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Optical Detection of Magnetic Resonance Measurements of the Effects of pH on the Triplet States of Benzimidazole and Purine

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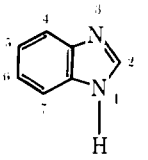
Abstract: The triplet states of benzimidazole, purine, their protonated cations, and the anion of purine have been investigated by optical detection of magnetic resonance (ODMR) in zero field. Large, measurable changes occur in the zero-field splitting (ZFS) parameters upon protonation and deprotonation. Microwave saturated phosphorescence decay measurements on benzimidazole, along with slow passage ODMR, have been used to obtain the ZFS parameters $D = 11.50$, $E = 3.25\ m^{-1}$ for benzimidazole, where the major principal axis is normal to the molecular plane. The spin-lattice relaxation pattern also was determined. Protonation, leading to C_{2v} symmetry, changes the ZFS to $D = 11.80$, $E = -1.32\ m^{-1}$. Probable D and E values are reported for the other species investigated. For purine, and its protonated cation, as well as 5′-adenylate, we assign $D < 3|E|$ (major principal axis as in benzimidazole) based upon comparison with theoretical calculations. Triplet decay lifetimes were measured. Protonation has little effect on the triplet lifetime of benzimidazole, whereas the lifetime of triplet purine lengthens considerably upon protonation and deprotonation. The purine species decay nonexponentially, while the benzimidazole species exhibit simple exponential phosphorescence decay. Benzimidazole is observed to be protonated in its excited singlet and triplet states in phosphate buffer at an apparent pH of 7. This effect may be associated with the formation of specific hydrogen bonded complexes with phosphate, since protonation is not observed in neutral buffer free solution.

I. Introduction

We have been interested recently in the effects of binding heavy metal ions such as Ag^+ and CH_3Hg^+ on the triplet state properties of the nucleic bases, and we have used optical detection of magnetic resonance (ODMR) to measure the effects of metal binding on the zero-field splittings (ZFS).[1,2] It has been found[3] that attachment of heavy closed-shell metal ions to aromatic chromophores such as the purines and pyrimidines results in an external heavy atom effect[4] which enhances the triplet yield, and shortens the triplet radiative lifetime. This effect greatly enhances the detection sensitivity of the ODMR method. In previous measurements of CH_3Hg-nucleoside and -nucleotide complexes,[2] we found large perturbations of the ZFS, as well as a dependence of the ZFS on the site of attachment. A heavy atom containing cation such as CH_3Hg^+ can perturb the ZFS through (1) enhanced spin-orbit coupling[4,5] and (2) alteration of the electron spin-spin dipolar interaction through electrostatic perturbation of the π-electron distribution. Electrophilic attack on various sites of the bases of DNA may be a significant step in chemical mutagenesis and carcinogenesis.[6,7]

In order to gauge the magnitude of the perturbation of the ZFS to be expected from purely electrostatic effects of cation addition, we have made ODMR and optical spectral measurements on the effects of protonation of the model compounds benzimidazole (**1**) and purine (**2**). Protonation of purine and benzimidazole is expected to occur at different sites,[8] primarily N(1) of purine and N(3) of benzimidazole. It was



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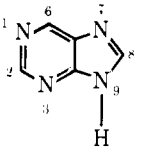

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of interest, therefore, to compare the effects of protonation of the six-membered ring vs. protonation of the imidazole ring on the measurable properties of the respective triplet states. Also, we were interested in verifying the presence of postulated tautomers of protonated purine, and possibly of purine itself. Proton tautomerism, which could affect base pairing, has been postulated as a mechanism for spontaneous mutations in DNA.[9,10]

Slow-passage ODMR measurements in zero applied magnetic field yield only the triplet sublevel energy splittings. In order to obtain information which could be applied to the problem of locating the principal axes in the molecular framework we have made kinetic measurements to obtain the individual triplet sublevel decay constants and to establish the sublevel radiative properties. These measurements allowed us to assign D and E values for benzimidazole and its cation with reasonable certainty. The unexpected kinetic behavior of the triplet state and the large apparent influence of spin-lattice relaxation at temperatures as low as 1.10 K precluded the unambiguous assignment of D and E values for the purine species.

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Table I. Luminescence of Benzimidazole and Purine[a]

compd	pH*	λ^{F}_{max}, nm	λ^{P}_{0-0}, nm[b,c]	τ_p, s[d]	τ_p, s[c,d]	τ_p, s[d,e]
benzimidazole	1.3	283.7	371.2	6.27		
	7.0[f]	284.2	371.0	6.43	6.70[g]	
	7.0[h]	289.0	373.7			
	11.2	288.7	373.4	6.22	6.47[g]	6.29
purine	1.0	328.5	387.0[i]	3.51 (54)	3.96 (41)	3.35 (65)
				2.23 (46)	2.46 (59)	1.82 (35)
	7.0	[j]	367.0	2.02 (49)	2.20 (47)	2.01 (55)
				1.25 (51)	1.24 (53)	1.20 (45)
	12.0	333.5	395.5	3.49 (73)	3.91 (50)	3.41 (82)
				2.46 (27)	2.52 (49)	1.53 (18)

[a] Concentration ca. 10^{-3} M in 50:50 (v/v) EG-H_2O glass. T = 77 K except where otherwise indicated. λ_{exc} = 260–280 nm. [b] Peak position of 0–0 band. Estimated error ±0.2 nm. [c] T = 4.2 K. [d] For nonexponential decay, preexponential factors (%) of component lifetimes given in parentheses. [e] T = 1.1–1.2 K. Decay measured during microwave saturation of all sublevel populations. [f] Solution buffered at pH* 7.0 with 0.1 M phosphate buffer. [g] Major component of nonexponential decay. Minor component (<10%) with shorter lifetime also is present. [h] Solvent unbuffered. pH* adjusted with NaOH. [i] 0–0 band is a poorly resolved shoulder. [j] No fluorescence detected.

