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Elimination of the Hydrogen Bonding Effect on the Solvatochromism of 3-Hydroxyflavones

Andrey S. Klymchenko,*[†,‡] Vasyl G. Pivovarenko,[‡] and Alexander P. Demchenko[†,§]

TUBITAK Research Institute for Genetic Engineering and Biotechnology, 41470 Gebze-Kocaeli, Turkey,
Department of Chemistry, Kyiv National Taras Shevchenko University, 01033 Kyiv, Ukraine, and
A. V. Palladin Institute of Biochemistry, 9 Leontovicha str., 01030 Kyiv, Ukraine

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We address the important, frequently discussed, and still unresolved question of the role of solute−solvent hydrogen bonding in modulating the excited-state intramolecular proton transfer (ESIPT) reaction in 3-hydroxyflavone (3HF) derivatives, which provides a dramatic variation of the relative intensities of normal (N*) and phototautomer (T*) emissive species. We synthesized a new 3HF derivative, 5,6-benzo-4′-diethylamino-3-hydroxyflavone (BFE), in which the additional benzene ring protects the 4-carbonyl from H-bonding with the protic solvents but allows the intramolecular bond with the 3-hydroxyl group, which is the pathway of ESIPT, to be maintained. The absorption and fluorescence properties of BFE and its parent analogue 4′-diethylamino-3-hydroxyflavone (FE) were studied in a set of 20 representative solvents. In aprotic media, these dyes in absorption and fluorescence spectra show similar solvatochromism, whereas in protic solvents dramatic differences are observed, which demonstrate that for BFE the effects of intermolecular H-bonding are eliminated. The elimination of the specific interaction of BFE with protic media in the ground state is shown by thin-layer chromatography. The most dramatic differences in spectroscopic properties between BFE and FE are observed for the intensity ratio of the two emission bands, I_{N*}/I_{T*}. Whereas for FE a linear correlation of $\log(I_{N*}/I_{T*})$ with the solvent polarity function $f(\epsilon)$ exists only in aprotic media and in protic solvents strong systematic deviations are observed, for BFE this linear correlation extends to all of the studied solvents. Therefore, we suggest that the protic solvents modulate ESIPT in 3-hydroxyflavone derivatives exclusively by proton-donor intermolecular H-bonding with 4-carbonyl, which occurs with the preservation of the intramolecular H-bond. Our results show how molecular design allows the elimination of the strong intermolecular H-bonding perturbation of the solvent polarity-dependent ESIPT reaction, which provides the means for constructing fluorescent probes with strong selectivity to universal solvent effects.

Introduction

Excited-state intramolecular proton transfer (ESIPT) reactions in organic fluorophores are among the fastest events known in chemistry.[1] They occur on the scale of 10^{-13} to 10^{-14} s between intramolecularly H-bonded donor and acceptor groups and result in the formation of tautomer species that possess dramatically Stokes-shifted fluorescence emission spectra.[2,3] Such extremely fast rates are particularly characteristic for ESIPT in 3-hydroxyflavone (3HF),[4,5] which is a classical example of this type of reaction.[6] Meanwhile, the steady-state fluorescence spectra of 3HF and its derivatives in solution allow for the common observation of the short-wavelength band belonging to the initially excited normal band (N*) in addition to the long-wavelength band of the tautomer (T*) form. The relative band intensities of these two forms may vary within the narrow range of solvent conditions in extremely broad ranges, up to the complete disappearance of one of the forms.[7−13] This makes 3HF derivatives very attractive as prototypes of molecular sensors and molecular electronic devices. They have already been applied as sensors of polarity[12,13] and electric fields[14] and as probes for structural variations in micelles[15,16] and biomembrane models.[17−19]

In view of the high ESIPT rate, which for 3HF and its derivatives is reported to be significantly higher than the emission rates,[4−7] the origin of the N* band in fluorescence spectra and the basic mechanism of two-band switching behavior are not clearly understood. This creates problems in the interpretation of spectroscopic data and in the design of new sensors. One of the factors that can hamper the ESIPT reaction is the hydrogen bonding with solvent molecules.[20−25] It is known that the intramolecular H-bond in 3HF is not very stable because it closes the five-membered ring between the 3-hydroxyl and 4-carbonyl groups.[25] Both of these groups can participate in hydrogen bonding with the solvent, and the intermolecular H-bonds can, in principle, compete with intramolecular bonds. This perturbation can be thought to result in the appearance of the N* band in emission.[20−25] However, 3HF derivatives with π-electron donor substituents at the 4′ position exhibit strong dependencies of the intensity ratios of the N* and T* forms, I_{N*}/I_{T*}, on solvent polarity that are unrelated to the H-bond perturbation effect.[7−9] Our present study[13] shows that for FE in aprotic solvents $\log(I_{N*}/I_{T*})$ exhibits a good linear correlation with the solvent polarity function[26] $f(\epsilon) = (\epsilon - 1)/(2\epsilon + 1)$. These results are consistent with the notion that the excited-state equilibrium N* ↔ T* is established on a very fast scale and that the polar solvent can provide dielectric stabilization of a more-polar N* form, shifting the equilibrium toward the

