SPECTRAL AND PHOTOCHEMICAL PROPERTIES OF ALLOXAZINES

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Introduction

Alloxazine derivatives are studied in our group because they are: the isomers of isoalloxazines, products of their chemical, photochemical and biochemical decomposition, despite of similarities they exhibit distincly different properties, they undergo interesting chemical and photochemical reactions under relatively mild conditions.

Results

7-cyanoalloxazine. To compare with the relatively well recognized properties of methyl derivatives of alloxazines (1,2) it was decided to check the impact of electron accepting substituents in the benzene ring of the molecule. As first the 7-cyanoalloxazine was synthesized and its spectral properties studied. This compound exhibits well resolved absorption spectra with maxima at 380.5, 320.5, 264.5, 247.5 and 218.5 nm (buffer pH 6.0), or 377.5, 317.0, 263.0 and 252 (shoulder) in dioxane (insoluble in less polar solvents). Along with the shifts due to change in solvent polarity most prominent in the region of minimum at 335 nm (shifts to 343 in dioxane) and a shoulder is appearing at about 307 nm. Maximum at longest wavelengths shows no distinct vibronic fine structure. Intensity

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ratio of the two maxima at longest wavelengths (*E* 9.2 and 6.0 $\times 10^3$ in dioxane) resembles spectra of isoalloxazines. Fluorescence emission maximum appears at about 435 nm. In presence of pyridine strong phototautomerism occurs (second emission maximum at about 536 nm) and negligible in presence of acetic acid. Mass spectral fragmentation shows characteristic for alloxazines sequence (m/e: 239, 196, 168, 141, 115, 114...) leaving the cyano group intact.

Photoreduction of alloxazines in viscous media. To estimate the angles between transition moments of the two low energy electronic transitions of alloxazine and its derivatives monomethylated in the benzene ring polarization of fluorescence was measured. The results obtained for solutions of alloxazines in glycerol at -10°C in different series were inconsistent and of poor reproducibility. Accidentaly it has been found that solutions of alloxazines in glycerol undergo efficient photoreduction even under dim day light or during measurements in a spectrofluorometer. More detailed studies on deaerated solutions have shown that photoreduction is equally efficient also for alloxazines methylated at N-1 and N-3 and 3-methyllumiflavin, the reaction is of pseudofirst order and fully reversible, its temperature dependence is low (decrease by about 20% when going from 20°C to -10°C), less efficient in 1,3-propanediol and 1,2-propanediol (10 times and 20 times, respectively), does not proceed in ethylene glycol. Most probably the non hydroxylic hydrogen at C-2 of glycerol molecule is the reactive one as in lecithine(3). Thus, use of glycerol should be avoided the more so due to low permeability of gases reoxidation even in vigorously shaked solution is very slow.

Phototautomerism. Studies on alloxazine-isoalloxazine phototautomerism (4) were continued. To avoid possible formation of anionic species solutions of 3-methyllumichrome containing different concentrations of acetic acid in mixture with methanol or dioxane were used. Reaction rate constants estimated using Stern-Volmer equation and the diffussion controlled reaction approach differ by more than order of magnitude (2.9x10^{8} to 9.4x10^{9} l.mol^{-1}.s at 20°C).Activation energies estimated by different methods also do not coincide. Inspite of distinct influence of solvent viscosity (in glycerol) equal phototautomeric efficiency (ratio of isoalloxazinic vs. alloxazinic fluorescence intensity) was observed for 3-undecyllumichrome in 1% acetic acid in cyclohexane and paraffine. In pure acetic acid the highest efficiency is usually reached at 190K but in some experiments it remains constant down to 77K. It is assumed that spatial arrangement of solute and solvent molecules are responsible for the observed phenomena.

Band shape analysis. The solvent polarity and substitution site in the benzene ring dependency of alloxazine absorption and emission spectra is very significant (1,4). Attempts were continued to find reliable and objective method for band shape analysis. To this aim an interface was designed for Cary 118C-spectrophotometer allowing for direct recording of spectra in digitalized form by a computer. Using 3-methyllumiflavin as a model compound and its solutions in dioxane-water mixtures the obtained spectra were smoothed, 2-nd and 4-th derivative calculated and used to control the fit. Both long walength maxima were resolved using Gaussian curves located initially at frequencies indicated by the fine structure, keeping decreasing spacing, and also broadening of half-widths with increasing frequency. Calculations were made using least squares method until the fit was better than 0.2%. Reproducible results give resolution of both lowest energy maxima into 5 subbands sets of different progression. The assumed 0-1 transition of the second maximum shifts by about 2000 cm^{-1} when going from water to dioxane (the other subbands of this progression less). Around 28 kK an irregularity appears which might be ascribed to excitation of a nonbonding electron (appropriate solvent polarity dependence). At frequencies from 29 to 34 kK regular discrepancies were found in more polar solvents, probably indicating the presence of differently solvated solute molecules.

Photodecomposition. Alloxazines are practically photostable in aqueous solutions but undergo slow photodecomposition under prolonged irradiation in alcohols, acetic acid and pyridine (5). Alloxazine and its monomethyl derivatives were irradiated using polychromatic light (250W high pressure Hg lamp, filter cutting of light below 350 nm).In alcohols all alloxazines decompose slowly to many products. In pyridine only 9-monomethyl alloxazine decomposes into 3 major fluorescent products (under anaerobic conditions much faster). All alloxazines undergo photoreaction in acetic acid (under anaerobic conditions photoreduction) but only 6-monomethylalloxazine yields a single photoproduct. This compound was isolated, and work on elucidation of its structure is in progress.

Quarternary flavinium salts. Quarternary flavinium salts and their reduced derivatives show considerable photoreactivity. Among the photoproducts formed during photolysis in different organic solvents no alloxazines were found.

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