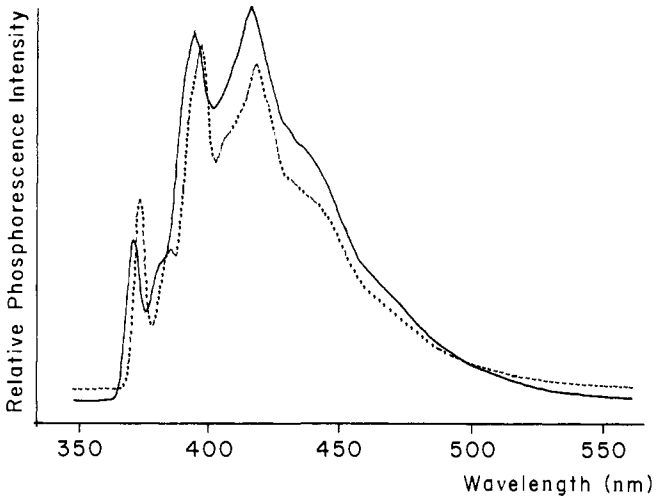


Figure 1. Phosphorescence spectra of benzimidazole in EG-H_2O. Cation, pH* 1.3 (—); neutral, pH* 11.2 (- - - -). T = 77 K. Relative intensity of spectra is arbitrary.

II. Experimental Section

Benzimidazole (Eastman, Inc.) was purified by recrystallization from doubly distilled water. Purine (Aldrich, Inc.) was found to contain no luminescent impurities and was used without further purification. Ethylene glycol (EG, Matheson Coleman and Bell, Inc., Chromatoquality) was used from a freshly opened container. All samples were dissolved in 50:50 (v/v) EG-water. Previously distilled water was deionized and redistilled in an all-quartz apparatus prior to use. The pH of the EG-water solutions was adjusted by addition of H_3PO_4 (Mallinckrodt, Inc., AR) to obtain a low pH solution, or NaOH to obtain solutions of pH 7 and above. The pH of the solution containing the sample (ca. 1 mM) was measured at ambient temperature with a pH meter (Beckman, Inc., Model 4500) employing a glass electrode. The effective pH measured in this manner will be referred to as pH*. In one set of measurements on benzimidazole, the solution was buffered at pH* 7.0 with 0.1 M phosphate buffer. The purine pH* 7 solution was buffered with 0.01 M phosphate. The solvent mixture was checked for the absence of extraneous luminescence prior to use.

Luminescence and phosphorescence spectra were measured at 77 K with a Perkin-Elmer, Inc., MPF-2A spectrofluorimeter. Phosphorescence decay measurements were made at 77, 4.2, and ca. 1.1 K. The phosphorescence decays were monitored over three decades of intensity, signal averaged, and deconvoluted as described previously.[11] Slow-passage ODMR measurements were made in the range 1.1–1.2 K in zero applied magnetic field according to previously described procedures.[11,12] The phosphorescence was monitored using a cooled EMI, Inc., 6256S photomultiplier at the peak of the 0–0 band, which was prominent in each sample except for purine at pH* 1; in the latter case the phosphorescence was monitored at the peak of the emission band (410 nm). The monitoring band-pass was 3.2 nm. The fluorescence background was suppressed by a rotating sector. Microwave sweep rates were low (10–20 MHz/s) to avoid fast passage effects as much as possible, and duplicate measurements were made with increasing and decreasing microwave frequency and the ODMR peak frequencies were averaged. The ascending and descending peak frequencies typically differed by about 50 MHz, but this difference increased rapidly with increasing sweep rates.

The apparent lifetimes of the triplet sublevels were measured at the lowest temperature obtainable in our apparatus, ca. 1.1 K, using the microwave-induced delayed phosphorescence (MIDP) method.[13] The MIDP experiments give the true lifetimes of the magnetic sublevels for $T_1 \rightarrow S_0$ processes only if spin-lattice relaxation is negligible.[13] In this paper we will use κ_i to designate the apparent decay constant of the ith triplet sublevel obtained from MIDP measurements, and reserve k_i for the true $T_i \rightarrow S_0$ rate constant. In spite of the complications arising from spin-lattice relaxation, MIDP nonetheless is extremely useful for establishing qualitatively the number of long-lived and of rapidly decaying sublevels, and their relative energy ordering.

In order to ascertain the importance of spin-lattice relaxation, and to determine the k_i, we have made a series of microwave saturated phosphorescence decay measurements[14] on benzimidazole. This method is described in Appendix I. These measurements were not attempted on purine since the phosphorescence decay was found to be nonexponential under conditions of rapid spin-lattice relaxation as well as microwave pumping of all sublevels, vitiating this method.

III. Results

1. Luminescence Spectra. The phosphorescence spectrum of benzimidazole as well as the lifetime of the triplet state was found to be surprisingly insensitive to protonation of the imidazole ring. The ground-state pK_as of benzimidazole are 5.53 and 13.2 at 20 °C.[15] The phosphorescence spectra measured at pH* 1.3 and 11.2 are shown in Figure 1. Table I gives the 77 K phosphorescence lifetimes, positions of the phosphorescence 0–0 peak, and the position of the fluorescence maximum at various pH*. It has been observed previously that in aqueous solution at room temperature the fluorescence maximum of benzimidazole shifts from 290 to 360 nm upon protonation,[16,17] a result of solvent relaxation in the excited state possibly involving solvent exciplex formation.[18] A fluorescence titration carried out by Börresen[16] showed, however, that the two fluorescence spectra have a midpoint at pH 5.3, which indicates very little change in the basicity of benzimidazole in the fluorescent state. In the rigid EG-water glass at 77 K we see only a small shift in the fluorescence peak wavelength upon protonation, which is consistent with Börresen's finding. The absence of a large shift in the well-resolved phosphorescence 0–0