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* Corresponding author. Current address: Faculté de Pharmacie, Université Louis Pasteur UMR 7034 du CNRS, BP 24, 67401 Illkirch, France. Fax: +33390244313. E-mail: aklymchenko@aspirine.u-strasbg.fr.

[†] TUBITAK Research Institute for Genetic Engineering and Biotechnology.

[‡] Kyiv National Taras Shevchenko University.

[§] A. V. Palladin Institute of Biochemistry.

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latter.[8,13,27] Thus, both the solvent-specific intermolecular H-bond perturbations and the general solvent effects may contribute to 3HF's emission, which complicates the data analysis.

The question arises of whether the H-bonding perturbation can be completely abolished by targeted chemical modification of 3HF molecule. Because the geometrical requirements for the formation of a H-bond with the 4-carbonyl in 3HF are rather stringent, its steric screening from the H-bonding with the solvent could be provided by the attachment of an additional benzene ring proximal to this group. In this report, we demonstrate that the new compound, 5,6-benzo-4′-diethylamino-3-hydroxyflavone (BFE), retaining the major spectroscopic properties of its parent analogue 4′-diethylamino-3-hydroxyflavone (FE) exhibits the property of the complete elimination of H-bonding effects. This allows us to observe a very good conformity of the I_{N*}/I_{T*} ratio to the solvent dielectric constant function that describes universal solute−solvent interactions.

Experimental Section

All of the solvents were of spectroscopic grade. Ethyl acetate, tetrahydrofuran, acetonitrile, and dimethylformamide were appropriately dried. 4′-Diethylamino-3-hydroxyflavone (FE) was synthesized and purified as described elsewhere.[7] 5,6-Benzo-4′-diethylamino-3-hydroxyflavone (BFE) was synthesized using a recently developed modification of the general two-step procedure.[28] 1-(2-Hydroxy-1-naphthyl)-1-ethanone and 4-diethylaminobenzaldehyde (0.7 g of each) were dissolved in 5 mL of dimethylformamide, and then 0.5 g of sodium methoxide was added. After 3 h, the resulting mixture was diluted with 40 mL of ethanol followed by 4.1 g of sodium methoxide and 5.8 mL of 35% hydrogen peroxide. Refluxing for 30 s afforded the product BFE, which was precipitated after the addition of water and neutralization. It was filtered, washed with water, and recrystallized from *n*-butanol after drying. Yield 0.54 g (40% including two steps). Orange needles, mp 211 °C; ^{1}H NMR (200 MHz, CDCl$_3$): δ 1.23 (6H, t, *J* 7.0), 3.45 (4H, q, *J* 7.0), 6.80 (2H, d, *J* 9.1), 7.51 (1H, s), 7.61 (1H, d, *J* 9.2), 7.62 (1H, dd *J* 6.9, 8.0), 7.77 (1H, dd, *J* 6.9, 8.0), 7.91 (1H, d, *J* 8.0), 8.05 (1H, d, *J* 9.2), 8.19 (2H, d, *J* 9.1), 10.04 (1H, d, 8.0); MS (EI) *m/z:* 359.1 (M$^+$).

Absorption spectra were recorded on a Cary 3 Bio spectrophotometer (Varian). Fluorescence spectra were recorded on a Quanta Master spectrofluorometer (Photon Technology International). The solutions of dyes for absorption and fluorescence spectroscopy were used in concentrations corresponding to an absorbance close to 0.1. Quantum yields φ were determined with respect to a solution of FE in ethanol as the reference ($\varphi = 0.52$).[7] The excitation wavelength for the fluorescence studies was 410 nm. All of the spectral measurements were performed at room temperature. In the cases when two bands overlapped, the fluorescence spectra were deconvoluted using the program Siano (a kind gift from Dr. A. O. Doroshenko from the Karazin University, Kharkov, Ukraine), as described previously.[12] Thin-layer chromatography (TLC) was performed on silica gel plates (0.25-mm thickness, Merck 60 F_{524}).

Results

Spectroscopic Properties of FE and BFE Are Similar in Aprotic Solvents. The results of the systematic analysis of light-absorption and fluorescence data for compounds FE and BFE in solvents of different polarities and H-bonding properties are presented in Table 1. In all aprotic solvents, the absorption maxima of FE and BFE are almost at the same position. This demonstrates that the electronic structure of the primary chromophore was not significantly perturbed with the introduction of a benzene ring.

