Bull. Chem. Soc. Jpn., ***76***, *1741–1746 (2003)*

Excited-State Dipole Moments of Indoles Using Solvatochromic Shift Methods: An Experimental and Theoretical Study

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The electronic absorption and fluorescence spectra of some indoles were recorded at room temperature in solvents of different polarities. In order to elucidate the solvent band shifts during relaxation of the excited states, the dipole moments of the excited singlet states were estimated from solvent-dependent Stokes shift data using a solvatochromic method based on a microscopic solvent polarity parameter ($E_{\mathrm{T}}^{\mathrm{N}}$). All indoles show a substantial increase in the dipole moment upon excitation to the emitting state. These results are generally consistent with the Austin model 1 (AM1) calculations, and are found to be quite reliable in view of the fact that the correlation of the solvatochromic Stokes shifts with the microscopic solvent polarity parameter ($E_{\mathrm{T}}^{\mathrm{N}}$) is superior to that obtained using bulk solvent polarity functions in almost all the systems studied here.

In continuation of our work on the photophysical properties of some natural products,[1–3] we report here on the excited-state dipole moments of some indole derivatives: indole (I), indol-5-ol (I5O), 5-hydroxyindole-2-carboxylic acid (5HI2C), 3-indoleacetic acid (3IA) and 5-hydroxytryptophan (5HT) (Fig. 1). The indole ring plays important photobiological and photochemical roles in reactions occurring in living cells.[4] The indole ring system is a good prototype for testing theoretical models, and it is common to many naturally occurring compounds; e.g., the essential amino acid tryptophan has been utilized as an intrinsic chromophore to study the optical properties of biomolecules, since 90% of the total fluorescence in proteins comes from tryptophan.[5] Because a great deal of information about protein conformational dynamics and the mechanism of function is potentially available through studies of the fluorescence characteristics of indole groups in proteins,[6–8] it was felt to be worthwhile to investigate its spectral behavior under different environments.

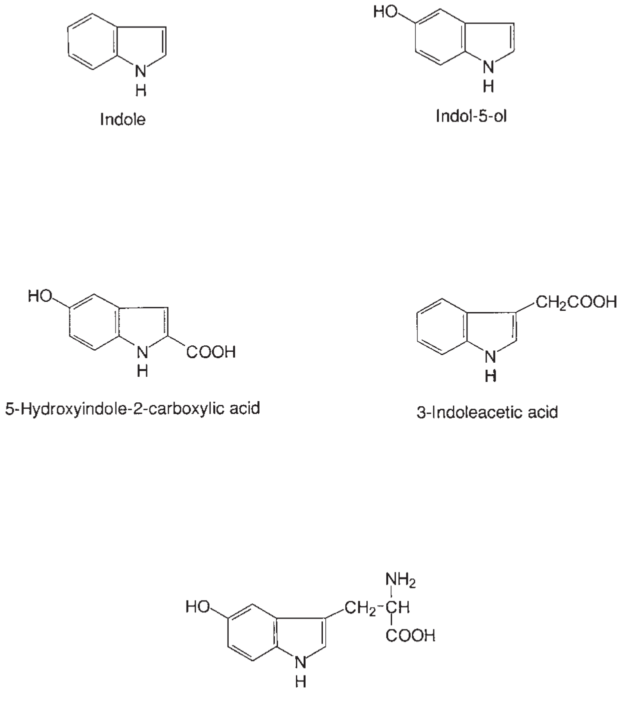

Fig. 1.

The solvent effects on the spectral properties of indoles have been extensively studied. The unusually large fluorescence Stokes shifts of indole and its derivatives in polar solvents have received much attention,[9–21] and have been explained based on the interactions with these solvents,[15] the solvent dielectric relaxation mechanism[14] or the formation of an exciplex between an excited indole and a polar solvent molecule,[16–20] suggesting a considerably higher dipole moment in the excited state than in the ground state.[14,22–24] A number of theoretical studies[25–28] on indole and its derivatives have been extensively reported along with experimental studies. It has been observed by several workers[21,29–32] that the lowest absorption band of indole is due to two electronic transitions, designated as $^1\mathrm{L_b} \leftarrow \mathrm{S_0}$ and $^1\mathrm{L_a} \leftarrow \mathrm{S_0}$; the $^1\mathrm{L_a}$ state is more polar than the $^1\mathrm{L_b}$ state.