Table II. Slow-Passage ODMR Signals of Benzimidazole and Purine[a]

compd	pH*[b]	ν_1, GHz ($\Delta\nu$)[c]	ν_2, GHz ($\Delta\nu$)[c]	ν_3, GHz ($\Delta\nu$)[c]
benzimidazole	1.3	0.79 (90)	3.18[d] (120)	3.90 (100)
	7.0[e]	0.79 (90)	3.20[d] (90)	3.89 (120)
	7.0[f]	1.95 (490)	2.58 (310)	4.31 (790)[g,h]
	11.2	1.83 (390)	2.59 (310)	4.26 (320)[g]
purine	1.0[i]	0.94 (62) 1.09 (66)[j]	3.40 (190)	4.40 (240)[k]
	7.0	1.03 (90)	3.88 (120)	4.86 (180)[k]
	12.0	1.15 (50)[l]	3.14 (160)	4.23 (110)[k]

[a] T = 1.1-1.2 K, λ_{exc} = 280 nm except purine, pH* 7.0 when λ_{exc} = 270 nm; phosphorescence monitored at 0-0 maximum (Table I) except as otherwise indicated. Solvent is 50:50 v/v ethylene glycol-water. Estimated error is ±10% of line width. [b] Apparent pH at 24 °C. [c] Full width at half-maximum line width (MHz) in parentheses. [d] Signal observed only by using EEDOR. ν_3 signal saturated. [e] Sample is 0.1 M in phosphate buffer. [f] Unbuffered sample. pH* adjusted to 7 using aqueous NaOH. [g] ODMR signal is extremely weak. [h] Signal reported is enhanced by EEDOR, saturating ν_1. [i] Phosphorescence monitored at 410 nm. See Figure 2. [j] Signal is a well-resolved doublet. [k] Observed only by EEDOR. ν_1 signal saturated. [l] Poorly resolved doublet. Average frequency and total signal width reported.

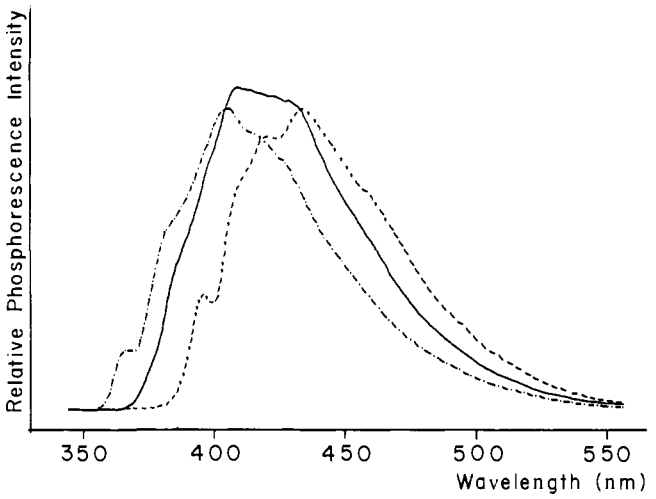


Figure 2. Phosphorescence spectra of purine in EG-H_2O at T = 4.2 K. Cation, pH* 1 (—); neutral, pH* 7 (- · · · -); anion, pH* 12 (- - - -). Relative intensity of spectra is arbitrary.

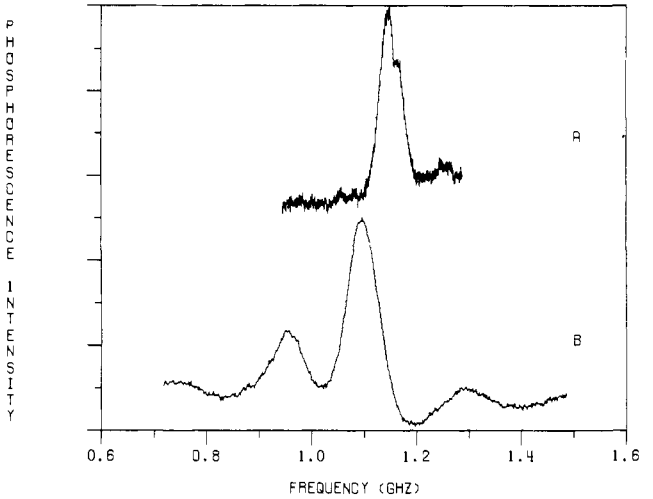


Figure 3. Slow-passage ODMR signals of the purine ν_1 transition at T = 1.2 K. Solvent is EG-H_2O. (A) anion, pH* 12; (B) cation, pH* 1.

peak upon protonation of benzimidazole is consistent with little change of pK_a in the triplet state in agreement with the conclusions reached earlier by Jackson and Porter.[19]

It is apparent from Table I that the luminescence properties of benzimidazole in unbuffered pH* 7 solution differ from those in pH* 7 phosphate-buffered solution. In fact, the luminescence in the buffered medium resembles that of the pH* 1.3 solution in which protonation certainly has taken place in the ground state, while the luminescence of the unbuffered pH* 7 solution resembles that of the pH* 11.2 solution in which benzimidazole is unprotonated and neutral. The ODMR measurements, to be described below, confirm that the triplet state in the pH* 7 phosphate buffer is protonated.

The pK_as of purine are reported to be 2.52 and 8.90.[15] The luminescence and phosphorescence decay data are given in Table I. Weak fluorescence is observed at 77 K from purine at pH* 1 and 12 where the major species are the protonated cation and the deprotonated anion, respectively. At pH* 7, no fluorescence is detected. The phosphorescence intensity is comparable at each pH*, however; the phosphorescence spectra are shown in Figure 2. The striking red shift of the phosphorescence of both the cation and anion relative to neutral purine is in marked contrast with the minor blue shift observed upon protonation of benzimidazole. Although the phosphorescence decay of benzimidazole is a single exponential over three decades of intensity at each measured pH*, the decay of the purine phosphorescence is nonexponential. The decay kinetics could be fitted in each case to two exponential components with comparable intensity as indicated in Table I. The triplet lifetime of purine is significantly shorter than that of either of its ions. It should be noted that our results for the fluorescence of purine are similar to those reported by Börresen[16] in water at room temperature, in that fluorescence is observed for both the cation and anion but not for the neutral species.

