135. Laser-Induced Luminescence of Flavinium Salts

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Summary

Flavinium salts dissolved in an ethanolic glass exhibit blue fluorescence and orange-red phosphorescence upon excitation with a UV. line from an argon ion laser equipped with UV. optics. This arrangement enables the wavelength distribution and the time-dependence of the phosphorescence to be measured in a relatively short time. Four cationic flavins were investigated. In spite of the small difference in the chemical structure of the compounds studied, large differences in the spectral shapes and in the ratio of quantum efficiencies of phosphorescence and fluorescence became apparent. The phosphorescence lifetimes were of the same order of magnitude, which indicated a similar rate of depletion of the lowest excited triplet state for all four cations. However, the efficiency of triplet formation (intersystem crossing) is affected by slight structural modifications in the pyrimidine subnucleus of the flavinium salt. The results point to a possible role of vibronic spin-orbit coupling in the intersystem crossing.

Introduction. – Flavins[3]) are widespread in nature. They function as redox catalysts in many biological electron transfer reactions [1]. The yellow pigments also play a role in photobiological processes like phototropism and photoreception (see e.g. [2] and references cited therein) and they can mediate in many *in vitro* photochemical reactions (for a review see [3]). Flavins in their excited states have been implicated in the primary photochemical events. Because of these considerations quite a number of spectroscopic studies on flavins have been carried out ([2] [4] and references therein).

In a previous paper we reported on the light absorption and fluorescence properties of neutral and cationic flavins [5]. The cations have in common that the fluorescence in aqueous solution is strongly quenched resulting in very short lifetimes and low quantum yields [5]. Despite small structural differences of the cat-

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[3]) Flavin = 7,8-dimethylisoalloxazine = 7,8-dimethyl-10-substituted-2,3,4,10-tetrahydrobenzo[*g*]-pteridine-2,4-dione.

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ions the spectral shapes of absorption and emission spectra in apolar solvents show distinct variations indicating changes in the vibronic transitions between ground and excited singlet states [5].

When an ethanediol/water solution of a flavin protonated on N(1) is frozen, fluorescence reappears and phosphorescence becomes visible [6]. As a continuation of the previous study [5] we have investigated the triplet state of the compounds by means of phosphorescence observed in a rigid glass at low temperature. For this study we have developed a sophisticated set-up for obtaining luminescence spectra and lifetimes. This system allows us to record both the total emission (fluorescence and phosphorescence) and phosphorescence spectra in a relatively short measuring time. This has the advantage that photodestruction associated with prolonged irradiation time can be minimized. An elaborate and schematic description of the experimental set-up is given. The combination of the argon-ion laser as the source of excitation with the sensitive photon-counting technique and the averaging capabilities makes this arrangement a very powerful method.

It turned out that the shape of the phosphorescence spectra of structurally closely related cationic flavins is markedly different, whereas the phosphorescence lifetimes are similar. The most significant result is the high phosphorescence/fluorescence ratio of one of the cations. This indicates that slight structural modifications in the pyrimidine subnucleus of the isoalloxazine moiety have significant effects on the intersystem crossing efficiency.

Materials and Methods. – *Materials and Sample Preparation.* The flavinium salts *(cf. Fig. 3)* were prepared according to [7]. They were dissolved in fluorescent grade ethanol *(Merck)* containing a trace amount of perchloric acid in order to prevent decomposition. The compound protonated on N(1) was obtained by dissolving 3-methyl-lumiflavin in ethanol acidified with a few drops of 6N $HClO_4$. Absorption spectra were obtained with a *Cary*-14 spectrophotometer. The absorbance at the exciting wavelength (363 nm) was adjusted to 1.0/cm at room temperature. The solutions were transferred to *Varian* quartz EPR. tubes, selected for low fluorescence background.

