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Electronic Spectra of the Various Hydrogen-Bonded Species of Pyrazine in a Mixed Hydroxylic Solvent

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Abstract: The 77°K phosphorescence, phosphorescence excitation, and absorption spectra of pyrazine in ethanol with up to 0.2 mol fraction water are presented. The phosphorescence origin of pyrazine in pure ethanol lies at 26,290 cm^{-1}. For pyrazine in an ethanol–water solvent two additional phosphorescent systems with origins at 26,620 and 27,370 cm^{-1} appear. These are assigned as due to pyrazine hydrogen bonded with one and two water molecules, respectively. All three species are found to have lifetimes of less than 21 msec indicating lowest triplet states of $n\pi^*$ character for each. The excitation spectra of the three species are presented. These spectra indicate that upon going from one species to another the shifts in the singlet–singlet and singlet–triplet $n \rightarrow \pi^*$ transition energies are identical. The nature of the shifts upon going from one species to another is discussed. The phosphorescence quantum yield of the hydrated species is found to be less than that of the unhydrated species. Deuteration of the solvent has an enhancing effect on the phosphorescence quantum yield of the hydrated species but does not effect the yield of the unhydrated species.

Solvent shifts of the $n \rightarrow \pi^*$ transitions of azines and ketones have been investigated by many workers.[1-5] These transitions generally show a blue shift on changing from a hydrocarbon to a hydroxylic solvent. Brealey and Kasha have examined the change in the $n \rightarrow \pi^*$ absorption spectra of pyridazine and benzophenone in various hexane–ethanol solvent mixtures. Their studies clearly indicated that the blue shift upon addition of alcohol is due to the formation of a hydrogen-bonded complex of the alcohol with the azine or ketone. The blue shift results because the hydrogen bonding are stronger in the ground state than in the excited state. The role of the Franck–Condon principle on the blue shifts was later discussed by Pimentel[6] and was shown by Krishna and Goodman to be important in the electronic spectra of pyrazine.[4]

In this paper we examine the electronic spectra of pyrazine in a mixed hydroxylic solvent of ethanol and water. We will show that three distinct hydrogen-bonded species exist in this solvent and will present their spectra. This system offers the unique opportunity of examining several hydrogen-bonded complexes of various strengths in the same solvent system. Pyrazine is chosen for study because it is a prototype molecule with well assigned lowest singlet and triplet excited states of B_{3u} ($n\pi^*$) character.[3,7,8]

Experimental Section

Pyrazine (Aldrich Chemical Co.) was purified by repeated zone melting. Distilled water and absolute ethanol (U. S. Industrial Chemical Co.) were used without further purification. "Superdry" ethanol was prepared by the method of Lund and Bjerrum.[9] D_2O (Matheson Coleman and Bell) and CH_3CH_2OD (Stohler Isotope Chemical Co.) were used without further purification. All solutions were placed in quartz tubes and rapidly immersed in liquid nitrogen in optical quartz dewars. Degassing was found to have no effect on the spectra or lifetimes and was not employed in subsequent measurements. It was found that ethanol–water solutions with up to 10% water by volume will form perfect glasses if narrow quartz tubes of 3–4-mm inside diameter are used. The spectra were found to be independent of whether or not the glass cracked.

Excitation and emission spectra were taken on a Baird-Atomic Model SF-1 fluorescence spectrometer. Some emission spectra were also taken on a Jarrell-Ash 0.75-m Czerney-Turner spectrometer (Model 78-460) with exciting light from a 150-W Xenon lamp filtered with a $NiSO_4$ solution and a Corning 754 filter. An RCA 1P28 photomultiplier tube was used with this spectrometer. The absorption spectra were taken on a Cary 15 spectrometer with the samples placed in contact with liquid nitrogen in a quartz dewar.

Lifetimes were measured by using a variable-speed rotating cam to chop the exciting light and sending the decay signal into the Jarrell-Ash monochrometer. The signal from the photo tube was displayed on a Textronix 5103 N storage oscilloscope.

