

FLUORESCENCE SPECTRA OF BIFLAVONES

ASHUTOSH SHARMA*, M. K. MACHWE AND V. V. S. MURTI†

Department of Physics and Astrophysics, University of Delhi, Delhi 110 007, India.

† Department of Chemistry

* Present address: Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, USA

ABSTRACT

Fluorescence excitation and emission spectra of apigenin trimethyl ether (I) and of the biflavones, cupressuflavone hexamethyl ether (II), hinokiflavone pentamethyl ether (III) and amentoflavone hexamethyl ether (IV) are reported in a few typical solvents. The effect of solvent on the positions and intensity of the excitation and emission maxima is explained. Also the solvent-dependent Stokes shifts are used to estimate the excited state dipole moments of the molecules. The change in the intensity and the shifts in the band maxima of the "dimers" of apigenin are used to assess the spectral transition polarization.

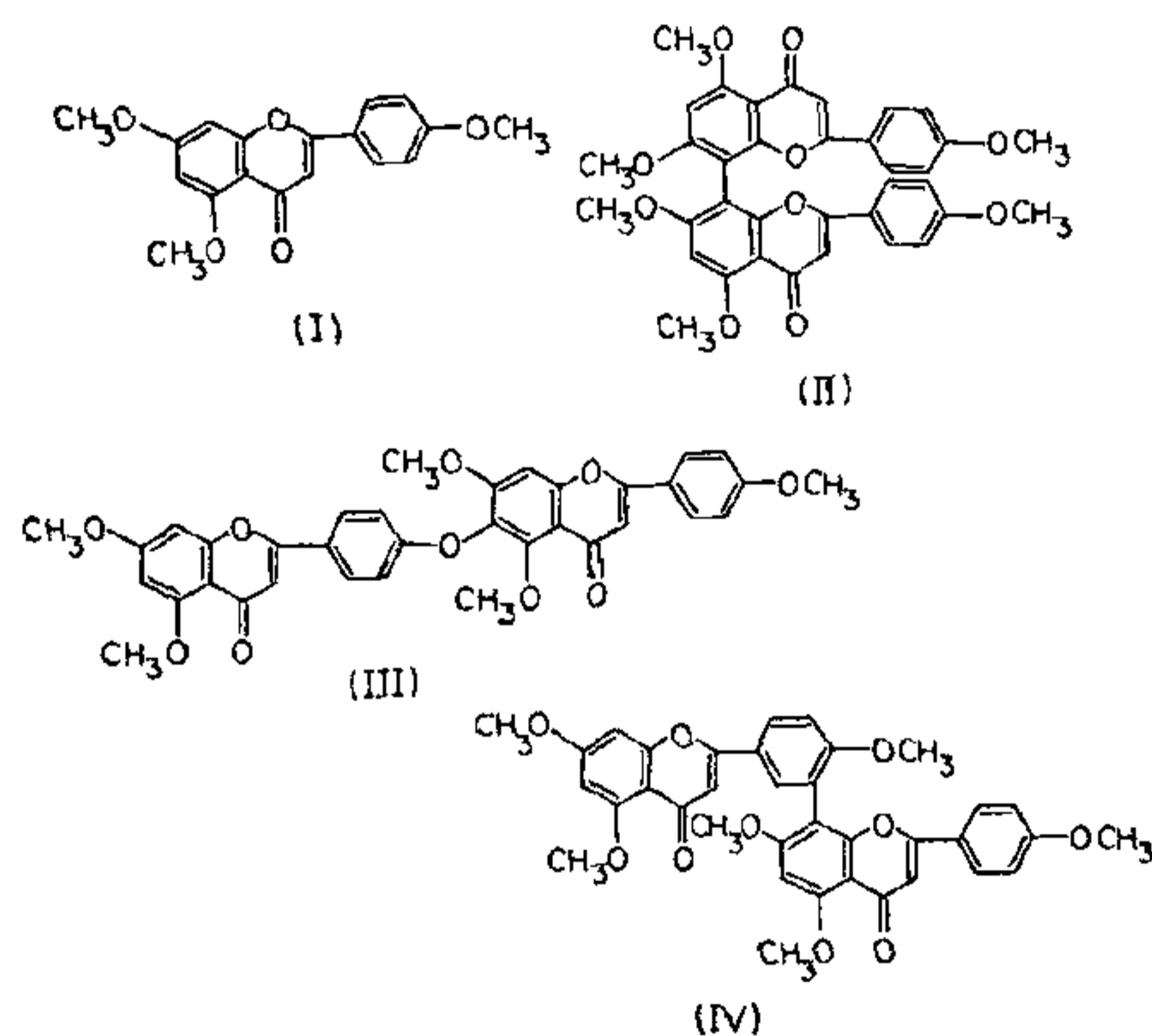
INTRODUCTION

COUMARINS, chromones and flavonoids are a class of natural colouring matters widely distributed in plants. A recent addition to this group is the biflavones¹, originally found in gymnosperms but now known to occur in angiosperms also. These compounds have a number of physiological activities in animals but their function in plants is not clear. An important property of these compounds is that many of them are fluorescent. It was considered to be of interest to study whether the fluorescence of these substances has any role in energy transfer in certain biological processes in plants. The study of fluorescence excitation and emission spectra gives valuable information regarding the behaviour of the complex molecules and changes produced in them upon excitation.

