Spectroscopy of the Lowest Phosphorescent State of Thiopyronine[1]

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Abstract: Epr and optical studies of randomly distributed molecules of thiopyronine photoexcited into the triplet state have been carried out at 77°K. The zero-field-splitting parameter D^* and the phosphorescence lifetime of the lowest triplet state decrease as the concentration is increased from 10^{-6} to 10^{-2} M. The decrease of this D^* value is discussed in terms of intramolecular charge transfer and the delocalization of the triplet electrons over adjacent molecules.

Valuable information on the lowest photoexcited triplet state of organic molecules has been obtained with electron paramagnetic resonance (epr) spectroscopy.[2] Only few experiments have been done with heteroaromatic molecules which are of theoretical and also of biological interest. Mostly molecules with two or three rings have been investigated, *e.g.*, quinoxaline,[3] phenoxazine,[4] and riboflavin.[5]

In the following, we report the study of the heteroaromatic molecule thiopyronine, which contains a sulfur bridge connecting two benzene rings (Figure 1) in a random matrix at 77°K, by optical and epr methods. The lowest triplet state of thiopyronine lies in the red spectral region. The lifetime of this triplet state and the zero-field-splitting (ZFS) constants are concentration dependent. Thiopyronine is of photobiological interest, since bacteria can be inactivated with visible light in the presence of thiopyronine, and photochemical lesions are induced in ultraviolet light absorbing deoxyribonucleic acid (DNA).[6]

Experimental Section

Thiopyronine was synthesized according to the method of Biehringer and Topaloff.[7] The material was recrystallized several times. The purity was checked by thin layer chromatography and the melting point determined. Thioxanthene, purchased from the Aldrich Chemical Co., was also recrystallized.

The absorption spectra of both compounds in ethanol were measured with a Cary 15 spectrometer using appropriate cells. The fluorescence spectra at room temperature and at 77°K were measured using an Aminco Bowman spectrophotometer with a 1P28 photomultiplier.

Phosphorescence spectra and lifetimes were measured with a phosphoroscope described earlier.[8] In the blue and red regions, appropriate Jarrell-Ash gratings and EMI 6256 and RCA 31000 E photomultipliers were used. Accurate phosphorescence lifetimes were determined with a Nuclear Data, Model ND-2200, multichannel analyzer system operated in the signal-averaging mode. Both degassed and nondegassed samples yielded the same results. All spectroscopic experiments were carried out by dissolving the sample in pure ethanol or phosphate buffer with ethylene glycol (3:2). The emission spectra were corrected for photomultiplier and grating efficiencies with a Sylvania tungsten lamp as a standard.

The epr spectra were recorded at 77°K with a Varian X-band epr spectrometer, Model 4502-15, with Mark II fieldial control. The magnetic field strength was calibrated with a Varian F-8 proton resonance fluxmeter and the frequency was monitored by a Hewlett-Packard frequency counter, Model 5245. Locking in on the $\Delta m = 2$ triplet signal, the lifetime of the triplet state was also measured with a slotted-disk light chopper, by feeding the chopped signal into the multichannel analyzer mentioned above.

For randomly distributed molecules, the fine-structure constants can be obtained from the following relations.[9,10]

$$D^{*2} = (^3/_4)[\delta^2 - 4(g\beta)^2 H_{min}{}^2] \quad (1)$$

$$D^{*2} = (D^2 + 3E^2) \quad (2)$$

$$D = -(^3/_2)Z \qquad E = {}^1/_2(X - Y) \quad (3)$$

$$X = (^1/_6\delta)(g\beta)^2[{}^3H_z{}^2 - {}^2H_z{}^2] \quad (4)$$

Y and Z are obtained from cyclic permutations of x, y, and z. The nomenclature is the standard one used in ref 9 and 10.