Results

Phosphorescence Spectra. The 77°K phosphorescence spectrum of pyrazine in pure ethanol is shown in Figure 1A. The 0,0 band is the most intense band in the spectrum and lies at 26,290 cm^{-1}. We will call this subspectrum I in what follows. The phosphorescent lifetime is 20.5 msec and is consistent with the $^3B_{3u}$ ($n\pi^*$) assignment of the phosphorescent state.

The phosphorescence spectra of pyrazine in ethanol–water mixtures with mole fractions of water of 0.12, 0.17, and 0.22 are shown in Figures 1B–D, respectively. All of the spectra in this figure were excited with continuous radiation below 3300 Å so that all species get excited. We notice the appearance of two new bands to the blue of the origin of subspectrum I. One band is located at 26,620 cm^{-1} (origin of subspectrum II),

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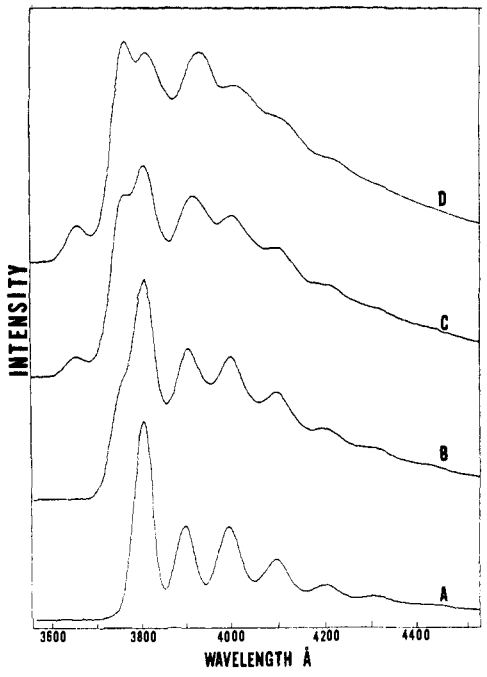

Figure 1. The 77°K phosphorescence spectra of pyrazine in ethanol with various amounts of water. The concentration of pyrazine was 2.0×10^{-3} M. The mole fractions of water are 0.0, 0.12, 0.17, and 0.22 in A, B, C, and D, respectively.

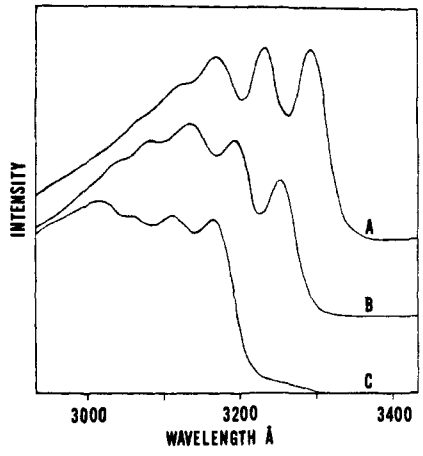

Figure 2. The 77°K phosphorescence excitation spectra of pyrazine in an ethanol–water solvent. The concentration of pyrazine was 1.0×10^{-4} M. Spectrum A is of pyrazine in pure ethanol using the origin of subspectrum I as the emission energy. Spectra B and C are in a solvent with a 0.22 mol fraction of water. The emission energies of these spectra are the origins of subspectra II and III, respectively.

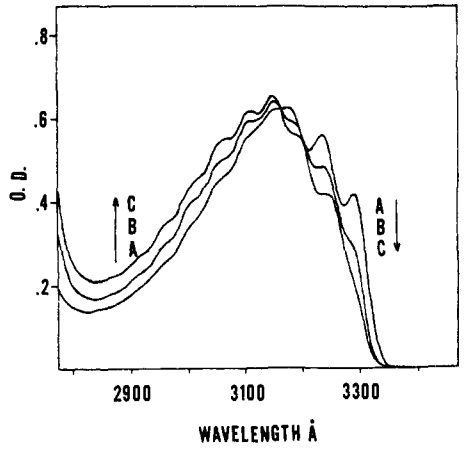

Figure 3. The 77°K absorption spectra of 2.38×10^{-3} M pyrazine in pure ethanol (A), ethanol–water with $X_{H_2O} = 0.026$ (B), and ethanol–water with $X_{H_2O} = 0.065$ (C). The path length was 3 mm and the pyrazine concentration is not corrected for solvent contraction.

and the other is located at 27,370 cm^{-1} (origin of subspectrum III). The relative intensities of these subspectra are found to be independent of pyrazine concentration over a range of 10^{-2}–10^{-4} M. The phosphorescence lifetime measured for the origins of subspectra I, II, and III are 20.5, 20.3, and 18.7 msec, respectively.