In the present work, we applied solvatochromic shift methods to estimate the excited-state dipole moments of some biologically important indoles, both experimentally as well as theoretically. It has already been observed that the correlation of the solvatochromic Stokes shifts with the microscopic solvent polarity parameter ($E_{\mathrm{T}}^{\mathrm{N}}$) is superior to that obtained using bulk solvent polarity functions.[1,2] A similar observation was made for all of the indoles studied here. The increase in the dipole moment has been explained based on the possible resonance structures.

1. Experimental

Indoles of laser grade were purchased from Sigma and used as such without further purification. Triply distilled water and spectroscopic-grade solvents were used after checking their purity.

Published on the web September 15, 2003; DOI 10.1246/bcsj.76.1741

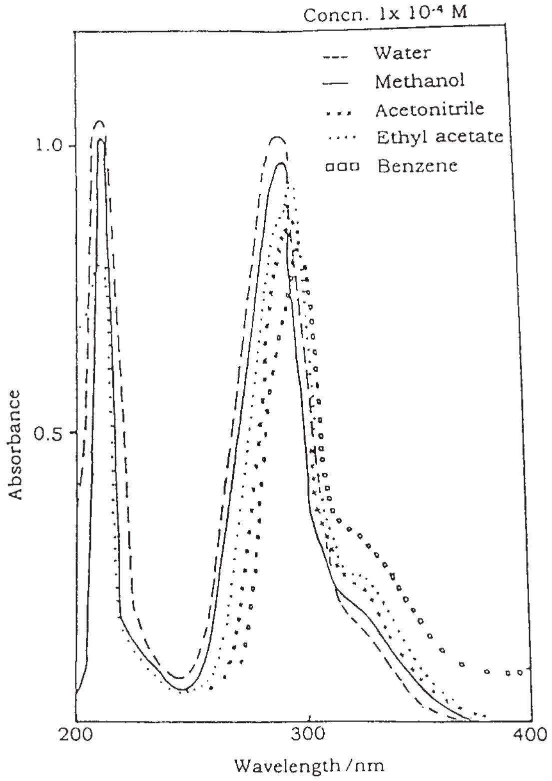

Fig. 2. Absorption spectra of 5-hydroxyindole-2-carboxylic acid in different solvents.

The absorption and fluorescence spectra were measured on a Shimadzu (260) spectrophotometer and a Jasco FP-770 spectrofluorimeter, respectively (Figs. 2 and 3). Austin Model 1 (AM1)[33] semiempirical molecular-orbital calculations were carried out to estimate the ground-state and excited-state dipole moments and the absorption maxima in the vapor phase. The Onsager cavity radii of all the molecules were determined theoretically using the optimized geometry. The package Spartan Version 4.0[34] was used for all of the theoretical calculations reported here.

1.1 Determination of Dipole Moments. Two methods, depending on the internal electric field (solvatochromism), were implemented in the present investigation, the details of which are given in our earlier paper.[1]

In the first method, the following Bakshiev[35] and Chamma and Viallet[36] equations (Eqs. 1 and 2) were applied to estimate the change in the dipole moment of a molecule upon excitation:

$$\bar{\nu}_a - \bar{\nu}_f = S_1F_1(D,n) + C_1, \tag{1}$$

$$1/2(\bar{\nu}_a + \bar{\nu}_f) = S_2F_2(D,n) + C_2, \tag{2}$$

where $\bar{\nu}_a$ and $\bar{\nu}_f$ are the absorption and fluorescence maxima, n and D are the refractive index and the dielectric constant of the solvents, respectively. The expression for the slopes (S_1 and S_2) and solvent polarity functions ($F_1(D,n)$ and $F_2(D,n)$) are described in a previous paper.[1]

Plots of the Stokes shifts (($\bar{\nu}_a - \bar{\nu}_f$) and $1/2(\bar{\nu}_a + \bar{\nu}_f)$) against the bulk solvent polarity functions ($F_1(D,n)$ and $F_2(D,n)$) for different solvents yield the slopes S_1 and S_2, respectively. Thus, the ratio of the excited-state to the ground-state dipole moments (μ^*/μ) can be obtained using

