



Cite this: *Photochem. Photobiol. Sci.*, 2016, **15**, 219

The kinetics and mechanism of photooxygenation of 4'-diethylamino-3-hydroxyflavone†

Zoltán Szakács,^a Márton Bojtár,^b László Drahos,^c Dóra Hessz,^d Mihály Kállay,^e Tamás Vidóczy,^d István Bitter^b and Miklós Kubinyi^{*a,d}

The photolysis reactions of 4'-diethylamino-3-hydroxyflavone (**D**), a versatile fluorescent probe showing excited-state intramolecular proton transfer (ESIPT), and the magnesium chelate of **D** (MgD^{2+}) have been studied in acetonitrile solution. Upon UV irradiation both species were oxidized into *O*-4-diethylaminobenzoyl salicylic acid, differently from the photoreaction of the parent compound 3-hydroxyflavone (3HF) which was described to undergo rearrangement to 3-hydroxy-3-phenyl-indan-1,2-dione. The photooxygenation of the Mg^{2+} complex was found to be significantly faster than the reaction of the pure dye. As the kinetic analysis of the absorption spectra of samples under irradiation showed, the rate coefficients for the oxygenations of the excited state dye and complex have close values, $k_{\text{ox}}(\text{D}^*) = 2.4 \times 10^7 \text{ min}^{-1}$, $k_{\text{ox}}(\text{MgD}^{2+*}) = 3.9 \times 10^7 \text{ min}^{-1}$; the difference arises from the higher photooxygenation quantum yield of the complex, $\Phi(\text{MgD}^{2+*}) = 2.3 \times 10^{-3}$, than the respective value for the pure dye, $\Phi(\text{D}) = 1.5 \times 10^{-4}$. The potential energy surface of the photooxygenation of **D** was calculated assuming a reaction path in which the phototautomer formed from **D** via ESIPT, reacts in its triplet state with triplet molecular oxygen O_2 , a mechanism similar to that suggested for the photoreaction of the parent 3HF. The moderate values for the transition state energies confirmed the plausibility of the hypothetical mechanism.

Received 29th September 2015,
Accepted 17th December 2015

DOI: 10.1039/c5pp00358j

www.rsc.org/ppp

Introduction

3-Hydroxy-flavones (3HFs) have two bands in their fluorescence spectra, one arising from the 'normal' (N^*) excited molecules, the other from the tautomeric (PT^*) species, the latter formed *via* excited state intramolecular proton transfer (ESIPT) from the hydroxyl to the carbonyl group of the N^* molecules.^{1,2} The positions of the two bands, as well as their intensity ratios are sensitive to the local environment, making many of these compounds attractive ratiometric fluorescent sensors.¹ Besides, as was demonstrated by Kasha *et al.*, 3HFs with their normal and tautomeric ground states (**N** and **PT**) and N^* and PT^* excited states can act as four-level laser materials.³

3-Hydroxy-flavones with dialkylamino substituents in their 4' positions (NR_2 -3HFs) proved to be particularly successful molecular fluorescent sensors. The intramolecular proton transfer cycle in 4'-diethylamino-3-hydroxyflavone, the title compound of this study, a prototype of these probes is exhibited in Scheme 1. The $\text{S}_0 \rightarrow \text{S}_1$ excitation of NR_2 -3HF probes is associated with a strong charge transfer.^{1,2} As a consequence, the position of their N^* band is highly sensitive to the polarity of the environment. Besides, NR_2 -3HF probes, as hydrogen bond acceptors *via* their CO groups, confer the additional advantage that the ratio of the fluorescence intensities of their N^* and PT^* forms characterizes the hydrogen bonding ability of their local environment.⁴ By exploiting the special spectroscopic features of NR_2 -3HF probes, detailed information can be obtained on their locations in phospholipid membranes.⁵⁻⁸ Attaching such fluorophores covalently to peptides and proteins, peptide-membrane interactions^{9,10} and structural changes of proteins¹¹ can be studied. Furthermore, NR_2 -3HFs may have important future applications as fluorescent sensors for the detection of adenosine triphosphate (ATP), as their excitation spectra are sensitive to ATP,^{7,12} and this effect is selective over the other nucleotides.¹³

The excitation of 3HFs may be followed by irreversible reactions (oxygenation, and rearrangement),¹⁴ the quantum yields of which characterize the photostability of these fluorescent probes, which is a key point when they are applied in analyti-

^aDepartment of Physical Chemistry and Materials Science, Budapest University of Technology and Economics, 1521 Budapest, Hungary. E-mail: kubinyi@mail.bme.hu

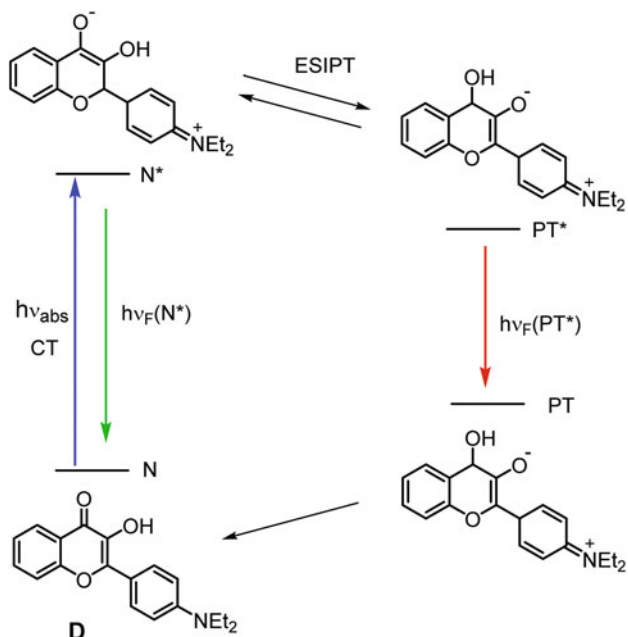
^bDepartment of Organic Chemistry and Technology, Budapest University of Technology and Economics, 1521 Budapest, Hungary

^cInstitute of Organic Chemistry, Research Center for Natural Sciences, Hungarian Academy of Sciences, 1519 Budapest, P.O. Box 286, Hungary

^dInstitute of Materials and Environmental Chemistry, Research Center for Natural Sciences, Hungarian Academy of Sciences, 1519 Budapest, P.O. Box 286, Hungary

^eMTA-BME Lendület Quantum Chemistry Research Group, Department of Physical Chemistry and Materials Science, Budapest University of Technology and Economics, 1521 Budapest, Hungary

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c5pp00358j



Scheme 1 Photoinduced intramolecular proton transfer cycle of 4'-diethylamino-3-hydroxyflavone (D).

