

Pyrene-1-Carboxylate in Water and Glycerol Solutions: Origin of the Change of pK Upon Excitation

Nathaniel V. Nucci · Bogumil Zelent ·
Jane M. Vanderkooi

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Abstract Pyrene-1-carboxy acid has a pK of 4 in the ground state, and a pK of 8 in the excited state. Fluorescence spectra of the acid and base forms are presented as a function of solvent and temperature. Ab initio quantum calculations indicate that the bond between the ring system and the carboxyl group has aromatic character that becomes stronger upon excitation. This stabilization helps to account for the increase in pK upon excitation.

Keywords Proton transfer · Quantum calculations · Cryogenic · Dipole

Introduction

Excited states of molecules are chemically distinct from the originating ground state molecules. A striking manifestation of the difference is seen in pK 's, which can be orders of magnitude different between the two electronic isoforms. For instance, 1-naphthol has a pK of 9.23 and a pK^* of 2.5. These respective values are 7.3 and 1 for 3-hydroxypyrene 5,8,10-trisulphonate [1, 2]. The change in pK upon excitation of some organic molecules provides a means to change proton ion concentration by excitation with light. Excited state proton donors, i.e., molecules that become more acidic in the excited state relative to the ground state, have been used in studies of protein and macromolecule

surfaces [3–6]. Consideration of the change in pK 's can also help explain the photochemistry of compounds. For example, the pK of the phenoxy group of tyrosine, a biologically important amino acid, changes from 10.3 in the ground state to 4.2 upon excitation [7].

Other aromatic compounds become more basic upon excitation. Again, the change in pK can be very large. The pK of ground state acridine is 5.45, whereas the excited state molecule has a pK of 10.3. The respective ground and excited state pK 's for 2-naphthoic acid are 4.2 and 11.5 and for pyrene-1-carboxylate are 4 and 8.1 [8]. These compounds can also be used to perturb the pK of a system [9]. Excited state proton acceptance reactions have had fewer applications for biological studies, but recently, we used pyrene-1-carboxylate as a probe of proton acceptance from commonly-occurring biological buffers [10]. We showed that excitation of pyrene-1-carboxylate leads to fluorescence from pyrene-1-carboxy acid when proton donation from a surrounding group occurs during the excited state lifetime.

A change in pK upon excitation reflects a change in the distribution of π electrons upon excitation, as pointed out by Weller [1, 11]. To explain changes in pK 's for excited and ground state hydroxy-aromatic compounds, Agmon carried out semi-empirical calculations, and he pointed out that unequal charge distribution over the aromatic ring accounts for the difference in pK 's of a series of substituted hydroxy-aromatic compounds [12, 13]. A similar analysis has not yet been carried out for carboxy aromatic compounds. In this paper, we characterize the spectra of pyrene-1-COO⁻ and pyrene-1-COOH in water and in glycerol/water. We carry out Hartree-Fock quantum mechanical calculations of pyrene-1-COO⁻ and pyrene-1-COOH to interpret the results.

N. V. Nucci · B. Zelent · J. M. Vanderkooi (✉)
Department of Biochemistry and Biophysics,
School of Medicine, University of Pennsylvania,
Philadelphia, PA 19104, USA
e-mail: vanderko@mail.med.upenn.edu

Experimental

Materials

Water was deionized and then glass distilled. Pyrene-1-carboxylic acid and pyrene were obtained from Fluka Chemie (Deisenhofen, Germany). Spectral grade glycerol was obtained from Sigma-Aldrich Company (St. Louis, MO). The pH's of solutions were measured using a glass electrode that was calibrated with standard buffers. All samples were equilibrated with atmosphere.

Spectroscopy

A Hitachi Perkin-Elmer U-3000 absorption spectrophotometer (Hitachi Instruments, Danbury, CT) was used to take UV-visible absorption spectra.

Fluorescence emission spectra were measured with a Fluorolog-3-21 Jobin-Yvon Spex Instrument SA (Edison, NJ) equipped with a 450 W Xenon lamp for excitation and a cooled R2658P Hamamatsu photomultiplier tube for detection. Slit width was set to provide a band-pass of 2 nm for excitation and for emission. The concentration of pyrene-1-carboxylate was adjusted to give an absorbance of 0.05 at the excitation wavelength, 333 nm. Measurements made at 20°C used 90° geometry. Temperature-dependent fluorescence spectra were measured with front surface geometry with the sample held between two quartz plates with a 100 micron spacer. An Omniplex closed-cycle helium cryostat from Advanced Research Systems (Allentown, PA) was used to regulate the temperature of the sample in the range from ambient temperature to 12 K.