We assign the three subspectra as due to the emission from the three possible pyrazine hydrogen-bonded species in this mixed solvent. Subspectrum I is due to pyrazine hydrogen bonded to ethanol at both nitrogens, subspectrum II is due to pyrazine hydrogen bonded to ethanol at one nitrogen and water at the other, and subspectrum III is due to pyrazine hydrogen bonded to water at both nitrogens. The shift in the origin band in going from the two ethanol species to the one water one ethanol species is 330 cm^{-1} and the shift in going from the two ethanol to the two water is 1080 cm^{-1}.

Phosphorescence Excitation Spectra. In order to further demonstrate that the three emission subspectra are due to three separate species, we have taken phosphorescence excitation spectra by monitoring the intensity of the origin of each subspectrum separately as a function of exciting wavelength. The phosphorescence excitation spectrum of pyrazine in pure ethanol at 77°K is shown in Figure 2A. The emission energy used to get this spectrum was the 26,290-cm^{-1} origin of subspectrum I. From this spectrum we find the origin of the singlet–singlet $^1B_{3u}$ ($n\pi^*$) ← $^1A_{1g}$ (ground) transition to be located at 30,270 cm^{-1}. In Figures 2B and 2C we see the excitation spectra of pyrazine in an ethanol–water solution which has a 0.17 mol fraction of water. In Figure 2B the origin of subspectrum II is monitored and in Figure 2C the origin of subspectrum III is monitored. The 0,0 excitation bands are located at 30,620 and 31,380 cm^{-1}, respectively. The fact that we get three different excitation spectra is therefore strong evidence that we are seeing three distinct pyrazine species in this mixed solvent rigid solution.

Absorption Spectra. The absorption spectrum of pyrazine in pure ethanol at 77°K is shown in Figure 3A. The positions of the peaks in this spectrum coincide with those of the phosphorescence excitation spectrum of species I. The effect of adding water to the solvent is shown in Figures 3B and 3C. We see a dramatic decrease in the intensity of the 0,0 band and an enhancement of the intensity in the high energy portion of the spectrum. The overall intensity of the spectrum is not effected by water indicating that the three species have similar absorbing powers.

It is difficult to get a good estimate of the decrease in concentration of species I with the addition of water from Figure 3. A good estimate of this can be obtained from Figure 4 which shows the phosphorescence spectrum of pyrazine in ethanol with various amounts of water. In this set of spectra the excitation wavelength has been set to correspond to the 0,0 absorption

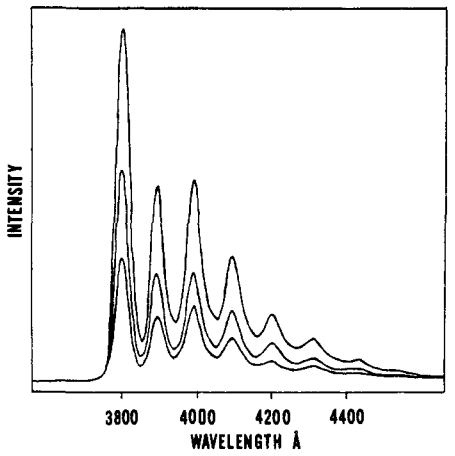

Figure 4. The 77°K phosphorescence spectra of 3.4×10^{-4} M pyrazine in ethanol with various amounts of water. The excitation wavelength is the absorption origin of pyrazine in pure ethanol. The mole fractions of water are 0.0, 0.033, and 0.065, respectively.

band of species I so that no other species are excited. The relative intensities of these spectra are therefore proportional to the concentration of species I. We see that by the time the mole fraction of water reaches 0.065 the concentration of species I has decreased to about a third of its initial value. It is interesting to note that at this concentration of water the phosphorescence spectrum of species I strongly dominates that of species II independent of the exciting wavelength (see Figure 1). Analysis of these results indicates that the quantum yield of phosphorescence for species II is less than a third of that for species I. In a solvent of CH_3CH_2OD and D_2O we find that the quantum yield of species II is about twice that of species II in the protonated solvent. The lifetimes are only slightly affected by solvent deuteration. Because of the small concentration of species III we are unable to determine the effect of deuteration on its quantum yield.

