its tautomer (**1**, **T**). The position and intensity of the bands are both dependent on the structure[2] of the given flavonol and on the nature of the medium,[3] which augurs well for the use of these compounds as fluorescent probes. ESIPT in flavonols has been thoroughly investigated by a number of other research groups.[4,5] Moreover, ESIPT has been detected in 2-[4-(diethylamino)phenyl]-3-hydroxy-4*H*-chromen-4-one (the diethylamino derivative of flavonol) (**2**)[6] and similar compounds − these appear to be the most sensitive of the flavonols to changes in solvent polarity and proton donating ability.[7−9] This feature has been utilised in research into the constitution of mixed liquid phases,[10] the nature of micelles,[11,12] and the properties of liposomes[13,14] and proteins.[15] A moiety of **2** also occurs in fluorescent cationic chelators, the spectra of which are very sensitive to the radius of the bonded cation.[16,17]

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Judging by the structures of 3,7-dihydroxy-2,8-diphenyl-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione (diflavonol) (**3**) and its derivatives, these compounds could be promising fluorescent probes, containing as they do two cross-conjugated chromophores. Diflavonols contain two oxo groups which on excitation accumulate a negative charge; this makes these compounds more responsive than flavonols to the properties of the medium. Two chromophores should roughly double the molar extinction coefficient. Enlargement of the condensed system in diflavonols should also bring about an increase in fluorescence quantum yields. Finally, the possible transfer of two protons should enhance the ability of the medium to influence the absorption and fluorescence spectra of these compounds.

The above premises have prompted us to synthesise diflavonols and investigate their spectral features. In this work attention is focused on 2,8-bis[4-(diethylamino)phenyl]-3,7-dihydroxy-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione (the diethylamino derivative of diflavonol) (**4**) − a compound that appears very promising with regard to applications.

Results and Discussion

Absorption Spectra and Structure of 4 in the Ground State

In the absorption spectra of **4** two bands always appear between 300 and 500 nm (Figure 1). At elevated solvent polarities, the long-wavelength band shifts towards the red, i.e. it exhibits a positive solvatochromic effect (Table 1), which indicates that the relevant excited state of the compound is more polar than the ground state. The absorption bands in the spectrum of **4** dissolved in hexane are relatively

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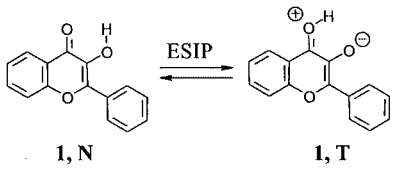

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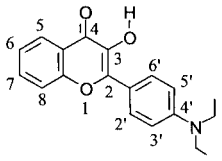

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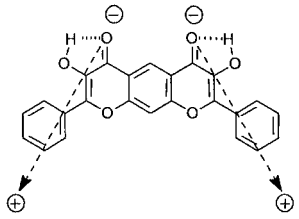

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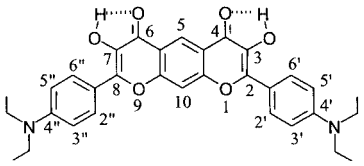

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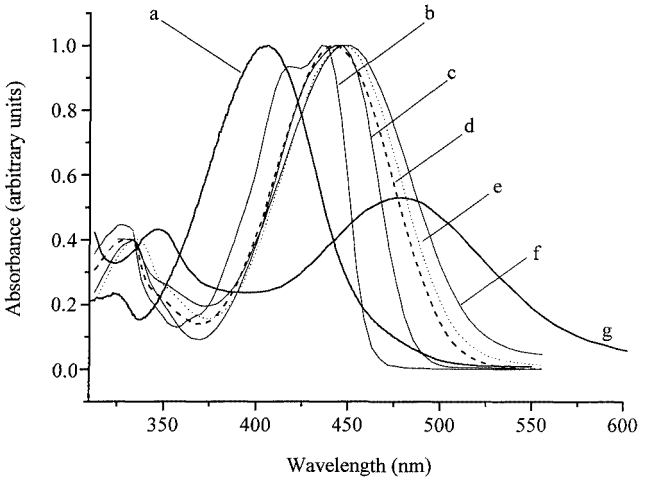