Results

Absorption Measurements. The absorption spectra of thiopyronine and thioxanthene are shown in Figure 1. Unlike thioxanthene, thiopyronine is characterized by a large peak in the visible region (λ_{max} 564 nm, ϵ 7.4 × 10^4).[11] As the concentration of thiopyronine is increased from 10^{-5} to 10^{-3} M, a shoulder appears at 530 nm and becomes more pronounced at higher concentrations, depending upon the solvent. In phosphate buffer–glycol (pH 7.6), the shoulder is detectable at a concentration of 5 × 10^{-6} M, in ethanol at 10^{-5} M. At pH 13 the 564-nm peak disappears.

Emission Properties. (a) Fluorescence. Thiopyronine and thioxanthene have fluorescence maxima at 590 and at 325 nm, respectively. The excitation spectra for both molecules are consistent with the absorption spectra.

(b) Phosphorescence. The phosphorescence spectra at 77°K are presented in Figure 2. The 703-nm peak was found to be highly concentration dependent as compared to the 460-nm peak (Table I). With increasing concentration, the peak in the red region of the spectra shifts to longer wavelengths while the lifetime decreases. The lifetime in ethanol is longer compared

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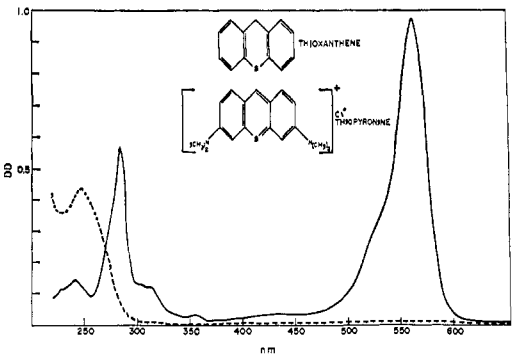

Figure 1. Absorption spectra: – – –, 10^{-5} M thioxanthene; ——, 10^{-5} M thiopyronine in ethanol.

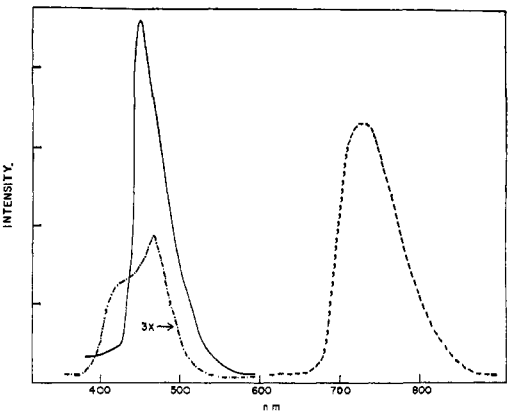

Figure 2. Phosphorescence spectra at 77°K of ——, thioxanthene; –·–·–, thiopyronine; and – – – – –, thiopyronine in ethanol at a concentration of 10^{-3} M.

to that in phosphate buffer–glycol (Table II). For a concentration of 10^{-2} M, the lifetime of 23 msec measured in phosphate buffer–glycol with epr techniques agrees with that determined optically. The results with the blue peak will be discussed in a later paper.

Table I. Corrected Phosphorescence Peaks and Relative Intensities of Thiopyronine in Ethanol, at 77°K, as a Function of Concentration

Concn, M	λ_{max}, nm, ±2	Intensity, arbitrary units	λ_{max}, nm, ±2	Intensity, arbitrary units
5×10^{-6}	703	10	460	1
5×10^{-5}	720	16	480	3
5×10^{-4}	760	41	480	4
5×10^{-3}	800	82		

Table II. Phosphorescence Lifetimes of Thiopyronine in Ethanol and Phosphate Buffer–Glycol (pH 7.6), Measured at 690 nm

	Lifetime, msec, ±10%	
Concn, M	Ethanol	Phosphate buffer–glycol
9×10^{-10}	350	
9×10^{-9}	321	
9×10^{-8}	233	228
9×10^{-7}	170	134
9×10^{-6}	157	107
9×10^{-5}	144	89
9×10^{-4}	127	81