2. Slow-Passage ODMR. The microwave frequencies at which ODMR responses are observed are given in Table II. The response is in each case an increase in phosphorescence intensity. Large ODMR frequency shifts occur in benzimidazole upon changing the solvent conditions from pH* 11.2 and 7 (unbuffered) to pH* 7 (buffered) and 1.3. The changes are attributed to the effects of protonation on the ZFS of benzimidazole. The close correspondence of the signal frequencies observed at pH* 1.3 and 7 (buffered) confirm the presence of a structure similar to protonated benzimidazole in the latter solvent. For both solvents, the ν_2 signal is unobservably weak and can be observed only by the EEDOR method,[12,20] whereas ν_3 is the strongest signal. On the other hand, in pH* 7 (unbuffered) and 11.2 solution, i.e., for unprotonated benzimidazole, ν_3 is the weakest signal, but can be enhanced by EEDOR, whereas ν_2 is the most intense signal. The ODMR frequency shifts upon changing the pH* of purine solutions are not as striking as those which occur in benzimidazole. The ν_3 signal is the weakest for cation, anion, as well as neutral purine, and could be observed only by the EEDOR method. The ν_1 signal of purine is a resolved doublet at pH* 1 and 12. These ODMR signals are shown in Figure 3. The splitting of the ν_1

Table III. Microwave Induced Delayed Phosphorescence Measurements of Benzimidazole and Purine[a]

compd	pH*	microwave transition	τ_f, s[b]	τ_s, s[c]	apparent decay constants s^{-1}[d] κ_1	κ_2	κ_3
benzimidazole	1.3	ν_1	e	7.6		0.13	0.08
		ν_3	e	12.5			
	7.0[f]	ν_1	e	9.3		0.11	0.06
		ν_3	e	18.0			
	11.2	ν_1	2.0	6.6	0.50	0.15	0.06
		ν_2	2.0	15.8			
purine	1.0	ν_1'[g]	1.71	9.0	0.66	0.13	0.10
		ν_1''[h]	1.40	9.8			
		$(\nu_1' + \nu_1'')$	1.41	9.8			
		ν_2	1.58[i]	7.9			
	7.0	ν_1	0.81	5.5	1.21	0.20	0.18
		ν_2	0.84	4.9			
	12.0	ν_1	1.24	7.3	0.86	0.14	0.09
		ν_2	1.08	11.5			

[a] T = 1.1-1.2 K except for purine, pH* 12, for which $T \sim 1.3$ K. [b] Decay lifetime of fast passage transient. Average of several MIDP responses. Some of the responses in purine were not single exponentials, in which case a weighted average of the deconvoluted decay constants was used. Standard deviation ~10% except as noted. [c] Decay lifetime of MIDP intensity. Estimated accuracy ±5%. [d] Arranged in decreasing order. No sublevel ordering is implied. [e] Not analyzed. [f] Phosphate buffered sample. [g] Microwave sweep over low-frequency component of ν_1 doublet only. See Figure 3. [h] Microwave sweep over high-frequency component of ν_1 doublet only. See Figure 3. [i] Standard deviation ±20%.

signal of purine at pH* 1 (~150 MHz) is considerably larger than that at pH* 12 (~16 MHz). Although the splitting at the low pH* can be due only to the presence of two emitting triplet states with slightly differing ZFS, the splitting at high pH is of the magnitude associated with ^{14}N quadrupole and second-order hyperfine interactions,[21] so that only one triplet species may be present. None of the other purine signals at pH* 1 reveals a splitting, although their larger overall width relative to the ν_1 signals may conceal an unresolved splitting. It is possible, also, that the nonexponential decay of the purine phosphorescence results from the presence of more than one triplet state at each pH*, in which case neutral purine would be thus characterized as well, even though no splitting of the ODMR lines is observed. We do not believe that an explanation for the nonexponential phosphorescence decay based upon the presence of more than one purine proton tautomer is reasonable since such an explanation is not tenable for the anion. Nonexponential phosphorescence decay of purines under rapid spin-lattice relaxation or microwave pumping conditions has been reported previously[17,22,23] but no convincing explanation for these observations has been offered. One possibility is that the triplet states of some molecules are characterized by sensitivity of their $T_1 \rightarrow S_0$ decay lifetimes to interactions with the solvent. The radiationless intersystem crossing rate constants in particular might be influenced by local solvent structure. A distribution of decay lifetimes in a heterogeneous system might lead to nonexponential phosphorescence decays such as those observed for purine.

3. MIDP Measurements. MIDP experiments were made in order to determine the triplet state sublevel radiative pattern, and to obtain an estimate of the individual sublevel decay lifetimes. A typical set of MIDP responses for the purine anion is given in Figure 3. Since it is likely that spin-lattice relaxation still affects the decay of the individual sublevels even at 1.1-1.2 K, the lifetimes associated with the MIDP analysis are not assumed to represent true lifetimes for the $T_1 \rightarrow S_0$ decay processes. A summary of the decay lifetimes obtained from the MIDP measurements is given in Table III. The long component was obtained from a least-squares fit of log (I) vs. t, where I is the intensity of the MIDP response normalized to the intensity of the decay at $t = 0$, and t is the time from the beginning of the decay to the occurrence of the fast passage response. Usually six to eight points are included spread over about one decade of I. The short component is obtained by deconvolution of the decay following the fast-passage response, and is the average of several individual analyses. The short component of the benzimidazole decay was analyzed only in the pH* 11.2 solution. MIDP signals were obtained only for those magnetic resonance transitions which did not require the EEDOR method for observation during slow-passage ODMR measurements. The transition which does not induce a response connects the long-lived, relatively nonradiative sublevels in each case. In benzimidazole we find that at pH* 1.3 and 7 (buffered) MIDP responses are produced at ν_1 and ν_3, but not at ν_2. At pH* 11.2, however, MIDP responses occur at ν_1 and ν_2, but not at ν_3. In purine, on the other hand, ν_1 and ν_2 produce MIDP responses at each pH*, while ν_3 does not. These results show that the shortest lived, most radiative sublevel is the intermediate one in energy for each of the species examined except protonated benzimidazole, where the shortest lived sublevel is either highest or lowest in energy.