Figure 1. Normalised absorption spectra of a $5 \cdot 10^{-6}$ M solution of **2** in methanol (a) and a $2 \cdot 10^{-6}$ M solution of **4** in hexane (b), toluene (c), acetonitrile (d), dimethyl sulfoxide (e), methanol (f), and a 0.1 M solution of NaOH in methanol (g)

narrow and the long-wavelength one is split at 1500 cm^{-1}. As the solvent polarity increases, these bands become broader. Broadening is most pronounced if the compound is dissolved in methanol or DMSO; this could be due to the formation of intermolecular hydrogen bonds with the solvent. However, hydroxy groups are not deprotonated in these cases, since spectrophotometric titration with alkali has indicated that the absorption spectra of anionic forms of **4** extend beyond 500 nm (Figure 1). The long-wavelength bands in the absorption spectra of **2** and **4** appear in roughly the same spectral region, which means that the energy gaps between the excited and ground electronic levels are similar in both compounds. The features of the electronic absorption spectra indicate that no tautomerisation of **4** takes place in the ground state, since the maxima relevant to tautomers − the more highly conjugated systems − would otherwise be situated at much longer wavelengths.

Further information on the structure of **4** in the ground state is provided by NMR spectroscopy. Analysis of NMR spectra shows that **2** and **4** have oxo groups in the *para* position relative to the oxygen heteroatom of the pyrone ring. The signals of the hydrogen atom in position 5 appear at low field (δ = 8.25 ppm in **2** and δ = 9.15 ppm in **4**), these spectra show that the strong anisotropic deshielding is caused by the two adjacent oxo groups. There is only one signal located at δ = 171.9 ppm in the ^{13}C NMR spectrum of **4**, due to the equivalent C=O groups. The closeness of the ^{1}H signals of the equivalent 2′-H and 6′-H atoms in **2** and **4** (δ = 8.17 and 8.19 ppm, respectively) demonstrates that the electron density distribution in the relevant parts of both molecules is identical. The smaller number of signals in the ^{1}H and ^{13}C NMR spectra of **4** provides further confirmation of the symmetrical structure of the molecule and the absence of tautomerisation in the ground state.

Fluorescence Excitation and Fluorescence Spectra − Influence of the Solvents

Only in nonpolar solvents (pentane, hexane) do the shape and position of bands in the fluorescence excitation spectra of **4** (Figure 2) coincide with those in the absorption spectra (Figure 1). In other solvents, bands coincide with respect to the position, but differ with respect to shape and intensity. The differences become more pronounced when solvent polarity increases. On the other hand, as regards to data collection, the shape and position of bands are independent of the selected wavelength in all the solvents.

The fluorescence spectra of **4** exhibit more than one band in most of the solvents (Table 1 and Figure 3). The bands are completely separated in the spectra recorded in nonpolar aprotic solvents. The position and ratio of band intensity do not depend on the compound concentration in the $10^{-8}-10^{-5}$ M range. This suggests that several entities (tautomers), formed as a result of ESIPT in **4**, exist in the excited state and emit radiation.

Two bands are always observed in the fluorescence spectra of **4** recorded in aprotic solvents. These bands exhibit positive solvatofluorochromy, i.e. a shift towards the red at elevated solvent polarities (Table 1, Figure 3). Plots of wavenumbers corresponding to emission maxima versus the solvent polarity parameter $E_T(30)$[18] are straight lines with

Table 1. Absorption and emission characteristics of **4** ($3\cdot10^{-6}$ M solutions)