TABLE 1: Spectral Characteristics of the Studied Flavone Derivatives[a]

	solvent	$f(\epsilon)$	dye	λ_{max}^{abs}	λ_{max}^{N*}	λ_{max}^{T*}	I_{N*}/I_{T*}	φ
1	hexane	0.1849	FE[b]	396	421	553	0.010	0.14
			BFE	395	444	548	0.007	0.27
2	toluene	0.2387	FE[b]	408	456	566	0.044	0.14
			BFE	407	478	566	0.039	0.33
3	carbon disulfide	0.2588	FE	423	459	578	0.049	0.16
			BFE	421	471	574.5	0.029	0.41
4	thiophene	0.2676	FE	412	466	569	0.087	0.16
			BFE	411	484	570	0.061	0.34
5	di-*n*-butyl ether	0.2887	FE	403	460	562.5	0.0685	0.09
			BFE	401	469	559	0.031	0.19
6	anisol	0.3413	FE	411	477	573	0.211	0.14
			BFE	412	505	574.5	0.204	0.37
7	chloroform	0.3563	FE[b]	413	481	560	0.669	0.19
			BFE	412	502	566	0.176	0.37
8	bromobenzene	0.3728	FE	416	476	573	0.178	0.17
			BFE	416	501	574	0.161	0.34
9	*tert*-pentanol	0.3810	FE	410	487	558.5[c]	2.22[c]	0.22
			BFE	407	505	563	0.259	0.28
10	ethyl acetate	0.3850	FE[b]	401	475	570	0.253	0.05
			BFE	403	510	572	0.281	0.22
11	tetrahydrofuran	0.4074	FE	405	478	575	0.240	0.05
			BFE	406	510	575	0.310	0.20
12	dichloromethane	0.4217	FE	411	492	568	0.621	0.17
			BFE	411	512	571	0.404	0.40
13	*n*-octanol	0.4276	FE	415	506	562.5[c]	3.136[c]	0.56
			BFE	411	526[c]	569[c]	0.53[c]	0.46
14	*tert*-butanol	0.4422	FE	411	499	559.5[c]	3.69[c]	0.33
			BFE	408	516[c]	566[c]	0.473[c]	0.38
15	*n*-butanol	0.4574	FE	416	518	567[c]	6.57[c]	0.58
			BFE	415	535[c]	572[c]	1.06[c]	0.40
16	acetone	0.4647	FE[d]	404	502	574	0.936	0.05
			BFE	405	531[c]	582[c]	1.30[c]	0.28
17	ethanol	0.4704	FE[b]	412	523	574[c]	10.4[c]	0.52
			BFE	409	539[c]	580[c]	1.27[c]	0.42
18	dimethylformamide	0.4798	FE	407	509	583[c]	1.78[c]	0.08
			BFE	409	541[c]	590[c]	1.96[c]	0.28
19	acetonitrile	0.4802	FE[b]	404	509	574[c]	1.72[c]	0.09
			BFE	406	538[c]	586[c]	1.85[c]	0.33
20	dimethyl sulfoxide	0.4842	FE[d]	411	514	584[c]	2.83[c]	0.13
			BFE	411	547[c]	595[c]	2.32[c]	0.26

[a] λ_{max}^{abs}: position of absorption maximum; λ_{max}^{N*} and λ_{max}^{T*}: positions of fluorescence maxima of the N* and T* forms. φ is the fluorescence quantum yield. [b] Data of ref 28. [c] The values were evaluated from the results of the deconvolution. [d] Data of ref 14.

Fluorescence spectra of dyes FE and BFE in a series of representative aprotic solvents (Figure 1) demonstrate two emission bands, the positions and relative intensities of which are strongly solvent-dependent. With the introduction of the fused benzene ring, the general regularities in fluorescence spectra characteristic of ESIPT in 4′-dialkylamino-substituted 3HFs are retained. As commonly observed,[7−13] in low-polarity solvents (hexane, toluene, thiophene), the N* band occupies the shortest-wavelength position and is of lowest intensity relative to the long-wavelength T* band. With the increase in polarity, the N* band shifts toward longer wavelengths with a dramatic increase in its relative intensity (Figure 1). The T* band shifts also, but to a lesser extent. In the case of BFE, a somewhat smaller resolution between the N* and T* bands is observed because of the red shift of the N* band (Table 1). The origin of

