ON THE EXPLANATION OF THE APPEARANCE OF TWO FLUORESCENCE BANDS OF THE BENZIMIDAZOLE CATION

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The benzimizadole cation in weak acidic media has two completely different fluorescence spectra with regard to the position (3700 Å and 2870 Å) and shape. Comparing them with the absorption spectrum of the cation we hawe supposed that in the excited state there is the intramolecular proton transfer from nitrogen (1) to nitrogen (3). At liquid nitrogen temperature the change of acidity of the medium causes the transformation of one form of fluorescence spectrum into the other.

The monocation–dication equilibriun constant in the first excited singlet state was determined by means of fluorometric titration.

Benzimidazole as antimetabolic [1] has attracted the attention of many authors and become the object of their research. The cation-molecule equilibrium constant (pK. 5.53), and molecule-anion equilibrium constant (pK 12.3) in the ground state were determined by ALBERT *et al.* [2]. ROGEPS *et al.* [3] have determined the cation-molecule equilibrium constant in the first singlet excited state (pk 5.3) by fluorometric titration and concluded that it is not considerably different from that in the ground state.

Using WELLEL'S method [4], LONGWORTH *et al.* [5] have calculated the difference between pK_1^* and pK_1 and obtained the value $\Delta pK = 6.3$. Already in the recorded absorption and fluorescence polarization spectra SCHÜTT *et al.* [6] have noticed that the fluorescence spectrum of the cation of benzimidazole at $-180\,^{\circ}C$ consists of two different bands (longwave and shortwave bands) and that the longwave fluorescence band corresponds to the absorption band ^1L_a, whereas the shortwave fluorescence band corresponds to the ^1L_b absorption band.

KONDO *et al.* [7] suppose that the ^1L_a state of the cation in the excited state is more polar than in the ^1L_a state. Therefore the ^1L_a state is strongly stabilized by the interaction with the polar solvent molecules, while the ^1L_b state is not, and that the reversal of the two excited energy levels can occur while the separation between them is small. The results is that in aqueous solutions at room themperature only the longwave fluorescence band of the benzimidazole cation appears. This supposition is not completely supported by experimental evidence.

Experimental

Benzimidazole of the quality "prissimum" was obtained from "Fluka" and used without further purification. Ethanol, sulphuric, perchloric, phosphoric, boric and acetic acids, as well as sodium hydroxide are p. a. commercial reagents without their own luminescence. The solvent of the aqueous solutions of benzimidazole was a Britton-Robinson buffer, whereas for super alcaline and super acidic media concentrated *NaOH* and concentrated H_2SO_4 in water, respectively, were used. Hammett's acidity function (H_0) and pH values of superalcaline solutions were determined by the well known methods [8, 9].

Absorption spectra were recorded on the VSU—1 type "Zeiss" spectrophotometer, whereas luminescence spectra were recorded on a Aminco. Bowman SPF with an ellipsoidal mirror condensing system. The photomultiplier 1P28 was used for detection, whereas low temperature was attained with liquid nitrogen using a quartz Dewar-cold finger assembly. Luminescence spectra were corrected on the response of spectral sensitivity of the apparatus and detector.

Results and discussion

The absorption spectra of benzimidazole dissolved in aqueous solutions are shown in Fig. 1. The isosbestic point for three forms of dissociation: cation molecule and anion

$$BH^+ \underset{H^+}{\overset{pK_1}{\rightleftharpoons}} B \underset{H^+}{\overset{pK_2}{\rightleftharpoons}} B^-$$

