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Mechanism of excitation and emission of papaverine molecule: fluorescence polarization spectroscopy study and MO calculation of transition moments

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Abstract

The polarization of the fluorescence spectra of papaverine and isoquinoline was studied. The electronic structure of ground and excited states of papaverine, isoquinoline, and 5,6-dimethoxy-1-methylisoquinoline were studied by MNDO-PM3-CI and CNDO-CI methods. Results of spectral investigations, concerning the mechanism of excitation and emission of papaverine are presented along with theoretical considerations. The results strongly suggest the existence of coupling between the isoquinoline and benzene ring in the papaverine molecule.

1. Introduction

Papaverine (6,7-dimethoxy-1-[3,4-dimethoxy benzyl]-isoquinoline) is an organic molecule (benzylisoquinoline alkaloid) with no symmetry, having substituted isoquinoline and benzene rings linked by a CH_2 group. It has a wide clinical use as a vasodilator [1–3], and as an inhibitor of pathological spasms in smooth muscles. The crystal structure of papaverine was determined [4], and its conformation in solution was studied by NMR [5]. The fluorescence of papaverine has been subjected to very few investigations [6]. It is obvious that two chromophores can be identified in this molecule: 6,7-dimethoxy isoquinoline and 3,4-dimethoxy phenyl groups, see Fig. 1.

The structure of papaverine is interesting, because the molecule consists of two conjugated rings (isoquinoline and benzene), separated by a CH_2 group. These two single bonds prevent the conjugation across the molecule. The distance between these two ring systems is not too large, and the papaverine may exhibit the behavior of a molecule with two interacting groups. Our goal was to elucidate the mechanism of excitation and relaxation of this complex structure.

In examining the excitation mechanism it was assumed that:

- there is a coupling of the electronic densities of the two rings and
- there is energy transfer between the chromophores upon excitation.

These assumptions were tested by investigating the spectral properties and comparing these with theoretical calculations. The determination of the excited states of the molecule can be done by examination of absorption and fluorescence spectra. To understand the mechanism of excitation and

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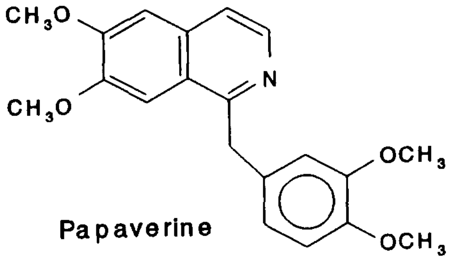


Fig. 1. Structure of papaverine.

relaxation it is useful to determine the transition moment directions (polarizations) of the electronic transitions. A major point in this study was the correlation of the transition moment directions, as microscopic values, with the fluorescence polarization, as macroscopic value.

2. Material and methods

Commercial papaverine and isoquinoline were used without further purification. Concentrations of samples in ethanol (Merck, p.a.) were 4 and 10 mmol/l for papaverine and isoquinoline, respectively. Absorption spectra were measured using a PYE Unicam SP8-100 spectrophotometer at room temperature. Fluorescence excitation and emission spectra were measured on an Aminco Bowman spectrophotofluorimeter, equipped with a 150 W xenon lamp and R446-S photomultiplier tube. The instrument was interfaced to an IBM AT 286 microcomputer for data acquisition and processing. Polarized fluorescence was measured at 77 K (liquid nitrogen) by using a pair of Glen's prisms. The degree of polarization (P) was determined according to [7]:

$$P = (I_p - I_n f)/(I_p + I_n f),$$

where I_p and I_n are components of the vector (electric field vector) of emission fluorescence parallel and perpendicular, to the vector of the incident light, respectively, and f is a correction factor, as defined by Azumi et al. [8]. All spectral data are the average of two or more spectra.

Theoretical self-consistent field calculations with configuration interaction (SCF CI) were carried out for isoquinoline, 6,7-dimethoxy-1-methylisoquinoline (DMMIQ) and papaverine. The calculations were performed using the all valence electron approximation by the CNDO method [9] (included in program CNDUV [10]) and MNDO-PM3 method [11] (included in program MOPAC [12]). The geometries of all molecules used in CI calculations was obtained by use of the MNDO-PM3 method. Calculations of various electronic states of molecules were performed with a multi-electron configuration interaction facility (MECI) in MOPAC, and with the extensive configuration interaction method in CNDUV. The number of electronic configurations (microstates) used in CI was 36 in MOPAC and 250 in CNDUV. Programs were installed by us on IBM 3090 and IBM AT 386 computers. Calculated results were compared with the observed spectra for isoquinoline and papaverine.

