ELECTRONIC STRUCTURE OF ETHIDIUM BROMIDE

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1. Introduction

Ethidium bromide (3,8 diamino-6-phenyl-5-ethyl phenantridinium bromide) is a fluorescent compound widely used in molecular biology because of its physical properties and biological activities. It is a trypanocidal dye [1] with antiviral properties [2] which intercalates between adjacent base pairs in double helix regions of nucleic acids [3].

By intercalation, ethidium bromide has its fluorescence quantum yield strikingly enhanced [4], and the complex it forms with DNA displays optical activity in the visible [5].

In this paper we are concerned with the electronic structure, excitation energies, and direction of transition moments of ethidium bromide. These quantities are useful to interpret experimental data obtained by measurements of circular dichroism and intermolecular energy transfer [5, 6], thus investigating the structure of nucleic acids.

2. Methods

Ethidium bromide cation can easily be treated by the methods of quantum mechanics if some simplifying hypotheses are made. It has long been known that phenyl and ethyl substituents have a very poor effect on the visible and near UV spectra of polynuclear aromatic hydrocarbons [7]. It can therefore be assumed that phenyl and ethyl groups do not affect the electronic structure of the phenantridinium ring, so that the calculation can reasonably be performed on the system of the atoms in the ring with the two nitrogen atoms at its extremities.

From now on such system will be called the main ring (fig. 1). The geometry of ethidium bromide is known from X-ray diffraction analysis [8]; we have checked that in the reference system of inertial axes of the main ring, the Z coordinates of the atoms in the main ring differ from zero by less than 0.1 Å. It is therefore reasonable to assume that the main ring is planar. We have considered the quaternary nitrogen atom, at position 5, as participating with only one electron to the π electronic system.

We have performed a Pariser–Parr calculation [9] on the system of 18 π electrons in the field of the main ring. We have used a C.D.C. 3600 computer program, kindly furnished by G. Bessis and O. Chalvet, of the Centre de Mécanique Ondulatoire Appliquée de Paris.

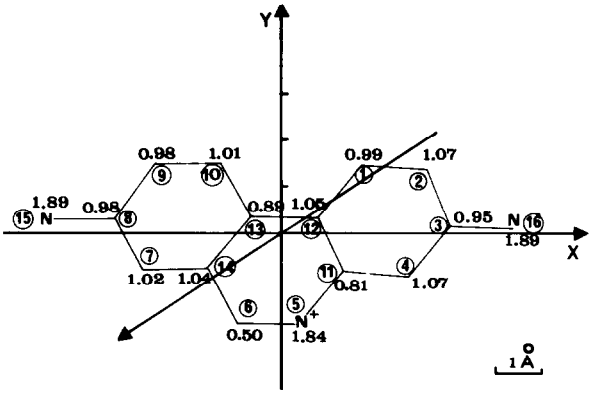

Fig. 1. Planar geometry of the main ring and charge distribution on it. The numbers in circles refer to atomic numeration. The other numbers refer to the net π electronic distribution on single atoms. The numbers greater than 1 indicate a negative charge, while the numbers smaller than 1 indicate a positive charge, on carbon atoms. On the other hand, for nitrogen atoms, a positive charge is indicated by a number smaller than 2. The arrow shows the direction of the first transition moment.

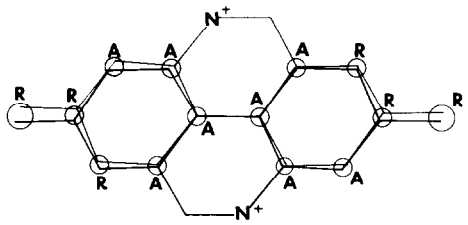

Fig. 2. Electrostatic attraction (A) and repulsion (R) sites in the stacking model proposed by Thomas and Roques.

