

A spectrophotometric study of the protonation processes of some *N*-[1-(benzimidazol)-1-yl]methylbenzamide derivatives

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The protonation of *N*-[1-(benzimidazol)-1-yl]methylbenzamide derivatives in aqueous acids (H₂SO₄) media was investigated, using a spectrophotometric method. The investigated compounds have two protonation processes. The first protonation process occurs in weakly acidic aqueous solutions (pH range) and refers to the protonation of the benzimidazole part of the molecule. The second protonation process occurs in concentrated sulfuric acid solutions and refers to protonation of the amide group. The protonation constants of the second process were calculated by the Hammett and Cox-Yates method. The effect of chemical structure on the ionisation constants is discussed. A correlation between the protonation constants and antimicrobial activity was established.

Keywords: spectrophotometry, *N*-[1-(benzimidazol)-1-yl]methylbenzamide derivatives, protonation constants, antimicrobial activity.

Benzimidazoles represent a well known group of physiologically active compounds. A range of benzimidazoles is now important as clinically useful drugs, such as human and veterinary anthelmintics, fungicides, antineoplastics, bactericides, antihistaminics, vasodilators, hypotensives, local anesthetics and spasmolytic agents.¹⁻⁷

Benzimidazoles and their derivatives act as weak organic bases in acidic media. A knowledge of the acidity constants of weakly basic substrates is of central importance to the study of reaction mechanisms taking place in acidic media. Biological activity often depends upon the protonation constants (pK_{BH^+}) of the investigated compounds, as one of the parameters in quantitative structure-activity relationships (QASR).^{8,9}

The aim of this work was to determine the protonation constants of a series of *N*-[1-(benzimidazol)-1-yl]methylbenzamide derivatives and to find whether they correlate with the biological activity.

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EXPERIMENTAL

Materials

The structures of the investigated compounds, synthesized by a procedure described elsewhere,^{10,11} are presented in Table I.

TABLE I. Investigated compounds

1.	<i>N</i> [(1-(benzimidazol)-1-yl)methyl]benzamide	R ₁
2.	<i>N</i> [(1-(benzimidazol)-1-yl)methyl-2-chlorobenzamide	R ₂
3.	<i>N</i> [(1-(benzimidazol)-1-yl)methyl-4-chlorobenzamide	R ₃
4.	<i>N</i> [(1-(benzimidazol)-1-yl)methyl-2-fluorobenzamide	R ₄
5.	<i>N</i> [(1-(benzimidazol)-1-yl)methyl-4-methoxybenzamide	R ₅
6.	<i>N</i> [(1-(benzimidazol)-1-yl)methyl-4-methylbenzamide	R ₆

The compounds were characterized by melting point determination and IR and NMR spectroscopy, as well as by elemental analysis. Stock substrate solutions (concentration of 10^{-3} mol dm⁻³) were prepared in ethanol. The concentration of the test aqueous solutions of the investigated compounds during the determination of the pK_{BH^+} values was $c = 5 \cdot 10^{-5}$ mol dm⁻³ in the weakly acidic solutions (pH range 1.00 – 6.20) and $c = 2 \cdot 10^{-5}$ mol dm⁻³ for the determination of $pK_{BH_2^{2+}}$ in the more acidic solutions (from 1.00–17.60 mol dm⁻³ H₂SO₄). The content of ethanol in the test aqueous solutions was 2 – 5 %. All other used chemicals were of analytical grade purity.

Spectral and pH measurements

Absorption spectra were taken on a VARIAN CARY 219 spectrophotometer in 1 cm quartz cells at 25 °C. The spectra were recorded in weakly acidic solutions (pH 6.2 to 1), as well as in solutions of 1 mol dm⁻³ to 17.60 mol dm⁻³ H₂SO₄. The reference was an acid solution which contained the same quantity of ethanol as the test solutions.

In the weakly acidic solutions, the ionic strength was kept constant by the addition of sodium perchlorate ($I = 0.1$ mol dm⁻³). The pH values of the solutions in the range of 2–7 were measured by an ISKRA MA-5704 pH-meter. The concentration of concentrated sulfuric acid solutions was determined from density measurements at 25 °C by precise density meter.¹² H_A^{13} and X^{14} acidity functions were used for the characterization of the concentrated sulfuric acid solutions.