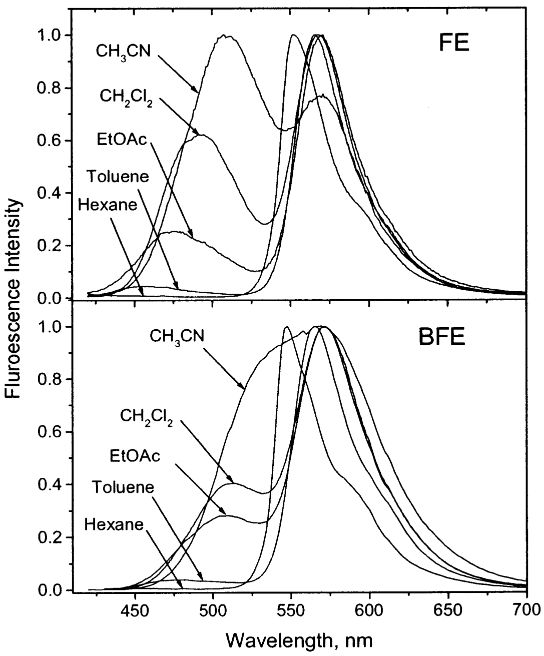
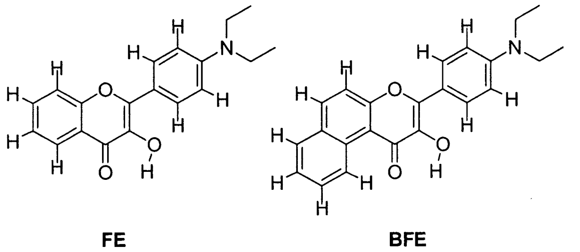


Figure 1. Fluorescence spectra of the studied flavones in different aprotic solvents. Excitation wavelength 410 nm.

CHART 1



the larger Stokes shift of the N* band in the case of BFE is not clear. This is probably related to the steric strain between 4-carbonyl and the proximal aromatic proton of the attached benzene ring (Chart 1), which results in some relaxation process occurring in the excited state. Importantly, the I_{N*}/I_{T*} ratios in aprotic solvents for these two compounds are very similar (Table 1). This means that the introduction of the benzene ring does not modulate the ESIPT reaction to any significant extent.

Protic Solvents Reveal Essential Spectroscopic Differences between FE and BFE. The comparison of solvent pairs with similar nonspecific solvatochromic properties (according to solvent polarity function $f(\epsilon)$) but with different H-bond donor abilities allows us to reveal the important differences in the spectroscopic behavior of FE and BFE (Figure 2). The most significant effects are observed for the relative intensity of the N* and T* emission bands, I_{N*}/I_{T*}. In chloroform, FE shows higher I_{N*}/I_{T*} values compared to those in more polar ethyl acetate (Table 1), whereas for BFE this effect is the opposite (Figure 2A). In *tert*-pentanol with respect to ethyl acetate, a solvent of almost the same polarity, FE shows dramatic growth of the I_{N*}/I_{T*} ratio, whereas in the case of BFE it does not change considerably (Figure 2B). These interesting spectroscopic differences between FE and BFE can be followed in all of the studied protic solvents (Table 1). This shows that in H-bonding donor solvents the ESIPT of FE is strongly hampered compared to that in aprotic solvents of similar polarity, whereas for BFE the protic nature of solvents does not effect this reaction.

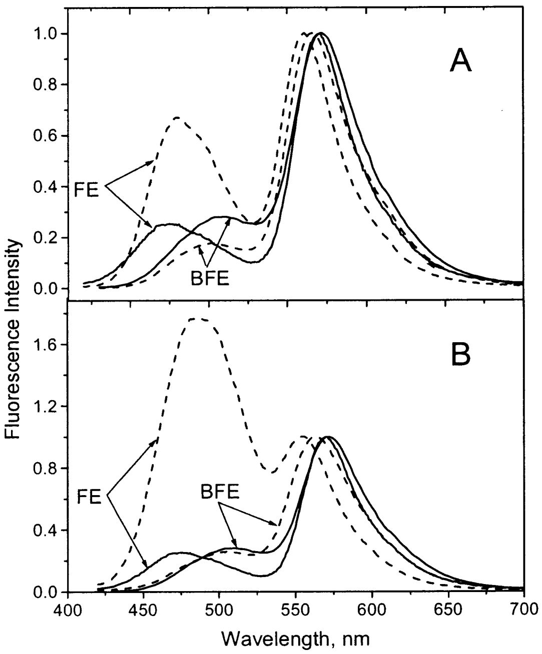


Figure 2. Fluorescence spectra of the studied flavones in solvent pairs of different H-bond donor ability: (A) ethyl acetate (—), chloroform (— — —); (B) ethyl acetate (—), *tert*-pentanol (— — —). Excitation wavelength 410 nm.