Angles (ζ) between the direction of the transition moment of the lowest energy singlet transition and directions of other singlet transition moments were calculated from degrees (extent) of polarization (P). These calculations were based on the assumption that Eq. (1) can be applied to a quantum mechanical value-transition moment.

$$P = \frac{3\cos^2\zeta - 1}{3 + \cos^2\zeta}. \tag{1}$$

Eq. (1) relates the degree of polarization and the angle between the absorption and emission dipoles in classical theory [7].

3. Results and discussion

Fig. 2 presents the absorption, the fluorescence excitation and emission spectra of papaverine, as well as polarization spectra for fluorescence emission and excitation. Emission spectra were measured by exciting the peak of the near UV band at 280 nm. The excitation spectra were obtained at the emission wavelength of 375 nm.

The excitation spectrum of papaverine closely resembles its absorption spectrum, and has near and far UV electronic transitions at 330, 285 and 243 nm. An emission band maximum was observed at 350 nm.

The excitation (absorption) spectrum of papaverine has roughly the same shape as the

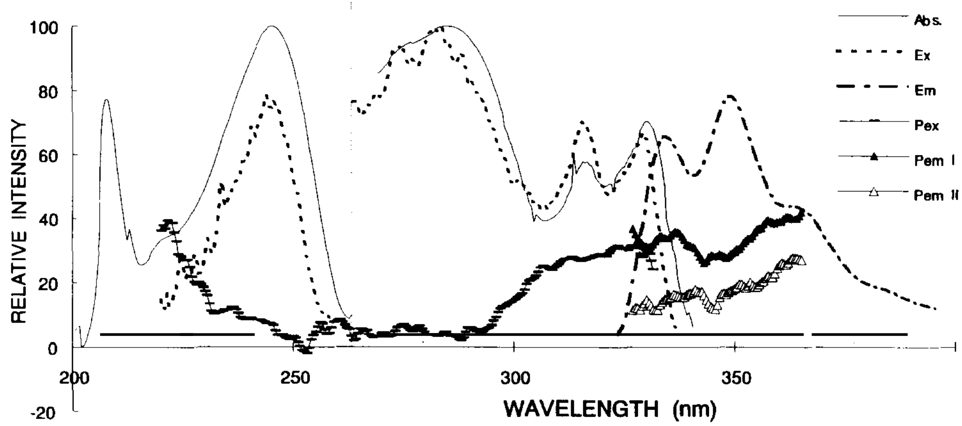


Fig. 2. Absorption (Abs), excitation (Ex) and emission (Em) fluorescence spectra of papaverine. Fluorescence polarization of excitation (Pex) was monitored at an emission wavelength of 360 nm. Emission polarization spectra were produced by excitation at 320 (Pem I) and 300 nm (Pem II). Fluorescence polarization was measured at 77 K. The long-wavelength segment of the absorption and excitation spectra is scaled by a factor of 10.

excitation (absorption) spectrum of isoquinoline. Comparing the papaverine spectra with the published spectrum of isoquinoline [13] the red shift has been observed. The close resemblance of spectra of these two compounds suggests that π electrons in the isoquinoline ring in the papaverine molecule could be involved in the excitation of papaverine. The same similarity has been found in emission spectra of papaverine and isoquinoline. Corollary, in literature the isoquinoline ring could be identified as the chromophore for emission, too.

Assignment of the bands in excitation (absorption) spectrum of the papaverine is based on the similarity with absorption spectrum of isoquinoline. Since, Baba et al. [14] have assigned bands in the isoquinoline spectrum with ^1L_b, ^1L_a and ^1B_b, in increasing order of band energy, we decided to assign bands in the same way.

Calculated energies of electronic transitions for all molecules as well as observed results are listed in Table 1. Comparison between the results of calculated and experimental data shows that the electronic transitions are well described by the theory (maximal difference is 7%).

It is well known that alkyl- and alkyloxy-substituents on the isoquinoline ring cause the red shift, but it is not known how strong the influence of the 1-[3,4-dimethoxybenzyl] group in papaverine is. Calculated energies of transitions of 6,7-dimethoxy-1-methylisoquinoline and papaverine are comparable in both MO methods used. Therefore, methoxy and methyl substituents on the isoquinoline moiety have a major influence on the degree of red shift of bands in the papaverine excitation (absorption) spectra. Hence, the influence of the 3,4-dimethoxy phenyl group on the transition energies could be neglected.