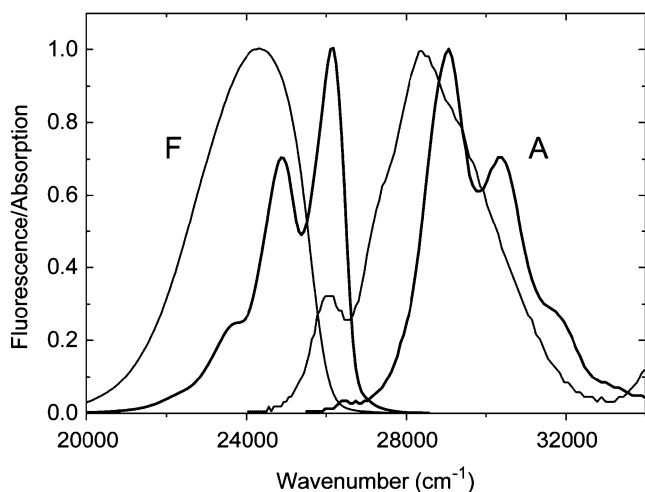


Fig. 1 Absorption (*A*) and fluorescence emission (*F*) spectra of pyrene-1-COO⁻ (*thick lines*) and pyrene-1-COOH (*thin lines*) measured at pH 9.6 and 1.2 respectively in 1:1 *v/v* glycerol/water at 20°C

Quantum mechanical calculations

Ground and excited state vacuum calculations of pyrene-1-COO⁻ and pyrene-1-COOH were performed using GAUSSIAN 98 [14]. Minimum ground state geometries were calculated for protonated and deprotonated carboxy-1-pyrene using a series of Hartree-Fock optimizations with increasingly complex basis sets. The reported ground state geometries were optimized with HF/6-31G(d,p) [15–18]. Frequency calculation showed these to be minimum structures. Excited state geometries were optimized with CIS/6-31G(d,p) with minimum geometries confirmed by frequency calculation. Potential distributions were calculated using the natural population analysis (NPA) approximation included in the NBO portion of the Gaussian package [19].

Results

Absorption and fluorescence of pyrene-1-COO⁻ and pyrene-1-COOH

In Fig. 1 absorption spectra of pyrene-1-COO⁻ in water and glycerol are shown as a function of wavenumber. The lowest energy absorption peak is at 26,060 cm⁻¹ for the carboxy acid, and at ~26,470 cm⁻¹ for the carboxylate. The extinction of the 0,0 band is low, which is also found for the parent compound pyrene [20]. The emission of the carboxy acid is broad and featureless, and the position of the 0,0 transition can not be determined. The peak position of the emission of the carboxylate is at 26,150 cm⁻¹.

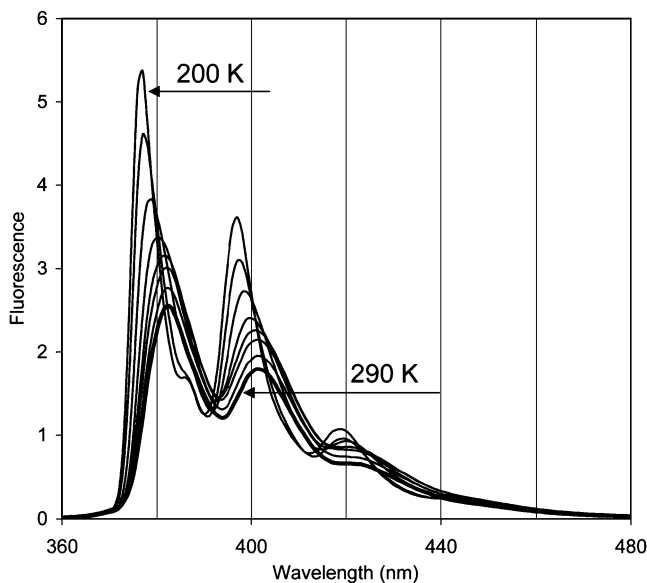


Fig. 2 Fluorescence emission spectra of 1 mM pyrene-1-COO⁻ at 290, 270, 250, 240, 230, 220 and 210 K. Excitation: 333 nm; spectral band pass: 2 nm. Solution was 1:1 *v/v* glycerol/water, pH 9