The important evidence for specific H-bonding interactions of FE with protic solvents is the observed red shifts of the absorption maximum with respect to aprotic solvents (Table 1). Importantly, for BFE, these shifts are much smaller,[29] demonstrating that in the ground state its H-bonding with protic solvents, if it exists, is significantly weaker. Much more pronounced differences between the dyes are observed in H-bonding effects on the positions of the emission maxima. Thus, the N* band of FE shows significant red shifts in the protic solvents compared with those in aprotic solvents of similar polarity, whereas with BFE this effect is almost not observed (Table 1). Furthermore, the T* band of FE is significantly blue-shifted in protic solvents, whereas for BFE the position of the T* band is very close to that in most of the solvents[30] (Table 1).

Solvent-Dependent Differences in Quantum Yields. Extremely interesting differences in the solvent-dependent behavior of dyes BFE and FE are observed for the fluorescence quantum yield (φ). FE shows an almost 10-fold increase in φ for protic solvents with respect to φ values for aprotic solvents of comparable polarity. Meanwhile, for BFE, this effect is not observed; in all of the solvents it exhibits similarly high φ values (Table 1). This result, together with the spectroscopic data, demonstrates clearly that all of the specific interactions of the excited FE molecule with protic solvents that dramatically affect its fluorescence properties are eliminated in the case of BFE.

Spectroscopic Study of Solvent Mixtures. To investigate further the spectroscopic effects of intermolecular H-bonding, we took two completely miscible solvents of very close polarities, ethyl acetate ($f(\epsilon) = 0.3850$) and *tert*-pentanol ($f(\epsilon) = 0.3810$), and performed the fluorescence studies of FE and BFE dyes in their binary mixtures of different composition. Because in our earlier studies we showed that $\log(I_{N*}/I_{T*})$ is an important photophysical parameter of the ESIPT reaction that demonstrates a good linear correlation with solvent polarity,[12,13,27] we used it for evaluating the present data. The results show that for FE the increase in alcohol concentration causes a strong and nonlinear increase in $\log(I_{N*}/I_{T*})$ in all concentration ranges up to 75 vol % (Figure 3). Meanwhile, for BFE compared with FE, the increase in $\log(I_{N*}/I_{T*})$ observed under the same

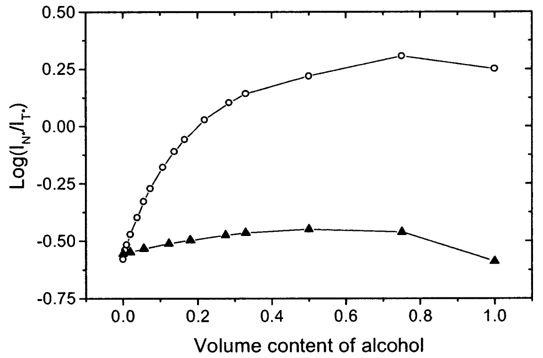


Figure 3. Logarithm of the fluorescence intensity ratio I_{N^*}/I_{T^*} for FE (○) and BFE (▲) vs the volume content of *tert*-pentanol in ethyl acetate.

conditions is negligible. The nonlinearity of the solvent effect for FE is additional evidence of the specific solvent–solute interaction in the presence of *tert*-pentanol—the formation of intermolecular H-bonds, which hampers the ESIPT reaction. The absence of considerable spectroscopic effects in the case of BFE is one more demonstration that an additional benzene ring prevents this interaction by screening the ESIPT system from the intermolecular H-bonding perturbations.

Data on Thin-Layer Chromatography. Finally, the presence or absence of intermolecular H-bonding in the ground state can be directly checked by thin-layer chromatography (TLC) on silica gel, which contains hydroxyl groups of polysilicic acids. We observe that in the mixture of aprotic solvents dichloromethane/hexane = 2:1 the chromatographic mobility, R_f, for BFE is much higher than that for FE, 0.45 and 0.13, respectively. This means that intermolecular H-bonding of BFE with the silica gel surface is much lower, assuming that nonspecific interactions of these compounds with the surface are similar. This condition can be tested in comparing R_f values for compounds such as 2-hydroxyacetophenone and 2-hydroxyacetonaphthone, which differ by the presence of the fused benzene ring. The results of TLC in the solvent mixture dichloromethane/hexane = 1:1 are as follows: R_f(2-hydroxyacetophenone) = 0.45; R_f(2-hydroxyacetonaphthone) = 0.41. They demonstrate that the affinities of the carrier surface for the tested compounds are similar, which signifies that nonspecific interactions for FE and BFE should also be similar and therefore their R_f differences should be attributed to H-bonding with the carrier.