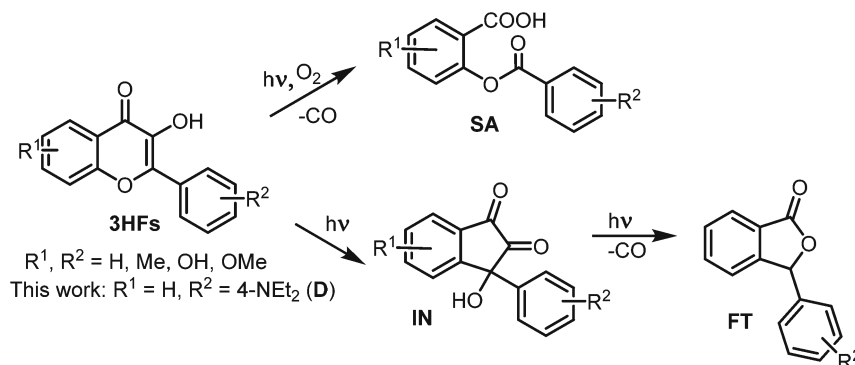
cal and imaging techniques. As no reports on such photoreactions of NR_2 -3HFs have been found in the literature, we performed this study on the photolysis of 4'-diethylamino-3-hydroxyflavone (D, see Scheme 1), involving the identification of the product(s), the determination of the photophysical and kinetic parameters, and the analysis of the reaction mechanism on the basis of quantum chemical calculations. Our study was extended to the photolysis of the Mg complex, MgD^{2+} , as an example for the photoinduced reaction of a chelate complex of the 4'-diethylamino derivative of 3HF.

The reactions of other 3HFs, induced by UV irradiation, especially the reactions of the parent compound 3HF, have widely been studied, and basically three kinds of products have been identified (Scheme 2). In the absence of O_2 , the photolysis of 3HFs resulted in 3-hydroxy-3-aryl-indan-1,2-dione

rearrangement products (IN) in apolar, polar aprotic as well as in polar protic solvents.^{15–18} Some INs lost CO, transforming into 3-aryl-phthalides (FTs).^{16,17} Under aerated conditions, in apolar solvents 3HFs underwent photooxygenation into *O*-benzoyl salicylic acid derivatives (SA) via oxidative decarbonylation.¹⁹

The presence of some metal ions (Mg^{2+} , Ca^{2+} , Hg^{2+} , Zn^{2+} , Pb^{2+}) did not affect the photooxygenation of 3HF in MeOH or in MeOH/ H_2O mixtures, the same products were obtained – IN or IN + FT as in the neat solvent, whereas other metal ions (Cu^{2+} , Ni^{2+} , Fe^{3+} , Co^{2+} , Al^{3+}) prevented the photoreaction.^{16,17} Recently, these investigations have been extended to a 3HF-tris(2-picolylamine) ligand pair which forms penta- and hexacoordinated chelates with a series of transition metal ions, and their photooxygenation to SA and degradation to FT products have been observed – the type of reaction depended on the solvent.^{20,21}

The mechanism of the photooxygenation of the parent compound, 3HF, observed in apolar solvents, has been studied in detail. In such solvents the formation of the S_1 state phototautomer, ${}^1PT^*$, from the excited normal molecules, ${}^1N^*$ via ESIPT is a fast process (a transient absorption signal with 35 fs rise time was assigned to ${}^1PT^*$),²² and the conversion is practically complete, as the less polar ${}^1PT^*$ form is more stable. The singlet phototautomer is converted into the triplet species, ${}^3PT^*$, with a quantum yield of 0.18 (in *n*-heptane),²³ and it is this ${}^3PT^*$ form of 3HF, which is oxidized by the triplet state oxygen, ${}^3\text{O}_2$.²⁴ The quantum yield of the photooxidation is only ~ 0.001 .²⁵ For the rate constant of the overall (physical + chemical) quenching of the T_1 state dye by ${}^3\text{O}_2$, $3.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ was obtained in *n*-heptane, a value close to 1/9 of the diffusion controlled rate, which is the theoretical limit for a triplet-triplet energy transfer, *i.e.* the quenching was dominantly a physical process.²³ In the photochemical reaction, an *exo*-peroxide and an *endo*-peroxide were reported as intermediates. The *exo*-peroxide was the photolysis product of 3HF in the O_2 matrix, and detected by infrared spectroscopy.²⁶ The *endo*-peroxide was obtained by photolysing 3HF on a silver electrode, and detected by surface enhanced Raman scattering.²⁷



Scheme 2 Photoreactions of 3-hydroxyflavones.

Materials and methods

Synthesis of D

D was synthesized in two steps, following the general procedure of Smith *et al.* for the synthesis of flavones.²⁸ First, 2-hydroxy-acetophenone was condensed in ethanol with 4-diethylamino-benzaldehyde to the corresponding chalcone,²⁹ which was oxidized then with H₂O₂ in alkaline ethanol to obtain **D** in the form of a yellow solid.³⁰ The product was identified by NMR spectroscopy and HRMS.³¹

Spectroscopic and photochemical experiments

The acetonitrile solvent was of HPLC grade and purchased from Merck. Mg(ClO₄)₂ was from Sigma Aldrich, and used without further purification.

The absorption spectra were recorded on an Agilent 8453 diode array UV-Vis spectrometer. The fluorescence spectra and the fluorescence decay curves were recorded with an FLS920 Edinburgh Instruments combined steady state and fluorescence lifetime spectrometer. Two pulsed diode lasers – an EPL-375 (emitting at 378 nm, pulse width ~90 ps) and an EPL-470 (emitting at 473 nm, pulse width ~100 ps) were used as light sources when the decay curves were recorded. NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer. The mass spectra were obtained using a Waters Q-TOF Premier mass spectrometer (Manchester, UK) in positive electrospray mode.

In the photolysis experiments, the samples were irradiated by using an Osram XBO OFR 150 W xenon arc lamp, placing a BG3/4g band-pass filter in front of the lamp, with a transmission range of ~300–420 nm. The photon flux incident on the samples was determined by chemical actinometry, *via* measuring the yield of photoconversion of *o*-nitrobenzaldehyde into *o*-nitrosobenzoic acid.^{32,33}

The optical spectroscopic and photochemical experiments were carried out at 25 °C, in aerated solutions.

Quantum chemical calculations

The theoretical calculations were carried out using the Gaussian09 program package^{34,35} at the density functional theory (DFT) and time-dependent DFT (TD-DFT)^{36–38} levels. We employed the M06-2X functional³⁹ with the 6-31++G** basis set for geometry optimizations and for vibrational frequency calculations as well. The solvent effects were described by the integral equation formalism polarizable continuum model (IEF-PCM) with acetonitrile as the solvent. The excited-state energy minimizations were started from the corresponding optimized ground state geometries. The transition state (TS) searches were executed in two steps. First, we used the quadratic synchronous transit (QST3)⁴⁰ method with a smaller 6-31G* basis set using the optimized structures of the reactants and products. Secondly, a TS optimization was carried out using the Bery algorithm with the 6-31++G** basis set, recomputing the Hessian in each geometry optimization cycle.