Solvent	$E_T(30)$[a] [kcal/mol]	Position of maxima in spectra [nm]		Relative integral fluorescence intensity (arbitrary units)[b][c]	Integral fluorescence intensity (I)[c] ratio (I_{TT}/I_{NN})	Stokes shift[d] [cm^{-1}]		Band half-width [cm^{-1}]	
		Absorption[e]	Emission			**NN** form	**TT** form	**NN** form	**TT** form
Pentane	30.9	425	456, 611	0.7	100	1500	7100		790
Hexane	30.9	427 (4.7)	455, 613	0.7	56	1500	7100	1900	820
CCl_4	32.5	442	475, 618	0.7	34	1600	6500	2010	940
Toluene	33.9	441	502, 631	0.7	7.0	2800	6800	2250	1050
Benzene	34.5	442	495, 624	0.8	6.6	2400	6600	2260	1040
Diethyl ether	34.6		510, 634	0.9	4			2680	1200
1,4-Dioxane	36		505, 635	0.9	7			2630	1150
THF	37.4		548, 647	0.8	0.33			3220	1210
Bromobenzene	37.5		533, 644	0.8	0.76			2680	1200
Ethyl acetate	38.1	435 (4.77)	542, 638	0.8	0.40	4550	7300	3020	1270
$CHCl_3$	39.1	447	522, 630	1	0.27	3200	6500	2450	1360
CH_2Cl_2	41.1	449 (4.73)	550, 635	1[f]	0.07	4000	6500	2590	
Acetone	42.2		580	0.3	ca. 0			3380	
$HCOOCH_3$	45		580	0.3	ca. 0			3340	
Acetonitrile	46	439 (4.73)	596	0.1	ca. 0	5800		3320	
1-Butanol	50.2		570	0.03	ca. 0			3480	
1-Propanol	50.7	449 (4.73)	568	0.018	ca. 0	4700		3420	
Methanol	55.5	450 (4.65)	564	0.003	ca. 0	4500		4260	

[a] Taken from ref.[18] [b] Relative integral fluorescence intensities represent values related to the fluorescence intensity in CH_2Cl_2 (equal to 1). [c] Excitation wavelength corresponds to the long-wavelength maxima in the absorption spectra; integral fluorescence intensity is given by the total area of the fluorescence band (or the area ascribed to the **NN** or **TT** forms). [d] Represents the difference in position of maxima of long-wavelength absorption and relevant fluorescence bands. [e] log ε is given in parentheses. [f] The fluorescence quantum yield determined using quinine sulfate in 1 M H_2SO_4[19] as standard was 0.64.

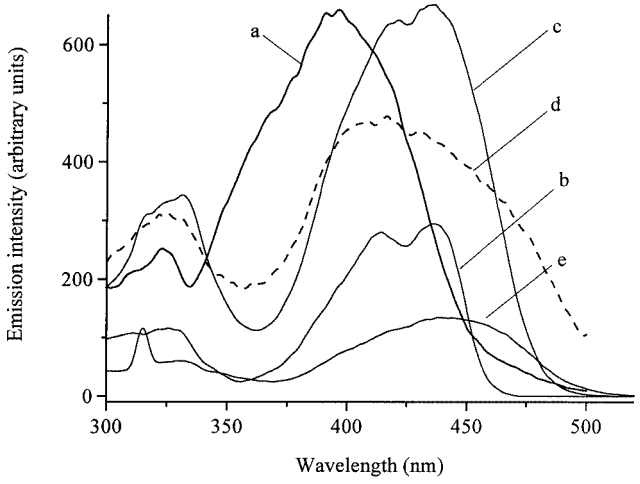


Figure 2. Fluorescence excitation spectra of a $5\cdot10^{-6}$ M solution of **2** in acetonitrile (a) and a $2\cdot10^{-6}$ M solution of **4** in pentane (b), 1,4-dioxane (c), 1-butanol (d), and 1,2-diacetoxyethane (e); in all cases emission was measured at the wavelength corresponding to the fluorescence spectrum maximum; the respective intensities of spectra in pentane and 1-butanol have been multiplied by 0.25 and 20