Additional evidence can be provided by a comparison of BFE and 5,6-benzoflavone, a compound without 4′-diethylamino and 3-hydroxyl groups. The latter molecule is obviously much less polar than BFE, and it can form only one H-bond with its protic surroundings. Therefore, if BFE can form at least one H-bond of similar strength with the silica gel carrier, it will exhibit a significantly lower R_f. Meanwhile, the results show that its R_f is much larger than that obtained for 5,6-benzoflavone (0.17), which demonstrates the absence of its H-bonding with the protic surface of silica gel. Interestingly, the R_f of FE is close to that of 5,6-benzoflavone, which indicates that the FE molecule interacts with the protic carrier probably by a single H-bond.

Correlation of the I_{N^*}/I_{T^*} Ratio with Solvent Polarity. Because the I_{N^*}/I_{T^*} ratio also depends on solvent polarity, we may expect that by eliminating the specific intermolecular H-bonding interactions we made the probe that senses only polarity $f(\epsilon)$ described by universal solute–solvent interactions. The comparative graphs of $\log(I_{N^*}/I_{T^*})$ versus solvent polarity $f(\epsilon)$ for 20 studied solvents (Figure 4) demonstrate that. Whereas for FE a systematic deviation in the direction of increasing large $\log(I_{N^*}/I_{T^*})$ values in protic solvents and chloroform is observed, a very good linear correlation of $\log(I_{N^*}/I_{T^*})$ with solvent polarity $f(\epsilon)$ exists in the case of BFE.

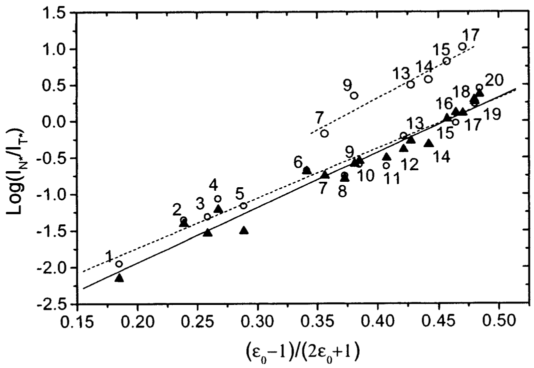


Figure 4. $\log(I_{N^*}/I_{T^*})$ for FE (○) and BFE (▲) vs the solvent polarity function $f(\epsilon)$. The solvents are numbered according to their sequence in Table 1. The solid line represents the linear fit for the BFE data, and the correlation equation is $\log(I_{N^*}/I_{T^*}) = 7.562f(\epsilon) - 3.455$. Correlation coefficient $r = 0.98$. The dashed lines are the linear fits of the data for FE in aprotic (lower) and H-bond donor (upper) solvents.

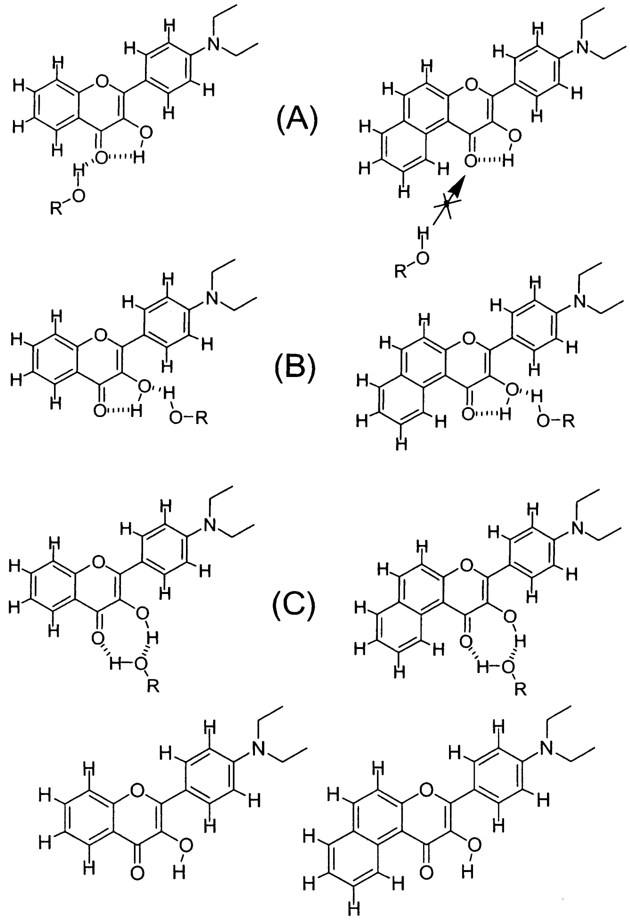
Discussion

The problem of accounting for the specific H-bonding solute–solvent interactions on the background of universal solvent effects (that are often expressed in terms of solvent polarity[31]) arises in all cases when the chromophore is capable of forming these interactions in the ground or excited states. It becomes especially important for the chromophores exhibiting ESIPT because this reaction occurs between proton donor and acceptor groups that are connected with intramolecular H-bonds.[1−3] Commonly, these groups are exposed to the solvent and are subjected to perturbations by hydrogen bonding with solvent molecules. This is particularly valid for 3-hydroxyflavone.[20−25]