Results and discussion

Optical spectra

The absorption and fluorescence spectra of **D** in pure solvent and in the presence of Mg(ClO₄)₂ in various concentrations are shown in Fig. 1. The fluorescence band at 510 nm belongs to the N*, the band at 575 nm to the PT* form of the dye.⁴¹ The large shifts of the absorption and N* fluorescence bands and the disappearance of the PT* band are typical of the Mg complexes of 3HF: the parent compound 3HF and the dimethyl analogue of **D**, 4'-NMe₂-3HF were reported to show similar spectral changes in the presence of Mg²⁺ ions.^{42,43} In contrast, the addition of Mg²⁺ ions to the solutions of the respective 3-methoxyflavones, did not induce such changes in the spectra. By a simple explanation, the hydroxyl protons of 3HFs dissociated and MgL⁺ chelates were formed (L = ligand). Recent combined conductometric, spectroscopic and theoretical studies have concluded that the parent compound 3HF forms a complex [MgClO₄(3HF)]⁺, *i.e.* an ion pair of the salt is bound by the ligand, in which the intramolecular CO...HO hydrogen bond is ruptured, but the proton is not dissociated.^{44,45}

A least-squares fitting of the absorbance data confirmed that ligand **D** and Mg²⁺ ions react in a 1 : 1 ratio, and yielded a value of $K_S = 110 \text{ M}^{-1}$ for the stability constant. For comparison, the stability constants of the other alkali earth metal com-

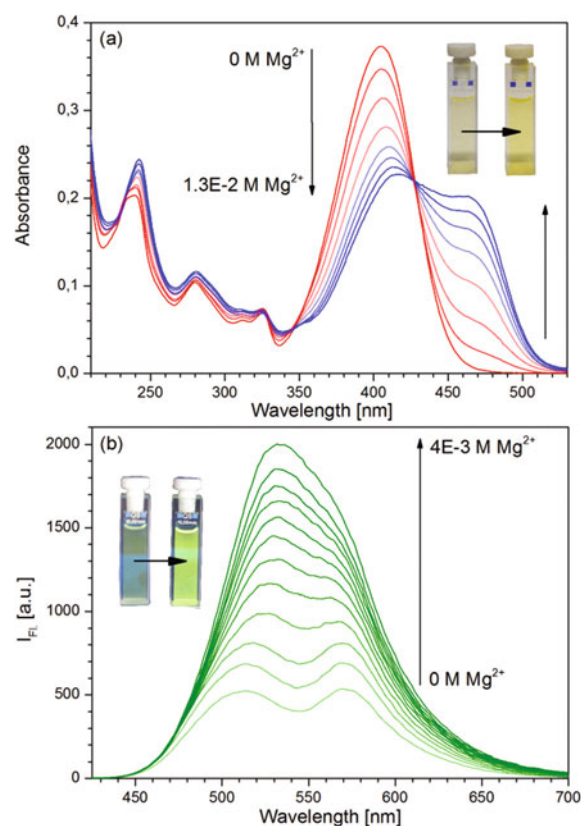


Fig. 1 (a) Absorption and (b) fluorescence ($\lambda_{\text{ex}} = 427 \text{ nm}$) spectra of **D** at various Mg(ClO₄)₂ concentrations. [D]₀ = 10⁻⁵ M.

Table 1 Fluorescence decay time constants of **D** and MgD^{2+}

	λ_{ex} (nm)	λ_{em} (nm)	τ_1 (ps) (rel. amplitude)	τ_2 (ps) (rel. amplitude)
D	378	470	65 (9%)	376 (91%)
		510	97 (7%)	383 (93%)
		630		376 (100%)
MgD^{2+}	473	535	3570 (100%)	

plexes of **D** have also been determined, and significantly lower values have been obtained (see Table S1 in the ESI†).

The fluorescence decay data of the pure dye and its Mg complex are presented in Table 1. The decay curves of the dye were taken at an emission wavelength on the rising edge of the N^* band (470 nm), on the falling edge of the PT^* band (630 nm) and at some intermediate wavelengths (510 nm is shown in Table 1). Biexponential curves were obtained, with a dominant ~ 380 ps slower component, which practically did not change with the wavelength, indicating quasi-equilibrium between the two excited state tautomers. Femtosecond fluorescence studies proved that the forward and reverse ESIPT reactions in **D** and related molecules are much faster than the deactivations of the N^* and PT^* forms.^{41,46}

The decay of the Mg complex was studied in a solution containing $\sim 52\%$ of the dye in the complexed state (the initial concentrations were $[\text{D}]_0 = 2.5 \times 10^{-6}$ M and $[\text{Mg}(\text{ClO}_4)_2]_0 = 10^{-2}$ M). The longer lifetime of the excited state complex, 3570 ps can be associated with the rigidity of the chelate structure and the lack of proton transfer as the deactivation channel.⁴³

Product of photolysis

The variation of the absorption spectrum of the aerated solution of the dye upon irradiation by UV light, measured in a 1 cm cell is shown in Fig. 2a. Like in the case of the parent compound, 3HF,³ photoinduced reaction was observed only in aerated solutions of **D**, and the samples unaerated by nitrogen bubbling were insensitive to UV light. The spectra obtained by irradiating a $\text{D-Mg}(\text{ClO}_4)_2$ mixture (aerated), in which $\sim 52\%$ of **D** was present in the complexed form, are exhibited in Fig. 2b.

A larger amount of the product, required for measuring the NMR spectra was obtained then by irradiating a 5×10^{-4} M solution of **D** in a vessel for 24 h, using the same band-pass filter and UV lamp, achieving a $\sim 70\%$ conversion by the UV-VIS spectrum. The product was isolated by column chromatography (Merck silica gel, toluene–methanol 9 : 1).

The ^1H NMR spectrum of the product (Fig. 3) is consistent with the salicylic acid derivative SA (see Scheme 2) and not with the 1,2-indanedione IN. The position of the doublet of the H5 protons (7.82 ppm) indicates the connection of an electron acceptor CO group, while the relatively low values of the H2 (6.83 ppm) and H4 (6.99 ppm) chemical shifts are indicative of an electron donating group (ester oxygen).