4. Microwave Saturated Phosphorescence Decay Measurements. The data of the microwave saturated phosphorescence decay measurements carried out on benzimidazole at pH* 11.2 are given in Table IV. The calculated decay constants based on the two possible absolute orderings of the sublevels appear in Table V. The method of calculation is described in Appendix I. The differences between the κ_i obtained from the MIDP measurements (Table III) and the k_i obtained from the microwave saturated phosphorescence decay measurements (Table V) illustrates the considerable influence of spin-lattice relaxation on the MIDP results even at 1.1 K. The dominant relaxation path connects the longest and shortest lived sublevels.

IV. Discussion

1. ZFS Parameters. The triplet states which we have investigated are $^3(\pi,\pi^*)$ states of planar aromatic molecules, based upon their long phosphorescence lifetimes (Table I). It is well known[24] that $^3(n,\pi^*)$ lifetimes generally fall in the millisecond regime as a result of more efficient spin-orbit mixing of radiative singlets with triplets relative to $^3(\pi,\pi^*)$ states. Since the molecules are assumed planar, one principal zero-field axis (defined as x) must lie normal to the molecular plane, while the two other orthogonal axes lie in the molecular plane.[25] The directions of the y and z principal axes are not known a priori unless the molecule has C_{2v} or higher symmetry, in which case z lies along the twofold molecular axis.[25] The molecules which we have studied have only C_s symmetry except for protonated benzimidazole, which is C_{2v}. The ZFS

Table IV. Microwave Saturated Phosphorescence Decay Data of Benzimidazole[a]

transition[b]	τ_1, s[c]	τ_2, s[c]	ρ_s, s^{-1}	ρ_p, s^{-2} ($\times 10^2$)	$\bar{\rho}$, s^{-1}
ν_1 ($j \leftrightarrow k$)	3.563	10.367	0.3771	2.707	
ν_2 ($i \leftrightarrow j$)	4.939	7.137	0.3426	4.965	
ν_3 ($i \leftrightarrow k$)	2.730	7.377	0.5098	2.837	
$\nu_2 + \nu_3$	6.287				0.1591

[a] $T = 1.15 \pm 0.03$ K. pH* 11.2 in EG-H_2O glass. See Appendix I for definition of symbols. [b] Transitions are numbered in order of increasing frequency; see Table II. Letters label the triplet sublevels saturated. i, j, k are ordered sequentially in energy. [c] Last figure is not significant. Estimated accuracy ±1%.

Table V. Benzimidazole Sublevel Decay and Spin-Lattice Relaxation Rate Constants from Microwave Saturated Phosphorescence Decay[a]

rate constant, s^{-1}	assumed energy ordering	
	$\epsilon_k > \epsilon_j > \epsilon_i$[b]	$\epsilon_i > \epsilon_j > \epsilon_k$
k_i	0.043	0.034
k_j	0.250	0.258
k_k	0.189	0.189
W_{ij}	0.077	0.086
W_{ji}	0.086	0.077
W_{ik}[c]	−0.003	−0.004
W_{ki}[c]	−0.004	−0.003
W_{jk}	0.008	0.008
W_{kj}	0.008	0.008
k	0.161	0.161

[a] Method of calculation outlined in Appendix I. Conditions given in Table IV in which the data used for the calculation are given. [b] This is the most probable sublevel ordering. See text. [c] Zero, within the estimated error of ± 0.005 s^{-1}.

parameters will be defined in relation to the zero-field spin Hamiltonian

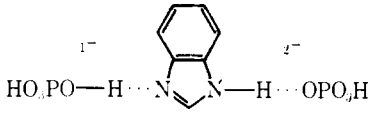
$$\mathcal{H}_s = D(S_x^2 - \tfrac{2}{3}) + E(S_z^2 - S_y^2) \quad (1)$$

The ZFS of $^3(\pi,\pi^*)$ states of aromatic hydrocarbons and azaaromatic molecules is dominated by electron spin-spin dipolar coupling, and many calculations of this interaction based upon reasonable π-electron wave functions are in excellent agreement with the observed ZFS.[26] In order to allow the use of the ODMR and triplet state kinetic data of the preceding section for assignment of D and E values, we make two assumptions regarding the general properties of $^3(\pi,\pi^*)$ states of the type we have investigated: (a) D is positive and larger than $|E|$; (b) T_x has the smallest of the sublevel $T_i \rightarrow S_0$ decay constants. (Sublevel T_i has the electron spin polarized in the molecular jk plane.[25]) Assumption (a), which makes the T_x sublevel lowest in energy, is based upon ZFS calculations of similar molecules such as naphthalene,[26,27] quinoxaline,[28] quinoline,[28] and indole,[29] which satisfy assumption (a) and are in good agreement with the *absolute values* of D and E obtained experimentally from single-crystal EPR measurements. Assumption (b) is based upon the theoretical model of spin-orbit coupling in aromatic hydrocarbon $^3(\pi,\pi^*)$ states[30] which can be extended to azaaromatic systems,[25] showing that mixing of singlet character into the T_x sublevel is far less efficient than for the T_y, T_z sublevels. It is also consistent with the observation[5] that phosphorescence of $^3(\pi,\pi^*)$ states is polarized normal to the molecular plane. Specifically, the phosphorescences of purine and its anion have been shown[31] to be polarized perpendicular to both the ^1L_a and ^1L_b absorption bands which are in-plane polarized and mutually perpendicular. Furthermore, the T_x sublevel of quinoxaline has been shown to be the longest lived in MIDP measurements in which spin-lattice relaxation has been demonstrated to be negligible.[13]

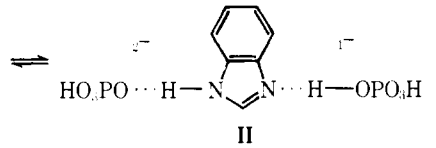
2. Benzimidazole. With the aid of the above assumptions, we can assign the absolute energy sublevel ordering of benzimidazole according to the first column of Table V, and identify the ith sublevel as T_x. This assignment requires that $D > 3|E|$, which is in disagreement with the assignment made recently by Harrigan and Hirota,[32] who made the opposite tentative assignment by analogy with indole. The ordering $D < 3|E|$ is well established for indole from magnetophotoselection[33] and single-crystal EPR measurements in high magnetic field.[32] Microwave saturated phosphorescence decay measurements on indole[14] verify that T_x is the longest lived sublevel. The D and E parameters for benzimidazole based upon our assignment are given in Table VI.