A systematic solvent-effect analysis which employs the internal electric field, provides a simple method for the determination of the excited state dipole moment of the fluorescing molecule²⁻⁷. There have been earlier studies on the fluorescence of coumarins and the other simple flavonoids⁸ but a systematic detailed study of this group of compounds, and of biflavones in particular, is still lacking. The present investigation concerns the fluorescence of typical naturally occurring biflavones, which are "dimers" of apigenin (scheme I). Because of the solubility problem, the complete methyl ethers have been employed, and the effect of different solvents and substitution pattern on the position and shape of the fluorescence excitation and emission spectra were studied.

EXPERIMENTAL

Apigenin trimethyl ether (I) and the biflavone methyl ethers (II, III, IV) were purified by preparative



TLC⁹. AR grade solvents (*n*-propanol, *iso*-amyl alcohol, methanol and formamide) were employed. Water, double glass distilled, was used. All the solvents were checked for any possible fluorescent contamination by recording their fluorescence emission spectra separately in blank experiments. Excitation and emission spectra were recorded on an Aminco-Bowman spectrophotofluorometer at $19 \pm 2^\circ \text{C}$.

The instrument was first calibrated with solutions of quinine sulphate in 0.1 H₂SO₄. The concentration of the solute in all the cases was kept low ($\sim 10^{-6}$ g/cc) to avoid inner filter effect. The nonlinear response of the photomultiplier tube (IP 21) and the non-uniform emission of the Xenon Lamp were corrected. The effect of scattering by the solvents was checked by blank experiment. This has been found low ($\sim 0.5\%$) and wherever necessary corrections were applied. The recorded wavelengths are correct within $\pm 2 \text{ nm}^3$.