The HRMS measurement (see Fig. S1 in the ESI†) confirmed this structure, as the measured mass $[\text{M} + \text{H}]^+ = 314.1396$

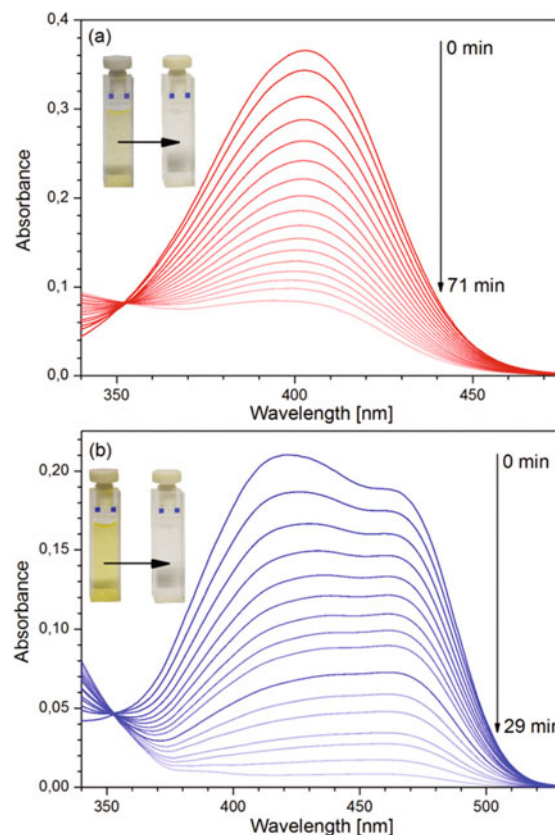


Fig. 2 Variation of the absorption spectra of solutions of **D** under irradiation by UV light. (a) Pure **D**, (b) $\text{D-Mg}(\text{ClO}_4)_2$ mixture. Initial concentrations: (a) $[\text{D}]_0 = 10^{-5}$ M, (b) $[\text{D}]_0 = 5 \times 10^{-5}$ M, $[\text{Mg}(\text{ClO}_4)_2]_0 = 10^{-2}$ M.

showed good agreement (1.9 ppm) with the calculated mass for the salicylic acid ester SA ($\text{C}_{18}\text{H}_{19}\text{NO}_4$, $\text{MH}^+ = 314.1392$), whereas the rearrangement product IN ($\text{C}_{19}\text{H}_{19}\text{NO}_3$, $\text{MH}^+ = 310.1443$) would have had a mass, identical to that of **D**.

The irradiation of a mixture with MgClO_4 , with $[\text{D}]_0 = 5 \times 10^{-4}$ M, and $[\text{Mg}(\text{ClO}_4)_2]_0 = 7 \times 10^{-3}$ M initial concentrations, led also to SA, as proved by the ^1H NMR spectrum of the product.

Kinetic analysis

The aim of the analysis has been to compare the photooxidation rates and quantum yields of the dye and its Mg complex. The photooxidation of the pure dye has been followed by recording the absorption spectra of the sample at 2 minute intervals for an hour. The instantaneous concentrations of the dye and the photooxidation product were obtained by resolving the spectra into the contributions of **D** and the product, **P**. The photoinduced processes in the solution of the pure dye were described by the set of equations:



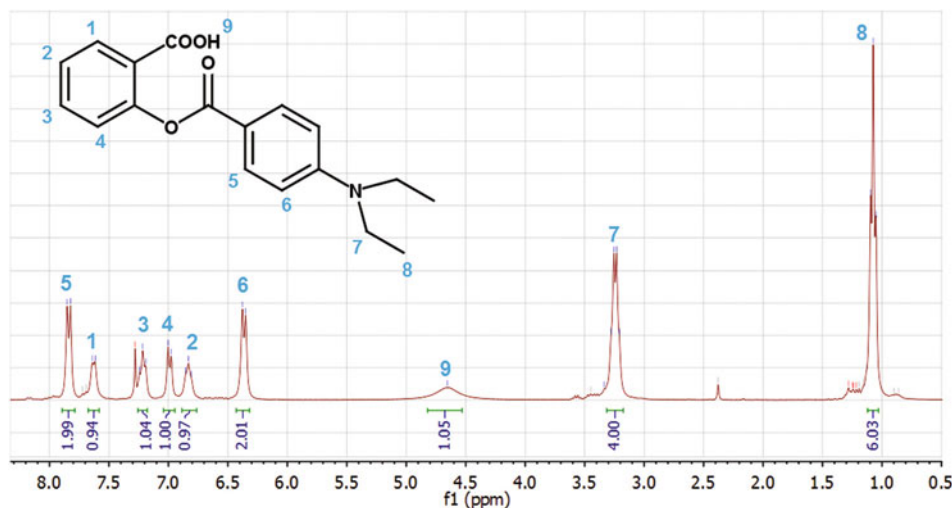


Fig. 3 $^1\text{H-NMR}$ spectrum of the photolysis product of **D**.



The rate of process (1), the excitation of the dye, expressed in mol photons $\text{L}^{-1} \text{s}^{-1}$, could be obtained as the fraction of the intensity of the incident light, absorbed by the dye, $I_{\text{abs}}^{\text{D}}$, related to the volume of the sample:

$$\nu_1 = (k_{\text{abs}}^{\text{D}}[\text{D}]) = \frac{I_{\text{abs}}^{\text{D}}}{V} \quad (4)$$

where

$$I_{\text{abs}}^{\text{D}} = \int_{\lambda_1}^{\lambda_2} \frac{\epsilon^{\text{D}}(\lambda)[\text{D}]}{\epsilon^{\text{D}}(\lambda)[\text{D}] + \epsilon^{\text{P}}(\lambda)[\text{P}]} \{1 - \exp[-A(\lambda)]\} I(\lambda) d\lambda \quad (5)$$

which is the extension of a formula derived for the absorption of monochromatic light by a single component of a multi-absorber solution,⁴⁷ to polychromatic light. In eqn (5) $\epsilon^{\text{D}}(\lambda)$ and $\epsilon^{\text{P}}(\lambda)$ are the absorption coefficients of the dye and the product, respectively, at wavelength λ , $A(\lambda)$ is the absorption spectrum of the sample, and $I(\lambda)$ is the spectrum of the irradiating light. ($I(\lambda)$ was derived from the spectra of the xenon arc lamp and the transmission filter applied, it is related to the intensity of the incident light, I^0 , determined by actinometry, as $I^0 = \int_{\lambda_1}^{\lambda_2} I(\lambda) d\lambda$.) The photooxidation of the dye, reaction (2), was treated as a first order reaction, because of the large excess of dissolved O_2 in the samples. (The reaction has been conducted under air-equilibrated conditions, with intensive stirring. The concentration of O_2 is $[\text{O}_2]_{\text{satd}}^{\text{air}} = 2.42 \times 10^{-3} \text{ M}$ in air-saturated acetonitrile,⁴⁸ two orders higher than the total concentration of **D**, a fraction of which are the excited state D^* molecules in reaction (2).) The rate coefficient of the oxidation was calculated from the (numerical) derivatives of the $[\text{D}] - t$ function:

$$-\frac{d[\text{D}]}{dt} = k_{\text{ox}}^{\text{D}}[\text{D}^*] \quad (6)$$

obtaining the concentration of the excited state dye from

$$\frac{d[\text{D}^*]}{dt} = \frac{I_{\text{abs}}^{\text{D}}}{V} - k_{\text{tau}}^{\text{D}}[\text{D}^*] - k_{\text{ox}}^{\text{D}}[\text{D}^*] \cong 0 \quad (7)$$

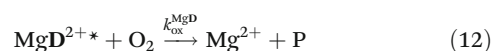
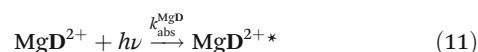
i.e. applying the quasi steady-state approximation for this species.