$$\frac{\mu^*}{\mu} = \frac{|S_1 - S_2|}{|S_1 + S_2|}. \tag{3}$$

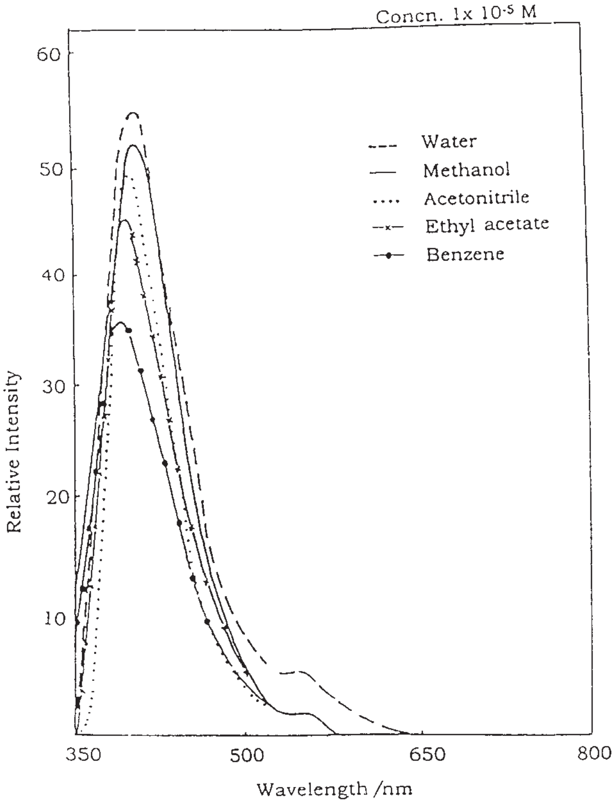

Fig. 3. Fluorescence spectra of 5-hydroxyindole-2-carboxylic acid in different solvents.

In the second method,[37] the problem associated with the error in estimating the Onsager radius (a) was minimized, since the ratio of two Onsager radii (a_B/a) is involved (Eq. 4). The excited-state dipole moment is determined by

$$\Delta\bar{\nu}_{af} = 11307.6[(\Delta\mu/\Delta\mu_B)^2(a_B/a)^3]E_T^N + \text{Constant}, \tag{4}$$

where $\Delta\bar{\nu}_{af}$ is the Stokes shift and E_T^N is the solvent-polarity parameter proposed by Reichardt,[38] which is based on the absorption wavenumber of a standard betaine dye in the solvent. Its value for different solvents is given in Table 1. $\Delta\mu_B$ (= 9 D) and a_B (= 6.2 Å) are the dipole-moment changes upon excitation and the Onsager cavity radius, respectively, of betaine dye,[37] whereas $\Delta\mu$ and "a" are the corresponding quantities for the molecule of interest. Thus, the plot of $\Delta\bar{\nu}_{af}$ (Stokes shifts) versus E_T^N (Eq. 4) can be used to obtain the change in the dipole moment ($\Delta\mu$) upon excitation.

The Onsager cavity radii of all molecules were taken to be 40% of the longest axis in the optimized geometry of the molecule, obtained by using semiempirical calculations. Thus, by knowing

Table 1. Calculated Values for Solvent Polarity Parameters

Solvent	E_T^N	$F_1(D,n)$	$F_2(D,n)$
Cyclohexane	0.015	0.040	0.290
Benzene	0.117	0.005	0.340
Ethyl acetate	0.228	0.060	0.490
Acetonitrile	0.472	0.860	0.650
Methanol	0.762	0.850	0.650
Water	1.000	0.910	0.680

Table 2. Wavenumbers (cm^{-1}) for the Absorption and Emission[a)] Maxima of Indoles in Different Solvents

Compound	Benzene	Cyclohexane	Ethyl acetate	Acetonitrile	Methanol	Water[b)]	V.P.[c)]
I	37593	37565	37147	37147	36900	36900	29412
	(32786)	(32467)	(31746)	(30395)	(29498)	(28169)	
I5O	36023	37037	37147	36873	37037	37009	28090
	(30211)	(30395)	(30030)	(30030)	(29761)	(29673)	
5HI2C	33760	—	33921	34036	34364	34482	28011
	(25380)	—	(25125)	(24752)	(24509)	(24390)	
3IA	35460	36544	35536	35587	35561	35511	29070
	—	(31545)	(30303)	(28735)	(28571)	(27700)	
5HT	—	—	36496	36310	36337	36469	29586
	—	(29411)	(29761)	(29673)	(29325)	(29325)	

a) In parentheses. b) Excluded in the plots due to strong H-bonding interactions. c) Absorption maxima in vapor phase calculated theoretically.

the ground-state dipole moment (μ) from theoretical calculations, the excited-state dipole moment (μ^*) can be estimated.