Antimicrobial investigations

The filter paper disc method was applied for the antimicrobial investigations. Each of the investigated isolates of bacteria was seeded in tubes with nutrient broth (NB). 1 cm³ of seeded NB was homogenized in the tubes with 9 cm³ of melted nutrient agar (NA). The tubes with NA were kept in a bath water at 45 °C. The homogenous suspension was poured out in Petri dishes.

The discs of filter paper (diameter 5 mm) were arranged on the cool medium. After cooling on the formed solid medium, 20 ml of the investigated compounds ($c = 10^{-4}$ to $c = 5 \cdot 10^{-2}$ mol dm⁻³ in dimethylformamide) were placed on the filter papers by micropipette. After 24 h incubation in a thermostat at 25 – 27 °C, the inhibition zone diameters were measured and the antibacterial activities of the investigated compounds estimated. Every test was done in triplicates. I_{50} values were determined in the way described in the literature.¹⁵

The antibacterial activities of investigated compounds were tested against *Erwinia Carotovora* subsp. *Carotovora* (bacterial soft rot of vegetable origin).

RESULTS AND DISCUSSION

Absorption spectra

The protonation of the *N*-[1-(benzimidazol)-1-yl]methylbenzamide derivatives ($R_1 - R_6$) was investigated by a spectrophotometric method, following the changes in the electronic absorption spectra of aqueous solutions of the investigated compounds in sulfuric acid.

The electronic absorption spectra of the investigated compounds in neutral media are characterized by the presence of absorption maxima: at ≈ 240 nm, ≈ 270 nm, ≈ 278 nm (Fig. 1, Table II). The absorption bands at ≈ 270 nm, ≈ 278 nm are characteristic for the benzimidazole part of the molecule.¹⁶ The absorption band at ≈ 240 nm is characteristic for the benzamide part of the molecule.¹⁷ With increasing acidity (pH 6.80 – 1.00), the absorption spectra show hypsochromic shifts of the absorption maxima (≈ 268 nm and ≈ 274 nm) of about 3 nm (Fig. 1, Table II). This hypsochromic shift of the absorption maxima was followed by the appearance of clear isosbestic points (≈ 220 nm, ≈ 255 nm and ≈ 275 nm). These spectral changes result from the protonation of the benzimidazole part of the molecule.^{18,19} Increasing the acidity from 1 mol dm⁻³ to 17 mol dm⁻³ H₂SO₄ shifts the absorption maximum (≈ 240 nm) to longer wavelengths with increasing absorption intensity (Fig. 2). These spectral changes result from the protonation of the benzamide part of the molecule.¹⁷

TABLE II. Ultraviolet spectral data of different ionic forms

Compound	Ionic form	Acidity	λ_{\max} /nm	$\epsilon_{\max} \cdot 10^{-4} / \text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$
R ₁	B	pH 6.80	233; 271; 279	2.51; 0.85; 0.62
	BH ⁺	pH 1.36	230; 269; 275	2.40; 1.25; 1.03
	BH ₂ ²⁺	$H_A = -5.63$	230; 260	0.92; 3.85
R ₂	B	pH 6.80	242; 269; 277	3.20; 1.65; 1.25
	BH ⁺	pH 1.54	240; 266; 273	2.55; 2.50; 2.02
	BH ₂ ²⁺	$H_A = -5.63$	240; 260	3.20; 5.15
R ₃	B	pH 6.80	243; 271; 279	4.15; 1.15; 0.80
	BH ⁺	pH 1.59	243; 266; 274	3.65; 1.65; 1.25
	BH ₂ ²⁺	$H_A = -5.28$	243; 268	1.55; 4.75
R ₄	B	pH 6.80	235; 270; 278	2.25; 0.90; 0.65
	BH ⁺	pH = 1.30	235; 268; 275	2.05; 1.50; 1.20
	BH ₂ ²⁺	$H_A = -5.89$	235; 260	1.20; 3.40
R ₅	B	pH 6.80	252; 269; 277	3.90; 2.62; 1.55
	BH ⁺	pH 1.42	252; 266; 273	3.50; 3.70; 2.85
	BH ₂ ²⁺	$H_A = -5.89$	252; 270	1.10; 3.00
R ₆	B	pH 6.80	251; 268; 279	3.45; 2.35; 1.20
	BH ⁺	pH 1.80	251; 266; 274	3.20; 2.95; 2.20
	BH ₂ ²⁺	$H_A = -5.89$	251; 281	1.25; 3.70

The plots of absorbancy against pH and acidity functions give sigmoid curves (Figs. 1. and 2.) which confirm the formation of two ionic forms.