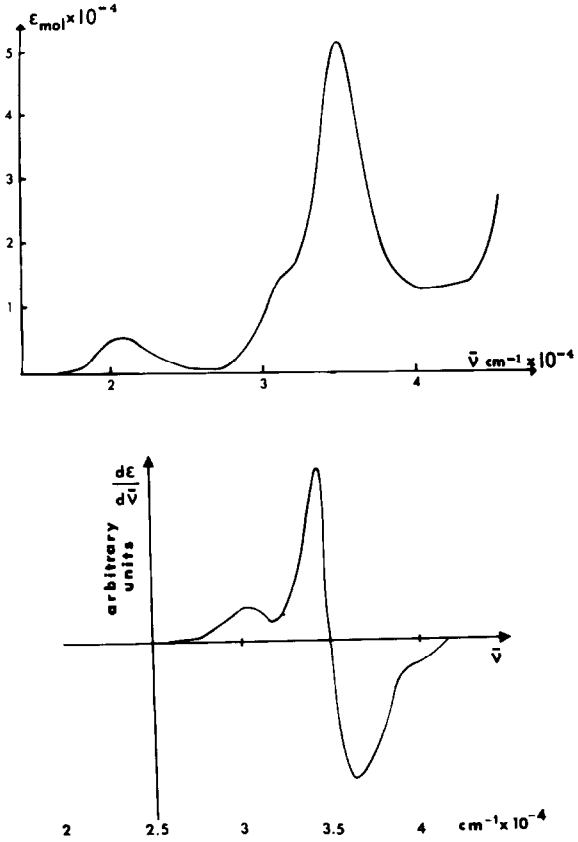

Fig. 3. Absorption spectrum (above), and its derivative plot (below), of ethidium bromide in aqueous solution. The shoulder at 31,250 cm^{-1}, attributed to transition 1–4 is clearly confirmed by the derivative plot.

In this program, monocentric and bicentric repulsion integrals have been computed following the method described by Mataga [10]. We have considered only singlet–singlet transitions and, for this purpose, we have invoked configuration interaction (27 configurations, of which 17 mono-excited and 10 bi-excited).

Table 1
Charge distribution.

Atom	Fundamental state	First exc. state	ΔQ
1	0.9907	0.9830	−0.0077
2	1.0755	1.0881	+0.0146
3	0.9466	0.8284	−0.1182
4	1.0698	1.0223	−0.0475
5	1.8423	1.8856	+0.0433
6	0.4994	0.8497	+0.3503
7	1.0179	1.0669	+0.0490
8	0.9827	0.9077	−0.0750
9	0.9831	1.1314	+0.1473
10	1.0096	0.9582	−0.0514
11	0.8079	0.7864	−0.0215
12	1.0527	0.8815	−0.1712
13	0.8927	1.0020	+0.1093
14	1.0458	1.0280	−0.0178
15	1.8945	1.8537	−0.0408
16	1.8886	1.7969	−0.0917

Corrected values of fluorescence polarization coefficients [11] were measured using a photon counting spectrofluorometer, described by J. Paoletti [12]. Emission wavelength was at 620 nm. Ethidium bromide was dissolved in bidistilled glycerol (0.6×10^{-5} M) at 10°. Absorption spectrum and its derivative plot were obtained with a Cary 17 spectrophotometer.

3. Results and discussion

The predicted electronic charge distributions of the fundamental and the first excited states are reported in table 1.

In the fundamental state, the greatest positive charge lies on the carbon atom at position 6, which Thomas and Roques [13] postulated as the site of reduction of ethidium bromide by sodium borohydride.

Furthermore, the model of stacking of ethidium bromide cations proposed by these authors, is strongly supported by the result of this calculation. If ethidium bromide cations were stacked, with the nitrogen atoms N5 lying symmetrically in relation to the major