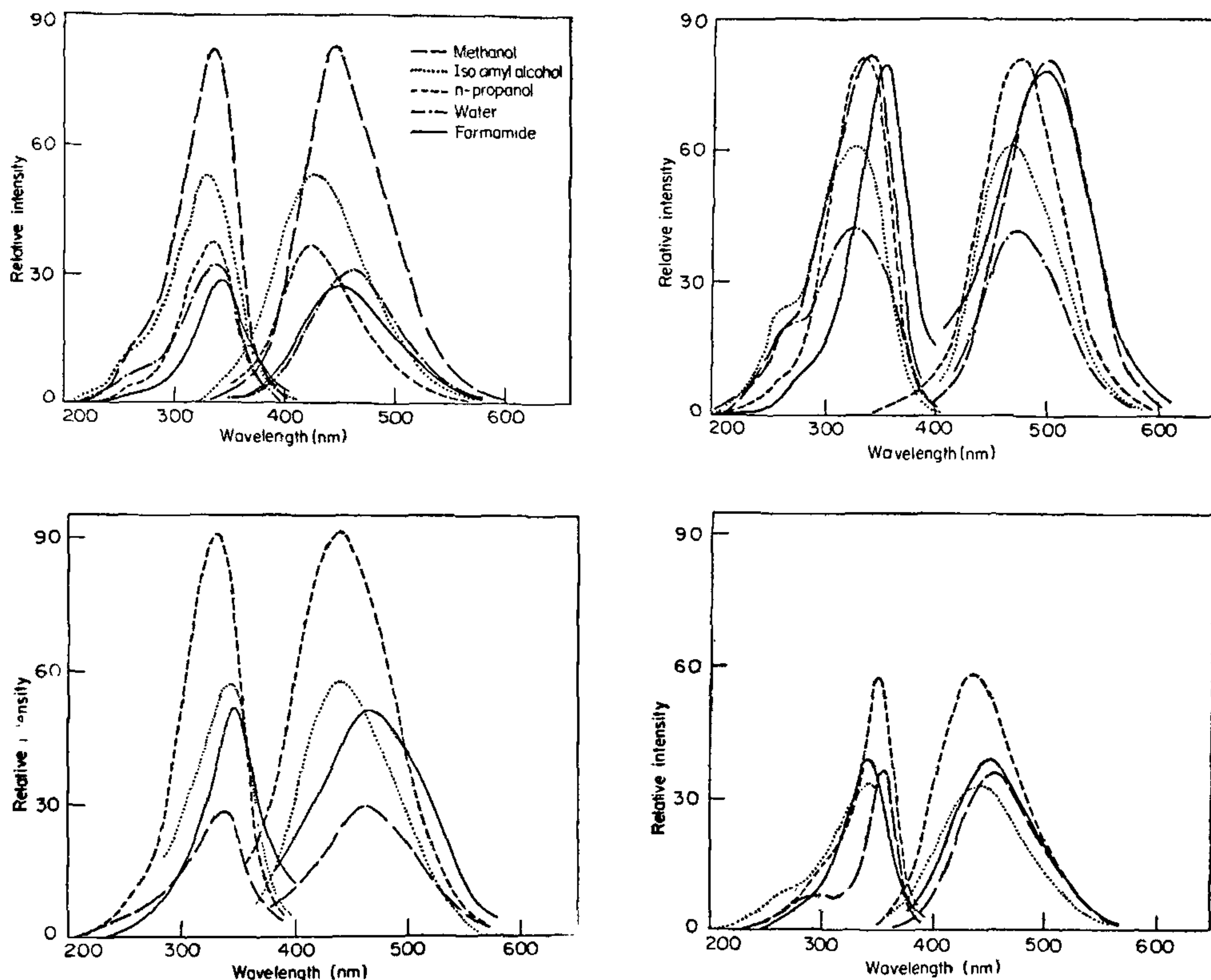


Figure 1-4. Excitation and fluorescence spectra in different solvents; 1. Apigenin trimethyl ether, 2. Cupressuflavone hexamethyl ether, 3. Hinokiflavone pentamethyl ether, 4. Amintoflavone hexamethyl ether. (1 & 2-top, 3 & 4-bottom).

RESULTS AND DISCUSSION

The fluorescence excitation and emission spectra of the compounds (I-IV) presented in figure 1 and table 1 show the solvent shift data. The intensity and the position of the excitation bands 1 and 2 at 315-360 nm and 250-290 nm respectively were affected by a change in solvent in all the cases, however the influence of the solvent on the position of the band 1 is less for compounds I and III than for the compounds II and IV. It may also be noticed from table 1 that the fluorescence emission peaks are shifted more than the excitation peaks by solvent changes. Bathochromic shifts, with a few exceptions, are observed with the increasing polarity of the solvent thus indicating $\pi \rightarrow \pi^*$ transitions.

