Intramolecular Interactions in Aromatic Amino Acids and Model Compounds

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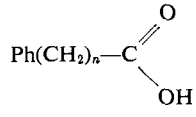
Abstract: The absorption and fluorescence spectra of a number of phenylalkylcarboxylic acids were investigated at room temperature as a function of pH. Changes in intensity as well as in the vibronic structure of the absorption band corresponding to the $^1A_{1g} \rightarrow {}^1B_{2u}$ benzene transition are attributed to the inductive effect of the carboxyl group. The quenching of fluorescence is interpreted in terms of intramolecular interaction of charge-transfer type between the carboxyl and phenyl groups. This is supported by an increase in the phosphorescence/fluorescence ratio and shortening of phosphorescence lifetime in going from toluene to phenylacetic acid. A proton-transfer mechanism is ruled out, since the ethyl ester has essentially the same absorption and luminescence characteristics as phenylacetic acid in its un-ionized form.

The aromatic amino acids (phenylalanine, tyrosine, tryptophane) absorb light in the near-ultraviolet and their luminescence can easily be observed. They can be used as intrinsic spectroscopic probes for the study of polypeptides and proteins.[1,2] It has been observed that some chemical groups, such as the carboxylic and amino groups present in the isolated amino acid, and the peptide bond when the amino acid is included in a polypeptide, can quench the fluorescence.[3]

The specific effect of the carboxylic group on the fluorescence yield of the aromatic ring[4] is best demonstrated when the pH of the medium is varied. A decrease in fluorescence yield is observed with the protonation of the carboxylate anion group. This quenching effect is evidence for interaction between the carboxylic and the aromatic groups in the amino acid.

To understand this interaction, we have studied the effect of the carboxylic group on absorption and emission properties in model substances. These model compounds are phenylalkylcarboxylic acids, where the alkyl chain is of different lengths



where n = 1–4.

Thus phenylalanine differs from phenylpropionic acid (n = 2) by the presence of the amino group. In each case, the spectroscopic properties of these compounds were compared to those of toluene. The ethyl ester of phenylacetic acid (n = 1) was also studied to examine the role of the carboxylic proton in fluorescence quenching of these compounds.

Experimental Section

(A) **Materials.** (1) Phenylalkylcarboxylic acids were purified by several crystallizations from alcohol–water mixtures and by vacuum sublimation. (2) Toluene and ethyl phenylacetate were purified by vacuum distillation. (3) Ethanol was purified by fractional distillation of absolute alcohol until all benzene was removed, and was distilled freshly when needed. This gave transparent nonluminescent ethanol

(B) **Methods.** (1) Absorption spectra were recorded on a Cary-15 spectrophotometer. (2) Emission spectra and phosphorescence lifetimes were obtained using an Amino-Keers spectrosphosphorimeter. pH values were measured using a Beckman pH meter.

Results

Absorption spectra of phenylalkylcarboxylic acids were measured between 300 and 200 nm. In this region, bands corresponding to $^1A_{1g} \rightarrow {}^1B_{2u}$ and $^1A_{1g} \rightarrow {}^1B_{1u}$ benzene transitions are observed. The $^1A_{1g} \rightarrow {}^1B_{2u}$ absorption band shows systematic changes as a function of pH. In the case of phenylacetic acid, where the carboxylic group is separated from the phenyl group by one methylene group, the effect is large (Figure 1). There is a decrease in the integrated intensity of the absorption band by as much as 22% and a small shift of 185 cm^{-1} to shorter wavelengths when the carboxylate anion group is protonated. There is also an effect on the detailed vibronic structure of the absorption band. If one compares the spectra of phenylacetic acid in its two forms to that of toluene,[5] one sees that all vibronic bands which do not include an excitation of the out-of-plane bending mode (528 cm^{-1}) decrease appreciably in intensity in the acid form; *i.e.*, the pure electric-dipole-allowed subsystem becomes much weaker in intensity. These two effects, while still present for the other acids of the series, decrease from phenylacetic (n = 1) to phenylvaleric acid (n = 4). The latter acid has an absorption spectrum essentially the same as that of toluene.

The $^1A_{1g} \rightarrow {}^1B_{1u}$ absorption band maximum shifts to shorter wavelengths by about 450 cm^{-1} when one goes from toluene, or phenylvaleric acid, to phenylacetic acid in its acid form. Moreover, an additional absorption between 220 and 235 nm appears on the long-wavelength shoulder of the $^1A_{1g} \rightarrow {}^1B_{1u}$ band of the phenylacetic acid (Figure 2). In all aspects the absorption spectrum of the ethyl ester of phenylacetic acid is indistinguishable from that of the protonated form of the corresponding acid.