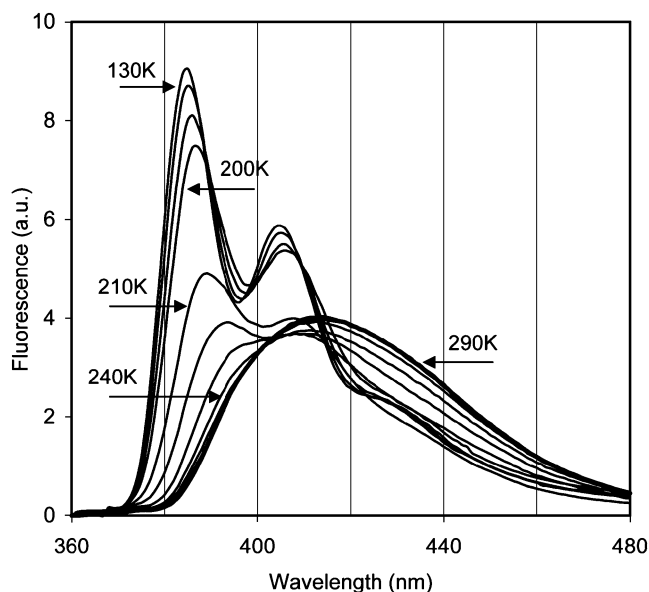


Fig. 3 Fluorescence emission spectra of 1 mM pyrene-1-COOH at pH 1 in glycerol/water (1:1 v/v) at 290, 270, 250, 240, 230, 220, 210, 200, 190, 170 and 130 K. Excitation: 333 nm; spectral band pass: 2 nm

For pyrene-1-COO⁻ emission maxima occur at 381 and 400 nm in water and 383 and 402 nm in 1:1 (v/v) glycerol/water at 290 K (Fig. 2). The peaks of the sample in glycerol/water shift to the blue and the peak-width narrows as temperature decreases. At 12 K the position maximum of the 0–0 transition band for pyrene-1-COO⁻ in 1:1 (v/v) glycerol/water is 375 nm.

Emission spectra of pyrene-1-COOH at various temperatures are shown in Fig. 3. The fluorescence spectra of pyrene-1-COOH are particularly sensitive to temperature, with the striking observation that only at low temperature, i.e. below the glass transition of glycerol/water at ~240 K,

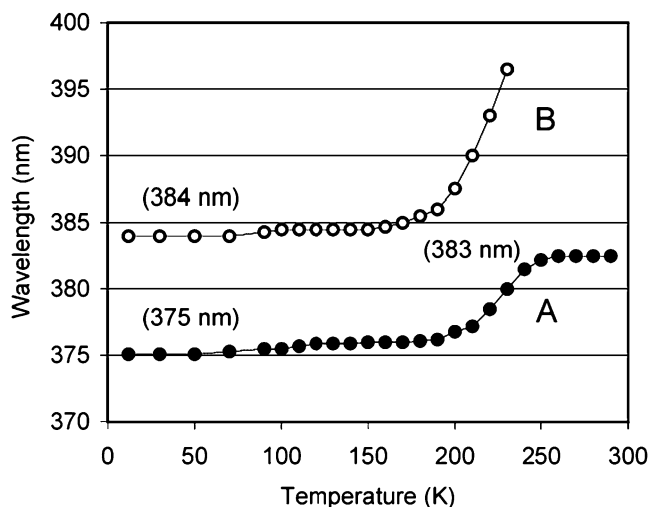


Fig. 4 Wavelength of the 0,0 transition as a function of temperature for pyrene-1-COO⁻ (A) and pyrene-1-COOH (B). Conditions given in Figs. 2 and 3, respectively

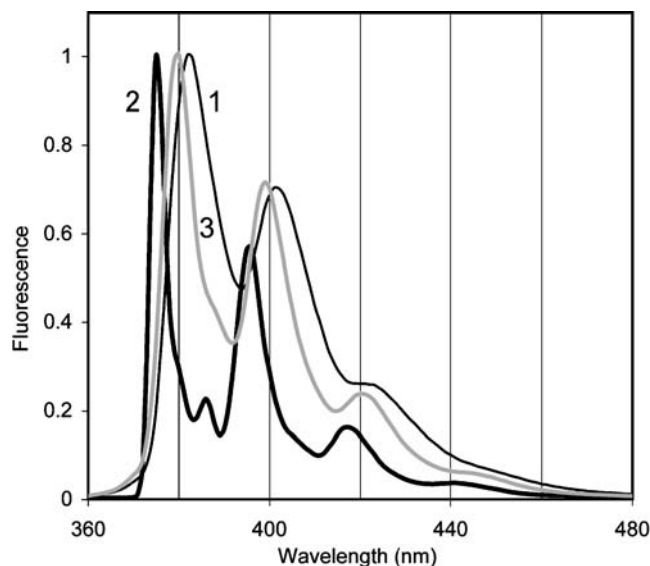


Fig. 5 Comparison of spectra of pyrene-1-COO⁻ in glycerol/water at 290 K (1) and 12 K (2) and in 95% methanol at 290 K (3)

is vibronic resolution seen. The vibronic resolution resembles the emission of pyrene-1-COO⁻ (cf. Fig. 2) but the emission maximum of the 0,0 transition at 12 K is 384 nm (26,041 cm⁻¹), lower in energy to the 0,0 transition of pyrene-1-COOH (375 nm, 26,666 cm⁻¹) by 625 cm⁻¹.