Ground-State Interactions. In principle, three different complexes of the ground-state molecule of FE with an alcohol molecule can be considered (Scheme 1): a proton of the alcohol molecule can form a H-bond with the lone electron pair of the 4-carbonyl (A) or the 3-hydroxyl group (B), and the hydroxyl group of the alcohol can also form a seven-membered cycle by double H-bonding with both the 4-carbonyl and 3-hydroxyl groups (C). In the case of BFE, the additional benzene ring provides steric screening of the lone electron pair of 4-carbonyl (Scheme 1). Thus, in the ^{1}H NMR spectra of this compound, one of the aromatic protons exhibits a resonance peak at an abnormally low field (10.04 ppm), which belongs to the proton of the attached benzene ring that is extremely close to 4-carbonyl overlapping with its lone electron pair. Since H-bonds formed by oxygen are strongly directional in space, which is determined by the symmetry of its lone electron pairs, it is evident that for the dye BFE the interaction between its 4-carbonyl group and alcohol (type A) is eliminated, whereas the other types, B and C, may not be affected (Scheme 1). Quantum chemical simulations showed that the 2-phenyl group in 3HF is out of the plane of the chromone heterocycle with an angle of ca. 28°, which is the result of steric hindrance produced by the lone electron pairs of 3-oxygen.[32,33] In this respect, it could be expected that for flavones FE and BFE the H-bonding of the alcohol with 3-oxygen (type B, Scheme 1,B) is sterically hindered by the 2-phenyl group. Therefore, only one possible interaction of BFE and alcohols can be considered, which requires the breaking of the intramolecular H-bond (Scheme 1, C).

Meanwhile, the chromatographic data provide proof that in the ground state the BFE molecule does not form H-bonds with

SCHEME 1: Possible Ground-State Interactions of Alcohols with 3HF Derivatives FE and BFE



FE BFE

the proton donor medium, and the FE molecule, the 4-carbonyl group of which is available for H-bonding of type A, can form only one H-bond with the protic medium. This specific interaction is observed in the case of FE as a red shift of the absorption spectra in protic solvents with respect to the other solvents.[30] The much smaller red shift observed for the case of BFE is additional evidence that the specific interactions of its ground-state molecules with protic solvents are strongly diminished. Thus, our data allow the exclusion of H-bonding interactions of type C for both of the dyes. Therefore, we can conclude that in the ground state the dye BFE does not interact specifically with protic solvents at the studied conditions, whereas for the case of FE only the interaction of type A is significant.

Excited-State Interactions. How can these results be extrapolated to excited molecules, in which the electronic density is shifted significantly from the 4′-diethylamino group to the 4-carbonyl and 3-hydroxy groups because of the intramolecular charge transfer?[7−9,34] For the case of the N* state, the H-bonding of negatively charged 4-oxygen with protic solvents should be much stronger. The latter should result in the effective stabilization of the N* state. In our experiments, this effect is observed as systematic red shifts of the N* emission band for FE in protic solvents with respect to aprotic solvents of similar polarity. Meanwhile, in the case of BFE, this interaction is not possible; therefore, no considerable shifts of the N* band are observed (Table 1).

The ESIPT product T* state possesses a very different electronic distribution, so the negative charge is shifted from 4-oxygen to 3-oxygen. In this respect, the H-bonding of protic solvents with 4-oxygen or 3-oxygen atoms may produce a destabilization of this state. In fluorescence spectra, this is observed for the dye FE in protic solvents with respect to aprotic solvents of similar polarity as a blue shift of the T* band (Table 1). Importantly, the blue shift of the T* band for the case of BFE is significantly smaller, which indicates that intermolecular H-bonding with 4-carbonyl plays an important role in the destabilization of this state in the case of FE.

The intensity ratio of the emission bands, I_{N*}/I_{T*}, is an important characteristic of the ESIPT reaction, which is the only spectroscopic parameter that is directly connected to the relative energies of the two excited states.[13,27] Our results show that steric hindrance of 4-carbonyl from the intermolecular H-bonding of type A is sufficient to eliminate all of the specific effects of protic solvents on the I_{N*}/I_{T*} ratio. Therefore, it can be suggested that only the H-bonding with 4-oxygen, which is realized in the case of FE excited molecules, may hamper ESIPT and result in a dramatic increase of the I_{N*}/I_{T*} ratio. Meanwhile, the H-bonding interactions in protic solvents with the participation of 3-oxygen (types B and C) either do not form or, being formed, do not affect the spectra.