Calculation of the first protonation constants

The first ionisation constants (pK_{BH^+}) were calculated from the dependencies of the absorbance vs. pH curves, at wavelengths characteristic for the protonation of the benzimidazole part of the molecules ($\gg 266$ nm and 274 nm, Fig. 1), according to a known spectrophotometric method²⁰:

$$pK_{BH^+} = \log I + m \cdot \text{pH} \quad I = \frac{A - A_B}{A_{BH^+} - A_B} = \frac{A - A_B}{A_{BH^+} - A_B} \quad (1)$$

under the condition that the slope parameter $m = 1$ (m – represent the number of protons bonded to a base molecule B). A_B is the absorbance of the unprotonated

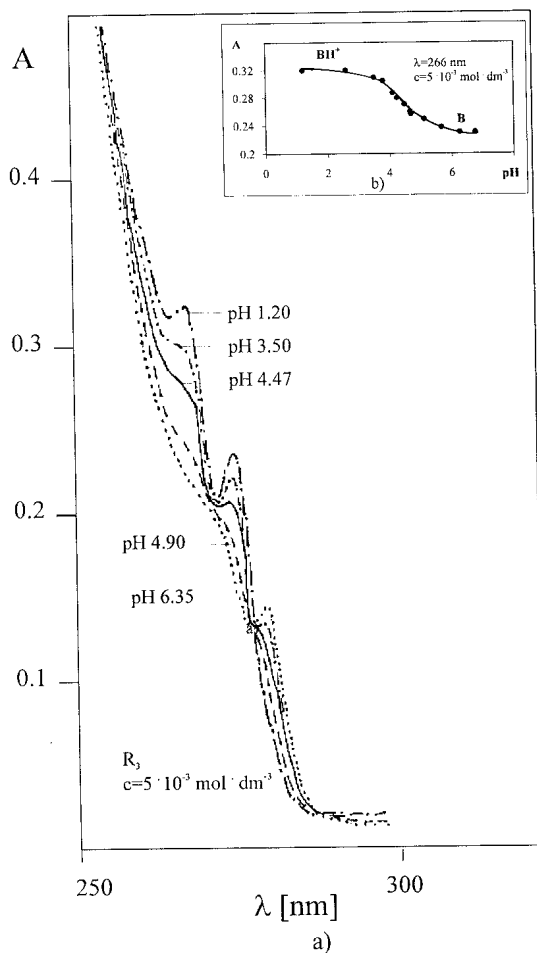


Fig. 1. a) Absorption spectra of *N*-[1-(benzimidazol)-1-yl]methyl-4-chlorobenzamide (R_3) in solutions of different acidity (pH) -protonation of the benzimidazole part of molecule. b) Dependence of A on pH.

form, A_{BH^+} (Fig. 1) is the absorbance of the protonated form and A is the absorbance of the solution at the given acidity and the same wavelength λ .

The protolytic equilibria in the weakly acidic media are separated by more than three pK_{BH^+} units from the equilibria in the more concentrated acid solutions. The first protonation constants were determined independently, as for monoprotic bases. The results are presented in Table III.

Calculation of the second protonation constants

The second protonation process occurs at high acidity levels, outside the pH scale, and presents the protonation of the amide groups. The $pK_{BH_2^{2+}}$ values were calculated from the changes in the absorption spectra vs. acidity. The increase of acidity from $c = 1 \text{ mol dm}^{-3}$ to 17 mol dm^{-3} H_2SO_4 shifts the absorption maximum at $\approx 240 \text{ nm}$ to longer wavelengths at $\approx 260 \text{ nm}$ with increasing intensity of the absorption (Fig. 2a). Plots of A vs. acidity for these characteristic absorption maxima show that full protonation of the investigated compounds was achieved (Fig. 2b). The absorbance changes vs. acidity for the characteristic absorption maxima ($\approx 240 \text{ nm}$ and $\approx 260 \text{ nm}$) were used for the calculation of the ionisation ratio I (Eq. 1).