The temperature dependence of peak positions as a function of temperature for the two species are summarized on Fig. 4. At ~240 K, the glass transition of glycerol/water, the peak of the carboxylate shifts to the blue. For pyrene-1-COOH the 0,0 transition could not be seen at high temperature, but as the solvent glassifies, resolution is seen. Changes in the spectra below 150 K were very small.

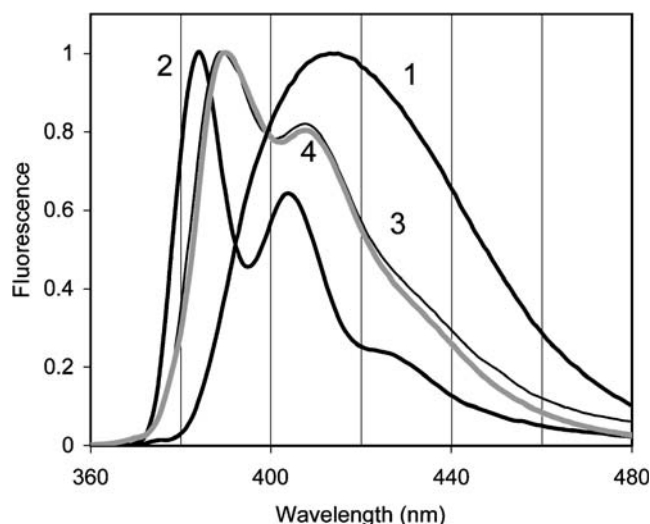


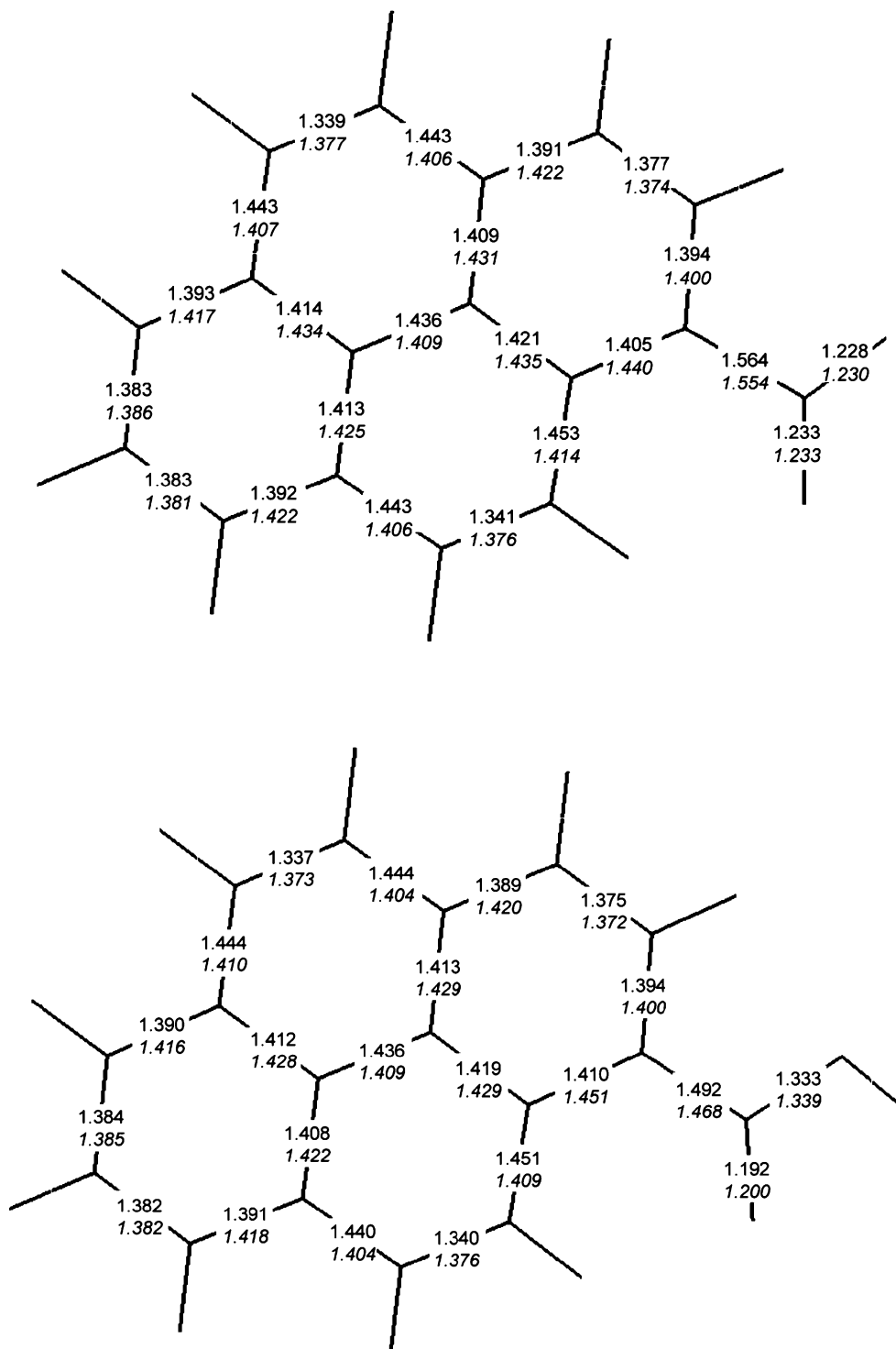
Fig. 6 Comparison of spectra of pyrene-1-COOH in glycerol/water at 290 K (1) 12 K (2) 210 K (3, thin line) and in 95% methanol (5% water) at 290 K (4, thick line)