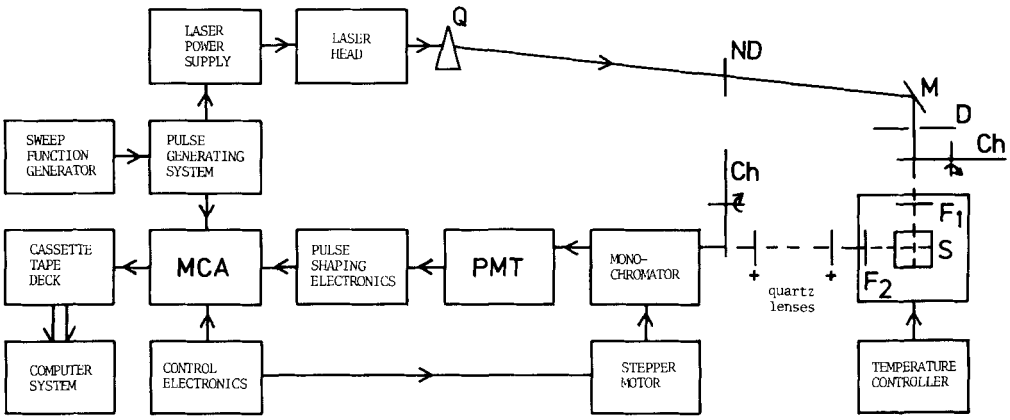

Fig. 1. *Schematic diagram of experimental set-up.* Q = quartz wedge; ND = neutral density filters; M = mirror; D = diaphragm; Ch = chopper blade; F_1 = excitation filters; S = sample tube; F_2 = emission filter; PMT = photomultiplier tube in cooled housing; MCA = multichannel analyzer.

Degassing was performed by bubbling helium through the solution for 15 minutes. All manipulations were carried out in the dark whenever possible.

Emission Spectra. A block diagram of the measuring system is given in *Figure 1*. For excitation, an argon-ion laser with UV. option, CR18UV, was used. With the UV. all-lines mirrors installed the output power was 3.9 W at 50 A discharge current. In the experiments to be described only a small fraction of this power was used. The output power was kept at a constant value of 200 mW all-lines UV. by the light stabilization electronics of the laser power supply. The laser beam was attenuated further with a neutral density filter (ND.). The 363.5 nm laser line was selected with an extra cavity quartz wedge (Q) and a diaphragm (D). Synchronous motors (50 Hz, 220 V) were used for driving the chopper blades (Ch) in excitation and emission light paths. The chopper blades could be adjusted to rotate in and out of phase. Also for minimizing background, a *Balzers* 363 nm interference filter (F_1) was placed in the input gate of the sample compartment (S). The quartz EPR. sample-tube was cooled down in this compartment to the desired temperature (usually 100 K) by a regulated flow of liquid nitrogen. A *Schott* KV418 cut-off filter (F_2) was placed in the emission light path for rejection of the excitation light. The detection wavelength was scanned with a 0.25 nm *Jarrel Ash* monochromator, type 82410, with a 1180 lines/nm grating blazed at 500 nm. The scan was performed by a stepper motor, which was driven by a stepper motor controller from *Applied Photophysics*. Emission light was detected with an EMI 9659QB photomultiplier tube (PMT.) in a dry-ice cooled housing from *Products for Research*. The photon counting technique was used by conditioning the photon pulses from the PMT. in a preamp (*Ortec* 113), spectroscopy amplifier (*Ortec* 451) and single channel analyzer (*Ortec* 420A). The photon counts were collected in a multi-channel analyzer (MCA), model 8001 from *Laben*, operating in multiscaling mode. Every phosphorescence spectrum consisted of 511 wavelength steps ($\Delta\lambda$), corresponding with the 512 channels of the MCA. During a time $\Delta\tau$, which can be selected with the control electronics, the photon counts from the (out of phase chopped) phosphorescence light were integrated in one channel of the MCA. At the end of time $\Delta\tau$ the detection wavelength was incremented with $\Delta\lambda$ by the control electronics and stepper motor drive. At the same time the MCA was triggered and the photon counts stored in the next channel. Total emission spectra were detected in a similar way, except that the chopper blades were rotating in phase. Every spectrum can be stored on cassette tapes and, if desired, can be corrected for the wavelength dependence of the sensitivity of the detection channel by computer processing. The corrected spectra can be plotted on a wavenumber scale as shown in *Figure 3*.