Discussion

Equilibrium in the Excited State. The fact that the phosphorescence excitation spectra at 77°K are so distinct for the three species indicates that the rate of equilibrium of pyrazine with ethanol and water is slow compared with the lifetime of an excited molecule. If this were not the case and the various species rapidly interconverted to one another in the excited state, the excitation spectrum would be independent of the emission wavelength used and the emission spectrum would be independent of excitation wavelength used.

Interpretation of the Spectral Shifts. The spectral shifts of the excitation origin bands on going from the no water species to the one water species and from the no water to the two water species are 350 and 1100 cm^{-1}, respectively. The 1100-cm^{-1} shift is identical with the shift observed by Mason[10] in the room temperature $n \rightarrow \pi^*$ absorption spectrum of pyrazine on going from ethanol to water as a solvent. The 350- and 1100-cm^{-1} shifts in the excitation spectra are identical within experimental error to the shifts observed for the phosphorescence origins. This result indicates that the singlet and triplet B_{3u} ($n\pi^*$) states of pyrazine form hydrogen bonds of similar strength as might be expected.

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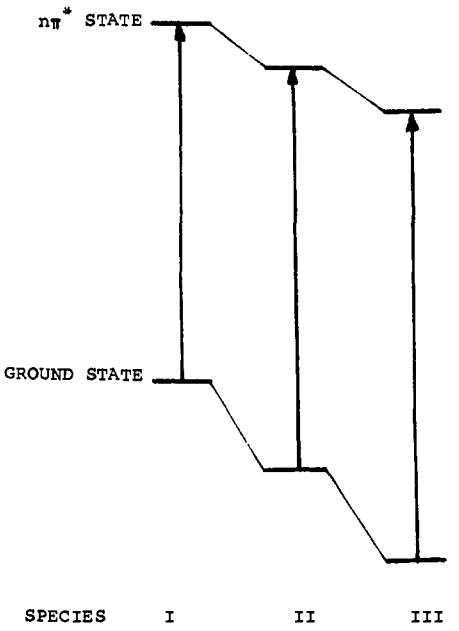

Figure 5. The effect of replacing weak hydrogen bonds with strong hydrogen bonds on the transition energies of pyrazine. Species I, II, and III are pyrazine hydrogen bonded to two ethanol, one ethanol and one water, and two water molecules, respectively.

The observation of blue shifts in pyrazine as hydrogen bonds with ethanol are replaced by hydrogen bonds with water can be rationalized with reference to Figure 5. When one ethanol is replaced by one water a weaker hydrogen bond is replaced by a stronger hydrogen bond. Because of this the ground state of species II is more stable than that of species I. In the $n\pi^*$ excited state we expect less "nonbonding" electron density at the nitrogens and as a result stabilization due to the formation of a stronger hydrogen bond should be less marked. The blue shift on going to species II is therefore due to the difference in the ground and excited-state stabilization energies of species II relative to species I. On going from species II to species III, the second weak hydrogen bond is replaced by a strong hydrogen bond and a blue shift again results.