Comparison of solvent and temperature effects

When a molecule is excited, the dipole moment changes, and dipolar molecules change position around the excited state molecule; this lowers the energy of the excited state molecule and produces a shift in the emission spectrum [21]. It follows that the shift is a function of the relaxation rates of the

dipolar molecules and the dipole nature of the solvent, and that lowering the temperature or lowering the dipole strength of the solvent can have the same effect. In Fig. 5, the emission spectrum of pyrene-1-COO⁻ is shown in methanol at room temperature. The peak is at 380 nm. This compares with a peak position of 382 and 375 nm for pyrene-1-COO⁻ in glycerol/water at high and low temperature.

Fig. 7 Bond lengths in Angstroms for C–C and C–O of ground and excited state pyrene-COO⁻ (*top*) and pyrene-COOH (*bottom*). Values for excited state molecule are shown in *italics*



The emission maximum of pyrene-1-COOH in glycerol is slightly red shifted relative to the spectrum observed for the fluorophore in water. The maximum in water is 416 and 413 nm in glycerol/water. The emission in methanol,

however, is blue shifted. As seen in Fig. 6 the spectrum of pyrene-1-COOH in glycerol/water at ~ 210 K is superimposed on a spectrum of pyrene-1-COOH in methanol at room temperature. The two spectra are approximately the same,

Fig. 8 Angles for ground and excited state pyrene-COO⁻ (*top*) and pyrene-COOH (*bottom*). Values for excited state molecule are shown in *italics*