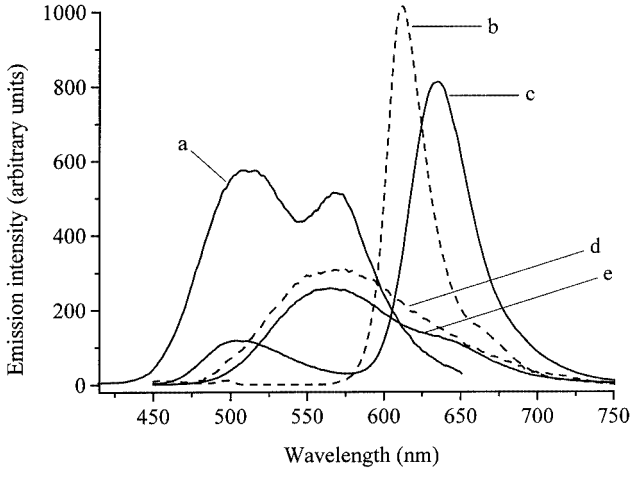


Figure 3. Fluorescence spectra of a $5\cdot10^{-6}$ M solution of **2** in acetonitrile (a) and a $2\cdot10^{-6}$ M solution of **4** in pentane (b), 1,4-dioxane (c), 1-butanol (d), and 1,2-diacetoxyethane (e); excitation wavelengths: 405 nm for **2**, and 430 nm for **4**; the intensity of the spectrum in 1-butanol has been multiplied by a factor of 20

slopes that are steeper for **4** than for **2** in the case of the short-wavelength band (Figure 4). This implies that the excited state of **4** is more polar than **2**. The emission bands of **4** in CH_2Cl_2 appear closer together, and in more polar solvents such as acetone and acetonitrile, the emission curve has one maximum only. Furthermore, the relationship of ν_{max} versus $E_T(30)$ for the diflavonol short-wavelength band exhibits two different slopes for solvents of high and low polarity. The slope for high-polarity solvents is the same as in the case of **2**; we think that this may be due to the overlapping emission of two different forms of diflavonol.

The short-wavelength emission bands of **4** increase in width 1.5−2 times in aprotic solvents of moderate and high polarity, i.e. 1,4-dioxane, THF, CH_2Cl_2, ethyl acetate, methyl formate, acetone and acetonitrile (Table 1). Most of these solvents are able to form intermolecular hydrogen bonds with the hydroxy groups of **4**. However, such consid-

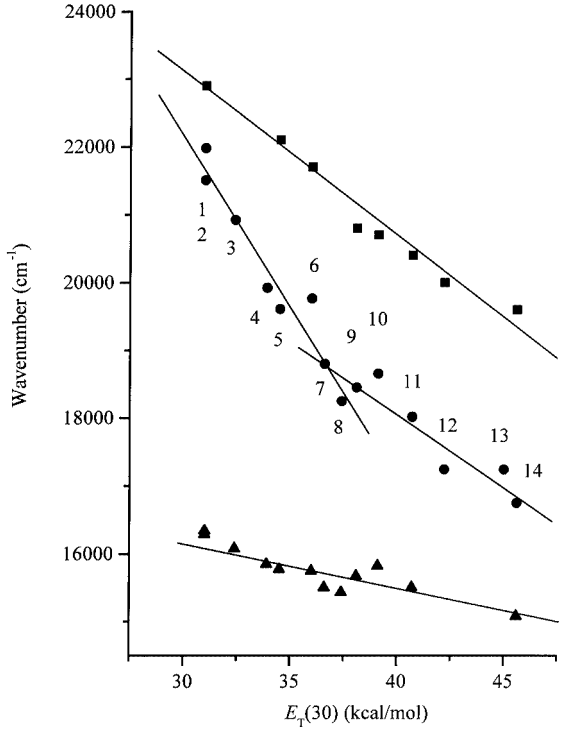


Figure 4. The position of the short-wavelength maximum in the fluorescence spectra of **2** (squares) and **4** (circles) as a function of the solvent polarity parameter, $E_T(30)$; the triangles indicate the same function for the long-wavelength maximum of **4**; solvents used: *n*-pentane (1), *n*-hexane (2), CCl_4 (3), toluene (4), diethyl ether (5), 1,4-dioxane (6), bromobenzene (7), THF (8), ethyl acetate (9), $CHCl_3$ (10), CH_2Cl_2 (11), acetone (12), methyl formate (13), and acetonitrile (14)

erable broadening of the fluorescence bands cannot be attributed solely to this effect. Both fluorescence and absorption bands of **4** in nonpolar solvents are shifted towards the red by 2000−3000 cm^{-1} relative to those of **2**; this effect testifies to the similar nature of the emitting forms of flavonol and diflavonol.

The fluorescence intensities of **4** are relatively high in solvents of low and moderate polarity, and decrease abruptly, by 1−2 orders of magnitude, in polar aprotic solvents (Table 1). In our opinion, the formation of intermolecular hydrogen bonds is one of the causes of this sudden reduction in emission ability in such solvents.