Phosphorescence Decay. For measuring the phosphorescence decay, the detection wavelength was set at a fixed wavelength corresponding to the maximum in the phosphorescence spectrum. The output of the argon-ion laser was 100% intensity modulated with an about 50% duty cycle by a pulse generating system from *Farnell (Fig. 2)*. The repetition frequency ($1/t_1 = 0.1$ Hz) was generated by a sweep function generator (*Wavetek* 185). The data collection in the MCA was initiated by direct triggering (by a pulse from the pulse generating system) of the timing electronics of the MCA (*Laben*, model 8190). Delayed control was provided by the pulse generating system to the laser power supply. By setting this delay (t_d in *Fig. 2*) the start point of the decay relative to the start point of the data collection can be chosen. In this way we can inspect both types of curves (build-up and decay of phosphorescence). In particular the end value of the

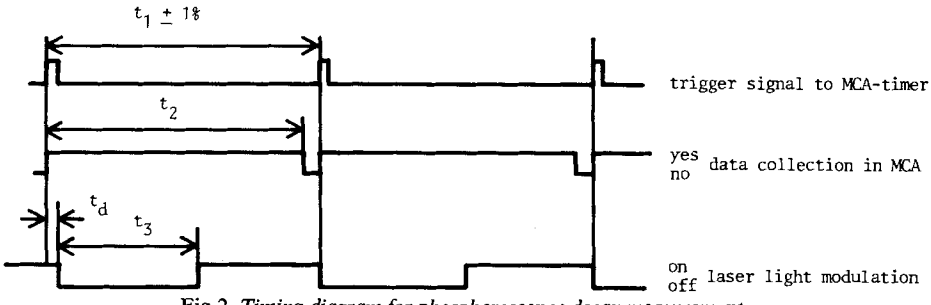

Fig. 2. *Timing diagram for phosphorescence decay measurements*

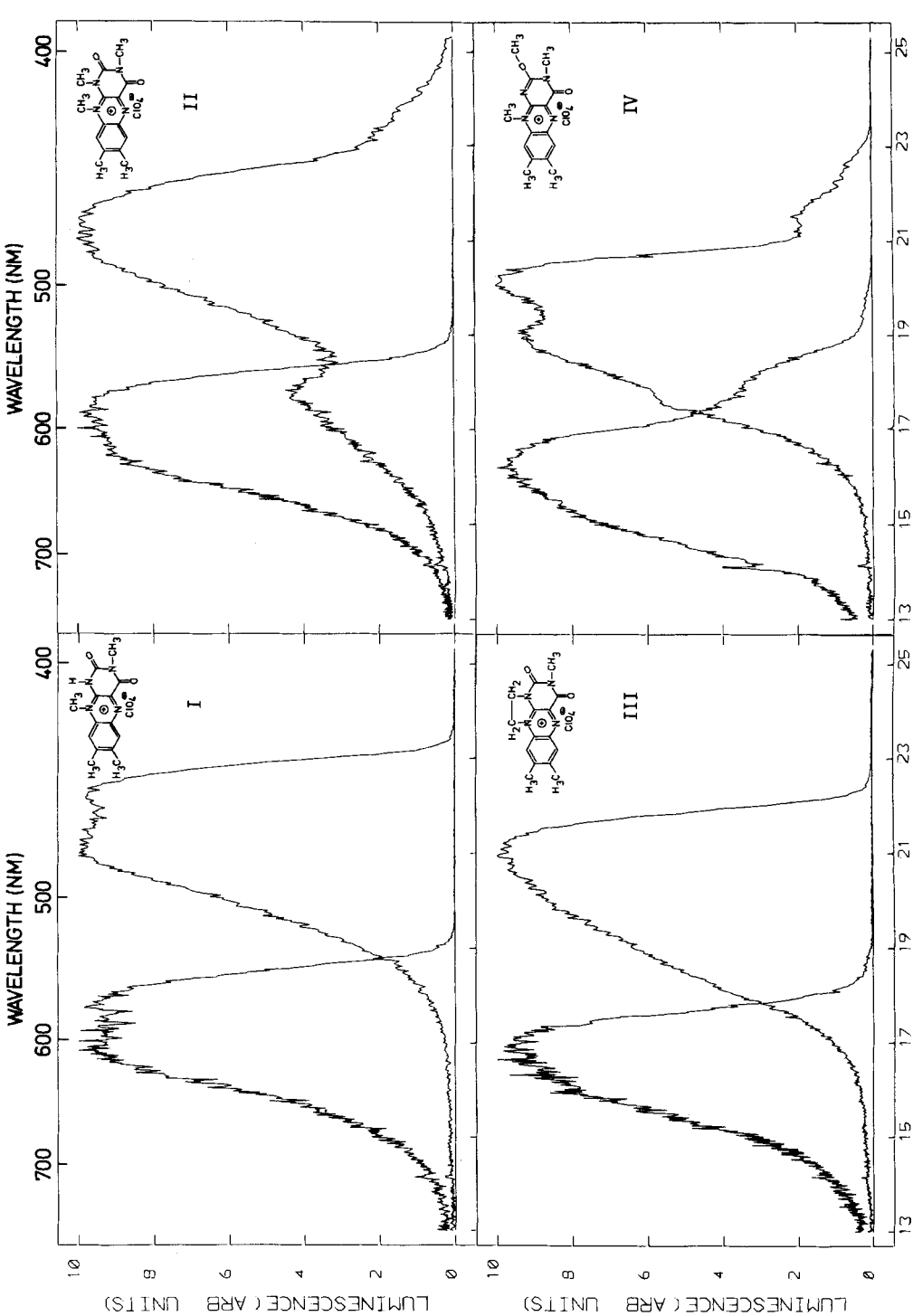

Fig. 3. *Normalized total emission and phosphorescence spectra of cationic flavins in an ethanolic glass at 100K.* Each spectrum was recorded in 250 seconds, corrected for variations in detector sensitivity with wavelength and is plotted on a wavenumber scale (KiloKayser). Details are given in the Methods section and in the *Table*.

build-up curve can be accommodated in the first few channels of the MCA. In our experiments *(Fig. 4)* only the phosphorescence decay curves are shown. The MCA was operating in the multiscaling mode and the step time from one channel to the next was controlled by the timer of the MCA. By choosing the time of data collection for the 512 channels (t_2 in *Fig. 2*) very close to the repetition period of the light modulation (t_1 in *Fig. 2*) an almost 100% data collection efficiency was obtained. The input counts for the MCA came from the *Ortec* modules, as described in the spectral measurements. Because