Table 2 and 3 show the fluorescence excitation and emission energy shifts in the "dimers"-II, and III and IV as compared with the parent compound apigenin trimethyl ether (I). As far as fluorescence emission shifts are concerned it is found that $\Delta \bar{\nu}_f$ for compound II > III > IV and also this shift in the case of compound IV decreases with increasing polarity of the solvent. The observed large value of $\Delta \bar{\nu}_f$ for compound II suggests that in the dimeric form this molecule is more compact than III and IV. In such a case, there is a greater possibility of "across the space", $\pi-\pi'$ interaction between the two side phenyl units causing large energy shifts. It can be noted that $\Delta \bar{\nu}_f$ (table 3) is always positive. This is possible if the excited state charge distribution in the solute molecule is markedly

TABLE 1
Fluorescence excitation and emission spectra of the compounds.

Compound solvent	λ_{exc} nm	λ_f nm	ν_{exc} cm ⁻¹	ν_f cm ⁻¹	ν_{exc} cm ⁻¹	ν_f cm ⁻¹	$1/2 (\nu_{exc} + \nu_f)$	$F(Dn)$	$F_2(Dn)$
I. Apigenine trimethyl ether (I)									
Iso-amyl alcohol	332	438	30120	22831	7289	26475	0.73	0.64	
<i>n</i> -propanol	332	428	30120	23364	6756	26742	0.78	0.51	
Methanol	326	448	30675	22321	8354	26498	0.85	0.66	
Formamide	338	450	29586	22222	7364	25904	0.89	0.75	
Water	328	462	30488	21645	8843	26068	0.91	0.68	
II Cupressuflavone hexamethyl ether (II)									
Iso-amyl alcohol	316	472	31646	21186	10460	26416	0.73	0.64	
<i>n</i> -propanol	322	478	31056	20921	10135	25988	0.78	0.51	
Methanol	328	510	30488	19608	10880	25048	0.85	0.66	
Formamide	336	510	29762	19608	10154	24685	0.89	0.75	
Water	312	476	32051	21008	11043	26529	0.91	0.68	
III. Hinokiflavone pentamethyl ether (III)									
Iso-amyl alcohol	335	452	29851	22124	7727	25987	0.73	0.64	
<i>n</i> -propanol	326	448	30675	22321	8354	26498	0.78	0.51	
Methanol	328	460	30488	21739	8749	26113	0.85	0.66	
Formamide	338	464	29486	21552	8034	25569	0.89	0.75	
IV. Amentoflavone hexamethyl ether (IV)									
Iso-amyl alcohol	326	446	29762	22422	7340	26092	0.73	0.64	
<i>n</i> -propanol	350	435	28571	22989	5582	25780	0.78	0.51	
Methanol	352	456	28409	21930	6479	25169	0.85	0.66	
Formamide	338	452	29586	22124	7462	25855	0.89	0.75	

TABLE 2

Flourescence excitation spectral shifts on dimerization of Apigenin trimethyl ether,

$$\Delta \bar{\nu}_{exc} = \bar{\nu}_{exc_{ATE}} - \bar{\nu}_{exc_{ATED}} \text{ cm}^{-1} \times 10^{-4}$$

Solvent Biflavone	<i>n</i> -propanol		Formamide		Methanol		Iso-amyl alcohol	
	band 1	band 2	band 1	band 2	band 1	band 2	band 1	band 2
Cupressuflavone hexamethyl ether (II)	-0.0936	-0.0726	0.0176	0	0.0187	0	-0.1526	0.1372
Hinokiflavone pentamethyl ether (III)	-0.0550	0	0	0	0.0187	-0.0726	0.0269	0.0699
Amentoflavone hexamethyl ether (IV)	0.1549	0.1372	0	0	0.2266	0.0699	0.0358	0

different from the ground state charge distribution and is such as to give rise to a stronger binding with the polar solvents in the excited state. This would be so if the dipole moment of the solute molecule is more in the excited state. In addition to the change due to dimerization, some energy shift in the case of fluorescence emission can also be due to specific interactions between solvent and solute molecules or their streng-

thening in the excited state. The similarity of excitation spectra for all the "dimers" relative to apigenin trimethyl ether, suggests that the "dimerization" does not contribute appreciably to the π -electron system of the compounds. Small energy shifts $\Delta \bar{\nu}_{exc}$ in peak excitation wavelength (table 2) also agree with this conclusion. Of the compounds studied the large values of $\Delta \bar{\nu}_{exc}$ in compounds II and IV as compared to III