In the polarization spectra of excitation for papaverine presented in Fig. 2, three regions with different degrees of polarization, can be identified. They correspond to three bands in the excitation (absorption) spectra. The degree of polarization and angles ζ calculated by Eq. (1) are presented in Table 2 for all three molecules.

Polarizations of the second and third electronic transitions of papaverine reveal that their transition moments are not parallel or perpendicular to the transition moment of the lowest electronic transition. Comparing the experimentally determined values of the degree of polarization for bands in papaverine and isoquinoline spectra (that match the previous published values [15]), one can observe the difference (opposite signs) in the short wavelength bands. This observation proves that substituents influence the excitation and emission mechanism in papaverine. Variation of excitation

Table 1
Calculated and observed electronic transition energies (eV) for isoquinoline, DMMIQ and papaverine

Molecule	$S_1[L_b]$			$S_2[L_a]$			$S_3[B_b]$		
	exp	CNDO	PM3	exp	CNDO	PM3	exp	CNDO	PM3
Isoquinoline	3.91	4.07	4.01	4.66	4.69	4.61	5.74	5.80	5.77
DMMIQ	–	3.91	3.90	–	4.51	4.07	–	5.31	5.49
Papaverine	3.76	3.88	3.88	4.35	4.41	4.06	5.11	5.25	5.16

Table 2
Calculated and observed angles between electronic transition moments, and observed polarizations for isoquinoline, 6,7-dimethoxy-1-methylisoquinoline (DMMIQ) and papaverine

Molecule	$S_1:S_1$			$S_2:S_1$			$S_3:S_1$		
	P	Angle		P	Angle		P	Angle	
		Exp	CNDO		Exp	CNDO		Exp	CNDO
Isoquinoline	0.30	33.0	0	0.05	51.3	122.1[a]	−0.10	61.6	59.0
DMMIQ	–	–	0	–	–	129.0[a]	–	–	126.8[a]
Papaverine	0.33	30.3	0	0.05	51.3	44.2	0.10	47.9	40.2

[a] To compare the overall directions of transition moments the values of 180° − ζ could be used. For example the direction given in the last cell in the second row in the table is actually 180° − 126.8° = 53.2°.

wavelength (Pem I and Pem II in Fig. 2) does not change the emission pattern. This indicates that photoemission results from joint action of two aromatic moieties in papaverine.

Since only minor differences between calculated angles ζ (CNDO-CI method) of isoquinoline and DMMIQ (considering only orientations, not directions of transition moments) were found, one can conclude that 6,7-dimethoxy and 1-methyl groups do not change the orientation of transition moments. Fig. 3 shows that all transitions in both molecules lie in the plane of isoquinoline ring, a characteristic feature of pure $\pi^* \leftarrow \pi$ transitions in the planar conjugated systems.

The calculated angle ζ for the third transition of papaverine is considerably different from those of isoquinoline and DMMIQ. The benzyl group has a profound effect on the orientation of transition moments of papaverine. This is the reason for the existence of the out-of-isoquinoline-ring-plane component of transition moments (Fig. 3), which proves the coupling of electron densities of two the unconjugated ring systems. It also explains the difference in experimentally determined polarizations.

Coupling of electron densities cannot be explained by mono-determinant molecular orbitals