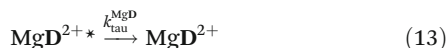
The quantum yield of the photooxidation of the dye was obtained then from k_{ox}^{D} and $k_{\text{tau}}^{\text{D}}$ as

$$\Phi_{\text{ox}}^{\text{D}} = \frac{k_{\text{ox}}^{\text{D}}[\text{D}^*]}{I_{\text{abs}}^{\text{D}}/V} = \frac{k_{\text{ox}}^{\text{D}}}{k_{\text{tau}}^{\text{D}} + k_{\text{ox}}^{\text{D}}} \quad (8)$$

When Mg^{2+} has been added to the reaction mixture in great excess (so that over 50% of **D** was complexed at the start of the reaction), the overall rate of photooxidation has increased considerably. Absorption spectra of the sample were recorded at 1 minute intervals for 15 minutes. The concentrations of the dye, the complex and the product, the latter identical as in the photolysis of the pure dye, were obtained by resolving the spectra of the reaction mixtures into the spectra of the three absorbing components, taking the known equilibrium constant for the complexation into consideration, *i.e.* ensuring that $[\text{MgD}^{2+}]/([\text{D}][\text{Mg}^{2+}])$ is constant all the time.

The reaction schemes (1)–(3) have been amended by the reactions:





The amount of light absorbed by MgD^{2+} was calculated according to the following equation:

$$I_{\text{abs}}^{\text{MgD}} = \int_{\lambda_1}^{\lambda_2} \frac{\epsilon^{\text{MgD}}(\lambda)[\text{MgD}]}{\epsilon^{\text{D}}(\lambda)[\text{D}] + \epsilon^{\text{MgD}}(\lambda)[\text{MgD}] + \epsilon^{\text{P}}(\lambda)[\text{P}]} \times \{1 - \exp[-A(\lambda)]\} I(\lambda) d\lambda \quad (14)$$

The rate coefficient of the oxidation of the excited complex was obtained then from the sum of the numerical derivatives of the $[\text{D}] - t$ and $[\text{MgD}] - t$ functions:

$$-\frac{d[\text{MgD}]}{dt} = k_{\text{ox}}^{\text{MgD}}[\text{MgD}^*] - k_{\text{assoc}}[\text{Mg}^{2+}][\text{D}] + k_{\text{dissoc}}[\text{MgD}] \quad (15)$$

$$-\frac{d[\text{D}]}{dt} = k_{\text{ox}}^{\text{D}}[\text{D}^*] + k_{\text{assoc}}[\text{Mg}^{2+}][\text{D}] - k_{\text{dissoc}}[\text{MgD}] \quad (16)$$

hence

$$-\frac{d[\text{D}]}{dt} - \frac{d[\text{MgD}]}{dt} = \left(\frac{d[\text{P}]}{dt}\right) = k_{\text{ox}}^{\text{D}}[\text{D}^*] + k_{\text{ox}}^{\text{MgD}}[\text{MgD}^*] \quad (17)$$

where k_{ox}^{D} was known from the previous experiment. The concentrations of the excited state species were obtained using the steady-state approximations (7) and

$$\frac{d[\text{MgD}^*]}{dt} = \frac{I_{\text{abs}}^{\text{MgD}}}{V} - k_{\text{tau}}^{\text{MgD}}[\text{MgD}^*] - k_{\text{ox}}^{\text{MgD}}[\text{MgD}^*] \cong 0 \quad (18)$$

Finally, the quantum yield of the photooxidation of the complex was obtained as

$$\Phi_{\text{ox}}^{\text{MgD}} = \frac{k_{\text{ox}}^{\text{MgD}}}{k_{\text{tau}}^{\text{MgD}} + k_{\text{ox}}^{\text{MgD}}} \quad (19)$$

The values obtained for the photooxidation rate coefficients and quantum yields are collected in Table 2. For **D** a lower Φ_{ox} value was obtained than $\Phi_{\text{ox}} \sim 0.001$, reported for the parent compound, 3HF.²⁵ The rate coefficients for the oxidations of the excited state dye and Mg complex have close values. The higher rate of the photoreaction in the presence of the Mg salt, observable through the change in the absorption spectrum, is associated with the higher quantum yield for the oxidation of the complex.

Table 2 Rate coefficients and quantum yields for photooxygenation

Reactant	$k_{\text{ox}}/\text{min}^{-1}$	Φ_{ox}
D	2.4×10^7	1.5×10^{-4}
MgD^{2+}	3.9×10^7	2.3×10^{-3}

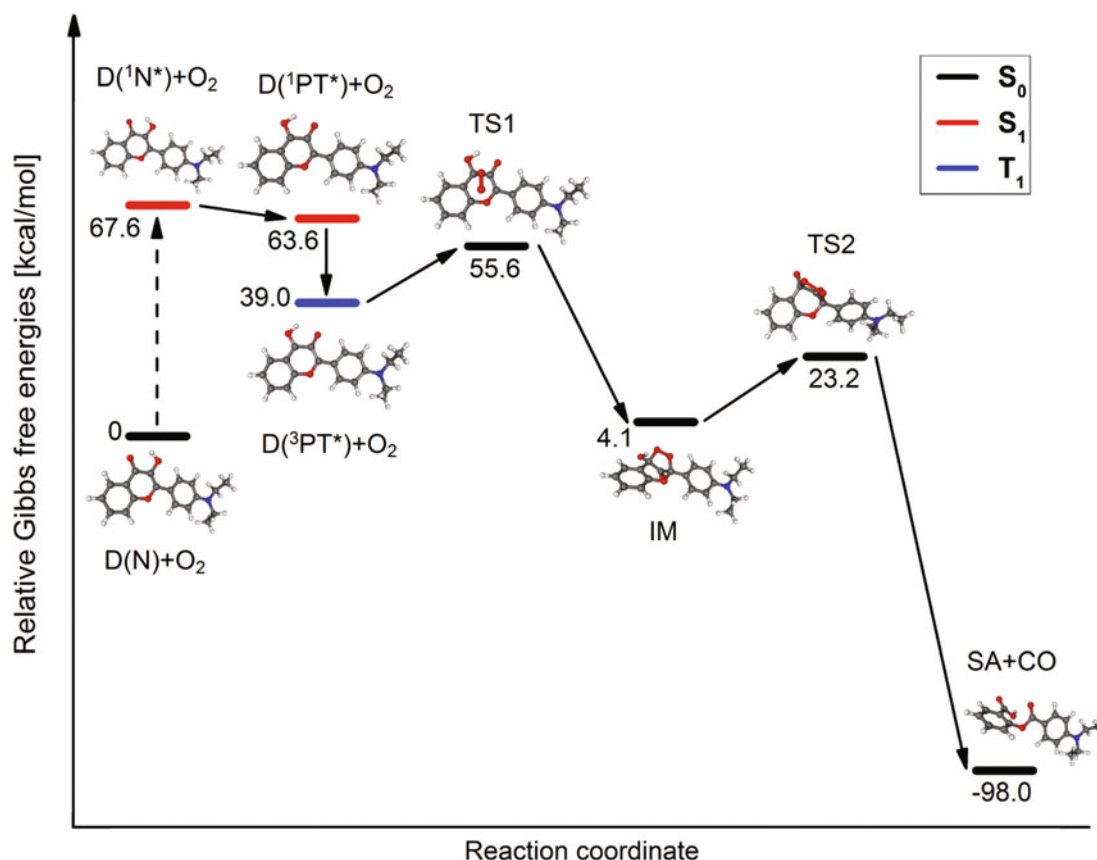


Fig. 4 The proposed reaction path of the photooxygenation.