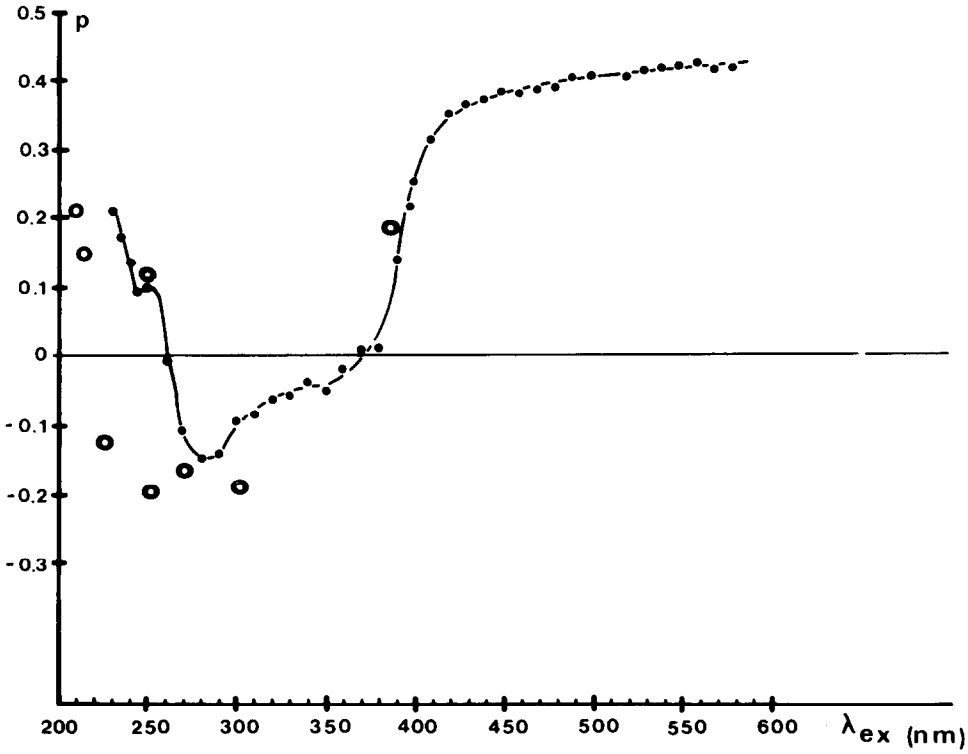

Fig. 4. Comparison of predicted and measured values of fluorescence polarization coefficients of ethidium bromide. No spectral shift has been taken into account. ○ Predicted values of p_λ for transitions 1–3 to 1–10. • Measured values of p_λ.

Table 2

Transition	1–2	1–3	1–4	1–5	1–6	1–7	1–8	1–9	1–10
Energy (nm)	445.1	385.1	301.3	271.9	250.9	249.7	226.0	211.0	202.9
Energy (eV)	2.78	3.22	4.11	4.56	4.94	4.96	5.48	5.86	5.97
Energy (cm^{-1})	22,463	25,964	33,180	36,777	39,854	40,042	44, 241	47,261	48,186
f	0.353	0.080	0.031	0.334	0.142	0.419	0.145	0.759	0.227
ϑ	214°	175°	116°	177°	179°	143°	145°	122°	190°

The symbol f refers to oscillator strength, while the symbol ϑ refers to the angle formed by the transition moment with the X axis in fig. 1; the angles are related to the trigonometric system.

axis, most interactions would be repulsive. On the other hand all eight atoms in the center of the main ring attract each other, if stacked as shown on fig. 2. Both extremities carry positive charges, and this can explain why the Br anion crystallizes near the amino groups [8].

Excitation to the first state results in a striking increase of charge density on atoms in position 13, 9 and 6, and in a corresponding decrease on atoms in the right cycle.

In table 2 we report excitation energies, oscillator strengths and directions of transition moments. As far as transitions 1–2, 1–4 and 1–5 are concerned, there is good agreement between prediction and measurements (fig. 3). The slight spectral red shift which is observed was expected from the bathochromic effect of phenyl and ethyl substituents [7]. The transition 1–3 at about 25900 cm^{-1}, undetected by ab-

sorption measurements, is clearly demonstrated by electric dichroism measurements [14]. The angles α between the moments of transitions of higher energy and the moment of the transition of the lowest energy can be determined from fluorescence polarization measurements through the well known equation [15]:

$$\frac{1}{p_\lambda} - \frac{1}{3} = \left(\frac{1}{p_0} - \frac{1}{3}\right)\left(\frac{2}{3\cos^2\alpha - 1}\right) \quad (1)$$

Where p_0 is the so called principal polarization of photoluminescence [16], and p_λ is the observed coefficient of polarization at different excitation wavelengths.

The values of α can be compared to the predicted ones to check the accuracy of the calculation. A quantitative comparison is often difficult because of the frequent overlapping of the different transitions caused by the solvent. In the present case, transition 1–3 is well isolated and is therefore suitable for such a comparison, if one takes into account a spectral shift of about 1000 cm^{-1}.

The predicted value of α for this transition (39°) is remarkably close to the measured one (35°).

The other transitions are energetically very close to each other and the analysis can only be semiquantitative.

Expected values of p_λ obtained from equation (1), taking α from table 2, are compared to measured ones in fig. 4.

The agreement between prediction and measurements is quite satisfactory, if one takes into account the relative strength of oscillators, reported in table 2.

The absolute values of oscillator strengths are not accurate: the measured value for the first transition is $f = 0.09$, while the predicted one is $f = 0.35$; they are nevertheless interesting for a comparison of relative probabilities of predicted transitions.

The same calculation has been performed for an ideal main ring (interatomic distances of 1.4 Å, bond angles of 120°) and the results are practically identical.

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