2. Results and Discussion

2.1 Solvent Effect on the Absorption and Fluorescence Spectra. The absorption and fluorescence maxima of indole and all of its derivatives in different solvents are given in Table 2. We used for analyses only those solvents for which the absorption and fluorescence spectra were recorded. Nonpolar solvents, like cyclohexane and benzene, were kept out of further analyses in the cases of 5HI2C and 5HT, respectively, due to their insolubility in these solvents.

The absorption spectrum in indoles above 260 nm is a superposition of bands due to two electronic transitions, $^1L_b \leftarrow S_0$ and $^1L_a \leftarrow S_0$;[21,30,31] however, the positions of these bands depend on the particular indole. While the ^1L_a band is very sensitive to the polarity of the solvent, there is a relatively small dependence on the solvent polarity for the ^1L_b band. The interaction between indole and polar solvents can bring the ^1L_a state below the ^1L_b state. In addition, the fluorescence emission from indoles in polar solvents is predominantly from the ^1L_a state,[12,21,22,25,39–42] whereas the structured fluorescence spectral shapes in a non-polar solvent, like cyclohexane, show a predominance of the ^1L_b character in the excited state. However, in some cases, the ^1L_b band considerably overlaps with the ^1L_a band, thus making it difficult to assign a particular electronic transition of the absorption spectra.[21]

The solvent-dependent Stokes shifts are more significant in the cases of I, 5HI2C and 3IA, indicating a large dipole-moment change upon excitation. In most of the systems studied here, the absorption and emission maxima are marked by a red shift along with an increase in the solvent polarity, except for I5O and 5HI2C, where a slight blue shift is observed.

In the case of 5HI2C, the effect of the solvent polarity on the absorption band at ~290 nm (see Fig. 2) is prominent, whereas the position of the weak absorption band at ~320 nm is negligibly affected (or unaffected) by the solvent polarity, which justifies the use of the band at ~290 nm to be a reasonable scale for measuring the solvatochromic Stokes shifts.

The absorption maxima of all the molecules in the vapor phase were determined theoretically, and were found to be different from those in solutions (Table 2). This difference is obvious, because there is almost no interaction between the molecules at low pressure. Depending upon the nature and extent of solute-solvent interactions, the spectral characteristics, viz. shape, maxima and intensity, change.

2.2 Determination of the Ground and Excited-State Dipole Moments. To determine the excited-state dipole moments of molecules, we recorded their absorption and fluorescence band maxima in a series of polar and nonpolar solvents (Table 2). Those solvents with a possibility of having strong specific interactions (e.g. intermolecular H-bonding) with the compounds were excluded. The shifts of the emission peaks with the solvent polarity changes are more pronounced than the shifts of the absorption peaks, indicating that the increase in the dipole moment of the molecule upon excitation, i.e. $\Delta\mu$ ($\mu^* - \mu$), is positive. The calculated values of the correlation coefficients R^2 of the Stokes shifts versus different solvent polarity parameters ($F_1(D,n)$, $F_2(D,n)$ and E_T^N) are given in Table 3 (Fig. 4). A reasonable improvement in R^2 the correlation coefficient was observed with the E_T^N parameter for most cases.

The Onsager cavity radii and ground-state and excited-state dipole moments were calculated theoretically for all of the systems, and are given in Table 4. This table also contains the $\Delta\mu$ values derived from the slope (m) obtained from plots of the Stokes shifts versus E_T^N (using Eq. 4, second method). The slopes S_1 and S_2 and the ratio of the dipole moments (μ^*/μ) calculated using the first method are given in Table 5.

It can be seen that the dipole moments of indoles are signif-

Table 3. Correlation Coefficients for Fits of the Stokes Shifts with Solvent Polarity Functions

Molecule	Correlation Coefficients		
	I[a)]	II[b)]	III[c)]
I	0.965	0.934	0.901
I5O	0.465	0.297	2E−06
5HI2C	0.986	0.807	0.932
3IA	0.854	0.990	0.997
5HT	0.916	0.407	0.448

a) Calculated from the plot of Stokes shifts versus E_T^N using Eq. 4. b) Calculated from the plot of Stokes shifts versus $F_1(D,n)$ using Eq. 1. c) Calculated from the plot of Stokes shifts versus $F_2(D,n)$ using Eq. 2.