TABLE III. The protonation constant values and antimicrobial activity against *Erwinia carotovora* subsp. *carotovora*

Compound	I. Benzimidazole group protonation		Antimicrobial activity
	pK_{BH^+}	m	$\log \frac{1}{c}$
R ₁	4.45–0.03	0.98	3.35
R ₂	4.10–0.06	1.03	2.25
R ₃	4.18–0.04	0.95	2.80
R ₄	4.29–0.05	1.05	2.60
R ₅	4.85–0.04	1.02	3.55
R ₆	4.63–0.07	0.92	3.40

Compounds	II. Amide group protonation			
	HAFM ¹		EAFM ²	
	$pK_{BH_2^{2+}}$	Slope parameter m	$pK_{BH_2^{2+}}$	Slope parameter m^1
R ₁	–3.25–0.05	0.89	–3.07–0.02	0.52
R ₂	–3.80–0.08	0.98	–3.39–0.07	0.54
R ₃	–3.76–0.05	0.93	–3.82–0.05	0.57
R ₄	–3.95–0.05	1.02	–3.97–0.08	0.58
R ₅	–2.82–0.06	1.01	–2.75–0.03	0.61
R ₆	–3.08–0.03	0.98	–2.78–0.02	0.65

¹Hammet acidity function method. ²"Excess acidity" function method.

The ionisation constants ($pK_{BH_2^{2+}}$) were determined using two methods:

1. Hammett acidity function method²⁰ (HAFM)

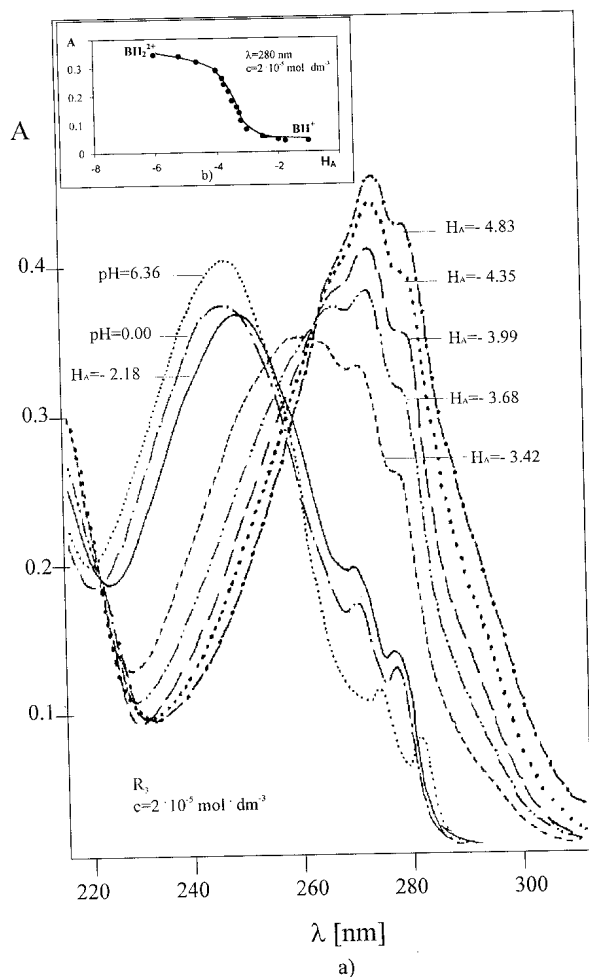


Fig. 2. a) Absorption spectra of *N*-[1-(benzimidazol)-1-yl]methyl-4-chlorobenzamide (R_3) in solutions of different acidity (H_A) -protonation of the amide group. b) Dependence of A on H_A .

$$pK_{BH_2^{2+}} = \log I + m H_x \quad (2)$$

where the H_A acidity function was used as H_x .²⁰ The slope parameter, m , of $\log I$ vs. H_A has the value $m = 1$, when the used method and the acidity function describe satisfactorily the protonation process.

2. The "Excess acidity" function method¹⁴ (EAFM), permits the accurate determination of pK_{BH^+} values for any base in any acidity region. This includes protonation of N, C, O and S atoms, and bases of any charge type: negative, positive and dipositive, as well as neutral. The ionisation constant is given as follows:

$$pK_{BH^n^{n+}} (\log I - \log c_{H^+}) - m^* \cdot X \quad (3)$$

where c_H^+ is the proton concentration and X the excess acidity, the values of which, as a function of weight percent composition, are available for the aqueous sulfuric acid system.¹⁴ The slope parameter m^+ expresses the sensitivity of the substrate to the changing acidity.