The fluorescence intensity of **4** in protic solvents is extremely low (Table 1), and special precautions have to be taken if the emission spectra are to be reliable. In all the solvents tested − methanol, 1-propanol, and 1-butanol − the fluorescence spectra are broad and exhibit a single maximum. In protic solvents, then, it is probably the hydrogen-bonded complexes of **4** with alcohol molecules bonded to oxo and/or hydroxy groups that fluoresce. The fluorescence of the deprotonated compound (anionic forms) should be disregarded, since titration with alkali causes emission to cease altogether.

It is interesting that the long-wavelength fluorescence of **4**, characteristic of aprotic solvents, becomes attenuated in alcohols, where only one broad emission band now appears (Table 1). In the case of **2**, both emission bands are always present, although their relative intensity varies. Presumably, then, emission in protic solvents takes place mainly from one excited state of **4**.

Photodestruction

Flavonols are known to undergo photochemical destruction.[20] We subjected emission decay and the formation of photo-destruction products to continuous scrutiny during the daily illumination of **4**, dissolved in pentane under ambient conditions (Figure 5). The fluorescence excitation and emission spectra of the product are very similar to those of **2**, which may suggest that photochemical reactions destroy one of the pyrone rings in **4**. This behaviour needs to be taken into account if the compound is to be used as a fluorescent probe.

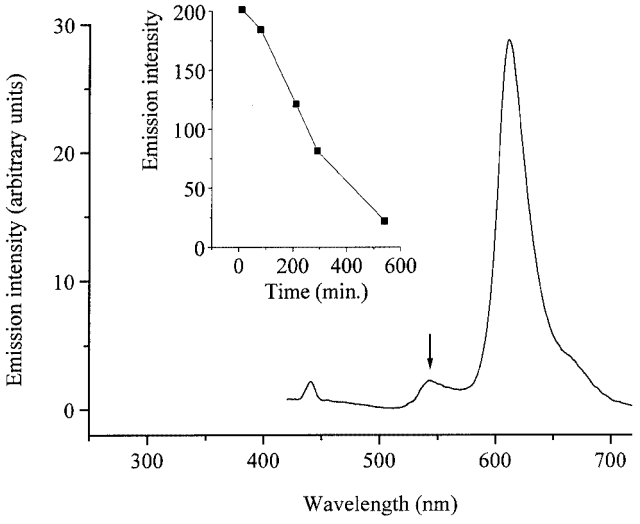


Figure 5. Fluorescence spectrum of a 10^{-7} M solution of **4** in pentane after 9 h of daily illumination; excitation wavelength 390 nm; photodestruction leads to a gradual decrease in emission intensity (graph at top left); the new product formed in this process has the same emission spectrum as **2** (indicated by the arrow)

Interpretation of Absorption and Fluorescence Spectra

The perfect symmetry of **4** facilitates interaction between the π-electron systems of the two halves of the molecule; the effect can be considered in terms of cross-conjugation. The compound can thus be regarded as a system of two mutually interacting chromophores.[21] One possible explanation for the absorption and emission features of this molecule is provided by the exciton model, developed for molecular aggregates by McRae and Kasha.[22,23] This model has been successfully used to describe the spectral properties of bichromophoric cyanine dyes.[24,25] In order to use this approach, a molecule with a single chromophore, **2**, may be regarded as a dye whose spectral features constitute a basis for examining the properties of bichromophoric **4**. According to the McRae−Kasha model, the interaction of the two chromophores in **4** causes the excited (S_1) state to split into two levels of higher, S_1^h, and lower, S_1^l, energy

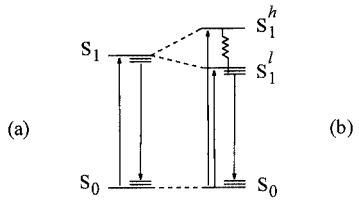
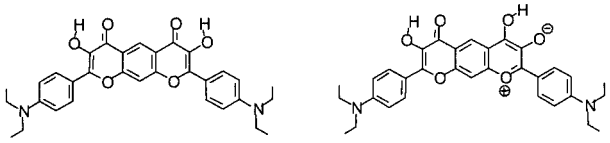