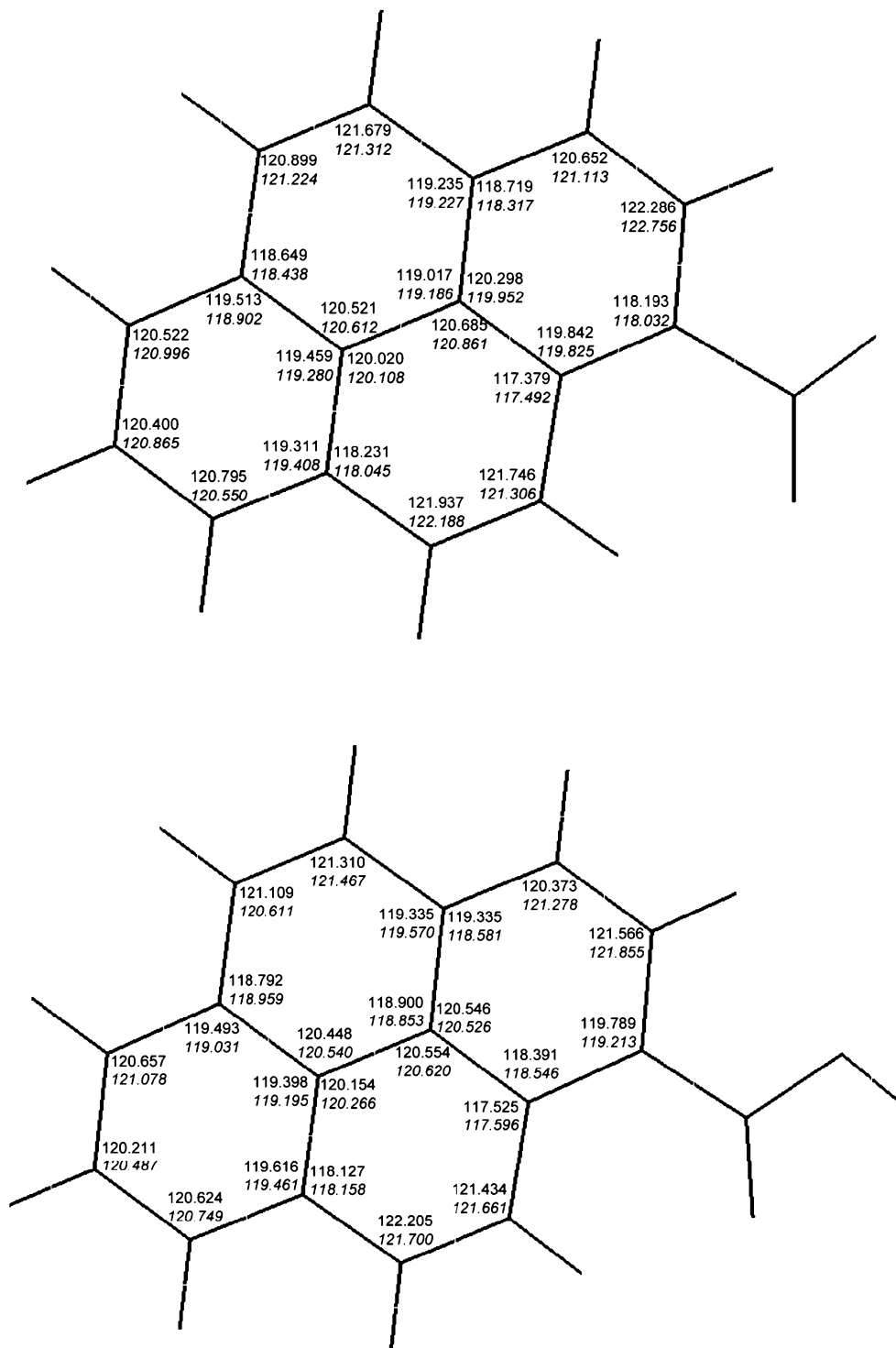
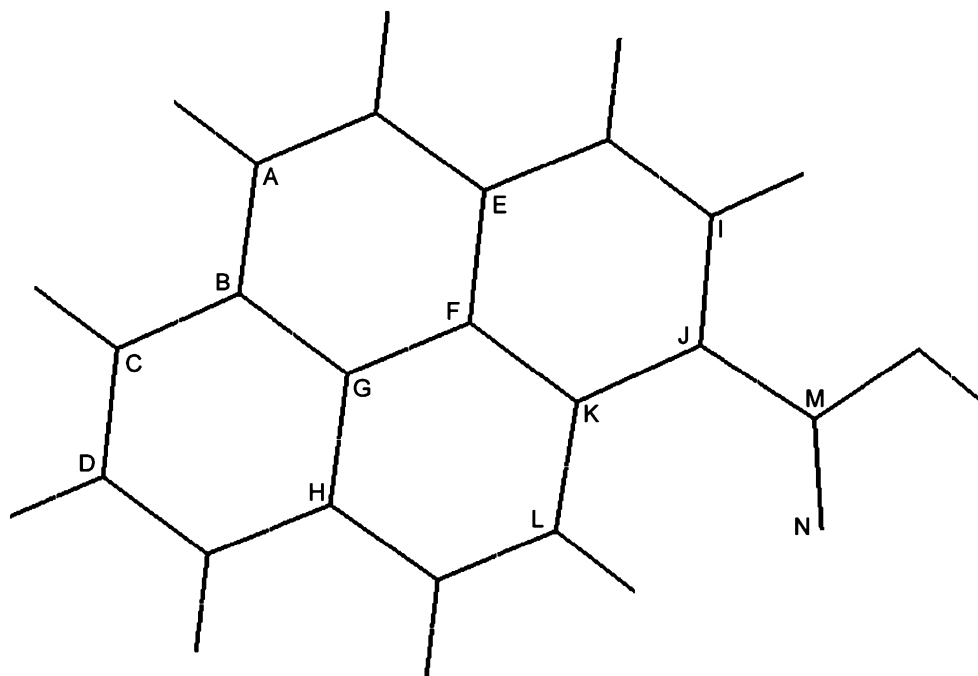


Fig. 9 Definition of dihedral angles for ground and excited state pyrene-1-COO⁻ and pyrene-1-COOH. Values are given in Table 1



with peak position at 390 nm. The shift with temperature and the shift with changing solvent are consistent with relaxation of the dipolar solvent molecules.