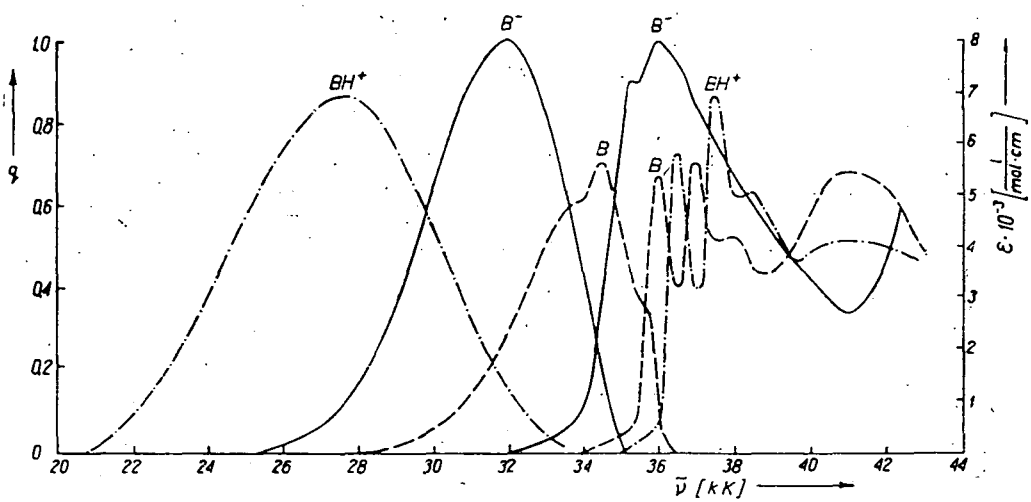

Fig. 1. Fluorescence and absorption spectra of benzimidazole (10^{-4} M) in aqueous solutions at 295 °K: cation (BH^+), pH 1.6; molecule (B), pH 8; and anion (B^-), pH 18.5

is at 39 400 cm^{-1}. Each form of dissociation has a corresponding fluorescence spectrum (Fig. 1). It is evident that in the cation fluorescence band the Stokes shift is too great so that it is completely separated from the cation absorption spectrum. The quantum yields of fluorescence in aqueous solutions at room temperature for cation, molecule and anion are 0.76, 0.80 and 0.06, respectively.

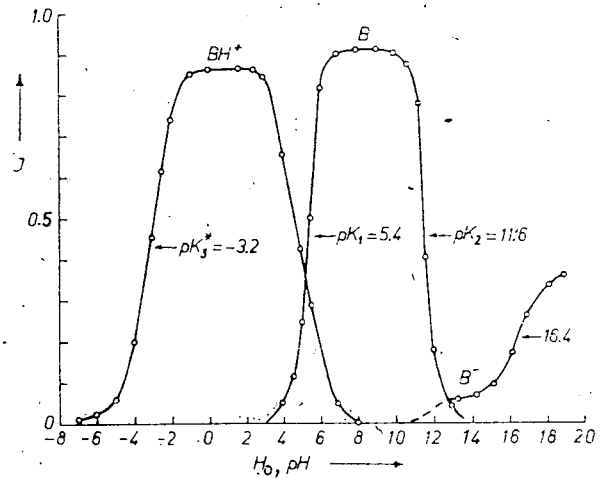

Fig. 2. Effect of H_0 and pH on relative fluorescence intensities of benzimidazole dissociation forms (10^{-4} M) activated by radiation of 2540 Å wavelength at 295 K. BH^+, B, and B^- represent the fluoremetric titration curves for monocation, molecule and anion, respectively

Fig. 2. shows the pH change effects upon the fluorescence characteristics of benzimidazole dissolved in water (10^{-4} M). The fluorescence intensities of various forms of dissociation are related in the same way as its quantum yields. The pK values of the cation-molecule and molecule-anion equilibria (pK_1 5.4 pK_2 11.6) obtained from the change in fluorescence intensity showed no real difference from those obtained from hydrogen ion titration (5.53, 12.3).

From the shift of the half-height of the longwave absoprtion band caused by by ionization, using Weller's equation, we obtained pK_1^* 4.2 for the cation-molecule equilibrium constant, and pK_2^* 10.2 for the molecule-anion equilibrium constant in the excited singlet state. The same constants calculated from the shift of fluorescence spectra caused by ionization have the following values: pK_1^* 16.2 and pK_2^* 11.1

Fig. 2. shows the fall of the cation fluorescence intensity with the half-height at H_0 −3.2. This process is reversible. Therefore it can bee supposed that the abovementioned fluorescence intensity change is caused by the protonation of monocation ion in the excited state, while dication is nonfluorescencent. According to this supposition, it follows that the monocation-dication equilibrium constant in the excited state is pK_3^* −3.2, and that the equilibrium is completetly established during the excited state of monocation. The corresponding eqiulibrium constant in the ground state is $pK_3 < -10$ because, as we have said, the absorption spectrum does not change

essentially up to H_0 −8.8. Thus the monocation in the excited state is a stronger base than in the ground state.