(a) Protonation of Benzimidazole. Protonation of benzimidazole leads to a change in the sublevel pattern in that the radiative, shortest lived sublevel is now the highest in energy. The possibility that the shortest lived sublevel is the lowest in energy is discarded on the basis of the previous discussion. Surprisingly, the overall triplet lifetime is scarcely affected by protonation. This, together with the long triplet lifetime of benzimidazole itself, indicates little effect of the N3 lone-pair electrons on spin-orbit mixing. This is in sharp contrast with the quinoxaline triplet state, whose short triplet lifetime (0.25 s)[34] is attributed to the influence of the lone pair electrons. D and E are known to have opposite signs in quinoxaline[34] as well as in naphthalene.[35] We are reasonably confident that this assignment can be made for protonated benzimidazole as well. We note from Table VI the good agreement of D and E values with those of naphthalene and quinoxaline, molecules of similar size and shape. Also, T_z is the shortest lived, most radiative sublevel in each case.[13]

Benzimidazole at pH* 7 in 0.1 M phosphate buffer has triplet state properties indistinguishable from those of the cation at pH* 1.3, whereas in pH* 7 unbuffered medium the triplet state is that of the neutral molecule. We suggest that specific hydrogen-bonded complexes with phosphate are present, possibly with the structures I and II. The properties



I



II

of the fluorescent and phosphorescent states would be represented by the symmetrical tautomer III, which might be stabilized in the excited state as a result of increased basicity of the benzimidazole hydrogen-bonded nitrogen atom, or possibly even in the ground state at low temperature if significant entropy reduction is associated with the equilibrium (I,II) ⇌ III.

Table VI. D and E Values for Benzimidazole, Purine, and Related Molecules[a]

compd	pH*	exptl D/hc, m^{-1}	exptl E/hc, m^{-1}	calcd D/hc, m^{-1}	calcd E/hc, m^{-1}	ref
naphthalene	*b,c*	10.12	−1.42	10.81	−0.93	*d*
quinoxaline	*b,e*	10.07	−1.82	10.2	−1.2	*f*
quinoline	*b,g*	10.30	−1.62	10.3	−1.0	*f*
indole	*h*	10.12	4.40	10.84	7.70	*i*
				12.0	2.5	*f*
benzimidazole	1.3[j]	11.80	−1.32			
	7.0[k]	11.50	3.25			
	11.2[k]	11.43	3.06			
purine	1.0[l]	9.07	5.68			
		13.00[m]	1.70[m]			
	7.0	9.81	6.47	11.9	6.5	*f,n*
		14.61[m]	1.67[m]			
	12.0[o]	12.35	1.86			
		8.96[m]	5.25[m]			
5′-adenylate	7.0[p]	10.54	4.94	11.55	5.35	*f,n,q*
				12.31	9.02	*i,q*

[a] D and E refer to eq 1 with z along long in-plane axis for molecules with higher than C_{2v} symmetry. D (experimental) is assumed to be positive. Only absolute value of E is given for molecules of C_s symmetry. [b] From high-field measurements in durene crystal. [c] Reference 35. [d] M. Godfrey, C. W. Kern, and M. Karplus, *J. Chem. Phys.*, **44**, 4459 (1966). [e] Reference 34. [f] Reference 36. [g] J. S. Vincent and A. H. Maki, *J. Chem. Phys.*, **42**, 865 (1965). [h] ODMR of trap emission in indole neat crystal. Reference 12. [i] Reference 33. [j] Protonated. Probable assignment; see text. [k] Neutral molecule, unbuffered solution. [l] Protonated species. [m] Assignment considered less likely than that above. [n] Principal axis system transformed to correspond to ours. [o] Anion. [p] R. J. Hoover, K. F. S. Luk, and A. H. Maki, *J. Mol. Biol.*, **89**, 363 (1974). Zero-field ODMR transitions assigned to give $D < 3|E|$. [q] Calculation is for 9(H)-adenine.

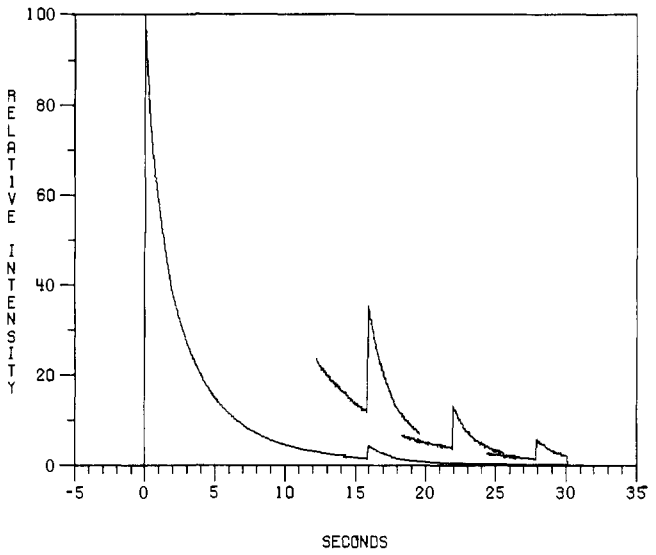
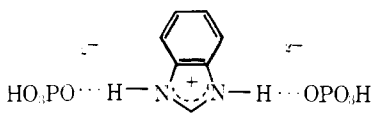


Figure 4. MIDP signals of purine anion at 1.3 K in EG-H_2O, pH* 12. ν_1 transition in pulsed after delays of 16, 22, and 28 s following shuttering of exciting light followed (2 ms) by opening of monitoring shutter in separate experiments. The original phosphorescence decay and the smaller response at t = 16 s are ×1. Signals offset from the decay base line each are ×8.