Upon H-bonding, the strong increase in the N* band relative emission (I_{N*}/I_{T*}) should be a result of the decrease in its energy relative to that of the T* state, and this is witnessed by the positions of the corresponding bands. In this respect, it is important that the H-bonding with protic solvents does not totally switch off the ESIPT in the FE molecule because the T* emission is still observed in all of the studied alcohols (Table 1). Furthermore, the H-bonded FE molecules preserve the ability to sense solvent polarity on a level of sensitivity that is similar to that of nonbonded ones because a linear correlation between $\log(I_{N*}/I_{T*})$ and $f(\epsilon)$ is also observed in protic solvents and its slope is close to that in aprotic solvents (Figure 4). This suggests that the same mechanism may also drive the response of the dye FE to polarity in protic solvents, which is an additional argument for the maintenance of the intramolecular H-bond in protic solvents both in the ground and excited states.

The intramolecular H-bond in 3-hydroxyflavone that closes the five-membered ring was considered to have relatively low stability.[25] Thus, low-temperature experiments on the parent 3HF in glasses and rigid matrixes showed that, indeed, protic additives can uncouple the ESIPT by breaking the intramolecular H-bond.[20−22] Meanwhile, the immobilization of solvent molecules on the formation of the seven-membered ring complex involving two H-bonds (type C) is associated with a decrease in the entropy, which in liquid solvents at room temperature may shift the equilibrium toward the dissociation of the complex with the restoration of intramolecular H-bonding. This explains why under the conditions of our experiments we do not observe the perturbation of intramolecular H-bonding by protic solvents. An additional factor is the presence of a π-electron donor, the 4′-diethylamino group in the 3-hydroxyflavones of the present study, which increases the basicity of 4-carbonyl. This should strengthen the intramolecular H-bonding and at the same time favor the intermolecular H-bonding by 4-carbonyl of type A.

These considerations lead to two important conclusions regarding the role of H-bonding in the spectroscopic behavior of the 4′-diethylamino-3HF system. (a) Hydrogen bonding with 4-carbonyl (Scheme 1, A) is the only mechanism of the specific interaction of FE with protic solvents that modulates its ESIPT and influences the spectroscopic properties. This means that at least for the emissive species of FE and BFE in protic solvents at room temperature the intramolecular H-bonds are never broken by intermolecular H-bonding with the solvent. This statement is in apparent disagreement with the commonly accepted mechanism of H-bonding perturbation of ESIPT in parent 3HF[21−25] and shows that the latter mechanism cannot be extended to FE and its analogues. (b) The response of the N* and T* states to intermolecular H-bonding is different. Whereas the N* state is stabilized upon H-bonding at 4-carbonyl, the T* state is destabilized, which is suggested by the different sign of the shifts in the correspondent emission bands. Such destabilization cannot be achieved by universal solvent−solute interactions because they always act as a stabilizing factor. This fact can explain why in protic solvents the I_{N*}/I_{T*} ratio is sharply increased, and this does not correlate with the effect of the solvent polarity function $f(\epsilon)$. The elimination in BFE of H-bonding with 4-carbonyl makes this dye insensitive to all H-bond perturbation effects. As a result, BFE shows very good linear correlation with $f(\epsilon)$ (Figure 4) that is independent of the solvent type.

Our results show how molecular design allows us to eliminate the strong intermolecular H-bonding perturbation of the solvent polarity-dependent ESIPT reaction. Thus, BFE, being selectively sensitive to universal interactions, can serve as the reference for FE, a compound with very similar ESIPT properties but with a strong sensitivity to H-bonding. And what is also very important is that the proposed design methodology may allow us to obtain new fluorescence molecular sensors in which the H-bonding perturbations by the environment can be either reinforced or eliminated. This opens new prospects in the study of microenvironmental properties using fluorescence probes.

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References and Notes

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(29) Recently, we demonstrated that the absorption spectrum of FE depends on the electronic polarizability of the solvent (ref 13) so that in aromatic solvents CS_2 and DMSO possessing a high refractive index the absorption maxima are shifted to the red. The same is observed for BFE (Table 1).

(30) The position of the T* band of FE, especially for BFE in acetone, acetonitrile, DMF, and DMSO, is significantly shifted to the red. We expect that these solvents, because of their strong H-bond accepting ability and the steric availability of their oxygen or nitrogen atoms, may form H-bonds with the proton of the T* state, resulting in the spectral effects opposite to those of the protic solvents.

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