In superalkaline aqueous solutions (pH 15—19) there is considerable increase of fluorescence intensity with half-height at pH 16.4 without a simultaneous change of the absorption spectrum of anion. The process is reversible and the fluorescence spectrum is not essentially different from that of anion which can be recorded in the region pH 13—15. However, there is little probability that this is the molecule-anion equilibrium in the excited state at pK_2^* 16.4, considering that by Weller's formula as has been said earlier, considerably lower values of pK_2^* (10.2 and 11.1) are obtained.

The fluorescence specrta of benzimidazole cation in the solution ethanol +0.01 N of sulphuric acid at room temperature and liquid nitrogen termperature are shown in Fig. 3. together with the absorption spectrum of the same solution at room temperature. These two fluorescence bands of benzimidazole cation are well known in literature [6, 7]. The longwave band (3700 Å) corresponds to that in aqueous solution in Fig. 1., with very great Stokes shift, whereas the shortwave band (2870 Å) has negligable Stokes shift and mirror symmetry with ^1L_b absorption band.

Our supposition for the appearance of two fluorescence bands of cation, so very far from each other (about 700 Å), is the following: In the excited singlet electronic state in the cation of benzimidazole, in weak acidic solutions at room temperature, there is the intramolecular proton transfer from nitrogen (1) to nitrogen (3), followed by fluorescence emission in the form of the longwave fluorescence band (3700 Å) of cation, as shown on the Diagram I.

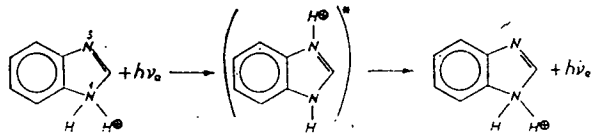

Diagram I.

Thus, the cation of one structure absorbs, whereas the cation of the other structure emits radiation. In the ground state the basicity of nitrogen (1) is higher than that of nitrogen (3), which results from the shift of the absorption ^1L_b band of cation and molecule, and the protonation of nitrogen (1) occurs. However, nitrogen (1) [N] in the excited state is a weaker base than in the ground state ($pK_1^* > pK_1$), whereas nitrogen (3) [N] in the excited state is a stronger base than in the ground state |4| ($pk_1^* > pK_1$). That is the cause of the proton transfer from nitrogen (1) to nitrogen (3) during the excited state of cation.

At room temperature in the aqueous solutions the process of proton transfer shown on Diagram I is practically completed. However, in ethanol solutions a shortwave fluorescence band (2870 Å) of weak intensity is seen, whereas in *n*-hexane solution these two fluorescence bands of cation are approximately of the same intensity (Fig. 3.). Thus with the increase of the polarity of the solvent there is an increase of the intensity of fluorescence band 3700 Å, while that of the band 2870 Å decreases. At liquid nitrogen temperature this proton transfer almost disappears during the excited state of cation, so that only 2870 Å band appears.