Reaction mechanism

The transition states and intermediates of the photooxidation of **D** were characterized with the help of potential energy surface (PES) calculations. A reaction path analogous to the experimentally established steps of the photooxidation of the parent compound, 3HF,^{23–27} was considered. The results are illustrated in Fig. 4. The reaction takes place with the PT* form, since the C=C–O(δ^-) and CH–OH(δ^+) functional groups as nucleophilic/electrophilic centers at the given positions in the tautomeric species (see Scheme 1), promote the addition of O₂.²⁴ The first steps are the S₀ → S₁ excitation of the N form, and the subsequent conversion of ¹N* to its tautomer, ¹PT*. We did not search for the TS of the ESIPT reaction on the S₁ PES since this phototautomerization is well documented in the literature.^{49,50} Since the triplet O₂ molecule most probably attacks triplet species for spin rules,^{23,24} we suppose that ¹PT* undergoes inter-system crossing to give ³PT*. The reaction of ³PT* with ³O₂ leads to an S₀ state *endo*-peroxide intermediate (IM in Fig. 4), which decomposes – on the S₀ surface – into the final product (an SA derivative, see Scheme 2) and CO. Although the approaches (deficiencies of the TD-DFT method, relatively small basis set, and continuum solvation model) introduced in the calculations allow only for qualitative results, the reasonable values for the Gibbs free energies of the intermediate, IM and transition states, TS1, TS2 confirm the plausibility of this hypothesized reaction mechanism.

The parent compound, 3HF was reported to show a ground-state solute–solvent proton transfer in DMSO, a hydrogen bond acceptor solvent.⁵¹ Such anion formation would obviously affect the reactivity of our derivative toward molecular O₂. The absorption spectrum of **D** in neat acetonitrile (Fig. S2 in the ESI†), however, does not indicate the occurrence of anionic species. On the other hand, the spectrum of the MgD²⁺ complex shows similarity to the spectrum of the anionic form of the dye, yet, its photolysis results in the same oxidation product, as the photolysis of the free dye. It is difficult to assess the reaction mechanism for the oxygenation of complexed **D**, but we note that the anionic form of 3HF was

found to exhibit significantly increased reactivity toward ¹O₂, related to the uncharged species.²⁴

To understand why the oxygenation is preferred in the excited state, it is instructive to inspect the frontier molecular orbitals (MOs) of **D** in its N and T forms, which are visualized in Fig. 5. As can be seen, the lowest unoccupied molecular orbitals (LUMOs) are localized with opposite phases on the carbons where the oxygen molecule binds, unlike the highest occupied molecular orbitals (HOMOs). Thus, considering the electronic structure of the oxygen molecule, the electronic excitation to the S₁ state (with the subsequent inter-system crossing) facilitates the formation of the T–O₂ complex.

Conclusions

In studies on the photolysis of 3-hydroxyflavone and its substituted derivatives, two types of reactions had been observed: photooxygenation leading to the respective *O*-benzoyl salicylic acid and rearrangement into the respective 3-hydroxy-3-aryl-indane-1,2-dione. The type of reaction depended on the substituents, the solvent and the aeration/deaeration of the sample. In our present work, it has been found that 4'-diethylamino-3-hydroxyflavone (**D**) which is a versatile fluorescent probe, undergoes the former reaction (photooxygenation) in acetonitrile. Our theoretical calculations on the S₀, S₁, and T₁ PESs of the system proved that the photooxygenation reaction is a possible reaction route. The excited molecule, after tautomerization, undergoes inter-system crossing, and the triplet-state species reacts with the oxygen molecule. Our study has been extended to the Mg chelate of **D** (MgD²⁺), the photolysis of which resulted in the same product as that of the pure dye. Analyzing the temporal change of the absorption spectra of the reaction mixtures and with the help of actinometric measurements, the quantum yields for the photoreactions of **D** and its Mg chelate and the rate coefficients for the oxidations of the excited state species (**D*** and MgD²⁺*) have been determined, striving for obtaining data, which are, in principle independent of the intensity of the irradiating light. It can be hoped that these results will be utilized in the development of further 3-hydroxychromone based and other fluorescent sensors, in particular in the evaluation of their photostability.

Acknowledgements

This work was financially supported by the Hungarian Scientific Research Fund (Grant K108752) and the National Office for Research and Innovation (Grant KMOP-1.1.2-07/1-2008-0002). The authors are grateful to Dr András Simon for the NMR experiments.

References

- 1 S. Protti and A. Mezzetti, Any colour you like. Excited state and ground state proton transfer in flavonols and appli-

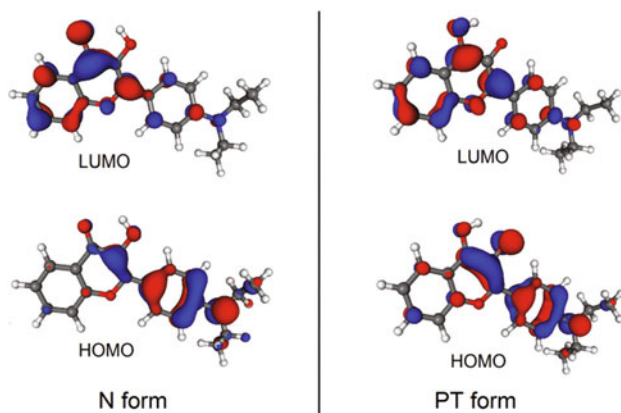


Fig. 5 Calculated molecular orbitals of the N and PT forms of **D**.