The values of $pK_{BH_2^{2+}}$ calculated from the spectrophotometric data using Eqs. (2) and (3) are summarized in Table III. The results for second protonation constants obtained by HAFM method (Table III) show that the present bases closely follow the H_A acidity function with m values near unity. The $pK_{BH_2^{2+}}$ values determined by the HAFM and EAFM methods show satisfactory agreement. The $pK_{BH_2^{2+}}$ values and the values of the slope parameter m^* (EAFM method) are in agreement with reported values for amide protonation.^{14,22} The obtained results show that the investigated compounds are weaker bases than benzimidazoles²¹ or benzamides.^{14,22,23}

Substituent effect

Plots of the ionisation constants values of the *N*-[1-(benzimidazol)-1-yl]methylbenzamide derivatives substituted in the *para* position, against Hammett's σ ²⁴ constants are linear (Fig. 3) and in good agreement with the Hammett equation²⁴:

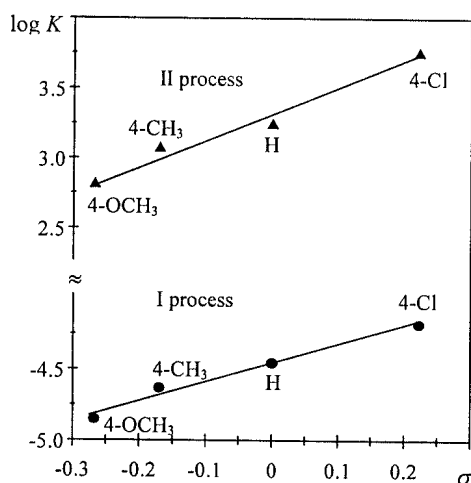


Fig. 3. Dependence of the protonation constant values ($\log K$) against Hammett's substituents constants, σ .

$$\log K_{BH^+} = r \cdot \sigma + \log K_{BH^+}^0 \quad (4)$$

where K_{BH^+} is the ionisation constant of the substituted compound and $K_{BH^+}^0$ is the ionisation constant of the unsubstituted compound. The parameters of the Hammett equation are listed in Table IV.

The large value of the reaction constant r for the second protonation process shows the large substituent effect on the protonation of the amide group.

TABLE IV. The values of the reaction constants τ

	I protonation process	II protonation	process
		HAFM	EAFM
τ	1.31–0.12	1.83–0.19	2.23–0.43
r	0.990	0.988	0.963

Structure activity relationship

The quantitative structure-activity relationships is based on the correlation of the observed potency of bioactivity with physicochemical, molecular and substituent parameters among a series of congeneric compounds.²⁵ The correlation of antibacterial activity of the *para* substituted derivatives (R_1 , R_3 , R_5 , R_6) towards *Erwinia carotovora subsp. Carotovora* and the protonation constants, pK_{BH^+} , as well as the substituent parameters (ρ^8 and s^{24}) are described by the equation:

$$\log \frac{1}{c} = 0.734 pK_{BH^+} - 0.2612 s - 0.222 \rho + 0.001 \quad (5)$$

$$(r = 0.998; s = 0.107)$$

Compounds with *ortho* substituents deviate from this Eq. (5). We assume that this deviation is caused by a steric effect.

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ИЗВОД

СПЕКТРОФОТОМЕТРИЈСКО ИСПИТИВАЊЕ ПРОЦЕСА ПРОТОНАЦИЈЕ НЕКИХ ДЕРИВАТА N-[1-(БЕНЗИМИДАЗОЛ)-1-ИЛ]МЕТИЛ-БЕНЗАМИДА

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Процес протонације деривата N-[1-(бензимидазол)-1-ил]метилбензамида је испитан у воденим растворима сумпорне киселине, применом спектрофотометријске методе. Испитивана једињења показују два процеса протонације. Први процес протонације се јавља у слабо киселим воденим растворима рН области и одговара протонацији бензимидазоловог дела молекула. Други процес протонације се јавља у растворима концентроване киселине (ван области рН) и одговара протонацији амидне групе. За други процес протонације, константе протонације су одређене применом Hammett-ове и Cox-Yates методе. Продискутован је утицај хемијске структуре на јонизационе константе.

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