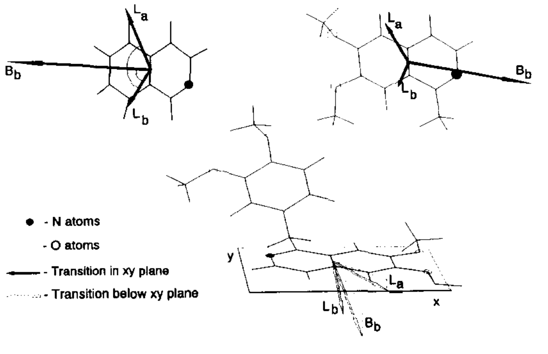
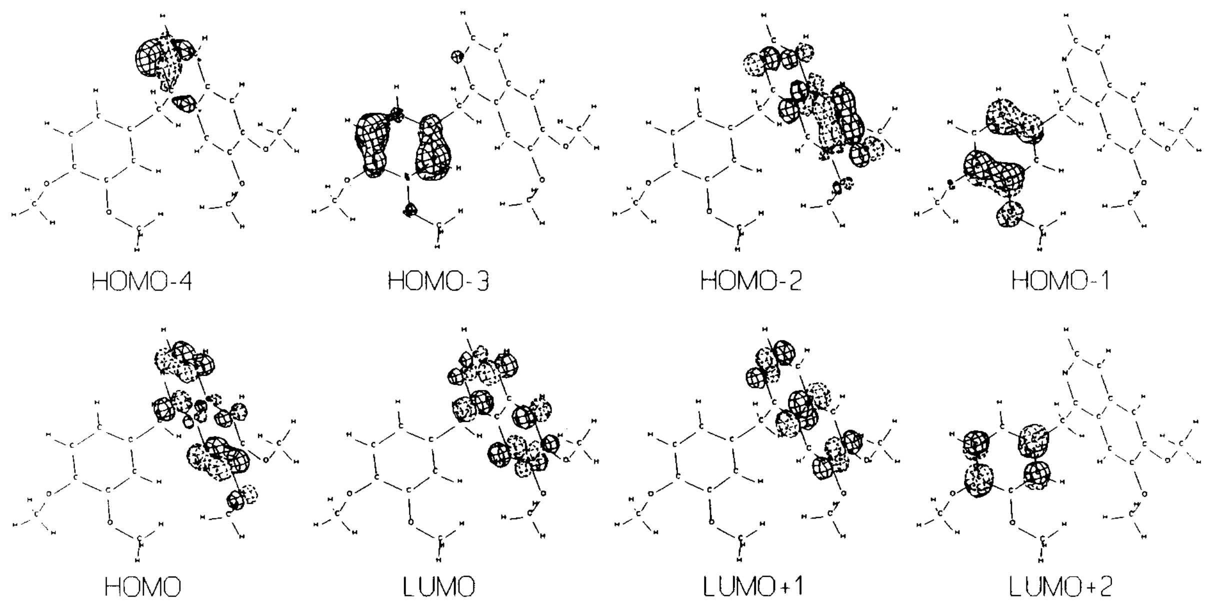


Fig. 3. Calculated transition moments for the main excitation (absorption) bands of isoquinoline, DMMIQ and papaverine.


HOMO-4 HOMO-3 HOMO-2 HOMO-1

HOMO LUMO LUMO+1 LUMO+2

Fig. 4. The SCF molecular orbitals (obtained by CNDO-CI method) of papaverine, involved in UV–VIS spectral transitions are shown.

Table 3
Microstate contributions to the excited state of papaverine, calculated by CNDO-Cl method

Microstate (single excitations)	Excited states (in ascending order of energy)				
	I[L$_b$][b]	II	III[L$_a$][b]	IV	V[B$_b$][b]
LUMO ← HOMO	0.641	− 0.269	0.431	0.415	0.195
LUMO ← HOMO − 1[a]	0.132	0.173		0.281	− 0.643
LUMO ← HOMO − 2	− 0.410	− 0.133	0.244	0.290	− 0.141
LUMO ← HOMO − 3[a]		0.116	0.169		0.166
LUMO + 1 ← HOMO	0.493	0.247	− 0.338	− 0.397	
LUMO + 1 ← HOMO − 1[a]	0.153		− 0.157		
LUMO + 1 ← HOMO − 2	0.305			0.157	− 0.244
LUMO + 2 ← HOMO[a]		0.312	0.176		
LUMO + 2 ← HOMO − 1		− 0.648	− 0.257		
LUMO + 2 ← HOMO − 3					− 0.130

[a] These microstates involve the electron transfer between chromophores.
[b] Transitions from the ground state to these excited states are found in electron spectrum of papaverine.

that spread over the entire molecule. The SCF calculations result in molecular orbitals that are localized either on the isoquinoline ring or on the benzyl group (Fig. 4). However, coupling is confirmed through configuration interaction. In these calculations of excited states the large number of single excitations is included. Many of these excitations involve the electron promotion from the filled molecular orbital localized on the isoquinoline ring to unfilled molecular orbitals localized on the benzyl group and vice versa.

In Table 3 the results of CI calculations on papaverine are presented. Every excited state is composed from a number of electronic microstates.

The microstate is defined as the promotion of an electron from one filled orbital to an empty (unoccupied) orbital. Numbers in the table specify the contribution of a particular microstate into a real excited state of the system.

Inspection of the CI matrix reveals that the matrix elements, relating to exchange of electrons between chromophores in papaverine, are relatively large. It means that significant charge-transfer between chromophores occurs during excitation. This mixing adds the out-of-plane component to the corresponding transition moments.

4. Conclusion

In this study, the initial assumption about the coupling of electronic densities of two non-conjugated chromophores in papaverine was confirmed. There is no experimental or theoretical evidence for the energy transfer in a secondary photophysical process, between two π electron moieties upon excitation. Every excitation affects both chromophores in the primary photophysical process, i.e. the papaverine molecule behaves as a single chromophore.

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