Figure 6. Scheme of energy levels of molecules with one (a) and two (b) chromophores

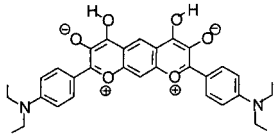
(Figure 6). The two long-wavelength bands in the absorption spectrum of **4** can thus be ascribed to $S_0 \rightarrow S_1{}^l$ and $S_0 \rightarrow S_1{}^h$ transitions (where S_0 denotes the ground state). The band intensities give an indication of how the transition moments of the chromophores are oriented.[21] The $S_0 \rightarrow S_1$ transition moment of **2** lies in the plane of the chromophore and is oriented from the pyrone ring to the lateral phenyl ring.[25] The angle between the vectors of the transition moments corresponding to the $S_0 \rightarrow S_1{}^l$ and $S_0 \rightarrow S_1{}^h$ transitions in **4** (450 and 330 nm, respectively), calculated from the band intensities,[21] is 120°, which suggests that electron density is being transferred from the nitrogen atoms in the lateral rings to the π-system of the central phenyl ring. The interaction of the chromophores causes the long-wavelength maximum in the absorption spectrum of **4** to shift by approximately 2000 cm^{-1} towards the red relative to the maximum in the spectrum of **2**.

The possibility that **4** exists in the $S_1{}^l$ and $S_1{}^h$ states may also provide an explanation for the presence of two bands in the fluorescence spectra. However, in these spectra there may sometimes be one band and sometimes more than two. It seems, therefore, that in order to achieve a comprehensive explanation of emission phenomena, one should assume the presence of three emitting states of **4**, which can be related to the electronically excited tautomers **NN**, **NT**, and **TT**.



NN

NT



TT

Tautomers **NN** and **TT** are symmetrical and have two identical chromophores. As the structure of these chromophores in **2** and **4** is identical, their solvation and Stokes shifts should be similar. This is the case with nonpolar aprotic solvents, where the emission bands of **4** exhibit the same Stokes shift as the **N** and **T** bands of **2**, i.e. about 2100 cm^{-1} towards the red. In view of this, the emission bands in the spectra of **4** in nonpolar aprotic solvents can be assigned to the **NN** (short-wavelength) and **TT** (long-wavelength) tautomers (Table 1). Since the chromophores of the **NT** form of **4** are different, the excited-state energy of this entity should be lower than the energy of **NN** but higher than that of **TT**. Therefore, under comparable conditions the emission maximum of **NT** should lie between the maxima of **NN** and **TT**, and close to the position of the maximum of the **T** form (long-wavelength) of **2** (Figure 3). In practice, this is the case in aprotic polar solvents, such as acetone, methyl formate or acetonitrile, in which emission is due mainly to the **NT** form and exhibits a Stokes shift of about 2000 cm^{-1} lower than in **2**. Moreover, the fluorescence spectra of **4** in aprotic polar solvents are broad with a complex pattern (e.g. in Figure 7), which suggests that the emission of **NT** overlaps that of **NN** and **TT** on the short- and long-wavelength shoulders, respectively. Indeed, deconvolution of a selected spectrum (Figure 7) shows that emission exhibits maxima at 605 nm (main), as well as at 546 and 662 nm (weaker). Fluorescence spectra in other polar aprotic solvents display a similar pattern, which reinforces the above assumption. The long-wavelength maxima in solvents less polar than acetonitrile exhibit positive solvatofluorochromy and are shifted to the 630−640 nm region. This lends further support to the idea that maxima of about 600 nm are due to the **NT** form. In ethyl acetate, THF, 1,4-dioxane or diethyl ether, emission from **NT** most probably occurs as well, this being manifested by the greater width of the short-wavelength band (Table 1).