- cations, in *Specialist Periodical Report: Photochemistry*, ed. A. Albani, Royal Society of Chemistry, Cambridge, 2012, vol. 40, pp. 295–322.
- A. P. Demchenko, K. C. Tang and P. T. Chou, Excited-state proton coupled charge transfer modulated by molecular structure and media polarization, *Chem. Soc. Rev.*, 2013, **42**, 1379–1408.
 - P. Chou, D. McMorrow, T. J. Aartsma and M. Kasha, The Proton-Transfer Laser. Gain Spectrum and Amplification of Spontaneous Emission of 3-Hydroxyflavone, *J. Phys. Chem.*, 1984, **88**, 4596–4599.
 - A. S. Klymchenko and A. P. Demchenko, Multiparametric probing of intermolecular interactions with fluorescent dye exhibiting excited state intramolecular proton transfer, *Phys. Chem. Chem. Phys.*, 2003, **5**, 461–468.
 - V. V. Shynkar, A. S. Klymchenko, Y. Mély, G. Duportail and V. G. Pivovarenko, Anion Formation of 4'-(Dimethylamino)-3-hydroxyflavone in Phosphatidylglycerol Vesicles Induced by HEPES Buffer: A Steady-State and Time-Resolved Fluorescence Investigation, *J. Phys. Chem. B*, 2004, **108**, 18750–18755.
 - A. S. Klymchenko, G. Duportail, A. P. Demchenko and Y. Mély, Bimodal Distribution and Fluorescence Response of Environment-Sensitive Probes in Lipid Bilayers, *Biophys. J.*, 2004, **86**, 2929–2941.
 - G. M'Baye, O. V. Martyloga, G. Duportail and V. G. Pivovarenko, 3-Hydroxy-4'-[di(2-hydroxymethyl)amino] flavone as a new step in search of an ideal membrane ratiometric fluorescent probe, *J. Photochem. Photobiol., A*, 2006, **184**, 113–124.
 - D. Ghosh, S. Batuta, S. Das, N. A. Begum and D. Mandal, Proton Transfer Dynamics of N,N'-Dimethylamino-3-hydroxyflavone Observed in Hydrogen-Bonding Solvents and Aqueous Micelles, *J. Phys. Chem. B*, 2015, **119**, 5650–5661.
 - V. Y. Postupalenko, V. V. Shvadchak, G. Duportail, V. G. Pivovarenko, A. S. Klymchenko and Y. Mély, Monitoring membrane binding and insertion of peptides by two-color fluorescent label, *Biochim. Biophys. Acta, Biomembr.*, 2011, **1808**, 424–432.
 - V. Y. Postupalenko, O. M. Zamotaiev, V. V. Shvadchak, A. V. Strizhak, V. G. Pivovarenko, A. S. Klymchenko and Y. Mély, Dual-Fluorescence L-Amino Acid Reports Insertion and Orientation of Melittin Peptide in Cell Membranes, *Bioconjugate Chem.*, 2013, **24**, 1998–2007.
 - S. V. Avilov, C. Bode, F. G. Tolgyesi, A. S. Klymchenko, J. Fidy and A. P. Demchenko, Heat perturbation of bovine eye lens alpha-crystallin probed by covalently attached ratiometric fluorescent dye 4'-diethylamino-3-hydroxyflavone, *Biopolymers*, 2005, **78**, 340–348.
 - D. A. Yushchenko, O. B. Vadzyuk, S. O. Kosterin, G. Duportail, Y. Mély and V. G. Pivovarenko, Sensing of adenosine-5'-triphosphate anion in aqueous solutions and mitochondria by a fluorescent 3-hydroxyflavone dye, *Anal. Biochem.*, 2007, **369**, 218–225.
 - V. G. Pivovarenko, O. B. Vadzyuk and S. O. Kosterin, Fluorometric Detection of Adenosine Triphosphate with 3-Hydroxy-(4'-dimethylamino)flavone in Aqueous Solutions, *J. Fluorescence*, 2006, **16**, 9–15.
 - M. Sisa, S. L. Bonnet, D. Ferreira and J. H. Van der Westhuizen, Photochemistry of Flavonoids, *Molecules*, 2010, **15**, 5196–5245.
 - T. Matsuura, T. Takemoto and R. Nakashima, Photo-induced reactions – LXXI. Photorearrangement of 3-hydroxyflavones to 3-aryl-3-hydroxy-1,2-indandiones, *Tetrahedron*, 1973, **29**, 3337–3340.
 - I. Yokoe, K. Higuchi, Y. Shirataki and M. Komatsu, Photochemistry of Flavonoids. III. Photorearrangement of Flavonols, *Chem. Pharm. Bull.*, 1981, **29**, 894–898.
 - S. Protti, A. Mezzetti, C. Lapouge and J.-P. Cornard, Photochemistry of metal complexes of 3-hydroxyflavone: towards a better understanding of the influence of solar light on the metal–soil organic matter interactions, *Photochem. Photobiol. Sci.*, 2008, **7**, 109–119.
 - S. Protti and A. Mezzetti, Solvent effects on the photophysics and photoreactivity of 3-hydroxyflavone: A combined spectroscopic and kinetic study, *J. Mol. Liq.*, 2015, **205**, 110–114.
 - P.-T. Chou and M. L. Martinez, Photooxygenation of 3-hydroxyflavone and molecular design of the radiation-hard scintillator based on the excited-state proton transfer, *Radiat. Phys. Chem.*, 1993, **41**, 373–378.
 - K. Grubel, A. R. Marts, S. M. Greer, D. L. Tierney, C. J. Allpress, S. N. Anderson, B. J. Laughlin, R. C. Smith, A. M. Arif and L. M. Berreau, Photoinitiated Dioxygenase-Type Reactivity of Open-Shell 3d Divalent Metal Flavonolate Complexes, *Eur. J. Inorg. Chem.*, 2012, 4750–4757.
 - K. Grubel, S. L. Saraf, S. N. Anderson, B. J. Laughlin, R. C. Smith, A. M. Arif and L. M. Berreau, Synthesis, characterization, and photoinduced CO-release reactivity of a Pb(II) flavonolate complex: Comparisons to Group 12 analogs, *Inorg. Chim. Acta*, 2013, **407**, 91–97.
 - S. Ameer-Beg, S. M. Ormson, R. G. Brown, P. Matousek, M. Towrie, E. T. J. Nibbering, P. Foggi and F. V. R. Neuwahl, Ultrafast measurements of excited state intramolecular proton transfer (ESIPT) in room temperature solutions of 3-hydroxyflavone and derivatives, *J. Phys. Chem. A*, 2001, **105**, 3709–3718.
 - W. E. Brewer, S. L. Studer, M. Standiford and P.-T. Chou, Dynamics of the Triplet State and the Reverse Proton Transfer of 3-Hydroxyflavone, *J. Phys. Chem.*, 1989, **93**, 6088–6094.
 - S. L. Studer, W. E. Brewer, M. L. Martinez and P.-T. Chou, Time-Resolved Study of the Photooxygenation of 3-Hydroxyflavone, *J. Am. Chem. Soc.*, 1989, **111**, 7643–7644.
 - M. L. Martinez, S. L. Studer and P.-T. Chou, Direct Evidence of the Triplet-State Origin of the Slow Reverse Proton Transfer Reaction of 3-Hydroxyflavone, *J. Am. Chem. Soc.*, 1990, **112**, 2427–2429.
 - P.-T. Chou, S. L. Studer, M. L. Martinez, E. Orton and M. Young, Photooxygenation of 3-hydroxyflavone in a 12 K O₂ matrix, *Photochem. Photobiol.*, 1991, **53**, 587–593.
 - M. Wang, T. Spataru, J. R. Lombardi and R. L. Birke, Time Resolved Surface Enhanced Raman Scattering Studies of