of the good stability of the modulation frequency of the sweep function generator, of the MCA-timer and of the synchronous chopper motors, our first experiments showed an interference pattern on the display of the MCA. Thus, we made the modulation frequency ($1/t_1$) semi-random relative to the chopping frequency and to that of the MCA-timer by slowly sweeping that modulation frequency about ±1%. The data collection time t_2, delay time t_d and laser light modulation time t_3 were kept at a constant value. This averaging procedure resulted in smooth decay curves.

Results and Discussion. – The luminescence spectra of the four different flavinium salts are given in *Figure 3*. The following features can be distinguished. Both fluorescence and phosphorescence are blue-shifted relative to the corresponding spectra of the neutral 3-methyllumiflavin [4]. The total as well as the phosphorescence spectra of all compounds are different in shape. The spectrum of compound IV exhibits the best resolved fine structure in both types of spectra. In cation IV the phosphorescence starts around 20,000 cm^{-1}, a shoulder is developed at about 18,000 cm^{-1} and a maximum is visible at about 16,000 cm^{-1}. The 0,0-transition ($T_1 \rightarrow S_0$) must therefore be located at about 19,000 cm^{-1}. In fluorescence a similar shoulder can be observed at 21,500 cm^{-1} ($S_1 \rightarrow S_0$) and therefore the singlet-triplet energy difference corresponds to about 2,500 cm^{-1}. The spectral shapes of the other flavinium ions are hardly resolved. The cation protonated at N(1) (compound I) exhibits both in fluorescence and in phosphorescence a dual maximum. In this particular case the 0,0- and 0,1-transitions can be distinguished (separation about 1,000 cm^{-1}). The other two compounds (II and III) show the least spectral resolution and therefore the peak locations are only indicative for the position of the electronic transitions. The positions of the phosphorescence and fluorescence transitions are given in the *Table*.