TABLE 3

Fluorescence emission spectral on dimerization of Apigen trimethyl ether

$$\Delta\bar{\nu}_f = \bar{\nu}_{fATE} - \bar{\nu}_{fATED} \text{ cm}^{-1} \times 10^{-4}$$

Solvent/ Biflavone	<i>n</i> -propanol	formamide	Methanol	Iso-amyl alcohol
Cupressuflavone hexamethyl ether (II)	0.2443	0.2614	0.2713	0.1645
Hinokiflavone pentamethyl ether (III)	0.1043	0.0660	0.0582	0.0707
Amentoflavone hexamethyl ether (IV)	0.0375	0.0098	0.0391	0.0404

may not be totally due to "dimerization" and may be specific interactions with the solute molecule in the ground state have to be taken into account. In short, the change in fluorescent properties with the change of solvent may be attributed to the distortion of electron densities in various energy states, due to the surrounding media as well as in some cases, due to specific interactions e.g. hydrogen-bonding. The shifts in emission spectra are larger than the corresponding shifts in the excitation spectra and indicate the changed molecular behaviour in the excited state. It may be inferred from the results that the molecules now studied have different electronic properties in the two combining energy states and are more polarizable in the excited state.

The shifts in excitation and fluorescence peaks with change of solvents can be used to find the dipole moment of the solute molecule in the first excited singlet state^{4,5}. In the present case it has not been possible to get absolute values of the dipole moments in the excited state as the Onsager's cavity radii are not known. However, the relative values of the excited state dipole moments of (I-IV) have been estimated and are $\mu_{eI} = 3.2 \mu_{gI}$, $\mu_{eII} = 1.7 \mu_{gII}$, $\mu_{eIII} = 2 \mu_{gIII}$ and $\mu_{eIV} \sim \mu_{gIV}$. Table 1 gives the functions $F_1(D,n)$ $F_2(D,n)$ which have been used to calculate the μ_e . It is clear that the dipole moments of (I-III) increase upon excitation, while (IV) seems to be an exception. It, therefore, follows that the excited states of compounds (I-III) are more polarized than the ground states. This conclusion agrees with the inference drawn above from the fact that the polarity of the

solvent affects fluorescence maxima more than the excitation maxima.

Energy shifts $\Delta\bar{\nu}_{exc}$ in the excitation bands 1 and 2 and their intensities suggest that the system 1 is long axis (l) polarized while system 2 is short axis (s) polarized. The band systems 1 and 2 correspond to Platt's L'_a and L'_b spectral band designation¹⁰.

16 February 1983; Revised 30 May 1983

1. Mabry, T. J., Mabry, H. and Harborne, J. B., *The flavonoids*, Academic Press, London, 1975.
2. Sun, M. and Song, P. S., *Photochem. Photobiol.*, 1977, **25**, 3.
3. Sharma, A. and Machwe, M. K., *Curr. Sci.*, 1982, **51**, 821.
4. Bakshiev, N. G., *Opt. Spectroskopiya*, 1964, **16**, 821.
5. Chamma, A. and Viallet, P., *C. R. Acad. Sci., Ser. C.*, 1970, **270**, 1901.
6. Arbeloa, I. L., *J. Chem. Soc., Faraday Trans.*, 1981, **77**, 1735.
7. Campomacchia, A. C., Kumar, V. and Brazzel, C., *Talanta*, 1982, **29**, 65.
8. Murti, V. V. S., Rajagopalan, S., Ramachandra Rao, L., *Proc. Indian Acad. Sci.*, 1951, **A34**, 319 and earlier references.
9. Chandramouli, N., *Synthetic and structural studies in the chemistry of biflavones*, Ph. D. thesis, Delhi University, 1973.
10. Platt, J. R., *J. Chem. Phys.*, 1949, **17**, 484.
11. Onsager, L., *J. Am. Soc.*, 1936, **58**, 1486.