- 3-Hydroxyflavone on a Ag Electrode, *J. Phys. Chem. C*, 2007, **111**, 3044–3052.
- 28 M. A. Smith, R. M. Neumann and R. A. Webb, A modification of the Algar-Flynn-Oyamada preparation of flavonols, *J. Heterocycl. Chem.*, 1968, **5**, 425–426.
- 29 S. Park and H.-J. Kim, Highly activated Michael acceptor by an intramolecular hydrogen bond as a fluorescence turn-on probe for cyanide, *Chem. Commun.*, 2010, **46**, 9197–9199.
- 30 S. M. Ormson, R. G. Brown, F. Vollmer and W. Rettig, Switching between charge-transfer and proton-transfer emission in the excited-state of a substituted 3-hydroxyflavone, *J. Photochem. Photobiol., A*, 1994, **81**, 65–72.
- 31 V. G. Pivovarenko, L. Jozwiak and J. Błażejowski, 2,8-Bis[4-(diethylamino)phenyl]-3,7-dihydroxy-4H,6H-pyrano[3,2-g]chromene-4,6-dione - A new liquid-phase-sensitive fluorescent probe utilising intramolecular one- or two-proton transfer phenomena, *Eur. J. Org. Chem.*, 2002, 3979–3985.
- 32 K. L. Willett and R. A. Hites, Chemical Actinometry: Using o-Nitrobenzaldehyde to Measure Light Intensity in Photochemical Experiments, *J. Chem. Educ.*, 2000, **77**, 900–902.
- 33 E. S. Galbavy, K. Ram and C. Anastasio, 2-Nitrobenzaldehyde as a chemical actinometer for solution and ice photochemistry, *J. Photochem. Photobiol., A*, 2010, **209**, 186–192.
- 34 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09, Revision D.01*, Gaussian, Inc., Wallingford, CT, 2009.
- 35 J. B. Foresman and A. E. Frisch, *Exploring Chemistry With Electronic Structure Methods*, Gaussian, Pittsburgh, 1996.
- 36 P. Hohenberg and W. Kohn, Inhomogeneous Electron Gas, *Phys. Rev.*, 1964, **136**, B864–B871.
- 37 W. Kohn and L. J. Sham, Self-Consistent Equations Including Exchange and Correlation Effects, *Phys. Rev.*, 1965, **140**, A1133–A1138.
- 38 F. Furche and D. Rappoport, Density functional methods for excited states: Equilibrium structure and electronic spectra, *Theor. Comput. Chem.*, 2005, **16**, 93–128.
- 39 Y. Zhao and D. G. Truhlar, The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals, *Theor. Chem. Acc.*, 2008, **120**, 215–241.
- 40 C. Y. Peng and H. B. Schlegel, Combining synchronous transit and quasi-newton methods to find transition-states, *Isr. J. Chem.*, 1993, **33**, 449–454.
- 41 P.-T. Chou, S.-C. Pu, Y.-M. Cheng, W.-S. Yu, Y.-C. Yu, F.-T. Hung and W.-P. Hu, Femtosecond Dynamics on Excited-State Proton/Charge-Transfer Reaction in 4'-N,N-Diethylamino-3-hydroxyflavone. The Role of Dipolar Vectors in Constructing a Rational Mechanism, *J. Phys. Chem. A*, 2005, **109**, 3777–3787.
- 42 A. D. Roshal, A. V. Grigorovich, A. O. Doroshenko, V. G. Pivovarenko and A. P. Demchenko, Flavonols and Crown-Flavonols as Metal Cation Chelators. The Different Nature of Ba²⁺ and Mg²⁺ Complexes, *J. Phys. Chem. A*, 1998, **102**, 5907–5914.
- 43 A. D. Roshal, A. V. Grigorovich, A. O. Doroshenko, V. G. Pivovarenko and A. P. Demchenko, Flavonols as metal-ion chelators: complex formation with Mg²⁺ and Ba²⁺ cations in the excited state, *J. Photochem. Photobiol., A*, 1999, **127**, 89–100.
- 44 V. N. Agieienko, Y. V. Kolesnik and O. N. Kalugin, Structure, solvation, and dynamics of Mg²⁺, Ca²⁺, Sr²⁺ and Ba²⁺ complexes with 3-hydroxyflavone, and perchlorate anion in acetonitrile medium: A molecular dynamics simulation study, *J. Chem. Phys.*, 2014, **140**, 194501.
- 45 V. N. Agieienko and O. N. Kalugin, Complexation of Ni(ClO₄)₂ and Mg(ClO₄)₂ with 3-Hydroxyflavone in Acetonitrile Medium: Conductometric, Spectroscopic, and Quantum Chemical Investigation, *J. Phys. Chem. B*, 2014, **118**, 12251–12262.
- 46 V. V. Shynkar, Y. Mély, G. Duportail, E. Piémont, A. S. Klymchenko and A. P. Demchenko, Picosecond time-resolved fluorescence studies are consistent with reversible excited-state intramolecular proton transfer in 4'-(dialkylamino)-3-hydroxyflavones, *J. Phys. Chem. A*, 2003, **107**, 9522–9529.
- 47 R. C. Michaelson and L. F. Loucks, Expressions for the Light Absorbed by a Single Component of a Multi-Absorber Solution, *J. Chem. Educ.*, 1975, **52**, 652–653.
- 48 C. Franco and J. Olmsted, Photochemical determination of the solubility of oxygen in various media, *Talanta*, 1990, **37**, 905–909.
- 49 R. Casadesús, O. Vendrell, M. Moreno, J. M. Lluch and K. Morokuma, On the intramolecular proton transfer of 3-hydroxyflavone in the first singlet excited state: A theoretical study, *Chem. Phys.*, 2006, **325**, 243–250.
- 50 C.-C. Lin, C.-L. Chen, M.-W. Chung, Y.-J. Chen and P.-T. Chou, Effects of Multibranching on 3-Hydroxyflavone-Based Chromophores and the Excited-State Intramolecular Proton Transfer Dynamics, *J. Phys. Chem. A*, 2010, **114**, 10412–10420.
- 51 S. Protti, A. Mezzetti, J.-P. Cornard, C. Lapouge and M. Fagnoni, Hydrogen bonding properties of DMSO in ground-state formation and optical spectra of 3-hydroxyflavone anion, *Chem. Phys. Lett.*, 2008, **467**, 88–93.