The most significant result was obtained for the flavinium salt II, where the phosphorescence was superimposed on the fluorescence spectrum. In contrast, the phosphorescence of the other related cations I and III was completely buried un-

Table. *Spectroscopic Properties of Cationic Flavins in an Ethanol Glass at 100 K*

Compound[a])	Luminescence maximum			
	Phosphorescence (cm^{-1})	Fluorescence (cm^{-1})	Q_p/Q_f[b])	τ_p[c]) (ms)
I	18,000	22,200	0.017	198
II	17,500	21,500	0.48	161
III	17,000	21,000	0.033	191
IV	19,000[d])	21,500[d])	0.066	173

[a]) For the structure, see *Figure 3*.
[b]) Ratio of quantum yield of phosphorescence and of fluorescence.
[c]) Observed phosphorescence lifetime.
[d]) Location of 0,0-transitions.

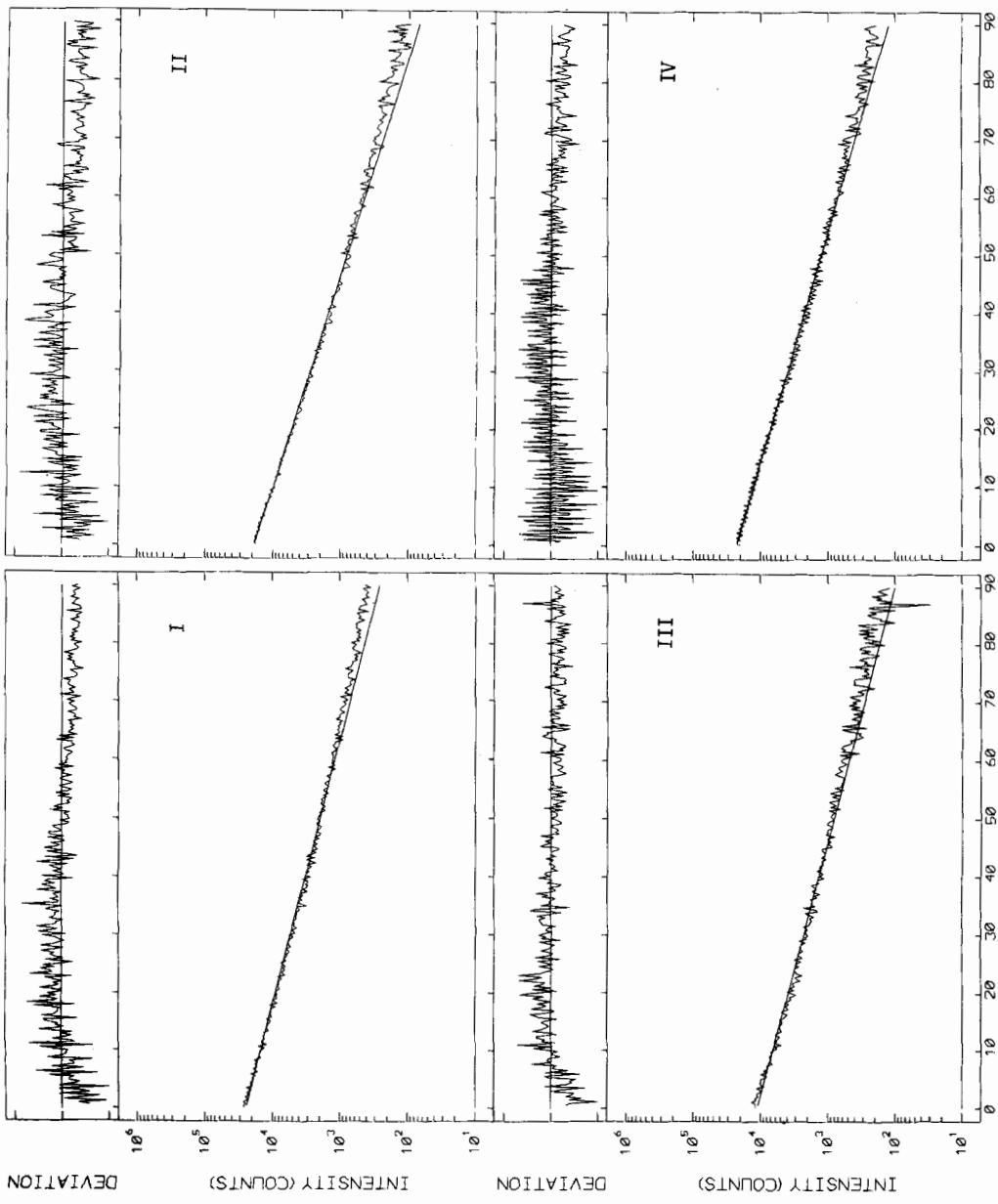

Fig. 4. *Phosphorescence decay patterns of cationic flavins.* Also shown is the fit with a single phosphorescence lifetime. The lifetimes are collected in the *Table*. The deviation in channel i of the MCA is defined as $DV(i)=[P_c(i)-P(i)]/\sqrt{P(i)}$, where P(i) is the phosphorescence content and $P_c(i)$ the calculated content in channel i. Details are given in the Methods section.

der the fluorescence. In compound IV the shoulder at 18,000 cm^{-1} in the total emission spectrum probably originates from the phosphorescence. Because of experimental difficulties we have not determined the quantum yield of the fluorescence at 100 K relative to a standard[4]). In general, however, the fluorescence appearing from the four samples is not widely different under comparable measuring conditions. At any rate the ratio between the quantum yields of fluorescence and phosphorescence of each individual sample can be determined with acceptable precision and the results obtained are collected in the *Table*. This ratio is by far largest for compound II[5]).

The phosphorescence decay patterns of the four cations are shown in *Figure 4* and the lifetimes calculated from these are given in the *Table*. It is evident that all cations can be characterized by the same lifetime of 160–200 ms. The deviations indicate a slight heterogeneity (*cf.* the tail of the decay curves in *Fig. 4*), which we did not investigate further. Phosphorescence reflects both radiative and radiationless processes. If the intersystem crossing to the ground state ($T_1 \rightarrow S_0$) becomes more efficient in one of the compounds the phosphorescence must decay more rapidly. This is not observed and it means that the rate of triplet depopulation of these cations is similar.