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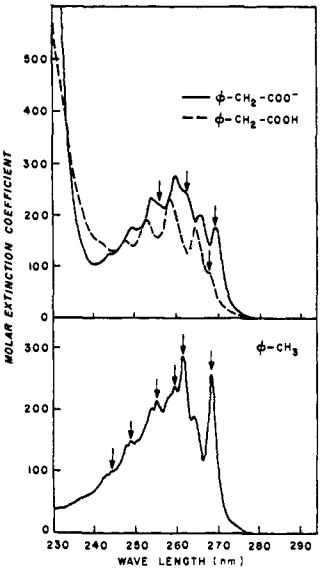

Figure 1. The $^1A_{1g} \rightarrow {}^1B_{2u}$ type absorption bands for toluene, phenylacetic acid, and sodium phenylacetate in ethanol solutions at room temperature. Arrows mark vibronic bands of the electronically allowed subsystem, *i.e.*, vibronic bands which do not include the 528-cm^{-1} out-of-plane bending mode. The spectrum of sodium phenylacetate undergoes a small blue shift (185 cm^{-1}) in going to the un-ionized form.

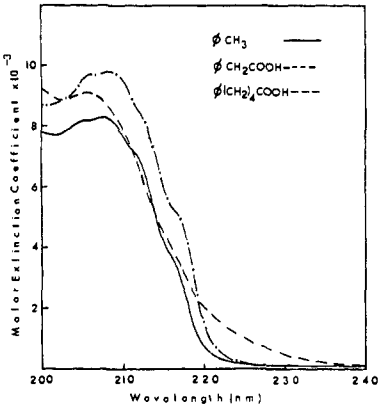

Figure 2. The $^1A_{1g} \rightarrow {}^1B_{1u}$ type absorption bands for toluene, phenylacetic acid, and phenylvaleric acid in ethanol solutions at room temperature.

Luminescence spectra of these compounds were studied. It was found that these acids in the conjugate base forms have essentially the same fluorescence yields as toluene. When the pH is decreased, the fluorescence yield undergoes a sharp drop at a pH value of about 4.5, corresponding to the appearance of the un-ionized form of the carboxyl group (Figure 3). The extent of this fluorescence quenching decreases with increasing the separation between the phenyl and the carboxylic group, *i.e.*, with increasing the number of methylene groups. The quenching of the fluorescence does not vary with the concentration of the acid, which shows that this quenching is essentially an intramolecular and not an intermolecular effect. At low temperature both phosphorescence and fluorescence can be measured.[6,7] The ratio of phosphorescence to fluorescence is higher for the protonated form than for the corresponding anion form. The ethyl ester of phenylacetic acid has essentially the same fluorescence yield in alcoholic solution as the un-ionized form of the corresponding acid.

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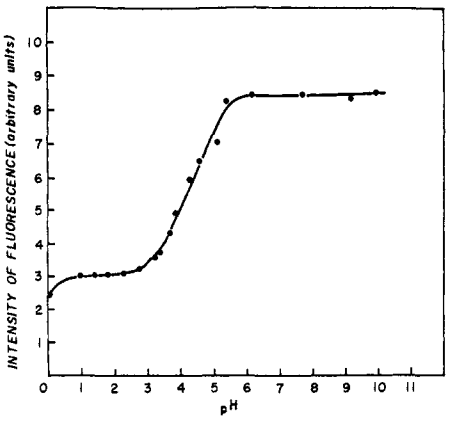

Figure 3. Effect of pH on the relative intensity of the fluorescence of phenylacetic in water at room temperature.

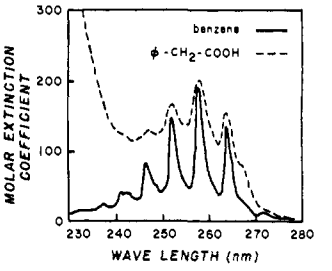

Figure 4. Room-temperature absorption spectra of benzene and phenylacetic acid in ethanol. The spectrum of benzene is displaced by 300 cm^{-1} to the right for the purpose of comparison.

Discussion

The experimental results show unambiguously that the carboxylic and ester groups have an effect on spectroscopic properties of toluene. By contrast, the carboxylate anion has little effect on the vibronic structure of the lowest absorption band and the fluorescence quantum yield of toluene. The protonation of the carboxylate group produces a decrease in the integrated absorption intensity of the $^1A_{1g} \rightarrow {}^1B_{2u}$ band, *i.e.*, a decrease of the oscillator strength of the corresponding transition. This effect can be explained by the inductive effect of the substituent.[8] Methyl substitution in benzene breaks down the D_{6h} symmetry of benzene, making the $^1A_{1g} \rightarrow {}^1B_{2u}$ transition electric-dipole allowed; thus, the $^1A_{1g} \rightarrow {}^1B_{2u}$ transition in toluene consists of two subsystems, namely the electric-dipole-allowed system and the vibrationally induced system. The latter is induced by the 528-cm^{-1} out-of-plane bending mode. In benzene, only the vibrationally induced system is present. In the phenylacetic acid molecule the -COOH group, being electron withdrawing, makes the methyl group less electron donating. Thus, the -COOH group decreases the effect of the methyl group on the aromatic ring, making the vibronic structure of the lowest absorption bands of phenylacetic acid and benzene very similar in shape, as shown in Figure 4. Configuration interaction of

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$^1B_{2u}$ and charge-transfer states is probably responsible in great part for the red shift of ~300 cm^{-1} for the $^1A_{1g} \rightarrow {}^1B_{2u}$ transition in phenylacetic acid relative to benzene. In the case of the phenylacetate anion, the carboxylate anion does not decrease and may even enhance the inductive effect of the methyl group, causing the vibronic structure of the toluene and phenylacetate anion lowest absorption bands to be similar. This also explains the small red shift of phenylacetate anion spectrum relative to that of toluene, as shown in Figure 1.