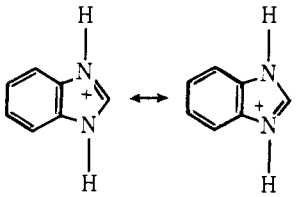
III

At present, we cannot rule out other explanations, such as nonspecific electrostatic effects of ionic strength influencing the protonic equilibrium of benzimidazole at reduced temperature.

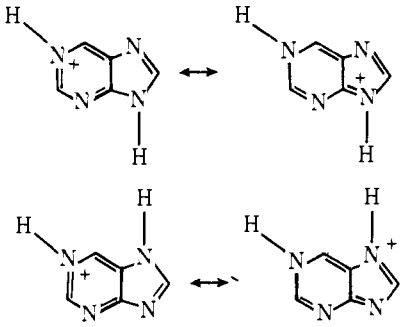
3. Purine. (a) Purine Anion. The problem of the nonexponential phosphorescence decay of purine and its ions unfortunately precluded interpretable microwave saturated phosphorescence decay measurements to obtain the sublevel decay constants. Thus, we are unable to identify T_x on the basis of relative decay constants. Furthermore, although the ordering of the κ_i obtained from MIDP measurements is the same as that of the k_i of benzimidazole, the two smaller κ_i's are quite similar for each of the purine species. With the possible exception of the anion, it is not possible to use the κ_i's to make an assignment of D and E for the purine species because of the possible influence of spin-lattice relaxation on the apparent decay constants. If it is assumed that spin-lattice relaxation does not alter the ordering of the two smallest decay constants of the anion, then κ_2 and κ_3 are in the order which leads to the assignment $D > 3|E|$. Deprotonation of purine produces an ion which deviates from C_{2v} symmetry only because of the unsymmetrical location of the nitrogen atoms of the pyrimidine ring. The protonation of benzimidazole, which leads to an ion of C_{2v} symmetry, results in the reduction of the magnitude of E as we have seen above. The assignment with $D > 3|E|$ thus seems again the more likely of the two possibilities, and we will use this as the preferred, tentative assignment for the anion.

(b) Neutral Purine Molecule, Adenine. The preferred assignment of the ZFS for purine must be based upon the results of theoretical calculations. Pullman[36] has carried out an extensive set of calculations of the ZFS of heterocycles including purine using SCF π-molecular orbitals obtained with a Pariser-Parr-Pople procedure. The results for purine, adenine, and indole are included in Table VI. The assignment $D < 3|E|$ leads to very reasonable agreement of the experimental values with the calculated ZFS for the cases of purine and adenine. Unfortunately, however, the agreement is not as satisfactory in the case of indole where $D > 3|E|$ is calculated. In spite of this, and because we do not think that E should be very much smaller than it is for indole and benzimidazole, we tentatively assign $D < 3|E|$ for both purine and adenine.

The large value of E for purine is attributed to the presence of the localized conjugated double bond of the five-membered ring.[32,36] Since indole and benzimidazole also have this mesomeric group, a large E is consistent with these triplet states as well, as is the reduction in E upon protonation of benzimidazole with its concomitant loss of the localization of the double bond by resonance.



(c) **Protonation of Purine.** Protonation of purine is thought to occur mainly at N1, although NMR evidence exists[37] for the presence of considerable amounts of the N7 and N3 protonated tautomers in equilibrium at room temperature. The ODMR spectrum of protonated purine does show the presence of more than one triplet state at helium temperature. Since the ZFS of the two triplets are very nearly the same, it is doubtful that they can be assigned to tautomers as different as 1*H*,9*H*- and 7*H*,9*H*-, for instance, which appear to be[37] the major tautomers at room temperature. Tautomeric triplet states cannot be ruled out, however. Tautomers such as 1*H*,9*H*- and 1*H*,7*H*- have similar bond structures, and their triplets could have similar ZFS, i.e.



No calculations of the ZFS of protonated purine have been made to our knowledge and thus there is little basis on which to make an assignment. We prefer also in this case the assignment $D < 3|E|$, based upon analogy with similar molecules having a localized mesomeric group which interacts strongly with a phenyl or pyrimidine ring.[32]

Recent evidence[38] based on NMR suggests that purine at room temperature is about 40% 7*H*- and 60% 9*H*-. Also, purine crystallizes as the 7*H*- tautomer.[39] We observe no evidence for such tautomerism in our system at low temperature, although we cannot rule it out since the ZFS of these tautomers could be too similar for us to resolve. Clearly, further ODMR measurements on model N-methylated purines might help resolve these questions.

V. Conclusions

Protonation of benzimidazole and purine, as well as the deprotonation of purine, leads to large, easily measurable changes in the ZFS of the triplet states. The ZFS perturbations occurring as a result of protonation are comparable to those found upon binding heavy atom cations such as CH_3Hg^+ to purine nucleic bases.[2] The latter, we believe, can be treated by electrostatic arguments without the need for involving spin–orbit perturbations.

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Appendix I. Microwave-Saturated Phosphorescence Decay Measurements[14]

In this method, the phosphorescence decay is monitored during microwave saturation of each of the zero-field magnetic resonance transitions in turn, and finally during microwave saturation of two zero-field transitions simultaneously. In the absence of microwave saturation and optical pumping, the time development of the populations of the individual triplet sublevels ($\dot{n}_i$), is given by the solutions of the three coupled differential equations

$$\dot{n}_i = -(k_i + W_{ij} + W_{ik})n_i + W_{ji}n_j + W_{ki}n_k \qquad \text{(A1)}$$