The data as presented do not enable us to draw firm conclusions about the intersystem crossing efficiency from excited singlet to the triplet manifold ($S_1 \rightarrow T_1$). To establish these efficiencies precisely one should employ the laser photolysis approach as outlined by *Grodowski et al.* [8]. To a first approximation one can determine *Kasha's* intersystem crossing ratio, $Q_p/Q_f = K_{isc}/K_f$ (K_f from reference [5]), which leads to K_{isc} values of 8.7, 1.3 and 3.0 MHz for compounds II, III and IV respectively.

In summary, the following conclusions can be drawn. The spectral shape is different in the three related cationic species, which implies that the *Franck-Condon* factors across the phosphorescence spectrum can be influenced by slight structural modifications. Methylation cannot be considered to be a large perturbation and would not be expected to introduce large spin-orbit coupling into the aromatic ring system (*cf.* the spectroscopic data of naphthalene and 1-methylnaphthalene cited in [9], p.272). When the bridged cation III is compared with compound II, methylated at N(1) and N(10), it is reasonable to suppose that the conformation of both cationic flavins differ somewhat. The bridge can impose some constraints on possible vibrational modes in the molecule, whereas the two methyl groups of compound II might show steric hindrance causing a slight distortion in the molecule. Since spin-orbit mixing is not expected to differ much, vibronic spin-orbit coupling ([9], p.409) may then provide an alternative mechanism to explain the enhanced phosphorescence. A detailed description of this interaction applied to a complicated molecule like these flavin cations falls outside the scope of this contribution. Recently, resonance *Raman* spectra of pro-

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[4]) The intensity of the signal depends, among other factors, on the position of the quartz tube in the dewar, on the quality of the glass, on fluctuations in temperature, *etc.*

[5]) Under any conditions the phosphorescence yield of compound II is much larger than that for the other compounds.

tonated FMN (on N(1)) and the bridged compound III were reported [10]. Although a limited assignment of the vibrations was made, the small differences, observed in the spectra of both cationic species, were not interpreted. A qualitative conclusion from this work is that small perturbations in the N(1)–N(10) region have important consequences for the photophysical properties of the flavinium salts.

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