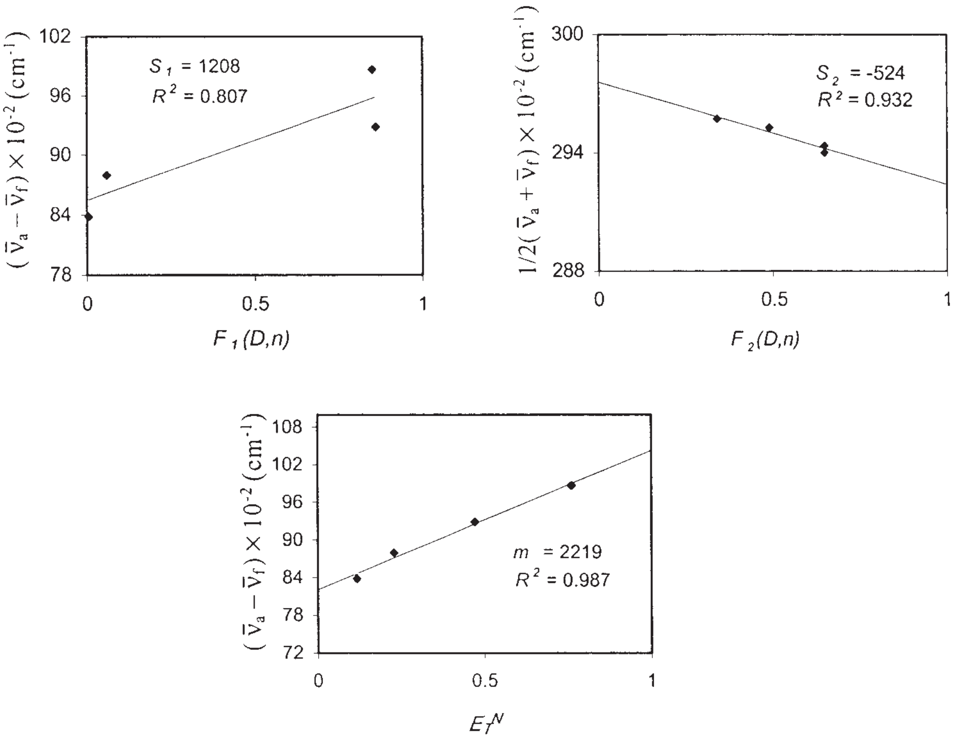

Fig. 4. Stokes shifts of 5-hydroxyindole-2-carboxylic acid versus the solvent polarity functions $F_1(D,n)$, $F_2(D,n)$ and E_T^N.

Table 4. Experimental and Theoretical Values of $\Delta\mu$ for Indoles

Molecule	Cavity Radius a/Å	m	$\mu^{a)}$/D	$\mu^{*a)}$/D	$\Delta\mu$/D	
					Experimental	Theoretical
I	3.370	4080	1.88	3.60	2.16 (2.98)[b)]	1.72
I5O	3.800	1440	3.05	3.09	1.54	0.04
5HI2C	4.395	2219	5.29	7.73	2.37	2.43
3IA	3.836	3511	3.02	3.81	2.44	0.79
5HT	5.395	880	0.82	5.81	2.03	4.99

a) Calculated theoretically using AM1 method.[33] b) Obtained from Ref. 14.

Table 5. Experimental and Theoretical Values of (μ^*/μ) for Indoles

Molecule	S_1	$-S_2$	μ^*/μ	
			Experimental	Theoretical[a)]
I	2419	4727	3.09	1.91
I5O	695	7	1.20	1.01
5HI2C	1208	524	2.53	1.46
3IA	2244	5344	2.44	1.26
5HT	342	781	2.55	7.05

a) Calculated theoretically using AM1 method.[33]

icantly higher in the excited singlet state than in the ground state, with the exception of I5O, which is relatively less polar in the excited singlet state. The increase in the dipole moment upon excitation varies between 1.54 D and 2.44 D. The experimental values of the dipole-moment change ($\Delta\mu$) are in fair agreement with the AM1 theoretical results, and also with the experimental result reported by Song and co-workers[14] (Table 4).

This significant change in the dipole moment upon excitation could be explained in terms of resonance structures. In Fig. 5, the resonance structures of indole are shown. Among all of the possible resonance structures, (b–e) are stable, but (b) is the most dominant structure of indole in the excited state.[17] The excited-state electron distribution for I5O is presented in Fig. 6. The excitation of I5O causes the hydroxy group to enhance the resonance interaction with the π-electron cloud of benzene ring, while placing a partial positive charge on the oxygen. In I5O, the structures (c–e) are found to be stable, out of which (c) is the most dominant. The average charge separation in I5O is smaller than that of indole, which explains the smaller change in the dipole moment of I5O as compared to indole upon excitation.