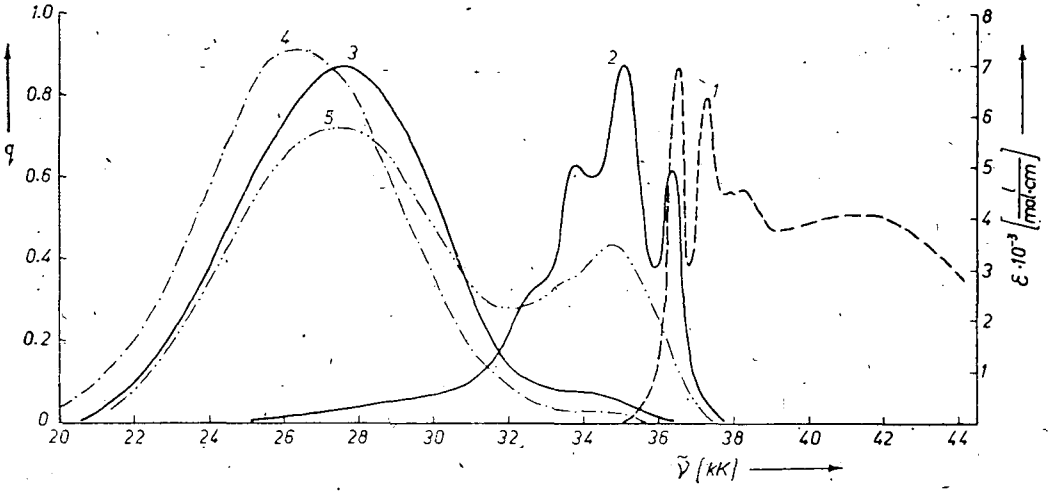

Fig. 3. Absorption and fluorescence spectra of benzimidazole monocation (10^{-4} M):
1. Absorption spectrum in ethanol+0.01 N H_2SO_4 solution at 295 K;
2 and 3. Fluorescence spectrum in ethanol+0.01 N H_2SO_4 solution at 77 K and 295 °K, respectively;
4. Fluorescence spectrum in aqueous solution (pH. 1.85) at 295 K;
5. Fluorescence spectrum in n-hexane+0.08 N CCl_3COOH solution at 295 K

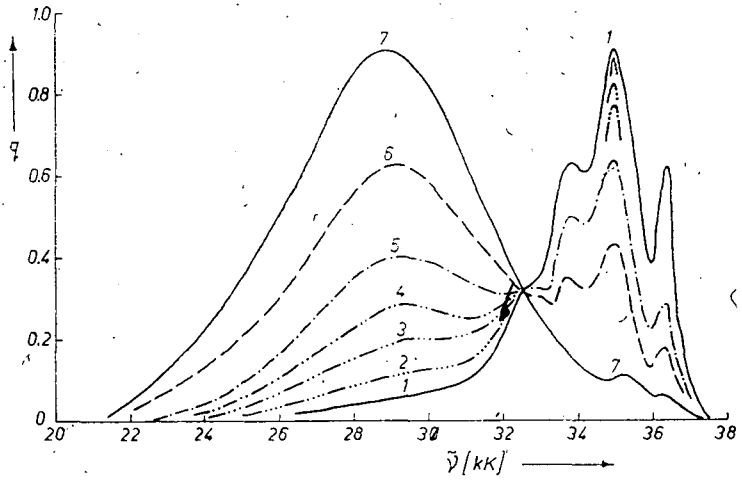

Fig. 4. Fluorescence spectra of benzimidazole (10^{-4} M) at 77 K in ethanol+H_2SO_4 of different concentrations of H_2SO_4:
1. — 10^{-2} M; 2. — 0.8 M; 3— 2.3 M: 4. — 4.3 M; 5. — 8.2 M: 6. — 12.3 M; 7. — 18 M

With the increase of the concentration of sulphuric acid in ethanol solution at liquid nitrogen temperature the fluorescence band 3700 Å appears along with the disappearance of the fluorescence band 2870 Å. (Fig. 4.) At the concentration of sulphuric acid of 18 M the band 2870 Å virtually disappears, whereas the band 3700 Å attains maximum intensity. Under the conditions of high concentrations of the hydrogen ions the solutions, during the excited state of cation, even in solid solutions, the synchronous process of deprotonation of nitrogen (1) and protonation of nitrogen (3) occurs.

The experimental results given in this paper concerning the fluorescence of benzimidazole monocation are not contradictory to the hypothesis of KONDO *ET AL.* [7], given for the explanation of the appearence of the longwave band (3700 Å) of the benzimidazole monocation.

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ОБ ОБЪЯСНЕНИИ ДВУХ ПОЛОС ФЛУОРЕСЦЕНЦИИ КАТИОНА БЕНЗИМИДАЗОЛА

И. Янич, П. Ристич и Й. Цайко

Бензимидазол в слабокислой среде имеет два различныеых, по длине волны (3700 Å и 2870 Å) и по форме спектра, флуоресценции. Сравнивая их со спектром поглощения катиона мы предполагаем, что в возбужденном состоянии имеет место внутримолекулярный перенос протона от азота (1) к азоту (3). При температуре жидкого азота изменение кислотности среды вызывает переход от одной формы спектра флуоресценции к другой. Путем флуориметрической титрации была определена константа равновесия монокатион-дикатион в первом синглетном состоянии.