To a first approximation, we might expect the stabilization energies on going from species I to II to be similar to those on going from species II to III, as indicated in Figure 5. However, this cannot be the case, since the observed shift on going from species I to II is less than half of that on going from species II to III. We believe that Figure 5 is correct for the ground state. In other words, the stabilization energy resulting in the replacement of the first weak hydrogen bond by a strong hydrogen bond is similar to the stabilization energy resulting from the replacement of the second weak hydrogen bond by a strong hydrogen bond. This is logical because the two nitrogens should have approximately the same "nonbonding" electron density independent of whether they are coordinated to ethanol or water. The small shift on going from species I to II is interpreted as being due to a larger excited-state stabilization energy for species II than is indicated in Figure 5. In species I and III the $n \rightarrow \pi^*$ promotion results in an equal decrease in the "nonbonding" electron density of the two nitrogens. This will not be the case for species

II. One would expect excited species II to have more "nonbonding" electron density on the nitrogen hydrogen bonded to water than on the nitrogen bonded to ethanol. By acquiring such a distribution upon going to the excited state, the molecule maintains as much as possible the stability due to the strong hydrogen bond with water while only sacrificing stability due to a weak hydrogen bond with ethanol. The result is a large excited-state stabilization energy on going from species I to species II and therefore a small blue shift in the spectrum of species II relative to species I. Although these arguments are not based on any theoretical evidence we feel that they are qualitatively sound. It is possible that the small shift upon going from species I to species II is in some way associated with the dipole moment of species II.[11] If this were so we would expect the peak of species II to be shifted as the dielectric constant of the solvent changes. Since we observe no shift in the species II peak as the water concentration changes or upon addition of up to 10^{-1} *M* LiCl to the solvent, we can rule out dipole moment contributions to the shift.

Low Phosphorescence Yield of Species II. The small phosphorescence quantum yield of species II relative to species I indicates either a smaller quantum yield of intersystem crossing from the excited singlet state or a more rapid rate of intersystem crossing from the triplet for species II. The latter can be ruled out since the lifetime of species II is similar to that of species I and because the protonated and deuterated species II have the similar lifetimes even though they have different phosphorescence yields. It is known that a $^3\pi\pi^*$ state lies between the lowest $^1n\pi^*$ and $^3n\pi^*$ states in pyrazine.[12] Upon going from species I to species II the energy gap between $^1n\pi^*$ and $^3\pi\pi^*$ should get larger and this may well result in a smaller intersystem crossing rate from the $^1n\pi^*$ state. The increased phosphorescence yield of species II in deuterated solvents is interesting and is probably associated with a reduction in the rate of internal conversion from the $^1n\pi^*$ state to the ground state. Internal conversion from $^1n\pi^*$ is known to be important in the azines.[13]

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Proton Magnetic Resonance Studies of Metal Complexation of Nucleosides in Dimethyl Sulfoxide

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Abstract: For the purpose of deciding whether the group IIa metal ions (Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) and the group IIb ones (Zn^{2+}, Cd^{2+}, and Hg^{2+}) have different biochemical characters with respect to formation of the complexes with nucleosides, we have carried out proton resonance studies. The nucleosides used were adenosine (Ad) and guanosine (Gu), in dimethyl sulfoxide medium. The dependences of the proton shieldings on the metal concentrations have been analyzed to yield limiting shifts and formation constants of the 1:1 metal–nucleoside complexes in equilibrium, and different effects were detected between the group IIa and IIb ions. The changes of the proton nuclear shielding by formations of the metal complexes are ascribed to the electrostatic field effect originating from the charges of the metal ions. For the group IIa–Gu complexes, the distances of the ion from the binding sites were estimated as 3.7 Å, using the Buckingham–Musher equation.

The nucleosides are of prime biological importance, and proton magnetic resonance (pmr) studies on the binding of metal ions to these have been reported by a number of investigators.[2-6] Studies of metal complexes of nucleosides may serve to some degree as models for the nucleic acid and metal ion interactions. Wang and Li[4a] measured the pmr shifts of the systems in which the metal salt is $ZnCl_2$ and the nucleosides are adenosine (Ad), guanosine (Gu), cytidine (Cy), and uridine (Ur) in dimethyl sulfoxide (DMSO) medium, and estimated the formation constants of the 1:1 Zn–nucleoside complexes. Kan and Li[5] found the formation constants of the 1:1 mercury(II) complexes with Ad, Gu, and Cy in DMSO by the same technique. Jordan and McFarquhar[6] reported evidence concerning the existence of a 1:1 Ca^{2+}–Gu complex.

Wacker, *et al.*,[7] found that the nucleic acids extracted from natural products, especially RNA, contain a considerable amount of metals Mg, Ca, Sr, Ba,

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