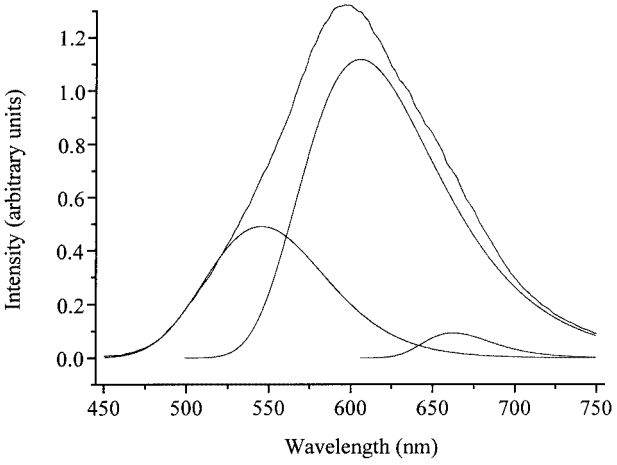


Figure 7. Fluorescence spectrum of a $2 \cdot 10^{-6}$ M solution of **4** in acetonitrile, and the result of the mathematical deconvolution of the spectrum into three components with λ_{max} = 546, 605, and 662 nm

Further evidence suggesting the existence of three emitting states in **4** is the dependence of the fluorescence maxima on the polarity parameter (Figure 4). This dependence is linear in the case of the short-wavelength band of **2** and the long-wavelength band of **4**, but is a combination of two straight lines with respect to the short-wavelength bands of

4. As far as this latter dependence is concerned, one line characterises nonpolar solvents from pentane to bromobenzene, the other polar solvents from bromobenzene to acetonitrile. The line for long-wavelength maxima corresponds to the **TT** form of **4**. The line with the steepest slope − characteristic of the least polar aprotic solvents − corresponds to the **NN** form, while the line for most polar solvents corresponds to the **NT** form of **4**. This last line has the same slope as that for **2**, which may suggest that the **N** form of **2** and the **NT** form of **4** have similar dipole moments. The first line, assigned to the most polar **NN** form, has the steepest slope. A similar assignment can be made with respect to the very weak emission of **4** in protic solvents, where it is the **NN** and **NT** forms that mainly emit radiation.

In summary, compound **4**, representing a new class of fluorescent dyes, has been synthesised, and the presence of two proton-transfer systems imparts unique fluorescent properties to the molecule. The fluorescence spectra of the compound exhibit two well-separated bands, the position and intensity of which are strongly dependent on the nature of the surroundings. It was found that emission spectra could be interpreted under the assumption that there exist three excited states in the molecule. The marked influence of the medium on the emitting features makes diflavonols convenient probes sensitive to selected properties of liquid phases.

Experimental Section

General: The following instrumentation was used: Melting points (uncorrected): PHMK apparatus ("VEB Analytik", Dresden). Elemental analyses: Eager 200 (Carlo Erba). ^{1}H and ^{13}C NMR spectra: Varian Mercury 400 MHz spectrometer (internal standard: tetramethylsilane). Electronic absorption spectra: Specord M40 spectrophotometer (Carl Zeiss, Jena). Fluorescence measurements: Perkin−Elmer LS-50 spectrofluorometer. The solvents used for the absorption and fluorescence measurements were of spectral grade (Merck) and used as supplied, with the exception of CH_2Cl_2 (reagent quality), which was purified by distillation from P_2O_5.[26,27] Deconvolution of the fluorescence spectra bands was carried out with the computer program developed by Doroshenko,[28] which uses the iterational nonlinear least-squares method based on the Fletcher−Pauell algorithm. The shapes of the individual emission bands were approximated by a log-normal function which, in contrast to the commonly applied Gauss and Lorentz functions, accounts for the asymmetry of the spectral bands. Materials: **2** and **4** were synthesised in accordance with the general procedure described by Smith et al.[29] The purity of both dyes was confirmed by thin-layer chromatography (50 × 150 mm Silufol UV-254 plates with 98:2 to 85:15% v/v chloroform/methanol eluent) and fluorescence spectra analysis (synchronous scans).