Geometry and charge distribution of pyrene-1-COO⁻ and pyrene-1-COOH

The *pK* of ground state pyrene-1-COOH is ~4 and the excited state *pK* is ~8 [22]. As noted in “Introduction,” because of the difference in *pK* between ground and excited state molecules, excitation of pyrene-1-COO⁻ leads to protonation of the excited state molecule, provided a proton donor is accessible within the excited state lifetime [10]. In order to rationalize why the *pK* changes upon excitation, changes in geometry and charge distribution of the molecules were considered.

Ground and excited state molecular geometries were computed for the carboxy-1-pyrene molecule. Fig. 7 gives bond distances, Fig. 8 labels bond angles, Fig. 9 defines dihedral angles with the values given on Table 1. Partial charges were assigned to each atom using natural bond orbital population analysis (Fig. 10).

Bond distances and angles change upon excitation for both forms of carboxy-1-pyrene (Fig. 7 and 8). For pyrene-1-COOH, the length of the C–C bond between the ring and the COOH substituent is 1.492 in the ground state, which changes to 1.468 Å upon excitation. The short distance is comparable to a C–C π bond, and the further shortening upon excitation indicates stronger bonding in the excited state. This bond length is longer for pyrene-1-COO⁻; the C–C bond in the carboxylate is calculated to be 1.564 and

1.554 Å, in ground and excited state respectively. This large distance shows a more aliphatic-type bond between the carboxylate and the aromatic ring.

Pyrene-1-COO⁻ is non-planar in the ground state, as indicated by dihedral angles that are less than 180°, and the carbonyl group is not in-plane. In contrast, this in vacuo calculation indicates that the COOH is planar to the ring for the ground and excited states. Note that the dihedral angle for IJMN is 180° for pyrene-COOH, whereas this angle changes from 162 to 191° upon excitation of the carboxylate form (Table 1). The planarity of the COOH to the ring is consistent with aromaticity.

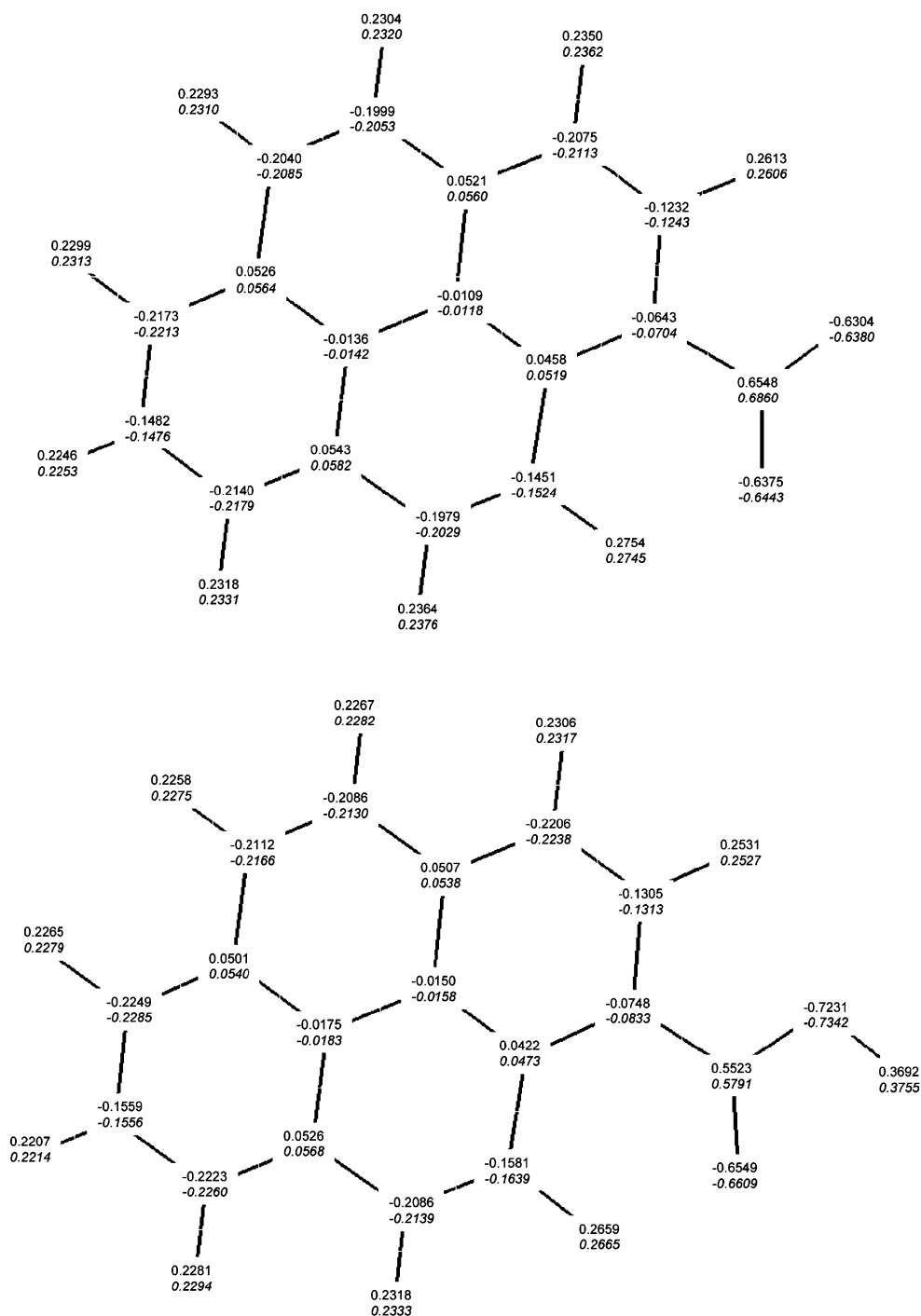
The calculated dipoles for carboxy-pyrene derivatives are given in Table 2. The dipole value is larger for the pyrene-1-COO⁻, consistent with the formal charge on the carboxylate, and the orientation of the dipole does not change with excitation. The dipole direction of pyrene-1-COOH changes somewhat with excitation. The overall