A large change in the dipole moment upon excitation in case of 5HI2C (Fig. 7) could be explained in two ways: (1) It is expected that introducing a –COOH group at the 2-positon in I5O generates two local dipoles in the molecule, resulting in a higher value of $\Delta\mu_{\text{exp}}$ in 5HI2C, as compared to I5O. (2) It has been suggested[4] that the –COOH group is planar to the indole ring, which makes it conjugated to the rest of the mole-

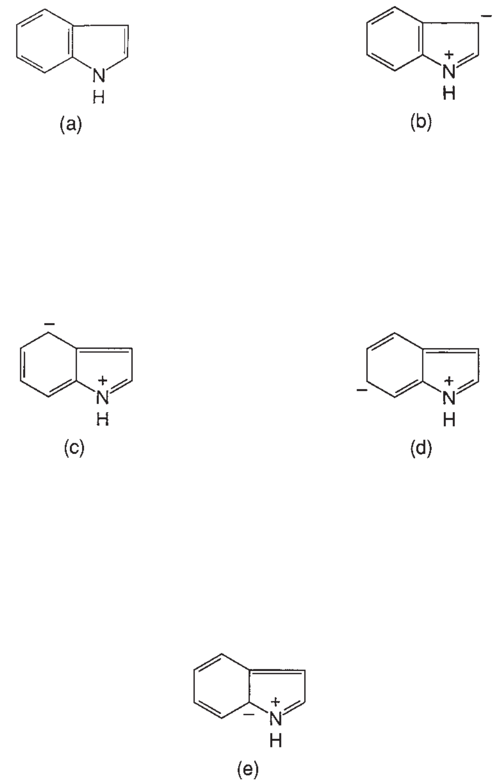

Fig. 5. Possible resonance structures for indole.

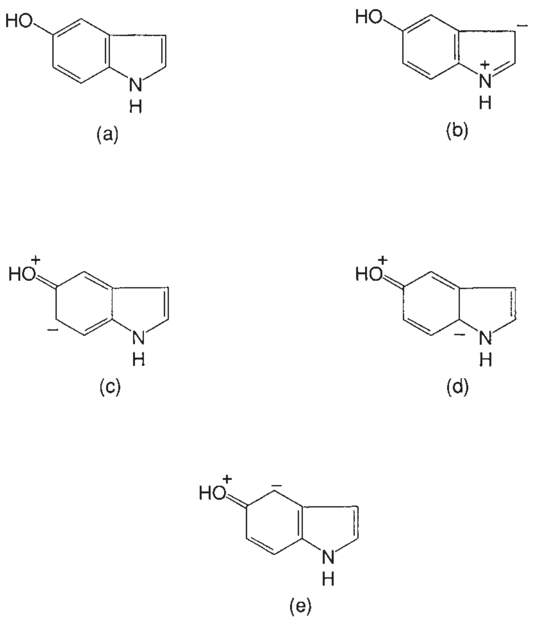

Fig. 6. Possible resonance structures for 5-hydroxy substituted indoles.

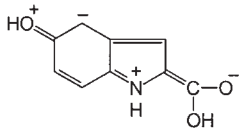

Fig. 7.

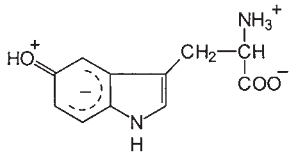

Fig. 8.

cule, resulting in an intramolecular charge transfer from the ring to the carbonyl oxygen, and thereby enhancing the polarizability of the molecule. This results in a considerable increase in the dipole moment of the molecule upon excitation.

Possible resonance structures of 5HT are similar to those of I5O (Fig. 6), but the dipole moment change in 5HT (Fig. 8) upon excitation is slightly higher as compared to I5O, which may be due to the development of an additional dipole caused by the zwitterionic nature of 5HT, thereby increasing the dipole moment slightly in the excited state.

3. Conclusion

This excited-state parameter may provide valuable information about the nature of the emitting state, thereby allowing a careful examination of various theoretical models for the electronic structure of the excited states, which is often useful in designing nonlinear optical materials. The experimental simplicity and the excellent correlation of experimental data, perhaps arising from the rigor associated with the representation of the solvent polarity at a microscopic level, were adequately illustrated in this study. Because the reliability of the AM1-predicted excited state dipole moments has not yet been firmly established, the discrepancies observed between the experimental and theoretical results in a few cases may require further investigations.

Neera Sharma and Sapan K. Jain gratefully acknowledges the financial assistance from University Grants Commission (UGC) and Council of Scientific and Industrial Research (CSIR), New Delhi.

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