2-[4-(Diethylamino)phenyl]-3-hydroxy-4*H*-chromen-4-one (2): The compound was prepared with a yield of 37%; m.p. 151−152 °C. $C_{19}H_{19}NO_3$ (309.37): calcd. C 73.77, H 6.19, N 4.53; found C 73.70, H 6.20, N 4.60. ^{1}H NMR ($CDCl_3$): δ = 1.24 (t, *J* = 7.0 Hz, 6 H, CH_3), 3.47 (q, *J* = 7.0 Hz, 4 H, CH_2), 6.80 (d, *J* = 9.1 Hz, 2 H, 3′-H and 5′-H), 6.80 (br. s, 1 H, OH), 7.38 (t, *J* = 7.9 Hz, 1 H, 6-H), 7.55 (d, *J* = 7.9 Hz, 1 H, 8-H), 7.63 (t, *J* = 7.9 Hz, 1 H, 7-H), 8.17 (d, *J* = 9.1 Hz, 2 H, 2′-H and 6′-H), 8.25 (d, *J* = 7.9 Hz, 1 H, 5-H).

2,8-Bis[4-(diethylamino)phenyl]-3,7-dihydroxy-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione (4): The compound was prepared in two steps. In the first step, the (2*E*)-3-[4-(diethylamino)phenyl]-1-[5-{(2*E*)-3-[4-(diethylamino)phenyl]prop-2-enoyl}-2,4-dihydroxyphenyl]prop-2-en-1-one was synthesised in the following manner. 1-(5-Acetyl-2,4-dihydroxyphenyl)ethanone (Aldrich) (0.8 g, 4.1 mmol) and 4-(diethylamino)benzaldehyde (0.85 g, 4.8 mmol) were dissolved in 20 mL of toluene. Morpholine (0.9 g, 10 mmol) was added, and the mixture was refluxed for 24 h. Then, ca. 15 mL of the solvent was evaporated, and the residue diluted with 20 mL of hexane. The red precipitate was filtered and recrystallised from a 1:4 chloroform/ethyl alcohol mixture to yield 1.28 g (61%) of the product, m.p. 249−251 °C. $C_{32}H_{36}N_2O_4$ (512.65): calcd. C 74.98, H 7.08, N 5.46; found C 75.00, H 7.10, N 5.60. ^{1}H NMR ([D$_6$]DMSO): δ = 1.17 (t, *J* = 7.0 Hz, 12 H, CH_3), 3.43 (q, *J* = 7.0 Hz, 8 H, CH_2), 6.39 (s, 1 H, 2-H), 6.80 (d, *J* = 8.0 Hz, 4 H, Ar), 7.81 (d, *J* = 8.0 Hz, 4 H, Ar), 7.82 to 7.90 (m, 4 H, propenoyl), 8.88 (s, 1 H, 5-H), 14.2 (s, 2 H, OH). (2*E*)-3-[4-(Diethylamino)phenyl]-1-(5-{(2*E*)-3-[4-(diethylamino)phenyl]prop-2-enoyl}-2,4-dihydroxyphenyl)prop-2-en-1-one (1.03 g, 2 mmol) was dissolved in 100 mL of 50% ethyl alcohol, sodium hydroxide (8 g, 0.2 mol) was added, and the mixture heated to boiling. After cooling to room temperature, 30% hydrogen peroxide (1.6 mL) was added and the mixture stirred for 24 h. The resulting red precipitate was filtered, washed successively with 10% acetic acid, water and ethyl alcohol, and recrystallised from chloroform to yield 65 mg (6%) of final product, m.p. 302−305 °C. $C_{32}H_{32}N_2O_6$ (540.62): calcd. C 71.10, H 5.97, N 5.18; found C 71.00, H 6.00, N 5.30. ^{1}H NMR ($CDCl_3$): δ = 1.25 (t, *J* = 7.0 Hz, 12 H, CH_3), 3.48 (q, *J* = 7.0 Hz, 8 H, CH_2), 6.80 (d, *J* = 9.1 Hz, 4 H, 3′-H, 5′-H, 3′′-H and 5′′-H), 6.85 (br. s, 2 H, OH), 7.66 (s, 1 H, 10-H), 8.19 (d, *J* = 9.1 Hz, 4 H, 2′-H, 6′-H, 2′′-H and 6′′-H), 9.15 (s, 1 H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.0 (CH_3), 44.8 (CH_2), 105.6 (C-10), 111.2 (C-3′, C-5′, C-3′′ and C-5′′), 116.9, 118.6, 125.0, 129.7 (C-2′, C-6′, C-2′′ and C-6′′), 136.3 (C-5), 147.4, 149.3, 156.7, 171.9 (CO).

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