The decrease in oscillator strength in going from toluene or phenylacetate anion to phenylacetic acid cannot alone explain the magnitude of the decrease (70%) in fluorescence yield. Since singlet–singlet radiationless transitions from the lowest singlet to the ground state are expected to be negligible in these molecules, this decrease in fluorescence yield implies that protonation of the carboxylate group increases the intersystem crossing rate. This is further supported by the larger phosphorescence/fluorescence ratio for phenylacetic acid compared to toluene, the measured phosphorescence/fluorescence ratios in ethanol glass being 2.05 and 0.96, respectively. We shall now consider different mechanisms which could lead to an enhancement of phosphorescence and quenching of fluorescence.

We have considered first the possibility of energy transfer from the aromatic group to the carboxylic group, and back. This transfer is impossible energetically from the aromatic singlet to the carboxylic singlet state, and improbable from the aromatic singlet to the carboxylic triplet state.

White[4] has shown that protons at very high concentrations in aqueous solutions can quench fluorescence of aromatic amino acids. This suggests that fluorescence could be quenched by a proton-transfer mechanism. The observation that the ethyl ester of phenylacetic acid has a similar fluorescence yield as phenylacetic acid eliminates a proton-transfer mechanism.

Tsubomura and Mulliken[9] have shown that charge-transfer interaction with oxygen can enhance the singlet–triplet transition probability for many organic molecules by borrowing intensity from the charge-transfer band, the intensity of which comes in part from allowed transitions of the donor. Considering the values for ionization potenitals and electron affinities of toluene and acetic acid molecules, the electron transfer would be from the aromatic group to the carboxylic acid group. Observation of charge-transfer absorption in phenylacetic acid would provide direct evidence for intramolecular interaction between the phenyl ring and the carboxylic group. It may be pointed out that in the case of benzoic acid, where the carboxylic group is directly conjugated to the phenyl ring, a charge-transfer band appears at 228 nm. The absorption on the long-wavelength shoulder of the $^1A_{1g} \rightarrow {}^1B_{1u}$ band in phenylacetic acid (between 220 and 235 nm) may correspond to such charge-transfer absorption. Such absorption is absent in both toluene and phenylvaleric acid, as shown in Figure 2.

The carboxylic acid is expected to have a higher electron affinity than the carboxylate anion. This would make the energy of the charge-transfer state lower for the phenylcarboxylic acid compared to the corresponding anion. One would therefore expect an enhancement of the singlet–triplet transition probability leading to fluorescence quenching in the phenyl carboxylic acid compared to the corresponding anion. Electron affinities of acetic acid and its ester are expected to be close, and this would explain the comparable quenching effects of these two groups.

Enhancement of singlet–triplet transition probability in phenylacetic acid is supported by our observation that the natural phosphorescence lifetimes (τ_p^0) of phenylacetic acid and its ester are shorter than those of toluene or phenylvaleric acid. The values of τ_p^0 obtained from lifetime and phosphorescence quantum yield measurements in ethanol glass at 77°K are: phenylacetic acid, 11.9 sec; ethyl phenylacetate, 11.5 sec; phenylvaleric acid, 17.4 sec; and toluene, 18.3 sec. These results imply that some overlap occurs between the π-electron systems of the aromatic ring and the carboxylic group in phenylacetic acid and its ester. In the case of benzoic acid where such overlap is larger, the measured value for τ_p^0 is 3.1 sec under the same conditions. This result is similar to that reported by Maria and McGlynn,[10] who reported a phosphorescence lifetime, τ_p, of 2.1 sec for benzoic acid and concluded that the lowest triplet state of benzoic acid does not involve an excitation completely localized on the benzene ring, but rather involves considerable delocalization onto the carboxyl group.

The effect of the substituent on luminescence is small but still noticeable when four methylene groups separate the ring from the substituent. This probably indicates that in phenylvaleric acid certain conformations in which the carboxyl group approaches the phenyl group are present in solution.

The problem of fluorescence quenching in aromatic amino acids has led to a more general problem of molecular spectroscopy, namely the possible effects of substituents on the luminescence properties of aromatic groups when the substituents are not directly substituted on the ring but are separated by one of several methylene groups. An extensive study of similar compounds with substituents of different electronic character is being carried out to examine the details of the charge-transfer mechanism proposed in this paper.

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