Epr Measurements. The D^* values of the lowest triplet state for different concentrations of thiopyronine in phosphate buffer–glycol and ethanol were calculated from eq 1 and are given in Table III. The $\Delta m = 1$ canonical peaks for thiopyronine could be obtained only at concentrations of the order of 5×10^{-3} M. They were positioned at 3178, 3392, 3651, 2959, 3739, and 2863 G for a microwave frequency of 9239 MHz. The fine-structure constants could be obtained directly from the relations 2, 3, and 4 and also by the method of iteration.[12] The latter procedure also provides the components of the g tensor (Table IV). Applying neutral density filters, the yield of the triplet state is proportional to the incident light intensity. Exciting directly into the 564-nm peak (Corning cutoff filters No. 3484 and 3486), no difference in lifetime and shape of the spectrum was found except for a 20% decrease in emission intensity.

Table III. ZFS Parameter of the Lowest Triplet State of Thiopyronine in Phosphate Buffer–Glycol (PBG) and in Ethanol as a Function of Concentration

Concn, M	Solvent	D^*, cm^{-1}
4×10^{-3}	Ethanol	0.0423 ± 0.001
1×10^{-3}	Ethanol	0.0446 ± 0.001
1×10^{-4}	Ethanol	0.0639 ± 0.002
1×10^{-5}	Ethanol	0.0656 ± 0.002
3×10^{-3}	PBG	0.0394 ± 0.001
6×10^{-4}	PBG	0.0401 ± 0.002
6×10^{-5}	PBG	0.0430 ± 0.002
6×10^{-6}	PBG	0.0434 ± 0.002

Table IV. ZFS Energies of g-Tensor Components Derived from the $\Delta m = 1$ Epr Spectra of Thiopyronine (3×10^{-3} M) in Phosphate Buffer–Glycol

Parameter	Value	Parameter	Value
X, cm^{-1}	0.0066 ± 0.0001	E, cm^{-1}	0.0075 ± 0.0002
Y, cm^{-1}	0.0216 ± 0.0004	g_{xx}	2.003
Z, cm^{-1}	0.0273 ± 0.0004	g_{yy}	1.994
D, cm^{-1}	0.0410 ± 0.0004	g_{zz}	1.999

For thioxanthene only the $\Delta m = 2$ transition was detectable and a D^* value of 0.1097 cm^{-1} was obtained.

Discussion

The strong absorption band of thiopyronine at 564 nm results from the interaction of the chromophore, *i.e.*, the dimethylamino groups, with the ring system. Compared with other structurally related molecules, the direction of the electric dipole oscillator of the long-wavelength absorption band is probably determined by the line joining the nitrogens.[13]

In a highly acidic medium, pH <0.5, the 564-nm peak disappears and hydration of thiopyronine in the carbon bridge is possible, a reaction similar to that of thioxanthene.[14] At pH >11, a nucleophilic attack by OH^- at the carbon bridge could lead to a disruption of the resonance structure. Starting from a thiopyronine so-

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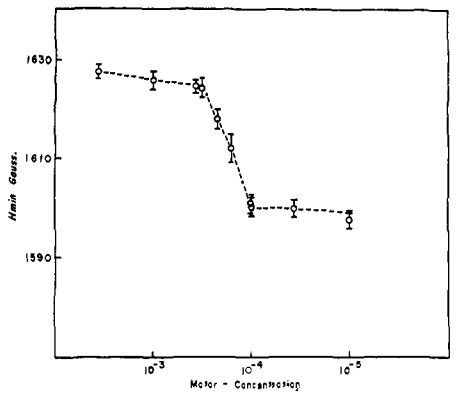

Figure 3. Variation of H_{min} with concentration of thiopyronine in ethanol at 77°K and a microwave frequency of 9239 MHz.

lution at high or low pH, the color determined by the 564-nm peak can be restored by neutralizing the acid or the base. However, this reaction is not completely reversible, since it depends on the time elapsed before neutralization, *e.g.*, if the solution is allowed to stand for 15 min at pH 13 and then neutralized, only 50% of the original intensity, measured at 564 nm, is restored.

