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Ionization and tautomerism of methyl fluorescein and related dyes



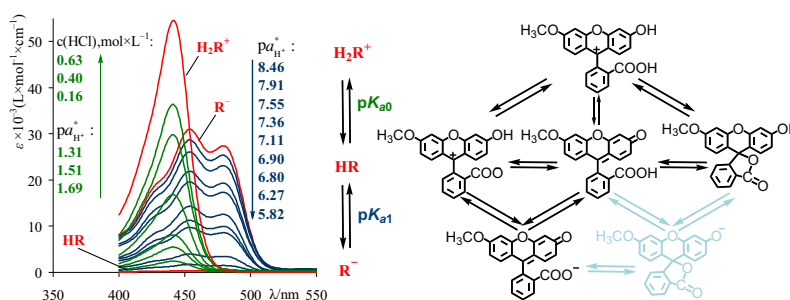
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HIGHLIGHTS

- The molecular form of fluorescein methyl ether in water is a mixture of tautomers.
- The fractions of the zwitter-ion, quinonoid, and lactone are 11%, 6%, and 83%.
- In aqueous ethanol, the colorless molecular lactone predominates.
- The lactone structure of the monoanion in solution is unlikely.

GRAPHICAL ABSTRACT



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ABSTRACT

The protolytic equilibrium of methyl ether of fluorescein is studied in water, aqueous ethanol, and in other solvents. The constants of the two-step dissociation are determined by spectrophotometry. In water, the fractions of the zwitterionic, quinonoid, and lactonic tautomers are correspondingly 11%, 6%, and 83%, as deduced from the UV–visible spectra. Corresponding study of the ionization of the methyl ether ester of fluorescein, fluorescein ethyl ester, and sulfonefluorescein allows testing the correction of the attribution of the microscopic dissociation constants of methoxy fluorescein. The results of nuclear magnetic resonance and infrared spectroscopy, as well as the X-ray analysis confirm the predomination of the lactonic structure of the molecular species in solid state and in DMSO. Contrary to it, the spectroscopic studies in both hydrogen-donor bond (HDB) and non-HDB solvents confirm that the presence of lactonic monoanion is atypical for the dye under study and, with high probability, also for the mother compound fluorescein.

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Introduction

Nowadays, much attention has been given to the structure, absorption, and fluorescence of fluorescein dyes in solution [1–9]. The recent studies of the structure and spectra in the gas phase are of special interest [10–14]. The evident reason of such activities

is the expanding applications of the abovementioned compounds in versatile fields, including biochemistry [15–18].

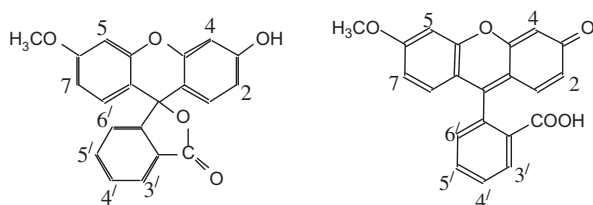
It should be mentioned, however, that the detailed ionization scheme of the mother compound, fluorescein, in water [4,6,19–23] and non-aqueous solvents was already studied in full and reported in a set of publications [24–29].

In order to further this research we studied the protolytic equilibrium of the monomethyl derivative of fluorescein, which may be represented as a colorless lactone, 3'-hydroxy-6'-methoxyspiro[isobenzofuran-1(3H),9'-(9H)-xanthene]-3-one, or as a quinonoid, 2-(6-methoxy-3-oxo-3H-xanthene-9-yl) benzoic acid:

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At first glance, this compound is of less interest for the physico-chemistry of xanthenes, because within the reasonable pH range it cannot generate a double charged brightly emitting anion. However, the examining of the ionic equilibrium of methyl ether of fluorescein is necessary for better understanding the (partly hidden) properties of the parent dye.

The detailed ionization equilibrium of fluorescein in solutions is given in Scheme 1.

The structures possessing identical or similar chromophore systems are designed by the same letters, namely, H_3Z^+ and H_2Z^\pm , H_2Q and HQ^- , HX^- and X^{2-} . Whereas the neutral form exists in solution as a mixture of three tautomers, zwitterion H_2Z^\pm , quinonoid H_2Q , and colorless lactone H_2L , only the carboxylate tautomer, HQ^- , is typical for the monoanion. The phenolate tautomer HX^- appears in small quantities only in pure non-hydrogen bond donor solvents, such as DMSO, acetonitrile, and acetone [29], but it predominates in the gas phase [10–14]. For the derivatives bearing halogen atoms in the xanthene moiety, such as eosin, the zwitterionic tautomer is less typical, whereas the monoanion exists predominantly as HX^- [25,28].

The values of the tautomerization constants K_T , K_T' , and K_T'' , and consequently the indices of the microscopic dissociation constants, pK (Scheme 1), were estimated for fluorescein in water and in several non-aqueous systems [6,7,20–22,28]. But for all that, some structures were excluded from consideration. For example, the anions–lactones HL^- and L^{2-} (not shown in Scheme 1) were regarded as less probable because in different solvents the variations of the maximal molar absorptivity of mono- and di-anions were rather of solvatochromic nature and occurred simultaneously with the shifts of the wavelength of the absorption band maximum. Such lactonic anions were registered only in the case of nitro derivatives of fluorescein [30].