where k_i is the first-order rate constant of the ith sublevel for $T_1 \rightarrow S_0$ decay, and W_{ij} is the rate constant for transitions from the ith to jth sublevel. Each sublevel decays as a linear superposition of three first-order decays with decay constants given by the eignevalues of the coefficient matrix of the right-hand side of eq A1. Only when all $W_{ij} = 0$ are the eigenvalues given by the k_i. The deconvolution of the phosphorescence decay into three exponential components, even if it could be done uniquely, would yield only the eigenvalues of A1, which is not enough information to fix the six (assuming that W_{ij} and W_{ji} are related by a Boltzmann factor) unique rate constants. Microwave saturation of a pair of levels during the triplet decay forces the system to behave as a pseudo-two-level system in which the rate constants of the saturated levels are averaged. The phosphorescence decay then is described by two coupled differential equations analogous to A1; now the decay will be a linear superposition of two exponential components instead of three, and the deconvolution is not difficult. Let $\rho_1{}^k$ and $\rho_2{}^k$ be the two decay constants observed when the ith and jth sublevels are kept saturated, and define their sum as $\rho_s{}^k$ and their product as $\rho_p{}^k$. It is easy to show that

$$\rho_s{}^k = \tfrac{1}{2}(3\bar{k} + k_k + W_{ik} + W_{jk} + 2W_{ki} + 2W_{kj}) \qquad \text{(A2)}$$

and

$$\rho_p{}^k = \tfrac{3}{2}\bar{k}(k_k + W_{ki} + W_{kj}) + \tfrac{1}{2}k_k(W_{ik} + W_{jk} - W_{ki} - W_{kj} - k_k) \qquad \text{(A3)}$$

where $\bar{k} = (k_i + k_j + k_k)/3$. Since three pairs of sublevels may be saturated in turn, eq A2 and A3 represent six equations in all. The six equations, however, are linearly dependent. In order to be able to determine the three k's and the three independent W's from A2 and A3, an additional measurement of the phosphorescence decay during microwave saturation of all sublevels (two microwave transitions simultaneously) is carried out to yield a single exponential decay constant $\bar{\rho}$. This experimental value is substituted for $\bar{k}$ in eq A2 and A3, and the resulting equations are solved for the best k's and W's by computer using a nonlinear least-squares procedure which minimizes the differences between observed and calculated ρ_s's and ρ_p's. The agreement between the experimental $\bar{\rho}$ and the calculated $\bar{k}$ serves as a check on the consistency of the experiment.

An absolute energy ordering of the sublevel energies must ba assumed in solving eq A2 and A3 since W_{ij} and W_{ji} are related by $W_{ij} = W_{ji}\exp(\epsilon_{ij}/kT)$, where $\epsilon_{ij} = \epsilon_i - \epsilon_j$. We find that the W_{ij} obtained from the least-squares procedure are independent of the choice of absolute level ordering, except for interchange of indices, whereas the k_i differ with the ordering assumed. In principle, it might be possible to establish the absolute level ordering from microwave saturated phosphorescence decay measurements at several temperatures to determine the one absolute level ordering which produces an invariant set of k's. In practice this is difficult, however, since the effect of interchange of absolute level order on a calculated k is only of the order $|W_{ji} - W_{ij}|$.

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Methylmercury as a Spin–Orbit Probe. Effect of Complexing on the Excited State Properties of Tryptophan, Tryptamine, and Benzimidazole

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Abstract: The binding of CH_3Hg^{II} with tryptophan (HTrp), tryptamine ($HTam^+$), and benzimidazole (HBIm) has been observed and formation constants (K_f) are reported. K_f for binding at the amine site of HTrp and $HTam^+$ is about 60 and 10 times, respectively, that predicted from the observed correlation of log K_f with the aminium pK_a for a series of simple primary amines. The enhancement is attributed to a specific interaction between CH_3Hg^{II} and the indole ring. NMR chemical shifts point to a location of the methyl group above the indole plane, and model building confirms the possibility of an unstrained geometry with CH_3Hg^{II} located parallel to and in close contact with the aromatic plane. In the complex with HBIm, $CH_3HgHBIm^+$, the NMR chemical shift of CH_3 is downfield, consistent with σ bonding of CH_3Hg^{II} at N3 of HBIm. CH_3Hg^{II} binding quenches the fluorescence of each compound, and enhances the radiative quantum yield of phosphorescence, that of CH_3HgTrp becoming 0.9 ± 0.1. Phosphorescence lifetime measurements at 77 K yield reduction factors of 6000 (HTrp), 8000 ($HTam^+$), and 40 (HBIm) relative to the unperturbed compound. Differences in the magnitude of the heavy atom effect as measured by phosphorescence lifetime reduction are attributed to differences in the position of CH_3Hg^{II} relative to the affected chromophore. Optically detected magnetic resonance measurements, and pulsed laser-excited phosphorescence decay measurements at 1.1–1.2 K show that the heavy atom effect differs for individual triplet sublevels. Complexing of HBIm with CH_3Hg^{II} has the same effect on the triplet state zero-field splittings as does protonation, suggesting that spin-orbit coupling and electron delocalization effects are not important.

Introduction

Methylmercury is a widely occurring and highly toxic environmental pollutant.[1] The neurotoxicity of CH_3Hg^{II} is well documented,[2] and mutagenic effects also have been observed.[3-6] There has been a continuing interest in the study of CH_3Hg^{II} binding with various bases, and the formation constants of many methylmercury complexes have been determined.[7-15]

We have been interested in the identification of specific base targets and binding sites of alkylmercury in polynucleotides using optical detection of magnetic resonance (ODMR) methods,[16] with which many properties of the phosphorescent state can be uncovered. The mercury atom, which is in effect a spin-orbit probe, induces a heavy atom effect[17] in the target molecule. Binding of the spin-orbit probe leads to fluorescence quenching by enhancement of the intersystem crossing rate, and results in a reduction of the triplet lifetime, frequently accompanied by an increase in the phosphorescence quantum yield.[18] The ODMR sensitivity increases with increasing triplet radiative quantum yield. Magnetic resonance passage conditions also can be made rapid enough to discriminate against signals from long-lived triplet states. We have found in numerous cases that ODMR signals from uncomplexed molecules can be effectively suppressed relative to those complexed with a spin-orbit probe, especially when the latter enhances the phosphorescence quantum yield.[19,20]

During recent ODMR investigations of CH_3Hg^{II} complexes of nucleosides and nucleotides,[20] we added equimolar CH_3HgOH to 1 mM solutions of indole in order to satisfy

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