At 77°K, with increasing concentration, the phosphorescence spectra of thiopyronine in the red spectral region shift to longer wavelengths with decreasing lifetimes. The dependence of the lifetime upon concentration (Table II) indicates quenching even at a concentration as low as 10^{-7} *M*. This concentration dependence of lifetime is accompanied by the appearance of a shoulder at 530 nm in the absorption spectra, which becomes more pronounced as the concentration is increased from 1×10^{-5} to 1×10^{-3} *M*. Aggregation of dye molecules causing new bands in the absorption spectra and changing the emission properties have been reported earlier in the case of structurally related molecules such as methylene blue and thionine.[15,16]

Flash photolysis experiments of aqueous solutions of thiopyronine (5.5×10^{-6} *M*) at room temperature resulted in triplet-state species with absorption peaks at 340, 370, 400, 425, 470, 480, and 690 nm.[17]

In contrast to the lifetime, which drastically depends on concentration, the ZFS parameters are not measurably influenced by adjacent randomly distributed molecules once they are a critical distance apart. Figure 3 shows that below a certain critical concentration in ethanol ($<10^{-4}$ *M*) the H_{min} does not change appreciably with concentration. The *D** value of 0.0656 cm^{-1} is probably that of a thiopyronine molecule isolated from adjacent thiopyronine molecules. From presently available theoretical and experimental data, the *D** values of heterocyclics and substituted aromatic compounds seem to be comparable with those of the aromatic analogs, *e.g.*, quinoxaline (*D** = 0.1055 cm^{-1})[3] and naphthalene (*D** = 0.1031 cm^{-1}).[18] Substituents such as amino and methyl groups do not seem to appreciably alter the ZFS parameters.[19] Thiopyronine in ethanol ($<10^{-4}$ *M*) has been found to have a *D** value of 0.0656 cm^{-1}, which is lower than that of its aromatic analog, anthracene (*D** = 0.0737 cm^{-1}).[20] Apparently the intramolecular interaction between the dimethylamino groups and the positively charged sulfur contributes to a further delocalization of the triplet electrons as compared to the aromatic analog. Presently available experimental data indicate that the stronger the charge-transfer characteristics, the greater is the delocalization of the triplet electrons.[12,21] *D** values of 0.0502 and 0.0772 cm^{-1} have been reported in the case of intermolecular charge-transfer complexes of 1,2,4,5-tetracyanobenzene with durene and mesitylene in a random matrix.[12]

Increasing the concentration ($>10^{-4}$ *M*), the decreasing *D** values indicate further delocalization of the triplet electrons contributing to the triplet state, probably over two or more adjacent molecules. This seems to be consistent with the fact that on increasing the concentration a transition from a random to a more ordered distribution of molecules takes place (Figure 3). However, it is also possible that the triplet electrons are more delocalized intramolecularly as a result of increased electrostatic intermolecular interactions at higher concentrations. A *D** value of 0.0064 cm^{-1} has been observed for crystalline ion-radical salts. This value has been attributed to the two triplet electrons being distributed over four molecules.[22]

At all concentrations measured, the *D** value of thiopyronine in phosphate buffer–glycol is lower than in ethanol (Table III). This is probably due to a greater degree of dimerization in aqueous solution as compared to that in ethanol. This is further confirmed by the observation that, for comparable concentrations, the phosphorescent lifetimes in phosphate bufler–glycol at 77°K are shorter than those in ethanol (Table II). Structurally related dyes, such as methylene blue and thionine, have been found to dimerize more strongly in aqueous solution than in ethanol.[16]

Epr experiments with single crystals would be valuable to elucidate further the electronic structure of thiopyronine.

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