The above-mentioned regularities of tautomerism of fluorescein dyes have been recently corroborated by quantum-chemical calculations [31].

The quantitative study of ionization and tautomerism of the methyl ether of fluorescein and several related dyes allows one to verify the assumptions used earlier and compare the pK values with those of fluorescein. The probable detailed protolytic equilibrium of methyl fluorescein is given in Scheme 2.

The probability of existence of the lactonic monoanion should be verified more directly than in the case of fluorescein, because here the R^- species predominates within a broad pH range. On the other hand, such a study allows elucidating how replacing of OH by OCH₃ influences the acidity of the COOH and the remaining OH group.

Some data on ethers and esters of fluorescein dyes are available in the literature [25–37]; Amat-Guerri et al. studied the ethers of eosin and rose bengal B and thus estimated the fractions of the lactonic monoanions of these dyes [37].

In this paper, we report the characterization of methyl ether of fluorescein and its methyl ester in solid (X-ray, IR spectra) and liquid (IR, ¹H NMR, and ¹³C NMR spectroscopy) states, the dissociation constants of the dyes in 50 mass% aqueous ethanol by means of vis-spectroscopy, and the results of examination of the

tautomeric equilibrium of methyl fluorescein. Two related dyes, sulfonefluorescein and ethyl ester of fluorescein, were also studied in order to compare all the pK values with those of fluorescein in the same mixed solvent.

Also, the protolytic equilibrium of methyl fluorescein was studied in water, despite the limited solubility of the neutral molecular species. This was necessary in order to compare the K_T , K_T' , K_T'' , and pK values with those for fluorescein, taking into account some colliding information concerning the tautomerism of fluorescein just in water.

Indeed, Nagase et al. [38] proposed all the three tautomers, whereas Scharf [39] and Zanker and Peter [40] considered the equilibrium between H_2L and H_2Q . Hioka and colleagues trend to such point of view in their recent paper [4].

Using the visible spectra of neutral species, Lindqvist [19,41] estimated the fractions of the tautomers: the ratio of H_2Z^\pm , H_2Q , and H_2L equals 2:1:5. We somewhat refined these approach; the percentages of the zwitterionic, quinonoidal, and lactoid tautomers was found to be 22%, 11%, and 67%, respectively [20]. Simultaneously a paper by Chen, Nakamura, and Tamura appeared where only the lactoid tautomer, H_2L , was presumed [32]. Later on, Tamura and co-workers found the fractions of all the three tautomers of fluorescein in water close to ours: 20%, 13%, and 67%, respectively [21]. The corresponding values of 15, 15, and 70% were then published by Klonis and Sawyer [22].

Alternatively, Fompeydie and Levillain [25,26] considered the zwitterion rather as a kind of a transient form, whereas Diehl et al. [42] assumed that the molecular species of fluorescein exist in water just as this tautomer. However, Sjöback et al. [23] considered the quinonoid tautomer as 'generally believed to be prevalent' in aqueous solutions.

Hence, the verification of the problem using the methyl ether of fluorescein as a model compound seems to be pertinent.

Experimental

Materials

Solvents for synthesis were purified according to standard methods. Solvents for visible spectroscopic measurements and pK_a determination were of analytical and spectroscopic grade. Buffer solutions components, i.e., phosphoric, acetic, hydrochloric acids, as well as sodium chloride were of analytical grade. Sodium hydroxide solution was prepared using CO₂-free water and kept protected from the CO₂-containing air. 1,8-Diazabicyclo[5.4.0]undec-7-ene, or DBU (Merck), was used as commercially obtained.

Synthesis of the dyes

Methyl ether ester of fluorescein

This compound was synthesized following Fischer and Hepp [43]. The product was recrystallized from the CCl₄/CHCl₃ (3:1 by volume) mixture: 2.75 g was dissolved in 40 mL of the mixed solvent under heating. Then the solution was filtered and cooled to give the orange precipitate (1.9 g). ¹H NMR ((CD₃)₂S=O) δ /ppm: 8.19 (1H, dd, $J = 7.1$, $J = 2.1$, 3'-H), 7.94–7.68 (2H, m, $J = 7.1$, $J = 2.1$, 4',5'-H), 7.47 (1H, dd, $J = 7.1$, $J = 2.1$, 6'-H), 7.21 (1H, d, $J = 2.1$, 4-H), 6.95–6.68 (3H, m, 1,2,8-H), 6.36 (1H, dd, $J = 9.5$, $J = 2.1$, 7-H), 6.21 (1H, d, $J = 2.1$, 5-H), 3.88 (3H, s, Ph-O-CH₃), 3.55 (3H, s, Ph-CO-O-CH₃). ¹³C NMR ((CD₃)₂S=O) δ /ppm: = 183.89, 165.21, 163.92, 158.39, 153.60, 150.14, 133.92, 133.24, 130.73, 130.40, 130.08, 129.39, 129.51, 128.88, 116.65, 113.60, 114.31, 104.60, 100.60. IR/cm⁻¹, selected bands: 1726, 1642, 1587, 1509, 1453, 1256, 1211, 1105.

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