Table 1 Calculated dihedral angles for ground and excited electronic states

	Pyrene-1-COO ⁻		Pyrene-1-COOH	
	Ground	Excited	Ground	Excited
ABCD	179.95	180.01	180.00	180.00
EFGH	179.47	180.18	180.00	180.00
IJKL	178.97	180.75	180.00	180.00
IJMN	162.69	191.62	180.00	180.00

Angles are defined in Fig. 8.

Fig. 10 Charges for pyrene-COO⁻ (*top*) and pyrene-COOH (*bottom*). Values for excited state molecule are shown in *italics*



dipole moment is smaller for pyrene-1-COOH than for pyrene-1-COO⁻, but the change upon excitation is larger. For calculation of the transition dipole moment a more extended analysis is required [23]. We also note that since the calculations are made in vacuo they do not simulate solvation effects.

Figure 10 presents the calculated NBO charges for ring atoms of the ground and excited state molecules. Agmon et

Table 2 Calculated permanent dipole moments for ground and excited state molecules

	Electronic state	Debye
Pyrene-1-COO ⁻	Ground	13.76
	Excited	14.80
Pyrene-1-COOH	Ground	3.09
	Excited	2.46

al. [13] discuss the changes in the degree of antiaromaticity of the ring system between ground and excited states to explain the photoacidity of naphthol derivatives. They relate these changes to redistributions of charge which cause the pK changes. For the carboxy-pyrene compounds we did not see evidence for this upon the NBO charges or the bond lengths and angles (Figs. 7 and 8).

Discussion

The calculations presented here indicate that the π electrons of the compound pyrene-1-COOH are partially delocalized on the carboxy-acid in the excited state. This stabilizes it relative to the excited state basic form, and this fact largely accounts for the change in pK in the excited state. The extra delocalization shifts the absorption and emission spectra to the red, relative to the basic form (Fig. 1).

The basic form has a small dipole moment change upon excitation and consequently, the fluorescence of pyrene-1-COO⁻ is relatively insensitive to dipolar relaxation of solute molecules, as seen from only small changes in fluorescence with temperature (Fig. 3). The lack of rearrangement of solvent is consistent with the observation that the fluorescence decay is described by a single exponential function [10]. This feature simplifies analysis of excited state reactions.

The acidic form, pyrene-1-COOH shows a larger shift in the spectrum than the basic form when exposed to temperature extremes and change in solvent. The emission spectrum is not resolved at room temperature, but at low temperature vibronic resolution becomes apparent. Nevertheless, even at the lowest temperature examined, 12 K, the spectrum is still broader than that observed for pyrene-1-COO⁻. A larger inhomogeneously broadened band can arise from greater interaction with solvent. In liquids, molecular dynamics and quantum calculations indicate that the solvent cage around each molecule has differences which result in a distribution of energies for the 0,0 absorption bands for the ensemble of molecules [24]. Furthermore distortions of the positions of the chromophore itself can lead to inhomogeneous broadening. This is true for both forms of the pyrene derivative. However, although the COOH group shares an aromatic type bonding with the ring, the absolute position of the carboxy group is not rigid. Because rotation of the group will change the coupling, a broadened spectral band will result.

In conclusion, properties of pyrene-1-COO⁻ and pyrene-1-COOH are compared. Spectra that are given show that pyrene-1-COO⁻ is less sensitive to solvent and temperature. Calculations indicate that all bond lengths change with protonation and excitation, but the striking effect is that the

carboxyl group is more conjugated in the acid form relative to the base form.

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